recurrence. The main molecular alterations defining high risk tumors were identified by Ingenuity Pathways Analyses software. We further validated the experimental approach and characterized the effects of TGF-β1 and the TGF-β1 receptor kinase inhibitor SB-431542 in an *in vitro* invasion assay with Hec1A endometrial cell line.
Results: Gene expression profiling identified a number of molecular pathways associated with high risk of recurrence in endometrial cancer, and designated a prominent role to TGF-β signaling in the acquisition of an aggressive phenotype. We showed that TGF-β1 promoted morphologic and molecular alterations consistent with an epithelial to mesenchymal transition in Hec1A cells. Moreover, TGF-β1 was able to promote Hec1A cells invasion and SB-431542 reversed these effects. We further demonstrated in a 3D inverted invasion assay that the TGF-β pathway represents a key molecular event in the initial steps of carcinoma invasion.
Conclusion: Our study indicates that the acquisition of a high risk of recurrence phenotype in endometrial carcinomas strongly relies on TGF-β1. The results highlight the promising utility of TGF-β pathway inhibitors for the development of targeted therapies in endometrial cancer.

160 POSTER

Cancer-associated IDH1 and IDH2 mutations: therapeutic opportunities

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Somatic mutations in the enzymes isocitrate dehydrogenase IDH1 and IDH2 are a common feature of more than 70% of grade II−III gliomas and secondary glioblastomas, 20−30% cytogenetically normal acute myeloid leukemia, and a variety of other malignancies at lower frequencies. These mutations are all heterozygous and occur at amino acid residues of the IDH substrate binding site, resulting in loss of the enzymes' ability to catalyze conversion of isocitrate to ±-ketoglutarate, and gain of function of a neoactivity to catalyze the NADPH-dependent reduction of ±-ketoglutarate to R(−)-2-hydroxyglutarate (2-HG). Elevated levels of R(−)-2-HG are found in tumors of malignant gliomas, and in malignant cells and serum of AML patients, that harbor IDH mutations. In addition, patients with a rare inherited neurometabolic disorder, 2-hydroxyglutaric aciduria, exhibit elevated levels of 2-HG in their CNS and are predisposed to the development of gliomas. Altogether these findings suggest the hypothesis that 2HG functions as an oncometabolite, and that the excess 2HG which accumulates in vivo may contribute to the formation and progression of cancers. We also demonstrate here that 2-HG metabolite is a tractable metabolic biomarker of mutant IDH enzyme activity in clinical samples. It is possible that small molecule inhibitors of mutant IDH enzymes may have therapeutic applicability in multiple cancers harboring IDH mutations.

161 POSTER

Localisation and characterisation of ET-1 binding to human colorectal cancers and evaluation of the orally active ETA receptor antagonist zibotentan (ZD4054)

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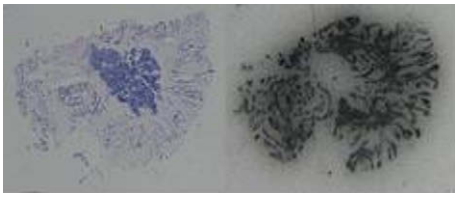
Background: Endothelin-1 (ET-1) acts via two endothelin receptors, ET_A receptor and ET_B receptor. ET-1 and ET_A receptor, which promote cancer growth and progression, are overexpressed in colorectal cancer tissues. We investigated the distribution of ET_A receptor and ET_B receptor in patient tissue sections. The affinity (K_d) and receptor density (B_{max}) of ET-1 was determined in whole tissue homogenates and colorectal fibroblasts. In addition the effect of the orally active ET_A receptor specific antagonist zibotentan (ZD4054) on ET-1 receptor binding (IC_{50}) was evaluated against subtype selective laboratory compounds.
Material and Methods: ET-1 receptor distribution and binding characteristics (K_d; B_{max}) were determined using *in vitro* autoradiography on patient sections, whole tissue homogenates and primary fibroblasts isolated from human colon tissues. Immunohistochemistry (IHC) was used to identify fibroblasts, endothelial cells and surrounding collagen type XI.
Results: ET-1 binding to cancer and normal colon tissue had similar characteristics. However there was greater ET_A receptor than ET_B receptor binding in colorectal cancer sections. Within both cancer and normal tissues, the strongest binding was to stromal cells, in particular fibroblasts, confirmed by immunohistochemistry. Further characterisation performed on primary fibroblasts revealed high density and affinity ET-1 binding in these cells (Bmax 3.03 ng/mg and K_d 213.6). Inhibition studies showed ET_A receptor antagonists (BQ123; zibotentan) were more effective at reducing ET-1 binding (IC_{50} values 0.1 μM, 10 μM respectively) than the ET_B receptor antagonist BQ788 (IC_{50}; 1 mM).
Conclusions: ET-1 binds strongly to receptors within colon cancer stroma structures, such as cancer-associated fibroblasts and endothelial cells, and is consistent with ET-1 signalling contributing to colorectal cancer growth, desmoplasia and neovascularisation. Furthermore, we have demonstrated that the orally active ET_A receptor antagonist zibotentan reduces ET-1 binding to colorectal cancer tissues. This study provides further evidence for the potential therapeutic use of the specific ET_A receptor antagonist zibotentan as an adjuvant treatment for colorectal cancer.



Tumour sections: Haematoxylin & eosin staining (left); ET-1 binding to colorectal cancer using autoradiography (right).

162 POSTER

MEK162 (ARRY-162), a novel MEK 1/2 inhibitor, inhibits tumor growth regardless of KRas/Raf pathway mutations

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MEK1/2, a dual specific kinase, is downstream of both Ras and Raf and required for the activation of ERK1/2. Mutated, oncogenic forms of Ras and Raf are commonly found in cancer and are implicated in uncontrolled cell proliferation. Tumors which harbor these mutated forms are reported to be highly sensitive to MEK inhibition. Interestingly, in a survey of 15 xenograft models, the greatest efficacy of MEK162 was observed in models which did not harbor these activating mutations. For example, treatment of BxPC-3 pancreatic carcinoma, CRC13B2 colon carcinoma, HT1080 fibrosarcoma and NCI-H1975 NSCL carcinoma (EGFR T790M) resulted in varying degrees of anti-tumor activity, including partial and complete regressions, in response to daily administration of 30–100 mg/kg MEK162. These models were chosen for further study into the underlying mechanisms of MEK antitumor activity in the absence of Ras/Raf mutation.
MEK inhibitors are reported to affect angiogenesis, through direct effects on endothelial cell proliferation, and tumor cell apoptosis, through increasing the pro-apoptotic protein BIM. The anti-angiogenic effect of MEK162 was first examined in an *in vivo* vascular endothelial cell growth factor (VEGF)- and basic fibroblast growth factor (bFGF)-induced matrigel invasion assay. MEK162 was a highly potent inhibitor of neoangiogenesis (100% inhibition, 10 mg/kg daily administration) and was equally efficacious as other known angiogenesis inhibitors (sunitinib, axitinib). Investigation of angiogenesis endpoints in xenografts, however, did not support a role for angiogenesis in the activity of MEK162. Established tumors from the above models were treated with 100 mg/kg MEK162 (5 days) and examined for VEGF and microvessel density (CD31 staining). No significant decreases were observed in these markers. Western blot analysis of tumor lysates confirmed that MEK was potently inhibited, as evidenced by profound pERK inhibition, and that BIM levels were increased. Taken together, these data suggest that while MEK162 is a potent inhibitor of neoangiogenesis, effects on established vascular systems are more complex and that stimulation of pro-apoptotic pathways may be the major contributor to the potent anti-tumor activity observed *in vivo*. These data further support the preclinical investigation of the effects of MEK inhibitors on apoptotic protein expression and the clinical investigation of MEK inhibitors in tumors with both wild-type and mutated Ras/Raf pathways.