

REVIEW

By: Prof. Dr Reneta Toshkova, PhD, Institute of Experimental Morphology, Pathology and Anthropology with Museum– Bulgarian Academy of Sciences (IEMPAM-BAS); Member of the Scientific Juri determined by order № ПД-15-62/ 26.05.2023 by the Director of the IEMPAM-BAS, Sofia

Regarding: Competition for the occupation of the Academic position "Associate Professor" in the Scientific specialty "Immunology" (01.06.23), in the Category of Higher education 4. "Natural Sciences, Mathematics and Informatics, Professional field 4.3 "Biological sciences", for the needs of the of "Pathology" Department at IEMPAM-BAS, announced in the State Gazette issue 38, page 79, dated 04/28/2023.

The only candidate for the announced competition is Dr. Rositsa Svetolik Milcheva, assistant professor in the "Pathology" Department at IEMPAM-BAS. The submitted documents on paper and electronic media were well systematized, in agreement with the requirements of the State Law on the Development of the Academic Staff, meeting all Institutional Regulations, and containing all applicable materials.

Biographical data and career profile of the candidate

Dr. Rositsa S. Milcheva was born in Sofia. In 2002 she graduated from the Faculty of Biology of the "St. Kliment Ohridski" University with a master degree in Molecular Biology. Since 2002, she has been working at the Institute of Experimental Pathology and Parasitology (IEPP) of the Bulgarian Academy of Sciences as a specialist and in 2005 she was selected a "Research associate". In the period 2010 - 2014 she held the academic position of "Assistant" and from 2014 - until now she is the "Assistant Professor" in the "Pathology" department at IEMPAM-BAS. She was a PhD student in the Department of "Pathology", of the Faculty of Medicine at the Comenius University in Bratislava, Slovakia for the period 2007 – 2011. In 2011, she successfully defended her dissertation on "Mechanisms of apoptosis in striated muscle fiber after invasion by *Trichinella spiralis*" and received the educational and scientific degree "Doctor". Dr. Milcheva specialized three times (2006, 2007 and 2008) at the Faculty of Medicine of the Comenius University in Bratislava, Slovakia. Other foreign specializations were in the Center for Glycobiology at the Imperial College (London, UK), in the University of Wales (Aberystwyth, UK), and in the Institute of Molecular Biosciences at the Karl Franz University (Graz, Austria). All specializations have a very positive effect in her professional development in the field of immunology, biochemistry, and cell biology. The

total scientific production includes 29 scientific articles, 21 of which are in journals indexed in WoS/Scopus, total number of citations - 74 and h-index 5 (Scopus).

Description of the presented scientific publications for the competition

Dr. Rositsa S. Milcheva participated in the current competition with a PhD Thesis Abstract and 19 articles, which do not repeat those presented for the acquisition of the Educational and Scientific Degree "Doctor" and the competition for "Assistant Professor". All scientific articles are published in journals indexed by Web of Science and/or SCOPUS and have a common impact factor (IF 24.242), including international journals such as: Gels, Acta Histochemica, Materials Science and Engineering, Open Life Sciences, Parasitology Research, Biologia, Acta Parasitologica, Helminthologia, Folia Parasitologica. Articles could be grouped into quartiles as follows: 4 articles with Q1; 3 articles with Q2; 10 articles with Q3; 2 articles with Q4 (according to Web of Science and SCOPUS international scientific databases). Of all the publications submitted for the competition, 15 were published in journals indexed in Web of Science and Scopus after being selected as an "Assistant Professor". The personal contribution of the candidate is indisputable - in 10 publications she is the first author, in 2 - second author, in 3 - third. Scientific studies have been carried out as a result of successfully implemented interdisciplinary national and international projects. Dr. Rositsa Milcheva was a head of 2 and a participant in 1 scientific projects financed by the National Fund for Scientific Research. She was a participant in 2 EBR projects of the BAS with the Latvian Academy of Sciences and is a head of 1 current project on the bilateral cooperation programme of the BAS with the Slovak Academy of Sciences. She participated in a project funded under the Operational Program "Development of Human Resources" to support the development of doctoral students, postdoctoral fellows and young scientists.

In her studies, Dr. Milcheva demonstrates extensive technical experience by using a wide range of classical and modern scientific approaches - lectin and immunohistochemistry, one- and two-dimensional electrophoresis, western and lectin blot, flow cytometry, cell culture, cell death analysis (AnnexinV/PI and TUNEL tests), RT-PCR, realtimePCR, gene expression analysis, statistical analyses, etc. The results of the scientific production became available to the scientific community with 16 reports and 6 posters in 22 scientific forums, of which 12 were international. A list of 32 citations of 9 articles in the Web of Science and Scopus databases is presented, of which 24 after holding the position of "Assistant professor".

Evaluation of the scientific-research and scientific-applied activity of the candidate (Contributions).

The research activity of Dr. Rositsa Milcheva corresponds to the profile of the competition. The contributions of the achieved results were focused in the field of

immunology, biochemistry, cellular and molecular biology, and experimental pathology and were described in details in the applied "author's reference" as a part of the relevant documents. The most important of them are related to *in vivo* and *in vitro* studies of immunological, morphological, genetic, biochemical and functional changes in an experimental tissue model of a Supporting cell ("Nurse cell") formed in a section of a muscle fiber (cell) of the transverse striated muscle tissue after infestation with the parasitic nematode *Trichinella spiralis* and aimed to elucidate the parasite-host relationship and the modulation of the host immune response.

Due to the course of the conducted studies, **Contributions of a fundamental and scientific-applied character** were achieved, systematized as follows:

1. The changes in glycosylation during the different developmental stages of *Trichinella* nematodes have been characterized, which contribute to the elucidation of parasite-host relationships and the modulation of the host immune response by *Trichinella spiralis*. The obtained results have the potential for application in the combat against allergies, autoimmune and malignant diseases.

2. The mechanisms of apoptosis as a tool for adaptation of *Trichinella* and the construction of an efficient habitat niche in striated muscle tissue have been studied. The results elucidate some of the mechanisms of suppression and evasion of the immune response of the *Trichinella*-infected host.

3. The mechanisms of apoptosis during the intestinal phase of *Trichinella* infection were investigated. The invasion of the gastrointestinal tract by *Trichinella* was found not to be associated with apoptotic mechanisms, but with modulation of inflammation as part of the host immune response.

4. Serum sialic acid levels have been found to be a reliable diagnostic marker of inflammation as part of the non-specific host immune response in experimental *Trichinella spiralis* invasion of the rat (*Rattus norvegicus*) as well as an indicator of inflammation in skin infection.

5. Up-regulation of two nuclear proteins poly-(ADP-ribose) polymerase 1 (PARP-1) and cell proliferation nuclear antigen (PCNA) was found in the process of transformation of invaded striated muscle cell into a Nurse cell of *T. spiralis*. It has been shown that the transformation process is associated with nuclear localization of the proteins apoptosis-inducing factor (AIF), Bcl-2 associated protein X (BAX) and caspase-3, which are normally located in the cell cytoplasm. Nuclear localization of excretory-secretory proteins of *T. spiralis* was established. The results demonstrate the high adaptability of the parasite and the ability of *T. spiralis* to regulate intracellular systems in striated muscle tissue, and contribute to a better understanding of host-parasite relationships.

6. It was found that invasion of striated muscle tissue by *T. spiralis* and the process of local de-differentiation of the affected area from the muscle cell into a Nurse cell were associated with increased intracellular biosynthesis of α -2,3- and α -2,6 -sialylated glycoproteins. It has been shown that α -2,3-sialylation in the Nurse cell results from increased expression of the enzyme β -galactoside α -2,3-sialyltransferase 6. A constant feature of all stages of the Nurse cell of *T. spiralis* is also the increased expression of α -dystroglycan and the enzyme UDP-H-acetylglucosamine 2-epimerase/H-acetylmannosamine. Also, the early stage of de-differentiation of the affected striated muscle cell in the Nurse cell of *T. spiralis* is associated with *de novo* expression of the enzyme H-acetylgalactosaminyl- α -2,6-sialyltransferase 1, which is not a characteristic of this type of tissue. These studies are of medical importance and relevance and contribute to a more detailed description of glycosylation, to a better understanding of the adaptive abilities of striated muscle tissue, which is relevant to the treatment of hereditary and congenital myopathies associated with defects in biosynthetic glycosylation apparatus.

7. One innovative and original research demonstrated that the muscle phase of *T. spiralis* infection and the de-differentiation of the affected areas of the striated muscle tissue into a Nurse cell of the parasitic nematode proceeds with a highly elevated protein and RNA-expression of the protein dystrophin. This protein is part of a dystrophin-glycoprotein associated complex, important for maintaining the integrity of muscle cells during contraction, and its incorrect biosynthesis (due to a genetic defect) leads to the development of progressive diseases known as muscular dystrophies.

8. An important point in the research is the complete and renewed characterization of the gene expression of enzymes from three families of sialyltransferases, with substrate specificity glycoproteins, in striated muscle tissue in models *in vivo* (mouse - *Mus musculus*) and *in vitro* (mouse cell line C2C12). The study demonstrates the differences in the expressions of each enzyme of the three families of sialyltransferases and elucidates the possibility of the C2C12 cell line replacing the use of laboratory animals for analysis, as well as its potential for application in the field of oncology.

9. Original group of studies was the research of the influence of Fumonisin B1 (FB1) and deoxynivalenol (DON) mycotoxins on the immune system of chickens and their effect *in vitro* on permanent cell lines. It was established that mycotoxins applied in natural concentrations (contained in the feed) and especially their combination affect the number and function of lymphocytes (proliferative activity, mitogenic response), macrophages, the structure of immune organs and the immune status and health of poultry, with possible adverse economic effect. In *in vitro* experiments, mycotoxins administered at low concentrations have a pronounced antiproliferative and cytotoxic effect on various cell lines (BALB/c 3T3, DEC 99, MDA-MB-231, MCF-7 and Hela cells).

10. Metacarn fixative was proven to preserve the morphology, protein immunoreactivity and RNA integrity of paraffin-embedded tissues to a much higher

extent in comparison with the traditionally used neutral-buffered formaldehyde. These properties make it the most suitable fixative for morphological and immunohistochemical studies, and gene expression analyzes performed on paraffin tissue sections, which is a sparing approach in the use of laboratory animals.

Another important focus of the candidate's research is in the field of nanotechnology.

11. New anti-biofilm coatings based on zinc oxide nanoparticles (ZnO NPs) conjugated with the enzyme amylase (degrading the biofilm matrix) were developed and applied to silicone urinary catheters. The antimicrobial properties of these particles were investigated in a 7-day rabbit (*Oryctolagus cuniculus*) model. The results of clinical, hematological and histological studies of excretory system organs showed that the nanofunctionalization of catheters with hybrid ZnO/AM coatings are a promising strategy for the prevention and control of catheter-related urinary infections in the clinical practice.

12. Another contribution of the candidate was the characterization of a new type of synthesized interpenetrating polymer network (IPN) - hydrogel (IPM), based on poly (carboxybetaine) methacrylate and poly (sulfobetaine) methacrylate. The following properties of the IPM hydrogel were demonstrated: the ability to change its volume under the influence of external stimuli characteristic of living systems (temperature, pH and salt concentration); good mechanical stability, lack of cytotoxicity and antibacterial activity against *Pseudomonas Aeruginosa*, *Acinetobacter Baumannii* and *Klebsiella Pneumoniae* and excellent *in vivo* biocompatibility. With these characteristics, IPM hydrogel is a unique biomaterial, with potential for application in the field of medicine, pharmacy and the production of smart materials, in the production of sensor devices.

Assessment of compliance of the applicant's indicators with the minimum national requirements according to Appendix 1 of the Regulations for the implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria.

The evidence presented in the table for the fulfillment of the minimum national requirements and the requirements of the regulations of IEMPAM-BAS, accurately indicate the number of collected points. A total number of the points for each criterion are presented in detail. The point estimate can be summarized in a table as follows:

Table - Minimum required points by group of indicators in accordance with the Law on the Development of the Academic Staff in the Republic of Bulgaria and Regulations for its application in the country, BAS and IEMPAM-BAS.			
Indicator	Minimum required	points	Number of points of the candidate, based on the

		evidence presented
A	50	50 PhD Thesis
B	-	-
B	100	100 , obtained from 5 refereed publications, with an impact factor (Scopus): 2 x Q1 = 50 1 x Q2 = 20 2 x Q3 = 30
Г	220	234 , obtained from 14 refereed publications, with an impact factor (Scopus): 2 x Q1 = 50 2 x Q2 = 40 8 x Q3 = 120 2 x Q4 = 24
Д	60	64 , obtained from 32 citations in foreign refereed publications 32 x 2 = 64
Minimum total number of points for indicators A, B, G and D	430	448 total number of points of the candidate for indicators A, B, G and D

The analysis of the quantitative criteria shows that Dr. Rositsa Milcheva meets the minimum requirements for each of the groups (A-D), exceeding them by indicators "G" and "D" and fully meets the requirements of the IEMPAM-BAS regulations, as and the national requirements according to the Law on the Development of the Academic Staff in the Republic of Bulgaria

Personal impressions and professional awards

I know Dr. Milcheva from the appointment and at the Institute. She is a hardworking and purposeful scientist, an excellent specialist in the field of immunology, cell biology and biochemistry. She has good organizational skills, a proven ability to work independently and in a team, and readily accepts new challenges. Dr. Milcheva has been awarded three prizes and two diplomas (best publication of a young scientist in IEPP-BAS, project leader with significant project funding in IEMPAM, best presentation at an international forum), which proves an excellent and high level of Professional training.

Conclusion

The scientific research activity of Assistant Professor Rositsa Svetolik Milcheva is entirely in the field of the announced competition and fully covers all the quantitative and qualitative criteria for awarding the scientific title "Associate Professor" in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the conditions and procedures for acquiring scientific degrees and holding academic positions at BAS and IEMPAM-BAS. A large part of the research has been published in international journals with an impact factor and an impact rank, they have been cited many times, which proves the international recognition of the scientific production. Original results have been obtained with an indisputable fundamental, methodological and scientific-applied contribution character in the medico-biological aspect and with possibilities for application in medical practice. I believe that Dr. Milcheva's academic growth will open up new horizons for her and will allow her to share knowledge and experience, and to develop her full potential as an erudite researcher for the further development of the scientific direction - immunology at the Institute.

Based on the above, I give a positive and high assessment of the overall research activity of the candidate and I confidently suggest to the members of the Scientific Jury and the honorable Scientific Council of IEMPAM-BAS to choose Assistant Professor, Dr. Rositsa Svetolik Milcheva, in the academic position "**Associate Professor**" in the Scientific speciality "**Immunology**" (01.06.23), in the Higher Education Category 4. "Natural Sciences, Mathematics and Informatics", Professional Field 4.3. "Biological sciences", for the needs of the "Pathology" Department at IEMPAM-BAS.

04.09.2023r.

Sofia

Sign:



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