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from

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on the thesis of the

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on topic:

THE ROLE OF NGF, BDNF AND THEIR RECEPTORS TrKA, TrkB AND p75NTR FOR INVASION AND METASTASISATION OF PROSTATE CARCINOMA

to award a degree

"DOCTOR OF MEDICAL SCIENCES"

Today, thanks to modern diagnostic methods, 90% of established prostate carcinomas are in the T1-T2 stage. However, there is a tendency for high risk for these patients from biochemical progression, metastasis and lethal outcome. Performing a histochemical assessment may relatively well improve prognosis for the outcome of the disease after the operative treatment. The assumed life expectancy and mortality in the coming years would allow individual choices of behavior in the post-operative period. In recent years, studies have been expanding on the correlation between obesity, prostate tumor volume and Gleason Score in prostate cancer patients. The mechanism by which overweight affects prostate cancer is still unclear. Most likely, the gland is affected by the carcinoma adipokines that regulate the tumor. A number of authors have explored the potential role of NGF and BDNF and the associated with them synthesis of neuroendorphins. The latter are released from periprostatic fattissue in patients with prostate cancer. The first described neurotrophin is NGF. It is mainly synthesized in the nervous system, but also in saliva, kidney and prostate. It plays an important role in the differentiation, growth and apoptosis not only in the nerve cells. It is necessary for the survival of sympathetic and sensory nerves.

Neurotrophins also include BDNF (Derived Neurotrophic Factor), Nevrotrophin-3 (NT-3) and Neurotrophin - 4/5. BDNF is mainly synthesized in the hypothalamus and the cerebral cortex. It is responsible for growth, differentiation of neurons and synapses and stimulation of axon

growth.

The major receptors for NGF are TrkA (Transmembrane Tyrosine kinase), p75 and p75 (LNGFR) (Low-Affinity Nerve Growth Receptor).

The discovery of NGF and BDNF initiated two new concepts in cell biology:

- Concept of Cell Growth Factors - differentiation and functions of different cell types are regulated by specific signal proteins called cell growth factors. Today they occupy a central place in the pathogenesis of a number of diseases.

- Concept of "neurotrophic theory" - effector cells secrete NGF, BDNF and other neurotrophins, that are transported retrogradely to the neuron nucleus. The genes responsible for the structural and functional biology of nerve cells are expressed there. Determination of Gleason Score is an important prognostic factor. It is a mandatory diagnostic indicator for staging of prostate cancer and determining the type of operative and conservative treatment.

There is a logical relationship between the expression density of proliferative neurotrophins and Gleason Score. Therefore, it is quite natural to seek a link between BDNF and NGF as participants in paracrine intracellular regulation, clinical stage and expression of the traceable neurotrophins.

In this respect are the studies of the author. The desire and the efforts of Assoc. Prof. N. Evtimov to pay great attention to this problem, which is relevant for the urological practice, is commendable. This deserves our positive assessment, because it is not the author's end in itself. Honestly and with inner conviction, we must appreciate his longstanding interest in the diagnosis and treatment of prostate cancer. Until now, our scientific literature lacks consistent scientific research and generalized results on the issue. In this sense, the present scientific work has an extremely significant scientific, practical and applied character.

The dissertation thesis, presented for review, is being developed for the first time in Bulgaria. It is written in 232 standard typographic printing pages, which include 44 tables and 58 figures, black and white, in chronological order in the text. The work has an information for the contributions. The bibliography includes 290 titles, including 27 in Cyrillic and 263 in Latin. All the Cyrillic alphabet titles are from Bulgarian authors. Authors are not listed in alphabetical order, but sequentially appearing in the text as required by the American Medical Association (AMA). This bibliography shows the author's high awareness of the problem. It is noteworthy that the majority of the cited literary sources have been in the last few years, making a good impression. The dissertation work is up-to-date and written in clear and professional language. In all chapters, the author's own opinion and attitude is related to the issues discussed.

The distribution of the material by chapter is as follows:

1. Introduction - 7 pages
2. Literary Review - 56 pages
3. Objective and tasks - 1 page
4. Material and Methods - 18 pages
5. Results - 74 pages
6. Discussion - 27 pages
7. Conclusions - 2 pages
8. Scientific contributions - 3 pages
9. Bibliography - 19 pages

Scientific publications on the thesis are 10 in referenced and 14 in not referenced journals.

Literature review is written professionally, profoundly, in detail and comprehensively. It consists of four sub-chapters.

In the first of them considered stromal - epithelial paracrine interactions that modulate the growth of the prostate. Serious and thorough attention is paid to studies of several authors, who established cell regulation in mesenchymal enlargement of the prostate. They performed immunohistochemical analysis of the cytoplasm of epithelial cells in the prostate acini. It was found that neuroendocrine cells produce neurotrophic growth factors. This confirms that local paracrine mechanism regulates prostate growth and differentiation. The author shows his personal opinion, attitude and vision in discussed problem.

In the second subchapter Assoc. Prof. N. Evtimov describes the role of NGF in the prostate. Studies in recent years have shown that the mediators of differentiation, proliferation and apoptosis were synthesized in periorgan fat tissue. He examines and draws attention to a number of growth factors and found that the least explored are NGF and BDNF. And last but not least, after examining the available literature he suggests, that BPH and prostate cancer are developing from one and the same disease, but the different manifestation of the disease is influenced by various genetic and epigenetic factors.

In the third subchapter the author makes a thorough review of neuroendocrine cells in the prostate. They are specific cells that regulate its hyperplasia under the influence of a number of growth factors such as EGF, IGF and FGF. It examines the assumption of some authors that individual growth factors of prostatic stromal cells stimulate proliferation of neoplasms in mice and men. Along with that discusses the experience of a number of contemporary authors and draws attention to the statement that neurotrophins determine programmed cell death of neurons.

In the fourth subchapter the author examines the neurotrophic receptors. He follows them up consistently. Especially important for urological practice are TrK (tropomyosin receptor kinase), and p75 receptor. Of interest are the studies of several authors of basal cellular architectonics of prostatic ducts, likely pathophysiological path of prostate cancer, the relationship tyrosine - kinase receptors and transmembrane molecules of the plasmalemma. Assoc. Prof. N. Evtimov scrutinizes and in details the hypothesis for development of prostate cancer and the mechanism of uncontrolled proliferation. He provides a selective list of adipokines - associated diseases and discusses the possible connection and pathogenesis between obesity and prostate cancer. And last but not least he pays attention to the predisposition genetic risk factors for its development and epigenetic changes in men. Everywhere his serious scientific approach is clear in the interpretation of scientific evidence from studies literature.

The literature survey is informative and balanced. It ends with an analysis and an emphasis by the author on the most important unsolved problems. The survey is supported by a large number of literature sources.

The aim of the thesis is formulated shortly, precisely and clearly. It is in line with the title of the evaluation study of immunohistochemical density of neurotrophins NGF and BDNF and their tyrosine kinases receptors. The author will document the expression of NGF and BDNF and their specific receptors as a system for paracrine regulation in prostate cancer and their likely role in the occurrence and metastasis of this disease.

The author sets 14 tasks that are described in details. They sound a bit cumbersome and difficult to some extent for the reader. On the other hand, they summarize in details his experience on the issue and respond to the questions posed in the literature review.

In chapter "Materials and Methods" Assoc. Prof. N. Evtimov follows up retrospectively the individual parameters of 257 patients. They were diagnosed with localized prostate cancer and radical prostatectomy performed in the Department of Urology at the Hospital "St. Anna", Varna between 2010 and 2016 year. Patients were divided into 2 groups according to the type of operation, the values of the PSA (20), TNM classification (pT2N0M0), staging of the tumor (G II) and Gleason Score (7).

He describes in details the clinical, morphological, immunohistochemical and statistical methods and data analysis. Data processing Assoc. Prof. N. Evtimov uses software package SPSS Version 07 and a great number of different statistical methods - variation, alternative, non-parametric and graphic analysis, coefficient of Wilcoxon - Wilks' Lambda and others. This ensures getting enough reliable information on the issue and realization of scientific research of the author in terms of its goals and objectives.

In chapter "**Results**" the author presents his research results, compare and discuss them and reaches appropriate conclusions. Clear desire of the author is to highlight and explain in more details the controversial and uncertainties in his investigations. In all discussed and unsolved problems is expressed his personal respect and suggests his opinion on their solutions.

Assoc. Prof. N. Evtimov performs a tremendous amount of work. He divides patients into two large main groups according to the type and approach of radical prostatectomy - 27 patients with laparoscopic access and 157 patients with open surgery. Patients of both groups were compared and analyzed sequentially according to the clinical parameters age, values of the PSA, the weight of the prostate gland, surgical criteria (duration of operation, intraoperative blood loss, pre- and postoperative complications, duration of urethral catheterization), average hospital stay. Presented is interesting and of great value comparative analysis of preoperative Gleason Score, the performed pTNM classification, histopathological evaluation of the prostatic tissue, the status of the resection line, the regional lymph nodes and others.

The author presents interesting results for the first time in our literature. He pays particular attention to his primary aim - to establish a correlation between the selected parameters and the expression level degree of neurotrophins. Very good impression makes the detailed comparative analysis of patients from the two surgical groups according to immunohistochemical parameters. Assoc. Prof. N. Evtimov presents interesting statistically reliable data from the correlation analysis between the expression density of BDNF, NGF, TrkA, TrkB, p75 and anthropometric parameters BMI, age, prostate volume, PSA, Gleason Score, clinical staging and grading of pTNM tumor. This makes it possible to perform a combined assessment of the above results, which is essential for the prognosis and treatment of prostate cancer in our country.

In chapter "**Discussion**" Assoc. Prof. N. Evtimov discuss in depth the two major groups of parameters tracked by him during the study - clinical and immunohistochemical. The scientific study has a significant practical and contribution character. He proves the correlation between BMI, prostate volume, PSA, Gleason Score and the expression density of neurotrophins and their combined use. This enables him to create a predictive model with a high positive predictive value. According to the author the combined evaluation allows him to define a group of patients

with low risk of lymph metastasis and therefore they do not require extended pelvic lymph node dissection.

Conclusions at the end of the thesis are 10 in number. They summarize the vast and profound work of the author.

The first four conclusions are on the correlation between anthropological characteristics of the patients (waist circumference, neck, hips and BMI) and change Gleason Score.

The fifth, sixth and seventh conclusions are on NGF expression in periprostatic and perirectal tissue and expression of BDNF in prostate epithelial cells, microvascular and glial tissues. The latter are associated with metastasis of solid tumors and is considered to be a negative prognostic indicator.

The eighth and ninth conclusions comment the high expression of TrkA and the low expression of TrkB in prostate carcinoma tissue.

The tenth conclusion focuses on the combined evaluation between the expression of NGF and BDNF and the three factors Gleason Score, PSA and pTNM and their positive correlation.

Scientific contributions of the thesis are 8 original and 5 confirmatory. No doubt they are important for urological practice and specialists, who deal with this problem. The most important of these original contributions are as follows:

1. For the first time in Bulgaria the author follows up the expression and density of NGF and BDNF and their receptors for use as a prognostic criterion for patients with prostate cancer.
2. For the first time is performed a correlation between the anthropometric characteristics of the patients and the expression of neurotrophins and their receptors in the prostate gland as well as a combined evaluation of their expression and the values of Gleason Score, PSA and pTNM.
3. For the first time is offered a possibility of using the density of the neurotrophins in the serum and urine of patients with prostate carcinoma as a prognostic marker for metastasis of prostate cancer.
4. For the first time is offered the use of neurotrophins as potential targets for the treatment of prostate cancer.

Along with everything mentioned up to here I want to strongly emphasize, that I am extremely satisfied with the final mode of the presented thesis.

CONCLUSION

Assoc. Prof. Nikolai Todorov Evtimov was born on 21/05/1960 in Varna. He is married with two children. In 1984 he graduated medicine at Medical University of Varna. Between 1985 and 1995 he worked consecutively as resident surgeon, Executive Director and Head of the Surgical Ward at the Regional Municipal Hospital in Tutrakan. Between 1995 and 2005 he worked as a plastic surgeon at the Clinic for Plastic surgery and burns at the Medical University, Varna and also as an urologist. Since 2005 until now he works as an urologist at the hospital "St. Anna", Varna and Clinic and Polyclinic of Urology at Martin Luther University, Halle, Germany. During this period he specialized twice in the University Clinic of Urology and Andrology, Salzburg, Austria. Since 1992 Assoc. Prof. N. Evtimov is board certified in surgery and since 1998 - in urology. In 2013 he defended a thesis on "Systemic inflammatory response in laparoscopic and classical radical prostatectomy in prostate cancer. Surgical and functional comparison" and acquire

scientific degree "doctor". On 10/06/2017 he was elected for Associated Professor at the Department of Surgical Medicine, Medical University "Prof. P. Stoyanov", Varna.

Assoc. Prof. Nikolai Evtimov is fluent in written and spoken English, German, French and Russian languages and has computer skills. He specialized in Germany and Austria in the field of laparoscopic and robot assisted urology.

He is a member of the Bulgarian Association of Urology. Actively participates with papers and presentations in numerous scientific events.

In conclusion I emphasize that the presented thesis of Assoc. Prof. N. Evtimov is really fundamental. He discussed extremely current medical and socially significant disease in men. The study is unique scientific and practical summary of novelties in the diagnostics and treatment of prostate cancer. The number of studied patients is enough to make scientifically and statistically reliable conclusions. Used clinical diagnostic methods, clinical material and complex approach in their interpretation are a guarantee of a good knowledge of the material and the important contributions of the author to this problem.

I had the opportunity and pleasure to personally follow up and monitor the scientific research and practical activities of Assoc. Prof. N. Evtimov over the years. His great practical experience and thorough scientific data processing are proof for the high quality of the thesis. These own contributions have scientific and applied value for urological practice at home.

My evaluation of the author's efforts and the results of the thesis is highly positive. All this gives me the right and moral grounds to recommend to the honorable members of the respected scientific jury to vote in favor and award Assoc. Prof. Nikolay Todorov Evtimov the degree of "**Doctor of Medical Sciences.**"

20/01/2019
Sofia

Reviewer:
(Prof. D. Mladenov, MD, PhD, DMSc)

