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, antisenesce approach, metalloproteinase 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


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[11]. Surgical resection has been accepted the best treatment for hepatocellular 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 model 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 etiologic factors of HCC, such as hepatitis B virus (HBV)[9-11], hepatitis C virus (HCV)[12], hemochromatosis[13], thiorotri[14], for the study of alpha fetoprotein (AFP)[15,16], and for other studies[17-20]. 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[21], in woodchuck[22,23], and in chicken[24] 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[25,26] at the authors’ institution reported two paper in 1998 and 1999, only one paper has yet been reported. Seki et al[27] (1999) established a human hepatocellular carcinoma cells with metastasis to lymph nodes.
Again, very few papers have been reported concerning animal HCC with metastatic behavior. Ogawa et al. (2001) reported the establishment of rat HCC cell lines with differing metastatic potential in nude mice. Reifnch et al. (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. (1976) reported the establishment of a series of human tumors in nude mice including HCC. The same group has used the human HCC nude mouse 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 (38). In 1995, Liu et al. (1995) established a nude mouse xenograft model from human HCC. In 1996, Leveille-Webster et al. (1996) 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. (1987) established a transplanted 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 metastatic behavior. In 1993, Aruga et al. (1993) 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. (1995) (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. (1996) established a human HCC model in nude mice using orthotopic transplantation, and malignant behavior (invasion of abdominal cavity) was observed. Tao et al. (1998) established a human HCC nude mouse model using SMMC-LTNM cell transplanted into abdominal cavity and liver, the lung metastatic rate was 59%. Genda et al. (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. (2000) established 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. (2001) established a human HCC model in nude mice with high metastatic rate in lymph node.

For animal HCC, Masui et al. (1997) reported a highly metastatic HCC in male F344 rats induced by chemical carcinogens. Li et al. (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 (35-40). Because either metastatic human HCC model in nude mice or human HCC cell line with metastatic potential was not available 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) (45,46) and human HCC cell line with high metastatic potential was reported in 1998 (in Chinese) and 1999 (in English) (38,39). 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 brain metastasis has been reported in Fidler’s group (61). In early 1990s, “metastatic models constructed in nude mice by orthotopic transplantation of histologically intact patient specimens” has been used in Hoffman’s group, and several such models including lung cancer, pancreatic cancer, ovarian cancer, etc have been reported (62,63). 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 focus 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 characteristics 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 (65-68).

**Biological characteristics of LCI-D20 and LCI-D35 models**

Remarkable difference was found between the LCI-D20 and LCI-D35 tumors: 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). 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). The Corneal micropocket model has been employed to investigate angiogenesis, it was found that the vascular response induced by high metastatic model LCI-D20 was stronger than in low metastatic model LCI-D35 (68). N-Acetylglucosaminyltransferase 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 metastasis 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 between GnT V activity and HCC metastasis (69).
Studies on a well

Heparin is inhibitory effect of the angiogenesis

Recently, interferon (1b was proved to expressed in HCC, and ICAM-1 is closely related to HCC

Gene transfer of dominant-negative flk-1 mutant has been studied and demonstrated in LCI-D20 nude mice model [74].

The following approaches have also been tried for the intervention of metastasis is in the LCI-D20 model. 1. Antisense H-ras: When antisense H-ras oligodeoxynucleotides (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 tumorigenicity (being 50% vs 100%) and metastatic potential (lung metastasis 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 heparin inhibited tumor growth (tumor size being 1.50±0.61 cm vs 2.98±0.50 cm in the control), inhibited lung metastasis is (being 20% vs 60%) and prolonged survival (50 days survival being 60% vs 0) [80]. 3. Metalloproteinase inhibitor-BB-94: Effect of BB-94 on tumor growth and metastasis in the LCI-D20 model was also observed, the tumor weight being 2.27 g 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 important model for the in vivo and in vitro study of mechanism of HCC metastasis, angiogenesis as well as intervention of HCC recurrence after resection.

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