SUPPLEMENTAL MATERIALS AND METHODS

DNA extraction

Blood samples (10 ml) were collected from each individual into the ethylenediaminetetraacetic acid tubes. The peripheral blood mononuclear cells (PBMC) were separated and genomic DNA extracted using the QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol.

Bioinformatical analysis and whole-genome exon sequencing

Genomic DNA was extracted from peripheral EDTA-anticoagu-lated blood samples by QIAamp DNA Blood Mini Kit (Qiagen). Exon libraries were prepared by KAPA HTP Library Preparation Kit and IDT Whole Exome and sequenced using paired-end, 150-cycle chemistry on the Illumina Sequencing platform (Illumina, San Diego, CA).The sequencing data were aligned to the reference human genome (GRCh37/hg19) using BWA and variant calls were generated using GATK tools. Each samples have the variants ranging from 271,229 to 328,220. Variants were then filtered based on family history (occur in all persons with disease but not in healthy individuals). The resulting variants (15,926) were further filtered for missense variants and predicted to be damaging or deleterious by PolyPhen-2 (>0.45) and SIFT (<0.05). Finally, we achieved 129 missense variants that predicted to be damaging or deleterious. Nonsense mutations were selected but not considered because only two genes (BX088651.1 and PABPC3) with nonsense mutations and they are unknown (BX088651.1) or rarely reported (PABPC3). We used DAVID Function Annotation to characterize these variant genes, and observed DMBT1 is belonged to OMIM_disease category related to Medulloblastoma.

Sanger sequencing

The identified missense variants (such as the DMBT1 mutation) were validated using Sanger sequencing for all subjects on an ABI3730 (Applied Biosystems, Foster city, CA, USA) using a standard Sanger sequencing protocol. The PCR primers used for amplification of DMBT1 coding sequence were 5 -CCCAATACTTGAGCTTCCA-3 and 5 -ACACCAGCGTCTTCGTGAT-3.

DMBT1 siRNA and transient transfection

To knockdown DMBT1 expression in thyroid cancer cell lines, we obtained separate plasmids containing two DMBT1 siRNAs and a negative control siRNA from Biotend (Shanghai, China) and then transiently transfected these constructs into thyroid cancer cells using Lipofectamine 3000 (Invitrogen, Shanghai, China) according to the manufacturers' instructions. In brief, cells were grown in 6-wells plates and transiently transfected with 50 nmol/L of siRNA for 48 h and efficiency of DMBT1 knockdown was verified by using qRT-PCR and Western blot.

Construction of plasmids carrying wild type and mutant DMBT1 cDNA and gene transfection

Plasmids carrying wild type and mutant DMBT1 cDNA were constructed by using PCR, gene cloning, amplification, and DNA-sequencing with standard gene cloning technologies. Specifically, we first amplified three DMBT1 cDNA fragments (1a, 1703 bp; 1b, 1413 bp; and 2, 2242 bp) using PCR and the primer sequences were

1a, 5 -AGAAGATTCTAGAGCTAGCGATGGGCATTTCCACAGTGAT-3 and 5 -AGGACAGCTCCACAGATAGCTCTCGTGGCCGGAACATCT-3; 1b, 5 -AGATGTTCCGGCCACGAGAGCTATCTGTGGAGCTGTCCT-3 and 5 -CCTATACAGCACCTCCACT-3; and 2, 5 -AGTGGAGGTGCTGTATAGGGGCAGCTGGGGAACCGTGTGCGACGACAG CTGGGACACCAATGATG-3 and 5 -GCAGATCCTTCGCGGCCGCGCTACCGAGGCTCCTCTTCTC-3. We then inserted them into a pCDH3.1 vector to obtain pCDH3.1-DMBT1 carrying the recombinant human DMBT1 cDNA by using the pEASY-Uni Seamless Cloning and Assembly Kit (Transgen Biotech, Shanghai, China) according to the manufacturer's protocol Furthermore, we also performed a site directed mutagenesis to obtainmutant*DMBT1* D1657E using the Mut Express II Fast Mutagenesis Kit V2 (Vazyme, Nanjing, China). The primers used for site-directed mutagenesis were 5 -TGTGCGACGAAAGCTGGGACACCAATGATGCCAATGTGGTCT-3 and 5 -TGTCCCAGCTTTCGTCGCACACGGTTCCCCAGCTGCCCCTA-3.

After that, cells growing in 6-wells plates were transfected with 2 μg of wild type or mutant plasmid DNA using Lipofectamine 3000 (Invitrogen) for 48 h and then assayed for DMBT1 expression using qRT-PCR and Western blot.

Cell lines and culture

Human medullary thyroid cancer cell line TT, human anaplastic thyroid cancer cell line Cal62, and human squamous thyroid cancer cell line SW579 were obtained from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences

(Shanghai, China). Human papillary thyroid cancer cell line TPC-1 and human follicular thyroid cancer cell line FTC133 were obtained from The Cell Bank of University of Colorado Cancer Center (Denver, Colorado, USA). Cal62 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (10,000 units penicillin, and 10 μg streptomycin per mL in 0.9% NaCl). SW579, TPC-1, TT, and FTC133 were grown in L-15, RPMI-1640, F12K, DMEM/F12 supplemented with 10% FBS, 1% penicillin-streptomycin in a humidified incubator with 5% CO_2 at 37°C.

RNA isolation and qRT-PCR

Total RNA was isolated using Trizol reagent (Invitrogen) and 1 μg each of these total RNA samples was reversely transcribed into cDNA using the PrimeScript^{TM} RT reagent kit (Takara, Dalian, China). Amplicons were then amplified in triplicate using the SYBR Premix Ex Taq^{TM} kit (Takara) according to the manufacturer's instructions. The primer used for DMBT1 were 5'-GCAGTTCCCCTCTCATTGCT-3' and 5'-AGCAGGTTGGTGCTGTCATT-3' and β-actin primers were 5'-TGACGTGGACATCCGCAAAG-3' and 5'-CTGGAAGGTGGACAGCGAGG-3'. The house keeping gene β-actin was used as internal control. The threshold cycle (Ct) values were analyzed using the comparative Ct (2-ΔΔCt) method to calculate relative level of DMBT1 mRNA.

Western blot

Total cellular protein was extracted from 1 × 10^6 cultured cells using the ProteoJET Mammalian Cell Lysis Reagent (Fermentas Inc., Thermo, Waltham, MA, USA) containing phenylmethanesulfonyl fluoride (Roche, Indianapolis, IN, USA); and PhosSTOP (Roche). After quantitation, 20 μg of each protein sample was separated in 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred on to polyvinylidene fluoride membranes (PVDF; Millipore, Billerica, MA, USA). After blocking in 5% nonfat milk solution in phosphate buffered saline (PBS)-Tween 20 (PBS-T), the membranes were incubated with an antibody against human DMBT1 (1:500; Abcam, Cambridge, MA, USA) or GAPDH (1:5000; Abcam) and then subsequently incubated with goat anti-rabbit or anti-mouse IgG (1:5000; Jackson ImmunoResearch Laboratories, Shanghai, China). Protein bands were visualized using the enhanced chemiluminescence reagents (Thermo Fisher Scientific) or the 1-stepTM NBT/BCIP reagent (Thermo Fisher Scientific) and detected by the Alpha Imager (Alpha Innotech, San Leandro, CA, USA).

Cell viability CCK8 assay

Cell viability after gene transfection was measured using the cell viability Cell Counting Kit-8 (CCK8; Dojindo Molecular Technologies, Gaithersburg, Maryland, USA). Briefly, cells were seeded into 96-well plates at 5×10^3 cells/well and grown up to different test time. Next, 10 μl of CCK-8 solution was added to each well and

further cultured for 4 h at 37°C and then optical density numbers were measured using a spectrophotometer (BioTek, Shanghai, China) at 450 nm. The experiments were conducted in five wells and repeated at least three times.

5-ethynyl-20-deoxyuridine incorporation assay

To assess cell proliferation, we performed the 5-ethynyl-20-deoxyuridine (EdU) incorporationassay using a kit fromRibobio (Shanghai, China). In particular, transfected tumor cells were grown overnight and then switched to a growth medium containing 50 μMEdU and cultured for 2 h at 37 °C. After that, cells were fixed in 4% formaldehyde at room temperature for 15 min and then incubated in 0.5% Triton X-100 at room temperature for 20 min. Cells were then washed in PBS three times and incubated with 100 μl of 1 x Apollo® reaction cocktail for 30 min in the dark at the room temperature. Subsequently, the EdU-positive cells were analyzed by a flow cytometer (BD Biosciences, FranklinLakes, NJ, USA).

Cell migration assay

Cells were grown and transiently transfected with DMBT1 cDNA, siRNA, or mutated DMBT1 cDNA for 48 h and then 1×10^4cells in 100 μl FBS-free culture medium were added into the chambers of 8 μm pore size (BD, Shanghai, China), while the bottom chambers were filled with 600 μl growth medium supplemented with 20% FBS. The cells were then cultured at 37°C for 24 h. The cells that migrated into the reverse side of the inserts were then fixed in 4% formaldehyde at room

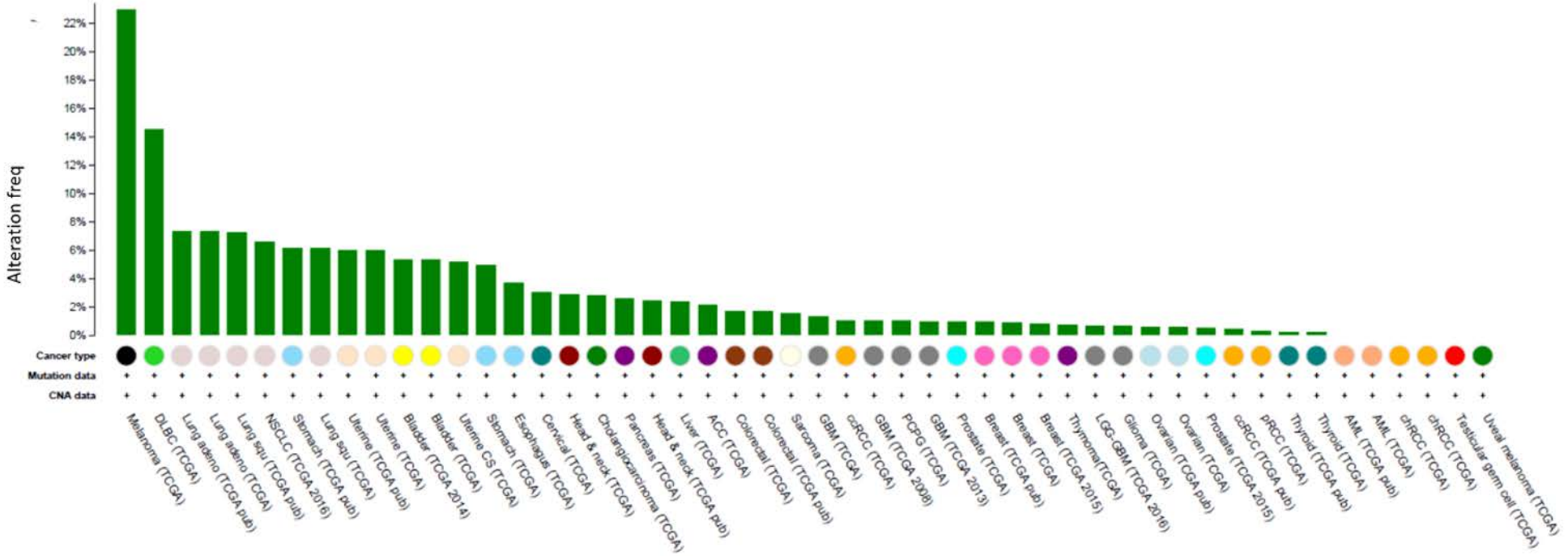
temperature for 30 min and stained with 0.05% crystal violet for 30 min. After, images of the migrated cells were captured under a light microscope and the migrated cells were counted and quantified.

Flow cytometric cell cycle distribution assay

After gene transfection, cells were harvested in ice-cold PBS and fixed in 70% ethanol for treatment with RNAse (2 μg/ml) at 37°C for 30 min. Cells were then stained with 400 μlpropidium iodide (PI; 10 μg/ml) for 40 min at room temperature. The cells were determined for DNA content using the FACS Calibur flow cytometer (BD Biosciences, FranklinLakes, NJ, USA) for cell cycle distribution.

Statistical analysis

In vitro data were summarized as the mean ± SEMof at least triplicate experiments and statistically analyzed using GraphPad Prism 5.1 (GraphPad Software, La Jolla, CA, USA). For comparison among the groups, we used a Student's *t* test or one- or two wayanalysis of variance (ANOVA). A $p < 0.05$ was defined as statistically significant at $*p < 0.05$, $**p < 0.01$, or $***p < 0.001$ level.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20%
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
Cancer type
Mutation data
CNA data
Melanoma (TCGA)
DLBC (TCGA)
Lung adeno (TCGA pub)
Lung adeno (TCGA)
Lung squ (TCGA pub)
NSCLC (TCGA 2016)
Stomach (TCGA pub)
Lung squ (TCGA)
Uterine (TCGA pub)
Uterine (TCGA)
Bladder (TCGA)
Bladder (TCGA 2014)
Uterine CS (TCGA)
Stomach (TCGA)
Esophagus (TCGA)
Cervical (TCGA)
Head & neck (TCGA)
Cholangiocarcinoma (TCGA)
Pancreas (TCGA)
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Liver (TCGA)
ACC (TCGA)
Colorectal (TCGA)
Colorectal (TCGA pub)
Sarcoma (TCGA)
GBM (TCGA)
ccRCC (TCGA)
GBM (TCGA 2008)
PCPG (TCGA)
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Prostate (TCGA 2015)
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Thyroid (TCGA)
Thyroid (TCGA pub)
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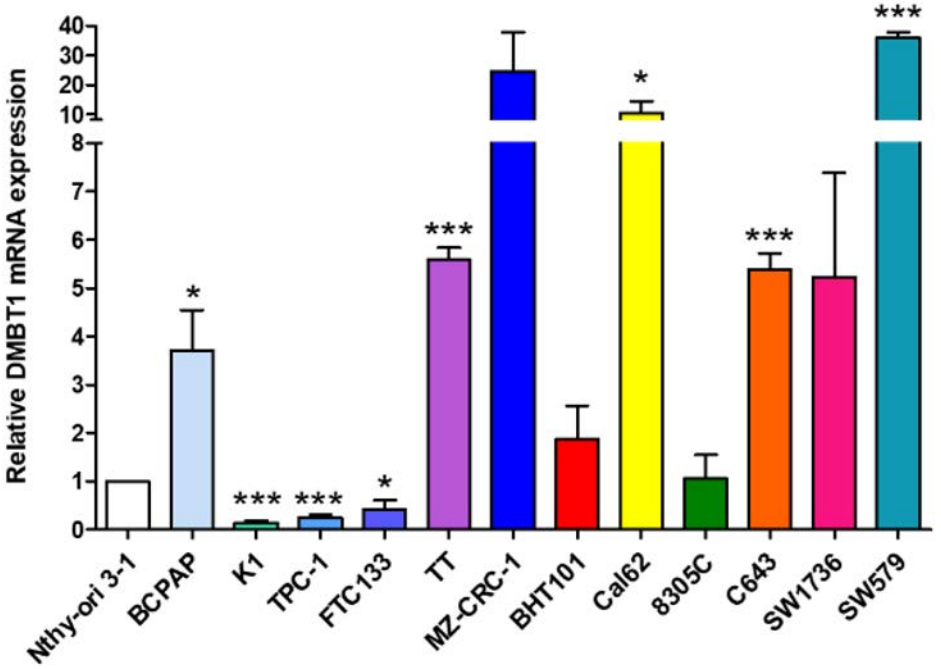
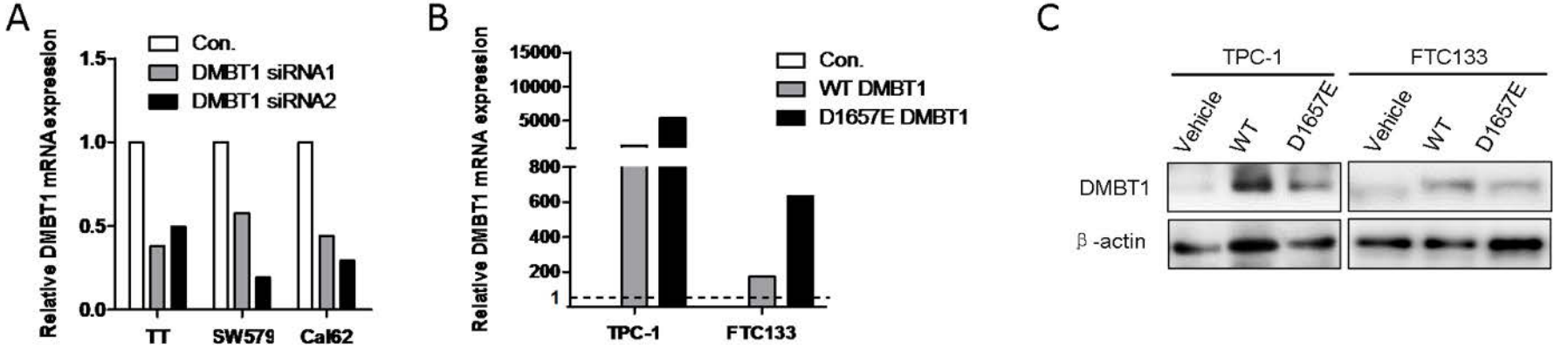

Relative DMBT1 mRNA expression
40
30
20
10
8
7
6
5
4
3
2
1
0
*
***
***
*
***
*
***
***
Nthy-ori 3-1
BCPAP
K1
TPC-1
FTC133
TT
MZ-CRC-1
BHT101
Cal62
8305C
C643
SW1736
SW579


A
Relative DMBT1 mRNA expression
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1.0
0.5
0.0
Con.
DMBT1 siRNA1
DMBT1 siRNA2
TT
SW579
Cal62
B
Relative DMBT1 mRNA expression
15000
10000
5000
800
600
400
200
1
Con.
WT DMBT1
D1657E DMBT1
TPC-1
FTC133
C
TPC-1
FTC133
Vehicle
WT
D1657E
Vehicle
WT
D1657E
DMBT1
β -actin

SupplementalTable S1. Demographic and clinical data of all family members with DMBT1 p.D1657E mutation.

Subject	Sex	Age (years)	Diagnosis
			Pedigree 1
II-5	Male	69	Familiar Mediastinal Neuroendocrine Cancer
III-2	Female	38	Familiar Mediastinal Neuroendocrine Cancer
III-8	Female	44	Familiar Mediastinal Neuroendocrine Cancer
III-9	Male	35	Familiar Mediastinal Neuroendocrine Cancer
IV-1	Female	22	None
IV-3	Female	2	None
			Pedigree 2
III-3	Female	55	Familiar Mediastinal Neuroendocrine Cancer
III-5	Female	52	Familiar Mediastinal Neuroendocrine Cancer
III-7	Female	48	None
III-11	Female	48	None
IV-1	Male	26	None
IV-4	Male	25	None

Supplemental Table S2. DNA sequencing profile of thyroid cancer cell lines for DMBT1 variant.

Cancer Cell type	Cell line	D1657E status
Papillary (PTC)	TPC-1	wt/wt
Follicular (FTC)	FTC133	wt/wt
Medullary (MTC)	TT	wt/wt
Anaplastic (ATC)	Cal62	wt/wt
Thyroid Squamous cell carcinoma	SW579	wt/wt