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Targeting of phosphatases (PTPases) by sodium stibogluconate (SSb) in WM-9 human melanoma for potentiation of Stat-1 phosphorylation and for antitumor effects

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PTPases are key enzymes in signaling and cell growth modulation. A gene array of WM-9 melanoma cells identified almost 20 constitutively expressed PTPases. Among the negative regulatory PTPases in cytokine signaling, only SHP-2 was identified in the gene array, presence of which in WM-9 cells was confirmed by Western blot. Using synthetic phosphatase substrates, SSb inhibited SHP-1 and SHP-2 at 10 μg/ml. In contrast, no inhibition of the MKP-1 phosphatase resulted, suggesting a specificity of inhibition. Direct cell growth inhibitory effects of SSb for WM-9 cells was demonstrated *in vitro* with approximately 50% growth suppression at 50 μg/ml when assessed either by MTT or SRB assays. When assessed in WM-9 cells transplanted subcutaneously in the nude mouse, SSb (0.5 mg/mouse/sq day) inhibited tumor growth approximately 55% with no obvious toxicity. Since SHP-2 targets tyrosine phosphorylation resulting from cytokines, effects on interferon (IFN)-induced signaling were assessed. At 10 μg/ml of SSb, phosphorylation of stat 1 was prolonged when assessed by Western blot using stat-1 anti-phosphotyrosine antibody. The direct antiproliferative effects were augmented with almost complete suppression of WM-9 growth with the combination of IFN-alpha2 (1000 units/ml) and SSb (10 μg/ml). *In vivo*, the combination of IFN-alpha2 and SSb resulted in eradication of preformed WM-9 tumors in the nude mouse, an effect not seen with either modality alone. Thus SSb had antitumor activity against melanoma possibly through inhibition of SHP-2 (absence of SHP-1 in WM-9 cells was identified both on the gene array and by Western blot). Targeting of signaling through the low molecular weight phosphatase inhibitor, SSb, may be a useful experimental and possibly clinical approach for prolonging cytokine signaling.

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Identification of genes associated with multiple myeloma and monoclonal gammopathy of undetermined significance using the myeloma microarray

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Gene expression profiling using DNA microarrays has great potential to improve the understanding, diagnosis, and management of multiple myeloma. The near completion of the human genome sequencing project further increases the analytical power of this technology. A cDNA microarray uniquely suitable for the analysis of multiple myeloma has been developed, the Myeloma microarray. To identify genes that are dysregulated in plasma cell disorders 6 subtracted libraries were constructed to enrich for myeloma or plasma cell specific cDNA sequences. cDNA pools from monoclonal gammopathy of undetermined significance (MGUS) (1 library), smouldering multiple myeloma (SMM) and multiple myeloma (MM) (3 libraries) and myeloma cell lines (2 libraries) were subtracted against cDNA pools of related normal hematopoietic tissues (including CD138+ selected normal bone marrow derived plasma cells) or normal non-hematopoietic tissues. 6,000 cDNA fragments were then analyzed using DNA microarray technology. Genes dysregulated in plasma cell disorders were identified using pairs of fluorescence-labeled cDNA probes synthesized from MGUS, SMM, MM and myeloma cell lines (n=35) and normal tissue poly A+ RNAs (n=35). Over 420,000 hybridization signals were analyzed. Gene cluster analyses demonstrated significant similarities between MGUS and SMM, clearly distinguishing the gene expression profile of these 2 diseases from that of multiple myeloma. Expression patterns of 52 genes overexpressed in multiple myeloma were confirmed and characterized further by Real Time PCR using a panel of cDNAs comprising of multiple myeloma (including CD138+ sorted myeloma cells), normal tissues and MACS sorted hematopoietic subpopulations. In addition to genes known to be associated with MM or plasma cells (including CD138, VGEF receptor, IL6 receptor), we identified 3 novel genes Ly1728, Ly1732 and Ly1851 which are highly overexpressed in the majority of multiple myeloma patients. Ongoing studies evaluate the diagnostic and prognostic value of these genes.

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The histone deacetylase inhibitor LAQ824 is selectively toxic to tumor cell lines including multidrug resistant cells

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Modulation of chromatin structure through histone acetylation is emerging as one critical mechanism of gene expression in normal and abnormal cell physiology. Histone acetylase transferases (HATs) and histone deacetylases (HDACs) regulate the steady-state acetylation state of histones. The histone deacetylase HDAC1 is overexpressed in prostate and gastric tumors and treatment of tumor cell lines with HDAC inhibitors results in increased expression of cell growth inhibitors such as p21 and p27, leading to cell differentiation, cell cycle arrest or apoptosis. We have developed a novel HDAC inhibitor NVP-LAQ824 and showed that it activates p21 expression and inhibits cell growth. In cell proliferation assays, NVP-LAQ824 selectively induces apoptosis in tumor cells. This provides a potential therapeutic window for anticancer therapy. Additionally, NVP-LAQ824 inhibits growth of established human tumor xenografts independent of the p53 tumor suppressor gene status. To further investigate the mechanism by which NVP-LAQ824 selectively inhibits cell growth, normal fibroblasts (NDHFs) and HCT116 colon tumor cell lines were treated with the compound and the effect on cell cycle distribution evaluated. FACS analysis revealed a G1 and and G2/M phase arrest in NDHFs but only a G1 phase arrest in the HCT116 cell lines. The drug-induced decrease in G2/M phase in HCT116 cells was accompanied by increased accumulation of cells in a sub-G1 phase reminiscent of apoptotic cells. This suggests the existence of a G2/M checkpoint mechanism in NDHFs that is absent in the HCT116 cells, leading to inappropriate G/2M progression. In support of a compromised G2/M checkpoint in NVP-LAQ824-sensitive cells, treatment of H1299 cells which are relatively resistant to NVP-LAQ824 and sensitive HCT116 cells with three HDAC inhibitors NVP-LAQ824, MS-275 or Trapoxin resulted in increased expression of the mitotic check point protein MAD1 in H1299 cells but not in HCT116 cells. Furthermore, to determine whether NVP-LAQ824 is subject to P-glycoprotein (P-gp) mediated multidrug resistance (MDR), we tested the compound against the MDR cell lines MDA/T0.1 and KB8511. MTS assays indicated similar sensitivity of the parental cells and their MDR derivatives to NVP-LAQ824. In contrast, paclitaxel, was approximately 200-fold less effective in the two MDR cell lines. In conclusion, NVP-LAQ824 has selective toxicity against cancer cells with potential utility against multidrug resistant tumors.

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Inactivation of VHL gene induces constitutive phosphorylation of MET protein in clear cell renal carcinoma: MET protein as a novel target for renal carcinoma therapy

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The MET tyrosine kinase receptor is a product of MET proto-oncogene on chromosome 7q. MET protein is phosphorylated and activated by stimulation of hepatocyte growth factor/scatter factor (HGF/SF). Activated MET protein binds to a variety of second messengers to activate these signal pathways, and regulates cell growth, morphology and motility. MET is expressed in a wide variety of organ and acts important roles in organ development, organ reconstruct, and oncogenicity. In kidney, it is expressed in renal proximal tubule epitherial cells (RPTEC), which is normal origin for renal carcinoma. We previously reported that mutations in the tyrosine kinase domain of the MET gene induced constitutive phosphorylation of MET protein and predispose to papillary renal carcinoma, which occupies about 5-10% of human kidney cancer. Clear cell renal carcinoma (CCRC) which occupies over than 80% of human kidney cancer is caused by inactivation of VHL gene, tumor-suppression gene. The mutations of MET gene were not detected in CCRC, but it was previously reported that overexpression of MET gene in CCRC. We monitored the phosphrylation of MET protein in CCRC cell lines and normal origin, renal proximal tubule epitherial cell (RPTEC) to understand a role of MET protein in oncogenicity of CCRC. It was detected that MET protein was overexpressed in CCRC cell lines compared with RPTEC. MET protein was activated constitutively in CCRC, although activation of MET in RPTEC need stimulations of HGF/SF. The constitutive activation of MET protein was inhibited by overexpression of exogeneous normal VHL genes. The constitutitve phosphorylation was not regulated by autocline roop by growth factors including HGF/SF. Additionally, the MET dephosphorylation regulated by exogeneous normal VHL gene was inhib-

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.