ORP9 promotes migration and invasion in Huh7 cells

Dongdong Liu [a], Boyan Huang

College of Life science and Technology, Jinan University, Guangzhou 510000, China
[a]54264783@qq.com

Abstract

Oxysterol binding protein related protein family (ORPs) is a kind of protein family that contains an oxysterol binding domain and widely exists in eukaryote cells. ORP9 is a member of the protein family, but so far, we knew little about the biological function of ORP9. OSBP-related protein 9 (ORP9) expresses highly in HCC tissues, and expresses lowly in normal liver tissues, but the mechanism that occurs remain obscure. To gain insight into function of ORP9 in HCC, we utilized wound healing, transwell assay and western blot assay analyses. Our results shows that overexpress ORP9 promotes the ability of migration and invasion in Huh7 cells, and knockdown ORP9 suppresses the ability of migration and invasion in Huh7 cells.

Keywords

ORP9, migration, invasion, Huh7 cells.

1. Introduction

Oxysterol binding protein related protein family (ORPs) is a kind of protein family that contains an oxysterol binding domain and widely exists in eukaryote cells[1-2]. Members of ORPs contain a conserved C-terminal OSBP homology domain that binds sterols[3], oxysterols and lipids, the pleckstrin homology (PH) domain and FFAT (two phenylalanines in an acidic tract) motif[4]. PH domain allows this protein to target Golgi apparatus by binding PI-4P, while FFAT motif binds Vesicle-associated membrane protein-associated protein (VAP) on endoplasmic reticulum (ER). Functional analysis indicated diverse roles of ORPs in the regulation of lipid metabolism, which has an impact on a variety of cellular biological functions, including vesicle transport, cell cycle, and differentiation[5-7].

ORP9 is a member of the protein family. There are two subclasses of ORP9 in mammals, ORP9L and ORP9s. Both of them are encoded by the ORP9 gene[8], and have FFAT motif and OSH domain that binds VAP protein. But so far, we have known little about the biological function of ORP9. All we know is that ORP9 expresses in Macrophage and has relationship with the metabolism of blood glucose and lipids[9].

In the present study, we found that ORP9 expresses highly in HCC tissues and weakly in normal liver tissues, prompting that ORP9 may make contribution to HCC cells. Besides, some current studies showed that one prominent symptom of HCC is distant metastasis related to the capability of migration and invasion of the cells [10,11]. Thus, we hypothesized that mediate the ability of migration and invasion in HCC cells.

2. Materials and methods

2.1 Materials

Rabbit antibodies against human ORP9 were purchased from Sigma-Aldrich;

Anti-actin monoclonal antibody was purchased from Proteintech;

siORP9 or control non-targeting siRNA were produced by Invitrogen;

ORP9 cDNA was constructed by our lab;

Lipofectamine 2000 was purchased from Invitrogen;

Matrigel was purchased from Corning;

Transwell insert was purchased from Corning;

Huh7 cells were maintained in DMEM containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 ℃ in a humidified incubator with 5% CO.

2.2 Methods

ORP9 Overexpression. Huh7 cells were seeded on 12-well plates at 80–90% confluency and then transfected with ORP9 cDNA or empty plasmid by using Lipofectamine 2000 for 24h.

RNA interference. Huh7 cells were seeded on 12-well plates at 30–50% confluency for 24 h and then transfected with siORP9 or control non-targeting siRNA (siORP9: GGA UGU CAC UUU CAA CUU A dTdT; siNT: UUC UCC GAA CGU GUC ACG U dTdT) by using Lipofectamine 2000 for 72h.

Western blot analysis. Cellular total protein samples were mixed with Laemmli sample buffer, boiled for 10 min, and subjected to SDS-PAGE. Western blot analysis was conducted as described.

Wound-Healing and Transwell invasion assays. For the wound-healing assay, ORP9- or empty vector transfected cells were cultured on a 35 mm dish until confluence and then wounded with a 200 μL pipette tip. Migration photos were captured at 0 and 48h hours after scratching. Invasion assay was performed with Matrigel following the manufacturer's instructions. The Matrigel membrane was stained with crystal violet and migrated cells were counted under a microscope. Both experiments were repeated in triplicate independently.

3. Results

3.1 ORP9 overexpression promoted migration in Huh7 cells

The results of wound healing showed that the ability of migration was significantly enhanced in overexpressed ORP9 cells compared with the cells with empty vector. Western blot assay indicated ORP9 overexpression efficiency were over 100%.

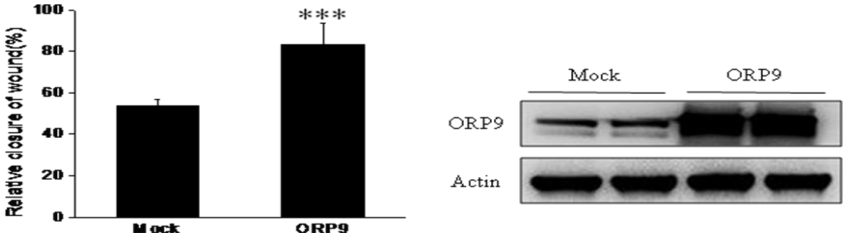

Fig. 1 ORP9 overexpression promotes migration in Huh7 cells (***p < 0.001; n=5)

3.2 ORP9 knockdown suppressed migration in Huh7 cells

The results of wound healing showed that the ability of migration was significantly reduced in knockdown ORP9 cells. Western blot assay indicated ORP9 knockdown efficiency were over 100%.

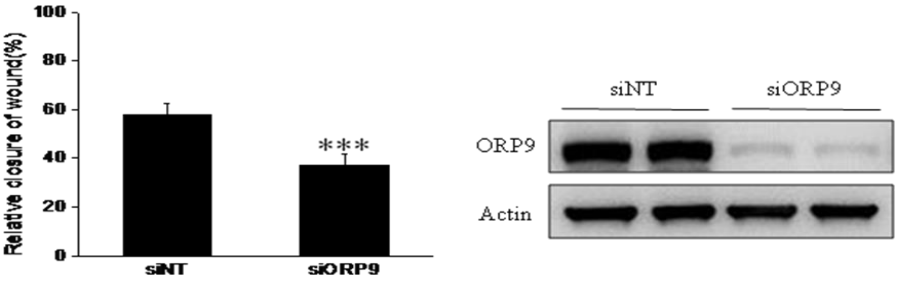

Fig. 2 ORP9 knockdown inhibites migration in Huh7 cells (***p < 0.001; n=5)

3.3 ORP9 overexpression promoted invasion in Huh7 cells

The results of transwell assay showed that the ability of invasion was significantly enhanced in overexpressed ORP9 cells. Western blot assay indicated ORP9 overexpression efficiency were over 100%.

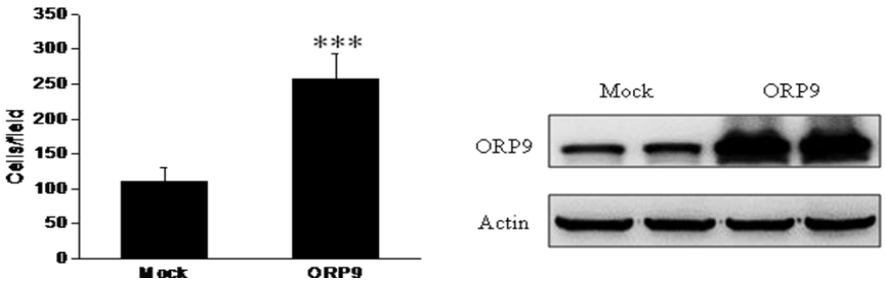

Fig. 3 ORP9 overexpression promotes invasion in Huh7 cells (***p < 0.001; n=5)

3.4 ORP9 knockdown suppressed invasion in Huh7 cells

The results of transwell assay showed that the ability of invasion was significantly reduced in knockdown ORP9 cells. Western blot assay indicated ORP9 knockdown efficiency were over 100%.

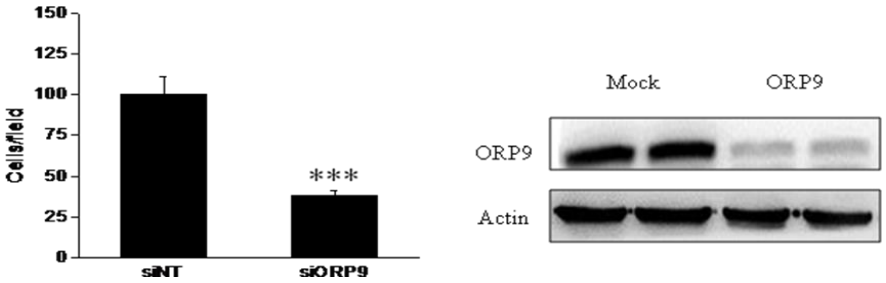

Fig. 4 ORP9 knockdown inhibites invasion in Huh7 cells (***p < 0.001; n=5)

4. Conclusion

These results offered primary clues that ORP9 mediates the migration and invasion ability in Huh7 cells, and it may offer a novel target to understand the function of ORP9 in Hepatocellular Carcinoma cells.

5. References

[1] Taylor F R, Saucier S E, Shown E P, et al. Correlation between oxysterol binding to a cytosolic binding protein and potency in the repression of hydroxymethylglutaryl coenzyme a reductase[J]. J Biol Chem, 1984, 259(20): 12382-12387.

[2] Dawson P A, Ridgway N D, Slaughter C A, et al. Cdna cloning and expression of oxysterol-binding protein, an oligomer with a potential leucine zipper[J]. J Biol Chem, 1989, 264(28): 16798-16803.

[3] Lehto M, Laitinen S, Chinetti G, et al. The osbp-related protein family in humans[J]. J Lipid Res, 2001, 42(8): 1203-1213.

[4] Loewen C J, Roy A, Levine T P. A conserved er targeting motif in three families of lipid binding proteins and in opi1p binds vap[J]. EMBO J, 2003, 22(9): 2025-2035.

[5] Jaworski C J, Moreira E, Li A, et al. A family of 12 human genes containing oxysterol-binding domains[J]. Genomics, 2001, 78(3): 185-196.

[6] Levine T P, Munro S. The pleckstrin homology domain of oxysterol-binding protein recognises a determinant specific to golgi membranes[J]. Curr Biol, 1998, 8(13): 729-739.

[7] Liu X, Ridgway N D. Characterization of the sterol and phosphatidylinositol 4-phosphate binding properties of golgi-associated osbp-related protein 9 (orp9)[J]. PLoS One, 2014, 9(9): e108368.
[8] Wyles J P, Ridgway N D. Vamp-associated protein-a regulates partitioning of oxysterol-binding protein-related protein-9 between the endoplasmic reticulum and golgi apparatus[J]. Exp Cell Res, 2004, 297(2): 533-547.
[9] Yan D, Olkkonen V M. Characteristics of oxysterol binding proteins[J]. Int Rev Cytol, 2008, 265: 253-285.
[10] Wang Y H, Sui X M, Sui Y N, et al. Brd4 induces cell migration and invasion in hcc cells through mmp-2 and mmp-9 activation mediated by the sonic hedgehog signaling pathway[J]. Oncol Lett, 2015, 10(4): 2227-2232.
[11] Zhang D, Zhou P, Wang W, et al. Microrna-616 promotes the migration, invasion and epithelial-mesenchymal transition of hcc by targeting pten[J]. Oncol Rep, 2016, 35(1): 366-374.