The Faculty Club

Recognizing Excellence in Translational Research

Celebrate Innovation in Basic Science and Clinical Research

Faculty Club Reception
Honoring Outstanding Current Research
May 1, 2008 6 to 8 p.m.
Joslyn Castle
3902 Davenport Street

Creighton UNIVERSITY
School of Medicine

Faculty Club Presentations

Innovations in Basic Science and Clinical Research

May 1, 2008
Joslyn Castle

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1. Dr. Mohammed Akhter
2. Dr. Olga Bajenova
3. Dr. Jason Bartz
4. Dr. Archana Chatterjee
5. Dr. Chris Destache
6. Dr. Naresh Dewan
7. Dr. Frank Dowd
8. Dr. Cristina Fernandez
9. Dr. Bernd Fritzsch
10. Dr. Zoran Gatalica
11. Dr. Thomas Guck
12. Dr. Richard Hallworth
13. Dr. Joseph Knezetic
14. Dr. Robyn Kondrack
15. Dr. Stephanie Maciejewski
16. Dr. Sumeet Mittal
17. Dr. Thomas Murray
18. Dr. Ruby Satpathy
19. Dr. Poonam Sharma
20. Dr. Xuedong Shen
21. Dr. David Smith
22. Dr. Patrick Swanson
23. Dr. Yaping Tu
24. Dr. Meera Varman
25. Dr. Dennis Wolff
26. Dr. Peng Xiao

Effects of Glucocorticoid on Bone Structure and Strength by Genotype

Manolides AS, Cullen DM, Akhter MP

Creighton University School of Medicine, Omaha, NE

Glucocorticoids (GCs) are used to treat several diseases, but long-term use is fraught with adverse effects, particularly osteoporosis. GCs suppress the canonical Wnt/β pathway, resulting in decreased expression of critical bone proteins. Current focus is on the characterization of the low-density lipoprotein receptor-related protein 5 (Lrp5) gene and its role in osteoporosis. A G171V substitution in the Lrp5 gene in transgenic mice results in high bone mass (HBM). This study examined the influence of GC treatment on bone structure and strength in HBM, Lrp5 heterozygous knockout (KO, +/-) and wildtype (WT) mice. 71 mice were treated with either prednisone or placebo, and L4 vertebral bodies analyzed by micro computed tomography (CT) for bone structure, and mechanically tested in compression (along cranial-caudal axis) to determine structural strength properties. Differences in all measured variables corresponding to GC treatment and genotype were analyzed using two-way ANOVA ($P < 0.05$). GC treatment caused decreased strength parameters and disruption of bone structure in vertebral bodies of HBM, but not KO or WT mice. Despite treatment related loss, both bone structure and strength parameters remained elevated as compared to KO and WT mice. Vertebral cortical shell thickness was not different between treated and control groups of any genotype (data not shown). Results suggest a possible non-linear relationship between bone strength/structure and Wnt activity. Also possible are GC and/or Lrp5 mutation effects on unidentified pathways. In vertebral bodies, GC treatment appears to have its negative impact primarily on trabecular bone in HBM mice.

The abstract/poster was presented at Midwest Student Biomedical Research Forum (MSBRF, March 1, 2008, Omaha). The poster won First Place Award and is qualified to be presented in National meeting (National Student Research Forum-2008, Houston, TX)

Interactions in Colorectal Carcinoma Cells

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Carcinoembryonic antigen (CEA) is a glycosylated protein that is used as a tumor marker for predicting recurrence in gastrointestinal malignancies. CEA has been implicated in the development of hepatic metastasis of human colorectal cancers. Although clinical and experimental data suggest that CEA plays a role in the formation of hepatic metastasis, the mechanism by which CEA causes an enhancement of metastasis has yet to be elucidated. Previously we discovered a CEA-binding protein in colorectal cancer cells and macrophages. This protein was identified as heterogeneous nuclear RNA-binding protein M (hnRNPM). In this study we analyze the effect of CEA and hnRNPM on the function of adherent junction proteins in colorectal carcinoma. Our data show that in CEA-deficient MIP101 cells hnRNPM interacts with both beta- and alpha-catenin proteins. CEA produced by MIP101 transfectants (clone 6 and clone 8) disrupts these interactions, as well as the interaction between beta-catenin and adherent junction protein E-cadherin. Silencing of hnRNPM by small hairpin RNA (shRNA) positively correlated with the down-regulation in the expression of E-cadherin, beta-catenin, snail, integrin α3, integrin α 5 and beta-tubulin genes that are involved in epithelial-mesenchimal transition (EMT). The data show that as an initial step of metastasis CEA-induced detachment of tumor cells from the primary malignancy relates to the loss of cell-cell adhesion, and the E-cadherin/beta-catenin complex appears to play an essential role in this process.

Neuroinvasion Following Nasal Cavity Infection in Hamsters

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Background: Some animal species that acquire prion diseases are olfactory-driven and use their sense of smell for a variety of basic behaviors. Therefore their nasal cavity could be an initial site of contact for any material in the environment that harbors the infectious agent. Recently it has been demonstrated that the nasal cavity is a more efficient route of infection than per os inoculation in hamsters. The diseases-associated form of the prion protein (PrPd) was detected in nasal associated lymphoid tissue and submandibular lymph nodes as early as 4 weeks after placement of infected brain homogenate inferior to the nostrils. Surprisingly, there was no evidence of PrPd in the olfactory epithelium or olfactory nerve fibers at any time after inoculation.

Objectives: The aim of this study was to determine the route by which PrPd was transported into the central nervous system following extranasal prion infection of hamsters.

Methods: The brain, spinal cord, trigeminal ganglia and superior cervical ganglia were collected at 2 week intervals following extranasal inoculation of infected brain homogenate and processed for immunohistochemical (IHC) detection of PrPd.

Results: PrPd was initially identified in the brainstem and the intermediolateral cell column of the spinal cord at 12 and 14 weeks post inoculation, respectively. In the brainstem PrPd was restricted to the sensory root of the trigeminal nerve (V), the spinal tract of V, the principal nucleus of V and the spinal nucleus of V at the earliest time points after inoculation. However, PrPd was not identified in the Vth ganglia or the superior cervical ganglia until 22 weeks post inoculation.

Conclusions: The results of this study suggest that neuroinvasion following prion exposure to the nasal cavity occurs via the trigeminal and sympathetic nerves. The lack of PrPd in the trigeminal and superior cervical ganglia prior to spread into the CNS suggests that the agent does not accumulate to any degree within these structures prior to neuroinvasion, remaining undetectable with IHC until just prior to the onset of clinical symptoms.

Evaluation of Surgical Antibiotic Prophylaxis at a Children's Hospital

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Background: Appropriate antimicrobial prophylaxis has been shown to reduce the incidence of surgical site infections (SSIs).

Objective: The purpose of this study was to evaluate the administration of antibiotic prophylaxis in surgical patients at Children's Hospital in Omaha, Nebraska.
Methods: The data were obtained through a retrospective chart review of medical records for surgeries performed at Children's Hospital in Omaha, Nebraska from January 1 to December 31, 2006. The types of procedures included general surgery (GS), cardiothoracic surgery (CTS), neurosurgery (NS), and orthopedic surgery (OS). The type and dose of antibiotic used, the time of initial administration of the antibiotic with respect to the time of incision, the time of intra-operative administration of antibiotics (if pertinent), and the duration of post-operative administration of antibiotics were recorded. Outcomes recorded included detection of an SSI before the patient was discharged, the patient's length of stay (LOS) in the hospital and in the Intensive Care Unit (ICU), and whether the SSI resulted in mortality. The four most common failures of compliance with prophylactic protocols including administering an inappropriate dose of antibiotic, selecting an inappropriate antibiotic for the specific surgery, inappropriate timing of pre-operative and post-operative administration of the antibiotic, and inappropriate administration of intra-operative antibiotics were noted. The degree of adherence to the CDC guidelines for surgical antibiotic prophylaxis were also recorded.

Results: A total of 1397 surgeries in the 4 selected categories were reviewed. There were 20 SSIs, and 7 mortalities. The LOS was increased due to an SSI in 6 of the 20 cases; the ICU stay was prolonged in 12 of the 20 cases. Of the 20 SSIs, 7 had no pathogen identified, 5 had Staphylococcus aureus, 4 had methicillin-resistant Staphylococcus aureus, 3 had coagulase-negative Staphylococcus, 3 had Gram-negative rods, and 2 had other organisms cultured from the surgical site. The SSIs were seen in the NS, GS and CTS and none resulted in mortality. There was complete compliance with the CDC guidelines only 46.1% of the time. Compliance with at least three of the four criteria occurred 90.7% of the time and compliance with at least two of the four criteria, 100% of the time. Inappropriate timing of antibiotic administration was noted in 39.1% of the procedures. There was no noted association between compliance with CDC guidelines and occurrence of SSIs.

Conclusions: There were few SSIs during the period of study but compliance with CDC guidelines was not consistent. Most notably, the antibiotics were given at an inappropriate time in more than one-third of the cases. Thus, the hospital could benefit from implementing a more strict practice of the correct timing in prophylactic antibiotic administration.

Ritonavir-, Lopinavir-, and Efavirenz-containing Nanoparticles: in vitro Release of Anti-Retroviral Therapy (ART)

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Ritonavir (RTV), lopinavir (LPV), and efavirenz (EFV) are considered appropriate HAART in the treatment of HIV-infected patients. We fabricated ART nanoparticles containing these 3 drugs and tested the release of the drugs from the nanoparticles over 28 days. Methods: RTV, LPV, and EFV (10 mg of each) were added to 200 mg poly-caprolactone polymer in methylene chloride. Pluronic-127 (200 mg) was dissolved in 2% poly vinyl alcohol. The two phases were combined in a water-in-oil-in water emulsion using a Teflon emulsion tip at 15 W (6 min). The organic phase was evaporated overnight, washed, frozen at -80C and lyophilized. Nanoparticles were tested for particle size and charge using photon correlation spectroscopy. Drug loading, entrapment efficiencies, in vitro release were determined ART nanoparticles (10 mg) were placed in PBS (pH 7.4 and 4.0) and 100 uL PBS was removed, centrifuged, and analyzed by HPLC for RTV, LPV and EFV concentrations. ART nanoparticles (10 mg) were placed in PBS (pH 7.4 and 4.0) and 9.9 ml were removed and replaced with 9.9ml of fresh PBS. Samples were taken every 2 hours for 8 hrs, and then 1, 2, 3, 4, 6, 8, 10, 14, 21 and 28 days. Drug levels were analyzed by HPLC. Intra-day and inter-day variability were both < 10%. Results are presented as mean + SEM. Results: Nanoparticles size was 369.2 + 83.9 nm. Mean zeta potential was -25.1 + 12.8. ART loading averaged 38.3%. Loading efficiency was 18.2%, 30.5%, 46.4% for RTV, LPV, and EFV, respectively. Entrapment efficacy averaged 97.4%. In vitro release of RTV, LPV, and EFV occurred over 28 days. Peak ART levels for pH 4.0 (simulating endozome pH) occurring at day 6 with levels of 5.0 + 0.3 mg/mL for RTV, 4.7 + 0.2 mg/mL for LPV and 4.6 + 0.6 mg/mL for EFV. Peak RTV, LPV, EFV levels at pH 7.4 were 6.3 + 1.0, 4.9 + 0.4, and 5.6 + 0.9 mg/mL, respectively occurred at 4 days. The ART levels at 28 days were all > 3.5 mg/mL. In vitro measurements where 9.9 mLs PBS was removed and replaced, the peak level (pH 4.0 and 7.4) were all > 4.0 mg/mL, at time 0 hr. The day 28 levels for RTV, LPV, and EFV were all > 0.3 mg/mL. Cellular assays demonstrate that NPs are readily taken up into human MDMs. Conclusions: Results of these experiments demonstrate that RTV, LPV, and EFV release from our nanoparticle formulation occurs over 28 days. ART nanoparticles could be a possible delivery system for multiple anti-retroviral agents in the future.

Presented at: Conference on Retrovirus and Opportunistic Infections, Boston, Feb 3-6th

Case/Self Management for COPD: a Randomized Controlled Trial

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Rationale: The aim of our study was to determine whether a limited case/self management program reduces hospital admissions and emergency department (ED) visits due to COPD exacerbations. Methods: This was a prospective multi-center, randomized, controlled, 1 yr study of case/self management (1hr educational and clinical assessment session, adjustment of respiratory medications according to guidelines, a written action plan with refillable prescriptions for antibiotics and prednisone bursts, and a monthly telephone call from the case manager) compared to usual care. Eligibility included a hospital admission, ED visit, systemic steroid use, or use of home O2 for COPD. Results: 743 patients were enrolled at 5 VA sites. Baseline characteristics were similar in both groups. The composite primary endpoint of hospitalizations and ED visits for COPD was 49.0/100 pt yr in the intervention group and 83.1 in the usual care group ($p < 0.0001$, 41% reduction; reduction in COPD hospitalizations 31%, ED visits 51%). The difference in St. Georges Respiratory Questionnaire scores at 1 yr was 4.9 in favor of case/self management ($p< 0.001$). Conclusions: A COPD case/self management program in a VA setting reduces hospital admissions and ED visits due to COPD exacerbation.

	Usual Care N=370	Self/Case Mgmt N=373	P (difference; 95% CI)
FEV1 % pred (baseline)	38	36	
Hospitalization or ED visit for COPD/100 pt yr Hospitalization	83.1	49.0	<.0001 (34.1; 22.1, 46.1)
Hospitalization	40.4	27.9	<0.01 (12.5; 3.9,21.2)
ED visit	42.7	21.1	<0.0001 (21.6; 13.2,29.9)

Funded By: VA VISN 23

Muscarinic Receptor Subtypes and ERK in the Mouse Parotid Gland

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[2] University of Washington, Seattle, USA

A previous study indicated that carbachol stimulated the ERK pathway independently of the EGF receptor pathway. Carbachol stimulation required protein kinase C but not calcium. Objectives: The purpose of this study was to determine the role of the 2 mouse parotid muscarinic receptor subtypes in the ERK signaling pathway. Methods: Mouse parotid gland cells were incubated with 5 μM carbachol (near EC50 value) with at least 7 different concentrations of subtype-selective muscarinic receptor antagonists as well as atropine. The cells were lysed and the supernatant subjected to electrophoresis and immunoblotting to determine the level of phosphorylated ERK relative to total ERK. Inhibition curves were analyzed by non-linear regression analysis and IC50 values were determined. Results: The following muscarinic receptor antagonists were used: pirenzepine (M1 selective), methoctramine (M2 selective), 4-DAMP (M3 selective) and atropine (non-selective). Antagonism of carbachol-stimulated ERK phosphorylation yielded near-parallel inhibition curves when comparing the 4 antagonists. The IC50 values (micromolar) for atropine, 4-DAMP, pirenzepine and methoctramine were 0.43±0.18, 0.75±0.26, 122.7±24.0, and 3527±725, respectively (n = 3 or 4 inhibition curves). This rank order of IC50 values closely matched the values for the antagonists in binding studies as well as the antagonists' effects on phosphoinositide turnover and cyclic AMP levels that we have previously reported. Conclusions: The results indicate that, just as the M3 receptor controls the calcium signaling pathway in the mouse parotid gland, the M3 receptor is the major muscarinic receptor controlling ERK. Supported by Grant DE05249 from NIDCR-NIH.

AN INNOVATIVE TOOL FOR TACKLING CHILDHOOD OBESITY

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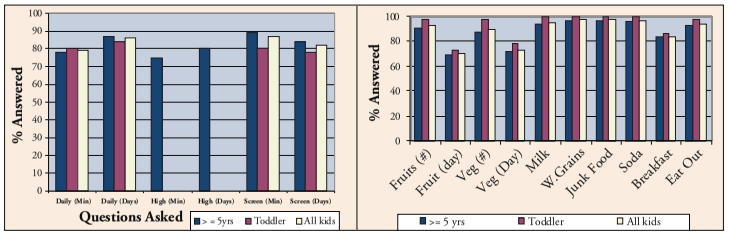
BACKGROUND

Childhood obesity is a growing epidemic throughout this country. Previous studies have shown that 33% of the children in Omaha, Nebraska are obese or at risk (BMI > 85th %). Creighton University Medical Center's Pediatrics encounters about 1200 health care maintenance visits annually.

Figure 1. Physical Activity and Nutrition Assessment Form.

PURPOSE

Working with the Nebraska Health and Human Services Department, a new clinical tool called the Physical Activity and Nutrition Assessment Form (PANAF) was developed to improve the efficiency of a health care maintenance visits **(Figure 1)**. The form is completed by the parent or child in the waiting room. If the patient is ready for a lifestyle change, the physician and patient use the form's responses to create a modification in the child's activity or diet.



Figures 2 and 3. Percentage of each question on form that was completed by respondents.

METHODS

The PANAF responses were analyzed from 200 health maintenance visits over a 3 month period. Physicians & office staff were trained on the form's dynamics –

- assess the patient's readiness for change
- make small changes (20-50%) from the original behavior
- make 1 to 2 changes at a time.

Visits were analyzed to assess patients' ability to complete the form, quality of answers provided, and what type of response the physician prescribed.

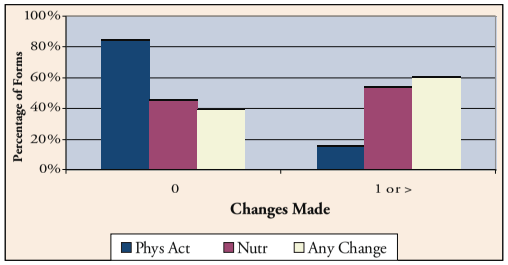
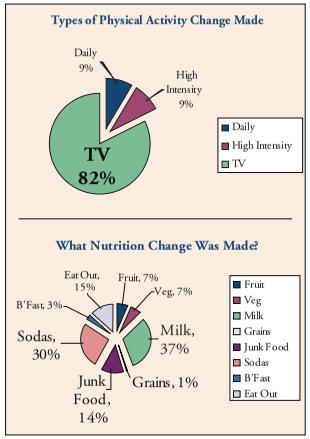


Figure 4 (Above). Percentage of recommendations made.

RESULTS

All of the questions were answered by at least 70% of respondents **(Figures 2 & 3)**.

- At least one change was recommended and agreed upon in 61% of participants **(Figure 4)**.
- A physical activity change was recommended in 16% of the patients.
- 54% of patients had one nutritional change made.
- 82% of responders agreed to a decrease in screen time (television, computer, text messaging) **(Figure 5)**.
- 37% agreed to changing their milk consumption **(Figure 6)**.



Figures 5 and 6. Types of physical activity changes (upper) and nutrition changes (lower).

CONCLUSION

The PANAF is a useful tool for creating a more efficient dialogue about improving overall health & nutrition of children in health care maintenance visits. The tool can be used in children starting at age 2 and does not need to be limited to merely obese or at risk children. A majority of respondents were able to complete the form. A majority were ready for a lifestyle change either in physical activity or nutrition.

SOX2 is Essential for Sensory Neuron Survival in the Inner Ear

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Inner ear function requires coordinated development of the sensory neurons and the sensory epithelia, which are composed of hair and supporting cells. Sox2 is expressed in the common precursors of these cell types and is essential for the specification of the sensory epithelia. Sox2 is also expressed in the sensory neurons as they differentiate, but its role in their development is unknown. We find that Sox2 is transiently expressed in delaminating otic neuroblasts concomitant with Neurogenin1 (Neurog1), a gene required for their specification. In the Sox2Lcc/Lcc regulatory mouse mutant, which lacks inner ear expression of the gene and sensory epithelia do not develop, neuroblasts delaminate and express Neurog1. Consistent with this expression, some sensory neurons develop and their processes project to areas where sensory epithelia would normally be present, but these neurons soon disappear. BDNF expression was absent in the Sox2Lcc/Lcc cochlea, implying loss of neurons in later stages is due to the absence of the sensory epithelia, which would normally secrete molecules promoting neuronal survival. In the hypomophic regulatory mutant Sox2Ysb/Ysb, BDNF was still expressed in patches of hair cells and nerve fiber bundling was disrupted to mirror those patches. Our data implies specification and initiation of differentiation of otic sensory neurons does not require Sox2 and is independent of the development of sensory epithelia. Sox2 is, presumably indirectly, required for maintenance of innervation through its effect on sensory epithelia development. Sox2 probably acts independently of Neurog1 because it is expressed in the sensory epithelia of Neurog1 null mice.

Adenoid Cystic Carcinoma of the Breast Expresses ER-A36
A Novel Alternatively Spliced Variant of Human Estrogen Receptor-A (ER-A66)

Zoran Gatalica, Hao Deng, Snjezana Grazio, Janez Lamovec, Juan Palazzo and Zhaoyi Wang

Background:
Adenoid cystic carcinoma (ACC) of the breast is considered a special type of breast carcinoma with favorable prognosis, that does not express classic estrogen receptor alpha (ER-α66), progesterone receptor (PR) and HER-2. We have investigated expression of a novel alternatively spliced variant of ER-α66, named ER-α36, which we recently identified and cloned (Wang ZY et al, PNAS 2006;103: 9063) and demonstrated that it mediates membrane initiated steroid signaling (MISS). MISS is dependent on ER interaction with receptor tyrosine kinases, and we hence investigated expression of epidermal growth factor receptor (EGFR) in ACC.

Methods:
14 patients with breast ACC and with long follow-up data (up to 27 years) were studied for expression of ER-α66, PR, HER-2, EGFR and ER-α36 using immunohistochemical methods. ER-α36-specific antibody was custom-made by the Alpha Diagnostic International (San Antonio, TX) against the unique 20 amino acids at the C-terminal of the ER-a36, other antibodies were from commercially available sources. Mammary carcinoma cell line (MDA-MB-231) that lacks expression of ER-α66, PR and HER2, but expresses ER-α36 and EGFR, was used as a positive control.

Results:
No lymph node metastasis (pN0) was seen in any of the ACC cases at the time of the surgery. However, five of 14 patients developed metastatic disease (between 6 and 23 years after the surgery), and two died of disease (7 and 27 years after the surgery). No tumor showed ER-α66, PR or HER2 expression, but 8/11 cases showed ER-α36 expression in membranous and cytoplasmic distribution. EGFR was expressed in 8/14 cases.
In an experimental model of triple negative mammary carcinoma (MDA-MB-231 cells), 17β-estradiol (E2) strongly induced rapid phosphorylation of the MAPK/ERK1/2, but had only a weak effect in ER-α36 siRNA knock-down MDA-MB-231 cells.

Conclusion:
Despite earlier claims that ACC rarely metastasize, this tumor type is a typical example of a "triple negative" breast carcinoma with significant mortality upon long follow-up. Although consistently negative for ER-α66 expression (using routinely available antibodies for diagnostic purposes), ACC expresses ER-α36, and it is this receptor variant that shows strong E2 signal transduction and an increased cell growth. This non-genomic estrogen signaling probably involves membranous interaction between EGFR and ER-α36, resulting in activation of mitogen activated protein kinase pathway.

Relationship Between Acceptance of HIV/AIDS and Functional Outcomes Assessed in a Primary Care Setting

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Individuals with HIV/AIDS are living longer; requiring a paradigm shift that views HIV/AIDS as not only an acute possibly fatal disease but also as a chronic condition in need of long term management. Acceptance, a concept which requires patients to reconceptualize their condition from an acute to a chronic perspective and then to function despite the condition, has been found important in the treatment of chronic pain and may have relevance for patients with HIV/AIDS. Two pain acceptance inventories were rewritten to measure acceptance of illness, the Illness and Impairment Relationship Scale (IAIRS) a single scale that measures the extent to which patients accept their illness yet remain functional despite it and the Chronic Illness Acceptance Questionnaire (CIAQ), consisting of two scales Activities Engagement (AI) and Illness Willingness (IW). Sixty-nine individuals with HIV/AIDS completed the two illness acceptance inventories along with the Short Form-12 (SF-12) a widely used objective measure of overall physical and mental health outcomes and a 13-item version the Beck Depression Inventory (BDI) during routine visits with their primary care physician. Excellent internal reliabilities obtained for the IAIRS and the AI and IW scales of the CIAQ were .81, .84, and .79 respectively. All three acceptance of illness scales were significantly correlated with the physical and mental health scales from the SF-12 and the BDI. Regression results indicated that the acceptance measures accounted for a significant portion of the variance beyond that found for demographic variables in the prediction of each of the outcome measures. These findings indicated that acceptance and its measurement can be applied to a chronic illness conditions such as HIV/AIDS. Successful medical treatments for HIV/AIDS leading to longer life may require attention to cognitive-behavioral issues such as acceptance so that patients can function well despite their illness.

Using Two-photon, Two-channel, Metabolic Imaging to Determine the Metabolic Status of the Cochlea

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Metabolism and mitochondrial dysfunction have been proposed to be involved in many different hearing disorders including noise induced hearing loss. We have employed two-photon fluorescence imaging of electron carriers to study the metabolic status of the different cell types in excised, intact mouse organ of Corti preparations. Reduced nicotinamide adenine dinucleotide (NADH) and the oxidized forms of flavoproteins (Fp) both fluoresce when excited by femtosecond pulses of 740-nm light. Since NADH fluoresces only when reduced and Fp only when oxidized these two intrinsic fluorophores have been used to determine the relative percentages of oxidized and reduced energy equivalents in cells. An evaluation of the hemicochlea and an intact cochlea explant was conducted. Stability of the explanted preparation was improved upon from previous experiments and will be discussed for different turns.

Supported by NIH DC 02053, National Science Foundation-EPSCoR EPS-0346476 (CFD 47.076) (to RH).

Pathobiology of Cancer: Polymorphisms in Folate Cycle Enzymes Influence Progression to Esophageal Adenocarcinoma

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Objective: To determine which Single Nucleotide Polymorphisms (SNPs) in folate pathway genes influence progression from reflux disease (GERD) to Barrett's esophagus (BE) and esophageal adenocarcinoma (BA).

Background: In BE, acid reflux is involved in replacement of normal squamous epithelium with columnar, intestinal type epithelium. The increased risk of adenocarcinoma development in patients diagnosed with BE is 30 to 40-fold. Experimentally, exposure to the bile salts present in the reflux contents has been shown to increase proliferation in BE tissues. Cellular proliferation may produce localized increased need for nutrients, including folate. Folate participates in the de novo synthesis of purines and pyrimidines, and folic acid deficiency restricts thymidylate synthesis, where conversion of uracil to thymidine is the rate limiting step in DNA synthesis. The resulting unbalanced nucleotide pool leads to mis-incorporation of nucleotides and DNA strand breaks, which can manifest in cancer development. Additionally, formation of S-adenosylmethionine (SAM) results from transfer of the methyl group in the folate cycle to the methionine cycle. SAM is the universal methyl donor in biosynthesis and DNA methylation. Methylation of DNA in CpG islands directly regulates gene expression and aberrant methylation patterns can lead to dysfunctional cell proliferation and cancer progression. Single nucleotide polymorphisms (SNPs) have been identified in the enzymes of both the folate and methionine cycles. These SNPs result in amino acid substitutions and influence enzyme activity.

Hypothesis: Alterations in the enzymes of the folate and methionine pathways influence the progression of esophageal reflux disease to Barrett's adenocarcinoma by altering the methylation patterns of key genes in cancer development. The targeted genes include tumor suppressor genes, genes involved in metastasis and invasion and DNA repair enzymes.
Design: Polymorphisms in folate cycle enzymes were evaluated in >300 patients, using either allele-specific TaqMan® probes on the SmartCycler II system (MTHFR 677C>T, MTHFR 1298A>C, MTR 2756A>G and MTRR 66G>A) or allele-specific TaqMan® MGB probes on the 7500 Fast Cycler (SHMT 1420C>T, MTHFD 401T>C, MTHFR 1958G>A). Experimental groups (>100 per groups) consisted of patients with BE or BA, and the age-matched control group consisted of >100 patients with GERD.

Results: For MTRR, a decrease frequency of 66A was detected in the BE/BA group (62.4%, controls 72.2%, Pearson's correlation, $p<0.05$). An increased frequency of MTR 2756G was detected in the BE/BA group (41.7%, controls 30.3%, Pearson's correlation, $p <0.025$). Together, the presence of the G allele (MTR variant and MTRR WT) was found in increased frequency for the BE/BA group (34.7%, controls 25.3%). No significant differences were detected in the frequency of the five MTHFR, MTHFD or SHMT polymorphisms.

Conclusion: Together, these results suggest a role of these polymorphisms in esophageal cancer development and progression. Given that the folate and methionine cycles intersect with the activity of MTR, polymorphisms in the methionine cycle may play a more substantial role in cancer development and progression, particularly with respect to DNA methylation. Further studies will investigate polymorphisms in the methionine cycle and CpG hypermethylation of target genes.

Cost Analysis of an Intensive Smoking Cessation Intervention in Cardiac Patients

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Introduction: Overwhelming evidence indicates that smoking cessation interventions (SCI) are associated with substantial quit rates in hospitalized smokers with cardiac disease. Most hospitals do not offer formal smoking cessation interventions which may result from concerns that such programs are not cost-effective. We recently demonstrated that our SCI not only reduced rates of smoking, but also reduced morbidity and mortality. A cost analysis of this study was performed.

Methods: The methods and outcomes of our SCI have been previously published (Chest 2007;131:446-452). Costs of the SCI and costs of direct medical care in the intervention (INT) and usual care (UC) groups were prospectively determined over the 5 years subjects were enrolled and treated. Costs were discounted by 5% per year, sensitivity analyses were conducted as well as a cost-effectiveness (CE) ratio.

Results: A total of 209 smokers hospitalized with acute cardiac disease were included in the analysis. Cost of the SCI in the INT group was $247,376 over the 5 years. Direct medical care costs in the intervention group were $625,000 compared to $1,025,000 in the UC group. The use of the SCI was cost saving with the INT group having $152,625 less in total costs than the UC group. Sensitivity analysis indicated that hospital cost would have to be discounted by 70% before the SCI was no longer cost saving. The CE ratio, if calculated only on the cost of providing the SCI ignoring subsequent hospital costs, was $1443 per life year gained.

Conclusion: An intensive SCI is actually cost saving in high risk cardiac smokers. The intervention is extremely cost-effective. This study indicates that health care insurance payers should cover intensive tobacco cessation interventions.

Meeting where work was or will be presented: American College of Cardiology, Spring 08.

Amiodarone Side Effects and Monitoring: Temporal Trends, Adherence and Clinical Outcomes

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Background: Amiodarone is an effective antiarrhythmic agent with a substantial risk of toxicity. Monitoring is recommended with the following minimum: 2 clinic visits per year including thyroid function tests (TFTs) and liver function tests (LFTs), annual eye exam (AEE) and chest x-ray (CXR).

Purpose: The objective of this study was to evaluate adherence to the minimum monitoring standards for amiodarone in two cohorts of patients (one treated between 1988-1992 and the other 2000-2004). Adherence to monitoring was correlated with adverse drug reactions and outcome of therapy.

Methods: Consecutive patients initiated on amiodarone during 1988-1992 and 2000-2004 were followed prospectively. Only patients remaining on amiodarone for ÿ 12 mos were included. Patients with a minimum of 2 clinic visits having TFTs and LFTs, AEE and CXR were considered to be adherent to monitoring. Outcomes were categorized as (1) discontinued for lack of efficacy (DC-LOE); (2) discontinued due to SE (DC-SE); (3) continued with SE (C-SE); and (4) continued without SE (C-NoSE). Results: 577 pts initiated on amiodarone during 1988-1992 and 553 during 2000-2004 were included. Mean follow-up was 22 months.

Outcomes for all 1130 patients include: DC-LOE 18%; DC-AE 38%; C-NoAE 22%; and C-AE 22%. The proportion of patients compliant with follow-up was significantly greater in 1988-1992 (67%) compared to 2000-2004 (55%; $p < 0.05$). Patients with the outcome of DC-AE were significantly more likely to be non-compliant with follow-up in both time periods ($p < 0.05$). The most common types of side effects in the DC-AE groups were pulmonary, hyperthyroidism, neurologic (tremor), and hepatic.

Conclusion: Pts compliant with follow-up are less likely to discontinue amiodarone due to side effects than non-compliant pts. It is hypothesized that compliant pts have side effects that can be managed clinically which reduces the severity of side effects and reduces the number requiring drug discontinuation.

Meeting where work was or will be presented at American College of Clinical Pharmacy Spring Forum.

Laparoscopic Repair of Intra-Thoracic Stomach

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Aim: Intra- thoracic stomach (ITS) represents the most extreme form of Para esophageal hernia (PEH). A high rate of recurrences after laparoscopic repair of PEH has prompted the widespread use of mesh for hiatal reinforcement. However mesh placement can have serious complications. We aim to show that mesh is not needed routinely as long as attention is paid to address short esophagus.

Methods: All patients undergoing laparoscopic repair of ITS by a single surgeon from January 2004 thru July 2007 are included. ITS is defined when nearly the entire stomach has herniated above the diaphragm. Objective evaluation for hiatal hernia recurrence was undertaken either by upright esophagram or upper endoscopy at least one year after surgery. Any symptomatic or asymptomatic recurrence was considered a failure.

Results: Forty-five patients underwent laparoscopic repair of ITS during the study period. Twenty-six (58%) were women. The mean age was 71.8 (range 48 to 88). Three (7%) patients required conversion to open including one patient who could not tolerate pneumo-peritoneum. Two conversions were for mediastinal bleeding .Four (9%) patients had mesh used for crus closure. Eleven patients (24%) were deemed to have short esophagus (SE) of which 5 underwent Collis-gastroplasty with fundoplication. Twenty-seven patients had a concomitant anti-reflux procedure performed. A total of 13 (29%) patients (6 with SE) had only sac excision and repair of hiatus defect. Twenty-eight patients had more than one year follow-up. Mean follow-up is 24.1 (range 12 to 43) months. One year follow-up was available in 96% (27/28) of patients; one patient has died in the interim due to unrelated causes. There was two (8%) anatomic failure both had a 1-cm asymptomatic hiatal hernia. All patients report a high degree of satisfaction with surgery (mean score 9.1 on scale of 1 to 10). There was no significant difference in satisfaction scores with/ without mesh use or with/without fundoplication.

Conclusion: The laparoscopic repair of ITS is safe and durable with high patient satisfaction at long term follow-up. The most important things to avoid the recurrence were meticulous dissection and to address the short esophagus. From our study, mesh is not needed routinely to achieve successful repair additionally role of fundoplication needs to be defined.

Experimental Biology 2008
American Society for Pharmacology and Experimental Biology
April 5-9, 2008

Brevetoxin Sensitizes Immature Cerebrocortical Neurons to NMDA Receptor Signaling Through Activation of Voltage-Gated Sodium Channels

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Brevetoxins (PbTx) are potent polyether neurotoxins that activate voltage-gated sodium channels. Inasmuch as [Na+]i has been shown to act as a positive regulator of NMDA receptor currents, we used immature cerebrocortical cultures to assess the influence PbTx-2 on NMDA receptor mediated calcium influx, excitotoxicity and neurite outgrowth. We first confirmed that PbTx-2 elevates [Na+]i in cerebrocortical neurons.PbTx-2 produced a concentration-dependent elevation of [Na+]i in DIV 2,4,6 and 9 cerebrocortical cultures.

PbTx-2 (30nM) leftward shifted the NMDA-induced Ca2+ influx concentration-response curves in cortical cultures.NMDA-induced excitotoxicity in DIV 4, 6 and 9, but not DIV 2, cerebrocortical cultures was also enhanced by PbTx-2. The most striking PbTx-2 potentiation of NMDA-induced [Ca2+]i elevation occurred in DIV 2 cultures. We used the specific Src kinase inhibitor PP2 to demonstrate that PbTx-2 potentiation of NMDA-induced elevation of [Ca2+]i was dependent on Src. Pharmacological studies showed that the PbTx-2 enhanced neurite outgrowth involves elevation of [Na+]i, enhancement of NMDA receptor signaling and engagement of the CaMKK pathway.

Left Ventricular Pacing Impedance is Related to Future Response to Cardiac Resynchronization Therapy

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Introduction: Cardiac resynchronization therapy (CRT) is an established treatment for patients with advanced heart failure, cardiomyopathy and intraventricular conduction delay with wide QRS complex >120 ms. However, many questions have yet to be answered, including how to better identify patients who are going to be CRT responders and thus increase the overall CRT response rate. The relationship between left ventricular pacing impedance during bi-ventricular pacemaker implantation and future response to CRT is largely unknown. Methods: We studied 108 consecutive patients (age 69.9± 9.7 years, mean EF 20%) with CRT. There were 69 patients with ischemic and 39 patients with non-ischemic cardiomyopathy. Echocardiography was performed in all patients before and at last follow up of CRT. Positive response to CRT was defined as a decrease in left ventricular end systolic volume (LVESV) by more than 15%. Implantation lead parameters were recorded. Results: During follow-up of 15± 10.9 months after CRT, forty-four percent of our patient population (48/108) had positive response to CRT (baseline and post CRT LVESV 173.8± 82.1 ml vs. 110.9± 56.8 ml, $p< 0.0001$). Left ventricular lead capture threshold and pacing impedance during implantation in responders and non-responders were 1.83±1.02 vs. 1.88±1.1 volts and 895±13 vs. 1008±18 ohms respectively. Adjusted by gender, age, and clinical characteristics, CRT response was related to pacing impedance ($p< 0.05$) using logistic regression analysis. However, capture threshold was not predictive of CRT response ($p= 0.73$). Conclusion: Lower left ventricular pacing impedance during implantation is related to positive response to CRT. This suggests that lead site myocardial specific factors may play an important role in predicting future CRT response and selecting a good lead position during implantation can increase the overall CRT response rate.

	CRT Responders	CRT Non-Responders	SE	Wald	p
Gender	30m, 18f	54m, 6f	0.84	1.17	0.28
Age (yrs)	69.2±10.5	70.5±9.1	0.04	0.69	0.41
LVEF(%)	20.5±6.6	20.1±6.6	0.06	1.63	0.20
LV capture threshold (Volts)	1.83±1.02	1.88±1.1	0.29	0.12	0.73
LV Pacing Impedance(Ohms)	895±13	1008±18	0.001	4.01	< 0.05

Meeting where work was or will be presented HRS 2008, San Francisco, CA

Expression Profile of MUC4 in Gastric Adenocarcinoma and its Functional Significance

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Introduction: MUC4 is a large, heavily glycosylated trans-membrane mucin, which is overexpressed in various cancers. It has been shown that MUC4 overexpression promotes tumorigenesis and metastasis in cancer. Based on this observation, we proposed that MUC4 may be overexpressed in gastric cancer and, may be associated with the aggressivenessofgastricadenocarcinomas.

Methods: We investigated MUC4 expression in gastric cancer cell lines and tissue. Tissue microarray (TMA) comprising of gastric cancer (n=83) and normal gastric tissue (n=45) was used to detect expression of MUC4 using immunohistochemistry. Expression of MUC4 was characterized in 3 gastric cancer cell lines (KATOIII, AGS & MKN45) using immunoblot assay. Confocal immunoflorescence microscopy was performed for exact localization of MUC4 protein in the cells. To further analyze the role of MUC4 in gastric carcinogenesis, MUC4 was ectopically expressed in AGS gastric cancer cell line, which is negative for MUC4 expression, using an engineered MUC4 cDNA construct (MUC4 minigene). The MUC4 overexpressed clones were used for in vitro functional studies(motility and aggregation assays).

Results: Immunohistochemical analysis of gastric cancer tissue microarray slides (TMA) showed a significant difference in MUC4 expression between normal and gastric adenocarcinoma ($p<0.001$). Out of three gastric carcinoma cell lines, MUC4 expression was found in the SRCC cell line (KATOIII) and absent in AGS and MKN45 cell lines. MUC4 expression was found on the cell membrane. MUC4 was ectopically expressed in AGS cell line (using MUC4 minigene) and used for in vitro motility and aggregation assay. MUC4 overexpressed clones showed significant increase in motility and a decrease aggregation when compared to the vector transfected cells ($p<0.005$).

Conclusions: Results show that MUC4 is overexpressed in gastric adenocarcinoma. Overexpression of MUC4 in gastric adenocarcinomas may have a role in promoting the aggressive properties of gastric cancer. MUC4 may have potential prognostic value as a marker of aggressive types and may serve as potential therapeutic target. Further, in vivo studies using animal models will clarify the role of MUC4 mucin in gastric cancer.

Presented at Gastrointestinal symposium, American Society of Clinical Oncology (ASCO) meeting, Orlando, Florida on 1/25/08.

Left Ventricular Restrictive Filling as a Predictor of Outcome in Patients with Cardiac Resynchronization Therapy

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We hypothesized that left ventricular restrictive filling (LVRF) is a predictor on responses to cardiac resynchronization therapy (CRT), mortality and regression of left ventricular mechanical dyssynchrony (LVMD). Method: We studied 100 consecutive patients on CRT (male 73, female 27, age 69.9± 9.6 years). Patients with atrial fibrillation and mitral valve stenosis were excluded. Patients were divided into normal or relaxation abnormality (Group I), pseudonormalization (Group II) and restrictive filling (Group III), according to the pattern of mitral flow and annulus motion by pulsed wave (PW) and tissue Doppler (TDI). The time difference (TPW-TDI) between QRS onset to the end of LV ejection by PW and QRS onset to the end of the systolic wave in basal segment with greatest delay by TDI was measured before CRT and 14.4 ± 10.5 months after CRT. TPW-TDI > 50ms was defined as LVMD. Positive response to CRT was defined as left ventricular end systolic volume decrease of ÿ15% after CRT. Results: Group I, II and III before and after CRT consisted of 29, 38 and 33 patients and 46, 28 and 26 patients. The percentage of CRT responders in Group III was lower than in Group I (p< 0.05). TPW-TDI in Group III did not decrease after CRT (p= 0.46, Table). Patients with LVRF either before or after CRT predicted a higher mortality compared to Group I (33% vs 7%, p= 0.03 and 42% vs 4%, p< 0.001) during follow-up of 17.0± 10.6 months.
Conclusion: Patients with LVRF predicted a lower rate of positive response to CRT and a higher mortality after CRT.

		Group I	Group II	Group III	p (Group I vs III)	p (Group II vs III)	p (among three)
LVEF (%)	Baseline	20.9±7	19.8±7	20.7±6.1	0.92	0.56	0.80
	After CRT	28.2±14.8	24.3±11.4	25.5±10.6	0.39	0.65	0.68
	p	0.02	0.04	0.03			
Responders (%)		62% (18/29)	37% (14/38)	33% (11/33)	< 0.05	0.95	<0.05
TPW-TDI (ms)	Baseline	90.1±53	72.5±42.1	63.6±49.3	0.05	0.41	0.03
	After CRT	44.9±23	44.9±29.5	55.4±39.2	0.21	0.2	0.69
	p	< 0.001	0.001	0.46			

Meeting where work was or will be presented: Scientific Session of American College in Cardiology 2008 in Chicago

Synthesis of a 4-Benzyl-l-Histidine Derivative for Solid Phase Peptide Synthesis

D. David Smith, Michael Mao, Hayley Young and Martin Hulce

Calcitonin Gene-Related Peptide (CGRP) is a potent vasodilator that is distributed throughout the central and peripheral nervous systems. The serendipitous discovery that N-α-benzyl-[4-benzyl-His10]CGRP(8-37) is a potent, competitive antagonist at CGRP receptors highlighted the important role of position 10 in antagonist binding to receptors. Unfortunately, this peptide antagonist was originally synthesized as a minor byproduct of the solid phase synthesis of N-α-benzyl-CGRP(8-37). Consequently, the synthesis of the 4-benzyl-l-histidine was undertaken. Diastereomeric 4-phenylspinacine made by Pictet-Spengler reaction of l-histidine was hydrogenated to give 4-benzyl-l-histidine. Esterification to the methyl ester, Boc-protection of the α-amino group followed by protection of the alkylated imidazole side chain with a benzyloxymethyl- group and saponification afforded the desired histidine derivative suitably protected for SPPS. This new histidine analogue together with other 4-arylmethyl-substituted histidine analogues will serve as novel tools to elucidate the role of His10 in high affinity binding of antagonists to CGRP receptors.

Evidence for Long-Range RAG1/2 Interactions with Coding Sequences in Discrete V(D)J Initiation Complexes Assembled on Coupled Cleavage Susbstrates

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Antigen receptor genes in lymphocytes are assembled from gene segments by V(D)J recombination. The RAG1/2 proteins initiate V(D)J recombination by forming a synaptic complex with two different gene segments through interactions with recombination signal sequences (RSS), and then catalyzing a DNA double strand break at each RSS. The DNA ends generated by RAG-mediated cleavage are subsequently processed and joined by the nonhomologous end-joining repair pathway. RAG protein interactions with the RSS are well established; however, contacts between the RAG proteins and coding DNA have been difficult to observe experimentally, but are presumably important to help guide appropriate and efficient repair. One possible explanation is that previous studies of RAG-RSS interactions have largely relied on oligonucleotide substrates, which may be too short to support stable long-range protein-DNA interactions. To address this shortcoming, we analyzed discrete RAG-DNA complexes assembled on long PCR-generated paired RSS substrates for their activity and pattern of protein-DNA contacts using in-gel cleavage and footprinting assays. We demonstrate that optimal substrate cleavage requires the presence of two different RSSs. We also observe unique patterns of hypersensitivity to the chemical nuclease 1,10 phenanthroline-copper (Cu-OP) both within and outside the RSS, even well into the coding DNA. Interestingly, RAG-mediated protection of the coding DNA increases after substrate nicking and/or cleavage. The implications of these results are discussed.

This research is supported by grants from the NIH (AI055599) and State of Nebraska LB692 biomedical research program.

This work was presented at the Keystone Symposia on DNA Replication and Recombination in Santa Fe, NM in Feb. 2008.

Proteasome-Dependent Degradation of Regulator of G Protein Signaling 4 (RGS4) Controls Breast Cancer Metastasis

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Background: Breast cancer is the most common malignancy among American women, with more than 40,000 deaths each year. Aberrant G-protein-coupled receptor (GPCR) signaling contributes to metastasis, the major cause of breast cancer death. Although RGS protein family inhibits GPCR signaling, the role and mechanisms of RGS proteins in breast cancer metastasis have not been identified.

Results: Using the gene microarray technique, we found that RGS4 mRNA was selectively up-regulated over 1800-fold in metastatic breast cancer cells. However, due to proteasome-dependent degradation, RGS4 protein was barely detectable in these cells despite this high level of RGS4 transcription. Proteasome inhibitor-induced blockade of endogenous RGS4 protein degradation attenuated metastatic ability of theses cells, which can be abolished by silencing endogenous RGS4 expression using RGS4-targeted short hairpin RNA. Similarly, transient expression of exogenous RGS4, but not other RGS proteins in breast cancer cells also caused marked inhibition of these indices of tumor metastasis. Stable expression of RGS4, but not its functional deficient N128A mutant, in metastatic breast cancer cells effectively attenuated cell invasiveness both in vitro and in a mouse xenograft model. In addition, RGS4 selectively inhibits Gi-dependent activation of Rac, a key activator of breast cancer metastasis. Finally, immunohistochemistry studies of human breast carcinoma specimens indicated a strong correlation between RGS4 protein down-regulation and the invasive phenotype.

Conclusion: Collectively, our findings establish RGS4 as a novel inhibitor of breast cancer metastasis and its proteasome-dependent degradation is an important step in breast cancer progression.

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Meeting presented: Experimental Biology -2008, April 5-9, 2008 at San Diego, CA

Pediatric Patients with Asthma Exacerbation Evaluated in an Academic Versus a Community Hospital Emergency Department

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Introduction: Asthma is the most prevalent pediatric illness and is a common cause of hospitalization. Objective: To compare the pediatric patients seen in the ER with asthma exacerbation at two institutions: community Children's Hospital of Omaha (CH) versus Creighton University Medical Center (academic center-AC).

Method: Retrospective chart review of all patient visits under 18 years of age between January 2005 and December 2006.

Results: The median length of stay for AC was 2.6 hours compared to 1.6 hours at CH. Male pediatric patients made up 116/228 (51%) of asthma exacerbation cases at the AC, compared to 180/261 (69%) at CH, $p<0.01$. The racial demographic at AC was 167/228 (73%) African American and 33/228 (14%) Caucasians. This contrasts with CH where 155/262 (59%) were Caucasians and 66/262 (25%) were African Americans, $p<0.01$. The average age for patients seen in the AC ER for asthma exacerbation was 9.4 years, whereas it was 5.6 years at CH, $p<0.01$. At AC, 87/228 (38%) of patients received a CXR, compared to 59/262 (23%) at CH. There were a greater number of patients seen for asthma exacerbation in the autumn months than other months of the year.

Conclusion: The median length of stay was longer at AC than CH. The average age of patients was higher at AC compared to CH. There was a statistically significant male predominance at CH. Patients seen at AC were predominately minority compared to CH. Also, at AC more patients received radiologic evaluation than CH.

PIP3-Dependent Rac Exchanger 1 (P-Rex1) Promotes Prostate Cancer Cell Migration

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Activation of Rac by guanine-nucleotide exchange factors (GEFs) has been suggested to play an important role in cell migration which is critical for tumor metastasis. Here we investigated the role of P-Rex1, a novel specific GEF for Rac, in human prostate cancer cell migration. PCR and immunofluorescent staining showed that the expression of P-Rex1 was undetectable in normal prostate epithelial cells (PrEC) and less metastatic LNCaP cells but significantly increased in more metastatic prostate cancer cell lines (DU-145, PC-3-LN4 and PC-3). Our study also revealed increased P-Rex1 protein level in human prostate tumor specimens compared to the corresponding normal prostate tissues. Using an in vitro transwell migration chamber assay, we found that NIH-3T3 conditioned media (3T3-CM) can induce prostate cancer cell migration which positively correlates with expression levels of P-Rex1. Exogenously expressed P-Rex1 enhanced Gi-coupled receptor-dependent migration of PC-3-LN4 cells up to four-fold in a dose-dependent manner. Knockdown of endogenous P-Rex1 by small interfering RNA reduced the migration of PC-3 cells. Furthermore, exogenous expression of P-Rex1 in PC-3-LN4 cells induced strong Rac-dependent lamellipodia. Our results suggest that up-regulation of P-Rex1 promotes prostate cancer cell migration and contributes to the metastasis of prostate cancer. (The Nebraska State LB595 to Y. Tu.)

In Vivo Genome-Wide Expression Study of Human Blood B Cells Suggests a Novel ESR1 and MAPK3 Network for Postmenopausal Osteoporosis

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Osteoporosis is characterized by low BMD resulting from bone resorption (by osteoclasts) exceeding bone formation (by osteoblasts). Estrogen deficiency evokes increased osteoclastic activity and bone loss.

Studies showed that B cells may participate in osteoclastogenesis via expression of osteoclast-related factors, such as NF-kappaB ligand (RANKL), transforming growth factor beta (TGFB), and osteoprotegerin (OPG). However, the role of B cells in bone metabolism and osteoporosis is still largely unknown, particularly at the systematic expression level in vivo.

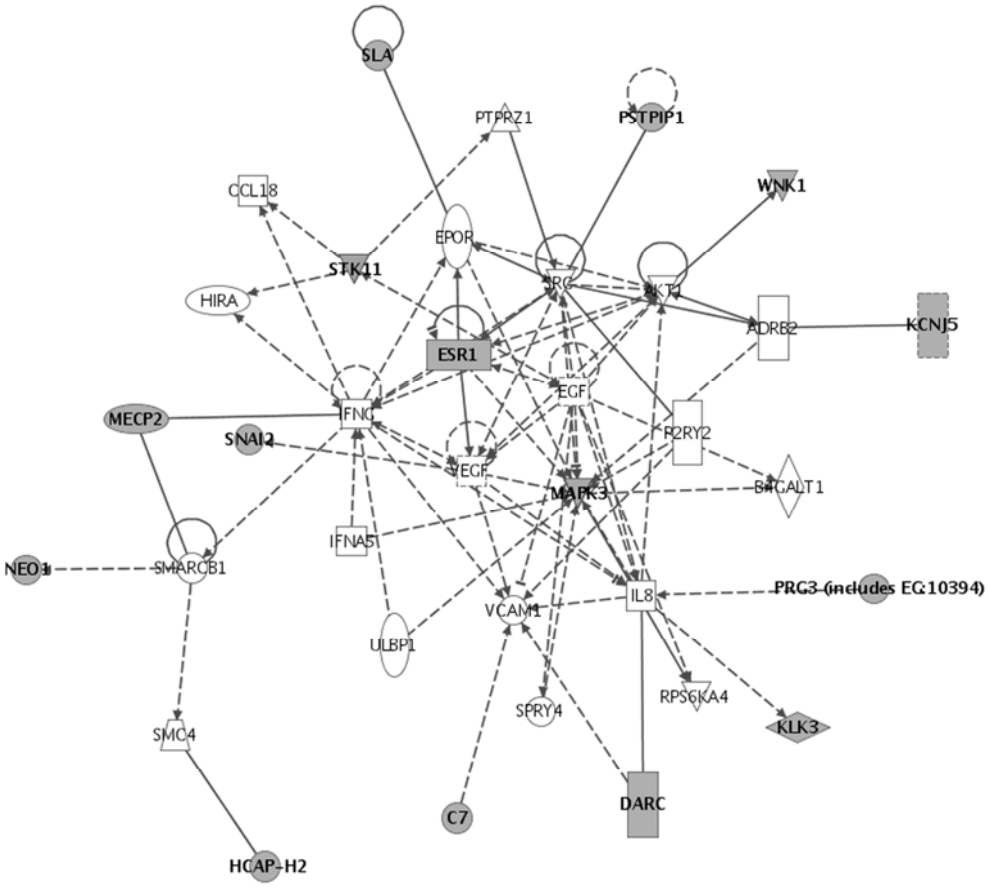
In this study, we recruited 20 unrelated postmenopausal Caucasian females aged 54 - 60, 10 with high (spine or hip Z-score > 0.84) and 10 with low BMD (spine or hip Z-score < -0.84). Total RNA of the freshly isolated blood B cells from those subjects were extracted and hybridized individually to Affymetrix HG-U133A GeneChip@ arrays to identify genes differentially expressed between low and high BMD subjects. Significance of differential expression was tested by t-test and adjusted with Benjamini and Hochberg (BH) procedure for multiple-testing.

Twenty-nine genes were down-regulated in the low vs. high BMD group. Those genes were further analyzed using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com) and a network involving estrogen receptor 1 (ESR1) and mitogen-activated protein kinase 3 (MAPK3) was identified (Fig. 1). Real-time RT-PCR confirmed the differential expression of 8 genes, including ESR1 ($p = 0.044$) and MAPK3 ($p = 0.002$).

This is the first in vivo genome-wide expression study on human B cells for osteoporosis. Our results suggest a novel mechanism for postmenopausal bone loss that downregulation of ESR1 and MAPK3 in B cells further regulates the secretion of factors leading to increased osteoclastogenesis or decreased osteoblastogenesis.

Presented in the 29th American Society of Bone and Mineral Research Annual Meeting, San Diego, LA, September 2008

Fig. 1


SLA
PTPRZ1
PSTPIP1
CCL18
WNK1
EPOR
STK11
HIRA
ESR1
ADRB2
KCNJ5
EGF
MECP2
IFNG
SNAI2
P2RY2
VEGF
B4GALT1
MAPK3
IFNA5
NEO1
SMARCB1
IL8
PRG3 (includes EG:10394)
VCAM1
ULBP1
RPS6KA4
SPRY4
KLK3
SMC4
DARC
C7
HCAP-H2