that impacts metastasis, KISS1 (products) are likely drug-able and may be useful for inhibiting colonization of tumors at secondary sites.
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254 INVITED

Tumour vascular diversity and its translation into targeted therapeutics

R. Pasqualini, W. Arap. *USA*

Abstract not received.

255 INVITED

Targeting the invasion-association integrin alphavbeta6 as an anti-carcinoma strategy

I. Hart, D. Dicara, J.F. Marshall. *Queen Mary's School of Medicine and Dentistry at Barts and The London, Institute of Cancer & CR-UK Clinical Centre, Centre for Tumour Biology, London, United Kingdom*

The epithelial-specific integrin αvβ6 usually is not expressed in resting tissues but is upregulated in wound-healing, inflammation and in many different cancers. Indeed our own immunohistochemical analyses have shown that in oral squamous cell carcinoma, for example, around 90% of tumours express high levels of αvβ6, often at their invasive margins. Interestingly the induced expression of αvβ6 in experimental systems has been shown to increase carcinoma cell invasion, possibly explaining why strong expression of αvβ6 correlates with a 69% reduction of patient survival in colon cancer (Bates RC, et al. J Clin Invest 2005; 115: 339–347). As an integrin hetrodimer αvβ6 obviously is expressed at the cell surface and thus represents a potential target for imaging and, possibly, therapy. Characterised as the prime receptor for Foot-and-Mouth-Disease virus αvβ6 is known to recognise the Arg-Gly-Asp (RGD) motif present in the G-H loop of the VP1 structural protein. We have designed peptide probes, based around this core RGD sequence, and shown that longer (20 mer) peptides are better antagonists of αvβ6 activity than shorter peptides (lead peptide A20FMDV2; IC_{50} = 1 nM). Structural analysis by NMR has shown that efficacy of these longer peptides corresponds with the presence of a helix immediately C-terminal to this critical RGD motif. Non-adjacent residues, brought into juxtaposition as a linear array on the outer face of the helix, also appear to interact with αvβ6. Using engineered human tumour cell lines which differ only in their expression of αvβ6 we were able to demonstrate specificity of binding using biotinylated peptides. *In vivo* specificity was demonstrated using nude mice bearing αvβ6-positive and -negative xenografts which were injected with ^{18}F-FBA-A20FMDV2; positive: negative tumour ratio >4:1 with MicroPET showing selective accumulation in size-matched β6-positive tumours. These data indicate that carcinoma-specific αvβ6 may represent a suitable target for these peptides; a possibility currently under investigation in this laboratory.

256 INVITED

Targeting hypoxia as an anti-metastatic strategy

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Metastases are the primary cause of cancer-related deaths. Several pre-clinical and clinical studies have demonstrated that the development of metastatic disease is linked with the extent of hypoxia in the primary tumour. From the data that has been amassed so far, it would appear that hypoxia, through a number of discreet mechanisms, can "breed" an aggressive disease phenotype. This is problematic given the fact that hypoxia has been identified in all solid tumours analysed to date and that hypoxic cells are resistant to standard radiotherapy and many forms of chemotherapy. On the other hand hypoxia is a tumour-specific physiological abnormality and as such it can be exploited as a difference between tumours and normal tissues. The association between hypoxia and the development of metastases suggests there may be a potential therapeutic window of opportunity in targeting primary tumour hypoxia to reduce metastatic dissemination. There are two ways to approach this. The first is to target the condition of hypoxia per se, using bio-reductive agents that are selectively cytotoxic towards hypoxic cells. The second is to identify hypoxia-dependent changes in gene expression that are pivotal in the transition to a metastatic phenotype. Examples will be given where these approaches have led to a significant reduction in metastatic burden in experiments.

Thursday 9 November

Poster Sessions

Bioreductive agents

257 POSTER

Tirapazamine disrupts vascular endothelial-cadherin, suggesting a mechanism behind its ability to cause central vascular dysfunction

L. Huxham, A. Kyle, A. Minchinton. *BC Cancer Research Centre, Medical Biophysics, Vancouver, Canada*

By mapping the microregional effects in HCT-116 tumour xenografts, we have shown that the hypoxic cytotoxin tirapazamine (SR 4233: 3-amino-1,2,4-benzotriazine 1,4-dioxide) unexpectedly causes central vascular dysfunction 1 day after treatment with a progression over the following 1–3 days to necrosis. Similar effects, but with different kinetics, have been seen after treatment with known vascular targeting agents such as combretastatin A4 phosphate which is a microtubule disrupting agent and has also been shown to interfere with the endothelial cell-specific junctional molecule vascular endothelial-cadherin (VE-cadherin).
To investigate the mechanism of action behind the vascular dysfunction caused by tirapazamine we have examined human umbilical vein endothelial cells under oxic and hypoxic conditions. Cells were seeded in 4 well glass slide chambers and grown until confluent. Chamber slides were then gassed with specific levels of oxygen. The confluent monolayers were treated with 100 μM tirapazamine for 1.5 hours and stained to show hypoxia (pimonidazole), DNA double strand breaks (γH2AX), microtubule fine structure (β-tubulin), and vascular endothelial cell adhesion junctions (VE-cadherin).
Under hypoxic conditions tirapazamine treated cells showed labeling for pimonidazole and an increase in γH2AX compared to cells in an oxic environment. Microtubule disruption was not seen after exposure to tirapazamine in either the oxic or hypoxic groups. However, under hypoxic conditions tirapazamine did cause disruption of VE-cadherin as seen by an absence of pseudopodia and by fragmentation of the structured cell membrane junctions.
We propose that this activity of tirapazamine *in vivo* is related to its effect on hypoxic tumour vasculature located in the centre of tumours. The observed central vascular dysfunction may be due to disruption of the cell adhesion junctions between endothelial cells in hypoxic regions, thereby damaging the vessel and leading to cessation of perfusion along the vessel.
This research is supported by the Canadian Institutes of Health Research and the Michael Smith Foundation for Health Research. Animals were maintained in accordance with the Canadian Council on Animal Care guidelines.

258 POSTER

Tricyclic triazine 1,4-dioxides: a new class of hypoxia-selective cytotoxins with improved extravascular transport compared to tirapazamine

M.P. Hay, K.O. Hicks, B.G. Siim, F.B. Pruijn, H.H. Lee, S. Yang, K. Pchalek, A. Blaser, W.R. Wilson, W.A. Denny. *The University of Auckland, Auckland Cancer Society Research Centre, Auckland, New Zealand*

Pharmacokinetic/pharmacodynamic (PK/PD) modelling has shown the in vivo activity of tirapazamine (TPZ), a bioreductive cytotoxin currently in Phase III clinical trial in combination with chemo-radiotherapy, to be limited by poor extravascular transport (EVT). EVT limitations result from slow diffusion and high bioreductive metabolism.
We present a new class of tricyclic triazine 1,4-dioxides (TTOs) as hypoxia-selective cytotoxins with improved EVT compared to TPZ. The indanetriazine core was designed to increase EVT by increasing lipophilicity and decreasing hypoxic metabolism through lowered electron affinity. The addition of a lipophilic amine side chain, attached via the 3-NH position, provided increased solubility while maintaining EVT. Replacement of the 3-NH linker with an alkyl linker improved diffusion by increasing lipophilicity and removing H-bond donors. The 3-alkyl substituents contributed to increased rates of metabolism which were balanced by the electron-donating nature of the indanetriazine core.
TTOs were screened for in vitro hypoxic cytotoxicity (IC_{50}) and hypoxic selectivity (HCR = aerobic IC_{50}/hypoxic IC_{50}) in human HT29 colon carcinoma cells. Diffusion coefficients (D_{MCL}) were calculated from diffusion studies in HT29 multicellular layers and rates of hypoxic metabolism (k_{met}) measured in single cell suspensions. Calculation of a 1-D transport parameter (X_*) allowed comparison of EVT between TTOs. PK/PD modelling predicted the plasma AUC required for 1 log of hypoxic cell killing