Polichemotherapy with paclitaxel, Bcl-2 silencing and starvation in P-TEN mutated prostate cancer cells

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Background: The critical role of PTEN in regulating the PI3K/Akt/mTOR signaling pathway raises the possibility that targeting downstream effectors of the PI3K pathway, such as Bcl-2, might be an effective anti-proliferative strategy for PTEN-deficient prostate cancer cells.

Methods: Four prostate cancer cell lines (LNCaP, PC3, DU145, 22Rv1) were assayed for their levels of total Akt and Ser473 phosphorylated Akt (p-Akt) by Western Blotting; their growth rates and sensitivity to different doses of paclitaxel were determined by cell counts after Trypan Blue dye exclusion assay. Cells were subjected to different combinations of starvation (growth factors and/or aminoacids withdrawal), paclitaxel treatment and Bcl-2 silencing by siRNA; cell viability was evaluated by Trypan Blue dye exclusion assay, Propidium Iodide (PI) and Annexin-V/PI staining.

Results: We assessed the sensitivity of different prostate cancer cell lines to starvation and we observed a differential response correlated to the levels of Akt activation. The four prostate cancer cell lines also showed different sensitivity to taxol treatments: LNCaP and 22Rv1 cells were more resistant to paclitaxel than DU145 and PC3 cells. Combining taxol with growth factors and aminoacids deprivation leaded to a more than additive reduction of cell viability compared to single treatments in PTEN-mutant LNCaP cells. Down-modulation of anti-apoptotic Bcl-2 protein by siRNA sensitized LNCaP cells to taxanes and starvation induced cell death.

Conclusion: Silencing Bcl-2 in PTEN-mutated prostate cancer cells enhances the apoptotic effects of combined starvation and taxol treatments, indicating that inhibition of Bcl-2 may be of significant value in PTEN-mutant tumor therapy.
P4.2

Cisplatin caused impairment of antioxidant system and electron transport chain in liver and kidney of Sprague Dawley rats

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Cisplatin (cis-diamminedichloroplatinum II) has been extensively used clinically for more than a generation and is one of the most important chemotherapeutic agents ever developed. It is used alone or in combination with other agents in the management of a variety of tumors. In spite of its potent antineoplastic activity, the clinical use of cisplatin is often limited by the risk of undesirable side effects on normal tissue cells. These effects are postulated to occur via different mechanisms. Therefore, cisplatin-induced mitochondrial injury has increasingly been investigated as a mediator of toxicity in normal tissues. In order to clarify the potential effect of cisplatin, the present study was designed to study: (1) uptake of cisplatin into liver and kidney tissues of Male Sprague Dawley adult rat, (2) alterations of the mitochondrial electron transport chain, succinate dehydrogenase (SDH) and cytochrome c oxidase (COX) activities and levels of adenine nucleotides upon cisplatin exposure, (3) catalase (CAT) activity and indicative marker of membrane damage, lipid peroxidation (LPO) levels in order to investigate the response of the antioxidant defence system and damage caused by cisplatin, and (4) whether capsaicin has a possible protective effect against the alterations and damage induced by cisplatin. ICP/MS analysis showed that platin levels in liver were similar to those observed in kidney on day 1 after single dose cisplatin injection. After 4 days of exposure, the levels decreased in kidney and liver compared to the 1st day. We found that cisplatin caused significant impairment of SDH, COX, and CAT activities, and nucleotide levels associated with membrane LPO in isolated mitochondria. It was determined whether capsaicin, as an antioxidant, has a possible protective role on all investigated parameters of liver and kidney induced by cisplatin. The results of capsaicin + cisplatin suggest that capsaicin has antioxidant capacity to scavenge ROS to prevent membrane damage.
P4.3

Genomic landscape of the PI3K-AKT pathway and the RAS-RAF-MEK-ERK pathway in ovarian cancer: A treatment strategy

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Background: Genetic alterations of the PI3K-AKT and RAS-RAF pathways in ovarian cancer (OC) are characteristically abundant and heavily diverse. These alterations upregulate oncogenic signals which have well-defined functional consequences on cell proliferation and thus, determine the therapeutic response to pathway-specific drugs.

Purpose: Alterations of genes belonging to the PI3K-AKT and RAS-RAF pathways are common in ovarian cancer. Based on our examination of genomic alterations in ovarian cancer patients at Avera Cancer Institute, SD, we have tested anti-tumor effects of pathway-specific inhibitor(s) either alone or in combination in the context of pathway-specific gene alterations.

Methods: Comprehensive genomic profiling from 33 OC patients (February 2014 through April, 2016) were analyzed. Patients were biopsied after consultation and samples were characterized for genomic [Foundation One] and proteomic analyses [Theranostics]). We also evaluated mutation distribution in cell free DNA via digital NGS using the Guardant 360 panel.

Results: In total 59 genes were altered in ovarian patients and the most frequent genetic alterations are TP53 (73%). The PI3K-AKT-mTOR pathway-specific genes and the RAS-RAF-MEK pathway-specific genes were altered in 45% and 33% of patients respectively. BRCA1/BRCA2 was altered 15% of patients. Herein we tested the anti-tumor efficacy of both single agent or combination therapy of isoform-specific PI3K inhibitor (alpha or beta) or an allosteric or kinase inhibitor of mTOR and a MEK1/2 inhibitor using ovarian cancer cell lines (PIK3CA mutated, HER2 amplified SKVO3, PIK3CA, PTEN and RAF mutated, A2780, TP53, PTEN, KRAS and ARID1A mutated, OVX18, BRCA1 null, UWB1.289 and TP53 mutated OVCAR3 cells) based model. Data show a combined effect of more than one PI3K-AKT-mTOR pathway-specific inhibitor (isoform-specific or pan-PI3K inhibitor or mTOR kinase or allosteric inhibitor) or a combination of PI3K pathway inhibitor plus MEK1/2 inhibitor as determined from the changes in proliferative and apoptotic signals as well as cell cycle arrest.

Conclusion: These preclinical data provide a conceptual framework for using the pathway-specific inhibitor(s) matched with pathway-specific gene altered patients. This strategy warrants further clinical investigation.
Navigating the Genomic Landscape of ER+ve BC in Search of PI3K-Signaling Algorithm: A Rational Combination in Precision Medicine

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Background: The characteristically abundant and diverse genetic alterations in ER+ breast cancers (BC) often affect oncogenic signal transduction pathway and thus determine therapeutic sensitivity to a drug. We have enriched the conventional approach of targeting ER+ve tumor cells with AI in combination with PI3K-pathway specific drugs by the major genetic alterations observed in our Avera patients towards the development of a better treatment rationale based on a signaling algorithm.

Methods: We retrospectively analyzed over 200 consecutive BC patients (neoadj., adj. and met.) admitted to our center from Feb. ’14 through April ’16. The samples were characterized by IHC, FoundationOne, and Theranostics. In cell line based models, a combination of the pan-PI3K pathway inhibitor (i), GDC-0941 or isoform-specific i, GDC-0032 / AZD6482 were tested with AI.

Results: A total of 123 genes were altered in 122 ER+ BC patients. The predominant pathway alterations were found in cell cycle and PI3K-mTOR pathways. Collective alterations in 13 nodes of PI3K-mTOR pathway (PIK3CA, PIK3C2B, PIK3R1, PIK3R2, AKT1, AKT3, PTEN, MDM2, MDM4, TSC2, mTOR, RICTOR, RPTOR) were observed in 102 among 122 ER+BC patients. Alterations in PIK3CA gene (45%) was predominant, followed by alterations in PTEN (13%) and MDM2/4 (11%) genes. The common co-occurrence of alterations being the PIK3CA with the MDM2/4 and PTEN genes. Different types of alterations in PIK3CA, PTEN, and MDM2/4 included deletions, point mutations, frameshifts, exon losses, and amplifications. A combination of the pan-PI3K pathway i, GDC-0941 or isoform-specific i (GDC-0032, AZD6482) along with AI blocked proliferative signals and enhanced apoptosis in ER+/PIK3CA mutated cells in contrast to ER+/PTEN mutated cells. In PIK3CA mutated MCF7 and T47D cells GDC-0941 and GDC-0032 alone or in combination with AI were effective in blocking pAKT while treatment with AI alone was ineffective. In contrast, p110beta isoform specific i AZD6482 was effective in blocking the proliferative signals while GDC-0032 failed to abrogate proliferative signals in PTEN mutated MDA-MB415 cells. These mechanistic results were confirmed by AnnexinV apoptosis, 3D ON-TOP assays, real-time proliferation and qRT-PCR.

Conclusion: A better therapeutic outcome can be designed by merging the (1) in-depth information about genetic alterations from the patient and (2) understanding of the associated cell signaling pathways about tumorigenesis.
EGFR Targeted EDV Delivery of Chemotherapeutics Facilitates Tumour Regression via Stimulation of the Innate Immune System

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Background: Tumour progression involves mechanisms which can push immune cell populations toward a pro-tumour phenotype. To counter this, cancer immunotherapies aim to reprogram the immune system to an antitumour phenotype. The EngeneIC Dream Vector (EDV™), a first in class cytoimmunotherapy for cancer treatment capable of carrying cytotoxic payloads directly to the tumour, is known to elicit an immune response, although the nature of this response is not fully understood. The aim of this study, was to examine the ability of EGFR targeted EDVs loaded with supercytotoxic PNU159682 (EGFREDVPNU15982), a drug which can overcome resistance, to “switch” innate immune cells, namely natural killer (NK) cells and macrophages, towards an antitumour phenotype.

Methods: Multi-drug resistant A549 or T84 xenografts were grown in Balb/c nude mice. EGFREDVPNU15982, EGFREDV, or EGFREDVDox, were administered 3x’s weekly via tail vein injection and 48-72 hours after the final injection, immune cells were isolated from tumour, spleen and blood. Immune cell phenotype and function was examined via flow cytometry and X-CELLigence RTCA.

Results/conclusion: In order to focus solely on innate immune cell populations, nude mice were selected for this study due to their lack of T-cells. EGFREDVPNU15982 treatment resulted in significant tumour regression in both xenografts. Phenotyping of NK cells isolated from EDV treated xenografts showed an increase in phenotypes associated with IFN secretion, proliferation, tumour trafficking, and cytolytic properties. Functional assays demonstrated a significant increase in cell lysis of target cells by these isolated NK cells. Macrophage populations in the spleen and tumour of EGFREDVPNU15982 treated mice exhibited a significant shift to the M1 antitumour phenotype and an increase in tumour infiltration. However, functional assays demonstrated that CD11b+ cells isolated from tumours were only effective in lysing target cells in tumours treated with EGFREDVPNU15982. In mice that stopped responding to treatment, tumour infiltrating NK and macrophage phenotypes returned to that of non EDV treated mice. This study provides evidence that EDV treatment can promote antitumour NK and macrophage phenotypes facilitating tumour regression.