vivo marks the uncommitted, proliferative cells in the small intestine of the mouse.
To address the physiological role of Notch signaling in intestinal homeostasis, the gene encoding Pofut1, a fucosyltransferase required for the activity of all Notch receptors, was deleted in the mouse intestinal epithelium, through Villin-Cre-mediated recombination.
Inhibition of canonical Notch signaling was confirmed by concomittant down- and up-regulation of Hes1 and Math1 mRNA levels, respectively. The body weight of Pofut1F/F:VillinCre mice was dramatically reduced compared to control littermates. Removal of Pofut1 led to a massive increase in commitment to the secretory cell lineage characterized by increased numbers of goblet cells, Paneth cells and enteroendocrine cells, as revealed by alcian blue, lysosyme and chromogranin A/B immunostaining, respectively. Consistent with this, the levels of mRNA encoding gut hormones (CCK, GIP, Glucagon), Paneth cell markers (MMP7) and mucus-secreting cell markers (MUC2, TFF3, FIZZ2) were enhanced, as determined by quantitative RT-PCR. Whereas, the specific allocation of Paneth and enteroendocrine cells was unchanged, goblet cells accumulated in the crypts. In parallel, microarray gene expression data revealed that absorptive cell markers (L-FABP, DPP4, ApoB) were repressed in intestinal epithelium lacking Pofut1. Interestingly, determination of cell renewal capacity in the intestinal mucosa, through Ki67 and BrdU immunostaining, revealed that the transit amplifying compartment was maintained in the upper crypts of the intestinal mucosa whereas decreased proliferating cells were detected in the colonic epithelium. In vitro, in human colon carcinoma HT29 Cl16E cells that spontaneously differentiate along the goblet cell lineage in culture, inactivation of Notch receptors activation led to cell cycle arrest in G1, and concomitant induction of expression of the MUC2 and TFF3 goblet cell markers. This later effect was mediated by Hath1 since its targeted down-regulation by specific siRNAs also inhibited MUC2 and TFF3 expression.
Therefore, we conclude that Notch signaling participates in the maintenance of intestinal progenitors and cancer cells in a highly proliferative state. Moreover, this study provides novel insight into the molecular mechanisms involved in intestinal cell fate specification, and maturation of secretory cell types in particular, induced by Notch.

304 ORAL

Early normalization of elevated baseline bone resorption marker levels by zoledronic acid and improved survival in patients with bone metastases from solid tumors

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Introduction: In patients with malignant bone disease, elevated N-telopeptide of type I collagen (NTX) levels are associated with significantly increased risks of skeletal-related events (SREs), disease progression, and death compared with normal NTX levels. Zoledronic acid reduces the risk of SREs and levels of NTX, parameters that have been associated with survival, in patients with malignant bone disease. This exploratory analysis investigated whether early normalization of urinary NTX correlated with a reduced mortality in patients with bone metastases from solid tumors.
Material and Methods: In this subset analysis, urinary NTX was measured at baseline and at 3 months in 3 randomized trials in patients with bone metastases from breast cancer (n = 379), hormone-refractory prostate cancer (n = 314), or lung cancer and other solid tumors (n = 204) who received zoledronic acid for up to 24 months. Patients were classified by baseline NTX levels (normal, <64 nmol/mmol creatinine; elevated, $\geqslant$64 nmol/mmol creatinine).
Results: Approximately 55% of patients had elevated NTX at baseline. Levels of NTX normalized within 3 months of zoledronic acid treatment in 76.2% of patients with elevated baseline NTX levels. Moreover, zoledronic acid-mediated NTX normalization reduced the risk of death by 48% in patients with breast cancer (risk ratio [RR] = 0.517; P = 0.002), 59% in patients with prostate cancer (RR = 0.410; P < 0.0001), and 58% in patients with lung cancer and other solid tumors (RR = 0.427; P = 0.012). Zoledronic acid-mediated normalization of NTX also significantly prolonged SRE-free survival in patients with breast cancer or prostate cancer (P < 0.001 for both) compared with persistently elevated NTX at 3 months. Further analyses revealed that there was a continuum of benefit in all cancer types tested dependent on the percentage decrease of NTX levels at 3 months, with the greatest survival benefit occurring in patients whose NTX levels decreased $\geqslant$ 75% (P < 0.01 for comparison between percentage reduction quartiles in all tumor types tested).
Conclusions: Among patients with elevated baseline NTX receiving zoledronic acid, those whose levels normalized by 3 months had better clinical outcomes, including prolonged SRE-free survival and overall survival, compared with patients whose NTX levels remained elevated. This finding held for all tumor types studied. New treatment strategies should be investigated in patients with persistently elevated NTX levels.

305 ORAL

Mapping of interstitial fluid pressure in solid tumours using dynamic contrast enhanced MRI – dream or reality?

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Background: Interstitial fluid pressure (IFP) of most solid tumours is increased relative to normal tissues creating a barrier for transvascular transport, thus compromising the delivery and efficacy of chemotherapy and macromolecules. Here we demonstrate that bevacizumab decreases IFP in HT29 (human rectal cancer xenografts) and assess if infusion dynamic contrast enhanced MRI (iDCE-MRI) kinetic parameters correlate with IFP or changes induced by bevacizumab.
Materials and Methods: 29 SCID mice bearing subcutaneous HT29 tumours of ~8.5 mm diameter received a single dose of 10 mg/kg bevacizumab intraperitoneally; controls received saline. iDCE-MRI was performed on days 1, 3 & 5, using a slow infusion rate (5.5 microlitres/min for 60 mins) of contrast agent [Gadopentetate dimeglumine; Gd-DTPA], sequential images before and during the infusion were acquired. The kinetic parameters (inflow rate (Kinf); max enhancement (Minf) and total Gd-DTPA delivery for 60 mins (AUC60) were estimated from changing T1 relaxation rates. Immediately after MRI, the IFP was measured directly using wick-in-needle technique.
Results: There was no correlation of IFP measurements and any kinetic MRI parameter. IFP was significantly lowered (p < 0.001) on day 5 only in treated tumours (mean $\pm$ SD 15.1$\pm$4.7 cf 36.9$\pm$5.6 mmHg). There were no significant differences in any kinetic MRI parameters between treated and control animals at day 1, 3 & 5.
Conclusions: Tumour IFP cannot be directly related to iDCE-MRI. Changes in IFP induced by bevacizumab on day 5 were not reflected by alterations in MRI parameters.

Poster presentations (Tue, 25 Sep, 14:00−17:00)
Basic science

306 POSTER

Methylnaltrexone-induced receptor tyrosine phosphatase mu (RPTP mu) activation regulates inhibition of VEGF-induced angiogenesis

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Angiogenesis or the formation of new blood vessels is important in the growth and metastatic potential of various cancers. Therefore, agents that inhibit angiogenesis have important therapeutic implications. We have previously shown that methylnaltrexone (MNTX), a peripheral mu opiate receptor (mOP-R) antagonist which has completed phase 3 trials for opioid-induced constipation in advanced illness, inhibits VEGF and opioid-induced endothelial cell (EC) proliferation and migration, two key components in tumor-associated angiogenesis (Microvasc Res 2006; 72(1−2): 3−11).
In this study, we examined the mechanism by which MNTX inhibits VEGF-induced angiogenic events. Our results indicate that treatment of human pulmonary microvascular EC with MNTX (100 nM), but not the uncharged mOP-R inhibitor, naloxone, increased Receptor Protein Tyrosine Phosphatase mu (RPTP mu) activity which was independent of mOP-R expression. Silencing RPTP mu expression (siRNA) in human EC inhibited MNTX protection from VEGF-induced proliferation and migration. Mechanistically, silencing RPTP mu increased VEGF-induced Src and RhoA activation as well as tyrosine phosphorylation (inactivation) of the negative regulator of RhoA, rhoGAP.

Taken together, our results suggest that MNTX activation of RPTP mu activity is central in inhibiting VEGF-induced Src and RhoA activation, rhoGAP inactivation, and angiogenesis. Our results indicate that MNTX may represent a potential therapeutic agent for the treatment of tumor-associated angiogenesis.

307 POSTER

Inducing apoptosis in colorectal tumor cells through inhibition of Aurora B kinase

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Background: Aurora kinases are serin/threonin kinases with oncogene potential. The overexpression of Aurora kinases was found in colorectal tumors and correlates with poor clinic prognosis. Recent studies showed that inhibition of aurora kinases inhibited the growth of colorectal tumors in vitro and in vivo. However, the underlying mechanism is still unknown. In this study, we examined the anti-tumoral signaling of aurora kinase inhibitor (AKI), ZM 447439.
Methods and Materials: The apoptosis and cells death were counted in Colo-205 and HCT-116^{wt} colorectal tumor cells, at different time points after administration of ZM447439, using Hoechst33342/propidium iodide double staining. Mitochondrial potential (TMRE fluorescence) and polyploidy formation (propidium iodide fluorescence) was examined using flow cytometry. Western-blotting was performed to assess phosphorylated histone H3 (Ser 10) and cleavage of caspases. To analyze the signal pathway of the Aurora kinase inhibitor, all assays were also undertaken in HCT-116$^{p53-/-}$, HCT-116$^{p21-/-}$, HCT-116$^{Bak-/-}$, HCT-116$^{Bax-/-}$ and HCT-116$^{Bak/Bax-/-}$ cells.
Results: ZM447439 inhibited phosphorylation of histone H3 (Ser 10), verifying its inhibitory capability to Aurora kinase. Inhibition of Aurora kinases with ZM447439 significantly stimulated increase of apoptosis and cell death in Colo-205 and HCT-116^{wt} cells in a concentration- and time-dependent manner. PARP was processed in HCT-116^{wt} cells treated with ZM447439. Knockout of p53, Bak, Bax or Bak/Bax in HCT-116 cells significantly reduced apoptosis and cell death induced by ZM447439, compared with wild-type ($p < 0.01$). A progressive dissipation of mitochondrial membrane potential was observed in HCT-116^{wt} cells 24 h (5.3%), 48 h (34.3%) and 72 h (58.3%) after treatment of 5μM ZM447439, normalized to control group respectively ($p < 0.01$). Knockout of p53, Bak, Bax or Bak/Bax in HCT-116 significantly protected cells from dissipation of mitochondrial membrane potential induced by ZM447439. Knockout of Bak, Bax or both Bak and Bax caused similar resistance to Aurora kinase inhibitor, while knockout of Bak and Bax showed a nonsignificant increase of the resistance. ZM447439 induced notable endoreduplication and polyploidy formation in all examined cell lines ($p < 0.01$), while HCT-116$^{p21-/-}$ was the most sensitive to undergo endoreduplication and form polyploidy.
Conclusion: The small molecular Aurora kinase inhibitor induced apoptosis and cell death in colorectal tumor cells in vitro and may be a new class of potential therapeutic agent for colorectal tumors. P53, Bak and Bax play important roles in Aurora kinase inhibitor induced apoptosis and cell death. Understanding the underlying mechanism may help to design new therapeutic concept.

308 POSTER

Celecoxib-induced apoptosis depends on Bak and is only marginally modified by Bcl-2 overexpression

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Background: Celecoxib and other selective cyclooxygenase-2 (COX-2) inhibitors are potent inducers of apoptosis and increase the efficacy of ionising radiation. Celecoxib induces apoptosis via a novel mitochondrial pathway requiring Apaf-1 and caspase-9. However, apoptosis induction is independent from expression level of anti-apoptotic Bcl-2 as demonstrated in our earlier investigations. We therefore wanted to evaluate the relevance of both crucial pro-apoptotic Bcl-2 proteins – Bak and Bax – for celecoxib-induced apoptosis in Jurkat T-Lymphoma cells.
Material and Methods: Induction of apoptosis was determined using COX-2- and Bax-negative Jurkat cell clones being (a) deficient for Bak (Jurkat Bak negative), (b) proficient for Bak (Jurkat Bak positive), or (c) proficient for Bak and overexpressing Bcl-2 (Jurkat Bcl-2). After treatment with 0, 50, 75 and 100 μM of celecoxib apoptosis was determined by flow cytometry (morphology, depolarisation of the mitochondrial membrane potential and nuclear fragmentation), fluorescence microscopy (chromatin condensation, nuclear fragmentation) and Western Blotting (caspase-activation, Bcl-protein-expression).
Results: Celecoxib induced substantial apoptosis in Jurkat cells expressing Bak (but not Bax). The extent of apoptosis was negligibly modified by overexpression of Bcl-2 in concordance with our earlier findings. But, apoptotis decreased substantially in Jurkat Bak negative cells lacking both proapoptotic multidomain Bcl-2 proteins, Bax and Bak. The apoptotic rates were clearly distinguishable from Bak positive cells even after increase of incubation time or concentration of celecoxib. Similarly, depolarisation of the mitochondrial membrane potential, activation of caspases-9, -8 and -3 as well as cleavage of the caspase-3 substrate PARP were only observed for Bak-positive Jurkat cells independent from Bcl-2 overexpression. In contrast, Bak-deficiency was sufficient to inhibit apoptotic changes.
Conclusions: Lack of Bak was sufficient to abrogate celecoxib-induced mitochondrial damage, caspase-activation and nuclear fragmentation. Anti-apoptotic Bcl-2 overexpression did marginally modify response to celecoxib. These data provide evidence for a Bak-dependent but COX-2-independent pro-apoptotic effect of celecoxib in Bax-negative Jurkat T-lymphoma cells in vitro.

309 POSTER

Serum matrix metalloproteinase 1 (MMP1) as a prognostic marker in bone metastases (BM) treated with bisphosphonates

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Background: Pre-clinical studies suggest that cancer cells in bone do express MMPs with collagenolytic activity (J Bone and Miner Res 2003;18:859−67). In human BM sections we found an higher expression of MMP1 in patients already treated with BP (ASCO 2002 abstract 1793). In this prospective study we determined whether the baseline activity of MMP1 was predictive of OS and SREs as defined by: pathologic fractures (PF), radiation to treat BM (RT) or spinal cord compression (SCC).
Methods: We studied 116 BM patients, median age: 64 years; 67% females; 61% breast cancer; 19% prostate cancer; and 20% other tumor types. All patients were classified according to x-ray pattern (lytic 54%, blastic 23%, or mixed 21%) and extent of BM (number of skeletal segments involved). At the time of study entry, 108 patients had serum levels of the MMP1 active form measured with enzyme
linked fluorescence immunoassay using reagents obtained from R&D Systems (limit of detection: 0.39 ng/mL). During the time period on study, patients received treatment with IV zoledronate (57%), IV pamidronate (28%), or more than one BP.
The proportional hazards model was used to investigate the correlation of MMP1 baseline level with OS and time to first SRE (TTSRE); and Poisson regression with the skeletal morbidity rate (SMR): number of SREs/person/year.
Results: The median follow-up was 21 months. The median value of MMP1 was 4.73 ng/mL (range: 0−32.3 ng/mL). During the time period on study, 38% had PF, 57% had RT, and 9.5% had SCC. Median TTSRE was 20 months and the SMR was 0.84. Median OS time was 29 months. Serum MMP1 levels were associated with increased mortality risk with a hazard ratio (HR) of 1.08, 95%CI 1.02−1.14, $p = 0.007$ (8% increased risk of death for each 1 ng/ML increase in serum MMP1). The correlation of MMP1 with OS was independent of the extent of BM (interaction test for MMP1 and the number of skeletal segments involved with bone metastases: $p = 0.8$). MMP1 serum levels were not associated with TTSRE (HR 1.01, 95%CI 0.94−1.08, $p = 0.64$) or SMR (HR 1.02, 95%CI 0.95−1.09, $p = 0.68$).
Conclusions: Serum levels of the active form of MMP1 at baseline is associated with decreased survival in BM patients on BP therapy. These results suggest that MMP1 might be a new target for the treatment of BM.