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A common variant rs2272804 in the 5'UTR of RIBC2 inhibits downstream gene expression by creating an upstream open reading frame

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Abstract. – OBJECTIVE: The RIB43A domain with coiled-coils 2 (RIBC2) encodes an uncharacterized vertebrate protein exhibiting similarity with Chlamydomonas protofilament ribbon proteins, required for ciliary motility. To date, no functional variants capable of triggering a change in the expression of RIBC2 have been reported.

PATIENTS AND METHODS: The genotypes of rs2272804 in 30 individuals were identified with Sanger sequencing to estimate allele frequencies. Dual-Luciferase and mutagenesis assays were carried out to investigate the impact of rs2272804 on transcriptional and translational levels. The microarray data of 7 types of cancer were obtained from the Gene Expression Omnibus (GEO) to explore the role of rs2272804 in those diseases.

RESULTS: In this study, we identified a common variant in the 5'UTR of RIBC2, rs2272804, which can create an upstream open reading frame (uORF) in the 5'UTR significantly inhibiting the expression of its host gene. Using Dual-Luciferase constructs, we found that this variant leads to an 85% reduction in translational efficiency, but only a 20% decrease was observed at the transcriptional level. In terms of population studies, mRNA levels of RIBC2 varied according to their rs2272804 genotypes. The "A" allele homozygotes, which created a uORF, showed the lowest transcriptional levels while the transcriptional activity of the "C" allele homozygotes without an uORF was the highest, consistent with the *in-vitro* studies. Furthermore, we explored its role in 7 types of cancer and identified RIBC2 as a significantly differentially expressed gene (DEG) in breast cancer (BRCA), ovarian serous cystadenocarcinoma (OV), and kidney renal clear cell carcinoma (KIRC). Finally, we showed that the overexpression of RIBC2 enhanced the expression of TRIM37 and down-regulated TRAF2. TRIM37 is a member of the tripartite motif (TRIM) family involved in developmental patterning and oncogenesis while TRAF2 is associated with the signal transduction from members of the TNF receptor superfamily.

CONCLUSIONS: Our reports identified a common variant that exerts a dramatic impact on expression efficiency and provides further functional insight into RIBC2.

Key Words:
RIBC2, UORF, CHIP-seq, TRIM37.

Introduction

An upstream open reading frame (uORF) is one of the most critical post-transcriptional regulatory elements underlying cellular regulation of gene expression[1]. It is characterized by an ORF sequence with its start codon preceding the translation initiation site (TIS) in the 5'UTR[2]. Commonly found in mature mRNA from mammal transcriptomes, UORFs typically reduce the protein expression of their host genes by blocking the efficiency of translation initiation or by triggering mRNA decay[2,3]. Therefore, mutations located in the 5'UTR can affect the transcriptional and translational efficiency by changing the number of uORFs or uORF-encoded peptides, which we term "uORF-altering mutations". To date, multiple works have linked uORF-altering mutations to human diseases. Occhi et al[4] reported a novel 4-bp deletion that caused a frameshift in the uORF of CDKN1B resulting in a MEN4 phenotype; Wen et al[5] identified a range of defects in the second uORF of HR that gave rise to increased translation of downstream main ORF leading to Marie

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Unna hereditary hypotrichosis[5,6]; and Hornig et al[7] described a recurrent germline mutation in the 5' untranslated region of androgen receptor (AR) genes with complete androgen insensitivity syndrome.

In this study, we focused on a common variant that creates an uORF that has a dramatic impact on the expression of RIBC2, which encodes a testis-specific ribbon protein that has rarely been explored. RIBC2, also known as TRIB or C22orf11, is required for ciliary motility and is clustered with some known ciliary beating components[8]. Previous RNA-sequencing data of different tissues from 95 people demonstrated the particularly high expression of RIBC2 in the testis[9]. Pathological research has shown that RIBC2 is hypermethylated in ulcerative colitis (UC) patients and associated with transcriptional repression[10]. Therefore, we attempted to find other genetic factors influencing the expression of RIBC2, and we present a preliminary functional study identifying potential interacting genes. After scanning all known variants of the 5'UTR of RIBC2 from dbSNP, rs2272804 was identified as an uORF-altering variant. This mutant allele created the only uORF in the 5'UTR of RIBC2, potentially by blocking the normal scanning of ribosomes on the main ORF of its host gene. We evaluated the impact of rs2272804 at the transcription and translation levels of RIBC2 using Dual-Luciferase assays.

Further, to explore its role in various types of cancer, we used microarray data to systematically identify all the differentially expressed genes (DEGs). We found RIBC2 to be a significant DEG in human breast cancer (BRCA), ovarian serous cystadenocarcinoma (OV), and kidney renal clear cell carcinoma (KIRC). Finally, a preliminary investigation of its interacting genes observed that overexpression of RIBC2 led to up-regulation of tripartite motif-containing 37 (TRIM37), a gene involved in developmental patterning and oncogenesis that can promote the growth and migration of pancreatic cancer cells[11]. The overexpression of RIBC2 also led to down-regulation of TNF receptor-associated factor 2 (TRAF2). TRAF2 can mediate the signal transduction from members of the TNF receptor superfamily, and plays multiple roles in TNF receptor-induced signaling to NF-κB, MAP kinases, and cell death[12]. Finally, we conducted ChIP-qPCR assays of the transcriptionally active regions of TRAF2 and TRIM37 identified by the ChIP-Seq data from Encode. We showed that RIBC2 commonly activated TRIM37 expression by directly binding to its promoter region. Based on our investigations, we suggest that this variant plays an initial role in oncogenesis by notably altering the expression of RIBC2.

Patients and Methods

Cell Culture and Luciferase Reporter Gene Assays

HEK-293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Hyclone, South Logan, UT, USA) with 10% fetal bovine serum (FBS; Hyclone, South Logan, UT, USA) and appropriate antibiotics. The full-length wild-type (WT) 5'UTR sequence of RIBC2 was amplified from human genomic DNA and cloned upstream of hRluc in reconstructed psiCHECK-2 vector (Promega, Madison, WI, USA). Meanwhile, the vector reconstruction steps were given in supplement. PCR-mediated mutagenesis was used to construct the mutant RIBC2 5'UTR sequence. For transfection investigations, 200 ng of each construct was introduced into cultured HEK-293T cells using the Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 24 h, cells were washed and lysed according to the manufacturer's protocol (Promega, Madison, WI, USA). The activity of firefly luciferase (Hluc) and *Renilla* luciferase (hRluc) was measured on the VICTOR x5 Multimode Plate Reader (Perkin-Elmer, Waltham, MA, USA) using the Dual-Luciferase® Reporter (DLR™) Assay System (Promega, Madison, WI, USA). Relative luciferase activity was measured by normalizing the activity against the Hluc luciferase.

Gene Expression Analysis

This investigation was approved by the local Research Ethics Committee and informed consent was obtained from all participants. Peripheral blood was collected for DNA and RNA extraction from 30 unrelated individuals. Quantitative PCR was performed on a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) using the GoTag® qPCR Master Mix (Promega, Madison, WI, USA). Total RNA of transfected HEK-293T cells was extracted with TRIzol® Reagent (Life Technologies, Gaithersburg, MD, USA). Reverse transcription was performed with a GoScript™ Reverse Transcription System

Table I. The ChIP-qPCR primers used in this study.

	5'Primers	3'Primers
TRIM37	CACTGAACTCCAGCCTGGG	GAGGTAATGCTCTGCAACCGG
TRAF2	ATGAACCTTCTGCACCAGGG	ACTTCAGAGCCACACCCGTAG
NC1	CATAACTGTAAATTTTCGCAGGA	TACTTCGTGGTTTTCTAGCTT
NC2	CCATCGGTACAGAATATGCTC	TTGGCTCTCAATTTGACCAC
GAPDH	AGAAGGCTGGGGCTCATTTG	AGGGGCCATCCACAGTCTTC

(Promega, Madison, WI, USA). The qPCR reactions were carried out in 20 μL volumes containing 10 μL GoTaq® qPCR Master Mix, 1 μL of each cDNA sample, 200-300 nMol of each primer according to their R-squared value and amplification efficiency, and Nuclease-Free Water bringing the total reaction volume to 20 μL. Thermal cycling consisted of denaturation (95°C for 30 s), annealing (60°C for 30 s), and extension (72°C for 25 s), performed in 40 cycle steps. A standard curve analysis for each candidate gene was done before the work. Hluc was used as the vector transcript for the normalization of PCR values, and glyceraldehyde-3-phosphate dehydrogenase was used as a negative control. qPCR analysis was repeated independently three times with triplicate technical samples. A two-tailed *t*-test was used to compare the values of the target samples and the control samples. A *p*-value of $p < .05$ was considered statistically significant. Sequences of primers are listed in Table I.

Microarray Data Mining and Interacting Gene Prediction

To investigate the interactions and functions of RIBC2, we assessed the consequences of its overexpression in HEK-293T. Full-length coding sequences of RIBC2 were amplified from human coding DNA and inserted into the pCDNA3.1+-FLAG expression vector (Clontech, Mountain View, CA, USA). The candidate genes were predicted and selected from Protein-Protein Interaction Database (PrePPI: http://bhapp.c2b2.columbia.edu/PrePPI) and Protein-Protein Interaction Networks (STRING: http://string-db.org)[13,14].

Microarray data were obtained from Gene Expression Omnibus (GEO). Seven types of cancer: 1. kidney renal clear cell carcinoma (KIRC); 2. osteosarcoma (OS); 3. human breast cancer (BRCA); 4. hepatocellular carcinoma (HCC); 5. ovarian serous cystadenocarcinoma (OV); 6. acute myeloid leukemia (AML), and 7. pancreatic adenocarcinoma (PAAD) were randomly chosen to investigate how the expression of RIBC2 affects tumor tissues compared with normal tissues. GEO2R and in-house scripts were used to identify differentially expressed genes. The procedure had 4 steps: 1. Scanning of basic information from the microarray data (including experiment information, case/control, single/binary channel(s), and density of probes). 2. Normalization by cleaning the experiment background and unification within groups. 3. Build a linear model to screen for differentially expressed genes using the Limma package downloaded from biocLite. 4. Affirm statistical significance by meeting the threshold ($p < 0.05$, Fold Change > 1, adj. $p < 0.05$). This method has previously been used to identify a 19-gene expression signature that serves as a predictor for survival in colorectal cancer[15].

To identify the transcriptionally active regions or the promoters of TRAF2 and TRIM37, ChIP-Seq data of H3K4me1 and H3K4me3 were downloaded from Encode, and the processed data were presented using IGV (Integrative Genomics Viewer) to analyze the binding peaks. ChIP assays were performed with the Simple ChIP Assay Kits (Cell Signaling Technology, Danvers, MA, USA). HEK-293 cells were first treated with 37% formaldehyde for 10 min at room temperature and subjected to ultrasonication on ice. The DNA-protein complex was immune-precipitated with the RIBC2 antibody (Abnova, 89207561, Taipei, Taiwan). The DNA fragments were then reversely released and amplified by specific PRC reactions. The primers used in the ChIP assays have been listed in Table I.

Results

Impact of Rs2272804 on Downstream Gene Expression

According to the RefSeq database, only one transcript (NM_015653) for RIBC2 was annotated, and the position of rs2272804 was chr22:45413743 (GRCh38/hg38). The total length of the 5'UTR for NM_015653 was 196bp. The "C" allele of rs2272804 was marked as the reference allele while the "A" allele created an uORF with 111bp,

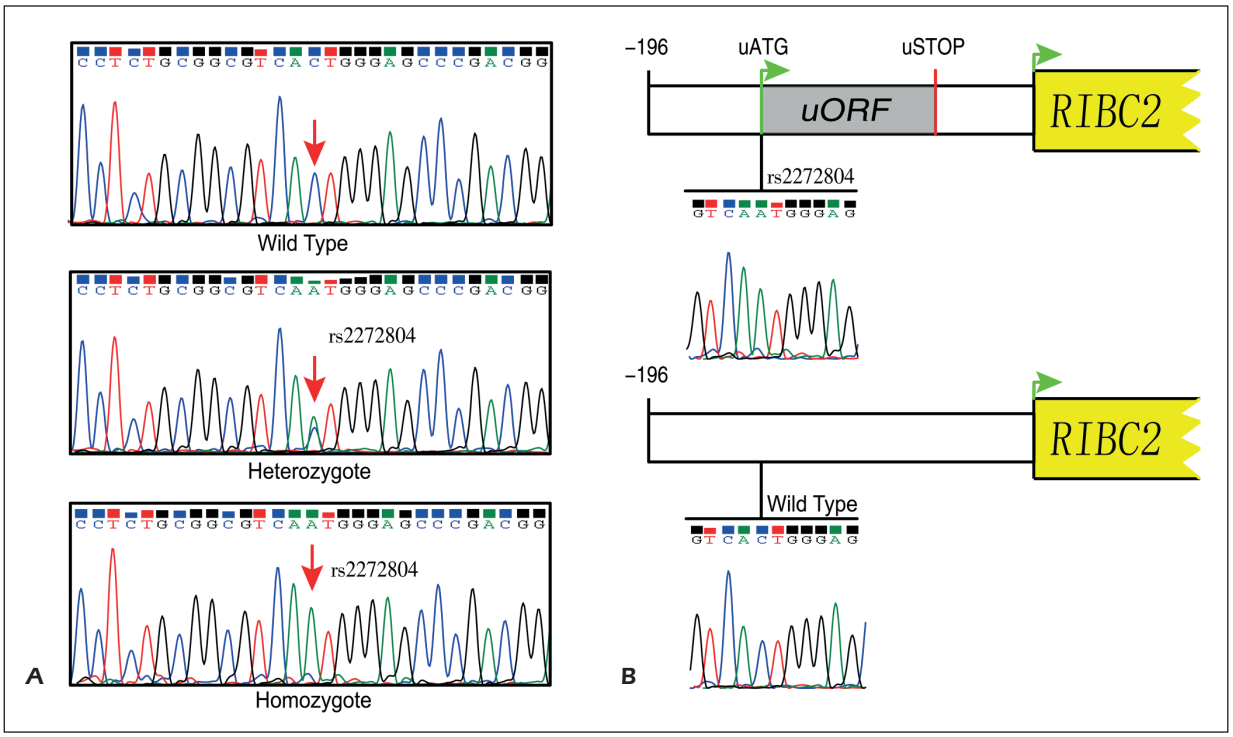

Figure 1. Rs2272804 created a fully upstream open reading frame on the 5'UTR of RIBC2. **A**, Sanger sequencing results of wild type, heterozygote, and homozygote of rs2272804; **B**, Atlas of structure in wild-type and mutant rs2272804 showed that the C > A mutation created the only uORF in 5'UTR of RIBC2.

turning the non-uORF transcript into a transcript with only one uORF (Figure 1). Both mRNA and protein levels were measured to determine whether the existence of this uORF in the 5'UTR region of RIBC2 influenced the expression of the gene. The 5'UTR sequences with wildtype and mutant rs2272804 were inserted into the psiCheck2 plasmid and verified using Sanger sequencing. At the protein level, the activity of firefly luciferase (Hluc) and *Renilla* luciferase (hRluc) were measured. The Dual-Luciferase assays showed that the hRluc activity (normalized using Hluc activity) dropped by about 85% after the "A" allele of rs2272804 was introduced, showing a significant inhibiting effect of the uORF-producing variant on post-transcriptional regulation ($p < 0.0001$) (Figure 2A). Next, to investigate the impact of this variant on the mRNA levels, total RNA of transfected HEK-293T cells was extracted and reverse-transcribed into cDNA. Quantitative PCR was used to evaluate the CT values of hRluc genes between the wild-type and mutant constructions (against Hluc expression). We found that the "A" allele of rs2272804 led to a decrease in mRNA level by about 20% ($p = 0.0205$) (Figure 2B).

Population Studies on Rs2272804

To investigate the *in-vivo* expression of RIBC2 in both heterozygous and homozygous genotypes of rs2272804, peripheral blood samples from 30 healthy participants were used. A pair of primers were designed to amplify the 5'UTR sequence of RIBC2, and the genotypes of rs2272804 were identified by sequencing (Figure 1). We identified 4 C/C homozygous, 15 A/C heterozygous, and 11 A/A homozygous samples, which was comparable with the frequencies recorded in dbSNP (A:C allele ratio = 0.539:0.461 in Asian populations). A Hardy Weinberg Equilibrium was performed on this SNP using Package "genetics" in R (Version: 3.5.1), and it passed the HWE-test ($p = 0.6973$). Furthermore, the mRNA levels of RIBC2 were measured by the Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and were consistent with the *in-vitro* results of Dual-Luciferase studies (Figure 2B). The results showed that the *in-vivo* expression of RIBC2 was significantly different among these three groups (C/C, C/A, and A/A) normalized to the expression of GAPDH. The A/A homozygous samples had the lowest mRNA levels of RIBC2 while the C/C homozygous samples, whose 5'UTR

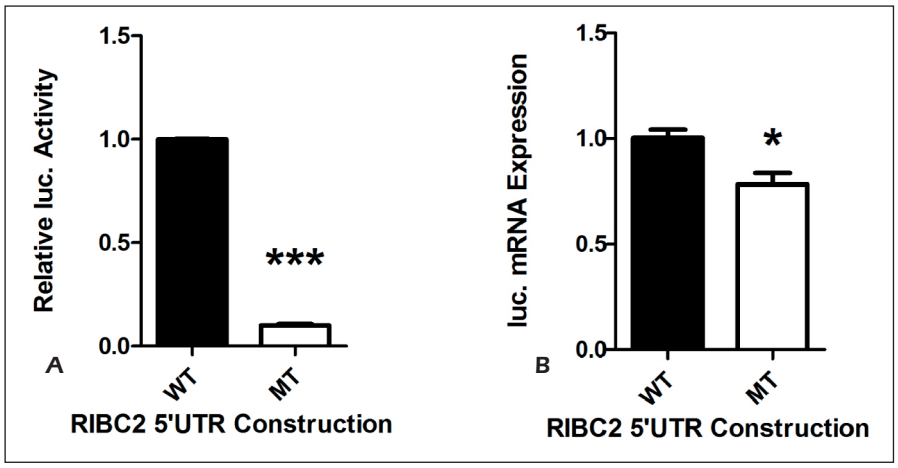

Figure 2. The impact of the rs2272804 variant on the transcriptional and translational levels of luciferase activities.

of RIBC2 was not embedded with any uORF, exhibited the highest mRNA levels on average (Figure 3). The inhibiting effect of the "A" allele was significant in homozygous and heterozygous groups (C/C-A/A $p < 0.0001$, C/C-C/A $p < 0.0001$, C/A-A/A $p = 0.0005$).

RIBC2 Expressions in Diverse Types of Cancer and its Interacting Genes

Since RIBC2 was not characterized well and our luciferase works showed that rs2272804 led to a reduction of RIBC2 expression at both mRNA and protein levels. We focused on whether the alteration in expression of RIBC2 was associated with oncogenesis. The expression values of RIBC2 in 7 types of cancer, above-mentioned, were evaluated using the open-source microarray data from GEO datasets. To avoid experimental bias from undersized sample space, we chose the series of works with no fewer than 20 samples. We found that RIBC2 was significantly up-regulated in BRCA ($p < 0.0001$) and down-regulated in OV ($p < 0.0001$) and KIRC ($p = 0.0004$) (Figure 4). The normalized expression of RIBC2 for each sample is shown in Figure 5. Next, genes that interact with RIBC2 were explored with in-silico studies and, using the STRING website and the PrePPI database (Figure 6, Table II), *TRAF2*, *TRIM37*, *VIM*, and

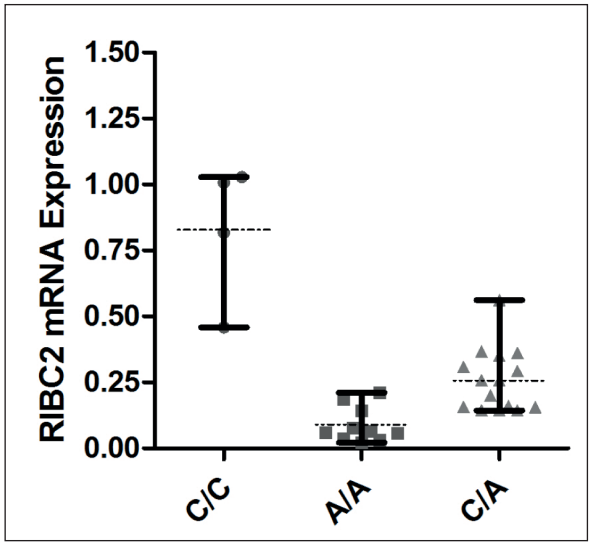

Figure 3. The differences in RIBC2 mRNA levels among individuals with CC, AA, and CA genotypes in rs2272804.

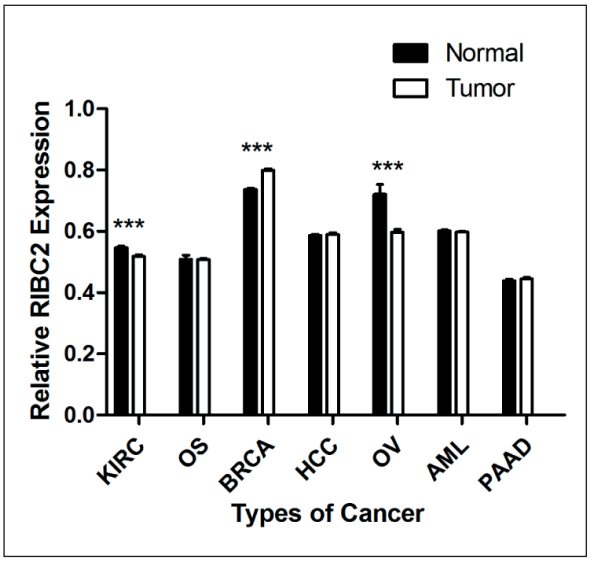

Figure 4. The expression of RIBC2 among 7 different tumor tissues and normal tissue. RIBC2 is up-regulated in BRCA while it's down-regulated in OV.

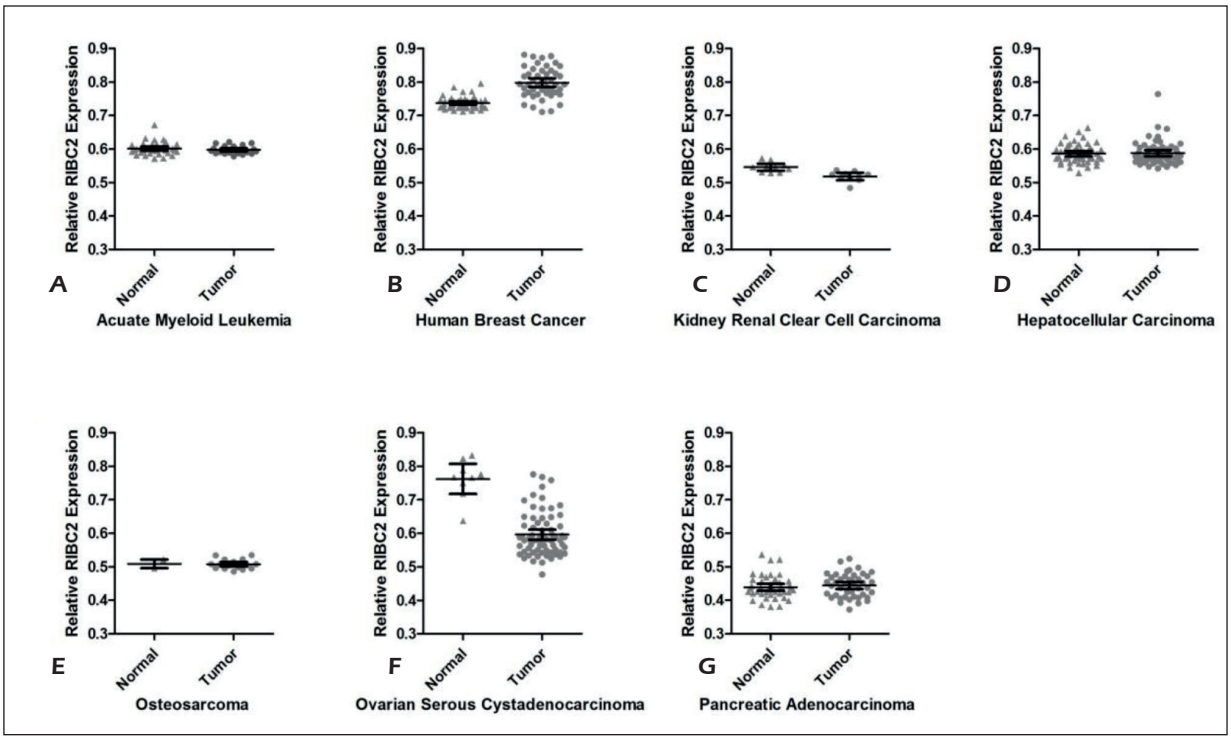

Figure 5. Relative RIBC2 expression in seven candidate tumors.

UBASH3B were selected as candidates that might interact with RIBC2. For experimental validation, full-length coding sequences of RIBC2 were synthesized and cloned into the pCDNA3.1+-FLAG expression vector, and that plasmid was transfected into HEK293T for overexpression. The overexpression of RIBC2 led to significant up-regulation of TRIM37 (p = .0491) and down-regulation of

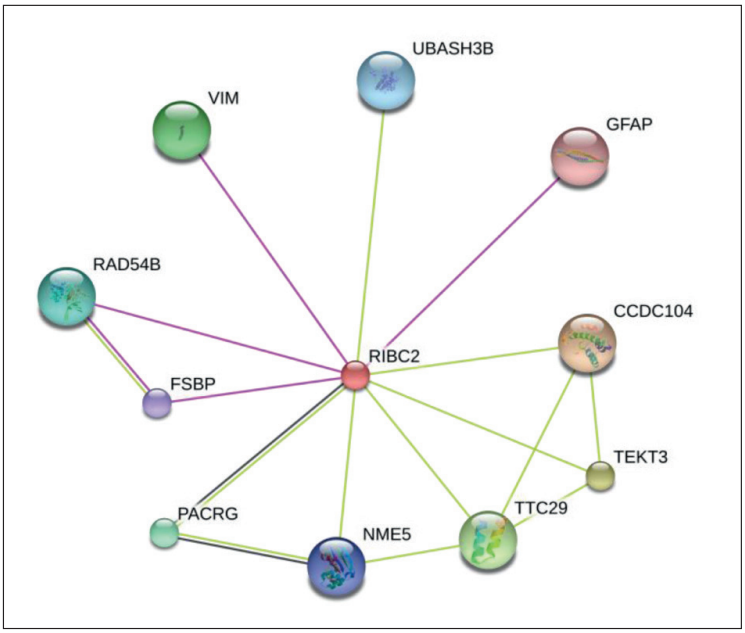

Figure 6. RIBC2 protein interaction network from STRING.

Table II. Interacting genes with RIBC2 predicted from PrePPI.

Uniprot_id	Gene_name	PrP	Max_S	OR	EP	PR	Pred_Score	Exp_Score	Final_Prob
Q12933	TRAF2	15.9043	15.9043	0	2.013	2.1077	67.4788	4625.64	0.998
O94972	TRIM37	15.9043	15.9043	0.5125	2.013	2.1077	34.5829	4625.64	0.996
Q15834	CCDC85B	0	0	0	0	0	0	4625.64	0.885
Q8WXE1	ATRIP	0	0	0	0	0	0	4625.64	0.885
Q9Y2I6	NINL	0	0	0	0	0	0	4625.64	0.885
P19012	KRT15	0	0	0	0	0	0	4625.64	0.885
P14136	GFAP	0	0	0	0	0	0	4625.64	0.885
Q96BY9	TMEM66	0	0	0	0	0	0	4625.64	0.885
Q5JR59	MTUS2	0	0	0	0	0	0	4625.64	0.885
Q9NSU2	TREX1	0	0	0	0	0	0	4625.64	0.885
P14923	JUP	0	0	0	0	0	0	4625.64	0.885
P08670	VIM	0	0	0	0	0	0	4625.64	0.885
P36406	TRIM23	0	0	0	0	0	0	4625.64	0.885
O95967	EFEMP2	0	0	0	0	0	0	4625.64	0.885

TRAF2 ($p = 0.0096$) normalized to the expression of GAPDH, while the expressions of VIM and UBASH3B remained stable despite the over-expressing of RIBC2 (Figure 7). The qPCR primers were designed for the binding peaks of H3K4me1 and H3K4me3 on TRIM37 and TRAF2 (Figure 8A). The ChIP-qPCR assays indicated significant enrichment of RIBC2 on the promoter region of TRIM37 (Figure 8B).

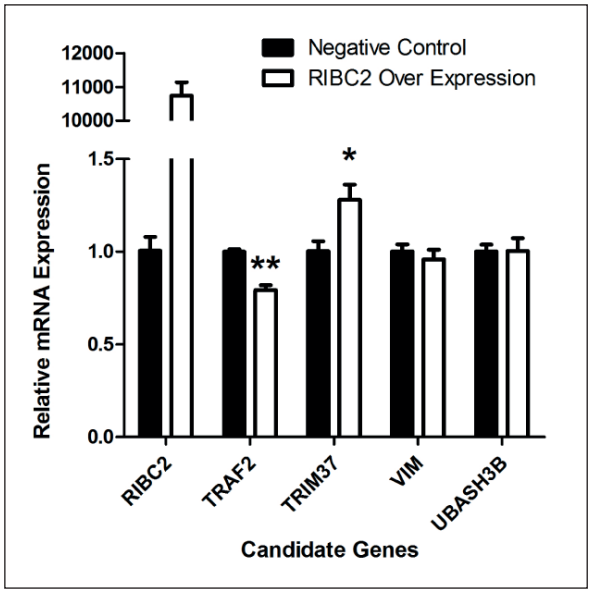

Figure 7. The overexpression of RIBC2 altered the expression of TRAF2 and TRIM37. The candidate genes for potential interaction with RIBC2 were identified by STRING.

Discussion

Identifying functional variants in untranslated regions is challenging, uORF-altering mutations, however, are a critical factor affecting the translation efficiency of their downstream main ORFs and the expression of genes. To date, both common or rare variants that create or remove uORFs, or in-frame AUG codons, have been reported to lead to an altered phenotype[16,17]. This highlights the importance of the comprehensive annotation of variants in 5'UTR from SNP databases produced by high-throughput sequencing technologies. In this work, we explored a common variant in RIBC2, rs2272804, whose function has been poorly reported. The "A" allele, which creates the only uORF in the 5'UTR region of RIBC2, has shown great diversity in its frequency among different races. The high incidence of that variant, which acts as a major inhibitor of RIBC2 expression, indicates that post-transcriptional regulation of this gene is widely-occurring in healthy individuals. This enables us to draw the conclusion that RIBC2 expression varies greatly among people based on their rs2272804 genotype. This has been partly verified by our population studies using qPCR assays. According to the Dual-Luciferase assays, the protein levels and the mRNA levels of RIBC2 are both reduced when an uORF occurred in the 5'UTR region. Johnstone et al[18] have shown that transcripts with uORFs tended to have lower mRNA levels than those with none. The protein levels of RIBC2 are expected to drop more than

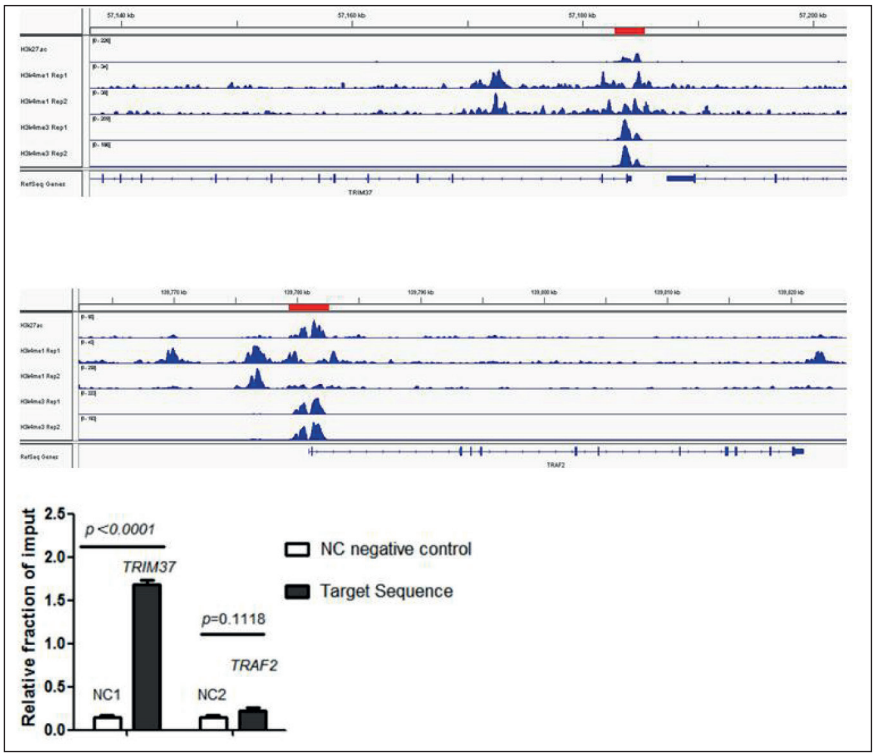

Figure 8. Data mining results from Encode to identify the transcriptionally active regions of TRAF2 and TRIM37 and the exploration of the regulatory role of RIBC2 on TRAF2 and TRIM37.

the mRNA levels. The results of *in-vitro* studies on the function of 'A' and 'C' alleles (with or without uORF) using Dual-Luciferase and qPCR assays are consistent with the RIBC2 quantification from the population research.

Due to the diversity in RIBC2 expression, we attempted to find out whether its expression level was correlated with the development or progression of cancer. Therefore, the microarray data from 7 types of cancer (kidney renal clear cell carcinoma (KIRC), osteosarcoma (OS), human breast cancer (BRCA), hepatocellular carcinoma (HCC), ovarian serous cystadenocarcinoma (OV), acute myeloid leukemia (AML), and pancreatic adenocarcinoma (PAAD)) were retrieved for a systematic identification of differentially expressed genes (DEGs). The RIBC2 expression was significantly up-regulated, or down-regulated, in OV, KIRC, and BRCA. However, considering the high incidence of rs2272804, the moderate changes may be explained by the different distributions of the variant among these patients. More effort towards understanding the genetic background of the recruited individuals is needed to fully understand the expression of RIBC2 in different cancer types. Substantial attempts have been made to elucidate the relation between the common SNPs and a number of specific diseases[19,20]. Therefore, further functional studies should be carried out to confirm whether the accumulation of the common SNPs alters the expression of these genes, potentially influencing the physiological states of their carriers, leading to an increased risk for known diseases. In summary, first, we reported on a functional variant in RIBC2 with a strong inhibition effect on protein expression but a relatively modest effect on mRNA levels. Moreover, a population study was carried out for genotyping and evaluating the mRNA levels of RIBC2, which showed trends consistent with the Dual-Luciferase researches. Besides, expression in seven types of cancer was examined, and RIBC2 was found to be up-regulated in OV and down-regulated in BRCA and KIRC. Finally, the overexpression of RIBC2 leads to a change in expression of the oncoprotein TRIM37 and the down-regulation of TRAF2, which is associated with signal transduction from members of the TNF receptor superfamily. However, according to the ChIP-qPCR results, the regulation of TRAF2 when altering the expression of RIBC2 might be indirect. Of note, TRIM37 plays an important role in a great diversity of cancers. In fact, TRIM37 promoted cell migration and metastasis in hepatocellular carci-

noma by activating the Wnt/β-catenin signaling. Also, it is a new histone H2A ubiquitin ligase and breast cancer oncoprotein[11,21]. Thus, the present work provides some insights into a functional, but common, variant in RIBC2, as well as a preliminary exploration of its potential roles in cancers.

Conclusions

We identified a common variant that exerts a significant impact on expression efficiency and provided further functional insights into RIBC2.

Conflict of Interests

The Authors declared that they have no conflict of interests.

References

1) Somers J, Poyry T, Willis AE. A perspective on mammalian upstream open reading frame function. Int J Biochem Cell Biol 2013; 45: 1690-1700.
2) Barbosa C, Peixeiro I, Romao L. Gene expression regulation by upstream open reading frames and human disease. PLoS Genet 2013; 9: e1003529.
3) Calvo SE, Pagliarini DJ, Mootha VK. Upstream open reading frames cause widespread reduction of protein expression and are polymorphic among humans. Proc Natl Acad Sci U S A 2009; 106: 7507-7512.
4) Occhi G, Regazzo D, Trivellin G, Boaretto F, Ciato D, Bobisse S, Ferasin S, Cetani F, Pardi E, Korbonits M, Pellegata NS, Sidarovich V, Quattrone A, Opocher G, Mantero F, Scaroni C. A novel mutation in the upstream open reading frame of the CDKN1B gene causes a MEN4 phenotype. PLoS Genet 2013; 9: e1003350.
5) Wen Y, Liu Y, Xu Y, Zhao Y, Hua R, Wang K, Sun M, Li Y, Yang S, Zhang XJ, Kruse R, Cichon S, Betz RC, Nothen MM, van Steensel MA, van Geel M, Steijlen PM, Hohl D, Huber M, Dunnill GS, Kennedy C, Messenger A, Munro CS, Terrinoni A, Hovnanian A, Bodemer C, de Prost Y, Paller AS, Irvine AD, Sinclair R, Green J, Shang D, Liu Q, Luo Y, Jiang L, Chen HD, Lo WH, McLean WH, He CD, Zhang X. Loss-of-function mutations of an inhibitory upstream ORF in the human hairless transcript cause Marie Unna hereditary hypotrichosis. Nat Genet 2009; 41: 228-233.
6) Girard M, Bizet AA, Lachaux A, Gonzales E, Filhol E, Collardeau-Frachon S, Jeanpierre C, Henry C, Fabre M, Viremouneix L, Galmiche L, Debray D, Bole-Feysot C, Nitschke P, Pariente D, Guettier C, Lyonnet S, Heidet L, Bertholet A, Jacquemin E, Henrion-Caude A, Saunier S. DCDC2 mutations cause neonatal sclerosing cholangitis. Hum Mutat 2016; 37: 1025-1029.
7) Hornig NC, de Beaufort C, Denzer F, Cools M, Wabitsch M, Ukat M, Kulle AE, Schweikert HU, Werner R, Hiort O, Audi L, Siebert R, Ammerpohl O, Holterhus PM. A recurrent germline mutation in the 5'UTR of the androgen receptor causes complete androgen insensitivity by activating aberrant uORF translation. PLoS One 2016; 11: e0154158.
8) Chung MI, Kwon T, Tu F, Brooks ER, Gupta R, Meyer M, Baker JC, Marcotte EM, Wallingford JB. Coordinated genomic control of ciliogenesis and cell movement by RFX2. eLife 2014; 3: e01439.
9) Fagerberg L, Hallstrom BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S, Danielsson A, Edlund K, Asplund A, Sjostedt E, Lundberg E, Szigyarto CA, Skogs M, Takanen JO, Berling H, Tegel H, Mulder J, Nilsson P, Schwenk JM, Lindskog C, Danielsson F, Mardinoglu A, Sivertsson A, von Feilitzen K, Forsberg M, Zwahlen M, Olsson I, Navani S, Huss M, Nielsen J, Ponten F, Uhlen M. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics 2014; 13: 397-406.
10) Kang K, Bae JH, Han K, Kim ES, Kim TO, Yi JM. A genome-wide methylation approach identifies a new hypermethylated gene panel in ulcerative colitis. Int J Mol Sci 2016; 17. pii: E 1291.
11) Jiang J, Yu C, Chen M, Tian S, Sun C. Over-expression of TRIM37 promotes cell migration and metastasis in hepatocellular carcinoma by activating Wnt/beta-catenin signaling. Biochem Biophys Res Commun 2015; 464: 1120-1127.
12) Barbosa C, Peixeiro I, Romao L. Gene expression regulation by upstream open reading frames and human disease. PLoS Genet 2013; 9: e1003529.
13) Zhang QC, Petrey D, Garzon JI, Deng L, Honig B. PrePPI: a structure-informed database of protein-protein interactions. Nucleic Acids Res 2013; 41: D828-D833.
14) Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ, von Mering C. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2015; 43: D447-D452.
15) Abdul Aziz N, Mokhtar NM, Harun R, Mollah MM, Mohamed Rose I, Sagap I, Mohd Tamil A, Wan Ngah W, Jamal R. A 19-Gene expression signature as a predictor of survival in colorectal cancer. BMC Med Genomics 2016; 9: 58.
16) Somers J, Wilson LA, Kilday JP, Horvilleur E, Cannell IG, Poyry TA, Cobbold LC, Kondrashov A, Knight JR, Puget S, Grill J, Grundy RG, Bushell M, Willis AE. A common polymorphism in the 5' UTR of ERCC5 creates an upstream ORF that confers resistance to platinum-based chemotherapy. Genes Dev 2015; 29: 1891-1896.
17) Girard M, Bizet AA, Lachaux A, Gonzales E, Filhol E, Collardeau-Frachon S, Jeanpierre C, Henry

C, Fabre M, Viremouneix L, Galmiche L, Debray D, Bole-Feysot C, Nitschke P, Pariente D, Guettier C, Lyonnet S, Heidet L, Bertholet A, Jacquemin E, Henrion-Caude A, Saunier S. DCDC2 mutations cause neonatal sclerosing cholangitis. Hum Mutat 2016; 37: 1025-1029.

18) Johnstone TG, Bazzini AA, Giraldez AJ. Upstream ORFs are prevalent translational repressors in vertebrates. EMBO J 2016; 35: 706-723.

19) de Luis DA, Izaola O, Primo D, Aller R. Single nucleotide polymorphisms at the ADIPOQ gene locus rs1501299 interact with different type of dietary fatty acids in two hypocaloric diets. Eur Rev Med Pharmacol Sci 2019; 23: 2960-2970.

20) Wang D, Zhang DF, Feng JQ, Li GD, Li XA, Yu XF, Long H, Li YY, Yao YG. Common variants in the PARL and PINK1 genes increase the risk to leprosy in Han Chinese from South China. Sci Rep 2016; 6: 37086.

21) Bhatnagar S, Gazin C, Chamberlain L, Ou J, Zhu X, Tushir JS, Virbasius CM, Lin L, Zhu LJ, Wajapeyee N, Green MR. TRIM37 is a new histone H2A ubiquitin ligase and breast cancer oncoprotein. Nature 2014; 516: 116-120.