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Effect of CD133-positive Stem Cells in Repeated Recurrence of Hepatocellular Carcinoma after Liver Transplantation : A Case Report

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Abstract

Liver transplantation (LT) is currently one of the best available strategies for treating multiple hepatocellular carcinoma (HCC) and decompensated liver cirrhosis. However, patients often undergo HCC recurrence after LT, with most HCC recurrences detected at 1-2 years. CD133 was the first identified member of the prominin family of pentaspan membrane proteins and is a marker of hepatic stem cells. Here, we report a unique case of seven repeated recurrences of HCC in the lungs after LT, with all HCC recurrences resected curatively by a thoracoscopic approach. Pathological examination revealed moderately differentiated HCC identical to that in the original histology of the liver tumor. Interestingly, no CD133 immunoreactivity was observed in cancerous lesions of the primary HCC and the 1st to 2nd recurrences, as indicated by immunohistochemistry. However, CD133 was strongly stained in the cancerous lesions from the 3rd to 7th recurrences. The patient survived and had no recurrence after 9 years of the initial living donor LT. In conclusion, we investigated an evocative case of seven repeated recurrences of HCC in the lungs to elucidate the significance of circulating CD133-positive hepatic stem cells. This case illustrates the need for further research to clarify the mutual effect of CD133-positive hepatic stem cells for the development of new therapeutic strategies.

Key words : CD 133・Prominin-1・Stem cell・Hepatocellular carcinoma・Repeated recurrence・Liver transplantation

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths in the world and its incidence has increased considerably over the past decade[1]. Among the various therapies for treating HCC and the underlying liver cirrhosis, the ultimate curative therapy is only liver transplantation (LT) when it restricts to multiple HCC or decompensated liver cirrhosis. However, HCC recurs in 10-60% of patients after LT[1)~4)]. Recurrence is generally associated with increased mortality, and the reported median survival after HCC recurrent ranges from 8.7 to 24.5 months after LT[1)~4)]. The majority of the tumor burden in recurrent HCC after LT is typically in extrahepatic legions. Extrahepatic recurrence has been reported in 38.5-53.0% of recurrences, although only 0-23% of recurrences are solely hepatic in origin[3)4)]. The lung is the most

Abbreviations
CT ; computed tomography, HCC ; hepatocellular carcinoma, LDLT ; living donor liver transplantation, LT ; liver transplantation

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common extrahepatic site of recurrence, with involvement in 43–56% of cases followed by bone with an 18–33% involvement rate[1)~4)]. The molecular mechanisms underlying the development and progression of extrahepatic HCC recurrences remain unclear.

Recently, the mechanism of normal stem cells has been applied to cancer cells. Cancer stem cells have the ability to self-renew and differentiate, thereby sustaining tumor growth[5)]. CD133, also known as prominin-1 and AC133, was the first identified member of the prominin family of pentaspan membrane proteins[6)]. In 1997, CD133 was reported as a marker of hematopoietic progenitor cells and was subsequently found to be expressed in epithelial and non-epithelial progenitor cells in murine and human tissues including brain, kidney, prostate, pancreas and skin[7)~11)]. The specific functions and ligands of CD133 have not been elucidated completely. There are no reports that demonstrate the effects of CD133-positive stem cells in HCC recurrence after living donor LT (LDLT). This unique evocative case may help to elucidate the significance of CD133-positive stem cells in persistent recurrences repeated seven times in the lung, which underwent repeated pneumonectomy (Fig. 1).

Case report

The patient was a 56-year-old woman with end-stage liver disease secondary to liver cirrhosis and HCC caused by hepatitis C virus infection. She had multiple intrahepatic HCC, indicating beyond Milan criteria. In addition, high levels of serum alpha-fetoprotein at 3157 ng/mL and serum des-gamma-carboxy prothrombin at 308 mAU/mL were noted. The patient underwent LDLT using a left lobe graft donated by her 22-year-old nephew. Histological analysis of the intrahepatic tumor revealed moderately differentiated HCC and no evidence of vascular invasion or satellite lesions in the liver. Postoperative immunosuppression was induced with basilixiimab and mycophenolate mofetil with cyclosporine without steroids, followed by maintenance with cyclosporine monotherapy. An increase in the HCV-RNA titer of more than 300 kIU/mL was noted at 11 months after LDLT. After confirming pathological recurrent hepatitis C by liver biopsy, the patient underwent antiviral therapy comprising poly ethyl glycol-interferon 2b (1.5 μg/kg/week) and ribavirin (400 mg/day). Unexpectedly, the patient underwent seven recurrences of HCC after LDLT, with surgery performed each time (Fig. 1). First, about 1 year and 5 months after LDLT, routine surveillance by chest computed tomography (CT) revealed three heterogeneously enhanced lesions in segment 3 and 5 of the right lung and segment 4/5 of the left lung. No recurrent HCC was detected in the whole body except the lung using various modalities such as abdominal CT, bone scintigraphy and upper gastrointestinal endoscopy. Thor-

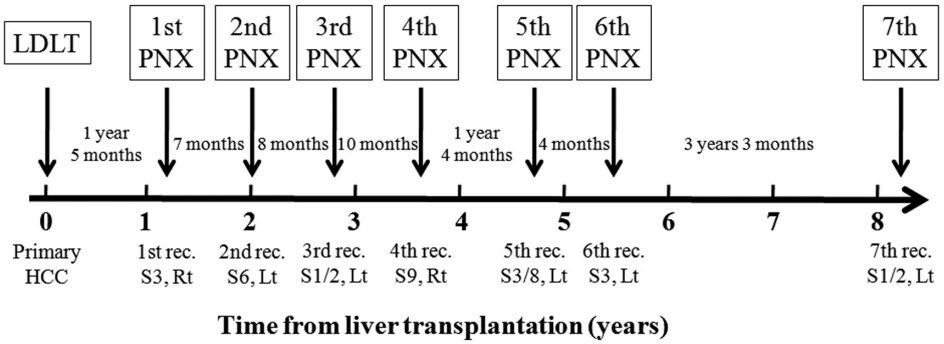

Fig. 1 Timeline of events
Abbreviations : PNX, pneumonectomy ; LDLT, living donor liver transplantation ; Lt, left ; Rec, recurrence ; Rt, right ; S, segment.

acoscopic partial pulmonary resection for this recurrence was performed and pathological examination revealed moderately differentiated HCC identical to that in the original histology of the liver tumor. Subsequent recurrences were as follows : segment 6 of the left lung at 7 months after the initial recurrence, segment 1 and 2 of the left lung at 8 months after the 2nd recurrence, segment 9 of the right lung at 10 months after the 3rd recurrence, segment 3/8 of the left lung at 1 year and 2 months after the 4th recurrence, segment 3 of the left lung at 4 months after the 5th recurrence, and the 7th recurrence in segment 1 and 2 of the left lung at 3 years and 3 months after the 6th recurrence. Thus, all HCC recurrences in the lung were resected curatively by a thoracoscopic approach, and pathological examination revealed moderately differentiated HCC identical to that in the original histology of the liver tumor. Interestingly, no CD133 immunoreactivity was observed in cancerous lesions from the primary HCC and the 1st to 2nd recurrences. However, CD133 was strongly stained in cancerous lesions from the 3rd to 7th recurrences (Fig. 2). The patient survived and had no recurrence at 7 months after the 7th diagnosis of HCC recurrence, indicating about 9 years survival after the initial LDLT.

Discussion

HCC is an aggressive malignancy mainly due to tumor metastasis or recurrence even after undergoing potentially curative treatment such as LT, and the recurrence of HCC after LT is a well-known complication associated with high mortality[1)~4)]. Faber et al. demonstrated that repeated liver resection is a valid and safe curative therapy option for treating recurrent HCC. However, they also argued that, due to impaired liver function, multifocal intrahepatic or extrahepatic recurrence repeated resection is only feasible for a minority of patients[12)]. The mechanism underlying the development and recurrence of HCC associated with stem cells remains unclear. Therefore, elucidation of the mechanism might be useful to improve anti-cancer treatments, such as LT, hepatectomy and chemotherapy, against HCC recurrence. In the present case, the patient underwent seven recurrences of HCC in her lungs after the initial LDLT, and pathological examinations of recurrent tumors showed moderately to poorly differentiated HCC identical to that in the original histology of the liver tumor. In addition, all subsequent recurrences after the 3rd HCC recurrence

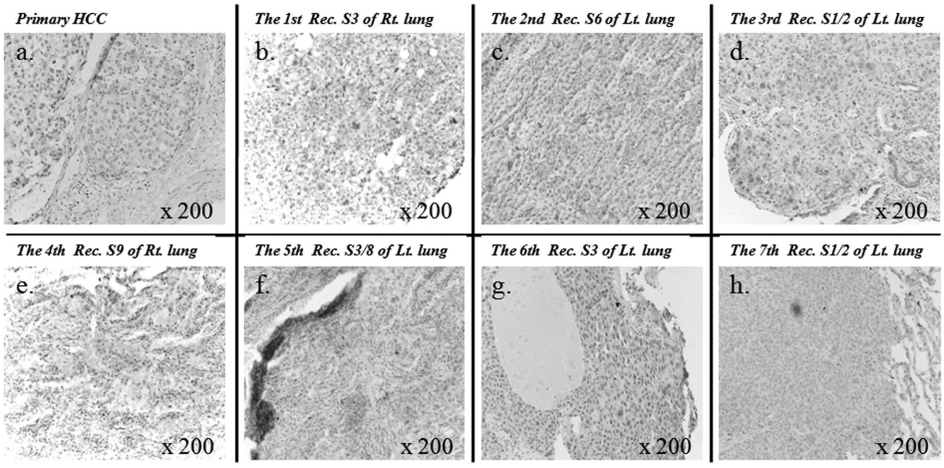

Fig. 2 CD133 expression by immunohistochemistry (x200)
Immunohistochemical staining was performed by using rabbit polyclonal antibody against CD133. No CD133 immunoreactivity was observed in cancerous lesions of (a) the primary HCC and (b) the 1st to (c) 2nd recurrences in lung. Whereas, CD133 was strongly stained in the cancerous lesions from the 3rd to 7th recurrences (d-h).
Abbreviations : HCC, hepatocellular carcinoma ; Lt, left ; Rec, recurrence ; Rt, right.

indicated the association of CD133-positive hepatic stem cells, as determined by immunohistochemistry. To our knowledge, this is the first case report to demonstrate repeated recurrences of HCC involving CD133-positive hepatic stem cells after LT, which is unique and highly informative to clarify the significance of hepatic stem cells in cancer progression.

In general, recurrent tumors arise from residual tumor cells disseminated in the remnant organ[5)6)]. Malignant tumor phenotypes, such as a large size, high levels of serum tumor markers and vascular invasion, are predictive of this type of recurrence, which is usually observed within 2 years following surgery[2)~4)]. However, the molecular mechanism of this recurrence appears to be related to their biological contexts and clinical courses, which remains unknown. Some reports have demonstrated the molecule mechanism of CD133, a cell surface marker of stem cells, in many solid tumors[13)14)]. Piao et al. demonstrated that CD133-positive cells contribute to the radioresistance of HCC by showing CD133-positive cells preferentially activate anti-apoptotic genes such as Bcl-2 in response to radiation treatment[13)]. In addition, Ma et al. demonstrated that CD133-postive HCC cells contribute to chemoresistance via preferential activation of Akt/protein kinase B and Bcl-2 cell survival responses[14)]. These observations suggest that CD133-positive cells have an increased DNA repair capability and faster cell cycle transition than those of CD133-negative cells. Targeting of these specific survival signaling pathways in CD133-positive cancer stem cells may provide a novel therapeutic model for the disease.

Although the mechanisms of liver carcinogenesis associated with activated CD133-positive cancer stem cells are still unclear, some reports have demonstrated that the increased turnover of hepatocytes, and inflammatory cell infiltration observed in chronic hepatitis and cirrhosis may lead to an accumulation of genetic alterations, which ultimately results in the development of HCC[15)]. In addition, CD133-positive cells are more tumorigenic than CD133-negative cells after treatment for tumor recurrence[13)14)16)]. Here, the clinical course of this case showed that HCC recurrence was repeated with CD133-positive HCC after the 3rd pulmonary resection, suggesting that CD133 may contribute to HCC recurrence in the lung. Therefore, we speculate that accumulated DNA damage causing genetic alterations might be associated with increased numbers of CD133-positive hepatic stem cells. Further investigation is required to determine the mechanisms that potentiate these stem cells with the genetic alterations cased by the accumulated DNA damage.

Most tumor cells can proliferate independently and form new tumors[15)]. The cancer stem cell hypothesis suggests that both cancer stem cells and mature cancer cells can migrate into the blood stream. However, cancer stem cells are more capable of surviving in circulation and depositing in distant organs or re-circulating back to the liver remnant[10)~13)]. In contrast, the half-life of mature cancer cells in blood circulation is very short as 1.0 to 2.4 hours[17)] and most cancer cells undergo apoptosis[18)]. Therefore, mature cancer cells are less likely to be responsible for metastasis. On the other hand, the cancer stem cell subset possesses indefinite self-renewal and extensive proliferation, and is capable of forming new tumors. Therefore, cancer stem cells are considered to be the "root" of tumors. Thus, in this case, we speculate that circulating cancer stem cells might be the cells responsible for repeated metastasis in the lung and particularly all subsequent recurrences after the 3rd HCC recurrence. In addition, because HCC is a highly vascular tumor, HCC cells migrate into blood vessels, indicating that CD133-positive hepatic stem cells might be involved in the high incidence of recurrence.

In conclusion, we reported a unique and evocative case of seven repeated recurrences of HCC to elucidate the significance of circulating

CD133-positive hepatic stem cells that were involved in all subsequent recurrences after the 3rd pneumonectomy, as indicated by immunohistochemistry. Thus, CD133-positive hepatic stem cells might be a target for eradication to prevent HCC recurrence after pneumonectomy. Further research is essential to clarify the mutual effect of such CD133-positive hepatic stem cells for the development of new therapeutic strategies.

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（和文抄録）

CD133 発現陽性細胞が出現した肝移植後の肝細胞癌再発の 1 例

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佐伯浩司[1)]，渡邊雅之[3)]，調　憲[1)]，前原喜彦[1)]

肝移植は非代償性肝硬変を合併した肝細胞癌の症例に対する治療戦略として確立している．しかしながら，肝移植後に肝細胞癌の再発をみとめることも希ではなく，そのほとんどが移植後 1〜2 年以内におこるとされている．また近年，CD133 は現在肝の幹細胞のマーカーとして注目されている．われわれは肝移植後に 7 回の肺転移を繰り返し，胸腔鏡下に切除可能であった症例を経験した．原発巣，初回，2 回目の再発まで癌部における CD133 は陰性であったのにも関わらず，3 回目以降の再発部には CD133 の発現は強陽性であった．患者は肝移植後 9 年の現在再発なく生存中である．本症例は肝移植後 7 回の再発を繰り返した．流血中の CD133 陽性幹細胞の肝癌再発における臨床的な意義を示唆する貴重な症例と考え報告する．