

MEETING ABSTRACT

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The interactome of the A_{2A} adenosine receptor *in vitro* and *in vivo*

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Background

The A_{2A} adenosine receptor is a prototypical G protein-coupled receptor. It is expressed in a wide variety of cells including as different types as neurons, platelets, cells of the immune system and muscle. The cell-specific expression of the A_{2A} adenosine receptor is controlled by at least three different upstream non-coding exons and their corresponding promoters. Compared to other G protein-coupled receptors the A_{2A} receptor holds an unusually long intracellular carboxy terminus, which consists of 122 amino acids. This C-terminus turned out to be the docking site for other proteins. Using a yeast-2-hybrid screen we have previously identified proteins interacting with the C-terminus including ARNO/cytohesin-2, SAP102 and USP4.

Materials and methods

To verify these interactions *in vivo* and to identify new interacting proteins of the A_{2A} adenosine receptor we chose a two-step proteomics approach: we first expressed tagged receptors in HEK293 fibroblasts using various TAP (tandem affinity purification)-tag variants; the differently tagged receptors were analyzed for expression, localization and their pharmacological properties (ligand binding and cAMP accumulation) to identify tags suitable to further analyze the receptor's interactome. These tagged receptors were then used to optimize the purification and to make the first initial screens using 2D-nano-LC-MS/MS approach.

Results and conclusions

We could identify two tags suitable for further analysis of the A_{2A} adenosine receptor interactome. One of the

tags kept the receptor to a large extent in the endoplasmic reticulum, while the second tag allowed surface expression. Pharmacological properties of the receptors were comparable to untagged versions of the receptor. LC-MS/MS analysis of the purified ER trapped version of the receptor revealed proteins putatively involved in the folding of the receptor like heat shock proteins. The A_{2A} adenosine receptor expressed at the cell surface will be used in the *in vivo* approach. To perform this we generate a transgenic mouse expressing the TAP-tagged version of the A_{2A} adenosine receptor under the control of its endogenous promoters (homologous knock-in). This will allow us to examine tissue and developmental specific interaction partners of the A_{2A} adenosine receptor.

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