系统性红斑狼疮儿童特异性全长抗体基因库的构建与分析(点击查看pdf全文)

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摘要: 目的构建系统性红斑狼疮（SLE）儿童个体特异性哺乳动物细胞表面展示的全长人抗体基因库。方法采集SLE确诊患
儿外周血，分离外周血淋巴细胞，提取外周血淋巴细胞总RNA，用RT-PCR扩增全长Kappa轻链（LCκ）和重链可变区（VH）基因、
构建抗体轻链基因库和抗体重链基因库，将抗体基因库转染293T细胞，流式细胞仪分析抗体在293T细胞表面的表达。结果以
0.8 μg总RNA为模板，用6套引物成功扩增全长Kappa轻链和重链可变区基因，插入表达载体，构建获得的重链基因库容量为
9.4×104，轻链基因库容量为8.4×104。随机挑选的10个重链克隆有8个含有正确的阅读框架，编码8个不同的氨基酸序列，10个
轻链克隆7个含有正确的阅读框架，编码7个不同的氨基酸序列。流式细胞仪分析挑选的单克隆和抗体基因库，均检测到全长
抗体在293T细胞表面的表达，理论上抗体库中可表达的抗体多样性可以达到109。结论以0.8 μg总RNA为模板，通过RT-PCR
扩增，成功构建SLE儿童特异性全长人抗体基因库，并在293T细胞表面成功展示SLE儿童个体抗体库中的全长抗体，为进一步
研究SLE儿童自身抗体的特点及自身抗体在SLE 发病机制中的作用及临床应用打下良好基础。

Abstract: Objective To construct a personalized full-length fully human antibody mammalian display library for children with
systemic lupus erythematosus (SLE). Methods The total RNA was isolated from the PBMCs of SLE children. The heavy chain
variable region and kappa light chain (VH and LCκ) of the antibody genes were amplified by RT-PCR and inserted into the
pDGB-HC-TM vector separately to construct the heavy chain and light chain libraries. The library DNAs were transfected into
293T cells and the expression of full-length fully human antibody on the surface of 293T cells was analyzed by flow cytometry.
Results Using 0.8 μg total RNA as the template, the VH and LCκ were amplified and the full-length fully human antibody

导航/NAVIGATE
本期目录/Table of Contents
下一篇/Next Article
上一篇/Previous Article

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mammalian display library was constructed. The VH and LCκ gene libraries had a size of 9.4×104 and 8.4×104, respectively.
Sequence analysis of 10 clones randomly selected from the VH and LCκ gene libraries each showed that 8 heavy chain clones
and 7 light chain clones contained correct open reading frames, and flow cytometry demonstrated that all the 15 clones express
full-length antibodies on 293T cell surfaces. 293T cells co-transfected with the VH and LCκ gene libraries expressed the
full-length antibodies on the cell surface. Conclusion The personalized full-length fully human antibody library for SLE
children constructed allows display of the full-length antibodies on mammalian cell surfaces, thus providing a valuable
platform for analyzing the autoantibodies, their etiological role, and their clinical implications in SLE.

参考文献/REFERENCES

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