Results: Both malignancies showed high HDAC1 expression ranging from 38.7% in DLBCL to 59.2% in CTCL (P = 0.10). Almost all samples (97%) showed moderate or high level of HDAC2 expression. High HDAC2 expression ranged from 31.2% in CTCL to 60.6% in DLBCL (P < 0.0001). In total, 21 CTCL showed high HDAC2 expression. These included 12 of 17 cases with aggressive histology or phenotype. HDAC6 showed low to moderate level of expression in both lymphomas. Low level of HDAC6 was observed in 29.4% of DLBCL and 30.7% of CTCL (P = 0.89). High level of acetylated H4 was more common in DLBCL (35.3%) compared to CTCL (14.3%) (P = 0.02). Furthermore, high H4 acetylation seemed to be more common in DLBCL belonging to the activated B-cell like (ABC) category (42.1%, 8 of 19) than in the less aggressive germinal center B-cell (GCB) type (10%, 1 of 10) (P = 0.12).
Conclusion: The prevalence of HDAC2 expression in both malignancies, and particularly in the more aggressive phenotypes of CTCL, suggest a possible involvement of HDAC2 in the development of malignant lymphomas. The low level of H4 acetylation in CTCL might be encouraging as a possible biomarker for HDACi response. These observations should however be validated in prospective studies.

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Hypermethylation induced SPARC, TIMP-3 and PENK down-regulation in endometrial cancer

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Background: Endometrial cancer remains the leading cause of gynecological cancer. This study was to investigate the genes down-regulated in the endometrial cancer.
Methods: Initially, we performed a genome-wide analysis of gene expression in a set of 55 sporadic endometrioid endometrial adenocarcinomas and compare this to 29 normal endometrium controls using microdissection and high density oligonucleotide microarray. To identify signaling pathways that are associated with cervical tumorigenesis, microarray expression data were imported into a PathwayAssist software. We further measured methylation of the three genes using methylation-specific polymerase chain reaction (MSP-PCR) in a total of 76 endometrioid endometrial cancers
Results: We obtained a dominant signaling pathway in which 25 genes were coordinately regulated in endometrial cancer. These genes encode for proteins that are part of a signaling pathway associated with cell cycle progression and invasion. Of the 25 genes, SPARC (5q31.3−q32), TIMP-3 (22q12.1−12.3) and PENK (8q23−q24) were down-regulated in cancer for 2.9-fold, 3.48-fold and 3.12-fold, respectively, when compared to normal. The results showed hypermethylation of SPARC, TIMP-3 and PENK in 99%, 25% and 95% of these cancers, respectively. Furthermore, we found the hypermethylation of TIMP-3 was correlated to clinical stage of the tumors and total survival of the patients.
Conclusions: The results obtained from this study indicate that hypermethylation induced down-regulation of SPARC, TIMP-3 and PENK might be related to the development and progression of endometrioid endometrial cancer. Demethylation and reactivation of the three genes may be as an adjunct therapy for the endometrial cancer.
Acknowledgment: Funded by Research Grants Council of the Hong Kong SAR (CUHK4427/03M).

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Secreted frizzled-related protein 4 inhibits proliferation and metastatic potential in prostate cancer

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Background: Activation of the Wnt signaling pathway is implicated in aberrant cellular proliferation in a variety of cancers. In prostate cancer, Wnt 3A signaling promotes cell growth through an androgen-dependent interaction with downstream beta-catenin signaling. Secreted Frizzled-Related Protein 4 (sFRP4) inhibits Wnt signaling by binding and sequestering the Wnt ligand. Our group has already demonstrated that increased expression of membranous sFRP4 predicts for a good prognosis in localized prostate cancer. Thus the aim of this project was to investigate the phenotype of sFRP4 overexpression in androgen-dependent and androgen-independent prostate cancer models.
Material and Methods: sFRP4-overexpressing androgen-dependent (LNCaP) and androgen-independent (PC3) prostate cancer models were established *in vitro*. Changes in proliferation and metastatic potential were assessed using Cell Titer 96® system, soft agar assays and matrigel invasion chambers. Immunofluorescence and immunohistochemistry were used to identify changes in adhesion molecules *in vitro* and *in vivo* respectively.
Results: sFRP4 overexpression in both cell line models results in a morphologic change to a more epithelioid cell type with increased membranous beta-catenin and cadherins (E-cadherin in LNCaP, N-cadherin in PC3) resulting in more adhesions between cells. Functionally, sFRP4 overexpression is associated with a decreased rate of proliferation (p = 0.0002) and decreased anchorage-independent growth in both systems (p < 0.0001) and decreased invasiveness of PC3 cells (p < 0.0001). Furthermore, in human localized prostate cancer (n = 224) increased membranous sFRP4 expression is associated with increased membranous beta-catenin expression (p = 0.02).
Conclusions: These data suggest that sFRP4 is an inhibitor of prostate cancer growth and metastasis independent of hormonal status with correlative evidence in human disease. Consequently, sFRP4 is a potential new therapeutic target for androgen-independent prostate cancer.

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The significance of Pyk2 in hepatocellular carcinoma invasiveness

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Background and Objective: Our previous study showed that proline rich tyrosine kinase (Pyk2) is over-expressed in tumor tissues as compared to their adjacent non-tumor tissue. Statistical analysis suggests that this over-expression is significantly correlated with tumor growth and venous invasion. Over-expression of Pyk2 is also significantly correlated with shorter overall and disease-free survival. However, the precise mechanism of Pyk2 on tumor invasiveness is still unclear due to the limited reports. In the current study, we aim to investigate the role of Pyk2 on the invasiveness of hepatocellular carcinoma cells by both *in vitro* functional study and *in vivo* animal models.
Materials and Methods: In the *in vitro* study, plasmids containing the full length or dominant negative form of Pyk2 was transfected into HCC cell line (PLC). After selection with antibiotics (G418), stable clones with the expression of full length or dominant negative form of Pyk2 was isolated. The invasiveness was compared according to their ability to adhere to the extracellular matrix, colony formation assay and wound healing assay. The mechanism of the Pyk2 signaling was investigated by western blotting and immunoprecipipation assay.
In vivo tumor models were done in athymic nude mice. Tumors produced from the different transfectants were implantanted into the liver of the mice. After 48 days the mice were sacrificed and tissue samples were collected. Tumor growth pattern including invasiveness was examined by H&E staining. Tumor cell proliferation (Ki67) and apoptosis (TUNEL) were compared among the groups of mice with liver tumor from different transfectants.
Results: The full length Pyk2 transfectant possessed the highest cell motility as compared to the vector control and C-terminal transfectants by wound healing assay. Pyk2 full length transfectant also presented significantly stronger adhesiveness towards collagen I, fibronectin and laminin by adhesion assay. It promoted the anchorage-independent growth as well as the anchorage dependent growth by the soft agar assay and colony formation assay. Western blotting and co-precipipation assays indicated that Pyk2 forms a signaling complex with c-Src. Phosphorylation of c-Src, MEK and ERK 1/2 were up-regulated in full length Pyk2 transfectants as compared to the vector control and C-terminal transfectants.
The tumors from the full length Pyk2 transfectants got the highest incidence of Ki67 positive staining tumor cells and least apoptotic cells as compared to empty vector control and C-terminal transfectants.
Conclusion: Over-expression of Pyk2 may contribute to an invasive phenotype of HCC.

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Peroxiredoxin II protects cancer cells in a way of proteasome inhibition

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Background: Peroxiredoxin II, a cytosolic isotype of human 2-Cys peroxiredoxin(Prx), can behave either as a peroxidase or as a molecular

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%,