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抗肝癌干细胞功能性单克隆抗体的研制 点此下载全文

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摘要：

摘 要? 目的：研制抗肝癌干细胞的功能性单克隆抗体，为肝癌干细胞的靶向治疗提供候选抗体药物。方法：从人肝癌组织中分离人肝癌干细胞样细胞（human liver cancer stem-like cells, hLCSLCs），免疫BALB/c裸鼠，采用脾细胞融合法制备大容量单抗库。应用细胞免疫荧光、无血清培养、裸鼠皮下成瘤等方法筛选、鉴定特异识别肝癌干细胞的单克隆抗体。流式细胞仪分选hLCSLCs侧群细胞（hLCSLCs side population cells, hLCSLCs-SP），无血清悬浮培养法和CCK-8法检测杂交瘤单抗对hLCSLC-SP自我更新和增殖能力的影响。结果：细胞融合后获得2 964株杂交瘤克隆，在能与hLCSLCs反应的237株克隆中，有116株单抗能与hLCSLCs的细胞膜结合，其中的33株杂交瘤单抗只与hLCSLC-SP反应（阳性率为2%~5%）、不与非hLCSLC-SP反应。该33株单抗中有6株能与CD133阳性细胞有不同比例的共染，并且与无血清悬浮培养的成球细胞呈阳性反应(阳性率为3%~26%)，明显高于hLCSLCs-SP。裸鼠皮下接种1×104个15D2单抗阳性的hLCSLCs，成瘤率为100%。功能性筛选实验发现，6株单抗中的4株能显著抑制hLCSLC-SP的增殖和成球生长，其抑制率分别为24%~42%和13%~50%。结论：采用自建的大容量单克隆抗体库技术，筛选获得了4株特异性识别hLCSLC-SP的功能性单抗，为肝癌干细胞的抗体靶向治疗奠定了基础。

关键词：肝肿瘤 肿瘤干细胞 单克隆抗体 靶向治疗

Preparation of functional monoclonal antibodies against human liver cancer stem cells Download Fulltext

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Abstract:

Abstract Objective:To prepare functional monoclonal antibodies(McAbs) against liver cancer stem cells, so as to provide candidate antibody drugs for stem cell-targeted therapy of liver cancer. Methods: Human liver cancer stem-like cells (hLCSLCs) were separated from human hepatocarcinoma tissues and were used to immunize BALB/c nude mice. Spleen cells from hLCSLCs-immunized mice were fused with SP2/0 cells to prepare large monoclonal antibody library. Hybridoma McAbs recognizing hLCSLCs were screened and identified by immunofluorescence, sphere formation culture and in vivo tumor formation assays. hLCSLCs side population cells (hLCSLCs-SP) were sorted by flow cytometry. The effects of hybridoma McAbs on self-renewal and proliferation of hLCSLCs-SP were identified by serum-free suspension culture and CCK-8 assay. Results: A total of 2 964 McAb clones were obtained by fusing immunized spleen cells with SP2/0 cells, and 237 McAbs could interact with hLCSLCs as detected by fixed-cell immunofluorescence; 116 of the 237 McAbs interacted with the membrane of hLCSLCs, and 33 McAbs specifically reacted with hLCSLCs-SP but not with non-hLCSLCs-SP, with positive rates being 2%-5%. Six of the 33 McAbs co-stained with CD133 on hLCSLCs-SP. Further investigation showed that the positive rates of these 6 McAbs were 3%-26% with sphere cells after serum-free suspension culture, which were significantly higher than those with hLCSLCs-SP. Tumor formation rate was 100% when 1×104 hybridoma clone 15D2-positive hLCSLCs were injected into nude mice. Functional study showed that 4 of these 6 McAbs significantly suppressed the proliferation and sphere formation ability of hLCSLCs-SP, with the inhibitory rates being 24%-42% and 13%-50%, respectively. Conclusion: We have successfully constructed the large McAb library against hLCSLCs, from which 4 hybridoma McAbs can specifically react with hLCSLCs-SP, laying a foundation for cancer stem cell-based antibody-targeted therapy for liver cancer.

Keywords:liver neoplasms cancer stem cell monoclonal antibody targeted therapy

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