A novel function of KPNA1/importinα in neuronal cells.

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Abstract

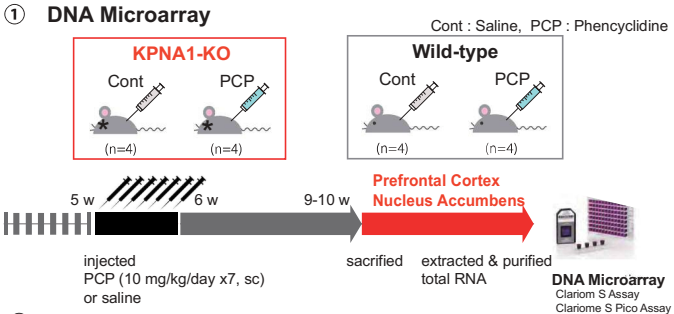
Schizophrenia, autism spectrum disorder (ASD), and attention deficit/hyperactivity disorder (ADHD) have been reported to be caused by intracellular transport deficits in neuronal cells and their migration disorder. However, the intracellular transport systems have not been studied in this context. Since importins mediate transportation from cytoplasm to nucleus, and from synapse to soma, perturbation of importin-dependent pathway may have significant neuronal consequences. Our behavioral tests using KPNA1 knockout (KO) mice revealed impairment of novel object recognition, tendency of depression, and increased sensitivity to phencyclidine (PCP), a non-competitive antagonist for NMDA glutamate receptor that causes schizophrenia-like symptoms. Coupling intracellular signals to behavioral output likely requires clarification of a novel functional role of KPNA1 in neuronal cells.

In this study, DNA microarray data provided insights into the possible gene expression alterations in parts of brain, such as prefrontal cortex (PFC), and nucleus accumbens (NA) of KPNA-KO mice. Principal components analysis (PCA) of KPNA-KO mice with PCP revealed different clusters in scatter plots, suggesting higher sensitivity and/or weakness to PCP. Notably, gene expression of cytoplasmic dynein components and their associated factors was found to be reduced in KPNA-KO mice, which was further, remarkably reduced in presence of PCP.

Live-cell imaging using FRAP (fluorescence recovery after photobleaching) and dual fluorescence tracking showed migration of KPNA1 both antero- and retrogradely, along with co-migration of cytoplasmic dynein. Endogenous KPNA1 accumulated near the centrosome and around the nucleus. Our findings suggested that KPNA1 functions in intracellular transport through microtubules (MTs) and in neuronal cell migration dependent on MT traction. This unexpected and intriguing discovery, related to axonal transport, may provide new insight into neuropsychiatric disorders.

Materials & Methods

① **DNA Microarray**

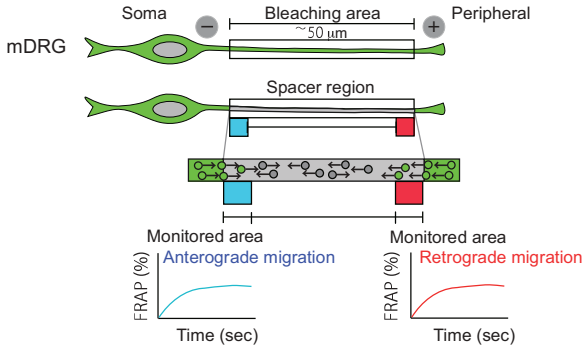


② **Time-lapse fluorescence imaging**

i) mDRG preparation

- dissociated DRGs from postnatal mice (P2-P5).
- transfected each vector to express GFP-protein.
- cultured in D-MEM/10%FBS/20 ng/ml 2.5SmNGF for 24-48 hrs.
- FRAP was carried out and monitored the fluorescence recovery with a confocal microscope (FV-1200, Olympus).

ii) Fluorescence Recovery after Photobleaching (FRAP)



a. Nucleus Accumbens (NAc) **b. Prefrontal Cortex (PFc)**

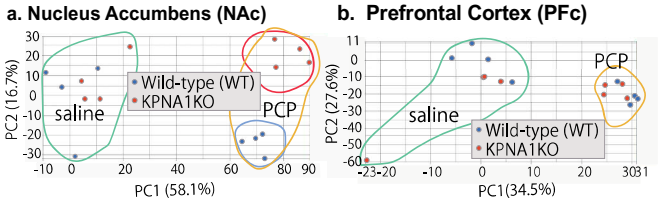


Figure 1. Principal componant analysis of DNA microarray data.

PCA summarized gene expression levels of Nucleus accumbens **(a.)** and Prefrontal cortex **(b.)** from KPNA1-KO and Wild -type mice.
Note: In Nucleus accumbens, KPNA-KO mice suggested higher sensitivity and/or weakness to PCP.

Results

Table. Comparison of gene expression levels by DNA microarray .

・KPNA1KO(Cont) vs WT (Cont)

KPNA1KO Avg	WT Avg	Fold Change	P-value(Welch's t test)	Gene
7.25	11.04	-13.83	1.32E-09	KPNA1
7.28	10.5	-9.35	0.0015	Cytoplasmic dynein heavy chain
8.15	9.31	-2.24	0.0007	Cytoplasmic dynein intermediate chain

・KPNA1KO(PCP) vs WT (PCP)

KPNA1KO Avg	WT Avg	Fold Change	P-value(Welch's t test)	Gene
7.4	11.52	-17.38	2.18E-08	KPNA1
6.61	10.96	-20.42	0.0005	Cytoplasmic dynein heavy chain
7.01	9.5	-5.63	0.0002	Cytoplasmic dynein intermediate chain
11.04	12.9	-3.62	0.0067	doublecortin

Note: Gene expression levels of dynein components were reduced in KPNA1-KO mice.

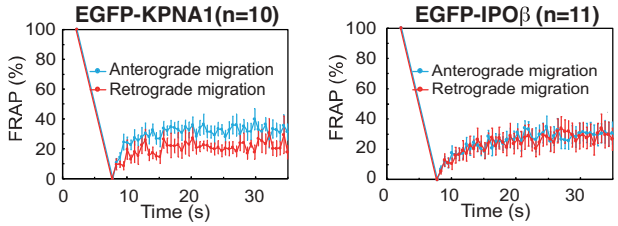


Figure 2. FRAP analysis data of EGFP-KPNA1 and EGFP-IPOβ.

Note: EGFP-KPNA1 and EGFP-IPOβ displayed bi-directional movements on MTs in mDRG.

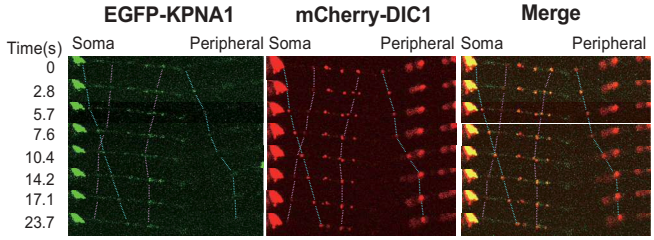


Figure 3. Direct visualization of the movement of fluorescence-tagged proteins in DRG using confocal microscopy.
(a) EGFP-KPNA1, **(b)** mCherry-DIC1 and **(c)** Merge .
Note: KPNA1 co-migrated with dynein, not only retrograde migration but also anterograde one of axonal transport in mDRG.

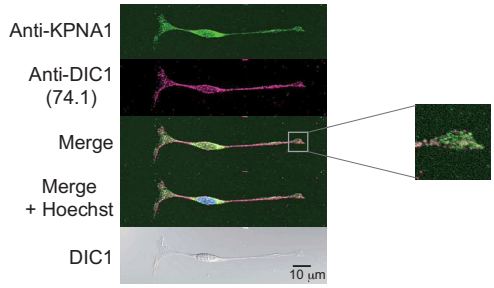


Figure 4. Subcellular localization of KPNA1/dynein in mDRG.

Immunocytochemistry data using anti-KPNA1 Ab(Sigma) and Anti-DIC Ab(Merk). **Note:** KPNA1 and dynein co-localized near th centrosome and around the nucleus.

Our Models