



## 稳定表达SAV细胞的建立及其相互作用蛋白的筛选

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**摘要** 目的 建立能稳定表达 SAV (Salrador Homolog) 的人胚胎肾细胞 HEK293 细胞池并从中纯化出SAV相互作用蛋白复合物。方法 从人胚胎肾细胞 HEK293 中提取 RNA, RT-PCR 扩增得到SAV基因片断, 构建表达质粒 pBabe-SBP-FLAG-SAV。将 pBabe-SBP-FLAG-SAV质粒和病毒包装质粒共同转染 HEK293T 细胞来产生病毒, 收获病毒后感染 HEK293 细胞。通过嘌呤霉素筛选直至没有细胞死亡并用 Western-blot 验证表达情况。用链酶亲和素 beads 从该稳定细胞池中纯化SAV相互作用蛋白并通过银染检测。结果 pBabe-SBP-FLAG-SAV真核表达载体构建成功, 包装病毒感染 HEK293 后通过 Western-blot 检测可见SAV蛋白的表达, FLAG beads 和链酶亲和素 beads 免疫沉淀进一步证明了该融合蛋白表达的正确性。链酶亲和素纯化出的SAV相互作用蛋白复合物在银染时可见。结论 成功构建了 pBabe-SBP-FLAG-SAV真核表达载体及稳定表达细胞池, 纯化得到SAV相互作用蛋白复合物。为阐明这些蛋白复合体在 Hippo 信号通路中的作用提供了可靠信息。

**关键词:** SAV HEK293 稳定细胞池 相互作用蛋白

**Abstract:** OBJECTIVE To Establish HEK293 cell model in which SAV is stably expressing and purification of SAV interacting protein complex. METHODS RT-PCR was used to amplify SAV gene segment in HEK293 cells and then the PCR product was cloned into pBabe-SBP-FLAG vector with BamH I and EcoR I restriction enzyme cutting sites. Co-transfection of pBabe-SBP-FLAG-SAV and the packing plasmids into HEK293T cells to produce retroviruses which will be used for infection of HEK293 cells. Stable cell pool was selected by puromycine for 2 weeks and SAV expression was detected by Western-blot. SAV interacting protein complex was purified by streptavidin beads from the stable cell pool and virulized by silver staining. RESULTS pBabe-SBP-FLAG-SAV expression vector was successfully constructed. FLAG-SBP tagged SAV in HEK293 stable cell pool was detected by straight Western-blot and IP-Western-blot. SAV interacting protein complex was captured by streptavidin beads and some specific bands purified from SAV stable cell pool was visualized compare to control in silver staining. CONCLUSION pBabe-SBP-FLAG-SAV eukaryotic expression plasmid and the stable cell pool is successfully constructed and the interacting protein complex is purified by streptavidin beads, which provide a foundation for further investigation of SAV.

**Keywords:** SAV, HEK293, stable cell pool, protein complex

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[1] EDGAR B A. From cell structure to transcription: Hippo forges a new path[J]. *Cell*, 2006, 124 (2) : 267-273.

[2] PAN D. Hippo signaling in organ size control[J]. *Genes Dev*, 2007, 21 (8) : 886-897.

[3] XU T, WANG W, ZHANG S, et al. Identifying tumor suppressors in genetic mosaics: The Drosophila LATS gene encodes a putative







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protein kinase [J]. *Development*, 1995, 121 (7) : 1053-1063.

- [4] CHAN E H, NOUSIAINEN M, CHALAMALASETTY R B, *et al.* The Ste20-like kinase Mst2 activates the human large tumor suppressor kinase LATS1[J]. *Oncogene*, 2005, 24 (6) : 2076-2086. 
- [5] ZHAO B, WEI X M, LI W Q, *et al.* Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control[J]. *Genes Dev*, 2007, 21 (21) : 2747-2761. 
- [6] ZHAO B, YE X, YU J, *et al.* TEAD mediates YAP-dependent gene induction and growth control[J]. *Genes Dev*, 2008, 22 (14) : 1962-1971. 
- [7] LEI Q Y, ZHANG H, ZHAO B, *et al.* TAZ promotes cell proliferation and epithelial-mesenchymal transition and is inhibited by the hippo pathway[J]. *Mol Cell Biol*, 2008, 28 (7) : 2426-2436. 
- [8] ZHANG H, LIU C Y, ZHA Z Y, *et al.* TEAD Transcription Factors Mediate the Function of TAZ in Cell Growth and Epithelial-Mesenchymal Transition[J]. *J Biol Chem*, 2008, 284 (12) : 13355-13362.
- [9] MCCLATCHEY A I, GIOVANNINI M. Membrane organization and tumorigenesis-the NF2 tumor suppressor, Merlin[J]. *Chem Genes Dev*, 2005, 19 (19) : 2265-2277.
- [10] OVERHOLTZER M, ZHANG J, SMOLEN G A, *et al.* Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon Proc[J]. *Natl Acad Sci*, 2006, 103 (24) : 12405-12410. 
- [11] CHAN S W, LIM C J, GUO K, *et al.* A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells[J]. *Cancer Res*, 2008, 68 (8) : 2592-2598. 
- [12] TAPON N, HARVEY K F, BELL D W, *et al.* Salvador promotes both cell cycle exit and apoptosis in Drosophila and is mutated in human cancer cell lines[J]. *Cell*, 2002, 110(4): 467-78.
- [13] CAO Y F, YANG L, TAN X H, *et al.* AQP9 Protein Phosphorylation Decreases Arsenic Influx in Mammal Cells[J]. *Chin Pharm J* (中国药学杂志), 2012, 47(9): 679-683.

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