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3137 Immunohistochemistry of Carbonic Anhydrase in Developing Rat Sublingual Gland. R. S. REDMAN*, F. D. PEAGLER, R. L. MCNUTT, & I. JOHANSSON (VA Medical Ctr., Wash., D.C., & Umeå Univ., Sweden).

Carbonic anhydrase (CA) has been immunohistochemically localized to many structures involved in bicarbonate transport, including the striated and excretory ducts (SD, ED) of the rat sublingual gland (Hennigar *et al., Anat Rec.* 207:605, 1983). The purpose of this study was to immunohistochemically assess developmental changes in the CA isozymes I, II and VI in the rat sublingual gland. Glands were excised from one or more rats of each sex from each of 4 litters at ages 1, 7, 14, 28, 35, 42 and 80 (adult standard) days, fixed in Helly's fluid for 3 hr, then 2% $K_2Cr_2O_7$ for 2 hr. and embedded in 56° C m.p. paraffin. Sections were cut at 6 µm and incubated in normal sheep serum, then in either polyclonal (pc) sheep anti-human CA I or CA II Ab-HRP (Biodesign Int'l), *or* in pc rabbit anti-human CA VI Ab (purified by Protein A-Sepharose CL-4B) followed by pc goat anti-rabbit Ab-HRP. The chromogen was DAB. CA II reactions at 1 day were +++ (scale of 0 to ++++) in ED and SD, ++ in the demilunes (DL) of acini (AC) , and + in the ID (intercalated ducts). These changed gradually to the adult pattern of ++++ in SD and ED and ++ in ID and DL by 28 days. Myoepithelium and mucous AC were near 0 at all ages. Muscle controls were ++++. Parenchymal cells were 0 with DAB alone or the goat anti-rabbit AB-HRP followed by DAB. CA I and VI followed the same pattern but generally were + and ++ lighter, respectively. The usefulness of CA isozymes as immunohistochemical markers of the functional differentiation of DL, SD and ED in the developing rat sublingual gland is CA II > I > VI, with VI not useful for DL. Prenatal samples will be required to encompass the age of onset. Supported by Department of Veterans Affairs and University of Umeå.

3138 Connexin Expression in Rat Submandibular and Sublingual Glands. T. MURAMATSU*, S. HASHIMOTO and M. SHIMONO (Department of Pathology, Tokyo Dental College, Chiba, Japan)

Previous studies have shown that gap junctions are well developed in exocrine glands, and are associated with regulation of secretion. The purpose of the present study is to examine expression and localization of the gap junction proteins (connexin32 and 43) in rat submandibular(SMG) and sublingual glands(SLG) by use of specific antibodies. Twenty-four adult male Sprague-Dawley rats weighing about 300g were sacrificed. Western blot analysis with anti-connexin32 and 43 antibodies showed bands of approximately 27kDa and 43kDa in both glands. Immunofluorescence microscopy demonstrated the presence of reactive spots for connexin32 between acinar cells in both glands. In contrast, reactive spots for connexin43 were observed at the periphery of the alveolar structures in both glands. No positive spots for both connexins were detected between ductal cells in either gland. The frequency of connexin32-positive spots in SMG was approximately equal to that in SLG. In contrast, the connexin43-positive spots in SLG were more frequent and larger than those in SMG, and the differences were statistically significant ($p<0.05$). Immuno-electron microscopy revealed that connexin32 were localized on the gap junctional membranes between acinar cells. Immunolabeling for connexin43 was located on the gap junctions between thin processes of myoepithelial cells. These results suggest that connexin32 is associated with regulation for secretory function of acinar cells and that connexin43 is associated with that for contraction of the myoepithelial cells in the rat salivary glands.

3139 Identification of Type I and Type III Salivary Cell Progenitors in the Developing Hamster Submandibular Gland. S MAHMOODIAN*, R FERNANDES, D COTANCHE and M A KUKURUZINSKA (Boston University School of Graduate Dentistry, Boston, MA)

During the development and differentiation of the rat submandibular gland (SMG), two transient cell types have been identified: Type I cells, which secrete protein-C, and Type III cells, producing B1-immuno-reactive secretory proteins, B1-IP, and implicated to be the progenitors of ductal and acinar cells, respectively. To initiate studies on the characterization of the salivary cell lineage in the developing hamster SMG, immuno-histochemical approaches were coupled with the confocal microscopy imaging (BioRad MRC 600 with krypton-argon laser) Phalloidin staining of the actin filaments in neonatal and adult SMGs provided clear three-dimensional images of acini and ducts, as well as transitional developmental structures, indicating that confocal imaging represents an excellent approach to studies of the salivary cell lineage. The neonatal (5 day-old) hamster SMGs cross-reacted in a specific manner with antibodies raised to the rat neonatal secretory markers, B1 and protein C, providing evidence that the B1 and C proteins are also expressed in the neonatal hamster SMG. Both proteins were virtually undetectable in the adult hamster tissue, indicating that, like in rat, they were developmentally regulated. These studies suggest the presence of Type I and Type III salivary cell progenitors in the neonatal hamster SMG and support the value of confocal imaging in the analysis of the salivary cell lineage. Supported by Grants DE10183, K04 DE00362 and T35 DE07268.

3140 Role of E-Cadherin in Submandibular Gland Cells. K. UCHIHASHI*, N. TAKAI, H. MIYAO, H. MURAKAMI and Y. YOSHIDA (Department of Physiology, Osaka Dental University, Osaka, Japan).

Maintenance of the intercellular junction in salivary gland cells depends on various adhesion mechanisms that separate the luminal spaces from intercellular and interstitial spaces. E-cadherin (E-CD), which is calcium-dependent cell-cell adhesion molecules, are one such class of molecules responsible for this mechanism. They have been observed in several laboratory studies. We examined the relationship between tight junctional permeability and the sealing mechanisms of E-CD in the submandibular gland cells of mature (9-week-old) and immature (1-2-days-old) mice. Male ICR mice were used. The mature and immature mice were divided into three groups respectively; controls, those treated with tunicamysin (TM), which blocks glicosylation of asparagine residue glycoproteins, and those receiving intraductal injection of anti-E-CD. Immunohistochemical procedures with a monoclonal antibody against E-CD were used to determine specify the ultrastructural features of the submandibular gland cells. The permeability of tight junctions was tested using microperoxidase as a tracer. There was strong expression of E-CD molecules on the cell-cell boundaries in acinar cells of the mature controls as well as in the TM treated mice. This was particularly true in the adherens region. The same results were observed in the immature mice. Although the tight junctions of both mature and immature glands treated with TM were impermeable to MP, they became permeable following intraductal injection of anti-E-CD antibody solution. These results indicate that the adhesion and sealing mechanisms are mediated by E-CD in the intercellular junctions. However, suger chains of E-CD do not take part in the reaction.

3141 Measurement of Basement Membrane mRNA species in Cultured Salivary Glands. C.P. MCARTHUR*, Y. WANG ,D. HERUTH and P. ROTHBERG1, and O. NARAYAN [2] (UMKC School of Dentistry, Kansas City). [1]Children's Mercy Hospital, K.C., [2]KU Medical Center, K.C.

There is little known about the role of basement membrane and matrix proteins in the pathogenesis of Sjogren's Syndrome (SS). Preliminary studies have suggested increased expression of laminin compared with fibronectin in minor salivary glands of patients with SS. Semiquantitative measurements of mRNA using in situ hybridization also showed increased laminin B chain mRNA. The objective of this study is to standardize a method for the quantification of laminin and fibronectin mRNA in labial salivary glands using the Ribonuclease Protection Assay(RPA). DNA templates were constructed from a whole brain cDNA library by cloning Laminin and Fibronectin PCR products into the pGEM-T vectors. The 164 bp laminin product was exon 21 of the laminin B1 chain and the 287 bp fibronectin product was the 3' untranslated segment. The orientation of the inserts was confirmed by DNA sequence analysis and LAM 9 and FIB 4 were chosen as forward orientation and LAM 7 and FIB 3 as reverse orientation. Plasmid minipreps were screened and DNA digested with PST1 and SAC11 and analyzed by PAGE. Sequence specific hybridization probes were used to detect each mRNA simultaneously from a single cultured salivary gland lysate. The study showed that protected fragments of laminin and fibronectin can be quantified in extremely small samples of tissues which are not amenable to northern blot analysis. Supported by NIH Grant DE11159-01.

3142 Cell Cycle Control in Isoproterenol Induced Murine Salivary Acinar Proliferation. T. Zeng*, H. Yamamoto and M. Humphreys-Beher (Dept. of Oral Biology, University of Florida, Gainesville, Florida, U.S.A.)

The eukaryotic cell cycle is a biochemical pathway resulting in both DNA replication and cell division. Cyclin Dependent Kinases (CDKs) control the cell cycle in all eukaryotes, while other proteins, known as cyclins, act as their regulatory subunits. The prototypic member of the CDK family, p34cdc2, controls entry into mitosis in all eukaryotes. Entry into G1 and S phase in mammalian cells is controlled by other members of the cdc2 kinase family: CDK2, CDK4, WEE1, etc. The study of chronically administered isoproterenol (ISO) induced acinar cell proliferation in rats and mice reveal changes in many signal transduction pathways. Since cell cycle control is the final mechanism that leads to cell division and proliferation, we have used ISO treated animal model to study the role of CDKs in salivary gland acinar cell proliferation. In this study we have surveyed, by immunoblotting, the protein levels of cell cycle components (CDC2, CDK2, CDK4, WEE1), and their regulatory subunits (cyclin A, B, D, E) in normal and ISO treated mouse and rat parotid and submandibular salivary gland acinar cells. The morphological and biochemical changes in the salivary glands were studied over a time-course from 0 to 144 hr. Our results show that the salivary gland undergoes a self confined proliferation with its peak at 72 hr ISO stimulation. The expression of CDKs and cyclins show a consistent relation to actual gland proliferation. Levels of CDC2 (mitosis key regulator), CDK2-cyclin E (S phase regulator), and CDK4-cyclin D (G1 entry key regulator) all increase over the first 72 hr. After 72 hr of ISO treatment the levels of CDKs and cyclins begin to decrease. These results are in accordance with other changes previously observed in the activation of phosphotyrosine signal transduction, and form a complete pathway from the activation of acinar cell membrane receptor to nuclear division. This method also provides a satisfactory method for the study of the cell cycle control. This work was supported by NIDR grant DE08778 to M. Humphreys-Beher and WHO fellowship HRF91 to T. Zeng.

3143 p53 Expression in Adenoid Cystic Carcinoma of Salivary Gland. Q.R. ZHU*, F.H. WHITE, G.L. TIPOE (Department of Anatomy, University of Hong Kong, Hong Kong).

Previous studies have suggested that the alteration of the p53 gene is the most common genetic abnormality in human cancer. The aims of the present study were to investigate and evaluate p53 immunostaining in the adenoid cystic carcinoma (ACC) of the salivary gland and to correlate the expression with patient survival. A total of 26 cases of ACC in parotid gland (n=12) and the minor palatine glands (n=14) were studied, with 10 cases each of normal parotid gland and of palatine gland as non-neoplastic controls. Staining was performed with mouse monoclonal antibody DO-7 against p53 (Dako, USA) using the ABC method. Stained nuclei irrespective of intensity or frequency were considered as positive. Positive nuclei were further evaluated as the percentage of the total nuclei of the reference epithelium. Clinical survival data were available for patients for periods up to 156 months. No normal tissues showed immunoreactivity with p53 protein. 13 of 14 (92.9%) cases of palatal and 3 of 12 (25.0 %) cases of parotid neoplasms stained with p53 protein and the percentage of positive nuclei ranged from 0.01 to 50.1%. The expression rate of p53 protein was more markedly elevated in palatal than in parotid neoplasms, supporting the traditional view that palatal ACCs are more malignant than parotid ACCs. The patients who died of tumours in the follow up period had a statistically higher positive expression rate (86%) than patients with no evidence of disease at the end of the follow-up period of between 60 to 156 months (23 %). p53 may play an important role in the development of ACCs of the salivary gland and p53 analysis may be a useful indicator of poor prognosis. Supported by Committee for Research and Conference Grants, University of Hong Kong.

3144 **Retrospective Study of Salivary Gland Lesions in Venezuelan Children.** C. ALVAREZ, C. COLANTONI, H. RIVERA, and R.A. OCANTO* (Universidad Central, Caracas, Venezuela; and Creighton University, Omaha, NE, USA)

Oral biopsies and clinical histories available from the Central Oral Histopathology Lab, Universidad Central de Venezuela (N_{UCV}=1315), and JM de los Rios Children's Hospital (N_{JMR}=336) were collected for children under 16, from 1968 to 1994, and from 1983 to 1993 respectively. These centers represent the two main referral places in Venezuela. Samples were analyzed according to age cohorts (0-4, 5-8, 9-12, and 13-16 years), sex, intraoral location, and histopathological diagnosis. A total of 294 lesions were classified as follows:

	Non-neoplastic	Neoplasms	TOTAL
UCV Histop. Lab	105 (87.5%)	15 (12.5%)	120 (40.8%)
Children's Hosp.	96 (55.2%)	78 (44.8%)	174 (59.2%)
TOTAL	201 (68.4%)	93 (31.6%)	294

Females showed a greater predilection (56%) than males, and prevalence was higher for the 9-12 age cohorts (UCV) and children under 8 (JMR). The most common intraoral location was in minor salivary glands and the lower lip. Non-neoplastic lesions constituted the majority, and Mucoceles represented the largest single entity in this category (60%). Pleomorphic Adenoma was the most frequent benign neoplasm and Mucoepidermoid Carcinoma was the most frequent of primary malignant tumors. Most non-epithelial neoplasms were represented by Hemangiomas. These results are similar to previously reported findings across various geographical regions and ethnic groups. This study was supported by a grant from the Consejo de Desarrollo Científico y Humanístico, Caracas, Venezuela.