ited by blocking cadherin, which acts an important role in cell-cell adhesion. From these results, it was suggested that constitutive phosphorylation of MET?protein in CCRC was induced by inactivation of VHL gene via cadherin. Finally, we show that NK4, antagonist for HGF/SF inhibited growth of CCRC cells.

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Prospective identification and isolation of breast cancer tumor initiating cells

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Using a model in which human breast cancer cells were grown in immunocompromised mice, we found that only a minority of breast cancer cells had the ability to form new tumors. Surface marker expression distinguished populations of cancer cells that were enriched or depleted for tumorigenic (tumor initiating) activity. We prospectively identified and isolated the tumor initiating cells as CD44 +CD24 -/lowLineage- in 8 of 9 patients. As few as 100 cells from this population were able to form tumors, while tens of thousands of other cancer cells failed to form tumors. These tumor initiating cells could be serially passaged, each time generating new tumors containing additional CD44 +CD24 -/lowLineage- tumorigenic cells as well as phenotypically mixed populations of non-tumorigenic cells. In one tumor that expressed EGF-R, tumor initiating cells that lacked detectable expression of this receptor formed tumors, suggesting that antibodies against this target would spare these cells. Inhibition of Notch 4-signaling by an anti-Notch4 antibody induced tumor initiating cells to undergo apoptosis. Effective treatment of breast cancer will require therapeutic strategies that target and eliminate the tumorigenic subset of cancer cells.

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Novel nitroimidazoyluracil prodrug derivatives as tumour-selective inhibitors of the angiogenic enzyme thymidine phosphorylase

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Introduction: An essential stage in the growth and metastasis of solid tumours is the development of new blood vessels (angiogenesis). Platelet-derived endothelial cell growth factor (PD-ECGF) has been implicated in a variety of angiogenic effects by promoting endothelial cell proliferation in a range of tumour cells. PD-ECGF is identical to the enzyme thymidine phosphorylase (TP, dThdPase, EC 2.4.2.4). TP catalyses the reversible phosphorylation of thymidine to thymine and 2-deoxyribose-1-phosphate, and it is proposed that the dephosphorylated 2-deoxyribose is responsible for the angiogenic stimulus of TP. TP/PD-ECGF has chemotactic activity *in vitro*, and angiogenic effects *in vivo* and its expression is an adverse prognostic indicator in breast, bladder, ovarian, and colorectal tumours. TP/PD-ECGF has been shown to be regulated by hypoxia and is focally expressed in the hypoxic regions of solid tumours. Thus, the hypoxic up-regulation of TP in many tumours and its angiogenic activity makes TP an attractive target for cancer chemotherapy. Hence, there would be substantial advantage in selectively inhibiting TP in the tumours where it is generating its angiogenic effects by promoting tumour growth.
Aims and Objectives: We report the design and synthesis of nitroimidazolyluracil analogues and their corresponding amino derivatives as potentially hypoxia-mediated bioreductively-activated TP inhibitors (figure). The presence of hypoxia in tumours will cause bioreductive 'activation' of the nitro prodrug to form the active amino species in areas of the tumour where TP is most highly expressed. Molecular modelling studies to the human TP predicted that the binding of the aminoimidazoyl uracil derivatives was energetically more favoured than that of their corresponding nitro counterparts. The compounds were evaluated for their inhibition against TP.
Results and Conclusion: The IC_{50} values for the 5-halo-6-[2-(aminoimidazo-1-yl)methyl]uracil analogues (B2 and B3) were ~20 nanomolar, as potent as the most effective inhibitor, 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl]-uracil hydrochloride (TPI). In contrast, the corresponding 5-halo-6-[2-nitroimidazo-1-yl)methyl]uracil analogues (A2 and A3) were approximately1000-fold less active with IC_{50} values of 22-24 micromolar (Table). This approach may be useful to selectively deliver TP inhibitors to the hypoxic areas of solid tumours.

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Expression of calmodulin-sensitive phosphodiesterase in rat tumour cell line and non-malignant astrocytes

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In many tumour cells low levels of cAMP have been observed concomitantly with an over expression of cyclic nucleotide phosphodiesterases (PDEs) of the subfamily PDE4 (Marko et al., 2000) Thus, inhibition of tumour specific or tumour associated PDEs, thereby manipulating intracellular cAMP levels, may be a promising way of tumour cell growth inhibition. In five of six highly malignant human glioblastoma cell lines originating from the 60 tumour cell line panel of the NCI, PDE1 was the predominant enzyme family, whereas PDE4 activity was minor. RT-PCR, using subtype-specific oligonucleotide primers indicated that the PDE1C subtype was predominantly expressed. In the rat glioblastoma cell line C6 we also found high PDE1C expression. PDE1C and PDE4 have affinities for cAMP in the low micro molar range. Therefore we speculated that PDE1C might play a pivotal role in cAMP homeostasis of CNS tumour cells in a similar fashion as PDE4 has been found for epithelial tumour cells. Signals for PDE1A-C transcripts were present in astrocytes, whereas in the C6 tumour cell line only signals for PDE1C were observed. Transcripts for PDE4A, B and D subtypes were detected in both cell types, however. Overall cAMP-specific PDE activity in non-malignant cells was markedly higher than in tumour cells. In tumour cells calcium/calmodulin-sensitive PDE1 appeared to be reduced, whereas the rolipram-sensitive PDE4 activity was significantly higher. Thus, despite high calcium/calmodulin-sensitive PDE activity, the expression of PDE1C isoenzymes is decreased in rat CNS tumour cells compared to primary astrocytes. Similar results were obtained from a comparison of PDE expression in human CNS tumour tissue and grey/white matter. On the other hand, PDE4 appeared to be predominantly expressed in C6 glioblastoma cells. This parallels our previous studies on human keratinocytes, where we have found a markedly higher PDE4 expression in malignant as compared to normal primary cells. Incubation of rat CNS cells with calmidazolium chloride, which is described as a PDE1 inhibitor, caused indiscriminate cell growth inhibition of non-malignant and malignant cells. The results reported here do not support the concept that PDE1 inhibition is a promising way for anticancer treatment in CNS tumours, whereas PDE4 remains a promising target.

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Eag1 potassium channel as cancer therapy target

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Potassium channels are implicated in rapid signaling processes. Their high variability and ubiquity lead to the generally accepted conclusion that they might be also implicated in many other functions. We have described a voltage-operated, potassium selective channel (Eag1) that is under strict control during the cell cycle. Eag1 is normally expressed in brain. Outside the blood-brain barrier, Eag1 expression can only be demonstrated in very restricted cell populations. This expression pattern is no longer valid for tumors, since a significant percentage of epithelial tumors show robust Eag1 expression. *In vitro* experiments allowed us to conclude that the channel is not only influenced by the cell cycle, but its overexpression can change the proliferation properties of the cells. Eag1-transfected cells grow faster than controls. They also lose contact inhibition, as well as growth factor-dependence and substrate attachment requirement In summary, Eag1-transfected cells show a transformed phenotype. Moreover, inhibition of the channel (either expression or function) in several human tumor cell lines by either antisense, small molecule, blocking antibody or short interfering RNA leads to a reduction in DNA synthesis and proliferation.