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.

Immunofluorescence and Image Analysis: Cells plated on coverslips were fixed with 3.7% ice-cold paraformaldehyde, permeabilized with 0.1% Triton X-100 for 10 min and incubated with primary antibody in 0.1% gelatin and incubated with the Alexa 488 goat anti-mouse IgG1 and 568 goat anti-rabbit IgG. Proteins were detected with a Nikon TE 2000S epifluorescence microscope equipped with a CCD camera by using a Nikon lamp shutter with a mercury lamp for excitation. In colocalization experiments, scanning was conducted with 25−30 optical series from the top to the bottom of the cell with a step size of 0.45 μm. A Z-stack was acquired using the MetaMorph software, and every two-color stack (red and green) acquired separately in black and white (B/W). Each stack is deconvolved using the AutoDeblur 9.1 function of AutoQuant and then merged by transforming the two channels corresponding to red (tetramethylrhodamine B isothiocyanate) and green (fluorescein isothiocyanate) into a single two color stack by using the "RGB merge" command of ImageJ software.
Results: Immunofluorescent staining using anti-EGFR and GFP-NHERF1 indicates that NHERF1 colocalized with EGFR upon EGF stimulation. Functional experiments with truncated and binding groove-mutated PDZ domain constructs demonstrated that NHERF1 regulates these interaction through its PDZ1 domain. Enhancing of the expression of NHERF1 by transfection of wild-type (wt) NHERF1 inhibited ligand-induced degradation of EGFR upon EGF stimulation, suggesting that NHERF1 plays an important role in regulation of EGFR degradation in cells.
Conclusions: Taken together, our studies suggest that NHERF1 senses signal of EGF and regulates ligand-induced degradation of EGFR

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The stability of matrix metalloproteinases and chemokines in blood stored under various conditions

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Background: It is highly important to understand the stability of proteins in blood if they are to be used as biomarkers of disease, particularly when the disease process may affect the stability of the protein of interest. In clinical trials biomarkers are measured using frozen blood samples, whereas clinical tests are conducted on fresh blood samples soon after collection. Rouy et al (2005), along with our observations (unpublished), have suggested that matrix metalloproteinases (MMP) and chemokines in blood may be particularly vulnerable to degradation when samples are frozen. The aim of this study was to evaluate the stability of MMPs and chemokines in serum from colorectal cancer patients and control subjects, by analysing fresh blood samples and aliquots stored at −80ºC or in liquid nitrogen.
Methods: Blood from 10 healthy controls was age and sex matched to pre-operative blood collected from 10 colorectal cancer patients. Immediately after processing the blood, plasma MMP-9 was quantified by ELISA, and MMP-1, -2, -3, -7, -8 was quantified in serum by multiplex assay (R&D Systems). Chemokines CXCL-2, -3, -4, -8 were analysed in serum by multiplex assay (R&D Systems). The serum and plasma was sub-aliquoted and stored at −80ºC and in liquid nitrogen. An aliquot was stored at 4ºC overnight and analysed on the following day. Stored samples were analysed at 1, 30 and 90 days following collection.
Results: Preliminary results show that only MMP-1 in normal and cancer samples significantly decreased in serum when stored at either −80ºC or in liquid nitrogen for up to 1 month ($p = 0.02$) in comparison to freshly analysed serum samples. No such trends are evident for any of the other biomarkers, although the data suggest that where such trends exist, they are more clearly visible in normal samples.
We intend on measuring biomarker levels up to a period of 18 months, at which time we will have sufficient data to be able to estimate the effect of time, storage condition, and whether these effects are different for normal or cancer samples.
Conclusions: Blood samples analysed fresh show similar levels of chemokines and MMPs (with the exception of MMP-1) as bloods that are analysed following storage at either −80ºC or in liquid nitrogen for up to 3 months.

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Gene signature and lymph node metastasis in patients with early stage cervical cancer

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Background: Pelvic lymph node metastases are the main prognostic factor for survival in early stage cervical cancer, yet accurate detection methods before surgery are lacking. In this study we examined whether gene expression profiling can predict the presence of lymph node metastasis in early stage squamous cell cervical cancer before treatment.
Methods: Tumour samples of 35 patients with early stage cervical cancer who underwent radical hysterectomy and pelvic lymph node dissection, 16 with and 19 without lymph node metastasis, were analyzed. We investigated differential expression and prediction of patient status for lymph node positive versus lymph node negative tumours. Classifiers were built by using a multiple validation strategy, enabling the assessment of both classifier accuracy and variability.
Results: Five genes (BANF1, LARP7, SCAMP1, CUEDC1, PEBP1) showed differential expression between tumour samples from patients with and without lymph node metastasis. However, the accuracy of class prediction is only 64.5% with a 95% confidence interval (CI) of 40−90%.
Conclusions: Expression profiling did not provide an accurate classification for lymph node status in early stage cervical cancer. Five genes were identified that may be attractive candidate markers for lymph node metastasis in early stage cervical cancer.

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Gene signature and early stage cervical cancer

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Background: Cervical cancer is caused mainly by infection with a high-risk group of human papilloma viruses (HPV's). However, HPV infection alone is not enough for triggering cervical cancer. Few patients infected with high-risk HPV develop cervical cancer with a long incubation time, suggesting that additional factors or cellular events are required for progression to cervical cancer. In this study we identified genes involved in cervical carcinogenesis.
Methods: Tumour samples of 35 patients with early stage cervical cancer and samples of five normal cervical tissues were analyzed. We investigated differential expression and prediction of patient status for healthy versus cervical cancer tissue. Classifiers were built by using a multiple validation strategy, enabling the assessment of both classifier accuracy and variability.
Results: A total of 9313 probes representing human genes and transcripts were differentially expressed between healthy cervical tissue and early stage cervical cancer tissue with a q-value $\leqslant 0.005$. There is considerable overlap between previous studies and our study (top 200 genes upregulated) in terms of genes differentially expressed between normal cervical tissue and cervical cancer. Biological processes involved in cervical cancer oncogenesis are related to cell cycle, cell division, response to DNA damage stimulus and chromosome segregation. Highly accurate class prediction was obtained for healthy versus early stage cervical cancer tissue, mean accuracy of 99.5% (95% CI of 90–100%).
Conclusions: Expression profiling provides an accurate classification for early stage cervical cancer. A subset of genes involved in cervical cancer was identified.

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HER2-amplified breast carcinomas: molecular characteristic and response to trastuzumab treatment.

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Background: Therapy with trastuzumab of HER2-positive breast carcinomas has been shown to be active in 50% of patients in 4 clinical trials involving more than 10,000 cases. Forest plot of these studies did not identify any factors predictive of response and therefore all patients with HER2-positive breast carcinomas must be treated with trastuzumab even though it is known that halph of these patients will not benefit from this treatment. The identification of predictive factors of response is therefore mandatory. The mechanism underlying the antitumor activity of trastuzumab in vivo is still controversial. Different mechanisms have been proposed to account for its therapeutic effect including down-modulation of HER-2, activation of apoptotic signals, impairment of angiogenesis and interaction with the immune system. Analysis in animal models as well as neo-adiuvant clinical trials suggested that trastuzumab activity may depends on engagement of the Fc receptor, suggesting that Fc-dependent antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity are critical for trastuzumab efficacy.
Methods: To investigate the gene profiling of HER2-positive tumors, we selected 42 HER2-amplified breast carcinomas potentially targets of trastuzumab therapy that were profiled using cDNA microarray technology.
Results: The two groups obtained by unsupervised hierarchical clustering showed different modulation of genes belonging to ECM. One group presented the upmodulation of extracellular matrix (ECM) molecules (collagens, fibronectin, laminins) other ECM structural genes (i.e. fibulins,