

Effects of Three-Dimensional Stiffness on the Proliferation, Stemness And Invasion of Hepatic Cancer Stem Cells

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Abstract: Hepatocellular carcinoma (HCC) is the third most common cancer in the world. Previous studies have shown that hard matrix promotes the proliferation of liver tumor cells. However, the role of matrix stiffness on hepatic cancer stem cells (HCSCs) is still unclear. Three-dimensional hydrogels with different stiffness were used to mimic the normal liver tissue (4kPa) and cancerous liver tissue (26kPa) stiffness. The proliferation, stemness and invasion properties of HCSCs under 3D different stiffness were detected. **METHOD:** HCSCs were screened and cultured by enrichment method, and the effect of matrix stiffness on HCSCs was studied by three-dimensional culture of HCSCs in hydrogels of different stiffness. CCK-8, clonal size measurement and QRT-PCR were used to examine the effects of different matrix stiffness on the proliferation of HCSCs. CD133 and CD90 were used to examine the effects of different matrix stiffness on the stemness of HCSCs. Immunofluorescence was used to detect epithelial mesenchymal transition-related proteins to investigate the effects of different matrix stiffness on the invasion ability of HCSCs. **RESULTS:** The matrix stiffness of 4 kPa significantly inhibited the proliferation, stemness maintenance and invasion ability of HCSCs. The matrix stiffness of 26 kPa significantly promoted the proliferation, stemness maintenance and invasion ability of HCSCs. Subsequently, we culture the HCSCs in 4kPa and 26kPa hydrogels for matrix stiffness exchange. The results showed that the stemness and invasive ability of HCSCs in 26kPa-4kPa decreased significantly, while the stem cell stemness of liver cancer within 4kPa-26kPa and the invasive ability is significantly increased. Animal experiments have further validated this result. **CONCLUSION:** The mechanical environment of HCSCs has a significant regulatory effect on their proliferation, stemness and invasion ability. The stiffness of pathological liver tissue plays a significant role in promoting the occurrence and development of tumor. Our study provides new ideas for further understanding the pathogenesis and treatment of liver cancer.

Keywords: Stemness; hepatic cancer stem cells; EMT; three-dimensional matrix stiffness.

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