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TLR4全长及其截断体重组腺病毒对Rf-6A细胞骨架的影响

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为了研究LPS受体TLR4全长及其胞内段缺失的TLR4截断体（delta-TLR4）的绿色荧光蛋白重组腺病毒对内皮细胞系Rf-6A骨架蛋白的影响，采用PCR方法扩增目的基因片段，亚克隆至腺病毒穿梭质粒pAdTrack中，用BJ5183细菌同源重组法将目的基因重组于腺病毒骨架载体，重组腺病毒质粒经Pac Ⅰ酶切线性化后，用脂质体法转染293细胞进行腺病毒的包装扩增，用重组腺病毒感染Rf-6A细胞，采用免疫荧光标记方法观察结果。免疫荧光标记结果表明Ad-delta-TLR4明显抑制了LPS引起的细胞骨架F-actin的解聚与重排，Ad-TLR4则使LPS引起的F-actin应力纤维产生增强。以上结果说明TLR4全长及其截断体的重组腺病毒感染内皮细胞对LPS诱导的细胞骨架变化具有不同的影响，Ad-delta-TLR4对LPS引起的内皮细胞骨架变化具有抑制作用。

EFFECTS OF RECOMBINANT ADENOVIRUS OF FULL-LENGTH AND TRANCATED FORM OF TOLL-LIKE RECEPTOR 4 ON THE ORGANIZATION OF F-ACTIN IN Rf-6A ENDOTHELIAL CELLS

To investigate the effects of recombinant adenovirus that express full-length and trancated form of toll-like receptor 4 (TLR4 and delta-TLR4) on the rganization of F-actin in cultured endothelial cell line Rf-6A, the sequences of TLR4 and delta-TLR4 were amplified by PCR from a TLR4 containing plasmid pcDNA3-TLR4 followed by subcloning of the fragments into a adenovirus shuttle vector pAdTrack to form transfer plasmids, pAdTrack-TLR4 and pAdTrack-delta-TLR4. After linearization with pme Ⅰ, pAdTrack-TLR4 and pAdTrack-delta-TLR4 were cotransformed into BJ5183 bacteria that was pretransformed with adenovirus genomic plasmid of pAdEasy-1. The positive recombinants adenovirus plasmid were digested with Pac Ⅰ and transfected into HEK293 cells for the packaging of recombinant adenovirus particles. After infections of the resultant viruses in Rf-6A cells, LPS-induced F-actin depolymerization and reorganization was detected with fluorescence staining with rodamine-phalloidin. The results indicated that LPS stimulation induced F-actin depolymerization and formation of stress fibers. Recombinant adenoviral vectors containing full-length TLR4 intensified LPS-induced F-actin depolymerization, while delta-TLR4 recombinant adenovirus inhibited the depolymerization of F-actin induced by LPS in Rf-6A cells significantly. These results revealed that the infection of recombinant adenovirus of full-length and trancated form of TLR4 in cells showed different effects on LPS-induced cellular response. Ad-delta-TLR4 showed the protection of cultured endothelial cells from injury of LPS-induced depolymerization and eorganization of F-actin.

关键词