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

Mingchun Wang¹, Xiangjun Sun²*, Yongjun Jiang³, Maogang Chen³

¹Surgical Operating Room, Linyi People’s Hospital, Linyi city, Shandong, PR China
²Department of Hepatobiliary Surgery, Linyi People’s Hospital, Linyi city, Shandong, PR China
³Department of Urology, Third People’s Hospital, Linyi city, Shandong, PR China

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.

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 SMCC-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