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Effects of PPI-2458, an inhibitor of methionine aminopeptidase type-2, on growth of melanoma and expression of MITF

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Background: The purpose of this study was to determine the anti-proliferative activity of PPI-2458, an orally available methionine aminopeptidase type-2 (MetAP-2) inhibitor, on the growth of human melanoma cells *in vitro* and in a mouse melanoma xenograft model. This study further investigated the mechanism(s) of PPI-2458 induced growth inhibition.
Material and Methods: Human melanoma UACC-62, A375 and M14 cell proliferation was measured by [^{3}H]-thymidine incorporation. The amount of MetAP-2 inhibited in cell lysates was measured with a MetAP-2 pharmacodynamic assay and the expression of the microphthalmia associated transcription factor (MITF) and MetAP-2 proteins was determined by Western blot. In the mouse UACC-62 xenograft model, PPI-2458 was administered orally at 30 mg/kg, every other day (QOD).
Results: PPI-2458 inhibited the growth of the UACC-62, A375 and M14 cell lines with GI_{50s} of 1, 5 and 2 nM, respectively. This growth inhibition was related to the amount of MetAP-2 enzyme inhibited in these cells, consistent with previous reports that show a link between PPI-2458 induced growth inhibition and the level of MetAP-2 inhibition. In mouse B16F10 melanoma cells, prolonged exposure of these cells *in vitro* to high concentrations of PPI-2458 did not result in resistance to this agent, but induced melanogenesis, a morphological feature of differentiated melanocytes. Here we show that melanogenesis was associated with the downregulation of MITF protein expression, an unexpected finding given the role of MITF as a positive regulator of this process. In contrast to B16F10 cells, prolonged exposure of human UACC-62, A375 and M14 cells to high concentrations of PPI-2458 did not induce melanogenesis, but moderate downregulation of MITF protein expression was detected. Moreover, transfection of MITF siRNA inhibited the growth of all three melanoma cell lines, albeit at different levels, and further sensitized these cells to treatment with PPI-2458. In the UACC-62 xenograft model PPI-2458 (PO, 30 mg/kg, QOD) inhibited tumor growth and almost completely inhibited MetAP-2 in the white blood cell and tumor compartments 24 hours after the final dose.
Conclusions: PPI-2458 inhibited the growth of human melanoma cells *in vitro* and *in vivo* and this activity was linked to the amount of MetAP-2 inhibited in these cells. Moreover, MITF may have a role as a downstream effector of PPI-2458 induced growth inhibition in melanoma cells.

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Class I histone deacetylase isoforms in prostate cancer: expression patterns in vivo and functional implications in vitro

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Background: Histone deacetylase (HDAC) inhibitors have been shown to act as supressors of tumor growth in vivo and in vitro by inducing growth arrest, differentiation and apoptosis of transformed cells. These effects are mediated by hyperacetylation of histones, which leads to transcriptional alteration of a small subset of genes. The different functions of specific HDAC isoforms in this background and their expression in tumor tissue are largely unknown.
Material and Methods: Expression patterns of HDAC1, HDAC2 and HDAC3 were studied by immunohistochemistry in a cohort of 192 prostate carcinomas and subsequently correlated with clinicopathological parameters and patient survival. Further, the effect on cell proliferation of the prostate carcinoma cell line PC-3 after treatment with valproic acid (VPA) and suberoylanilide hydroxamic acid (SAHA) as well as isoform specific RNA interference (RNAi) was investigated.
Results: We could show an overexpression of all three HDAC isoforms in the majority of prostate cancer cases (HDAC1: 69.8%, HDAC2: 74%, HDAC3: 94.8%) compared to non-neoplastic prostate parenchyma. Overexpression of HDAC1 and HDAC2 correlated with tumor dedifferentiation as determined by Gleason Grade (HDAC1: $p = 0.006$, HDAC2: $p = 0.047$). In addition, expression of HDAC2 but not of HDAC1 and HDAC3 had an independent prognostic effect on relapse-free patient survival. A significant growth arrest in PC-3 cells could be achieved by the use of VPA and SAHA as well as selective HDAC3 RNAi. Both SAHA and VPA induced a dose dependent decrease of HDAC protein levels for all three HDAC isoforms.
Conclusions: Our study shows that HDAC isoform expression is prognostically important in prostate cancer and suggest that specific HDAC inhibition might serve as an interesting approach for novel targeted chemotherapy in this tumor entity.

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Activity of the histone deacetylase (HDAC) inhibitor PXD101 in preclinical prostate cancer studies

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Background: PXD101 is a novel hydroxamate that potently inhibits the enzymatic activity of histone deacetylase (HDAC) at nanomolar concentration. In the present investigation, we examined the growth-inhibitory activity of PXD101 on human prostate cancer cell lines in vitro and in vivo.
Material and Methods: Four human prostate cancer cell lines were used for the in vitro analysis: PC-3, DU145, LNCaP and 22RV1. Growth-inhibition was determined using CellTiter-Glo readout and cell-cycle analysis was performed via FACS following staining with propidium iodide. In vivo anti-tumor activity was assessed using an orthotopic prostate cancer model following implantation of PC-3 cells into the posterior lobe of the prostates of athymic nude mice.
Results: In vitro, PXD101 inhibited the growth of all four cell lines at high nanomolar potency following three days of exposure. Under these conditions, PXD101 appeared to be cytotoxic on some cell lines (e.g. 22RV1), but primarily cytostatic on other cell lines (e.g. PC-3). Cell-cycle analyses demonstrated that PXD101 induced G2/M arrest in PC-3 cells. Washout and timecourse experiments indicated that PC-3 cells required exposure to PXD101 for >12h for complete growth-inhibition and that PXD101 demonstrated dose-dependent cytotoxicity on PC-3 cells following five days of exposure. In the orthotopic tumor model, PXD101 administered i.p. twice daily at 20 mg/kg and 40 mg/kg inhibited tumor growth by 27% and 35%, respectively, and administered thrice daily at 40 mg/kg inhibited tumor growth by 43%. Moreover, metastatic lung lesions were observed in 47% (8/17) of vehicle-treated animals, but in none of the animals administered PXD101. None of the treatment groups exhibited body weight losses greater than 10%.
Conclusions: These results indicate that PXD101 is active in preclinical prostate cancer models and support the clinical evaluation of PXD101 for the treatment of this disease.

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Development of web-based bioinformatics tools to reveal relationships between gene expression, gene function and anticancer drug response

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A large and growing body of research demonstrates the value of gene expression data to fundamentally improve our understanding of cancer progression, disease susceptibility, drug resistance and drug response. Microarrays provide for the identification of cancer biomarkers for early cancer detection, identification of drug molecular targets, indications for cancer outcome and recurrence, as well as patient response to treatment. However, scientists seeking to harness the potential of microarrays can be rapidly overwhelmed during the data analysis process because the statistically appropriate handling of these data is specialized and multidimensional. Because an enormous harvest of information is obtained from each microarray experiment, specialized bioinformatics tools are required to best discriminate meaningful biological information from these data. Integrating intuitive software that allows users to capture, manage, and analyze anticancer drug development information from microarray experiments will be critical to deriving novel scientific insight from experimental results. Our objective is to make available to clinicians and researchers user-friendly, web-based visualization and mining of microarray data to facilitate the discovery of unknown relationships regarding gene function and drug response. In order to accomplish this task the following elements are required: a customized relational database infrastructure for the basic storage, search, management, and retrieval of microarray data; Specialized web-based microarray analysis tools to better identify novel candidate biomarkers; OMIM, gene ontology and pathway links to enable researchers to better estimate the functional significance of candidate biomarkers; and a user-friendly Graphical User Interface (GUI). As an example to illustrate the input, output and analysis process, we compared the gene expression profiles of HT29 cells grown to 70% confluency in McCoys 5A media with 10% FBS and 1% penicillin/streptomycin. The cells were treated with 1616 nM of 5-FU for 48 hrs. By allowing users to link expression patterns, like the ones illustrated, with data from other domains, such as OMIM, gene ontology, molecular pathways, and clinical outcomes, we hope to stimulate new insights into the biological significance of microarray expression results and promote the development of new hypothesEs into the mechanisms of anticancer drug response.