339

Characterisation of CA-IX expression and activity in human tumour cell lines

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CA-IX is a transmembrane member of the a class of carbonic anhydrases (CA) that catalyse the reversible hydration of carbon dioxide and serve a wide range of physiological functions including acid-base regulation and respiration. CA-IX is tumour-associated and may have potential roles in oncogenesis. CA-IX is upregulated by hypoxia, which is associated with increased aggressiveness, metastasis and poor prognosis. It has been postulated that CA-IX is involved in maintaining the extracellular acidification common to solid tumours that is assumed to be related to enhanced proliferation, invasiveness and has implications for chemotherapeutic drug uptake and activity. The aims of the study were to assess the effect of anoxia for inducing CA-IX protein expression and activity in a panel of human tumour cell lines, to identify if expression of CA-IX is related to cell proliferation and finally determine whether CA-IX expression can reflect itself in changes in drug uptake and toxicity. A panel of 16 cell lines was characterised for CA-IX protein expression under both aerobic and anoxic conditions by western blotting. An electrometric method assessed the CA activity of aerobic and anoxic membrane fractions. The growth of MDA435 cells transfected with CA-9 and empty vector controls was assessed under aerobic and anoxic conditions by a MTT proliferation assay. The levels of activity and protein expression of CA-IX varied between cell lines from very low in MDA435 and RT112 cells to high in SW480 cells in aerobic conditions. A subset of the panel exhibited substantial anoxic induction of CA-IX protein and activity (e.g. HT29, MDA231, and SW480). CA activity in the majority of cell lines was abrogated in the presence of the CA inhibitor acetazolamide in air and anoxia. Incubation of CA-IX expressing MDA435 cells with acetazolamide in air and anoxia induced similar dose-dependent inhibition of proliferation compared to controls. The majority of tumour cell lines tested expressed CA-IX protein and showed activity to varying degrees but not all exhibited induction by anoxia. Expression of CA-IX did not appear to provide a growth advantage to tumour cells under aerobic or anoxic conditions. Further *in vitro* and *in vivo* studies will be carried out in which a selected panel of cells with high and low CA-IX expression will be treated with acetazolamide in combination with anticancer agents.

340

ZD4054: a specific endothelin A receptor antagonist with potential utility in prostate cancer and metastatic bone disease

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Blockade of the endothelin A receptor represents an exciting new therapeutic target in the treatment of both prostate cancer and metastatic bone disease. Specific blockade of the endothelin A receptor may produce most benefit, since concomitant inhibition of the endothelin B receptor may affect clearance of ET-1 (Fukuroda et al., Biochem Biophys Res Comm 1994; 199: 1461-1465), leading to an anti-diuretic effect and subsequent oedema (Clavell et al., Am J Physiol 1995; 268: F455-F460). ZD4054 binds to the human endothelin A receptor with an affinity of 5.4 nM but does not displace ET-1 from the human endothelin B receptor at concentrations up to 10 μM. Using blockade of ET-1 mediated vasoconstriction as an endpoint, ZD4054 is active at a dose of 0.03 mg/kg i.v. in the anaesthetised dog, with a dose of 0.1 mg/kg being active for at least 7 hours. In addition, selective endothelin B agonist-induced vasodilation is not inhibited by ZD4054 at a dose of 1.0 mg/kg. ZD4054 is orally absorbed, has good bioavailability in the rat and the dog (>70%) and has a favourable toxicity profile, a single oral dose of 2 g/kg is well tolerated in the rat. In conclusion, ZD4054 has an endothelin A specific action, good physical properties, is well tolerated preclinically, and may provide a new therapeutic option in prostate cancer and metastatic bone disease. It is currently in phase I trials.

341

Extracellular matrix of human osteosarcoma as a potential new therapeutic target

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Introduction: Osteosarcoma is the most common primary malignancy of bone in childhood and adolescence. Improvement of its treatment might be expected from novel therapeutic targets identified by extracellular matrix (ECM) investigation. Extracellular matrix construction is principally different in tumours from normal tissue. Aims: In this study involvement of extracellular matrix and its components in osteosarcoma cell proliferation and invasion was investigated.
Materials and methods: A stabile osteosarcoma cell line (OSCORT) was established from the biopsy fragments of a 17-year-old boy. Osteosarcoma cells were cultured conventionally, or on extracellular matrix gel (ECM-gel), as well as on the matrix produced by them (OSCORT-ECM). Cell proliferation, cell cycle analysis, expression of proliferation-related proteins and beta-1 integrin, and activity of matrix metalloproteinases was compared within these culture conditions.
Results: ECM-gel and osteosarcoma matrix increased cell proliferation (cell numbers and thymidine incorporation), which was confirmed by increased cyclin D1 and PCNA protein expression as well. Among ECM components heparan sulfate proteoglycan (HSPG, supposed to be agrin) and fibronectin was the most responsible for these effects. HSPG and fibronectin increased beta-1 integrin expression as well. Oppositely, type IV collagen decreased proliferation, and the proportion of cells in G1 or S-phase, while increased the ratio of cells in G2-phase. This effect was further proved with increase of cyclin B1 and Ki-67 expression, and of topoisomerase II activity. ECM-gel, OSCORT-ECM and HSPG also increased 92 kD matrix-metalloproteinase-9 activity.
Conclusion: We conclude that ECM has significant role in maintenance of malignant phenotype of human osteosarcoma, and a unique heparan sulfate proteoglycan produced by osteosarcoma cells may partly be responsible for this effect, which can be addressed as potential therapeutic target in the future.

342

In vivo cellular and molecular multicolor imaging with GFP and RFP

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Strong fluorescent labeling with green fluorescent protein (GFP) along with inexpensive video detectors, positioned external to the mouse, allows the monitoring of details of tumor growth, angiogenesis, and metastatic spread in mouse models. Opening a reversible skin-flap in the light path increases detection sensitivity to the single-cell level on internal organs. Single tumor cells, expressing GFP, seeded on the brain can be imaged through a scalp skin-flap. Lung tumor micro-foci representing a few cells are viewed through a skin-flap over the chest wall while contralateral micrometastases were imaged through the corresponding skin-flap. Pancreatic tumors and their angiogenic micro-vessels were imaged via a peritoneal wall skin-flap. A skin-flap over the liver allowed imaging of physiologically relevant micrometastases originating in an orthotopically implanted tumor. Single tumor cells on the liver arising from intraportal injection were also detectable (Yang, M., et al. Proc. Natl. Acad. Sci. USA 99, 3824-3829, 2002). Using the anti-apoptotic gene *bcl-2*, dominant-negative *caspase 9* (C9DN), p53 and $p16^{INK4a}$, we showed by whole-body GFP imaging that disruption of the apoptosis and senescence programs confer tumor agressiveness and drug resistance of in lymphomas which arose in E μ-myc transgenic mice (Schmitt, C.A., et al. Cancer Cell 1, 289-298, 2002; Schmitt, C.A., et al. Cell 109, 335-346, 2002). HT-1080 human fibrosarcoma labeled with GFP or RFP were injected i.v. into SCID mice. This resulted in individual green or red clones growing on the lung visualized by dual color imaging in living mice. These results indicate the possibility of color-coding cells of different genotypes *in vivo* for molecular imaging studies such as those described above.