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论著

抗人基质金属蛋白酶-2的单抗制备及功能研究

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摘要 目的：在体外原核表达MMP-2纤维连接蛋白样片段(MFD),制备抗MMP-2特异性的单克隆抗体(McAb),以期得到能够有效抑制MMP-2活性的单抗。 方法： 运用原核表达的MFD蛋白纯化浓缩后免疫BALB/C小鼠，尾静脉加强后取脾细胞融合，ELISA法筛选阳性克隆，制备腹水。运用Western blotting对抗体进行鉴定，并观察抗体与天然MMP-2的反应情况, 进行亚型鉴定，组织特异性鉴定同时进行功能实验:增殖实验、侵袭实验以及体外血管新生实验。 结果： 小鼠脾细胞与杂交瘤细胞融合后筛选获得一株持续分泌单克隆抗体的细胞株,命名为SZ-117。 ELISA法测定上清和腹水效价分别为2×10-3和2×10-5。抗体为IgG1亚型,可以与天然的MMP-2相结合，与血细胞表面没有交叉反应。免疫组化提示：胃、胆囊、脾脏、卵巢、前列腺、输卵管以及淋巴结的间质中都有阳性表达，而在甲状腺、小肠、肝脏等组织器官未见阳性表达。该抗体可以有效地抑制血管内皮细胞株Eahy926细胞以及胰腺癌细胞株1990细胞的侵袭行为，同时还可以抑制Eahy926细胞在体外的血管生成。 结论： 抗MMP-2特异性抗体的获得为MMP-2测定提供了一个有效工具,并且该抗体还可以有效地抑制内皮细胞在体外的血管生成和肿瘤细胞的侵袭行为，有望成为一种新的抗肿瘤物质。

关键词 基质金属蛋白酶； 抗体,单克隆

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Preparation and study of monoclonal antibody to matrix metalloproteinase-2

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Abstract

<FONT face=Verdana>AIM: To obtain a McAb that can inhibit the function of matrix metalloproteinase-2 (MMP-2), we expressed the fibronectin-like domain of MMP-2 (MFD) in vitro and prepared a McAb against MMP-2. METHODS: The purified MFD protein was used to immunize BALB/C mouse three times. Then the spleen of mouse was taken out and hybridized with hybridoma cells SP2/0. The positive cell clones were screened with ELISA method. The subtype and tissue specificity of the McAb were identified and its effect on endothelial cell migration and tube-formation was analyzed. RESULTS: After the spleen cells of the mouse and hybridoma cells SP2/0 were hybridized, a piece of cells that continuously secreted McAb against MMP-2 was obtained and named SZ-117. The titers of this McAb in culture supernatants and ascites were 2×10－3 and 2×10－5, respectively. The heavy chain of the McAb belongs to IgG1 subclass. The McAb identified native MMP-2. MMP-2 existed in the stromal tissue of stomach, cholecystis, spleen, ovarian, prostate, salping and lymph node. It inhibited the invasion behavior of endothelial cells Eahy926 and pancreatic carcinoma cells 1990 and inhibited the tube-formation of Eahy926 cells. CONCLUSION: A useful tool for testing MMP-2 is obtained and it will be helpful to look for a kind of new anti-tumor material.</FONT>

Key words Matrix metalloproteinases Antibodies monoclonal

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