Rectal Cancer

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Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, the cancer can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the rectal cancer.
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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.

In the U.S., according to the National Cancer Institute in 2010, the most common cancers (excluding non-melanoma skin cancers) are listed below.

Cancer type	Estimated new cases	Estimated deaths
Bladder	70,530	14,680
Breast (female-male)	207,090-1,970	39,840-390
Colon and rectal (combined)	142,570	51,370
Endometrial	43,470	7,950
Kidney (renal cell)	53,581	11,997
Leukemia	43,050	21,840
Lung (including bronchus)	222,520	157,300
Melanoma	68,130	8,700
Non-Hodgkin lymphoma	65,540	20,210
Pancreatic	43,140	36,800
Prostate	217,730	32,050
Thyroid	44,670	1,690

Literatures:

Agostini, M., L. M. Pasetto, et al. (2008). "Glutathione S-transferase P1 Ile105Val polymorphism is associated with haematological toxicity in elderly rectal cancer patients receiving preoperative chemoradiotherapy." Drugs Aging **25**(6): 531-9.

BACKGROUND: Increasing evidence suggests that common gene polymorphisms may influence the toxicity of various cytotoxic agents used in the treatment of cancer. OBJECTIVE: To evaluate the predictive value of acute toxicity of methylenetetrahydrofolate reductase 677T polymorphism, glutathione S-transferase P1 (GSTP1) substitution of isoleucine with valine at codon 105 (Ile105Val) polymorphism and the tandem repeat polymorphism in the thymidylate synthase gene promoter in elderly patients with rectal cancer receiving preoperative chemoradiotherapy (CRT). METHOD: From 1994 to 2002, 166 Caucasian patients underwent surgery following CRT for mid-low rectal cancer at a single institution, 42 (male-to-female ratio, 25 : 17) of whom were aged > or =65 years (median age 70 years, range 65-79). The pre-treatment clinical stage was tumour (T) stage 3-4 in 38 patients and node (N)-positive in 29 patients. Patients received external-beam radiotherapy with conventional fractionation and fluorouracil-based chemotherapy. Blood samples were used to extract and amplify DNA. Gene polymorphisms were determined by polymerase chain reaction and restriction enzyme digestion. Acute toxicity to preoperative therapy was reported according to the National Cancer Institute Common Toxicity Criteria, version 2. Univariate and multivariate analyses were performed using one-way analysis of variance and linear regression, respectively. RESULTS: Haematological toxicity (grade 1-2) was observed in 15 of 40 patients for whom toxicity data were available and gastrointestinal toxicity (grade 1-4) in 24 of these same 40 patients. At univariate analysis, female sex ($p = 0.036$) and GSTP1 Ile105Val ($p = 0.0376$) were associated with haematological toxicity. At multivariate analysis, GSTP1 Ile105Val polymorphism ($p = 0.041$) was the only factor found to be associated with haematological toxicity. Patients carrying the Val/Val genotype in the GSTP1 gene had a lower risk of haematological toxicity (odds ratio = 0.322, 95% CI 0.101, 0.957) than patients with the Ile/Ile genotype. CONCLUSION: GSTP1 Ile105Val polymorphism is a promising marker of potential

haematological toxicity in elderly patients with rectal cancer receiving preoperative CRT.

Arbea, L., I. Coma-Canella, et al. (2007). "A case of capecitabine-induced coronary microspasm in a patient with rectal cancer." World J Gastroenterol **13**(14): 2135-7.

5-Fluorouracil (5-FU) is the most frequently used chemotherapy agent concomitant with radiotherapy in the management of patients with rectal cancer. Capecitabine is an oral fluoropyrimidine that mimics the pharmaconkinetics of infusional 5-FU. This new drug is replacing 5-FU as a part of the combined-modality treatment of a number of gastrointestinal cancers. While cardiac events associated with the use of 5-FU are a well known side effect, capecitabine-induced cardiotoxicity has been only rarely reported. Here, we reviewed the case of a patient with rectal cancer who had a capecitabine-induced coronary vasospasm. The most prominent mutation of the dihydropyrimidine dehydrogenase gene was also analyzed.

Atkin, G. K., F. M. Daley, et al. (2006). "The impact of surgically induced ischaemia on protein levels in patients undergoing rectal cancer surgery." Br J Cancer **95**(7): 928-33.

The goal of targeted therapy has driven a search for markers of prognosis and response to adjuvant therapy. The surgical resection of a solid tumour induces tissue ischaemia and acidosis, both potent mediators of gene expression. This study investigated the impact of colorectal cancer (CRC) surgery on prognostic and predictive marker levels. Tumour expression of thymidylate synthase, thymidine phosphorylase, cyclin A, vascular endothelial growth factor (VEGF), carbonic anhydrase-9, hypoxia inducible factor-1alpha, and glucose transporter-1 (GLUT-1) proteins was determined before and after rectal cancer surgery. Spectral imaging of tissue sections stained by immunohistochemistry provided quantitative data. Surgery altered thymidylate synthase protein expression (P=0.02), and this correlated with the change in the proliferation marker cyclin A. The expression of hypoxia inducible factor-1alpha, VEGF, and GLUT-1 proteins was also different following surgery. Colorectal cancer surgery significantly impacts on intratumoral gene expression, suggesting archival specimens may not accurately reflect in situ marker levels. Although rectal cancer was the studied model, the results may be applicable to any solid tumour undergoing extirpation in which molecular markers have been proposed to guide patient therapy.

Baba, S., H. Ogiwara, et al. (1994). "Extended lymphadenectomy and the quality of life in rectal cancer patients." Int Surg **79**(1): 23-6.

For a better quality of life in rectal cancer patients, high dose radiotherapy following abdominoperineal resection of the rectum with a pelvic partition is another surgical option replacing extended abdominoperineal resection. In addition to pelvic partition with polyglycolic acid mesh, the tissue expander was inserted into the pelvic cavity to support the intestine upward and the bladder forward. The mean total radiation dosage was 5040 cGy. Between 1989 and 1991, 10 patients were treated according to this method. Out of 10 patients 9 were free of recurrence, and only one had hepatic metastasis. In addition, postoperatively, the average residual urine by this method was 39.1 ml and was statistically different compared to a figure of 200 ml in conventional abdominoperineal resections ($p < 0.001$, "t"-test). In order to individualize the operative procedures among a variety of surgical options, the molecular biological technique was utilized. In p53 stain analysis of 114 colorectal cancer patients, patients with p53 positive staining reached a higher stage than those with p53 negative staining ($p < 0.05$, chi 2 analysis). Therefore, we surmised that the positivity of the p53 stain could be one of the factors gauged as an indication of postoperative high-dose radiation. In conclusion, high-dose postoperative radiotherapy was thought to be one of the treatment modalities to improve the survival and quality of life of advanced rectal cancer in selected cases.

Bedrosian, I., G. Giacco, et al. (2006). "Outcome after curative resection for locally recurrent rectal cancer." Dis Colon Rectum **49**(2): 175-82.

PURPOSE: Few biologic markers have been studied as prognostic factors in recurrent rectal carcinoma patients. We sought to determine the influence of clinical, pathologic, and biologic (p53, bcl-2, and ki-67) variables on survival after curative resection of locally recurrent rectal cancer. METHODS: Retrospective review of patients with locally recurrent rectal cancer who received surgery with curative intent. RESULTS: From 1988 to 1998, 134 patients with locally recurrent rectal cancer underwent operative exploration. Curative resection was performed in 85 patients. Median follow-up was 43 (range, 1.3-149) months. On multivariate analysis, negative predictors of overall survival included an elevated carcinoembryonic antigen level (P=0.02; hazard ratio 2.41; 95 percent confidence interval, 1.19-4.89) and an R1 resection margin (P = 0.01; hazard ratio, 2.81; 95 percent confidence interval, 1.27-6.21). In 26 patients for whom biologic variables were available, p53, bcl-2, and ki-67 did not

significantly impact disease-specific survival or overall survival. Five-year disease-specific survival, overall survival, and pelvic control rates were 46, 36, and 51 percent respectively. Of the 50 patients who relapsed, time to second local recurrence was longer than time to development of metastasis (median, 16.5 vs. 9 months). Median survival for patients with metastatic recurrence was 26.1 vs. 41.5 months for those with a subsequent local recurrence alone. CONCLUSIONS: Approximately two-thirds of patients with locally recurrent rectal cancer can be resected for cure. Preoperative carcinoembryonic antigen and an R0 resection margin were the only significant predictors of overall survival. p53, bcl-2, and ki-67 did not impact survival outcomes.

Bengala, C., S. Bettelli, et al. (2009). "Epidermal growth factor receptor gene copy number, K-ras mutation and pathological response to preoperative cetuximab, 5-FU and radiation therapy in locally advanced rectal cancer." Ann Oncol **20**(3): 469-74.

BACKGROUND: Cetuximab improves activity of chemotherapy in metastatic colorectal cancer (mCRC). Gene copy number (GCN) of epidermal growth factor receptor (EGFR) has been suggested to be a predictive factor of response to cetuximab in patients (pts) with mCRC; on the contrary, K-ras mutation has been associated with cetuximab resistance. PATIENTS AND METHODS: We have conducted a phase II study with cetuximab administered weekly for 3 weeks as single agent and then with 5-fluorouracil and radiation therapy as neo-adjuvant treatment for locally advanced rectal cancer (LARC). EGFR immunohistochemistry expression, EGFR GCN and K-ras mutation were evaluated on diagnostic tumor biopsy. Dworak's tumor regression grade (TRG) was evaluated on surgical specimens. RESULTS: Forty pts have been treated; 39 pts are assessable. TRG 3 and 4 were achieved in nine (23.1%) and three pts (7.7%) respectively; TRG 3-4 rate was 55% and 5.3% in case of high and low GCN, respectively (P 0.0016). Pts with K-ras mutated tumors had lower rate of high TRG: 11% versus 36.7% (P 0.12). In pts with wild-type K-ras, TRG 3-4 rate was 58.8% versus 7.7% in case of high or low GCN, respectively (P 0.0012). CONCLUSIONS: In pts with LARC, EGFR GCN is predictive of high TRG to cetuximab plus 5-FU radiotherapy. Moreover, our data suggest that a wild-type K-ras associated with a high EGFR GCN can predict sensitivity to cetuximab-based treatment.

Bongaerts, B. W., A. F. de Goeij, et al. (2006). "Alcohol and the risk of colon and rectal cancer with mutations in the K-ras gene." Alcohol **38**(3): 147-54.

The first metabolite of alcohol, acetaldehyde, may trigger replication errors and mutations in DNA, which may predispose to developing colorectal cancer (CRC). In a prospective study on colon and rectal cancer, we investigated the following hypotheses: alcohol consumption is associated with an increased risk of mutations in the K-ras oncogene, and beer consumption is associated with an increased risk of G-->A mutations in this gene. Therefore, we studied the associations between consumption of alcohol and alcoholic beverages and the risk of CRC without and with specific K-ras gene mutations. In 1986, 120,852 men and women, aged 55-69 years, completed a questionnaire on risk factors for cancer. The case-cohort approach was used for data processing and analyses. After 7.3 years of follow-up, excluding the first 2.3 years, complete data from 4,076 subcohort members, 428 colon and 150 rectal cancer patients, were available for data analyses. Incidence rate ratios (RRs) and corresponding 95% confidence intervals (95% CIs) were estimated using Cox proportional hazards models. Compared to abstaining, a total alcohol consumption of 30.0 g/day and more was associated with the risk of colon and rectal cancer with and without a K-ras mutation in both men and women. Independent from alcohol intake, liquor consumption when compared to nonliquor consumption was associated with an increased risk of rectal cancer with a wild type K-ras in men (RR: 2.25, 95% CI: 1.0-5.0). Beer consumption was not clearly associated with the risk of colon and rectal tumors harboring G-->A mutations in the K-ras gene in men. This association could not be assessed in women because of sparse beer consumption. In conclusion, alcohol does not seem to be involved in predisposing to CRC through mutations in the K-ras gene, and specifically beer consumption is not associated with colon and rectal tumors harboring a G-->A mutation.

Brink, M., M. P. Weijenberg, et al. (2005). "Meat consumption and K-ras mutations in sporadic colon and rectal cancer in The Netherlands Cohort Study." Br J Cancer **92**(7): 1310-20.

Case-cohort analyses were performed on meat and fish consumption in relation to K-ras mutations in 448 colon and 160 rectal cancers that occurred during 7.3 years of follow-up, excluding the first 2.3 years, and 2948 subcohort members of The Netherlands Cohort Study on diet and cancer. Adjusted incidence rate ratios and 95% confidence intervals were computed for colon and rectal cancer and for K-ras mutation status subgroups. Total fresh meat, most types of fresh meat and fish were not associated with colon or rectal cancer, neither overall nor with K-ras mutation status. However, several weak associations were observed for tumours with a

wild-type K-ras, including beef and colon tumours, and an inverse association for pork with colon and rectal tumours; for meat products, an increased association was observed with wild-type K-ras tumours in the colon and possibly with G>A transitions in rectal tumours.

Brink, M., M. P. Weijenberg, et al. (2005). "Dietary folate intake and k-ras mutations in sporadic colon and rectal cancer in The Netherlands Cohort Study." Int J Cancer **114**(5): 824-30.

We studied the association between dietary folate and specific K-ras mutations in colon and rectal cancer in The Netherlands Cohort Study on diet and cancer. After 7.3 years of follow-up, 448 colon and 160 rectal cancer patients and 3,048 sub-cohort members (55-69 years at baseline) were available for data analyses. Mutation analysis of the K-ras gene was carried out on all archival adenocarcinoma specimens. Case-cohort analyses were used to compute adjusted incidence rate ratios (RR) and 95% confidence intervals (CI) for colon and rectal cancer overall and for K-ras mutation status subgroups according to 100 mug/day increased intake in dietary folate. Dietary folate intake was not significantly associated with colon cancer risk for men or women, neither overall nor with K-ras mutation status. For rectal cancer, folate intake was associated with a decreased disease risk in men and was most pronounced for K-ras mutated tumors, whereas an increased association was observed for women. Regarding the K-ras mutation status in women, an increased association was observed for both wild-type and mutated K-ras tumors. Specifically, folate intake was associated with an increased risk of G>T and G>C transversions in rectal tumors (RR = 2.69, 95% CI = 1.43-5.09), but inversely associated with G>A transitions (RR = 0.08, 95% CI = 0.01-0.53). Our data suggest that the effect of folate on rectal cancer risk is different for men and women and depends on the K-ras mutation status of the tumor.

Cascinu, S., F. Graziano, et al. (2002). "An analysis of p53, BAX and vascular endothelial growth factor expression in node-positive rectal cancer. Relationships with tumour recurrence and event-free survival of patients treated with adjuvant chemoradiation." Br J Cancer **86**(5): 744-9.

Tumours of patients with node-positive rectal cancer were studied by immunohistochemistry for p53, BAX and vascular endothelial growth factor expressions. Results were correlated to the relapse rate, the pattern of relapse and the event-free survival after radical surgery and adjuvant chemoradiation. After a median follow-up of 60 months, 39 patients remained disease-free and 40 patients relapsed (18 local relapses and 22 distant metastases). The majority of disease-free patients showed p53 negative and vascular endothelial growth factor negative tumours. Local relapses occurred more frequently in patients with p53 overexpressing tumours (P<0.01), while distant metastases were in patients with vascular endothelial growth factor positive tumours (P<0.003). Patients with p53 negative or vascular endothelial growth factor negative tumours showed better event-free survival than patients with p53 positive or vascular endothelial growth factor positive tumours. BAX analysis did not show any association with patients' outcome and it was unrelated to the p53 status. Adjuvant treatment strategies for node-positive rectal cancer may be improved by identifying categories of high-risk patients. In this study, vascular endothelial growth factor and p53 expressions correlated with recurrent disease, pattern of relapse and poor event-free survival.

Chen, Y., K. J. Chang, et al. (2002). "Establishment and characterization of a rectal cancer model in mice: application to cytokine gene therapy." Int J Colorectal Dis **17**(6): 388-95.

BACKGROUND AND AIMS: We established an orthotopic animal model of rectal cancer in mice and applied this model to the study of the antitumor effects of cytokine-assisted tumor vaccine. MATERIALS AND METHODS: The CT-26 murine colon adenocarcinoma cells were inoculated into the submucosa of the rectum of the mice to induce the rectal tumor. The tumor growth rate and the survival time of the mice were observed. The cDNA of granulocyte-macrophage colony-stimulating factor (GM-CSF) was transduced to the CT-26 cell line via a retroviral vector, and the therapeutic effects of irradiated GM-CSF secreting tumor vaccine on the rectal tumor were investigated. RESULTS: All the mice implanted with the wild-type tumor cells had tumor growth in the rectum and died. The mean survival time of the mice was 28.9 days. Two doses of irradiated GM-CSF secreting tumor vaccine administered on days 0 and 3 after tumor cell implantation significantly prolonged the survival of the mice with rectal tumor compared with that of the control groups (P<0.0001). In contrast, no antitumor effect was observed when the treatment with GM-CSF secreting tumor vaccine was delayed to 3 days after tumor cell implantation (P>0.17). CONCLUSION: The results suggest that cytokine gene therapy exerts an antitumor effect on small tumors and may be considered as an adjuvant immunotherapy of rectal cancers and prevention of reimplantation of tumor cells disseminated during or following surgery. The orthotopic animal model of the rectal cancer in mice

could be applied to the in vivo experimental studies of rectal cancer.

Curtin, K., W. S. Samowitz, et al. (2009). "Somatic alterations, metabolizing genes and smoking in rectal cancer." Int J Cancer **125**(1): 158-64.

Cigarette smoking has been identified as a risk factor for rectal cancer. Our investigation evaluates associations between active and passive smoking and TP53, KRAS2, and BRAF V600E mutations, microsatellite instability (MSI), and CpG Island Methylator Phenotype (CIMP) in rectal tumors. We examine how genetic variants of GSTM1 and NAT2 alter these associations in a population-based, case-control study of 750 incident rectal cancer cases and 1,201 controls. Detailed tobacco exposure data were collected in an extensive questionnaire. DNA from blood was examined for GSTM1 and NAT2 variants. Tumor DNA was assessed to determine TP53 (exons 5-8), KRAS2 (codons 12-13) and BRAF mutations, MSI (BAT26 and TGFbetaRII analysis), and CIMP (methylation of CpG islands in CDKN2A, MLH1, MINT1, MINT2 and MINT31). Cigarette smoking (>20 pack-years, relative to nonsmokers) was associated with increased risk of TP53 mutations (OR = 1.4, 95% CI 1.02-2.0), BRAF mutations (OR = 4.2, 95% CI 1.3-14.2) and MSI (OR = 5.7, 95% CI 1.1-29.8) in rectal tumors. Long-term environmental tobacco smoke (ETS) exposure of >10 hr/wk was associated with increased risk of KRAS2 mutation (OR = 1.5, 95% CI 1.04-2.2). All smoking indicators were suggestive of increased risk in CIMP+ rectal cancer. GSTM1 and NAT2 were generally not associated with rectal tumor alterations; however, we observed an interaction of ETS and NAT2 in TP53-mutated tumors ($p < 0.01$). Our investigation shows active smoking is associated with increased risk of TP53, BRAF and MSI+ in rectal tumors and is suggestive of increased risk of CIMP+ tumors. ETS may increase risk of KRAS2 mutations; association with TP53 mutations and ETS may be influenced by NAT2.

Daemen, A., O. Gevaert, et al. (2008). "Integrating microarray and proteomics data to predict the response on cetuximab in patients with rectal cancer." Pac Symp Biocomput: 166-77.

To investigate the combination of cetuximab, capecitabine and radiotherapy in the preoperative treatment of patients with rectal cancer, fourty tumour samples were gathered before treatment (T0), after one dose of cetuximab but before radiotherapy with capecitabine (T1) and at moment of surgery (T2). The tumour and plasma samples were subjected at all timepoints to Affymetrix microarray and Luminex proteomics analysis, respectively. At surgery, the Rectal Cancer Regression Grade (RCRG) was registered. We used a kernel-based method with Least Squares Support Vector Machines to predict RCRG based on the integration of microarray and proteomics data on To and T1. We demonstrated that combining multiple data sources improves the predictive power. The best model was based on 5 genes and 10 proteins at T0 and T1 and could predict the RCRG with an accuracy of 91.7%, sensitivity of 96.2% and specificity of 80%.

de Bruin, E. C., C. J. van de Velde, et al. (2008). "Epithelial human leukocyte antigen-DR expression predicts reduced recurrence rates and prolonged survival in rectal cancer patients." Clin Cancer Res **14**(4): 1073-9.

PURPOSE: The development of local and distant recurrences is a major problem in the treatment of rectal cancer patients. In this study, we investigated whether epithelial human leukocyte antigen-DR (HLA-DR) expression allowed discrimination between high and low tumor recurrence rates, and analyzed the mechanism behind its expression. EXPERIMENTAL DESIGN: The role of IFNgamma in HLA-DR expression was studied in rectal cancer cell lines and tumors by promoter-specific analyses of class II transactivator (CIITA). The predictive value of epithelial HLA-DR expression was investigated by immunohistochemical evaluation of 1,016 rectal tumors, obtained from a large prospective trial. Associations with recurrences and survival were determined by univariate and multivariate log-rank testing. RESULTS: HLA-DR was induced by IFNgamma in rectal cancer cell lines. Activity of the IFNgamma-inducible pIV-CIITA promoter correlated with epithelial HLA-DR expression in rectal tumors. Patients with HLA-DR-positive tumors developed less frequent local and distant recurrences [1.6% versus 9.1% ($P = 0.0015$) and 15.3% versus 29.9% ($P < 0.0001$), respectively, after 5 years of follow-up] and had better survival (78.6% versus 61.3%; $P < 0.0001$) than patients with HLA-DR-negative tumors. Epithelial HLA-DR was more often found in lower tumor-node-metastasis (TNM) stages. Next to TNM and circumferential resection margin, HLA-DR expression was independently associated with lower distant recurrence rates and prolonged survival. CONCLUSIONS: Epithelial HLA-DR expression can be used as a marker to discriminate patients with high or low risk of developing recurrences. The possible involvement of IFNgamma, the relationship with lower TNM stages, and the independent effect on recurrence development together suggest that the host immune response plays an important role in controlling tumor cells.

de Heer, P., E. C. de Bruin, et al. (2007). "Caspase-3 activity predicts local recurrence in rectal cancer." Clin Cancer Res **13**(19): 5810-5.

PURPOSE: Radiotherapy followed by total mesorectal excision surgery has been shown to significantly reduce local recurrence rates in rectal cancer patients. Radiotherapy, however, is associated with considerable morbidity. The present study evaluated the use of biochemical detection of enzymatic caspase-3 activity as preoperative marker for apoptosis to preselect patients that are unlikely to develop a local recurrence to spare these patients from overtreatment and the negative side effects of radiotherapy. EXPERIMENTAL DESIGN: Nonirradiated freshly frozen tissue samples from 117 stage III rectal cancer patients were collected from a randomized clinical trial that evaluated preoperative radiotherapy in total mesorectal excision surgery. Additional frozen archival tissues from 47 preoperative biopsies and corresponding resected colorectal tumors were collected. Level of apoptosis was determined by measuring the enzymatic activity of caspase-3 in a biochemical assay. RESULTS: In tumor tissue, caspase-3 activity lower than the median was predictive of 5-year local recurrence (hazard ratio, 7.4; 95% confidence interval, 1.7-32.8; P = 0.008), which was unaffected by adjustment for type of resection, tumor location, and T status (adjusted hazard ratio, 7.5; 95% confidence interval, 1.7-34.1; P = 0.009). Caspase-3 activity in preoperative biopsies was significantly correlated with caspase-3 activity in corresponding resected tumors (r = 0.56; P < 0.0001). CONCLUSION: Detection of tumor apoptosis levels by measuring caspase-3 activity, for which a preoperative biopsy can be used, accurately predicted local recurrence in rectal cancer patients. These findings indicate that caspase-3 activity is an important denominator of local recurrence and should be evaluated prospectively to be added to the criteria to select rectal cancer patients in which radiotherapy is redundant.

de Maat, M. F., C. J. van de Velde, et al. (2008). "Quantitative analysis of methylation of genomic loci in early-stage rectal cancer predicts distant recurrence." J Clin Oncol **26**(14): 2327-35.

PURPOSE: There are no accurate prognostic biomarkers specific for rectal cancer. Epigenetic aberrations, in the form of DNA methylation, accumulate early during rectal tumor formation. In a preliminary study, we investigated absolute quantitative methylation changes associated with tumor progression of rectal tissue at multiple genomic methylated-in-tumor (MINT) loci sequences. We then explored in a different clinical patient group whether these epigenetic changes could be correlated with clinical outcome. PATIENTS AND METHODS: Absolute quantitative assessment of methylated alleles was used to assay methylation changes at MINT 1, 2, 3, 12, 17, 25, and 31 in sets of normal, adenomatous, and malignant tissues from 46 patients with rectal cancer. Methylation levels of these biomarkers were then assessed in operative specimens of 251 patients who underwent total mesorectal excision (TME) without neoadjuvant radiotherapy in a multicenter clinical trial. RESULTS: Methylation at MINT 2, 3, and 31 increased 11-fold (P = .005), 15-fold (P < .001), and two-fold (P = .02), respectively, during adenomatous transformation in normal rectal epithelium. Unsupervised grouping analyses of quantitative MINT methylation data of TME trial patients demonstrated two prognostic subclasses. In multivariate analysis of node-negative patients, this subclassification was the only predictor for distant recurrence (hazard ratio [HR], 4.17; 95% CI, 1.72 to 10.10; P = .002), cancer-specific survival (HR, 3.74; 95% CI, 1.4 to 9.43; P = .003), and overall survival (HR, 2.68; 95% CI, 1.41 to 5.11; P = .005). CONCLUSION: Methylation levels of specific MINT loci can be used as prognostic variables in patients with American Joint Committee on Cancer stage I and II rectal cancer. Quantitative epigenetic classification of rectal cancer merits evaluation as a stratification factor for adjuvant treatment in early disease.

Debucquoy, A., K. Haustermans, et al. (2009). "Molecular response to cetuximab and efficacy of preoperative cetuximab-based chemoradiation in rectal cancer." J Clin Oncol **27**(17): 2751-7.

PURPOSE: To characterize the molecular pathways activated or inhibited by cetuximab when combined with chemoradiotherapy (CRT) in rectal cancer and to identify molecular profiles and biomarkers that might improve patient selection for such treatments. PATIENTS AND METHODS: Forty-one patients with rectal cancer (T3-4 and/or N+) received preoperative radiotherapy (1.8 Gy, 5 days/wk, 45 Gy) in combination with capecitabine and cetuximab (400 mg/m2 as initial dose 1 week before CRT followed by 250 mg/m2 /wk for 5 weeks). Biopsies and plasma samples were taken before treatment, after cetuximab but before CRT, and at the time of surgery. Proteomics and microarrays were used to monitor the molecular response to cetuximab and to identify profiles and biomarkers to predict treatment efficacy. RESULTS: Cetuximab on its own downregulated genes involved in proliferation and invasion and upregulated inflammatory gene expression, with 16 genes being significantly influenced in microarray analysis. The decrease in proliferation was confirmed by immunohistochemistry for Ki67 (P = .01) and was accompanied by an

increase in transforming growth factor-alpha in plasma samples (P < .001). Disease-free survival (DFS) was better in patients if epidermal growth factor receptor expression was upregulated in the tumor after the initial cetuximab dose (P = .02) and when fibro-inflammatory changes were present in the surgical specimen (P = .03). Microarray and proteomic profiles were predictive of DFS. CONCLUSION: Our study showed that a single dose of cetuximab has a significant impact on the expression of genes involved in tumor proliferation and inflammation. We identified potential biomarkers that might predict response to cetuximab-based CRT.

Elsaleh, H., P. Robbins, et al. (2000). "Can p53 alterations be used to predict tumour response to pre-operative chemo-radiotherapy in locally advanced rectal cancer?" Radiother Oncol **56**(2): 239-44.

PURPOSE: To examine whether p53 tumour suppressor gene alterations can be used to predict tumour response to pre-operative chemo-radiation in locally advanced rectal cancer in terms of reduction in tumour size and local failure. METHODS: p53 alterations were studied in pre-treatment biopsy specimens of rectal carcinomas from 48 patients by immunohistochemistry (IHC) and polymerase chain reaction/single strand conformation polymorphism (PCR-SSCP) gene mutation analysis. Pre-operative pelvic radiotherapy was delivered with four fields, 45 Gy to the ICRU point in 25 fractions over 5 weeks. A radio-sensitising dose of 5-fluorouracil (500 mg/m(2)) was delivered concurrently for 6 days of the 5-week schedule (days 1, 2, 3 and days 22, 23 and 24). Total meso-rectal excision was planned 4 to 6 weeks from completion of pre-operative treatment. Response to therapy was assessed by macroscopic measurement of the surgical specimen by a pathologist who was unaware of the pre-treatment tumour size or of the p53 status. RESULTS: IHC evidence of p53 protein accumulation was found in 40% of tumours, p53 gene mutation in 35% and p53 alteration (either or both changes) in 46%. The average reduction in tumour size was 53% in the group with 'wild-type' p53 (IHC-/SSCP-) and 63% in the group with altered p53 (either IHC+ or SSCP+; P=0.18). No significant differences in tumour size reduction or local failure were observed in the groups with p53 overexpression or p53 mutation compared with normal. CONCLUSIONS: p53 alteration detected by IHC or SSCP analysis is not a clinically useful predictor of local response to pre-operative adjuvant therapy in advanced rectal carcinoma.

Esposito, G., S. Pucciarelli, et al. (2001). "P27kip1 expression is associated with tumor response to preoperative chemoradiotherapy in rectal cancer." Ann Surg Oncol **8**(4): 311-8.

BACKGROUND: Our aim was to ascertain whether or not the response to preoperative chemoradiotherapy for rectal cancer is associated with p27kip1 and p53 protein expression. METHODS: Thirty-eight patients (27 male, 11 female) with a mean age of 59 years (age range 33-87) and stage II-III rectal cancer received preoperative chemoradiotherapy (45-50.4 Gy; 5-FU 350 mg/m2/day and leucovorin 10 mg/m2/day). Thirty-one underwent low anterior resection; seven underwent abdominoperineal excision. Endoscopic tumor biopsies, performed before adjuvant therapy, were evaluated for: histologic type, tumor differentiation, mitotic index, and p27kip1 and p53 protein expression which were immunohistochemically determined. p53 expression was graded as: a) absent or present in < or =10% of tumor cells; b) present in 11-25%; c) present in 26-75%; and d) present in >75% of tumor cells. p27kip1 expression was assessed using both light microscopy (percent of stained cells x10 HPF) and cytometry with an image analysis workstation. Tumor response, ascertained with histology, was classified using a scale from 0 (no response) to 6 (complete pathologic response). RESULTS: The mitotic index for the endoscopic biopsies was low in 14 cases, moderate in 17 cases, and high in 7 cases. p53 protein expression was found in 21 (a), 3 (b), 3 (c), and 11 (d) cases. The mean percentage of cells expressing the p27kip1 protein was 34 (range 0-77.14%). A close correlation was found between cytometric and light microscopy findings for p27kip1 (r2 = 0.92, P = .0001). Tumor differentiation was good in 5 cases, poor in 2 cases, and moderate in the remaining 31 cases. While the response to adjuvant therapy was good/complete in 25 (65.78%) cases, it was absent/poor in 13 (34.21%) cases. Univariate analysis associated type of adjuvant therapy (chemoradiotherapy, P = .0428) and p27kip1 protein lower expression (P = .0148) with a poor response to adjuvant treatment. Stepwise linear regression found overexpression of p53 and p27kip1 and young age to be independent variables that were linked to a good response to adjuvant therapy. CONCLUSIONS: Lack of p27kip1 and p53 protein expression in rectal cancer is associated with a poor response to preoperative adjuvant therapy.

Fernebro, E., B. Halvarsson, et al. (2002). "Predominance of CIN versus MSI in the development of rectal cancer at young age." BMC Cancer **2**: 25.

BACKGROUND: Development of proximal and distal colorectal cancers involve partly different mechanisms associated with the microsatellite instability (MSI) and the chromosomal instability (CIN) pathways. Colorectal cancers in patients under

50 years of age represent about 5% of the total number of tumors and have been associated with an increased frequency of MSI tumors. However, MSI and CIN may play different roles in the development of colon cancer and rectal cancer, and we have specifically investigated their contribution to the development of rectal cancer at young age. METHODS: Thirty rectal cancers diagnosed before the age of 50 were characterized for DNA-ploidy, MSI, mutations of KRAS and CTNNB1 and immunohistochemical expression of p53, beta-catenin and of the mismatch repair (MMR) proteins MLH1 and MSH2. RESULTS: DNA aneuploidy was detected in 21/30 tumors, KRAS mutations in 6 tumors, no mutations of CTNNB1 were detected but immunohistochemical staining for beta-catenin showed nuclear staining in 6 tumors, and immunohistochemical expression of p53 was detected in 18 tumors. MSI was detected in 3/30 tumors, all of which showed and immunohistochemical loss of staining for the MMR protein MSH2, which strongly indicates a phenotype associated with hereditary nonpolyposis colorectal cancer (HNPCC). CONCLUSIONS: MSI occurs only in a small fraction of the tumors from young patients with rectal cancer, but when present it strongly indicates an underlying HNPCC-causing mutation, and other mechanisms than HNPCC thus cause rectal cancer in the majority of young patients.

Figer, A., R. Shtoyerman-Chen, et al. (2001). "Phenotypic characteristics of colo-rectal cancer in I1307K APC germline mutation carriers compared with sporadic cases." Br J Cancer **85**(9): 1368-71.

The I1307K APC germline mutation is associated with an increased risk to colo-rectal cancer (CRC). Whether and to what extent the phenotype of CRC in mutation carriers differs from sporadic cases, remains unknown. To gain insight into this issue, we analysed 307 unselected Israeli patients with CRC, who were treated in a single medical centre, for harbouring the I1307K mutation. Twenty-eight mutation carriers (9.1%) were detected. Two of 28 mutation carriers (7.1%) and 93/277 (33.6%) of non-carriers, were of non-Ashkenazi origin ($P < 0.01$). In 74/278 (26.6%) of the sporadic cases, and only 1/28 (3.6%) of mutation carriers (3.6%) the tumour was located in the right colon ($P < 0.01$). Mutation carriers had a more advanced disease stage (14/28 - 50% Dukes C), as compared with 60 (19.5%) of non-carriers ($P = 0.02$). The mean age at diagnosis was similar: 65 (+/- 9.7) years and 66.3 (+/- 11.6) years, for mutation carriers and non-carriers, respectively. No statistical differences were noted between the two groups in sex distribution, tumour grade, and family history of cancer. We conclude that early age at diagnosis and family history of cancer cannot be used to predict who is likely to harbour the I1307K APC germline mutation carriers. However, the tumours in patients with this mutation appear different than those without, are less likely to be proximal and more likely to be advanced than tumours in non-carriers.

Funke, S., A. Risch, et al. (2009). "Genetic Polymorphisms in Genes Related to Oxidative Stress (GSTP1, GSTM1, GSTT1, CAT, MnSOD, MPO, eNOS) and Survival of Rectal Cancer Patients after Radiotherapy." J Cancer Epidemiol **2009**: 302047.

Radiotherapy exerts part of its antineoplastic effect by generating oxidative stress, therefore genetic variation in oxidative stress-related enzymes may influence survival of rectal cancer patients. We hypothesized that genetic polymorphisms associated with higher amounts of reactive oxygen species (ROS) that exaggerate cytotoxic activity could improve survival after radiotherapy. We followed 114 rectal cancer patients who received radiotherapy for an average of 42.5 months. Associations between genotypes (GSTP1, GSTM1, GSTT1, CAT, MnSOD, MPO and eNOS) and overall survival were assessed using Kaplan-Meier curves and Cox proportional hazards regression. As hypothesized, patients carrying low ROS producing eNOS Glu298Asp asparagine allele showed an increased hazard of death compared to homozygous carriers of the glutamine allele (hazard ratio (HR): 2.10, 95% confidence interval (CI): 1.01-4.38). However, carriers of low ROS producing MPO G463A A allele had a decreased hazard of death compared to patients homozygous for the G allele (HR: 0.44, 95% CI: 0.21-0.93) although patients homozygous for the A allele had a slightly increased hazard (HR: 1.12, 95% CI: 0.25-5.08). This explorative study provides first results and highlights the need for further, larger studies to investigate association between genetic variation in oxidative stress genes and survival of rectal cancer patients who received radiotherapy.

Gassler, N., I. Herr, et al. (2004). "Wnt-signaling and apoptosis after neoadjuvant short-term radiotherapy for rectal cancer." Int J Oncol **25**(6): 1543-9.

Recent surgical concepts for primary rectal cancer include the combination of surgery and short-term neoadjuvant radiotherapy (STNR). This is usually given in a dose of 25 Gy over five days in order to reduce local recurrence rates. Clinical studies have shown that local recurrence is found in some patients despite STNR. We identified molecular patterns of the Wnt- and apoptosis pathways as well as expression of junction-associated molecules in rectal cancer specimens of patients who received STNR and in those who did not. Expression patterns were examined by immunohistochemistry and molecular

techniques such as LightCycler RT-PCR and Western blot analysis in 25 sporadic rectal adenocarcinoma specimens derived from STNR-patients or non-pretreated donors, respectively. The molecular pattern in response to STNR was heterogeneous and was reflected by responders who show activation of apoptosis and cellular remodeling, whereas the group of non-responders from STNR did not show such reaction and was very similar to untreated controls. Enhanced expression of beta-catenin was generally mediated by STNR, but exclusively in the responder group impaired expression of c-Myc and junction-associated molecules as well as cleavage of poly-ADP-ribose polymerase and of the caspase substrate cytokeratin 19 were found. The molecular profile suggests that STNR interferes with Wnt-signaling and c-Myc expression. STNR in its present form is not suitable to fully complete the sequence of apoptosis in all rectal adenocarcinomas.

Gimbel, M. I. and P. B. Paty (2004). "A current perspective on local excision of rectal cancer." Clin Colorectal Cancer **4**(1): 26-35; discussion 36-7.

Local excision of rectal cancer is appealing because of its technical ease and excellent functional results, but concern over inadequate pathologic staging and inferior treatment outcomes when compared with radical surgery remain a major hurdle for its widespread use. Local failure rates in modern series for local excision are 4%-18% for T1 rectal cancers and 22%-67% for T2 cancers, and cancer cure rates are only 70%-80%. In addition, data from the past decade suggest that preoperative staging with endorectal ultrasound, use of postoperative adjuvant chemotherapy/radiation therapy, and aggressive salvage surgery have not been reliable methods of limiting local tumor recurrence or improving long-term cure rates. At present, highly stringent criteria for patient selection are recommended, yet such stringency decreases the utility of the procedure. What are needed are new approaches to an old problem. Novel strategies under evaluation include enhanced imaging modalities for lymph node metastases, neoadjuvant chemotherapy/radiation therapy, and more liberal use of immediate salvage resection for high-risk pathologic features. Molecular profiling of tumors with genetic markers and better integration of traditional and gene-targeted systemic therapy are promising approaches for the future. This review of the literature evaluates the recent successes and failures of local excision of rectal cancer and provides a current perspective on the expanded use of local excision without compromising care.

Giralt, J., M. de las Heras, et al. (2005). "The expression of epidermal growth factor receptor results in a worse prognosis for patients with rectal cancer treated with preoperative radiotherapy: a multicenter, retrospective analysis." Radiother Oncol **74**(2): 101-8.

BACKGROUND AND PURPOSE: Expression of epidermal growth factor receptor (EGFR) is observed in 50-70% of colorectal carcinoma and is associated with poor prognosis. The aim of this study was to determine the prognostic value of EGFR status before radiotherapy in a group of patients with locally advanced rectal cancer treated with preoperative radiotherapy. PATIENTS AND METHODS: Eighty-seven patients were studied retrospectively. Treatment consisted of pelvic radiotherapy, in 50 patients with concomitant chemotherapy and surgical resection. Immunohistochemistry for EGFR was determined at the preradiation biopsy and in the resected specimens. Immunohistochemical analysis for EGFR expression was evaluated according to extension and staining intensity. We defined positive staining (EGFR positive), when extension was 5% or more. RESULTS: A total of 52 of 87 tumors showed EGFR positive status at biopsy (60%) and EGFR expression was associated neither with clinical tumor stage nor with clinical nodal stage. EGFR positive expression was linked to a lack of pathologic complete response to preoperative radiotherapy (P=0.006). Disease-free survival was lower among patients with EGFR positive status before radiotherapy (P=0.003). In a multivariate analysis EGFR expression at biopsy was a statistically significant predictor of disease-free survival, RR=2.88(1.1-7.8), P=0.036. CONCLUSIONS: EGFR is expressed in a significant number of rectal tumors. EGFR-positive expression before radiotherapy is an indicator for poor response and low disease-free survival.

Gordon, M. A., J. Gil, et al. (2006). "Genomic profiling associated with recurrence in patients with rectal cancer treated with chemoradiation." Pharmacogenomics **7**(1): 67-88.

PURPOSE: Stage II and III adenocarcinoma of the rectum has an overall 5-year survival rate of approximately 50%, and tumor recurrence remains a major problem despite an improvement in local control through chemotherapy and radiation. The efficacy of chemoradiation therapy may be significantly compromised as a result of interindividual variations in clinical response and host toxicity. Therefore, it is imperative to identify those patients who will benefit from chemoradiation therapy and those who will develop recurrent disease. In this study, we tested whether a specific pattern of 21 polymorphisms in 18 genes involved in the critical pathways of cancer progression (i.e., drug metabolism, tumor microenvironment, cell cycle regulation, and

DNA repair) will predict the risk of tumor recurrence in rectal cancer patients treated with chemoradiation. PATIENTS AND METHODS: A total of 90 patients with Stage II or III rectal cancer treated with chemoradiation were genotyped using polymerase chain reaction (PCR)-based techniques for 21 polymorphisms. RESULTS: A polymorphism in interleukin (IL)-8 was individually associated with risk of recurrence. Classification and regression tree analysis of all polymorphisms and clinical variables developed a risk tree including the following variables: node status, IL-8, intracellular adhesion molecule-1, transforming growth factor-beta, and fibroblast growth factor receptor 4. CONCLUSION: Genomic profiling may help to identify patients who are at high risk for developing tumor recurrence, and those who are more likely to benefit from chemoradiation therapy. A larger prospective study is needed to validate these preliminary data using germline polymorphisms on tumor recurrences in rectal cancer patients treated with chemoradiation.

Gunther, K., T. Brabletz, et al. (1998). "Predictive value of nuclear beta-catenin expression for the occurrence of distant metastases in rectal cancer." Dis Colon Rectum **41**(10): 1256-61.

PURPOSE: Adenomatous polyposis coli protein, glycogen synthetase kinase-3-beta, T cell transcription factor/lymphoid enhancer-binding factor, and beta-catenin modulate cell differentiation and proliferation via the expression of effector genes. It has recently been postulated that beta-catenin is a potent oncogene of sporadic colorectal carcinogenesis and a prognostic tumor marker. Our aim was to investigate whether the nuclear overexpression of beta-catenin, possibly caused by mutations in exon 3 of beta-catenin (CTNNB1), is correlated with distant metastatic spread or disease-free survival in rectal carcinoma. METHODS: Immunohistochemical analysis was performed with an anti-beta-catenin-monoclonal antibody on paraffin sections of two groups of patients (n = 2 x 77) with rectal carcinoma curatively treated by surgery alone. The patients selected were all free of local disease, to exclude surgical influence. Patient groups were matched for age, gender, International Union Against Cancer stage, and year of operation (1982 to 1991) and differed only in subsequent metachronous distant metastatic spread. Follow-up was prospective (median, 9.6 years). Three staining patterns were defined: membranous (normal), diffuse cytoplasmic (pathologic), and intense nuclear staining (pathologic). When intense nuclear staining was defined, the specimen was microdissected. Then, DNA was isolated, polymerase chain reaction-amplified, and sequenced to detect mutations in exon 3. RESULTS: Nuclear overexpression of beta-catenin correlated neither with distant metastatic spread (chi-squared, 0.37; P = 0.79) nor with disease-free survival (log-rank with trend, P = 0.62). No mutations were found in the area of the serine/threonine-kinase glycogen synthetase kinase-3-beta-phosphorylation site in exon 3 (CTNNB1) of beta-catenin. CONCLUSION: Although beta-catenin seems to play an important role in early colorectal carcinogenesis, its value as a prognostic marker is questionable. It must be assumed that metastatic ability is determined by other factors than the disturbance of the beta-catenin T cell transcription factor/lymphoid enhancer-binding factor cascade and that other mechanisms might cause the observed nuclear translocation of beta-catenin.

He, Y., L. J. Van't Veer, et al. (2009). "PIK3CA mutations predict local recurrences in rectal cancer patients." Clin Cancer Res **15**(22): 6956-62.

PURPOSE: Identifying rectal cancer patients at risk for local recurrence would allow for refinement in the selection of patients who would benefit from preoperative radiotherapy. PIK3CA, KRAS, and BRAF mutations are commonly found in colon cancers, but their prevalence has not been clearly assessed in rectal cancer. In this study, we aim to determine the mutation frequencies of PIK3CA, KRAS, and BRAF and to investigate whether a mutation may be used as a prognostic parameter in rectal cancer patients. EXPERIMENTAL DESIGN: We evaluated DNA mutations in PIK3CA, KRAS, and BRAF in 240 stage I to III rectal tumors obtained from nonirradiated patients from the Dutch Total Mesorectal Excision trial. RESULTS: PIK3CA, KRAS, and BRAF mutations were identified in 19 (7.9%), 81 (33.9%), and 5 (2.1%) rectal cancers. Patients with PIK3CA mutations developed more local recurrences (5-year risks, 27.8% versus 9.4%; P = 0.006) and tended to develop these recurrences more rapidly after surgery (median local recurrence-free interval since surgery: 7.9 versus 19.6 months; P = 0.07) than patients without PIK3CA mutations. In multivariate analysis, PIK3CA mutations remained as an independent predictor for the development of local recurrences (hazard ratio, 3.4; 95% confidence interval, 1.2-9.2; P = 0.017), next to tumor-node-metastasis stage. CONCLUSION: PIK3CA mutations can be used as a biomarker in identifying rectal cancer patients with an increased risk for local recurrences. Currently, our findings suggest that prospective evaluation of PIK3CA mutation status could reduce overtreatment by preoperative radiotherapy for the low-risk patients who might otherwise only experience the side effects.

Ho-Pun-Cheung, A., E. Assenat, et al. (2007). "Cyclin D1 gene G870A polymorphism predicts response to neoadjuvant radiotherapy and prognosis in rectal cancer." Int J Radiat Oncol Biol Phys **68**(4): 1094-101.

PURPOSE: To investigate whether CCND1 genetic variations associated with a constitutive nuclear protein may influence either the pathologic response to preoperative RT or the prognosis in a series of rectal cancer patients. METHODS AND MATERIALS: Seventy rectal cancer patients treated by neoadjuvant radiotherapy were included in the study. CCND1 exon 5 mutations were screened, and the G870A polymorphism was assessed for correlation with clinical variables, tumor response, and patient outcome. RESULTS: No exon 5 mutation was found. Concerning the G870A polymorphism, the A/A variant was significantly associated with radiosensitivity ($p = 0.022$). Moreover, patients harboring the A allele were correlated with a lower risk of local failure ($p = 0.017$). Also, combination of the G870A polymorphism with the post-therapeutic lymph node status allowed the elaboration of a prognostic index, which accurately distinguished subgroups of patients with predictable recurrence-free ($p = 0.003$) and overall ($p = 0.044$) survival. CONCLUSIONS: Although CCND1 exon 5 mutations are rare in rectal cancer, G870A polymorphism is a frequent variation that may predict radiosensitivity and prognosis.

Ho-Pun-Cheung, A., C. Bascoul-Mollevi, et al. (2009). "Validation of an appropriate reference gene for normalization of reverse transcription-quantitative polymerase chain reaction data from rectal cancer biopsies." Anal Biochem **388**(2): 348-50.

Gene expression quantification using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) requires data normalization using an invariable reference gene. Here we assessed the stability of 15 housekeeping genes in 31 tumor and normal rectal samples to validate a reliable reference gene for rectal cancer studies. Our data show that 18S and 28S RNA are stably expressed in all samples. Moreover, when used for normalization, 18S, but not 28S, greatly reduced unspecific variations of gene expression due to RNA degradation. These results demonstrate that 18S is an appropriate reference gene for normalization of RT-qPCR data from rectal cancer samples.

Horisberger, K., P. Erben, et al. (2009). "Topoisomerase I expression correlates to response to neoadjuvant irinotecan-based chemoradiation in rectal cancer." Anticancer Drugs **20**(6): 519-24.

Biomarkers may help predict the efficacy of neoadjuvant chemoradiation in patients with rectal cancer. We hypothesized that the expression of topoisomerase I (Topo I) and thymidylate synthase (TS) may help predict the treatment response in patients undergoing irinotecan and capecitabine-based chemoradiation. Patients with rectal cancer (cT3/4Nx or Tx/N+) received neoadjuvant chemoradiotherapy within clinical studies with irinotecan and capecitabine. Samples of normal and tumour tissues were collected before the start of the treatment and during surgical resection. Topo I and TS were measured using real-time PCR. The results of gene expression levels were compared between responders (defined as ypT0-2 ypN0) and nonresponders (ypT3-4 or ypN1/2). A total of 38 patients were analysed, 18 of them were responders. The biopsies of the untreated tumour tissue of responding patients showed a significant higher expression of Topo I compared with nonresponding patients ($P = 0.015$). Normal tissue did not show this difference ($P = 0.126$). During chemoradiation, the Topo I expression in tumour tissue of responders decreased significantly. TS did not show any differences between responders and nonresponders before treatment, but a significant decrease in the tumour tissue of responders was noted at the end of the treatment. Our data suggest that Topo I expression in rectal tumour mucosa might serve as a predictor of response to the neoadjuvant irinotecan-based chemoradiation, and hence might be a factor contributing to the development of individualized treatment.

Ibi, I., Y. Saito, et al. (1999). "Biological effects of preoperative radiotherapy on metastatic lymph nodes from rectal cancer." Am Surg **65**(5): 427-30.

The quantitative description of the proliferative activity of cancer cells correlates with the aggressiveness of malignant tumors. The aim of this retrospective study was to determine the biological effect of adjuvant therapy on metastatic lymph nodes from rectal cancer and to compare the results between patients treated with surgery alone and patients treated with preoperative radiotherapy. Expression of the proliferating cell nuclear antigen (PCNA) was examined in metastatic lymph node samples of 12 rectal cancer patients receiving and 14 patients not receiving preoperative radiotherapy. PCNA immunostaining was performed by an avidin-biotin complex immunoperoxidase technique. The results of the mean proliferation index (PI) between the two groups were compared. A semiquantitative PCNA grading system was also estimated. In patients receiving preoperative radiotherapy, the PI was 22.8 per cent, and only one patient had high proliferative grade. On the contrary, the PI in nonirradiated patients

was 67.6 per cent, and nine patients showed high proliferative grade. Although not sufficient to reach significance in terms of prognosis, the present study confirms the clinical value of radiation therapy, and it supports the suggestion to treat Dukes' C patients with preoperative radiotherapy to decrease the risk of local recurrence.

Inoue, Y., K. Tanaka, et al. (2009). "Microdissection is essential for gene expression analysis of irradiated rectal cancer tissues." Oncol Rep **22**(4): 901-6.

Microdissection is a reliable technique and is extensively used in many cancer studies. We sought to verify the importance of the microdissection technique in molecular analysis of irradiated rectal cancer specimens. Forty patients with rectal cancer underwent 5-fluorouracil based chemoradiotherapy followed by curative surgery. We compared gene expressions that had previously been shown to be involved in chemotherapy or radiation effects; one obtained using RNA extracted from cancer cells by microdissection, and the other from bulky cancer tissues in all patients. More than 50% regression of the primary tumor was seen in 16 patients (40.0%). There was no significant difference in candidate gene expression profiles between tumor and stromal cells except for thymidine phosphorylase (TP). Without microdissection, there was no significant association between distant recurrence and gene expression in specimens. With microdissected sample analysis, however, patients who developed distant recurrence were found to have significantly higher intratumoral thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD) and orotate phosphoribosyl transferase (OPRT) compared with patients without recurrence. It is possible that microdissection is essential for gene expression analysis of clinically irradiated rectal specimens because preoperative chemoradiotherapy for rectal cancer affects the tumor-stroma balance in irradiated rectal cancer specimen.

Jakob, C., D. E. Aust, et al. (2004). "Thymidylate synthase, thymidine phosphorylase, dihydropyrimidine dehydrogenase expression, and histological tumour regression after 5-FU-based neo-adjuvant chemoradiotherapy in rectal cancer." J Pathol **204**(5): 562-8.

Pre-operative 5-fluorouracil (5-FU)-based chemoradiotherapy in locally advanced rectal cancer (UICC-II/III) may significantly reduce local tumour mass. Response to pre-operative treatment, however, varies significantly. Thymidylate synthase (TS), thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD) are thought to be important predictors for the efficiency of 5-FU-based treatment. The aim of this study was to determine the correlation between TS-, TP-, and DPD-gene expression and the response to 5-FU-based long-term pre-operative chemoradiotherapy assessed by histopathological tumour regression. Additionally, the predictive value of intra-tumoural TS-, TP-, and DPD-gene expression in pre-operative rectal tumour biopsies was assessed by correlation with the histopathological regression grade. Formalin-fixed, paraffin wax-embedded pre-operative biopsies (n = 14) and surgical resection specimens (n = 40) from patients with rectal carcinoma (clinical UICC stage II/III) receiving neo-adjuvant 5-FU-based chemoradiotherapy were studied for TS-, TP-, and DPD-gene expression by quantitative TaqMan real-time PCR after laser microdissection. Results were compared with standardized histopathological tumour regression analysis. There was a significant association between low TS-gene expression in pre-operative tumour biopsies and tumour response ($p = 0.02$). TS- and TP-gene expression was significantly lower in resection specimens of responders than of non-responders ($p = 0.02$) when microdissection was used. Statistical significance was even higher when TS and TP were combined ($p = 0.0001$). For the DPD gene, no significance was found at all. In conclusion, this study shows that TS gene expression in a pretreatment biopsy predicts the response of local rectal cancer to neo-adjuvant 5-FU-based chemoradiotherapy in a high percentage. Moreover, intra-tumoural TS- and TP-gene expression in surgical rectal specimens after neo-adjuvant chemoradiotherapy correlates significantly with histopathological tumour regression when microdissection is applied.

Jakob, C., T. Liersch, et al. (2005). "Immunohistochemical analysis of thymidylate synthase, thymidine phosphorylase, and dihydropyrimidine dehydrogenase in rectal cancer (cUICC II/III): correlation with histopathologic tumor regression after 5-fluorouracil-based long-term neoadjuvant chemoradiotherapy." Am J Surg Pathol **29**(10): 1304-9.

In locally advanced rectal cancer, neoadjuvant 5-fluorouracil (5-FU)-based long-term chemoradiotherapy leads to marked tumor reduction and decrease of local recurrence rate. Thymidylate synthase (TS), thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD) are known to be important biomarkers to predict tumor response to 5-FU-based therapy. The aim of this study was to examine the correlation between TS, TP, and DPD protein expression and histopathologic tumor regression after neoadjuvant chemoradiotherapy. The results were compared with the recently published mRNA data. Preoperative biopsies (n = 25) and resection specimens (n = 40) from patients with rectal

carcinoma (clinical UICC stage II/III) receiving neoadjuvant 5-FU-based chemoradiotherapy were studied for TS, TP, and DPD protein expression by immunohistochemistry using three different scoring systems (intensity, pattern, intensity + pattern). Results were compared with histopathologic tumor regression. A significant correlation between protein expression and tumor response was only seen when both staining intensity and staining pattern were considered. With this method, a significant association was seen between high TS expression in tumor biopsies as well as resection specimens and nonresponse of the tumor to therapy (P = 0.04). Furthermore, low TP expression in the resection specimens was significantly associated with lack of response (P = 0.02). For DPD no significant correlations were found at all. In conclusion, these results suggest that immunohistochemistry like RT-PCR is a suitable method to determine the correlation between TS, TP, and DPD expression and histopathologic tumor regression. However, precise results can only be achieved if staining intensity as well as staining pattern within the tumors are evaluated.

Jakob, C., T. Liersch, et al. (2006). "Prognostic value of histologic tumor regression, thymidylate synthase, thymidine phosphorylase, and dihydropyrimidine dehydrogenase in rectal cancer UICC Stage II/III after neoadjuvant chemoradiotherapy." Am J Surg Pathol **30**(9): 1169-74.

Histologic tumor regression (TR) in rectal cancer after preoperative chemoradiotherapy (CT/RT) may be useful as a surrogate end point for early treatment efficacy, but little is known about its prognostic value. The aim of this follow-up study was to evaluate whether TR is able to predict prognosis in rectal cancer patients. Furthermore, the prognostic value of thymidylate synthase (TS)-gene, thymidine phosphorylase (TP)-gene, and dihydropyrimidine dehydrogenase (DPD)-gene expression after neoadjuvant CT/RT was determined. Forty patients with rectal cancer cUICC stage II/III, receiving preoperative 5-fluorouracil (5-FU) based CT/RT were studied for therapy-induced TR and categorized as "responders" or "nonresponders" according to their TR-grade. Posttherapeutical TS-gene, TP-gene, and DPD-gene expression on surgical resection specimens was quantified by TaqMan real-time PCR after microdissection. During a median follow-up of 58 months, cancer recurrence occurred in 28%. A significant correlation was seen between disease-free survival and lymph node status (P<0.001). All patients, who developed cancer recurrence had a posttherapeutical positive lymph node status. The majority of patients with cancer recurrence were "responders" (91%) after CT/RT. There was a significant correlation between posttherapeutical TS-gene expression and cancer recurrence within the subgroup of "responders." TS-gene expression was significantly higher in patients with cancer recurrence than in those, who are disease-free up to date (P=0.028). In conclusion, lymph node status remains the most important prognostic marker in rectal cancer patients, whereas posttreatment TR by itself has no prognostic significance. Furthermore, measurement of posttherapeutical TS-gene expression may help to identify patients at higher risk for cancer recurrence.

Jakob, C., T. Liersch, et al. (2008). "Predictive value of Ki67 and p53 in locally advanced rectal cancer: correlation with thymidylate synthase and histopathological tumor regression after neoadjuvant 5-FU-based chemoradiotherapy." World J Gastroenterol **14**(7): 1060-6.

AIM: To investigate the predictive value of Ki67 and p53 and their correlation with thymidylate synthase (TS) gene expression in a rectal cancer patient cohort treated according to a standardized recommended neoadjuvant treatment regimen. METHODS: Formalin fixed, paraffin embedded pre-therapeutical tumor biopsies (n = 22) and post-therapeutical resection specimens (n = 40) from patients with rectal adenocarcinoma (clinical UICC stage II/III) receiving standardized neoadjuvant 5-fluorouracil (5-FU) based chemoradiotherapy were studied for Ki67 and p53 expression by immunohistochemistry and correlated with TS mRNA expression by quantitative TaqMan real-time PCR after laser microdissection. The results were compared with histopathological tumor regression according to a standardized semiquantitative score grading system. RESULTS: Responders (patients with high tumor regression) showed a significantly lower Ki67 expression than non-responders in the pre-therapeutical tumor biopsies (81.2% vs 16.7%; P < 0.05) as well as in the post-therapeutical resection specimens (75.8% vs 14.3%; P < 0.01). High TS mRNA expression was significantly correlated with a high Ki67 index and low TS mRNA expression was significantly correlated with a low Ki67 index in the pre-therapeutical tumor biopsies (corr. coef. = 0.46; P < 0.01) as well as in the post-therapeutical resection specimens (corr. coef. = 0.40; P < 0.05). No significant association was found between p53 and TS mRNA expression or tumor regression. CONCLUSION: Ki67 has, like TS, predictive value in rectal cancer patients after neoadjuvant 5-FU based chemoradiotherapy. The close correlation between Ki67 and TS indicates that TS is involved in active cell cycle processes.

Jiang, Q., K. Chen, et al. (2005). "Diets, polymorphisms of methylenetetrahydrofolate reductase, and the susceptibility of colon cancer and rectal cancer." Cancer Detect Prev **29**(2): 146-54.

The aim of this study was to investigate the association of environmental factors (dietary folate, methionine and drinking status) and polymorphisms in the methylenetetrahydrofolate reductase (MTHFR C677T and A1298C) gene, as well as the combination of these factors, with the risk of colon cancer and rectal cancer. A case-control study of 53 colon cancer patients, 73 rectal cancer patients and 343 healthy controls was conducted. Genotypes of C677T and A1298C polymorphisms were analyzed by PCR-RFLP. The dietary folate and methionine intakes were assessed using food-frequency questionnaires and food consumption tables. Unconditional logistic regression was applied to estimate the odds ratios (ORs) and their 95% confidence intervals (CIs). The frequency of MTHFR 677T and 1298C alleles in healthy population were 39.4 and 17.2%, respectively. After adjustment for specific variants, the MTHFR 677TT genotype showed a significantly reduced risk of colon cancer compared with the wild type (OR=0.22, 95% CI: 0.50-0.98), and 1298C allele-carrier showed an inverse association with the risk of rectal cancer compared to the wild type (OR=0.52, 95% CI: 0.28-0.98). Adequate intake of folate was a protective factor from colon cancer (OR=0.32, 95% CI: 0.12-0.88) and MTHFR C677T polymorphism showed a statistically significant effect (OR=0.25, 95% CI: 0.06-0.93), reducing the risk of colon cancer in groups that have an intake of folate exceeding 115.64ng per 1000kcal per day. This study suggests that MTHFR C677T and A1298C polymorphisms are associated with the reduced risk of colon and rectal cancers, respectively. Adequate folate intake shows an inverse association with the risk of colon cancer. There is a significant interaction between MTHFR C677T polymorphism and folate intake in reducing the risk of colon cancer.

Kandioler, D., R. Zwrtek, et al. (2002). "TP53 genotype but not p53 immunohistochemical result predicts response to preoperative short-term radiotherapy in rectal cancer." Ann Surg **235**(4): 493-8.

OBJECTIVE: To evaluate and compare the predictive power of p53 gene analysis versus p53 immunohistochemical staining in terms of response to preoperative short-term radiotherapy using 25 Gy in operable rectal cancer. SUMMARY BACKGROUND DATA: Recent studies show that p53 may be a determinant of radiosensitivity being required for induction of apoptosis in case of radiation-induced DNA damage. METHODS: Preirradiation biopsy samples of 64 patients with rectal carcinoma were analyzed. Genetic alterations of the p53 gene were detected by complete direct sequencing of exons 2 to 10. Expression of the nuclear phosphoprotein p53 was assessed by immunohistochemical staining. Results were correlated with histopathology of resected specimens and follow-up data, respectively. RESULTS: Mutations of the p53 gene were present in 45% of tumors. Patients with a normal p53 gene had a significant survival advantage. Comparing pre- and postradiotherapy T category, a reduction was seen in patients with normal p53 genotype only. A mutant p53 genotype was highly specific in indicating stable disease concerning T category after irradiation. Protein overexpression was detected in 61%. Overexpression of the p53 protein was not related to survival or response. The concordance between immunohistochemistry and sequencing was only 0.51. CONCLUSIONS: The authors show that downstaging after short-term radiation may occur but is seen in tumors with normal p53 gene only. Moreover, p53 genotype but not p53 immunohistochemistry is predictive for response to preoperative short-term radiotherapy and patient survival.

Kapiteijn, E., G. J. Liefers, et al. (2001). "Mechanisms of oncogenesis in colon versus rectal cancer." J Pathol **195**(2): 171-8.

Observations support the theory that development of left- and right-sided colorectal cancers may involve different mechanisms. This study investigated different genes involved in oncogenesis of colon and rectal cancers and analysed their prognostic value. The study group comprised 35 colon and 42 rectal cancers. Rectal cancer patients had been treated with standardized surgery performed by an experienced rectal cancer surgeon. Mutation analysis was performed for p53 in eight colon cancers and for APC and p53 in 22 rectal cancers. MLH1, MSH2, Bcl-2, p53, E-cadherin and beta-catenin were investigated by immunohistochemistry in all colorectal tumours. APC mutation analysis of the MCR showed truncating mutations in 18 of 22 rectal tumours (82%), but the presence of an APC mutation was not related to nuclear beta-catenin expression (p=0.75). Rectal cancers showed significantly more nuclear beta-catenin than colon cancers (65% versus 40%, p=0.04). p53 mutation analysis corresponded well with p53 immunohistochemistry (p<0.001). Rectal cancers showed significantly more immunohistochemical expression of p53 than colon cancers (64% versus 29%, p=0.003). In rectal cancers, a significant correlation was found between positive p53 expression and worse disease-free survival (p=0.008), but not in colon cancers. Cox regression showed that p53-expression (p=0.03) was an

independent predictor for disease-free survival in rectal cancers. This study concluded that rectal cancer may involve more nuclear beta-catenin in the APC/beta-catenin pathway than colon cancer and/or nuclear beta-catenin may have another role in rectal cancer independently of APC. The p53-pathway seems to be more important in rectal cancer, in which it also has independent prognostic value. When prognostic markers are investigated in larger series, differences in biological behaviour between colon and rectal cancer should be considered.

Kim, H. R., Y. J. Kim, et al. (2000). "Change of telomerase activity in rectal cancer with chemoradiation therapy." J Korean Med Sci **15**(2): 167-72.

Telomerase, an enzyme associated with cellular immortality, is expressed by most malignant cells and is inactive in most normal somatic cells, with the exception of proliferative stem cells, germ cells and activated lymphocytes. Measuring telomerase activity clinically may provide useful diagnostic and prognostic information of cancer. The purpose of this study was to investigate the change in telomerase activity following chemoradiation in rectal cancer, which almost always produces positive enzymatic activity. A total of 24 tumor tissue samples were used in this study, consisting of 12 paired specimens before and 4 weeks after chemoradiation. Telomerase activity was determined by PCR-based telomeric repeat amplification protocol (TRAP) assay. The telomerase activity was positive in 10 out of 12 patients (83%) in pre-irradiated and post-irradiated states. The levels of telomerase activity was decreased in 8 out of 10 patients after chemoradiation (80%) and two cases showed no change in enzymatic activity. One case showed no activity in either sample. The other case showed no enzymatic activity in the pre-irradiated sample, but showed weak activity in the post-irradiated sample. These data indicate that telomerase activity in rectal cancer is reduced after neoadjuvant chemoradiation therapy, possibly suggesting a mechanism of downstaging following chemoradiation therapy in cancer.

Kim, I. J., S. B. Lim, et al. (2007). "Microarray gene expression profiling for predicting complete response to preoperative chemoradiotherapy in patients with advanced rectal cancer." Dis Colon Rectum **50**(9): 1342-53.

PURPOSE: Preoperative chemoradiotherapy is widely used to improve local control and sphincter preservation in patients with locally advanced rectal cancer. In the present study, we investigated whether microarray gene expression analysis could predict complete response to preoperative chemoradiotherapy in rectal cancer. METHODS: Tumor tissues were obtained from 46 patients with rectal cancer (31 for training and 15 for validation testing). All patients underwent preoperative chemoradiotherapy involving 50.4 gray radiotherapy, followed by surgical excision 6 weeks later. Response to chemoradiotherapy was evaluated according to Dworak's tumor regression grade. Tumor regression Grades 1, 2, and 3 were considered partial responses, and tumor regression Grade 4 was considered a complete response. By using the 31 training samples, genes differentially expressed between partial response and complete response were identified, and clustering analysis was performed. Prediction analysis of response to chemoradiotherapy was performed on the 31 training samples by using a selected set of 95 "predictor" genes. Those findings were validated by independent analysis of the 15 test samples. RESULTS: The 31 training samples comprised 20 partial response and 11 complete response cases. A primary set of 261 genes was identified as differentiating between partial response and complete response. By supervised clustering using these 261 genes, 30 of 31 training samples were clustered correctly according to tumor response. A gene set comprising the top-ranked 95 genes displaying differential expression between partial response and complete response was applied to predict response to chemoradiotherapy. Complete response and partial response were accurately predicted in 84 percent (26/31) of training samples and 87 percent (13/15) of validation samples. CONCLUSIONS: Microarray gene expression analysis was successfully used to predict complete responses to preoperative chemoradiotherapy in patients with advanced rectal cancer.

Kobayashi, H., Y. Hashiguchi, et al. (2007). "Absence of cyclooxygenase-2 protein expression is a predictor of tumor regression in rectal cancer treated with preoperative short-term chemoradiotherapy." Dis Colon Rectum **50**(9): 1354-62.

PURPOSE: Neoadjuvant chemoradiotherapy followed by total mesorectal excision has become the standard of care for patients with locally advanced rectal cancer. This study was designed to determine whether pretreatment cyclooxygenase-2 and p53 protein expression were predictors of histopathologic response in patients with rectal cancer treated with preoperative short-term chemoradiotherapy. METHODS: Fifty-two patients with low rectal cancer received short-term preoperative chemoradiotherapy (20 Gy given in 5 daily doses of 4 Gy and concurrent administration of Tegafur/Uracil 400 mg/day), followed by total mesorectal excision. Cyclooxygenase-2 and p53 protein expression were measured by immunohistochemistry before and at the

time of resection. Tumor regression grading was evaluated according to the criteria by Rodel (Grade 4, complete regression; Grade 3, regression >50 percent; Grade 2, 25-50 percent; Grade 1,<25 percent; and Grade 0, no regression). RESULTS: Two patients had a pathologic complete response. Good response (Grade 3 + 4) was found in 57.7 percent of the resected specimens. Cyclooxygenase-2 was expressed in 80.8 percent of patients before chemoradiotherapy and in 100 percent after chemoradiotherapy. The rates of good response (Grade 3 + 4) were significantly associated with lack of cyclooxygenase-2 expression before chemoradiotherapy (P = 0.021). However, there was no correlation between p53 protein expression and tumor regression grading. CONCLUSIONS: Patients with tumor lacking cyclooxygenase-2 expression before chemoradiotherapy are more likely to demonstrate good response to treatment. Cyclooxygenase-2 protein expression may be a marker for response to chemoradiotherapy in patients with rectal cancer.

Krishnamurthi, S. S., Y. Seo, et al. (2007). "Adjuvant therapy for rectal cancer." Clin Colon Rectal Surg **20**(3): 167-81.

Patients with stage II and III rectal cancer benefit from a multidisciplinary approach to treatment. Studies of postoperative adjuvant therapy consistently demonstrate decreases in locoregional recurrence with the use of radiation therapy. The use of postoperative chemotherapy results in improved disease-free survival and overall survival in certain studies. Preoperative radiation therapy decreases locoregional recurrence and in one study demonstrated an improvement in survival. The addition of chemotherapy to preoperative radiation results in improved locoregional control, but not survival. Preoperative chemoradiation is the standard of care for patients with clinical stage II and III rectal cancer in the United States due to improved local recurrence, acute and late toxicity, and sphincter preservation compared with postoperative chemoradiation. Promising approaches include the incorporation of new chemotherapeutic and biologic agents into chemoradiation and adjuvant chemotherapy regimens; new radiation techniques, such as the use of intraoperative radiation therapy and an accelerated concomitant radiation boost; and gene and protein expression profiling, to better predict response to treatment and prognosis.

Kristensen, A. T., J. Bjorheim, et al. (2004). "DNA variants in the ATM gene are not associated with sporadic rectal cancer in a Norwegian population-based study." Int J Colorectal Dis **19**(1): 49-54.

BACKGROUND AND AIMS: A large number of DNA single-nucleotide polymorphisms (SNPs) have been discovered following the Human Genome Project. Several projects have been launched to find associations between SNPs and various disease cohorts. This study examined the possible association between the reported SNPs and sporadic rectal cancer. It has been proposed that SNPs in the ataxi-telangiectasia mutated (ATM) gene modulate the penetrance of some cancers. The investigated target sequence harbors three polymorphisms (IVS38-8 T/C in intron 38, 5557 G/A and 5558 A/T in exon 39), resulting in eight possible microhaplotypes at the DNA level. Furthermore, the two exonic SNPs are sited next to each other, allowing four possible amino acids in the same codon. METHODS: We report on a new method analyzing SNPs and microhaplotypes based on theoretical thermodynamics and migration of variant fragments by cycling temperature capillary electrophoresis. Fluorophore-labeled PCR products were analyzed without any post-PCR steps on a standard 96 capillary-sequencing instrument under denaturing conditions. RESULTS: More than 7000 alleles were microhaplotyped based on peak migration patterns of individual samples and sequencing results. The ATM polymorphisms and microhaplotypes examined did not significantly differ between sporadic rectal cancer and normal population. CONCLUSION: No associations were found between the IVS38-8 T/C, 5557 G/A and 5558 A/T polymorphisms and microhaplotypes in the ATM gene with respect to sporadic rectal cancer.

Kristensen, A. T., J. N. Wiig, et al. (2008). "Molecular detection (k-ras) of exfoliated tumour cells in the pelvis is a prognostic factor after resection of rectal cancer?" BMC Cancer **8**: 213.

BACKGROUND: After total mesorectal excision (TME) for rectal cancer around 10% of patients develops local recurrences within the pelvis. One reason for recurrence might be spillage of cancer cells during surgery. This pilot study was conducted to investigate the incidence of remnant cancer cells in pelvic lavage after resection of rectal cancer. DNA from cells obtained by lavage, were analysed by denaturing capillary electrophoresis with respect to mutations in hotspots of the k-ras gene, which are frequently mutated in colorectal cancer. RESULTS: Of the 237 rectal cancer patients analyzed, 19 had positive lavage fluid. There was a significant survival difference (p = 0.006) between patients with k-ras positive and negative lavage fluid. CONCLUSION: Patients with k-ras mutated cells in the lavage immediately after surgery have a reduced life expectation. Detection of exfoliated cells in the abdominal cavity may be a useful diagnostic tool to

improve the staging and eventually characterize patients who may benefit from aggressive multimodal treatment of rectal cancer.

Kumar, A., H. Collins, et al. (2002). "Effect of preoperative radiotherapy on matrilysin gene expression in rectal cancer." Eur J Cancer **38**(4): 505-10.

Matrilysin, a member of matrix metalloproteinase family, is believed to play a significant role in the growth and proliferation of colon cancer cells. Overexpression of the matrilysin gene has been shown to correlate with Dukes' stage and increased metastatic potential in colorectal cancer. The aim of this study was to evaluate the effect of preoperative high-dose radiotherapy (25 Gy in five fractions over 5 days) on matrilysin (MMP-7) gene expression, in patients with resectable rectal cancer, by a quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Biopsy samples of tumour (n=30) and distant normal mucosa (n=12) from 15 patients were obtained pre- and post-radiotherapy. Messenger (m)RNA was extracted from all of the tissue samples and reverse transcribed to double-stranded cDNA. Quantitative RT-PCR was performed to study the effect of preoperative radiotherapy on matrilysin gene expression in both the tumour and normal mucosal specimens. Matrilysin mRNA values were expressed relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for each sample. In 14 out of 15 cases, matrilysin mRNA was detected in the cancerous tissue. Although all six normal mucosal specimens expressed matrilysin mRNA, the levels were approximately 10-fold lower compared with those seen in the paired tumour samples. Preoperative radiotherapy led to a significant 6- to 7-fold increase (P=0.001) in the expression of matrilysin mRNA in rectal cancer tissue. In contrast, there was no significant change in the matrilysin mRNA expression of normal mucosal specimens post-radiotherapy. Preoperative high-dose radiotherapy upregulates matrilysin gene expression in rectal cancer. Matrilysin inhibition may be a useful preventive or therapeutic adjunct to radiotherapy in rectal cancer.

Li, M., J. Y. Li, et al. (2007). "Colorectal cancer or colon and rectal cancer? Clinicopathological comparison between colonic and rectal carcinomas." Oncology **73**(1-2): 52-7.

AIMS: The aim of this study was to compare features of colon and rectal cancers such as prognosis, clinicopathological features and tumor markers, namely carcinoembryonic antigen (CEA), matrix metalloproteinase (MMP)-2 and p27(kip1). METHODS: Two hundred and thirty patients with stage I-III colon or rectal cancer were retrospectively assessed with the endpoint of recurrence or metastasis after curative operation. CEA, MMP-2 and p27(kip1) were studied by immunohistochemistry in cancer tissues of all patients. RESULTS: The disease-free 3-year survival rate after operation of the total 230 patients was 63.0%. The prognosis of colon cancer was significantly better than that of rectal cancer (70.6 vs. 57.0%; p = 0.017), especially for stage III (p = 0.0059). Multivariate analysis also demonstrated that tumor location in the colon or rectum, differentiation, venous invasion and the expression of CEA were independent factors for prognosis. The hazard of recurrence and metastasis in rectal cancer was 1.564 times that in colon cancer. In both groups, there were no statistical differences in age, gender, tumor size, tumor gross type, mucin production, tumor differentiation, venous invasion, MMP-2 and p27(kip1). CONCLUSION: We investigated prognosis, clinicopathological factors, oncogenes and tumor suppressor gene production in colon and rectal cancers. The prognosis of colon cancer is better than that of rectal cancer, especially for stage III. This study shows some differences between colon and rectal cancer.

Li, S., B. Yu, et al. (2005). "Combined liposome-mediated cytosine deaminase gene therapy with radiation in killing rectal cancer cells and xenografts in athymic mice." Clin Cancer Res **11**(9): 3574-8.

PURPOSE: The aim of this study was to assess the antitumor efficacy of combination of cytosine deaminase (CD) suicide gene therapy with radiation and to grope for new therapeutic strategy for local recurrent rectal cancer. EXPERIMENTAL DESIGN: HR-8348 cell line of human rectal cancer was used to assess efficiency of transfection with plasmid pEGFP-N1 and PXJ41-CD. The cells were exposed to radiation followed by liposome-mediated transfection. Cell inhibition assay was done with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. Antitumor efficacy of combined liposome-mediated CD suicide gene therapy with radiation was determined by treatment of nude mice bearing HR-8348 cancer cell xenograft. RESULTS: The efficiency of liposome-mediated CD gene transfection can be improved by radiation. With radiation at 2, 4, 6, and 8 Gy, the efficiency of liposome-mediated transfection increased from 21.3% to 62.2%, 78.0%, 83.2%, and 87.8%, respectively. CD expression was enhanced as well. Cancer cell inhibition experiment showed that combined liposome-mediated CD gene therapy with radiation had much stronger antitumor effect. With HR-8348 tumor xenograft model, suppression of tumor xenograft was observed. Compared with control

group, tumor volume was inhibited by 81.5%, 48.5%, and 37.4%, respectively, in the combined CD/5-fluorocytosine with radiation group, CD/5-fluorocytosine group, and radiation group and the wet weight of tumor was decreased by 80%, 41.7%, and 37.7%, respectively. CONCLUSION: These findings suggested that combination of liposome-mediated CD gene therapy with radiation is a safer and efficient anticancer method. Its therapeutic efficacy may meet clinical treatment on local recurrent rectal cancer.

Liang, J. T., Y. M. Cheng, et al. (1999). "Reappraisal of K-ras and p53 gene mutations in the recurrence of Dukes' B2 rectal cancer after curative resection." Hepatogastroenterology **46**(26): 830-7.

BACKGROUND/AIMS: Recurrence of rectal cancer remains a major clinical problem. This study was conducted to evaluate the impact of K-ras and p53 mutations on the recurrence of rectal cancer. METHODOLOGY: A total of 166 resected Dukes' B2 stage rectal carcinomas were collected between January 1990 and April 1994. The stored frozen tissues were retrieved for immunocytochemistry of p53 and genomic analysis of K-ras and p53 genes. The data of K-ras and p53 gene mutations were correlated with clinicopathological variables. The concordance of immunocytochemistry with genomic analysis in the survey of p53-mutations was examined. The follow-up data were analyzed by Kaplan-Meier estimator. RESULTS: Sixty-nine patients (41.6%) developed recurrent tumor. A significantly higher recurrence rate ($p = 0.0013$) and shorter median recurrence time were noted in p53 mutated than non-mutated cancers. Mutations in K-ras gene do not significantly increase the risk of tumor recurrence ($p = 0.1702$). K-ras and p53 mutations are not associated with clinicopathological parameters ($p > 0.05$). Kappa statistic indicates highly significant reproducibility between immunocytochemistry and genomic analysis for p53 mutations ($p < 0.0001$). CONCLUSIONS: Presence of p53 mutation significantly increases the recurrence rate and shortens the recurrence time of the resected rectal cancers. Pre-operative routine check for p53 mutations by immunocytochemistry may be beneficial in choosing the optimal surgical strategy for rectal cancer.

Liersch, T., M. Grade, et al. (2009). "Preoperative chemoradiotherapy in locally advanced rectal cancer: correlation of a gene expression-based response signature with recurrence." Cancer Genet Cytogenet **190**(2): 57-65.

Preoperative chemoradiotherapy is recommended for locally advanced rectal cancer (UICC stage II/III). We recently demonstrated that responsive and nonresponsive tumors showed differential expression levels of 54 genes. In this follow-up study, we investigated the relationship between this gene set and disease-free (DFS) and overall survival (OS). Pretherapeutic biopsies from 30 participants in the CAO/ARO/AIO-94 trial of the German Rectal Cancer Study Group were analyzed using gene expression microarrays. Statistical analysis was performed to identify differentially expressed genes between recurrent and nonrecurrent tumors and to correlate these changes with disease recurrence and outcome. After a median follow-up of 59 months, seven of eight patients with recurrent disease was a nonresponder, and one responsive tumor recurred. Response to chemoradiotherapy was significantly correlated with an improved DFS (log rank $P=0.028$), whereas OS did not differ significantly ($P=0.11$). Applying a class comparison analysis, we identified 20 genes that were differentially expressed between recurrent and nonrecurrent tumors ($P<0.001$). Analyzing the first two principal components of the 54 genes previously identified to predict response, we observed that this response signature correlated with an increased risk of cancer recurrence. These data suggest that the genetic basis of local response also affects the genetic basis of tumor recurrence. Genes that are indicative of nonresponse to preoperative chemoradiotherapy might also be linked to an increased risk of tumor recurrence.

Liersch, T., C. Langer, et al. (2006). "Lymph node status and TS gene expression are prognostic markers in stage II/III rectal cancer after neoadjuvant fluorouracil-based chemoradiotherapy." J Clin Oncol **24**(25): 4062-8.

PURPOSE: According to the CAO/ARO/AIO-94 trial of the German Rectal Cancer Study Group, preoperative combined fluorouracil (FU) -based long-term chemoradiotherapy (CT/RT) is recommended for patients with International Union Against Cancer (UICC) stage II/III rectal cancer. However, despite the local benefit of neoadjuvant treatment, the overall prognostic value remains uncertain in comparison with adjuvant CT/RT. Furthermore, the prognostic value of molecular biomarkers, such as thymidylate synthase (TS), thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD), all of which are involved in the FU metabolism, is unknown in neoadjuvant settings. We assessed the impact of standardized preoperative CT/RT and intratumoral TS, TP, and DPD levels on patient outcome. PATIENTS AND METHODS: Forty patients with rectal cancer pretherapeutic UICC stage II/III, receiving preoperative FU-based CT/RT (CAO/ARO/AIO-94 trial) followed by standardized surgery, including total mesorectal excision, were investigated. Downsizing, downstaging, tumor

regression, as well as TS, TP, and DPD gene expression of post-treatment surgical specimens were correlated with disease-free survival (DFS) and overall survival (OS). RESULTS: Significant downsizing (P < .001) and downstaging (P = .001) were achieved with preoperative CT/RT. During a median follow-up of 49 months (95% CI, 43 to 58 months), the cancer recurrence rate was 28.2%. DFS and OS were significantly increased in patients with downstaging (P < .001 and P = .003, respectively), compared with patients without downstaging. All patients who developed cancer recurrence had a persistent positive lymph node status after preoperative CT/RT (P < .001) and a significantly higher TS gene expression (P = .035) compared with those patients without recurrence. CONCLUSION: Persistent positive lymph node status and high intratumoral TS expression after preoperative CT/RT are predictive of an unfavorable prognosis in rectal cancer UICC stage II/III.

Lin, L. C., H. H. Lee, et al. (2006). "p53 and p27 as predictors of clinical outcome for rectal-cancer patients receiving neoadjuvant therapy." Surg Oncol **15**(4): 211-6.

Our aim was to examine whether certain molecular markers, specifically p53, p21, p27, and Bcl-2, could be used to predict the tumor response of rectal cancer to neoadjuvant therapy and determine the overall and disease-free survival rates of patients following neoadjuvant therapy. Seventy-seven patients with rectal cancers were used in this study. All of them received neoadjuvant therapy and 53 of them were given radical surgery. Immunohistochemical tests were performed for the four markers mentioned above using biopsy specimens obtained from 70 of the patients prior to radiation. The identical tests were performed for the same markers using excised specimens from the patients after radical surgery. For the pre-radiation specimens, the positive rate for having p27 and Bcl-2 markers was 32.7% and 16.6%, respectively. This rate increased to 73.5% and 41.6% (p=0.001 and 0.012, respectively) in the specimens obtained after the surgery. With respect to "fair response (FR)" of patients, the pre-radiation biopsy specimens showed significant difference for the p53 (-) and p27 (+) markers (p=0.006). Patients with a 3-year overall survival rate were found to have, from their surgical specimens, 92% of the p27 (+) and 75% of p27 (-) markers (p=0.0058). Our study showed: first, the rate of positive identification of molecular markers, p27 and Bcl-2, increased following neoadjuvant therapy. Second, either the p53 (-) or p27 (+) status was a good predictor for FR in the pre-radiation biopsy specimens. Third, patients with p27 (+) markers in the surgical specimens lived longer at 3 years.

Liu, H. Y., B. Zhou, et al. (2007). "Association of E1AF mRNA expression with tumor progression and matrilysin in human rectal cancer." Oncology **73**(5-6): 384-8.

OBJECTIVE: To examine E1AF mRNA expression and to determine whether it is correlated with tumor progression and matrilysin in human rectal cancer. METHODS: Real-time RT-PCR was used to determine E1AF and matrilysin expression in 100 matched rectal cancers and normal tissues. RESULTS: Among the 100 rectal cancers, 69 cases of E1AF mRNA overexpression were observed. E1AF mRNA overexpression correlated well with matrilysin. In carcinomas, E1AF mRNA overexpression correlated significantly with depth of invasion, lymph node metastasis, venous involvement and advanced pTNM stage. CONCLUSIONS: E1AF was correlated significantly with tumor progression of human rectal cancer and may be an important factor in rectal cancer progression.

Loof, J., D. Pfeifer, et al. (2009). "Significance of an exon 2 G4C14-to-A4T14 polymorphism in the p73 gene on survival in rectal cancer patients with or without preoperative radiotherapy." Radiother Oncol **92**(2): 215-20.

BACKGROUND AND PURPOSE: An exon 2 G4C14-->A4T14 polymorphism in the p73 gene was shown to be related to survival in several types of cancers, including colorectal cancer. The purpose was to investigate if this polymorphism was related to survival in rectal cancer patients with or without preoperative radiotherapy. MATERIALS AND METHODS: DNA extracted from tissue of 138 rectal cancer patients that received preoperative radiotherapy or had surgery alone was typed for the polymorphism by PCR using confronting two-pair primers. RESULTS: Among patients, 69% had GC/GC genotype, 27% had GC/AT and 4% had AT/AT. In the radiotherapy group, patients carrying the AT (GC/AT+AT/AT) allele had stronger expression of p53 (p=0.001) and survivin protein (p=0.03) than those carrying the GC/GC genotype. Further, among patients receiving preoperative radiotherapy the GC/GC genotype tended to be related to better survival (p=0.20). Patients with GC/GC genotype, along with negative p53 and weak survivin expression showed better survival than the other patients (p=0.03), even after adjusting for TNM stage and tumor differentiation (p=0.01, RR, 7.63, 95% CI, 1.50-38.74). In the non-radiotherapy group, the polymorphism was not related to survival (p=0.74). CONCLUSIONS: Results suggest that the p73

G4C14-->A4T14 polymorphism could be one factor influencing outcome of preoperative radiotherapy in rectal cancer patients.

Lopez-Crapez, E., F. Bibeau, et al. (2005). "p53 status and response to radiotherapy in rectal cancer: a prospective multilevel analysis." Br J Cancer **92**(12): 2114-21.

The aim of this study was to evaluate, in a prospective study, the predictive role of p53 status analysed at four different levels in identifying the response to preoperative radiotherapy in rectal adenocarcinoma. Before treatment, 70 patients were staged and endoscopic forceps biopsies from the tumour area were taken. p53 status was assessed by total cDNA sequencing, allelic loss analysis, immunohistochemistry, and p53 antibodies. Neoadjuvant treatment was based on preoperative radiotherapy or radiochemotherapy. Response to therapy was evaluated after surgery by both pathologic downstaging and histologic tumour regression grade. In all, 35 patients (50.0%) had p53 gene mutations; 44.4% of patients had an allelic loss; nuclear p53 overexpression was observed in 39 patients (55.7%); and p53 antibodies were detected in 11 patients (16.7%). In the multilevel analysis of p53 status, gene mutations correlated with both nuclear protein overexpression ($P < 0.0001$) and loss of heterozygosity ($P = 0.013$). In all, 29 patients (41.4%) were downstaged by pathologic analysis, and 19 patients (29.2%) were classified as tumour regression grade 1. Whatever the method of evaluation of treatment response, no correlation between p53 alterations and response to radiotherapy was observed. Our results do not support the use of p53 alterations alone as a predictive marker for response to radiotherapy in rectal carcinoma.

Luchtenborg, M., M. P. Weijenberg, et al. (2005). "Meat and fish consumption, APC gene mutations and hMLH1 expression in colon and rectal cancer: a prospective cohort study (The Netherlands)." Cancer Causes Control **16**(9): 1041-54.

OBJECTIVE: The aim of this study was to investigate the associations between meat and fish consumption and APC mutation status and hMLH1 expression in colon and rectal cancer. METHODS: The associations were investigated in the Netherlands Cohort Study, and included 434 colon and 154 rectal cancer patients on whom case-cohort analyses (subcohort n = 2948) were performed. RESULTS: Total meat consumption was not associated with the endpoints studied. Meat product (i.e. processed meat) consumption showed a positive association with colon tumours harbouring a truncating APC mutation, whereas beef consumption was associated with an increased risk of colon tumours without a truncating APC mutation (incidence rate ratio (RR) highest versus lowest quartile of intake 1.61, 95% confidence interval (CI) 0.96-2.71, p-trend = 0.04 and 1.58, 95% CI 1.10-2.25, p-trend = 0.01, respectively). Consumption of other meat (horsemeat, lamb, mutton, frankfurters and deep-fried meat rolls) was associated with an increased risk of rectal cancer without a truncating APC mutation (RR intake versus no intake 1.79, 95% CI 1.10-2.90). No associations were observed for meat consumption and tumours lacking hMLH1 expression. CONCLUSIONS: Our data indicate that several types of meat may contribute differently to the aetiology of colon and rectal cancer, depending on APC mutation status but not hMLH1 expression of the tumour.

Luna-Perez, P., J. Segura, et al. (2000). "Specific c-K-ras gene mutations as a tumor-response marker in locally advanced rectal cancer treated with preoperative chemoradiotherapy." Ann Surg Oncol **7**(10): 727-31.

BACKGROUND: Forty percent of patients with colorectal cancer develop mutations in the K-ras gene. OBJECTIVE: Our objective was to evaluate whether the presence of c-K-ras gene mutations is a useful tumor-response marker in patients with locally advanced rectal cancer treated with preoperative chemoradiotherapy. MATERIAL AND METHODS: Thirty seven patients with locally advanced rectal cancer were treated with preoperative chemoradiotherapy. Four to six weeks later, surgery was performed. Specimens were classified according to the UICC-AJC classification. A segment of the tumor was obtained to analyze specific c-K-ras gene mutations. Restriction fragment length polymorphism (RFLP) and single strand confirmation polymorphism (SSCP) techniques were used with a set of probes to detect specific c-K-ras mutations in codons 12, 13, and 61. The 37 patients were divided into Group A (with mutations) and Group B (without mutations). RESULTS: All 37 patients completed the scheduled treatment. Group A consisted of 12 patients, whose tumors were classified and specific c-K-ras mutations were located as follows: eight in codon 12, two in codon 13, and one in codon 61. Group B consisted of 25 patients. The tumors were classified and there were more early-stage tumors in Group A, whereas in Group B there were more advanced-stage tumors ($P = .05$, respectively). The mean follow-up was 36.2+/-18.3 months. All Group A patients survived, whereas 8 of the 25 patients in Group B died due to progressive metastatic disease. Survival in Group A was 100%, whereas in Group B it was 59% ($P = .03$). CONCLUSIONS: The presence of specific c-K-ras mutations is an indicator of tumor response in patients

with locally advanced rectal cancer treated with preoperative chemoradiotherapy and surgery. Therefore, responding patients may be more amenable to less radical surgical procedures based on c-K-ras mutations.

Lynch, H. T., G. S. Schuelke, et al. (1984). "Genetics of rectal cancer." Bull Cancer **71**(1): 1-15.

Two operational subdivisions of hereditary colorectal cancer susceptibility are those with and those without premalignant adenomatous colonic polyp expression. In both of these subdivisions, reliable biomarkers of gene carriage would be of value in patient management as we have previously emphasized. Consideration must also be given to the familial (hereditary) occurrence of inflammatory bowel diseases associating with colorectal cancer susceptibility. The occurrence of rectal cancers should therefore alert the physician to investigate the possibility of a significant family medical history in order to fully elucidate the genetic heterogeneity of susceptibility to this disease. Clinicians should also be alert to the possibility of extracolonic malignancies where probable genetic colorectal cancer susceptibility is evident. Whenever possible, all potential biomarkers should be investigated to aid in definition of genetic heterogeneity.

Marquardt, F., F. Rodel, et al. (2009). "Molecular targeted treatment and radiation therapy for rectal cancer." Strahlenther Onkol **185**(6): 371-8.

BACKGROUND: EGFR (epidermal growth factor receptor) and VEGF (vascular endothelial growth factor) inhibitors confer clinical benefit in metastatic colorectal cancer when combined with chemotherapy. An emerging strategy to improve outcomes in rectal cancer is to integrate biologically active, targeted agents as triple therapy into chemoradiation protocols. MATERIAL AND METHODS: Cetuximab and bevacizumab have now been incorporated into phase I-II studies of preoperative chemoradiation therapy (CRT) for rectal cancer. The rationale of these combinations, early efficacy and toxicity data, and possible molecular predictors for tumor response are reviewed. Computerized bibliographic searches of Pubmed were supplemented with hand searches of reference lists and abstracts of ASCO and ASTRO meetings. RESULTS: The combination of cetuximab and CRT can be safely applied without dose compromises of the respective treatment components. Disappointingly low rates of pathologic complete remission have been noted in several phase II studies. The K-ras mutation status and the gene copy number of EGFR may predict tumor response. The toxicity pattern (radiation-induced enteritis, perforations) and surgical complications (wound healing, fistula, bleeding) observed in at least some of the clinical studies with bevacizumab and CRT warrant further investigations. CONCLUSION: Longer follow-up (and, finally, randomized trials) is needed to draw any firm conclusions with respect to local and distant failure rates, and toxicity associated with these novel treatment approaches.

Matsuo, K., N. Hamajima, et al. (2002). "Aldehyde dehydrogenase 2 (ALDH2) genotype affects rectal cancer susceptibility due to alcohol consumption." J Epidemiol **12**(2): 70-6.

BACKGROUND: Epidemiologic studies have shown the association between alcohol consumption and colorectal cancer, especially for rectal cancer. The alcohol related enzyme encoding gene ALDH2 has polymorphism Glu487Lys, and 487Lys allele is closely linked with phenotypic loss of enzyme activity. MATERIALS AND METHODS: A hospital-based case-control study was conducted with 72 colon and 70 rectal cancer cases and 241 non-cancer controls to evaluate the alcohol consumption and ALDH2 Glu487Lys polymorphism. The logistic regression model was applied to estimate the odds ratios (ORs). RESULT: The crude ORs for Glu/Lys and Lys/Lys genotype relative to Glu/Glu for colon and rectal cancer were not statistically significant. However, with the rectal cancer analysis, the ORs for high alcohol consumption were greater with 487Glu/Lys genotype compared with Glu/Glu, albeit not. CONCLUSIONS: These observations suggested rectal cancer risk might be influenced by ALDH2 gene polymorphism. The prevention effect by alcohol reduction might differ by ALDH2 genotype.

Matsuo, S., S. Eguchi, et al. (2001). "Attenuated familial adenomatous polyposis associated with advanced rectal cancer in a 16-year-old boy: report of a case." Surg Today **31**(11): 1020-3.

We herein present a case of attenuated familial adenomatous polyposis (AFAP) with advanced rectal cancer in a 16-year-old boy. His mother and younger brother both had subcutaneous soft tissue tumors in the back and sparse-type colorectal polyposis. His mother also had dental anomalies and gastric fundic gland polyposis. The patient was admitted to our hospital for investigation of bloody stools. Barium enema and colonofiberscopy revealed advanced rectal cancer and sparse (<50) colorectal polyps. He also had dental anomalies, a subcutaneous soft tissue tumor in the back, and gastric fundic gland polyposis as extracolonic manifestations. A total proctocolectomy and ileoanal anastomosis were performed, and histological examination of the resected specimens confirmed moderately

differentiated adenocarcinomas of the rectum with metastases to the regional lymph nodes. The other colorectal polyps were tubular adenomas with no evidence of malignancy. Germline mutations in the APC gene were observed in codons 486, 545, 1493, and 1556. This case serves to demonstrate that a total proctocolectomy with ileoanal anastomosis should be the procedure of choice for young patients found to have advanced rectal cancer associated with FAP.

McDowell, D. T., F. M. Smith, et al. (2009). "Increased spontaneous apoptosis, but not survivin expression, is associated with histomorphologic response to neoadjuvant chemoradiation in rectal cancer." Int J Colorectal Dis **24**(11): 1261-9.

PURPOSE: Survivin has been shown to be an important mediator of cellular radioresistance in vitro. This study aims to compare survivin expression and apoptosis to histomorphologic responses to neoadjuvant radiochemotherapy (RCT) in rectal cancer. MATERIALS AND METHODS: Thirty-six pre-treatment biopsies were studied. Survivin mRNA and protein expression plus TUNEL staining for apoptosis was performed. Response to treatment was assessed using a 5-point tumour regression grade. RESULTS: Survivin expression was not found to be predictive of response to RCT (p = NS). In contrast, spontaneous apoptosis was significantly (p = 0.0051) associated with subsequent response to RCT. However, no association between survivin expression and levels of apoptosis could be identified. CONCLUSIONS: This in vivo study failed to support in vitro studies showing an association between survivin and response to chemotherapy and radiation therapy. These results caution against the translation of the in vitro properties of survivin into a clinical setting.

Meng, W. J., L. Wang, et al. (2007). "Novel mutations and sequence variants in exons 3-9 of human T cell factor-4 gene in sporadic rectal cancer patients stratified by microsatellite instability." World J Gastroenterol **13**(27): 3747-51.

AIM: To establish the role of human T Cell Factor-4 (hTCF-4) gene exons 3-9 mutation status in association with sporadic rectal cancer with microsatellite instability (MSI). METHODS: Microsatellite markers were genotyped in 93 sporadic rectal cancer patients. Eleven cases were found to be high-frequency MSI (MSI-H). Sequence analysis of the coding region of the exons 3-9 of hTCF-4 gene was carried out for the 11 MSI-H cases and 10 controls (5 microsatellite stability (MSS) cases and 5 cases with normal mucosa). The sequencing and MSI identification were used. RESULTS: Several novel mutations and variants were revealed. In exon 4, one is a 4-position continuous alteration which caused amino acid change from Q131T and S132I (391insA, 392 G > A, 393 A > G and 395delC) and another nucleotide deletion (395delC) is present in MSI-H cases (5/10 and 4/10, respectively) but completely absent in the controls. CONCLUSION: Novel mutations in exon 4 of hTCF-4 gene were revealed in this study, which might be of importance in the pathogenesis of sporadic rectal cancer patients with MSI-H.

Menko, F. H., G. L. Kaspers, et al. (2004). "A homozygous MSH6 mutation in a child with cafe-au-lait spots, oligodendroglioma and rectal cancer." Fam Cancer **3**(2): 123-7.

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant condition due to heterozygous germline mutations in DNA mismatch repair genes, in particular MLH1, MSH2 and MSH6. Recently, a syndrome was recognized in which children develop haematological malignancies, solid tumours and signs of neurofibromatosis type 1 due to bi-allelic MMR gene mutations in MLH1, MSH2 and PMS2. Here we describe the child of healthy consanguineous parents who had cafe-au-lait spots, oligodendroglioma, and rectal cancer. The patient was homozygous for the MSH6 mutation c.3386_3388delGTG in exon 5 which has a predicted pathogenic effect. Germline NF1 gene mutation testing was negative. The rectal tumour showed microsatellite instability and absence of MSH6 staining, whereas the brain tumour was MSI stable and showed normal immunohistochemical expression of MSH6. Apparently, not only MLH1, MSH2 and PMS2, but also MSH6 is involved in the syndrome of childhood cancer and signs of neurofibromatosis type 1.

Milosavljevic, T. (1998). "Rectal cancer--from genetics to the therapy." Acta Chir Iugosl **45**(2 Suppl): 23-7.

Cancer of the colon and rectum is major cause of cancer-associated morbidity and mortality. Globally, it is the third most common cancer in males and fourth most common in females. Colorectal cancer is primarily a genetic disease with lesions being either somatically induced by environmental agents or inherited through the germline. Familial adenomatous polyposis and hereditary non-polyposis colorectal cancer are the main types of inherited colorectal cancer. Sporadic colorectal cancer is though to involve several genes in a multistep pathway. Colorectal cancer occurs as a result of a series of genetic alterations in normal tissue that lead to disorganization in the molecular mechanisms that control growth. Most of the evidence incriminating

diets as a major factor in the genesis of colorectal cancer. Case control studies and complex statistical analyses implicate both causative and protective dietary factors. The aim of screening is to detect neoplasia of the colon and rectum before it reaches an advanced stage, survival being directly related to the stage at presentation. The most sensitive evaluation of the whole colon is by colonoscopy. The way forward continues to rest with research into more sensitive occult fecal blood tests, more cost-effective endoscopic protocols and greater knowledge of the biological factors triggering dysplastic changes in large bowel mucosa.

Mohiuddin, M. and M. M. Ahmed (1997). "Critical issues in the evolving management of rectal cancer." Semin Oncol **24**(6): 732-44.

Evolving trends in the management of rectal cancer have focused on organ preservation, improved quality of life, and survival of patients. A significant shift is underway in our thinking about what constitutes the true rectum and defining the "proximal" and "distal" segments of the rectum. Tumor mobility remains a dominant prognostic factor in patient selection and choice of surgery. A clinical staging with tumor location in the rectum provides a logical algorithm for treatment decision making with either chemoradiation therapy or surgery as initial treatment of choice. Current rectal cancer management has largely focused on postoperative adjuvant radiation strategies with improvement reported for T3 and N+ cases. Recent data from Europe suggests that preoperative radiation has a significant advantage over surgery alone or postoperative treatment. This appears to be borne out by institutional studies of high-dose preoperative radiation (>45 Gy) in the United States. Aggressive preoperative combined chemoradiation has also led to significant downstaging of cancer with pathological complete response rates of 20% to 30%. This offers new options for surgical management of residual disease with endocavitary radiation or local excision. The development of new agents Gemcitabine, paclitaxel, and CPT-11 may also prove beneficial. New treatment strategies need to be coordinated with evolving knowledge of the biological behavior of the tumor based on its genetic fingerprints. c-Ki-ras and C-myc mutations have been implicated in tumor initiation and progression. A number of other tumor suppressor genes, APC gene, p53, and DCC have also been implicated in colorectal tumor carcigenesis. The modification of biological behavior by mutations in these genes is currently under study. This may guide new treatment strategies significantly reducing the death rates from rectal cancer and improving functional results of treatment.

Moore, H. G., J. Shia, et al. (2004). "Expression of p27 in residual rectal cancer after preoperative chemoradiation predicts long-term outcome." Ann Surg Oncol **11**(11): 955-61.

BACKGROUND: Compared with surgery alone, preoperative radiotherapy and 5-fluorouracil-based chemotherapy (combined-modality therapy; CMT) improves outcomes in patients with locally advanced rectal cancer. Although numerous studies have focused on identifying molecular markers of prognosis in the primary rectal cancer before CMT, our aim was to identify markers of prognosis in residual rectal cancer after preoperative CMT. METHODS: Sixty-seven patients with locally advanced (T3-4 and/or N1) rectal cancer were treated with preoperative radiotherapy (median, 5040 cGy) with or without 5-fluorouracil-based chemotherapy. Residual tumor in the resected specimen, available for 52 patients, was analyzed for tumor-node-metastasis stage, lymphovascular and/or perineural invasion, and immunohistochemical expression of p27, p21, p53, Ki-67, retinoblastoma gene, cyclin D1, and bcl-2. Recurrence-free survival (RFS) was determined by the Kaplan-Meier method and compared by the log-rank test. RESULTS: With a median follow-up of 69 months, the overall 5-year RFS was 74%. RFS was significantly worse for patients with positive p27 expression (P = .005), T3-4 tumors (P = .02), and positive lymph nodes (P = .04) in the irradiated specimen. On multivariate analysis, positive p27 expression remained an independent negative prognostic factor for RFS (P = .04). None of the other proteins was significantly associated with RFS. CONCLUSIONS: Our results indicate that positive p27 expression in rectal cancer after preoperative chemoradiation is an independent negative predictor of RFS. Expression of p27 in the residual rectal cancer may therefore identify patients with disease likely to be refractory to standard therapy and for whom investigational approaches should be strongly considered.

Murtaugh, M. A., K. Curtin, et al. (2007). "Dietary intake of folate and co-factors in folate metabolism, MTHFR polymorphisms, and reduced rectal cancer." Cancer Causes Control **18**(2): 153-63.

Little is known about the contribution of polymorphisms in the methylenetetrahydrofolate reductase gene (MTHFR) and the folate metabolism pathway in rectal cancer alone. Data were from participants in a case-control study conducted in Northern California and Utah (751 cases and 979 controls). We examined independent associations and interactions of folate, B vitamins, methionine, alcohol, and MTHFR polymorphisms (MTHFR C677T and

A1298C) with rectal cancer. Dietary folate intake was associated with a reduction in rectal cancer OR 0.66, 95% CI 0.48-0.92 (>475 mcg day compared to < or = 322 mcg) as was a combination of nutrient intakes contributing to higher methyl donor status (OR 0.79, 95% CI 0.66-0.95). Risk was reduced among women with the 677 TT genotype (OR 0.54, 95% CI 0.30-0.9), but not men (OR 1.11, 95% CI 0.70-1.76) and with the 1298 CC genotype in combined gender analysis (OR 0.67, 95% CI 0.46-0.98). These data are consistent with a protective effect of increasing dietary folate against rectal cancer and suggest a protective role of the MTHFR 677 TT genotype in women and 1298 CC in men and women. Folate intake, low methyl donor status, and MTHFR polymorphisms may play independent roles in the etiology of rectal cancer.

Murtaugh, M. A., C. Sweeney, et al. (2006). "Vitamin D receptor gene polymorphisms, dietary promotion of insulin resistance, and colon and rectal cancer." Nutr Cancer **55**(1): 35-43.

Modifiable risk factors in colorectal cancer etiology and their interactions with genetic susceptibility are of particular interest. Functional vitamin D receptor (VDR) gene polymorphisms may influence carcinogenesis through modification of cell growth, protection from oxidative stress, cell-cell matrix effects, or insulin and insulin-like growth factor pathways. We investigated interactions between foods (dairy products, red and processed meat, and whole and refined grains) and dietary patterns (sucrose-to-fiber ratio and glycemic index) associated with insulin resistance with the FokI polymorphism of the VDR gene and colon and rectal cancer risk. Data (diet, anthropometrics, and lifestyle) and DNA came from case-control studies of colon (1,698 cases and 1,861 controls) and rectal cancer (752 cases and 960 controls) in northern California, Utah, and the Twin Cities metropolitan area, Minnesota (colon cancer study only). Unconditional logistic regression models were adjusted for smoking, race, sex, age, body mass index, physical activity, energy intake, dietary fiber, and calcium. The lowest colon cancer risk was observed with the Ff/ff FokI genotypes and a low sucrose-to-fiber ratio. Rectal cancer risk decreased with greater consumption of dairy products and increased with red or processed meat consumption and the FF genotype. Modifiable dietary risk factors may be differentially important among individuals by VDR genotype and may act through the insulin pathway to affect colon cancer risk and through fat, calcium, or other means to influence rectal cancer risk.

Nilbert, M. and E. Fernebro (2000). "Lack of activating c-SRC mutations at codon 531 in rectal cancer." Cancer Genet Cytogenet **121**(1): 94-5.

Cellular SRC (c-SRC), which is the human homolog of the Rous sarcoma viral oncogene, v-src, is highly activated in colorectal cancer. Recently, a subset of colon cancers have shown a nonsense mutation at codon 531, which truncates c-SRC directly C-terminal to the c-SRC kinase regulatory domain. This specific mutation has been demonstrated to be activating, transforming, and tumorigenic and to promote metastasis. We investigated 100 rectal cancers, half of which had tumor spread outside the rectum, for this mutation by using direct sequencing. None of these tumors displayed any genetic alteration at this locus, and we thus conclude that the codon 531 mutation is a rare cause of c-SRC activation in rectal cancer.

Nilbert, M., M. Planck, et al. (1999). "Microsatellite instability is rare in rectal carcinomas and signifies hereditary cancer." Eur J Cancer **35**(6): 942-5.

We analysed microsatellite instability (MSI) in a consecutive series of 165 rectal carcinomas. Data on a personal and/or family history of cancer were collected from all patients and revealed metachronous cancer in 9 patients, 2 of whom had developed colorectal cancer, and a suspected familial aggregation of colorectal cancer in three families. Only three of the 165 (2%) rectal cancers showed MSI. The patients whose tumours displayed MSI had clinical histories suggesting hereditary cancer--a family history of colorectal cancer and/or synchronous colorectal cancers. Denaturing gradient gel (DGGE) analysis was used to screen the MSI+ patients for mutations in the hMLH1 and hMSH2 genes and revealed two new germline mutations; a 1 bp deletion in exon 10 of hMSH2 creating a premature stop-codon and a splice donor site mutation in intron 16 of hMLH1. Considering colorectal carcinomas as a group, MSI has been reported to occur in approximately 10-20% of the tumours and thus can not, per se be used for clinical detection of hereditary tumours. This study shows, however, that MSI is rare in rectal carcinomas and when present strongly suggests a hereditary predisposition for colorectal cancer development.

Nilbert, M. and E. Rambech (2001). "Beta-catenin activation through mutation is rare in rectal cancer." Cancer Genet Cytogenet **128**(1): 43-5.

Increased transcriptional activation through beta-catenin stabilization plays a central role in colorectal tumorigenesis. Alterations of phosphorylation sites within the CTNNB1 gene, which codes for beta-catenin has been reported to occur in about one-half of colorectal tumors without

APC-gene mutations. We assessed the importance of mutations in the regulatory domain, located within exon 3 of CTNNB1, in 103 rectal carcinomas and correlated these data with presence of microsatellite instability, somatic frame-shift alterations of the TCF-4 gene, and APC-gene mutations in the tumors. No mutation was detected in exon 3 of the CTNNB1 gene and our results thus demonstrate that beta-catenin activation through mutation rarely contributes to the development of sporadic and microsatellite instability stable rectal cancer.

Nitta, N., M. Ochiai, et al. (1987). "Amino-acid substitution at codon 13 of the N-ras oncogene in rectal cancer in a Japanese patient." Jpn J Cancer Res **78**(1): 21-6.

The activation of proto-oncogenes in colorectal cancers in Japanese patients was studied using a mouse NIH3T3 cell transfection assay system. Of thirty-five colorectal cancers examined, one rectal cancer showed an unusually high transformation efficiency and, in this rectal cancer, the N-ras oncogene was found to be activated. Nucleotide sequence analysis of the activated N-ras showed a single G----C point mutation at the first letter of codon 13, resulting in the coding of arginine instead of glycine. This amino-acid substitution at codon 13 may be responsible for the efficient induction of transformants of NIH3T3 cells in vitro.

Ojima, E., Y. Inoue, et al. (2007). "Effectiveness of gene expression profiling for response prediction of rectal cancer to preoperative radiotherapy." J Gastroenterol **42**(9): 730-6.

BACKGROUND: Our aim was to determine whether the expression levels of specific genes could predict clinical radiosensitivity in human colorectal cancer. METHODS: Radioresistant colorectal cancer cell lines were established by repeated X-ray exposure (total, 100 Gy), and the gene expressions of the parent and radioresistant cell lines were compared in a microarray analysis. To verify the microarray data, we carried out a reverse transcriptase-polymerase chain reaction analysis of identified genes in clinical samples from 30 irradiated rectal cancer patients. RESULTS: A comparison of the intensity data for the parent and three radioresistant cell lines revealed 17 upregulated and 142 downregulated genes in all radioresistant cell lines. Next, we focused on two upregulated genes, PTMA (prothymosin alpha) and EIF5a2 (eukaryotic translation initiation factor 5A), in the radioresistant cell lines. In clinical samples, the expression of PTMA was significantly higher in the minor effect group than in the major effect group (P = 0.004), but there were no significant differences in EIF5a2 expression between the two groups. CONCLUSIONS: We identified radiation-related genes in colorectal cancer and demonstrated that PTMA may play an important role in radiosensitivity. Our findings suggest that PTMA may be a novel marker for predicting the effectiveness of radiotherapy in clinical cases.

Okoshi, K., S. Nagayama, et al. (2009). "A case report of pathologically complete response of a huge rectal cancer after systemic chemotherapy with mFOLFOX6." Jpn J Clin Oncol **39**(8): 528-33.

A 54-year-old man was referred to our hospital because of a huge, unresectable rectal cancer occupying his entire pelvic space with a solitary liver metastasis. He had undergone a laparotomy for surgical resection, but ended up with a sigmoid colostomy due to possible invasion into the urinary bladder and pelvic wall. At the completion of seven cycles of FOLFOX regimen, radiographic examination revealed remarkable reduction of the primary rectal tumor and regional lymph nodes, and also a complete response (CR) of the liver metastasis. The tumor was extirpated without any macroscopic residues by a low anterior resection of the rectum, along with a partial resection of the urinary bladder and seminal vesicles. Since pathological and immunohistochemical examinations showed no viable cancer cells in any parts of the resected specimens, the lesion was regarded as a pathologically CR. Analysis for single-nucleotide polymorphisms in the genes involved in nucleotide excision repair, excision repair cross-complementing group 1 and xeroderma pigmentosum group D, showed a genotypic pattern sensitive to oxaliplatin. To our knowledge, this is a rare case of an initially unresectable primary rectal cancer, which was down-staged to a pathologically CR by FOLFOX chemotherapy instead of chemoradiotherapy.

Pan, Z. Z., D. S. Wan, et al. (2004). "Co-mutation of p53, K-ras genes and accumulation of p53 protein and its correlation to clinicopathological features in rectal cancer." World J Gastroenterol **10**(24): 3688-90.

AIM: To determine the accuracy of p53 gene mutations predicted by overexpression of p53 protein immunohistochemically, and to investigate the co-mutation of p53 and K-ras genes in rectal cancer and its effect on promoting malignant biologic behaviors of tumors. METHODS: Ninety-seven specimens of rectal cancer were surgically resected in our hospital from August 1996 to October 1997. The hot mutation areas of p53 gene (in exons 5-8) and K-ras gene (in codon 5/12 and 13) were detected with polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), and overexpression of p53 protein was detected with immunohistochemistry

(IHC) in the 97 specimens of rectal cancer. Correlation between gene mutations and tumor clinicopathologic factors was studied, and survival analysis was penfomed as well. RESULTS: There were 36 cases of p53 gene mutations in 61 p53 protein positive cases, and 21 cases of p53 gene non-mutation in 36 p53 protein negative cases respectively. The coincidence rate of p53 gene mutation by IHC method with PCR-SSCP method was 58.8% (57/97). The mutation rate of p53 gene was 52.6% (51/97), while K-ras gene mutation was observed in codons 12 and 13 in 61 cases with a mutation rate of 62.9% (61/97). Single gene mutation of p53 or K-ras was found in 32 cases. Both p53 and K-ras gene mutation were found in 48 cases. Statistical analysis showed that p53 and K-ras gene mutations were not related to the clinicopathologic factors, including tumor size, gross tumor type, histological classification, differentiation, invasion to intestinal veins, lymphatics and nerves, invasive depth to wall, lymph node metastasis, and Dukes' stages (P>0.05). The survival in patients with no gene mutation, single gene mutation and both gene mutations were similar (P>0.05). CONCLUSION: IHC has a certain false positive and false negative rate in detecting p53 gene mutations. Malignant biological behaviours of rectal cancer are not enhanced by p53 and K-ras gene mutations. Co-mutation of p53 and K-ras gene has neither synergic carcinogenesis-promoting effect, nor prognostic effect on rectal cancer.

Peng, J., J. J. Lu, et al. (2008). "Prediction of treatment outcome by CD44v6 after total mesorectal excision in locally advanced rectal cancer." Cancer J **14**(1): 54-61.

BACKGROUND: The purpose of this study was to investigate the significance of CD44 variant 6 (CD44v6) in predicting the treatment outcome of locally advanced adenocarcinoma of the rectum after total mesorectal excision (TME). METHODS: Expression of CD44v6 protein was detected using immunohistochemistry in 179 patients with pathologically confirmed stage II or III rectal adenocarcinoma. All patients were treated with TME, and neither neoadjuvant nor adjuvant radiotherapy were used. The correlation between the expression of CD44v6 and other disease-related characteristics with treatment outcome was investigated. RESULTS: The 5-year overall survival and disease-free survival rates were 66.75% and 65.77%, respectively, and the overall locoregional recurrence rate was 8.13% for the entire group of patients. CD44v6 was present in 41.9% of all patients. Multivariate analysis revealed that CD44v6 status and pelvic nodal metastasis were independent risk factors for the rate of distant metastases (P = 0.036 and 0.035, respectively), disease-free survival (P = 0.009 and 0.016, respectively), and overall survival (P = 0.048 and 0.034, respectively). Lymph node metastasis was the only independent risk factor for locoregional recurrence (P = 0.048), and a trend was found for CD44v6 on predicting the locoregional recurrence (P = 0.06) with both stage II and III diseases. CD44v6 is significantly associated with locoregional recurrence in stage III rectal cancer (hazard ratio 6.02, 95% confidence interval 1.25-29.0; P = 0.018), and the overall locoregional recurrence was significantly higher for patients with positive expression of CD44v6 than for those with negative expression (17.63% vs 6.62%; P = 0.026). CONCLUSION: CD44v6 expression in cancer cells is a sensitive marker for predicting the treatment outcome in patients with stage II and III adenocarcinoma of the rectum after TME and may be used to determine the necessity of adjuvant treatment. However, further investigations are needed to determine the clinical application of CD44v6 and its reliability.

Peng, J. J., S. J. Cai, et al. (2007). "Predicting prognosis of rectal cancer patients with total mesorectal excision using molecular markers." World J Gastroenterol **13**(21): 3009-15.

AIM: To explore the prognostic variables in rectal cancer patients undergoing curative total mesorectal excision and the effect of postoperative chemotherapy in advanced rectal cancer. METHODS: A total of 259 consecutive rectal cancer patients treated with curative total mesorectal excision between 1999 and 2004 were collected. p53, p21, PCNA, and CD44v6 were examined using immunohistochemistry (IHC). The correlation between clinicopathological or molecular variables and clinical outcomes, including local recurrence, metastasis, disease-free survival and overall survival, was analyzed. RESULTS: The median follow-up was 44 mo. Five-year survival rates and 5-year disease free survival rates were 75.43% and 70.32%, respectively. Multi-analysis revealed TNM staging, preoperative CEA, and CD44v6 level were independent risk factors predicting overall survival or disease free survival. The hazard ratio of peroperative CEA was 2.65 (95% CI 1.4-5) and 3.10 (95% CI 1.37-6.54) for disease free survival and overall survival, respectively. The hazard ratio of CD44v6 was 1.93 (95% CI 1.04-3.61) and 2.21 (95% CI 1.01-4.88) for disease free survival and overall survival, respectively. TNM staging was the only risk factor predicting local recurrence. Postoperative chemotherapy without radiotherapy did not improve patients' outcome. CONCLUSION: TNM staging, preoperative CEA and CD44v6 were independent prognostic factors for rectal cancer patients with total mesorectal excision. Postoperative

chemotherapy may be only used together with radiotherapy for rectal cancer patients.

Pufulete, M., P. W. Emery, et al. (2003). "Folate, DNA methylation and colo-rectal cancer." Proc Nutr Soc **62**(2): 437-45.

Prospective cohort and case-control studies suggest an association between low folate intake and increased risk of colo-rectal adenoma and cancer. Some, but not all, animal studies indicate that folate supplementation protects against the development of colo-rectal neoplasms, although supraphysiological folate doses have been shown to enhance tumour growth. Folate is a methyl donor for nucleotide synthesis and biological methylation reactions, including DNA methylation. A low dietary folate intake may increase the risk of colo-rectal neoplasia by inducing genomic DNA hypomethylation, which can affect the expression of proto-oncogenes and tumour suppressor genes associated with the development of cancer. Common polymorphisms in genes involved in the methylation pathway, such as methylenetetrahydrofolate reductase and methionine synthase, have been shown to influence risk of colo-rectal neoplasia, with interactions dependent on folate status and/or alcohol intake, which is known to antagonise methyl group availability. There is some evidence to show that DNA from normal-appearing colo-rectal mucosa in individuals with colo-rectal cancer is hypomethylated. In a case-control study DNA methylation in normal-appearing colo-rectal mucosa has been shown to be lower in individuals with colo-rectal cancer (P = 0.08) and colo-rectal adenoma (P = 0.009) than in controls free of colo-rectal abnormalities. Human intervention trials to date suggest that supraphysiological doses of folate can reverse DNA hypomethylation in colo-rectal mucosa of individuals with colo-rectal neoplasia. In a double-blind randomised placebo-controlled study folate supplementation at physiological doses has been shown to increase DNA methylation in leucocytes (P = 0.05) and colonic mucosa (P = 0.09). Further studies are required to confirm these findings in larger populations and to define abnormal ranges of DNA methylation.

Reerink, O., A. Karrenbeld, et al. (2004). "Molecular prognostic factors in locally irresectable rectal cancer treated preoperatively by chemo-radiotherapy." Anticancer Res **24**(2C): 1217-21.

PURPOSE: The aim of this study was to determine the relationship between survival and value of molecular markers in the primary tumour in a group of patients with irresectable rectal cancer, treated with preoperative chemo-radiotherapy. MATERIALS AND METHODS: Immunohistochemistry for p53, p21, bcl-2 and Ki-67 was performed on pre-treatment biopsy specimens of 34 patients with irresectable rectal cancer. Preoperative treatment consisted of pelvic irradiation of 45-56 Gy, combined with 5FU and leucovorin (350/20 mg/m2 x 5 d; in weeks 1 and 5 during radiotherapy). The median follow-up was 38 months. Endpoints were pathological T-stage and survival after surgery. RESULTS: Expression of p21 correlated significantly with survival (p=0.005). Survival and p21 expression also correlated significantly, when adjusted for tumour response (p=0.005, RR=4.8 (1.6-14.7)). CONCLUSION: Expression of p21 predicts a worse survival in irresectable rectal cancer treated with preoperative chemo-radiotherapy. No relationship was found between tumour response in chemo-radiotherapy and p53, bcl-2 or Ki-67.

Reymond, M. A., O. Dworak, et al. (1998). "DCC protein as a predictor of distant metastases after curative surgery for rectal cancer." Dis Colon Rectum **41**(6): 755-60.

PURPOSE: The aim of this study was to determine the value of DCC (deleted in colorectal cancer) protein for predicting metachronous distant metastases after curative surgery for rectal cancer. The DCC protein--for which a gene has been located on chromosome 18q--has recently been reported to have a prognostic value in colorectal cancer. This finding might have implications for treatment of International Union Against Cancer Stage II rectal carcinoma, in which distant metastases will develop in 14 percent of patients despite optimal surgery. METHODS: Paraffin-embedded tissues from 85 patients who developed distant metastases, but no local recurrence, after curative surgery for rectal cancer were matched with 85 samples from patients who remained disease-free. Matching criteria were tumor stage, age, gender, and date of surgery. Expression of DCC protein was assessed using immunohistochemistry. End points of follow-up were recurrence of disease and death. Mean follow-up was 9.6 years. No patient received either local or systemic adjuvant therapy. RESULTS: The DCC protein was found to be expressed in 64.9 percent of tumor samples. Nonexpression of DCC protein had an negative influence on survival (P = 0.03). For all tumor stages together, sensitivity of the test for subsequent occurrence of distant metastases was 42 percent and specificity was 71 percent. In Stage II cancers, the positive predictive value was 19 percent, and the negative predictive value was 88 percent. CONCLUSIONS: Our results confirm that DCC protein is a useful prognostic marker in patients with rectal carcinomas, but the positive predictive value of DCC protein for occurrence of metachronous metastases does not appear to be sufficient to justify

adjuvant therapeutic measures in Stage II rectal cancer.

Rimkus, C., J. Friederichs, et al. (2008). "Microarray-based prediction of tumor response to neoadjuvant radiochemotherapy of patients with locally advanced rectal cancer." Clin Gastroenterol Hepatol **6**(1): 53-61.

BACKGROUND & AIMS: Neoadjuvant chemoradiotherapy has become a standard treatment of locally advanced rectal carcinomas, even though the responsiveness varies from complete response to resistance. The aim of the study was to evaluate the capacity of gene expression signatures to identify responders and nonresponders pretherapeutically. METHODS: By using microarray technology we generated gene expression profiles of 43 biopsy specimens of locally advanced rectal carcinomas. The transcription profile then was compared with histopathologic response and used to identify a set of genes discriminating responders from nonresponders. RESULTS: We identified a gene expression signature of 42 genes, mostly encoding proteins that either play a role in the nucleus, such as the transcription factor ETS2, or are associated with transport function, such as the solute carrier SLC35E1, or the regulation of apoptosis, such as caspase-1. In leave-one-out cross-validation the correct classification of a responder was 71%, the specificity of the analysis for a correct classification of a nonresponder was 86%. By applying an additional statistical method of 200 successive splittings into training and test data sets we generated an individual prediction accuracy measure for each predicted response. CONCLUSIONS: Our study shows that pretherapeutic prediction of response of rectal carcinomas to neoadjuvant chemoradiotherapy is feasible, and may represent a new valuable and practical tool of therapeutic stratification.

Rodel, F., J. Hoffmann, et al. (2002). "High survivin expression is associated with reduced apoptosis in rectal cancer and may predict disease-free survival after preoperative radiochemotherapy and surgical resection." Strahlenther Onkol **178**(8): 426-35.

BACKGROUND: Spontaneous apoptosis has been shown to predict tumor response to preoperative radiochemotherapy in rectal cancer. It remains to be elucidated, however, which genetic profile determines whether a tumor is more or less prone to apoptosis. Recently, a novel member of the inhibitor of apoptosis family, designated survivin, was identified. In the present study, we investigated the impact of survivin on tumor cell apoptosis and the risk to develop distant metastases or local failure after preoperative radiochemotherapy and surgical resection. PATIENTS AND METHODS: The expression of survivin, p53, bcl-2 and the apoptotic index was evaluated by immunohistochemistry on pretreatment biopsies of 54 patients with locally advanced adenocarcinoma of the rectum. Survivin expression was correlated to clinical and histopathological markers, the levels of spontaneous apoptosis, p53 and bcl-2, as well as to disease-free survival, 5-year rates of local failure and distant disease after preoperative radiochemotherapy and surgical resection. RESULTS: Survivin expression inversely correlated with the apoptotic index: High survivin expression was found in 56% of rectal carcinoma biopsies with a median apoptotic index of 1.22%. Conversely, low survivin expression in tumor cells was associated with a high median apoptotic index (2.29%, p = 0.0001). High survivin expression also segregated with bcl-2 overexpression (65% bcl-2+ in tumors with high survivin expression as compared to 35% bcl-2+ in tumors with low survivin expression), but the difference was not statistically significant (p = 0.1). Low survivin expression was significantly related to an increased disease-free survival rate (77% vs 18% at 5 years in tumors with high survivin expression, p = 0.02) and to a reduced risk for distant metastases (18% vs 78% at 5 years in tumors with high survivin expression, p = 0.05) and local failure (6% vs 37% at 5 years in tumors with high survivin expression, p = 0.07). CONCLUSION: An inverse correlation between survivin expression and the level of spontaneous apoptosis in pretreatment biopsies suggests that survivin strongly inhibits tumor cell apoptosis in rectal cancer. Survivin expression may provide a novel predictive indicator for disease-free survival after preoperative radiochemotherapy and surgical resection in rectal cancer.

Rogers, C. G., M. L. Gonzalgo, et al. (2006). "High concordance of gene methylation in post-digital rectal examination and post-biopsy urine samples for prostate cancer detection." J Urol **176**(5): 2280-4.

PURPOSE: We evaluated the concordance between post-digital rectal examination and post-prostate biopsy urine samples using conventional methylation specific polymerase chain reaction analysis of 3 gene promoters in patients with suspected or confirmed prostate cancer. MATERIALS AND METHODS: Voided urine specimens were collected from 17 men after 15-second digital rectal examination and again after transrectal ultrasound guided biopsy of the prostate for suspected malignancy or for followup biopsy as part of an expectant management protocol. Urine sediment DNA was isolated and subjected to bisulfite modification. Methylation of GSTP1, EDNRB and APC promoters was determined by conventional methylation specific polymerase chain reaction analysis in post-digital

rectal examination and post-biopsy samples, and correlated with clinical information. RESULTS: Prostate cancer was detected on prostate biopsy in 12 of 17 patients (71%). Promoter methylation was detected in post-digital rectal examination urine specimens for GSTP1 (24%), APC (12%) and EDNRB (66%). Promoter methylation was detected in post-biopsy urine specimens for GSTP1 (18%), APC (18%) and EDNRB (77%). The concordance between post-digital rectal examination and post-biopsy urine samples was 94% for GSTP1 and APC, and 82% for EDNRB. Overall 100% of patients with biopsy proven prostate cancer had at least 1 gene methylated in urine vs 60% of those without evidence of prostate cancer on biopsy. CONCLUSIONS: Gene analysis using conventional methylation specific polymerase chain reaction is a reliable method for detecting abnormal DNA methylation in voided urine samples obtained following digital rectal examination or prostate needle biopsy. The concordance between post-digital rectal examination and post-biopsy urinary samples for promoter methylation is high (82% to 94%), suggesting that urine collected after digital rectal examination may be used for genetic analysis with results similar to those in post-biopsy urine samples.

Rooney, P. S., P. A. Clarke, et al. (1994). "DNA content of rectal mucosa and rectal mucosal proliferation in individuals at high risk of colorectal cancer." Eur J Cancer Prev **3**(1): 57-61.

Genetic changes are important in the development of colorectal cancer. Ploidy and rectal mucosal proliferation were measured in histologically normal rectal mucosa of 85 individuals (mean age 59 years, range 29-74) who had a total colonoscopy. Fifty-one subjects had an adenoma or were undergoing adenoma surveillance. Twenty-two subjects had a strong family history of colorectal cancer and 12 individuals comprised a control group who had a normal colonoscopy without a family history of colorectal cancer. An abnormal DNA content (aneuploidy) was found in the normal mucosa of nine (10.6%) individuals. There was no significant difference in rectal mucosal proliferation with those who had aneuploidy and those who had diploidy. There was a trend towards increased proliferation in those with aneuploidy and adenomas, compared with controls. Of the 35 individuals undergoing adenoma surveillance, eight had recurrent adenomas, and three of these expressed aneuploidy. In the other 27, in whom no adenomas were found, no individual expressed aneuploidy (P = 0.01, Fisher's exact test). Aneuploidy within histologically normal mucosa is an unusual feature, which requires further investigation, particularly in patients developing adenomas.

Saigusa, S., K. Tanaka, et al. (2009). "Correlation of CD133, OCT4, and SOX2 in rectal cancer and their association with distant recurrence after chemoradiotherapy." Ann Surg Oncol **16**(12): 3488-98.

BACKGROUND: Cancer stem cells are associated with metastatic potential, treatment resistance, and poor patient prognosis. Distant recurrence remains the major cause of mortality in rectal cancer patients with preoperative chemoradiotherapy (CRT). We investigated the role of three stem cell markers (CD133, OCT4, and SOX2) in rectal cancer and evaluated the association between these gene levels and clinical outcome in rectal cancer patients with preoperative CRT. METHODS: Thirty-three patients with rectal cancer underwent preoperative CRT. Total RNAs of rectal cancer cells before and after CRT were isolated. Residual cancer cells after CRT were obtained from formalin-fixed paraffin-embedded (FFPE) specimens using microdissection. The expression levels of three stem cell genes were measured using real-time reverse-transcription polymerase chain reaction (RT-PCR). The association between these gene levels and radiation was evaluated using colon cancer cell lines. Immunohistochemical staining of these markers after CRT was also investigated. RESULTS: There were significant positive correlations among the three genes after CRT. Patients who developed distant recurrence had higher levels of the three genes compared with those without recurrence in residual cancer after CRT. These elevated gene levels were significantly associated with poor disease-free survival. The radiation caused upregulation of these gene levels in LoVo and SW480 in vitro. Immunohistochemically, CD133 staining was observed in not only luminal surface but also cytoplasm. CONCLUSIONS: Expression of CD133, OCT4, and SOX2 may predict distant recurrence and poor prognosis of rectal cancer patients treated with preoperative CRT. Correlations among these genes may be associated with tumor regrowth and metastatic relapse after CRT.

Sasajima, K., Y. Yamanaka, et al. (1993). "Multiple polyps of esophagus, stomach, colon, and rectum accompanying rectal cancer in a patient with constitutional chromosomal inversion." Cancer **71**(3): 672-6.

BACKGROUND: It has been reported that colorectal carcinomas are caused by a multistage process. In patients with familial adenomatous polyposis, carcinoma of the colorectum frequently develops and occasionally polyps develop in the upper gastrointestinal tract. Chromosomal deletion often is found for chromosomes 5, 17, and 18, on which tumor suppressor genes are located. Furthermore, loss of the

alleles of loci on chromosome 3 has been reported in renal cell carcinoma, small cell lung carcinoma, and mixed salivary gland tumor in hereditary and sporadic cases. These data support the concept of a recessive mechanism for the development of human tumors. PATIENTS AND RESULTS: The authors report the case of a 48-year-old woman with rectal cancer accompanied by multiple polyps in the esophagus, stomach, and colorectum. Histologically, the polypoid lesions in the esophagus, stomach, and colorectum showed a thickened mucosa, hyperplastic polyps, and mixed hyperplastic adenomatous polyps, respectively. Karyotype analysis showed 46, xx, inv(3)(p12.2q25.3) in all 20 inspected peripheral lymphocytes. By Southern blot with a c-raf probe, one allele of the c-raf-1 gene, which has been mapped on chromosome 3p25, was deleted from the rearranged chromosome 3 in the peripheral lymphocytes, intact colonic mucosa, and cancer tissue. CONCLUSIONS: These results suggest that the development of hyperplastic polyps and carcinoma of the rectum results from the allelic loss in chromosome 3p, as has been reported for solid tumors at other sites.

Saw, R. P., M. Morgan, et al. (2003). "p53, deleted in colorectal cancer gene, and thymidylate synthase as predictors of histopathologic response and survival in low, locally advanced rectal cancer treated with preoperative adjuvant therapy." Dis Colon Rectum **46**(2): 192-202.

PURPOSE: Adjuvant therapy, either preoperatively or postoperatively, and modifications of surgery have been used to try to improve outcome of surgery for rectal cancer in regard to both local recurrence and survival. Assessment of prognosis in patients after resection is currently primarily based on clinicopathologic factors. These predict the subsequent behavior of the tumor only imperfectly. The aim of this study was to evaluate three potential molecular genetic markers of prognosis (p53, deleted in colorectal cancer gene, and thymidylate synthase) in Dukes Stage B and C low rectal tumors treated with adjuvant therapy and to determine whether they correlate with survival, local recurrence, or the pathologic response to adjuvant therapy (assessed by extent of tumor regression and tumor down-staging). METHODS: Sixty locally advanced low rectal tumors resected after preoperative chemoradiotherapy or radiotherapy alone were studied by immunohistochemical staining for p53, deleted in colorectal cancer gene, and thymidylate synthase. In addition, p53 gene mutations were sought by polymerase chain reaction-single-strand conformation polymorphism analysis. These results were correlated with survival, local recurrence, and pathologic response to adjuvant therapy. RESULTS: Lack of thymidylate synthase staining by immunohistochemistry was associated with tumor down-staging after preoperative chemoradiotherapy but not after radiotherapy or for these two combined groups. There was no correlation between p53, deleted in colorectal cancer gene, or thymidylate synthase immunohistochemical staining or between p53 polymerase chain reaction-single-strand conformation polymorphism and local recurrence or survival in locally advanced low rectal cancers treated with preoperative adjuvant therapies. CONCLUSION: Prediction of prognosis in patients with locally advanced low rectal cancers treated with preoperative adjuvant therapies continues to be problematic. Thymidylate synthase immunohistochemistry appears to be the most promising factor of those assessed in predicting tumor down-staging after preoperative chemoradiotherapy for locally advanced low rectal cancers.

Schneider, S., D. J. Park, et al. (2006). "Gene expression in tumor-adjacent normal tissue is associated with recurrence in patients with rectal cancer treated with adjuvant chemoradiation." Pharmacogenet Genomics **16**(8): 555-63.

Recurrence is a significant clinical problem for patients with rectal cancer treated with adjuvant chemoradiation. Previous studies have suggested that determining intratumoral gene expression of key genes may be helpful in predicting clinical outcome of patients with gastrointestinal malignancies undergoing chemotherapy. The role of molecular predictors for prediction of recurrence in the setting of adjuvant chemoradiotherapy is not well established. The present study was designed to identify a genetic profile that would be associated with recurrence in patients with rectal cancer treated with adjuvant chemoradiation therapy. A retrospective study with a longitudinal cohort and a cross-sectional cohort of 67 patients with locally advanced rectal cancer who underwent cancer resection, followed by 5-fluorouracil (5-FU) plus pelvic radiation was conducted. Total RNA was extracted from formalin-fixed, paraffin-embedded, laser-captured-microdissected tissue. We determined mRNA levels of genes involved in the 5-FU pathway (thymidylate synthase, dihydropyrimidine dehydrogenase), DNA-repair (excision-repair cross-complementing factor 1, Rad51), angiogenesis/radiation sensitivity [vascular endothelial growth factor (VEGF)] and radio-sensitivity [epidermal growth factor receptor (EGFR)] in tumor tissue and tumor-adjacent normal tissue by quantitative reverse transcriptase-polymerase chain reaction. In univariate analysis, only intratumoral gene expression level of VEGF ($P = 0.055$) was associated with recurrence, whereas elevated mRNA expression

levels of thymidylate synthase (P = 0.008), VEGF (P = 0.023) and EGFR (P = 0.004) in tumor-adjacent normal tissue were significantly associated with recurrence. Multivariate analysis using recursive partitioning indicated that distinct groups of recurrence could be defined by elevated mRNA expression levels of VEGF, EGFR in tumor-adjacent normal tissue, and Rad51 in tumor tissue. These data suggest that the genetic profile of the tumor-adjacent normal tissue may be associated with treatment failure, indicating that tumor microenvironment may be more important in the development of recurrence of rectal tumors than formerly expected.

Slattery, M. L., K. Curtin, et al. (2006). "PPARgamma and colon and rectal cancer: associations with specific tumor mutations, aspirin, ibuprofen and insulin-related genes (United States)." Cancer Causes Control **17**(3): 239-49.

We hypothesize that the peroxisome proliferator-activated receptor-gamma (PPARgamma) is associated with colorectal cancer given its association with insulin, diabetes, obesity, and inflammation. In this study, we evaluated the association between colorectal cancer and specific tumor mutations and the Pro12Ala (P12A) PPARgamma polymorphism. We also evaluated interactions between the PPARgamma gene and other insulin-related genes and use of aspirin and non-steroidal anti-inflammatory drug use. Data were available from 1,577 cases of colon cancer that were matched to 1,971 population-based controls and 794 cases of rectal cancer that were matched to 1,001 population-based controls. Colon tumors from the case subjects were evaluated for p53 and Ki-ras mutations and microsatellite instability (MSI). Insulin-related genes evaluated were the Bsm1, polyA, and Fok1 polymorphisms of the VDR gene; the G972R IRS1 polymorphism; the G1057D IRS2 polymorphism; the 19CA repeat polymorphism of the IGF1 gene; and the -200A>C IGFBP3 polymorphism. The odds ratio (OR) between the PA/AA genotypes and proximal tumors was 0.83 (95% CI: 0.69-1.01); for distal tumors was 1.00 (95% CI: 0.83-1.21); and for rectal tumors was 1.04 (95% CI: 0.86-1.25). Evaluation of specific types of tumor mutations showed that colon cancer cases with the PA or AA genotypes were less likely to have p53 tumor mutations (OR 0.78; 95% CI: 0.62-0.99), specifically transition mutations (OR 0.74; 95% CI: 0.56-0.97). Colon cancer cases also were less likely to have a tumor with MSI if they had the PA or AA PPARgamma genotype (OR 0.68; 95% CI: 0.47-0.98); differences in Ki-ras mutations were not seen in colon tumors by PPARgamma genotype. Those who did not take ibuprofen-type drugs and had the PA or AA genotypes were at a significantly greater risk of rectal cancer (OR 2.11; 95% CI: 1.52-2.92; p interaction 0.03) than people with the PP genotype regardless of ibuprofen-type drug use. There was a significant interaction between the -200A>C IGFBP3 polymorphism and the Pro12Ala PPARgamma polymorphism and risk of colon cancer (p for interaction = 0.02) with individuals being at significantly lower risk if they had both the CC IGFBP3 genotype and the PA/AA PPARgamma genotype. For rectal cancer there was a significant interaction between the Bsm1/polyA polymorphisms (p = 0.001) of the VDR gene and the PA/AA Pro12Ala PPARgamma polymorphism with the highest risk group being those with both the PA/AA Pro12Ala PPARgamma and the BB/SS VDR genotypes. These data suggest that PPARgamma may be associated with many aspects of colorectal cancer including insulin- and inflammation-related mechanisms.

Slattery, M. L., S. Edwards, et al. (2003). "Associations between smoking, passive smoking, GSTM-1, NAT2, and rectal cancer." Cancer Epidemiol Biomarkers Prev **12**(9): 882-9.

Cigarette smoking has been identified as a risk factor for colon cancer, however, much less is known about the association between cigarette smoking and rectal cancer. The purpose of this article is to evaluate the associations between rectal cancer and active and passive cigarette smoking and other forms of tobacco use. We also evaluate how genetic variants of GSTM-1 and NAT2 alter these associations. A population-based case-control study of 952 incident rectal cancer cases and 1205 controls was conducted. Detailed tobacco use information was collected as part of an interviewer-administered questionnaire. DNA was extracted from blood to examine genetic variants of GSTM-1 and NAT2. Cigarette smoking was associated with an increased risk of rectal cancer in men [odds ratio (OR)=1.5, 95% confidence interval (CI), 1.1-2.1 for current smokers; OR=1.7, 95% CI, 1.3-2.3 for smoking >20 pack-years of cigarettes relative to never-smokers]. After adjusting for active smoking, exposure to cigarette smoke of others also was associated with increased risk among men (OR=1.5, 95% CI, 1.1-2.0). Neither GSTM-1 genotype nor NAT2-imputed phenotype was independently associated with rectal cancer. However, the risk associated with smoking cigarettes among those who were GSTM-1 null relative to those who never smoked and had the GSTM-1 present genotype was OR=2.0 (95% CI, 1.2-3.3). This interaction was of borderline significance (P=0.08). Men who had the combined GSTM-1 present genotype and who were rapid acetylators had no increased risk from cigarette

smoking. There were no significant associations between cigarette smoking and rectal cancer among women. This study shows that men who smoke cigarettes, especially those who smoke >20 pack-years, are at increased risk of rectal cancer. This association may be influenced by GSTM-1 genotype. Furthermore, exposure to cigarette smoke of others may increase risk of rectal cancer among men who do not smoke.

Slattery, M. L., M. Murtaugh, et al. (2005). "Energy balance, insulin-related genes and risk of colon and rectal cancer." Int J Cancer **115**(1): 148-54.

Energy balance, or the ability to maintain body weight by balancing energy intake with energy expenditure, appears to be important in the etiology of colon cancer. One possible mechanism whereby energy balance may be associated with colorectal cancer is through its association with insulin. In our study, we evaluate the interaction between polymorphisms in 4 genes thought to be involved in insulin-related functions and components of energy balance with risk of colorectal cancer. Data from 2 population-based case-control studies of colon and rectal cancer conducted in Utah and Northern California were used to evaluate associations between body mass index (BMI), physical activity, energy intake and sucrose-to-fiber ratio and a CA repeat polymorphism of the IGF1 gene, the A/C polymorphism at nucleotide -202 of the IGFBP3, the G972R polymorphism of the IRS1 gene and the G1057D polymorphism of the IRS2 gene. A total of 1,346 incident colon cancer cases and 1,544 population-based controls and 952 incident rectal cancer cases and 1,205 controls were available for analysis. Inconsistent associations were identified between BMI, physical activity, energy intake and insulin-related genes. The 192/192 IGF1 genotype was associated with significant reduction in colon cancer risk among those with high physical activity (odds ratio [OR] 0.57; 95% confidence interval [CI] 0.39-0.83; p interaction 0.01). Although there was no significant pattern of interaction between either BMI or energy intake and polymorphisms assessed, specific sources of energy did appear to be more related to colon cancer risk in the presence of specific IRS2 and IGF1 genotypes. A high sucrose-to-fiber ratio increased risk of colon cancer in men who had the IRS2 DD genotype and among men who did not have the 192/192 IGF1 genotype. In summary, these data support the importance of components of energy balance in risk of colorectal cancer. Obesity, physical activity and energy intake appear to alter risk of colorectal cancer; however, the risk appears to be minimally influenced by genetic variants evaluated.

Slattery, M. L., W. Samowtiz, et al. (2004). "CYP1A1, cigarette smoking, and colon and rectal cancer." Am J Epidemiol **160**(9): 842-52.

Cytochrome P-450 (CYP) is involved in the activation and metabolism of polycyclic aromatic hydrocarbons in tobacco products. The authors evaluated the association of two polymorphisms in the CYP1A1 gene--the noncoding Msp I polymorphism in the 3'-untranslated region and the Ile462Val polymorphism in exon 7--with colon and rectal cancer. The authors used data from two incident case-control studies of colon cancer (1,026 cases and 1,185 controls) and rectal cancer (820 cases and 1,036 controls) conducted in California and Utah (1991-2002). CYP1A1 genotype was not associated with colon or rectal cancer. Having GSTM1 present, a CYP1A1 variant allele, and the rapid-acetylator NAT2 imputed phenotype was associated with increased risk of colon cancer (odds ratio = 1.7, 95% confidence interval: 1.2, 2.3). Among men, the greatest colon cancer risk was observed for having any CYP1A1 variant allele and currently smoking (odds ratio = 2.5, 95% confidence interval: 1.3, 4.8; Wald chi(2)test: $p < 0.01$). Assessment of GSTM1 and CYP1A1 and rectal cancer in men showed a twofold elevation in risk for more than 20 pack-years of smoking, except among those with GSTM1 present who had a variant CYP1A1 allele. These data support the association between smoking and colon and rectal cancer. Smoking may have a greater impact on colorectal cancer risk based on CYP1A1 genotype; this might further be modified by GSTM1 for rectal cancer risk.

Slattery, M. L., C. Sweeney, et al. (2006). "Associations between vitamin D, vitamin D receptor gene and the androgen receptor gene with colon and rectal cancer." Int J Cancer **118**(12): 3140-6.

The transcriptional activity of the vitamin D receptor (VDR) gene is regulated, at least in part, by the androgen receptor (AR) gene. We evaluate how the number of polyglutamine (CAG) repeats of the AR gene influence colorectal cancer in conjunction with vitamin D, sunshine exposure and VDR. Studies of colon (1,580 cases and 1,968 controls) and rectal (797 cases and 1,016 controls) cancer were used. Vitamin D intake and average hours of sunshine exposure interacted with AR genotype in men. Men with low vitamin D intake or low levels of sunshine exposure who had 23+ CAG repeats of the AR gene had the greatest risk of colon cancer. ORs for men with 23 or more CAG repeats of the AR gene and in the lowest tertile of vitamin D intake or sunshine exposure were 1.71 (95% CI 1.14, 2.56) and 1.51 (95% CI 1.09, 2.09). Men with high levels of sunshine exposure were at reduced risk of developing rectal cancer if they had 23 or more CAG repeats (OR 0.62 95% CI 0.39, 0.97)

than if they had fewer than 23 CAG repeats. The FF genotype of the Fok1 VDR gene was associated with reduced risk of colon cancer among women with any allele of 23+ CAG repeats (OR 0.62 95% CI 0.44, 0.88), whereas men with the LL/bb VDR genotypes were at reduced risk of rectal cancer if they also had 23+ CAG repeats (OR 0.71 95% CI 0.48, 1.05) relative to men with fewer than 23 CAG repeats of the AR gene. These data provide support for the role of vitamin D and sunshine exposure in the etiology of colorectal cancer and suggest that AR gene may modulate the association.

Slattery, M. L., C. Sweeney, et al. (2005). "Associations between apoE genotype and colon and rectal cancer." Carcinogenesis **26**(8): 1422-9.

Apolipoprotein E (apoE) plays a major role in the metabolism of bile acids, cholesterol and triglycerides, and has recently been proposed as being involved in the carcinogenic process. Given the potential role of bile acids in colorectal cancer etiology, it is reasonable that colorectal cancer risk might be modified by apoE genotype. We used data collected from a case-control study of colon cancer (n=1556 cases and 1948 controls) and rectal cancer (n=777 cases and 988 controls). The absence of an e3 apoE allele significantly increased the risk of colon cancer (OR=1.37 95% CI 1.00-1.87), particularly among those diagnosed when older than 64 years (OR=1.88 95% CI 1.17-3.04; P interaction between age and apoE genotype equal to 0.05). A significant three-way interaction was detected for family history of colorectal cancer, age at diagnosis and apoE genotype (P = 0.05), in those diagnosed when older, not having an e3 allele and having a significantly increased risk of colon cancer with family history of colorectal cancer (OR=3.93 95% CI 1.23-12.6). This was compared with the risk associated with family history of colorectal cancer among those diagnosed when older, with an e3 allele of 1.61 (95% CI 1.17-2.23) or those diagnosed when younger without an e3 allele (OR=2.40 95% CI 0.56-10.3). Among those diagnosed when older than 64 years, associations of BMI and prudent diet with colon cancer were stronger among individuals without an e3 allele, although the P for interaction was not significant. We did not detect any significant associations between apoE genotype and rectal cancer, survival after diagnosis with colorectal cancer, stage of disease at diagnosis or type of tumor mutation. These findings suggest those apoE genotypes that do not include the e3 allele, the same genotypes that are associated with increased risk of coronary heart disease, may influence development of colon cancer among those who are older at diagnosis.

Slattery, M. L., C. Sweeney, et al. (2005). "Associations between ERalpha, ERbeta, and AR genotypes and colon and rectal cancer." Cancer Epidemiol Biomarkers Prev **14**(12): 2936-42.

Estrogen and androgens are thought to be involved in the etiology of colorectal cancer. We evaluate genetic variants of the estrogen receptor genes (ERalpha and ERbeta) and the androgen receptor gene (AR). We use data from two large case-control studies of colon (n = 1,580 cases and 1,968 controls) and rectal (n = 797 cases and 1,016 controls) cancer. We evaluated the 351A >G XbaI polymorphism of ERalpha, the 1,082 G >A and CA repeat polymorphisms of ERbeta, and the CAG repeat of AR. Having two 25 or more CA repeats in ERbeta was associated with an increased relative risk of colon cancer in women [odds ratio (OR), 2.13; 95% confidence interval (95% CI), 1.24-3.64] but not in men (P(interaction) relative excess risk from interaction < 0.01; multiplicative = 0.03). Increasing number of AR CAG repeats was directly associated with colon cancer among men (OR, 1.28; 95% CI, 1.06-1.54), but not women (OR, 0.83; 95% CI, 0.68-1.02); the interaction P value for AR gene x sex was <0.01. Taking hormone replacement therapy (HRT) was associated with a reduced risk of colon cancer in the presence of the R allele of the ERbeta gene, whereas an R allele was associated with increased risk among postmenopausal women who did not take HRT. Postmenopausal women not using HRT who had > or =25 CA repeats of the ERbeta gene had over a 6-fold increased risk of colon cancer (OR, 6.71; 95% CI, 2.89-15.6). Our results suggest that the ERbeta gene is more important than ERalpha in the etiology of colorectal cancer.

Slattery, M. L., R. K. Wolff, et al. (2009). "Tumor markers and rectal cancer: support for an inflammation-related pathway." Int J Cancer **125**(7): 1698-704.

Inflammation may be a key element in the etiology of colorectal cancer. In our study, we examine associations between factors related to inflammation and specific rectal cancer mutations. A population-based study of 750 rectal cancer cases with interview and tumor DNA were compared to 1,205 population-based controls. Study participants were from Utah and the Northern California Kaiser Permanente Medical Care Program. Tumor DNA was analyzed for TP53 and KRAS2 mutations and CpG Island methylator phenotype. We assessed how these tumor markers were associated with use of anti-inflammatory drugs, polymorphisms in the IL6 genes (rs1800795 and rs1800796) and dietary antioxidants. Ibuprofen-type drugs, IL6 polymorphisms (rs1800796) and dietary alpha-tocopherol and

lycopene significantly altered likelihood of having a TP53 mutation. This was especially true for TP53 transversion mutations and dietary antioxidants (OR for beta-carotene 0.51 95% CI 0.27, 0.97, p trend 0.03; alpha-tocopherol 0.41 95% CI 0.20, 0.84, p trend 0.02) Beta-carotene and ibuprofen significantly altered risk of KRAS2 tumors. The associations between lutein and tocopherol and TP53 and KRAS2 mutations were modified by IL6 genotype. These results suggest that inflammation-related factors may have unique associations with various rectal tumor markers. Many factors involved in an inflammation-related pathway were associated with TP53 mutations and some dietary factors appeared to be modified by IL6 genotype.

Slattery, M. L., R. K. Wolff, et al. (2007). "IL6 genotypes and colon and rectal cancer." Cancer Causes Control **18**(10): 1095-105.

Inflammation appears to play a key role in the development of colorectal cancer (CRC). In this study we examine factors involved in the regulation of inflammation and risk of CRC. Data from a multi-center case-control study of colon (N = 1579 cases and N = 1977 controls) and rectal (N = 794 cases and N = 1005 controls) cancer were used to evaluate the association between the rs1800795 and rs1800796 IL6 polymorphisms and CRC. We evaluated the joint effects of IL6 single nucleotide polymorphisms and regular use of aspirin/NSAIDs and vitamin D receptor (VDR) genotype. Having a C allele of the rs1800796 IL6 polymorphisms and the GG genotype of the rs1800795 IL6 polymorphisms was associated with a statistically significantly reduced the risk of colon (OR 0.76 95% CI 0.57, 1.00), but not rectal (OR 1.49 95% CI 1.02,2.16) cancer. Both IL6 polymorphisms were associated with significant interaction with current use of aspirin/NSAIDs to alter risk of colon cancer: individuals with a C allele in either polymorphism who were current users of aspirin/NSAIDs had the lowest colon cancer risk. CRC risk also was associated with an interaction between VDR and IL6 genotypes that was modified by current use of aspirin/NSAIDs. This study provides further support for inflammation-related factors in the etiology of CRC. Other studies are needed to explore other genes in this and other inflammation-related pathways.

Smith, F. M., J. V. Reynolds, et al. (2006). "Pathological and molecular predictors of the response of rectal cancer to neoadjuvant radiochemotherapy." Eur J Surg Oncol **32**(1): 55-64.

AIMS: The prediction of sensitivity and resistance to neoadjuvant therapy has great potential value for many tumour sites. A neoadjuvant regimen is increasingly the gold standard in rectal cancer management and the aim of this review was to highlight predictive markers currently assessed and evaluate their clinical utility. METHODS: A systematic search of Medline was conducted using the following keywords 'colorectal', 'neoadjuvant', 'molecular', 'predict' and 'radiotherapy'. Original manuscripts from all relevant listings were sourced. These were hand searched for further articles of relevance. RESULTS: Conventional indices including tumour stage and grade were unable to predict histological response. Immunohistochemical assessment of P53 gene, Bcl 2, Bax and microsatellite instability are of no predictive value. Studies utilising molecular response predictors from archival pre-treatment tumour tissues have identified several promising predictive markers including p21, spontaneous apoptosis and direct sequencing of the p53 gene. Global gene expression from fresh pre-treatment tissue using cDNA microarray has only recently been assessed but identified expression differences between 54 genes and was able to predict response with 78% sensitivity and 86% specificity. CONCLUSIONS: Currently there are no clinically useful predictors of response based on standard pathological assessment and immunocytochemistry. Direct gene sequencing of p53, studies of apoptosis and global gene sequencing may hold promise.

Speake, W. J., R. A. Dean, et al. (2005). "Radiation induced MMP expression from rectal cancer is short lived but contributes to in vitro invasion." Eur J Surg Oncol **31**(8): 869-74.

AIMS: Matrix metalloproteinase (MMP) activity is increased after radiation. The aims of this study were to assess the time course of this increase and its effects on malignant cell invasion. METHODS: Colorectal cancer (HCT 116, LoVo, C 170 HM 2, CaCO-2), fibroblast (46-BR.IGI, CCD-18 Co) and fibrosarcoma (HT1080) cell lines were irradiated at 4 gray (4 Gy) and matrix metalloproteinase gene and protein expression examined over a 96 h period by real time polymerase chain reaction and gelatin zymography. Invasion was assessed on Matrigel. Human rectal tumour MMP expression was compared before and after long course radiotherapy. RESULTS: Radiation increased MMP gene expression of tumour cell lines, and resulted in increased MMP protein activity in the HT1080 line. HT1080 and HCT 116 in monoculture and LoVo in co-culture were more invasive after radiation at 48 h in vitro, but long course radiotherapy did not result in a consistent increase in MMP expression from human rectal tumour biopsies. CONCLUSIONS: Radiation results in increased MMP expression for a limited time period. This results in an early increase in cell

line invasion. Further clinical research is required to clarify if MMP inhibition given perioperatively following radiotherapy decreases local recurrence rates.

Speer, G., O. Dworak, et al. (2000). "Vitamin D receptor gene BsmI polymorphism correlates with erbB-2/HER-2 expression in human rectal cancer." Oncology **58**(3): 242-7.

Apart from the regulation of calcium metabolism, 1, 25-dihydroxyvitamin D(3) plays an essential role in cell proliferation and differentiation in several tissues. The vitamin D receptor (VDR) gene shows polymorphisms in humans that appear to be clinically significant in some pathological conditions. In the present study, the BsmI polymorphism of the VDR gene was studied in 59 Caucasian patients with rectal cancer (mean follow-up: 48 months). The presence of the VDR B allele significantly correlated with the overexpression of the erbB-2 oncogene. There was no difference in the VDR genotype between cancer and normal mucosal cells. Coexpression of erbB-2, pan-ras, p53 and EGFR internal and external domains was significantly higher in cancer cells than in normal mucosa. There was no significant correlation between VDR genotypes and age, gender, tumor infiltration depth, number and site of lymph node metastases and lymphatic or blood vessel infiltration. The VDR genotype alone did not influence survival. Overexpression of erbB-2 and EGFR was associated with a poor prognosis. In patients expressing only one oncogene in cancer cells, the presence of the VDR B allele showed a tendency to a poor prognosis. In conclusion, VDR gene BsmI polymorphism might affect the development and prognosis of rectal cancer by influencing erbB-2 oncogene expression.

Spindler, K. L., J. N. Nielsen, et al. (2006). "Prediction of response to chemoradiation in rectal cancer by a gene polymorphism in the epidermal growth factor receptor promoter region." Int J Radiat Oncol Biol Phys **66**(2): 500-4.

PURPOSE: Epidermal growth factor receptor (EGFR) has been associated with radioresistance in solid tumors. Recently a polymorphism in the Sp1 recognition site of the EGFR promoter region was identified. The present study investigated the predictive value of this polymorphism for the outcome of chemoradiation in locally advanced rectal cancer. METHODS AND MATERIALS: The study included 77 patients with locally advanced T3 rectal tumors. Treatment consisted of preoperative radiation therapy at a total tumor dose of 65 Gy and concomitant chemotherapy with Uftoral. Blood samples from 63 patients were evaluated for Sp1 -216 G/T polymorphism by polymerase chain reaction analysis. Forty-eight primary tumor biopsies were available for EGFR immunostaining. Patients underwent surgery 8 weeks after treatment. Pathologic response evaluation was performed according to the tumor regression grade (TRG) system. RESULTS: Forty-nine percent had major response (TRG1-2) and 51% moderate response (TRG 3-4) to chemoradiation. The rates of major response were 34% (10/29) in GG homozygote patients compared with 65% (22/34) in patients with T containing variants (p=0.023). Fifty-eight percent of biopsies were positive for EGFR expression (28/48). The major response rates with regard to EGFR immunostaining were not significantly different. EGFR-positive tumors were found in 83% of the GG homozygote patients compared with 38% of patients with TT or GT variants (p=0.008). CONCLUSIONS: There was a significant correlation between EGFR Sp1 -216 G/T polymorphism and treatment response to chemoradiation in locally advanced rectal cancer. Further investigations of a second set of patient and other treatment schedules are warranted.

Stein, U., B. Rau, et al. (1999). "Hyperthermia for treatment of rectal cancer: evaluation for induction of multidrug resistance gene (mdr1) expression." Int J Cancer **80**(1): 5-12.

Environmental stress factors, such as heat, may induce multidrug resistance gene (mdr1) expression, which could result in the disadvantageous multidrug resistance (MDR) phenotype. To evaluate this possibility in a clinical situation, we investigated mdr1 gene expression in patients with locally advanced rectal cancer who underwent preoperative radio-chemo-thermo-therapy (RCTT). Patients were classified into groups according to the treatment schedule of RCTT vs. radio-chemo-therapy (RCT) without hyperthermia (control group). Expression of the mdr1 gene was analyzed in tumors and normal rectal tissues prior to and post-treatment (RCTT or RCT, respectively) by means of semi-quantitative and quantitative reverse transcription-polymerase chain reaction (RT-PCR). The data were correlated with therapeutic response and survival parameters. Based on our evaluation criteria, in 2 of 19 tumors of the RCTT group, mdr1 gene expression was increased more than 2-fold; in 3 of 19 tumors of this group, however, mdr1 expression was decreased more than 2-fold. In the patient control group, levels of mdr1 gene expression were reduced in 2 of 8 tumors. Thus, hyperthermia combined with RCT (RCTT) in comparison with RCT alone does not lead to an increase in mdr1 gene expression in patients with locally advanced rectal cancer within the preoperative treatment schedule. The risk of inducing the classical multidrug resistance phenotype by hyperthermia was

thus minimal in this clinical setting. Subsequent adjuvant chemotherapy should thus not be hindered.

Stoehlmacher, J., E. Goekkurt, et al. (2008). "Thymidylate synthase genotypes and tumour regression in stage II/III rectal cancer patients after neoadjuvant fluorouracil-based chemoradiation." Cancer Lett **272**(2): 221-5.

PURPOSE: According to the CAO-/ARO-/AIO-94 trial of the German Rectal Cancer Study Group, pre-operative 5-fluorouracil (5-FU)-based long-term chemoradiotherapy (CT/RT) is recommended for patients with rectal cancer UICC stage II/III. However, despite the local benefit of neoadjuvant treatment, the overall prognostic value remains uncertain in comparison to adjuvant CT/RT. We assessed the impact of standardized pre-operative CT/RT and intratumoural mRNA levels and polymorphisms of the TS gene on histopathological tumour regression. PATIENTS AND METHODS: 40 patients with rectal cancer UICC stage II/III, receiving pre-operative 5-FU-based CT/RT followed by standardized surgery, including total mesorectal excision, were investigated. TS gene expression and TS polymorphisms of surgical specimens were correlated with the grade of histopathological tumour regression (0-4). Patients achieved regression grades 2-4 were determined as responders. RESULTS: TS polymorphisms (5'-28bp repeat+G/C SNP and TS1494del6) could be determined in 39/40 (97.5%) and in 38/40 (95%) patients, respectively. Quantification of TS mRNA expression was successful in 36/40 (90%) patients. There was a highly significant linkage disequilibrium between 5'- and 3'-TS polymorphisms (p=0.0013). Interestingly, the majority of patients (82.1%) with 5'-TS genotypes known to be associated with low mRNA expression (2R/2R, 2R/3RC, 3RC/3RC) also possessed the TS1494del6 +6bp/+6bp genotype correlating with high TS mRNA expression. TS1494del6 polymorphism was significantly associated with TS mRNA expression. Patients with TS1494del6 -6bp/-6bp or -6bp/+6bp genotypes showed significantly lower mean TS mRNA expression with 0.55 (range:0.33;0.84) as compared to +6bp homozygotes with a mean expression of 0.90 (range:0.20;1.91) (p=0.025). Furthermore, all patients with TS 3'-UTR -6bp/-6bp or -6bp/+6bp genotype (11/11) were responders as compared to only 20/26 (77%) of patients with TS 3'-UTR +6bp/+6bp genotype (p=0.082). TS 5'-polymorphisms were not associated with neither tumour regression nor gene expression. CONCLUSION: Our data suggest that the TS1494del6 polymorphism may be an important predictor for histopathological tumour regression in UICC II/III rectal cancer patients receiving neoadjuvant 5-FU-based CT/RT.

Szeliga, J., Z. Sondka, et al. (2008). "NOD2/CARD15 polymorphism in patients with rectal cancer." Med Sci Monit **14**(9): CR480-4.

BACKGROUND: Reports published in the past several years have not provided conclusive evidence regarding a relationship between the development of colorectal cancer and NOD2 gene mutations, though some geographic variability has been shown. It was found that the aforementioned mutations were more frequent among the colorectal cancer patients and that the presence of the 1007fs variant might also be associated with young patient age. CONCLUSIONS: The analysis of the material does not allow presenting a conclusive answer as to whether the 1007fs, G908R, and R702W mutations or P268S polymorphism contribute to the development of sporadic colorectal cancer in the Polish population. Patients in some populations could likely benefit from instituting earlier colorectal cancer screening studies following the detection of the 1007fs mutation.

Terrazzino, S., M. Agostini, et al. (2006). "A haplotype of the methylenetetrahydrofolate reductase gene predicts poor tumor response in rectal cancer patients receiving preoperative chemoradiation." Pharmacogenet Genomics **16**(11): 817-24.

OBJECTIVE: The objective of the present study was to evaluate whether germline methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms as well as polymorphisms in the thymidylate synthase gene promoter, namely the variable number tandem repeat polymorphism (TS VNTR) and the intrarepeat G to C single nucleotide polymorphism (TS SNP), are predictive markers of tumor regression in rectal cancer patients following preoperative chemoradiotherapy. BASIC METHODS: Blood samples from 125 patients with primary adenocarcinoma of the mid-low rectum who received 5-fluorouracil-based chemotherapy and external beam radiotherapy (median dose 48.4 Gy), 125 patients (women n=45, men n=80; median age 60 years, range 31-79 years) were genotyped. Response to preoperative treatment was evaluated employing the Tumor Regression Grade criteria. On the basis of the pathologic response, patients were classified as responders (TRG 1-2, n=48) and non-responders (TRG 3-5, n=74). Three patients were excluded because of insufficient data. MAIN RESULTS: Among the polymorphic variants examined, the MTHFR 677T-1298A haplotype was, upon univariate analysis, the only variable found associated with tumor regression (P=0.004). Moreover, at multivariate analysis, the MTHFR 677T-1298A haplotype was an

independent predictor of tumor regression. Patients not carrying the MTHFR 677T-1298A haplotype (odds ratio 0.29, 95% confidence interval 0.13-0.64, P=0.002) displayed a higher response rate than patients with the MTHFR 677T-1298A haplotype. CONCLUSIONS: Unlike TS VNTR and SNP polymorphisms, MTHFR 677T-1298A haplotype in genomic DNA has the potential to be a predictive marker of tumor response in rectal cancer patients submitted to preoperative chemoradiotherapy.

Theodoropoulos, G. E., A. C. Lazaris, et al. (2006). "Hypoxia, angiogenesis and apoptosis markers in locally advanced rectal cancer." Int J Colorectal Dis **21**(3): 248-57.

BACKGROUND AND AIMS: Hypoxia-inducible factor 1alpha (HIF-1alpha) is a critical regulatory protein of cellular response to hypoxia. HIF-1alpha triggers the angiogenic process through activation of the vascular endothelial factor (VEGF) gene. The bcl-2 anti-apoptotic and the death promoting p53 genes, regulate the apoptotic cell death. We investigated the relationship between hypoxia, angiogenesis and apoptosis and their prognostic impact in patients with advanced rectal cancer. PATIENTS AND METHODS: The immunohistochemical expression of HIF-1alpha, VEGF, p53 and bcl-2 and the determination of microvessel density (MVD), apoptotic index (AI) were carried out in tumour tissue samples obtained from 92 patients with locally advanced rectal cancer (LARC) (T3,4/N+/-). RESULTS: HIF-1alpha high reactivity and VEGF overexpression were noted in 47.8 and 44.6% of the examined cases, respectively. They significantly correlated with lymph node metastasis (P<0.001) and low rectal location (P=0.016). HIF-1alpha expression was directly correlated with VEGF up-regulation (P<0.001) and MVD (P<0.001). VEGF expression was closely interrelated with MVD (P<0.001). In univariate analysis advanced grade, infiltrative pattern of tumour growth, vascular invasion, positive lymph node status, HIF-1alpha expression and VEGF upregulation were related to decreased disease-free and overall survival. In multivariate analysis, only high HIF-1alpha reactivity and positive lymph node status emerged as independent variables of adverse prognostic significance. CONCLUSION: HIF-1alpha and VEGF may play an important predictive and prognostic role in patients with LARC.

Tian, C., Z. G. Zhou, et al. (2007). "Overexpression of connective tissue growth factor WISP-1 in Chinese primary rectal cancer patients." World J Gastroenterol **13**(28): 3878-82.

AIM: To clarify the expression change of Wnt-induced secreted protein-1 (WISP-1) in human rectal cancer and to determine whether it is correlated with invasion and metastasis of human rectal cancer. METHODS: Eighty-six paired samples of rectal cancer and surgically resected distant normal rectal tissue were collected and allocated into cancer group and control group respectively. WISP-1 mRNA was detected by relative quantitative real-time RT-PCR and WISP-1 protein was examined by immunohistochemical staining. RESULTS: WISP-1 gene overexpression was found in 65% (56/86) primary rectal cancers, 2-30 times that of the level in normal matched rectal tissues (P = 0.001). The mRNA expression level was correlated with Duke's staging, histological differentiation grade and lymph node status. The WISP-1 protein expression was in accordance with mRNA expression level. The positive degree of immunohistochemical staining in the cancer group (1.40 +/- 0.35) was different from that in control group (1.04 +/- 0.08, P < 0.001). Moreover, in cancer group the positive staining degree in high-level mRNA cancers (1.46 +/- 0.37, n = 56) was higher than that in low-level mRNA (1.28 +/- 0.28, n = 30, P = 0.018). CONCLUSION: Aberrant levels of WISP-1 expression may play a role in rectal tumorigenesis. WISP-1 may be used as a specific clinical diagnosis and prognosis marker in rectal cancer.

Tsuji, T., T. Sawai, et al. (2003). "Genetic analysis of radiation-associated rectal cancer." J Gastroenterol **38**(12): 1185-8.

Genetic aberrations in radiation-associated colorectal cancer have not been studied in detail. We analyzed genetic aberrations in five rectal cancers that developed long after radiotherapy had been performed for cervical cancer. Microsatellite instability (MSI) in tumors was examined at five loci: D2S123, D3S966, TP53, DCC, and BAT26. Mutation of simple repeat sequences within the hMSH3, BAX, and transforming growth factor Beta type II receptor (TGFBetaRII) genes was examined by polymerase chain reaction and single-strand conformation polymorphism (PCR-SSCP). Mutation of p53 exons 5-8 was examined by PCR-SSP and direct sequencing. Mutations of the K-ras gene were analyzed by two-step PCR. No MSI was found in tumor specimens at any of the loci examined, and no mutations in the target genes were observed. K- ras mutation was detected in two carcinomas, but not in their irradiated normal mucosa, while p53 mutation was observed in another two carcinomas, but not in their irradiated normal mucosa. Our results suggest that the radiation-associated rectal carcinomas examined in this study did not develop through the mutator phenotype pathway; rather, tumorigenesis was

probably mediated through the multistep carcinogenesis pathway.

Ulrich-Pur, H., B. M. Erovic, et al. (2005). "Changes in Mcl-1 expression in rectal cancer in relation to neo-adjuvant radiotherapy." Wien Klin Wochenschr **117**(4): 136-40.

BACKGROUND: Expression of the antiapoptotic protein myeloid cell leukemia-1 (Mcl-1) may be disordered in malignancies of the rectum. High levels of Mcl-1 may correlate with unfavourable clinical outcome. AIM OF THE STUDY: The aim of the study was to determine the biologic significance and the prognostic value of the protein Mcl-1 in a group of patients with rectal cancer using immunohistochemical staining in archival specimens. PATIENTS AND METHODS: Expression of the Bcl-2 family member Mcl-1 was determined in 23 rectal malignancies. Half of the patients with rectal cancer were treated with preoperative short-term radiation therapy of 25 Gy followed by radical surgery; the other patients were treated just with radical surgery. Differences in Mcl-1 expression between irradiated and non-irradiated rectal cancer cells were analysed immunohistochemically, and Mcl-1 expression was correlated with overall survival. Induction of Mcl-1 expression by irradiation versus control in colorectal cancer cells was detected using Western blot. RESULTS: Mcl-1 was expressed at high levels in 35% of all specimens. Significantly stronger expression was detected in specimens of irradiated rectal cancer compared with non-irradiated tissues (p-value: 0.005). No association was seen between marker expression patterns and clinicopathological data of the respective patients. CONCLUSION: Our findings indicate that irradiated rectal cancer produces significantly higher levels of the antiapoptotic protein Mcl-1 than non-irradiated rectal carcinoma. The data also suggest that the high level of Mcl-1 was induced by the radiotherapy. As Mcl-1 is an antiapoptotic regulator, its over-expression in irradiated rectal cancer could constitute a detrimental development antagonizing the potential benefit of adjuvant radiotherapy. Further evaluation of the correlation between Mcl-1 expression and overall survival seems warranted.

Villafranca, E., Y. Okruzhnov, et al. (2001). "Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer." J Clin Oncol **19**(6): 1779-86.

PURPOSE: Thymidylate synthase (TS) is an important target enzyme for the fluoropyrimidines. TS gene promoter possesses regulatory tandemly repeated (TR) sequences that are polymorphic in humans, depending on ethnic factors. These polymorphisms have been reported to influence TS expression. TS expression levels affect tumor downstaging after preoperative fluoruracil (5-FU)-based chemoradiation. Tumor downstaging correlates with improved local control and disease-free survival. The aim of this study is to correlate TR polymorphisms with downstaging and disease-free survival. PATIENTS AND METHODS: Sixty-five patients with rectal cancer underwent tumor resection after preoperative 5-FU-based chemoradiation. Tumor downstaging was evaluated by comparing the pretreatment T stage with the pathologic stage observed in the surgical specimen. TS polymorphism genotype was determined by polymerase chain reaction amplification of the corresponding TS promoter region, and products of amplification were electrophoresed, obtaining products of 220 bp (2/2), 248 bp (3/3), or both (2/3). The TS polymorphism genotype results were subsequently compared with the downstaging observed and with disease-free survival. RESULTS: Patients who were homozygous for triple TR (3/3) had a lower probability of downstaging than patients who were homozygous with double TR or heterozygous patients (2/2 and 2/3): 22% versus 60% (P =.036; logistic regression). Furthermore, a trend toward improved 3-year disease-free survival was detected in the 2/2 and 2/3 groups, compared with that in the 3/3 group (81% v 41%; P =.17). CONCLUSION: This preliminary study suggests that TS repetitive-sequence polymorphisms are predictive for tumor downstaging. TR sequences in TS promoter may be useful as a novel means of predicting response to preoperative 5-FU-based chemoradiation.

Wagsater, D., A. Hugander, et al. (2004). "Expression of CXCL16 in human rectal cancer." Int J Mol Med **14**(1): 65-9.

Local immunoregulation mediated by infiltration of inflammatory cells into colorectal adenocarcinomas is important for tumour progression. Tumour-associated macrophages and T cells are predominant components of chemokine-guided infiltrate of most colorectal tumours. CXCL16 is a newly discovered CXC chemokine expressed by antigen presenting cells attracting Th1, Tc and NK T cells. In this study, which is the first report on expression of the chemokine CXCL16 in human rectal cancer, CXCL16 gene and protein expression were analysed in cancer and normal adjacent tissue. Immunohistochemistry revealed CXCL16 expression in macrophages in normal tissue. The CXCL16 was found to a very limited extent in tumour-associated macrophages. Western blot analysis showed a suppression of CXCL16 protein in rectal cancer

compared to non-cancer tissue in 83% of the patients (n=23, P=0.003). However, with real-time PCR mRNA was not down-regulated in the cancer compared to normal tissue, which may depend on regulated factor(s) at the level of translation and/or post-translation. The results may reflect one of the immunological mechanisms underlying carcinogenesis.

Watanabe, T., Y. Komuro, et al. (2006). "Prediction of sensitivity of rectal cancer cells in response to preoperative radiotherapy by DNA microarray analysis of gene expression profiles." Cancer Res **66**(7): 3370-4.

Preoperative radiotherapy has been widely used to improve local control of disease and to improve survival in the treatment of rectal cancer. However, the response to radiotherapy differs among individual tumors. Our objective here was to identify a set of discriminating genes that can be used for characterization and prediction of response to radiotherapy in rectal cancer. Fifty-two rectal cancer patients who underwent preoperative radiotherapy were studied. Biopsy specimens were obtained from rectal cancer before preoperative radiotherapy. Response to radiotherapy was determined by histopathologic examination of surgically resected specimens and classified as responders or nonresponders. By determining gene expression profiles using human U95Av2 Gene Chip, we identified 33 novel discriminating genes of which the expression differed significantly between responders and nonresponders. Using this gene set, we were able to establish a new model to predict response to radiotherapy in rectal cancer with an accuracy of 82.4%. The list of discriminating genes included growth factor, apoptosis, cell proliferation, signal transduction, or cell adhesion-related genes. Among 33 discriminating genes, apoptosis inducers (lumican, thrombospondin 2, and galectin-1) showed higher expression in responders whereas apoptosis inhibitors (cyclophilin 40 and glutathione peroxidase) showed higher expression in nonresponders. The present study suggested the possibility that gene expression profiling may be useful in predicting response to radiotherapy to establish an individualized tailored therapy for rectal cancer. Global expression profiles of responders and nonresponders may provide insights into the development of novel therapeutic targets.

Watwe, V., M. Javle, et al. (2005). "Cyclooxygenase-2 (COX-2) levels before and after chemotherapy: a study in rectal cancer." Am J Clin Oncol **28**(6): 560-4.

OBJECTIVES: Induction of cyclooxygenase-2 (COX-2) by inflammatory mediators, oncogenes, and carcinogens has been demonstrated in preclinical models. However, there are limited clinical data regarding COX-2 induction by chemotherapy or radiation. Experimental data suggest cross-talk between the EGFR and COX-2 pathways. The aim of this study was to analyze the expression of COX-2 before and after chemoradiation (CRT) and correlate the same with tumor (T) down-staging and survival. Similar data were obtained for EGFR expression before and after chemoradiation. METHODS: Archival paraffin-embedded tumor specimens from patients undergoing CRT between 1995 and 2001 were analyzed. COX-2 expression was measured by immunohistochemistry (IHC), using the 160112 COX-2 mouse monoclonal antibody. For EGFR, we used mouse monoclonal Ab-10. Standard immunoperoxidase technique was used to detect the avidin- biotin peroxidase complex. Staining in tumor tissue was visually scored and confirmed by an image analyzer (ACIS; ChromaVision Medical Systems, Inc, San Juan Capistrano, CA). RESULTS: Twenty pretreatment biopsy samples from rectal cancer patients and their paired, post-CRT surgical specimens (n = 17) were analyzed. Three cases had no primary tumor after CRT. COX-2 expression was noted in 19 of 20 pretreatment samples and 17 of 17 surgical specimens. EGFR expression was noted in 10 cases pretreatment. Six patients with weakly positive COX-2 expression pretreatment had increased COX-2 expression after CRT, whereas in 1 patient the expression decreased after CRT. No EGFR induction was noted. There was no statistical association between EGFR and COX-2 expression in this data set. Median survival for the entire cohort was 38.9 months. There was no difference in survival between the COX-2 induced and noninduced groups. CONCLUSIONS: COX-2 induction was seen with CRT in this population of rectal cancer patients. Prognostic significance of this induction remains to be defined in a larger cohort.

Weijenberg, M. P., M. Luchtenborg, et al. (2007). "Dietary fat and risk of colon and rectal cancer with aberrant MLH1 expression, APC or KRAS genes." Cancer Causes Control **18**(8): 865-79.

OBJECTIVE: To investigate baseline fat intake and the risk of colon and rectal tumors lacking MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) repair gene expression and harboring mutations in the APC (adenomatous polyposis coli) tumor suppressor gene and in the KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) oncogene. METHODS: After 7.3 years of follow-up of the Netherlands Cohort Study (n = 120,852), adjusted incidence rate ratios (RR) and 95% confidence intervals (CI) were computed, based on 401 colon and 130 rectal cancer patients. RESULTS:

Total, saturated and monounsaturated fat were not associated with the risk of colon or rectal cancer, or different molecular subgroups. There was also no association between polyunsaturated fat and the risk of overall or subgroups of rectal cancer. Linoleic acid, the most abundant polyunsaturated fatty acid in the diet, was associated with increased risk of colon tumors with only a KRAS mutation and no additional truncating APC mutation or lack of MLH1 expression (RR = 1.41, 95% CI 1.18-1.69 for one standard deviation (i.e., 7.5 g/day) increase in intake, p-trend over the quartiles of intake <0.001). Linoleic acid intake was not associated with risk of colon tumors without any of the gene defects, or with tumors harboring aberrations in either MLH1 or APC. CONCLUSION: Linoleic acid intake is associated with colon tumors with an aberrant KRAS gene, but an intact APC gene and MLH1 expression, suggesting a unique etiology of tumors with specific genetic aberrations.

Yang, D., S. Schneider, et al. (2006). "Gene expression levels of epidermal growth factor receptor, survivin, and vascular endothelial growth factor as molecular markers of lymph node involvement in patients with locally advanced rectal cancer." Clin Colorectal Cancer **6**(4): 305-11.

BACKGROUND: Patients diagnosed with locally advanced rectal cancer usually receive surgical resection and adjuvant chemoradiation therapy. Lymph node involvement is an important clinical prognostic factor affecting recurrence and survival. Few studies have explored molecular markers associated with lymph node involvement of rectal cancer. PATIENTS AND METHODS: Tissue was obtained from 59 patients with locally advanced rectal cancer who were treated with adjuvant chemoradiation therapy. We assessed messenger RNA (mRNA) levels of genes involved in pathways of angiogenesis (vascular endothelial growth factor [VEGF], cyclooxygenase-2), apoptosis (survivin), tumor growth and epidermal growth factor receptor (EGFR), DNA repair (ERCC1, Rad51), and the DNA synthesis in tumor tissue and tumor-adjacent normal tissue from paraffin-embedded samples using laser-capture microdissection methods. RESULTS: Twenty-four patients had no involvement of regional lymph nodes and 35 had lymph node metastases. In univariate analysis, patients with lymph node involvement had higher mRNA levels of VEGF and survivin in tumor tissue and EGFR in tumor-adjacent normal tissue compared with patients with no lymph node involvement ($P < 0.1$; t test). Multivariate analysis using recursive partitioning showed that mRNA levels of EGFR, survivin, and Rad51 are primarily responsible for delineating node positive from node negative. CONCLUSION: Gene expression of VEGF, survivin, and EGFR could be associated with lymph node involvement in patients with locally advanced rectal cancer. Further independent studies of those gene expression levels and lymph node involvement are warranted to better characterize the associations.

Yang, L., Z. G. Zhou, et al. (2006). "Quantitative analysis of PPARdelta mRNA by real-time RT-PCR in 86 rectal cancer tissues." Eur J Surg Oncol **32**(2): 181-5.

AIM: The purpose of this study is to clarify the expression change of PPARdelta gene in human colorectal cancer tissues. METHODS: Applying real-time RT-PCR, we quantified PPARdelta mRNA in a series of 86 tissues from excised primary rectal cancers. In each case, accompanying normal mucosa was collected for comparison. RESULTS: Among the 86 rectal cancer tissues, 48 cases showed PPARdelta overexpression: 39 tumours gave an expression level 1.5-5 times, five tumours 10-20 times, and four tumours more than 20 times relative to normal mucosa. However, the general level of PPARdelta mRNA in rectal cancer tissues is not statistically different from normal mucosa. CONCLUSIONS: The expression of PPARdelta gene in rectal cancers is not statistically different from normal mucosa.

Yoshikawa, R., H. Yanagi, et al. (2002). "Prognostic values of radiation-induced p53 in adjacent normal mucosa and p21WAF1/CIP1 expression in rectal cancer patients." Int J Oncol **21**(6): 1223-8.

DNA damage induces p53-mediated cell cycle arrest in which p21WAF1/CIP1, a cyclin-dependent kinase inhibitor, may play a critical role by being regulated via wild-type p53. Although adjuvant preoperative radiotherapy in rectal carcinoma is generally believed to improve the prognosis, it remains unclear which factors control the response. We investigated the interactions between the underlying mechanisms of cell cycle perturbation in response to radiotherapy, and local recurrence and distant metastasis in patients undergoing radical surgery for rectal carcinoma. A retrospective review was carried out in which 63 cases of Dukes' B or C, well or moderately differentiated rectal carcinomas in the lower two-thirds of the rectum, with or without preoperative radiotherapy, were immunohistochemically analyzed using antibodies to p53 and p21WAF1/CIP1. Induced p53 expression in adjacent normal mucosa, as seen in seven of 35 cases with radiotherapy, and mutually exclusive p21WAF1/CIP1 immunoreactivity, was strongly associated with local recurrence (P=0.0001). Furthermore, high p21WAF1/CIP1 expression was associated with a lack of distant metastasis (P=0.032).

Our data suggest that there are some cases in which p53 overexpression in adjacent normal mucosa induced by radiotherapeutic treatment might heighten the risk of local recurrence, and that p21WAF1/CIP1 induction independent of the status of the p53 gene showing radiosensitivity might lead to a less distant metastasis.

Zhang, R., S. Takahashi, et al. (1998). "p53 gene mutations in rectal cancer associated with schistosomiasis japonica in Chinese patients." Cancer Lett **131**(2): 215-21.

Mutations in p53 tumor suppressor gene were examined in 44 Chinese patients with rectal cancer, including 22 cases with advanced schistosomiasis japonica and 22 cases without schistosomiasis. In schistosomal rectal cancer (SRC), 13 mutations were found in 10 cases, which included 11 base-pair substitutions and two deletions. Of 11 base substitutions, nine were transitions and two were transversions and seven of them were located at CpG dinucleotides. In non-schistosomal rectal cancer (NSRC), 13 mutations were found in nine cases, all of which were base-pair substitutions. Of 13 substitutions, 10 were transitions and three were transversions and three of them were located at CpG dinucleotides. The proportion of base-pair substitutions at CpG dinucleotides was higher in SRC patients than in NSRC patients, although this was not statistically significant (P = 0.054). Point mutation was frequent at codon 248 in SRC. A higher frequency of arginine missense mutations was observed in SRC than in NSRC. These observations suggest that the mutations in SRC are the result of genotoxic agents produced endogenously through the course of schistosomiasis japonica.

Zhang, W., D. J. Park, et al. (2005). "Epidermal growth factor receptor gene polymorphisms predict pelvic recurrence in patients with rectal cancer treated with chemoradiation." Clin Cancer Res **11**(2 Pt 1): 600-5.

An association between epidermal growth factor receptor (EGFR) signaling pathway and response of cancer cells to ionizing radiation has been reported. Recently, a polymorphic variant in the EGFR gene that leads to an arginine-to-lysine substitution in the extracellular domain at codon 497 within subdomain IV of EGFR has been identified. The variant EGFR (HER-1 497K) may lead to attenuation in ligand binding, growth stimulation, tyrosine kinase activation, and induction of proto-oncogenes myc, fos, and jun. A (CA)(n) repeat polymorphism in intron 1 of the EGFR gene that alters EGFR expression in vitro and in vivo has also been described. In the current pilot study, we assessed both polymorphisms in 59 patients with locally advanced rectal cancer treated with adjuvant or neoadjuvant chemoradiation therapy using PCR-RFLP and a 5'-end [gamma-(33)P]ATP-labeled PCR protocol. We tested whether either polymorphism alone or in combination can be associated with local recurrence in the setting of chemoradiation treatment. We found that patients with HER-1 497 Arg/Arg genotype or lower number of CA repeats (both alleles <20) tended to have a higher risk of local recurrence (P = 0.24 and 0.31, respectively). Combined analysis showed the highest risk for local recurrence was seen in patients who possessed both a HER-1 497 Arg allele and <20 CA repeats (P = 0.05, log-rank test). Our data suggest that the HER-1 R497K and EGFR intron 1 (CA)(n) repeat polymorphisms may be potential indicators of radiosensitivity in patients with rectal cancer treated with chemoradiation.

References

1. Agostini, M., L. M. Pasetto, et al. (2008). "Glutathione S-transferase P1 Ile105Val polymorphism is associated with haematological toxicity in elderly rectal cancer patients receiving preoperative chemoradiotherapy." Drugs Aging **25**(6): 531-9.
2. Arbea, L., I. Coma-Canella, et al. (2007). "A case of capecitabine-induced coronary microspasm in a patient with rectal cancer." World J Gastroenterol **13**(14): 2135-7.
3. Atkin, G. K., F. M. Daley, et al. (2006). "The impact of surgically induced ischaemia on protein levels in patients undergoing rectal cancer surgery." Br J Cancer **95**(7): 928-33.
4. Baba, S., H. Ogiwara, et al. (1994). "Extended lymphadenectomy and the quality of life in rectal cancer patients." Int Surg **79**(1): 23-6.
5. Bedrosian, I., G. Giacco, et al. (2006). "Outcome after curative resection for locally recurrent rectal cancer." Dis Colon Rectum **49**(2): 175-82.
6. Bengala, C., S. Bettelli, et al. (2009). "Epidermal growth factor receptor gene copy number, K-ras mutation and pathological response to preoperative cetuximab, 5-FU and radiation therapy in locally advanced rectal cancer." Ann Oncol **20**(3): 469-74.
7. Bongaerts, B. W., A. F. de Goeij, et al. (2006). "Alcohol and the risk of colon and rectal cancer with mutations in the K-ras gene." Alcohol **38**(3): 147-54.
8. Brink, M., M. P. Weijenberg, et al. (2005). "Dietary folate intake and k-ras mutations in sporadic colon and rectal cancer in The Netherlands Cohort Study." Int J Cancer **114**(5): 824-30.
9. Brink, M., M. P. Weijenberg, et al. (2005). "Meat consumption and K-ras mutations in sporadic colon and rectal cancer in The Netherlands Cohort Study." Br J Cancer **92**(7): 1310-20.
10. Cascinu, S., F. Graziano, et al. (2002). "An analysis of p53, BAX and vascular endothelial growth factor expression in node-positive rectal cancer. Relationships with tumour recurrence and event-free survival of patients treated with adjuvant chemoradiation." Br J Cancer **86**(5): 744-9.
11. Chen, Y., K. J. Chang, et al. (2002). "Establishment and characterization of a rectal cancer model in mice: application to cytokine gene therapy." Int J Colorectal Dis **17**(6): 388-95.
12. Daemen, A., O. Gevaert, et al. (2008). "Integrating microarray and proteomics data to predict the response on

cetuximab in patients with rectal cancer." Pac Symp Biocomput: 166-77.
13. de Bruin, E. C., C. J. van de Velde, et al. (2008). "Epithelial human leukocyte antigen-DR expression predicts reduced recurrence rates and prolonged survival in rectal cancer patients." Clin Cancer Res **14**(4): 1073-9.
14. de Heer, P., E. C. de Bruin, et al. (2007). "Caspase-3 activity predicts local recurrence in rectal cancer." Clin Cancer Res **13**(19): 5810-5.
15. de Maat, M. F., C. J. van de Velde, et al. (2008). "Quantitative analysis of methylation of genomic loci in early-stage rectal cancer predicts distant recurrence." J Clin Oncol **26**(14): 2327-35.
16. Debucquoy, A., K. Haustermans, et al. (2009). "Molecular response to cetuximab and efficacy of preoperative cetuximab-based chemoradiation in rectal cancer." J Clin Oncol **27**(17): 2751-7.
17. Elsaleh, H., P. Robbins, et al. (2000). "Can p53 alterations be used to predict tumour response to pre-operative chemo-radiotherapy in locally advanced rectal cancer?" Radiother Oncol **56**(2): 239-44.
18. Esposito, G., S. Pucciarelli, et al. (2001). "P27kip1 expression is associated with tumor response to preoperative chemoradiotherapy in rectal cancer." Ann Surg Oncol **8**(4): 311-8.
19. Fernebro, E., B. Halvarsson, et al. (2002). "Predominance of CIN versus MSI in the development of rectal cancer at young age." BMC Cancer **2**: 25.
20. Figer, A., R. Shtoyerman-Chen, et al. (2001). "Phenotypic characteristics of colo-rectal cancer in I1307K APC germline mutation carriers compared with sporadic cases." Br J Cancer **85**(9): 1368-71.
21. Funke, S., A. Risch, et al. (2009). "Genetic Polymorphisms in Genes Related to Oxidative Stress (GSTP1, GSTM1, GSTT1, CAT, MnSOD, MPO, eNOS) and Survival of Rectal Cancer Patients after Radiotherapy." J Cancer Epidemiol **2009**: 302047.
22. Gassler, N., I. Herr, et al. (2004). "Wnt-signaling and apoptosis after neoadjuvant short-term radiotherapy for rectal cancer." Int J Oncol **25**(6): 1543-9.
23. Gimbel, M. I. and P. B. Paty (2004). "A current perspective on local excision of rectal cancer." Clin Colorectal Cancer **4**(1): 26-35; discussion 36-7.
24. Giralt, J., M. de las Heras, et al. (2005). "The expression of epidermal growth factor receptor results in a worse prognosis for patients with rectal cancer treated with preoperative radiotherapy: a multicenter, retrospective analysis." Radiother Oncol **74**(2): 101-8.
25. Gordon, M. A., J. Gil, et al. (2006). "Genomic profiling associated with recurrence in patients with rectal cancer treated with chemoradiation." Pharmacogenomics **7**(1): 67-88.
26. Gunther, K., T. Brabletz, et al. (1998). "Predictive value of nuclear beta-catenin expression for the occurrence of distant metastases in rectal cancer." Dis Colon Rectum **41**(10): 1256-61.
27. He, Y., L. J. Van't Veer, et al. (2009). "PIK3CA mutations predict local recurrences in rectal cancer patients." Clin Cancer Res **15**(22): 6956-62.
28. Ho-Pun-Cheung, A., C. Bascoul-Mollevi, et al. (2009). "Validation of an appropriate reference gene for normalization of reverse transcription-quantitative polymerase chain reaction data from rectal cancer biopsies." Anal Biochem **388**(2): 348-50.
29. Ho-Pun-Cheung, A., E. Assenat, et al. (2007). "Cyclin D1 gene G870A polymorphism predicts response to neoadjuvant radiotherapy and prognosis in rectal cancer." Int J Radiat Oncol Biol Phys **68**(4): 1094-101.
30. Horisberger, K., P. Erben, et al. (2009). "Topoisomerase I expression correlates to response to neoadjuvant irinotecan-based chemoradiation in rectal cancer." Anticancer Drugs **20**(6): 519-24.
31. Ibi, I., Y. Saito, et al. (1999). "Biological effects of preoperative radiotherapy on metastatic lymph nodes from rectal cancer." Am Surg **65**(5): 427-30.
32. Inoue, Y., K. Tanaka, et al. (2009). "Microdissection is essential for gene expression analysis of irradiated rectal cancer tissues." Oncol Rep **22**(4): 901-6.
33. Jakob, C., D. E. Aust, et al. (2004). "Thymidylate synthase, thymidine phosphorylase, dihydropyrimidine dehydrogenase expression, and histological tumour regression after 5-FU-based neo-adjuvant chemoradiotherapy in rectal cancer." J Pathol **204**(5): 562-8.
34. Jakob, C., T. Liersch, et al. (2005). "Immunohistochemical analysis of thymidylate synthase, thymidine phosphorylase, and dihydropyrimidine dehydrogenase in rectal cancer (cUICC II/III): correlation with histopathologic tumor regression after 5-fluorouracil-based long-term neoadjuvant chemoradiotherapy." Am J Surg Pathol **29**(10): 1304-9.
35. Jakob, C., T. Liersch, et al. (2006). "Prognostic value of histologic tumor regression, thymidylate synthase, thymidine phosphorylase, and dihydropyrimidine dehydrogenase in rectal cancer UICC Stage II/III after neoadjuvant chemoradiotherapy." Am J Surg Pathol **30**(9): 1169-74.
36. Jakob, C., T. Liersch, et al. (2008). "Predictive value of Ki67 and p53 in locally advanced rectal cancer: correlation with thymidylate synthase and histopathological tumor regression after neoadjuvant 5-FU-based chemoradiotherapy." World J Gastroenterol **14**(7): 1060-6.
37. Jiang, Q., K. Chen, et al. (2005). "Diets, polymorphisms of methylenetetrahydrofolate reductase, and the susceptibility of colon cancer and rectal cancer." Cancer Detect Prev **29**(2): 146-54.
38. Kandioler, D., R. Zwrtek, et al. (2002). "TP53 genotype but not p53 immunohistochemical result predicts response to preoperative short-term radiotherapy in rectal cancer." Ann Surg **235**(4): 493-8.
39. Kapiteijn, E., G. J. Liefers, et al. (2001). "Mechanisms of oncogenesis in colon versus rectal cancer." J Pathol **195**(2): 171-8.
40. Kim, H. R., Y. J. Kim, et al. (2000). "Change of telomerase activity in rectal cancer with chemoradiation therapy." J Korean Med Sci **15**(2): 167-72.
41. Kim, I. J., S. B. Lim, et al. (2007). "Microarray gene expression profiling for predicting complete response to preoperative chemoradiotherapy in patients with advanced rectal cancer." Dis Colon Rectum **50**(9): 1342-53.
42. Kobayashi, H., Y. Hashiguchi, et al. (2007). "Absence of cyclooxygenase-2 protein expression is a predictor of tumor regression in rectal cancer treated with preoperative short-term chemoradiotherapy." Dis Colon Rectum **50**(9): 1354-62.
43. Krishnamurthi, S. S., Y. Seo, et al. (2007). "Adjuvant therapy for rectal cancer." Clin Colon Rectal Surg **20**(3): 167-81.
44. Kristensen, A. T., J. Bjorheim, et al. (2004). "DNA variants in the ATM gene are not associated with sporadic rectal cancer in a Norwegian population-based study." Int J Colorectal Dis **19**(1): 49-54.
45. Kristensen, A. T., J. N. Wiig, et al. (2008). "Molecular detection (k-ras) of exfoliated tumour cells in the pelvis is a prognostic factor after resection of rectal cancer?" BMC Cancer **8**: 213.
46. Kumar, A., H. Collins, et al. (2002). "Effect of preoperative radiotherapy on matrilysin gene expression in rectal cancer." Eur J Cancer **38**(4): 505-10.
47. Li, M., J. Y. Li, et al. (2007). "Colorectal cancer or colon and rectal cancer? Clinicopathological comparison between colonic and rectal carcinomas." Oncology **73**(1-2): 52-7.
48. Li, S., B. Yu, et al. (2005). "Combined liposome-mediated cytosine deaminase gene therapy with radiation in killing

rectal cancer cells and xenografts in athymic mice." Clin Cancer Res **11**(9): 3574-8.
49. Liang, J. T., Y. M. Cheng, et al. (1999). "Reappraisal of K-ras and p53 gene mutations in the recurrence of Dukes' B2 rectal cancer after curative resection." Hepatogastroenterology **46**(26): 830-7.
50. Liersch, T., C. Langer, et al. (2006). "Lymph node status and TS gene expression are prognostic markers in stage II/III rectal cancer after neoadjuvant fluorouracil-based chemoradiotherapy." J Clin Oncol **24**(25): 4062-8.
51. Liersch, T., M. Grade, et al. (2009). "Preoperative chemoradiotherapy in locally advanced rectal cancer: correlation of a gene expression-based response signature with recurrence." Cancer Genet Cytogenet **190**(2): 57-65.
52. Lin, L. C., H. H. Lee, et al. (2006). "p53 and p27 as predictors of clinical outcome for rectal-cancer patients receiving neoadjuvant therapy." Surg Oncol **15**(4): 211-6.
53. Liu, H. Y., B. Zhou, et al. (2007). "Association of E1AF mRNA expression with tumor progression and matrilysin in human rectal cancer." Oncology **73**(5-6): 384-8.
54. Loof, J., D. Pfeifer, et al. (2009). "Significance of an exon 2 G4C14-to-A4T14 polymorphism in the p73 gene on survival in rectal cancer patients with or without preoperative radiotherapy." Radiother Oncol **92**(2): 215-20.
55. Lopez-Crapez, E., F. Bibeau, et al. (2005). "p53 status and response to radiotherapy in rectal cancer: a prospective multilevel analysis." Br J Cancer **92**(12): 2114-21.
56. Luchtenborg, M., M. P. Weijenberg, et al. (2005). "Meat and fish consumption, APC gene mutations and hMLH1 expression in colon and rectal cancer: a prospective cohort study (The Netherlands)." Cancer Causes Control **16**(9): 1041-54.
57. Luna-Perez, P., J. Segura, et al. (2000). "Specific c-K-ras gene mutations as a tumor-response marker in locally advanced rectal cancer treated with preoperative chemoradiotherapy." Ann Surg Oncol **7**(10): 727-31.
58. Lynch, H. T., G. S. Schuelke, et al. (1984). "Genetics of rectal cancer." Bull Cancer **71**(1): 1-15.
59. Marquardt, F., F. Rodel, et al. (2009). "Molecular targeted treatment and radiation therapy for rectal cancer." Strahlenther Onkol **185**(6): 371-8.
60. Matsuo, K., N. Hamajima, et al. (2002). "Aldehyde dehydrogenase 2 (ALDH2) genotype affects rectal cancer susceptibility due to alcohol consumption." J Epidemiol **12**(2): 70-6.
61. Matsuo, S., S. Eguchi, et al. (2001). "Attenuated familial adenomatous polyposis associated with advanced rectal cancer in a 16-year-old boy: report of a case." Surg Today **31**(11): 1020-3.
62. McDowell, D. T., F. M. Smith, et al. (2009). "Increased spontaneous apoptosis, but not survivin expression, is associated with histomorphologic response to neoadjuvant chemoradiation in rectal cancer." Int J Colorectal Dis **24**(11): 1261-9.
63. Meng, W. J., L. Wang, et al. (2007). "Novel mutations and sequence variants in exons 3-9 of human T cell factor-4 gene in sporadic rectal cancer patients stratified by microsatellite instability." World J Gastroenterol **13**(27): 3747-51.
64. Menko, F. H., G. L. Kaspers, et al. (2004). "A homozygous MSH6 mutation in a child with cafe-au-lait spots, oligodendroglioma and rectal cancer." Fam Cancer **3**(2): 123-7.
65. Milosavljevic, T. (1998). "Rectal cancer--from genetics to the therapy." Acta Chir Iugosl **45**(2 Suppl): 23-7.
66. Mohiuddin, M. and M. M. Ahmed (1997). "Critical issues in the evolving management of rectal cancer." Semin Oncol **24**(6): 732-44.
67. Moore, H. G., J. Shia, et al. (2004). "Expression of p27 in residual rectal cancer after preoperative chemoradiation predicts long-term outcome." Ann Surg Oncol **11**(11): 955-61.
68. Murtaugh, M. A., C. Sweeney, et al. (2006). "Vitamin D receptor gene polymorphisms, dietary promotion of insulin resistance, and colon and rectal cancer." Nutr Cancer **55**(1): 35-43.
69. Murtaugh, M. A., K. Curtin, et al. (2007). "Dietary intake of folate and co-factors in folate metabolism, MTHFR polymorphisms, and reduced rectal cancer." Cancer Causes Control **18**(2): 153-63.
70. Nilbert, M. and E. Fernebro (2000). "Lack of activating c-SRC mutations at codon 531 in rectal cancer." Cancer Genet Cytogenet **121**(1): 94-5.
71. Nilbert, M. and E. Rambech (2001). "Beta-catenin activation through mutation is rare in rectal cancer." Cancer Genet Cytogenet **128**(1): 43-5.
72. Nilbert, M., M. Planck, et al. (1999). "Microsatellite instability is rare in rectal carcinomas and signifies hereditary cancer." Eur J Cancer **35**(6): 942-5.
73. Nitta, N., M. Ochiai, et al. (1987). "Amino-acid substitution at codon 13 of the N-ras oncogene in rectal cancer in a Japanese patient." Jpn J Cancer Res **78**(1): 21-6.
74. Ojima, E., Y. Inoue, et al. (2007). "Effectiveness of gene expression profiling for response prediction of rectal cancer to preoperative radiotherapy." J Gastroenterol **42**(9): 730-6.
75. Okoshi, K., S. Nagayama, et al. (2009). "A case report of pathologically complete response of a huge rectal cancer after systemic chemotherapy with mFOLFOX6." Jpn J Clin Oncol **39**(8): 528-33.
76. Pan, Z. Z., D. S. Wan, et al. (2004). "Co-mutation of p53, K-ras genes and accumulation of p53 protein and its correlation to clinicopathological features in rectal cancer." World J Gastroenterol **10**(24): 3688-90.
77. Peng, J. J., S. J. Cai, et al. (2007). "Predicting prognosis of rectal cancer patients with total mesorectal excision using molecular markers." World J Gastroenterol **13**(21): 3009-15.
78. Peng, J., J. J. Lu, et al. (2008). "Prediction of treatment outcome by CD44v6 after total mesorectal excision in locally advanced rectal cancer." Cancer J **14**(1): 54-61.
79. Pufulete, M., P. W. Emery, et al. (2003). "Folate, DNA methylation and colo-rectal cancer." Proc Nutr Soc **62**(2): 437-45.
80. Reerink, O., A. Karrenbeld, et al. (2004). "Molecular prognostic factors in locally irresectable rectal cancer treated preoperatively by chemo-radiotherapy." Anticancer Res **24**(2C): 1217-21.
81. Reymond, M. A., O. Dworak, et al. (1998). "DCC protein as a predictor of distant metastases after curative surgery for rectal cancer." Dis Colon Rectum **41**(6): 755-60.
82. Rimkus, C., J. Friederichs, et al. (2008). "Microarray-based prediction of tumor response to neoadjuvant radiochemotherapy of patients with locally advanced rectal cancer." Clin Gastroenterol Hepatol **6**(1): 53-61.
83. Rodel, F., J. Hoffmann, et al. (2002). "High survivin expression is associated with reduced apoptosis in rectal cancer and may predict disease-free survival after preoperative radiochemotherapy and surgical resection." Strahlenther Onkol **178**(8): 426-35.
84. Rogers, C. G., M. L. Gonzalgo, et al. (2006). "High concordance of gene methylation in post-digital rectal examination and post-biopsy urine samples for prostate cancer detection." J Urol **176**(5): 2280-4.
85. Rooney, P. S., P. A. Clarke, et al. (1994). "DNA content of rectal mucosa and rectal mucosal proliferation in individuals at high risk of colorectal cancer." Eur J Cancer Prev **3**(1): 57-61.
86. Saigusa, S., K. Tanaka, et al. (2009). "Correlation of CD133, OCT4, and SOX2 in rectal cancer and their association with distant recurrence after chemoradiotherapy." Ann Surg Oncol **16**(12): 3488-98.

87. Sasajima, K., Y. Yamanaka, et al. (1993). "Multiple polyps of esophagus, stomach, colon, and rectum accompanying rectal cancer in a patient with constitutional chromosomal inversion." Cancer **71**(3): 672-6.
88. Saw, R. P., M. Morgan, et al. (2003). "p53, deleted in colorectal cancer gene, and thymidylate synthase as predictors of histopathologic response and survival in low, locally advanced rectal cancer treated with preoperative adjuvant therapy." Dis Colon Rectum **46**(2): 192-202.
89. Schneider, S., D. J. Park, et al. (2006). "Gene expression in tumor-adjacent normal tissue is associated with recurrence in patients with rectal cancer treated with adjuvant chemoradiation." Pharmacogenet Genomics **16**(8): 555-63.
90. Slattery, M. L., C. Sweeney, et al. (2005). "Associations between apoE genotype and colon and rectal cancer." Carcinogenesis **26**(8): 1422-9.
91. Slattery, M. L., C. Sweeney, et al. (2005). "Associations between ERalpha, ERbeta, and AR genotypes and colon and rectal cancer." Cancer Epidemiol Biomarkers Prev **14**(12): 2936-42.
92. Slattery, M. L., C. Sweeney, et al. (2006). "Associations between vitamin D, vitamin D receptor gene and the androgen receptor gene with colon and rectal cancer." Int J Cancer **118**(12): 3140-6.
93. Slattery, M. L., K. Curtin, et al. (2006). "PPARgamma and colon and rectal cancer: associations with specific tumor mutations, aspirin, ibuprofen and insulin-related genes (United States)." Cancer Causes Control **17**(3): 239-49.
94. Slattery, M. L., M. Murtaugh, et al. (2005). "Energy balance, insulin-related genes and risk of colon and rectal cancer." Int J Cancer **115**(1): 148-54.
95. Slattery, M. L., R. K. Wolff, et al. (2007). "IL6 genotypes and colon and rectal cancer." Cancer Causes Control **18**(10): 1095-105.
96. Slattery, M. L., R. K. Wolff, et al. (2009). "Tumor markers and rectal cancer: support for an inflammation-related pathway." Int J Cancer **125**(7): 1698-704.
97. Slattery, M. L., S. Edwards, et al. (2003). "Associations between smoking, passive smoking, GSTM-1, NAT2, and rectal cancer." Cancer Epidemiol Biomarkers Prev **12**(9): 882-9.
98. Slattery, M. L., W. Samowtiz, et al. (2004). "CYP1A1, cigarette smoking, and colon and rectal cancer." Am J Epidemiol **160**(9): 842-52.
99. Smith, F. M., J. V. Reynolds, et al. (2006). "Pathological and molecular predictors of the response of rectal cancer to neoadjuvant radiochemotherapy." Eur J Surg Oncol **32**(1): 55-64.
100. Speake, W. J., R. A. Dean, et al. (2005). "Radiation induced MMP expression from rectal cancer is short lived but contributes to in vitro invasion." Eur J Surg Oncol **31**(8): 869-74.
101. Speer, G., O. Dworak, et al. (2000). "Vitamin D receptor gene BsmI polymorphism correlates with erbB-2/HER-2 expression in human rectal cancer." Oncology **58**(3): 242-7.
102. Spindler, K. L., J. N. Nielsen, et al. (2006). "Prediction of response to chemoradiation in rectal cancer by a gene polymorphism in the epidermal growth factor receptor promoter region." Int J Radiat Oncol Biol Phys **66**(2): 500-4.
103. Stein, U., B. Rau, et al. (1999). "Hyperthermia for treatment of rectal cancer: evaluation for induction of multidrug resistance gene (mdr1) expression." Int J Cancer **80**(1): 5-12.
104. Stoehlmacher, J., E. Goekkurt, et al. (2008). "Thymidylate synthase genotypes and tumour regression in stage II/III rectal cancer patients after neoadjuvant fluorouracil-based chemoradiation." Cancer Lett **272**(2): 221-5.
105. Szeliga, J., Z. Sondka, et al. (2008). "NOD2/CARD15 polymorphism in patients with rectal cancer." Med Sci Monit **14**(9): CR480-4.
106. Terrazzino, S., M. Agostini, et al. (2006). "A haplotype of the methylenetetrahydrofolate reductase gene predicts poor tumor response in rectal cancer patients receiving preoperative chemoradiation." Pharmacogenet Genomics **16**(11): 817-24.
107. Theodoropoulos, G. E., A. C. Lazaris, et al. (2006). "Hypoxia, angiogenesis and apoptosis markers in locally advanced rectal cancer." Int J Colorectal Dis **21**(3): 248-57.
108. Tian, C., Z. G. Zhou, et al. (2007). "Overexpression of connective tissue growth factor WISP-1 in Chinese primary rectal cancer patients." World J Gastroenterol **13**(28): 3878-82.
109. Tsuji, T., T. Sawai, et al. (2003). "Genetic analysis of radiation-associated rectal cancer." J Gastroenterol **38**(12): 1185-8.
110. Ulrich-Pur, H., B. M. Erovic, et al. (2005). "Changes in Mcl-1 expression in rectal cancer in relation to neo-adjuvant radiotherapy." Wien Klin Wochenschr **117**(4): 136-40.
111. Villafranca, E., Y. Okruzhnov, et al. (2001). "Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer." J Clin Oncol **19**(6): 1779-86.
112. Wagsater, D., A. Hugander, et al. (2004). "Expression of CXCL16 in human rectal cancer." Int J Mol Med **14**(1): 65-9.
113. Watanabe, T., Y. Komuro, et al. (2006). "Prediction of sensitivity of rectal cancer cells in response to preoperative radiotherapy by DNA microarray analysis of gene expression profiles." Cancer Res **66**(7): 3370-4.
114. Watwe, V., M. Javle, et al. (2005). "Cyclooxygenase-2 (COX-2) levels before and after chemotherapy: a study in rectal cancer." Am J Clin Oncol **28**(6): 560-4.
115. Weijenberg, M. P., M. Luchtenborg, et al. (2007). "Dietary fat and risk of colon and rectal cancer with aberrant MLH1 expression, APC or KRAS genes." Cancer Causes Control **18**(8): 865-79.
116. Yang, D., S. Schneider, et al. (2006). "Gene expression levels of epidermal growth factor receptor, survivin, and vascular endothelial growth factor as molecular markers of lymph node involvement in patients with locally advanced rectal cancer." Clin Colorectal Cancer **6**(4): 305-11.
117. Yang, L., Z. G. Zhou, et al. (2006). "Quantitative analysis of PPARdelta mRNA by real-time RT-PCR in 86 rectal cancer tissues." Eur J Surg Oncol **32**(2): 181-5.
118. Yoshikawa, R., H. Yanagi, et al. (2002). "Prognostic values of radiation-induced p53 in adjacent normal mucosa and p21WAF1/CIP1 expression in rectal cancer patients." Int J Oncol **21**(6): 1223-8.
119. Zhang, R., S. Takahashi, et al. (1998). "p53 gene mutations in rectal cancer associated with schistosomiasis japonica in Chinese patients." Cancer Lett **131**(2): 215-21.
120. Zhang, W., D. J. Park, et al. (2005). "Epidermal growth factor receptor gene polymorphisms predict pelvic recurrence in patients with rectal cancer treated with chemoradiation." Clin Cancer Res **11**(2 Pt 1): 600-5.
121. PubMed (2013). http://www.ncbi.nlm.nih.gov/pubmed.
122. Cancer. Wikipedia. (2013) http://en.wikipedia.org/wiki/Cancer.

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