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In Vivo Mn-Enhanced MRI for Early Tumor Detection and Growth Rate Analysis in a Mouse Medulloblastoma Model[1,2]

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Abstract

Mouse models have increased our understanding of the pathogenesis of medulloblastoma (MB), the most common malignant pediatric brain tumor that often forms in the cerebellum. A major goal of ongoing research is to better understand the early stages of tumorigenesis and to establish the genetic and environmental changes that underlie MB initiation and growth. However, studies of MB progression in mouse models are difficult due to the heterogeneity of tumor onset times and growth patterns and the lack of clinical symptoms at early stages. Magnetic resonance imaging (MRI) is critical for noninvasive, longitudinal, three-dimensional (3D) brain tumor imaging in the clinic but is limited in resolution and sensitivity for imaging early MBs in mice. In this study, high-resolution (100 μm in 2 hours) and high-throughput (150 μm in 15 minutes) manganese-enhanced MRI (MEMRI) protocols were optimized for early detection and monitoring of MBs in a *Patched-1* (*Ptch1*) conditional knockout (CKO) model. The high tissue contrast obtained with MEMRI revealed detailed cerebellar morphology and enabled detection of MBs over a wide range of stages including pretumoral lesions as early as 2 to 3 weeks postnatal with volumes close to 0.1 mm^3. Furthermore, longitudinal MEMRI allowed noninvasive monitoring of tumors and demonstrated that lesions within and between individuals have different tumorigenic potentials. 3D volumetric studies allowed quantitative analysis of MB tumor morphology and growth rates in individual *Ptch1*-CKO mice. These results show that MEMRI provides a powerful method for early *in vivo* detection and longitudinal imaging of MB progression in the mouse brain.

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Abbreviations: 2D, two-dimensional; 3D, three-dimensional; Cb, cerebellum; CKO, conditional knockout; GCP, granule cell precursor; IHC, immunohistochemistry; MB, medulloblastoma; MEMRI, Mn-enhanced MRI; Mn, manganese; MRI, magnetic resonance imaging; *Ptch1*, *Patched-1*; *SHH*, *Sonic Hedgehog*; T1w, T1-weighted; T2w, T2-weighted; Td, tumor doubling time (in weeks)

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Introduction

Pediatric central nervous system tumors are the leading cause of cancer-related mortality in childhood. In this age group, medulloblastoma (MB) is the most frequent malignant brain tumor accounting for approximately 20% of all cases. MB is an embryonal tumor found in the cerebellum (Cb) that typically appears between the ages of three and nine but is also found in infants and adults [1,2]. With advances in aggressive treatment strategies combining surgery, cranio-spinal radiotherapy (in children ≥3 years of age), and chemotherapy, overall survival rates for patients with MB approaches 70% to 80% [3–5]. In spite of this progress in patient outcome, clinical management of MB still remains a challenge since patients who do survive often suffer devastating effects of the multimodal therapy, such as major long-term neurocognitive and neuroendocrine sequelae and a significant risk of developing secondary tumors [6–9]. For this reason, characterization of MB pathogenesis is the focus of intense research in neuro-oncology, with the hope that a greater understanding of the biologic pathways disrupted in this disease will lead to the development of novel and less harmful therapies specifically targeted to the abnormal molecular signatures of this developmental cerebellar tumor.

Significant progress has been made in our understanding of the pathogenesis of MB as a result of genome-wide profiling of human MB samples [10–12]. There is now clear evidence that MB is not a single tumor entity but that it comprises at least four subgroups, including those related to mutations in the Wingless and Sonic Hedgehog (SHH) signaling pathways, each associated with distinct gene expression signatures, transcriptomes, histopathologic phenotypes, and prognoses [13,14]. Furthermore, mouse models have been reported relevant to all four known MB subgroups [3,15–20]. From both the clinical and preclinical studies, consensus is forming that identification of the subgroup status and further subclassification [21] of these subgroups will enable treatment strategies tailored to individual tumors, which should translate into improved patient outcomes.

Despite this progress, detailed understanding of the differences between these MB subgroups and the degree of heterogeneity that exists within and between subgroups is still unclear. For example, the SHH-MB subgroup, consisting of approximately 30% of human MBs, is one of the most studied subtypes and has been recapitulated in several genetic mouse models [3,15] and was recently subdivided into three human subtypes [21]. SHH is an essential pathway that normally regulates the proliferation of one of the major cell populations within the developing Cb, the granule cell precursors (GCPs) [22,23]. Several studies have demonstrated a connection between increased SHH signaling and MB tumorigenesis, most notably due to loss-of-function mutations in *Patched-1* (*Ptch1*) [24–26] and activating mutations in *Smoothened* [27,28], the two key receptors of the pathway. Likewise, it has been substantially shown that GCPs are susceptible to malignant transformation and subsequent MB formation through oncogenic activation of the SHH pathway, revealing GCPs as the primary cell of origin of SHH-MBs [29,30]. Interestingly, there is emerging clinical evidence of complex molecular heterogeneity within MBs, including within this well-characterized SHH subgroup [21,31–35], which could indicate distinct cellular etiologies, specific altered signaling pathways, or differences in the timing and location of the cells of origin and genetic mutations driving tumor formation and progression. Therefore, it seems likely that additional uncharacterized MB subgroupings could be present and that additional large human and preclinical studies are needed to further dissect their biologic basis and degree of clinical relevance.

Given the importance of mouse models in this research, there is a clear need for efficient, high-throughput methods for analyzing MB phenotypes. Analysis of current mouse MB models has been mostly limited to static, two-dimensional (2D) information acquired with traditional histologic methods. Complementing these data, *in vivo* imaging modalities should provide a powerful approach for noninvasive tumor detection in mouse models, which are often limited by incomplete penetrance. Additionally, sensitive imaging techniques could allow the characterization and monitoring of tumor progression and the volumetric quantification of changes in response to novel therapies. In particular, detection and analysis of the *early* stages of tumorigenesis is especially important for brain tumors like MB, given that early stages of disease are asymptomatic and advanced-stage tumors may not accurately reflect the most relevant initiating or driver mutations for tumor progression.

Due to its excellent tissue contrast and high penetration and resolution, clinical magnetic resonance imaging (MRI) has played a major role in the diagnosis and management of human brain tumors. However, there are certain challenges for the use of MRI in preclinical brain tumor research. For instance, the utility of *in vivo* MRI in mouse tumor systems relies on the development of imaging protocols for high-throughput screening in models that traditionally have been limited by incomplete penetrance, as well as high-resolution *in vivo* analysis even at the early tumor stages. In addition, the establishment of MRI protocols for longitudinal imaging over extended periods of time is crucial for dynamic qualitative and quantitative analysis of tumor progression, which should provide a better stratification of experimental cohorts based on tumor phenotype and behavior. Previous studies have used conventional MRI techniques for morphologic brain tumor imaging in mouse MB models, but similar to most human studies [36–39], mouse MB MRI reports have largely focused on detection and monitoring of tumor burden at relatively advanced tumor stages. For example, T2-weighted (T2w) MRI has been used to define the location and extent of advanced MBs in mouse models [40–42], while gadolinium-enhanced T1-weighted (T1w) imaging has been used to evaluate breakdown of the blood-brain barrier (BBB) and leakage of the contrast agent into MB tumors [40]. Because gadolinium enhancement depends on tumor type and stage and is often observed at later stages of tumor progression, gadolinium-enhanced imaging has not been used extensively for early tumor detection in the clinic, especially for MB.

The overall goal of the current study was to implement an *in vivo* MRI protocol for early detection and characterization of tumor progression in a mouse model of SHH-MB. Manganese (Mn)–enhanced MRI (MEMRI) has been used extensively for both anatomic and functional neuroimaging studies in mice. MEMRI relies on the properties of paramagnetic Mn^{2+} ions to produce MR contrast. Besides multiple roles in normal physiology, Mn^{2+} can function as a calcium (Ca^{2+}) analog, entering neurons through voltage-gated Ca^{2+} channels and resulting in positive enhancement on T1w MR images of the tissues where it accumulates. Once in neurons, Mn^{2+} is also transported along axons and can cross synapses to accumulate in adjacent neurons [43–48]. We have previously demonstrated the utility of MEMRI for detailed analysis of brain and Cb development in normal and mutant mice, from embryonic to adult stages [49–54]. Given the increased signal and high degree of anatomic definition in the Cb obtained with MEMRI [53], we reasoned that this would be a good potential method for analyzing abnormalities in cerebellar anatomy caused by the presence of MB lesions.

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