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HPMA copolymer-TNP-470 (caplostatin) and Avastin show synergistic inhibition of human tumor growth in mice

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Inhibition of angiogenesis, a concept proposed by Judah Folkman in 1971, is one of the most promising approaches for treating human tumors. The formation of capillaries from preexisting blood vessels is now considered to be a key point for tumor growth beyond a critical size of approximately 1 mm^3. Solid tumors can trigger this complex process by expression of angiogenic factors. Of particular clinical interest is the vascular endothelial growth factor (VEGF); its expression correlates with vessel density and poor prognosis in various tumors. Therapies directed against VEGF or its receptors are showing efficacy in cancer treatment. Recently, this modality has received validation in a large, Phase III clinical trial in metastatic colorectal cancer patients. Monoclonal antibody to VEGF, Avastin, plus chemotherapy resulted in a highly significant longer time to progression and greater survival than chemotherapy alone. Avastin is approved for clinical use in 28 countries.
TNP-470, a broad spectrum angiogenesis inhibitor, has also shown promise in clinical trials, however, doses necessary for tumor regression, showed signs of neurotoxicity. We recently described the synthesis and characterization of a novel non-toxic, water-soluble N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-TNP-470 conjugate, now called **caplostatin** [Nature Medicine 10, 255 (2004)]. Conjugation of TNP-470 to HPMA copolymer eliminated its neurotoxicity while retaining its antiangiogenic and anti-tumor activity. Moreover, caplostatin has an improved pharmacokinetic profile, all of which could facilitate its return to clinical trials.
Here we describe the combination of two angiogenesis inhibitors, caplostatin and Avastin with the goal of augmenting the effects of either drug alone. Targeting the endothelial cell, rather than the tumor itself, avoids issues of accessibility to the tumor interior and drug resistance may be less likely. We propose that the antitumor spectrum and efficacy of Avastin can further increase when administered in combination with caplostatin. Our findings demonstrate the high antiendothelial/antitumoral efficacy of the concurrent administration of caplostatin and Avastin *in vitro*. Furthermore, we show the synergistic effect of the combination on 6 different subcutaneous and orthotopic tumor models with a 50% of tumors undergoing complete regression in the COLO-205 human colon carcinoma model. A potential explanation for the favorable combination would be that Avastin inhibits tumor growth by targeting VEGF while VEGF is only one of many angiogenesis stimulators. TNP-470 which has the broadest spectrum of any known antiangiogenic/anti-cancer agent, may target multiple pathways. Our data suggest that combining two non-toxic angiogenesis inhibitors can have increased synergistic anti-tumor effect and no toxicity. The clinical translation of this novel combination is warranted.

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Regulation and targeting of the hypoxia-inducible factor pathway

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Hypoxia-inducible factor-1 (HIF-1) is a transcriptional complex consisting of HIF-1a and HIF-1b subunits and is central to regulating genes involved in cell survival and proliferation, vascularisation and metabolic adaptation. HIF-1 activity is dependent on the availability of the a subunit, which is regulated by cellular oxygen and growth factors. Upon induction, HIF-1a localises to the nucleus, where it binds to HIF-1b to form the HIF-1 complex. Overexpression of HIF-1a has been observed in many human cancers and correlates with an aggressive tumour phenotype Therefore, HIF-1 and components of the HIF-1 pathway are attractive therapeutic targets.
To identify small molecule inhibitors of the HIF-1 pathway, we developed and implemented a cell-based assay that we used recently in a high-throughput screen [Chau et al. Cancer Research 2005; 65(11): 4918-28]. We identified two novel hit compounds, CCT001268 and CCT002847 that inhibited HIF-1 activity and HIF-1a protein induced by the hypoxia mimetic agent, deferoxamine mesylate without significantly inhibiting constitutive luciferase activity in our control cells. CCT001268 but not CCT002847 inhibited HIF-1 activity and HIF-1a protein induced by hypoxia and significantly inhibited VEGF and Glut-1 expression. Interestingly, both CCT001268 and CCT002847 inhibited HIF-1a protein induced by insulin-like growth factor-1. Furthermore, we have shown that CCT001268 is a potent inhibitor of VEGF-mediated angiogenesis *in vitro*, has favourable pharmacokinetic properties *in vivo*, and have identified its potential mechanism of action.
HIF-1a belongs to a growing family of structurally related proteins. HIF-2a, like HIF-1a is sensitive to multiple stimuli and can be constitutively expressed. However, the relative contribution of HIF-1a and HIF-2a to HIF-regulated target genes under different conditions is unclear. Using siRNA techniques to knockdown HIF-1a and/or HIF-2a expression, we found that HIF-1a primarily regulates transcriptional activation of VEGF in response to hypoxia and IGF-1 as compared with HIF-2a in MCF-7 cells. In renal carcinoma cells that constitutively express both HIF-1a and HIF-2a due to loss of von Hippel-Lindau (pVHL) E3 ligase function, we found that high basal VEGF, GLUT-1, uPAR and PAI-1 expression was predominantly dependent on HIF-2a. Furthermore, we found a reciprocal relationship between HIF-1a and HIF-2a expression in these cells.
Our observations suggest that HIF-targeted gene expression is stimulus and cell-type dependent, and that small molecule inhibitors of the HIF pathway ideally should target both HIF-1a and HIF-2a expression. Accordingly, we found that CCT001268 blocked constitutive HIF-1a, HIF-2a and VEGF expression in renal carcinoma cells. Our data have important implications for how we target the HIF pathway therapeutically.

55 INVITED

The MET oncogene drives a genetic program linking cancer to haemostasis

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The close relationship between activation of blood coagulation and cancer is an old enigma. In 1865 *migrans trombophlebitis* (a condition of the blood that predisposes it to spontaneous coagulation) was described as forewarning of occult malignancy (Trousseaus sign). This pioneer observation emphasized the existence of haemostasis disorders associated with cancer onset, since then extensively reported in clinical and epidemiological studies, but so far orphan of a mechanistic explanation. Here we report a mouse model of sporadic tumorigenesis based on genetic manipulation of somatic cells. Targeting the activated *MET* oncogene to adult liver caused slowly progressing hepatocarcinogenesis. Surprisingly, this was preceded and accompanied by a syndrome manifesting first with blood hypercoagulation (venous thromboses), and then evolving towards fatal internal haemorrhages. The pathogenesis of this syndrome is driven by the transcriptional response to the oncogene, including prominent up-regulation of plasminogen activator inhibitor type 1 (PAI-1) and cyclooxygenase-2 (COX-2) genes. *In vivo* analysis showed that both proteins support the thrombohaemorrhagic phenotype, thus providing direct genetic evidence for the long-sought link between oncogene activation and haemostasis.

56 EACR Award

The European Association for Cancer Research (EACR) Young Cancer Research Award – Molecular regulation of an invasion-related molecule: options for tumor staging and clinical strategies

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The urokinase-receptor (u-PAR) promotes the invasive and metastatic phenotype and has been shown to be associated with early relapse and poor prognosis in enumerous types of cancers. In addition, we have shown that expression of the u-PAR on minimal residual tumor cells in the bone marrow of patients with gastric cancer indicates a metastatically relevant phenotype of these early disseminated tumor cells, and that the detection of u-PAR on these cells at the time of primary surgery can predict the development of a clinically relevant minimal residual disease component, early relapse, and poor prognosis. From our and other studies we know that high u-PAR gene expression in carcinoma cells is largely due to the transcriptional regulation of the gene. We have characterized two cis-elements (−152/−135, bound with an AP-2-like protein, Sp1, and Sp3; −190/−171, bound with AP-1-transcription factors) of the u-PAR promoter which are decisive for diverse means of u-PAR-gene expression in highly invasive colon cancer cells: The AP-2/Sp1 element is mediating constitutive and PMA-induced gene expression, and, more importantly, induction of u-PAR-mediated proteolysis brought about by c-Src, and suppression brought about by the newly characterized tumor suppressor Pdcd4. The AP-1-motif is mediating constitutive gene expression, as well as induction of u-PAR-mediated proteolysis brought about by K-Ras. U-PAR-mediated proteolysis could be inhibited by specific Src-and Ras-inhibition, as well as transcriptionally by dominant-negative AP-2.
The lecture will focus on the differential binding of transcription factors to both u-PAR promoter elements *in vivo*, having been investigated in a large series of resected tumor and normal tissue of colorectal and gastric cancer patients. We will demonstrate that, depending on the transcription