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BM-derived cells that foster tumor growth and their manipulation

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Myeloid-lineage cells that infiltrate tumors are more likely to promote tumor growth than to they are to mount effective anti-tumor responses. We studied the contribution of bone marrow-derived cells to transplanted and endogenous tumor models, and evaluated the recruitment and functional importance of hematopoietic cells and endothelial progenitors in the process of tumor angiogenesis. By using transcriptionally targeted lentiviral vectors and conditional cell elimination strategies, we demonstrated that bone marrow-derived myeloid cells, but not endothelial progenitors, play important roles during tumor vascularization. In particular, we showed that tumor angiogenesis and growth are dependent on a small subset of circulating and tumor-homing monocytes, which express the angiopoietin receptor Tie2 (Tie2-expressing monocytes, TEMs). Both mouse and human TEMs belong to the "resident monocyte" subset, a monocyte population that does not participate in inflammatory responses. We will present data that highlight the molecular and functional features of TEMs, and their relationship with other myeloid cell subsets previously implicated in tumor growth, such as tumor-associated macrophages (TAMs) and the so-called "myeloid-derived suppressor cells".

Given their tumor-specificity, we speculated that TEMs could be used as gene delivery vehicles for the selective transport of gene therapy to tumors. By transplanting hematopoietic stem cells transduced by lentiviral vectors with TEM-restricted expression, we targeted interferon-alpha (IFN-α) delivery to tumors and achieved substantial anti-tumor activity in mouse tumor models, including orthotopic, spontaneous and metastatic tumor models. In a spontaneous breast carcinoma model (MMTV-PyMT), we achieved significant inhibition of the mammary tumor burden in early (incipient tumors) and late (established tumors) intervention trials. The treated tumors were massively infiltrated by T cells and activated macrophages, suggesting the occurrence of an immune cell-mediated anti-tumor response. Remarkably, prevention trials achieved near-complete suppression of metastatic outgrowth in the lungs. Importantly, TEM-mediated IFN-α delivery did not impair hematopoiesis or wound healing detectably in the mice. Conversely, expression of IFN-α broadly in hematopoietic cells or in the plasma were highly toxic and, paradoxically, poorly effective. These results illustrate the therapeutic potential of gene- and cell-based IFN-α delivery, and should allow developing IFN-based treatments that more effectively treat cancer.

07 July 2008 14:35 - 16:35

SYMPOSIUM

Molecular pathology

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Gene expression profiling of breast cancer

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Breast cancer is presently classified based on tumor diameter, histologic type and grade, lymph node status and estrogen receptor, progesterone receptor and HER2 status. A more refined classification should be possible based on genetic alterations and gene expression profiles.

We have previously defined a gene expression profile of 70 genes that is predictive for a short interval to distant metastases (<5 yrs) in lymph node negative (LN0) patients. We have subsequently validated the prognostic value of this 70 gene profile in a cohort of 295 stage I and II breast cancer patients younger than 53 years of age.

To test whether gene expression profiling can be used in clinical practice, we have performed a study in 16 hospitals in the Netherlands. For 427 lymph node negative breast cancer patients, the 70 gene prognosis profile was assessed; 50% of the tumors were shown to have a good prognosis profile.

To identify gene expression profiles associated with response to chemotherapy, we are conducting neoadjuvant chemotherapy studies. Gene expression profiles are generated from core needle biopsies obtained before treatment and correlated with the response of the primary tumor to the chemotherapy administered. To date, no gene expression profile predicting the response of primary breast carcinomas has been identified, but we are currently expanding the series of patients in this neoadjuvant chemotherapy study.

We conclude that gene expression profiling is a method that will lead to improved classification of breast cancer by incorporating novel diagnostic tests that can be reliably implicated in clinical practice.

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2. Hannemann J, Oosterkamp HM, Bosch CAJ, et al. Changes in gene expression associated with response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2006 23: 3331-3342.

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Breast cancer phenotype and genetic alterations: how are they connected?

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Breast cancer is a heterogeneous disease, encompassing a number of histological entities with distinct biological features, pathological characteristics, and most importantly, clinical behaviour. In recent years, expression profiling analysis has demonstrated that breast cancers can be systematically classified into reproducible molecular subgroups according to their expression profile. Although still heterogeneous, these subgroups have been shown to be of prognostic significance. Several studies have suggested that this phenotypic variation may be explained by their cell of origin. However, there is increasing evidence in support of the concept that molecular subtypes and special histological types of breast cancer are characterised by distinct genetic aberrations, which may explain their phenotypic diversity and clinical behaviour. Firstly, our group and others have demonstrated that sporadic basal-like breast carcinomas, although known not to harbour BRCA1 somatic mutations, not only phenocopy tumours arising in BRCA1 germline mutation carriers, but also have remarkably similar genetic profiles. Secondly, tubular, cribriform, low grade invasive ductal and invasive lobular cancers have been shown to have a luminal phenotype, to evolve from the same precursors and to harbour remarkably similar genetic aberrations (i.e. gains of 1q and deletions of 16q). The main difference between lobular carcinomas and other tumours pertaining to this low grade luminal group has been shown to be the target gene of 16q deletions; whilst in lobular cancers it is the CDH1 gene that encodes E-cadherin, in other tumours pertaining to this molecular subgroup, the target gene is yet to be identified. Further evidence for this concept stems from the reported increased risk of lobular breast cancer in patients with CDH1 gene mutations and from K14cre;$Cdh1^{F/F}$;$Trp53^{F/F}$ engineered mice, which consistently develop tumours with histological features of lobular breast cancer. Finally, we demonstrate that other special types of breast cancer, such as pleomorphic lobular carcinomas and micropapillary carcinomas, are characterised by a constellation of genetic changes that differentiate them from oestrogen receptor and histological grade matched invasive ductal carcinomas. Taken together, the above findings support the concept that to some extent the phenotypic characteristics of breast cancers are underpinned by specific patterns of genetic aberrations.

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The genetic future of pathology: of pancreatic cancer and other malignancies

No abstract received

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Tumor metastasis: mechanistic insights and clinical challenges

No abstract received

07 July 2008 17:30 - 18:30

PLENARY LECTURE

Inflammation and cancer

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Inflammation and cancer

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There is amply epidemiological and mechanistic evidence that inflammation and inflammatory processes, such as these that lead to activation of NF-κB, play critical role in early tumor promotion and the growth and progression of primary tumors. Little information, however, exists regarding

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