In vivo ^{1}H MRS detection of choline compounds in pancreas of MEN1 knock-out mice

Min-Hui Cui[1,2], Ziqiang Yuan[3], Sean Cahill[4], Asha Adem[3], Steven K. Libutti[3], and Craig A. Branch[1,2]

[1]*Gruss Magnetic Resonance Research Center, Albert Einstein College of Medicine, Bronx, New York, United States,* [2]*Radiology, Albert Einstein College of Medicine, Bronx, New York, United States,* [3]*Surgery, Albert Einstein College of Medicine, Bronx, New York, United States,* [4]*Biochemistry, Albert Einstein College of Medicine, Bronx, New York, United States*

Introduction. Proton MR spectroscopy (^{1}H MRS) is a powerful noninvasive tool for biochemically characterizing normal and abnormal tissues in vivo. In vivo ^{1}H MRS has been applied to differentiate between benign and malignant lesions based on the quantitative measurement of the signal intensity detected from total choline-containing compounds (tCho) in brain cancers (1), breast cancers (2) and prostate cancers (3) etc. Recently, Su et al (4) demonstrated the feasibility of acquiring in vivo ^{1}H MRS data from the normal human pancreas. To assess the potential role of in vivo ^{1}H MRS in the diagnosis and treatment of pancreatic cancer, we performed in vivo ^{1}H MRS and ex vivo ^{1}H NMR in an endocrine neoplasia type 1 conditional knock-out (MEN1 KO) mouse model which develops pancreatic neuroendocrine tumors (PNETs). This mouse model leads to the selective development of a single insulinoma in the pancreata (5).

Methods. Animals: Eleven *Men1* KO mice (6F/5M. Age: 11.1 ± 2.7 m, 7 ~ 15 m. Weight: 28.7 ± 6.9 g, 18 ~ 39 g), and two WT littermates (2F, 9 and 15 month old, weight = 31.5 ± 0.7 g) were studied. In vivo MRI/MRS: In vivo MRI/MRS were performed on a 9.4 T Varian Direct Drive animal MRI/MRS system (Agilent Technologies, Inc. Santa Clara, CA) with a 35-mm ID quadrature ^{1}H volume coil (m2m Imaging Co., Cleveland, OH). Mouse was supinely placed in a plastic holder with extremities and tail taped. Anesthesia was achieved with isoflurane mixed with room air. An anesthesia adjusted breathing rate of ca. 50 per minute was found to be optimal. Surface body temperature was maintained at 34~35°C using warm air. MRI/MRS data acquisition was gated to the respiratory cycle. Multi-slice coronal scout images were acquired using a gradient-echo imaging sequence. Water-suppressed ^{1}H MRS data were acquired *via* a LASER sequence on a voxel placed in pancreas (TR = ca. 3 s, TE = 36 ms, 256 transients, complex points = 1024, SW = 4006 Hz). Shimming routinely resulted in unsuppressed water signal line width of 20 ~ 40 Hz. To provide an internal reference, non-suppressed water spectra (TR > 10 s, TE = 36 ms, nt =16) were also acquired from the same metabolic voxel. tCho signal intensity was represented relative to water signal from the same voxel after T1 correction of saturation factors. Ex vivo ^{1}H NMR: Homogenized pancreatic tissues from *Men1* KO and WT mice were extracted with 50:50 CH_3CN and water. After lyophilizing, the powder was dissolved in 550 μL D_2O and 5 μL 10-mM TSP stock. Standard ^{1}H NMR spectra of pancreatic tissue extracts were acquired on a Bruker DRX 600-MHz spectrometer with a 5-mm TXI probe. Choline integrals were normalized to TSP integral. All data are presented as mean ± SD.
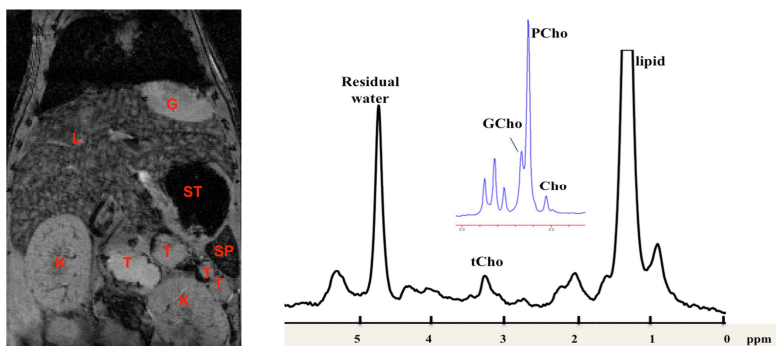

FIG.1 (Left) MRI image of a *Men1* KO mouse showing gallbladder (G), kidney (K), liver (L), stomach (ST), spleen (SP) and pancreatic tumors (T). (Right) In vivo ^{1}H MRS spectrum on pancreatic tumor of *Men1* KO mouse showing total choline (tCho) peak at 3.2 ppm. The insert, a high-resolution ^{1}H NMR spectrum of KO mouse pancreatic tumor extract, showing free choline (Cho), phosphocholine (PCho), and glycerophosphocholine (GPCho) peaks at 3.207 ppm, 3.226 ppm, and 3.234 ppm, respectively.

Results. Choline was observed in pancreas of all *Men*1 KO mice (Fig.1), but was not detectable in pancreas of WT littermates. The choline methyl proton peak at 3.2 ppm in in vivo ^{1}H MRS represents the total choline including free choline (Cho), phosphocholine (PCho), and glycerophosphocholine (GPCho), which can be differentiated in ex vivo ^{1}H NMR spectrum of pancreas extracts as shown in insert in Fig.1, but not in in vivo spectra. The total choline levels in pancreas of *Men*1 KO mice ranged from 6.7 to 54.1 as listed in Table 1. Ex vivo ^{1}H NMR data from pancreas extracts indicated that elevated choline levels in KO mice are predominantly due to increased PCho and GPCho levels as listed in Table 2.

Table 1. Total choline levels (tCho/H_2O) in pancreas of *Men1* KO and WT mice estimated *via* in vivo ^{1}H MRS.

	Sex	Age (m)	Weight (g)	tCho/H_2O ($\times 10^{-4}$)
KO	6F/5M	11.1 ± 2.7	28.7 ± 6.9	15.0 ± 14.4
WT	2F	12.0 ± 4.2	31.5 ± 0.7	Not detected

Discussion and Conclusion. In vivo ^{1}H MRS-visible choline-containing compounds may act as a long-term second-messenger system for cellular proliferation (1). Elevated total choline levels in the pancreas of *Men*1 KO mice in comparison to WT mice, especially increased phophocholine and glycerophophocholine levels (Table 2), may reflect accelerated phosphorylation of choline or synthesis of membrane phospholipid precursors during intensified tumor cell replication (1). To conclude, findings in our study demonstrate the feasibility of in vivo ^{1}H MRS estimation of choline in pancreas of the *Men*1 KO mouse model. This approach may allow assessment of tumor proliferation in follow-up studies of pancreatic tumor treatments.

Table 2. Relative choline metabolite integrals in ^{1}H NMR spectra of pancreatic tissue extracts from WT and *Men1* KO mice.

	Cho/TSP	PCho/TSP	GPCho/TSP	tCho/TSP
WT (n=5)	0.09 ± 0.03	0.52 ± 0.10	0.40 ± 0.11	1.01 ± 0.24
KO (n=6)	0.24 ± 0.06	1.66 ± 0.40	1.43 ± 0.19	3.09 ± 0.58
p values	0.10	0.04	< 0.01	0.02

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