compared with ZA (HR 0.77; 95% CI: 0.66–0.89; P = 0.001). Rates of adverse events were similar for both treatment arms. Osteonecrosis of the jaw occurred infrequently (2.0% denosumab, 1.4% ZA; P = 0.39). AEs potentially associated with renal toxicity occurred in 4.9% of the denosumab arm and in 8.5% of the ZA arm. In conclusion, an antitumor effect of ZA has been recently suggested in breast cancer whereas denosumab appears to be superior to ZA in delaying or preventing SREs in breast cancer.

Thursday, 25 March 2010 15:30–17:00

CLINICAL SCIENCE SYMPOSIUM

Stroma and microenvironment

223 Invited

A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer

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To identify factors that predict the response to chemotherapy, we have developed an approach based on the use of multiple regression to reduce the complexity of gene expression data sets. We tested the approach on tumour biopsies from individuals with estrogen receptor-negative breast cancer treated with 5-fluorouracil, epirubicin and cyclophosphamide (FEC) in the EORTC 10994/BIG 00−01 trial. We report that increased stromal gene expression predicts resistance to preoperative chemotherapy with FEC. The predictive value of the stromal signature was validated in two independent cohorts that received chemotherapy but not in an untreated control group, indicating that the signature is a predictive marker for drug response rather than a prognostic marker for innate tumour aggressiveness. Several models could explain the result, the simplest being reduced access of drugs to the tumour cells or crosstalk between tumour and stroma leading to secretion of survival factors by the stromal cells. These findings suggest that antistromal agents may offer a new way to overcome resistance to FEC.

224 Invited

Epigenetics of carcinoma-associated myofibroblasts: implications for anti-cancer therapies

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It is increasingly recognized that the non-neoplastic stromal compartment in most solid cancers plays an active role in tumor proliferation, invasion and metastasis. Cancer associated fibroblasts (CAFs) are one of the most abundant cell types in the tumor stroma, and these cells can be strongly pro-tumorigenic. Evidence that CAFs are epigenetically and possibly also genetically distinct from normal fibroblasts is beginning to define these cells as potential targets of anti-cancer therapy. In particular, our lab has shown that CAFs in gastric carcinomas have global DNA hypomethylation, with focal gains of methylation in gene promoters (Jiang, Gonda, et al, Cancer Res, 2008; Gonda et al., Semin Cell Dev Biol., 2009). We now find that this phenomenon generalizes to other common types of human carcinomas including prostate cancers and some types of breast cancer. For anti-cancer therapy we are testing the hypothesis that further decreases in DNA methylation induced in CAFs by the hypomethylating drug 5aza-dC may lead to a functional crisis in these cells, thus impairing their ability to support tumor growth. In a triple transgenic mouse model of aggressive pancreatic carcinoma (p53/Brca1/K-ras) we find that single-agent 5aza-dC has striking anti-tumor efficacy when initiated at the stage of pancreatic intraepithelial neoplasia. Our current experiments are aimed at dissecting whether this effect is due to functional inhibition of CAFs, or to direct inhibition of neoplastic epithelial cell proliferation, or both.

225 Invited

Oxidative stress promotes myofibroblast differentiation and tumour spreading

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JunD regulates genes involved in anti-oxidant defense. We took advantage of the chronic oxidative stress resulting from junD deletion to examine the role of Reactive-Oxygen-Species (ROS) in tumor development. In a model of mammary carcinogenesis, junD inactivation increased tumor incidence and revealed an associated reactive stroma. junD-inactivation in the stroma was sufficient to shorten tumor free survival rate and enhance metastatic spread. ROS promoted conversion of fibroblasts into highly migrating myofibroblasts through accumulation of the HIF-1a transcription factor and the CXCL12 chemokine. Accordingly, treatment with an antioxidant reduced the levels of HIF and CXCL12 and, subsequently, numerous myofibroblast features. Interestingly, CXCL12 accumulated in the stroma of HER2− human breast adenocarcinomas. Moreover, stroma of HER2 tumors exhibited a high proportion of myofibroblasts, which was correlated to high rate of nodal metastases. Finally, this subset of tumors revealed an associated oxido-reduction signature, further demonstrating the relevance of our findings in human cancers. Collectively, our data uncover a new mechanism by which oxidative stress increases the migratory properties of stromal fibroblasts, which in turn may potentiate metastatic dissemination.

226 Proffered paper oral

HOXB9, a gene overexpressed in breast cancer, induces angiogenesis, invasion, and lung metastasis

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Background: The mechanisms underlying tumoral secretion of signaling molecules into the microenvironment, which modulates tumor cell fate, angiogenesis, invasion and metastasis, are not well understood. Aberrant expression of transcription factors, which has been implicated in the tumorigenesis of several types of cancers, can constitute a mechanism that induces the expression of growth and angiogenic factors in tumors leading to their local increase in the tumor microenvironment to favor tumor progression. We recently observed that transcription factor, HOXB9, is deregulated in breast cancer and enhanced expression correlated with high tumor grade. A role for elevated HOXB9 expression in breast tumor progression is demonstrated by its ability to activate the ErbB and TGF-B pathways which influence aggressive tumor phenotypes and to induce angiogenesis in the tumor microenvironment.

Materials and Methods: To quantify HOXB9 expression in breast cancer, we analyzed cDNAs generated from laser captured, purified populations of tumor cells and adjacent normal mammary epithelial cells from 40 clinically and pathologically annotated cases of breast cancer. Next, we introduced the HOXB9 construct into MCF10A to test the functional consequence of HOXB9 overexpression. Furthermore, we stably introduced the activated G12V H-Ras allele into HOXB9-MCF10A cells and investigated their ability to form tumors and the metastatic potential.

Results: Overexpression of HOXB9 was found in 43% of primary breast cancer by RT-PCR and in situ hybridization and correlated with high tumor grade. Ectopic expression of HOXB9 in MCF10A mammary epithelial cells induced EMT accelerating cellular migration and invasion. It also increased the expression of angiogenic factors, which enhance the formation of new vessels in mouse dorsal air sac model. Conversely, genetic ablation of endogenous HOXB9 in MDA-MB-231 breast cancer cells suppresses their motility and angiogenic potential. Further, we confirmed that HOXB9-induced tumor phenotypes arise through the activation of both ErbB-AKT and TGFβ signaling pathways. Finally, in mouse xenograft model, we observed that HOXB9 cooperates with activated H-Ras to transform mammary epithelial cells leading to large, vascularized tumors showing highly metastatic potential to the lung.

Conclusions: Our findings imply that overexpression of HOXB9 in human breast cancer contributes to tumor progression through activation of signaling pathways that alter both tumor-specific cell fates and tumor-stromal microenvironment, leading to increased invasion and metastasis.