

## **OPINION**

by Professor Peter Ivanov Ghenev, MD, PhD

Department of General and Clinical Pathology, Forensic Medicine and  
Deontology, Medical University - Varna

on the dissertation work of Dr. Nevena Zhelyazkova Yanulova; on the  
topic: "APOPTOSIS AND NECROPTOSIS IN RENAL CELL  
CARCINOMA", for awarding the educational and scientific degree  
"DOCTOR", in the scientific specialty "Pathology and cytopathology".

### **I. Procedure data**

By order No. R-109-357/25.10.2024 of the Rector of the Medical  
University - Varna, I was elected as an internal member of the Scientific jury,  
and on the basis of protocol No. 1/4.11.2024 I am assigned to prepare an  
opinion.

Attached herein, I present: Opinion on the dissertation work for the award of the  
educational and scientific degree "Doctor" to Dr. Nevena Yanulova in the  
Department of Higher Education: 7. Health and Sports, Professional Direction  
7.1. Medicine, Scientific specialty "Pathology and cytopathology", for the needs  
of the Department of General and Clinical Pathology, Forensic Medicine and  
Deontology, Faculty of Medicine of the Medical University - Varna.

### **II. Biographical data and career development of the candidate**

Dr. Nevena Yanulova was born on February 21, 1987. She graduated  
from High school in 2006 at the "Ivan Vazov" Science and Mathematics High  
School in Dobrich. In 2013, she graduated from the Medical University - Varna,  
majoring in "Medicine" and joined the Department of Pathology, Dobrich  
Hospital, as a resident doctor. In 2014, she started specializing in Pathology at  
the Clinic of the same name at the "Saint Marina" University Hospital - Varna  
and acquired the specialty "Pathology and cytopathology" in January, 2020.

Dr. Yanulova is an assistant in the Department of General and Clinical  
Pathology at the Medical University - Varna from September 2019, initially as  
a deputy, and later - from March 2020 on employment contract. During these  
years, she participated in the educational work of the Department - she

conducted practical classes in all disciplines with students of medicine and dentistry and participated in the diagnostic process in the hospital, mastering all the basic methods of biopsy diagnostics. She is involved in the scientific activity of the Department, showing interest in tumor pathology and kidney diseases. In 2020, Dr. Yanulova was enrolled as a doctoral student in the Self preparing program for the acquisition of the scientific and educational degree "Doctor" in the scientific specialty "Pathology and cytopathology". Dr. Yanulova worked on the thesis, using the materials and the database of the Clinic for General and Clinical Pathology at the "Saint Marina" University Hospital - Varna and with the assistance of the Doctoral School at the MU-Varna. Dismissed with the right of defense by Order of the Rector of MU-Varna.

Dr. Yanulova has more than 10 years of work experience in the specialty; she is a member of the Bulgarian Society of Pathology and the Bulgarian Medical Union.

For participation in the competition, the candidate has submitted a set of materials in electronic format, which are in accordance with the requirements of MU-Varna.

### **III. Structure of the dissertation**

Dr. Yanulova submitted for review a Thesis written in 171 standard pages, structured according to standard requirements and containing as follows: Table of Contents and Abbreviations (7 pages), Introduction (2 pages), Literature Review (49 pages), Aim and objectives (1 p.), Material and methods (7 p.), Results and discussion (80 p.), Conclusions and contributions (3 p.) and References (20 p.). The thesis is illustrated with 65 figures and 80 tables. Photomicrographs are of high quality and allow for interpretation. The bibliography covers 226 literary sources, of which 6 are in Cyrillic.

### **Relevance of the topic**

Renal cell carcinoma is less common than carcinoma of the prostate gland and bladder, but is more malignant and its progression causes death in about 40% of patients. The reasons for this are different in nature. First of all, the tumor is detected late, because it develops for a long time without symptoms, or if there are clinical complaints, they are extremely uncharacteristic. It also exhibits resistance to standard therapeutic procedures.

These characteristic features give current importance to scientific research aimed at elucidating biological behavior and the search for factors with prognostic and predictive significance.

In this sense, Dr. Janulova sets the goal of this work - the morphological study of markers for apoptosis and necroptosis on biopsy materials, with a view to determining their prognostic significance.

Apoptosis, as a biological process, has long been proven to play a role in tumor transformation and progression. Both in experimental settings and in oncological practice, the induction of apoptosis in cancer cells is directed against their survival and is therefore considered a therapeutic goal.

Necroptosis was described later than apoptosis, as an alternative form of cell death that is triggered when the process of apoptosis is inhibited. Necroptosis is also involved in a number of stages of oncogenesis - transformation, progression, phenotypic variants and metastatic signature, but has a controversial role in neoplasms different in location, type and stage.

The combined morphological study of markers of apoptosis and necroptosis in renal cell carcinoma is a well-thought-out in-depth study, relevant to the modern requirements.

### **Literature review**

The literature review covers 226 literary sources, which are mostly from the last decade. Introduces the subject consistently, outlines the problems, examines in detail the epidemiology, etiology, risk factors, genetics and already known factors for the appearance, course and morphological diagnosis of renal malignant tumors. The histological classification, the novelties in it and in the differentiation and stage reporting systems are presented in detail. It is noteworthy, that Dr. Yanulova was able to analyze and summarize the literature information in order to highlight unclear or contradictory data and accordingly to determine the aim and formulate the tasks of the study. Her personal attitude to the problem is also evident, especially in those areas where it has its own results.

### **Purpose, tasks, materials and methods**

The aim is clearly formulated: on well-selected, morphologically diagnosed biopsy cases of renal cell carcinoma, to semi-quantitatively analyze

the immunohistochemical expression of established and promising markers for apoptosis - AIF; and for necroptosis - RIPK3, both in primary foci and in metastases. The cases were divided into three groups according to the histological variant of the carcinoma: 20 cases with papillary carcinoma, 21 patients with chromophobe and 39 with clear cell carcinoma of the kidney.

The obtained results are interpolated according to morphological indicators: histological type, degree of differentiation, localization and stage, presence and area of tumor necrosis, presence of tumor-infiltrating lymphocytes, vascular invasion. All morphologic findings were matched with clinical characteristics of the patients, such as sex, age, and survival. The obtained data were statistically processed.

## **Results and Discussion**

Own results are presented following the order of formulated tasks. After the presentation of the results for each of the tasks, a discussion and comparison with the data from the literature was made. Arguments and new scientific facts confirming the achievement of the goal are indicated, which are shaped as conclusions.

The conclusions are too numerous and to some extent this makes it difficult to highlight the essential achievements of the doctoral thesis. For example, the poorly studied intracellular localization (cytoplasmic and nuclear) of the two studied markers - AIF and RIPK3.

Dr. Yanulova found that the cytoplasmic expression of AIF in tumor cells was higher than the nuclear one, but there was no difference compared to peritumor tissues. Nuclear expression of AIF did not correlate with any of the examined clinico-morphological indicators, while cytoplasmic expression was dependent on age, stage and the absence of tumor emboli. In addition, papillary renal cell carcinoma had statistically significant high cytoplasmic expression.

With RIPK3 also a cytoplasmic level was found in tumor cells, which was higher than the nuclear content, but was lower compared to the peritumor tissue. Moreover, the cytoplasmic expression of RIPK3 (in contrast to AIF) did not show a dependence on clinico-morphologic parameters.

In summary, cytoplasmic expression of AIF is related to lymphovascular invasion and tumor progression, whereas, cytoplasmic expression of RIPK3 is associated with differentiation and the onset of lymphovascular invasion.

Dr. Yanulova has achieved good results, which give her the opportunity for more interpretations in the future. The applicant modestly notes the contributions of the thesis, divided into original scientific-applied and confirmatory.

Dr. Yanulova submitted for review an abstract (68 pages with tables and colored figures), designed according to the requirements of the normative documents. Although in an abbreviated form, it accurately reflects the content and gives an overall idea of the dissertation work.

Dr. Yanulova participated in the competition with two publications in Bulgarian scientific journals, printed during the doctoral studies, all strictly on the topic, in which she was the first or only author.

#### **IV. Conclusion**

The submitted documentation and thesis meet the procedural requirements and normative documents of the Medical University - Varna. The candidate's scientific output corresponds to the required criteria. I accept the reference for scientific contributions without remarks. I highly value the professional qualities of the candidate, as well as the opportunity for career development.

For these reasons, I am particularly pleased to fulfill my duties as a member of the Scientific Jury and to vote positively for Dr. Nevena Yanulova to be awarded the educational and scientific degree "Doctor" in the field of higher education 7. Health care and sports, professional direction 7.1 Medicine and the scientific specialty "Pathology and cytopathology".

16.XII.2024

Reviewer:

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

(Prof. Peter Ghenev, MD, PhD)

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For these reasons, I am particularly pleased to fulfill my duties as a member of the Scientific Jury and to vote positively for Dr. Novina Yandova to be awarded the educational and scientific degree "Doctor" in the field of higher education 7. Health care and sports, professional direction 7.1. Medicine and the scientific specialty "Pathology and cytology".



Reviewer:

16/11/2024

(Prof. Peter Ghenov, MD, PhD)