Keynote Presentations

Novel Strategies for Checkpoint Blockade Inhibition: Targeting CD39 Ectonucleotidase and Correcting Aberrant Purinergic Signaling in Cancer

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Abstract

Immunotherapies are newly developing interventions that modify the patient’s immune system to fight cancer, by stimulating rejection-type processes and/or by blocking suppressive pathways. ATP can be released at high levels from malignant cells, particularly after injury caused by radiotherapy or chemotherapy. Extracellular ATP provokes inflammation by “purinergic signals” and promote anti-tumor responses. Many tumors are proficient at converting ATP into the product adenosine, through the expression of eectonucleotidases, such as CD39 and CD73 inter alia, on cancer cells, regulatory immune cells, scar tissue and the vasculature. Several tumor cells (e.g. hematological malignancies, lymphoproliferative disease and multiple myeloma) also express CD39 intrinsically. The nucleoside derivatives, such as adenosine, interfere with immune responses to the cancer by serving as a “checkpoint inhibitor”, act in concert with other immunosuppressive factors e.g. PD-1; and also promote tumor blood supply by “neoangiogenesis”.

We have shown that mAb to CD39 or chemical inhibitors to block the phosphohydrolytic functions, impede extracellular ATP scavenging and prevent generation of the derivative adenosine. We are developing panels of innovative, monoclonal antibodies (mAb) and testing small molecule inhibitors to block CD39, to abrogate immunosuppressive T regulatory cell functionality and suppressive functions in vitro. These planned approaches to neutralize CD39 will also have major impacts on cancer angiogenesis, blocking neovascularization of tumors as modeled in vivo. Our approach has the potential to provide new checkpoint blockade inhibitors, substantively bolster immunostimulatory effects to cancer and serve as adjunctive treatments in settings of other immunomodulatory approaches, radiotherapy and/or chemotherapy.

miRNAs at Cell-Cell Junctions: Role in Cancer and Implications for Therapy

Panos Z. Anastasiadis*, Antonis Kourtidis, Jennifer M. Carr, Irene K. Yan, Tushar Patel and E. Aubrey Thompson
Mayo Clinic Comprehensive Cancer Center, FL, USA

Abstract

Cell-cell junction formation at the apical zonula adherens (ZA) is critical for epithelial tissue development or maintenance and is perturbed during cancer progression. E-cadherin (Ecad) and p120 catenin (p120) are core components of the ZA in epithelial cells. Recently, a novel Ecad-p120 partner called PLEKHA7 was identified to support the integrity of the ZA. Here, we reveal that the ZA, via PLEKHA7, associates with the RNA interference (RNAi) machinery, to suppress pro-tumorigenic signaling via miRNAs. PLEKHA7 co-localizes and co-precipitates with a non-nuclear subset of the core microprocessor proteins DROSHA and DGCR8. In addition, PLEKHA7 associates with all the major RISC components, including Ago2, GW182 and PABPC1, at the ZA. Recruitment of DROSHA, DGCR8 and Ago2 to the ZA is PLEKHA7- and p120/cadherin-dependent,
opposed by active Src, and independent of microtubules. The PLEKHA7-associated microprocessor complex co-precipitates with primary miRNAs (pri-miRNAs) and possesses pri-miRNA processing activity. As a result, PLEKHA7 regulates the levels of a specific set of miRNAs, including miR-24, miR-30a, miR-30b and let-7g, and suppresses the expression of pro-tumorigenic markers, including SNAI1, MYC, and CCND1. The presence of RISC, as well as of miR-30b and its target SNAI1 mRNA at the ZA suggests that PLEKHA7-mediated mRNA silencing happens at the junctions. Consistent with a tumor-suppressing role, loss or mislocalization of PLEKHA7 expression is common in breast and renal cancer. The present work uncovers a novel miRNA-mediated mechanism through which adhesion complexes regulate cellular behavior and tumor progression.

The ACTION Study: An Initiative to Address the Socio-Economic Burden of Cancer in South-East Asia

Mark Woodward
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Abstract
One of the biggest obstacles to developing policies in cancer care in Southeast Asia is the lack of reliable data on the disease burden and economic consequences. To address this shortfall, in 2012, a study of new cancer patients in the Association of Southeast Asian Nations (ASEAN) region – the Asean CoSTi in Oncology (ACTION) study – was mounted to assess the socio-economic impact of cancer. Simultaneously, a series of roundtable meetings of key stakeholders and experts was launched, with the broad aim of producing advice for governments in ASEAN to take appropriate account of issues relating to cancer, as well as to generate knowledge and interest through engagement with the media. An important product of these roundtables has been the Jakarta Call to Action on Cancer Control.

ACTION is a prospective longitudinal study of 9513 consecutively recruited adult patients with an initial diagnosis of cancer from Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Thailand and Viet Nam. The mean age of participants was 52 years; 64% were women. Twelve months after diagnosis, deaths and incidences of household financial catastrophe (out-of-pocket medical costs exceeding 30% of annual household income) were recorded. The effect on these two outcomes of a range of socio-demographic, clinical and economic predictors was estimated using a multinomial regression model (Kimman et al, 2015; BMC Medicine 2015). The study adds compelling, and much needed, evidence to the argument for policies that improve access to care and provide adequate financial protection from the costs of illness in the ASEAN region.

Molecular Targeting for Brain Tumors Treatment

Waldemar Debinski
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Abstract
Glioblastoma (GBM) is the most common primary brain tumor in adults and represents an unmet need in medicine. We discovered that interleukin 13 receptor alpha 2 (IL-13RA2) and EphA2 receptors are over-expressed in most patients with GBM, but not in normal brain, and also in spontaneous canine high-grade gliomas; the combined over-expression is ~90%. IL-13RA2 and EphA2 are targets for multiple therapeutic approaches currently in the clinic or under pre-clinical evaluation. In a further search for targets in GBM, we came across the EphA3 receptor. Our research and others' has shown that IL-13RA2, EphA2, and EphA3 are widely present in various compartments of GBM tumors microenvironment: i) all three receptors are expressed in tumor cells of the core of GBM tumors and on tumor cells-infiltrating normal brain parenchyma, while EphA2 is also over-expressed in tumor neovasculature, and ii), IL-13RA2, EphA2, and EphA3 are associated with, and/or play important roles in the pathobiology of glioma stem-like cells (GSC). The EphA3 receptor can be also readily detected in GBM-infiltrating cells of bone marrow origin. Thus, collectively, IL-13RA2, EphA2, and EphA3 are over-expressed and are functional in several GBM compartments involved in tumor progression and/or resistance to therapies. We are currently pursuing the novel idea of targeting all four receptors with one targeted cytotoxic compound (QUAD-CTX). The agent delivers a catalyst to GBM tumors, specifically killing transformed cells and abnormal cells of the tumor environment. This "molecular resection", unlike surgery, is expected to result also in an "in-situ vaccination".
Targeting the Unfolded Protein Response to Overcome Therapeutic Resistance in Cancer

Hari K. Koul
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Abstract

Each year over half a million men and women will die of cancer in US, mostly due to therapy resistant disease. Solid tumors in general respond poorly to current chemotherapeutic regiments. Due to high proliferative potential, the cancer cells incur a lot of stress due to nutritional deprivation, hypoxia etc. As a result, tumor microenvironment becomes limiting and cancer cells up-regulate survival pathways to continue cell growth and evade apoptosis. Treatment resistant tumors frequently harbor gain of function mutations in KRAS and activation of aberrant signaling cascades. Thus it is no surprise that these pathways have been actively targeted, albeit unsuccessfully, for therapeutic intervention. Moreover, aggressive cancer cells have very high demand for protein synthesis, as such protein synthetic machinery is highly active in cancer cells. The newly synthesized proteins need to be properly folded, and sorted for them to function properly. UPR is a collection of signaling pathways elicited in response to accumulation of unfolded proteins in the ER lumen whose downstream effectors are responsible for alleviating ER stress. However, UPR can also tip the balance to cell death if ER stress is beyond remedy. Thus we believe that targeting components of UPR response may help push the ER stress to its limit and promote cell death rather than cell survival. Based on these considerations we hypothesize that dual targeting components in translational machinery and components of UPR could offer a new paradigm to overcome therapy resistance in cancer in general and in solid tumors in particular.

Pre-clinical Vetting of Novel Treatment Strategies for Brain Tumors Using PDX Models

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Abstract

Minimal progress has been made in developing effective molecularly targeted therapies for primary or metastatic brain tumors over the past decade. To enhance pre-clinical testing of potential therapies, the Mayo Clinic has developed a panel of over 100 brain tumor patient-derived xenograft (PDX) models for both glioblastoma (GBM) and brain metastases. In comparison to the original patient tumor, derivative PDX models faithfully preserve the genetic, epigenetic, and phenotypic characteristics of the original tumor. Using a subset of 25 PDX models, the efficacy of a brain penetrant PARP inhibitor, veliparib, combined with temozolomide (TMZ) was evaluated. These results demonstrated profound combinatorial activity in a subset of GBM with promoter hypermethylation within the DNA repair gene MGMT. In contrast, evaluation of a brain impenetrant PARP inhibitor, rucaparib, using the same dosing regimen, demonstrated efficacy only in flank tumor models and not orthotopic models. Based on these data, the combination of veliparib with TMZ was taken forward in a randomized Phase II/III clinical trial testing the combination only in patients with MGMT promoter hypermethylation, and the Phase II portion of the study is nearing completion. Collectively, these studies highlight the importance of evaluating novel therapies in the context of orthotopic tumors, which have at least a partially intact blood-brain barrier, and illustrate how evaluating treatment responses across a relatively large panel of PDX models can be used to define biomarker enrichment strategies for clinical trials.

Glioblastoma Heterogeneity Modelling In–vitro and In–vivo

Tamara Lah Turnšek
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Abstract

Glioblastoma multiforme (GBM) is the most lethal brain cancer, consisting of heterogeneous mixture of functionally distinct cancer cells, leading to tumour progression. This autonomous cancer cell heterogeneity is superimposed by non-autonomous heterogeneity, due to the presence of various normal cells infiltrating the tumour mass (also called stromal cells) altogether comprising tumour microenvironment. Among these are also mesenchymal stem cells (MSCs), recruited most likely
from the bone marrow. Their multiple interactions can be studied in the *in vitro* and *in vivo* (animal) models, dissecting and thereafter superimposing various bilateral interactions. First, we examined GBM cell lines U87 and U373, resembling the neural and mesenchymal GBM phenotypes, respectively, in the *in vitro* indirect co-cultures to reveal the impact of their cross-talk on their behavioral alterations. The effects on cells' transcriptome revealed altered expression of 264 genes in U87, being associated with proliferation, inflammation, migration and adhesion and 221 in U373 cells, mainly associated with apoptosis, cell cycle, differentiation and migration. Gene expression changes observed in the co-cultured U87 and U373 cells resulted in altered invasiveness of GBM cells, being the key characteristics of GBM and the major reason for poor GBM patients' survival. Contrary to the increased invasion of U373 cells observed upon U87 co-culturing in both, 2D and 3D invasion assays, the invasion of U87 cells appeared highly dependent on the matrix substrate and the spatial set up: U87 cell invasion decreased in the indirect 2D co-culture on matrigel, whereas it increased in 3D-collagen embedded U87 spheroids exposed to U373 culture medium. Indirect co-culturing of GBM cells also oppositely affected their temozolomide resistance: We found that that U373 cells protect neuronal-like U87 cells from TMZ cytotoxicity and oppositely, U87 cells seem to sensitize U373 cells to TMZ. Moreover, we speculated that these two cell types seem to differentially responded to the presence of stromal cells, such as the bone-marrow derived MSCs and thus, we studied the impact of direct interaction between MSCs and these two distinct GBM cell lines (2). We were mostly interested into the cell invasion *in vitro*, as well as *in vivo*, using zebrafish embryo model (3). We demonstrated that the effect of MSC/GBM cellular cross-talk on GBM cell invasion is GBM cell type specific; namely, MSCs decreased the invasion of U87 cells, whereas they increased the invasion of U373 cells (2), being of mesenchymal GBM phenotype. This was observed *in vitro* and the *in vivo* zebrafish embryo. In contrast, both GBM cell lines increased the invasiveness of MSCs upon direct cell to cell interaction. Moreover, we observed that U373 cell invasion correlated with increased expression of cathepsin B, calpain 1, uPA/uPAR, MMP-9 and MMP-14, all involved in the protease signalling cascade, leading to enhanced GBM invasion (3). Using selective inhibitors in a 3D-invasion model *in vitro* we confirmed involvement of these enzymes in MSC-enhanced invasion of U373 cells. In contrast, candidate proteases were not altered or were even down-regulated in U87 cells upon direct contact with MSCs. Taken together, our findings are the first to suggest that the response of heterogeneous tumour (GBM) cells' population to stromal cells (MSCs) strongly depends on the tumour cell subtypes, and that these are affecting each other in a paracrine manner, favouring the mesenchymal GBM subtypes aggressiveness. These occurs even in the tumours of the same histopathological appearances and clinical diagnosis. In conclusion, defining transcriptional alterations of distinct GBM cells upon co-culturing leads to better understanding of the mechanism of GBM heterogeneity.

**Special Talks**

**The Role of Zinc in the Development of Cancers, and Its Implications for Treatment and Prevention**

Leslie C. Costello and Renty Franklin

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**Abstract**

It has been well established since 1952 that zinc is markedly decreased in virtually all cases of prostate cancer, and that the decrease in zinc occurs early in the development of malignancy. The decrease in zinc results from the down regulation of ZIP1 zinc uptake transporter. The decrease is essential to prevent the cytotoxic effects that zinc exhibits in the premalignant and malignant cells. A similar relationship is evident in hepatocellular cancer and in pancreatic adenocarcinoma. Other cancers also exhibit decreased zinc, although not well established. Consequently, decreased zinc is a common relationship in many cancers. The cytotoxicity in the premalignant/malignant cells by the higher zinc levels that exist in the normal cells, offers a plausible target to prevent the development of malignancy, and/or termination of progressing malignancy. This could be achieved by an approach that will restore the increased zinc level in the premalignant/malignant cells; thereby exhibiting cytotoxic/tumor suppressor effects. However, it must be recognized that these cancers are ZIP-deficient malignancies; which poses the issue of a mechanism that will facilitate the uptake of zinc by the premalignant/malignant cells. One approach is to employ a zinc ionophore such as clioquinol; which we have shown to be highly effective in suppressing the growth of ZIP1-deficient prostate xenograft tumors in mice. Moreover, such effective treatment has minimal systemic contraindications. The untreatable status and high death rate of many of these cancers dictate that barriers, which have prevented progress in the development of such zinc treatment approaches and clinical trials, should be removed.
Plant Extracted Solasodine Rhamnosides, a New Era for Cancer Therapy?

Bill Cham
Australasian Institute of Medical Research, Vanuatu

Abstract
Solasodine rhamnosides extracted from plant material are promising anticancer drugs. Studies have shown that these agents offer gains in specificity, efficacy, safety, tolerability, non-resistance and convenience in the treatment of patients with cancer. Our group was the first to describe the antineoplastic properties of these molecules. Substantive data are now available that cover preclinical development of solasodine rhamnosides for a large range of cancers and the clinical development, comprising of Phases I to IV trials of a specific topical formulation of solasodine rhamnosides, for the treatment of skin cancers. Further studies are ongoing for the treatment of terminal cancers. This presentation will cover all the necessary aspects starting from drug extraction, identification, preclinical and clinical studies. Examples of patient’s treatment outcomes will be exhibited.

Featured Presentations

Lung Cancer Stemness and Metastasis: Novel Mechanisms and Therapeutic Targets

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Abstract
Background: Non-small cell lung cancer (NSCLC) is highly correlated with smoking. Our studies have shown that nicotine, the non-carcinogenic component of tobacco smoke, can promote cell proliferation, tumor growth and metastasis. Further, nicotine could enhance the self-renewal properties of stem-like cells from NSCLC, by inducing the SOX2 transcription factor, through the involvement of YAP1 and E2F1. Targeting SOX2 appears to be a viable strategy to combat stemness, angiogenesis and tumor growth.

Objective: Identify molecular mechanisms underlying self-renewal of stem-like cells and to develop strategies to eliminate them. Such agents can be expected to have anti-tumor activities.

Methods: A series of in vitro studies supplemented by xenograft experiments were conducted on NSCLC cells. Techniques used include double immunofluorescence experiments, proximity ligation assays, in vitro binding assays, IP-western blots etc.

Results: Our studies show that SOX2 is a major regulator of stem-like functions in NSCLC cells and it is upregulated by components of the Hedgehog and Hippo signaling cascades. We find that YAP1, the oncogenic component of the Hippo signaling pathway, as well as Gli1 transcription factor, are major regulators of SOX2, stemness and vascular mimicry in NSCLC. The fact that nicotine could enhance stemness through the involvement of SOX2 adds a new dimension to the mechanisms by which smoking initiates and promotes NSCLC. Agents that target SOX2 could be beneficial in combating NSCLC.

Discussion and Conclusions: It has been suggested that eliminating stem-like cells would be necessary to combat tumor growth, drug resistance and metastasis. Significant efforts have been made to identify signaling cascades and specific molecules that promote stem-like functions. Our finding that oncogenic components of the Hippo signaling pathway as well as the Hedgehog signaling pathway contribute to stemness of NSCLC cells is a step in this direction. Agents that can suppress SOX2 expression appear to be effective in inhibiting self-renewal as well as vascular mimicry of stem-like cells, and can be expected to have significant anti-cancer activities.
Effects of Anticancer Drug on Chromosome Instability (CIN) and New Clinical Implications for Tumor-Suppressing Therapies

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Abstract

Whole-chromosomal instability (CIN), manifested as unequal chromosome distribution during cell division, is a distinguishing feature of most cancer types. CIN is generally considered to drive tumorigenesis, but a threshold level exists whereby further increases in CIN frequency in fact hinder tumor growth. While this attribute is appealing for therapeutic exploitation, drugs that increase CIN beyond this therapeutic threshold are currently limited. In our previous work, we developed a quantitative assay for measuring CIN based on the use of a non-essential human artificial chromosome (HAC) carrying a constitutively expressed EGFP transgene. Here, we used this assay to rank 62 different anticancer drugs with respect to their effects on chromosome transmission fidelity. Drugs with various mechanisms of action such as antimitotubule activity, histone deacetylase (HDAC) inhibition, mitotic checkpoint inhibition, and targeting of DNA replication and damage responses were included in the analysis. Ranking of the drugs based on their ability to induce HAC loss revealed that paclitaxel, gemcitabine, dactylolide, LMP400, talazoparib, olaparib, peloruside A, GW843682, VX-680, and cisplatin were the top ten drugs demonstrating HAC loss at a high frequency. Therefore, identification of currently used compounds that greatly increase chromosome mis-segregation rates should expedite the development of new therapeutic strategies to target and leverage the CIN phenotype in cancer cells.

Application of a Subclass of Camptothecin Analogues with Novel Mechanism of Action for Targeted and Personalized Cancer Treatment

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Abstract

An anticancer agent, designated as FL118, was recently identified as a novel camptothecin (CPT) analogue that possesses exceptional antitumor efficacy in animal models of human tumor. The known mechanism of action (MOA) for the CPT analogues including irinotecan and topotecan (two FDA-approved CPTs) is the inhibition of the topoisomerase I (Top1) activity. FL118 exhibits much better antitumor activity than irinotecan and topotecan, and effectively overcomes irinotecan- or topotecan-resistant human tumors. The question is whether FL118 is a better Top1 inhibitor. Our studies indicate that FL118 is not a better Top1 inhibitor in comparison with SN-38 (the active metabolite of irinotecan) shown in the in vitro biochemical catalytic assay, and Top1 mutations increase irinotecan and topotecan resistance for 100-1000 folds, while it much less affects FL118 efficacy. Furthermore, it is well documented in the literature that Top1 expression level is positively associated with CPTs’ antitumor sensitivity. Low Top1 expression is linked to CPT resistance. In contrast to these findings, FL118 can exhibit high antitumor efficacy in in human tumors without Top1 expression, while tumors with high Top1 expression can be resistant to FL118. This indicates that FL118 antitumor activity does not require Top1 expression in the tumor cells. Instead, we found that FL118 selectively inhibits the expression of multiple anti-apoptotic proteins (survivin, Mcl-1, XIAP, cIAP2) and the oncogenic protein MdmX. Importantly, genetic modulation of these oncogenic proteins demonstrate their role in FL118 function. These observations indicate that FL118 possesses the antitumor MOA distinct from other CPT compounds including irinotecan and topotecan.

New Therapeutic Delivery Approaches in Neuro-oncology

Graeme Woodworth*, David Hersh, Anthony Kim, Jeff Winkles, Victor Frenkel and Howard Eisenberg
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Abstract

A long-standing goal in treating patients with glioblastoma (GB), the most common and deadly primary brain cancer, is linking tumor specific features with effective anti-tumor therapies to generate long-term treatment responses. Core principles
Advancement of these concepts is likely to turn GBM from a uniformly fatal cancer into a chronic disease with the potential for cure. Magnetic resonance imaging-guided focused ultrasound (MRFUS) is an emerging and exciting technology to aid in this advancement process. The technology was recently FDA-approved for the treatment of Essential Tremor and is being evaluated in clinical trials for numerous other conditions. MRFUS has the potential to radically alter the treatment of GB and other devastating neurological malignancies.

**Discovery of a Novel Therapeutic Target in Multistage Tumorigenesis**

Fiona Haxho¹, Ronald J. Neufeld² and Myron R. Szewczuk³

¹Departments of Biomedical and Molecular Sciences, Kingston, Ontario, Canada
²Department of Chemical Engineering, Queen’s University, Kingston, Ontario, Canada

**Abstract**

Several of the growth factors and their receptor tyrosine kinases (RTK) such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF) and insulin are promising candidate targets for cancer therapy. Indeed, tyrosine kinase inhibitors (TKI) have been developed to target these growth factors and their receptors, and have demonstrated dramatic initial responses in cancer therapy. Yet, most patients ultimately develop TKI drug resistance and relapse. It is essential in the clinical setting that the targeted therapies are to circumvent multistage tumorigenesis. Here, we identify a novel receptor signaling platform linked to EGF, NGF, insulin and TOLL-like receptor (TLR) activations, all of which are known to play major roles in tumorigenesis. The importance of these findings signify an innovative and promising entirely new targeted therapy for cancer. The role of mammalian neuraminidase-1 (Neu1) in complex with matrix metalloproteinase-9 and G protein-coupled receptor tethered to RTKs and TLRs is identified as a major target in multistage tumorigenesis. Evidence exposing the link connecting growth factor-binding and immune-mediated tumorigenesis to this novel receptor-signaling paradigm will be reviewed in its current relationship to cancer.

**A Novel β-Catenin Signaling Pathway Activated by Interleukin 1β Leads to the Onset of Epithelial-Mesenchymal Transition in Breast Cancer Cells and Drug Resistance**

Isaura Meza*, Eloy A. Perez-Yepez, Jorge T. Ayala Sumuano, Aura Jimenez-Garduno, Daniel Urrutia-Cabrera and Maria del Carmen Dominguez-Robles

Department of Molecular Biomedicine, Centro de Investigacion y de Estudios Avanzados del IPN (CINVESTAV), Mexico City, Mexico

**Abstract**

Interleukin 1β has been associated with tumor development, invasiveness and metastasis in various types of cancer. However, the molecular mechanisms underlying this association have not been clearly elucidated. The present study is the first to show, in breast cancer cells, that an IL-1β/IL-1RI/β-catenin signaling pathway induces β-catenin nuclear accumulation due to GSKββ inactivation by Akt phosphorylation. Translocation to the nucleus of the accumulated β-catenin and formation of the TCF/Le/β-catenin complex induce sequential expression of c-MYC, CCDN1, SNAIL1 and MMP2, leading to up-regulation of proliferation, migration and invasion. All of these processes have been shown to be required in cancerous cells to initiate the transition from a non-invading to an invasive phenotype. Higher levels of IL-1β are known to induce in ERα cells and MSC the release of cytokines and chemokines that enhance the inflammatory microenvironment and metastasis. We report here that IL-1β induction of the breast cancer cells to an invasive phenotype also leads to Estrogen Receptor-α gene methylation through TWIST1 overexpression, increasing the resistance of cells with an invasive phenotype to anticancer endocrine therapy drugs such as tamoxifen.
Identification of Novel Cancer-Relevant PARP1 Inhibitor Targets by Chemical Proteomics

Claire Knezevic1, Gabriela Wright1, Lily Remsing Rix1, Woosuk Kim2, Brent Kuenzi1, Yunting Luo1, January Watters1, John Koomen1, Eric Haura1, Alvaro Monteiro1, Caius Radu2, Harshani Lawrence1 and Uwe Rix1*

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Abstract

PARP1 inhibitors are a new drug class, which shows interesting anticancer activity in a wide range of tumors, particularly BRCA1/2-mutant breast and ovarian cancers. Most PARP1 inhibitors target the NAD+ binding pocket within PARP1. However, NAD+ is a co-factor required by many enzymes and the individual drug target profiles beyond the PARP family are unknown. Importantly, off-targets can result in differential clinical properties, such as efficacy and side effects. Using an unbiased, mass spectrometry-based chemical proteomics approach, we performed a direct comparison of four clinical PARP inhibitors, namely olaparib, veliparib, niraparib, and rucaparib, and generated proteome-wide target maps in CAL51 breast cancer cells. PARP1 inhibitors as a drug class were found to display high target selectivity. However, in addition to the canonical targets PARP1, PARP2 and several of their complex binding partners, we identified hexose-6-phosphate dehydrogenase (H6PD) and deoxycytidine kinase (DCK) as novel targets of rucaparib and niraparib, respectively. Subsequent biochemical and cell-based functional validation suggested that inhibition of DCK by niraparib could have detrimental effects if combined with nucleoside analogue pro-drugs, such as cytarabine or gemcitabine, as bioactivation of these pro-drugs depends on DCK. H6PD gene silencing, on the other hand, caused apoptosis and further sensitized cells to PARP1 inhibitors suggesting that H6PD may be, in addition to its established role in metabolic disorders, a new anticancer target. Importantly, the rucaparib structure could also serve as a novel starting point for developing more potent and selective inhibitors of H6PD for anticancer therapy.

IL-15 in the Life and Death of Lymphocytes: Implications for the Treatment of Cancer

Thomas A. Waldmann

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Abstract

Interleukin-15 (IL-15) acts through a heterotrimeric receptor involving the specific IL-15R alpha subunit and IL-2/IL-15R beta and γc subunits shared with IL-2. IL-15 plays a pivotal role in the maintenance of natural killer cells and the induction of stem, central and especially effector memory CD8 T-cells. In rhesus macaques continuous intravenous infusion of Escherichia coli processed rhIL-15 at 20 μg/kg/day was associated with an approximately 10-fold increase in the numbers of circulating NK cells and monocytes, and 80 to 100-fold increase in the number of effector memory CD8 T-cells. IL-15 has proven effective in the treatment of a number of murine syngeneic tumor models. Clinical trials involving rhIL-15 given by bolus infusion, by subcutaneous or by continuous intravenous infusions have been completed in patients with metastatic malignancy. Furthermore, clinical trials have been initiated that employ the combination of IL-15 with IL-15R alpha+/- IgFc. In translation of the effects of IL-15 on NK cells and monocytes, combination therapy of IL-15 with anticancer monoclonal antibodies has shown augmented antibody-dependent, cell-mediated cytotoxicity action and antitumor efficacy in murine models, an observation that is being translated into clinical trials. Furthermore, IL-15 is being evaluated in association with agents such as anti-CTLA4 and anti-PD1 that relieve checkpoints on the immune system. It is hoped with these diverse approaches IL-15 will take a central place in the combination treatment of cancer.

Targeting Immune Suppression in the Cancer Microenvironment

Dennis Klinman

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Abstract

The ability of tumor-specific cytotoxic T cells and natural killer cells to eliminate cancers is hindered by the immunosuppressive cells present in the tumor microenvironment. Monocytic myeloid-derived suppressor cells (mMDSC) constitute the most
active of these tumor infiltrating leukocytes and are key contributors to the immunosuppressive milieu. mMDSC arise in the bone marrow from myeloid progenitors and are present at high frequency in patients with cancer.

Murine mMDSC express TLR9 and respond to stimulation with CpG oligonucleotides (TLR9 agonists) by differentiating into tumoricidal macrophages. In vivo administration of CpG ODN slows/prevents the growth of tumors in mice, an outcome linked to the increased activity of tumoricidal T cells. Human mMDSC express TLRs 2, 7 and 8 (but not 9) and are induced to differentiate into macrophage when stimulated via the relevant Toll-like receptors. Agonists targeting TLR 1/2 (such as PAM3) induce mMDSC to mature into immunosuppressive M2-like macrophage whereas agonists targeting TLR 7/8 (such as R848) cause the same precursors to mature into tumoricidal M1-like macrophages. The cytokines responsible for mediating these differences in maturation pathway and activity will be described.

A Role of IL-18 and Innate Immune Cells in Cancer Immunotherapy

Haruki Okamura
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Abstract
Recent breakthrough in cancer immunotherapy brought about a great progress in cancer therapy. The strategy of this therapy is mainly to re-activate immune system against tumor cells, targeting acquired immune cells. In the present study, we tried to explore the contribution of innate immune lymphocytes to the defense against tumors. In mice of peritoneal disseminating tumor model using CT26 cell, a colon cancer cell, and of lung metastasis model by B16 melanoma cells, treatment with immune checkpoint inhibitors such as anti CTLA4, anti-PD-L1, and anti-PD-1 Abs significantly suppressed tumor growth and prolonged survival of animals. Administration of IL-18 in combination with these inhibitors far more effectively prolonged the survival, and under some conditions, it completely eliminated tumors. Notably, combination of IL-18 and the inhibitors markedly increased the population of a subset of NK cells with phenotypes of pre-mNK, B220 high MHC-II high NKG2D high, gδ T cells, and CD8+ T cells. To the contrary, it reduced CD25 high FOXP3+ Treg cells in the peritoneal exudate cells of peritoneal dissemination model mice. Thus, IL-18 was shown to enhance expansion of effector cells and to suppress regulatory cells. The results of analysis on the cellular components in the lung of metastasis model will be presented. Roles of IL-18 in activation of NK cells will also be discussed.

Combination of CART19 Immunotherapy with Inhibition of BTK, B-cell-expressed Kinase, in Lymphoma

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Abstract
Purpose: Mantle cell lymphoma (MCL) is a B-cell malignancy that remains incurable despite development of novel therapeutic agents such as the BTK inhibitor Ibrutinib. Furthermore, responses of MCL to therapy with chimeric antigen receptor T cells recognizing CD19 (CART19, CTL019) remain essentially undefined. We, therefore, evaluated the combination of CART19 cells with Ibrutinib in pre-clinical models of MCL to determine if this combination of active immunotherapy with cell signaling inhibition proves particularly efficacious. This combination study has been the first-in-kind for lymphoma of any type.

Experimental Design: Patient-derived MCL cell lines and primary MCL samples were exposed to CART19 cells and ibrutinib, either separately or in combination, both in vitro and in vivo, in mouse xenograft model.

Results: MCL cells strongly activated multiple CART19 cell effector functions and the anti-MCL cytotoxic effect was enhanced in the presence of ibrutinib. In a xenograft MCL model, response to CART19 cells has proved superior as compared to ibrutinib (median survival not reached versus 95 days, p<0.005) but the mice receiving CART19 cell monotherapy eventually developed MCL tumors. In contrast, 80% of mice treated with the CART19 cell/ibrutinib combination remained in long-term remission as compared to 0% of mice treated with CART19 cells alone (p<0.05).

Conclusions: Combination of CART19 cells with ibrutinib, two potent therapeutic modalities with very different mode of action, is highly active in the pre-clinical models of MCL. Our findings pave the way for this two-pronged therapeutic strategy in patients with MCL and, possibly, other types of B-cell lymphoma.
Use of Dual-Mode Cellular Imaging in Cancer Vaccine Development

Jeff W. M. Bulte
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Abstract

Further clinical optimization of the dose, route, vaccine composition, and use of immunoadjuvants could greatly benefit from clinical imaging approaches that can interrogate the biological fate of cells repeatedly and non-invasively without the need for obtaining biopsies. By pre-labeling DCs with superparamagnetic iron oxide nanoparticles as an MRI contrast agent, it is not only possible to follow their migration to nearby lymph nodes, but also to verify if the injections have been performed accurately. Surprisingly, in our first clinical DC MRI cell tracking study, we showed that the target lymph node was routinely misinjected in 50% of stage IV melanoma patients. A different kind of cancer vaccine developed at our institute is GVAX, which consists of lethally irradiated tumor cells engineered to secrete GM-CSF. By pre-labeling GVAX with SPIO, we developed “magnetovaccination” as a novel MRI technique to monitor serially over time DC antigen capture and subsequent homing to draining lymph nodes. Using magnetoGVAX and MRI for serially monitoring the afferent arm of the immune response (DCs), and bioluminescent imaging (BLI) for monitoring the efferent arm (T cells), we applied dual-mode imaging to better understand the time course of antigen capture, lymph node delivery, and clonal T cell expansion. Depending on the timing of administration, toll-like-receptor (TLR) agonists either reduced or enhanced antigen capture and delivery to the lymph nodes. The lack of antigen delivery to lymph nodes was consistent with the lack of T cell BLI signal in the lymph nodes. In those cases, a massive extra nodal T cell proliferation occurred in the liver and spleen. Our studies show how dual-mode imaging can be used to evaluate and optimize combinatorial cancer vaccines.

Tumour Microenvironment: Promising Target of Successful Anticancer Therapy

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Abstract

Epithelial-mesenchymal interaction is important in the morphogenesis, development, wound healing and also in cancer behavior. It is dependent on the range of cytokines, chemokines, growth, proliferating and transforming factors produced by both the counterpartners. Tumor stroma contains, except the mesenchyme, also blood vessels and the inflammatory cells. We focused our research on cancer-associated fibroblasts (CAF). They are producers of extracellular components, which are necessary to formation bioactive cancer microenvironment and are able to influence the biology of tumor predominantly the differentiation status of tumor cells and their migratory potential. It is agreement with hypothesis of the British surgeon Stephen Paget postulated 100 years ago, that cancer cells need a special environment in a similar way to seeds needing fruitful soil. CAF from squamous/basal cell carcinomas significantly influence phenotype of normal epithelial cells to be similar to cancer cells. Interestingly, CAF isolated from the different types of tumors (squamous/basal cell carcinoma, melanoma, breast carcinoma) are biologically active to breast cancer cell line that suggest the tumor type nonspecific activity of CAF. We also tested the biological activity of their products on normal epithelial cells. These experiments clearly demonstrated that blocking of selected growth factors and chemokines enable to alter the microenvironment and to stimulate the differentiation of epithelial cells. In summary, presented results demonstrate that the epithelial-mesenchymal interaction represents the important part of tumour microenvironment also in case of tumours originated from squamous cell epithelia with therapeutic consequences of manipulation of these interactions using antibodies blocking specific growth factors/chemokines/chemokines.

How Cancer Cells Program the Tumor Microenvironment of Pancreatic Cancer

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Abstract

Tumor microenvironment (TME) plays an important and complex role in supporting cancer initiation, progression, and
metastasis. Emerging evidence has suggested that tumor cells can transcriptionally reprogram stromal cells through hormonal and cytokine signals. However, the molecular mechanism of reprogramming remains unknown. It is conceivable that epigenetic regulation will provide a dynamic and reversible modulation of stromal cells. Focusing on pancreatic cancer, we showed that tumor cells reprogram stromal fibroblasts fundamentally in the form of DNA methylation conferring both dynamic and stable modification on stromal cells. As a result, tumor cells convert the TME into a tumor supportive environment. Similar mechanisms may occur universally in other stroma components such as macrophages in the TME as well as in other tumor types. Therefore, our study has described a novel mechanism that mediates the role of TME in cancer development. Therapeutic agents targeting DNA methylation are being actively developed; however, so far, they are primarily focused on treating tumor cells. DNA methylation is a reversible process, thus targeting the methylation process in the host of the cancer provides a new strategy for cancer treatment and prevention.

Neoadjuvant Immunotherapy in High Risk Melanoma: A New Justified Approach

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Abstract

Intratumoral administration (ITAd) of granulocyte macrophage-colony stimulating factor (GM-CSF) did result in 50% complete response (CR)in in-transit metastases. Lesions that failed GM-CSF therapy had CR to ITAd of interleukin-2 (IL-2). While GM-CSF can activate dendritic cells which are antigen presenting cells (APC), IL-2 activates cytotoxic T cells. Therefore, the sequential administration of these two cytokines can complement one another. Furthermore, cutaneous melanoma is an immunogenic tumor, but also very heterogeneous. Therefore, an effective adjuvant therapy must be autogenic and specific. Utilizing patient own tumor, before its excision, as the source for tumor specific antigens, sequential ITAd of low dose GM-CSF followed by low dose IL-2 in deeply invasive melanoma, one week prior to its resection “Neoadjuvant Approach” did induce massive antitumor response at the injection sites characterized by complete tumor necrosis with massive histiocyteosis. In addition, there was an overexpression of great number of immune cells at the injection sites as well as in some regional lymph nodes which included cytotoxic T cells (CD8+) and helper cells (CD4+) cells. The duration of response ranged from 31-over 60 months in patients with in-transit metastases as well in those with primary invasive melanoma. This autologous nontoxic approach seemed to overcome tumor heterogeneity with excellent response. In conclusion, in vivo autoimmunization of melanoma sites by ITAd of these two cytokines seemed to induce an immense antitumor response without major side effects, and such an immune response was transmitted initially via the lymphatics. Such an approach seemed to prolong patient survival.

Nano-diamino-tetrac (Nanotetratec, NDAT), A Thyroid Hormone Antagonist at Integrin αvβ3, Causes Necrosis via Anti-Angiogenesis and Induces Apoptosis in Human Glioblastoma Xenografts

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Abstract

Emerging clinical and in vitro cell culture evidence indicates that glioblastoma multiforme (GBM) is a thyroid hormone-dependent tumor. L-thyroxine (T4) induces proliferation of glioma/GBM cancer cells via a multifunctional cell surface receptor for T4 on the extracellular domain of cancer cell integrin αvβ3. This action of physiologic concentrations of free T4 is blocked by nanoparticulate tetraiodothyroacetic acid (Nanotetratec, Nano-diamino-tetrac, NDAT) in which tetrac is covalently bound to poly(lactic-co-glycolic acid) (PLGA) nanoparticles. We have examined histopathologically the induction by NDAT of devascularization, necrosis and apoptosis in U87MG human GBM cell xenografts in nude mice. Treatment was 1 mg tetrac equivalent/kg body weight s.c. as NDAT daily X10 d, begun 2 d following tumor implantation. Tumor volumes in treated animals at sacrifice were decreased 60% vs. control (PLGA only) and xenograft weight was reduced by 50% (P<0.01). Tumor area in histologic sections was reduced by 80% in treated animals (P<0.001 vs. controls). Blinded histopathological interpretation
of slides from xenografts revealed essentially complete loss of tumor vascularity with NDAT (P<0.001 vs. control), but without hemorrhage. Eighty percent of cell populations in grafts was necrotic or apoptotic (P<0.001 vs. control). In summary, NDAT devascularized GBM xenografts with resultant widespread necrosis. Graft shrinkage in treated animals was 50%-80% in weight/volume/histological area vs. controls. The thyroid hormone receptor on αβ in U87MG cells is a single endocrine target with multiple downstream actions; pharmacologic inhibition of this receptor by NDAT caused non-cytotoxic GBM cell death and loss of xenograft vascularity.

NOSH-NSAIDS a New Class of Anti-Inflammatory Pharmaceuticals as Chemotherapeutic Agents

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are chemopreventive, but their long-term use may lead to significant gastrointestinal (GI), cardiovascular (CV) and renal side effects. In our search for a “better NSAID”, we developed hybrids that release NO and H,S, two gaseous signaling molecules of biological relevance. The rationale for their use was based on the observations that both modulated some components of the local mucosal defense systems, leading to reduced GI toxicity. Also, these gas transmitters have protective roles in the CV and renal systems. We have reported on the synthesis and some general characterizations of NOSH-aspirin, NOSH-naproxen, and NOSH-sulindac. All three have enhanced GI safety profiles yet retain all the basic pharmacological activities of their respective native NSAID. They have potent anti-inflammatory characteristics coupled with anti-pyretic, analgesic, and anti-platelet properties. These compounds are orders of magnitude more potent than their corresponding NSAID in inhibiting the growth of adenomatous, epithelial, and lymphocytic cancer cell lines but have minimal effects on normal cells. In vivo, NOSH-aspirin was shown to have chemo-preventive properties that were superior to aspirin and NOSH-naproxen was shown to have chemotherapeutic properties. NOSH-aspirin showed synergistic activity with 5-flourouracil, an established chemotherapeutic agent, in inhibiting colon cancer cell growth. Mechanistically, these agents a) reduced proliferation, b) caused G0/G1 cell cycle arrest, c) increased apoptosis, d) reduced NF-kB, FoxM1, and b-catenin expressions in tumors, e) activated caspase-3 enzyme activity, f) increased reactive oxygen species, and g) increased p53 expression. Some of these proteins were modified through S-nitrosylation (S-NO) and S-sulfhydration (S-SH).

Anti-cancer Chemotherapy Specific to Acidic Cancer Nests

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Abstract

The acidification of solid cancer nests is now well known, but the chemotherapy under acidic conditions has not been focused. The acidosis causes the internal acidification of cancer cells, which affects the function of the target molecule(s) of anti-cancer drugs, leading us to argue that the efficacy of drugs may be altered in acidic cancer nests. We have examined the anti-proliferation activity of approximately 280 inhibitors under acidic conditions in vitro, and found that 4 compounds, lovastatin, cantharidin, manumycin A, and ionomycin, inhibited cancer cell proliferation strongly at acidic pH, but the inhibition was very low in medium of pH around 7.4. There have been many clinical reports to suggest that statins are effective on suppression of cancer progression, but statins are not standard for the present. One reason is that no effect of statins on cancer progression has been reported in some clinical trials. Our results imply that the efficacy of statins is low at the early stage of cancer development and/or in small cancer nests where acidosis is not enough. Despite the limitation that statins are useful only for progressed acidic cancer nests, statins are superior because of less effect on normal tissues. In fact, statins are now prescribed as an anti-hyperlipidemia medication, and no critical side effects, such as dysfunction of immune system, pain, diarrhea, nausea, and hair loss, have been reported. Therefore, chemotherapy with acidosis-dependent drugs may reduce mortality of many cancer patients, in association with amelioration of the serious conditions emerged in current anti-cancer chemotherapy.
Intratumor Heterogeneity in Human Tumors: Beyond the Genes

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Abstract

Background: For decades, pathologists have been describing new anatomopathological entities emphasizing the great intra and interheterogeneity. Genomic studies have identified a vast number of genetic alterations throughout the whole tumor and metastasis becoming a perplexing puzzle. To address tumor heterogeneity, we studied DNA mutations and RNA expression in three different areas of the tumors, as well as protein expression of receptors and signaling factors in whole lung carcinomas sections.

Material and Methods: In 130 lung adenocarcinomas the istopathological features and the expression of ALK, HER2, ki6, pMAPK, pAkt, pmtor, pS6 4E-BP1, p4E-BP1, eIF4E, peIF4E, and Epithelial-Mesenchymal Transition (EMT) factors (N-cadherin, YB1, pYB1) was assessed in whole paraffin sections. In 30 adenocarcinomas, molecular studies were run in three different histological cores with acinar, papilar and micropapilar cell growth patterns. EGFR, KRAS, ALK and ROS alterations were analyzed by PCR or FISH. RNA expression of 110 genes was screened using NanoString technology. Levels of expression were evaluated by a histo-score and correlations were analyzed using Kruskal-Wallis and Kaplan Meier (log Rank) statistical tests.

Results: The predominant histological subtypes were acinar, papillary and solid, with the latter associated with higher histological grade and more necrosis. pAkt, pmtor, pS6 showed a patchy tissue distribution while p4E-BP1 and peIF4E displayed a homogeneous and diffuse protein expression in about 80% of tumors. High expression of eIF4E, 4EBP1 and YB1 was associated with higher recurrence rate, histological grade and metastases. Over 90% cases showed RNA expression heterogeneity, a heterogeneous distribution of EGFR mutations in about 20% cases and a homogeneous pattern of KRAS mutations.

Conclusions: In lung adenocarcinomas, there is intratumor heterogeneity of mRNA expression, of EGFR mutations in a significant number of cases and a striking diversity in protein expression. With this data, we may conclude: first, the importance to study several areas of the tumors to assess tumor heterogeneity both at molecular and protein levels; second, the patchy expression of signaling factors, third, in most cases peIF4E and p4E-BP1 displayed a homogeneous expression supporting its role as a therapeutic target and fourth, pathologists need to integrate the molecular data, protein and histopathological data and apply systems biology.

Neuropeptides as Autocrine Growth Factors in Lung Cancer

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Abstract

Neuropeptides and their receptors are present in high concentrations in SCLC cells. Peptide G protein-coupled receptors (GPCR) and the EGFR are widely expressed in NSCLC cells. Peptide receptors for bombesin (BB), endothelin (ET), neotensin (NTS), and pituitary adenylate cyclase activating polypeptide (PACAP) regulate transactivation of the EGFR. Addition of BB, ET-1, NTS or PACAP to NSCLC cells cause phosphatidylinositol (PI) turnover within seconds after addition to NSCLC cells. After 2 minutes the tyrosine phosphorylation of the EGFR and ERK are dramatically increased. The transactivation of the EGFR results from increased secretion of TGFα from NSCLC cells. The release of TGFα and the transactivation of the EGFR is inhibited by MMP and Src inhibitors such as GM6001 and PP2, respectively. Activation of GPCR increases reactive oxygen species (ROS). The increase in ROS as well as EGFR transactivation is inhibited by Tiron and N-acetylcysteine. The ROS may impair phosphotyrosine phosphatase activity, resulting in increased EGFR tyrosine phosphorylation. The ETA-R regulates the formation of EGFR homodimers or EGFR-HER2 heterodimers (Moody et al., Proc. AACR 2016; 57: 461). The growth of NSCLC cells is inhibited by ZD4054 (ETA-R antagonist) or gefitinib (TKI). ZD4054 and gefitinib are synergistic at inhibiting the proliferation of NSCLC cells with wild type EGFR or secondary T790M mutations. Hours after addition of ET-1 to NSCLC cells, COX-2 and vimentin are dramatically increased whereas E-cadherin is decreased, suggesting that ET-1 facilitates epithelial mesenchymal transitions (EMT). Because the EMT caused by ET-1 addition to NSCLC cells is reversed by ETA-R antagonists, gefitinib or lapatinib, the ET-1 may facilitate EMT in an EGFR/HER2 dependent manner. Pacitaxel and/or macitentan (ETA-R and ETβR antagonist) decreased migration of NSCLC cells into the mouse brain. The results indicate that neuropeptides may play an important role in NSCLC proliferation and migration.
Targeted Therapies for Pediatric Cancers

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Abstract

Targeted molecular cancer therapeutics have revolutionized adult cancer care, but have had little impact on pediatric cancer care. One contributing factor for this inequality is most pediatric cancers are driven by oncogenic events that are currently not directly druggable. Two prominent examples are MYCN-amplified neuroblastoma and EWS-FLI1 translocated Ewing’s Sarcoma, both of which are driven by aberrantly expressed transcription factors (MYCN and FLI1, respectively). Alternative targeted therapeutic strategies to combat these deadly pediatric cancers are an important avenue of investigation to improve patient outcomes. Herein, we describe a pharmacogenomics strategy to uncover combination targeted therapies designed to specifically target genetically-defined subsets of pediatric cancers. This approach is effective at finding sometimes occult vulnerabilities of these cancers and exploiting them for therapeutic gain. Data is presented demonstrating how this approach may be successful at developing specifically-effective targeted therapy combinations for clinical trial testing in subsets of pediatric cancer.

Interaction Between ZBP-89 and Oncogenic Molecules in Hepatocellular Carcinoma

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Abstract

This study was to explore how ZBP-89, a transcription factor, interacted with oncogenic molecules, resulting in the inhibition of hepatocellular carcinoma (HCC) which is frequently resistant to chemo- and radio-therapies. HCC cell lines and mouse models were used to study how ZBP-89 induced the death of HCC cells. Tumor cell death was determined by MTT assay and TUNEL method. The potential binding to the promoter was analyzed by gel shift assay. HCC mouse models were established to confirm in vitro results. Our results showed that ZBP-89 induced apoptosis in HCC cells, enhanced HCC cells to other anti-HCC treatments, and inhibited the growth of tumor cells both in vitro and in vivo. Mechanically, ZBP-89 could function as a suppressor of HCC via multiple channels. 1) binds to the promoter region of pro-apoptotic Bak to increase its expression; 2) interacts with some p53 mutants to re-install the normal function of p53; 3) participates in gene methylation by inhibiting DNA methyltransferase 1 (DNMT1) and histone deacetylases 3 (HDAC3); 4) interacts with other transcriptional factors such as SP-1 to promote the expression of pro-apoptotic molecules or/and to suppress the expression of anti-apoptotic molecules. Collectively, our data demonstrate that ZBP-89 is able to facilitate the death of HCC cells via several mechanisms, leading to the inhibition of HCC growth in vitro and in vivo. (This study was supported by SRFDP and RGC ERG Joint Research, No: M-CUHK406/13 and the National Natural Science Foundation of China, No. 81472339).

Extracellular Ubiquitin: Relevance in Understanding Haematological Cancer

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Abstract

The extracellular role of ubiquitin in haematopoiesis was first reported by our group. We demonstrated the involvement of ubiquitin in homing and adhesion of hematopoietic progenitor stem cells using the classical Till and McCulloch assay. Incubation with exogenous ubiquitin at higher concentration can induce differentiation of HL-60 and K-562 cells. Recently, it has been reported by others that Inactivation of the transcription factor p53, through either direct mutation or aberration in one of its many regulatory pathways, is a hallmark of virtually every tumour. We propose that presence of extracellular free ubiquitin may compete with MDM2 to prevent p53 ubiquitination. In zebra fish, varying concentration of injected ubiquitin caused developmental delay and phenotypic aberrations. In transgenic embryos with rfp tag, concentration of fluorescence was observed, possibly by displacement of HSC’s from their niche. Expression of pall gene, implicated in hematopoietic malignancies, was checked relative to housekeeping beta-actin gene. Expression of pall decreased in rfp+ embryos. Beta-actin expression was higher in rfp- embryos unlike being standard throughout as expected of a housekeeping gene. This could be due
to over-expression of housekeeping genes due to stress condition stimulated by the extracellular ubiquitin. The down regulation of tal1, however, could be attributed to epigenetic silencing by ubiquitin. In rpf- embryos, tal1 was upregulated, possibly because some cells might have been in intermediate stages of maturation. We propose that ubiquitin can be used as a tool to understand the mechanism of haematological cancers in-vitro and in-vivo.

**Targeting the Wnt/beta-catenin-N-glycosylation Axis in Head and Neck Cancer**

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**Abstract**

Head and neck cancer is the 6th most common cancer in the United States with majority of cases presenting as oral squamous cell carcinoma (OSCC). Aggressive cancers are thought to arise from populations of cancer stem-like cells that drive tumor progression and resistance to therapy. The Wnt/beta catenin signaling cascade and the metabolic pathway of protein N-glycosylation function in a positive feedback loop and play important roles in stem cell phenotypes, and their aberrantly increased activities track with OSCC progression in the cancer genome atlas (TCGA). Here, we show that disrupting nuclear catenin interaction with the cAMP-responsive element binding (CREB)-binding protein (CBP), inhibited OSCC aggressive phenotypes in cellular, zebrafish and murine models. Targeting b-catenin-CBP interaction with a small molecule inhibitor, ICG-001, abolished cell proliferation, downregulated N-glycosylation and enhanced intercellular adhesion. Analyses of ICG-001 gene expression signatures revealed greater response in metastatic compared to non-malignant cells. Gene set enrichment analyses identified several pro-tumorigenic signaling pathways inhibited by ICG-001, and its higher inhibition score tracking with lower overall survival of OSCC patients in TCGA. In addition, ICG-001 inhibited orthotopic tumor growth and metastases and promoted capsular phenotypes in nude mice. Further, ICG-001 inhibited aggressive OSCC cells-driven tumors, isolated by FACS, in zebrafish. Similarly, ICG-001 interfered with growth and aggressive cell phenotypes in immune-competent mice. These results suggest that aberrant activation of beta -catenin/CBP signaling in oral tissues drives a stem-like state and OSCC progression and that ICG-001 may represent a novel therapeutic for the treatment of OSCC in humans.

**Dendrimer-based Selective Proteostasis-inhibition Strategy to Control NSCLC Growth and Progression**

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**Abstract**

Elevated valosin containing protein (VCP/p97) levels promote the progression of non-small cell lung carcinoma (NSCLC). Although many VCP inhibitors are available, most of these therapeutic compounds have low specificity for targeted tumor cell delivery. Hence, the primary aim of this study was to evaluate the in vitro efficacy of dendrimer-encapsulated potent VCP-inhibitor drug in controlling non-small cell lung carcinoma (NSCLC) progression. The VCP inhibitor(s) (either in their pure form or encapsulated in generation-4 PAMAM-dendrimer with hydroxyl surface) were tested for their in vitro efficacy in modulating H1299 (NSCLC cells) proliferation, migration, invasion, apoptosis and cell cycle progression. Our results show that VCP inhibition by DBeQ was significantly more potent than NMS-873 as evident by decreased cell proliferation (p<0.0001, MTT-assay) and migration (p<0.05; scratch-assay), and increased apoptosis (p<0.05; caspase-3/7-assay) as compared to untreated control cells. Next, we found that dendrimer-encapsulated DBeQ (DDNDBeQ) treatment increased ubiquitinated-protein accumulation in soluble protein-fraction (immunoblotting) of H1299 cells as compared to DDN-control, implying the effectiveness of DBeQ in proteostasis-inhibition. We verified by immunostaining that DDNDBeQ treatment increases accumulation of ubiquitinated-proteins that co-localizes with an ER-marker, KDEL. We observed that proteostasis-inhibition with DDNDBeQ significantly decreased cell migration rate (scratch-assay and transwell-invasion) as compared to the control-DDN treatment (p<0.05). Moreover, DDNDBeQ treatment showed a significant decrease in cell proliferation (p<0.01, MTT-assay) and increased caspase-3/7 mediated apoptotic cell death (p<0.05) as compared to DDN-control. This was further verified by cell cycle analysis (propidium-iodide-staining) that demonstrated significant cell cycle arrest in the G2/M-phase (p<0.001) by DDNDBeQ treatment as compared to control-DDN. Moreover, we confirmed by clonogenic-assay that DDNDBeQ
treatment significantly (p<0.001) inhibits H1299 colony-formation as compared to control/DDN. Overall, encapsulation of potent VCP-inhibitor DBeQ into a dendrimer allows selective VCP-mediated proteostasis-inhibition for controlling NSCLC-tumor growth and progression to allow tumor-targeted sustained drug delivery.

Astrocytes Promote Brain Metastases of Breast Cancer via Induction of Autophagy

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Abstract

Data from an MDA231Br animal breast cancer model as well as staining of brain metastases developed from breast cancer revealed an attenuated expression of KISS1 tumor suppressor in the metastatic cells seeded in the brain. Co-culture of breast cancer cells with astrocytes decreases expression of KISS1. The array data of chemokines expressed presented in astrocytes during co-culture with breast cancer cells suggests involvement of CXCL12 in KISS1 regulation. Treatment by CXCL12 chemokine induces expression of cellular microRNA-345 in the breast cancer cells that inversely correlates with KISS1 expression. Decreasing KISS1 expression leads to activation of autophagy and autophagy-mediated cancer cell invasion. As presented in a diagram during this presentation, KISS1 inhibition suggests a new mechanism of cancer cell dissemination in the brain.

Diagnosis of Lung Tumor Types Based on Metabolomic Profiles in Lymph Node Aspirates

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Abstract

Lung cancer is the leading cause of cancer-related deaths in the US and worldwide. The majority of lung cancer patients are diagnosed with an advanced stage, precluding curable surgical resection, and therapy is limited to thoracic radiation and chemotherapy. The efficacies have improved in recent years due to patient pre-selection based on the cytology of fine needle aspirates of lymph nodes, which harbor circulating tumor cells. We report herein the evaluation of lymph node aspirates for the presence of metabolite features that are suitable to distinguish patients with lung cancer from patients without malignancies and to separate patients with adenocarcinoma from patients with squamous cell carcinoma. The results provide evidence that patient groups can be separated based on their metabolite profiles. Multivariate statistical analyses (PLS and OPLS) produce a set of consensus features that can be further developed into clinical biomarkers. Two of the features were identified as kynurenine and oxidized glutathione, and their identities were confirmed in a second larger sample set. In addition, the accompanying metabolites glutamate, glutamine and tryptophan were included in the validation experiment to utilize relative metabolite ratios as indicators for corresponding enzyme activities. The validation results demonstrate significant differences in metabolites and metabolite ratios between lymph node aspirates harboring a circulating tumor cell and aspirates negative for malignancy. Together the results demonstrate that metabolite profiles in lymph node aspirates are suitable to differentiate patients with adenocarcinoma from patients with squamous cell carcinoma.
Using Gene Editing to Investigate the Role of MicroRNA and RNA Splicing in Cancer

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Abstract

We have constructed TALEN to knockout microRNA-21, a microRNA linked to various cancers and fibrotic diseases. HeLa cells without miR-21 were phenotypically less transformed in vitro and in vivo, and they were also more sensitive to genotoxic drugs. We have also used TALEN to knockout ZRSR2, a myelodysplastic syndrome splicingosomal gene. Splicing of U12-type introns, but not U2-type introns, is partially inhibited in ZRSR2-null cells, suggesting an unrealized splicing function for the homologous ZRSR1.

We have designed and constructed a library of gRNAs to target most of the annotated human miRNA stem-loops. The oligo pool was cloned into a lentiviral vector, and amplicon-sequencing confirmed 99.9% of the gRNAs are represented in the library. By transducing HeLa and examining the change in the prevalence of each gRNA after 14 cell divisions, we identified a set of pro-growth miRNAs. By mining the GEO database, we found several of those microRNAs are over-expressed in cervical tumors as compared to matched controls. By analyzing the TCGA database, we found patients with high expression of certain microRNA have poorer survival. We have also constructed a lenti-sgRNA-Cas9-RFP library for screening miRNA function in primary cells or in PDX models.

Dendritic Cell Derived Exosomes for Cancer Therapy

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Abstract

Peptide loaded exosomes are promising cancer treatment vehicles, however, low T cell responses in human clinical trials indicate a need to further understand exosome-induced immunity. We have used mouse models to understand the mechanisms for immune stimulation by exosomes. We have demonstrated that antigen-loaded exosomes carry whole protein antigens and require B cells for induction of antigen-specific T cells. In the presentation, our recent findings on how to boost T cell responses and tumor rejection in the B16 mouse melanoma model, and also effects of allogeneic exosomes will be discussed and the data demonstrate ways to increase the feasibility of exosome-based therapeutic approaches in cancer.

KRAS: Signaling and Cancer

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Abstract

Ras proteins are small GTPases that act as signal transducers between cell surface receptors and several intracellular signaling cascades. KRAS is among the most frequently mutated oncogenes in human tumors. Ras proteins consist of highly homologous catalytic domains, and flexible C-terminal hypervariable regions (HVRs) that differ significantly across Ras isoforms. We have been focusing on key mechanistic questions in oncogenic Ras biology from the structural and signaling standpoints. These include whether Ras’ disordered hypervariable region (HVR) has a role beyond membrane anchoring; Does Ras form dimers, and if so what is their structural landscape and how they help in activating Raf; What are Ras’ redundant pathways
The Oncogenic Properties of the Redox Inflammatory Protein Inducible Nitric Oxide Synthase in ER(-) Breast Cancer. Development of Models for Poor Outcome

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Abstract

Inflammation generates reactive chemical species that induce conditions of oxidative nitrosative stress as emerged as factor in poor outcome of many cancers. Our recent finding show that in the inflammatory protein inducible nitric oxide synthase (iNOS) is a strong predictor of poor outcome in ER(-) patients. Furthermore 46 genes, of which 23 were associated with basal like breast cancer, were elevated when iNOS high. In vitro studies using ER(-) cell lines showed that fluxes of nitric oxide (NO) delivered by NO donors surprising mimic this relationship in the patient cohort. Using this model, we show that NO at different specific concentration stimulate pro-oncogenic mechanisms such as AKT, ERK, NFkB, AP-1, and HIF-1 that lead to increase of metastatic and cancer stem cells proteins. In addition, we show that tumor suppressor gene BRCA1 and PP2A are inhibited by these NO levels. Similarly, other studies show that these concentrations of NO increase immunosuppressive proteins TGFβ and IL-10 in leukocytes to decrease efficacy of some anticancer therapies further contributing to pro-tumorigenic environment. Using this model, we have identified several new compounds that have efficacy in xenographic models. These finding have provided a model that shows how NO can affect numerous mechanism that leads to a more aggressive phenotype. These models have provided us a new opportunity to examine the effects of different strategies that target the more aggressive phenotype while being less toxic to the host.

Tumor-Targeting with Antibody-Based Agents

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Abstract

Antibody–toxin fusion proteins, here called immunotoxins, employ an antibody fragment to deliver a potent bacterial toxin to cancer cells. Immunotoxin binding is directed to surface antigens expressed preferentially on malignant cells. For immunotoxins that are made with truncated variants of Pseudomonas exotoxin (PE), the antibody delivers the toxin to the cell surface and from there, via endocytosis and trafficking to the endoplasmic reticulum, to the cytosol where it ADP-ribosylates elongation factor 2, leading to inhibition of protein synthesis and ultimately, cell death. Basic science, pre-clinical research and clinical testing of PE-based immunotoxins have highlighted enormous potential for this platform. We target CD22 on B-cell malignancies and mesothelin or EGFR on various epithelial cancers. Clinically, a pivotal Phase III trial in patients with hairy cell leukemia is on-going. Other trials, targeting epithelial cancers expressing mesothelin, are also being conducted (see clinicaltrials.gov). Two aspects of immunotoxin biology present challenges for development: immunogenicity of the toxin (in patients with intact immune systems) and cytostatic rather than cytotoxic responses by some cancers. To reduce immunogenicity, key epitopes can be altered via mutagenesis or the co-administration of immunosuppressants. When immunotoxins are cytostatic, combination treatments can enhance cytotoxicity and lead to cell death. Results of drug screens to identify suitable immunotoxin-enhancing combinations will be discussed.
Optimizing Therapeutic Strategies for Lung Cancer Treatment by Targeting Mitochondrial and Heme Function

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Abstract

Mitochondrial respiration and oxidative phosphorylation are crucial for providing cellular energy for the development and progression of many cancers. Heme is a central factor in oxygen utilization and oxidative phosphorylation. Heme serves as a prosthetic group in many enzymes and proteins involved in oxygen utilization, including complexes II, III, and IV of the mitochondrial respiratory chain. Our recent study showed that heme plays essential roles in lung cancer cell function. We found that the rates of heme synthesis and uptake are increased significantly in a variety of lung cancer cells, compared to the normal lung cells. Additionally, we showed that the levels of the rate-limiting heme synthetic enzyme ALAS and heme uptake proteins are highly elevated in many lung cancer cells and tumors. Evidently, both heme biosynthesis and uptake are intensified to enhance heme availability for the production of oxygen-utilizing hemoproteins and enhanced oxygen consumption in cancer cells. Conversely, depleting heme in cancer cells causes a lack of hemoproteins, leading to reduced oxygen consumption and cellular energy generation. Further, lowering heme levels strongly suppresses NSCLC cell proliferation, colony formation, and migration. Together, our results showed that heme availability is significantly increased in cancer cells and tumors, which leads to elevated production of hemoproteins, resulting in intensified oxygen consumption and cellular energy production for fueling cancer cell progression. We will present data from mouse models to show that inhibiting heme function can be an effective strategy to delay tumor growth.

Dietary Nutrients Induced Epigenetic Changes in Triple Negative Breast Cancer

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Abstract

The prevalence of TNBC is significantly higher in premenopausal African-American and African women compared to Caucasians. It is established that BRCA1 deficiency is one of the major genetic alteration and it is occurring in ~50% of TNBC patients. Epigenetic silencing of BRCA1 by promoter methylation occurs commonly in TNBC and this is associated with poor outcome with standard chemotherapy. The present study focused to elucidate the activity of different epigenetic modifiers (different natural products) on modulating the expression of BRCA1 in TNBC cells. Studies detected that wild type BRCA1 is less expressed in TNBC cell lines (MDA-MB-231 and MDA-MB-468) compared to ER positive cell line. Quercetin/curcumin increased mRNA and protein expression levels of BRCA1, and the combination of quercetin and curcumin exhibited a significant increase in BRCA1 expression. Promoter methylation studies revealed curcumin/quercetin decreased wild type BRCA1 promoter methylation. The decrease in DNA methyl transferase 1 (DNMT1) expression was lower with curcumin/quercetin compared to DNMT1 inhibitor 5-Aza-deoxycytidine. Further, there was no significant alteration on global methylation with curcumin/quercetin treatment. Quercetin/curcumin treatment significantly enhanced the histone H3 lysine 9 acetylation of BRCA1 promoter in TNBC cell lines. These studies indicate that quercetin/curcumin effect is specifically exhibited on epigenetically modified tumor suppressor gene. The pretreatment of quercetin/curcumin before chemo/radiation treatment may be effective to reduce the TNBC tumor burden. Further TNBC animal studies are warranted in order to conclude the curcumin/quercetin induced epigenetic alteration of BRCA1.

Single Cell Heterogeneity in Response to Therapy

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Abstract

Glioma cells treated with Temozolomide (TMZ) activate several mechanisms, including cell cycle arrest, apoptosis,
autophagy and senescence. Some of these mechanisms are activated in subpopulation of cells whereas others are activated in the same cell, such as autophagy and senescence, but the causal relationship between these mechanisms is not clear. We measured the levels of autophagy and senescence in single glioma cells after treatment with TMZ and observed that autophagy and senescence were triggered in a very heterogeneous manner. Cells with low levels of autophagy 3 days after TMZ treatment were more likely to die from apoptosis while cells with high levels of autophagy were more likely to progress to senescence. The inhibition of autophagy with 3MA triggered apoptosis and decreased senescence, while its activation with rapamycin increased temozolomide–induced senescence. Combination of rapamycin prior to TMZ with 3MA 3 days after TMZ produced the largest reduction in cell number in vitro and reduced tumor growth in an in vivo model.

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Signaling of Oncogenic K-Ras Through Calmodulin

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Abstract

K-Ras, a membrane-associated GTPase, is frequently mutated in cancer. Oncogenic mutations impair GTP hydrolysis and reduce K-Ras interaction with GTPase activating proteins, leaving K-Ras GTP-bound. K-Ras-GTP signals to promote cell proliferation, survival, and motility. The most common in cancer G12D and G12V mutants of K-Ras activate distinct signaling pathways and differ in tumorigenic potential and resistance to chemotherapy. Structural features distinguishing G12V from G12D K-Ras are expected but X-ray crystallography did not reveal significant differences. K-Ras mutants may exhibit structural variations in solution or upon association with binding partners, while only one conformer exists in the crystal. Our results suggest that G12V K-Ras-GDP exhibits distinct conformational dynamics in solution. This conformational flexibility enhances G12V K-Ras-GDP association with the calcium modulator protein calmodulin. Ca2+-calmodulin removes K-Ras from the plasma membrane, reduces activation of Raf, and promotes cell migration. The K-Ras-calmodulin complex activates Wnt by suppressing its non-canonical Ca2+-dependent branch, promotes the stem cell-like phenotype of K-Ras-driven cancer cells and enhances their survival. Understanding the mechanisms of K-Ras activation by oncogenic mutations and interactions with Ca2+-calmodulin may lead to development of novel anti-cancer therapeutics.

Regulation of ABC Transporters by COX-2: Potential Use of Celecoxib in MDR Cancers

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Abstract

Multidrug resistance (MDR) is one of the major issues to be addressed in treating many diseases including cancers. One of the well-established causes for the multidrug resistance involves over expression of members of ATP binding cassette (ABC) proteins. In the light of time and cost incurred in the novel drug development, identifying drugs as adjuvants might be a useful alternate treatment strategy for cancer. Others and we have shown that Cyclooxygenase-2 (COX-2) over-expression induces the expression of MDR-1 (multidrug resistance protein 1), which causes multidrug resistance, suggesting that COX-2 inhibition might reduce the chemo resistance phenotype. We have also demonstrated that Celecoxib, a COX-2-specific inhibitor, reverses drug resistance in Chronic myeloid leukemia (CML) and hepatocellular carcinoma cells. Recently we showed that COX-2 not only regulates MDR1 but also many other ABC transporters leading to chemoresistance. Furthermore, the RNA expression levels of COX-2 and few other ABC transporter genes are elevated in the CML patients undergoing imatinib therapy. Also there was an increase in the percent of patients with higher COX-2 and ABC transporter gene RNA levels with increase in imatinib dosage indicating the role of COX-2 in chemoresistance and potential use of celecoxib in overcoming chemoresistance in cancer.
Oxidative Stress–Induced Sulfiredoxin Promotes Colorectal Cancer Development

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Abstract

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer death in both men and women. Sulfiredoxin (SrX) is a novel oxidoreductase that restores the peroxidase activity of peroxiredoxin (Prx) to maintain cellular redox balance, and is also a regulator of cellular redox-related signaling. The function and mechanisms of SrX in cancer development are not well understood. We found that SrX is preferentially expressed in human colorectal cancer cells but not in normal colon epithelial cells. Loss-of-function studies demonstrate that knockdown of SrX in poorly differentiated colorectal cancer cells not only leads to the inhibition of colony formation and cell invasion in vitro, but also reduces tumor xenograft growth and represses metastasis to distal organs in a mouse orthotopic implantation model. Notably, exactly opposite effects were observed in gain-of-function experiments when SrX was ectopically expressed in well-differentiated colorectal cancer cells. Mechanistically, expression of SrX enhances the activation of mitogen activated protein kinase (MAPK) signaling through increasing the activation of the epithelial growth factor receptor (EGFR). Our studies indicate that SrX is a critical oncogenic protein that can be used as a molecular target to develop therapeutics for patients with metastatic colorectal cancer.

Hypoxia-Inducible Factors in Cancer Stem Cells and Inflammation: Implications in Leukemia Therapy

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Abstract

Hypoxia-inducible factors (HIF) mediate metabolic switch in cells in hypoxic environments, including those in both normal and malignant tissues with limited supplies of oxygen. Paradoxically, recent studies have shown that cancer stem cells and activated immune effector cells exhibit high HIF activity in normoxic environments and that HIF activity is critical in maintenance of cancer stem cells as well as differentiation and function of inflammatory cells. Since inflammation and cancer stem cells are two major barriers to effective cancer therapy, targeting HIF may provide a new approach for the ultimate challenges. Here we will review the function of HIF pathway as it relates to leukemia and present our data showing therapeutic effect of HIF inhibitor in meeting challenges associated with leukemia.

Augmented Telomerase Activity, Reduced Telomere Length and the Presence of Alternative Lengthening of Telomere in Renal Cell Carcinoma: Plausible Predictive and Diagnostic Markers

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Abstract

In this study, we analyzed 100 cases of renal cell carcinoma (RCC) for telomerase activity, telomere length and alternative lengthening of telomeres (ALT) using the TRAP assay, TeloTTAGGG assay kit and immunohistochemical analysis of ALT associated promyelocytic leukemia (PML) bodies respectively. A significantly higher (P = 0.000) telomerase activity was observed in 81 cases of RCC which was correlated with clinicopathological features of tumor for instance, stage (P = 0.008) and grades (P = 0.000) but not with the subtypes of RCC (P = 0.355). Strikingly, the telomere length was found to be significantly shorter in RCC (P = 0.000) to that of corresponding normal renal tissues and it is well correlated with grades (P = 0.016) but not with stages (P = 0.202) and subtypes (P = 0.669) of RCC. In this study, telomere length was also negatively correlated with the age of patients (r2 = 0.528; P = 0.000). ALT associated PML bodies containing PML protein was found in telomerase negative
cases of RCC. It suggests the presence of an ALT pathway mechanism to maintain the telomere length in telomerase negative RCC tissues which was associated with high stages of RCC, suggesting a prevalent mechanism for telomere maintenance in high stages. In conclusion, the telomerase activity and telomere length can be used as a diagnostic as well as a predictive marker in RCC. The prevalence of ALT mechanism in high stages of RCC is warranted for the development of anti-ALT inhibitors along with telomerase inhibitor against RCC as a therapeutic approach.

**Biological Observations on the Chemotherapy Curable Malignancies; Unique Genetic Events, Frozen Development, Natural Apoptosis and Absent Cancer Stem Cells**

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**Abstract**

The list of metastatic malignancies curable with chemotherapy; trophoblast tumours, germ cell tumours, acute leukemia, high grade lymphoma and the rare childhood malignancies, is unchanged from the 1970s. This lack of progress in curing other metastatic cancers with chemotherapy presents a number of major clinical and scientific challenges. The paradigm of cancer cells being sensitive to chemotherapy as a result of rapid growth and then developing chemotherapy resistance is well established, however we would like to present an alternate hypothesis. The new hypothesis relates to the biological properties of the cancer cells, specifically the observation that each of the chemotherapy curable malignancies arise from cells that undergo natural DNA manipulations that are intrinsically associated with high levels of natural apoptosis. Trophoblast tumours arise from the cells of conception, which have just undergone nuclear fusion. Germ cell tumours arise from pre-malignant precursor cells that are subject to pressures to undergo meiosis and mitosis. In the B cell malignancies, acute leukaemia that arises from pro-B cells during VDJ rearrangement of the immunoglobulin genes and diffuse large B cell lymphoma is closely linked to somatic hyper mutation. The B cell malignancies that arise at other points in the B cell development pathway that do not involve DNA manipulations are not chemotherapy curable. The other key biological difference the chemotherapy curable malignancies have is that their unusual developmental pathway means that they are not linked to any conventional hierarchical cancer stem cells. Research into these natural apoptotic sensitivity pathways may lead to novel therapeutic avenues.

**New Targeted Treatments of Non-melanoma Skin Cancer**

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**Abstract**

Non melanoma skin cancer (NMSC) comprises basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) and is regarded to be the most common cancer in man. Various traditional therapies options have been used for many decades including surgical, topical, and radiation treatments. During the last years' various new treatment options to target NMSC have been developed: In advanced local and metastatic BCC the oral Smoothened inhibitors Vismodegib, Sonidegib, and Taladegib have been stablished as effective treatments. In SCC the oral tyrosinase inhibitors Erlotinib and Gefitinib which address epidermal growth factor receptor (EGRF) show supporting data in recent trials. Furthermore, treatments for dual combination oral therapy targeting both EGRF and insulin-like growth factor 1 receptor (IGF-1) are under investigation. Actinic keratoses are regarded as in-situ SCC. Various topical treatment options have been developed to more precisely target cancer cells: Imiquimod binds to the Toll-like receptor 7 and promotes anti-tumor inflammatory reactions. Photodynamic treatment (PDT) uses the accumulation of protoporphyrine IX in tumors cells and the subsequent induction of singlet oxygen by light. A transformation process in NMSC treatment has been initiated. Latest therapies directly target tumor cells and precursors avoiding unnecessary collateral damage.
Inducing Differentiation of Premalignant Cells as a Novel Therapeutic Strategy in Hepatocarcinoma

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Abstract

Hepatocellular carcinoma (HCC) represents the second leading cause of cancer-related deaths and is reported to be resistant to chemotherapy caused by tumor-initiating cells. These tumor-initiating cells express stem cell markers. An accumulation of tumor-initiating cells be found in 28-50% of all HCC and is correlated with a poor prognosis. Mechanisms that mediate chemoresistance include drug export, increased metabolism and quiescence. Importantly, the mechanisms that regulate quiescence in tumor-initiating cells have not been analyzed in detail so far. In the present research we have developed a single cell tracking method to follow up the fate of tumor-initiating cells during chemotherapy. Thereby, we were able to demonstrate that mCXCL1 exerts cellular state specific effects regulating the resistance to chemotherapeutics. mCXCL1 is the mouse homolog of the human Interleukin 8, a chemokine which correlates with poor prognosis in HCC patients. We found that mCXCL1 blocks differentiation of premalignant cells and activates quiescence in tumor-initiating cells. This process depends on the activation of the mTORC1 kinase. Blocking of the mTORC1 kinase induces differentiation of tumor-initiating cells and allows their subsequent depletion using the chemotherapeutic drug doxorubicin. Our work deciphers the mCXCL1-mTORC1 pathway as crucial in liver cancer stem cell maintenance and highlights it as a novel target in combination with conventional chemotherapy.

Conditioned Medium from Adipose-derived Stem Cells (ADSCs) Promotes Epithelial-to-Mesenchymal-like Transition (EMT-Like) in Glioma Cells In Vitro

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Abstract

Mesenchymal stem cells (MSCs) have recently been described to home to brain tumors and to integrate into the tumor-associated stroma. Understanding the communication between cancer cells and MSCs has become fundamental to determine whether MSC-tumor interactions should be exploited as a vehicle for therapeutic agents or considered a target for intervention. Therefore, we investigated whether conditioned medium from adipose-derived stem cells (ADSCs-CM) modulate glioma...
tumor cells by analyzing several cell biology processes *in vitro*. C6 rat glioma cells were treated with ADSCs-CM, and cell proliferation, cell cycle, cell viability, cell morphology, adhesion, migration, and expression of epithelial-mesenchymal transition (EMT)-related surface markers were analyzed. ADSCs-CM did not alter cell viability, cell cycle, and growth rate of C6 glioma cells but increased their migratory capacity. Moreover, C6 cells treated with ADSC-CM showed reduced adhesion and underwent changes in cell morphology. Up-regulation of EMT-associated markers (vimentin, MMP2, and NRAS) was also observed following treatment with ADSC-CM. Our findings demonstrate that the paracrine factors released by ADSCs are able to modulate glioma cell biology. Therefore, ADSC-tumor cell interactions in a tumor microenvironment must be considered in the design of clinical application of stem cell therapy.

**Dissecting the Roles of *Etv5* and Ascl1 in Oligodendrogliomagenesis**

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**Abstract**

Oligodendrogliomas (ODG) are a devastating class of brain tumor that have poor prognosis and are ultimately fatal. ODG is associated with CIC loss and IDH gain-of-function mutations. We propose to characterize genes downstream of CIC that are associated with ODG transformation, focusing on *Etv5*, a transcriptional regulator that is upregulated in ODG. We are particularly interested in the role of *Etv5* in regulating proliferation and specifying an oligodendrocyte precursor cell (OPC) fate, since NPCs are considered to be the developmental cell of origin in ODG. Our earlier work indicated that *Etv5* is a key regulator of a glioblastoma, acting in the context of elevated RAS/ERK signaling to induce OPC differentiation. Our working hypothesis is that CIC normally represses the ability of *Etv5* to promote proliferation and an OPC identity. We are testing this model in normal cortical development and in ODG cell lines that are unique to the Chan lab. First, using a conditional *Etv5* knock-out, we found that the loss of *Etv5* results in the production of fewer cortical neurospheres from E12.5 progenitors. Second, knock-down of *Etv5* in BT88 cells derived from an ODG tumor also reduced neurosphere number. Taken together, these studies suggest that *Etv5* is an essential regulator of proliferation in both normal cortical development and in ODG tumorspheres. Future studies will assess the role of *Etv5* in OPC fate specification. By elucidating the importance of *Etv5* in normal OPC development and in ODG formation, we may identify druggable targets for ODG treatment, aiding future preclinical studies, while also building our foundational knowledge of ODG.

**Reirradiation in Recurrent Head and Neck Carcinomas: Why, When and How**

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**Abstract**

Locoregional recurrence remains the major cause of failure in head and neck malignancies after radical treatment. Every attempt should be made in case of an unresectable recurrent disease or a second primary disease in a medically fit patient to achieve some form of locoregional control. Local control (LC) is an important prognostic factor during primary irradiation and most of the failures occur at local site only. A conformal technique of reirradiation is preferable in the form of brachytherapy, radiosurgery, neutron therapy or IMRT, to allow dose escalation and reduce normal tissue toxicity. Differences in the location and extent of recurrent tumor, initial radiation treatment parameters, elapsed time since prior treatment, extent of normal tissue sequelae, and relatively sparse data on acute and late normal tissue recovery from prior treatment and tolerance to reirradiation pose a significant challenge in the formulation of broadly applicable guidelines for reirradiation pertaining to target volumes and dose of radiation. Limited reirradiation volumes that omit elective reirradiation of nodal areas have been proven to be sufficient. A greater likelihood of local control has been seen with administration of at least 50 to 60 Gy of reirradiation. Reirradiation in recurrent HNC holds promise for long-term survival for appropriately selected patients such as those who undergo surgical salvage but are found to have high-risk features or those who are medically suitable for curative-intent interventions but are not amenable to curative-intent resection.
Symbiotic Bacteria Provide Chemoprevention Against Hepatitis B Virus Mediated Hepatocellular Carcinoma in Hepatitis B x Transgenic Mice

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Abstract

Chronic infection with hepatitis B virus (HBV) is associated with the development of progression of chronic liver disease (CLD) and the appearance of hepatocellular carcinoma (HCC). HCC is a prevalent cancer worldwide with few treatment options. Given that HCC develops most often on the background of chronic inflammation, experiments were designed to test the hypothesis that selected probiotic bacteria that suppress inflammation could be used as a simple and inexpensive means to prevent or delay the appearance of HCC. To test this, hepatitis B x (HBx) transgenic mice, which develop progressive liver lesions that culminate in HCC, were treated with a mixture of probiotic bacteria (Synbiotic 2000). The results showed a significant reduction in the number and size of dysplastic and HCC nodules compared to control transgenic mice. Microarray analysis of selected immune and cancer associated markers showed a strong reduced expression in the liver of mice treated with Synbiotic 2000 compared to controls. The results also highlighted the strong connection between HBx expression, CLD, and the development of HCC. Thus, Synbiotic 2000 attenuates the pathogenesis of HCC, and may be useful in cancer chemoprevention, not only for HCC, but perhaps against other cancers that often develop on the background of chronic inflammation.

Next Generation Sequencing for Routine Clinical Tumor Genotyping: A Vital Link for Bench-to-Bedside Transition of Cancer Genomics

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Abstract

Routine screening of tumors in a clinical molecular diagnostic laboratory is vital for screening of clinical markers of prognostic and predictive significance. Recently, the next-generation sequencing (NGS) technologies have come to fore as the preferred technologies for genotyping of tumors. Their capability of massively parallel sequencing enables screening multiple markers in multiple samples simultaneously from a single and often low input of nucleic acid. However, implementing these complex genotyping platforms is challenging due to the need for adequate optimization and validation of the technology, streamlining the interpretation, reporting and storage of results. Additional financial and ethical considerations also add to the challenges. The talk will highlight the advantages and challenges in implementing high-complexity NGS testing for routine screening of tumors in a high-volume clinical molecular diagnostic laboratory.

Mass Spectrometry-based Proteomics: A Powerful Tool in Prostate Cancer Research

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Abstract

Proteins are the effectors of most cellular reactions and constitute the targets of many drugs. Thus, a broad characterization of
the changes in protein expression occurring during prostate cancer development would allow the identification of novel targets for therapeutic intervention as well as prognostic biomarkers of disease aggressiveness and predictive biomarkers of treatment response. We have used quantitative mass spectrometry-based proteomics to characterize the prostate cancer proteome. We used formalin-fixed paraffin-embedded (FFPE) specimens, the most common archival form, which allows retrospective studies of clinical material with valuable medical information associated. Over 6,000 proteins on average were identified and quantified per sample for a combined amount of more than 9,000 proteins. Comparison between cancer and adjacent benign prostate epithelial tissue revealed increased expression of proteins involved in fatty acid and protein synthesis and ribosomal biogenesis among others, in malignant cells. The expression of proteins located at the mitochondria was also upregulated and correlated with elevated oxidative phosphorylation capacity of the cancer cells. Additionally, we also identify a novel prognostic biomarker of disease aggressiveness that can potentially help to early stratification of patients. Overall, system-wide characterization of protein expression from FFPE samples may contribute, together with other “omic” technologies, to the identification of processes and pathways altered during cancer progression helping to develop of alternative, more efficient therapies.

Repositioning for the Treatment of Thyroid Cancer

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Abstract

Repositioning of established non-cancer pharmacotherapeutic agents with well-known activity and side-effect profiles is a promising avenue for the development of new treatment modalities for multiple cancer types. Our group has focused on analyzing some of the medications with mechanism of action that may have relevance to thyroid cancer (TC). Experimental in vitro and in vivo evidences, as well as results of clinical studies, have indicated that molecular targets for medications currently available for the treatment of mood disorders, sexually transmitted diseases, metabolic disorders, and diabetes may be active and relevant in TC. For instance, the anti-diabetic agent, metformin, is able to inhibit ERK, which is commonly activated in TC cells. Also, the HIV protease inhibitor Nelfinavir targets the heat shock protein (HSP) 90 chaperone, which is required for folding and stability of RET mutants in Medullary Thyroid Cancer. We will present the in vitro results generated in our lab on all those agents. Repositioning of established medications for the treatment of TC could broaden the scope of current therapeutic strategies. These diverse treatment choices could allow physicians to provide an individualized approach to optimize treatment for patients with TC.

Proteasome Inhibition in Cancer Treatment: Therapeutic Potentials and Side Effects

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Abstract

Cancer treatment is a complex process that should target the critical pathways to achieve cellular damage. During the therapeutic process many different macromolecules are damages and proteins are among them. Protein damage may occur via the oxidation of proteins, which cause inactivation of proteins. Limited pathways reduce oxidized proteins and mainly they are removed from the cell by proteasomal system. The removal of oxidized proteins is generally a required process for healthy organisms to keep the integrity while in cancer cells the situation may be different. Cancer cells have higher proteasome activity compared to normal cells. This increased activity of proteasome may decrease efficacy of the cancer therapy and therefore targeting proteasome is an effective way to decrease the therapy resistance. Proteasomal system has been focused since 1999 as a target of chemotherapy since it has crucial roles in the turnover of oxidatively damaged proteins and also many signaling
proteins. Bortezomib is the first generation proteasome inhibitor, which is being used in the treatment of many cancer types. Thousands of studies have been focused on bortezomib and its potential role on different pathways and clinical trials resulted in hopeful outcomes in patients. Besides bortezomib, other proteasome inhibitors have been developed as second-generation drugs. In our laboratory, cell lines such as breast, prostate, hippocampal tumor and colon cancer have been tested from different aspects of proteasomal activity. Recently proteasome inhibitors bortezomib and carfilzomib have been tested on neural stem cells to investigate the mechanisms of peripheral neuropathy induced by these inhibitors.

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Glycolytic Inhibition Modulates LD Biogenesis in Ovarian Cancer to Inhibit Cell Growth and Promotes Chemosensitivity

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Abstract

PFKFB3, a critical enzyme in aerobic glycolysis has recently emerged as a contributing factor to the growth and survival of several solid tumors including lung, breast, and colon. We had identified the overexpression of activated form of PFKFB3 in primary ovarian tumor and in several chemoresistant cell lines compared to their sensitive counterparts. PFK-158 (ACT), a novel PFKFB3 inhibitor is shown to be effective against solid tumors in phase-1 clinical trials. Previously, we found that PFK-158 suppressed cell proliferation, reduced glucose uptake, lactate release and induced cell death by autophagy and apoptosis in several ovarian cancer cells. It also synergized with cis/carboplatin and paclitaxel in the resistant cells and inhibited tumor growth in vivo when treated alone or in combination with these drugs. Our recent data shows accumulation of lipid droplets (LDs) in chemoresistant cells which are reduced upon PFK-158 treatment. LDs are cellular organelles associated with lipid metabolism and cancer progression. Autophagic inhibition rescued PFK-158 mediated synergy and depletion of LDs suggesting a critical role of autophagy in PFK-158 mediated anti-cancer effects. We show downregulation of PLA2G3 (a secretory phospholipase A2; LD associated protein and connected with inflammatory pathway) along with LD depletion upon PFK158 treatment. Further, we demonstrate that PLA2G3 knockdown in resistant cells inhibits colony formation, reduction in cell growth, depletion in LDs, and an increase in carboplatin sensitivity. To summarize, our findings establish a crucial role of PFKFB3 along with an association between glycolytic and lipid metabolism in ovarian tumor promotion and drug resistance.

Prevention of Mammary Carcinogenesis by Trianthema portulacastrum: Preclinical Evidence and Molecular Mechanisms

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Abstract

Breast cancer is the most frequently diagnosed cancer among women worldwide and there is an urgent need for effective and safe drugs. The utility of various natural and dietary products to reduce the risk of breast cancer is well documented. Trianthema portulacastrum Linn. (Aizoaceae), an Indian medicinal and dietary plant, has been found to possess various health benefits. The present study was initiated to investigate mechanism-based chemopreventive potential of a well characterized ethanolic extract of T. portulacastrum (TPE) against 7,12-dimethylbenz(a)anthracene (DMBA)-initiated rat mammary tumorigenesis, an experimental model that closely resembles human breast cancer. The animals had free access to a basal diet mixed with TPE to yield three dietary doses (i.e., 50, 100 and 200 mg/kg body weight) throughout the experimental period (18 weeks). Following 2 weeks of TPE treatment, mammary tumorigenesis was induced by oral administration of DMBA (50 mg/kg body weight). At the end of the study (16 weeks after DMBA treatment), TPE registered a drastic reduction of DMBA-induced mammary tumor incidence, total tumor burden and average tumor weight as well as reversed histopathological changes. TPE dose-dependently reduced the expression of proliferating cell nuclear antigen and cyclin D1, triggered apoptosis, upregulated Bax, downregulated Bcl-2, and suppressed Wnt/β-catenin signaling. TPE also downregulated cyclooxygenase-2 and heat shock protein 90, blocked the degradation of inhibitory κBα, interfered with the translocation of nuclear factor-κB (NF-κB) from...
cytosol to nucleus and upregulated the expression and nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2). These results suggest that TPE prevents DMBA-inflicted breast carcinoma, at least in part, by anti-inflammatory mechanisms mediated through simultaneous and differential modulation of two interconnected molecular circuits, namely NF-κB and Nrf2 signaling pathways.

**Paradigm for Natural Product-based Medicinal Discovery and Recent Innovations**

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**Abstract**

Introduction of a novel paradigm of medicinal discovery based on principles of ethnobotany, evochemistry and evidence-based methodologies is provided. This paradigm is contrasted with principles and methods of conventional drug discovery and applications of the paradigm leading to development of health supportive and medical therapies from botanical sources are described. Recent innovations including the development of a supercritical CO₂ extract from Curcuma amada (CA) rhizomes as a direct and adjuvant anticancer agent is introduced. Specially, anticancer activity is evaluated with *in vitro* and *in vivo* tumor models; alveolar rhabdomyosarcoma (SJRH30) and glioblastoma (U-87MG). Synergistic activity between CA and chemotherapeutic drugs is assessed using the Compusyn analytic software. Another novel research track that is introduced involves the application of CA with compounds such as 2-deoxyglucose that target energy metabolism in cancer models.

**Anti-cancer Activity of Fisetin, a Flavonoid from Mother Nature**

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**Abstract**

Treatment of cancer has considerably improved over the past several years. Much of this success is attributed to an improved use of chemotherapeutic drugs which affect rapidly dividing cells in general and in the process also result in toxicity to healthy tissue. Therefore, identification and development of novel non-toxic agents for targeted prevention and treatment of cancer is needed. Using Mother Nature as a source, we have identified many polyphenols that interact with multiple targets. Recently we have been investigating the role of fisetin, a dietary tetrahydroxyflavone, in inhibition of multiple oncogenic pathways in a variety of cancer types both *in vitro* and *in vivo*. Fisetin is found in strawberry, apple, persimmon, kiwi, onion and cucumber where it serves as a coloring agent. Fisetin induces apoptosis and inhibits invasion in pancreatic cancer AsPC-1 cells through suppression of DR3-mediated NF-κB activation. In COX-2-overexpressing human colon cancer HT29 cells fisetin treatment induced apoptosis associated with down-regulation of COX-2 protein expression. Fisetin physically interacts with the mTOR molecule and docks at two sites with a binding energy of -8 Kcal/mol. We also found that fisetin treatment leads to induction of autophagic-programmed cell death. Fisetin enhances tubulin polymerization with activity superior to paclitaxel and sensitizes prostate cancer cells to the chemotherapeutic effects of cabazitaxel. These observations suggest that fisetin could be a useful chemotherapeutic agent that could be used either alone or as an adjuvant with conventional chemotherapeutic drugs for the management of cancer and underscore the need to test its activity in the clinic.

**Tea Polyphenols as Modulators of Nrf2: Implication in Chemoprevention and Chemotherapeutics**

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**Abstract**

Redox-sensitive transcription factor, Nrf2 is a double edged sword. In normal cells it positively regulates antioxidants, xenobiotic detoxification enzymes, drug efflux pumps and confers cytoprotection against toxicants where as in cancer cells overexpression of Nrf2 and its downstream targets offer survival advantage and chemoresistance. Tea polyphenols are well known modulators of Nrf2. So we tried to investigate whether green tea polyphenol epigallocatechin gallate (EGCG) and
black tea extract (BT) had any cytoprotective effect against carcinogen, arsenite (As III) in normal human skin keratinocytes (HaCaT). Secondly, we were interested to find whether EGCG and BT could be used as effective inhibitors Nrf2 in lung cancer cells (A549). EGCG and BT could efficiently improve the cell viability of As-III treated HaCaT cells. As III induced cell proliferation with low doses and apoptosis with high doses, both of which were efficiently modulated by EGCG and BT. Tea polyphenols facilitated reduction of nuclear expression and increase of cytoplasmic expression of Nrf2 in HaCaT cells. In lung cancer cells A549, Nrf2 and its downstream targets were found to be differentially modulated in a dose and time dependent manner which in turn induced cytotoxicity. Therefore, it may be indicated that EGCG and BT were effective in maintaining an optimum expression of Nrf2 in As III treated HaCaT cells which offered cytoprotection against As III. Where as in lung cancer cells tea polyphenols inhibited Nrf2 and rendered the cells more susceptible to cytotoxicity.

**Novel VEGFR2 Inhibitors for Treating Solid Tumors and Brain Metastasis**

**Appu Rathinavelu**

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**Abstract**

The Vascular Endothelial Growth Factor (VEGF) plays a central role and is frequently expressed in high levels in cancers. Therefore, efforts to develop anti-angiogenic therapies have largely focused on inhibiting VEGF or its receptors. We have discovered two new anti-angiogenic agents codenamed F16 and JFD, and both of them have received the US patent approval in 2011. Through systematic in vitro testing, both JFD and F16 were found to specifically block VEGFR2 phosphorylation and disrupt the network formation of the endothelial cells in the Matrigel® assay. As anticipated, in the in vivo experiments also these compounds were able to significantly inhibit the growth of the tumors without any significant change in the body weight or toxicity during the study period. However, the combination of JFD or F16 with Taxol produced the maximum anti-tumor effect compared to monotherapy. In addition, the plasma levels of MUC1, which is a biomarker for the assessment of breast cancer growth, was found to be elevated prior to the treatment in the experimental animals and the level was significantly decreased along with the reduction in the tumor burden following the treatment. When F16 was administered by intraperitoneal injections into Balb-C mice, to measure the extent of absorption and distribution of F16 in Plasma, Urine, Feces, as well as in tissue samples prepared from Brain, Liver, and Kidney, the basic Pharmacokinetics (PK) profile was established. Interestingly, a new formulation that was used for preparing and injecting F16 a dose of 200 mg/kg body weight showed a high level accumulation of F16 in the brain after 12 hrs of drug injection. So far, our results indicate that F16 can become an excellent anti-angiogenic inhibitor for the treatment of breast prostate, lung, colorectal and brain cancers both in monotherapy as well as in combination. (Research was supported by the Royal Dames of Cancer Research Inc., Ft. Lauderdale, Florida and by the grant from the Community Foundation of Broward, Ft. Lauderdale, Florida).

**Bitter Melon’s Sweet Promise: Role in Head and Neck Cancer**

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**Abstract**

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer and leading cause of cancer related mortality worldwide, despite the advancement in treatment procedures. Therefore, new strategies to achieve a favorable response for the improvement in the prognosis of HNSCC are urgently needed. One approach is through dietary means. Here, we examined the role of bitter melon extract (BME) in HNSCC tumor microenvironment. Mouse head and neck cancer cells were subcutaneously injected into the flanks of syngeneic mice. We observed that oral gavage of BME significantly inhibits the tumor growth in mice as compared to control group. Further study suggested that BME inhibits cell proliferation as evident from low expression of proliferating cell nuclear antigen (PCNA) and c-Myc in the tumors of BME fed mice as compared to that of control group. We next investigated the role of BME as an immunomodulator in HNSCC model. Forkhead box protein P3+ (FoxP3+) T cells suppress tumor immunity. Our data suggested that BME treatment decreases the infiltrating regulatory T (Treg) cells by inhibiting FoxP3+ populations in the tumors and in spleens. Additionally, BME treatment reduces Th17 cell population in the tumor. However, BME treatment did not alter Th1 and Th2 cell populations. Together, our findings offer a new insight into how bitter melon extract inhibits head and neck tumor growth by modulating cell proliferation and Treg populations, with implications for how to control tumor-infiltrating lymphocytes and tumor progression.
Importance of the Integration of African Traditional Medicine into the Existing Health Care System for Inflammatory Diseases and Cancer

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Abstract

Background: It has been estimated that in Africa, there is one traditional health practitioner for every 200–400 people, whereas the availability of trained medical personnel is typically one per 20,000 people. In the light of this statistical data and the growing of epidemics in Africa, there is an urgent need for more research into the efficacy and safety of the herbal medicines being used by the majority of the population, and how they can be improved and further developed.

This study would like to investigate another approach for an integration of traditional medicine into health system using the support of those community-based organizations.

Methods: Cancer bush (CB, Sutherlandia frutescens) and Devil’s claw (Harpagophytum procumbens) have been selected for their traditional use and efficacy to treat cancer and inflammatory diseases. Various methods including chemical and biological analysis, have been used to evaluate successively their antioxidant, antimutagenic, chemopreventive, anti-tumor and anti-metastatic activities.

Results: Our data have demonstrated that CB and DEV possess anti-mutagenic, anti-oxidant activities. They displayed substantial chemo-preventive/anti-inflammatory activity by inhibiting TPA-induced COX-2 expression in mouse skin through the reduction of catalytic activity of ERK. Both extracts inhibited TPA induced expression of c-Fos and DNA-binding of AP-1. Anti-metastatic activity of DEV has been demonstrated in its suppressive effects on MMP-7 protein (Matrix Metalloproteinase-7) in HT-29 cells.

Conclusion: Understanding the mechanism underlying activity of traditional preparations will lead to a better management of patients in their local communities and to drug discovery and development for an integration of traditional medicine into health system.

Targeting Ubiquitin-proteasome System with Natural Compounds: Implications in Anti-cancer Therapy

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Abstract

Ubiquitin Proteasome System (UPS) plays critical role in normal cellular functions. It is exploited by the cancer cells for survival and proliferation making UPS a valid therapeutic target. Therefore, efforts were taken to develop proteasome inhibitors. So far, only 2 agents namely, bortezomib and carfilzomib got approval by Food and Drug Administration for the treatment of multiple myeloma. Unfortunately, some patients are either inherently resistant to proteasome inhibitors or they eventually develop resistance to it. Additionally, bortezomib may cause toxicities such as peripheral neuropathy in cancer patients. There is an urgent need for novel proteasome inhibitors that are better than existing agents in terms of safety and efficacy. Natural compounds could be of great help in designing such agents. Indeed, various phytochemicals have shown promise as proteasome inhibitors. These natural compounds could be used as it is or as templates to design and develop novel proteasome inhibitors and warrant further in depth screening as well as studies.
Resveratrol-copper: A Novel Extracellular DNA-degrading Agent in the Treatment of Cancer

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Abstract
Recent research from our laboratory has shown that circulating cell-free nucleic acids (cfNAs) isolated from sera of cancer patients can freely enter into healthy cells, accumulate in their nuclei, trigger a DNA damage repair response (DDR) and integrate into host cell genomes by an unique mechanism (http://www.ias.ac.in/article/fulltext/jbsc/040/01/0091-0111; http://f1000research.com/articles/4-924/v1). Similarly, at the tissue level, locally generated cfNAs from dead cells can be taken-up by healthy bystander cells to induce DDR that facilitates their integration into recipient cell genomes. cfNAs from cancerous cells can induce dsDNA breaks, inflammation, chromosomal instability and oncogenic transformation of the recipient cells. It follows, therefore, that degrading cfNAs might be beneficial in preventing the initiation and metastasis of cancer. Resveratrol (R) is a plant polyphenolic antioxidant which, paradoxically, acts as a pro-oxidant in the presence of copper (Cu) by reducing Cu (II) to Cu (I) thereby generating a free radical. R-Cu has been shown to be capable of cleaving plasmid DNA via this pro-oxidant property. Our research has shown for the first time that R-Cu can also degrade genomic DNA and can degrade cfNAs in vivo. We have further shown that the pro-oxidant property of R-Cu with respect to DNA degradation can be retained even when the molar concentration of Cu is reduced more than thousand-fold with respect to that of R (http://f1000research.com/articles/4-1145/v2). In view of our new findings concerning oncogenic properties of cfNAs, R-Cu seems a promising agent in preventing initiation and metastasis of cancer. We have also discovered that the toxic side-effects of chemotherapy are largely due to DNA damage and cell-death induced by cfNAs derived from dead cells and that chemotherapy-induced toxicity can be prevented by concurrent treatment with R-Cu. If this finding can be successfully translated to humans, it would be a boon to the millions of patients that receive chemotherapy every day. We are on the threshold of embarking on a clinical trial on R-Cu in patients with cancer.

Bioactives from Cinnamomum tamala with Potential Antitumor Role

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Abstract
Indian spice, Cinnamomum tamala also known as tejpat, was assessed in our present studies. Petroleum ether fraction of crude methanol extract of leaves of Cinnamomum tamala (CT) showed potential cytotoxic activity against cancer cells in vitro. Administration of petroleum ether fraction of methanol extract of CT [CT (A)] showed significant tumor volume and tumor weight reduction in mouse fibrosarcoma induced in Swiss albino mice. In an attempt to guesstimate probable bioactive antitumor compounds in petroleum ether fraction CT (A), GC-MS analysis of crude fraction was done. Eugenol and α-Caryophyllene – two compounds most probably present in active fraction of Cinnamomum tamala leaves, were identified. In silico molecular docking analysis of Eugenol and α-Caryophyllene was carried out on several anticancer drug targets using the software Maestro (GLIDE, Schrödinger 9.0, LLC, NY). Docking pattern and binding interactions of the components of the active fraction gave valuable insights into their mechanism/s of action. Eugenol and α-Caryophyllene showed interaction pattern similar to that of Camptothecin - standard drug with Topoisomerase I; when both components were docked together. This suggests probable additive influence of Eugenol and α-Caryophyllene in inhibiting Topoisomerase I and eventual antitumor effect. However, further studies are required to confirm the same.
Resveratrol Driven Suicide Gene Therapy for Non-small Cell Carcinoma

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Abstract

A novel suicide gene therapy vector, pE9NS.G45α, was engineered by cloning growth arrest and DNA damage inducible 45 alpha (GADD45α) cDNA downstream to the synthetic CaRg promoter E9NS containing nine repeats of [CCATATAAGG]9 (CaRg) element with modified core A/T sequence of early growth response-1 promoter. GADD45α is a nuclear protein often upregulated by environmental stresses and DNA-damage agents to induce growth arrest and apoptosis, and CaRg elements are the chemo/radio-responsive region in the Egr-1 promoter. By being connected with the inducible promoter, the expression of therapeutic target could be nicely controlled to perform cytotoxic effects. Here, we evaluated the efficacy of our suicide gene therapy vector with cisplatin, resveratrol or radiation in three NSCLC cell lines: H1299 (deleted p53), A549 (wild-type p53) and H23 (mutated p53). All the combinations successfully activated promoter E9NS to drive the expression of GADD45α, and subsequently reduced cell viability and induced apoptosis regardless of p53 status. Our study demonstrates that GADD45α-targeted suicide gene therapy sensitizes NSCLC cells to cisplatin, resveratrol and radiation.

Identification and Functional Analysis of Choline Transporter in Tongue Cancer: A Novel Molecular Target for Tongue Cancer Therapy

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Abstract

We examined the functional characteristics of choline uptake in human tongue carcinoma using the cell line HSC-3. Furthermore, we explored the possible correlation between the inhibition of choline uptake and apoptotic cell death. Both choline transporter-like protein 1 (CTL1) and CTL2 mRNAs and proteins were expressed, and were located in plasma membrane and mitochondria, respectively. Choline uptake was saturable and mediated by a single transport system, which is pH-dependent. Several cationic drugs inhibited cell viability and [3H] choline uptake. Choline uptake inhibitors and choline deficiency inhibited cell viability and increased caspase-3/7 activity. We conclude that extracellular choline is mainly transported via a CTL1 that relies on a directed H+ gradient as a driving force. The functional inhibition of CTL1 by cationic drugs could promote apoptotic cell death. Furthermore, CTL2 may be the major site for the control of choline oxidation in mitochondria and hence for the supply of endogenous betaine and S-adenosyl methionine, which serves as a major methyl donor. Identification of this CTL1- and CTL2-mediated choline transport system provides a potential new target for tongue cancer therapy.

IL-1β Induces Chemoresistance to Cisplatin in Breast Cancer Cells Through Expression of Apoptosis-evading Genes

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Abstract

Breast cancer is the most common diagnosed malignancy in the World and the first cause of death in women. Approximately 90% of breast cancer deaths are caused by local invasion, metastasis, and chemoresistance to treatment of the tumor cells. In the
tumor microenvironment the inflammatory interleukin-1β (IL-1β) has been associated with tumor development, invasiveness, metastasis and initiation of the epithelial-mesenchymal transition (EMT). IL-1β-induced EMT triggers activation of PI3K/Rac pathways leading to expression of markers associated with cell migration and invasion. Recently, we reported that in breast cancer cells highly responsive to IL-1β a novel pathway, IL-1R1/β-catenin, is activated. The activation leads to increased transcription of SNAIL, c-MYC and MMP2, that are genes involved in the progression to an invasive phenotype. However, it is still undefined the possible association between IL-1β-induced-EMT and the acquisition of chemoresistance in these cells. Analysis of the expression and participation of cell survival genes in an IL-1β-highly sensitive MCF-7 clone showed gene up-regulation in a RNA-seq analysis of cells stimulated with IL-1β. The expression of genes CDKN1A, TP63 and BCL2 was validated by real-time-PCR. When stimulated cells were challenged with Cisplatin, drug resistance correlated with increased expression of such genes. Our data showed that IL-1β stimulus induced resistance to cisplatin through expression of cell survival genes.

Identification of a New Reactivator of Mutant P53 with In vivo Antitumor Activity

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Abstract

The p53 tumor suppressor is a transcription factor inactivated in all human cancers due to mutations in the p53 protein or to the overexpression of endogenous negative regulators of wild-type (wt) p53. The high prevalence, and the often observed increased drug resistance of mutant p53-expressing tumors, make mutant p53 a highly appealing target for novel anticancer therapies. In the present work, a yeast-based assay consisting of Saccharomyces cerevisiae cells expressing human mutant p53R280K was used to screen for reactivators of this mutant p53 with high clinical relevance. Using this approach, a chemical library of enantiopure tryptophanol-derived oxazoloisoindolinones was tested, and SLMP53-1 was selected as a potential reactivator of mutant p53R280K. The molecular mechanism of SLMP53-1 was further validated in human breast adenocarcinoma MDA-MB-231 cells expressing mutant p3R280K. In these cells, SLMP53-1 exhibited a p53-dependent growth inhibitory effect associated with apoptosis. Contrary to the majority of known p53 activators, no genotoxicity and in vivo toxicity were observed with SLMP53-1. Additionally, the p53-dependent antitumor activity of SLMP53-1 was validated in vivo using human xenograft mice models. Recently, the direct binding of SLMP53-1 to mutant p3R280K was confirmed by MicroScale Thermophoresis. Collectively, several evidences are provided supporting the identification of the first reactivator of mutant p53R280K with promising applications as anticancer drug candidate. We thank European Union (FEDER funds POCI/01/0145/FEDER/007728) and National Funds (FCT/MEC) under the Partnership Agreement PT2020 UID/MULTI/04378/2013, UID/DTP/04138/2013 (iMed.ULisboa), FCT project PTDC/DTP-FTO/1981/2014.

Roy-Bz: A New Small-Molecule Selective Activator of Protein Kinase C with Anticancer Activity

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Abstract

The protein kinase C (PKC) is a family of serine/threonine kinases grouped into three major subfamilies: classical, novel and atypical. PKC isozymes are widely recognized therapeutic targets in cancer. Particularly, PKCδ-selective activators are promising anticancer agents due to the well-known pro-apoptotic functions of PKCδ. However, to date, PKCδ-selective small-molecule
activators are still missing. Using a yeast PKC screening assay, consisting of Saccharomyces cerevisiae cells individually expressing PKC isoforms, the compound Roy-Bz was identified as potential PKCd-selective activator, from a library of semi-synthetic derivatives of a natural diterpenoid. The direct binding and selectivity of Roy-Bz to PKCδ was confirmed using an \textit{in vitro} PKC assay and recombinant PKC proteins. In human colon carcinoma cell lines (HCT116), Roy-Bz exhibited a potent growth inhibitory effect (IC50 of 0.63 ± 0.05 μM) through activation of a p53-mediated mitochondrial apoptotic pathway involving p53 phosphorylation at Ser46. All these effects were dependent on PKCd activation, since either its inhibition by the PKCδ-selective inhibitor rottlerin, or its knock-down, abolished the Roy-Bz-induced apoptotic effects. Roy-Bz is a non-genotoxic compound, and has \textit{in vivo} antitumor activity in human xenograft mice models without apparent toxicity. Collectively, several data are provided supporting the identification of the first PKCδ-selective small-molecule activator with promising anticancer properties. We thank European Union (FEDER funds POCI/01/0145/FEDER/007728) and National Funds (FCT/MEC) under the Partnership Agreement PT2020 UID/MULTI/04378/2013, and FCT project PTDC/DTP-FTO/1981/2014. FCT fellowship: SFRH/BD/87109/2012.

\textbf{Atypical Antipsychotics for the Treatment of Brain Tumors}

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\textbf{Abstract}

\textbf{Background:} Brain tumors are some of the most difficult types of cancers to treat in part due to the paucity of effective therapies able to cross the blood brain barrier. Drug “repositioning” is an attractive idea because the use of existing FDA-approved drugs can bypass or shorten critical steps of drug development. Antipsychotic drugs (APDs), both typical and atypical, are primarily used for the treatment of schizophrenia and mood disorders. However, they may also have potential for CNS cancers. Compared to typical APDs, atypical APDs may be especially suitable for this purpose due the lower frequency of extrapyramidal side effects (EPS), while also causing side effects that may actually benefit patients suffering from cancer. A primary side effect of APDs is metabolic disturbance resulting in weight gain. Along with reduced nausea often experienced by patients treated with atypical APDs, these effects may actually benefit cancer patients undergoing radio and chemotherapy. In addition, CNS cancer patients often experience depression and other psychiatric symptoms that may be reduced or eliminated with the use of atypical APDs. Quetiapine, in particular, is well known for effectively treating depression and mood disturbances. Furthermore, recent data suggests that these drugs may possess anti-cancer effects. APDs have been shown to induce growth inhibition in lymphoma, neuroblastoma, non-small cell lung cancer and leukemia as we as potentiate the effects of some commonly used chemotherapeutic agents in leukemia. Here we sought to investigate the effects of clozapine, risperidone, and quetiapine on brain tumor cell lines.

\textbf{Design/Methods:} Anti-cancer effects of APDs on Medulloblastoma (DAOY) and glioblastoma cell lines (U87), including patient-derived glioblastoma stem cells (GSCs). Viability was determined using MTS assay. Cells were exposed to increasing concentrations (10–100 uM) for 72 hr or (0.5–10 uM) for 144 hr.

\textbf{Results:} All 3 APDs induced dose-dependent brain tumor cell death at 72 hr treatment, with clozapine being the most effective. However, these concentrations are not clinically achievable, therefore we exposed the cells to lower doses 5–25 uM for 6 days. Surprisingly these concentrations of clozapine had no effect on cell viability while both risperidone and quetiapine reduced cell viability. We then sought to determine the effects of risperidone and quetiapine on patient-derived glioblastoma stem-like cells (GSC’s). At 6 days of treatment 10 uM of risperidone or quetiapine reduced GSC viability to < 50%.

\textbf{Conclusion:} Patients suffering from brain tumors often experience a myriad of symptoms including nausea, loss of appetite and psychiatric aspects. APD’s such as quetiapine may not only improve the quality of life but may also negatively affect tumor growth. Here we demonstrated that clinically relevant concentrations of quetiapine reduce GSC viability. Ongoing experiments will evaluate the molecular mechanisms of APD’s on these and examine the potential of combining APD’s with the current standard of care.
**Cyclooxygenase-2 Regulates TGFβ-induced Cancer Stemness in Triple-negative Breast Cancer**

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**Abstract**

Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer with poor prognosis and limited therapeutic options. These types of tumors show high risk of recurrence and exhibit resistance to conventional therapies, due to the fact they are enriched in breast cancer stem cells (BCSCs). Previous studies showed that BCSCs are regulated by the TGFβ signaling pathway, but the molecular mechanisms underlying this process remain to be elucidated. In this study, we investigated the role of the cyclooxygenase-2 (COX-2), a downstream target of TGFβ, in regulating BCSCs in TNBC. Bioinformatics analysis revealed that COX-2 is highly expressed in TNBC and that its expression significantly correlated with poor survival outcome in basal subtype of breast cancer. We also found TGFβ-mediated COX-2 expression to be Smad3-dependent and to be required for BCSC self-renewal and expansion in TNBCs. Indeed knocking down COX-2 expression, using COX-2 shRNA lentiviral particles strikingly blocked TGFβ-induced tumor sphere formation and TGFβ-induced enrichment of the CD24lowCD44high stem-like cell population. Blockade of COX-2 activity using a pharmacological inhibitor, celecoxib, also prevented TGFβ-induced BCSCs self-renewal, implicating COX-2 as a critical player downstream of TGFβ-mediated regulation of cancer stemness in breast cancer. Moreover, we found COX-2 to be required for TGFβ-induced expression of mesenchymal and basal breast cancer markers. Together, our results describe a novel and essential role for COX-2 in mediating the TGFβ effects on BCSC self-renewal in TNBC, and imply that therapeutic targeting of COX-2 may prove useful for the treatment of TNBC by eliminating BCSCs.

**Dissecting the Role of Etv5 in Oligodendrogliomagenesis**

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**Abstract**

Oligodendrogliomas (ODG) are a devastating class of brain tumor that have poor prognosis and are ultimately fatal. ODG is associated with CIC loss and IDH gain-of-function mutations. We propose to characterize genes downstream of CIC that are associated with ODG transformation, focusing on Etv5, a transcriptional regulator that is upregulated in ODG. We are particularly interested in the role of Etv5 in regulating proliferation and specifying an oligodendrocyte precursor cell (OPC) fate, since OPCs are considered to be the developmental cell of origin in ODG. Our earlier work indicated that Etv5 is a key regulator of a glioblast fate, acting in the context of elevated RAS/ERK signaling to induce OPC differentiation. Our working hypothesis is that CIC normally represses the ability of Etv5 to promote proliferation and an OPC identity. We are testing this model in normal cortical development and in ODG cell lines that are unique to the Chan lab. First, using a conditional Etv5 knock-out, we found that the loss of Etv5 results in the production of fewer cortical neurospheres from E12.5 progenitors. Second, knock-down of Etv5 in BT88 cells derived from an ODG tumor also reduced neurosphere number. Taken together, these studies suggest that Etv5 is an essential regulator of proliferation in both normal cortical development and in ODG tumorspheres. Future studies will assess the role of Etv5 in OPC fate specification. By elucidating the importance of Etv5 in normal OPC development and in ODG formation, we may identify druggable targets for ODG treatment, aiding future pre-clinical studies, while also building our foundational knowledge of ODG.

**APOBEC3G Governs Cellular Oncogenic Transformation**

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**Abstract**

The oncogenic potential of APOBEC3G gene was recently appreciated by its inhibitory influence of APOBEC3G upon miRNA-mediated repression of the gene responsible for hepatic metastasis. The present study employed human PBMCs, as an
archetype model to understand the role of APOBEC3G in regulation of gene involved in oncogenesis. Such a study revealed that APOBEC3G transrepresses KLF4 by binding to its mRNA at 3’UTR. This phenomenon was paralleled by the sustained expression of the cellular SP1 that ensured overexpression of genes coding for c-myc, Bmi-1, BCL-2 and MDM2 coupled with downregulation of p53 in PBMCs thereby creating a favorable situation for oncogenic transformation. Additionally, cell cycle regulators like Cyclin D, B and E, etc., were found to be upregulated, along with increase in S-phase of the cell cycle. Furthermore, study revealed the role of APOBEC3G in tailoring immune response that could help tumor growth through escape of immune surveillance. This study unambiguously revealed that APOBEC3G has the inherent capacity to upregulate genes coding for STAT3, CCL5, IL-6, IL-4, and NF-kB coupled with downregulation of IL-10, IL-17. Based upon these results, we propose that increased expression of APOBEC3G could have the inherent capacity to ensure the alterations of gene expression in such a way that leads to cellular proliferation, hallmark of oncogenesis as well as make the environment conducive for tumor progression and immune escape through its ability to ensure sustained chronic inflammation. This may add a new dimension to understand oncogenesis in general, and the novel role of APOBEC3G in the oncogenic process in particular.

**Anti-inflammatory Effects of Novel 2,5-disubstituted-1,3,4-Oxadiazoles with Glycine/ Alanine Hybrids**

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**Abstract**

Histone deacetylases (HDACs) are known to be involved in many pathological conditions including cancer, inflammatory diseases, neurodegenerative disorders etc. Previously, we designed, synthesized and evaluated the in vitro histone deacetylase (HDAC) inhibitory activity of a series of novel 2,5-disubstituted 1,3,4-oxadiazoles containing glycine and alanine amino acids as class I HDAC inhibitors, specifically with moderate selectivity towards HDAC8a. All the compounds synthesized (10a-j) showed anti-tumor activity. Further the anti-inflammatory potency of the compounds was evaluated on *E. coli*-infected mouse macrophage, RAW 264.7, cells. All the compounds inhibited the proliferation of the infected macrophages. However, compounds 10f and 10h showed high efficacy. Compounds 10f and 10h reduced the bacterial growth in the macrophages as determined by counting the number of colony forming units (CFU). Both the compounds inhibited the pro-inflammatory cytokines (IL-12, TNF-α, IFN-γ and IL-6) production and induced the anti-inflammatory cytokine (IL-2) production. Compounds 10f and 10h also inhibited the COX-2 inflammatory protein levels. The results of the study clearly demonstrate that compounds 10f and 10h with HDAC inhibitory activity might be promising lead molecules with potent anti-inflammatory and anti-bacterial activity.

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