**Basic Science**

Elevating the reactive oxygen species threshold in prostate cancer stem cells using VK3-OCH3 as a strategy to target Prostate cancer

**Background:**

Prostate cancer (PCa) is the second most leading cause of cancer related deaths in American men. According to American Cancer Society, in the year 2020, there will be an estimate of 191,930 new cases and 33,330 deaths from PCa. Although several cancer treatments are available, these have a number of drawbacks and can leave the patient with cardio-toxicity, loss of libido and infertility. Androgen deprivation therapy (ADT) is a standard promising therapy for metastatic PCa patients. However, patients within 18-24 months suffer from relapse and progresses into castration resistant prostate cancer (CRPC). Experimental evidences suggest that, cancer stem cells (CSC) which constitute a sub-population of cancer cells, play a pivotal role in carcinogenesis, metastasis and chemo-resistance. Therefore, targeting CSC can decrease the PCa recurrence rates and can help to offer better treatment options.

**Hypothesis:**

We hypothesize that targeting prostate cancer stem cells (PCSC) with VK3-OCH3 could be an alternative and effective strategy to treat CRPC.

**Study design:**

The anti-cancer effects of VK3-OCH3 were explored in PCSC. Cell viability assay (MTT) was performed to evaluate the Vitamin K analogue and the effect of VK3-OCH3 on metastatic and tumorigenic properties by performing colony formation assay, wound healing and trans-well migration assay. Annexin V – FITC/PI and cell cycle assay were performed to detect apoptosis and cell cycle arrest when treated with VK3-OCH3 using flow cytometry. Furthermore, to evaluate the mechanism of action, real-time PCR and western blot along with confocal immunofluorescence microscopy analysis were carried out.

**Results:**

Our data revealed that VK3-OCH3 was more effective against PCSC compared to other Vitamin K analogues. Subsequently, colony formation assay and Trans-well migration assays indicate that VK3-OCH3 exhibits anti-tumorigenic and anti-metastatic properties against PCSC. Further studies showed that antioxidants such as NAC and GSH antagonized the effects of VK3-OCH3 on PCSC survival, suggesting activation of oxidative stress by VK3-OCH3. Reactive oxygen species (ROS) generation in VK3-OCH3 treated cells were confirmed by microscopic imaging for ROS fluorescent probe (DCFDA) activation. VK3-OCH3 treatment also induced marked elevations in mitochondrial ROS generation in PCSC as determined via FACS analysis which correlated with the significant apoptotic cell death. Furthermore, we tested the effect of VK3-OCH3 on cell cycle progression using FACS and observed a significant G0 cell cycle arrest. By using an oncogene array, it was found that survivin, an apoptotic inhibitor, was down-regulated by VK3-OCH3, which was further confirmed by Western blot analysis. Our subsequent confocal studies showed that, VK3-OCH3 down-regulates the expression levels of EMT marker vimentin. Furthermore, confocal microscopy imaging analysis revealed that VK3-OCH3 induces LC-3 puncta formation indicating autophagy activation. In addition, the western blot results showed the conversion of LC-3I
to LC-3II with concurrent up-regulation in the expression levels of p62 and Atg 16 which confirms the induction of autophagy with increasing doses of VK3-OCH3. Of note, VK3-OCH3 treated PCSCs had a significant reduction in stem cell marker ALDH1 as determined through confocal image analysis.

Conclusion:

Our results suggest that VK3-OCH3 could be developed as a potential ROS targeted anti-CSC strategy in treating PCa.

**Basic Science**

**Evaluation of the potency and efficacy of a human lymphatic filariasis vaccine, rBmHAXT (LFGuardTM)**

**Background:**

Lymphatic filariasis (LF) is a neglected tropical disease caused by Wuchereria bancrofti, Brugia malayi, and Brugia timori affecting around 120 million people around the world. LF is transmitted when mosquitoes, having had a blood meal from an infected individual, take a subsequent blood meal from a healthy subject, transmitting infective larvae into their bloodstream. The clinical manifestations of LF include damage and obstruction of the lymphatic vessels resulting in a severe local inflammation and irreversible disfiguration. Currently there is no vaccine available for LF. Studies in our laboratory have demonstrated that the multivalent recombinant BmHAXT protein, comprising four B. malayi antigens (HSP12.6, ALT-2, TPX-2, and TSP) is a promising vaccine candidate for LF, conferring up to 88% and 57% protection against challenge LF infections in mice and primates respectively.

**Hypothesis/Aims:**

For the transition of the vaccine candidate to human clinical trials, the histidine tag bound to the protein, which aids in its purification, needs to be removed. In the present study, we aim to assess the potency and efficacy of a GLP manufactured tag-free multivalent fusion protein vaccine, rBmHAXT (LFGuardTM) for human LF in outbred Mongolian gerbils and CD1 mice and inbred Balb/c mice. We hypothesize, that the tag-free recombinant vaccine will demonstrate no significant difference in its safety, potency and efficacy when compared to the his-tagged recombinant vaccine used in previous studies.

**Methods/Study Design:**

For the potency testing, gerbils and Balb/c mice were immunized with a low dose (5 ug), medium dose (15 ug), and high dose (40 ug) of the vaccine plus alum adsorbed GLA (AL019) as adjuvant given subcutaneously four times at monthly interval. The CD1 mice were immunized s.c. four times with a range of doses from 0.5 to 40 ug/dose.

**Results:**

For the gerbils, 40 ug dose resulted in the maximum titer of antigen-specific IgG antibodies (1:40,000). However, in CD1 mice, 15ug was sufficient to give the significant titer (1:20,000) (p<0.05). All vaccinated mice had significantly (p<0.05) high levels of antigen specific IgG1, IgG2a, IgG2b and IgG3 antibodies compared to control animals. Two weeks after the last dose of the vaccine, all mice were challenged with 10-15 infective third
stage larvae of Brugia malayi using a micropore chamber method. Our results showed that the rBmHAXT vaccine conferred 78.88% (±9.79) protection in CD1 mice and 91.30% (±6.81) protection in Balb/c mice which was significantly (p<0.05) higher than the control animals for which it conferred 15.1% (±5.43) and 4.0% (±8.94) for CD1 and Balb/c mice respectively. Correlates of vaccine-induced protection showed an increase in the percentages of antigen-specific memory T cells in the spleen of vaccinated mice.

Conclusions/Implications:

Our results show that the tag-free vaccine candidate is able to confer a significant amount of protection against LF challenge infections. Potency studies suggest that the dose required to confer an optimal amount of protection for CD1 animals is 15ug of the protein, while the optimal dose for Mongolian gerbils and Balb/c mice is 40ug. These findings suggest that LFGuardTM could be developed as the first human LF vaccine.
Case Report

Plant Protein Diet and Kidney Function: Can lifestyle change prevent the need for dialysis?

In Rockford, there are five Dialysis centers independent of the hospital dialysis facilities. According to the NIH, 750,000 patients per year are affected by end stage renal disease (ESRD). In 2018 there were over 100,000 patients on the kidney transplant waiting list, but only 21,000 donor organs available. Medicare spent $35 billion on ESRD in 2016. Patients living with ESRD make up 1% of the Medicare populations but account for 7% of the Medicare Budget. Patients on dialysis have a 20-25% mortality rate after one year.

Clinical Issue/Case importance

ESRD is highly prevalent, expensive and current dialysis treatment has a high mortality rate. This case illustrates introducing a plant protein diet has potential to prevent progression or reverse chronic renal disease.

Hypothesis/Aims

Changing to a plant protein diet improved the renal function of a patient with Chronic Kidney Disease on the dialysis waiting list.

Methods/Study Design

A 70 year-old female with hypertension, diabetes, coronary artery disease and pre-dialysis progressive renal failure was referred to a therapeutic lifestyle change program by her physician. During her participation in the Complete Health Improvement Program (CHIP) her cholesterol, renal function, weight, blood pressure and medications were recorded from September 2009-June 2012. Medical records from primary care and specialty care were reviewed. Qualitative information about the patient was obtained by a live interview at 4 years and 10 years. Quantitative data from the medical record was used to determine clinical outcome, medication use and laboratory values.

Results

Blood urea nitrogen decreased from 52 mg/dL to 22 mg/dL and creatinine decreased from 2.3 mg/dL to 1.4 mg/dL from September 2009-June 2012. Glomerular filtration rate improved, from 23mL/min to 42 mL/min. The patient was removed from the dialysis waiting list. The patient experienced a 57pound weight loss. Blood pressure changed from 162/64 mmHg to 112/70mmHg. Retinal laser treatments decreased from 4 a year to none. Insulin, hydrochlorothiazide and nifedipine were discontinued. These outcomes have remained stable over a ten-year period.

Conclusion/Implications

Therapeutic lifestyle change programs emphasizing a plant protein diet can be used to improve renal function in patients with pre-clinical and chronic progressive kidney disease. The side effect of her therapeutic lifestyle change was a simultaneous improvement in her other chronic medical conditions. This case study supports the need for randomized control trials to demonstrate the role a plant protein diet could play in prevention and treatment of pre-dialysis and late stage kidney disease. This treatment may decrease the need for dialysis, resulting in improved patient care, quality of life and lower medical cost.
Clinical
Non-opioid utilization reduces opioid requirements for outpatient laparoscopic procedures: a retrospective chart review.

Background
As one aspect of a multi-faceted response to the Opioid Epidemic, efforts in healthcare have been aimed at reducing the amount of opioids prescribed by providers. These changes are being made to reduce the adverse effects of opioids for the individual patient and decrease excess opioids in the community. We plan to demonstrate the efficacy of a non-opioid pain management protocol for minimally invasive outpatient surgery in order to promote further change in opioid prescribing.

Methods
We carried out a retrospective chart review of 234 patients undergoing outpatient laparoscopic inguinal hernia repair and laparoscopic cholecystectomy whose pain was managed with either opioid or non-opioid pain management protocol. These cases were analyzed for follow-up pain ratings, requests for refills of pain medication, and readmissions. Readmissions were further analyzed for cause and determined to be related, unrelated, or possibly related to the surgery performed or pain management protocol used.

Results
Our patient population was mainly middle-aged, Caucasian, overweight adults in rural Northwest Illinois. A total of 234 cases were reviewed for post-op pain ratings, requests for refills at follow-up, and readmission after 1- and 6-months. 167 cases were managed with opioids while 67 were managed with the non-opioid pain management protocol. No significant difference was found in the post-operative pain scales reported by patients for either protocol (opioid mean=1.09, SD=1.98; non-opioid mean=1.18, SD=2.30). There was no increase in requests for pain medication refills when managed with non-opioids compared to opioids (non-opioid=2.99%, opioid=4.19%). There was, however, an increased correlation of readmission when patients were managed with opioids (1-month=14.97%, 6-month=29.94%) compared to non-opioids (1-month=8.96%, 6-month=22.39%). Furthermore, there was an increased incidence of readmission determined to be related to the surgery or pain management protocol used when patients were managed with opioids for pain. This was true for confirmed related readmissions at 1-and 6-months (OR=2.07, 95% CI= 0.44 to 9.79) as well as for possibly related and related readmissions at 1-month (OR=2.74, 95% CI= 0.78 to 9.58) and 6-months (OR=2.27, 95% CI= 0.75 to 6.87).

Conclusions/Implications
Our results indicate that non-opioid pain management for these elective laparoscopic procedures adequately manages patients’ pain while reducing the incidence of readmission. There was no difference in the post-operative pain ratings or requests for pain medication when either protocol was used. This means that patients who were managed with non-opioids did not return complaining of uncontrolled pain or pain that required more medication. In addition, patients managed without opioids were less likely to present to the emergency department overall and were less likely to be readmitted for reasons related to their surgery or medications. Although these results were not found to be statistically significant (most likely due to the small sample size in a
rural town), they demonstrate that there was no increased need for medication or increased occurrence of readmission to the hospital when the pain management protocol was only non-opioid management. These results should be used to give providers the confidence to change their post-operative pain regimens for elective laparoscopic procedures and should prompt further research into more invasive procedures.
Community

The relationship between Eviction and Health Outcomes in Rockford IL

Background

Eviction generates housing instability and fosters the cyclic nature of poverty. While some socioeconomic disparities in the U.S. gain consistent media coverage, there is another widening disparity quietly affecting impoverished Americans, eviction. There were 2.3 million eviction filings in the U.S. in 2016 (a rate of four every minute). Rockford IL had the highest eviction rate of all large cities in IL in 2015 at 4.97% with 1,287 evictions or an average of 3.53 evictions per day. The eviction rate for Rockford in 2015 was dramatically higher than Illinois’ eviction rate of 1.72% in 2015 (77.36 evictions per day) and was 2.59% higher than the U.S. average for that year. Due to lack of data, there has been minimal research on the relationship between eviction, as an additional socioeconomic factor, and health outcomes.

Hypothesis/Aims

Our aim is to show additional socioeconomic disparities that may be contributing to poor health outcomes that have not been previously addressed or identified. Stable housing is a basic necessity that every individual deserves access to, however housing is not always accessible. Results will help provide information and data for strengthening efforts to aid individuals who are lacking in stable housing.

Study Design

Descriptive analyses will be used to examine eviction rates and five measures of health outcomes in 53 Rockford census tracts in 2015. Due to the small sample size, 53 census tracts in Rockford, we cannot draw any solid conclusions from statistical analyses focusing on Rockford alone. Therefore, our goal is to describe the disparities in the relationship between eviction rate and our health outcomes in areas of Rockford that are more adversely affected by evictions versus those that are not. We used data from the Eviction Lab, the Centers for Disease Control and Preventions’ 500 Cities Project and the U.S. Census Bureau. Healthcare measures from the 500 Cities Project included 1) lack of access to healthcare and 2) annual physical checkups as measures of healthcare utilization; 3) diabetes and 4) hypertension as measures of chronic disease; and 5) obesity as a measure of unhealthy behavior. The data from Eviction Lab is described as rates per 100 and the data from 500 Cities Project is described as prevalence, each for the 2015 year and per census tract. This is a preliminary analysis before these relationships will be examined for 1238 census tracts in urban Illinois, which will give us a large enough sample to draw conclusions from more advanced statistical analyses.

Results

Plotting eviction rates for 53 Rockford census tracts showed that in 2015 most census tracts with higher eviction rates experience a higher prevalence of hypertension, diabetes, and obesity. Census tracts with higher eviction rates tended to have a lower prevalence of annual checkups with physicians and a higher prevalence of lack of access to healthcare. Further analyses also showed that census tracts that experienced higher rates of eviction also experienced higher rates of ‘rent burden’ defined as the percent of household income that is used for rent. Our data also showed that while some census tracts have a household income of around $20,000 for 2015,
these census tracts were paying as much as or above the average rent price in Rockford while also experiencing higher eviction rates and more adverse health outcomes.

Conclusions/Implication

Our results outline a stark disparity in Rockford that could be contributing to adverse health outcomes. Some census tracts in Rockford that experience disproportionately higher eviction rates and rent burden also suffer from a higher prevalence of adverse health outcomes. Low socioeconomic status has already been linked to higher rates of adverse health outcomes. But how are individuals supposed to manage chronic health problems without a home? Lack of consistent refrigeration, cooking necessities or even electricity, as provided by stable housing, could easily be contributing to or worsening the health outcomes in these impoverished census tracts. Stable housing shouldn't be a privilege, it is a basic necessity that is not provided to some members of our society.

Community

Using the Rural Active Living Assessment Tools to Support a Rural Wellness Program

Introduction

The Enhanced-Win With Wellness program is a three year community-based intervention aiming to reduce chronic disease risk through supporting healthy lifestyles Stephenson and Carroll counties. Research shows that rural communities are at a higher risk for chronic disease because of the physical environment, policies, and poor programming. The purpose of this part of the program was to use the Rural Active Living Assessment (RALA) to identify ways to promote an environment conducive to healthy living.

Methods

Assessments were performed using the Town-Wide Assessment (TWA) and Program & Policy Assessment (PPA) components of the RALA. Three team members conducted the assessments and used the TWA to assess 18 characteristics of each community scoring them from 0-100. The PPA assesses 11 town level policy and program items that support physical activity and scores each town on a scale from 0-100.

Results

The TWA scores ranged from 69-96 (mean - 82.25). Most of the amenities were in good/excellent condition. Three of the four towns had limited recreational opportunities during the winter. It was difficult identifying some amenities because online resources lacked information.

The PPA scores ranged from 47-70 (mean - 59.75). None of the towns had a walk to school program and only two had policies requiring new projects to include sidewalks. Except for one town, there was very little physical activity programming and limited public transportation.
Conclusion

The TWA and PPA are easy to use tools which can be used to identify gaps in recreational opportunities and help develop policies or programs supporting physical activity. Future steps include locally disseminating this information, supporting polices promoting physical activity, and sharing this information with E-WWW participants.