1H MRS Metabolite Profiles of Medulloblastomas in Transgenic SMO Mice

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INTRODUCTION

Medulloblastomas, that are believed to arise from granule-cell precursors (GCP), are the most frequently diagnosed pediatric brain tumours in children [1]. It has become obvious that genetic and molecular abnormalities underpinning human medulloblastomas are numerous [2]. One commonly observed genetic alteration concern the sonic hedgehog –dependent signaling involving approximately 30% of human medulloblastomas [3]. The sonic hedgehog (Shh)-Patched (Ptch) signaling pathway plays a crucial role in mitogenic regulation of GCP [4]. Recently, a genetically modified (GEM) mouse line has been generated overexpressing smoothened receptor (SMO) in GCPs with very high incidence of medulloblastomas after the age of 2 months [5]. *In vivo* [6, 7] and *ex vivo* [8] ^{1}H MRS studies point to high taurine concentration in human medulloblastomas. A recent high resolution magic angle spinning (HR-MAS) analysis of medulloblastoma specimens revealed that creatine, glutamine, phosphorylcholine (PC), glycine and *scyllo*-inositol are higher in these tumours than in other pediatric brain tumour originating from neuronal cell lines [8]. In the present study we have used SMO mice to characterize metabolite profiles both *in vivo* and *ex vivo* in the SMO model of medulloblastoma with known molecular pathology.

METHODS

The SMO mice were obtained from Fred Hutchinson Cancer Research Center (FHCRC, Seattle, WA). The SMO mice of 118±42 days (range 68 -180 days) from both genders were scanned for MRI and ^{1}H MRS. C57Bl/6 mice were used as age matched wild type (WT) controls. Animals were anaesthetized with 4% isofluorane in 40% O_2 (maintenance isofluorane 1-1.5% through nosecone), placed in a custom –built head holder with a bite bar and ear pins using a single turn surface coil for transmission and reception. All MR scans were performed in a 7T horizontal Varian Unity-Inova system. A fast spin echo sequence was used for T_2-weighted MRI (TR = 2,500 ms, 8 echoes, interecho delay 15 ms, effective TE = 60 ms, FOV 25 mm^2, 256x128 data matrix, slice thickness 0.75 mm). Single spin echo sequence with TR = 700 ms and TE = 9 ms (other acquisition parameters as above) was used to obtain T_1-weighted images. Single voxel ^{1}H MRS were acquired with the LASER sequence [9] with TR = 2,500 ms, TE = 27 ms, SW 3 kHz covered with 5k data points, voxel dimensions set according to the T_2-hyperintensity in cerebellum of SMO mice, in the WT mice the voxel (2x2x3 mm^3) was positioned to the basal cerebellum. Tissue specimens from SMO and WT mice for HR-MAS were frozen in liquid N_2 and stored at -70 ^{o}C until transferred into zirconia MAS rotors on dry ice. A 500 MHz Bruker Avance2+ system equipped with a ^{1}H HR MAS probe was used with rotor temperature 27^{o}C and the spinning rate at 5kHz, 1D NOESY preset for water suppression, SW 8 kHz covered with 32k data points. TARQUIN [10] was used for both *in vivo* and *ex vivo* spectral analyses. Metabolites are referenced to total creatine at 3.03 ppm.

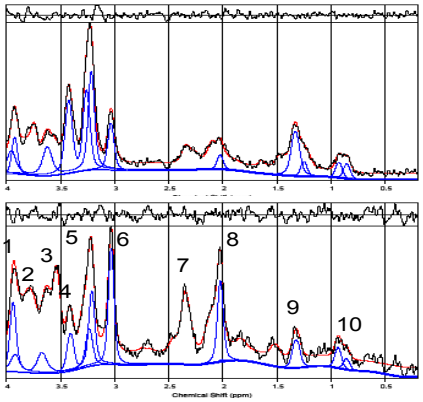

Fig 1. ^{1}H MR spectrum of cerebellar voxel from a SMO (**A**) and WT (**B**) mouse, echo time was 27ms. Peaks are assigned as follows: 1; Creatine+phosphocreatine, 2; glutamate+glutamine, 3; myo-inositol+glycine, 4; taurine, 5; choline-containing compounds+taurine, 6; creatine+phosphocreatine, 7; glutamate, 8; N-acetyl-asparate, 9; lactate+mobile lipids+macromolecules and 10; mobile lipids+macromolecules.

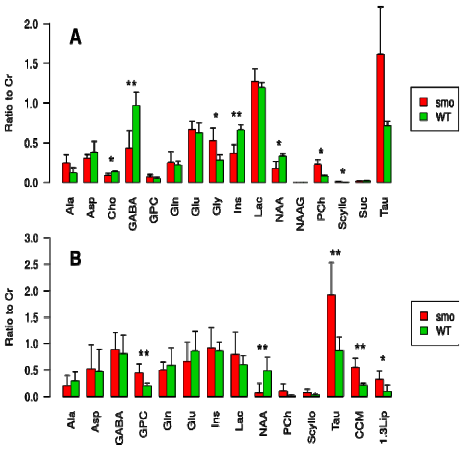

Fig. 2. Metabolile ratios referenced to total Creatine peak From *ex vivo* (A) and *in vivo* (B) MRS. Key: Ins = myo-inositol; Suc = succinate; CCM = GPC + PC; 1.3Lip = lipid peaks at 1.3 ppm

RESULTS

In a cohort of 13 SMO mice scanned three types of cerebellar appearances in T_2-weigthed MRI were seen as follows: (a) no obvious abnormality (n=5); (b) focal hyperintensity (n=2) and (c) widespread hyperintensity, large-sized ceberellum and absence of foliage (n=6). T_1 signal enhancement after Magnevist injection (0.2 mmlo/kg ip) was detected in T_2-hyperintense cerebellar tumours in the SMO mice. ^{1}H MRS was acquired from 8 animals with abnormality in T_2-MRI as sign of medulloblastoma. Typical spectra from a SMO medulloblastoma (Fig. 1A) and WT cerebellum (Fig. 1B) are shown. *In vivo* metabolite concentrations revealed that NAA is strongly decreased, whereas choline containing metabolites (CCM), taurine and 1.3 ppm lipid were increased in the SMO medulloblastomas (Fig. 2B). HR-MAS analyses of cerebellar specimens showed that in addition to the *in vivo* MRS changes glycine, phosphocholine and *scyllo*-inositol are elevated, whereas GABA and *myo*-inositol concentrations are decreased in the medulloblasoma tissue (Fig. 2A).

CONCLUSIONS

Medulloblastomas in the SMO mice show metabolite profiles which are characterized by very low NAA, low GABA and *myo*-inositol and high taurine, CCM, *scyllo*-inositol and glycine. Interestingly, a large body of these metabolic alterations is reported from human medulloblastomas with heterogeneous molecular pathology [8]. It appears that taurine, CCM (especially PC) and *scyllo*-inositol are potential common MRS biomarkers for medulloblastomas, whereas *myo*-inositol, GABA and glycine may be more associated with tumours with aberrant SMO signaling in GCPs.

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