chaperone upon exposure to oxidative stress and many Prx isozymes are overexpressed in a variety of human diseases including cancers. Although biochemical properties of Prx isozymes have been extensively studied, their physiological role in human cancer cells remains obscure and certainly warrants further study. Here we demonstrated that human (h) Prx II, as functions as a molecular chaperone for cancer cell survival, and that this function is associated with its inhibition of proteasome.
Materials and Methods: On human cervical cancer cell line HeLa, a stable transformant with h Prx II DNA construct that overexpressing prx II protein has been developed. MTT assay and immunoblotting including ubiquitination were done.
Results: Stably transformed HeLa cells overexpressing prx II protein (HeLa-prx), compared with parental cells(HeLa) showed a significant resistance to cytotoxic assaults by drug treatments including doxorubicin and taxol. With treatment of taxol, cyclin D1, a major oncoprotein to drive cell division, was less decreased in HeLa-prx cells compared with parental cells. The fragment of PARP, an indicator of apoptosis was less observed in HeLa-prx cells with treatment of taxol, suggesting prx II makes cancer cells to be more resistant to cytotoxic agent. With treatment of proteasome inhibitor MG-132, the protein level of cyclin D1 was recovered, showing that it is regulated by proteasome. In HeLa-prx cells, compared with parental cells, protein ubiquitinations were significantly less occurred either in control state or in taxol treatment. This result suggests that Prx II protein inhibits the function of proteasome.
Conclusion: Prx II protein trigger chaperone functional switch to inhibit the function of proteasome. This change is primarily protects certain kind of oncoprotein such as cyclin D1. The chaperone function finally protects HeLa cells from cytotoxic drug-induced cell death in cells overexpressing Prx II protein.

393 POSTER

The multidrug transporter MRP4/ABCC4 is a powerful marker of poor prognosis in neuroblastoma and a target for therapeutic suppression

M.D. Norris[1], J. Smith[1], C. Flemming[1], L. Lyn[2], J. Allen[2], S.L. Cohn[3], W.B. London[4,5], A. Buxton[5], G.M. Marshhall[1], M. Haber[1]. *[1]Children's Cancer Institute Australia, Sydney, Australia; [2]Centenary Institute of Cancer Medicine and Cell Biology, Sydney, Australia; [3]Northwestern University School of Medicine, Chicago, USA; [4]University of Florida, Gainesville, USA; [5]Children's Oncology Group Statistics Department, Gainesville, USA*

Several members of the Multidrug Resistance-associated Protein (MRP/ABCC) family of transporters are associated with cytotoxic drug resistance which may contribute to chemotherapy failure. We have previously shown, both retrospectively and prospectively, that high-level expression of MRP1 is strongly predictive of poor outcome in neuroblastoma (NEJM, 334:231–8, 1996; JCO, 24:1546−53, 2006), and that MRP1 can be regulated by the MYCN oncogene (Oncogene 23:753−62, 2004). We have further shown MRP4 expression to be prognostic of outcome in a small cohort of neuroblastomas (MCT, 4:547−53, 2005). We have now examined expression of MRP2, 3, 4 and 5 in a large prospectively accrued cohort ($n = 209$) of primary untreated neuroblastomas from patients enrolled on POG biology protocol 9047. Real-time PCR was used to determine gene expression. Older age, advanced stage, and MYCN amplification were all predictive of poor outcome. Amongst MRP2–5, only MRP4 (ABCC4) expression was significantly higher in poor-prognosis MYCN–amplified versus non-amplified tumors ($p < 0.0001$). Unlike MRP2, 3 and 5, high levels of MRP4 were also highly predictive of decreased event-free-survival (EFS) ($p < 0.0001$) and overall survival (OS) ($p < 0.0001$). Following adjustment for the effect of MYCN amplification and other prognostic indicators by multivariate analysis, MRP4 expression retained significant prognostic value for both EFS (hazard ratio 2.7; $p = 0.0141$) and OS (hazard ratio 2.7; $p = 0.0180$), whereas MYCN amplification lost prognostic significance. These data, together with the close correlation observed between expression of MYCN and MRP4 ($r < 0.830$; $p < 0.0001$), suggested that MYCN regulates MRP4 expression. Support for this was obtained from promoter analysis studies and analysis of MRP4 levels in tet-regulated MYCN-inducible cells. Collectively, these data confirm MRP4 expression as a powerful prognostic marker in childhood neuroblastoma and indicate that MRP4 is a target for therapeutic suppression. Since clinically relevant modifiers of MRP4 are lacking, we have screened a focussed chemical small molecule library and isolated a number of novel specific inhibitors of this drug transporter with potential clinical utility, which are currently undergoing characterisation. These small molecule inhibitors have the potential to be used in the treatment of this disease and in other cancers in which MRP4 has a clinically relevant role.

394 POSTER

Hydroxamate histone deacetylase inhibitor selectively degrades Aurora A via HDAC6/Hsp90 pathway

J.H. Park[1], J.H. Lee[1], D.Y. Oh[1,2], S.A. Im[1,2], Y.J. Bang[1,2], T.Y. Kim[1,2]. *[1]National Research Laboratory for Cancer Epigenetics, Cancer Research Institute, Seoul, Korea; [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea*

Background: Histone deacetylase inhibitor (HDI) is emerging as a promising anticancer therapy based on its potent antiproliferation activity and tumor-selectivity. The molecular mechanisms underlying the cytotoxicities of HDIs against cancer cells remain poorly understood. Aurora A and Aurora B kinases are essential for the regulation of chromosome segregation and cytokinesis during mitosis. The overexpression or amplification of Aurora kinase leads to genetic instability and its inhibition has shown significant antitumor effects. Here, we report that structurally related hydroxamate LAQ824 and SK-7068 induce tumor-selective mitotic defects by depleting Aurora A.
Materials and Methods: Antitumor activities of HDIs were analyzed by using MTT assay, cell cycle analysis, MPM2 staining, and immunofluorescence microscopy. Expressions and localizations of Aurora kinases were analyzed by using western blotting and immunofluorescence microscopy. Histone deacetylase (HDAC) 6/Heat shock protein (Hsp) complex dependent regulation of Aurora A was analyzed by using co-immunoprecipitation assay, immunofluorescence microscopy, MALDI-TOF mass spectrometry, and HDAC inhibition assay.
Results: We found that HDI-treated cancer cells, unlike normal cells, exhibit defective mitotic spindles. Following HDI, Aurora A was selectively downregulated in cancer cells, whereas Aurora B remained unchanged in both cancer and normal cells. LAQ824 or SK-7068 treatment inhibited HDAC6 present in Aurora A/Hsp90 complex. Inhibition of HDAC6 acetylated Hsp90, and resulted in dissociation of acetylated Hsp90 from Aurora A. As a result, Hsp70 binding to Aurora A was enhanced in cancer cells, leading to proteasomal degradation of Aurora A. On the other hand, no complex formation was observed between Aurora B and HDAC6.
Conclusions: In conclusion, these data suggest that mitotic abnormality in cancer cells could be a target of HDI. By reducing centrosomal Aurora A in cancer cells, HDI induces mitotic cell death, which is linked with its tumor-selective cytotoxicity. The outcome of cancer treatment depends on the defects of cancer cells, which include genetic and epigenetic changes. Therefore, given that both epigenetic silencing and mitotic abnormalities such as Aurora A overexpression are common in malignancies, our data indicate that hydroxamate HDI such as LAQ824 or SK-7068 is likely to be a more effective HDI in cancer cells overexpressing Aurora A.

395 POSTER

CpG island methylator phenotype (CIMP): a novel biomarker to predict new therapy for breast cancer

Y. Yu[1], W. Feng[1], L. Shen[2], D.G. Rosen[3], J. Liu[3], Y. Shen[4], K.K. Hunt[5], R. Orlandi[6], J.-P. Issa[2], R.C.J. Bast[1]. *[1]University of Texas, M.D. Anderson Cancer Center, Experimental Therapeutics, Houston, USA; [2]University of Texas, M.D. Anderson Cancer Center, Leukemia, Houston, USA; [3]University of Texas, M.D. Anderson Cancer Center, Pathology, Houston, USA; [4]University of Texas, M.D. Anderson Cancer Center, Biostatistics, Houston, USA; [5]University of Texas, M.D. Anderson Cancer Center, Surgical Oncology, Houston, USA; [6]Istituto Nazionale Tumori, Milan, Italy*

Background: Although clonal, epigenetic changes are potentially reversible. The classic DNMT inhibitors, 5-aza-C and 5-aza-dC, are cytosine analogs that are incorporated into replicating DNA. One potential consequence of incorporation is the reactivation of previously methylated tumor suppressor genes that have been transcriptionally silent. These analogs have been used clinically for treatment of cancer patients. A CpG island methylator phenotype (CIMP) is defined as concordant methylation of multiple genes. CIMP has been reported in leukemia, colon cancer and lung cancer, and used to predict response to demethylating therapy, but it has not been defined in breast cancer. To investigate if CIMP does exist in breast cancer and play a role in tumorigenesis, we studied the methylation profile in normal/tumor breast tissues.
Material and Methods: We have screened 10 known tumor suppressor genes (ARHI, RASSF1A, hMLH1, HIN-1, CDH13, RIL, E-cadherin, p16, 14−3–3 sigma, RIZ1) in 6 breast cancer cell lines, 2 normal breast epithelial cells, 91 pairs of breast cancers and adjacent normal breast tissues, and 8 pairs of primary and metastasis breast cancer tissues using a new technique known as Pyrosequencing Methylation Assays.
Results: Pyrosequencing has been shown to be a quantitative and reliable technique. Four of ten tumor suppressor genes, RASSF1A, RIL, CDH13 and HIN-1, were frequently methylated (49%, 47%, 36% and 42%,