MRI CHARACTERIZATION OF A NOVEL MOUSE MODEL OF SPORADIC MEDULLOBLASTOMA

Giselle Alexandra Suero-Abreu[1,2], G. Praveen Raju[3], Diane Pham[3], Luis Barraza[4], Kamila U. Szulc[1,2], Edward J. Houston[2], Alexandra Joyner[4], and Daniel H. Turnbull[1,2]

[1]Biomedical Imaging Department, NYU School of Medicine, New York, NY, United States, [2]Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, NY, United States, [3]Department of Pediatrics, Weill Cornell Medical College, New York, NY, United States, [4]Developmental Biology Program, Memorial Sloan Kettering Institute, New York, NY, United States

Introduction: Significant progress has been made in our understanding of the pathogenesis of brain tumors partly due to the development of genetically engineered mouse models that recapitulate the human disease. MRI has been used previously in preclinical brain tumor studies to non-invasively screen for tumor presence, characterize tumor progression and to monitor treatment response [1]. However, one of the greatest translational applications of such preclinical models is the ability to investigate tumor etiology and to draw insights on the molecular pathways altered in these cancers [2,3]. Since studies performed in advanced-stage tumors may not accurately reflect the genetic alterations critical for tumorigenesis, there is a clear need for more sensitive imaging protocols that allow the analysis of the early stages of tumor development. In this study we optimized an *in vivo* high resolution Mn-enhanced MRI (MEMRI) protocol for the characterization of different stages of tumorigenesis in a novel mouse model of sporadic medulloblastoma (MB), the most common malignant pediatric brain tumor originating in the cerebellum (Cb).

Methods:

Animal Model: One of the most relevant mouse models of *Shh*-induced MB is the *Patched (Ptc1)* mutant mouse [4]. In our study we generated a new variant of this model (referred as *Ptc1*-CKO) by combining $Ptf1a^{cre/+}$ mice with mice homozygous for a floxed allele of the *Patched* gene ($Ptc1^{fl/fl}$). Using this approach, *Ptc1* was deleted in *very few Ptf1a*-expressing granule cell progenitors (GCPs) compared to the extensive mutation of *numerous* GCPs seen in other models [5]. Based on our imaging, tumors in *Ptc1*-CKO mice likely initiate from a single mutated granule cell progenitor and thus better reflect the likely clonal origin of sporadic human MBs. ***Imaging:*** MRI experiments were performed 24h after intraperitoneal (IP) injection of $MnCl_2$ in isotonic saline (0.4-0.6 mM/kg) using a 7T Bruker Biospec system [4]. A 25-mm (ID) quadrature Litz coil (Doty) was used to acquire 3D T1-weighted gradient echo images. The sequence parameters were: TE/TR=3.6/50 ms, flip angle=40°, matrix=256^3, time ~2h for high resolution; and TE/TR=4/15 ms, FA=18°, matrix=128^3, time ~15min for high throughput imaging. Image analysis including registration, segmentation and 3D rendering were performed using AMIRA software (Visage Imaging). After imaging, mice were cardio-perfused and tumors extracted for histological (H&E) and immunohistochemical analysis using *Ki67* and *NeuN* staining as markers of proliferation and neuronal differentiation, respectively.

Results: The contrast enhancement obtained with MEMRI showed detailed Cb morphology and successfully allowed detection of MB tumors in *Ptc1*-CKO mice (Fig 1). Our imaging protocol had sufficient sensitivity to detect pre-neoplastic lesions as early as postnatal day (P) 14, as validated with histology (Fig 2). *In vivo* longitudinal imaging allowed the noninvasive assessment of tumor progression, including tumor volume and growth rate, as well as following the fates of individual early pre-neoplastic lesions (Fig 1). Advanced MBs in *Ptc1*-CKO mice showed two distinct morphological and immunohistochemical (molecular) patterns (Fig 3). In ongoing experiments, MRI images are providing spatial information for the dissection of tumor tissue for further gene expression analysis using DNA microarrays (data not shown).

Discussion: We used MEMRI to characterize a novel genetic mouse model of sporadic MB and showed that tumors display at least two distinct imaging and molecular phenotypes contrary to previous reports that all *Shh*-driven MBs converge to a common molecular endpoint [6]. Our *in vivo* MEMRI imaging protocol allowed the detection of early pre-neoplastic lesions and the analysis of advanced-stage MB tumors. Furthermore, longitudinal MEMRI studies showed that only one pre-neoplastic lesion develops into a tumor and that each pre-neoplastic lesion likely arises from a single mutant cell. This novel protocol should be useful for noninvasive volumetric analysis of individual growth rates and morphologic features in this and other mouse models. We also expect that applying our imaging approach to this unique preclinical MB model will help us better understand MB pathogenesis and will ultimately serve as a platform to test current and novel therapies.

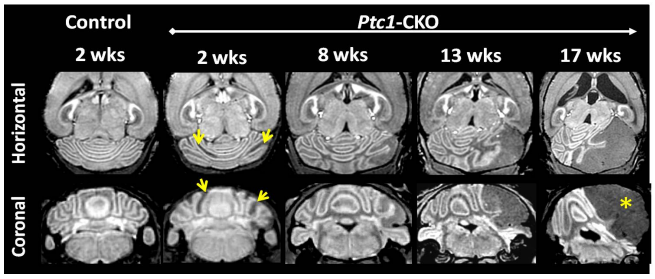

Fig.1 Longitudinal imaging showing detection of tumors in *Ptc1*-CKO mice compared to a control. *In vivo* monitoring of tumor progression showed that multiple lesions were observed at the early time points (arrows at 2 wks), however at a later time point (17 wks) only one lesion per mouse progressed to an advanced MB (*) while the additional early lesions regressed or had delayed progression.

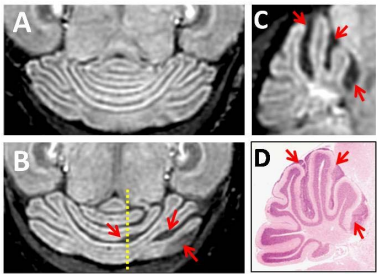

Fig.2 Horizontal images showing multiple tumor lesions (arrows) in the Cb of a *Ptc1*-CKO mouse (B) compared to control (A). Matching sagittal MRI (C) and H&E section (D) from the mouse in (B) at the Cb paravermis (yellow line) showed excellent correlation between areas of negative contrast enhancement and the early tumor.

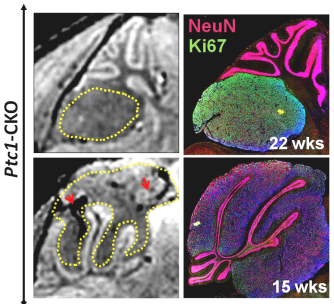

Fig.3 *Left:* MRI distinguishes advanced MBs as focal *(top)* and diffuse tumors (bottom) that could present heterogeneities in the tumor mass (arrows) *Right:* IHC experiments revealed distinct **undifferentiated NeuN-/Ki67+** (top) and **differentiated NeuN+/Ki67 low** *(bottom)* staining patterns in these two tumor subtypes.

References: [1] Fomchenko EI Holland, EC.. Clin Cancer Res. 2006;12;18:5288-97.[2] Wechsler-Reya R,et al. Annu Rev Neurosci.2001;24:385-428. [3] Gilbertson R.J & Ellison D.W. Annu. Rev. Pathol.2008; 3: 341-365 [4] Hoshino et al. Neuron. 2005;47(2):201-13. [5] Wadghiri YZ et al. NMR Biomed. 2004;17(8):613-9.[6] Schuller U et al.Cancer Cell. 2008;14(2):123-34. ***Acknowledgements:*** This work was supported by NIH grants R01NS038461 and RO1HL07866 to DT and a Geoffrey Beene Cancer Res Center grant 21680 and R01CA128158 to ALJ.