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基础研究 BASIC RESEARCH

TFPI-2基因体外诱导胰腺癌Panc-1细胞的凋亡

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Apoptosis of Panc-1 pancreatic cancer cells induced by the TFPI-2 gene *in vitro*

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Abstract

AIM: To study the effect of the TFPI-2 gene on apoptosis of human pancreatic cancer cells.

METHODS: Recombinant pEGFP-C1-TFPI-2 was transfected into Panc-1 cells using liposomes. Positive cells were selected by G418. Expression of the TFPI-2 gene was determined by Reverse transcription-polymerase chain reaction(RT-PCR) and Western blotting. Cell growth and apoptosis were also studied.

RESULTS: Expression of TFPI-2 mRNA and protein was detected in Panc-1 cells transfected with pEGFP-C1-TFPI-2. Comparing Panc-1 cells transfected with vector and untransfected cells, the growth rate was lower and the apoptotic index of G418-resistant Panc-1 cells with the expression of TFPI-2 protein was faster(n = 9, P = 0.02).

CONCLUSION: The TFPI-2 gene can inhibit the growth of Panc-1 cells and cause cells to enter apoptosis.

Key Words: Pancreatic neoplasms; Apoptosis; TFPI-2; Western blotting; Reverse transcription-polymerase chain reaction

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摘要

目的: 将TFPI-2基因转染胰腺癌细胞系Panc-1细胞, 研究其对胰腺癌细胞凋亡的影响.

方法: 将重组质粒pEGFP-C1-TFPI-2通过脂质体介导转染胰腺癌细胞系Panc-1细胞, G418筛选获得阳性细胞克隆后, 用逆转录-聚合酶链反应(RT-PCR)和免疫印迹(Western blot)技术分别检测转染细胞中TFPI-2 mRNA及相应蛋白的表达, 同时测定转染细胞的生长曲线和凋亡情况.

结果: 同转染空载体及未转染细胞相比, 转染成功的Panc-1细胞可以检测到TFPI-2 mRNA和相应蛋白的表达, 细胞生长受到抑制(n = 9, P = 0.02), DNA琼脂糖凝胶电泳显示凋亡细胞特有的“梯状”条带.

结论: 外源性TFPI-2基因能够抑制胰腺癌细胞生长增殖、诱导胰腺癌细胞凋亡.

关键词: 胰腺肿瘤; 细胞凋亡; 组织因子途径抑制物2; 免疫印迹; 逆转录-聚合酶链反应

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0 引言

细胞凋亡(apoptosis)又称细胞程序性死亡(PCD), 是由基因调控的细胞自主的有序性死亡. PCD异常可导致多种疾病如肿瘤的发生[1]. TFPI-2基因位于人染色体7q22, 与多种肿瘤的发生有关[2-6]. 我们将外源性TFPI-2基因转染到

■背景资料
胰腺癌是消化道恶性程度最高的肿瘤之一, 其发病机制的研究成为近年来的热点. 组织因子途径抑制物2是新近发现的一种丝氨酸蛋白酶抑制剂, 在调控肿瘤细胞侵袭及转移、抑制肿瘤新生血管的形成及诱导肿瘤细胞凋亡中起重要作用. 近年来, 有关TFPI-2诱导肿瘤细胞凋亡的研究尚处于起步阶段, TFPI-2能否诱导胰腺癌细胞凋亡的实验研究国内外目前相关报道不多. 而且实验结果也不尽相同.

■相关报道
近年来研究发现, VEGF能够上调TFPI-2的表达, 进而降解丝氨酸的蛋白产物以结合细胞凋亡受体, 引导细胞程序性死亡, 这一过程中, 凋亡蛋白BAX、细胞色素C及半胱天冬酶3的表达水平增高, 而XIAP(X-linked inhibitor of apoptosis)、抗凋亡蛋白Bcl-2的表达水平降低, 从而导致肿瘤细胞凋亡率增加, 发挥其抗肿瘤效应.

人胰腺癌细胞系Panc-1细胞, 观察其对胰腺癌细胞凋亡的影响, 探讨TFPI-2基因抑制肿瘤细胞增殖的机制.

1 材料和方法

1.1 材料 人胰腺癌细胞系Panc-1购自中科院细胞库; TFPI-2基因真核表达载体pEGFP-C1-TFPI-2由本实验室构建; DMEM培养基购自Hyclone公司; RNA抽提试剂TRIzol Regent购自Gibco BRL公司; RT-PCR试剂盒购自Promega公司; DNA聚合酶pfu, dNTP 购自申能博彩公司; Lipofectamine2000转染试剂盒购自Invitrogen公司; 多克隆抗兔TFPI-2抗体、DBA显色试剂盒购自Santa Cruz公司; DNA抽提试剂盒购自Qiagen公司.

1.2 方法 细胞培养及转染Panc-1细胞用含100 g/L胎牛血清的DMEM培养基在37℃, 50 mg/mLCO_2的培养箱传代培养. 取对数生长期的细胞, 接种于12孔培养板, 每孔1×10^5, 培养24 h后, 用无血清的DMEM培养基洗涤两次以备转染. 采用Lipofectamine2000脂质体介导的基因转移技术将重组表达载体pEGFP-C1-TFPI-2转染到Panc-1细胞, G418筛选稳定表达TFPI-2的细胞株, 命名为Panc-1-TFPI-2. 转染空载体和未转染细胞为对照组, 分别命名为Panc-1-V和Panc-1-P. 人TFPI-2基因PCR扩增的上下游引物分别为: 5'CAGAATTCTATGGACCCCGCTCGCCCC3'和5'CAGTCGACTTAAAATTGCTTCTTCCG3', 扩增产物为708 bp. β-actin作为内参照, 上下游引物分别为5'GCTCGTCGTCGACAACGGCT3'和5'CAAACATGATCTGGGTCATCTTCTC3', 扩增产物为353 bp. 按照TRIzol说明书分别从3组细胞中提取总RNA, 紫外分光光度计检测其浓度和纯度. 采用两步法RT-PCR合成TFPI-2 DNA, 取扩增产物进行凝胶电泳, 紫外透视仪检测结果并摄像.

1.2.1 Western blot检测 动分别提取3组细胞的细胞外基质(ECM)蛋白[7], 测定蛋白浓度, 制备120 g/L SDS-PAGE凝胶, 并进行蛋白质电泳, 电泳分离后, 恒压转移到PVDF膜上, 用含5 g/L脱脂牛奶的TBST缓冲液封闭, 4℃过夜. TBST1/200稀释抗TFPI-2多抗, 室温培育1.5 h, TBST洗5次, 再用HRP标记的抗鼠二抗培育1 h, 显色, 显影. β-actin作为内参.

1.2.2 细胞生长曲线测定 将3组细胞Panc-1-TFPI-2, Panc-1-V和Panc-1-P无血清培养24 h, 使细胞同步化. 取2×10^4细胞接种于24孔板, 每隔24 h消化3组细胞并计数, 取平均值. 共检测7 d, 以细胞数为纵坐标, 时间为横坐标绘制生长曲线.

1.2.3 DNA片段化实验 分别收集3组细胞约1×10^7, 用PBS洗1次, 随后加入含蛋白酶K和RNase的细胞裂解液500 μL, 混匀后于50℃水浴5 h; 加酚：氯仿：异戊醇0.5 mL抽提; 离心后上清移至另一离心管, 加0.5 mL氯仿：异戊醇抽提; 离心后上清移至另一离心管, 然后加3 mol/L乙酸钠10 μL和预冷无水乙醇1 mL, 混匀后置液氮5 min, 沉淀DNA, 离心去上清, 经700 mL/L乙醇洗涤后置室温干燥10 min, 加TE缓冲液30 μL溶解DNA. 20 g/L琼脂糖凝胶电泳, EB染色观察, 凝胶图像分析仪拍摄照片[8].

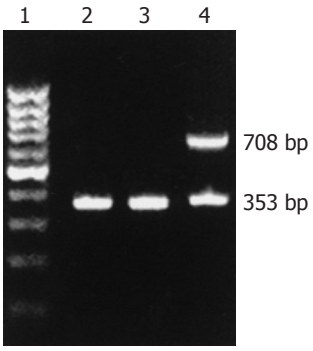

图 1 转染后TFPI-2 mRNA表达. 1: 100 bp DNA Ladder Marker; 2: Panc-1-V; 3: Panc-1-P; 4: Panc-1-TFPI-2.

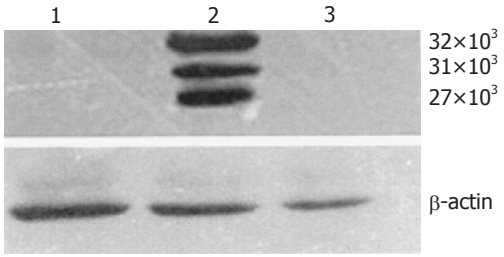

图 2 转染pEGFP-C1-TFPI-2细胞中TFPI-2蛋白表达. 1: Panc-1-P; 2: Panc-1-TFPI-2; 3: Panc-1-V.

2 结果

2.1 TFPI-2 mRNA和蛋白的检测 TRIzol法提取总RNA, 采用RT-PCR检测3组细胞中TFPI-2基因的表达. 结果显示, Panc-1-TFPI-2组细胞有708 bp的扩增条带, 而Panc-1-V和Panc-1-P组细胞未见明显条带(图1). Panc-1-TFPI-2组细胞经Western blot证实有32 kDa, 31 kDa和27 kDa 3种分子质量的TFPI-2蛋白的表达, 而Panc-1-V和Panc-1-P组细胞无任何一种蛋白的表达(图2).

2.2 TFPI-2基因对胰腺癌细胞生长的影响 Panc-1-TFPI-2组细胞较Panc-1-V和Panc-1-P组细胞生长明显减慢(图3).

2.3 Panc-1细胞凋亡 Panc-1-TFPI-2组细胞抽提

■创新盘点
本研究证实TFPI-2能够在体外诱导胰腺癌细胞Panc-1的凋亡, 为进一步研究TFPI-2在胰腺癌细胞增殖中的作用奠定了基础.

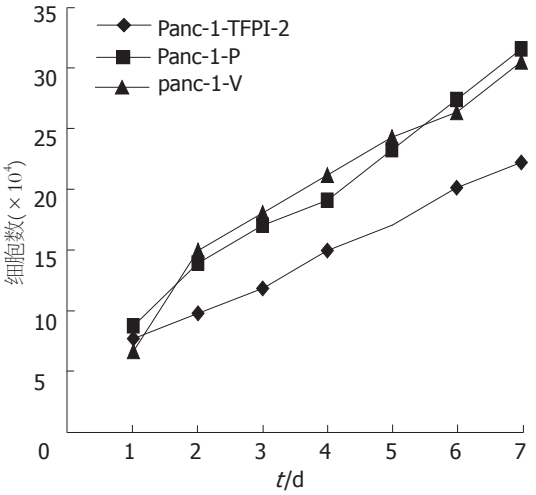

图 3 Panc-1细胞的生长曲线.

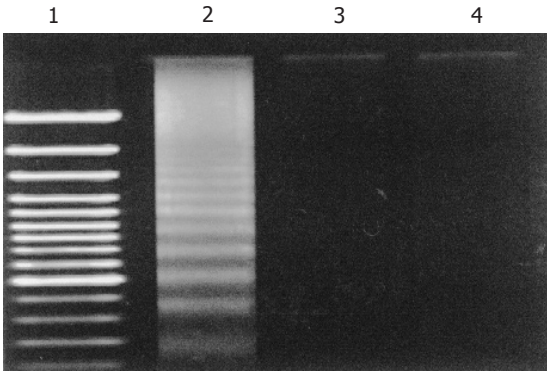

图 4 DNA片段化实验. 1: DNA Maker; 2: Panc-1-TFPI-2; 3: Panc-1-P; 4: Panc-1-V.

■应用要点

TFPI-2能够诱导胰腺癌细胞的凋亡, 这为进一步研究其作用机制, 为研发新的抗肿瘤药物及其在抗癌领域的临床应用奠定了实验基础.

DNA, 琼脂糖凝胶电泳, 出现“梯状”条带, 而Panc-1-V和Panc-1-P组细胞仅在电泳加样孔附近出现基因组条带, 未出现“梯状”条带, 说明转染细胞存在细胞凋亡现象(图4).

3 讨论

TFPI-2基因位于人染色体7q22, 其编码蛋白也称胎盘蛋白-5, 是新近发现的一种丝氨酸蛋白酶抑制物, 能降解多种丝氨酸蛋白[9-13]. 通过对神经胶质细胞瘤、绒毛癌、肺小细胞癌等肿瘤的研究, 发现TFPI-2具有抗肿瘤和抑制肿瘤血管生成等生物学功能[14-19]. 有实验证实将组织金属蛋白抑制物(tissue inhibitor of metalloproteinase, TIMP)转染细胞后可明显增加细胞的凋亡率[20-22], 而同只有胶原酶抑制作用TIMP相比, TFPI-2的蛋白酶抑制谱更广, 抑制能力更强, 有可能在诱导癌细胞凋亡中发挥重要作用. 细胞凋亡与多种基因改变有关, 在肿瘤的发生发展过程中起重要作用. 其发生受两条独立的信号转导途径的调节: 一条是通过Fas和TNF受体介导的级联反应的调节; 另一条是通过线粒体依赖的caspase级联反应的调节. 在这一过程中, 细胞色素C易位, Bax/Bcl-2蛋白比例发生改变, Bax蛋白对凋亡的执行来说是必需的, 其与Bcl-2蛋白比例的变化对该途径具有调节作用[23-26]. 另有研究表明抗凋亡基因XIAP在肿瘤发生过程中表达上调, 从而促进肿瘤的恶性生物学行为. Baker *et al*[27]研究证实, TFPI-2可以降解丝氨酸的蛋白产物以结合细胞凋亡受体, 引导细胞程序性死亡. TFPI-2能够调节白介素8的表达, 在TNF诱导下, 白介素8通过核因子kappaB信号途径调控肿瘤细胞的凋亡. 此外, TFPI-2亦可通过caspase途径调控肿瘤细胞的凋亡, 在这一过程中, 凋亡蛋白BAX、细胞色素C及半胱天冬酶3的表达水平增高, 而XIAP(X-linked inhibitor of apoptosis)、抗凋亡蛋白Bcl-2的表达水平降低, 从而导致肿瘤细胞凋亡率增加[28-30].

胰腺癌是常见的恶性肿瘤, 近年来发病率逐年增加. 有关胰腺癌发病机制目前还不十分清楚. 随着分子生物学技术的发展, 与胰腺癌有关的凋亡基因不断被发现和克隆, 为胰腺癌发病机制的探讨、诊断和治疗提供了新靶点. 我们通过脂质体介导将外源性TFPI-2基因转染到胰腺癌细胞系Panc-1细胞, 经RT-PCR及Western blot方法证实TFPI-2基因mRNA和相应蛋白可以在转染细胞内稳定表达. 说明能成功的把TFPI-2基因转染到胰腺癌细胞Panc-1中, 建立稳定、高表达TFPI-2蛋白的胰腺癌细胞模型. 转染成功细胞生长曲线显示, 细胞生长速度减慢, 细胞生长受到抑制; 抽提DNA电泳呈典型的凋亡细胞特有的不连续“梯状”条带, 说明TFPI-2基因能诱导人胰腺癌细胞的凋亡, 从而起到抑癌作用, 其具体机制有待进一步研究. TFPI-2基因诱导细胞凋亡的研究尚少, 具有广阔的应用前景. 在后续的实验中, 我们将继续研究TFPI-2基因在胰腺癌实体瘤中的表达, 进一步探讨TFPI-2基因在胰腺癌细胞凋亡中的作用及其机制.

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■同行评价

本文通过脂质体介导将外源性TFPI-2基因转染到胰腺癌细胞系Panc-1细胞, 用RT-PCR和Western blot技术分别检测转染细胞中TFPI-2 mRNA及相应蛋白的表达情况, 同时测定转染细胞的生长曲线和细胞凋亡情况, 说明TFPI-2基因能诱导人胰腺癌细胞的凋亡, 从而起到抑癌作用. 该试验选题符合该领域的研究热点, 设计科学、合理, 作者掌握了相关的实验基础理论和专门实验技术, 正确统计学方法, 结论可靠.

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