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酵母双杂交筛选与弓形虫毒力因子ROP18 相互作用的宿主蛋白

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Screening of Host Cell Proteins that Interact with Toxoplasma gondii ROP18 via Yeast Two-hybrid System

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摘要目的利用酵母双杂交方法,筛选与弓形虫毒力因子ROP18相互作用的宿主蛋白。 方法 RT-PCR扩增弓形虫RH株速殖子ROP18基因片段,扩增产物经双酶切后插入酵母双杂交诱饵载体pGBKT7中,将重组质粒导入酵母AH109菌株中,检测诱饵载体有无毒性和自激活作用,利用酵母双杂交系统从人胚胎脑cDNA文库中筛选与ROP18相互作用的宿主蛋白。 结果 构建了诱饵载体pGBKT7?鄄ROP18,ROP18具有自激活功能。用ROP1825-251aa为诱饵,筛选获得一系列与ROP18相互作用的宿主蛋白: DNA损伤特异性结合蛋白1(DDB1)、torsin A相互作用蛋白1(TOR1AIP1)、整联蛋白β1(integrinβ1)、溶质运载蛋白3(SLC3A2)、酪氨酸硫化转移酶2(TPST2)、Derl样域家族成员2(DERL2)和OCIA结构域蛋白1(OCIAD1)。 结论 通过酵母双杂交技术筛选出与弓形虫毒力因子ROP18相互作用的多种宿主蛋白。

关键词: 弓形虫 ROP1825-251aa 酵母双杂交 相互作用 宿主蛋白

Abstract: Objective To screen the host cell proteins that can interact with Toxoplasma gondii ROP18 by using yeast two-Hybrid system. Methods The ROP18 gene fragments were amplified by RT-PCR from mRNA of T. gondii RH strain. The product of RT-PCR was digested with double restriction enzyme and was subcloned into the bait vector pGBKT7. The recombinant plasmid was transferred into yeast AH109 strain. Its toxicity and the autonomous activating activity were tested. The human fetal brain cDNA library was screened with pGBKT7-ROP1825-251aa as the bait plasmid by yeast two-hybrid system. Results The bait was constructed and AH109/PGBKT7-ROP18 showed an autonomous activity. The yeast strain AH109/pGBKT7-ROP1825-251aa line was then mated with the Mate & PlateTM Human Fetal Brain cDNA library. Using the selection procedures, eight novel host cell proteins were obtained: damage-specific DNA binding protein 1 (DDB1), torsin A interacting protein 1 (TOR1AIP1), integrin beta 1, solute carrier family 3 (SLC3A2), tyrosylprotein sulfotransferase (TPST2), OCIA domain containing 1 (OCIAD1), Der1-like domain family member 2 (DERL2), in addition to Homo sapiens activating transcription factor 6 beta (ATF6). Conclusion Eight novel host cell proteins have been obtained via yeast two-hybrid system, which can interact with TgROP18.

Keywords: Toxoplasma gondii; ROP1825-251aa; Yeast two-hybrid; Interaction; Host protein

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