The impact of modern immunohistochemistry and molecular biology techniques in the diagnosis, prognosis, and treatment of solid cancers

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Abstract

This habilitation thesis is a synthesis of the most important results from my postdoctoral scientific research activity. It also aims to reveal future research directions and career development plans. In this thesis, the subchapters are organized in accordance with the main research themes I have addressed throughout my career. These research themes have gradually led to the publication of scientific articles that have made a significant impact on my professional development. At this moment, I am the main author of 14 scientific articles published in ISI-indexed journals with impact factor (11 of which I am the first author) and a co-author of 12. The results that we have published have made an impact in the scientific community, leading to 182 citations in the ISI Web of Knowledge database (Hirsch index 8). My cumulative impact factor as the main author (FCiAP) is 24.635.

After defending my PhD thesis (2012), my research activity was initially focused on the pathology of the thyroid gland. In a first step, I carried on with the main hypotheses and research topics that stood at the basis of my PhD thesis, namely the identification of immunohistochemical markers that could serve as a complement tool for the histopathological diagnosis of thyroid carcinomas. The past half century has seen a significant number of advances in thyroid pathology. In particular, the classification of thyroid tumors of follicular cell-origin has undergone major changes, with a considerable impact on the treatment and management of many patients. The introduction of NIFTP ("Non-invasive follicular thyroid neoplasm with papillary-like nuclear features") in 2016 was one of the most remarkable changes, a true "paradigm shift" in thyroid pathology. More than ever, the importance of molecular biology and the value of biomarkers that may aid diagnosis and provide prognostic information are emphasized. Over the last 12 years I have addressed in my scientific papers all these important and relevant issues: from morphological criteria and immunohistochemical markers that may aid the diagnosis of thyroid tumors, to the impact of the (new) 2017 WHO/2016 TNM classification of the thyroid tumors on their epidemiological trends and pathological characteristics, moving further to their molecular profile, the molecular reclassification into *BRAF*-like *versus* *RAS*-like subtypes, and assessing the prognostic value of *BRAF*V600E mutation in papillary thyroid carcinomas.

Working with formalin-fixed paraffin-embedded tissues (FFPE), the first challenge in performing molecular studies on thyroid tumors was DNA extraction from these samples. The isolated genomic DNA needed to be adequate (in terms of both quality and quantity) to be successfully used for further genetic amplification techniques. With the support and warm guidance of Professor Angela Borda (Head of the Histology Department), I bought all the necessary equipment and consumables needed to implement this technique (DNA extraction from FFPE tissues) in our lab (Histology Laboratory, UMFST Târgu-Mureș). Setting up the DNA extraction technique from FFPE samples represented the starting point for several research projects. I completed studies focused on the technical aspects, such as the challenges of setting up DNA extraction from FFPE tissues and the influence of pre-analytical factors on obtaining an adequate DNA from these specimens, as well as studies in which this technique enabled us to determine and study the molecular profile for different types of tumors.

I further continued with research projects focused on assessing the performance and value of PD-L1 (Programmed Death Ligand-1) immunohistochemical (IHC) expression in urothelial carcinomas (UC). Immunotherapy by checkpoint blockade targeting PD-1/PD-L1 complex has revolutionized the treatment of advanced cancer around the world, showing remarkable clinical activity in several tumor types. However, the use of immunotherapy is limited to patients with PD-L1-positive tumors, as assessed by IHC. In 2019, I started a large project aiming to evaluate and study PD-L1 IHC expression in a large series of UCs using different clones: QR1 (Quartett), 22C3 (Dako), and SP263 (Ventana). This was a multi-institutional project carried out in collaboration with the Urology and Pathology Departments from Mureș County Clinical Hospital, Romania, and Lyon Sud Hospital, Centre Hospitalier Lyon Sud, France, respectively.

Between 2019 and 2022, I carried on with my research activity in a new domain, as a member of an international project coordinated by Prof. Dr. Giulio Alessandri (University of Brescia, Italy). The aim of this project was to investigate the role of micro-fragmented adipose tissue (MFAT) as a drug delivery (DD) system for chemotherapeutic drugs in different types of tumor proliferations, like pancreatic adenocarcinoma and glioblastoma. I was directly involved and had responsibilities in conducting the experimental *in vivo* and *in vitro* studies carried out at our university, using the research infrastructure from the Regenerative Medicine Laboratory at the Center for Advanced Medical and Pharmaceutical Research (CCAMF). This project has been completed, with some of the results already published and others pending publication.

Since April 2021, I have become the coordinator of the Normal and Pathological Morphology Laboratory (MORFO) at CCAMF. I have made my contribution for good management and progress of the research activity in this lab. Today, this laboratory is fully functional, equipped with an infrastructure that allows performance of basic histopathological techniques as well as more advanced laboratory techniques (e.g., automated IHC, laser microdissection, etc.). There is also qualified personnel fully in charge of all the technical aspects related to the research activities. My aim in the future is to further advance and expand the research activities within this lab.

My career development plans and future research directions follow naturally my past professional achievements and scientific activity. The research directions I intend to persuade in the future are as follows:

- the molecular profile of the thyroid tumors: completing and continuing the studies aiming to assess the impact of these data on the management and prognosis of patients with thyroid cancer (studying C228T and C250T mutations of the *TERT* gene);

- investigation and validation of IHC PD-L1 expression in various solid tumors;

- MFAT and its devitalized variant (D-MFAT): their applications as DD systems in oncology.

Through these research projects, my aim is to reinforce the knowledge and expertise I have gained in these fields of interest, and most importantly, to bring new scientific data with relevant clinical impact, which could translate into solutions and elements of progress in the diagnosis, prognosis, and management of oncological patients.