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Anti-angiogenesis approach to genitourinary cancer treatment

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Abstract

Angiogenesis plays a crucial role in the survival, proliferation, and metastatic potential of several tumors, including genitourinary (GU) cancers. Over the last decade, increasing basic science and clinical research have led to the approval of several angiogenesis inhibitors. GU tumors are unique in its pathogenesis whereby specific pathways, such as involvement of the Von Hippel-Lindau gene in clear cell renal cell cancer and aberrant overexpression of vascular endothelial growth factor in prostatic cancers and transitional cell bladder cancers, allow for potential targeting using angiogenesis inhibitors. This review discusses the biologic pathways as well as the rationale for using angiogenesis inhibitors in renal cell, prostate, and transitional cell bladder cancers. This review also focuses on pivotal trials and emerging data on the use of these inhibitors.

Keywords

Renal cell cancer; Prostate cancer; Bladder cancer; Bevacizumab; Sorafenib; Sunitinib; anti-angiogenesis

1. Introduction

The term angiogenesis was coined over a century ago [1], but was not fully elucidated until the 1960's when the late Dr. Judah Folkman found that tiny tumors grew to about 1 mm in size and stopped expanding in the absence of neovascularization [2]. Since then, several investigators have examined various *in vivo* and *in vitro* bioassays, mechanisms of angiogenesis, proangiogenic molecules, and eventually, inhibitors against these molecules, that have been translated into clinical practice [3]. The angiogenic process in the tumor microenvironment involves the complex interplay of free angiogenic growth factors with their cognate receptors, endothelial cell activation, and vascular remodeling. However, as specific angiogenic inhibitors are discovered, unique challenges exist in the application of these inhibitors and how best to measure the effects in a clinically meaningful way. The most impressive anti-cancer results today are with agents targeting vascular endothelial growth factors (VEGF). For instance, bevacizumab, a monoclonal antibody against VEGF, is the first Food and Drug Administration (FDA) approved targeted angiogenesis inhibitor (first and second-line with chemotherapy in metastatic colon cancer) [4]. It has gained approval in combination with cytotoxic agents in several other solid tumors, including lung and breast

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cancer. This review will discuss key angiogenic pathways and therapeutic strategies involved in common genitourinary (GU) tumors, specifically clear cell renal cell cancer (RCC), prostate cancer, and transitional cell cancer (TCC) of the bladder.

2. Pathways involved in the angiogenic process

Various mechanisms are involved in the angiogenic process with convergence of these signals permitting transduction and subsequent activation of pathways that promote tumor proliferation, migration, invasion, and ultimately, survival and metastasis. The disruption of the balance between pro- and anti-angiogenic growth factors, favoring the former; disruption of endothelial cell adhesion, as well as hypoxic regulation of various molecular and cellular systems, contributes to genetic transcription leading to angiogenesis.

2.1. Pro-angiogenic growth factors

The family of VEGF has been the most extensively studied proangiogenic factor with more than seven family members described to date [5-7]. VEGF-A is the main ligand involved for tumor angiogenesis [8-10]. Three VEGF receptors have also been described, VEGFR1 (Flt-1: Fms-like tyrosine kinase-1), VEGFR2 (KDR: kinase-insert Domain-containing Receptor in humans; Flk-1: Fetal-liver kinase-1 in mice), and VEGFR3 (Flt4) [11-15]. VEGF-A binds the receptors VEGFR1 and 2, transducing major signals for angiogenesis. VEGF-A is critical in early survival of the embryo [16] and is also known as the vascular permeability factor because of its specific activity [17]. The tumor cell and its supporting infiltrating macrophages and mesenchymal cells have been shown to secrete VEGF-A [18], which contributes to increased tumor growth and metastasis. Other members of the VEGF family bind and activate varying receptors. For instance, the placenta growth factor (PlGF) binds and activates only VEGFR1 [7], while VEGF-C and D binds VEGFR3, which regulates lymphatic growth. Thus, the VEGF system functions in a paracrine manner, where surrounding cells secrete VEGF and VEGF activates its cognate receptors on endothelial cells, to promote angiogenesis.

2.2 Disruption of endothelial cell adhesion

Endothelial cells are part of the vascular system responsible for the integrity of the capillary system. Once an angiogenic phenotype is triggered, endothelial cell activation occurs, which describes a series of events that brings about the invasive, migratory, and proliferative capacity of the endothelial cell [19]. Central to these cell adhesion mechanisms is the integrins, which are the cell surface receptors for the extracellular matrix (ECM). Integrins are a family of heterodimer transmembrane glycoproteins consisting of an α and β subunit [20]. Preclinical studies show that genetic ablation or disruption of various integrins result in early embryonic death thought to be secondary to defects in vascular patterning [21]. These integrins bind several natural ligands, including laminin, fibronectin, vitronectin, fibrinogen, fibrin, thrombospondin, matrix metalloproteinase (MMP-2), and fibroblast growth factor 2 [22]. The integrins mediate signaling events by activating the integrin-linked kinase (ILK), protein kinase B (PKB/Akt), mitogen-activated protein kinase (MAPK), Raf or nuclear factor kappa B (NF- κ B) pathways [22], in conjunction with other growth factor receptors, resulting in disruption of cell adhesion, tumor proliferation and migration, and survival.

2.3 Hypoxic regulation of molecular systems

Variations in oxygen tension results in activation of different genes that are similarly regulated in cancer. One of the important mechanisms involved in the regulation of VEGF is via the Von Hippel-Lindau (VHL) protein-induced degradation of the hypoxia-inducible factor 1 (HIF-1 α). HIF is a heterodimeric transcription factor composed of an alpha and beta 1 subunit with HIF-1 α initially identified as a transcription factor regulating erythropoietin production in the kidney especially during times of hypoxia [23,24]. In normoxic conditions, HIF-1 α

interacts with the VHL protein, which functions as the recognition site of the E3 ubiquitin ligase, to allow degradation by 26S proteasomes. However, in times of hypoxia, the hydroxylation of HIF-1 α is reduced, thereby allowing the 2 subunits to combine at nuclear hypoxic response elements of target genes, which encodes for angiogenesis [25]. In cells that are deficient in VHL, inappropriate accumulation of HIF-1 α occurs even in normoxic conditions. In addition, loss of function of the VHL protein leads to avoidance of HIF-1 α degradation, thereby leading to constitutive activation of the target genes VEGF, PDGF and transforming growth factor beta (TGF- β) responsible for angiogenesis, proliferation, and survival.

3. Targeting angiogenesis in GU cancers: renal cell carcinoma, prostate cancer, and bladder Cancer

Angiogenesis plays a pivotal role in the pathogenesis of GU cancers, thus providing a rational drug target for using angiogenic inhibitors in these tumors. There is a strong biologic basis for targeting angiogenesis in clear cell RCC, the most common histologic type of RCC. In clear cell RCC, at least 60% of tumors have inactivation of the VHL gene [25]. Mechanisms of inactivation of the VHL gene include deletions, methylation, or mutation [26-28]. The resultant mutation of the VHL gene, which functions as a tumor suppressor gene, causes oversecretion of VEGF by clear cell RCC. This mutant VHL gene can be seen not only in hereditary forms, but also in sporadic RCC. With hypoxia, tumor-associated macrophages also migrate towards the hypoxic center of the tumor [29]. Although VEGF-A is the most widely studied ligand in activation of clear cell RCC, other mechanisms may be operative that are independent of the VHL pathway, involving other ligands like VEGF-B and C [30].

Similarly, in prostate and bladder cancer, neovascularization with angiogenesis has been described. Histological studies measuring the microvessel density (MVD) in prostate cancer has been used as a prognostic factor for predicting aggressiveness and metastasis [31]. The same has been shown in bladder cancer with MVD being associated with significant differences in disease-free survival (DFS) and overall survival (OS) [32,33]. Recurrence was also lowest for those with the lowest MVD count and highest for those with the highest MVD. Although MVD is not ideal as a sole prognostic factor [34], there is emerging clinical evidence of the value of using strategies of angiogenesis inhibition in prostate cancer [35-39], either alone or in combination with cytotoxic chemotherapy. In addition, higher baseline urine VEGF levels correlated with worse survival in 100 patients with prostate cancer enrolled in a Cancer and Leukemia Group B (CALGB) study undergoing therapy with suramin, a growth factor antagonist [40].

It is also increasingly being recognized that TCC of the bladder has divergent genetic defects [41]. Non-invasive, low-grade papilloma tumors are characterized by *HRAS* activating mutations and fibroblast growth factor 3 (FGFR3) gene mutations resulting in constitutive activation of the receptor tyrosine kinase-Ras pathway. High grade invasive tumors often involve the p53 and retinoblastoma protein tumor-suppressor pathway, along with changes in the microenvironment influenced by the imbalance between pro-angiogenic and anti-angiogenic factors potentially contributing to decreased survival in those with overexpression of pro-angiogenic factors [42,43].

4. Clinical translation of angiogenesis inhibitors in GU cancers

4.1 Bevacizumab

Bevacizumab is the first angiogenesis inhibitor to gain FDA approval [4]. It is a recombinant humanized monoclonal antibody composed of human protein sequences (93%) and a small murine (7%) protein sequence, developed by combining the complementarity-determining

region of the mouse anti-VEGF antibody muMAb VEGF A.4.6.1 into the human IgG1 region, developed against human vascular endothelial growth factor (VEGF) [44]. Neutralization of VEGF has been shown to result in *in vivo* tumor inhibition [8]. The consequent overexpression of downstream targets such as VEGF with HIF-1 α accumulation in loss-of-function VHL seen in RCC brought forth a logical target to inhibit tumor growth. The first trial that validated the use of anti-angiogenesis in RCC [45] was a randomized placebo-controlled phase II study in previously treated clear cell RCC [46]. One hundred sixteen patients, of whom majority (93%) had progressed from prior high-dose interleukin-2 (IL-2) treatment, were randomized to either placebo or bevacizumab at doses of either 3 or 10 mg per kilogram of body weight, given every 2 weeks. Time to progression (TTP) and response rates were the primary endpoint of the trial. Results showed a longer TTP in patients receiving 10 mg/kg of bevacizumab than in those receiving placebo (4.8 versus 2.5 months, $P < 0.001$, log-rank test) and a trend towards improved TTP in those patients who received low dose bevacizumab (3 months versus 2.5 months for placebo, $P = 0.041$). However, overall survival was not significantly different in this trial not powered for survival (all P values were greater than 0.20) [47]. The promising results of this phase II study with a doubling of TTP brought forth a multicenter, randomized, double-blind, phase III trial. Six hundred forty-nine previously untreated, nephrectomized metastatic RCC patients were randomized to receive interferon alfa-2a (9 million units subcutaneously three times weekly) and bevacizumab (10 mg/kg every 2 weeks; $n = 327$) or placebo and interferon alfa-2a ($n = 322$) [48]. The primary endpoint was overall survival (OS). Secondary endpoints included progression-free survival (PFS), response rate, and safety. Results showed a non-significant, yet improved trend towards OS in the bevacizumab and interferon group ($P = 0.0670$) which may have been confounded since interferon alfa-2a patients were encouraged to receive bevacizumab after demonstration of positive interim results [49]. There was an improvement in the median PFS in the bevacizumab plus interferon arm (10.2 months) compared to the control group (5.4 months); HR 0.63, 95% CI 0.52-0.75; $P = 0.0001$. There was a similar improvement in the overall response rate (ORR) with the bevacizumab plus interferon arm compared to the interferon alone arm (31% versus 13%, respectively, $P = 0.0001$), although complete response was rare (1% for the combination arm). One observation with the use of bevacizumab in RCC trials is that response rates may not be the best indicator of activity and endpoints such as PFS may be more appropriate endpoints [45]. Similar results were observed in another phase III trial conducted by the CALGB [50]. This study enrolled 732 previously untreated patients with metastatic clear cell RCC and randomized them into 2 arms, either bevacizumab (10 mg/kg every 2 weeks) plus interferon (9 million units subcutaneously three times weekly) or interferon alone at the same schedule. Results showed a median PFS of 8.5 months (95% CI, 7.5 to 9.7 months) in the combined arm versus 5.2 months (95% CI, 3.1 to 5.6 months) in the interferon monotherapy arm, (log-rank $P < .0001$). Among the 639 patients with measurable disease, superior ORR was also observed in the combination arm, 25.5% (95% CI, 20.9 - 30.6%) versus the interferon arm of 13.1% (95% CI, 9.5-17.3%); $P < .0001$.

Single agent bevacizumab was initially studied in prostate cancer but failed to show significant clinical activity [51]. However, given the encouraging responses seen in other tumor types in combination with chemotherapy, the CALGB combined bevacizumab with docetaxel and estramustine leading to a 77% PSA decline rate (defined as PSA decline of $>50\%$ in 58 of 75 patients with sufficient PSA data) [52]. Results are also awaited of another CALGB trial, 90401, which compared overall survival between men with chemotherapy-naïve metastatic castration-resistant prostate cancer (CRPC) treated with docetaxel and prednisone and those treated with docetaxel, prednisone and bevacizumab [53]. The combination of the anti-angiogenic agent thalidomide with bevacizumab has also showed promise in CRPC. Prior work with thalidomide as a single agent [36,37] in metastatic prostate cancer demonstrated modest activity. The addition of thalidomide to docetaxel, led to an improvement in median OS in a phase II trial of 75 chemotherapy-naïve metastatic CRPC patients [39,54]. The combination arm ($n = 50$) had a median OS of 25.9 months versus the docetaxel-alone arm ($n = 25$) of 14.7

months, $P2 = .0407$. This trial led to the combination of a four-drug regimen, bevacizumab, thalidomide, docetaxel, and prednisone, leading to an estimated median PFS of 18.2 months and a PSA decline rate of approximately 90% for the 60 patients enrolled [55]. Bevacizumab is also being studied in metastatic Transitional Cell Cancer (TCC) of the bladder in combination with gemcitabine and cisplatin [56].

4.2 Sorafenib

Sorafenib functions not only as a multi-tyrosine kinase inhibitor that targets wild-type and mutant b-Raf and c-Raf kinase isoforms *in vitro*, but also inhibits angiogenesis via inhibition of VEGFR-2, VEGFR-3, and/or platelet-derived growth factor receptor-beta (PDGFR- β) [57,58]. The US Food and Drug Administration (FDA) approval in GU cancers has been limited to RCC for sorafenib [59,60], although it has also been studied in prostate cancer, and TCC.

An initial randomized phase II discontinuation trial conducted in 202 metastatic RCC patients showed a longer PFS in those treated with sorafenib compared to placebo. Further randomization of patients ($n=65$) who achieved stable disease after the initial run in of 3 months of sorafenib therapy [61] led to a median PFS of 24 weeks for the sorafenib versus 6 weeks for the placebo group, $P=0.0087$. A subsequent randomized phase III trial called TARGET (Treatment Approaches in Renal Cancer Global Evaluation Trial) enrolled 903 patients and evaluated sorafenib at a 400 mg twice daily dose versus placebo in cytokine refractory patients. There was a significantly longer PFS for the sorafenib group with median PFS of 5.5 versus 2.8 months in the placebo group [62]. Although final OS was not significantly different, some have suggested that this was due to the cross-over of patients since censoring of patients who crossed over to sorafenib did show a survival advantage for the sorafenib group.

Several phase II trials have been conducted in the US and Europe to evaluate the role of sorafenib in metastatic CRPC [63-65]. These trials showed some modest stabilization of disease but with few significant prostate-specific antigen (PSA) declines. For instance, a PSA decline rate of 3.6% [95% confidence interval (CI) 0.1% to 18.3%], defined as PSA decline of $\geq 50\%$ for at least 4 weeks, was observed in one trial that enrolled 28 chemo-naïve patients [63]. PSA may not be a good biomarker for this drug as it has been shown to increase PSA secretion *in vitro* [65]. It is currently being studied in combination with docetaxel in this patient population [66].

Sorafenib has been studied as a single agent in advanced TCC of the bladder in both the second-line [67], as well as first-line setting [68], but found to have minimal activity. Efforts are under way to determine whether there is an improvement in TTP in advanced TCC with the combination of sorafenib with cytotoxic chemotherapy (gemcitabine and cisplatin) [69].

4.3 Sunitinib

Sunitinib is another small molecule tyrosine kinase inhibitor that targets VEGFR1 and 2, PDGFR- α and β , c-KIT, and the FLT-3 and RET kinases [70]. Sunitinib exhibited more pharmacologic stability and efficacy than its predecessor compound SU5416 [71,72]. Several single arm phase II trials were conducted that have showed promising response rates in metastatic RCC [73-75]. In the phase III trial of 750 patients with good and intermediate risk clear cell RCC, 375 patients in each arm were randomized to either sunitinib at a dose of 50 mg on a 4 out of 6 weeks schedule versus interferon alpha (IFN- α) at 9 million units thrice weekly subcutaneously [76]. Improved PFS was observed in those receiving sunitinib at a median of 11 months versus 5 months, in those treated with IFN at a HR of 0.42; $P<0.001$. This remained significant upon further follow-up of the study [77]. Similar to sorafenib, there was a survival trend for the sunitinib group, which was statistically significant upon censoring of patients who crossed over to the sunitinib arm, with a median overall survival for patients

on sunitinib of 26.4 months versus 20 months with patients on IFN- α who did not cross-over ($P=0.0362$, log-rank test).

Sunitinib in prostate cancer is currently being investigated in a phase I/II trial in combination with docetaxel with the primary objective of characterization of pharmacokinetics, safety, tolerability, and anti-tumor activity of this combination [78]. Another phase I trial evaluating the safety of combining sunitinib and bevacizumab has preliminarily shown that the combination is feasible and has activity [79]. This trial enrolled 32 patients with solid tumors, 11 patients of whom had GU tumors, with sunitinib at a 4 weeks on and 2 weeks off schedule and bevacizumab given on days 1, 15, 29 of every 42-days cycle with doses of sunitinib ranging from 25 mg to 50 mg, in combination with bevacizumab at doses ranging from 5 mg/kg to 10 mg/kg body weight. Of 23 evaluable patients, 7 achieved a partial response (PR), of whom 3 patients had RCC and 2 had bladder cancer. Another 11 patients achieved stable disease. Several toxicities were reported including a dose limiting toxicity (DLT) of grade 4 hypertension, although the regimen was felt to be tolerable in general, without excess unexpected toxicities.

4.4 Other agents under study

4.4.1. AZD2171—AZD2171 is an oral, potent, indole-ether quinazoline ATP-competitive small molecule that inhibits proliferation via inhibition of all VEGF receptors [80,81]. It been used in RCC [82] and metastatic prostate cancer [83], with encouraging results. A phase I dose-escalation study was performed in prostate cancer and the maximal tolerated dose (MTD) was defined at 20 mg with DLTs occurring at the 30 mg dose [84]. An objective response was observed in one patient and 4 patients were observed to have PSA reductions after drug discontinuation. There is currently an ongoing phase II trial of AZD2171 using 20 mg dose at the National Cancer Institute that has enrolled 18 of a planned 35 patients with metastatic CRPC who have progressed after docetaxel, with encouraging responses [83]. Of the eleven patients with measurable disease, 2 had PR. Notable shrinkage of lymph nodes, lung, liver, and bony metastases were also observed although the PSA levels have not corresponded with imaging responses.

Similarly, AZD2171 has been studied in RCC with encouraging results [82]. The overall tumor response rates for 32 evaluable out of 43 enrolled patients was 84% (95% CI: 67 -95%), including a PR in 12 out of 32 (38%) patients and stable disease in 15 out of 32 (47%) pts, with a median PFS of 8.7 months (95% CI: 5.1-not reached).

5. Response assessment using angiogenesis inhibitors

One remaining challenge with the use of these anti-angiogenic therapies is how to evaluate activity. For instance, in prostate cancer, the traditional biomarker using PSA has been found to be an inadequate marker for certain agents [65,85]. Furthermore, the use of traditional measurements of response, such as complete or partial response, are at best problematic, since anti-angiogenic therapy seldom result in tumor shrinkage compared to what has been seen using cytotoxic chemotherapy agents. Therefore, attempts have been made to use functional imaging modality, which may detect early response to antiangiogenic therapy [86]. Magnetic Resonance Imaging (MRI) modality has been used to demonstrate the anti-angiogenic effects of tyrosine kinase inhibitors in RCC [87]. Further refinement using dynamic contrast enhanced magnetic resonance imaging (DCE-MRI), has shown a concomitant decrease in the parameter for measuring vascular permeability, perfusion and blood vessel area, called the K^{trans} using a vascular targeting agent [88]. However, variability is high and may not be predictive of clinical response or PFS [89]. Therefore, refinement of the techniques used to evaluate response is needed over time.

6. Future directions

Challenges remain in devising schedules, combining with cytotoxic chemotherapy, assessing response, and using biologic or functional imaging. Development of these agents has improved the outcome of patients for diseases such as renal cell cancer and increased our understanding of the biology of cancer. Hopefully the ongoing robust studies using angiogenesis inhibitors in the clinic will lead to a broader application of these agents in GU malignancies.

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