**Withaferin A in combination with Cisplatin suppresses mucin family proteins in Epithelial Ovarian Cancer Cells**

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## Abstract

Ovarian cancer is the leading cause of death among gynecological cancers and is the fifth leading cause of cancer death in women. Currently, ovarian cancer is diagnosed with MUC16 (CA-125), a transmembrane glycoprotein, used as a serum biomarker. Initially, ovarian cancer is treated with a combination of cytoreductive surgery and platinum/taxane based chemotherapy which is effective in 70-80% of the cases. After first round of treatment, serum MUC16 level is a good indicator of survival. An increase in MUC16 level in serum indicates recurrent epithelial ovarian cancer in which platinum/taxane based chemotherapy is only 30% effective. Various investigators have reported that the mucin family, particularly MUC1, 4 and 16 in ovarian cancer play a role in not just cancer cell proliferation but also metastasis which may be part of reoccurrence of cancer. We hypothesize that Withaferin A in combination with Cisplatin can sensitize EOC to cisplatin as a result of down regulation of expression of MUC1, MUC 4 and MUC 16. In the present study, we studied the effects of Withaferin A (WFA), cisplatin (CIS) both alone and in combination on expression of MUC1, MUC4 and MUC16 both in epithelial ovarian cancer cell line A2780 and orthotopic ovarian tumors generated in mice by injecting A2780 cells directly into ovaries. Western blot and immunohistochemical analysis were used to determine the expression of MUC1, MUC 4 and MUC 16 in ovarian tumors. In contrast, WFA alone and in combination with Cisplatin suppressed the expression of MUC1, MUC 4 and MUC 16 in A2780 cells as well as in tumors. Combination of WFA and CIS was found to be highly effective in suppression of MUC1, MUC 4 and MUC 16, suggesting that the WFA and CIS combination therapy may be a potential therapy for ovarian cancer.

## Role of Mucins in Ovarian Cancer

<table>
<thead>
<tr>
<th>MUC 1</th>
<th>MUC 4</th>
<th>MUC 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell growth and adhesion by affecting E-cadherin [1]</td>
<td>Protein EMF by decreasing expression of E-cadherin and vimentin</td>
<td>Protein EMF by decreasing expression of E-cadherin and vimentin</td>
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<tr>
<td>Cell growth and proliferation by affecting cell signaling pathways within cell [5]</td>
<td>Decreasing expression of nucleus cadherin and vimentin</td>
<td>Decreasing expression of EMT transcription factors (TWIST1, SNAI2)</td>
</tr>
<tr>
<td>• Inhibitory factors to tumorigenesis of cancer cells</td>
<td>• Positive metastasis by interacting with matrilysin [2]</td>
<td>• Protein EMF by decreasing expression of E-cadherin and vimentin</td>
</tr>
<tr>
<td>• Positive metastasis by decreasing expression of mesenchymal [3]</td>
<td>• Inhibitory factors to tumorigenesis of cancer cells</td>
<td>• Negative EMF to tumorigenesis of cancer cells</td>
</tr>
</tbody>
</table>

## Results

### Fig 1. MUC 1 expression in (a) A2780 Cell line treated with WFA, Cis or Combination [Lane 1: Control, 2: WFA 0.5 μM, 3: WFA 1.5 μM, 4: WFA 5.0 μM, 5: CIS 20 μM, 6: CIS 100 μM, 7: WFA 1.5 μM + CIS 20 μM, 8: WFA 1.5 μM + CIS 20 μM (b) Ovarian tumor sections isolated from nude mice treated with WFA, CIS or combination (c) Ovarian tumor [Lane 1: Control, 2: WFA 2 mg/kg, 3: CIS 6 mg/kg, 4: WFA 2 mg/kg + CIS 6 mg/kg].

### Fig 2. MUC 4 expression in (a) A2780 Cell line treated with WFA, Cis or Combination [Lane 1: Control, 2: WFA 0.5 μM, 3: WFA 1.5 μM, 4: WFA 5.0 μM, 5: CIS 20 μM, 6: CIS 100 μM, 7: WFA 1.5 μM + CIS 20 μM, 8: WFA 1.5 μM + CIS 20 μM (b) Ovarian tumor sections isolated from nude mice treated with WFA, CIS or combination (c) Ovarian tumor [Lane 1: Control, 2: WFA 2 mg/kg, 3: CIS 6 mg/kg, 4: WFA 2 mg/kg + CIS 6 mg/kg].

### Fig 3. MUC 16 expression in (a) A2780 Cell line treated with WFA, Cis or Combination [Lane 1: Control, 2: WFA 0.5 μM, 3: WFA 1.5 μM, 4: WFA 5.0 μM, 5: CIS 20 μM, 6: CIS 100 μM, 7: WFA 1.5 μM + CIS 20 μM, 8: WFA 1.5 μM + CIS 20 μM (b) Ovarian tumor sections isolated from nude mice treated with WFA, CIS or combination (c) Ovarian tumor [Lane 1: Control, 2: WFA 2 mg/kg, 3: CIS 6 mg/kg, 4: WFA 2 mg/kg + CIS 6 mg/kg].

## Conclusion

- Withaferin A alone and in combination with Cisplatin decreases expression of MUC 1, MUC 4, and MUC 16.
- Cisplatin alone increases the expression of MUC1, MUC 4 and MUC 16 in orthotopic tumors by increasing number of cancer cells expressing each gene.
- Combination of Withaferin A and Cisplatin at higher concentration significantly decreases expression of MUC 1, MUC 4 and MUC 16 in orthotopic tumors and A2780 cell line.
- Combination of Withaferin A and Cisplatin at higher concentrations decreases expression of MUC 1, MUC 4 and MUC 16 and is correlated with a decrease in cancer stem cells [6].
- Withaferin A and Cisplatin may work through MUC members to decrease cancer stem cells as shown by previous study [6].

## References


## Acknowledgements

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Kakar Lab – Taylor Johnson & Ria Jain
**Introduction**

- In the 1990s, two major cancer susceptibility genes were identified for breast cancer: BRCA1 and BRCA2. After a point, genetic linkage studies in high incidence breast cancer families did not identify any more predisposing mutations. (Source)
- Studies have been conducted testing the cancer suppressor effect of a rat quantitative trait locus (QTL) named Mammary carcinoma susceptibility 1b (Mcs1b).
- Mapping and sequencing of the Mcs1b QTL have revealed candidate causal variants.
- CRISPR/Cas9, or Clustered Regularly Interspaced Short Palindromic Repeats/Cas9, is an RNA-guided nuclease bacterial system involved in the defense against invading phages and plasmids.
- The CRISPR/Cas9 system can be engineered to facilitate genome editing in mammalian cells. The Cas9 nuclease enzyme can make double stranded breaks at specific locations in the DNA because guide RNAs (gRNAs) recruit the nuclease to cut at specific genomic locations.
- These double-strand breaks in DNA may be repaired by non-homologous end joining (NHEJ) DNA-repair. NHEJ repair often results in insertion or deletion mutations.
- Cells can also use an alternative repair system to correctly repair itself, which is typically less error prone, called homology-directed repair.

**Hypothesis**

Candidate variant A74-UL-SNV-18 is involved in regulating transcript levels of Mcs1b genes.

**Objective**

- Successfully clone plasmids containing gRNA sequences designed to target A74-UL-SNV-18.

**Translational Relevance**

Rat Mcs1b is orthologous to breast cancer risk associated human locus 8q11.2. Understanding this locus in rats could help to understand breast cancer risk in humans. Further, this work could also identify potential breast cancer prevention pathways.

**Methods**

1. Growing Bacteria
2. Extract and Amplify Plasmid DNA using Wizard SV Gel and PCR Cleanup System
3. Insert oligonucleotides in Plasmid; Ligate and Transform Cells
4. Screen Transform Cells using Diagnostic Double-restriction-enzyme Digest
5. Extract and Purify DNA from successfully cloned cells

**Results**

1. A map of the plasmid ordered from Addgene. After cloning, plasmids will contain sequences for gRNAs to target Cas9 nuclease to rat A74-UL-SNV-18. 2. A figure of how the gRNAs were selected and scored. Sequences 1, 3 and 6 were chosen. 3. A chart of the purchased oligonucleotide pairs and the corresponding sequences. These were inserted into the plasmid, and the first sequence in each pair was used to verify the sequence was inserted correctly.

**Discussion**

The gRNA sequences were successfully inserted into plasmids, and those plasmids were transformed into a bacteria. Approximately 94% of the oligonucleotides were inserted correctly as seen in figure 4. There was one oligonucleotide sequence that did not insert correctly. This sequence should read 5 CAC CGG TCA CGG CTT GGT CAT CCT CGG G 3', but instead it reads CAC CGG TCA CGG CTT GGT CAT CCT CGG C 3'. This could be because the result of a mutation caused during bacterial replication. This also could be because of the way the plasmid inserted.

**Future Directions**

- Transiently transfect CRISPR gRNA sequence containing plasmids into rat mammary epithelial cells to target A74-UL-SNV-18.
- Compare Mcs1b gene transcript levels between gene-edited and unedited cell lines.

**Acknowledgements**

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The Effects of Ligand Treatment on the Dimerization of EGFR-GFP and ErbB3-dsRED in Chinese Hamster Ovary Cells

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Abstract

Purpose. To better understand the effects of homo- and heterodimerization between two ErbB receptor kinase family members, epidermal growth factor receptor (EGFR) and ErbB3, on the biological function of the cell.

Methods. Chinese hamster ovary (CHO) cells stably and transiently express EGFR fused with a green fluorescent protein (GFP), and ErbB3 fused with a red fluorescent protein (dsRED). CHO cells were serum-starved and underwent dose-response using three separate ligands – EGF, BTC, and NRG1. Immunoblotting and widefield fluorescent imaging observed the presence of activity of EGFR-GFP and ErbB3-dsRED in CHO cells.

Results. Immunoblotting and widefield fluorescent imaging of transiently transfected CHO cells demonstrate that EGFR and ErbB3-dsRED are stably present in close domains in CHO cells after transcription. Immunoblotting of phosphorylated EGFR suggests that concentrations of 4 nM and 16 nM of ligand result in significantly increased activity of the receptor kinase. Furthermore, widefield fluorescent imaging of CHO EGFR-GFP cells shows EGFR-GFP colocalized into endosomes after EGF and BTC treatments.

Conclusions. EGFR-GFP and ErbB3-dsRED are stably present in CHO cells. EGF and BTC treatment at concentrations of 4 nM and higher promotes an increase in phosphorylation of EGFR. GFP, ErbB3-dsRED demonstrates little to no phosphorylation activity under ligand treatment when no other ErbB family dimer partner is present in the cell.

Figure 1

A) GFP Treatment

B) NRG1 Treatment

C) BTC Treatment

Figure 2

Figure 2. Widefield fluorescent imaging of EGFR-GFP, ErbB3-dsRED, Parental, GFP, EGF, ErbB3-GFP, ErbB3-dsRED, CHO E85-dsRED cells were transiently transfected with EGFR-GFP using Lipofectamine 2000, and then incubated overnight at 37°C. CHO cell lines were serum-starved for two hours at 37°C, and treated with 16 nM EGF, NR1, or BTC for fifteen minutes. Coverslips were then fixed with 4% paraformaldehyde. Coverslips were mounted to slides using prolong with DAPI, and then imaged using widefield white light imaging. Scale bar represents 10 µm. n = 3

Figure 3

Figure 3. Schematic of the hypothetical relationship between EGFR and ErbB3 under ligand treatment. A) EGFR has been known to bind to EGF (Hynes and MacDonald, ScienceDirect). Whether BTC can bind to ErbB3 is unknown. B) It is hypothesized that BTC treatment will induce primarily EGFR homoforms, NRG1 treatment will produce ErbB3 homoforms, and BTC treatment will result in the heterodimerization of EGFR and ErbB3.

Background

ErbB receptor tyrosine kinases are a four-membered family that exhibit the same basic structure: an extracellular domain, an intracellular domain, and a transmembrane section. The extracellular portion has two ligand-binding domains and two cysteine-rich domains (I–IV). Once a ligand binds to this region, it activates the kinase domain located in the intracellular portion and causes the receptor kinase to form dimers with other ErbB family members. The activated kinase domain trans-phosphorylates the tyrosine residues of its dimer partner.

Although similar in structure, EGFR and ErbB3 demonstrate key differences in kinase domain activity and ligand-binding domains. EGFR has a functional kinase domain and can phosphorylate the tyrosine residues of its dimer partner. ErbB3, however, has diminished kinase activity. This results in less phosphorylation to its partner, and therefore less docking sites to create a signaling cascade that elicits a cellular response. The homo- and heterodimerization between ErbB3 family members ultimately determines which tyrosine residues are phosphorylated, and therefore which effectors interact with the receptor. If EGFR can heterodimerize with ErbB3, most phosphotyrosines will be present on the ErbB3 receptor. ErbB3 has been shown to be an important contributor to migration response in cancer cells. We hypothesize that if the EGFR-ErbB3 complex will result in an interaction with effectors that promote cellular migration, which could be linked to the development of metastasis in carcinoma cells.

Hypothesis

EGFR and ErbB3 heterodimerization could potentially contribute to the progression and metastasis expressed in cancer cells

Questions

Can EGFR-GFP and ErbB3-dsRED be stably expressed in CHO cells?

Is there a ligand specific redistribution of EGFR-GFP and ErbB3-dsRED in CHO cells?

Conclusion

Chinese Hamster Ovary cells express stably transfected EGFR-GFP and ErbB3-dsRED in separate cell lines. EGFR-GFP is capable of being activated after treatment of ligands EGF and BTC in CHO EGFR-GFP cells, particularly at concentrations of 4 nM and higher. Ligand treatment appears to have little to no effect on the activity of ErbB3-dsRED in CHO ErbB3-dsRED cells, most likely due to diminished kinase activity of ErbB3. Furthermore, EGF and BTC treatment to CHO cells containing EGFR-GFP and transiently transfected CHO cells containing both EGFR-GFP and ErbB3-dsRED demonstrates minimal localization and compartmentalization of receptor.

Future directions: Create a stably dual-transfected CHO cell lines containing varying levels of both EGFR-GFP and ErbB3-dsRED in order to determine how receptor density affects heterodimerization formation between these receptor kinases. Induce heterodimerization between EGFR-GFP and ErbB3-dsRED and observe how this effects the proliferation/migration of the cell.

Acknowledgements

R25 Cancer Education Program, Ceresa Lab Members

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Design and Synthesis of Polymer Blend Electrospun Fibers for Sustained Release of siRNA to the Female Reproductive Tract

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Introduction

Efficacious preventative and therapeutic agent delivery to the female reproductive tract (FRT) is challenging due to the harsh microenvironment of the FRT, causing rapid degradation and clearance—especially of biological agents including genes and proteins. To overcome these challenges, nanotechnologies, such as electrospun fibers (EFs), can be customized and utilized for encapsulation, specific targeting, and sustained delivery of biologics and chemotherapeutics. Factors including surface morphology and fiber diameter can be altered, enabling high encapsulation, prolonged release, and subsequently efficacious biodistribution. Due to this versatility, nanofibers prove to be an exciting and promising platform for gene and drug delivery to the FRT. The immediate goal of this study was to engineer poly(lactic-co-glycolic acid)-polyether (PLGA-PEO) and poly(lactone-co-caprolactone)-polyether (PLCL-PEO) blend fibers to achieve high encapsulation efficiency and sustained release of siRNA. We evaluated EF morphology, size, loading, and controlled-release to be a better understanding of factors that modulate EF properties and delivery to the FRT. The long-term goal of this study is to evaluate the most promising formulation of the above EFs to deliver siRNA targeting the H19/BEa oncogene in Hela cells in vitro. We hypothesize that these siRNA EFs will provide an efficacious delivery platform to initially protect and sustainably deliver siRNA in the unique microenvironment of the FRT. We provide a preliminary assessment of the novel EFs to encapsulate and release siRNA from our polymer blended and non-blended EF formulations. The results of this study will allow us to rationally design formulation(s) to provide the most effective siRNA delivery to decrease expression of our desired target Bea oncogene in future studies.

Methods

EF Formulations: In this study, we designed and synthesized the following EF formulations:

- PLGA (50:50 Lactic Acid:Glycolic Acid)
- PLLA (80:20 Lactone Caprolactone)
- 100% PEO
- 90:10 PEO:PLGA
- 75:25 PEO:PLGA
- 20% PLGA 75:25 PEO
- 40% PLGA 75:25 PEO
- 60% PLGA 75:25 PEO
- 90:10 PLGA 75:25 PEO
- 100% PLGA
- 90:10 PEO:PLCL
- 75:25 PEO:PLCL
- 20% PEO 75:25 PLCL
- 40% PEO 75:25 PLCL
- 60% PEO 75:25 PLCL
- 90% PEO 75:25 PLCL

Encapsulant: siMimic oligonucleotide sense and antisense strands were annealed and complexed with spermine prior to electrospinning.

EF Fabrication:

1. Polymer is dissolved in solvent.
2. Polymer solution is incubated and drawn up into syringe.
3. High Voltage DC
4. Finished fiber left to desiccate.

EF Characterization: We performed scanning electron microscopy (SEM) to evaluate EF surface morphology and size. We used PicoGreen to measure the amount of siMimic we encapsulated in our EFs. To determine the amount of siMimic released from our EFs, we evaluated controlled release in both phosphate-buffered saline (PBS) and simulated vaginal fluid (SVF).

Results

Figure 1. SEM images of: (A) 15% PLGA:PEO and (B) 9% PLGA:PEO. Scale bar = 10 µm.

Effect of Solvent on Polymer Blend Formulation

Figure 2. SEM images of: (A) PLGA:PEO in HFIP, (B) PEO in DCM 4:1 DMF, and (C) PLGA:PEO EFs in HFIP 4:3 Water. Scale bar = 10 µm.

Figure 3. SEM images of: (A) PLGA, (B) PLGA 90:10 PEO, (C) PLGA 72:25 PEO, (D) PEO, (E) PEO 90:10 PEO, (F) PLCL 72:25 PEO EFs with siMimic and spermidine. Scale bar = 1 µm.

Figure 4 & Table 1. Loading and encapsulation efficiency (EE) of siMimic in EFs.

Conclusion

- All EF formulations displayed well-delineated morphology and high encapsulation efficiencies (1~80%, greater than that of nanoparticles, which have an EE of ~10-40%).
- PLGA 90:10 PEO EFs displayed the most release of siMimic and appear to be the most promising for future gene delivery.
- Incorporation of PEO significantly enhances release for PLCL EFs, while having less effect on PLGA EFs.

Future Studies

- Apply EF polymer blend formulations to synthesize and fully characterize actual siRNA EFs.
- Examine the efficacy of the “optimal” EF platform to distribute siRNA to Hela cell monolayers (2-D) and tumor spheroids (3-D).
- Determine the efficacy of E6 siRNA EFs on mRNA expression in Hela monolayers and spheroids.

Acknowledgments

This research is supported by the University of Louisville Cancer Education Program NCI R25-CA134283. Thank you, Dr. Stuart Williams for allowing us to use your electrospinning apparatus.
Perceived survivorship needs in patients with human papillomavirus (HPV)-positive and (HPV)-negative head and neck cancer
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Introduction
As the survivorship rates for cancer patients increase, the need for survivorship resources is also increasing. The demographics of head and neck cancer are changing with the emerging role of HPV-induced cancers. Patients diagnosed with HPV-positive oropharyngeal cancers are, on average, 10 years younger than patients with tobacco and/or alcohol-induced cancers. In addition, men with HPV-induced tumors outnumber women 3:1. We hypothesize that the survivorship needs of patients with HPV-positive tumors will differ significantly from those with tumors induced by smoking and/or alcohol.

Methods
For this study, patients were asked to partake in a survey. We included patients with squamous cell carcinoma of a mucosal site of the head and neck treated with curative intent. Patients with other histologies and those treated with palliative intent were excluded. Collected data included demographics (age, gender, race, income level, cancer site, stage, tobacco and alcohol history), and planned or completed treatment regimens. Patients were asked about which phase of treatment they were in (haven’t started, in the midst, completed/how long ago?), and then to rank their concerns after treatment in order of importance to them. They were also asked about various resources, and how useful they felt those resources would be.

Results
A total of 20 surveys have been distributed and returned thus far. Of those completed, 40% were patients with HPV+ head and neck cancer. Regardless of HPV status, the most commonly cited survivorship concerns were physical side effects, followed by cancer recurrence, cosmetic side effects, and emotional side effects. With regard to survivorship resource utilization, 50% of patients reported or anticipated pain after completion of treatment. In addition to pain management, patients also reported an interest in nutritional counseling, support groups, and complementary/alternative therapies. In this small sample size, this did not differ between patients with HPV+ and patients with HPV- tumors.

Figure 1: Patient-reported concerns after treatment

Figure 2: Survivorship resources patients reported they were most likely to use

Table 1: Patient demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n, %)</th>
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<tbody>
<tr>
<td>HPV</td>
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<tr>
<td>Positive</td>
<td>8, 40%</td>
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<tr>
<td>Negative</td>
<td>12, 60%</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
<td>14, 70%</td>
</tr>
<tr>
<td>Female</td>
<td>6, 30%</td>
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<tr>
<td>Cancer Site*</td>
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<tr>
<td>Tongue</td>
<td>8, 40%</td>
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<tr>
<td>Tonsil</td>
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<tr>
<td>Neck</td>
<td>5, 25%</td>
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<tr>
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<td>71-85</td>
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</table>

*Cancer site is reported as listed by the patient. Neck is a site of regional metastasis.

Conclusions
In this preliminary analysis, physical side effects of treatment and risk of cancer recurrence dominate survivorship concerns of patients with head and neck cancer, regardless of tumor HPV status. Of various survivorship resources available, patients reported they were most likely to use pain management services, support groups, complementary medicine, and nutritional counseling. Only 10% of patients surveyed reported that they would not utilize any of these resources. Data collection and survey administration are still ongoing.

Acknowledgements
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Mechanistic Insight Into Vinyl Chloride-Induced Liver Injury: Role of Dietary Fatty Acids

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Abstract

H&E

LPUFA + CIEOH

HPFU + CIEOH

Summary

Dietary polyunsaturated fatty acids blunt:
1. Liver damage
2. Inflammation
3. Lipid accumulation
4. ER Stress

after CIEOH

FIGURE 6: Hepatic mRNA Expression after ER Stress and CIEOH. Markers were treated and real-time PCR for CIEOH, LPS, and TNFα were performed as described in Materials and Methods.

MATERIALS AND METHODS

Animals and treatments. Eight week old C57Bl/6j mice were obtained from Jackson Laboratory (Bar Harbor, ME). Animals were administered a corn diet and some were injected with a dose of chloroethanol (50 mg/kg, i.p. dosages). Animals were sacrificed and blood and tissue were collected for further analyses.

Cell culture and treatments. Hep2 cells from ATCC (Manassas, VA) were exposed to [1, 2, 5, 10, 25, 50, 80, 100 μM] of chloroethanol for 48 hours (34°C). Cells were plated at 200,000 cells per well on 12-well plates. Cells were harvested after 4 hours.

Lipids were extracted from the livers of the treated mice. The lipids were diluted 1:10 and sonicated for the triglyceride assay. The lipids were then sonicated for the cholesteral assay.

Blood and tissue samples were collected for further analyses.

Lipids were extracted from the livers of the treated mice. The lipids were diluted 1:10 and sonicated for the triglyceride assay. The lipids were then sonicated for the cholesterol assay.

Figure 1. Mice were treated as described in Materials and Methods. Representative photographs of hematoxylin & eosin (H&E) and Oil Red-O staining are shown.

**Background**

Vinyl chloride (VC, 1) is a known carcinogen that has been associated with hepatic steatosis, steatohepatitis, and hepatocellular carcinoma in humans.\(^1\) While most studies on the health effects of vinyl chloride have focused on the toxic effect of VC on high-dosed, and taking into consideration VC exposure in low-dosed with risk-modifying factors. It has been shown that certain types of diet can affect the biotransformation of vinyl chloride (VC, 1) in particular, nonalcoholic fatty liver diseases. Bioactive oxidized fatty acids metabolites (DiACAMs) play a critical role in the development/growth of hepatic inflammation and injury in the context of steatosis. We hypothesize that VC exposure may synergize with other pollutants that cause inflammation.

**Methods**

Mice were administered a bolus dose of chloroethanol or vehicle (DMSO) 10x after being fed a low alcohol diet (LPS, 42% kcal from ALCOHOL, 42% kcal from fat, 14% kcal from protein). Animals were sacrificed 24-48 hours after CIEOH exposure. Samples were harvested for determination of liver damage, inflammation, oxidative and ER stress.

Livers in UFD-fed mice, chloroethanol caused no detectable liver damage or inflammation. In HPFA-fed mice, chloroethanol increased HPFA-induced liver damage, steatosis, inflammatory cells and hepatic expression of pro-apoptotic cytokines and genes. Furthermore, chloroethanol altered protein expression in key genes involved in ER stress.

Taken together, VC and HPFA cause liver damage, inflammation and ER stress markers. This serves as proof-of-concept that liver hepatosteatosis may be modified by a low alcohol diet and red wine. These data implicate exposure to VC as a risk factor in the development of liver disease in susceptible populations.

**Conclusions**

VC hepatosteatosis may be prevented by a low alcohol diet and red wine. These data implicate exposure to VC as a risk factor in the development of liver disease in susceptible populations.

Figure 2. Mice treated as described in Materials and Methods. Hepatic mRNA expression was assessed by real-time PCR for CIEOH, LPS, and TNFα.

**Future directions**

It is likely that VC exposure will have a modulating effect on the development of liver disease in susceptible populations. Further studies are needed to determine the mechanisms by which VC exposure affects liver inflammation.

**Funding**

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**References**


I3C Decreases Cyclin E Expression and Represses Cancer Cell Growth
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Abstract

Introduction: A product of glucobrassicin breakdown; Indole-3-Carbinol (I3C) is one compound among several derived from cruciferous vegetables that have been identified for their anticancer effects. Previous studies have shown that I3C induces G1, phase arrest, and works synergistically with Adenovirus to slow cancer cell replication and upregulate apoptosis, and may play a role in prevention and combination therapy for the treatment of tumors. Overexpression of cyclin E has been linked to tumorigenesis as it aids cell cycle transition into S phase where DNA replication can occur. ED1 cells have been transgenically induced to overexpress cyclin E causing tumorigenesis. Thus, ED1 mice should serve as a novel murine intermediate of cellular and human models, and serve as an effective in vivo model for Ad and I3C combination therapy.

Methods: In order to investigate the preventative and therapeutic potential of I3C, the current study utilizes MTT assay, crystal violet staining, and immunoblot analyses to understand the effect of I3C on cancer replication.

Results: I3C inhibits cell proliferation and metabolic activity in a dose-dependent manner. I3C also downregulates the expression of cell cycle proteins cyclin E, CDK2, and pRb.

Conclusion: I3C downregulates the expression of cell cycle proteins, which inhibits the proliferation of cancer cells. ED-1 cells are particularly susceptible to I3C treatment and should serve as a good model for the effects of I3C on human-type cyclin E in a murine model.

Conclusions

- Cyclin E-overexpressed ED1 cells are most sensitive to I3C
- I3C represses cancer cell growth at high concentrations
- I3C downregulates cyclin E, CDK2, pRb

Future Directions

- Kinase activity assay
- Cell cycle proteins
- CDK activity
- Murine models with Ad infection

Acknowledgements

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