Communication

Cloning of an Apobec-1-binding Protein That Also Interacts with Apolipoprotein B mRNA and Evidence for Its Involvement in RNA Editing*

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Apolipoprotein (apo)B mRNA editing is mediated by a multiprotein editosome complex. Apobec-1 is the catalytic component of this complex, but other proteins involved in editing have not been identified. We used the yeast two-hybrid system to identify an apobec-1-interacting protein, ABBP-1. ABBP-1 contains 331 amino acid residues and is identical to a previously reported human type A/B hnRNP except for a 47-residue insertion at its N-terminal region. Northern blot analysis indicates that ABBP-1 mRNA is distributed in multiple human tissues. By deletion analysis, we mapped the apobec-1-binding region to the glycine-rich domain. ABBP-1 also binds to apoB mRNA transcripts around the editing site and can be UV-cross-linked to them in vitro. Immuno-depletion of ABBP-1 from an active apoB mRNA editing transcript inhibits its editing activity. Down-regulation of ABBP-1 in an apobec-1-expressing HepG2 cell line by transfection with an antisense ABBP-1 cDNA construct leads to inhibition of endogenous apoB mRNA editing. We conclude that ABBP-1 is an apobec-1-interacting protein that may play an important role in apoB mRNA editing.

Apolipoprotein (apo)B mRNA editing is a novel mechanism for the posttranscriptional regulation of gene expression in mammals (reviewed in Refs. 1 and 2). It consists of a C → U conversion of the first base of the codon CAA, encoding glutamine 2153, to UAA, an in-frame stop codon, in apoB mRNA (3, 4). ApoB-100 and apoB-48 are the translation products of the unedited and edited mRNA, respectively. Although apoB-48 corresponds to the N-terminal 48% of apoB-100, the two proteins have drastically different properties and entirely different physiological functions (5).

ApoB mRNA editing is an intranuclear function (6). It is mediated by a multiprotein editosome complex, the catalytic component of which has been cloned (7–11). This component, known as apobec-1, consists of a 27–28-kDa protein that shows substantial sequence similarity to cytidine deaminase (11, 12). In fact, it has cytidine deaminase activity (12) that mediates the sequence-specific deamination of C-6666 in apoB-100 mRNA to a uridine residue in apoB-48 mRNA.

Apobec-1 exists as a spontaneous homodimer (10). It is active against synthetic human apoB mRNA substrates in vitro only in the presence of complementation factors (7). These factors are widely distributed in mammalian tissues, including many that do not synthesize apoB mRNA (8, 13). None of these factors have been characterized or cloned.

In order to understand the mechanism by which apobec-1 edits apoB mRNA and the protein composition of the editosome complex, we have used the yeast two-hybrid system to identify proteins that specifically interact with apobec-1 in vitro and in vivo. By this technique, we cloned an apobec-1-binding protein that appears to be a variant of hnRNP type A/B. This protein, named ABBP-1 (apobec-1-binding protein-1), demonstrates many properties that suggest that it plays an important role in apoB mRNA editing.

MATERIALS AND METHODS

Two-hybrid Cloning—The cDNA of human apobec-1 (10) was subcloned into PAS-2 and used as bait to screen several human two-hybrid libraries (Clontech, Inc.). About 60 positives were obtained from 6 × 10^6 Trp-Leu colonies. Five passed the mating assay (14) and consist of independent clones. One of these clones, isolated from a human placenta library, contains a partial ABBP-1 cDNA. The 5’ end of the ABBP-1 cDNA sequence was cloned by using the Clontech’s 5’ rapid amplification of cDNA ends cDNA kit.

In Vitro Binding—The glutathione S-transferase (GST)-A2B1B fusion protein was used for in vitro binding experiments. In 1 ml of phosphate-buffered saline, 0.2% Nonidet P-40 solution, 100 µl of the GST beads (Amersham Corp) was incubated with the appropriate amount of [32P]cytidine-labeled apobec-1 (obtained by coupled transcription-translation using a TnT kit from Promega, Inc.) at 4°C for 1 h with gentle rocking. The beads were washed five times and were then boiled to denature in SDS loading buffer and analyzed by SDS-PAGE (15). Truncation of the GST-A2B1B fusion protein was done by polymerase chain reaction subcloning. The subclones were confirmed by complete sequencing and the size of the truncated proteins analyzed by SDS-PAGE.

RNA Band Mobility Shifting and UV Cross-linking—RNA gel mobility shift assay and SDS-PAGE analysis of UV-cross-linked protein-RNA complex were performed as described previously (16). 200 ng of [32P]-labeled human apoB mRNA, a 295-mer (bases 6560–6854), was incubated with the appropriate amount of GST-A2B1B fusion protein in a total volume of 20 µl of editing buffer (17) at room temperature for 30 min. 10 µl was digested with T1 ribonuclease and loaded on a 5% nondenaturing polyacrylamide gel in 50 mM Tris-glycine (pH 7.9) buffer. The other 10 µl was exposed to UV light for 15 min with a short-wave UV lamp and digested with T1 ribonuclease. The pooled samples were loaded on 12.5% SDS-PAGE and the gels exposed to x-ray as described (16).

In Vitro Editing Extracts and Effect of Immunodepletion—HepG2 cells transfected with human apobec-1 cDNA using the vector pFLAG-CMV-2 (International Biotechnologies, Inc., New Haven, CT) were used to generate the S-100 fractions for the in vitro editing assays as described (18). Protein A-agarose purified rabbit IgG fraction against the GST-A2B1B fusion protein was covalently immobilized to agarose beads (Bio-Rad) and used to incubate with the S-100 fractions at 4°C overnight in a slow rocking platform. A typical reaction contained 1 ml of phosphate-buffered saline, 0.2% Triton solution. The beads were boiled on ice, and the cleared supernatant was collected for the editing assays. In vitro editing and primer extension assays were performed as described (19).

Transfection of ApoB-1-1 Antisense DNA and Selection of Transfected

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Cloning of an Apobec-1-binding Protein

RESULTS

Cloning, Structure, and Expression of ABBP-1—Using the yeast two-hybrid system and apobec-1 as bait, we have identified five independent cDNA clones. The in vivo interactions in the yeast system were confirmed by binding in vitro to human apobec-1 (see below). Partial sequence analysis revealed that one of these clones, ABBP-1, contains RNA-binding motifs (RNP motifs, also known as RNA recognition motifs, or RNA-binding domain (20)) that suggest that it may be involved in apoB mRNA editing. The complete deduced amino acid sequence of ABBP-1 is shown in Fig. 1. It contains 331 amino acid residues with a calculated Mr 36,615 and is identical in structure to human type A/B hnRNP (21) except for an 47-amino acid insert (double-underlined). The two pairs of RNP2 (hexamer) and RNP1 (octamer) sequences are boxed. The ATP/GTP fold is overlined.

Cells—The ABBP-1 cDNA construct was subcloned in an antisense orientation in pHook-3 (Invitrogen Co., San Diego, CA). This construct was transfected into stable apobec-1 expressing HepG2 cells (10) by electroporation. Selection of transfected cells was performed 48 h later using the Capture-Tec pHook-3 kit according to the manufacturer’s instructions. This method allows the specific selection of antisense ABBP-1-transfected cells. Total RNA was prepared from the selected cells and used for analysis of the extent of the apoB mRNA editing. Cells transfected with pHook-3 without ABBP-1 cDNA insert were used as a control.

Miscellaneous Methods—Antibodies against GST-ABBP-1 fusion protein were generated by direct injection of the glutathione-conjugated GST-ABBP-1 beads intradermally into New Zealand rabbits. Booster shots were done with the gel-purified GST-ABBP-1 protein. Human tissue RNA Northern blots were purchased from Clontech, Inc. Hybridization was performed in 50% formamide (w/v) 5 × SSPE (1 × SSPE = 0.18 M NaCl, 10 mM NaPO4, pH 7.7, 1 mM EDTA), 10 × Denhardt’s 2%, SDS, and 100 μg/ml Escherichia coli DNA and washed as described previously (10).

A potential ATP/GTP bindingifold is present in the C-terminal region immediately following the 47-amino acid insertion. Northern blot analysis of RNAs isolated from various human tissues indicates that ABBP-1 mRNA expression is widely distributed; a ~2-kilobase band was identified in all the tissues examined, including spleen, thymus, prostate, testis, ovary, small intestine, pancreas, colon, leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, and kidney (data not shown).

Mapping of ABBP-1 Binding to Human Apobec-1—The ability of ABBP-1 to bind to apobec-1 synthesized in vitro was examined by using full-length and various deletion mutants of ABBP-1 conjugated at the N terminus to GST. As shown in Fig. 2, full-length GST-ABBP-1 binds to human apobec-1 in vitro, confirming the yeast two-hybrid interactions in vitro. This binding is mediated by the C-terminal region of the molecule, since removal of this part of the molecule completely abolishes binding (mutant 4). The 47-amino acid insertion (mutants 8 and 9) by itself appears competent in binding, but is not required for binding because its deletion from ABBP-1 (in mutant 2 and 3) does not impair binding. A stretch of 4 glutamine residues upstream of the glycine-rich domain does not by itself confer apobec-1 binding (mutant 4, which contains this stretch at its C terminus, does not bind). The ATP/GTP fold immediately downstream to the 47-amino acid insertion is not required for binding (mutant 3, which misses this fold, binds well). Taken together, it is evident that sequences rich in glycine in ABBP-1 mediate apobec-1 binding.

ABBP-1 Binds to Human ApoB mRNA Fragment Around the Edited Site and Can Be UV Cross-linked to It—Since ABBP-1 contains typical RNA-binding motifs, we tested whether it would bind to apoB mRNA in vitro. Binding of 32P-labeled human apoB mRNA (containing nucleotides 6560–6854) to GST-ABBP-1 was examined by band mobility-shift analysis. As
shown in Fig. 3A, ABBP-1 binds to $^{32}$P-apoB mRNA. Binding was effectively competed in the presence of excess unlabeled apoB mRNA, but not yeast RNA. Furthermore, ABBP-1 could be specifically cross-linked to $^{32}$P-apoB mRNA by UV light (Fig. 3B). UV cross-linking showed the same specificity as the gel-shift analysis.

**Down-regulation of ABBP-1 Inhibits ApoB mRNA Editing**—We transfected a human apobec-1 cDNA vector into the human hepatoma cell line HepG2. S-100 extracts from these cells are competent in editing human apoB mRNA in vitro (Fig. 4A). To examine the potential role of ABBP-1 in apoB mRNA editing, we examined the effect of immunodepletion with anti ABBP-1 IgG on in vitro editing activity (Fig. 4A). We found that immunodepletion of ABBP-1 in the editing extracts results in a significant inhibition of editing; the extent of editing is reduced from $14.75 \pm 4.97\%$ in control extracts to $4.43 \pm 1.95\%$ ($p < 0.05$) in immunodepleted extracts. The use of preimmune serum and sham incubation of the extract under the same conditions resulted in minimal changes in editing. This experiment suggests that ABBP-1 is a component of the apoB mRNA editing complex in vitro.

To further investigate the role of ABBP-1 in editing-competent HepG2 cells (produced by stable transfection with apobec-1 cDNA (10)) under in vivo conditions, we transfected them with either an ABBP-1 antisense cDNA expression vector clone or with the expression vector (pHook-3) only (Fig. 4B). Total RNA was isolated from successfully transfected cells (selected by magnetic bead-coupled hapten capture of pHook-3-directed antigen expression) and assayed for the extent of apoB mRNA editing. We found that transfection with antisense ABBP-1 led to a marked inhibition in endogenous apoB mRNA editing in these cells from $10.83 \pm 2.84\%$ to $3.87 \pm 1.79\%$ ($p < 0.05$), a $-75\%$ reduction. Transfection with the vector alone had no effect. Thus, down-regulation of ABBP-1 expression by antisense expression inhibits endogenous apoB mRNA editing in vivo.

**DISCUSSION**

The search for complementation proteins for apoB mRNA editing started with the demonstration of a 40-kDa protein (16) and later both a $\sim 44$-kDa and a $\sim 66$-kDa protein (24, 25) that interact with synthetic apoB mRNA sequences as demonstrated by UV cross-linking in vitro. The role of these proteins in apoB mRNA editing, if any, is unknown. Recently, Schock et al. (26) demonstrated the presence of a 240-kDa protein antigen in a 27 S putative editosome complex. This protein was not

**Fig. 3.** **Binding of ABBP-1 to $^{32}$P-apoB mRNA** A, band mobility shift analysis. B, UV cross-linking. Binding and UV cross-linking were performed with ABBP-1 and synthetic human $^{32}$P-apoB mRNA (nucleotides 6560–6854) as described under “Materials and Methods.”

**Fig. 4.** **Effect of ABBP-1 down-regulation on apoB mRNA editing.** A, effect of anti-ABBP-1 IgG-beads on apoB mRNA editing activity. In vitro tissue editing extract was prepared from HepG2 cells transfected with a human apobec-1 cDNA. Immunodepletion and editing reaction and primer extension assays are as described under “Materials and Methods.” Lane 1, fresh control extract; lane 2, control extract, sham incubated overnight; lane 3, extract incubated overnight with beads only; lane 4, extract incubated overnight with preimmune beads; lanes 5–7, extracts incubated overnight with anti-ABBP-1 beads. B, effect of antisense ABBP-1 cDNA expression on endogenous apoB mRNA editing in HepG2 cells expressing apobec-1. Transfection and selection of successfully transfected cells and primer extension assays are as described under “Materials and Methods.” Lane 1, HepG2 cells that were mock-transfected; lane 2, HepG2 cells stably transfected with pCMV; lane 3, HepG2 cells stably transfected with apobec-1 cDNA. Lanes 4–7 were studied in apobec-1-expressing HepG2 cells produced by stable transfection (10). Lane 4, transfected with vector pHook-3; lanes 5–7, different transfections using antisense ABBP-1 cDNA subcloned in a pHook-3 vector.
demonstrated to interact with either apobec-1 or apoB mRNA and its role in apoB mRNA editing in vivo remains to be determined.

In this study we have used the yeast two-hybrid technique to identify a protein, ABBP-1, that specifically binds to apobec-1. The deduced amino acid sequence of ABBP-1 indicates that it is a RNA-binding protein that shows sequence identity with a human hnRNP type A/B protein (21), except for a 47-amino acid insertion produced by alternative RNA splicing. The previously described hnRNP protein was identified as an RNA-binding protein of unknown function in HeLa cells. Here we show that this protein interacts specifically with apobec-1. We have identified two isoforms of ABBP-1 proteins are typically involved in protein-protein interactions (23), and in ABBP-1 they appear to mediate its binding to apobec-1. We have identified two isoforms of ABBP-1 produced by alternatively spliced mRNAs. Both forms seem to be competent in binding to apobec-1 in vitro. ABBP-1 also binds to apoB mRNA and can be UV-cross-linked to it in vitro. Immunodepletion of ABBP-1 in editing extracts appears to inhibit their editing activity. Most important, expression of antisense ABBP-1 transcript by transfection reduces endogenous apoB mRNA editing in vitro. This protein interacts specifically with apobec-1. We speculate that ABBP-1 may be involved in recruiting and possibly disrupting the secondary structure of apoB mRNA and bringing it to the vicinity of apobec-1 (and possibly other complementation proteins) for editing. We note that ABBP-1 is the first apobec-1-interacting protein identified and cloned. Furthermore, to date, other than apobec-1 itself, it is the only protein whose down-regulation seems to affect both the editing activity of a cellular editing extract assayed in vitro and the level of endogenous apoB mRNA editing in a cell. Future research in this area will be aimed at the identification of other factor(s) involved in apoB mRNA editing and their possible interaction with apobec-1 and ABBP-1.

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