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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