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New coordination between F-actin and microtubules

Cells maintain vesicles transportation, cell migration, cell polarity, mitosis, cell shape and even cell signaling through cytoskeleton, which is the most conservative and essential structures in diverse organism. F-actin and microtubule, two components of the cytoskeleton, have been investigated individually or simultaneously for decades of years. Crosstalk between F-actin and microtubule occurs mainly through signaling pathway such as RhoA/Rac1 activation/inactivation, or through direct structure-based contact mediated by microtubule-actin cross-linkers, motors binding with or without actin and microtubule associated proteins. However, whether Noncentrosomal microtubule and F-actin coordinate with each other and the underlying mechanism have never been investigated.

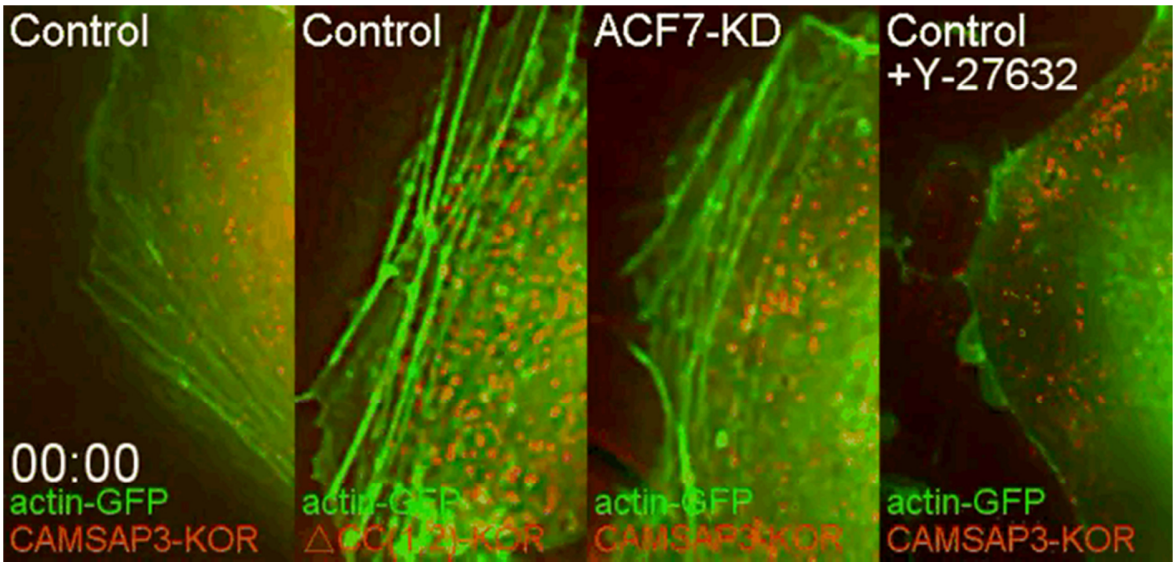

Fig. 1. The Nezha/CAMSAP3 retrograde flow.
Time-lapse movie Nezha/CAMSAP3-KOR and actin-GFP expressed in control or ACF7-KD cells, and ΔCC (1, 2)-KOR and actin-GFP expressed in control cells, and then treated with DMSO or Y-27632 (Rho-associated kinase inhibitor). Images were acquired at 30-s intervals for 14.5min.

Noncentrosomal microtubules mainly exist in plants, epithelia cells as well as neurons. They are thought to be generated either through release from centrosome, cut off from pre-existing microtubules or nucleated from cytoplasm Noncentrosomal MTOCs. Recently, Nezha (also called CAMSAP3) has been identified locating at the minus-end of Noncentrosomal microtubules, and stabilizes the minus ends through protecting microtubule from depolymerization. The minus-ends of noncentrosomal microtubules always stay very still in the cytoplasm compared with dynamic plus-ends, however, the mechanism remains unclear, not to mention the crosstalk between the noncentrosomal microtubules and F-actin.

Nezha/CAMSAP3 exists at the minus-ends of over 90% noncentrosomal microtubules in Caco2 epithelial cells, contains CH domain in its N-terminal, three coiled-coil domains, and the CKK domains responsible for its minus-end localization. CC(1,2) domain is proved required for the stable anchor of

Nezha/CAMSAP3, and interacts with the 19th spectrin domain of ACF7 (also called microtubule-actin crosslinking factor 1).

ACF7 contains two CH-domains in its N-terminal for binding to F-actin, spectrin repeats domains in the central region, and C-terminal microtubule and EB1 binding domains. ACF7 is detected localizing at the minus-ends of the noncentrosomal microtubules depending on Nezha/CAMSAP3, and is required for the anchor of noncentrosomal microtubule minus ends as Nezha/CAMSAP3 fail to anchor stably in the cells through depletion of ACF7, similar to deletion of CC(1,2) domain.

Noncentrosomal microtubule minus-end is anchored at F-actin through ACF7. When depletion of ACF7 or CC(1,2) domain, Nezha/CAMSAP3 fails to anchor at F-actin but can be rescued by linking with N-terminal two CH domains of ACF7. This indicates noncentrosomal microtubules minus-ends are bridged at the F-actin through Nezha/CAMSAP3-ACF7 complex.

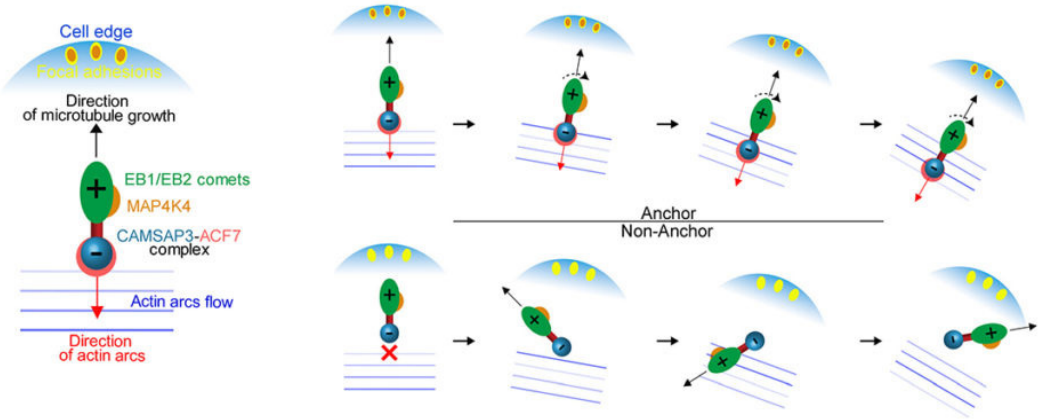

Fig. 2. Noncentrosomal Microtubule Minus Ends Anchor at Actin Filaments via the CAMSAP3-ACF7 Complex and Contribute to Cell Migration
Noncentrosomal microtubule minus-ends are tethered to actin filaments by the CAMSAP3-ACF7 complex under normal conditions. CAMSAP3 and microtubules undergoing retrograde flow with actin arcs are transported from the cell periphery toward the inside of cell. This retrograde movement of actin arcs applies a tractive force to noncentrosomal microtubules, orients these microtubules perpendicular to the cell edge, regulates perpendicular peripheral microtubule length, and maintains uniform orientation of EB1/2 emerging from CAMSAP3. When microtubule minus-end protein CAMSAP3 fails to anchor at actin filaments, either upon ACF7 knockdown or depletion of CC (1, 2) domain, CAMSAP3 loses the ability to adjust and stabilize microtubule orientation relative to actin filaments, which leads to unstable CAMSAP3 clusters, disorganized growth of microtubules, and decreased targeting frequency of EB2 to FAs. As a downstream result, MAP4K4 accumulation at FAs is reduced and cell migration is compromised.

Myosin II facilitate actin filaments to form into stress fibers including actin arcs which are parallel to the cell edges, and forces actin arcs to retrograde flow from cell edges into the inside. In this process, Nezha/CAMSAP3 also undergoes retrograde flow together with actin arcs which depends on ACF7 and Myosin II activity (Fig. 1). This allows Noncentrosomal microtubules entering into the inside of the cell and

also keeps microtubules directions perpendicular to the cell edge. Noncentrosomal microtubules grow from anchored CASMAP3 sites and maintain constant direction in control instead of ACF7 knock-down cells. The crosslink between noncentrosomal microtubules and F-actin through Nezha/CAMSAP3-ACF7 complex allows F-actin to regulate microtubule retrograde flow and constant growth direction.

Microtubule is guided by F-actin to target to focal adhesion (FAs) and regulate FAs dynamics to facilitate cell migration. In this process, MAP4K4 is transported to FAs through EB2 and active Arf6 by interacting with IQSEC1, a guanine nucleotide exchange factor of Arf6. Coordination of Noncentrosomal microtubules and F-actin through Nezha/CAMSAP3-ACF7 complex facilitates microtubules targeting to FAs and MAP4K4 localizing at FAs to regulate FAs size and cell migration. Exquisite cooperation exists between noncentrosomal microtubules and F-actin to form an intricate network and build the physical basis for their cellular function. Functions regarding to this new crosslink between noncentrosomal microtubules and F-actin in other biological process and cell types including neurons and their in-vivo role remain to be further investigated (Fig. 2).

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