the role of inflammation in metastatic progression, which until recently was mainly attributed to genetic changes intrinsic to the cancer cell. Using a mouse model of prostate cancer metastatic progression, the TRAMP mouse, we found that activation and nuclear translocation of IκB kinase α (IKKα) within prostate cancer (CaP) cells in a critical event in metastatogenesis as it is required for repression of the potent metastasis suppressor maspin. Activation of IKKα in CaP cells, however, depends on interaction with inflammatory cells that are recruited into the growing tumors and produce IKKα activating cytokines such as RANK ligand.

To understand how inflammatory cells are recruited into growing tumors to promote metastatic progression we screened carcinoma lines for their ability to produce soluble factors that activate macrophages and induce cytokine production. We identified such factors which activate macrophages through TLR2 to produce TNF-α and other inflammatory cytokines. Most importantly, the ability of carcinoma cells that produce such factors to establish lung and liver metastasis is strongly dependant on TLR2 activation and TNF-α production by host bone-marrow derived cells.

These results strongly support the notion that metastatic progression is highly dependant on dynamic and reciprocal interactions between cancer cells and inflammatory cells, which are recruited into growing tumors to produce pro-metastatic cytokines.

POSTER SESSION

Cell and tumour biology 2

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Inhibition of reactive stroma by platelet derived growth factor receptor (PDGF-R) tyrosine kinase inhibitor reduces growth and lymph node metastasis of human colon carcinoma

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The stroma constitutes a large part of most solid tumors, and the tumor-stroma interaction contributes to tumor growth and progression. Stromal reaction (desmoplasia) is observed in carcinomas but not in non-invasive adenomas. We have previously reported that desmoplastic stromal cells within colon carcinoma express high levels of platelet derived growth factor receptor (PDGF-R), whereas colon cancer cells do not. In this study, we determined whether inhibition of PDGF-R tyrosine kinase signaling by imatinib affects the stromal reaction and inhibits the growth and metastasis of human colon cancer cells growing in the subcutis or cecal wall of nude mice. KM12SM human colon cancer cells were injected into the subcutis (ectopic implantation) or the cecal wall (orthotopic implantation) of nude mice. KM12SM cells were also injected into the spleen of nude mice to produce liver metastases. Groups of mice (n=10) received saline (control), imatinib, the cancer chemotherapeutic irinotecan, or a combination of imatinib and irinotecan. The tumor stroma was then stained with antibodies against alpha smooth muscle actin and collagen I. Four weeks of treatment with imatinib and irinotecan significantly inhibited tumor growth (relative to control or single-agent therapy) in the cecum and liver but not in the subcutis. In the cecum and liver, tumors induced active stromal reaction, whereas in the subcutis, stromal reaction was minimal. Combination therapy completely inhibited lymph node metastasis and tumor cell growth at the abdominal wall wound. Imatinib alone or in combination with irinotecan inhibited phosphorylation of PDGF-R in tumor-associated stromal cells. Combination therapy also significantly decreased stromal reaction and tumor cell proliferation and increased apoptosis in both tumor cells and tumor-associated stromal cells. These data indicate that administration of a PDGF-R tyrosine kinase inhibitor in combination with irinotecan impairs the progressive growth of orthotopically implanted colon cancer cells in nude mice by blocking PDGF-R signaling in tumor-associated stromal cells.

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Identification of bone metastasis markers in prostate cancer

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Background: Presently, bone scintigraphy is the mainstay of diagnosis of bone metastases. Since this technique relies on the osteoblastic reaction, early metastases may sometimes be missed. To identify a candidate biomarker for bone metastases, we analysed serum protein expressions in patients with prostate cancer.

Methods: The study population comprised 10 untreated patients with prostate cancer. Of these, 4 patients had bone metastases (M1) while 6 patients did not (M0); metastasis was confirmed by bone scanning and magnetic resonance imaging. The mean Gleason score was 8.4 (range, 7–9), and the mean pre-treatment PSA level was 146.5 ng/ml (range, 13.8–630 ng/ml). All the patients received androgen deprivation therapy as the initial treatment. Plasma samples were collected before prostate biopsy, and the PSA value in these samples showed a decrease to <0.1 ng/ml after treatment. The samples were analysed by the microflow liquid chromatography/tandem mass spectrometry (μLC-MS/MS) system. All MS/MS data were evaluated quantitatively (differences in protein expressions between the M0 and M1 groups) and qualitatively (protein identification). After aligning the MS/MS data sets with the i-OPAL algorithm, peptide signal intensities between the M0 and M1 groups were compared. The results were assessed statistically with Student's t test. Furthermore, the MASCOT MS/MS ion search program was used for protein identification from amino acid sequences.

Results: The μLC-MS/MS analysis provided approximately 10000 MS/MS spectra for each sample. We tentatively set the peptide score to more than 30 and ranked it as the first criterion for protein identification. Analysis of the pre-treatment plasma samples led to the identification of 31 differentially expressed proteins between the M0 and M1 groups. The signal intensities of 25 proteins were higher in the M1 group than in the M0 group; these proteins included apolipoprotein (APO)-A1, APO-A2, APO-A4, alpha2 macroglobulin, legumain, ceruloplasmin, serine proteinase inhibitor, transferrin and vitamin D-binding protein (DBP). On the one hand, 10 proteins were identified in the post-treatment plasma samples; these proteins did not include alpha2 macroglobulin, legumain and DBP.

Conclusions: These differentially expressed proteins, namely, alpha2 macroglobulin, legumain and DBP, are probably related to bone metabolism and may be useful as biomarkers for bone metastases.

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Role of a soluble form of urokinase plasminogen-activator receptor in the control of human prostate cancer cell growth and invasion

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Introduction: Urokinase-type plasminogen activator (uPA) and its specific membrane receptor (uPAR) control extracellular matrix proteolysis, cell migration, invasion and cell growth in several cancers. The uPAR released from human cancers is detected in blood as soluble uPAR (suPAR). No information is available on the mechanism(s) of action of suPAR on prostate cancer (PCa) cells growth and invasion.

Materials and methods: In order to clarify this issue, we tested the effect of a treatment with the human recombinant suPAR (comprising amino acids I-303) on the proliferation, migration and invasion of DU145 cells, a PCa cell line expressing a potent autocrine uPA-uPAR signalling system.

Results: The results indicate that suPAR significantly inhibits cell growth, promotes apoptosis and decreases both migration and MatrigelTM invasion of DU145 cells. The mechanism of action of suPAR seems to be linked to a decrease of ERK and FAK activation. Cleavage of suPAR by chymotripsin (CsuPAR) reverses these effects. When added to the uPA negative LNCaP cells, suPAR was ineffective; on the contrary, when LNCaP cells were cultured on fibronectin-coated plates in order to stimulate uPA expression, suPAR significantly decreases cell proliferation.

Conclusions: In conclusion, our data suggest that suPAR can function as a potent molecule scavenger for uPA in these human PCa cells characterized by high levels of uPA/uPAR, as in DU145 cells, while it is ineffective in uPA-deficient LNCaP cells. The molecular mechanism(s) through which suPAR participates to the control of PCa progression may possibly correlate with the long-term goal to identify new therapeutic targets aimed at silencing tumour in vivo.

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HDAC2 is overexpressed in pancreatic ductal adenocarcinoma and involved in anti-apoptotic signaling

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Background: Histone deacetylases (HDACs) and acetyl transferases (HATs) are two counteracting enzyme families which affect gene expression through their influence on chromatin conformation. Although it

is known that inhibitors of HDACs (HDACi) induce cell cycle arrest and apoptosis of pancreatic cancer cells, the contribution of the individual HDACs to the carcinogenesis of pancreatic cancer is unknown so far.

Materials and methods: Expression of HDAC2 in pancreatic ductal adenocarcinomas was investigated using immunohistochemistry of tissue microarrays. Function of HDAC2 was analyzed using RNA interference in pancreatic cancer cell lines. Results were reproduced using the selective class I HDACi valproic acid. Proliferation and viability was measured using BrdU and MTT assays, respectively. Apoptosis was analyzed using Hoechst stains and PARP western blots.

Results: Immunohistochemistry and scoring reveals for the first time the overexpression of nuclear HDAC2, especially in undifferentiated pancreatic ductal adenocarcinomas. The knockdown of HDAC2 neither impaired proliferation nor reduced viability of pancreatic cancer cell lines. Instead we observed a sensitization towards death receptor (TRAIL)- and chemotherapy (etoposide)-induced apoptosis. In line, the selective class I HDACi valproic acid sensitizes pancreatic cancer cells towards death receptor (TRAIL)- and chemotherapy (etoposide)-induced apoptosis in a time- and dose-dependent fashion, without change of proliferation and viability.

Conclusions: Taken together, these data suggest a pivotal role of HDAC2 in regulating anti-apoptotic signaling and therapeutic resistance in pancreatic ductal adenocarcinoma. Therefore, targeting HDAC2 could be a promising future approach for the treatment of this dismal disease.

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Kallikrein-related peptidase 6 overexpression promotes non-small cell lung cancer cell proliferation and is associated with poor patient outcome

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Background: The human kallikrein-related peptidases (KLK) are a family of serine proteases that are often aberrantly expressed in common human malignancies and contribute to neoplastic progression through multifaceted roles.

Materials and Methods: We evaluated KLK6 expression in the tumoral and normal adjacent lung tissue of 56 patients with Non-Small Cell Lung Cancer (NSCLC) by real-time RT-PCR and immunohistochemistry. To determine the impact of KLK6 overexpression on the growth of lung cancer cells, we integrated the cDNA encoding the complete sequence of KLK6, through homolog recombination, in a NSCLC line (A549 Flp-In) and determined the growth rate of two independent clones. Progression of the KLK6- and parental cells inside the cell cycle was assessed by flow cytometry following synchronization of cells at the end of the G1 phase with starvation and hydroxyurea treatments. Key regulator proteins of the cell cycle were analyzed by Western blot in synchronized and unsynchronized cells.

Results: We found KLK6 transcript up-regulation in tumor tissues from patients with NSCLC and association of KLK6 status with low patient survival. KLK6 immunoreactivity was restricted to epithelial cells of normal bronchi and detected in most of cancer samples, in which KLK6 signal intensity correlated with well differentiated tumors. Ectopic expression of KLK6 dramatically enhanced NSCLC cell growth. Analysis of cell cycle progression revealed that promotion of cell growth caused by KLK6 results from an acceleration of cell cycle progression through G1/S transition, which was accompanied with a marked increase of cyclin E and repression of p21. In addition, expression of KLK6 in NSCLC cells was associated with an increase of c-Myc that is well-know to promote cell-cycle progression via regulation of cyclin D/E activation and down-regulation of p21.

Conclusion: In conclusion, ectopic expression of KLK6 facilitates cell cycle progression, certainly through alteration of c-Myc and downstream key regulators, and thus promotes cell proliferation. Moreover, KLK6 is overexpressed in NSCLC and associated with poor prognosis. Altogether, those findings suggest that KLK6 might play a central role in NSCLC development and progression.

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Antiproliferative effect of GNRH-III and GNRH-II peptide derivatives on MCF-7, T47-d and HT-29 cells

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The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH-I; <EHWSYGLRPG-NH_2) is the central regulator of reproductive system through the stimulation of the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Some kinds of tumour cells (e.g. breast, prostate, colon) produce GnRH and express its receptors. GnRH-II (<EHWSHGWYPG-NH_2) is also expressed in humans and it has more inhibitory effect to tumour cell replication than GnRH-I. GnRH-III (<EHWSHDWKPG-NH_2) is a weak GnRH agonist. It has antiproliferative effect without significant endocrine effect therefore it could be used as a selective antitumour agent in the cancer therapy. We have found that symmetric dimmer derivatives of GnRH-III were more potent for the inhibition of tumour growth in vitro and in vivo, but their endocrine effect was even lower than the monomer had.

Our aim was to synthesise new asymmetric dimer derivatives of GnRH-I or GnRH-II and GnRH-III which increased receptor binding activity and can enhance the antiproliferative effect of GnRH-III (20%).

GnRH-derivatives were synthesized by solid phase synthesis using mixed Fmoc and Boc strategies. Different natural GnRH-derivatives were coupled to directly or through a spacer each other forming asymmetric dimers. The following dimers were produced in solution via thioeter linkage: GnRH-I-GnRH-III([VD-1]; [VD-12]; [VD-3]; [VD-31]) and GnRH-II-GnRH-III ([VD-2]; [VD-22]).

The receptor binding assay was used for determination of LH secretion of different GnRH derivatives on rat pituitary cells. We found that the asymmetric dimers retained the endocrine activity of natural GnRH-I or GnRH-II. The coupling of GnRH-III to the modified GnRH-I or GnRH-II did not disorder this effect.. Dimers VD-2 and VD-22 evoked LH release only in the higher concentration, but dimers VD-1 and VD-12 were more potent in the lower concentration. VD-31 dimer had the highest endocrin activity.

The in vitro cytostatic and antiproliferative effect of GnRH-derivatives were studied on MCF-7 and T-47D human breast cancer, HT-29, human colon carcinoma cell lines. We found that no cytostatic effect of the asymmetric dimers was observed on MCF-7 and HT-29 cell lines in the studied concentration range.

The asymmetric dimers had different antiproliferative effect on MCF-7, T-47D and HT-29.The most sensitive cell was T-47D for these asymmetric dimers. Dimer [VD-12] was the most potent (80%) on T-47D cells.

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Identification of DUSP1/MKP1 mediated pathways in lung cancer progression

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Mitogen-activated protein kinase (MAPK) signalling pathways are activated in response to a diverse array of extracellular stimuli, cell proliferation, differentiation or transformation. MAPK and JNK/SAPK activation requires phosphorylation of both threonine and tyrosine residues, that are dephosphorylated by protein phosphatases, resulting in inactivation of MAP kinases. The enzymes involved in this inactivation are the dual specific family of protein phosphatases (DUSP). DUSP1 was the first one DUSP to be identified and is encoded by an immediate early gene that has been shown to be stimulated under conditions of inflammation and stress, oxidative stress or growth factors. In addition, we have shown previously that DUSP1inhibition decreases tumor growth and sensitizes cancer cells to conventional chemotherapy, resulting in a NSCLC tumor size arrest.

The aim of this work is to gain insight into the cellular pathways involving DUSP-1 actions by using a double strategy that combines siRNA and microarray technologies. This strategy will provide a differential expression profile of genes regarding functionality of DUSP1.

The present study is based on a comparative analysis of RNA expression of the NSCLC H460 and H460-siDUSP1 cell lines. Total RNA from both cell lines was extracted, reverse-transcribed and hybridized into an array platform containing the whole human genome (affymetrix Human Genome U133 Plus 2.0). After data normalization, we selected 136 genes at least 3-fold up and down regulated comparing the interfered versus wild type cell lines (H460 and H460-siDUSP1). Posterior gene ontology analysis identified some of specific biological pathways related to angiogenesis, MAP kinase phosphatase activity, cell-cell signalling and growth factor activity. We validated the gene expression by real time PCR and pathways obtained by the gene ontology study were confirmed by the next complementary assays: