[1]唐波,黄定德,郑磊,等.噬菌体展示肽库筛选VPAC1高亲和力结合多肽[J].第三军医大学学报,2013,35(01):10-14.

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噬菌体展示肽库筛选VPAC1高亲和力结合多肽(PDF)

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Title: Screening of a high-affinity peptide specific for VPAC1 from phage display peptide library

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关键词: VPAC1; 稳定转染; 噬菌体展示肽库; 十二肽; 分子探针

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摘要: 目的 通过噬菌体展示肽库技术筛选VPAC1高亲和力结合十二肽配体，并进行特异性和亲和力鉴定。 方法 建立稳定表达VPAC1的真核细胞系；利用噬菌体肽库展示技术进行全细胞差减筛选，随机挑取40个噬菌体克隆进行测序，初步鉴定确定阳性克隆，明确外源十二肽序列，固相合成该十二肽；利用体外竞争结合ELISA及流式细胞分析，鉴定目标多肽与VPAC1受体结合的特异性及亲和力。 结果 成功构建稳定表达VPAC1细胞系；经过4轮差减筛选，回收噬菌体逐轮得到富集，随机挑取40个克隆测序，共得到15条序列不同的多肽，经ELISA鉴定有7个克隆与细胞有特异性高结合能力；阳性噬菌体克隆与细胞结合通过多肽VP1介导；VP1能特异高亲和地与VPAC1受体结合。 结论 通过噬菌体展示肽库技术成功筛选得到与VPAC1高亲和力特异结合十二肽。

Abstract: Objective To screen a high-affinity peptide specific for vasoactive intestinal peptide receptor VPAC1 using phage display peptide library, and to identify the specificity and affinity of the selected peptide. Methods A eukaryotic cell line stably expressing VPAC1 was established, four-round subtractive panning using phage display peptide library was conducted, and then forty phage clones were randomly picked out for sequencing. The positive phage clones were identified using cellular ELISA, and exogenous dodecapeptide was determined

and synthesized. *In vitro* competitive binding analysis and flow cytometry were performed to evaluate the binding specificity and affinity of target dodecapeptide. Results VPAC1 was stably expressed on transfected CHO-K1/VPAC1 cells. A significant enrichment was obtained through four rounds of panning, and fifteen different peptide sequences were identified from forty clones. Seven phage clones had specific and high affinity binding with positive cells through cellular ELISA analysis. The positive phage clones bound to CHO-K1/VPAC1 through candidate peptide VP1, and VP1 could specifically bind to VPAC1. Conclusion A high-affinity dodecapeptide specific for VPAC1 is obtained using phage display peptide library.

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唐波，黄定德，郑磊，等· 噬菌体展示肽库筛选VPAC1高亲和力结合多肽[J]·第三军医大学学报,2013,35(1):10-14.

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