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"GEORGE EMIL PALADE" UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE, AND TECHNOLOGY OF TÂRGU MUREȘ - SCHOOL OF DOCTORAL STUDIES

Summary of the doctoral thesis:

Morphological, immunophenotypic and molecular aspects of melanoma

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Melanoma (MM) is a malignant tumor of melanocytic origin that can be found in the skin, mucosa, choroid, cochlea, midbrain or meninge. Primary cutaneous MM is a malignant tumor with a relatively high incidence, being the 21st most frequently diagnosed malignant tumor worldwide. Non-melanocytic cutaneous malignancies are about 4 times (6.2%) more common than MM, which accounts for about 1.7% of all malignancies diagnosed annually worldwide, with a doubled mortality over the past two decades. Due to the increasing of his incidence and variable heterogeneity, further studies are needed to molecularly characterize MM which is also underlined by the need to validate classical diagnostic and prognostic markers, as well as to highlight new useful markers in differentiating benign from malignant melanocytic lesions and in identifying aggressive MM at risk of relapse or metastasis, in order to provide effective and targeted therapeutic solutions in both primary and metastatic MM.

The aim of this study was to identify cases of MM diagnosed between 1999 and 2018 in order to assess general clinicopathological parameters and to highlight the possible prognostic impact on overall survival (OS). We relied on performing an "in silico" analysis of international public databases, verifying the protein expression of classical diagnostic markers and possible prognostic markers, correlated with their genomic and transcriptomic expression, in order to flourish their immunohistochemical (IHC) expression both from a diagnostic and prognostic point of view.

In the first chapter we built a retrospective database that included the clinicopathological aspects of primary MM over the last two decades to highlight the prognostic aspects related to gender, age, location, histological type, Breslow index, ulceration, lymphovascular and perineural invasions, growth phase and pT stage. In situ lesion and distant metastases without data about primary lesion as well as inoperable cases or positive surgical excision margins, were excluded. The relatively small number of studies on the prognostic impact of generic and MM specific clinicopathological factors among Caucasian, led us to perform this study. Female gender has

been shown to act as a favorable prognostic factor in the progression of cutaneous MM, highlighting the gender and location as independents prognostic factors. Cutaneous and mucosal MM were more frequently diagnosed in females whereas ocular MM was more frequently encountered in males. Location of cutaneous MM was more commonly found in the limbs for women and in the trunk for men. The highest mortality rate was reported for head and neck located MM. Breslow index, ulceration, lymphovascular invasion, growth phase and pT stage were also shown to act as independent unfavorable prognostic factors.

The second chapter consists of two sub-chapters. On one hand, in the first sub-chapter we have addressed the identification of specific and sensitive diagnostic markers of cutaneous MM, useful also in differentiating benign vs. malignant melanocytic lesions or markers with a possible prognostic role. Starting from the hypothesis that the inter-relationship between SOX10 and SOX11 during embryogenesis could be the starting point of possible tumorigenic pathways, they were first studied from an IHC point of view, together with classical diagnostic markers, and the results were also validated through "in silico" analysis of international databases (genomic, transcriptomic and proteomic). For routine diagnosis of cutaneous MM, using S100 protein IHC, associated with at least one of the conventional melanoma-cocktail markers: HMB-45, Melan-A or Tyrosinase is recommended and when there are cocktail-negative cases MITF and SOX10 associated with Ki-67 proliferation index are recommended to outline the certainty of malignancy. SOX10 and S100 protein are the most sensitive IHC markers of MM, but their specificity is really low, which requires the identification of a suitable cocktail or a more effective IHC marker. We concluded that identification of the melanocytic origin of tumor cells can be successfully achieved using the conventional cocktail together with S100, but in difficult cases adapting the cocktail by replacing Melan-A with SOX10 suggests an increased success rate in differentiating malignant versus benign lesion. SOX11 is a useful marker of discrepancy between malignant and benign melanocytic lesions which also identifies aggressive cutaneous MM at risk for lymphatic dissemination and may be considered as an indicator of circulating tumor cells.

On the other hand, sub-chapter two started from the premise of KIT, RAS and BRAF inter-relationship in the MAPK/MEK pathway, and quantification of gene interactions was performed trough "in silico" analysis followed by IHC validation of the results. Another objective of the study was to assess the subcellular localization of IHC expression, in particular for BRAF expression, which was reported with translocated expression in other tumor categories. We concluded that subcellular BRAF expression (exclusively nuclear, exclusively cytoplasmatic or mixed) among cutaneous MM is an independent prognostic factor. Nuclear BRAF expression (exclusively nuclear or mixed) being specific for aggressive MM with a likely increased risk of anti-BRAF therapy resistance. KIT- positive MM show a more favorable outcome, outlining the fact that KIT expression may predict a milder MM behavior.