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Molecular cloning and identification of mouse *Cklfsf2a* and *Cklfsf2b*, two homologues of human *CKLFSF2*

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Abstract

Human chemokine-like factor superfamily (*CKLFSF*) is a novel gene family comprising *CKLF* and *CKLFSF1–8*. Among them, *CKLFSF2* is highly expressed in testis and may play important roles in male reproduction. Besides, it is very active during evolution and has two counterparts in mouse. For further study, we cloned the two mouse genes by EST assembly and RT-PCR methods and designated them as mouse *Cklfsf2a* and *Cklfsf2b*. Their predicted open-reading frames (ORFs) that encode 169 and 210 amino acids, respectively, were obtained; and their predicted full-length molecular sizes that are approximately 1.2 kb for mCklfsf2a and 0.9 kb for mCklfsf2b were confirmed by Northern blot analysis. Mouse Cklfsf2a and Cklfsf2b show similarities with human CKLFSF2 in the expression patterns that are abundant in testis, hematopoietic and immune tissues; as well as in the chromosome localizations that neighbor *CKLFSF1* and *3*. Their putative protein products have 47.6 and 45.5% identities with hCKLFSF2, respectively; both of them contain four potential transmembrane regions and MARVEL domains, which are also similar with hCKLFSF2. Functionally, they all can affect the transcriptional activity of androgen receptor in PC-3 and HeLa cells, but mCklfsf2a is a repressor while mCklfsf2b and hCKLFSF2 are enhancers. Taken together, we conclude that mouse *Cklfsf2a* and *Cklfsf2b* are two homologues of human *CKLFSF2*. Studies on them would provide much help in further investigation of the latter.

Keywords: CKLFSF; Homologue; Androgen receptor; Male reproduction

1. Introduction

CKLFSF is a novel protein family that provides a structural and functional link between chemokines and members of the transmembrane 4 super family (TM4SF). It may have important functions across a wide range of physiological and pathological processes. In human, nine genes, *CKLF* and *CKLFSF1–8* encode the CKLFSF proteins. Among them, *CKLF*, *CKLFSF1* and *CKLFSF2* are tightly linked on human chromosome 16q22.1, which may suggest their close relationship (Han et al., 2003). *CKLF* has four RNA splicing forms: CKLF1–4. CKLF1 has a CC motif and exhibits chemotactic effects on a wide spectrum of leukocytes both in vitro and in vivo (Han et

Abbreviations: CKLFSF, chemokine-like factor super family; *CKLF*, *chemokine-like factor*; *CKLFSF1–8*, *chemokine-like factor super family member 1–8*; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; ORF, open-reading frame; MGD, mouse genome database; PCR, polymerase chain reaction; RT-PCR, reverse transcription PCR; TM4SF, transmembrane 4 super family; EST, expressed sequence tag; UTR, untranslated region; MARVEL, MAL and related proteins for vesicle trafficking and membrane link; AR, androgen receptor; ARR19, androgen receptor corepressor-19 kDa; MMTV, mouse mammary tumor virus; Luc, luciferase

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al., 2001). CKLF2, the full-length cDNA product encoding 152 amino acids, has four putative transmembrane regions and a stimulating effect on the proliferation and differentiation of C2C12 cells (Xia et al., 2002). Rat and mouse Cklfs have RNA splicing forms and functions similar to those of human CKLFs (Lou et al., 2003; Rui et al., 2003). Human *CKLFSF1* is a complicated gene, having at least 23 isoforms designated as CKLFSF1-v1-v23 whose protein products are predominantly expressed in human testis and most abundant in the spermatocytes (primary and secondary) as well as in the tissue fluid surrounding spermatogonia and spermatocytes. The above facts indicate that they may exert critical functions in gene regulation and tissue differentiation during spermatogenesis (Wang et al., 2004). Our recent studies show that human CKLFSF2 with four putative transmembrane regions can be detected in the spermatogonium and can also be secreted into the tissue fluid of human testis at the same time (Shi et al., 2005). Such evidences serve to indicate that human CKLFSF2 may have an important function in the context of germ-cell development.

On the other hand, *CKLFSF2* is active during evolution; and in mouse, it has two counterparts, mouse *Cklfsf2a* and *Cklfsf2b*. According to our preliminary studies, human CKLFSF2 can promote the transcriptional activity of AR (unpublished data). However, Jeong et al. (2004), reported a novel androgen receptor (AR) corepressor named androgen receptor corepressor-19 kDa (ARR19), which is in fact the mouse Cklfsf2a. They found that ARR19 represses AR transactivation in a dose-dependent manner and that it directly associates with AR through the N-terminal and leucine zipper-containing regions (Jeong et al., 2004). These provide interesting clues to explore the activities of CKLFSF2 in male reproductive functions and its evolution across different species.

To assist the exploration, we cloned and characterized mouse *Cklfsf2a* and *Cklfsf2b* in the present study. They exhibit similar expression patterns and chromosomal localizations with human *CKLFSF2*. On the overall amino acid level, they show 47.6 and 45.5% sequence identities with human CKLFSF2, respectively, and share similar characteristics with the latter. Luciferase assays indicated an opposite function of mCklfsf2a with hCKLFSF2 in AR transactivation as mentioned above; but mCklfsf2b was observed to enhance the transcriptional activity of AR just like hCKLFSF2. This work is expected to be of considerable benefit in investigating the functions of human CKLFSF2 and studying the evolution of the gene family.

2. Materials and methods

2.1. Blast-based searches and EST assembly of mouse Cklfsf2a and 2b

Using the mouse Cklf2 cDNA as a query sequence, we performed BLASTN searches against the public database of ESTs (dbEST) (Banfi, Guffanti, & Borsani, 1998), thereby retrieving a set of related ESTs (listed in Table 1) from mouse EST databases. These ESTs were assembled into a contig from overlapping ESTs through manual alignment, which was named mouse *Cklfsf2a*. Subsequently we used this contig as the query sequence to perform repeated BLASTN searches, whereupon we found an additional set of mouse ESTs (listed in Table 1). Following EST assembly, another contig homological to mouse *Cklfsf2a* was obtained and designated as mouse *Cklfsf2b*.

2.2. Molecular cloning of mouse Cklfsf2a and 2b

Based on the predicted sequences of mouse *Cklfsf2a* and *2b*, a series of gene-specific primers were designed for the purpose of amplifying their ORFs by nested RT-PCR. These oligonucleotides are as follows: 5′-GTT GAG AGC CAC CCT TGA ACA G-3′ (P1), 5′-CAC AGC TTG GCA TGG GTC C-3′ (P2), 5′-ACA ACC ATG GCA GCA CCG-3′ (P3), 5′-CAC AGT TAC CAC TTC CTT AAC C-3′ (P4), 5′-TTG AAC AGG TCA GGA GAC ACC AG-3′ (P5), 5′-GAT GGA GAA CTC TGC TGG AAA TC-3′ (P6), 5′-AAC AAC CAT GGC AGC GCC-3′ (P7), and 5′-GCT GCG CAG TCA CCA TCC AG-3′ (P8). Among them, P1 and P2, P5 and P6 are the primers for amplifying the full-length cDNA sequence of m*Cklfsf2a* and *2b*, respectively; while P3 and P4, P7 and P8 are primers for amplifying the coding region of m*Cklfsf2a* and *2b*, respectively. The total RNA for RT-PCR was extracted from Balb/c mouse testis using Trizol (Life Technologies Inc., Rockville, MD) according to the manufacturer's instructions. First-strand

Table 1
Accession numbers of ESTs for mouse *Cklfsf2a* and *2b* assembly

mCKLFSF2a	*mCKLFSF2b*
BY706860	BU945940
BU605057	AV209880
BQ840031	BY706101
BU743969	AV266820
AA108901	CA464807
BE626217	BU962007
AI449770	AV208542
BU583340	