

Original Article

shRNA-mediated silencing of *PKHD1* gene promotes proliferation, migration and invasion of human intrahepatic cholangiocarcinoma HuCCT-1 cells

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Abstract: Intrahepatic cholangiocarcinoma (ICC), a type of cholangiocarcinoma, is characterized by insidious onset and lack of typical clinical symptoms at early onset, and the lack of effective treatments results in a poor prognosis. Identification of novel biomarkers and treatment targets is therefore of great significance to improve the survival for ICC patients. Polycystic kidney and hepatic disease 1 (*PKHD1*), a gene responsible for autosomal recessive polycystic kidney disease (ARPKD), has been linked to cancers, and mutation of the *PKHD1* gene may cause abnormal proliferation and differentiation of bile duct epithelial cells. However, the role of the *PKHD1* gene in the biological behaviors of ICC cells remains unknown until now. The present study was therefore designed to examine the effects of *PKHD1* knockdown on the proliferation, migration and invasion of human ICC HuCCT-1 cells and investigate the underlying mechanisms. We transfected a lentiviral vector LV3-PKHD1 that contained the short hairpin RNA (shRNA)-mediated silencing of the *PKHD1* gene into HuCCT-1 cells, while GFP lentiviral vector LV3NC-transfected cells served as negative controls. The cell proliferation, migration and invasion were measured, and the ultrastructure of primary cilium was observed using scanning electron microscopy (SEM). The expression of PI3K/Akt signaling proteins was determined with Western blotting. qRT-PCR assay determined down-regulation of *PKHD1* mRNA expression and Western blotting analysis revealed reduced FPC expression in HuCCT-1 cells post-transfection with LV3-PKHD1, which validated the effective silencing of the *PKHD1* gene in HuCCT-1 cells. Wound scratch assay showed that the LV3-PKHD1 transfected HuCCT-1 cells had a greater healing ability of the scratch than the LV3NC-transfected and nontransfected cells at 24 and 48 h, and CCK-8 assay revealed that the LV3-PKHD1 transfected HuCCT-1 cells exhibited a greater proliferative ability than the LV3NC-transfected and nontransfected cells ($P < 0.01$). In addition, Transwell migration assay showed significantly more LV3-PKHD1 transfected HuCCT-1 cells penetrating through the Transwell chamber than the LV3NC-transfected and nontransfected cells ($P < 0.01$), and Transwell invasion assay revealed more LV3-PKHD1 transfected HuCCT-1 cells crossing the Matrigel than the LV3NC-transfected and nontransfected cells ($P < 0.01$). Moreover, Western blotting assay detected significant up-regulation of PI3K, Akt, p-Akt, and NF- κ B expression in LV3-PKHD1 transfected HuCCT-1 cells as compared to that in the LV3NC-transfected and nontransfected cells ($P < 0.05$), and SEM displayed shorter length, less number and lower distribution density of primary cilium on the surface of LV3-PKHD1 transfected HuCCT-1 cells relative to LV3NC-transfected cells. The results of this study demonstrate that the silencing of the *PKHD1* gene promotes the proliferation, migration and invasion of human ICC HuCCT-1 cells via the PI3K/Akt signaling pathway.

Keywords: Intrahepatic cholangiocarcinoma, PKHD1, proliferation, migration, invasion, PI3K/Akt signaling, gene silencing

Introduction

Cholangiocarcinoma is a primary bile tract malignant tumor that originates from the bile duct epithelial cells [1]. The incidence of cholangiocarcinoma is estimated to rank second

only to hepatocellular carcinoma (HCC) in all hepatobiliary malignancies and comprises approximately 3% of all gastrointestinal tumors [2]. This malignancy is found to be highly prevalent in people at ages of approximately 70 years, with a bit higher incidence in men than in