University of Bucharest
Doctoral School of Biology

Habilitation thesis

Summary

Title

Multimodal approaches in autoimmunity and cancer - skin pathologies from an immunobiological perspective

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THESIS SUMMARY

The habilitation thesis entitled "Multimodal approaches in autoimmunity and cancer - skin pathologies from an immunobiological perspective" reflects the my scientific, professional and academic activity in the field of Immunology, carried out over a period of 20 years since obtaining the title of Doctor of Sciences in the field of Biology (2006).

The thesis is based on original studies and review articles published in the period from the completion of the doctorate until 2025, in ISI or BDI indexed journals, in book chapters, with support from national and international research projects in which I was director or project responsible, or projects in which I was involved as a member of the research team.

The thesis is organized into three main sections, with specific chapters and subchapters as follows:

***Part I Scientific Achievements** describes the main scientific contributions in my field of activity, embodied in the most representative publications. The section dedicated to the Immunobiology of the antitumor immune response presents some concepts related to the tumor transformation cycle, from healthy tissue subjected to damage when innate immune cells (neutrophils and M1 macrophages) will infiltrate the tissue and generate an acute inflammation that will trigger the repair cascade, returning the tissue to its initial state. If the inflammation is sustained and has chronic characteristics, the tissue is also infiltrated by adaptive immune cells (Th1, Tc, Th17) and the precancerous lesion can return to its normal, undamaged state. When the chronic inflammation persists, then the precancerous lesion transforms into a primary tumor. The primary tumor may have infiltrating M1, Th1, Tc, Th17 cells, and the antitumor activity is intense and can defeat tumorigenesis, but when the infiltrating cells are M2 macrophages, Th2 lymphocytes, Treg, a pro-tumorigenic environment is amplified, and the primary tumor becomes aggressive and starts to metastasize. This tumor transformation cycle is therefore based on the inflammatory process that stands out as a critical feature in tumor development. In the section Immunobiology in cutaneous tumorigenesis, we emphasized that in non-melanoma NMSC tumors (squamous carcinoma SCC and basal cell carcinoma BCC) there are several deregulated signaling pathways that contribute to the initiation of neoplastic transformation and keratinocytes are the main cells of the epidermis whose deregulated proliferation could initiate NMSC. In melanoma, melanocytes are the affected cell population and we also recapitulate the steps by which normal melanocytes subjected to various factors induce genetic instability and inflammatory conditions and can undergo malignant transformation, and we add new data from the field to this framework.*

Immunobiological evaluations in skin cancer - Cutaneous melanoma, is a section in which I have presented original results regarding certain immunobiological parameters in experimental cellular and animal models of melanoma, as well as in patients diagnosed with cutaneous

melanoma. Thus, we developed a protocol based on mass spectrometry (SELDI-TOF-MS) to identify protein biomarkers with the potential to differentiate between melanoma patients and controls (healthy) and to highlight patients with good clinical outcomes from patients with unfavorable outcomes. This approach through SELDI technology thus provided us with a useful proteomic framework in the management of melanoma, contributing to the development of biomarkers in deciphering crucial cellular and molecular events related to advanced stages of the disease.

Subsequently, in an experimental model of cutaneous melanoma in mice I have presented the evaluation of inflammatory cytokines as markers of disease progression and to test our experimental model we used low doses of dacarbazine (DTIC), the first cytostatic approved by the FDA for metastatic melanoma; DTIC was still used at the time of our study in human therapeutic approaches. In the group of female mice, the circulating levels of IL-1-beta, MIP-1-alpha and KC (CXCL1) had a 10-fold increase after 7 days after inoculation, while IL-6, IL-10 and MCP-1 statistically doubled their value. GM-CSF was recorded with a statistically significant increase of 42%, while the increase in IL-12 was not statistically significant compared to the control group, as was the 30% decrease in TNF-alpha. The inflammatory pattern of male mice at 7 days after inoculation was completely different. We did not register for any of the cytokines/chemokines tested the same increase as in the female group. Therefore, circulating KC increased 4-fold, while IL-1-beta and TNF-alpha statistically doubled their circulating values. The increases in circulating IL-6, as well as the decrease in IL-10, were not statistically different. IL-12, GM-CSF, MCP-1 and MIP-1 were practically not affected by tumor evolution in the male mice group. Further studies on melanoma development in women, compared to men, would open a new area of investigation for evaluating the efficacy of the (immune)therapy.

The changes suffered by the local microenvironment and the its complex role in tumor progression and therapy personalization, were approached from the point of view of proteomic technologies that advance knowledge, support clinical investigation and biomarker validation.

In the topic addressed since my doctoral studies, I brought new contributions in the years that followed obtaining the PhD title. Thus, I proposed a potential mechanism for the generation of reactive oxygen species following photodynamic therapy (PDT) with fullerene-porphyrin compounds as photosensitizing agents (Ps) in cancer. Through transmission electron microscopy, we confirmed the apoptotic characteristics of cells subjected to PDT with metallated Ps compounds. Investigating the proliferative capacity of tumor cells treated in vitro, we showed that cells that evade the irradiation protocol present a reduced proliferative capacity probably associated with a delayed effect of PDT that acts more slowly on certain cellular subpopulations.

We have highlighted by confocal microscopy aspects related to the intracellular localization of a photosensitizer (exemplified by a Ga^{3+} Ps compound) thus contributing to a deeper understanding of the mechanism of cellular localization and light activation of the compound in

PDT. We emphasize the possibility of using diamagnetic metallated Pc for PDT as a therapy for invasive brain tumors, adding new players in the therapeutic approach to this disease. In addition, our results have highlighted new mechanisms that should be investigated, since the localization of Ps and the long-term delayed decline effect post-PDT indicate certain complex intracellular pathways that trigger tumor cell death.

The study of the immune system and antitumor responses in cancer, with reference to skin cancers as the main research area of our laboratory, clearly outlines the role of immunobiology as a new omic actor with the potential to decipher mechanisms that still remain unanswered (e.g., resistance to therapy) and to offer new, personalized therapeutic approaches. Exploring the specific role of immune cell populations in melanoma and non-melanoma skin cancers in identifying new biomarkers and new immune networks will be a continuing concern of my research profession.

The original section of the thesis continues with the most recent studies on autoimmune interrelationships in skin pathology: psoriasis. In this section, I described experimental models to study the complexity of Pso pathogenesis, namely the establishment of a murine model of psoriasiform dermatitis. This murine model was analyzed by performing a basic cellular profile at three levels: peripheral blood, spleen and skin. The evaluation of the cellular immune framework in this experimental model was useful for further studies to identify the etiopathogenic mechanism and/or for studies on targeted therapies. In the context of Pso therapeutics, I addressed a topic related to nutritional strategies with impact in the psoriasis, microbiome and obesity axis; I have studied an integrated model comprising the link between Pso and obesity, the analysis of the microbiota model in obesity, as well as autoimmune diseases. We addressed a complex animal model of Pso and obesity that highlights new molecular mechanisms governing these processes, as well as pointing out updated nutritional strategies that may contribute to new adjuvant therapies in Pso.

We were concerned with identifying mechanisms that could support adjuvant therapies in improving psoriatic lesions and normalizing immune parameters: IgY generated against pathological human antibiotic-resistant bacteria administered orally to Pso-C57 BL/6 mice led to the normalization of serum cytokine/chemokine levels in relation to peripheral immune cell populations. The obvious result that emerged from our study was the improvement of anti-inflammatory cytokine levels as well as the normalization of circulating immune cells in the Pso group treated with IgY, the healing process being more efficient in the IgY-treated group compared to the natural healing group and was statistically supported by an increased normalization of the investigated parameters. These results suggest the need for further detailed studies on the intimate mechanism governing the gut microbiota and influencing skin-related autoimmune reactions.

We have deepened our molecular explorations by investigating microbial signatures in obesity-exacerbated psoriasis. In this model, we have shown associations with inflammation and metabolic stress (prolonged fat intake) in a murine model of Pso. The obesity-exacerbated Pso signature (Pso-W) consisted of increased abundance of the genera Ruminococcus, Clostridium, Lachnoclostridium, Desulfovibrio, and Enterorhabdus. The same pathobionts overrepresented in obese Pso mice showed positive correlations with indicators of metabolic stress and proinflammatory factors, indicating potential biomarkers of disease severity. In addition, we highlighted some metabolic features in the therapeutic exploration of psoriatic arthritis: showing that alterations in lipid metabolism exacerbate inflammation by modulating immune cell function, both systemically and locally, amplifying disease severity.

Further in our analysis, we focused on critical regulators of the interrelationship between autoimmunity, chronic inflammation and lipid homeostasis, namely dermal mesenchymal stem cells (MSCs). We corroborated data that converge on the networks of soluble factors, immune and non-immune cells and that mediate the inflammatory state imprinted by MSCs in psoriatic arthritis. Moreover, we signaled the possibility of a vicious circle between MSCs and keratinocytes in psoriatic skin, resulting in a cascade of cellular and molecular events that fuel disease progression and that needs to be explored in the future. We emphasized that metabolic explorations could outline an adjuvant reprogramming approach to help psoriasis patients undergoing biological therapy.

In the third section of my scientific achievements, I have integrated data and results generated by the COVID-19 pandemic which represented an unpredicted challenge for society, and has incited the scientific community and indeed all aspects of life. We highlighted features of the immunological memory developed towards the S protein of the SARS-CoV-2 virus, in the antigenic variability context of the S protein (related to the viral variants that circulated in the population). We emphasized elements of the humoral immune response to SARS-CoV-2 infection, a response mediated by IgG antibodies in tandem with those of the IgA type, and these aspects were mentioned for the first time nationally at the time of publication of the mentioned articles. The appearance of IgA in the bloodstream after immunization by intramuscular vaccination with the approved mRNA platform vaccine was also mentioned for the first time.

Very important both at that time and now, we detected and published the phenomenon of decline in the humoral immune response via IgG and IgA antibodies, thus aligning with the efforts that were taking place internationally regarding the elucidation of the immunological foundations of SARS-CoV-2 infection.

The pandemic period showed me that research carried out in the biomedical field is of paramount importance in the translation of results into clinical implementation and, last but not least, for raising awareness among the general public regarding the importance of research results in unprecedented situations, such as the COVID-19 pandemic.

*The **Part II** of the thesis, entitled **Professional and Academic achievements** I have presented my professional and academic achievements that are directly correlated with the scientific ones, my professional career in the last 20 years, after obtaining my PhD, recording important milestones that were registered in the following areas:*

- *project management as project manager or director*
- *mentoring and guiding students, doctoral researchers or postdoctoral fellows in projects with structural funds*
- *participation in national and international conferences as a guest speaker*
- *publishing articles and book chapters as well as submitting patent applications*
- *membership as a member of academic and professional societies (SIR, EADO, OBBCSSR)*
- *activity as an evaluator in research projects (project offers and progress evaluation)*
- *activity as a peer-reviewer for journals in my field of activity*

A separate chapter of my academic career refers to the coordination of research teams as well as training and mentoring activities that I carried out during the development of projects with European funds where I worked as an expert.

In the period since obtaining my PhD, I have been promoted to a higher academic degree, CSII (in 2017) and then CSI (in 2019), with the next stage of academic and professional progress to be obtaining the habilitation certificate.

*The thesis continues with **Part III Plans for career development**, which includes directions for career development in research and teaching, guidance and mentoring of young researchers.*

The bibliography of each section includes the main bibliographical references in which I presented the personal results cited throughout the paper, as well as references that support the current state of knowledge presented in the habilitation thesis.

In addition to research activity, I am attached to the idea that a research laboratory remains a fundamental crucible for the training of young students, doctoral students or postdoctoral researchers in the biomedical field, a site where young scientists learn the skills necessary for "knowledge production" as the primordial definition of research.