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Original article

IMMUNOHISTOCHEMICAL EXPRESSION OF NGF/TRKA AND BDNF/TRKB IN TUMOR PARIENCHYMA AND PERIPROSTATIC ADIPOSE TISSUE, DEPENDING ON THE PATHOANATOMIC STAGE OF PROSTATE CANCER

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ABSTRACT

Introduction: For more than 30 years, the idea of inhibiting growth factors has taken over the minds of different generations of scientists since the progression of the neoplastic process is determined by uncontrolled proliferation differentiation and apotosis and the metastasis of the neoangiogenesis process.

The aim: of this work is to show the density of immunohistochemical expression of NGF/TrkA and BDNF/TrkB A Trk B in tumor parienchyma and periprostatic adipose tissue. The study ability is to use it as a prognostic indicator for metastasis of prostate cancer.

Material and methods: During the period 2018-2022, 184 patients were diagnosed with radical prostateectomy on the occasion of localized prostate cancer with PSA < or > 20 nmoll/ml, Gleason< or >7, G< or >II. study of the density of expression besch determined by a semi-quantitative method and was established by counting epithelial cells associated with a monocronal antibody, calculated on 20 fields.

Results: The study showed expression of Trk receptors in epithelial cells in all tissue samples. NGF/TrkA has poor expression in adipose tissue in areas perirectally and ventrally in the pathoanatomical stage G1, and it becomes moderately positive as the process of undifferentiation progresses. The same tendency is observed in the expression of neurotrophin in prostate-epithelial cells.

Conclusions: The increased density of NGF, as well as the preoperative determination of the expression of BDNF and TrK a,b receptors in the pathoantomic preparation, can be used as a negative prognostic indicator of the aggressiveness of the CaP tumor, as well as its possible metastasis.

Keywords: pathogenesis of prostate cancer, immunohistochemistry, growth factors, NGF, BDNF,

INTRODUCTION:

Prostate cancer is the most common diagnosis of malignant neoplasms in the male population. With age, the possibility of developing prostate cancer increases. [1] We know that all growth factors can be synthesized by various mature and embryonic cells. [2]. In addition to EGF, other growth factors are isolated. Fibroblast growth factor (FGF), transforming growth factor (TGF-á.â) EGF, and bFGF promoting cell division [3] are separated from the ventral part of the prostate while transforming growth factor (TGF-beta) inhibits proliferation (Nikajaer et al. 2012). The initial lesions in the prostate are due to fibromuscular nodes. Therefore, in order to clarify the cause of BPH and the formation of lesions in malignant degeneration, efforts should be directed to studying changes in the interaction between stroma and epithelial cells of the prostate gland [4-6]. While the role of EGF, BFGF, KGF, TGF-beta has been studied, no studies documenting the expression of NGF and BDNF in both the prostate and the main paracrine organ in the human bode–adipose tissue – have been described in the literature [6-8].

We know that all growth factors can be synthesized by various mature and embryonic cells. In the prostate, the main growth factor is the epidermal growth factor [9]. Prostate-specific antigen is produced only by the epithelial cells of the gland, so in epithelial hyperplasia, serum PSA levels are increased in contrast to stroma hyperplasia since epidermal growth factor, which is directly influenced by DHT (dehydrotestosterone) directly induces cell proliferation and differentiation of epithelial prostate cells [10-12].

From the accumulated literature on the expression of NGF and BDNF in both the prostate and the main paracrine organ in the human body – adipose tissue, there are very few studies. Based on this, we suggest that nerve growth factor (NGF) should play a fundamental role in the pathogenesis of prostate cancer.

We propose that BPH and PC is a development of the same disease, but the different manifestation of the disease is influenced by different genetic and epigenetic factors. Nerve growth factor – as a protein secreted by stro-

mal cells and is responsible for paracrine regulation of epithelial tumor of the prostate gland *In vitro.* In addition, relevant NGF receptors are detected on the epithelial cells of the beak prostate *in vivo* and may be representative of a simple, specific form, the production of which is determined by a specific gene. It is logical to assume the dependence between receptors and the role of NGF, which is secreted by stroma cells of the prostate and their function in paracrine regulation for both the enlargement of the prostate in BPH and the occurrence of prostate carcinoma.

NGF is the first known neurotrophin that plays a role in nervous system development [12-14]. It stimulates the proliferation differentiation and suppresses the apoptosis of the nerve cell [15-16].

Neurvotrophins are activated through two classes of receptors located on the complement of the membrane called Trk receptors and panneorotrophin receptor p75 ^{ntr}. NGF preferentially binds to the Trk A receptor, but BDNF binds to Trk B. All neurotrophins bind with panneutrophen receptor p75$^{ntr.}$

The role of neurotrophins in the following neoplastic diseases has been directly proven. Thyroid carcinoma, prostate carcinoma, melanoma, myeloma disease, pancreatic carcinoma, ovarian carcinoma and hepatocellular carcinoma [16-20].

The AIM of this work is to show the density of immunohistochemical expression of NGF/TrkA and BDNF/TrkB A Trk B in tumor parienchyma and periprostatic adipose tissue, depending on the pathological stage of the disease and to study its ability to use it as a prognostic indicator for metastasis of prostate cancer.

MATERIAL AND METHODS :

During 2018-2021. on 184 patients underwent radical prostateectomy in case of localized prostate carcinoma with PSA < or > 20 nmoll/ml, Gleason< or >7, G< or >II. Patients were operated on two techniques: 151 conventional and 27 LRPVE. Tab. 1.

In all patients, the expression of NGF, BDNF,p75 and their TrK receptors in periprostatan adipose tissue and prostate gland by rabbit polyclonal antibodies production of (Santa Cruz Biotech) for NGF, BDNF, TrkA was examined, but for p75 is with mouse monoclonal antibodies (DAKO) St. Barbara.

The density of expression was determined by a semiquantitative method, which was established by counting epithelial cells associated with antigens, in which the density was calculated on 20 fields. This is a modified method of Habib F, Chicholm G. Department of Surgery/Urology Western General Hospital Edinburgh Scotland. (table 1)

Table 1. Deviding the patients into groups:

Patient groups:	Group I (Gleason score 7, PSA<20, G I-II, cTNM 2b-c, age<60)	Group II (Gleason score >7, PSA>20, G III-IV, cTNM>2c, age>60)	P
	76	75	
Number of patients (257)	179	78	
Average age	62.9 (42 - 74)	64.8 (52 - 76)	
Weight	86 kg	76 kg	
Height	174.7 cm	173 cm	
Average circumference of the neck	40.9	40	
Average circumference of hips	104.2	102	
Average circumference of the waist	99	90	
Average value of PSA	7.9 (2.4 – 10.2)	7.25 (4,4 - 11,3)	
Average value of Gleason score	5,7	8	
Preoperative clinical stage			
T1a	0	6	
T1c	79	75	
T2a	14	12	
T2b	7	7	
Average time of the duration of surgery in min	180 (120 - 240)	120 (80 - 190)	< 0.05
Blood loss/ml	200 (100 - 700)	550 (200 - 1900)	< 0.05
Transfusions %	3%	9%	
Complications %			

Rectum leisure	1,8	1,6	
Lymphocele	3,2	2,9	
Suporation	3,1	3,4	
Revision	1,25	2,5	
Duration of the cateterisation in days	8,9	10,2	< 0.05
Use of analgetics	33	35	
Average hospital stay	9,4	11,2	
Hystological parameters			
Average weight of the prostate in grams	37 (18 - 72)	42,3 (20 - 120)	
Average score of Gleason score	6,4	5,7	
Pathological stage %			
pT2a	16%	19%	
pT2b	27%	22%	
pT2c	23%	25%	
pT3a/b	33%	34%	
Positive regional lymph knots %	0	1,67 %	
Positive resection line %			
pT2a/b/c	9,8 patients	12,6 patients	
pT3a/b	29 patients	31 patients	

RESULTS AND DISCUSSION:

I. Correlation analysis between the expression densities of BDNF, NGF, TrkA, TrkB, p75 and clinical stage pTNM < > II

49 patients were in Stage < T2M0N0 18 of which were in Stage < T1cM0N0; 17 in Stage < T2aM0N0; 14 in Stage < T2bM0N0. In the other group were a total of 26 patients: 17 in stage < cT3aM0N0; 9 in stage < cT3bM0N0. Expression of NGF at Stage < T2M0N0 is enhanced with the change in the pathoanatomical stage. The density of expression in the perirectal prostate tissue relative to the periventral tissue is distinct. The opposite is the dependence of BDNF expression and its specific receptor TrkB. In BDNF, depending on the change stage < > T2M0N0, the dependence of correlation is the opposite.

TrkA expresses strongly in differentiated processes and decreases in undifferentiated patients. TrkB stage < > T2M0N0. In this dependence, the correlation value is reversed; in undifferentiated tumors, TrkB expression is absent. (table 2), (table 3).

Table 2. The expression of NGF depending of the stage < > T2M0N0

Number of patients	**Patoanatomical stage under T2M0N0**	**Expression of NGF in Adipose tissue**			**Expression of NGF In prostate**			
49		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
18	cT1cN0M0	2	2	0	2	3	3	3
17	cT2aN0M0	2	2	0	2	3	3	3
14	cT2bN0M0	3	3	0	2	3	3	3
Number of patients	**Patoanatomical stage above T2M0N0**	**Expression of NGF in Adipose tissue**			**Expression of NGF In prostate**			
26		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
17	cT3aN0M0	2	2	0	3	3	3	3
9	cT3bN0M0	3	3	0	3	3	3	3

Table 3. Expression of BDNF in dependence of the stage <> T2M0N0

Number of patients	Patoanatomical stage under T2M0N0	Expression of BDNF in Adipose tissue			Expression of BDNF In prostate			
49		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
18	cT1cN0M0	2	2	0	3	3	3	2
19	cT2aN0M0	2	2	0	3	3	3	2
14	cT2bN0M0	2	2	0	3	3	3	3
Number of patients	**Patoanatomical stage avove**	**Expression of BDNF in adipose tissue**			**Expression of BDNF in prostate**			
26		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
17	cT3aN0M0	2	2	0	2	2	2	2
9	cT3bN0M0	3	3	0	3	3	3	3

Immunohistochemical analysis of normal prostate and biopsy material from patients with CaP showed expression of Trk receptors in epithelial cells in all tissue samples. They are localized in the epithelial components of the ductus, basal and lumen cells. Analyses show that there is expression of Trk A in basal ductal cells in normal prostate and no expression of Trk B.

To determine the role of the Trk family of receptors for prostate carcinoma, it is necessary to approach and analyze the expression of Trk receptors in stromal smooth muscle cells in a normal prostate and one with carcinoma. There is an expression of only Trk C in a normal prostate, and in prostate carcinoma there is an expression of Trk A and TrkB receptors (fig.1) (table 4).

Fig. 1. Expression of BDNF adipose tissues and vessels

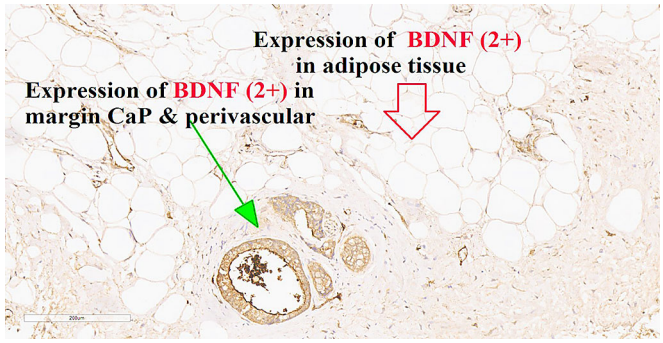

Table 4. Expression of TrkA and TrkB in correlation of stage < > T2M0N0 TrkA

Number of patients	Patoanatomical stage under T2M0N0	Expression of TrkB in adipose tissue			Expression of TrkB in prostate			
49		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
18	cT1cN0M0	0	0	0	2	2	2	3
17	cT2aN0M0	0	0	0	2	2	2	2
14	cT2bN0M0	2	2	0	3	3	3	3
Number of patients	**Patoanatomical stage above T2M0N0**	**Expression of TrkB in adipose tissue**			**Expression of TrkB in prostate**			
26		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
17	cT3aN0M0	3	3	3	3	3	3	3
9	cT3bN0M0	0	0	0	0	0	0	0

II. Correlation analysis between the expression density of BDNF, NGF, TrkA, TrkB, p75 and pathological stage Grading d" e" GII

Expression of NGF in Grading < G2: 43 patients were included, of which 19 were with Grading G1 and 34 patients with G2. In the group with Grading > G2 fell 17 with stage G3. From the analysis presented in Tab. 4, 42 it can be seen that NGF has weak expression in adipose tissue in the areas located perirectally and ventrally in pathoanatomical stage G1, and it becomes moderately positive as the indifferentiation of the process progresses. The same trend is observed in the expression of neurotrophin in prostate-epithelial cells. It is weak in highly differentiated tumor cells, intensifies to moderate in G2, and is highly positive in stage G2, G3 (table 5), (table 6), (table 7).

Table 5. Expression of NGF in the correlation of Grading < > G2

Number of patients	**Grading ≤ G2**	**Expression of NGF in adipose tissue**			**Expression of NGF in prostate**			
43		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
19	G1	1	1	0	1	1	1	0
34	G2	2	2	0	3	3	3	3
Number of patients	**Grading > G2**	**Expression of NGF in adipose tissue**			**Expression of NGF in prostate**			
		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
17	G3	2	2	0	3	3	3	3

Table 6. Expression of BDNF in correlation of Grading < > G2, TrkA and TrkB Grading < > G2.

In dependence of the value of the correlation is weak and reverse.
BDNF Grading < > G2. In this dependence, the value of the correlation is moderate in < G2 and strong in > G3

Number of patients	**Grading ≤G2**	**Expression of BDNF in adipose tissue**			**Expression of BDNF in prostate**			
43		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
19	G1	2	2	0	2	3	2	2
34	G2	2	2	0	3	3	3	3
Number of patients	**Grading > G2**	**Expression of BDNF in adipose tissue**			**Expression BDNF in prostate**			
		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
17	G3	2	2	0	2	2	2	2

Table 7. Expression of TrkA and TrkB depending on Grading < > G2; BDNF Grading < > G2. In this dependence the correlation value is moderate at < G2 and strong at > G2, 3

Number of patients	**Grading ≤G2**	**Expression of TrkA in adipose tissue**			**Expression of TrkA in prostate**			
43		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
19	G1	1	1	0	2	2	2	2
34	G2	2	2	2	3	3	3	3
Number of patients	**Grading > G2**	**Expression of TrkA in adipose tissue**			**Expression of TrkA in prostate**			
		M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
17	G3	2	2	0	2	2	2	2

TrkB

Number of patients	**Grading ≤G2**	**Expression of Trk B in adipose tissue**			**Expression of Trk B in prostate**			
43		Ì.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH
19	G1	0	0	0	2	2	2	3
34	G2	2	2	0	3	3	3	3
Number of patients	**Grading > G2**	**Expression of Trk B in adipose tissue**			**Expression of Trk B in prostate**			
	M.PV	M.PR	M.K	P.PV	P.PR	Ca.P	BPH	
17	G3	3	3	3	3	3	3	3

CONCLUSION:

1. Expression of NGF in Stage < > T2M0N0 and Grading > G2 NGF is expressed simultaneously in adipose perirectal and periprostatic tissue, expressed strongly in epithelial and stromal-prostate cells. NGF expresses poorly in adipose tissue at PSA < 20, located perirectally. NGF expresses poorly in prostate tissue when we have less than 5% carcinoma in the material under study. NGF was not express in the control sample.

2. BDNF expression depending on stage < T2M0N0 and Grading < G2. BDNF is expresses only in prostate epithelial cells and poorly in adipose tissue. It is extremely strong in areas with invasive prostate cancer and is absent in the glands with BPH. There was no BDNF expression in stromal tissue and control sample. This suggests that BDNF expression density would be a prognostic factor in the aggressiveness of the process.

3. BDNF expression depending on stage>T2M0N0 and Grading >G2. BDNF expresses extremely strongly in microvascular and glial tissues. It is associated with the metastasis of solid tumors and is considered a negative prognostic factor. The mean number of microvascular BDNF expression was 76.8 microvessels (median 66); a much higher number of 39.2 microvessels compared to BDNF expression in BPH tissues.

4. Grading > G2 TrkA expresses strong, and in Grading d" G2 TrkA expresses in prostate carcinoma tissue, located rectally and weakly in adipose tissue. There was no expression in BPH prostate tissue, and the control had weak expression in the perirectal adipose tissue.

5. Grading > G2 TrkB receptor in adipose tissue is absent, and in ganglion and carcinoma tissues, expression is weak. In carcinoma tissue, BDNF expression was weak Grading d" G2 weak and lacked expression of its specific receptor TrkB.

In conclusion, the increased density of NGF, as well as the preoperative determination of the expression of BDNF and Trk A, Trk B receptors in the pathoantomic sample, which is observed in our material, can be used as a negative prognostic indicator of the aggressiveness of the PaC tumor, as well as its possible metastasis. This immunohistochemical analysis can also be applied to the TRU-Cut biopsy material and is an indicator of the aggressiveness of the tumor process. This is established by the increased expression of the Trk family. This allows planning and adequate therapy, provision of nerve-sparing surgery for radical prostatectomy, a secure plan of surgery. The development of standardized models for the treatment of patients and prediction of results is determined by immunohistochemical tests such is the Stockholm3 test, is blood and combines biomarkers, clinical variables and genetic markers the risk of development of prostate carcinoma. They are based on the combination of disease course and standardized clinical and pathoanonotmic parameters, such as preoperative PSA, Gleason score. In this way, we can make predictions of life expectancy.

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