



MARCH6 Promotes Papillary Thyroid Cancer Development By Destabilizing DHX9



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Importance

Recent evidence has shown that some Membrane-associated ring-CH-type finger (MARCH) proteins play important roles in cancer development. However, the role of MARCH6 in tumorigenesis, including thyroid tumorigenesis, remains unknown.

Objective

To investigate the functional roles of MARCH6 in the progression of thyroid cancer.

Methods

Cell thyroid cancer cell lines BCPAP, K1 and TPC-1

Patients and specimens clinical samples were histopathologically diagnosed by 2-3 pathologists

Others Immunohistochemical staining, Lentivirus-mediated MARCH6 knockdown and overexpression, CCK-8 assay, colony formation assay, Transwell assay, cell cycle and cell apoptosis assay, In vivo xenograft study, qRT-PCR and western blot were used.

Results

- Immunoblotting analysis, qRT-PCR and western blot results all showed that MARCH6 are overexpressed in the majority of PTCs.

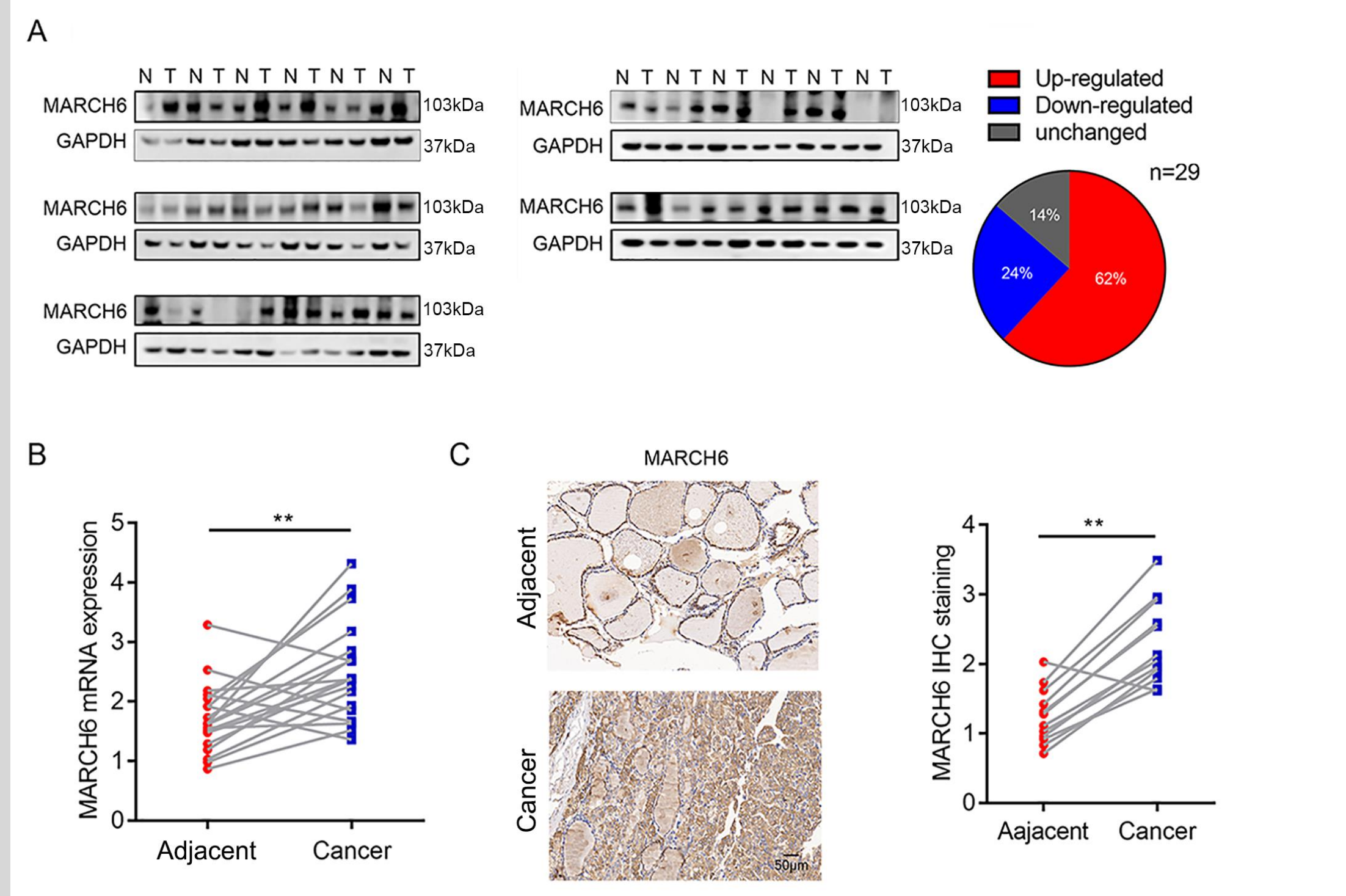


Figure 1 mRNA and protein abundance of MARCH6 is increased in PTCs.

(A) Western blot analysis of MARCH6 in 29 pairs of PTCs and normal tissues. 62% of PTCs had upregulated MARCH6. 24% of PTCs had downregulated MARCH6. 14% of PTCs had unchanged MARCH6. (B) qRT-PCR analysis of MARCH6 in 19 pairs of PTCs and normal tissues. **p<0.01. (C) IHC staining of MARCH6 in 12 pairs of PTCs and normal tissues. **p<0.01.

- Lentivirus-mediated MARCH6 knockdown and overexpression, results showed that ectopic expression of MARCH6 promoted the growth and proliferation, enhanced the colony formation of thyroid cancer cells. Otherwise, reverse.

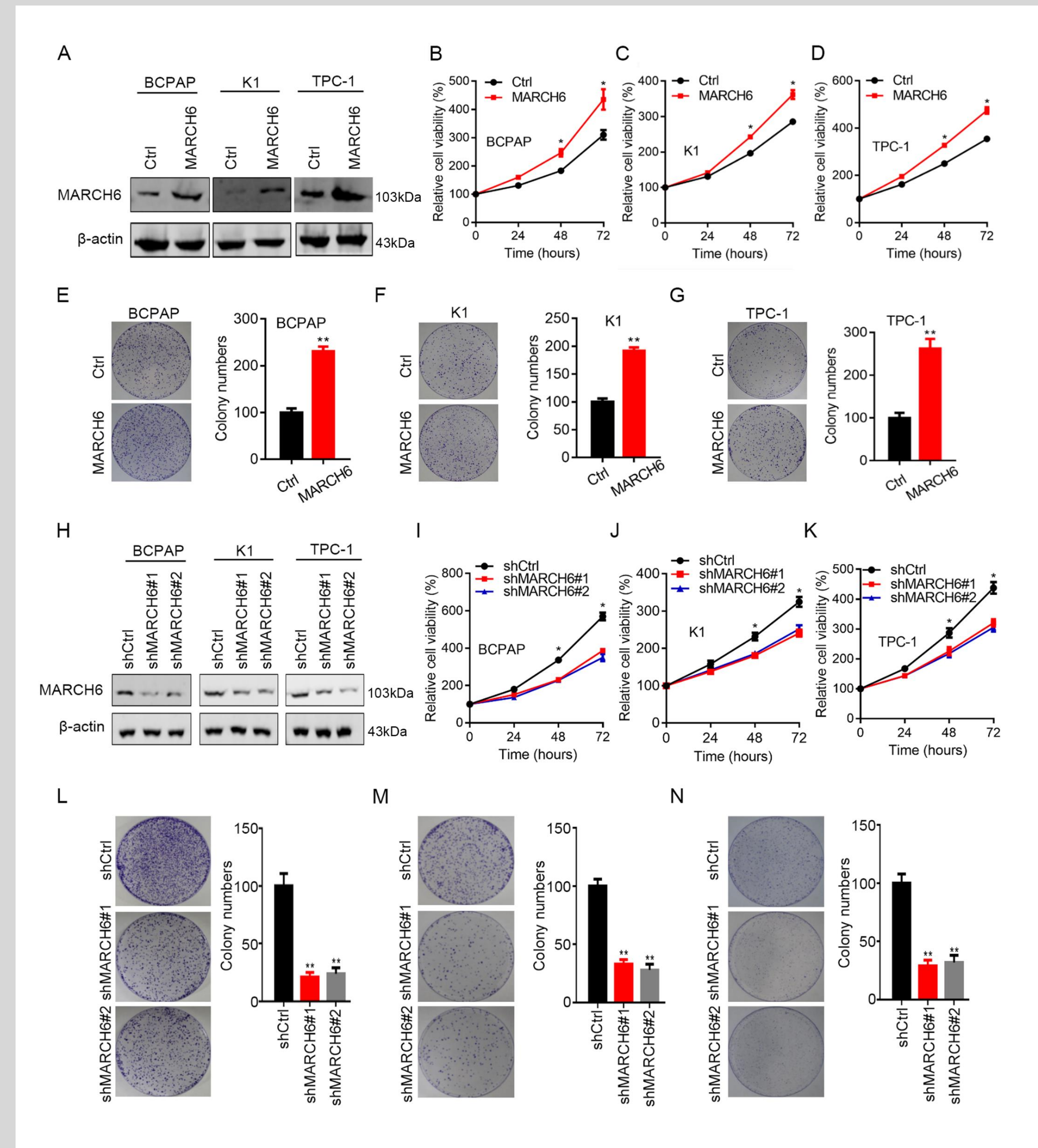


Figure 2 MARCH6 promotes the proliferation and colony growth in thyroid cancer cells.

(A) Western blot analysis of MARCH6 in Ctrl and MARCH6 overexpressed thyroid cancer cells. (B-D) Cell proliferation was detected by CCK8 assay in Ctrl and MARCH6 overexpressed BCPAP (B), K1 (C) and TPC-1 (D) cells. *p<0.05. (E-G) Colony formation assay was performed in Ctrl and MARCH6 overexpressed BCPAP (E), K1 (F) and TPC-1 (G) cells. **p<0.01. (H) Western blot analysis of MARCH6 in shCtrl, shMARCH6#1 and shMARCH6#2 thyroid cancer cells. (I-K) Cell proliferation was detected by CCK8 assay in shCtrl, shMARCH6#1 and shMARCH6#2 BCPAP (I), K1 (J) and TPC-1 (K) cells. *p<0.05. (L-N) Colony formation assay was performed in shCtrl, shMARCH6#1 and shMARCH6#2 BCPAP (L), K1 (M) and TPC-1 (N) cells. **p<0.01.

- MARCH6 interacts with and destabilizes DHX9 in thyroid cancer cells though regulating the ubiquitination levels of DHX9.

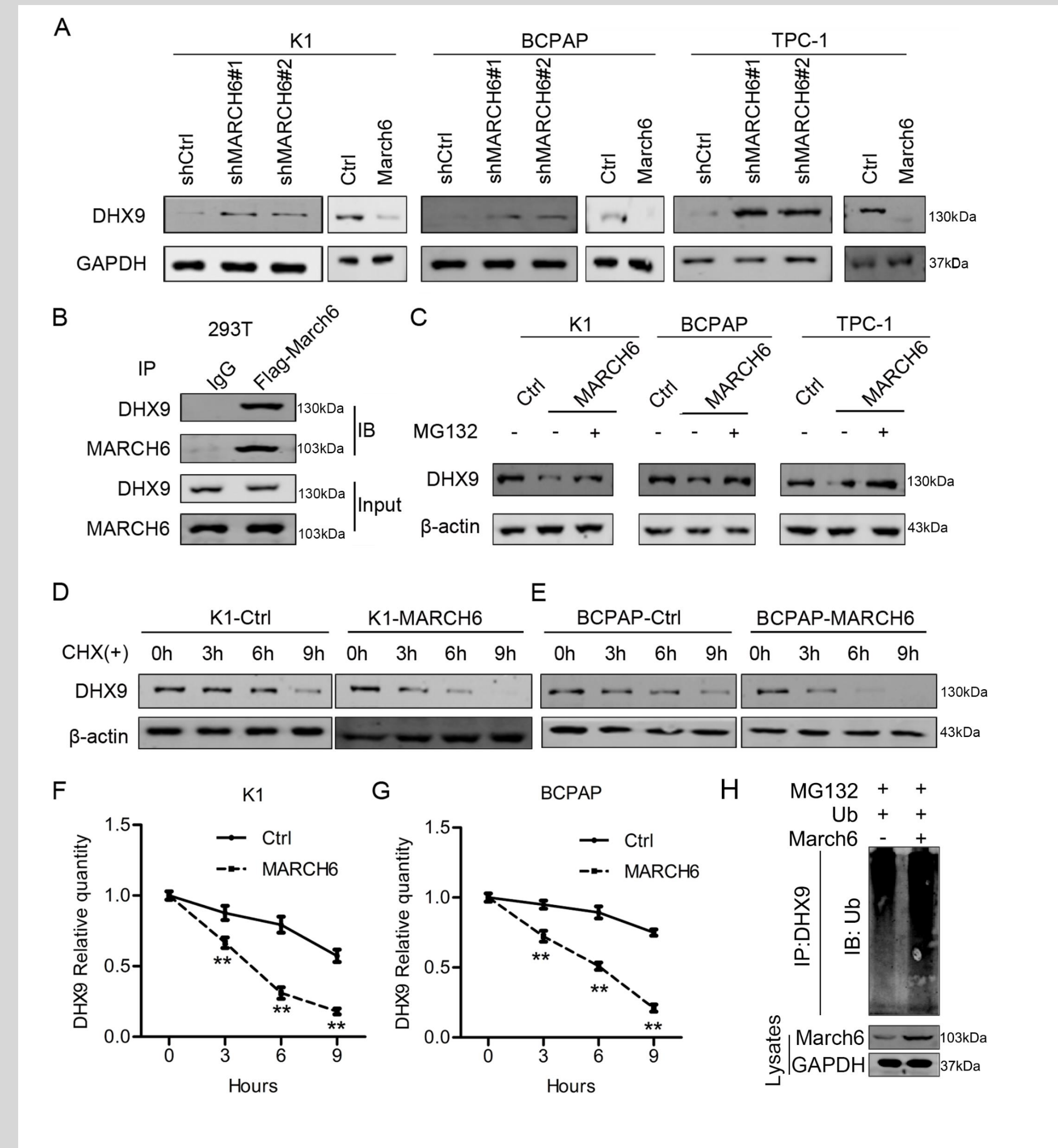


Figure 3 MARCH6 downregulates DHX9 via ubiquitination.

(A) Immunoblotting analysis of DHX9 in MARCH6-knockdown and MARCH6-overexpressing thyroid cancer cells. (B) MARCH6-Flag was overexpressed in HEK293 cells. Cell lysates were subjected to immunoprecipitation with IgG and Flag antibodies and immunoblotting with MARCH6 and DHX9. (C) Ctrl and MARCH6-overexpressing cells were treated with MG132. Immunoblotting was used to detect DHX9. (D-G) Ctrl and MARCH6-overexpressing cells were treated with cycloheximide. Immunoblotting was used to detect DHX9. **p<0.01. (H) Ubiquitin was simultaneously overexpressed in Ctrl and MARCH6 overexpressing TPC-1 cells. The cells were then subjected to IP with DHX9 antibody. Ubiquitination levels were analyzed by immunoblotting assay.

- MARCH6 activates AKT/mTOR signaling through downregulation of DHX9

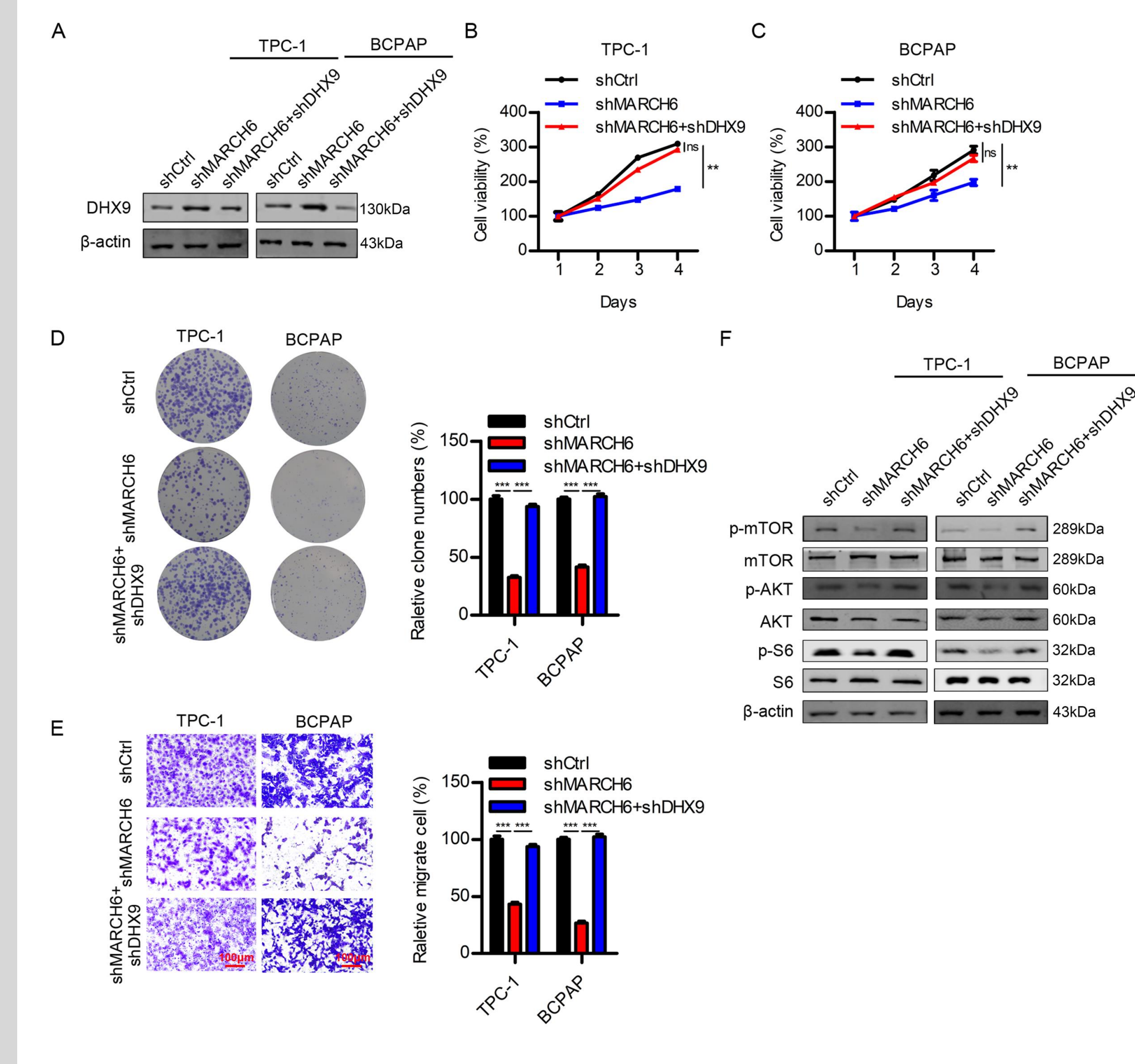


Figure 4 MARCH6 regulates the AKT/mTOR signaling pathway via DHX9.

(A) shCtrl, shMARCH6 and shMARCH6+shDHX9 TPC-1 and BCPAP cells were subjected to immunoblotting analysis of DHX9. (B and C) Cell proliferation was detected by the CCK-8 assay in the cells shown in A. ns, no significance. **p<0.01. (D) The cells were subjected to colony formation analysis. ***p<0.001. (E) The cells were subjected to Transwell migration assays. Scale bars = 100 μm. ***p<0.001. (F) The cells were subjected to immunoblotting with the indicated antibodies.

Conclusions

MARCH6 significantly promotes thyroid cancer growth and migration by interacting with DHX9 and activating the AKT/mTOR pathway.

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