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Identification of MMP-2 as a novel enhancer of cerebellar granule cell proliferation

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ABSTRACT

During the first postnatal days in the mouse, granule cells (GCs) undergo massive proliferation, which then gradually decreases. Matrix metalloproteinase-2 (MMP-2), a Zn^{2+}-dependent proteolytic enzyme, is involved in a wide variety of pathological and physiological pathways. Evidence for a role of this proteinase in cell proliferation is emerging, reporting its involvement in pathological proliferation, as well as during neurogenesis and developmental proliferation of non-CNS tissues. In this study, MMP-2 protein expression was observed within the early postnatal cerebellar cortex, predominantly in Purkinje cells and within the GC proliferative zone, i.e. the superficial external granular layer (EGL). Consistently, the spatiotemporal MMP-2 mRNA and protein profiles highly correlated with the peak of GC precursor (GCP) proliferation and detailed morphometric analyses of MMP-2 deficient cerebella revealed a thinner EGL due to a decreased GCP proliferation. BrdU cumulative experiments, performed to measure the length of different cell cycle phases, further disclosed a transiently prolonged S-phase in MMP-2 deficient GCPs during early cerebellar development. In consequence, MMP-2 deficient animals displayed a transient delay in GC migration towards the IGL. In conclusion, our findings provide important evidence for a role for MMP-2 in neuronal proliferation and cell cycle kinetics in the developing CNS.

Introduction

The well-known and rather simple cytoarchitecture of the mammalian cerebellum has turned it into a widely used model system to study developmental processes. During development, a highly foliated cerebellar cortex is formed via stereotyped neuronal patterning of distinct subsets of neuronal cell types, among which granule cells (GCs) are the most prevalent. Embryonically, the ventricular zone along the fourth ventricle gives rise to Bergmann glial fibers, Purkinje cells (PCs), neurons of the deep cerebellar nuclei and a subset of interneurons mediating output from the PCs, while GCs originate from the rhombic lip. In mice, GC precursors (GCPs) start to proliferate at embryonic day 10 (E10), and around E13, they migrate tangentially to form the external granular layer (EGL). In the superficial EGL, GCPs undergo massive proliferation during the first postnatal days until approximately postnatal day 15 (P15), when the EGL gradually disappears. This process coincides with a gradual decrease in GCP proliferation rate and thus an increase in cell cycle duration, which can range from 15 h to 29 h (Espinosa and Luo, 2008; Schilling et al., 2008). Proliferation of GCPs depends on different mitogens, of which Sonic hedgehog (Shh) seems the most potent one (Behesti and Marino, 2009; Vaillant and Monard, 2009; Wechsler-Reya and Scott, 1999). Shh is secreted from PCs and distributed along the molecular layer (ML) and EGL. In the superficial EGL, Shh binds to the GCP cell membrane via local extracellular matrix (ECM) cues and subsequently activates and represses different cell cycle regulators. In the deep EGL, this Shh activity is counteracted by ECM molecules, such as vitronectin, thereby resulting in GCP cell-cycle exit (Pons et al., 2001; Vaillant and Monard, 2009). Postmitotic GCs then start to migrate tangentially along previously formed GC axons or parallel fibers until reaching the border of the ML. Hereafter, they migrate radially towards the internal granular layer (IGL) along Bergmann glial fibers, where they receive input from mossy fibers and make the appropriate synaptic connections with PC dendrites (Roussel and Hatten, 2011).

Matrix metalloproteinases (MMPs) have been characterized as important regulators of ECM turnover by proteolytic degradation of matrix components (Klein and Bischoff, 2011; Woessner, 1991). However, they also contribute to non-ECM substrate cleavage of, for instance, cell surface receptors and growth factors, thereby influencing important signaling pathways and biological functions (Sternlicht and Werb, 2001). Moreover, they are also able to target intracellular and intranuclear proteins, e.g. transcription factors, relating them to apoptotic or proliferative events (Cauwe and Opdenakker, 2010).

Together with MMP-9, MMP-2 (gelatinase-A, 72-kDa type IV collagenase) belongs to the gelatinase subfamily (for a review of all

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MMP families see (Jackson et al., 2010)), and is often implicated in detrimental processes like tumor metastasis (Brooks et al., 1996; Itoh et al., 1997; Stetler-Stevenson, 1999), blood-brain-barrier breakdown and demyelination (Gijbels et al., 1992; Matsumura et al., 2005). Conversely, MMP-2 has also been attributed a role in several physiological and developmental processes, e.g. embryonic neovascularization, skeletal development and epithelial growth (Alexander et al., 1997; Bruni-Cardoso et al., 2010; Duong and Erickson, 2004; Mosig et al., 2007), and its involvement in central nervous system (CNS) development, plasticity and repair is emerging (Verslegers et al., 2013). Besides, based on its expression and activity pattern, MMP-2 is also assumed to contribute specifically to cerebellar development in rodents (Ayoub et al., 2005; Vaillant et al., 1999). MMP-2 deficient (MMP-$2^{-/-}$) mice have often been used (Asahi et al., 2001; Berglin et al., 2003; Itoh et al., 1998; Ohno-Matsui et al., 2003) to study MMP-2 functionality, but studies in the CNS and during brain development in these mice are limited (Mizoguchi et al., 2010; Mizoguchi et al., 2011).

Here, we report a functional implication of MMP-2 in neuronal precursor proliferation during CNS development. Detailed morphological analyses in MMP-$2^{-/-}$ pups revealed a transiently reduced proliferation rate of GCPs due to S-phase lengthening during early postnatal cerebellar development, thereby leading to a decreased and/or delayed GC production. Consequently, the arrival of differentiated GCs in the IGL was transiently delayed in these MMP-$2^{-/-}$ animals.

Results

MMP-2 expression and distribution correlates with the peak of GCP proliferation

Real-time PCR experiments revealed a high cerebellar MMP-2 mRNA expression during the first postnatal week, which peaks around P3, steeply decreases towards P8 and then progressively decreases until P21 (Fig. 1A). Immunostainings for MMP-2 at different postnatal ages revealed a diffuse and weak MMP-2 immunoreactivity at P0 (Fig. 1B), which became more restricted thereafter. A prominent MMP-2 protein expression was found within the superficial EGL during early postnatal development (P3–P12) (Figs. 1C–F, white arrowheads), within the Purkinje cell layer (PCL) at P3 (Fig. 1C) and in PC somata and proximal dendrites from P6 on (Figs. 1D–G, arrows). In the young adult cerebellum (P30), MMP-2 protein distribution became more restricted to PCs and was also sporadically observed in the cytoplasm of interneurons in the ML (Fig. 1G, red arrowheads). Additional double stainings for MMP-2 and the neuronal marker NeuN at P3, clearly confirmed a prominent MMP-2 expression in the ECM surrounding GCPs in the superficial proliferative EGL (Fig. 1H). To investigate production of MMP-2 by GCPs, gelatin-based zymography was performed on conditioned medium of cultured primary immature GCs harvested at P3, when most of the EGL consists of proliferating GCPs,

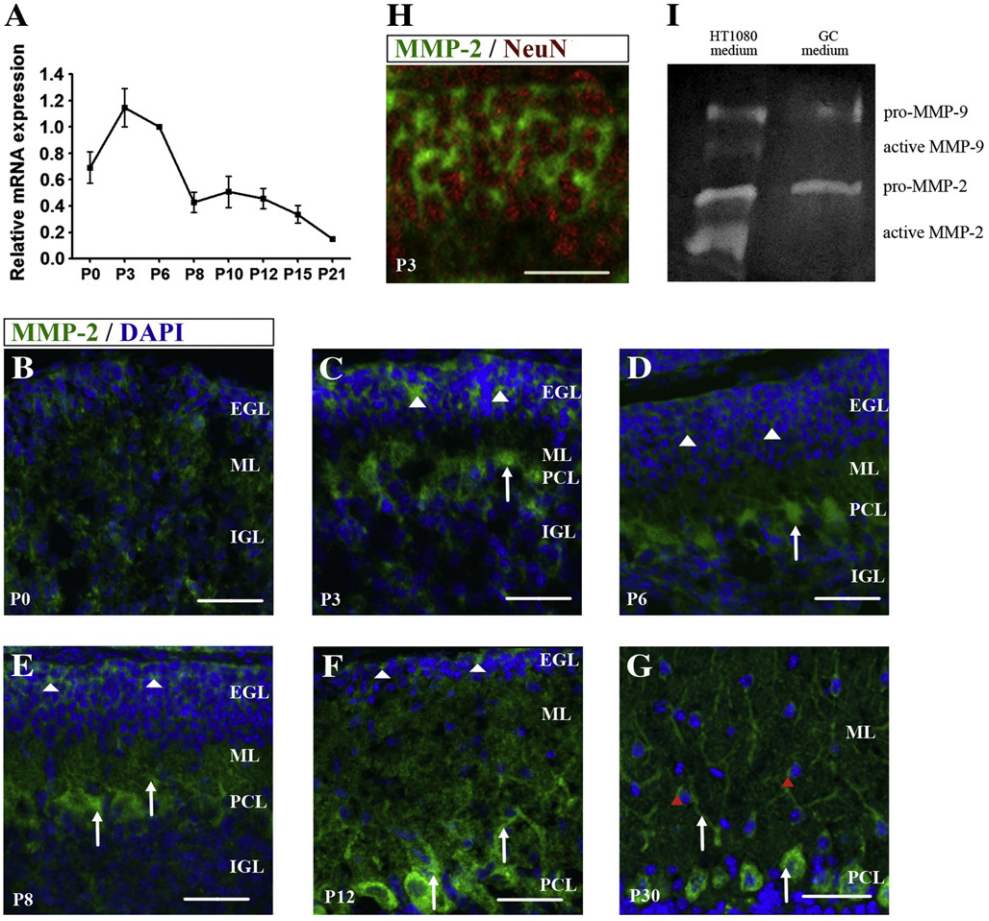

Fig. 1. MMP-2 spatiotemporal expression and activity pattern in the cerebellar cortex of WT pups. (A) Quantitative real-time PCR on cerebellar extracts revealed a maximal MMP-2 mRNA expression at P3, which rapidly decreased thereafter. (B–G) Immunohistochemical stainings for MMP-2, performed on WT sagittal vermal cerebellar sections at various developmental ages, showed an evenly distributed MMP-2 immunoreactivity in the cerebellar cortex and white matter at P0 (B). MMP-2 becomes predominantly expressed in the matrix of the EGL from P3 onwards (arrowheads C–F). Furthermore, MMP-2 signal is strongly detected in the PCL at P3, and also in PC somata and primary dendrites from P6 on (arrows, C–G). In the young adult cerebellum (P30), MMP-2 is also present in some scattered ML interneurons (G, red arrowhead). (H) A higher magnification of a double staining for NeuN (red) and MMP-2 (green) at P3 showed high MMP-2 protein expression in the ECM surrounding the GCPs in the EGL. (I) Gelatin zymography revealed a clear band at approximately 72 kDa, corresponding to pro-MMP-2, in serum-free culture medium of primary GCs at P3. Serum-free medium of stimulated HT1080 cells, used as a positive control, showed pro- and active MMP-2. EGL = external granular layer, ML = molecular layer, PCL = Purkinje cell layer, IGL = internal granular layer, P = postnatal day. Scale bar: 50 μm in panels A–G, I and 25 μm in panel H. Data are represented as mean ± s.e.m., n ≥ 3.