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Tropism of liver epithelial cells toward hepatocellular carcinoma *in vitro* and *in vivo* with altering gene expression of cancer stem cells

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ABSTRACT

Background: Rat liver epithelial (RLE) cells could inhibit the proliferation and invasiveness of hepatoma cells *in vitro*. This study is to understand the tropism and the effect of RLE cells on mouse hepatoma cells both *in vitro* and *in vivo*.
Methods: RLE cells were isolated from new-born rats and characterized their stem cell markers. Co-culture and HCC mouse model was established to detect therapeutic effect of RLE cells.
Results: RLE cells (including Thy-1$^+$ RLE cells, Thy-1$^-$ RLE cells, RLE cells) displayed a selective tropism toward ML-1 hepatoma cells both *in vitro* and *in vivo*. They altered the gene expression of some cancer stem cell markers in the liver tumor.
Conclusion: Liver epithelial cells have a selective tropism toward HCC *in vitro* and *in vivo*. They could alter the gene expression of cancer stem cells.

1. Introduction

Hepatocellular carcinoma (HCC) is a leading malignancy in Asia where hepatitis B or C is prevalent.[1,2] The prognosis for those who are beyond the indications for resection or transplantation is usually poor.[3–5] Heterogeneity of HCC contributes to progression.[6,7] The effect of the current systemic therapy or target therapy is limited.[8] Therefore, investigation of a novel strategy for those patients is important.

Bone marrow-derived mesenchymal stem cells (MSC) can inhibit the growth and invasiveness of HCC cells.[9–11] However, rapid obtaining of such stem cells from human bone marrow has difficulties in clinical practice. It would be more feasible and practical to use liver progenitor cells as a weapon to treat HCC.

Rat liver epithelial (RLE) cells from WB-F 344 rats have some characteristics of progenitor cells or stem cells.[12–14] We have previously reported that RLE cells can inhibit the proliferation and invasiveness of hepatoma cells (HL-IIE-C3 cells) *in vitro*.[14] However, two main characteristics of RLE cells need be elucidated *in vitro* or *in vivo*. One is whether these RLE cells might move towards the tumor, and the other is whether they might inhibit tumor growth or change tumor gene expressions. Therefore, these two characteristics of RLE cells were examined.

2. Material and methods

2.1. Animals

Pathogen-free Fisher (F344) rats and C57BL/6 mice were purchased from the National Laboratory Animal Center, Taiwan. Rats and mice were separately housed in our institute. All animal studies were performed in accordance with the guidelines for the care and use of laboratory animals and in accordance with the approval protocols of the Institutional Animal Care and Use Committee of our hospital (IACUC approval No: 99-1-43-C1).

2.2. Hepatoma cells

The mouse hepatoma cell line ML-1 cells were originally

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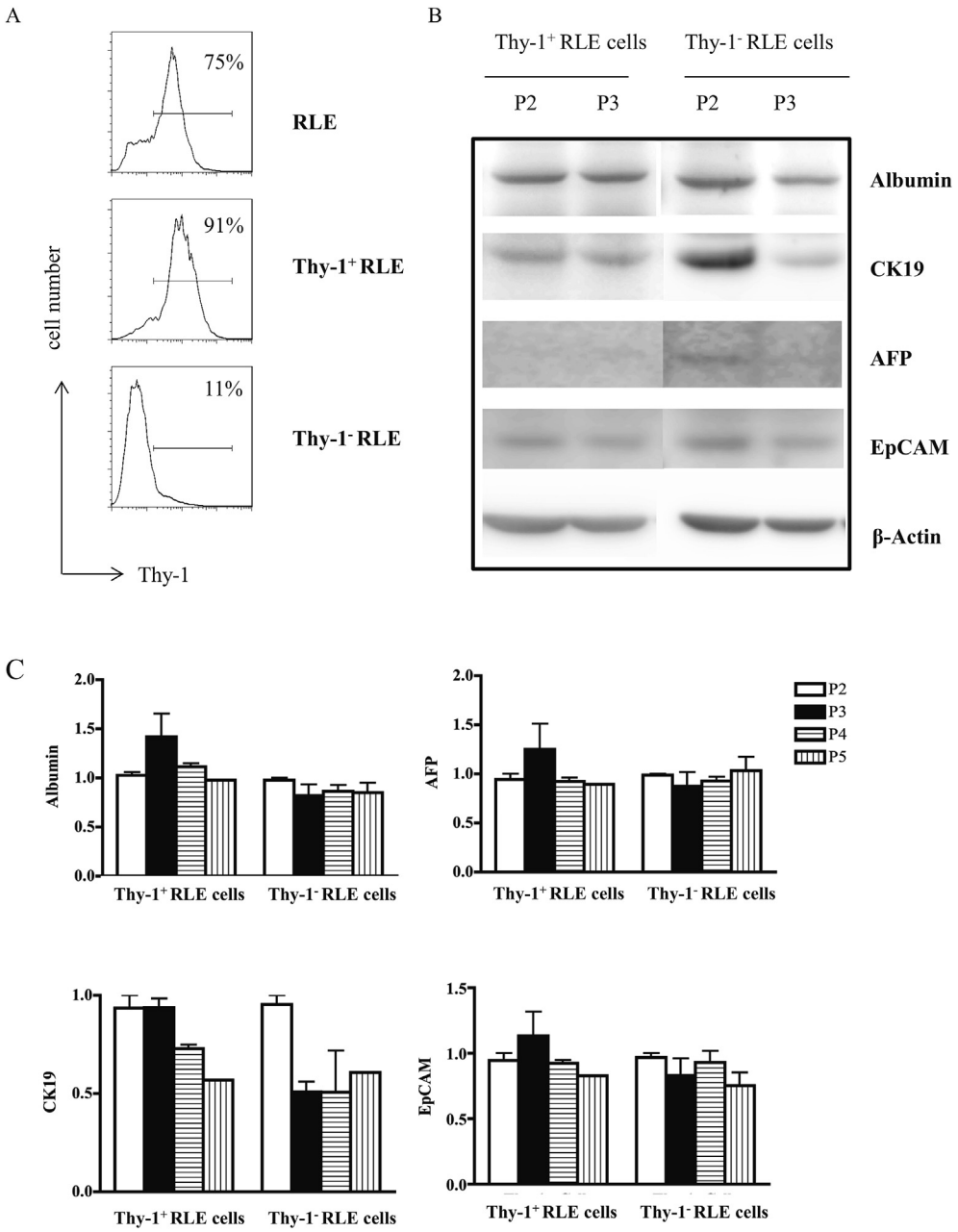

Fig. 1. The expressions of the progentior markers in sorted Thy-1$^+$ RLE cells and Thy-1$^-$ RLE cells were similar. (A) Thy-1 expression in RLE cells was detected by flow cytometry. The gates represent the percentages of Thy-1$^+$ cells. (B) The protein expression of albumin, CK19, AFP and EpCAM in the second passage (P2) and the third passage (P3) of Thy-1$^+$ RLE cells and Thy-1$^-$ RLE cells were detected by Western blotting,. (C) Normalized protein expression of albumin CK19, AFP and EpCAM are presented in the relative mean with standard error. (P2: the second pasage; P3: the third passage; P4: the forth passage; P5: the fifth passage).

established from hepatocytes of Balb/C mice by hepatitis Bx DNA transfection.[15] Hepa1-6 cells (BCRC #60051) were purchased from Biosource Collection and Research Center, Taiwan. Both cell lines were cultured in the Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (Gibco, Carlsbad, CA, USA) at 37 °C in a humidified 5% CO2 incubator, and were routinely sub-cultured with 0.05% trypsin in phosphate-buffer at 80–90% confluence.

2.3. Isolation and cell culture of RLE cells

Pathogen-free ten-day old Fisher (F344) rats were purchased from the National Laboratory Animal Center (Taipei, Taiwan). Rats were housed at our hospital. They were used to isolate RLE cells (under anesthesia). Liver pieces were incubated in a DMEM/F12 containing 10 mM HEPES (both from Gibco), 1 mg/ml type IV collagenase (Sigma, St. Louis, MO, USA) and 1% penicillin/streptomycin at 37 °C for 20 min. RLE cells were plated on collagen I-coated culture dishes incubated at 37 °C in a humidity incubator with 5% CO2. Cells were grown in a stem cell medium containing DMEM/F12, 2% FBS, 10 mM HEPES, 0.1% ITS Premix (Corning, NY, USA), 1×10^{-7} M dexamethasone (Sigma St. Louis, MO, USA), 10 ng/ml mouse stem cell factor (SCF; eBioscience, San Diego, CA, USA), 20 ng/ml epidermal growth factor (EGF) (Sigma, St. Louis, MO, USA) and penicillin/streptomycin.

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