

REVIEW

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Regarding dissertation for awarding the educational and scientific degree "Doctor" of Dr. Martina Georgieva Stoeva, PhD student at the Department "General and clinical pathology, forensic medicine and deontology" in the Faculty of Medicine at Medical University of Varna, on topic "Immunohistochemical expression of the necroptosis marker RIPK3 in breast cancer" with supervisor prof. Maria Tzaneva, MD, PhD

The proposed for review by me of dissertation on topic "Immunohistochemical expression of the necroptosis marker RIPK3 in breast cancer" with author Dr. Martina Georgieva Stoeva, is topical. According to the literature, breast cancer is the most commonly diagnosed malignancy in women and one of the most common causes of cancer-related mortality due to tumor progression and metastasis. A number of risk factors, including family history, some genetic mutations, hormonal, reproductive, and other environmental factors, are involved in the oncogenesis of breast carcinoma, but the exact mechanisms remain unclear. In routine oncology practice, clinical and morphological parameters (histological type, degree of differentiation, TNM stage of the tumor), as well as the molecular profile of the tumor (expression of estrogen and progesterone receptor / ER and PR, and epidermal growth receptor factor HER2) play a key role in determining patients' prognosis and choice of therapy. Modern oncology is constantly striving to improve diagnostic and treatment methods in patients with neoplastic diseases by studying new molecules that would be important as prognostic factors and as potential target molecules for targeted therapy. In recent years, the role of necroptosis in the regulation of biological features of malignant tumors with different localization has been the subject of intensive study. Necroptosis is a form of programmed cell death which is similar in mechanism to apoptosis and has morphological similarities to necrosis. Key mediators of necroptosis are RIPK1 and RIPK3 (Receptor Interacting Serine / Threonine Kinase 1 and 3), and MLKL (Mixed Lineage Kinase Domain Like Pseudokinase). Accumulated data show that necroptosis plays an important role in tumor immunity and in the progression, and metastasis of malignant tumors with different localization. Literature data on the role

of necroptosis in tumor biology and the prognosis of patients with breast cancer are scarce. Studying the expression of necroptosis mediators would help to elucidate its role in the development, metastasis and future treatment of breast cancer patients.

In this direction is the dissertation work of Dr. Martina Stoeva. The presented dissertation meets the requirements for structure and volume of the Faculty of Medicine, MU-Varna. It is written on 134 pages, designed in the following sections: Introduction (2 pages) Literature review (42 pages), Aim and purposes (1 page), Material and methods (8 pages), Results and discussion (57 pages), Conclusions (2 pages), Contributions (1 pages), Publications related to the dissertation (1 page) and References (17 pages).

The literature review is devoted to the breast cancer and synthesizes the state of scientific knowledge at the moment. Dr. Stoeva focuses in detail on the epidemiology and risk factors for the development of breast cancer, the morphological classification and diagnosis of individual histological variants; the modern criteria for determining the degree of differentiation and staging of breast cancer, as well as morphological molecular markers with prognostic and predictive value. Much of the review is devoted to the accumulated data in the literature on necroptosis, its molecular mechanisms and its role in tumor progression and metastasis in malignant tumors of different localization, including breast cancer. The bibliography contains 204 literature sources, of which only 4 - in Bulgarian. The literature citations are up-to-date as over 50% of them are from the last 10 years.

The aim of the dissertation is clearly formulated, namely was to investigate the immunohistochemical expression of the necroptosis marker RIPK3 and its relation to clinical and morphological parameters, receptor status, proliferative marker Ki 67 and survival without progression of patients with breast cancer. There are 6 purposes that fully meet the aim. To perform the set purposes, in the study are included 79 patients with breast cancer and 19 patients with fibrocystic disease as control group, histologically diagnosed at the Department "General and clinical pathology, forensic medicine and deontology", MU-Varna. The diagnosis and stage of 71 of the cases of breast cancer have been determined according to the 5th edition of the WHO on breast tumors from 2019 on surgical biopsies and in the rest 8 cases - the diagnosis was made histologically on a tru-cut biopsy, and the stage was determined by imaging. The molecular profile of breast cancer was assessed according to the

immunohistochemical expression of ER, PR, HER2 and Ki 67. In 58 cases, subsequent hormone, radiation and / or chemotherapy was performed at the University Hospital "St. Marina" - Varna and their disease-free survival was determined. Immunohistochemical cytoplasmic and nuclear expression of RIPK3 was assessed in all studied patients (with breast cancer and fibrocystic disease) using the H-score (histological score). The methods used for statistical analysis, as well as the morphological methods of research are adequate to the set aim and purposes, and all the necessary conditions for their implementation were observed.

It was obtained original results, illustrated with 40 figures and 44 tables. An exhaustive analysis of the individual clinical and morphological characteristics of the patients with breast cancer was performed. It has been found that breast cancer is most common in female in the age group 61-70, as 66% of them are aged above 65 yrs. In 80% of the cases the histological type was ductal carcinoma and in the rest 20% - lobular carcinoma. Regarding the T-stage, the histological degree of differentiation and the proliferative activity of the tumor, assessed by immunohistochemical expression of Ki 67, it was found that the proportions of cases with T2- stage, moderately differentiated (G2) carcinoma and high Ki 67 index are the highest. The reported mean progression-free survival of the studied breast cancer patients who received adjuvant chemotherapy and radiation therapy and achieved remission was 113.8 months.

The results of the immunohistochemical study of the marker of necroptosis RIPK3 showed significant differences in the level of cytoplasmic and nuclear expression of the marker when comparing the group of breast cancer and control group of fibrocystic disease - the cytoplasmic expression of RIPK3 in mammary carcinoma tumor tissue is lower, while nuclear expression is higher than in the control group. The performed statistical analyzes show that the cytoplasmic expression of RIPK3 in lobular carcinoma is higher compared to ductal carcinoma, as well as in highly differentiated breast carcinomas compared to low-differentiated tumors. In breast carcinomas with pronounced intensity and high overall score of ER or PR, or low Ki 67 expression, intense cytoplasmic expression of RIPK3 is most common. In contrast, in triple-negative carcinomas, the mean cytoplasmic expression of RIPK3 as assessed by H-score was the lowest. Cytoplasmic expression of RIPK3 in tumor tissue did not show an association with the age of breast cancer patients, T-stage, nodal status, HER2 status of the tumor and was not associated with progression-free


survival of the patients. Regarding nuclear expression of RIPK3 in mammary carcinoma tumor tissue, high nuclear expression of RIPK3 was found to be associated with lymph node metastases (N1 stage) and lower progression-free survival in breast cancer patients. Nuclear expression of RIPK3 in breast carcinoma tumor tissue showed no association with other clinical and morphological parameters such as patient age, histological type of carcinoma, T-stage, degree of differentiation, and tumor molecular profile. It should be noted that all the results reported in this dissertation are very well interpreted and compared with those of other authors.

The 12 conclusions made by Dr. Stoeva fully correspond to the obtained results. The indicated contributions of the dissertation work are of original or scientific-applied nature and are of direct importance for the modern morphological diagnosis of breast cancer in everyday clinical practice.

The thesis summary of Dr. Martina Stoeva meets the requirements according to the Rules for the terms and conditions for obtaining scientific degrees of MU-Varna and reflects the main elements of the research and the results in the dissertation. In connection with the dissertation, the PhD student Dr. Stoeva has 2 publications (the both in the scientific journal "Varna Medical Forum") as well as 1 scientific communication at a national scientific forum. Dr. Stoeva is the first author of the both publications, which is proof of its leading role in the research.

In summary, the dissertation of Dr. Martina Stoeva is fully completed, with clear aim and tasks, original results and conclusions about the cytoplasmic and nuclear immunohistochemical expression of the necroptosis marker RIPK3 and their relations with the clinico-morphological characteristics, molecular profile and survival without progression of the patients with breast cancer. It meets the requirements of the Law for the development of the academic staff in our country, the Rules for its implementation and the terms and conditions for obtaining scientific degrees of Medical University of Varna. Therefore, I recommend to the honoured members of the Scientific Jury for conducting the defense to give a positive vote of Dr. Martina Georgieva Stoeva for the award of the educational and scientific degree "Doctor" in the scientific specialty "Pathology and Cytopathology".

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Reviewer: 
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