S.IV.1.

Targeting growth factor and inflammation signaling pathways for the prevention of prostate cancer

John DiGiovanni

Division of Pharmacology & Toxicology, College of Pharmacy and Department of Nutritional Sciences, College of Natural Sciences, The University of Texas at Austin, 1400 Barbara Jordan Blvd, Austin, TX 78723 USA
e-mail: john.digiovanni@austin.utexas.edu

The effects of dietary energy balance on PCa progression using the HiMyc mouse model have been studied to gain greater insight into the mechanisms and to identify potential targets for prevention strategies. Thirty percent calorie restriction (CR) significantly reduced PCa progression whereas, diet-induced obesity (DIO) led to more rapid progression of PCa in this mouse model, findings similar to that seen in men with PCa. DIO also significantly increased (and 30% CR decreased) numbers of T-lymphocytes and macrophages in the ventral prostate (VP) compared with overweight control. Further experiments have shown that the mRNA expression of inflammatory and angiogenesis mediators in RNA samples from VP was significantly increased in the DIO group compared with both the overweight control and CR diet groups. Further analyses showed that enhanced growth factor (Akt/mTORC1 and STAT3) and inflammatory (NF-kappaB and cytokines) signaling in the tumors may play a role in dietary energy balance effects on PCa progression in HiMyc mice. Using a procedure to separate the stromal-vascular fraction (SVF) [containing adipose stromal cells (ASCs), inflammatory cells and endothelial cells] we have analyzed the production of a number of inflammatory cytokines and chemokines as well as growth factors. Further studies have suggested that CXCL12/CXCR4 signaling may play an important role in driving PCa progression in HiMyc mice during obesity through selective effects on PCa stem cells. Based on these data and other data to be presented, we have evaluated several agents (e.g. metformin, rapamycin, 6-shogaol, ursolic acid and others for their ability to prevent PCa development and/or progression using the HiMyc model. Overall, these studies are leading to a greater understanding of the impact of dietary energy balance on PCa development and progression as well as identifying novel strategies for PCa prevention and for offsetting the effects of obesity on PCa progression.

Key words: prostate cancer; dietary energy balance; prevention

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S.IV.2.

Lysine specific demethylase 1 (LSD1) as a novel target for prostate cancer prevention and treatment

Xiaolin Zi[1,2,3], Noriko N. Yakoyama[1], Xuesen Li[1], Yichao Zheng[4], Yichao Duan[4], Hongmin Liu[4]

[1]Departments of Urology and [2]Pharmacology and [3]Chao Family Comprehensive Cancer Center, University of California, Irvine, 101 The City Drive S, Orange, CA, USA; [4]School of Pharmaceutical Sciences, Zhengzhou University, P. R. China
e-mail: xzi@uci.edu

Histone lysine-specific demethylase 1 (LSD1) that removes the methyl group of Histone 3 lysine 4 (H3K4) for regulation of gene transcription is overexpressed in many cancers. In prostate cancer, LSD1 is an important androgen-receptor (AR) coactivator for promoting expression of AR targeting genes. We have shown that kawain, the main component of kava root extracts, inhibited LSD1 enzyme activity, enhanced H3K9 dimethylation and decreased the mRNA expression of androgen receptor target genes, including PSA and TMPRSS2, in LNCaP cells. In addition, dietary feeding of kava root extract reduced tumor burden and inhibited the development of high-grade prostatic intraepithelial neoplasia and adenocarcinomas in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. Based on these results, we have further designed and synthesized novel series of 1, 2, 3-triazole-dithiocarbamate hybrids via "click" chemistry. A lead LSD1 chemical inhibitor (named LHM101) with an IC_{50} value of 2.11 μM and selectivity over MAO-A up to 1250 fold has been screened out from this focused small molecule library. We also have shown that LHM101 preferentially inhibited the growth of castration resistant prostate cancer cell line C4-2B and 22Rv1 with an IC_{50} of around 1μM via induction of apoptosis with minimal effect on normal prostate epithelial cells. Biochemical analysis revealed that LHM101 was a reversible and flavin adenine dinucleotide (FAD) competitive LSD1 inhibitor. Molecular modeling predicted that LHM101 reasonably docked into the FAD pocket of LSD1.LHM101 also inhibited the expression of AR target genes and some androgen synthesis genes in castration-resistant prostate cancer, reduced tumor growth in a prostate cancer xenograft model, and had a synergistic effect with second-generation anti-androgen drug Enzalutamide. Taken together, these results suggested a novel epigenetic approach for both prostate cancer prevention and treatment by targeting LSD1.

Key words: LSD1; kava; prostate cancer

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S.IV.3.

Inhibition of ATR kinase activity for the treatment of lung cancer

Christopher J. Bakkenist

Departments of Radiation Oncology and Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
e-mail: bakkenistcj@upmc.edu

ATR and ATM are protein kinases activated at stalled and collapsed replication forks and DNA double-strand breaks (DSBs), respectively, where they function to maintain genome integrity by mediating cell cycle checkpoints and DNA repair. ATM has been widely studied since ataxia telangiectasia individuals who express no ATM protein are the most radiosensitive humans identified. It has therefore been postulated that ATM kinase inhibitors (ATMi's) will increase the efficacy of radiotherapy. ATR has also been widely studied, but advances have been complicated by the finding that ATR is an essential protein in mice and mammalian cells. Nevertheless, pharmacologic ATR and ATM kinase inhibitors have been identified and these sensitize cancer cells to ionizing radiation (IR) in tissue culture. ATR kinase inhibitors (ATRi's) also synergize with cisplatin to induce cell death in tissue culture. Further, withaferin A disrupts ATR kinase signaling in cells cultured *in vitro*. Since concurrent cisplatin and radiation is used as standard of care for locally advanced and metastatic NSCLC patients, ATR kinase inhibition may significantly improve the efficacy of first line treatment in tens of thousands of patients in the USA every year. Until recently, however, *in vivo* studies have been limited by the absence of bioavailable ATR and ATM kinase inhibitors.

Here we describe orally active and bioavailable ATR and ATM kinase inhibitors and show that, in contrast to expectations, ATRi is surprisingly well tolerated. We show that cisplatin-ATRi induces a complete response in ATM-deficient lung cancer xenografts and potentiates the effect of cisplatin in $p16^{INK4A}$-deficient lung cancer xenografts. We also show that conformal radiation-ATRi and radiation-ATMi induce profound responses in an autochthonous $Kras^{G12D}$/Twist1 mouse model of lung adenocarcinoma, and that the efficacy of radiation-ATRi for the treatment of lung cancer appears to be better than that of radiation-ATMi by virtue of lower toxicity. Finally, we show that withaferin A impacts ATR kinase signaling in cells cultured *in vitro*.

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S.IV.4.

Web server tools for structure-based therapeutic design: modeling of protein structure, flexibility, aggregation properties and interactions

Sebastian Kmiecik, Michal Jamroz, Maciej Blaszczyk, Mateusz Kurcinski, Agata Szczasiuk, Andrzej Kolinski

Department of Chemistry, University of Warsaw, Pasteura 1, Warsaw, Poland
e-mail: sekmi@chem.uw.edu.pl

Recently, we developed a series of molecular modeling tools for *structure-based studies of protein* functions and interactions. These tools are publicly available as web servers that can be *easily operated via web browser interface, even by non-specialists:* CABS-fold server for protein structure prediction [1]; CABS-flex server for modeling of protein structure flexibility [2]; Aggrescan3D server for prediction of protein aggregation propensities and rational design of protein solubility [3]; and CABS-dock server for prediction of peptide binding sites and peptide docking [4]. The web servers are freely available from the laboratory website: http://biocomp.chem.uw.edu.pl/tools

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Key words: protein modeling, protein structure prediction, protein flexibility, protein aggregation, protein-peptide docking

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