CANCER

C1 Disruption of Choline Acetyltransferase Activity Suppresses Lung Adenocarcinoma Growth in Smokers

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The clinicopathological profile of lung adenocarcinoma (LAC) differs in smokers and non-smokers. LAC in smokers is relatively resistant to therapies. For example, targeted therapeutic agents like EGFR-inhibitors (erlotinib and gefitinib) are highly effective in LACs which develop in non-smokers. However, such targeted therapies display much lower anti-tumor activity in LACs in patients who are active smokers. Similarly, lung cancer patients (who are active smokers) show a lower response to chemotherapy than those who are non-smokers. However, the majority of LAC patients are smokers. This underlines the need to identify novel molecular targets relevant for LAC therapy in patients who are exposed to cigarette smoke via active smoking or exposure to secondhand smoke. Several convergent studies show that nicotine (the addictive component of cigarette smoke) accelerates the growth of lung cancers, as well as confers resistance to chemotherapy. One of the mechanisms underlying the biological activity of nicotine is that it promotes the secretion of the neurotransmitter acetylcholine (ACh) from LAC cells. ACh is known to be an autocrine growth factor for LAC cells and is synthesized by the enzyme choline acetyltransferase (ChAT). We investigated the feasibility of ChAT as a molecular target for LAC in smokers. We find that ChAT levels are up-regulated in human LAC cell lines and tissues in a smoking history–dependent manner. Finally, the ChAT inhibitor BW813U causes robust apoptosis in human LAC cells. The magnitude of BW813U-induced apoptosis is similar across LAC cell lines (irrespective of smoking history); however, the concentration of BW813U that causes apoptosis is lower in LAC cell lines belonging to heavy smokers. Our studies validate choline acetyltransferase (ChAT) as a viable drug target for the majority of the population of lung cancer patients who are smokers.

C2 Anti-angiogenic Activity of Memantine—a dual α7-nAChR/NMDAR antagonist—in Human Small Cell Lung Cancer

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Non–small-cell lung cancer (NSCLC) and small cell lung cancer (SCLC) bear a strong etiological association with smoking habits. Nicotine, the addictive component of cigarettes, promotes angiogenesis in lung cancer via the α7-nicotinic acetylcholine receptor (α7-nAChR) subunit in human lung endothelial cells. Therefore, we hypothesized that α7-nAChR-antagonists should display potent anti-angiogenic and antitumor activity in lung cancers. Memantine is a dual α7-nAChR/*N*-methyl-D-aspartate receptor (NMDAR) antagonist that is used in the clinic for the treatment of patients suffering from mild-to-moderate Alzheimer disease. Receptor binding assays have shown that the affinity of memantine for α7-nAChR is greater than NMDAR. Memantine attenuated nicotine-induced angiogenesis in human microvascular endothelial cells of the lung (HMEC-Ls). Most interestingly, the levels of α7-nAChR in tumor-associated endothelial cells were greater than normal lung endothelial cells. The anti-angiogenetic activity of memantine was mediated by the α7-nAChR (and not by NMDARs) on lung endothelial cells. Furthermore, the α7-nAChR antagonist memantine displayed potent anti-angiogenic activity in the chicken chorioallantoic membrane model. Our studies show that α7-nAChR antagonists may be useful anti-tumor agents relevant for the treatment of human lung cancer.

C3 Capsaicin Sensitizes Human Small Cell Lung Cancer Cells to the Proapoptotic Activity of Camptothecin

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Platinum-based chemotherapy is the cornerstone of small cell lung cancer (SCLC) treatment. Chemotherapy initially shows excellent outcomes with 60% to 80% patients achieving remission. However, patients relapse within a year and after that, the tumor does not respond to chemotherapy or radiation. Camptothecin (topotecan) is the only Food and Drug Administration (FDA)-approved drug for relapsed SCLC. Camptothecin has been shown to display an objective response rate of about 3%. Therefore, agents that can increase camptothecin response rate should be of considerable benefit to SCLC patients. We tested the combinatorial effects of the nutritional compound capsaicin with camptothecin in human SCLC. Caspase-3 activity assays reveal that the combination of camptothecin and capsaicin displayed greater apoptotic activity than any of the compounds used alone. Statistical analysis using the Chou-Talalay isobologram showed that capsaicin displayed synergistic apoptotic activity with camptothecin. Chicken chorioallantoic membrane assays confirmed the combinatorial effects of capsaicin and camptothecin. The synergistic effects of capsaicin and camptothecin are mediated by the calpain pathway and involve the proteolytic cleavage of the proapoptotic protein Bax to generate a more potent proapoptotic Bax fragment protein. The results of our studies will foster the hope of novel second-line treatment regimens for human SCLC.

C4 StarD10 as a Novel Colon Cancer Diagnostic Marker

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Background: The early detection of colorectal cancer (CRC) significantly improves the prognosis andis a key factor to reduce the mortality from CRC. The steroidogenic acute regulatory protein StarD10 is a lipid transfer protein with selective binding site to phosphatidylcholine and phosphatidylethanolamine. Screening of a cDNA expression library from a human colon carcinoma patient with autologous serum identified autoantibodies against an expressed sequence tag corresponding to StarD10. This finding indicates that StarD10 may be a target for anti-tumor immune response and may play a role in the development of colon cancer. Our aim was to investigate the role of StarD10 in colon cancer and its possible use as a blood diagnostic biomarker. **Methods:** Plasma samples, cancer, and corresponding surrounding non-tumorous tissue from surgical resection for primary colon cancer were used. Gene and protein expression were measured by real-time PCR and Western blot analyses. **Results:** Secretome P2.09 server predicted that StarD10 is a non-classically secreted protein. Down-regulation of StarD10 protein level was found in plasma of patients that received surgical resection for primary colon cancer by 56.38% compared with plasma from healthy patients. Surprisingly, the molecular weight of StarD10 was approximately 54kDa, greater than expected for a 291-amino acid nascent protein, suggesting post-translational modifications during StarD10 secretion. In addition, StarD10 immunohistochemistry showed that it is exclusively expressed in transverse and sigmoid colon tissues. We found that StarD10 was highly expressed in sigmoid colon cancer by 1.4-fold and 2.5-fold compared with normal mucosa mRNA and protein levels, respectively. **Conclusions:** We demonstrate for the first time that serologically detected StarD10 may be a biomarker in colon cancer. Plasmatic down-regulation of StarD10 may result in inverted correlation between secreted and retained StarD10 that could cause aberrant lipid signaling contributing to cellular transformation.

C5 Membrane Protein from Infective *Leishmania donovani* Induces Apoptosis in HepG2 cells: Involvement of Reactive Oxygen Species–Dependent P53-Mediated Mitochondrial Death Cascade

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Liver cancer is the fifth most intermittently diagnosed cancer in men and the second most frequent cause of cancer death, whereas in women it is the seventh most common cancer and sixth leading cause of death due to cancer. Current anticancer therapies like surgical resection and chemotherapy are not found to be satisfactory for several types of cancer and several substitutive strategies are being adopted for the treatment of cancers that are resistant to therapies in use. Several microbial communities involving live, attenuated, or genetically-altered microbes or their cellular components or synthesized products, have gained increasing attention to be evaluated as therapeutic agents against various pathophysiological conditions of disease including cancer. A novel membrane protein isolated from the protozoan parasite *Leishmania donovani* strain AG83 may aid in growth inhibition of human hepatocellular carcinoma cell line HepG2. We explored the underlying mechanisms. The cytotoxic effect of the membrane protein on HepG2 cells is associated with increased DNA fragmentation, accession in number of annexinV positive cells, and cell cycle arrest, but it did not affect normal human peripheral blood mononuclear cells. The detection of up-regulated levels of caspases 3 and 9, cytosolic cytochrome C, proapoptotic proteins Bax, and Bad, along with the observed down-regulation of antiapoptotic proteins Bcl-2 and Bcl-XL and loss of mitochondrial membrane potential (MMP) indicated the involvement of mitochondrial pathway. Up-regulated reactive oxygen species (ROS) and p53 levels promote the apoptosis of HepG2 cells. Inhibiting p53-mediated transactivation by pifithrin-α resulted in failure to induce apoptosis by the membrane protein. In conclusion, the *Leishmania donovani* membrane protein efficiently induces apoptosis in HepG2 cells through ROS-mediated p53dependent mitochondrial pathway and can be used as an effective chemopreventive agent for cancer bio therapy.

C6 Targeting Neuropilin-2 Prevents Pancreatic Ductal Adenocarcinoma Progression

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Pancreatic cancer is the third leading cause of cancer-related deaths. Therefore, a novel treatment for pancreatic cancer is urgently needed. Neuropilins (NRPs) are cell-surface receptors found in tumor and endothelial cells. Arteries express NRP1, whereas veins, capillaries, and lymphatics express NRP2. Neuropilins bind competitively to vascular endothelial growth factor (VEGF) family members or to inhibitory semaphorin-3F (SEMA3F) ligands. Human pancreatic ductal adenocarcinoma (PDAC) cells express NRP2; *NRP2* silencing in PDAC cells attenuated tumorigenesis *in vivo*. We hypothesized that Nrp2 expression in the tumor vasculature is necessary for PDAC growth and progression in mice. The endothelium in the adult mouse pancreas and liver are devoid of Nrp2 expression; however, Nrp2 is dramatically up-regulated in tumor-associated blood vessels in pancreatic tumors in human orthotopic xenograft models, in spontaneous transgenic (pdx-*cre*;kras$^{+/+}$; p53$^{+/-}$) mouse models, and in human patient samples. To investigate the dependence of pancreatic cancer growth on host vascular Nrp2 expression, a C57BL/6 syngeneic mouse PDAC cell line was injected orthotopically into the pancreas of *Nrp2*$^{+/+}$, *Nrp2*$^{+/-}$, and *Nrp2* $^{-/-}$ (knockout) mice. Panc0H7 tumors were significantly smaller in *Nrp2*-deficient mice as compared to *Nrp2*-intact mice. Tumor microvessel density was significantly lower in *Nrp2*-knockout mice compared to wild-type littermates. We next investigated the inhibitory SEMA3F ligand as a potential therapy for pancreatic cancer in preclinical prevention and intervention trials. Specifically, control- or SEMA3F-adenovirus, which actively encodes SEMA3F protein, were injected intravenously into mice before or after orthotopic injection of syngeneic PDAC cells. Results demonstrated that pancreatic tumors harvested from SEMA3F-treated mice were significantly smaller than the tumors from the control mice. Metastases were detected in the livers of mice treated with control virus but not in the SEMA3F-treated group. Overall our results suggest that targeting the NRP2 axis may be a potential treatment strategy that inhibitsthe growth and metastasis of pancreatic tumors.

C7 Differential Roles of β-Catenin and Yap during Development of Hepatoblastomas in Mice

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The molecular mechanisms underlying hepatoblastoma (HB) development are poorly understood. Yap1 and β-catenin are activated in most HB. Overexpression of yap1 and β-catenin in livers of mice leads to HB. In the current study, instead of co-delivering yap1 and β-catenin (Y1B1), we delivered either yap followed 3 days or 7 days later by β-catenin (Y1B2) or vice-versa (Y2B1) via sleeping-beauty transposon/transposase-based hydrodynamic tail vein injection. Mouse livers were harvested at 6 weeks (6w), 7w, and 9w post-second injection for assessment of tumor size, phenotype, proliferation, and signaling pathways. There was no difference in the liver phenotype between Y1B1) and Y1B2 models. However, the tumor size was significantly smaller in Y2B1 model at early time-point (6w to 7w); there was no difference at late stage (9w). Immunohistochemistry and Western blot showed no differences in GS, CCND1, or β-catenin expression. However, Ki-67 expression was comparably higher in Y1B2 and Y1B1 livers compared to Y2B1. mTORC1 (p-mTOR2448) and mTORC2 (p-mTOR2481) showed higher expression in Y1B2 and Y2B1 than Y1B1 livers at different times. mTOR downstream effectors such as p-S6, p-EIF4, p-4EBP showed higher expression at 6w in Y1B1, but no differences at later stages for the three groups. c-Myc showed higher levels at early stages in Y1B1 group only. Erk pathway was activated in the co-injection group and Akt only at late stage of Y1B2 and Y2B1 livers. Such results imply that yap and β-catenin have differential roles in HB initiation and progress. Yap might play a more important role in HB initiation, and β-catenin in HB progression. mTOR, Myc, and Akt signaling may not be the mechanisms behind differential roles of the two pathways in HB pathogenesis. Further studies combining laser capture microdissection of early nodules may yield novel mechanistic insights.

C8 Endoscopic Ultrasound Guided Fine Needle Aspiration/Brush in Cytopathology Diagnosis: A 15-Month Study

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Introduction: EUS-guided fine needle aspiration/Brush (EUS-FNA/Brush) has become increasingly popular for the diagnosis and staging of gastrointestinal and peri-gastrointestinal lesions. **Objective:** To evaluate the diagnostic accuracy and spectrum of lesions in gastrointestinal EUS-FNA., **Methods**: 124 EUS-FNA during the period from August 2015 to November 2016 were studied. **Results**: Age ranged from 13 to 80 years with a slight female predominance. Common bile duct was the most common site with 47 cases amongst which were nine adenocarcinoma and seven cases were suspicious for malignancy. Pancreatic EUS-FNA showed five adenocarcinoma, two solid pseudopapillary epithelial neoplasms, one case each of neuroendocrine tumor, anaplastic carcinoma, and non-Hodgkin lymphoma (NHL). Amongst esophageal lesions, three cases were suspicious for malignancy and four were inflammatory, four showed squamous cell carcinoma, one case each adenocarcinoma and leiomyoma. Among gastric lesions there was—one case each of adenocarcinoma, granulomatous inflammation, and gastrointestinal stromal tumor. Periportal lymph nodes were the commonest nodes and there were 11 necrotizing granulomatous