REVIEW

by Professor Dr. Peter Ivanov Ghenev, Ph.D.

Department of General and Clinical Pathology, Forensic Medicine and Deontology,

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on the dissertation of Dr. Lilyana Nikolova Petkova, on the topic:

"Expression of Cyclin D1, BCL2, p53 and other melanocyte markers in malignant melanoma of the skin and melanocyte nevi - a comparative analysis of immunohistochemical expression, morphological profiles, their importance for diagnosis and tumor progression",

for the educational-scientific degree "DOCTOR", in the scientific specialty "Pathoanatomy and Cytopathology".

By decision of the Faculty Council at the Faculty of Medicine, Medical University - Varna (Protocol № 57/21.12.2021) and by Order № P-109-594/31.12.2021 of the Rector of the Medical University – Varna, I was elected internal member and Chairman of the scientific jury, and on the basis of Protocol №1/07.01.2022 I have been appointed to prepare a review

Please find attached: Review on the Ph.D. thesis of Dr. Lilyana Petkova, with scientifical supervisor Prof. Bogomila Gateva Manevska, MD, DSc

Field of higher education: 7. Health and sports.

Professional field 7.1. Medicine.

Scientific specialty "Pathoanatomy and Cytopathology", for the needs of the Department of General and Clinical Pathology, Forensic Medicine and Deontology, Faculty of Medicine at the Medical University - Varna.

I. Details of the procedure

Dr. Petkova has a main employment contract in the Department of General and Clinical Pathology at the Medical University - Varna and is enrolled as a doctoral student in independent training for the scientific and educational degree "Doctor" in the scientific specialty "Pathology and Cytopathology" with Order of the Rector of MU-Varna № P-109-77/1.02.2019.

Dr. Petkova is working on the thesis, using the materials and the database of the Clinic of General and Clinical Pathology at the University Hospital "St. Marina" - Varna and with the assistance of the Doctoral School at MU-Varna. She presents protocols for successfully passed exams for doctoral minimum - in the specialty and in English. She has completed the education with the right to defense - at the Department Council (Protocol №386/ 6.12.2021 and Order R-109-594 / 31.12.2021 of the Rector of MU-Varna.

The candidate has submitted for participation in the competition a set of materials on electronic media, which are in accordance with the requirements of MU-Varna.

II. Biographical data and career development

Dr. Lilyana Petkova was born on September 23, 1961. She graduated in 1979 from the Mathematical High School in Dobrich. In 1988, she graduated from the Medical University - Varna. She began working as a doctor and part-time assistant in the Department of General and Clinical Pathology at the Medical University - Varna, Dobrich branch. She acquired specialty "Pathology and Cytopathology" at MU - Sofia (Certificate series AC № 01342/1995) and later - professional qualification "Cytopathology" (Certificate series C № 0027/2000).

In 2014, she started working at the Clinic of General and Clinical Pathology of the University Hospital "St. Marina" Varna and later - after a successful

competition as an assistant at the Department. During these years she participated in the educational activities – she conducted practical classes in all disciplines with students of medicine and dentistry and participated in the diagnostic process in the hospital, mastering all basic methods of biopsy diagnosis and showing interest in pigmented tumors, breast pathology, immunohistochemical methods and cytological examinations, for which she has in-depth knowledge and practical skills.

Dr. Petkova has more than 32 years of experience as a pathologist; she is a member of the Bulgarian and European Society of Pathology, the Bulgarian Medical Union and the Union of Scientists in Bulgaria.

III. Structure of the dissertation

Dr. Petkova presented for review a dissertation written in very good Bulgarian on 148 standard pages, structured according to standard requirements and distributed as follows: Contents and abbreviations (6 pages), Introduction (2 pages), Literary review 41 pages), Objectives and tasks (1 page), Material and methods (8 pages), Results (57 pages), Discussion (14 pages), and Conclusions and contributions (3 pages). It is illustrated with 41 figures and 30 tables. The bibliography includes 126 literary sources, 10 in Cyrillic and 116 in Latin.

Relevance of the topic

Malignant melanoma is one of the most malignant tumors in human pathology. Its aggressive biological potential is derived from the rapid rate of growth and metastatic spread - through lymphatic and blood vessels. This makes the prognosis for patients extremely unfavorable. The disease is heterogeneous and its treatment is based on objective criteria, most of which are determined by biopsy.

A specific problem for malignant melanoma is the presence of many variants of benign melanocyte lesions, which are widespread and long-standing, but some of

them have the potential for malignant transformation. In order to accurately diagnose and determine the biological behavior of the tumor, more and more research is focused on the etiology, pathogenesis and spread of malignant melanoma, to identify reliable prognostic and predictive factors, and to distinguish it from benign precursors. The dissertation treats exactly these problems in detail and this makes it extremely relevant.

Review of literature

The literature review is based on 126 literature sources, mostly from the last ten years. It introduces into the topic consistently, outlines the problems, examining in detail the epidemiology, etiology, risk factors, genetics and already known factors for the appearance, course and morphological diagnosis of melanocyte tumors. The histological classification of pigmented neoplasms - benign and malignant, as well as modern methods for their diagnosis are presented in detail. It is noteworthy that Dr. Petkova has skillfully analyzed and summarized the literary information so as to highlight unclear or contradictory data and, accordingly, to determine the aim of the study and formulate the tasks. The personal attitude of the doctoral student to the problem is also evident, especially in those areas where he has his own results.

Aim and tasks of the dissertation

The aim is clearly stated: on well-selected, morphologically diagnosed biopsy cases of benign and malignant pigmented tumors, to analyze the immunohistochemical expression of a set of established and promising melanocyte markers to propose criteria to help morphological diagnostics, especially in distinguishing border-line lesions from malignant melanoma, as well as the possible prediction of biological behavior in these tumors and the determination of appropriate therapy. To achieve this aim, five specific tasks have been formulated.

Material and methods of research

The study was performed on biopsy materials from a total of 91 patients with skin pigment lesions, including 57 benign melanocyte nevi, 10 atypical nevi and 24 malignant melanomas. Microscopic evaluation of each lesion was performed on serial topographic sections, covering the entire area and the edges at the periphery and in depth, according to accepted standards for morphological diagnosis of pigmented lesions. The expression of S100, HMB 45, Ki 67, p53, BCL2 oncoprotein and Cyclin D1 was immunohistochemically assessed and evaluated qualitatively and semi-quantitatively in intensity and distribution. The obtained data were processed with various statistical methods.

Results

The results are presented in the order of the tasks. First of all, all cases were divided into three diagnostic groups according to their histological type, respectively - benign nevi, atypical nevi and melanoma. Demographic indicators (gender and age) and topographic location were then studied. Malignant melanomas were analyzed according to Breslow thickness, Clark invasion level, mitotic activity, ulceration, satellite, and tumor-infiltrating lymphocytes. The only case of melanoma regression is described in detail. Within the three groups, the results of the immunohistochemically tested expression of Cyclin D1, BCL2, S100 protein, p53, HMB45 and Ki 67 were compared.

The expression of Cyclin D1, p53 protein and HMB45 was reported according to an eleven-model scheme introduced by Dr. Petkova, which includes a combined assessment of the intensity, character and location of the reaction. When considering the immunoreactivity of HMB45, the doctoral student includes a new criterion - gradient in expression.

A comparative study of immunohistochemical markers of malignant potential shows that the combination of Cyclin D1, p53 protein and HMB 45 has the highest statistical reliability. In this aspect, other morphological features of the pigmented lesions are considered, such as ulcerations, microsatellites and tumor-infiltrating lymphocytes, for which no significant patterns have been established.

Particularly interesting and innovative is the approach applied by the PhD student in assessing the expression gradient of HMB 45. The results show a high level of association between the expression gradient of HMB45 and benign nevi, a tendency to reduce the expression gradient in atypical nevi and no gradient in melanomas. This is a new scientific fact of great importance for the differential diagnosis of difficult cases.

Regression in malignant melanoma is the subject of special attention, although only one case is described in the sample for the thesis. Spontaneous regression is a rare phenomenon and this makes in-depth study difficult. According to the literature, the correlation of regression with the prognosis is contradictory. Establishing the molecular mechanisms of regression would allow the identification of targets for its therapeutic induction.

Discussion

In the discussion, the own results are analyzed in detail and compared with the data from the literature. Arguments and new scientific facts confirming the achievement of the goal are presented, which are formed as conclusions. The doctoral student modestly notes the contributions of the thesis, divided into original scientific-applied and confirmatory.

Dr. Petkova presented for review an abstract (111 pages with tables and color figures), designed according to the requirements. Although in abbreviated form, it accurately reflects the content and gives a complete picture of the dissertation.

Dr. Petkova participates in the competition with four publications in Bulgarian scientific journals, published in the period of doctoral studies, all strictly on the topic, in which she is the first or only author.

IV. Conclusion

The presented documentation and dissertation work meet the procedural requirements according to the normative documents of the Medical University - Varna. The scientific production of the candidate meets the required criteria. I accept the reference for scientific contributions without remarks. I highly appreciate the professional qualities of the candidate, as well as her opportunity for career development.

For these reasons, I am particularly pleased to fulfill my duties as a member of the Scientific Jury and to vote positively for Dr. Lilyana Petkova to be awarded the educational and scientific degree "Doctor" in higher education 7. Health and Sports, Professional 7.1 Medicine and the scientific specialty "Pathoanatomy and Cytopathology".

10.II.2022 Reviewer:

(Prof. Peter Ghenev, MD, PhD)