[1]贾茜,高建,张小丽,等.HepG2、Hep3B细胞中肿瘤干细胞相关标志分子的表达[J].第三军医大学学报,2012,34(09):852-856.

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# HepG2、Hep3B细胞中肿瘤干细胞相关标志分子的表达

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Title: Expression of cancer stem cell-associated markers in liver cancer cell lines HepG2 and Hep3B

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关键词: 肝癌干细胞; CD90; CD133; Oct4; ABCG2; 耐药性

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摘要: 目的　富集培养肝癌干细胞，研究肝癌干细胞相关标志物CD90、CD133、八聚体4（Oct4）和ATP结合盒转运蛋白ABCG2在肝癌细胞HepG2、Hep3B中的表达，并初步分析其意义。　方法　使用流式细胞仪从肝癌细胞（检测HepG2、Hep3B两种细胞系）中分选肝癌干细胞并行无血清成球培养。设肝癌细胞组为对照组。单细胞克隆形成实验检测细胞增殖能力。分别给予肝癌细胞及肝癌干细胞阿霉素处理，MTT法测阿霉素处理后各组细胞的存活率，Real-time PCR及Western blot分别检测各组细胞CD90、CD133、Oct4和ABCG2 mRNA及蛋白水平的表达。　结果　肝癌干细胞单个细胞增殖能力强于肝癌细胞，克隆形成分析显示培养第14天克隆形成率Hep3B细胞组低于Hep3B CSCs组[8/27（30%）*vs* 12/23（52%），*P*<0.05]；HepG2细胞组克隆形成率也低于HepG2 CSCs组[7/38（18%）*vs* 9/26（35%），*P*<0.05]。用阿霉素处理肝癌细胞及肝癌干细胞48 h后，MTT法测定结果显示肝癌干细胞组细胞活性显著高于对照组(*P*<0.05)：HepG2细胞组细胞活性为（38.17±6.92）%、HepG2 CSCs组为（69.88±5.43）%；Hep3B细胞组（50.16±4.89）%，Hep3B CSCs组为（78.53±7.86）%。Real-time PCR结果显示肝癌干细胞组中CD90、CD133、Oct4及ABCG2基因mRNA表达较亲代细胞组显著上调（*P*<0.05）。Western blot结果显示肝癌干细胞组Oct4及ABCG2蛋白水平显著上调，与亲代细胞组间表达差异有显著性（*P*<0.05）。　结论　肝癌干细胞相关标志CD90、CD133、Oct4及ABCG2均高表达于肝癌干细胞，并且Oct4及ABCG2 基因的高表达有可能与肝癌干细胞耐药性相关。

Abstract: Objective　To explore the expression of cancer stem cell (CSC)-associated markers CD90, CD133, octamer 4 (Oct4) and ATP-binding cassette transporter G2 (ABCG2) in liver cancer cell lines HepG2 and Hep3B, and to preliminarily analyze the significance.　Methods　Liver CSCs were separated from the HepG2 and Hep3B

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cell lines by flow cytometry, and were cultured in serum-free medium to form spheres. The liver cancer cells were assigned as a control group. Cell proliferation capacity was examined by single-cell clone formation assay. Cell viability was detected by MTT assay after treated with doxorubicin. The mRNA and protein expression levels of CD90, CD133, Oct4 and ABCG2 were detected by real-time PCR and Western blotting, respectively. Results The single cell proliferation capacity of the liver CSCs was stronger than that of the liver cancer cells. The single-cell clone formation assay showed that the colony formation rate of the Hep3B cells was lower than that of the Hep3B CSCs after cultured for 14 d [8/27 (30%) *vs* 12/23 (52%), *P*<0.05], and the colony formation rate of the HepG2 cells was also lower than that of the HepG2 CSCs [7/38 (18%) *vs* 9/26 (35%), *P*<0.05]. MTT assay showed that the cell viability significantly increased in the liver CSCs compared with that in the liver cancer cells after treated with doxorubicin for 48 h [HepG2 cells (38.17±6.92)% *vs* HepG2 CSCs (69.88±5.43)%, *P*<0.05; Hep3B cells (50.16±4.89)% *vs* Hep3B CSCs (78.53±7.86)%, *P*<0.05]. The Real-time PCR results showed that the mRNA expression levels of CD90, CD133, Oct4 and ABCG2 were significantly increased in the liver CSCs compared with those in the parental cells (*P*<0.05). Western blotting results showed that the protein expression levels of Oct4 and ABCG2 significantly increased in the liver CSCs compared with those in the parental cells (*P*<0.05). Conclusion The CSC-associated markers CD90, CD133, Oct4 and ABCG2 are highly expressed in liver CSCs, and the high expression of Oct4 and ABCG2 may be closely associated with the CSCs drug resistance to chemotherapy.

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