Identification of a Novel AIF3 Isoform

Published date: Feb. 24, 2017

Technology description

ABSTRACT

Apoptosis-inducing factor (AIF) is a bifunctional flavoprotein with a vital function in bioenergetics within mitochondria and a lethal function in cell death when it moves to the nucleus. We identified a novel AIF isoform AIF3 in mouse brain by 5′ RACE. AIF3 lacks exons 2 and 3, which contain a hydrophobic mitochondrial inner membrane sorting signal. When we removed floxed AIF exon 3 in the forebrain postnatally, AIF3 is induced in mouse brain. Our preliminary data indicate that AIF3 might play an important role in neurodegeneration.

FEATURES

1) AIF3 is a completely new isoform in addition to previously known isoforms, including AIF2 and several other short AIF forms.

2) The role of AIF3 is completely unknown. We have the AIF3 mouse model, which will allow studying the role of AIF3 in neurodegeneration and general cell death in other systems, and lymph node.

DESCRIPTION DETAILS

In order to identify novel AIF isoforms in adult mouse brain, 5′-RACE was performed using the cDNA prepared from C57BL/6 mice according to the manufacturer′s recommendation (Invitrogen). For the 5′-RACE, the first round of polymerase chain reaction (PCR) amplification was done using the adaptor primer (5′-CGACTGGAGCACGAGGACACTGA-3′) and Aif gene specific reverse primer GSP-Re (5′-GCTTGTGTATTCCACGATTGGGATTCA-3′). The nested PCR was done with the nested adaptor primer (5′- GGACACTGACATGGACTGAAGGAGTA-3′) and Aif gene specific reverse primer GSP-Nested-Re (5′-GAGGAGGTCGCATGTATGGCAGTTCAG-3′). PCR products were then cloned using zero blunt TOPO PCR cloning kit (Invitrogen) and sequenced. From the 5′ RACE assay, we have identified a novel AIF3 isoform.

AIF3 mice were made the following way. The targeting vector for producing the AIFflox allele consists of an 11 kb SphI fragment of the AIF genomic DNA including 7.8 kb upstream and 3.2 kb downstream from the AIF exon 3, two loxP sites at 114 bp upstream and 190 bp downstream of the AIF exon 3. The G418 resistance gene (NEO) flanked by frt sites and the herpes simplex thymidine kinase (TK) gene

inserted 5’ of the targeting construct were used for positive and negative selection, respectively. The targeting construct was electroporated into 129/SV embryonic stem (ES) cells. Homologous recombination introduced a floxed exon 3 along with the Neo/frt cassette in a correctly recombined ES clone. FlpE recombinase was overexpressed in the selected targeted 129/SV ES cells to remove the neomycin cassette and Southern analysis was performed to screen for clones with the Neo cassette deletion. The selected clones were expanded and injected into C57BL/6 blastocysts to yield chimeric mice. AIFflox mice were backcrossed >8 times onto the C57BL/6 background prior to initiation of the current studies. For neuron-specific deletion of AIF exon 3, we crossed AIFflox/AIFflox female with male mice expressing iCre recombinase under the control of the CamKII➔ promoter (CamKII➔-iCre) (Dragatsis and Zeitlin, 2000; Casanova et al., 2001) or Cre recombinase under the transcriptional control of the dopamine transporter promoter (DAT-Cre) (Zhuang et al., 2005). Alternatively, we crossed female (AIFflox/AIF+, CamKII➔-iCre+) mice with male (AIFflox/Y, CamKII➔-iCre-) mice.

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