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