

## REVIEW

by Prof. Jordan Atanasov Doumanov DSc,

Professor of Biochemistry at the Department of Biochemistry, Faculty of Biology, Sofia University "St. Kliment Ohridski", appointed as a 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 of "Professor", Professional field 4.3. Biological sciences (Biochemistry), field of higher education 4. "Natural sciences, mathematics and informatics", announced in State Gazette No. 7/23.01.2024 for the needs of the Faculty of Pharmacy, Department of Biochemistry, Molecular Medicine and Nutrigenomics

At the announced competition, the only candidate who submitted documents is **Associate Professor Dr. Maria A. Radanova** from the Department of "Biochemistry, Molecular Medicine and Nutrigenomics", Faculty of Pharmacy, Medical University - Varna. According to the documents presented by the "Career Development" department, Associate Professor Radanova has more than 23 years of experience in the area, which fully satisfies the requirements set out in the Law and the Regulations for its application, as well as 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.

### **Brief biographical data of the applicant**

Associate Professor Maria Radanova was born in 1975 in Ruse. She completed her higher education at Sofia University "St. Kliment Ohridski" in 1999, majoring in "Molecular Biology" and Master's degree in "Clinical Chemistry". After graduation, she went to Varna, where from 2007 to 2011 she was a doctoral student on independent training in the department of "Biochemistry, molecular medicine and nutrigenomics", specialty "Biochemistry", MU "Prof. Dr. Paraskev Stoyanov". In 2011, she successfully defended her thesis, entitled "Investigation of molecular effects of C1q inhibition in patients with lupus nephritis". In 2015, she acquired an additional specialization in Biochemistry.

In the years between 2000 and 2006, she was appointed first as a biologist at the Institute of Reproductive Biology and Immunology "Acad. Kiril Bratanov", BAS, and then successively as a biologist, assistant professor and senior assistant professor at the University of Ruse "Angel Kanchev" - branch office - Silistra. In 2006, she started working as an assistant professor in the Department of Biochemistry, Molecular Medicine and Nutrigenomics at the Medical University "Prof. Dr. Paraskev Stoyanov", Varna. In this department, the professional development of Associate Professor Radanova continues, where she successively passes through the positions of assistant professor, senior assistant professor, chief assistant professor and associate professor (2016 - until now). Since 2017, she is also an Associate Professor at "Sveta Marina" University Hospital, Varna. Over the years, Associate Professor 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.

### **Scientific production and analysis of scientometric data**

The total scientific output of Associate Professor Radanova includes 69 publications. The data analysis I have done is based on the publications from the Academic report presented in the competition documentation.

The scientific output, from the presented Academic report of Associate Professor Radanova, includes 28 publications, 2 published book chapters, 1 chapter in a published university book, 2 published university textbooks, 1 dissertation and 15 participations with reports and posters in national and international scientific forums.

All scientific articles have been published in international journals with IF and Q-rank, among which stand out prestigious journals such as Journal of Biological Chemistry, International Journal of Molecular Sciences, The Journal of Immunology, Molecular Immunology, Clinical & Experimental Immunology, etc. Eight of these articles were published in journals belonging to the highest quartile (Q1) according to the Scimago Journal Rank (SJR). The total impact factor of the presented scientific production is over 70, and the citation reference shows over 150 titles without self-citations. According to Scopus data, Associate Professor Radanova's Hirsch index is 10.

For her participation in the current competition, Associate Professor Radanova presented 14 scientific publications that were not reviewed in previous competitions for the awarding of scientific degrees or academic positions. Scientific articles on this competition make up 50% of the output presented in the Academic Directory, and the analysis of publication activity over the years shows that after her habilitation in 2016, she published an average of 2 articles per year. This certainly indicates a very intensive research activity, especially for a university associate professor who also has a significant teaching activity.

Articles for the current competition were published in journals with a total impact factor of 44,892, of which 4 (28.6%) were in journals with an impact rank of Q1, 7 (50%) in Q2 and 3 (21.4%) in Q3. This distribution well illustrates not only the intensity, but also the high quality of the scientific research conducted by the candidate.

The scientific production presented and the scientometric data achieved fully correspond and, in all indicators, exceed the minimal requirements for awarding the academic position of "Professor". Data for meeting the requirements by indicators are presented as follows: 102 points from Group B (required 100 points), 210 points for a Group Γ (required 200 points) and 238 points for a Group Δ (required 100 points). Associate Professor Radanova actively participates in the training of doctoral students and the development of scientific projects. In the presented materials, the participation in 10 projects (2 international and 8 national) is noted. The reference indicates that she was the head of 5 of them. The total amount of funds attracted by the candidate for the projects is over BGN 290,000. As a result of this intensive work, Associate Professor Radanova collected 316.98 points from group E, out of the required 150 points. An indicator of the high quality and value of scientific projects is the INSERM-funded international project "Anti-complement auto-antibodies: Relevance for immune dysregulation and kidney injury in lupus nephritis (AutoCompLN)", together with the world-famous immunological laboratory of Dr. Lubka Roumenina.

### **Analysis of scientific contributions**

Associate Professor Radanova's scientific interests and published scientific results are entirely in the field of the announced competition, being concentrated in three scientific directions: **I.**

Diagnostic and prognostic biomarkers in solid tumors; **II.** Molecular effects of C1q inhibition in patients with autoimmune diseases; **III.** Pathological dysregulation of metabolism. The results published in the so far unused publications are in groups B,  $\Gamma$  and E, according to tables 1 and 2, Decision No. 26/February 13, 2019 of the Ministerial Council on professional field 4.3. Biological Sciences. I will consider the presented articles as they are systematized by the candidate, and in a summarized form I will present the most important scientific achievements from which the scientific contributions follow.

**I. Diagnostic and prognostic biomarkers in solid tumors**

The biological functions of non-coding RNAs (circular RNAs, circRNAs and microRNAs, miRNAs) are related to the maintenance of tissue differentiation, transcriptional and post-transcriptional regulation, epigenetic regulation, chromatin remodeling, protein stabilization, affecting the functions of other non-coding RNAs and others. Dysregulation of non-coding RNAs is closely related to the pathogenesis of many diseases, including the development of solid tumors, which determines their potential for use as clinical non-invasive biomarkers.

Research in this direction is presented in 5 publications that are in group B (2 belong to Q1, 2 to Q2 and 1 to Q4) and 1 publication in group E, summarizing the results of studies that present circular RNAs with their unique features as potential prognostic and diagnostic biomarkers in colorectal carcinoma (CRC). Radanova's team showed that the plasma expression levels of the four circRNAs: has\_circ\_0001445, hsa\_circ\_0003028, hsa\_circ\_0007915 and hsa\_circ\_0008717 in CRC patients are significantly increased, and hsa\_circ\_0001445 has prognostic significance for CRC metastases. Additional studies revealed that the circulating microRNA - miRNA-618 can be used as a prognostic biomarker in metastatic colon cancer (mCC) and that patients carrying the AC rs2682818 genotype have a reduced risk of colon cancer. In other studies, Radanova suggested that at least three microRNAs: miRNAs-SNPs – miR-146a rs2910164, miR-27a rs895819 and miR-608 rs4919510 are promising as prognostic and diagnostic biomarkers for CRC. To this line of research group  $\Gamma$  includes 4 more publications (1 belong to Q1, 1 to Q2 and 2 to Q3). Research is again related to the analysis and evaluation of biomarkers. Radanova found out that high levels of RIPK3 protein expression in metastatic colorectal carcinoma are associated with a lower risk of disease progression, better response to therapy, higher progression-free survival and better overall survival. In addition, the team showed the relatively high frequency of somatic mutations in the

KRAS gene in Bulgarian patients with colorectal cancer, namely 44.92%. The association between neutrophil/lymphocyte ratio (NLR) in patients with non-small cell lung carcinoma was evaluated. Patients with higher NLR levels and with sarcopenia before immunotherapy were found to be more likely to develop hyperprogressive disease subsequently and to have a shorter overall survival. And last but not least, Radanova is tracking the frequency and predictive value of PIK3CA mutations in Bulgarian patients with metastatic breast carcinoma. The study shows that PIK3CA mutations are frequent in the studied group of Bulgarian patients (HR+/HER2-) 29.2%. The presence of a mutation is associated with the presence of metastatic disease at diagnosis.

## **II. Molecular effects of C1q inhibition in patients with autoimmune diseases**

The complement system is an important part of the immune response. Its normal functioning is mandatory for the protection of the body from pathogens, as well as for the removal of immune complexes, apoptotic cells and cell debris.

The scientific output in this direction, in which Associate Professor Radanova is involved, are dissertation thesis for the acquisition of an educational and scientific degree "Doctor" (indicator A) and 3 publications (2 from Q2 and 1 chapter from the book) from group Γ. The main results in this direction are related to the assessment of the association of five single nucleotide polymorphisms (SNPs): rs665691, rs682658, rs172378, rs292001 and rs294179, found in the C1q gene cluster, with rheumatoid arthritis and its clinical and immunological characteristics. The minor G-allele and the GG genotype of rs172378 as well as the AA genotype of rs292001 were found to be associated with the risk of developing rheumatoid arthritis. Here, Radanova also presents a detailed protocol for the detection of plasma anti-C3 IgG autoantibodies, which recognize the active C3b fragment of C3 and have functional effects. They are found in about 30% of patients with lupus nephritis, correlate with disease severity, and have diagnostic and prognostic potential as biomarkers.

## **III. Pathological dysregulation of metabolism**

Metabolic syndrome is directly related to fatty liver disease, obesity and diabetes, which determines the relevance of this scientific direction.

The reports in this direction, with which Radanova participates, are 3 publications from group Γ (1 from Q1, 2 from Q2) and 2 with an impact factor, but outside the list of publications evaluated under the current competition (1 from Q2, 1 from Q3).

The results of fatty liver studies of rats on a high fructose diet (HFD) and S-adenosylmethionine (S-AdoMet) administration showed that the expression of hemoxygenase-1 (HO-1), the levels of malondialdehyde and SH-groups in liver as well as plasma triglycerides were significantly increased on HFD. S-AdoMet intake suppresses the increase in lipid peroxidation and triglyceride levels and is associated with a significant increase in hepatic HO-1 expression to prevent the development of fatty liver disease. The increased serum levels of cytokeratin-18 in the high-fructose diet group and their association with liver histological changes and biochemical parameters indicate the key role of apoptosis in the pathogenesis of high-fructose-induced liver injury, as well as the reliability of CK-18 as a biomarker for assessing liver damage in metabolic syndrome.

Separate from the metabolic syndrome, but related to metabolic dysregulation, are the results regarding the identification of 15 genes responsible for the production of dynein polypeptide chains, as well as factors for its assembly. Variants in these genes are associated with an increased risk of male infertility.

The last 2 publications in this direction are studies on the antioxidant and anti-inflammatory effect of the Varna sulfur mineral waters. Radanova and colleagues found that these waters led to a significant increase in total glutathione and thiols, with increased expression of  $\gamma$ -glutamylcysteinyl ligase and sICAM-1, indicative of improved redox status, and that mean plasma creatinine levels and high-sensitivity C-reactive protein (hs-CRP) decreased, and glomerular filtration rate (eGFR) and diuresis increased significantly.

**The contributions of these studies can be summarized as follows:**

1. A database of circular RNAs with oncogenic function in colorectal carcinoma was created.
2. An original interpretation of the mechanisms by which transcribed ultraconserved regions regulate gene expression is presented.

3. Two novel colorectal carcinoma circRNAs have been identified that distinguish patients in IV from patients in stage III disease, one of which has been evaluated with prognostic significance in metastatic patients.
4. The prognostic potential of a novel microRNA for colon carcinoma has been established.
5. Single nucleotide polymorphisms in miRNA genes have been found to be associated with the prognosis of patients with metastatic colorectal carcinoma and with the risk of developing the disease.
6. The predictive and prognostic role of RIPK3 in metastatic colon cancer was evaluated for the first time.
7. An assessment of the frequency of KRAS mutations in Bulgarian patients with advanced and metastatic colorectal carcinoma has been published for the first time.
8. For the first time, it has been shown that patients with high-NLR non-small cell lung cancer and sarcopenia who progress on platinum-based chemotherapy are at higher risk of developing hyperprogressive disease after immunotherapy as second-line treatment.
9. Data on the frequency of PIK3CA mutations in Bulgarian patients with metastatic breast carcinoma (HR+/HER2-) are presented for the first time.
10. A functional deficiency of C1q was discovered for the first time, indicating the critical role of functional regions located in the collagen stem region of the protein in maintaining tolerance to self structures.
11. The functional consequences of the binding of autoantibodies and hemolysis products to the globular domains of C1q have been established, which could explain the insufficient "clearance" of immune complexes and CRP-opsonized apoptotic cells in lupus nephritis.
12. For the Bulgarian cohort, an association between individual and nonequilibrium SNPs located in the C1q gene cluster was found with the risk of developing rheumatoid arthritis, and this association was not determined by plasma C1q levels.
13. A cytoprotective effect of S-adenosylmethionine on hepatocytes was established by increasing the expression of hemoxygenase-1 enzyme in states of fructose-induced disorder.
14. Serum levels of SC-18 (cytokeratin-18) have been found to correlate with histological and biochemical changes in liver damage and can be used as a biomarker for non-invasive assessment of its progression in metabolic syndrome.

15. The first meta-analysis evaluating the association between variants in dynein-related genes and male infertility was conducted. The study is the first to distinguish pathogenic variants in dynein-related genes from harmless variants. The study also pioneered the description of dynein-related genes as molecular targets for future studies of sperm motility problems.

### **Teaching activity**

As a qualified teacher, Associate Professor Radanova is also engaged in active teaching activities. She currently gives lectures in Biochemistry for students from the specialties "Dental Medicine", "Physical Therapist" and "Medical Laboratory Technician".

Contribution to her educational activity are the 2 published chapters of the book, as well as the co-authored university book and 2 university textbooks. Associate Professor Radanova was the supervisor of four doctoral students, three of whom have already successfully defended their theses.

The presented data characterize Associate Professor Radanova as a teacher with intensive teaching in the field of biochemistry and molecular biology, covering all aspects of this activity - from the development and teaching of the academic disciplines, through the writing of books and textbooks necessary for the training of students, to individual work with them. Associate Professor Radanova enjoys the name of a highly qualified and erudite teacher.

### **Conclusion**

I have the pleasure of personally knowing Associate Professor Maria Radanova. She is an established teacher and specialist with high professional qualifications, who develops active scientific activity in the field of biochemistry. Her scientific output is significant in quantity and quality, exceeding the requirements for awarding the academic position of "Professor" defined in the regulatory framework. She has extensive experience in leadership and teamwork, teaching doctoral students, competences and skills in shaping ideas, concepts and projects, as well as realizing scientific publications. This gives me the reason to confidently give my positive assessment and recommend to the Scientific Jury to elect Associate Professor Dr. Maria A. Radanova as a "Professor" in professional direction 4.3. Biological Sciences, with a scientific specialty "Biochemistry".



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**Reviewer:**

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**Prof. Jordan Doumanov DSc**