



Review

of the thesis “Micro RNAs influence on autophagy and innate immune processes signalling in tumor pathogenesis”,
submitted by assistant Radostina Tsvetankova
in partial fulfillment of the requirements for the
“Doctor of Philosophy” research degree
supervisor Prof. Kasimira Todorova, PhD, DSc

General presentation of the documents and procedure

The announcement for current “Doctor of Philosophy” research degree thesis and the procedure for arriving at a decision are based on the following documents: *i*) Act on Development of the Academic Staff in the Republic of Bulgaria, amend. 12.III.2021; *ii*) Regulations on the Implementation of the Development of Academic Staff in the Republic of Bulgaria Act, amend. 19.II.2019; *iii*) The Rules on the Conditions and Procedure for Acquiring Science Degrees and Holding Academic Positions in Bulgarian Academy of Sciences, amend. 31.05.2021 (tech amend. 14.06.2021); и *iv*) Decision of the Scientific Council of Institute of Biology and Immunology of Reproduction №122 от 28.11.2023 г.

The presented set of materials (all in PDF) comply with the requirements and are well structured. Furthermore, the *necessary procedure detailed in the points above has been followed.*

Compliance with the “minimum national requirements” for the “Doctor of Philosophy” research degree .

The information contained within the documents is enough to gain sufficient insight in candidate’s achievements¹. Documents analysis shows that Tsvetankova *satisfies*

¹There is a comprehensive report on compliance with the requirements of the BAS credit system, as well as a signed “declaration of originality”.

the required criteria as detailed below²:

| група | показател | | точки |
|-------|-----------|--|-------|
| A | 1 | A PhD thesis manuscript | 50 |
| Г | 7 | Publications in scientific journals, which are referenced and indexed in renowned scientific databases | 30 |

The thesis

The first ten pages of the *review* discuss the epidemiology, diagnosis and prognosis of prostate carcinoma (PC). I don't agree with the proportions in the exposition, but that's a subjective opinion. The text reveals a good general medical knowledge. The following pages are a brief presentation of studies on a relatively large set of molecules whose aberration is associated with disease and are therefore potential biomarkers. There are conflicting facts and this has been objectively noted — a good premise for biomarker searches to continue in the micro RNA (miRNA) ³.

The actual review begins on page 24. MiRNA and specifically their putative role in prostate carcinogenesis are briefly discussed (page 26)⁴. Attention is paid (p. 33) to the autophagy — generally and specifically in PC; as well as innate immune signalling in PC (p. 42). The literature review ends with a summary (p. 44) that accurately summarizes the issues and fully justifies the set *aim* (p. 45). All *tasks* are formulated exactly (p. 46).

The experimental set-up can be summarized as follows:

- two PC cell lines isolated from lymphatic metastasis and bone marrow metastasis were compared;
- cells were transfected with a synthetic mimic or inhibitor of miRNA-141, or with a synthetic analogue of MAPK1-specific small interfering RNA;
- cells were cultured in different conditions and analysed by different methods.

I have no notes on the *Materials and Methods* section (pp. 47-60). Where commercial kits are used, the manufacturer protocol has been followed and the reader is directed to it. The description of the few “home lab” protocols is comprehensive enough.

The graphical representation of the *results* is of high quality and standardized, allowing easy comparison between experiments. In the text below the figures, the

²Group designation is according to the Bulgarian alphabet

³Perhaps a definition of the term *biomarker* would be helpful to the reader.

⁴I am sceptical of the suggestion that the so-called “liquid biopsy” (which is simply biological fluid testing) will replace classical biopsy as the gold standard.

description is complete. In the block diagrams, however there is some excess of textual information (repeated captions) in fine print and this makes them illegible (eg Fig. 3).

Some data are results of a new and/or specific experimental techniques. The description starts with explanation of the reaction principles. This is a good approach. Attempts to digitally stack photomicrographs (which is the correct term!) have not yielded a definitive result. Nevertheless, the doctoral student quite correctly presents and comments on it.

In the *discussion* there is a critical comparison of the results with the literature. Four diagrams are included in an attempt to relate the observed dependencies into a summary scheme. A peculiar citation style is noticeable in places (p. 113), but all bibliographical information is available and no references are missing from the list of sources.

I approve of the *conclusions* drawn on page 128 with two standing out:

- miRNA-141 has a positive regulatory effect on the autophagy marker LC3 in both cell lines;
- Cell migration was affected after siMAPK1 silencing, and this was more pronounced in the bone metastasis model.

I noted some questions in the manuscript and handed over to the student. The currently available results and comments are complete, sufficient and of high quality.

Additional comments

I have many *grammatical and stylistic objections* being aware that this is not a fundamental factor in a biomedical dissertation as it would be in a linguistic, legal or social work. Such a “illiterate phenomenon” is observed more and more often and this manuscript is no exception. Academic style avoids verbosity, uses precise terms, rules for abbreviations and unambiguous expressions.

I can accept the typing of the Greek letter μ as u. However, there is no reason why a “liter” should be represented by a capital L rather than an l, as is required in the SI (p. 86 and many other places).

It is not appropriate to consider the costs related to PC treatment in experimental paper (p. 11).

The graphs in the panel of Fig. 16 are wrongly named *histograms*. No statistical information is provided for some of the experiments presented (e.g. in Fig. 20, Fig. 26; for comparison, Fig. 23 and Fig. 27 are correctly indicated for credibility). Observed differences can therefore be assumed to be a random event and should not be commented on.

The submitted “abstract project” reflects the essence of the dissertation.

I have no personal observations on the laboratory skills of the student.

I have had good impressions of her presentations during the discussions of the results obtained. I assume Tsvetankova has considerable personal contribution in both publications.

Conclusion

Radostina Tsvetankova is a promising student, with proven competency in the area of immunology. The manuscript includes a considerable volume of laboratory results achieved. The discussion has allowed the creation of scientific conclusions. In essence, the paper meets the requirements for a doctoral dissertation. The obtained results were published in two papers with an impact factor. According to the documentation and my personal impressions, the educational part of the program has been fully implemented.

As a member of the jury, I confidently express my *positive opinion* to grant assistant Radostina Tsvetankova “Doctor of Philosophy” research degree .

February 5, 2024

Reviewer: Stephan Radoslavov Lolov
Sofia
2024.02.05 20:39:48 +02'00'