Clinical Effect of Enoxaparin on INR following Hepatobiliary & Gastroesophageal Resection
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Background
- Enoxaparin inactivates factor Xa via a complex formed after binding to circulating anti-thrombin III.
- This mechanism is reported to not alter hemostatic measures such as clotting time, PT or PTT.
- To date, no clinical trials have shown a causal relationship between the clinical or pharmacological effects of enoxaparin on INR.
- The aim of our study is to show the clinical effect of enoxaparin on International Normalized Ratio (INR).

Methods
- Three hundred fifty cases were reviewed from IRB approved databases of patients undergoing gastroesophageal or hepatobiliary surgeries for malignancy.
- Forty-two of the patients received 30mg of enoxaparin daily and 247 received 40mg daily for prophylaxis against venous thromboembolism (VTE) starting post-operative day 1.
- 61 patients who did not receive enoxaparin were used as controls.
- INR levels were recorded for 6 days: pre-operative to 5 days after surgery.

Results
- Median preoperative INR was 1.0 in the 30mg, 40mg, and control groups.
- The average post-operative INR in both the 30 mg and 40 mg groups were both significantly higher than the average post-operative INR of the control group (P=0.015 and P=0.00075 respectively).

Conclusions
- This is the first clinical evidence of the effect of enoxaparin on INR in patients undergoing abdominal surgeries.
- We demonstrate an increase in the INR for patients who received enoxaparin for post-operative VTE prophylaxis.
- Future studies, will evaluate if these increase in INR that occur are clinically relevant or related to lab abnormalities.

Acknowledgements
I would like to thank the NIH R25 Education Grant (R25 CA134203) for funding this research, my mentor Dr. Robert C.G. Martin for his guidance, and Travis Shutt and Jack Rostas for their assistance in collecting the data.

Table 1: Laboratory Measurements (mean ± 95% CI)

<table>
<thead>
<tr>
<th>Drug (mg)</th>
<th>Pre-OP</th>
<th>POD1</th>
<th>POD2</th>
<th>POD3</th>
<th>POD4</th>
<th>POD5</th>
<th>Average</th>
<th>Post-OP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.00 ± 0.22</td>
<td>10.98 ± 0.23</td>
<td>10.97 ± 0.24</td>
<td>10.96 ± 0.25</td>
<td>10.95 ± 0.26</td>
<td>10.94 ± 0.27</td>
<td>10.93 ± 0.28</td>
<td>10.92 ± 0.29</td>
</tr>
<tr>
<td>30 mg</td>
<td>11.05 ± 0.24</td>
<td>10.99 ± 0.25</td>
<td>10.98 ± 0.26</td>
<td>10.97 ± 0.27</td>
<td>10.96 ± 0.28</td>
<td>10.95 ± 0.29</td>
<td>10.94 ± 0.30</td>
<td>10.93 ± 0.31</td>
</tr>
<tr>
<td>40 mg</td>
<td>11.06 ± 0.26</td>
<td>10.99 ± 0.27</td>
<td>10.98 ± 0.28</td>
<td>10.97 ± 0.29</td>
<td>10.96 ± 0.30</td>
<td>10.95 ± 0.31</td>
<td>10.94 ± 0.32</td>
<td>10.93 ± 0.33</td>
</tr>
</tbody>
</table>

Pre-OP vs Control: P=0.008 30 mg vs control: P=0.026 40 mg vs control: P=0.006

Figure 1: All Patient INR (mean with 95% CI)
**Introduction**
Hepatocellular Carcinoma (HCC) is the second leading cause of cancer-related deaths worldwide. Evidence indicates Cancer Stem Cells (CSCs) play a key role for HCC therapeutic failure and recurrence. Previous work in our group used gold nanorods (GNRs) loaded curcumin to target and kill cancer cells in vitro and in vivo. This study aims to target CSCs with GNR based BBs in 24 hrs. GNRs loaded curcumin to target and kill cancer cells in vitro and in vivo.

**Objective and Hypothesis**
- Assess efficacy of BBs in vitro and in vivo and determine downstream cellular events they illicit when inducing HCC cell death.

**Methods**
- In vitro HCC cell lines: HepG2 and Hep3B
- Spheroid assay: HCC cells → HCC CSCs
- XTT Cell Viability Assay: to determine metabolically active cells after treatment to find appropriate treatment time for efficacy
- TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling): stains for DNA fragmentation
- Western Blot of Caspase-3, -8, & -9; β-catenin; GSK3β; Cyclin D1
- In vivo: HCC tumor-induced murine model
- Ultrasound: show tumor progression
- H&E, Silver, and CD133 staining marks presence of CSC in HCC region prior to laser, and shows lack of CSC in HCC region post laser. This is graphically presented below.

**Results**
- BB+Laser treatment is more effective in killing HCC-CSCs in 24hrs than TUR and ADR alone in HepG2 cells.
- BB+Laser treatments induce DNA fragmentation, a hallmark of apoptosis, as shown with the TUNEL assay.
- Western analysis indicates the extrinsic pathway is the main apoptotic signaling that BB+Laser treatments invoke when killing HCC-CSCs.
- HCC orthotopic mouse model shows the efficacy of BB+Laser treatments in vivo, as well as their ability to specifically target HCC-CSCs.

**Conclusions**
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**Acknowledgements**
- Research was supported by the NCI R25-CA134283 grant
**LINE-1 ORF-1 protein concentration in healthy women population and endometrial cancer patients**

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### Abstract

**Introduction**

Endometrial cancer is the most common gynecological cancer diagnosed in the U.S. It is of great importance for clinicians to find a reliable biomarker that can be measured in blood in order to diagnose these patients earlier and have a better prognosis. Interspersed nuclear element 1 (LINE-1) is the most abundant retrotransposon in the human body. LINE-1 hyper-activation has been demonstrated in many types of cancer including, colon, prostate, lung, breast and endometrial. LINE-1 activation results in production of Open Reading frame 1 and 2 (ORF-1 and ORF-2).

**Objective and hypothesis**

In this study serum was obtained from normal and endometrial cancer patients and the amount of ORF1p was quantified. The hypothesis was that ORF1p is going to be higher in cancer patients compare to normal patients.

**Methods**

Healthy and cancer patients were enrolled from the University of Louisville OB/GYN Physician Outpatient Center and James Graham Brown Cancer Center (controls= 24 cases= 2). Blood was collected and serum was separated by spinning down. ORF-1p was measured using a quantitative in-house ELISA with a polyclonal antibody in the department of pathology at University of Louisville.

**Results**

The mean ORF-1 protein concentration in the normal population (n=24) was 9.31 (SD=8.25) ng/ml. The age rage The cancer patients (n=2) ORF-1 protein concentration was 0 ng/ml.

**Conclusions**

The results do not indicate that ORF-1 protein is increased in endometrial cancer patients. However, this study provides a foundation for future research in this field. Disadvantages of this study include a small sample population because of a lack of time to recruit healthy women and endometrial cancer patients. This research is the beginning of a series of new studies which are going to focus in finding the relationship between different pathological conditions and the ORF-1 protein concentration.

**Acknowledgements**

Research supported by a grant from R25-CA 134283 and the School of Medicine Summer Research Scholar Program.
Comparison Of DNA Damage Response To Hexavalent Chromium In Alligator And Human Lung Fibroblasts

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Aim 1: Differences in homologous repair after 24-hour exposure

Aim 3: Comparing amount of intracellular uptake

Research Question

How we did it:

Broblasts experience greater inhibition of homologous recombination during prolonged and has been previously shown to cause lung cancer in humans. Additionally, these data further support the one environmental health approach (A)

What we found:

What does it mean?

Future Work

Take Home Message

Acknowledgments

Research was supported by the NCI R25 University of Louisville Cancer Education Program (R25-CA134283) and by NIEHS grant number ES016893 (JPW). The content is solely the responsibility of the presenters and does not necessarily represent the official views of the National Institutes of Health.

Additional funding was provided by Integrated Mission Support Services (IMSS).

Further Reading


Research into the biological mechanisms of hexavalent chromium reveals its potential to cause cancers in humans. A One Environmental Health Approach (A)

We pursue a one environmental health approach, focusing on genomic instability. This study aimed to investigate the genotoxic effects of hexavalent chromium on human and alligator lung fibroblasts. Specifically, we examined the ability of these cells to repair DNA damage induced by chromium, with a focus on homologous recombination.

Future Work

This study explored the potential for alligator lung fibroblasts to serve as a biomonitor for chromium exposure. Additional research is needed to further investigate the role of alligators in monitoring chromium pollution and its health effects. The findings suggest that alligator lung fibroblasts could be a useful model for studying chromium-induced genotoxicity and its implications for human health.

Take Home Message

This research highlights the potential of alligators as a biological indicator of chromium exposure, offering insights into the genotoxic effects of this pollutant on lung fibroblasts. The results support the use of alligators for biomonitoring chromium pollution, potentially aiding in the assessment of environmental risk associated with chromium exposure.

Acknowledgments

Research was supported by the NCI R25 University of Louisville Cancer Education Program (R25-CA134283) and by NIEHS grant number ES016893 (JPW). The content is solely the responsibility of the presenters and does not necessarily represent the official views of the National Institutes of Health.

Additional funding was provided by Integrated Mission Support Services (IMSS).

Further Reading


Research into the biological mechanisms of hexavalent chromium reveals its potential to cause cancers in humans.
Oncolytic Ability of Mutated Adenoviruses

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University of Louisville School of Medicine

Abstract

Introduction: Lung cancer is the leading cause of cancer deaths worldwide killing more patients than any other form of cancer. Novel lung therapeutic strategies are needed in order to combat this deadly disease. One new approach to target cancerous lung cells is the use of oncolytic adenoviruses to induce the release of cancer associated antigens for subsequent uptake by dendritic cells and immune response. The adenovirus has been shown to be safe clinically, however their therapeutic efficacy is restricted by their limited viral spread and a minute natural immune response.

Objective: To determine the mutated adenovirus with the greatest cytopathic effect on each cell line as well as the effect of a Histone Deacetylase inhibitor on virus spread in vitro.

Methods: AdWT, AdUV, Adm3, Adm60, Adm117 and AdCycE adenoviruses were compared in this study on A549, ED-1 and ED-6 lung cancer cell lines. Additionally, the effect of the Histone Deacetylase Inhibitor, Panobinostat, was conducted using AdUV on A549 and ED-6 cell lines. Virus titer was determined using the TCD50 method. Adenovirus spread and cell viability was quantified via crystal violet staining. Total protein concentration was determined using BSA assay reading at a wavelength of 590 nm and protein expression of Cyclin E and CDK was observed by western blot analysis.

Results: Adm and AdUV were shown to lyse A549 most efficiently, Adm117 and Adm60 were shown to lyse ED-1 most efficiently, and Adm and Adm60 were shown to lyse ED-6 lung cancer cells most efficiently compared to the other mutated Adenoviruses. With the addition of 10-20nMol of HDACi a significant difference was observed compared to the control. However, at concentrations greater than 40nMol there was no difference observed between the control and the wells with the virus added.

Conclusions: These studies provide the groundwork that will be used in future experiments on these cell lines, as well as show the potential increase in cytopathic ability of the adenovirus with the addition of a Histone Deacetylase Inhibitor.

Introduction

• Lung Cancer:
  - Is the leading cause of cancer death.
  - Is in need of novel therapeutics in order to better treat the disease.

• Oncolytic therapy:
  - The adenovirus has been shown to be safe to use in the clinical setting
  - Therapeutic efficacy has so far been restricted by limited virus spread and minute natural immune response

These studies:

- Have shown certain mutated adenoviruses have different oncolytic potential in distinct lung cancer cell lines.
- Paint to which mutated adenoviruses should be used in future studies on the A549, ED-1 and ED-6 cell lines.
- Reveal the potential enhancement of adenoviruses oncolytic ability with the addition of a Histone Deacetylase Inhibitor.

Conclusions

- Adm and AdUV were shown to have the highest oncolytic ability in the A549 cell line.
- Adm117 and AdUV were shown to have the highest oncolytic ability in the ED-1 cell line.
- Adm and Adm60 were shown to have the highest oncolytic ability in the ED-6 cell line.
- The addition of 10-20nMol of a Histone Deacetylase Inhibitor revealed to enhance the oncolytic ability of the AdUV virus, while concentrations greater than 40nMol had no difference compared to the control.
Changes in MiR-200 Family and RASSF2 Expression in Colorectal Cancer and Normal Adjacent Epithelium

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University of Louisville School of Medicine¹, Price Institute of Surgical Research², Department of Pathology³

Introduction

- Colorectal Cancer (CRC) is the second leading cause of cancer-related death in the U.S.A.
- MicroRNAs (miRNA) are small non-coding RNA molecules that are involved in RNA silencing and posttranscriptional gene regulation.
- The miR-200 family (miR-200a, -200b, -200c, -141, and -429) has been widely studied in cancer, and is associated with blocking the epithelial to mesenchymal transition (EMT) in metastasis.
- One of the targets of the miR-200 family is RASSF2, which negatively regulates K-Ras, an oncogenic signaling protein.
- We have observed that the miR-200 family has been upregulated in colon cancer cell lines and that modulating miR-200 family expression in cell culture can influence K-Ras expression and cell proliferation.
- However, data from cell culture should be taken with caution, as its generalizability to physiologic conditions can be variable.
- We aimed to validate our in vitro findings by looking at miR-200 and RASSF2 expression in fresh frozen CRC tissue and histologically normal adjacent epithelial tissue.

Methods

- Following informed consent, tissue samples were taken from resection specimens from 5 patients with stage III CRC from the University of Louisville surgical biorepository.
- Tissue was cut and mounted on charged Histogen glass slides.
- One slide per sample was stained with hematoxylin and eosin (H&E), and cancer and normal epithelial cells were identified and marked by Pathology.
- Remaining fresh frozen tissue slides were stained and dehydrated using Arcturus® HistoGene® Frozen Section Staining Kit.
- Specific cells of interest were extracted using the ArcturusXTM Laser Capture Microdissection System using H&E slides as reference.
- RNA was extracted and isolated from tissue using the Arcturus® PicoPure® Frozen RNA Isolation Kit for LCM.
- Quantity and Quality of purified RNA was assessed using the NanoDrop2000™ (260/280 of 1.8-2.0 was considered pure).
- Reverse Transcription followed by quantitative real-time polymerase chain reaction (qRT-PCR) was performed using specific primers and probes to the miRNAs and mRNA of interest.
- Protein was extracted from the patient resection specimens and assayed using a Western Blot.
- Data was analyzed using paired t-tests after normalizing raw data to U6 for endogenous miRNA expression, 18S for mRNA, and β-actin for protein.

Acknowledgements

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Results

Fig. 1: H&E staining of malignant tumor tissue (1) and adjacent normal tissue (2) used as a guide in microdissection. In the tumor samples, only hypoxic, dystrophic tissue was excised, while in normal epithelial tissue, only the epithelial cells were excised.

Fig. 2: Sample LCM capture of normal adjacent colon epithelium. Tissue is stained with Arcturus® HistoGene® Frozen Section Staining Kit. One glandular structure containing absorptive epithelium and goblet cells was selectively captured while stroma was left on the slide.

Conclusions

- Although our data concerning RASSF2 are consistent with what we observed in cell culture, that for the miR-200 family are not.
- RASSF2 is a negative regulator of K-Ras, an oncogenic signaling protein. It is advantageous for cancer cells to downregulate RASSF2 expression in any environment.
- MiR-200, on the other hand, has two dichotomous roles. Upregulation may be able to block RASSF2, but downregulation helps induce EMT.
- In cell culture, there is no advantage in promoting EMT and metastasis, but this advantage exists in vivo, particularly in later or more aggressive cancers.
- Further investigations considering earlier stages of CRC may shed light on this discrepancy.
- Although we were able to modulate RASSF2 expression, K-Ras activation, and cell proliferation by inducing or repressing miR-200 expression in cell culture, this study identifies limitations of using miR-200 as a therapeutic target for CRC in clinical practice.
Development of novel diagnostic methodologies for diagnosis and monitoring in melanoma

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Department of Medicine, University of Louisville; J.B. Speed School of Engineering, University of Louisville; School of Public Health and Information Sciences, University of Louisville; Department of Biomedical Informatics, College of Medicine, The Ohio State University

INTRODUCTION

Our lab studies multiple cancers, autoimmune and other diseases using a thermal-physical technique called differential scanning calorimetry (DSC). DSC studies changes in heat capacity over a specific temperature range of patient plasma and other biofluid samples providing a characteristic plot that we call a thermogram. Previous studies have shown that thermograms can delineate between patients with active disease and patients with no evidence of disease (NED). In this study, we applied DSC to the analysis of melanoma patient plasma samples to determine the diagnostic utility of DSC for the detection and longitudinal monitoring of melanoma. In addition, we applied mass spectrometry and ELISA analyses to determine specific biomarkers in melanoma patient samples that cause the significant changes in the bulk plasma proteome detected by DSC.

METHODS

DSC

- Plasma thermogram data were stratified into two model groups: NED (28 patients, 86 samples) and Active disease (28 patients, 30 samples)
- Median thermograms were constructed and visualized for differences between peak 1 and active disease
- Thermogram differences were quantified through the calculation of summary metrics: thermogram area (area), maximum peak height (height), first moment temperature (Cp1), heat capacity maximum of the first peak ~62°C (Cp1 peak 1), heat capacity maximum of the second peak ~70°C (Cp1 peak 2), peak 1: peak 2 ratio (Cp1 peak 1 / Cp1 peak 2) and temperature of the peak maximum (Tm)
- Diagnostic performance of the summary metrics was determined by the construction of receiver operating characteristic (ROC) curves
- R software analyses was used to calculate metrics and ROC curves

Mass Spectrometry:

- High-resolution, high mass accuracy LCMS data collected using Proxeon EASY nLC UHPLC system and Orbitrap Elite mass spectrometry in a top-10 Decision Tree approach
- LCMS data were assigned using Proteome Discoverer v1.4.0.288 with Mascot v2.5.1 and Proxeon nLC MALDI-TOF Mass Spectrometry: high resolution, high mass accuracy LCMS data collected using Proxeon EASY nLC UHPLC system and Orbitrap Elite mass spectrometry in a top-10 Decision Tree approach
- Mean thermograms were constructed and visualized for comparison by Scaffold Q+S v4.3.4 using an intensity based absolute quantification (iBAQ) approach.

ELISA:

- Plasma concentrations of macrophage receptor with collagenous structure (MARCO) and complement C4b were measured via ELISA in 32 patient samples (8 NED, 8 active disease, 8 prostate cancer, 8 ovarian cancer, 8 melanoma)

RESULTS

SI. Median thermograms showed a substantially lower peak 1 and a slight right shift in peak 2 for the active disease group compared to the NED group.

2. Statistically significant differences (p<0.01) were found for area, Cp1 peak 1 and Tm.

3. ROC curves showed moderate discrimination (AUC values of 0.7 or lower) for height, Cp1 peak 1, Cp1 peak 2/Cp2 peak 2 and Tm. This demonstrates that single thermogram metrics have low diagnostic performance.

4. A total of 362 proteins were identified across three SEC fractions of 10 patient samples. 37 proteins were identified as statistically different between melanoma and ovarian cancer samples including 33 with higher abundance in melanoma and ovarian cancer samples compared to NED with higher abundance in ovarian cancer samples. Mean iBAQ scores were compared between melanoma and melanoma cancer samples with values interpreted with consideration to the relative abundance in the control sample. Two proteins were selected for further analysis: MARCO and complement C4b.

5. MARCO ELISA: concentration decreased as tumor load increased.

6. Complement C4b ELISA: concentration increased as tumor load increased

CONCLUSIONS

DSC shows potential for quantitatively characterizing the bulk proteome of melanoma plasma samples. Mass spectrometry analysis identified two candidate biomarkers, MARCO and complement C4b, where plasma differential abundance of discriminated melanoma patient samples from ovarian cancer samples and a healthy control. Measurement of plasma concentrations of MARCO and complement C4b and ELISA analysis showed the correlation of MARCO and C4b plasma levels with tumor load.