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肝癌干细胞样细胞的分离及其耐药性受PI3K/Akt通到：

《第三军医大学学报》[ISSN:1000-5404/CN:51-1095/R] 卷: 35 期数: 2013年第02期 页码: 99-104
栏目: 论著 出版日期: 2013-01-30

Title: Isolation of HCC cancer stem-like cells and chemo-resistance mediated by PI3K/Akt pathway

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关键词: 肝细胞癌; 肿瘤细胞; 培养的; 肿瘤干细胞; 表柔比星; 耐药性; PI3K/Akt

Keywords: hepatocellular carcinoma; tumor cells; cultured; neoplastic stem cells; epirubicin; chemoresistance; PI3K/Akt

分类号: R73-351;R730.23;R735.7

文献标志码: A

摘要: 目的　分离肝癌干细胞样细胞，初步探讨PI3K/Akt通路调节其对化疗药物阿霉素的敏感性。　方法　将人肝癌细胞株PLC、HepG2、Hep3B置于无血清条件培养基中培养，形成细胞球，选用PLC细胞株进行后续实验。采用流式细胞仪、克隆形成实验、SCID小鼠体内成瘤实验鉴定PLC细胞球（肝癌干细胞样细胞）的肿瘤干细胞特性。MTT法、流式细胞仪测定PLC细胞球对化疗药物阿霉素的敏感性。流式细胞仪分析加入PI3K/Akt通路特异性抑制剂LY294002、阿霉素共同孵育细胞球后，其凋亡率的变化。Western blot法比较PLC细胞球、PLC贴壁细胞中p-Akt1(Ser473)蛋白分子表达量及加入抑制剂LY294002作用于细胞球后，p-Akt1(Ser473)、Akt1蛋白分子表达量的变化。结果　肝癌干细胞标志物CD90在细胞球中的表达较贴壁细胞显著升高（P<0.01）。细胞球的克隆形成数目（123.00±28.48）为贴壁细胞（56.33±7.37）的2.18倍（P<0.05）。同样细胞数接种于SCID小鼠皮下7周后，细胞球的致瘤率明显大于贴壁细胞。以5 μg/mL的阿霉素分别处理细胞球和贴壁细胞48 h后，细胞球的增殖率明显高于贴壁细胞[（71.83±12.30）% *vs* （45.68±5.95）%, P<0.05]，凋亡率显著低于贴壁细胞[（11.73±3.77）% *vs* （41.22±6.73）%, P<0.01]，而以阿霉素5 μg/mL和LY294002共同孵育细胞球后，其凋亡率[（35.44±6.65）%]显著增加（P<0.01）。Western blot检

测到细胞球的p-Akt1(Ser473)蛋白分子表达量显著高于贴壁细胞（$P<0.01$），加入抑制剂LY294002处理细胞球后，p-Akt1(Ser473)蛋白表达量明显降低($P<0.05$)，Akt1的表达量无明显变化($P>0.05$)。　结论　肝癌干细胞样细胞对化疗药物阿霉素具有耐药性，其耐药机制与Akt信号通路第473位点磷酸化Akt1分子有关。

Abstract: Objective To isolate hepatocellular carcinoma (HCC) cancer stem-like cells and investigate the role of PI3K/Akt pathway in the sensitivity of liver cancer stem-like cells to chemotherapeutic drug doxorubicin (DOX). Methods Human HCC cell lines PLC, HepG2, and Hep3B were cultured in serum-free condition medium to form tumor spheres, and PLC cell line was finally selected to proceed the subsequent experiments. Fluorescence-activated cell sorting (FACS) and colony formation assay and SCID mice tumorigenicity experiments *in vivo* were used to identify the traits of CSCs in PLC spheres（liver cancer stem-like cells）. The sensitivity of the cancer stem-like cells to DOX was detected with MTT assay and FACS. The apoptotic rate of the cancer stem-like cells was analyzed with FACS after the treatment of DOX and LY294002, an inhibitor specific to PI3K/Akt signaling pathway. Western blotting was used to detect the expression of p-Akt1Ser473 and Akt1 protein in PLC spheres and in PLC monolayer cells in present or absent of the inhibitor LY294002. Results The expression of liver CSCs marker CD90 in the obtained spheres was obviously up-regulated as compared to monolayer cells ($P<0.01$). The cloning number of spheres （123.00 ± 28.48）was 2.18 times higher than that of the monolayer cells （56.33 ± 7.37, $P<0.05$). The tumorigenicity of spheres was evidently greater than the monolayer cells when same number of cells was subcutaneous injected into SCID mice after 7 weeks. The proliferation rate was greatly elevated after 48 hour-treatment of 5 μg/mL DOX in the spheres than in the monolayer cells [（71.83 ± 12.30）% *vs* （45.68 ± 5.95）%, $P<0.05$], while the apoptosis rate was sharply reduced [（11.73 ± 3.77）% *vs* （41.22 ± 6.73）%, $P<0.01$]. But the apoptotic rate of spheres was sharply increased after the treatment of 5 μg/mL DOX and LY294002. （35.44 ± 6.65）%, $P<0.01$). The expression of p-Akt1Ser473 protein significantly exceeded in spheres than monolayer cells (P<0.01) and the p-Akt1Ser473 expression at protein levels was obviously decreased after addition of LY294002 ($P<0.05$). There was no change in the expression of Akt1 protein ($P>0.05$). Conclusion The liver cancer stem-like cells demonstrate drug resistance to chemotherapeutic drug DOX, and the mechanism is related to Akt signaling pathway molecule Akt1 phosphorylated at Ser473.

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