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MEETING ABSTRACT

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Investigating the molecular mechanisms underlying the differential subcellular targeting of the metabotropic glutamate receptor 1 in the cerebellar cortex

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Background: Type 1 metabotropic glutamate ($mGlu_1$) receptors play a pivotal role in different forms of synaptic plasticity in the cerebellar cortex, e.g. long-term depression at excitatory synaptic inputs to Purkinje cells (PCs) and rebound potentiation at inhibitory postsynaptic potentials. These various forms of plasticity might depend at least in part on subsynaptic arrangement of the receptor that can be regulated by protein–protein interactions. To elucidate the molecular mechanisms implicated in the differential subcellular targeting and the physiological functions of $mGlu_1$ receptors in the cerebellar cortex, we have searched for novel interaction partners of these receptors. To this aim, we have selected an unbiased proteomic approach, namely co-immunoprecipitation (Co-IP) followed by liquid chromatography–tandem mass spectrometry (LC-MS/MS).
Methods: Co-IP of $mGlu_{1(\alpha)}$ receptor was performed from C57BL/6N mouse cerebellar homogenates using highly specific polyclonal antibodies. Confirmation of the specificity was obtained using tissue obtained from *Grm1* knockout (KO) mice. High-speed membrane fractions were solubilized in buffer containing 1% non-ionic and 0.1% ionic detergents. Co-IP was carried out using affinity-purified guinea-pig or rabbit polyclonal antibodies directed against the carboxy-terminal tail of the $mGlu_{1(\alpha)}$ receptor and protein A conjugated to magnetic beads. Eluted proteins were then resolved on SDS-PAGE and analyzed by quantitative mass spectrometry.
Results: LC-MS/MS analysis of the Co-IP eluates identified a number of well-known direct and indirect interactors such as Homer proteins, TRP channels and GluD2 receptors. A novel putative interaction partner, namely KCTD12, was identified in all LC-MS/MS analyses performed on wild-type eluates, but not on *Grm1*-KO ones. KCTD12 is already known as a $GABA_B$ receptor auxiliary subunit, which is involved in the desensitization of $GABA_B$ receptor responses, and shows a distinct expression pattern in PCs. To investigate whether the KCTD family of proteins are common interaction partners of group 1 mGlu receptors, we are currently performing Co-IP for $mGlu_5$ receptors from mice hippocampal homogenates. In order to elucidate the mechanisms that regulate the coupling between $mGlu_1$ receptors and KCTD12, we have generated a recombinant mammalian cell line stably expressing KCTD12. Heterologous co-expression of different $mGlu_1$ receptor mutants is ongoing to investigate the type and site of interaction besides the role that KCTD12 might play on $mGlu_1$ receptor trafficking and function.
Discussion: Our findings suggest a novel $mGlu_1$ receptor interaction partner, namely KCTD12, in the mouse cerebellum. Further investigations will elucidate the role that KCTD12 might play on $mGlu_1$ receptor localization and function in the cerebellar cortex.

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