

Opinion

from

Prof. Ilko Georgiev Bakardzhiev, MD, PhD

Head of the Board of Medical Cosmetologists, Medical College, Medical University - Varna
of a dissertation of Dr. Plamen Petrov Vassilev, on the topic "Apoptosis and necroptosis in basal cell and spinocellular skin carcinoma", for awarding the scientific and educational degree "*Doctor*", in the scientific specialty "Pathologyanatomy and Cytopathology".

Supervisor: Prof. Maria Angelova Tsaneva, MD, PhD

By Order No. R-109-427/06.10.2025 of the Rector of MU-Varna, I have been elected as a member of the scientific jury, and on the basis of Protocol No. 1/14.10.2025, I have been appointed to prepare an opinion on the procedure for acquiring the educational and scientific degree "*Doctor*" of Dr. Plamen Petrov Vassilev, a full-time PhD student, at the Department of General and Clinical Pathology, Forensic Medicine and Deontology", MU-Varna. The presented set of documents is in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation and the Regulations for the Development of the Academic Staff at the Medical University of Varna.

Malignant skin diseases are a heterogeneous group of tumors that can be with melanocytic and non-melanocytic origin, an unfavorable prognosis and an increasing incidence worldwide. Skin carcinomas originating in the epidermis are two main histological types (basal cell and spinocellular or squamous cell carcinoma). Risk factors for the development of these tumors are patients with first and second phototypes of skin, sunburn, especially in childhood, prolonged use of photosensitizing substances, genetic and somatic mutations, hereditary factors, immunosuppression, environmental characteristics. Therapeutic behavior in these neoplasms includes their surgical removal, targeted radiotherapy and chemotherapy. The latter two therapies induce apoptosis, a natural process of programmed cell death, through which tumor cells are removed. Sometimes the induction of apoptosis is not possible due to congenital or acquired resistance to these tumors. The most manifest sign of the early and late stages of apoptosis is the activation of cysteine proteases (caspases). Necroptosis is a relatively new form of cell death that is stimulated by classical cell death receptors, identical to those of the external pathway of apoptosis. It combines the morphological characteristics of necrosis and part of the molecular regulatory mechanisms of apoptosis. It is mediated by the receptor family of interacting protein kinases: RIP1 and RIP3, which are involved in the formation of a complex called necrosome. where, through cascade phosphorylation and conformational changes, the permeability of the cell membrane is affected. Induction of necroptosis in defective apoptosis is a potential therapeutic strategy. In addition to the clinical and histological criteria for the precise diagnosis of neoplasms, markers are increasingly sought, which are even more

important for the early diagnosis and success of treatment and prognosis of these diseases, as all studies using modern methods are up-to-date and a fully scientifically grounded necessity for our country. The detailed study of the expression of Caspase-3 and RIPK3 in patients with basal cell and squamous cell carcinoma in the future may be used as more powerful diagnostic and prognostic tools.

Structure, characteristics and evaluation of the dissertation:

The presented dissertation is written on 139 standard typewritten pages distributed as follows: content and abbreviations- 8 pages, introduction- 2 pages, literary review- 54 pages, purpose and objectives - 1 page, material and methods - 5 pages, results and discussion - 43 pages, conclusion - 1 page, contributions- 1 page. It is illustrated with 66 tables and 35 figures. The bibliography includes 355 literary sources, 6 in Cyrillic and 349 in Latin.

The **literature review** describes epidemiological and risk factors for the occurrence of basal cell and squamous cell carcinoma of the skin. The main signaling pathways during the embryonic period of skin development are considered. Special attention is paid to hereditary, genetic factors and somatic mutations in tumorigenesis. Morphological characteristics, staging, prognostic and predictive histological parameters of BCC and SCC are presented. The different types of cell death - apoptosis, necroptosis, their molecular mechanisms and their role in the tumor process are discussed. The literature review is built on the basis of a large volume of literature and reflects excellent knowledge of the studied problems by the doctoral student.

The purpose of the dissertation is clearly and precisely defined. For its implementation, 7 correctly formulated tasks are set.

Material and methods are methodologically correct developed on a sufficient clinical contingent. The present study is retrospective and includes 91 patients, 46 with basal cell carcinoma of the skin and 45 with squamous cell carcinoma for the period 2015 - 2021. Different criteria were used to categorize each parameter - localization, histological type of tumor, TNM staging, tumor infiltrating lymphocytes, vascular and perineural invasion. As specific research methods, an indirect immunoperoxidase method for immunohistochemical analysis using mini KIT high Ph DAKO K8024 was used.

The following methods of statistical analysis are applied: descriptive statistical analysis, Student test for two independent samples, Variance Analysis (ANOVA). For the statistical analysis of the data, the specialized software package STATISTICA (StatSoft Inc, USA, STATISTICA Manual (Data analysis software system), Version 13.0, 2022) was used. The graphs are built in Microsoft Excel for Windows. The research methods are selected according to the tasks set and are very well described and informative.

Results and discussion

The results of the studies are explained in detail, systematized, illustrated precisely, statistically confirmed and presented in tables and figures. They give a clear answer to the tasks set. The clinical and morphological characteristics of patients with basal cell and squamous cell carcinomas have been studied. The doctoral student conducts a comparative analysis between

the average values of Caspase-3 expression in tumor tissue and adjacent non-tumor tissue in patients with BCC and SCC. It was found that Caspase-3, the marker of apoptosis, is expressed equally in tumor and non-tumor tissue of BCC. The cytoplasmic and nuclear expression of Caspase-3 in tumor and non-tumor tissue in SCC is investigated and increased level in the cytoplasm of tumor cells is reported. An elevated level may indicate that the apoptotic pathway is active in SCC. Cytoplasmic and nuclear expression of Caspase-3 in the tumor tissue of BCCs and SCCs are not affected by age and the gender of the patients. The obtained results show an inverse relationship between cytoplasmic and nuclear expression of Caspase-3 and differentiation of SCCs. As tumor differentiation decreases, Caspase-3 expression increases. Dr. Vassilev found that in BCC and SCC, cytoplasmic and nuclear expression of RIPK3 are directly correlated with tumor-infiltrating lymphocytes (TILs). High expression of the antibody is associated with a higher intensity of TILs in the tumor tissue. The obtained results support the understanding of the immunogenic properties of necroptosis because the necroptosis marker RIPK3 is elevated in both skin cancers when the intensity of TILs is highest. The obtained data are perfectly compared with the known, used from the literature sources. Larger studies and the experience of other researchers are cited in detail, and their results are compared and discussed.

16 conclusions have been formulated, which give an idea of the scientific and research work carried out at all stages of the conducted studies. The contributions are divided into 2 scientific contributions of original nature and 8 scientific contributions of practical applied nature. In principle, I accept the contributions indicated by the candidate. In connection with the dissertation, two full-text publications in Bulgarian scientific journals are presented. The abstract reflects the structure and main elements of the dissertation.

Conclusion:

The presented dissertation of Dr. Plamen Petrov Vassilev is original and up-to-date in its content and interpretation, methodologically correctly developed, with a large number of studies carried out. It fully meets the criteria of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation and the Regulations for the Development of the Academic Staff at the Medical University of Varna for obtaining the scientific and educational degree "*Doctor*" in the scientific specialty *pathological anatomy and cytopathology*. The conducted studies are scientific and practical, provide an answer of the tasks set and provide new information about the expression and localization of Caspase-3 and RIPK3 expression in basal cell and squamous cell carcinoma of the skin. The outstanding merits and contributions give me grounds to vote positively and confidently recommend for a positive assessment by the esteemed members of the specialized scientific jury.

Varna

12.11.2025

Prof. Dr. Ilko Bakardzhiev, MD, PhD

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