

REVIEW

by Prof. Radostina Ivaylova Alexandrova, MSc, PhD (Pathology Section, Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences) - member of the Scientific Jury according to order No. 123/ 29.11.2023 of the Director of the Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov" - Bulgarian Academy of Sciences (IBIR-BAS)

Regarding Dissertation work entitled: "Impact of specific micro RNAs in tumor pathogenesis by altering the processes of autophagy and innate immune signaling";

for awarding the educational and scientific degree "DOCTOR" in scientific specialty "Immunology", code 01.06.23, professional field "Biological Sciences" 4.3., field of higher education 4. "Natural Sciences, Mathematics and Informatics";

of PhD student Radostina Petkova Tsvetankova, Laboratory "Reproductive OMICS Technologies", IBIR-BAN, Sofia, Bulgaria;

with scientific supervisor: Prof. Krasimira Todorova - Hayrabedyan, MSc, PhD, DSc, "Reproductive OMICS Technologies" Laboratory, IBIR-BAN.

1. Relevance and significance of the dissertation work

The topic of the presented dissertation is extremely relevant, but also very brave. It is enough to mention the three key words micro RNA, autophagy and innate immune signaling on the one hand and prostate cancer on the other.

Cancers, and prostate carcinoma in particular, are among the leading medical and societal challenges of our time. And it is not surprising, given that neoplasias are in second place as a cause of death or permanent disability, ranking in this sad ranking right after cardiovascular diseases. As for prostate cancer, according to the Global Cancer Observatory, as of 2020, it was the most frequently diagnosed malignant tumor in men in 112 out of 185 countries, and it was the fifth leading cause of death.

Age is among the main risk factors for the development of prostate cancer, and with the increase in life expectancy, its importance in health, social and economic aspects is expected to increase. This once again emphasizes the urgent need for a better study of it and, above all, to improve the possibilities of its control.

Undoubtedly, this cannot happen without a thorough understanding of the molecular genetic, epigenetic and immunological molecules and pathways involved in its pathogenesis, in order to find new opportunities to improve diagnostic, prognostic and therapeutic approaches. What are the main factors involved in carcinogenesis; under what circumstances, how and why the cell goes to its death or survival; when and where autophagy intervenes and what is the significance of the innate immune signaling. Studying each of these "players" separately is complex enough, and searching for and delineating the relationships between them, identifying the molecules and mechanisms that condition them, outlining the overall puzzle that will, in short, determine the fate of not only the cell, and of a human, is an extremely

important and attractive, but also an infinitely difficult task. Therefore, the decision of the doctoral student and the supervisor to go in this direction during the preparation of the dissertation deserves respect.

The topic of the dissertation is in line with the objectives of the National Strategy for the Development of Scientific Research in the Republic of Bulgaria 2017-2030 as well as with the priorities of the Horizon Europe program of the EC, as it will contribute to improving health and increasing the quality of life, helping to solve global challenges such as cancer and creating a prerequisite for achieving scientific excellence.

2. Overview of the dissertation work

The dissertation is written on 160 pages and includes: Title page (1 page), Table of Contents (3 pages), List of used abbreviations (1 page), Introduction (1 page), Literature review (38 pages), Aims and objectives (2 pages), Materials and methods (14 pages), Results (47 pages), Discussion (20 pages), Conclusions (1 page), Contributions (1 page), Bibliography (24 pages .), Appendices (2 pages), List of publications and contributions associated with the PhD thesis (2 pages), Additional contributions, publications and projects during the doctoral studies (2 pages), Acknowledgments (1 page). It is illustrated with 4 schemes (3 in the "Literature review" and 1 in the "Results"), 33 figures (32 in the "Results" section, 1 in Appendices) and 5 diagrams (1 in the "Results" and 4 in the "Discussion" - color or black and white, but all in high quality).

The **Introduction** reveals the motives that provoked interest in the topic of the dissertation, its relevance and significance. Written in a short, concise and reasoned manner, it immediately grabs the reader's attention. Sounds like a trailer for an interesting movie that is definitely worth watching.

The **Literature review** is carried out in the same spirit. In it, the excellent awareness of the author in the field of the dissertation (and not only!), her ability to synthesize and summarize, her desire to present the multifaceted image of the considered problems in an accessible and fascinating way meet. The Literature Review consistently presents the biology and behavior of prostate carcinoma and the questions and problems it poses to the biomedical community and society as a whole; the status and capabilities of diagnostic and prognostic methods and currently available biomarkers for this disease, with their advantages and disadvantages; the targeted therapy that has gained momentum in other oncological diseases, such as breast cancer and a number of oncohematological diseases, and through which biomedical science turns the "strengths" of malignant tumors responsible for the increased proliferation, invasiveness and survival of cancer cells into their "Achilles heel" and a target for selective attack using monoclonal antibodies and kinase inhibitors. However, in order for targeted therapy to "take over" prostate cancer, a thorough study of the molecular genetic, epigenetic and immunological mechanisms that cause it is needed. It is to them that the second part of the Literary Review is dedicated. In it, the PhD student focuses on the nature and role of microRNAs in the course of carcinogenesis and their potential application as reliable diagnostic and predictive biomarkers in prostate carcinoma. Moreover, they can be isolated not only by tissue biopsy, but also in non-invasive ways from available body fluids (blood, serum, plasma, but also urine and ejaculate). The latter makes them particularly attractive for clinical practice, as it would allow repeated sampling and early diagnosis and/or monitoring of disease progression and response to applied therapy. Special attention is paid to the role of autophagy in prostate carcinoma as well as innate immune signaling.

The critical view of the doctoral student is impressive, as she objectively interprets the information found in the available literature, touching on the "contradictory" nature of the data and the challenges we face. The literature review ends with a short summary, which once again points to the necessity of the conducted research. His focus is on the gene MAPK1 (mitogen-activated protein kinase) and miLNK-141 - key players in carcinogenesis and tumor progression, their role, however, still hides a number of unknowns. Depending on the range of genes it affects and the genes that affect it, miRNA-141 can perform a tumor suppressor as well as an oncogenic function and poses more than one question to specialists. Answers must be sought because miRNA-141 may serve as a convenient biomarker (detected in readily available biological fluids, including urine) and a promising therapeutic strategy. However, for this to happen, we need quite a few more pieces of the puzzle, and the idea of the PhD student and the research supervisor is to find some of them.

The literature review is written in a high scientific style, but at the same time it is easy to read. Behind its preparation lies a lot of knowledge, thought, work, professional interest and curiosity, love for the chosen specialty - all of which have turned it into a valuable source of modern information for a wide range of specialists. It is worth thinking about how to disseminate it more successfully.

Goals and tasks. The objective (1 item) is clearly and precisely formulated. The tasks (9 in number) are described step by step and sound like a concrete and well thought out plan of action. The PhD student and the supervisor have started the study after serious preliminary work on the preparation of the research strategy.

The **Materials and Methods** section is detailed and well described. Two human cell lines derived from metastatic prostate carcinoma were used as model systems: from bone metastases (line PC3) and metastases in the left subclavicular lymph node (line LNaCaP). The two cell lines also differ in a number of other parameters, including presence/absence of hormone receptors, the p53 gene, Y chromosome, sensitivity to androgen therapy, DNA methylation levels in the miRNA-141 promoter. A wide range of modern molecular/molecular genetic, biochemical, bioinformatics and immunological methods as well as cell culturing was used.

The methods and techniques are presented in detail, and information on the reagents and apparatus used is given, which would be useful to anyone carrying out similar studies.

The **Results** follow the course of the set goals and objectives. They are detailed and well illustrated with 32 figures and 1 high-quality diagram that make them easy to understand.

During the preparation of the dissertation, original data were obtained on the role of microRNA-141 in prostate cancer and more specifically in the regulation of mitophagy and macrophagy in the background of complex relationships with key participants in other signaling pathways (TAK1-NF- κ B, TAK1- p38MAPK) as well as for its importance in DNA methylation. New data were also revealed on the impact of MAPK1 on autophagy in cell model systems of various metastatic forms of prostate carcinoma, cell migration activity and proinflammatory signaling.

The obtained original results enrich our knowledge of the biology and behavior of metastatic prostate carcinoma and the role of epigenetic regulation and autophagy, revealing new diagnostic, prognostic and therapeutic possibilities.

The **Discussion** section is undoubtedly the most challenging part of writing a dissertation. Not just an exam, but a test of the author's theoretical preparation and critical

thinking, his ability to compare data and facts, to see order in chaos, but also inconsistencies in otherwise ordered things at first glance, to draw the red line connecting what was done by the choice of title, through the formulation of the purpose and objectives, all the way to the final conclusion and contribution. In this case, the doctoral student more than successfully coped with this challenge, which is also a kind of certificate for an excellent job done by the research supervisor in his role as a mentor. The obtained own results are analyzed in detail against the background of the information found in the available literature. The most valuable thing in the case is that the role and fate of the individual participants in the studied events are not considered separately as individual soloists, but a successful attempt is made to hear the music of the whole orchestra, to see the interrelationships between the paths in which they participate. And this is not at all easy, considering how diverse the studied genes/molecules and processes are, and how different and even opposite sides they can manifest depending on the circumstances in which they occur - in the context of the specific microenvironment and situation, under the influence of a number of factors, many of which are still unknown. And only thanks to the small steps made by such ambitious (and brave) studies will help to put the whole puzzle in order and take a really big step forward, namely – a breakthrough in the prevention, diagnosis and treatment of cancers in general and prostate carcinoma in particular.

Based on the obtained results, 9 **conclusions** have been systematized, which I fully accept. I also agree with the two original **Contributions**, indicating the innovative elements in the dissertation work.

The cited **Literature** covers a total of 359 titles (2 are in Cyrillic and the rest in Latin), and 165 of them (~46%) have been published since 2013.

3. Publications and participation in scientific forums of the doctoral student in the topic of the PhD thesis

On the subject of the dissertation, two articles were published in publications that are referenced and indexed in world-renowned scientific information databases (Scopus, Web of Science): "Comptes rendus de l'Académie bulgare des Sciences" (with an impact factor of 0.326; Q3) and "Biotechnology and Biotechnological Equipment" (with an impact factor of 1.762; Q3). Radostina Tsvetankova is the first author in both publications. The obtained results are presented with 5 announcements at 4 scientific forums, 3 of which are international.

4. Assessment of the correspondence between the Abstract of the PhD thesis and the dissertation

The abstract is prepared in full accordance with the dissertation work and presents in a shortened version the essence of the study, the sequence of the experimental work, the results obtained and the conclusions and contributions made on their basis.

5. Critical notes and comments, recommendations, questions to the PhD student

The dissertation work would only benefit if a few more figures were included in the Literary Review to illustrate the presented information.

The two cell lines used as model systems also differ in the methylation level of the miRNA-141 promoter - it is good to mention this difference already in their presentation in the "Materials and methods" section.

In the text, I noticed some technical and grammatical errors: incorrectly used definite or indefinite articles; the word order of some expressions / sentences in Bulgarian literally follows the English word order; foreigners (from Russian and English); inclusion of the authors of Cyrillic among those of Latin, and not separately, etc.

The listed omissions in no way reduce the quality of the dissertation work.

I have the following questions for the PhD student:

- It would be interesting to compare the behavior of the main "players" studied in the thesis and their relationships in prostate carcinoma cells, benign neoplasms and normal tissues. Science and human inquisitiveness are endless, but the time to prepare a dissertation and the budget for its implementation are extremely limited. So my question is whether such a comparison can be made based on the available literature / experimental data and whether similar studies are planned in the future
- In what direction does she think research in this area should continue, what are the next pieces of the puzzle to be found?
- What are the main challenges researchers will face in finding them?.

6. Autobiographical data and personal impressions of the PhD student

Radostina Tsvetankova graduated from a class with a profile of natural sciences at "Nesho Bonchev" secondary school in the city of Panagyurishte in 2011. In 2015, she obtained a bachelor's degree in "Bioinformatics" at "Paisiy Hilendarski" University in Plovdiv, in 2014-2015 she received postgraduate education as a "Teacher of Biology", and in 2017 she became a "Master" in "Medical Biology" at the same University. She worked as a registrar and technical assistant at MBAL Uni Hospital OOD in Panagyurishte, as a teacher and medical biologist in clinical laboratories. In 2018, after successfully passing a competitive exam, she was enrolled as a PhD student in the "Productive OMICS Technologies" laboratory at IBIR-BAS, where she was appointed as an assistant in June 2022. She went through training seminars and courses, held in Sofia (IBIR, Military Medical Academy), Plovdiv and Varna. She is the author of 3 publications and participates in the team of 4 scientific research projects.

8. Conclusion

The presented dissertation is dedicated to an important and significant topic, the goals and tasks set in it have been fully achieved, at a high professional level, and the obtained original results are an indisputable contribution to the field of tumor immunology, molecular genetics, epigenetics, biochemistry and bioinformatics.

In the course of the preparation, the PhD student Radostina Tsvetankova has improved her biomedical knowledge, mastered a wide range of modern methods and techniques, and grew as a capable and promising scientist. She has been actively involved in the preparation and successful implementation of an ambitious research project, which, with its conception and scope, contributes to increasing the authority of Bulgarian science in the European and world scientific space. And the most important thing: the new results obtained during the implementation of the dissertation work contribute to the fight against one of the leading medical and societal challenges of the 21st century - cancers and, more specifically, prostate carcinoma. The efforts made by the doctoral student and the supervisor in the preparation of the dissertation work and the resulting final product deserve high praise.

All this gives me reason to believe that the presented dissertation fully complies with the Law on the Development of the Academic Staff in the Republic of Bulgaria, as well as with the regulations for its application in IBIR - BAS, for awarding the educational and scientific degree "Doctor". I confidently give my positive assessment and recommend the members of the Honorable Scientific Jury to award Radostina Petkova Tsvetankova the educational and scientific degree "Doctor" in the scientific specialty "Immunology", code 01.06.23, professional direction "Biological Sciences" 4.3., field of higher education education 4. "Natural sciences, mathematics and informatics".

30.01. 2024 г.



/Prof. R. Alexandrova/