Aurora-A-elicited Astrin phosphorylation regulates mitotic progression

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ABSTRACT

Astrin is a spindle protein regulating several aspects of mitosis including the maintenance of bipolar spindle pole, regulation of sisterchromatid cohesion and chromosome alignment. However, it remains unclear how to regulate Astrin protein in mitosis. Our present study has shown that Astrin behaves as a mitotic phosphoprotein and was directly phosphorylated by the mitotic kinase Aurora-A in an *in vitro* kinase assay. Furthermore, the mitosis dependent phosphorylation of Astrin was blockaded by immunodepletion or shRNA-mediated knockdown of Aurora-A. It indicates that Astrin acts as an *in vitro* and *in vivo* substrate of Aurora-A. The phosphorylation site was subsequently mapped to serine 115 of Astrin. And site-directed mutagenesis analysis revealed that Astrin S115A mutants, no longer serving as a substrate of Aurora-A, delayed the mitosis progression and induced chromosome misalignment and DNA lagging along the mitotic progression. By contrast, Astrin S115D, which mimics Aurora-A elicited phosphorylation, accelerated the mitosis progression and slightly deceased the level of DNA lagging. The binding activity of Astrin S115A mutant to its mitotic interaction proteins LC8 and CLASP-1alpha was remarkably reduced, leading to the mislocalization of the sequential two proteins and the misalignment of chromosome. In conclusion, we are the first to demonstrate that Aurora-A, a well-characterized oncokinase controlling a wide range of mitotic events by phosphorylating numerous mitotic factors, phosphorylates Astrin *in vitro* and *in vivo*, which guides the binding of Astrin to its cellular partners, and ensures accurate chromosome alignment and segregation and thereby proper mitosis progression.

Key words: Astrin, Aurora-A, phosphorylation, chromosome misalignment