more, head-to-head trials of linifanib versus sorafenib and brivanib versus sorafenib for advanced HCC are ongoing globally. Finally, second-line trials of brivanib, RAD001, axitinib, and ramucimubab for sorafenib failure are also ongoing as global clinical trials [50, 60]. These trial results are expected to yield better outcomes for different stage of HCCs. If positive results are obtained by these clinical trials, the life expectancy in each stage is expected to be much prolonged based on theoretical calculations using hazard ratios of OS incorporated from the SHARP trial [50].

In Japan, although a phase III study in HCC patients following TACE was revealed to be a negative [61], an investigator-sponsored trial investigating the efficacy and tolerability of a combination of TACE with sorafenib is underway (TACTICS trial). In addition, a phase III trial for HCC of acyclic retionid, a vitamin A analog, after resection or RFA has been completed and was presented at the American Society of Clinical Oncology Meeting in 2010. However, the results did not meet the primary end point.

A global phase III trial of sorafenib as an adjuvant therapy after surgery or ablation is currently underway (STORM trial) and a global phase II trial of sorafenib as a maintenance therapy with a combination of TACE is also ongoing (SPACE trial). These results are expected to confirm its usefulness in daily clinical practice. In addition, use of sorafenib in combination with hepatic arterial infusion chemotherapy (SILIUS trial) is also ongoing in Japan. A paradigm shift in HCC treatment may be induced if positive results are obtained by these currently ongoing sorafenib trials.

Conclusion

Recent progress in HCC, including issues from pathogenesis to molecular targeted therapy for HCC, has been described in this article. It is strongly expected that this supplement issue will enhance the most up-to-date knowledge on HCC of the readers of *Oncology*.

Disclosure Statement

The author declares that he has no financial conflict of interest.

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Signaling Pathways Governing Tumor Angiogenesis

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Key Words
Stromal cell · Vascular endothelial growth factor · c-Jun N-terminal kinase

Abstract
Angiogenesis is regulated by the highly coordinated function of various proteins with pro- and antiangiogenic functions. Proangiogenic factors include vascular endothelial growth factor (VEGF), fibroblast growth factor, platelet-derived growth factor, insulin-like growth factor, transforming growth factor, angiopoietins, and several chemokines; antiangiogenic factors include thrombospondin-1, angiostatin, and endostatin. Matrix metalloproteinases display a dual role in vascular development. Notch signaling affects remodeling of the primary vascular network of uniformly sized vessels into functionally and morphologically distinct arteries, veins, and capillaries. Tumors, described as 'wounds that never heal', lose the appropriate balance among these factors. Although VEGF-targeted therapies are showing promise, new angiogenesis targets are needed to make additional gains. Here, we highlight recent advances in our understanding of the regulation of tumor angiogenesis and discuss the potential of molecular targeting as a new therapeutic approach.

Introduction

Angiogenesis, which is the process of new blood vessel growth from preexisting vessels, is imperative in malignant tumor growth. It is regulated by a balance of proangiogenic and angiostatic factors which, upon the switch of tumor cells to an angiogenic phenotype, leads to tumor growth and progression [1]. Since the enormous boost of angiogenesis research after the early 1990s, various angiogenesis regulators have been discovered. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) were identified as positive regulators of angiogenesis. Interferon-α, angiostatin, and endostatin are examples of the first generation of angiogenesis inhibitors, while compounds such as bevacizumab, sunitinib, and erlotinib are examples of current clinically used compounds [2].

Fundamental research as well as these clinical applications have demonstrated that tumor endothelial cells are considered a suitable target for cancer therapy as they play an essential role in angiogenesis. However, angiogenesis is now recognized as the product of evolving crosstalk between different cell types within the tumor and its stroma [3]. There is substantial evidence that the proinflammatory response at the tumor stroma could be rerouted in a tumor-promoting direction by stimulating angiogenesis and tissue remodeling [4]. In this review, we discuss the current literature regarding the molecular mechanisms by which tumor angiogenesis is regulated.

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Vascular Endothelial Growth Factor

VEGF and its receptors represent one of the best-validated signaling pathways in angiogenesis [5]. The VEGF family comprises 5 VEGF glycoproteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E) and placental growth factors 1 and 2, among which VEGF-A is the best characterized. VEGF-A and its receptor, VEGFR-2, are key targets of antiangiogenic agents. VEGF primarily targets endothelial cells and is secreted by cancer cells to mediate tumor angiogenesis [6]. VEGF is upregulated by hypoxia-inducible factor (HIF) 1α and platelet-derived growth factor (PDGF) B and can also be released from the extracellular matrix (ECM) by matrix metalloproteinase (MMP) 9 to initiate an angiogenic switch that promotes tumor growth. In hepatocellular carcinomas (HCCs), expression of VEGF is decreased by a disruption of c-Jun N-terminal kinase (JNK), a member of mitogen-activated protein kinase (MAPK) [7]. JNK may regulate VEGF transcription through AP-1, whereas the effect of JNK could be quite indirect and exerted through factors such as IL-1β and oxygen tension. In epithelial cancers, E-cadherin is downregulated by an HIF-1α-dependent mechanism via the transcription factor Snail, thus promoting epithelial-mesenchymal transition [8]. VEGF elicits epithelial-mesenchymal transition via an autocrine loop [9], suggesting that VEGF involves not only tumor angiogenesis but also the early dissemination of malignant cells outside the epithelial layer.

VEGFRs comprise an extracellular component and an intracellular domain with a consensus tyrosine kinase sequence. VEGFR-1 and VEGFR-2 were found to be predominantly expressed in endothelial cells and VEGFR-3 is primarily associated with lymphangiogenesis [10]. The VEGF family members each bind these receptors with different affinities. VEGFR-1 is a receptor for VEGF-B and placenta growth factor. During pathologic conditions such as tumorigenesis, it is a potent, positive regulator of angiogenesis. VEGFR-2 mediates most of the cellular effects of VEGF-A during angiogenesis, including microvascular permeability, endothelial cell proliferation, migration, and invasion [5]. VEGFR-3 binds with the highest affinities to VEGF-C and VEGF-D and drives lymphangiogenesis. Like VEGFR-2, VEGFR-3 signaling can contribute to angiogenesis in tumors, in which the receptor is expressed on tumor blood vessels as well as on lymphatics. Neuropilins 1 and 2 serve as coreceptors for VEGF by increasing the binding affinity of ligands to VEGFRs [11].

Targeting the tumor vasculature is a particularly attractive strategy because of the presumed genetic stability of endothelial cells [12]. Indeed, the current FDA approved antiangiogenic agents that inhibit the VEGF pathway. These agents include bevacizumab, a humanized anti-VEGF-A monoclonal antibody [13], and two small molecule inhibitors targeting VEGFR2, sorafenib and sunitinib [14, 15]. In combination with other anticancer agents, the addition of bevacizumab significantly increased the progression-free survival and the median overall survival in colorectal cancer and non-small cell lung carcinoma (NSCLC) [16, 17]. It has been shown that systemic administration of sorafenib, a tyrosine kinase inhibitor targeting the VEGF and ERK pathways, significantly extends the survival of patients with advanced stage HCC [18, 19]. However, antiangiogenic treatments targeting a single pathway such as VEGF-A rarely induce durable tumor responses, both in mice and in patients with cancer [20], and may also favor metastasis in selected tumor models [21, 22]. The mechanism for this acquired resistance is not well described but appears to be due in part to expansion or expression of redundant alterations in maturing vasculature [23] and epigenetic mechanisms [24]. Recently, tumor resistance or recurrence after antiangiogenic therapy was causally linked to the recruitment of bone marrow-derived myeloid cells [25]. Damaging the tumor vasculature indeed enhances tumor hypoxia, which in turn upregulates the expression of several myeloid cell chemoattractants that rouse the influx of myeloid cells to treated tumors [20]. Once recruited to the tumors, myeloid cells promote angiogenesis by releasing angiogenic and tissue-remodeling factors [26] and also stimulate tumor cell intravasation, dissemination, and metastasis [27, 28]. New antiangiogenesis targets need to be explored.

Fibroblast Growth Factor

The fibroblast growth factor (FGF) family includes 18 ligands. FGFs interact with 4 main receptors, i.e. FGFRs [29, 30]. The FGF ligands are among the earliest angiogenic factors reported and are involved in promoting the proliferation, migration, and differentiation of vascular endothelial cells [31, 32]. Overexpression of various FGF ligands in different types of tumors has been documented [30]. While the prototype members, FGF-1 and FGF-2, are devoid of a signal peptide and thus are poorly secreted [33], most members of the family have a signal peptide and are efficiently secreted [34]. FGFs have been reported to promote angiogenesis inde-

pendently of VEGF [29], and FGF-2 in particular has been shown to possess potent angiogenic activity [35]. FGFRs are often overexpressed in tumors, and mutations of the FGFR genes have been found in human cancers, making it particularly significant that FGFR activation in endothelial cell culture and animal models leads to angiogenesis [29, 30].

Platelet-Derived Growth Factor

The family of PDGFs consists of 5 members (PDGF-A, PDGF-B, PDGF-AB, PDGF-C, and PDGF-D), and there are 3 types of PDGF receptors (PDGFR-α, PDGFR-β, and PDGFR-αβ) [29]. PDGF signaling promotes cell migration, survival, and proliferation and regulates angiogenesis indirectly by inducing VEGF transcription and secretion [36]. PDGF is involved in vessel maturation and the recruitment of pericytes [37]. PDGF is expressed by endothelial cells and generally acts in a paracrine manner, recruiting PDGFR-expressing cells, particularly pericytes and smooth muscle cells, to the developing vessels [38]. Deletion of *PDGF* and *PDGFR* in mice results in hemorrhage and tissue edema in early embryos as a consequence of defective vascular development [37]. Mutations involving upregulation of PDGF and/or PDGFR have been described in human cancers [38], indicating a likely role for the PDGF pathway in carcinogenesis.

Transforming Growth Factor-β

Transforming growth factor-β (TGF-β) and the corresponding receptors are produced by nearly every cell type, although each of the 3 isoforms of TGF-β (TGF-β1, TGF-β2, and TGF-β3) demonstrates a different tissue expression pattern [39]. TGF-β participates in angiogenesis, cell regulation and differentiation, embryonic development, and wound healing and also has potent growth inhibition properties [39]. TGF-β receptors are classified as type I, II, or III. Type I and II receptors contain serine/threonine kinase domains in their intracellular protein regions, whereas type III does not possess kinase activity but is believed to participate in transferring TGF-β ligands to type II receptors. TGF-β ligands bind to and stimulate type II receptors that recruit, bind, and phosphorylate type I receptors, activating downstream signaling proteins known as SMADs. TGF-β is believed to have both proangiogenic and antiangiogenic properties, depending on the levels present. Low levels of TGF-β contribute to angiogenesis by upregulating angiogenic factors and proteases, whereas high doses of TGF-β stimulate basement membrane reformation, recruit smooth muscle cells, increase differentiation, and inhibit endothelial cell growth [40].

Angiopoietins and TIE Receptors

Angiopoietins (ANGs), important angiogenic molecules, have been identified as ligands for TIE2. ANG1 activates TIE2, leading to receptor autophosphorylation upon binding, and it mediates a range of effects such as tightening cell junctions to inhibit cell permeability and inflammation and activating endothelial cell migration and survival through the PI3K-AKT signaling pathway [41, 42]. ANG1 is a vasculogenic factor which is signaled through the endothelial and bone marrow cell-specific TIE2 receptor tyrosine kinase and promotes endothelial cell survival and vascular maturation by increasing endothelial cell-pericyte interaction [41, 42]. ANG2 is upregulated by hypoxia and may trigger angiogenesis via an autocrine loop in endothelial cells, which express TIE2. ANG2-TIE2 axis promotes angiogenesis in tumors by destabilizing the blood vessels and sensitizing endothelial cells to proliferation signals mediated by other proangiogenic factors such as VEGF [41, 42]. However, in the absence of VEGF, ANG2 promotes endothelial cell apoptosis and consequent blood regression [41–43]. Genetic or pharmacological targeting of ANG2 reduced tumor angiogenesis and delayed the growth of subcutaneous tumors to a variable extent in different studies [44–46]. The role of ANG2 in tumor angiogenesis and growth remains controversial and poorly defined. Expression of the ANG receptor TIE2 is not restricted to endothelial cells. TIE2 is weakly expressed by some circulating monocytes and is significantly upregulated upon their homing to tumors and differentiation into a subset of perivascular macrophages [27, 47]. These TIE2-expressing macrophages have features of M2-polarized tumor-associated macrophages (TAMs) [48], promote tumor angiogenesis [49], and are required for the formation of tumor blood vessels [26, 47]. Because tumor-infiltrating TAMs promote vascular regrowth following therapy-induced vascular damage [50], targeting these cells might increase the efficacy of antiangiogenic treatment by counteracting myeloid cell-mediated angiogenesis and resistance to therapy [20]. The ANG2-TIE2 pathway regulates the proangiogenic activity of TAM.

Delta/Jagged-Notch Signaling

Delta/Notch signaling mediates cell-cell communication and regulates cell fate determination as well as tumor angiogenesis. The family of Notch receptors consists of 4 members (Notch1–4) and there are 3 types of their transmembrane ligands Delta-like (Dll1, Dll3, and Dll4) and 2 members of Jagged (Jagged1 and Jagged2). Dll4 and Notch signaling regulate the cellular actions of VEGF [51]. Dll4 regulates excessive VEGF-induced vessel branching, allowing vessel formation to occur at a productive and efficient rate [52]. Inhibition of Dll4 leads to an increase in tumor vascular density. However, the vascular network is very poorly formed and essentially nonfunctional, and a significant decrease in tumor size was observed. In addition, the decrease in tumor size was noted even in tumor models that are resistant to VEGF blockade [53, 54]. Expression of Jagged1 is dependent on MAPK signaling. Overexpression of Jagged1 in head and neck squamous carcinoma cells leads to increased vascularization and tumor growth, suggesting that Jagged1 promotes angiogenesis [55]. Inhibition of specific components of the Notch signaling pathway, such as Dll4 or Jagged1, may prove to be effective for inhibiting functional angiogenesis in tumors.

Matrix Metalloproteinases

MMPs consist of a multigene family of zinc-dependent ECM-remodeling endopeptidases implicated in pathological processes such as carcinogenesis. MMPs are able to proteolytically process substrates in the extracellular milieu and, in so doing, promote tumor progression. In tumor vasculature, MMPs display a dual role because they can act as both positive and negative regulators of angiogenesis depending on the time point of expression during tumor angiogenesis and vasculogenesis as well as the availability of the substrates. The key players of the MMP family that participate in tumor angiogenesis are mainly MMP-2, MMP-9, and MMP-14 [56].

For tumor growth, it is necessary to eliminate the physical barriers by ECM degradation and subsequently to generate proangiogenic factors. Indeed, MMP-9 participates in the angiogenic switch because it increases the bioavailability of important factors in this process, such as VEGF and bFGF, by degradation of extracellular components, such as collagen type IV [57]. The angiogenic balance is tightly regulated by MMPs, which also downregulate blood vessel formation through the generation of degradation fragments that inhibit angiogenesis such as tumstatin, endostatin, and angiostatin, which are generated via cleavage of collagen types IV and XVII, plasminogen, and perlecan [58–60].

Chemokines

Structurally, chemokines are grouped into 4 families (designated CC, CXC, C, and CX_3C) based on the location of conserved cysteine residues near their amino terminus. In the CC subgroup the first two cysteine residues are adjacent, whereas in the CXC subgroup the first 2 cysteine residues are separated by a nonconserved amino acid, constituting the Cys-X-Cys or 'CXC' motif.

The CXC chemokines, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 (IL-8), promote the migration and proliferation of endothelial cells and are potent promoters of neovascularization. All of these CXC chemokine ligands in the mouse signal via the receptor, CXCR2, whereas in humans they signal through CXCR1 and CXCR2. CXCR2 is the primary receptor for angiogenesis in humans. In a syngeneic tumor model of lung cancer and renal cell carcinoma, *CXCR2* knockout mice had decreased tumor growth, increased necrosis, and decreased angiogenesis and metastases compared to wild-type mice [61, 62]. In colorectal cancer, in vivo tumor growth is induced by increased expression of CXCL1 [63]. CXCL1, CXCL2, and CXCL3 were shown to be highly expressed in patients with malignant melanoma [64]. A direct relationship was found between tissue levels of CXCL5 in surgical specimens of NSCLC and the extent of capillary density consistent with tumor angiogenesis [65]. The expression of CXCL8 (IL-8) in human prostate, lung, and gastric cancer cells is associated with tumorigenicity and neovascularization [66, 67]. Induction of CXCL8 (IL-8) preserved the angiogenic response in HIF-1α-deficient colon cancer cells, indicating that IL-8 mediates angiogenesis independently of VEGF signaling pathways [68]. In addition to the CXC chemokine family, 3 members of the CC chemokine family, CCL2 (MCP-1), CCL11, and CCL16, have also been implicated in neovascularization. Human endothelial cells express CCR2 and respond to MCP-1, resulting in angiogenesis and tumor progression [69].

CXCL4, the first chemokine shown to block angiogenesis [70], is a potent inhibitor of endothelial cell chemotaxis and proliferation and has been shown to inhibit the angiogenic effect of VEGF and bFGF [71]. CXCL9, CXCL10, and CXCL11 are also potent inhibitors of angio-

genesis. Their angiostatic effect appears to be mediated via CXCR3 [72], the expression of which is strongly induced by IL-2.

Conclusion

The complex molecular pathways that govern tumor angiogenesis are logical targets for pharmacological manipulation given the important role they play in the growth and development of cancers. Tumor cells are genetically unstable and biologically heterogeneous, which is considered the principal cause of the failure of systemic chemotherapies. It is believed that endothelial cells in tumor stroma are genetically stable and that these cells will not become drug resistant in response to antivascular therapy. However, recent studies showed that endothelial cells are aneuploid and that they express neoplastic markers [73]. Signals from different stromal cell types have been shown to modulate tumor growth and their responsiveness to therapies in a variety of models, raising the possibility that drugs interfering with these pathways could provide additional therapeutic strategies. Future research regarding the role of critical mediators altering tumor microenvironment involved in tumor angiogenesis may lead to novel therapeutic applications.

Disclosure Statement

The authors disclose no conflicts.

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Adjuvant Therapy after Curative Treatment for Hepatocellular Carcinoma

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Key Words

Adjuvant therapy · Curative treatment · Hepatocellular carcinoma · Prevention of recurrence · Improvement of survival · Interferon molecular targeted agents

Abstract

It is widely accepted that hepatocellular carcinoma (HCC) has an annual recurrence rate of approximately 15–20% even after potentially curative treatment, with the 5-year recurrence rate reaching 80–90%. This recurrence rate is also known to be similar after various curative treatments including resection, percutaneous ethanol injection therapy, and radiofrequency ablation. Generally, in treating patients with HCC associated with hepatitis C or liver cirrhosis, aggressive efforts to prevent secondary carcinogenesis are necessary rather than simply observing the clinical course after treatment. Presently, a combination of peg-interferon and ribavirin is known to be highly effective in patients with difficult-to-treat hepatitis C with a high viral load and genotype I virus. Therefore, indications of these treatments must be considered to prevent secondary carcinogenesis in patients with hepatitis C. Recently, long-term follow-up of low-dose, long-term maintenance therapy using pegylated interferon-α2a for cirrhotic patients clearly showed a preventive effect on HCC occurrence and recurrence. Preventing secondary carcinogenesis by suppressing inflammation employing the same treatment as that against primary carcinogenesis is also important. The molecular targeted agent sorafenib markedly suppresses the serine/threonine kinases of Raf in the MAP kinase cascade and inhibits the tyrosine kinases of angiogenesis factor receptors such as vascular endothelial growth factor and platelet-derived growth factor receptors. It thus simultaneously prevents the proliferation of tumors and inhibits angiogenesis. A clinical trial to examine the recurrence-preventing effect of sorafenib by administration of it after curative treatment such as resection or ablation is in progress (STORM trial: http://clinicaltrials.gov.com, NCT00692770). Treatments to prevent recurrence (including intrahepatic metastasis and multicentric carcinogenesis) as well as early detection and early curative treatment are extremely important to improve the prognosis of patients with HCC. Thus, further research on this issue should be carried out, especially in relation to molecular targeted therapy.

Introduction

It is widely accepted that hepatocellular carcinoma (HCC) shows an annual recurrence rate of approximately 15–20% even after potentially curative treatment, with the 5-year recurrence rate reaching 80–90%. This recurrence rate is also known to be similar after various curative treatments including resection, percutaneous ethanol injection therapy, and radiofrequency ablation. Prevention of the recurrence rate is one of the unmet clinical needs in the treatment of HCC. In addition, of the recurrence rate

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of 15–20%, about 7% is considered to be due to multicentric carcinogenesis, and the remaining about 10–15% is though to be due to the regrowth of remaining microscopic intrahepatic metastatic foci. Therefore, treatments to prevent recurrence should be conducted with these mechanisms of recurrence in mind. Generally, HCC is considered to recur more frequently from remaining intrahepatic foci within 1–2 years of curative treatment and from multicentric carcinogenesis after 2 or more years.

Interferon Therapy for Viral Eradication in Patients after Curative Treatment for HCV-Related HCC

Generally, in treating patients with HCC associated with hepatitis C or liver cirrhosis, aggressive efforts to prevent secondary carcinogenesis are necessary rather than simple observation of the clinical course after treatment. If a diagnosis of chronic hepatitis C can be made clinically on the basis of the laboratory findings, pegylated interferon (IFN) ribavirin combination therapy to attempt eradication of the hepatitis C virus should be performed. Such patients did not used to be treated aggressively because the majority of them had genotype Ib and a high viral load, which made them 'difficult-to-treat patients'. Naturally, IFN therapy to eradicate the virus is easy after curative treatment for liver cancer in patients with a low viral load of genotype I virus. In the literature, complete viral eradication has been established to exhibit favorable effects on the subsequent lower recurrence and improved outcome [1]. Presently, a combination of peg-interferon and ribavirin is known to be highly effective in patients with difficult-to-treat hepatitis C with a high viral load and genotype I viral. Therefore, indications of these treatments must be considered to prevent secondary carcinogenesis in patients with hepatitis C.

A total of 8 prospective randomized controlled trials (RCTs) of IFN therapy after curative treatment for HCC have been reported despite differences in the type of IFN used and its dose or administration period [1–8]. Five were on HCV-related HCC [1–5], 2 on HBV- and HCV-related HCC [6, 7], and 1 on HBV-related HCC [8]. Of the 5 reports on HCV-related HCC, 4 were from Japan and 1 was from Europe. Four of these reports were about IFN therapy aimed toward viral eradication [1, 3–5], and the duration of IFN administration was 48–104 weeks. The sample size was the largest in a recent report by Mazzafero et al. [5] (n = 150), following a report by Shiratori et al. [4] (n = 74). Intention-to-treat analysis of the results of these studies led to the conclusion that the first recurrence cannot be reduced significantly by IFN therapy, but that an improvement in the outcome is clear in patients in whom the virus was successfully eradicated. However, in 4 reports on the long-term administration of IFN (36 months [2], 26 months [3], 26 months [1], and 24 months [6]), the therapy was clearly effective in decreasing the recurrence rate and improving the outcome.

Recently, long-term follow-up of low-dose, long-term maintenance therapy using pegylated IFN-α2a for cirrhotic patients clearly showed a preventive effect of HCC occurrence although those data are a subset analysis of the HALT-C Study [9].

Low-Dose, Long-Term IFN Therapy (IFN Maintenance Therapy) to Prevent Recurrence and Improve Survival after Curative Treatment

Recently, IFN has been intentionally administered at a low dose and over a long period to improve the ALT level and in expectation of its direct anticancer effect rather than to eliminate the virus. The RCTs reported by Ikeda et al. [2], Kubo et al. [1, 3], and Lin et al. [6] are considered to have been conducted based on this concept. Also, low-dose, long-term IFN therapy has also been reported in case-control studies involving subjects matched for age, platelet count, tumor factors, and hepatic functional reserve [10–15].

The direct anticancer effect of IFN has been demonstrated in vitro [16], and the idea of using IFN at a low dose and over a long period without interruption as a maintenance therapy staring immediately after curative treatment for HCC on the basis of its direct anticancer or carcinostatic effect to reduce the recurrence rate or delay of recurrence has also been proposed [10, 12]. The direct anticancer effect of IFN has been speculated to be derived from: (1) suppression of the cell cycle via IFN receptors, (2) induction of apoptosis via cell-mediated immunity, and (3) inhibition of angiogenesis (fig. 1). The IFN administration period is the longest, with a median duration of 4.7 years, and the sample size is largest (n = 127) in 'Maintenance Interferon Therapy' published in *Oncology* [10], in which the IFN therapy is continued for as long as possible, in principle. In this report, the results of the first low-dose, long-term maintenance IFN therapy using pegylated IFN are presented [10].

Of the case-control studies that have been reported, 5 are related to hepatitis C [11–13] and 2 are related to hepatitis B-related HCC [15, 17]. In all of these studies, the recurrence or survival rate was evaluated similarly to

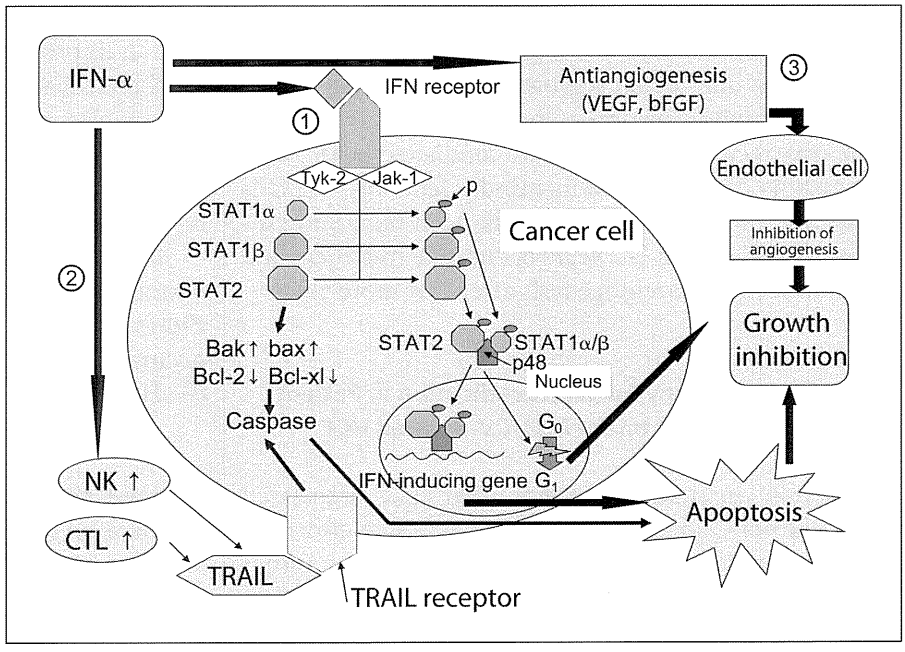

Fig. 1. Hypothesis of the direct antitumor growth inhibition effect of IFN.

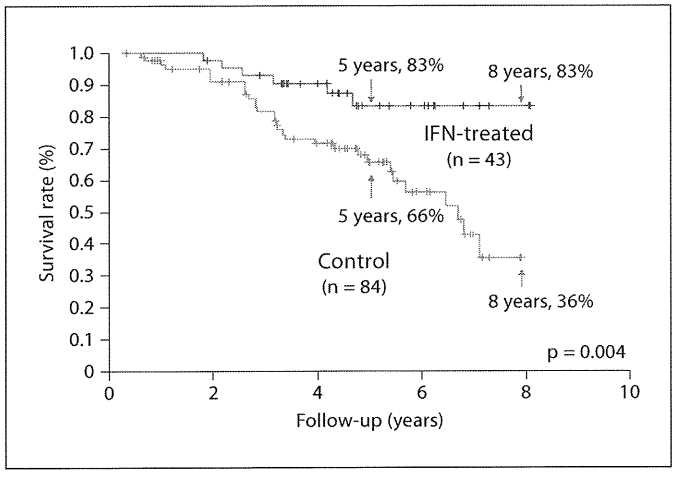

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Fig. 2. Overall survival rates after RFA treatment. Kaplan-Meier analysis of cumulative survival rates after curative RFA in the IFN maintenance treatment and control groups. The survival of the maintenance IFN group was significantly better than that of the control group (p = 0.004). Multivariate analysis clearly showed that maintenance IFN therapy is an independent prognostic factor (cited from Kudo et al. [10] with permission).

RCTs, and significant differences were found in the survival rate with a prolonged duration of IFN administration or the observation period (fig. 2). Improvements in the survival rate were observed in 2 studies with an administration period of 6 months [11, 13] and in 1 study with a median administration period of 4.7 years [10]. A significant difference in the recurrence rate (including the rates of the second and third recurrences) was noted in all 3 studies (fig. 3–5).

It has also recently been reported that recurrence is significantly suppressed, and survival is prolonged, in patients administered IFN for 2 years or longer [18]. Also, the recurrence rate of HBV-related HCC as well as the hazard ratio have been reported to be lower in an IFN-treated group than in a non-IFN-treated group [15]. IFN administration has also been reported to increase the overall survival rate [17]. These results suggest that IFN prevents recurrence by suppressing inflammation or by its direct anticancer effect not only in hepatitis C patients but also in patients with hepatitis B.

From these observations, IFN administration at a low dose and for as long as possible is considered to somewhat delay the first recurrence, prevent second and third recurrences, and, consequently, improve survival [4, 19]. According to Jeong et al. [14], during the follow-up period the Child-Pugh score showed no difference in the IFN-treated group but there was a significant worsening in the control group, and the percentage of patients who could not be candidates for cancer treatment because of the wide spread of cancer or worsening of the liver function was higher in the control than in the IFN-treated group.

To summarize these results, low-dose, long-term, or maintenance IFN therapy is considered to significantly

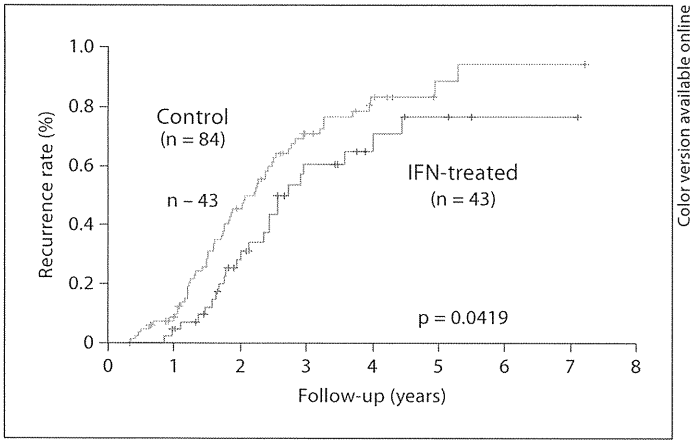

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Fig. 3. Cumulative first recurrence rate of HCC. Kaplan-Meier analysis of the cumulative first recurrence rate after curative RFA treatment in the IFN maintenance and control groups. The first recurrence rate was significantly lower in the IFN maintenance group than in the control group (p = 0.04). However, multivariate analysis showed that IFN is not an independent factor (cited from Kudo et al. [10] with permission).

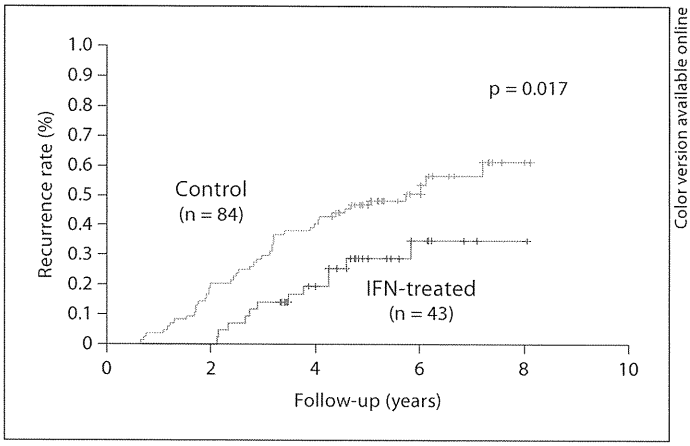

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Fig. 4. Cumulative second recurrence rate of HCC. Kaplan-Meier analysis of the cumulative second recurrence rate after curative RFA treatment in the IFN maintenance and control groups. The second recurrence rate was significantly lower in the IFN maintenance group than in the control group (p = 0.017). Multivariate analysis also showed that IFN is a significant independent factor (cited from Kudo et al. [10] with permission).

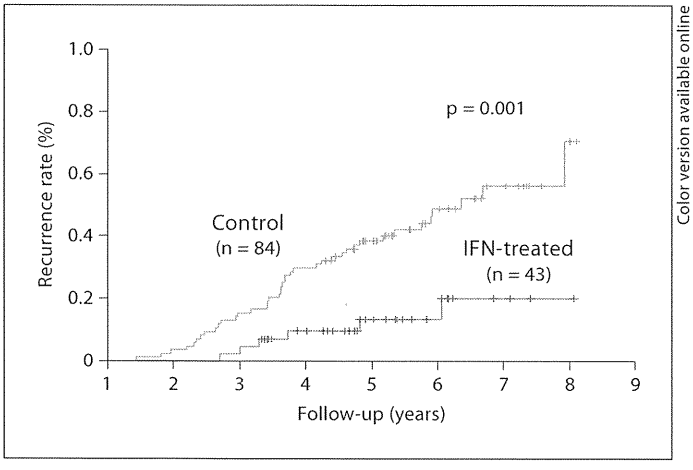

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Fig. 5. Cumulative third recurrence rate of HCC. Kaplan-Meier analysis of the cumulative third recurrence rate after curative RFA treatment in the IFN maintenance and control groups. The third recurrence rate was significantly lower in the IFN maintenance group than in the control group (p = 0.001). Multivariate analysis also showed that IFN is a significant independent factor (cited from Kudo et al. [10] with permission).

prevent or delay the recurrence of HCC or secondary carcinogenesis by maintaining the liver function in an adequate state and by its direct anticancer effect, making it possible to detect a slight recurrence while the disease is still in its early stages and to improve the survival rate by making it possible to attempt curative treatment again. The decrease in the need to perform noncurative treatments such as transarterial chemoembolization which worsen the liver function and the liver function-preserving effect of IFN are also considered to contribute to the improvement of the survival rate [19].

Meta-Analyses of the Recurrence-Preventing or Survival-Prolonging Effect of IFN after Curative Treatment for HCC

Recently, 2 reviews [19, 20] and 6 meta-analyses [21–26] were published regarding the recurrence-preventing or survival-prolonging effect of IFN therapy after the resection or ablation of HCC. All of them suggested that IFN is effective in preventing recurrence or improving survival.

Anti-Inflammation Treatments to Prevent Recurrence

It is well known that carcinogenesis from chronic hepatitis or liver cirrhosis can be significantly prevented by lowering AST and ALT at low levels, i.e. suppression of inflammation. Preventing secondary carcinogenesis by suppressing inflammation employing the same treatment as that against primary carcinogenesis is also im-

portant. Since keeping the ALT level low with Strong NeoMinophagen C [27] and ursodeoxycholic acid [28] in addition to low-dose IFN therapy is generally considered to contribute to the prevention of carcinogenesis, it is recommended to conduct these anti-inflammation therapies as well.

Clinical Studies in Progress

The SHARP Study, carried out primarily in Western countries in 2007, showed that the molecular targeted agent sorafenib significantly prolonged the survival of patients with advanced HCC [29]. The Asia Pacific Study also showed similar results [30], and sorafenib is presently regarded as the standard of care for advanced HCC. Sorafenib markedly suppresses serine/threonine kinases of Raf in the MAP kinase cascade and inhibits tyrosine kinases of angiogenesis factor receptors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors. It thus simultaneously prevents the proliferation of tumors and inhibits angiogenesis. A clinical trial to examine the recurrence-preventing effect of sorafenib by administration of it after curative treatment such as resection or ablation is in progress (STORM trial: http://clinicaltrials.gov, NCT00692770). It is designed to validate the working hypothesis that sorafenib has a preventive effect against recurrence by suppressing the angiogenic switch in the progression of a premalignant tumor to a malignant tumor and also in intrahepatic metastatic lesions in which angiogenesis has been established via suppression of angiogenesis and proliferation. If the recurrence-preventing effect of sorafenib is demonstrated, the survival of patients after curative treatments is expected to be prolonged by several years [31, 32].

Other Recurrence-Preventing Drugs

Peretinoin [33, 34] and vitamin K may be effective for the prevention of secondary carcinogenesis and have an inhibitory effect on the formation of portal tumor thrombi [35]. Concerning vitamin K, a large-scale clinical trial was carried out, but it was discontinued as it was judged ineffective by a data monitoring committee at the interim analysis because no significant recurrence-preventing effect could be demonstrated. However, the results may have been different if the end point had been set as the overall survival rate or the time until the appearance of portal tumor thrombi.

Regarding peretinoin, a large-scale multicenter clinical trial was completed, and the results were reported in the annual meeting of American Society of Clinical Oncology (ASCO) in 2010 [36]. Unfortunately, a significant decrease in the recurrence rate, which was the primary end point, could not be demonstrated in the groups administered peretinoin at 300 and 600 mg compared with a placebo group. However, as a 600-mg dose of peretinoin was suggested to be effective compared with the placebo in combination with 300 mg, the positive results of future clinical trials are eagerly expected.

Conclusion

Treatments for the prevention of recurrence after curative treatments of HCC were reviewed. Treatments to prevent recurrence (including intrahepatic metastasis and multicentric carcinogenesis) as well as early detection and early curative treatment are extremely important to improve the prognosis in patients with HCC. In addition, even though HCC recurs, the recurrent lesion is often solitary and detected in a stage in which curative treatment is still possible in patients in whom recurrence-preventing treatment by IFN is continued, possibly because of the antiproliferative effect, antiangiogenic effect, and apoptosis-inducing effect of IFN [19]. Therefore, the prognosis will be markedly improved by such recurrence-preventing treatments as well as efforts for the early detection followed by treatment of recurrence. Finally, the results of clinical trials of adjuvant therapy using the molecular targeted agent sorafenib are also eagerly awaited.

Disclosure Statement

The author declares that he has no financial conflict of interest.

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Diagnostic Imaging of Hepatocellular Carcinoma: Recent Progress

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Key Words

Hepatocellular carcinoma · Contrast-enhanced ultrasound · Sonazoid · Defect reperfusion imaging · Double contrast ultrasound · Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging · Sonazoid contrast-enhanced ultrasound

Abstract

The diagnostic imaging of hepatocellular carcinoma (HCC) has recently undergone marked progress. The advent of the ultrasound (US) contrast agent Sonazoid, approved in January 2007, and magnetic resonance imaging (MRI) with the liver-specific MRI contrast agent gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA-MRI), approved in January 2008, are of particular significance. Sonazoid contrast-enhanced US (Sonazoid-CEUS) is useful not only for the diagnosis of HCC, but also for guiding treatment and assessing treatment response. Sonazoid-CEUS has proven to be particularly effective for screening and staging, which used to be considered impossible with CEUS, through the introduction of the newly developed diagnostic technique of defect reperfusion imaging. It is still not possible if other vascular agents such as SonoVue and Definity are used. In particular, Gd–EOB-DTPA-MRI has been suggested to be much more reliable in the differentiation of early HCC from precancerous dysplastic nodules than any other modalities such as multidetector raw computed tomography, dynamic MRI, and superparamagnetic iron oxide-MRI. A decrease in contrast uptake in the hepatocyte phase observed on EOB-MRI is strongly suggestive of cancer, and the absence of early staining in the arterial phase suggests early HCC. The differential diagnostic capacity of Gd-EOB-DTPA-MRI is considered to far exceed that of what were previously the most useful imaging techniques, computed tomography (CT) during hepatic arteriography or CT during arterial portography, and to be comparable to that of the pathological diagnosis by pathologists specialized in liver.

Introduction

Hepatocellular carcinoma (HCC) is the 6th most common cancer and 3rd most common cause of cancer death worldwide.

The diagnostic imaging of HCC has recently undergone marked progress. The advent of the second-generation ultrasound (US) contrast agent Sonazoid (manufactured and distributed by Daiichi Sankyo; provided by

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GE Healthcare), approved exclusively in Japan in January 2007, and magnetic resonance imaging (MRI) with the liver-specific MRI contrast agent gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA-MRI), approved in January 2008, are of particular significance. Sonazoid contrast-enhanced US (Sonazoid-CEUS) is useful not only for the diagnosis of HCC, but also for guiding treatment and assessing treatment response. Sonazoid-CEUS has proven to be particularly effective for screening and staging, which used to be considered impossible by CEUS, through the introduction of the newly developed diagnostic technique, defect reperfusion imaging [1–3]. In addition, Gd-EOB-DTPA-MRI has been suggested to be much more reliable than other modalities such as multidetector raw computed tomography (MDCT), dynamic MRI, and superparamagnetic iron oxide (SPIO)-MRI in areas in which conventional imaging techniques have been ineffective, i.e. the differentiation of early HCC from precancerous dysplastic nodules. The differential diagnostic capacity of Gd-EOB-DTPA-MRI is considered to far exceed that of conventional imaging techniques and be comparable to that of the pathological diagnosis. In this article, the latest advances in imaging techniques for HCC are reviewed.

Advances in US

B-Mode US

B-mode US possesses the advantages that it is noninvasive, can be performed at the bedside, and shows excellent temporal and spatial resolutions. It has therefore been used extensively as a screening method for high-risk patients with HCCs. Indeed, US is recommended as the initial screening modality in the evidence-based [4] and consensus-based [5] Clinical Practice Guidelines for HCC as proposed by the Japan Society of Hepatology (JSH), the practice guidelines on the management of HCC of the American Association for the Study of Liver Disease [6] and the practice guidelines of the European Association for the Study of the Liver [7]. However, the limitations of conventional B-mode US for the definitive diagnosis of HCC have declined considerably in the light of recent improvements in the temporal and spatial resolutions of computed tomography (CT) and MRI, and in MRI contrast agents. The role of US is thus currently limited, unless CEUS, as described below, is employed.

Table 1. Role of imaging in the management of HCC

Diagnosis
1 Screening (lesion detection)
2 Differential diagnosis (confirmation)
3 Diagnosis of malignant grade (dedifferentiated grade)
4 Staging
Treatment
5 Evaluation of treatment response after TACE or RFA
6 Treatment aid (treatment guidelines)
7 Identification of local recurrence or viable lesion
8 Detection of intrahepatic distant recurrence in entire liver

TACE = Transarterial chemoembolization.

Sonazoid-CEUS

Characteristics of Sonazoid-CEUS

Sonazoid is a second-generation US contrast agent that was first approved in Japan in January 2007, in advance of its approval in other countries. It is characterized by its ability to allow real-time blood-pool images to be obtained at a low acoustic pressure. More importantly, it is also taken up by Kupffer cells in the postvascular phase or Kupffer phase (starting 10 min postinjection) and provides extremely stable Kupffer images suitalble for repeated scanning from 10 to about 120 min after injection [2, 3, 8–11].

Table 1 shows the roles of imaging modalities in the diagnosis and treatment of HCC. Despite improvements in imaging modalities such as US, CT, and MR, until recently there have been many limitations associated with the diagnosis and treatment of HCC; this includes issues related to screening, staging, evaluation of therapeutic response, treatment guidance, identification of the site of local tumor progression after ablation, and diagnosis of intrahepatic distant recurrence after treatment (table 2). CEUS using Levovist was clinically useful in differential diagnosis [1, 12, 13], evaluation of the degree of malignancy [14], evaluation of the therapeutic response of transcatheter arterial embolization [15–17], and as a treatment guidance for radiofrequency ablation (RFA) [18, 19], but it is associated with limitations in the evaluation of treatment responses to RFA [20], screening, and staging [1].

Before the introduction of Sonazoid to the clinical use, the expectations of Sonazoid-CEUS were simply that it would 'be more effective and convenient for vascular imaging than Levovist. Imaging will be possible without

Table 2. Problems that should be solved in the management of HCC

Screening	Some tumors cannot be depicted by B-mode US due to coarse liver parenchyma
Staging	Difficult by B-mode or CEUS (using Levovist or SonoVue)
Evaluation of treatment response	CT is frequently used after RFA; difficult to determine the safety margin if Lipiodol is not injected
Localization of the local recurrence nodule after RFA	Difficult by CEUS Not possible by B-mode US alone
Treatment guidance	Difficult for the nodules which are not shown by B-mode US
Detection of recurrence	MDCT every 3 months (cost, X-ray exposure, etc.)

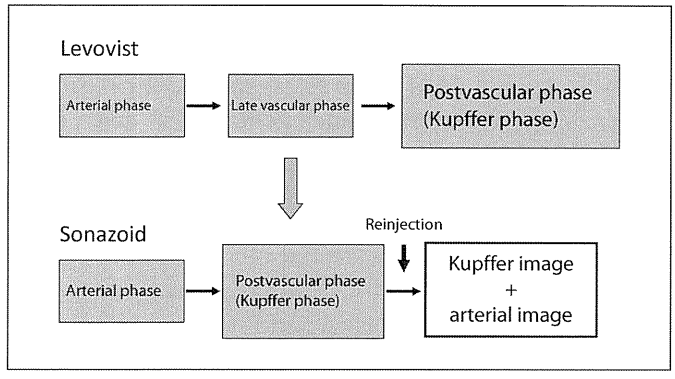

Fig. 1. Defect reperfusion imaging: basic concept. Defect reperfusion imaging of tumors that are unclear on B-mode US. Typical HCC shows arterial vascularity in the Kupffer defect after reinjection.

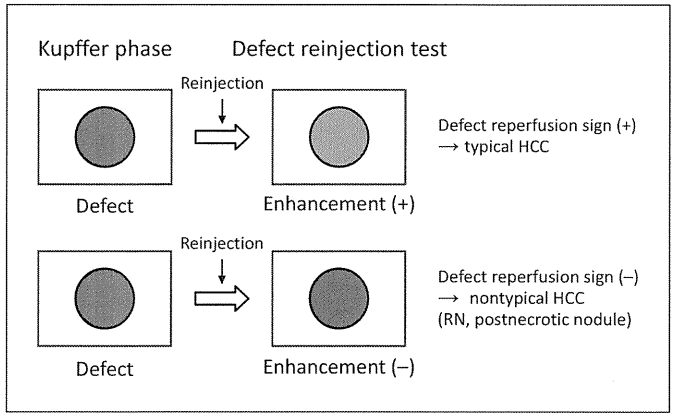

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Fig. 2. Value of defect reperfusion imaging in B-mode-undetectable nodules. Defect reperfusion imaging makes possible the confirmation of HCCs that are unclear on B-mode US but detectable on dynamic CT, local recurrence after RFA, nodules on screening, and a definitive diagnosis of HCC. RN = Regenerative nodule.

high-end equipment, leading to reducing the skill- or equipment-dependence and greater popularity of CEUS than at least in the Levovist era.' Regarding Kupffer imaging, several negative views were expressed, such as: 'It may provide very stable Kupffer imaging, but, considering that its major objective is staging of metastatic liver cancer, Kupffer imaging will not be used widely in Japan, and, in that sense, Sonazoid is unlikely to cause marked changes in the clinical practice concerning HCC.' However, these views have changed markedly over the approximately 3 years since its introduction on the market on January 10, 2007. Sonazoid-CEUS has emerged as an innovative breakthrough technology capable of revolutionizing the clinical approach to HCC through the development of an epoch-making technique of 'defect reperfusion imaging' [11] or 'double-contrast US' [10].

Defect reperfusion imaging is achieved by reinjecting Sonazoid into areas shown to be defects in the Kupffer phase [10, 11]. The introduction of this technique has resolved most of the limitations associated with the diagnosis and treatment of HCC, including those shown in table 2.

Development of Defect Reperfusion Imaging (Double-Contrast US)

We developed defect reperfusion imaging [2, 11] in February 2007, by applying Kupffer imaging, which is extremely stable and suitable for repeated scanning and real-time blood flow imaging. This represents a breakthrough technique in determining the sites of tumors and is a treatment aid for HCCs that are not distinctly delineated by B-mode US but show a typical vascular pattern on dynamic CT or MRI (fig. 1, 2) [2, 11].

CEUS is performed at a mechanical index value of 0.2. Sonazoid is intravenously injected at 0.01 ml/kg, early arterial enhancement can be evaluated in the vascular phase, and the presence or absence of defects was evaluated in the Kupffer phase from 10 min after injection. A probe is then applied to areas identified with defects in the Kupffer phase, after which an additional dose of Sonazoid (0.01 ml/kg) is injected, and the presence or absence of arterial blood flow into the defects can be evalu-

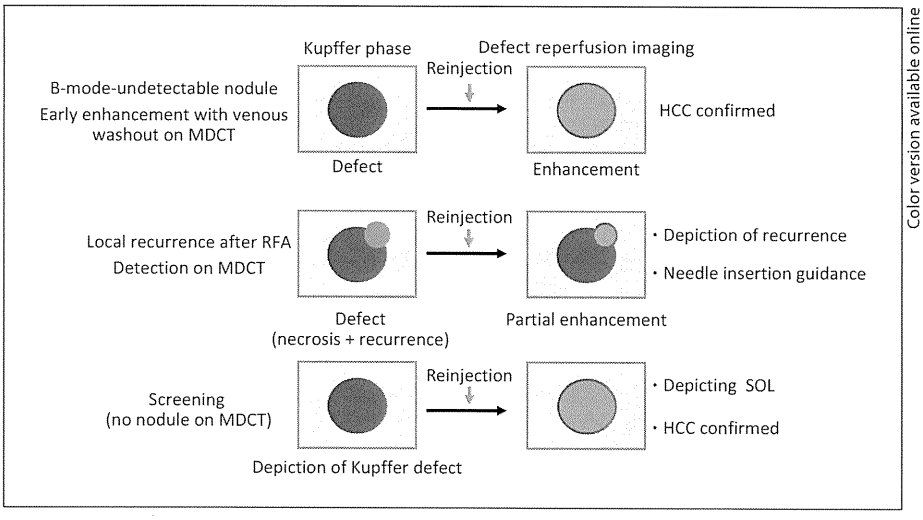

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Fig. 3. Defect reperfusion imaging. In addition to B-mode-undetectable nodules, detection of area of recurrence followed by needle insertion guidance and screening is possible by defect reperfusion imaging. SOL = Space occupying lesion.

ated by defect reperfusion imaging of the stable Kupffer phase and the reinjection method [11]. By assuming that the defect reinjection sign was positive when arterial vascularity was noted inside a Kupffer defect, nearly 100% of typical HCCs could be diagnosed (fig. 1, 2). Moreover, when the tumor sites are not identified with B-mode US, vascular-phase CEUS is not useful at all. However, when clear defects are observed in the Kupffer phase, reinjection of Sonazoid into these defects facilitates the confirmation of HCC, leading to a precise treatment guidance at the RFA needle placement (fig. 3).

This method first uses stable Kupffer imaging 10 min postinjection without vascular imaging to detect tumors undetectable by B-mode US, and the reinjection of Sonazoid confirms whether or not these tumors receive an arterial supply (fig. 1–3) [2, 3]. A characteristic of this technique is that tumors exhibiting typical CT images, arterial enhancement with venous washout can be correctly confirmed as defects first in the Kupffer phase, and their arterial enhancement is then demonstrated by the reinjection of Sonazoid, requiring no special equipment or analytical procedures (fig. 1, 2). This novel technique allows the identification of tumors that present as typical CT images but are not identifiable on B-mode US with an almost 100% confidence. If a tumor is detected as a Kupffer defect, but shows no enhancement by the reinjection of Sonazoid, i.e., hypovascular nodule, it may be confirmed to be a different nodule from a hypervascular tumor, which was originally detected by CT (fig. 2). This technique is therefore expected to be a highly significant addition to the treatment guidance for HCC.

Defect reperfusion imaging also has a wide range of applications, allowing screening for HCC in cirrhotic livers with coarse hepatic parenchyma [11], diagnosis of malignant grade, identification of the site of local tumor progression after treatment [21, 22], and US-guided needle puncture [22–25] (fig. 3).

Impact of Defect Reperfusion Imaging on the Diagnosis and Treatment of HCC

Screening

To screen patients in the super-high-risk group (viral hepatitis B- or C-related liver cirrhosis), Sonazoid is injected intravenously in the outpatient clinic at 0.01 ml/kg. Patients then walk to the US department where they are examined by a US technologist, 10–60 min after the injection. Kupffer phase screening with Sonazoid-CEUS can be conducted simply as a routine examination, and the examiner can concentrate on depicting Kupffer defects in the enhanced normal liver parenchyma in the Kupffer phase, which is extremely simple. It can more easily pick up the abnormal Kuppfer defect as compared to B-mode US, which is much more difficult because of coarse liver parenchymal echo. If a defect is found, dual-phase fusion images, involving superimposed images of different phases (Kupffer and arterial phases), can be obtained by reinjecting Sonazoid, thus providing information on both the Kupffer function and arterial blood flow in one tomographic image (fig. 1). This dual-phase fusion imaging or double-contrast US allows HCC to be detected and definitively diagnosed with an accuracy of 100%. Sonazoid will also markedly improve the efficiency of HCC screening [10]. CEUS has conventionally been con-

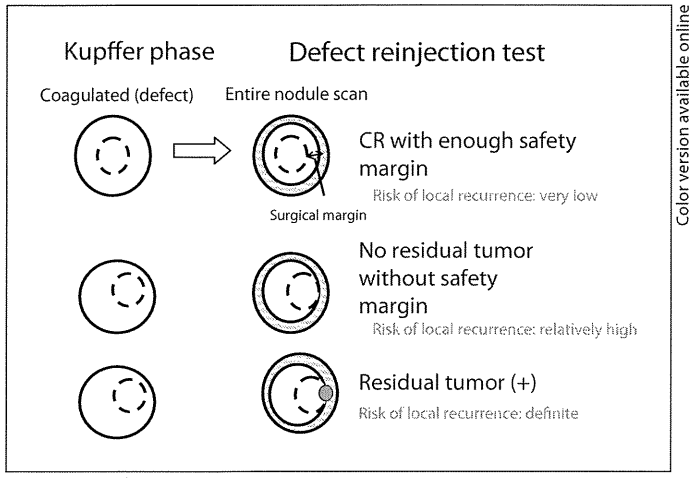

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Fig. 4. Value of defect reperfusion imaging (reinjection test) in the evaluation of treatment response by RFA. Evaluation of treatment response by defect reperfusion imaging with Sonazoid. CR = Complete response.

sidered as suitable for tumors already detected by B-mode US as in the case of SonoVue or Definity, rather than for screening or staging, but this concept has been completely changed by the introduction of defect-reperfusion imaging using Sonazoid. At present a prospective randomized study comparing which is the better modality for surveillance – B-mode US or Kupffer phase US – is ongoing (NCT trial No. 01214343).

Staging

The introduction of Sonazoid is also expected to result in breakthroughs in HCC staging. CT hepatic arteriography (CTHA) and CT arterial portography (CTAP) have previously been the most sensitive modalities for staging, but Sonazoid-CEUS allows the presence or absence of Kupffer defects unlike the main tumor to be evaluated by careful scanning of the entire liver in the Kupffer phase. If defects are detected, the possibility of intrahepatic metastases can be confirmed [2, 3], making accurate staging possible which is comparable to dynamic CT.

Evaluation of Therapeutic Response

The therapeutic response of HCC by RFA has usually been evaluated by CT [26]. However, the comparison with pretreatment CT images is necessary to determine the safety/ablative margin, and judgments based on CT images tend to be inaccurate when Lipiodol is not injected into the liver at the angiographic procedure. In addition, it is very difficult to evaluate the therapeutic effect using CEUS alone, because the margin of the original nodule becomes blurred after RFA [27]. However, defect reperfusion imaging allows the safety/ablative margin to be determined accurately, by delineating the treated nodule in the Kupffer phase, determining the coagulation area, and then after reinjection of Sonazoid scanning the entire tumor confirms whether the safety margin is large enough or not by assessing the distance of the arterial flow area and the ablated area (fig. 4). Further evaluation is needed to establish the percentage of patients in whom this procedure can be applied, i.e., how often the margin of the tumor can be correctly determined after RFA treatment using this procedure. The current situation is that evaluation CT can be omitted in about half of patients according to our experience [unpubl. data]. Sonazoid-CEUS is also more favorable than CT from the point of view of cost-effectiveness. In addition, Sonazoid-CEUS has several important advantages, including lower cost, reduced X-ray exposure, no allergy to the contrast medium, and applicability in patients with renal failure or bronchial asthma, in whom dynamic CT/MRI cannot be performed.

Treatment Guidance

Defect reperfusion imaging is most effective in the field of treatment guidance. Real-time virtual sonography [28, 29] or puncture under Levovist-enhanced US guidance [18, 19] used to be performed in tumors unidentifiable on B-mode US. However, real-time virtual sonography requires CT volume data and special equipment, and it is often difficult to examine exactly the same cross section. Levovist is technically difficult to use, in that the puncture must be performed during a very short period of time of the early vascular phase [18, 19], and it has thus failed to gain wide acceptance in this field.

In contrast, Sonazoid-CEUS makes it possible to accurately detect even the B-mode-unidentifiable nodules with the use of defect reperfusion imaging and then facilitates accurate needle placement after having confirmed their viability. After having confirmed the viability, needle placement can be performed during the long-lasting, stable Kupffer phase, allowing the operator more time for the procedure [21–25, 30].

US Imaging of Locally Recurrent Foci

Sonazoid-CEUS is also effective in determining the site of local tumor progression of previously ablated HCC. It has previously not been possible to guide the puncture needle or to determine the site of local tumor progression by B-mode US alone. This was because the previously co-