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硕 士 学 位 论 文

肝细胞核因子 4 α 在肾癌上皮间质转化中的
作用

Epithelial-mesenchymal Transition in Renal Cell Carcinoma:
The Role of Hepatocyte Nuclear Factor 4 α

谭伟

指导教师姓名: 刘荣福 教授

专 业 名 称: 外科学 (泌尿外科)

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摘要

【背景及目的】

肾癌 (renal cell carcinoma, RCC) 是起源于肾小管上皮细胞的恶性肿瘤, 占肾脏恶性肿瘤的 80%~90%, 近年来发病率呈上升趋势。除传统手术治疗外, 缺乏其它的有效治疗方法。上皮间质转化(epithelial-mesenchymal transition, EMT) 是上皮细胞在细胞结构、形态、功能等方面获得间质细胞特性的过程, 在肿瘤的发生、浸润、转移过程中具有重要作用, 核心为上皮标志物 E-cadherin 表达降低和间质标志物表达增高。肝细胞核因子 4 α (hepatocyte nuclear factor 4 α , HNF4 α)是调节多种基因转录的重要核因子, HNF4 α 参与维护肝细胞脂肪代谢、白蛋白合成、药物解毒、能量代谢、胆汁酸合成等重要功能, 也是调控与维持肝细胞上皮表型的重要基因。我们通过研究 HNF4 α 在肾癌患者及其癌旁正常组织的表达情况, 发现肾癌组织中 HNF4 α 表达较正常组织中明显下降, 且晚期肾癌患者下降水平显著。为了研究 HNF4 α 在肾癌组织中表达水平降低的意义, 本课题将着重探讨 HNF4 α 对肾癌生物学行为的影响及其分子机制, 为 HNF4 α 防治肾癌的进一步研究奠定基础。

【实验方法】

本研究通过 Real-time PCR 及免疫组化染色分别检测肾癌组织及正常组织中 HNF4 α 在 mRNA 水平及蛋白水平表达的变化, 分析其表达差异及与肾癌分期等临床病理特征的关系。进而构建过表达 HNF4 α 基因的慢病毒载体 pBobi-puro-HNF4 α , 与对照质粒 pBobi-puro-GFP 在 293FT 细胞中进行病毒包装获得病毒颗粒, 分别用于感染人肾癌细胞株 (OS-RC-2)。利用细胞划痕实验、Transwell 侵袭实验分别观察 HNF4 α 表达上调对肾癌细胞的细胞形态及细胞迁移、侵袭等生物学行为的影响, 流式细胞术检测癌细胞凋亡情况, 并用 Western Blotting 技术进一步分析 EMT 相关标志物及 Snail、MMP-7、Cleaved-parp 等基因表达水平的改变。

【实验结果】

约 73.3%的患者肾癌样本中 HNF4 α mRNA 表达水平较癌旁正常组织降低, 免疫组化染色显示 HNF4 α 在正常肾脏组织肾小管细胞核内高表达, 而肾癌组织

中正常肾小管结构紊乱甚至消失，癌细胞核内 HNF4 α 表达明显降低，在晚期患者中尤其明显。通过将外源 HNF4 α 基因导入肾癌细胞后，肾癌细胞出现了迁移、侵袭能力的降低，细胞形态发生改变并诱发了癌细胞凋亡，与其相关的 MMP-7、Cleaved-Parp 基因表达出现相应改变，EMT 的重要促进因子 Snail 表达下降，同时伴有 EMT 上皮标志物 E-cadherin 表达增高及间质标志物 Vimentin 表达降低。

【结论】

1. HNF4 α 在肾癌上皮间质转化过程具有非常重要的意义，肾癌组织中 HNF4 α 表达的下降与肾癌临床分期及组织学分级相关。
2. 在肾癌细胞中过表达 HNF4 α 可以明显改善肾癌细胞的恶性生物学行为，使之呈现类似 MET（间质上皮转化）的表现。

关键词：肝细胞核因子 4 α (HNF4 α) 肾癌 (RCC) 上皮间质转化 (EMT)

Abstract

【Background and Objective】

Renal cell carcinoma (RCC) is the most common malignant tumor arising in the kidney, which originates from renal tubular epithelial cells. Its incidence rate showed an upgrade trend in recent years. The common therapeutic approach is conventional surgery, but it has been lack of other effective therapies for a long time. Epithelial-mesenchymal transition (EMT) is a complex process which make epithelial cell acquire characteristic of mesenchymal cell and change cytoarchitecture, cell morphology and cell function. EMT is extremely important for tumorigenesis, tumor invasion and tumor metastasis, The key incident are the reduction of epithelial marker E-cadherin and increase expression of mesenchymal markers such as Vimentin. Hepatocyte nuclear factor 4 α (HNF4 α) is a member of nuclear receptor superfamily and regulates transcription of a variety of genes. The important role of HNF4 α is found in hepatic fat metabolism, serum protein production, xenobiotic detoxification, energy metabolism, bile acid biosynthesis. HNF4 α also plays vital role in regulating and maintaining the epithelial phenotype of hepatocyte. We have analyzed the HNF4 α expression between renal carcinoma tissue and adjacent normal renal tissue, and the result showed that markedly reduction of HNF4 α expression was found in renal carcinoma tissue in comparison with normal renal tissue, and more obvious decrease was observed in those advanced stage cases. To clarify the significance and mechanism of the lower expression of HNF4 α in RCC, our research will focus on the effects of HNF4 α on biological behaviors of RCC and provide a foundation for further work.

【Methods】

This study detected the mRNA and protein expression of HNF4 α in paired neoplastic and noncancerous renal parenchyma samples by real-time PCR and immunocytochemical staining respectively, and analyzed the expression difference of HNF4 α and its relationship with RCC clinical stage and other clinicopathologic

features. Furthermore, The recombinant lentiviral vector pBobi-puro-HNF4 α was constructed to over-express HNF4 α gene in RCC cells (OS-RC-2) , and recombinant lentiviral vector pBobi-puro-GFP was used as control. Those recombinant lentiviral vectors were transfected in packaging cell 293FT cells to produce lentivirus. When OS-RC-2 cells were infected by lentivirus expression of HNF4 α , the morphology changes was observed by microscope, migration and invasion ability of OS-RC-2 cells was tested by wound healing and transwell invasion assay respectively, flow cytometry was used to detect the cell apoptosis. Finally, the expression of EMT marker, Snail, MMP-7 and Cleaved-Cparp were detected by western blotting.

【Result】

About 73.3% of the tissue samples with renal cell carcinoma show a significant decrease in HNF4 α mRNA expression compared to noncancerous renal tissue samples, immunocytochemical staining showed that changes of the expression of HNF4 α protein and HNF4 α mRNA were similar. HNF4 α protein was high expression in normal renal tubules, but in tumor samples, normal structures of renal tubules were disorganized or disappeared, which replaced by tumor cells, and the HNF4 α expression in nucleus was lower than normal tissue in most of cases. These changes were more obvious in some advanced cases. The ectopic expression of HNF4 α in RCC cells led to inhibitory effect of cell migration and cell invasion, notable alter cellular morphology and induce apoptosis. The relative genes such as MMP-7, Cleaved-Parp were showed corresponding change. Snail is a major repressor of E-cadherin and promote EMT, the decline expression of Snail was detected, accompanied by the increase of epithelial marker E-cadherin and reduction of mesenchymal marker vimentin.

【Conclusion】

1. HNF4 α has crucial significance in the progress of epithelial-mesenchymal transition in renal cell carcinoma, and the reduction of HNF4 α in RCC samples are associated with tumor staging and histological grade.
2. Over-expression of HNF4 α in RCC cells could inhibit its malignant biological behavior and result in phenotype transition similar to mesenchymal-epithelial

transition (MET) .

Key Words: Hepatocyte nuclear factor 4 α (HNF4 α); Renal cell carcinoma (RCC); epithelial-mesenchymal transition (EMT)

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