is a glycosyl-phosphatidylinositol-linked 70-kD molecules expressed in various cell surface. Enzymatic activity of 5'-Nucleotidase is catalyses the dephosphorylation of purine and pyrimidine ribo and deoxyribonucleotide monophosphates to their corresponding nucleoside. A possible function for CD73 is to regulate the availability of adenosine for interaction with cell surface adenosine receptor by converting AMP to adenosine. Although expression of CD73 in lymphocyte and vascular endothelium has been well-investigated, immunohistochemical and enzymehistochemical expression of CD73 in the thyroid tissue has not been studied yet. Materials and Methods: Frozen thyroid sections (normal thyroid, nodular goiter, follicular adenoma and papillary carcinoma fixed with 4% paraformaldehyde were used for CD73 immunohistochemistry with indirect method. Demonstration of 5'-nucleotidase activity was according to Wachstein and Meisel with modifications using cryostat thyroid sections. Results: Strong immunoreactivity of CD73 was found at the apical cell surface in papillary thyroid carcinoma, however, CD73 was negative or focally faint positive in normal thyroid, nodular goiter and follicular adenoma. In enzymehistochemistry, 5'-nucleotidase activity was localized in the apical cell surface corresponding to CD73 immunoexpression and strong enzyme reactivity was found in papillary thyroid carcinoma. Conclusion: CD73 will be a useful diagnostic marker for papillary thyroid carcinoma.

P4-28

Immunocytochemical Study of Proliferative C-Cell Disorders in the Rat

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In rats, the frequency of spontaneous C-cell tumors is very high and is both age- and gender-dependent. Throughout their life-span the three specific stages of neoplastic progression can be seen: diffuse C-cell hyperplasia, focal C-cell hyperplasia and C-cell tumours. Taking advantage of the availability of this theoretical model of the human medullary thyroid carcinoma, we have carried out an immunohistochemical study to verify the existence of any relationship between the expression of different markers and the successive steps of tumor development. Eighty Wistar rats of both sexes were killed at various ages, ranging from 3 to 24 months, and their thyroid glands were fixed in Bouin's solution, embedded in paraffin and serially sectioned. After studying the pattern of distribution of C cells, 40 thyroid glands were selected (10 normal C-cell pattern; 10 diffuse C-cell hyperplasia; 10 focal C-cell hyperplasia and 10 C-cell tumors). Using the streptavidin-biotin-peroxidase method, the presence of chromogranin, calcitonin (CT), CGRP and somatostatin (SS) was studied. A characteristic immunohistochemical pattern in neoplastic C-cells in comparison with normal and hyperplastic C-cells was found, particularly for CT and SS, with no differences related to the gender of the animals under study. Specifically, a considerable heterogeneity for CT was only displayed by C-cell carcinomas, being less pronounced in C-cell adenomas. As for SS, this regulatory peptide was found in a minor subpopulation of CT-positive cells in normal and hyperplastic glands, but in a few C-cell adenomas and most C-cell carcinomas nearly all immunoreactive cells for CT were also positive for SS. We conclude that rat C-cell neoplasms constitute a very particular tumor entity which shares many but not all immunohistochemical features with the human disease.

P4-29

Cell-by-Cell Comparison of Zirconyl Hematoxylin and Alcian Blue Staining of Paget Cells in a case of Extramammary Paget's Disease

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Despite its occasional unavailablity, alcian blue has long been the stain of choice for the pathognomonic mucin-secreting cells of extramammary Paget's disease. Zirconyl hematoxylin is a recently developed substitute for alcian blue made from common laboratory materials. We have stained sections from three different areas of a single case of extramammary Paget's disease with zirconyl hematoxylin, destained with 7% HCl in ethanol, restained with alcian blue at pH 2.5, destained with 2.5% trifluoroacetic acid in dichloromethane, and restained with zirconyl hematoxylin. Each staining procedure stained the same cells.

P4-30

Comparison of HER2 mRNA Amplification with the Results of Immunohistochemistry in Human Breast Cancer Using Laser Assisted Microdissection Technique

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In human breast cancer, over expression of HER2 protein (score 3+) means a good indication for Herceptin administration and a favorable prognosis. In this study, we examined the correlation between the expression of HER2 protein and the status of mRNA amplification in human breast cancer.

Materials and Methods: Twenty breast cancers were subjected for this study. The surgical specimens were handled under enough informed consent. Fresh tumor tissues were cut no more than 10×5×5 mm, freeze immediately in Hexane/dry-ice acetone. Eight-micrometer thick sections were cut and mounted on a membrane film-coated slide glass, then fixed in methanol and stained with toluidine blue. Microdissection was carried out by using a laser assisted microdissection instrument (LS 337 laser scissors instrument: Cell Robotics Inc. USA, Meiwa Inc. Japan). RNA was extracted from the microdissected tumor tissues by means of guanidium isothiocyanate (GITC) method, then transcribed to cDNA. HER2 and GAPDH (internal control) mRNAs were analyzed by real time polymelase chain reaction (PCR) based on fluorogenic TaqMan method. Amplification of HER2 mRNA was standardized with the copy number of GAPDH mRNA. Immunohistochemistry for HER2 protein was carried out using LSAB (labeled streptavidine biotin) method.

Results and Discussion: Regarding the status of HER2 mRNA amplification, we obtained 4 cases of invalid score and 4 cases of distinct and unequivocal amplification. The status of HER2 mRNA amplification of these 8 cases was well correlated with the expression of HER2 protein, evaluated as score 0 and score 3+, respectively. The cases evaluated as score 1+ and score 2+ in the immunohistochemistry showed a relatively wide range of HER2 amplification. This result suggests that theses groups (evaluated immunohistochemically as score 1+ and 2+) are quite heterogenous and may include candidates for Herceptin therapy.

Conclusion: We described that a combination of laser assisted microdissection technique and real time PCR is a powerful and reliable method for the quantitative analysis of mRNA. Our results disclosed that the necessity for further and precise investigation of the groups evaluated as score 1+ and 2+ in the immunohistochemistry.