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Original Article

Immunohistochemical Detection of Possible Cellular Origin of Hepatic Histiocytic Sarcoma in Mice

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Histiocytic sarcoma (HS) spontaneously arises in the liver in mice ; however, the cellular origins of hepatic HS have not been fully clarified. In this study, we immunohistochemically analyzed 18 cases of hepatic HS from the archives of our previous experiments. In all cases, the tumor cells showed positive reactions for the macrophage-specific markers F4/80 and CD68. The cells were negative for mesenchymal cell and lymphoid cell markers, suggesting that germ cell tumor or lymphoma components do not coexist in the neoplasm. We detected scattered $Ly6C^{+}F4/80^{-}$ macrophage precursors in the extramedullary hematopoietic foci and liver tissue around the HS lesions. We also showed that certain populations of HS cells express the Ly-6C antigen. These findings suggest that Ly-$6C^{+}$ macrophage progenitor cells are a possible cellular origin of murine hepatic HS. Our study identified a novel phenotype of murine HS in two of 18 cases. These cases showed the nodular accumulations of tumor cells with cohesive cytoplasm mimicking the features of epithelioid granuloma. In agreement with the expression of CD204 in epithelioid cells in granulomatous diseases, these HS cells hardly expressed CD204, although the common type HS cells were strongly positive for this antigen. These data suggest that hepatic HS may stem from Ly-$6C^{+}$ macrophage precursors. Furthermore, a subset of hepatic HS cases can possibly differentiate into epithelioid cell-like phenotypes. 〔*J Clin Exp Hematopathol 52(3) : 171-177, 2012*〕

Keywords: epithelioid variant, histiocytic sarcoma, immunohistochemistry, Ly-6C antigen, mouse liver

INTRODUCTION

Histiocytic sarcoma (HS) is an aggressive neoplasm that is characterized by malignant proliferations of histiocytes and is of unknown molecular etiology. Human HS is extremely rare ; however, spontaneous HS is occasionally observed in mice.[1,2] The liver is the most common site of murine HS, although the disease may develop in other organs, such as the uterus, lungs, spleen and lymph nodes.[2] However, the reasons why the incidence of murine HS is so high and why the liver is the predominant site remain unclear.

The cellular origins of hepatic HS have not been fully elucidated. Pluripotent germ cells are considered to be a possible origin of HS because teratocarcinoma cells can differentiate into hematopoietic cells, including macrophages.[3] However, Feldman *et al*. proposed that a common clonal origin of follicular lymphoma may transdifferentiate to HS.[4] Lacroix-Triki *et al*. demonstrated a significant association between murine HS and hepatic extramedullary hematopoiesis.[2] Barker *et al*. indicated that a high incidence and early onset of HS are observed in Hertwig's anemia mice with liver myelopoiesis.[5] These findings suggest that hepatic HS may originate from hematopoietic cells, including macrophage precursors. In this report, we ascertained whether a possible origin of hepatic HS is a macrophage precursor or whether pluripotent germ cells differentiate into various mesenchymal cells. In the present study, we investigated 18 cases of murine primary hepatic HS from the archives of a previous study[6] using immunohistochemical analyses of antibodies against the Ly-6C antigen, a specific marker of macrophage precursors and immature monocytes,[7,8] lymphoid cell markers and various mesenchymal cell markers.

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MATERIALS AND METHODS

Animals

HS occurred in 177 of 3,984 specific pathogen-free *B6C3F1* mice that were exposed to very low doses of gamma rays.[6] However, since autopsies were performed after waiting for natural death, postmortem decomposition was severe in

Table 1. A list of the primary antibodies

Antibody	Recognized antigen	Positive cells	Source
F4/80	a 160 kD cell surface glycoprotein : a member of the EGF-TM7 family	wide range of mature tissue macrophages	Serotec, Oxford, UK
2F8	CD204 : class A scavenger receptor (SR-A) type I and II	tissue macrophages, hepatic sinusoidal endothelial cells	Serotec, Oxford, UK
FA-11	macrosialin (CD68) : a heavily glycosylated transmembrane protein of 87-115kD	monocytes, tissue macrophages, Langerhans cells	Serotec, Oxford, UK
ER-MP20	Ly-6C : a 14kD differentiation antigen	macrophage/dendritic cell precursors, endothelial cells, subpopulations of B- and T-lymphocytes	Serotec, Oxford, UK
B220	CD45R antigen	B lymphocytes, subsets of NK cells	BD Pharmingen, San Diego, CA
anti-CD5	mouse CD5 (Ly-1) : a cell surface glycoprotein	T lymphocytes	BD Pharmingen, San Diego, CA
anti-desmin	desmin : a intermediate filament	smooth muscle cells	Dako, Glostrup, Denmark
anti-α-SMA*	α-isoform of smooth muscle actin	smooth muscle cells, myoepithelial cells	Dako, Glostrup, Denmark
anti-factor VIII	factor VIII-related antigen	endothelial cells	Dako, Glostrup, Denmark
anti-PCNA	a nuclear protein vital for cellular DNA synthesis	proliferating cells	Dako, Glostrup, Denmark

*SMA, smooth muscle actin ; PCNA, proliferating cell nuclear antigen

many cases. Because only 18 cases of hepatic HS had a well-preserved morphology, immunohistochemical analyses were performed in these cases.

Immunohistochemistry

The liver tissue specimens were embedded in paraffin wax after fixation, and hematoxylin and eosin staining was performed for histopathological observation.[6] Table 1 provides a list of the primary antibodies used in this study. Thin (3 μm) sections were deparaffinized in xylene and rehydrated in a graded ethanol series, and then incubated with proteinase K for FA-11 and ER-MP20 staining or subjected to microwave pretreatment with either a pH9.0 Target Retrieval Solution (Dako, Glostrup, Denmark) for 2F8 and anti-CD5 staining or a pH6.0 citrate buffer for other antibody staining. Next, these sections were immersed in methanol containing 0.3% hydrogen peroxide for 30 min to inhibit endogenous peroxidase activity. After the reaction of the primary antibodies was complete, the samples were incubated with horseradish peroxidase-labeled goat anti-rat or anti-rabbit antibodies (Nichirei, Tokyo, Japan). Because anti-human-desmin, α-smooth muscle actin (α-SMA) and proliferating cell nuclear antigen antibodies cross-react with mouse antigens,[9] the HISTOFINE mouse stain kit was used for immunostaining according to the manufacturer's protocol (Nichirei). The immunoreactions were visualized using a diaminobenzidine substrate kit (Nichirei). All sections were counterstained with Mayer's hematoxylin. We used rat or rabbit IgG (Dako) as the negative control.

RESULTS

The HS cells were positive for macrophage markers, but not for lymphoid or mesenchymal markers

Abnormal enlargement of the liver and the formation of tumorous lesion (range of liver weight : 1,894-12,060 mg) were observed in 18 HS-bearing mice.[6] Lung metastasis of hepatic HS occurred in 15 of 18 cases. Histological examination revealed that large clusters of atypical histiocytic cells with abundant eosinophilic cytoplasm and hyperchromatic nuclei were present in all samples (Fig. 1a). Multinucleated giant cells (MGCs) appeared in 10 cases, and necrosis was observed in seven cases. Immunohistochemical analysis showed that these tumor cells were positive for F4/80, one of the most specific antibodies against murine macrophages,[10] and weakly to moderately positive for FA-11 (CD68) in all cases (Fig. 1b & 1c, Table 2). The proportion of proliferating cell nuclear antigen-positive proliferating tumor cells was approximately 10-40%. The tumor cells were negative for other cell markers of lymphocytes (CD45R, CD5), smooth muscle cells (α-SMA, desmin) and endothelial cells (factor VIII) in all cases (Fig. 1d-1f, Table 2), suggesting that lymphoma or mesenchymal tumor components do not exist in murine hepatic HS. The histological characteristics and immunohistochemical reactions of HS were not found to correlate with low-dose irradiation (Table 2).

Numerous Ly-6C$^+$ macrophage progenitor cells were observed in the tumorous tissue of murine hepatic HS

We next examined the distribution of Ly-6C$^+$ macrophage precursors using immunohistochemistry. Approximately 40% of tumor cells with low F4/80 expression were positive for Ly-6C in hepatic HS (Fig. 2a & 2b). Although Ly-6C is also known to be a marker of endothelial cells,[8] the Ly-6C$^+$

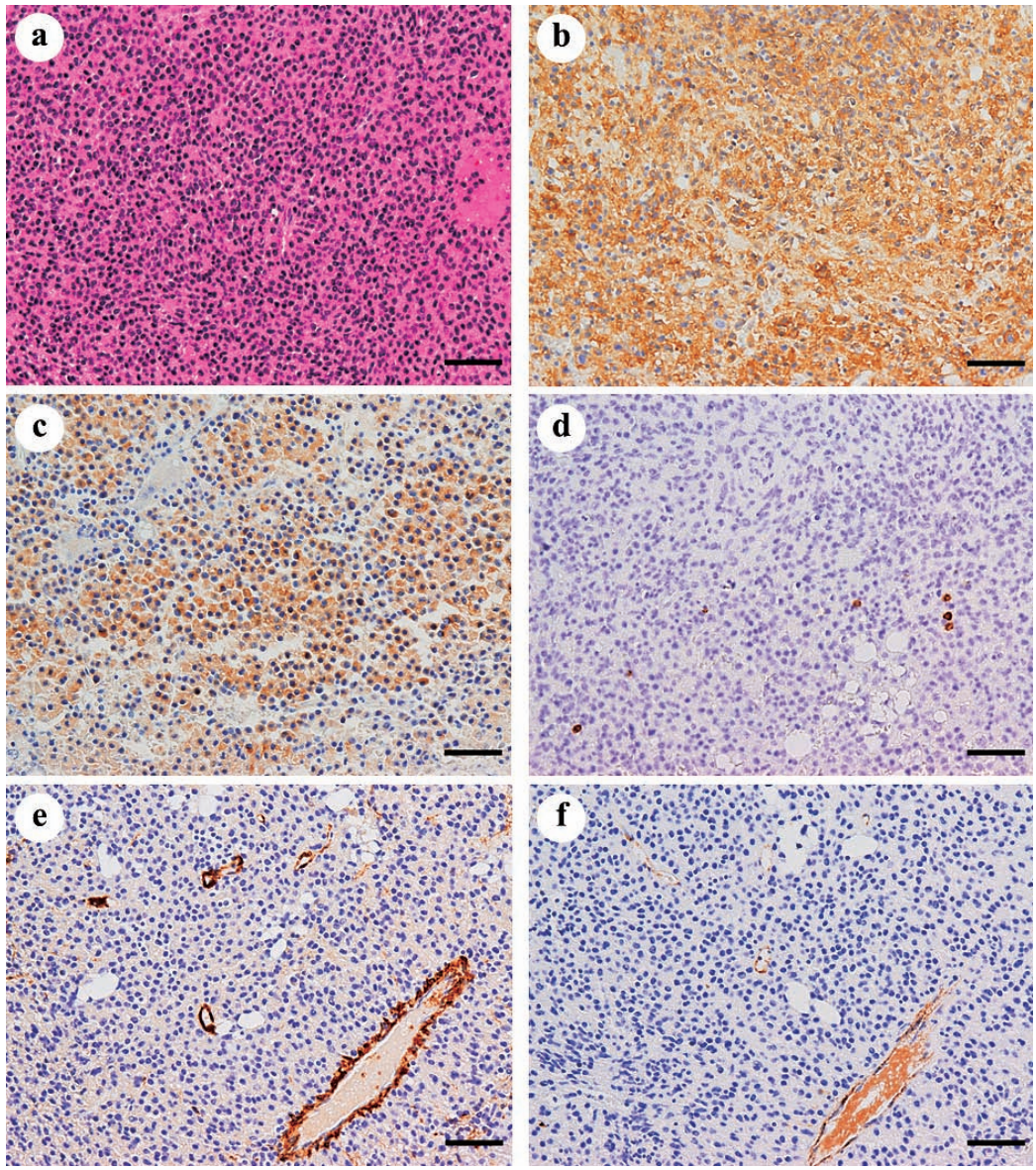

Fig. 1. Histological evaluation of hepatic histiocytic sarcoma (HS). (***1a***) HS features were indicated by hematoxylin and eosin staining. (***1b-1f***) Immunohistochemical findings of HS. The tumor cells were strongly positive for F4/80 (*1b*) and weakly to moderately positive for CD68 (*1c*) in all cases. The tumor cells were negative for CD45R (*1d*), α-smooth muscle actin (*1e*) and factor VIII (*1f*) in all cases. The scale bars represent 50 μm.

tumor cells were negative for another endothelial cell marker (factor VIII) (Fig. 1f). We confirmed that many extramedullary hematopoietic foci exist in the livers of mice bearing HS (Fig. 2c), similar to the results of a previous report.[2] It is interesting to note that scattered Ly-6C$^+$F4/80$^-$ macrophage progenitor cells were also detected in the hematopoietic foci and non-tumorous liver tissue around the HS lesions (Fig. 2a & 2d). These findings suggest that Ly-6C$^+$ macrophage progenitor cells might be a cellular origin of hepatic HS.

Two cases of murine hepatic HS differentiated into epithelioid granuloma-like variants

Most cases of hepatic HS in this study (16 of 18 samples) showed a sinusoidal infiltration growth pattern in addition to diffuse proliferation or partial nodal involvement (Fig. 3a). These tumor cells strongly expressed CD204 (class A scavenger receptor types I and II), a macrophage-restricted molecule, as well as F4/80 (Fig. 3b, Table 2). Interestingly, another two HS cases showed different histological features.

Table 2. Histology and immunohistochemistry of hepatic histiocytic sarcoma

Case	Sex	Dose (mGy/day)	Histological pattern	F4/80	CD68	CD204	CD45R	CD5	α-SMA	Desmin	Factor VIII
1	F	0	Diffuse and sinusoidal	++	±	++	−	−	−	−	−
2	F	0	Nodular and sinusoidal	++	+	++	−	−	−	−	−
3	F	0	Nodular and sinusoidal	+	+	++	−	−	−	−	−
4	M	0	Nodular and sinusoidal	++	+	++	−	−	−	−	−
5	M	0	Nodular and sinusoidal	++	+	++	−	−	−	−	−
6	M	0	Diffuse and sinusoidal	++	+	++	−	−	−	−	−
7	F	0.05	Nodular and sinusoidal	++	+	++	−	−	−	−	−
8	F	0.05	Nodular and sinusoidal	++	±	++	−	−	−	−	−
9	M	0.05	Diffuse and sinusoidal	+	±	++	−	−	−	−	−
10	M	0.05	Epithelioid granulomatous	++	+	±	−	−	−	−	−
11	F	1.1	Nodular and sinusoidal	++	+	++	−	−	−	−	−
12	M	1.1	Diffuse and sinusoidal	++	±	++	−	−	−	−	−
13	M	1.1	Nodular and sinusoidal	+	±	++	−	−	−	−	−
14	M	1.1	Nodular and sinusoidal	++	+	++	−	−	−	−	−
15	F	1.1	Epithelioid granulomatous	++	+	−	−	−	−	−	−
16	F	21	Diffuse and sinusoidal	++	±	++	−	−	−	−	−
17	M	21	Nodular and sinusoidal	++	+	++	−	−	−	−	−
18	M	21	Nodular and sinusoidal	++	+	++	−	−	−	−	−

++ ; more than 75% positive immunoreactive cells ; + ; 25% to 75% positive ; ± ; 1% to 25% positive ; − ; less than 1% positive ; α-SMA, α-smooth muscle actin. *Dose ; exposure dose of gamma rays per day

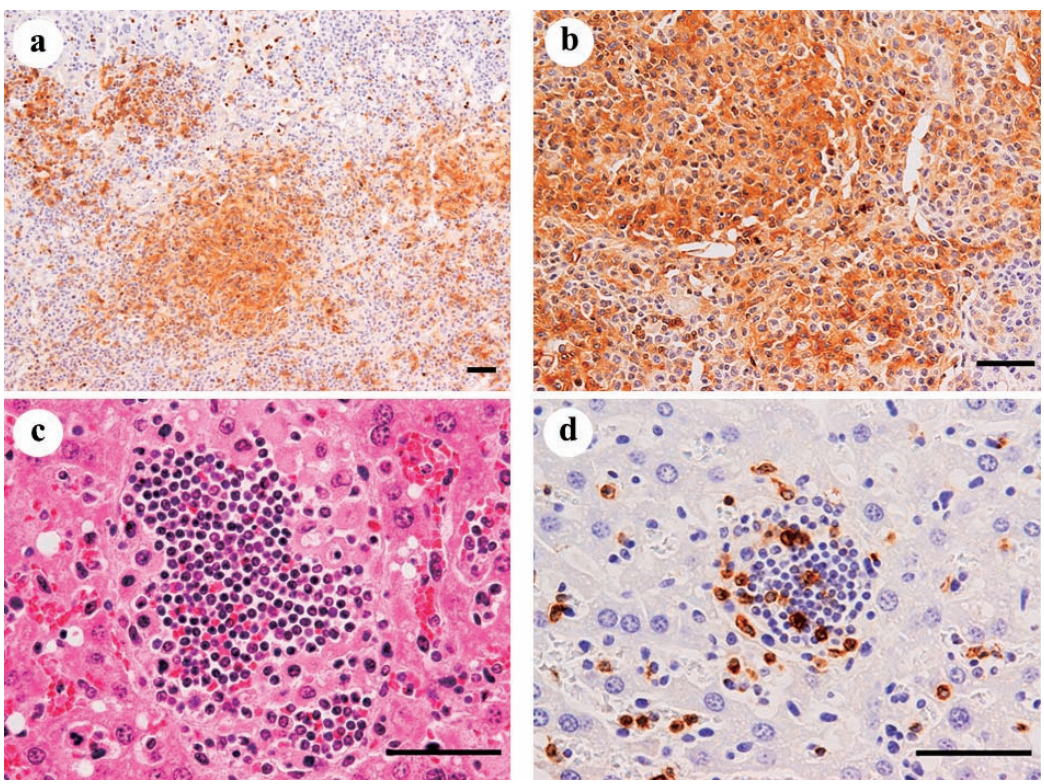

Fig. 2. An immunohistochemical demonstration of Ly-6C-positive precursors in hepatic histiocytic sarcoma (HS) lesions. Scattered and clustered Ly-6C-positive cells were observed in tumorous tissue (*2a*, *2b*). Hematopoietic foci were observed in non-tumorous tissue surrounding HS lesions (*2c*, hematoxylin and eosin stain). Ly-6C-positive cells were also observed in the hematopoietic foci (*2d*). The scale bars represent 50 μm.

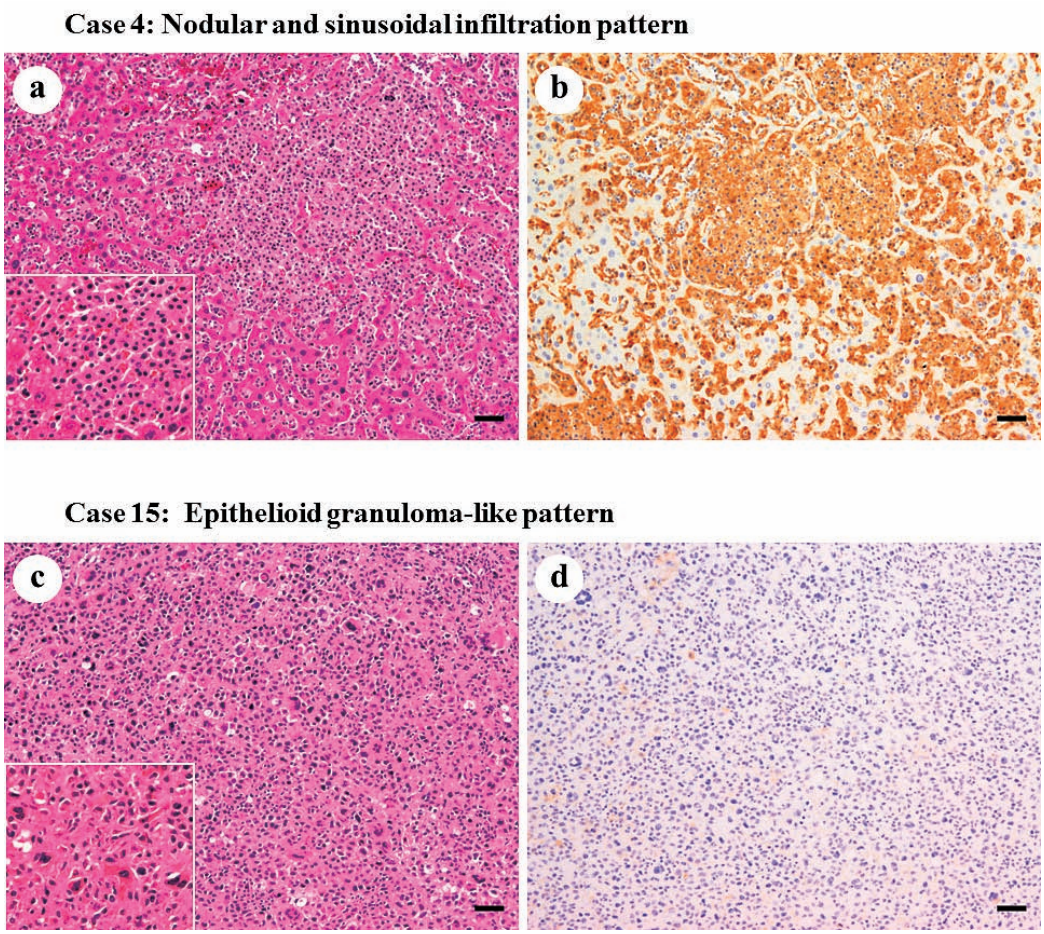

Fig. 3. Histological variants of hepatic histiocytic sarcoma (HS). (***3a***) Common type HS features were indicated by hematoxylin and eosin (HE) staining. ***Inset***, high-power view of HS cells. (***3b***) The tumor cells in the common type HS were strongly positive for CD204. (***3c***) Another variant of HS showed epithelioid granuloma-like features on HE staining. Inset, high-power view of HS cells. (***3d***) The tumor cells in epithelioid cell-like HS were barely positive for CD204. The scale bars represent 50 μm.

Specifically, these tumor cells formed clearly demarcated nodular proliferations mimicking epithelioid granulomas and also showing slight expression of CD204 (Fig. 3c & 3d, Table 2). The epithelioid cell-like tumor cells were negative for Ly-6C (data not shown). These findings suggest that murine hepatic HS cells might therefore be able to differentiate into epithelioid cell-like variants with a very low expression of CD204.

DISCUSSION

The cellular origins of HS have not been fully elucidated, although many researchers are attempting to uncover them. Both pluripotent germ cells and hematopoietic progenitor cells are thought to be possible candidates for cells of HS origin.[2,3] A subset of human HS cases occur in patients with mediastinal malignant germ cell tumors.[11] Culture experiments have revealed that teratocarcinoma cells may differentiate among hematopoietic cells,[3] thus indicating that HS may arise from germ cells. The relationship between HS and germ cell tumors has been observed in mice as well as humans.[12] In contrast, other studies have reported some human HS cases to be associated with follicular lymphoma, diffuse large B-cell lymphoma or mantle cell lymphoma.[4,13-15] Similarly, previous reports described that several HS cases coexist with B-cell lymphoma, which might have a common clonal origin of the HS in mice,[16,17] thus indicating that HS may originate from clonal hematopoietic progenitor cells of lymphoma. Our study showed that germ cell tumors and lymphoma components do not coexist in murine hepatic HS cells using immunohistochemical analysis, thus suggesting that HS occurrence may stem from an independent origin in the liver rather than from the transformation of other tumors.

The incidence of HS in a certain type of mice is far higher

than that in humans. Moreover, the liver is the most commonly involved site.[2] This strongly suggests that a unique origin of HS may exist in the murine liver. For instance, Kupffer cells, which constitute the majority of liver macrophages, are considered to be a cellular origin of HS.[2,17] Interestingly, Lacroix-Triki *et al*. and Barker *et al*. proposed that extramedullary hematopoietic foci in the liver are strongly associated with the involvement of murine hepatic HS.[2,5] We also confirmed the presence of many hematopoietic foci in the livers of HS-bearing mice. These findings indicate that myeloid progenitor cells in hematopoiesis are reasonable as origins of HS. Therefore, we postulate that murine hepatic HS may originate in macrophage precursors of extramedullary hematopoiesis. We detected scattered Ly-6C^{+} F4/80^{-} cells corresponding to macrophage precursors/immature monocytes, not to Kupffer cells, in the hematopoietic foci and liver tissue surrounding the HS lesions. We also showed that approximately 40% of HS cells express Ly-6C as well as F4/80, indicating that Ly-6C^{+} immature tumor cells differentiate into F4/80^{+} mature macrophage phenotypes. These findings suggest that Ly-6C^{+} macrophage precursors in extramedullary hematopoiesis might be a convincing cellular origin of murine hepatic HS. Because previous studies demonstrated that the incidences of HS are increased in Bax/ARF-double null mice[15] and glycidol-treated haploinsufficient p16^{INK4A}/p19^{ARF} mice,[18] some genetic abnormality in Ly-6C^{+} macrophage precursors may cause HS. Interestingly, a previous study revealed that B-lymphocyte progenitor cells have the potential to differentiate into CD5^{+} macrophages.[19] Although the hepatic HS cases in our examination were negative for CD5, a certain type of HS case with B-cell lymphoma[4, 12-15,17] might originate in the common progenitor cells of B cells.

In this study, we observed that hepatic HS appeared morphologically to be very similar to epithelioid granuloma in two of 18 cases. These tumor cells hardly expressed CD204, a macrophage class A scavenger receptor of types I and II, although the tumor cells in the other 16 cases were strongly positive for this antigen. Since epithelioid cells in granulomatous diseases such as tuberculosis or sarcoidosis decrease their expression of CD204,[20] tumor cells with very weak CD204 expression in HS might be the cells that differentiate toward epithelioid cells. It is interesting to note that CD204^{+} mononuclear tumor cells surround CD204^{-} MGCs in tumorous clusters. CD204 plays pivotal roles in granuloma formation in early stages and is then attenuated after differentiation into epithelioid cells or MGCs,[20,21] thus suggesting that phenotypic changes in CD204^{+} tumor cells occur during MGC formation. Little has been reported on these subtypes of murine HS, although previous reports have described HS with epithelioid features in *Sprague-Dawley* rats.[22] Our present findings suggest the possibility that murine hepatic HS can differentiate into epithelioid cell-like phenotypes.

In the present study, we have made the following findings : Ly-6C^{+} macrophage progenitor cells in extramedullary hematopoiesis and liver tissue surrounding the HS lesions

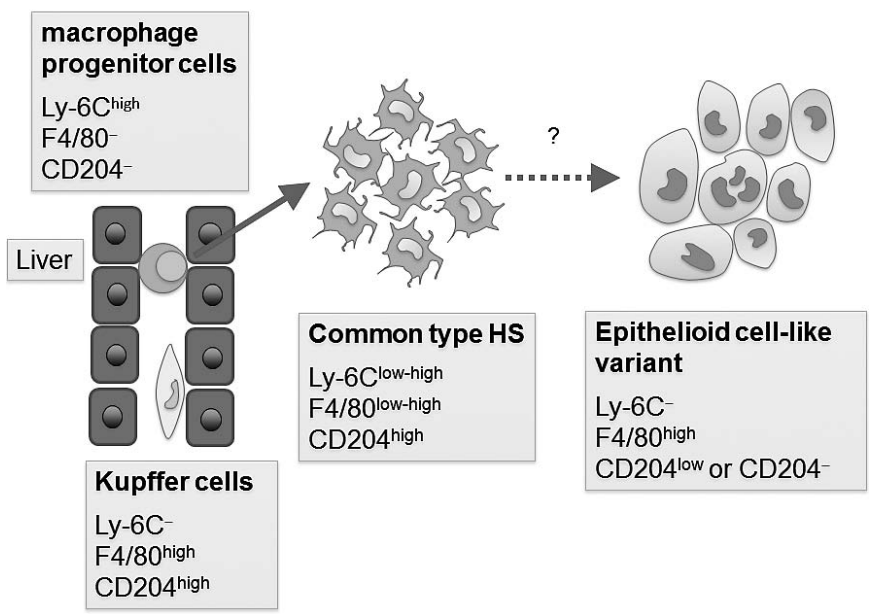

Fig. 4. A scheme of a possible cellular origin and a novel histological subtype of murine hepatic histiocytic sarcoma (HS). HS tumor cells may originate in Ly-6C^{+} macrophage progenitor cells, and a part of HS cells might differentiate into epithelioid cell-like variants.

may be the cellular origin of mouse hepatic HS ; a subset of murine HS has new phenotype tumor cells that differentiate toward epithelioid cell-like features (Fig. 4). Further molecular biological studies are necessary to clarify the origins and differentiation of murine hepatic HS in detail.

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