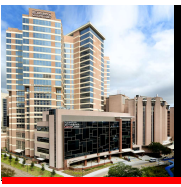

Yap1 Hydroxylation Suppress Prostate Cancer Metastasis

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Abstract

Yes-associated protein 1 (YAP1), a key transcriptional coactivator in the Hippo pathway, is an important driver in cancer development and progression (1). It plays an oncogenic role in various cancer types. However, multiple studies also support a tumor-suppressive function for YAP1 (2-8). Thus, the functions of YAP1 are likely context-dependent (9). On the cellular level, YAP1 promotes prostate cancer cell proliferation (10-13). Yet, the role of YAP1 in prostate cancer cell invasion, migration, and metastasis is not well defined. Through functional, transcriptomic, epigenomic, and proteomic analyses, we showed that prolyl hydroxylation of YAP1 plays a critical role in the suppression of cell migration, invasion, and metastasis in prostate cancer (14). Knockdown (KD) or knockout (KO) of YAP1 led to an increase in cell migration, invasion, and metastasis in prostate cancer cells. Microarray analysis showed that the EMT pathway was activated in Yap1-KD cells. ChIP-seq analysis showed that YAP1 target genes are enriched in pathways regulating cell migration. Mass spectrometry analysis identified P4H prolyl hydroxylase in the YAP1 complex and YAP1 was hydroxylated at multiple proline residues. Proline-to-alanine mutations of YAP1 isoform 3 identified proline 174 as a critical residue, and its hydroxylation suppressed cell migration, invasion, and metastasis. KO of P4ha2 led to an increase in cell migration and invasion, which was reversed upon Yap1 KD. Our study identified a novel regulatory mechanism of YAP1 by which P4HA2-dependent prolyl hydroxylation of YAP1 determines its transcriptional activities and its function in prostate cancer metastasis.

Results

YAP1 Deletion/mutation is Associated with Poor Survival and Metastasis

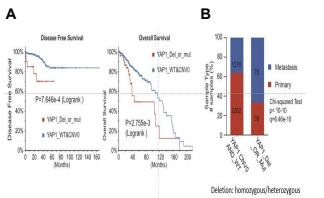

Figure 1. Yap1 deletion/mutation is associated with poor survival and metastasis. (**A**) Disease free (Left) and Overall (Right) survival curve of patients with Yap1 WT or deletion/mutation. (**B**) Percentage of primary and metastasis tumors in patients with Yap1 WT or deletion/mutation.

YAP1 Suppresses Cell Migration/Invasion and Metastasis

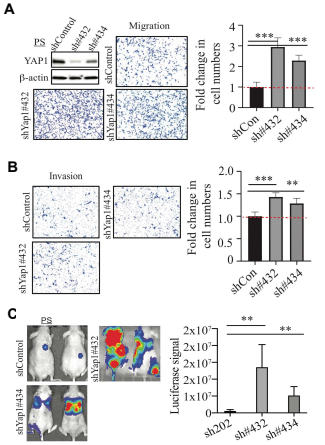

Figure 2. Yap1 suppresses cell migration/invasion and metastasis. (A, B) Cell migration and invasion assay using Pten/Smad4-deficieint prostate cancer (PS) cells transduced with control shRNA and Yap1 shRNAs. The Yap1 KD efficiency was confirmed by WB analysis. **(C)** Luciferase imaging in mice injected with PS cells transduced with control shRNA and Yap1 shRNAs through the tail vein.

YAP1 Suppresses Pathways Involved in Cell Mobility and Metastasis

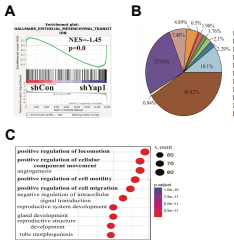

Figure 3. YAP1 suppresses pathways involved in cell mobility and metastasis. (A) GSEA analysis of microarray data from PS cells transduced with doxycycline-inducible Yap1 shRNA identified EMT as the top pathway activated in Yap1-KD cells. **(B, C)** ChIP-seq analysis identified YAP1 binding sites and YAP1-regulated pathways.

YAP1 Interacts with P4HA2

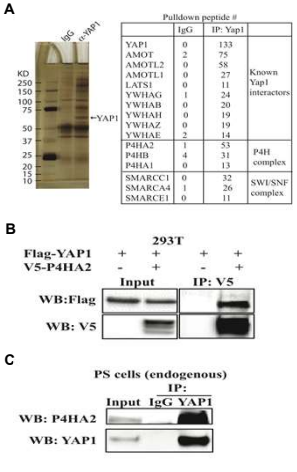

Figure 4. YAP1 Interacts with P4HA2. (A) Immunoprecipitation-mass spectrometry analysis identified known YAP1-interacting proteins and novel YAP1-interacting proteins. **(B)** Exogenous YAP1 interacts with exogenous P4HA2 when overexpressed in 293T cells by transfection of the indicated plasmids for co-immunoprecipitation experiments. **(C)** Endogenous YAP1 interacts with endogenous P4HA2 in PS cells.

YAP1 is Hydroxylated at Multiple Proline Residues

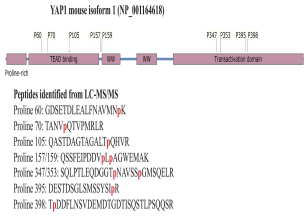

Figure 5. YAP1 is hydroxylated at multiple proline residues. Multiple prolyl hydroxylation sites were identified in peptides of YAP1 isoform 3 from the LC- MS/MS analysis.

YAP1 Hydroxylation-defective Mut4-5 (PA) Promotes Cell Migration/invasion and Metastasis

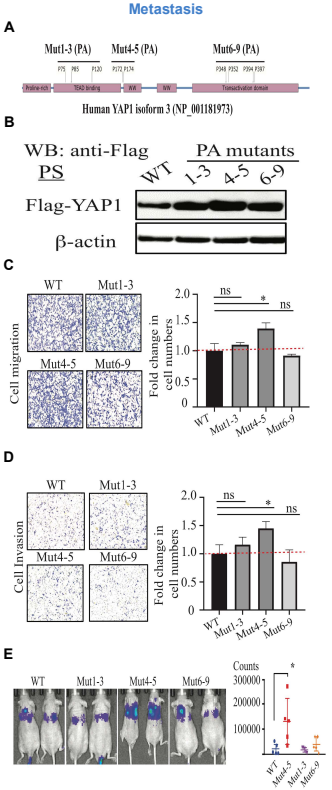

Figure 5. YAP1 hydroxylation-defective Mut4-5 (PA) promotes cell migration/invasion and metastasis. (A) Scheme showing the strategy to generate prolyl hydroxylation–defective YAP1 mutants by mutating proline to alanine (PA): Mut1-3 (P75/85/120A), Mut4-5 (P172/174A), and Mut6-9 (P348/352/394/397). Human YAP1 isoform 3 was used. **(B)** Expression of YAP1 WT and PA mutants in Yap1-KO PS cells with overexpression of YAP1 WT and PA mutants. **(E)** Tail vein injection of Yap1-KO cells with overexpression of YAP1 WT and PA mutants.

YAP1 P174 is Critical for Suppressing Cell Migration/invasion

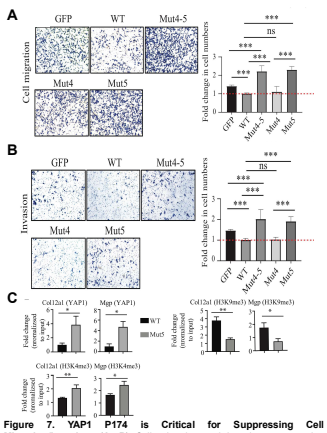

Figure 7. YAP1 P174 is Critical for Suppressing Cell Migration/invasion. (A, B) Cell migration and invasion assay using Yap1-KO PS cells with overexpression of GFP, YAP1 WT, Mut4-5, Mut4, and Mut5. **(C)** ChIP-qPCR analysis of YAP1, H3K4me3, and H3K9me4 binding sites in Col12a1 and Mgp.

P4HA2 Suppresses Cell Migration/invasion

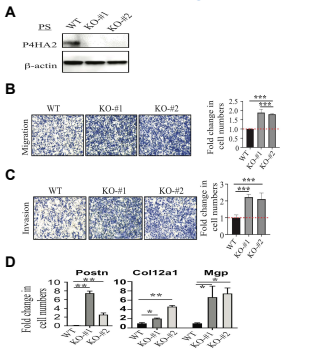

Figure 8. P4HA2 suppresses cell migration/invasion. (A) WB analysis of P4HA2 in P4ha2-WT and P4ha2-KO PS cells. **(B, C)** Cell migration and invasion using P4ha2-WT and P4ha2-KO PS cells. **(D)** qPCR analysis of YAP1 target genes (Postn, Col12a1, and Mgp) in P4ha2 KO and WT cells.

P4HA2 Suppresses Cell Migration/invasion through YAP1

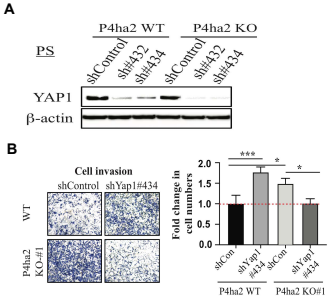

Figure 9. P4HA2 Suppresses Cell Migration/invasion through YAP1. (**A**) WB analysis of YAP1 in P4ha2-WT and P4ha2-KO PS cells transduced with Yap1 shRNAs. (**B**) Cell invasion assay in P4ha2-WT and P4ha2-KO PS cells transduced with shYap1#434.

Proposed Model for Tumor Suppressive Role of YAP1 in Metastasis

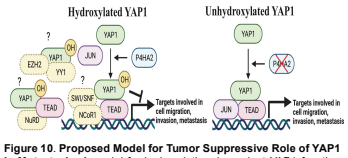

Figure 10. Proposed Model for Tumor Suppressive Role of YAP1 in Metastasis. A model for hydroxylation-dependent YAP1 function in cell migration, invasion, and metastasis (Created with BioRender.com). Left: P4HA2-mediated hydroxylation of YAP1 may impair its interactions with transcription factors such as JUN or enhance the recruitment of corepressor, such as SWI/SNF-NCoR1, NuRD, and EZH2/YY1, which results in a decrease in the expression of genes involved in cell migration, invasion, and metastasis. Right: In the absence of P4HA2, non-hydroxylated YAP1 may efficiently interact with transcription factors such as JUN to activate genes involved in cell migration, invasion, and metastasis.

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