

技术与方法

抗兔辅酶 II 依赖性视黄醇脱氢 / 还原酶单克隆抗体的制备和鉴定

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收稿日期 2008-1-17 修回日期 2008-2-27 网络版发布日期:

摘要 背景与目的: 制备抗兔辅酶 II 依赖性视黄醇脱氢 / 还原酶[NADP(H)-dependent retinol dehydrogenase / reductase, NRDR] 的单克隆抗体(mAb), 并鉴定其特性。材料与方法: 以基因工程重组兔 NRDR 为抗原免疫 BALB / c 小鼠, 用杂交瘤技术建立稳定分泌兔 NRDR mAb 的细胞株。以间接 ELISA 法筛选阳性克隆、鉴定 Ig 亚类、细胞培养上清及腹水效价, 同时采用 Western blot 方法检测 mAb 的特异性。结果: 获得 3 株可分泌特异性 mAb 的杂交瘤细胞(NR1、NR2 和 NR5)。其抗体亚类均为 IgG1, 细胞培养上清效价依次为 1:20、1:40 和 1:20, 腹水效价分别为 1:106、1:107 和 1:106。Western blot 结果显示 NR1、NR2 和 NR5 抗体均能有效识别兔肝组织中的 NRDR 及重组表达的兔 NRDR。结论: 成功地建立了稳定分泌兔 NRDR mAb 的杂交瘤细胞株, 为 NRDR 生物学功能的进一步研究打下了基础。

关键词 [兔辅酶 II 依赖性视黄醇脱氢 / 还原酶](#); [单克隆抗体](#); [杂交瘤](#)

Preparation and Characterization of Monoclonal Antibody against Rabbit NADP(H)-dependent Retinol Dehydrogenase / Reductase

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Abstract BACKGROUND AND AIM: To prepare specific monoclonal antibody (mAb) against rabbit NADP(H)-dependent retinol dehydrogenase / reductase (NRDR) for further exploration of its structure and function. MATERIALS AND METHODS: The BALB/c mice were immunized with the recombinant rabbit NRDR expressed by genetic engineering. Splenocytes of immunized mice were collected and fused with the mouse myeloma cell line NS-1 cells. Hybridomas that secreted rabbit NRDR mAb were cloned with limited dilution method. Characteristics of mAb(Ig subclasses, titers and specificities) were identified and determined by indirect ELISA and Western blot. RESULTS: From over 80 positive hybridomas which secreted anti-rabbit-NRDR mAbs, three clones of hybridoma were obtained, and designated as NR1, NR2 and NR5. They were all of IgG1 subclass. The cell culture supernatant titers of NR1, NR2 and NR5 were 1:20, 1:40 and 1:20, respectively. Ascite titers of NR1, NR2 and NR5 mAb were 1:106, 1:107 and 1:106, respectively. Western blot analysis showed that mAbs had specific binding abilities with NRDR in rabbit liver and recombinant rabbit NRDR. CONCLUSION: The hybridomas secreting mAbs to rabbit NRDR were established successfully and primarily identified, which could lay the basis for further research on the biological structure and function of NRDR.

Keywords [rabbit NRDR](#) [monoclonal antibody\(mAb\)](#) [characterization](#); [retinoic acid](#)

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