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.

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

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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.

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

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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.

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

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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%,

respectively) in breast cancer tissues, but not or low in normal breast tissues. Significant association was found between methylation of HIN-1 and of RASSF1A (R = 0.48, P < 0.00001) and of RIL and CDH13 (R = 0.23, p = 0.025). In addition, global hypomethylation (LINE) was correlated with RIL and RASSF1A hypermethylation (P < 0.05). In these 91 cases, 22 (24.2 %) had 3 or 4 genes methylated (CIMP ++), 33 (36.2%) had 2 genes methylated (CIMP +), 19 (20.9%) had 1 gene methylated (CIMP ±) and 17 (18.7%) had 0 gene methylated (CIMP −). The data indicate that, similar to colon cancer and leukemia, CIMP does exist in breast cancer.
Based on first 37 cases with clinical information available, we found that the CIMP positive group has more cases with late stage, larger tumor size, and positive lymph nodes than does the CIMP negative group. Further more, in 4 of 8 cases of paired primary/metastasis tumor samples, which have equal percent of tumor cells, we have found that methylation levels of HIN-1 or RIL in metastatic tumors was higher than their primary tumors. Hypermethylation of tumor suppressor genes may play a role in metastases of breast cancer.
Conclusions: Consequently, our data support that CIMP is a novel biomarker for clinical classification of breast cancer. Hypermethylation of multiple tumor suppressor genes, especially in matastatic tumors may predict new demethylating therapy. Our studies provide the rational for large, adequately powered examination of this clinical issue.

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Broad spectrum and potent anti tumor activity of YM155, a novel small molecule survivin suppressant, against a large scale panel of human tumor cell lines

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Survivin is one of major transcriptomes in human cancers, and its multiple essential functions give large impacts on cancers to survive and progress. Therefore, survivin stands out as an attractive target for novel cancer therapeutics. YM155 is a novel small molecule survivin suppressant which is entering into phase II trials in various cancers. YM155 induces massive tumor regressions in experimental human hormone refractory prostate cancer (HRPC) and non-small cell lung cancer (NSCLC) models, and is expected to show antitumor activity as a novel type of chemotherapeutic options with a clear survivin suppressive activity (AACR-NCI-EORTC 2005, Abstract #B203, AACR Annual Meeting 2006, Abstract #5671). In this study, we evaluated the antiproliferative activities of YM155 against a panel of 127 human tumor cell lines using the sulforrhodamine B assay. Survivin mRNA level in each tumor cell line was also quantified by an real time PCR with an ABI PRISM® 7900. YM155 showed antiproliferative activity against 123 cell lines with mean log GI50 values of −7.85 (14 nM), and was markedly potent against a majority of cell lines of the different tumor types, especially the HRPC, melanoma, NSCLC, breast cancer, ovarian cancer, sarcoma, lymphoma and leukemia cell lines. All the tested cell lines expressed high levels of survivin, but the survivin expression levels were marginally correlated with the GI50 values of YM155. Its antiproliferative activities were also not related to p53 status, and YM155 showed almost equal drug sensitivities against the cell lines with normal and mutated (or truncated) p53. SHP-77, MCF-7/ADR, MCF-7/mdr1 and A549/R cell lines expressing the multi-drug resistance (MDR) phenotype were resistant to YM155. In A375, and SK-MEL-5 human malignant melanoma xenograft models, 3-day continuous infusions of YM155 showed potent antitumor activities, including tumor regressions at doses ranging from 1 to 10 mg/kg with no decrease in body weight. These results clearly demonstrate that YM155 shows broad spectrum and potent antiproliferative activities against various human cell lines, and also suggest that the drug sensitivity of YM155 is not simply explicable with the survivin expression levels or p53 status. Further extensive studies on multiple factors to explain how YM155 achieves broad therapeutic prospects in various type of cancers are necessary to characterize the most sensitive tumor types to YM155.

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Potentiation of the antitumor activity of bortezomib, a proteasome inhibitor, by the combination with EGFR inhibitors in human cancer cell lines

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Methods: The antiproliferative activity of bortezomib alone or in combination with gefitinib, ZD6474, or cetuximab was evaluated in human lung (A549, GLC-82, Calu3), colon (GEO, HCT-15, HCT-116, HT-29), pancreatic (MiaPaca2), and esophageal (KYSE-30) cancer cell lines which possess a functional TGF-EGFR autocrine pathway, by using MTT and soft agar colony formation assays. Combination effects were analyzed according to the Chou and Talalay method. Cell cycle distribution and apoptosis were quantitated by flow cytometry. Effects on protein expression were determined using western blotting techniques.
Results: Bortezomib determined a dose-dependent growth inhibition in the nine cancer cell lines (IC_{50} values, range 6 to 42 nM). A significant synergistic antiproliferative effect was observed with the combination of bortezomib with either gefitinib, cetuximab, or ZD6474 in all nine cancer cell lines (combination index values, range 0.10−0.55). This effect was accompanied by a significant induction in apoptosis by the combined treatment with bortezomib and each EGFR inhibitor. Western blot analyses demonstrated that bortezomib induced a reduction in total and phosphorylated (P)-EGFR expression, an induction in P-MAPK and in p27 expression, with no changes in the expression of total and P-akt, MAPK, bcl-2, bcl-xL, and p21. In contrast, the combined treatment with bortezomib and each EGFR inhibitor caused an efficient suppression in P-EGFR, P-MAPK and P-akt levels with a parallel significant increase in p27 protein.
Conclusions: These results provide the rationale basis to translate in a clinical setting the combination of a proteasome inhibitor with an EGFR inhibitor as a multi-targeted treatment for human cancer.

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Discovery and characterization of a small molecule inhibitor of human Cdc7 kinase

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Background: Cdc7 kinase plays a pivotal role in regulating DNA replication in eukaryotic organisms. Genetic evidences indicate that Cdc7 inhibition can cause selective tumor cell death in a p53 independent manner supporting the rational for developing Cdc7 small molecule inhibitors for the treatment of cancers. Here we described the first small molecule selective Cdc7 inhibitor: PHA-767491A.
Material and Methods: The activity of PHA-767491A as a kinase inhibitor and as anti-proliferative agent, was determined using conventional biochemical and cellular assays. The effects of PHA-767491A on DNA replication were studied using DNA combing technique. Anti-tumor activity was determined in mice bearing sub-cutanously implanted tumours.
Results: We have identified PHA-767491A as a low nanomolar inhibitor of human Cdc7 kinase and found that PHA-767491A blocks proliferation of multiple cell lines at low micromolar concentration.
Molecular studies indicate that the primary target of PHA-767491A is the initiation reaction of DNA replication since origin firing but not replication fork speed is decreased in treated cells. Furthermore phosphorylation levels of Mcm2 protein at specific Cdc7 dependent phospho-sites drop upon drug treatment. Consistent with specific inhibition of Cdc7 kinase, PHA-767491A does not affect transition through mitosis once DNA replication is already completed.
Similarly to Cdc7 depletion by siRNA, pharmacological inhibition of the kinase causes p53 independent apoptosis in several tumor cell lines while it only causes reversible cell cycle arrest in primary fibroblasts. In this model a p53 dependent pathway is important to maintain viability during compound treatment. PHA-767491A has antitumor activity as single agent that is more pronounced in a leukaemia derived xenograft model.
Conclusions: PHA-767491A is the first small molecule that specifically inhibits the initiation of DNA replication through a mechanism that is consistent with the inhibition of Cdc7 kinase. Characterization of PHA-767491A mechanism of action support the notion that pharmacological inhibition of Cdc7 kinase may provide novel tools to tackle DNA replication in a variety of tumors.