

Original Article

Bcl-2-associated transcription factor 1 interacts with fragile X-related protein 1

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The absence of fragile X mental retardation protein (FMRP) causes fragile X syndrome (FXS), which is the leading cause of hereditary mental retardation. Fragile X-related protein 1 (FXR1P), which plays an important role in normal muscle development, is one of the two autosomal paralogs of FMRP. To understand the functions of FXR1P, we screened FXR1P-interacting proteins by using a yeast two-hybrid system. The fragile X-related gene 1 (FXR1) was fused to pGBKT7 and then used as the bait to screen the human fetal brain cDNA library. The screening results revealed 10 FXR1P-interacting proteins including Bcl-2-associated transcription factor 1 (BTF). The interaction between FXR1P and BTF was confirmed by using both β -galactosidase assay and growth test in selective media. Co-immunoprecipitation assay in mammalian cells was also carried out to confirm the FXR1P/BTF interaction. Moreover, we confirmed that BTF co-localized with FXR1P in the cytoplasm around the nucleus in rat vascular smooth muscle cells by using confocal fluorescence microscopy. These results provide clues to elucidate the relationship between FXR1P and FXS.

Keywords fragile X-related protein 1; Bcl-2-associated transcription factor 1; protein interaction

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Introduction

Fragile X syndrome (FXS) is an X-linked genetic disease, which is the leading cause of inherited intellectual disability. It was first described by Martin and Bell [1]. It was reported that FXS affects ~1 in 2000 men and 1 in 4000 women [2] and 2.8% of the Chinese mental retardation (MR) population screened by DNA analysis had the fragile X full mutation [3]. FXS is caused by the mutations of the fragile X mental retardation gene 1 (*FMR1*), which is located on the X chromosome and whose locus at Xq27.3 coincides with the folate-sensitive fragile site [4,5]. The syndrome is characterized by MR, macroorchidism, typical facial appearance, and

various degrees of autistic behavior [6]. Fragile X-related gene 1 (*FXR1*) belongs to the *FXR* gene family including *FMRI* and *FXR2*, and the sequence of *FXR1* is highly conservative [7]. The coding products of *FXR1* and *FXR2* are fragile X-related protein 1 (FXR1P) and FXR2P, respectively. They share 60% identical amino acids with fragile X mental retardation protein (FMRP), which is encoded by *FMRI* [8,9]. These three proteins share the same characteristic functional domains of RNA-binding proteins (RBPs), i.e. two KH domains and one RGG box, which bind to a variety of RNAs [10] and associate with the 60S ribosomal subunits [11]. They also have a nuclear localization signal and a nuclear export signal, which suggests that they can shuttle between the nucleus and the cytoplasm [10,12].

FXR1P has seven isoforms [13,14], including one cardiac-specific isoform [15] and three muscle-specific isoforms [14]. FXR1P is essential for postnatal survival. Inactivation of FXR1P in mice leads to impaired myogenesis and finally causes the death of neonates shortly after birth, which most probably is due to an abnormal development of the myocardial or respiratory muscle [14]. In *Xenopus*, reduction of FXR1P disrupted the rotation and segmentation of somitic myotomal cell and hindered normal myogenesis [16]. In zebrafish, knockdown of *FXR1* caused abnormalities in striated muscle and severe cardiomyopathy and led to heart failure in the embryos [17]. Finally, in humans, altered expression of muscle-specific isoforms of FXR1P had been implicated in facioscapulohumeral muscular dystrophy. These patients had abnormal expression patterns of three different FXR1P muscle-specific isoforms in the myoblasts and the myotubes [18]. In a word, these studies suggested that FXR1P plays important roles in normal muscle development.

FMRP has been identified as the key cause of FXS. Moreover, the high degree of similarity in protein structure and the relationship among members of the *FXR* family suggests that FXR1P and FXR2P may both play important roles in the pathogenesis of FXS. However, the function of FXR1P and FXR2P in FXS is far from clear. To gain further insights into the function of these proteins, we aim to

identify novel interacting proteins of FXR1P. In this study, we used the full coding sequence of human FXR1P as the bait in a yeast two-hybrid system. Because FXR1P is expressed in fetal brain [19], we chose a human fetal brain cDNA library for screening the FXR1P-interacting proteins [20] and identified novel FXR1P-interacting proteins that could define the function of FXR1P.

Materials and Methods

Plasmids

The full open reading frame of the human *FXR1* (GenBank NC_000003.11) gene was inserted into the pGBKT7 vector [15] to generate recombinant vector pGBKT7-FXR1 (Table 1), which was used as the bait plasmid in yeast two-hybrid screening. In the co-immunoprecipitation assay, the full open reading frame of the human *FXR1* gene and human *BTF* gene were inserted into the pCMV-HA vector [15] and the pCMV-Myc vector [21], respectively, to generate recombinant vector pCMV-HA-FXR1 and pCMV-Myc-BTF (Table 1). Similarly, in the co-localization assay, the full open reading frames of the human *FXR1* gene and human *BTF* gene were inserted into the pEGFP-C1 vector [22] and pDsRed-Monomer-N1 vector [23] to generate the expression vector pEGFP-N1-FXR1 and pDsRed-Monomer-N1-BTF (Table 1), respectively.

Strains and growth conditions

Escherichia coli strains used were DH5 α and Top10. *Saccharomyces cerevisiae* strains were Y187 and AH109. *E. coli* was grown in Luria–Bertani medium (1% tryptone, 0.5% yeast extract, 1% NaCl, and pH 7.0) at 37°C. *Saccharomyces cerevisiae* strains were cultured at 30°C in yeast peptone dextrose medium (2% tryptone, 1% yeast extract, and 2% glucose) or SC medium (0.67% yeast nitrogen base without amino acids and 2% glucose) supplemented with amino acid mixture without the indicated amino acids for selection. Rat vascular smooth muscle cells (VSMCs) were cultured in Dulbecco's modified Eagle's

medium (DMEM) supplemented with 10% fetal calf serum in a humidified 5% CO₂ incubator at 37°C.

Yeast two-hybrid screening

Human Fetal Brain Matchmaker cDNA library (Clontech, Palo Alto, USA) cloned into the vector pGADT7 was used to transfect the yeast strain Y187, and the transfected strain was used for two-hybrid screening [24]. The pGBKT7-FXR1 recombinant plasmid was transfected into the yeast strain AH109, which was then used as the bait to screen the library. All of the procedures were performed according to the manufacturer's protocol (Clontech). The yeast strain AH109-pGBKT7-FXR1 was mated to Y187 pre-transformed with the cDNA library and then cultured for 20 h in yeast peptone dextrose adenine medium with kanamycin to produce diploid zygotes. The transfected products were plated onto the appropriate selective medium SD/Trp⁻/His⁻/Leu⁻, and incubated for 3 days at 30°C. A total of 1 × 10⁸ transformants were plated. The colonies appearing on SD/Trp⁻/His⁻/Leu⁻ medium were collected and assayed for β -galactosidase activity. The β -gal filter lift assay was conducted according to the manufacturer's instructions (Clontech). Subsequently, the library from the positive colonies (positive growth in SD/Trp⁻/His⁻/Leu⁻ medium and positive β -gal activity) were extracted and digested with *Hind*III. Plasmid fragment sizes were analyzed by agarose gel electrophoresis to segregate the colonies. DNA purified from the positive yeast colonies was used to transfect *E. coli* Top10. DNA sequence analysis and BLAST were performed at www.pubmed.com.

Retesting the interaction between BTF and FXR1 in the yeast two-hybrid system

The yeast strain Y187 was transfected with plasmids pGADT7-BTF and then plated on SD/Leu⁻ medium [25]. The yeast strains Y187-pGADT7-BTF and AH109-pGBKT7-FXR1 were tested for autonomous activation, and none of the constructs showed any self-activation. Subsequently, AH109-pGBKT7-FXR1 was mated to Y187-pGADT7-BTF. The

Table 1. Plasmids used in this study

Plasmid name	Vector	Insert (s)	Insertion site	Primer sequence
pGBKT7-FXR1	pGBKT7	FXR1	<i>Eco</i> RI	ctagaattcatgctggacgtgacggtgctagaattctatgaaacaccattc
pCMV-HA-FXR1	pCMV-HA	FXR1	<i>Eco</i> RI, <i>Xho</i> I	tatgaattcggatgctggagctgacggtggaggcgtcaggtatgaaacaccattcaggac
pCMV-Myc-BTF	pCMV-Myc	BTF	<i>Sal</i> I, <i>Not</i> I	gtcgaccatgggtcgtccaattctattctcagaattttgtcttcaaaagtttgctactgc atctcagcggcgcttattctctttctcttgc
pEGFP-N1-FXR1	pEGFP-N1	FXR1	<i>Xho</i> I, <i>Hind</i> III	tatctcgaggatgctggagctgacgatcaagcttgaaacaccattcaggac
pDsRed-Monomer-N1-BTF	pDsRed-Monomer	BTF	<i>Hind</i> III, <i>Kpn</i> I	atcaagcttatgggtcgtccaattcttctcagaattttgtcttcaaaagtttgctactgca tctcacatggtacctattctttctctctgc

transformants were cultured in yeast peptone dextrose adenine medium for 20 h and then plated onto the appropriate selective medium (SD/Trp⁻/His⁻/Leu⁻). After the colonies appeared on the plates, β -galactosidase assays were performed. The interaction was then confirmed if the colonies showed β -galactosidase activity and grew on the SD/His⁻/Trp⁻/Leu⁻ medium [26].

Co-immunoprecipitation assay

VSMCs were grown in DMEM with 10% fetal calf serum, 100 U/ml penicillin, and 100 mg/ml streptomycin. When the cells reached 80% confluence, they were placed in six-well plates with basal DMEM without fetal calf serum or antibiotics and co-transfected with the mammalian expression plasmids pCMV-HA-FXR1 and pCMV-Myc-BTF by Lipofectamine 2000 (Invitrogen, Carlsbad, USA) [27,28]. The transfected cells were incubated for 5 h, and then the medium was replaced with complete DMEM containing 10% fetal calf serum, 100 U/ml penicillin, and 100 mg/ml streptomycin. At 48 h, the cells were harvested. Approximately 2 mg of total protein extract was used in each co-immunoprecipitation [29]. The samples were incubated with either 1 μ g of anti-Myc or anti-HA antibody, and pulled down with protein A/G agarose for 2 h at 4°C. The relative molecular mass of the proteins was estimated by pre-stained protein markers. The pulled-down proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes (Whatman, Piscataway, USA). The transfer conditions were 100 V for 1 h at 4°C. Different antibodies (Santa Cruz Biotechnology, Santa Cruz, USA) were used to detect the corresponding proteins.

Cellular co-localization of FXR1P and BTF

When the confluence of the VSMCs reached 80%, they were placed in six-well plates with DMEM without fetal calf serum and antibiotic. The mammalian expression plasmids

pEGFP-N1-FXR1 and pDsRed-Monomer-N1-BTF were, respectively, single-transfected or co-transfected into the VSMCs by Lipofectamine 2000. The transfected cells were incubated for 5 h, and then the medium was replaced with complete DMEM containing 10% fetal calf serum, 100 U/ml penicillin, and 100 mg/ml streptomycin. The cells were harvested after transfection for about 48 h, then washed twice with PBS, and fixed with 4% paraformaldehyde. Then, the nucleus was stained with 4', 6-diamidino-2-phenylindole (Beyotime, Nantong, China) for 10 min. A laser confocal microscope (Carl Zeiss, Oberkochen, Germany) was used to observe the localization and the co-localization of BTF and FXR1P in the cells.

Results

Ten of the 156 positive colonies containing BTF interacted with FXR1P by yeast two-hybrid system screening

To identify the putative FXR1P-interacted proteins, we performed a yeast two-hybrid screening [30]. A yeast expression vector (pGBKT7) containing the full open reading frame of human *FXR1* fused to the DNA-binding domain (BD) of the yeast transcription factor Gal4 was used as the bait. A human fetal brain matchmaker cDNA library in the vector pGADT7 was used as prey to transfect the yeast strain Y187. After mating AH109-pGBKT7-FXR1 with Y187-pGADT7-cDNA, we performed selection by SD lacking histidine, tryptophan, and leucine (SD/Trp⁻/His⁻/Leu⁻) medium and β -galactosidase assay. Among the 156 positive colonies grown in the SD/Trp⁻/His⁻/Leu⁻ medium, 10 different colonies showed positive results in the β -galactosidase assay (Fig. 1). These 10 colonies were sequenced (Fig. 2) and the results were analyzed by BLAST as the main tool for homology analysis (<http://www.ncbi.nlm.nih.gov>).

After examining, we found one colony from the colonies that expresses a protein highly identical to human

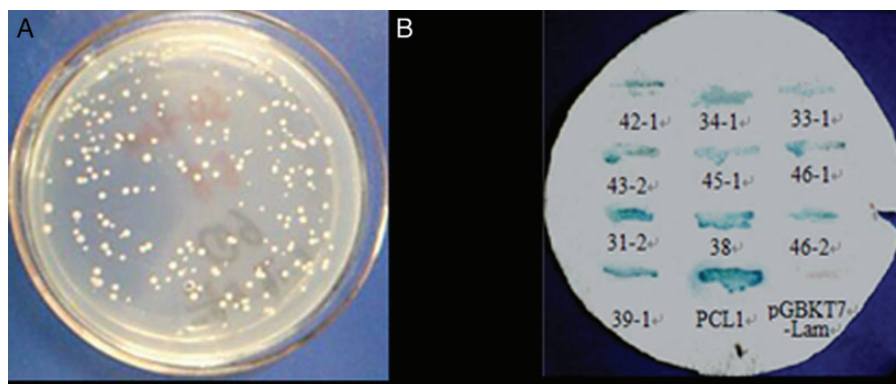


Figure 1. Screening FXR1P-interacting proteins by the yeast two-hybrid system (A) After mating AH109-pGBKT7-FXR1 with Y187-pGADT7-cDNA, we screened with SD lacking histidine, tryptophan, and leucine (SD/Trp⁻/His⁻/Leu⁻) medium and obtained 156 positive colonies. (B) After the colonies appeared on the plates, β -galactosidase assays were performed and we obtained 10 different colonies.

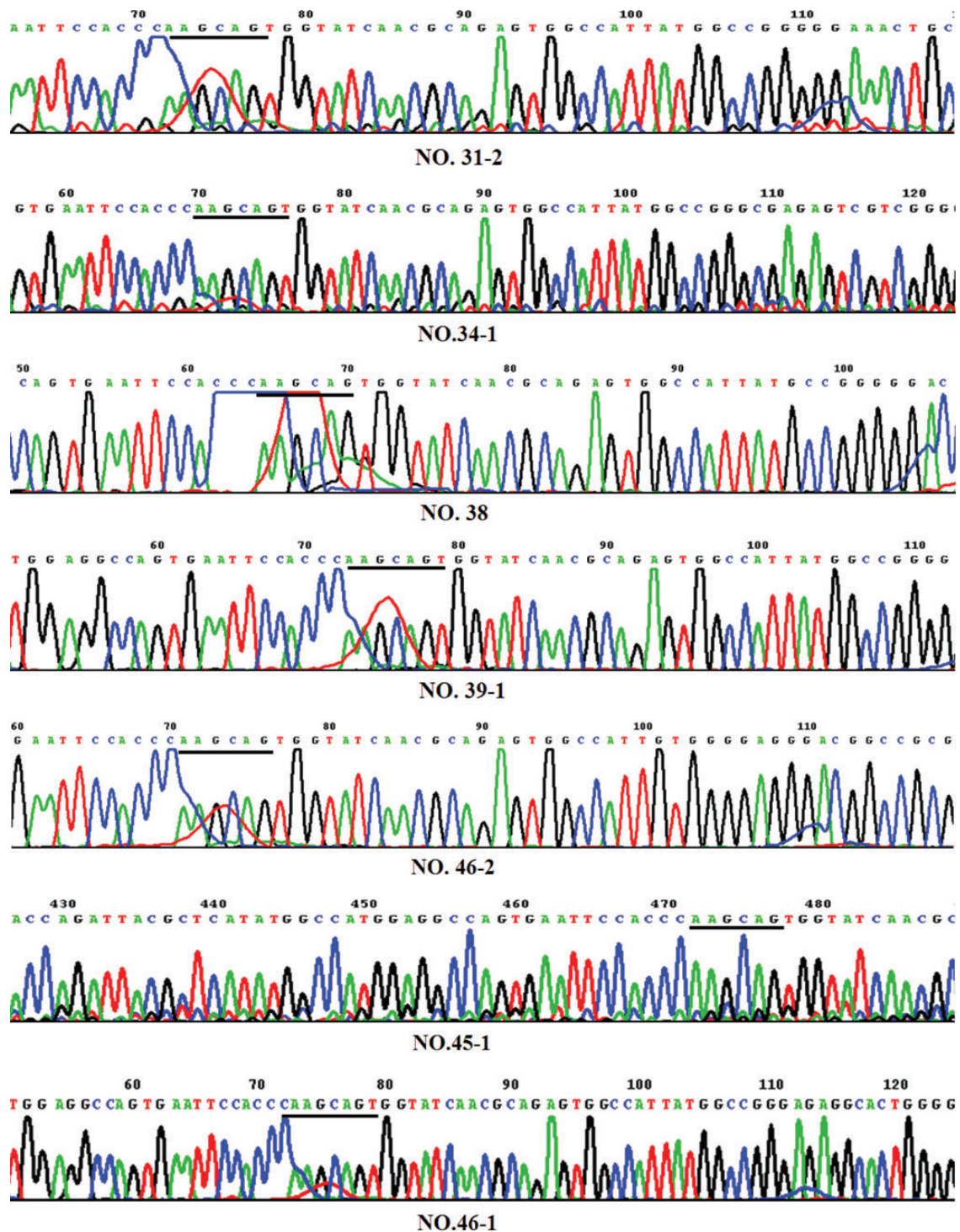


Figure 2. Sequencing result of the BTF positive colonies The 10 positive colonies were sequenced.

Bcl-2-associated transcription factor 1 (BTF) (100% identity) (Table 2), a protein with 921 amino acids.

Direct retesting of the interaction between FXR1P and BTF by using a yeast two-hybrid system

To test the ability of BTF to interact with FXR1P, the two library plasmids were used to transfect Y187, and PcL1 as

the control vector. Mating was performed between various Y187-pGADT7-cDNA colonies and AH109-pGBKT7-FXR1. The transformants were cultured on the SD/His⁻/Trp⁻/Leu⁻ medium. Then, the colonies appeared on the media, and the β-galactosidase assay showed positive results. In addition, no β-galactosidase activity was observed in the yeast strain transformed with only the bait plasmid pGBKT7-FXR1.

Table 2. Bioinformatics analysis result

Clone number	Gene name	GenBank number	Homology
31-2	Bcl-2-associated transcription factor 1 (BTF)	NM014739.1	100%
34-1	<i>Homo sapiens</i> ferritin, heavy polypeptide 1 (FTH1)	NM_002032	99%
38	<i>Homo sapiens</i> hydroxysteroid (17-beta) dehydrogenase 1 (HSD17B1)	NM_000413.1	100%
39-1	<i>Homo sapiens</i> scaffold attachment factor B (SAFB)	NM_002967.2	98%
46-2	CMP-N-acetylneuraminic acid synthetase (CMAS)	NM_018686.3	99%
45-1	<i>Homo sapiens</i> Golgi autoantigen, Golgi subfamily a, 4 (GOLGA4)	NM_002078.3	96%
46-1	Chorionic somatomammotropin hormone 1 (CSH1)	NM_022640.2	100%
43-2	<i>Homo sapiens</i> zinc finger protein 787 (ZNF787)	Non-coding sequence	
42-1	Zinc finger, CCHC domain contain 7	Non-coding sequence	
33-1	<i>Homo sapiens</i> myosin heavy polypeptide 10 (MYH10)	Non-coding sequence	

FXR1P and BTF have direct interaction in rat VSMCs

To confirm the direct interaction between FXR1P and BTF, we performed co-immunoprecipitation assay in the VSMCs. pCMV-Myc-BTF was co-transferred with pCMV-HA-FXR1 into the VSMCs. The cell extracts from the co-transfected cells were immunoprecipitated with either the polyclonal anti-HA antibody or the anti-Myc antibody and protein A/G agarose, and then detected by immunoblotting. After being precipitated with the anti-HA antibody and the protein A/G agarose, the protein could be detected by the anti-Myc antibody. After being precipitated with the anti-Myc antibody and the protein A/G agarose, the protein could be detected by the anti-HA antibody. In our study, a positive control was obtained by the anti-HA antibody or the anti-Myc antibody. A negative control was obtained by IgG. FXR1P and BTF exhibited positive results (Fig. 3). The results indicated that there was an interaction between FXR1P and BTF in the VSMCs.

BTF and FXR1P co-localized mainly in the cytoplasm around the nucleus

To understand the functional role of FXR1P in the muscle cell, we used VSMCs. A eukaryotic expression vector encoding the full coding sequence of human *BTF* fused to red fluorescent protein (RFP) and a eukaryotic expression vector encoding the full coding sequence of human *FXR1* fused to green fluorescent protein (GFP) were used in the transfection experiments. The RFP or enhanced GFP (EGFP) expressed red or green, respectively, however, the co-expression of RFP and EGFP was yellow. The expression and subcellular distribution of these proteins in the transfected cells were analyzed by laser confocal microscope. It showed that the fusion proteins FXR1P and BTF were located in the cytoplasm (Fig. 4). After co-transfection, the positive signal (yellow) was visible in the cytoplasm, which showed that BTF and FXR1P were co-localized mainly in the cytoplasm around the nucleus in the VSMCs (Fig. 5). In this

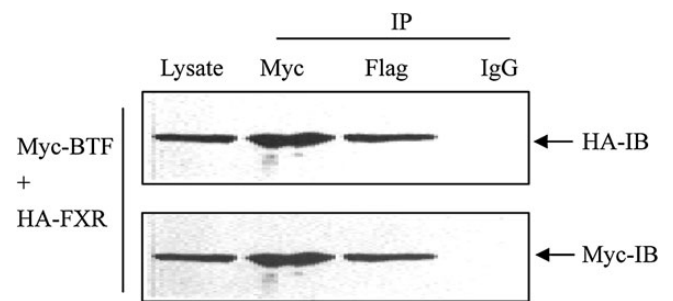


Figure 3. Co-immunoprecipitation of Myc-BTF and HA-FXR1P FXR1P interacts with BTF *in vitro*. Rat VSMCs were co-transfected with pCMV-Myc-BTF and pCMV-HA-FXR1. Then, the lysates were incubated with a mouse anti-HA antibody (or a mouse anti-Myc antibody) and the protein A/G-Sepharose beads. The complex was segregated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and analyzed by immunoblotting with anti-Myc (or anti-HA) antibody. The expressions of BTF and FXR1P were detected with anti-Myc and anti-HA antibody, respectively.

experiment, the transfection rate of the VSMCs was >90%, and the transfection efficiency is the best when the cell density is about 60%.

Discussion

FXS is caused by the expansion of a highly polymorphic CGG repeat in the 5' untranslated region of the *FMR1* gene [31] and abnormal methylation of the adjacent CpG island [32]. FMRP, FXR1P, and FXR2P share some similar structure and domains with known function, but their function is not completely known. It has been confirmed that animal models with *FMR1* and *FXR1/2* knockout showed both similarities and differences in phenotype [18,33]. Thus, whether FXR1P plays roles in the pathogenesis of FXS or other diseases remains unclear.

Protein–protein interactions or protein complexes are integral in nearly all of the cellular processes, ranging from metabolic to structural. Protein–protein interactions are

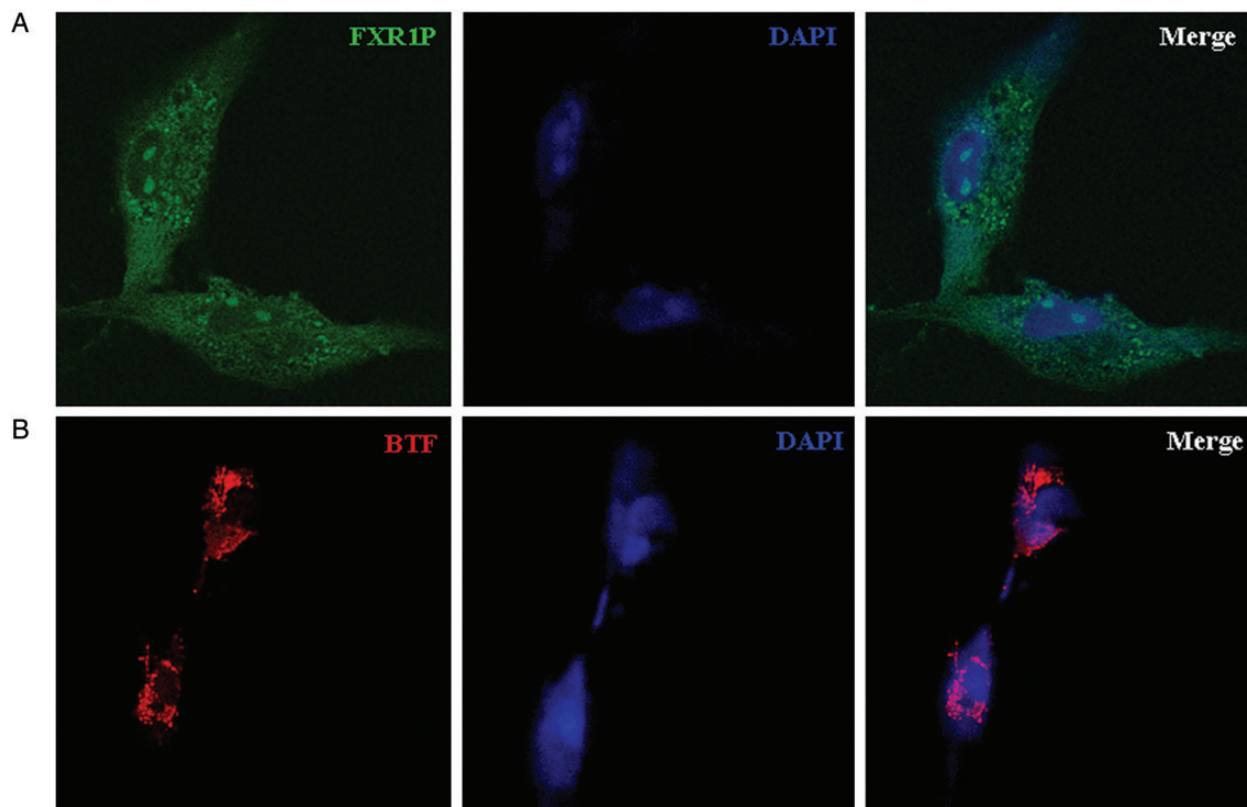


Figure 4. Subcellular co-localization studies of FXR1P and BTF (A) Left: rat VSMCs were transfected with pEGFP-N1-FXR1. FXR1P (green) is located in the cytoplasm. (B) Left: rat VSMCs were transfected with pDsRed-Monomer-N1-BTF. BTF (red) is located in the cytoplasm. The nuclei were stained by DAPI (middle; panels A and B). All of the samples were visualized by laser confocal microscope 48 h after transfection.

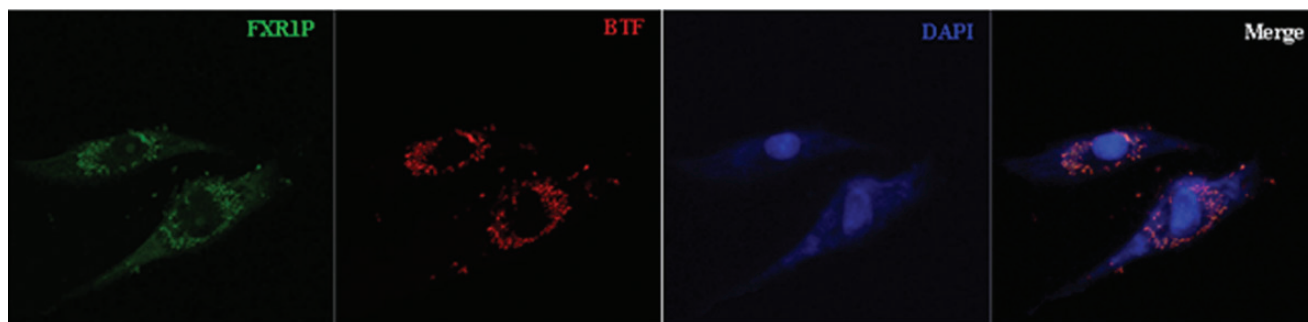


Figure 5. Co-localization studies of FXR1P and BTF pEGFP-N1-FXR1 and pDsRed-Monomer-N1-BTF were co-transfected together into the rat VSMCs. All of the samples were tested after transfection for about 48 h. FXR1P (green) and BTF (red) are visible around the nucleus. Positive signal (yellow) is visible in the cytoplasm. Yellow indicated the co-localization of pEGFP-FXR1P with pDsRed-BTF. The nuclei (blue) were stained by DAPI.

involved in DNA replications and transcriptions, RNA splicing, protein translations and modifications, biological responses, signal transduction, and several other metabolic processes [34]. Finding the interacting partners of a ‘protein of unknown function’ can provide far more insights into the actual function than sequence-based predictions, and can provide a platform for future research. FMRP forms homo- and hetero-multimer with FXR1P and FXR2P *in vitro* as well as *in vivo* [10]. Furthermore, cytoplasmic FMRP interaction protein 2 (CYFIP2) can interact with FMRP as well as FXR1P and FXR2P [35,36].

The yeast two-hybrid system is one of the methods for identifying protein–protein interactions [37]. In the two-hybrid system, two proteins are expressed, respectively, one (a bait protein) fused to the Gal4 DNA BD and the other (a prey protein) fused to the Gal4 transcriptional activation domain. In the Y2HGold yeast strain, activation of the reporters (AUR1-C, ADE2, HIS3, and MEL1) only occurs in a cell containing proteins that interact and bind to the Gal4-responsive promoter [38]. However, the yeast two-hybrid system tends to identify a high proportion of false positives [39]. In this study, we finally obtained 10 different colonies

via the yeast two-hybrid system and chose 1 colony named BTF to be further confirmed by the co-immunoprecipitation and the subcellular co-localization experiments.

Immunoprecipitation is a useful method for studying protein–protein interactions [40]. It can isolate the proteins of interest from the cellular extracts via specific antibodies. By using immunoprecipitation assay, we can determine the interaction of the interested proteins via western blot analysis. Interestingly, after co-immunoprecipitation, the result showed that FXR1P and BTF were co-precipitated. Thus, the two proteins either can directly interact or combine to a link factor to form a protein complex. This evidence may confirm the interaction between BTF and FXR1P.

In fluorescence microscopy, co-localization refers to the observation of the spatial overlap between two (or more) different fluorescent labels. Because each label exhibits a separate emission wavelength, it can be used to determine whether the different ‘targets’ are located in the same area of the cells or very near to one another. This definition can be split into two different phenomena: co-occurrence, which refers to the presence of two (possibly unrelated) fluorophores in the same pixel; and correlation, a much more significant statistical relationship between the fluorophores indicative of a biological interaction [41]. This technique is important in many biological and physiological studies trying to demonstrate a relationship between two proteins. In our study, co-localization is used together with co-immunoprecipitation to characterize protein–protein interactions in a true mammalian environment. As FXR1P plays an important role in normal muscle development, and its absence causes muscular abnormalities in mice, frog, and zebrafish, we used rat VSMCs in the subcellular co-localization experiments. We found that BTF and FXR1P were, respectively, located in the cytoplasm in the transfection assays. However, when the cells were co-transfected with *BTF* and *FXR1P*, we found the merged signal in the cytoplasm around the nucleus. Since the interaction between the two proteins was confirmed by co-immunoprecipitation *in vivo*, the overlapping staining indicated an interaction between the FXR1P and BTF in mammalian cells, but not co-occurrence. However, evidence showed that the distribution space of BTF and FXR1P can completely overlap with each other in the cells. Thus, this proves indirectly that there is an interaction between these two proteins.

BTF is a death-promoting transcriptional repressor [42] and implicated in DNA damage repair and apoptosis [43]. It was first found in human acute myeloid leukemia cell line KG-1, and it locates on human chromosome 6q22-23 [44]. Although linked to the Bcl-2 family members, BTF does not share structural similarities with these proteins [45]. The most prominent feature of BTF is the presence of an arginine-serine (RS)-rich region located near the N-terminus. RS domain-containing proteins are typically linked with

pre-mRNA biogenesis and processing, such as pre-mRNA splicing [46–48]. In 2007, Merz *et al.* [49] examined the composition of human mRNPs by using LC-MS/MS and discovered the presence of BTF among newly identified mRNP proteins. A subsequent study identified BCLAF1 in hmRNP that mediated cyclin D1 message stability with SNIP1, SKIP, TAP150, and Pinin [50].

The most important role of BTF is to function as a regulator in apoptosis, and the protein is widely expressed in some tissues, such as the brain, heart, kidney, liver, lung, and so on, acting as an induction factor for apoptosis. Our results suggested that *in vivo* FXR1P and BTF play a biological role in the forming of a complex or the correlation between them. *FXR1* is a housekeeping gene. FXR1P, the protein encoded by *FXR1*, plays an important role in cell growth, proliferation, and differentiation. Moreover, high expression of FXR1P occurs not only in the brain and the gonad, but also in muscle and heart. Thus, FXR1P is essential to maintain the morphology, structure, and function of these cells.

As FXR1P and BTF are both proteins, and they play key roles in the growth and development of cells, we speculated that there may be two possible types of interaction: (i) when the cells of the body are in the repair or embryonic period and a large number of cells need repair, FXR1P restrains the function of BTF; (ii) when the growth of the cells is excessive and it needs to inhibit this growth to maintain the balance of the cells, FXR1P maybe promotes the function of BTF. Finally, to make BTF and FXR1P attain the dynamic equilibrium, we hypothesized that the interaction between BTF and FXR1P can influence the apoptosis and growing development of the cells.

Moreover, it is worth noting that FXR1P is an RBP. Recent research showed that FXR1P can interact with RNA [51–56] and affect its stability [51], which suggested that FXR1P can participate in regulating gene expression with two pathways: one is to utilize the miRNA pathway to regulate target mRNA expression [57,58]; the other is to play a role in post-transcriptional regulation by interacting with mRNA directly and affecting the stability of mRNA [51]. Although we have not yet known the specific mechanism and interaction between FXR1P and BTF, we can be sure that BTF is a new member of the regulatory networks of FXR1P, and plays a certain role in the entire regulatory system. Meanwhile, FXR1P may also take part in the apoptosis pathway through the regulation by the Bcl-2 protein family and play a role in the process of regulating the growth and development of the cells. Further studies are necessary to clarify the specific mechanism.

We screened 10 FXR1P-interacted proteins including BTF by a yeast two-hybrid system. The encoded product of *BTF* is Bcl-2-associated transcription factor 1. The interaction between FXR1P and BTF was confirmed not only by retesting in yeast using both a β -galactosidase assay

and growth studies on selective media, but also by a co-immunoprecipitation assay and a subcellular co-localization assay in mammalian cells. Furthermore, BTF was co-localized with FXR1P in the cytoplasm of mammalian cells. The present findings suggested that FXR1P may play an important role in the regulation of the cell apoptosis mechanism and in damage recognition and repair by interacting with BTF. In the future, it will be important to explore the roles of FXR1P in cell apoptosis and of FXR1P interaction with BTF in FXS.

In summary, this study, for the first time, showed that FXR1P interacts with BTF *in vivo* and proved that FXR1P and BTF can co-localize mainly in the cytoplasm around the nucleus.

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