
Inflammatory Microenvironment in Prostate Carcinogenesis

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1. Introduction

The association between prostate cancer and inflammation was first formally addressed in the nineteenth century and since then many authors have confirmed the biological and clinical evidence of this association. However, the molecular mechanism involved is yet to be deciphered.

There are two well established pathways linking inflammation and cancer: the extrinsic pathway from conditions that cause non-resolving smouldering inflammatory responses and the intrinsic pathway where the misregulation of oncogenes and tumor suppressor genes switch on the expression of inflammation-related programs.

Prostate cancer is a complex and progressive disease. Over time the cells become resistance to hormonal therapies that are designed to block the release and/or the uptake of androgens. During this stage androgen receptor (AR) mutants are able to bind promiscuous steroids, and may convert AR antagonists to agonists. Other hormones and their receptors are involved in the abnormal growth of the gland. Particularly, oestrogens and oestrogen receptors defined a subclass of prostate cancer with a very aggressive clinical phenotype (such as the TMPRSS2-ERG fusion). In addition, other signaling cascades are switched on bypassing the androgen/AR axis and favoring tumor progression. Among them, cyclooxygenase-2 (COX-2), neuroendocrine differentiation and the loss of the tumor suppressor phosphatase and tensin homolog (PTEN), with the concomitant inhibition of the PI3K/Akt, resulting in Bcl-2 overexpression and the burst of pro-inflammatory cytokines, chemokines and other growth factors production, contributing all to the progression to the hormonal-resistance disease. As in other malignancies in prostate cancer, reactive oxygen species (ROS) cause ox-

oxidative damage to macromolecules in epithelial cells and can react with other cellular components initiating a free radical chain reaction, thus sustaining the prostate carcinogenic process and its progression.

The molecular mechanisms that prime the pathogenesis of cancer-related inflammation are complex and involve a delicate interplay between tumor and its microenvironment. In prostate tumors, the switch to an angiogenic phenotype is known to be critical for its progression. Unless a tumor can stimulate the formation of new blood vessels, it remains restricted to a microscopic size. Inflammation and hypoxia are widely accepted as key elements in the induction of angiogenesis.

Dissection of the diversity of cancer-related inflammation is critical for the design of innovative diagnostic and therapeutic strategies in prostate cancer.

Specifically, the following topics and molecular events are reviewed and discussed in this chapter:

- The cytokine and chemokine orchestration and the associated downstream genetic events that cause neoplastic transformation in the prostatic tissue.
- Acknowledging the oxidative stress imbalance in the tumoral niche as key mediators of signaling cascades.
- The relevance of microRNAs as oncogenes and tumor suppressor genes and how microRNA expression profiles can be used for markers of prostate cancer prevention and therapeutics.
- The potential of prostate tumoral cells in the inflammatory microenvironment to express an endothelial-like phenotype and mimic vasculogenic networks.

2. Body

2.1. The cytokine & chemokine orchestration in prostate cancer: Strategies, avenues and traits

Cytokines are a family of cell-signaling protein molecules that are secreted by various cell types and are a category of signaling molecules used extensively in intercellular communication. Cytokines can be classified as proteins, peptides, or glycoproteins. A variety of cytokines are secreted by cells in the tumor microenvironment and can impact on prostate cancer growth. These cytokines can then act in a paracrine fashion on tumor cells to stimulate a variety of physiological activities including cell proliferation, invasion, migration, chemoresistance, etc.

The tumor inflammatory microenvironment is characterized by immune cell infiltration: tumor-associated macrophages, mast cells, dendritic cells, natural killer cells, neutrophils, eosinophils and lymphocytes. These cells produce a variety of cytotoxic mediators such as ROS and reactive nitrogen species (RNS), serine and cysteine proteases, matrix metallopro-

teinase (MMP), tumor necrosis factor α (TNF α), interleukins, interferons and enzymes, as COX-2, lipooxygenase-5 and phospholipase A2, which activate or are activated by transcription factors such as nuclear factor κ B (NF- κ B) and signal transducers and activators of transcription-3 (STAT3), activator protein 1 and hypoxia-inducible factor 1 α (HIF-1 α) that mediate tumor cell proliferation, transformation, metastasis, survival, invasion, angiogenesis, chemoresistance and radioresistance.

Present discoveries highlight chemokines and their receptors as relevant factors for inflammation. The directed migration of a cell toward the source of a secreted protein signal, known as chemotaxis, has been commonly associated to the leukocyte trafficking triggered by infection and to secondary lymphoid organs. Although extensively studied as part of the immune system, chemokines have lately been investigated as mediators of tumor development. Chemokines, the executors of chemotactic signals, are constitutively expressed in destined cell types and tissues maintaining the homeostasis of the hematopoietic and the immune system. However, inflammatory chemokines, either produced by the tumor cells or by tumor-associated cells, behave differently and their expression is induced upon inflammatory stimuli promoting proliferation and angiogenesis, contributing to the malignant progression. They certainly modify the sensitivity of prostate cancer cells to environmental stresses such as hypoxia, oxidative stress, DNA damage, altering several pathways crosstalk and producing hormone-refractory aggressive tumors. In addition to the classical roles described above, their pleiotropic effects include: potentiating the production of growth factors, inducing growth signals, attenuating apoptosis, further linking the cytokine signaling to the hypothesis that inflammation and inflammatory mediators rise as the seventh hallmark of cancer [1]. In this section we will focus on some of the several cytokines implicated in the prostate cancer microenvironment given that there are too many factors to describe.

2.1.1. The chemokine family acquaintance

To date, over 50 chemokines and 20 chemokine receptors have been recollectored. These are grouped into four categories, C, CC, CXC and CX3C, according to the location of the main cysteine residues near the N-terminal domain of these proteins [2]. Chemokine binding to their corresponding seven transmembrane-domain G-protein-coupled receptors causes the activation of signal transduction networks leading to chemotaxis. These receptors have been implicated in the migration of breast, prostate and lung cells to secondary sites in the bone [3]. Up to date the most relevant chemokine receptors in prostate cancer dissemination, are CXCR4, CXCR7 and CXCR6 [3].

The CXCR4/CXCL12 axis exerts multifactorial effects and has been related to both, the homing of tumor cells to specific organs and the growth of tumor cells at specific locations. CXCL12, also known as SDF-1 (stromal derived factor 1), is considered a homeostatic chemokine which regulates the hematopoietic cell trafficking and secondary lymphoid tissue architecture. It is constitutively expressed in several organs including lung, liver, skeletal muscle, brain, kidney, heart, skin, bone marrow and its secretion is linked to tissue damage. CXCR4 is expressed in endothelial cells and pericytes of hypoxic, injured, or pathological tissues. Of note, endothelial precursor cells also express and secrete CXCL12. In turn,

CXCR4 is widely expressed on hematopoietic cells including CD34⁺ hematopoietic stem cells, T- and B-lymphocytes, monocytes and macrophages, neutrophils and eosinophils as well as by brain, lung, colon, heart, kidney, liver endothelial and epithelial cells, microglia, astrocytes, neuronal cells, and progenitor cells including endothelial and smooth muscle progenitors. Functional CXCR4 is expressed on embryonic pluripotent stem cells and several types of tissue-committed stem cells. These cells with functional CXCR4 expression migrate and/or invade along CXCL12 gradients. CXCR4⁺ pro-angiogenic cells include immature and mature hematopoietic cells, endothelial precursor cells, and smooth muscle cell progenitors, which have direct or indirect pro-angiogenic properties. Interestingly, CXCL12 plays a role in the mobilization and recruitment of these cells to the neo-angiogenic niches supporting revascularization of ischemic tissue and tumor growth [4]. This axis has been strongly implicated in prostate cancer tumorigenesis and progression [5].

2.1.2. Chemokines and their relevance in the metastatic behavior of prostate cancer

Metastases is a multistep process including: invasion of the primary tumor cells to adjacent tissue, intravasation, dissemination through the blood or lymph, extravasation and seeding, adapting to a different tissue microenvironment and finally proliferating in such distant organs. This process involves both the selection of features that favor cancer cells growth and the concomitant alteration of the stroma generating a “fertile soil” which facilitates invasion, anchoring and survival of metastatic cells [6].

Prostate neoplasms have a striking tendency to metastasize to bone. The molecular mechanisms underlying the bone homing behavior have yet to be decoded. However, such mechanisms may include signaling cascades that induce a vascular pathway, that produce the trigger of chemotactic factors by bone marrow stromal cells and the production of growth factors within the bone, reinforcing the survival and proliferation of tumoral cells. It is of common knowledge that hematopoietic stem cells are directed to the bone during bone marrow transplantation and human fetal development [7, 8] and CXCL12/CXCR4 appears in this scene as key molecules in bone seeding. Metastatic prostate cancer cells may use a similar pathway to localize to the bone. Several human prostate cancer cell lines express functional CXCR4 and differential levels of its ligand alter physiological processes of these cells such as adhesion, migration and invasion, assigning a role for this axis in prostate advanced disease. It is worth mentioning some controversial reports regarding the expression of this receptor and its ligand in prostate cancer. Mochizuki *et al.* [9] reported that the expression of CXCR4, but not its ligand, was increased in prostate carcinoma indicating that prostate cancer cells may also be affected by exogenous SDF-1. However, other authors showed high expression of both, ligand and receptor [10].

Interestingly, the blockade of CXCR4 inhibited the expression of vascular endothelial growth factor (VEGF) and the concomitant angiogenesis and even reduced significantly bone metastasis *in vivo* [11]. Furthermore, CXCR4 is positively regulated by AR [12]. Androgen-induced CXCR4 expression was functional in TMPRSS2-ERG-positive prostate cancer cells, further indicating the relevance of this chemokine in prostate cancer metastasis [13]. The immunohistochemical pattern of CXCR4 expression in patients with metastatic prostate

cancer has shown that high expression of this chemokine in tumors had poorer cancer-specific survival than patients with low expression of CXCR4. This receptor expression has proved to be a useful prognostic factor for patients with metastatic prostate cancer treated with androgen-withdrawal therapy [14].

Strikingly, regulation of CXCL12 expression in the tumor microenvironment has been poorly studied. Some reports indicate that hypoxia may induce its expression in endothelial cells and in prostate tumor cells [5]. Could CXCL12 have an additional role to its chemo-attractant properties? Could it also act as a growth factor or prevent the apoptosis of tumoral cells enabling metastasis to take place? These questions still need to be answered.

CXCR7 (RDC1), a second receptor for CXCL12, regulates a spectrum of normal and pathological processes but fails to couple to G-proteins and to induce the typical chemokine receptor mediated cellular responses. It also binds to CXCL11 and dimerizes with CXCR4. This receptor with dual specificity is up-regulated in many tumors, but its function within the tumoral niche needs further clarification [15]. Studies show that CXCR7 expression provides proliferation and survival advantages and increased adhesion properties between prostate cancer cells and the host endothelial cells. It is also more highly expressed in prostate metastases (specially those to the bone) compared to primary tumors and elevated levels of CXCR7 correlate with the aggressiveness of the disease. In the vasculature, the expression of CXCR7 is elevated in endothelial cells associated with tumors [16] and this chemokine receptor has been further linked to tumor angiogenesis *in vivo* [17].

Other inflammatory mediators may regulate CXCR7 function. Of note, high serum levels of IL8 have been reported in patients with advanced metastatic prostate cancer. In primary prostate carcinoma tissues, IL8 strongly correlates with biochemical prostate specific antigen (PSA) recurrence and CXCR7 expression is induced by IL8 in prostate tumor cells. As survival following androgen deprivation is a critical step in the emergence of castration-resistant tumors, IL8-induced up-regulation of CXCR7 may enhance the survival and proliferation properties of those tumor cells. Thus the up-regulation of CXCR7 induced by IL8 emerges as a promoter of castration-resistant tumors survival [15]. Moreover, CXCR7-depleted tumors showed significantly reduced levels of relevant factors for prostate tumorigenesis like cyclin D1, VEGF and phosphorylated epidermal growth factor receptor [15]. There is also additional evidence for a potential role of CXCR7 as a CXCL12 scavenger, suggesting that this receptor in turn modulates the activity of CXCR4 in tumor formation and is critical for the fine-tuning of the motility of hematopoietic cells in the bone marrow and lymphoid organs.

However, the blockade of the CXCR4 and CXCR7 only partially impaired the metastatic behavior of prostate cancer *in vivo*, arguing that other functional chemokine/chemokine receptor pairs may be involved in prostate cancer progression [18].

The third chemokine receptor noteworthy in prostate cancer is CXCR6, displaying high expression not only in prostate cancer cell lines but also in prostate tissues [19]. This receptor is also known as Bonzo, STRL33 or TYMSTR. In humans, Bonzo is expressed by small subsets of T cells and CD16+ cells, but not by B cells, monocytes or dendritic cells [20].

CXCL16 is one of the two known transmembrane chemokines. It is also constitutively expressed on fibroblasts, keratinocytes and cancer cells of various origin tissues [19]. CXCL16 was identified as the ligand for this receptor and was found to signal through NF- κ B via heterotrimeric G proteins/PI3K/PDK-1/Akt/IKK/I κ B [21]. It was also reported to signal through the Akt/mTOR pathway [22]. A variety of chemokines contain a conserved sequence motif (ELR, glutamic acid-leucine-arginine) that precedes the first cysteine residue near the amino-terminal end which is critical for the receptor binding, for the chemotactic activity and for the promotion of angiogenesis. Intriguingly, although lacking an ELR motif in the chemokine domain, CXCL16 appears as proangiogenic. CXCR6 was shown to regulate blood vessel formation by an autocrine/paracrine loop established between prostate cancer and endothelial cells and was observed that both IL8 and IL6 levels were altered in response to changes in CXCR6 expression [18]. The striking similarities between CXCL16 and CXCL12 are likely to result in additive effects [23]. Moreover, CXCL12 and CXCL16 were observed in tissues enriched with plasma cells and in cultured human bone marrow stromal cells [23]. Thus, plasma cells are likely to be recruited to bone marrow and other target tissues via CXCR4 and CXCR6 [18]. CXCL16 not only attracts T cells and natural killer T cells toward dendritic cells but also supports their firm adhesion to dendritic cells [24]. Taken together, high CXCL16/CXCR6 expression may be strongly related to aggressive cancer behavior, and particularly, high-secreted ligand expression to bone metastases of prostate cancer [19].

While it is well accepted that chemokines promote tumor development, these molecules may in turn be used to the benefit of cancer patients, acting in the recruitment of dendritic cells and /or effector cells or for their angiostatic properties. However, chemokine-mediated recruitment of immature dendritic cells within tumors, due to factors produced by the tumor milieu, may induce immune tolerance. In this context, the balance between positive and negative effects should be examined when designing novel strategies to eradicate tumors based on chemokine targeting.

2.1.3. Role of IL8 and IL6 in the transition to hormone refractory prostate cancer

Prostate cancer cells and the surrounding stroma are exposed to a plethora of interleukins and chemokines, receiving their signaling stimuli, re-enforcing tumor-promoting functions. Similar to other chemokines that recognize and bind G-protein-coupled receptors, IL8 acts through CXC receptors. The expression of CXCL8 (also known as IL8), one of the best-characterized members of the chemokine family, has been described as a key effector in prostate cancer. Normal prostate epithelial cells and tissues produce low amount of IL8, whereas prostate cancer cells from primary and metastatic tumors produce progressively greater amounts [25]. High levels of CXCL8 also correlate to an elevated adherence of the prostate tumor cells to the endothelium, hence increasing angiogenesis, tumorigenicity and lymph node metastasis *in vivo* [26, 27]. Even more, CXCL8 is a transcriptional target of NF- κ B and its expression is elevated in androgen independent prostate cancer, contributing to the transition to a castration-resistant state and to resistance to standard chemotherapeutic drugs [28]. To date, the chemotherapy strategy utilized for advanced prostate cancer disease is

based on the combination of docetaxel (a cytostatic drug) with prednisone (a glucocorticoid prodrug). However, this therapeutic strategy shows a modest survival benefit over palliative care, where many patients respond initially, but eventually develop a resistance to docetaxel. Among other factors, increased IL8 production decreases the sensitivity of hormone-resistant cells to the cytotoxic chemotherapeutic agents and also reduces prostate cancer cell apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). In experimental prostate cancer a naphthalimide was shown to decrease IL8 expression and to enhance taxol activity when co-administered with this compound. Thus, negative regulators of this chemokine could emerge as second line treatment for patients with docetaxel-resistant advanced prostate cancer [29].

One of the most interesting mediators clearly implicated in prostate cancer is IL6, a multi-functional cytokine, produced by inflammatory cells, osteoblasts and even prostate cancer cells. There are multiple lines of clinical and experimental evidence preponderantly showing that IL6 contributes to prostate cancer progression. Both, patients with prostate cancer and patients with advanced metastatic disease display high expression levels of IL6 and its soluble receptor in the circulating plasma [30]. These observations have led to study whether this axis could predict biochemical recurrence in radical prostatectomy patients [31] providing a rationale for the clinical relevance of IL6 as a prognostic factor. In particular, a phase II study assessed the efficacy of siltuximab, in men with castrate resistant prostate cancer that had been treated with one prior chemotherapy with the primary endpoint being PSA response rate (defined by a 50% reduction of PSA) [32]. This drug, also known as CNTO 328, is a human-mouse chimeric monoclonal neutralizing IL6 antibody. The response rate was small and no men with disease had a Response Evaluation Criteria in Solid Tumors (RECIST) response. This criterion defines a set of rules that assesses whether a patient improves ("responds"), stays the same ("stabilizes"), or worsens ("progression") during treatments. The results obtained evidenced the lack of a beneficial therapeutic effect of IL6 neutralization in patients with advanced androgen resistant disease. However, there are still some positive prospects for IL6 neutralization, providing an additional benefit to other chemotherapy regimens, especially in light of its anti-apoptotic effects [33].

In addition to the clinical observations, *in vitro* studies have provided evidence that IL6 modulates prostate cancer cell growth of hormone-refractory cells, but had no effect on the growth of hormone-dependent cell lines [33].

IL6 has also been implicated in other aspects of prostate cancer pathophysiology such as tumorigenesis in the prostate microenvironment. IL6 foremost effect is the activation of Janus kinase (JAK) signaling and of signal transducers and activators of transcription (STAT) proteins, especially STAT3. Through this signaling pathway, IL6 stimulates autocrine activation of insulin-like type I growth factor receptor (IGF-IR) to confer tumorigenesis [34]. Depending on the cellular context, IL6 can also signal through MAPK and phosphatidylinositol-3 kinase (PI3K) pathways [35, 36].

This cytokine can be produced autocrinally in castrate resistant prostate cells and can transactivate the AR in those cells. However, the AR status as well as other interacting signaling cascades will define the role of IL6 on ligand-independent AR activation, tumor formation,

and subsequent growth. Additionally, IL6 has been proposed to initiate an intracrine signaling pathway, alternative to the androgen receptor axis, affecting metabolic enzyme levels. Surprisingly, testosterone plasma levels were significantly increased when IL6 overexpressing prostate cancer cells were inoculated in castrated mice, showing that this cytokine regulates the expression of steroidogenic genes in tumoral cells [5].

Overall, IL6 strongly correlates with more advanced stages of the disease, therapy resistance, poor prognosis and can be predictive of recurrence after treatment of localized cancer. Based on all the clinical and preclinical evidence, further exploration for IL6 inhibition is justified; however, its efficacy may greatly depend on the stage of disease or other individualized factors.

2.1.4. Tumor Necrosis factor: Linking inflammation to prostate cancer

TNF was named for its ability to induce rapid haemorrhagic necrosis of experimental cancers [37]. However, it soon became noticeable that this cytokine presented anti-tumoral activity and cytotoxicity against several tumoral cells [38]. Currently, TNF is considered as a relevant player in host defense and inflammation with several activities extending far beyond its original anti-tumoral action. Among its effects, TNF signaling may lead to both, cell apoptosis and necrosis, and also to tumor progression and metastasis by switching on survival genes [39].

TNF signals through TNF receptor 1 (TNF-R1) and TNF-R2. While TNF-R1 is expressed constitutively in most tissues, TNF-R2 is modulated and is mostly found on immune system cells. TNF binds to the death domain containing TNF-R1 to recruit TNF receptor-associated death domain (TRADD), Fas-associated death domain (FADD) and caspase-8, forming the death-inducing signaling complex [40]. Interestingly, when TNF-R1 is activated, it also recruits receptor-interacting protein (RIP) and TNF receptor-associated factor 2 (TRAF2) and activates NF- κ B, involved in cell survival, proliferation, anti-apoptotic activity and highly implicated in the inflammatory response [41].

TNF α plays critical roles in cancer pathophysiology building an elaborate association between inflammation and cancer. It functions as a key regulator of the tumor microenvironment, promoting tumor progression, even in the absence of invading inflammatory cells [42]. It facilitates cancer development acting directly on neoplastic cells or indirectly through endothelial and other inflammatory cells [43]. However, the mechanisms by which TNF α enables these events are not fully described. A recent publication from Davis *et al.* [44] explains the dichotomy of TNF α effect on the control of apoptosis in prostate cancer cells. These authors propose a physiologic role for TNF α in prostate regression after androgen withdrawal. This factor is required for castration-induced prostate regression, but membrane-bound TNF α protein and stromal cell specific TNF α mRNA levels increase in rat prostate after castration, which is coincident with a paracrine effect of TNF α in prostate cancer regression. However, when wild-type non-castrated mice were treated with TNF α no regression of the gland was observed [44]. All these evidences showed that this cytokine acts in the context of supplemental castration-induced signals.

Summarizing, the chemokine scene displays a vast crosstalk of pathways involved in the day-to-day dialogue between the cancer cells and the inflammatory microenvironment. The challenge relies in identifying the homeostatic target/targets that govern this setting in order to successfully re-direct the therapeutic efforts against prostate cancer.

2.2. The oxidative stress imbalance in the prostate tumor: Gearing the journey to cancer

The development of cancer is a complex process. Cancer cells associate, both in primary as well as in secondary colonization sites with resident stromal fibroblasts, smooth muscle cells, macrophages, endothelium, neurons and migrating cells at metastatic niches and phenotypically and genotypically activate them, triggering different signaling mechanisms. During this process, the cancer cells and cells in the cancer microenvironment “co-evolve” in part due to oxidative stress, and acquire the ability to mimic other cell types (which can be termed osteomimicry, vasculomimicry, neuromimicry and stem cell mimicry), and undergo transition from epithelium to mesenchyme with definitive behavioral modifications. Prostate cancer cells co-evolve in their genotypic and phenotypic characters with stroma and acquire osteomimetic properties allowing these cells to proliferate and survive in the skeleton as bone metastasis [45]. ROS, RNS and other factors implicated in oxidative and nitrosative stress alters the homeostatic milieu, affecting macromolecules and damaging cell membranes, altering organelles permeability and function. Thus co-targeting different players in this complex scenario will be an effective treatment alternative for prostate cancer progression.

2.2.1. The prostate and its oxidative defense barriers

The normal prostate epithelium consists of prostatic ducts that contain basal cells, stem cells, secretory luminal cells and neuroendocrine cells. The stromal component consists of smooth muscle, fibroblasts, vascular endothelial cells, nerve cells, inflammatory cells, insoluble matrix and soluble factors. Inflammation is clearly associated to the early stages of prostate carcinogenesis [46]. The macrophages in the tumor microenvironment produce ROS and RNS. The increase in reactive radicals such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^{\bullet}), etc. produces DNA damage, causes genetic mutations and initiates/promotes cancer progression. Some molecules implicated in prostate atrophy include p53 and AR mutations, hypermethylation of the CpG island of the promoter of glutathione S transferase-P1 (GSTP1), decreased activity of manganese superoxide dismutase (MnSOD) and increased expression of NADPH oxidase 1, which initiate high grade prostatic intraepithelial neoplasia (PIN) and progressive prostate cancer [45].

The prostate gland depends on the androgen/AR signaling for growth. Activation of this axis in advanced prostate cancer has been attributed to various mechanisms, including AR hypersensitivity, *de novo* intraprostatic androgen synthesis, promiscuous AR activation via adrenal androgens, non-androgenic steroids and non-canonical AR activation via growth factors and cytokines through intracellular signal-transduction pathways [47]. These mechanisms may result from abnormalities in the AR status (e.g., mutation, splice variants) and/or

in the levels of its co-regulators. Furthermore, some AR splice variants have been identified with constitutive effects in the absence of ligands [48].

ROS are endogenously generated during cellular metabolic processes. It can also come from external sources. Thus, excessive ROS production or impairment of antioxidant defense systems can induce oxidative stress. This increase in ROS levels may contribute to the initiation and development of various cancers, including prostate cancer, because oxidative stress regulates cellular fate in various systems. ROS are considered to be tumor initiators/promoters given the potential for induction of DNA damage. Furthermore, signaling pathways in response to intracellular changes in ROS levels may trigger proliferation, apoptosis and senescence, events highly implicated in all the stages of the carcinogenic process. However, little is known about the exact molecular machinery that mediates ROS function in the tumorigenic process. Several transcription factors that regulate AR activity/transcription are implicated in oxidative stress, among them, NF- κ B, c-Myc, CREB, Sp1 and Foxo3a [49]. Interestingly, castration-induced oxidative stress in prostate cancer cell lines increased AR levels through the overexpression of an oncogene member of the basic helix-loop-helix transcription factor Twist 1, which regulates the expression of AR by binding to E-boxes in its promoter, resulting in a gain of castration resistant phenotype [50] and being responsible of metastasis [51]. Evidently, there is a connection between oxidative stress and androgen deprivation in prostate cancer, which is also supported by previous observations of increased oxidative damage associated to the development of malignancies [52]. Of interest, when comparing the expression profile of castration resistant prostate cancer gene with the genetic landscape of hormonal sensitive tumors, the endogenous antioxidant defense system is clearly repressed, in particular MnSOD, which regulates ROS production by converting superoxide to a less reactive species, acting as a ROS scavenger. Hence, MnSOD in advanced prostate cancer could be mechanistically linked to AR reactivation. An array for transcription factor DNA binding activity showed that AR (among other transcription factors) binds to DNA after MnSOD knocked-down [53]. These findings correlate with a clear transcriptional repression of stress-related genes [54].

2.2.2. Is oxidative stress governing the co-regulators of nuclear receptors?

Co-regulators of transcription orchestrate the action of nuclear receptors. Each tissue has a "quantitative finger print" of co-activators based on the relative inherited concentrations of these molecules. When the cellular concentration of a co-activator is altered, genetic dysfunction usually leads to a pathologic outcome. Co-regulators contain the potential to efficiently promote cellular pathologies by coordinately misdirecting multiple independent functions such as oncogenesis. During the development and progression of prostate tumors there are a misregulation of AR co-activators, many of them play a critical role in redox maintenance protecting cells from cytotoxicity produced by oxidative stress. That is the case with peroxiredoxin (Prx), a gene elevated in cancer with anti-oxidant capacity. Prx1, a co-activator that facilitates the binding of androgen to the AR, is regulated by nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2), a transcription factor also induced by oxidative stress. Another member of this family, Prx2, is also regulated by oxidative stress but in this

case through Foxo3a, another transcription factor implicated in AR transcription and cellular responses to oxidative stress and overexpressed in the castrate resistant-disease. Remarkably, the subcellular distribution of co-regulators seems to be relevant in the regulation of the AR activity. While cytoplasmic Prx2 enhances AR transactivation, its nuclear localization decreases the receptor activity, suggesting that the redox status of the nucleus and cytoplasm might affect AR signaling through this co-regulator [55].

2.2.3. Oxidative stress and tumor-stroma co-evolution

Since the initial seed and soil hypothesis elaborated by Paget in 1889 [56], the relevance of the tumor microenvironment in the carcinogenic process is continuously on scene. Tissue recombination experiments with mixed prostate stromal/epithelial cell xenografts surprisingly revealed that transformation of epithelial cells is accompanied by a transdifferentiation of fibroblasts. Prostate stroma is mainly composed of fibroblasts and smooth muscle cells, and an intermediate cell type described as myofibroblast. The highly proliferative stromal cells immediately surrounding malignant glands have been described as “reactive stroma” or “carcinoma-associated fibroblast” (CAF) [57]. Wound repair exhibits a fibroblastic switch to a myofibroblast-like phenotype, with the subsequent extracellular matrix (ECM) remodeling through angiogenesis and increased protease activity [58]. The “reactive stroma” of a malignant tumor may parallel the granulation tissue of a healing wound in many ways, behaving as wounds that never heal. This “reactive stroma” comprises multiple cell types, which have been altered from their original state to become permissive of prostate cancer cell progression. In human prostate cancers, the “reactive stroma” displays increased number of myofibroblasts, amplification of ECM proteins, and increased local vascular density, properties almost identical to those seen in granulation tissue. Intriguingly, there is still no effective marker of “reactive stroma” available. The receptors activated by serine proteases (PARs) are good candidates as PARs play key roles in tissue remodeling and cancer invasion. Other key signaling mediators also involved in the “reactive stroma” phenotype include tumor growth factor beta (TGF β), partly responsible for fibroblast transdifferentiation. Other fibroblastic and smooth muscle markers participate in the transformation phenomena, such as vimentin and smooth muscle α -actin. However, TGF β also affects the cancer cell itself, accomplishing contrary roles in the different stages of cancer evolution. Even in precancerous PIN lesions elevated TGF β expression was detected in epithelial cells. In addition to TGF β , chronic inflammation has also been the focus in the development of prostate cancer. Several characteristics of chronic inflammation are increased, such as the induction of the proinflammatory enzyme COX-2 and production of ROS and RNS. In turn, the infiltration of macrophages and leukocytes together with COX-2 activation, further enhances the burst of oxidative stress, promoting a more aggressive phenotype.

2.2.4. Oxidative stress triggers metabolic reprogramming

Mounting evidence recollected in the last paper of Hanahan and Weinberg [59] display compelling data on oxidative stress as a scaffold of the well-established hallmarks of cancer. Oxidative stress players are expressed abnormally in tumors, positively affecting compulsory

stages of the carcinogenic process, by stimulating cell proliferation and anchorage independent cell growth, causing insensitivity to apoptosis, sustaining *de novo* angiogenesis, and by altering the migration/invasion program through metabolic and epigenetic mechanisms. ROS mediates ligand-independent transactivation of receptor tyrosine kinase and ERK activation affecting proliferation, promoting tissue invasion and metastatic dissemination due to MMP secretion/activation. Furthermore, ROS induce the release of VEGF and angiopoietin promoting angiogenesis and evading apoptosis/anoikis [60-62].

In cancer cells, high levels of ROS can result from increased basal metabolic activity, mitochondrial dysfunction due to hypoxia or mitophagy, peroxisome activity, uncontrolled growth factor of cytokines signaling and oncogene activity, as well as from enhanced activity of known ROS sources as NADPH oxidase, COX or lipoxygenases [62]. It is well accepted that the activity of oxidants on tumors depends on their mutagenic potential, their capacity to rule the intracellular signaling pathways governing cellular homeostasis and their recognized role in stromal reactivity, mandatory for cancer development and dissemination [63, 64].

Cell vulnerability appears as a consequence of the oxidative status of their constituents promoting spontaneous and therapy induced cell death. Thus, resistance to oxidative stress is positioned as a major mechanism of tumor chemo- and radio-defense.

The tumor hypoxic microenvironment as well induces this "reactive stroma", affecting the cancer cells motility, and consequently generating a more aggressive tumor, which can metastasize to the bone. Hypoxia generates ROS production and likewise anti-oxidants agents have shown to suppress hypoxia induced epithelial to mesenchymal transition (EMT), impairing the metastatic phenotype [65]. The "reactive stroma" recruitment to the cancer foci begins early during carcinogenesis and its co-evolution is predictive of human cancer progression, which is facilitated by tumor-stroma interactions.

It is of particular significance that many genes, which are regulated by oxidative stress, are targets of NF- κ B [66]. NF- κ B is constitutively activated in human prostate carcinoma and correlates with disease progression [67]. NF- κ B is an inducible transcription factor that belongs to the Rel/NF- κ B family. Increasing evidence suggests that inhibition of NF- κ B activity in prostate cancer cells can suppress angiogenesis, invasion and metastasis by down-regulating the expression of NF- κ B downstream target genes, such as VEGF, plasminogen activator type urokinase and MMP-9 [68]. Additionally, heme-oxygenase 1 (HO-1), the rate-limiting enzyme in heme degradation, confers cytoprotection against oxidative stress and inflammation [69]. This protein exerts vital metabolic functions limiting the axis of heme degradation and maintaining the cellular homeostasis. Several signaling molecules are implicated in the cytoprotection conferred by HO-1, including NF- κ B and PI3K/Akt [70]. Although classical recognized as a microsomal protein, its presence has been detected in other subcellular compartments [71, 72]. Recent studies have reported that HO-1 suffers a proteolytic degradation in its hydrophobic C-terminal domain, which would facilitate its entrance to the nucleus [73]. It has been proposed that HO-1 possesses in the nucleus a non-catalytic canonical function participating in the regulation of the activity of several nuclear transcription factors and also regulating its own transcription [72,

73]. Moreover, it has been documented HO-1 nuclear expression in human primary prostate carcinomas [71]. It has also been reported that it impairs prostate tumor growth *in vivo* and down-regulates the expression of target genes associated with inflammation and angiogenesis [74, 75]. However, clinical data demonstrated a statistically significant difference in HO-1 epithelial expression between benign, high-grade PIN, localized prostate cancer, and advanced prostate cancer, where castration resistant disease presented the highest HO-1 expression followed by benign tissue. This work provides experimental evidence for a cross talk between epithelial HO-1 expression and PTEN deletions, which are associated with adverse clinical outcome [76].

Altogether these findings may indicate that the oxidative stress imbalance may strongly influence the prostate carcinogenic process and may also cooperate in the bone homing of prostate cancer, the most clinically significant aspect of this disease. The stromal–epithelial interaction gains therapeutic relevance, as prostate carcinoma cells must induce the hospitality of bone cells in order to take up residence in an osseous microenvironment.

2.3. MicroRNAs as emerging key players in the etiology and progression of prostate cancer – Clinical implications

MicroRNAs (miRNAs or miRs) are short non-coding RNAs (18-24 nucleotides) regarded as a novel class of regulatory molecules that suppress gene expression at the post-transcriptional level. miRNA genes are, in general, regulated and transcribed in the same manner as a protein-coding gene. They are transcribed by the RNA polymerase II into long primary transcripts (pri-miRNAs) that can contain the precursors of one to several clustered miRNAs. These primary transcripts are then cleaved by endonucleases (Drosha) to produce the pre-miRNAs which consist of ~70-nucleotide hairpin structures. The pre-miRNAs are further processed in the cytoplasm by the Dicer complex into the mature miRNAs which are incorporated into the RNA-induced silencing complex (RISC) that execute the regulatory activity through the binding to the 3' untranslated region (3'UTR) of target mRNAs having complementary sequences. The formation of the mRNA/miRNA duplexes, lead to mRNA degradation, inhibition of translation, or a combination of both.

At present, there are more than 1,600 human miRNAs entries in the miRBase release 19 [77]. Each of these molecules may regulate the expression of hundreds of genes within one cell, and one particular target may be regulated by several miRNAs via different binding sites, creating an extremely complex regulatory network for gene expression. Indeed, it has been estimated that about 60% of the protein-coding genes are targets of miRNAs [78]. In recent years, rapidly growing evidence has established the significance of miRNAs in different physiological processes such as development and differentiation, cell cycle, metabolism, homeostasis and apoptosis [79].

On the contrary, an altered expression of these regulators play an important role in diseases, including carcinogenesis [80]. Quantitative alterations, either genetic or epigenetic, may modify the expression levels of miRNAs, and are associated with tumor development and progression in various tumors. More than half of the deregulated miRNAs map at, or near to, cancer-associated *loci* prone to deletions, amplifications and translocations [81]. Qualita-

tive changes can also arise when there are mutations that disrupt or create miRNA recognition sites. Therefore, miRNAs may contribute to carcinogenesis acting as oncogenes, called oncomirs, if they promote tumor growth when they are over-expressed. They may also act as tumor suppressors when they stimulate cancer development and progression when they are down-regulated. As a general rule, oncomirs target tumor-suppressor gene mRNAs (e.g. miR-21 regulates PTEN), and tumor-suppressor miRNAs target proto-oncogene mRNAs (e.g. let-7 regulates KRAS).

miRNAs, as well as mRNAs, display tissue-specific expression profiles and, therefore, they may have different roles in cells from different origins. An example of this disparity is miR-125b which can have a tumor suppressor activity in ovarian and breast cancers but act as an oncomir in prostate cancer, thyroid cancer, neuroblastoma and glioblastoma [82]. The study of the global miRNA expression levels (miRNAome) has been rising in the past years and abundant miRNAome data are currently available for several cancers. The miRNA expression patterns in different types of tissues have been reported to be more predictive of tumor origin and differentiation status than mRNA profiles because, unlike mRNA expression, a modest number of miRNAs (~200 in total) might be sufficient to classify human cancers [83]. In prostate cancer, the expression of several miRNAs and their target mRNAs are altered and involved in development, invasion and metastasis. Nevertheless, the data on miRNA expression in prostatic tumors are still conflicting and, at present, a conclusive miRNA profile cannot be recognized. In this section we describe miRNAs that have been studied in the context of prostate cancer and summarize their possible application in disease diagnosis and prognosis.

2.3.1. miRNAs associated to prostate cancer

The expression of miR-21 is up-regulated in many types of cancers, including prostate cancer, glioblastoma, lymphoma, pancreatic cancer, and lung cancer, among others [84, 85]. miR-21 can act as an oncomir that contributes to prostate tumor growth, resistance to apoptosis, invasiveness and metastasis. Its regulatory activity probably involves the down-regulation of the tumor-suppressor gene PTEN (commonly lost or down-regulated) programmed cell death 4 (PDCD4), tropomyosin 1 alpha (TPM1), and myristoylated alanine-rich proteinase kinase C substrate (MARCKS), among other genes. miR-21 was found to be over-expressed in androgen-independent prostate cancer cell lines but its expression is low in androgen-dependent prostate cancer cells; therefore, it may be responsible, at least in part, for the development of castrate-resistant tumors. AR can bind to miR-21 promoter resulting in an androgen-dependent transcriptional regulation of miR-21; consequently androgen-dependent miR-21 expression may contribute to prostate cancer pathogenesis. In support of these findings, an *in vivo* study showed that miR-21 is over-expressed in human prostate tumor samples compared to the matching normal tissue, and tumor growth was accelerated in xenograph models when miR-21 expression was elevated [86].

miR-221 and miR-222 are two highly homologous oncomirs that are frequently over-expressed in different cancers. In primary prostate carcinomas and cell lines, these two miRNAs inversely correlate with the expression of the tumor suppressor gene p27, which is a

well-established marker of poor prognosis in prostate cancer and other types of tumors [87]. *In vitro* and *in vivo* experiments link these two miRNAs to prostate cancer development and progression. Furthermore, miR-221 and miR-222 contribute to the growth and maintenance of castration-resistant prostate cancer (CRPC) through mechanisms that comprise the AR signaling.

Another oncomir, miR-125b, was reported to be over-expressed in androgen-independent prostate cancer lines and was also implicated in the hormone independent growth. The mRNA of the pro-apoptotic protein Bak1, which was found down-regulated in CRPC, is a target of miR-125b. However, this miRNA was also suggested to act as a tumor suppressor in a different context because it was found to be down-regulated in CRPC and in breast cancer where it silences the expression of HER-2/*neu* [88]. Interestingly, it was also reported that HER-2/*neu* is over-expressed in the progressing prostate tumors [89]. Therefore, the relevance of miR-125b in prostate cancer progression needs further investigation to assess its role in prostate carcinogenesis.

miR-101-1 and miR-101-2 map in two *locus* (1p31.3 and 9p24.2, respectively) that are commonly deleted in localized and metastatic prostate cancer. In addition, the loss of miR-101-1 or -2 is associated with the over-expression of EZH2, a histone methyltransferase enzyme that is a direct target of miR-101. The up-regulation of this miRNA reduced the proliferation and the invasive potential of the DU145 cell line. COX-2 is another target of miR-101, linking the miRNAs portray to chronic inflammation and tumor development via the COX-2/prostaglandins pathway [90]. *In vitro* studies have shown that there is an inverse correlation between miR-101 and COX-2 in different prostate-derived cell lines, and the over-expression of miR-101 reduces the proliferation rate of the COX-2-associated benign prostatic hyperplasia cell line [91]. Similarly, experimental models by inoculation of cells into BALB/c athymic nude mice demonstrated that the miR-101 over-expressing clone showed a slower tumor growth. Furthermore, the treatment of the tumorigenic BPH1 cell line (BPH^{CAFTD}) with exogenous miR-101 resulted in an inhibition of prostate cancer growth *in vitro* and *in vivo* [91]. Similarly, the over-expression of miR-128a reduced invasion capability of the androgen independent prostate tumor cell line, DU145, and was found to be progressively decreased in tissues from benign prostatic hyperplasia, to localized prostate cancer and to distant metastasis [92].

Another tumor suppressor miRNA that was reported to play a role in prostate cancer progression to CRPC is miR-146. This miRNA is down-regulated in androgen-independent cell lines and CRPC tissues compared to androgen-dependent cell lines and non-tumor epithelial tissues [93]. The mechanism of action of miR-146 consists of the inhibition of the expression of ROCK1 (Rho-activated protein kinase 1), which is a member of the hyaluronan/CD168 pathway involved in prostate cancer invasion and metastasis.

PKC ϵ (protein kinase C epsilon) and ZEB2 (zinc finger E-box binding homeobox 2) are two proteins involve in the migration and invasion capabilities of prostate cancer cells and their expression is regulated, at least in part, by miR-205. This miRNA was reported to be down-regulated in prostate cancer cell lines and carcinomas compared to the non-tumorigenic cell line RWPE-1 and normal prostate tissues, respectively. miR-205 also induces genes involved

in cell-cell junctions and down-regulates genes associated with prostate cancer progression such as IL6, caveolin-1, EZH2, ERBB3, E2F1 and E2F5.

This list is just a small part of all miRNA alterations found in prostate cancer (For a more complete list of miRNAs in prostate cancer, the reader may refer to the review written by Coppola *et al.*[85] and Pang *et al.*[94]), but other players cannot be discarded.

2.3.2. miRNAs as biomarkers for prostate cancer diagnosis and prognosis

Based on the evidence that miRNAs may be deregulated in different pathologies in a tissue-specific manner, multiple studies have investigated the potential use of the miRNAome as a biomarker. As a consequence, a growing amount of evidence proposes that the miRNAome can be used as a tool to better define pathological signatures and, in turn, to accurately differentiate tumors according to their origin and cellular lineage. In addition, miRNAs meet other important requisites that may allow their use as biomarkers for cancer diagnosis and prognosis: 1) miRNAs are remarkably stable molecules in different types of clinical samples, including formalin-fixed paraffin-embedded (FFPE) tissues which is the standard technique used for long-term conservation of biological samples, 2) they can be analyzed by simple methods such as quantitative retro-transcriptase polymerase chain reaction (qRT-PCR), and 3) the lack of intricate transcriptional and translational regulation compared to mRNA.

The tumoral expression of miR-1 and miR-133a correlates with tumor progression. Interestingly, the relapse-free survival of patients with prostate cancer can be predicted by the expression of miR-1 in the tumor specimens. Patients with tumors having low miR-1 expression are more likely to have a biochemical relapse than patients with tumors having high miR-1 expression [95].

Besides their intracellular function, miRNAs can also be released by cells and circulate in the blood stream. Consequently, miRNAs can be isolated from serum and plasma; evenmore, they can be isolated from other body fluids such as urine, saliva and semen. The discovery of circulating miRNAs opened up intriguing possibilities to use the circulating miRNAome as one additional biomarker to improve cancer diagnosis, determine tumor staging more accurately and predict prognosis. Some reports demonstrate that miRNA levels in body fluids may change under certain pathological conditions, including prostate cancer [96]. For this reason, within the past years, studies on miRNAs in cancer have burst onto the scene, and evidence that miRNAs may represent new diagnostic and prognostic molecules in human cancers is rapidly accumulating. However miRNA levels as tools for diagnosis and prognosis in prostate cancer are still limited [96].

Although, serum and plasma levels of miR-141 seems to be one of the most promising markers for prostate cancer diagnosis because they are consistently increased in men diagnosed with this carcinoma compared to healthy individuals; the differences are statistical significant only when the comparisons are made between healthy persons and advanced prostate cancer patients [96]. miR-141 is also elevated in prostatic tumor specimens, suggesting that the raise of this molecule in the body fluids is originated by the tumor cells and increases as

disease progresses. Serum levels of other miRNAs are also altered in specimens from men with prostate cancer when compared to healthy individuals (e.g. miR-21, miR-200, miR-221, miR-375, and others), but results are inconsistent among reports.

miR-141 was also studied as a predictor factor for prostate cancer classification. One study showed increased levels of serum miR-141 and miR-375 in high-risk patients (Gleason score ≥ 8 or N1) compared to low-risk patients (Gleason score 7 or N0) [97]. Another study found that serum miR-21 is increased in patients with CRPC resistant to docetaxel, opening the possibility to use serum miRNAs as markers of therapeutic response as well [98]. Unfortunately, the specificity and sensitivity of miRNAs when used as single markers for prostate cancer diagnosis and prognosis are similar to the specificity and sensitivity of other markers currently used (e.g. PSA).

In summary, miRNAome from serum or plasma samples may not add much information for prostate cancer diagnosis, outcome and response to therapy when used as a single biomarker. In addition, it is unlikely to achieve the desired level of accuracy for prostate cancer diagnosis or prognosis, because one miRNA may be altered in many different diseases. Furthermore, one mRNA can be affected by several miRNAs. Therefore, circulating miRNAome should be considered an additional tool to improve the accuracy of current diagnostic molecules such as PSA, and other diagnostic tests such as the digital rectal exam, echography and others. Similarly, the tumor miRNAome may help to improve the pathological classification of prostate tumors. Up to date the miRNA profile cannot substitute other clinical tools, but can efficiently supplement them.

2.3.3. Targeting miRNAs as therapeutic strategies

The discovery of miRNAs a decade ago and the subsequent study of their role in the pathogenesis of disease, unveiled a new scenario where miRNA modulators could be used in order to restore the homeostasis of an altered cell or tissue. Recently, a novel class of synthetic inhibitory molecules (antagomirs) that compete with target mRNAs for the binding of miRNAs, allowing mRNA translation, has been introduced as silencers of oncomirs. The antagomirs uncover the way to miRNA-based therapeutic strategies. As the number of *in vivo* studies that analyze the use of miRNAs as therapeutic molecules is restricted to a very small number, further investigations are needed. In spite of all the data being generated, the knowledge and understanding of miRNA in prostate cancer is still at the early stage. Once the normal/pathological role of each alteration is deciphered, and the results validated in a vast cohort of patients, the selected miRNAs might be attractive candidates for prostate cancer diagnosis, patients' management and therapeutic strategy.

2.4. The nuts and bolts of prostate cancer survival, mastering the tumoral vasculature: angiogenesis, vasculogenic mimicry or vessel co-option?

2.4.1. Angiogenesis as a hallmark of cancer

The hallmarks of cancer define distinctive and complementary capabilities that allow tumors to grow and disseminate. One of those capacities is the induction of angiogenesis. This

process specifically refers to the sprouting of new blood vessels from pre-existing ones, involving proliferation of endothelial cells and migration towards pro-angiogenic molecules. The expansion of the existing vasculature also relies on the accumulation of circulating endothelial progenitor cells. The latter are immature endothelial cells, typically arising in the bone marrow, with the capacity to extravasate in response to pro-angiogenic factors and promote new vessel formation known as vasculogenesis. This process also takes place in the tumor microenvironment; however, it is generally associated with embryogenesis and development and involves the birth of new endothelial cells and their assembly into tubes in addition to the sprouting. Following this morphogenesis, the normal vasculature results in a quiescent action, becoming in the adult only an active process in wound healing events and in female reproductive cycling, but only transiently.

The tumor and its microenvironment display a completely different scenario, allowing pro-inflammatory molecules to switch on the angiogenic process enabling the tumor to grow, persist and disseminate. The tumor-associated angiogenesis was previously considered to be important in growing macroscopic tumors; however, the clinical evidence show that it directly contributes to the microscopic premalignant phase of neoplastic progression, further securing its position as an integral hallmark of cancer. This angiogenic switch is governed by angiogenic regulators that bind to stimulatory or inhibitory cell-surface receptors displayed by vascular endothelial cells. The well-known inducers of angiogenesis include among others: VEGF-A, TGF β and IL8; while inhibitors include: thrombospondin-1 (TSP-1) and angiostatin, among others. In tumors, these molecules support the rapid division of tumor cells [59]. VEGF signaling occurs via three main subtypes of receptor tyrosine kinases known as VEGFR1, VEGFR2 and VEGFR3. Its expression can be upregulated both by hypoxia and by oncogene signaling [99, 100]. Additionally, VEGF ligands can be sequestered in the ECM in latent forms that can then be activated by ECM-degrading proteases such as MMP9. Also the fibroblast growth factor (FGF) family is capable of activating VEGF and has been implicated in sustaining tumor angiogenesis. TSP-1 emerges as a counterpart of the angiogenic process, that when activated suppresses proangiogenic stimuli [101]. Of note, *Ras* and *Myc*, dominant oncogenes can also upregulate angiogenic factors in the tumoral microenvironment, and these signals can also be produced indirectly by immune inflammatory cells.

It is of particular interest the fact that angiogenesis inhibitors, such as TSP-1, angiostatin and endostatin offer natural barriers to tumor angiogenesis. This was described by Ribatti *et al.* [102], followed by several studies reporting other endogenous inhibitory agents. Most of these molecules appear to derive from proteolytic cleavage of structural proteins that are not angiogenic regulators *per se*, and some can be detected in normal mice and human plasma. These agents serve under normal circumstances as physiologic rheostats modulating angiogenesis during tissue remodeling and wound healing but may also act as intrinsic barriers to the sustained angiogenesis in emerging neoplasias.

How do these counterpart molecules behave in the tumoral process? How can we decipher the cross talk of this aberrant mix of proangiogenic signals? A massive amount of information describes the features of a cancer cell. However, it is wise to acknowledge the differen-

tial concepts of causes, oncogenic events, signal transduction programs, and hallmarks to show that there is a complexity under this network of interrelations that dynamically changes in different cells, between cells, and most importantly at different times in any given cell. Cancer is an evolving, heterogeneous system, hence the intricacy of the forming vasculature supporting tumor growth and progression.

2.4.2. Intussusception and vessel co-option

While sprouting angiogenesis requires VEGF for endothelial cells to proliferate, migrate and mature into new vessels, in the absence of this factor, the blood vessels split into new vessels without the need of endothelial cell proliferation. This phenomena is termed intussusception and has been demonstrated in various tumors [103]. Intussusception cannot be stopped by anti-VEGF strategies.

Intussusceptive microvascular growth refers to vessel network formation by insertion of connective tissue columns, called tissue pillars, into the vessel lumen and to the subsequent growth of these pillars, resulting in the sub-division of the vessel lumen. Intussusception is observed in a variety of normal and malignant tissues. It is faster and more inexpensive than sprouting, occurring within hours or even minutes and besides its autonomy from endothelial cell proliferation, it also becomes independent from basement membrane degradation, or even invasion of the connective tissue. However, intussusceptive microvascular growth displays a limiting factor: it can only work on existing vessel networks. Therefore intussusceptive microvascular growth has the ability to increase the complexity and density of the tumor microvessel mesh already built by sprouting. Although the molecular networks underlying this vascularization mechanism are poorly understood, the role of some local stimuli, such as intravascular shear stress, may induce a cascade of physiological or pathological reactions in endothelial cells, such as new capillary development by tissue pillar formation [104].

The absence of intense endothelial cell proliferation in intussusceptive microvascular growth implies that neovascularization by this mechanism would be resistant to angiostatic treatment in itself. Clinically, accumulation of tumor blood vessels by intussusceptive vessel growth is associated with a poor outcome for various types of cancers [105].

Until recently, vascularization of malignant tumors was considered the exclusive result of directed capillary ingrowth (endothelial sprouting). However, recent advances have been made in identifying the processes involved in angiogenesis and vascular remodeling. Consequently, the simplistic model of an invading capillary sprout has been deemed insufficient to describe the entire spectrum of morphogenic and molecular events required to form a neovascular network. Cancer tissue can acquire its vasculature by co-option of pre-existing vessels, intussusceptive microvascular growth, postnatal vasculogenesis, glomeruloid angiogenesis, or vasculogenic mimicry [103, 105].

Before discussing the different ways a tumor is vascularized, we should highlight that these mechanisms may not be mutually exclusive; the literature has shown that in most cases there is a cross-talk between these systems, participating in conjunction in physiological as

well as in pathological angiogenesis. Although the various types of cancer vascularization may share similar molecular signaling cascades and may be controlled partly by almost identical regulatory factors, a significant variety of differences also prevail.

It is widely accepted that the primary tumors and metastases have an initial avascular growth stage and then the angiogenic switch is turned on to support the exponential tumor growth. Tumor-induced angiogenesis and tumor cell vessel interactions are one of the most important events during all the stages of tumor development. However, it is not fully understood what is exactly happening before or during the initiation of vascularization of the primary tumor and the micrometastasis. In the beginning malignant cells may associate with and grow preferentially along pre-existing microvessels, prior to building their own vasculature. This process is called vessel co-option and was first proposed by Holash *et al.* [106]. Although at first, it is limited to the early stages of human tumorigenesis, morphological evidence suggests that co-option of pre-existing blood vessels might persist during the entire period of primary or metastatic tumor growth. During solid tumor growth, no signs of directed vessel ingrowth can be appreciated; instead, these tumors decide to develop by co-opting the massive vascular plexus present in the peritumoral connective tissue. Several controversies have been raised regarding how tumors progress, whether microtumors may initiate growth by exploiting pre-existing vessels without inducing angiogenesis or initiating through the induction of angiogenic sprouts from host vessels [107]. These discrepancies may have aroused given the differences in vascular niches in applied experimental models. Although unresolved from a mechanistic point of view, this uncertainty may raise important challenges when outlining a rationale for therapeutic strategies. This implies that, whereas compounds may be efficient inhibitors of angiogenesis and tumor growth in angiogenesis-dependent tumors (such as subcutaneous tumor xenografts), their effects may be limited in tumors growing in tissues with an intrinsic vascular density that allows for co-option by infiltrative tumors or other forms of neo-vasculature.

Based on this knowledge, new ways to inhibit the various vascular modalities have been developed in the past decade. When applying these targeted therapies, there are several aspects to take into consideration: the stage of tumor progression, the type of vascularization of the cancerous tissue and the molecular signaling networks behind the vascularization process.

What are the key aspects in determining the vascularization patterns of tumors? First, the local microenvironment, important during tumor initiation. Second, the cell number, subsidizing microtumors ability of inducing angiogenesis. Moreover, to trigger exponential growth, tumors must depend on vascularization through angiogenesis, which is much more powerful than vessel co-option to increase the tumoral mass and to acquire nutrition and oxygen from the host circulation system. If possible, tumors will prefer this kind of vascularization pattern. Alternatively, another choice is the strategy of co-opting host vessels in order for tumor cells to survive when they cannot acquire enough support from its niche and have no capacity to establish intrinsic vessels through angiogenesis. This is consistent with the observations that anti-angiogenic therapies result in an increase of vascular co-option

[108]. Third, the co-option and migration along host vessels will be inhibited once angiogenic sprouts begin to be induced.

Of note in liver metastases of human colorectal carcinomas, different growth patterns can be observed, depending on the degree of differentiation. These liver metastases represent a truly heterogeneous group and their growth patterns (replacement, pushing and desmoplastic) predict the fraction of immature blood vessels, the fraction of proliferating endothelial cells and the fraction of apoptotic tumor cells. The replacement growth pattern expands mainly by co-opting the stroma with the sinusoidal blood vessels of the liver [109].

The use of anti-vascular endothelial growth factor antibodies have been used for the abrogation of angiogenesis and growth of human prostate carcinoma microtumors and even metastasis in orthotopic prostate cancer xenografts. Although up to date there are no reports suggesting that vessel co-option is also an alternative route for growth and dissemination of prostate tumors, the contribution of this vascular route to prostate tumorigenesis needs further exploration; specifically, the involvement of this survival tool for growth of microtumors [110, 111].

Many studies have reported the close association between host vessels and extravasated cells during the onset of metastases. The co-opting manner makes these tumoral cells cover vessel surface area as much as possible and obtain the necessary support from host, such as nutrients or oxygen, with remarkable vessel-like pseudopodia. As Weinberg articulated for this kind of behaviour "tumor cells require effective interactions with the vasculature in order to acquire nutrients and to shed metabolic waste products and carbon dioxide.... In some normal tissues with an especially high metabolic activity, most cells enjoy direct contact with at least one capillary. This intimate association means that their access to oxygen and critical nutrients not dependent on the diffusion of these molecules over large distances and through densely packed cell layers" [112].

The tumoral vascular picture clearly displays differential contributions of vessel co-option and angiogenesis at the earliest stage of tumor initiation and metastasis. While angiogenesis appears as a key player for tumor exponential growth, the strategy of co-opting host vessels seems indispensable for cancer cell survival. Future anti-vascular therapies should seriously take into consideration the alternative ways in which a tumor disseminate and evades conventional anti-angiogenic treatments.

2.4.3. Vasculogenic mimicry

How can we distinguish normal angiogenesis from tumor-associated angiogenesis? Tumor neovasculature is marked by precocious capillary sprouting, convoluted and excessive vessel branching, distorted and enlarged vessels, erratic blood flow, leakiness leading to blood lakes, and distorted levels of endothelial cell proliferation and apoptosis [59]. Also, certain types of cancer cells have the capacity to mimic the activities of endothelial cells and to participate in processes that involve the formation of a fluid-conducting, matrix-rich meshwork, metamorphosing into vessels that either carry blood or connect to the host's blood supply. This new mechanism, by which some aggressive tumors may acquire a blood supply, was

first described by Maniotis and coworkers [113] and was termed 'vasculogenic mimicry'. However, it cannot be considered a vasculogenic event as true vasculogenesis involves de novo formation of endothelial cell-lined vessels. Since its discovery, vasculogenic mimicry has been catalogued in several types of tumors. How does vasculogenic mimicry contribute to tumor growth and progression, and can it be targeted by therapeutic agents?

Several interpretations of vasculogenic mimicry have evolved since tumor angiogenesis was recognized as not the only mechanism of blood supply for tumor microcirculation. Vasculogenic mimicry describes the ability of aggressive tumoral cells to express endothelium-associated genes and to form ECM-rich vasculogenic-like networks in three-dimensional (3D) cultures. These new vessels have no endothelial lining and are mainly composed of basement membrane-like material. The formation of these networks, seem to mimic the embryonic development of vasculogenic meshes and they were associated with the distinctly patterned ECM-rich networks that are observed in aggressive tumors. Since its discovery, vasculogenic mimicry has been described in several kinds of tumors, including melanoma, synovial sarcoma, rhabdomyosarcoma, osteosarcoma, breast carcinoma and ovarian carcinoma. Most of these studies correlate the aggressiveness of the tumor with angiogenesis or vasculogenic mimicry proliferation [114]. But how do they form and what is their contribution to tumorigenesis?

In the beginning, researchers observed in xenograft models and human biopsies, patterned loops and arcs that confined spheroidal clusters of tumoral cells. These loops and arcs formed networks that were lined with cancer cells and contained laminin and other components of the ECM yet not explored. Studies of tumor-tissue sections showed that the spheroidal tumor clusters contained either small, channel-like spaces between them, or seemed to be partially or totally juxtaposed by ECM. Some of these channel-like spaces were originally defined as 'vascular channels', because they were found to contain erythrocytes and plasma and were thought to provide a perfusion mechanism and a dissemination path within the tumor that might work independently or together with angiogenesis or vessel co-option.

Blood lakes within the tumor are another physiological phenomena that also draw attention. These are large collections of extravascular erythrocytes lining tumor spaces or channels. As hemorrhage is a manifestation of the defective endothelial barrier function in tumors the reason as why some tumors are bloodier than others, might rely on the balance between erythrocyte extravasation and the vessel wall stability. Rapid endothelial cell proliferation and defective pericyte coverage might contribute to the instability of tumor vessel walls leading to this hemorrhage. Pericytes are supporting cells that are closely apposed to the outer surfaces of the endothelial tubes in normal tissue vasculature, providing mechanical and physiologic support to the endothelial cells and have been associated with the maintenance of a functional neo-vasculature of most if not all tumors [115].

The literature on vasculogenic mimicry in prostate cancer is scarce, although therapeutic implications of it have been described in aggressive prostate cancer *in vitro* [116]. The prognostic value of vasculogenic mimicry remains debatable as there is at least one study showing that there is no significant correlation between vasculogenic mimicry channels and histological grading of prostate cancer [117].

Interestingly, Liu *et al.* [114] looked at this correlation in human tissue samples to determine clinical pathology, prognosis and a possible molecular mechanism. They statistically correlated histological with clinicopathological data from prostate carcinoma cases confirming that vasculogenic mimicry was more often seen in those patients with seminal vesicle invasion, lymph node metastasis, distant metastasis tissues or shorter PSA doubling time (PSADT), all important clinical prognostic factors of prostate cancer. They concluded that vasculogenic mimicry mainly exists in the high-risk prostate cancer patients and is a new independent marker of poor prognosis of the disease. Though more studies with larger sample sizes are needed to further confirm the correlation of vasculogenic mimicry and prostate cancer prognosis, these results might explain why some anti-angiogenesis treatments remain clinically less effective.

2.4.4. Molecular signaling

The identification of molecules that are uniquely expressed on the surface of endothelial cells of tumor vessels has been a holy grail of vascular biology. Such molecules could serve as therapeutical targets. Although there is no molecule truly associated to tumor vessels, several show higher expression in tumors. Among those relevant in prostate cancer we find: endoglin (CD105), VEGF/VEGFR-2 complexes, thrombospondin-1 receptor (CD36), Thy-1 cell surface antigen (Thy-1), phosphatidylserine, prostate-specific membrane antigen (PSMA), MMP, Her2/Neu and multiple tumor endothelial markers. The absence of absolute specificity of these molecules for tumor vessels drives the search for better targets [118]. Of note, Her2/Neu plays an important role in the spreading of prostate carcinomas to the bone and its high expression is associated with a poorer prognosis in patients with bone metastases. The Her2/Neu receptor is part of a molecular signaling cascade that involves Akt and MMP-9 activation, enabling the cancer cell to penetrate the matrix and facilitating angiogenesis.

It is wise to recognize the lead role of MMP in facilitating the invasiveness of prostate cancer. These molecules are important in the degradation of the ECM, allowing tumoral cells to metastasize to distant sites throughout the body. This protease activity, not only allows for cell migration, but also facilitate angiogenesis, providing the tumor with nutrition and further proliferation [119]. Of note, MMP-2 plays an important role in the preliminary stages of the vasculogenic mimicry genesis, degrading collagen IV. Reports showed that human prostate carcinoma samples positive for vasculogenic mimicry had a significantly higher MMP-2 expression levels compared to vasculogenic mimicry-negative patients. Metastat, an inhibitor of MMP, decreased the formation of vasculogenic mimicry networks in aggressive prostate tumors. However, further studies are needed to elucidate the mechanism of formation of vasculogenic mimicry in detail [114].

In bone metastases, the prostate metastatic tissue might allow for angiogenesis via the MMP9 derived from osteoclasts. Interesting, some MMP have a higher expression with higher Gleason's scores. This fact has led to the revamping of the MMP as possible prognostic factors and even more, as valid candidates for therapy. However, the MMP field is at a crossroad; in the last few years, accumulating evidence from experimental models of cancer,

knockout mice and proteomics studies has challenged our views on how MMP function in the tumoral process. This challenge has been compounded by the fact that the clinical trials with MMP inhibitors failed to show therapeutic efficacy in cancer patients. MMPs have a vast repertoire of substrates not limited to the ECM components, and multiple proteins can be potentially targeted by MMPs and may be important for the anti-tumor activity of the host. This may partly explain why broad-spectrum synthetic MMP inhibitors failed to show clinical efficacy.

The MMP picture is not simple and reveals a complex contribution to cancer progression, putting aside the long-held view of MMP as a family that promotes cancer metastasis. Today, the evidence shows that members of the MMP family may promote or inhibit cancer development. Moreover, an individual MMP may act positively or negatively on tumor progression depending on other factors, on the tumor stage, tumor site (primary, metastasis), enzyme localization (tumor vs. stromal) and substrate profile [120]. In the *-omics era*, the identification of the substrates targeted by MMP in biological samples, known as degradomics, promises to become an important tool for defining the role of MMP in cancer. Establishing correlations, particularly in advanced prostate carcinomas, may assist in better patient stratification.

2.4.5. Cell plasticity and cancer stem cells

In fact, more questions than answers have been raised about the relevance of the *in vivo* studies on tumor vasculature. Is there a morphological and functional connection between prostate tumor-cell-lined networks and endothelium-lined vasculature? Is it possible for aggressive prostate cancer cells to form functional vessels when placed in an ischaemic, non-tumor microenvironment? What is the potential relevance of a 'plastic' tumor-cell phenotype, and how can we identify and target tumor cells that can masquerade as other cell types? Many of the biological properties that are relevant to embryogenesis are also important for tumor growth. For example, during embryonic development, the formation of primary vascular networks occurs by the process of vasculogenesis (the differentiation of mesodermal progenitor cells (angioblasts and hemangioblasts) to endothelial cells) and their organization into a primitive network [121]. The remodeling of the vasculogenic network into a more refined microvasculature occurs through angiogenesis in the same way as tumors require a blood supply for growth and also use the blood supply for metastatic dissemination [122].

Cells capable of vasculogenic mimicry display a high degree of plasticity, causing them to resemble dedifferentiated cell types. A stem cell is considered the most dedifferentiated cell, holding the capacity to generate various novel cell types. However, a new concept comes into the picture, the cancer stem cells (CSCs). These cells hold the capacity to self-renew, differentiate and proliferate indefinitely, being the latter a key event in tumor growth. Tumoral vasculogenic mimicry is characterized by an undifferentiated molecular signature together with embryonic-like differentiation plasticity implying a link between cancer stem cells and aggressive tumor cells capable of vasculogenic mimicry. Moreover, these two cell types

share the potentiality of unlimited proliferation capacity, cellular plasticity and the expression of a gene signature responsible of maintaining pluripotency.

Among the signaling molecules known to influence stem cell renewal and differentiation in aggressive forms of prostate cancer, we find: Wnt, Src, BMP (bone morphogenic proteins) and TGF β [5]. Other transcription factors are also involved in bone metastasis. HIF1 α in tumor cells, inhibits osteoblasts differentiation, induces osteoclasts differentiation and promotes tumor growth. Hypoxia and TGF β signaling in parallel drive the development of tumor bone metastases and regulate a common set of tumor genes stimulating the production of VEGF and CXCR4 in both tumor cells and bone microenvironment to enhance angiogenesis and tumor homing. VEGF, a target gene of Runx2, facilitates tumor growth and both the osteolytic and the osteoblastic disease [123, 124]. Additionally, prostate cancer cell lines express mediators of tumor growth and bone destruction, among them IL8, IL6 and PTHrP. Runx2 is also a key regulator of metastasis related genes and its presence in the primary tumor could be critical for the diagnosis of prostate cancer bone metastasis [125].

The Notch signaling pathway is now recognized as an important player in tumor angiogenesis. Two key Notch ligands have been implicated in this process, Delta-like 4 (Dll4) and Jagged1. Notch appears to be very attractive because specifically, bone metastases from prostate cancer patients expressed Notch-1 protein in the osteoblastic lesions. Correspondingly, Notch ligand Jagged-1 was found to be highly-expressed in metastatic prostate cancer compared to localized disease or benign prostate tissues, and high Jagged-1 expression in a subset of clinically localized tumors was found to be significantly associated with tumor recurrence [5]. Although the molecular mechanism of Notch signaling is not completely understood, silencing of Notch-1 inhibits MMP9, uPA and VEGF expression, given support to the effect of Notch in invasion [126, 127]. Moreover, Wang et al [126] recently proposed a down-regulated signaling cascade downstream of Notch-1, with reduced Akt and mTOR phosphorylation and inactivated NF- κ B signaling. The interplay between these pathways provides a balance between self-renewal and differentiation. Dll4 expression activates Notch resulting in restriction of new sprout development. In agreement with this activity, inhibition of Dll4-mediated Notch signaling in tumors results in hyper sprouting of nonfunctional vasculature [128]. This Dll4 inhibition may paradoxically lead to increased angiogenesis but poor tumor growth because the newly growing vessels are not functional. In contrast, Jagged1 has been described as a Notch ligand expressed in tumor cells that may influence tumor angiogenesis by activating Notch on tumor endothelium. Of note, Notch activation is also critical for the maintenance of stem cell self-renewal potency in several stem cell microenvironments. These results indicate that Notch signaling can have diverse signaling outcomes dependent on the cellular niche, as it is able to induce (endothelial) differentiation in some cases, while promoting self-renewal potency in others [128].

TGF β signaling also draws our attention given that it is a key molecule in the maintenance of an undifferentiated state in human embryonic stem cells. Various components of the TGF β signaling cascade are highly expressed in stem cells, including Nodal and its regulators Cripto and LEFTY1/2 [101,102]. However little is known about signaling cascades governing the pluripotent state [129]. Taken together multiple stimuli provided by prostate

tumors and their effective microenvironment can trigger differential signaling cascades that in turn will define the fate of the host. Thus a variety of therapeutic venues may have to co-exist in order to be translated into clinical utility.

2.4.6. *Clinical significance*

Undoubtedly, there are more questions than answers at this time regarding the functional significance of vasculogenic networks and vascular marker expression by prostate cancer cells. If tumor vasculogenesis can be demonstrated in experimental models, does it occur concomitantly with angiogenesis or as a remodeling of angiogenesis in aggressive tumors? Is vessel co-option involved? Is tumor cell vasculogenesis an alternative angiogenic switch in aggressive tumors? Regardless of the terms employed to describe the expression and mimicry of vascular-like gene by aggressive prostate cancer tumor cells, this area of research is worthy of analysis. It is wise to consider that in addition to the current anti-vascular treatments, the novel therapeutic approaches against tumor vasculature must be harmonized with the stage of tumor progression and with the molecular mechanism responsible for the angiogenic phenotype.

In our perspective the challenge relies in combining the anti-vascular strategies with the existing therapeutic regimes. The rational application of antivascular agents must be tagged along with the notion that these therapies must be individually tailored for the different types of cancer cells. The clinical management of prostate cancer would benefit greatly from the better understanding of the diverse vascularization mechanisms helping to fine-tune these novel anti-cancer strategies.

3. Conclusions

It is clear that multiple host and environmental factors contribute to prostate cancer and that inflammation sets the scene for the appearance of a reactive stroma, providing growth factors, chemokines and proteins that stimulate among other things, invasion. In return, this cancer finds a fertile soil to proliferate and disseminate in the bone, which acts as a specialized niche for prostate cancer cells. Moreover, the vascular compartment contributes significantly to prostate cancer growth through provision of oxygen and nutrients. Prostate cancer cells break into the scene co-opting blood vessels, by intussusception or even enhancing angiogenesis, attracting endothelial cells, promoting their growth in the tumor microenvironment and even transdifferentiating through the EMT. The intricacy relies on deciphering the diabolic liaison of all these factors and physiological processes. How can successful therapeutic strategies be designed if there are still so many hidden molecular variables waiting to be unveiled? The path in building promising clinical action plans will depend on unraveling the rheostat molecules that control the metabolic reprogramming of tumoral cells and the tumor microenvironment. Who are the key players controlling all the biochemical reactions producing ROS and RNS within cancer cells? Even more who are their exact targets? Several microRNA signatures are identified and described in the inflammatory milieu associated to

prostate cancer, hence are miRNA-base therapeutic strategies a promising option for the disease? The possibility to target cancer cell malignancy by intervention on both its metabolic reprogramming and its interplay with environmental factors is in truth captivating. The key molecules and pathophysiological process outlined throughout this chapter drive home the concept that the tumor microenvironment enhanced by an inflammatory wand offers interesting homeostatic targets for prostate cancer therapy. In this synopsis, blocking the sustained inflammatory network will offer new promising avenues to achieve significant therapeutic gains in the treatment of prostate cancer.

Abbreviations

AR→androgen receptor

COX-2→cyclooxygenase-2

CXCR4→C-X-C chemokine receptor type 4, bonzo, STRL33 or TYMSTR

CXCR7→C-X-C chemokine receptor type 7, RDC1

ECM→extracellular matrix

ELR→glutamic acid-leucine-arginine motif

EMT→epithelial mesenchymal transition

HIF-1 α →hypoxia-inducible factor 1 alpha

HO-1→heme-oxygenase 1

IFNs→interferons

IGF-IR→insulin-like type I growth factor receptor

IL6→interleukin 6

IL8→interleukin 8

miRNAs or miRs→microRNAs

MMP →matrix metalloproteinase

MnSOD→manganese superoxide dismutase

NF- κ B→nuclear factor κ B

Nrf2→nuclear factor (erythroid-derived 2)-like 2

RNS →reactive nitrogen species

PARs→serine proteases

PI3K →phosphatidylinositol-3 kinase

PIN→prostatic intraepithelial neoplasia→

Prx → peroxiredoxin

PSA → prostate specific antigen

PTEN → phosphatase and tensin homolog

RECIST → Response Evaluation Criteria in Solid Tumors

RNS → reactive nitrogen species

ROS → reactive oxygen species

SDF-1 → stromal derived factor 1

STAT3 → signal transducers and activators of transcription-3

TGF β → transforming growth factor beta

TMPPSS2-ERG → transmembrane protease, serine 2 – ets related gene

TNF α → tumor necrosis factor alpha

TRAIL → tumor necrosis factor-related apoptosis-inducing ligand

VEGF → vascular endothelial growth factor

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