Gastric Cancer Literature

Mark H Smith

Queens, New York 11418, USA
mark20082009@gmail.com

Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, the cancer can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the gastric cancer.
[Smith MH. **Gastric Cancer Literature.** *Cancer Biology* 2012;2(3):75-132]. (ISSN: 2150-1041). http://www.cancerbio.net. 7

Keywords: cancer; biology; life; disease; research; literature; gastric

1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

Literatures

Al-Moundhri, M. S., M. Al-Nabhani, et al. (2009). "Gastric cancer risk predisposition and prognostic significance of vascular endothelial growth factor (VEGF) gene polymorphisms--a case-control study in an Omani population." Mol Carcinog **48**(12): 1170-6.

Vascular endothelial growth factor (VEGF) plays a central role in angiogenesis, tumor growth, and metastasis. We investigated the associations between VEGF gene polymorphisms and gastric cancer (GC) risk predisposition and prognostic characteristics in an Omani population, an ethnic group which has not been studied previously. We analyzed three VEGF polymorphisms (+405 G/C, -460 T/C, and +936 C/T) by the extraction of genomic DNA from peripheral blood of 130 GC patients and 130 control subjects followed by VEGF genotyping using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis. There were no significant associations between the VEGF polymorphisms and GC risk. There were significant correlations between the +405 C/C genotype and both poor tumor differentiation (P = 0.007) and lymph node metastasis (P = 0.03) and between the -460 T/T genotype and poor tumor differentiation (P = 0.03) with a statistical trend toward lymph node involvement (P = 0.05). VEGF gene polymorphisms had no significant effects on survival, but the VEGF +405 G/G genotype had a statistical trend toward lower survival rate with a hazard ratio of 1.6 [95% CI, 0.9-2.9] compared with the VEGF +405 CC/GC combined genotype (P = 0.049). Multivariate analysis showed that disease stage at diagnosis and the +405 G/G genotype were independent variables of adverse prognostic significance. There were no associations between the six common haplotypes identified and both GC risk predisposition and survival. The current study suggests that VEGF polymorphisms have no role in GC risk predisposition, but may have prognostic significance in GC patients.

Alvarez, C. J., M. Lodeiro, et al. (2009). "Obestatin stimulates Akt signalling in gastric cancer cells through beta-arrestin-mediated epidermal growth factor receptor transactivation." Endocr Relat Cancer **16**(2): 599-611.

Obestatin was identified as a gut peptide encoded by the ghrelin gene that interacts with the G protein-coupled receptor, GPR39. In this work, a sequential analysis of its transmembrane signalling pathway has been undertaken to characterize the intracellular mechanisms responsible for Akt activation. The results show that Akt activation requires the phosphorylation of T308 in the A-loop by the phosphoinositide-dependent kinase 1 (PDK1) and S473 within the HM by the mammalian target of rapamycin (mTOR) kinase complex 2 (mTORC2: Rictor, mLST8, mSin1, mTOR kinase) with participation neither of G(i)(/o)-protein nor Gbetagamma dimers. Obestatin induces the association of GPR39/beta-arrestin 1/Src signalling complex resulting in the transactivation of the epidermal growth factor receptor (EGFR) and downstream Akt signalling. Upon administration of obestatin, phosphorylation of mTOR (S2448) and p70S6K1 (T389) rise with a time course that parallels that of Akt activation. Based on the experimental data

obtained, a signalling pathway involving a beta-arrestin 1 scaffolding complex and EGFR to activate Akt signalling is proposed.

Ando, T., T. Ishikawa, et al. (2009). "Synergistic effect of HLA class II loci and cytokine gene polymorphisms on the risk of gastric cancer in Japanese patients with Helicobacter pylori infection." Int J Cancer **125**(11): 2595-602.

It has been reported that polymorphisms of human leukocyte antigen (HLA) genes and several cytokine genes are associated with an increased risk of developing gastric cancer (GC). However, the results of studies from different geographic regions, ethnic groups and study groups are inconsistent. The aim of this study was to evaluate the influence of H. pylori infection and host genetic factors on GC susceptibility in Japanese patients with GC. We analyzed genotypes for HLA class I and II, tumor necrosis factor alpha, interleukin (IL)-1beta, IL-1 receptor, IL-4, IL-4Ralpha and IL-10 in 330 H. pylori-infected noncardia patients with GC and 190 H. pylori-infected nonulcer dyspeptic controls. Haplotype analyses indicated that the frequencies of the HLA DRB1*0405 and DQB1*0401 alleles were increased in the patients with intestinal-type GC when compared with controls (both DRB1*0405 and DQB1*0401: p = 0.015, OR = 1.57, 95% CI = 1.09-2.26), but the changes were not statistically significant after correction for multiple comparisons. None of the cytokine gene polymorphisms were associated with GC susceptibility, whether patients with GC were analyzed as a group according to the histological subtype. Of interest was the comparison of controls and patients with intestinal-type GC. The frequency of an IL-10-592AA homozygote showing concomitant carriage of the HLA DRB1*0405-DQB1*0401 haplotype was significantly higher in patients with intestinal-type GC (chi(2) = 6.369, p = 0.0116, p(c) = 0.0464, OR = 2.43, 95% CI = 1.21-4.48). Our results suggest that the HLA class II and IL-10-592A/C polymorphisms synergistically affect the susceptibility to GC development of H. pylori-infected individuals in the Japanese population.

Argent, R. H., R. J. Thomas, et al. (2008). "Toxigenic Helicobacter pylori infection precedes gastric hypochlorhydria in cancer relatives, and H. pylori virulence evolves in these families." Clin Cancer Res **14**(7): 2227-35.

PURPOSE: Helicobacter pylori infection by virulent strains is associated with gastric adenocarcinoma. We aimed to determine whether infection with virulent H. pylori preceded precancerous gastric hypochlorhydria and atrophy in gastric cancer relatives and quantify the extent of virulence factor evolution. EXPERIMENTAL DESIGN: H. pylori strains from 51 Scottish gastric cancer relatives were characterized by genetic fingerprinting and typing the vacuolating cytotoxin gene (vacA), the cytotoxin-associated gene (cagA), and housekeeping genes. We phenotyped strains by coculture with gastric epithelial cells and assessing vacuolation (microscopy), CagA tyrosine phosphorylation (immunoblot), and interleukin-8 secretion (ELISA). RESULTS: Toxigenic (vacA type s1/m1) H. pylori was associated with precancerous gastric hypochlorhydria (P<0.01). Adult family members with this type of H. pylori had the same strain as currently noncohabiting adult family members in 68% cases, implying acquisition during childhood from each other or a common source. We analyzed different isolates of the same strain within families and showed that H. pylori commonly microevolved to change virulence: this occurred in 22% individuals and a striking 44% cases where the strain was shared within families. Microevolution in vacA occurred by extragenomic recombination and in cagA by this or duplication/deletion. Microevolution led to phenotypic changes in virulence. Passage of microevolved strains could be tracked within families. CONCLUSIONS: Toxigenic H. pylori infection precedes and so likely causes gastric hypochlorhydria, suggesting that virulent H. pylori increases cancer risk by causing this condition. Microevolution of virulence genes is common within families of gastric cancer patients and changes H. pylori virulence.

Belair, C., F. Darfeuille, et al. (2009). "Helicobacter pylori and gastric cancer: possible role of microRNAs in this intimate relationship." Clin Microbiol Infect **15**(9): 806-12.

Chronic infection by Helicobacter pylori is a major risk factor for gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. H. pylori possesses a set of virulence factors, including the CagA effector, which interferes with intracellular signalling pathways and mediates phenotypic alterations, strongly evoking neoplasic transformation. MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression involved in development, cell proliferation and immune responses. miRNAs are frequently altered in cancers, revealing their functions as oncogenes or tumour suppressors. However, the role, if any, that miRNAs play in the host cell responses to H. pylori remains unknown. This review considers the possible involvement of some miRNAs, including miR-146, miR-155, miR-21, miR-27a, miR-106-93-25 and miR-221-222 clusters and the miR-200 family in H. pylori-induced infection and gastric cancers. Further exploration of miRNA-mediated gene silencing,

taking into account the relationship between host targets and bacterial effectors, will most certainly bring new insights into the control of gene expression in human gastric cells chronically infected by H. pylori.

Bertazza, L., S. Mocellin, et al. (2009). "Survivin gene levels in the peripheral blood of patients with gastric cancer independently predict survival." J Transl Med **7**: 111.

BACKGROUND: The detection of circulating tumor cells (CTC) is considered a promising tool for improving risk stratification in patients with solid tumors. We investigated on whether the expression of CTC related genes adds any prognostic power to the TNM staging system in patients with gastric carcinoma. METHODS: Seventy patients with TNM stage I to IV gastric carcinoma were retrospectively enrolled. Peripheral blood samples were tested by means of quantitative real time PCR (qrtPCR) for the expression of four CTC related genes: carcinoembryonic antigen (CEA), cytokeratin-19 (CK19), vascular endothelial growth factor (VEGF) and Survivin (BIRC5). RESULTS: Gene expression of Survivin, CK19, CEA and VEGF was higher than in normal controls in 98.6%, 97.1%, 42.9% and 38.6% of cases, respectively, suggesting a potential diagnostic value of both Survivin and CK19. At multivariable survival analysis, TNM staging and Survivin mRNA levels were retained as independent prognostic factors, demonstrating that Survivin expression in the peripheral blood adds prognostic information to the TNM system. In contrast with previously published data, the transcript abundance of CEA, CK19 and VEGF was not associated with patients' clinical outcome. CONCLUSIONS: Gene expression levels of Survivin add significant prognostic value to the current TNM staging system. The validation of these findings in larger prospective and multicentric series might lead to the implementation of this biomarker in the routine clinical setting in order to optimize risk stratification and ultimately personalize the therapeutic management of these patients.

Chen, C. N., C. C. Chang, et al. (2009). "Identification of calreticulin as a prognosis marker and angiogenic regulator in human gastric cancer." Ann Surg Oncol **16**(2): 524-33.

The purpose of this study was to identify genes of interest for a subsequent functional and clinical cohort study using complementary (c)DNA microarrays. cDNA microarray hybridization was performed to identify differentially expressed genes between tumor and nontumor specimens in 30 gastric cancer patients. Subsequent functional studies of the selected gene were carried out, including cell cycle analysis, cell migration analysis, analyses of vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF), and oligo-microarray studies using two pairs of stable cell lines of the selected gene. Another independent cohort study of 79 gastric cancer patients was conducted to evaluate the clinical significance of the selected gene in human gastric cancer. Calreticulin (CRT) was selected for further investigation. Two pairs of stable cell lines of CRT overexpression and CRT knockdown were constructed to perform functional studies. CRT enhanced gastric cancer cell proliferation and migration. Overexpressed CRT upregulated the expression and secretion of PlGF and VEGF. CRT had a reciprocal effect on connective tissue growth factor (CTGF) expression. Positive immunohistochemical staining of calreticulin was significantly correlated with high microvessel density (MVD) ($p = 0.014$), positive serosal invasion ($p = 0.013$), lymph node metastasis ($p = 0.002$), perineural invasion ($p = 0.008$), and poor patient survival ($p = 0.0014$). Multivariate survival analysis showed that CRT, MVD, and serosal invasion were independent prognosticators. We conclude that CRT overexpression enhances angiogenesis, and facilitates proliferation and migration of gastric cancer cells, which is in line with the association of CRT with MVD, tumor invasion, lymph node metastasis, and survival in gastric cancer patients.

Chen, W., L. Wang, et al. (2008). "The role of IGFBP3 functional polymorphisms in the risk of gastric cancer in a high-risk Chinese population." Eur J Cancer Prev **17**(2): 82-7.

Insulin-like growth factors (IGFs) and their receptors play a crucial role in regulating cell proliferation, differentiation, and apoptosis. Insulin-like growth factor-binding protein-3 is the most abundant insulin-like growth factor receptor in the serum and binds the majority of insulin-like growth factors. Studies reported that circulating level of insulin-like growth factor-binding protein-3 was modulated by functional genetic variants of insulin-like growth factor-binding protein-3 and, therefore, maybe associated with the risk of gastric cancer. In this case-control study of 576 gastric cancer cases and 647 cancer-free control participants in a high-risk Chinese population, we tested the hypothesis that functional polymorphisms A-202C and Gly32Ala of insulinlike growth factor-binding protein-3 are associated with risk of gastric cancer. We found that the variant 32Ala allele was associated with a significantly increased risk of gastric cancer (adjusted odds ratio=1.84, 95% confidence interval=1.45-2.33 for 32Gly/Ala and odds ratio=2.39, 95% confidence interval=1.47-3.90 for 32Ala/Ala, respectively),

compared with the wild-type homozygote 32Gly/Gly. Although the A-202C variant was not significantly associated with gastric cancer risk in the single locus analysis, we found a significant locus-locus interaction between insulin-like growth factor-binding protein-3 A-202C and Gly32Ala loci on gastric cancer risk (Pint<0.001). These findings suggest that functional variants of insulin-like growth factor-binding protein-3 might be important markers for gastric cancer susceptibility and further studies are warranted to characterize the functional relevance of the locus-locus interaction of this gene.

Chen, Z., J. Q. Fan, et al. (2009). "Promoter hypermethylation correlates with the Hsulf-1 silencing in human breast and gastric cancer." Int J Cancer **124**(3): 739-44.

The HSulf-1 gene is an important factor that modulates the sulfation status of heparan sulfate proteoglycans (HSPGs) in the extracellular matrix, resulting in disturbance of HSPG-related signal transduction pathways. Recently, HSulf-1 has been reported to be down-regulated in several human cancers. In this study, we first cloned and characterized the 5' promoter region of the HSulf-1 gene (around 400 bp) that contained high basal promoter activity. We also found that this functional promoter region was hypermethylated in a number of human cancer cell lines. Furthermore, we found that hypermethylation in this promoter region correlated with the down-regulation of the HSulf-1 expression in human breast and gastric cancer cell lines and tissue samples. These results suggest that the promoter hypermethylation may be one of the mechanisms of the HSulf-1 gene silencing in human breast and gastric cancers. Finally, we demonstrated that the HSulf-1 promoter was more frequently (p<0.05) methylated in cell-free DNA extracted from serum samples of human breast and gastric cancer patients than that of healthy people (76.2%, 55.0% and 19.0%, respectively), indicating that detection of the HSulf-1 promoter methylation in serum samples may have clinical implications in early detection and diagnosis of human breast and gastric cancers.

Dar, A. A., A. Belkhiri, et al. (2009). "The aurora kinase A regulates GSK-3beta in gastric cancer cells." Oncogene **28**(6): 866-75.

Aurora kinase A (AURKA) is located at 20q13, a region that is frequently amplified in gastric cancer. In this study, we have investigated the role of AURKA in regulating glycogen synthase kinase (GSK)-3beta and beta-catenin/TCF complex in gastric cancer cells. Our results demonstrate a significant increase in the phosphorylation of GSK-3beta at Ser 9 following the overexpression of AURKA in AGS cells. The immunoprecipitation with antibodies specific for AURKA and GSK-3beta indicated that the two proteins coexist in the same protein complex. The recombinant human AURKA protein phosphorylated the GSK-3beta protein at Ser 9 in a concentration-dependent manner, in vitro. The phosphorylation of beta-catenin (Ser33/37/Thr41) by GSK-3beta is known to target beta-catenin towards degradation. In line with our findings, the increase in phospho-GSK-3beta level was accompanied by a significant decrease in beta-catenin phosphorylation (Ser33/37/Thr41) and accumulation of beta-catenin protein. The knockdown of AURKA reversed the phosphorylation of GSK-3beta and the beta-catenin protein levels. The immunofluorescence analysis demonstrated colocalization of AURKA and GSK-3beta proteins and a significant increase in the nuclear beta-catenin levels in cells overexpressing AURKA. The beta-catenin/TCF transcription activity was measured using the pTopFlash and its mutant pFopFlash luciferase reporter vectors. Indeed, AURKA overexpression led to a significant increase in the pTopFlash reporter activity, whereas kinase dead AURKA mutant (D274A) had no effect. Consistent with these findings, we detected a significant mRNA up-regulation of several direct targets of the beta-catenin/TCF transcription complex (cyclin D1, c-MYC, c-MYC-binding protein, CLDN1, FGF18 and vascular endothelial growth factor), and a two-fold increase in the proliferation rate in AURKA overexpressing cells. We conclude that the AURKA/GSK-3beta interaction is important in regulating beta-catenin, underscoring a novel oncogenic potential for AURKA in gastric tumorigenesis.

De Feo, E., R. Persiani, et al. (2009). "A case-control study on the effect of p53 and p73 gene polymorphisms on gastric cancer risk and progression." Mutat Res **675**(1-2): 60-5.

The p53 protein and its functional homologue p73 share several functions in modulating cell-cycle control and apoptosis. Based on the functional interaction between p53 and p73 in carcinogenesis, we investigated the combined effect of p73 G4C14-to-A4T14 and p53 gene polymorphisms and their interaction with selected environmental factors, on the risk for gastric cancer in a hospital-based case-control study conducted in Italy. The effect of these polymorphisms on cancer progression was also investigated. One hundred and fifteen gastric cancer cases and 295 hospital controls were genotyped for p73 G4C14-to-A4T14, and p53 exon 4 (Arg72Pro), intron 3 and intron 6 polymorphisms. An increased risk for gastric cancer was found to be associated with the inheritance of the p73 homozygous variant genotype among the gastric cancer intestinal histotype

(odds ratio (OR)=6.75; 95% confidence interval (95% CI)=1.88-24.24). An effect modification of the p73 variant allele by gender was observed [(OR=2.82; 95%CI=1.24-6.40) among females, versus an OR of 0.70 (95%CI=0.32-1.54) among males; p-value for homogeneity among strata estimates =0.03]. Gene-gene interaction analyses demonstrated that individuals with combined p53 exon 4 and intron 6 variant alleles are borderline significantly protected from gastric cancer (OR=0.52; 95% CI=0.26-1.07; p-value for interaction =0.005), which was confirmed by the haplotype analysis. Finally, a poorer survival was observed among carriers of the variant allele of p53 intron 6 if compared with those carrying both wild-type alleles (p-value for log-rank test =0.02). This study shows that the p73 G4C14-to-A4T14 polymorphism may be a risk factor for gastric cancer, as reported from other studies in different tumour sites among Caucasians. Along with the protective effect of p53 exon 4-intron 6 allelic variants, already noted for breast and lung cancer, our results require confirmation from larger studies.

Falchetti, M., C. Saieva, et al. (2008). "Gastric cancer with high-level microsatellite instability: target gene mutations, clinicopathologic features, and long-term survival." Hum Pathol **39**(6): 925-32.

Gastric cancer is one of the leading causes of cancer death worldwide, and although the incidence has decreased in Western countries, specific high-risk areas are present in Italy. Gastric cancer with high-level microsatellite instability (MSI-H) represents a well-defined subset of carcinomas showing distinctive clinicopathologic features. We examined clinicopathologic associations and long-term survival in a series of 159 gastric cancer cases from a high-risk population in Tuscany (central Italy). MSI-H was associated with antral location of the tumor (P = .001), intestinal type according to Lauren classification (P = .002), expanding type according to Ming classification (P = .0001), and mucinous histologic type according to the Japanese Research Society for Gastric Cancer classification (P = .002). In addition, MSI-H was strongly associated with a higher survival at 15 years (P = .01) and with loss of hMLH1 expression, evaluated by immunohistochemistry (P = .001). Multivariate analyses showed a significant association between the absence of hMLH1 reactivity and the expanding tumor type (P = .002). We also investigated the MSI-H-related genetic changes by analyzing coding repeats within target genes involved in pathways that control cell growth (TGFbetaRII, IGFIIR, RIZ, TCF4, DP2), apoptosis (BAX, BCL10, FAS, CASPASE5, APAF1), and DNA repair genes (hMSH6, hMSH3, MED1, RAD50, BLM, ATR, BRCA2, MRE11). Gastric cancer cases with MSI-H were found to accumulate heterozygous mutations affecting multiple molecular pathways and multiple genes within each pathway. Intriguingly, in this subset, TGFbetaRII mutations appeared to be inversely related to BLM mutations (P = .006), whereas RAD50 mutation carriers showed significantly reduced survival (P = .03).

Feng, M. Y., K. Wang, et al. (2009). "Gene expression profiling in TWIST-depleted gastric cancer cells." Anat Rec (Hoboken) **292**(2): 262-70.

TWIST is an important transcription factor during embryonic development and has recently been found to promote the epithelial-mesenchymal transition (EMT) phenomenon seen during the initial steps of tumor metastasis. To further investigate the potential targets and interacting genes of TWIST in human gastric cancer, we performed microarray analysis to compare the gene expression profiles in HGC-27 cells, with or without small interfering RNA (siRNA)-mediated depletion of TWIST. Our results showed that NF1, RAP1A, SRPX, RBL2, PFDN4, ILK, F2R, ERBB3, and MYB were up-regulated, whereas AKR1C2, FOS, GDF15, NR2F1, ATM, and CTPS were down-regulated after TWIST depletion. Moreover, TWIST-depleted HGC-27 cells showed a reversal of the morphologic and molecular changes associated with EMT. These results provide evidence that TWIST regulates the expression of several genes involved in the differentiation, adhesion, and proliferation of gastric cancer cells. The role of TWIST in the development of certain types of gastric cancer is discussed.

Feng, M. Y., K. Wang, et al. (2009). "Metastasis-induction and apoptosis-protection by TWIST in gastric cancer cells." Clin Exp Metastasis **26**(8): 1013-23.

TWIST, a basic helix-loop-helix transcription factor, has been recently reported to play an important role in tumorigenesis of human cancer through converting the early stage tumors into invasive malignancies. Upregulation of TWIST is often found in cancer patients, especially those with shorter survival period and poor response to chemotherapy. Here we studied the functions of TWIST on regulating migration rate, apoptosis, and gene expression in gastric cancer cells. TWIST expression is elevated in MGC-803 and HGC-27 cells that exhibit high invasive potential; whereas it is reduced in BGC-823 and SGC-7901 cells that possess relatively low invasive content. To evaluate functional consequences of TWIST induction, we examined the effect of TWIST on cell migration and apoptosis. Overexpression of TWIST in BGC-823 cells resulted in increased migration content and decreased

sensitivity to the arsenic oxide-induced cell death. Moreover, small interference RNA-mediated TWIST ablation in MGC-803 and HGC-27 cells showed suppressed migration ability, increased induction of apoptosis in response to arsenic oxide, and elevated cell cycle arrest. Furthermore, we found a negative correlation between the TWIST level and p53 level, probably due to transcriptional regulation. Our results have identified TWIST as a critical regulator of gastric cancer cell proliferation and migration, suggesting a potential therapeutic approach to inhibit the growth and metastasis of gastric cancer through inactivation of TWIST.

Forte, G. I., C. Cala, et al. (2008). "Role of environmental and genetic factor interaction in age-related disease development: the gastric cancer paradigm." Rejuvenation Res **11**(2): 509-12.

The association of Helicobacter pylori (Hp) infection with gastric cancer is well known and might be considered a paradigmatic example of the role that interaction among environmental factors and individual background might play in inducing age-associated disease. To evaluate the role of interaction of Hp infection with genetic background, gastric cancer and chronic gastritis patients as well as random selected controls were typed for five inflammation-related polymorphisms of IL-1 and IL-10 cytokine genes. No association among IL-10 or IL-1 variants with an increased risk of gastric cancer was found, whereas an Hp-independent association of IL-1beta -511T positive genotypes to an increased risk of chronic gastritis was found (Hp-/511T+ OR 1.89, 95% CI: 1.01-3.54; Hp+/-511T+ OR 1.83, 95% CI: 1.05-3.19). Stratification of gastric cancer group according to Hp infection does not allow finding a statistically significant association of Hp+ to the higher histological grading (G3) of gastric cancer (OR 1.54, 95% CI: 0.46-5.11). Our findings seem to confirm that cytokine genetic variants might contribute to determining the background for inflammaging in which H. pylori infection might facilitate cancer development.

Fu, H., Z. Hu, et al. (2009). "TGF-beta promotes invasion and metastasis of gastric cancer cells by increasing fascin1 expression via ERK and JNK signal pathways." Acta Biochim Biophys Sin (Shanghai) **41**(8): 648-56.

Transforming growth factor-beta (TGF-beta) is involved in actin cytoskeleton reorganization and tumor progression. Fascin1, an actin-binding protein, increases cell invasiveness and motility in various transformed cells. To determine whether fascin1 is an important mediator of the tumor response to TGF-beta, we applied the small interfering RNA (siRNA) technique to silence fascin1 in gastric cancer (GC) cells MKN45. Results showed that the effects of TGF-beta1 on GC cells invasion and metastasis were mediated by tumor production of fascin1; furthermore, it was found that TGF-beta1- induced fascin1 expression was suppressed by the specific inhibitors of JNK and ERK pathways, SP6001125 and PD98059, respectively, but not by transient transfection of Smad2 and Smad4 siRNA. Our data for the first time demonstrated that fascin1 is an important mediator of TGF-beta1-induced invasion and metastasis of GC cells, which involves JNK and ERK signaling pathways.

Gorouhi, F., F. Islami, et al. (2008). "Tumour-necrosis factor-A polymorphisms and gastric cancer risk: a meta-analysis." Br J Cancer **98**(8): 1443-51.

Inflammation is one of the early phases in the development of gastric cancer. Therefore, several studies have examined the association of polymorphisms in tumour-necrosis factor-A gene (TNF-A) with gastric cancer risk. This meta-analysis reviews and summarises published evidence for these associations. Searching several databases yielded 24 independent studies that reported on the associations between TNF-A polymorphisms and gastric cancer risk. We analysed available data for the most commonly investigated polymorphisms: TNF-A -308G>A (23 studies), TNF-A -238G>A (9 studies), and TNF-A -857C>T (5 studies). Summary odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated in the random-effects model using the DerSimonian-Laird method. Q-statistic and I(2)-statistic were calculated to examine heterogeneity, and funnel plots were plotted to examine small study effects. The overall ORs (95% CIs) for AG and AA genotypes vs GG genotype for TNF-A -308 were 1.09 (0.94-1.27) and 1.49 (1.11-1.99), respectively. For TNF-A -238, the corresponding ORs (95% CIs) were 1.05 (0.84-1.33) and 1.25 (0.30-5.26), respectively. The overall ORs (95% CIs) for CT and TT genotypes (vs CC) for TNF-A -857 were 1.06 (0.89-1.27) and 1.57 (0.91-2.70), respectively. The statistically significant association between TNF-A -308GG and gastric cancer was limited to western populations. This association showed little heterogeneity (I(2)=0) and remained consistently strong when analyses were limited to anatomic and histologic subtypes of gastric cancer, or limited to studies in which genotype frequencies were in Hardy-Weinberg equilibrium, or limited to larger studies. These same subgroup analyses did not change results associated with other polymorphisms. In conclusion, TNF-A -308AA genotype was associated with a statistically significant increased risk of gastric cancer, whereas other studied polymorphisms were not. The association between

TNF-A -857TT genotype and gastric cancer was near significant, and may become significant if more studies are published.

Gravalos, C. and A. Jimeno (2008). "HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target." Ann Oncol **19**(9): 1523-9.

Gastric cancer is the second leading cause of cancer mortality in the world and its management, especially in advanced stages, has evolved relatively little. In particular, no targeted modality has so far been incorporated to its treatment armamentarium. HER2 overexpression is increasingly recognized as a frequent molecular abnormality, driven as in breast cancer by gene amplification. There is mounting evidence of the role of HER2 overexpression in patients with gastric cancer, and it has been solidly correlated to poor outcomes and a more aggressive disease. Additionally, preclinical data are showing significant antitumor efficacy of anti-HER2 therapies (particularly monoclonal antibodies directed towards the protein) in in vitro and in vivo models of gastric cancer. As a result, several clinical trials are exploring in different settings and with diverse designs the potential of anti-HER2 therapies in gastric cancer patients. This review summarizes the rationale, preclinical evidence, retrospective clinical analyses, and the interim clinical data pertaining HER2 therapies in gastric cancer.

Guan, X., H. Zhao, et al. (2009). "Polymorphisms of TGFB1 and VEGF genes and survival of patients with gastric cancer." J Exp Clin Cancer Res **28**: 94.

BACKGROUND: Some TGFB1 and VEGF polymorphisms are believed to be functional. Given that these genes are involved in tumor growth and progression including angiogenesis, dissemination, and invasiveness, we hypothesized that these polymorphisms would be associated with survival in patients with gastric cancer. METHODS: We genotyped TGFB1 -509 C>T, +1869 T>C, and +915 G>C and VEGF -1498T>C, -634G>C, and +936C>T in 167 patients with gastric cancer. Using the Kaplan and Meier method, log-rank tests, and Cox proportional hazard models, we evaluated associations among TGFB1 and VEGF variants with overall, 1-year, and 2-year survival rates. RESULTS: Although there were no significant differences in overall survival rates among all polymorphisms tested, patients with TGFB1+915CG and CC genotypes had a poorer 2-year survival (adjusted hazard ratio (HR), 3.06; 95% confidence interval (CI), 1.09-8.62; P = 0.034) than patients with the GG genotype had. In addition, patients heterozygous for VEGF -634CG also had a poorer 1-year survival (adjusted HR, 2.08; 95% CI, 1.03-4.22; P = 0.042) than patients with the -634GG genotype. CONCLUSION: Our study suggested that TGFB1+915CG/CC and VEGF -634CG genotypes may be associated with short-term survival in gastric cancer patients. However, larger studies are needed to verify these findings.

Guan, X., H. Zhao, et al. (2009). "The VEGF -634G>C promoter polymorphism is associated with risk of gastric cancer." BMC Gastroenterol **9**: 77.

BACKGROUND: Both TGF-beta1 and VEGF play a critic role in the multiple-step process of tumorgenesis of gastric cancer. Single nucleotide polymorphisms (SNPs) of the TGFB1 and VEGF genes have been associated with risk and progression of many cancers. In this study, we investigated the association between potentially functional SNPs of these two genes and risk of gastric cancer in a US population. METHODS: The risk associated with genotypes and haplotypes of four TGFB1 SNPs and four VEGF SNPs were determined by multivariate logistic regression analysis in 171 patients with gastric cancer and 353 cancer-free controls frequency-matched by age, sex and ethnicity. RESULTS: Compared with the VEGF-634GG genotype, the -634CG genotype and the combined -634CG+CC genotypes were associated with a significantly elevated risk of gastric cancer (adjusted OR = 1.88, 95% CI = 1.24-2.86 and adjusted OR = 1.56, 95% CI = 1.07-2.27, respectively). However, none of other TGFB1 and VEGF SNPs was associated with risk of gastric cancer. CONCLUSION: Our data suggested that the VEGF-634G>C SNP may be a marker for susceptibility to gastric cancer, and this finding needs to be validated in larger studies.

Guo, J., Y. Miao, et al. (2009). "Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues." J Gastroenterol Hepatol **24**(4): 652-7.

BACKGROUND AND AIM: MicroRNAs (miRNAs) play important roles in carcinogenesis. The global miRNA expression profile of gastric cancer has not been reported. The purpose of the present study was to determine the miRNA expression profile of gastric cancer. METHODS: Total RNA were first extracted from primary gastric cancer tissues and adjacent non-tumorous tissues and then small isolated RNAs (< 300 nt) were 3'-extended with a poly(A) tail. Hybridization was carried out on a microParaflo microfluidic chip (LC Sciences, Houston, TX, USA). After hybridization detection by fluorescence labeling using tag-specific Cy3 and Cy5 dyes, hybridization images were collected using a laser scanner and digitized using Array-Pro image analysis software (Media Cybernetics, Silver Spring, MD, USA). To validate the results and investigate the biological

meaning of differential expressed miRNAs, immunohistochemistry was used to detect the differential expression of target genes. RESULTS: The most highly expressed miRNAs in non-tumorous tissues were miR-768-3p, miR-139-5p, miR-378, miR-31, miR-195, miR-497 and miR-133b. Three of them, miR-139-5p, miR-497 and miR-768-3p, were first found in non-tumorous tissues. The most highly expressed miRNAs in gastric cancer tissues were miR-20b, miR-20a, miR-17, miR-106a, miR-18a, miR-21, miR-106b, miR-18b, miR-421, miR-340*, miR-19a and miR-658. Among them, miR-340*, miR-421 and miR-658 were first found highly expressed in cancer cells. The expression of some target genes (such as Rb and PTEN) in cancer tissues was found to be decreased. CONCLUSION: To our knowledge, this is the first report about these miRNAs associated with gastric cancer. This new information may suggest the potential roles of these miRNAs in the diagnosis of gastric cancer.

Guo, X., N. Ma, et al. (2008). "Increased p38-MAPK is responsible for chemotherapy resistance in human gastric cancer cells." BMC Cancer **8**: 375.

BACKGROUND: Chemoresistance is one of the main obstacles to successful cancer therapy and is frequently associated with Multidrug resistance (MDR). Many different mechanisms have been suggested to explain the development of an MDR phenotype in cancer cells. One of the most studied mechanisms is the overexpression of P-glycoprotein (P-gp), which is a product of the MDR1 gene. Tumor cells often acquire the drug-resistance phenotype due to upregulation of the MDR1 gene. Overexpression of MDR1 gene has often been reported in primary gastric adenocarcinoma. METHODS: This study investigated the role of p38-MAPK signal pathway in vincristine-resistant SGC7901/VCR cells. P-gp and MDR1 RNA were detected by Western blot analysis and RT-PCR amplification. Mitgen-activated protein kinases and function of P-gp were demonstrated by Western blot and FACS Aria cytometer analysis. Ap-1 activity and cell apoptosis were detected by Dual-Luciferase Reporter Assay and annexin V-PI dual staining. RESULTS: The vincristine-resistant SGC7901/VCR cells with increased expression of the multidrug-resistance 1 (MDR1) gene were resistant to P-gp-related drug and P-gp-unrelated drugs. Constitutive increases of phosphorylated p38-MAPK and AP-1 activities were also found in the drug-resistant cells. Inhibition of p38-MAPK by SB202190 reduced activator protein-1 (AP-1) activity and MDR1 expression levels and increased the sensitivity of SGC7901/VCR cells to chemotherapy. CONCLUSION: Activation of the p38-MAPK pathway might be responsible for the modulation of P-glycoprotein-mediated and P-glycoprotein-unmediated multidrug resistance in the SGC7901/VCR cell line.

Guo, X., H. Oshima, et al. (2008). "Stromal fibroblasts activated by tumor cells promote angiogenesis in mouse gastric cancer." J Biol Chem **283**(28): 19864-71.

Myofibroblasts, also known as activated fibroblasts, constitute an important niche for tumor development through the promotion of angiogenesis. However, the mechanism of stromal fibroblast activation in tumor tissues has not been fully understood. A gastric cancer mouse model (Gan mice) was recently constructed by simultaneous activation of prostaglandin (PG) E2 and Wnt signaling in the gastric mucosa. Because both the PGE2 and Wnt pathways play a role in human gastric tumorigenesis, the Gan mouse model therefore recapitulates the molecular etiology of human gastric cancer. Microvessel density increased significantly in Gan mouse tumors. Moreover, the expression of vascular endothelial growth factor A (VEGFA) was predominantly induced in the stromal cells of gastric tumors. Immunohistochemistry suggested that VEGFA-expressing cells in the stroma were alpha-smooth muscle actin-positive myofibroblasts. Bone marrow transplantation experiments indicated that a subset of gastric myofibroblasts is derived from bone marrow. Importantly, the alpha-smooth muscle actin index in cultured fibroblasts increased significantly when stimulated with the conditioned medium of Gan mouse tumor cells, indicating that gastric tumor cells activate stromal fibroblasts. Furthermore, conditioned medium of Gan mouse tumor cells induced VEGFA expression both in embryonic and gastric fibroblasts, which further accelerated the tube formation of human umbilical vein endothelial cells in vitro. Notably, stimulation of fibroblasts with PGE2 and/or Wnt1 did not induce VEGFA expression, thus suggesting that factors secondarily induced by PGE2 and Wnt signaling in the tumor cells are responsible for activation of stromal fibroblasts. Such tumor cell-derived factors may therefore be an effective target for chemoprevention against gastric cancer.

Hara, M., H. Nakanishi, et al. (2008). "Interleukin-2 potentiation of cetuximab antitumor activity for epidermal growth factor receptor-overexpressing gastric cancer xenografts through antibody-dependent cellular cytotoxicity." Cancer Sci **99**(7): 1471-8.

Cetuximab, a chimeric monoclonal antibody to epidermal growth factor receptor (EGFR), has been proved to have clinically significant antitumor activity against advanced colorectal cancers, but its therapeutic activity for gastric cancers remains unclear. In the present study, we investigated the

antitumor effect and action mechanism of cetuximab using EGFR high-expressing (MKN-28) and EGFR low-expressing (GLM-1) gastric cancer cell lines without gene amplification. Cetuximab showed neither significant growth inhibition nor induction of apoptosis in either cell line in vitro, and only slightly inhibited ligand-induced phosphorylation of protein kinase B and extracellular signal-regulated kinase in MKN-28 cells. In contrast, cetuximab significantly inhibited subcutaneous and intraperitoneal tumor growth of MKN-28 cells, but not GLM-1 cells, in nude mice. This antitumor activity was significantly enhanced and diminished in nude mice by treatment with interleukin-2 (IL-2) and antiasialo GM1 antibody, which can expand and deplete natural killer (NK) cells, respectively. Antibody-dependent cellular cytotoxicity (ADCC) of cetuximab, as measured by (51)Cr release assay, was significantly higher in MKN-28 than in GLM-1 cells. This ADCC activity was enhanced by IL-2 and reduced by heat-aggregate of human immunoglobulin G, an inhibitor for FcR-III of NK cells. These results suggest that cetuximab in combination with IL-2 shows significant antitumor activity against EGFR high-expressing gastric cancer mainly through NK cell-mediated ADCC. Combination therapy with cetuximab and IL-2 would thus offer a new potential therapeutic approach for a subset of EGFR-overexpressing gastric cancers.

Hayashi, M., M. Inokuchi, et al. (2008). "High expression of HER3 is associated with a decreased survival in gastric cancer." Clin Cancer Res **14**(23): 7843-9.

BACKGROUND: The role of human epidermal growth factor receptor (HER) 3 and HER4 has been elucidated in gastric cancer. HER1 and HER2 overexpression are regarded as prognostic factors and targets of treatment. The dimerization of the HER family receptors activates downstream signal pathways and promotes tumor progression. This study investigated the positive correlation between HER1 and HER4 expression and the prognosis of patients with gastric cancers. EXPERIMENTAL DESIGN: Tumor samples were obtained from gastric adenocarcinomas of 134 patients who underwent a gastrectomy from 1999 to 2002. The expression of each HER was analyzed in the tumor by immunohistochemical staining. Parametric correlations were done between HER expression and the clinicopathologic findings. A multivariate analysis was done with the overall survival. RESULTS: HER3 expression was significantly associated with parameters involved with tumor progression, including the depth of tumor invasion (T1 versus T2-T4; P = 0.000), involved lymph nodes (P = 0.000), distant metastasis (P = 0.008), tumor stage (P = 0.000), and recurrent disease (P = 0.000). HER1 was also significantly associated with those factors excluding distant metastasis. A significant relationship was observed between the expression of HER1 and HER3 (P = 0.000). HER3 overexpression was associated with a significantly worse survival (P = 0.0000) and was an independent prognostic factor in the multivariate analysis (hazard ratio, 2.382; 95% confidence interval, 1.009-5.625; P = 0.048). CONCLUSIONS: HER3 overexpression is strongly associated with tumor progression and poor prognosis of patients with gastric cancer. It may become a new prognostic factor and a target of treatment.

He, X. W., T. Liu, et al. (2008). "Calcium carbonate nanoparticle delivering vascular endothelial growth factor-C siRNA effectively inhibits lymphangiogenesis and growth of gastric cancer in vivo." Cancer Gene Ther **15**(3): 193-202.

A nonviral gene carrier, calcium carbonate (CaCO3) nanoparticle, was evaluated for efficient in vitro and in vivo delivery of small interfering RNA (siRNA) targeting vascular endothelial growth factor-C (VEGF-C). The chemically synthesized CaCO3 nanoparticle has a 58 nm diameter and +28.6 mV positive surface charge. It is capable of forming a CaCO3 nanoparticle-DNA complex and transferring DNA into targeted cells with high transfection efficiency while effectively protecting the encapsulated DNA from degradation. Furthermore, the CaCO3 nanoparticle-DNA complex has no obvious cytotoxicity for SGC-7901 cells, while a liposome-DNA complex exhibited measurable cytotoxicity. SGC-7901 cells transfected with a VEGF-C-targeted siRNA via CaCO3 nanoparticle exhibit significantly reduced VEGF-C expression as measured by real-time PCR and enzyme-linked immunosorbent assay; whereas no decrease in VEGF-C expression is observed in cells treated by control transfection. Transfection of SGC-7901 cells with VEGF-C siRNA via CaCO3 nanoparticle also dramatically suppresses tumor lymphangiogenesis, tumor growth and regional lymph-node metastasis in subcutaneous xenografts. Significant downregulation of VEGF-C messenger RNA expression in a subcutaneous xenograft derived from VEGF-C siRNA-treated SGC-7901 cells was confirmed by real-time PCR as compared to controls. We conclude that CaCO3 nanoparticle is a novel and nonviral system for effective delivery of siRNA for cancer gene therapy.

Hishida, A., K. Matsuo, et al. (2009). "Associations of a PTPN11 G/A polymorphism at intron 3 with Helicobacter pylori seropositivity, gastric atrophy and gastric cancer in Japanese." BMC Gastroenterol **9**: 51.

BACKGROUND: Previous studies have revealed the significance of Helicobacter pylori (H. pylori) infection as a risk factor of gastric cancer. Cytotoxin-associated gene A (cagA) positivity has been demonstrated to determine the clinical outcome of H. pylori infection in the presence of SHP-2 (src homology 2 domain-containing protein tyrosine phosphatase-2). This study aimed to examine the formerly reported association of G/A PTPN11 (protein-tyrosine phosphatase, nonreceptor-type 11) polymorphism (rs2301756) with gastric atrophy, as well as the association with gastric cancer in a Japanese population using a large sample size. METHODS: Study subjects were 583 histologically diagnosed patients with gastric cancer (429 males and 154 females) and age- and sex-frequency-matched 1,636 non-cancer outpatients (1,203 males and 433 females), who visited Aichi Cancer Center Hospital between 2001-2005. Serum anti-H. pylori IgG antibody and pepsinogens were measured to evaluate H. pylori infection and gastric atrophy, respectively. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by a logistic model. RESULTS: Among H. pylori seropositive non-cancer outpatients, the age- and sex-adjusted OR of gastric atrophy was 0.82 (95% CI 0.62-1.10, P = 0.194) for G/A, 0.84 (95% CI 0.39-1.81, P = 0.650) for A/A, and 0.83 (95% CI 0.62-1.09, P = 0.182) for G/A+A/A, relative to G/G genotype, and that of severe gastric atrophy was 0.70 (95% CI 0.47-1.04, P = 0.079), 0.56 (95% CI 0.17-1.91, P = 0.356), and 0.68 (95% CI 0.46-1.01, P = 0.057), respectively. Among H. pylori infected subjects (H. pylori seropositive subjects and seronegative subjects with gastric atrophy), the adjusted OR of severe gastric atrophy was further reduced; 0.62 (95% CI 0.42-0.90, P = 0.012) for G/A+A/A. The distribution of the genotype in patients with gastric cancer was not significantly different from that for H. pylori infected subjects without gastric atrophy. CONCLUSION: Our study results revealed that those with the A/A genotype of PTPN11 rs2301756 polymorphism are at lower risk of severe gastric atrophy, but are not associated with a decreased risk of gastric cancer, which partially supported our previous finding that the polymorphism in the PTPN11 gene encoding SHP-2 was associated with the gastric atrophy risk in H. pylori infected Japanese. The biological roles of this PTPN11 polymorphism require further investigation.

Howlett, M., T. R. Menheniott, et al. (2009). "Cytokine signalling via gp130 in gastric cancer." Biochim Biophys Acta **1793**(11): 1623-33.

Cytokine signalling pathways that depend on gp130 are dysregulated in several epithelial cancers including gastric cancer. It has been established that blockade of SHP2 activation of MAPK signalling results in hyperactivation of STAT3 resulting in increased cell proliferation, angiogenesis, inflammation and inhibition of both immunocyte and epithelial cell apoptosis. Additionally, key genes regulated downstream of gp130 via MAPK activation such as the stomach-specific tumor suppressor gene tff1 are suppressed, contributing to the oncogenic outcome. The main cytokine driver of gp130 signalling in the stomach is IL-11, with IL-6 having little activity in the antral stomach in which most pathology initiates. IL-11 is up-regulated in both mouse and human gastric cancer and in pre-neoplastic mucosa. A characteristic gene signature specifically associated with IL-11 drive has been observed, although the prognostic value of the signature has not yet been assessed. Infection of human or mouse stomach with Helicobacter pylori, especially that expressing the CagA cytotoxin, produces constitutive MAPK activation, but also activated STAT3 and increases IL-11 expression. The possibility of designing and utilising small molecule inhibitors of either IL-11 or STAT3 activation may be worthwhile in developing new cancer therapeutics.

Huang, W., L. F. Yu, et al. (2008). "Angiotensin II type 1 receptor expression in human gastric cancer and induces MMP2 and MMP9 expression in MKN-28 cells." Dig Dis Sci **53**(1): 163-8.

Angiotensin II (Ang II), a main effector peptide in the renin-angiotensin system, acts as a growth-promoting and angiogenic factor via angiotensin II receptor1 (AT1R). In this study, we investigated the expression of angiotensin II type1 receptor (AT1R) in gastric cancer and the effects of Ang II on the expression of MMP2 and MMP9 in the human gastric cancer cell line MKN-28 cells. The expression of the Ang II type I receptor was examined by western and immunocytochemistry in gastric cancer cell lines and detected by real-time PCR and immunohistochemistry in normal and gastric cancer tissues. The expression of MMP2 and MMP9 were detected by real-time PCR and western after treatment with Ang II and/or AT1R antagonist. AT1R were expressed in all human gastric cancer lines and the expression of AT1R was significantly higher in cancer tissues than that in normal gastric tissues ($P < 0.01$). Furthermore, Ang II promoted the expression of MMP2 and MMP9 in MKN-28 cells, and the stimulatory effects of Ang II could be blocked by AT1R antagonist. These results suggest that AT1R is involved in the progression of gastric cancer and may promote the angiogenesis of gastric cancer cell line (MKN-28), and these effects may be associated with the upregulation of MMP2 and MMP9.

Ishido, K., M. Azuma, et al. (2009). "Evaluation of prognostic factors for the response to S-1 in patients with stage II or III advanced gastric cancer who underwent gastrectomy." Pharmacogenet Genomics **19**(12): 955-64.

OBJECTIVES: Many studies have reported that low intratumoral mRNA expression of thymidylate synthase (TS) is an important biomarker of response to chemotherapy in patients with unresectable advanced gastric cancer. However, the role of gene expression profile of patients who received postoperative adjuvant chemotherapy remains unclear. In this study, we evaluated how TS and other associated genes related to outcome. METHODS: Seventy-nine patients with stage II or III advanced gastric cancer who underwent gastrectomy were analyzed. Thirty-nine patients received adjuvant chemotherapy with S-1 after surgery (S-1 group) and 40 patients underwent surgery only (surgery group). Formalin-fixed, paraffin-embedded tumor tissues were dissected by the laser-captured microdissection technique and analyzed for target gene expressions using a quantitative real-time polymerase chain reaction. RESULTS: There were no significant differences between the S-1 group and the surgery group in gene expressions except TS (P=0.034). In the S-1 group, recurrence-free survival (RFS) and overall survival (OS) were significantly longer in patients with low TS expression compared with patients with high TS expression (P=0.021 and 0.016), whereas there were no correlations in the surgery group. Furthermore, RFS and OS were both correlated with extent of lymph node metastasis (N) (P=0.038 and 0.020) and TS expression (P=0.021 and 0.032). On multivariate analysis it was found that TS expression and N were significant independent prognostic factors of RFS and OS (TS: P=0.027 and 0.050, N: P=0.048 and 0.032). CONCLUSION: Our results suggested that intratumoral TS expression is an independent prognostic factor in patients with gastric cancer who received postoperative adjuvant chemotherapy with S-1.

Ishigami, S., S. Natsugoe, et al. (2008). "HLA-class I expression in gastric cancer." J Surg Oncol **97**(7): 605-8.

PURPOSE: We investigated the clinical impact of HLA-class I tumor cells in gastric cancer. MATERIALS AND METHODS: HLA-class I expression was immunohistochemically evaluated in specimens from 141 gastric cancer patients. The correlation between HLA-class I expression and clinical factors was analyzed. RESULTS: HLA-class I was identified in 96 (68.1%) gastric carcinomas. The loss of HLA-class I significantly correlated with the depth of invasion ($P < 0.01$), nodal involvement ($P < 0.05$) and tumor histology ($P < 0.01$). According to the positivity of HLA-class I, shallow depth and the absence of nodal metastasis increased. HLA-class I expression was a significant prognostic factor in gastric cancer ($P < 0.02$); however, HLA-class I was not an independent prognostic factor by multivariate analysis. CONCLUSIONS: Our data may suggest that loss of HLA-class I in gastric cancer did not directly reflect immunological escape from tumor antigen-specific cytotoxic T lymphocytes, unlike in other cancers.

Ivanauskas, A., J. Hoffmann, et al. (2008). "Distinct TPEF/HPP1 gene methylation patterns in gastric cancer indicate a field effect in gastric carcinogenesis." Dig Liver Dis **40**(12): 920-6.

BACKGROUND: Aberrant methylation of the transmembrane protein containing epidermal growth factor and folistatin domains/hyperplastic polyposis 1 gene was recently reported in hyperplastic colon polyps, colorectal adenomas and carcinomas. However, there are only limited data on significance of transmembrane protein containing epidermal growth factor and folistatin domains/hyperplastic polyposis 1 gene methylation in gastric adenocarcinomas. AIM: The aim of this study was to determine the prevalence of transmembrane protein containing epidermal growth factor and folistatin domains/hyperplastic polyposis 1 promoter methylation in gastric adenocarcinomas. PATIENTS: Study population consists of 48 patients with gastric cancer and 11 dyspeptic patients. METHODS: Using the Methylight assay, transmembrane protein containing epidermal growth factor and folistatin domains/hyperplastic polyposis 1 gene methylation was assessed in fresh frozen cancer tissue and matched tumoural-free area of patients with gastric cancer and in the gastric mucosa of dyspeptic patients. RESULTS: Transmembrane protein containing epidermal growth factor and folistatin domains/hyperplastic polyposis 1 promoter gene methylation was observed in 35 of 48 (73%) gastric adenocarcinomas, and in 27 of 48 (56%) matched tumoural-free area cases (p=0.087). In contrast, the occurrence of transmembrane protein containing epidermal growth factor and folistatin domains/hyperplastic polyposis 1 methylation was much lower in gastric mucosa of dyspeptics (1 of 11; 9%) and the difference was significant in comparison with both tumoural tissue (p=0.0001) and tumoural-free area (p=0.0047) of cancer patients. Transmembrane protein containing epidermal growth factor and folistatin domains/hyperplastic polyposis 1 gene expression was significantly reduced in adenocarcinomas in comparison with matched tumoural-free area (p=0.022). CONCLUSION: Our

data suggest that methylation of transmembrane protein containing epidermal growth factor and folistatin domains/hyperplastic polyposis 1 is present in the majority of gastric adenocarcinomas and in the surrounding tumoural-free area, indicating that this epigenetic change may point to a field effect in the gastric mucosa.

Jones, K. R., Y. M. Joo, et al. (2009). "Polymorphism in the CagA EPIYA motif impacts development of gastric cancer." J Clin Microbiol **47**(4): 959-68.

Helicobacter pylori causes diseases ranging from gastritis to peptic ulcer disease to gastric cancer. Geographically, areas with high incidences of H. pylori infection often overlap with areas with high incidences of gastric cancer, which remains one of the leading causes of cancer-related deaths worldwide. Strains of H. pylori that carry the virulence factor cytotoxin-associated gene A (cagA) are much more likely to be associated with the development of gastric cancer. Moreover, particular C-terminal polymorphisms in CagA vary by geography and have been suggested to influence disease development. We conducted a large-scale molecular epidemiologic analysis of South Korean strains and herein report a statistical link between the East Asian CagA EPIYA-ABD genotype and the development of gastric cancer. Characterization of a subset of the Korean isolates showed that all strains from cancer patients expressed and delivered phosphorylatable CagA to host cells, whereas the presence of the cagA gene did not strictly correlate to expression and delivery of CagA in all noncancer strains.

Jung, S. W., M. Sugimoto, et al. (2009). "homB status of Helicobacter pylori as a novel marker to distinguish gastric cancer from duodenal ulcer." J Clin Microbiol **47**(10): 3241-5.

The hom family of Helicobacter pylori outer-membrane proteins, especially the homB gene, has been suggested as a novel virulence factor; however, the clinical association and function of this gene are still unclear. We evaluated the presence of the homA, homB, and cagA genes in 286 strains isolated from patients in the U.S. and Colombian populations (126 with gastritis, 96 with duodenal ulcer, and 64 with gastric cancer) by PCR. The results were compared with the clinical presentation and gastric injury. The prevalence of the homB gene was significantly higher in strains isolated from gastric-cancer patients (71.9%) than in those from duodenal ulcer patients (52.1%) ($P = 0.012$). In a multivariate analysis, the presence of the cagA gene significantly increased the risk for developing gastric cancer and duodenal ulcer, with the presence of the homB gene acting as a factor that could distinguish gastric cancer from duodenal ulcer (adjusted odds ratio, 3.033; 95% confidence interval, approximately 1.37 to approximately 6.73). cagA status was correlated with homB status ($r = 0.323$; $P < 0.01$). A histological analysis showed that cagA status was associated with inflammation and atrophy both in the antrum and in the corpus, while homB status was associated with inflammation and atrophy in the corpus. homB gene status might be susceptible to gastric-cancer development such that the homB gene is used as a factor for discriminating the risk of gastric cancer from that of duodenal ulcer.

Karam, R., J. Carvalho, et al. (2008). "The NMD mRNA surveillance pathway downregulates aberrant E-cadherin transcripts in gastric cancer cells and in CDH1 mutation carriers." Oncogene **27**(30): 4255-60.

Germline mutations in the gene encoding the tumour suppressor E-cadherin (CDH1) are the underlying genetic defect responsible for hereditary diffuse gastric cancer (HDGC). A remarkably high percentage (approximately 80%) of CDH1 mutations in HDGC patients and carriers generate premature termination codons (PTCs). Here, we examined whether CDH1 transcripts harbouring PTCs are downregulated by nonsense-mediated decay (NMD), an RNA surveillance pathway that degrades PTC-bearing transcripts. Using an allele-specific expression (ASE) assay to differentiate between mutated and wild-type CDH1 alleles, we found that PTC-bearing CDH1 mRNAs are strongly downregulated in normal gastric tissue from several CDH1 mutation carriers. We show that NMD is responsible for this robust downregulation, as CDH1 transcripts harbouring PTCs in the KATO-III gastric tumour cell line were upregulated in response to protein synthesis inhibitors or depletion of the NMD factors UPF1 and eIF4AIII. Analysis of HDGC patients harbouring CDH1 alleles with PTCs at a wide variety of different positions indicates an association of their predicted ability to induce NMD and an earlier age of onset of gastric cancer. This suggests that NMD may be detrimental for HDGC patients and therefore NMD is a potentially useful therapeutic target for CDH1 mutation carriers.

Katoh, M. and M. Katoh (2007). "Comparative integromics on JMJD1C gene encoding histone demethylase: conserved POU5F1 binding site elucidating mechanism of JMJD1C expression in undifferentiated ES cells and diffuse-type gastric cancer." Int J Oncol **31**(1): 219-23.

Epigenetic modifications of genomic DNA and histones alter the chromatin structure to regulate the accessibility of transcription factors to the promoter or enhancer regions. In 2003, we identified and characterized JMJD1C (TRIP8) consisting of TRI8H1 domain with C2HC4-type zinc finger-like

motif, TRI8H2 domain with thyroid hormone receptor beta-binding region, and JmjC domain. JMJD1A (TSGA), JMJD1B (5qNCA) and JMJD1C with the common domain architecture are histone H3K9 demethylases implicated in the nuclear hormone receptor-based transcriptional regulation. Here, comparative integromics on JMJD1C gene is reported. JMJD1C variant 1, previously reported, consists of exons 1, 2 and 3-26, while JMJD1C variant 2 characterized in this study was transcribed from novel exon 1B located 5' to exon 3. Four human JMJD1C ESTs were transcribed from exon 1, while 14 human JMJD1C ESTs from exon 1B. All of 26 mouse Jmjd1c ESTs were transcribed from exon 1b. These facts indicate that JMJD1C variant 2 transcribed from exon 1B was the major transcript. Human JMJD1C variant 2 with TRI8H1, TRI8H2, and JmjC domains showed 85.7% total-amino-acid identity with mouse Jmjd1c. Human JMJD1C mRNA was expressed in undifferentiated embryonic stem (ES) cells, pancreatic islet, diffuse-type gastric cancer, and other tissues or tumors. Mouse Jmjd1c mRNA was expressed in fertilized egg, blastocyst, undifferentiated ES cells, embryonic germ cells, c-Kit+/Sca-1+/Lin- hematopoietic stem cells, pancreatic islet, and other tissues. Comparative genomics analyses revealed that binding sites for POU5F1 (OCT3/OCT4), AP-1, and bHLH transcription factors within the promoter region located 5' to exon 1B of human JMJD1C gene were conserved in chimpanzee, cow, mouse and rat JMJD1C orthologs. POU5F1-mediated expression of JMJD1C histone demethylase is implicated in the reactivation of silenced genes in undifferentiated ES cells, pancreatic islet, and diffuse-type gastric cancer.

Katoh, Y. and M. Katoh (2007). "Conserved POU-binding site linked to SP1-binding site within FZD5 promoter: Transcriptional mechanisms of FZD5 in undifferentiated human ES cells, fetal liver/spleen, adult colon, pancreatic islet, and diffuse-type gastric cancer." Int J Oncol **30**(3): 751-5.

Canonical WNT signals are transduced through Frizzled (FZD) family receptor and LRP5/LRP6 co-receptor to upregulate FGF20, JAG1, DKK1, WISP1, CCND1 and MYC genes for cell-fate determination, while non-canonical WNT signals are transduced through FZD family receptor and ROR2/PTK7/RYK co-receptor to activate RHOA/RHOU/RAC/CDC42, JNK, PKC, NLK and NFAT signaling cascades for the regulation of tissue polarity, cell movement, and adhesion. We previously reported molecular cloning and characterization of human FZD5, which showed six amino-acid substitutions with human Hfz5. FZD5, functioning as WNT5A receptor, is the key molecule in the fields of oncology, regenerative medicine, cardiology, rheumatology, diabetology, and gastroenterology. Here, comparative integromics analyses on FZD5 orthologs were performed by using bioinformatics (Techint) and human intelligence (Humint). Chimpanzee FZD5 and cow Fzd5 genes were identified within NW_104292.1 and AC166656.2 genome sequences, respectively. FZD5 orthologs were seven-transmembrane proteins with extracellular Frizzled domain, leucine zipper motif around the 5th transmembrane domain, and cytoplasmic DVL- and PDZ-binding motifs. Ser523 and Ser529 around the DVL-binding motif of FZD5 orthologs were putative aPKC phosphorylation sites. POU5F1 (OCT4)-binding site linked to SP1-binding site within the 5'-promoter region of human FZD5 gene was evolutionarily conserved among mammalian FZD5 orthologs. POU5F1 was more related to POU2F and POU3F subfamily members. POU5F1 was preferentially expressed in undifferentiated human embryonic stem (ES) cells, pancreatic islet, and diffuse-type gastric cancer. POU2F1 (OCT1) was expressed in ES cells, fetal liver/spleen, adult colon, POU2F2 in ES cells, fetal liver/spleen, and POU2F3 in diffuse-type gastric cancer. Multiple SP1/KLF family members, other than KLF2 or KLF4, were expressed in undifferentiated human ES cells. Together, these facts indicate that POU5F1 and POU2F subfamily members play a pivotal role for the FZD5 expression in undifferentiated human ES cells, fetal liver/spleen, adult colon, pancreatic islet, and diffuse-type gastric cancer.

Ke, Q., J. Liang, et al. (2008). "Potentially functional polymorphisms of the vascular endothelial growth factor gene and risk of gastric cancer." Mol Carcinog **47**(8): 647-51.

Vascular endothelial growth factor (VEGF), the key mediator of angiogenesis, plays an important role in the development of different kind of tumors, including gastric cancer (GC). The aim of this study is to test the hypothesis that genetic variants of VEGF are associated with risk of GC. We genotyped four potentially functional polymorphisms (-2578C > A, -1498T > C, -634G > C, and +936C > T) of the VEGF gene in a population-based case-control study of 540 GC cases and 561 frequency-matched cancer-free controls in a high risk Chinese population. We found that none of the four polymorphisms or their haplotypes achieved significant difference in their distributions between GC cases and controls. Multiple logistic regression analyses revealed that GC risk was not significantly associated with the variant genotypes of the four VEGF polymorphisms as compared with their wild-type genotypes. In conclusion, our data did not support a significant association between VEGF SNPs and the risk of GC.

Kim, H. Y., G. S. Park, et al. (2008). "Secretion of biologically active recombinant human granulocyte-macrophage colony-stimulating factor by transduced gastric cancer cells." Yonsei Med J **49**(2): 279-87.

PURPOSE: Gastric cancer has the highest incidence rate among cancers in Asia. The advanced type of signet ring cell carcinoma has poor prognosis compared to other types of gastric cancer. The immuno-gene therapy with cytokine-based tumor vaccines has not yet been investigated for gastric cancer. The granulocyte macrophage colony-stimulating factor (GM-CSF)-based tumor vaccine has been demonstrated as the most potent stimulator for specific and long-lasting systemic tumor immunity. MATERIALS AND METHODS: In the present study, KATO III cells, the human signet ring cell gastric carcinoma cell line, were genetically modified by the transduction with the human GM-CSF cDNA or the modified hGM-CSF in replication-deficient retroviruses. The genomic integrations and mRNA expressions of the transgenes were determined by Southern and Northern blot analyses. RESULTS: Wild type (wt) or modified hGM-CSF was integrated into the genome of KATO III cells. The modified hGM-CSF mRNA was more stable than that of wt. The KATO III cells with the modified hGM-CSF produced higher level of hGM-CSF (12.4-19 ng/10(6)cells/48hrs) than that with wt hGM-CSF, when determined by enzyme-linked immunosorbent assay (ELISA). The secreted recombinant hGM-CSF could support the proliferation of the GM-CSF-dependent cell line, indicating that the hGM-CSF secreted by the transduced KATO III cells has biological activities. Irradiated, transduced KATO III cells continued to secret hGM-CSF without proliferation. CONCLUSION: Our results suggest that GM-CSF secreting KATO III cells could be tested for the treatment of gastric cancer as an allogeneic tumor vaccine as a part of immunotherapeutic treatment.

Kim, J. G., S. K. Sohn, et al. (2007). "Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with gastric cancer." Ann Oncol **18**(6): 1030-6.

BACKGROUND: The present study analyzed vascular endothelial growth factor (VEGF) gene polymorphisms and their impact on the prognosis for patients with gastric cancer. PATIENTS AND METHODS: Five hundred and three consecutive patients with surgically resected gastric adenocarcinoma were enrolled in the present study. The genomic DNA was extracted from paraffin-embedded tissue and four VEGF (-460T > C, -116G > A, +405G > C, and +936C > T) gene polymorphisms were determined using a polymerase chain reaction-restriction fragment length polymorphism assay. RESULTS: The survival analysis showed no association of three VEGF gene polymorphisms with the prognosis. For the +936C > T polymorphism, the T/T genotype, however, had a worse overall survival (OS) compared with the C/C genotype (P = 0.037). The -460 T/C or C/C genotype was a poor prognostic factor in patients with stage 0 or I gastric cancer (OS: hazard ratio (HR) = 3.96, disease-free survival (DFS): HR = 4.87). In the haplotype analysis, the CACC haplotype was associated with a significantly worse survival when compared with the TGGC haplotype (OS: HR = 1.72, DFS: HR = 1.73). CONCLUSIONS: VEGF gene polymorphisms were found to be an independent prognostic marker for patients with gastric cancer. Consequently, the analysis of VEGF gene polymorphisms can help identify patient subgroups at high risk of a poor disease outcome.

Kim, J. S., M. A. Kim, et al. (2009). "Biomarker analysis in stage III-IV (M0) gastric cancer patients who received curative surgery followed by adjuvant 5-fluorouracil and cisplatin chemotherapy: epidermal growth factor receptor (EGFR) associated with favourable survival." Br J Cancer **100**(5): 732-8.

The aim of this study was to analyse the impact of epidermal growth factor receptor (EGFR), thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP), aurora kinase (ARK) A/B, and excision repair cross-complementing gene 1 (ERCC1) on the efficacy of adjuvant chemotherapy with 5-fluorouracil and cisplatin (FP) after curative gastric resection. Normal and cancer tissue were separately obtained from gastrectomy samples of 153 patients with AJCC stage III-IV (M0) who subsequently treated with adjuvant FP chemotherapy. TS, DPD, TP, ERCC1, and ARK proteins were measured by immunohistochemistry (IHC). EGFR expression was investigated using a standardized IHC with the EGFR PharmDx assay. Amplification of EGFR gene was analysed using fluorescent in situ hybridisation (FISH). In multivariate analysis, stage, ratio of positive to removed lymph nodes, and EGFR expression were significant prognostic factors for overall survival. Patients with higher EGFR expression had better overall survival than those with lower expression (relative risk: 0.475 (95% confidence interval, 0.282-0.791, P=0.005). Low EGFR expression might be a predictive marker for relapse in curative resected stage III-IV (M0) gastric cancer patients who received adjuvant FP chemotherapy.

Kim, K. E., H. Song, et al. (2009). "Expression of ADAM33 is a novel regulatory mechanism in IL-18-

secreted process in gastric cancer." J Immunol **182**(6): 3548-55.

IL-18 has recently been reported to play a critical role in tumor migration, invasion, and metastasis. Because IL-18 has various biological activities after its secretion as an 18 kDa mature form, the regulation of the IL-18 secretion process is an important step in tumor progression. This study investigated the implication of IL-18 in vascular endothelial growth factor (VEGF)-D-regulated migration, along with the role of the IL-18 secretion process. VEGF-D enhanced cell migration, which was then blocked by inhibiting IL-18. VEGF-D increased IL-18 expression and secretion, suggesting that IL-18 is a critical mediator for VEGF-D-enhanced migration. VEGF-D induced a disintegrin and metalloprotease 33 (ADAM33) expression, which has a metalloproteinase domain. VEGF-D-enhanced IL-18 secretion and cell migration were inhibited by ADAM33 knock-down. Moreover, cell proliferation was considerably reduced in ADAM33 small interfering RNA transfectants. In conclusion, ADAM33 has a key role in gastric cancer pathogenesis by up-regulating IL-18 secretion process, resulting in increased cell migration and proliferation.

Kim, K. K., J. J. Lee, et al. (2008). "Macrophage inhibitory cytokine-1 activates AKT and ERK-1/2 via the transactivation of ErbB2 in human breast and gastric cancer cells." Carcinogenesis **29**(4): 704-12.

Macrophage inhibitory cytokine-1 (MIC-1) is a member of the transforming growth factor-beta superfamily, which is overexpressed in a variety of human cancers, including breast and gastric cancer. The function of MIC-1 in cancer remains controversial and its signaling pathways remain poorly understood. In this study, we demonstrate that MIC-1 induces the transactivation of ErbB2 in SK-BR-3 breast and SNU-216 gastric cancer cells. MIC-1 induced a significant phosphorylation of Akt and ERK-1/2, and also effected an increase in the levels of tyrosine phosphorylation of ErbB1, ErbB2 and ErbB3 in SK-BR-3 and SNU-216 cells. The treatment of these cells with AG825 and AG1478, inhibitors specific for ErbB2 tyrosine kinase, resulted in the complete abolition of MIC-1-induced Akt and ERK-1/2 phosphorylation. Furthermore, the small-interfering RNA-mediated downregulation of ErbB2 significantly reduced not only the phosphorylation of Akt and ERK-1/2 but also the invasiveness of the cells induced by MIC-1. Our results show that ErbB2 activation performs a crucial function in MIC-1-induced signaling pathways. Further investigations revealed that MIC-1 induced the expression of the hypoxia inducible factor-1alpha protein and the expression of its target genes, including vascular endothelial growth factor, via the activation of the mammalian target of rapamycin (mTOR) signaling pathway. Stimulation of SK-BR-3 with MIC-1 profoundly induces the phosphorylation of mTOR and its downstream substrates, including p70S6K and 4E-BP1. Collectively, these results show that MIC-1 may participate in the malignant progression of certain human cancer cells that overexpress ErbB2 through the transactivation of ErbB2 tyrosine kinase.

Kim, M. and H. C. Chung (2009). "Standardized genetic alteration score and predicted score for predicting recurrence status of gastric cancer." J Cancer Res Clin Oncol **135**(11): 1501-12.

PURPOSE: To build a standardized genetic alteration score (SGAS) based on genes that are related to a patient's recurrence status, and to obtain the predicted score (PS) for predicting a patient's recurrence status, which reflects the genetic information of the gastric cancer patient. METHODS: SGAS was constructed using linear combinations that best account for the variability in the data. This methodology was fit to and validated using cDNA microarray-based CGH data obtained from the Cancer Metastasis Research Center at Yonsei University. RESULTS: When classifying cancer patients, the accuracy was 92.59% in the leave-one-out validation method. CONCLUSIONS: SGAS provided PS for the risk of recurrence, which was capable of discriminating a patient's recurrence status. A total of 59 genes were found to have a high frequency of alteration in either the recurrence or non-recurrence status. SGAS was found to be a significant risk factor on recurrence and explained 31% variability of the 59 genes.

Kim, M. H., J. S. Park, et al. (2008). "Lysophosphatidic acid promotes cell invasion by up-regulating the urokinase-type plasminogen activator receptor in human gastric cancer cells." J Cell Biochem **104**(3): 1102-12.

There is a strong correlation between the overexpression of urokinase-type plasminogen activator receptor (uPAR) and gastric cancer invasion. This study examined the effect of phospholipid lysophosphatidic acid (LPA) on uPAR expression in human gastric cancer AGS cells and the underlying signal transduction pathways. Treating human gastric AGS cells with LPA induced the expression of uPAR mRNA and promoter activity in both a time- and dose-dependent manner. Small interfering RNA targeting for LPA receptors, dominant negative Rho-family GTPase (RhoA, Rac1, and Cdc42) and an expression vector encoding a mutated c-jun (TAM67) partially blocked the LPA-induced uPAR expression. Site-directed mutagenesis and electrophoretic mobility

shift studies showed that the transcription factors activation protein-1 (AP-1) and nuclear factor (NF)-kappaB are essential for the LPA-induced uPAR transcription. In addition, AGS cells treated with LPA showed enhanced invasion, which was partially abrogated by the uPAR-neutralizing antibodies and inhibitors of Rho kinase, JNK, and NF-kappaB. This suggests that LPA induces uPAR expression through the LPA receptors, Rho-family GTPase, JNK, AP-1 and NF-kappaB signaling pathways, which in turn stimulates the cell invasiveness of human gastric cancer AGS cells.

Kim, S., M. G. Choi, et al. (2009). "Silibinin suppresses TNF-alpha-induced MMP-9 expression in gastric cancer cells through inhibition of the MAPK pathway." Molecules **14**(11): 4300-11.

Tumor necrosis factor (TNF)-alpha is one of the pro-inflammatory cytokines highly expressed in Helicobacter pylori that inhibits gastric acid secretion. In this study we determined the effect of silibinin on TNF-alpha-induced MMP-9 expression in gastric cancer cell lines. MMP-9 mRNA and protein expression was dose-dependently increased by TNF-alpha in SNU216 and SNU668 gastric cancer cells. On the other hand, TNF-alpha-induced MMP-9 expression was dose-dependently suppressed by silibinin. To verify the regulatory mechanism of silibinin on TNF-alpha-induced MMP-9 expression, the gastric cancer cell lines were pretreated with silibinin prior to TNF-alpha. TNF-alpha-induced MMP-9 expression was inhibited by the MEK1/2 specific inhibitor, UO126. Finally, we investigated the effect of adenoviral constitutively active (CA)-MEK and CA-Akt on MMP-9 expression. The expression of MMP-9 was significantly increased by CA-MEK overexpression, but not by CA-Akt overexpression. Taken together, we suggest that silibinin down-regulates TNF-alpha- induced MMP-9 expression through inhibition of the MEK/ERK pathway in gastric cancer cells.

Kim, T. Y., I. S. Kim, et al. (2008). "Transcriptional induction of DLC-1 gene through Sp1 sites by histone deacetylase inhibitors in gastric cancer cells." Exp Mol Med **40**(6): 639-46.

We previously reported that trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, induced DLC-1 mRNA expression and accumulated acetylated histones H3 and H4 associated with the DLC-1 promoter in DLC-1 non-expressing gastric cancer cells. In this study, we demonstrated the molecular mechanisms by which TSA induced the DLC-1 gene expression. Treatment of the gastric cancer cells with TSA activates the DLC-1 promoter activity through Sp1 sites located at -219 and -174 relative to the transcription start site. Electrophoretic mobility-shift assay (EMSA) revealed that Sp1 and Sp3 specifically interact with these Sp1 sites and showed that TSA did not change their binding activities. The ectopic expression of Sp1, but not Sp3, enhances the DLC-1 promoter responsiveness by TSA. Furthermore, the TSA-induced DLC-1 promoter activity was increased by p300 expression and reduced by knockdown of p300. These results demonstrated the requirement of specific Sp1 sites and dependence of Sp1 and p300 for TSA-mediated activation of DLC-1 promoter.

Kim, W. H., S. H. Lee, et al. (2009). "Neuropilin2 expressed in gastric cancer endothelial cells increases the proliferation and migration of endothelial cells in response to VEGF." Exp Cell Res **315**(13): 2154-64.

The structure and characteristics of the tumor vasculature are known to be different from those of normal vessels. Neuropilin2 (Nrp2), which is expressed in non-endothelial cell types, such as neuronal or cancer cells, functions as a receptor for both semaphorin and vascular endothelial growth factor (VEGF). After isolating tumor and normal endothelial cells from advanced gastric cancer tissue and normal gastric mucosa tissues, respectively, we identified genes that were differentially expressed in gastric tumor endothelial (TEC) and normal endothelial cells (NEC) using DNA oligomer chips. Using reverse transcriptase-PCR, we confirmed the chip results by showing that Nrp2 gene expression is significantly up-regulated in TEC. Genes that were found to be up-regulated in TEC were also observed to be up-regulated in human umbilical vein endothelial cells (HUVECs) that were co-cultured with gastric cancer cells. In addition, HUVECs co-cultured with gastric cancer cells showed an increased reactivity to VEGF-induced proliferation and migration. Moreover, overexpression of Nrp2 in HUVECs significantly enhanced the proliferation and migration induced by VEGF. Observation of an immunohistochemical analysis of various human tumor tissue arrays revealed that Nrp2 is highly expressed in the tumor vessel lining and to a lesser extent in normal tissue microvessels. From these results, we suggest that Nrp2 may function to increase the response to VEGF, which is more significant in TEC than in NEC given the differential expression, leading to gastric TEC with aggressive angiogenesis phenotypes.

Kitajima, Y., K. Ohtaka, et al. (2008). "Helicobacter pylori infection is an independent risk factor for Runx3 methylation in gastric cancer." Oncol Rep **19**(1): 197-202.

Runx3, a member of the human runt-related transcription factor family, is known as a possible tumor suppressor gene for gastric cancer. Runx 3 expression is frequently suppressed by the promoter hypermethylation in gastric cancer cell lines and tissues. However, the precise mechanism of the induction of Runx3 methylation, which is considered to be a critical step in gastric carcinogenesis, remains to be elucidated. In the present study, we evaluated runx3 gene methylation in 57 resected early gastric cancer specimens. Then, we correlated Runx3 methylation in the cancer tissue specimens with clinicopathological factors as well as the mucosal backgrounds, such as intestinal metaplasia surrounding the cancer cells and Helicobacter pylori (H. pylori) infection. Runx3 methylation was observed in 30 of the 57 (52.6%) cancer specimens, whereas methylation was detected in 10 of the 57 (17.5%) corresponding non-cancerous mucosae. In comparison to the clinicopathological factors, Runx3 methylation was significantly correlated with both age and tumor location. A multivariate analysis demonstrated that age and tumor location as well as H. pylori infection were independent risk factors for Runx3 methylation. We demonstrated for the first time that H. pylori infection contributes to Runx3 methylation in gastric cancer tissues. When a persistent infection by H. pylori continues in the middle/lower stomach for a long period, Runx3 methylation may be induced and the subsequent loss of Runx3 expression may therefore affect gastric carcinogenesis.

Kodama, M., Y. Kitadai, et al. (2008). "Vascular endothelial growth factor C stimulates progression of human gastric cancer via both autocrine and paracrine mechanisms." Clin Cancer Res **14**(22): 7205-14.

PURPOSE: Vascular endothelial growth factor (VEGF)-C induces lymphangiogenesis by activating the VEGF receptor (VEGFR)-3, which is expressed by lymphatic endothelial cells. VEGFR-3 has also been detected on several malignant cells, but the significance of VEGFR-3 expression on malignant cells remains unclear. In this study, we examined the expression and function of VEGFR-3 in gastric carcinoma cells. EXPERIMENTAL DESIGN: We examined the expression of VEGFR-3 by four human gastric carcinoma cell lines and in 36 surgical specimens of gastric carcinoma. We also used cDNA microarrays to examine the effect of VEGF-C on gene expression in VEGFR-3-expressing KKLS cells. To stimulate VEGF-C/VEGFR-3 signaling in an autocrine manner, the VEGF-C expression vector was transfected into KKLS cells, and stable transfectants were established. These cells were then transplanted into the gastric walls of nude mice. RESULTS: Two of the four gastric carcinoma cell lines expressed VEGFR-3 mRNA. In 17 of 36 gastric carcinoma specimens, VEGFR-3-specific immunoreactivity was detected on tumor cells. In vitro treatment of KKLS cells with VEGF-C stimulated cell proliferation and increased expression of mRNAs encoding cyclin D1, placental growth factor, and autocrine motility factor. Following inoculation of VEGF-C-transfected and control cells into the gastric walls of nude mice, tumor growth of the VEGF-C-transfected cells was greatly accelerated in comparison with that of control cells. Greater angiogenesis and lymphangiogenesis were also detected in VEGF-C-transfected tumors than in control tumors. CONCLUSIONS: Gastric carcinoma cells express VEGF-C and VEGFR-3. VEGF-C may play a role in the progressive growth of human gastric carcinoma through both autocrine and paracrine mechanisms.

Kolev, Y., H. Uetake, et al. (2008). "Lactate dehydrogenase-5 (LDH-5) expression in human gastric cancer: association with hypoxia-inducible factor (HIF-1alpha) pathway, angiogenic factors production and poor prognosis." Ann Surg Oncol **15**(8): 2336-44.

BACKGROUND: Lactate-dehydrogenase-5 (LDH-5) is an important isoenzyme converting pyruvate to lactate under hypoxic conditions and might play an important role in the development and progression of malignancies. However, the role of LDH-5 in gastric cancer is still unclear. In this study, we investigated the clinical significance of LDH-5 expression in gastric carcinoma. METHODS: LDH-5 expression in 152 patients with different grade and stage gastric carcinoma was analyzed by immunohistochemistry. In addition, hypoxia-inducible factor 1alpha (HIF-1alpha) as a marker of tumor hypoxia, as well as vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) as angiogenesis parameters were also assessed in this study. Correlations between the expression of investigated proteins and various clinicopathological factors including survival were determined. RESULTS: There were 94 cases (61.8%) showing high LDH-5 expression, and 95 patients (62.5%) had high HIF-1alpha expression. Positive correlation was found between LDH-5 expression and HIF-1alpha, VEGF, and COX-2. The overexpression of LDH-5 was more prevalent in advanced tumors having positive vessel invasion. Patients with overexpression of LDH-5 showed far lower disease-free (63.5% vs 82.7%) and overall (56.3% vs 78.4%) survival rates compared with patients with low LDH-5 expression. HIF-1alpha expression was shown to have no significance on survival. In multivariate analysis, high LDH-5 expression kept its independence as a negative prognostic indicator. CONCLUSION: The results of

the current study show that LDH-5 expression may be a useful prognostic factor for patients with gastric carcinoma.

Kosaka, Y., K. Mimori, et al. (2007). "Identification of the high-risk group for metastasis of gastric cancer cases by vascular endothelial growth factor receptor-1 overexpression in peripheral blood." Br J Cancer **96**(11): 1723-8.

Identification of an isolated tumour cell with metastatic ability is important for predicting the recurrence and prognosis of gastric cancer. A biological marker for evaluating the metastatic ability of gastric cancer cells has not yet been identified. We assessed vascular endothelial growth factor receptor-1 mRNA expression by quantitative real-time reverse transcriptase-polymerase chain reaction. Vascular endothelial growth factor receptor-1 mRNA in peripheral blood was more highly expressed in perioperative metastasis-positive and postoperative recurrence cases than in normal control cases, early cancer cases and nonmetastatic advanced cancer cases. The peripheral blood vascular endothelial growth factor receptor-1 mRNA-positive group was associated with advanced clinical stage, deep invasion beyond the muscularis propria, lymphatic involvement, vascular involvement, lymph node metastasis, positive peritoneal lavage cytology, preoperative metastasis and postoperative recurrence. Flow cytometry analysis disclosed that vascular endothelial growth factor receptor-1 expressing cells in the peripheral blood were more abundant in cancer cases with metastases than in cases without metastases. Our data suggest that the amount of positive cells may provide information on the clinical features of gastric cancer, especially in regard to gastric cancer metastasis.

Kunii, K., L. Davis, et al. (2008). "FGFR2-amplified gastric cancer cell lines require FGFR2 and Erbb3 signaling for growth and survival." Cancer Res **68**(7): 2340-8.

We have identified a critical role for amplified FGFR2 in gastric cancer cell proliferation and survival. In a panel of gastric cancer cell lines, fibroblast growth factor receptor 2 (FGFR2) was overexpressed and tyrosine phosphorylated selectively in FGFR2-amplified cell lines KatoIII, Snu16, and OCUM-2M. FGFR2 kinase inhibition by a specific small-molecule inhibitor resulted in selective and potent growth inhibition in FGFR2-amplified cell lines, resulting in growth arrest in KatoIII cells and prominent induction of apoptosis in both Snu16 and OCUM-2M cells. FGFR2-amplified cell lines also contained elevated phosphotyrosine in EGFR, Her2, and Erbb3, but the elevated phosphorylation in EGFR could not be inhibited by gefitinib or erlotinib. We show that the elevated EGFR, Her2, and Erbb3 phosphotyrosine is dependent on FGFR2, revealing EGFR family kinases to be downstream targets of amplified FGFR2. Moreover, shRNA to Erbb3 resulted in a loss of proliferation, confirming a functional role for the activated EGFR signaling pathway. These results reveal that both the FGFR2 and EGFR family signaling pathways are activated in FGFR2-amplified gastric cancer cell lines to drive cell proliferation and survival. Inhibitors of FGFR2 or Erbb3 signaling may have therapeutic efficacy in the subset of gastric cancers containing FGFR2 amplification.

Lai, K. C., H. C. Chiang, et al. (2008). "Artificial neural network-based study can predict gastric cancer staging." Hepatogastroenterology **55**(86-87): 1859-63.

BACKGROUND/AIMS: Primary gastric cancer is a multi-factorial disease comprising many low-penetrance clinicopathological factors and genetic predisposition. Preoperative prediction of tumor staging can be made by artificial neural network (ANN)-based study using clinic-pathological datasets and genetic susceptibility testing. METHODOLOGY: A hospital-based, retrospective, randomized control study was conducted for 121 patients who had recently developed primary gastric cancer. Clinical data and pathological findings were collected and genetic polymorphisms of candidate genes were evaluated. ANN-based study was conducted to predict tumor staging and to evaluate the relative impact of each factor. RESULTS: The best training method was the Quick method, which had an accuracy of 81.82%. The most important factors associated with tumor staging were age and polymorphisms of genes p21, IL-1, IL-4 and p53. CONCLUSIONS: Analysis of genetic polymorphisms of candidate genes by ANN using clinicopathological datasets is a promising method for predicting human gastric cancer staging. This strategy can identify the important genetic, clinical and pathological factors, determine their relative impact, and aid in the development of a prognostic staging system that is useful in individualized patient care.

Lan, M., Y. Shi, et al. (2007). "KCl depolarization increases HIF-1 transcriptional activity via the calcium-independent pathway in SGC7901 gastric cancer cells." Tumour Biol **28**(3): 173-80.

BACKGROUND: Hypoxia-inducible factor 1alpha (HIF-1alpha) has been reported to be expressed aberrantly in gastric cancer cells. Stability and transactivation of HIF-1 were associated with the change of intracellular calcium. We hypothesized that KCl depolarization may modulate HIF-1 activity in

gastric cancer cells through calcium involvement. METHODS: HIF-1alpha expression and its transcriptional activity were determined in SGC7901 gastric cancer cells treated with KCl and/or CoCl2 under normoxia. KCl induced change in the intracellular free calcium concentration and its effect on HIF-1 activity was investigated subsequently. RESULTS: Exposure of SGC7901 cells to KCl (50 mM) could induce HIF-1alpha expression and its nucleus accumulation under normoxic conditions, reaching the peak at 8 and 2 h, respectively. KCl could also induce transactivation of the HIF-1 reporter gene and its target gene VEGF secretion at 8 h. Further experiments confirmed that depolarization of SGC7901 cells with KCl caused an increase in intracellular free calcium concentration. Chelation of intracellular calcium by BAPTA [1,2-bis (2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid] induced HIF-1alpha accumulation and HIF-1 activity. However, elevation of cytosolic calcium level by ionomycin, a calcium ionophore, failed to induce HIF-1 transcriptional activity. CONCLUSIONS: KCl depolarization would act through the calcium-independent pathway leading to enhanced HIF-1 transcriptional activity in gastric cancer cells.

Lee, H. J., S. W. Kim, et al. (2009). "Chemokine receptor CXCR4 expression, function, and clinical implications in gastric cancer." Int J Oncol **34**(2): 473-80.

The chemokine receptor CXCR4 is associated with the biological behavior of cancer, but few studies have addressed the expression and function of CXCR4 in human gastric cancer and its impact on disease prognosis. We studied the expression of CXCR4 using RT-PCR, Western blotting, flow cytometry, and confocal microscopy in five gastric cancer cell lines. We also examined cell proliferation, migration, and anti-apoptotic activity in response to stromal cell-derived factor (SDF)-1alpha and evaluated SDF-1alpha/CXCR4 signaling pathways. Furthermore, we investigated the correlation between CXCR4 expression and the clinical features of 221 gastric cancer tissue samples. CXCR4 transcripts and proteins were detectable in all five gastric cancer cell lines. However, MKN-28, MKN-45, MKN-74, and SNU16 cells did not express membrane CXCR4. In contrast, KATO III cells expressed membrane CXCR4. In these cells, SDF-1alpha-induced migration was observed and was blocked by AMD3100, a specific inhibitor of CXCR4. SDF-1alpha induced rapid phosphorylation of Erk1/2 MAPK but did not promote phosphorylation of Stat3 or Akt. Gastric cancer tissue samples expressed CXCR4 with variable intensities. Strong CXCR4 expression was significantly associated with lymph node metastases (P=0.028) and higher stages III/IV (P=0.047), and further tended to be correlated with a reduced 5-year survival rate (42.6% vs. 53.9%; P=0.1). In conclusion, CXCR4 expression is associated with gastric cancer cell migration in vitro, and strong expression of CXCR4 by gastric cancer cells is significantly associated with lymphatic metastasis in patients with gastric cancer, suggesting that CXCR4 plays an important role during gastric cancer progression.

Lee, K. A., J. H. Park, et al. (2007). "Interaction of polymorphisms in the interleukin 1B-31 and general transcription factor 2A1 genes on the susceptibility to gastric cancer." Cytokine **38**(2): 96-100.

Proinflammatory genotypes of the IL-1 (interleukin-1) gene have been associated with an increased gastric cancer risk in Caucasians, whereas some studies in Asian populations did not find such association. Furthermore, the risk genotypes differed somewhat between Caucasian and Asian populations. These findings might reflect more complex genetic mechanisms in Asian compared with Caucasian populations. Therefore, we examined a polymorphism (rs1864169) in the general transcription factor 2A1 (GTF2A1) gene as a test of the hypothesis that this transcription factor and IL-IB gene polymorphisms interact in the effects on the gastric cancer risk due to the possible biological relationship between the two genes. Genotyping of the 515 control and 342 case samples was performed by primer extension assay and SNaPshot assays. We found an association between carriage of the IL1B-31C allele and gastric cancer among Koreans, which was observed only in subjects with GTF2A1 GG genotype. The GTF2A1GG/IL1B-31C carrier genotype combination showed stronger association with diffuse type gastric cancer cases. These findings indicate that the effect of the two genetic polymorphisms on risk of gastric cancer is synergistic. Our results also suggest that an additional host genetic factor acting epistatically may differentially contribute to the histogenesis of the diffuse and intestinal subtypes.

Lee, K. H., E. Y. Choi, et al. (2008). "Hepatocyte growth factor promotes cell survival by phosphorylation of BAD in gastric cancer cells." Oncol Res **17**(1): 23-32.

Hepatocyte growth factor (HGF) is one of the survival factors with a potent ability to promote cell survival by inhibiting apoptosis. However, the mechanism by which HGF inhibits apoptosis is not completely understood. To explore the genes associated with stomach cancer cell survival by HGF, we used cDNA microarray technology and selected 26 genes up- or downregulated in NUGC-3 cells during

HGF treatment. Among them, BAD was confirmed to be upregulated at the RNA and protein levels by HGF treatment. We investigated the effect of BAD induced by HGF on cell survival. HGF treatment inhibited apoptosis induced by BAD overexpression and enhanced BAD phosphorylation. Pretreatment of NUGC-3 cells with PI3K inhibitors, LY 294002, decreased HGF-induced BAD phosphorylation on Ser136 whereas an MEK inhibitor, PD 98059, decreased BAD phosphorylation on Ser112. In conclusion, increases in BAD levels as well as BAD phosphoryation by HGF might contribute to HGF-mediated cell survival in NUGC-3 cells.

Lee, S. E., J. W. Lim, et al. (2009). "Activator protein-1 mediates docosahexaenoic acid-induced apoptosis of human gastric cancer cells." Ann N Y Acad Sci **1171**: 163-9.

Docosahexaenoic acid (DHA) shows anti-inflammatory and/or anticancer effects in some cells. Activator protein-1 (AP-1) regulates cellular proliferation and apoptosis. Although recent studies demonstrate the association between gastric cancer risk and DHA, the exact molecular mechanism has not been clarified. We investigated whether AP-1 mediates DHA-induced apoptosis of gastric cancer cells. We found that DHA induced cell death and DNA fragmentation in parallel with the activation of extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinases (JNK) as well as AP-1. DHA increased the protein levels of p53, cytochrome c, and Bax in gastric cancer cells. DHA-induced DNA fragmentation and protein levels of p53, cytochrome c, and Bax were inhibited in the cells transfected with c-jun dominant-negative mutant (TAM67). Because JNK and ERK are upstream signaling for AP-1 activation, we suggest that DHA-induced activation of AP-1 may mediate apoptosis of gastric cancer cells by inducing the expression of apoptotic genes in gastric cancer cells.

Lee, S. H., J. Kim, et al. (2009). "Hypoxic silencing of tumor suppressor RUNX3 by histone modification in gastric cancer cells." Oncogene **28**(2): 184-94.

RUNX3 is a tumor suppressor that is silenced in cancer following hypermethylation of its promoter. The effects of hypoxia in tumor suppressor gene (TSG) transcription are largely unknown. Here, we investigated hypoxia-induced silencing mechanisms of RUNX3. The expression of RUNX3 was downregulated in response to hypoxia in human gastric cancer cells at the transcriptional level. This downregulation was abolished following treatment with the histone deacetylase (HDAC) inhibitor trichostatin A (TSA) and cytosine methylation inhibitor 5-aza-2-deoxycytidine (5-Aza), suggesting that an epigenetic regulatory mechanism may be involved in RUNX3 silencing by hypoxia. DNA methylation PCR and bisulfite-sequencing data revealed that hypoxia did not affect the methylation of RUNX3 promoter. A chromatin immunoprecipitation (ChIP) assay revealed increased histone H3-lysine 9 dimethylation and decreased H3 acetylation in the RUNX3 promoter following hypoxia. Hypoxia resulted in the upregulation of G9a histone methyltransferase (HMT) and HDAC1; additionally, overexpression of G9a and HDAC1 attenuated RUNX3 expression. The overexpression of G9a and HDAC1, but not their mutants, inhibited the nuclear localization and expression of RUNX3. Diminished mRNA expression and nuclear localization of RUNX3 during hypoxia was abolished by siRNA-mediated knockdown of G9a and HDAC1. This study suggests that hypoxia silences RUNX3 by epigenetic histone regulation during the progression of gastric cancer.

Li, D., J. Ding, et al. (2009). "Fibronectin promotes tyrosine phosphorylation of paxillin and cell invasiveness in the gastric cancer cell line AGS." Tumori **95**(6): 769-79.

AIMS AND BACKGROUND: Paxillin is a central protein within the focal adhesion and serves as a critical transducer of signals from fibronectin. Although abnormal expression of fibronectin and paxillin is often observed during the development of human malignancies, the relationship between paxillin and cell invasion in gastric cancer is still unclear. The current study was designed to investigate the potential role and mechanisms of fibronectin in tyrosine phosphorylation of paxillin and in the invasiveness of gastric cancer cells. METHODS: Expression of paxillin in human gastric cancer samples was examined by immunohistochemical staining. A gastric cancer cell line, AGS, was stimulated by fibronectin with gradient concentrations, and expression of paxillin and phosphorylation of paxillin tyrosine 118 (tyr118) was detected by immunoprecipitation and Western blotting. The invasiveness of AGS cells was measured by the modified Boyden chamber assay. Small interfering RNA (siRNA) targeting paxillin was used to establish the role of paxillin (tyr118) in the process of cell invasion enhanced by fibronectin. siRNA targeting focal adhesion kinase (FAK) was used to verify the effect of FAK tyrosine 397 (tyr397) on phosphorylation of paxillin (tyr118). RESULTS: Positivity for paxillin staining in human gastric cancer was associated with tumor stage. AGS cell showed dose dependence on fibronectin for invasiveness and phosphorylation of paxillin (tyr118). Invasiveness and phosphorylation of paxillin (tyr118) in AGS cells reached their peak when the concentration of fibronectin reached 100 nmol/L. siRNA targeting

paxillin decreased the phosphorylation of paxillin (tyr118) and the invasiveness of AGS cells significantly as compared with controls. Blockage of FAK (tyr397) can inhibit phosphorylation of paxillin (tyr118) stimulated by fibronectin. CONCLUSIONS: Fibronectin promotes paxillin (tyr118) phosphorylation and invasiveness of AGS cells. Paxillin silencing by RNA interference inhibits the cell invasiveness stimulated by fibronectin. Paxillin is a key factor in the fibronectin-stimulated invasiveness of AGS cells.

Li, K., Y. Zhang, et al. (2009). "Association of the hypoxia inducible factor-1alpha gene polymorphisms with gastric cancer in Tibetans." Biochem Genet **47**(9-10): 625-34.

To determine how single nucleotide polymorphisms (SNPs) in the hypoxia inducible factor-1alpha (HIF-1alpha) gene coding regions affect gastric cancer, the authors conducted an association study of the HIF-1alpha polymorphisms C1772T and G1790A for a Tibet population. DNA was extracted from peripheral blood of 87 gastric cancer patients and 106 controls and analyzed using the polymerase chain reaction/ligase detection reaction test for HIF-1alpha polymorphisms. There was a significant increase in the frequency of the GA 1790 genotype in patients with gastric cancer compared with healthy controls (OR 2.93; 95% CI 1.06-8.06). The genotype frequency of the HIF-1alpha G1790A allele A is higher in gastric cancer groups than in controls (OR 2.78; 95% CI 1.03-7.45). As for the C1772T polymorphism, no positive correlation was found between gastric cancer patients and controls (P = 0.06). Our results suggest that the HIF-1alpha G1790A polymorphism may be associated with gastric cancer in Tibetans.

Li, Q., N. Zhang, et al. (2009). "Critical role and regulation of transcription factor FoxM1 in human gastric cancer angiogenesis and progression." Cancer Res **69**(8): 3501-9.

The mammalian forkhead box (Fox) transcription factor FoxM1b is implicated in tumorigenesis. However, the presence of expression and role of FoxM1b in gastric cancer remain unknown. Therefore, we investigated FoxM1b expression in 86 cases of primary gastric cancer and 57 normal gastric tissue specimens. We further investigated the underlying mechanisms of altered FoxM1b expression in and the effect of this altered expression on gastric cancer growth and metastasis using in vitro and animal models of gastric cancer. We found weak expression of FoxM1b protein in the mucous neck region of gastric mucosa, whereas we observed strong staining for FoxM1b in tumor cell nuclei in various gastric tumors and lymph node metastases. A Cox proportional hazards model revealed that FoxM1b expression was an independent prognostic factor in multivariate analysis (P < 0.001). Experimentally, overexpression of FoxM1b by gene transfer significantly promoted the growth and metastasis of gastric cancer cells in orthotopic mouse models, whereas knockdown of FoxM1b expression by small interfering RNA did the opposite. Promotion of gastric tumorigenesis by FoxM1b directly and significantly correlated with transactivation of vascular endothelial growth factor expression and elevation of angiogenesis. Given the importance of FoxM1b to regulation of the expression of genes key to cancer biology overall, dysregulated expression and activation of FoxM1b may play important roles in gastric cancer development and progression.

Li, T., B. W. Cao, et al. (2008). "Correlation of transforming growth factor beta-1 gene polymorphisms C-509T and T869C and the risk of gastric cancer in China." J Gastroenterol Hepatol **23**(4): 638-42.

BACKGROUND AND AIM: As an important cytokine that modulate the cell cycle, the involvement of transforming growth factor beta-1 (TGF-beta1) in carcinogenesis has been extensively studied for many years. Literatures have demonstrated that TGF-beta1 gene polymorphisms may alter the risk of various cancers, such as lung, prostate and breast. To investigate whether polymorphisms of the TGF-beta1 gene can modify the risk of gastric cancer, we conduct this hospital-based, case-control study. METHODS: One hundred and sixty-seven cases and 193 gender, age-matched healthy controls were enrolled in this case-control study. TGF-beta1 polymorphisms C-509T and T + 869C were identified by PCR-RFLP and ARMS-PCR protocols, respectively. RESULTS: Significantly different distributions of both genes were demonstrated between the case and control. Variant genotypes -509CT, -509TT, +869TC and +869CC were associated with increased risk of gastric cancer (P = 0.001, OR = 2.54; P = 0.016, OR = 2.09; P < 0.001, OR = 3.46; P < 0.001, OR = 4.04, respectively). With haplotype analysis, wild type CT (-509C and +869T) led to a lower frequency in case than that in control (P < 0.001), while haplotype TC was more frequent in case than in control (P < 0.001). Multiple logistic regression analysis revealed that individuals with haplotype TC had an increased likelihood of developing gastric cancer (OR = 3.19, 95%CI = 1.72-5.90). CONCLUSIONS: Our findings imply that -509C > T and +869T > C gene polymorphisms in TGF-beta1 may be a critical risk factor of genetic

susceptibility to gastric cancer in the Chinese population.

Li, W., Z. Ge, et al. (2008). "CIP2A is overexpressed in gastric cancer and its depletion leads to impaired clonogenicity, senescence, or differentiation of tumor cells." Clin Cancer Res **14**(12): 3722-8.

PURPOSE: Cancerous inhibitor of protein phosphatase 2A (CIP2A) is an oncogenic factor stabilizing c-MYC protein and driving cellular transformation. We determine whether CIP2A expression can serve as marker for gastric cancer and investigate the mechanism underlying CIP2A-mediated transformation and cell proliferation. EXPERIMENTAL DESIGN: Normal and malignant gastric tissues derived from 37 patients with gastric cancer were analyzed for CIP2A expression using reverse transcription-PCR and immunohistochemical staining. Gastric and other cell lines with different p53 and pRB backgrounds were used to inhibit CIP2A expression using small interfering RNA and then examined for clonogenic potentials, senescence, or differentiation. RESULTS: CIP2A mRNA was present in 34 of 37 (90%) of tumor specimens but absent in 27 of 37 (73%) of matched normal gastric mucosa. In 10 adjacent normal tissues with detectable CIP2A mRNA, 6 of them exhibited much weaker levels of CIP2A compared with their corresponding tumors. Thus, a total of 32 (87%) gastric cancer samples overexpressed CIP2A. CIP2A protein expression was readily detectable in the tumor tissues but absent in normal gastric mucosa. Depleting CIP2A expression substantially inhibited growth and clonogenic capabilities of tumor cell lines independently of p53 and pRB pathways. Gastric cancer-derived AGS cells underwent senescence following the inhibition of CIP2A expression. Moreover, CIP2A depletion triggered partial differentiation of leukemic HL60 cells. CONCLUSION: CIP2A in tumor cells is required for sustained proliferation by preventing cell growth arrest, senescence, or differentiation and its expression is significantly ($P < 0.001$) discriminatory between normal and cancerous gastric tissue.

Li, X., Z. C. Yue, et al. (2008). "Elevated serum level and gene polymorphisms of TGF-beta1 in gastric cancer." J Clin Lab Anal **22**(3): 164-71.

Transforming growth factor (TGF)-beta1, as a candidate tumor marker, is currently of interest. In this study, serum TGF-beta1 levels in gastric cancer (GC) patients and healthy volunteers were measured using enzyme-linked immunosorbent assay (ELISA). In addition, single nucleotide polymorphisms (SNPs) of the TGF-beta1 gene at codon 10 and codon 25 were identified by means of amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) and sequence analysis. Our results indicated that serum concentrations of TGF-beta1 in GC patients were significantly higher than those in the control, and positively correlated with tumor mass, invasion, metastasis, and clinical stage. The serum TGF-beta1 levels of patients recovering from radical resection were markedly lower than those before surgery. Meanwhile, no deoxyribonucleic acid (DNA) sequence variation at codon 25 of the TGF-beta1 gene was found and a TGF-beta1 gene polymorphism at codon 10 did not show obvious correlations with either TGF-beta1 expression or clinicopathological parameters of GC. Our evidence suggested that serum concentration of TGF-beta1 might be a novel tumor marker for GC and the polymorphisms of TGF-beta1 gene did not play a role as a determinant of serum TGF-beta1 concentration or as a genetic risk factor in the gastric carcinogenesis and progression.

Li, Z. Q., W. P. Yu, et al. (2007). "Association of gastric cancer with tyrosine hydroxylase gene polymorphism in a northwestern Chinese population." Clin Exp Med **7**(3): 98-101.

Gastric cancer (GC) is a common and complex disease caused by multifactors. The aim of our study was to investigate the association of the common polymorphisms detected in insulin-like growth factor (IGF)-II, IGF-1 receptor, insulin-like growth factor binding protein 1 (IGFBP1), insulin (INS) and tyrosine hydroxylase (TH) with susceptibility to GC in a northwestern Chinese population. One hundred and fifty-four GC patients and 166 healthy controls were investigated in our study. The genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism. The frequencies of CC and CT genotypes of TH were significantly higher in GC patients than in controls, as the odds ratios were 3.03 (95%CI 1.438-6.362, P=0.003) and 1.97 (95%CI 1.218-3.167, P=0.005), respectively. No association was found between the polymorphisms of IGF-II ApaI, insulin-like growth factor-1 receptor MnlI, IGFBP1 Bgl II and INS-23HphI and the development of GC. The presence of CC and CT genotypes of TH was associated with a significantly increased risk of GC. But the polymorphisms of other genes detected did not indicate an increased risk of GC in the investigated population.

Lin, M. T., C. C. Chang, et al. (2007). "Elevated expression of Cyr61 enhances peritoneal dissemination of gastric cancer cells through integrin alpha2beta1." J Biol Chem **282**(47): 34594-604.

Cysteine-rich 61 (Cyr61/CCN1) is involved in human gastric cancer development and progression. Nonetheless, the role of Cyr61 as regards peritoneal

dissemination of such cancers has not yet been completely characterized. We used liposome-mediated transfection to establish Cyr61, or antisense Cyr61, expression vectors into gastric cancer AGS or MKN45 cell lines. Transfectants were tested by means of a cancer-cell adhesion assay in vitro and ex vivo. Furthermore, a functional integrin fluorescence-activated cell sorting assay, reverse transcription-PCR, and an AP-1 reporter assay were performed to investigate the potential signaling pathway of Cyr61. It was shown that stable transfection of Cyr61 into the AGS cell line strongly enhanced its adhesion ability. The overexpression of Cyr61 within AGS cells significantly increased the functional expression of integrin alpha(2)beta(1). Function-neutralizing antibody to integrin alpha(2)beta(1) effectively suppressed the Cyr61-mediated enhanced adhesion of AGS cells to peritoneal tissue. Promoter assays of integrin alpha2 gene further revealed that the AP-1 pathway was evidently activated within Cyr61-expressing AGS cells. Animal studies have revealed that mice injected with Cyr61-overexpressed AGS cells featured a greater number of peritoneal seeding nodules and a lower survival rate than the Neo control cell lines, and when such cells were treated with functional blocking antibody to integrin alpha(2)beta(1), they were able to elicit a decline in the peritoneal dissemination. The data suggest that Cyr61 may contribute to the peritoneal dissemination of gastric cancer by promoting tumor-cell adhesion ability through the up-regulation of the functional integrin alpha(2)beta(1) via an AP-1-dependent pathway.

Lin, M. T., I. H. Kuo, et al. (2008). "Involvement of hypoxia-inducing factor-1alpha-dependent plasminogen activator inhibitor-1 up-regulation in Cyr61/CCN1-induced gastric cancer cell invasion." J Biol Chem **283**(23): 15807-15.

Cysteine-rich 61 (Cyr61/CCN1), one of the members of CCN family, has been implicated in the progression of human malignancies. Previously, our studies have demonstrated that Cyr61/CCN1 has a role in promoting gastric cancer cell invasion, but the mechanism is not clear yet. Here, we found that hypoxia-inducing factor-1alpha (HIF-1alpha) protein, but not mRNA, expression was significantly elevated in gastric cancer cells overexpressing Cyr61. Supportively, a profound reduction of endogenous HIF-1alpha protein was noted in one highly invasive cell line, TSGH, when transfected with antisense Cyr61. By comparison, the induction kinetics of HIF-1alpha protein by recombinant Cyr61 (rCyr61) was distinct from that of insulin-like growth factor-1 and CoCl(2) treatment, both well known for induction of HIF-1alpha. Using cycloheximide and MG132, we demonstrated that the Cyr61-mediated HIF-1alpha up-regulation was through de novo protein synthesis, rather than increased protein stability. rCyr61 could also activate the PI3K/AKT/mTOR and ERK1/2 signaling pathways, both of which were essential for HIF-1alpha protein accumulation. Blockage of HIF-1alpha activity in Cyr61-expressing cells by transfecting with a dominant negative (DN)-HIF-1alpha strongly inhibited their invasion ability, suggesting that elevation in HIF-1alpha protein is vital for Cyr61-mediated gastric cancer cell invasion. In addition, several HIF-1alpha-regulated invasiveness genes were examined, and we found that only plasminogen activator inhibitor-1 (PAI-1) showed a significant increase in mRNA and protein levels in cells overexpressing Cyr61. Treatment with PAI-1-specific antisense oligonucleotides or function-neutralizing antibodies abolished the invasion ability of the Cyr61-overexpressing cells. Transfection with dominant negative-HIF-1alpha to block HIF-1alpha activity also effectively reduced the elevated PAI-1 level. In conclusion, our data provide a detailed mechanism by which Cyr61 promoted gastric cancer cell invasive ability via an HIF-1alpha-dependent up-regulation of PAI-1.

Lin, M. T., B. R. Lin, et al. (2007). "IL-6 induces AGS gastric cancer cell invasion via activation of the c-Src/RhoA/ROCK signaling pathway." Int J Cancer **120**(12): 2600-8.

Interleukin-6 (IL-6) is a multifunctional cytokine that is associated with the disease status and outcomes of gastric cancer. Nonetheless, the underlying mechanism of how IL-6 promotes the spread of gastric cancer is still unclear. In this study, we used a modified Boyden chamber assay to test the invasion ability of different gastric cancer cell lines. Liposome-mediated transfection was used to introduce an IL-6 expression vector into AGS cells, and the transfectants were further examined for the expression of active RhoA and phosphorylated Src using a pull-down assay and coimmunoprecipitation/Western blot analysis. Furthermore, RhoA expression in gastric adenocarcinoma specimens was investigated immunohistochemically. We documented that IL-6 could promote AGS cell motility and invasiveness, and inhibition of RhoA expression by dominant negative RhoA, C3 transferase, or dominant negative Src expressing plasmids could effectively decrease the invasiveness of IL-6 transfectants. We also documented an interaction between active RhoA and phosphorylated-Src following IL-6 treatment. Gastric cancers displaying high expression of RhoA are highly correlated with aggressive lymph node metastasis, more advanced tumor stage, histologically diffuse type and poorer survival. In conclusion, IL-6

induces AGS gastric cancer cell invasion via activation of the c-Src/RhoA/ROCK signaling pathway and RhoA expression could be used as a prognostic factor in patients with gastric adenocarcinoma.

Liu, B., H. Sun, et al. (2009). "Adenovirus vector-mediated upregulation of spermidine /spermine N1-acetyltransferase impairs human gastric cancer growth in vitro and in vivo." Cancer Sci **100**(11): 2126-32.

Spermidine/spermine N(1)-acetyltransferase (SSAT) is the rate-limiting step in polyamine catabolism. In a previous study, we constructed a recombinant adenovirus, Ad-SSAT, which can express human SSAT. In the present study, we investigated the effect of upregulated and downregulated SSAT on gastric cancer cells. We found that upregulated SSAT could inhibit the growth of MGC803 and SGC7901 cells, whereas adverse results were found with downregulated SSAT. We further analyzed cell cycle profiles and the expression levels of the major cell cycle regulatory proteins of S phase. The results showed that the growth inhibition was caused by S phase arrest. Ad-SSAT suppressed the expression of cyclin A and nuclear factor E2F1 in MGC803 and SGC7901 cells. We observed the E2F promoter activity caused by Ad-SSAT using a reporter gene assay. We also investigated the antitumorigenicity of upregulated SSAT by Ad-SSAT using a SGC7901 xenograft model in nude mice. Our results suggest that the upregulation of SSAT by Ad-SSAT infection inhibited the growth of gastric cancer in vitro and in vivo. Ad-SSAT arrested gastric cancer cells in S phase, which was mediated through downregulation of the cyclin A-E2F signaling pathway.

Liu, L. Y., Y. C. Han, et al. (2008). "Expression of connective tissue growth factor in tumor tissues is an independent predictor of poor prognosis in patients with gastric cancer." World J Gastroenterol **14**(13): 2110-4.

AIM: To examine the expression of connective tissue growth factor (CTGF), also known as CCN2, in gastric carcinoma (GC), and the correlation between the expression of CTGF, clinicopathologic features and clinical outcomes of patients with GC. METHODS: One hundred and twenty-two GC patients were included in the present study. All patients were followed up for at least 5 years. Proteins of CTGF were detected using the Powervision two-step immunostaining method. RESULTS: Of the specimens from 122 GC patients analyzed for CTGF expression, 58 (58/122, 47.5%) had a high CTGF expression in cytoplasm of gastric carcinoma cells and 64 (64/122, 52.5%) had a low CTGF expression. Patients with a high CTGF expression showed a higher incidence of lymph node metastasis than those with a low CTGF expression (P = 0.032). Patients with a high CTGF expression had significantly lower 5-year survival rate than those with a low CTGF expression (27.6% vs 46.9%, P = 0.0178), especially those staging I + II + III (35.7% vs 65.2%, P = 0.0027). CONCLUSION: GC patients with an elevated CTGF expression have more lymph node metastases and a shorter survival time. CTGF seems to be an independent prognostic factor for the successful differentiation of high-risk GC patients staging I + II + III. Over-expression of CTGF in human GC cells results in an increased aggressive ability.

Liu, R., Z. Li, et al. (2009). "Mechanism of cancer cell adaptation to metabolic stress: proteomics identification of a novel thyroid hormone-mediated gastric carcinogenic signaling pathway." Mol Cell Proteomics **8**(1): 70-85.

Gastric cancer is the second most common cancer worldwide and has a poor prognosis. To determine the mechanism of adaptation to metabolic stress in cancer cells, we used gastric cancer as a model system to reveal the potential signaling pathways involved. Two-dimensional polyacrylamide gel electrophoresis coupled with ESI-Q-TOF MS/MS analysis was used to identify differentially expressed proteins between gastric tumor tissues and the corresponding noncancerous tissues. In total, 107 spots with significant alteration (+/-over 2-fold, $p < 0.05$) were positively identified by MS/MS analysis. Altered expression of representative proteins was validated by RT-PCR and Western blotting. Cluster analysis of the changed proteins revealed an interesting group of metabolic proteins, which suggested accumulation of triiodothyronine (T(3); the major functional component of thyroid hormone) and overexpression of hypoxia-induced factor (HIF) in gastric carcinoma. These observations were further confirmed by electrochemiluminescence immunoassay and immunohistochemistry. T(3)-induced expression of HIF1-alpha and vascular endothelial growth factor was further verified using a gastric cancer cell line and in vivo mouse model. Because the early accumulation of HIF1-alpha was found to be independent of de novo transcription, we also found that the cytosolic cascade phosphatidylinositol 3-kinase/Akt pathway sensitive to T(3) stimulus was involved. Furthermore we demonstrated that T(3)-induced overexpression of HIF1-alpha was mediated by fumarate accumulation and could be enhanced by fumarate hydratase inactivation but inhibited by 2-oxoglutarate. These results provide evidence for alteration of metabolic

proteins and dysfunction of thyroid hormone regulation in gastric tumors, and a novel thyroid hormone-mediated tumorigenic signaling pathway is proposed. Our findings are considered a significant step toward a better understanding of adaptations to metabolic stress in gastric carcinogenesis.

Liu, Y., Q. Y. Zhang, et al. (2007). "Relationship between LAPTM4B gene polymorphism and susceptibility of gastric cancer." Ann Oncol **18**(2): 311-6.

BACKGROUND: A novel gene called LAPTM4B (lysosome-associated protein transmembrane 4beta) was mapped to 8q22, and contains seven exons. The 2.25-kb messenger RNA of the gene encodes a putative lysosome-associated protein with four transmembrane regions. There are two alleles of the gene, named as LAPTM4B*1 and LAPTM4B*2. Allele *1 differs from allele *2 in that it contains only one copy of a 19-bp sequence in the 5' untranslated region (UTR), whereas this sequence is duplicated and tandemly arranged in allele *2. Studies showed that the allelic variation of LAPTM4B was associated with the genetic susceptibility of hepatocellular carcinoma but not with that of esophageal squamous cell carcinoma. This study was designed to investigate the possible association between the allelic variation of LAPTM4B and the genetic susceptibility of gastric cancer. Materials and methods: The genotype of LAPTM4B was analyzed in 350 unrelated healthy adult individuals and 214 patients with gastric cancer by utilizing polymerase chain reaction based on specific primers. The genotypic distribution of LAPTM4B was analyzed by chi(2) test. RESULTS: The allelic frequencies of the *2 were 33.88% and 24.14% in the gastric cancer group and the healthy control group, respectively, which was significantly different between the two groups ($P < 0.001$). There was a significant difference in the overall genotypic distribution between the patients and the controls ($P = 0.023$). The risk of suffering from gastric cancer was increased 1.819 times in the individuals of the *1/2 genotype [95% confidence interval (CI) 1.273-2.601] and 2.387 times in the individuals of the *2/2 genotype of LAPTM4B (95% CI 1.195-4.767) compared with the *1/1 genotype. No association between the genotypic distribution of LAPTM4B and the clinical information on patients of gastric cancer such as age, pathological type, differentiation classification of TNM, and infection of hepatitis B virus was shown. CONCLUSION: This study indicated that allele *2 of LAPTM4B might be the risk factor of gastric cancer, which could be associated with genetic susceptibility of gastric cancer.

Lo, S. S., J. H. Chen, et al. (2009). "Functional polymorphism of NFKB1 promoter may correlate to the susceptibility of gastric cancer in aged patients." Surgery **145**(3): 280-5.

BACKGROUND: Activated nuclear factor-kappaB (NF-kappaB) is associated reportedly with the pathogenesis of numerous malignancies. This study investigated whether a common insertion (ins)/deletion (del) polymorphism (-94 ins/del ATTG) in the NFKB1 promoter is associated with susceptibility to gastric cancer and its tumor behavior. METHODS: Blood samples from 182 gastric cancer patients and 116 controls were examined by polymerase chain reaction-based genotyping. Allelotype and genotype (polymorphism) of NFKB1 promoter in gastric cancer patients were analyzed with controls and patients' clinicopathologic factors to evaluate their association using a multivariate analytical model. RESULTS: The mean ages of patients and controls were 65.7 +/- 12.8, and 64.9 +/- 8.8 years old, respectively. Sex ratios (male to female) were 2.7:1 and 2.2:1, respectively. Insertion allelotype, genotypes with ins/ins, as well as ins allele carrier (ins/ins+ ins/del) were significantly greater in gastric cancer patients than in controls, especially in patients >65 years old, but not in younger patients. The polymorphism did not correlate with clinicopathologic factors and patient survival. CONCLUSION: NFKB1 could be a susceptible gene for gastric cancer and its functional polymorphism in promoter is associated with the risk of gastric cancer, particularly in aged patients.

Ma, J., M. Chen, et al. (2008). "Pancreatic duodenal homeobox-1 (PDX1) functions as a tumor suppressor in gastric cancer." Carcinogenesis **29**(7): 1327-33.

AIM: Pancreatic duodenal homeobox-1 (PDX1) is a transcription factor of homeobox genes family important in differentiation and development of the pancreas, duodenum and antrum. This study aims to clarify the putative role of PDX1 in gastric carcinogenesis. METHODS: PDX1 expression was detected in gastric tissues with chronic gastritis and cancer as well as gastric cancer cell lines by immunohistochemistry, western blot, reverse transcription-polymerase chain reaction (RT-PCR) or quantitative real-time RT-PCR assays. The effects of PDX1 on cell proliferation, apoptosis, clone formation and migration were evaluated using cancer cell lines after transient or stable transfection with PDX1-expressing vector. The ability of PDX1 stable transfectant in tumor formation in xenograft mice was assessed. RESULTS: PDX1 was strongly expressed in normal gastric glands, but was absent in 29 of 39 of human gastric cancer and most gastric cancer cell lines. Negative correlation between PDX1 and Ki-67

expression was found in both gastric tissues and cell lines. Ectopic overexpression of PDX1 significantly inhibited cell proliferation and induced apoptosis, accompanied by the activation of caspases 3, 8, 9 and 10. Overexpression of PDX1 also impaired the ability of cancer cells in clonal formation and migration in vitro. Furthermore, stable transfection with PDX1 reduced the ability of cancer cells in tumor formation in nude mice. CONCLUSIONS: PDX1 expression is lost in gastric cancers. Its effect on cell proliferation/apoptosis, migration and tumor formation in vitro and in vivo suggested that this protein functions as a putative tumor suppressor in gastric cancer.

Marrelli, D., C. Pedrazzani, et al. (2009). "Negative Helicobacter pylori status is associated with poor prognosis in patients with gastric cancer." Cancer **115**(10): 2071-80.

BACKGROUND: Recent studies have suggested that Helicobacter pylori (H. pylori) infection may be related to better prognosis in patients with gastric cancer, but to the authors' knowledge, this finding has not yet been validated. In the current study, the association between H. pylori status and clinical outcome was investigated in a large cohort of patients. METHODS: Frozen non-neoplastic gastric mucosa and serum samples obtained from 297 patients who underwent surgery for primary gastric cancer between 1988 and 2004 were retrieved from the serum and tissue bank of the study department. H. pylori status was defined by means of polymerase chain reaction (PCR) analysis for the vacA gene in gastric mucosa and by serologic assay of H. pylori and CagA antibodies. Univariate and multivariate analyses were used for the association between clinicopathologic variables and long-term outcome. RESULTS: Positivity for H. pylori infection was observed in 256 of 297 patients (86%), whereas in 41 patients (14%), PCR for vacA and both serologic tests were negative. Negative H. pylori status was found to be significantly associated with cardia location, advanced pT classification, noncurative surgery, and a lower 5-year survival rate after R0 resection (24% vs 57%; P < .001). Multivariate survival analysis confirmed H. pylori status as a significant prognostic factor (hazards ratio, 2.47; 95% confidence interval, 1.40-4.35 [P = .002]). The influence of H. pylori status on long-term survival was observed in patients with early as well as advanced pT classifications. CONCLUSIONS: Negative H. pylori status appears to be an indicator of poor prognosis in patients with gastric cancer, and is independent of other well-known clinical and pathologic prognostic variables.

Matsubara, J., T. Nishina, et al. (2008). "Impacts of excision repair cross-complementing gene 1 (ERCC1), dihydropyrimidine dehydrogenase, and epidermal growth factor receptor on the outcomes of patients with advanced gastric cancer." Br J Cancer **98**(4): 832-9.

Using laser-captured microdissection and a real-time RT-PCR assay, we quantitatively evaluated mRNA levels of the following biomarkers in paraffin-embedded gastric cancer (GC) specimens obtained by surgical resection or biopsy: excision repair cross-complementing gene 1 (ERCC1), dihydropyrimidine dehydrogenase (DPD), methylenetetrahydrofolate reductase (MTHFR), epidermal growth factor receptor (EGFR), and five other biomarkers related to anticancer drug sensitivity. The study group comprised 140 patients who received first-line chemotherapy for advanced GC. All cancer specimens were obtained before chemotherapy. In patients who received first-line S-1 monotherapy (69 patients), low MTHFR expression correlated with a higher response rate (low: 44.9% vs high: 6.3%; P=0.006). In patients given first-line cisplatin-based regimens (combined with S-1 or irinotecan) (43 patients), low ERCC1 correlated with a higher response rate (low: 55.6% vs high: 18.8%; P=0.008). Multivariate survival analysis of all patients demonstrated that high ERCC1 (hazard ratio (HR): 2.38 (95% CI: 1.55-3.67)), high DPD (HR: 2.04 (1.37-3.02)), low EGFR (HR: 0.34 (0.20-0.56)), and an elevated serum alkaline phosphatase level (HR: 1.00 (1.001-1.002)) were significant predictors of poor survival. Our results suggest that these biomarkers are useful predictors of clinical outcomes in patients with advanced GC.

Matsubara, J., Y. Yamada, et al. (2008). "Impact of insulin-like growth factor type 1 receptor, epidermal growth factor receptor, and HER2 expressions on outcomes of patients with gastric cancer." Clin Cancer Res **14**(10): 3022-9.

PURPOSE: Expression levels of insulin-like growth factor type 1 receptor (IGF-IR), epidermal growth factor receptor (EGFR), and HER2 expressions have been linked to clinical outcomes in several solid tumors. However, the clinical significance of these biomarkers in gastric cancer (GC) remains unclear. This study was designed to delineate the clinical implications of these three biomarkers in GC. EXPERIMENTAL DESIGN: The study group comprised 87 patients who underwent gastrectomy at National Cancer Center Hospital and subsequently received chemotherapy for recurrent or residual tumors. Using immunohistochemical techniques, we analyzed the expressions of IGF-IR, EGFR, and HER2 on formalin-fixed paraffin-embedded specimens of surgically removed primary

tumors. RESULTS: IGF-IR expression (defined as >10% membranous staining) was found in 67 tumors (77%), EGFR expression in 55 (63%), and HER2 expression in 16 (18%). Positive coexpression of IGF-IR and EGFR was found in 48 tumors (55%), that of IGF-IR and HER2 in 16 (18%), and that of EGFR and HER2 in 13 (15%). Multivariate survival analysis showed that IGF-IR-positive expression [hazard ratio (HR) 2.14, 95% confidence interval (95% CI) 1.20-3.82; P = 0.01], performance status 1 or 2 (HR 1.83, 95% CI 1.15-2.91; P = 0.01), and diffuse type tumors (HR 1.71; 95% CI 1.08-2.70; P = 0.02) were significant predictors of poor survival. CONCLUSIONS: IGF-IR expression in surgical GC specimens, poor performance status, and diffuse type tumors are significant predictors of poor outcomes in patients with GC. Our data suggest that anti-IGF-IR strategies may prove valuable in such patients.

Matsubara, J., Y. Yamada, et al. (2008). "Clinical significance of insulin-like growth factor type 1 receptor and epidermal growth factor receptor in patients with advanced gastric cancer." Oncology **74**(1-2): 76-83.

OBJECTIVE: To better understand the clinical implications of insulin-like growth factor type 1 receptor (IGF-1R), epidermal growth factor receptor (EGFR) and HER2 expressions in gastric cancer (GC). METHODS: The study group comprised 86 patients who received first-line chemotherapy for advanced GC at the National Cancer Center Hospital. Using laser-captured microdissection and a real-time RT-PCR assay, we quantitatively evaluated mRNA levels of IGF-1R, EGFR and HER2 in paraffin-embedded cancer specimens of surgically removed primary tumors. RESULTS: In univariate analysis of the study group as a whole, patients with low expression of both IGF-1R and EGFR (n = 13) had a significantly longer overall survival than the other patients (n = 51; median, 24.6 vs. 12.8 months; log-rank p = 0.013). Multivariate survival analysis demonstrated that high EGFR expression [hazard ratio, HR: 2.94 (95% confidence interval, CI: 1.40-6.17), p = 0.004] and poor performance status [HR: 1.96 (95% CI: 1.12-3.42), p = 0.018] were significant predictors of poor survival. In patients given first-line S-1 monotherapy (n = 29), low IGF-1R (p = 0.002) and low EGFR (p = 0.035) gene expression correlated with a better response, without a significant prolongation of survival. CONCLUSION: Our data warrant further investigations on the strategy of co-targeting IGF-1R and EGFR in GC.

Matsumura, S., N. Oue, et al. (2007). "DNA demethylation of vascular endothelial growth factor-C is associated with gene expression and its possible involvement of lymphangiogenesis in gastric cancer." Int J Cancer **120**(8): 1689-95.

Previous studies have indicated that lymphangiogenesis in solid tumors is associated with lymphatic metastasis. Overexpression of Vascular endothelial growth factor (VEGF)-C plays a major role in lymphangiogenesis in cancers. In the present study, DNA methylation and expression of the VEGF-C gene was investigated in gastric cancer (GC). Four GC cell lines (MKN-45, MKN-74, HSC-39 and HSC-43) showed no expression of VEGF-C, and the VEGF-C gene was found to be methylated in these cells. In contrast, 7 GC cell lines (MKN-1, MKN-7, MKN-28, TMK-1, KATO-III, SH101-P4 and HSC-44PE) expressed VEGF-C, and the VEGF-C gene was found to be unmethylated in these cell lines. In addition, expression of VEGF-C mRNA was retrieved by treatment with a demethylating agent, Aza-2'-deoxycytidine. In GC tissue samples, bisulfite DNA sequencing analysis revealed that VEGF-C was not methylated in 9 (29.0%) of 31 GC samples, whereas demethylation was not observed in corresponding non-neoplastic mucosa samples. Overexpression of VEGF-C mRNA was observed in 16 (51.6%) of 31 GC samples by quantitative reverse transcription-polymerase chain reaction. Of the 9 GC cases with VEGF-C demethylation, 8 (88.9%) overexpressed VEGF-C. In contrast, of the 22 GC cases without VEGF-C demethylation, 8 (36.4%) overexpressed VEGF-C (p = 0.0155). Furthermore, lymphatic vessel density determined by immunostaining of podoplanin in GC tissues was associated with overexpression of VEGF-C (p < 0.0001). These results suggest that demethylation and activation of the VEGF-C gene is likely involved in lymphangiogenesis in GC.

Matsuzaki, S., F. Tanaka, et al. (2009). "Clinicopathologic significance of KIAA1199 overexpression in human gastric cancer." Ann Surg Oncol **16**(7): 2042-51.

BACKGROUND: KIAA1199 is an inner-ear-specific gene which encodes KIAA1199 protein, the function of which is unknown. KIAA1199 might be a novel, positively regulated target of Wnt signaling. The aim of this study was to examine the expression of KIAA1199 in surgical specimens of gastric cancer to evaluate the clinical outcome. METHODS: The expression of KIAA1199 mRNA was studied by semiquantitative reverse-transcription polymerase chain reaction (RT-PCR), and the expression status was analyzed from the viewpoint of clinical and pathological factors. Univariate and multivariate analyses were performed. In addition, an immunohistochemical study was performed in the selected samples. RESULTS: A significantly higher expression of KIAA1199 messenger RNA (mRNA)

was recognized in tumor tissue compared with that of paired normal tissues (P < 0.01). The cases were divided into high- (n = 39) and low-expression (n = 71) groups according to KIAA1199 expression status in the tumor. The overall 5-year survival rate was significantly better in the KIAA1199 low-expression group (61.2%) than in the high-expression group (29.6%) (P < 0.05). Clinicopathological factors such as well and moderately tumor differentiation, positive lymph node metastasis, positive distant metastases, and positive peritoneal dissemination were more frequently observed in the high-expression group than in the low-expression group (P = 0.02, 0.08, 0.01, and 0.03, respectively). KIAA1199 expression was an independent prognostic factor (P = 0.03). CONCLUSIONS: KIAA1199 was highly expressed in gastric cancer, and was associated with prognosis and lymph node metastasis in multivariate analyses. Taken together, KIAA1199 may be a novel gene that plays an important role in progression of gastric cancer.

Mattioli, E., P. Vogiatzi, et al. (2007). "Immunohistochemical analysis of pRb2/p130, VEGF, EZH2, p53, p16(INK4A), p27(KIP1), p21(WAF1), Ki-67 expression patterns in gastric cancer." J Cell Physiol **210**(1): 183-91.

Although the considerable progress against gastric cancer, it remains a complex lethal disease defined by peculiar histological and molecular features. The purpose of the present study was to investigate pRb2/p130, VEGF, EZH2, p53, p16(INK4A), p27(KIP1), p21(WAF1), Ki-67 expressions, and analyze their possible correlations with clinicopathological factors. The expression patterns were examined by immunohistochemistry in 47 patients, 27 evaluated of intestinal-type, and 20 of diffuse-type, with a mean follow up of 56 months and by Western blot in AGS, N87, KATO-III, and YCC-2, -3, -16 gastric cell lines. Overall, stomach cancer showed EZH2 correlated with high levels of p53, Ki-67, and cytoplasmic pRb2/p130 (P < 0.05, and P < 0.01, respectively). Increased expression of EZH2 was found in the intestinal-type and correlated with the risk of distant metastasis (P < 0.05 and P < 0.01, respectively), demonstrating that this protein may have a prognostic value in this type of cancer. Interestingly, a strong inverse correlation was observed between p27(KIP1) expression levels and the risk of advanced disease and metastasis (P < 0.05), and a positive correlation between the expression levels of p21(WAF1) and low-grade (G1) gastric tumors (P < 0.05), confirming the traditionally accepted role for these tumor-suppressor genes in gastric cancer. Finally, a direct correlation was found between the expression levels of nuclear pRb2/p130 and low-grade (G1) gastric tumors that was statistically significant (P < 0.05). Altogether, these data may help shed some additional light on the pathogenetic mechanisms related to the two main gastric cancer histotypes and their invasive potentials.

May, F. E., S. M. Griffin, et al. (2009). "The trefoil factor interacting protein TFIZ1 binds the trefoil protein TFF1 preferentially in normal gastric mucosal cells but the co-expression of these proteins is deregulated in gastric cancer." Int J Biochem Cell Biol **41**(3): 632-40.

The gastric tumour suppressor trefoil protein TFF1 is present as a covalently bound heterodimer with a previously uncharacterised protein, TFIZ1, in normal human gastric mucosa. The purpose of this research was firstly to examine the molecular forms of TFIZ1 present, secondly to determine if TFIZ1 binds other proteins apart form TFF1 in vivo, thirdly to investigate if TFIZ1 and TFF1 are co-regulated in normal gastric mucosa and fourthly to determine if their co-regulation is maintained or disrupted in gastric cancer. We demonstrate that almost all human TFIZ1 is present as a heterodimer with TFF1 and that TFIZ1 is not bound to either of the other two trefoil proteins, TFF2 and TFF3. TFIZ1 and TFF1 are co-expressed by the surface mucus secretory cells throughout the stomach and the molecular forms of each protein are affected by the relative abundance of the other. TFIZ1 expression is lost consistently, early and permanently in gastric tumour cells. In contrast, TFF1 is sometimes expressed in the absence of TFIZ1 in gastric cancer cells and this expression is associated with metastasis (lymph node involvement: p=0.007). In conclusion, formation of the heterodimer between TFIZ1 and TFF1 is a specific interaction that occurs uniquely in the mucus secretory cells of the stomach, co-expression of the two proteins is disrupted in gastric cancer and expression of TFF1 in the absence of TFIZ1 is associated with a more invasive and metastatic phenotype. This indicates that TFF1 expression in the absence of TFIZ1 expression has potentially deleterious consequences in gastric cancer.

Mejias-Luque, R., S. Peiro, et al. (2008). "IL-6 induces MUC4 expression through gp130/STAT3 pathway in gastric cancer cell lines." Biochim Biophys Acta **1783**(10): 1728-36.

The gastric mucosal levels of the pro-inflammatory cytokine Interleukin 6 (IL-6) have been reported to be increased in Helicobacter pylori-infected subjects and, in gastric adenocarcinomas, the up-regulation of intestinal mucin genes (MUC2 and MUC4) has been detected. To analyse the regulatory effects of IL-6 on the activation of intestinal mucins, six gastric cancer cell lines were treated for different times with several concentrations of IL-6, and the

expression of MUC2 and MUC4 was evaluated. IL-6 induced MUC4 expression, detected by quantitative RT-PCR, Western blot and immunofluorescence, and MUC2 expression was not affected. MUC4 mRNA levels decreased after blocking the gp130/STAT3 pathway at the level of the receptor, and at the level of STAT3 activation using the AG490 specific inhibitor. MUC4 presents two putative binding sites for STAT factors that may regulate MUC4 transcription after a pro-inflammatory stimulus as IL-6. By EMSA, ChIP and site-directed mutagenesis we show that STAT3 binds to a cis-element at -123/-115, that conveys IL-6 mediated up-regulation of MUC4 transcriptional activity. We also demonstrated that p-STAT3 binds to MUC4 promoter and a three-fold increase in p-STAT3 binding was observed after treating GP220 cells with IL-6. In conclusion, IL-6 treatment induced MUC4 expression through the gp130/STAT3 pathway, indicating the direct role of IL-6 on the activation of the intestinal mucin gene MUC4 in gastric cancer cells.

Mimori, K., T. Fukagawa, et al. (2008). "Hematogenous metastasis in gastric cancer requires isolated tumor cells and expression of vascular endothelial growth factor receptor-1." Clin Cancer Res **14**(9): 2609-16.

PURPOSE: Recent studies of cancer metastasis have focused on the role of premetastatic gene expression and circulating tumor cells. We did a blind prospective study in gastric cancer to assess the significance of isolated tumor cells (ITC) and to test the hypothesis that vascular endothelial growth factor receptor-1 (VEGFR-1) is expressed within the bone marrow at tumor-specific, premetastatic sites. EXPERIMENTAL DESIGN: Both bone marrow and peripheral blood samples from 810 gastric cancer patients were collected at the Central Hospital, National Cancer Center (Tokyo, Japan). The samples were transferred to Kyushu University Hospital (Beppu, Japan) where they were analyzed by quantitative real-time reverse transcription-PCR for three epithelial cell markers, carcinoembryonic antigen, cytokeratin-19, and cytokeratin-7, as well as VEGFR-1. RESULTS: ITCs were observed in peripheral blood and bone marrow even in early stages of gastric cancer. The frequency of ITC in bone marrow was significantly associated with the stage of disease by ANOVA ($P < 0.01$). Gastric cancer metastasized when ITCs were observed in the presence of VEGFR-1. In the 380 patients who were ITC negative and showed low VEGFR-1 expression, synchronous (at the time of surgery) and heterochronous (recurrent) metastases were not observed. CONCLUSIONS: ITCs circulate even in early stages of disease. Furthermore, elevated expression of VEGFR-1 facilitates the establishment of hematogenous metastases in gastric cancer. This study indicates that the simultaneous presence of ITC and VEGFR-1 expression at premetastatic sites is clinically significant for disease progression.

Mita, H., M. Toyota, et al. (2009). "A novel method, digital genome scanning detects KRAS gene amplification in gastric cancers: involvement of overexpressed wild-type KRAS in downstream signaling and cancer cell growth." BMC Cancer **9**: 198.

BACKGROUND: Gastric cancer is the third most common malignancy affecting the general population worldwide. Aberrant activation of KRAS is a key factor in the development of many types of tumor, however, oncogenic mutations of KRAS are infrequent in gastric cancer. We have developed a novel quantitative method of analysis of DNA copy number, termed digital genome scanning (DGS), which is based on the enumeration of short restriction fragments, and does not involve PCR or hybridization. In the current study, we used DGS to survey copy-number alterations in gastric cancer cells. METHODS: DGS of gastric cancer cell lines was performed using the sequences of 5000 to 15000 restriction fragments. We screened 20 gastric cancer cell lines and 86 primary gastric tumors for KRAS amplification by quantitative PCR, and investigated KRAS amplification at the DNA, mRNA and protein levels by mutational analysis, real-time PCR, immunoblot analysis, GTP-RAS pull-down assay and immunohistochemical analysis. The effect of KRAS knock-down on the activation of p44/42 MAP kinase and AKT and on cell growth were examined by immunoblot and colorimetric assay, respectively. RESULTS: DGS analysis of the HSC45 gastric cancer cell line revealed the amplification of a 500-kb region on chromosome 12p12.1, which contains the KRAS gene locus. Amplification of the KRAS locus was detected in 15% (3/20) of gastric cancer cell lines (8-18-fold amplification) and 4.7% (4/86) of primary gastric tumors (8-50-fold amplification). KRAS mutations were identified in two of the three cell lines in which KRAS was amplified, but were not detected in any of the primary tumors. Overexpression of KRAS protein correlated directly with increased KRAS copy number. The level of GTP-bound KRAS was elevated following serum stimulation in cells with amplified wild-type KRAS, but not in cells with amplified mutant KRAS. Knock-down of KRAS in gastric cancer cells that carried amplified wild-type KRAS resulted in the inhibition of cell growth and suppression of p44/42 MAP kinase and AKT activity. CONCLUSION: Our study highlights the utility of DGS for identification of copy-number alterations.

Using DGS, we identified KRAS as a gene that is amplified in human gastric cancer. We demonstrated that gene amplification likely forms the molecular basis of overactivation of KRAS in gastric cancer. Additional studies using a larger cohort of gastric cancer specimens are required to determine the diagnostic and therapeutic implications of KRAS amplification and overexpression.

Mitsuno, M., Y. Kitajima, et al. (2007). "Aberrant methylation of p16 predicts candidates for 5-fluorouracil-based adjuvant therapy in gastric cancer patients." J Gastroenterol **42**(11): 866-73.

BACKGROUND: Aberrant methylation of some cancer-related genes has been reported to correlate with sensitivity to chemotherapeutic agents. The present study was designed to determine whether DNA methylation in six cancer-related genes affects recurrence of gastric cancer in patients who received 5-fluorouracil-based adjuvant chemotherapy. METHODS: The methylation status of six genes, MGMT, CHFR, hMLH1, p16INK4a, E-cadherin, and Runx3, was analyzed in 56 surgically resected gastric cancer tissue specimens by methylation-specific polymerase chain reaction. Of the 56 patients who underwent surgical resection, 38 received 5-fluorouracil (5-FU)-based adjuvant chemotherapy postoperatively (adjuvant group), whereas the other 18 (32%) did not (surgery group). RESULTS: There were no significant differences between the two groups with respect to sex, cancer differentiation, depth of tumor invasion, lymph node metastasis, lymphatic invasion, vascular invasion and tumor stage. Among the genes, methylation of p16INK4a showed a significant correlation with longer survival in the 38 patients of the adjuvant group, but not in the 18 patients of the surgery group. A multivariate analysis identified p16INK4a methylation to be an independent factor predicting a longer recurrence-free period under 5-FU-based adjuvant chemotherapy. CONCLUSIONS: The present study demonstrated for the first time that gastric cancer patients with p16INK4a methylation specifically benefit from 5-FU-based adjuvant chemotherapy.

Miyagawa, K., C. Sakakura, et al. (2008). "Overexpression of RegIV in peritoneal dissemination of gastric cancer and its potential as A novel marker for the detection of peritoneal micrometastasis." Anticancer Res **28**(2B): 1169-79.

BACKGROUND: Regenerating gene type IV (RegIV) is a candidate marker for cancer and inflammatory bowel disease. In this study, its potential as a novel marker for the detection of gastric cancer peritoneal micrometastases was examined. PATIENTS AND METHODS: RegIV mRNA levels in the peritoneal washes of 95 gastric cancer patients and 22 with benign disease were quantified by real-time RT-PCR. To examine whether expression of RegIV enhance tumorigenicity or not, thirty two mice were injected intraperitoneally or subcutaneously with RegIV transfectants of TMK-1 cells, parental TMK-1 cells, or neomycin control transfectants. RESULTS: RegIV expression was markedly higher in patients with peritoneal metastases compared to those without. The level of RegIV mRNA in gastric cancer patients was related to the extent of wall penetration. A cut-off value for RegIV-positive expression was based on an analysis of negative control patients with benign disease, and gastric cancer patients above the cut-off value constituted the micrometastasis (MM+) group. Based on this criteria, 3 out of 43 T1 or T2 cases were MM+ (93% specificity). Among 15 patients with peritoneal dissemination (7 out of 15 cases were positive by cytology), 14 cases were positive for RegIV expression (93% sensitivity), while analysis of carcinoembryonic antigen (CEA) mRNA failed to detect micrometastases in 4 cases (73% sensitivity). Combined analysis of CEA and RegIV improved the accuracy of diagnosis to 100%. The prognosis of RegIV-positive cases was significantly worse than that of RegIV-negative cases. Multivariate analysis using the Cox proportional hazards model suggested that RegIV may be an independent prognostic factor. Stable expression of RegIV significantly enhanced peritoneal metastasis in an animal model of gastric cancer. CONCLUSION: These findings suggest that RegIV mRNA expression has the potential to serve as a novel marker for detecting peritoneal dissemination in gastric cancer.

Mori, K., T. Suzuki, et al. (2007). "Detection of minimal gastric cancer cells in peritoneal washings by focused microarray analysis with multiple markers: clinical implications." Ann Surg Oncol **14**(5): 1694-702.

BACKGROUND: Peritoneal cytology is an important prognostic factor of gastric cancer. However, peritoneal cytology requires great skill, which may explain its low prevalence. A reverse transcriptase-polymerase chain reaction-based assay with multiple marker genes or immunocytochemistry was assessed as an alternative method of gathering the same kind of data as cytology. METHODS: Peritoneal washings from 179 patients with gastric cancer were analyzed by multiplex reverse transcriptase-polymerase chain reaction with 10 marker genes and subsequent hybridization to a customized oligo-nucleotide array. Results with this assay were either validated as a prognostic factor or confirmed by demonstrating the presence of cancer cells by immunocytochemical cytology. RESULTS: Only 1

(2.2%) of 44 disease-free cases was shown to be positive by the microarray assay, whereas 13 (93%) of 14 conventional cytology-positive cases were found to be positive. This assay further detected approximately one-third of cytology-negative patients either with peritoneal recurrence (7 of 20, 35%) or with non-peritoneal recurrence (6 of 22, 27%). A high concordance between the microarray assay and immunocytochemical cytology with five antibodies against CK20, FABP1, MUC2, TFF1, and MASPIN was confirmed. The clinical outcome of the microarray assay-positive cases was poor, as was that of the cytology-positive cases. CONCLUSIONS: Our assay, though time-consuming and requiring special equipment, demonstrated a specificity and sensitivity equal to or better than cytology in our institutes. The minimal free peritoneal cancer cells detected by the microarray assay may provide the same clinical information as larger amounts of cancer cells for patients with gastric cancer. An anti-MASPIN antibody may be helpful in peritoneal cytology of gastric cancer.

Mori, Y., H. Kataoka, et al. (2007). "Subcellular localization of ATBF1 regulates MUC5AC transcription in gastric cancer." Int J Cancer **121**(2): 241-7.

Human gastric epithelium has a unique mucin gene expression pattern, which becomes markedly altered in gastrointestinal disorder. This alteration in mucin expression, including the mucin MUC5AC, may be related to the development and prognosis of gastric cancers, and MUC5AC-positive gastric cancer has been reported to be poor prognosis. However, the molecular mechanism of MUC5AC transcriptional regulation has not been fully elucidated. AT motif-binding factor 1 (ATBF1) is a homeotic transcriptional regulatory factor recently identified as a tumor suppressor gene, and its subcellular localization suggests a link to cell proliferation and differentiation. We investigated the mechanism of MUC5AC transcriptional regulation by ATBF1. In 123 gastric cancer lesions, ATBF1 expressed in the nucleus significantly suppressed MUC5AC expression, as determined by immunohistochemistry. In addition, analysis of the MUC5AC promoter region revealed an AT motif-like element. This element was found to be essential for ATBF1 suppression of MUC5AC promoter activity as shown in a dual luciferase-reporter assay. Over-expressed ATBF1 also significantly suppressed endogenous MUC5AC protein expression in gastric cancer cells. Chromatin immunoprecipitation demonstrated that ATBF1 binds to the AT motif-like element in the MUC5AC promoter. These results indicate that ATBF1 in the nucleus negatively regulates the MUC5AC gene in gastric cancer by binding to an AT motif-like element in the MUC5AC promoter.

Moss, S. F., J. W. Lee, et al. (2008). "Decreased expression of gastrokine 1 and the trefoil factor interacting protein TFIZ1/GKN2 in gastric cancer: influence of tumor histology and relationship to prognosis." Clin Cancer Res **14**(13): 4161-7.

PURPOSE: Transcriptional profiling showed decreased expression of gastrokine 1 (GKN1) and the related trefoil factor interacting protein (TFIZ1/GKN2) in Helicobacter pylori infection. Decreased GKN1 and GKN2 mRNA expression has been reported in gastric adenocarcinoma. We have examined GKN1 and GKN2 protein expression in a large gastric cancer series, correlated expression with tumor subtype, and evaluated their utility as prognostic biomarkers. EXPERIMENTAL DESIGN: GKN1, GKN2, and the trefoil factors TFF1 and TFF3 were examined in tissue microarrays from 155 distal gastric adenocarcinomas. Immunohistochemical expression was correlated with clinical outcome. GKN1 and GKN2 expression was measured by real-time PCR and Western analysis in samples of gastric cancer and adjacent nonneoplastic mucosa. RESULTS: GKN1 was lost in 78% of diffuse and 42% of intestinal cancers ($P < 0.0001$, diffuse versus intestinal). GKN2 expression was lost in 85% of diffuse and 54% of intestinal type cancers ($P < 0.002$). GKN1 and GKN2 down-regulation were confirmed by Western and real-time PCR analysis. Loss of either protein was associated with significantly worse outcome in intestinal-type tumors by univariate analysis; and GKN2 loss remained a predictor of poor outcome in multivariate analysis ($P < 0.033$). TFF1 was lost in >70%, and TFF3 was expressed in approximately 50% of gastric cancers. CONCLUSIONS: Loss of GKN1 and GKN2 expression occurs frequently in gastric adenocarcinomas, especially in the diffuse subtype. GKN1 and GKN2 loss are associated with shorter overall survival in the intestinal subtype.

Moutinho, C., A. R. Mateus, et al. (2008). "Epidermal growth factor receptor structural alterations in gastric cancer." BMC Cancer **8**: 10.

BACKGROUND: EGFR overexpression has been described in many human tumours including gastric cancer. In NSCLC patients somatic EGFR mutations, within the kinase domain of the protein, as well as gene amplification were associated with a good clinical response to EGFR inhibitors. In gastric tumours data concerning structural alterations of EGFR remains controversial. Given its possible therapeutic relevance, we aimed to determine the

frequency and type of structural alterations of the EGFR gene in a series of primary gastric carcinomas. METHODS: Direct sequencing of the kinase domain of the EGFR gene was performed in a series of 77 primary gastric carcinomas. FISH analysis was performed in 30 cases. Association studies between EGFR alterations and the clinical pathological features of the tumours were performed. RESULTS: Within the 77 primary gastric carcinomas we found two EGFR somatic mutations and several EGFR polymorphisms in exon 20. Six different intronic sequence variants of EGFR were also found. Four gastric carcinomas showed balanced polysomy or EGFR gene amplification. We verified that gastric carcinoma with alterations of EGFR (somatic mutations or copy number variation) showed a significant increase of tumour size ($p = 0.0094$) in comparison to wild-type EGFR carcinomas. CONCLUSION: We demonstrate that EGFR structural alterations are rare in gastric carcinoma, but whenever present, it leads to tumour growth. We considered that searching for EGFR alterations in gastric cancer is likely to be clinically important in order to identify patients susceptible to respond to tyrosine kinase inhibitors.

Mroczko, B., M. Lukaszewicz-Zajac, et al. (2009). "Expression of tissue inhibitors of metalloproteinase 1 (TIMP-1) in gastric cancer tissue." Folia Histochem Cytobiol **47**(3): 511-6.

Degradation of extracellular matrix (ECM) is an essential step of invasion and metastasis of gastric cancer. The proteolysis of basement membranes depends on the balance between activities of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). The aim of the study was to assess the expression of TIMP-1 in gastric cancer (GC) and interstitial inflammatory infiltrate cells within GC tissue in relation to clinico-pathological features of tumor and to estimate the prognostic significance of TIMP-1 expression for patients' survival. The presence of TIMP-1 in 54 cases of gastric cancer samples was investigated by immunohistochemistry. The expression of TIMP-1 in cancer and interstitial inflammatory infiltrate cells was evaluated in semi-quantitative scale. The immunoreactivity of TIMP-1 in cancer and inflammatory cells was positive in 100% of cases and varied from weak to intense reaction. The intensity of TIMP-1 expression increased with more advanced tumor stages and in patients who died of cancer during 2-year observation. TIMP-1 expression in interstitial inflammatory infiltrate cells was the independent prognostic factor for patients' survival. The results suggest the role of TIMP-1 in gastric tumorigenesis, although this issue requires further investigtions.

Murray, D., G. Horgan, et al. (2008). "NET1-mediated RhoA activation facilitates lysophosphatidic acid-induced cell migration and invasion in gastric cancer." Br J Cancer **99**(8): 1322-9.

The most lethal aspects of gastric adenocarcinoma (GA) are its invasive and metastatic properties. This aggressive phenotype remains poorly understood. We have recently identified neuroepithelial cell transforming gene 1 (NET1), a guanine exchange factor (GEF), as a novel GA-associated gene. Neuroepithelial cell transforming gene 1 expression is enhanced in GA and it is of functional importance in cell invasion. In this study, we demonstrate the activity of NET1 in driving cytoskeletal rearrangement, a key pathological mechanism in gastric tumour cell migration and invasion. Neuroepithelial cell transforming gene 1 expression was increased 10-fold in response to treatment with lysophosphatidic acid (LPA), resulting in an increase in active levels of RhoA and a 2-fold increase in cell invasion. Lysophosphatidic acid-induced cell invasion and migration were significantly inhibited using either NET1 siRNA or a RhoA inhibitor (C3 exoenzyme), thus indicating the activity of both NET1 and RhoA in gastric cancer progression. Furthermore, LPA-induced invasion and migration were also significantly reduced in the presence of cytochalasin D, an inhibitor of cytoskeletal rearrangements. Neuroepithelial cell transforming gene 1 knockdown resulted in AGS cell rounding and a loss of actin filament organisation, demonstrating the function of NET1 in actin organisation. These data highlight the importance of NET1 as a driver of tumour cell invasion, an activity mediated by RhoA activation and cytoskeletal reorganisation.

Nakamura, J., Y. Kitajima, et al. (2009). "Hypoxia-inducible factor-1alpha expression predicts the response to 5-fluorouracil-based adjuvant chemotherapy in advanced gastric cancer." Oncol Rep **22**(4): 693-9.

Hypoxia frequently occurs in various solid tumors, thereby accelerating cancer progression and treatment resistance. Hypoxia-inducible factor-1alpha (HIF-1alpha) plays a central role in tumor hypoxia by up-regulating the gene expression related to angiogenesis, cancer invasion and anti-apoptosis. The present study immunohistochemically investigated HIF-1alpha expression in 63 gastric cancer specimens. Those specimens were obtained from 44 patients that received 5-FU chemotherapy post-operatively whereas the remaining 19 patients did not. The immunostaining pattern of HIF-1alpha was classified into 3 patterns: diffuse-positive within the tumor (DP), positive at the invasive front of the tumor (FP) and

negative (N). Thirty-six of 63 (57.1%) patients exhibited DP, 24 (38.1%) revealed FP and the remaining 3 (4.8%) patients were judged as N. The HIF-1alpha expression pattern grouped into DP and FP/N correlated with the clinicopathological factors and survival. As a result, the HIF-1alpha expression did not show a significant correlation with the clinicopathological factors, such as the depth of invasion, lymph node metastasis and tumor stage, nor patient survival in the 63 patients. However, in the 44 patients that underwent chemotherapy, patients with the FP/N pattern showed longer survival than those with the DP pattern. On the other hand, no significant difference in survival was found between the 2 patterns among 19 patients without the chemotherapy. These results indicated that the diffuse expression of HIF-1alpha in gastric tumors might lead to drug resistance against adjuvant chemotherapy using 5-FU. In conclusion, the assessment of the HIF-1alpha expression in the resected tissues might predict the drug response to adjuvant 5-FU chemotherapy in advanced gastric cancer patients.

Nakamura, Y., T. Migita, et al. (2009). "Kruppel-like factor 12 plays a significant role in poorly differentiated gastric cancer progression." Int J Cancer **125**(8): 1859-67.

Gastric cancer is the second common malignant neoplasia in Japan, and its poorly differentiated form is a deadly disease. To identify novel candidate oncogenes contributing to its genesis, we examined copy-number alterations in 50 primary poorly differentiated gastric cancers using an array-based comparative genomic hybridization (array-CGH). Many genetic changes were identified, including a novel amplification of the 13q22 locus. Several genes are located in this locus, and selective knockdown of one for the Kruppel-like factor 12 (KLF12) induced significant growth-arrest in the HGC27 gastric cancer cell line. Microarray analysis also demonstrated that genes associated with cell proliferation were mostly changed by KLF12 knockdown. To explore the oncogenic function of KLF12, we introduced a full length of human KLF12 cDNA into NIH3T3 and AZ-521 cell lines and found that overexpression significantly enhanced their invasive potential. In clinical samples, KLF12 mRNA in cancer tissue was increased in 11 of 28 cases (39%) when compared with normal gastric epithelium. Clinicopathological analysis further demonstrated a significant correlation between KLF12mRNA levels and tumor size ($p = 0.038$). These data suggest that the KLF12 gene plays an important role in poorly differentiated gastric cancer progression and is a potential target of therapeutic measures.

Nikiteas, N. I., N. Tzanakis, et al. (2007). "Vascular endothelial growth factor and endoglin (CD-105) in gastric cancer." Gastric Cancer **10**(1): 12-7.

BACKGROUND: Vascular endothelial growth factor (VEGF) overexpression has been associated with advanced stage and poor survival in several cancers. Additionally, CD-105 (endoglin) was proposed as a marker of neovascularization in solid malignancies. The aim of the present study was to (1) evaluate the VEGF and CD-105 expression in gastric carcinoma, (2) determine the role of VEGF gene sequence variations in VEGF expression in gastric carcinoma, and (3) correlate the results of VEGF and CD-105 expression with other standard prognostic parameters, such as size, grade, stage of the disease, metastases, and patient survival. METHODS: VEGF and CD-105 expression were evaluated in 100 unrelated gastric cancer patients using immunohistochemistry. For the genotyping, DNA was isolated from the blood of the gastric cancer patients and from 100 healthy individuals. The genotyping was performed by polymerase chain-restriction fragment length polymorphism analysis. RESULTS: VEGF protein was strongly expressed in the cytoplasm of 36% of the gastric carcinoma samples tested. In all cases, high VEGF expression was accompanied with high endoglin expression. Our results revealed no statistical significant association of any VEGF gene polymorphism with the VEGF and endoglin expression. The correlation of VEGF/CD-105 expression with the clinicopathological parameters of gastric cancer showed that the high expression of VEGF/CD015 was correlated only with lymph node metastasis ($P = 0.028$). The Kaplan-Meier survival curves have shown a clear association of overall survival after diagnosis of gastric cancer with high VEGF, as well as high CD-105 expression. CONCLUSION: Our results support that VEGF and CD-105 are closely relevant to lymph node metastasis and act as two valuable indicators of prognosis.

Ning, X., S. Sun, et al. (2007). "Calcyclin-binding protein inhibits proliferation, tumorigenicity, and invasion of gastric cancer." Mol Cancer Res **5**(12): 1254-62.

Calcyclin-binding protein/Siah-1-interacting protein (CacyBP/SIP), a target protein of the S100 family, which includes S100A6, S100A1, S100A12, S100B, and S100P, has been identified as a component of a novel ubiquitinylation complex leading to beta-catenin degradation. However, the function of CacyBP/SIP in gastric cancer has not been elucidated. In the present study, we prepared CacyBP/SIP overexpressing and knockdown cell lines of gastric cancer. Forced CacyBP/SIP expression inhibited the proliferation of gastric cancer cells,

suppressed tumorigenicity in vitro, and prolonged the survival time of tumor-bearing nude mice. In addition, increased CacyBP/SIP repressed the invasive potential of gastric cancer cells. Conversely, the down-regulation of CacyBP/SIP by RNA interference showed the opposite effects. Further studies showed that depressed CacyBP/SIP increased the expression of total and nuclear beta-catenin at the protein level and elevated the transcriptional activity of Tcf/LEF. Taken together, our results suggest that CacyBP/SIP may be a potential inhibitor of cell growth and invasion in the gastric cancer cell, at least in part through the effect on beta-catenin protein expression and transcriptional activation of Tcf/LEF.

Nojima, M., H. Suzuki, et al. (2007). "Frequent epigenetic inactivation of SFRP genes and constitutive activation of Wnt signaling in gastric cancer." Oncogene **26**(32): 4699-713.

Activation of Wnt signaling has been implicated in gastric tumorigenesis, although mutations in APC (adenomatous polyposis coli), CTNNB1 (beta-catenin) and AXIN are seen much less frequently in gastric cancer (GC) than in colorectal cancer. In the present study, we investigated the relationship between activation of Wnt signaling and changes in the expression of secreted frizzled-related protein (SFRP) family genes in GC. We frequently observed nuclear beta-catenin accumulation (13/15; 87%) and detected the active form of beta-catenin in most (12/16; 75%) GC cell lines. CpG methylation-dependent silencing of SFRP1, SFRP2 and SFRP5 was frequently seen among GC cell lines (SFRP1, 16/16, 100%; SFRP2, 16/16, 100%; SFRP5, 13/16, 81%) and primary GC specimens (SFRP1, 42/46, 91%; SFRP2, 44/46, 96%; SFRP5, 30/46, 65%), and treatment with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine rapidly restored SFRP expression. Ectopic expression of SFRPs downregulated T-cell factor/lymphocyte enhancer factor transcriptional activity, suppressed cell growth and induced apoptosis in GC cells. Analysis of global expression revealed that overexpression of SFRP2 repressed Wnt target genes and induced changes in the expression of numerous genes related to proliferation, growth and apoptosis in GC cells. It thus appears that aberrant SFRP methylation is one of the major mechanisms by which Wnt signaling is activated in GC.

Oue, N., K. Sentani, et al. (2009). "Serum olfactomedin 4 (GW112, hGC-1) in combination with Reg IV is a highly sensitive biomarker for gastric cancer patients." Int J Cancer **125**(10): 2383-92.

Gastric cancer (GC) is 1 of the most common human cancers. Early detection remains the most promising approach to improving long-term survival of patients with GC. We previously performed Serial Analysis of Gene Expression (SAGE) on 4 primary GCs and identified several GC-specific genes including Reg IV. Of these genes, olfactomedin 4 (OLFM4, also known as GW112 or hGC-1) is a candidate gene for cancer-specific expression. In this study, we examined the expression of olfactomedin 4 in human GC by immunohistochemistry. We also assessed serum olfactomedin 4 levels in GC patients by enzyme-linked immunosorbent assay. 94 (56%) of 167 GC cases were positive for olfactomedin 4 by immunostaining. Olfactomedin 4 staining was observed more frequently in stage I/II cases than in stage III/IV cases. The serum olfactomedin 4 concentration in presurgical GC patients (n = 123, mean +/- SE, 36.3 +/- 3.5 ng/mL) was significantly higher than that in healthy individuals (n = 76, 16.6 +/- 1.6 ng/mL). In patients with stage I GC, the sensitivity of serum olfactomedin 4 (25%) and Reg IV (35%) was superior to that of CA19-9 (5%) or CEA (3%). Furthermore, in patients with stage I GC, the combination of olfactomedin 4 and Reg IV elevated the diagnostic sensitivity to 52%. These results suggest that serum olfactomedin 4 is a useful marker for GC and its measurement alone or in combination with Reg IV has utility in the early detection of GC.

Pang, R. P., J. G. Zhou, et al. (2007). "Celecoxib induces apoptosis in COX-2 deficient human gastric cancer cells through Akt/GSK3beta/NAG-1 pathway." Cancer Lett **251**(2): 268-77.

In this study, we analyzed the mechanisms of the apoptotic effects of celecoxib on COX-2 deficient gastric cancer cell line, MGC-803. We found celecoxib treatment induced caspase-dependent apoptosis in MGC-803 cells. Celecoxib inhibited Ser473 Akt and Ser9 GSK3beta phosphorylation and induced upregulation of nonsteroidal anti-inflammatory drugs-activated gene-1 (NAG-1) expression. The effects of celecoxib on NAG-1 expression were abolished by pretreatment with GSK3beta inhibitor, SB216763. Furthermore, GSK3beta gene silencing by siRNA inhibited the celecoxib-induced NAG-1 expression. Our study demonstrated that Akt/GSK3beta/NAG-1 signal pathway may represent as the major mechanism of the COX-2-independent effects of celecoxib on gastric cancer cells.

Park, J. Y., K. H. Park, et al. (2007). "CXCL5 overexpression is associated with late stage gastric cancer." J Cancer Res Clin Oncol **133**(11): 835-40.

PURPOSE: Chemokines play multiple roles in the development and progression of many different tumors. Our cDNA array data suggested that

chemokine CXCL5 was upregulated in gastric cancer. Here, we analyzed CXCL5 protein expression in gastric cancer and investigated the clinical implications of CXCL5 upregulation. METHODS: Immunostaining for CXCL5 was performed on gastric tissue microarrays of tissue specimens obtained by gastrectomy. The intensity of immunostaining in tumor tissue was considered strong when tumor tissue staining was more intense than in normal tissue; the intensity was null when staining was weaker in the tumor than in normal tissue; and the intensity was weak when staining was similar in both tissues. Serum CXCL5 levels and microvascular density in tumor tissue were measured by ELISA and monoclonal antibody to Factor VIII. RESULTS: Strong CXCL5 expression correlated with tumor stage. CXCL5 expression did not correlate with T stage. However, N stage positively correlated with CXCL5 expression. Serum CXCL5 levels in late stage (IIIB, IV) gastric cancer patients were higher than in patients with benign conditions. Microvascular density was higher in tumors with strong CXCL5 expression, but the correlation with CXCL5 was not linear. Multiple logistic regression analyses showed that, compared to no or weak expression, strong expression of CXCL5 was a significant risk factor for high N stage (N2, N3). CONCLUSIONS: CXCL5 overexpression was associated with late stage gastric cancer and high N stage. These results suggest a role for CXCL5 in the progression of gastric cancer, specifically in lymph node metastasis.

Park, M. J., K. H. Kim, et al. (2008). "Bile acid induces expression of COX-2 through the homeodomain transcription factor CDX1 and orphan nuclear receptor SHP in human gastric cancer cells." Carcinogenesis **29**(12): 2385-93.

The caudal-related homeobox gene, CDX1, encodes for an intestinal-specific transcription factor and is involved in the induction of intestinal metaplasia (IM) of the stomach in gastric cancer. Gastric IM induced by bile reflux is a precancerous gastric adenocarcinomal lesion and has been associated with the induction of cyclooxygenase-2 (COX-2). In this study, we demonstrate the molecular mechanisms underlying the transcriptional regulation of COX-2 by bile acid in gastric cells. We noted that the ectopic expression of CDX1 enhanced COX-2 gene expression and that bile acid was associated with the induction of CDX1 expression. Furthermore, the induction of CDX1 by bile acid was mediated by the orphan nuclear receptor, small heterodimer partner (SHP). Finally, it was verified that the expression of COX-2, CDX1, SHP and CCAAT element-binding protein beta messenger RNA in human IM lesions were significantly higher than in lesions associated with gastritis. Collectively, these results reveal that bile acid induces an increase in the gene expression of COX-2 via the sequential transcriptional induction of SHP and CDX1 in precancerous lesions of human gastric cancer.

Park, S., J. H. Kim, et al. (2007). "Aberrant hypermethylation of the FGFR2 gene in human gastric cancer cell lines." Biochem Biophys Res Commun **357**(4): 1011-5.

We have previously shown that fibroblast growth factor receptor 2 (FGFR2) plays an important role in gastric carcinogenesis. In this study, we assessed DNA methylation status in the promoter region of FGFR2 gene in gastric cancer cell lines, and indicated that this region was highly methylated, compared with FGFR2-expressing gastric cancer cell lines. Moreover, the restoration of FGFR2 expression by treating methylated cells with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine strongly suggests that the loss of FGFR2 expression may be due to the aberrant hypermethylation in the promoter region of the FGFR2 gene. Thus, our results suggest that the epigenetic silencing of FGFR2 through DNA methylation in gastric cancer may contribute to tumor progression.

Peek, R. M. (2008). "Prevention of Gastric Cancer: When is Treatment of Helicobacter Pylori Warranted?" Therap Adv Gastroenterol **1**(1): 19-31.

Chronic gastritis induced by Helicobacter pylori (H. pylori) is the strongest known risk factor for adenocarcinoma of the distal stomach, yet the effects of bacterial eradication on carcinogenesis remain unclear. H. pylori isolates possess substantial genotypic diversity, which engenders differential host inflammatory responses that influence clinical outcome. H. pylori strains that possess the cag pathogenicity island and secrete a functional cytotoxin induce more severe gastric injury and further augment the risk for developing distal gastric cancer. Carcinogenesis is also influenced by host genetic diversity, particularly involving immune response genes such as interleukin-1ss and tumor necrosis factor-alpha. Human trials and anima studies have indicated that eradication of H. pylori prior to the development of atrophic gastritis offers the best chance for prevention of gastric cancer. However, although the timing of intervention influences the magnitude of suppression of premalignant and neoplastic lesions, bacterial eradication, even in longstanding infections, is of clear benefit to the host. It is important to gain insight into the pathogenesis of H. pylori-induced gastritis and adenocarcinoma not only to develop more effective treatments for gastric cancer, but also because it might serve as a paradigm

for the role of chronic inflammation in the genesis of other malignancies that arise within the gastrointestinal tract.

Petrocca, F., R. Visone, et al. (2008). "E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer." Cancer Cell **13**(3): 272-86.

Deregulation of E2F1 activity and resistance to TGFbeta are hallmarks of gastric cancer. MicroRNAs (miRNAs) are small noncoding RNAs frequently misregulated in human malignancies. Here we provide evidence that the miR-106b-25 cluster, upregulated in a subset of human gastric tumors, is activated by E2F1 in parallel with its host gene, Mcm7. In turn, miR-106b and miR-93 regulate E2F1 expression, establishing a miRNA-directed negative feedback loop. Furthermore, upregulation of these miRNAs impairs the TGFbeta tumor suppressor pathway, interfering with the expression of CDKN1A (p21(Waf1/Cip1)) and BCL2L11 (Bim). Together, these results suggest that the miR-106b-25 cluster is involved in E2F1 posttranscriptional regulation and may play a key role in the development of TGFbeta resistance in gastric cancer.

Rohwer, N., S. Lobitz, et al. (2009). "HIF-1alpha determines the metastatic potential of gastric cancer cells." Br J Cancer **100**(5): 772-81.

Gastric adenocarcinoma is characterised by rapid emergence of systemic metastases, resulting in poor prognosis due to vanished curative treatment options. Better understanding of the molecular basis of gastric cancer spread is needed to design innovative treatments. The transcription factor HIF-1alpha (hypoxia-inducible factor 1alpha) is frequently overexpressed in human gastric cancer, and inhibition of HIF-1alpha has proven antitumour efficacy in rodent models, whereas the relevance of HIF-1alpha for the metastatic phenotype of gastric adenocarcinoma remains elusive. Therefore, we have conducted a comprehensive analysis of the role of HIF-1alpha for pivotal metastasis-associated processes of human gastric cancer. Immunhistochemistry for HIF-1alpha showed specific staining at the invading tumour edge in 90% of human gastric cancer samples, whereas normal gastric tissue was negative and only a minority of early gastric cancers (T1 tumours) showed specific staining. Hypoxia-inducible factor 1alpha-deficient cells showed a significant reduction of migratory, invasive and adhesive properties in vitro. Furthermore, the HIF-1alpha-inhibitor 2-methoxy-estradiol significantly reduced metastatic properties of gastric cancer cells. The accentuated expression at the invading edge together with the in vitro requirement of HIF-1alpha for migration, invasion and adherence argues for a pivotal role of HIF-1alpha in local invasion and, ultimately, systemic tumour spread. These results warrant the exploration of HIF-1alpha-inhibiting substances in clinical treatment studies of advanced gastric cancer.

Ruzzo, A., E. Canestrari, et al. (2007). "Polymorphisms in genes involved in DNA repair and metabolism of xenobiotics in individual susceptibility to sporadic diffuse gastric cancer." Clin Chem Lab Med **45**(7): 822-8.

BACKGROUND: Gastric cancer is the second highest cause of cancer mortality in the world, despite declining rates of incidence in many industrialized countries. We carried out a case-control study to evaluate whether polymorphisms of DNA repair and glutathione S-transferase (GST) genes modulate the risk of developing diffuse gastric cancer. METHODS: ERCC1 118 T/C, XRCC1 399 G/A, XPD 312 G/A, XPD 751 A/C, XRCC3 241 C/T, MS 919 A/G, GSTP1 105 A/G, GSTM1-null/positive and GSTT1-null/positive genotypes were obtained for a series of 126 Helicobacter pylori-negative diffuse gastric cancer patients and 144 Helicobacter pylori-negative controls sampled from the population of Marche, an area with high gastric cancer risk in central Italy. RESULTS: GSTP1 105 A/G and GSTP1 105 G/G genotypes were identified as protective factors, with odds ratio (OR) of 0.4 (95% CI 0.17-0.81, p=0.01) and OR=0.58 (95% CI 0.33-1, p=0.05), respectively. GSTT1-null genotype was identified as a protective factor, with OR=0.48 (95% CI 0.22-0.99, p=0.04). There was no significant difference between cases and controls for XPD 751 A/C, ERCC1 118 T/C, XRCC3 241 C/T, XRCC1 399 G/A, XPD 312 G/A, GSTM1-null/positive and MS 919 A/G polymorphisms. CONCLUSIONS: This study suggests that GSTP1 105A/G and GSTT1-null/positive genotypes might be associated with a reduced risk for sporadic diffuse gastric cancer. Clin Chem Lab Med 2007;45:822-8.

Saeki, N., D. H. Kim, et al. (2007). "GASDERMIN, suppressed frequently in gastric cancer, is a target of LMO1 in TGF-beta-dependent apoptotic signalling." Oncogene **26**(45): 6488-98.

Defining apoptosis-regulatory cascades of the epithelium is important for understanding carcinogenesis, since cancer cells are considered to arise as a result of the collapse of the cascades. We previously reported that a novel gene GASDERMIN (GSDM) is expressed in the stomach but suppressed in gastric cancer cell lines. Furthermore, in this study, we demonstrated that GSDM is expressed in the mucus-secreting pit cells of the gastric epithelium and frequently silenced in primary gastric cancers. We

found that GSDM has a highly apoptotic activity and its expression is regulated by a transcription factor LIM domain only 1 (LMO1) through a sequence to which Runt-related transcription factor 3 (RUNX3) binds, in a GSDM promoter region. We observed coexpression of GSDM with LMO1, RUNX3 and type II transforming growth factor-beta receptor (TGF-betaRII) in the pit cells, and found that TGF-beta upregulates the LMO1- and GSDM-expression in the gastric epithelial cell line and induces apoptosis, which was confirmed by the finding that the apoptosis induction is inhibited by suppression of each LMO1-, RUNX3- and GSDM expression, respectively. The present data suggest that TGF-beta, LMO1, possibly RUNX3, and GSDM form a regulatory pathway for directing the pit cells to apoptosis.

Sangodkar, J., J. Shi, et al. (2009). "Functional role of the KLF6 tumour suppressor gene in gastric cancer." Eur J Cancer **45**(4): 666-76.

Gastric cancer is the second most common cancer and a leading cause of cancer-related death worldwide. The Kruppel-like factor 6 (KLF6) tumour suppressor gene had been previously shown to be inactivated in a number of human cancers through loss of heterozygosity (LOH), somatic mutation, decreased expression and increased alternative splicing into a dominant negative oncogenic splice variant, KLF6-SV1. In the present study, 37 gastric cancer samples were analysed for the presence of loss of heterozygosity (LOH) of the KLF6 locus and somatic mutation. In total, 18 of 34 (53%) of the gastric cancer samples analysed demonstrated KLF6 locus specific loss. Four missense mutations, such as T179I, R198G, R71Q and S180L, were detected. Interestingly, two of these mutations R71Q and S180L have been identified independently by several groups in various malignancies including prostate, colorectal and gastric cancers. In addition, decreased wild-type KLF6 (wtKLF6) expression was associated with loss of the KLF6 locus and was present in 48% of primary gastric tumour samples analysed. Functional studies confirmed that wtKLF6 suppressed proliferation of gastric cancer cells via transcriptional regulation of the cyclin-dependent kinase inhibitor p21 and the oncogene c-myc. Functional characterisation of the common tumour-derived mutants demonstrated that the mutant proteins fail to suppress proliferation and function as dominant negative regulators of wtKLF6 function. Furthermore, stable overexpression of the R71Q and S180L tumour-derived mutants in the gastric cancer cell line, Hs746T, resulted in an increased tumourigenicity in vivo. Combined, these findings suggest an important role for the KLF6 tumour suppressor gene in gastric cancer development and progression and identify several highly cancer-relevant signalling pathways regulated by the KLF6 tumour suppressor gene.

Sawabu, T., H. Seno, et al. (2007). "Growth arrest-specific gene 6 and Axl signaling enhances gastric cancer cell survival via Akt pathway." Mol Carcinog **46**(2): 155-64.

Activation of tyrosine kinases is an important factor during cancer development. Axl, one of the receptor tyrosine kinases, binds to the specific ligand growth arrest-specific gene 6 (Gas6), which encodes a vitamin K-dependent gamma-carboxyglutamyl protein. Although many receptor tyrosine kinases and their ligands are involved in gastric carcinogenesis, whether Gas6-Axl signaling is involved in gastric carcinogenesis has not been elucidated. The aim of this study was to investigate the expression of Gas6 and Axl in gastric cancer and also their roles during gastric carcinogenesis. mRNA and protein of Gas6 and Axl were highly expressed in a substantial proportion of human gastric cancer tissue and cell lines, and Gas6 expression was significantly associated with lymph node metastasis. With recombinant Gas6 and a decoy-receptor of Axl in vitro, we demonstrated that Gas6-Axl signaling pathway enhanced cellular survival and invasion and suppressed apoptosis via Akt pathway. Our results suggests that Gas6-Axl signaling plays a role during gastric carcinogenesis, and that targeting Gas6-Axl signaling could be a novel therapeutic for gastric cancer.

Saxena, A., K. Nath Prasad, et al. (2008). "Association of Helicobacter pylori and Epstein-Barr virus with gastric cancer and peptic ulcer disease." Scand J Gastroenterol **43**(6): 669-74.

OBJECTIVE: Helicobacter pylori and Epstein-Barr virus (EBV) infections are common world-wide. Though H. pylori infection is a major factor in gastroduodenal diseases, its role in association with EBV infection is unknown. We prospectively studied the association of H. pylori and EBV in patients with gastric cancer (GC) and peptic ulcer disease (PUD). MATERIAL AND METHODS: A total of 348 adult patients (non-ulcer dyspepsia (NUD) 241, PUD 45, GC 62) undergoing upper gastrointestinal endoscopy between September 2003 and May 2007 were enrolled in the study. H. pylori infection was diagnosed by rapid urease test, culture, histopathology and polymerase chain reaction (PCR). EBV DNA was detected by non-polymorphic Epstein-Barr nuclear antigen-1 (EBNA-1) gene-based PCR and sequence analysis. RESULTS: The rate of H. pylori infection was higher in patients with PUD than in those with GC (80% versus 56.5%, p=0.01) and NUD (80% versus 55.2%, p=0.002). In patients with

GC and PUD, EBV DNA was detected more often than in those with NUD (GC versus NUD - 82.3% versus 37.3%, p<0.001; PUD versus NUD - 75.5% versus 37.3%, p<0.001). H. pylori infection and EBV DNA detected in different groups of patients was as follows: 62.2% in PUD, 46.8% in GC and 29.5% in NUD. PUD and GC were significantly associated (p<0.001 and <0.05, respectively) with the presence of H. pylori infection and EBV DNA as compared with NUD. CONCLUSIONS: EBV DNA either alone or in combination with H. pylori infection was significantly associated with GC and PUD, suggesting that EBV might play an important role in gastroduodenal pathology.

Seidl, C., M. Port, et al. (2007). "213Bi-induced death of HSC45-M2 gastric cancer cells is characterized by G2 arrest and up-regulation of genes known to prevent apoptosis but induce necrosis and mitotic catastrophe." Mol Cancer Ther **6**(8): 2346-59.

Tumor cells are efficiently killed after incubation with alpha-emitter immunoconjugates targeting tumor-specific antigens. Therefore, application of alpha-emitter immunoconjugates is a promising therapeutic option for treatment of carcinomas that are characterized by dissemination of single tumor cells in the peritoneum like ovarian cancer or gastric cancer. In diffuse-type gastric cancer, 10% of patients express mutant d9-E-cadherin on the surface of tumor cells that is targeted by the monoclonal antibody d9MAb. Coupling of the alpha-emitter (213)Bi to d9MAb provides an efficient tool to eliminate HSC45-M2 gastric cancer cells expressing d9-E-cadherin in vitro and in vivo. Elucidation of the molecular mechanisms triggered by alpha-emitters in tumor cells could help to improve strategies of alpha-emitter radioimmunotherapy. For that purpose, gene expression of (213)Bi-treated tumor cells was quantified using a real time quantitative-PCR low-density array covering 380 genes in combination with analysis of cell proliferation and the mode of cell death. We could show that (213)Bi-induced cell death was initiated by G(2) arrest; up-regulation of tumor necrosis factor (TNF), SPHK1, STAT5A, p21, MYT1, and SSTR3; and down-regulation of SPP1, CDC25 phosphatases, and of genes involved in chromosome segregation. Together with morphologic changes, these results suggest that (213)Bi activates death cascades different from apoptosis. Furthermore, (213)Bi-triggered up-regulation of SSTR3 could be exploited for improvement of the therapeutic regimen.

Sekikawa, A., H. Fukui, et al. (2008). "REG Ialpha protein mediates an anti-apoptotic effect of STAT3 signaling in gastric cancer cells." Carcinogenesis **29**(1): 76-83.

Signal transducer and activator of transcription 3 (STAT3) signaling plays roles in inflammation-associated carcinogenesis. Regenerating gene (REG) Ialpha protein, an interleukin (IL)-6-inducible gene, is suggested to be involved in the gastritis-gastric cancer sequence. We investigated the involvement of IL-6/STAT3 signaling in REG Ialpha protein expression and examined whether REG Ialpha protein mediates an anti-apoptotic effect of STAT3 signaling in gastric cancer cells. The effects of IL-6/STAT3 signaling on REG Ialpha protein expression were examined using a STAT3 small interfering RNA system in gastric cancer cells. The element responsible for IL-6-induced REG Ialpha promoter activation was determined by a promoter deletion assay. The anti-apoptotic effects of STAT3 signaling and its induced REG Ialpha protein were examined by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphatase nick-end labeling and caspase assay in vitro. Human gastric cancer specimens were analyzed by immunohistochemistry for phosphorylated signal transducer and activator of transcription 3 (p-STAT3) and REG Ialpha protein. IL-6 treatment enhanced the expression of REG Ialpha protein through STAT3 activation in gastric cancer cells. The IL-6-responsive element was determined to lie in the sequence from -142 to -134 of the REG Ialpha promoter region. REG Ialpha protein mediated the anti-apoptotic effects of STAT3 signaling in gastric cancer cells by enhancing Akt activation, Bad phosphorylation and Bcl-xL expression. The expression of REG Ialpha protein was significantly correlated with that of p-STAT3 in gastric cancer tissues. REG Ialpha protein may play a pivotal role in anti-apoptosis in gastric tumorigenesis under STAT3 activation.

Seno, H., K. Satoh, et al. (2007). "Novel interleukin-4 and interleukin-1 receptor antagonist gene variations associated with non-cardia gastric cancer in Japan: comprehensive analysis of 207 polymorphisms of 11 cytokine genes." J Gastroenterol Hepatol **22**(5): 729-37.

BACKGROUND AND AIM: Helicobacter pylori (H. pylori)-induced chronic atrophic gastritis is a high-risk factor for gastric cancer. Immune responses to H. pylori are involved in gastric mucosal inflammation, and might affect clinical outcome, including the development of gastric cancer. The present study examines the significance of gene polymorphisms of various cytokines in the development of gastric cancer following H. pylori infection. METHODS: One hundred Japanese non-cardia gastric cancer patients and 93 dyspeptic patients as controls were enrolled in the study (age range 50-75 years). All patients were positive for H. pylori. Genomic DNA was extracted from peripheral

whole blood leukocytes, and we comprehensively analyzed 207 single nucleotide polymorphisms (SNP) in 11 cytokine genes; interleukin (IL)-1alpha, IL-1beta, IL-1 receptor antagonist (RN), IL-4, IL-4R, IL-8, IL-10, IL-12, TNF-alpha, TNF-beta, and IFN-gamma, using either invader assay (163 SNP), direct sequencing (22 SNP), or PCR-restriction fragment length polymorphism (22 SNP). RESULTS: Among the 207 SNP examined, the IL-4 gene diplotypes (984 and 2983 AA/GA) had a significant negative association with gastric cancer development (odds ratio =0.3, 95% confidence interval =0.1-0.9). When we adopted the dyspeptic patients over 66 years of age as the controls, the IL-1RN gene diplotypes (-1102 and 6110 CG/GA) also had a significant negative association (odds ratio =0.2, 95% confidence interval =0.1-0.7). CONCLUSION: A comprehensive analysis of 207 SNP of 11 cytokine genes revealed that variations in IL-4 and IL-1RN genes are negatively associated with the risk of developing gastric cancer following H. pylori infection. Distinct host cytokine responses in the gastric mucosa might have a role in H. pylori-induced carcinogenesis.

Seto, M., M. Ohta, et al. (2009). "Regulation of the hedgehog signaling by the mitogen-activated protein kinase cascade in gastric cancer." Mol Carcinog **48**(8): 703-12.

The hedgehog and mitogen-activated protein kinase (MAPK) signaling pathways regulate growth in many tumors, suggesting cooperation between these two pathways in the regulation of cell proliferation. However, interactions between these pathways have not been extensively studied. We assessed cross-talk between hedgehog and MAPK signaling in the regulation of cell proliferation in gastric cancer. We showed that PTCH expression was significantly correlated with extracellular signal-regulated kinase (ERK) 1/2 phosphorylation (P = 0.016) as well as SHH expression (P = 0.034) in the 35 gastric cancers assessed by immunohistochemistry. Indeed, MAPK signaling increased the GLI transcriptional activity and induced the expression of hedgehog target genes in gastric cancer cells. The inductive effect of activated KRAS and mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK) 1 was blocked by the suppressor of fused (SUFU), indicating that MAPK signaling regulates GLI activity via a SUFU-independent process. Moreover, the deletion of the NH2-terminal domain of GLI1 gene resulted in reduced response to MEK1 stimulation. Our results suggest that the KRAS-MEK-ERK cascade has a positive regulatory role in GLI transcriptional activity in gastric cancer.

Shanks, A. M. and E. M. El-Omar (2009). "Helicobacter pylori infection, host genetics and gastric cancer." J Dig Dis **10**(3): 157-64.

Helicobacter pylori infects half the world's population and is responsible for a considerable global health burden, including peptic ulcer disease and gastric cancer. The infection causes a chronic gastritis, the severity and distribution of which determine the clinical outcome. Bacterial, environmental and host genetic factors combine to define the degree of gastric damage. Most patients have a limited mild pan-gastritis with no significant clinical consequences. Antral-predominant gastritis is associated with high gastric acid output and an increased risk of duodenal ulcers. Corpus-predominant gastritis is associated with a reduction in gastric acid, multifocal gastric atrophy and an increased risk of gastric cancer. Host genetic factors are particularly important in defining the severity and extent of Helicobacter-induced gastritis. The most relevant and consistent genetic factors uncovered thus far are in the interleukin-1 and tumor necrosis factor-A gene clusters. These cytokines appear to play a key role in the pathophysiology of gastric cancer and their roles have been confirmed in animal models that mimic human gastric neoplasia. More genetic factors have also been uncovered and, with advancing technology, there is every prospect of defining a full genetic risk profile in the next decade. This will aid in targeting the testing and treatment of Helicobacter pylori, which offers a true opportunity to prevent and defeat this global killer.

Shibata, T., T. Arisawa, et al. (2009). "Selenoprotein S (SEPS1) gene -105G>A promoter polymorphism influences the susceptibility to gastric cancer in the Japanese population." BMC Gastroenterol **9**: 2.

BACKGROUND: Inflammation is a key factor in the process of carcinogenesis from chronic gastritis induced by Helicobacter pylori. Selenoprotein S (SEPS1) is involved in the control of the inflammatory response in the endoplasmic reticulum (ER). Recently the -105G>A polymorphism in the promoter of SEPS1 was shown to increase pro-inflammatory cytokine expression. We examined the association between this polymorphism and the risk of gastric cancer. METHODS: We took stomach biopsies during endoscopies of 268 Japanese gastric cancer patients (193 males and 75 females, average age 65.3), and 306 control patients (184 males and 122 females, average age 62.7) and extracted the DNA from the biopsy specimens. All subjects provided written informed consent. For the genotyping of the SEPS1 promoter polymorphism at position -105G>A, PCR-RFLP methods were used and the PCR products were digested with PspGI. Logistic-regression analysis was used to estimate odds ratios (OR) and 95% confidence

intervals (CI), adjusting for age, sex, and H. pylori infection status. RESULTS: Among cases, the distribution of genotypes was as follows: 88.4% were GG, 11.2% were GA, and 0.4% were AA. Among controls, the distribution was as follows: 92.5% were GG, 7.2% were GA, and 0.3% were AA. Among males, carrying the A allele was associated with an increased odds of gastric cancer, compared with the GG genotype (OR: 2.0, 95% CI 1.0-4.1, p = 0.07). Compared with the GG genotype, carrying the A allele was significantly associated with increased risks of intestinal type gastric cancer (OR: 2.0, 95%CI 1.0-3.9, p < 0.05) as well as of gastric cancer located in the middle third of the stomach (OR: 2.0, 95%CI 1.0-3.9, p < 0.05). CONCLUSION: The -105G>A promoter polymorphism of SEPS1 was associated with the intestinal type of gastric cancer. This polymorphism may influence the inflammatory conditions of gastric mucosa. Larger population-based studies are needed for clarifying the relation between inflammatory responses and SEPS1 polymorphism.

Solcia, E., C. Klersy, et al. (2009). "A combined histologic and molecular approach identifies three groups of gastric cancer with different prognosis." Virchows Arch **455**(3): 197-211.

The limited prognostic value of currently used histologic classifications of gastric cancer and their failure to account for the complexity of the disease as revealed by more recent investigations prompted a combined reinvestigation of histologic, molecular, and clinicopathologic patterns in 294 extensively sampled, invasive gastric cancers representing all main histotypes and stages of the disease and followed for a median of 150 months. Among histologic parameters tested, only cellular atypia, angio-lympho- or neuroinvasion, Ki67 proliferation index, expansile/infiltrative type growth, and T8 cell-rich high lymphoid intra-/peritumor response (HLR) proved to be stage-independent predictors of patient survival. Among molecular tests, p53 gene exon 7 (loop 3) and 8 (loop-sheet-helix motif and S-10 band), but not p53 protein overexpression, TP53 LOH or 18qLOH, were found to worsen prognosis. Microsatellite DNA instability was a favorable prognostic factor when coupled with HLR. Patient survival analysis of the main histotypes and their subtypes confirmed the favorable prognosis of HLR, well-differentiated tubular, muconodular, and low grade diffuse desmoplastic cancers, and highlighted the worse prognosis of anaplastic and infiltrative-lymphoinvasive mucinous cancers compared to ordinary cohesive and diffuse cancers. Distinct roles of individual morphologic and molecular factors in tumor progression of the different histotypes have been recognized. The combination of survival-predictive histotypes and individual histologic or molecular parameters allowed us to develop a classification of all gastric cancers into three grades of increasing malignancy which proved to be of high prognostic value.

Song, I. S., N. S. Oh, et al. (2009). "Human ZNF312b promotes the progression of gastric cancer by transcriptional activation of the K-ras gene." Cancer Res **69**(7): 3131-9.

Gastric cancer ranks second among the most common causes of cancer deaths worldwide. Recent studies reported target molecules that are candidates for new therapeutic interventions; however, their molecular mechanism has not been clearly defined. In this study, we found that ZNF312b plays a role in tumor progression and metastasis in gastric cancer via transcriptional activation of the K-ras oncogene. ZNF312b seems to be specifically overexpressed in gastric cancer tissues and cell lines. The overexpression of ZNF312b induces cancer-like phenotypes, including accelerated proliferation and increased tumor masses in nude mice, which are completely reversed by its knockdown in gastric cancer cell lines, implying direct involvement in gastric tumor progression. From analyses using deletion mutants of ZNF312b and K-ras promoter-driven luciferase reporters, we found that it translocates into the nucleus via the proline-rich domain of its COOH terminus to activate transcription of the K-ras gene, resulting in an enhancement of the extracellular signal-regulated kinase signaling pathway that governs cell proliferation. Taken together, these results suggest that ZNF312b contributes to the promotion of gastric cancer by triggering K-ras oncogene expression. The current study is the first to report that ZNF312b, a novel transcription factor, was associated with tumorigenicity of gastric cancer. This might be a valuable target that could provide new insight into the development of new therapeutic modalities for patients with gastric cancer.

Song, I. S., A. G. Wang, et al. (2009). "Regulation of glucose metabolism-related genes and VEGF by HIF-1alpha and HIF-1beta, but not HIF-2alpha, in gastric cancer." Exp Mol Med **41**(1): 51-8.

Hypoxia-inducible factors (HIFs) are transcription factors that activate the transcription of target genes involved in crucial aspects of cancer development. This study investigated the expression of HIFs and their contribution to the regulation of target genes related to angiogenesis and glucose metabolism in gastric cancer. The data showed that HIFs were over-expressed in gastric cancer and that activation of the target genes was observed mainly in

the early stages. Moreover, the results of the present study revealed that only HIF-1alpha, but not HIF-2alpha dimerizes with HIF-1beta and then regulates expression of target genes in response to hypoxia. The results of the present study demonstrate that HIF-1alpha and HIF-1beta enhances expression of VEGF and glucose metabolism-related genes in response to hypoxia in gastric cancer. These data offer important information regarding HIF pathways in the development of gastric cancer.

Suganuma, M., K. Yamaguchi, et al. (2008). "TNF-alpha-inducing protein, a carcinogenic factor secreted from H. pylori, enters gastric cancer cells." Int J Cancer **123**(1): 117-22.

TNF-alpha inducing protein (Tip alpha) is secreted from Helicobacter pylori (H. pylori): it is a potent inducer of TNF-alpha and chemokine genes, mediated through NF-kappaB activation, and it also induces tumor-promoting activity in Bhas 42 cells. To investigate the carcinogenic mechanisms of H. pylori with Tip alpha, we first examined how Tip alpha acts on gastric epithelial cells. We found that fluorescent-Tip alpha specifically bound to, and then entered, the cells in a dose- and temperature-dependent manner, whereas deletion mutant of Tip alpha (del-Tip alpha), an inactive form, neither bound to nor entered the cells, suggesting the presence of a specific binding molecule. Mutagenesis analysis of Tip alpha revealed that a dimer formation of Tip alpha with a disulfide bond is required for both specific binding and induction of TNF-alpha gene expression. A confocal laser scanning microscope revealed some Tip alpha in the nuclei, but del-Tip alpha was not present, which indicated that an active form of Tip alpha can penetrate the nucleus and may be involved in the induction of TNF-alpha gene expression. Examination of Tip alpha production and secretion in 28 clinical isolates revealed that H. pylori obtained from gastric cancer patients secreted Tip alpha in significantly higher amounts than did H. pylori from patients with chronic gastritis, suggesting that Tip alpha is an essential factor in H. pylori inflammation and cancer microenvironment in the human stomach. Tip alpha is thus a new carcinogenic factor of H. pylori that can enter the nucleus through a specific binding molecule, and its mechanism of action is completely different from that of CagA.

Sugimoto, M., T. Furuta, et al. (2007). "Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer." J Gastroenterol Hepatol **22**(1): 51-9.

BACKGROUND AND AIM: In Western countries, polymorphism of pro-inflammatory cytokine genes is associated with the development of gastric cancer and duodenal ulcer. The aim of this study was to clarify the association of polymorphisms of interleukin (IL)-1beta and tumor necrosis factor (TNF)-alpha with susceptibility to peptic ulcer diseases and gastric cancer in Japan. METHODS: The IL-1beta-511/-31 and TNF-alpha-308/-857/-863/-1031 genotypes were determined in Helicobacter pylori-positive patients with gastritis only (n = 164), gastric ulcers (n = 110), duodenal ulcers (n = 94), or gastric cancers (n = 105), and in H. pylori-negative controls (n = 172). RESULTS: Carriage of the alleles TNF-alpha-857 T (odd ratio [OR], 1.826; 95% confidence interval [CI], 1.097-3.039), TNF-alpha-863 A (OR, 1.788; 95% CI, 1.079-2.905) and TNF-alpha-1031 C (OR, 1.912; 95% CI, 1.152-3.171) was associated with increased risk for gastric ulcer development. Carriage of the alleles TNF-alpha-857 T (OR, 1.686; 95% CI, 1.003-2.832), TNF-alpha-863 A (OR, 1.863; 95% CI, 1.118-3.107) and TNF-alpha-1031 C (OR 2.074; 95% CI, 1.244-3.457) was also associated with increased risk of gastric cancer development. There was no relationship between the development of H. pylori-related diseases and polymorphisms of IL-1beta-511/-31 and TNF-alpha-308. The simultaneous carriage of three different high-producer alleles of TNF-alpha-857/-863/-1031 significantly increased the risk of gastric ulcer (OR, 6.57; 95% CI, 2.34-18.40) and gastric cancer (OR, 5.20; 95% CI, 1.83-14.78). CONCLUSIONS: Polymorphisms in TNF-alpha rather than IL-1beta are associated with increased risk for gastric ulcers and gastric cancer in Japan. The simultaneous carriage of more than one high-producer allele of TNF-alpha further increased the risks for gastric ulcer and cancer.

Suzuki, G., H. Cullings, et al. (2007). "Low-positive antibody titer against Helicobacter pylori cytotoxin-associated gene A (CagA) may predict future gastric cancer better than simple seropositivity against H. pylori CagA or against H. pylori." Cancer Epidemiol Biomarkers Prev **16**(6): 1224-8.

BACKGROUND: To investigate the IgG antibody titer against Helicobacter pylori CagA as a risk factor for future noncardia gastric cancer. METHODS: A nested case-control study was done in the longitudinal cohort of atomic bomb survivors using stored sera before diagnosis (mean, 2.3 years). Enrolled were 299 cancer cases and 3 controls per case selected from cohort members matched on age, gender, city, and time and type of serum storage and countermatched on radiation dose. RESULTS: H. pylori IgG seropositive with CagA IgG low titer was the strongest risk factor for noncardia gastric cancer [relative risk (RR), 3.9; 95% confidence interval (95% CI), 2.1-7.0; $P < 0.001$], especially for intestinal-type

tumor (RR, 9.9, 95% CI, 3.5-27.4; P < 0.001), compared with other risk factors, H. pylori IgG seropositive with CagA IgG negative (RR, 2.2; 95% CI, 1.3-3.9; P = 0.0052), H. pylori IgG seropositive with CagA IgG high titer (RR, 2.0; 95% CI, 1.3-3.2; P = 0.0022), chronic atrophic gastritis (RR, 2.4; 95% CI, 1.8-3.3; P < 0.001), current smoking (RR, 2.3; 95% CI, 1.4-3.5; P < 0.001), or radiation dose (RR, 2.1; 95% CI, 1.2-3.1; P = 0.00193). Current smoking showed significantly higher risk for diffuse-type than intestinal-type tumors (P = 0.0372). Radiation risk was significant only for nonsmokers, all noncardia, and diffuse-type gastric cancers. CONCLUSIONS: A low CagA IgG titer is a useful biomarker to identify a high-risk group and it also provides a clue to understanding host-pathogen interaction.

Suzuki, G., H. Cullings, et al. (2009). "LTA 252GG and GA genotypes are associated with diffuse-type noncardia gastric cancer risk in the Japanese population." Helicobacter **14**(6): 571-9.

BACKGROUND: There are limited numbers of reports on the association of lymphotoxin-alpha (LTA) genotypes with gastric cancer. METHODS: A nested case-control study was carried out in the longitudinal cohort of atomic bomb survivors using stored sera before diagnosis (mean, 2.3 years) and blood cells. Enrolled were 287 cases with noncardia gastric cancer of diffuse and intestinal types and three controls per case selected from cohort members matched on age, gender, city, and time and type of serum storage and counter-matched on radiation dose. RESULTS: LTA 252GG and GA genotypes were associated with the prevalence of Helicobacter pylori IgG seropositivity and higher antibody titer against H. pylori cytotoxin-associated gene A (CagA) protein in controls and they were an independent risk factor for noncardia gastric cancer of diffuse type (RR = 2.8 (95% CI: 1.3-6.3), p = .01, and RR = 2.7 (95% CI: 1.5-4.8), p < .001), but not for intestinal type, after adjusting for H. pylori IgG seropositivity, CagA antibody titers, chronic atrophic gastritis, smoking, and radiation dose. Cessation of smoking (RR = 0.4 (95% CI: 0.2-0.7), p < .001) and never smoking (RR = 0.4 (95% CI: 0.3-0.6), p < .001) were both protective for future noncardia gastric cancer. Radiation dose was associated with noncardia gastric cancer in subjects with both the LTA 252G-allele and never smoking/quit smoking histories (RR = 3.8 (95% CI: 1.7-5.9), p = .009). CONCLUSION: The LTA 252 genotype is associated with noncardia gastric cancer of diffuse type in Japan and interacted with radiation dose.

Tahara, T., T. Shibata, et al. (2009). "Effect of polymorphisms in the 3' untranslated region (3'-UTR) of vascular endothelial growth factor gene on gastric cancer and peptic ulcer diseases in Japan." Mol Carcinog **48**(11): 1030-7.

A complex interaction of host genetic and environmental factors may be relevant in the development of Helicocobacter pylori-related gastric carcinogenesis. We investigated the effect of vascular endothelial growth factor (VEGF) gene polymorphisms on the risk of gastric cancer (GC) and peptic ulcer diseases in a Japanese population. The G1612A(rs10434) and C936T(rs3025039) polymorphisms in the 3' untranslated region (3'-UTR) of VEGF gene were genotyped in a total of 844 subjects including 385 GC, 143 ulcer including 98 gastric ulcer (GU), 45 duodenal ulcer (DU), and 316 nonulcer subjects. The 1612A carrier held a significantly higher risk of GC when compared to both noncancer and nonulcer (overall noncancer vs. GC; OR = 1.61, 95% CI = 1.17-2.21, P = 0.0038, nonulcer vs. GC; OR = 1.54, 95% CI = 1.07-2.22, P = 0.0197). The 1612A carrier was more closely associated with an increased risk of noncardiac cancer (OR = 1.64, 95% CI = 0.17-2.21, P = 0.0038), lower third cancer (OR = 1.97, 95% CI = 1.30-3.00, P = 0.002), and Lauren's diffuse-type cancer (OR = 1.75, 95% CI = 1.24-2.46, P = 0.001), while the same genotype was not associated with the progression of GC. The C936T genotype was not associated with a risk of GC and its progression. Both the G1612A and C936T genotypes were not associated with the risk of peptic ulcer diseases. Our data suggest that the G1612A, but not C936T polymorphisms in the 3'-UTR of VEGF gene is associated with the susceptibility to GC in the Japanese population.

Takahata, M., Y. Inoue, et al. (2009). "SKI and MEL1 cooperate to inhibit transforming growth factor-beta signal in gastric cancer cells." J Biol Chem **284**(5): 3334-44.

Chromosomal amplification occurs frequently in solid tumors and is associated with poor prognosis. Several reports demonstrated the cooperative effects of oncogenic factors in the same amplicon during cancer development. However, the functional correlation between the factors remains unclear. Transforming growth factor (TGF)-beta signaling plays important roles in cytostasis and normal epithelium differentiation, and alterations in TGF-beta signaling have been identified in many malignancies. Here, we demonstrated that transcriptional co-repressors of TGF-beta signaling, SKI and MDS1/EVI1-like gene 1 (MEL1), were aberrantly expressed in MKN28 gastric cancer cells by chromosomal co-amplification of 1p36.32. SKI and MEL1 knockdown synergistically restored TGF-beta responsiveness in MKN28 cells and reduced tumor

growth in vivo. MEL1 interacted with SKI and inhibited TGF-beta signaling by stabilizing the inactive Smad3-SKI complex on the promoter of TGF-beta target genes. These findings reveal a novel mechanism where distinct transcriptional co-repressors are co-amplified and functionally interact, and provide molecular targets for gastric cancer treatment.

Taniguchi, H., Y. Fujiwara, et al. (2007). "Gene therapy using ets-1 transcription factor decoy for peritoneal dissemination of gastric cancer." Int J Cancer **121**(7): 1609-17.

The ets-1 transcription factor plays an important role in cell proliferation, differentiation, apoptosis and tissue remodeling. Aberrant ets-1 expression correlates with aggressive tumor behavior and poorer prognosis in patients with various malignancies. This study evaluated the efficacy of double-stranded decoy oligonucleotides targeting ets-1-binding cis elements for the suppression of ets-1 in treatment of a peritoneal dissemination model of gastric cancer. In vitro, MTT assay was performed to evaluate the effect of the ets-1 decoy on cell growth. Electrophoretic mobility shift assay (EMSA) was performed to determine ets-1 activity. In vivo, the effect of the ets-1 decoy was investigated in the peritoneal dissemination nude mice model. Disseminated nodules were analyzed immunohistochemically. Ets-1 decoy, but not scrambled decoy, significantly inhibited cell growth in 2 gastric cancer cell lines, which showed overexpression of ets-1 protein by inhibiting the binding activity of ets-1. In the peritoneal dissemination model, the ets-1 decoy significantly suppressed the disseminated nodules, and tended to prolong the survival rate. PCNA index, microvessel density and VEGF expression were also reduced in peritoneal tumors treated with ets-1 decoy. Intraperitoneal injection of ets-1 decoy inhibited peritoneal dissemination of gastric cancer in a nude mice model. The results indicate that the decoy strategy for ets-1 offers a promising therapy for patients with incurable peritoneal dissemination of gastric cancer, most of which show overexpression of ets-1 protein.

Tu, S. P., A. L. Chi, et al. (2009). "p53 inhibition of AP1-dependent TFF2 expression induces apoptosis and inhibits cell migration in gastric cancer cells." Am J Physiol Gastrointest Liver Physiol **297**(2): G385-96.

Overexpression of trefoil factor 2 (TFF2) is associated with increased cell migration, resistance to apoptosis, and possibly increased gastric cancer invasion. Dysregulation of p53 is frequently observed in preneoplastic conditions of the stomach. Here, we investigated the effect of p53 on the expression and function of TFF2 in gastric cancer cell lines. Gene expression was determined by reverse transcription-polymerase chain reaction, and promoter activity was assessed by dual luciferase reporter assays. Apoptosis was detected by flow cytometry, and cell migration was evaluated by the Boyden chamber assay. Exogenous expression of p53 dose dependently inhibited endogenous TFF2 mRNA, protein, and promoter activity and resulted in induction of cell apoptosis and inhibition of cell migration. Downregulation of TFF2 by small interfering RNA sensitized gastric cancer cells to drug-induced p53-dependent apoptosis. Addition of human TFF2 peptide reversed p53-dependent apoptosis and inhibition of cell migration. The p53-responsive element was mapped to an AP-1-like cis-element at -182 bp upstream of the TFF2 transcription start site. Mutation of this AP-1-like element abrogated p53-mediated inhibition of TFF2 promoter activity. Gel shift and chromatin immunoprecipitation assays demonstrated that c-Jun and c-Fos bind to this AP-1-like element. Ectopic expression of c-Jun/c-Fos or p300 or treatment of cells with phorbol 12-myristate 13-acetate (PMA) stimulated endogenous TFF2 mRNA expression and promoter activity, and p53 inhibited the effects of AP-1 and PMA on TFF2. p53 induces cell apoptosis and inhibits cell migration in part by downregulating TFF2 expression through an AP-1-like site, suggesting that TFF2 may be an important downstream target of p53.

Tu, S. P., P. Liston, et al. (2009). "Restoration of XAF1 expression induces apoptosis and inhibits tumor growth in gastric cancer." Int J Cancer **125**(3): 688-97.

XAF1 (XIAP-associated factor 1) is a novel XIAP binding protein that can antagonize XIAP and sensitize cells to other cell death triggers. Our previous results have shown that aberrant hypermethylation of the CpG sites in XAF1 promoter is strongly associated with lower expression of XAF1 in gastric cancers. In our study, we investigated the effect of restoration of XAF1 expression on growth of gastric cancers. We found that the restoration of XAF1 expression suppressed anchorage-dependent and -independent growth and increased sensitivity to TRAIL and drug-induced apoptosis. Stable cell clones expressing XAF1 exhibited delayed tumor initiation in nude mice. Restoration of XAF1 expression mediated by adenovirus vector greatly increased apoptosis in gastric cancer cell lines in a time- and dose-dependent manner and sensitized cancer cells to TRAIL and drugs-induced apoptosis. Adeno-XAF1 transduction induced cell cycle G2/M arrest and upregulated the expression of p21 and downregulated the expression of cyclin B1 and cdc2. Notably, adeno-XAF1

treatment significantly inhibited tumor growth, strongly enhanced the antitumor activity of TRAIL in a gastric cancer xenograft model in vivo, and significantly prolonged the survival time of animals bearing tumor xenografts. Complete eradication of established tumors was achieved on combined treatment with adeno-XAF1 and TRAIL. Our results document that the restoration of XAF1 inhibits gastric tumorigenesis and tumor growth and that XAF1 is a promising candidate for cancer gene therapy.

Udhayakumar, G., V. Jayanthi, et al. (2007). "Interaction of MUC1 with beta-catenin modulates the Wnt target gene cyclinD1 in H. pylori-induced gastric cancer." Mol Carcinog **46**(9): 807-17.

Beta-catenin can function as an oncogene when it is translocated to the nucleus, binds to T-cell factor (TCF) or lymphoid enhance factor and transactivate its target gene. The mechanism responsible for the activation of Wnt signaling pathway in the Cytotoxin-associated antigen A (CagA) Helicobacter pylori (H. pylori)-infected gastric carcinoma has not been elucidated. We hypothesize that whether interaction of MUC1 with beta-catenin modulates the Wnt signaling and its target gene cyclinD1 in CagA H. pylori-infected gastric carcinoma. The result demonstrate that binding of MUC1 CT with Protein Kinase C delta (PKC delta), tyrosine phosphorylation of MUC1 CT, and CagA are strongly associated with the interaction of MUC1 with beta-catenin in CagA H. pylori-infected gastric carcinoma. A statistically significant difference (chi(2) = 24.49; P < 0.001) was found when the binding of MUC1 CT and beta-catenin was compared to subcellular localization of beta-catenin. We also observed significant statistical correlation (chi(2) = 14.885; P < 0.001) between the cyclinD1 overexpression and the subcellular localization of beta-catenin. The overexpression of cyclinD1 was significantly higher (chi(2) = 13.785; P < 0.002) in advanced gastric carcinoma with CagA H. pylori infection. In addition cyclinD1 overexpression was significantly higher (chi(2) = 37.267; P < 0.001) with the interaction of MUC1 CT with beta-catenin in advanced gastric cancer. These findings indicate that MUC1 CT plays a role in the intracellular signaling through its interaction with beta-catenin and upregulate the Wnt target gene cyclinD1 in CagA H. pylori-infected gastric carcinoma.

Wanajo, A., A. Sasaki, et al. (2008). "Methylation of the calcium channel-related gene, CACNA2D3, is frequent and a poor prognostic factor in gastric cancer." Gastroenterology **135**(2): 580-90.

BACKGROUND & AIMS: The calcium channel voltage-dependent alpha2delta subunit consists of 4 genes, CACNA2D1 to CACNA2D4, of which CACNA2D2 and CACNA2D3 are located on 3p21.3 and 3p21.1, respectively. Here, we examined the relation between alpha2delta subunit gene alterations and gastric carcinogenesis. METHODS: The expression and methylation status of the alpha2delta subunit genes were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and methylation-specific PCR in gastric cancers (GCs). The effects of CACNA2D3 expression were examined by cell proliferation and adhesion assays, and they predicted target gene alterations. RESULTS: Aberrant methylation of CACNA2D1 and CACNA2D3 mostly corresponded to their expression status in GC cell lines. CACNA2D1/3 methylation was detected in 10 (12.5%) and 24 (30%) of the 80 GC cases, respectively, but no CACNA2D2 methylation was seen in 32 cases. CACNA2D3 methylation was more frequently found in diffuse type than in intestinal type (16/38 [42.1%] vs 8/42 [19.0%]; P = .025) GCs. Among the 53 patients with advanced GCs, patients with cancers showing CACNA2D3 methylation had a significantly shorter survival time than patients without this methylation (P = .003). Exogenous CACNA2D3 expression strongly inhibited cell growth and adhesion and up-regulated p21 and p27 expression in HEK-293T and NUGC4 cells. Inverse effects were seen by CACNA2D3 small interfering RNA treatment in the CACNA2D3-positive cell lines, indicating that CACNA2D3 may have tumor suppressive functions. CONCLUSIONS: Loss of CACNA2D3 expression through aberrant promoter hypermethylation may contribute to gastric carcinogenesis, and CACNA2D3 methylation is a useful prognostic marker for patients with advanced GC.

Wang, H. L., H. Bai, et al. (2007). "Rationales for expression and altered expression of apoptotic protease activating factor-1 gene in gastric cancer." World J Gastroenterol **13**(38): 5060-4.

AIM: To elucidate the relationship between apoptotic protease activating factor-1 (Apaf-1) gene and gastric cancer. METHODS: Thirty-five postoperative cancer and adjacent normal tissue samples were collected in the present study. Expression of the Apaf-1 gene in these samples was analyzed by semi-quantitative RT-PCR. Loss of heterozygosity (LOH) was used to determine whether there was loss of Apaf-1 gene in domain of 12q22-23 in the samples. Promoter methylation of Apaf-1 gene in the samples was analyzed by methylation specific (MSP) PCR. RESULTS: The expression of Apaf-1 mRNA in gastric cancer tissue samples was 51%. The LOH frequency of D12S346, D12S1706, D12S327, D12S1657 and D12S393 was 33%, 8%, 58%, 12%

and 42%, respectively. Fifty percent LOH was found at two sites and 17% LOH at three sites. Apaf-1 mRNA expression decreased significantly in 13 cases (rs=0.487, P=0.003). The rate of Apaf-1 promoter methylation was 49% in gastric cancer tissue samples and 23% in para-cancerous tissue samples. Promoter methylation occurred significantly in 16 of 18 gastric cancer tissue samples with decreased expression of Apaf-1 mRNA rs=0.886, P=10(-6)). CONCLUSION: The expression of Apaf-1 gene is low in gastric cancer tissues. Methylation of Apaf-1 gene promoter and LOH in domain of 12q22-23 are the main reasons for the expression and altered expression of Apaf-1 gene.

Wang, L., X. Guan, et al. (2008). "Targeted inhibition of Sp1-mediated transcription for antiangiogenic therapy of metastatic human gastric cancer in orthotopic nude mouse models." Int J Oncol **33**(1): 161-7.

Overexpression of the transcription factor Sp1 may play a critical role in human gastric cancer angiogenesis. In the present studies, we determined whether targeting Sp1 has a therapeutic benefit. Treatment with mithramycin A (MIT) suppressed the expression of Sp1 and its downstream target genes in both human gastric cancer cell culture and tumors growing in nude mice. The molecular responses were accompanied by a significant inhibition of gastric cancer angiogenesis, growth and metastasis. Conversely, treatment with bevacizumab (BVZ), a neutralizing antibody against VEGF A, suppressed human gastric cancer growth in nude mice in a dose-dependent manner. Gene expression analyses revealed that treatment with low dose of BVZ substantially upregulated the expression of Sp1 and its downstream target genes, including VEGF and EGFR, in tumor tissues, whereas it did not have this effect on gastric cancer cells in culture. Combined treatment with BVZ and MIT produced synergistic tumor suppression, which was consistent with suppression of the expression of Sp1 and its downstream target genes. Thus, treatment with BVZ may block VEGF function but activate the pathway of its expression via positive feedback. Collectively, Sp1 is an important regulator of the expression of multiple angiogenic factors and functional status of Sp1 signaling pathway may profoundly affect the angiogenic phenotype of and effectiveness of antiangiogenic strategies for human gastric cancer.

Wang, Q., Y. Huang, et al. (2007). "siRNA targeting midkine inhibits gastric cancer cells growth and induces apoptosis involved caspase-3,8,9 activation and mitochondrial depolarization." J Biomed Sci **14**(6): 783-95.

Midkine (MK), a heparin-binding growth factor, is expressed highly in various malignant tumors, so it acts as attractive therapeutic target. In the present study, we used siRNA targeting MK to downregulate human MK expression in human gastric cancer cell line BGC823 and SGC7901 so as to determine the advantages of this anticancer therapeutic. The cell proliferation was evaluated by a WST-8 (4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1, 3-benzene disulfonate sodium salt) assay and colony formation assay. Apoptosis was determined by flow cytometer analysis and colorimetric assay. Our results showed that the BGC823 and SGC7901 cell growth were significantly inhibited by knockdown of MK gene. The loss of mitochondrial membrane potential, release of cytochrome c from the mitochondria into cytosol and increased activity of caspase-3, 8 and 9 occurred concomitantly with inhibition of MK gene. These results indicated that siRNA targeting MK gene can inhibit gastric cancer cells growth and induce apoptosis via mitochondrial depolarization and caspase-3 activation. MK siRNA may be a promising novel and potential therapeutic strategy for the treatment of gastric cancers.

Wang, S. L., D. Z. Zheng, et al. (2008). "Increased expression of hLRH-1 in human gastric cancer and its implication in tumorigenesis." Mol Cell Biochem **308**(1-2): 93-100.

Altered signaling pathways or deregulated transcription factors represent an important category of molecular events leading to aberrant gene regulation in gastric cancer, among which the role of WNT/beta-catenin pathway remains unclear. LRH-1 is a critical transcription factor in controlling cell proliferation via crosstalk with the beta-catenin signaling pathway. In order to gain a knowledge of the expression of hLRH-1v1 and hLRH-1 in gastric cancer, a Q-PCR analysis was carried out. Our results showed that in about 50 and 47.6% of 42 tested patients with gastric cancer, the mRNA expression of hLRH-1v1 and hLRH-1 was significantly upregulated, as compared with self-paired normal control, respectively. Besides, overexpression of hLRH-1 was shown to promote the proliferation of gastric adenocarcinoma cell SGC-7901 via induction of cyclin E1. Taken together, our present study demonstrated for the first time the increased expression of hLRH-1v1 and hLRH-1 in human gastric cancer, an alteration which may implicate in tumorigenesis.

Wang, Z., S. R. Cai, et al. (2009). "High expression of PRL-3 can promote growth of gastric cancer and

exhibits a poor prognostic impact on patients." Ann Surg Oncol **16**(1): 208-19.

High expression of PRL-3 had been implicated in lymph node metastasis of gastric cancer. In the present study, we detected the expression of PRL-3 in primary gastric cancer tissue, and evaluated its role in gastric cancer growth and the prognostic impact on patients. PRL-3 phosphatase expression was measured in 137 gastric tumor samples by using the immunohistochemistry method, and the overall survival rate was compared between the patients with high PRL-3 expression (n = 85) and those with moderate or low PRL-3 expression (n = 52). RNA interference, mediated by recombinant lentivirus expressing artificial PRL-3 miRNA, was used to knockdown PRL-3 expression in SGC7901 cell line. MTT assay and animal experiment were conducted to determine the role of PRL-3 in the proliferation of SGC7901 cells and tumor growth. PRL-3 expression was more frequently detected in tumors with a diameter >40 mm and in advanced stages. Furthermore, the overall survival rate of high PRL-3 expression was significantly lower than that of moderate or low PRL-3 expression ($P < 0.001$), and multivariate analysis showed that PRL-3 expression level independently influences the survival of patients ($P = 0.024$). Importantly, knockdown of PRL-3 significantly suppressed the proliferation of SGC7901 cells and slowed the tumor growth compared with controls ($P < 0.05$). PRL-3 is associated with gastric cancer progression. High PRL-3 expression in the primary lesion had a negative impact on prognosis. PRL-3 plays a key role in the control of gastric cancer growth. PRL-3 should be considered as a potential therapeutic target and a prognostic factor.

Wang, Z., Y. L. He, et al. (2008). "Expression and prognostic impact of PRL-3 in lymph node metastasis of gastric cancer: its molecular mechanism was investigated using artificial microRNA interference." Int J Cancer **123**(6): 1439-47.

High PRL-3 expression had been reported to have close association with lymph node metastasis (LNM) of gastric cancer. However, the prognostic significance of highly expressing PRL-3 in LNM of human gastric cancer and the role in the metastasis remain unclear. Our study examined PRL-3 expression both in the LNM (n = 107) and in the primary lesion (n = 137) of gastric cancer, and compared the overall survival rates. RNA interference, induced by recombinant plasmid pcDNA.rPRL3-miR expressing artificial PRL-3 miRNA, was employed to knockdown PRL-3 expression in human SGC7901 gastric cancer cells. Invasion assay and migration assay in vitro were conducted to determine the role of PRL-3 in the metastasis. The role of PRL-3 in the proliferation of SGC7901 cells and tumor growth were also determined. We observed that high PRL-3 expression was more frequently detected in the LNM than in the matched primary lesion (72.9 vs. 47.7%, $p < 0.001$). Furthermore, the overall survival rate of the patients with high expression of PRL-3 in the LNM was significantly less than those with moderate/low expression ($p = 0.003$). Importantly, knockdown of PRL-3 can significantly reduce both invasion and migration potencies of SGC7901 cells ($p < 0.001$), and significantly suppressed the proliferation of SGC7901 cells and slowed down the tumor growth ($p < 0.001$). It was concluded that high expression of PRL-3 in the LNM had a negative impact on the prognosis of the patients, and plays important roles in LNM of gastric cancer and the tumor growth, which can be a potential therapeutic target and a prognostic factor.

Wex, T., J. Bornschein, et al. (2009). "Host polymorphisms of immune regulatory genes as risk factors for gastric cancer." Minerva Gastroenterol Dietol **55**(4): 395-408.

The infection of the stomach with the gram-negative bacterium Helicobacter pylori is the main risk factor for the development of gastric cancer (GC). This led to the classification of this germ as "definite carcinogen" by the World Health Organization in 1994. The current model of gastric carcinogenesis is based on the interaction of multiple risk factors including virulence factors of the bacterium (e.g. CagA, VacA), environmental factors (diet, smoking) and host factors (gene polymorphisms). The complex interplay among these factors determines the clinical outcome of the infection leading to at least one of three major diseases in 1 out of 7 infected persons, namely ulcer disease, GC and "mucosa-associated lymphoid tissue" lymphoma in 15 %, 1% and 0.1% of all persons infected with H. pylori, respectively. Recently, an increasing number of genomic polymorphisms, mostly single nucleotide polymorphisms have been identified as risk factors for gastric cancer. Among them are genes encoding for cytokines, pattern recognition receptors, cell cycle-regulators, proteases, HLA-molecules, and enzymes for detoxification. In the last years it has become clear that an uniform "genomic risk pattern" for all GC patients does not exist. Most of these host factors are restricted either to the histological type (intestinal vs. diffuse), ethnical background (particularly Caucasian vs. Asian) and tumor localization (non-cardia vs. cardia cancer). Here, we review the current knowledge about the role of host factors for the gastric carcinogenesis focusing on immune-regulatory genes, in particular on the cytokine interleukin-1beta.

Wex, T., M. P. Ebert, et al. (2008). "Gene polymorphisms of the NOD-2/CARD-15 gene and the risk of gastric cancer in Germany." Anticancer Res **28**(2A): 757-62.

BACKGROUND: NOD-2 is involved in the intracellular recognition of bacterial muramyl dipeptides, and three independent polymorphisms of this gene have been identified as risk factors for the development of Crohn's disease. PATIENTS AND METHODS: To study the role of NOD-2 in gastric carcinogenesis, NOD-2 mutations (SNP5: P268S, SNP8: R702W, SNP12: G908R, and SNP13: 3020insC) were genotyped in 171 patients with gastric cancer and 153 controls. RESULTS: Applying a numerical model, SNP5 was found to carry a slightly increased risk (OR = 2.25, 95% CI: 1.05-2.17, p = 0.027) for the development of gastric cancer, whereas SNP8 was similarly distributed between controls and patients with gastric cancer. SNP5 and 8 were found to be genetically linked in both groups ($p < 0.02$). The allele frequency of SNP12 and 13 were rare and therefore analyzed in subgroups only and not statistically analyzed due to the lack of statistical power. CONCLUSION: NOD-2 is not a risk factor for gastric carcinogenesis in the Caucasian population.

Wu, C. W., J. Yu, et al. (2009). "Peroxisome proliferator-activated receptor delta and gastric cancer (Review)." Oncol Rep **22**(3): 451-7.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily which form heterodimers with retinoid X receptors (RXRs) in nucleus and bind to the PPAR response elements (PPREs) of target genes, leading to a wide spectrum of physiological functions. With an improved understanding of its physiological role, PPARdelta and its agonist have been gaining attention in cancer research in recent years. Despite the paucity of research concerning the direct relationship between PPARdelta and gastric cancer, there is substantial evidence that PPARdelta may play a role in the development of gastric cancer. This review focuses on recent literature describing the role of PPARdelta, especially in its association with nuclear factor-kappaB (NF-kappaB), interleukin-1beta (IL-1beta), cyclooxygenase-2 (COX-2) and Wnt-beta-catenin/TCF-4 pathways on gastric tumorigenesis and highlights critical discrepancies that need to be resolved for a more comprehensive understanding of how this receptor modulates gastric tumorigenesis. The potential role of PPARdelta as a therapeutic target in the treatment of gastric cancer deserves further research focus.

Wu, C. Y., M. S. Wu, et al. (2007). "Elevated plasma osteopontin associated with gastric cancer development, invasion and survival." Gut **56**(6): 782-9.

OBJECTIVE: Osteopontin (OPN) has been found to be valuable in diagnosis and predicting the prognosis of a variety of malignancies. The aims of the present study are to evaluate the usefulness of plasma OPN level for predicting gastric cancer development, invasion and survival. PATIENTS AND METHODS: One hundred and thirty two gastric cancer patients and 93 healthy controls were enrolled. Real-time quantitative reverse-transcription polymerase chain reaction and immunohistochemical staining were used to detect OPN expression in gastric cancer tissues. Plasma levels of OPN were measured by enzyme-linked immunosorbent assay. Plasma OPN levels were compared with gastric cancer development, clinicopathological features and outcomes. RESULTS: Expression of OPN mRNA was significantly higher in gastric cancer tissues compared with non-tumour tissues. Most OPN immunoactivity was localised to cancer cells. The median plasma OPN level was significantly higher in patients than in controls ($p<0.0001$), and significantly higher in patients with advanced stages, serosal invasion, lymph node metastasis, lymphatic invasion, venous invasion and liver metastasis. Logistic regression showed that high plasma OPN level (greater than 67.3 ng/ml) is significantly associated with advanced stages, serosal invasion, lymph node metastasis, lymphatic invasion, venous invasion and liver metastasis. Plasma OPN level demonstrated significant association with patient survival ($p<0.0001$), especially in the subgroups with invasive phenotypes. On Cox multivariate analysis, elevated plasma OPN level was an independent risk factor for poor survival ($p<0.0001$). CONCLUSIONS: Elevated plasma OPN level is significantly associated with gastric cancer development, invasive phenotypes and survival. Plasma OPN level may have potential usefulness as a diagnostic and prognostic factor for gastric cancer.

Wu, D., Y. Tian, et al. (2009). "Genetic variants in the Runt-related transcription factor 3 gene contribute to gastric cancer risk in a Chinese population." Cancer Sci **100**(9): 1688-94.

Runt-related transcription factor 3 (RUNX3) is a well known gene for its functions in gastric cancer suppression, but the effect of its genetic variations on the risk of gastric cancer remains unclear. In this study, ten tagging single nucleotide polymorphisms (tSNPs) of the RUNX3 gene were selected and genotyped in a hospital-based case-control study of 312 gastric cancer patients and 329 cancer-free controls in a Chinese population. In the single-locus analysis, three RUNX3 intronic tSNPs associated with significantly increased risk of gastric cancer were

observed: the SNP3 rs11249206 CC genotype (adjusted odds ratio [OR] = 1.75, 95% confidence interval [CI] = 1.03-2.99), compared with the TT genotype; the SNP7 rs760805 AA genotype (adjusted OR = 1.82, 95% CI = 1.14-2.92), compared with the TT genotype; and the SNP8 rs2236852 GG genotype (adjusted OR = 1.69, 95% CI = 1.05-2.72), compared with the AA genotype. In the combined analyses of these three tSNPs, we found that the combined genotypes with four to six variant (risk) alleles (i.e. SNP3 C, SNP7 A, and SNP8 G alleles) were associated with an increased risk of gastric cancer compared with those with one to three variant (risk) alleles (adjusted OR = 2.00, 95% CI = 1.41-2.85), and this increased risk was more pronounced among subgroups of age > or =65 years, never smokers, and never drinkers. However, no significant association was observed in the clinicopathological features analyses. In conclusion, the RUNX3 genetic variants may modulate the risk of gastric cancer in a Chinese population. Further larger and functional studies are warranted to validate the findings.

Wu, K., Y. Nie, et al. (2009). "Molecular basis of therapeutic approaches to gastric cancer." J Gastroenterol Hepatol **24**(1): 37-41.

Gastric cancer is the top lethal cancer in Asia. As the majority of cases present with advanced disease, conventional therapies (surgery, chemotherapy, and radiotherapy) have limited efficacy to reduce mortality. Emerging modalities provide promise to combat this malignancy. Target-protein-based cancer therapy has become available in clinical practice. Numerous molecules have been shown potential to target specific pathways for tumor cell growth. Cyclooxygenase-2 (COX-2) is overexpressed in and correlated with gastric cancer, and knockdown of COX-2 or administration of COX-2 inhibitors suppresses tumor formation in models of gastric cancer. Induction of apoptosis, reduction of angiogenesis, and blocking of potassium ion channels may present new mechanisms of COX-2 inhibition. Runt-related transcription factor 3 (RUNX3) is a candidate tumor suppressor gene whose deficiency is causally related to gastric cancer. RUNX3 is downregulated in metastatic gastric cancer. RUNX3 activation inhibits angiogenesis in xenograft tumors in nude mice. Tumor microenvironment modulation also provides a powerful tool to inhibit cancer development and progress; details of the potential roles of angiopoietins are discussed in this review. Osteopontin is a secreted protein involved in stress response, inflammation, wound healing, and immune response. Inhibition of osteopontin by RNA interfering technique suppressed tumorigenesis as well as angiogenesis in gastric cancer. Immunotherapy remains another important choice of adjuvant therapy for cancer. A tumor-specific antigen MG7-Ag has been identified with great potential for inducing immune response in gastric cancer. Using HLA-A-matched allogeneic gastric cancer cells to induce tumor-specific cytotoxic T lymphocytes appeared to be an alternative option of immunotherapy for gastric cancer.

Wu, W. K., T. T. Tse, et al. (2009). "Expression of ErbB receptors and their cognate ligands in gastric and colon cancer cell lines." Anticancer Res **29**(1): 229-34.

BACKGROUND: ErbB receptors and their cognate ligands are implicated in cancer progression. Their expression in gastrointestinal cancer, however, has not been systemically studied. MATERIALS AND METHODS: The expression of four ErbB receptors and a panel of ErbB ligands were determined by reverse transcription-PCR in two gastric (TMK1, MKN-45) and two colon (SW1116, HT-29) cancer cell lines. Cell proliferation was measured by MTT assay while gene knockdown was achieved by RNA interference. RESULTS: ErbB1, ErbB2 and ErbB3 receptors and five known or putative ErbB ligands, namely, epiregulin, epidermal growth factor (EGF), heparin-binding EGF, transforming growth factor alpha (TGFalpha) and neuroglycan-C were expressed in all four cell lines. Knockdown of neuroglycan-C, however, did not affect cell proliferation. CONCLUSION: This study profiles the expression of ErbB receptors and their cognate ligands in gastric and colon cancer cells. These findings might lay the basis for the development of ErbB pathway-directed therapeutics for gastrointestinal cancer.

Wu, Z. Q., R. Zhang, et al. (2007). "Histone deacetylase inhibitor trichostatin A induced caspase-independent apoptosis in human gastric cancer cell." Chin Med J (Engl) **120**(23): 2112-8.

BACKGROUND: Histone deacetylase inhibitors (HDACIs) have been reported to induce apoptosis in cancer cells. The effects of trichostatin A (TSA) on gastric cancer cells have not been well characterized. This study was aimed to explore the effects and mechanisms of TSA on human gastric cancer SGC-7901 cells. METHODS: The cells were treated with TSA and analyzed by cell proliferation assay, Western blot, TUNEL assay, flow cytometry by fluorescein isothiocyanate (FITC) conjugated with Annexin V and PI staining, immunofluorescence analysis, analysis of subcellular fractionation, gene chips and real time polymerase chain reaction (PCR). RESULTS: TSA could inhibit cell growth and induced apoptosis in gastric cancer SGC-7901 cells

through the regulation of apoptosis-related genes, such as Bcl-2, Bax and survivin. Further study indicated that the pan-caspase inhibitor z-VAD-fmk did not inhibit the apoptosis induced by TSA, and we did not observe the cleavage of poly ADP ribose polymerase (PARP) after TSA treatment too. In addition, apoptosis inducing factor (AIF) and EndoG were found to translocate from mitochondria to nucleus in the immunofluorescence assay and the Western analysis of subcellular fractionation confirmed the result of immunofluorescence assay. CONCLUSIONS: The apoptosis induced by TSA in gastric cancer SGC-7901 cells involves a caspase-independent pathway.

Xiao, Q., L. Li, et al. (2007). "Transcription factor E2F-1 is upregulated in human gastric cancer tissues and its overexpression suppresses gastric tumor cell proliferation." Cell Oncol **29**(4): 335-49.

The E2F family members play a critical role in cell cycle regulation and other biological processes in the cell. To better understand the involvement of E2F-1 in the development and progression of gastric tumors, we investigated the mutation and expression of E2F-1 in human gastric cancer tissues and the effect of E2F-1 overexpression on the proliferation of gastric carcinoma cells. In this study, 80 pairs of gastric cancer specimens and paratumor tissues from different patients and 40 stomach mucosa specimens from healthy individuals were examined. PCR-SSCP analysis demonstrated that mutations were not detected in any of the gastric cancer and normal tissue specimens. In addition, the results of an immunohistochemistry assay revealed higher expression rates of E2F-1 ($P<0.01$) in gastric cancer tissues (72.5%) than in paratumor tissues (30.0%) of the same individuals and stomach mucosa from healthy individuals (22.5%). However, no correlation was observed between the E2F-1 levels and patients' clinical features, such as sex, age, histological types, lymph node metastasis, and clinical stages ($P>0.05$). Finally, the influence of E2F-1 overexpression on the growth of human gastric carcinoma MKN-45 cells in vitro was assessed by measuring colony formation, cell survival, and cell cycle progression. Our data clearly showed that cell growth and proliferation were significantly inhibited in MKN-45 tumor cells transfected with the expression vector encoding E2F-1 in comparison with nontransfected cells or cells transfected with empty vector. These findings suggest that E2F-1, a stable and conservative gene during the oncogenesis and progression of stomach cancers, may potentially serve as a biomarker for clinical diagnosis of gastric carcinomas and as a target for the development of novel therapeutic interventions to treat this disease.

Xie, Y., Y. Yin, et al. (2009). "Short interfering RNA directed against the E2F-1 gene suppressing gastric cancer progression in vitro." Oncol Rep **21**(5): 1345-53.

Gastric cancer is the third most common cancer in China. The sustained overexpression of E2F-1 is a characteristic feature of gastric cancer. RNA interference (RNAi), which has been proven to be a powerful tool for suppressing gene expression, may provide a promising way forward in gastric cancer therapy. In this study, we constructed the recombinant Psilencer 4.1- E2F-1 siRNA plasmids and transfected them into gastric cancer MGC-803 cells in vitro. Our data demonstrated that E2F-1 siRNA led to inhibition of endogenous E2F-1 mRNA and protein expression as determined by real-time quantitative RT-PCR and Western blotting. Furthermore, simultaneous silencing of E2F-1 resulted in a reduction of tumor cell proliferation activity and a higher percentage of apoptotic cells. The inhibition of migration and invasion potential of tumor cells was investigated in vitro. In summary, siRNA targeting of E2F-1 can effectively inhibit gastric cancer progression and may be used as a potent therapy.

Xing, C. G., B. S. Zhu, et al. (2009). "Effects of LY294002 on the invasiveness of human gastric cancer in vivo in nude mice." World J Gastroenterol **15**(40): 5044-52.

AIM: To investigate the effects of class I phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002 on the invasiveness and related mechanisms of implanted tumors of SGC7901 human gastric carcinoma cells in nude mice. METHODS: Nude mice were randomly divided into model control groups and LY294002 treatment groups. On days 5, 10 and 15 after treatment, the inhibitory rate of tumor growth, pathological changes in tumor specimens, expression levels of matrix metalloproteinase (MMP)-2, MMP-9, CD34 [representing microvessel density (MVD)] and vascular endothelial growth factor (VEGF), as well as apoptosis indexes in tumor samples were observed. RESULTS: In this study, we showed that treating the tumors with LY294002 could significantly inhibit carcinoma growth by 11.3%, 29.4% and 36.7%, after 5, 10 and 15 d, respectively, compared to the control group. Hematoxylin & eosin staining indicated that the rate of inhibition increased progressively (23.51% +/- 3.11%, 43.20% +/- 3.27% and 63.28% +/- 2.10% at 5, 10 and 15 d, respectively) along with apoptosis. The expression of MMP-2 was also downregulated (from 71.4% +/- 1.6% to 47.9% +/- 0.7%, 31.9% +/- 0.9% and 7.9% +/- 0.7%). The same effects were observed in MMP-9 protein expression (from 49.4% +/- 1.5% to 36.9% +/- 0.4%,

23.5% +/- 0.9% and 7.7% +/- 0.6%), the mean MVD (from 51.2% +/- 3.1% to 41.9% +/- 1.5%, 30.9% +/- 1.7% and 14.9% +/- 0.8%), and the expression of VEGF (from 47.2% +/- 3.1% to 25.9% +/- 0.5%, 18.6% +/- 1.2% and 5.1% +/- 0.9%) by immunohistochemical staining. CONCLUSION: The class I PI3K inhibitor LY294002 could inhibit the invasiveness of gastric cancer cells by downregulating the expression of MMP-2, MMP-9, and VEGF, and reducing MVD.

Xu, Q., Y. Yuan, et al. (2009). "Risk of gastric cancer is associated with the MUC1 568 A/G polymorphism." Int J Oncol **35**(6): 1313-20.

Identifying the genetic variants that alter MUC1 protein expression may further our understanding of the risk for development of gastric cancer (GC). We used PCR-SSPs to identify the genotype of MUC1 A/G polymorphism at its 568 site of exon 2 and immunohistochemistry to detect MUC1 protein expression in GC patients and non-cancer subjects and analyzed the association between this polymorphism and MUC1 protein expression. We found that the frequency of AA genotype was significantly high in the GC patients and the risk for GC in AA genotype carriers increased 1.81-fold. Moreover, we found a significant underexpression of MUC1 protein in GC as compared to non-cancer subjects, which was negatively correlated to AA genotype of MUC1 (r=-0.1790, P=0.004). Furthermore, this study provides a possible mechanistic insight that the MUC1 A/G polymorphism at its 568 site disrupts the physiological functions of MUC1 which is important to the physiological protection of gastric mucosa. Thus we have provided evidence that may identify the MUC1 A/G polymorphism at 568 site, as a potential genetic factor which leads to an increase in susceptibility for GC through alteration of MUC1 gene and MUC1 expression in the population that carry the A allele.

Xu, W. H., Y. L. Ge, et al. (2007). "Inhibitory effect of vascular endothelial growth factors-targeted small interfering RNA on proliferation of gastric cancer cells." World J Gastroenterol **13**(14): 2044-7.

AIM: To examine the effects of vascular endothelial growth factor (VEGF)-targeted small interfering RNA (siRNA) on proliferation of gastric cancer cells in vitro. METHODS: Several siRNAs were transfected into human gastric cancer cell line SGC-7901 with Lipofectamine 2000. Cells not transfected with Lipofectamine 2000 or scrambled (SCR) siRNA served as controls. The inhibitory effect of siRNA on the expression of VEGF mRNA and protein was detected by RT-PCR and ELISA. MTT assay was used to examine the inhibition rate of cell growth. The change in cell cycling of siRNA-treated cells was detected by flow cytometry. RESULTS: siRNA targeting human VEGF effectively inhibited the proliferation of gastric cancer cell line SGC-7901 and the distribution of cell cycle. The percentage of G(0)/G(1) phase was significantly higher in siRNA(1)- and siRNA(2)-transfected cells than in control cells. The expression of VEGF mRNA was significantly inhibited in siRNA(1)- and siRNA(2)-transfected cells compared with that in control cells. VEGF protein notably decreased in siRNA-transfected cells, but had no effect on SCR siRNA. CONCLUSION: VEGF siRNA inhibits proliferation of gastric cancer cells in vitro.

Xu, Y., X. Qu, et al. (2009). "Midkine positively regulates the proliferation of human gastric cancer cells." Cancer Lett **279**(2): 137-44.

Midkine (MDK), a heparin-binding growth factor, modulates the proliferation and migration of various cells, is often highly expressed in many malignant tumors, and may act as an oncoprotein. We found that MDK is overexpressed in clinical human gastric cancer tissues relative to its expression in adjacent noncancerous tissues. To further investigate the biological activities of MDK in gastric cancer, we introduced the MDK gene into human SGC7901 gastric cancer cells, where it contributed to the proliferation of SGC7901 cells in vitro and in vivo. Conversely, the knockdown of MDK expression by siRNA resulted in significantly reduced proliferation of BGC823 cells. Our study also shows that MDK activates both the Akt and ERK1/2 pathways and upregulates the expression of several cell-cycle-related proteins, including cyclin A, cyclin D1, Cdk2, Cdk4, and Cdk6, which in part explains the contribution of MDK to gastric cancer cell survival and growth. These results demonstrate that MDK contributes to gastric cancer cell proliferation and suggest that it plays an important role in the development of human gastric cancer.

Yamada, H., K. Shinmura, et al. (2009). "Absence of germline mono-allelic promoter hypermethylation of the CDH1 gene in gastric cancer patients." Mol Cancer **8**: 63.

BACKGROUND: Germline mono-allelic promoter hypermethylation of the MLH1 or MSH2 gene in families with hereditary nonpolyposis colorectal cancer has recently been reported. The purpose of this study was to evaluate if germline promoter hypermethylation of the tumor suppressor gene CDH1 (E-cadherin) might cause predisposition to gastric cancer. METHODS: We prepared two groups of samples, a group of blood samples from 22 patients with familial gastric cancer or early-onset

gastric cancer selected from among 39 patients, and a group of non-cancerous gastric tissue samples from 18 patients with sporadic gastric cancer showing loss of CDH1 expression selected from among 159 patients. We then investigated the allele-specific methylation status of the CDH1 promoter by bisulfite sequencing of multiple clones. RESULTS: Although there was a difference between the methylation level of the two alleles in some samples, there was no mono-allelic promoter hypermethylation in any of the samples. CONCLUSION: These results suggest that germline mono-allelic hypermethylation of the CDH1 promoter is not a major predisposing factor for gastric cancer.

Yamamoto, H., Y. Kitadai, et al. (2009). "Laminin gamma2 mediates Wnt5a-induced invasion of gastric cancer cells." Gastroenterology **137**(1): 242-52, 252 e1-6.

BACKGROUND & AIMS: Wnt5a expression stimulates in vitro migration and invasion of cultured gastric cancer cells by an unknown mechanism and is also correlated with aggressiveness of gastric tumors. The aim of this study was to show that Wnt5a is involved in metastasis of gastric cancer cells in vivo and to explore the molecular mechanism by which Wnt5a regulates migration and invasion. METHODS: In an experimental liver metastasis assay, Wnt5a-knockdown gastric cancer cells were injected into the spleens of nude mice. Microarray analyses were used to compare expression patterns between mouse fibroblast L cells that stably express wild-type and a mutant form of Wnt5a to investigate Wnt5a-dependent gene expression. The expression of genes found to be regulated by Wnt5a was investigated in cultured gastric cancer cells. Immunohistochemical analyses were performed to measure levels of Wnt-regulated gene products in 153 gastric cancer samples. RESULTS: Knockdown of Wnt5a in gastric cancer cells reduced the number of liver metastases that formed in nude mice. Microarray analyses indicated that Wnt5a activity induced expression of the gene encoding laminin gamma2, a subunit of the epithelial basement membrane protein laminin-5. Wnt5a induced the expression of laminin gamma2 through the activation of protein kinase C and c-Jun-N-terminal kinase. The invasive activity of gastric cancer cells depended on laminin gamma2; Wnt5a expression levels correlated with those of laminin gamma2 in diffuse-scattered type gastric tumor samples from patients. CONCLUSIONS: Wnt5a contributes to gastric cancer progression by increasing metastatic potential. Wnt5a up-regulates laminin gamma2 to mediate gastric cancer cell aggressiveness.

Yamamura, Y., W. L. Lee, et al. (2008). "Role of TAp73alpha in induction of apoptosis by transforming growth factor-beta in gastric cancer cells." FEBS Lett **582**(17): 2663-7.

Transforming growth factor-beta (TGF-beta) is implicated as a tumor suppressor because it eliminates cancer cells from normal tissues by inhibiting cell growth and inducing apoptosis. Although p53 tumor suppressor is required for TGF-beta-induced p21 WAF1 expression and cell growth inhibition, its role in TGF-beta-induced apoptosis remains unclear. Here, we report that TAp73alpha, which is a member of the p53 family, binds to p53-binding sites in the promoters of proapoptotic Bax and Puma to activate their transcription, and mediates TGF-beta-induced apoptosis in gastric cancer cells. Our findings reveal a novel role of TAp73alpha in the induction of apoptosis by TGF-beta in cancer cells.

Yang, J. J., K. P. Ko, et al. (2009). "The role of TNF genetic variants and the interaction with cigarette smoking for gastric cancer risk: a nested case-control study." BMC Cancer **9**: 238.

BACKGROUND: The aim of this study was to investigate the role of TNF genetic variants and the combined effect between TNF gene and cigarette smoking in the development of gastric cancer in the Korean population. METHODS: We selected 84 incident gastric cancer cases and 336 matched controls nested within the Korean Multi-Center Cancer Cohort. Six SNPs on the TNF gene, TNF-alpha-238 G/A, -308 G/A, -857 C/T, -863 C/A, -1031 T/C, and TNF-beta 252 A/G were genotyped. The ORs (95% CIs) were calculated using unconditional logistic regression model to detect each SNP and haplotype-pair effects for gastric cancer. The combined effects between the TNF gene and smoking on gastric cancer risk were also evaluated. Multi dimensionality reduction (MDR) analyses were performed to explore the potential TNF gene-gene interactions. RESULTS: TNF-alpha-857 C/T containing the T allele was significantly associated with an increased risk of gastric cancer and a linear trend effect was observed in the additive model (OR = 1.6, 95% CI 1.0-2.5 for CT genotype; OR = 2.6, 95% CI 1.0-6.4 for TT genotype). All haplotype-pairs that contained TCT or CCC of TNF-alpha-1031 T/C, TNF-alpha-863 C/A, and TNF-alpha-857 C/T were associated with a significantly higher risk for gastric cancer only among smokers. In the MDR analysis, regardless of smoking status, TNF-alpha-857 C/T was included in the first list of SNPs with a significant main effect. CONCLUSION: TNF-alpha-857 C/T polymorphism may play an independent role in gastric carcinogenesis and the risk for gastric cancer by TNF genetic effect is pronounced by cigarette smoking.

Yang, L., H. J. Gu, et al. (2008). "Tissue inhibitor of metalloproteinase-2 G-418C polymorphism is associated with an increased risk of gastric cancer in a Chinese population." Eur J Surg Oncol **34**(6): 636-41.

AIMS: To examine the effect of the TIMP-2 G-418C polymorphism on gastric cancer risk. METHODS: We conducted a hospital-based, case-control study using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method in 412 individuals (206 gastric cancer patients and 206 age, sex matched cancer-free controls). RESULTS: The genotype and allele frequencies were significantly different (P = 0.007 and 0.005, respectively) between cases and controls. Further analysis showed that the variant TIMP-2 genotypes (CC+GC) had a 51% increased risk of gastric cancer compared with GG [adjusted odds ratio (OR) 1.51, 95% confidence interval (CI) 1.00-2.26, P = 0.049]. The elevated gastric cancer risk was especially evident in younger individuals (age < 58 years old) (adjusted OR 2.21, 95% CI 1.18-4.16) and smokers (adjusted OR 2.61, 95% CI 1.01-6.72). However, no significant association was observed between the variant genotypes and clinicopathological features of gastric cancer. CONCLUSIONS: These findings suggest that the TIMP-2 G-418C polymorphism is a genetic predisposing factor for gastric cancer.

Yang, S., H. C. Jeung, et al. (2007). "Identification of genes with correlated patterns of variations in DNA copy number and gene expression level in gastric cancer." Genomics **89**(4): 451-9.

To identify DNA copy number changes that had a direct influence on mRNA expression in gastric cancer, cDNA microarray-based comparative genomic hybridization (aCGH) and gene expression profiling were performed using 17 K cDNA microarrays. A set of 158 genes showing Pearson correlation coefficients over 0.6 between DNA copy number changes and mRNA expression level variations was selected. In an independent gene expression profiling of 60 tissue samples, the 158 genes were able to distinguish most of the normal and tumor tissues in an unsupervised hierarchical clustering, suggesting that the differential expression patterns displayed by this specific group of genes are most likely based on the gene copy number changes. Furthermore, 43 statistically significant (P<0.01) genes were selected that correctly distinguished all of the tissue samples. The copy number changes detected by aCGH can be verified by fluorescence in situ hybridization and real-time polymerase chain reaction. The selected genes include those that were previously identified as being tumor suppressors or deleted in various tumors, including GATA binding protein 4 (GATA4), monoamine oxidase A (MAOA), cyclin C (CCNC), and oncogenes including malignant fibrous histiocytoma amplified sequence 1 (MFHAS1/MASL1), high mobility group AT-hook 2 (HMGA2), PPAR binding protein (PPARBP), growth factor receptor-bound protein 7 (GRB7), and TBC1 (tre-2, BUB2, cdc16) domain family, member 1 (TBC1D1).

Yang, Z., X. Zhang, et al. (2007). "Up-regulation of gastric cancer cell invasion by Twist is accompanied by N-cadherin and fibronectin expression." Biochem Biophys Res Commun **358**(3): 925-30.

Twist, a newly found EMT-inducer, has been reported to be up-regulated in those of diffuse-type gastric carcinomas with high N-cadherin level. We show here MKN45, a cell line derived from undifferentiated carcinomas cells, expresses high levels of Twist. Down-regulation of Twist, using an antisense Twist vector in MKN45 cells, inhibits cell migration and invasion, companied with a morphologic changes associated with MET. Suppression of Twist also decreases the expressions of N-cadherin and fibronectin, but not of E-cadherin in MKN45. In contrast, overexpression of Twist in MKN28, a cell line derived from moderate differentiated carcinomas, results in up-regulation of N-cadherin and fibronectin, companied with down-regulation of E-cadherin. Taken together, our results suggest that Twist regulates cell motility and invasion in gastric cancer cell lines, probably through the N-cadherin and fibronectin production.

Yano, K., T. Imaeda, et al. (2008). "Transcriptional activation of the human claudin-18 gene promoter through two AP-1 motifs in PMA-stimulated MKN45 gastric cancer cells." Am J Physiol Gastrointest Liver Physiol **294**(1): G336-43.

Claudin-18 (CLDN18), a member of the claudin family of proteins that are structural components of tight junctions, has two alternatively spliced variants, claudin-18a1 and claudin-18a2, which are highly expressed in lung and stomach, respectively. Downregulation of claudin-18a2 is associated with gastric cancers of an intestinal phenotype; however, the mechanisms regulating its expression have not been defined. Here, we found that phorbol 12-myristate 13-acetate (PMA) treatment of MKN45 human gastric cancer cell line increased claudin-18a2 expression. In addition, this study aimed to characterize the human CLDN18a2 promoter. Using reporter gene assays and deletion analysis, we mapped the critical promoter region of the PMA-stimulated claudin-18a2 expression to the -923/-286 region. Electrophoretic mobility shift assays and mutational analyses revealed that two activator protein

(AP)-1 binding sites played an important role in the expression of claudin-18a2 in PMA-stimulated MKN45 cells. Protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) inhibitors suppressed the upregulation of claudin-18a2. These results indicate that the PKC/MAPK/AP-1 dependent pathway regulates claudin-18a2 expression in gastric cells.

Yasui, W., N. Oue, et al. (2009). "Transcriptome dissection of gastric cancer: identification of novel diagnostic and therapeutic targets from pathology specimens." Pathol Int **59**(3): 121-36.

Gastric cancer is the fourth most common malignancy in the world, and mortality due to gastric cancer is second only to that from lung cancer. 'Transcriptome dissection' is a detailed analysis of the entire expressed transcripts from a cancer, for the purpose of understanding the precise molecular mechanism of pathogenesis. Serial analysis of gene expression (SAGE) is a suitable technique for performing transcriptome dissection. Gastric cancers of different stages and histology were analyzed on SAGE, and one of the largest gastric cancer SAGE libraries in the world was created (GEO accession number GSE 545). Through SAGE, many candidate genes have been identified as potential diagnostic and therapeutic targets for the treatment of gastric cancer. Regenerating islet-derived family, member 4 (Reg IV) participated in 5-fluorouracil (5-FU) resistance and peritoneal metastasis, and its expression was associated with an intestinal phenotype of gastric cancer and with endocrine differentiation. GW112 expression correlated with advanced tumor stage. Measurement of Reg IV and GW112 levels in sera indicated a sensitivity of 57% for detection of cancer. SPC18 participated in tumor growth and invasion through transforming tumor growth factor-alpha upregulation. Palate, lung, and nasal epithelium carcinoma-associated protein (PLUNC) was a useful marker for gastric hepatoid adenocarcinoma. Expression of SOX9, HOXA10, CDH17, and loss of claudin-18 expression were associated with an intestinal phenotype of gastric cancer. Information obtained from transcriptome dissection greatly contributes to diagnosis and treatment of gastric cancer.

Zhang, J., C. Dou, et al. (2008). "Polymorphisms of tumor necrosis factor-alpha are associated with increased susceptibility to gastric cancer: a meta-analysis." J Hum Genet **53**(6): 479-89.

We conducted a meta-analysis to assess the association between tumor necrosis factor-alpha (TNF-alpha) gene TNFA-308 (G > A) and TNFA-857 (C > T) polymorphisms and gastric cancer (GC) susceptibility. We also performed subgroup analyses based on ethnicity (Caucasian, east Asian, and other populations) and tumor location [noncardia gastric cancer (NCGC)]. There were 3,335 GC patients and 5,286 controls for TNFA-308, and 1,118 GC patients and 1,591 controls for TNFA-857 in our analysis. Overall, allele contrast (A vs. G) of TNFA-308 polymorphism produced significant results in worldwide populations [Pheterogeneity = 0.05, random-effects (RE) odds ratio (OR) 1.19; 95% confidence interval (CI) 1.03-1.37, P = 0.02] and Caucasian populations (Pheterogeneity = 0.15, fixed-effects (FE), OR 1.27; 95% CI 1.11-1.45, P = 0.0005). Similar results were also obtained in recessive models and homozygote contrasts. No significant association was observed in NCGC and east Asian subgroup analysis. T variant of TNFA-857 produced significant results only in allele contrast (Pheterogeneity = 0.38, FE OR 1.17; 95% CI 1.01-1.35, P = 0.04). In conclusion, TNFA-308 locus of TNF-alpha would be a risk factor for GC, especially in Caucasian populations. Besides, TNFA-857 locus may be related to GC risk, which demonstrated changeability of results in different contrasts.

Zhang, Y. J. and J. Y. Fang (2008). "Molecular staging of gastric cancer." J Gastroenterol Hepatol **23**(6): 856-60.

Gastric cancer has traditionally been staged using purely histological methods, but these methods provide little information about the biology of gastric cancer and have limited predictive power. Recent studies have shown that clinically relevant gastric cancer subtypes have distinct gene expression profiles. This approach, termed molecular staging, can lead to the discovery of novel diagnostic and prognostic biomarkers of gastric cancers. This update reviews advances in molecular staging of gastric cancer and discusses their implications for the prognosis and diagnosis of this complex disease. Technologies used in molecular staging as well as future directions for the optimization of molecular staging of gastric cancer are also discussed.

Zhang, Z., Z. Li, et al. (2008). "miR-21 plays a pivotal role in gastric cancer pathogenesis and progression." Lab Invest **88**(12): 1358-66.

Gastric cancer causes nearly one million deaths worldwide per year. Although Helicobacter pylori infection is the main risk factor, in about 80% or more of gastric cancers, the molecular pathway underlying H. pylori infection leading to the development of gastric cancers remains unclear. Recently accumulating evidence suggests that microRNAs (miRNAs) may regulate diverse biological processes and may be important in

tumorigenesis. miR-21 has been frequently observed to be aberrantly overexpressed in various tumors. Using TaqMan quantitative real-time PCR, we confirmed that miR-21 was significantly overexpressed in human gastric cancer tissues and cell lines. Remarkably, miR-21 was also significantly overexpressed in H. pylori-infected gastric mucosa, implying that overexpression of miR-21 in gastric cancer may be due in part to H. pylori infection. More importantly, we showed that forced expression of miR-21 significantly enhanced cell proliferation and invasion in AGS cells, a human gastric cancer cell line, whereas knockdown of miR-21 by inhibitor caused a significant reduction in cell proliferation and a significant increase in apoptosis. Furthermore, we demonstrated that knockdown of miR-21 significantly decreased cell invasion and migration of AGS cells. Finally, we showed that RECK, a known tumor suppressor in gastric cancer, is a bona fide target of miR-21. Taken together, miR-21 may be important in the initiation and progression of gastric cancers as an oncomiR, likely through regulating RECK. Our findings suggest a potential regulatory pathway in which H. pylori infection upregulates expression of miR-21, which in turn downregulates RECK, and then leads to the development of gastric cancer.

Zhu, B. H., W. H. Zhan, et al. (2007). "(-)-Epigallocatechin-3-gallate inhibits growth of gastric cancer by reducing VEGF production and angiogenesis." World J Gastroenterol **13**(8): 1162-9.

AIM: To investigate the effect of (-)-epigallocatechin-3-gallate (EGCG) on growth of gastric cancer and its possible mechanism. METHODS: Heterotopic tumors were induced by subcutaneously injection of SGC-7901 cells in nude mice. Tumor growth was measured by calipers in two dimensions. Tumor angiogenesis was determined with tumor microvessel density (MVD) by immunohistology. Vascular endothelial growth factor (VEGF) protein level and activation of signal transducer and activator of transcription 3 (Stat3) were examined by Western blotting. VEGF mRNA expression was determined by RT-PCR and VEGF release in tumor culture medium by ELISA. VEGF-induced cell proliferation was studied by MTT assay, cell migration by gelatin modified Boyden chamber (Transwell) and in vitro angiogenesis by endothelial tube formation in Matrigel. RESULTS: Intraperitoneal injection of EGCG inhibited the growth of gastric cancer by 60.4%. MVD in tumor tissues treated with EGCG was markedly reduced. EGCG treatment reduced VEGF protein level in vitro and in vivo. Secretion and mRNA expression of VEGF in tumor cells were also suppressed by EGCG in a dose-dependent manner. This inhibitory effect was associated with reduced activation of Stat3, but EGCG treatment did not change the total Stat3 expression. EGCG also inhibited VEGF-induced endothelial cell proliferation, migration and tube formation. CONCLUSION: EGCG inhibits the growth of gastric cancer by reducing VEGF production and angiogenesis, and is a promising candidate for anti-angiogenic treatment of gastric cancer.

References

1. Al-Moundhri, M. S., M. Al-Nabhani, et al. (2009). "Gastric cancer risk predisposition and prognostic significance of vascular endothelial growth factor (VEGF) gene polymorphisms--a case-control study in an Omani population." Mol Carcinog **48**(12): 1170-6.
2. Alvarez, C. J., M. Lodeiro, et al. (2009). "Obestatin stimulates Akt signalling in gastric cancer cells through beta-arrestin-mediated epidermal growth factor receptor transactivation." Endocr Relat Cancer **16**(2): 599-611.
3. Ando, T., T. Ishikawa, et al. (2009). "Synergistic effect of HLA class II loci and cytokine gene polymorphisms on the risk of gastric cancer in Japanese patients with Helicobacter pylori infection." Int J Cancer **125**(11): 2595-602.
4. Argent, R. H., R. J. Thomas, et al. (2008). "Toxigenic Helicobacter pylori infection precedes gastric hypochlorhydria in cancer relatives, and H. pylori virulence evolves in these families." Clin Cancer Res **14**(7): 2227-35.
5. Chen, C. N., C. C. Chang, et al. (2009). "Identification of calreticulin as a prognosis marker and angiogenic regulator in human gastric cancer." Ann Surg Oncol **16**(2): 524-33.
6. Chen, W., L. Wang, et al. (2008). "The role of IGFBP3 functional polymorphisms in the risk of gastric cancer in a high-risk Chinese population." Eur J Cancer Prev **17**(2): 82-7.
7. Chen, Z., J. Q. Fan, et al. (2009). "Promoter hypermethylation correlates with the Hsulf-1 silencing in human breast and gastric cancer." Int J Cancer **124**(3): 739-44.
8. Dar, A. A., A. Belkhiri, et al. (2009). "The aurora kinase A regulates GSK-3beta in gastric cancer cells." Oncogene **28**(6): 866-75.
9. De Feo, E., R. Persiani, et al. (2009). "A case-control study on the effect of p53 and p73 gene polymorphisms on gastric cancer risk and progression." Mutat Res **675**(1-2): 60-5.
10. Falchetti, M., C. Saieva, et al. (2008). "Gastric cancer with high-level microsatellite instability: target gene mutations, clinicopathologic features, and long-term survival." Hum Pathol **39**(6): 925-32.
11. Feng, M. Y., K. Wang, et al. (2009). "Gene expression profiling in TWIST-depleted gastric cancer cells." Anat Rec (Hoboken) **292**(2): 262-70.
12. Feng, M. Y., K. Wang, et al. (2009). "Metastasis-induction and apoptosis-protection by TWIST in gastric cancer cells." Clin Exp Metastasis **26**(8): 1013-23.
13. Forte, G. I., C. Cala, et al. (2008). "Role of environmental and genetic factor interaction in age-related disease development: the gastric cancer paradigm." Rejuvenation Res **11**(2): 509-12.
14. Fu, H., Z. Hu, et al. (2009). "TGF-beta promotes invasion and metastasis of gastric cancer cells by increasing fascin1 expression via ERK and JNK signal pathways." Acta Biochim Biophys Sin (Shanghai) **41**(8): 648-56.
15. Gorouhi, F., F. Islami, et al. (2008). "Tumour-necrosis factor-A polymorphisms and gastric cancer risk: a meta-analysis." Br J Cancer **98**(8): 1443-51.

16. Gravalos, C. and A. Jimeno (2008). "HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target." Ann Oncol **19**(9): 1523-9.
17. Guan, X., H. Zhao, et al. (2009). "Polymorphisms of TGFB1 and VEGF genes and survival of patients with gastric cancer." J Exp Clin Cancer Res **28**: 94.
18. Guan, X., H. Zhao, et al. (2009). "The VEGF -634G>C promoter polymorphism is associated with risk of gastric cancer." BMC Gastroenterol **9**: 77.
19. Guo, J., Y. Miao, et al. (2009). "Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues." J Gastroenterol Hepatol **24**(4): 652-7.
20. Guo, X., H. Oshima, et al. (2008). "Stromal fibroblasts activated by tumor cells promote angiogenesis in mouse gastric cancer." J Biol Chem **283**(28): 19864-71.
21. Guo, X., N. Ma, et al. (2008). "Increased p38-MAPK is responsible for chemotherapy resistance in human gastric cancer cells." BMC Cancer **8**: 375.
22. Hara, M., H. Nakanishi, et al. (2008). "Interleukin-2 potentiation of cetuximab antitumor activity for epidermal growth factor receptor-overexpressing gastric cancer xenografts through antibody-dependent cellular cytotoxicity." Cancer Sci **99**(7): 1471-8.
23. Hayashi, M., M. Inokuchi, et al. (2008). "High expression of HER3 is associated with a decreased survival in gastric cancer." Clin Cancer Res **14**(23): 7843-9.
24. He, X. W., T. Liu, et al. (2008). "Calcium carbonate nanoparticle delivering vascular endothelial growth factor-C siRNA effectively inhibits lymphangiogenesis and growth of gastric cancer in vivo." Cancer Gene Ther **15**(3): 193-202.
25. Hishida, A., K. Matsuo, et al. (2009). "Associations of a PTPN11 G/A polymorphism at intron 3 with Helicobactor pylori seropositivity, gastric atrophy and gastric cancer in Japanese." BMC Gastroenterol **9**: 51.
26. Howlett, M., T. R. Menheniott, et al. (2009). "Cytokine signalling via gp130 in gastric cancer." Biochim Biophys Acta **1793**(11): 1623-33.
27. Huang, W., L. F. Yu, et al. (2008). "Angiotensin II type 1 receptor expression in human gastric cancer and induces MMP2 and MMP9 expression in MKN-28 cells." Dig Dis Sci **53**(1): 163-8.
28. Ishido, K., M. Azuma, et al. (2009). "Evaluation of prognostic factors for the response to S-1 in patients with stage II or III advanced gastric cancer who underwent gastrectomy." Pharmacogenet Genomics **19**(12): 955-64.
29. Ishigami, S., S. Natsugoe, et al. (2008). "HLA-class I expression in gastric cancer." J Surg Oncol **97**(7): 605-8.
30. Ivanauskas, A., J. Hoffmann, et al. (2008). "Distinct TPEF/HPP1 gene methylation patterns in gastric cancer indicate a field effect in gastric carcinogenesis." Dig Liver Dis **40**(12): 920-6.
31. Jones, K. R., Y. M. Joo, et al. (2009). "Polymorphism in the CagA EPIYA motif impacts development of gastric cancer." J Clin Microbiol **47**(4): 959-68.
32. Jung, S. W., M. Sugimoto, et al. (2009). "homB status of Helicobacter pylori as a novel marker to distinguish gastric cancer from duodenal ulcer." J Clin Microbiol **47**(10): 3241-5.
33. Karam, R., J. Carvalho, et al. (2008). "The NMD mRNA surveillance pathway downregulates aberrant E-cadherin transcripts in gastric cancer cells and in CDH1 mutation carriers." Oncogene **27**(30): 4255-60.
34. Katoh, M. and M. Katoh (2007). "Comparative integromics on JMJD1C gene encoding histone demethylase: conserved POU5F1 binding site elucidating mechanism of JMJD1C expression in undifferentiated ES cells and diffuse-type gastric cancer." Int J Oncol **31**(1): 219-23.
35. Katoh, Y. and M. Katoh (2007). "Conserved POU-binding site linked to SP1-binding site within FZD5 promoter: Transcriptional mechanisms of FZD5 in undifferentiated human ES cells, fetal liver/spleen, adult colon, pancreatic islet, and diffuse-type gastric cancer." Int J Oncol **30**(3): 751-5.
36. Ke, Q., J. Liang, et al. (2008). "Potentially functional polymorphisms of the vascular endothelial growth factor gene and risk of gastric cancer." Mol Carcinog **47**(8): 647-51.
37. Kim, H. Y., G. S. Park, et al. (2008). "Secretion of biologically active recombinant human granulocyte-macrophage colony-stimulating factor by transduced gastric cancer cells." Yonsei Med J **49**(2): 279-87.
38. Kim, J. G., S. K. Sohn, et al. (2007). "Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with gastric cancer." Ann Oncol **18**(6): 1030-6.
39. Kim, J. S., M. A. Kim, et al. (2009). "Biomarker analysis in stage III-IV (M0) gastric cancer patients who received curative surgery followed by adjuvant 5-fluorouracil and cisplatin chemotherapy: epidermal growth factor receptor (EGFR) associated with favourable survival." Br J Cancer **100**(5): 732-8.
40. Kim, K. E., H. Song, et al. (2009). "Expression of ADAM33 is a novel regulatory mechanism in IL-18-secreted process in gastric cancer." J Immunol **182**(6): 3548-55.
41. Kim, K. K., J. J. Lee, et al. (2008). "Macrophage inhibitory cytokine-1 activates AKT and ERK-1/2 via the transactivation of ErbB2 in human breast and gastric cancer cells." Carcinogenesis **29**(4): 704-12.
42. Kim, M. and H. C. Chung (2009). "Standardized genetic alteration score and predicted score for predicting recurrence status of gastric cancer." J Cancer Res Clin Oncol **135**(11): 1501-12.
43. Kim, M. H., J. S. Park, et al. (2008). "Lysophosphatidic acid promotes cell invasion by up-regulating the urokinase-type plasminogen activator receptor in human gastric cancer cells." J Cell Biochem **104**(3): 1102-12.
44. Kim, S., M. G. Choi, et al. (2009). "Silibinin suppresses TNF-alpha-induced MMP-9 expression in gastric cancer cells through inhibition of the MAPK pathway." Molecules **14**(11): 4300-11.
45. Kim, T. Y., I. S. Kim, et al. (2008). "Transcriptional induction of DLC-1 gene through Sp1 sites by histone deacetylase inhibitors in gastric cancer cells." Exp Mol Med **40**(6): 639-46.
46. Kim, W. H., S. H. Lee, et al. (2009). "Neuropilin2 expressed in gastric cancer endothelial cells increases the proliferation and migration of endothelial cells in response to VEGF." Exp Cell Res **315**(13): 2154-64.
47. Kitajima, Y., K. Ohtaka, et al. (2008). "Helicobacter pylori infection is an independent risk factor for Runx3 methylation in gastric cancer." Oncol Rep **19**(1): 197-202.
48. Kodama, M., Y. Kitadai, et al. (2008). "Vascular endothelial growth factor C stimulates progression of human gastric cancer via both autocrine and paracrine mechanisms." Clin Cancer Res **14**(22): 7205-14.
49. Kolev, Y., H. Uetake, et al. (2008). "Lactate dehydrogenase-5 (LDH-5) expression in human gastric cancer: association with hypoxia-inducible factor (HIF-1alpha) pathway, angiogenic factors production and poor prognosis." Ann Surg Oncol **15**(8): 2336-44.
50. Kosaka, Y., K. Mimori, et al. (2007). "Identification of the high-risk group for metastasis of gastric cancer cases by vascular endothelial growth factor receptor-1 overexpression in peripheral blood." Br J Cancer **96**(11): 1723-8.
51. Kunii, K., L. Davis, et al. (2008). "FGFR2-amplified gastric cancer cell lines require FGFR2 and Erbb3 signaling for growth and survival." Cancer Res **68**(7): 2340-8.
52. Lai, K. C., H. C. Chiang, et al. (2008). "Artificial neural network-based study can predict gastric cancer staging." Hepatogastroenterology **55**(86-87): 1859-63.
53. Lan, M., Y. Shi, et al. (2007). "KCl depolarization increases HIF-1 transcriptional activity via the calcium-independent

pathway in SGC7901 gastric cancer cells." Tumour Biol **28**(3): 173-80.
54. Lee, H. J., S. W. Kim, et al. (2009). "Chemokine receptor CXCR4 expression, function, and clinical implications in gastric cancer." Int J Oncol **34**(2): 473-80.
55. Lee, K. A., J. H. Park, et al. (2007). "Interaction of polymorphisms in the interleukin 1B-31 and general transcription factor 2A1 genes on the susceptibility to gastric cancer." Cytokine **38**(2): 96-100.
56. Lee, K. H., E. Y. Choi, et al. (2008). "Hepatocyte growth factor promotes cell survival by phosphorylation of BAD in gastric cancer cells." Oncol Res **17**(1): 23-32.
57. Lee, S. E., J. W. Lim, et al. (2009). "Activator protein-1 mediates docosahexaenoic acid-induced apoptosis of human gastric cancer cells." Ann N Y Acad Sci **1171**: 163-9.
58. Lee, S. H., J. Kim, et al. (2009). "Hypoxic silencing of tumor suppressor RUNX3 by histone modification in gastric cancer cells." Oncogene **28**(2): 184-94.
59. Li, D., J. Ding, et al. (2009). "Fibronectin promotes tyrosine phosphorylation of paxillin and cell invasiveness in the gastric cancer cell line AGS." Tumori **95**(6): 769-79.
60. Li, K., Y. Zhang, et al. (2009). "Association of the hypoxia inducible factor-1alpha gene polymorphisms with gastric cancer in Tibetans." Biochem Genet **47**(9-10): 625-34.
61. Li, Q., N. Zhang, et al. (2009). "Critical role and regulation of transcription factor FoxM1 in human gastric cancer angiogenesis and progression." Cancer Res **69**(8): 3501-9.
62. Li, T., B. W. Cao, et al. (2008). "Correlation of transforming growth factor beta-1 gene polymorphisms C-509T and T869C and the risk of gastric cancer in China." J Gastroenterol Hepatol **23**(4): 638-42.
63. Li, W., Z. Ge, et al. (2008). "CIP2A is overexpressed in gastric cancer and its depletion leads to impaired clonogenicity, senescence, or differentiation of tumor cells." Clin Cancer Res **14**(12): 3722-8.
64. Li, X., Z. C. Yue, et al. (2008). "Elevated serum level and gene polymorphisms of TGF-beta1 in gastric cancer." J Clin Lab Anal **22**(3): 164-71.
65. Li, Z. Q., W. P. Yu, et al. (2007). "Association of gastric cancer with tyrosine hydroxylase gene polymorphism in a northwestern Chinese population." Clin Exp Med **7**(3): 98-101.
66. Lin, M. T., B. R. Lin, et al. (2007). "IL-6 induces AGS gastric cancer cell invasion via activation of the c-Src/RhoA/ROCK signaling pathway." Int J Cancer **120**(12): 2600-8.
67. Lin, M. T., C. C. Chang, et al. (2007). "Elevated expression of Cyr61 enhances peritoneal dissemination of gastric cancer cells through integrin alpha2beta1." J Biol Chem **282**(47): 34594-604.
68. Lin, M. T., I. H. Kuo, et al. (2008). "Involvement of hypoxia-inducing factor-1alpha-dependent plasminogen activator inhibitor-1 up-regulation in Cyr61/CCN1-induced gastric cancer cell invasion." J Biol Chem **283**(23): 15807-15.
69. Liu, B., H. Sun, et al. (2009). "Adenovirus vector-mediated upregulation of spermidine /spermine N1-acetyltransferase impairs human gastric cancer growth in vitro and in vivo." Cancer Sci **100**(11): 2126-32.
70. Liu, L. Y., Y. C. Han, et al. (2008). "Expression of connective tissue growth factor in tumor tissues is an independent predictor of poor prognosis in patients with gastric cancer." World J Gastroenterol **14**(13): 2110-4.
71. Liu, R., Z. Li, et al. (2009). "Mechanism of cancer cell adaptation to metabolic stress: proteomics identification of a novel thyroid hormone-mediated gastric carcinogenic signaling pathway." Mol Cell Proteomics **8**(1): 70-85.
72. Liu, Y., Q. Y. Zhang, et al. (2007). "Relationship between LAPTM4B gene polymorphism and susceptibility of gastric cancer." Ann Oncol **18**(2): 311-6.
73. Lo, S. S., J. H. Chen, et al. (2009). "Functional polymorphism of NFKB1 promoter may correlate to the susceptibility of gastric cancer in aged patients." Surgery **145**(3): 280-5.
74. Ma, J., M. Chen, et al. (2008). "Pancreatic duodenal homeobox-1 (PDX1) functions as a tumor suppressor in gastric cancer." Carcinogenesis **29**(7): 1327-33.
75. Marrelli, D., C. Pedrazzani, et al. (2009). "Negative Helicobacter pylori status is associated with poor prognosis in patients with gastric cancer." Cancer **115**(10): 2071-80.
76. Matsubara, J., T. Nishina, et al. (2008). "Impacts of excision repair cross-complementing gene 1 (ERCC1), dihydropyrimidine dehydrogenase, and epidermal growth factor receptor on the outcomes of patients with advanced gastric cancer." Br J Cancer **98**(4): 832-9.
77. Matsubara, J., Y. Yamada, et al. (2008). "Clinical significance of insulin-like growth factor type 1 receptor and epidermal growth factor receptor in patients with advanced gastric cancer." Oncology **74**(1-2): 76-83.
78. Matsubara, J., Y. Yamada, et al. (2008). "Impact of insulin-like growth factor type 1 receptor, epidermal growth factor receptor, and HER2 expressions on outcomes of patients with gastric cancer." Clin Cancer Res **14**(10): 3022-9.
79. Matsumura, S., N. Oue, et al. (2007). "DNA demethylation of vascular endothelial growth factor-C is associated with gene expression and its possible involvement of lymphangiogenesis in gastric cancer." Int J Cancer **120**(8): 1689-95.
80. Matsuzaki, S., F. Tanaka, et al. (2009). "Clinicopathologic significance of KIAA1199 overexpression in human gastric cancer." Ann Surg Oncol **16**(7): 2042-51.
81. Mattioli, E., P. Vogiatzi, et al. (2007). "Immunohistochemical analysis of pRb2/p130, VEGF, EZH2, p53, p16(INK4A), p27(KIP1), p21(WAF1), Ki-67 expression patterns in gastric cancer." J Cell Physiol **210**(1): 183-91.
82. May, F. E., S. M. Griffin, et al. (2009). "The trefoil factor interacting protein TFIZ1 binds the trefoil protein TFF1 preferentially in normal gastric mucosal cells but the co-expression of these proteins is deregulated in gastric cancer." Int J Biochem Cell Biol **41**(3): 632-40.
83. Mejias-Luque, R., S. Peiro, et al. (2008). "IL-6 induces MUC4 expression through gp130/STAT3 pathway in gastric cancer cell lines." Biochim Biophys Acta **1783**(10): 1728-36.
84. Mimori, K., T. Fukagawa, et al. (2008). "Hematogenous metastasis in gastric cancer requires isolated tumor cells and expression of vascular endothelial growth factor receptor-1." Clin Cancer Res **14**(9): 2609-16.
85. Mita, H., M. Toyota, et al. (2009). "A novel method, digital genome scanning detects KRAS gene amplification in gastric cancers: involvement of overexpressed wild-type KRAS in downstream signaling and cancer cell growth." BMC Cancer **9**: 198.
86. Mitsuno, M., Y. Kitajima, et al. (2007). "Aberrant methylation of p16 predicts candidates for 5-fluorouracil-based adjuvant therapy in gastric cancer patients." J Gastroenterol **42**(11): 866-73.
87. Miyagawa, K., C. Sakakura, et al. (2008). "Overexpression of RegIV in peritoneal dissemination of gastric cancer and its potential as A novel marker for the detection of peritoneal micrometastasis." Anticancer Res **28**(2B): 1169-79.
88. Mori, K., T. Suzuki, et al. (2007). "Detection of minimal gastric cancer cells in peritoneal washings by focused microarray analysis with multiple markers: clinical implications." Ann Surg Oncol **14**(5): 1694-702.
89. Mori, Y., H. Kataoka, et al. (2007). "Subcellular localization of ATBF1 regulates MUC5AC transcription in gastric cancer." Int J Cancer **121**(2): 241-7.
90. Moss, S. F., J. W. Lee, et al. (2008). "Decreased expression of gastrokine 1 and the trefoil factor interacting protein

TFIZ1/GKN2 in gastric cancer: influence of tumor histology and relationship to prognosis." Clin Cancer Res **14**(13): 4161-7.
91. Moutinho, C., A. R. Mateus, et al. (2008). "Epidermal growth factor receptor structural alterations in gastric cancer." BMC Cancer **8**: 10.
92. Mroczko, B., M. Lukaszewicz-Zajac, et al. (2009). "Expression of tissue inhibitors of metalloproteinase 1 (TIMP-1) in gastric cancer tissue." Folia Histochem Cytobiol **47**(3): 511-6.
93. Murray, D., G. Horgan, et al. (2008). "NET1-mediated RhoA activation facilitates lysophosphatidic acid-induced cell migration and invasion in gastric cancer." Br J Cancer **99**(8): 1322-9.
94. Nakamura, J., Y. Kitajima, et al. (2009). "Hypoxia-inducible factor-1alpha expression predicts the response to 5-fluorouracil-based adjuvant chemotherapy in advanced gastric cancer." Oncol Rep **22**(4): 693-9.
95. Nakamura, Y., T. Migita, et al. (2009). "Kruppel-like factor 12 plays a significant role in poorly differentiated gastric cancer progression." Int J Cancer **125**(8): 1859-67.
96. Nikiteas, N. I., N. Tzanakis, et al. (2007). "Vascular endothelial growth factor and endoglin (CD-105) in gastric cancer." Gastric Cancer **10**(1): 12-7.
97. Ning, X., S. Sun, et al. (2007). "Calcyclin-binding protein inhibits proliferation, tumorigenicity, and invasion of gastric cancer." Mol Cancer Res **5**(12): 1254-62.
98. Nojima, M., H. Suzuki, et al. (2007). "Frequent epigenetic inactivation of SFRP genes and constitutive activation of Wnt signaling in gastric cancer." Oncogene **26**(32): 4699-713.
99. Oue, N., K. Sentani, et al. (2009). "Serum olfactomedin 4 (GW112, hGC-1) in combination with Reg IV is a highly sensitive biomarker for gastric cancer patients." Int J Cancer **125**(10): 2383-92.
100. Pang, R. P., J. G. Zhou, et al. (2007). "Celecoxib induces apoptosis in COX-2 deficient human gastric cancer cells through Akt/GSK3beta/NAG-1 pathway." Cancer Lett **251**(2): 268-77.
101. Park, J. Y., K. H. Park, et al. (2007). "CXCL5 overexpression is associated with late stage gastric cancer." J Cancer Res Clin Oncol **133**(11): 835-40.
102. Park, M. J., K. H. Kim, et al. (2008). "Bile acid induces expression of COX-2 through the homeodomain transcription factor CDX1 and orphan nuclear receptor SHP in human gastric cancer cells." Carcinogenesis **29**(12): 2385-93.
103. Park, S., J. H. Kim, et al. (2007). "Aberrant hypermethylation of the FGFR2 gene in human gastric cancer cell lines." Biochem Biophys Res Commun **357**(4): 1011-5.
104. Peek, R. M. (2008). "Prevention of Gastric Cancer: When is Treatment of Helicobacter Pylori Warranted?" Therap Adv Gastroenterol **1**(1): 19-31.
105. Petrocca, F., R. Visone, et al. (2008). "E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer." Cancer Cell **13**(3): 272-86.
106. Rohwer, N., S. Lobitz, et al. (2009). "HIF-1alpha determines the metastatic potential of gastric cancer cells." Br J Cancer **100**(5): 772-81.
107. Ruzzo, A., E. Canestrari, et al. (2007). "Polymorphisms in genes involved in DNA repair and metabolism of xenobiotics in individual susceptibility to sporadic diffuse gastric cancer." Clin Chem Lab Med **45**(7): 822-8.
108. Saeki, N., D. H. Kim, et al. (2007). "GASDERMIN, suppressed frequently in gastric cancer, is a target of LMO1 in TGF-beta-dependent apoptotic signalling." Oncogene **26**(45): 6488-98.
109. Sangodkar, J., J. Shi, et al. (2009). "Functional role of the KLF6 tumour suppressor gene in gastric cancer." Eur J Cancer **45**(4): 666-76.
110. Sawabu, T., H. Seno, et al. (2007). "Growth arrest-specific gene 6 and Axl signaling enhances gastric cancer cell survival via Akt pathway." Mol Carcinog **46**(2): 155-64.
111. Saxena, A., K. Nath Prasad, et al. (2008). "Association of Helicobacter pylori and Epstein-Barr virus with gastric cancer and peptic ulcer disease." Scand J Gastroenterol **43**(6): 669-74.
112. Seidl, C., M. Port, et al. (2007). "213Bi-induced death of HSC45-M2 gastric cancer cells is characterized by G2 arrest and up-regulation of genes known to prevent apoptosis but induce necrosis and mitotic catastrophe." Mol Cancer Ther **6**(8): 2346-59.
113. Sekikawa, A., H. Fukui, et al. (2008). "REG Ialpha protein mediates an anti-apoptotic effect of STAT3 signaling in gastric cancer cells." Carcinogenesis **29**(1): 76-83.
114. Seno, H., K. Satoh, et al. (2007). "Novel interleukin-4 and interleukin-1 receptor antagonist gene variations associated with non-cardia gastric cancer in Japan: comprehensive analysis of 207 polymorphisms of 11 cytokine genes." J Gastroenterol Hepatol **22**(5): 729-37.
115. Seto, M., M. Ohta, et al. (2009). "Regulation of the hedgehog signaling by the mitogen-activated protein kinase cascade in gastric cancer." Mol Carcinog **48**(8): 703-12.
116. Shanks, A. M. and E. M. El-Omar (2009). "Helicobacter pylori infection, host genetics and gastric cancer." J Dig Dis **10**(3): 157-64.
117. Shibata, T., T. Arisawa, et al. (2009). "Selenoprotein S (SEPS1) gene -105G>A promoter polymorphism influences the susceptibility to gastric cancer in the Japanese population." BMC Gastroenterol **9**: 2.
118. Solcia, E., C. Klersy, et al. (2009). "A combined histologic and molecular approach identifies three groups of gastric cancer with different prognosis." Virchows Arch **455**(3): 197-211.
119. Song, I. S., A. G. Wang, et al. (2009). "Regulation of glucose metabolism-related genes and VEGF by HIF-1alpha and HIF-1beta, but not HIF-2alpha, in gastric cancer." Exp Mol Med **41**(1): 51-8.
120. Song, I. S., N. S. Oh, et al. (2009). "Human ZNF312b promotes the progression of gastric cancer by transcriptional activation of the K-ras gene." Cancer Res **69**(7): 3131-9.
121. Suganuma, M., K. Yamaguchi, et al. (2008). "TNF-alpha-inducing protein, a carcinogenic factor secreted from H. pylori, enters gastric cancer cells." Int J Cancer **123**(1): 117-22.
122. Sugimoto, M., T. Furuta, et al. (2007). "Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer." J Gastroenterol Hepatol **22**(1): 51-9.
123. Suzuki, G., H. Cullings, et al. (2007). "Low-positive antibody titer against Helicobacter pylori cytotoxin-associated gene A (CagA) may predict future gastric cancer better than simple seropositivity against H. pylori CagA or against H. pylori." Cancer Epidemiol Biomarkers Prev **16**(6): 1224-8.
124. Suzuki, G., H. Cullings, et al. (2009). "LTA 252GG and GA genotypes are associated with diffuse-type noncardia gastric cancer risk in the Japanese population." Helicobacter **14**(6): 571-9.
125. Tahara, T., T. Shibata, et al. (2009). "Effect of polymorphisms in the 3' untranslated region (3'-UTR) of vascular endothelial growth factor gene on gastric cancer and peptic ulcer diseases in Japan." Mol Carcinog **48**(11): 1030-7.
126. Takahata, M., Y. Inoue, et al. (2009). "SKI and MEL1 cooperate to inhibit transforming growth factor-beta signal in gastric cancer cells." J Biol Chem **284**(5): 3334-44.
127. Taniguchi, H., Y. Fujiwara, et al. (2007). "Gene therapy using ets-1 transcription factor decoy for peritoneal dissemination of gastric cancer." Int J Cancer **121**(7): 1609-17.

128. Tu, S. P., A. L. Chi, et al. (2009). "p53 inhibition of AP1-dependent TFF2 expression induces apoptosis and inhibits cell migration in gastric cancer cells." Am J Physiol Gastrointest Liver Physiol **297**(2): G385-96.
129. Tu, S. P., P. Liston, et al. (2009). "Restoration of XAF1 expression induces apoptosis and inhibits tumor growth in gastric cancer." Int J Cancer **125**(3): 688-97.
130. Udhayakumar, G., V. Jayanthi, et al. (2007). "Interaction of MUC1 with beta-catenin modulates the Wnt target gene cyclinD1 in H. pylori-induced gastric cancer." Mol Carcinog **46**(9): 807-17.
131. Wanajo, A., A. Sasaki, et al. (2008). "Methylation of the calcium channel-related gene, CACNA2D3, is frequent and a poor prognostic factor in gastric cancer." Gastroenterology **135**(2): 580-90.
132. Wang, H. L., H. Bai, et al. (2007). "Rationales for expression and altered expression of apoptotic protease activating factor-1 gene in gastric cancer." World J Gastroenterol **13**(38): 5060-4.
133. Wang, L., X. Guan, et al. (2008). "Targeted inhibition of Sp1-mediated transcription for antiangiogenic therapy of metastatic human gastric cancer in orthotopic nude mouse models." Int J Oncol **33**(1): 161-7.
134. Wang, Q., Y. Huang, et al. (2007). "siRNA targeting midkine inhibits gastric cancer cells growth and induces apoptosis involved caspase-3,8,9 activation and mitochondrial depolarization." J Biomed Sci **14**(6): 783-95.
135. Wang, S. L., D. Z. Zheng, et al. (2008). "Increased expression of hLRH-1 in human gastric cancer and its implication in tumorigenesis." Mol Cell Biochem **308**(1-2): 93-100.
136. Wang, Z., S. R. Cai, et al. (2009). "High expression of PRL-3 can promote growth of gastric cancer and exhibits a poor prognostic impact on patients." Ann Surg Oncol **16**(1): 208-19.
137. Wang, Z., Y. L. He, et al. (2008). "Expression and prognostic impact of PRL-3 in lymph node metastasis of gastric cancer: its molecular mechanism was investigated using artificial microRNA interference." Int J Cancer **123**(6): 1439-47.
138. Wex, T., J. Bornschein, et al. (2009). "Host polymorphisms of immune regulatory genes as risk factors for gastric cancer." Minerva Gastroenterol Dietol **55**(4): 395-408.
139. Wex, T., M. P. Ebert, et al. (2008). "Gene polymorphisms of the NOD-2/CARD-15 gene and the risk of gastric cancer in Germany." Anticancer Res **28**(2A): 757-62.
140. Wu, C. W., J. Yu, et al. (2009). "Peroxisome proliferator-activated receptor delta and gastric cancer (Review)." Oncol Rep **22**(3): 451-7.
141. Wu, C. Y., M. S. Wu, et al. (2007). "Elevated plasma osteopontin associated with gastric cancer development, invasion and survival." Gut **56**(6): 782-9.
142. Wu, D., Y. Tian, et al. (2009). "Genetic variants in the Runt-related transcription factor 3 gene contribute to gastric cancer risk in a Chinese population." Cancer Sci **100**(9): 1688-94.
143. Wu, K., Y. Nie, et al. (2009). "Molecular basis of therapeutic approaches to gastric cancer." J Gastroenterol Hepatol **24**(1): 37-41.
144. Wu, W. K., T. T. Tse, et al. (2009). "Expression of ErbB receptors and their cognate ligands in gastric and colon cancer cell lines." Anticancer Res **29**(1): 229-34.
145. Wu, Z. Q., R. Zhang, et al. (2007). "Histone deacetylase inhibitor trichostatin A induced caspase-independent apoptosis in human gastric cancer cell." Chin Med J (Engl) **120**(23): 2112-8.
146. Xiao, Q., L. Li, et al. (2007). "Transcription factor E2F-1 is upregulated in human gastric cancer tissues and its overexpression suppresses gastric tumor cell proliferation." Cell Oncol **29**(4): 335-49.
147. Xie, Y., Y. Yin, et al. (2009). "Short interfering RNA directed against the E2F-1 gene suppressing gastric cancer progression in vitro." Oncol Rep **21**(5): 1345-53.
148. Xing, C. G., B. S. Zhu, et al. (2009). "Effects of LY294002 on the invasiveness of human gastric cancer in vivo in nude mice." World J Gastroenterol **15**(40): 5044-52.
149. Xu, Q., Y. Yuan, et al. (2009). "Risk of gastric cancer is associated with the MUC1 568 A/G polymorphism." Int J Oncol **35**(6): 1313-20.
150. Xu, W. H., Y. L. Ge, et al. (2007). "Inhibitory effect of vascular endothelial growth factors-targeted small interfering RNA on proliferation of gastric cancer cells." World J Gastroenterol **13**(14): 2044-7.
151. Xu, Y., X. Qu, et al. (2009). "Midkine positively regulates the proliferation of human gastric cancer cells." Cancer Lett **279**(2): 137-44.
152. Yamada, H., K. Shinmura, et al. (2009). "Absence of germline mono-allelic promoter hypermethylation of the CDH1 gene in gastric cancer patients." Mol Cancer **8**: 63.
153. Yamamoto, H., Y. Kitadai, et al. (2009). "Laminin gamma2 mediates Wnt5a-induced invasion of gastric cancer cells." Gastroenterology **137**(1): 242-52, 252 e1-6.
154. Yamamura, Y., W. L. Lee, et al. (2008). "Role of TAp73alpha in induction of apoptosis by transforming growth factor-beta in gastric cancer cells." FEBS Lett **582**(17): 2663-7.
155. Yang, J. J., K. P. Ko, et al. (2009). "The role of TNF genetic variants and the interaction with cigarette smoking for gastric cancer risk: a nested case-control study." BMC Cancer **9**: 238.
156. Yang, L., H. J. Gu, et al. (2008). "Tissue inhibitor of metalloproteinase-2 G-418C polymorphism is associated with an increased risk of gastric cancer in a Chinese population." Eur J Surg Oncol **34**(6): 636-41.
157. Yang, S., H. C. Jeung, et al. (2007). "Identification of genes with correlated patterns of variations in DNA copy number and gene expression level in gastric cancer." Genomics **89**(4): 451-9.
158. Yang, Z., X. Zhang, et al. (2007). "Up-regulation of gastric cancer cell invasion by Twist is accompanied by N-cadherin and fibronectin expression." Biochem Biophys Res Commun **358**(3): 925-30.
159. Yano, K., T. Imaeda, et al. (2008). "Transcriptional activation of the human claudin-18 gene promoter through two AP-1 motifs in PMA-stimulated MKN45 gastric cancer cells." Am J Physiol Gastrointest Liver Physiol **294**(1): G336-43.
160. Yasui, W., N. Oue, et al. (2009). "Transcriptome dissection of gastric cancer: identification of novel diagnostic and therapeutic targets from pathology specimens." Pathol Int **59**(3): 121-36.
161. Zhang, J., C. Dou, et al. (2008). "Polymorphisms of tumor necrosis factor-alpha are associated with increased susceptibility to gastric cancer: a meta-analysis." J Hum Genet **53**(6): 479-89.
162. Zhang, Y. J. and J. Y. Fang (2008). "Molecular staging of gastric cancer." J Gastroenterol Hepatol **23**(6): 856-60.
163. Zhang, Z., Z. Li, et al. (2008). "miR-21 plays a pivotal role in gastric cancer pathogenesis and progression." Lab Invest **88**(12): 1358-66.
164. Zhu, B. H., W. H. Zhan, et al. (2007). "(-)-Epigallocatechin-3-gallate inhibits growth of gastric cancer by reducing VEGF production and angiogenesis." World J Gastroenterol **13**(8): 1162-9.
165. PubMed (2012). http://www.ncbi.nlm.nih.gov/pubmed.
166. Cancer. Wikipedia. (2012) http://en.wikipedia.org/wiki/Cancer.

238/2012