axons had one or two myelin lamellae as observed by electron microscopy. Oligodendrocytes with such thin myelin sheaths were immunostained by both anti-Rip and anti-β-MBP antibody, but not by anti-Nogo-A antibody. While an extensive myelination had begun by oligodendrocytes before at P12, Nogo-A was not detected in oligodendrocytes during this period. Nogo-A was first detected in oligodendrocytes with the adult-type myelin sheaths at P21. Immunostaining of Nogo-A was found in the cytoplasm, but not on the surface plasma membrane of oligodendrocytes, nor in the myelin sheaths. These results indicate that Nogo-A is expressed in correlation with development of oligodendrocytes, and manifested in the mature oligodendrocytes.

P3-44

Detection of oligodendrocytes in tissue sections using PCR digoxigenin-labeled myelin cDNAs

M. Said Ghourouj, Walid Jalalh, Mirela Cerghet and Robert P. Skoff
UMR 7004 CNRS/ULP, Faculté de Médecine, Strasbourg, France and Department of Anatomy and Cell Biology, School of Medicine, Wayne State University, Detroit, Michigan, USA

Oligodendrocytes, the myelin forming cells in the central nervous system, were visualized with high resolution in situ hybridization at light microscopic level. Digoxigenin (DIG)-tagged cDNA probes were synthesized and efficiently labeled by PCR. Specific probes were made by RT from the brain total RNAs followed by PCR with designed specific primers to myelin genes. Probes specific to a major protein of the myelin, proteolipid protein alone (PLP), PLP and its isoform DM20 together (PLP/DM20) and a minor myelin protein, myelin oligodendrocyte glycoprotein (MOG), were synthesized and labeled with digoxigenin. In situ hybridization was then applied on 50 and 100 micron thick free floating tissue sections from mouse brains and spinal cords using standard in situ hybridization and developed with NBT-BCIP. Despite a low expression of MOG and PLP mRNAs in newborn brain and in the adult, oligodendrocyte were detected in similar pattern and sensitivity as with the riboprobes. This versatile and easy method for synthesis and labeling of specific probes to oligodendrocytes can be also applied to detect many other mRNAs in the nervous system and in other tissues.

P4-01

Vimentin-Positive Fibroblasts and Extracellular Matrix Components in Human Gingival Hyperplasias: A Immunohistochemical Approach

Carlos J. Saboia-Dantas, Sandra I. L. Seixas, Igor I. C. Silva, Renato M. Salgado, Terezinha J. Sirotheau-Correia
Fluminense Federal University

Gingival hyperplasias are proliferative lesions characterized by abnormal clinic enlargement of the gingivae, with increased extracellular matrix (ECM) deposits in the lamina propria associated with cellular proliferation. In the present work, the distribution of the vimentin-positive gingival fibroblasts, collagen I and tenascin-C, were analysed in gingival surgical specimens from control, edematous or fibrotic chronic inflammatory hyperplasia, and phenytoin users. The specimens were fixed in 4% buffered formal, dehydrated and embedded in paraffin. Serial sections were labeled with anti-vimentin (DAKO), anti-collagen I and anti-tenascin-C antibodies (BIOHIT), using a biotinylated secondary and ExtrAvidin-peroxidase reagent (SIGMA). The vimentin-positive fibroblasts were increased in number in all groups, when compared with control group, and co-localized with dense type I-collagen fiber bundles associated with positive reactivity for tenascin-C. In phenytoin-associated hyperplasia, the number of vimentin-positive cells, collagen I and tenascin-C density were increased, when compared with edematous and fibrotic inflammatory hyperplasia. The fibroblasts in fibrotic areas were fusiforms, have many prolongaments, were localized along collagen I bundles and associated with expression of tenascin-C in ECM. The results suggests a critical role of gingival cells and ECM components in the maintenance of the proliferative and fibrotic process in gingival tissue, influencing the reversibility degree of such lesions, mainly in phenytoin-associated hyperplasia. The relationships between gingival fibroblasts sub-populations and ECM assembly are critical in proliferative pathological process to determine the reversibility or not of such lesions.

P4-02

Histopathological and immunohistochemical studies of 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced hamster tongue carcinogenesis

Ryochi Sakamoto, Tetsuya Nitta, Yoshiaki Kamiwaka, Kazumasa Sugihara, Sachie Matsushita*, Kazuhisa Hasui*, Shinichiro Tsuyama*, Fusayoshi Murata*

The First Department of Oral and Maxillofacial Surgery, Kagoshima University Dental School

The purpose of this study was to investigate cell proliferation as assessed by 5-bromo-deoxyuridine (BrdU) incorporation during 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced carcinogenesis of hamster tongue. 1.0% acetone solution of DMBA was applied to lingual mucosa of hamsters after scratching with a root canal broach three times a week. The procedures were continued until the end of 14th week. One hour before sacrifice, all animals were injected BrdU intraperitoneally. Tongues were excised, fixed in 10% neutral-buffered formalin, processed routinely and embedded in paraffin. Sections were cut for hematoxylin and eosin staining and BrdU immunohistochemical staining. BrdU immunohistochemical staining was achieved using the EnVision system (Dako). Leukoplakia was found in treated tongue from 4 week. Histopathologically, there was hyperkeratosis and acanthosis. The degree of hyperplasia increased with time. Squamous cell carcinoma (SCC) was observed from 10 week. Histopathologically, there was an invasive proliferation of well-differentiated squamous cell carcinoma into the muscle layer in the lingual mucosa. EnVision system produced fast clear and reliable staining results. Immunohistochemically, in non-treated tongue and treated tongue which histological findings showed mild-dysplasia, BrdU positive cells were mainly expressed in the basal cell. On the other hand, in SCC, BrdU positive cells were expressed in basal cell, prickle cell and the periphery of tumor cell nests. In addition to this study, the results of immunohistochemistry of cell proliferation-associated antigens and cell adhering molecules will be reported together.

P4-03

Basal cell adenocarcinoma and basal cell adenoma of the salivary gland: an immunohistochemical and ultrastructural study

Tetsuya Nitta1, Ryochi Sakamoto1, Yoshiaki Kamiwaka1, Kazumasa Sugihara1, Kazuhisa Hasui1, Shin-ichiro Tsuyama2, Fusayoshi Murata1

First Department of Oral and Maxillofacial Surgery, Kagoshima University of Dental School,1 Department of Anatomy, Faculty of Medicine, Kagoshima University.2

Basal cell adenocarcinoma (BCAC) has cytological characteristics of basal cell adenoma (BCA) and is a low-grade malignant tumor of the salivary gland. The purpose of this study was to compare the immunohistochemical and ultrastructural characteristics of BCAC and BCA. Study design: Case 1: A 60-year-old man presented in 2000 with a mass in right buccal mucosa. Case 2: A 73-year-old woman presented in 1996 with a mass in left parotid gland. Surgically resected two