

Developmental Neurobiology and Regeneration



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Purkinje cell dendritic development and vascular plasticity in the Central Nervous System

The dendritic tree reflects the neuronal cell type and the synaptic connections of a given neuron. During development the shape of dendrites is determined by outgrowth and retraction of dendritic processes. Our laboratory is interested in how neuronal activity may be linked to the shaping of the dendritic tree of Purkinje cells, the principal cells of the cerebellar cortex. The dendritic tree of Purkinje cells is well developed in organotypic slice cultures (Fig. 1). In previous work we have shown that glutamate receptor activation and increased protein kinase C activity strongly inhibit dendritic development of Purkinje cells. Their inhibitory action is mediated by changes in the calcium equilibrium of the Purkinje cells. The control of intracellular calcium is an important link between functional activity and dendritic growth in Purkinje cells we want to further elucidate how changes in functional activity are converted to changes in dendritic growth.

For the long-term control of the calcium equilibrium in Purkinje cells calcium clearing mechanisms are essential. We have investigated the role of the plasma membrane Ca^{2+} -ATPase 2 (PMCA2) which is highly expressed in Purkinje cell dendritic spines. When we applied chronic pharmacological inhibition of PMCA2 function with carboxyeosin we found a mild reduction of the size of Purkinje cell dendritic trees similar to the observation in PMCA2-deficient mice. However, when PMCA2 inhibition was combined with mGluR stimulation it had a partial rescuing effect similar to that seen after blockade of voltage-gated calcium channels. We are currently studying the role of the sodium calcium exchanger (NCX), another important calcium clearing mechanism in Purkinje cells. In addition to controlling dendritic development, protein kinase C activity in Purkinje cells is also linked to SCA-14, a hereditary cerebellar disease with cerebellar dysfunction and Purkinje cell degeneration. SCA-14 is a particular interesting model because its phenotype and clinical settings are indistinguishable from other types of SCA which are caused by polyglutamine expansions. SCA-14, in contrast, is caused by point mutations in the coding region of the PKCgamma gene. The mechanisms by which the mutations in the PKCgamma gene lead to Purkinje cell degeneration in SCA-14 are still rather unclear. We have generated a transgenic mouse model in which a mutated form of protein kinase C gamma is expressed specifically in Purkinje cells. In the human disease this mutation causes Purkinje cell dysfunction, degeneration and death. We could show that the presence of the mutated protein in mouse Purkin-

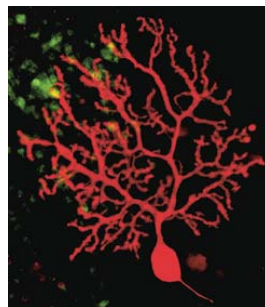


Fig. 1: View of a Purkinje cell in an organotypic slice culture after 12d *in vitro*. Anti-calbindin staining for Purkinje cells is shown in red. The elaborate dendritic tree of this cell has developed almost entirely during the culture period.

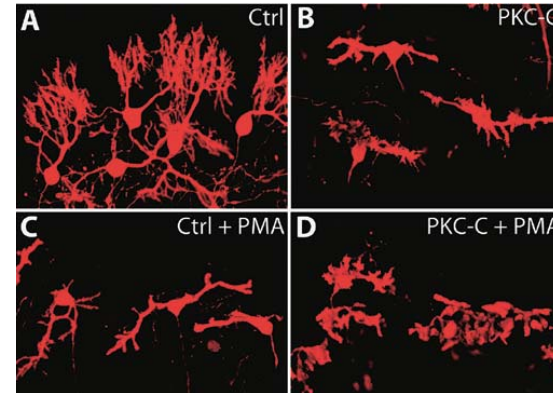


Fig. 2: The Purkinje cell dendritic tree in slice cultures derived from mice with transgenic expression of a mutated PKCgamma from SCA14 (B) is severely compromised compared to control (A) and resembles strongly that of Purkinje cell with pharmacological activation of PKCgamma (C). Further pharmacological activation of PKCgamma in mutant Purkinje cells results in only a minor additional reduction of the dendritic tree (D). Modified from Ji *et al.* 2014.

je cells induces a striking reduction of Purkinje cell dendritic outgrowth in organotypic slice cultures indicating that the mutated protein in the Purkinje cells acts like a constitutive active kinase and is biologically active in the Purkinje cells (Fig. 2). Due to the Purkinje cell-specific expression of the transgene we have taken advantage of this mouse model for identifying molecules signalling downstream of increased PKCgamma activity. Using a gene chip array approach we have identified several potential candidate molecules with increased mRNA expression in Purkinje cells. Out of those we have further studied carbonic anhydrase 8 (CA8) and have identified it as a potential mediator of PKCgamma activity and Purkinje cell dendritic development (Fig. 3). Our studies have provided a better understand Purkinje cell dysfunction and may be helpful for developing novel therapeutic strategies for cerebellar diseases.

Neuronal survival is also dependent on an intact blood supply and an intact blood brain barrier. Our group has analysed neurovascular interactions in a slice culture setting. This has allowed us to challenge neurons in organotypic slice cultures by ischemia or excitotoxic compounds and study neuronal survival and the blood vessel architecture simultaneously under these conditions. Our work so far has revealed complex interactions between neuronal survival and maintenance of blood vessels under ischemic conditions. This work has provided novel insights in the yet rather poorly explored neurovascular interactions.

Selected Publications

- Shimobayashi E, Wagner W, Kapfhammer JP. (2015) Carbonic Anhydrase 8 Expression in Purkinje Cells Is Controlled by PKC Activity and Regulates Purkinje Cell Dendritic Growth. *Mol. Neurobiol.* [Epub ahead of print], doi: 10.1007/s12035-015-9444-3
- Ji J, Hassler ML, Shimobayashi E, Paka N, Streit R, Kapfhammer JP. (2014) Increased protein kinase C gamma activity induces Purkinje cell pathology in a mouse model of spinocerebellar ataxia 14. *Neurobiol. Dis.* 70: 1–11, doi: 10.1016/j.nbd.2014.06.002
- Chip S, Zhu X, Kapfhammer JP. (2014) The Analysis of Neurovascular Remodeling in Entorhino-hippocampal Organotypic Slice Cultures. *J. Vis. Exp.*, e52023, doi: 10.3791/52023
- Sherkhane P, Kapfhammer JP. (2013) The plasma membrane Ca^{2+} -ATPase2 (PMCA2) is involved in the regulation of Purkinje cell dendritic growth in cerebellar organotypic slice cultures. *Neural Plast.* 2013: 321685
- Chip S, Nitsch C, Wellmann S, Kapfhammer JP. (2013) Subfield-specific neurovascular remodeling in the entorhino-hippocampal-organotypic slice culture as a response to oxygen-glucose deprivation and excitotoxic cell death. *J Cereb Blood Flow Metab.* 33:508–518

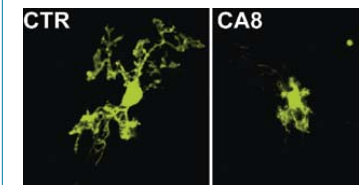


Fig. 3: For transfection studies Purkinje cells were grown in dissociated cultures. After transfection with a control GFP plasmid Purkinje cells develop a typical dendritic tree in such cultures (A). In contrast, after transfection with CA8, the dendritic tree is smaller and dendritic development is compromised (B) indicating that CA is involved in controlling Purkinje cell dendritic growth. Modified from Shimobayashi *et al.* 2015.