Ex Vivo Metabolic Imaging for Parotid Tumors: Implications for Precise Diagnosis and Customized Treatment

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Background incl. aims
Primary parotid neoplasms necessitate specialized attention due to their diverse histological and clinical features [1,2]. Accurate classification and treatment selection rely on a thorough comprehension of both the phenotypic and molecular attributes of these tumors. Molecular markers and interactions within the tumor microenvironment critically influence their behavior. Therefore, identifying new markers using routine techniques such as needle aspiration to visualize tissue metabolism in vivo is essential for refining diagnostic accuracy and treatment strategies. Our study aims to preliminarily delineate the complex metabolic profiles of parotid gland tissues, especially focusing on distinguishing between healthy subjects and patients with squamous cell carcinoma, employing both Fluorescence Ratio (FRIM) and Fluorescence Lifetime (FLIM) Imaging Microscopy for precise quantification.

Methods
In our investigation, we conduct ex vivo analysis using advanced two-photon metabolic imaging techniques to examine the morphological, molecular, and functional aspects of parotid neoplasms. Fine needle aspiration biopsy is utilized to obtain cells for analysis, bypassing the need for invasive procedures and minimizing patient discomfort. Through this approach, we analyze metabolic markers such as NADH and FADH2, employing Fluorescence Ratio Imaging Microscopy (FRIM) and Fluorescence Lifetime Imaging Microscopy (FLIM) for precise quantification [3].

Results
Through the application of Metabolic Imaging techniques to analyze NADH and FADH2 levels in ex vivo samples, our objective is to complement traditional morphological assessments with a comprehensive evaluation of tissue metabolic states. We employed both microscopy imaging techniques to analyze the behavior of these two molecules in both healthy and diseased subjects. For instance, in the case of FRIM, we introduced an innovative workflow pipeline (depicted in Figure 1), which commences with autofluorescence imaging to derive redox ratio images by analyzing the blue and red channels. A redox ratio value approaching 0 indicates a state of reduction, while nearing 1 signifies oxidation. Subsequently, mitochondrial and cytoplasmic masks are applied to these images, enabling the assessment of tissue metabolic activity.

Figure 2 displays images in the blue channel, red channel, and the RR image generated by the analysis pipeline respectively for the healthy subject (top) and the subject affected by squamous cell carcinoma (bottom). These latter images feature a scale bar indicating areas of heightened metabolic activity within the tissue.

As shown in Figure 3, controls exhibit higher RR_mit values compared to patients, suggesting greater oxidative activity in healthy tissue.
RR_mit indicates the balance between reduced and oxidized pixels in relation to mitochondria, reflecting metabolic activity and cell viability. A higher RR suggests greater

metabolic activity and more viable cells, while a higher RR low may signal metabolic alterations, as observed in squamous cell carcinoma. So, the higher RR_mit in healthy subjects compared to patients highlights significant disparities in tissue metabolic health.

We delved into metabolic dynamics by identifying the quantity of mitochondria exhibiting reduced and oxidized states within both healthy and diseased tissue, leveraging suitable algorithms for precise analysis.

Conclusion

Introduction of an innovative pipeline for autofluorescence image analysis has yielded significant findings. Notably, the results reveal a distinct contrast between healthy and diseased tissues. Moving forward, these insights are poised to influence diagnostic protocols and personalized treatments, with a focus on enhancing patient well-being. Such advancements hold promise for refining our understanding of tissue pathology and optimizing therapeutic strategies tailored to individual patient needs.

Graphic:

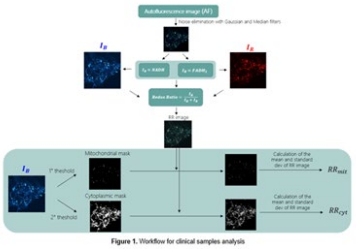

Figure 1. Workflow for clinical samples analysis

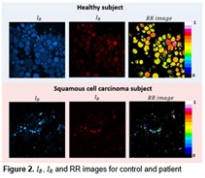

Figure 2. I_R, I_B and RR images for control and patient

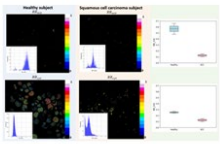
Figure 3. RR images results for control and patient

Keywords:

Metabolic Imaging, parotid carcinomas,metabolic markers

Reference:

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