ABSTRACT

MT nucleation from γ-tubulin complexes, located at centrosome, is an essential step in the formation of MT cytoskeleton. In mammalian cells, γ-tubulin is encoded by two genes. We functionally characterized two γ-tubulin proteins and have found that both are functionally equivalent. γ-Tubulin 2 is able to substitute for γ-tubulin 1 in MT nucleation. However, we revealed that unlike TUBG1, TUBG2 expression is downregulated in mouse preimplantation development.

Mast cells represent effectors of the allergy reaction. Their activation by antigen induces number of cellular processes such as degranulation, proliferation and cytoskeleton rearrangements. The regulatory mechanisms of MT reorganization during mast cell activation are unknown. We identified new signaling proteins, GIT1 and βPIX that interact with γ-tubulin. Depletion of GIT1 or βPIX leads to changes in MT nucleation. GIT1 is phosphorylated on tyrosine and associates with γ-tubulin in a Ca^{2+}-dependent manner. Our data suggested a novel signaling pathway for MT rearrangement in mast cells where tyrosine kinase-activated GIT1 and βPIX work in concert with Ca^{2+} signaling to regulate MT nucleation. We tested the capability of GIT1 and βPIX to influence γ-tubulin function in more cell types. We found out that GIT1/βPIX signaling proteins together with associated PAK1 kinase regulate MT nucleation in osteosarcoma and retinal epithelial cells. In these cells, GIT1 with PAK1 represent positive and βPIX negative regulators of MT nucleation via changes in γ-tubulin accumulation at centrosome. GIT1/βPIX signaling proteins are phosphorylated by PAK1 and directly interact with γ-tubulin. We propose that GIT1/βPIX signaling proteins with PAK1 kinase represent novel regulatory mechanism of MT nucleation in interphase cells.

MTs are known chemotherapeutic targets due to their crucial role in mitosis. As cells can get resistance to used tubulin-binding agents, new compounds interfering with MTs are synthetized. We tested panel of steroidal derivatives in their cytotoxicity, effect on MT polymerization and dynamics. We revealed that estradiol dimer is capable of MT destabilization associated with cell death.