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Fund “Nauka” Project № 19005 Resume – Competition-Based Session 2019:

“Comparative analysis of immunohistochemical expression of Cyclin D1, BCL2, and P53 in melanocytic nevi and skin melanomas, to comprise a diagnostic algorithm”

Project leader: Chief assist. prof. Ina Georgieva Kobakova, MD, PhD

Scientists enrolled in the project:

- Project leader: chief assistant professor Ina Georgieva Kobakova, MD, Ph.D., Department of Medico-Biological sciences, Faculty of Pharmacy, Medical University – Varna;
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In humans, skin melanoma is one of the most aggressive malignant tumors, giving frequent recurrence and being capable of metastasis both in the lymphogenic and hematogenic pathways to almost all organs. From the side of the organism, the immune reaction is weak or non-existent, allowing for the fast progression of the condition.

Risk factors and reasons for the development of skin melanoma are various; however, ultraviolet radiation and solar burns, especially in people with light skin, are the main factors. Other factors contributing to the development of melanoma are genetic determination and age of 50 and upwards. Often melanoma arises from predicting nevi, with multiple nevi increasing the risk further.

The development and development of the neoplasm is associated with a disruption in the balance of cell growth and cell death, a process swayed by multiple protein factors.

Cyclin D1 is a protein, which is part of the cyclin-dependent kinases complex of CDK4 and CDK6. Cyclin D1/CDK4 and Cyclin D1/CDK6 activate (phosphorylate) the Rb

(retinoblastoma) protein, a tumor suppressor gene and the cell progresses from the G1 to the S phase of the cell cycle. Mutations, amplification, and overexpression of the cyclin D1 gene increase the speed with which the cell cycle passes through the phases of cell division, characteristic of tumors and oncogenesis.

The Bcl-2 protein is antiapoptotic, allowing for cell survival by inhibiting pro-apoptotic genes and their protein products.

The P53 protein is a transcription factor regulating the cell cycle utilizing suppression in malignancies and therefore can be coined as the "guardian" of the genome.

Publication on the expression of Cyclin D1 and BCL-2 in melanomas and benign pigment lesions (nevi) show significant differences in expression in the blue nevi and the mimicking melanoma. In healthy skin, there is Cyclin D1 expression benign nevi have zonal expression, dysplastic nevi overexpression, with the expression correlating with the cytological atypia. According to Zainal Abidin, acral melanomas have significant expression of Cyclin D1, unlike non-acral ones. Eduard R. Sauter defines expression in both acral melanomas and lentigo maligna and superficially spreading melanoma. BCL2 immunoreactivity in both nevi and melanoma is positive, however, it declines in the malignant lesion. According to other authors, BCL2 expression is identical in nevi and melanoma, with the result not showing a significant. P53 protein expression in Spitz nevi, ordinary nevi and melanoma manifest as a determining factor according to Reuven Bergman, with high expression in melanomas.

Goals:

The goal of the current project is to evaluate the immunohistochemical expression of Cyclin D1, BCL2 and P53 in conventional nevi and skin melanomas, with the task of establishing their pathogenic role in the progression of melanocytic lesions and their progression and define a diagnostic algorithm. To define the melanocytic origin of poorly pigmented, achromatic melanoma, S100 protein, and melanosome clone HMB45 will also be used. The first marker is highly sensitive with low specificity, with most of the melanocytic lesion expressing it both in the cytoplasm and cell nucleus. Melanosome clone HMB45 is a highly sensitive and specific marker for melanin-producing cells and will be used to define the S100 positive tumors as melanocytic and differentiate from non-melanocytic ones.

Research tasks:

To study the expression of Cyclin D1 in conventional pigmented nevi, atypical nevi, malignant melanoma and control of healthy skin.

To study the expression of BCL2 in conventional pigmented nevi, atypical nevi, malignant melanoma and control of healthy skin.

To evaluate the expression of P53 in conventional pigmented nevi, atypical nevi, malignant melanoma and control of healthy skin.

Study design:

A retrospective approach was chosen for the study. The materials used for the study with being archived tissue blocks embedded in paraffin, from which 3-4 micrometer thick sections will be prepared and stained with Hematoxylin and Eosin to evaluate the histological changes as well as stained on a Dako Autostainer Link48 with the defined antibodies. Evaluation of the histological slides and the expression of the antibodies will be carried out on a Leica light microscope. The stained slides will be scanned and archived using the Leica Aperio AT2 automated scanned system. A total of four groups will be included in the study – conventional nevi, atypical nevi, melanomas and control of healthy skin. The material is selected from the archive of DCC Dobrich Ltd. and the Department of General and Clinical Pathology, St. Marina University Hospital – Varna. These will include a target group of 80 nevi and melanomas and 20 healthy controls.

Methodology, including statistical analysis of data.

The study will make use of classical methods – a retrospective analysis of tissue blocks stained with hematoxylin and Eosin and immunostained with the aforementioned antibodies and evaluated under a light microscope.

Statistical analysis:

- descriptive
- non-parametric Pearson test
- parametric analysis
- regression analysis

Assessment by the lead researcher of the benefits of the study:

The defined problems have a direct impact on the diagnostic process of a melanocytic lesion, which is difficult to define as malignant on histology. Therefore, the results can define early diagnostic features and therefore improvement in treatment. Theoretically, the study can also define key processes in the development of melanoma from preceding lesions. The results will enrich the knowledge of histological criteria and define risk factors and cohorts of patients.

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Achieved results:

In-depth analysis of the immunohistochemical expression of Cyclin D1, BCL2, and p53 and HMB-45 in benign, atypical, and malignant melanocytic neoplasms performed during the study. Based on the type and gradient of expression, an eleven-point scale for assessing the risk of malignancy was compiled with a very high degree of statistical reliability.