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NET-1 knockdown inhibits proliferation and promotes apoptosis of hepatocellular carcinoma cells by regulating apoptosis-related proteins and the PI3K/Akt signaling pathway.

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Abstract

Background: Neuroepithelial transforming gene-1 (*NET-1*) is a guanine nucleotide exchange factor that activates Rho family proteins. We aimed to evaluate the effect of siRNA-mediated knockdown of *NET-1* on proliferation and apoptosis of hepatocellular carcinoma (HCC) cells and the underlying mechanisms.
Material and methods: *NET-1* mRNA and protein levels were detected in four HCC cell lines MHCC97-L, MHCC97-H, SMCC7721, and HepG2, and the normal liver cell line L-02 using RT-PCR and western blot. Cell proliferation was evaluated by the CCK-8 assay, and apoptosis was assessed by flow cytometry. Protein levels of apoptosis-related proteins and PI3K/Akt pathway proteins were evaluated by western blot.
Results: *NET-1* levels were significantly higher in the four HCC cell lines than those in the normal liver cell line L-02; the highest levels were observed in MHCC97-H cells. Knockdown of *NET-1* by siRNA inhibited proliferation and promoted apoptosis of HCC cells. In addition, *NET-1* knockdown decreased *Bax* and *cyclin D1* expression, but increased *Bcl-2* and caspase-3 levels in HCC cells. The PI3K/Akt pathway was blocked by knockdown of *NET-1*.
Conclusion: *NET-1* knockdown inhibits proliferation and promotes apoptosis of HCC cells by regulating the expression of apoptosis-related proteins including *Bax*, *Bcl-2*, *cyclin D1*, and caspase-3, and the PI3K/Akt pathway.

Keywords: *NET-1*, Hepatocellular carcinoma, Apoptosis, Proliferation.

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and the third leading cause of cancer-related death worldwide [1]. It is an increasingly important public health problem and its overall incidence remains alarmingly high in the developing world [2]. In the past two decades, despite extensive research and significant advancements in the diagnosis and treatment of HCC, the prognosis of the disease remains poor [3]. The process of HCC cell proliferation and apoptosis consists of a complex of series of sequential steps involving coordination of diverse signal transduction pathways [4,5]. A better understanding of the mechanism of proliferation and apoptosis of HCC will help to develop novel therapeutic strategies and improve patient survival.

Neuroepithelial transforming gene-1 (*NET-1*) is a member of the guanine nucleotide exchange factor (GEF) family that activates Rho family proteins. It was originally identified as an oncogene in neuroepithelial cells. Several recent studies showed dysregulation of *NET-1* expression in HCC. Shen et al. reported that the mRNA expression of *NET-1* was markedly up-regulated in human HCC tissues in comparison to matched paracarcinoma tissues, and *NET-1* expression was significantly higher in TNM III-IV HCC tissues than that of TNM I-II HCC tissues [6]. In addition, *in vitro* studies demonstrated that knockdown of *NET-1* expression by short interfering RNA (siRNA) significantly inhibited proliferation of HepG2 and SMMC-7721 HCC cells [7-9]. A recent study by Ye et al. showed that *NET-1* knockdown effectively decreased migration, invasion and metastasis of the HCC MHCC-97H cell line through interactions with merlin, the product of the neurofibromatosis type 2 gene [10]. Despite of these findings, our understanding of the relation of *NET-1* with HCC remains limited.

In this study, we aimed to evaluate the effect of siRNA-mediated knockdown of *NET-1* on proliferation and apoptosis of HCC cells and the underlying mechanisms. We assessed *NET-1* mRNA and protein levels in four HCC cell lines and