The Filamin A Actin Binding Domain Structure and Function:

Implications for a gain-of-function mechanism for the otopalatodigital syndrome spectrum disorders

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Abstract

The filamin family act as scaffolding proteins associating with actin filaments, acting through a highly conserved actin binding domain (ABD). The ABD of the filamins is homologous to that found in other F-actin binding proteins such as dystrophin. Mutations in the filamin A gene cause a wide range of disease symptoms in humans reflecting the diversity of the roles that filamin A has in cell structure and signalling pathways. The diseases fall into two separate phenotypic groups. Periventricular nodular heterotopia (PVNH) generally results from the complete loss of filamin A protein, and affects the central nervous system. The clinically separate otopalatodigital disorders (OPD) spectrum disorders are skeletal disorders and were hypothesised to be gain of function phenotype diseases. At the beginning of this work, there was very little structural data available for the human filamins, and none for the crucial highly conserved actin binding domain. This lack of structural data limited the interpretation of the biochemical and genetic data and constrained our understanding of the disease associated mutations that cluster in this domain. These studies aimed to provide insights into the structure and mechanism of actin binding domains, and thus provide a better understanding of the diseases caused when this domain is mutated.

A secondary structural analysis and crystal structures of the wildtype and OPD2 associated mutant ABDs were obtained. The overall fold of the three proteins was equivalent as determined by circular dichroism spectroscopy and x-ray crystallography. The ABD from filamin A E254K showed 3.7 fold increased F-actin affinity, accompanied by a reduced thermostability (of 5.6 °C). Western blotting of OPD2, frontometaphyseal dysplasia (FMD) and PVNH patient fibroblast lysates showed similar levels of filamin A compared to the control cells. In addition the OPD and PVNH patient fibroblasts were able to adhere to fibronectin and migrate with an equivalent rate to control cells.

Together these results have allowed correlations to be developed between structure, protein stability, actin affinity, cellular phenotype and the overall clinical phenotype. Showing that, at least in one example, OPD2 may be due to an increased actin affinity providing further evidence for a gain of function mechanism of OPD2.

Διά τὸ θαυμάζειν ἡ σοφία

Wisdom begins in wonder

Socrates

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Abbreviations

ABD	Actin binding domain
ABS	Actin binding sequence
Amp	Ampicillin
ADP	Atomic displacement parameter
BCA	Bicinchoninic acid
BLAST	Basic local alignment search tool
BMD	Becker muscular dystrophy
BSA	Bovine serium albumin
CaM	Calmodulin
cDNA	Complementary DNA
CD	Circular dichroism
CH	Calponin homology domain
C-terminal	Carboxyl terminal
CV	Column volume
DMD	Duchenne muscular dystrophy
DMEM	Dulbecco's modified eagle media
DNA	Deoxyribonucleic acid
dNTP	Deoxy-nucleotide tri-phosphate
DTT	Dithiothreitol
E. coli	*Escherichia coli*
EDTA	Ethylene diamine tetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
F-actin	Filamentous actin
FMD	Frontometaphyseal dysplasia
g	Gravitational field, unit of
G-actin	Globular (monomeric) actin
GOF	Gain of function
IPTG	Isopropyl-β-D-thiogalactopyranoside
kb	Kilobase pairs (of DNA)
K_d	Dissociation constant
LOF	Loss of function

M2	Human melanoma derived cell lines
MCS	Multiple cloning site
MEF	Mouse embryonic fibroblasts
MNS	Melnick Needles syndrome
NCS	Non crystallographic symmetry
N-terminal	Amino terminal
OMIM©	Online mendelian inheritance in man
OPD	Otopalatodigital syndrome
ORF	Open reading frame
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDB	Protein database
PIP_2	Phosphotidylinositol 4,5-bisphosphate
PVNH	Periventricular nodular heteropia
RMSD	Root mean square deviation
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulfate
TEMED	N, N, N', N'-tetramethylethylenediamine
Tris	Tris-(hydroxymethyl)-aminomethane
V	volts
WT	Wildtype

Related Publications

Clark, A. R., Sawyer, G. M., Robertson, S. P., Sutherland-Smith, A. J. (2009). Skeletal dysplasias due to filamin A mutations result from a gain-of-function mechanism distinct from allelic neurological disorders. Human Molecular Genetics Advance Access published September 22, 2009, 10.1093/hmg/ddp442