Review

Synthèse

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The role of angiogenesis in prostate and other urologic cancers: a review

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Abstract

ANGIOGENESIS IS A PROCESS CRITICAL TO both tumour growth and metastasis. It is a dynamic integrated process involving basement membrane degradation, endothelial cell proliferation and migration, and capillary tubule formation. Under normal circumstances, the microvasculature is maintained in a quiescent state. The acquisition of the angiogenic phenotype depends on the outcome of stimulatory and inhibitory regulation by the tumour and its microenvironment. There are markers of angiogenesis that potentially could provide prognostic information in addition to that gained from conventional clinicopathologic data, and antiangiogenic therapy for urologic cancers has potential advantages over current therapeutic strategies. Promising preclinical studies have led to the initiation of phase I studies of antiangiogenic therapy in combination with chemotherapy, which may lead to novel treatments for urologic malignant tumours and may identify new intermediate markers for the response to therapy.

Prostate adenocarcinoma (PCa) is estimated to be the most commonly diagnosed noncutaneous malignant disease and the second leading cause of cancer deaths in men, whereas bladder transitional cell carcinoma (TCC) and renal cell carcinoma (RCC) are estimated to be the sixth and ninth most commonly diagnosed malignant diseases respectively.[1] Despite significant improvements in local and systemic therapies, most deaths from cancers are due to metastases that resist conventional therapies.[2–4] A major barrier to effective treatment is the biologic heterogeneity of cancer cells exhibited by genetic, biochemical, immunologic and biologic characteristics, such as cell-surface receptors, enzymes, karyotypes, cell morphology, growth parameters and drug resistance. Because of this heterogeneity, continued empiricism in the treatment of urologic cancers is unlikely to produce significant clinical improvements in patient outcomes.[4] Rather, treatments directed toward critical processes that are involved in tumorigenesis and metastasis are more likely to result in significant advances in the treatment of urologic cancers. There are several lines of evidence that carcinogenesis is a multistep process reflecting genetic alterations that occur in the transformation of normal cells into highly malignant derivatives.[5] Similarly, the process of angiogenesis appears to be acquired in discrete steps during tumour development and is critical to tumorigenicity and metastasis.[5] In this article, we review the compelling evidence that supports the importance of angiogenesis and its clinical relevance in urologic cancers.

Angiogenesis

Tumour growth and metastasis depend upon the development of a neovasculature in and around the tumour.[6–10] This process, called angiogenesis, is regulated by the balance between stimulatory and inhibitory factors released by the tumour and its microenvironment (Table 1).[8,11–15] Angiogenesis facilitates progressive tumour growth by providing adequate oxygenation to the tumour through a series of interrelated steps, including endothelial cell proliferation, motility of endothelial cells through the extracellular matrix toward angiogenic stimuli, and capillary differentiation (Fig. 1).

Much of our knowledge of the biology of PCa, TCC and RCC arises from animal experiments in which human tumour cells have been implanted into immune-

deficient nude mice. The angiogenic, tumorigenic and metastatic potential of these human xenografts grown in nude mice is enhanced by implanting these tumour cells and growing them within the orthotopic site rather than a heterotopic site.[16–20] Thus, mechanisms regulating angiogenesis are tissue specific,[21] and the angiogenic phenotype is regulated by the differential expression of cytokines and growth factors within the microenvironment of the organ.[22] This concept of organ-specific angiogenesis is important for the interpretation of preclinical studies evaluating angiogenesis and antiangiogenic therapies for human cancers, because heterotopic tumour models will not reflect accurately the interactions between the microenvironment of the organ and the tumour.

Prognostic markers

A biomarker is biologic material that can be used to enhance the detection of disease or provide prognostic information. The ideal prognostic biomarker would be noninvasive and would provide predictive information regarding the natural history of the disease or the response to treatment in addition to that gained from conventional clinicopathologic parameters. Markers of angiogenesis, including angiogenesis factor expression and microvessel density (MVD), have been evaluated as prognostic markers for urologic cancers.[23]

Antiangiogenic therapies

Because tumour growth and metastasis depend on angiogenesis, a great deal of attention has been focused on therapy that can interrupt this process. Antiangiogenic therapy can target endothelial cells directly; inhibit the production or action, or both, of proangiogenic peptides by the tumour cells or host; or enhance the expression of angiogenesis inhibitors within the tumour.[24] Antiangiogenic therapy that targets endothelial cells, rather than tumour cells directly, has been evaluated as a novel therapeutic strategy for malignant diseases.[25,26] The theoretical advantage to this therapy is that endothelial cells are unlikely to acquire mutations that lead to drug resistance.[27] Endothelial cells are also readily exposed to bloodborne agents, circumventing the problem of drug delivery. Although it was widely assumed that antiangiogenic therapy was antiproliferative, it has recently been found that antiangiogenic therapy can induce apoptosis and tumour regression.[28]

Prostate cancer

Angiogenesis

Early studies reported that conditioned medium from cultures of human PCa cells stimulated endothelial cells, suggesting that these cells produce proangiogenic factors,[29] and both basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) proteins have been measured in the urine of patients with PCa.[30] Subsequently, it was reported that human PCa produces VEGF and interleukin-8 (IL-8) protein.[31] These and other proangiogenic factors are believed to enhance the tumorigenicity and metastasis of PCa, because xenografts of the highly metastatic PC-3M-LN4 PCa cell line overexpressed bFGF, IL-8 and matrix metalloproteinase-9 (MMP-9)

Table 1: Factors implicated as angiogenic promoters and inhibitors in prostate, bladder and kidney cancers

Cancer	Promoter	Inhibitor
Prostate	Basic fibroblast growth factor (bFGF)	**Endogenous inhibitor**
	Vascular endothelial growth factor (VEGF)	Interleukin-10 (IL-10)
	Interleukin-8 (IL-8)	Tissue inhibitor of metalloproteinase-1
	Matrix metalloproteinase-9 (MMP-9)	Angiostatin
	Matrix metalloproteinase-2 (MMP-2)	Prostate-specific antigen
	Transforming growth factor-β_1	Interferon
	Platelet-derived endothelial growth factor	**Pharmaceutical agent**
	Nitric oxide	Anti-VEGF antibody (A4.6.1)
		Fumagillin analogue, 0-(chloroacetyl-carbamoyl) fumagillol (TNP-470)
		Linomide
		Carboxyamido-triazole
		Diet
		Energy-reduced diet
		Soy products
Bladder	bFGF	**Endogenous inhibitor**
	VEGF	Thrombospondin
	Midkine	Interferon
	Thymidine phosphorylase	**Pharmaceutical agent**
	Hepatocyte growth factor	Antiepidermal growth factor receptor antibody (C225)
	Hyaluronic acid fragments	DC101
	IL-8	Halofuginone
	Transforming growth factor-β_1	**Diet**
	Hyaluronidase	Soy products
	MMP-9	
	Angiogenin	
Kidney	bFGF	**Endogenous inhibitor**
	VEGF	Endostatin
	Thymidine phosphorylase	Interferon
	MMPs	Vitamin D_3
	Urokinase-type plasminogen activator (uPA)	**Pharmaceutical agent**
	Transforming growth factor-β_1	Fumagillin analogue, 0-(chloroacetyl-carbamoyl) fumagillol (TNP-470)
	Hepatocyte growth factor	

mRNA compared with the poorly metastatic parental PC-3M cell line from which it was selected.[32] Similarly, the highly metastatic LNCaP-LN3 cell line overexpressed VEGF compared with either the parental LNCaP cell line or LNCaP-Pro5, which was selected for its tumorigenicity in the prostate.[33] LNCaP-LN3 was also more metastatic and overexpressed VEGF mRNA and protein and VEGF receptor protein, with enhanced tumour-induced angiogenesis, compared with either LNCaP or LNCaP-Pro5, after implantation into the prostate of athymic nude mice.

The importance of IL-8 as a mediator of PCa angiogenesis was shown by experiments in which PC-3P cells with lower metastatic potential were transfected with sense IL-8, whereas highly metastatic PC-3M-LN4 cells were transfected with antisense IL-8 constructs.[34] Following orthotopic implantation, the sense-transfected PC-3P cells overexpressed IL-8 and MMP-9 and were highly angiogenic, tumorigenic and metastatic compared with parental PC-3P cells or controls. Antisense transfection of the PC-3M-LN4 cells reduced the expression of IL-8 and MMP-9 and tumour-induced angiogenesis, resulting in inhibited tumorigenicity and metastasis. Therefore, IL-8 also seems to be involved in the regulation of angiogenesis and tumour growth in PCa, in part by induction of MMP expression.

The angiogenic and metastatic phenotype of PCa was also shown to be directly related to the expression of transforming growth factor-β_1 and MMP-2 and inversely related

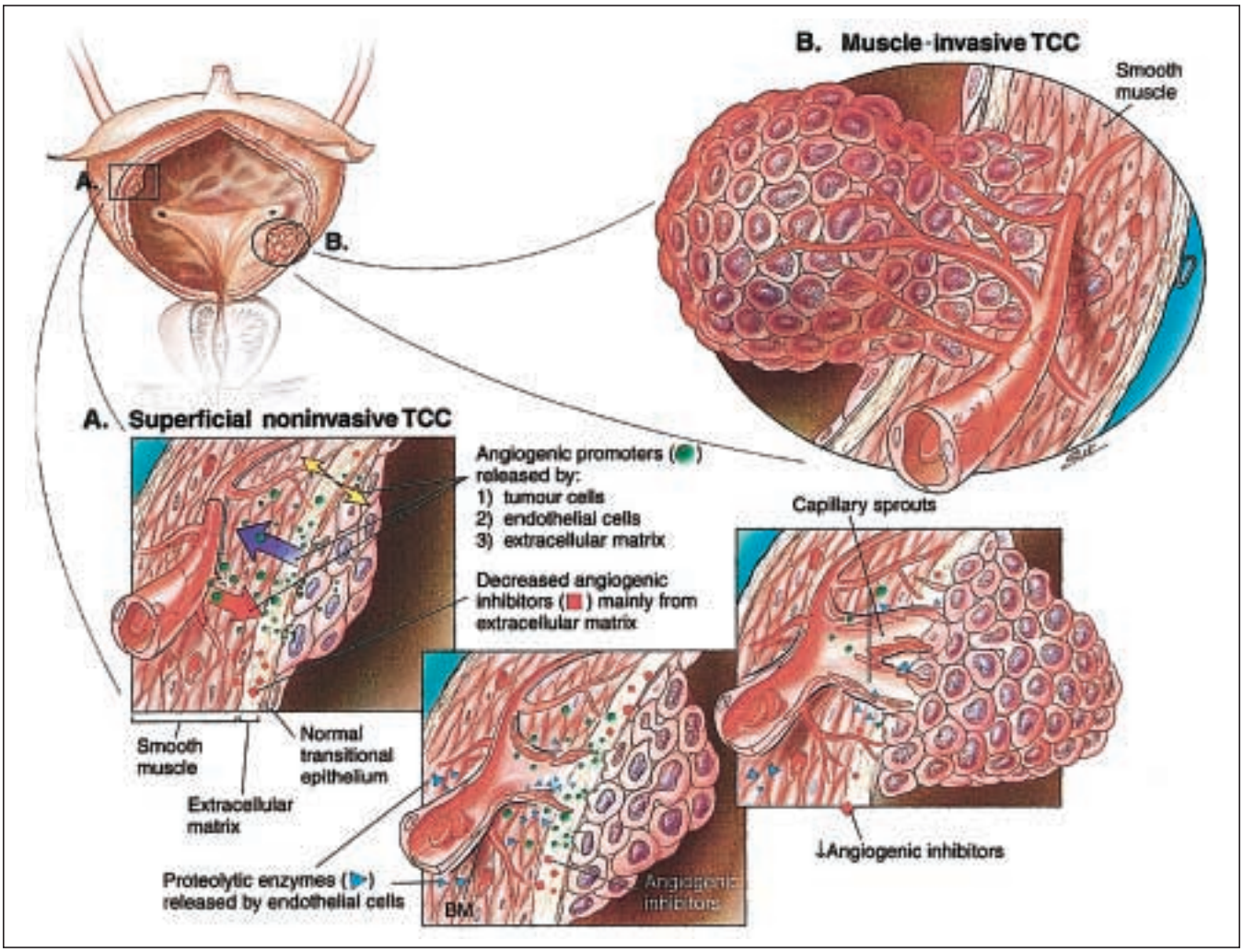

Fig. 1: Angiogenesis in (A) superficial and (B) muscle-invasive transitional cell carcinoma of the bladder is, in general, illustrative of the process of tumour neovascularization in other cancers. The multiple, sequential and interrelated steps by which angiogenesis is hypothesized to occur involve angiogenic stimuli or lack of endogenous angiogenic inhibitors, or both; the inciting by endothelial cells within venules of local proteolysis to degrade the basement membrane; the protrusion of endothelial cells through the wall of the venule; degradation of the interstitial matrix; continuing movement of endothelial cells toward the angiogenic stimulus; formation by endothelial cells of capillary sprouts that form a lumen; proliferation within sprouts; joining of tips of sprouts; blood flow; and formation of new basement membrane and incorporation of microvascular pericytes. TCC = transitional cell carcinoma, BM = basement membrane.

to the expression of IL-10, which upregulated a natural tissue inhibitor of metalloproteinase-1.[35,36]

Androgens seem to regulate VEGF expression in PCa cells and prostatic fibroblasts.[37,38] Hypoxia also induces VEGF transcription through the mediator, hypoxia-inducible factor-1,[39] whose promoter activity is inhibited by protein kinase C inhibitors.[40] Therefore, hypoxia, which can occur when tumours outgrow their blood supply, is a likely explanation for the induction of angiogenesis in PCa.

Several human PCa cell lines express enzymes that convert plasminogen or plasmin to the potent antiangiogenic product angiostatin.[41,42] Furthermore, Fortier and colleagues found that prostate-specific antigen has dose-dependent antiangiogenic activity and inhibits endothelial cell response to both bFGF and VEGF.[43] This antiangiogenic activity may explain the slow progression of certain PCas; however, levels of prostate-specific antigen studied by Fortier and coworkers were, for the most part, much higher than those observed in the serum of patients with PCa. Aberrant nitric oxide production by prostate tumour or host cells may also be a factor in tumour progression because of stimulation mediated by nitric oxide of cancer cell migration and invasiveness.[44–46]

Although the biology of PCa has been further characterized by the identification of relevant angiogenic factors, additional studies are needed to fully elucidate the multiple mechanisms involved in PCa angiogenesis.

Prognostic markers

Both MVD and the expression of various angiogenic factors have been evaluated as prognostic factors for patients with PCa. MVD is a potential prognostic factor that has been correlated with clinical stage, pathologic stage, metastasis, and histopathologic grade and is a significant predictor of disease-specific survival and progression after therapy.[23,47–51] However, MVD has not been consistently shown to correlate independently with outcome after radical prostatectomy[52–56] and, therefore, the prognostic value of MVD needs to be confirmed in prospective studies prior to widespread clinical use.

Both VEGF and bFGF have also been evaluated as prognostic factors in PCa, however, neither is prognostic and their detection may merely reflect the presence of benign prostatic hyperplasia, which is usually present in most patients with PCa.[30,57–59] In preliminary studies, transforming growth factor-β_1 correlated with tumour grade, MVD, bone metastases and a shorter median cancer-specific survival,[60] whereas platelet-derived endothelial growth factor correlated with increased MVD.[61]

Antiangiogenic therapies

Most studies that have evaluated antiangiogenic therapy for PCa have been in the preclinical setting, and the efficacy demonstrated suggests that these agents may be useful clinically. Systemic therapy with the anti-VEGF antibody A4.6.1 inhibited neovascularization, the local growth of human PCa xenografts[62] and lung metastasis.[63] Treatment of the PC-3 and Dunning R-3327 cell lines growing subcutaneously in athymic mice with the synthetic fumagillin analogue 0-(chloroacetyl-carbamoyl) fumagillol (TNP-470) inhibited tumour growth and metastasis in a dose-dependent manner.[63,64] Another antiangiogenic agent, linomide, inhibited tumour-induced angiogenesis and growth of PC-3 tumours implanted subcutaneously in nude mice,[65,66] and it appeared to act directly on tumours with no significant effect on VEGF or bFGF expression relative to controls.[67]

Interferon-α (IFN-α) and IFN-β are cytokines with antiangiogenic activity. Transfected PCa cells with the murine IFN-β gene may have decreased tumorigenesis and metastasis resulting from bystander effects (the effect of interferon on murine inflammatory cells and stroma), resulting in decreased tumour angiogenesis.[68] Although interferon has been relatively ineffective in the treatment of most solid tumours clinically, the delivery of optimal doses using effective schedules[69] and local delivery via gene therapy[68] may significantly improve the results of interferon therapy.

In a phase II clinical trial, the antiangiogenic agent carboxyamido-triazole was administered to 15 patients with androgen-independent PCa, but no significant clinical response was seen.[70] PCa angiogenesis is thought to be androgen dependent; however, in a study evaluating neoadjuvant androgen deprivation, no effect on MVD was observed in 80 patients who had undergone radical prostatectomy.[71]

Consumption of energy-reduced diets by mice with human PCa xenographs resulted in antiangiogenic activity and decreased VEGF expression,[72] whereas the consumption of soy products enhanced apoptosis and decreased tumour cell proliferation, MVD and tumour volume.[73]

Thus far, no reported antiangiogenic therapies have demonstrated significant efficacy in clinical studies. Promising novel and more specific molecules such as the anti-VEGF receptor monoclonal antibody DC101, used as monotherapy or adjuvantly with chemotherapy with or without androgen deprivation, are undergoing preclinical studies.

Bladder cancer

Angiogenesis

The role of angiogenesis in TCC is well established.[74–85] Although the expression of bFGF is variable, it appears to be an important mediator of angiogenesis in TCC. Highly metastatic variants of the human 253J TCC cell line expressed high levels of bFGF mRNA compared with the parental cell line and were highly angiogenic and metastatic.[86] However, the balance between proangiogenic peptides and inhibitors ultimately determines the angiogenic phenotype of the tumour cell, and downregulation of the endogenous angiogenic inhibitor thrombospondin seems to be another key event in bladder carcinogenesis.[87]

Thymidine phosphorylase has angiogenic activity and may also play a role in the pathogenesis of TCC.[88] Hyaluronidase degrades hyaluronic acid into small fragments that stimulate angiogenesis, and urinary levels of hyaluronic acid, hyaluronidase and hyaluronic acid fragments are elevated in patients with TCC.[89–91]

Prognostic markers

MVD has been extensively evaluated as a prognostic factor for TCC and correlated with lymph node metastasis, disease recurrence and survival, and it stratified the risk of recurrence attributed to p53 expression in muscle-invasive TCC.[92–96] However, MVD was not predictive of disease progression in patients with superficial lamina propria–invasive tumours.[97] It appears that MVD can be used to identify patients with locally advanced TCC who are at risk of developing metastatic disease after definitive therapy.

VEGF and bFGF expression by human TCC has also been evaluated, and thus far no definitive conclusions can be reached regarding their prognostic value.[77,98–103] However, bFGF may be a marker of chemoresistance, because the HT1376 TCC cell line, following transfection with the bFGF sense cDNA, demonstrated cisplatin resistance.[104] Overexpression of bFGF mRNA in radical cystectomy specimens also independently predicted poor outcome for patients who received neoadjuvant chemotherapy.[95]

Angiogenin is another proangiogenic factor that is overexpressed in muscle-invasive TCC relative to superficial TCC, and increased serum angiogenin levels were associated with a lower overall survival rate.[105] The loss of expression of thrombospondin protein has also been suggested to be an independent predictor of disease recurrence and overall survival in patients undergoing radical cystectomy.[85] Invasive tumours have also been found to overexpress the heparin-binding growth factor midkine[80] and hepatocyte growth factor.[81,82] Expressions of several angiogenic factors and MVD appear to be potential prognostic markers for TCC, but again prospective studies are necessary before their widespread clinical use.

Antiangiogenic therapies

Several antiangiogenic therapies have demonstrated efficacy for TCC in preclinical studies. Subcutaneous IFN-α therapy inhibited tumour growth and decreased MVD and the expressions of bFGF and VEGF in human TCC growing orthotopically in nude mice.[106] This effect was observed in tumours that were resistant to the antiproliferative effect of interferon, indicating that the inhibition of tumour growth was dependent upon the antiangiogenic effect of interferon. Subsequently, it has been demonstrated that the optimal antiangiogenic effect and efficacy of IFN-α is highly dependent upon the dose and schedule of administration.[69]

Antiangiogenic activity has been observed following therapy for human bladder cancer xenografts with the antiepidermal growth factor receptor monoclonal antibody C225. Therapy with C225 caused the regression of established human xenografts by downregulating the expression of VEGF, bFGF and IL-8 with concomitant inhibition of growth and metastasis.[107]

Similarily, therapy with DC101, in combination with paclitaxel, inhibited tumour growth and metastasis and improved the survival of mice with human bladder cancer xenografts growing orthotopically within the bladder of athymic nude mice.[108] In a heterotopic murine model for bladder TCC, both the pharmaceutical agent halofuginone and dietary soy products have demonstrated antiangiogenic effects.[109,110]

Renal cell carcinoma

Angiogenesis

RCC is among the most vascular of solid tumours, which suggests a prominent role for neovascularization in its pathogenesis, and experiments have demonstrated that human RCC cells induced angiogenesis.[111] VEGF may play a prominent role in RCC angiogenesis, because several studies have demonstrated higher expression levels of VEGF mRNA and protein in tumours relative to normal renal tubules[112–114] and benign oncocytomas,[114] and VEGF appears to act on RCC endothelial cells in a paracrine fashion.[112,115] VEGF has 5 isoforms and expresses variable levels of each, and the increased expression of VEGF-189 in RCC has been associated with higher stage tumours.[116] The role of bFGF in RCC angiogenesis and tumorigenesis is less clear.[113,117]

The activity of MMPs is regulated by the balance of expression with tissue inhibitors of metalloproteinase, whose inactivation by methylation has been observed in RCC.[118] Urokinase-type plasminogen activator (uPA) is a serine protease that activates plasminogen to form plasmin, resulting in alterations of the extracellular matrix degradation, thereby facilitating endothelial cell migration during angiogenesis. Overexpression of the uPA receptor has been observed in RCC.[119] Levels of uPA correlate with inactivation of the von Hippel-Lindau gene, a tumour suppressor gene inactivated in the majority (75%) of sporadic RCCs.[120,121] This suggests that the uPA system plays an important role in angiogenesis, tumorigenicity and metastasis in RCC. The von Hippel-Lindau protein may also be involved in several alternative pathways affecting angiogenesis in RCC by regulating VEGF production and causing regression of the angiogenic mediator transforming growth factor-β_1.[122–124]

Prognostic markers

MVD and the expression of angiogenic factors have been evaluated as prognostic markers for RCC, but it is unclear whether either contributes any additional prognostic information in addition to that obtained from current clini-

copathologic parameters.[125–131] Thymidine phosphorylase overexpression correlated with MVD and was an independent prognostic factor for survival in RCC.[125]

Angiogenesis factors have also been identified in the serum of patients with RCC. VEGF levels were significantly higher in patients with RCC relative to control patients, but they did not remain a prognostic factor upon multivariate analysis, which included tumour grade and stage.[132] Associations between bFGF or VEGF levels and tumour grade, stage or overall survival have differed among studies.[132,133] The VEGF receptor (FLT-1) has also been evaluated as a prognostic factor in RCC, and increased FLT-1 mRNA expression was observed in RCC compared with normal renal tissue.[134] No angiogenic factor appears to be a candidate prognostic marker for RCC.

Antiangiogenic therapies

Several antiangiogenic therapies for RCC have shown promise in preclinical studies and are currently being evaluated in clinical trials. Systemic administration of the endogenous angiogenic inhibitor endostatin suppressed tumour growth in RCC in a murine model,[135] and endostatin is now undergoing evaluation in a multi-institutional phase I clinical trial for patients with solid malignant tumours. TNP-470 demonstrated promising results in preclinical studies, however, a phase II clinical study of 33 patients with metastatic RCC found that TNP-470 had manageable toxic effects, but led to only one partial response of short duration.[136–138]

The results of interferon therapy for locally advanced or metastatic RCC have been disappointing. In a phase II clinical study, subcutaneous IFN-α-2b was used to treat 12 patients with progressive metastatic RCC.[139] Its efficacy was minimal, although higher responses were observed in patients with higher bFGF serum concentrations. Two well-designed, randomized prospective studies of patients with metastatic RCC did not demonstrate a survival advantage with IFN-α-2a or IFN-γ-1b.[140,141] Several other phase II or III clinical trials, with less rigorous study designs, have also demonstrated modest responses or no efficacy for interferon therapy with or without chemotherapeutic agents or other immunotherapies in the treatment of metastatic RCC.[142–151] Significant toxicity was experienced in these trials. Only 2 trials have shown that interferon provided a modest survival advantage compared with chemotherapy or hormonal therapy alone.[152,153] Optimal doses, scheduling and the type of systemic interferon administered may improve the antiangiogenic effect and clinical efficacy.[68] Vitamin D_3 agents also inhibited angiogenesis, tumorigenicity and metastases in animal xenografts of RCC.[154] Currently, clinical trials are evaluating the efficacy of the angiogenesis inhibitor thalidomide in patients with advanced RCC.

Conclusion

The mechanisms regulating the acquisition of the angiogenic phenotype of urologic cancers are undergoing vigorous study but have not, as yet, been fully elucidated. Angiogenesis plays a critical role in tumorigenesis and metastasis in prostate, bladder and renal cancers. Several angiogenic biomarkers have been implicated as prognostic markers for urologic malignant diseases in preliminary reports, but these results need to be confirmed in prospective studies. Antiangiogenic therapy targets pathways regulating tumour growth and metastasis, and promising preclinical studies have led to the initiation of phase I trials for patients with muscle-invasive TCC using the antiangiogenic agents C225 with chemotherapy and interferon with chemotherapy in a neoadjuvant setting. These antiangiogenic strategies, especially in combination with cytoreductive chemotherapy, may lead to novel treatments for urologic cancers and may identify new intermediate markers for the response to therapy.

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