Introduction

- Pancreatic cancer is the fourth leading cause of cancer death in the United States and has a five-year survival rate of less than five percent.
- The number of pancreatic resections performed each year has increased with mortality decreasing to acceptable over the past 10–20 years.
- However, morbidity remains high.
- Contemporary reviews cite post-operative morbidity at 30%–60%, with pancreatic fistula being the most common morbidity at 5%–30%.
- Pancreatic fistula is one of the most morbid and costly complications associated with pancreatic resection.
- Many strategies, both technical and pharmacological, have been put forth to attenuate this morbidity, but no consensus for treatment has been reached.

Methods

- A review of a prospectively collected hepatopancreatico-biliary database was performed.
- Patients were included if they underwent pancreatic resection between 10/01/91 and 05/16/2012.
- Patients received somatostatin prophylactically at the discretion of their surgeon.
- Data were analyzed using univariate and multivariate analysis to determine if somatostatin had any effect on pancreatic fistula formation, fistula severity, length of stay (LOS), and readmission rates.

Purpose of Study

- Evaluate the efficacy of somatostatin in preventing pancreatic fistulas and improving post-surgical outcomes after pancreatic resection.

Results

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No Somatostatin</th>
<th>Somatostatin</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>295 (58%)</td>
<td>215 (42%)</td>
<td></td>
</tr>
<tr>
<td>Fistula/leak</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>23 (8%)</td>
<td>7 (3%)</td>
<td>0.031</td>
</tr>
<tr>
<td>B</td>
<td>11 (4%)</td>
<td>2 (1%)</td>
<td>0.051</td>
</tr>
<tr>
<td>C</td>
<td>10 (3%)</td>
<td>4 (2%)</td>
<td>0.413</td>
</tr>
<tr>
<td>B and C</td>
<td>21 (7%)</td>
<td>6 (3%)</td>
<td>0.031</td>
</tr>
<tr>
<td>Readmission</td>
<td>30 (10%)</td>
<td>27 (13%)</td>
<td>0.398</td>
</tr>
<tr>
<td>LOS</td>
<td>9 (2-81)</td>
<td>9 (2-48)</td>
<td>0.462</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: A comparison of patients receiving prophylactic somatostatin to patients not receiving prophylactic somatostatin</th>
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</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>Somatostatin Use</td>
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<tr>
<td>PMH Cardiac</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3: Multivariate predictors of post-operative pancreatic fistulas</th>
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<tr>
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</tr>
<tr>
<td>Somatostatin Use</td>
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<tr>
<td>PMH Cardiac</td>
</tr>
</tbody>
</table>

Conclusions

- Somatostatin associated with a statistically significant decrease in both the rate of fistula formation and the number of clinically significant fistulas in our pancreatic resection patients.
- Somatostatin use post-pancreatectomy is beneficial in preventing pancreatic fistulas and improving some post-surgical outcomes.

Acknowledgements

University of Louisville Cancer Education Program NIH/NCI (R25 CA134283)
Tobacco-Induced Dysregulation of Matrix Metalloproteinases in HL-60 cells.

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Introduction

- Matrix metalloproteinases (MMPs) are a family of approximately 18 secreted and membrane-bound zinc-endopeptidases involved in angiogenesis and tissue remodeling, two key determinants of cancer growth.
- In tumor metastasis, MMPs can break down extracellular components, release bioactive molecules, and induce epithelial-mesenchymal transitions.
- There are distinct correlations between smoking prevalence and cancers such as lung, oral and blood cell cancers.
- Nicotine (3-(1-methyl-2-pyrrolidiny1) pyridine), a key toxic component of tobacco, is thought to dysregulate matrix metalloproteinase secretion in innate immune cells in an α, nicotinic acetylcholine receptor (nAChR)-dependent manner.
- HL-60 cells were derived from an individual with acute promyelocytic leukemia and are commonly employed as model of innate immune cell differentiation and function.
- We set out to examine the influence of nicotine on MMP production by HL-60 cells.

Materials and Methods

- HL-60 cells were cultured in RPMI 1640 medium in an atmosphere of 5% CO2 and 100% humidity.
- HL-60 cells were differentiated (Figure 1) into neutrophils in by 1.3% DMSO or into monocytes by 16 nM PMA.

Results

- Promyelocytic and DMSO-differentiated HL-60 cells express α,3-acetylcholine nicotinic receptors (α, nAChR).
- (A) α, nAChR Western blot, (B) α, nAChR Immunofluorescence staining (x1000) (C) Negative control for α, nAChR Immunofluorescence staining (x1000).

Conclusions

- Nicotine suppressed MMP-8 release from TLR-stimulated neutrophilic HL-60 cells in an α-bungarotoxin manner.
- Future studies will assess the influence of nicotine on the expression of membrane-bound MMP species as well as MMP expression and secretion in HL-60 cells differentiated into the monocytic lineage.
- The relevance of nicotine exposure to MMP expression and cancer development in innate leukocytes is currently unclear.

Acknowledgements

NCI Cancer Education Program at the University of Louisville, Director: Dr. David Hein (HB)
NIDR R01DE019826 (DAS)
Evaluation of AFP Staging System for Hepatocellular Carcinoma in Non-cirrhotic Patients

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Kelly McMasters, MD, PhD, Glenda Callender, MD, Ryan Anderson, BS, Robert CG Martin II, MD, PhD
Department of Surgery, Division of Surgical Oncology, University of Louisville, Louisville, KY

• Hepatocellular carcinoma (HCC) is one of the top five most common cancers in the world and one of the top three most common causes of mortality worldwide
• One of the primary staging systems is the Barcelona Clinic Liver Cancer (BCLC) stage based on Child-Pugh score.
• Alpha-fetoprotein (AFP) is used as a biomarker for HCC but until recently its prognostic value remained uncertain.
• In Kentucky, a significant number of patients present without cirrhosis, yet BCLC stage continues to be used to help determine treatment and prognosis.

Results
• Patients presenting with HCC in the absence of cirrhosis appear to have different characteristics than patients with cirrhosis. Staging according to AFP level is an appropriate predictor of prognosis in non-cirrhotic patients with HCC.
• While BCLC stage cannot differentiate between cirrhotic and non-cirrhotic patients; AFP stage does.

Conclusions
• Patients presenting with HCC in the absence of cirrhosis appear to have different characteristics than patients with cirrhosis. Staging according to AFP level is an appropriate predictor of prognosis in non-cirrhotic patients with HCC.
• While BCLC stage cannot differentiate between cirrhotic and non-cirrhotic patients; AFP stage does. However, both reached statistical significant in predicting overall survival at 1 and 5 years. Neither BCLC stage or AFP stage alone is optimal. Rather, both factors should be taken into account to determine appropriate management for patients.
Granulocyte Colony Stimulation Factor (G-csf) Switches Müller Glia from a Gliosis Pathway to a Photoreceptor Progenitor Pathway in Vitro

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University of Louisville School of Medicine

Introduction

The leading cause of blindness in western countries is retinal degeneration. Loss of photoreceptors leads to permanent vision deficits. In lower vertebrates, Müller glia proliferate in response to retinal damage and dedifferentiate to retinal photoreceptor progenitors, which in turn replace lost photoreceptors. This Müller cell dedifferentiation is triggered by signaling factors which activate the Stat3 transcription factor. In mammals this Müller cell pathway is not significantly activated leaving higher vertebrates susceptible to retinal damage and disease. Here, we investigated the effects of Granulocyte Colony Stimulation Factor (G-csf) on Müller cells in vitro. G-csf is a well known activator of Stat3. It is widely used to boost bone marrow stem cell proliferation and differentiation during chemotherapy. But, recent evidence demonstrates that G-csf has functions beyond its role in chemotherapy—it can stimulate proliferation of cardiac progenitors and neural stem cells to add new differentiated cells to damaged heart and CNS. Moreover, its receptor is expressed on Müller cells in the retina.

Methods

Mouse Müller cells were cultured on Matrigel in DMEM complete medium with 10% FBS and penicillin/streptomycin. The cells were treated with 100 ng/ml G-csf for 5 or 10 days, then the cells were fixed and immunostained for pax6 (retinal progenitor cells marker), nestin (retinal progenitor cells marker), GFAP (gliosis marker), GS (Müller cells marker), β-tubulin (neuronal cells marker), and recoverin (photoreceptor marker).

Results

Fig.1. (a) Cross-section of a human eye. The retina is located at the posterior portion of the eye indicated by the arrow. (b) Diagram of cell layers of the retina. (c) Progenitor cell originating from Müller cell in Fig.1(b). (d) Rod photoreceptor differentiated from the progenitor cell in Fig.1(c).

Fig.2. G-csf causes an increase in the number of pax6 positive cells and a decrease in the number of GFAP positive cells. There is significant difference in the percentage of cells with and without these markers between G-csf treated and untreated populations.

Fig.3 (a) More spheres developed in the G-csf treated group than in the untreated group. (b) Over 90% of cells in culture stained positive for glutamine synthetase (GS), a Müller cell marker. (c-d) After 5 days in culture cells within the spheres stained positive for β-tubulin and pax6, retinal progenitor cell markers. (e) After 5 days in culture cells stained positive for nestin, a retinal progenitor cell marker. (f) After 10 days in culture cells stained positive for recoverin, a photoreceptor marker.

Conclusions

Müller glia can progress along two distinct pathways following retinal injury and disease. Cells marked by GFAP contribute to gliosis and retinal damage. Cells in which GFAP is diminished and pax6 and nestin are induced can contribute to cells expressing photoreceptor markers. Our results suggest that G-csf can switch Müller glia from a gliosis pathway to a retinal progenitor pathway.

Acknowledgements

Research supported by grants from Research to Prevent Blindness, National Cancer Institute R25 grant - Cancer Education Program NIH/NCI (R25-CA134283), and the School of Medicine Summer Research Scholar Program.
BCL6 Expression as a Prognostic Indicator in a Subset of DLBCL.

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1Department of Biochemistry and Molecular Biology, University of Louisville School of Medicine
2Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston TX

Background

Diffuse large B-cell lymphoma (DLBCL) is an aggressive lymphoid neoplasm that accounts for about 40% of all newly diagnosed non-Hodgkin’s lymphomas each year. Activated B-cell (ABC) DLBCL possesses gene expression patterns that resemble in-vitro activated peripheral B-cells and is characterized by NF-κB activation, while germinal center (GCB) DLBCL shows expression patterns similar to non-malignant germinal center B-cells and carries the better prognosis. TP53 is a gene coding the tumor suppressor p53, a transcription factor that acts as a sensor of cellular stressors. It functions by regulating various downstream target genes, thus promoting cell cycle arrest, initiating DNA repair, inhibiting angiogenesis, triggering senescence, or initiating apoptosis if cellular damage is irreparable. BCL6 is a gene coding for a transcriptional repressor that acts in mature GC B-cells through DNA binding and recruitment of co-repressors. It prevents premature activation and differentiation of GC B-cells, and fine-tunes the DNA damage response during somatic hypermutation and class switch recombination. Double-hit B-cell lymphomas are a subset of lymphomas defined by chromosomal breaks involving MYC and BCL2 and carry a poor prognosis.

Methods

Gene status and expression percentage was determined by sanger-based sequencing, fluorescence in-situ hybridization (FISH), and immunohistochemistry. Kaplan-Meier survival proportions were determined for various combinations of BCL6 and TP53 gene expression and status.

Results

After analyzing the data of BCL6 and TP53 for the potential of a Double-Hit Lymphoma, no significant relationship was found between concurrent mutations and survival. However, we found that in a subset of patients with wild-type but overexpressed TP53, expression of BCL6 may act as a prognostic indicator in ABC-DLBCL but not GCB-DLBCL. Based on Kaplan-Meier analysis, overexpression of BCL6 in the ABC subtype was found to predict better survival (P=0.0207), while overexpression of BCL6 in the GCB subtype was found to predict poor survival but was not significant (P=0.2945).

Conclusions

I would like to thank Michael Gordon and Yong Li for their help and support throughout this project. I would also like to thank Ken H Young of the Department of Hematopathology at the University of Texas MD Anderson Cancer Center and all the members of the DLBCL consortium.

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References


Basso K: Roles of BCL6 in normal and transformed germinal center B cells. Immunological Reviews 247, 2012

Off-Target Drug Effects: The Identification of Natural Products and FDA-Approved Cancer Drugs as G-Quadruplex DNA-Interacting Agents

John D. Gettelfinger1,3, Huy T. Le2,3, Lynn W. Deleuw1,3, Jonathan B. Chaires1,2,3, John O. Trent1,2

1Department of Medicine, 2Department of Biochemistry & Molecular Biology, 3James G. Brown Center for Cancer Research, University of Louisville, Louisville, KY 40202

Introduction
Guanine-rich oligonucleotidesequences capable of folding into G-quadruplexes, unique quadruplex helical tertiary structures, are located in functionally important sites of the human genome (e.g., the loci of retrotransposons, promoters of oncogenes, and 5’-untranslated and 3’-untranslated regions of several disease-modifying genes). Because of this, G-quadruplexes have emerged as attractive drug targets for cancer and other diseases. In addition to the classical DNA-binding agents (e.g., daunomycin, distamycin) it is particularly interesting that certain classes of protein-DNA mimics have been shown to directly interact with G-quadruplex structures. In the current work, we examined the off-target drug effects of several well-characterized and widely-known compounds by screening the National Cancer Institute Natural Product Set II (126 compounds) and Approved Oncology Drugs Set III (70 compounds) for G-quadruplex-interacting agents.

Methods
Oligonucleotide Preparation and Annealing and Small Molecule Acquisition
The human telomere (hTel) G-quadruplex sequence with a 6-fluorescent (FAM) molecule, FRET donor, attached at the 5’ end and a tetramethylrhodamine (TAMRA) molecule, FRET acceptor, at the 3’ end was designed and synthesized by Integrated DNA Technologies (Corvallis, OR). The sequences and the concentration were as follows:

5’-4FAM-AAGGTTAGGGTTAGGGTTAGGG-TAMRA-3’

Unlabelled hTel G-quadruplex sequences, polyA duplex hairpin sequence, and polyGC duplex hairpin sequences were obtained from Integrated DNA Technologies.

5’-AGGGTATAGGGTTAGGGTTAGGG-3’
5’-AGGGTATAGGGTTAGGGTTAGGG-3’

Stock solutions of the FRET-labeled oligonucleotide (250 μM) and unlabelled oligonucleotides (1000 μM) were made by reconstituting the lyophilized DNA in buffer, which is composed of teratoamine bromide ammonium (10 mM) and EDTA (10 μM) pH 7.0. Prior to annealing, the DNA was further diluted in buffer supplemented with 1 mM KCl (final concentration 20 mM) and DMSO (10 %). For the initial screening, FRET DNA was diluted to a concentration of 0.8 μM while unlabelled DNA was diluted to a concentration of 160 μM. The oligonucleotide samples were annealed in a water bath at 96°C, followed by the sample at temperatures for 10 minutes, and then cooling to room temperature overnight for the hTel sequences and on ice for 10 minutes for the duplex sequences.

The Natural Product Set II (NPS) and Approved Oncology Drug Set III (AODSIII) were obtained from the National Cancer Institute Developmental Therapeutics Program Open Chemical Repository (http://dtp.cancer.gov) in 96-well plate format. NPSII was reconstituted following accompanied instruction while AODSIII was used as supplied. For the screening experiments, compounds were further diluted 150 fold in buffer and supplemented with 1 mM KCl (final concentration 25 mM).

Fluorescent Resonance Energy Transfer (FRET) Melting Assay
FRET melting of the FAM was carried out on 96-well plates using Applied Biosystems (Foster City, CA) StepOnePlus PCR System. For the initial screen, annealed-FRET hTel was mixed with diluted compounds (final) or buffer (control) in a 1:1 ratio. In the FRET-selectivity screen, annealed-FRET hTel was mixed with unlabelled DNA and compounds in a 1:2 ratio. For all experiments, the reaction volume was 20 μL, DNA in 0.25 μM with 0.25 μM of the duplex. Fluorescence readings taken every 0.2°C. Results were analyzed using Applied Biosystems Proteins Thermal Shift™ Software.

Isothermal Titration Calorimetry (ITC)
ITC was performed using a Microcal VP-ITC microcalorimeter. The unlabeled hTel was dissolved, dialyzed and aliquoted in 10 μL 10 μM acetic acid/10 mM sodium phosphate pH 7.0 to a final concentration of 500 μM. Compounds were desorbed in dithiothreitol buffer to appropriate concentrations. ITC parameters were 15 μL injections at 5 minute intervals, reference power 15 μcalories, stirring speed 300 rpm. Data processing was performed using Origin 7.0 software supplied with the instrument.

3D Fluorescence Spectroscopy DNA Melt
3D DNA melt was performed on a Jasco FP-65000 Spectrophotometer. The FRET-hTel was diluted from the stock solution to a concentration of 1 μM with 1 μM EDTA, 25 mM KCl, pH 7.0 in a final concentration of 3 μM. Compounds were desorbed in dithiothreitol buffer to appropriate concentrations. Temperature was controlled over a temperature range of 25°C to 95°C with a ramp rate of 1°C/min. Fluorescence emission spectra (350-650 nm) were collected at various temperatures. The excitation wavelength was 492 nm with maximum emission at 502 nm.

Results

Figure 1. 3D Fluorescence Spectroscopy DNA Melt

Figure 2. Results of Initial Screening of NPS and AODSIII

Figure 3. Results of G-Quadruplex-Selectivity Screen

Figure 4. Isothermal Titration Calorimetry Results for Inhibitors Binding to hTel

Figure 5. Unfolding Assay Demonstrated Stabilization of hTel

Conclusions
Using a FRET-based melting assay, we were able to identify several novel DNA-binding agents whose actions were previously thought to occur either through an unknown mechanism or through a predominantly protein-binding mechanism. While DNA is a fundamentally attractive drug target, it is often damaged in drug design and development. As such, DNA is rarely, if at all, considered for off-target drug effects. Of the 27 compounds identified as quadruplex-binding hits from the 210 unique compounds in the initial screen, only 4 had previously been reported as DNA-interacting agents. 20 of the 27 compounds were new, and the study is the first to demonstrate direct binding interactions between these compounds and DNA. Moreover, 7 of the top 12 compounds exhibited a preference for binding one form of DNA (quadruplex) over another (duplex). Significantly, 5 of the top 12 compounds were tautomeric inhibitors, whereas all of these drugs, already approved by the FDA, interact with DNA in a significant and indirect fashion. Thus, DNA should be considered for potential off-target effects in drug design and development.

Acknowledgements
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