Effectiveness of Reducing Home VOC Measurements using One Inch Carbon Furnace Filters
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Exposure to high levels of carcinogens may lead to kidney, liver, or central nervous system damage. Volatile Organic Compounds (VOC’s) such as Acrolein, Carbon Tetrachloride, Benzene, and Chloroform are carcinogenic at high levels.

- Benzene is in paints and industrial solvents.
- Acrolein is in gasoline and cooking oils.
- Chloroform is in tap water, swimming pools, and drinking water.
- Carbon Tetrachloride is in aerosol propellants, dry cleaning, varnish, lacquer, plastic glue, plastic bonders.

The hypothesis was supported only by findings from the home of Participant A for Total VOC ppb, Chloroform ppb from the home of Participant B, and Acrolein from the home of both participants. Findings from these two case studies do not support the use of 1” carbon filters to consistently reduce carcinogenic VOCs. Future studies should investigate the use of 4” carbon filters and carbon air filtration systems in reducing VOCs in homes. Future studies should increase the number of participants and extend the evaluation time to at least 1 month.

Introduction

Objective

This study used one inch carbon furnace filters to determine if there is a difference between baseline and postliminary carcinogenic VOC readings in homes of older adults with asthma.

Preliminary Data

In an ongoing study examining the homes of older adults with asthma for asthma triggers, eighty-five VOC’s including Carbon Tetrachloride, Benzene, Chloroform, and Acrolein were measured over 24 hours.

Methods

- A case study approach was used with 2 participants in the asthma study volunteered their home for VOC data collection using 1” carbon furnace filters.
- A One Inch Filtrete Allergen Plus Odor Reduction Air and Furnace Filter that contains activated carbon was carefully placed in the furnace of each home.
- Activated carbon removes impurities in the air from unwanted chemicals, including VOCs.
- Grab samples were obtained at 8-day and 15-day period using the Summa Canisters (See picture).
- VOC samples were analyzed by gas chromatography/mass spectrometry in full scan mode according to US EPA method TO-15, using a quadruple GC (HP 6890) with a HP 5973 Mass Selective Detector.
- Data were graphically depicted to determine changes in ppb of total VOCs, Acrolein, Carbon Tetrachloride, Benzene, and Chloroform from baseline to postliminary data.

Conclusions

- The hypothesis was supported only by findings from the home of Participant A for Total VOC ppb, Chloroform ppb from the home of Participant B, and Acrolein from the home of both participants.
- Findings from these two case studies do not support the use of 1” carbon filters to consistently reduce carcinogenic VOCs.
- Future studies should investigate the use of 4” carbon filters and carbon air filtration systems in reducing VOCs in homes.
- Future studies should increase the number of participants and extend the evaluation time to at least 1 month.

Acknowledgements

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Healthy Lifestyle Impact on Breast Cancer-Specific and All-Cause Mortality

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Introduction

Despite recent advancements in early detection and treatment, breast cancer still remains the second leading cause of death for women in the U.S. [1]. Individual lifestyle factors have long been associated with cancer mortality, with national organizations like the American Cancer Society (ACS) going so far as to issue cancer prevention recommendations for many modifiable factors, including dietary and physical activity, based on data from the Nurses’ Health Study and diet [2]. Several studies have evaluated the combined impact of these factors on cancer mortality through the creation of a Healthy Lifestyle Index (HLI), however associations between the index and mortality among cancer survivors have been inconsistent [3-6]. Few studies have attempted to use the HLI to evaluate the impact of these factors on mortality in minority cancer populations. Hispanic (H) women have a higher risk of breast cancer-specific mortality than Non-Hispanic White (NHW) women, making this U.S. population one of particular interest [7].

The primary objective of this study was to evaluate the combined effect of healthy lifestyle factors on breast cancer-specific and all-cause mortality in NHW and H women included in the New Mexico site of the 4-Corners Women’s Health Study through the construction of a HLI. Based on the findings of past studies, we hypothesized the HLI would be associated with breast cancer-specific and all-cause mortality in both ethnic groups [3-6].

Methods

New Mexico Site of 4-Corners Women’s Health Study (1993-2003)

Data Collection

• To gain a better understanding of the etiology of breast cancer in Hispanic and NHW women
• Self-reported Hispanic, American, Indian, or NHW ethnicity
• Native American (TA) or Canadian
• 25-79 years of age
• Histologically confirmed first primary in situ or invasive breast cancer diagnosis
• Breast cancer diagnosis between October 1993 and May 2004
• Initial ascertainment from state registries (SEER) with subsequent screenings to confirm breast cancer
• Comprehensive diet and lifestyle data for the year prior to diagnosis collected by HBI components via structured computerized questionnaires
• Weight, height, and waist circumference measured at time of interview
• Blood/serum collected at time of interview

Statistical Analysis (using SAS Version 9.4; Cary, NC)

The dataset was restricted to cases with regional or distant cancer for the analytic sample (n=87), excluding outliers (n=4) and in situ cases (n=151). Descriptive statistics for demographic and prognostic variables were calculated and compared by HBI quartiles; differences were assessed using chi-square test. Cox proportional hazards multivariable modeling was used to estimate hazard ratios (HR) and 95% confidence intervals (95% CI) for HBI quartiles, adjusting for stage at diagnosis and education. Effect modification was evaluated by self-reported ethnicity and stage at diagnosis.

A Kaplan-Meier curve was constructed over time by HBI quartiles and a log-rank p-value was used to determine differences in survival time.

Table 1: Descriptive statistics for demographic and prognostic variables by healthy behavior index quartiles

Variable | Definition | Description | NHW (n=106) | H (n=74)
--- | --- | --- | --- | ---
Mortality | Deceased any cause | Outcome | 97 (92.4) | 69 (93.2)
Breast-cancer specific mortality | | | 71 (67.0) | 51 (68.9)
ICD-0-2 | C05-C09 | | 26 (24.5) | 18 (24.3)
ISC | | | 0=Never | 2=Current
Smoking | | | 7 (6.6) | 5 (6.8)
Body mass index (kg/m²) | | | 1=Normal, <25 | 2=Overweight, ≥25
| | | 21.4 (5.4) | 23.0 (4.6)
Hip to waist ratio (inches) | | | 1=Overweight, ≥25 | 2=Obese, ≥28
| | | 0.80 (0.17) | 0.85 (0.14)
Weight to hip ratio (inches) | | | 1=Normal, <25 | 2=Overweight, ≥25
| | | 67.0 (9.8) | 68.6 (9.7)
Physical activity index | | | 1=Low | 2=High
| | | 19.0 (5.1) | 20.1 (4.3)
Menopausal status | | | 0=Premenopausal | 1=Postmenopausal
| | | 42 (40.0) | 27 (36.5)
Smoking Status | | | 1=Former | 2=Current
| | | 28 (26.4) | 18 (24.3)
Total cholesterol (mg/dl) | | | 400-599 | 600-799
| | | 172 (16.1) | 173 (23.0)
HBI: 0-3 | 0.775 | 0.0001 | 0.0002 | 0.0000
HBI: 4-6 | 0.845 | 0.0001 | 0.0002 | 0.0000
HBI: 7-9 | 0.879 | 0.0001 | 0.0002 | 0.0000
HBI: ≥10 | 0.953 | 0.0002 | 0.0002 | 0.0000

Table 2: Associations between healthy behavior index and breast cancer-specific and all-cause mortality, stratified by race

All Women | NHW | H | Overall (N=180)
--- | --- | --- | ---
Healthcare Index | Adjusted HR | 95% CI | Adjusted HR | 95% CI | Adjusted HR | 95% CI
--- | --- | --- | --- | --- | --- | ---
HBI: 0-3 | 3.02 | 1.48-6.13 | 1.40 | 0.64-3.10 | 2.31 | 1.13-4.73
HBI: 4-6 | 4.07 | 2.02-8.21 | 1.80 | 0.74-4.36 | 3.19 | 1.67-6.11
HBI: 7-9 | 5.54 | 2.63-11.67 | 2.49 | 1.09-5.67 | 4.31 | 2.07-8.95

Conclusions

• Survival rates were lowest for HBI Q4 for breast cancer survival and overall survival (77% and 53%, respectively) and significantly differed from other HBI quartiles for overall survival (log-rank p=0.0005).
• An increased risk of breast cancer-specific mortality for HBI Q4 compared to Q1 was present, but was not statistically significant after adjusting for stage at diagnosis and education. A significant trend was seen across HBI quartiles. 
• A significant increase across HBI quartiles was observed among all women, NHW women, and those diagnosed with localized or regional/distant stage of disease for AC mortality.
• An increasing number of unhealthy lifestyle factors influences AC mortality among breast cancer survivors, which can primarily be attributed to cardiovascular and pulmonary diseases.
• Interventions for breast cancer survivors should address the combination of lifestyle factors and their effect on prognosis, recurrence, and second primaries.
Investigating HGPRT as a Component of an AS1411 Prodrug Mechanism
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Abstract
AS1411 is a guanine-rich oligonucleotide with antiproliferative activity in numerous cancer cell lines. It has previously been tested in human clinical trials and has induced dramatic clinical responses in a few patients.1,2 AS1411 acts as an aptamer that binds to nucleolin, a protein expressed selectively on the surface of cancer cells, but its precise mechanism of action is not yet fully understood.1 Recent literature has suggested that degradation of AS1411 to its constituent nucleotides may play a role. In particular, guanine-based purine compounds (GBPcs) are capable of significantly inhibiting cancerous growth in vivo,3,4 suggesting that AS1411 may function as a "prodrug" for guanine. It has been shown previously that the antiproliferative effects of GBPcs are dependent on the activity of hypoxanthine-guanine phosphoribosyltransferase (HGPRT), an enzyme involved in the purine salvage pathway.4 We reasoned that if AS1411 is acting as a prodrug for guanine then its activity should also depend on HGPRT. To test this possibility, AS49 lung cancer cells were left untreated, transfected with HGPRT siRNA, or transfected with a negative control siRNA prior to treatment with either AS1411, CRO (a cytosine-rich guanine, 6-methylguanine, and thymine; vehicle control) in concentrations of either 5 or 10 μM as indicated in the figure legends. AS49 and CRO in the detailed form were purchased from Integrated DNA Technologies (Coralville, IA). Cells transfected with 48 or 24 hours before being subjected to a MTT colorimetric assay to evaluate levels of proliferation.

Methods

Cell Culture & HGPRT siRNA Knockdown
AS49 cells were cultured in DMEM containing 10% FBS and 1% Penicillin/Streptomycin. Cells were grown to ~70% confluence and plated onto 96 well plates with 1000 cells per well. Cells were allowed to adhere for 24 hours and transferred to antibiotic-free media. Transfection was performed with Lipofectamine 2000 (Fisher Scientific) as described in the next section. Cells were then either untreated, transfected with HGPRT siRNA (siHGPRT #8, siRNA Technologies), or transfected with Negative Control #1 siRNA. After 4 hours media was replaced with complete media. Cells were then incubated for 24 or 48 hours as indicated by figure legends. After transfection, cells were treated with either AS1411, CRO (cytosine-rich negative control oligonucleotide), or sterile water (vehicle control) in concentrations of either 5 or 10 μM as indicated in the figure legends. AS49 and CRO controls were maintained in the detailed form were purchased from Integrated DNA Technologies (Coralville, IA). Cells transfected for 48 hours before being subjected to a MTT colorimetric assay to evaluate levels of proliferation.

BACA Protein Analysis and Western Blot Development Protocol
Cells lyses were prepared on ice with RIPA buffer containing protease and phosphatase inhibitors (Calbiochem, San Diego, CA) for 5 min at 4°C and clarified by centrifugation for 10 min at 14,000 rpm at 4°C. Protein concentrations were determined using the Pierce™ BCA Protein assay (Fisher Scientific, Waltham, MA) with bovine serum albumin (BSA) standards (Fisher Scientific). Samples for electrophoresis were prepared with 25 μg of protein, 4x loading buffer with 10% β-mercaptoethanol, and distilled water.

Sample were resolved by SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Fisher Scientific) in Tris-glycine transfer buffer (Life Technologies, Grand Island, NY) containing 20% methanol. Membranes were either blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.5% tween-20 (TBS-T) for 1 hour. The following dilutions were used for primary antibodies: HGPRT 1:500 and α-Tubulin 1:1000. Membranes probed for HGPRT were detected using SuperSignal® West Dura ECL (Fisher Scientific) while α-Tubulin was detected using Peroxidase ECL Western Blotting Substrate (Fisher Scientific). Chemiluminescence was visualized using Amersham Hyperfilm™ ECL Healthcare, Little Chalfont, UK) and exposure times are noted in the figure legends.

Results & Summary of Findings

Figure 1. Demonstration of HGPRT siRNA Knockdown at 48 Hours

Figure 2. Results of Initial HGPRT siRNA MTT Colorimetric Assay

Figure 3. Results of MTT Colorimetric Assay with Doubled Concentration of siRNAs

Figure 4. Exploration of HGPRT siRNA Knockdown Longevity

Figure 5. Results of MTT colorimetric assay with transfection time reduced from 48 to 24 hours

Summary of Findings:
• We successfully used two different siRNAs to knockdown the expression of HGPRT relative to negative control siRNAs.
• HGPRT silencing did not exhibit strong toxic effects on control groups as shown in proliferation assays.
• Concentrations of HGPRT siRNAs utilized were sufficient to induce silencing from 24 to 120 hours post-transfection.
• Despite repeated protocol augmentation, HGPRT silencing did not exhibit any significant effects on AS49's anti-proliferative activity.
• The data suggests that AS4111 effects are not dependent on HGPRT activity.

Future Directions & Acknowledgements
Future Directions:
Although these experiments suggest that AS4111 is not dependent upon HGPRT activity in this cell line under a variety of conditions, more experiments are necessary for
• Confirm that knockdown of HGPRT reduces the antiproliferative activity of GBPcs
• Test in different cell lines, including those that are more sensitive to AS49 (AS49 cells are only moderately sensitive).

Acknowledgements:
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References
Transport and Distribution of Stealth and Cell Penetrating Nanoparticles in Cervical Cancer Tissue Mimics

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Introduction

Background: Cervical cancer is highly prevalent in developing countries, due to insufficient access to health care. Inadequate screening combined with a lack of vaccines often leads to undetected tumors and elevated mortality rates. Relative to preventative options, cervical cancer treatments are often invasive and painful procedures that include surgery, chemotherapy, and radiation. For systemic chemotherapy in particular, it is challenging to achieve distribution within the tumor, thereby harming normal nonmalignant cells in the process. As an alternative, polymeric nanoparticles (NPs) may be used as drug and gene delivery vehicles to target and/or enhance the distribution of therapeutic agents in cervical cancer tumors. However, currently there is a lack of in vitro methods available to measure and predict therapeutic distribution. To date, in vivo studies are the primary method of evaluating distribution; but require limited patient samples and expensive animal models. To circumvent this challenge, three-dimensional (3D) cell culture models can be utilized to create a more physiologically relevant in vitro system to assess and predict NP distribution. Objective: In this study, our goal was to evaluate the penetration and distribution of stealth and cell penetrating NPs through three types of 3D tumor models: liquid overlay spheroids, hanging drop spheroids, and multicellular layers (MCLs). We used these 3D models of different cervical cancer cell lines (HeLa, CaSki, and SiHa) to represent different stages of cancer progression: nascent tumors, mid-stage anocutaneous tumors, and stratified epithelial layered tumors. Hypothesis: Based on previous studies performed with HeLa cells, we hypothesized that NP co-treatment would offer the greatest penetration and distribution within the tumors, relative to unmodified NPs. Methods: To test our hypothesis, we utilized confocal microscopy to image the 3D tumors, and analyzed the images with ImageJ software to evaluate NP distribution within the different tumor types and cell lines. Results: We found that MPG and MPG-PEG co-treatment NPs often offered the greatest distribution within the 3D tumor models relative to unmodified NPs. However, NP distribution in the tumors varied based on cell and tumor types, due to their differing sizes and morphologies. Conclusions: NP co-treatments offer a promising method to enhance delivery to, and the treatment of cervical cancer. However, tumor composition and morphology must be considered in the early stages of therapeutic screening and development to establish the best treatment type.

Methods

(1) Nanoparticle (NP) Synthesis
(2) Liquid Overlay Spheroid
(3) Hanging Drop Spheroid
(4) Multicellular Layer (MCL)
(5) 3D Tumor Nanoparticle Distribution Analysis

(1) HeLa Liquid Overlay Spheroid
(2) CaSki Liquid Overlay Spheroid
(3) SiHa Liquid Overlay Spheroid
(4) HeLa Hanging Drop Spheroid

Results: Spheroid Cross-Sections and Composite Images

Figure 3. Cross-Sections of Liquid overlay spheroids treated with NPs. Imaged using confocal microscopy.

Figure 4. Composite images of Liquid overlay spheroids treated with NPs. Imaged using confocal microscopy.

Figure 5. Cross-Sections of Hanging drop spheroids treated with NPs. Imaged using confocal microscopy.

Figure 6. Composite images of Hanging drop spheroids treated with NPs. Imaged via confocal microscopy.

Figure 7. Composite images of Multicellular layers. Imaged via confocal microscopy.

*Note: CaSki and SiHa cells did not form viable multicellular layers.

Results: MCL Composite Images

Figure 8. Quantified distribution and penetration of MPG-PEG & Unmodified NPs in: (1) HeLa, (2) CaSki, and (3) SiHa liquid overlay and hanging drop spheroids.

Results: Quantified Distribution and Penetration of NPs

Conclusions & Future Directions

> Between different cell types, NPs in CaSki tumors typically penetrated and distributed less than those in HeLa and SiHa tumors.
> CaSki tumors were smaller and more compact, making NP penetration difficult; whereas, SiHa and HeLa tumors formed more leaky interstitial space, allowing greater distribution of the NPs.
> Hanging drop spheroids had a greater amount of NP distribution and penetration, most likely due to the small size of the tumor models.
> Overall, MPG & PEG NP co-treatment (1/2 dose of each individually administered) demonstrated enhanced distribution and penetration in the 3D tumor models relative to unmodified NPs. *Note: In the CaSki liquid overlay spheroids, unmodified NPs had greater distribution than then MPG & PEG NP co-treatment.
> Since we observed a variation in NP penetration and distribution based on cell line/3D tumor type, tumor composition and morphology are important to consider when evaluating treatment options.
> We are currently testing efficacy with chemotherapeutic nanoparticles in spheroids.
> We are in the process of revising our MCL growth protocol. However, HeLa cells are the primary candidate to move forward in MCL experiments. Once complete, we will test MCL tumor models with MPG, PEG NPs, and MPG & PEG NPs.
> We are currently assessing NP distribution in HeLa MCLs.
> In the future, we plan to evaluate NP distribution and efficacy in in vivo cervical cancer models.

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Identification of Aberrant Wnt/β-catenin Signaling on Cancer Stem Cell Activation in Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the most common type of liver cancer that, when diagnosed at an advanced stage, has a 5-year survival rate of less than 12%. Alarmingly, the recurrence rate of HCC after curative and palliative treatment is 70% in the first 14 months. The most common causes of HCC include HBV, HCV, diabetes, fatty liver disease, and alcoholism. In fact, 18% of patients presenting with cirrhosis in the United States progress to HCC every year. Only 20% of patients qualify for curative treatment (tumor resection) as it is the only treatment option for very early-stage HCC that is typically asymptomatic. Treatment options for intermediate and advanced stage HCC include chemotherapy with Doxorubicin and Sorafenib. Cancer Stem Cells (CSCs) are a subpopulation in the tumor mass. Accumulating evidence suggests that the CSC subpopulation can initiate cancer and drive tumor recurrence, drug resistance, and metastasis. Here, we discuss a fundamental question that is not yet completely understood. The Wnt/β-catenin pathway is a cardinal pathway contributing to stem-cell organogenesis during embryonic development. Multiple studies have identified dysregulation of the Wnt/β-catenin signaling components in epithelial tumors such as HCC. Understanding CSC activation demands attention from the cancer community to identify therapeutic targets for clinical patients and improve patient outcome.

Introduction

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Aim

Hypothesis: We hypothesize that “β-catenin regulates CSCs activity in HCC via the canonical Wnt/β-catenin pathway.” We will test this hypothesis with the following experiments:

1. Stabilizing β-catenin in the canonical Wnt pathway will increase oncogenic Wnt downstream products and subsequently will increase cancer stem cell properties in HCC cells.

2. Inhibiting Wnt/β-catenin signaling will decrease oncogenic Wnt downstream products and subsequently will decrease cancer stem cell properties in HCC cells.

Working Hypothesis

Method

Ligand (LiCl): Phosphorylates Serine-32, 33, and 37 of β-catenin

LiCl treatment: LiCl phosphorylates Serine-9 position of GSK3β and inactivates it stabilizing β-catenin.

LiCl and XAV939 treatment: XAV939 stabilizes Axin1 and destabilizes the complex to inhibit β-catenin.

LiCl treatment in Control and Spheroid-Free Hepa1-6 cells

• Inducing spheroid formation (CSC property) by spheroid medium increases β-catenin accumulation and its downstream targets expression.

• LiCl treatment stabilizes β-catenin expression.

• Increases β-catenin expression and downstream targets compared to control and XAV939 treatments in both the control and spheroid culture cells.

XAV939 stabilizes the destruction complex in the HCC cells.

• Decreases β-catenin and downstream targets expression compared to control and XAV939 treatments in both control and spheroid culture cells.

• XAV939 and LiCl treatments in both control and spheroid culture cells.

Figure 1: Optimization of LiCl dose. MG132 inhibits proteasomal degradation and stabilizes β-catenin. (A) MG132 induces spheroid formation in a dose dependent manner. Images taken at 10X magnification. Black arrows indicate spheroids. Bar = 200 μM. (B) Hep3B cells showed a dose dependent increase in β-catenin levels up to 2.5 μM. (C) Hepa1-6 cells showed dose dependent increase in β-catenin levels.

Figure 2: Optimization of LiCl dose. LiCl inhibits GSK3β and stabilizes β-catenin. Western blot analysis showed, (A) dose dependent increase in β-catenin expression in Hepa1-6 cell line, and (B) dose dependent increase in β-catenin expression in Hepa1-6 cell line. 5 μM is the lowest dose effective in both cell lines.

Figure 3: Canonical Wnt Inhibitors affect β-catenin levels in spheroid forming CSC cells. (A) Western blot data showed increase in β-catenin and downstream targets expression for 5 μM LiCl treated cells in both control and spheroid culture cells. Spheroid culture cells showed increased β-catenin and downstream targets expression relative to control cells. 2 μM XAV939 treated caused decreased β-catenin in spheroid culture but not in control. (B) β-catenin expression was semi-quantified and averaged across three replications and then normalized to both control and GAPDH expression. (C) Downstream targets ABCG2, c-MYC, and Cyclin D1 were semi-quantified and normalized.

Results

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Conclusion

• Inhibiting proteasome degradation in the Canonical Wnt/β-catenin pathway induces an increase in β-catenin expression and spheroid formation characteristic of cancer stem cells in Hep3B and Hepa1-6.

• Inhibiting GSK3β, a key component of the Canonical Wnt/β-catenin pathway, induces an increase in both β-catenin expression as well as an increase in downstream targets of the Wnt/β-catenin pathway that are oncogenic properties of cancer stem cells in the Hep3B cell line.

• The unexpected results with respect to the XAV939 treatment in control cells can be attributed to either (1) a lack of optimized treatment dose and time or (2) alternate pathways associated with β-catenin.

References


Combining natural compound β-glucan with immune checkpoint inhibitor therapy to promote antitumor immunity

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Abstract

Although immune checkpoint inhibitors are a promising approach for facilitating antitumor responses, anti-PD-1 is clinically effective in a limited fraction of advanced lung cancer patients. Therefore, we examined whether β-glucan treatment could act as a potential supplementary therapy to anti-PD-1 immunotherapy, expanding the applicable patient population. The goal of our investigation was to determine whether whole glucan particle (WGP) treatment coupled with PD-1 inhibition would result in an enhanced antitumor response compared to either treatment alone. Our in vitro data showed that there was greater proliferation of CD8+ T-cells in the Combination Group than in single treatment or PBS Group. In the in vivo data showed that anti-PD-1 and WGP Groups resulted in reduced tumor burden. Both IFN-γ producing CD4+ and CD8+ T-cells were elevated in the single treatment groups in the tumor draining lymph node tissue. However, the Combination Group did not result in synergistic antitumor effect within the treatment period. Further studies are underway to examine whether survival benefits will be provided by WGP and anti-PD-1 combination therapy.

Methods

In vitro: M2 BMM and OT-1 spleen cell functional assay

In vivo: 3-week treatment of tumor-bearing mice

Results

Figure 1. OT-1 T-cell proliferation in co-culture with M2 BMM.

Figure 2. Tumor diameters of mice treated with PBS, WGP, anti-PD-1, or combination regimens.

Table 1. Comparison of tumor diameters among different treatment groups.

Table 2. Summary of frequency of Treg and IFN-γ producing CD4+ and CD8+ T-cells.

Conclusions

Future Directions

References

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The Impact of Complex Interactions of Chemokine Sequence Variants on Prostate Cancer Risk among men of African Descent.

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INTRODUCTION
Role of Chemokines in Inflammatory/Immune Response and Cancer

Chemokines belong to a family of small chemoattractant cytokines that mediate their effects by binding to protein- coupled receptors.

- Chemokine-chemokine receptor pairs trigger leukocyte production, which promotes cell survival and metastasis.
- Certain chemokine-chemokine receptor pairs are elevated in cancer
- Chemokines have several roles:
  - Lure cancer cells and chemokine receptor bearing immune cells (T and dendritic cells) to an inflamed site to promote lymph node metastasis
  - Mediate acute and chronic inflammation
  - Promote chemotaxis (cell movement), tumor growth and metastasis
  - Facilitate dendritic cell functions
- Regulate angiogenesis
- Genetic alterations detected in coding and regulatory regions of chemokine associated genes may alter macromolecules (mRNA/protein expression, chemokine-chemokine receptor production/function, protein-protein interactions), cellular behavior and ultimately modify PCa risk.

RESEARCH GAP
The impact of two or more genetic alterations detected in chemokine and chemokine receptor genes on prostate cancer (PCa) susceptibility remains understudied.

RESEARCH OBJECTIVES
Evaluate the individual and joint modifying effects of chemokine associated sequence variants in relation to PCa risk among men of African Descent.

HYPOTHESIS
Men who inherit two or more sequence variants (linked with a pro- inflammatory response, cell survival, proliferation, immune/tumor cell migration, chemotaxis, invasion, angiogenesis, and lymph node metastasis) have increased PCa risk relative to those with the wildtype genotype.

MATERIALS AND METHODS
Study Design
- Using a case-control study design, we evaluated the independent and joint effects of chemokine variants detected in chemokine associated genes in relation to PCa risk.
- 814 Men of African Descent (279 cases, 535 controls) were recruited from cancer screening programs, hospitals, or cancer centers located in the Washington D.C., South Carolina, and Kingston, Jamaica.
- Germ line DNA samples were obtained in germ-line DNA using Illumina’s Veracode genotyping system.
- Genetic data was generated by Expression Analysis, Inc.

Statistical Design
- Compared the frequency distribution of genotypes between cases and controls using the chi-square test.
- Risk estimates associated with inheritance of at least one minor chemokine-associated sequence variant allele were expressed as odds ratios (ORs) and corresponding 95% Confidence Intervals (95%CI) using unconditional multivariate LR models, adjusted for age.
- MDR was used to evaluate individual and joint modifying effects of innate immunity SNPs in relation to PCa risk (http://epistasis.org/).
- Combined effects were restricted to the total and U.S. population.
- Individual and gene combination effects were performed using SAS 9.4 and multi-factor dimensionality reduction (http://apistasis.org/), respectively.
- P-values for multiple hypothesis testing were made using false discovery rate (FDR) and permutation testing.

RESULTS
Table 2. Association between selected Chemokine-Related Sequence Variants and PCa Risk among Men of African Descent.

<table>
<thead>
<tr>
<th>Gene/Genotype</th>
<th>Controls n (% Total)</th>
<th>Cases n (% Total)</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intron 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCR5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1799988 T/T</td>
<td>166 (27.53)</td>
<td>93 (15.42)</td>
<td>0.570</td>
<td>0.22, 1.42</td>
</tr>
<tr>
<td>rs11574752 T/T</td>
<td>166 (27.53)</td>
<td>93 (15.42)</td>
<td>0.530</td>
<td>0.25, 1.11</td>
</tr>
<tr>
<td>rs3817655 T/T</td>
<td>166 (27.53)</td>
<td>93 (15.42)</td>
<td>0.510</td>
<td>0.25, 1.05</td>
</tr>
<tr>
<td>rs223895 A/A</td>
<td>166 (27.53)</td>
<td>93 (15.42)</td>
<td>0.679</td>
<td>0.44, 1.05</td>
</tr>
<tr>
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<td>166 (27.53)</td>
<td>93 (15.42)</td>
<td>0.679</td>
<td>0.44, 1.05</td>
</tr>
<tr>
<td>Exon 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2302004 C/C</td>
<td>166 (27.53)</td>
<td>93 (15.42)</td>
<td>2.66</td>
<td>1.10, 6.41</td>
</tr>
</tbody>
</table>

Table 3. Association between Selected Chemokine-Related Sequence Variants and PCa Risk among U.S. Men of African Descent.

<table>
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<th>Gene/Genotype</th>
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<th>Odds Ratio (OR)</th>
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Table 4. Association between selected Chemokine-Related Sequence Variants and PCa Risk among Jamaican Men

<table>
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<th>Gene/Genotype</th>
<th>Controls n (% Total)</th>
<th>Cases n (% Total)</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval</th>
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</table>

SUMMARY
- A complex interaction along the CCR5-CXCR5-CCL2 axis was identified as the best 3-factor PCa predictor, following MDR.
- Upon closer inspection of entropy graphs, this 3-factor model did not reveal synergistic effects in relation to PCa. However, we cannot rule out the possibility of additive effects.
- None of the pairwise combinations (CXCR5-CXCL, CXCR5-CCL2, CCL2-CCL5) provided any more information than each SNP considered alone based on analysis of individual and combined information gain scores.
- Assessment of the CCR5 rs227010, CR5 rs2136687 and CR7 rs323604 were not individually related to PCa.

FUTURE DIRECTIONS
- Identify and validate novel chemokine-associated SNPs as effective predictors of prostate cancer risk, disease progression, death/biologic recurrence, and overall survival within a larger and ethnic diverse sub-population.
- In vivo and in vitro studies are needed to understand the mechanism by which chemokine associated genes (e.g., CCL5, CXCR5 and CCL2) alter prostate cancer outcomes.

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1Department of Pharmacology & Toxicology and James Graham Brown Cancer Center

http://apistasis.org/

http://epistasis.org/