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Cancer and Environmental Factors

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GENERAL INFORMATION

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CME / CPD Accreditations

	CME / CPD points awarded
Hong Kong College of Obstetricians & Gynaecologists	Pending
Hong Kong College of Paediatricians	Pending
Hong Kong College of Pathologists	5.5 (Participation)
Hong Kong College of Physicians	Pending
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Medical Laboratory Technologists Board	1.5 (AM) / 1.5 (PM)

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SPEAKERS INFORMATION

Geoffrey FARRELL

Geoffrey Farrell graduated in medicine from University of Tasmania in 1970, and trained in gastroenterology and hepatology at Royal Prince Alfred and Royal Brisbane Hospitals. After completing his M.D., he was awarded an NHMRC CJ Martin Fellowship for postdoctoral research at UCSF. In 1980, he returned to Sydney to establish a Liver Research Group at Westmead Hospital which became the Storr Liver Unit, of which he was Director until Jan 2006. He then accepted his current position of Professor of Hepatic Medicine within ANU Medical School. Among many leadership roles, he has been President of Australian Society for Medical Research 1987, President of Gastroenterological Society of Australia (GESA) 2000-2001, and Head, Department of Medicine, University of Sydney 1996-1997. He is currently Editor-in-Chief of the Journal of Gastroenterology and Hepatology.

His research interests are in non-alcoholic steatohepatitis, ischemia-reperfusion injury, hepatocellular carcinoma, viral hepatitis and drug-induced liver injury. He has published 3 books, including the first on NAFLD, and written more than 200 scientific papers and 130 reviews / chapters / editorials. His work is very highly cited, with an H-index > 60, more than 30 articles cited > 100 times, average citation 33/paper, and 15 articles cited as the subject of editorials. He has been CIA on NHMRC Program Grants, an NIH RO1 for study of hepatitis C pathogenesis, and has held more than 25 NHMRC project grants. Among several honours and awards, he has received the Distinguished Research Prize of GESA, the Eric Susman Prize for Medical Research, RACP, and delivered the inaugural Hy Zimmerman lecture at AASLD 2002 as well as 6 other named orations. He has supervised more than 25 Doctoral Research Students (Ph.D. or M.D.), eight of whom have won highly competitive Young Investigator Awards.

Yuet-kin LEUNG

Yuet-kin Leung is a highly promising young investigator in the field of hormone and cancer. He received his Ph.D. in Biochemistry at the Chinese University of Hong Kong focusing on experimental therapeutics of liver cancer. He completed his postdoctoral training in estrogen receptor and prostate cancer at the University of Massachusetts Medical School (UMass). During his tenure at UMass, he made one of the most important discoveries in the field of estrogen action through the identification and characterization of four isoforms of estrogen receptor beta, a paper he published in *PNAS* in 2006. As an Assistant Professor of the Division of Environmental Genetics and Molecular Toxicology, the Department of Environmental Health at the University of Cincinnati, his research continues to flourish. His current research focuses on the differential actions of the various estrogen receptor isoforms in breast and prostate cancer. His recent work centers around the exciting topics of developmental origin of cancer risk and the impact of environmental estrogens on epigenetics reprogramming. He is currently a full member of American Association for Cancer Research and Endocrine Society. He was awarded twice as a Next Generation Biomedical Investigator by Center of Environmental Genetics at the University of Cincinnati. He is a co-Investigator of two National Institute of Environmental Health Sciences-funded projects focusing on bisphenol A and cancer.

Eric R. PROSSNITZ

Eric R. Prossnitz completed his Ph.D. at the University of California at Berkeley and subsequently carried out post-doctoral studies at the Scripps Research Institute. He joined the faculty of the Scripps Research Institute in 1994 and was recruited to the University of New Mexico in 1997. He is currently Director of the Women's Cancers Program at the UNM Cancer Center and a co-leader of the UNM Center for Molecular Discovery. His major areas of research are in the function of G protein-coupled receptors, including chemoattractant receptors and the estrogen receptor GPR30/GPER. His studies on the mechanisms of estrogen action and GPER have elucidated novel functions in the immune, vascular and endocrine systems as well as in cancer. His efforts also led to the identification of the first selective agonist and antagonist of GPER, both of which are now widely used to assess functions of GPER.

Narci TEOH

Narci Teoh is an Associate Professor of Medicine at the Australian National University and Senior Staff Specialist, Department of Gastroenterology and Hepatology, the Canberra Hospital, Australia. Her clinical and research interests include viral hepatitis, hepatocellular carcinoma, hepatocarcinogenesis and liver ischaemia reperfusion injury.

Shu-tian ZHANG

Shu-tian Zhang, M.D., Ph.D., is the Director of Gastroenterology and Vice President of Beijing Friendship Hospital, Capital Medical University. He is also the Vice Chairman of Chinese Association of Gastroenterology, Chinese Society of Gastroenterologists and Hepatologists, Chinese Society of Digestive Endoscopy and Dean of Beijing Digestive Disease Center.

Franky L. CHAN

Franky L. Chan, Ph.D., is the Professor and Theme Chief of the “Cancer and Inflammation” Theme at the School of Biomedical Sciences, the Chinese University of Hong Kong.

His research focuses on the hormonal carcinogenesis of prostate cancer. His current studies include: (1) roles of some orphan nuclear receptors, (2) calcium channels and signaling, (3) epithelial-mesenchymal-transition, (4) hypoxic growth regulation in the development and progression of prostate cancer, (5) identification and characterization of molecules in hormone-independent prostate cancer, (6) development of *in vitro* and *in vivo* models of prostate cancer. He serves as the academic editor of *PLoS One* and editorial board member of *Scientifica*.

Wei-hua JIA

Wei-hua Jia graduated from Peking Union Medical College (Peking, China) with her M.D. and Ph.D. in 1998. She furthered her professional development as a postdoctoral fellow until 2000 at Cancer Center, Sun Yat-sen University (Guangzhou,

China), where she was promoted to Professor of Oncology and Molecular Medicine in 2006. She then experienced as a visiting scholar in Johns Hopkins University during 2005. Currently, she undertakes both the PI of cancer molecular epidemiology and the director of tumor bank department in Sun Yat-sen University Cancer Center.

Her major researches focus on cancer epidemiology, tumor markers, molecular mechanisms of tumor invasion and metastasis. She has made remarkable achievement in genetic susceptibility and gene-environment interaction in nasopharyngeal cancer. She had published more than 30 articles in peer-reviewed scientific journals including *Nature Genetics* and *Cancer Research*.

She has won support from the National Natural Science Foundation of China and other six national research projects. She has been the committee member of tumor epidemiology and tumor aetiology, Chinese Association of Anti-cancer and also vice-president of biological sample bank, China Medicinal Biotech Association. In 2009, she was awarded "Program for New Century Excellent Talents in University" by the Ministry of Education of China.

Kin-mang LAU

Kin-mang Lau is an Assistant Professor at the Chinese University of Hong Kong. He received his B.Sc. degree in Biological Sciences from the University of Hong Kong in 1991 and M.Phil. degree in Pathological Sciences from the Chinese University of Hong Kong in 1993. After graduation, he pursued post-graduate trainings in cancer research in Kimmel Cancer Institute, Pennsylvania and the Tufts University, Massachusetts in United States for 4 years and obtained a Ph.D. at the University of Massachusetts Medical School at Worcester, Massachusetts in 2002. Upon completion, he returned to Hong Kong and worked as Scientific Officer at Hong Kong Hospital Authority in charge of Molecular diagnostic services in Prince of Wales Hospital, Hong Kong. In 2005, he joined the Chinese University of Hong Kong as Assistant Professor. His research interests include hormonal carcinogenesis in prostate cancer, immune evasion in nasopharyngeal carcinoma, and genetic alterations in medulloblastoma.

Yung-hou WONG

Yung-hou Wong obtained his Ph.D. degree at the Cambridge University, and completed his postdoctoral training at the University of California, San Francisco. He joined the faculty at the Hong Kong University of Science and Technology (HKUST) in 1992 and is currently a Chair Professor of the Division of Life Science. He is also the Director of Biotechnology Research Institute and Molecular Neuroscience Center at HKUST. His research interests are in the molecular pharmacology of G protein-coupled receptors and the mechanisms of drug actions. By utilizing a combination of biochemical, immunological, and molecular biology approaches, he has made significant contributions to our understanding of how activation of drug receptors leads to cellular responses, including those involved in the development of drug addiction and cancer. His work on the structure and function of the G proteins has resulted in the creation of universal G protein adapters that serve as powerful molecular tools for drug discovery. As a highly accomplished researcher, he has published over 175 scientific papers in leading journals and holds one patent. He is the Co-Editor-in-Chief of the *Journal of Molecular Signaling* and the Associate Editor of *NeuroSignals*. He also serves on the editorial boards of several international journals, including *Journal of Neurochemistry*, *Current Medicinal Chemistry*, and *Open Medicinal Chemistry*. He was awarded the Croucher Senior Research Fellowship in 2001. Along with his research, he also actively contributes to the community. He serves as the Chairman, Research and Testing Committee of the Consumer Council of Hong Kong and is a Member of the Program Committee for the Asia-Pacific International Molecular Biology Network.

ABSTRACTS FOR SYMPOSIUM LECTURES

Obesity, non-alcoholic fatty liver disease (NAFLD) and risk of hepatocellular carcinoma

Geoffrey FARRELL
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Hepatocellular carcinoma (HCC) causes more than 500,000 deaths each year, the third leading cause of cancer mortality worldwide, and is the most rapidly increasing fatal malignancy in western countries. Obesity and its complications of type 2 diabetes mellitus (T2D) and metabolic syndrome are a world-wide pandemic; Chinese people are particularly susceptible. The liver association is with non-alcoholic fatty liver disease (NAFLD), a highly prevalent chronic disorder in developed countries, such as 27% in a recent community-based study in Hong Kong employing proton magnetic resonance imaging. NAFLD encompasses a spectrum from simple steatosis, to non-alcoholic steatohepatitis (NASH) which causes liver fibrosis that can progress to cirrhosis and HCC. NAFLD, and particularly when associated with obesity and T2D, is increasingly associated with HCC. Thus, in regions like New Zealand and Northern United Kingdom, HCC attributable only to metabolic risk factors was rare in 2000 but now approaches 5-30% of cases, while in Japan, HCC is the commonest fatal malignancy in patients with T2D. Several epidemiological studies have confirmed that obesity (BMI > 30 kg/sq m in European populations) increases relative risk of HCC by 1.5 to 2.5-fold. T2D has a similar and possibly more important effect, while data from Taiwan indicate interaction between obesity and diabetes on HCC risk in those with chronic HBV or HCV infection may surpass 10-fold increased RR. With hepatitis C, obesity, most likely through mediation of insulin resistance, increases fibrotic progression to cirrhosis and risk of HCC; T2D is an independent risk factor for HCC, as well as for disease recurrence after resection and onset of HCC among those with a sustained antiviral response to treatment.

Although obesity, possibly insulin resistance and definitely T2D have been established as important risk factors for HCC, the underlying of mechanisms are not yet clear.

This field has employed murine models to reflect HCC development in humans, particularly diethyl nitrosamine (DEN) injection at day 12-15. There are several overlapping domains of interest involving dietary factors, obesity and its metabolic and inflammatory consequences. *First*, obesity induces a state of chronic, low-grade inflammation in adipose tissue as well as liver (NASH); some evidence based on elegant molecular knockout models infers this could play a role in hepatocarcinogenesis. Park *et al*. (2010) have shown that obesity induced by high fat diet or genetic modifications increases rates of HCC compared to lean mice, and our own unpublished studies confirm earlier HCC onset in DEN model (6mo vs. 9mo). Park *et al*. considered that IL-6 and TNF signalling are potential mediators for the development and progression of obesity-induced HCC through activation of the JAK/STAT3 and ERK pathways. However, not all models show these changes. *Second*, leptin (which also signals via STAT3) and adioponectin (which opposes hepatocarcinogenic) could also be involved; serum leptin increases in obesity while adiponectin falls with T2D and NASH. Saxena *et al*., (2010) showed that adiponectin inhibited both *in vitro* and *in vivo* HCC tumorigenesis, and negatively correlated with tumor size in human HCC samples. *Third*, insulin resistance is common to obesity, T2D and NASH. Resultant hyperinsulinemia increases production and biological activity of insulin-like growth factor 1 (IGF1), a peptide capable to stimulating growth of liver cells through cellular proliferation and inhibition of apoptosis (Starley, Calcagno, and Harrison 2010). IGF1 and insulin receptor signalling intermediates, particularly Akt and mTOR, could therefore be implicated in obesity-related hepatocarcinogenesis, but data are few. *Finally*, NASH and cirrhosis both associate with increased reactive oxygen species (ROS) production and oxidative stress; this could facilitate DNA damage with subsequent chromosomal instability that initiates hepatocarcinogenesis. Increased ROS production may also promote hepatocarcinogenesis by inducing p53 mutation, which occurs in the majority of human HCCs.

In conclusion, emerging data from epidemiological studies provides convincing evidence of an association between obesity, T2D and development of HCC, with and without other liver diseases such as hepatitis C. In the context of NAFLD, up to 50% of cases may not involve the usual intermediary of cirrhosis, inferring importance of other etiopathogenic factors. Experimental work to elucidate potential underlying mechanisms of obesity-induced HCC are in the early stages. Dietary factors as well

as factors integral to obesity and its complications, such as inflammation, adipokines and growth factors/insulin receptor signalling pathways could each play a role, as could oxidative stress, DNA damage and repair. Information from ongoing studies will be valuable to develop effective dietary or pharmacological agents to prevent or treat HCC in obese or diabetic patients.

EGFR activation results in enhanced expression of COX-2 and tumor growth through activation of β_2-adrenergic receptor in esophageal squamous cell carcinoma

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Background

Esophageal cancer is one of the most common malignant tumors in the world. The etiology and molecular mechanism of esophageal squamous cell carcinoma (ESCC) is still unclear. The overexpression and activation of EGFR can lead to uncontrolled cell growth by promoting cell proliferation and inhibiting apoptosis in the tumor tissue. EGFR-mediated signaling directly contributes to angiogenesis, cell invasion and metastasis that are related to tumor progression. However, the role of EGFR in the pathogenesis of ESCC has not been elucidated.

Our previous study found that, the proliferation of ESCC cells induced by EGFR activation can be inhibited by β-adrenergic receptor blockers. Further mechanistic investigation revealed that the cellular release of epinephrine and the expression of its synthesizing enzyme tyrosine hydroxylase were induced by EGF. The expression of β-adrenoceptor and the downstream signal transducer protein kinase A were also up-regulated to promote cell proliferation. Existing research suggest that metabolism of arachidonic acid is involved in tumor promotion caused by β-adrenergic receptor activation. Our previous studies confirmed the β_2-adrenergic receptor / extracellular signal-regulation kinase (ERK) / COX-2 pathway to promote the proliferation of ESCC cells. Therefore, we hypothesized that EGFR activation promote tumor growth by the β_2-adrenergic receptor / COX-2 pathway.

This study aimed to explore whether EGFR activation can promote the COX-2 expression and tumor growth by β_2-adrenergic receptor pathways in ESCC cells and nude mouse model, and investigate the relationship of EGFR and COX-2 expression in human ESCC specimens.

Methods

(1) Human ESCC cell line KYSE30 was treated with EGF, EGFR inhibitor (AG1478), β_2-selective antagonist (ICI 118551) and cyclooxygenase-2 inhibitor (nimesulide). Cell survival was tested with 3-(4, 5-dimethylthiazol-2yl)-2, 5 diphenyltetrazolium (MTT) assay. The expression of COX-2 was detected by western blot and real-time reverse transcription polymerase chain reaction (PCR).

(2) Human ESCC xenograft in nude mice was administered with EGF combining or not combining EGFR inhibitor, β_2-selective antagonist and cyclooxygenase-2 inhibitor. Tumor growth was observed and COX-2 expression was detected by western blot and real-time reverse transcription PCR.

(3) Western blotting was used to test 40 pairs of ESCC and adjacent noncancerous tissue, the tissue samples were divided into two groups according to the level of EGFR protein expression, then COX-2 expression was detected. Immunohistochemistry was applied to detect the expression of EGFR and COX-2 in 78 pairs of ESCC tissue, then the relationship between the EGFR and COX-2 expression was evaluated.

Results

(1) EGFR, β_2-adrenergic receptor and COX-2 was expressed in KYSE 30 cells. EGF stimulated KYSE30 cell proliferation in a dose-dependent manner. AG1478 (EGFR inhibitor), ICI 118551 (β_2-selective antagonist) and nimesulide (highly selective cyclooxygenase-2 inhibitor) attenuated cell proliferation induced by EGF. AG1478 and ICI 118551 also abrogated EGF-induced upregulation of COX-2 expression in the mRNA and protein level.

(2) Animal model indicated that EGF significantly stimulated the growth of human ESCC xenograft in nude mice, which was attenuated by AG1478, ICI 118551 and nimesulide. Moreover, AG1478 and ICI 118551 abrogated EGF-induced upregulation of COX-2 expression in the mRNA and protein level.

(3) Western blotting confirmed that the expression of COX-2 in EGFR high expression group was higher than that in EGFR low expression group. Immunohistochemistry showed that EGFR and COX-2 expression of the specimens of ESCC was positively correlated.

Conclusions

These data provided the first evidence that EGFR activation resulted in enhanced expression of COX-2 and tumor growth through activation of β_2-adrenergic receptor in ESCC. β_2-adrenergic receptor was a critical link between EGFR activation and COX-2 expression in human ESCC. This novel finding shed new light on combination of β-blocker and COX-2 inhibitor for the treatment of ESCC.

Key words: esophageal squamous cell carcinoma (ESCC), epidermal growth factor receptor (EGFR), β-adrenergic receptor, cyclooxygenase-2 (COX-2)

Environmental risks and HCC development: 'best fit' experimental models in liver carcinogenesis

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Hepatocellular carcinoma (HCC) is a fatal malignancy that ranks amongst the top 3 causes of cancer deaths worldwide. While the main risk factors for HCC have been unambiguously identified, such as hepatitis B and C virus infection, alcohol dependence and increasingly, obesity and type 2 diabetes, the key pathogenic and molecular mechanisms underlying liver carcinogenesis remain poorly defined. Research into HCC (or any cancer) requires appropriate and relevant models which incorporate the use of risk factors associated with tumour development, and those which accurately mimic the natural history, molecular and pathobiological characteristics of HCC, as well as the tissue microenvironment in which it arises. 'Hepatoma' cancer cell lines, derived from human or animal HCCs, enable mechanistic studies into genetic alterations causing abnormalities in hepatocyte proliferative and cell death signalling pathways, malignant transformation and the acquisition of metastatic potential. While the choice of using cell lines reflect their ease of availability and convenience, they bear little resemblance and relevance biologically to HCC *in situ*. In contrast, *in vivo* models of HCC better reflect human disease but vary in their derivation, establishment and ability to recapitulate the multi-step process of liver carcinogenesis, histopathology, the cirrhotic environment in which majority of human HCCs arise, as well as the molecular features of such tumours. This lecture will critically review a suite of different experimental models commonly utilised in liver cancer research, in particular the advantages and pitfalls underlying xenograft models, syngeneic animal model systems, carcinogen-induced rodent models, hepadnaviral-induced HCCs as well as 'classical' transgenic and tetracycline-regulated transgenic approaches. Selecting the 'best-fit' models to elucidate the pathogenesis of HCC and ultimately, to test the efficacy of therapies against HCC is of critical importance.

Environmental contributions to the risk of nasopharyngeal carcinoma (NPC) and Epstein-Barr virus activation — From epidemiologic evidence to biological mechanisms

Wei-hua JIA
Sun Yat-sen University Cancer Center, Guangzhou, China

Nasopharyngeal carcinoma (NPC) is rare in most parts of the world but is prevalent in southern China, especially in Guangdong province. The distinctive geographic distribution of NPC suggests that some environmental factors inherent in the traditional southern Chinese culture may play important roles in its development.

A hospital-based case-control study was conducted to explore the effect of dietary and lifestyle factors on the risk of NPC[1,2]. Some previously established dietary risk factors in the Cantonese population are still stable and have contributed to the incidence of NPC. The results suggested the consumption of canton-style salted fish during childhood and cigarette smoking were independent risk factors for NPC, while fresh fruit, Canton-style herbal tea and herbal slow-cooked soup demonstrated protective effects against NPC. Alcohol consumption represented a J-shape dose response trend, with NPC risk decreasing when light drinking and increasing when heavy drinking.

Epstein-Barr virus (EBV) infection is generally recognized as a major risk factor of nasopharyngeal carcinoma (NPC)[3]. EBV infection is highly prevalent and pandemic, while NPC incidence is low in most parts of the world. Consequently it is hypothesized that some specific factors among the confirmed or potential life-style risk factors may act as inducers for carcinogenic transforming potential of EBV in NPC occurrence. Epidemiological study showed that cigarette smoking was the only factors linked to seropositivity of the EBV VCA-IgA antibody, with a dose-response relationship in Guangdong healthy population ($P_{trend} < 0.001$).

To investigate the mechanism underlying the relationship between smoking and EBV serological reactivation, we furthered out study by cell biological assay. The cigarette smoke extract (CSE) was found to act as a chemical inducer to cause EBV

reactivation. CSE promoted EBV replication, induced the expression of the immediate-early transcriptional activators (Zta and Rta), and increased transcriptional expression levels of BFRF3 and gp350 in the lytic phase.

Our research confirmed the effect of smoking in EBV reactivation, which may finally cause NPC. Further studies are needed to search for other environmental inducers and illustrate the related mechanism. These foundings will have great value in NPC prevention in high-risk areas.

1. Jia WH, Luo XY, Feng BJ, Ruan HL, Bei JX, Liu WS, Qin HD, Feng QS, Chen LZ, Yao SY *et al*: **Traditional Cantonese diet and nasopharyngeal carcinoma risk: a large-scale case-control study in Guangdong, China**. *BMC Cancer*, **10**:446.
2. Ruan HL, Xu FH, Liu WS, Feng QS, Chen LZ, Zeng YX, Jia WH: **Alcohol and tea consumption in relation to the risk of nasopharyngeal carcinoma in Guangdong, China**. *Front Med China*, **4**(4):448-456.
3. Chien YC, Chen JY, Liu MY, Yang HI, Hsu MM, Chen CJ, Yang CS: **Serologic markers of Epstein-Barr virus infection and nasopharyngeal carcinoma in Taiwanese men**. *N Engl J Med* 2001, **345**(26):1877-1882.

Roles of the G protein-coupled estrogen receptor in carcinogenesis

Eric R. PROSSNITZ
University of New Mexico Cancer Center, Albuquerque, USA

Estrogen is a critical hormone in the human body, regulating multiple processes in many tissues. It is involved in the growth, development and homeostasis of tissues and organs, with the best understood being mammalian female reproduction and breast development. The importance in the stimulation and regulation of breast growth makes estrogen a critical factor in the initiation, progression and metastasis of breast cancer. Estrogen initiates multiple cellular responses ranging from the regulation of gene expression to survival and proliferation to adhesion and migration. The first described estrogen receptor (termed ER and later ERα) was reported over 40 years ago. The discovery in 1996 of a second highly homologous estrogen receptor ERβ advanced yet complicated our understanding of estrogen action. These traditional estrogen receptors are soluble proteins (located predominantly in the nucleus) that classically mediate their effects at the genomic level regulating transcription. Nevertheless, many reports over the years have characterized rapid cellular responses to estrogen, including the generation of second messengers and the activation of kinases, suggesting the existence of additional non-genomic signaling pathways and/or additional estrogen receptor(s).

In 2005, we characterized the G protein-coupled receptor (GPCR) GPR30 (now named GPER for G protein-coupled estrogen receptor) as an E2-binding receptor, structurally and functionally distinct from the classical ERs. Some perplexing effects of estrogen in many areas of cell biology and physiology that were not easily attributable to the classical ERs have now been in part explained by activities of this third ER. GPER is a 7-transmembrane spanning receptor of the GPCR superfamily that activates multiple cellular pathways including calcium mobilization, ERK and PI3K via transactivation of the epidermal growth factor receptor. As estrogen is capable of activating all three ERs (ERα, ERβ and GPER), selective agonists/antagonists were needed to elucidate the functional roles of the individual receptors, particularly GPER. We therefore embarked on a major screening effort that resulted in the identification of the first and still only GPER-selective agonist G-1 in 2006 and the first and only

GPER-selective antagonist in 2009. These structurally related compounds show >10^4 fold selectivity for GPER over the classical ERs, providing a unique opportunity, in combination with GPER-deficient mice, to unravel the specific contributions of GPER to estrogen biology and in particular breast and other cancers.

Novel interactions between signal transducing G proteins and the Fhit tumor suppressor

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The *FHIT* tumor suppressor gene is one of the most commonly altered genes in cancer since it is inactivated in about 60% of human tumors. Transgenic mice carrying one or two inactivated Fhit alleles are viable and long-lived, but they show increased rates of spontaneous and carcinogen-induced cancers, whereas the development of carcinogen-induced tumors in these mice can be prevented by administration of Fhit-expressing viral vectors. The Fhit protein is a member of the ubiquitous histidine triade proteins which hydrolyze dinucleoside polyphosphates such as Ap_3A. Despite the fact that Fhit functions as a tumor suppressor, the pathway through which Fhit inhibits growth of cancer cells remains largely unknown. Previous studies have demonstrated that Fhit phosphorylation by Src tyrosine kinases provides a linkage to growth factor signaling. Since many G protein-coupled receptors can regulate cell proliferation through multiple signaling components including Src, we explored the relationship between various $G\alpha$ subunits and Fhit. Interestingly, several members of the $G\alpha_q$ subfamily ($G\alpha_{16}$, $G\alpha_{14}$, and $G\alpha_q$) were found to co-immunoprecipitate with Fhit in their GTP-bound active state in HEK293 cells. The interaction appeared to exhibit $G\alpha$ subunit specificity because Fhit failed to associate with $G\alpha_{11}$ or $G\alpha_{i2}$. The binding of activated $G\alpha_q$ members to Fhit appeared to be direct and was detectable in native DLD-1 colon carcinoma cells. However, such interaction did not affect either Ap_3A binding and hydrolysis by Fhit, or the ability of $G\alpha_{q/16}$ to regulate downstream effectors including phospholipase $C\beta$, Ras, ERK, STAT3, and IKK. Functional mutants of Fhit including the H96D, Y114F, L25W and L25W/I10W showed comparable abilities to associate with $G\alpha_q$. Interestingly, receptor-induced activation of $G\alpha_q$, but not $G\alpha_s$ or $G\alpha_i$, led to an increase in Fhit phosphorylation at Tyr^{114} and simultaneously stimulated the *de novo* synthesis of Fhit in a PKC/MEK-dependent manner. These results demonstrate that activated $G\alpha_q$ subunits can interact with Fhit and modulate its phosphorylation and expression.
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Estrogens, environmental estrogens, and cancer

Yuet-kin LEUNG
Department of Environmental Health, University of Cincinnati, Cincinnati, USA

Estrogens regulate development, reproduction, metabolism, cognitive function, and many other vital functions through interaction with its specific receptors. Disruption of estrogen levels or expression programs of estrogen receptors during susceptible life-stages increases risks for disease development either acutely or in a delayed manner. In our laboratory, we investigate the role of estrogens in prostate and breast carcinogenesis and seek therapeutic opportunities by targeting this signaling axis. In the aging male, the incidence of prostate cancer rises exponentially, coinciding with a decline in the levels of testosterone and a rise in both circulating and tissue estrogen influence. These changes lead to a skewed estrogen to androgen ratio favoring the female hormone, which may be the underlying cause of prostate carcinogenesis. To better understand the mechanism, we have studied the major estrogen receptor subtype, estrogen receptor beta, in benign and malignant prostate tissues of aged men. The roles of the wild type and isoforms of estrogen receptor-beta are under intense investigation. We discover functional divergence between the wild type receptor and its isoforms during prostate cancer progression, which will be discussed in this presentation. The possibility that estrogens may not be the endogenous ligand for estrogen receptor beta will also be discussed. Additionally, the early-life reprogramming effect of estradiol-17 beta and its mimic, bisphenol A (BPA), in modifying the risk of breast cancer in adulthood through epigenetics regulation will also be highlighted using *in vivo* DMBA-induced rat mammary tumorigenesis model.

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Hypoxic growth prostate cancer: regulation of hypoxic transcription factor HIF-1α by novel O_2-independent mechanisms

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It is well known that adaptation of growth in a hypoxic tumor microenvironment is one of the major growth capacities and hallmarks of cancers, and this growth feature is being enabled by a number of growth signaling pathways, particularly induction of angiogenesis and reprogramming of energy metabolism to aerobic glycolysis in cancer cells. The effects of adaptive growth in hypoxia not only facilitate the survival of cancer cells in a microenvironment with reduced O_2 availability but also render cells resistance to various cancer therapies. The major adaptive response to hypoxia by cancer cells is the transcriptional regulation mediated by the hypoxia-inducible factor 1 (HIF-1). HIF-1 is a heterodimeric transcription factor consisting of HIF-1α subunit, its expression and transcriptional activity are tightly regulated by cellular O_2 level, and HIF-1β subunit, which is constitutively expressed. HIF-1 signaling is mostly controlled by protein levels of HIF-1α subunit, which undergoes continuous degradation in normoxia and is tightly regulated by a well-characterized O_2-dependent mechanism mediated by prolyl hydroxylase (PHD), von Hippel-Lindau (VHL) / Elongin-C / Elongin-B E3 ubiquitin ligase complex and proteasome. Recent advances also show that HIF-1α stability is also regulated by an O_2-independent mechanism mediated by binding of heat-shock protein 90 (HSP90) and a multi-functional scaffold protein RACK1 to HIF-1α. In this lecture, two new mechanisms on the regulation of HIF-1α stability recently characterized in prostate cancer cells will be discussed: (a) a Ca^{2+}- and ubiquitin-dependent mechanism regulated by a Ca^{2+} channel TRPM8 via a RACK1-HIF-1α-dependent regulatory pathway, and (b) a nuclear receptor-dependent mechanism regulated by direct physical interaction between HIF-1α and an orphan nuclear receptor ERRα (NR3B1). Potential implications for these novel regulatory mechanisms on prostate cancer development and treatment will be discussed.

Significances of a putative membranous estrogen receptor GPR30 in prostate cancer

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Prostate cancer (PCa) is the most common male cancer in western countries. In Hong Kong, a rapid increasing incidence is observed from 5.9 to 41.5 new cases per 100,000 persons (1983-2008). Currently it ranks the third common male cancer, posing significant impacts on Hong Kong men's health. It is well-established that estrogens play important roles in PCa. Use of a synthetic estrogen (diethylstibestrol) for metastatic PCa treatment was first established by Huggins and Hodges in 1941, primarily described as androgen deprivation via interference of androgen production in testis. However, most neoplasms ultimately become androgen-refractory, at which time virtually no effective therapies are available. Patients usually die of hormone-refractory metastasis. Therefore, there is a strong demand for alternatives to the treatment of androgen-insensitive PCa. Later, discovery of ERβ in normal rodent prostate, in addition to ERα, prompted to suggest the direct estrogenic actions on the tissue. We studied the biological significances of ERβ by various estrogenic and antiestrogenic agents and demonstrated the cell growth inhibition of ICI-182,780 (ICI) in DU145 prostate cancer cells via an ERβ-dependent pathway. Based on our preclinical data, several clinical trials were conducted and demonstrated that the treatment was able to induce biochemical responses (i.e. decrease of PSA level) without any major toxicity in the patients. Furthermore, emerging evidences indicate that estrogens can also trigger rapid signaling responses initiated at plasma membrane and an orphan G-protein-coupled receptor (GPR30) was identified at plasma membrane and endoplasmic reticulum with high affinity and low capacity binding to estrogens as a putative membranous estrogen receptor. GPR30 mediates nongenomic signaling of estrogen to regulate cell growth. We here demonstrated for the first time, in contrast to the reported promoting action of GPR30 on the growth of breast and ovarian cancer cells, that activation of GPR30 by the receptor-specific, non-estrogenic ligand G-1 inhibited growth of androgen-dependent and -independent prostate cancer (PCa) cells *in vitro* and PC-3 xenografts *in vivo*. Treatment of PC-3

cells with G-1-induced cell-cycle arrest at the G2 phase and reduced the expression of G2-checkpoint regulators (cyclin A2, cyclin B1, cdc25c, and cdc2) and the phosphorylation of their common transcriptional regulator NF-YA in PC-3 cells. With the extensive use of siRNA knockdown experiments and the MEK inhibitor PD98059 in the present study, we dissected the mechanism underlying G-1-induced inhibition of PC-3 cell growth, which was mediated through GPR30, followed by an activation of Erk1/2 and a c-jun/c-fos-dependent upregulation of p21, resulting in the arrest of PC-3 growth at the G2 phase. The discovery of this signaling pathway lays the foundation for future development of GPR30-based therapies for PCa.

ABSTRACTS FOR STATE KEY LABORATORY IN ONCOLOGY IN SOUTH CHINA POSTER PRESENTATION

(1) Chemokine C-C motif ligand 2 and chemokine C-C motif receptor 2 are metastatic predictors for nasopharyngeal carcinoma

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Purpose: To evaluate the prognostic potential of chemokine C-C motif ligand 2 (CCL2) and chemokine C-C motif receptor 2 (CCR2) expression in nasopharyngeal carcinoma (NPC) patients before treatment by analyzing the expression of these two markers.
Experimental design: Both CCL2 and CCR2 were prospectively detected in 105 NPC patients with immunohistochemistry before treatment. Meanwhile, their associations with clinical characteristics and survival of NPC patients were analyzed.
Results: For CCL2, the 7-year overall survival (OS) and 7-year distant metastasis free survival (DMFS) of high expression group and low expression group were 47% vs. 71% ($p = 0.013$) and 69% vs. 82% ($P = 0.041$), respectively. For CCR2, the 7-year OS and 7-year DMFS of high expression group and low expression group were 45% vs. 75% ($p = 0.004$) and 63% vs. 88% ($P = 0.016$), respectively. The 7-year OS and 7-year DMFS for both positive patients, one marker positive patients and both negative patients were 45% vs. 47% vs. 86% ($P = 0.001$) and 67% vs. 64% vs. 94% ($P = 0.014$), respectively. The Cox proportional hazards model analysis showed that both of them were independent predictors of OS and DMFS respectively.
Conclusions: High expression levels of CCL2 and CCR2 predict posttreatment distant metastases and poor overall survival in NPC patients.

(2) A randomized trial of induction chemotherapy plus concurrent chemoradiotherapy versus induction chemotherapy plus radiotherapy for locoregionally advanced nasopharyngeal carcinoma

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The aim of this randomized study was to compare the efficacy of induction chemotherapy plus concurrent chemoradiotherapy (IC+CCRT) versus induction chemotherapy plus radiotherapy (IC+RT) for patients with locoregionally advanced nasopharyngeal carcinoma. From August 2002 to April 2005, 408 patients were randomly divided into 2 groups: an IC + CCRT group and an IC + RT group. Patients in both groups received the same induction chemotherapy: 2 cycles of floxuridine (FuDR) + carboplatin (FuDR, 750 mg/m^2, d1-5; carboplatin, area under the curve [AUC] = 6). The patients received radiotherapy 1 week after they finished the induction chemotherapy. The patients in the IC + CCRT group also received carboplatin (AUC = 6) on days 7, 28, and 49 of radiotherapy. Eight patients did not meet the inclusion criteria, and the remaining 400 cases were analyzed. Grade III/IV toxicity was found in 28.4% of the patients in the IC + CCRT group and 13.1% of those in the IC + RT group ($P < 0.001$). 5-year overall survival rates were 70.3% and 71.7% ($P = 0.734$) in the IC + CCRT and IC + RT groups, respectively. No significant differences in failure-free survival, locoregional control, and distant control were found between the 2 groups. Compared with the IC + RT program, the IC + CCRT program used in the present study did not improve the overall survival and failure-free survival in patients with locoregionally advanced nasopharyngeal carcinoma. Using carboplatin in the concurrent chemoradiotherapy was not suitable for nasopharyngeal carcinoma.
Key words: induction chemotherapy, concurrent chemoradiotherapy, radiotherapy, nasopharyngeal carcinoma

(3) Downregulation of six microRNAs is associated with advanced stage, lymph node metastasis and poor prognosis in small cell carcinoma of the cervix

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Background: Small cell carcinoma of the cervix (SCCC) is very rare, and due to the long time period required to recruit sufficient numbers of patients, there is a paucity of information regarding the prognostic factors associated with survival. MicroRNAs (miRNAs) have been used as cancer-related biomarkers in a variety of tumor types, and the objective of this study was to determine whether microRNA expression profiles can predict clinical outcome in SCCC.
Methodology / Principal findings: Forty-four patients with SCCC who underwent radical hysterectomy between January 2000 and October 2009 were enrolled. Using the GeneCopoeia All-in-One™ Customized Human qPCR Primer Array, the expression profiles of 30 miRNAs associated with tumor metastasis was obtained from the formalin-fixed paraffin embedded samples of all 44 patients. Seven miRNAs, has-let-7c, has-miR-10b, has-miR-100, has-miR-125b, has-miR-143, has-miR-145 and has-miR-199a-5p were significantly down-regulated in advanced stage SCCC patients (FIGOIB2-IV) compared to early stage SCCC patients (FIGOIB1). Among, downregulation of six miRNAs, has-let-7c, has-miR-100, has-miR-125b, has-miR-143, has-miR-145 and has-miR-199a-5p were significantly associated with lymph node metastasis and reduced survival in SCCC. Kaplan-Meier survival analyses revealed that SCCC patients with low expression of has-miR-100 (P = 0.019) and has-miR-125b (P = 0.020) projected a significant tendency towards poorer prognosis.
Conclusions / Significance: This study demonstrates that downregulation of 7 miRNA associated with advanced stage, 6 miRNAs with metastasis and 2 with poor prognosis in SCCC. Functional analysis of these miRNAs may enhance our understanding of SCCC, as altered expression of specific miRNAs may regulate the metastatic pathway and provide novel targets for therapy.
Key words: small cell carcinoma, uterine cervix, microRNA, expression profile, prognosis

(4) Enhanced glyceraldehyde-3-phosphate dehydrogenase expression in human pancreatic carcinoma

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Increased aerobic glycolysis in cancer, a phenomenon known as the Warburg effect has been observed in various tumor cells. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key enzyme in glycolysis and has been known as a housekeeping molecule. In the present study, we found that GAPDH expression was significantly up-regulated in human pancreatic carcinoma tissues compared to the adjacent normal tissues. Importantly, further evaluated expression was observed in late pathological stage pancreatic cancer tissues, suggesting that high expression of GAPDH might play an important role in pancreatic cancer development and prognosis. 3-bromopyruvate propyl ester (3-BrOP) preferentially inhibited GAPDH activity. And 3-BrOP at low concentrations inhibited pancreatic cancer cells and a higher concentration was required to inhibit normal epithelial cells pancreas, which suggest that 3-BrOP killed the pancreatic cancer cells selectively. As we know, the prognosis of patients with pancreatic cancer is still very poor because of its aggressiveness and lack of effective therapies. Our study identified GAPDH as a potential target for pancreatic cancer therapeutics.
Key words: Warburg effect, GAPDH, 3-BrOP, pancreatic cancer, prognosis

(5) CCDC158 is a potential tumor suppressor gene in hepatocellular carcinoma

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In our previous study, we found that loss of heterozygosity (LOH) frequency of D4S2964 located at

4q21 in hepatocellular carcinoma (HCC) was as high as 50% (37/74) and correlated with HBV infection, suggesting that this locus comprises one or more tumor suppressor genes (TSG). In our present study, CCDC158 gene located in this locus was found to have high frequency (45.5%, 25/55) of LOH by a SNP microarray fabricated in house and DNA copy loss (54.5%, 61/112) by quantitative polymerase chain reaction (PCR) in HCC. CCDC158 LOH was also found in HCC cell lines SK-hep-1 and hepG2 by fluorescence in situ hybridization (FISH). Downregulation of CCDC158 mRNA and protein levels was documented in approximately 62.5% of the primary HCCs examined. Clinical association analysis showed that CCDC158 downregulation was associated with poor clinical outcomes and as an independent prognostic predictor of HCC. Functional studies revealed that over-expression of CCDC158 could effectively suppressed the malignant properties of HCC cells *in vitro* and *in vivo*, including inhibition of tumor cell growth, colony formation in soft agar, foci formation and tumor growth in nude mice. Molecular analyses revealed that CCDC158 could downregulate survivin and upregulated PTEN, leading to subsequent induction of cell apoptosis. Together, our findings suggest human CCDC158 gene is a novel TSG in HCC, and the reduced expression of this gene contributes to poor outcomes in this deadly disease.
Key words: hepatocellular carcinoma, tumor suppressor gene, CCDC158

(6) Decreased expression of the *ARID1A* gene is associated with poor prognosis in primary gastric cancer

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Background: The *ARID1A* gene encodes adenine-thymine (AT)-rich interactive domain-containing protein 1A, which participates in chromatin remodeling. *ARID1A* has been showed to function as a tumor suppressor in various cancer types. In the current study, we investigated the expression and prognosis value of *ARID1A* in primary gastric cancer.
Methodology / Principal findings: To investigate the role of *ARID1A* gene in primary gastric cancer pathogenesis, real-time quantitative PCR and western blotting were used to examine the *ARID1A* expression in paired cancerous and noncancerous tissues. Results revealed decreased *ARID1A* mRNA ($P = 0.0029$) and protein ($P = 0.0015$) expression in most tumor-bearing tissues compared with the matched adjacent non-tumor tissues. To further investigate the clinicopathological and prognostic roles of *ARID1A* expression, we performed immunohistochemical analyses of the 224 paraffin-embedded gastric cancer tissue blocks. Data revealed that the loss of *ARID1A* expression was significantly correlated with T stage ($P = 0.001$) and grade ($P = 0.006$). Consistent with these results, we found that loss of *ARID1A* expression was significantly correlated with poor survival in gastric cancer patients ($P = 0.003$). Cox regression analyses showed that *ARID1A* expression was an independent predictor of overall survival ($P = 0.029$).
Conclusions / Significance: Our data suggest that *ARID1A* may play an important role in gastric cancer and may serve as a valuable prognostic marker and potential target for gene therapy in the treatment of gastric cancer.
Key words: *ARID1A*, gastric cancer, expression, immunohistochemistry, prognosis

(7) The prognostic value of tumor-infiltrating neutrophils in gastric adenocarcinoma after resection

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Background: Several pieces of evidence indicate that tumor-infiltrating neutrophils (TINs) are correlated to tumor progression. In the current study, we explore the relationship between TINs and clinicopathological features of gastric adenocarcinoma patients. Furthermore, we investigated the prognostic value of TINs.
Patients and methods: The study was comprised of two groups, training group (115 patients) and test group (97 patients). Biomarkers (intratumoral CD15+ neutrophils) were assessed by immunohistochemistry. The relationship between clinicopathological features and patient outcome

were evaluated using Cox regression and Kaplan-Meier analysis.
Results: Immunohistochemical detection showed that the tumor-infiltrating neutrophils (TINs) in the training group ranged from 0.00-115.70 cells/high-power microscopic field (HPF) and the median number was 21.60 cells/HPF. Based on the median number, the patients were divided into high and low TINs groups. Chi-square test analysis revealed that the density of CD15+ TINs was positively associated with lymph node metastasis (p = 0.024), distance metastasis (p = 0.004) and UICC (International Union Against Cancer) staging (p = 0.028). Kaplan-Meier analysis showed that patients with a lower density of TINs had a better prognosis than patients with a higher density of TINs (p = 0.002). Multivariate Cox's analysis showed that the density of CD15+ TINs was an independent prognostic factor for overall survival of gastric adenocarcinoma patients. Using another 97 patients as a test group and basing on the median number of TINs (21.60 cells/HPF) coming from the training group, Kaplan-Meier analysis also showed that patients with a lower density of TINs had a better prognosis than patients with a higher density of TINs (p = 0.032). The results verify that the number of CD15+ TINs can predict the survival of gastric adenocarcinoma surgical patients.
Conclusions: The presence of CD15+ TINs is an independent and unfavorable factor in the prognosis of gastric adenocarcinoma patients. Targeting CD15+ TINs may be a potential intervenient therapy in the future.

(8) Reduced expression of *TCEAL7* is associated with gastric adenocarcinoma prognosis

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Background: *TCEAL7* has been proved to be a putative tumor suppressor gene. In the current study, we investigated the expression and prognosis value of *TCEAL7* in primary gastric adenocarcinoma.
Methodology / Principal findings: *TCEAL7* expression was analyzed using real-time quantitative PCR, western blotting, and immunohistochemical staining methods on tissue samples from a consecutive series of 406 gastric adenocarcinoma patients who underwent resections between 2003 and 2006. The relationship between *TCEAL7* expression, clinicopathological factors, and patient survival was investigated. RT-qPCR results showed that the expression of *TCEAL7* mRNA was reduced in tumor tissue samples, compared with expression in matched adjacent non-tumor tissue samples (P = 0.03); this finding was confirmed by western blotting analysis (P = 0.01). Immunohistochemical staining data indicated that *TCEAL7* expression was significantly decreased in 172 of 406 (42.4%) gastric adenocarcinoma cases; reduced *TCEAL7* expression was also observed in patients with poorly differentiated tumors (P = 0.01) and total gastric carcinomas (P = 0.02). Kaplan-Meier survival curves revealed that reduced expression of *TCEAL7* was associated with poor prognosis in gastric adenocarcinoma patients (P = 0.000). Multivariate Cox analysis identified *TCEAL7* expression as an independent prognostic factor for overall survival (95% CI = 0.518 - 0.953, P = 0.023).
Conclusions / Significance: Our data suggest that *TCEAL7* plays an important role in tumor progression and that reduced *TCEAL7* expression independently predicts an unfavorable prognosis in gastric adenocarcinoma patients.

(9) Chromogranin A (CgA) as poor prognostic factor in patients with small cell carcinoma of the cervix: results of a retrospective study of 293 patients

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Background: Small cell carcinoma of the cervix (SCCC) is a very rare tumor. Due to its rarity and the long time period, there is a paucity of information pertaining to prognostic factors associated with survival. The objective of this study was to determine whether clinicopathologic finings or immunohistochemical presence of molecular markers predictive of clinical outcome in patients with SCCC.
Methodology and findings: We retrospectively reviewed a total of 293 patients with SCCC (47 patients from Cancer Center of Sun Yat-sen University in China, 71 patients from case report of China journal, 175 patients from case report in PubMed database). Of those 293 patients with SCCC, the

median survival time is 23 months. The 3-year overall survival rates (OS) and 3-year disease-free survival rates (DFS) for all patients were 34.5% and 31.1%, respectively. Univariate and multivariate analysis showed that FIGO stage (Ⅱb-Ⅳ vs. Ⅰ-Ⅱa, Hazard Ratio (HR) = 3.08, 95% confidence interval (CI) of ratio = [2.05, 4.63], $P < 0.001$), tumor mass size (≥ 4 cm vs. < 4 cm, HR = 2.37, 95% CI = [1.28, 4.36], $P = 0.006$) and chromogranin A (CgA) (Positive vs. Negative, HR = 1.81, 95% CI = [1.12, 2.91], $P = 0.015$) were predictive of poor prognosis. CgA stained positive was found to be highly predictive of death in early-stage (FIGO Ⅰ-Ⅱa) patient specifically.
Conclusions: Patients with SCCC have poor prognosis. FIGO stage, tumor mass size and CgA stained positive may act as a surrogate for factors prognostic of survival. CgA may serve as a useful marker in prognostic evaluation for early-stage patients with SCCC.
Key words: cervical cancer, small cell carcinoma, chromogranin A, prognosis

(10) Cigarette smoking extract stimulates Epstein-Barr virus reactivation by modulating SP1 transcription factor binding to the *BRLF1*-promoter in B cells

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In previous epidemiology study, we first observed that tobacco smoking is significantly associated with elevated EBV titer in Cantonese population of EBV positive. Furthermore, cell biology experiments also suggested that cigarette smoking extract (CSE) could efficiently induce EBV reactivation. We hypothesized that environmental factors stimulate EBV reactivation as the first step of carcinogenesis, and then nasopharynx epithelial cells could be able to establish the latent infection, eventually, EBV is configured to promote carcinogenesis persistently in the epithelial cells. But the reason underlying EBV reactivation is still unknown. In this study, to evaluate the ability of CSE as an inducer for EBV reactivation, we systematically validated that CSE could efficiently stimulate EBV lytic life cycle. Furthermore, we investigated the molecular mechanisms involving in this process. Our results suggested that variations in SP1 binding sites in the promoter of immediate-early gene *BRLF1* could modulate EBV reactivation by CSE. By constructing serial site-directed mutants of *BRLF1* promoter (Rp) in luciferase assays, we found substitution mutations in SP1 binding sites reduced CSE-mediated activation of Rp. It is indicated that CSE reactivates EBV lytic life cycle via the mechanism of modulating SP1 binding to Rp.

(11) A matched cohort study comparing chemo-radiotherapy versus radiotherapy alone in elderly patients with advanced nasopharyngeal carcinoma

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Objectives: To compare the survival and treatment toxicity of combined chemo-radiotherapy (CRT) in the elderly patients with advanced nasopharyngeal carcinoma (NPC) with those of radiotherapy (RT) alone.
Design: Matched cohort study using historical data from the Cancer Center of Sun Yat-sen University. Matching characteristics were gender, age, histological type, T and N stages, radiotherapy dose to primary tumor and neck nodes, days of radiotherapy.
Setting: Retrospective analysis of medical records uniformly collected over a 6-year period in our institution.
Participants: A group of 87 patients aged 60 and older with advanced NPC treated with combined chemo-radiotherapy and a matched group treated with radiotherapy alone.
Measurements: 498 cases of patients between 1998 and 2002 were abstracted, and the radiotherapy doses and time of each group, the follow-up time were estimated. The overall survival rates (OS), cancer-specific survival rates (CSS), failure-free survival rates (FFS), locoregional failure-free survival rates (LR-FFS), distant failure-free survival rates (D-FFS), and treatment related toxicities of CRT and RT groups were assessed.
Results: The 5-year OS, CSS, FFS, and LR-FFS for CRT and RT groups were 62% vs. 40% ($P = 0.013$), 67% vs. 47% ($P = 0.018$), 65% vs. 53% (log-rank: $P = 0.064$, Breslow: $P = 0.048$), and 88% vs. 72%, ($P = 0.019$), respectively. There was no significant difference in 5-year D-FFS between the two groups (75% vs. 73%, $P = 0.456$). The CRT group experienced significantly more Grade ≥ 3 acute

mucositis (46.0% vs. 28.7%, *P* = 0.019).
Conclusion: Combined chemo-radiotherapy improves OS, CSS, FFS, and LR-FFS for elderly patients with advanced NPC, but not reduces distant metastases.

(12) BLLF1 gene variation of Epstein-Barr virus is dominant in the environment of South China

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Epstein-Barr virus (EBV) exists everywhere in the environment, and is one member of human herpesvirus 4 family. 95% of the world population will be infected during their adulthood. In addition, it is reported that EBV infection is related to several malignancies, e.g, Burkitt's Lymphoma, Hodgkin Disease, nasopharyngeal carcinoma (NPC) and gastric carcinoma, as well as some lymphoproliferative diseases such as infectious mononucleosis (IM) and post transplant lymphoproliferative disorder (PTLD), etc. NPC is of high incidence in South China and Southeast Asian. We planned to study the relationship between endemic NPC in South China and specific EBV type like GD1 (Guang Dong 1) in the environment. Referring to EBV gene BLLF1 which expresses one major envelope protein gp350 (glycoprotein 350), there are 4 deletion mutations (two 21bp, one 27bp and one 84bp deletions) according to B95.8. The molecular weight of gp350 is 350kDa and it is highly glycosylated that exists outside the membrane of EBV. Gp350 is crucial in EBV infection because it binds with CD21 (CR2), the membrane protein of B cell that causes virus entry. BLLF1 expresses gp350/220 two isoforms because of different splicing pattern when transcript into mRNA in the host cell nucleus, and the small isoform gp220 perhaps pulls EBV closer to B cell surface when binding with CD21. This indicates that 4 deletions in GD1 gp350 may shorten the molecule or change the conformational structure that could increase the affinity with CD21 molecule and enhance infection.
We have detected the four deletions type of BLLF1 in 389 Guangdong healthy people and 200 Shandong healthy people, as well 46 Guangdong NPC patients and 20 Shandong NPC patients by Sanger sequencing. The GD1-like type was found in 73.01% of Guangdong healthy people (284/389) compared to 25% of Shandong healthy people (50/200) ($P < 0.01$), and 80.43% of Guangdong NPC patients (37/46) compared to 15% of Shandong NPC patients (3/20). We demonstrated that EBV BLLF type is endemic rather than disease specific. In the future study, we will plan to figure out the reason this EBV type dominates in Guangdong environment.

(13) PEITC: a novel strategy to overcome platinum-resistance in cancer cells with stem like property

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Platinum (Pt)-based chemotherapy is an important regimen in the clinical treatment of cancer, but development of drug resistance presents a major challenge. One key mechanism involved in resistance to Pt drugs is the decrease of cellular Pt accumulation through efflux by the GSH-mediated export, and this is particularly significant in cancer cells with stem-like properties. In the present study, we showed that two Pt-resistant human cancer cell lines exhibited stem-like EMT properties, had high cellular GSH and accumulated much less Pt compared to their parental cells, and failed to undergo apoptosis when exposed to Pt at the drug concentrations that were toxic to the parental cells. Importantly, we found that the natural compound β-phenylethyl isothiocyanate (PEITC) was able to effectively abolish this drug resistant mechanism by effective depletion of cellular GSH, leading to a significant increase in cellular Pt as well as DNA-bound Pt. A combination of PEITC and Pt showed a striking synergistic anticancer activity both *in vitro* and *in vivo*, as evidenced by an increase in drug-induced apoptosis, a loss of colony formation capacity, and significant suppression of tumor growth in mice. Taken together, our study shows a promising therapeutic strategy to overcome drug resistance to platinum-based chemotherapy and may potentially have broad implications in clinical treatment of cancer.

(14) Clinicopathological characteristics and prognostic analysis of Lauren classification in gastric adenocarcinoma: data from a single institution in China over 10 years

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Background: According to the Lauren classification, gastric adenocarcinomas are divided into diffuse and intestinal types. The causative attribution explaining the dismal prognosis of diffuse-type remains unknown.
Methods: We examined the archive of 1,000 patients with gastric adenocarcinomas who received radical gastrectomy in Cancer Center of Sun Yat-sen University and assessed the effect of the Lauren classification on survival in a multivariate approach. Moreover, we compared the variation of clinical features between the diffuse-type and intestinal-type and revealed the contributing factors for the prognostic difference.
Results: There were 805 patients for the final analysis. Diffuse-type comprised 48.7% among the gastric carcinoma in our group and showed poorer prognosis than intestinal-type (P = 0.013). Multivariate analysis revealed that independent prognostic factors for gastric carcinoma patients were TNM stage (P < 0.001), tumor size (P < 0.001) and Lauren classification (P = 0.005). The 5-year overall survival rate of patients with diffuse-type and intestinal type were 44.1% and 52.7%, respectively. For the clinical features, diffuse-type was significantly associated with younger age (p < 0.001), female preponderance (p < 0.001), distal location (P < 0.001), advanced pN (p < 0.001), advanced TNM stage (p = 0.027) and poorly differentiated subtypes (P < 0.001). Among intestinal-type carcinoma, 146 (41.5%) were younger than 60 years old compared to 246 (62.1%) of patients with diffuse-type carcinoma (P < 0.001). The ratio of male to female was significantly higher in the intestinal-type carcinoma group than that in the diffuse-type group, 3.1 vs. 0.86 (P < 0.001).
Conclusions: The higher percentage of patients with advanced pN, advanced TNM stage and poorly differentiated subtypes appear to be associated with the poor prognosis of patients with resected diffuse-type gastric carcinoma.
Key words: China, clinical characteristics, gastric adenocarcinoma, Lauren classification, prognosis

(15) Exome sequencing in colorectal cancer with high quality of biospecimen

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Background: Studies of cancer genomic investigation and personalized therapy are proceeding greatly in worldwide. However, the quality and quantity of biospecimen, including qualified clinical study cohort with detailed pathological information, standard operating procedure (SOP) of specimen collection, conservation, and utilization remain the most fundamental restraint factors to unravel the mystery of cancer-specific genome.
Methods: In order to pick out genes of which we can explain how colorectal cancer emerges and develops, we performed a whole exome re-sequencing in colorectal specimen. 10 pathological diagnosed primary human colorectal tumors and their paired peripheral bloods were collected from Sun Yat-sen University Cancer Center, a WHO collaborative hospital. These biospecimen were selected according to: 1) newly diagnosed as colorectal cancer without chemo-/radio- therapy histories; 2) mono-origin in anatomy, representing a single histological type or subtype; 3) no necrosis in the tumor specimen which weighed more than 200mg; 4) with cancer cells account more than 70% in hematoxylin-eosin staining sections. The biospecimen were transported and reserved in liquid nitrogen within half an hour after resection. The paired peripheral bloods were then collected intravenously on the next morning after operation and then separated of lymphocyte, reserved in -80°C refrigerator subsequently.
Results: Sequenced by the Illumina high-throughput platform, 378 novel somatic mutations in which non-synonymous mutated genes such as TP53, APC, KRAS were caught with extremely high frequency, another 6 genes which hadn't been related with colorectal cancer before were found mutated in more than two cases as well. Uniting these bioinformatics findings with related clinical / pathological information, analysis of gene oncology, critical involved pathways, and specific biomarkers can be precisely performed.
Conclusions: Compare with other influences, biospecimen research plays an indispensable role in the biomedical and translational medicine researches. The quality and quantity of biospecimen can

largely decide what we can perform in the future research program. It sets a footstone at the beginning of research and leads to a substantial and prospective development of research.

(16) Stem-like cancer cells are identified and inducible by increasing genomic instability in human nasopharyngeal carcinoma cells

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The existence of cancer stem cells (CSCs) or stem-like cancer cells (SLCCs) is regarded as the cause of tumor formation and recurrence. However, the origin of such cells remains controversial with two competing hypotheses: CSCs are either transformed from tissue adult stem cells or dedifferentiated from transformed progenitor cells. In a previous study, we scanned side population (SP) cells from five nasopharyngeal carcinoma (NPC) cell lines and investigated stem cell characteristics, such as self-renewal and differentiation, using SP cells from the widely-used CNE-2 NPC cell line. We observed a strong tumorigenesis ability of SP cells by *in vivo* transplantation into immunodeficient mice. Immunofluorescence revealed that cytokine 19 was highly expressed on SP cells. SP cells were found to be more resistant to chemotherapy and radiotherapy and this was related to the ATP-binding cassette half transporter member 2 of G family protein and smoothened protein expression, respectively. Compelling evidence has determined the chromosomal aneuploidy to be one of the hallmarkers for cancer cells, indicating genome instability plays an important role in tumorigenesis which CSCs are believed to be the initiator. To gain direct evidence that genomic instability is involved in the induction of SLCCs, we utilized multiple approaches to enhance genomic instability and monitored the percentage of SLCCs. Using SP cells as a marker for SLCC in human NPC, we found that DNA damage inducers, UV or mitomycin C, are capable of increasing SLCCs in NPC CNE-2 cells. Likewise, either overexpression of a key regulator of cell cycle, Mad2, or knocking-down of Aurora B, an important kinase in mitosis, or Cdh1, a key E3 ligase in cell cycle, resulted in a significant increase of SP cells in CNE-2. More interestingly, enrichment of SLCCs was observed in the recurrent tumor tissues as compared with the primary tumor in the same NPC patients. Our study thus suggests that, beside transformation of tissue stem cells leading to CSC generation, genomic instability could be another potential mechanism resulting in SLCC formation, especially at tumor recurrence stage.

(17) The quantities of HKII and PKM2 in different gastric cancer

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Purpose: The application of 18F-FDG PET/CT in diagnosing primary gastric cancer is controversial because of the different detection rates in different subtypes. HexokinaseII (HKII) and pyruvatekinase isoenzyme type M2 (PKM2) are two important isoforms which are involved in the glycolysis of gastric cancer. This study aims to find out whether the quantities of HKII and PKM2 varied in different gastric cancers, and predict if this quantitation can elucidate the biochemistry basis of the different uptakes 18F-FDG in different gastric cancers.
Materials and methods: This retrospective study involved 42 patients with gastric cancer, including 31 adenocarcinomas (papillary adenocarcinoma and tubular adenocarcinoma), eight signet-ring cell carcinomas and three mucinous adenocarcinomas, with either newly diagnosed or recurrent tumors. Tumor samples and normal tissue obtained by excision were eligible for ELISA. The quantities of HKII and PKM2 were assessed and compared. Four patients underwent 18F-FDG PET/CT before surgery.
Results: The gastric cancer tissues HKII quantities for adenocarcinoma, signet-ring cell carcinoma and mucinous adenocarcinoma were 19.2 IU/ml, 20.83±6.04 IU/ml and 12.74±2.24 IU/ml respectively. Normal gastric tissue HKII quantities were 12.77±3.31 IU/ml. The cancer tissue HKII quantities of adenocarcinoma and signet-ring cell carcinoma were comparable. Mucinous adenocarcinoma HKII levels were lower, but there was no significant difference ($P > 0.05$). The gastric cancer tissue PKM2 quantities of adenocarcinoma, signet-ring cell carcinoma and mucinous adenocarcinoma were 4.85 IU/ml, 4.74 IU/ml and 5.01 IU/ml. The normal tissue's PKM2 quantities were 4.23±0.66 IU/ml. The PKM2 quantities of subtypes were comparative with each other and minimally higher than the normal

gastric mucosa, but there was no significant difference ($P > 0.05$).
Conclusions: The HKII quantities of adenocarcinoma and signet-ring cell carcinoma were minimally more than the normal tissue, and those of mucinous adenocarcinoma were comparative with the normal tissue. The quantities of PKM2 in gastric cancer tissue did not significantly differ from the normal gastric mucosa.
Key words: gastric cancer, hexokinaseII (HKII), pyruvatekinase isoenzyme type M2 (PKM2), PET/CT, ^{18}F-FDG, ELISA

(18) Combined expression of c-Met, OPN and p27 as a prognostic indicator for gastric carcinoma

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Background and aims: The aim of this study was to detect the expression of c-Met, osteopontin (OPN) and p27 in tumors from a large cohort of gastric carcinoma patients and to analyze the prognostic value of their combined expression.
Methods: The expression of these three markers was detected in tumor tissues from a cohort of 306 gastric carcinoma patients using immunohistochemistry, which was then used to assess the prognostic merit of the single or combined expression of these markers.
Results: Among the 306 gastric carcinoma cases analyzed, we found expression of c-Met, OPN and p27 in 61.4%, 53.5% and 51.9% of cases, respectively. The expression of each of these markers significantly correlated with the major clinicopathological features and prognoses of the patients, and a multivariate analysis revealed that these were independent prognostic indicators. The analysis that was performed for the combination of these three markers yielded a final expression model, MOP, which includes the four categories A, B, C and D. The final expression model MOP could predict the prognosis of the entire cohort of patients with high accuracy ($P < 0.001$). In addition, the prognosis of patients based on the Tumor, Node, Metastasis (TNM) system staging II/III, the grade of differentiation and the Lauren classification could be precisely predicted using this expression model. The 5-year overall survival rates of patients according to models A, B, C and D were 84.4%, 52.0%, 33.9% and 6.5%, respectively, and the 5-year disease-free survival rates were 81.2%, 47.0%, 31.2% and 6.5%, respectively. Among stage II patients, the overall survival rates according to models A, B, C and D were 100%, 78.9%, 66.7% and 25.0%, respectively, and those patients at stage III had overall survival rates of 64.3%, 34.4%, 22.0% and 5.6%, respectively.
Conclusions: The combined analysis of c-Met, OPN and p27 expression can predict patient prognosis more precisely than the analysis of independent expression, and the combined expression model is an independent prognostic indicator. Furthermore, the expression model yielded excellent prognostic classification of patients with the same TNM stage, grade of differentiation or Lauren classification.
Key words: c-Met, OPN, p27, immunohistochemistry, multivariate analysis, prognosis, gastric carcinoma

(19) Smoking associated with Epstein-Barr virus reactivation

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Background: Elevated levels of antibodies against Epstein-Barr virus (EBV) antigens reflect the reactivation of EBV. Various chemicals have been found to induce the EBV lytic cycle in laboratory. Furthermore, in our recent study, cigarette smoking was confirmed to as an environmental risk factor positively correlate with the IgA antibodies against EB virus capsid antigens (VCA-IgA), it suggested that cigarette smoking can reactivate EBV and then the role of reactivation was verified by cell biological assays. In this study, we use another essential biomarker of EBV reactivation, the IgA antibodies against Zta (Zta-IgA), to validate the association between cigarette smoking and EBV reactivation.
Methods: A community-based population including 1,498 healthy males from 21 regions of Guangdong in South China was recruited from October 2005 to October 2007. Eight categories of putative risk factors were interviewed and blood specimen were collected. Serostatus of the Zta-IgA

antibody was detected by ELISA and used as an index of EBV reactivation. Logistic regression models were used to assess the relationship between environmental factors and the Zta-IgA antibody serostatus.
Results: Of the 1,498 men, 217 were Zta-IgA seropositive and 1,281 were Zta-IgA seronegative. Among the eight potential risk factors, cigarette smoking was the only risk factor that significantly linked to Zta-IgA serostatus. Those who started smoking below 20 years old, the risk to present Zta-IgA seropositive was nearly two fold than those who never smoked (OR = 1.79, 95% CI = 1.72 - 2.61). There was also a significant dose-response relationship between cumulative exposure amount and Zta-IgA with ORs (95% CI) of 1.15 (0.78 - 1.69), 1.23 (0.79 - 1.91) and 3.01 (1.86 - 4.87) for cumulative exposure amount of < 20, 20 - 40 and ≥ 40 pack-years, respectively (P_{trend} < 0.0001). However, the relationship was not significant in the former smokers when compared to never smokers.
Conclusions: Cigarette smoking is positively associated with EBV reactivation. While quitting smoking, the risk of being Zta-IgA positive would greatly decrease.

(20) The accumulation and prognosis value of tumor infiltrating IL-36 producing cells in hepatocellular carcinoma

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Background: The role of IL-36 producing cells in tumors is controversial. In the present study, we investigated the prognostic value of measuring tumor infiltrating IL-36 producing cell levels in human hepatocellular carcinoma.
Methodology / Principal findings: Immunohistochemical staining was performed to investigate the levels of IL-36+ tumor infiltrating lymphocytes (TILs), as well as CD8+ cytotoxic T lymphocytes (CTLs) and CD57+ natural killer (NK) cells from 165 hepatocellular carcinoma patients. The prognostic value of measuring the densities of IL-36+ TILs and the correlation with CTLs and NK was evaluated. IL-36 producing cells were detected in hepatocellular carcinoma tissues. The median level of IL-36+ TILs was 5.90 cells/high power microscopic field (HPF). The higher densities of tumor infiltrating IL-36+ lymphocytes were associated with better overall survival (P = 0.023). Furthermore, we found that there were positive correlations between levels of IL-17 producing cells and the densities of CD8+ cells, as well as CD57+ cells (r = 0.178, P = 0.006 for CD8+ cells and r = 0.282, P = 0.003 for CD57+ cells, respectively). The prognosis analysis also showed that the higher levels of CD8+ CTLs and CD57+ NK cells correlated with better overall survival of hepatocellular carcinoma patients.
Conclusions: These findings show that tumor infiltrating IL-36 producing cells in hepatocellular carcinoma patients may have protective roles in the tumor microenvironment and may be treated as a prognostic marker for hepatocellular carcinoma patients.

(21) MicroRNA-497 functions as a tumor suppressor in human hepatocellular carcinoma

Rong-rong WEI, Yin XIE, Bin-kui LI, Guo-liang HUANG, Mei-yin ZHANG, Yun-fei YUAN, Ming SHI, Xiao-qian CHEN, Long HUANG, Hui-zhong ZHANG, Li HE, Bi-jun HUANG, Xiao-feng ZHENG, Ying ZHANG, Hui-yun WANG. Department of Experimental Research, Sun Yat-sen University Cancer Center, Guangzhou, China

Based on microarray data, 20 differently expressed miRNAs were found between 60 paired hepatocellular carcinoma (HCC) and non-cancerous liver (NC) tissues. Among the down-regulated miRNAs in HCC, the expression of miR-497 was remarkably low and selected to be analyzed by qRT-PCR in total 131 paired HCC and NC tissues. The result demonstrated that the expression of miR-497 was significantly lower in HCC than in NC (P < 0.001). Kaplan-Meier survival analysis showed that the low expression of miR-497 was significantly correlated with poor prognosis of HCC patients. Multivariate Cox regression analysis revealed that miR-497 was an independent predictor for HCC patients (HR = 0.56, 95% CI = 0.316 - 0.980, P = 0.042). Importantly, ectopic expression of miR-497 in HCC cells remarkably suppressed cell growth and colony formation, arrested cell cycle in G1 phase and induced apoptosis. Besides BCL2 that has been reported as the target of miR-497, we also found another two novel target genes. Luciferase assay showed a significant decreased activity, which is resulted from miR-497 binding to the 3'UTR of the two target genes (P < 0.001). Exogenous miR-497 could repress the mRNA and protein expression of the three target genes, while antagonism

of endogenous miR-497 resulted in the up-regulation of the three proteins. Furthermore, in the nude mouse model, up-regulated miR-497 could suppress tumor formation of HepG2 cells. These results indicate that miR-497 is a tumor suppressor and provide a new molecular prognostic marker and potential molecular target for HCC therapy.
Key words: miR-497, hepatocellular carcinoma, prognosis, BCL2

(22) Pre-treatment serum C-reactive protein as a predictor of distant metastasis and survival for patients with nasopharyngeal carcinoma

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Purpose: We investigate the value of pre-treatment serum C-reactive protein (CRP) in prognostication of nasopharyngeal carcinoma (NPC).
Patients and methods: A total of 335 new patients with NPC, who were curatively treated by radiation therapy from October 2006 to October 2007, were recruited. Pre-treatment serum CRP was measured, and the relation between the serum CRP and the clinicaopathological factors and the prognosis of the patients was investigated.
Results: The median pre-treatment CRP levels for this cohort were 1.52 mg/L. Levels of CRP were significantly elevated in patients with locoregionally advanced disease ($P < 0.001$). The ROC curve analysis-generated pre-treatment CRP cutoff point for OS was 2.46 mg/L, respectively. The subset of low CRP levels predicted a significant survival advantage over the high subset of high CRP levels for overall survival (OS), disease-free survival (DFS), and distant metastasis free survival (DMFS), with p values = 0.011, $p = 0.021$, and $p = 0.001$ in the univariate analysis. In the multivariate analysis, the prognostic impact of patient CRP levels was found to be statistically significant for rate of DMFS ($p = 0.004$), and boardline significant for rates in DFS and OS ($p = 0.041$, $p = 0.049$, respectively).
Conclusion: The current results showed that the pre-treatment serum CRP level is an independent and significant indicator predictive of poor prognosis and more distant metastasis in patients with NPC.

(23) Dose-response relationship between cigarette smoking and nasopharyngeal carcinoma: A meta-analysis

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Background and objective: Whether an association between cigarette smoking and nasopharyngeal carcinoma (NPC) risk exists is still an open question. In order to provide quantification of this issue, we conducted a meta-analysis of published epidemiological studies.
Design: We searched the MEDLINE database and the bibliography of retrieved articles for relevant studies published in English between January 1, 1966 and January 31, 2012 that met pre-established inclusion criteria, with no language restriction. Related data for effect estimates were extracted from each study by two investigators using a standardized protocol. Study-specific effect estimates were pooled using the DerSimonian and Laird random-effects models. Variation in individual study results was evaluated using the Q-statistic, inconsistency index (I^2), and subgroup and meta-regression analysis. Potential publication bias was assessed with funnel plots and Egger's test. Quality assessments of included studies adhere to the Newcastle-Ottawa Scale (NOS).
Results: We included 29 independent studies containing 9,403 cases and 41,452 comparison subjects. The pooled RR was 1.57 (95% CI = 1.34 - 1.84) for ever smokers vs. non-smokers, and 1.28 (95% CI = 1.08 - 1.53) after publish bias adjusted. Subgroup analysis show that OR of smoking for NPC was higher in the west countries (1.76, 95% CI = 1.47 - 2.10 with mild heterogeneity $p = 0.84$) than in southern China (1.22; 95% CI = 1.12 - 1.33; heterogeneity, $p < 0.01$). The pooled risk ratio for squamocellular cell carcinoma was significant higher than that for undifferentiated carcinoma (OR, 2.20, 95% CI = 1.63 - 2.98 vs. OR, 1.27; 95% CI = 0.98 - 1.66). Pooled analyses also found a dose-response between NPC risk and pack-years of smoking.
Conclusion: There was a dose-response relationship between cigarette smoking and NPC risk. And the effect of smoking on squamocellular cell carcinoma and undifferentiated carcinoma may be different.

(24) SIRT1 promotes myeloid leukemogenesis through derepression of HOXA cluster genes

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SIRT1 (silent mating type information regulation 2 homolog *S. cerevisiae*) is a NAD-dependent histone deacetylase (HDAC) and a key regulator of cell survival and aging. SIRT1 is prominently upregulated in primary acute myeloid leukemia (AML) cells and probably related to drug resistance of AML. We report that SIRT1 plays a positive role in leukemia development and progression. We detect the high expression level of SIRT1 in primary AML and myeloid leukemia cell lines (K562, THP1 and U937). In primary AML, K562, THP1 and U937 knocking-down of SIRT1 causes proliferation suppression. We show that decreased SIRT1 expression level increases the sensitivity of primary AML and myeloid leukemia cell lines to anti-cancer treatment. Mechanistically, high level of SIRT1 is directly related with the increased HOX gene expression. Enforced expression of HOXA5, A7 and A9 contribute to myeloid leukemogenesis. Hematopoietic cell commitment to myeloid or erythroid lineages which gradually loses self-renewal is accompanied by global downregulation of HOX gene expression. Decreased SIRT1 expression level leads to significant global repression of HOXA1, A5, A6, A7, A9, A10, A11 and A13 in K562 and THP1. These data provide direct evidence for that SIRT1 promotes myeloid leukemogenesis and derepresses HOXA cluster genes.

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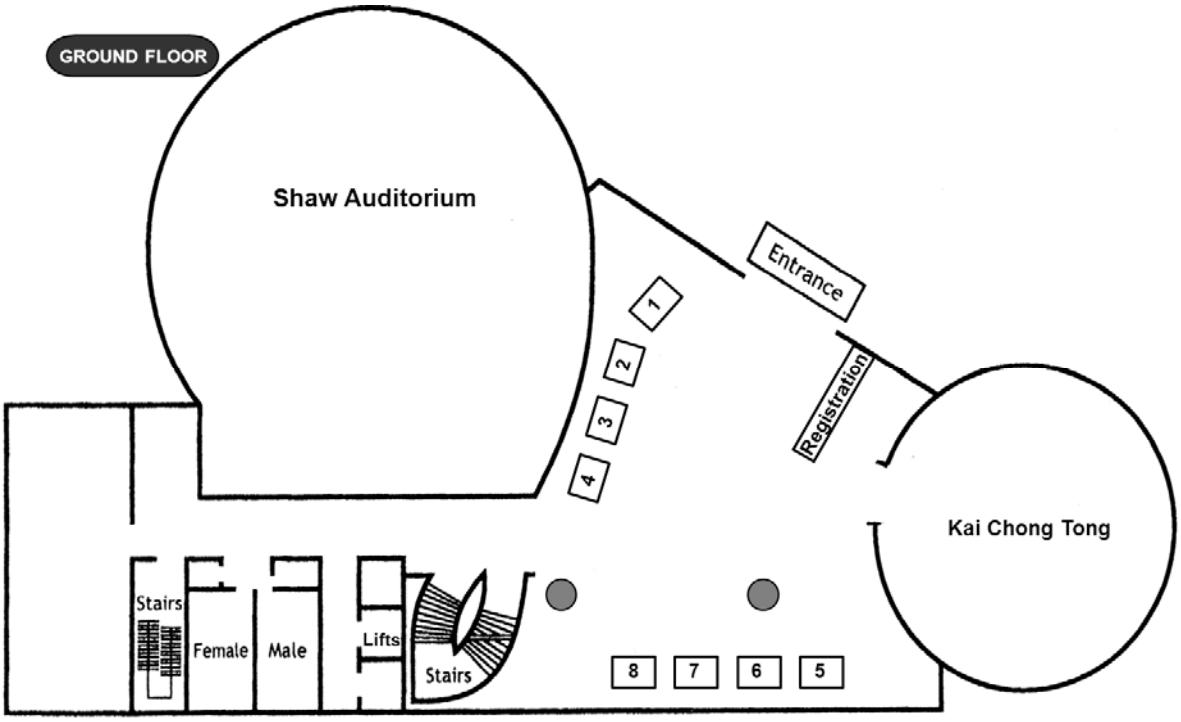

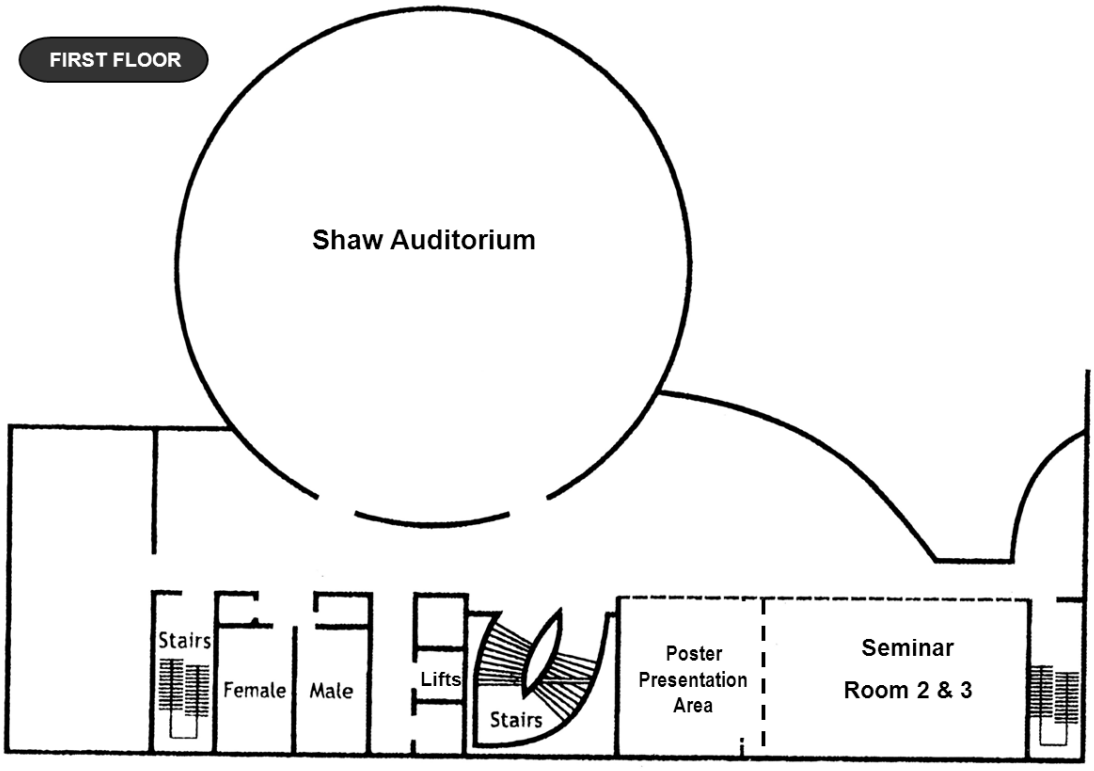

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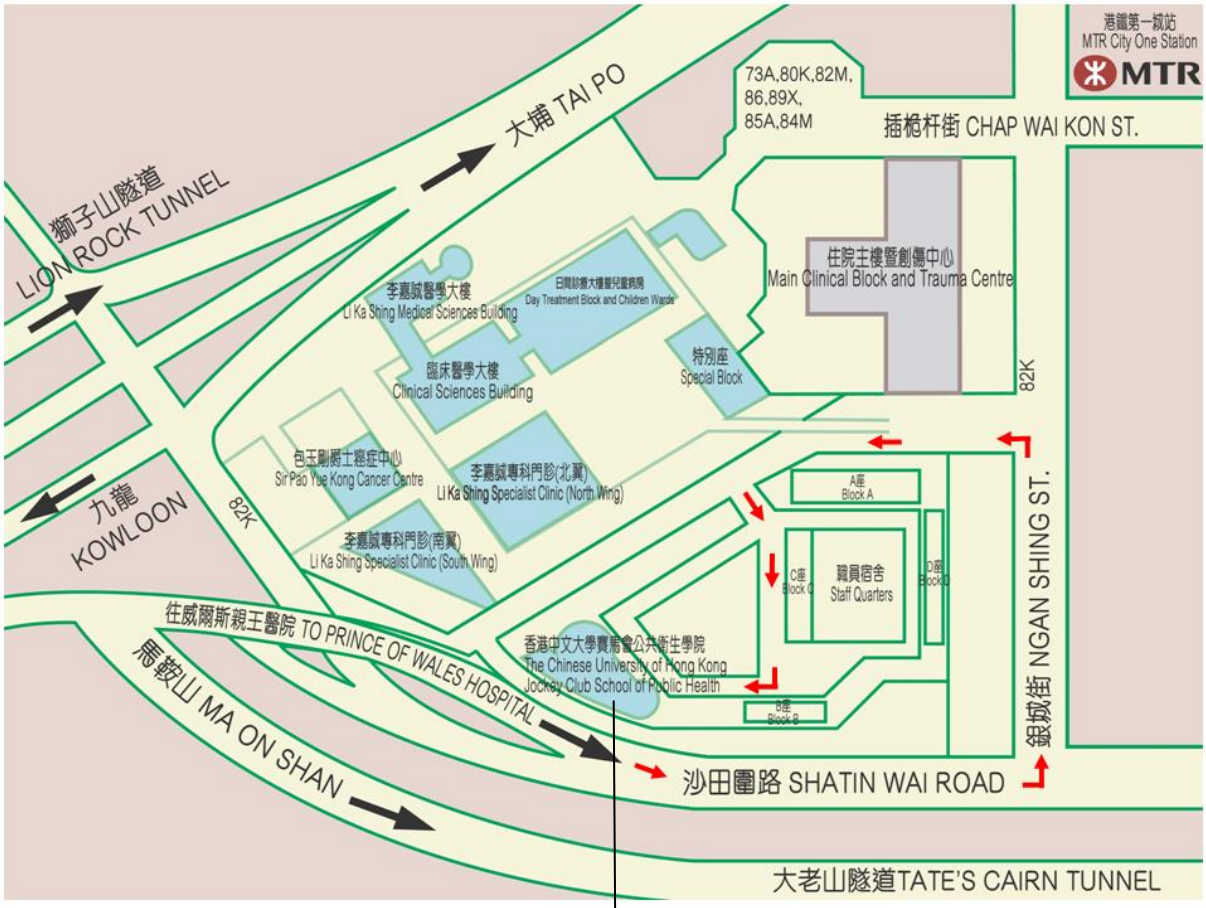

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