**Summary**

The dissertation for the degree

Doctor of science

in speciality "Urology"

of

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**The role of NGF, BDNF and** **their receptors TrkA, TrkB and p75NTR**

**for occurrence and metastasis of prostate carcinoma**

 **Introduction**

Prostate carcinoma is the most common diagnosis of malignant neoplasms among the male population.

Already enough evidence has been collected to show the presence of intracellular interactions between stromal cells and epithelial cells of the prostate gland. These proteins serve to connect and play an important role in the enlargement and the growth of tumor cells in carcinoma of the prostate gland.

The concept of intercellular interactions is of more than thirty years, but the demonstration of these substances, called neurotrofins becomes possible only in recent years. The main credit for this has the work of Rita Levi Montalcini 1987. These small proteins were first found in nerve cells, hence their name. Later their synthesis was found in other cells of the human body. These signal molecules play the fundamental role for life processes, such as cell growth, cell differentiation and apoptosis.

Neurotrofins activation is mediated by two classes of receptors in the cell. These are Trk neurotrophin receptors and receptor p75/ntr, later established that to belong to the Tumor Necrosis Factor receptor. NGF preferentially binds to TrkA, BDNF to TrkB while NT4/5, NT-3 binds to primarily TrkA and TrkB , but they are less specific. All neurotrofins are connected and activated with p75 ntr.

The original idea was probably borrowed from the properties of stem cells. Stem cells are undifferentiated cells with specific characteristics. In the fetal period, individual stem cells of various tissues differentiate into separate bodies. In sexually mature organisms, stem cells are found in different organs, as their function is most likely replacement of cells, as a result of their physiological exchange or pathological .

**The aim of this study is**

to present and assess the immunohistochemcal density of the expression of BDNF and NGF neurotrofins and tyrosine kinase receptors TrkA, TrkB and p75, which regulate epithelial-stromal interactions and their fundamental role in the occurrence and progression of the prostate cancer, according to the stage of the cancer patient in pTNM periprostate, front perirectal , adipose tissue and epithelial cells in patients with prostate carcinoma.

**Materials and methods**

During the period 2010-2016 in the Urology Clinic of Hospital “ St. Anna " Varna was carried out radical prostatectomy for localized prostate carcinoma of 257 patients. The patients were operated by two different techniques-laparoscopic and open approach.

The study is retrospective, monocentric on individual parameters, while erectile dysfunction is prospective.

The patients were divided in two groups depending on the PSA values<>20 nmol|ml pT2NOM0>< pT2N0M0, GII> < GII and Gleason score < > 7.

**Methods for demonstrating the expression of neurotrofins**

Tissue preparations were routinely treated for H&E and coloring peroksidaz/antiperoksidaz Immunohistochemistry with Polyclonal rabbit antibodies, producing of Santa Cruz Biotech.

Expression of NGF- Trk A, BDNF- Trk B is proved with secondary monoclonal antibody producing EnVision Flex Vis Syst; with DAKO has proved the expression of p75.

Density of expression of NGF and BDNF is established by means of

acount down of epithelial cells, associated with the antigen, in which the density is calculated on 20 fields. Determined is density of expression of the growth factors of material taken from patients with intraoperative prostate carcinoma.

257 patients were study with carcinoma of the prostate gland by the formula was set the volume of the sample and the assessment of the relative partition. Finally in the study, remained a group of 179 patients where radical prostatectomy was performed.

**Histological research methods**

The morphological diagnosis (typing, determining the depth of infiltration and vascular histological, grading and perinevral invasion) under examination material is fixed in 10% buffered formalin solution, as well as the material, taken for the study of rezection line. The results are examined in a laboratory to patoanatomy at University Hospital „ St. Marina "-Varna.

**Immunohistochemical research methods**

For the determination of immunohistochemical expression of BDNF and NGF neurotrofins and their receptors, TrkA,B and p75 used monoclonal antibodies of the company Dako En Vision , and standard immunohistochemical technique.

The density of the expression of neurotrofins and their receptors was determined visually semi-quantitative, in which were three degrees of expression.

Negative expression is marked with (0).

Positive expression, is marked with (+).

Medium strong expression is marked with (++) and strong expression with (+++).

A quantitative counting of cells was done under a microscope at 100 x magnification, as that area of the Visual field is 2 mm2. Density of expression in carcinoma tissue is compared against the power of expression in benigne prostatic tissue. We used the cells score with an area of 0.25 mm2. The count down was manual on the computer image,

**Statistical methods.**

Variational analysis – to test the normality of the distribution is used the test of Kolmogorov-Smirnov describing quantitative indicators with a normal or close to normal distribution.

Alternative analysis – a method for evaluating the frequency of occurrence and relative size in quality signs;

Wan Warden the method for evaluation of samples.

T-criterion for the evaluation of the hypothesis of the presence of a statistically significant difference between the parameters. The significance level used the zero hypothesis p < 0.05. A critical area for the following values of ( p) is a two-tailed distribution of the data.

Neparametrics analysis – comparing an empirically and theoretically expected distribution of the data. In testing of hypotheses about the failures of an accidental impact factor a test was used (Fischer, exact test) in the four fold tables and criterion 2 x in multiple tables. For comparing pairs of values in the groups was used nonparametrics analysis.

**Results**

**1. Comparison of patients in the two groups, depending on the clinical parameters.**

The patients were divided into two groups depending on the type of procedure as follo ws:

Patients are divided into two groups depending on the type of surgical procedure. In 27 patients were ELRP and in the RP – 157 patients. In the first group are patients with an average age 62.9 and the other – 64.8 g. Average weight of patients is 86 kg, respectively – 76 kg. Average height – 174.8 cm against 173 cm. Circumference of the neck – 40.9 cm, respectively, in the other group – 40 cm. Circumference of the hips – 102 cm to 104.2 cm. Waist – 99 cm, respectively – 90 cm. average values for PSA preoperativno – 7.9 (2.4 – 10.2), compared with 7.25 (4.4-11.3 ).

**2. Comparison of patients in both groups according to objective parameters**

For immunohistochemical survey patients were divided into two groups depending on the basic variables.

This was the main objective of our survey – to establish a relationship between the chosen parameters and the degree of expression of neurotrofins.

Of a total of 257 patients tested with proven localized prostate carcinoma patients were divided into two groups depending on the Gleason score, the value of PSA, the clinical stage, age of the patient .

The first group included 179 patients with Gleason score ≤ 7, PSA ≤ 20 G, I-II, c-2b TNM, age ≤ 60 years, BMI ≤ 25, volume of the prostate under 59 g. in the second group included 78 patients with Gleason score ≥7, PSA ≥ 20 , G III-IV, TNM c ≥ 2, aged ≥ 60 years, BMI ≥ 25, prostate volume over 59 g.

**Immunohistochemical parameters**

All patients were divided in two groups according to the age 60 years < > BMI 25 < > volume of prostate, 59 g < > pTNM stage II < > PSA values 20 nmol < >/ml and Grading G < > II. then perform analysis of the following dependencies:

2.1. Correlation analysis between the density of the expression of BDNF, NGF, TrkA, TrkB, p75 and 2 time anthropometric medical parameters.

 2.2. Correlation analysis between the density of the expression of BDNF, NGF, TrkA, TrkB, p75 and BMI.

2.3. Correlation analysis between the density of the expression of BDNF, NGF, TrkA, TrkB, p75 and the age of patients 60 years < >.

 2.4 correlation analysis between the density of the expression of BDNF, NGF, TrkA, TrkB, p75 and prostate volume 60 g < >.

2.5. Correlation analysis between the density of the expression of BDNF, NGF, TrkA, TrkB, p75 and PSA < > 10.

 2.6. Correlation analysis between the density of the expression of BDNF, NGF, TrkA, TrkB, p75 and Gleason score < > 7.

2.7. Correlation analysis between the density of expression. BDNF, NGF, TrkA, TrkB, p75 and pTNM. < > III

2.8. Correlation analysis between the density of the expression of BDNF, NGF, TrkA, TrkB, p75 and G < > II.

2.9. A combined evaluation was done between immunohistochemical expression of BDNF and NGF depending on the PSA values , cTNM ,Gleason score. The patients were divided into the following groups: depending on the values for PSA 10-20, PSA ≤ 10 > = 20, Gleason score ≥7 ≤, ≥ TNM T2M0N0.

**3.Correlation analysis between anthropometric and oncological parameters**

Our results indicate of the hips circumference and the Gleason score, p < 0.005, it means that if you change the circumference of the hips with 1 cm, respectively, Gleason score changes with the regression coefficient in 0.023 this is a weak dependence but positive with a tendency to increase. To increase the circumference of the neck with 1 cm Gleason score is increased by 0.39, which is also a positive trend.

**4. Correlation** **between density of expression of NGF, BDNF, TrkA, TrkB, p75 BMI ≥ ≤ 25**

With the rise in values of BMI increased expression of these neurotrofins, as for at 25 patients it is three +, as in the tissues located ventral and tissues situated perirectal, there is weak expression in a control sample of adipose tissue. When values of BMI > 25 kg/m2 for a total of 24 patients (Min – 25 kg/m2; Max – 34 kg/m2 Med – 28.2 kg/m2). In these patients the expression of NGF is highly positive. It is two plus (+ +) with values between 25-28 and three (+++) in BMI values over 28.

At high values of body mass index a highly positive expression is observed both in carcinoma tissue and cells with only benign prostatic hyperplasia . With averages of BMI of 25 kg/m2  we have the strongest expression in prostate tissue, placed rectally. In rising values of the BMI, the density of expression of this receptor decreases.

**5.Correlation analysis between the density of the expression of NGF, BDNF, TrkA, TrkB, p75 and prostate volume ≤ ≥ 60g**

In this group of patients the expression of NGF is strong throughout the studied material, with the exception of the control. In a study of the expression of BDNF in prostate weight 60 g < > this relationship is preserved and the value of the correlation is directly proportional to the volume of the prostate gland with increasing volume, expression of BDNF increases.

**6.Correlation analysis between the density of the expression of NGF, BDNF, TrkA, TrkB, p75 and PSA < > 10 nmol/mL**

There is expression of NGF both in adipose tissue and the tissue in carcinoma. It is weak in the hyperplastic tissue . Its density is not amplified and in PSA values are greater than 10 nmol/mL. In PSA values > 10 expression of NGF is retained, but decreases the expression of TrkA receptor . Expression of BDNF depending on the values of the PSA 10 nmoll/mL: At low values of PSA density of expression of BDNF is about 60% across the studied material from us; it reaches 100% in the case of values above 25 nmol/mL.

**6.Correlation analysis between the density of the expression of NGF, BDNF, TrkA, TrkB, p75 and Gleason score ≤ ≥ 7.**

With Gleason score rise increases the density of the expression of NGF.

Expression of NGF's (3 +) in the Gleason score (3 + 3 = 6) in periprostatic adipose tissue, suggests a more powerful production of this neurotrofin with the strengthening of the non-differential carcinoma tissue .

**7.Correlation analysis between the density of the expression of NGF, BDNF, TrkA, TrkB, p75 and clinical stage .**

Patients pTNM < > in pT1c tend to change towards a more undifferentiated stage, established in post-op pT 2c made post-mortem stage.

The distribution of stage in the group subject to open operating technique, was mostly in the neoplasm, but post operation stage pT1c in pT2c stage was observed. In this group the results obtained from the byopse study is established mean value with standard deviation1.24 .In the pathological stage pTÌc. In a study of preparations made post-mortem, the stage was changing in pT2c at an average value with standard deviation 1.42.

Post operation in the operated group in traditional 12 patients patoanatomical stage was established over the border in pT3a stage/ in the group subject to laparoscopic operating procedure, it is observed in 5 patients. Tracked were 49 patients in the Stage of which: < T2M0N0 18 patients in Stage 17 < T1cM0N0.

**8.The combined score of the expression of BDNF and NGF depending on the values of the Gleason score + PSA + cTNM**

With a strong expression of NGF and BDNF, Gleason score ≥7, PSA ≥ 20, T2M0N0 probability of biological progression of CaP is large and it would have to change the therapeutic decisions.

Conversely, patients with values of the Gleason score ≤ 7, PSA 20, TNM < < T2M0N0 and weak expression of NGF and BDNF in the case of localized CaP operative treatment will be enough. They don't need hormonal therapy or chemotherapy, x-ray therapy.

The combined score in these patients makes it possible to determine the Group of patients with a low risk of lymph metastasis, so they do not need advanced pelvic lymph nods dissection.

Because the study demonstrated that the expression of neurotrofins in patients with a high Gleason score is very strong (+ + +) not only in the tumor, and the nerves themselves, this means that the increased expression in the ganglion is sure and this factor is associated with increased risk of lymph nodes metastasis. It follows that the expression of NGF and BDNF is a predictor for development of lymph nodes metastasis.

**Inferences**

1. If the circumference of the waist change with 1 cm to the circumference of the hips, the Gleason score changes with 0.023, the coefficient of regression is 0.313. This is a positive addiction.

2.Increase the circumference of the neck with 1 cm, the Gleason score is increased by 0.39. Verification of adequacy is zero, which indicates an alternative value.

3.The correlation between the three indicators to anthropometric medical – tour of the waist, neck, hips, Gleason score is increased by 0.034. The trend is towards an increase in the patoanatomical stage, but this correlation is true only under the joint action of all three variables. Considered individually, these variables do not display according to the change of the pathological stage.

4.The relationship between the change of the Gleason score depending on BMI as a correlation is weak which is not statistically reliable. Due to the fact that the BMI is relation between weight and height that is relatively constant for life.

6. NGF is expressed at the same time in adipose periprostate, perirectal tissue. Also expressed in epithelial and high-stromal cells . NGF is weakly expressed in adipose tissue in PSA< 20 perirectal. NGF is expressed poorly in prostate tissue when we have less than 5% carcinoma research material. NGF is not expressed in the control sample.

7. BDNF is expressed in epithelial cells and low in fat tissue. It is extremely strong in areas with invasive prostate camcer and lacking in the glands with BPH. There is no expression of BDNF in tissue stromal and control sample. This implies that the density of the expression of BDNF would be prognostic factor for the aggressiveness of the process.

8. Strongly expressed in BDNF extremely in microvesicals and glial tissue. It connects with metastasis of solid tumors and is considered to be a negative prognostic indicator. The average number of microvescals expression of BDNF is 76.8 microvascular (median 66); a much larger number of 39.2 microvascular in the CP.

9.Strongly expressed in Trk A carcinoma prostate tissue, and low in rectal adipose tissue. There is no expression in prostatic tissue and adenoma tissue and in control samples has low expression in perirectal fat.

10.Trk A receptor in adipose tissue and in the ganglion and carcinoma fabric is weak. In carcinoma tissue expression of BDNF is the most weak and lacking expression of specific receptor Trk B.

11.The combined score between NGF and BDNF expression and the three factors Gleason score, PSA, pTNM indicates that expression of neurotrofins is enhanced with increased serum levels of PSA, Gleason score and patoanatomical stage. The expression of receptors is however different for NGF and retains the opposite tendency for BDNF .

When Gleason score ≥7, PSA > 20, cTNM > T2M0N0 and have strong expression of NGF and BDNF, the likelyhood of biological progression of CaP is large and it would have to change the therapeutic decisions.

Back to the values of the Gleason score ≤ 7, PSA < 20, TNM < c T2M0N0 . and weak expression of NGF and BDNF in these patients it pertains to localized CaP and operative treatment will be enough.

They will not have additional hormonal therapy, radiotherapy or chemotherapy. The combined score makes it possible to determine the group of patients with a low risk of lymph nodes metastasis, which is why they do not need advanced pelvic lymph dissection.

**Discussion**

It is important to note that after therapy is initiated with antiandrogen emergence of hormone-refractory resistance is inevitable .There are already new nosological group "resistant prostate cancer“, which occurs in patients where hormonal therapy is applied separately.

The combined hormone therapy and cytostatics is also limited in its capabilities. This leads to the emergence of new strategies, enabling the overcoming of resistance by attacking target with medication of endothelial epithelial and stromal cells with little or no inherited mutations .Specific strategies used for targeting angiogenesis in the CP, include blocking the proangiogenic factors such as NGF, BDNF, VENGF. This is introduced into practical systemic therapy, even in patients with metastatic prostate carcinoma (m CP). So now attempts are made to phase and sequence therapy at the advanced stage of the disease.

The choice of treatment should be modified according to molecular phase of hormone sensitivity, simptomatc and volume of metastatic disease and the history of the use of cytotoxic drugs. Then in the therapy enter growing factors or those molecules that affect the function of the high prevalence of effectory cells. In our study we wanted to study and possibly introduce yet another extremely sensitive and subjective method for diagnosis, prognosis, and why not as a therapeutic option in this common

among male population disease.

We proved correlation between BMI, volume of prostate, PSA, Gleason score and the density of expression of neurotrofins. We tried to explain the patoanatomical time of occurrence of prostate carcinoma. We introduced our personal assumptions about likely treatment through effects on growing factors or their receptors. We also know that combined Gleason score with PSA and clinical stages are used for prognostic criteria of the risk of progression .

The choice of treatment should be modified according to molecular phase of hormone sensitivity, symptomatic and volume of metastatic disease and the history of the use of cytotoxic drugs. Prostate carcinogenesis is manifested as a consequence of the activation of oncogenesis mutations of tumor suppressor genes and errors in DNA replication, which activate the tissue growth factors. The enzyme glutation-S-transferase-π (GST-π) has a major role in the development of prostate carcinoma, as this enzyme is "cell cleaner." It protects the cells through the conversion of free radicals into harmless water soluble products. It is available in normal cells and nearly-misses in high carcinoma cells. The absence or systematic destruction of GST-π increases the risk of development of CaP.

**Conclusion**

Target therapy is increasingly used for the treatment of neoplastic processes. Use of inhibitors of growing factors is rapidly becoming a promising therapeutic strategy in various solid tumors. In our study, we assume that the expression of NGF and BDNF neurotrofins, specific receptor TrkA and TrkB also would contribute to the accurate diagnosis and subsequent behavior in the therapy of the disease.

The strong expression of NGF and BDNF is probably related to the invasive and neoplastic processes. Increased expression of neurotrofins would also suggest increased proliferative activity.

To future prospects we discussed and the possibility to detect these neurotrofins in urine by the method of massaging the prostate and exfoliation of the prostate cells in urine ,and then with monoclonal antibodies to detect the density of the expression of NGF and BDNF.

Study on serum levels of NGF and BDNF could also contribute to early diagnosis and follow-up of prognostics of CaP.

Already several markers are available for potential therapy target: tyrosine-kinazes receptor, EGFR, angiogennics, VEGF, target P13K mTOR and prostatospecific membrane antigen.Observations support the growing focus on BDNF and NGF as therapeutic opportunity.

 Probably the creation of molecules, blocking receptors of BDNF and NGF, would constrain the development of neoplastic processes.