AADR March 2011 Abstracts (Summer 2010 Students)

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HPV Oncogenes E6 and E7 Directly Promote Human β-Defensin-3 Expression

Friday, March 18, 2011: 2 p.m. - 3:15 p.m.
Location: Hall C (San Diego Convention Center)
Presentation Type: Poster Session

B. CHOW, A. WEINBERG, and G. JIN, Dept of Biological Sciences, Case Western Reserve University, Cleveland, OH

Objectives: Human β-defensin-3 (hBD-3) is overexpressed in human papilloma viral (HPV) infection and carcinoma *in situ*; both involving the stratum basale of the oral mucosa. Since preliminary studies have demonstrated that HPV 16 E6 protein promotes hBD-3 expression in oral epithelial cells, our objective was to determine if HPV oncogenes E6 and E7 could contribute to overexpression of hBD-3 in oral cancer.
Methods: Oral tumor cells (Hsc-3) were infected with retroviral vectors containing E6 and E7 genes (24h), followed by RT-PCR and gel electrophoresis (2% agar) to determine changes in hBD-3 transcript levels of expression. Also, a 2.5kb hBD-3 promoter luciferase reporter was co-transfected (for 24h) with either an E6 or E7 expression vector into human embryonic kidney cells (HEK 293). Luciferase activity was measured after adding 50µl luciferin (10sec) to determine if E6 and E7 directly modulate hBD-3 gene expression.
Results: Hsc-3 cells infected with E6- or E7-containing retroviruses exhibited stronger expression bands for hBD-3 in the gel. Moreover, HEK293 samples transfected with either E6 or E7 (n=3/gp) showed higher luciferase activity (t-test, $p<0.05$).
Conclusions: Our *in vitro* studies demonstrate that both HPV type 16 E6 and E7 oncoproteins induce the expression of hBD-3 in oral squamous carcinoma cells. Moreover, HPV E6 and E7 proteins stimulate promoter activity of the hBD-3 gene, indicating that HPV oncogenes are able to up-regulate hBD-3 expression in oral cancer cells.
This work was supported by a Grant #IRG-91-022-15 from the American Cancer Society (GJ) and NIH/NIDCR P01DE019759 (AW)

Birth Factors and Presence of Teeth in Infants

Thursday, March 17, 2011: 2 p.m. - 3:15 p.m.
Location: Room 24B (San Diego Convention Center)
Presentation Type: Poster Discussion Session

M. EDGERTON[1], S. NELSON[2], M. HEIMA[3], L. KALKE[4], B. HATCH[5], G. FERRETTI[6], E. GRADY[6], K. KIRCHER[6], J. ALBERT[7], M. COLLIN[6], M. HACK[6], and P. MILGROM[8], [1]Community Dentistry, Case Western Reserve University, Cleveland, OH, [2]Dept. of Community Dentistry, Case Western Reserve University, Cleveland, OH, [3]Dept. of Pediatric Dentistry, Case Western Reserve University, Cleveland, OH,

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Objective: This study reports on whether birth factors were related to the presence of teeth in a cohort of 8 month old pre-term very low birth weight (VLBW) and full-term normal birth weight (NBW) infants recruited as part of an ongoing longitudinal study investigating the relationship between birth weight and early childhood caries (ECC). **Methods**: Data from a total of 237 (151 VLBW: mean birthweight 1051+/-267gms, 86 NBW: 3390+/-383 gms) infants who were seen at approximately 8 month corrected/chronological age follow-up visit was utilized. The infants were 66% African-American, 65% low socio-economic status, and 54% male. The caregivers were predominantly biological mothers with a mean age of 26.0 +/- 6.1 years, 53% with 12 years or less education, and 67% single. Birth factors were abstracted from medical charts and included birth group (VLBW and NBW), 1 and 5 minute Apgar scores, total days of hospitalization, feeding plan (breast milk, formula, both), gender, prenatal care, and maternal smoking and alcohol use. The infants received a visual dental exam to assess the presence/absence and the number of teeth at age 8 months. **Results**: Overall 22% of the infants did not have any teeth at age 8 months. The birth group, 1 and 5 minute Apgar scores, total days of hospitalization, feeding plan, gender, prenatal care, maternal smoking and alcohol use were statistically similar for infants with and without teeth. The mean number of teeth present was also similar in VLBW (2.9 +/- 2.9) and NBW (3.4 +/- 2.9) infants. **Conclusion**: VLBW infants who are medically compromised at birth are similar to NBW infants in the presence of teeth at 8 months. The post-partum medical factors associated with pre-term VLBW infants are not associated with delayed tooth eruption.
This research was supported by NIDCR R01DE017947 and CTSC UL1RR024989.

Assessment of implementation of a CAMBRA based program

Thursday, March 17, 2011: 3:30 p.m. - 4:45 p.m.
Location: Hall C (San Diego Convention Center)
Presentation Type: Poster Session
T. GUTBERG, S.T. TEICH, W. AL-RAWI, C. DEMKO, and R. JENSEN, Community Dentistry, Case Western Reserve University, Cleveland, OH

Objective: To examine the effect of the implementation of a CAMBRA based risk assessment program in an educational clinical setting.
Methods: Sixty-eight adult patients with at least moderate caries risk were recalled for a follow-up caries risk assessment (CRA). A CRA form was completed, including bitewings. We calculated change scores for high risk and protective factors for comparison by fluoride varnish receipt, initial caries risk (CR)status and patient demographics. CR status categories were compared with risk and protective factors preciously recorded on the CRA forms.
Results: Median patient age was 60 years (range, 24-84yrs); 47% were male and 63% were high caries risk per the first CRA. 44% received a fluoride varnish (FV+) application since the first CRA, (median=10 months, range 1-25 months). Moderate and high risk patients did not differ by age, time between CRA or receipt of fluoride varnish. A decrease in total high risk factors was significantly associated with more high risk factors at initial assessment (rho=.577) and more protective factors reported at follow-up (rho=.390), but not specifically with FV. 25% (17/68) of initial CR statuses were categorized lower than completed chart factors indicated; underestimates occurred most often when current caries was not a factor or when current caries was the only factor (11/17). Recommendations, particularly prescriptions, were more often recorded in progress notes than on the CRA form.
Conclusions: 1)There was a tendency to underestimate the CR status and students do not perceive the CRA form as being an important documentation tool for recommendations provided. Additional training should ensure better calibration of students and faculty. 2)FV is underutilized as a treatment modality for the population of patients seen in the clinic. 3)Within the limitations of this project, FV alone had limited effect on caries risk reduction.

Adolescent Overweight Status and Periodontal Changes Over Two Years

Friday, March 18, 2011: 3:30 p.m. - 4:45 p.m.
Location: Hall C (San Diego Convention Center)
Presentation Type: Poster Session

R. MEHTA[1], I. OUKHALOV[1], and L. BAHL-PALOMO[2], [1]Periodontics, Case Western Reserve University, Cleveland, OH, [2]Periodontology, Case Western Reserve University, Cleveland, OH

It is suggested that overweight status is a risk for periodontitis in older adults. It is also known that plaque is the primary etiology of periodontitis and although periodontal inflammation is common in adolescents. Bone loss is rare, but known to occur if the patient is susceptible to periodontitis.
Objectives: Do overweight adolescents experience worsening periodontal parameters (plaque index, inflammation, and bone loss) versus healthy counterparts.
Methods: 128 adolescent patients (62 overweight and 66 normal, based on CDC guidelines) of record were randomly selected for dental chart review in this IRB approved study. Periodontal parameters (plaque index, inflammation, bone loss, and patient treatment time) over a two year period were recorded from existing dental charts. Inflammation and plaque score were obtained from clinical photographs using a modified gingival index and plaque index respectively based on gingival index by Sillness and Loe. Bone height was measured from the CEJ to the bone crest using CBCT and a proven reliable and valid state-of-the-art software program and reported in milimeters.
Results: Inter- and intra-examiner calibration was completed at start of data collection: p=0.1352, CI=95% and p=0.6451, CI=95% respectively. Changes over two years in plaque index in overweight vs healthy was -0.0031 +/- 0.4506 and -0.0661 +/- 0.3212 respectively; inflammation index was 0.6305 +/- 0.4299 vs. 0.4757 +/- 0.4035; bone height was 0.2134 +/- 0.1666 and 0.1540 +/- 0.1785 respectively. The two year change in plaque score, inflammation and bone height between overweight and healthy adolescents were insignificant($p<.05$).
Conclusion: Overweight adolescents do not experience worsening periodontal parameters versus healthy weight counterparts when followed over two years.

Inflammation in overweight versus healthy females during orthodontic therapy

Thursday, March 17, 2011: 2 p.m. - 3:15 p.m.
Location: Hall C (San Diego Convention Center)
Presentation Type: Poster Session

I. OUKHALOV[1], R. MEHTA[1], and L. BAHL-PALOMO[2], [1]Periodontics, Case Western Reserve University, Cleveland, OH, [2]Periodontology, Case Western Reserve University, Cleveland, OH

Objectives: Is there a relationship between overweight status and changes in periodontal inflammation parameters in females during orthodontic therapy.
Methods: Records of 40 female patients (20 overweight, 20 normal based on CDC BMI guidelines) aged 16-25 who had completed orthodontics, were randomly selected for chart review. Inter and intra-examiner calibration was completed prior to data collection. Chart review was used to record changes in the following periodontal parameters pre- and post-treatment: plaque score, inflammation, bone level, and treatment time. Plaque score and inflammation were measured using pre and post-treatment photographs and indexed using Indexes by Löe and Silness. Bone height was measured from CEJ to bone crest in millimeters using pre and post-treatment CBCT, and treatment time was measured in days.
Results: During orthodontic treatment, plaque score change in overweight vs. normal females was 0.082 +/- 0.531 vs. -0.079 +/- 0.396, inflammation change was 0.840 +/- 0.419 vs. 0.672+/- 0.398 and bone height change was 0.307 +/- 0.139 vs. 0.212 +/- 0.217 respectively. Treatment time for overweight was 893 +/- 198 days vs. 813 +/- 197 days for normal patients. During treatment, change in plaque score, inflammation and bone height between overweight and healthy were insignificant ($p<.05$).
Conclusion: Overweight and healthy females undergoing orthodontic therapy had similar changes in plaque score, inflammation, and bone height; treatment times were similar. While being overweight has been

associated with periodontitis risk, no such relationship was found when comparing overweight and healthy females undergoing orthodontic therapy.

Characterization of the Salivary Proteome/Peptidome in Diabetics and Healthy Controls

Thursday, March 17, 2011: 2 p.m. - 3:15 p.m.
Location: Hall C (San Diego Convention Center)
Presentation Type: Poster Session

J. SALMERON, Biological Sciences, School of Dental Medicine, Case Western Reserve University, Cleveland, OH, K. LUNDBERG, Case Center for Proteomics and Bioinformatics, Case Western Reserve University, Cleveland, OH, and R. JUREVIC, Department of Biological Sciences, School of Dental Medicine, Case Western Reserve University, Cleveland, OH

The constellation of oral effects of diabetes includes xerostomia, increased susceptibility to infection, complications in wound healing, increased incidence of periodontal disease, and other defect associated with epithelial barrier function. Proteomic and peptidomic analysis of saliva could lead to the identification of potential biomarkers for both diagnosis and monitoring of disease progression and therapeutic outcomes.
Objectives: Expression proteomics analysis using a label-free approach was performed on whole un-stimulated saliva obtained from affected persons and controls.
Methods: A total of 3 saliva samples per subject were prepared from each group, which consisted of 20 individuals and was fractionated using a 3K cut off filter to isolate the peptidome from the proteome. Samples were prepared for Lys-C digestion. Digests were analyzed by LC/MS/MS via capillary liquid chromatography and a LTQ-FT. Automated differential quantification of peptides was accomplished using Rosetta Elucidator. Peptide and protein identifications were integrated with these quantifications and used for statistical analysis via one way ANOVA.
Results: Utilizing label free protein expression enabled effective fractionation of a complex sample and robust protein quantification, leading to the identification and quantification of approximately 130 proteins and peptides.
Conclusion: Further analysis may uncover a relationship between some of these proteins and peptides with the diabetic condition.
Supported by grants from NIH/NIDCR: 1 K23DE016110-1, P01DE019759-01

Association of Salivary Adrenomedullin, Calprotectin and LL-37 Levels With Age

Thursday, March 17, 2011: 2 p.m. - 3:15 p.m.
Location: Hall C (San Diego Convention Center)
Presentation Type: Poster Session

T. VANYPEREN[1], C. DEMKO[2], S.K. GHOSH[1], and A. WEINBERG[3], [1]Case Western Reserve University, Cleveland, OH, [2]Community Dentistry, Case Western Reserve University, Cleveland, OH, [3]Biological Sciences, Case Western Reserve University, Cleveland, OH

Antimicrobial peptides (AMPs) are natural antibiotics that provide a first line of defense against a wide spectrum of pathogens. These peptides may be particularly important in the saliva, where members of the microbial flora are present in high numbers.
Objective: To investigate age-related associations in salivary levels of antimicrobial peptides, adrenomedullin (ADM), calprotectin (S100A8/A9) and LL-37 (cathelicidin) in a cross-sectional study.
Methods: 4ml of un-stimulated saliva was collected from 146 healthy volunteer patients, age 8 to 78 years. The levels of calprotectin and LL-37 were measured using ELISA Kit from Hycult Biotech (Canton, MA) and the levels of ADM were measured using EIA kit from Phoenix Pharmaceuticals (Burlingame, CA). Concentrations of the AMPs were normalized with total salivary protein. Decayed/Missing/Filled Teeth (DMFT) and gingival index (GI) were recorded on all patients; periodontal measures of the 6 Ramjford teeth were obtained on adult

patients. Association of the three AMPs with each other, age and oral health measures was analyzed with Pearson's or Spearman's correlation.
Results: Median levels (ug/mg of protein, range) of ADM were 3.6 (.2 – 22), calprotectin 3.7 (0 – 130), and LL-37 7.9 (0 – 228). Calprotectin and LL-37 were more likely to have 'outlier' values. Inter-personal variability was observed across the full age range of subjects. LL37 was significantly correlated with both calprotectin (rho=.343) and ADM (rho=.367). Calprotectin level was inversely correlated with age (r =-.194, P=.02). Adjusted for age, higher calprotectin was associated with less caries experience, but with a higher proportion of teeth with pocket depths greater than 4mm.
Conclusions: Calprotectin appears most influenced by age, with highest levels in the pre-adolescent group and lowest in patients ≥55 years. Additional ontogeny related studies are underway to associate more salivary AMPs with age, emphasizing birth through 2 years of age.
Supported by NIH/NIDCR RO1DE16334 and RO1DE17334.

Susceptibility of Gut Fusobacterium nucleatum Isolates to HBD-2 and -3

Saturday, March 19, 2011: 1:45 p.m. - 3 p.m.
Location: Hall C (San Diego Convention Center)
Presentation Type: Poster Session
B. PURT[1], Z. FENG[1], J. STRAUSS[2], E. ALLEN-VERCOE[2], and A. WEINBERG[1], [1]Biological Sciences, Case Western Reserve University, Cleveland, OH, [2]University of Guelph, Guelph, ON, Canada

Previously, we showed that certain oral Fusobacterium nucleatum (Fn) strains are resistant to human beta defensins -2 and -3 (hBD-2; hBD-3), while others are sensitive, and that resistant ones induce hBDs in human oral epithelial cells (HOECs) better than the sensitive ones (IADR/AADR 2004, abstract#2727).
Objective: To determine the sensitivity of gut Fn strains to hBD-2 and -3.
Methods: Fn strains EAVG_003 and EAVG_012 isolated from gut mucosal biopsy specimens from colon cancer screen patients, and EAVG_002, EAVG_005 and EAVG_018, isolated from gut mucosal biopsy specimens taken from inflammatory bowel disease lesions (Anaerobe, 2008), along with the oral type strain Fn 25586 were plated on blood agar plates and incubated in anaerobic conditions (37° C) for 1-3 days, after which, they were inoculated into enriched tryptic soy broth (ETSB), supplemented with Hemin and vitamin K. Bacteria were pelleted and washed with phosphate buffer saline (PBS) and 10mM phosphate buffer (PB). Bacteria were resuspended in PB supplemented with 1% ETSB (v/v), and adjusted to $2x10^6$ CFU/ml. Each Fn strain was mixed with various concentrations (0, 2.5, 5, 10 µM) of hBD-2 and hBD-3, respectively, and incubated anaerobically (37°C) for 3 hr. Bacteria were serially diluted and plated on blood agar plates. Colonies were counted after 3 days of incubation.
Results: All gut strains tested were resistant to hBD-2 up to 10µM, while all except one (strain 012) were sensitive to hBD-3 at 2.5 µM. EAVG_012 was resistant up to 5µM hBD-3. Fn 25586 was resistant to both hBD-2 and hBD-3 at all the concentrations tested.
Conclusion: We recently discovered the fusobacterial associated beta defensin inducer (FAD-I) in Fn 25586 that promotes hBD expression in HOECs (J Biol Chem, 2010). Comparing FAD-I in sensitive vs resistant Fn strains will provide important clues for structure-function studies going forward.
Supported by NIH/NIDCR R01DE016334.