MED-18

Title: Clinical Evaluation of Somatostatin Use in Pancreatic Resections: Clinical Efficacy or Limited Benefit? [2012]

Authors: Ryan Anderson, B.S.,[1] Erik Dunki-Jacobs, M.D.,[2] Glenda Calender, M.D.,[2] Nick Burnett, B.A.,[1] Charles Scoggins, M.D. M.B.A.,[2] Kelly McMasters, M.D. PhD,[2] Robert Martin, M.D. PhD.[2] Medicine[1] and Surgery.[2]

Keywords: Somatostatin, pancreatic cancer, pancreaticoduidenectomy, Whipple, distal pancreatectomy, pancreatic fistula, octreotide

Abstract:

Background: The benefit of somatostatin for the prevention of pancreatic fistula has been widely debated in the literature. The aim of this study is to evaluate the efficacy of somatostatin in preventing pancreatic fistulas and improving post-surgical outcomes after pancreatic resection.

Hypothesis: Somatostatin improves post-surgical outcomes after pancreatectomy.

Methods: A review was performed of a prospectively collected 2002 hepato-pancreatico-biliary database was performed. Patients were included if the underwent pancreatectomy 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, and readmission rates.

Results: We identified 510 patients who underwent pancreatectomy. Overall, 30 (5.9 %) patients developed postoperative pancreatic fistulas and 27 (5.1%) fistulas were of clinical significance (grade B or C). Somatostatin was administered prophylactically to, 215 (42.2%) patients. Pancreatic fistula developed in 7 (3.3%) patients who received somatostatin versus 23 (7.8%) patients who did not receive somatostatin (p=0.031). Among patients receiving somatostatin, 6 (2.8%) fistulas were of clinical significance versus 21 (7.1%) fistulas for patients who did not receive somatostatin (p=0.031).

Conclusion: Somatostatin associated with a statistically significant decrease in both the rate of fistula formation and the number of clinically significant fistulas in our pancreatectomy patients. Somatostatin use post-pancreatic resection is beneficial in preventing pancreatic fistulas and improving some post-surgical outcomes. More thought needs to be put into this treatment modality, possibly by examining a larger patient population or through a randomized clinical trial where the distribution of receiving somatostatin to not receiving somatostatin was more evenly distributed.

Acknowledgements

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University of Louisville, Department of Surgery, Division of Surgical Oncology

MED-23

Title: Evaluation of AFP Staging System for Hepatocellular Carcinoma in Non-cirrhotic Patients [2012]

Authors: Nicolas Burnett, BA,[1] Erik Dunki-Jacobs, MD,[1] Charles Scoggins, MD,[1] Kelly McMasters, MD, PhD,[1] Ryan Anderson, BS,[1] Robert Martin, MD, PhD.[1] Surgical Oncology.[1]

Keywords: Hepatocellular carcinoma, AFP, BCLC stage, KY Hepatoma

Abstract:

Background

The Barcelona Clinic Liver Cancer (BCLC) staging classification is one of the primary systems used for staging hepatocellular carcinoma (HCC). This staging systems assumes the coexistence of cirrhosis and stratisfies stages based on the degree of cirrhosis. In the South-Midwest, a significant proportion of HCC presents in patients with non-cirrhotic livers. Despite the absence of cirrhosis, these patients are currently staged according to the BCLC classification system. Recently, an alternative staging system was proposed which classifies patients according to AFP level. The AFP staging system has been shown to be predictive of disease free survival following liver transplant for HCC. The aim of this study was to apply the AFP staging system to non-cirrhotic HCC patients and to compare its ability to predict overall survival against the more commonly used BCLC system.

Methods

A prospective hepatopancreaticobiliary (HPB) database from 1/2000 to 6/2012 was reviewed for all patients with a diagnosis of HCC. Patients were staged based on traditional BCLC classification as well as by AFP stage according to four levels: <10ng/ml, 10-150ng/ml, 150-500ng/ml, and >500ng/ml. Cirrhotic patients were compared to non-cirrhotic patients in terms of patient demographics and HCC stage. Kaplan-Meier analysis of overall survival (OS) was performed for non-cirrhotic patients according to both the BCLC staging and AFP staging systems.

Results

A total of 518 patients were diagnosed and treated for HCC. Cirrhotic and non-cirrhotic patients differed significantly in terms of median age at presentation (64 vs 70, $p < 0.001$) and gender (76% male vs 65% male, $p = 0.006$). BCLS staging classification did not distinguish between cirrhotics and non-cirrhotics ($p = 0.733$), while AFP staging demonstrated a significant difference between the two ($p < 0.0001$). Kaplan-Meier analysis of overall survival for non-cirrhotic patients with HCC was significant for both the BCLC staging classification and the AFP staging system ($p = 0.003$ vs $p < 0.0001$, respectively).

Conclusion

Patients presenting with HCC in the absence of cirrhosis appear to have different characteristics than patients with cirrhosis. Staging according to AFP level may be a more accurate predictor of prognosis in non-cirrhotic patients with HCC.

MED-29

Title: Granulocyte Colony Stimulation Factor (G-csf) Switches Müller Glia from a Gliosis Pathway to a Photoreceptor Progenitor Pathway in vitro [2012]

Authors: Laurie Conrad,[1] Yongqing Lui, Henry J. Kaplan, Douglas C. Dean, Wei Wang. Medicine[1] and Ophthalmology & Visual Sciences.[2]

Keywords: M&#65533;ller, Granulocyte colony, retina, photoreceptor, gcsf

Abstract:

Granulocyte Colony Stimulation Factor (G-csf) Switches Müller Glia from a Gliosis Pathway to a Photoreceptor Progenitor Pathway in vitro

Purpose: 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 dedifferentiaon is triggered by signaling factors that 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 and 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/mg g-csf for 5 or 10 days, then the cells were fixed and immunostained for pax6, GFAP, GS, β-tubulin, and recoverin.

Results: Following g-csf treatment, Müller cells significantly increase expression of the retinal progenitor marker pax6 and decrease expression of GFAP which marks cells involved in gliosis. More spheres developed in the g-csf treated group than in the untreated group. After 5 days in culture cells within the spheres stained positive for β-tubulin and pax6, and nestin, After 10 days in culture cells stained positive for recoverin.

Conclusions: Müller glia can progress along two distinct pathways following retina injury and disease. Cells marked by GFAP contribute to gliosis and retinal damage, whereas cells where 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 gliotic pathway to a retinal progenitor pathway.

Research supported by grants from Research to Prevent Blindness, National Cancer Institute R25 grant for Cancer Education Program NIH/NCI (R25- CA134283), and the University of Louisville School of Medicine Summer Research Scholar Program.

MED-35

Title: BCL6 Expression as a Prognostic Indicator in a Subset of DLBCL [2012]

Authors: Noura Estephane,[1] Michael Gordon,[1] Yong Li, PhD.[1] Biochemistry and Molecular Biology.[1]

Keywords: TP53, BCL6, DLBCL, B-cell, Lymphoma

Abstract:

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. Using a cohort of 506 DLBCL patients including clinical, gene expression and sequencing data, we tested our initial hypothesis that concurrent *BCL6* translocation and *TP53* mutation may also constitute a double-hit lymphoma. Gene status and expression percentage was determined by Sanger-based sequencing, fluorescence in-situ hybridization, and immunohistochemistry, and Kaplan-Meier survival proportions were determined for various combinations of *BCL6* and *TP53* gene expression and status. No significant relationship was found between concurrent *BCL6* and *TP53* mutation. However, we found that in patients with wild-type but overexpressed *TP53*, expression of *BCL6* may act as a prognostic indicator in both ABC-DLBCL and 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).

Title: Off-Target Drug Effects: The Identification of Natural Products and FDA-Approved Cancer Drugs as G-Quadruplex-Interacting Agents [2012]

Authors: John Gettelfinger,[1] Huy Le, Lynn Deleeuw, Jonathan Chaires, John Trent. Medicine, James G. Brown Cancer Center,[1] Biochemistry & Molecular Biology, James G. Brown Cancer Center[2] and Medicine, Biochemistry & Molecular Biology, James G. Brown Cancer Center.[3]

Keywords: quadruplex, cancer, G-quadruplex

Abstract:

Guanine-rich oligonucleotide sequences capable of folding into G-quadruplex tertiary structures are localized to functionally important sites of the human genome (e.g. the telomere, promoters of oncogenes, 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. We screened the National Cancer Institute Natural Product Set II (120 compounds) and Approved Oncology Drugs Set IV (101 compounds) for G-quadruplex-interacting agents. For the primary screen, we employed a fluorescence resonance energy transfer (FRET) melting assay of the G-quadruplex-forming sequence d[$AG_3(T_2AG_3)_3$] from the human telomere. G-quadruplex-selective compounds identified in the primary screen were confirmed through the secondary screen using circular dichroism, fluorescence, and UV/Vis spectroscopy as well as isothermal titration calorimetry. Several of the positive hits (e.g. daunorubicin, doxorubicin, mitoxantrone, and quinacrine) have previously been identified as DNA intercalators and known to interact with G-quadruplex structures. However, most of the positive hits (i.e. raloxifene, ergocristine, imatinib, vandetanib, sunitinib, sorafenib, and crizotinib) have not been previously demonstrated to bind to nucleic acids. Raloxifene is a selective estrogen receptor modulator. Ergocristine is an ergot alkaloid used to produce lysergic acid diethylamine. The other compounds mentioned are tyrosine kinase inhibitors. Imatinib is particularly important in that it is the first compound to be "rationally designed" for its specific target and is often cited as a model of targeted therapy. Our findings suggest novel mechanisms for off-target drug effects and warrant further investigations into the possible implications of the therapeutic uses of these compounds.

MED-42

Title: Retrospective Experience with Medullary Thyroid Carcinoma [2012]

Authors: Farrah Harden, B.S.,[1] Amy Quillo, M.D.,[1] Elsa Stephen, B.A.,[1] Kelsey Lewis, B.S.,[1] Michael Flynn, M.D.,[1] Jeffrey Bumpous, M.D.,[1] Richard Goldstein, M.D. Ph.D.,[1] Glenda Callender, M.D.[1] Surgery.[1]

Keywords: Surgery, Oncology, Endocrine, Thyroid, Genetics, Carcinoma, MEN2, Rare

Abstract:

Introduction

Medullary Thyroid Carcinoma (MTC) is a relatively rare cancer that occurs both sporadically and in the setting of inherited *RET* protooncogene mutations associated with Multiple Endocrine Neoplasia Type 2. Our goal was to review our local experience with MTC.

Methods

A retrospective study was performed of all patients undergoing thyroidectomy for a diagnosis of thyroid cancer in a single institution from 01/01/2001-12/31/2011. Charts were reviewed for demographic variables, operative notes, pathology reports, and biochemical data.

Results

A total of 352 patients were identified who underwent thyroidectomy for thyroid cancer. Of these, 14 (4%) patients had a diagnosis of MTC. All but one patient underwent initial thyroidectomy at our high-volume thyroid center; that patient underwent thyroid lobectomy in the community for a thyroid nodule and was referred for further management once the diagnosis of MTC was made. One patient underwent thyroid lobectomy at our center for unilateral goiter; when MTC was diagnosed on final pathology, the patient did not follow up for additional surgical management. One patient underwent prophylactic thyroidectomy for known MEN2A; the remainder of the patients underwent therapeutic thyroidectomy and appropriate lymph node dissection. Results of genetic testing for MEN2 were available for 5 patients; 4 tested positive for MEN2A and 1 tested negative.

Discussion

Although our experience with MTC is small, we found that MTC represents approximately 5% of all thyroid cancers treated at our center. This is in line with the incidence rates seen in the literature. Interestingly, for 9 patients, we were not able to conclusively determine whether or not they underwent genetic testing for MTC; further study into the barriers to genetic testing is indicated.

MED-49

Title: Assessing the Harvard Scale of Breast Cosmesis: An Analysis of Patients Treated with Breast Conserving Therapy on a Phase II Clinical Trial [2012]

Authors: Allison Hunter, BA,[1] Anthony Dragun, MD.[2] Medicine[1] and Radiation Oncology.[2]

Keywords: Harvard Scale , Breast Cancer, Cosmesis, Cosmetic Outcome

Abstract:

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Purpose: Cosmetic outcome is frequently used to assess quality of life (QOL) outcomes in patients that have undergone treatment for breast cancer. As such, an evaluation of the quality and objectivity of the Harvard Scale, a commonly-used assessment tool utilized to evaluate cosmetic outcome in breast cancer patients, is important in gauging objective and congruent QOL expectations following breast cancer treatment.

Materials and Methods: Seventeen (17) patients enrolled on a Phase II protocol, involving Accelerated Hypofractionated Radiotherapy after Breast Conserving Surgery for early stage breast cancer treatment, were assessed at four post-operative time points within a one-year window following radiation treatment. Each time point assessment was completed by the treating physician using the four-tier Harvard Scale (Excellent, Good, Fair, Poor) to compare the treated breast to the controlled breast. A survey including the same de-identified images was then distributed to forty-four (44) medical students and residents in hopes of assessing the validity of the Harvard Scale.

Results: Of the 44 medical student and resident respondents, there was equal distribution between male (23) and female (21), with the majority of respondents describing themselves as single (82%), Caucasian (70%), and less than twenty-nine (29) years of age (89%). The physician's assessment of cosmetic outcome differed somewhat from the respondent averages: Excellent (43% vs. 27%), Good (34% vs. 34%), Fair (20% vs. 28%) and Poor (3% vs. 11%). Congruence between the physician and the respondents in the same four categories showed to be 49%, 37%, 44%, and 86%, respectively. Finally, when combining the categories into a bimodal representation of Excellent/Good (70%) versus Fair/Poor (72%), there was a significant increase in congruence between the treating physician and the respondents.

Conclusion: Evaluation of cosmetic outcome following treatment for early stage breast cancer can be a subjective process, often differing from evaluator to evaluator. Our results suggest that a two point scale in place of the four-point Harvard Scale would be a better method to evaluate cosmetic outcome due to the significant improvement in congruity among evaluators.

MED-55

Title: Identifying urine exosome miRNA profiles in melanoma patients [2012]

Authors: Daniel Kmetz, BS,[1] Deyi Xiao, MD,[1] Michael Egger, MD,[1] Sabine Weigel, MS,[2] Wolfgang Zacharias, PhD,[2] Hongying Hao, MD, PhD,[1] Kelly McMasters, MD, PhD.[1] Surgery[1] and Microarray Facility.[2]

Keywords: exosome, melanoma

Abstract:

Melanoma is a highly heterogeneous malignant tumor with a wide range of prognoses among stages based on the American Joint Committee on Cancer (AJCC) groupings. A need exists for developing biomarkers that can aid in melanoma diagnosis, prognosis, and follow-up. Exosomes are 40-100nm diameter membrane vesicles released from tumors into the body fluids such as urine. Unique miRNA in tumor exosomes contributes to malignant progression through post-translational silencing of mRNA. This leads to the possibility of characterizing differences in expression of exosomal miRNA of melanoma and non-melanoma patients in the search for a biomarker for assessment of the disease process.

This study used urine collected from non-melanoma, Stage I, and Stage IV melanoma patients to isolate exosomes. Western blot and electron microscopy were used to assess purity of exosome. Urine exosome RNA were isolated and applied to Affymetrix miRNA array 2.0. Differentially expressed miRNAs across the three sample groups were analyzed by ANOVA.

The results showed that stage I melanoma patients had 10% greater yield of exosome RNA than that of our control group, and Stage IV patients had 20% higher yield of RNA than that of Stage I patients. 33 differentially expressed miRNAs were found in Stage I vs Control group. Among them were miR-3201, miR-3128, miR-3148, miR-378, miR-320a, and miR-320b. 17 differentially expressed miRNAs were identified in Stage IV vs control group, such as miR-548a-3p, miR-103, miR-30c, miR-93, and miR-191. In comparison of Stage IV vs Stage I melanoma patients, there were 5 miRNAs (miR-1825, miR-1972, miR-1184, miR-3201, and miR-940) with significant changes. There were 9 significant expressed miRNAs detected in both Stage I vs Control and Stage IV vs Control groups. A database search of these miRNAs' function revealed some of the miRNAs have been previously implicated in cancer process, but many have no known function.

To our knowledge this is the first attempt to analyze urine exosomal RNA profiles in melanoma patients. These distinctive urine exosomal miRNA signatures may be used as diagnostic and prognostic markers for melanoma patients. This research could lead to translation into a non-invasive method for identifying diagnostic and prognostic biomarkers in melanoma patients.

MED-61

Title: Effect of angiotensin converting enzyme inhibition on outcomes in retrospective study in consecutive patients with head and neck cancer [2012]

Authors: Callie Linden,[1] Dr. Rebecca Redman.[1] Medicine.[1]

Keywords: ACE Inhibitors, angiotensin II, head and neck cancer

Abstract:

Introduction: The main aim of this study is to explore the potential effects of ACE inhibition on outcomes in patients with head and neck cancer undergoing treatment with radiation and/or chemotherapy, both in terms of cancer-specific survival as well as toxicity of treatment (including short and medium-term follow-up). ACE inhibitors reduce the production of angiotensin II which appears to directly stimulate vascular endothelial growth factor (VEGF) which promotes angiogenesis. ACE inhibitors and ARBs have been shown in vitro and in vivo to decrease tumor growth and inhibit metastasis[3,4]. In addition to improving cancer-specific survival and outcomes, ACE inhibitors may also help to mitigate the toxicities of cancer treatment.

Methods: This is a retrospective chart review of consecutive patients with head and neck cancer who were treated with radiation and/or chemotherapy at the James Graham Brown Cancer Center between September 2002 and December 2009. Data collected will be demographic data, cancer site of origin and stage, HPV expression, medications, indication for ACE inhibitor where applicable, treatment data, disease-related outcomes, and toxicities.

Results:

Conclusion:

MED-85

Title: CaMKII represses CaMKIV transcription to promote leukemia cell proliferation [2012]

Authors: Lauren Strait,[1] Cuibo Yang,[2] Maddalena Illario, MD, PhD,[3] Uma Sankar, PhD.[2] Medicine,[1] Pharmacology and Toxicology, University of Louisville[2] and Dipartimento Biologia e Patologia Cellulare e Molecolare, Federico II Uniververisty.[3]

Keywords: calcium, calmodulin, kinase, myeloid leukemia

Abstract:

Background: Calcium/Calmodulin dependent protein kinases (CaMK) play an important role in cell signaling cascades by linking increases in intracellular calcium with biological processes. In myeloid leukemia cells, calmodulin dependent protein kinase II (CaMKII) promotes cell proliferation by binding to RAS/RAF and stabilizing the mitogen-activated protein kinase (MAPK) pathway. CaMKII also binds to and phosphorylates retinoic acid receptor (RAR), a nuclear hormone receptor transcription factor. This phosphorylation of RAR increases its interaction with co-repressors causing an inhibition of RAR-mediated gene transcription. CaMKIV, on the other hand is expressed at very low levels in myeloid leukemia cells. However, pharmacological inhibition of CaMKII enhances CaMKIV mRNA and protein expression, potentially through the de-repression of the *CaMK4* transcription. The *CaMK4* promoter contains RAR response element (RRE) that can be activated by retinoic acid. Since CaMKII can inhibit RAR-mediated transcription, we hypothesize that increased CaMKII can repress CaMKIV transcription. In this study, we investigated the potential cross talk between CaMKII and CaMKIV in multiple myeloid leukemia cell lines.

Methods: Chromatin Immunoprecipitation (ChIP) assay was performed on U937 and K562 cells. First, U937 and K562 cells were cultured in RPMI-1640 media. Cells were then treated with 10% formaldehyde to a final concentration of 0.75% for 10 minutes to cross-link the proteins to the DNA. Glycine was then added to a final concentration of 125 mM for 5 minutes to terminate the cross-linking reaction. Cells were then washed twice with cold PBS and resuspended in lysis buffer. The lysate was sonicated over a time course and samples were taken at 5, 10, 15, and 20 minutes during sonication. The samples were analyzed on a 1.5% agarose gel to determine optimal conditions to obtain a desired fragment length of 200-800 base pairs. The 15 minutes sample was chosen and an immunoprecipitation (IP) was performed using an Abcam ChIP kit and histone H3 and RAR-alpha antibodies. After the IP was performed the DNA was purified and a quantitative PCR was performed for analysis using histone and CaMKIV promoters.

Results and Conclusion: Repression of CaMK4 transactivation by CaMKII has previously been proven in our lab using HEK 293 cells as the transfected target cell. The purpose of this study was to investigate whether this occurs in vivo, in leukemia cells. The U937 and K562 cell lines cannot be easily transfected, so a ChIP assay was utilized instead. Using the ChIP assay we established the baseline for RAR-alpha binding on CaMKIV promoter in the myeloid leukemia cell lines. The next step in this process is to inhibit CaMKII using KN-93 and to see how this effects CaMKIV expression. Also, our lab has previously demonstrated that CaMKIV suppresses proliferation in U937 cells and is now re-testing this relationship in K562 and HL-60 myeloid leukemia cells.

MED-90

Title: Determining the relationship between ubiquilin 1 and insulin-like growth factor 1 receptor in HEK 293T cells. [2012]

Authors: Jara Vega Velez, BS,[1] Levi Beverly, PhD.[2] Medicine[1] and Pharmacology and Toxicology.[2]

Keywords: Ubiquilin, UBQLN1, UBA, IGF1R

Abstract:

Ubiquilin1 (UBQLN1) is a protein that binds to ubiquitinated proteins and either takes the protein to the proteasome for degradation or protects the protein from degradation. UBQLN1has been shown to play a role in protection and degradation of different proteins that may play a role in cancer. Insulin-like growth factor 1 receptor (IGF1R) activity has been linked to cancer and we have shown that IGF1R interacts with UBQLN1. IGF1R is a tyrosine kinase receptor that is activated by insulin-like growth factor 1 and insulin-like growth factor 2. Upon stimulation, IGF1R is phosphorylated and begins a signaling cascade that is vital for cell proliferation and survival. To explore UBQLN1's interaction with IGF1R, 293T cells were transfected with UBQLN1 deletion constructs that express UBQLN1 proteins lacking various domains. We show that increased expression of UBQLN1 lacking an intact Ubiquitin-associated domain (UBA) is capable of stabilizing phosphorylated IGF1R following serum starvation. This result indicates the loss of the UBA domain somehow increases the persistence of phosphorylated IGF1R following removal of growth factors. This relationship is relevant because many cancers show an overexpression and/or hyper-activation of IGF1R. Normally, phosphorylated IGF1R may be targeted for protein degradation by UBQLN1. However, if a mutation changes this regulatory step, phosphorylated IGF1R may become stabilized and not targeted for degradation. The persistence of activated IGF1R prolongs the signal for cell proliferation and survival, favoring the development of a tumor and cancer. The data presented suggest that UBQLN1 may be involved in the physiological regulation of IGF1R signaling and alterations in UBQLN1 expression may alter IGF1R signaling in cancer. Therapeutic treatments would involve targeting UBQLN1 for degradation or preventing the interaction with phosphorylated IGF1R.

D-1

Title: Tobacco-Induced Dysregulation of Matrix Metalloproteinases in HL-60 cells. [2012]

Authors: Harrison Black, BS,[1] Diane Renaud, PhD,[1] David Scott, PhD.[1] Department of Oral Health and Systemic Disease Research.[1]

Keywords: Tobacco, Nicotine, Cancer, Immunology, Matrix Metalloproteinases

Abstract:

Background: Matrix metalloproteinases (MMPs) are proteins that play a major part 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. Nicotine (3-(1-methyl-2-pyrrolidinyl) pyridine), a key toxic component of tobacco, is thought to dysregulate matrix metalloproteinase secretion in innate immune cells in a α7 nicotinic acetylcholine receptor (nAChR)-dependent manner. HL60 cells were derived from an individual with acute promyelotic leukemia and are commonly employed as model of innate immune cell differentiation and function. **Aim:** We set out to examine the influence of nicotine on MMP production by HL60 cells. **Methods:** HL60 cells were differentiated into neutrophils in the presence of 1.3% DMSO and stimulated with *E. coli* LPS with and without preincubation with nicotine and the nAChR antagonist, α-bungarotoxin. MMP-9, -8 and -2 secretions were measured by ELISA at 1 and 24 hrs. **Results:** MMP-2 was not detected, while nicotine did not influence MMP-9 release ($p > 0.05$). MMP-8 release at 24 hr was suppressed by nicotine in an α-bungarotoxin-senstive nAChR-dependent manner ($p < 0.01$). **Conclusion:** Nicotine suppressed MMP-8 release from TLR-stimulated neutrophilic HL60 cells. Future studies will assess the influence of nicotine on the expression membrane-bound MMP species as well as MMP expression and secretion in HL60 cells differentiated into the monocytic lineage.

D-13

Title: Translocase Activity in Parotid Acinar Cells [2012]

Authors: McKinley Soult, DMD,[1] Venkatesh Srirangapatnam,[1] Anne Carenbauer,[1] Douglas Darling.[1] Dept. of Oral Health and Rehabilitation.[1]

Keywords: Translocase, Parotid

Abstract:

Background: Salivary glands have a critical role in maintaining the dynamic equilibrium of the oral cavity. Saliva has antimicrobial properties, lubricates food for swallowing and initiates carbohydrate digestion. Salivary gland cancers occur in approximately one in every 50,000 people each year in the United States. Recent studies suggest that translocases (adenine nucleotide translocase) or flippases /floppases (P-glycoprotein; MDR1; ABCB1) are factors in eliciting cancer cell death and promising therapeutic targets. Additionally, lipid flippases/floppases or scramblases may play a role in sorting of salivary secretory proteins in the parotid gland. Our objective was to identify which flippases are present in the parotid gland and determine their location in the acinar cell. **Methods**: Translocase expression was determined with PCR and immunocytochemistry. Total RNA was isolated from the parotid glands of healthy adult Sprague-Dawley rats, and cDNA analyzed by RT-PCR. Primers for specific flippases/floppases or translocases were designed from mRNA sequences selected from NCBI nucleotide databases. PCR products were visualized by agarose gel electrophoresis. The mRNA levels for the selected proteins were quantitated by real-time PCR using the QuantiTect SYBR Green kit protocol and the expression normalized to GAPDH. Immunohistochemistry on paraffin embedded parotid sections was performed following antigen retrieval, to localize some of the identified proteins. The sections were imaged by confocal microscopy. **Results:** Previous microarray analyses in the lab suggested the presence of the flippases/floppases ABCA1, ABCB6, ABCD3, ABCF1, ABCG1, ATP11A, ATP11B, CABC1 and the scramblases PLSCR1 and PLSCR2, during parotid gland development. In the present investigation, PCR analysis of these proteins demonstrated strong bands for ABCA1, ABCG1, ATP11A and CABC1 genes and weak bands for ABCB6, ABCD3, ABCF1, ATP11B, PLSCR1 and PLSCR2 genes. Quantitative PCR analysis showed a high level of expression for ATP11A suggesting that it could be the major flippase of the parotid gland, followed by CABC1. ABCA1, ABCB6 and ABCF1 were also strongly expressed. ATP11B, ABCG1, PLSCR1 and PLSCR2 were weakly expressed. Immunocytochemistry revealed strong expression of ABCA1 towards the lumen of the acinus as well as on the cell membrane. ABCG1 was not detected. **Conclusion:** Several flippases/floppases or scramblases that could be potentially involved in the secretory process are found in the healthy parotid gland. ATP11A and CABC1 have the highest levels of mRNA expression of those studied. Further analysis of these proteins will help elicit their role in protein sorting in the parotid gland.