[1]郑怡麟,何莹,陈安,等.抗人降钙素原特异性单克隆抗体的制备鉴定及初步应用[J].第三军医大学学报,2013,35(06):523-526.

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抗人降钙素原特异性单克隆抗体的制备鉴定及初步

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Title: Preparation and identification of monoclonal antibodies against human procalcitonin

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摘要: 目的 获得抗人降钙素原（procalcitonin，PCT)单克隆抗体，并鉴定其基本特性。 方法 从NCBI数据库中获得PCT编码序列，以原核表达系统表达PCT重组蛋白，经镍亲和层析柱纯化，获得筛选单克隆抗体的筛选原；综合应用多种生物信息学分析软件预测 PCT 中潜在的B 细胞表位肽，用合成肽对BALB/c小鼠进行免疫，纯化单克隆抗体，通过间接ELISA、Western blot和免疫组化鉴定获得的单克隆抗体。将获得的单克隆抗体作为捕获抗体，经过配对建立检测重组PCT的ELISA体系，并初步对临床血清进行了检测。 结果 成功获得3株PCT特异性单克隆抗体，分别为2-D7、2-H12和4-H12，亚类分别为IgG1和IgG2a，最高效价为1∶1 024 000，亲和力最高可达到 1.3×10^9 L/mol；选取2-D7经Western blot鉴定能够检测重组PCT蛋白，经免疫组化证实能特异性识别甲状腺组织中PCT蛋白，经过配对成功建立了检测重组PCT的ELISA体系，运用建立体系检测临床血清PCT含量时发现正常组与细菌培养阳性组之间具有显著差异。 结论 成功表达了人PCT重组蛋白，获得了3株高特异性和高亲和力的PCT单克隆抗体，成功建立了检测重组PCT的ELISA体系，并初步用于临床样本检测。

Abstract: Objective To prepare and characterize the monoclonal antibodies (mAbs) against human procalcitonin (PCT). Methods Human PCT protein was expressed with prokaryotic expression system, and the purified recombinant PCT was applied as the antigen for screening the mAbs. Based on bioinformatics analysis, the potential B cell epitopes within human PCT were predicted and

synthesized. BALB/c mice were immunized using the synthesized peptides, which were conjugated with bovine serum albumin. Enzyme-linked immunosorbent assay (ELISA), Western blotting and immunohistochemistry were employed to characterize the mAbs, and the ELISA system that can detect PCT in serum was established. Results The PCT recombinant protein was successfully highly expressed and the purity of the recombinant PCT reached about 95% after purification. Three hybridoma cell lines secreting antibodies against PCT, 2-D7, 2-H12 and 4-H12 were obtained. The subtypes of the mAbs belonged to IgG1 and IgG2a. The monoclonal antibody 2-D7 has the highest titer of 1∶1 024 000 and affinity constant of 1.3×10^9 L/mol. Western blotting showed that 2-D7 only reacted with recombinant PCT and the native PCT in human thyroid gland tissues, but not with unrelated protein H-FABP. Using 2-D7 and the purchased antibody as capturing and detecting antibody, respectively, an ELISA system measuring recombinant PCT was developed. The ELISA system was applied to detect PCT in clinical samples, and significant difference was found between the two groups. Conclusion Three monoclonal antibodies against human PCT with high titer and specificity have been successfully prepared, and an ELSIA system for detecting human PCT has been developed, which can be preliminarily used for clinical detection of PCT.

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