

REVIEW

by Prof. Dr. Lazar Jelev Slavov, MD

**Department of Anatomy, Histology and Embryology, Medical University - Sofia
of the Dissertation for awarding the Educational and Scientific Degree "Doctor"**

Professional field: 4.3 Biological Sciences

PhD Program: Genetics

Author: Georgi Dimitrov Blazhev

Form of Doctoral Study: Full-time

Primary Unit: Department of Genetics, Faculty of Biology at Sofia University "St. Kliment
Ohridski"

Title: *MULTI-OMICS APPROACH FOR ANALYSIS OF BIOLOGICAL AND CLINICAL
HETEROGENEITY OF SOME RARE MALIGNANCIES*

Scientific Supervisor: Assoc. Prof. Velizar Stefanov Shivarov, MD, PhD

Medical University – Pleven

1. General Presentation of the Procedure and the Doctoral Candidate

The submitted set of materials for the procedure, both in paper and electronic format, complies with the requirements of the procedure for acquiring the educational and scientific degree "Doctor" according to the Law for the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of LDASRB, and the Regulations on the Conditions and Procedures for Acquiring Scientific Degrees and Occupying Academic Positions at Sofia University "St. Kliment Ohridski" (last amended on 28.06.2023).

2. Brief Biographical Data of the Doctoral Candidate

The doctoral candidate, Georgi Dimitrov Blazhev, was born in Pleven on March 31, 1995. He completed his secondary education in his hometown in 2014. In 2018, he earned a Bachelor's degree in Molecular Biology from Sofia University, and in 2020, a Master's degree in Cell and Gene Engineering from Sofia University. In July of the same year, he was enrolled in a full-time doctoral program in Genetics (4.3 Biological Sciences) after a competitive examination. In July 2023, he was officially cleared for defense, having completed his work on the dissertation titled "Multi-omics Approach for Analysis of Biological and Clinical Heterogeneity of Some Rare Malignancies". The doctoral candidate is proficient in English and Spanish and has advanced knowledge and skills in bioinformatics. During his doctoral studies, he conducted mandatory exercises in "General Genetics" for undergraduate biology students at Sofia University during the academic year 2021/2022.

3. Relevance of the Topic and Appropriateness of the Objectives and Tasks

It is a well-known fact that malignant diseases are and will continue to be one of the leading causes of mortality in developed countries in the coming decades. On the other hand, malignant diseases are a heterogeneous group with diverse pathogenesis, clinical course, and prognosis. It is known that the majority of cancer cases affect only a few organ sites, such as lung carcinoma, colorectal carcinoma, breast cancer, and prostate cancer, which to some extent allows for the unification of approaches for these patients. However, about $\frac{1}{4}$ of all cancer cases fall under the so-called rare types of cancer, which have different localizations and result from rarer genetic changes, which does not allow or significantly limits the unification of treatment approaches due to the inability to accumulate sufficient data on the biology of the respective subgroups and, of course, to conduct sufficiently large clinical trials with standard or innovative oncological drugs. In this regard, all studies on the group of rare cancers that aim to integrate existing data from various datasets to optimize the diagnosis, prognostic stratification, and treatment of these patients are relevant and address important medical problems. Naturally, the greatest unmet medical needs are found in rare cancers associated with the worst prognosis, i.e., the shortest overall survival. Such a disease is certainly malignant pleural mesothelioma (MPM), which is the subject of the presented dissertation. The dissertation essentially demonstrates the possibilities of systems biology to integrate large arrays of existing omics data to derive clinically significant prognostic and/or predictive biomarkers/models, which also reflect certain biological characteristics of the

disease. Therefore, the dissertation is multidisciplinary with links to various medical and biological specialties such as pathology, oncology, immunology, genetics, and bioinformatics, which enhances the relevance of the presented work.

4. Characteristics and Evaluation of the Dissertation

The dissertation is written in the commonly accepted structure and includes the following parts: introduction (4 pages); literature review (26 pages); objectives and tasks (2 pages); materials and methods (8 pages); results (32 pages); discussion (8 pages); conclusion, findings, and contributions (4 pages); appendices (5 pages). The dissertation, with a total volume of 110 pages, is illustrated with 38 figures (all in color, most with more than one subsection) and 8 tables. The work refers to 173 items in English, with most of the articles published in the last 15 years with the advancement of omics technologies. Overall, the structure and content of the dissertation are well-connected and balanced in terms of the volume of information. The main sections are formulated logically according to the chosen objectives and are adequate to the content of the set tasks.

Although the title is generally formulated in relation to some rare types of cancer, the dissertation is entirely focused on MPM, which is justified in the introductory part. Furthermore, including more rare types of cancer would significantly expand the dissertation and would be beyond the educational goals of the doctorate, which had to be fulfilled within the statutory 3-year period.

The literature review continues the direction set by the introduction by examining various aspects of the etiology, pathogenesis, and genetic aspects of MPM. I believe that considering the fact that the doctorate is in the field of "Biological Sciences" and the specialty of "Genetics," it is appropriate that purely medical aspects of MPM, such as diagnosis and treatment, are not detailed. On the other hand, the doctoral candidate has presented some basic principles of omics technologies and their application in oncology. This logically leads to the formulation of some current questions about MPM and derives the objectives and tasks of the dissertation.

The selected bioinformatics methods logically address the set tasks and, according to the cited sources, are current and adequate. Figure 8 presents a flowchart of the overall analytical approach in the dissertation, which strengthens impression of a systematic biological

approach to the work and facilitates its understanding even by interested parties who are not specialists in the field of omics technologies.

A very good impression is made by the selected and well-described approach for systematic selection of sources for analytical data. I agree that for the purposes of this work, the chosen source of information – PubMed – is adequate, contemporary, and sufficient. Furthermore, the doctoral candidate has followed the established global standard for selecting a source of biomedical information – the so-called PRISMA standard (professionally presented in Figure 10). The derivation and validation of the two-gene prognostic score follow the generally accepted standard for using training and validation data sets. The elegance and innovation of the work lie in the primary selection of indicators (in this case, genes) to be used for deriving the score. This was done through the initial selection of validated genes known on which MPM cell lines depended in various gene knock-down screens. The method for deriving the prognostic score is simple and commonly used in the literature. Additionally, it shows excellent reproducibility of its prognostic value in both validation sets of transcriptomic data. A plus of the work is that one of the validation data sets is from RNA sequencing, and the other from a standard hybridization microchip, suggesting that the derived score is likely independent of the method used to determine gene expression levels. Subsequently, the work investigates whether the defined prognostic groups of MPM patients reflect certain biological characteristics of the disease. For this purpose, a widely accepted method for GSEA was used, with the doctoral candidate utilizing standardized gene lists known to define some of the characteristic features of cancer. It is demonstrated that in all three data sets, the high prognostic score defines similar groups of patients regarding the expression of genes from these lists. Examples of such gene lists and a summary of the overlapping lists are provided (Figures 26, 27). It is interesting that this similarity in expression profiles is likely weakly dependent on epigenetic factors such as DNA methylation, as shown in the dissertation in Figures 28-34. Essentially, these are negative data but lead to the above conclusion and show the thorough investigation of the problem in the dissertation, so I agree that their inclusion in the final version of the dissertation is acceptable. Likely influenced by his scientific advisor, who is also a specialist in immunology, the doctoral candidate investigated whether the 2-PS correlated with infiltration by certain immune cells. To determine the degree of infiltration, a quantitative bioinformatics approach was again used, showing the relative proportion of such immune cells in the overall transcriptomic profile of the tumor tissue. The observed positive correlation with the degree of macrophage infiltration is interesting and should be interpreted

in the context of their role in the pathogenesis of MPM as described in the literature review section of the thesis. Finally, an attempt was made to find some evidence for the potential predictive value of the 2-PS by calculating it for different MPM cell lines and then correlating it with their drug sensitivity data. Some statistically significant and clinically relevant correlations were identified, confirming the rationale of the chosen approach for the primary selection of genes for composing the 2-PS.

The discussion is presented concisely and logically, comparing the obtained results with other similar studies. As a biologist, the doctoral candidate could probably have examined the biological function of the two genes included in the 2-PS in more detail and presented more in-depth rational hypotheses about their involvement in the pathogenesis of MPM.

The conclusions drawn are logical and correspond to the fulfillment of the research tasks. I have no remarks on them. A good impression is made by the formulation of guidelines for possible future research.

5. Contributions and significance of the development for science and practice

I accept the formulated original and affirmatively scientific contributions. Particularly important is the demonstration of an original rational approach for the primary selection of genes in malignant diseases, which are used to develop prognostic models/scores based on transcriptomic data. It is evident that the doctoral candidate had the opportunity to acquire in-depth knowledge on how to integrate existing omics data to draw clinically and biologically significant conclusions for a specific disease.

6. Evaluation of publications related to the dissertation

Presented as related to the dissertation are two publications in international journals, one of which has an impact factor. According to the journal ranking, the minimum required points for defending a PhD in Professional Direction 4.3 Biological Sciences are fulfilled. This proves that the doctoral candidate has acquired sufficient skills under the guidance of their supervisor not only to generate contemporary scientific data but also to publish it. It is recommended that in the future, the doctoral candidate deepen their independent research and publish it in international scientific journals.

7. Abstract

The presented abstract in Bulgarian and English correctly reflects the essence and content of the main dissertation and can be used as a concise reference summary. The abstract for the dissertation is made according to the requirements and sufficiently details the main parts, including the results achieved in the dissertation.

CONCLUSION

In conclusion, regarding the content and related scientific publications, the dissertation of Georgi Dimitrov Blazhev on the topic "Multi-omics approach for analyzing the biological and clinical heterogeneity of some rare malignancies" represents a significant original contribution to science in the sense of LDASRB. Furthermore, the doctoral candidate has completed the training program in regular form of doctoral studies according to the requirements of Sofia University "St. Kliment Ohridski". For these reasons, I express a positive opinion on awarding the Doctor of Science degree to Georgi Dimitrov Blazhev in the Professional Direction 4.3 Biological Sciences and scientific specialty "Genetics". I propose that the other members of the scientific committee also vote positively in the procedure.

Sincerely,

(Prof. Lazar Jevlev Slavov, MD, PhD)

22.05.2024