ABSTRACT

Microtubules (MTs) are highly dynamic structures essential for the spatio-temporal intracellular organization and transport, signal propagation, cell differentiation, motility and division. To perform these roles, MTs create arrangements capable of fast and precise adaptation to various signals. MTs are under the control of many factors regulating MT nucleation, stability and dynamics. Bone marrow-derived mast cells (BMMCs) are important immune system cells, which can cause serious diseases if their functions are deregulated. Although MT reorganization during BMMC activation is well established, the molecular mechanisms that control their remodelling are largely unknown. In the presented thesis we functionally characterised GIT1/βPIX signalling proteins, PAK1 kinase, and Ca^{2+} signalling in the regulation of MT nucleation in BMMCs and other cell types. We also elucidated the function of miltefosine (hexadecylphosphocholine), a promising candidate for the treatment of mast cell-driven diseases.

We found that GIT1/βPIX signalling proteins are γ-tubulin-interacting proteins associating with centrosomes in BMMCs. MT nucleation is positively regulated by GIT1 and Ca^{2+}, whereas βPIX is a negative regulator of MT nucleation in BMMCs. Cytosolic Ca^{2+} affects γ-tubulin properties and stimulates the interaction of γ-tubulin with GIT1 and γ-tubulin complex proteins. Moreover, γ-tubulin forms complexes with tyrosine-phosphorylated GIT1 in activated BMMCs. Our data suggest a novel mechanism for the concerted action of tyrosine kinases, GIT1/βPIX proteins and Ca^{2+} in the regulation of MT nucleation in activated BMMCs.

We showed that GIT1/βPIX signalling proteins, together with PAK1 kinase regulate MT nucleation from interphase centrosomes in different cell types. GIT1 and PAK1 represent positive, and βPIX negative regulators of this process. The level of MT nucleation correlates with the amount of γ-tubulin at centrosomes. We proved that GIT1 and βPIX are phosphorylated by PAK1 kinase and determined their binding domains for the interaction with γ-tubulin. Based on obtained results, we propose that GIT1/βPIX and PAK1 represent a novel regulatory mechanism of MT nucleation in interphase cells.

We showed for the first time that miltefosine modulates BMMCs both at the plasma membrane and in the cytosol. It inhibits the degranulation, MT reorganization and antigen-induced chemotaxis. While the aggregation and tyrosine phosphorylation of high-affinity IgE receptors is suppressed in activated BMMCs treated with miltefosine, Ca^{2+} influx is not inhibited. Besides that, miltefosine modulates intracellular movement of granules, MT dynamics in Ca^{2+}-dependent manner and attenuates the phosphorylation of proteins interacting with MT plus-end binding protein EB1. Miltefosine's action at multiple sites in BMMCs could explain its effectivity in the treatment of mast cell diseases.