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臨床組－醫師

下調NRPL2蛋白表現透過mTOR途徑及抑制自噬促使肝細胞癌生長

Knock-downing NPRL2 protein enhanced the development of hepatocellular carcinoma via activity of mTOR pathway and inhibition of autophagy

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a highly invasive malignancy that results in the second-leading cause of cancer-related mortality. Recently, GATOR 1 complexes consists of NPRL2, NPRL3, and DEPDC5, and plays an important role in regulating tumor growth through amino acid-deficient conditions for inhibition of mTORC1 signaling. The impact of NPRL2 on the development of HCC remains largely unknown and deserves to be elucidated.

AIMS

This study aims to investigate the role of GATORC1 in the prognosis and the mechanism of HCC through mTOR and autophagy signal pathway.

MATERIAL & METHODS

We established HepG2 cells knocked down for NPRL2 using shRNA systems. We used a mouse xenograft model. We enrolled 300 HCC patients who underwent resection to evaluate survival and tumor recurrence.

RESULTS

ShRNA-mediated NPRL2 downregulation significantly reduced the expression of NPRL2, NPRL3 and DEPDC5 in HepG2 cells; Efficient downregulation of NPRL2 protein expression by both shRNA and siRNA systems enhanced the proliferation, migration, and colony formation *in vitro*. NPRL2 downregulation significantly increased HCC growth in a mouse xenograft model. NPRL2 downregulation increased the AKT and mTOR activation and inhibited autophagy *in vitro* and *in vivo*. Low NPRL2, NPRL3, DEPDC5, and LC3, and high p62 and mTOR protein expression was significantly associated with disease-free survival and overall survival in HCC patients.

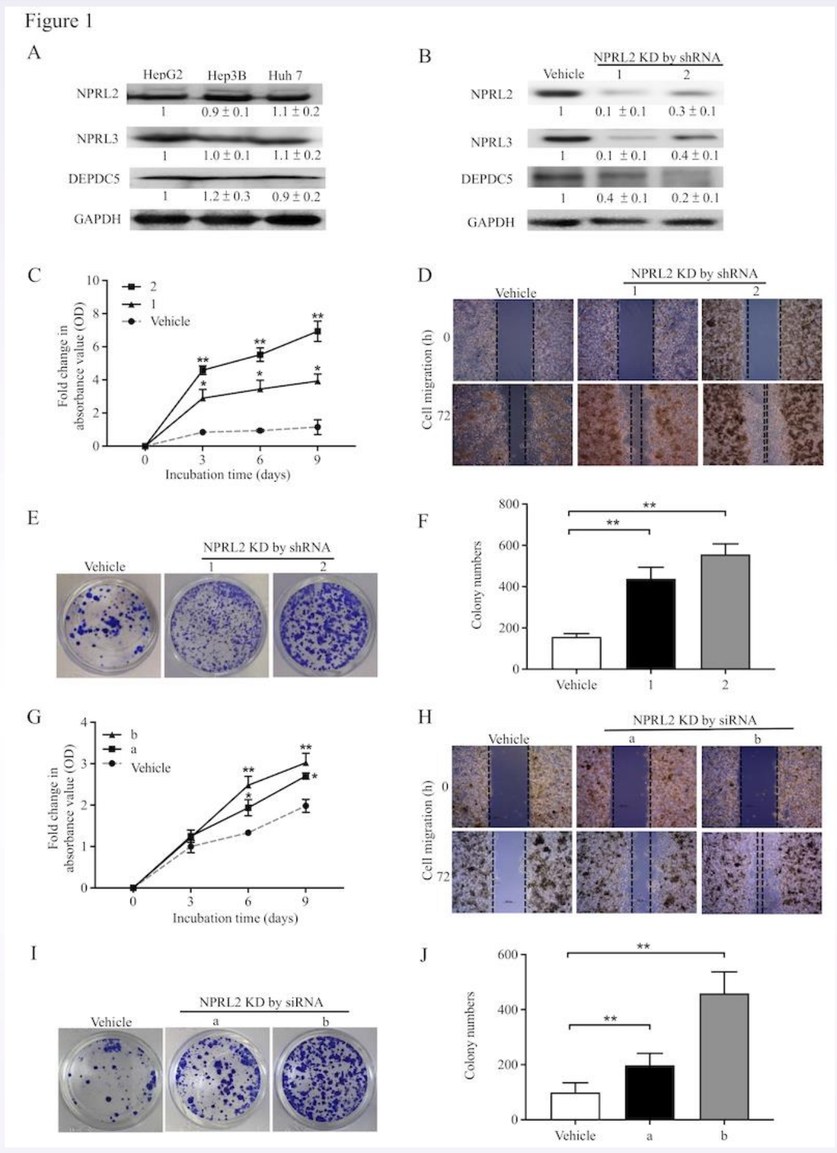

Figure 1. Efficient downregulation of NPRL2 promoted the proliferation, migration, and colony formation of HCC cells. (A) The protein expression levels of NPRL2, NPRL3 and DEPDC5 were relatively high and similar in HCC cell lines. (B) We knocked down NPRL2 in HepG2 cells using shRNA systems. NPRL2, NPRL3, and DEPDC5 protein expression was significantly reduced in HepG2 cells (n=3). (C and G) Downregulation of NPRL2 protein by the shRNA and siRNA system significantly increased the cell proliferation rate compared to that in the vehicle group (n=3). (D and H) The migration of HepG2 cells significantly increased after NPRL2 downregulation by shRNA and siRNA for 72 h (Scale bar: 200 μm; *n* = 3). (E-F and I-J) HepG2 cells with NPRL2 downregulation by the shRNA and siRNA system increased colony formation ability compared to that in the vehicle group (n=3).

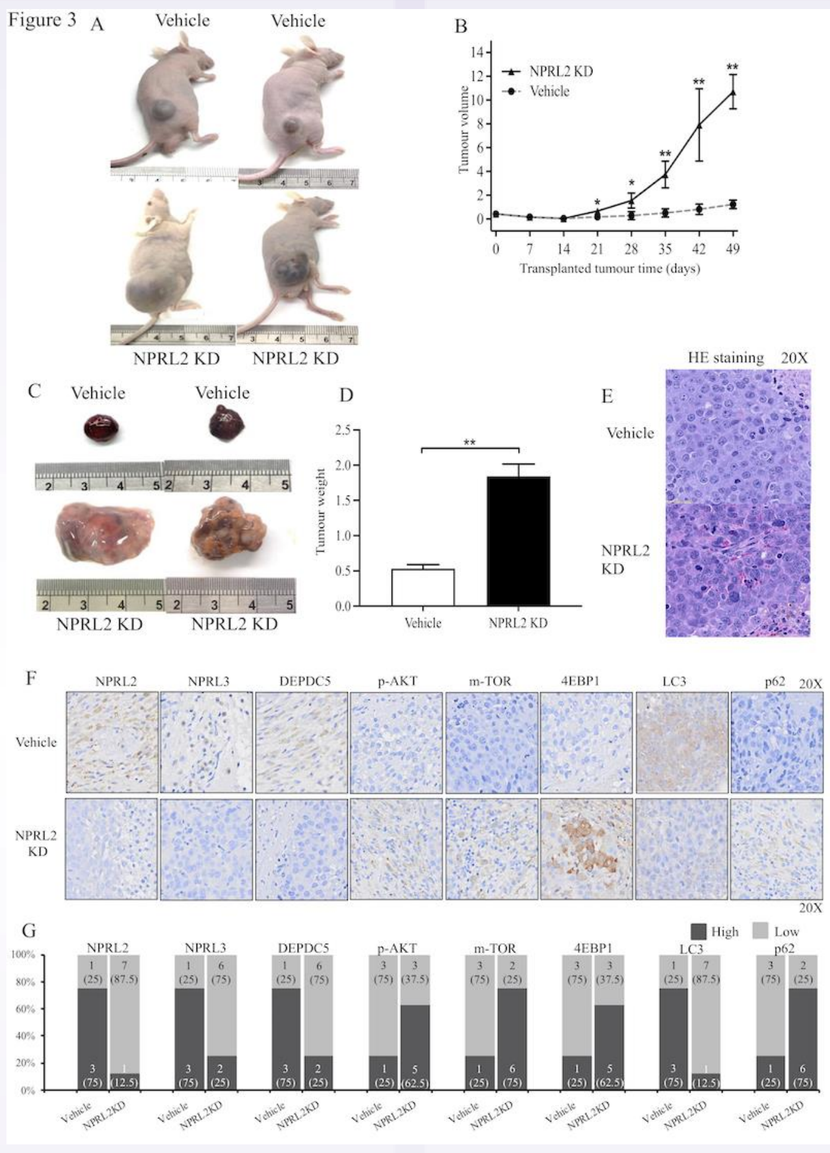

Figure 3. Knockdown of NPRL2 in human HepG2 cells promoted tumor growth *in vivo*. (A-B) In the NPRL2 downregulation group, the tumor growth curve was significantly increased and tumor volume had increased by up to 8-fold on day 30 in xerographic mice (n=4-8). (C-D) Tumor weight in the NPRL2 downregulation group had significantly increased by up to 3-fold that of the vehicle group at the end of the study. (E) Haematoxylin and eosin staining of the transplanted tumors in both groups indicated malignant tumors. (F-G) The protein expression levels of NPRL2, NPRL3, DEPDC5, and LC3 were significantly lower and those of p-AKT, mTOR, 4EBP1, and p62 were significantly higher in the NPRL2 KD group than in the counterpart group.

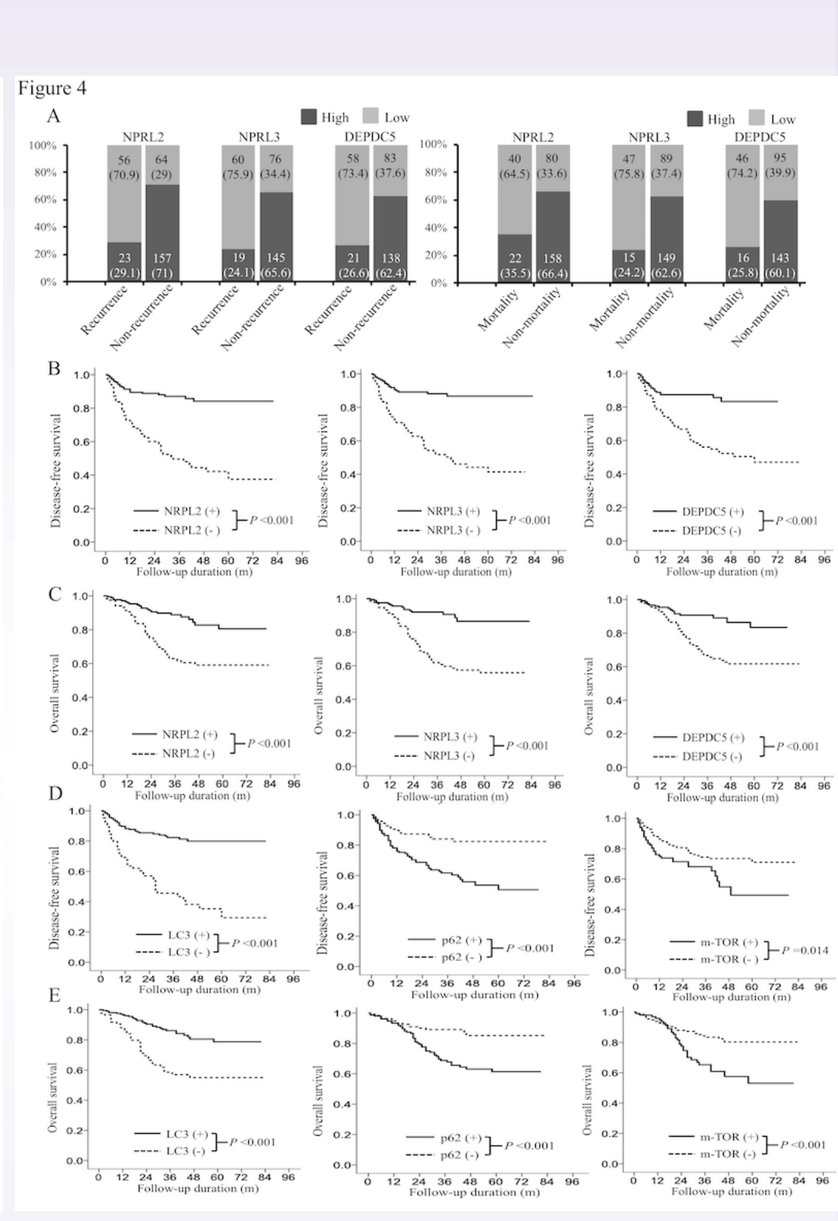

Figure 4. NPRL2, NPRL3, DEPDC5, LC3, p62, and mTOR were predictive factors for DFS and OS in HCC patients after surgical resection. (A) In 300 HCC patients, 79 patients occurred recurrence and 62 patients experienced mortality. Low NPRL2, NPRL3, and DEPDC5 protein expression was significantly associated with higher recurrence and mortality (P<0.05). Data are shown as number (%). (B-C) In patients, low NPRL2, NPRL3, and DEPDC5 protein expression was significantly associated with worse DFS and OS as revealed by Kaplan-Meier analysis (P<0.05). (D-F) Low LC3, high p62, and high mTOR protein expression in patients was significantly associated with worse DFS and OS according to Kaplan-Meier analysis (P<0.05).

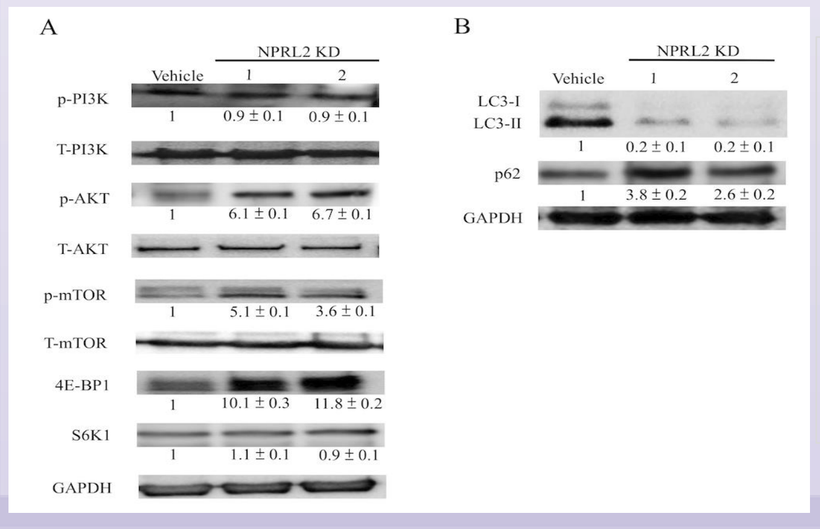

Figure 2. Knockdown of NPRL2 significantly increased AKT and mTOR activity via 4E-BP1 and inhibited autophagy in HCC cells.

CONCLUSION

Efficient downregulation of NPRL2 significantly increased HCC proliferation, migration, and colony formation *in vitro*, and increased HCC growth *in vivo*. Low NPRL2 protein expression was closely correlated with poorer clinical outcomes in HCC patients. These results provide a new mechanistic understanding of HCC and aid the development of treatments for HCC.

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