New strategy of antiangiogenic therapy for hepatocellular carcinoma
Minireview

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Hepatocellular carcinoma (HCC) is a hypervascular tumor, and tumor progression and prognosis is associated with angiogenesis. **E**xtracellular matrix remodeling and inflammation play important roles in hepatocarcinogenesis. Some ingredients of extracellular matrix such as endostatin and sulfated polysaccharide, some immunomodulatory agents and cox-2 inhibitor suppress the angiogenesis of HCC. Because vasculogenic mimicry is associated with high tumor grade, some differentiation agents are used to inhibit antiagiogenesis. Besides suppressing the proliferation directly, somatostatin inhibits angiogenesis to suppress growth indirectly. Copper chelator prevents copper from functioning as a cofactor in angiogenesis. The renin-angiotensin system is frequently activated in patients with chronic liver diseases. Perindopril, an angiotensin converting enzyme inhibitor, inhibits angiogenesis by reducing vascular endothelial growth factor (VEGF) production. Kinase inhibitors of VEGF and epidermal growth factor receptors are expected to be of benefit for some patients. Following transarterial embolisation and/or resection, antiangiogenic therapy could prevent the recurring and metastasis. Hypoxia enhances the proliferation, suppresses the differentiation and apoptosis, and induces multidrug resistance of HCC. Because antiangiogenic therapies induce hypoxia, it should be borne in mind the side affects of antiangiogenic therapy. Because long-acting antiangiogenent are needed to control cancer, it needs more clinical studies to confirm the drug resistance of antiangiogenetic therapy.

Key words: angiogenesis, hepatocellular carcinoma, hypoxia, vascular endothelial growth factor

Hepatocellular carcinoma (HCC) is a common malignancy globally. The only curative treatments are surgical resection or liver transplantation, but only a few patients are eligible for these procedures. Local ablative treatments such as ethanol injection and radiofrequency ablation can lengthen survival in selected patients. Unfortunately, most patients are suitable only for palliative treatment because of the extent of their tumor or background liver disease or both [1]. The presence of liver cirrhosis and the associated volume expansion, electrolyte imbalances, decreased liver synthetic and metabolic reserve, and portal hypertension has made the design of systemic therapy for HCC a becombing a major challenge [2].

HCC is a hypervascular tumor, and blood support comes from new branch vessels of hepatic artery [3]. Experimental and clinical data indicate that in human HCC tumor progression is associated with angiogenesis and that an increase in microvascular density (MVD) is associated with a poor prognosis [4, 5]. Such clinicopathological characteristics are not only useful for imaging diagnosis but are also applicable to the treatment of HCC. Because transarterial embolisation (with or without chemotherapy), which aim at tumor vessel, has become one of standard treatments for HCC, antiagiogenic therapy is expected to be benefit for some patients. Here we summarize the new strategy of antiangiogenic therapy for HCC.

1 Kinase inhibitors of vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) receptors. Recent researches have discovered many important angiogenic factors involved in the regulation of angiogenesis in HCC, although the exact molecular pathways are far from clear. Current data suggest that VEGF plays a critical role in angiogenesis of HCC [6]. Tumor expression of VEGF has been shown to correlate with tumor invasiveness and prognosis in patients with HCC [7, 8]. Thus VEGF is an important molecular target for antiangiogenic therapy of HCC.

PTK 787, a tyrosine kinase inhibitor of VEGF receptors, significantly inhibited the growth of tumor xenografts derived from inoculation of PLC and Hep3B HCC cells, resulting in a suppression of tumor MVD [9]. Recently, it has been demonstrated that ZD6474, an inhibitor of VEGF receptor, may

directly inhibit HCC cell proliferation, adhesion, migration and invasion, and the expression of phosphorylated EGFR [10].As the phase II study of GEMOX-B (gemcitabine, oxaliplatin and bevacizumab, an anti-VEGF antibody), the objective response rate was 20%, and 27% of patients had stable disease. Median overall survival was 9.6 months and median progression-free survival (PFS) was 5.3 months; the PFS rate at 3 and 6 months were 70% and 48%, respectively [11].

The ubiquitous Raf serine/threonine kinases are critical molecules within the Raf mitogen extracellular kinase (MEK) extracellular signal-related kinase (ERK) signaling pathway, which regulates cellular proliferation and survival. Raf kinase isoforms (wild-type Raf-1 or the b-raf V600E oncogene) are overactivated in HCC. Sorafenib (BAY 43-9006) is the first oral multi-kinase inhibitor to be developed that targets Raf kinases and receptor tyrosine kinases associated with angiogenesis (VEGF receptor and platelet-derived growth factor receptor)[12]. Sorafenib suppress MVD of PLC/PRF/5 human tumor xenografts in vivo [13]. According to the phase I clinical trial, Sorafenib was effective for certain patients with advanced HCC [14]. As phase II study, 2.2% patients achieved a partial response, 5.8% patients had a minor response, and 33.6% patients had stable disease for at least 16 weeks. Investigator-assessed median time to progression was 4.2 months, and median overall survival was 9.2 months [15].

Betacellulin is a member of the EGF family, and its signal action is mediated through EGF receptors (EGFR). Overexpression of betacellulin by HCC cells and EGFR by tumor endothelial cells enhance vascularity in a paracrine manner [16]. Gefitinib (ZD1839) is a selective EGFR tyrosine kinase inhibitor that has been used successfully to treat lung cancer. Oral administration of gefitinib inhibited angiogenesis of HCC. Effect of gefitinib on HCC-induced angiogenesis depends on its inhibition of the production of VEGF, probably involving a PTEN/Akt signaling pathway [17]. Moreover, gefitinib inhibited the production of active matrix metalloproteinase 9 by HCC [18].

2 Somatostatin. Somatostatin is a small peptide, which exerts inhibitory effects on various types of tumors, including HCC. The effect of somatostatin is mediated by its specific receptor(SSTR). In normal liver, both hepatocytes and hepatic stellate cells (HSCs) are negative for all five SSTR, while cirrhotic liver and hepatoma cells (HepG2, HuH7) express SSTRs [19]. It was reported that the somatostatin receptor was positive in 41% of HCC, and the predominant receptor was receptor 2 [20]. Dramatically, SSRs are not expressed in some human HCC cell lines, such as MHCC97-H, and MHCC97-L [21].

The vessels which provide a route for supply of nutrient and oxygen sustain cell growth. Now that somatostatin could inhibit cell proliferation directly, does somatostatin inhibit angiogenesis to inhibit the cell growth indirectly? Interestingly, somatostatin analogue octreotide is able to inhibit angiogenesis induced by HCC in vivo [22, 23]. Human umbilical vein endothelial cells (HUVECs) expressed the somatostatin receptor subtype SSTR3. In vitro, octreotide inhibited the proliferation, invasion, and differentiation of HUVECs elicited by VEGF. Accordingly MHCC97-H and MHCC97-L cells were insensitive to octreotide at concentrations that significantly inhibited endothelial cells proliferation in vitro. Moreover, octreotide was sufficiently potent to suppress nude mice corneal neovascularization induced by tumor tissues from LCI-D20. Systemic administrations of octreotide produced significant suppression of the growth of LCI-D20 [24].

Although long-acting octreotide is safe in advanced HCC, the efficacy of the somatostatin analogue octreotide remains controversial [25–31]. Most clinical trial showed no survival benefit for HCC patients treated with long-acting octreotide. According to a randomized placebo-controlled tria, a significantly higher survival time was observed for the octreotide group (49 +/- 6 wk) as compared to the control group (28 +/- 1 wk) and to the SSTR negative group (28 +/- 2 wk), while in another research,no clear relationship between SSTR with clinical outcomes was identified [30, 32]. High quality prospective randomized multicenter control trials, with large number of patients and correct clinical sample selection, must be conducted to identify whether the expression of SSTRs in HUVECs and / or HCC cells could prognosticate the effect of octreotide.

3 Endostatin. Malignant transformation of hepatocytes may occur in the context of chronic liver injury, regeneration and cirrhosis. Extracellular matrix (ECM) surrounds hepatocyte as a capsule. Proliferation of hepatocyte and production of ECM are response for the injury and closing the wound. When the injury is limited in time, the result of the repair is restoration of normal tissue structure. When the injury is persistent, however, there is net accumulation of ECM, resulting in cirrhosis. Furthermore, multiple cycles of injury and repair are the setting for unregulated growth and neoplasia [33]. ECM remodeling plays an important role in the carcinogenesis and progression of HCC. Hepatocarcinoma cells create a permissive soil by **e**xtracellular matrix remodeling, result in high proliferation, low differentiation, apoptosis block, invasion and metastasis [34, 35].

Collagen XVIII is an ingredient of ECM. Endostatin, a carboxy-terminal, 20-kDa fragment of the noncollagenous 1 domain of collagen XVIII, is an endogenous angiogenesis inhibitor [36]. In liver cancer, both long-form and short-form of endostatin can be detected in HCC cells and cancerous stromal cells [37]. Lower tissue collagen XVIII mRNA level in HCC is associated with larger tumor size and higher MVD. Moreover, collagen XVIII mRNA is also related to recurrence after resection [38]. Inspiringly, endostatin inhibit tumor growth and metastasis in a nude mice model bearing human HCC [39, 40]. Recombinant endostatin inhibited angiogenesis, and showed synergistic effects with doxorubicin in down-regulating VEGF expression, inhibiting the proliferation of endothelial cells, angiogenesis and the growth [41]. Furthermore, a phase I clinical trial of recombinant human endostatin showed no significant toxicities [42–44].

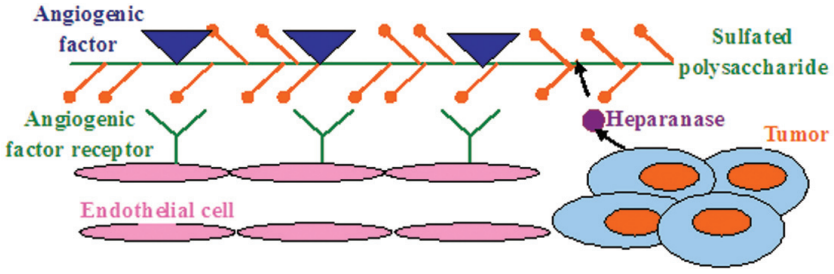

Figure 1 The inhibition on angiogenesis of sulfated polysaccharide
Sulfated polysaccharide could bind VEGF and fibroblast growth factor but was unable to present VEGF and fibroblast growth factor to its high-affinity receptors. Moreover, sulfated polysaccharides could inhibit heparanase, which cleaves heparan sulfate chains of heparan sulfate proteoglycans and causes release of growth factors sequestered by heparan sulfate chains.

4 Sulfated oligosaccharides and polysaccharides. Tissue damage, coagulation and angiogenesis occur in wound healing orderly, and angiogenesis could be triggered by coagulation. Tumor stroma generation is similar to wound healing, and tumor-mediated activation of the hemostatic system has been implicated in both the formation of tumor stroma and the promotion of hematogenous metastasis [45, 46]. Generally, sulfated polysaccharides are the ingredients of ECM and have the action of anticoagulation. Furthermore, sulfated polysaccharide is an endogenous angiogenesis inhibitor. Sulfated polysaccharide could bind VEGF and fibroblast growth factor but was unable to present VEGF and fibroblast growth factor to its high-affinity receptors [47, 48]. Moreover, sulfated polysaccharides could inhibit heparanase, which cleaves heparan sulfate chains of heparan sulfate proteoglycans and causes release of growth factors sequestered by heparan sulfate chains (Fig 1) [47–50]. Downregulating the expression of heparanase inhibits the angiogenesis of HCC cells both in vitro and in vivo [51].

Phosphomannopentaose sulfate (PI-88) has the important property of inhibitors of in vitro angiogenesis and heparanase activity [52]. Dose-limiting toxicity consisted of thrombocytopenia and pulmonary embolism. Other toxicity was generally mild and included prolongation of the activated partial thromboplastin time and injection site echymosis [53]. Recently, Progen Industries announced positive preliminary results from its Phase II clinical trial of PI-88 for the treatment of patients with primary liver cancer following surgical resection of the tumor. The trial demonstrated that PI-88 increased time to tumor recurrence by 76%.The patient group treated with 160 mg of PI-88 had a substantial delay in tumor recurrence compared to those not receiving PI-88 (30 weeks compared with 17 weeks).The final data for this Phase II trial (at 48 weeks — comprising 36 weeks of treatment and a 12-week follow-up period) are expected to be available by the second quarter of 2007 [54, 55]. Recently, our group separated sulfated polysaccharide from Gekko swinhonis Guenther, which has been used as the anti-cancer drug in traditional Chinese medicine for hundreds of years [56]. Gekko sulfated polysaccharide (Gepsin) could suppress the secretion of IL-8 by HCC (unpublication data).

5 Cytokines. As abnormal repair for chronic tissue injury, HCC may occur in the context of chronic liver inflammation [57]. Tissue damage, immune response and angiogenesis occur in wound healing orderly, and angiogenesis could be triggered by inflammation [58, 59]. Previous reports demonstrated the association of HCC angiogenesis with inflammatory cytokines such as macrophage migration inhibitory factor (MIF) and tumor necrosis factor (TNF) families [60–63]. Interferon (IFN)-alpha, one of the potent antiinflammatory cytokines, has been used for the treatment of viral hepatitis. In addition to preventing hepatocarcinogenesis by clearance of the hepatitis virus, IFN-alpha exerts its antiangiogenesis function through inhibition of VEGF and fibroblast growth factor (FGF)-beta [64–67]. Moreover, the lowest dose (1000 IU) of IFN-beta could inhibit bFGF secretion and angiogenesis [68]. Interleukin 12 could activate natural killer cells and cytotoxic T lymphocytes and inhibit angiogenesis induced by HCC [69–71]. MDA-7/IL-24, a unique multifunctional cytokine in the IL-10 family, is endogenously expressed in NK cells, B cells and dendritic cells [72]. The "bystander" effects proposed for MDA-7/IL-24 protein include immune stimulation, apoptosis and antiangiogenesis [72, 73]. Recently, Wand et al reported that IL-24 could reduce angiogenesis of HCC [74].

6 COX-inhibitors. Recent evidence indicates that cyclooxygenase-2 (COX-2) is an important molecular target for anticancer therapies. Its expression is undetectable in most normal tissues, and is highly induced by pro-inflammatory cytokines and growth factors. COX-2 is a well-known target of non-steroidal anti-inflammatory drugs (NSAIDs). Many epidemiological studies demonstrate that treatment with NSAIDs reduce the incidence and mortality of malignancies.

It is now well-established that COX-2 is chronically overexpressed in HCC, and COX-2 expression in liver is correlated with the presence of inflammatory cells [75]. Increased expression of COX-2 in noncancerous liver tissue has been significantly associated with shorter disease-free survival in patients with HCC. Up-regulation of COX-2 is correlated with

VEGF expression and tumor angiogenesis in HCC [76–78]. Inhibition of Cox-2 expression suppressed the proliferation, migration, and differentiation of HUVECs in vitro and neovascularization in vivo. Evidence indicates that COX-2-derived prostaglandins (PGs) contribute to tumor growth by inducing angiogenesis. These inhibitory effects of Cox-2 inhibitor could be partially reversed by the addition of exogenous PGE [79]. Furthermore, Cox-2 inhibition led to the down-expression of proangiogenic factors such as VEGF, hepatocyte growth factor, FGF and angiopoietins in HCC. Cox-2/PGE-2/VEGF pathway possibly contributes to tumor angiogenesis in HCC [79].

Conventional NSAIDs non-selectively inhibit both the constitutive form COX-1, and the inducible form COX-2. Experimental studies on animal models of liver cancer have shown that NSAIDs, including both selective and non-selective COX-2 inhibitors, exert chemopreventive as well as therapeutic effects. Thus COX-inhibitors may use both COX-2-dependent and COX-2-independent mechanisms to mediate their antitumor properties [80].

7 Angiotensin-converting enzyme inhibitors. Angiotensin converting enzyme (ACE) inhibitor is used widely as an antihypertensive agent. The renin-angiotensin system (RAS) is frequently activated in patients with chronic liver diseases. Angiotensin-II (AT-II) is produced by ACE. AT-II induces VEGF in HCC [81]. Among ACE inhibitors, perindopril appeared to be a potent inhibitor of tumor development and angiogenesis, whereas angiotensin-II type 1 receptor [AT (1)-R] antagonists did not exert such an inhibitory effect. The level of VEGF, in the tumor was significantly suppressed by perindopril. In vitro studies showed that perindopril-derived active form, perindoprilat, suppressed the endothelial cell tubule formation. Perindoprilat treatment also significantly inhibited VEGF mRNA expression in BNL-HCC cells in vitro [82, 83]. Thus perindopril inhibited tumor development and angiogenesis independent from AT (1)-R blockage, and that VEGF alternation may be involved in the mechanism of this inhibitory effect.

Treatment with both vitamin K2 and perindopril markedly inhibited the development of preneoplastic lesions in association with suppression of neovascularization in the liver. The combination treatment with vitamin K2 and perindopril exerted a more potent inhibitory effect as compared with the single agent treatments. The in vitro study demonstrated that vitamin K2 and perindopril inhibited the endothelial cell tubular formation. Vitamin K2 also suppressed endothelial cell proliferation in a dose-dependent manner [84]. Vitamin K2 and ACE-I treatment resulted in a marked increase of apoptosis in the tumor, whereas tumor cell proliferation itself was not altered [85]. After one-year treatment of oral administration of vitamin K and ACE-I, not only the serum levels of AFP and AFP-L3 returned to the normal ranges, but also the liver dysplastic nodule in a old woman with liver cirrhosis disappeared [86].

8 Copper chelator. Serum copper levels are upregulated in many human tumors and correlated with tumor burden and prognosis. Copper chelators reduce tumor growth and MVD in animal models. Recent studies have revealed that copper is an important cofactor for several angiogenic agents [87]. Trientine dihydrochloride (trientine) is used in clinical practice as an alternative copper-chelating agent for patients with Wilson's disease of penicillamine intolerance. Copper plays a pivotal role in tumor development and angiogenesis in the murine HCC cells [88, 89], and Copper chelators, especially trientine, could inhibit angiogenesis and enhance apoptosis in the tumor with consequent suppression of the tumor growth in vivo [90–92]. The production of interleukin-8 (IL-8) from the tumor was suppressed by trientine. Thus the chelating effect of trientine prevented copper from functioning as a cofactor in angiogenesis, which resulted in reducing IL-8 level, an angiogenic factor produced by hepatoma cells [93].

9 Thalidomide. Thalidomide was developed in the 1950's and originally marketed as a sedative but was withdrawn after its teratogenic effects were recognized in 1964. Thalidomide has been used in the treatment of the malignancy diseases, such as multiple myeloma, lymphoma, renal cell carcinoma, *etc.* The effects may come from its potent inhibitor of angiogenesis and immune response-modifying properties [94–98].

With a 1.5%-2.6% complete response (CR) rate, a 2.6%-6.1% partial response (PR) rate, and a 7.7%-29.7% stable disease (SD) rate, the results indicate that thalidomide mostly may offer HCC patients disease stabilization (Table 1) [99–106]. Patt *et al* re-

Table 1. Response rate of HCC treated with thalidomide

	Dose (mg/day)	N	CR/PR	SD	PR+SD
Hsu *et al.* 2003 [99]	200 to 600*	63	1/3		10(16%)**
Wang *et al.* 2004 [100]	150 to 300	99	0/6		
Schwartz *et al.* 2005 [101]	200 38	1/1	5	7(18%)	
Patt *et al.* 2005 [102]	100037	0/1	11	12(32%)	
Zhu *et al.* 2005 [103]	200 to 800	26	0/1	2	3(12%)
Han *et al.* 2006 [104]	200 8	0/1	9	5(62.5%)	
chiou *et al.* 2006 [105]	200 42	0/2	6	11(26%)	
Chuah *et al.* 2007 [106]	100 to 800	37	0/1	5	7(19%)

HCC: hepatocellular carcinoma, CR: complete response, PR: partial response, SD: stable disease.

* All responders responded at a dose equal to or less than 300 mg per day.

**Another 6 of the 42 patients with elevated alpha-FP levels before treatment had a more than 50% decrease in their alpha-FP levels after thalidomide treatment.

ported that high doses of thalidomide (1000mg/d) achieved a high response rate, while Hsu *et al* reported that all patients responded at a dose equal to or less than 300 mg per day [99, 102]. Chiou *et al* reported that high total doses of thalidomide achieved a high response rate [105]. Low-dose thalidomide is safe while high doses thalidomide have significant toxicity [99, 102, 107]. In view of the significant neurologic toxicity, thalidomide monotherapy at the high doses studied cannot be recommended for the treatment of HCC [102]. Furthermore, Chiou *et al* reported that Thalidomide therapy is most likely to be effective in patients with cirrhosis and early stage small HCC (< 5 cm) [105].

10 Combination of antiangiogenic therapy with other anticancer therapies. Preliminary results of the clinical studies suggest that single-agent antiangiogenic therapy is poorly active in advanced tumors, because antiangiogenic agents aiming at single targets can be neutralized by up-regulation of other proangiogenic factors [108, 109]. Therefore, combined approaches addressing at least two angiogenic targets should be more effective. sFlt-1 is soluble VEGF receptor 1 as an indirect inhibitor of angiogenesis. Combined expression of sFlt-1 and endostatin effectively suppresses HCC growth under in vivo conditions [110]. Moreover, combination of antiangiogenic therapy with other anticancer therapies is expected to have a good action in advanced tumors [109].

Several researches showed that certain antiangiogenic therapeutics increase the activity of cytotoxic anticancer treatments in preclinical models. The combination treatment of 5-FU and perindopril showed a more inhibitory effect on HCC growth and neovascularization than treatment with 5-FU or perindopril alone. [111]. Zhu *et al* reported that combination of epirubicin and thalidomide (from 200 mg to 800 per day) was well tolerated and 41% patients (7/19) had stable disease [112].

Immunotherapy could induce the immune response for cancer cell to eliminate the small size tumor and prevent the metastasis. Single treatment with either IFN or perindopril significantly attenuated the development of HCC, and the combination of IFN and perindopril nearly abolished hepatocarcinogenesis and neovascularization [113, 114]. However, combination thalidomide and low-dose IFN-alpha2a is neither safe nor efficacious [102].Gene transfer of vasostatin inhibited the proliferation of aortic endothelial cells and angiogenesis, whereas B7H3 therapy activated CD8 (+) and NK cells and increased their infiltration into tumors, and enhanced the levels of circulating IFN-gamma. B7H3 and vasostatin combination gene therapy caused the complete regression of HCC [115].

Preoperative serum endostatin had a significant inverse correlation with MVD, and the serum endostatin level was related to better prognosis after curative resection in HCC patients [116]. Collagen XVIII mRNA is also related to recurrence after resection [38]. Thus antiangiogenic therapy may prevent recurrence after resection of HCC. Endostatin prevents metastasis after resection of HCC in an animal model [117]. All data suggest that endostatin can be used as an adjuvant agent after resection of HCC. IFN-alpha inhibits metastasis and recurrence of human HCC after curative resection in nude mice mediated by antiangiogenesis through downregulating expression of vascular endothelial growth factor but not basic fibroblast growth factor [66]. TNP-470 is a fumagillin analogue that can inhibit endothelial cell proliferation [118–120]. However, the side effects of TNP-470 on liver regeneration after partial hepatectomy are controversial [121, 122]. It should be kept in mind when TNP 470 is used after hepatectomy, especially in cirrhotic patients. Phosphomannopentaose sulfate (PI-88), which has the important property of inhibitors of in vitro angiogenesis and heparanase activity, increases time to tumor recurrence by 76% on patients with HCC following resection [56].

Transcatheter arterial chemoembolization (TACE) could contribute to angiogenesis of HCC and high serum VEGF level has been shown to predict poor response and survival of patients with inoperable HCC undergoing TACE treatment [123, 124]. PTK787 is a tyrosine kinase inhibitor which could block VEGF receptor. PTK787 combined with hypoxia achieved a better therapeutic efficacy than hypoxia alone through enhancing hypoxia-induced antitumor cell effect and preventing the activation of angiogenic process [125]. VEGF antisense oligodeoxynucleotides (ODNs) can inhibit VEGF expression of Walker-256 cells. VEGF antisense ODNs mixed with lipiodol embolizing liver cancer is better in inhibiting liver cancer growth, VEGF expression and MVD than lipiodol alone [126]. Mugitani's study suggested that TNP 470 treatment was more effective when combined with hepatic artery ligation, which had more clinical significance in patients receiving TACE [127].

11 The challenge of antiangiogenic therapy for HCC. HCC is developed through liver cirrhosis which demolishes normal liver blood system. The catastrophe of the normal liver blood system leads to the shortage of blood circulation in the liver and causes hypoxia. Generally, hypoxia could suppress the proliferation of cells. Unfortunately, HCC cells show resistance to hypoxia [128].

Hypoxia enhances the proliferation, suppresses the differentiation and apoptosis of HCC [129–134]. The hypothesis is that HCC cells develop the ability of survival and proliferation in the hypoxia microenvironment because of longtime hypoxia. Now that antiangiogenic therapies induce hypoxia, it should be kept in mind the side effects of antiangiogenic therapy for HCC [135].

The expression of multidrug resistance related genes mdr1, multidrug resistance transporter P-glycoprotein and LRP in HCC is under the control of HIF-1[131]. Apoptosis of HIF-1alpha transfected cells was inhibited when they were exposed to 5-Fu. Hypoxia might be one of the causes for the formation of multidrug resistance in HCC via hypoxia-elicited multidrug resistance related protein expression [136, 137]. The attractive question is whether antiangiogenic therapies induce multidrug resistance of HCC.

Arsenic trioxide (As_2O_3) inhibited HCC growth and angiogenesis, down-regulated the expression of VEGF, and

enhanced tumor cell apoptosis at doses greater than 1 mg/kg, but low doses (<1 mg/kg) of As_2O_3 promoted tumor growth, up-regulated the expression of VEGF and tumor angiogenesis [138, 139]. Clearly, appropriate dosages of some antiangiogenents are required to treat patients to avoid undesirable side effects.

Wang *et al* reported a case with high AFP and lung metastasis had a good response to thalidomide (serum level of AFP dropped and lung lesions became disappeared). Unfortunately, the AFP increased again and lung lesion recurred after 9 months thalidomide continuous therapy. That means the resistant may develop if patients take thalidomide for enough long time [99]. Now that long-acting antiangiogenent are needed to control cancer, it needs more clinical study to confirm the drug resistance of antiangiogenetic therapy.

Conclusion

HCC is a hypervascular tumor, and tumor progression and prognosis is associated with angiogenesis. Kinase inhibitors of VEGF and EGF receptors, somatostatin, endostatin, copper chelator, angiotensin converting enzyme inhibitors, Cox-2 inhibitor, sulfated polyssacharide, differentiation agents, cytokines, are used to inhibit antiagiogenesis–induced by HCC.

Antiangiogenic agents aiming at single targets can be neutralized by up-regulation of other proangiogenic factors. Therefore, combined approaches addressing at least two angiogenic targets should be more effective. Combined with antiangiogenesis therapy and immune therapy should be more effective for prevention of metastasis. Because transarterial embolisation and resection could contribute to angiogenesis of HCC, antiangiogenic therapy following transarterial embolisation and/or resection may provent the recurring and metastasis.

Hypoxia enhances the proliferation, suppresses the differentiation and apoptosis of HCC. Moreover, hypoxia might be one of the causes for the formation of multidrug resistance in HCC. Now that antiangiogenic therapies induce hypoxia, it should be kept in mind the side effects of antiangiogenic therapy for HCC. Because long-acting antiangiogenent are needed to control cancer, it needs more clinical study to confirm the drug resistance of antiangiogenetic therapy.

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