Involvement of calcium in regulation of gamma-tubulin properties

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Keywords: *Calcium, Gamma-tubulin, Microtubule nucleation*

Abstract

Activation of mast cells initiates signaling events leading to calcium influx and release of allergic mediators from cytoplasmic granules. Although microtubules rearrange after activation, molecular mechanisms that control their remodeling are largely unknown. For microtubule nucleation, essential complexes are formed by gamma-tubulin and gamma-tubulin complex proteins (GCPs).Here we report that in a bone marrow-derived mast cells (BMMCs), gamma-tubulin interacts with p21-activated kinase interacting exchange factor (βPIX) and G protein-coupled receptor kinase-interacting protein 1 (GIT1). Microtubule regrowth experiments revealed that the depletion of βPIX in BMMCs stimulated microtubule nucleation, while depletion of GIT1 led to the inhibition of nucleation when compared to control cells. Phenotypic rescue experiments confirmed that βPIX and GIT1 represent, respectively, negative and positive regulators of microtubule nucleation. Live-cell imaging disclosed that both proteins are associated with centrosomes. Immunoprecipitation and pull-down experiments revealed that enhanced level of calcium affects gamma-tubulin properties and stimulates the association of GIT1 and GCPs with gamma-tubulin.. Moreover, in activated cells, gamma-tubulin forms complexes with tyrosine-phosphorylated GIT1.

We provide a possible mechanism for the concerted action of tyrosine kinases, GIT1/βPIX proteins and calcium in the propagation of signals leading to microtubule nucleation in activated mast cells. Supported by grants P302/12/1673 (GACR) and LD13015 (MSMT).