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Molecular cloning and characterization of two novel truncated isoforms of human Na^+/Ca^{2+} exchanger 3, expressed in fetal brain☆

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Abstract

The human gene encoding the Na^+/Ca^{2+} exchanger family member 3 (NCX3) undergoes extensive alternative splicing, with four variants previously identified. In this study, we report two novel alternative transcripts encoding two N-terminally truncated NCX3 proteins specifically expressed in human fetal brain. The identified transcripts, designated NCX3-tN.1 and NCX3-tN.2, are approximately 2.8 kb and 2.9 kb, respectively. The open reading frames (ORFs) are predicted to encode separately a 284 and a 298 amino acid (aa) polypeptide. Sequence analysis and bioinformatics reveal that NCX3-tN.1 and NCX3-tN.2 are the result of alternative splicing of the NCX3 gene. They have their own potential start codons and unique 5′ untranslated regions (UTRs) that are different from those of the known NCX3 variants. The variants include a part of intron 2 of the original gene organization as their first exon (exon "a") at the 5′ end of the novel transcripts. NCX3-tN.2 consists of six exons including exon "a" and exons 4, 6, 7, 8 and 9 of NCX3, while NCX3-tN.1 lacks exon 4, but is otherwise similar to NCX3-tN.2. Expression studies show that both variants can be translated into protein and NCX3-tN.1 seems more efficiently translated. Based on their structural features, NCX3-tN.1 and NCX3-tN.2 proteins are potentially involved in regulation of Na^+/Ca^{2+} homeostasis.

Keywords: Alternative splicing; CNS; NCX3; *SLC8A3*

1. Introduction

Intracellular calcium is an important messenger of many physiological functions and multiple pathways regulate its concentration. The sodium–calcium exchanger (NCX) plays an important role in the maintenance of the intracellular Ca^{2+} homeostasis and is present in the plasma membrane of most cells. The primary function of NCX is to transport Ca^{2+} in exchange for Na^+, in an inward or outward direction across the plasma membrane. The direction of transport depends on the prevailing Na^+ and Ca^{2+} electrochemical gradients, with an exchange of three Na^+ ions for one Ca^{2+} ion (for review, see Philipson and Nicoll, 2000; Quednau et al., 2004).

Three mammalian members of the exchanger family have been identified, NCX1 (GenBank accession no. NM_021097), NCX2 (GenBank accession no. XM_375633) and NCX3 (GenBank accession no. AF508982). Although all three members arise from separate genes on different chromosomes, they share high amino acid identity (about 70%), especially in the hydrophobic regions (Nicoll et al., 1990, 1996b; Li et al., 1994). NCX1 was first cloned from canine cardiac sarcolemma (Nicoll et al., 1990) and later the

Abbreviations: aa, amino acid(s); bp, base pair(s); cDNA, DNA complementary to RNA; DD-PCR, differential display-polymerase chain reaction; dNTP, deoxyribonucleoside triphosphate; EST, expressed sequence tag; EtdBr, ethidium bromide; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; kb, kilobase(s) or 1000 bp; MTN, Multiple Tissue Northern (Blot); NCX, Na^+/Ca^{2+} exchanger; nr, non-redundant; ORF, open reading frame; RACE, rapid amplification of cDNA ends; SDS, sodium dodecyl sulfate; *SLC8A*1, 2 and 3, solute carrier family 8 (sodium–calcium exchanger) genes, members 1, 2 and 3; SSC, 0.15 M NaCl/0.015 M Na_3 citrate pH 7.6; TM, *trans*-membrane domain; u, unit(s); UTR, untranslated region(s); XIP, exchanger inhibitory protein.

☆ The cDNA sequences described in this work have been deposited to GenBank under the accession numbers AJ745101 and AJ745102.

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corresponding human gene, *SLC8A1* was mapped to chromosome 2p22.1 (Kraev et al., 1996). NCX1 is best-characterized and nearly ubiquitously expressed, but with a high level in heart, brain and kidney (Philipson et al., 1996). NCX2 and NCX3 seem to have similar properties as NCX1 but their expression appears to be restricted to brain and skeletal muscle (Li et al., 1994; Nicoll et al., 1996b). The NCX2 gene, *SLC8A2*, is located on chromosome 19q13.2 (Kikuno et al., 1999), while the *SLC8A3* gene, encoding NCX3, maps on chromosome 14q24.2 (Gabellini et al., 2002).

The mature exchanger protein consists of an extracellular N-terminal signal sequence and two sets of hydrophobic domains, separated by a large central hydrophilic cytoplasmic loop and followed by an intracellular C-terminal region. The hydrophobic domains comprise five *trans*-membrane domains (TMs) in the N-terminal region, TM1–TM5, and four TMs in the C-terminal region, TM6–TM9 (Nicoll et al., 1999; for review, see Nicoll et al., 2002).

The large intracellular hydrophilic loop is involved in regulation of the exchanger, but is not essential for transport (Nicoll et al., 1999). The 60 amino acids (aa) long and similar β-1 and β-2 repeats are two conserved regions in the loop, with no suggested regulatory function (Philipson and Nicoll, 2000). The endogenous exchanger inhibitory peptide (XIP) is a 20 aa long region, located near the TM5, in the intracellular loop (Li et al., 1991). The XIP might have an auto regulatory function and may also be involved in Na^+-dependent inactivation of the exchanger (Li et al., 1991; Matsuoka et al., 1997).

Further, in the N- and C-terminal halves of all the three NCX members, there are highly conserved internal repeat sequences, the α-1 and α-2 repeats (Nicoll et al., 1996a; Schwarz and Benzer, 1997). These repeats are located in between the TM2 and TM3 (α-1) and the TM7 and TM8 (α-2), formed by gene duplication during evolution and considered to be involved in ion binding and translocation of the exchanger (Nicoll et al., 1996a; Schwarz and Benzer, 1997). The function of the exchanger is highly sensitive to mutations in the α-repeat regions (Nicoll et al., 1996a).

Alternative splicing is common among the NCX genes. The NCX1 mRNA is alternatively spliced and at least 12 distinct variants have been described in various species and tissues (Furman et al., 1993; Kofuji et al., 1994; Lee et al., 1994; Reilly and Lattanzi, 1996; Quednau et al., 1997; Van Eylen et al., 2001). In addition, three alternative tissue-specific promoters have been reported (Lee et al., 1994; Barnes et al., 1997; Nicholas et al., 1998). Recently, several alternative transcripts of NCX3 have been characterized in different tissues, mainly generated by alternative splicing of exons encoding the large cytoplasmic loop (Gabellini et al., 2002).

The NCX3 and the NCX1 genes have comparable arrangements of exons, for instance, both contain a large exon 2 and have a similar number of nucleotides between corresponding exons (Kraev et al., 1996; Gabellini et al., 2002). The numbering of NCX1 and NCX3 exons differ, however, since the NCX3 gene includes only nine exons (numbered 1 to 9) (Gabellini et al., 2002) compared to the 12 exons of NCX1. Exons 2–5 of the NCX3 are homologous to the corresponding exons of NCX1, but exons 6–9 of NCX3 correspond to exons 9–12 of NCX1 (Kraev et al., 1996). The predicted NCX3 protein is coded by eight exons (2 to 9), with and without either exon 3 or 4. The start codon is predicted to be located in the 5′ end of exon 2 and the stop in exon 9 (Gabellini et al., 2002).

In spite of the NCX3 gene lacking the commonly alternatively spliced exons 6–8 of NCX1 (Kraev et al., 1996; Gabellini et al., 2002), alternative splicing of NCX3 does occur. Four alternatively expressed variants of NCX3 have been described, in both human (Gabellini et al., 2002) and rat (Quednau et al., 1997) (Fig. 1). The variants NCX3.1, NCX3.2 (found in human brain and neuroblastoma) and NCX3.3 (found in human skeletal muscle) are derived from alternative splicing in the region of exons 3–5 (Quednau et al., 1997; Gabellini et al., 2002). NCX3.4, found in human skeletal muscle, is a truncated isoform produced by skipping of exons 3–5, leading to a frame shift and generating a short NCX3 variant with a deletion of the C-terminal portion of the β-2 repeat domain and the whole hydrophobic C-terminal half of the protein (Gabellini et al., 2002). Since it has been reported that the C-terminal part of the protein is not crucial for ion transport (Li and Lytton, 1999; Van Eylen et al., 2001), NCX3.4 should be functional.

In this study, we report two novel alternative transcripts encoding two N-terminally truncated NCX3 proteins from human fetal brain, designated as NCX3-tN.1 and NCX3-tN.2. Genomic sequence analysis revealed that they result from alternative splicing of the NCX3 gene localized on chromosome 14. Sequence analysis showed that NCX3-tN.1 and NCX3-tN.2 have their own start codons and unique 5′ untranslated regions (UTRs) that are different from the 5′ UTRs of the currently known NCX3 variants. The new variants both start from a novel exon, designated as exon "a", located in the intron 2 of the previously described gene organization. The expression of the two truncated mRNAs is likely to be induced from an alternative promoter of the NCX3 gene and is brain-specific in examined fetal tissues. NCX3-tN.2 consists of six exons including exon "a", exon 4 and exons 6–9, while NCX3-tN.1 lacks exon 4, but is otherwise similar to NCX3-tN.2.

2. Materials and methods

2.1. Differential display-PCR—isolation of 47-1 cDNA fragment from human glioma cells

A 281 base pair (bp) long cDNA (DNA complementary to RNA) fragment (47-1) was isolated as differentially expressed in the two clonal human malignant glioma cell lines, U-343 MG and U-343 MGa Cl 2:6 (Nister et al.,