

斑马鱼VEGF基因的siRNA表达载体构建、有效序列筛选及其对基因表达的干预性研究

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为探索小干扰RNA (small interfering RNA, siRNA) 表达质粒在研究斑马鱼血管内皮生长因子 (vascular endothelial growth factor, VEGF) 基因调控网络中的应用, 构建了4个以斑马鱼VEGF基因为靶点的siRNA表达载体pS1-VEGF、pS2-VEGF、pS3-VEGF及pS4-VEGF。通过显微注射的方法将载体导入1-2细胞期斑马鱼体内, 于胚胎发育的48 h采用RT-PCR的方法检测VEGF基因的表达量, 研究不同干扰序列对VEGF基因表达的干涉作用。结果显示, 成功地构建了siRNA表达载体。针对不同位点的寡核苷酸序列抑制VEGF基因表达的效率有显著差异, 其中注射了pS1-VEGF的胚胎出现了心包膜水肿、血流速度减慢、循环红细胞堆积等症状, 同时肠下静脉、节间血管以及其它血管出现不同程度的发育缺陷。实验结果说明, pS1-VEGF可引起斑马鱼胚胎血管发育缺陷。

Construction and screening efficient VEGF siRNA expression vector in zebrafish

Four siRNA expression vectors targeting various locations of zebrafish VEGF (vascular endothelial growth factor) gene were constructed. The expression vectors were introduced into zebrafish embryos by pressure injection the solution to the blastodiscs of embryos at 1-2 cell stage, and the expression level of VEGF gene was determined by RT-PCR at 48 hpf (hours post fertilization). siRNAs targeting different locations showed diverse gene silence effects. Pericardial edema and erythrocyte accumulation, with reduced numbers of circulating red blood cells were observed following the injection of pS1-VEGF. Subintestinal veins and intersegmental vessels development defects were observed, too. It is suggested that delivery of pS1-VEGF resulted in vascular development defects in zebrafish.

关键词

RNA干扰(RNA interference); 斑马鱼(Zebrafish); 血管生成(Vascular development); 血管内皮生长因子(Vascular endothelial growth factor)