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HPIP基因siRNA对TGF-β1诱导的肾小管上皮细胞上皮-间充质转化及凋亡的影响

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[摘　要]　**目的：**探讨抑制HPIP基因表达对TGF-β1诱导的肾小管上皮细胞上皮-间充质转化(epithelial-mesenchymal transition，EMT)及凋亡的影响。**方法：**10 ng/mL的TGF-β1刺激HK-2细胞24，48 h，Western印迹法检测HPIP蛋白表达。将HK-2细胞随机分为空白组、TGF-β1组和TGF-β1+si-HPIP组，处理24 h后，Western印迹法检测HPIP，E-cadherin，α-SMA，N-cadherin，Snail，Twist，t-AKT，p-AKT和Bax蛋白表达；流式细胞术检测细胞凋亡率。**结果：**TGF-β1刺激HK-2细胞24，48 h后HPIP蛋白表达均显著高于对照组(0 h) ($P<0.05$)。TGF-β1组E-cadherin蛋白表达显著低于空白组，α-SMA，N-cadherin，Snail，Twist，p-AKT和Bax蛋白表达及细胞凋亡率均显著高于空白组($P<0.05$)，而TGF-β1+si-HPIP组E-cadherin蛋白表达显著高于TGF-β1组，α-SMA，N-cadherin，Snail，Twist，p-AKT和Bax蛋白表达及细胞凋亡率均显著低于TGF-β1组($P<0.05$)。**结论：**抑制HPIP基因表达可延缓肾小管上皮细胞EMT进程和降低细胞凋亡率，机制可能与下调PI3K/AKT信号有关。

[关键词]　肾小管上皮细胞；HPIP基因；TGF-β1诱导；上皮-间充质转化；凋亡

Effect of HPIP gene siRNA on epithelial-mesenchymal transition and apoptosis induced by TGF-β1 in renal tubular epithelial cells

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Abstract　**Objective:** To investigate the effect of HPIP gene expression was inhibited on the epithelial-mesenchymal transition (EMT) and apoptosis of renal tubular epithelial cells induced by TGF-β1. **Methods:** After HK-2 cells

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were stimulated with 10 ng/mL TGF-β1 for 24 h and 48 h, Western blot was used to detect the expression of HPIP protein. HK-2 cells were randomly divided into a blank group, a TGF-β1 group and a TGF-β1 + si-HPIP group. The cells were treated with 24 h, Western blot were used to detect HPIP, E-cadherin, α-SMA, N-cadherin, Snail, Twist, t-AKT, p-AKT and Bax protein expression. The apoptosis rate of cells was detected by flow cytometry. **Results:** The expression of HPIP protein in HK-2 cells stimulated by TGF-β1 for 24 h and 48 h was significantly higher than that in the control group (0 h) (P<0.05). The expression of E-cadherin protein in TGF-β1 group was significantly lower than that in the blank group, the expression of α-SMA, N-cadherin, Snail, Twist, p-AKT, Bax protein and cells apoptosis were significantly higher than those in the blank group (P<0.05), but the expression of E-cadherin protein in TGF-β1+si-HPIP group was significantly higher than those in TGF-β1 group, the expression of α-SMA, N-cadherin, Snail, Twist, p-AKT protein and cells apoptosis were significantly lower than those in TGF-β1 group (P<0.05). **Conclusion:** Inhibition of HPIP gene expression can reduce the EMT process and apoptosis rate of renal tubular epithelial cells, which may be related to downregulation of PI3K/AKT signaling.

Keywords renal tubular epithelial cells; HPIP gene; TGF-β1 induction; epithelial-mesenchymal transition; apoptosis

肾间质纤维化(renal interstitial fibrosis，RIF)是多种慢性肾脏疾病进展至终末期肾衰竭的共同病变过程，其轻重程度可反映肾脏疾病肾功能的恶性程度[1]。小管上皮细胞损伤和损伤后反应可能是推动和启动RIF的一个主要因素[2]。肾小管上皮-间充质转化(epithelial-mesenchymal transition，EMT)是RIF发生的中心环节，受细胞因子、生长因子等的调节[3]。TGF-β1是引起肾小管上皮细胞转分化的一个重要的细胞因子[4]。此外，肾小管上皮细胞凋亡在RIF发生发展中也发挥重要作用，TGF-β1可诱导肾小管上皮细胞凋亡[5]。因此，抑制肾小管上皮细胞转分化及凋亡具有重要意义。人造血相关的PBX相互作用蛋白质(hematopoietic PBX-interacting protein，HPIP)是以前B淋巴细胞白血病转录因子(pre-B-cell leukemia transcription factor 1，PBX1)为诱饵，通过酵母双杂交技术，从胎儿肝cDNA文库中筛选获得而命名，目前在肿瘤中研究较多，其表达与人类肿瘤发生发展存在密切关系[6-7]。HPIP在RIF中的研究极少，miR-152可靶向HPIP抑制小管上皮细胞EMT进程[8]。本研究旨在探讨抑制HPIP表达对肾小管上皮细胞EMT及凋亡的影响及机制。

1 材料与方法

1.1 细胞株

人肾小管上皮细胞株HK-2购自美国ATCC公司。细胞常规复苏后，用含10%FBS的DMEM/F12培养基，于37 ℃，5%CO_2培养箱中传代培养。

1.2 试剂与仪器

DMEM/F12培养液、胰酶均购自美国Gibco公司；重组人TGF-β1购自美国R&D System公司；Annexin V-FITC/PI试剂盒购自江苏碧云天生物技术研究所；HPIP，E-cadherin，α-SMA，N-cadherin，Snail，Twist，t-AKT，p-AKT和Bax抗体均购自美国Cell Signaling公司；流式细胞仪购自美国Beckman Coulter公司。

1.3 TGF-β1诱导的HK-2细胞HPIP蛋白表达

10 ng/mL的TGF-β1刺激HK-2细胞0，24和48 h后，采用适量的放射免疫沉淀法(radio-immunoprecipitation assay，RIPA)裂解液提取细胞总蛋白，二喹啉甲酸(bicinchoninic acid，BCA)法检测上清液中蛋白浓度，取40 μg蛋白上样，每孔道等量，经10%SDS-PAGE分离蛋白，半干法转蛋白至PVDF膜，5%脱脂奶粉封闭膜1 h，加HPIP(1∶500)和内参GAPDH(1∶1 000)抗体，4 ℃孵育过夜，洗膜，加1∶3 000稀释的羊抗鼠辣根过氧化物酶(horseradish peroxidase，HRP)标记的抗体，洗膜，增强型化学法(enhanced chemiluminescence，ECL)作用5 min，曝光10 s~3 min，冲片，图片分析系统分析杂交信号吸光度值。实验重复3次。

1.4 实验分组及处理

常规培养HK-2细胞，细胞达75%~85%汇合度后，无血清培养基同步处理细胞24 h，随机分为3个处理组：正常对照组(空白组)、TGF-β1组(使用10 ng/mL的TGF-β1刺激细胞)和TGF-β1+si-HPIP

组(培养液中加入TGF-β1后转染si-HPIP)。处理24 h后，收集细胞，用于后续实验研究。

1.5 siRNA转染HK-2细胞

以1×10^5个/mL浓度接种HK-2细胞于6孔细胞培养板，于37 ℃，5%CO_2培养箱中培养24 h，观察到培养板铺满80%的细胞时，弃掉培养基，每孔加无血清及双抗的DMEM/F12培养基过夜。按照脂质体Lipofectamine 2000说明书，制备si-HPIP-Lip 2000混合物，阴性对照siRNA(NC)与Lipofectamine 2000混合物制备与此相同，将混合物加入至6孔板相应孔中，仅加入Lipofectamine 2000的为空白组，轻摇6孔板使之混匀，于37 ℃，5%CO_2培养箱中孵育4~6 h，弃掉培养液继续培养。

1.6 HPIP，E-cadherin，α-SMA，N-cadherin，Snail和Twist，t-AKT，p-AKT和Bax蛋白表达检测

方法参照1.3。

1.7 细胞凋亡率检测

采用Annexin V-FITC/PI双染法，通过流式细胞仪检测细胞凋亡率。接种HK-2细胞于6孔板，细胞数达70%~80%时，换为无血清的DMEM/F12培养基培养细胞24 h，再换为含血清的培养基，按照1.4分组及方法处理细胞，每组6个复孔，24 h后收集细胞，PBS洗涤细胞，胰酶消化后重悬细胞，取细胞悬液加195 μL Annexin V-FITC结合液(1×)，轻轻重悬细胞，再加5 μL Annexin V-FITC，轻柔混匀，避光室温(20 ℃~25 ℃)孵育10 min，离心，加190 μL Annexin V-FITC结合液(1×)轻轻重悬细胞，再加10 μL的PI，轻柔混匀，冰浴避光放置，随即使用流式细胞仪进行检测。实验重复3次。

1.8 统计学处理

实验数据采用SPSS 21.0软件进行分析，计量资料用均数±标准差($\bar{x}\pm s$)表示，多组差异比较采用单因素方差分析，两两比较采SNK-q检验，$P<0.05$为差异有统计学意义。

2 结果

2.1 TGF-β1诱导的HK-2细胞HPIP蛋白表达

TGF-β1刺激HK-2细胞24，48 h后HPIP蛋白表达分别为0.341±0.036和0.472±0.053，均显著高于实验前水平(0.063±0.008，$P<0.05$；图1)。

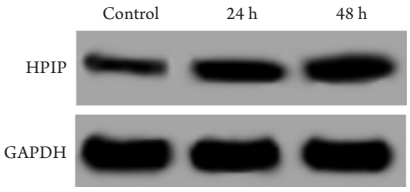

图1 TGF-β1诱导的HK-2细胞HPIP蛋白表达

Figure 1 Expression of HPIP protein in HK-2 cells induced by TGF-β1

2.2 siRNA转染HK-2细胞效果

si-HPIP组HPIP蛋白表达显著低于空白组($P<0.05$)，而NC组与空白组HPIP蛋白表达差异无统计学意义($P>0.05$；图2)。

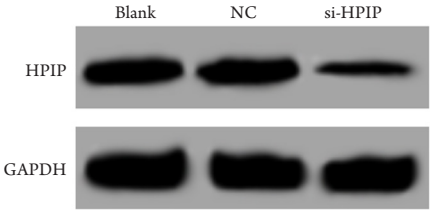

图2 si-HPIP转染HK-2细胞后HPIP蛋白表达

Figure 2 Expression of HPIP protein after si-HPIP was transfected into HK-2 cells

2.3 si-HPIP降低TGF-β1诱导的HK-2细胞EMT过程

TGF-β1组E-cadherin蛋白表达显著低于空白组，α-SMA，N-cadherin，Snail和Twist蛋白表达显著高于空白组($P<0.05$)，而TGF-β1+si-HPIP组E-cadherin蛋白表达显著高于TGF-β1组，α-SMA，N-cadherin，Snail和Twist蛋白表达显著低于TGF-β1组($P<0.05$，图3，表1)。

2.4 si-HPIP降低TGF-β1诱导的HK-2细胞凋亡率

TGF-β1组(25.21±1.11)%细胞凋亡率显著高于空白组(1.83±0.16)%，而TGF-β1+si-HPIP组(11.47±0.78)%细胞凋亡率显著低于TGF-β1组($P<0.05$；图4)。

2.5 si-HPIP下调TGF-β1诱导的HK-2细胞PI3K/AKT信号

TGF-β1组p-AKT和Bax蛋白表达均显著高于空白组($P<0.05$)，而TGF-β1+si-HPIP组p-AKT和Bax蛋白表达均显著低于TGF-β1组($P<0.05$)。3组间t-AKT蛋白表达差异无统计学意义($P>0.05$；表2，图5)。

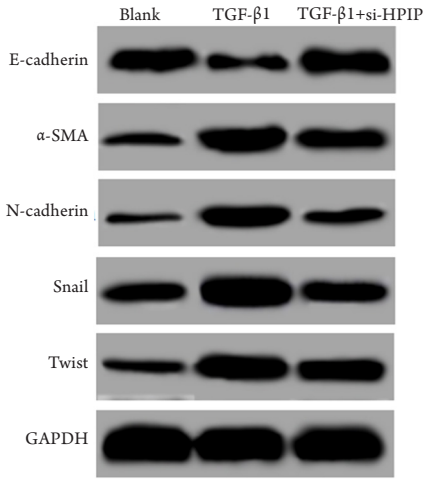

图3 si-HPIP对TGF-β1诱导的HK-2细胞EMT过程的影响

Figure 3 Effect of si-HPIP on EMT process induced by TGF-β1 in HK-2 cells

表1 E-cadherin，α-SMA，N-cadherin，Snail和Twist蛋白相对表达量

Table 1 relative expressions of E-cadherin, α-SMA, N-cadherin, Snail, and Twist proteins

组别	E-cadherin	α-SMA	N-cadherin	Snail	Twist
空白组	0.382 ± 0.042	0.051 ± 0.008	0.035 ± 0.004	0.152 ± 0.017	0.062 ± 0.008
TGF-β1组	0.120 ± 0.014*	0.411 ± 0.045*	0.319 ± 0.028*	1.243 ± 0.087*	0.555 ± 0.048*
TGF-β1+si-HPIP组	0.353 ± 0.038#	0.224 ± 0.023#	0.114 ± 0.016#	0.267 ± 0.031#	0.302 ± 0.032#
F	54.542	111.439	183.128	366.722	161.258
P	<0.001	<0.001	<0.001	<0.001	<0.001

*P<0.05 vs 空白组；#P<0.05 vs TGF-β1组。

*P<0.05 vs blank group; #P<0.05 vs TGF- β1 group.

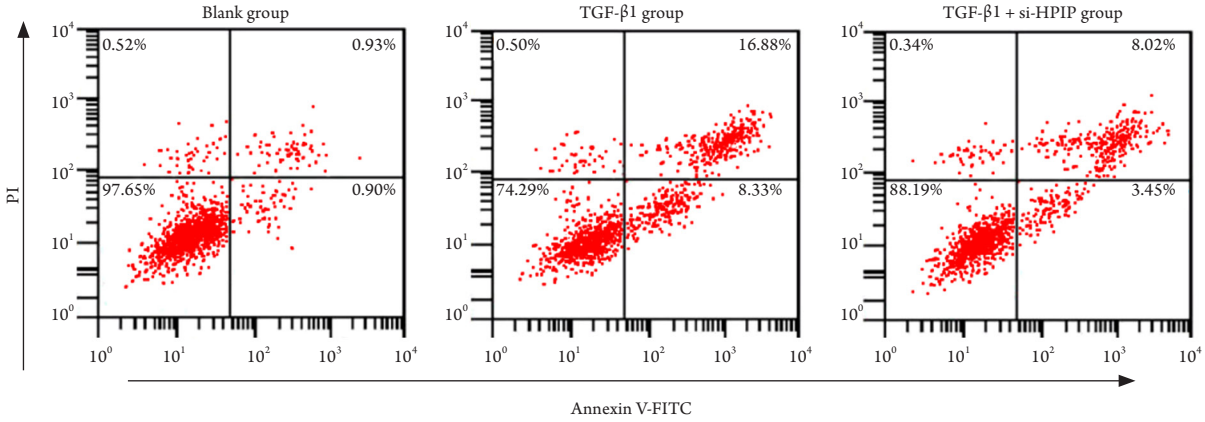

图4 si-HPIP对TGF-β1诱导的HK-2细胞凋亡的影响

Figure 4 Effect of si-HPIP on apoptosis of HK-2 cells induced by TGF-β1

表2 p-AKT，t-AKT和Bax的蛋白相对表达量

Table 2 Relative expression of p-AKT, t-AKT, and Bax proteins

组别	p-AKT	t-AKT	Bax
空白组	0.052 ± 0.007	0.411 ± 0.038	0.104 ± 0.015
TGF-β1组	0.175 ± 0.021*	0.434 ± 0.043	0.382 ± 0.039*
TGF-β1+si-HPIP组	0.094 ± 0.012#	0.451 ± 0.045	0.136 ± 0.016#
F	55.491	0.682	104.014
P	<0.001	0.541	<0.001

*P<0.05 vs 空白组；#P<0.05 vs TGF-β1组。

*P<0.05 vs blank group; #P<0.05 vs TGF- β1 group.

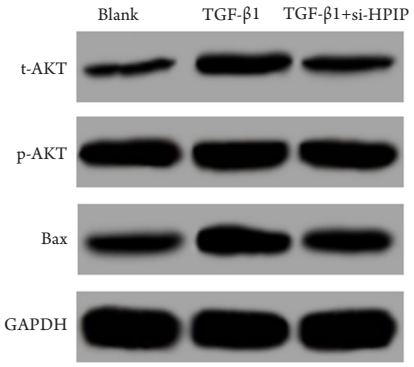

图5 si-HPIP对TGF-β1诱导的HK-2细胞PI3K/AKT信号的影响

Figure 5 Effect of si-HPIP on PI3K/AKT signal induced by TGF-β1 in HK-2 cells

3 讨论

TGF-β是一个可发挥多重生物学效应的细胞因子，可引起细胞的增殖及细胞外基质沉积，其表达高低与由多种因素引起的RIF存在密切关系，已被认为是目前引起RIF的一个最重要因子[9]。HPIP是近期发现的人类蛋白，编码的基因位于1p21.3染色体上，近些年的研究多集中于肿瘤方面。已有多项研究[10-11]显示HPIP在肝癌、肺癌等肿瘤有超表达，其表达影响肿瘤发生、浸润、迁移等。HPIP在RIF发生发展中的作用研究较少。TGF-β1可诱导HPIP上调表达，miR-152可通过靶向HPIP调控TGF-β1诱导的小管上皮细胞EMT进程[8]。本研究首先用10 ng/mL的TGF-β1刺激HK-2细胞24和48 h，细胞中HPIP的表明均明显升高，这与前人研究[8]结果一致。

EMT的主要表现为：上皮细胞表型标志物E-cadherin，cytokeratin等丢失；vimentin，间质细胞表型标志α-SMA，N-cadherin及collagen I等增加[12]。E-cadherin是EMT的关键分子，α-SMA是EMT的主要分子标志之一，TGF-β1可通过调控EMT相关蛋白表达，从而诱导肾小管上皮细胞EMT[13]。此外，TGF-β1可诱导肾小管上皮细胞凋亡，而过表达Sirt1可抑制细胞凋亡[14]。RNA干扰(RNAi)是近些年发展起来的一项用于研究基因功能的新技术，由于其对基因表达抑制的高效性、特异性而迅速成为重要的基因沉默方法[15]。本研究结果显示：TGF-β1可上调肾小管上皮细胞α-SMA，下调E-cadherin表达，并诱导细胞凋亡，而通过RNAi技术抑制HPIP表达后细胞中E-cadherin表达升高，α-SMA，N-cadherin，Snail和Twist表达降低，细胞凋亡率降低。本研究结果提示抑制HPIP表达可能通过降低肾小管上皮细胞EMT进程及抑制细胞凋亡，从而对减弱RIF损伤。

近几年的研究[16-17]表明：在TGF-β1诱导的EMT过程中需要一些重要的信号途径参与，如Smad，Notch，Wnt，PI3K/AKT信号等。PI3K是生长因子受体超家族信号转导过程的一个重要成员，AKT是PI3K重要的下游因子，其被激活后可通过影响与细胞增殖相关的foxo，凋亡相关的Bax，Bcl-2及新陈代谢相关的GSK3β等一系列细胞因子，进而影响细胞生存、凋亡、代谢等过程[18-19]。PI3K/AKT信号与TGF-β1的一些功能存在密切关系，如TGF-β1刺激后的肾小球系膜细胞p-AKT表达明显升高，而PI3K/AKT信号抑制剂能降低TGF-β1诱导的I型胶原表达的升高[20]。抑制PI3K/AKT信号可降低肾小管上皮细胞凋亡[21]。本研究结果显示抑制HPIP表达可降低磷酸化的AKT及Bax表达，提示HPIP对肾小管上皮细胞EMT进程及凋亡的影响可能与PI3K/AKT信号有关。

综上所述，本研究发现抑制HPIP表达可降低肾小管上皮细胞EMT进程及细胞凋亡率，机制可能是下调PI3K/AKT信号。本研究可能为RIF的基

因治疗提供了一定的理论基础。目前关于HPIP在肾病方面的研究较少，值得进一步深入探讨。

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