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The expression patterns of *Tetratricopeptide repeat domain 36* (*Ttc36*)

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ABSTRACT

Tetratricopeptide repeat domain 36 (*Ttc36*), whose coding protein belongs to tetratricopeptide repeat (TPR) motif family, has not been studied extensively. We for the first time showed that *Ttc36* is evolutionarily conserved across mammals by bioinformatics. Rabbit anti-*mouse Ttc36* polyclonal antibody was generated by injecting synthetic full-length peptides through "antigen intersection" strategy. Subsequently, we characterized *Ttc36* expression profile in *mouse*, showing its expression in liver and kidney both from embryonic day 15.5 (E15.5) until adult, as well as in testis. Immunofluorescence staining showed that *Ttc36* is diffusely expressed in liver, however, specifically in kidney cortex. Thus, we further compare *Ttc36* with proximal tubules (PT) marker Lotus Tetragonolobus Lectin (LTL) and distal tubules (DT) marker Calbindin-D28k respectively by double immunofluorescence staining. Results showed the co-localization of *Ttc36* with LTL rather than Calbindin-D28k. Collectively, on the basis of the expression pattern, *Ttc36* is specifically expressed in proximal distal tubules.

1. Introduction

Tetratricopeptide repeat domain 36 (Ttc36) codes a hypothetical protein that is predicted to hold a tetratricopeptide repeat (TPR) domain, composed of three adjacent TPR motifs. These TPR motifs, each of which typically possesses a characteristic 34-amino acid sequence conserved in evolution (Zeytuni and Zarivach, 2012), were originally identified in yeast cell division cycle proteins (SSikorski et al., 1990; Hirano et al., 1990), and successively found in a wide range of functionally irrelevant proteins, occurring in different organisms including bacteria, plants, animals and humans (Jernigan and Bordenstein, 2015). The conserved 34-amino acid sequence folds to shape a helix-turn-helix motif and adjacent motifs present in a parallel manner form a right-handed superhelix, whose groove structure can accommodate the complementary domain of various ligands and further mediate protein-protein interactions (Das et al., 1998; D'Andrea and Regan, 2003). Relying on the basic function of mediating protein-protein interaction, TPR-containing proteins, as scaffolds, are implicated in a variety of biological functions such as chaperone, cell-cycle, kinetochore localization, transcription and splicing, mitochondrial and endoplasmic reticulumal protein transport, cilia formation and function and bacterial pathogenesis (Xiol et al., 2012; Nijenhuis et al., 2013; Katibah et al., 2014; Sunryd et al., 2014; Xu et al., 2015; Cerveny et al., 2013). The expression profile, structure as well as function of Ttc36 remains unknown to date. Our previous document indicated that, while the function of Ttc36 was not known, this gene is expressed in kidney doubtlessly (Liu et al., 2014). However the expression of Ttc36 in other organs remains to be determined, and its precise localization within the cells is likewise to be confirmed.

Abbreviations: Ttc36, Tetratricopeptide repeat domain 36; TPR, tetratricopeptide repeat; PT, proximal tubules; LTL, Tetragonolobus Lectin; DT, distal tubules Tamm–Horsfall protein (THP); THP, Tamm–Horsfall protein; EDTA, Ethylene Diamine Tetraacetic Acid; EGTA, Ethylene Glycol Tetraacetic Acid; PVDF, Polyvinylidene Fluoride; FITC, fluorescein isothiocyanate; DAPI, 6-diamidino-2-phenylindole.

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In the present report, in order to characterize the expression profile and localization of Ttc36 in mouse, we generated a pAb of Ttc36 protein by an"antigen intersection"strategy of immunisation and purification (Jacquot et al., 2004). Subsequently Western Blot using the anti-mouse Ttc36 pAb we produced, revealed that TTC36 protein distribution. Further, we showed that Ttc36 expression was confined to tubules in renal cortex. Based on the application of Co-immunofluorescence staining of Ttc36 and LTL, a classic marker of renal proximal tubules (Lam et al., 2014), and hardly no expression of Ttc36 in distal tubules by Co-immunofluorescence staining with Clabindin-D28K, also called Clabindin or Clab1, a distal tubules marker (Georgas et al., 2008), we initial speculate Ttc36 as a novel renal proximal tubules maker, which could be useful in vivo.

2. Results

2.1. Ttc36 is evolutionarily conserved

We analyzed its sequence of various species based on the database in GeneBank. Then a molecular phylogenetic tree of Ttc36 gene was produced by Clustalx1.83 and MEGA5.0. The orthologs of Ttc36 are found in various species, including Mammalia HUMAN (*Homo sapiens*, NM_001080441.1); BOVIN (*Bos taurus*, NM_001040515.2); DOG (*Canis lupus* familiaris, XM_546500.5); MOUSE (*Mus musculous*, NM_138951.1); RAT (*Rattus norvegicus*, NM_001005546.1); RABBIT (*Oryctolagus cuniculus*, XM_002722197.2)), Aves (CHICK (*Gallus gallus*, NM_001278062.1)), Amphibian (XENTR (Xenopus tropicalis, NM_001015772.1), Actinopterygii (DANRE (*Danio rerio*, NM_001007388.1)) and Insecta (DROME (*Drosophila melanogaster*, NM_140387.2)). And the results showed that Ttc36 gene is evolutionarily conserved across species, especially in mammal, e.g., human, bovin, dog, rabbit, mouse and rat (Fig. 1a), while no orthologs are found in fungi, plants, and lower organisms. To make the potentially conserved tetratricopeptide repeat (TPR) residues clear, we deduced the comparison of the amino acid sequences of Ttc36 proteins above mentioned 10 different species. The alignment of Ttc36 homologues reveals conserved residues in their respective C-terminal domains and TPR motifs of Ttc36 especially in mammals (Fig. 1b). Subsequently, the spatial structure of Ttc36 was predicted by SWISS-MODEL online software based on template-2y4Tb (Fig. 1c), and there are three TPR motifs in Ttc36. What's more, the TPR1 and TPR2 (black color in Fig. 1c) were predicted interact with other proteins. These data indicated that Ttc36 is evolutionarily conserved, especially in the domains of three TPR motifs.

2.2. The generation of Anti-Ttc36 rabbit polyclonal antibody

Anti-mouse Ttc36 antibody was generated through rabbit immunization by an "antigen intersection" strategy of immunization and purification (Davis et al., 1999; Arora et al., 2014). Firstly, GST-Ttc36 and 6His-Ttc36 fusion protein are successfully expressed from prokaryotic expression system to purification (Fig. 2a and b). After immunization of GST-Ttc36, Western blotting and ELISA have been done and demonstrated that there exists specific binding sites for Ttc36 in antiserum (Fig. 2e) and shows off a high titer (1: 65536 in Fig. 2c). Then the antiserum was purified by 6His-Ttc36 coupled antigen affinity, and Western blotting together with ELISA showed the specificity was increased after the purification and therefore the unspecific binding is decreased (Fig. 2c–e).

2.3. Temporal and spatial expression patterns of Ttc36

To represent the expression profiles of Ttc36 in mouse, we adopted Western blotting with the histiocyte lysate of brain, bladder, heart, intestine, kidney, liver, testis, skeletal muscle, spleen and lung respectively, and results displayed Ttc36 is confined to express in the kidney, liver and testis, albeit lower expression level in the testis (Fig. 3a). The temporal expression in liver was analyzed by Western blot with the total protein collected from the histiocyte lysate of liver at different embryonic period and adult stages, indicating that Ttc36 is expressed firstly in embryonic day 15.5 (E15.5) liver (Fig. 3b). The spatial expression of Ttc36 in liver is diffused by immunofluorescence staining (Fig. 3c).

Further, we exposed the expression patterns of Ttc36 in kidney. Semi-quantitative RT-PCR were performed with total RNA extracted from mouse kidney at different developmental stages, which manifested that Ttc36 is firstly expressed in E15.5 kidney (Fig. 4a), and their expression is obviously increased after E15.5. Analogously, Western blotting tissue sample from developing embryonic kidney or adult kidney at different stages, with the purified Anti-Ttc36 antibody revealed that Ttc36 protein also firstly appear in E15.5 kidney, as well as markedly raise after that (Fig. 4b). More importantly, there was no perceptible difference between male and female adult kidney. Whereafter, to determine spatial distribution of Ttc36 protein expressed in kidney development, indirect immunofluorescence staining was carried out in mouse whole kidney sections which was gathered from P0 and W6 mouse. Just as shown in Fig. 4c, the expression of Ttc36 is specific in kidney. Collectively, the location of Ttc36 is mainly in renal cortical tubules.

2.4. Ttc36 specific expression in PT rather than DT

To locate exactly Ttc36 expression in kidney, we made kidney sections from different stages (E14.5, E15.5, E17.5 and 6 weeks) and co-staining with LTL, the classic markers of proximal tubules (Lam et al., 2014). Antibodies to LTL were used to label PT. Results from double immunofluorescence staining with anti-Ttc36 polyclonal antibodies and anti-proximal tubules markers LTL validated that Ttc36 is localized in proximal tubules and showed that Ttc36 protein is firstly expressed in proximal tubules in E15.5 kidney, later than LTL which is already expressed in E14.5 kidney (Fig. 5a). Interestingly, Ttc36 is co-expressed with LTL in the proximal tubules of adult kidney (Fig. 5a). In addition, Proximal tubules, as well as distal tubules, both originate from metanephric mesenchyme and there are similarities between them in morphology (Little and McMahon, 2012). Thus, distal tubules marker Calbindin-D28k was used to identify if Ttc36 localization is separate to distal tubules (Fig. 5b). Double-labeled tissue with primary antibodies to Ttc36 and Calbindin-D28k demonstrated that Ttc36 is isolated to distal tubules. Taken together, Ttc36 is specifically expressed in proximal tubules, rather than distal tubules.

3. Discussion

As a new gene, Ttc36 has seldom been reported in the past several decades. We for the first time performed bioinformatic analysis which showed that Ttc36 is evolutionarily conserved in mammals. In our study, the Ttc36 coding sequence was amplified from the cDNA which was reverse transcribed by RNA isolated from C57BL/6J mouse kidney. We revealed the expression distribution of Ttc36, showing that this gene was restricted to express in liver, kidney and testis, and its intracellular localization to cytoplasm. Furthermore, we determined spatio-temporal expression in liver and kidney, its expression both from embryonic day 15.5 (E15.5) until adult without gender differences. Immunofluorescence staining showed that the expression of Ttc36 is dispersive in liver, however, specific in kidney cortex, thus we further demonstrated its precise localization by comparing Ttc36 with proximal tubules marker Lotus Tetragonolobus Lectin (LTL) and distal tubules marker