

## Position

**by Assoc. Prof. Dr. Anastas Dimitrov Pashov, Department of Immunology, Institute of Microbiology "Acad. Stefan Angelov", Bulgarian Academy of Sciences**

**Subject:** Doctoral thesis for the acquisition of the educational and scientific degree "Doctor" in Professional Field: 4.3. "Biological Sciences", Specialization: "Immunology"

**Title of the thesis:** *"The Influence of Specific MicroRNAs on Tumor Pathogenesis through Modulation of Autophagy Processes and Innate Immune Signaling"*

**Author of the thesis:** Radostina Petkova Tsvetanova, regular PhD student in the "Reproductive Omics Technologies" laboratory at the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences. **Supervisor:** Prof. Krasimira Todorova-Hayrabedyan, PhD.

Prostate cancer is the most common oncological disease in adult men and ranks fifth in terms of mortality among male tumors. Serum PSA is often used as a diagnostic marker, but it is not always accurate due to falsely elevated or normal values in different conditions. MicroRNAs hold promise for the diagnosis and therapy of prostate cancer, as they are stable in biological fluids and regulate important cellular processes. They are associated with tumorigenesis and can serve as reliable noninvasive biomarkers. Studies show that microRNAs regulate over 60% of human genes and can have therapeutic role in the fight against cancer. Therefore, the topic of the dissertation is extremely relevant.

The presented dissertation consists of 160 pages with 4 schemes, 33 figures, 3 tables, and 5 diagrams. The bibliography includes an impressive 359 literary sources.

The literature review is rich in information and adequately presents the state of the scientific problem. It is divided into the following main thematic sections: Diagnosis and prognosis of prostate cancer, Targeted therapy in prostate cancer, Molecular-genetic mechanisms in prostate cancer, MicroRNAs as reliable biomarkers for prostate cancer diagnosis, Autophagy in prostate cancer, and Innate immune signaling in prostate cancer. Each section is a thorough review of the individual components of the problem. The review of the role of microRNAs as a source of new biomarkers is particularly detailed and comprehensive, which is also the focus of the dissertation. All this demonstrates the doctoral student's ability to find, analyze, and synthesize scientific information sources.

The objective of tracking the autophagy processes and innate immune signaling after MAPK1 gene silencing and modulation of microRNA-141 levels in prostate carcinogenesis is well formulated and represents an important scientific task. There are 9 adequately formulated tasks summarizing the main methodological approaches to achieving the goal.

The applied methods are from molecular biology, cellular biology, and immunology, including the modern method of nanopore sequencing and analysis of the transcriptome data, thus, obtained. The statistical data processing is adequate.

The results are summarized in subsections, according to the set tasks. The experiments are logically planned according to the research goals, and the obtained results are significant and original. The analysis and formulated secondary hypotheses, which outline the future development of this research program, demonstrate a profound understanding of the molecular-biological basis of the observed phenomena, as well as the mechanisms and possibilities of the used methodology.

The discussion is extremely thorough and critical. The discussion shows the acquired ability of the doctoral student to critically analyze the data in the context of the current scientific information. It logically follows the conclusions and contributions. There are 9 conclusions accordingly. The large number of conclusions adequately reflects the richness and diversity of the results. The careful adherence to the data is impressive. There are 2 contributions with an original character, including: 1) The key role of microRNA-141 in the regulation of mitophagy has been proven, and 2) Selective inhibition of MAPK1 in combination with the restoration of microRNA-141 levels can be potentially used as an adjunct therapy to chemotherapy.

**Questions and comments:**

Are there statistical data for decreased expression of ATG16L after MAPK1 gene silencing in LNCaP cells? Figure 1 does not convincingly present this fact.

What is the reason for the low quality of the reads in nanopore sequencing of cells transfected with microRNA-141 mimic, but not in those transfected with the corresponding inhibitor?

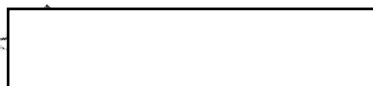
**Evaluation of the Abstract of the thesis:** The abstract is well structured and in line with the dissertation, reflecting the objectives, conducted research, obtained results with discussions, and the conclusions made. It includes references to the contributions and information about the scientific activity of the PhD student.

Radostina Petkova Tsvetankova has submitted all necessary documents in accordance with the procedure on paper and electronic media, in accordance with the Regulations on the terms and procedure for acquiring scientific degrees and taking academic positions at the Bulgarian Academy of Sciences and the Institute of Biology and Immunology of Reproduction. She presented her results and dissertation project for discussion at a seminar at IBIR-BAS. The results of the dissertation have been included in two publications with an impact factor. The results have been reported in 5 presentations. Based on the credits earned, the doctoral candidate has successfully completed the prescribed educational and scientific program with 412 points, exceeding the minimum requirement of 130 points. The dissertation serves as evidence that Radostina Tsvetankova has developed the necessary competencies for obtaining the educational degree of a doctor, which include theoretical background, methodological knowledge, independence and experience in planning experiments, and the ability to conduct in-depth analysis of results and literature.

**CONCLUSION**

The content and relevance of the dissertation meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the internal regulations of the Bulgarian Academy of Sciences and the Institute of Biology and Immunology of Reproduction. I evaluate the work of Radostina Petkova Tsvetankova positively and confidently recommend granting the educational and scientific degree of "Doctor."

Anastas Pashov



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