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.