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Hypothesis: Blood for determination of blood-based biomarkers for detection of colorectal neoplasia is often drawn at the time of colonoscopy. This may introduce biomarker result biases resulting from bowel preparation of the colonoscopy patients.
Objectives: To evaluate the influence of bowel preparation on protein and epigenetic biomarker levels in blood samples from subjects provided diagnostic colonoscopies.
Methods: Subjects ($N=126$) were recruited and blood samples collected before and after bowel preparation (immediately prior to colonoscopy). Concentrations of AFP, CA19-9, CEA, hs-CRP, CyFra21-1, ferritin, galectin-3, and TIMP-1 were determined in plasma using the Abbott ACHITECT® i2000 automated immunoassay platform. The epigenetic biomarkers including histone-modification H4K20m3, nucleosome-bound methylated DNA (5-mc) and total nucleosome levels were determined in serum with the Volition TECAN automated immunoassay platform. The galectin-3 ligand (an aberrantly glycosylated form of haptoglobin produced by neoplastic cells) was determined in serum using both a plate-based ELISA and a micro-bead platform.
Result: Pre-colonoscopy bowel preparation did not influence the levels of plasma AFP, CA19-9, CEA, hs-CRP or TIMP-1 (all p values >0.30), while the preparation had an insignificant reduction on plasma levels of Ferritin ($p=0.07$) and Galectin-3 ($p=0.08$). However, a highly significant decrease in the plasma levels of CyFra21-1 (28.7 %; $p<0.001$) was shown from pre- to post bowel preparation. An association between the decrease in plasma CyFra21-1 levels and morbidity or co-morbidity could not be shown. Bowel preparation did not influence the levels of nucleosomes (all p-values >0.50) or galectin-3 ligand levels (p-values >0.80).
Conclusions: The present results demonstrated that pre-colonoscopy osmotic bowel preparation (Moviprep®) had a significant influence on the levels of plasma CyFra21-1, which decreased by 28.7 %, but did not affect levels of eight other proteins and three nucleosomes.
Funding Source: N/A

111 Evaluation of Circulating Long Non-Coding RNA H19 and Cell-free Nucleosomes in Plasma of Patients with Gastric Cancer

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Hypothesis: Gastric cancer (GC) is one of the most common cancers and is thought to develop as a result of environmental factors and the accumulation of genetic and epigenetic alterations. Long non-coding RNAs (lncRNAs) are emerging molecules and have been shown to be involved in cancer development and progression. LncRNA H19 has been associated with GC. In recent years liquid biopsy draws a remarkable attention as a non-invasive procedure and is considered a potential source of the detection of cancer biomarkers. We hypothesize that the H19 in blood circulation could serve a biomarker in GC.
Objectives: In the present study we aimed to assess the impact of H19 and cell-free nucleosomes in GC. Fort this purpose, we measured plasma levels of H19 and nucleosomes at diagnosis of GC patients and compared to non-cancer individuals. We also evaluated these two markers after surgery of GC patients.
Methods: Study participants consisted of patients with GC ($N=38$), individuals with gastric inflammation ($N=24$) and cancer- and inflammation-free control individuals ($N=18$), as verified by gastroscopy. Plasma samples were taken before gastroscopy for all participants and also after surgery (7–12 days) for a subset of GC patients ($N=21$). H19 was measured by real-time PCR while nucleosomes were determined by ELISA-based approach.
Result: Our findings reveal that plasma levels of H19 did not differ between GC (median: 48,57) and control subjects (median: 93,05) (Mann–Whitney test, $p>0,05$) while significantly higher in GC patients compared to inflammatory disease (median: 6,34) ($p=0,006$). Comparing pre- and postoperative levels of H19 in GC, we detected significantly lower levels of H19 expression after tumor removal (median:15,15) (Wilcoxon Signed Ranks test, $p=0,002$). We found no significant differences in nucleosomes levels between study groups (Kruskal-Wallis, $p>0,05$). Nucleosomes levels did also not differ between pre- and postoperative plasma samples in GC patients (t test, $p>0,05$).
Conclusions: In summary, the results of this pilot study suggest that circulating H19 might be a potential marker in GC and warrant further research on this issue.
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112 Glad-PCR Assay of DNA Methylation Markers Associated with Colorectal Cancer

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Hypothesis: Colorectal cancer (CRC) is one of the major malignancies leading to a high incidence and cancer death worldwide. Hypermethylation of the gene regulatory regions is shown for many cancer diseases including CRC. The detection of epigenetic biomarkers is one of the most promising diagnostic and prognostic tools. On the basis of recently discovered unique methyl-directed site-specific DNA endonuclease GlaI we developed a GLAD-PCR assay (GlaI hydrolysis and Ligation Adapter Dependent PCR) allowing quick and inexpensive estimation of 5′-R(5mC)GY-3′ site in a definite position of human genome without bisulfite conversion.
Objectives: It is known that an aberrant DNA methylation in cancer cells is catalyzed by DNA methyltransferases Dnmt3a and Dnmt3b, which predominantly recognize and methylate RCGY sequences with formation of R(5mC)GY sites. Recently, based on a new methyl-directed DNA endonuclease GlaI, we developed a GLAD-PCR assay, which allows to determine R(5mC)GY site in a definite position of the genomic DNA. In this work we have applied GLAD-PCR assay for identification of the methylated RCGY sites in the regulatory regions of some downregulated genes associated with colorectal cancer (CRC). This list includes ADHFE1, ALX4, CNRIP1, EID3, ELMO1, ESR1, FBN1, HLTF, LAMA1, NEUROG1, NGFR, RARB, RXRG, RYR2, SDC2, SEPT9, SFRP2, SOCS3, SOX17, THBD, TMEFF2, UCHL1 and VIM genes. GLAD-

PCR analysis of selected RCGY sites within the regulatory regions of some of these genes demonstrates a good prognostic potential with relatively high sensitivity and specificity of CRC detection in tumor DNA.
Methods: Briefly, GLAD-PCR assay is carried out in three steps. At the first step GlaI cleaves R(5mC)GY sites in genomic DNA. Then the obtained DNA fragments are ligated with the unique oligonucleotide adapter. The last step is a PCR with TaqMan probe and a genomic primer, which are complementary to target DNA fragment, and a so-called hybrid primer, which is complementary to the adapter and partially to the genomic sequence at the cleavage point of the site of interest. As a result, despite of the presence of a huge number of different DNA fragments obtained after GlaI hydrolysis and a ligation step, PCR takes place specifically from the target region of DNA.
Result: GLAD PCR assay of DNA from a colorectal adenocarcinoma cell line SW837 was used to reveal R(5mC)GY sites in selected regions of 23 genes (26 studied regulation regions in total) where sensitivity and specificity have been determined for each site. Tissue samples ($n = 21$) of colorectal adenocarcinomas of varying degree of differentiation and 9 paired normal colon mucosa samples were studied by GLAD-PCR assay. The data revealed a high level of methylation in the selected methylated sites among all tumor showing maximal number of unmethylated RCGY site in HLTF and SDC2 regulation regions. At the same time GLAD PCR assay showed an absence of the methylation of the selected RCGY sites for SDC2, FBN1(3.3), FBN1(3.1), SEPT9, THBD and VIM genes. Surprisingly, we observe methylation of selected RCGY sites in all normal samples in case of TMEFF2 and SOX17 regulation regions and in the most of normal samples in EID3 and ESR1 genes.
Conclusions: In this study we have applied a new GLAD-PCR assay to identify an aberrantly de novo methylated RCGY sites in several down-regulated genes in CRC. The analysis of their methylation status demonstrated a good prognostic potential with relatively high sensitivity and specificity of CRC detection in the tissue DNAs. Moreover, we suppose that these sites may serve as suitable predictive markers for noninvasive blood and stool screening for CRC by GLAD-PCR as well.
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113 Immunotherapy in Endocrine Dependent Metastatic Breast Cancer

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Hypothesis: Hormonetherapy is advised for ER+ metastatic breast cancer patients because of its efficacy concomitant with low toxicity, however in most patients the occurrence of resistance is a not yet well understood hurdle to overcome.
In these patients during clinical benefit from conventional antiestrogens the addition of cycles of sequential immunotherapy could prolong the benefit and delay the arising of acquired hormone resistance.
Objectives: In order to validate this hypothesis in 1992 we started an open exploratory clinical trial and more times during the study we have reported on results and the potential rationale. They both are here updated.
Methods: Forty-two operated breast cancer patients with distant metastases and in clinical benefit during first line antiestrogen salvage therapy were recruited. Beta-interferon (INF beta) 3,000.000 IU im/day 3 days/week, weeks 1–4 and successively recombinant interleukin-2 (IL-2) 3,000.000 IU sc/day 3 days/week, weeks 5–8 were added to antiestrogens until to progression. The immunotherapy cycle lasted 10 weeks and the patient continued antiestrogens only during weeks 9–10, the 11th week being the first week of the successive cycle.
Result: The pattern of laboratory data showed an immune stimulation during clinical benefit and that during clinical benefit from combined hormone-immunotherapy differently than at the progression no relevant immune inhibition occurred. The addition of INFbeta-IL-2 sequence significantly prolonged clinical benefit and overall survival from conventional antiestrogens.
Conclusions: To further confirm these promising results of a more general immunological approach to delay acquired hormone resistance a multicenter prospective phase II trial is going to be launched by the Cancer Center Institute of Tuscany in Italy.
Funding Source: N/A

114 Selected Growth Factors and Different Gastric Neoplasms in Humans

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Hypothesis: Abnormal interactions between various growth factors may be an overlooked mechanism linking the development of different types of gastric neoplasms in humans. Therefore, in this study we i)compared levels of selected growth factors among patients with gastric neoplasms and healthy volunteers; ii)verified the potential associations between systemic levels of examined substances and staging of gastric cancer; and iii)estimated potential diagnostic benefits that can be derived from measurements of systemic levels of growth factors in patients with lesions detected within the gastric tissue.
Objectives: For this study 75 patients with gastric neoplasms (cancer, gastrointestinal stromal tumors, neuroendocrine neoplasms, lymphomas) and 40 healthy volunteers were included.
Methods: Systemic levels of hepatocyte, fibroblast and vascular-endothelial growth factors (HGF, FGF, and VEGF, respectively), as well as, of insulin-like growth factor-1 (IGF-1) were measured using commercially available ELISA tests.
Result: The results obtained demonstrated, that patients with gastric cancer have significantly higher systemic levels of HGF, FGF and VEGF, as well as lower concentrations of IGF-1, in comparison to both healthy volunteers and patients diagnosed with other types of gastric neoplasms ($p < 0.05$ for all). In patients with gastric cancer systemic levels of only HGF significantly correlated with cancer staging evaluated according to the TNM classification. Based on receiver operating characteristic curve analysis systemic levels of examined growth factors did not appear to hold diagnostic potential in confirming or excluding the presence of gastric cancer in humans (area under curve values of 0.48–0.65, and $p > 0.08$ for all).
Conclusions: In patients diagnosed with gastric cancer, an abnormal systemic biochemical balance in multiple growth factors occurs. This phenomenon exists as soon as on the earlies stages of gastric cancer development in humans and seems to be specific only for gastric cancer. Furthermore, systemic levels of examined growth factors do not seem to possess sufficient diagnostic value to be used as independent markers of gastric cancer in humans.
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