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研究报告

重组人纤溶酶原Kringle1-5的制备及其

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摘要 为了研究重组人纤溶酶原 Kringle1-5(K1-5)的抗血管生成活性及其对内皮细胞增殖的影响，通过PCR扩增人纤溶酶原K1-5 cDNA，定向克隆于原核表达载体pET30a(+)中,构建重组表达载体pET-K1-5，转化E.coli BL21(DE3)，IPTG诱导表达,SDS-PAGE 和Western杂交检测K1-5的表达。鸡胚尿囊膜 (CAM) 实验和MTT实验分别检测重组人纤溶酶原Kringle1-5对鸡胚新生血管生成和内皮细胞的抑制作用。结果表明,IPTG诱导原核表达载体pET-K1-5在E.coli BL21(DE3)中的表达量约占菌体总蛋白量的32%，K1-5主要以包涵体形式存在,包涵体经过洗涤、溶解、Ni-spin 亲合柱层析纯化以及蛋白质复性等步骤后,获得了纯度约为96%的重组K1-5蛋白。CAM实验表明，原核表达的重组人K1-5能有效地按剂量依赖的方式抑制鸡胚新生血管的形成。MTT实验结果显示，重组人K1-5特异地抑制内皮细胞的增殖，而对非内皮细胞无抑制作用。

关键词 Kringle1-5；纤溶酶原；肿瘤血管生成抑制剂；血管抑素；原核表达

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Preparation of Human Recombinant Kringle 1-5 and Its Bioactivity

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Abstract

To investigate antiangiogenesis activity and effects on endothelial cell proliferation of human recombinant K1-5 expressed in E.coli BL21, the cDNA of human K1-5 obtained from a cloning vector pUC57K1-5 by PCR, was inserted into an expression vector pET30 (+) to construct a prokaryotic expression vector pET-K1-5. Recombinant K1-5 efficiently expressed in E.coli BL21 after IPTG induction was monitored by SDS-PAGE and Western blotting with an anti-angiostatin monoclonal antibody. The expressed K1-5 accounted for approximately 32% of the total bacterial proteins as estimated by densitometry, and existed mainly as inclusion bodies. The inclusion bodies were washed, lysed and purified to a purity of 96% by the nickel affinity chromatography. Refoled K1-5 protein was tested on chicken CAMs, and a large number of newly formed blood vessels were significantly regressed. In the present study, we demonstrated that bacterial-expressed K1-5 effectively inhibited angiogenesis of the chicken embryo in a dose-dependent manner through CAM assay. In addition, human recombinant K1-5 potently inhibited endothelial cell proliferation with no inhibition on non-endothelial cells. Taken together, these findings demonstrated that human recombinant K1-5 effectively inhibited angiogenesis of the chicken embryo in a dose-dependent manner and specially suppressed in vitro the proliferation of human umbilical vein endothelial cells.

Key words Kringle1-5 plasminogen antiangiogenesis inhibitor angiostatin prokaryotic expression

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