Life Science Seen from Molecular Motor

body forming molecular motor
How molecular motor works

2006.11.27.
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KIF3 Complex
= KIF3A + KIF3B + KAP3

kif3B Knockout

kif3A Knockout

The KIF3 motor transports N-cadherin and organizes the developing neuroepithelium

Teng et al. Nature Cell Biol. 7:474-, 2005
A Schematic view of gene targeting
Hypertrophy and invading tumor-like rosette phenotype of kap3 cKO mouse
KAP3-deficient leads to malignant transformation of neuroepithelium
Enlargement of the Neural Progenitor Pool in cKO Mouse Brain
N-cadherin and beta-catenin levels are reduced from cell periphery in cKO brain
Establishment of kap3fl/fl and kap3-/- embryonic fibroblast cell lines

- E13.5 kap3fl/fl mouse embryos
- kap3fl/fl fibroblasts
- pSV3neo G418
- Immortalization of kap3fl/fl (control)
- pCre-Pac puromycin
- kap3-/- (KO)
Impaired Ca\textsuperscript{2+}-dependent cell adhesion in kap3-/- MEFs
Reduced N-cadherin and Beta-catenin levels from cell periphery
Diminished arrival of newly synthesized N-cadherin to the plasma membrane
Impaired plasma membrane targeting of N-cadherin, Beta-catenin, and p120 in KAP3-deficient Cells
KIF3A/3B/KAP3 heterotrimer associated with N-cadherin complex
Colocalization of KIF3/KAP3 with N-cadherin containing vesicles
KAP3 and N-cadherin moving together in A431D cells
KAP3 and N-cadherin moving together in A431D cells
Mechanism of phenotype of cKO brain formation

- KIF3 motor-deficient
  - Impaired post-Golgi transport of N-cadherin
  - Loss balance between proliferation and cell-cell adhesion
  - Expand progenitor pool
  - Decreased adhesion of neuroepithelium
  - Rosette
KIF 4 and Activity Dependent Neuronal Survival
KIF4 regulates activity-dependent neuronal survival by suppressing PARP-1 enzymatic activity

R. Midorikawa et al.
Structural view of KIF4

- Tissue distribution: ubiquitous (in juvenile stage)
- Intracellular localization: nuclei and cytosol
Screening of KIF4-binding protein using GST-pull down

(A) Pull Down using GST-KIF4 tail
(B) Pull down using only GST

<table>
<thead>
<tr>
<th></th>
<th>1.0 M NaCl</th>
<th>2.0 M NaCl</th>
<th>Beads</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP</td>
<td>200</td>
<td>116</td>
<td>97</td>
</tr>
<tr>
<td>66</td>
<td>45</td>
<td>31</td>
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</table>

GST

PARP : Poly (ADP-ribose) polymerase
Structural view of PARP

Poly ADP-ribosylation for acceptor protein by PARP

- activity of various transcription factors
- chromatin structure
## In-vitro binding assay

### Binding between tissue-purified PARP and GST-KIF4 tail

<table>
<thead>
<tr>
<th>Purified PARP (μg)</th>
<th>GST-KIF4 tail</th>
<th>GST</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>DNA(-)</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>DNA(+)</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>DNA(+)</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>DNA(-)</td>
<td></td>
</tr>
</tbody>
</table>

**PARP**

- **GST-KIF4 tail**
- **GST**
Overexpression of KIF4 deletion mutants in NIH3T3 cells

**Effect of KIF4 tail on PARP automodification**

<table>
<thead>
<tr>
<th>+ KIF4 tail domain (μg)</th>
<th>0</th>
<th>2</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>Automodified PARP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PARP</td>
<td></td>
<td></td>
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<tr>
<td>KIF4 tail domain</td>
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</table>

Full length KIF4

Headless KIF4

Tailless KIF4

GFP only

Poly (ADP ribose) proteins Merged
PARP activity of wild-type and kif4 KO ES cells

Northern blot

Western blot

(kb) WT KO

(kDa) WT KO
ES cells

WT neurons
In vitro differentiation into excitatory neurons

kif4 KO neurons
Percentages of apoptic cells

PARP activity of WT and kif4 KO neurons

Graphs showing the percentages of apoptic cells and the relative PARP activity for WT and kif4 KO neurons under different conditions.
Survival of CGCs (cerebellar granule cells) after knockdown of PARP by RNAi

- Percentages of apoptic cells

![Graph showing survival rates of CGCs after knockdown of PARP by RNAi]
Knockdown of KIF4 in CGCs by RNAi

Anti-PAR labeling for CGCs knock down of KIF4

[Images showing experiment results]

Knockdown of KIF4 in CGCs by RNAi

[Table showing relative intensity of KIF4 bands after different treatments]
Survival of CGCs after knockdown of KIF4 by RNAi
Binding between PARP and KIF4 after depolarization
Localization of KIF4 and PARP in ES-derived neurons after depolarization
Localization of KIF4 in nocodazol treated neurons
Electrical stimulation applied for CGCs. 
(CAMK II mediates depolarization-dependent PARP-1 activation)
Localization of KIF4 after electrical stimulation in CGCs

None (a)  50 Hz, 1hr (b)  50Hz, KN-62(+), 1hr (c)

Anti-KIF4 (14.3±1.8%)  Anti-KIF4 (53.8±4.5%)  Anti-KIF4 (14.0±1.4%)
Expression of KIF4 deletion mutants in the brain cortex by in utero electropolation
Density of headless-KIF4 and tailless KIF4-positive cells around CA1 region
(3 days and 8 days after electroporation)
Schematic diagram of the involvement of KIF4 in the regulation of the survival of developing neurons.
The Kinesin Superfamily

All Family of KIFs

M-kinesins

KIF2C = MCAK
XKCM1, XKIF2 in Xenopus
KIF2A

Motor domain

Globular domain

Motor domain

Stalk domain

Abundant in juvenile brain
Generation of \textit{kif2a\textsuperscript{-/-}} mice

Construction

\textbf{Vector}\hfill \textbf{WT}\hfill \textbf{KO}

\begin{itemize}
  \item PBS
  \item \textit{ßgeo}
  \item pBS P+1
  \item BB
  \item BB
  \item 1kb P+1
  \item \textit{ßgeo}
  \item BB
  \item B
  \item 2.5kb
  \item B
  \item P+1 B
  \item 1kb B
\end{itemize}

\textbf{Southern}\hfill \textbf{Western}

\begin{itemize}
  \item +/+ +/−
  \item 5.0kb
  \item 2.5kb
  \item 116kD
  \item 97kD
  \item \textbf{KIF2A}
  \item Homma et al. Cell 114:229—, 2003
\end{itemize}
kif2a<sup>-/-</sup> mice

kif2a<sup>-/-</sup> mice died in the day without suckling milk.
Laminary defects in *kif2a*<sup>−/−</sup> brain

<table>
<thead>
<tr>
<th>Cortex</th>
<th>Hippocampus</th>
</tr>
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<tbody>
<tr>
<td><img src="image1.png" alt="Cortex image" /></td>
<td><img src="image2.png" alt="Hippocampus image" /></td>
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<table>
<thead>
<tr>
<th>Cerebellum</th>
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</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Cerebellum image" /></td>
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</table>

Legend:
- egl: external granule layer
- gr: granule layer

Scale: 200 μm
Migratory defects in \textit{kif2a}\(^{-/-}\) brain

+/

E14+/

E14-/

-/-
Axon branching abnormality in \textit{kif2a}^{-/-} cortex

More horizontal neurites are observed in \textit{kif2a}^{-/-} cortex than in \textit{kif2a}^{+/+} cortex.
Abnormal axonal branching

MZ
CPs
CPi
SP
IZ
VZ
Abnormal arborization in *kif2a*−/− hippocampal neuron
Time-lapse Imaging

+/+

-/-
KIF2A depolymerizes MT *in vitro*

Tubulins are recovered from the supernatant in the presence of ATP as a result of MT depolymerization by KIF2A.
Movement of individual MTs

+/-

-/-
KIF2A function in growth cone

+/-

+/-