Poster abstracts

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FOXO1A and plasma low molecular weight proteins determination: a promising diagnostic approach and biomarker for colorectal tumors

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Background: Familial adenomatous polyposis (FAP) has an autosomal dominant inheritance caused by mutations in the APC (adenomatous polyposis coli) tumor suppressor gene located on chromosome 5. Pathogenetic germline mutations in the APC gene are nonsense and frameshift in about 90% of affected individuals and a genotype-phenotype relationship has been observed. Missense mutations have also been found in a few cases, even if their role in FAP is still unknown. An association between a missense mutation APC I1307K and the risk of sporadic colorectal cancer (CRC) has been reported.
In order to improve the knowledge about the genetic effect of APC I1307K on the phenotype, we tried a new approach using the Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI/MS).
Methods: A non-Jewish lady with attenuated familial adenomatous polyposis (A-FAP) without a family history of tumors and her son a APC mutation I1307K was found. In order to evaluate whether the presence and abundance of the ionic species are related to the presence of cancer or the presence of mutation, comparative analyses of 9 healthy clean colon subjects, 9 subjects with sporadic polyps, 10 patients with stage IV colorectal cancer without polyps, and 2 FAP patients, carriers of a frameshift mutation 2713–2714del15ins5 in APC gene, were evaluated.
The profiles of LMW plasma proteins of samples were obtained with MALDI/MS using a Voyager-DE PRO instrument (Applied Biosystems, Foster City, CA, USA), according to standardized protocols.
Results: Comparative analysis of plasma proteins profiles of the index patient and her healthy son, FAP and sporadic colorectal cancer patients and subject with preneoplastic lesion, showed a characteristic abundance ionic species at m/z 905, whereas it was not present in healthy controls. Two peptides were found from MALDI/MS/MS spectra of m/z 905 belonging to the Kininogen-1 precursor and human Forkhead box protein 01A (FOXO1A). FOXO1A was present in only two subjects carrying I1307K, not in others patients.
Conclusions: Our findings suggest a relationship between m/z 905, FOXO1A and the development and growth of colorectal cancer. FOXO1A determination in the plasma with MALDI/MS might be a promising approach for early detection of colon carcinoma or for the development of targeted therapies.

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Expression of topoisomerase IIa and chromosome 17 instability in intraoperative specimens in NSCLC

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Background: Intraoperative imprints and FNAs offer a fast and accurate method of diagnosis and also a qualitative substrate for immunocytochemical and molecular studies – especially preserved samples by liquid phase cytology methods – because of the high conservation of the nucleus features. Aberrations of chromosome 17 are common in many cancers including NSCLC. Topoisomerase family which includes Topo I (20q11), Topo IIa (17q21), Topo IIb (3p24) is a class of enzymes in the nucleus of all living cells, which affect the topological structure of DNA. Cells die when Topoisomerase is inhibited and for this reason is a target of chemotherapy
Methods: Using Tissue Microarray Technology (Chemicon TMArrayer ATA100), we created a 40 tumours TMA. 40 NSCLC (20 Adenocarcinomas and 20 Squamous carcinomas) and 10 normal lung epithelia (as control group) were obtained and embedded into a single paraffin block (core diameter: 1 mm). Immunohistochemical stain for anti-Topoisomerase IIa (Ki S1-DACO Corp) combined with CISH for the detection of chromosome 17 instability (Chr 17 Centromeric probe, Zymed kit) and specific gene status (Topo IIa Amplification probe Zymed kit) was performed in 2 and 5 μm sections and in intraoperative imprints. Finally using a semi-automated Image Analysis System we evaluated the nuclear features of number and optical density and the number of signals of chromosome 17 centromeres and gene copies per nucleus. Statistical analysis was performed by SPSS version 11.0 software.
Results: A significant proportion of NSCLC showed over expression of the marker (18/40) and CISH application showed Topo IIa amplification (high or low level) or deletion of one allele in 11/18 cases. Co-evaluating chromosome 17 instability we observed that 10/18 only amplification while the last 4/18 displayed deletion. We observed that chromosome 17 instability co-appearing with Topo IIa amplification correlates with low differentiation and poor prognosis ($p < 0.001$).
Conclusions: The results indicate that Topo IIa amplification or deletion is a critical genetic event correlating with biological behaviour in NSCLC and determining chemo sensitivity. Also intraoperative imprints and FNAs appeared to be more accurate at the evaluation of centromeric and specific gene signals because of the nucleus integrity.

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The Aurora kinase inhibitor AZD1152 inhibits cell proliferation, modifies the cell cycle and enhances chemotherapeutic activity in in vitro models

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Background: AZD1152-HQPA is the active moiety of AZD1152, a specific inhibitor of Aurora kinase, selective for Aurora B. Our previous studies showed cell growth inhibition of 40−60% in several cell lines after 1−3 days drug exposure then 1−3 days washout. Cell cycle modulation was seen with a marked increase in cells with 4N DNA, dependent on period of drug exposure (#4359 AACR 2007). These results led us to investigate AZD1152-HQPA action after 1 day drug exposure then 1−4 days washout.
Methods: Colon cancer cell lines, HCT116, Colo205 and HT29, were treated with AZD1152-HQPA 30 and 300nM for 1 day, followed by 0−2 days washout, 3 and 4 days washout for less sensitive lines. Cell number was evaluated at $t = 0$ and after treatment. Percentage of proliferation was determined and cell cycle modulation investigated in HCT116 and Colo205 utilising the regimen previously reported. MiaPaCa-2, a pancreas cancer cell line, was also analysed. In combination studies, Colo205 and MiaPaCa-2 cells were exposed to 1 day AZD1152-HQPA (30 and 300nM) followed by 1 day oxaliplatin (IC50) or 3 days gemcitabine (IC50), respectively.
Results: The sensitivity of colon cancer cell lines to AZD1152-HQPA increased from HT29 to Colo205 to HCT116. The percentage of HCT116 cell proliferation decreased progressively over time with AZD1152-HQPA from 40 to 85%. The decrease in Colo205 was 15−50% while HT29 cells recovered the ability to proliferate during washout. At AZD1152-HQPA 300nM proliferation was drastically reduced. Results obtained with MiaPaCa-2 cells were similar to those with Colo205. Exposure to AZD1152-HQPA also induced extreme modification of cell structure, with a marked increase in size. 24h exposure to AZD1152-HQPA led to a marked dose-dependent increase in cells with 4N DNA. The rate of reversibility increased from HCT116 to Colo205 to HT29. Preliminary evaluation of combined AZD1152-HQPA plus oxaliplatin, in Colo205 and plus gemcitabine, in MiaPaCa-2, confirmed previous data, and showed that activity of the conventional drugs increased when given after AZD1152-HQPA.
Conclusions: AZD1152-HQPA induced significant effects on cells in vitro, with drastic reduction of cell proliferation and cell cycle modification. Preliminary results of combination with conventional chemotherapeutics suggest that AZD1152 may have a role in combination with chemotherapy. AZD1152-HQPA is a trademark of the AstraZeneca group of companies

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The Na+/H+ exchanger regulator factor (NHERF1) is a component of EGF receptor signaling complex and regulates EGF receptor degradation

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Background: The Na+/H+ exchanger regulator factor (NHERF1) is a post-synaptic density 95/disc-large/zona occludens (PDZ) domain-containing protein that recruits membrane receptors/transporters and cytoplasmic signaling proteins into functional complexes. NHERF1 expression is altered in breast cancer and we have demonstrate is an important player in mammary tumor progression and could be validate as a tumor marker (Mol Biol Cell. 2007). Here we report that NHERF1 interacted with epidermal growth factor receptor (EGFR) upon EGF stimulation.
Methods: Transfection of NHERF1 cDNAs: Human mammary cell lines were transiently transfected with wild-type mouse NHERF1 cDNA inserted into the pcDNA vector, using LipoTAXI reagent and the experiments were conducted 48h later. Coimmunoprecipitation: After treatment monolayers were lysed in ice-cold coimmunoprecipitation lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 100 microM Na_3VO_4, and 1 mM NaF, protease inhibitors). 150 μg of total cellular protein was incubated at 4ºC with 1 μg of primary antibody and protein A/G Plus-Agarose overnight. Immunoprecipitates were resuspended in SDS sample buffer, run on 10% SDS-PAGE, analyzed by Western blotting.