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• REVIEW •

Metastatic human hepatocellular carcinoma models in nude mice and cell line with metastatic potential

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Abstract

Metastatic human HCC model is needed for the studies on mechanism and intervention of metastatic recurrence. By using orthotopic implantation of histologically intact tissues of 30 surgical specimens, a patient-like metastatic model of human HCC in nude mice (LCI-D20) and a low metastatic model of human HCC in nude mice (LCI-D35) have been established. All mice with transplanted LCI-D20 tumors exhibited extremely high metastatic ability including spontaneous metastasis to liver, lungs, lymph nodes and peritoneal seeding. Remarkable difference was also found in expression of some of the invasiveness related genes and growth factors between the LCI-D20 and LCI-D35 tumors. PAI-1 increased gradually following tumor progression in LCI-D20 model, and correlated with tumor size and AFP level. Phasic expression of tissue intercellular adhesion molecule-1 in this model was also observed. Using corneal micropocket model, it was demonstrated that the vascular response induced by LCI-D20 tumor was stronger than that induced by LCI-D35 tumor. Similar report on metastatic human HCC model in nude mice and human HCC cell line with metastatic potential was rarely found in the literature. This LCI-D20 model has been widely used for the studies on intervention of metastasis, including anti-angiogenesis, antisense approach, metallopro teinase inhibitor, differentiation inducer, etc. It is concluded that the establishment of metastatic human HCC model in nude mice and human HCC cell line with metastatic potential will provide important models for the *in vivo* and *in vitro* study of HCC invasiveness, angiogenesis as well as intervention of HCC recurrence.

Subject headings hepatocellular carcinoma; metastasis; metastatic model; nude mice; cell line; experimental intervention; angiogenesis

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INTRODUCTION

Liver cancer is the 4th most common cause of death from cancer and the 3rd most common in men. The highest age-standardised mortality rate was in China (34.7/100000), which alone accounts for 53% of all liver cancer deaths worldwide[1]. Surgical resection has been accepted the best treatment forhepatocellular carcinoma (HCC), the most common type of primary liver cancer in China. However, recurrence and metastasis remain the major obstacles for further prolonging survival after resection. Even after curative resection of small HCC, the recurrent rate remained high[2,3]. Therefore, studies on metastasis and recurrence will be an important issue in the 21st century. To this end, metastatic human HCC mod el in nude mice and cell line with metastatic potential are needed for the studies on mechanism, angiogenesis and intervention of metastatic recurrence.

Brief review of literature

In 1963, the first human HCC cell line (BEL-16) was established by Chen[4]. At the authors' institution, the human HCC model in nude mice (LTNM) was established in 1982, but metastasis was not found in this model[5]. Human HCC nude mice model and human HCC cell line with metastatic potential were rarely reported in the literature.

Hepatocellular carcinoma cell line

In the recent three decades, a good number of human HCC cell lines have been established. Shen *et al*[6], after the establishment of the first human HCC cell line in 1963, reported a series of human HCC cell line (BEL-7402, BEL-7404, BEL-7405) in the ensuing years. In 1973, Alexander *et al*[7] established the famous human HCC cell line (PLC/PRF/5) which produces HBsAg. Dong[8] established the human HCC cell line (SMMC-7721) in 1977, which remains one of the human HCC cell line that currently used in China.

Many human HCC cell lines have been established for the studies on etiological factors of HCC, such as hepatitis B virus (HBV)[9-14], hepatitis C virus (HCV)[15], hemochromatosis[16] , thorotrast[17], for the study of alpha fetoprotein (AFP)[18,19], and for other studies[20-30]. Unfortunately, of the above human HCC cell lines, metastatic potential was rarely mentioned or demonstrated.For animal HCC cell lines, the establishment of such cell lines in rat[31], in woodchuck[32-34], and in chicken[35] have been reported.

Hepatocellular carcinoma cell line with metastatic potential

Human HCC cell line with metastatic potential was rarely reported in the literature. Besides Tian *et al*[36,37] at the authors' institution reported two paper in 1998 and 1999, only one paper has yet been reported. Seki *et al*[38] (1999) established a human hepatocellular carcinoma cells with metastasis to lymph nodes.

Again, very few papers have been reported concerning animal HCC with metastati c behavior. Ogawa *et al*[39] (2001) reported the establishment of rat HCC cell lines with differing metastatic potential in nude mice. Reichner *et al*[40] (1996) reported that interleukin-6 production by rat hepatocellular carcinoma cells is associated with metastatic potential.

Hepatocellular carcinoma model in animal

In 1976. Shimosato *et al*[41] reported the establishment of a series of human tumors in nude mice including HCC. The same group has used the human HCC nude mice model for the study of alpha fetoprotein in relation to tumor growth [42]. As had mentioned, at the authors' institution, a human HCC model in nude mice has been reported in 1982[5]. In 1995, Liu *et al*[43] established a nude mice xenograft model from human HCC. In 1996, Leveille-Webster *et al*[44] established an intrahepatic xenografts of human HCC in severe combined immunodeficiency mice for the study of multidrug resistance.

For animal HCC model, Qian *et al*[45] (1987) established a transplan table HCC model in 615-strain mice (H 615).

Hepatocellular carcinoma model in animal with metastatic behavior

In this paper, we need to focus to human HCC model in nude mice with metastati c behavior. In 1993, Aruga *et al*[46] reported the establishment and characterization of liver metastatic model of human hepatoma in nude mice, metastasis was mainly found in the liver of this subcutaneous tumor model. Sun *et al*[47,48] (1995 and 1996) at the authors' institution reported the first patient-like metastatic human HCC model in nude mice with 100% of spontaneous metastasis to lung, lymph node and liver. Peng *et al*[49] (1996) established a human HCC model in nude mice using orthotopic transplantation, and malignant behavior (invasion of abdominal cavity) was observed. Tao *et al*[50] (1998) established a human HCC nude mice model using SMMC-LTNM tumor transplanted into abdominal cavity and liver, the lung metastatic rate was 59%. Genda *et al*[51] (1999) reported the construction of metastatic models using orthotopic implantation of human HCC cell lines into the livers of SCID mice, two of the 5 cell lines injected showed vascular tumor thrombi and intrahepatic metastasis. Zheng *et al*[52] (2000) establ ished an orthotopic transplantation tumor model from the subcutaneous model of human HCC in nude mice, the spontaneous metastatic rate was 57.8%. Shi *et al*[53] (2001) established a human HCC model in nude mice with high metastatic rate in lymph node.

For animal HCC, Masui *et al*[54] (1997) reported a highly metastat ic HCC in male F344 rats induced by chemical carcinogens. Li *et al*[55] (1998) established a lymph node metastatic model of mouse HCC Hca-F cells in C3H/Hej mice.

A synopsis of related studies at Liver Cancer Institute of Fudan University

At the authors' institution, studies on recurrence and metastasis of HCC have been conducted since 1993[56-60]. Because either metastatic human HCC model in nude mice or human HCC cell line with metastatic potential was not availuable at that time, therefore, efforts have been made for the establishment of such model and cell line. At the authors' institution, the establishment of metastatic human HCC model in nude mice was reported in 1995 (in Chinese) and 1996 (in English)[47,48] and human HCC cell line with high metastatic potential was reported in 1998 (in Chinese) and 1999 (in English)[36,37]. These might probably be the first metastatic human HCC model in nude mice and cell line with metastatic potential. A Synopsis on the establishment and studies of these models at the Liver Cancer Institute of Fudan University is reported herein.

Establishment of metastatic human HCC in nude mice

In 1988, development of *in vivo* models for studies of brain metastasis has been reported in Fidler's group[61]. In early 1990s, "metastatic models constructed in nude mice by orthotopic transplantation of histologically inta ct patient specimens" has been used in Hoffman's group, and several such models including lung cancer, pancreatic cancer, ovarian cancer, etc have been reported[62-64]. However, patient-like human HCC model in nude mice with metastatic behavior was not found.

At the authors' institution, by using orthotopic implantation of histologically preserved metastatic tumor tissues of 30 surgical specimens, a highly metastatic model of human HCC in nude mice (LCI-D20) has been established. This model was obtained through *in vivo* clonal selection by repeated "lung foci to liver". All mice with transplanted LCI-D20 tumors in the liver exhibited 100% transplantability and metastatic ability as well as various manifestations of tumor behaviour in HCC patients. These included: local growth, regional invasion , spontaneous metastasis to liver, lungs, lymph nodes and peritoneal seeding. The high metastatic ability maintained up to 120 passages. Histological characte ristics of LCI-D20 tumor were similar to those of the original tumor. Karyotype analysis revealed heteroploid cells. Expression of AFP and HBxAg was shown using immunohistochemistry. The duration between two passages was around 20 d. At the same period, using orthotopic implantation of histologically preserved metastatic tumor tissues, a low metastatic model of human HCC in nude mice (LCI-D35) has also been established as a control. Invasion to the liver and peripheral organs was not found. Pathological findings revealed no metastasis in the liver, lung and lymph node. The duration between the two passages was around 35d. The biological characteristics of this LCI-D35 model remained unchanged up to 59 passages. Karyotype analysis revealed diploid cells[47,48].

Biological characteristics of LCI-D20 and LCI-D35 models

Remarkable difference was found between the LCI-D20 and LCI-D35 tumors: ① High expression of some of the invasiveness related genes, such as c-*fos*, c-*jun*, N-ras, H-ras and P53 mutation was found in LCI-D20 tumor but not in LCI-D35 tumor[65]. ② Using comparative genomic hybridization (CGH) technique, we have demonstrated that chromosome 8p deletion was associated with HCC metastasis[66]. When comparison was made between LCI-D20 and LCI-D35 using CGH, it was shown that 8p deletion remains one of the important alterations[67]. ③ Corneal micropocket model has been employed to investigate angiogensis, it was found that the vascular response induced by high metastatic model LCI-D20 was stronger than in low metastatic model LCI-D35[68]. ④ N-Acetylglucosaminyltra nsferase V (GnT V) activity was much higher in LCI-D20 model when compared with LCI-D35, indicating the close relation between GnT V activity and HCC metastas is[69].

It was observed that both serum and tissue PAI-1 content increased gradually following tumor progression in LCI-D20 model, PAI-1 correlated with tumor size and AFP level and provided potential clinical impact as prognostic marker[70]. Phasic expression of tissue intercellular adhesion molecule-1 (ICAM-1) in this model was also observed, ICAM-1 increased

with the progressio n of LCI-D20 tumor, and markedly increased when metastasis occurrs[71].

Establishment of human HCC cell line with high metastatic potential

Metastatic behavior was not reported in human HCC cell lines that commonly use d in China when inoculated into nude mice, such as BEL-7402[6], PLC/PRF/5[7] and SMMC-7721[8].

In order to conduct in vitro study for metastatic recurrence, a human HCC cell line with metastatic potential (MHCC97) was established from a subcutaneous xenograft of the above LCI-D20 tumor using alternating *in vivo* and *in vitro* cultivation. The MHCC97 cells appear as polygonal epithelial cells. The doubling time was 31 h. Karyotype analysis revealed that the number of chromosome was 59-65, the median range of chromosome number was 60-61, which accounted for 73%. Aberrant chromosomes i(1)(q) and der(4) (pter→q35::?) were its chromo some markers, which might be related to carcinogenesis and progression of HCC. Secretion of AFP was demonstrated in MHCC97 cells. HBsAg and HBxAg were detected using PCR. Upon intrahepatic inoculation in nude mice, the xenograft grew and metastasized to the lungs, with metastatic rate up to 100% at 5th week. The cancer cells of lung foci were AFP positive. The latency period of tumor nodule formation after inoculation was 15d-20d. Invasion to the liver, diaphragm an d abdominal wall was observed after intrahepatic inoculation. The biological cha racteristics remained stable after in vitro passages for 2 years. The MHCC97 cell line was preserved in liquid nitrogen at the 120 passages. RT-PCR products for integrin α5 and β1, uPA-R, VEGF and nm23-H1 mRNA from MHCC97 cell line were positive. Immunostaining showed strongly positive for c-Met, uPA-R in both of xenografts and lung me tastatic lesions. However, integrin α5 and β1 were positive only in xenograft but not in lung metastatic lesions. E-cadherin was not expressed either in xenograft or in the metastatic lesions. Mutation of p53 at codon 249 was also observed in MHCC9 7 cells, but not in LCI-D35 cells (low metastatic model), indicating p53 mutati on might relate to HCC metastasis[36,37,72].

Experimental intervention for metastasis using LCI-D20 model

The LCI-D20 model, a patient-like metastatic human HCC model, is a useful model for the studies of experimental intervention. Two kinds of approach have been used: ① Studies on a well established model to observe the tumor inhibition rate and lung metastatic rate. ② Studies on intervention after curative resection of the established liver xenograft, which mimicked to that of curativ e resection of HCC in patient, to observe the recurrent rate and lung metastatic rate.

Several anti-angiogenic agents have been studied in nude mice bearing LCI-D20 tumor. ① Suramin was shown to inhibit tumor growth and metastasis of human HCC in nude mice, when compared with control, the tumor volume (cm^3) was 7.5 *vs* 10.8, and lung metastasis found in 62.5% and 100% respectively[73]. ② Inhibitory effect of the angiogenesis inhibitor TNP-470 on tumor growth (tumor weight being 0.97 g *vs* 2.04 g) and lung metastasis (being 8% *vs* 50%) was also demonstrated in LCI-D20 nude mice model[74]. ③ Gene transfer of dominant-negative flk-1 mutant has been studied and showed inhibition of angiogenesis, growth and metastases in LCI-D20 model, the tumor was 10 folds smaller than the control, lung metasta sis being 20% *vs* 100%, and vessels were hardly visible as compared with rich neovascularization in the control[75]. ④ Endostatin, a potent anti-angiogenic agent, has been shown to inhibit tumor growth in LCI-D20 model, and the combination with cisplatin enhanced the response, the mean tumor volume (mm^3) was 8376 for control, 3777 for endostatin, 1629 for cisplatin, and 463 for endostatin + cisplatin[76]. ⑤ Cytostatic calcium influx inhibitor carboxyamido- triazole (CAI) was also proved of effect for anti-angiogenesis[77]. ⑥ Recently, interferon (1b was proved effective to prevent the recu rrence in the liver and inhibit lung metastasis after resection of liver tumor in a dose-dependent manner. The mechanism was mediated by anti-angiogenesis[78].

The following approaches have also been tried for the intervention of metastas is in the LCI-D20 model. ① Antisense H-ras: When antisense H-ras oligodeoxyn ucleotides (ODNs) was used, specific inhibition of H-ras expression observed. Antisense H-ras ODNs induced apoptotic cell death, inhibited the growth rate of LCI-D20 cells in vitro and *in vivo*, and alter *in vivo* tumorigenesity (being 50% *vs* 100%) and metastatic potential (lung metastases being 0% *vs* 100%)[79]. ② Heparin is structurally and functionally similar to that of heparan sulfate, metabolite of suramin, therefore the role of heparin on metastasis was studied in LCI-D20 model. It has been demonstrated that hepa rin inhibited tumor growth (tumor size being 1.50±0.61 cm *vs* 2.98±0.50 cm in the control), inhibited lung metastas is (being 20% *vs* 60%) and prolonged survival (50 days survival being 60% *vs* 0)[80]. ③ Metalloproteinase inhibitor-BB94: Effect of BB-94 on tumor growth and metastasis in the LCI-D20 model was also observed, the tumor weight being 2.27g *vs* 3.13 g, lung metastasis being 44% *vs* 100%, and survival on day 45 being 100% and 56%[81]. ④ PD-ECGF that expressed in HCC, and particularly in tumor thrombus, is able to convert more prodrug (such as Furtulon and Xeloda-Capecitabine) into 5-Fu. Using capecitabine, prevention of lung metastasis as well as inhibition of tumor growth was observed in nude mice model of LCI-D20, thus will of potential as "targeting chemotherapy"[82]. ⑤ ICAM-1 is closely related to HCC metastasis. It was demonstrated that β peptide (a polypeptide designed by authors' institution, which is able to block ICAM-1) can inhibit recurrence in the liver and lung metastasis in LCI-D20 model after resection of tumor at early stage and advanced stage. However, the metastatic recurrent rate in the liver after resection in the early stage was lower than that after resection in the advanced stage, being 0%(0/5) versus 60%(3/5), and 100%(5/5) in the control[83]. ⑥ Retinoid acid was not effective in controlling tumor growth and metastasis in this particular LCI-D20 model.

The conclusion is that the establishment of metastatic human HCC model in nude mice and human HCC cell line with metastatic potential will provide an importan t model for the *in vivo* and *in vitro* study of mechanism of HCC metastasi s, angiogenesis as well as intervention of HCC recurrence after resection.

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REFERENCES

1 Pisani P, Parkin M, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. *Int J Cancer*, 1999;83:18-29
2 Tang ZY, Yu YQ, Zhou XD. An important approach to prolonging survival fur ther after radical resection of AFP positive hepatocellular carcinoma. *J Exp Clin Cancer Res*, 1984;3:359-366

3 Zhou XD, Tang ZY, Yu YQ, Yang BH, Lu JZ, Lin ZY, Ma ZC, Zhang BH. Recurrence after resection of alpha fetoprotein positive hepatocellular carcinoma. J Cancer Res Clin Oncol, 1994;120:369-373 4 Chen RM. The establishment of a strain of human liver cell carcinoma in vit ro and some preliminary observations. *Chin Med J*, 1963; 82:228-237

5 Tang ZY, Ma ZC, Xue Q, Lin ZY, Ying YY, Xu YD, Sun LS, Yuan XH. Transp lantation of human hepatocellular carcinoma in nude mice. Establishment of model and its serological and morphological features. *Shanghai Yike Daxxue Xue bao*, 1982;9:21-26

6 Shen DW, Chen RM. Human hepatocellular carcinoma cells cultivated in vitro. In: Tang ZY (ed) Subclinical hepatocellular carcinoma. *Berlin: Springer*, 1985:336-349

7 Alexander J, Bey E, Macnab G. Establishment of human hepatoma cell line whic h produces hepatitis B surface antigen (HbsAg).In: Neiburgs HE (ed) Prevention and detection of cancer. New York: Marcel Dekker, 1977:321-331

8 Dong RC. Establishment of a human hepatocarcinoma cell line SMMC-7721 and in itial observations on its biologic characteristics. In: Tang ZY, Wu MC, Xia SS (eds) Primary liver cancer. *Berlin: Springer*, 1989:145-153

9 Lee Y, Bong Y, Poo H, Lee Y, Park J, Oh S, Sohn M, Lee S, Park U, Kim N, Hyu n S. Establishment and characterization of cell lines constitutively expressing hepatitis B virus X-protein. *Gene*, 1998;207: 111-118

10 Le Jossic C, Glaise D, Corcos L, Diot C, Dezier JF, Fautrel A, Guguen-Guillouzo C. Trans-acting factors, detoxication enzymes and hepatitis B virus replication in a novel set of human hepatoma cell lines. *Eur J Biochem*, 1996;238:400-409

11 Bagnarelli P, Devescovi G, Manzin A, Bearzi I, Bonazzi P, Carloni G, Clementi M. Growth-factor independence of a new differentiated hepatitis B vir us DNA-negative human hepatoma cell line. *Hepatology*, 1990;11:1024-1032

12 Saito H, Morizane T, Watanabe T, Kagawa T, Iwabuchi MN, Kumagai N, Ina gaki Y, Tsuchimoto K, Tsuchiya M. Establishment of a human cell line (HCC-T) from a patient with hepatoma bearing no evidence of hepatitis B or A virus infection. *Cancer*, 1989;64:1054-1060

13 Das PK, Nayak NC, Tsiquaye KN, Zuckerman AJ. Establishment of a human hepatocellular carcinoma cell line releasing hepatitis B virus surface antigen. *Br J Exp Pathol*, 1980;61:648-654

14 Lee JH, Ku JL, Park YJ, Lee KU, Kim WH, Park JG. Establishment and cha racterization of four human hepatocellular carcinoma cell lines containing hepat itis B virus DNA. *World J Gastroenterol*, 1999;5: 289-295

15 Aoki Y, Aizaki H, Shimoike T, Tani H, Ishii K, Saito I, Matsuura Y, Mi yamura T. A human liver cell line exhibits efficient translation of HCV RNAs pro duced by a recombinant adenovirus expressing T7 RNA polymerase. *Virology*, 1998;250:140-150

16 Homma S, Nagamori S, Fujise K, Hasumura S, Sujino H, Matsuura T, Shimizu K, Niiya M, Kameda H. Establishment and characterization of a human hepatocel lular carcinoma cell line JHH-7 producing alpha-fetoprotein and carcino-embryonic antigen-changes in secretion of AFP and CEA from JHH-7 cells after h eat treatment. *Hum Cell*, 1990;3:152-157

17 Dippold WG, Dienes HP, Knuth A, Sachsse W, Prellwitz W, Bitter-Suermann D, Meyer zum Buschenfelde KH. Hepatocellular carcinoma after thorotrast exposure: establishment of a new cell line (Mz-Hep-1). *Hepatology*, 1985;5:1112-1119

18 Nohara T. Establishment of an AFP-producing serum-independent culture cell line of human liver cancer and changes in protein synthesis associated with the absence of serum (First Part). *Gastroenterol Jpn*, 1987;22:722-729

19 Sing GK, Pace R, Prior S, Scott JS, Shield P, Martin N, Searle J, Batt ersby C, Powell LW, Cooksley WG. Establishment of a cell line from a hepatocellular carcinoma from a patient with hemochromatosis. *Hepatology*, 1994;20:74-81

20 Utsunomiya I, Iemura A, Yano H, Akiba J, Kojiro M. Establishment and characterization of a new human hepatocellular carcinoma cell line, HAK-3, and its response to growth factors. *Int J Oncol*, 1999;15: 669-675

21 Yano H, Iemura A, Fukuda K, Mizoguchi A, Haramaki M, Kojiro M. Establi shment of two distinct human hepatocellular carcinoma cell lines from a single nodule showing clonal dedifferentiation of cancer cells. *Hepatology*, 1993;18:320-327

22 Tokiwa T, Kusaka Y, Endo A, Namba M. Primary culture of liver cancer tissues with or without transcatheter arterial embolization and establishment of a cell strain. *Cell Biol Int Rep*, 1992;16:259-267

23 Hasumura S, Sujino H, Nagamori S, Kameda H. Establishment and characterization of a human hepatocellular carcinoma cell line JHH-4. *Hum Cell*, 1988;1:98-100

24 Sasaki F, Kameda H, Hata Y, Uchino J. Establishment of the human hepatocellular carcinoma cell line and its characteristics. *Hum Cell*, 1988;1:89-91

25 Nohara T. Establishment of an AFP-producing serum-independent culture cell line of human liver cancer and changes in protein synthesis associated with the absence of serum (second part). *Gastroenterol Jpn*, 1988;23:23-28

26 Murakami T, Yano H, Maruiwa M, Sugihara S, Kojiro M. Establishment and characterization of a human combined hepatocholangiocarcinoma cell line and its heterologous transplantation in nude mice. *Hepatology*, 1987;7:551-556

27 He L, Isselbacher KJ, Wands JR, Goodman HM, Shih C, Quaroni A. Establi shment and characterization of a new human hepatocellular carcinoma cell line. *In Vitro*, 1984;20:493-504

28 Watanabe T, Morizane T, Tsuchimoto K, Inagaki Y, Munakata Y, Nakamura T, Kumagai N, Tsuchiya M. Establishment of a cell line (HCC-M) from a human he patocellular carcinoma. *Int J Cancer*, 1983; 32:141-146

29 Chang C, Lin Y, O-Lee TW, Chou CK, Lee TS, Liu TJ, P'eng FK, Chen T Y, Hu CP. Induction of plasma protein secretion in a newly established human hep atoma cell line. Mol Cell Biol, 1983;3:1133-1137

30 Wang JB. Establishment and some characteristics of a hepatoma cell line (QGY-7703). *Zhonghua Zhongliu Zazhi*, 1981;3:241-244

31 Novicki DL, Jirtle RL, Michalopoulos G. Establishment of two rat hepat oma cell strains produced by a carcinogen initiation, phenobarbital promotion pr otocol. *In Vitro*, 1983;19:191-202

32 Unoura M, Kobayashi K, Fukuoka K, Matsushita F, Morimoto H, Oshima T, Kaneko S, Hattori N, Murakami S, Yoshikawa H. Establishment of a cell line from a woodchuck hepatocellular carcinoma. *Hepatology*, 1985;5:1106-1111

33 Ohnishi S, Aoyama H, Shiga J, Itai Y, Moriyama T, Ishikawa T, Sasaki N, Yamamoto K, Koshimizu K, Kaneko S. Establishment of a new cell line from a woodchuck hepatocellular carcinoma. *Hepatology*, 1988;8:104-107

34 Abe K, Kurata T, Yamada K, Okumura H, Shikata T. Establishment and cha racterization of a woodchuck hepatocellular carcinoma cell line (WH44KA). *Jpn J Cancer Res*, 1988;79:342-349

35 Kawaguchi T, Nomura K, Hirayama Y, Kitagawa T. Establishment and chara cterization of a chicken hepatocellular carcinoma cell line, LMH. *Cancer Res*, 1987;47:4460-4464

36 Tian J, Tang ZY, Ye SL, Liu YK, Lin ZY. Establishment of a human hepat ocellular carcinoma (HCC) cell line with high metastatic potential (MHCC97) and its biological characteristics. *Zhonghua Zhongliu Zazhi*, 1998;20:405-407

37 Tian J, Tang ZY, Ye SL, Liu YK, Lin ZY, Chen J, Xue Q. New human hepat ocellular carcinoma (HCC) cell line with highly metastatic potential (MHCC97) an d its expression of the factors associated with metastasis. *Br J Cancer*, 1999;81:814-821

38 Seki S, Kitada T, Kawada N, Sakaguchi H, Kadoya H, Nakatani K, Satake K, Kuroki T. Establishment and characteristics of human hepatocellular carcinoma cells with metastasis to lymph nodes. *Hepatogastroenterology*, 1999;46:2812-2817

39 Ogawa K, Nakanishi H, Takeshita F, Futakuchi M, Asamoto M, Imaida K, Tatematsu M, Shirai T. Establishment of rat hepatocellular carcinoma cell lines with differing metastatic potential in nude mice. *Int J Cancer*, 2001;91:797-802

40 Reichner JS, Mulligan JA, Palla ME, Hixson DC, Albina JE, Bland KI. Interleukin-6 production by rat hepatocellular carcinoma cells is associated wi th metastatic potential but not with tumorigenicity. *Arch Surg*, 1996;131:360-365

41 Shimosato Y, Kameya T, Nagai K, Hirohashi S, Koide T, Hayashi H, Nomura T. Transplantation of human tumors in nude mice.*J Natl Cancer Inst*, 1976;56:1251-1260

42 Hirohashi S, Shimosato Y, Kameya T, Koide T, Mukojima T, Taguchi Y, Kageyama K. Production of alpha-fetoprotein and normal serum proteins by xenot ransplantation human hepatomas in relation to their growth and morphology. *Cancer Res*, 1979;39:1819-1828

43 Liu X, Hu J, Cui H, Yang X, Li S. Establishment and biological charact eristics of the nude mice xenograft model from human hepatocellular carcinoma. *Zhongguo Yixue Kexueyuan Xuebao*, 1995;17:98-103

44 Leveille-Webster CR, Arias IA. Establishment and serial quantificati on of intrahepatic xenografts of human hepatocellular carcinoma in severe combined immunodeficiency mice, and development of

therapeutic strategies to overcome multidrug resistance. *Clin Cancer Res*, 1996;2:695-706
45 Qian ZC, Cheng YZ, Xu GY, Tan HN, Deng XJ. Establishment and experimental study of a transplantable hepatocellular carcinoma model in 615-strain mice (H 615). *Zhonghua Zhongliu Zazhi*, 1987;9: 187-189
46 Aruga A, Takasaki K, Hanyu F. Establishment and characterization of liver metastatic model of human hepatoma in nude mice. *Int Hepatol Commun*, 1993;1:138-145
47 Sun FX, Tang ZY, Liu KD, Ye SL, Xue Q. Growth pattern and metastatic behavior of orthotopically metastatoc modelo human hepatocellular carcinoma in nude mice. *Zhonghua Yixue Zazhi*, 1995;75: 673-675
48 Sun FX, Tang ZY, Liu KD, Ye SL, Xue Q, Gao DM, Ma ZC. Establishment of a metastatic model of human hepatocellular carcinoma in nude mice via orthotopi c implantation of histologically intact tissues. *Int J Cancer*, 1996;66:239-243
49 Peng BG, Ding J, Lv MD, Chen LJ, Huang JF, Lu LJ. A model of transplan tation of human hepatocellular carcinoma in mouse: its establishment and charact erization. *Zhongshan Yike Daxue Xuebao*, 1996;17(Suppl):7-10
50 Tao WZ, Zheng WQ, Jun ZJ. The tumor invasion and metastasis in the transplantation of transplanted human hepatocellular carcinoma into nude mice abdominal cavity and orthotopic hepatic tissue. *Dier Junyi Daxue Xuebao*, 1998;19:54-56
51 Genda T, Sakamoto M, Ichida T, Asakura H, Kojiro M, Narumiya S, Hiroha shi S. Cell motility mediated by rho and Rho-associated protein kinase plays a critical role in intrahepatic metastasis of human hepatocellular carcinoma. *Hepatology*, 1999;30:1027-1036
52 Zheng JM, Tao WZ, Zheng WQ, Bao JZ, Wang YL, Zhu MH. Establishment of the orthotopic transplantation tumor model from the subcutaneous model of human hepatocellular carcinoma in nude mice. *Dier Junyi Daxue Xuebao*, 2000;21:456-459
53 Shi CH, Wang XW, Wang WY, Shi XY, Zhu DS, Li LJ. Experimental study on a human hepatocellular carcinoma model using orthotopic transplantation. *Zhongguo Shiyan Dongwu Xuebao*, 2001;9:57-61
54 Masui T, Nakanishi H, Inada K, Imai T, Mizoguchi Y, Yada H, Futakuchi M, Shirai T, Tatematsu M. Highly metastatic hepatocellular carcinomas induced in male F344 rats treated with N-nitrosomorpholine in combination with other hepatocarcinogens show a high incidence of P53 gene mutations along with alt ered mRNA expression of tumor-related genes. *Cancer Lett*, 1997;112:33-45
55 Li HF, Ling MY, Xie Y, Xie H. Establishment of a lymph node metastatic model of mouse hepatocellular carcinoma Hca-F cells in C3H / Hej mice. *Oncol Res*, 1998;10:569-573
56 Wang XM, Tang ZY, Xue Q, Hong XY, Bao WH, Zou HQ. Transforming growth factor a induces proliferation and expression of epidermal growth factor receptor in hepatocellular carcinoma cells. *J Exp Clin Cancer Res*, 1995;14:179-184
57 Qin LX, Tang ZY, Liu KD, Ye SL, Zhou G. p53 mutations may be related to tumor invasiveness of human hepatocellular carcinoma in China. *Oncol Rep*, 1995;2:1175-1179
58 Tang ZY, Qin LX, Wang XM, Zhou G, Liao Y, Weng Y, Jiang XP, Lin ZY, Li u KD, Ye SL. Alterations of oncogenes, tumor suppressor genes and growth factors in hepatocellular carcinoma: with relation to tumor size and invasiveness. *Chin Med J*, 1998;111:313-318
59 Tang ZY, Zhou XD, Lin ZY, Yang BH, Ma ZC, Ye SL, Wu ZQ, Fan J, Liu YK , Liu KD, Qin LX, Tian J, Sun HC, He B, Xia JL, Qiu SJ, Zhou J. Surgical treatme nt of hepatocellular carcinoma and related basic research with special reference to recurrence and metastasis. *Chin Med J*, 1999;112:887-891
60 Tang ZY. Surgery of hepatocellular carcinoma with special reference to studies on metastasis and recurrence. *Gastroenterol Today*, 2000;4: 191-195
61 Schackert G, Fidler IJ. Development of in vivo models for studies of brain metastasis. *Int J Cancer*, 1988;41:589-594
62 Fu X, Guadagni F, Hoffman RM. A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically intact patient specimens. *Proc Natl Acad Sci USA*, 1992;89:5645-5649
63 Wang X, Fu X, Hoffman RM. A patient-like metastasizing model of human lung adenocarcinoma constructed via thoratomy in nude mice. *Anticancer Res*, 1992;12:1399-1401
64 Fu X, Hoffman RM. Human ovarian carcinoma metastatic models constructe d in nude mice by orthotopic transplantation of histologically intact patient specimens. *Anticancer Res*, 1993;13:283-286
65 He B, Liu YK, Tang ZY, Xue Q. Gene mutation and expression in metastat ic model of human hepatocellular carcinoma in nude mice established by orthotopic implantation. *Zhonghua Ganzangbing Zazhi*, 1997;5:245-245
66 Qin LX, Tang ZY, Sham JST, Ma ZC, Ye SL, Zhou XD, Wu ZQ, Trent JM, Guan XY. The association of chromosome 8p deletion and tumor metastasis in human he patocellular carcinoma. *Cancer Res*, 1999; 59: 5662-5665
67 Qin LX, Tang ZY, Ye SL, Liu YK, Ma ZC, Zhou XD, Wu ZQ, Lin ZY, Sun FX, Tian J, Guan XY, Pack SD, Zhuang ZP. Chromosome 8p deletion is associate with metastasis of human hepatocellular carcinoma when high and low metastatic models are compared. *J Cancer Res Clin Oncol*, 2001;127:482-488
68 Sun HC, Li XM, Xue Q, Chen J, Gao DM, Tang ZY. Study of angiogenesis induced by metastatic and non-metastatic liver cancer by corneal micropocket model in nude mice. *World J Gastroenterol*, 1999; 5:116-118
69 Shao DM, Wang QH, Chen C, Shen ZH, Yao M, Zhou XD, Tang ZY, Gu JX. N-Actylglucosaminyltransferase V activity in metastatic models of human hepatoce llular carcinoma in nude mice. *J Exp Clin Cancer Res*, 1999;18:331-335
70 Zheng Q, Tang ZY, Xue Q. Changes of PAI-1 and biological behaviour of human hepatocellular carcinoma in metastatic model of nude mice. *Zhonghua Wai ke Zazhi*, 1998;36:687-689
71 Sun JJ, Zhou XD, Zhou G, Liu YK. Expression of intercellular adhesive molecule-1 in liver cancer tissues andliver cancer metastasis. *World J Gastroenterol*, 1998;4:202-205
72 Tian J, Tang ZY, Xue Q, Ye SL, Liu YK, Lin ZY, Chen J. Expression of the metastasis-associated factors of a new human hepatocellular carcinoma cell line with highly metastatic potential. *Zhonghua Yixue Zazhi*, 1999;79:470-472
73 Li XM, Tang ZY, Xue Q, Liu YK, Ye SL, Zhang XR, Zhao ZH. Suramin inhib its growth and metastasis of human hepatocellular carcinoma in nude mice. *Zhonghua Shiyan Waike Zazhi*, 1998;15:3-4
74 Xia JL, Yang BH, Tang ZY, Sun FX, Xue Q, Gao DM. Inhibitory effect of the angiogenesis inhibitor TNP-470 on tumor growth and metastasis in nude mice bearing human hepatocellular carcinoma. *J Cancer Res Clin Oncol*, 1997;123:383-387
75 Li XM, Tang ZY, Chen FG. Gene transfer of dominant-negative flk-1 mut ant inhibits angiogenesis, growth and metastases of human hepatocellular carcino ma in nude mice. *Zhongguo Zhongliu Shengwu Zhiliao Zazhi*, 1998;5:160-162
76 Sun HC, Zhang M, Tang ZY, Li XM, Wang L, Gao DM, Xue Q, Chen J. Inhibi tion of growth of LCI D20 hepatocellular carcinoma by Endostatin. *Zhonghua Shiyan Waike Zazhi*, 1999;16:491-492
77 Li XM, Sun HC, Tang ZY, Zhou J. Cytostatic calcium influx inhibitor carboxyamido-triazole inhibited angiogenesis induced by human hepatocellular carcinoma. *Zhonghua Shiyan Waike Zazhi*, 1999;16:489-490
78 Wang L, Tang ZY, Qin LX, Wu XF, Sun HC, Xue Q, Ye SL. High-dose and long-term therapy with interferon-alfa inhibits tumor growth and recurrence in nude mice bearing human hepatocellular carcinoma xenografts with high metastatic potential. *Hepatology*, 2000;32: 43-48
79 Liao Y, Tang ZY, Ye SL, Liu KD, Sun FX, Huang Z. Modulation of apoptosis, tumorigenesity and metastatic potential with antisense H-ras oligodeoxynuc leotides in a high metastatic tumor model of hepatoma: LCI-D20. *Hepatogastroe nterology*, 2000; 47:365-370
80 Zheng Q, Tang ZY, Xue Q. Role of heparin on tumor growth and metastasis in model of human hepatocellular carcinoma in nude mice. *Zhonghua Ganzangbing Zazhi*, 1998;6:151-152
81 Bu W, Tang ZY, Sun FX, Ye SL, Liu KD, Xue Q, Chen J, Gao DM. Effects o f matrix metalloproteinase inhibitor BB-94 on liver cancer growth and metastasis in a patient-like orthotopic model LCI-D20. *Hepatogastroenterology*, 1998;45:1056-1061
82 Zhou J, Tang ZY, Fan J, Wu ZQ, Ji Y, Wang L, Bao WH, Qiu SJ. Study on inhibition of the growth and metastasis of LCI-D20 hepatocellular carcinoma by capecitabine. *Zhonghua Shiyan Waike Zazhi*, 2000;17: 403-405
83 Sun JJ, Zhou XD, Liu YK, Tang ZY, Sun RX, Zhao Y, Uemura T. Inhibitory effects of synthetic â peptide on invasion and metastasis of liver cancer. *J Cancer Res Clin Oncol*, 2000;126:595-600

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