SUPPLEMENTARY MATERIAL

PET Tracer ^{18}F-Fluciclovine Can Detect Histologically Proven Bone Metastatic Lesions: A Preclinical Study in Rat Osteolytic and Osteoblastic Bone Metastasis Models

Shuntaro Oka[1], Masaru Kanagawa[1], Yoshihiro Doi[1], David M. Schuster[2], Mark M. Goodman[2], Hirokatsu Yoshimura[1]

[1] Research Center, Nihon Medi-Physics Co., Ltd., Chiba, Japan

[2] Division of Nuclear Medicine and Molecular Imaging, Department of Radiology and Imaging Sciences, Emory University, Atlanta, GA, USA

MATERIALS AND METHODS

Gene expression analyses

RNA isolation from cultured cells

Total RNA was isolated from cultured AT6.1 and MRMT-1 cells using RNeasy Mini Kit (catalog number: 74104; QIAGEN, Tokyo, Japan) and QIAshredder (catalog number: 79656; QIAGEN), based on the manufacturer's protocol. The concentration of total RNA was calculated from the 260-nm absorbance, as measured by the NanoDrop 2000 spectrophotometer (ThermoFisher Scientific, Kanagawa, Japan).

Reverse transcription-polymerase chain reaction

One microgram of extracted total RNA was reverse-transcribed using Transcriptor First Strand cDNA Synthesis Kit (catalog number: 04379012001; Roche Diagnostics, Tokyo, Japan), based on the manufacturer's protocol. The primers (see Supplementary Table S1) were synthesized by Nihon Gene Research Laboratories. Reverse transcription-PCR (RT-PCR) was performed using Mx3000P QPCR system (Agilent Technologies Japan, Tokyo, Japan) and FastStart Universal SYBR Green Master (Rox) (catalog number: 04913922001; Roche Diagnostics) with the following thermal cycles: 1 cycle for enzyme activation (95°C, 10 min), and appropriate cycles (exponential phase of PCR for all genes) for the osteoblastic-related genes (Supplementary Table S1) or 24 cycles (exponential phase of PCR for ASCT1, ASCT2, LAT1, and 4F2hc) for amino acid transporter (AAT)-related genes for denaturing and extension (95°C for 15 seconds and then 60°C for 30 seconds). The PCR products and 20 bp DNA ladder (catalog number: 3409A/B; Takara Bio, Shiga, Japan) were electrophoresed at 100 V for 80 minutes on ice by 3% agarose gel containing Tris-borate-EDTA buffer (TBE buffer; 89 mM Tris, 89 mM borate, and 1 mM EDTA) using Mupid-2plus (Mupid, Tokyo, Japan). The gel was then incubated with 1 μg/mL ethidium bromide in TBE buffer for 30 minutes, after washing the gel twice with TBE buffer for 5 minutes. The bands of the PCR products were detected using Gel Doc EZ system (Bio-Rad, Tokyo, Japan).

Table S1. The primer sets, product sizes, and cycle numbers for the reverse transcription-PCR of the osteoblastic-related genes and the amino acid transporter-related genes.

Gene	Accession No.	Forward (5'-3')	Reverse (5′-3′)	Product size (bp)	Cycle number
Osteoblastic-related genes					
GAPDH	NM_017008.3	TGGGAAGCTGGTCATCAAC	GCATCACCCCATTTGATGTT	78	28
ALP	NM_013059.1	GCACAACATCAAGGACATCG	TCAGTTCTGTTCTTGGGGTACAT	72	41
BSP	NM_012587.2	AGCATGCCTACTTTTATCCTCCT	CTCCGAACTATCGCCATCTC	92	38
OC	NM_013414.1	CTACCTCAACAATGGACTTGGA	GAGCTCACACACCTCCCTGT	76	39
RUNX2	NM_053470.2	CCGTGTCAGCAAAACTTCTTT	CTCACGTCGCTCATCTTGC	95	38
Col1	NM_053304.1	ATGTTCAGCTTTGTGGACCTC	GCAGCTGACTTCAGGGATGT	93	32
Keratin	NM_053976.1	AAGCTTGAGGCGGAGATTG	TCTGGACAGTTTGCATGGAG	106	41
Amino acid transporter-related genes					
ASCT1	NM_198763.1	AACCAGTTACACAGTGGTCGTC	CAGTGACGACGGGGATCT	74	24
ASCT2	NM_175758.3	TTCCCCTCCAATCTGGTGT	CTCTGTGGACAGGCACCAC	65	24
LAT1	NM_017353.1	ATGTGGCTCCGCTTTAAGAA	GGAGGGCCAGATTCACCT	61	24
LAT2	NM_053442.1	TCCTATGTCAAGGACATCTTCG	CACCAGCACAGCAATCCATA	66	40
LAT3	NM_001107742.1	TCACATTTCCTGGAATCAAGC	CCAGACCACGTGAACATGA	73	38
LAT4	NM_001105812.1	GTCGGAGGGCTTTTACTCCT	CTCGACTGTGCCGTTAGTGA	64	30
4F2hc	NM_019283.1	CAGCTATGGGGATGAGCTTG	TCATTCCACAGCATGAATGG	81	24

ALP, alkaline phosphatase; ASCT, alanine-serine-cysteine transporter; BSP, bone sialoprotein (i.e., osteopontin); Col1: collagen-1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; Keratin, cytokeratin-18; LAT, L-type amino acid transporter; OC, osteocalcin; RUNX2, runt-related transcription factor 2.

RESULTS

Gene expression analyses

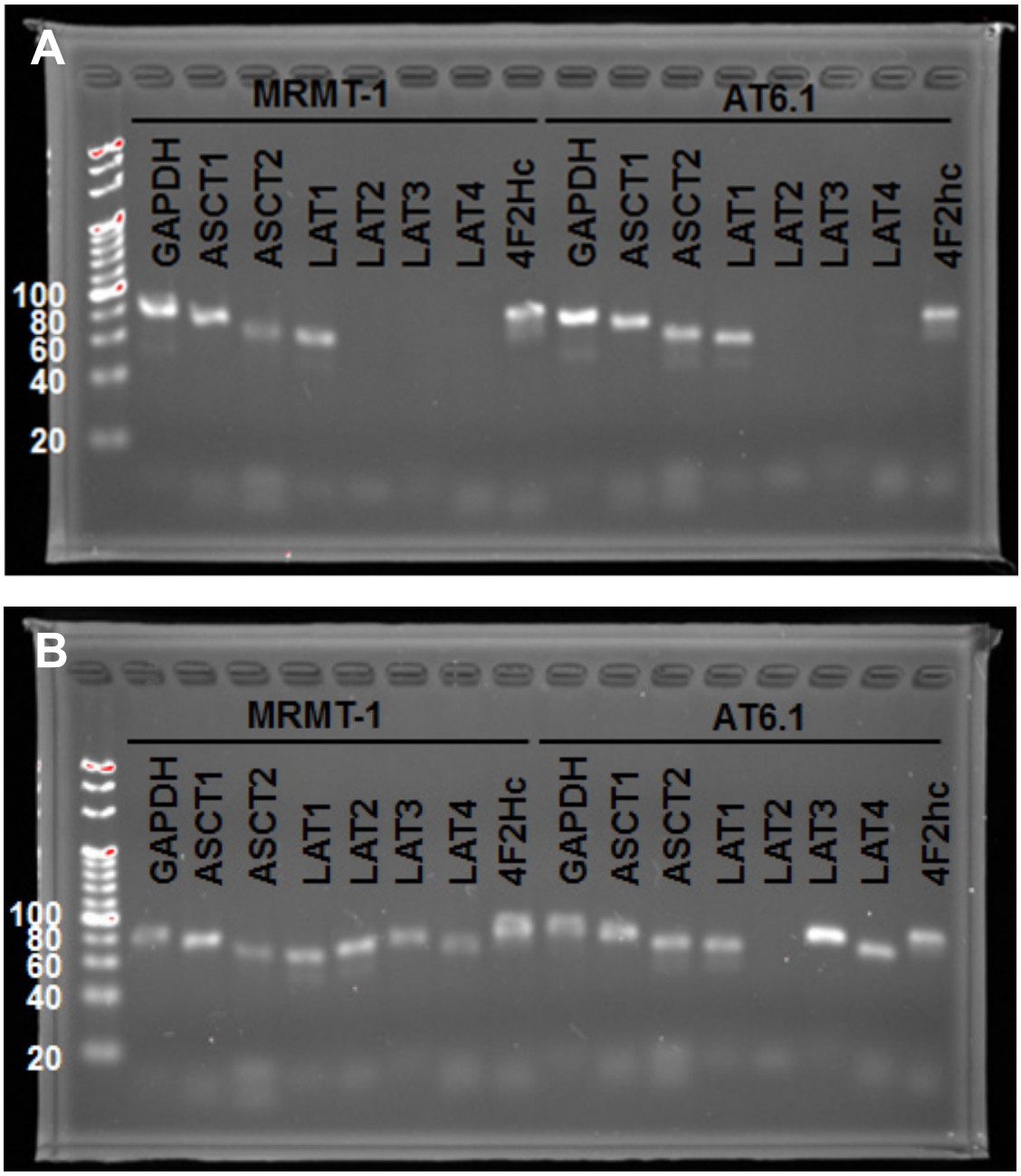

Figure S1. Electrophoresis of the RT-PCR products of AAT-related genes in MRMT-1 and AT6.1. (A) The MRMT-1 and AT6.1 cells expressed systems ASC (ASCT1, ASCT2) and L (LAT1, LAT2, LAT3, LAT4), and 4F2hc, which forms heterodimer with LAT1 and LAT2 in 24 cycles (exponential phase of PCR for these AAT genes) of a set of denaturing and extension in RT-PCR. (B) If the cycles were set for appropriate cycles for LAT2 (40 cycles), LAT3 (38 cycles), and LAT4 (30 cycles), then LAT2−4 in MRMT-1 and system L AAT, except LAT2 in AT6.1, were detected. ASCT, alanine-serine-cysteine transporter; 4F2hc, 4F2 heavy chain; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; LAT, L-type amino acid transporter; RT-PCR, reverse transcription polymerase chain reaction.

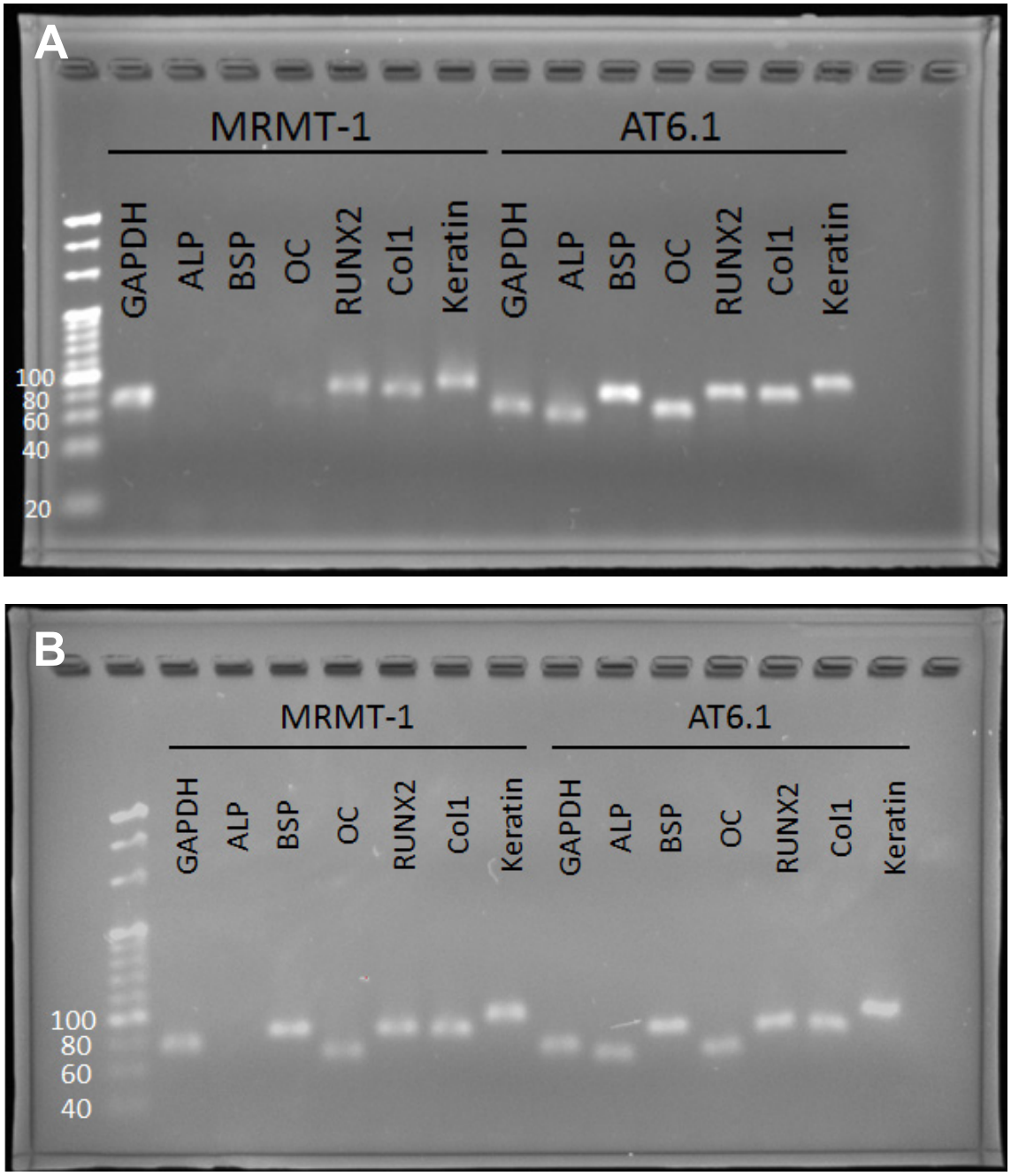

Figure S2 Electrophoresis of the RT-PCR products of osteoblastic-related genes in MRMT-1 and AT6.1. (A) The AT6.1 cells, but not MRMT-1 cells, expressed all osteoblastic-related genes we tested. (B) If the cycles were set for 50 cycles for all genes (i.e., saturation phase), BSP and OC, but not ALP, were detected in MRMT-1. ALP, alkaline phosphatase; BSP, bone sialoprotein (i.e., osteopontin); Col1, collagen type 1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; Keratin, cytokeratin-18; OC, osteocalcin; RUNX2, runt-related transcription factor 2.