Complexity of Neural Component of Tumor Microenvironment in Prostate Cancer

Aleksandra Sejda^a Dawid Sigorski^b Jacek Gulczyński^c Wojciech Wesołowski^d
Joanna Kitlińska^e Ewa Iżycka-Świeszewska^c

^aDepartment of Pathomorphology, Collegium Medicum, University of Warmia and Mazury, Olsztyn, Poland;

^bDepartment of Oncology, Collegium Medicum, University of Warmia and Mazury, Olsztyn, Poland; ^cDepartment of Pathology and Neuropathology, Medical University of Gdańsk, Gdańsk, Poland; ^dEL-PAT Pathology Co, Elbląg, Poland; ^eDepartment of Biochemistry and Molecular and Cellular Biology, Georgetown University Medical Center, Washington, DC, USA

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contribution of neoplastic cell interactions with the nervous system and the neural TME component, also to search for potential therapeutic targets in malignant tumors such as PC.

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Abstract

The tumor microenvironment (TME) plays an essential role in the development and progression of neoplasms. TME consists of the extracellular matrix and numerous specialized cells interacting with cancer cells by paracrine and autocrine mechanisms. Tumor axonogenesis and neurogenesis constitute a developing area of investigation. Prostate cancer (PC) is one of the most common malignancies in men worldwide. During the past years, more and more studies have shown that mechanisms leading to the development of PC are not confined only to the epithelial cancer cell, but also involve the tumor stroma. Different nerve types and neurotransmitters present within the TME are thought to be important factors in PC biology. Moreover, perineural invasion, which is a common way of PC spreading, in parallel creates the neural niche for malignant cells. Cancer neurobiology seems to have become a new discipline to explore the

Introduction

Research on the interactions between tumor cells and the surrounding stroma is now being conducted for numerous cancer types, not only for better understanding their role in carcinogenesis and tumor growth but also as promising and attractive therapeutic option [1–3]. The involvement of stroma in cancer development and progression was first mentioned in the early 1970s [4]. It is currently widely recognized that tumor stroma or tumor microenvironment (TME) plays a crucial role in cancer biology. Consequently, this phenomenon has been incorporated into the “hallmarks of cancer” [5, 6]. Stroma can have suppressing and/or promoting effects in solid tumors and also hematogenic neoplasms [7, 8]. TME consists of the extracellular matrix (ECM) composed of

structural, specialized proteins (collagen, elastin, fibrillin, fibronectin elastin) and stromal cells such as cancer-associated fibroblasts (CAF), specialized mesenchymal cell types (adipocytes, myogenic cells), mesenchymal stem cells, immune cells, endothelial cells as well as extravasated blood cells [2, 9]. TMA includes also exosomes – the vesicular extracellular transporters of microRNAs, mRNAs, DNA fragments and other functional or signaling particles which are secreted by fibroblasts, endothelial cells, neuronal cells, immune cells and cancer cells [2, 10]. A number of cytokines, growth and ECM-modulating factors are secreted by stromal cells to communicate with each other and with cancer cells, driving diverse feedback responses between disarrayed compartments of neoplastic tissue [11, 12]. Cancer stroma development might be compared to wound healing and repair or regeneration. However, during normal healing, proliferation, migration and ECM modifications are self-limited and underlay multistep control [1, 13]. In both situations, fibroblasts must be recruited and activated following ECM remodeling, inflammatory response, and angiogenesis stimulation. In cancer, reactive stromal cells develop a specific population that can exert prosurvival signals, increasing the motility and invasiveness of neoplastic cells, and modulating gene expression [14]. Regeneration and cancer share some similarities also in regard to the stimulatory role of nerves within the microenvironment, since nerves and individual axons infiltrate the blastema and are necessary for tissue growth and remodeling supervision [15]. During wound healing, nerves sprouting from preexisting nerve fibers form a neural network within the microenvironment [16]. The neural tissue elements, such as axons and neurons of different types, are important components of TME creating specific intra/peritumoral neural milieu [17–20]. The knowledge on neural component of TME function and significance is still low but neurobiology of cancer has become a new discipline in oncology.

Both clinical and in vitro experiments showed that nerve fibers found within and around the tumor mass release neurotrophins, transmitters and neuropeptides directly acting on receptors expressed by cancer cells and modulating signaling [19, 21]. The nerves are also involved in angiogenesis and inflammatory/immune response. Activated sensory nerves have the ability to send proinflammatory signals to the central nervous system, where in turn neuromediators influencing local immune cells are released. Immune system regulation by neural tissue is supported mainly by the β -adrenergic pathway. In lung and breast in vitro cancer models, stimulation of

β -adrenergic receptors resulted in increased metastatic potential of cancer cells among others via NK-cell, macrophages signaling or osteoblast stimulation [22]. Moreover, sympathetic nervous system regulates pathological gene expression in human tumors leading to DNA damage repair inhibition, oncogene activation, apoptosis and anoikis suppression [23]. Proper innervation is critical for tumorigenesis as shown by experimental studies and clinical data. For instance, vagotomy reduces the risk of gastric cancer [24]. Direct invasion of cancer cells into neighboring tissues precedes lymphatic and hematogenic metastases. The distinct way of such a spread is perineural invasion (PNI), which is a neoplastic invasion of nerves and related histological structures. PNI is not only the form of tumor infiltration but it also creates the neural TME and tumor promoting perineural niche [25]. The occurrence of PNI was found to be factor indicating poor prognosis correlated with decreased overall and disease-free survival time in many cancers including prostatic, colon, pancreatic, gastric and head and neck carcinoma [26–29].

In the last few years, tumor innervation/axonal network and PNI have become a new area of interest, and several landmark studies in this field were performed on prostate cancer (PC) [30–34]. The presence of axons within the tumors results from the cancer invasion into surrounding tissue or axonal outgrowth from pre-existing nerves in the process of axonogenesis [30–32]. Moreover, the number of infiltrating neurons in PC may result from neurogenesis – the process of new neuron formation. Two alternative theories on neurogenesis in TME have been identified: neuronal neurogenesis and stem cells neurogenesis [31, 35]. Recent in vitro studies showed that the other source of new neurons in PC tumor tissue might be the central nervous system. The neuronal progenitors from brain subventricular zone were shown to reach the tumor through bloodstream and differentiate into new neurons from early stages of cancer development. Doublecortin positive-progenitors are able to migrate from subventricular zone thorough blood-brain barrier and settle in prostate stroma where can differentiate into adrenergic neurons [36].

Prostate Gland, PC, and Its Microenvironment

Basic Histology and Innervation of Prostatic Gland

Histologically normal prostatic gland is composed of glandular epithelium and typical organ stroma. In general, benign glands together with ductal structures consist

Fig. 1. Schematic presentation of the histology of the normal prostate with contribution of innervation. Prostatic glands surrounded with basal membrane are composed of basal and luminal cells with accompanying few neuroendocrine cells. Cholinergic fibers supply mainly glandular component of the prostate while adrenergic nerves are presented within the stroma. ACh, Acetylcholine; nNOS, Neural nitric oxide synthase; VAcHT, vesicular ACh transporter; NA, Noradrenaline; TH, Tyrosine hydroxylase; NPY, Neuropeptide Y.

of luminal secretory cells, basal cells, and few neuroendocrine cells. Neuroendocrine cells are the source of numerous peptide hormones, neuropeptides and biogenic amines acting on other components. The glandular structures surrounded with basal lamina are embedded in prominent fibromuscular stroma composed of smooth muscle cells admixed with fibroblasts and blood vessels [37–39]. Growth of stroma in a normal prostatic gland is positively regulated directly by growth factors such as epidermal growth factor and fibroblast growth factor. Signaling depending on the transforming growth factor (TGF) may lead to a stimulation of fibroblasts, but on the other hand, it inhibits the proliferation of normal prostate epithelium [40].

In the stroma, there are also efferent and afferent axons of the autonomic nervous system, which innervate stromal components and glandular cells [40]. Prostate gland receives sympathetic and parasympathetic autonomic nerve supply from the hypogastric nerve and the pelvic nerve respectively [41]. The majority of nerve bundle passes to the capsule with decreasing nerve density in the transition and central zone. In the posterior capsule, the number of nerves is higher than that in the anterior capsule [42]. Parasympathetic nerves are relatively evenly dispersed in the prostatic base and apex, whilst the total number of sympathetic nerves decreases from the base to the apex. The majority of sympathetic nerve fibers is located dorso-laterally [41, 42]. In a normal prostatic gland, both the sympathetic and parasympathetic innervation have an impact on the surrounding structures through

secreted neurotransmitters (NT; Fig. 1). Prostatic epithelium is innervated by cholinergic fibers, while the stroma receives predominantly noradrenergic innervation [43, 44]. Cholinergic muscarinic receptors M1 are numerous on epithelial cells, whereas M2 receptors are more common on stromal muscle cells. Their activation leads to the release of acetylcholine (ACh), which takes part in the secretory function of the prostate [43]. Sympathetic nervous system α and β receptors are located mostly in the stroma. Activation of α 1- and β 2-receptors mediates contraction of the smooth muscles and secretion from the luminal cells respectively [44]. One of the sympathetic NT produced by ganglion cells as a co-transmitter of noradrenaline, and expressed by neuroendocrine cells and some prostatic glandular cells is neuropeptide Y (NPY) [45]. Transcriptome-based study showed that prostatic epithelial cells express numerous proneuronal genes. Interestingly, basal cells overexpressed genes associated with neural development, neurogenesis and axonal guidance molecules, whereas luminal cells expressed genes involved in neural signal response and processing [46].

PC Pathoclinical Data

PC is one of the most commonly occurring neoplasm in males and the fifth most common cause of cancer-related death worldwide. The majority of patients are above 60 years of age at the time of diagnosis [47]. Tumors usually arise peripherally, in posterolateral part of the gland, close to the capsule and the neuro-vascular bundle. In 85% cases, prostatic cancer is multifocal and individual

tumors differ histologically. PC typically spreads by local extension into the periprostatic fat tissue, with PNI and involvement of seminal vesicles. In advanced stages, it also infiltrates the urinary bladder, pelvic soft tissue, or rectum. Metastases to the regional lymph nodes occur in 5–12% of patients. In the disseminated phase, multiple osteoblastic metastases into bones are typical [48]. The histopathological diagnosis of PC relies on architectural and nuclear features of neoplastic glands. In some cases, immunohistochemistry is necessary for differential diagnosis with benign lesions. Grading in PC is assessed with Gleason score (GS) recommended by WHO, with prognostic significance. It is assigned by 2 predominant architectural patterns. In course of time, several changes were introduced into GS. In 2014, a new grading system of Grade Groups, based on GS, was incorporated by ISUP and WHO 2016 classification [49].

There are many therapeutic options for PC based mainly on the tumor stage, histologic grade, patient's age, and PSA level, enclosed in special nomograms. Early-stage PC is successfully treated by prostatectomy or radiation therapy; in parallel, some patients can live with a tumor under active surveillance [50]. Because PC is typically androgen-dependent, androgen deprivation therapy is used in the advanced and metastatic stage of the disease. However, despite the initial sensitivity to antiandrogen therapy, in a different period of time, PC becomes castration resistant prostate cancer and needs other therapeutic options. In some cases, prostatic cancer, especially castration resistant prostate cancer might undergo the yet not well understood neuroendocrine conversion with incurable metastatic progression [50, 51]. Hence, new therapeutic algorithms and strategies are needed for better PC control. Although the true impact of microenvironment in PC pathogenesis and progression is not known, it is clear that targeting the stromal components may represent a promising approach for the treatment of PC [17, 52].

PC Stroma and Its Neural Component

In PC, the neoplastic glandular component devoid of basement membrane is directly embedded in stroma composed of smooth muscle cells admixed with specific fibroblasts and blood vessels [37, 38]. The most prominent TME components are activated fibroblasts having myofibroblastic phenotype called CAF. They can guide cancer cell growth, PNI, dissemination or cancer pain through the release of cytokines, chemokines and growth factors [1, 53]. CAFs are mostly controlled in many ways via the TGF- β (TGF β) signaling pathway. TGF β upregu-

lation promotes angiogenesis and generation of reactive stroma by matrix production (fibronectin, elastin, collagen type I) and release factors responsible for extracellular remodeling (metalloproteinases and their inhibitors). Moreover, TGF β activation induces epithelial to mesenchymal transition [54]. One of the best studied chemokine stromal cell-derived factor 1 (SDF-1/CXCL12) might be secreted by CAFs and might stimulate expression of its receptors CXCR4 on benign prostatic cells, which is connected to tumor development. CXCL12/CXCR4 interaction promotes inflammatory response, androgen-dependent cancer cell proliferation and bone metastases [2, 55, 56]. Soluble proteins play an important role in autocrine and paracrine interactions between cancer cells and TME enhancing tumor progression. Elevated level of IL-6 secretion from CAFs is thought to be a mechanism of androgen-independent progression of PC via the PI3K-Akt, STAT3 and MAPK/ERK pathways [35]. Activated stromal cells and immune cells react with each other. Cells of innate inflammatory response (mostly M2 macrophages and dendrite cells) recognize atypical epithelial cells and are responsible for establishing an immunosuppressive pro-TME. Macrophages secrete fibroblast growth factor and epidermal growth factor, which promote the development of reactive stroma. CD4 and CD8 T cells produce tumor-promoting cytokines and Treg lymphocytes suppress anti-cancer immune response [57, 58]. PC enriched in stroma with a high number of fibroblasts was found to be more aggressive and androgen-resistant [8]. Study on PC xenografts distinguished a stromal-derived metastatic gene expression signature, which can be used to separate indolent PC from tumors with metastatic potential [59]. Reactive stromal grading might be used independently of classical Gleason grading to evaluate patient prognosis. The stromogenic carcinoma (reactive stromal grading grade 3) is associated with positive lymph node metastasis, extracapsular extension, reduced recurrence-free survival and time to biochemical recurrence (BCR) [60, 61].

In the last years, it was found that also neural tissue is an active element of the TME of PC. The PNI is the best known form of cancer-nerve crosstalk in PC. Recently, there is increasing data pointing to the role of axonogenesis and neurogenesis in PC biology [31, 62, 63]. The other related topics are molecules involved in tumor-neural TME interactions, with 3 main classes of proteins: neurotrophic factors, axon guidance molecules and NT including neuropeptides. Their sources are cancer cells, tumor-associated neuroendocrine

Fig. 2. Schematic presentation of histology of the PC with axonogenesis. PC composed of neoplastic glands devoid of basal membrane, which are intermixed or surrounded with the complex stroma containing axonal branches of autonomic nervous system secreting specific NT. NT, neurotransmitters; NGF, nerve growth factor; ACh, Acetylcholine; nNOS, Neural nitric oxide synthase; VAcHT, vesicular ACh transporter; NA, Noradrenaline; NPY, Neuropeptide Y; TH, Tyrosine hydroxylase.

Fig. 3. Schematic presentation of complex cancer microenvironment with neural component and its impact on tumor growth. Cancer microenvironment composed mainly of CAF, myofibroblasts, inflammatory cells and blood vessels admixed with neural component made of Schwann cells and axons acting on neoplastic cells and stromal cells via numerous growth factors and NT. They stimulate cancer growth by regulation of different processes including angiogenesis, axonogenesis, and modification of immune response. CAF, cancer associated fibroblasts.

cells, and stromal elements [17]. Experiments on PC cell lines showed that PC androgen-resistant cell culture have their genetic profile consistent with the so-called brain signature in 28% [51].

There are several important clinical observations and implications connecting the role of neural stimulation or even neural dependence of PC. PC develops mainly in peripheral subcapsular best innervated regions of the gland and PNI is a very frequent phenomenon in this tumor [64]. The incidence of PC is significantly lower in patients with myelopathy or spinal cord injuries, especially below T11 level [65, 66]. Furthermore, research on animal models revealed that chemical or surgical dener-

vation caused a decrease in the tumor size. In the same study, the gene expression signature was checked in PC from denervated rat prostates and human PC in patients with spinal cord injuries. These results showed significant changes in gene profile when compared to normal prostatic tissue; especially genes involved in the cellular energy metabolism were downregulated [33]. The evidence collected in recent years suggests that neural stimulation is the second most important factor after hormonal androgenic signaling which control evolution of PC. Figures 2 and 3 illustrate the main aspects of histology of PC in the context of epithelial-stromal composition and TME neural component.

PNI in PC

PNI is a multistep active process. Several definitions exist, including the most widely used one, according to which PNI is an existence of cancer cells within any of the nerve sheath layers [67]. The other definitions are more strict or morphometric, for example, the criterion for PNI as minimum of 33% invasion of nerve circumference [25]. PNI occurs in many tumors being common pathological finding in gastrointestinal tract tumors, as in pancreatic carcinoma it reaches even 100% [67]. A meta-analysis of 24 studies on the incidence and prognostic significance of PNI in gastric cancer showed its 6.8–75.6% occurrence and confirmed its impact on the overall survival rate [28]. In head and neck squamous cell carcinomas, PNI is reported in 25–80% of cases with relation to worse prognosis and increased risk of regional recurrences. In these groups of tumors, several parameters were measured, such as the number of involved nerves, nerve diameter or extratumoral versus intratumoral PNI occurrence [68].

In PC, the incidence of PNI depends on the type of analyzed material; however in prostatectomy series it is found in about 75% of cases and is the most significant extracapsular spreading pathway [69, 70]. PNI is an important indicator of the patient outcome, which should be mentioned in the reports of histopathological evaluation of needle biopsy specimens [71]. The association of PNI in prostatectomy cases with ISUP grades and a higher pT stage was revealed by Lubig et al. [70]. Interestingly, in this work, prognostic significance of PNI was not found in dichotomic division into positive versus negative PNI status. However, a more precise analysis and counting of invaded nerves showed that presence of <1 nerve with PNI on 5 high powered fields correlates with a better prognosis [70]. Zhang et al. [69] in their meta-analysis of 19 studies demonstrated that PNI is associated with higher risk of BCR after surgery or radiotherapy and could serve as an independent predictor for BCR in patients with PC.

The relevance of nerves infiltration for tumor dissemination was previously believed to be a mostly passive process in which nerves act as roots for cancer cell migration. However, studying the role of neurotrophic factors and NT secreted by axons and nerve endings, and also the active role of Schwann cells, has shed a new light on this mechanism [19, 21, 22]. In parallel, cancer cells are also able to release neurogenic factors and axon guidance molecules to promote new axon development within the tumors, in part similar to the process of angiogenesis. The

next question is the role of formation of specific nerve-cancer synapse in tumor progression. One of the first studies to support the above-mentioned hypothesis was performed on human PC cell lines. Studies on the PC-3 line showed that these cancer cells secrete a nerve growth factor (NGF), brain-derived nerve factor, pleiotrophin or neuregulin, which facilitate cancer innervation [52, 72]. Using immunohistochemical methods, proNGF was detected in PC cells with an overexpression comparable to benign prostatic hyperplasia and correlated positively with a higher GS [73]. Similarly, a high expression of brain-derived nerve factor and its receptor TrkB has been observed in PC [74].

To the family of axon guidance cues belong also netrins, ephrins, semaphorins, and slits. The best-known molecule in this group is netrin-1, which is involved in tumor cell motility, proliferation, apoptosis, angiogenesis and PNI. In most of the examined cancer types, netrin-1 expression is upregulated, together with its receptors downregulation [75]. In PC netrin-1 expression is generally reduced, but upregulation of netrin-1 mRNA in PC-3 cell line was found in hypoxia [76, 77].

Perineural niche forms a specific background for cancer cells providing a favorable microenvironment for their survival and development. Cancer cells in perineural space are metabolically active with a higher proliferation ability and reduced apoptosis [18, 73].

Axonal Network in TME and Nerve Fiber Density in PC

In vitro studies revealed that axonal stimulation may be related to more aggressive behavior and formation of distant metastases [22, 78]. A study by Magnon et al. [30] was pioneering in this area. Using mouse models these authors observed that sympathetic fibers of autonomic nervous system, acting through stromal β 2- and β 3-adrenergic receptors, promote tumor cell survival, whereas parasympathetic fibers play a role in tumor migration and dissemination through stromal muscarinic cholinergic receptor M3. Sympathetic nervous system signals are probably the most important in the early stages of carcinogenesis, while cholinergic stimulation promotes metastatic potential. The analysis performed on mice xenografts injected with PC-3 human PC cell line revealed that both tumor-infiltrating sympathetic fibers and intratumoral parasympathetic fibers arise from normal prostate tissue [30]. Moreover, sympathetic stimulation via β 2-adrenergic receptors seems to have a great impact on an-

Fig. 4. **a** Small nerves and dense axonal network in prostate stroma surrounding solid PC infiltrate. Note the sparse intratumoral axons visible in this case. Normal prostate tissue situated at the bottom of the picture (PGP9.5, 200 $\times$). **b** Multiple dispersed axons are presented within the cancer stroma between neoplastic glands and inflammatory infiltrate (PGP9.5, 400 $\times$). **c** Specific adrenergic fi-

bers dispersed mainly within tumor stroma of PC. They are not visible inside cancer infiltrate (TH, 200 $\times$). **d** PNI with active delamination of one of the nerve trunks. Cancer cells surround nerves and disintegrate their structure. Tumor stroma with irregular adrenergic nerve fibers network (TH, 200 $\times$).

giogenesis, which in part explains anticancer activity of beta-blockers [18].

Nerve density is one of the parameters of tumor innervation and has been examined in some carcinomas including prostate, pancreatic, and breast cancer. The methods of determining nerve density differ between authors and the results are difficult to compare. Different immunohistochemical markers are used for the visualization of axons in tissue. The most commonly used are pan-neuronal antibodies against protein gene products 9.5 (PGP 9.5; Fig. 4a, b) and S100. The sympathetic and para-

sympathetic nerves are usually evaluated by anti-tyrosine hydroxylase (TH; Fig. 4c, d) and anti-vesicular ACh transporter antibodies respectively. The axons or nerve branches could be counted manually under an optical microscope or with the assistance of digital image programs processing neural density or area [30, 32, 62, 79].

In the study of Magnon et al. [30], nerve fiber density analysis of PC samples from prostatectomy specimens showed adrenergic fibers mostly located in the surrounding peripheral stromal tissue. Cholinergic fibers, however, were found in high density at the center of the tumor

around cancer cells. Interestingly, both were correlated with a higher proliferation index, but the extraprostatic extension was more frequent in patient with a high density of cholinergic fibers. Having compared nerve density in human PC with matched normal controls, it was found that in cancer the number of nerves is higher. Moreover, there is evidence that axonogenesis starts at the beginning of cancerous progression and nerve fiber density is increased even in prostatic intraepithelial neoplasia [33]. Some studies have shown that neurons are also involved in cancer-related neurogenesis and the number of ganglia and the number of neurons in ganglia are significantly higher in PC than in normal tissue [31]. Chemical sympathectomy performed on Hi-Myc transgenic mice showed that the amount of high grade prostatic intraepithelial neoplasia was reduced by 83% after the procedure in comparison to non-treated controls. Ablation of adrenergic fibers does not show any impact on tumor invasion. In the same study, adrenergic fibers were found to develop around high-grade prostatic intraepithelial neoplasia acini, which supports the idea that adrenergic signals play a crucial role in the early cancer development [30].

Little is known about the prognostic relevance of nerve density in solid tumors. The main reasons are methodological problems and different research criteria. Some of the few performed studies demonstrated that in breast and colorectal cancer, the presence of PGP 9.5 positive fibers correlates with lymph node metastases [73, 80]. Moreover, in colorectal cancer, a high number of axons was found to be an independent prognostic factor for poor outcome [80]. In breast cancer, the association of nerve density with the grade of invasive carcinoma and patient survival was also confirmed [62]. Interestingly, recently published data showed that breast cancer recurrences correlated with a higher density of sympathetic nerve fibers and with lower density of parasympathetic innervation [81]. In pancreatic adenocarcinoma, higher parasympathetic nerve density correlated positively with tumor budding and early recurrences [79].

There are only a few analyses of axon density and clinicopathological features of PC. Olar et al. [32] found only a weak correlation between the axon density and the lymph node status. These authors did not observe correlation with the preoperative PSA level, patient age, clinical staging and GS. In another work, an increased number of nerves was reported to be associated with extracapsular extension, whereas seminal vesicle invasion was correlated with a higher number of neurons in ganglia [31]. Reeves et al. [34] found that pure sympathetic non-PNI nerve count demonstrates a statistically significant cor-

relation with pathological margin status. No correlation with patient age, preoperative PSA level, ISUP Grade Groups or GS was established.

Increased nerve density evaluated by PGP 9.5 was a predictor of time to biochemical cancer recurrence established by measuring serum PSA level [32]. Similarly, in another work, TH-positive nerve density of the normal tissue surrounding PC areas correlated positively with BCR [30]. Ayala et al. [31] found that the median area of axons was lower in the non-recurrent tumors than in the patients with recurrence. Reeves et al. [34] showed that the total nerve counts in both the PNI and non-PNI nerves did not predict a biochemical relapse after prostatectomy, but the non-adrenergic, non-nitrergic nerves number was predictive of BCR. Magnon et al. [30] group assessed neural areas in PC clinical sections. They revealed that TH-positive nerve areas per field of normal tissue ($>2,000 \mu\text{m}^2$ per field) and vesicular ACh transporter-positive nerve areas in tumor tissue ($>300 \mu\text{m}^2$ per field) were associated with higher recurrence rates.

Molecular pathways connected with cancer-related axonogenesis have not been discovered in detail. In vitro models suggest a potential role of semaphorin family genes (ligands for neuropilins) such as S4F in the induction of PNI. S4F immunohistochemical cytoplasmatic overexpression correlated positively with nerve density and PNI diameter in PC cells. Interestingly, the intensity of staining was higher in cases with high nerve density and a higher PNI diameter [31, 82]. Studies on prostatectomy specimens showed an increased number of axons to be correlated with the expression of proteins involved in pro-survival pathways such as PTEN/Akt or NFkB activation. Increased nerve density was associated with upregulation of the androgen receptor and estrogen receptor alpha [32].

All these data show that cancer-related axonogenesis and neurogenesis are involved in the PC biology.

NT in PC

NT control normal tissue homeostasis. They might be separated into 2 groups: classic (epinephrine, norepinephrine, ACh, dopamine, aspartate, glutamate, glycine, gamma-aminobutyric acid, serotonin, histamine) and small polypeptides known as neuropeptides (bombesin, substance P, neurotensin, NPY, enkephalin, endorphin). Many receptors for NT have been found on cancer cells demonstrating the role of neurotransmitter secretion in cancer evolution [21]. The cancer cell-nerve synapse reflects the junction between these 2 compartments. In nor-

Fig. 5. a NPY cytoplasmic immunoreactivity within the PC cells and strong expression within the axons in the stroma. At the bottom of the image, small nerve with PNI is visible (NPY, 600×). **b** Strong heterogeneous NPY cytoplasmic expression in PC cells, foci of PC neuroinvasion with associated perineural inflammatory infiltrate (NPY, 400×).

mal prostate gland and PC, neuroendocrine cells produce some neuropeptides including NPY, in parallel NPY expression has been found in normal luminal cells (low) and cancer cells [83].

NPY is widely expressed in the central and peripheral nervous system, influencing via its Y receptors many physiological processes, such as cortical excitability, stress and immune response, food intake, circadian rhythms, cardiovascular function, glucose metabolism. NPY is also involved in biology of some tumors, controlling angiogenesis, tumor growth, metastasis and cancer cell metabolism [84]. An *in vitro* study revealed that Y1 and Y2 receptors are present in the androgen-independent human PC-3 cell line and that their activation by NPY stimulates cancer cell proliferation, which suggests its impact on castration-resistant regulation of PC [83, 85]. NPY and its receptors (Y1R, Y2R, Y5R) were found to be expressed in PC cells and neuronal fibers but not in the surrounding stroma [86]. Our own results (data not published) showed a higher expression of NPY, Y1R, Y2R, and Y5R in PC tissue than in matched controls of benign prostatic hyperplasia and significantly higher expression of the NPY system in areas with neuroinvasion and extraprostatic infiltration (Fig. 5a, b) [87]. The correlation between NPY expression and tumor innervation is not very well recognized. However, in PC tumors with low axonal density, the NPY level was higher [88]. A recent study divided PC into 2 subclasses: one with low NPY mRNA expression coupled with ERG

translocation, and the opposite type – high NPY without ERG fusion. The first group appeared to be more aggressive with a higher rate of metastatic disease, whereas the second group represented patients with a more favorable prognosis [89]. On the contrary, in another work, high proNPY expression was found to be correlated with an increased risk of prostate-cancer-specific mortality. Moreover, using quick enrichment of small targets for mass spectrometry, NPY was selected as a promising specific biomarker for early PC detection in plasma, which may improve the PSA test [90]. Available data about expression level of NPY and its clinical correlations are inconclusive. Further studies are required to determine the role of NPY – both synthesized in cancer cells and released from sympathetic nerves – in PC initiation and progression.

Neuroendocrine differentiation (NED) is a well-recognized phenomenon observed in prostatic carcinoma. In advanced and hormone-resistant PC the number of neuroendocrine cells is increased (neuroendocrine transdifferentiation) and probably this paracrine stimulation is partially responsible for the antiandrogen treatment failure. This phenotypic switch is mostly related to androgen receptor resistance and poorer patient outcome [91–93]. Studies on androgen-independent PC cell lines showed antiproliferative activity of $\alpha 1$ -adrenoreceptors agonists [94]. The mechanism responsible for NED is probably N-myc overexpression, which stimulates the AKT signaling pathway. N-myc stabilization is determined by aurora kinases A and

B, which are found to be amplified in PC with NED. Some cytokines, especially interleukin 6, might induce NED by activation of PI3K/Etk/Bmx and STAT3 pathways [92, 95]. Neural transcription factor BRN2 is thought to be required for NED driving neuroendocrine phenotype, especially in castration-resistant PC cells. In in vitro models, inhibition of the AR pathway increases the expression of BRN2 [96].

Neural TME as a Potential Therapeutic Target

Neural cancer microenvironment presents itself as a promising future target area for anti-cancer therapy. There are some potential molecular strategies for personalized treatment related to axonogenesis and neuronogenesis [18, 52]. Anti-neurogenic therapies have already been tested in neurological disorders and there is feasibility of inhibiting NGF/proNGF as a therapeutic approach for PC [19]. Semaphorins are thought to be overexpressed in PC and inhibition of SEMA3C with small molecules, monoclonal antibodies, and antisense oligonucleotides appear as promising anticancer therapeutic modality [97]. NT and their inhibition present another attractive target for anti-cancer therapy. Several clinical trials are going to determine the clinical value of beta blockers as neoadjuvant treatment (NCT03152786, NCT02944201). Resistance to immunotherapy developing in some initially responding tumors is now a clinical problem. Some evidence demonstrated that adrenergic stress inhibits immune response and its blockage might increase therapeutic efficacy of immunotherapy [98]. It was already studied that beta-adrenergic blockers reduced PC-related mortality [99]. In vitro studies have shown that selective Y1 receptors might reduce cell growth dependent on NPY stimulations. This implicates a potential strategy for PC therapy using NPY axis inhibitors [100]. An interesting option seems to be PC treatment with botuline toxin before prostatectomy. Phase I/II clinical trial (NCT01520441) showed some morphological changes in tissue taken from botox-injected patients, such as atrophic and degenerative features, reduced cytoplasm, and pyknotic nuclei of cancer cells. Moreover, more extensive apoptosis and lower nerve density were found in tumors treated with botox before surgery [33].

Conclusions and Future Directions

Neural microenvironment and cancer-related axonogenesis constitute new fields for investigation that require further exploration in PC and other neoplasms [18–22,

25–29]. Future studies are needed to advance current understanding of molecular mechanism and neural signaling pathways that mediate neurogenesis and their impact on cancer development and progression. Besides, the role of nerve-endothelium interaction within the TME is still unclear and should be clarified. Translating neural involvement in cancer progression into clinical practice is a big challenge. Clinicopathological correlations of tumor innervation and use of widely interpreted aspects of tumor innervation as therapeutic approach appear as one of key objectives in cancer research. The studies focused on PC nerve density are still very few, but results published so far suggest that there are some clinical implications of nerve density count on patient outcome [30–32, 34]. Much remains to be clarified, especially the prognostic role of nerve density in PC. Some NT, such as NPY act in a paracrine and autocrine way in PC, and appear to be potential cancer biomarkers as diagnostic and therapeutic tools [90]. Little is known about how cellular components of neural niche such as Schwann cells trigger cancer invasion.

The possible role of neural TME as a target in PC treatment is on very early stage of investigations. There are mostly preliminary in vitro studies and ongoing clinical trials looking for molecular targets such as NGF, NPY or semaphorins [19, 97, 100]. The application of genome-wide expression analysis will help to discover new molecular strategies for cancer treatment. Beta-blockers are now investigated in some clinical trials whether they could prevent prostatic cancer development or its progression. Moreover, it was suggested that in breast cancer, autonomic nerves have an impact on modulation of expression of immune checkpoint molecules, which is important in the prediction response for immunotherapy [81]. Neuronal-dependent immune response and their role in cancer cell escape from immune surveillance appear as next interesting direction.

All studies to elucidate and accelerate our understanding of the neural regulation in prostatic cancer warrant thorough investigation. Cancer neurobiology seems to be an emerging discipline that opens new perspectives in oncology. With more consecutive studies, critical clinical significance of neural TME will be confirmed.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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