

OPINION

by Professor Dr. Rumiana Dimitrova Tzoneva, Institute of Biophysics and Biomedical Engineering, BAS

regarding a competition for "Associate Professor" in area 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty "Immunology", code 01.06.23, published in the State Gazette, issue 38 of 28. 04. 2023, for the needs of the section "Pathology", IEMPAM - BAS.

Assistant Professor Dr. Rositsa Milcheva is the only candidate in the announced competition. The documents provided by the candidate for holding the academic position "Associate Professor" are prepared in accordance with the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation.

Rositsa Milcheva graduated from Sofia University "St. Kliment Ohridski", Faculty of Biology in 2002 as a master's degree in Molecular Biology, specialization Clinical Chemistry, diploma A-2002 SU No. 160673. From 2002 to 2010, she worked consecutively as a specialist and assistant at IEMPAM-BAN. Between 2007-2011, she was enrolled as a doctoral student at Comenius University in Bratislava, Slovakia, Faculty of Medicine, Department of Pathology, code 7.1.21 pathology and forensic medicine.

In 2011, he successfully defended his doctoral dissertation on "*Mechanisms of apoptosis in striated muscle fiber after invasion by Trichinella spiralis*" under the supervision of Prof. Dr. Pavel Babal, diploma UK*4410.

From 2014 to the present, Dr. Rositsa Milcheva has been working as a Assistant Professor at IEMPAM-BAN.

In the current competition, Assistant Professor Dr. Rositsa Milcheva participated with 19 publications outside the publications included in the doctoral dissertation. Of these, 15 articles have been published in international journals with an impact factor, and four in journals with SJR. Of them, Q1 is 4 (four), Q2 is 3 (three), Q3 is 10 (ten), and Q4 is 2 (two). In 10 of the articles Dr. Rositsa Milcheva is the first author. The above papers have 32 citations in international journals, and the results of scientific research have been reported at 10 international and 12 national/foreign scientific forums. So far, Dr. Milcheva has a total of 29 publications, from which 20 are in SCOPUS, which have been cited 55 times without auto-citations of all authors (h-index = 4 - according to SCOPUS).

The research activity of Assistant Professor Dr. Rositsa Milcheva is in the field of immunology, cell biology, biochemistry, molecular biology and experimental pathology and partly biomaterials.

The main contributions to the research activity of Assistant Professor Dr. Rositsa Milcheva are related to the changes of an immunological, morphological, genetic, biochemical and functional nature in the so-called supporting cell (Nurse cell), formed as a result of invasion of the parasitic nematode *Trichinella spiralis* in a section of a cell (muscle fiber) of a transverse striated muscle tissue.

The studies are mainly aimed at elucidating the parasite-host relationship and the modulation of the immune response by *Trichinella spiralis*, which have potential applications in the fight against allergies, inflammatory reactions, autoimmune and malignant diseases. Data were obtained on reliable molecules - indicators of inflammation, as well as on the mechanisms of de-differentiation of the affected area from the muscle cell into a supporting cell and the occurrence of apoptotic processes. An additional contribution in this field is the elucidation of the adaptive capabilities of striated muscle tissue, which is relevant to the fight against a heterogeneous group of diseases such as hereditary and congenital myopathies associated with defects in the biosynthetic glycosylation apparatus. Brand new data are also shown that point to the mechanisms of regeneration of the affected muscle cell.

Approaches have also been developed to monitor the health and immune status of poultry, as well as the degree of cytotoxicity when using mycotoxins.

Developments using the metacarn tissue fixator have scientific, diagnostic and laboratory-technical potential.

Developments related to detection and identification, and phylogenetic analysis of six of the most common viruses in honey bees in Bulgaria are a known contribution.

The research carried out in the field of biomaterials has its contribution in dealing with various hospital infections and the development of highly biocompatible materials with application in the field of medicine and pharmacy and the production of intelligent materials involved in the production of sensor devices.

I. Contributions of a fundamental and scientific-applied nature

Field of immunology and experimental pathology

- a. The changes in glycosylation during the different stages of the development of nematodes of the genus *Trichinella* have been characterized (publication 6.1._B and 7 - list of citations).
- b. In an experimental invasion with *Trichinella spiralis* in a rat model, it was found that serum levels of sialic acid can serve as a reliable indicator of inflammation as part of the non-specific immune response (*Rattus norvegicus*) (publication 6.5._B and 7 - list of citations).
- c. A correlation was established between the 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 supporting cell, which 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 supporting cell results from increased expression of the enzyme β -galactoside α -2,3-sialyltransferase 6, α -dystroglycan and the enzyme UDF-H-acetylglucosamine 2-epimerase/H- acetylmannosamine (HNE). It was also found that the early stage of de-differentiation of the affected striated muscle cell in a support cell of *T. spiralis* was characterized by *de novo* expression of the enzyme H-acetylgalactosaminyl- α -2,6-sialyltransferase 1, which is not characteristic of this type of fabric. The originality of the obtained results stems from the fact that they are completely new for the specific scientific field and contribute to a more detailed description of glycosylation in striated muscle tissue - an area in which there is still not enough accumulated scientific data. (Posts 6.4._G, 6.7.G, 6.8.G, 6.12._G).

d. By studying the influence of the mycotoxins Fumonisin B1 (FB1) and deoxynivalenol (DON) on the immune system of chickens, it was found that they (added to the feed) lead to a decrease in the number of lymphocytes in chickens, their proliferative activity and mitogenic response. Mycotoxins in small concentrations have been found to have a pronounced antiproliferative and cytotoxic effect on various cell lines. (Publications 6.3.G, 6.5._G, 6.9._G and 7 - list of citations).

e. The alcohol-based fixative metacarn was found to successfully preserve the morphology, protein immunoreactivity and RNA integrity of paraffin-embedded tissues. These original results have potential application in various developments of a scientific, diagnostic and laboratory-technical nature. (publication 6.4._B-1 and 7 - list of citations).

Field of cell biology, biochemistry, molecular biology

f. The mechanisms of the occurrence of apoptosis in *Trichinella* as a means of adaptation and the construction of an effective habitat niche in the striated muscle tissue have been studied. The results are original and contribute to elucidating the mechanisms of suppression and evasion of the immune response of the *Trichinella*-infected host (publication 6.2.B and 7 - list of citations).

g. The mechanisms of apoptosis during the intestinal phase of *Trichinella* infection were investigated. It has been established that invasion of the gastrointestinal tract by *Trichinella* is not associated with apoptotic mechanisms, but that invasion by *Trichinella* in the intestinal phase is a modulator of inflammation as part of the host immune response (publication 6.4.B and 7 - list of citations).

h. In the process of transformation of an invaded striated muscle cell by *T. spiralis* into a supporting cell, an upregulation of two nuclear proteins - poly-(ADP-ribose) polymerase 1 (PARP-1) and cell proliferation nuclear antigen (PCNA). It has been shown that the transformation process is also associated with nuclear localization of apoptosis-inducing factor (AIF), Bcl-2 associated protein X (BAX) and caspase-3, which normally reside in the cell cytoplasm. Nuclear localization of excretory-secretory proteins of *T. spiralis* was established. (Publication 6.2.G).

i. Molecular biological studies were carried out for detection and identification, as well as phylogenetic analysis of six of the most common viruses in honey bees in samples from different regions of the country (Apis Mellifera (Deformed wing virus (DWV) - deforming wing virus; Acute bee paralysis virus (ABPV) - acute paralysis virus; Chronic bee paralysis virus (CBPV) - chronic paralysis virus; Sacbrood virus (SBV) - blister rot virus; Kashmir bee virus (KBV) - Kashmir virus; Black queen cell virus (BQCV) - black queen bee virus)(Publications 19, 20 and 22).

j. A complete and updated characterization of the gene expressions of enzymes from three families of sialyltransferases, with substrate specificity glycoproteins, in mouse (*Mus musculus*) striated muscle tissue and the mouse cell line C2C12 was performed in order to elucidate differences in the expressions of each of the three enzymes families of sialyltransferases and to clarify to what extent the C2C12 cell line can replace the use of laboratory animals for analyzes in the particular scientific field. This research finds its application in studies and in the field of oncological diseases (publication 6.10._G and 7 - list of citations).

k. A relationship was established between the muscle phase of *T. spiralis* infection and the de-differentiation of affected areas of striated muscle tissue into supporting cells of the

parasitic nematode on the one hand, highly elevated protein and RNA expression of the dystrophin protein, suggesting that is this a mechanism of regeneration of the affected muscle cell, or is it the result of the proven ability of *T. spiralis* to modulate the genetic identity of the host microenvironment (publication 6.13._ G).

Biomaterials area (nanomaterials and hydrogels)

l. The antimicrobial properties of zinc oxide nanoparticles (ZnO NPs) applied to silicone urinary catheters were investigated in a 7-day rabbit (*Oryctolagus cuniculus*) model. The results show that the nanofunctionalization of catheters with hybrid ZnO@AM coatings appears as a promising strategy for the prevention and control of acute infections in the clinic (publication 6.11._ G and 7 - list of citations).

m. The mechanical properties and antibacterial properties of a new type of interpenetrating polymer network - a hydrogel based on components poly (carboxybetaine) methacrylate and poly (sulfobetaine) methacrylate under the influence of external stimuli - temperature, pH and salt concentration - are characterized (publication 6.14_ G).

As an attestation for the professional growth of Assistant Professor Dr. Rositsa Milcheva over the years has also participated in various specializations - specialization in the Faculty of Medicine at Comenius University in the city of Bratislava, Slovak Republic; - studying at the Imperial College in London, Great Britain; - specialization at the Institute of Biological, Environmental and Agricultural Sciences at the University of Wales in Aberystwyth, Great Britain; - specialization at the Institute of Molecular Biosciences at the Karl Franz University in Graz, Austria, as well as the received awards and certificates from international and national events.

Dr. Rositsa Milcheva has participated in the implementation of 7 research projects, having been the head of 2 projects (funded by FNI) and 1 project (program "International cooperation within the framework of inter-academic contracts and agreements", in cooperation with the Slovak Academy of Sciences). Participated in 4 more projects (1 funded by the National Institute of Scientific Research and 3 under other programs).

Dr. Milcheva has participated in the training of students, doctoral students and postdoctoral fellows.

Conclusion: From the presented scientific works of Assistant Professor. Dr. Rositsa Milcheva shows that the overall scientific output of the candidate fully satisfies the requirements of the Law on Academic Development in the Republic of Bulgaria and the criteria for acquiring the scientific title "Associate Professor" at IEMPAM - BAS for professional field "Biological Sciences", scientific specialty "Immunology".

These scientific contributions have both fundamental and scientific-applied significance for the development primarily of immunology and experimental pathology, cell biology, biochemistry, molecular biology and partly biomaterial sciences.

Taking into account all the above, I will confidently vote positively in the scientific jury for the award of Dr. Rositsa Milcheva to the scientific title "Associate Professor".

05.09.2023 r.


Prof. Dr. Rumiana Tzoneva