RBM47-regulated alternative splicing of TJP1 promotes cell migration during epithelial-to-mesenchymal transition

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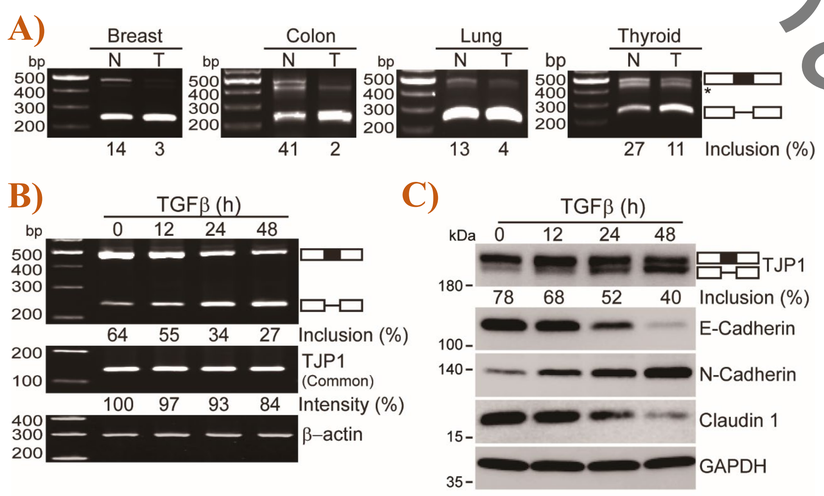
1 ABSTRACT

Epithelial–mesenchymal transition (EMT) is an important process in embryonic development, cancer metastasis, and organ fibrosis. Morphological and functional changes during the EMT process have been demonstrated to be regulated by alternative splicing. However, splicing factors involved in EMT and their regulatory mechanisms are largely unknown. Here, we showed that an isoform of tight junction protein 1 (TJP1), which is lacking exon 20, is predominantly expressed in cancer tissues, and that transforming growth factor-β (TGF-β) induced EMT in A549 cells. In addition, the expression level of RNA binding motif protein 47 (RBM47) was downregulated by TGF-β treatment. We found that alternative splicing of exon 20 of TJP1 was regulated by RBM47. Biochemical studies revealed that RBM47 promoted the inclusion of exon 20 via its direct binding to (U)GCAUG sequence in the downstream intronic region of exon 20. We confirmed that RBM47 competes with Rbfox2 for the (U)GCAUG sequence, which has been demonstrated to be a binding motif for the Rbfox family proteins, even though Rbfox2 has been shown to be irrelevant in the regulation of TJP1 splicing. Furthermore, we examined several genes that contain the (U)GCAUG sequence in a downstream intronic region of an alternative exon. The inclusion of these alternative exons were increased due to the overexpression of RBM47. Finally, we found that phenylalanine residues (amino acid 115 in RBM47) within the first RRM domain among three RRM domains were critical for its binding to the pre-mRNA of TJP1. Taken together, we demonstrated the regulatory mechanism for the alternative splicing of TJP1 pre-mRNA by RBM47 during EMT, thus providing a basis for studies related to EMT modulations in diseases involving cancer metastasis and fibrosis.

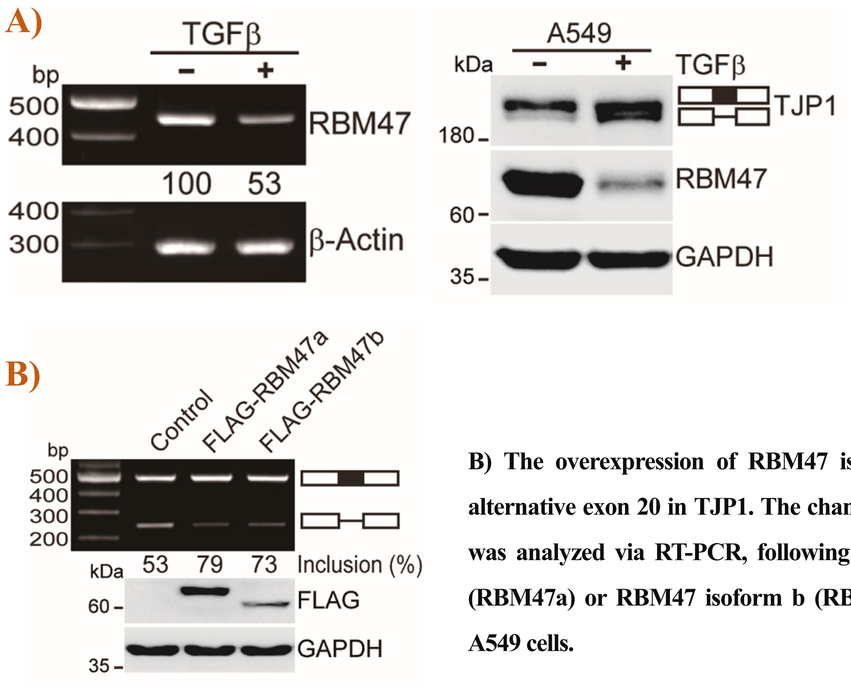
2 TGF-β induces alternative splicing change of TJP1.



A) An isoform of TJP1 lacking alternative exon 20 is predominantly expressed in tumor tissues. The upper and lower bands represent inclusion and exclusion of alternative exon 20 of TJP1, respectively. The TJP1 splicing patterns were analyzed by RT-PCR.

B) An inclusion of TJP1 alternative exon 20 decreases by TGF-β treatment in A549 cells. An exon in common region of TJP1 isoforms was used to identify expression levels of TJP1 mRNA by TGF-β treatment. (C) The protein expression of TJP1 alpha (-) isoform, which results from exclusion of TJP1 alternative exon, increases by TGF-β treatment in A549 cells. The E-Cadhrein, Claudin 1, and N-Cadherin were used to represent the TGF-β induced EMT

3 RBM47 regulates alternative splicing of TJP1.

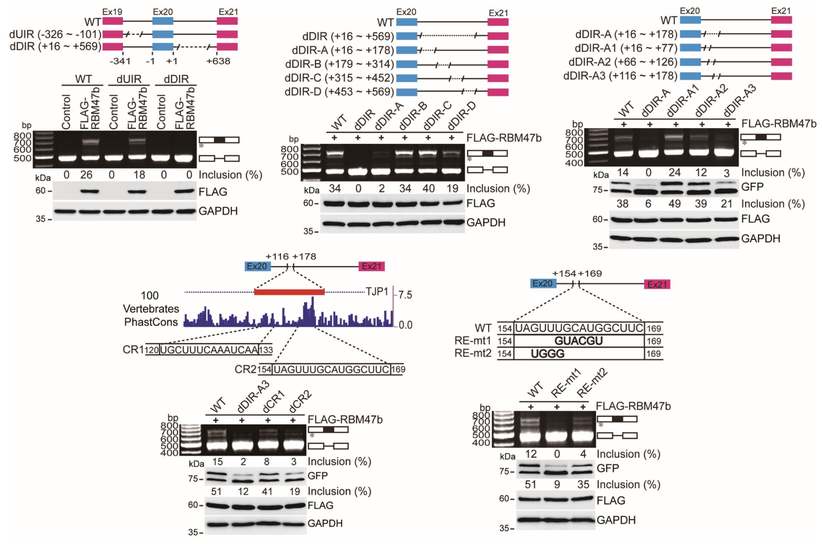


A) An expression of RBM47, alternative splicing factor, decreases transcriptionally and translationally by TGF-β treatment. The expression of RBM47 was measured by RT-PCR and immunoblot, following 5 ng/ml of TGF-β treatment for 48h in A549 cells.

B) The overexpression of RBM47 isoforms increases an inclusion of alternative exon 20 in TJP1. The changes of alternative splicing of TJP1 was analyzed via RT-PCR, following FLAG-tagged RBM47 isoform a (RBM47a) or RBM47 isoform b (RBM47b) transient transfection into A549 cells.

4 RBM47 enhances an inclusion of alternative exon in response to UGCAUG sequence in downstream intronic region of TJP1 exon 20.

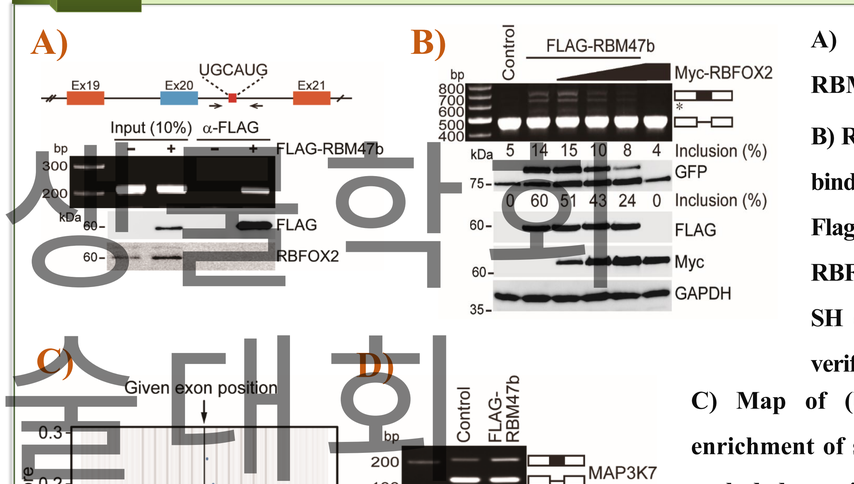
Deletion or mutation constructs of intronic regions in TJP1 minigene



An UGCAUG sequence in downstream intronic region of exon20 is identified as *cis*-regulatory elements for RBM47b.

The minigene construct contains genomic segment, which includes alternative exon 20 (Ex20), flanking constitutive exon 19 (Ex19) and 21 (Ex21), and upstream and downstream introns of exon 20, of TJP1 gene. The exon 19 fused with enhanced GFP (EGFP) for transcription. To investigate the response element of RMB47 in TJP1 was subjected to RT-PCR.

5 RBM47 binds directly to (U)GCAUG sequence in intronic region for regulation of alternative splicing



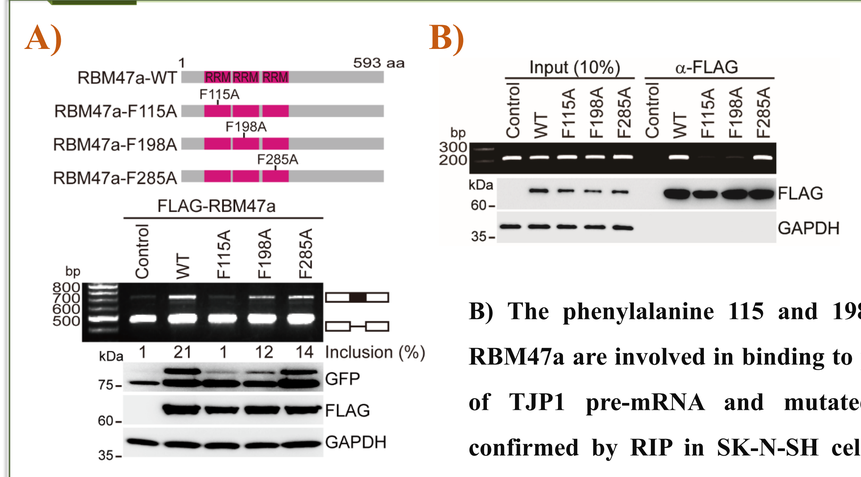
A) RIP analysis was shown that RBM47b binds to pre-mRNA of TJP1.

B) RBM47b competes with RBFOX2 for binding to (U)GCAUG sequence. After Flag-tagged RBM47b and Myc-tagged RBFOX2 were cotranfected into SK-N-SH cells, the changes of TJP1 AS was verified using RT-PCR.

C) Map of (U)GCAUG motif represents an enrichment of sequence in downstream intron of excluded exon in RBM47 knockdown H358 cells.

D) The inclusions of alternative exon by RBM47 were validated with genes involving (U)GCAUG sequences in downstream intron of alternative exon.

6 The first RRM domain of RBM47 plays a crucial role for its binding to pre-mRNA of TJP1.

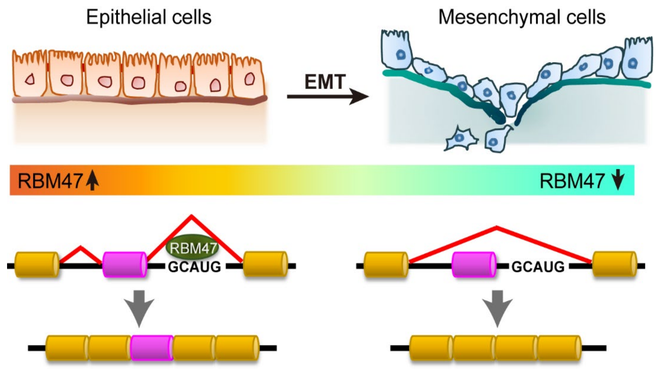


A) The phenylalanine 115 residue of RBM47 isoforms is involved in the inclusion of TJP1 alternative exon 20. Diagram represents mutation constructs of RBM47a

B) The phenylalanine 115 and 198 residues in RRM domains of RBM47a are involved in binding to pre-mRNA of TJP1. The binding of TJP1 pre-mRNA and mutated FLAG-tagged RBM47a was confirmed by RIP in SK-N-SH cells using anti-FLAG M2 affinity agarose gel.

7 SUMMARY

A model for alternative splicing regulation by RBM47



In epithelial cells, the RBM47 is dominantly expressed, allowing to bind directly to (U)GCAUG in downstream intronic region of alternative exon and promote exon inclusion. By contrast, in mesenchymal cells, the expression of RBM47 decreased remarkably, and an alternative exon excluded due to absence of RBM47.