

by Prof. Dr. Ivan Yankov Dechev, MD

Head of Clinic of Urology, UMHAT "St. Georgi "EAD, Plovdiv

Head of Department of Urology and General Medicine, Medical University, Plovdiv

Subject: Review of dissertation thesis *"The role of NGF and BDNF and their receptors Trk-A, Trk-B, p 75 for the onset and metastasis of prostate carcinoma"* presented by Assoc. Prof. Dr. Nikolay Todorov Evtimov, PhD, for awarding the Doctor of medical sciences "under the specialty" Urology "

1. General presentation of the procedure and dissertation work

By order № P-109-662 dated 14.12.2018 of the Rector of the Medical University - Varna I have been appointed as an external member of the scientific jury and I have been appointed by the Scientific Jury meeting with a protocol 1 / 14.12.2018 to issue an opinion under the procedure. The presented dissertation work has a volume of 231 pages and includes own results and their discussion and a list of scientific papers (publications and scientific activity from participation in international and national scientific forums) in connection with the doctoral work. The structure of the dissertation is classic and the clinical material is stratified in two groups depending on the operative approach - open or laparoscopic radical prostatectomy for localized prostate carcinoma. A precise and correct comparison of the immunohistochemical parameters was performed in the two groups and in the experimental part the density of expression of selected neurotrophins and their receptors was studied separately for various important clinical and pathological characteristics of the primary tumor (prostate volume, oncological parameters such as pathological local Gleason histology differentiation, PSA value) as well as some anthropometric parameters of patients (age, weight, height, neck circumference, hip and waist) with diagnostic and prognostic value. The conclusions and contributions are summarized at the end of the dissertation thesis and are followed by a list of used literature.

Author's summary of his dissertation is in a volume of 112 pages and reflects in a synthesized form a complete dissertation work.

2. Actuality of the problem solved in the dissertation in scientific and scientific application

Prostate cancer is among the top ten malignant neoplasms in men. It is the most commonly diagnosed solid malignant tumor and the second most common cause of cancer-related mortality in men worldwide, which identifies it as a significant medical problem. The accumulated evidence in the scientific literature as well as the obtained own results convincingly suggest that the expression of NGF and BDNF and their specific TrkA, B receptors in the fatty (periprostatic, peri-rectal) tissue and the density of expression of these neurotrophins in carcinoma and benign prostate tissue is prognostic a marker for the type

of histological differentiation of the tumor and its clinical aggressiveness. The quest to find specific and characteristic morphological characteristics for histological typing and predicting the biological behavior of this process has been a complex and challenging task for decades. Knowledge in this area will scientifically and post-localized prostate carcinoma adjuvant treatment and outline the prospects for new treatment strategies and in particular for castrate resistant prostate carcinoma that in their integrity can substantially slow the progression of the disease and improve quality the life of this patient group and the outcome of the disease. That is why I find the topic of dissertation work, chosen by Assoc. Prof. Evtimov, interesting, up-to-date, practically oriented and at the same time provocative in scientific terms.

3. Degree of knowledge of the state of the problem and of the literary material

The literature review as a structure and content deserves a high degree of appreciation and demonstrates the author's excellent awareness of the issues studied. This knowledge is based on 273 literary sources, the majority of which have been published since 2000. At the end of the literary review, Assoc. Prof. Evtimov clearly outlines the white fields regarding the insufficiently studied role of NGF, BDNF and their receptors TrkA, Trk B and p75NTR the development of human prostate carcinoma, the expression of growth factors and their respective receptors in both stromal and cellular membranes in human prostate carcinoma and the role of periprostatic adipose tissue as a source of paracrine signaling NGF and BDNF.

The aim of the dissertation thesis is to establish and evaluate the immunohistochemical density of neurotrophin expression NGF and BDNF as well as their tyrosine kinase receptors Trk (TrkA, TrkB, p75NTR) that regulate stromal-epithelial interactions and their fundamental role in the occurrence and progression of prostate carcinoma according to the pTNM stage of the patient in the periprostatic, anterior pericardial adipose tissue and gland in prostate cancer patients, and the tasks to accomplish this goal.

In the context of comprehensive preliminary information, Assoc. Prof. Evtimov logically links the immunohistochemical localization of neurotrophins and their receptors into prostate (benign and malignant) and periprostatic tissues and the interaction of immunoreactivity with different parameters (clinical and pathological) of prostate carcinoma.

The hypotheses are expressed and confirmed that:

- NGF is expressed both in fat (peri-perctal and periprostatic) tissue and strongly in epithelial and stromal prostate cells, this expression is dependent on the PSA value and prostate spread in the prostate but is not expressed in the control sample.
- BDNF is expressed only in prostatic epithelial cells and poorly in adipose tissue; expression is extremely pronounced in areas of invasive prostate cancer and absent in benign prostate tissue glands in the prostate stroma and the control sample suggesting that the expression density of BDNF is a possible prognostic factor for tumor aggressiveness.
- BDNF is expressed extremely strongly in the microvascular and glial tissues (average BDNF microvascular expression is 76.8 microvolts), is associated with metastasis of solid tumors and is defined as a negative predictive indicator.

In conclusion, the combined assessment between NGF expression and BDNF expression and the three Gleason score, PSA, pTNM factors indicates that neurotrophin expression is enhanced by increases in serum PSA levels, Gleason score and pathoanamic stage, but expression of the receptors is different for NGF and retains its trend, and for BDNF it is the opposite.

In the light of these experimentally proven dependencies, reasonable conclusions can be made about the clinical aggressiveness and progression of the tumor, as well as about the likelihood of regional lymphatic metastases, which justifies the volume of pelvic lymph dissection.

I am of the opinion that the scientific work proposed by Assoc. Prof. Evtimov, devoted to the major problems for the NGF and BDNF and their receptors (TrkA, TrkB, p75NTR), is one of the first fundamental and in-depth studies in our country on modern oncology problems and is undoubtedly a contribution to our specialized medical literature. Dissertation is obvious evidence that urology is not just a craft but also a developing science.

4. Conformity of the selected research methodology with the aim and tasks of the dissertation

The balanced literature review and demonstrated competence, which analyzes the results obtained so far, have allowed the author to clearly formulate the purpose and the specific tasks of the dissertation. The 14 tasks that are set are tailored to the definition of the goal and actually contribute to its realization. Methodologically, the dissertation work is built correctly. The experimental methods used (a combination of routine histological and immunohistochemical methods) are well-chosen, reliable (no false-positive reaction is obtained and the structure of the test tissues is not disturbed) and adequate to the research tasks. The detailed experimental results found in the dissertation talk not only about their authenticity but also about the precision with which the methods have been developed, which is a guarantee of their reliability and repeatability by other authors. It is an impression and good knowledge of statistical methods of data processing. The breadth of the scientific knowledge shown by the author is also emphasized by the accurate analysis of the relationship between the studied growth factors and their receptors with a panel of anthropometric and clinical parameters.

The design of dissertation works greatly facilitates readers, especially those who are not familiar with the subject of this dissertation in depth, to understand and summarize for themselves the rich information they offer. The text is fascinating and, in the most accessible and interactive way, it outlines the main achievements in the theory and ideas of the author in an area that is difficult to understand for non-specialists. In this sense, the work has not only a great scientific but also educational value for those who want to get acquainted with and get into the topic related to the morphological aspects of neurotrophins and their specific receptors for the occurrence and metastasis of prostate carcinoma.

The experimental material of the study is sufficient in volume, well documented, which guarantees credibility of the results and leads to correct and reliable conclusions. A significant number of immunohistochemical characteristics have been found and proven to be used for the complex assessment of biological behavior and the prognosis of prostate cancer in each individual patient.

5. Contributions from dissertation work

Experimental studies presented by Assoc. Prof. Evtimov are the result of many years of scientific and publishing activity of the author in the field of intercellular communications as a science and practice. There are 13 contributions I agree with. Contributions are correctly stratified as contributions of an original national value and confirmatory contributions.

6. Assessment of the dissertation papers

The basis of the dissertation is 24 scientific efforts, published in the period 2012-2018, which cover the quantitative scientific indicators (not less than 10% full publications, actually printed in scientific journals) for the scientific degree Doctor of Medical Sciences "In accordance with the Academic Staff Regulations at the Medical University - Varna. The publications reveal the nature and complexity of the dissertation and emphasize the leading expert role of Assoc. Prof. Evtimov in the published research. He has participated in international and national scientific forums on urology issues that are publicly disclosed in the dissertation. There are 15 references to scientific papers by Assoc. Prof. Evtimov, which speaks of their scientific significance.

Conclusion

I highly commend the dissertation thesis presented to me by Assoc. Prof. Dr. Nikolay Evtimov. The modern morphological methods used, the comparative evaluations, the results analysis and the conclusions drawn, present to me an erudite and established scientist who has gone beyond the narrow subject of urology. The dissertation work fully complies with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRARSR), the Regulations for the implementation of the ZARRPB and the Regulations for the Development of the Academic Staff at the Medical University - Varna. It contains original scientific, applied and applied results obtained with the author's leading role.

On the basis of this analysis, I give a **positive assessment** of the dissertation work and I think it is reasonable to propose that **Assoc. Prof. Dr. Nikolay Todorov Evtimov** should acquire the scientific degree "Doctor of Medical Sciences" in the field of higher education 7. "Health and Sport" Strand 7.1. "Medicine" in the scientific specialty "Urology".

11 February 2019

Member of the Scientific Jury:.....

(Prof. Ivan Yankov Dechev, PhD)