新乡医学院

科技活动月 研究成果展

The formation of TDP43/14-3-3ζ complex promotes in cell proliferation and migration, which maintain the p-EMT state in MHCC cell lines

背景介绍

Proliferation, metastasis and invasiveness are main factors in HCC malignant progression. TDP43 execute its classical function in different cancers, such as regulated mRNA alternative splicing, sustained mRNA stability or participated in microRNA biogenesis. Thus, TDP43 may also plays many nonclassical functions in cancer by binding with different partners. However, the real mechanism linking the interaction of TDP43 with its partner was needed further exploration in HCC.

作者简介

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研究方法

TDP43-affnity-LC-MS/MS was used to identity many potential clients of TDP43. Interestingly, 14-3-3 family as a kind of client of TDP 43 cached our attention because of every member was identified using this assay, moreover, 14-3-3ζ had a strongest binding force with TDP43. Immune blot assay identified the expression of TDP43 and 14-3-3ζ in HCC tissue and its paracancerous; Multiple TDP43 Co-IP assays analyzed the interaction of TDP 43 with 14-3-3ζ. Edu assay also verified the cell proliferation in upregulation of TDP43 in MHCCLM3 ($LM3^{TDP43\text{-}OE}$). Silencing to 14-3-3ζ in MHCCLM3 wild type verified the ability of cell proliferation; Immunofluorescence and Nucleoplasm separation assay analyzed the nuclear entrance of YAP. Silencing of 14-3-3ζ and downregulation of TDP43 in MHCCLM3 analyzed the cell proliferation, migration and invasion and E-Cadherin / Vimentin ratio of p-EMT state.

研究结果

1.TDP43 promotes cell proliferation by inhibiting hippo signaling pathway in HCC cell lines;
2. The interaction of TDP43 with 14-3-3ζ unlash the nuclear translocation of YAP;
3. TDP-43 upregulation facilitates the nuclear translocation and transcript activity of YAP in MHCCLM3 cell line;
4. Downregulation of 14-3-3ζ promotes YAP nuclear translocation of in MHCCLM3
5. TDP-43 promotes nuclear translocation of YAP via hijacking cytoplasmic retention of 14-3-3ζ ;
6. TDP-43 promotes the malignant progression of HCC via 14-3-3ζ mediated YAP nuclear translocation

研究结果

Figure1:

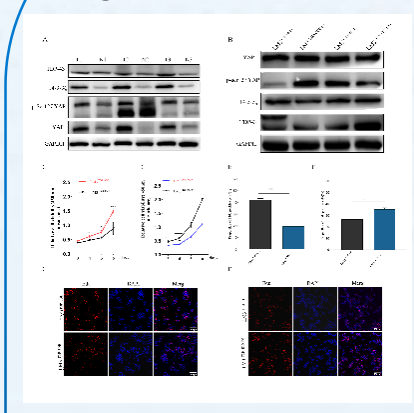

Figure1 ：TDP43 promotes cell proliferation by inhibiting hippo signaling pathway in HCC cell lines （A-B）Analysis of the expression of 14-3-3ζ, TDP-43, YAP in 3 HCC tissues and MHCCLM3 mimic cells, CCK8 assay. The cell proliferation ability of TDP-43 in LM3; (C-D) CCK-8 assay in MHCCLM3 cells with stably overexpressed TDP-43 were infected with lentivirus; (E-H) EdU assay in MHCCLM3 cells infected with lentivirus stably down regulation of TDP-43. (scale bar 100 μm) All experiments were repeated at least three times. Error bars represent mean+ SD. *P < 0.05, **P < 0.01, *** P <0.001, **** P < 0.0001 by 2-tail Student's t-test.

Figure2:

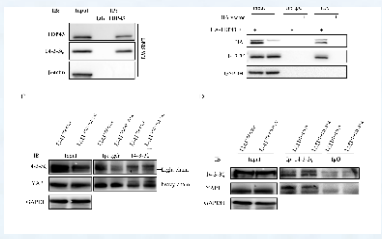

Figure2:The interaction of TDP43 with 14-3-3ζ unlash the nuclear translocation of YAP. (A) TDP-43 co ip assay displayed that TDP-43 interact with 14-3-3ζin LM3 wild type cell line; (B) HA co ip assay showed that there is a direct interaction between TDP-43 and 14-3-3ζ in 293T cell line; (C) 14-3-3ζco ip assay was identified that upregulation of TDP 43 impaired the interaction of 14-3-3ζwith YAP1;(D) Down-regulation of TDP-43 promoted the interaction of 14-3-3ζ with YAP1.

Figure3:

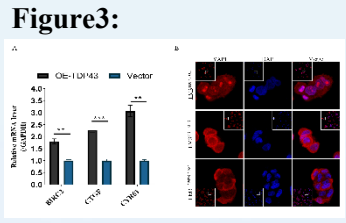

Figure3 ： TDP-43 upregulation facilitates the nuclear translocation and transcript activity of YAP in MHCCLM3 cell line. (A) Up-regulated TDP-43 promoted the activation ；（B）IF assay display that TDP-43 promote the entrance of nuclear of YAP1

Figure4:

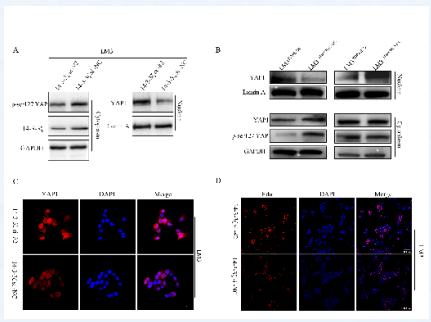

Figure4 ： Downregulation of 14-3-3ζ promotes YAP nuclear translocation of in MHCCLM3
(A) nucleocytoplasmic separation assay demonstrated 14-3-3ζ RNAi#2 promote YAP1 translocation of the nucleus; (B) Up-regulation of TDP-43 expression also increase YAP1 entry into the nucleus, down-regulation of TDP-43 expression is adverse; (C) IF showed 14-3-3ζ RNAi#2 promote the entry of YAP1 into the nuclear, （scale bar 20 μm）; (D) EDU assay identified that 14-3-3ζ RNAi#2 promote cell proliferation,（scale bar 100 μm）. All experiments were repeated at least three times

Figure5:

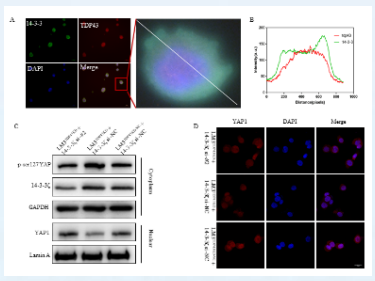

Figure5: TDP-43 promotes nuclear translocation of YAP via hijacking cytoplasmic retention of 14-3-3 ζ . (A)Immunofluorescence assay demonstrated that TDP 43 and 14-3-3 ζ co-localized in the cytoplasm in LM3 WT cell line; (B) Superimposed the fluorescence intensity of TDP 43 and 14-3-3ζ (C) nucleocytoplasmic separation assay demonstrated TDP-43 promote the entry of YAP1 into the nucleus via the participation of 14-3-3ζ; (D) Immunofluorescence assay showed down-regulation of TDP-43 didn't promote the translocation of YAP1 from cytoplasm to nucleus, independently. （scale bar 10 μm）

Figure6:

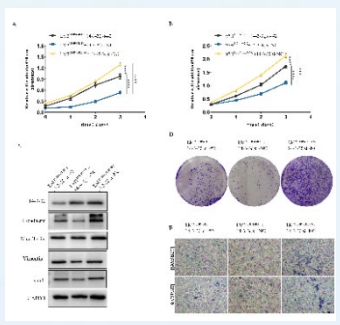

Figure6: TDP-43 promotes the malignant progression of HCC via 14-3-3ζ mediated YAP nuclear translocation. (A-B) The cell proliferation ability CCK 8 assay in MHCCLM3 and MHCC97H cells; (C) Relative EMT biomarker was identified in the above cell lines; (D) colony formation assay identified the cell proliferation ability in MHCC LM3 cell lines; (E) Trans well and Invasive assay were used to analysis the ability of migration and invasiveness in MHCC cell lines; All experiments were repeated at least three times. Error bars represent mean+ SD. *P < 0.05, **P < 0.01, *** P <0.001, **** P < 0.0001 by 2-tail Student's t-test

Figure7:

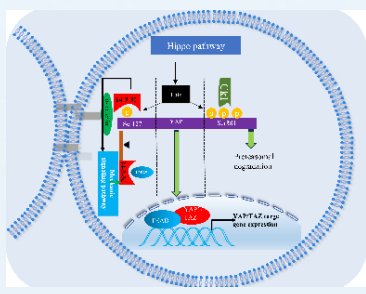

Figure7: Work model of TDP43/14-3-3ζ complex
TDP43 competes binding with YAP to 14-3-3ζ, mediating YAP nuclear localization and activating its target gene transcription, sustaining partial EMT status of tumor cells to promote HCC proliferation, migration and invasion, which kind of interaction regulated the balance between hippo signal and proteasomal degradation.

结 论

These data showed the complex of TDP43/14-3-3ζ unleashes YAP in the cytoplasm and drives its nuclear translocation, thus, activates its downstream target genes that may maintain the p-EMT state in MHCC cell lines. The TDP43/14-3-3ζ signal axis can be used as a new therapeutic target, which provides a new idea and experimental basis for the development of specific diagnostic markers in early liver cancer.

致 谢

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