Jpn J Clin Oncol. 37(12) 924-929. 2007

3) Nakata B, Sowa M, Tsuji A, Kamano T, Sasaki K, Fukunaga Y, Takahashi M, Tsujitani S, Mikami Y, Mitachi Y, Nishimura S, Araki H, Yamamitsu S, Hirakawa K, Tominaga S, Shirasaka T, Inokuchi K.: Continuous infusion of 5-fluorouracil with versus without low-dose, consecutive administration of cisplatin in advanced colorectal cancer. A prospective randomized phase II study. J Exp Clin Cancer Res. 26(1) 51-60 2007

２．学会発表

なし

H. 知的財産権の出願・登録状況

(予定を含む。)

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

別紙４

研究成果の刊行に関する一覧表レイアウト

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
1) 小尾俊太郎	肝癌に対する全身化学療法、肝動注化学療法のエビデンスは？	跡見裕、上村直美、白鳥敬子、正木尚彦	臨床に直結する肝・胆・膵疾患のエビデンス	文光堂	東京	2007	106-108
2) 板本敏行，他	HCCに対する肝移植の現況と展望	林紀夫	Annual Review消化器2008	中外医学社	東京	2008	231-236
3) Nakamura H	Hepatoma-derived growth factor in cancer development and progression.	Mohan RM	Research Advances in Cnacer	Global Research Network	India	2007	91-100

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
1) 野田剛広, 他	【消化器がんの化学療法　外科の立場から】肝がん(解説/特集)	MEDICO	39 (1)	10-14	2008
2) Nagano H, et al.	Hepatic resection followed by IFN-alpha and 5-FU for advanced hepatocellular carcinoma with tumor thrombus in the major portal branch.	HepatoGastroenterology	54(73)	172-179	2007
3) Yamamoto T, et al.	Successful treatment of multiple hepatocellular carcinoma with tumor thrombi in the major portal branches by intraarterial 5-fluorouracil perfusion chemotherapy combined with subcutaneous interferon-alpha and hepatectomy.	Int J Oncol	12(2)	150-154	2007
4) Yang Y, et al.	Patterns and clinicopathologic features of extrahepatic recurrence of hepatocellular carcinoma after curative resection.	Surgery	141(2)	196-202	2007
5) Damdinsuren B, et al.	Interferon alpha receptors are important for antiproliferative effect of interferon-alpha against human hepatocellular carcinoma cells.	Hepatology Research	37(1)	77-83	2007
6) Arai I, et al.	Overexpression of MT3-MMP in hepatocellular carcinoma correlates with capsular invasion.	Hepatogastroenterology	54(739)	167-171	2007
7) Wada H, et al.	Combination therapy of interferon-alpha and 5-fluorouracil inhibits tumor angiogenesis in human hepatocellular carcinoma cells by regulating vascular endothelial growth factor and angiopoietins.	Oncol Rep	18(4)	801-809	2007
8) Damdinsuren B, et al.	Combined intra-arterial 5-fluorouracil and subcutaneous interferon-alpha therapy for highly advanced hepatocellular carcinoma.	Hepatology Research	37(2)	238-250	2007
9) Nagano H, et al.	Interferon-alpha and 5-fluorouracil combination therapy after palliative hepatic resection in patients with advanced hepatocellular carcinoma, portal venous tumor thrombus in the major trunk, and multiple nodules.	Cancer	110(11)	2493-2501	2007
10) Wada H, et al.	Complete remission of hepatocellular carcinoma with portal vein tumor thrombus and lymph node metastases by arterial infusion of 5-fluorouracil and interferon-alpha combination therapy following hepatic resection.	J Gastroenterol	42(6)	501-506	2007
11) Uyama H, et al.	New chemotherapy for patients with advanced hepatocellular carcinoma: Pilot study of beta-interferon and doxorubicin one-shot intra-arterial chemotherapy.	Hepatol Res	37(12)	1018-1025	2007
12) Nakamura M, et al.	Role of the Fas/FasL pathway in combination therapy with interferon-alpha and fluorouracil against hepatocellular carcinoma in vitro.	J Hepatol	46(1)	77-88	2006
13) 村上昌裕, 他	Doxorubicin/IFN-β併用化学療法と肝切除術により長期生存し得た右心房内腫瘍栓を伴う進行肝細胞癌の1例	癌と化学療法	34 (12)	2087-2089	2007

14) 永野浩昭, 他	下大静脈内に連続する腫瘍栓を伴う肝細胞癌に対する肝右葉切除術	消化器外科	30 (1)	67-75	2007
15) 永野浩昭, 他	肝細胞癌に対するインターフェロンの抗腫瘍効果-臨床的検討から-	日本消化器病学会雑誌	104 (5)	654-659	2007
16) 太田英夫, 他	門脈内腫瘍栓を伴う混合型肝癌の1切除例	日本臨床外科学会雑誌	68 (1)	172-178	2007
17) 和田浩志, 他	I. 術前治療 1. 術前肝動脈塞栓術 (TAE)	外科	69 (5)	497-503	2007
18) 永野浩昭, 他	特集：肝胆膵がんと化学療法のすべて－5FUとインターフェロン	肝胆膵	55 (5)	823-831	2007
19) 左近賢人, 他	インターフェロン/5FU動注療法	肝胆膵	53 (5)	707-712	2006
20) 左近賢人, 他	7. 肝細胞がん薬物治療の新たな動き. 1) 肝細胞がんに使用される抗がん剤とその効果 (IFN併用も含めて)	臨床腫瘍プラクティス	2 (4)	401-404	2006
21) 小尾俊太郎	【腫瘍栓のすべて】 切除以外の治療 インターフェロンの併用動注療法 (2)	外科	70 (2)	197-201	2008
22) 金井文彦, 他	【肝胆膵がんと化学療法のすべて】 肝疾患 薬剤からみた化学療法 作用機序、理論と投与方法、成績、副作用 肝細胞癌に対する分子標的薬の開発状況	肝・胆・膵	55 (5)	861-866	2007
23) 小尾俊太郎	肝細胞癌	癌と化学療法	34 (10)	1559-1562	2007
24) 金子順一, 他	門脈本幹腫瘍栓で発症した肝細胞癌に対してインターフェロン/5-FU動注療法後、腫瘍栓消失し、生体肝移植を施行した1例	Liver Cancer	13 (1)	43-50	2007
25) Tashiro H, et al.	Reconstruction of the middle hepatic vein tributaries draining segments V and VIII of a right liver graft with the recipient's own middle hepatic vein using vascular closure staples.	Surg Today	38(3)	289-291	2008
26) Tashiro H, et al.	Biliary complications after duct-to-duct biliary reconstruction in living-donor liver transplantation: causes and treatment.	World J Surg	31(11)	2222-2229	2007
27) Ohdan H, et al.	Microsurgical hepatic artery reconstruction during living-donor liver transplantation by using head-mounted surgical binocular system.	Transpl Int.	20(11)	970-973	2007
28) Itamoto T, et al.	Repeat hepatectomy for recurrent hepatocellular carcinoma.	Surgery	141(5)	589-597	2007
29) Ando E, et al.	Surveillance program for early detection of hepatocellular carcinoma in Japan: result of specialized department of liver disease.	J Clin Gastroenterol	40	942-948	2006
30) Murashima S, et al.	A decrease in AFP level related to administaration of interferon in patients with chronic hepatitis C and a high level of AFP	Dig Dis Sci	51	808-812	2006
31) Nakashima Y, , et al.	Portal vein and intrahepatic micrometastasis in small hepatocellular carcinoma by gross type.	Hepatol Res	26	142-147	2003
32) Ando E, et al.	Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: analysis 48 cases.	Cancer	95	588-595	2002
33) 久保正二, 他	特集「局所再発癌に対する外科治療；適応とコツ」大血内進展を伴う進行再発肝細胞癌に対する外科治療	消化器外科	31 (2)	161-168	2008

34) 久保正二，他	肝細胞癌に併存する結節性病変のうち治療の対象となる病変は	消化器外科	30 (5)	589-600	2008
35) Kubo S, et al.	Effect of lamivudine on outcome after liver resection for hepatocellular carcinoma in patients with active replication of hepatitis B virus.	Hepatology Research	37(2)	94-100	2007
36) Kubo S, et al.	Risk factors for massive blood loss during liver resection for hepatocellular carcinoma in patients with cirrhosis.	Hepatogastroenterology	54(75)	830-833	2007
37) Kubo S, et al.	Surgical treatment for hepatocellular carcinoma detected after successful interferon therapy.	Surgery Today	37(4)	285-290	2007
38) Wakasa T, et al.	A histopathological study on combined hepatocellular and cholangiocarcinoma: cholangiocarcinoma component is originated from hepato- cellular carcinoma.	Hepatogastroenterology	54(74)	508-513	2007
39) Tamori A, et al.	Potential role of vitamin K2 as a chemopreventive agent against hepatocellular carcinoma.	Hepatology Research	37	S303-S307	2007
40) 高台真太郎，他	肝切除後に総肝動脈リンパ節転移を来たした肝細胞癌の１例	日本消化器外科学会雑誌	40 (1)	50-55	2007
41) 婆 正寛，他	生体肝移植術後、タクロリムスおよびシクロスポリンによる脳症をきたした１例	移植	42 (5)	464-469	2007
42) 久保正二，他	【一目でわかる 消化器外科主要手術・治療のケア21】 主要治療肝動脈塞栓療法	消化器外科NURSING	12 (1)	81-83	2007
43) Nakamura H, et al.	New chemotherapy for patients with advanced hepatocellular carcinoma: a pilot study of interferon-beta and doxorubicin one-shot intra-arterial chemotherapy.	Hepatology Research	37(12)	1018-1025	2007
44) Nakamura H, et al.	Vitamin K2 inhibits the proliferation of HepG2 cells through up-regulating the transcription of p21 gene.	Hepatology Research	37(5)	360-365	2007
45) Morita S, et al.	A phase I study of combination therapy of the oral fluorinated pyrimidine compound S-1 with low-dose cisplatin twice-a-week administration (JFMC27-9902 Step2) in patients with advanced gastric cancer using a continual reassessment method.	Jpn J Clin Oncol.	37(12)	924-929	2007
46) Nakata B, et al.	Continuous infusion of 5-fluorouracil with versus without low-dose, consecutive administration of cisplatin in advanced colorectal cancer. A prospective randomized phase II study.	J Exp Clin Cancer Res.	26(1)	51-60	2007
47) 辻晃仁	外来がん化学療法と地域連携	治療学	41 (11)	50-51	2007

肝癌

肝癌に対する全身化学療法，肝動注化学療法のエビデンスは？

小尾俊太郎・今村　潤

肝細胞癌に対する化学療法は，切除，経皮的局所療法，肝動脈塞栓術などの適応基準から逸脱した症例に行われている．肝機能因子と腫瘍因子で化学療法の適応基準も定められるが，現状では明確な基準はなく，個々のStudyで規定されている．一般的にはperformance status 2～3，コントロール不能の腹水，腫瘍占有率50%以上，Vp4，総ビリルビン2.0mg/dL以上の症例は除外されていることが多い．本項は肝細胞癌に対する化学療法のエビデンスを検討した．

全身化学療法のエビデンス

●単剤による全身化学療法のエビデンス（表1）

薬剤	症例数	奏効率	報告年	Study design	Evidence level
doxorubicin	35	28	1983 [1]	RCT	II
doxorubicin	52	11	1984 [2]	Cohort Study	IV
doxorubicin	66	24.5	1985 [3]	CCT	IV
mitoxantrone	74	8	1987 [4]	CCT	IV
etoposide	18	0	1987 [5]	Case Series	IV
doxorubicin	106	3.3	1988 [6]	RCT	I
etoposide	24	5	1989 [7]	Cohort Study	IV
CDDP	42	2.3	1993 [8]	Case Series	IV
CDDP	28	15.4	1993 [9]	Cohort Study	IV
vindesine	16	0	1995 [10]	Cohort Study	IV
5-FU	25	28	1995 [11]	Cohort Study	IV
topotecan	36	13.9	1997 [12]	Cohort Study	IV
paclitaxel	20	0	1998 [13]	Cohort Study	IV
gemcitabine	28	17.8	2000 [14]	Case Control Study	IV
IFN	58	6.6	2000 [15]	RCT	II
epirubicin	52	9	2001 [16]	Case Control Study	IV
tegafur/uracil	48	17.8	2001 [17]	RCT	II
gemcitabine	17	0	2001 [18]	Cohort Study	IV
irinotecan	14	7	2001 [19]	Cohort Study	IV
5-FU	20	0	2002 [20]	Cohort Study	IV
imatinib	17	0	2005 [21]	Cohort Study	IV
erlotinib	38	8	2005 [22]	Cohort Study	IV
thalidomide	37	5	2005 [23]	Cohort Study	IV
thalidomide	42	5	2006 [24]	Cohort Study	IV
irinotecan	29	0	2006 [25]	Cohort Study	IV

(表1) つづき

薬剤	症例数	奏効率	報告年	Study design	Evidence level
docetaxel	15	6	2006[26]	Cohort Study	Ⅳ
sorafenib	137	2.2	2006[27]	Cohort Study	Ⅳ
arsenic trioxide	29	3	2007[28]	Cohort Study	Ⅳ

●多剤による全身化学療法のエビデンス（表2）

薬剤	症例数	奏効率	報告年	Study design	Evidence level
5-FU＋IFN	29	18	1993[29]	Cohort Study	Ⅳ
doxorubicin＋IFN	22	10	1994[30]	Cohort Study	Ⅳ
CDDP＋IFN	56	13.3	1996[31]	CCT	Ⅲ
epirubicin＋etoposide	36	39	1997[32]	Cohort Study	Ⅳ
gemcitabine＋doxorubicin	50	11.8	2002[33]	Cohort Study	Ⅳ
epirubicine＋CDDP＋5-FU	21	14.5	2002[34]	Cohort Study	Ⅳ
CDDP＋IFN＋doxorubicin＋5-FU	188	20.9	2005[35]	RCT	Ⅱ
gemcitabine＋CDDP	30	20	2005[36]	Cohort Study	Ⅳ
5-FU＋mitoxantrone＋CDDP	51	27	2005[37]	Cohort Study	Ⅳ
CDDP＋IFN＋doxorubicin＋5-FU	26	15	2005[38]	Cohort Study	Ⅳ
epirubicin＋thalidomide	19	0	2005[39]	Cohort Study	Ⅳ
gemcitabine＋oxaliplatin＋bevacizumab	33	20	2006[40]	Cohort Study	Ⅳ
doxorubicin＋CDDP＋capecitabine	29	24	2006[41]	Cohort Study	Ⅳ

抗腫瘍効果や延命効果が確実な抗癌剤やそのレジメンは明らかにされていない．このためさまざまな薬剤が単剤あるいは多剤併用で用いられてきた．いずれも少数例を対象としたパイロット的研究であり，いまだ臨床研究段階といえる．1980年代はdoxorubicinが試みられ，90年代にはCDDP，5FUが試みられ，2000年になると血管新生阻害薬，分子標的薬が試みられている．それぞれの研究結果を論文上の奏効率として比較すると単剤での奏効率は0～28％であり，単剤では効果が期待できない．そこで多剤併用の研究を検討したところ単剤にIFNの併用，多剤併用，最近では多剤併用に血管新生阻害薬や分子標的薬の併用が行われている．これらの奏効率は0～39％であり，単剤よりは奏効率が高い傾向がある．ただし動注化学療法と比較すると，薬剤が全身に均等に流れるため副作用に十分注意する必要がある．本邦の肝細胞癌患者は85％がC型慢性肝炎由来であることを勘案すると，肝硬変合併症例でも安全に施行できて効果の高いレジメンが切望される．

動注化学療法のエビデンス

全身化学療法に比較して肝動注化学療法は有効か？

全身化学療法に比較して肝動注化学療法は有効という十分な科学的根拠はない．単剤多剤ともに，既存論文の奏効率を全身化学療法と比較すると，動注化学療法の方が優れている．薬剤は5-FU，CDDP，IFNが中心となっている．各研究によって薬剤の組み合わせ，投与量，投与時間が設定されているため，標準化されていない．また，動注化学療法の有効性を論じた論文が少ないため海外では認められていない．全身化学療法と動注化学療法を比較した論文は，奏効率は44％ vs. 60％で動注

㉓単剤による動注化学療法のエビデンス（表3）

薬剤	症例数	奏効率	報告年	Study design	Evidence level
epirubicin	20	8	1997[42]	CCT	Ⅲ
ifosfamide	19	37.5	1997[43]	Cohort Study	Ⅳ
doxorubicin	72	60	1999[44]	RCT	Ⅱ
CDDP	67	37	2002[45]	Cohort Study	Ⅳ

㉔多剤による動注化学療法のエビデンス（表4）

薬剤	症例数	奏効率	報告年	Study design	Evidence level
etoposide＋CDDP＋5-FU＋doxorubicin	28	46	1992[46]	Cohort Study	Ⅳ
CDDP＋5-FU	21	14.3	1995[47]	Cohort Study	Ⅳ
CDDP＋5-FU	9	44.4	1997[48]	Cohort Study	Ⅳ
CDDP＋5-FU	52	71	1999[49]	Case Control Study	Ⅳ
CDDP＋IFN	68	33	2000[50]	RCT	Ⅱ
oxaliplatin＋topotecan	13	77	2002[51]	Cohort Study	Ⅳ
CDDP＋etoposide	26	38	2002[52]	Cohort Study	Ⅳ
CDDP＋5-FU	7	33	2002[53]	Cohort Study	Ⅳ
CDDP＋5-FU	48	48	2002[54]	Case Control Study	Ⅳ
CDDP＋5-FU＋leucovorin	19	56	2002[55]	RCT	Ⅱ
5-FU＋IFN	11	73	2002[56]	Cohort Study	Ⅳ
CDDP＋5-FU＋IFN＋leucovorin＋methotrexate	34	45	2002[57]	Cohort Study	Ⅳ
CDDP＋mitomycin C＋leucovorin＋5-FU	53	28.3	2004[58]	Cohort Study	Ⅳ
5-FU＋IFN	28	21.5	2005[59]	Cohort Study	Ⅳ
5-FU＋IFN	156	51	2006[60]	Historical Cohort Study	Ⅲ

化学療法が優れていたが生存期間では有意差を証明できなかった．今後，遠隔転移への効果に関する検討，そして何よりも全身化学療法との比較を行い，その有効性を証明していかなければならない．

肝癌診療ガイドライン

平成14～15年度の厚生労働省診療ガイドライン支援事業により，科学的根拠に基づく肝癌診療ガイドライン作成に関する研究班が組織され，ガイドラインが取りまとめられた[61]．化学療法では8つのリサーチクエスチョンがあった．①化学療法の適応，②動注化学療法の有用性，③有効な薬剤，④インターフェロン併用の有用性，⑤効果予測．予後因子以上の5項目についての検討結果はすべて化学的根拠のある推奨はないとなっている．また⑥経口化学療法は有効という科学的根拠がなく推奨されていない．⑦ホルモン療法，⑧インターフェロン単独の2項目は無効なので推奨しないと結論されている．

現時点における肝癌に対する全身化学療法，肝動注化学療法に対する考え方

●進行肝細胞癌に対する化学療法はいまだ確立しておらず，標準的なものはない．今後エビデンスレベルの高い研究を重ね，早期に標準治療を確立する必要がある．

4. HCCに対する肝移植の現況と展望

広島大学大学院医歯薬総合研究科先進医療開発科学講座外科学准教授　板本敏行
同　教授　浅原利正

key words　hepatocellular carcinoma, liver transplantation, Milan criteria, UCSF criteria, down-staging

動　向

肝細胞癌 hepatocellular carcinoma (HCC) の肝移植に関するcontroversyは，ミラノ基準からの適応拡大と，移植前のdown-stagingの有効性などである．

適応拡大に関しては，ドナーソースとその社会的背景が脳死と生体移植で異なり，別々に議論されなければならない．適応のGold standardはミラノ基準で，この基準内で移植を行えば脳死，生体肝移植にかかわらず5年生存率70%以上，累積再発率15%以下は保証されている．脳死の場合，臓器分配の公平性からも，肝癌以外の疾病に対する肝移植と同等の成績でなければならない．しかし，生体では何%までの5年生存率，再発率なら許容されるのか，といった議論もされている．欧米ではミラノ基準外の症例に生体肝移植を行うことを推奨している[1,2]．ミラノ基準以外の適応基準も提唱されているが，これらはいずれも摘出肝の肉眼的・病理組織学的検査を後ろ向きに検討した結果から提唱されたものであり，術前の画像診断に適用すると5年生存率が10～15%低下する．近年の画像診断の進歩にもかかわらず，術前評価と摘出肝の病理結果の乖離を改めて指摘する論文もみられる[3,4]．

移植待機中のdown-stagingに関しては以前より後ろ向き研究で検討されてきたが，大規模な無作為比較試験はない．最近では肝動脈化学塞栓療法 transarterial chemoembolization (TACE) に加えラジオ波焼灼療法が積極的に行われるようになり，その効果が期待されている．しかし，down-stagingが移植後の予後をも改善するかどうかはcontroversialである．

A. 脳死肝移植の成績と適応

ミラノ基準が提唱されて10年が経過し，この基準の妥当性が検証されている一方で，同基準が厳格すぎるとの批判もある．しかし，米国肝臓学会からでたHCCに対する治療のガイドラインでは，脳死肝移植の適応基準としてやはりミラノ基準を推奨している．その理由として，適応拡大の可能性を提唱した報告は，摘出肝の病理学的検査結果に基づいており，その結果を移植前の適応基準として適用することは好ましくないとしている[1]．

適応拡大の代表的なUCSF (University of California, San Francisco) 基準 (6.5cm以下単発，4.5cm以下2～3個で合計8cm以下) の妥

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．

文献

1) Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology. 2005; 42(5): 1208-36.
2) Schwartz M, Roayaie S, Konstadoulakis M. Strategies for the management of hepatocellular carcinoma. Nat Clin Pract Oncol. 2007; 4(7): 424-32.
3) Freeman RB, Mithoefer A, Ruthazer R, et al. Optimizing staging for hepatocellular cancer before liver transplantation. Liver Transpl. 2006; 12(10): 1504-11.
4) Shah SA, Tan JC, McGilvray ID, et al. Accuracy of staging as a predictor for recurrence after liver transplantation for hepatocellular carcinoma. Transplantation. 2006; 81(12): 1633-9.
5) Leung JY, Zhu AX, Gordon FD, et al. Liver transplantation outcomes for early-stage hepatocellular carcinoma: results of a multicenter study. Liver Transpl. 2004; 10(11): 1343-54.
6) Onaca N, Davis GL, Goldstein RM, et al. Expanded criteria for liver transplantation in patients with hepatocellular carcinoma: a report from the International Registry of Hepatic Tumors in Liver Transplantation. Liver Transpl. 2007; 13(3): 391-9.
7) Decaens T, Roudot-Thoraval F, Hadni-Bresson S, et al. Impact of UCSF criteria according to pre- and post-OLT tumor features: analysis of 479 patients listed for HCC with a short waiting time. Liver Transpl. 2006; 12(12): 1761-9.
8) Grasso A, Stigliano R, Morisco F, et al. Liver transplantation and recurrent hepatocellular carcinoma: predictive value of nodule size in a retrospective and explant study. Transplantation. 2006; 81(11): 1532-41.
9) Llovet JM. Expanding HCC criteria for liver transplant: the urgent need for prospective, robust data. Liver Transpl. 2006; 12(12): 1741-3.
10) Llovet JM, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. Semin Liver Dis. 2005; 25(2): 181-200.
11) Todo S, Furukawa H. Japanese Study Group on Organ Transplantation. Living donor liver transplantation for adult patients with hepatocellular carcinoma: experience in Japan. Ann Surg. 2004; 240(3): 451-9.
12) Takada Y, Ueda M, Ito T, et al. Living donor liver transplantation as a second-line therapeutic strategy for patients with hepatocellular carcinoma. Liver Transpl. 2006; 12(6): 912-9.
13) Soejima Y, Taketomi A, Yoshizumi T, et al. Extended indication for living donor liver transplantation in patients with hepatocellular carcinoma. Transplantation. 2007; 83(7): 893-9.
14) Jonas S, Mittler J, Pascher A, et al. Living donor liver transplantation of the right lobe for hepatocellular carcinoma in cirrhosis in a European center. Liver Transpl. 2007; 13(6): 896-903.
15) Malagó M, Sotiropoulos GC, Nadalin S, et al. Living donor liver transplantation for hepatocellular carcinoma: a single-center preliminary report. Liver Transpl. 2006; 12(6): 934-40.
16) Gondolesi GE, Roayaie S, Muñoz L, et al. Adult living donor liver transplantation for patients with hepatocellular carcinoma: extending UNOS priority criteria. Ann Surg. 2004; 239(2): 142-9.
17) Fisher RA, Kulik LM, Freise CE, et al. Hepatocellular carcinoma recurrence and death following living and deceased donor liver transplantation. Am J Transplant. 2007; 7(6): 1601-8.
18) Freeman RB Jr. Transplantation for hepatocellular carcinoma: the Milan criteria and beyond. Liver Transpl. 2006; 12(11): S8-13.
19) Cillo U, Vitale A, Grigoletto F, et al. Intention-to-treat analysis of liver transplantation in selected, aggressively treated HCC patients exceeding the

Milan criteria.. Am J Transplant. 2007; 7(4): 972-81.

20) Yao FY, Kinkhabwala M, LaBerge JM, et al. The impact of pre-operative loco-regional therapy on outcome after liver transplantation for hepatocellular carcinoma. Am J Transplant. 2005; 5(4): 795-804.

21) Yao FY, Hirose R, LaBerge JM, et al. A prospective study on downstaging of hepatocellular carcinoma prior to liver transplantation. Liver Transpl. 2005; 11(12): 1505-14.

22) Otto G, Herber S, Heise M, et al. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. Liver Transpl. 2006; 12(8): 1260-7.

23) Porrett PM, Peterman H, Rosen M, et al. Lack of benefit of pre-transplant locoregional hepatic therapy for hepatocellular cancer in the current MELD era. Liver Transpl. 2006; 12(4): 665-73.

24) Lesurtel M, Müllhaupt B, Pestalozzi BC, et al. Transarterial chemoembolization as a bridge to liver transplantation for hepatocellular carcinoma: an evidence-based analysis. Am J Transplant. 2006; 6(11): 2644-50.

25) Zavaglia C, De Carlis L, Alberti AB, et al. Predictors of long-term survival after liver transplantation for hepatocellular carcinoma. Am J Gastroenterol. 2005; 100(12): 2708-16.

26) Pawlik TM, Gleisner AL, Anders RA, et al. Preoperative assessment of hepatocellular carcinoma tumor grade using needle biopsy: implications for transplant eligibility. Ann Surg. 2007; 245(3): 435-42.

27) Cillo U, Vitale A, Bassanello M, et al. Liver transplantation for the treatment of moderately or well-differentiated hepatocellular carcinoma. Ann Surg. 2004; 239(2): 150-9.

28) Sutcliffe R, Maguire D, Portmann B, et al. Selection of patients with hepatocellular carcinoma for liver transplantation. Br J Surg. 2006; 93(1): 11-8.

29) Ramos E, Llado L, Serrano T, et al. Utility of cell-cycle modulators to predict vascular invasion and recurrence after surgical treatment of hepatocellular carcinoma. Transplantation. 2006; 82(6): 753-8.

30) Lin WY, Tsai SC, Hung GU. Value of delayed 18F-FDG-PET imaging in the detection of hepatocellular carcinoma. Nucl Med Commun. 2005; 26(4): 315-21.

31) Sugiyama M, Sakahara H, Torizuka T, et al. 18F-FDG PET in the detection of extrahepatic metastases from hepatocellular carcinoma. J Gastroenterol. 2004; 39(10): 961-8.

32) Yang SH, Suh KS, Lee HW, et al. The role of 18F-FDG-PET imaging for the selection of liver transplantation candidates among hepatocellular carcinoma patients. Liver Transpl. 2006; 12(11): 1655-60.

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Hepatoma-derived growth factor in cancer development and progression

Hideji Nakamura
Division of Hepatobiliary and Pancreatic Medicine, Department of Internal Medicine, Hyogo College of Medicine, Mukogawa-cho 1-1, Nishinomiya, Hyogo 663- 8501, Japan

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.

Correspondence to: Hideji Nakamura,
E-mail: nakamura@hyo-med.ac.jp

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