ANGIOGENESIS IN PROSTATE CANCER

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Introduction

Prostate cancer is the most common form of cancer in the male. It is the second leading cause of cancer deaths in men in the UK and USA, after lung cancer [1]. The prevalence of prostate cancer is so high that it could almost be considered a normal age-related phenomenon. An estimated 164,690 new cases will be diagnosed, with 29,430 deaths in the United States in 2018 [2]. Clinical and experimental observations suggest that genetic, environmental, hormonal factors and acquired somatic mutations have roles in pathogenesis of prostate cancer. The detection of localized disease with digital rectal examination, serum prostate specific antigen (PSA) measurement and transrectal ultrasound guided biopsies is the most realistic opportunity for cure. With the various treatments available, 90% of patients with localized disease expect to live for 15 years, whereas patients with disseminated cancer have only a 10-40%-10 year survival rate [3,4]. There are several classes of prognostic factors in this disease like clinical T stage, serum PSA, pathological staging and grading, various molecular markers and angiogenesis factors. Radical prostatectomy, radiotherapy and hormonal therapy are the main conventional treatments for prostate cancer. However, several gene based approaches have been devised including inhibition of angiogenesis [5,6]. With this recent advent of targeted biological therapy, various prognostic factors need to be studied and worth analysed. This article reviews pathophysiology and role of angiogenesis in prostatic cancer along with its prognostic and therapeutic value.

Tumour angiogenesis

In the 1970s it was first proposed that tumors depended on the establishment of a microcirculation in order to grow beyond a few millimeters. Thereafter, the search to prove this hypothesis increased strongly and by the end of the 1980s, evidence was

given that tumors were angiogenesis-dependent and metastatic cells were only established its microcirculation. The process of neovascularization is regulated by numerous growth factors, vascular endothelial cells, and matrix proteins released from host stromal cells such as macrophages and mast cells. The process of tumor growth and metastasis involves tumor cell-host cell and cell-matrix interactions [7]. Angiogenesis is the process by which new blood vessels are formed from an existing vascular network and involves endothelial cells in such vessels undergoing migration, proliferation and differentiation into new capillary-like structures, followed by the formation of a surrounding basement membrane and deposition of extracellular matrix (ECM) [8]. Angiogenesis is a prominent feature of malignant tumors and the extent of this in a given tumor depends on the balance between pro-angiogenic and anti-angiogenic factors released by malignant cells and stromal cells present within the tumor [9]. Solid tumors do not grow beyond 2 mm without angiogenesis as there is only a limited diffusion of oxygen and nutrients in avascular tumors [8]. New blood vessels also promote the metastasis of cells in the tumor mass to distant sites [10]. In tumor angiogenesis, these newly formed vessels are poorly organized and are prone to collapse, leading to intermittent blood flow. Indeed, these new blood vessels exhibit a number of structural abnormalities, including an incomplete endothelial cell lining and basement membrane, lack of smooth muscle, blind ends and arteriovenous shunting [11,12,13]. Moreover, high oxygen consumption by tumor cell proliferation outstrips the development of new blood vessels in a given area. Together, these give rise to the phenomenon of tumor hypoxia. Expanding tumors become hypoxic and tumor cells express transcription factors, such as the hypoxia-inducible factor (HIF), which induce the release of proangiogenic growth factors such as vascular endothelial growth factors (VEGF) and transforming growth factors that promote the formation of new capillaries by recruiting, activating, and stimulating endothelial cells. Activated endothelial cells secrete matrix metalloproteases, which degrade the basement membrane, extracellular matrix, and adhesion receptors such as integrins, which allow their migration into the extracellular matrix toward the tumor cells [14]. Angiogenesis is regulated by both activator and inhibitor molecules. More than a dozen different proteins have been identified as angiogenic activators such as growth factors, VEGF & angiostatin and

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inhibitors such as endostatin & tissue inhibitors of metallproteinase. Levels of expression of angiogenic factors reflect the inhibitors should help to reduce both morbidity and mortality from carcinomas. Thousands of patients have received antiangiogenic therapy [15, 16]. Despite their theoretical efficacy, antiangiogeic treatments have not proved beneficial in terms of long-term survival. There is an urgent need for a new comprehensive treatment strategy combining antiangiogenic agents with conventional cytoreductive treatments in the control of cancer.

aggressiveness of tumor cells. The discovery of angiogenic

Vascular endothelial growth factor (VEGF)

Tumor cells express a number of cytokines and enzymes involved in regulating tumor angiogenesis. Of these, VEGF [17,18], basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), matrix metalloproteases (MMPs) [19,20], insulin like growth factor (ILGF), platelet derived growth factor (PDGF) [21,22] and angiopoietins 1 and 2 (Ang-1 & -2) [23,24] are amongst the most prominent. VEGF is also called vascular permeability factor (VPF) as it is known to increase the permeability of new and existing blood vessels in tumors [25,26]. It induces the proliferation and migration of endothelial cells [27], and activates proteolytic enzymes that enhance tumor invasion [28] (Figure 1). Vascular endothelial growth factor (VEGF) plays a major role, and VEGF inhibition is a promising therapeutic approach to some tumoral diseases [29]. VEGF is regulated by hypoxia as well as androgens and other growth factors such as epidermal growth factor (EGF), tumor necrosis factor (TNF-α), bFGF and ILGF [30,31,32,33]. Six members of the VEGF family have been isolated: VEGF-A, VEGFB, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PIGF). Five splice variants, encoded by a single VEGF gene, give rise to five forms of VEGF-A protein of different length (121, 145, 165, 189 & 206 amino acids). Among these, 121 & 165 are the most prominent forms in human tumors. VEGF121, VEGF145 and VEGF165 do not bind to heparin, while VEGF189 & VEGF206 bind to heparin with great affinity and are mainly stored in the extracellular matrix bound to proteoglycans [34,35]. Three high affinity tyrosine kinase receptors for VEGF family members are known. VEGF-R1 is derived from the flt-1 gene (fms-like tyrosine kinase-1), VEGF-R2 is derived from the flk-1/KDR gene (fetal liver kinase-1/kinase domain region), and VEGF-R3 from the

flt-4 gene (fms-like tyrosine kinase-4) [36,37]. These receptors are selectively expressed on endothelial cells. VEGF-A of its effects through VEGF-R2. VEGF-B & PlGF bind only VEGF-R2. On the other hand, VEGFC& D bind to VEGF-R3, and to some extent VEGF-R2. VEGF-C & D are important regulators of lymphangiogenesis. VEGF-R2 appears to be the most dominant receptor in VEGF-induced mitogenesis while the role of VEGF-R1 in endothelial cell function is much less clear and it may negatively regulate the activity of VEGF-R2 [38,39,40,41]. Salven, *et al*., (1997) [42] showed that VEGF-A plasma levels are binds to both VEGF-R1 & VEGF-R2 but is thought to exert most

higher in patients with widely disseminated cancer than those with it confined to the primary site. Moreover, VEGF inhibits the maturation of dendritic cells (antigen presenting cells), suggesting that tumors expressing high levels of VEGF disseminate more effectively due to greater vascular permeability as well as reduced immunosurveillance [43]. Therefore, a serum marker for active angiogenesis may be of value in the diagnosis of cancer and follow-up of tumor response to cancer therapy.

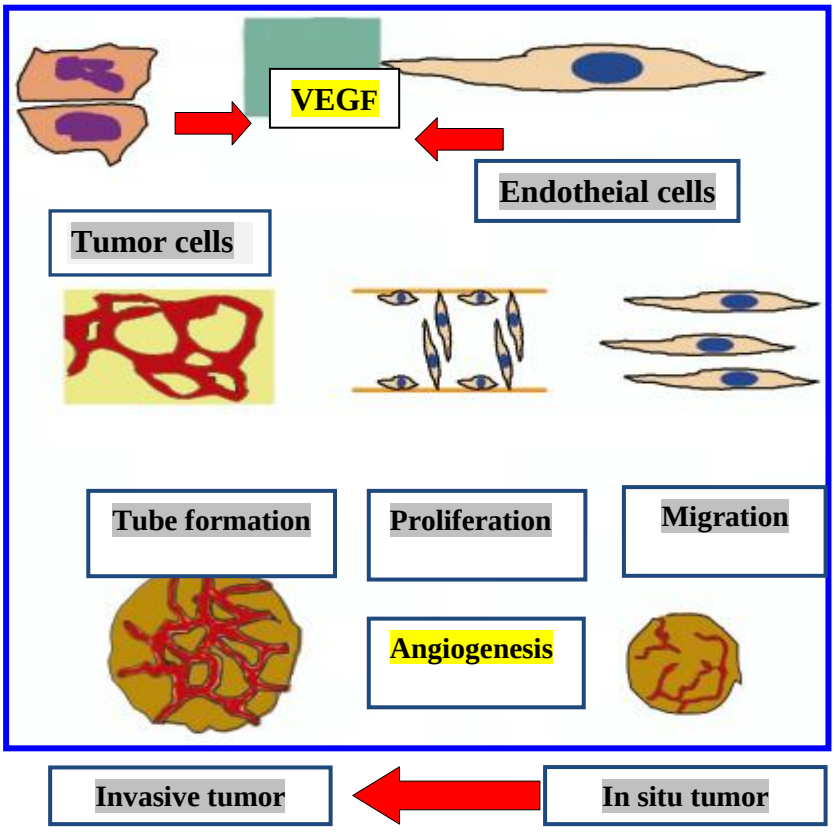

Figure 1: Angiogenesis facilitate the growth and the spreading of tumor

Angiogenesis and prostate cancer

Angiogenesis is a complex multistep process involving close orchestration of endothelial cells, extracellular matrix, and soluble factors. Essentially every step has been found to be regulated by inducers and inhibitors [44]. Prostate cancer has the ability to produce angiogenic factors such as metalloproteinases, vascular endothelial growth factor, fibroblast growth factor 2, transforming growth factor-beta and cyclooxygenase-2. On the other hand several endogenous inhibitors of angiogenesis have been described in prostate cancer e.g., angiostatin, endostatin, prostate specific antigen (PSA), thrombospondin-1, interleukin 10, interferons and retinoids [45]. VEGF & VEGFR-2 expression levels were significantly higher on prostate tumor cells at the site of bone metastasis as compared to the original prostate tumors from the same individual. This finding implies that VEGF/VEGFR-2 is involved in the process of metastasis development [46]. Moreover, Kaushal *et al*., (2005) [47] showed that cancer cells from patients with early stage prostate cancer predominantly expressed the angiogenic growth factor VEGF-A and its activated receptor VEGFR-1. Whereas, the majority of patients with advanced stage prostate tumours expressed higher levels of the lymphangiogenic growth factor VEGF-D and its activated receptors VEGFR-2 and VEGFR-3. This suggests that lymphangiogenesis plays a role in the progression of prostate cancer and that VEGF-D may provide a useful marker of advanced-stage disease. Several studies have demonstrated a negative correlation between tumor angiogenesis and prognosis in prostate cancer [48,49]. Moreover, Strohmeyer *et al*., (2000) [50] showed that intraductal microvessel density (IMVD) positively correlates with VEGF expression by tumor cells, but inversely with survival, in prostate cancer. Histopathologic techniques of microvascular density indexes require invasive tissue sampling and need to be standardized. Hemodynamic characteristics of immature neovessels can be noninvasively assessed by dynamic contrast-enhanced magnetic resonance imaging or computed tomography. Tissue enhancement depends on arterial input function, kinetic of distribution of blood into the capillary bed, leakage across the capillary walls, and volume of the interstitial space [51]. Looking at angiogenesis in prostate cancer specimens may help predict treatment outcomes. Cancers that stimulate many new vessels to grow are harder to treat and have a poorer outlook. New drugs are being studied that may be useful in stopping prostate cancer growth by keeping new blood vessels from

forming. A number of anti-angiogenic factors have emerged such as thrombospondin-1 (TSP-1), interferon-alpha (INF-α), tissue inhibitor of metalloproteinase-1 (TIMP-1), endostatin and angiostatin. These can inhibit tumor angiogenesis, and thus the growth of tumors, when administered to tumor-bearing mice [44,52]. In addition, combined antiangiogenic and immune therapy produced a significant anti-tumor effect in murine prostate tumor [53]. Because antiangiogenic treatment is cytostatic rather than cytotoxic, patients will need long-term therapy to prevent regrowth of the tumor. Due to the need for long term administration of the inhibitors, gene therapy has become an alternative which theoretically ensures a sustained availability of the anti-angiogenesis agents [54]. Prostate cancer is an ideal tumor for antiangiogenic studies because of the availability of a reliable tumor marker, PSA, the indolent clinical course of this cancer and the low rate of proliferation even in metastatic sites. Furthermore, clinical studies showed limited side effects, which is advantageous in this elderly patient group [45]. More than 20 anti-angiogenic agents are now in various stages of clinical trials [16,55]. For example, a randomised phase II study evaluated the combination of Thalidomide (anti-VEGF) with docetaxel (chemotherapeutic agent) to docetaxel alone. The combination therapy achieved higher PSA response rates (53% *versus* 37%) and longer median progression free survival (5.9 months *versus* 3.7 months) than single therapy alone [56]. More recently a randomized phase III trial has been conducted comparing the combined use of docetaxel, prednisone (DP) and bevacizumab (Bev) (anti-VEGF) with DP alone [57]. The bevacizumab group exhibited superiority in outcome measures such as progression free survival and PSA response rate. Several anti-angiogenic drugs are already being tested in clinical trials like thalidomide and bevacizumab (Avastin). It is now being tested in combination with hormone therapy and chemotherapy in men with advanced prostate cancer. The benefit of angiogenesis inhibitors has become a reality in several tumor types, with significant potential in prostate cancer (58).

CONCLUSIONS:

The tumour angiogenesis plays a pivotal role in the growth of tumors and its metastasis. It is currently a leading theme in prostate cancer management, and new drugs targeting the tumor neoangiogenic process are under clinical practice as well as development. Evaluation of angiogenesis can be used as a prognostic

marker to evaluate the aggressiveness of tumor and as a potential predictive marker of antiangiogenic treatment response.

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