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.