

Abstract of PhD dissertation

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Title of dissertation: „Functional characterization of the striatin family of proteins”

Microtubules (MTs) are components of the eukaryotic cytoskeleton. These tubular polymers of tubulin are responsible for a wide variety of cellular processes including maintenance of the cell shape, motility and intracellular transport. Microtubules are also involved in the formation of mitotic spindle and segregation of chromosomes during the cell division. MTs are highly dynamic structures and their polymerization/depolymerization rate depends on a family of proteins called microtubule-associated proteins (MAPs). The best known representatives of MAPs include MAP2, tau and MAP4. Phosphorylation of MAPs is the major mechanism of their regulation. The protein phosphatase 2A (PP2A) is an enzyme responsible for dephosphorylation of MAP2, the process by which the MAP2-MTs binding is enhanced and as the result, the MTs are stabilized (Gong 2000). PP2A is a serine/threonine phosphatase, composed of the catalytic (C), structural (A) and regulatory (B) subunits. Striatin (STRN), an ubiquitous protein expressed mainly in the central and peripheral nervous system, is one of the regulatory subunits of PP2A (Moreno 2000). The exceptional feature of striatin is the presence of four protein-protein interaction domains. As a consequence, striatin acts as a molecular scaffold that organizes the large signaling complexes (Hwang J 2014).

The main aim of these studies was to verify whether the PP2A-striatin complex is involved in regulation of phosphorylation of MAP2, and thus controls the MTs stability. Previously, it was shown using immunofluorescent imaging, that striatin and MTs co-localize in CHO cells (Kaźmierczak 2015). First, the expression of STRN, MAP2 and tau proteins was analyzed and confirmed in HEK293T cells, by immunoblotting. Subsequently, co-immunoprecipitation experiments indicated that endogenous MAP2 and STRN form a complex in HEK293T. However, the formation of a striatin/tubulin complex was not unequivocally confirmed.

In the next experiments, it was found that silencing of striatin via RNA interference, results in hyperphosphorylation of MAP2 in comparison to untreated cells and cells treated with control siRNA. In addition, the decrease in STRN expression caused a modest destabilization of MTs in HEK293T. Even tenuous alterations in microtubules stability or dynamics can have harmful effects, cause cell cycle arrest, inhibit the cell proliferation of cells and, eventually, cell death. Therefore, in the next step it was examined whether the downregulation of striatin would affect HEK293T cell proliferation. Cells were transfected with a striatin siRNA and the relative number of viable cells was determined in the MTT assay. Inhibition of striatin expression reduced the proliferation of HEK293T cells compared to cells transfected

with control siRNA. Likewise, the activity of caspases 3 and 7 was lower in cells with the silenced striatin gene compared to cells transfected with control siRNA.

In the next step of this work it was investigated whether the effect of striatin downregulation on cell-cycle progression. It occurred that inhibition of the striatin subtly affects HEK293T cells cycle, resulting in accumulation of cells in the G0/G1 phase and lowering the number of cells in the S phase.

In the last stage of this study it was examined whether the simultaneous downregulation of striatin, SG2NA and zinedin would have stronger effect on HEK293T cell proliferation, activity of caspases 3 and 7 and cell-cycle progression than downregulation of STRN only. The result indicate that: (i) simultaneous downregulation of STRN, SG2NA, ZIN has a stronger effect on proliferation of HEK293T cells than has silencing of striatin alone; (ii) cells with silenced STRN, SG2NA, ZIN showed slightly higher activity of caspases 3/7; (iii) simultaneous downregulation of all striatin family members resulted in the higher number of HEK293T cells that accumulated in G0/G1 phase when compared to cells with downregulated striatin.

Taking together it was hypothesized that striatin, most likely in PP2A/striatin complex modulates the microtubules dynamics via regulation of MAP2 phosphorylation.