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MEETING ABSTRACT

A3.1

Mad2, a novel player in clathrin-mediated endocytosis, interacts with monoamine transporters

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Background: Monoamine transporters conduct the reuptake of serotonin (SERT), dopamine (DAT) and norepinephrine (NET) following neurotransmission. Surface levels and subcellular localization of transporter proteins can be regulated by clathrin-mediated endocytosis (CME), during which cargo proteins internalize as part of surface-derived membrane vesicles. This process requires adaptor protein 2 (AP-2), which links cytosolic domains of cargo proteins to a cage of clathrin proteins. This leads to the formation of intracellular clathrin-coated vesicles. It is currently unknown how monoamine transporters connect to clathrin, as their intracellular domains do not provide any known interaction motif for AP-2 binding. Nevertheless, the carboxy-terminus of the transporter was shown to play a central role for its internalization [1]. Recent work shows that the insulin receptor (IR) interacts with AP-2 via a heterodimer of two mitotic spindle assembly checkpoint (SAC) proteins: Mad2 and Bubr1 [2]. A classical Mad2-interacting motif (MIM) in the IR C-terminus is crucial for AP-2 recruitment. Inspection of monoamine transporter C-termini reveals putative MIMs, similar to those found in other Mad2-interacting proteins (IR; CDC20; Mad1). Considering this similarity and the acknowledged but opaque role of the transporter C-terminus for endocytosis, it is reasonable to hypothesize that Mad2 initiates clathrin-mediated endocytosis of neurotransmitter transporters.
Methods: A combination of biochemical methods (GST pulldown, co-immunoprecipitation) was used to study a putative SERT–Mad2 interaction. Consequences of Mad2 depletion on SERT surface expression and subcellular localization were investigated using siRNA-mediated knock-down.
Results: The conducted experiments clearly show an interaction between Mad2 and monoamine transporters at the cellular surface. This interaction is dependent on the Mad2-interacting motif in the transporter C-terminus. Interestingly, the cognate transporter GAT1 misses Mad2 interaction. Mad2 depletion in YFP-SERT-expressing cells causes significant increase of SERT surface expression and differential SERT glycosylation. Furthermore, Mad2 knock-down depletes intracellular membrane compartments from YFP-SERT, indicating disturbed endocytosis.
Discussion: These results suggest a role for Mad2 during endocytosis of monoamine transporters. Since Mad2 shows marked expression in the brain, it is plausible to assume that the investigated interaction also occurs in a native biological system. Hence, this work could provide an answer to the puzzling question of the interplay between the monoamine transporter C-terminus and the endocytic machinery.
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