



Review

of Prof. Dr. Ivaylo Stefanov Stefanov, VMD, Faculty of Medicine, Faculty of Medicine, Burgas University "Prof. Dr. Assen Zlatarov", Chairman of the Scientific Jury of the competition for the academic position "Professor" under Order No. RD - 134 /12.04. 2024 of the Rector of the "Prof. Dr. Assen Zlatarov" University, Burgas.

Subject: Competition for the academic position "Professor" in the scientific specialty "Pathologoanatomy and Cytopathology", in the field of higher education 7. Health and Sports, professional area 7.1. "Medicine", announced in the Official Gazette, issue 13 /13.02. 2024 for the needs of Department of "Anatomy, Histology and Embryology, Pathology, Forensic Medicine and Deontology", Medical Faculty, "Prof. Dr. Assen Zlatarov" University, Burgas.

Materials for participation in the competition were submitted only by Prof. Dr. Maya Vladova Gulubova, MD.

The set of materials submitted by the candidate is in full compliance with the national regulatory requirements for holding academic positions and with the Regulations for the terms and conditions for acquiring scientific degrees and for holding academic positions at Burgas University "Prof. Dr. Asen Zlatarov".

I. Candidate's career profile

Professor Dr. Maya Gulubova graduated in 1983 at Medical Academy of Sofia, specialty "Medicine". She specialized in pathology in 1984 in Svishtov. In the same year, she began her professional career as a teacher and researcher in the position of " Assistant Professor " in the Department of General and Clinical Pathology at Higher Medical Institute - Stara Zagora. In the period 1993-2002, she held the position of "Chief Assistant Professor" at the Faculty of Medicine of Trakia University, after which she was promoted to the position of Associate Professor, and in 2013 she held the position of Professor. In 1989, she completed a specialization in liver pathology at the Moscow Medical Institute I "I. Sechenov". In 1999, she received the Educational and Scientific degree "Doctor" in the scientific specialty Pathologoanatomy and Cytopathology" after completing a PhD thesis on the topic "Role of sinusoidal cells Ito and pit cells in fibroplastic and neoplastic process in the liver ". Professor Dr. Maya Gulubova has extensive administrative experience covering the period 2004-2024, in the capacity of head of the Department of General and Clinical pathology, Forensic Medicine and Deontology and Dermatovenerology (2003-2023), deputy Dean for research at the Medical Faculty (2004-2007), Dean of the Medical Faculty (2007-2019) at the Trakia University, and current Head of the Clinic for General and Clinical Pathology at the "Prof. Dr. Kirkovich" University Hospital, Stara Zagora. The impressive administrative qualities of Prof. Gulubova found expression in the implementation of program accreditation of a number of medical specialties, accreditation of

doctoral programs (25 current, 16 in process, and 6 new ones). She has actively involved in the development of research activities in the Medical Faculty by assisting in the equipment of laboratories in Molecular Biology, Immunology, Biochemistry, Biophysics, Physiology, Molecular Pathology, Immunohistochemistry, Confocal Microscopy, as well as in the purchase of equipment for training and scientific activity at University Multiprofile Hospital for Active Treatment Prof. Dr Stoyan Kirkovich.

The scientific research activity of the candidate develops in several main morphological directions, which fully correspond to the field of higher education "7. Health care and Sports", professional area 7.1. "Medicine", scientific specialty "Pathologoanatomy and Cytopathology":

- Colorectal, rectal, gastric, endometrial, lung and thyroid carcinoma, with an emphasis on cytokines and immune cells - myeloid and plasmacytoid dendritic cells, T-helpers, NK and NKT cells using precise and modern methods.
- Liver pathology including hepatocellular carcinoma, liver metastases, hepatic sinusoids, biliary tract pathology, peliosis, integrins, adhesion molecules, extracellular matrix.
- Experimental diabetes with special attention on insulin-producing cells in bile ducts, pancreatic duct and liver.
- Tertiary lymphoid structures induced by tumor, infectious agents and autoimmune processes.

An important part of Prof. Dr. Gulubova's career development is her participation in the management and training of 22 doctoral students. She also participates in the training of specialists in the clinical specialty "General and Clinical Pathology". She won 4 research projects of the Ministry of Education and Science for Medical Faculty, corresponding to the scientific specialty "Pathologoanatomy and cytopathology".

The original scientific results obtained have been published in high indexed international journals.

II. General description of the submitted materials for the competition

Prof. Dr. Gulubova has submitted 21 articles in prestigious international and Bulgarian refereed journals for participation in the competition, 18 of them have an impact factor (IF= 22.385, and from 1984 to today the total IF of all publications is 106.006) and 2 with SJR, Quartiles: 2 with Q1, 4 with Q2, 13 with Q3, 1 with Q4. The extremely high H-index = 21 is an important indicator of the scientific significance of her scientific work. The candidate's personal contribution to scientific research is highlighted by her participation in 10 publications where she is the leading author, in 4 publications she is in second place, in 1 - in third place, and in the rest - in fourth and subsequent places. A list of 32 citations in foreign specialized journals was presented. The total number of citations at the moment - 1621 reflected in the SCOPUS database is impressive.

After being promoted to the position of "Professor", the candidate has participated in the implementation of 10 research projects corresponding to the scientific specialty "Pathologoanatomy and Cytopathology", 9 of which she has been the head of.

The significant contribution of Prof. Dr. Galabova in the field of pathology gives her a deserved place in the prestigious ranking of Stanford University (USA, California), which is based on a complex analysis of her scientific output and determines Prof. Dr. Galabova together with another 48 Bulgarian scientists as one of the best scientists in the world.

Evaluation of the candidate's scientific work

Prof. Dr. Galabova has attached a very well-prepared detailed report on the contributions from the scientific works, which accurately reflects her achievements in various scientific fields of important practical importance. The main scientific contributions of the candidate are related to:

I. Studies on bile ducts:

They include routine electron microscopy of endocrine cells and mast cells, light and electron microscopic immunohistochemistry in patients with extrahepatic cholestasis. The established tryptase, chymase VIP and SP immunopositive mast cells, adjacent to NSE positive nerve fibers and serotonin positive endocrine cells in the lower part of the common bile duct give reason to assume that they are involved in the regulation of motility, bile evacuation and hormonal secretion in the gastrointestinal tract. It has been established that in acute cholangitis, the tight adhesion of inflammatory cells to the vascular endothelium is mediated by adhesion molecules ICAM-1/LFA-1 and ICAM-1/Mac-1, ensuring the migration of primarily neutrophils to the focus of inflammation. Through light microscopy and ultrastructural immunohistochemistry, gastrin, somatostatin, secretin, serotonin, chromogranin and synaptophysin positive endocrine cells were determined in the lower third of the human choledochus, which is related to their participation in the regulation of physiological and pathological processes in this place.

Another part of the research is related to liver studies of extrahepatic cholestasis. The deposition of type III and type IV collagen in sinusoids and portal spaces in livers of patients with extrahepatic cholestasis was studied by light microscopy and ultrastructural immunohistochemistry. For the first time in the Bulgarian literature, information about the expression of the adhesion molecule ICAM-1 in the liver sinusoids in cholestatic hepatitis was given, and for the first time in the world literature, the expression of ICAM-1 on the cell membrane of Ito cells was documented, which means that these cells are involved in the transport of inflammatory cells through the space of Disse to hepatocytes. For the first time, the expression of type III and type IV collagen in the liver of reactive biliary hepatitis was investigated and an initial capillarization of the sinusoid was established.

II. Studies on liver sinusoids around metastatic and primary tumors by light and electron microscopic immunohistochemistry and flow cytometry.

Examination of mast cells in the liver around and in metastases showed increased numbers of these cells in extratumoral tissue, around liver metastases and in the metastases

themselves compared to control liver tissue. For the first time, electron microscopic differentiation was made between the granules of tryptase positive and chymase positive mast cells. The established changes in the sinusoids in the presence of liver metastases from various gastrointestinal tumors are an original contribution. Studies on extracellular matrix proteins and their integrin receptors show increased expression of tenascin and $\alpha 9\beta 1$ integrin, which are markers of perisinusoidal fibrosis and sinusoidal transformation. The deposition of tenascin in primary colorectal carcinomas and in simultaneously occurring liver metastases was investigated. Tenascin, fibronectin, laminin, collagen type IV, $\alpha 9\beta 1$, and $\alpha 5\beta 1$ integrins were found to be highly expressed in the noncapsular model, and patients with a capsule around liver metastases had longer survival. Original data on adhesion molecules, their ligands and some cytokines in hepatic sinusoids are presented. For example, it was found for the first time that VCAM-1 E-selectin adhesion molecules are expressed on the sinusoidal endothelium and on the endothelium of tumor vessels in sinusoids around metastases. In this place, the expression of the ligands of adhesion molecules LFA-1, Mac, VLA-4 on neutrophils and lymphocytes also increased.

Immunohistochemical and flow cytometric studies of the immune cells in the liver containing metastases from gastrointestinal carcinomas presented data on CD83+ - S100+ dendritic cells in the liver containing metastases from gastrointestinal tumors. Higher numbers of S100+ dendritic cells in metastases and liver sinusoids were found to correlate with longer patient survival. Flow cytometric typing of T lymphocytes (CD4+, CD8+, NKT CD3+/ CD56+, NK) was performed in a homogenate isolated from a human liver resected for metastasis. The immunohistochemical study of pit (NK/NKT) cells in human and rat liver allowed presenting original data on the stimulating effect of Polyerga on the proliferation and activation of rat NK/pit cells. Original data on the morphological features of human NK/pit cells in the liver of patients with primary malignant tumors of the gastrointestinal tract with and without metastases were presented.

Hepatic sinusoids were studied in other diseases, for example in patients with β thalassemia with mutations in codons 39, 8/9 and 110. Electron microscopic examination showed deposition of hemosiderin granules in hepatocyte, canal of Hering, Kupffer cell and sinusoidal endothelial cell.

The peptidergic innervation of the hepatic sinusoids was studied by immunohistochemical expression of SP, NPY, CGRP, SOM, SER in the liver of patients with primary malignant tumors of the gastrointestinal tract and liver cirrhosis. The importance of peptidergic innervation in the regulation of sinusoidal blood flow and in the regulation of Ito cell transformation, reduction of peptidergic nerves in portal spaces and septa in cirrhosis, involvement of somatostatin positive nerve fibers in increasing hepatocyte sensitivity to alcohol is highlighted for the first time. .

III. Immunohistochemical and genetic studies in colorectal carcinoma.

Immunohistochemical expression of GST-p in tumor tissue in primary colorectal carcinoma showed that when it was elevated, patients had shorter survival and resulted in

reduced efficacy of antitumor therapy. Studies were conducted to determine the genetic polymorphisms of the genes encoding the protein expression of GST-p. Other studies have identified microsatellite instability in colorectal carcinoma. Endocrine cells and enzymes involved in antioxidant defense against free radicals GST-p, SOD1, SOD2, and the cytokine VEGF in tumor tissue in primary colorectal carcinoma were investigated. In survival analyses, carcinomas containing endocrine cells have been shown to have a worse prognosis. An original contribution was the expression of enzymes involved in the antioxidant defense of GST-p SOD1 and SOD2 in endocrine colorectal carcinoma cells.

III. Studies of pathological processes in the uterus, prostate and bladder.

They can be divided into; studies on hypertrophy and carcinoma of the prostate gland, clinical and immunohistochemical studies on preneoplastic lesions of the cervix. On biopsy, it was found that HPV infection was most often associated histologically with flat papilloma, inverted papilloma, and exophytic papilloma. Tenascin immunoreactivity was found to be more prominent in the stroma surrounding solid tumor nests and weaker in the infiltrative tumor type, which was associated with a worse prognosis for patients. A significant contribution is that p16^{ink4a} can be successfully used to detect high grades of CIN. Microsatellite instability in endometrial carcinoma was investigated and found to be associated predominantly with the endometrioid histological type of carcinoma.

V. Immunohistochemical studies in gastric carcinoma.

They allow the distribution of immature (CD1a+ and S100+) and mature (CD 83+) dendritic cells to be determined. In patients with regional lymph node metastases, the number of CD1a+ and CD 83+ dendritic cells was greatly reduced. Patients with low CD 83+ dendritic cell counts and those expressing TGF- β had shorter survival. Increased HER2/new expression in tumor cells was observed, which correlated with shorter patient survival. VEGF expression in tumor cells correlates with the presence of distant metastases.

VI. Immunohistochemical studies in thyroid carcinoma.

The involvement of the Th2 cytokine TGF- β 1 and molecules from the TGF- β 1 signaling pathway Smad4, Smad7, TGF- β RII as well as dendritic cells in the tumor tissue of primary thyroid carcinomas has been studied.

VII. Rare cases.

Several rare cases were studied. A case of fibrosarcoma in the thyroid gland, a case of papillary cystadenoma with borderline malignancy and a high operative risk, a case of seronegative spondyloarthropathy complicated by HCV and a case of Churg-Strauss syndrome, a case of goblet cell carcinoid in the appendix and cecum, a case of heterotopic and gastrointestinal cyst located between the neck of the gallbladder and choledochal, three cases of hepatic peliosis.

The teaching activity of Prof. Dr. Gulubova includes conducting a significant number of hours of practical lessons and lectures for the period 1984-2024 in the following disciplines:

- "General and clinical pathology" for 3rd and 4th - year students, specialty Medicine.
- "Somatopathology and oncopathology" of 2nd-year students, specialty Social activities - full-time and part-time education.
- „Clinical pathology" of first-year students, specialty "Nurse" and specialty "Midwife"

Prof. Gulubova has a total teaching experience of 39 years, and her total annual workload varies from 360 to 480 hours.

The complex analysis of the teaching and research activity shows that the candidate has fulfilled the required criteria for occupying the academic position of "Professor", laid down in the Regulations for the terms and conditions for acquiring scientific degrees and for occupying academic positions at Burgas University "Prof. Dr. Assen Zlatarov", which are included in a group of indicators A, B, D, D and E. It is important to note that the points of the indicators for groups D and E significantly exceed the minimum required points.

III. Critical notes and recommendations

I have no comments on the presented scientific works and materials.

IV. Conclusion

The career development of Prof. Dr. Maya Gulubova, her scientometric indicators, the contributions of her scientific research activity fully correspond to the requirements specified in the Regulations for the terms and conditions for acquiring scientific degrees and for holding academic positions at Burgas University "Prof. Dr. Asen Zlatarov" regarding the academic position of "Professor".

The accumulated rich professional experience defines the candidate not only as a successful researcher but also as a leader of research teams, doctoral students and specialists.

For these reasons, I confidently give my positive assessment to Prof. Dr. Maya Vladova Gulubova and recommend to the members of the honorable Scientific Jury to support her selection for the academic position of of "Professor" in the scientific specialty "Pathologoanatomy and Cytopathology", in the field of higher education area of higher education 7. Health care and Sports", professional area 7.1. "Medicine" for the needs of Department of "Anatomy, Histology and Embryology, Pathology, Forensic Medicine and Deontology", Medical Faculty, University "Prof. Dr. Asen Zlatarov".

Signature:

19.06.2024r.

/ Prof. Dr. Ivaylo Stefanov Stefanov, VMD /