当性について検証した成績も報告されている．Leungらは，術前画像診断でUCSF基準内であれば5年生存率，累積再発率はそれぞれ59%，19%で，非HCC症例の予後と有意差がなくUCSF基準は妥当であるとしている[5]．Onacaらは International Registry of Hepatic Tumors in Liver Transplantationのデータベースを用いて1992年から2005年10月までのHCC移植1206症例を解析した．ミラノ基準内・外の5年無再発生存率はそれぞれ61.8%，42.8%とミラノ基準外で有意に予後不良であった．しかし，ミラノ基準外であっても5.1～6cm単発または5cm以下2～4個の症例では，3.1～5.0cm単発または3cm以下2～3個の症例とほぼ同等の無再発生存率であり，6cm以下単発，5cm以下4個まで適応拡大可能であるとしている[6]．ただし，腫瘍径，個数の評価は術前画像診断ではなく摘出肝で行っている．

フランスのグループは，移植前の画像診断からUCSF基準の妥当性をintention-to-treat方式で検証したところ，移植待機期間の中央値が4カ月と比較的短く，待機リストからの離脱率が2.5%と低いにもかかわらず，ミラノ基準外かつUCSF基準内症例（いわゆる適応拡大で恩恵を得るグループ）の5年生存率は45.6%であり，適応拡大を容認できないとしている[7]．Grassoらは，移植後再発を規定する因子は摘出肝における腫瘍最大径だけで，そのカットオフ値は3.5cmだとしており，腫瘍径を適応拡大すると再発率が上がると警告している[8]．適応拡大により移植待機期間が延長し，intention-to-treat方式でみるとさらなる予後低下が危惧されている[9]．一般に，欧米ではミラノ基準内にある待機患者の離脱率は年間15～30%で，intention-to-treat方式で評価すると5年生存率は10～15%低下する[10]．このように適応基準拡大に対して否定的な意見が優勢であり，ミラノ基準外の症例に対しては生体肝移植が行われている．

B. 生体肝移植の成績と適応

臓器の公共性を前提とする脳死肝移植に比べて，親族からの臓器提供を原則とする生体肝移植では，ミラノ基準外のHCCに対しても適用可能であり，待機期間が短縮されるメリットもある．しかし，適応拡大によって低下する生存率の許容下限をどこに設定するのか，あるいはその妥当性を証明することも困難であるが，5年生存率50%が許容下限という意見もある[1]．

わが国では2007年6月厚生労働省より，移植前の画像診断でミラノ基準内であれば摘出肝の病理組織学的検査の結果にかかわらず保健適応内とする通達が出された．全国集計316例の56%がミラノ基準外（摘出肝の病理組織学的検査）で，3年生存率はミラノ基準内79.4%，基準外60.0%，3年累積再発率はそれぞれ2.5%，36.6%であった[11]．最近の653例の集計では48%がミラノ基準外で，5年（無再発）生存率はミラノ基準内77.8% (75.6%)，基準外60.4% (47.1%) であった．特に腫瘍径5cmを超えるものでは5年生存率は40%，5年累積再発率は60%を超えており，腫瘍径に関する適応拡大を支持する成績は出ていない．Takadaらはミラノ基準内，外で4年生存率はそれぞれ68%，59%と差はなく，術後早期合併症の有無に予後が左右されたが，4年再発率はそれぞれ15%，35%とミラノ基準外で有意に高率であったと報告している．特に，腫瘍径5cm以上や肝癌治療歴が3回以上の場合，再発は有意に高率であった[12]．Soejimaらはミラノ基準外の3年無再発生存率は74%と良好な成績を報告した．やはり腫瘍径5cm以上では再発が高率で，腫瘍径での適応拡大は難しいが，腫瘍個数の制限を緩和可能であることを示唆している[13]．

ヨーロッパからの報告では，ミラノ基準外，UCSF基準外でも3年生存率はそれぞれ62%，53%と比較的良好であり，ミラノ基準が提唱された1990年代に比べて画像診断が飛躍的に進歩していることからも，適応拡大の可能性を示唆している[14]．米国肝臓学会のガイドラインでは，移植待機期間が長くなり，その間の腫瘍進展により待機リストから外れる可能性が高い場合には生体肝移植を行うことも推奨している[1]．一方で，Malagoらは34例中8例（23.5%）に術後3カ月以内の合併症による在院死を認め，60歳以上に対する生体肝移植の適応は慎重であるべきだとしている．また，ミラノ基準外の3年無再発生存率は47%と不良である[15]．Gondolesiらの報告によると，36例に対して62日という短い待機期間で生体肝移植が可能で，53%がミラノ基準外であった．またミラノ基準外の2年無再発率は74%と脳死移植例と同等であったとしているが，やはり36例中8例（22%）に術後合併症による在院死を認めている[16]．米国のAALD2 studyによると，生体肝移植例の待機期間は脳死例に比べて短く，進行例の割合も多く，肝癌再発が高率にみられ，ミラノ基準内に限っても移植後再発率は脳死0%に対して生体肝移植は26%であった．その理由として，生体肝移植例では，短い待機期間のために癌の生物学的悪性度を見極めることができなかったこと，下大静脈を温存して全肝摘出を行う生体肝移植では，肝脱転に伴う腫瘍の揉みだしが起こる可能性が高いとしている[17]．

C. 待機期間中のdown-staging

移植前に局所療法（TACE，ラジオ波焼灼療法など）を行う目的として以下の3点があげられる[18]．1）待機中の癌の進展を制御し待機リストからの離脱率を下げる，2）適応基準外の進行肝癌を適応基準内にdown-stagingする，3）移植後の予後向上を期待する．欧米では，移植待機中の離脱率をできるだけ抑え，なおかつintention-to-treat下での予後向上，適応拡大を目指してdown-stagingが積極的に試みられているが，移植後の予後向上に寄与しているかどうかはcontroversialである[18]．Cilloらは，移植禁忌（腫瘍栓，遠隔転移，低分化癌）でなければ腫瘍径，個数にかかわらず待機リストから外さないという方針でdown-stagingを行い，前向き研究の結果を報告した．離脱率はミラノ外でも年率4%と低率であった．さらに，観察期間の中央値21カ月で，ミラノ外全例（待機リスト上，および外れた症例，移植例すべて含む）の5年生存率73%で，ミラノ内症例と差はなかったと報告している．さらにUCSF基準逸脱全例の5年生存率も76%と良好であったとし，待機期間中の積極的なdown-stagingの有効性を報告している[19]．Yaoらは，pT2またはpT3 HCC移植患者に対して，待機中のTACEを含む局所治療の有効性について後ろ向きに検討している．pT2では前治療の有用性は証明されなかったが，pT3（ミラノ基準外）は移植前治療群の5年生存率は85.9%で無治療群の51.4%に比べて有意に良好であったと報告している[20]．同じくYaoらは，T2を超えるHCCに対して移植待機中にラジオ波焼灼療法，TACE，肝切除によりdown-stagingを前向き試験で試みた．30例中21例（70%）でdown-stagingに成功し16例（53%）に肝移植が行われ，14例（47%）に病理組織学的検査で完全壊死を含むdown-stagingが確認された．2例に移植前治療による肝不全死がみられたが，観察期間の中央値16カ月で移植例に再発はなく，30例の2年生存率は81.8%であったと良好な成績を報告している[21]．Ottoらは，待機期間中のTACEをdown-stagingの目的ではなく，その治療効果を癌の生物学的悪性度の指標として評価している．すなわち，ミラノ基準内・外にかかわらずTACEに治療効果の

あったものは待機期間中にTACEを可能な限り反復し，治療効果が継続していた症例の移植後の5年無再発率は94.5%であったと報告している[22]．

一方，PorrettらはT2までの症例で移植前のTACEを含む局所治療に予後改善効果を認めなかったとし[23]，Lesurtelらもmeta-analysisにより，移植前のTACEは，移植後の長期予後を向上しない（grade C），適応拡大に寄与しないばかりか待機リストからの離脱率も減少しないが（grade C），移植後の合併症増加もみられない（grade C），と報告している．いずれにしても待機リスト上にあるHCC患者に対してTACEの有効性を証明するためには，大規模な無作為比較試験が必要であると強調している[24]．このように現時点ではdown-stagingの有効性に関する結論は出ていない．

D. 移植後の予後予測

予後規定因子として，腫瘍径，腫瘍個数，組織学的脈管侵襲[25]，腫瘍分化度[25]などがあげられる．前2者はミラノ基準，UCSF基準で採用されている．組織学的脈管侵襲の頻度，分化度は腫瘍径と相関するが，画像診断や腫瘍マーカーなどを用いても正確に評価ができないため，腫瘍生検の是非についても議論されている[26-28]．Pawlikらは，肝切除または肝移植前のHCC患者120例に対して腫瘍生検を行い，摘出肝癌組織の病理検索の結果と対比している．54.8%に腫瘍分化度の不一致がみられ，さらに術前腫瘍生検による低分化癌の診断の特異度は92.5%と高率であったが，感度は34.6%ときわめて低率であり，HCC組織の不均一性に起因する腫瘍生検の限界を指摘している[26]．HCCの生物学的悪性度を評価するために分子生物学的手法による検討もなされている．Ramosらは，細胞周期を調節する遺伝子に着目し，移植に際して得られた標本の免疫組織学的検討を行った．その結果，腫瘍径3cm以上でpRb強発現例の80%に組織学的脈管侵襲がみられ，pRb陰性または低発現例では移植後再発が11%であったのに対して，強発現例では全例に移植後再発がみられた．腫瘍生検により得られた組織からpRb発現の程度を検索することにより，生物学的悪性度を評価でき，再発の予測，患者選択に有用であると報告している[29]．

E. 画像診断

HCCに対する^{18}F-fluoro-2-deoxy-d-glucose positron emission tomography（FDG-PET）の診断能と肝移植についての報告が散見される．感度50%前後と他臓器癌に比べると感度が低く，その有用性についてはcontroversialである[30]．しかし1cm以上の遠隔転移の感度は83%と高く，偽陽性もなく移植の適応決定にも有用である[31]．Yangらによると，HCCで肝移植予定の38例に対してFDG-PETを行い，原発巣に関してはわずか34%の陽性率であった．陽性率は組織学的脈管侵襲陽性例（78%），血清α-フェトプロテイン値＞200ng/ml（82%）に高率であったと報告している[32]．注目すべき点は，ミラノ基準内でFDG-PET陰性例（n＝20）に移植後再発がない一方で，ミラノ基準内でもFDG-PET陽性6例中4例（67%）に再発を認めたとしている[32]．FDG-PETは生物学的悪性度を反映している可能性があり適応決定の一助となりうる．

むすび

移植の適応決定に有用な指標は依然として腫瘍径と腫瘍個数であるが，今後のさらなる画像診断の進歩により，既存の適応基準が見直されるであろう．一方で，HCCの生物学的悪性度をよく反映する因子が分子生物学的手法によって明らかにされ，適応決定の補助的手段として導入されてく

る可能性もある．生体肝移植に関しては，今後，さらに症例を重ねていくに従い術後早期の合併症死が減少し，腫瘍因子のみが予後規定因子となれば，適応基準もより明確になる．わが国では，肝移植に至るまでにさまざまな前治療が行われ，保健適応の明確な基準も示されている．したがって，肝切除，ラジオ波焼灼療法，TACEに肝移植も含めた集学的治療を行う中で，移植時期の判断に迷うことも多い．今後，各治療法の最新の治療成績が明らかになるにつれていっそう明確な指針が示されるものと期待している．

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Hepatoma-derived growth factor in cancer development and progression

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Abstract

Hepatoma-derived growth factor (HDGF) was purified and cloned from a human hepatocellular carcinoma (HCC) cell line. HDGF and other five HDGF-related proteins belong to a new protein family with a significant homology in their amino terminus. HDGF is a nuclear targeted mitogen containing nuclear localization signals, and the ability of trafficking to the nucleus is essential to display the mitogenic activity, however, exogenously supplied HDGF stimulated the cellular proliferation. HDGF was highly expressed in various organs including liver, kidney, heart, lungs and gut in the fetal stage, and significantly decreased near birth and adult stage. HDGF was strongly expressed in cancer cells, including liver, lung and colon cancer cells, compared with the adjacent tissues, and exogenously supplied and endogenously over-expressed HDGF enhanced the proliferation of cancer cells. In mouse hepatocarcinogenesis model, HDGF was induced in the liver tissues at an early stage before liver tumor development. HDGF-over-expressed cells generated tumors and enhanced tumor growth in nude mice. HDGF also stimulated cell migration and tubule formation as well as the proliferation of human endothelial cells. HDGF induced tumorigenesis *in vivo* through both its direct angiogenic activity and induction of VEGF. Down-regulation of endogenous HDGF of cancer cells suppressed their proliferation, invasive activity and anchorage-independent growth in soft agar *in vitro*. The higher expression of HDGF showed more malignant potential for cancer progression. By immunohistochemistry, HDGF may be a useful prognostic factor for disease-free and/or overall survival in patients who have undergone the resection of HCC, non-small cell lung cancer, gastric cancer, esophageal cancer and pancreatic cancer. This review will describe the current knowledge about the molecular characteristics and physiological functions of HDGF in cancer development and progression, and its possible clinical utility in cancer regulation.

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Cancers develop by the accumulation of dysregulated gene expression from multistep genetic mutations of oncogenes and/or suppressor genes.

These oncogenic proteins and tumor suppressor proteins generally include growth factors, their receptors, intracellular signal transduction molecules and transcriptional regulatory factors. Over-expression of several factors and/or down-expression of some growth suppressors induced carcionogenesis and cancer progression. In one view, cancer cells display immature features and up-regulate gene expression with aberrant expressions of genes that are inactive in normal adult tissue. Genes that are reduced significantly during organ development are frequently absent from the adult tissues, and these developmentally regulated genes may be reactivated in human cancers. Some gene products expressed exclusively in tumors and in developing embryos are called onco-fetal proteins, which are useful for clinical cancer management as tumor markers. Furthermore, if it is possible to regulate the expression and activity of these proteins, new effective tools would be developed for cancer regulation.

Hepatoma-derived growth factor (HDGF) is a heparin-binding protein purified from the conditioned medium of the human well-differentiated hepatocellular carcinoma (HCC) cell line, HuH-7, which can proliferate autonomously in a serum-free chemically-defined medium (1,2). HDGF is highly expressed in several cancer cells (2-5).HDGF was also more highly expressed in various fetal organs than in adult organs, while it is ubiquitously expressed in various organs in humans and rodents (2-10). Thus, HDGF is one of the developmentally regulated genes which are abundantly expressed in cancer cells. HDGF is a major member of HDGF family proteins consisted of itself and five HDGF-related proteins (HRP) (6,11). HDGF is a unique nuclear targeting growth factor, which can traffic to the nucleus and resides dominantly in nucleus (12,13). Recently, some interesting experimental and clinical approaches for HDGF have clarified the possible roles of HDGF on tumor development and progression. In the following review we will describe the roles of HDGF on carcinogenesis and cancer progression and its potential clinical utility in cancer regulation.

Characteristics of HDGF molecule

HDGF is an acidic 26kDa protein consisting of 230 aminoacids. HDGF stimulated the proliferation of fibroblasts, endothelial cells, vascular smooth muscle cells, pulmonary epithelial cells and hepatocytes, as well as HCC, lung cancer and colon cancer cells (1-5,7-15). HDGF has high affinity to the glycoaminoglycans heparin and heparan sulphate (1,2,11). HDGF has no hydrophobic signal sequence in its N-terminus, although it was first identified and purified from the conditioned medium of HuH-7 cells. Conversely, amino acid sequence analysis demonstrated the presence of two nuclear localization signals (NLS) in the molecule of HDGF. The first functional nuclear localization signal (NLS1) resided in the *hath* region (described below) of the N-terminal region and the second NLS (NLS2) in gene-specific regions of the C-terminal region of the HDGF molecule (12). HDGF can traffic to the nucleus using these NLSs, especially the NLS2 in its gene-specific region. Immunohistochemical studies which used anti-HDGF antibody revealed that HDGF was dominantly localized in the nucleus, rather than the cytoplasm. The ability for trafficking to the nucleus is essential to display growth stimulating activity in HDGF-over-expression cells. In particular, the gene-specific region of HDGF, at least the bipartite NLS sequence and both the N-and C-terminal neighboring portions, is essential for the mitogenic activity (12). HDGF's mitogenic activity depends on its nuclear targeting. HDGF is a unique factor that is categorized in the nuclear targeting growth factors.

In contrast, exogenously supplied HDGF stimulated the proliferation of fibroblasts, endothelial cells and fetal hepatocytes. These facts suggest that receptor-mediated signal transduction systems work to exert HDGF activity to some degree in some physiological conditions. A possible receptor-binding site is estimated to be residing at amino acid residues 81-100 within the *hath* region, however, HDGF which had deleted these 20 amino acid residues still had proliferation activity (16). Exogenous HDGF stimulated the Erk phosphorylation in endothelial cells

(15). These findings suggest that HDGF exerts its proliferating activity via two different pathways; 1) via a plasma membrane-located HDGF receptor for which signaling depends on the *hath* region, especially amino acid residues 81-100, resulting in MAP kinase activation, and 2) via targeting to the nucleus by NLS. Thus, another membrane receptor for HDGF should be present in the plasma membrane for HDGF, although a probable receptor has not yet been identified.

HDGF has been mapped to a locus of chromosome 1q22 by computer analysis of human genome data. The HDGF gene has been found to consist of 6 exons and 5 introns in humans and mice from the analysis of human and mouse genome sequence data (17).

HDGF Family

HDGF is the first member of the HDGF family proteins. The N-terminal region of HDGF was highly conserved among the other five HDGF-related proteins (HRP) (6,11,18-20). This region is called *hath* (homologous to the amino terminus of HDGF) region. HDGF family members are characterized based on whether they contain the *hath* region and NLS in their gene-specific regions and are targeting the nucleus (17). HDGF seems to be divided into two or three subgroups, by molecular weight and isoelectric point (11). Of the HDGF family proteins, HRP-3, HRP-4 and les epithelial cell derived growth factor (LEDGF, HRP-5) have the mitogenic activity for epithelial cells and fibroblasts as well as HDGF (11,18,19). LEDGF is identical to p54/72, which is an RNA-binding protein and transcriptional cofactor for regulating general transcriptional factors (20). Thus, HDGF may be a unique and interesting bi-functional factor in the exertion of its function via signaling pathway from cell membrane binding and its direct action on DNA after nuclear translocation.

Hath region, which is well-conserved in the HDGF family proteins contains the PWWP domain (21,22). The PWWP motif was first described in a candidate gene WHSC1 in Wolf-Hirschhorn syndrome. Among the HDGF family proteins, the PWWP domain in the *hath* region is well-conserved, but a clear divergence in the PWWP domains can be detected among the HDGF family and the other PWWP domain containing proteins. NMR analysis of PWWP domain demonstrates that PWWP in HDGF shows a high degree of similarity to the PWWP domain structures from other PWWP-containing proteins, Dnmt3b and mHRP, suggesting that HDGF may function as a non-specific DNA-binding domain (23). Another NMR spectroscopic study revealed that PWWP domain of HDGF consisted of a five-stranded beta barrel with a-PWWP-specific long loop connecting beta 2 and beta 3 followed by a helical region including two alpha-helices, and also revealed that its structure had a characteristic solvent-exposed hydrophobic cavity, suggesting that the PWWP domains of the HDGF family bind to some component of chromatin via this cavity (24). Furthermore, surface plasma resonance analysis shows that this *hath* domain is primarily responsible for heparin binding (25). As described above, the putative receptor-binding site is considered to reside in the *hath* region, however, the proper function of the *hath* domain has not yet been clarified, and extensive research on the function of the *hath* domain should be performed in the future.

Developmentally regulated expression of HDGF

In the fetus, HDGF was abundantly expressed in the liver, heart, kidney, lungs, and gut.

HDGF was highly expressed in fetal liver of the mid-gestation stage, and was markedly decreased near birth. HDGF expression was significantly decreased with differentiation in fetal hepatocytes induced by oncostatin M treatment in *in vitro* primary culture differentiation system (9,26). Adenoviral introduction of HDGF antisense cDNA into the fetal hepatocytes suppressed their proliferation, and the inhibitory effects of the HDGF antisense virus were recovered by exogenous HDGF (9). The oval cell is a progenitor cell with bipotential activity for differentiating into hepatocytes and bile duct cells (27). Furthermore, HCC is considered to be developed from oval cells

induced in regeneration process after liver injury. HDGF was highly expressed in oval cells induced in rat acethyl aminofluorene/partial hepatectomy model (personal communication). HDGF expression was strongly detected in an oval cell line, Oc 15-5, by immunohistochemistry, which was established from the liver of Long-Evans-Cinnamon rats (28). These findings suggested that HDGF play important roles in the proliferation of immature hepatocytes and hepatic progenitor cells including oval cells, showing significant involvement of HDGF in liver development, regeneration and carcinogenesis.

In the fetus, HDGF was also expressed abundantly in the cardiovascular systems, including heart and aorta (8,10). HDGF was expressed in endothelial cells from fetal rat aorta, and disappeared in adult aorta. HDGF is highly expressed in the fetal conotruncus and heart by Northern analysis, and HDGF protein was first detected in atrial myocytes, hindgut epithelia and the notochord of the E10 rat, and then by E12 its expression had broadened to include the ventricular myocytes, endocardial cells, and cells of the ventricular outflow tract (8). HDGF is also one of the important factors involved in vascular smooth muscle cell growth during vascular development and repair in response to vascular injuries (10).

HDGF was reported to be widely distributed in the renal anlage at the early stages of renal development and disappeared from the adult kidney except for a small portion of the renal distal tubules (7). HDGF mRNA was most abundant at sites of nephron morhogenesis and ureteric bud cells in embryonic kidneys. HDGF was the most likely candidate among the endothelial growth factors secreted by metanephrogenic mesenchymal cells for involvement in nephrogenesis. These findings show that HDGF is a potent angiogenic factor in the kidneys.

HDGF was induced by airway pressure during lung development in the *in vitro* murine fetal lung model with airway ligation (29). Immunohistochemical studies revealed that HDGF was highly expressed in the endothelial cells of non-muscularized, forming blood vessels of the fetal lung (15). HDGF expression was enhanced dominantly in the bronchial and alveolar epithelial cells including type II pneumocytes by bleomycin-instillation in mice (4). Exogenously supplied HDGF promoted the proliferation of rat alveolar epithelial cells and bronchial epithelial cell line. Interestingly, *in vivo* intra-tracheal instillation of recombinant HDGF induced significant proliferation of bronchial and alveolar epithelial cells without causing marked interstitial inflammation (4). HDGF may play a role in the growth and construction of the bronchus and distal lung by stimulating the proliferation of bronchial epithelial cells and type II alveolar cells.

HDGF was also highly expressed in the gut in the fetal stage. Immunocytochemistry revealed HDGF in hind gut epithelia as well as atrial myocytes in the E10 rat (8). Furthermore, HDGF was expressed in the nucleus of the colonic epithelial cells, dominantly in the bottom of the intestinal crypts by immunohistochemical analysis (3). The so-called intestinal stem cells reside in the bottom of the crypts and proliferate to supply the epithelial cells. Recombinant HDGF stimulated the proliferation of colonic epithelial cells, and polyclonal anti-HDGF antibody suppressed their proliferation (3).

Roles of HDGF in cancers

HDGF is expressed more abundantly in various cancers including that of the liver, lung, stomach, esophagus, colon and pancreas than in non-malignant tissues. HDGF significantly stimulates the proliferation of HCC, lung cancer and colon cancer cells.

HDGF in carcinogenesis

The Fatty Liver Shionogi (FLS) mouse is an inbred strain that develops spontaneous fatty liver without obesity. In these mice, liver tumors develop at 40 weeks after birth, with the number and size increasing with age to about 45% in 52 weeks and 90% at 72 weeks after birth in male mice; these tumors have been histologically diagnosed as hepatocellular adenoma and carcinoma (30,31). In the liver of FLS

mice, Northern analysis revealed that HDGF expression increased gradually from the age of 24 weeks at the basal expression through to 52 weeks after birth, showing that HDGF expression had already increased at an early stage before the tumors developed microscopically in the liver (32). HDGF is more dominantly expressed in hepatocytes with fat droplets than the non-parenchymal cells. In the non-tumorous liver with abundant fatty change, the foci that expressed HDGF appeared at 24 weeks of age, and the number of these foci increased with age. These high HDGF-expressing foci were the activated macrophage clusters with enhanced DNA synthesis and droplets (32). Studies on the FLS mouse model suggest that HDGF may be induced and secreted or released from the hepatocytes and/or these foci, enhancing the cell cycle progression of hepatocytes, inducing their transformation, and promoting the proliferation of HCC cells in an intracrine, autocrine and/or paracrine manner. Furthermore, HDGF is highly expressed in oval cells, which are considered to be a candidate progenitor cell developing to HCC cells. By differential subtractive chain reaction from strong anchorage-independent growth to its negative HCC cells, HDGF was cloned as one of the genes related to anchorage-independency (33). These findings strongly suggest that HDGF potentially participates in hepatocarcinogenesis and in the early stage of HCC.

HDGF expression is dramatically increased in human colorectal cancers, especially in tumors proficient in DNA mismatch repair, and HDGF expression in fetal intestine explants inhibits maturation, suggesting a significant and important role in epithelial differentiation (5). HDGF was more highly expressed in colon cancer cells than non-transformed intestinal epithelial cells (5). Conversely, down regulation of HDGF by use of HDGF-siRNA has minimal effect on anchorage-dependent growth but reduces significantly anchorage-independent growth of NSCLC cells in soft agar (34). HDGF may also play a role in colon and lung cancer development.

HDGF-over-expressing NIH3T3 cells generated sarcomatous tumors in nude mice. HDGF-over-expressing NIH3T3 cells did not show significant anchorage-independent growth in soft agar assay, however, HDGF-over-expressing NIH3T3 cells developed more small colonies in soft agar than parent or neomycin-resistant cells (14). Thus, these findings suggest that HDGF is an oncogenic protein.

HDGF in cancer progression

HDGF protein was abundantly expressed in various human HCC cell lines. Indeed, HDGF expression was higher than in the adjacent liver tissues in humans and rodents (32,35). The HDGF-over-expressing hepatoma cell line HepG2 proliferated more rapidly than parent or neomycine-resistant cells (12). Recombinant HDGF stimulated the growth of HCC cells, and antisense HDGF oligonucleotides suppressed their growth (36). Recombinant HDGF also stimulated the proliferation of colon cancer cell lines, while polyclonal anti-HDGF antibody suppressed their proliferation (3). Exogenously supplied HDGF promoted the proliferation of bronchial squamous cell carcinoma cell line, A549 cells, while by use of HDGF-specific small interfering RNA (siRNA), knock-down expression of HDGF in NSCLC cells significantly showed more slow growth, less colony formation in soft agar and lesser *in vitro* invasion activity across a Matrigel membrane barrier (4,34). Furthermore, HDGF-over-expressing HepG2 cells produced larger tumors, showing more rapid growth, in nude mice than neomycin-resistant HepG2 cells *in vivo* (personal communication). In an *in vivo* mouse model, A549 showing reduced expression of HDGF by HDGF-siRNA grew significantly slower than the cells with negative control siRNA (34). The higher expression of HDGF showed more malignant potentials for cancer progression.

HDGF protein increased in melanoma cell lines compared with melanocytes as shown by Western blotting, and was strongly expressed in early and late

stage melanomas but low in melanocytes and non-tumorigenic nevi in human by immunohistochemistry (37). Proteomic differential display analysis for the expression of the intracellular proteins by two dimensional gel electrophoresis and mass spectrometry showed that HDGF was down regulated in regressive cancer cells as compared with that in inflammatory cell-promoting progressive cells of the murine fibrosarcoma cell line, suggesting HDGF is a candidate factor for cancer progression (38).

Thus, HDGF may be one potent factor intrinsically related to cancer development and progression.

HDGF in angiogensesis

HDGF is intrinsically related to angiogenesis and vasculogenesis. HDGF expression was induced in the regenerating process of vascular vessels in wound repair, and is highly expressed in the fetal stage of cardiovascular system (10,39). Additionally, HDGF was reported to be a candidate endothelial growth factor for involvement in glomerulus formation (7). These findings suggest that HDGF is a potent angiogenic factor. Tumors developed from HDGF-over-expressing NIH3T3 cells inoculated in nude mice were macroscopically red-colored and were histologically rich in vasculature (14). HDGF-over-expressing HepG2 cells also produced red tumors in nude mice, showing more rich vasculature in tumors as compared to parental and neomycin-resistant HepG2 cells (personal communication). Immunohistochemical analysis by anti-CD31 antibody showed prominent new vessel formation induced by HDGF. Indeed, HDGF stimulated the proliferation and tubule formation of human umbilical vein endothelial cells (14). Moreover, HDGF stimulated the proliferation and migration of human pulmonary microvascular endothelial cells *in vitro* (15). Using chick chorioallantoic membrane (CAM) as a biological assay for angiogenesis, recombinant HDGF stimulated blood vessel formation, and stimulated cellular reorganization within the CAM from a loose network into a more compact, linear alignment reminiscent of tube formation (15). Furthermore, in tumors developed by HDGF-over-expressing NIH3T3 cells, a potent angiogenic factor; vascular endothelial growth factor (VEGF), was strongly detected immunohistochemically (14). Western blotting using anti-VEGF antibody showed a significant induction of VEGF in HDGF-over-expressing NIH3T3 fibroblasts, and reporter assay using VEGF promoter revealed that HDGF significantly induced VEGF expression in NIH3T3 fibroblasts (14). Conversely, in pulmonary microvascular endothelial cells, VEGF was not induced by HDGF and VEGF treatment suppressed HDGF expression (15). It is suggested that HDGF stimulates the proliferation of endothelial cells by mechanisms distinct from VEGF. HDGF shows potent angiogenic activity via its own direct stimulation of the proliferation of endothelial cells and vascular smooth muscle cells, and by VEGF secreted from the surrounding fibroblasts induced by HDGF. The growth speed of tumors produced by inoculation of HDGF-over-expressing HepG2 cells in nude mice seems to be more prominent than the proliferating activity of HDGF-over-expressing HepG2 cells in cell culture *in vitro*, compared to neomycin-resistant cells. The more potent growth stimulating activity of HDGF *in vivo* than *in vitro* must be brought on by both the direct cell growth activity and the angiogenic activity induced by its own and VEGF-inducing activity. Thus, HDGF works as an angiogenic factor by its own endothelial growth promoting activity and through the induction of VEGF in the nucleus.

HDGF as a prognostic factor for patients with cancers.

By immunohistochemical estimation of HDGF expression, the relationship between HDGF expression and clinicopathological variables and its prognostic value for determining cancer recurrence and overall survival has been analyzed in patients with various types of cancers. The correlation between the expression of HDGF and disease-free and/or overall survival was shown in patients with liver, lung,

gastric, esophageal and pancreatic cancer. The relationship between the differentiation degree of cancer cells and HDGF expression level was only demonstrated in HCC, however, that was not shown in other types of cancers.

Hepatocellular carcinoma

In patients with chronic hepatitis, HDGF was more highly expressed in HCC than in the adjacent liver as shown by Northern blotting. Immunohistochemical analysis by use of specific anti-C terminus of HDGF antibody revealed that HDGF was more strongly and frequently expressed in the nucleus and cytoplasm of HCC cells than in the adjacent normal hepatocytes (32,35). Statistical analysis of the relation between HDGF expression and other clinicopathological features in HCC showed that the HDGF expression level by immunohistochemistry was significantly correlated only to the differentiation of HCC. HDGF expression was higher in well-differentiated carcinomas than in poorly-differentiated carcinomas in our study (35). In contrast, Hu *et al.* reported that HDGF was higher in poorly-differentiated HCC than in well-differentiated HCC (40). One possible explanation for the discrepancy between the two groups may be due to the specificity of the anti-HDGF antibody used for immunostaining. However, a more satisfactory explanation will be shown by a larger scale study. Conversely, in both our and their studies, the patients with higher HDGF expression in HCC showed an earlier recurrence and a poorer overall survival rate than those with lower expression after hepatectomy for HCC (35,40). Multivariate analysis showed that HDGF expression was an independent prognostic factor for disease-free and overall survival in patients who underwent a hepatectomy for HCC. These findings suggest that HDGF is a candidate for use as a prognostic factor for disease-free and overall survival of patients with HCC.

Gastric cancer

In gastric cancer, the patients with high and strong expression of HDGF by immunohistochemistry showed significantly higher rates of infiltrative tumor growth, vascular and lymphatic invasion, compared to those with lower expression (41). However, there is no significant correlation between HDGF expression and tumor differentiation stages. Furthermore, these patients with higher expression of HDGF showed significantly poorer disease-free and overall survival than those with lower expression. Multivariate analysis revealed HDGF expression level to be an independent prognostic factor for disease-free and overall survival in patients with gastric cancer.

Esophageal cancer

HDGF is highly expressed in esophageal cancers. Immunohistochemical classification of HDGF expression in esophageal cancer cells showed that patients with higher expression of HDGF showed poorer disease-free and overall survival compared to those with lower expression (42). There is no significant correlation between HDGF expression and other clinicopathological factors including tumor clinical stages and differentiation stages. HDGF expression level was a clinically used as a prognostic factor for esophageal cancers, especially for patients in the early stage of the disease (pT1-2). Another interesting piece of evidence is the possible association of HDGF with the radiosensitivity of esophageal cancer cells. HDGF was highly expressed in radiosensitive esophageal cancer cells, yet was rarely expressed in radioresistant cells (43). Radiotherapy was more effective in patients with esophageal cancer of high HDGF mRNA expression than those with low expression. HDGF may play an important role in radiosensitivity, although the mechanism remains to be clarified, and could be a novel marker predicting the effectiveness of radiotherapy in patients with cancer.

Pancreatic cancer

In pancreatic cancer cell lines, HDGF is abundantly expressed at a similar degree to HCC cell lines by Western blotting. By immunohistochemical analysis, 54% and 56% of patients who underwent curative

resections for primary ductal carcinomas showed high expression of HDGF for the nucleus and the cytoplasm, respectively (44). There was no significant relationship between HDGF expression and any clinicopathologic variables including lymph node metastasis or venous and neural invasion. Patients with higher nuclear HDGF-LI showed a poorer 5-year survival rate, although no significant difference was observed by the cytoplasmic HDGF expression (44). Multivariate analysis also revealed nuclear HDGF-LI to be an independent prognosticator for overall survival in patients with pancreatic ductal carcinomas.

Lung cancer

HDGF was found to be a mitogen for lung epithelial cells *in vitro* and *in vivo*. In non-small cell lung cancer (NSCLC) cells, HDGF expression was strongly-detected in the nucleus as well as other cancer cells, and HDGF labeling index (LI) was 20-95% by immunohistochemical analysis (45). In patients with early stage NSCLC who underwent curative surgery, the disease-free and disease-specific survival and overall survival were lower in those with higher HDGF expression indexes than in those with lower HDGF indexes (46). The high expression of HDGF showed distant metastasis and shortened survival time in patients with NSCLC. Iwasaki *et al.* also reported similar results in Japanese patients with completely resected NSCLC that patients with a high HDGF-LI (>/= 65%) had significantly poorer overall and disease-free survival than those with a low HDGF-LI (45). HDGF-expression in NSCLC correlated with DNA synthesis and intratumoral microvessel density analyzed by CD31 staining, which coincided with the characteristics of HDGF revealed by *in vitro* experiments. In this study, there was no significant association between HDGF-expression and clinicopathological variables. In patients with NSCLC, HDGF is a significant independent prognostic factor, also being more powerful than the pathological stage by multivariate analysis. Conversely, the relationship between HDGF expression and disease-free and overall survival remains to be clarified in patients with small cell lung cancers.

HDGF is a unique nuclear targeted growth factor, which is expressed abundantly in cancer cells and stimulates their proliferation. HDGF generated tumors and promoted their growth *in vivo* via its mitogenic activity and angiogenic activity deriving from both its own direct angiogenic activity and the induction of VEGF. Multivariate analysis of the relationship of HDGF expression and recurrence-free and overall survival in patients with HCC, NSCLC, gastric cancer, esophageal cancer and pancreatic cancer confirmed that HDGF has the potential to become a significantly efficacious prognostic marker for cancer patients. It will be expected in the future that any tool regulating HDGF expression or HDGF signal pathways may be a useful candidate for the suppression of carcinogenesis and cancer progression.

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特集　消化器がんの化学療法－外科の立場から

肝がん

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はじめに

肝細胞がんに対する化学療法の適応は，肝切除やradiofrequency ablation(RFA)等の局所治療による治療効果が期待しえない進行肝がんや，肝外転移病巣変とされる。しかしながら，肝細胞がんは一般的に抗がん剤の感受性が低く[1]，併存する肝障害によって十分量の抗がん剤が投与できないという問題点もある。このため，標準治療としての肝細胞がんに対する化学療法における標準的治療はいまだ確立されていない[2]。その一方で，肝細胞がんは，肝切除によって肉眼的治癒切除し得たとしても，高率に肝内再発をきたすため，さらなる肝細胞がんの切除成績向上のためには，術後の肝内転移再発を制御することが極めて重要である。切除後肝内再発の抑制を目的として，術前肝動脈(化学)塞栓術(Transcatheter Arterial(Chemo) Embolization：TAE/TACE)や術後補助化学療法などの治療が試みられてきた。

本稿では，肝細胞がんに対する化学療法の現況を，外科の立場から，①肝切除術後再発巣に対する化学療法，②術前肝動脈化学塞栓術，③術後補助化学療法の3項目について概説する。

1. 肝切除術後再発巣に対する化学療法

1)肝動注化学療法

再発肝細胞がんのうち，TAE/TACEが効を奏さない門脈内腫瘍栓を有する症例や広範囲にわたる多発肝内転移症例などを対象に，肝動注化学療法が施行されてきた。最近の肝細胞がんに対する肝動注化学療法の使用薬剤とその治療成績を表1に示した。肝動注化学療法における投与方法は，One-shot動注および持続動注がある。One-shot動注においては，濃度依存性の高いdoxorubicin(ADR)やcisplatin(CDDP)などが適している。一方，持続動注では時間依存性の高い5-fluorouracil(5-FU)を機軸とし，CDDPの間欠的投与が中心となっている。単剤投与における奏効率は15～60%と単純には比較できないが，全身化学療法より良好な成績であると考えられる[3]。また，多剤併用による肝動注化学療法の奏効率は，7～51%であると報告されている。

最近では，肝動注化学療法にInterferon(IFN)-αを併用することで，良好な治療成績が報告されている。IFNは生体内サイトカインの1種であり，生体内においてさまざまな生物学的作用を持つ。抗がん剤の作用を増強させるmodulatorの作用に加えて，自身が抗腫瘍効果を有している。Kanekoらの報告では，門脈内腫瘍栓を伴った進行肝細胞がん29例に対して，5-FU，CDDP，methotrexate(MTX)の3剤による肝動注投与とIFN-αとLV(leucovorin)の全身投与を併用し，奏効率45%と良好な結果を示している[4]。また，IFN-αと5-FU持続肝動注化学療法は，門脈内腫瘍栓を伴った進行肝細胞がん症例を対象として，8例のComplete Response(CR)症例を含めて，奏効率が48%と極めて良好な結果[5-7]が報告されている。さらに，その後の他施設における追試においても，ほぼ同程度の抗腫瘍効果を確認しており[8]，極めて有望な治療法と考えられる。

2)全身化学療法

切除後再発症例の中で，肺・副腎・リンパ節などの肝外病巣に対して全身化学療法が施行される。現在までの，肝細胞がんに対する単剤もしくは多剤併用による全身化学療法の治療成績を表2に示す。各種消化器がんと同様に，ADR，5-FU，CDDP，mitomycin C(MMC)などの薬剤が使用されているが，単剤での十分な効果は期待できない[9]。ADRは，もっとも肝細胞がんに感受性の高い薬剤であるが，ADRと他の薬剤との併用に関しては，

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表1　肝動注化学療法

報告者	使用薬剤	症例数	奏効率(%)
Olweny et al(1980)	ADR	10	60
Ikeda et al(1992)	ADR, CDDP, MMC	76	51
Nagasue et al(1986)	Epi-ADR	53	15
Takayasu et al(2000)	Epi-ADR, CDDP, VP-16	30	30
Onohara et al(1988)	CDDP	33	55
Ansfield et al(1971)	5-FU	11	27
Tanaka et al(2000)	5-FU, CDDP	77	45
Ando et al(2002)	5-FU, CDDP	58	43
Kaneko et al(2002)	IFN-α(sc.), 5-FU, CDDP, MTX, LV(i.v.)	29	45
Ota et al(2005)	IFN-α(sc.), 5-FU	55	48
Enjoji et al(2005)	IFN-α(sc.), 5-FU	28	57
Obi et al(2006)	IFN-α(sc.), 5-FU	116	52

ADR：doxorubicin, CDDP：cisplatin, MMC：mitomycin C, Epi-ADR：epirubicin, VP-16：etoposide
5-FU：5-fluorouracil, IFN：interferon, MTX：methotrexate, LV：leucovorin
sc.：subcutaneous infusion, i.v.：intra-venous infusion

表2　全身化学療法

報告者	使用薬剤	症例数	奏効率(%)
Chlebowski et al(1984)	ADR	52	11
Al-Idrissi et al(1982)	ADR, 5-FU, MMC	40	13
Yang et al(2002)	ADR, GEM	28	12
Park et al(2006)	ADR, CDDP, Capecitabine	29	24
Hochster et al(1985)	Epi-ADR	18	17
Kim et al(2006)	Epi-ADR, CDDP, UFT, LV	53	17
Tetef et al(1995)	5-FU, LV	15	1
Lozano et al(2000)	Capecitabine	37	13
Ikeda et al(2004)	5-FU, CDDP, MIT	51	27
Nakamura et al(in press)	S-1, IFN-α	12	25
Chao et al(1998)	Paclitaxel	20	0
Hebbar et al(2006)	Docetaxel	15	7
O'Reilly et al(2001)	Irinotecan	14	7
Kim et al(2004)	GEM, Docetaxel	21	10
Taieb et al(2004)	GEM, Oxaliplatin	26	15
Zhu et al(2006)	GEM, Oxaliplatin, Bevacizumab	33	18
Philip et al(2005)	Erlotinib	38	8
Eckel et al(2005)	Imatinib	17	0
Abou-Alfa et al(2006)	Sorafenib	137	2
Llovet et al(2007)	Sorafenib	299	2

ADR：doxorubicin, 5-FU：5-fluorouracil, MMC：mitomycin C, GEM：gemcitabine, CDDP：cisplatin,
Epi-ADR：epirubicin, UFT：uracil-tegafur, LV：leucovorin, MIT：mitoxantrone, IFN：interferon

表3　術前肝動脈(化学)塞栓療法

報告者	使用薬剤	症例数	結　果
Imaoka et al(1989)	CDDP	37	有効(Ts10cm以下)
Monden et al(1989)*	ADR	71	有意差なし
Adachi et al(1993)	ADR, MMC	46	有効(完全壊死例, Ts5cm以下)
Wu et al(1995)*	Epi-ADR	24	有害
Yamasaki et al(1996)*	なし	50	有意差なし
Harada et al(1996)	Epi-ADR, MMC	98	有効(完全壊死例)
Sugo et al(2003)	Epi-ADR	113	有効(StageⅢ, Ⅳ)

CDDP：cisplatin, ADR：doxorubicin, MMC：mitomycin C, Epi-ADR：epirubicin, Ts：Tumor Size
*：ランダム化比較試験

第Ⅱ相試験における奏効率は12～24%であり，今後はランダム化比較試験における検証が必要である。Epirubicin(Epi-ADR)は，単剤での全身投与における奏効率は，ADRを上回るものではなかった。5-FUも肝細胞がんに対して古くより使用されてきた抗がん剤の1つであるが，近年の第Ⅱ相試験において，5-FUとCDDP，mitoxantrone(MIT)の3剤併用により27%の奏効率が報告されている[10]。S-1は，5-FU系の薬剤であり，他の消化器がん(胃がん，大腸がん，膵がん等)において高い有効性を示すと報告されている。肝細胞がんについても，S-1とIFNの併用により，25%の奏効率が報告されている[11]。その他，paclitaxel，docetaxel，irinotecanなどについても臨床試験が実施されているが，有望とはいえない。gemcitabine(GEM)は当初，奏効率が18%と良好な結果が報告されたが，その後の追試ではその効果は確認されなかった。GEMとoxaliplatinとの併用が試みられたが，奏効率は20%以下であった。さらに，GEMとoxaliplatinに加えて，分子標的治療薬である抗血管内皮増殖因子(VEGF)レセプター抗体のbevacizumabの3剤併用投与の第Ⅱ相試験の結果は，bevacizumabの上乗せ効果は認められなかった[12]。化学療法とは厳密にはその定義から少し外れるが，その他の分子標的治療薬に関しては，RAFやVEGFレセプターなどを標的とするマルチキナーゼ阻害薬のsorafenibは第Ⅱ相試験における奏効率は2.2%であったが[13]，近年の第Ⅲ相試験(SHARP Trial)において，生存期間において対照群の7.9か月と比較して10.7か月と有意な延長が認められた[14]。sorafenib投与群における治療効果の内訳は，partial response(PR) 2.2%，stable disease(SD)71%，progression disease(PD)18%であった。本治療は肝細胞がんに対する分子標的治療の中で標準的治療となる可能性があるものの，sorafenib単独の奏効率は2.2%と低率であり，このことから，単剤では肝細胞がんの増殖を抑制し得ても根治し得ないと考えられる。肝細胞がん患者の予後向上のためには，他の抗がん剤との併用による抗腫瘍効果の改善が必要であろう。

2. 術前肝動脈化学塞栓術

現在，手術可能な肝細胞がんに対する術前治療の選択肢として，主にTAE/TACEが選択される。TAE/TACEは，栄養動脈より抗がん剤と塞栓物質を注入することにより，肝動脈末梢部を塞栓し腫瘍を壊死に陥らせる治療法である。本邦では，反復治療が可能であり，肝機能に及ぼす影響も比較的少ないため，肝内多発症例に対する標準的治療として位置づけられている[2]。術前にTAE/TACEを施行する目的は，肝切除施行時にすでに存在する肝内微小転移や術前の画像診断により描出できない病巣の治療および制御にある。これまでに，諸家により報告されている術前TAE/TACEの効果を表3に示す。それぞれの報告により，肝内再発抑制に有用である，再発予防効果は認めない，肝機

表4　術後補助化学療法

報告者	使用薬剤	治療期間	症例数	結　果
Izumi et al(1994)	動注ADR+MMC+Lip	1回のみ	23	有効(進行がん)
Lai et al(1998)	静注Epi-ADR+動注CDDP+Lip	4年	30	有害
Tanaka et al(2005)	動注CDDP+5-FU	1か月	7	有効(進行がん)
Hasegawa et al(2007)	経口UFT	1年	79	有害
Nagano et al(2007)	動注5-FU+皮下注IFN-α	3か月	15	有効(進行がん)

ADR：doxorubicin, MMC：mitomycin C, Lip：Lipiodol, Epi-ADR：epirubicin, CDDP：cisplatin, 5-FU：5-fluorouracil, UFT：uracil-tegafur, IFN：interferon

能障害により生存期間に負の影響を及ぼすなどさまざまであり，一定の見解は得られていない[16]。多くの報告は，Retrospective Studyであるが，肝細胞がんの中で肝切除の対象となる全症例に術前TAE/TACEを施行することは，有益ではないと考えられる。しかし，術前TAE/TACEの対象とする症例を選別することにより，目的とする肝内転移再発を抑制し，無再発生存期間や全生存期間の延長に寄与する可能性はあると思われる。今後は，術前TAE/TACEの方法，回数，使用薬剤の統一と標準化や対象症例を腫瘍径やStageなどにより選別したランダム化比較試験が必要である。

3. 術後補助化学療法

肝細胞がん切除後の補助化学療法の目的は，術後の高頻度の肝内再発を抑制することである。肝細胞がん根治切除後の早期再発形式の大多数は，肝内転移に起因する残肝再発である[10]。表4に，これまでの主な補助化学療法の結果を示す。それぞれの報告によって，結果はさまざまであり，一定の見解は得られていない。また，統計学的な症例数の設定のもとに，十分な症例数を集積できた臨床試験は2件しかなく，この2件のいずれの報告においても，補助化学療法の有効性は示されていない[17,19]。よって，現時点で肝細胞がん切除後の補助化学療法として有効なレジメンはないと考えられる。しかしながら，この2件の臨床試験は両者とも，腫瘍の進展度に関して早期がんから進行がんまでのあらゆる症例を対象としているため，補助化学療法の有効性が示されなかった可能性もある。門脈内腫瘍栓や全肝に多発する肝内転移を有する進行がんを対象とした臨床試験においては，症例数が少ないながらも，補助化学療法の有効性が示されており[19-21]，今後の課題としては，多施設におけるランダム化比較試験などにより，臨床腫瘍統計上評価しうる症例数を十分に集積した上での検討が必要である。

おわりに

肝細胞がんの切除成績向上のためには，術後の肝内転移再発の抑制を目的とする術前・術後治療，および肝外転移病巣に対する全身化学療法の確立が急務である。これまで進行肝細胞がんに対するさまざまなレジメンが試みられており，その中でもIFN併用化学療法は高い奏効率を示すことが報告されており，極めて有望な治療法と考えられる。また，近年の分子生物学の進歩により，分子標的治療薬におけるsorafenib等の標準的治療となる可能性のある薬剤も開発されてきている。今後は，妥当性のある臨床試験において抗腫瘍効果を検証することが重要課題となる。

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