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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-