Okur-Chung Neurodevelopmental Syndrome-linked CK2 α mutations have reduced kinase activity

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Research Article

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Abstract

The Okur-Chung Neurodevelopmental Syndrome, or OCNDS, is a newly discovered rare neurodevelopmental disorder. It is characterized by developmental delay, intellectual disability, behavioral problems (hyperactivity, repetitive movements and social interaction deficits), hypotonia, epilepsy and language/verbalization deficits. OCNDS is linked to de novo variants in CSNK2A1, that lead to missense or deletion/truncating mutations in the encoded protein, the protein kinase CK2a. Eighteen different missense CK2a mutants have been identified to date, however no biochemical or cell biological studies have yet been performed to clarify the functional impact of such mutations. Here, we show that 15 different missense CK2a mutations lead to varying degrees of loss of kinase activity as recombinant purified proteins and when mutants are ectopically expressed in mammalian cells. We further detect changes in the phosphoproteome of three patient derived fibroblast lines and show that the subcellular localization of CK2a is altered for some of the OCNDS-linked mutants and in patient derived fibroblasts. Our data argue that reduced kinase activity and abnormal localization of CK2a may underlie the OCNDS phenotype.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Tables

Mutant	Kinase activity (average, % of Wt)			Expression (average, % of Wt)
	Myc- CK2 α	GST- CK2 α	GST- CK2 α + His- CK2 β	in Cos-7
R47G	70	30	=	-
Y50F	60	10	40	-
F197I	40	0	15	-
E27K	30	45	=	-
P231R	30	10	40	-
R47Q	20	40	=	30
S51R	20	0	=	-
R80H	20	5	=	-
R191Q	20	50	=	-
V73E	15	50	=	-
D175G	15	0	=	-
I174M	10	10	20	-
K198R	10	20	=	-
R312W	0	10	=	40
R312Q	NT	25	=	15

Table 1. Comparison of CK2 α mutant activity and expression levels.

Table compiles average data from the activity and expression experiments in the article for ease of comparison. All data is expressed as % of Wt.

=: same activity in GST-CK2 α + CK2 β as in GST-CK2 α

- : same expression levels as wildtype

Figures

Figure 1

Expression of CK2 proteins in patient derived fibroblasts and CK2α knockout mice. Lysates from control and patient derived fibroblasts (R47G, D156E, K198R) were separated by SDS PAGE and used for Western blot analysis with anti-CK2α, anti-CK2α' and anti-CK2β antibodies as well as anti-tubulin (A). Results from 4 independent experiments are plotted (B). Western blotting analysis using antibodies against pS129Akt, pS473Akt, pERK (T202/Y204) and total Akt or ERK is shown. 3 experiments were performed and

quantified (C, D). Lysates from CK2α WT and KO mice (22 somite pairs) were analyzed with antibodies against pS473Akt and total Akt, pERK, total ERK and tubulin. Representative images of 3 independent experiments are shown and quantification is plotted (E, F). Quantification of mRNA expression of CK2 genes in control and patient derived fibroblasts (CSNK2A1, CSNK2A2, CSNK2B) (H).

Figure 2

Subcellular localization of CK2α in patient derived fibroblasts. Cells were fixed and stained with anti-CK2α antibody, DAPI and Texas red phalloidin (A). Quantification of CK2α subcellular localization. For all cell lines, more than 200 cells (from 3 different experiments) were counted (B). Quantification and categorization of F-actin fibers. For all lines, more than 140 cells (from 3 different experiments) were counted. White bar represents 30μm (C,D).

Figure 3

Phosphoproteomic analysis of patient derived fibroblasts. Mass spectrometrical analysis of phosphopeptides of lysates from control and patient fibroblasts was performed. Numbers of significantly altered phosphopeptides ($\log_2(FC) > \pm 0.5$), in three patient lines are shown as pie charts (A-C) and peptides categorized, depending on the presence or absence of a canonical or non-canonical CK2 consensus (D). Gene Ontology analysis (<https://go.princeton.edu/>) based on 26 proteins which were identified with significantly altered detection prevalence in mutant versus control fibroblasts. The number of identified proteins for cellular functional categories is presented in the bar graph (E).

Figure 4

CK2α sequence and functional domains. Scheme of mutants used in this study (A). Alignment of CK2α protein sequences from various vertebrate species (B). Residues used in this study that are mutated in

OCNDS are marked in grey.

A

B

C

Figure 5

Kinase activity of GST-fusion CK2α mutants. Purified GST-CK2α wt and mutant proteins were used in an in vitro kinase assay using CK2 peptide as substrate. After the reaction, samples were separated by SDS-PAGE and Coomassie staining was performed (A). Purified GST-CK2α and purified His-tagged CK2β were used in the kinase assay and Coomassie stain was performed after reaction (B). Quantification of 4

different experiments, normalized by GST-CK2α present in each reaction (C). Assays marked with # were not performed.

Figure 6

Kinase activity of ectopically expressed CK2α mutants. CK2α wt and mutants in pCMV-myc vectors were expressed in COS-7 cells and expression determined by western blotting using a-myc antibody. Results from 3 experiments were quantified, normalized by tubulin and plotted (A,B). 500 μg of COS-7 lysates

were used for immunoprecipitation followed by a kinase assay performed with immunoprecipitates. Results from 3 independent experiments are shown (C,D).

Figure 7

Subcellular localization of OCNDS causing CK2α mutants. All mutants were expressed in COS-7 cells and immunofluorescence analysis was performed using anti-myc antibody, DAPI and Texas red phalloidin. White bar represents 30μm (A, B). Quantification of the predominant localization of CK2α in COS-7 cells, at least 100 cells were counted. White bar represents 15μm(C).

Supplementary Files

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