

Opinion on the degree of Doctor of Philosophy

OPINION

IN THE COMPETITION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN MU-VARNA, DEPARTMENT OF GENERAL AND CLINICAL
PATHOLOGY, FORENSIC MEDICINE AND DEONTOLOGY, FACULTY OF
MEDICINE

From Assoc. Prof. Silvia Nikolaeva Genova, MD, PhD

Department of General and Clinical Pathology

Medical University of Plovdiv

of dissertation for obtaining an educational and scientific degree "**Doctor**"

Professional field: Medicine

Scientific specialty: *"Pathology and Cytopathology"*, code 03.01.03

Author: Martina Georgieva Stoeva, MD

Form of doctoral studies: full-time doctoral studies

Department: "General and Clinical Pathology, Forensic Medicine and Deontology", MU-Varna

Topic: IMMUNOHISTOCHEMICAL EXPRESSION OF THE NECROPTOSIS
MARKER RIPK3 IN BREAST CANCER

Supervisor: Prof. Maria Angelova Tsaneva, MD, PhD, Department of General and Clinical
Pathology, Forensic Medicine and Deontology, MU "Prof. Dr. Paraskev Stoyanov" Varna.

1. General presentation of the procedure and the doctoral student

The presented set of materials on paper and electronic media is in accordance with Art. 70 (1) of the Procedure for Acquisition of the degree of Doctor of Philosophy in MU - Varna; Regulations for the development of the academic staff of MU-Varna from 15.09.2020 and includes all necessary documents.

The doctoral student has submitted two full-text articles in a Bulgarian peer-reviewed journal and one participation in a Bulgarian forum on the topic of the dissertation.

Martina Georgieva Stoeva was born on February 22, 1988, in Blagoevgrad. She acquired a master's degree in Medicine in 2012 at the Medical University "Prof. Dr. Paraskev Stoyanov" Varna. Since 2017 she has been an assistant professor at the Department of General and Clinical Pathology, Forensic Medicine and Deontology, MU "Prof. Dr. Paraskev Stoyanov" Varna.

The total professional work experience of Dr. Stoeva in the department is 4 years and 8 months.

Dr. Stoeva acquired a specialty in General and Clinical Pathology in 2017. Certificate of recognized specialty № 021479 / 20.02.2018, MUV Series № 3942, Medical University "Prof. Dr. P. Stoyanov", Varna.

Academic Career Development: Since 2017 Dr. Stoeva is an assistant professor at MU-Varna. She conducts exercises, lectures and exams for students in "Medicine", "Dental Medicine", "Pharmacy", "Medical Laboratory Technician" (English and Bulgarian language program). She also conducts courses for specialists in General and Clinical Pathology and FED for medical students. Dr. Stoeva has participated in three research projects with significant scientific contributions.

Dr. Stoeva is a member of the Faculty Council at the Faculty of Medicine. She is fluent in English, written and spoken, C1 level.

2. Assessment of the personal participation of the doctoral student in the dissertation

The topic of the dissertation is relevant and does not repeat other studies in our country in connection with the expression of the marker for necroptosis RIPK3 in breast cancer.

The dissertation contains 133 standard pages and is illustrated with 50 tables and 42 figures. The literature includes 205 literature sources, of which 4 in Cyrillic and 201 in Latin.

The PhD student examines the various forms of cell death with morphological signs of necrosis, which is regulated in a programmed way through certain signaling pathways. To understand the molecular mechanism of necroptosis, most studies have focused on tumor necrosis factor alpha (TNF α), receptor-interacting protein kinase 3 (RIPK3) and caspase-8, and molecular activation and deactivation pathways, as well as of the interaction between the components of necroptosis in the cell. The passage of necroptotic signaling components through the nucleus is a mechanism for regulating the formation of cytosolic necrosomes as a form of regulation of necroptotic cell death. Inhibition of cell death is a hallmark of malignant tumors.

The doctoral student shows a thorough knowledge of the state of the problem and uses the literature material creatively, making a literature review on 42 pages with 205 literature sources, 4 of which are by Bulgarian authors.

The introduction presents the problems related to breast cancer, etiology, pathogenesis and the main therapeutic methods in patients with breast cancer, skillfully pointing to unresolved scientific, diagnostic and prognostic problems on the subject. The literature review includes the epidemiology and social significance of breast cancer, forms of programmed cell death, necroptosis, which is similar in mechanism to apoptosis and morphologically similar to necrosis, and signaling pathways mediated mainly by Receptor Interacting Serine/Threonine Kinase 1 (RIPK1), Receptor Interacting Serine/Threonine Kinase 3 (RIPK3) and Mixed Lineage Kinase Domain Like Pseudokinase (MLKL). Finally, emphasis is placed on necroptotic pathway mediators, alone or in combination that may potentiate metastasis and progression in malignant tumors. The dilemma that necroptosis also serves as a mechanism that prevents the development of a tumor process when apoptosis is compromised allows for in-depth research.

The literature review begins with the epidemiology, etiology and risk factors for the development of breast cancer. The family burden is considered; individual characteristics; genetic predisposition; reproductive factors; nutrition, obesity and physical activity; environmental factors such as radiation and exposure to diethylstilbestrol. Emphasis is placed on the discussion of different classification approaches such as: Molecular classification; Integrative cluster classification; Histopathological classification, where new aspects are given in the classification of epithelial tumors of the mammary gland. The classifications are illustrated with tables, which helps for accurate and fast orientation in the subject.

Prognostic and predictive factors are discussed on 10 pages, which include the problem of necroptosis and types of cell death.

The analysis of the literature review is discussed as a conclusion and notes that data on the influence of necroptosis on the development and progression of breast cancer are very scarce. Based on isolated reports that low RIPK3 expression is associated with aggressive clinical features, while high RIPK3 expression is associated with better survival, with the necroptosis marker being an independent prognostic factor, the doctoral student noted the need for further in-depth studies to investigate the role of necroptosis in the development, metastasis and future treatment of breast cancer patients.

Purpose and tasks: The aim is clear and precise, namely to study the expression of the necroptotic marker RIPK3 in relation to clinical and morphological parameters, receptor status, proliferative marker Ki67 and survival of patients without progression to breast cancer. For the purpose of the research 6 tasks have been formulated.

The first three tasks study the clinical and morphological characteristics of patients, receptor status: ER, PR, HER2 and the proliferative marker Ki67 in the tumor tissue of patients with breast cancer, as well as the survival of patients with breast cancer. Tasks 3 to 6 correspond to and contribute to the achievement of the formulated goal.

Retrospectively, a total of 98 patients were included, 79 of whom were diagnosed with breast cancer and the remaining 19 were controls. The material used in the study is sufficient to obtain statistically reliable results. The patients from the selected cases were biopsied and/or operated at the University Hospital "St. Marina" - Varna. The research methods are modern and their reporting, description and comments show that the dissertation skillfully uses them.

Carcinomas are divided into two groups including 16 cases of lobular carcinoma and 63 cases of ductal carcinoma. The controls were presented by 10 cases with non-proliferative type of fibrocystic breast disease (FCBD) and 9 cases of proliferative type, fully meeting the target and allowing for statistical processing. The criteria for staging, degree of differentiation and molecular classification of tumors are clearly defined. The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation. The H-score was used to assess the nuclear and cytoplasmic expression of RIPK3. The data were processed and analyzed using SPSS ver. 23. It should be noted the exceptionally good statistical processing of the materials, which gives reliability to the obtained results.

The "Results" chapter is presented on 55 standard pages, illustrated with 42 figures and 50 tables.

The obtained results from the research are well explained, correctly described, logically arranged and accompanied by well-structured tables, figures and photos. The results are summarized in tables and illustrated with appropriate bar, dot and line graphs. The excellent quality of the photo material makes a good impression. The cytoplasmic and nuclear expression of RIPK3 in the tumor tissue of all 79 patients was determined according to the set task of the dissertation work. The mean cytoplasmic and nuclear expression of RIPK3 was determined by H-score. The PhD student also determined the expression of RIPK3 in 19 patients with FCBD,

divided into two groups (10 cases with non-proliferative type of FCBD and 9 cases with proliferative type FCBD), as cytoplasmic and nuclear reactions were found in both types of FCBD. Comparative analysis of the cytoplasmic and nuclear expression of RIPK3 was determined depending on the age of the patients, the histological type of the tumor, the T-stage of the tumors, the molecular profile, the proliferative marker Ki67 and the survival of patients with breast cancer.

A detailed discussion of the results structured according to the formulated tasks is made in "Chapter V". The PhD student made a detailed comparative analysis of the cytoplasmic expression of RIPK3 depending on the age of the patients, the histological type of the tumor, the T-stage of the tumors, the molecular profile, metastases, the proliferative marker Ki67 and the survival of patients with breast cancer. The comparative analysis of cytoplasmic expression of RIPK3 was determined depending on: the area of expression in tumors (Proportion score - PS); the intensity of expression in tumor cells (Intensity score - IS); total score in tumor cells (Total score - TS) in ER and PR positive cells. A parallel has been made between the data in the literature, the world experience and the achieved own results. I would like to emphasize again that the correct choice of the used statistical methods contributes to the correctness of the made conclusions. Each result is accompanied by an explanation and statistical conclusions. The discussion is at a very good level, with clear and precise scientific and statistical language and style.

From the studies, research and results made in this way, 12 conclusions were formulated, 2 contributions with original character and 3 scientific contributions with practical-applied character. The conclusions are based on the statistical correlation of the studied cases and the practical interpretation of the results according to the set tasks. Regarding the contributions, the doctoral student determined the prognostic and predictive value of RIPK3 in patients with breast cancer for the first time in Bulgaria. For the first time a complex clinico-morphological and immunohistochemical analysis of the receptor status and RIPK3, a marker for necroptosis in patients with breast cancer, was performed and an analysis of the immunohistochemical expression of the marker for necroptosis RIPK3 was performed to elucidate its prognosis and survival in patients with breast cancer. The most significant of the results was that high nuclear expression of RIPK3 in tumor tissue was associated with lymph node metastasis and low progression-free survival in breast cancer patients. For the first time, breast cancer was analyzed for disease-free survival versus nuclear expression of RIPK3. Only a few research papers on the problem can be found in the world English-language literature.

Publications and personal contribution of the doctoral student. Dr. Stoeva has presented two full-text articles in a Bulgarian peer-reviewed journal and one participation in a Bulgarian forum on the topic of the dissertation.

From the dissertation presented in this way, I believe that Dr. Stoeva has independently developed the topic, and the results show her as a responsible, diligent and consistent young scientist who handles scientific sources and facts very well, is able to analyze, compare and summarize the results and has analytical and practical thinking.

It is obvious that Dr. Stoeva shows enviable consistency and ambition to work in the field of science and to develop in the chosen professional field.

Thesis summary

The thesis summary is written on 75 pages and is made according to the requirements, including the main parts of the dissertation, the required volume of tables, figures and photographs. The content of the thesis summary gives an excellent idea of the overall dissertation and reflects the main results, conclusions and contributions.

CONCLUSION

In conclusion, I would like to say that in the presented dissertation the doctoral student studies for the first time in our country necroptosis, nuclear and cytoplasmic expression of RIPK3 in breast cancer and makes an extensive analysis of the expression of the marker for necroptosis in tumor cells according to histological type of the tumor, T-stage, molecular profile, metastases, proliferative marker Ki67 and survival of patients with breast cancer. A great deal of research has been done, even exceeding the standard requirements for doctoral work. The dissertation contains original scientific, scientific-applied and applied results, which represent an original contribution to science and meet all the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria, The Regulations for application of the Law for the Development of Academic Staff in the Republic of Bulgaria and the Regulations of the Medical University of Varna. The submitted materials and dissertation results fully comply with the specific requirements adopted in connection with the Regulations of MU - Varna for application of the Law for the Development of Academic Staff in the Republic of Bulgaria.

Based on the above, I believe that the dissertation of Martina Georgieva Stoeva is a fully completed scientific work. I confidently give my positive assessment by voting "For" the awarding of Martina Georgieva Stoeva the degree of Doctor of Philosophy. I call on the other members of the esteemed scientific jury to vote in favor.

16.02.2022

Prepared the opinion: 

Assoc. Prof. Dr. Silvia Nikolaeva Genova, PhD