ACADEMIC REVIEW

From: **Prof. Dr. Yulian Rumenov Ananiev, MD, PhD**

Dean of the Medical faculty, Trakia university,

Head of the Department of General and Clinical Pathology, Forensic Medicine and Deontology, and Dermatovenereology

Subject: PhD-thesis for the award of an educational and scientific degree

"PhD"

To **Dr. Nevena Zhelyazkova Yanulova, MD**

PhD-thesis topic: **"APTOPOSIS AND NECROPTOSIS IN RENAL CELL CARCINOMA"**

Professional direction: 7.1. **Medicine**

PhD program: **"Pathologic anatomy and cytopathology"**

Scientific supervisor: Prof. Dr. Maria Tsaneva, MD, PhD

Brief details of the PhD student.

Dr. Nevena Zhelyazkova Yanulova was born on 21.02.1987. In 2006, she graduated from the School of Natural Sciences and Mathematics "Ivan Vazov" in Dobrich, and the following year she was accepted to study Medicine at MU-Varna. She graduated in 2013 and since 2014 has been a resident in General and Clinical Pathology with a specialization base at the University Hospital "St. Marina" in Varna. In 2019, she acquired a specialty. In the period between 2013-2014, she was a medical doctor in the Department of Pathology at the University Hospital "St. Marina" in Varna, and since 2019 - in the Clinic of General and Clinical Pathology of the University Hospital "St. Marina" in Varna. She has been successively a part-time assistant and assistant in the Department of General and Clinical Pathology, Forensic Medicine and Deontology of MU-Varna. She is a member of the Bulgarian Medical Union and the Bulgarian Society of Pathology. She has a high level of English.

Description, relevance and importance of the topic.

The PhD-thesis of Dr. Nevena Yanulova is a profound and interesting scientific work, emphasizing the importance of a largely clarified mechanism of cell death (apoptosis) and one that is currently being studied at different levels (necroptosis) in a relatively uncommon form of tumor pathology, but with extremely interesting progression, diagnostic and therapeutic challenges – renal cell carcinoma (RCC). The presented material consists of the following parts: introduction, literature review, aim and objectives, material and methods, results and discussion, conclusions, contributions of the thesis, publications related to the dissertation, used literature.

At the beginning, the dissertation begins with an **introduction**, in which Dr. Yanulova provides a brief overview of the problem of "renal cell carcinoma", the types of cell death, as well as the importance of the RIPK3 signaling pathway and its relationship to this disease and the processes associated with it.

The following is a **literature review** in which the PhD-candidate carefully presents the statistics related to her studies of carcinoma according to GLOBOCAN, discussing data for different countries and Bulgaria. She describes in an orderly manner the etiological and risk factors with an emphasis on: genetic predisposition and the relationship of some gene alterations, and known syndromes in the development of BCC, as well as socially significant and chronic inflammatory diseases. In the part related to morphology, the author describes the macroscopic features of the tumor, the modern histological classification, and then some of the most important features related to the prognosis of the disease.

At the next stage, Dr. Yanulova examines the process of apoptosis, as the most important form of cell death, along with the two pathways for its induction - extrinsic and intrinsic. After presenting in detail the molecular mechanisms and the importance of the apoptosis-inducing factor, she again makes reference to its importance in various neoplasias. The following is a description of necroptosis and receptor-interacting protein kinases (RIPK), presenting some of the most recent understanding and processes in the implementation of this mechanism of cell death, cellular pathways, and morphological features. The involvement of RIPK3 in cell signaling and its tumor-promoting and tumor-repressive functions is carefully described.

The chapter ends with a brief conclusion, in which Dr. Yanulova makes a reference to the next chapter, which concludes with the hypothesis of the need for further in-depth studies on the

role of RIPK3 in tumor development, progression, metastasis, recurrence, as well as anti-tumor immunity in BCC, as well as its correlation with clinico-morphological indicators.

The **aim** of the PhD-thesis is to ivestigate the immunohistochemical expression of the apoptosis marker, apoptosis-inducing factor (AIF) and the necroptosis marker, Receptor interacting protein kinase 3 (RIPK3) in patients with renal cell carcinoma and to determine their prognostic value. Six objectives are presented – directly related to the aim of the thesis, as task 1 is determined to be carried out on the basis of a documentary study, tasks 2, 3, 4 and 5, include morphological and immunohistochemical assessment in tissue samples, and task 6 aims to analyze the apoptosis marker, AIF and the necroptosis marker, RIPK3 in relation to patient survival and to determine their prognostic role in renal cell carcinoma. The aim and objectives are selected adequately and accurately, their planning is fully comparable with the accents and debatable points described in the literature review.

In the presented **methods and materials**, the PhD-candidate uses cases from the Department of General and Clinical Pathology, Forensic Medicine and Deontology of the Medical University - Varna, as well as information from the electronic database "MultiLab" of the University Hospital "St. Marina" - Varna. Eighty patients with RCC were selected, divided into three groups, and the histological type, presence and area of tumor necrosis, tumor-infiltrating lymphocytes (TILs), vascular invasion and degree of differentiation, TNM stage were described. In addition, the levels of immunohistochemical expression of AIF and RIPK3 were examined in all three groups of patients, including fourteen of the histologically verified distant metastases. Criteria for categorizing each indicator are carefully indicated, the immunohistochemical methodology, components and protocols are described, as well as a methodology for their reporting. At the end, a description of the selected statistical research methods is given, as well as the selected software product.

The chapter "**Results and Discussion**" follows, where the presented data logically follow the set aim and objectives. The results concerning the clinico-morphological characteristics of patients with renal cell carcinoma are described, and the different types of findings are discussed, compared with the studies described in the literature. Statistically reliable differences were sought in these comparisons, such as those found in T-stage and its comparison with tumor differentiation, area of necrosis, tumor-infiltrating lymphocytes, and vascular invasion. The relationship between tumor necrosis and the histological variant of the carcinoma, tumor-

infiltrating lymphocytes and vascular invasion in patients with RCC and localization was also confirmed. Relationship was also established between tumor-infiltrating lymphocytes and the gender of the patients, vascular invasion and localization of RCC, the degree of differentiation, histological variant of RCC and vascular invasion. In addition, with regard to patient survival, a relationship with age, gender, the extent of tumor necrosis and vascular invasion, as well as the T-stage of BCC was confirmed. Some tendencies, as well as results showing a lack of reliability, but with clinical and prognostic significance, were also discussed visually with a large number of charts.

At the next stage, the findings related to the expression of AIF in the tumor tissue of RCC, in the adjacent non-tumor tissue and in distant metastases were discussed, and an analysis and discussion of the average values of AIF expression in the tumor tissue and adjacent, non-tumor tissue was performed. A number of significant results and trends were established, all of which were illustrated with photographs of the IHC reaction, diagrams and tables. The comparisons were made on the basis of cytoplasmic and nuclear expression and correlated with all indicators collected by the participant.

And in the following subchapter, the information obtained from the study of RIPK3 expression in the tumor tissue of BCC, in the adjacent non-tumor epithelial tissue and in distant metastases is presented and analyzed in the same way, and here too, comparisons between the adrenal and cytoplasmic expression of the marker and the clinico-morphological findings and survival in patients are taken into account.

In the chapter "**Conclusions**" Dr. Yanulova formulates 18 conclusions, all of which are based on the previously discussed results, a significant part of which are significant.

The **contributions** of the thesis are divided into two groups: original scientific contributions, where the author has presented two contributions, as well as practical-applied scientific contributions - a total of five contributions, and here we can point out that both they and the previous two can be used in the routine assessment, monitoring and prediction of these patients.

In summary, we can conclude that the dissertation is relevant, interesting and in-depth, and its topic, the description of the scientific plan, as well as the implementation, proves the significance of the chosen topic and the application of the conclusions and contributions made.

Two more scientific publications are presented, in which Dr. Yanulova is the first author.

The PhD-thesis is written on a total of 169 pages and is illustrated with 65 figures and 80 tables. The bibliography includes 266 literary sources, 5 of which are in Cyrillic and the rest in Latin.

Based on the above, I believe that Dr. Yanulova's PhD-thesis represents **an extremely thorough, interesting and fully completed scientific work with significant results and conclusions, as well as applicability in diagnostic and clinical practice.**

I give my positive assessment and will vote "Positive" awarding the ESD "PhD" to Dr. Nevena Zhelyazkova Yanulova in the Field of Higher Education: 7. Health and Sports, Professional Field: 7.1. Medicine, PhD Program: "Pathoanatomy and Cytopathology and I also recommend the honorable members of the scientific jury to vote positively.

Заличено на основание чл. 5, §1, б. „В" от Регламент (ЕС) 2016/679

9.12.2024

Stara Zagora   **Prof. Dr. Yulian Rumenov Ananiev, MD, PhD**