



The role of HIF in angiogenesis, lymphangiogenesis, and tumor microenvironment in urological cancers

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Abstract

Typically associated with solid tumors, hypoxia contributes to tumor angiogenesis and lymphangiogenesis through various molecular mechanisms. Accumulating studies indicate that hypoxia-inducible factor is the key transcription factor coordinating endothelial cells to respond to hypoxia in urological cancers, mainly renal cell carcinoma, prostate cancer, and bladder cancer. Moreover, it has been suggested that tumor hypoxia in tumor microenvironment simultaneously recruits stromal cells to suppress immune activities. This review summarizes the mechanisms by which HIF regulates tumorigenesis and elaborates on the associations between HIF and angiogenesis, lymphangiogenesis, and tumor microenvironment in urological cancers.

Keywords HIF · Angiogenesis · Lymphangiogenesis · Tumor microenvironment · Urological cancers

Introduction

Urological tumors, mainly renal cell carcinoma (RCC), prostate cancer (PCa) and bladder cancer (BCa), make up 13.1% of newly diagnosed cases and account for 7.9% of all cancer-related deaths [1]. Urological tumors are solid tumors in which hypoxia is generated due to their rapid growth and inadequate nutrient supply. Hypoxia-inducible factors (HIFs) regulate various biological processes induced by hypoxia [2]. Angiogenesis and lymphangiogenesis are two major processes that depend on HIFs to respond to hypoxia. Through these processes, new vessels are formed from pre-existing vessels, and these vessels communicate with stromal components of tumor microenvironment (TME), promoting immunosuppression. This review

presents an overview of HIF-dependent angiogenesis, lymphangiogenesis, and the association between such processes with tumor microenvironment in urologic cancers and discusses novel applications and challenges of targeted therapy development for clinical translation.

HIF activity in urologic cancers

Regulation of HIF

HIFs, including HIF-1, HIF-2, and HIF-3, constitute a group of transcription factors mediating hypoxia-related responses. HIF-1 was first discovered through an analysis of the human EPO gene. It was later confirmed that HIF-1 consists of two elements: HIF-1 α and HIF-1 β [3].

HIFs are heterodimers with two subunits, and the stimulation of HIF signalling pathways requires the assembly of alpha units (HIF1 α , HIF2 α , and HIF3 α) and beta units (HIF1 β /ARNT1, ARNT2, and ARNT3) [4]. The homeostasis of HIFs is regulated by a family of hydroxylases and the hydroxylation reaction depends on oxygen.

Under normoxic conditions, prolyl hydroxylases are enzymes that facilitate the hydroxylation of HIF-1/2 α , and then the ubiquitin ligase complex comprising Von Hippel–Lindau (VHL) recognizes HIF-1/2 α hydroxylated proline residues, triggering the ubiquitination of HIF-1/2 α

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[5]. Finally, the 26 S proteasome degraded HIF-1/2 α . The hydroxylation of asparagine residues by Factor Inhibiting HIF-1 prevents HIF from engaging with p300 and CREB-binding protein, thereby inhibiting HIF-1/2 α transactivation [6]. A hypoxic environment inhibits hydroxylation reactions, resulting in the accumulation of HIF-1/2 α . When two subunits of HIF-1/2 bind to cis-acting elements of target genes, a complex is formed, which promotes transcription of the target genes [3].

HIF-1 α and HIF-2 α target different gene transcripts. HIF-1 α can upregulate the glycolytic enzymes expression, while HIF-2 α participates in iron metabolism [7]. HIF-3 α is an isoform of HIF- α that has been relatively less studied; however, the role of HIF-3 α is known to be distinct from that of HIF- α . Inhibitory PAS domain protein, a spliced variant of HIF-3 α , negatively regulates HIF-1 α , whereas HIF-2 α activates the HIF signalling pathway [8].

RCC

In clear cell RCC (ccRCC) patients, deletions or mutations of the VHL gene are connected with high levels of HIF expression, and elevated HIF levels are linked with poor outcomes [9]. On chromosome 3, the VHL gene is located at cytoband 3p25-26. One mutant allele of VHL is present in individuals with VHL disease, and tumorigenesis is largely dependent on the simultaneous inactivation of both alleles of VHL [10]. Recently, the somatic biallelic deactivation of VHL has been a common occurrence in most sporadic ccRCC. Somatic mutations of VHL have been reported at varying rates, with studies describing frequencies as high as 91% [11].

As part of the ubiquitin ligase complex, the VHL gene product (pVHL), is indispensable for HIF signaling. Loss of pVHL leads to VHL failure to recognize HIF- α subunits. Therefore, the stability of HIF- α is increased, consequently enhancing the transcriptional activities of the HIF target genes under normoxic conditions.

HIF-1 and HIF-2 appear to play distinct roles in ccRCC. ccRCCs that exclusively express HIF-2 exhibit a higher degree of malignancy compared to ccRCCs expressing both HIF-1 and HIF-2 [12]. Phenotype assays showed that HIF-1 α functions as a tumor suppressor and HIF-2 α functions as a tumor-promoting factor. HIF-1 α suppresses the expression of proto-oncogenes, but HIF-2 α upregulates oncogenic genes [13]. This is in contrast to the majority of tumors, where HIF-1 and HIF-2 are commonly considered oncogenes. In this particular scenario, there could potentially be an interplay between HIF-1 and HIF-2 contributing to this phenomenon.

BCa

In primary BCa samples, HIF-1 α has been observed via nuclear HIF-1 α immunostaining in a vast majority of tumor cells. No HIF-1 α expression has been observed in normal tissue [14]. HIF-1 α positively correlates with histological grade of tumors, VEGFR levels, and microvessel density (MVD). BCa patients with HIF-1 α overexpression showed poor survival outcomes [14]. The heightened expression of HIF-1 α induced by hypoxia stimulates the transcription of downstream genes, regulating activities like angiogenesis, nutrient metabolism, and cell cycle progression [15]. HIF-1 and HIF-2 exhibit a synergistic effect in bladder cancer. HIF-2 has been observed to exhibit staining within the stromal region of tumors, and its heightened expression has been correlated with inferior histological grades [16].

PCa

HIF-1 α is elevated in both PCa samples and human PCa cell lines [17]. Upregulated HIF-1 α has been reported to be correlated to increased biochemical recurrence, metastasis, and castration resistance in PCa [18, 19]. PCa patients with elevated HIF-2 expression also have poor disease-specific survival [20].

HIF is intricately regulated through genetic and epigenetic mechanisms in PCa. The gene amplification that has been detected by comparative genomic hybridization in PC3 cell lines may partially explain the overexpression of HIF-1 α [17]. Single nucleotide polymorphisms (SNPs) may contribute to the upregulation of certain genes. Two SNPs, C1772T and G1790A, identified in castration-resistant PCa (CRPC), may result in the collection of the HIF-1 α protein [21].

In general, both HIF-1 and HIF-2 play similar roles in promoting tumor growth in prostate cancer and bladder cancer. However, in renal tumors, HIF-1 and HIF-2 exhibit mutual antagonism, suggesting a regulatory relationship between them and resulting in differential effects on downstream target genes and pathways.

HIF and angiogenesis

Compact neoplasms initially lack vasculature and rely on oxygen diffused from host vessels. The proliferation or hypertrophy of tumor cells leads to elevated oxygen consumption by tumor cells, which culminates in diminished oxygen accessibility, causing a reduction in hydroxylase function and enhanced HIF transcription. Angiogenesis is an adjustable reaction to hypoxia, and it is commandeered by malignant cells to induce their own oxygen supply and

sustain neoplasm expansion. HIFs function as important angiogenic gene regulators. Activation of HIF in tumor and stromal cells leads to an escalation in mRNA level of vascular endothelial growth factor (VEGF) [2, 22]. Other genes involved in angiogenesis regulated by HIF include placental growth factor, platelet-derived growth factor (PDGF), stromal-derived factor, stem cell factor and angiopoietin 2. These factors interact with receptors on the cell membrane, activating specific signalling pathways that lead to the formation of new blood vessels, promoting tumor progression [2, 22]. Hypoxia-induced effects mainly influence two cell types, endothelial cells (ECs), the endothelial layer of blood vessels, and pericytes, which encircle blood vessels. During angiogenesis, endothelial cells respond to VEGF by initiating new blood vessel sprouting. ECs then differentiate into specialized vascular endothelial cells to enhance vessel formation. They became hypoxic because new vessels are located far from well-perfused blood vessels. The hypoxic microenvironment results in metabolic shift involving elevated glycolysis in ECs, and increasingly, fatty acid oxidation in biological activities [23]. ECs serve as barriers preventing the invasion of malignant cells, and the hypoxia modulators HIF-2 and HIF-1 generate opposite implications on barrier function in an iNOS-dependent manner [24]. Pericytes, which form a layer on the exterior of endothelial cells, control the contractility and permeability of blood vessels. PDGF impairs the adhesion of pericytes to blood vessels during neovascularization [25].

Angiogenesis is a complex biological process, and the VEGF family plays a dominant role in regulating angiogenesis. In VEGF family, VEGF-A is the most angiogenic relevant component, and it interacts with VEGFR-2 to impact neovascularization [26].

Angiogenesis in RCC

Heterogeneity is a characteristic of RCC and can be categorized into three histopathologic types: chromophobe RCC (chRCC), papillary RCC (pRCC), and ccRCC. Type 1 pRCC is more likely to arise from familial mutations of c-Met, whereas Type 2 pRCC is correlated to mutations in fumarate hydratase. Those suffering from Birt–Hogg–Dubé syndrome are prone to developing chRCCs. In most ccRCCs, both alleles of the VHL gene are inactivated because of mutations, chromosomal aberrations, or VHL promoter hypermethylation [9]. Mutations in TCEB1, encoding the elongin C module involved in ubiquitination, have been recently identified in ccRCCs [11]. The loss of pVHL and elongin C function leads to the accumulation of HIF-1 α plus HIF-2 α .

In normal tubular cells, only the HIF-1 α isoform has been detected via immunohistochemical approach [9, 27]. Both

HIF-1 α as well as HIF-2 α have been found in precancerous lesions of VHL-deficient kidney disease patients. In ccRCC tumors, around 30% of patients do not express HIF-1, and its expression is inversely correlated with tumor malignancy. Survival analysis also indicates a favorable prognosis associated with HIF-1 expression [9, 28, 29]. HIF-1 and HIF-2 reciprocally inhibit each other at the epigenetic and protein levels, indicating a regulatory relationship between them [13, 30]. Hoefflin et al. demonstrated that HIF-1 α exerted an indispensable effect on the formation of VHL mutant ccRCC mouse model, and deletion of HIF-2 α exerted little impact on tumor onset [31].

VEGF binds to VEGFR and promotes angiogenesis. In ccRCC, loss of pVHL correlates with increased expression of VEGF, which results in higher TNM stages and worse prognosis in patients. HIF-2 α knockdown alleviated VEGF expression in 786-O VHL-deficient cells, supporting an angiogenic role for VEGF in renal tumors [27]. In addition, in HIF-1 α deficient cells, targeting HIF-2 can regulate the expression of VEGF [32].

These results suggested that the two HIF isoforms facilitate cancer progression in different oncogenic periods and HIF-2 plays a central role in angiogenesis. Presently, targeted therapies for RCC predominantly focus on HIF-2, such as Belzutifan and PT2399. However, targeting the interaction between HIF-1 and HIF-2 can be a promising approach. When combined with drugs targeting HIF-2, it may enhance the efficacy of anti-angiogenic treatment.

Angiogenesis in BCa

The isoforms of HIF- α seemed to exert merged effects to accelerate the vascularization and progression of BCa. HIF-1 α was correlated with VEGF and MVD in BCa [33]. HIF-2 α was discovered in the stroma area around tumors, especially in the regions surrounding necrotic tumors, and was positively associated with VEGF expression [16]. Mesenchymal cells secrete cytokine hepatocyte growth factor (HGF), and HGF signalling is often simultaneously activated in hypoxic conditions. Koga et al. reported that HGF interacted with Met, the receptor of HGF, to enhance the synthesis of HIF-1 α via the MAPK and PI3K pathways in T24 cells. These up-regulation pathways may be attributed to the activation of VEGF, leading to the its binding with VEGFR [34]. Heat shock protein 90 is essential in HIF-1 α folding and maturation. The heat shock protein 90 inhibitor geldanamycin substantially decreased hypoxia-induced VEGF expression in T24 cells [34]. Xia et al. discovered that pyruvate kinase M2 bound with STAT3 to form a protein complex, which was subsequently translocated into the nucleus where it stimulated HIF activity to induce

angiogenesis in an oncogenic HRas-driven urothelial tumor mouse model [35].

Currently, targeted therapy for bladder cancer has shown limited efficacy. However, HGF, HSP90, pyruvate kinase M2, and STAT3 all hold potential as therapeutic targets for bladder cancer, as they play a role in anti-angiogenesis.

Angiogenesis in PCa

In PCa, the degradation of HIF is reduced under hypoxic conditions, and cytokines and growth factors regulate HIF levels through mitogen-activated protein kinase (MAPK) or phosphatidylinositol 3-kinase (PI3K) pathways. By activating PI3K, the mammalian target of rapamycin (mTOR) phosphorylates p70 S6 kinase (p70S6K). The ribosomal S6 protein undergoes phosphorylation by p70S6K, which triggers HIF-1 α synthesis. Triggering the MAPK pathway, extracellular signal-regulated kinase also phosphorylates p70S6K, as well as eukaryotic translation initiation factor 4E-binding protein 1. Phosphorylated 4E-binding protein 1 fails to combine with eukaryotic translation initiation factor 4E and induces the translation of HIF. PTEN, a tumor suppressor gene and is often mutated in PCa [36]. Fang et al. suggested that as a PI3K inhibitor, PTEN restrained the expression of both HIF and VEGF to inhibit angiogenesis in PCa [37]. Ci et al. discovered that transcription factor Krüppel-like factor 5 inhibited PI3K/AKT activity to reduce the rate of HIF and VEGF synthesis [38]. Sphingosine kinase 1 (SphK1) was stimulated in low oxygen conditions and activated Akt/glycogen synthase kinase-3B to prevent VHL-mediated HIF degradation [39, 40]. Then, SphK1 induced the accumulation of VEGF to promote angiogenesis in PCa. Insulin has been confirmed to enhance the creation of reactive oxygen species, such as H₂O₂, to activate p70S6K, leading to the upregulation of HIF and VEGF. Catalase has been demonstrated to abolish insulin-induced p70S6K activation [41]. Prostaglandin E2 (PGE2) is transported into cells by prostaglandin uptake transporter. Intracellular PGE2 interacts with intracellular EP2 receptors to exert its proangiogenic effect through the increased expression of HIF-1 α [42]. It has been confirmed that HIF could induce the transition from respiratory metabolism to glycolytic metabolism, and an increased glycolytic rate promoted lactate production [43]. Luo et al. indicated that lactic acid produced in a hypoxic tumor environment was transported into cells by Monocarboxylate Transporter 1, and lactate inhibited HIF degradation in a hypoxia-independent manner [44]. In the process of angiogenesis, tumor microenvironment can be divided into hypoxic regions and normoxic regions. Lactate produced in hypoxic regions is transported to normoxic regions through blood vessels and could be absorbed by surrounding cells [45]. Intracellular

lactate promotes HIF lactylation to stabilize HIF-1 α , which enhances the transcription of KIAA1199. KIAA1199 has been found to depolymerize hyaluronic acid and initiate angiogenesis by stimulating VEGFA and inhibiting semaphoring 3 A action [44].

Current targeted therapies for prostate cancer appear to have limited effectiveness. The VEGF-A monoclonal antibody, Bevacizumab, has failed to provide PSA relief in CRPC as a monotherapy and has not achieved the survival endpoint when used in combination with other drugs for metastatic CRPC. Moreover, drugs targeting VEGF and its receptors have been shown to develop resistance after achieving certain therapeutic effects, indicating the need for novel targets such as HIF, KIAA1199, and metabolic pathways, which should be given due attention.

HIF and Lymphangiogenesis

During embryonic development, lymphangiogenesis occurs later than angiogenesis, and VEGF-C, VEGF-D, as well as VEGFR-3 pathways dominate lymphangiogenesis [46, 47]. Under hypoxic conditions, both VEGF-C and VEGF-D are regulated by HIF. A vasodilator-vasoconstrictor imbalance triggered by hypoxia leads to malignant behavior of lymphatic endothelial cells (LECs). Nitric oxide is a common vasodilator, and the inhibition of nitric oxide synthesis can block lymphatic hyperplasia [48]. Endothelin-1, a vasoconstrictor, is upregulated under hypoxic conditions by the transactivation of activator-protein-1 in LECs. Endothelin-1 binds to the endothelin B receptor located on LEC surfaces to induce HIF-1 α expression and causes lymphatic cell proliferation mediated through the MAPK and PI3K pathways, leading to vessel branching [49]. In addition, hypoxic conditions increase the expression of Prospero-related homeobox1 (PROX1), which controls the key transformation of blood vascular endothelial cells into LECs in the embryonic period and, in turn, binds to the promoter of VEGFR-3 to enhance the expression of VEGFR-3 on LEC membrane [50, 51].

Hypoxia modulates macrophage action in both inflamed areas and malignant tumors. Macrophages in the circulation system are recruited to tumor microenvironment and located in the low-oxygen region [52]. HIF-1 α links macrophages with this hypoxic environment, regulating its recruitment. Macrophages in hypoxic regions display abnormal metabolic patterns and overexpress HIF-1 α and VEGF [48]. Macrophages secrete several cytokines, such as interleukin-1 β (IL-1 β), Migration-inhibitory factor (MIF), and VEGF-C/D in hypoxic conditions. MIF expressed by macrophages is stimulated under hypoxic conditions, and through the interactions with CD74, resulting in the

accumulation of HIF-1 α [53]. IL-1 β modulates HIF-1 α upregulation and activates target gene VEGF transcription through nuclear factor kappa B pathway [54]. IL-1 β triggered VEGF-C/-D release in macrophages to promote lymphangiogenesis in mouse airways [55]. Tumor-associated macrophages respond to hypoxia to induce HIF-1 α , which results in lymphangiogenesis.

Cyclooxygenase-2 is highly expressed in tumor cells. Its synthesized product, prostaglandin E2 binds to the EP1 receptor to promote the expression of VEGF-C, thereby facilitating lymphangiogenesis [56]. Additionally, VEGF-D inhibits 15-PDGH to block the degradation of prostaglandins to induce lymphatic vessel hyperplasia, which is engaged in tumor lymphatic metastasis [57]. The induction of lymphangiogenesis by HIF-1 α is not mediated solely through VEGF signaling. Hypoxia increases chemokine CXCL12/CXCR4 transcription, and HIF-1 α mediates CXCR4 production via VEGF-C in LECs. CXCL12 promotes lymphangiogenesis independent of VEGF-C/-D pathways [58].

Lymphangiogenesis is commonly considered as a mechanism that promotes tumor metastasis. VEGF-C stimulates the formation of new lymphatic vessels, allowing tumor cells to bypass lymph nodes and rapidly spread to distant organs [59]. In RCC, the impact of VEGF-C on survival is somewhat contradictory. In patients without metastasis and with lower histological grades of the tumor, higher levels of VEGF-C are associated with better prognosis, manifested by longer DFS and OS. However, in metastatic patients, higher expression of VEGF-C is associated with poorer prognosis [60].

This duality may be attributed to the inherent characteristics of the lymphatic system. During the early stages of RCC development, generated lymphatic vessels drain tumor cells to the lymph nodes, allowing immune cells to recognize the tumor cells more quickly and trigger an anti-tumor immune response. From this perspective, VEGF-C plays an anti-tumor role in the initial stages of tumor development, leading to improved patient survival. However, when tumor cells invade the lymph nodes, VEGF-C-induced lymphatic vessel formation accelerates tumor metastasis and recruits suppressive immune cells, impairing the anti-tumor immune response [61].

In RCC, the regulation of VEGF-C by HIFs can vary. HIF-1 binds to the promoter of VEGF-C in normal kidney tissue, promoting transcription and increasing lymphatic vessel formation [62]. As mentioned earlier, most RCCs have high expression of HIF-2. Ndiaye et al. demonstrated that HIF-2 promoted the production of VEGF-C protein but inhibited its transcription in VHL-inactivated RCC cell lines [60]. Previous studies have shown that hypoxia can reduce the VEGF-C mRNA but enhance protein expression of VEGF-C by affecting intra-ribosomal entry sites within

its 5'UTR [63]. Additionally, the activity of VEGF-C's promoter is mainly influenced by the binding strength of NF κ B. Hypoxia-induced dephosphorylation of NF κ B can interfere with its binding to the promoter, thereby inhibiting transcription and potentially causing a decrease in mRNA levels [60]. The regulatory patterns described above suggest that VEGF-C may be influenced by different mechanisms at the mRNA and protein levels, necessitating further experiments to elucidate the regulatory relationship between them Fig. 1.

HIF and Tumor Microenvironment

The characteristics of tumor microenvironment are complexity and continuous variability. It comprises different types of cells, including immune cells, blood vessels, stromal cells, and lymphatic vessels. The communication between malignant cells and other components in the TME promotes the malignancy of cancers. With the rapid growth of cancer cells, nutrients are inadequately provided by the existing vessel systems. Hypoxia and increased acidity are typical characteristics of tumor microenvironment. To overcome these challenges, TME cells secrete proangiogenic factors to facilitate angiogenesis and lymphangiogenesis, resulting in the significant infiltration of lymphocytes. These molecules and cells exert negative effects on antitumor immunity, which may eventually lead to an immunosuppressive microenvironment [64].

Hypoxia within the TME can lead to immune suppression. HIF-1 α signalling mediates PD-L1 expression in several immune-suppressive cells [65]. Macrophages in hypoxic regions of tumors are polarized into the M2-like phenotype [66]. The hypoxic TME also recruits regulatory T cells (Tregs) via the secretion of CC-chemokine ligand 28 [67].

Hypoxia-induced HIF-1 α accumulation activates fibroblast through the Sonic Hedgehog (Shh) pathway. Connective tissue growth factor and transforming growth factor- β of fibroblasts are upregulated by HIF-1 α [68]. Activated fibroblasts undergo contraction, proliferation, and overproduction of extracellular matrix (ECM) components to promote desmoplasia and fibrosis [68]. In addition, hypoxia causes the generation of blood and lymphatic vessels. Thus, fibroblasts and excessive ECM cause physical stress and compress the newly formed vessels. The compressed blood vessels exhibit impaired blood perfusion, which may aggravate hypoxia in the TME [69]. Constricted lymphatic vessels also show impaired drainage, leading to elevated interstitial fluid pressure, which further reduces blood flow [48, 70]. Hypoxia eventually compromises antitumor immunity. Cancer-associated fibroblasts (CAFs) directly induce immunosuppression. Feig et al. demonstrated that

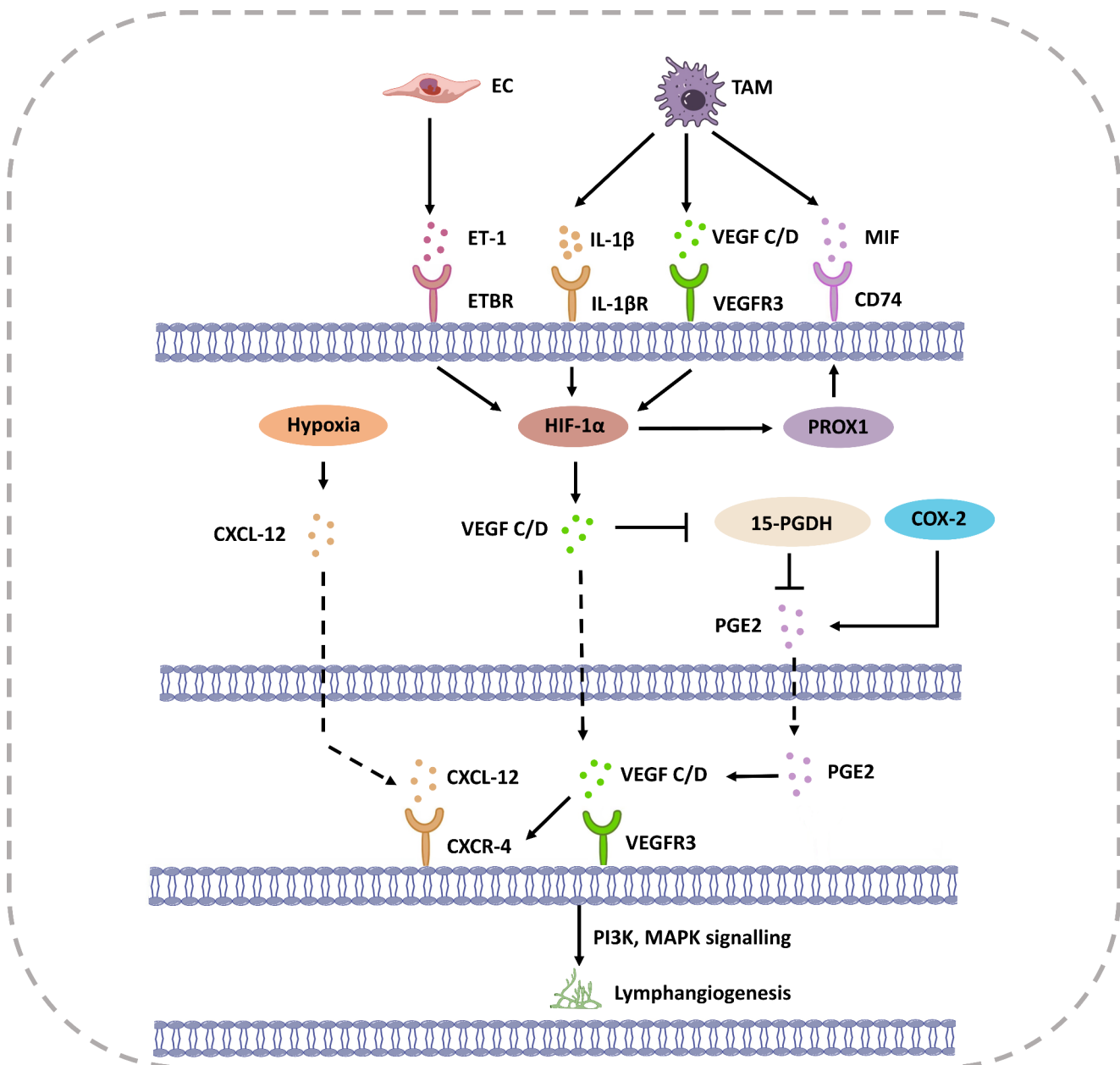


Fig. 1 Regulation of tumor-associated lymphangiogenesis by HIF-1 α

FAP+CAFs secreted CXCL12 and inhibited T lymphocyte activities [71]. CAFs also impaired NK cell cytotoxic activities by disrupting cell receptor expression and reducing cytokine production [68, 72].

The neovascularization of tumors often leads to structural anomalies and vessel leakiness. Endothelial cells display irregularities and polarity lost, detaching from the vascular wall and stacked together. Pericytes detach from ECs and lose their capacity to regulate blood flow and permeability [73]. The basement membrane of newly formed vessels forms pores and shows uneven thickness, compromising the normal positioning of endothelial cells and pericytes

[74]. The loss of tumor vascular integrity results in vessel leakiness. Additionally, the newly formed lymphatic vessels may lack functionality, and hyperplasia of these vessels can result in insufficient valve closure, leading to drainage obstruction [48]. Blood vessel leakage and obstructed lymphatic drainage exacerbate hypoxia within the TME.

The VEGF signalling pathway facilitates immune suppression through interactions with other stromal elements. VEGF binds to VEGFR2 on T cells to increase the expression of immune checkpoints. This process also significantly inhibits the cytotoxicity of effector T cells [75, 76]. VEGF inhibits monocyte differentiation into dendritic cells and

their maturation, dampening dendritic cell antigen presentation [77]. VEGF recruits Tregs to tumors and promotes the polarization of macrophages into M2 tumor-associated macrophages (TAMs) [78, 79]. Hypoxia-induced angiogenesis has been positively correlated with TAM infiltration in BCa [16]. Kusmartsev et al. reported that VEGFR-1 enhanced the inhibitory capacity of myeloid-derived suppressor cells in RCC [80]. Wang et al. reported that the FOXA1/HIF-1 α axis was connected with the epithelial-mesenchymal transition (EMT) and TAM infiltration in PCa [81] Fig. 2.

Targeted therapy

As mentioned previously, HIF is crucially involved in the progression of tumors, and the upregulation of HIF-1 α and HIF-2 α has been proved to indicate an unfavorable prognosis in numerous cancer patients. Therefore, targeting HIF and its downstream signalling may be potential strategies in clinical therapy. The Food and Drug Administration has approved vorinostat, a histone deacetylase inhibitor, for treating cutaneous T-cell lymphoma. Hutt et al. stated that vorinostat inhibits the translation of HIF-1 α and may be a potential approach in cancer treatment [82]. Belzutifan (MK-6482) is a HIF-2 α inhibitor and has led to a favourable

objective response rate and relatively mild adverse effects in patients with nonmetastatic RCC [83].

Although inhibition of lymphangiogenesis is considered a potential strategy target for cancer treatment, only a few drugs have been applied specifically to inhibit this process thus far. Lymphangiogenesis is triggered mainly by VEGF-C and VEGF-D pathways. IMC-3C5 is an anti-VEGFR-3 monoclonal antibody that is under evaluation in a Phase I clinical trial with colorectal cancer patients. Although the drug showed good tolerability, its antitumor efficacy was limited [84]. VGX-100, a VEGF-C-blocking antibody, is currently evaluated in an advanced solid tumor clinical trial for which the results have not yet been published (ClinicalTrials.gov number, NCT01514123).

Targeted therapy often involves targeting the VEGF signalling pathway to suppress angiogenesis. Although long-term antiangiogenic therapy may exacerbate tumor hypoxia, recent research has indicated that after a period of antiangiogenic drug use, tumor vasculature was normalized, which increased tumor cell sensitivity to immunotherapy or chemotherapy [24, 68]. Therefore, antiangiogenesis-targeted therapy is often combined with immune checkpoint inhibitors in the therapy of metastatic ccRCC. Cabozantinib plus nivolumab, axitinib plus pembrolizumab, and lenvatinib plus pembrolizumab have become standards of care for

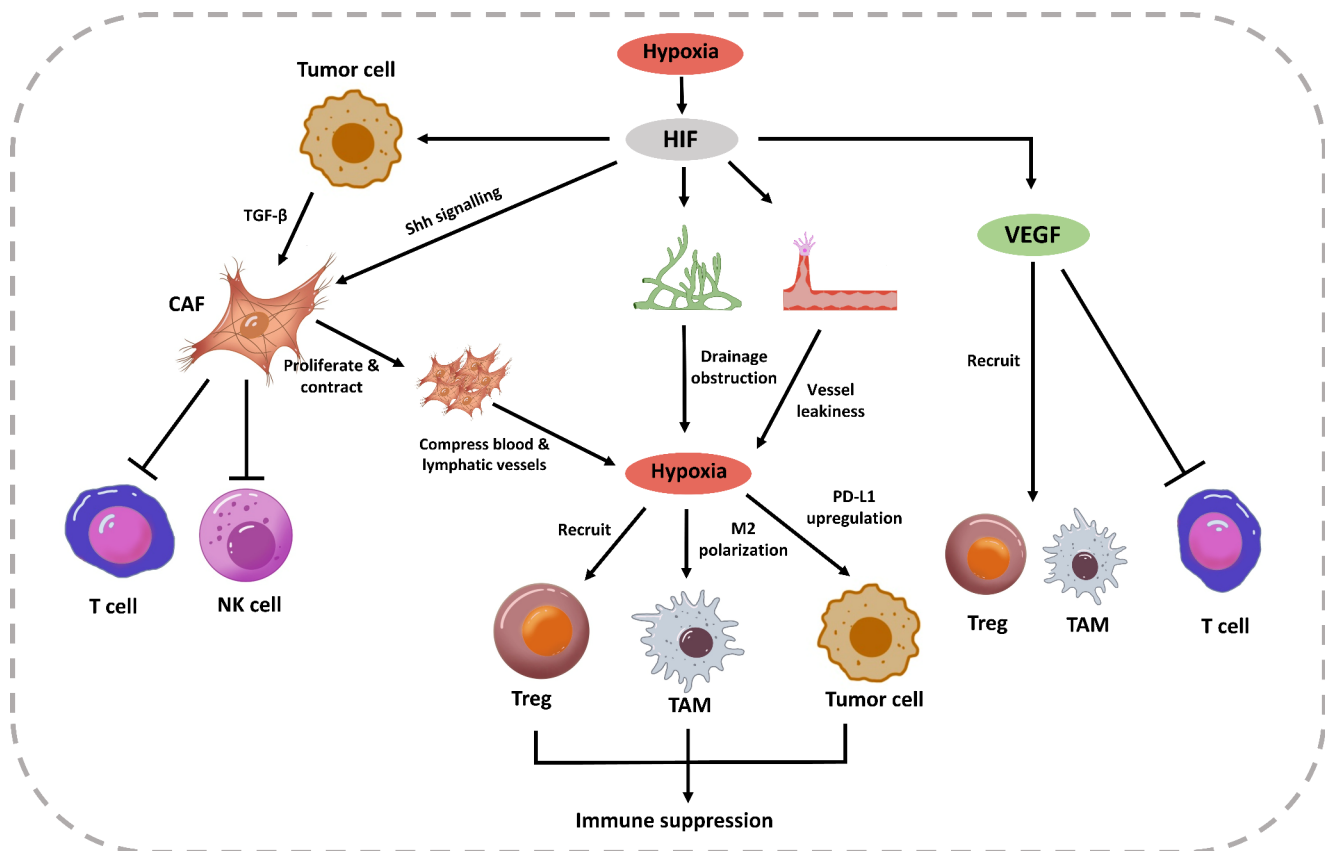


Fig. 2 Schematic overview of HIF-induced angiogenesis, lymphangiogenesis and immune suppression

metastatic ccRCC, according to European Association of Urology recommendations. Other combination therapies are currently in clinical trials, which are listed in Table 1.

Antiangiogenic monotherapy has led to relatively few clinical benefits in metastatic BCa [85]. A recent Phase III trial assessed the effectiveness of adding bevacizumab to gemcitabine and cisplatin in advanced BCa patients, and no improvement in overall survival (OS) was observed [86]. Further exploration and clinical evidence are required to determine the potency of antiangiogenic therapy and other combination therapies in BCa.

Although antiangiogenic therapy has shown limited efficacy in PCa, new targeted monotherapies or combination therapies are under development. ESK981 (CEP-11,981) is a multityrosine kinase inhibitor targeting VEGFR-1/2 and Tie-2 and is in a Phase II clinical trial to evaluate its potency in treating CRPC [87]. Atezolizumab plus cabozantinib has been entered into a Phase II clinical trial for treating CRPC patients whose tumors have progressed in the soft tissue during or after new androgen pathway targeting agents treatment for metastatic disease.

Discussion

The regulation of HIFs in response to hypoxia is a crucial process in tumorigenesis. Among the HIF family, HIF-1 and HIF-2 are the most extensively studied molecules, and they are often found to act as oncogenes in many types of tumors, including bladder cancer and prostate cancer. However, in ccRCC, HIF-1 exhibits tumor-suppressive effects, while HIF-2 promotes tumor growth. Interestingly, a study conducted by Hoefflin et al. revealed that HIF-1 is essential for the initiation formation of ccRCC, suggesting its potential role in carcinogenesis [31]. These seemingly contradictory findings can be attributed to a couple of factors. Firstly, the study was performed using a mouse model, which may not fully replicate the TME in human beings. Secondly, HIF-1 and HIF-2 may exert their effects at different periods of tumor development, indicating a dynamic balance between them. The inactivation of VHL leads to the elevation of HIF-1/2 in precancerous lesions. Increased levels of HIF-1 activate tumor formation, while elevated levels of HIF-2 result in suppression of HIF-1.

In bladder cancer and prostate cancer, the majority of research on angiogenesis has focused on the impact of HIF-1 on VEGF and its receptors. However, the role of HIF-2 and HIF-3 in these cancers remains largely unexplored. It

Table 1 Overview of drugs in clinical testing which target angiogenesis pathways and drug combinations for systemic therapy

Drug name	Target	Cancer Type	Current status	NCT number
Sunitinib + Abemaciclib	VEGFR-1/2/3 + CDK4/6	mRCC	Phase I	NCT03905889
XL092 + Nivolumab	VEGFR-2 + PD-1	RCC	Phase III	NCT05678673
Axitinib + Toripalimab	VEGFR-1/2/3 + PD-1	mRCC	Phase III	NCT04394975
Bevacizumab + Pazopanib	VEGF-A + VEGFR-1/2/3	mRCC	Phase I/II	NCT01684397
Pazopanib + Bevacizumab + Everolimus	VEGFR-1/2/3 + VEGF-A + mTOR	mRCC	Phase II	NCT01217931
Sunitinib + Temozolimus	VEGFR-1/2/3 + mTOR	mRCC	Phase II	NCT01517243
Axitinib + Avelumab	VEGFR-1/2/3 + PD-L1	mRCC	Phase III	NCT02684006
Anlotinib + TQB2450	VEGFR-1/2/3 + PD-L1	mRCC	Phase III	NCT04523272
Lenvatinib + Envafolelimab	VEGFR-1/2/3 + PD-L1	mRCC	Phase I/II	NCT05024214
Bevacizumab + TRK-950	VEGF-A + CAPRIN-1	mRCC	Phase I	NCT03872947
Trebananib + Pembrolizumab	Ang-2 + PD-1	mRCC	Phase I	NCT03239145
ESK981	VEGFR-1/2 + Tie-2	mCRPC	Phase II	NCT03456804
Sunitinib + Abiraterone + Prednisone	VEGFR-1/2/3 + CYP17A1	mPCa	Phase II	NCT01254864
Sunitinib + Leuprolide	VEGFR + LH, FSH	localized PCa	Phase II	NCT00329043
cabozantinib + atezolizumab	VEGFR-1/2/3 + PD-L1	CRPC, RCC and BCa	Phase I/II	NCT03170960
Resveratrol	VEGF + FGF-2	BCa	Preclinical	-
Carnosine	VEGFR-2	BCa	Preclinical	-
EV-CTSB	VEGF-A	BCa	Preclinical	-

Abbreviations: VEGFR, vascular endothelial growth factor receptor; CDK, cyclin-dependent kinase; mRCC, metastatic renal cell carcinoma; PD-1, programmed cell death protein 1; RCC, renal cell carcinoma; VEGF, vascular endothelial growth factor; PD-L1, programmed death-ligand 1; mTOR, mammalian target of rapamycin; CAPRIN-1, cytoplasmic activation/proliferation-associated protein-1; Ang-2, angiopoietin-2; Tie-2, tyrosine kinase with immunoglobulin-like and EGF-like domains 2; mCRPC, metastatic castration-resistant prostate cancer; CYP17A1, cytochrome p450 17a1; mPCa, metastatic prostate cancer; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PCa, prostate cancer; BCa, bladder cancer; FGF2, fibroblast growth factor 2.

appears that HIF-1 and HIF-2 work in synergy to promote tumor development, but the dynamic regulation between them, as observed in renal cancer, still requires further experimental exploration.

HIFs are multifunctional transcription factors that can target multiple downstream pathways to promote angiogenesis. VEGF and angiopoietin 2 bind to receptors located on the surface of endothelial cells, while stromal-derived factor 1 and stem cell factor bind to receptors on bone marrow cells to facilitate vascularization. In urologic tumors, angiogenesis is primarily associated with the VEGF pathway, neglecting the contribution of these targets to angiogenesis. However, focusing on these targets collectively may lead to discoveries in anti-angiogenic therapy.

Lymphangiogenesis in urologic tumors has not been thoroughly investigated, and existing studies primarily focus on the major regulatory factors VEGF-C/D and their interplay in tumor development. In human breast cancer tissue, the localization of HIF-1 and PDGF-B is proximal. PDGF-B has been found to promote immigration and growth of lymphatic endothelial cells in a mouse fibrosarcoma cell line [88], and in breast cancer cell lines, HIF-1 can enhance lymphangiogenesis by upregulating PDGF-B, indicating the significant influence of PDGF-B in lymphatic vessel formation [89]. Further mechanistic research and clinical trials are needed to validate its role in genitourinary tumors.

The presence of VHL mutations in RCC directly leads to elevated expression of HIFs and anti-angiogenic therapy has achieved encouraging results in the treatment of RCC. Targeted therapy and immunotherapy have been extensively employed in the management of metastatic RCC with ongoing clinical trials aiming to explore the most effective drug combinations.

The response to immunotherapy is determined by different immune microenvironments.

Bladder cancer, characterized by a high tumor mutation burden, demonstrates a favorable response to immunotherapy. Nivolumab has achieved notable efficacy in the DFS of muscle-invasive bladder cancer [90]. However, when it comes to monotherapy with anti-angiogenic agents, no benefits have been observed. Prostate cancer patients have limited benefits from immunotherapy targeting PD-1/PD-L1. This is related to the low tumor mutation burden, limited generation of neoantigens, and the presence of immunosuppressive immune cells in prostate cancer. On the other hand, anti-angiogenic therapy plays a crucial role in restoring abnormal tumor vasculature to a normal functional state. It could enhance the sensitivity to immunotherapy. However, VEGF blockade encounters resistance in prostate cancer. These challenges call for new targets that may bring about a breakthrough in targeted therapy and immunotherapy for prostate cancer.

In conclusion, we present a thorough explanation of how HIFs serve as critical transcription factors in driving tumorigenesis, angiogenesis, and lymphangiogenesis, and describe their interaction with stromal elements of tumor microenvironment. Furthermore, we discuss the application of targeted therapies against HIFs and downstream pathways, as well as their combination therapies in urological cancers. Our goal is to establish a foundation for future studies on hypoxia-related molecular mechanisms and identify new therapeutic targets for urological tumors.

Abbreviations

BCa	Bladder cancer
CAF	cancer-associated fibroblast
CRPC	castration-resistant PCa
chRCC	chromophobe RCC
EC	endothelial cell
EMT	epithelial-mesenchymal transition
ECM	extracellular matrix
HGF	hepatocyte growth factor
HIF	hypoxia-inducible factor
IL-1 β	interleukin-1 β
LEC	lymphatic endothelial cell
mTOR	mammalian target of rapamycin
MVD	microvessel density
MAPK	mitogen-activated protein kinase
OS	overall survival
pRCC	papillary RCC
PI3K	phosphatidylinositol 3-kinase
p70S6K	phosphorylates p70 S6 kinase
PDGF	platelet-derived growth factor
PROX1	prospero-related homeobox 1
PGE2	prostaglandin E2
PCa	prostate cancer
Treg	regulatory T cell
RCC	renal cell carcinoma
SNP	single nucleotide polymorphism
Shh	sonic hedgehog
SphK1	sphingosine kinase 1
TME	tumor microenvironment
TAM	tumor-associated macrophage
VEGF	vascular endothelial growth factor
pVHL	VHL gene product
VHL	Von Hippel–Lindau

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Declarations

Conflict of interest The authors declare no conflict of interest.

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References

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global Cancer statistics 2020: GLOBOCAN estimates of incidence and Mortality Worldwide for 36 cancers in 185 countries. *Cancer J Clin* 71:209–249. <https://doi.org/10.3322/caac.21660>
- Schito L, Semenza GL (2016) Hypoxia-inducible factors: Master regulators of Cancer Progression. *Trends in Cancer* 2:758–770. <https://doi.org/10.1016/j.trecan.2016.10.016>
- Semenza GL (2014) Oxygen sensing, Hypoxia-Inducible factors, and Disease Pathophysiology. *Annu Rev Pathol Mech Dis* 9:47–71. <https://doi.org/10.1146/annurev-pathol-012513-104720>
- Wang GL, Jiang BH, Rue EA, Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci USA* 92:5510–5514. <https://doi.org/10.1073/pnas.92.12.5510>
- Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG Jr (2001) HIF α targeted for VHL-Mediated Destruction by Proline Hydroxylation: implications for O₂ sensing. *Science* 292:464–468. <https://doi.org/10.1126/science.1059817>
- Mahon PC, Hirota K, Semenza GL (2001) FIH-1: a novel protein that interacts with HIF-1 α and VHL to mediate repression of HIF-1 transcriptional activity. *Nature* 15:2675–2686. <https://doi.org/10.1101/gad.924501>
- Albadari N, Deng S, Li W (2019) The transcriptional factors HIF-1 and HIF-2 and their novel inhibitors in cancer therapy. *Expert Opin Drug Discov* 14:667–682. <https://doi.org/10.1080/17460441.2019.1613370>
- Mahon PC, Hirota K, Semenza GL, Makino Y, Cao R, Svensson K, Bertilsson G, Asman M, Tanaka H, Cao Y, Berkenstam A, Poellinger L (2001) Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. *Nature* 414:550–554. <https://doi.org/10.1038/35107085>
- Schödel J, Grampp S, Maher ER, Moch H, Ratcliffe PJ, Russo P, Mole DR (2016) Hypoxia, hypoxia-inducible transcription factors, and Renal Cancer. *Eur Urol* 69:646–657. <https://doi.org/10.1016/j.eururo.2015.08.007>
- Gossage L, Eisen T, Maher ER (2015) VHL, the story of a tumour suppressor gene. *Nat Rev Cancer* 15:55–64. <https://doi.org/10.1038/nrc3844>
- Sato Y, Yoshizato T, Shiraishi Y, Maekawa S, Okuno Y, Kamura T, Shimamura T, Sato-Otsubo A, Nagae G, Suzuki H, Nagata Y, Yoshida K, Kon A, Suzuki Y, Chiba K, Tanaka H, Niida A, Fujimoto A, Tsunoda T, Morikawa T, Maeda D, Kume H, Sugano S, Fukayama M, Aburatani H, Sanada M, Miyano S, Homma Y, Ogawa S (2013) Integrated molecular analysis of clear-cell renal cell carcinoma. *Nat Genet* 45:860–867. <https://doi.org/10.1038/ng.2699>
- Gordan JD, Lal P, Dondeti VR, Letrero R, Parekh KN, Oquendo CE, Greenberg RA, Flaherty KT, Rathmell WK, Keith B, Simon MC, Nathanson KL (2022) HIF- α effects on c-Myc Distinguish two subtypes of sporadic VHL-Deficient clear cell renal carcinoma. *J Exp Clin Cancer Res* 281:435–446. <https://doi.org/10.1016/j.jccr.2008.10.016>
- Raval RR, Lau KW, Tran MGB, Sowter HM, Mandriota SJ, Li J-L, Pugh CW, Maxwell PH, Harris AL, Ratcliffe PJ (2005) Contrasting properties of Hypoxia-Inducible factor 1 (HIF-1) and HIF-2 in Von Hippel-Lindau-Associated Renal Cell Carcinoma. *Mol Cell Biol* 25:5675–5686. <https://doi.org/10.1128/MCB.25.13.5675-5686.2005>
- THEODOROPOULOS V (2004) Hypoxia-inducible factor 1 α expression correlates with angiogenesis and unfavorable prognosis in Bladder Cancer. *Eur Urol* 46:200–208. <https://doi.org/10.1016/j.eururo.2004.04.008>
- Yang X, Yin H, Zhang Y, Li X, Tong H, Zeng Y, Wang Q, He W (2018) Hypoxia-induced autophagy promotes gemcitabine resistance in human Bladder cancer cells through hypoxia-inducible factor 1 α activation. *Int J Oncol*. <https://doi.org/10.3892/ijo.2018.4376>
- Onita T, Ji PG, Xuan JW, Sakai H, Kanetake H, Maxwell PH, Fong G-H, Gabriel MY, Moussa M, Chin JL (2002) Hypoxia-induced, Perinecrotic expression of endothelial Per-ARNT-Sim domain Protein-1/Hypoxia-inducible Factor-2 α correlates with Tumor Progression, Vascularization, and focal macrophage infiltration in bladder Cancer1. *Clin Cancer Res* 8:471–480
- Ranasinghe WKB, Baldwin GS, Bolton D, Shulkes A, Ischia J, Patel O (2015) HIF1 α expression under Normoxia in Prostate Cancer— which pathways to target? *J Urol* 193:763–770. <https://doi.org/10.1016/j.juro.2014.10.085>
- Vergis R, Corbishley CM, Norman AR, Bartlett J, Jhavar S, Borre M, Heeboll S, Horwich A, Huddart R, Khoo V, Eeles R, Cooper C, Sydes M, Dearnaley D, Parker C (2008) Intrinsic markers of tumour hypoxia and angiogenesis in localised Prostate cancer and outcome of radical treatment: a retrospective analysis of two randomised radiotherapy trials and one surgical cohort study. *Lancet Oncol* 9:342–351. [https://doi.org/10.1016/S1470-2045\(08\)70076-7](https://doi.org/10.1016/S1470-2045(08)70076-7)
- Fraga A, Ribeiro R, Príncipe P, Lobato C, Pina F, Maurício J, Monteiro C, Sousa H, Calais da Silva F, Lopes C, Medeiros R (2014) The HIF1A functional genetic polymorphism at locus +1772 associates with progression to metastatic Prostate cancer and refractoriness to hormonal castration. *Eur J Cancer* 50:359–365. <https://doi.org/10.1016/j.ejca.2013.09.001>
- Nanni S, Benvenuti V, Grasselli A, Priolo C, Aiello A, Mattiussi S, Colussi C, Lirangi V, Illi B, D'Eletto M, Cianciulli AM, Gallucci M, De Carli P, Sentinelli S, Mottolese M, Carlini P, Strigari L, Finn S, Mueller E, Arcangeli G, Gaetano C, Capogrossi MC,

- Donnorso RP, Bacchetti S, Sacchi A, Pontecorvi A, Loda M, Farsetti A (2009) Endothelial NOS, estrogen receptor β , and HIFs cooperate in the activation of a prognostic transcriptional pattern in aggressive human Prostate cancer. *J Clin Invest* 119:1093–1108. <https://doi.org/10.1172/JCI35079>
21. Chau CH, Permenter MG, Steinberg SM, Retter AS, Dahut WL, Price DK, Figg IHW (2005) Polymorphism in the hypoxia-inducible factor 1 α gene may confer susceptibility to androgen-independent Prostate cancer. *Cancer Biol Ther* 4:1222–1225. <https://doi.org/10.4161/cbt.4.11.2091>
22. Semenza GL (2010) Vascular responses to Hypoxia and Ischemia. *ATVB* 30:648–652. <https://doi.org/10.1161/ATVBAHA.108.181644>
23. Polet F, Feron O (2013) Endothelial cell metabolism and tumour angiogenesis: glucose and glutamine as essential fuels and lactate as the driving force. *J Intern Med* 273:156–165. <https://doi.org/10.1111/joim.12016>
24. LaGory EL, Giaccia AJ (2016) The ever-expanding role of HIF in tumour and stromal biology. *Nat Cell Biol* 18:356–365. <https://doi.org/10.1038/ncb3330>
25. Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK, McDonald DM (2002) Abnormalities in Pericytes on blood vessels and endothelial sprouts in tumors. *Am J Pathol* 160:985–1000
26. Carmeliet P, Jain RK (2011) Molecular mechanisms and clinical applications of angiogenesis. *Nature* 473:298–307. <https://doi.org/10.1038/nature10144>
27. Mazumder S, Higgins PJ, Samarakoon R (2023) Downstream targets of VHL/HIF- α signaling in Renal Clear Cell Carcinoma Progression: mechanisms and therapeutic relevance. *Cancers* 15:1316. <https://doi.org/10.3390/cancers15041316>
28. Gordan JD, Lal P, Dondeti VR, Letrero R, Parekh KN, Oquendo CE, Greenberg RA, Flaherty KT, Rathmell WK, Keith B, Simon MC, Nathanson KL (2008) HIF- α effects on c-Myc Distinguish two subtypes of sporadic VHL-Deficient clear cell renal carcinoma. *Cancer Cell* 14:435–446. <https://doi.org/10.1016/j.ccr.2008.10.016>
29. Gordan JD, Lal P, Dondeti VR, Letrero R, Parekh KN, Oquendo CE, Greenberg RA, Flaherty KT, Rathmell WK, Keith B, Simon MC, Nathanson KL, Wu M, Huang Y, Chen T, Wang W, Yang S, Ye Z, Xi X, Zhu M, Chen Q, Liu X, Sun Q, Zhao X, Deng R, Wang Y, Huang J, Xu M, Yan J, Yu J, Keith B, Johnson RS, Simon MC, Roobol MJ, Carlsson SV, Mirzaei S, Paskes MDA, Okina E, Gholami MH, Hushmandi K, Hashemi M, Kalu A, Zarrabi A, Nabavi N, Rabiee N, Sharifi E, Karimi-Maleh H, Ashrafzadeh M, Kumar AP, Wang Y (2012) HIF1 α and HIF2 α : sibling rivalry in hypoxic tumour growth and progression. *J Exp Clin Cancer Res* 12:9–22. <https://doi.org/10.1038/nrc3183>
30. Xu J, Wang B, Xu Y, Sun L, Tian W, Shukla D, Barod R, Grillari J, Grillari-Voglauer R, Maxwell PH, Esteban MA (2012) Epigenetic regulation of HIF-1 α in renal cancer cells involves HIF-1 α /2 α binding to a reverse hypoxia-response element. *Oncogene* 31:1065–1072. <https://doi.org/10.1038/ncr.2011.305>
31. Hoefflin R, Harlander S, Schäfer S, Metzger P, Kuo F, Schönenberger D, Adlesic M, Peighambari A, Seidel P, Chen C, Consenza-Contreras M, Jud A, Lahrmann B, Grabe N, Heide D, Uhl FM, Chan TA, Duyster J, Zeiser R, Schell C, Heikenwalder M, Schilling O, Hakimi AA, Boerries M, Frew IJ (2020) HIF-1 α and HIF-2 α differently regulate tumour development and inflammation of clear cell renal cell carcinoma in mice. *Nat Commun* 11:4111. <https://doi.org/10.1038/s41467-020-17873-3>
32. Shinojima T, Oya M, Takayanagi A, Mizuno R, Shimizu N, Murai M (2007) Renal cancer cells lacking hypoxia inducible factor (HIF)-1 expression maintain vascular endothelial growth factor expression through HIF-2. *Carcinogenesis* 28:529–536. <https://doi.org/10.1093/carcin/bgl143>
33. Chai C-Y, Chen W-T, Hung W-C, Kang W-Y, Huang Y-C, Su Y-C, Yang C-H (2008) Hypoxia-inducible factor-1 expression correlates with focal macrophage infiltration, angiogenesis and unfavourable prognosis in urothelial carcinoma. *J Clin Pathol* 61:658–664. <https://doi.org/10.1136/jcp.2007.050666>
34. Koga F, Tsutsumi S, Neckers LM (2007) Low dose Geldanamycin inhibits hepatocyte growth factor- and Hypoxia-Stimulated Invasion of Cancer cells. *Cell Cycle* 6:1393–1402. <https://doi.org/10.4161/cc.6.11.4296>
35. Xia Y, Wang X, Liu Y, Shapiro E, Lepor H, Tang M-S, Sun T-T, Wu X-R (2022) PKM2 is essential for Bladder Cancer growth and maintenance. *Cancer Res* 82:571–585. <https://doi.org/10.1158/0008-5472.CAN-21-0403>
36. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliareis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997) *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and Prostate Cancer. *Science* 275:1943–1947. <https://doi.org/10.1126/science.275.5308.1943>
37. Fang J, Ding M, Yang L, Liu L-Z, Jiang B-H (2007) PI3K/PTEN/AKT signaling regulates prostate Tumor angiogenesis. *PLoS ONE* 19:2487–2497. <https://doi.org/10.1016/j.cellsig.2007.07.025>
38. Ci X, Xing C, Zhang B, Zhang Z, Ni JJ, Zhou W, Dong J-T (2015) KLF5 inhibits angiogenesis in PTEN-deficient Prostate cancer by attenuating AKT activation and subsequent HIF1 α accumulation. *Mol Cancer* 14:e50394. <https://doi.org/10.1186/s12943-015-0365-6>
39. Ader I, Brizuela L, Bouquerel P, Malavaud B, Cuvillier O (2008) Sphingosine kinase 1: a New Modulator of Hypoxia Inducible factor 1 α during Hypoxia in Human Cancer cells. *Cancer Res* 68:8635–8642. <https://doi.org/10.1158/0008-5472.CAN-08-0917>
40. Cho S-Y, Lee H-J, Jeong S-J, Lee H-J, Kim H-S, Chen CY, Lee E-O, Kim S-H (2011) Sphingosine kinase 1 pathway is involved in melatonin-induced HIF-1 α inactivation in hypoxic PC-3 Prostate cancer cells. *J Pineal Res* 51:87–93. <https://doi.org/10.1111/j.1600-079X.2011.00865.x>
41. Zhou Q, Liu L-Z, Fu B, Hu X, Shi X, Fang J, Jiang B-H (2007) Reactive oxygen species regulate insulin-induced VEGF and HIF-1 expression through the activation of p70S6K1 in human Prostate cancer cells. *Carcinogenesis* 28:28–37. <https://doi.org/10.1093/carcin/bgl085>
42. Fernández-Martínez AB, Lucio-Cazaña J (2015) Intracellular EP2 prostanoid receptor promotes cancer-related phenotypes in PC3 cells. *Cell Mol Life Sci* 72:3355–3373. <https://doi.org/10.1007/s00018-015-1891-5>
43. Samanta D, Semenza GL (2018) Metabolic adaptation of cancer and immune cells mediated by hypoxia-inducible factors. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* 1870:15–22. <https://doi.org/10.1016/j.bbcan.2018.07.002>
44. Luo Y, Yang Z, Yu Y, Zhang P (2022) HIF1 α lactylation enhances KIAA1199 transcription to promote angiogenesis and vasculogenic mimicry in Prostate cancer. *Int J Biol Macromol* 222:2225–2243. <https://doi.org/10.1016/j.ijbiomac.2022.10.014>
45. Pisarsky L, Bill R, Fagiani E, Dimeloe S, Goosen RW, Hagmann J, Hess C, Christofori G (2016) Targeting metabolic symbiosis to Overcome Resistance to anti-angiogenic therapy. *Cell Rep* 15:1161–1174. <https://doi.org/10.1016/j.celrep.2016.04.028>
46. Tammela T, Alitalo K (2010) Lymphangiogenesis: Molecular mechanisms and Future Promise. *Cell* 140:460–476. <https://doi.org/10.1016/j.cell.2010.01.045>
47. Ny A, Koch M, Vandevelde W, Schneider M, Fischer C, Diez-Juan A, Neven E, Geudens I, Maity S, Moons L, Plaisance S, Lambrechts D, Carmeliet P, Dewerchin M (2008) Role of VEGF-D and VEGFR-3 in developmental lymphangiogenesis, a chemogenetic study in *Xenopus* tadpoles. *Blood* 112:1740–1749. <https://doi.org/10.1182/blood-2007-08-106302>

48. Ji R-C, Li L, Ameri AH, Wang S, Jansson KH, Casey OM, Yang Q, Beshiri ML, Fang L, Lake RG, Agarwal S, Alilin AN, Xu W, Yin J, Kelly K (2014) Hypoxia and lymphangiogenesis in Tumor microenvironment and Metastasis. *Cancer Lett* 346:6–16. <https://doi.org/10.1016/j.canlet.2013.12.001>
49. Spinella F, Garrafa E, Di Castro V, Rosanò L, Nicotra MR, Caruso A, Natali PG, Bagnato A (2009) Endothelin-1 stimulates lymphatic endothelial cells and lymphatic vessels to grow and invade. *Cancer Res* 69:2669–2676. <https://doi.org/10.1158/0008-5472.CAN-08-1879>
50. Flister MJ, Wilber A, Hall KL, Iwata C, Miyazono K, Nisato RE, Pepper MS, Zawieja DC, Ran S (2010) Inflammation induces lymphangiogenesis through up-regulation of VEGFR-3 mediated by NF- κ B and Prox1. *Blood* 115:418–429. <https://doi.org/10.1182/blood-2008-12-196840>
51. Yoshimatsu Y, Miyazaki H, Watabe T (2016) Roles of signaling and transcriptional networks in pathological lymphangiogenesis. *Adv Drug Deliv Rev* 99:161–171. <https://doi.org/10.1016/j.addr.2016.01.020>
52. Qian B-Z, Pollard JW (2010) Macrophage diversity enhances Tumor Progression and Metastasis. *Cell* 141:39–51. <https://doi.org/10.1016/j.cell.2010.03.014>
53. Gaber T, Schellmann S, Ereku KB, Fangradt M, Tykwinska K, Hahne M, Maschmeyer P, Wagegg M, Stahn C, Kolar P, Dziurla R, Löhning M, Burmester G-R, Buttgerit F (2011) Macrophage Migration Inhibitory factor counterregulates dexamethasone-mediated suppression of Hypoxia-Inducible Factor-1 α function and differentially influences Human CD4+ T cell proliferation under Hypoxia. *J Immunol* 186:764–774. <https://doi.org/10.4049/jimmunol.0903421>
54. Jung Y-J, Isaacs JS, Lee S, Trepel J, Neckers L (2003) IL-1 β mediated up-regulation of HIF-1 α via an NF κ B/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis. *FASEB j* 17:1–22. <https://doi.org/10.1096/fj.03-0329fje>
55. Baluk P, Hogmalm A, Bry M, Alitalo K, Bry K, McDonald DM (2013) Transgenic overexpression of Interleukin-1 β induces persistent Lymphangiogenesis but not angiogenesis in Mouse Airways. *Am J Pathol* 182:1434–1447. <https://doi.org/10.1016/j.ajpath.2012.12.003>
56. Su J-L, Shih J-Y, Yen M-L, Jeng Y-M, Chang C-C, Hsieh C-Y, Wei L-H, Yang P-C, Kuo M-L (2004) Cyclooxygenase-2 induces EP1- and HER-2/Neu-Dependent vascular endothelial growth Factor-C Up-Regulation. *Cancer Res* 64:554–564. <https://doi.org/10.1158/0008-5472.can-03-1301>
57. Stacker SA, Williams SP, Karnezis T, Shayan R, Fox SB, Achen MG (2014) Lymphangiogenesis and lymphatic vessel remodelling in cancer. *Nat Rev Cancer* 14:159–172. <https://doi.org/10.1038/nrc3677>
58. Zhuo W, Jia L, Song N, Lu X, Ding Y, Wang X, Song X, Fu Y, Luo Y (2012) The CXCL12–CXCR4 chemokine pathway: a Novel Axis regulates Lymphangiogenesis. *Clin Cancer Res* 18:5387–5398. <https://doi.org/10.1158/1078-0432.CCR-12-0708>
59. Alitalo A, Detmar M (2012) Interaction of Tumor cells and lymphatic vessels in cancer progression. *Oncogene* 31:4499–4508. <https://doi.org/10.1038/onc.2011.602>
60. Ndiaye PD, Dufies M, Giuliano S, Douguet L, Grépin R, Durivault J, Lenormand P, Glisse N, Mintcheva J, Vouret-Craviari V, Mograbi B, Wurmser M, Ambrosetti D, Rioux-Leclercq N, Maire P, Pagès G (2019) VEGFC acts as a double-edged sword in renal cell carcinoma aggressiveness. *Theranostics* 9:661–675. <https://doi.org/10.7150/thno.27794>
61. Lund AW, Duraes FV, Hirosue S, Raghavan VR, Nembrini C, Thomas SN, Issa A, Hugues S, Swartz MA (2012) VEGF-C promotes Immune Tolerance in B16 Melanomas and Cross-presentation of Tumor Antigen by Lymph Node Lymphatics. *Cell Rep* 1:191–199. <https://doi.org/10.1016/j.celrep.2012.01.005>
62. Guo Y-C, Zhang M, Wang F-X, Pei G-C, Sun F, Zhang Y, He X, Wang Y, Song J, Zhu F-M, Pandupuspitasari NS, Liu J, Huang K, Yang P, Xiong F, Zhang S, Yu Q, Yao Y, Wang C-Y (2017) Macrophages regulate unilateral Ureteral obstruction-Induced Renal Lymphangiogenesis through C-C motif chemokine receptor 2–Dependent phosphatidylinositol 3-Kinase-AKT–Mechanistic target of Rapamycin Signaling and Hypoxia-Inducible Factor-1 α /Vascular endothelial growth Factor-C expression. *Am J Pathol* 187:1736–1749. <https://doi.org/10.1016/j.ajpath.2017.04.007>
63. Morfioise F, Kuchnio A, Frainay C, Gomez-Bouchet A, Delisle M-B, Marzi S, Helfer A-C, Hantelys F, Pujol F, Guillermet-Guibert J, Bousquet C, Dewerchin M, Pyronnet S, Prats A-C, Carmeliet P, Garmy-Susini B (2014) Hypoxia induces VEGF-C expression in metastatic Tumor cells via a HIF-1 α -Independent translation-mediated mechanism. *Cell Rep* 6:155–167. <https://doi.org/10.1016/j.celrep.2013.12.011>
64. Anderson NM, Simon MC (2020) The Tumor microenvironment. *Proc Natl Acad Sci USA* 30:R921–R925. <https://doi.org/10.1016/j.cub.2020.06.081>
65. Noman MZ, Desantis G, Janji B, Hasmin M, Karray S, Dessen P, Bronte V, Chouaib S, Mimura K, Kono K, Takahashi A, Kawaguchi Y, Fujii H (2014) PD-L1 is a novel direct target of HIF-1 α , and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *J Exp Med* 211:781–790. <https://doi.org/10.1084/jem.20131916>
66. Engblom C, Pfirschke C, Pittet MJ (2016) The role of myeloid cells in cancer therapies. *Nat Rev Cancer* 16:447–462. <https://doi.org/10.1038/nrc.2016.54>
67. Facciabene A, Peng X, Hagemann IS, Balint K, Barchetti A, Wang L-P, Gimotty PA, Gilks CB, Lal P, Zhang L, Coukos G (2011) Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and Treg cells. *Nature* 475:226–230. <https://doi.org/10.1038/nature10169>
68. Martin JD, Seano G, Jain RK (2019) Normalizing function of Tumor vessels: Progress, opportunities, and challenges. *Annu Rev Physiol* 81:505–534. <https://doi.org/10.1146/annurev-physiol-020518-114700>
69. Stylianopoulos T, Martin JD, Chauhan VP, Jain SR, Diop-Frimpong B, Bardeesy N, Smith BL, Ferrone CR, Hornicek FJ, Boucher Y, Munn LL, Jain RK (2012) Causes, consequences, and remedies for growth-induced solid stress in murine and human tumors. *Proc Natl Acad Sci USA* 109:15101–15108. <https://doi.org/10.1073/pnas.1213353109>
70. Khan KA, Kerbel RS (2018) Improving immunotherapy outcomes with anti-angiogenic treatments and vice versa. *Nat Rev Clin Oncol* 15:310–324. <https://doi.org/10.1038/nrclinonc.2018.9>
71. Feig C, Jones JO, Kraman M, Wells RJB, Deonarine A, Chan DS, Connell CM, Roberts EW, Zhao Q, Caballero OL, Teichmann SA, Janowitz T, Jodrell DI, Tuveson DA, Fearon DT (2013) Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in Pancreatic cancer. *Proc Natl Acad Sci USA* 110:20212–20217. <https://doi.org/10.1073/pnas.1320318110>
72. Öhlund D, Elyada E, Tuveson D (2014) Fibroblast heterogeneity in the cancer wound. *J Exp Med* 211:1503–1523. <https://doi.org/10.1084/jem.20140692>
73. Stylianopoulos T, Munn LL, Jain RK (2018) Reengineering the physical microenvironment of tumors to Improve Drug Delivery and Efficacy: from Mathematical modeling to bench to Bedside. *Trends Cancer* 4:292–319. <https://doi.org/10.1016/j.trecan.2018.02.005>
74. Chauhan VP, Stylianopoulos T, Martin JD, Popović Z, Chen O, Kamoun WS, Bawendi MG, Fukumura D, Jain RK (2012) Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. *Nat Nanotech* 7:383–388. <https://doi.org/10.1038/nnano.2012.45>

75. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet A-L, Latreche S, Bergaya S, Benhamouda N, Tanchot C, Stockmann C, Combe P, Berger A, Zinzindohoue F, Yagita H, Tartour E, Taieb J, Terme M, Kusmartsev S, Eruslanov E, Kübler H, Tseng T, Sakai Y, Su Z, Kaliberov S, Heiser A, Rosser C, Dahm P, Siemann D, Vieweg J (2015) VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. *J Exp Med* 212:139–148. <https://doi.org/10.1084/jem.20140559>
76. Gavalas NG, Tsiatas M, Tsitsilonis O, Politi E, Ioannou K, Zio-gas AC, Rodolakis A, Vlahos G, Thomakos N, Haidopoulos D, Terpos E, Antsaklis A, Dimopoulos MA, Bamias A (2012) VEGF directly suppresses activation of T cells from Ascites secondary to Ovarian cancer via VEGF receptor type 2. *Br J Cancer* 107:1869–1875. <https://doi.org/10.1038/bjc.2012.468>
77. Mimura K, Kono K, Takahashi A, Kawaguchi Y, Fujii H (2007) Vascular endothelial growth factor inhibits the function of human mature dendritic cells mediated by VEGF receptor-2. *Cancer Immunol Immunother* 56:761–770. <https://doi.org/10.1007/s00262-006-0234-7>
78. Peterson TE, Kirkpatrick ND, Huang Y, Farrar CT, Marijt KA, Kloepper J, Datta M, Amoozgar Z, Seano G, Jung K, Kamoun WS, Vardam T, Snuderl M, Goveia J, Chatterjee S, Batista A, Muzikansky A, Leow CC, Xu L, Batchelor TT, Duda DG, Fukumura D, Jain RK (2016) Dual inhibition of Ang-2 and VEGF receptors normalizes Tumor vasculature and prolongs survival in glioblastoma by altering macrophages. *Proc Natl Acad Sci USA* 113:4470–4475. <https://doi.org/10.1073/pnas.1525349113>
79. Hansen W, Hutzler M, Abel S, Alter C, Stockmann C, Kliche S, Albert J, Sparwasser T, Sakaguchi S, Westendorf AM, Schaden-dorf D, Buer J, Helfrich I (2012) Neuropilin 1 deficiency on CD4+Foxp3+regulatory T cells impairs mouse Melanoma growth. *Proc Natl Acad Sci USA* 209:2001–2016. <https://doi.org/10.1084/jem.20111497>
80. Kusmartsev S, Eruslanov E, Kübler H, Tseng T, Sakai Y, Su Z, Kaliberov S, Heiser A, Rosser C, Dahm P, Siemann D, Vieweg J (2008) Oxidative stress regulates expression of VEGFR1 in myeloid cells: link to Tumor-Induced Immune suppression in renal cell carcinoma. *J Immunol* 181:346–353. <https://doi.org/10.4049/jimmunol.181.1.346>
81. Jiang J, Chen S, Tsou T, McGinnis CS, Khazaei T, Zhu Q, Park JH, Strazhnik I-M, Hanna J, Chow ED, Sivak DA, Gartner ZJ, Thomson M, Wang X, Brea L, Lu X, Gritsina G, Park SH, Xie W, Zhao JC, Yu J (2022) FOXA1 inhibits hypoxia programs through transcriptional repression of HIF1A. *Oncogene* 41:4259–4270. <https://doi.org/10.1038/s41388-022-02423-6>
82. Hutt DM, Roth DM, Vignaud H, Cullin C, Bouche-careilh M (2014) The histone deacetylase inhibitor, Vorinostat, Represses Hypoxia Inducible Factor 1 alpha expression through transla-tional inhibition. *PLoS ONE* 9:e106224. <https://doi.org/10.1371/journal.pone.0106224>
83. Jonasch E, Donskov F, Iliopoulos O, Rathmell WK, Narayan VK, Maughan BL, Oudard S, Else T, Maranchie JK, Welsh SJ, Thame S, Park EK, Perini RF, Linehan WM, Srinivasan R, MK-6482-004 Investigators (2021) Belzutifan for Renal Cell Carcinoma in Von Hippel–Lindau Disease. *N Engl J Med* 385:2036–2046. <https://doi.org/10.1056/NEJMoa2103425>
84. Saif MW, Knost JA, Chiorean EG, Kambhampati SRP, Yu D, Pytowski B, Qin A, Kauh JS, O’Neil BH (2016) Phase 1 study of the anti-vascular endothelial growth factor receptor 3 mono-clonal antibody LY3022856/IMC-3C5 in patients with advanced and refractory solid tumors and advanced Colorectal cancer. *Cancer Chemother Pharmacol* 78:815–824. <https://doi.org/10.1007/s00280-016-3134-3>
85. Mazzola CR, Chin J (2015) Targeting the VEGF pathway in met-astatic Bladder cancer. *Cell Death Dis* 24:913–927. <https://doi.org/10.1517/13543784.2015.1041588>
86. Rosenberg JE, Ballman KA, Halabi S, Atherton PJ, Mortazavi A, Sweeney C, Stadler WM, Teply BA, Picus J, Tagawa ST, Katragadda S, Vaena D, Misleh J, Hoimes C, Plimack ER, Flaig TW, Dreicer R, Bajorin D, Hahn O, Small EJ, Morris MJ (2021) Randomized Phase III Trial of Gemcitabine and Cisplatin with Bevacizumab or Placebo in patients with Advanced Urothelial Carcinoma: results of CALGB 90601 (Alliance). *JCO* 39:2486–2496. <https://doi.org/10.1200/JCO.21.00286>
87. Qiao Y, Choi JE, Tien JC, Simko SA, Rajendiran T, Vo JN, Dele-hta AD, Wang L, Xiao L, Hodge NB, Desai P, Mendoza S, Juck-ette K, Xu A, Soni T, Su F, Wang R, Cao X, Yu J, Kryczek I, Wang X-M, Wang X, Siddiqui J, Wang Z, Bernard A, Fernandez-Salas E, Navone NM, Ellison SJ, Ding K, Eskelinen E-L, Heath EI, Klionsky DJ, Zou W, Chinnaiyan AM (2021) Autophagy inhibi-tion by targeting PIKfyve potentiates response to immune check-point blockade in Prostate cancer. *Nat Cancer* 2:978–993. <https://doi.org/10.1038/s43018-021-00237-1>
88. Cao R, Björndahl MA, Religa P, Clasper S, Garvin S, Galter D, Meister B, Ikomi F, Tritsaris K, Dissing S, Ohhashi T, Jackson DG, Cao Y (2004) PDGF-BB induces intratumoral lymphangiogenesis and promotes lymphatic Metastasis. *Cancer Cell* 6:333–345. <https://doi.org/10.1016/j.ccr.2004.08.034>
89. Schito L, Rey S, Tafani M, Zhang H, Wong CC-L, Russo A, Russo MA, Semenza GL (2012) Hypoxia-inducible factor 1-dependent expression of platelet-derived growth factor B pro-motes lymphatic Metastasis of hypoxic Breast cancer cells. *Proc Natl Acad Sci USA* 109:E2707–2716. <https://doi.org/10.1073/pnas.1214019109>
90. Bajorin DF, Witjes JA, Gschwend JE, Schenker M, Valder-rama BP, Tomita Y, Bamias A, Lebre T, Shariat SF, Park SH, Ye D, Agerbaek M, Enting D, McDermott R, Gajate P, Peer A, Milowsky MI, Nosov A, Neif Antonio J, Tupikowski K, Toms L, Fischer BS, Qureshi A, Collette S, Unsal-Kacmaz K, Brough-ton E, Zardavas D, Koon HB, Galsky MD (2021) Adjuvant Nivolumab versus Placebo in muscle-invasive Urothelial Car-cinoma. *N Engl J Med* 384:2102–2114. <https://doi.org/10.1056/NEJMoa2034442>

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