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LEARNING THE PATHOLOGY OF THYROID CANCER and HUMAN NEOPLASM APPLYING 3D MICROSCOPY

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Introduction
Over the past few years, Light Sheet Fluorescence Microscopy (LSFM) has been providing 3D reconstructions of fluorescent-labeled biological samples. The most important feature in 3D microscopy to be observed with Light-sheet fluorescent microscope (LSFM) or with Confocal Microscopy (CoM) is the clarification of organs, since samples need to be fully transparent, and the penetration of the antibodies. In thyroids glands, the application is particularly difficult due to its high vascularization and the presence of an extracellular material known as colloid, which hinders transparency and increases nonspecific staining and background due to being iodized. Hollow round cellular thyroid structures, called follicles, store inside the colloid composed of heavily glycosylated thyroglobulin, with iodide covalently attached to tyrosine.

Objective : Our aim is to standardize antibodies like HBME-1 using 3D microscopy, learning its expression and localization, also to reliably differentiate benign thyroid lesions from thyroid carcinomas.

Methodology: Several markers such as Cytokeratin Antibody anti-human, Vio R667, REAfinity (^{TM}) Clone REA831, Cytokeratin 19 Antibody, anti-human, PE, REAdye lease (™) clone REAL822 have been used and designated as useful markers although each of these antibodies has specific staining properties.In the present abstract we present the initial results using the most common neoplastic lesions, so-called Multinodular Goiter (MNG) from patients' tissues.We developed a protocol containing specific perfusion, photobleaching, staining and observation steps, able to register mouse thyroid in 3D. We applied this protocol to human clinical pathology thyroid tissues. DAPI (4´,6 -diamino-2-phenylindole) was used as a counterstain for nuclei. Samples were recorded with UltraMicroscope II-SuperPlan (Miltenyi) and SP5 Confocal (Leica). Imaris 9.2 software was used for reconstruction. Human bone marrow endothelium marker-1 (HBME-1) was used as a common molecular marker of tumors. The monoclonal anti-human HBME-1 antibodies were used.

Conclusions: By detecting the expression of HBME-1 revealed micronests of papillary carcinomas. The reactivity of HBME-1 in the papillary carcinoma's variant was apical. HBME-1 is perhaps of additional value in the diagnosis of thyroid malignancy. Our results also showed papillary carcinomas lack typical cytological features, such as nuclear grooves and optically clear nuclei present in benign thyroid lesions.

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