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Analyzing miRNAs in ductal adenocarcinomas of the pancreas: which method to choose?

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Background: MicroRNAs (miRNAs) have gained attention as an epigenetic component involved in the development of pancreatic ductal adenocarcinoma (PDAC). Therefore, miRNA expression was determined using different and partially redundant methods to correlate these methods for optimization of miRNA analysis in PDAC.
Methods: For 16 human PDAC cell lines with different metastatic potential miRNA expression was analyzed by miRNA microarray and Taqman Low Density Arrays (TLDA). Single tube quantitative RT-PCR was used to validate these results. The data from different methods were statistically evaluated and tested for intermethodic consistence and reliability of the results. Finally, the miRNA expression status and the cell lines' ability to metastasize were correlated.
Results: Comparing low and high metastatic cells, miRNA-microarrays identified fewer differentially expressed and only upregulated miRNAs (n = 27; 27 upregulated) compared to TLDAs (n = 54; 19 up- and 35 downregulated). Evaluating miRNAs that target tumor suppressor genes, expression of all single tube qRT-PCR validated miRNAs was detected to be significantly altered in TLDA analysis (100%). MiRNA microarrays detected only 25% of qRT-PCR validated miRNAs. Results from TLDA analysis correlated well with data from qRT-PCR and presented $\Delta\Delta$Ct values from 3.5 ± 1.86 (range: 0.8−5.62) compared to 3.74 ± 1.86 (range: 0.78−5.95) in qRT-PCR.
Conclusion: In PDAC notable differences comparing data obtained from different screening methods were found. While TLDA and qRT-PCR correlated well in quantity and quality of measured miRNAs, several tumor suppressor gene targeting and downregulated miRNAs were not detected by miRNA-microarrays. This heterogeneity shows that care must be exercised when comparing results from different methods in PDAC.

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Analysis of KIT and PDGFR-a mutations and microsatellite DNA alterations in gastrointestinal stromal tumours

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Background: Activating mutations in KIT and PDGFR-α tyrosine kinases are central to the pathogenesis of gastrointestinal stromal tumors (GISTs) and are associated with different clinical behaviour. GISTs are highly genetically instable and genetic alterations are proposed to have biologic and prognostic value in their development. Aim of the study was to identify *KIT* and *PDGFRA* mutations and additional chromosomal aberrations in GISTs with various types of *KIT* and *PDGFRA* mutations.
Material and Methods: 141 GISTs were tested for *KIT* (exons 9, 11, 13, 17) and *PDGFRA* (exons 12, 14, 18) mutations by direct sequencing of PCR products. Most of DNA samples were isolated from paraffin-embedded tissues. DNA from 45 GISTs were screened for loss of heterozygosity (LOH) in 11 microsatellite loci on 1p, 9p, 14q, 15q and 22q.
Results: 115 (81%) GISTs had *KIT* mutations, of them 96 (68%) harbored mutations in KIT exon 11. Mutations in *KIT* exons 9, 13 and 17 were found in 15, 2, and 2 GIST samples respectively. 16 (11%) GISTs had mutations in *PDGFRA* exon 18, of them five tumors had mutation D842V. Ten GISTs (7%) revealed wild type *KIT* and *PDGFRA*, among them gastric GIST with Carney's triad. 87% of 45 GISTs had LOH of at least one locus and 67% had LOH of two or more loci. LOH frequencies varied from 54% on 14q to 21% on 1p. LOH on 14q was more frequent in gastric GISTs, while LOH on other loci was higher in the intestinal tumors. Analysis of LOH in GISTs with mutations of *KIT* exon 11 revealed the significant increase in LOH on 22q in GISTs with deletions, while LOH on 14q was significantly more frequent in less aggressive GISTs with point mutations. There was no difference in LOH frequency on 14q between primary tumors and GISTs with metastases, but statistically significant difference between them was shown for 15q and 22q. GIST samples with duplications in *KIT* exon 11 or with *PDGFRA* mutations as well as with wild type *KIT* and *PDGFRA* appeared to be much more genetically stable, then GISTs with mutations in KIT exon 11.
Conclusions: Vast majority of GISTs demonstrated various mutations of *KIT* or *PDGFRA* and LOH of multiple chromosome loci. Specific types of mutations with a certain prognostic value in GIST were associated with allelic deletions on different chromosomes. Frequency of specific allelic losses correlated with the higher risk of tumor progression. Our findings support the increasing prognostic significance of molecular analysis of GISTs.

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Myc-dependent regulation and prgonostic role of Myc stabilizing protein, CIP2A, in human gastric cancer

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Background: Cancerous Inhibitor of Protein Phosphatase 2A (CIP2A) is a recently identified human oncoprotein overexpressed in head and neck squamous cell carcinoma (HNSCC) and in colon cancer. Importantly, it promotes malignant cell growth, cellular transformation and c-Myc protein expression (Junttila et al, 130, *Cell 2007*). However, the mechanisms by which CIP2A expression is induced in human malignancies have not been studied thus far.
Methods: The effects of c-Myc and CIP2A on each other's expression, and on cell proliferation, were investigated in several gastric cancer cell lines using small interfering RNAs (to CIP2A and to c-Myc) and immunoblotting. In addition, to evaluate the role of c-Myc in CIP2A regulation, an inhibitor of c-Myc – Max heterodimerization, 10058-F4, and an inducible MycER models were used. Tissue microarrays consisting of 223 gastric adenocarcinoma specimens were evaluated for the presence of CIP2A using immunohistochemistry and compared to patient survival data using Kaplan-Meier curves and two-sided statistical tests.
Results: This study (Khanna et al, *JNCI in Press*) identifies c-Myc as the first positive regulator of CIP2A expression. Depletion of c-Myc reduces CIP2A expression levels (mRNA and protein) and c-Myc activation results in increased CIP2A mRNA expression levels. CIP2A also promotes c-Myc's stability in gastric cancer cells. Furthermore, proliferation of gastric cancer cells is dependent on both CIP2A and c-Myc. Importantly, 10058-F4, inhibits CIP2A expression (mRNA and protein). Additionally, CIP2A and c-Myc immunopositivity associates in gastric cancer specimens (P = 0.021). Furthermore, CIP2A immunopositivity associates with poor prognosis in certain subgroups of gastric cancer patients i.e. those with small tumors ($\leqslant$5 cm, P = 0.001), advanced disease (pT3−4, P = 0.044), and p53 immunopositive carcinomas (P = 0.017). Importantly, depletion of CIP2A in gastric carcinoma cells (AGS, MKN-28, and KATO-III) inhibits their anchorage-independent growth.
Conclusions: This study reveals a novel positive feedback mechanism between CIP2A and c-Myc, wherein they promote each other's expression and gastric cancer cell proliferation. Additionally, CIP2A immunopositivity is a predictor of survival for some subgroups of gastric cancer patients and inhibition of CIP2A could be a viable therapeutic approach in gastric cancer patients with CIP2A and c-Myc positive tumors.

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Bag-1: a novel prognostic marker for adenocarcinoma of the pancreatic head

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Background: Pancreatic cancer is one of the most lethal forms of cancer with an expected 5-year survival of 5%. New prognostic markers may help to identify those who are most likely to benefit from aggressive surgical treatment and patients who require new treatment modalities, such as neoadjuvant therapy. In addition, markers that display prognostic significance offer the potential to become targets of intervention in themselves. A marker that might be of particular interest is the multifunctional protein Bag-1 (Bcl-2 associated anthanogen-1). Bag-1 has been investigated in multiple forms of cancer, its prognostic role however remains controversial. In the current study we aimed to clarify its role in the outcome of pancreatic cancer.
Patients and Methods: Bag-1 protein expression was studied by immunohistochemistry on original paraffin embedded tissue from 217 patients with microscopic radical resection (R0) of adenocarcinoma of the pancreatic head (n = 102) or periampullary region (n = 115). Expression was assessed for associations with time to recurrence (TTR) and overall survival (OS).
Results: Nuclear immunostaining for Bag-1 was present in approximately 80% of patients. In roughly 40% of patients Bag-1 resided in the cytosol, which was almost exclusively associated with nuclear expression. Presence

of nuclear Bag-1 was significantly associated with longer TTR ($p \leqslant 0.001$) and improved OS ($p = 0.001$) in patients treated for adenocarcinoma of the pancreatic head. Moreover, twenty-four percent and 19% of patients with nuclear Bag-1 were recurrence free and alive respectively 5 years following surgery compared with none of the patients lacking expression. In fact, for this tumor type, nuclear Bag-1 proved the only independent prognostic factor for outcome in multivariate analysis after adjustment for conventional prognostic factors, such as tumor extension, nodal involvement and differentiation. In periampullary tumors however Bag-1 failed to demonstrate an association with outcome. This observation is suggestive for different pathways in both types of pancreatic cancer.
Conclusion: The present study shows that patients treated for adenocarcinoma of the pancreatic head, whose tumors fail to express nuclear Bag-1, are more likely to develop recurrent disease and experience decreased survival than those with tumors expressing this biomarker. Nuclear Bag-1 thus seems to hold promise as a prognostic marker in this type of pancreatic cancer and could provide new leads in therapy.

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Comparison of fluorescence in situ hybridization and dual colour chromogenic in situ hybridization for the assessment of HER2 status on gastric cancer biopsies

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Background: HER2 over-expression and/or amplification are present in 9−38% of gastric or gastro-oesophageal junction (GEJ) cancers and correlate with poor outcome and more aggressive disease. It is well known that immunohistochemistry can give conflictive results, especially on small histological samples. Therefore, it has been recently proposed in breast carcinomas that up-front *in situ* hybridization may be the best option for assessing HER2 status. The aim of this study is to evaluate the concordance between *HER2* gene amplification determined by fluorescence *in situ* hybridization (FISH) and a new dual colour cromogenic *in situ* hybridization (CISH) in a series of gastric cancer patients. The results of a pilot study are presented herein.
Material and Methods: 30 gastric adenocarcinoma diagnosed by either endoscopically or surgically obtained biopsies were selected from our files. Dual colour FISH (Dako, Glostrup) and dual colour CISH (Dako, Glostrup) were performed in each case. Scoring of the FISH and CISH slides was identical, counting *HER*2 and CEN-17 signals from 30 tumour nuclei per case. All cases were evaluated in a blinded manner by 2 different physicians. Finally, the gene to CEN-17 ratio was calculated using the cut-off value of *HER*2/CEN-17 ratio >2 as amplified.
Results: All 30 specimens were analyzed successfully by CISH and FISH. A high concordance was found between FISH and CISH in the assessment of HER2 status. 9 cases were amplified and counted easily with both techniques, showing similar ratios. No Polysomy was detected with any technique in these 30 cases.
Discussion: Given the previous experience with the quality of HER2 testing in breast carcinoma in situ hibridation may be an accurate alternative for HER2 testing in gastric carcinomas. CISH allows for a better concurrent analysis of morphology, which is particularly important when studying small samples. A final report on >100 samples will be available at presentation.

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Exploratory study of the subcutaneous fat gene expression profile in patients with metastatic pancreatic carcinoma treated with standard gemcitabine chemotherapy regimen

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Background: Most clinical trials are designed to assess the antitumor effect of the chemoterapeutic intervention. There are few examples where the endpoint is to assess the biology of the host response to the treatment of the tumor. A large number of patients with pancreatic cancer present features of the cachexia syndrome and specially a marked weight loss. It has been postulated that a "cytokine storm" is the cause of the profound effect that this cancer has on distant tissues. This trial analyzed changes in the subcutaneous fat gene expression profile in relation with the clinical benefit variable with standard gemcitabine (G) treatment.
Methods: Patients with histology confirmed advanced pancreatic cancer, adequate organ function and written informed consent. Eligible pts were intended for a subcutaneous fat biopsy pretreatment and after 7 weeks of gemcitabine 1000 mg/m^2 together with response assessment. Clinical benefit (CB) (pain, analgesic consumption, Karnofsky and weight), QLQ-C30, serum cytokines and tumor markers were evaluated pretreatment, at 4 and 8 weeks. Fat gene expression profile was analyzed using Affimetrix U133Plus2.0 with the corresponding bioinformatic software. Serum cytokines where analyzed with xMAP technology with the Luminex 200 platform.
Results: 16 pts [8 m, 8 f, median age 62 yrs (range 47−72)]. Median weight change −0.75 kg (range −4.5 to 2). Nine pts had pre and post treatment biopsies and 7 only pretreatment. Three pts achieved CB at 8 weeks. Objective responses: 0 CR, 0 PR, 31% SD and 68%PD. Toxicity was similar to the one reported in gemcitabine's label. It was possible to extract quality RNA for microarray from subcutaneous fat use from all samples but 1. The limited number of samples precluded to obtain genes clearly involved in cachexia, however the IL-8 expression ($p = 0.03$) was significantly correlated with CB response either to gene and serum profile.
Conclusions: It is feasible to study prospectively the impact of cancer treatment on different tissue biomarkers and correlated with standard antitumor evaluation system. The reduced number of samples in this exploratory trial precludes producing significant biological conclusions.

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Immune response to gastrin-17 is an independent covariate for survival in colorectal, gastric and pancreatic cancers

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Many gastrointestinal (GI) cancers are sensitive to the mitogenic effects of autocrine/endocrine gastrin-17 (G17). The novel autologous immune stimulator, Polyclonal Antibody Stimulator (PAS), elicits antibodies that neutralize G17, thereby blocking its proliferative activity. Early research suggested clinical benefit for patients who mounted an immune response. We analyzed the data from more than 1200 patients from 5 monotherapy and combination chemotherapy studies in three GI cancers to define the relationship between immune-response and clinical efficacy and determine the dependence of this effect to baseline characteristics related to patient's health status.
PAS immune responders were defined by enzyme-linked immunosorbent assay. Relationships between demographics and baseline disease characteristics and immune response were examined by using a logistic regression analysis; relationships between immune response and survival were analyzed using Cox regression analysis.
In Stage II-IV pancreatic cancer patients, overall median survival (MS) was 111 days; MS was 176 days for immune responders and 63 days for non-responders; patients who received placebo had MS of 83 days ($p = 0.028$, log-rank). Stage IV pancreatic responder patients had higher MS (167 days versus 104 days). Similarly, Stage I-III pancreatic responders had higher MS (179 days versus 146 days in non-responders). For advanced gastric cancer patients who received PAS in combination with cisplatin and 5-FU, overall MS was 265 days. Those considered anti-G17 immune responders had a MS of 303 days compared to 70 days for non-responders ($p < 0.001$, log-rank). Under monotherapeutic conditions in colorectal studies, patients who were considered responders showed better survival (267 days) than non-responders (192 days). In metastatic colorectal cancer patients who had progressed after an irinotecan-based chemotherapy regimen, overall MS was 227 days; MS of PAS responders was 249 days versus 119 days for non-responders ($p < 0.001$, log rank).
Overall, patients who generated antibodies following immunization with PAS (between 57% and 100% of patients receiving PAS) had a significantly prolonged survival rate compared to those who did not. This effect was independent of various covariates that predicted the health status of the patients at baseline. The survival benefit for antibody responders and highly favorable safety profile indicate that PAS has exciting prospects for an improved anti cancer treatment regimen for various GI cancers.

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The emerging role of the novel serum marker GOLPH2 in detecting and monitoring of hepatocellular carcinoma

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In patients with HCC, surveillance strategies during the course of the disease are necessary, especially when testing the efficacy of novel