Cancer Biology Research Literatures

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Abstract: 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. This article introduces recent research reports as references in the related studies.
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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. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Anbazhagan, R., et al. (1999). "The S387Y mutations of the transforming growth factor-beta receptor type I gene is uncommon in metastases of breast cancer and other common types of adenocarcinoma." Cancer Res **59**(14): 3363-3364.

Recently, mutations of the transforming growth factor-beta receptor type I gene have been reported to occur at high frequency in breast cancer metastases, with all mutations being an identical C to A transversion at nucleotide 1160 of the gene (T. Chen et al, Cancer Res., 58: 4805-4810, 1998). This mutation would result in a serine to tyrosine substitution at codon 387 (S387Y) and would reportedly disrupt receptor function. Because this mutation reportedly occurred at high frequency in breast cancer metastases (42%) and much less frequently in primary breast cancer tumors (6%), this would seem to represent a pivotal genetic alteration in breast cancer progression. To further investigate the possible role of this specific genetic alteration in the progression of breast cancer and other forms of adenocarcinoma, we analyzed 20 breast cancer metastases, 15 lung adenocarcinoma metastases, and 13 colorectal cancer metastases for possible mutations at this site. Using both single-strand conformation polymorphism screening and sequencing, we found no mutations of this gene in any of our samples. Our results suggest the S387Y mutation of the transforming growth factor-beta receptor type I gene is not common in these types of human cancers.

Andreou, A., et al. (2012). "Adjuvant chemotherapy with FOLFOX for primary colorectal cancer is associated with increased somatic gene mutations and inferior survival in patients undergoing hepatectomy for metachronous liver metastases." Ann Surg **256**(4): 642-650.

OBJECTIVE: We hypothesized that metachronous colorectal liver metastases (CLM) have different biology after failure of oxaliplatin (FOLFOX) compared to 5-fluorouracil (5-FU) or no chemotherapy for adjuvant treatment of colorectal cancer (CRC). BACKGROUND: It is unclear whether patients treated with liver resection for metachronous CLM after adjuvant FOLFOX for CRC have worse outcomes than those who received 5-FU or no chemotherapy. METHODS: We identified 341 patients who underwent hepatectomy for metachronous CLM (disease-free interval >/=12 months, 1993-2010). Mass-spectroscopy genotyping for somatic gene mutations in CLM was performed in a subset of 129 patients. RESULTS: Adjuvant treatment for primary CRC was FOLFOX in 77 patients, 5-FU in 169 patients, and no chemotherapy in 95 patients. Node-positive primary was comparable between FOLFOX and 5-FU but lower in the no-chemotherapy group (P < 0.0001). Median metastasis size was smaller in the FOLFOX group (2.5 cm) than in the 5-FU (3.0 cm) or no-chemotherapy (3.5 cm) groups, (P = 0.008) although

prehepatectomy chemotherapy utilization, metastases number, and carcinoembryonic antigen levels were similar. Disease-free survival (DFS) and overall survival (OS) rates after hepatectomy were worse in patients treated with adjuvant FOLFOX [DFS at 3 years: 14% vs 38% (5-FU) vs 45% (no-chemo), OS at 3 years: 58% vs 70% (5-FU) vs 84% (no-chemo)]. On multivariate analysis, adjuvant FOLFOX was associated with worse DFS ($P < 0.0001$) and OS ($P < 0.0001$). Mutation analysis revealed >/=1 mutations in 57% of patients (27/47) after FOLFOX, 29% (12/41) after 5-FU, and 32% (13/41) after no chemotherapy ($P = 0.011$). CONCLUSIONS: Adjuvant FOLFOX for primary CRC is associated with a high rate of somatic mutations in liver metastases and inferior outcomes after hepatectomy for metachronous CLM.

Avery-Kiejda, K. A., et al. (2017). "Genome-wide miRNA, gene and methylation analysis of triple negative breast cancer to identify changes associated with lymph node metastases." Genom Data **14**: 1-4.

Triple negative breast cancer (TNBC) is a particularly important breast cancer subtype with an aggressive clinical phenotype that is associated with a higher likelihood of metastasis. This subtype is characterized by an absence of the estrogen (ER) and progesterone (PR) receptors, as well as the human epidermal growth factor receptor 2 (HER2/HER neu). The absence of the three receptors significantly reduces targeted treatment options for patients with TNBC and as such, there is an urgent need to identify novel treatment targets. Here, we provide detailed information regarding the design of a multi-platform dataset that describes genome-wide assessment of miRNA (assessed by microarray, GSE38167) and gene expression (assessed by microarray, GSE61723), as well as methylation (assessed by Illumina HM450K BeadChip, GSE78751) in TNBCs, matched normal adjacent tissues and matched lymph node metastases. The use of this multi-platform dataset is likely to uncover novel markers and key pathways involved in progression to lymph node metastasis in TNBC.

Badawy, O. M., et al. (2018). "Concordance Between FISH Analysis of Her-2/Neu Gene in Breast Duct Carcinoma and Corresponding Axillary Nodal Metastases: Egyptian National Cancer Institute Experience." Appl Immunohistochem Mol Morphol.

BACKGROUND: Breast cancer is a major health problem in Egypt. Her-2/Neu gene is routinely assessed for all breast cancer patients primarily by immunohistochemistry. At National Cancer Institute (NCI), Cairo University, Flourescence In Situ hybridization (FISH) analysis of Her-2/Neu gene is carried out for Her-2/Neu score 2 and for some cases of score 3 (particularly those assessed outside NCI). The test is performed essentially on the primary tumor. However, some situations require testing on corresponding lymph node metastases. There is a debate about the concordance between Her-2/Neu status in the primary tumor and synchronous lymph node metastases in various studies. AIM OF THE STUDY: The aim of this study was to test for the concordance between Her-2/Neu status in the primary breast tumor and corresponding axillary nodal metastases. MATERIALS AND METHODS: This is a retrospective study in which FISH analysis of Her-2/Neu was carried out simultaneously on archived material of 50 cases previously diagnosed as invasive duct carcinoma and the corresponding nodal metastases from the Pathology Department, NCI. RESULTS: There was complete concordance between Her-2 status in the primary tumor and the corresponding axillary lymph node metastatic deposits in which Her-2 was amplified in 44% of the studied cohort of Egyptian patients. CONCLUSIONS: Her-2/Neu gene assessed by FISH analysis on synchronous lymph node metastases is strongly correlated with the primary tumor. Hence, it is justified to carry out the Her-2/Neu test on synchronous lymph nodes to decide on whether to carry out anti-Her-2/Neu target therapy. Further studies on other metastatic sites is recommended.

Bekar, A., et al. (2007). "Investigation of mutations and expression of the FHIT gene in Turkish patients with brain metastases derived from non-small cell lung cancer." Tumori **93**(6): 604-607.

AIMS AND BACKGROUND: Brain metastases occur in 20-40% of patients with cancer, and their frequency has increased over time. Lung, breast and skin (melanoma) are the most common sources of brain metastases. Recent studies show that several genes such as CD44 and PTEN have roles in the suppression of metastatic growth. Although it has been determined that there is a relationship between the FHIT gene and several primary tumors, its role in the initiation and progression of brain tumors has not yet been entirely explained. Furthermore, it is not known whether the FHIT gene has a role in the formation of brain metastases. PATIENTS AND METHODS: The present study investigated mutations of the FHIT gene in Turkish patients with brain metastases derived from non-small cell lung cancer (NSCLC). Single-strand conformational polymorphism and sequencing analysis of the coding exons (5-9) of the FHIT gene were performed on 26 tissues. Furthermore, the level of Fhit protein expression of 36 tumor tissues was identified by immunohistochemistry. RESULTS: Using single-

strand conformational polymorphism and sequencing analyses, no point mutations of the FHIT gene were detected in brain metastases derived from NSCLC. However, it was observed that Fhit protein expression was reduced in 88.9% of subjects. CONCLUSIONS: We suggest that the FHIT gene may be turned off in brain metastases via other genetic/epigenetic mechanisms rather than mutations.

Bertorelle, R., et al. (1995). "Association of p53 gene and protein alterations with metastases in colorectal cancer." Am J Surg Pathol **19**(4): 463-471.

Using monoclonal antibody PAb 1801, p53 protein was detected in the neoplastic cells of 39 (46.9%) of 83 colorectal carcinomas studied. Patients with p53+ tumors showed a higher incidence of lymph node and liver metastases ($p = 0.035$); in patients whose tumors were located in the rectosigmoid, p53 expression also correlated with a more advanced stage according to Dukes' classification ($p = 0.015$) as well as nodal ($p = 0.006$) and liver ($p = 0.019$) metastases. Following amplification of exons 5 to 8 of the p53 gene by means of the polymerase chain reaction technique, single-strand conformation polymorphism analysis disclosed an anomalous migration pattern in 23 of the 39 p53+ tumors and in four of the 35 p53- tumors analyzed. Sequence analysis showed G:C-->A:T transitions in 63.6%, G:C-->T:A and G:C-->C:G transversions in 18.2%, deletions and insertions in 13.6%, and A:T-->G:C transitions in 4.6% of the cases. Loss of heterozygosity was studied in the DNA of 79 patients; allelic loss was found in 29 (49.1%) of the 59 informative patients. Loss of heterozygosity was correlated with p53 overexpression ($p = 0.0002$) as well as with the presence of mutations as detected by single strand conformation polymorphism analysis ($p = 0.0024$).

Brown, R. S., et al. (2002). "Amplification of the androgen receptor gene in bone metastases from hormone-refractory prostate cancer." J Pathol **198**(2): 237-244.

The aim of this study was to examine the prevalence of androgen receptor (AR) amplification in metastases to bone and other sites in patients with hormone-refractory prostate cancer (HRPC) and to compare these findings with those in pretreatment primary tumour samples from the same patients. Tissue from 24 patients with HRPC was available for study, together with 13 primary tumour specimens. AR gene amplification and copy number for X-chromosome were assessed by fluorescence in situ hybridization (FISH) using a SpectrumOrange-labelled probe at locus Xq11-13 for the AR gene and a SpectrumGreen-labelled alpha-satellite probe for the X-chromosome (Vysis, UK, Ltd.). A minimum of 20 nuclei were scored in each of three tumour areas by two independent observers. Samples from 18/24 patients with HRPC (12 bone marrow biopsies, three local tumour recurrences, and three lymph nodes) and nine primary tumour specimens were adequate for FISH analysis. Results were expressed as a mean ratio of AR gene copy number : mean X-chromosome number, with a ratio of greater than 1.5 defined as amplification. AR gene amplification was seen in 9/18 (50%) cases of HRPC and in none of the primary (untreated) tumour specimens ($p = 0.0048$, Fisher's exact test). For the 12 bone marrow samples, AR gene amplification occurred in 5/12 (38%) cases. Elevated copy number for chromosome X occurred in 3/18 (17%) HRPC and 4/9 (44%) matched primary tumours. This study shows for the first time that AR gene amplification can be demonstrated by FISH in bone metastases from HRPC patients. Because bone marrow biopsies can be obtained from most patients with HRPC, the findings provide a rational basis for the routine selection of patients who may respond more favourably to second-line anti-androgen therapy.

Christgen, M., et al. (2018). "Activating human epidermal growth factor receptor 2 (HER2) gene mutation in bone metastases from breast cancer." Virchows Arch.

In addition to amplification, point mutations of the human epidermal growth factor receptor 2 (HER2) gene (ERBB2) have been shown to activate the corresponding signaling pathway in breast cancer. The prevalence of ERBB2/HER2 mutation in bone metastasis of breast cancer and the associated phenotype are not known. In this study, bone metastases from breast cancer patients ($n = 231$) were analyzed for ERBB2/HER2 mutation. In 7 patients (3%; median age 70 years, range 50-83 years), gain-of-function mutations of ERBB2/HER2 were detected. The most frequent mutation was p.L755S (71%). In 29% of mutated cases, p.V777L was found. Lobular breast cancer was present in 71% of mutated cases ($n = 5$) and in 49% of all samples ($n = 231$; $p = 0.275$). Mutation frequency was 4.4% in the lobular subgroup and 17.4% in the pleomorphic subtype of lobular cancer ($n = 23$), respectively. All but one mutated lobular cancers were of the pleomorphic subtype ($p = 0.006$). Mutated cancers belonged either to the luminal ($n = 4$) or to the triple-negative types ($n = 3$). With regard to protein expression and gene amplification, HER2 was negative in all mutated cases. Among the 14% of metastatic luminal cancers with estrogen receptor gene (ESR1) mutation, conveying resistance against aromatase inhibitors, no concomitant ERBB2/HER2 mutation occurred. We conclude that activating HER2 mutation is present in

about 3% of bone metastases from breast cancers, with significantly higher rates in the pleomorphic subtype of lobular cancer. Since mutated cases appear to be HER2-negative by conventional testing, the opportunity for specific anti-HER2 therapy may be missed.

Delliaux, C., et al. (2018). "TMPRSS2:ERG gene fusion expression regulates bone markers and enhances the osteoblastic phenotype of prostate cancer bone metastases." Cancer Lett **438**: 32-43.

Prostate cancers have a strong propensity to metastasize to bone and promote osteoblastic lesions. TMPRSS2:ERG is the most frequent gene rearrangement identified in prostate cancer, but whether it is involved in prostate cancer bone metastases is largely unknown. We exploited an intratibial metastasis model to address this issue and we found that ectopic expression of the TMPRSS2:ERG fusion enhances the ability of prostate cancer cell lines to induce osteoblastic lesions by stimulating bone formation and inhibiting the osteolytic response. In line with these in vivo results, we demonstrate that the TMPRSS2:ERG fusion protein increases the expression of osteoblastic markers, including Collagen Type I Alpha 1 Chain and Alkaline Phosphatase, as well as Endothelin-1, a protein with a documented role in osteoblastic bone lesion formation. Moreover, we determined that the TMPRSS2:ERG fusion protein is bound to the regulatory regions of these genes in prostate cancer cell lines, and we report that the expression levels of these osteoblastic markers are correlated with the expression of the TMPRSS2:ERG fusion in patient metastasis samples. Taken together, our results reveal that the TMPRSS2:ERG gene fusion is involved in osteoblastic lesion formation induced by prostate cancer cells.

Driouch, K., et al. (1997). "Classical gene amplifications in human breast cancer are not associated with distant solid metastases." Br J Cancer **76**(6): 784-787.

To determine the relationship between breast cancer progression and gene amplification, we screened 62 distant metastases and 122 primary breast tumours for the amplification of the proto-oncogenes MYC and ERBB2 and the 11q13 chromosomal region. Surprisingly, solid metastases showed an absence of gene amplification. These results suggest that the amplification of the proto-oncogenes MYC and ERBB2 and the 11q13 chromosomal region seem to be involved mainly in the genesis of the primary breast tumour rather than its progression.

Duchnowska, R., et al. (2015). "Predicting early brain metastases based on clinicopathological factors and gene expression analysis in advanced HER2-positive breast cancer patients." J Neurooncol **122**(1): 205-216.

The overexpression or amplification of the human epidermal growth factor receptor 2 gene (HER2/neu) is associated with high risk of brain metastasis (BM). The identification of patients at highest immediate risk of BM could optimize screening and facilitate interventional trials. We performed gene expression analysis using complementary deoxyribonucleic acid-mediated annealing, selection, extension and ligation and real-time quantitative reverse transcription PCR (qRT-PCR) in primary tumor samples from two independent cohorts of advanced HER2 positive breast cancer patients. Additionally, we analyzed predictive relevance of clinicopathological factors in this series. Study group included discovery Cohort A (84 patients) and validation Cohort B (75 patients). The only independent variables associated with the development of early BM in both cohorts were the visceral location of first distant relapse [Cohort A: hazard ratio (HR) 7.4, 95 % CI 2.4-22.3; $p < 0.001$; Cohort B: HR 6.1, 95 % CI 1.5-25.6; $p = 0.01$] and the lack of trastuzumab administration in the metastatic setting (Cohort A: HR 5.0, 95 % CI 1.4-10.0; $p = 0.009$; Cohort B: HR 10.0, 95 % CI 2.0-100.0; $p = 0.008$). A profile including 13 genes was associated with early (</=36 months) symptomatic BM in the discovery cohort. This was refined by qRT-PCR to a 3-gene classifier (RAD51, HDGF, TPR) highly predictive of early BM (HR 5.3, 95 % CI 1.6-16.7; $p = 0.005$; multivariate analysis). However, predictive value of the classifier was not confirmed in the independent validation Cohort B. The presence of visceral metastases and the lack of trastuzumab administration in the metastatic setting apparently increase the likelihood of early BM in advanced HER2-positive breast cancer.

Erin, N., et al. (2009). "Altered gene expression in breast cancer liver metastases." Int J Cancer **124**(7): 1503-1516.

We previously developed a highly aggressive cell line from heart metastases of 4T1 breast carcinoma (designated 4THM), which produced liver metastases (designated 4TLM). In this study, gene array analysis (GAEA) compared gene expression profiles in 4TLM with profiles in 4T1 and 4THM primary tumors. GAEA demonstrated that 4T1 and 4THM tumors differed in about 250 genes. Over 1,000 genes, however, were expressed differently in 4TLM compared with primary tumors. A cohort of 16 genes showed significantly decreased

expression in 4THM tumors, which decreased even further in 4TLM. Many of these genes have been implicated in breast cancer, and many are involved in cell adhesion and junctional complexes. Expression of multiple tight and adherence junction proteins was either downregulated or disappeared in 4TLM; downregulation of claudin 4, claudin 7 and gamma-catenin was confirmed by quantitative polymerase chain reaction, immunoblot, and immunocytochemical (ICC) analyses. At the protein level, intact ZO-1 was also observed in 4T1 tumors, but was not expressed in 4THM or 4TLM tumors. ICC demonstrated expression of gamma-catenin at the plasma membrane with 4T1 tumors, whereas staining appeared to be nuclear/perinuclear in 4THM tumors. Claudin 7 staining was also seen in monocyte/pmacrophage-like cells in liver around metastatic lesions by ICC, and it appeared that larger 4TLM tumors apparently reexpressed claudin 7 RNA and protein. Our results demonstrate that decreased or abnormal expression of a number of cell adhesion/junctional proteins, including claudin 4, 7, ZO-1 and gamma-catenin, correlates with liver metastases, and that cell adhesion molecules in the microenvironment are also altered.

Evans, J. C., et al. (2017). "Formulation and Evaluation of Anisamide-Targeted Amphiphilic Cyclodextrin Nanoparticles To Promote Therapeutic Gene Silencing in a 3D Prostate Cancer Bone Metastases Model." Mol Pharm **14**(1): 42-52.

In recent years, RNA interference (RNAi) has emerged as a potential therapeutic offering the opportunity to treat a wide range of diseases, including prostate cancer. Modified cyclodextrins have emerged as effective gene delivery vectors in a range of disease models. The main objective of the current study was to formulate anisamide-targeted cyclodextrin nanoparticles to interact with the sigma receptor (overexpressed on the surface of prostate cancer cells). The inclusion of octaarginine in the nanoparticle optimized uptake and endosomal release of siRNA in two different prostate cancer cell lines (PC3 and DU145 cells). Resulting nanoparticles were less than 200 nm in size with a cationic surface charge (approximately +20 mV). In sigma receptor-positive cell lines, the uptake of anisamide-targeted nanoparticles was reduced in the presence of the sigma receptor competitive ligand, haloperidol. When cells were transfected in 2D, the levels of PLK1 mRNA knockdown elicited by targeted versus untargeted nanoparticles tended to be greater but the differences were not statistically different. In contrast, when cells were grown on 3D scaffolds, recapitulating bone metastasis, targeted formulations showed significantly higher levels of PLK1 mRNA knockdown (46% for PC3 and 37% for DU145, $p < 0.05$). To our knowledge, this is the first time that a targeted cyclodextrin has been used to transfect prostate cancer cells in a 3D model of bone metastasis.

Faleel, F. D., et al. (2016). "Modified mismatch polymerase chain reaction-restriction fragment length polymorphism detected mutations in codon 12 and 13 of exon 2 of K-ras gene in colorectal cancer patients and its association with liver metastases: Data from a South Asian country." J Cancer Res Ther **12**(4): 1272-1277.

AIM: Mutations in K-ras codon 12 and 13 of exon 2 are known to affect prognosis and impart resistance to anti-epidermal growth factor monoclonal antibody therapy in colorectal carcinoma (CRC). Our aim was to investigate the utility value of modified mismatch polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay to detect mutation in K-ras codons of CRC patients and to relate the mutational status to liver metastasis. METHODOLOGY: Mismatch PCR-RFLP was developed to detect K-ras mutations in DNA isolated from paraffinized tumor tissue of thirty CRC patients. All patients had 5 year follow-up data to detect liver metastasis. Cross-tabulations were generated between K-ras mutations and the metastatic status. The Chi-square test was used to indicate statistical significance of the association. RESULTS: Of the 30 CRC patients investigated, K-ras mutations of codons 12 and/or 13 of exon 2 were detected in 14 (46.6%). Meanwhile, 13 patients (43.3%) were observed to have developed liver metastases. There was a significant association between the presence of the K-ras mutation in codon 12 and the occurrence of liver metastasis (chi2 = 4.693, P = 0.030) on the contrary to the mutation in codon 13 to which such occurrence of liver metastases was not seen (chi2 = 1.884, P = 0.169). CONCLUSION: Codon 12 of exon 2 of K--ras gene detected by modified mismatch PCR-RFLP assay is significantly associated with liver metastasis in CRC patients during the first 5 years after surgery. Thus, modified mismatch PCR-RFLP protocol is a suitable method in this setting to detect K-ras gene mutations predicting liver metastasis in CRC patients.

Fertig, E. J., et al. (2015). "Analysis of gene expression of secreted factors associated with breast cancer metastases in breast cancer subtypes." Sci Rep **5**: 12133.

Breast cancer is a heterogeneous disease, having multiple subtypes with different malignant phenotypes. The triple-negative breast cancer, or basal breast cancer, is highly aggressive, metastatic,

and difficult to treat. Previously, we identified that key molecules (IL6, CSF2, CCL5, VEGFA, and VEGFC) secreted by tumor cells and stromal cells in basal breast cancer can promote metastasis. It remains to assess whether these molecules function similarly in other subtypes of breast cancer. Here, we characterize the relative gene expression of the five secreted molecules and their associated receptors (GP130, GMRA, GMRB, CCR5, VEGFR2, NRP1, VEGFR3, NRP2) in the basal, HER2 (human epidermal growth factor receptor 2) positive, luminal A, and luminal B subtypes using high throughput data from tumor samples in The Cancer Genome Atlas (TCGA) and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC). IL6 and CCL5 gene expression are basal breast cancer specific, whereas high gene expression of GP130 was observed in luminal A/B. VEGFA/C and CSF2 mRNA are overexpressed in HER2 positive breast cancer, with VEGFA and CSF2 also overexpressed in basal breast cancer. Further study of the specific protein function of these factors within their associated cancer subtypes may yield personalized biomarkers and treatment modalities.

Fitzgerald, K. A., et al. (2015). "The use of collagen-based scaffolds to simulate prostate cancer bone metastases with potential for evaluating delivery of nanoparticulate gene therapeutics." Biomaterials **66**: 53-66.

Prostate cancer bone metastases are a leading cause of cancer-related death in men with current treatments offering only marginally improved rates of survival. Advances in the understanding of the genetic basis of prostate cancer provide the opportunity to develop gene-based medicines capable of treating metastatic disease. The aim of this work was to establish a 3D cell culture model of prostate cancer bone metastasis using collagen-based scaffolds, to characterise this model, and to assess the potential of the model to evaluate delivery of gene therapeutics designed to target bone metastases. Two prostate cancer cell lines (PC3 and LNCaP) were cultured in 2D standard culture and compared to 3D cell growth on three different collagen-based scaffolds (collagen and composites of collagen containing either glycosaminoglycan or nanohydroxyapatite). The 3D model was characterised for cell proliferation, viability and for matrix metalloproteinase (MMP) enzyme and Prostate Specific Antigen (PSA) secretion. Chemosensitivity to docetaxel treatment was assessed in 2D in comparison to 3D. Nanoparticles (NPs) containing siRNA formulated using a modified cyclodextrin were delivered to the cells on the scaffolds and gene silencing was quantified. Both prostate cancer cell lines actively infiltrated and proliferated on the scaffolds. Cell culture in 3D resulted in reduced levels of MMP1 and MMP9 secretion in PC3 cells. In contrast, LNCaP cells grown in 3D secreted elevated levels of PSA, particularly on the scaffold composed of collagen and glycosaminoglycans. Both cell lines grown in 3D displayed increased resistance to docetaxel treatment. The cyclodextrin.siRNA nanoparticles achieved cellular uptake and knocked down the endogenous GAPDH gene in the 3D model. In conclusion, development of a novel 3D cell culture model of prostate cancer bone metastasis has been initiated resulting, for the first time, in the successful delivery of gene therapeutics in a 3D in vitro model. Further enhancement of this model will help elucidate the pathogenesis of prostate cancer and also accelerate the design of effective therapies which can penetrate into the bone microenvironment for prostate cancer therapy.

Gotley, D. C., et al. (1996). "The deleted in colon cancer (DCC) gene is consistently expressed in colorectal cancers and metastases." Oncogene **13**(4): 787-795.

The DCC (deleted in colorectal cancer) gene was originally identified as a candidate tumour suppressor gene in colon carcinogenesis on the basis of allelic losses in chromosome 18q.21 in 70% of colon cancers. Reverse transcriptase polymerase chain reaction (RT-PCR) of DCC mRNA suggests that DCC expression may also be reduced in colon cancers. We have used monoclonal antibodies generated against the DCC immunoglobulin-like domain to investigate DCC isoforms and DCC protein expression during colon cancer progression. Normal mucosa and colonic tumour specimens representative of the range of colonic tumour progression from benign adenomatous polyps to metastases were compared by Western blot analyses. We show that while M(r) 194 000 DCC is present in normal colonic mucosa and adenomatous polyps, it is also similarly expressed in colorectal carcinomas and colonic metastases in the liver. The presence of DCC protein is consistent with the presence of DCC mRNA transcripts in the same tissue specimens. Notably DCC was not completely lost in any colonic tumour specimens examined, even those that had progressed to metastatic cancers. Quantitation of DCC protein expression in tissue specimens by densitometry demonstrated that both normal and malignant specimens exhibit a wide range of DCC protein levels and there was no significant correlation between diminished DCC protein expression and colon cancer progression. These results demonstrate the pattern of expression of the DCC gene product in

colonic tumour progression and show that absence of DCC expression is not associated with colonic tumour progression.

Granja Nde, M., et al. (2005). "Potential use of loss of heterozygosity in pleural effusions of breast cancer metastases using the microsatellite marker of the 16q22.1 region of the CDH1 gene." Anal Quant Cytol Histol **27**(2): 61-66.

OBJECTIVE: To evaluate the presence of allelic loss in 16q22.1, including the locus of E-cadherin, in pleural effusions in breast cancer patients. STUDY DESIGN: Molecular analysis of DNA was performed using a DNA extraction kit (NucleoSpin, Macherey-Nagel, Germany). Loss of heterozygosity (LOH) in primary tumors and pleural effusions was analyzed using a microsatellite marker of the CDH1 gene, D16S265, described in previous studies. LOH was evaluated by radioactive polymerase chain reaction assay in 17 samples of pleural effusions and breast tissues (primary tumors and nonneoplastic adjacent tissue) from breast cancer patients: 7 positive for neoplastic cells, 6 suspected and 4 cases without evidence of neoplastic cells in the effusions. RESULTS: Thirteen cases (76%) were informative. LOH was detected in 5 cases (38.5%). In 3 of them LOH was detected only in the cytologic sample, and in 2 of them LOH was detected in the primary tumor and cytologic sample. CONCLUSION: Results show that LOH in the CDH1 gene can identify tumor cells in pleural effusions when morphologic analysis is difficult.

Habib, N. and S. Jensen (2003). "[Gene therapy in the treatment of colorectal cancer liver metastases]." Bull Acad Natl Med **187**(5): 893-897.

In the early 1990s gene therapy had the promise of a new therapeutic modality for many types of cancer including colorectal liver metastases. At the end of that decade the outlook was less encouraging following the death of a patient receiving adenovirus in Pennsylvania. Today the prospect is very bleak following the discovery of leukaemia in children treated for SCiD syndrome in France. Does this mean the start and fall of gene therapy? In this article the concept of gene therapy for colorectal liver metastases will be reviewed. Studies in this field will be cited. It is clear that there are several obstacles to surmount. It is difficult to foresee whether gene therapy will succeed in the clinics in patients with colorectal liver metastases. In this article the author will describe the systematic approach which might lead to success of gene therapy in these patients. In short, gene therapy so far has failed in patients with colorectal liver metastases. However it might succeed in the future. The jury is still out!

Hartung, F., et al. (2017). "A core program of gene expression characterizes cancer metastases." Oncotarget **8**(60): 102161-102175.

While aberrant expression or splicing of metastasis genes conveys to cancers the ability to break through tissue barriers and disseminate, the genetic basis for organ preference in metastasis formation has remained incompletely understood. Utilizing the gene expression profiles from 653 GEO datasets, we investigate whether the signatures by diverse cancers in various metastatic sites display common features. We corroborate the meta-analysis in a murine model. Metastases are generally characterized by a core program of gene expression that induces the oxidative metabolism, activates vascularization/tissue remodeling, silences extracellular matrix interactions, and alters ion homeostasis. This program distinguishes metastases from their originating primary tumors as well as from their target host tissues. Site-selectivity is accomplished through specific components that adjust to the target micro-environment. The same functional groups of gene expression programs are activated in the metastases of B16-F10 cells to various target organs. It remains to be investigated whether these genetic signatures precede implantation and thus determine organ preference or are shaped by the target site and are thus a consequence of implantation. Conceivably, chemotherapy of disseminated cancer might be more efficacious if selected to match the genetic makeup of the metastases rather than the organ of origin by the primary tumor.

He, Q., et al. (2016). "Comparison of KRAS and PIK3CA gene status between primary tumors and paired metastases in colorectal cancer." Onco Targets Ther **9**: 2329-2335.

PURPOSE: In metastatic or recurrent colorectal cancer (MRCRC), the concordance of Kirsten rat sarcoma viral oncogene homolog (KRAS) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) mutation status between the primary tumors and metastases is still controversial. The purpose of this study was to evaluate the association between KRAS and PIK3CA mutational status and various clinicopathologic features, and compare their genotype in primary tumors with that of the paired metastatic tumors. METHOD: We compared the mutation status of KRAS and PIK3CA between the primary tumors and the paired metastases of 59 MRCRC patients with available tissues (resection or biopsy). The presence

of KRAS and PIK3CA mutations were determined by direct sequencing analysis. RESULTS: Seventeen patients (28.8%) had the KRAS mutation and 46 patients (80.0%) had the PIK3CA mutation when considering both the primary and metastatic sites. KRAS mutation was observed in ten primary tumors and eleven related metastases (16.9% vs 18.6%), while PIK3CA mutation was found in 26 primary tumors and 32 related metastases (44.1% vs 54.2%). KRAS status was concordant between primary and metastatic sites in 45 patients (76.3%, kappa =0.157), while the concordance of PIK3CA status was only found in 25 patients (42.4%, kappa =-0.141). The PIK3CA status discordance rate was significantly higher in 40 patients undergoing metachronous resection of primary tumor or metastasis, compared with that in 19 patients with synchronous resection of primary tumor or metastasis (67.5% [27/40] vs 36.8% [7/19]; P=0.026). CONCLUSION: Our results demonstrate that low concordance of KRAS and high discordance of PIK3CA mutational status exist between the primary tumors and paired metastasis, and these findings remind us to have second thoughts about the need to evaluate metastatic tumors separately rather than only based on the primary tumor data when targeted therapy is considered.

Heys, S. D., et al. (1998). "NM23 gene product expression does not predict lymph node metastases or survival in young patients with colorectal cancer." Oncol Rep **5**(3): 735-739.

NM23 gene product is a putative metastases suppressor gene which has structural homology to a nucleoside diphosphate kinase. Previous studies examining the relationship between NM23 gene product expression and survival in patients with colorectal cancer have revealed conflicting results. However, no study has focused on young patients with colorectal cancer. This study was carried out to determine if expression of the NM23 gene product was correlated with metastatic potential and survival in young patients (45 years and under) with colorectal cancer. Eighty- one patients with colorectal cancer were studied and the presence of the NM23 gene product (H1) was detected using standard immunohistochemical techniques. NM23 gene product expression did not correlate with tumour stage, lymph node involvement by tumour, presence of distant metastases, extramural vascular invasion or degree of tumour differentiation. Independent risk factors for overall survival were: Dukes' stage (p=0.00001) and extramural vascular invasion (p=0.003). NM23 expression was not an independent prognostic indicator (p=0.55). Therefore, NM23 expression does not correlate with existing indicators of tumour aggressiveness and behaviour nor is it an independent predictor of survival in young patients with colorectal cancer.

Ishiguro, T., et al. (2010). "Gene trapping identifies chloride channel 4 as a novel inducer of colon cancer cell migration, invasion and metastases." Br J Cancer **102**(4): 774-782.

BACKGROUND: To date, there are few reports on gene products contributing to colon cancer progression. METHODS: We used a gene trap comprised of an enhanced retroviral mutagen (ERM) cassette that includes a tetracycline-responsive promoter upstream of a haemagglutinin (HA) tag and a splice donor site. Integration of the ERM within an endogenous gene yields a tetracycline-regulated HA-tagged transcript. We transduced RKO colon cancer cells expressing a tetracycline trans-activator-off with the ERM-encoding retrovirus and screened for enhanced migration. RESULTS: One clone showed fivefold enhanced migration with tetracycline withdrawal. Rapid amplification of cDNA ends identified the trapped gene as the chloride channel 4 (CLCN4) exchanger. Stable expression of a CLCN4 cDNA enhanced motility, whereas cells knocked down or null for this transcript showed reduced migration/invasion. CLCN4-overexpressing RKO colon cancer cells were more resistant than controls to proton load-induced cytotoxicity, consistent with the H(+)-extruding function of this antiporter. Intra-splenic delivery of RKO-CLCN4 transfectants, but not controls, yielded liver metastases, and transcript levels were higher in colon cancer metastases to the liver when compared with primary tumours. CONCLUSIONS: CLCN4 is a novel driver of colon cancer progression.

Isman, F. K., et al. (2012). "Association between SDF1-3'A or CXCR4 gene polymorphisms with predisposition to and clinicopathological characteristics of prostate cancer with or without metastases." Mol Biol Rep **39**(12): 11073-11079.

In the present study, we aimed to investigate the association between SDF1-3'A and CXCR4 gene polymorphisms and the susceptibility and clinicopathological development of prostate cancer. SDF1-3'A and CXCR4 gene polymorphisms were assessed by polymerase chain reaction restriction-fragment length polymorphism (PCR-RFLP) in 149 healthy subjects and 152 patients with prostate cancer. There were no significant differences in the distributions of SDF-1 and CXCR4 genotypes between controls and prostate cancer patients. However, the patients with AA genotype of SDF1-3'A gene presented a higher risk for developing an advanced disease status as compared to patients with GG homozygotes (aOR = 2.02; 95 % CI = 1.05-3.90;

P = 0.035). In addition, the distribution of AA genotype of SDF1-3'A gene was found significantly increased in the patients with bone metastasis in comparison to those without bone metastasis (aOR = 2.94; 95 % CI = 1.26-6.82; P = 0.012). On the other hand, CXCR4 gene polymorphism was not associated with the clinicopathological characteristics of prostate cancer. Our results suggest that SDF1-3'A and CXCR4 gene polymorphisms may not be risk factors for the susceptibility to prostate cancer. However, SDF1-3'A gene polymorphism may be associated with the progression and bone metastasis of prostate cancer in a Turkish men population.

Iwamoto, M., et al. (2004). "Overexpression of E2F-1 in lung and liver metastases of human colon cancer is associated with gene amplification." Cancer Biol Ther **3**(4): 395-399.

We have shown previously that metastatic tumors of human colorectal cancer in lung as compared to liver have high levels of thymidylate synthase (TS) mRNA expression that correlated with high levels of E2F-1 mRNA expression. We now report that Comparative Genomic Hybridization (CGH) and DNA PCR analyses of lung and liver metastases of human colon cancer show frequent gains in the region of chromosome 20q and have an increase in gene copy number of E2F-1. In as much as TS is transcriptionally regulated by E2F-1, these results provide an explanation for the high levels of TS mRNA noted in some tumor samples.

Kalender, M. E., et al. (2010). "Association between the Thr431Asn polymorphism of the ROCK2 gene and risk of developing metastases of breast cancer." Oncol Res **18**(11-12): 583-591.

The objective of this study was to analyze the genotype distributions and allele frequencies for ROCK2 Thr431Asn and Arg83Lys polymorphisms among breast cancer patients. In this case-control study, 223 patients with breast cancer were recruited and divided into two groups according to metastases (n = 128) and without metastases (n = 95). Genomic DNA from the patients and the control cases (n = 150) was analyzed by real-time PCR using a Light-Cycler. Neither genotype distributions nor the allele frequencies for the Arg83Lys polymorphism showed a significant difference between the groups. Although no marked changes were observed with nonmetastatic group, a statistically significant association was found between the control and metastatic group for the Thr431Asn polymorphism. Although homozygous carriers of the Thr431Thr genotype were more frequent, heterozygous carriers of the Thr431Asn genotype were less frequent among the metastatic patients than among controls. There was also an increase in Thr431 allele (60.5% in patients vs. 51.7% in controls) and decrease in Asn431 allele frequencies (48.3% in control vs. 39.5% in metastatic patients) in metastatic groups (p = 0.036). Our results demonstrate that Thr431Asn polymorphism of the ROCK2 gene could be a risk factor for the metastases of the breast cancer, and may help in predicting the prognosis.

Kim-Fuchs, C., et al. (2014). "The silencing of N-myc downstream-regulated gene-1 in an orthotopic pancreatic cancer model leads to more aggressive tumor growth and metastases." Dig Surg **31**(2): 135-142.

BACKGROUND: The understanding of molecular mechanisms leading to poor prognosis in pancreatic cancer may help develop treatment options. N-myc downstream-regulated gene-1 (NDRG1) has been correlated to better prognosis in pancreatic cancer. Therefore, we thought to analyze how the loss of NDRG1 affects progression in an orthotopic xenograft animal model of recurrence. METHODS: Capan-1 cells were silenced for NDRG1 (C(sil)) or transfected with scrambled shRNA (C(scr)) and compared for anchorage-dependent and anchorage-independent growth, invasion and tube formation in vitro. In an orthotopic xenograft model of recurrence tumors were grown in the pancreatic tail. The effect of NDRG1 silencing was evaluated on tumor size and metastasis. RESULTS: The silencing of NDRG1 in Capan-1 cells leads to more aggressive tumor growth and metastasis. We found faster cell growth, double count of invaded cells and 1.8-fold increase in tube formation in vitro. In vivo local tumors were 5.9-fold larger (p = 0.006) and the number of metastases was higher in animals with tumors silenced for NDRG1 primarily (3 vs. 1.1; p = 0.005) and at recurrence (3.3 vs. 0.9; p = 0.015). CONCLUSION: NDRG1 may be an interesting therapeutic target as its silencing in human pancreatic cancer cells leads to a phenotype with more aggressive tumor growth and metastasis.

Kodura, M. A. and S. Souchelnytskyi (2015). "Breast carcinoma metastasis suppressor gene 1 (BRMS1): update on its role as the suppressor of cancer metastases." Cancer Metastasis Rev **34**(4): 611-618.

BRMS1 was discovered over a decade ago as a potential tumor suppressor gene. In this review, we summarize the recent findings about the structure of BRMS1, mechanisms of its action and a role of BRMS1 in the cancer progression. As a suppressor of metastasis, BRMS1 has demonstrated a variety of ways to act on the cell functions, such as cell migration, invasiveness, angiogenesis, cell survival, cytoskeleton rearrangements, cell adhesion, and immune recognition. This variety of effects is a likely

reason behind the robustness of anti-metastatic influence of BRMS1. Intracellular signaling mechanisms employed by BRMS1 include regulation of transcription, EGF/HER2 signaling, and expression of NF-kB, fascin, osteopontin, and IL-6. Recently reported clinical studies confirm that BRMS1 can indeed be used as a prognostic marker. Approaches to employ BRMS1 in a development of anti-cancer treatment have also been made. The studies reviewed here with respect to BRMS1 structure, cellular effects, intracellular signaling, and clinical value consolidate the importance of BRMS1 in the development of metastasis.

Koehler, A., et al. (2004). "Gene expression profiling of colorectal cancer and metastases divides tumours according to their clinicopathological stage." J Pathol **204**(1): 65-74.

Gene expression profiling of matched colorectal carcinomas and metastases could reveal key molecular events involved in tumour progression and metastasis. Expression profiles have been created from 25 colorectal carcinomas (CRCs, pT1-4), corresponding normal colonic mucosa, and 14 liver metastases using cDNA arrays containing 1176 cancer-related genes (Clontech). Hierarchical clustering clearly distinguished carcinomas from non-cancerous tissues, separated tumours into high-stage (pT4 and extensive lymph node or distant metastases) and low-stage (< or =pT3) groups, and correlated with the histopathological classification in 87% (33/38 cases). Most primary tumours and matched liver metastases clustered on terminal branches of the dendrogram. Statistical analysis (Mann-Whitney U-test) revealed 40 tumour-specific genes (29 up-regulated, 11 down-regulated) which allowed identification of malignant tissue samples by cluster analysis. A specific expression signature in matching metastases was not found, but a set of 23 classifier genes with statistically significant expression patterns in high- and low-stage tumours was identified. These genes may represent important targets in colorectal carcinogenesis and might provide useful clinicopathological tools in the management of colorectal cancer.

Koumura, H., et al. (1997). "[Significance in gene expression of matrix metalloproteinase-9, urokinase-type plasminogen activator and tissue inhibitor of metalloproteinase for metastases of gastric and/or colo-rectal cancer]." Gan To Kagaku Ryoho **24 Suppl 2**: 324-331.

In order to clarify the role of matrix metalloproteinase-9 (MMP-9), urokinase-type plasminogen activator (uPA) and tissue inhibitor of metalloproteinase (TIMP) in metastases of gastroenterological cancer, their gene expression in the primary lesions on 47 gastric or 48 colorectal cancer patients was examined by RT-PCR method. 1) The expression of MMP-9, uPA, and TIMP was observed in 55.3%, 66.0% and 87.2% of gastric cancer and in 54.2%, 70.8%, and 89.6% of colorectal cancer, respectively. 2) In the cases with either peritoneal dissemination or lymph node metastases, the incidence of gene expression of MMP-9 was significantly higher in comparison to the cases without those metastases. The same result was observed as for uPA. 3) In the cases with liver metastases, the incidence of gene expression of MMP-9 was significantly higher in comparison to the cases without liver metastasis. The same result was observed as for uPA. The above results indicate that MMP-9 and uPA might play important roles in the peritoneal and lymph node metastases in gastric cancer and in liver metastasis in colorectal cancer. Therefore the investigation of their gene expression in the primary lesions of cancer could be one of the useful methods for the prediction of metastasis, leading to the best decision as to the treatment.

Kwong, Y. L., et al. (1996). "Adenoviral-mediated suicide gene therapy for hepatic metastases of breast cancer." Cancer Gene Ther **3**(5): 339-344.

Metastases of breast cancer are a major cause of treatment failure. To evaluate the therapeutic efficacy of suicide gene therapy in metastatic breast cancer, we used the herpes simplex virus thymidine kinase (HSV-tk) gene followed by ganciclovir (GCV) administration to treat breast cancer, generated by an adenocarcinoma cell line MOD in syngeneic mice. The bystander effect of HSV-tk + GCV on tumor cell killing was illustrated by demonstrating complete regression of subcutaneous tumors consisting of 90% parental tumor cells and 10% HSV-tk transformed tumor cells. To establish a model of breast cancer metastases in the liver, tumors were generated by intra-hepatic implantation of MOD cells in syngeneic animals. Two weeks after tumor cell implantation, replication defective adenoviral vectors expressing HSV-tk (ADV.tk), or beta-galactosidase (ADV. beta-Gal) were injected intratumorally, followed by buffer or GCV administration. Treatment with ADV.tk + GCV resulted in significant regression of tumor ($P < .001$), as assessed by computerized morphometric analysis of residual tumor. This was reflected as a significant prolongation of survival in treated animals ($P < .001$). These results demonstrate that ADV-mediated suicide gene therapy in vivo can be incorporated in a comprehensive treatment strategy for liver metastases of breast cancer.

Langsenlehner, U., et al. (2015). "Association of vascular endothelial growth factor--a gene polymorphisms and haplotypes with breast cancer metastases." Acta Oncol **54**(3): 368-376.

BACKGROUND: Vascular endothelial growth factor (VEGF-A) is a key regulator of tumor-induced angiogenesis and essential for tumor growth and distant tumor spread. The aim of the present study was to evaluate the role of VEGF-A polymorphisms and haplotypes for metastatic progression in breast cancer patients. MATERIAL AND METHODS: We performed a prospective study including 801 breast cancer patients. Occurrence of metastases was examined in regular follow-up investigations. Seven VEGF-A polymorphisms were selected and determined by 5'-nuclease assays (TaqMan). The selection of VEGF-A variants was based upon their location (promoter or UTR) as well as a minor allele frequency of at least 0.10. Haplotypes and linkage disequilibrium were determined using the Haploview program. RESULTS: Within a median follow-up time of 84 months, 165 (21%) patients developed distant metastases. In univariate analysis, carriers of the CCCCC haplotype formed by five polymorphisms upstream the coding region were at decreased risk of distant metastases [hazard ratio (HR)=0.743; 95% CI 0.579-0.953; p=0.019]. Univariate analysis also revealed a decreased risk of distant metastases for postmenopausal patients carrying the -634G>C polymorphism (HR 0.704; 95% CI 0.514-0.965; p=0.029) and the CCCCC haplotype (HR=0.645; 95% CI 0.464-0.898; p=0.009). After adjustment for other co-variates, the HR for distant metastases was 0.651 (95% CI 0.447-0.948) for postmenopausal carriers of the -634G>C polymorphism (p=0.025; corrected p-value=0.262), and 0.586 (95% CI 0.393-0.873) for postmenopausal patients with the CCCCC haplotype (p=0.009, corrected p-value=0.189). CONCLUSION: The results from univariate and multivariate analyses suggest an influence of VEGF-A gene variants on the development of distant metastases in breast cancer patients. However, none of the observed associations reached statistical significance after correction for the effects of multiple testing. Additional prospective and sufficiently powered studies are essential before firm conclusions about the role of VEGF-A gene variants for distant progression in breast cancer can be drawn.

Lesoon-Wood, L. A., et al. (1995). "Systemic gene therapy with p53 reduces growth and metastases of a malignant human breast cancer in nude mice." Hum Gene Ther **6**(4): 395-405.

We report on an in vivo delivery system that attenuates the growth, in nude mice, of a malignant human breast cancer cell line containing a p53 mutation. Nude mice, inoculated with breast carcinoma cells, were injected every 10-12 days with a liposome-p53 complex via the tail vein. A significant reduction of greater than 60% in primary tumor volume was observed as compared to the control groups. Furthermore, when individual growth patterns of the tumors were assessed, we found that primary tumor size regressed in the majority of p53-treated animals (8/15), whereas only one tumor in the control groups (1/22) regressed. The eight tumors that regressed with the liposome-p53 complex showed no evidence of relapse for 1 month after the cessation of treatment. We also determined that the administration of the liposome-p53 complex reduced the incidence of metastases. The MDA-MB-435 tumor cells, transduced with the lacZ gene, facilitated quantitation of beta-galactosidase activity and tumor burden in the lungs. The number of metastatic cells in the lung was significantly lower in the p53-treated group (0.53 +/- 0.43 x 10(6), $p < 0.01$) than in either the vector-treated (8.1 +/- 3.7 x 10(6)) or untreated control groups (15.8 +/- 5.9 x 10(6)). Thus, systemic administration of the liposome-p53 complex reduced not only the size of the primary tumors but, more importantly, prevented the relapse and metastases of these tumors.

Levine, E. A., et al. (2012). "Gene expression profiling of peritoneal metastases from appendiceal and colon cancer demonstrates unique biologic signatures and predicts patient outcomes." J Am Coll Surg **214**(4): 599-606; discussion 606-597.

BACKGROUND: Treatment of peritoneal metastases from appendiceal and colon cancer with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) shows great promise. Although long-term disease-free survival is achieved in some cases with this procedure, many patients have recurrence. Oncologists have treated such recurrences of appendiceal cancer similarly to colorectal carcinoma, which has been largely ineffective. This study uses gene expression analysis of peritoneal metastases to better understand these neoplasms. STUDY DESIGN: From a prospectively maintained database and tissue bank, 41 snap frozen samples of peritoneal metastases (26 appendiceal, 15 colorectal) from patients undergoing HIPEC with complete cytoreduction and more than 3 years of follow-up underwent global gene expression analysis. Distinct phenotypes were identified using unsupervised hierarchical clustering based on differential gene expression. Survival curves restratified by genotype were generated. RESULTS: Three distinct phenotypes were found, 2 consisting of predominantly low grade appendiceal samples (10 of 13 in Cluster 1 and 15 of 20 in Cluster 2) and 1

consisting of predominantly colorectal samples (7 of 8 in Cluster 3). Cluster 1 consisted of patients with good prognosis and Clusters 2 and 3 consisted of patients with poor prognosis (p = 0.006). Signatures predicted survival of low- (Cluster 1) vs high-risk (Cluster 2) appendiceal (p = 0.04) and low-risk appendiceal (Cluster 1) vs colon primary (Cluster 3) (p = 0.0002). CONCLUSIONS: This study represents the first use of gene expression profiling for appendiceal cancer, and demonstrates genomic signatures quite distinct from colorectal cancer, confirming their unique biology. Consequently, therapy for appendiceal lesions extrapolated from colonic cancer regimens may be unfounded. These phenotypes may predict outcomes guiding patient management.

Li, L., et al. (2015). "Pituitary tumor-transforming gene 1 enhances metastases of cervical cancer cells through miR-3666-regulated ZEB1." Tumour Biol.

Early cancer metastases often occur in cervical cancer (CC) patients, resulting in poor prognosis and poor therapeutic outcome after resection of primary cancer. Hence, there is a compelling requirement for elucidating the molecular mechanisms underlying the CC cell invasiveness. Recently, the role of microRNAs (miRNAs) and pituitary tumor-transforming gene 1 (Pttg1) in the carcinogenesis of CC has been reported. Nevertheless, the relationship between miRNAs and Pttg1 remains ill-defined. Here, we showed that the levels of miR-3666 were significantly decreased and the levels of zinc finger E-box binding homeobox 1 (ZEB1) and Pttg1 were significantly increased in the CC specimens from patients, compared to the paired non-tumor tissue. Moreover, the levels of miR-3666 and ZEB1 inversely correlated. Bioinformatics analyses showed that miR-3666 targeted the 3'-untranslated region (3'-UTR) of ZEB1 messenger RNA (mRNA) to inhibit its translation, which was confirmed by luciferase reporter assay. Moreover, Pttg1 overexpression inhibited miR-3666 and subsequently increased ZEB1 and cell invasion, while Pttg1 depletion increased miR-3666 and subsequently decreased ZEB1 and cell invasion. Together, our data suggest that Pttg1 may increase CC cell metastasis, possibly through miR-3666-regulated ZEB1 levels.

Liang, J., et al. (2018). "CDK8 selectively promotes the growth of colon cancer metastases in the liver by regulating gene expression of TIMP3 and matrix metalloproteinases." Cancer Res.

Unresectable hepatic metastases of colon cancer respond poorly to existing therapies and are a major cause of colon cancer lethality. In this study, we evaluated the therapeutic viability of targeting the Mediator kinase CDK8, an early clinical stage drug target, as a means to suppress metastasis of colon cancer. CDK8 was amplified or overexpressed in many colon cancers and CDK8 expression correlated with shorter patient survival. Knockdown or inhibition of CDK8 had little effect on colon cancer cell growth but suppressed metastatic growth of mouse and human colon cancer cells in the liver. This effect was due in part to inhibition of already established hepatic metastases, indicating therapeutic potential of CDK8 inhibitors in the metastatic setting. In contrast, knockdown or inhibition of CDK8 had no significant effect on the growth of tumors implanted subcutaneously, intrasplenically, or orthotopically in the cecum. CDK8 mediated colon cancer growth in the liver through downregulation of matrix metalloproteinase (MMP) inhibitor TIMP3 via TGFbeta/SMAD-driven expression of a TIMP3-targeting microRNA, miR-181b, along with induction of Mmp3 in murine or MMP9 in human colon cancer cells via Wnt/beta-catenin-driven transcription. These findings reveal a new mechanism for negative regulation of gene expression by CDK8 and a site-specific role for CDK8 in colon cancer hepatic metastasis. Our results indicate the utility of CDK8 inhibitors for the treatment of colon cancer metastases in the liver and suggest that CDK8 inhibitors may be considered in other therapeutic settings involving TGFbeta/SMAD or Wnt/beta-catenin pathway activation.

Makohon-Moore, A. P., et al. (2017). "Limited heterogeneity of known driver gene mutations among the metastases of individual patients with pancreatic cancer." Nat Genet **49**(3): 358-366.

The extent of heterogeneity among driver gene mutations present in naturally occurring metastases-that is, treatment-naive metastatic disease-is largely unknown. To address this issue, we carried out 60x whole-genome sequencing of 26 metastases from four patients with pancreatic cancer. We found that identical mutations in known driver genes were present in every metastatic lesion for each patient studied. Passenger gene mutations, which do not have known or predicted functional consequences, accounted for all intratumoral heterogeneity. Even with respect to these passenger mutations, our analysis suggests that the genetic similarity among the founding cells of metastases was higher than that expected for any two cells randomly taken from a normal tissue. The uniformity of known driver gene mutations among metastases in the same patient has critical and encouraging implications for the success of future targeted therapies in advanced-stage disease.

Martinet, O., et al. (2002). "T cell activation with systemic agonistic antibody versus local 4-1BB ligand gene delivery combined with interleukin-12 eradicate liver metastases of breast cancer." Gene Ther **9**(12): 786-792.

We have shown that interleukin-12 (IL-12) generated a strong, albeit transient, anti-tumor response, mostly mediated by natural killer (NK) cell. T cell participation, in addition to NK cells, was essential for persistence of the anti-tumor response. Ligation of 4-1BB, a co-stimulatory receptor expressed on activated T cells, is known to amplify T cell-mediated immunity. In this study, we compared the effect of a systemically delivered agonistic anti-4-1BB monoclonal antibody (anti-4-1BB mAb) with intra-tumoral adenoviral-mediated gene transfer of the 4-1BB ligand (ADV/4-1BBL) to liver metastases in a syngeneic animal model of breast cancer. Both treatments induced a dramatic regression of pre-established tumor. When combined with intra-tumoral delivery of the IL-12 gene, both anti-4-1BB mAb and ADV/4-1BBL were synergistic and led to survival rates of 87% and 78%, respectively. The anti-tumor immunity is mainly mediated by CD4+ T cells in IL-12 plus 4-1BB ligand-treated animals, and CD8+ T cells in IL-12 plus anti-4-1BB mAb-treated animals. However, only long-term survivors after treatment with IL-12 and 4-1BBL genes have showed significantly potent, systemic, and tumor-specific T cell-mediated immunity.

Martiniello-Wilks, R., et al. (2004). "Purine nucleoside phosphorylase and fludarabine phosphate gene-directed enzyme prodrug therapy suppresses primary tumour growth and pseudo-metastases in a mouse model of prostate cancer." J Gene Med **6**(12): 1343-1357.

Gene-directed enzyme prodrug therapy based on the E. coli purine nucleoside phosphorylase (PNP) gene produces efficient tumour cell killing. PNP converts adenosine analogs into toxic metabolites that diffuse across cell membranes to kill neighbouring untransduced cells (PNP-GDEPT). Interference with DNA, RNA and protein synthesis kills dividing and non-dividing cells, an important consideration for slow-growing prostate tumours. This study examined the impact of administering PNP-GDEPT into orthotopically grown RM1 prostate cancers (PCas) on the growth of lung pseudo-metastases of immunocompetent mice. C57BL/6 mice bearing orthotopic RM1 PCas received a single intraprostatic injection of OAdV220 (10(10) particles), a recombinant ovine atadenovirus containing the PNP gene controlled by the Rous Sarcoma virus promoter, followed by fludarabine phosphate (approximately 600 mg/m(2)/day) administered intraperitoneally (ip) once daily for 5 days. Pseudo-metastases were induced 2 days after intraprostatic vector administration by tail-vein injection of untransduced RM1 cells. Mice given PNP-GDEPT showed a significant reduction both in prostate volume (approximately 50%) and in lung colony counts (approximately 60%). Apoptosis was increased two-fold in GDEPT-treated prostates compared with controls ($P < 0.01$), but was absent in the lungs. Staining for proliferating cell nuclear antigen (PCNA) indicated that proliferation of both RM1 prostate tumours ($P < 0.01$) and lung colonies ($P < 0.01$) was significantly suppressed after GDEPT. Although prostate tumour immune cell infiltration did not differ significantly between treatments, immunostaining for Thy-1.2 (CD90) showed that GDEPT promoted Thy-1.2(+) cell infiltration into the prostate tumour site. This study showed that a single course of PNP-GDEPT significantly suppressed local PCa growth and reduced lung colony formation in the aggressive RM1 tumour model.

Masago, K., et al. (2010). "Good Clinical Response to Erlotinib in a Non-Small Cell Lung Cancer Patient Harboring Multiple Brain Metastases and a Double Active Somatic Epidermal Growth Factor Gene Mutation." Case Rep Oncol **3**(2): 98-105.

Recently, 2 small molecule kinase inhibitors (TKIs), targeting epidermal growth factor receptor (EGFR), have proven effective in the treatment of non-small cell lung cancer. However, it is unknown whether the EGFR double activating mutation of L858R in exon 21 and the in-frame deletion in exon 19 is a predictor of the effectiveness of EGFR-TKIs. We report for the first time a case of non-small cell lung cancer with central nervous system metastases harboring a rare EGFR double activating mutation who showed a good clinical response to erlotinib, regardless of his poor performance status, as swallowing is not possible. Therefore, we suggest that erlotinib may become a therapeutic choice in cases of central nervous system metastases even with poor performance status.

Matsuyama, R., et al. (2006). "Predicting 5-fluorouracil chemosensitivity of liver metastases from colorectal cancer using primary tumor specimens: three-gene expression model predicts clinical response." Int J Cancer **119**(2): 406-413.

We identified genes related to 5-fluorouracil (5-FU) sensitivity in colorectal cancer and utilized these genes for predicting the 5-FU sensitivity of liver metastases. Eighty-one candidate genes involved in 5-FU resistance in gastric and colon cancer cell lines were previously identified using a cDNA microarray. In this study, the mRNA

expression levels of these 81 selected genes and the genes of 5-FU-related enzymes, including thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD) and orotate phosphoribosyltransferase (OPRT), were measured using real-time quantitative RT-PCR assays of surgically resected materials from primary colorectal tumors in 22 patients. Clinical responses were estimated by evaluating the effects of 5-FU-based hepatic artery injection (HAI) chemotherapy for synchronous liver metastases. Four genes (TNFRSF1B, SLC35F5, NAG-1 and OPRT) had significantly different expression profiles in 5-FU-nonresponding and responding tumors ($p < 0.05$). A "Response Index" system using three genes (TNFRSF1B, SLC35F5 and OPRT) was then developed using a discriminate analysis; the results were well correlated with the individual chemosensitivities. Among the 11 cases with positive scores in our response index, 9 achieved a reduction in their liver metastases after 5-FU-based chemotherapy, whereas only 1 of the 11 cases with negative scores responded well to chemotherapy. Our "Response Index" system, consisting of TNFRSF1B, SLC35F5 and OPRT, has great potential for predicting the efficacy of 5-FU-based chemotherapy against liver metastases from colorectal cancer.

Maurer, C. A., et al. (1999). "Reduced expression of the metastasis suppressor gene KAI1 in advanced colon cancer and its metastases." Surgery **126**(5): 869-880.

BACKGROUND: The expression of the KAI1 gene and its gene product were studied in metastatic and non-metastatic human colorectal cancer to evaluate its role in the metastatic process. METHODS: KAI1 mRNA and protein expression was examined in 36 primary colorectal carcinomas and 6 liver metastasis using Northern blot and Western blot analyses. Forty-six normal colonic tissue samples served as controls. The exact site of KAI1 expression was analyzed by in situ hybridization and by immunohistochemistry in primary tumors, in the corresponding normal tissues, in lymph node metastases and liver metastases. RESULTS: Densitometric analysis of Northern blots revealed overexpression of KAI1 mRNA in 87% of colonic cancer tissues in comparison with the corresponding normal colonic tissues. This increase was 9.1-fold in median ($P < .001$). KAI1 mRNA expression was strongly dependent on tumor stage. Colorectal cancer at stages II and III revealed significantly higher KAI1 mRNA levels than stage IV tumors ($P < .03$ and $P < .015$, respectively) or normal controls. In addition, liver metastases showed reduced KAI1 mRNA expression when compared with their corresponding primary tumor. In situ hybridization confirmed the stage-dependent expression results obtained by Northern blots, in which the KAI1 mRNA signal was exhibited almost exclusively in the epithelial cells. Lymph node and liver metastases were largely devoid of KAI1 mRNA. Western blot analysis showed a highly significant increase of KAI1 protein level in stage II cancers in comparison with the normal colon ($P < .001$) but also in comparison with the more advanced tumor stages III and IV ($P < .03$) and $P < .02$, respectively), when metastases were already present. In accordance, KAI1 immunostaining decreased successively with the advance of the tumor stage and was absent in lymph node and liver metastases. CONCLUSIONS: These data demonstrate that the KAI1 mRNA expression and the KAI1 protein level increase in an earlier tumor stage of colorectal cancer, decrease in advanced stages, and are lost in metastases. The loss of KAI1 might favor the ability of colorectal cancer cells to metastasize.

Mayer-Kuckuk, P., et al. (2003). "Imaging of dihydrofolate reductase fusion gene expression in xenografts of human liver metastases of colorectal cancer in living rats." Eur J Nucl Med Mol Imaging **30**(9): 1281-1291.

Radionuclide imaging has been demonstrated to be feasible to monitor transgene expression in vivo. We hypothesized that a potential application of this technique is to non-invasively detect in deep tissue, such as cancer cells metastatic to the liver, a specific molecular response following systemic drug treatment. Utilizing human colon adenocarcinoma cells derived from a patient's liver lesion we first developed a nude rat xenograft model for colorectal cancer metastatic to the liver. Expression of a dihydrofolate reductase-herpes simplex virus 1 thymidine kinase fusion (DHFR-HSV1 TK) transgene in the hepatic tumors was monitored in individual animals using the tracer [(124)I]2'-fluoro-2'-deoxy-5-iodouracil-beta- d-arabinofuranoside (FIAU) and a small animal micro positron emission tomograph (microPET), while groups of rats were imaged using the tracer [(131)I]FIAU and a clinical gamma camera. Growth of the human metastatic colorectal cancer cells in the rat liver was detected using magnetic resonance imaging and confirmed by surgical inspection. Single as well as multiple lesions of different sizes and sites were observed in the liver of the animals. Next, using a subset of rats bearing hepatic tumors, which were retrovirally bulk transduced to express the DHFR-HSV1 TK transgene, we imaged the fusion protein expression in the hepatic tumor of living rats using the tracer [(124)I]FIAU and a microPET. The

observed deep tissue signals were highly specific for the tumors expressing the DHFR-HSV1 TK fusion protein compared with parental untransduced tumors and other tissues as determined by gamma counting of tissue samples. A subsequent study used the tracer [(131)I]FIAU and a gamma camera to monitor two groups of transduced hepatic tumor-bearing rats. Prior to imaging, one group was treated with trimetrexate to exploit DHFR-mediated upregulation of the fusion gene product. Imaging in the living animal as well as subsequent gamma counting of tissue samples showed increased signal and tracer accumulation, respectively, as compared to the group not treated with the antifolate. It is concluded that the two examined nucleotide imaging methods are feasible techniques for monitoring of DHFR-HSV TK fusion protein expression in hepatic colorectal tumor tissue in living animals.

Medimegh, I., et al. (2014). "Wild-type genotypes of BRCA1 gene SNPs combined with micro-RNA over-expression in mammary tissue leading to familial breast cancer with an increased risk of distant metastases' occurrence." Med Oncol **31**(11): 255.

Germ line deleterious mutations of BRCA1 gene are not the unique factor that could inactivate BRCA1 protein which leads to familial breast cancer onset with distant metastases' occurrence. The present research explores the role that could be assigned to BRCA1 SNPs to inactivate BRCA1 protein and therefore to the occurrence of familial breast cancer with an increased risk of distant metastases' occurrence. The presence or the absence of BRCA1 protein was first analyzed by applying the immunohistochemistry technique to the tumors with sporadic and familial breast cancer. Then, a case-control study was conducted including 40 patients with familial breast cancer, 46 ones with sporadic breast cancer and 34 healthy controls based on the genotyping of nine BRCA1 SNPs (c.442.58delT, c.2082C>T, c.2311T>C, c.2612C>T, c.3113A>G, c.3119G>A, c.3548A>G, c.4308T>C and 4837A>G) via direct sequencing. Finally, the functional role that could be assigned to these SNPs was focused upon. miRbase site was used as a bioinformatics tool to predict potential micro-RNAs (miRs) targeting SNPs that are associated with familial breast cancer according to the results of this research. These predicted miRs were confirmed by Q-PCR analysis and correlated with BRCA1 protein expression among patients along with potential distant metastases. Clinical outcome showed that distant metastasis concerned 45 % of familial breast cancer patients and 19.5 % with sporadic breast cancer. Analysis of BRCA1 protein expression revealed a negative staining among 46.6 % of familial breast cancer patients and only 16.6 % within sporadic breast cancer ones. The association of four variants was identified within BRCA1 gene (c.442.58 delT, c.2311T>C, c.2612C>T and c.4308T>C) to familial breast cancer across their wild genotypes. miR-1179 was selected as potential miR that targets the region of BRCA1 mRNA containing the c.2311T>C variant within the TT genotype. The expression of miR-1179 was significantly associated with familial breast cancer patients without BRCA1 deleterious mutations compared to those with sporadic breast cancer according to TT genotype along with BRCA1 negative staining and according to the occurrence of distant metastases. Combination between TT genotype of c.2311T>C and miR-1179 over-expression could generate a lack of BRCA1 protein leading to a high risk of familial breast cancer with distant metastases.

Miyagi, M., et al. (2007). "The TIMP-1 gene transferred through adenovirus mediation shows a suppressive effect on peritoneal metastases from gastric cancer." Int J Clin Oncol **12**(1): 17-24.

BACKGROUND: It has become clear in recent years that peritoneal metastasis takes place as the result of a multistep process involving attachment, invasion, proliferation, and angiogenesis. The aim of the present study was to evaluate the suppressive effect of tissue inhibitor of metalloproteinase-1 (TIMP-1) gene transfer on peritoneal dissemination. METHODS: We established a high-potential peritoneal metastasis cell line (MKN-45P), using the gastric cancer cell line MKN-45, and developed a peritoneal metastasis model in nude mice. The TIMP-1 gene was transferred to MKN-45 or MKN-45P by adenoviral transfection, and we performed an in vitro invasion assay and an in vivo study, using the peritoneal metastasis model. The TIMP-1 transfected group was compared with a non-virus group and a Lac-Z transfected group. RESULTS: The in vitro invasion assay showed that the number of invasive cells was significantly reduced in the TIMP-1 transfected group compared with that in the non-virus group and the Lac-Z transfected group, Moreover, the in vivo studies showed that the number and the weight of the peritoneal nodes in the TIMP-1 transfected group were significantly less than those in the Lac-Z transfected group, and less than those in the non-viral group. No bloody ascites was recognized in the TIMP-1 transfected group. The mean number of tumor vessels in the non-virus group and the Lac-Z group was significantly higher than that in the TIMP-1 group. CONCLUSION: TIMP-1 demonstrated an inhibitory effect on angiogenesis, and may be worthwhile investigating for use as a future therapy for peritoneal dissemination.

Mori, M., et al. (2004). "S100A11 gene identified by in-house cDNA microarray as an accurate predictor of lymph node metastases of gastric cancer." Oncol Rep **11**(6): 1287-1293.

Gastric cancer is one of the most common malignancies in the world, and in Asian countries its incidence and mortality rates are very high. Worldwide, Japan ranks first in the incidence of this type of cancer for both sexes. To shed light on the mechanisms underlying the development and/or progression of gastric cancer, we compared the expression profiles in gastric cancer cells obtained from surgical dissection of 20 gastric adenocarcinoma specimens with those in the corresponding non-cancerous mucosa, by cDNA microarray analysis. In total, 8,000 cDNA clones were randomly picked up and their 5'-end nucleotide sequences were determined. On the basis of sequence information, 4,608 independent clones were selected and used to produce the cDNA microarray. We identified 26 genes that were commonly up-regulated and 44 genes that were commonly down-regulated in cancerous tissues. To validate the cDNA microarray analysis, real-time PCR was performed. We found that gene S100A11 expression was associated with the development of lymph node metastases. S100A11 gene expression was clearly up-regulated in specimens from patients with lymph node metastases relative to those from patients without lymph node metastases. S100A11 gene expression status was useful to distinguish gastric cancers with lymph node metastases from those without lymph node metastasis. This genome-wide information contributes to an improved understanding of molecular changes during the development of gastric cancers. It may also help clinicians predict the development of lymph node metastases and assist researchers in identifying novel therapeutic targets for patients with gastric cancer.

Naruke, A., et al. (2015). "Comparison of site-specific gene expression levels in primary tumors and synchronous lymph node metastases in advanced gastric cancer." Gastric Cancer **18**(2): 262-270.

BACKGROUND: Many malignant tumors consist of heterogeneous subpopulations of cells. This heterogeneity is associated with genetic characteristics. However, it remains unclear whether gene expression levels differ among specific sites of tumors in gastric cancer. METHODS: We studied differences in gene expression levels among specific sites of primary tumors and synchronous lymph node metastases, using formalin-fixed, paraffin-embedded specimens resected surgically from 48 patients with previously untreated advanced gastric cancer. Specimens were obtained by laser-captured microdissection from five regions: (1) nonneoplastic mucosa, (2) surface layer (mucosa) of the primary tumor (surface sections), (3) middle layer (submucosa) of the primary tumor (middle sections), (4) the deepest layer of the primary tumor (muscularis propria or deeper) at the site of deepest invasion (deep sections), and (5) level 1 synchronous lymph node metastasis (lymph node metastases). Expression levels of the following target genes were determined by quantitative real-time polymerase chain reaction: thymidylate synthase (TS), thymidine phosphorylase (TP), dihydropyrimidine dehydrogenase (DPD), epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), and hypoxia-inducible factor-1alpha (HIF1alpha). RESULTS: TP, DPD, EGFR, and HIF1alpha gene expression levels were significantly higher in deep sections than in surface sections. TP, EGFR, VEGF, and HIF1alpha gene expression levels were significantly higher in lymph node metastases than in surface sections. TP, DPD, EGFR, VEGF, and HIF1alpha gene expression levels were positively correlated with the specific samples harvested from the tumors. CONCLUSIONS: Our results show that the expression levels of some genes in tumor cells can change in specific sites of tumors and can become higher in association with tumor progression.

Nasu, Y., et al. (1999). "Adenovirus-mediated interleukin-12 gene therapy for prostate cancer: suppression of orthotopic tumor growth and pre-established lung metastases in an orthotopic model." Gene Ther **6**(3): 338-349.

Interleukin-12 (IL-12) can elicit potent antitumoral effects that involve the recruitment of specific immune effector cells. We investigated the efficacy of a single injection of a recombinant adenovirus expressing murine IL-12 (AdmIL-12) directly into orthotopic mouse prostate carcinomas generated from a poorly immunogenic cell line (RM-9) derived from the mouse prostate reconstitution system. Significant growth suppression (> 50% reduction of tumor weight) and increased mean survival time (23.4 to 28.9 days) were observed compared with controls. Suppression of pre-established lung metastases was also observed following the injection of AdmIL-12 into the orthotopic tumor. Cytolytic natural killer cell activity was markedly enhanced 1-2 days after virus injection. Immunohistochemical analysis showed significantly elevated intratumoral infiltration of CD4+ and CD8+ T cells 7 days after virus injection. However, splenocyte-derived cytotoxic T lymphocytes were not detected during the 14 days following treatment. Increased numbers of nitric oxide synthase-positive

macrophages were seen in the AdmIL-12 treated group 7 days following injection. Systemic inhibition of natural killer cells with antiasialo-GM1 serum led to increased numbers of lung metastases in AdmIL-12-treated orthotopic tumors but did not affect local tumor growth. In this model system the antitumor effects of a single injection of adenovirus-mediated IL-12 appears to be based to a large extent on the activation of nitric oxide synthase in macrophages and possibly T cell activities, whereas the relatively early cytolytic activity of natural killer cells are largely but not exclusively responsible for the antimetastatic effects.

Nicos, M., et al. (2016). "Sensitive methods for screening of the MEK1 gene mutations in patients with central nervous system metastases of non-small cell lung cancer." Clin Transl Oncol **18**(10): 1039-1043.

BACKGROUND: The mitogen-activated protein kinases 1 and 2 (MEK1, MEK2) are fundamental partners in the RAS-RAF-MEK-ERK pathway that is involved in regulation of cell proliferation, differentiation and survival. Downregulation of the MEK cascades has been implicated in acquiring of the malignant phenotype in various cancers. Somatic mutations in MEK1 gene (substitutions K57N, Q56P, D67N) were described in <1 % of non-small cell lung cancer (NSCLC) and they were more commonly reported in adenocarcinoma patients with current or former smoking status. MATERIALS AND METHODS: In the following study, we assessed the MEK1 gene mutations in 145 FFPE tissue samples from central nervous system (CNS) metastases of NSCLC using HRM-PCR and ASP-qPCR techniques. The studied group was heterogeneous in terms of histopathology and smoking status. The prevalence of the MEK1 gene mutation was correlated with the occurrence of mutations in KRAS, EGFR, DDR2, PIK3CA, NRAS, HER2, AKT1 and PTEN genes. RESULTS: Using HRM and ASP-qPCR methods we identified one (0.7 %; 1/145) MEK1 substitution (Q56P) in CNS metastases of NSCLC. The mutation was identified in a single, 50-year-old, current smoking men with adenocarcinoma (1.25 %; 1/80 of all adenocarcinomas). CONCLUSIONS: According to the current knowledge, the incidence of MEK1 gene mutation in CNS metastatic lesion of NSCLC is the first such report worldwide. The analysis of gene profile in cancer patients may extend the scope of molecularly targeted therapies used both in patients with primary and metastatic tumors of NSCLC.

Nicos, M., et al. (2017). "Evaluation of ALK gene rearrangement in central nervous system metastases of non-small-cell lung cancer using two-step RT-PCR technique." Clin Transl Oncol **19**(12): 1447-1453.

PURPOSE: RT-PCR technique has showed a promising value as pre-screening method for detection of mRNA containing abnormal ALK sequences, but its sensitivity and specificity is still discussable. Previously, we determined the incidence of ALK rearrangement in CNS metastases of NSCLC using IHC and FISH methods. MATERIALS: We evaluated ALK gene rearrangement using two-step RT-PCR method with EML4-ALK Fusion Gene Detection Kit (Entrogen, USA). The studied group included 145 patients (45 females, 100 males) with CNS metastases of NSCLC and was heterogeneous in terms of histology and smoking status. RESULTS: 21% of CNS metastases of NSCLC (30/145) showed presence of mRNA containing abnormal ALK sequences. FISH and IHC tests confirmed the presence of ALK gene rearrangement and expression of ALK abnormal protein in seven patients with positive result of RT-PCR analysis (4.8% of all patients, 20% of RT-PCR positive patients). RT-PCR method compared to FISH analysis achieved 100% of sensitivity and only 82.7% of specificity. IHC method compared to FISH method indicated 100% of sensitivity and 97.8% of specificity. In comparison to IHC, RT-PCR showed identical sensitivity with high number of false positive results. CONCLUSION: Utility of RT-PCR technique in screening of ALK abnormalities and in qualification patients for molecularly targeted therapies needs further validation.

Nicos, M., et al. (2014). "Sensitive methods for detection of the S768R substitution in exon 18 of the DDR2 gene in patients with central nervous system metastases of non-small cell lung cancer." Med Oncol **31**(10): 176.

Discoidin death receptor 2 (DDR2) receptor belongs to a DDR family that shows a tyrosine kinase activity. The somatic mutations in DDR2 gene, reported in non-small cell lung cancer (NSCLC), are involved in up-regulation of cells' migration, proliferation and survival. A S768R substitution in DDR2 gene was commonly reported in squamous cell lung carcinoma. Clinical data of patients carrying the DDR2 gene mutation suggest that its presence can be independent of gender and age. The effectiveness of an oral dual-specific (Src and Abl) multikinase inhibitors-dasatinib-was observed in different cell lines and in some NSCLC patients with identified DDR2 mutation. In the present study, we have used three molecular methods (ASP-real-time PCR, ASP-DNA-FLA PCR and direct sequencing) to detect the DDR2 gene mutation in 143 patients with

NSCLC metastases to the central nervous system (CNS). The prevalence of the DDR2 gene mutation was correlated with the occurrence of mutations in the EGFR, KRAS, HER2 and BRAF genes. We identified three patients (2.1% of studied group) with DDR2 mutation. The mutation was observed in two patients with low differentiated squamous cell lung cancer and in one patient with adeno-squamous cell carcinoma (ADSCC). In ADSCC patients, DDR2 mutation coexisted with G12C substitution in KRAS gene. According to the current knowledge, examination of the presence of the DDR2 gene mutation in metastatic lesion is the first such report worldwide. The information, that these driver mutations are present in CNS metastases of NSCLC, could broaden therapeutic choices in such group of patients.

Niedergethmann, M., et al. (2007). "Gene expression profiling of liver metastases and tumour invasion in pancreatic cancer using an orthotopic SCID mouse model." Br J Cancer **97**(10): 1432-1440.

The prognosis of pancreatic adenocarcinoma is affected by early metastases and local tumour invasion beyond surgical margins. Gene expression profiling in pancreatic cancer tissue is complicated due to the high amount of RNAses being present in human tissue and that of suitable models. In order to demonstrate early metastases, the models should take into account the anatomical environment of the tumour. Using the orthotopic transplantation of pancreatic tumour cells in SCID (severe combined immunodeficiency) mice, these interactions are taken into consideration. In order to identify genes associated with local tumour invasion and metastases in ductal pancreatic cancer, we investigated a human pancreatic tumour cell line derived from an orthopic pancreatic tumour model in SCID mice. Differential gene expression was performed on the basis of microarray technique. The human MiaPaca-2 cell line was implanted orthotopically in SCID mice. Transcriptional profiling was performed on fresh frozen tissue derived from the primary tumour, the tumour invasion front and the liver metastases. Differentially expressed genes were identified using statistical analyses, and were validated with external databases and with immunohistochemistry. A total of 1066 of 14 500 genes were significantly differentially expressed. Comparing the primary tumour with the tumour invasion front, there were 614 statistically significant up- and 348 downregulated genes. Twenty-five statistically significant up- and 181 downregulated genes were identified comparing the liver metastases with the primary tumour. Eight genes (PAI-1, BNIP3l, VEGF, NSE, RGS4, HSP27, GADD45A, PTPN14) were chosen and validated in a semi-quantitative immunohistochemical analysis, which revealed a positive correlation to the array data. Overrepresentation analyses revealed a total of 66 significantly regulated pathways associated with cell proliferation, cell stress, cell communication metabolic and cytokine function. In conclusion, model marker genes for local invasion and liver metastases can be identified using transcriptional profiling in the SCID mouse. Overrepresentation analysis secures a good and fast overview about the significantly regulated genes and can assign genes to certain pathways. These marker genes can be related to the apoptotic cascade, angiogenesis and cell interaction.

Pantaleo, M. A., et al. (2008). "Gene expression profiling of liver metastases from colorectal cancer as potential basis for treatment choice." Br J Cancer **99**(10): 1729-1734.

At present no reports on gene expression profiling of liver metastases from colorectal cancer are available. We identified two different signatures using Affymetrix platform: epidermal growth factor receptor pathway was upregulated in metachronous lesions, whereas the pathway mainly related to angiogenesis was in synchronous lesions. Synchronous or metachronous liver metastases could be treated differently on the basis of different molecular pathways.

Powrozek, T., et al. (2014). "The application of real-time PCR technique to detect rare cell clones with primary T790M Substitution of EGFR gene in metastases of non-small cell lung cancer to central nervous system in chemotherapy naive patients." Pathol Oncol Res **20**(4): 945-951.

The time-limited efficacy of reversible EGFR-TKIs in patients with advanced non-small cell lung cancer (NSCLC) with EGFR gene activating mutations is associated with development of treatment resistance after some period of therapy. This resistance predominantly results from secondary mutations located in EGFR gene, especially T790M substitution. There is limited information available concerning the prevalence of primary T790M mutations in patients with metastatic NSCLC tumors before treatment with EGFR-TKIs. The aim of work was to assess the prevalence of de novo T790M mutations in EGFR gene in tissue samples from NSCLC metastatases in central nervous system (CNS) in both chemotherapy and EGFR-TKI naive NSCLC patients. We analyzed DNA samples isolated from paraffin-embedded tissue from CNS metastases for T790M mutations using real-time PCR and TaqMan probe against the T790M mutant sequence. The tissue samples were taken during palliative

neurosurgery in 143 NSCLC patients. Amplification of the T790M-specific sequence was detected in 25 patients (17.5 %). The quantity of mutated DNA was less than 1 % in all samples with amplification, and in vast majority (20 patients, 14 % of all samples) it was even less that 0.1 %. In 5 patients (3.5 %) quantity of mutated DNA ranged from 0.1 to 1 % and true positive results of T790M mutation presence in these patients were most possible. Amplification of this sequence was not concurrent with common EGFR mutations and was not associated with sex, smoking status and pathological type of cancer. There is a possibility to detect the primary T790M mutation in brain metastases of NSCLC in EGFR-TKIs naive patients.

Preusser, M., et al. (2013). "ALK gene translocations and amplifications in brain metastases of non-small cell lung cancer." Lung Cancer **80**(3): 278-283.

BACKGROUND: Increased incidence of brain metastases (BM) in non-small cell lung cancer (NSCLC) with ALK translocations was postulated, however, ALK gene aberrations in NSCLC-BM have not been investigated so far. METHODS: We investigated ALK and EML4 gene aberrations (amplifications, translocations, inversions) by fluorescent in situ hybridization (FISH) (n=175) and ALK and EML4 protein expression by immunohistochemistry (n=221) in NSCLC BM and corresponding primary tumors. RESULTS: ALK translocations were found in 4/151 (2.6%; 3 of them involving EML4) of BM of adenocarcinomas (AC), 1/9 (11.1%) of adenosquamous carcinomas (ASC), 0/5 of squamous cell carcinomas (SCC) and 0/10 of large cell carcinomas (LCC). Rearrangement of ALK without involvement of EML4 was seen in 1 AC-BM and rearrangement of EML4 without involvement of ALK in 3 AC-BM, 1 ASC-BM and 1 LCC. ALK amplifications without gene rearrangements were found in BM of 16/151 (10.6%) AC, 2/5 (40%) SCC, 0/9 ASC and one LCC. ALK translocation status was constant between BM and primary tumors in 16 evaluable cases including two cases with ALK-EML4 translocations Among these 16 cases ALK amplification was seen in two BM and none of the primary tumors. All cases with translocations but not with amplifications of ALK showed protein expression. We found no association of ALK gene status with patient age, gender or overall survival time. CONCLUSIONS: ALK translocations and amplifications are found in approximately 3% and 11% of NSCLC-BM, respectively. While ALK translocations appear to be constant between primary tumors and BM, amplifications seem to be more prevalent in BM. ALK translocation, but not ALK amplification is associated with ALK protein overexpression. Further studies are needed to determine whether NSCLC-BM patients with ALK gene aberrations may benefit from specific inhibitor therapy.

Rocken, C., et al. (2005). "The number of lymph node metastases in gastric cancer correlates with the angiotensin I-converting enzyme gene insertion/deletion polymorphism." Clin Cancer Res **11**(7): 2526-2530.

PURPOSE: In the present study, we aimed to substantiate the putative significance of angiotensin I-converting enzyme (ACE) on gastric cancer biology by investigating the influence of its gene polymorphism on gastric cancer progression. EXPERIMENTAL DESIGN: Genomic DNA was purified from peripheral blood mononuclear cells or tissue specimens. Amplified ACE gene fragments were separated on agarose gels. D or I alleles were identified by the presence of 190- or 490-bp fragments, respectively. Local expression of ACE was investigated by immunohistochemistry. RESULTS: Twenty-four of 113 (21%) gastric cancer patients had the II, 57 (51%) the ID, and 32 (28%) the DD genotype. The distribution of the ACE genotypes did not differ significantly from the control group of 189 patients without gastric cancer. However, the ACE genotypes correlated with the number of lymph node metastases and the Unio Internationale Contra Cancrum (UICC) tumor stage. Patients with the II genotype had a highly significantly smaller number of lymph node metastases ($P < 0.001$) and a significantly lower UICC tumor stage ($P = 0.01$) than patients with the DD genotype. No correlation was found between tumor type, tumor location, local tumor growth, distant metastases, and the ACE genotype. The expression of ACE in gastric cancer was investigated by immunohistochemistry in 100 of 113 patients. ACE was expressed by endothelial cells in all (100%) specimens and by tumor cells in 56 (56%) specimens. CONCLUSIONS: Our study shows that ACE is expressed locally in gastric cancer and that the gene polymorphism influences metastatic behavior.

Sadanandam, A., et al. (2011). "A cross-species analysis of a mouse model of breast cancer-specific osteolysis and human bone metastases using gene expression profiling." BMC Cancer **11**: 304.

BACKGROUND: Breast cancer is the second leading cause of cancer-related death in women in the United States. During the advanced stages of disease, many breast cancer patients suffer from bone metastasis. These metastases are predominantly osteolytic and develop when tumor cells interact with bone. In vivo models that mimic

the breast cancer-specific osteolytic bone microenvironment are limited. Previously, we developed a mouse model of tumor-bone interaction in which three mouse breast cancer cell lines were implanted onto the calvaria. Analysis of tumors from this model revealed that they exhibited strong bone resorption, induction of osteoclasts and intracranial penetration at the tumor bone (TB)-interface. METHODS: In this study, we identified and used a TB microenvironment-specific gene expression signature from this model to extend our understanding of the metastatic bone microenvironment in human disease and to predict potential therapeutic targets. RESULTS: We identified a TB signature consisting of 934 genes that were commonly (among our 3 cell lines) and specifically (as compared to tumor-alone area within the bone microenvironment) up- and down-regulated >2-fold at the TB interface in our mouse osteolytic model. By comparing the TB signature with gene expression profiles from human breast metastases and an in vitro osteoclast model, we demonstrate that our model mimics both the human breast cancer bone microenvironment and osteoclastogenesis. Furthermore, we observed enrichment in various signaling pathways specific to the TB interface; that is, TGF-beta and myeloid self-renewal pathways were activated and the Wnt pathway was inactivated. Lastly, we used the TB-signature to predict cyclopenthiazide as a potential inhibitor of the TB interface. CONCLUSION: Our mouse breast cancer model morphologically and genetically resembles the osteoclastic bone microenvironment observed in human disease. Characterization of the gene expression signature specific to the TB interface in our model revealed signaling mechanisms operative in human breast cancer metastases and predicted a therapeutic inhibitor of cancer-mediated osteolysis.

Schuler, M., et al. (2016). "First-Line Afatinib versus Chemotherapy in Patients with Non-Small Cell Lung Cancer and Common Epidermal Growth Factor Receptor Gene Mutations and Brain Metastases." J Thorac Oncol **11**(3): 380-390.

INTRODUCTION: Metastatic spread to the brain is common in patients with non-small cell lung cancer (NSCLC), but these patients are generally excluded from prospective clinical trials. The studies, phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations (LUX-Lung 3) and a randomized, open-label, phase III study of BIBW 2992 versus chemotherapy as first-line treatment for patients with stage IIIB or IV adenocarcinoma of the lung harbouring an EGFR activating mutation (LUX-Lung 6) investigated first-line afatinib versus platinum-based chemotherapy in epidermal growth factor receptor gene (EGFR) mutation-positive patients with NSCLC and included patients with brain metastases; prespecified subgroup analyses are assessed in this article. METHODS: For both LUX-Lung 3 and LUX-Lung 6, prespecified subgroup analyses of progression-free survival (PFS), overall survival, and objective response rate were undertaken in patients with asymptomatic brain metastases at baseline (n = 35 and n = 46, respectively). Post hoc analyses of clinical outcomes was undertaken in the combined data set (n = 81). RESULTS: In both studies, there was a trend toward improved PFS with afatinib versus chemotherapy in patients with brain metastases (LUX-Lung 3: 11.1 versus 5.4 months, hazard ratio [HR] = 0.54, p = 0.1378; LUX-Lung 6: 8.2 versus 4.7 months, HR = 0.47, p = 0.1060). The magnitude of PFS improvement with afatinib was similar to that observed in patients without brain metastases. In combined analysis, PFS was significantly improved with afatinib versus with chemotherapy in patients with brain metastases (8.2 versus 5.4 months; HR, 0.50; p = 0.0297). Afatinib significantly improved the objective response rate versus chemotherapy in patients with brain metastases. Safety findings were consistent with previous reports. CONCLUSIONS: These findings lend support to the clinical activity of afatinib in EGFR mutation-positive patients with NSCLC and asymptomatic brain metastases.

Sfakianakis, S., et al. (2015). "A network-based approach to enrich gene signatures for the prediction of breast cancer metastases." Conf Proc IEEE Eng Med Biol Soc **2015**: 6497-6500.

Despite the multiplicity of the gene expression analysis studies for the identification of genomics based origins of cancerous diseases, the presented gene signatures have generally little overlap. The genes do not function in isolation and therefore a more holistic approach that takes into account the interactions among them is needed. In this study we present a stepwise refinement methodology where starting from some initial set of biomarkers we expand and enrich this set taking into account existing biological information. In particular, we start with a 27 gene signature previously identified as indicative of the presence of circulating tumor cells (CTCs) in peripheral blood of breast cancer patients. We use the manually curated HINT database of protein-protein interactions as the background biological network to locate the network-based similarity of the input genes and how they connect to each other. The result is an enriched connected set of genes that is subsequently expanded

to form an even bigger network based on the ability of the surrounding genes to strongly correlate with the phenotypes of a training set of breast cancer patient cases. The induced network is then used as a new gene signature for the classification of breast brain metastases in an independent dataset. The results are encouraging for the validity of this method.

Shirota, Y., et al. (1999). "[Dihydropyrimidine dehydrogenase gene expression in patients with hepatic metastases from colorectal cancer]." Gan To Kagaku Ryoho **26**(12): 1729-1731.

Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme for 5-FU catabolism. Recently, much interest has been taken in the relation between the antitumor effect of 5-FU and DPD expression in gastrointestinal cancers. In this study, we compared DPD mRNA of 11 hepatic metastatic foci with that of 50 primary foci in colorectal cancer patients. DPD mRNA levels in hepatic metastatic foci were significantly higher than those in primary foci (median DPD/GAPDH ratio 0.79 vs 0.44, p = 0.035). Even in 6 cases available to compare DPD mRNA expression in matched primary and metastatic foci, the same significant difference was obtained (median DPD/GAPDH ratio 0.80 vs 0.36, p = 0.028). Our results suggested that the efficacy of intra-arterial infusion for metastatic liver tumor is mainly due to the fact that the high concentration of 5-FU is enough to overcome the high clearance of 5-FU, which is caused by DPD.

Simone, G., et al. (2010). "Chromogenic in situ hybridization to detect EGFR gene copy number in cell blocks from fine-needle aspirates of non small cell lung carcinomas and lung metastases from colo-rectal cancer." J Exp Clin Cancer Res **29**: 125.

BACKGROUND: Several studies demonstrated that epidermal growth factor receptor (EGFR) gene copy number (GCN) correlates to the response to tyrosine kinase inhibitors in non small cell lung cancer (NSCLC) and to anti-EGFR monoclonal antibodies (MoAbs) in metastatic colorectal cancer (CRC). In the presence of lung nodules, cytology is often the only possible diagnostic approach. Chromogenic in situ hybridization (CISH) is an alternative technique to fluorescence in situ hybridization (FISH), but its feasibility in detecting EGFR GCN in cell blocks from fine-needle aspiration cytology (FNAC) of lung nodules has not yet been established. METHODS: We evaluated the feasibility of CISH on 33 FNAC from 20 primary NSCLC (5 squamous carcinomas, 8 large cell carcinomas and 7 adenocarcinomas) and 13 lung metastases from CRC. RESULTS: Of the 33 FNAC analyzed by CISH, 27 (82%) presented a balanced increase in EGFR gene and chromosome 7 number: 10 cases (30%) showed a low polysomy, 15 (45%) a high polysomy and 2 (6%) NSCLC were amplified. No significant differences between NSCLC and CRC lung metastases were found in relation to disomic or polysomic status. In addition, no correlation between EGFR GCN and EGFR immunohistochemical overexpression was found. Furthermore, we compared CISH results with those obtained by FISH on the same samples and we found 97% overall agreement between the two assays (k = 0.78, p < 0.0001). Two cases were amplified with both assays, whereas 1 case of NSCLC was amplified by FISH only. CISH sensitivity was 67%, the specificity and positive predictive value (PPV) was 100%, and the negative predictive value (NPV) was 97%. CONCLUSIONS: Our study shows that CISH is a valid method to detect EGFR GCN in cell blocks from FNAC of primary NSCLC or metastatic CRC to the lung.

Singhi, A. D., et al. (2012). "MYC gene amplification is often acquired in lethal distant breast cancer metastases of unamplified primary tumors." Mod Pathol **25**(3): 378-387.

In breast cancer, amplification of MYC is consistently observed in aggressive forms of disease and correlates with poor prognosis and distant metastases. However, to date, a systematic analysis of MYC amplification in metastatic breast cancers has not been reported. Specifically, whether the MYC amplification status may change in metastases in comparison to the corresponding primary breast tumor, and potential variability among different metastases within the same patient have also not been assessed. We generated single patient tissue microarrays consisting of both primary breast carcinomas and multiple matched systemic metastases from 15 patients through our previously described rapid autopsy program. In total, the 15 tissue microarrays contained 145 primary tumor spots and 778 spots derived from 180 different metastases. In addition, two separate tissue microarrays were constructed composed of 10 matched primary breast cancers and corresponding solitary metastases sampled not at autopsy but rather in routine surgical resections. These two tissue microarrays totaled 50 primary tumor spots and 86 metastatic tumor spots. For each case, hormone receptor status, HER2/neu, EGFR and CK5/6 expression were assessed, and the cases were characterized as luminal, basal-like or HER2 based on published criteria. Both fluorescence in situ hybridization and immunohistochemistry for MYC was performed on all cases. Of the 25 cases, 24 were evaluable. While 4 of 24 primary tumors (16%) demonstrated MYC amplification, an additional 6 (25%

of total evaluable cases) acquired MYC amplification in their systemic metastases. Of note, there was remarkably little heterogeneity in MYC copy number among different metastases from the same patient. MYC immunoreactivity was increased in metastases relative to matched primaries in the surgical cohort, although there was no perfect correlation with MYC amplification. In conclusion, amplification of MYC is a frequent event in breast cancer, but occurs more frequently as a diffuse, acquired event in metastatic disease than in the corresponding primary. These observations underscore the importance of MYC in breast cancer progression/metastasis, as well as its relevance as a potential therapeutic target in otherwise incurable metastatic disease.

Snoeren, N., et al. (2012). "Exploring gene expression signatures for predicting disease free survival after resection of colorectal cancer liver metastases." PLoS One **7**(11): e49442.

BACKGROUND AND OBJECTIVES: This study was designed to identify and validate gene signatures that can predict disease free survival (DFS) in patients undergoing a radical resection for their colorectal liver metastases (CRLM). METHODS: Tumor gene expression profiles were collected from 119 patients undergoing surgery for their CRLM in the Paul Brousse Hospital (France) and the University Medical Center Utrecht (The Netherlands). Patients were divided into high and low risk groups. A randomly selected training set was used to find predictive gene signatures. The ability of these gene signatures to predict DFS was tested in an independent validation set comprising the remaining patients. Furthermore, 5 known clinical risk scores were tested in our complete patient cohort. RESULT: No gene signature was found that significantly predicted DFS in the validation set. In contrast, three out of five clinical risk scores were able to predict DFS in our patient cohort. CONCLUSIONS: No gene signature was found that could predict DFS in patients undergoing CRLM resection. Three out of five clinical risk scores were able to predict DFS in our patient cohort. These results emphasize the need for validating risk scores in independent patient groups and suggest improved designs for future studies.

Stark, A. M., et al. (2010). "Expression of metastasis suppressor gene maspin is reduced in breast cancer brain metastases and correlates with the estrogen receptor status." Neurol Res **32**(3): 303-308.

OBJECTIVES: The suppressor gene maspin (Serpin B5) is a promising candidate for future treatment. We have examined the messenger RNA (mRNA) and protein expression of maspin in normal breast tissue, breast cancer primaries, brain metastases and breast cancer cell lines. Results were compared to hormone receptor expression and proliferation index. METHODS: Maspin mRNA expression was examined by real-time polymerase chain reaction in fresh frozen human samples and breast cancer cell lines MCF-7, T47-D and MDA-MB-231. Maspin protein, estrogen and progesterone receptor expression as well as Ki-67 proliferation index were detected by immunohistochemistry from 16 patients with breast cancer primaries and breast cancer brain metastases. RESULTS: In relation to normal breast tissue, maspin mRNA expression was decreased in primary tumors and again decreased in brain metastases. Normalized C(T) values were 1 (normal tissue), 0.3 (primary tumors) and 0.13 (brain metastases). Immunohistochemistry revealed same tendencies. In comparison to poorly invasive breast cancer cell lines, maspin mRNA expression was decreased in highly invasive and metastatic 231-parental cell lines. In contrast, maspin mRNA expression was increased in 231-brain, and it was not detectable in 231-bone. Patients with maspin-positive primary tumors showed longer survival. DISCUSSION: This finding adds maspin to the list of metastasis suppressor genes possibly involved in the formation of breast cancer brain metastases.

Stark, A. M., et al. (2005). "Reduced metastasis-suppressor gene mRNA-expression in breast cancer brain metastases." J Cancer Res Clin Oncol **131**(3): 191-198.

PURPOSE: Brain metastases are an increasingly common complication in breast cancer patients. The Metastasis Suppressor Genes (MSG) Nm23, KISS1, KAI1, BRMS1, and Mkk4 have been associated with the metastatic potential of breast cancer in vitro and in vivo. METHODS: The mRNA expression of Nm23, KISS1, KAI1, BRMS1, and Mkk4 in fresh frozen tissue samples of brain metastases from ductal invasive breast cancer specimens was examined in relation to primary tumors. In a first step, mRNA expression screening was carried out using a semi-quantitative RT-PCR approach, in a second step quantitative real-time RT-PCR was performed on selected specimens. By immunohistochemical staining, gene products were visualized on the protein level. RESULTS: Semi-quantitative RT-PCR revealed reduced mRNA expression of Nm23, KISS1, KAI1, BRMS, and Mkk4 in brain metastases. Results for KISS1, KAI1, BRMS, and Mkk4 were confirmed by real-time RT-PCR. In detail, mRNA expression reduction in breast cancer brain metastases was tenfold. Expression of MSG could be confirmed by immunohistochemical staining on protein level. CONCLUSIONS: Our

investigations revealed significantly reduced mRNA expression of metastases suppressor genes KISS1, KAI1, BRMS1, and Mkk4 in breast cancer brain metastasis. Particularly, in the case of KISS1 and Mkk4, an important role for future treatment of patients with breast cancer brain metastatic lesions can be assumed.

Sun, L., et al. (2011). "Comparison of KRAS and EGFR gene status between primary non-small cell lung cancer and local lymph node metastases: implications for clinical practice." J Exp Clin Cancer Res **30**: 30.

BACKGROUND: Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) have been widely used for the treatment of non-small cell lung cancer (NSCLC). KRAS and EGFR somatic mutations in NSCLC may predict resistance and responsiveness to TKI, respectively. Nevertheless, most research to date has been conducted on samples from primary tumors. For many patients with advanced disease, their samples can only be obtained from metastases for test. The molecular characteristics of metastasized tumors may be different from those of primary tumors. MATERIALS AND METHODS: Mutation status of KRAS and EGFR between primary tumors and local lymph node metastases of 80 Chinese patients with NSCLC were analyzed by direct sequencing. Five of them were given gefitinib as neoadjunvant treatment after the EGFR-TKI sensitive mutations were detected in their biopsies of mediastinal lymph nodes metastases. McNemar's test was used to compare the EGFR and KRAS mutation status between primary tumors and corresponding local lymph node metastases. Data evaluation was carried out with SPSS_13.0 statistical software. RESULTS: Among the 160 samples, one primary tumor and seven metastases were identified with KRAS mutations and 21 primary tumors and 26 metastases were found to have EGFR mutations. KRAS and EGFR mutation status was different between primary tumors and corresponding metastases in 6 (7.5%) and 7 (8.75%) patients, respectively. One patient with no TKI sensitive mutations detected in the primary tumor showed disease progression. CONCLUSION: Our results suggest that a considerable proportion of NSCLC in Chinese population showed discrepancy in KRAS and EGFR mutation status between primary tumors and corresponding metastases. This observation may have important implication for the use of targeted TKI therapy in the treatment of NSCLC patients.

Takahashi, H., et al. (2006). "Classification of intramural metastases and lymph node metastases of esophageal cancer from gene expression based on boosting and projective adaptive resonance theory." J Biosci Bioeng **102**(1): 46-52.

Esophageal cancer is a well-known cancer with poorer prognosis than other cancers. An optimal and individualized treatment protocol based on accurate diagnosis is urgently needed to improve the treatment of cancer patients. For this purpose, it is important to develop a sophisticated algorithm that can manage a large amount of data, such as gene expression data from DNA microarrays, for optimal and individualized diagnosis. Marker gene selection is essential in the analysis of gene expression data. We have already developed a combination method of the use of the projective adaptive resonance theory and that of a boosted fuzzy classifier with the SWEEP operator denoted PART-BFCS. This method is superior to other methods, and has four features, namely fast calculation, accurate prediction, reliable prediction, and rule extraction. In this study, we applied this method to analyze microarray data obtained from esophageal cancer patients. A combination method of PART-BFCS and the U-test was also investigated. It was necessary to use a specific type of BFCS, namely, BFCS-1,2, because the esophageal cancer data were very complexity. PART-BFCS and PART-BFCS with the U-test models showed higher performances than two conventional methods, namely, k-nearest neighbor (kNN) and weighted voting (WV). The genes including CDK6 could be found by our methods and excellent IF-THEN rules could be extracted. The genes selected in this study have a high potential as new diagnosis markers for esophageal cancer. These results indicate that the new methods can be used in marker gene selection for the diagnosis of cancer patients.

Takahashi, Y., et al. (2006). "Detection of aberrations of ubiquitin-conjugating enzyme E2C gene (UBE2C) in advanced colon cancer with liver metastases by DNA microarray and two-color FISH." Cancer Genet Cytogenet **168**(1): 30-35.

Using DNA microarrays, the expression profiles of 1,700 genes in the primary tumor, liver metastases and paired normal tissue obtained from nine patients with advanced colorectal cancer was studied. Twenty genes were upregulated and only one gene was downregulated in the primary tumors. In the liver metastases, 39 genes were upregulated and only three genes were downregulated. There was no significant difference in gene expression between the primary tumors and the liver metastases. The most highly overexpressed gene in both the primary tumors and the liver metastases was the ubiquitin-conjugating enzyme E2C gene (UBE2C), located at

20q13.1. Additionally, two-color FISH analysis using probes for the region 20q13.1 and the chromosome 20 centromere revealed that amplification at 20q13.1 had occurred in 5 of 10 (50%) colon cancers. Comparison between the levels of gene expression and FISH results revealed that UBE2C expression is significantly changed by amplification at 20q13.1, suggesting genomic amplification as one mechanism of increased UBE2C expression. Our results showing aberrations in levels of gene expression and locus copy number of UBE2C suggest that this gene may play an important role in tumor progression leading to advanced colon cancer with liver metastasis.

Teng, Y., et al. (2010). "Inactivation of the WASF3 gene in prostate cancer cells leads to suppression of tumorigenicity and metastases." Br J Cancer **103**(7): 1066-1075.

BACKGROUND: The WASF3 protein is involved in cell movement and invasion, and to investigate its role in prostate cancer progression we studied the phenotypic effects of knockdown in primary tumors and cell lines. METHODS: ShRNA was used to knockdown WASF3 function in prostate cell lines. Cell motility (scratch wound assay), anchorage independent growth and in vivo tumorigenicity and metastasis were then compared between knockdown and wild-type cells. RESULTS: Increased levels of expression were seen in high-grade human prostate cancer and in the PC3 and DU145 cell lines. Inactivation of WASF3 using shRNAs reduced cell motility and invasion in these cells and reduced anchorage independent growth in vitro. The loss of motility was accompanied by an associated increase in stress fiber formation and focal adhesions. When injected subcutaneously into severe combined immunodeficiency (SCID) mice, tumor formation was significantly reduced for PC3 and DU145 cells with WASF3 knockdown and in vivo metastasis assays using tail vain injection showed a significant reduction for PC3 and DU145 cells. The loss of the invasion phenotype was accompanied by down-regulation of matrix metalloproteinase 9. CONCLUSIONS: Overall, these observations demonstrate a critical role for WASF3 in the progression of prostate cancer and identify a potential target to control tumorigenicity and metastasis.

Tobin, N. P., et al. (2015). "Molecular subtype and tumor characteristics of breast cancer metastases as assessed by gene expression significantly influence patient post-relapse survival." Ann Oncol **26**(1): 81-88.

BACKGROUND: We and others have recently shown that tumor characteristics are altered throughout tumor progression. These findings emphasize the need for re-examination of tumor characteristics at relapse and have led to recommendations from ESMO and the Swedish Breast Cancer group. Here, we aim to determine whether tumor characteristics and molecular subtypes in breast cancer metastases confer clinically relevant prognostic information for patients. PATIENTS AND METHODS: The translational aspect of the Swedish multicenter randomized trial called TEX included 111 patients with at least one biopsy from a morphologically confirmed locoregional or distant breast cancer metastasis diagnosed from December 2002 until June 2007. All patients had detailed clinical information, complete follow-up, and metastasis gene expression information (Affymetrix array GPL10379). We assessed the previously published gene expression modules describing biological processes [proliferation, apoptosis, human epidermal receptor 2 (HER2) and estrogen (ER) signaling, tumor invasion, immune response, and angiogenesis] and pathways (Ras, MAPK, PTEN, AKT-MTOR, PI3KCA, IGF1, Src, Myc, E2F3, and beta-catenin) and the intrinsic subtypes (PAM50). Furthermore, by contrasting genes expressed in the metastases in relation to survival, we derived a poor metastasis survival signature. RESULTS: A significant reduction in post-relapse breast cancer-specific survival was associated with low-ER receptor signaling and apoptosis gene module scores, and high AKT-MTOR, Ras, and beta-catenin module scores. Similarly, intrinsic subtyping of the metastases provided statistically significant post-relapse survival information with the worst survival outcome in the basal-like [hazard ratio (HR) 3.7; 95% confidence interval (CI) 1.3-10.9] and HER2-enriched (HR 4.4; 95% CI 1.5-12.8) subtypes compared with the luminal A subtype. Overall, 25% of the metastases were basal-like, 32% HER2-enriched, 10% luminal A, 28% luminal B, and 5% normal-like. CONCLUSIONS: We show that tumor characteristics and molecular subtypes of breast cancer metastases significantly influence post-relapse patient survival, emphasizing that molecular investigations at relapse provide prognostic and clinically relevant information. CLINICALTRIALS.GOV: This is the translational part of the Swedish multicenter and randomized trial TEX, clinicaltrials.gov identifier nct01433614 (http://www.clinicaltrials.gov/ct2/show/nct01433614).

Tran, T. N., et al. (2016). "Alterations of MET Gene Copy Number and Protein Expression in Primary Non-Small-Cell Lung Cancer and Corresponding Nodal Metastases." Clin Lung Cancer **17**(1): 30-38 e31.

INTRODUCTION: Mesenchymal epithelial transition factor (MET) is a promising therapeutic target in non-small-cell lung cancer (NSCLC) but there are limited data about MET alterations in treatment-naive NSCLC and whether or not these changes are consistent between primary tumors and metastases. We aimed to investigate concordance, clinicopathological correlations, and prognostic value of MET alterations in primary NSCLC and corresponding nodal metastases. MATERIALS AND METHODS: MET gene copy number (GCN) status was evaluated using fluorescent in situ hybridization (FISH) and MET protein expression using immunohistochemistry (IHC) in tissue microarray sections from a retrospective cohort of 300 surgically resected NSCLCs including 93 cases with nodal metastases. RESULTS: Primary NSCLCs were MET IHC positive in 28 (10.3%) of cases and MET FISH positive (high polysomy or amplification) in 22 (8.1%) but only 1 (0.4%) showed amplification. In metastases, high MET GCN (18.3%) and protein expression (21.3%) was more frequent compared with primary tumors. The status of MET in lymph nodes significantly correlated with MET status in the corresponding primary tumors. Squamous cell carcinomas showed lower MET overexpression compared with nonsquamous tumors but there were no other associations with clinicopathological characteristics. Patients with tumors that were either MET FISH positive or IHC positive had a significantly better overall survival in univariate and multivariate analyses. CONCLUSION: Alterations of MET are more commonly seen in nodal metastases than primary tumors and this might have implications for their utility as predictive biomarkers to select patients for MET inhibition. MET overexpression and MET high polysomy occur in a low proportion of primary NSCLCs and is associated with a good prognosis.

Trevisiol, C., et al. (2006). "Prognostic value of circulating KRAS2 gene mutations in colorectal cancer with distant metastases." Int J Biol Markers **21**(4): 223-228.

While tissue KRAS2 mutations have been extensively investigated, the role of circulating mutant KRAS2 gene in patients with colorectal carcinoma remains obscure. The aim of the present study was to explore the prognostic significance of circulating KRAS2 gene mutational status in subjects undergoing primary treatment for colorectal cancer. Codon 12 KRAS2 mutations were examined in DNA samples extracted from the serum of 86 patients with colorectal cancer and were compared with the KRAS2 status of their primary tumors. Tissue and serum KRAS2 status was compared with other clinicopathological variables (including CEA and CA 19-9 levels) and with cancer-related survival. KRAS2 mutations were found in tissue samples of 28 patients (33%); serum KRAS2 mutations were detected in 10 of them (36%). Serum KRAS2 status was significantly associated with Dukes' stage D (p=0.001) and with preoperative CA 19-9 levels (p=0.01). At multivariate analysis, cancer-related survival was associated with Dukes' stage (p<0.0001), CEA level (p=0.02), and mutant circulating KRAS2 (p=0.01). All 7 stage D patients with serum KRAS2 mutations died of the disease within 24 months of primary treatment; cancer-related survival was significantly better in 9 stage D patients without serum KRAS2 mutations, with 5 patients (56%) alive after 24 months and 1 patient (13%) alive after 44 months. Residual disease after surgery was evident in all 7 stage D patients with mutant circulating KRAS2, and in 5 out of 9 stage D patients without serum mutations. Serum KRAS2 status may impact substantially on the management of stage D colorectal carcinoma, since it appears to cor-relate with prognosis in this patient subgroup.

Tsaur, I., et al. (2015). "PCA3 and PSA gene activity correlates with the true tumor cell burden in prostate cancer lymph node metastases." Cancer Biomark **15**(3): 311-316.

BACKGROUND: Extent of pelvic lymph node (LN) dissemination is a critical prognostic feature for patients with prostate cancer (PCa) maintaining extended pelvic lymphadenectomy (LAD) as the gold standard for LN-staging. Unfortunately, conventional histopathological assessment may miss micrometastasis and recently presented immunocytochemical approach of the single cell analysis is still intricate. OBJECTIVE: To comparatively assess the potential of Prostate cancer gene 3 (PCA3) and prostate specific antigene (PSA) to perform as markers for tumor cell load. METHODS: Patients with high risk PCa for LN metastasis undergoing either a sentinel LN-guided staging LAD or retropubic radical prostatectomy with sentinel-guided pelvic LN dissection were included. LNs were investigated by routine histopathology. Tumor cell load was quantified by %immunocytochemistry. immunocytochemical single cell analysis. Gene activity was determined by qRT-PCR. RESULTS: Twenty four out of 226 LNs were positive in routine histopathology and 51 in single cell analysis. PSA mRNA level correlated with tumor cell density in patients with a positive immunocytochemistry. Gene activity of PCA3 was upregulated in metastatic LNs and correlated with tumor cell density in patients with tumor-invaded LNs as detected by immunocytochemistry.

CONCLUSIONS: PCA3 gene expression discriminates LN metastasis and might outperform PSA gene activity in reflecting tumor cell burden in pelvic LNs of PCa patients.

Tsuda, H., et al. (1998). "A prospective study of the significance of gene and chromosome alterations as prognostic indicators of breast cancer patients with lymph node metastases." Breast Cancer Res Treat **48**(1): 21-32.

In 150 surgically resected primary breast carcinomas that had axillary lymph-node metastases, we examined the incidence of loss of heterozygosity on chromosomes 16p, 16q, 17p, 17q, and 18q, point mutation of the p53 tumor-suppressor gene, nuclear immunoreaction of p53 protein, and amplifications of the c-erbB-2 and int-2 oncogenes by Southern blotting, single-strand conformation polymorphism analysis, and immunohistochemistry. We analyzed the association of these factors and conventional prognostic parameters with outcome of the patients, using Cox's univariate and multivariate analyses. The univariate analysis revealed that nuclear p53 immunoreaction, p53 mutation, and c-erbB-2 amplification as well as the number of metastatic lymph nodes, histological grade, and hormone-receptor statuses were significant prognostic indicators for both recurrence and cancer death. p53 immunoreaction was correlated more strongly with a poor prognosis than p53 mutations. The combination of p53 and c-erbB-2 effectively identified the high-risk patient group, and even among Grade 3 cases the subgroup with these alterations tended to have poorer clinical outcomes. The multivariate analysis including p53, c-erbB-2, and conventional factors. Lymph node status, grade, and p53 had independent impacts on the survival of patients. Under identical adjuvant systemic therapies, prognoses differed between the patient groups with and without alterations of p53 or c-erbB-2. Appropriate combinations of conventional factors with nuclear p53 immunoreaction and c-erbB-2 amplification would help to identify highly aggressive node-positive breast carcinomas and would aid stratification of patient groups in randomized clinical trials of adjuvant systemic therapies.

Ueda, K., et al. (2004). "Adenoviral-mediated gene transduction of the hepatocyte growth factor (HGF) antagonist, NK4, suppresses peritoneal metastases of gastric cancer in nude mice." Eur J Cancer **40**(14): 2135-2142.

The competitive inhibitory effects of NK4 (a specific hepatocyte growth factor (HGF)-antagonist) on the interaction between HGF and the c-Met/HGF receptor has been shown in HGF-mediated invasion of some distinct types of human cancer cells. Furthermore, NK4 has inhibitory effects on the angiogenic pathways driven by basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), as well as by HGF. In this study, to evaluate the therapeutic efficacy of adenoviral-mediated NK4 gene treatment, we employed animal models of peritoneal metastasis using two gastric cancer cell lines, the strongly c-Met expressing MKN45 cell line and the weakly c-Met-expressing cell line, TMK1. In both models, the total number and weight of peritoneal tumours per mouse and ascites treated early with AxCANK4 (administered 3 times 2, 7 and 12 days after the tumour inoculation) were significantly reduced compared with those treated with phosphate-buffered solution (PBS) and AxCALacZ ($P < 0.05$). In Factor-VIII-related-antigen-stained sections from peritoneal metastatic tumours, the inhibition of intratumour vessels was observed in tissues from tumours of MKN45 and TMK1 treated with AxCANK4. We also compared the therapeutic effect of early AxCANK4 treatment with that of late treatment (at 7, 12 and 17 days). Peritoneal metastases and ascites treated late with AxCANK4 showed less of an improvement than those treated early with AxCANK4 in both models. In addition, the inhibitory effect of cisplatin (CDDP) on peritoneal metastasis was significantly enhanced by AxCANK4, suggesting that the combination of intraperitoneal (i.p.) chemotherapy with NK4 gene therapy might be effective, even in cases of advanced peritoneal metastasis from gastric cancer. To conclude, these results show clearly that NK4 gene therapy inhibits peritoneal metastases from gastric cancer, regardless of the level of c-Met/HGF receptor expression in the tumour cells, and especially in the early stages of peritoneal metastasis.

Urnauer, S., et al. (2017). "EGFR-targeted nonviral NIS gene transfer for bioimaging and therapy of disseminated colon cancer metastases." Oncotarget **8**(54): 92195-92208.

Liver metastases present a serious problem in the therapy of advanced colorectal cancer (CRC), as more than 20% of patients have distant metastases at the time of diagnosis with less than 5% being cured. Consequently, new therapeutic approaches are of major need together with high-resolution imaging methods that allow highly specific detection of small metastases. The unique combination of reporter and therapy gene function of the sodium iodide symporter (NIS) may represent a promising theranostic strategy for CRC liver metastases allowing non-invasive imaging of functional NIS expression and therapeutic application of (131)I. For targeted NIS gene transfer polymers containing linear polyethylenimine (LPEI),

polyethylene glycol (PEG) and the epidermal growth factor receptor (EGFR)-specific ligand GE11 were complexed with human NIS DNA (LPEI-PEG-GE11/NIS). Tumor specificity and transduction efficiency were examined in high EGFR-expressing LS174T metastases by non-invasive imaging using (18)F-tetrafluoroborate ((18)F-TFB) as novel NIS PET tracer. Mice that were injected with LPEI-PEG-GE11/NIS 48 h before (18)F-TFB application showed high tumoral levels (4.8+/-0.6% of injected dose) of NIS-mediated radionuclide uptake in comparison to low levels detected in mice that received untargeted control polyplexes. Three cycles of intravenous injection of EGFR-targeted NIS polyplexes followed by therapeutic application of 55.5 MBq (131)I resulted in marked delay in metastases spread, which was associated with improved animal survival. In conclusion, these preclinical data confirm the enormous potential of EGFR-targeted synthetic polymers for systemic NIS gene delivery in an advanced multifocal CRC liver metastases model and open the exciting prospect of NIS-mediated radionuclide therapy in metastatic disease.

Van Den Eeden, S. K., et al. (2018). "A Biopsy-based 17-gene Genomic Prostate Score as a Predictor of Metastases and Prostate Cancer Death in Surgically Treated Men with Clinically Localized Disease." Eur Urol **73**(1): 129-138.

BACKGROUND: A 17-gene biopsy-based reverse transcription polymerase chain reaction assay, which provides a Genomic Prostate Score (GPS-scale 0-100), has been validated as an independent predictor of adverse pathology and biochemical recurrence after radical prostatectomy (RP) in men with low- and intermediate-risk prostate cancer (PCa). OBJECTIVE: To evaluate GPS as a predictor of PCa metastasis and PCa-specific death (PCD) in a large cohort of men with localized PCa and long-term follow-up. DESIGN, SETTING, AND PARTICIPANTS: A retrospective study using a stratified cohort sampling design was performed in a cohort of men treated with RP within Kaiser Permanente Northern California. RNA from archival diagnostic biopsies was assayed to generate GPS results. OUTCOME MEASUREMENTS AND STATISTICAL ANALYSIS: We assessed the association between GPS and time to metastasis and PCD in prespecified uni- and multivariable statistical analyses, based on Cox proportional hazard models accounting for sampling weights. RESULTS AND LIMITATIONS: The final study population consisted of 279 men with low-, intermediate-, and high-risk PCa between 1995 and 2010 (median follow-up 9.8 yr), and included 64 PCD and 79 metastases. Valid GPS results were obtained for 259 (93%). In univariable analysis, GPS was strongly associated with time to PCD, hazard ratio (HR)/20 GPS units=3.23 (95% confidence interval [CI] 1.84-5.65; p<0.001), and time to metastasis, HR/20 units=2.75 (95% CI 1.63-4.63; p<0.001). The association between GPS and both end points remained significant after adjusting for National Comprehensive Cancer Network, American Urological Association, and Cancer of the Prostate Risk Assessment (CAPRA) risks (p<0.001). No patient with low- or intermediate-risk disease and a GPS of<20 developed metastases or PCD (n=31). In receiver operating characteristic analysis of PCD at 10 yr, GPS improved the c-statistic from 0.78 (CAPRA alone) to 0.84 (GPS+CAPRA; p<0.001). A limitation of the study was that patients were treated during an era when definitive treatment was standard of care with little adoption of active surveillance. CONCLUSIONS: GPS is a strong independent predictor of long-term outcomes in clinically localized PCa in men treated with RP and may improve risk stratification for men with newly diagnosed disease. PATIENT SUMMARY: Many prostate cancers are slow growing and unlikely to spread or threaten a man's life, while others are more aggressive and require treatment. Increasingly, doctors are using new molecular tests, such as the17-gene Genomic Prostate Score (GPS), which can be performed at the time of initial diagnosis to help determine how aggressive a given patient's cancer may be. In this study, performed in a large community-based healthcare network, GPS was shown to be a strong predictor as to whether a man's prostate cancer will spread and threaten his life after surgery, providing information that may help patients and their doctors decide on the best course of management of their disease.

Wang, A. Z., et al. (2008). "[Association of TP53 gene polymorphisms with genetic susceptibility to liver metastases of colorectal cancer]." Zhonghua Yi Xue Yi Chuan Xue Za Zhi **25**(2): 168-171.

OBJECTIVE: To investigate the possible association between the single nucleotide polymorphisms (SNPs) (C-8343G, C-1863T and R72P) in TP53 gene and susceptibility to liver metastases of colorectal cancer (CRC) in a Chinese population. METHODS: The genotypes of each SNP in TP53 gene were determined by either TaqMan assays or PCR-based restriction fragment length polymorphism (RFLP) method in 121 colorectal cancer patients with liver metastases and sex-, age-matched 280 cases with nonmetastatic CRC as a control. Immunohistochemical staining for P53 was performed on paraffin-embedded sections. Odds

ratios (ORs) for colorectal liver metastases and 95% confidence intervals (CIs) from unconditional logistic regression models were used to evaluate relative risks. RESULTS: No significant association of C-8343G or C-1863T with colorectal liver metastases risk was observed. However, the R allele of the TP53 R72P polymorphism was more frequently found in metastatic cases than in nonmetastatic cases (P= 0.037). When compared with PP homozygotes, the ORs of metastases for RP heterozygotes was 2.21 (95% CI: 1.13-4.33), for RR homozygotes was 2.26 (95% CI: 1.03-4.94), and for carriers of the 72R allele (RP or RR genotype) was 2.22 (95% CI: 1.16-4.26). Stratified analysis indicated that carrying the 72R allele had a more pronounced increase in colorectal liver metastases risk among patients with positive P53 expression tumors (OR= 3.28, 95% CI: 1.21-8.88), whereas no significantly increased metastases risk was found in patients with negative P53 expression tumors (OR= 1.37, 95% CI: 0.52-3.62). CONCLUSION: The R allele of the TP53 R72P polymorphism may contribute to the etiology of liver metastases in CRC patients, particularly among those with positive P53 expression tumors. Both TP53 C-8343G and C-1863T may be not associated with colorectal liver metastases risk.

Xia, Z. J., et al. (2005). "[Relation of ME491/CD63 gene and integrin alpha5 in the invasion and metastases of ovarian cancer]." Zhonghua Fu Chan Ke Za Zhi **40**(11): 765-769.

OBJECTIVE: To explore the expression and significance of ME491/CD(63) and integrin alpha5 protein and mRNA in ovarian cancer. METHODS: The expression levels of ME491/CD(63) and integrin alpha5 proteins and mRNA were evaluated by using RT-PCR and hybridization in situ (HIS) in normal ovarian tissues (G(1)), ovarian benign tumor tissues (G(2)), ovarian borderline tumor tissues (G(3)) and ovarian cancer tissues (G(4)). The expression levels of ME491/CD(63) genes and proteins were analyzed by multivariant analysis and their effects on the invasion and metastases of ovarian cancer were explored and their correlation with age at surgery, metastatic sites, operation fashions, lymph status, operation pathological staging and pathological typing was studied. RESULTS: The results showed the exact biological effects of ME491/CD(63) and integrin alpha5 in ovarian cancer tissues. The tendency of two methods is coincidence on the whole. There was positive expression of ME491/CD(63) and integrin alpha5, and ME491/CD(63) mRNA expression levels were 1.7 +/- 0.3 and 1.5 +/- 0.3, and integrin alpha5 mRNA expression levels were 1.7 +/- 0.3 and 1.5 +/- 0.3 in G(1) and G(2); ME491/CD(63) is 1.1 +/- 0.5, and integrin alpha5 is 1.1 +/- 0.5 in G(3); ME491/CD(63) is 0.6 +/- 0.4, integrin alpha5 is 0.6 +/- 0.4 in G(4); There was no significant relationship between gene expression and age at surgery or clinical-pathological staging ($P > 0.05$). There was significant difference between G(1), G(2) and G(3) ($P < 0.01$), while no difference between G(1) and G(2) ($P > 0.05$). Low expression levels in G(4) (III, IV) were observed. Significant differences were noted between expression levels in G(4) (III, IV) and in G(2), G(3) or G(4) (I, II; $P < 0.01$). There was significant difference between expression levels in tissues with lymph metastases and tissues without lymph metastases ($P < 0.01$). CONCLUSIONS: ME491/CD(63) and integrin alpha5 are lowly expressed and negatively correlated with ovarian cancer. Different expression levels exist in tissues of late ovarian cancer, earlier ovarian cancer, benign ovarian tumor and borderline tumor. The changes of two genes are correlated with tumor differentiation degree, but not pathologic typing.

Yamada, Y., et al. (1991). "p53 gene mutations in gastric cancer metastases and in gastric cancer cell lines derived from metastases." Cancer Res **51**(21): 5800-5805.

Structural alterations of the p53 gene were investigated in tissue specimens of gastric and cervical cancers and in cell lines of gastric, esophageal, and cervical cancers, by polymerase chain reaction-single-strand conformation polymorphism analysis. Two of the four gastric cancer metastases and four of the eight cell lines originally established from gastric cancer metastases were found to have p53 gene alterations in the exon 5 to 11 region; point mutations and amino acid replacements were detected in a liver and an ovary metastasis at exon 7, in the TMK1 and MKN1 cell lines at exon 5, and in the OKAJIMA cell line at exon 10. The normal allele was not found in these cell lines. In the KATO-III cell line, gross deletion and rearrangement of the p53 gene were noted. However, no p53 mutations were identified in 19 primary lesions of gastric cancer, suggesting that the p53 gene abnormality preferentially occurs in the advanced stages of gastric cancer. In contrast to the gastric cancer, none of the 13 esophageal cancer cell lines, including two cell lines established from metastases, and none of the four cervical cancer cell lines showed any aberration in exons 5 to 11 of the p53 gene. During the course of the study, a novel polymorphism in intron 7 of the p53 gene was found, which can be recognized by restriction enzyme digestions of the polymerase chain reaction product.

Yano, S., et al. (1999). "Combined therapy with anti-P-glycoprotein antibody and macrophage colony-

stimulating factor gene transduction for multiorgan metastases of multidrug-resistant human small cell lung cancer in NK cell-depleted SCID mice." Int J Cancer **82**(1): 105-111.

Our aim was to determine the antimetastatic potential of anti-P-glycoprotein (P-gp) antibodies (Abs) against multidrug-resistant (MDR) human small cell lung cancer (SCLC) cells expressing P-gp. Human SCLC cells H69 (P-gp negative) and its etoposide-resistant variant H69/YP (P-gp positive) were used. H69 and H69/VP cells injected i.v. metastasized to the liver, kidneys and systemic lymph nodes of NK cell-depleted severe combined immunodeficient (SCID) mice. H69/VP cells, but not H69 cells, were resistant to treatments with vindesine. Treatment with mouse-human chimeric anti-P-gp Ab (MH162) and its mouse counterpart (MRK-16) reduced metastasis of H69/VP cells in various organs and prolonged the survival of tumor-bearing mice, although they were less effective if injected at late times (after 28 days). Treatment with another mouse anti-Pgp Ab, MRK-17, was effective only against liver metastasis. MH162 and MRK-16 efficiently induced Ab-dependent cellular cytotoxicity (ADCC) by peritoneal macrophages against H69/VP cells in vitro, but MRK-17 was less effective, in accordance with their in vivo antimetastatic potential. Gene transfection of macrophage colony-stimulating factor (M-CSF) into H69/VP cells to augment macrophage-mediated ADCC resulted in inhibition of metastasis to the liver and lymph nodes, but not kidneys. Combined treatment with a low dose of MRK-16 completely cured metastasis of M-CSF transfectant, but not of the mock transfectant. Our findings suggest that while anti-P-gp Abs had antimetastatic potential against SCLC cells expressing P-gp, combined treatment with M-CSF gene transduction to augment the therapeutic efficacy of anti-P-gp Abs may be beneficial for eradicating metastatic MDR SCLC in humans.

Zabaleta, J., et al. (2014). "The Presence of Mutations in the K-RAS Gene Does Not Affect Survival after Resection of Pulmonary Metastases from Colorectal Cancer." ISRN Surg **2014**: 157586.

Introduction. Our objective was to identify mutations in the K-RAS gene in cases of pulmonary metastases from colorectal cancer (CRC) and determine whether their presence was a prognostic factor for survival. Methods. We included all patients with pulmonary metastases from CRC operated on between 1998 and 2010. K-RAS mutations were investigated by direct sequencing of DNA. Differences in survival were explored with the Kaplan-Meier method log-rank tests and multivariate Cox regression analysis. Results. 110 surgical interventions were performed on 90 patients. Factors significantly associated with survival were disease-free interval (P = 0.002), age (P = 0.007), number of metastases (P = 0.001), lymph node involvement (P = 0.007), size of the metastases (P = 0.013), and previous liver metastasis (P = 0.003). Searching in 79 patients, K-RAS mutations were found in 30 cases. We did not find statistically significant differences in survival (P = 0.913) comparing native and mutated K-RAS. We found a higher rate of lung recurrence (P = 0.040) and shorter time to recurrence (P = 0.015) in patients with K-RAS mutations. Gly12Asp mutation was associated with higher recurrence (P = 0.022) and lower survival (P = 0.389). Conclusions. The presence of K-RAS mutations in pulmonary metastases does not affect overall survival but is associated with higher rates of pulmonary recurrence.

Zeng, Z. S., et al. (1994). "High level of Nm23-H1 gene expression is associated with local colorectal cancer progression not with metastases." Br J Cancer **70**(5): 1025-1030.

This study aimed to determine the expression of Nm23-H1 in colorectal cancer and liver metastases and to correlate Nm23-H1 expression with clinicopathological variables. Specimens from 59 primary colorectal cancers and five liver metastases were studied using Northern blot hybridisation. The mean +/- s.e. of tumour/normal (T/N) ratio of Nm23-H1 RNA expression was 4.3 +/- 0.4 ($P < 0.001$) and 5.1 +/- 0.90 ($P < 0.01$) for colorectal cancer and liver metastases respectively. No significant relationship was observed between the level of Nm23-H1 RNA and the patient's age, sex, tumour location, differentiation, presence of lymph node involvement or distant metastases. Nm23-H1 RNA level was 2.6 +/- 0.5 for tumour size less than 3.0 cm and 4.6 +/- 0.5 for those > or = 3.0 cm (P = 0.05). There appeared to be a trend between increasing relative Nm23-H1 RNA and bowel wall invasion, irrespective of metastatic status (T1 = 1.9 +/- 0.3, T2 = 4.1 +/- 0.6, T3 = 4.1 +/- 0.5 and T4 = 6.4 +/- 1.6). This difference was statistically significant when T1 was compared against > or = T2 lesions (P = 0.01). Western blot analysis reveals two Nm23H-1 bands (17.0 kDa and 18.5 kDa). In 16 colorectal patients, the T/N fold-increase in protein expression was 2.66 +/- 0.46 ($P < 0.001$) and 2.40 +/- 0.32 ($P < 0.001$) for the 17.0 and 18.5 kDa band respectively. Both Nm23-H1 RNA and protein levels in primary colorectal cancers do not appear to correlate with synchronous regional or distant metastases. Since Nm23-H1 RNA expression is associated with increasing tumour size and tumour local invasion, Nm23-H1 RNA expression may be associated with local disease progression.

Zeng, Z. S., et al. (2008). "c-Met gene amplification is associated with advanced stage colorectal cancer and liver metastases." Cancer Lett **265**(2): 258-269.

The c-Met proto-oncogene encodes a receptor tyrosine kinase (TK) that promotes invasive tumor growth and metastasis. Recent studies show that the presence of c-Met gene amplification is predictive for selective c-Met TK inhibitors in gastric cancer and lung cancer. In this study, we utilized a highly quantitative PCR/ligase detection reaction technique to quantify c-Met gene copy number in primary colorectal cancer (CRC) (N=247), liver metastases (N=147), and paired normal tissues. We identified no differences in c-Met gene copy number between normal colonic mucosa and liver tissue. However, mean c-Met gene copy number was significantly elevated in CRC compared with normal mucosa (P<0.001), and in liver metastases compared with normal liver (P<0.001). Furthermore, a significant increase in c-Met was seen in liver metastases compared with primary CRC (P<0.0001). c-Met gene amplification was observed in 2% (3/177) of localized cancers, 9% (6/70) of cancers with distant metastases (P<0.02), and 18% (25/147) of liver metastases (P<0.01). Among patients treated by liver resection, there was a trend toward poorer 3-year survival in association with c-Met gene amplification (P=0.07). Slight increases in c-Met copy number can be detected in localized CRCs, but gene amplification is largely restricted to Stage IV primary cancers and liver metastases. c-Met gene amplification is linked to metastatic progression, and is a viable target for a significant subset of advanced CRC.

Zhao, P., et al. (2010). "E10A, an adenovirus carrying human endostatin gene, in combination with docetaxel treatment inhibits prostate cancer growth and metastases." J Cell Mol Med **14**(1-2): 381-391.

E10A, a replication-defective adenovirus carrying human endostatin gene, has finished Phase I clinical trials for solid cancers. We assessed whether the combination of E10A with docetaxel would enhance antiangiogenic activities and inhibit prostate cancer growth and metastases. Combination use of conditioned medium from prostate cancer cells infected by E10A and docetaxel exerted synergistic inhibition of HUVECs proliferation, migration and tube formation, compared with either agent alone. In prostate cancer s.c. xenograft models, combined therapy resulted in significant tumor growth inhibition and survival improvement. The antitumoral effect was tightly correlated with a remarkable decrease in tumor cell proliferation, microvessel, especially immature vasculature and significant increase in apoptosis induction. Systemic administration of E10A and docetaxel also effectively inhibited orthotopic growth and metastases of prostate cancer and achieved better in vivo antiangiogenic effects than either agent alone. Our data indicate that E10A in combination with docetaxel exert enhanced antiangiogenic activities and inhibit prostate cancer growth and metastases. Therefore, this approach may be an effective treatment for advanced prostate cancer and deserves more extensive investigation.

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