



REVIEW

On the submitted works for participation in a competition for the academic position of "Professor" in the field of Higher Education 4. Natural sciences, mathematics and informatics, professional area 4.3. Biological sciences (Biochemistry), announced by Medical University – Varna in State Gazette, issue No. 7/23.01.2024

Reviewer: Svetla Dimitrova Petrova PhD

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Member of the Scientific Jury, according to order No. P-109-96/21.03.2024 of the Rector of Medical University - Varna, in a competition for the academic position "Professor", Professional area 4.3. Biological sciences (Biochemistry), Higher education field 4. "Natural sciences, mathematics and informatics", published in SG No. 7/23.01.2024 for the needs of the Faculty of Pharmacy, Department of Biochemistry, Molecular Medicine and Nutrigenomics

Candidate: Maria Atanasova Radanova PhD

Associate professor at the Department of "Biochemistry, Molecular Medicine and Nutrigenomics", Faculty of Pharmacy, Medical University - Varna.

According to the documents presented by the "Career Development" Department, Dr Maria Radanova has more than 23 years of work experience in the biology speciality and fully satisfies the requirements set out in the Law for the Development of Academic Staff in the Republic of Bulgaria (LDASRB) and the Regulations for its application, as well as she has an acquired specialty in Biochemistry as an additional condition for the competition. The materials submitted by the applicant are precisely prepared and completed in accordance with the legal requirements. I declare that I have no common publications with the candidate submitted for this competition.

1. Brief biographical details of the applicant

Assoc.prof. Maria Radanova graduated with honors at the Faculty of Biology of Sofia University "St. Kliment Ohridski" in 1999, majoring in "Molecular Biology" and received Master's degree in "Clinical Chemistry" with professional qualification - molecular biologist. After that she immediately started working as a biologist at IBIR "Acad. K. Bratanov" - BAS. Since 2001 her professional development continued at the University of Ruse "Angel Kanchev" (branch – Silistra), where after a competition she successively held the positions of assistant and senior assistant. After 2006, Maria Radanova's scientific and professional development was associated with the Medical University "Prof. Dr Paraskev Stoyanov" - Varna and the Department of Biochemistry, Molecular Medicine and Nutrigenomics, where she started her PhD studies in Biochemistry on independent training and where she successively passes through the positions of assistant professor, senior assistant prof., chief assistant prof. and associate professor (2016 - until now).

Since 2017, she is an assoc.prof. at "Sveta Marina" University Hospital, Varna.

My personal impressions of Maria Radanova's demonstrated ability to work, persistence, responsibility and, above all, her definite focus on research work, are from her time as a graduate student at the Laboratory of Molecular Immunology of the Department of Biochemistry at SU, where she worked on a current scientific problem related to the role of the C1q component of the complement in the autoimmune diseases. Maria Radanova continues research on establishing the mechanisms of intermolecular interactions of complement components, their role in systemic *lupus nephritis* and the possibilities for their clinical impact in her dissertation thesis in Biochemistry, entitled: "*Study of molecular effects of C1q inhibition in patients with lupus nephritis*", defended in 2012.

Maria Radanova's immunological knowledge research and experience are her personal contribution to the integral scientific development of the Department of Biochemistry, Molecular Medicine and Nutrigenomics. As a teacher and doctoral student at MU-Varna, Maria Radanova also acquires an additional specialization in medical biochemistry, required by Medical Universities, which essentially elevates her to a higher teaching level. Over the years, assoc. prof. Radanova periodically raised her qualifications, attending a number of courses, trainings and seminars organized by prestigious scientific institutions such as EMBO, UCL, EMQN CIC, University of Pittsburgh, Mendel Institute, etc.

2. General assessment of the applicant scientific activity

For participation in the competition for the Academic position "Professor", Dr Maria Radanova submitted an Academic Reference of scientific works that fully cover, both the National and those of the Medical University – Varna, requirements, including 14 publications (referred and indexed in Scopus and Web of Science), 2 published book chapters, 1 chapter in a published university textbook, 2 published university textbooks, 1 dissertation and 15 presentations and posters in national and international scientific forums.

- ✓ indicator group A1 (50 pts) - PhD thesis;
- ✓ indicator group B4 (102 pts) - 5 scientific publications (2 - Q1; 2 - Q2; and 1 - Q4) in publications referenced and indexed in Scopus and Web of Science;
- ✓ indicator group Г7 (210 pts) - 9 scientific publications (2-Q1; 5-Q2; and 2-Q3), in editions referenced and indexed in Scopus and Web of Science, but beyond those equivalent to habilitation work;
- ✓ indicator Г8 (30 pts) - 2 published book chapters;
- ✓ indicator Д11 (238 pts) – 119 citations of the presented scientific publications
- ✓ indicator E13 (100 pts) – 3 successfully defended PhD theses of her doctoral students
- ✓ indicators E14-E18 (207 pts) – leadership of (4) and participation (4) in scientific and educational projects
- ✓ indicator E19 – 1 chapter in a published university textbook;
- ✓ indicator E20 - 2 published university textbooks, with Maria Radanova being the scientific editor of one of them.

Assoc. prof. Dr Maria Radanova is the corresponding and/or first author in 7 publications, and in 5 she is the last, with a leading role and personal contribution to scientific research, showing that Maria Radanova is an established scientist. For the period from 2017 to 2023 (scientific papers in indicators B4, Г7 and Г8) she has a total Impact Factor of 44,892, total Scimago Journal Rank 10,732 and 119 citations (according to NACID reference with reg. no. 94-06-26 dated 29.02.2024), which illustrates well the intensity and high quality of the scientific research carried out by the candidate.

Assoc. prof. Dr Radanova also presents 2 full-text publications (marked with П1 and П2 - 35 pts) in scientific publications, referenced and indexed in global databases with scientific information, beyond the minimum scientometric requirements for the academic position "professor". The results of Dr

Radanova's research (presented for the competition), have been reported at 3 national and 11 international scientific forums.

3. Assessment of scientific and applied research activities

Assoc. Dr Maria Radanova presented research results, whose original scientific contributions were defined in three categories - fundamental, scientific-applied and methodical. I would define the contributions as interrelated elements of the integral research in several modern biology fields in which she worked in the period 2017-2023:

I. Diagnostic, prognostic and predicative biomarkers in solid tumors

(5 publications (equivalent to habilitation work - B4.1.; B4.2.; B4.3.; B4.4.; B4.5.; Г8.2.; E19 and 6 international scientific forums with published abstracts).

- **Non-coding RNAs** (ncRNAs) and single nucleotide polymorphisms (**SNPs**) as diagnostic and prognostic biomarkers

Assoc.prof. Radanova is the leader of two interdisciplinary research projects and one doctoral dissertation defended in 2022, which investigate patterns of impaired gene expression and regulation in colorectal carcinoma. The contributions are related to the study of the role of some ncRNAs (circRNA, miRNAs) affecting the processes of cell differentiation, gene expression, chromatin remodeling and epigenetic regulation, stabilization of functionally active proteins and the functions of other active non-coding RNAs, whose disorders unlock various pathological conditions and carcinogenesis. The results of these studies are related to the: *discovery of new ncRNAs* with differential expression in the plasma of patients with metastatic disease (compared to healthy controls); *found 4 new circRNAs, of which two are specific* (with potential for the early detection of metastasis), distinguishing patients who underwent adjuvant chemotherapy in stage III disease from patients with metastatic disease in stage IV; *creating a database of specific circRNAs* with oncogenic function, *aiming circRNAs to be targets for inhibiting tumor activity in colorectal carcinoma*; *establishing the role of one of the detected circRNAs* (hsa_circ_0001445) as an independent factor for poor prognosis in colon carcinoma by applying multivariate Cox regression analysis with prognostic purpose; *detection of miRNAs* whose levels show dependence and correlation with the expression levels of the discovered circRNAs and also have prognostic potential in patients with metastatic colorectal cancer; *identified certain SNPs* in the genes of the detected miRNAs, with patients carrying the AC rs2682818 genotype having a reduced risk of colon cancer instead of homozygote AA genotype of rs353293 in the miR-143/145 gene cluster and of the TT genotype of rs7372209 in the miR gene -26a-1; and at least 3 *microRNAs* (miRNAs-SNPs – miR-146a rs2910164, miR-27a,rs895819 and miR-608 rs4919510) have been defined as promising as prognostic and diagnostic biomarkers for colorectal carcinoma.

Dr Radanova raises an interesting *mechanistic hypothesis*, according to which the levels of the investigated circRNAs and miRNAs are high in patients with metastatic colorectal carcinoma not only due to the changed expression and regulation in tumor cells, but also due to the function of immune cells from the tumor microenvironment, neovascularization, vascular induced necrosis and the influence of resident immune cells in metastasized organs. I am sure that her narrow immunological specialization has a great contribution to the analysis and interpretation of the obtained results. The published book chapter (Г8.2) presents an original interpretation of the mechanisms by which transcribed ultraconservative regions (T-UCRs) regulate gene expression and how their own expression is regulated.

- **Protein and genetic biomarkers** with prognostic and predicative significance (D7.1.; D7.2.; D7.3. and D7.4.)

I think that the participation of Prof. Radanova as an immunologist and molecular biologist in a large-scale integrated study in collaboration with teams of oncologists, pathologists, geneticists, immunologists and biochemists, has a significant impact on her growth as a scientist with a wide-range view of various pathological states, which is important for the scientific activity of the Department of Biochemistry, molecular medicine and nutrigenomics.

In this scientific area, several important contributions, made for the first time, stand out: the *predicative and prognostic role of RIPK3* in metastatic colon cancer; *assessment of the frequency of KRAS mutations* in Bulgarian patients with advanced and metastatic colorectal carcinoma; *analysis and assessment of high NLR ratio* (neutrophil/lymphocyte) and sarcopenia in non-small-cell lung carcinoma in patients progressing on platinum-based chemotherapy, which immediately informs for the higher risk of developing hyper-progressive disease after immunotherapy; received *data on the frequency of PIK3CA mutations (29.2%)* in Bulgarian patients with metastatic breast carcinoma (HR+/HER2-), correlating with the presence of metastatic disease, and at the same time the presence of the mutation does not affect endocrine therapy.

II. Molecular effects of inhibition of C1q – the first component of the complement system – in patients with autoimmune diseases (A1.; Г7.8.; Г7.9. and Г8.1.).

The immunological research of assoc. prof. Maria Radanova is a prolongation in-depth of her initial scientific interests, most fully reflected in her PhD thesis (A1.), her long-term international cooperation with some of the most world-known scientists in the area of the complement system and were realized within the framework of an international research project (Г8.1.), as well as the preparation of two PhD theses under her supervision (Г7.8. and Г7.9.).

Here, the contributions relate to the study of the structure-function relationship of the complement subcomponent C1q, which activates the classical pathway and whose functional deficiency correlates with the critical role of the collagen regions of the complex C1q protein in maintaining tolerance to self-structures. Formation of complexes between autoantibodies and/or hemolysis products with the globular domains of C1q was established, which affected the removal of immune complexes and CRP-opsonized apoptotic cells in lupus nephritis (A1.). Single nucleotide polymorphisms (SNPs: rs665691, rs682658, rs172378, rs292001 and rs294179) in the C1q gene cluster that may be associated with risk of developing rheumatoid arthritis are found (eg, G-allele and GG genotype of rs172378, as well as the AA genotype of rs292001). *Methodically*, a detailed protocol (ELISA method) was created and presented for the routine *detection of anti-C3 autoantibodies in plasma*, which have diagnostic and prognostic potential as biomarkers, because in about 30% of patients with lupus nephritis, these autoantibodies and their detected concentration correlate with the severity of the disease (Г8.1.).

III. Pathological dysregulation of metabolism (Г7.5.; Г7.6.; Г7.7.; II1.; II2.)

The relevance of this scientific area is indisputable and is directly related to a number of metabolic disorders based on obesity, hepatic steatosis and diabetes. The main original scientific contributions of the research can be attributed to studies of the cytoprotective effect of S-adenosylmethionine (SAM) on hepatocytes, which were placed in conditions of fructose-induced disorder in rats with fatty liver (Г7.5). Different concentrations of SAM showed increased expression levels of hemoxygenase-1 (HO-1), malondialdehyde (MDA), SH-groups in their livers, and increased levels of plasma triglycerides (TAGs). Serum levels of cytokeratin-18 (SK-18) are used as a biomarker for non-invasive evaluation of

liver damage in metabolic syndrome (Г7.6) and indicate the key role of apoptosis in the pathogenesis of liver damage induced by high fructose concentrations.

The study of the relationship between variants in dynein-related genes and male infertility (Г7.7) is based on dysregulation of metabolism, disorders in the synthesis of dynein polypeptide chains and their assembly. The scientific team identified variants of 15 genes that correlate with an increased risk of male infertility. The study is also pioneering in describing dynein-related genes as molecular targets for future research on sperm motility problems.

Studies on the antioxidant and anti-inflammatory effects of Varna sulfur mineral waters are a special focus of the Department of "Biochemistry, molecular medicine and nutrigenomics". The scientific team with the participation of assoc. prof. Radanova found that the mineral waters lead to a significant increase in total glutathione (GSH) and thiols (-SH) and the expression of γ -glutamylcysteinyl ligase, which is an indicator of improved redox status. On the other hand, mean plasma levels of creatinine and high-sensitivity C-reactive protein (hs-CRP) decreased, and glomerular filtration rate (eGFR) and diuresis increased significantly.

4. The educational and teaching activities and the scientific tuition of students

As a full-time teacher at MU-Varna, assoc. prof. Dr Maria Radanova actively participates in the education of students and doctoral students, carrying out lectures in Biochemistry for students in the specialties "Dental Medicine", "Physical Therapist" and "Medical Laboratory Technician". The academic report from the "Educational Activity" Department shows a serious classroom workload of ~ 215 hours/year (lectures and exercises) in Biochemistry for the last four academic years. Three PhD theses under her supervision are evidence of her scientific teaching and training of students in the laboratories. She is the co-author of a textbook - *"Biology of liver metastasis in colorectal carcinoma."* Biliary-hepatic and pancreatic surgery" issued by Military Medical Publishing House (VTU Kableshkov EOOD), as well as two textbooks in practical biochemistry - *"Tasks and practical exercises in biochemistry"* (for students of medicine and kinesitherapy), issued by MU "Prof. Dr Paraskev Stoyanov" - Varna.

5. Scientific projects

Assoc. prof. Dr Maria Radanova presents a rich and intensive project activity, participating in 8 projects, leading 4 of them (financed by the "Science" Fund - Varna and the „National Scientific Fund”), including one under the bilateral cooperation program Bulgaria – France (E17.1) and INSERM-funded international project *"Anti-complement auto-antibodies: Relevance for immune dysregulation and kidney injury in lupus nephritis (AutoCompLN)"* (E18.4), together with the team of prof. Lubka Roumenina's immunological laboratory in Paris.

The total amount of project funds attracted by the candidate is more than BGN 290,000. As a result of this intensive work, assoc. prof. Radanova collected 316.98 points from group E, out of the required 150 points, which is a serious contribution to the scientific activity at MU-Varna.

I accept the certificate of fulfillment of the minimum national requirements under Art. 2b of ZRASRB, for the scientific field 4. Natural sciences, mathematics and informatics and professional area 4.3 Biological Sciences (Biochemistry), in which assoc. prof. Dr Maria Radanova exceeds the points for group of indicators Г, Д and E.

7. Conclusion

As a member of the Scientific Jury for the announced competition, I support the Academic growth of assoc. prof. Dr Maria Radanova. My personal impressions of Dr Maria Radanova define her as an

established highly qualified interdisciplinary specialist, with very strong motivation and active research activity in the area of biochemistry, molecular biology and immunology, demonstrating the need of integral modern biological science. Assoc.prof. Maria Radanova is an established highly qualified teacher - lecturer and supervisor of doctoral students.

The presented scientific publications contain contributions of a fundamental, methodical and applied nature, in terms of quantity and quality they exceed the requirements for awarding the academic position of "professor", and are in the area of the announced competition, fully meeting the requirements of LDASRB. I am sure that Prof. Radanova has the competences and skills to raise scientific concepts, she has acquired extensive experience in the tuition of doctoral students and team-working, which are essential for the realization of innovative research and scientific projects. I confidently give my positive assessment and recommend the Scientific Jury to elect Dr Maria Radanova as "Professor" in professional area 4.3. Biological Sciences, with a scientific specialty "Biochemistry" at the Department of "Biochemistry, Molecular Medicine and Nutrigenomics" of the Faculty of Pharmacy, Medical University "Prof. Dr Paraskev Stoyanov" - Varna.

Reviewer: prof. Svetla Petrova PhD

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