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Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

Cell culture and treatment

Human CTCL cell lines MJ, Myla, Sz4, PB2B, Hut78 were gifts from Dr Youwen Zhou (Department of Dermatology and Skin Science, University of British Columbia, Canada). All CTCL cell lines except MJ were cultured in RPMI-1640 medium (Sigma-Aldrich, St Louis, MO, USA) with 10% foetal bovine serum (Gibco, Carlsbad, CA, USA), 100 U/ml penicillin, and 0.1 mg/ml streptomycin. MJ cells were cultured in IMDM (Sigma-Aldrich, St Louis, MO, USA) with 20% foetal bovine serum (Gibco), 100 U/ml penicillin and 0.1 mg/ml streptomycin.

Lentivirus vector mediated gene knockdown and overexpression

A lentivirus shRNA vector was constructed by oligonucleotides encoding shRNAs against IKZF2 to U6-MCS-CBh-gcEGFP vector (GV493) between AgeI and EcoRI sites. Constructs with oligonucleotides encoding scrambled shRNA were used as controls. IKZF2 lentivirus vector was constructed by ligating a 1.6 kb or 1.2 kb fragment respectively containing full-length or tumour cell-type isoform IKZF2 cDNA to the Ubi-MCS-CMV-EGFP vector (GV409) between BamHI and NheI sites, and empty GV409 vector was used as control. All constructs were verified by DNA sequencing. Lentivirus production and transduction were performed as described previously. Oligonucleotides encoding shRNA were listed in **STable I**.

Next generation RNA-sequencing and gene expression analysis

Total RNA was extracted from Hut78 cells with IKZF2 silencing and corresponding vector as control using EZNA total RNA kit II (Omega Bio-tek, Norcross, GA, USA). Finally, 25~40 µl diethyl pyrocarbonate (DEPC)-treated water was added to dissolve the RNA. Subsequently, total RNA was qualified using a Nano Drop and Agilent 2100 bioanalyzer (Thermo Fisher Scientific, Waltham, MA, USA). Oligo(dT)-attached magnetic beads were used to purified mRNA. Purified mRNA was fragmented into small pieces with fragment buffer at appropriate temperature. Then First-strand cDNA was generated using random hexamer-primed reverse transcription, followed by a second-strand cDNA synthesis. Subsequently, A-tailing mix and RNA-index adapters were added by incubating to end repair. The cDNA fragments and products were purified by Ampure XP Beads, then dissolved in EB solution. The product was validated on the Agilent Technologies 2100 bioanalyzer for quality control. The double-stranded PCR products were heated denatured and circularized by the splint oligo sequence to get the final library. The single-strand circle DNA was formatted as the final library. The final library was amplified with phi29 to make DNA nanoball, which had more than 300 copies of 1 molecular, DNA nanoball were loaded into the patterned nanoarray and single end 50 bases reads were generated on BGIseq500 platform (BGI-Shenzhen, China). Sequencing raw reads were pre-processed by filtering out rRNA reads, sequencing adapters, short-fragment reads and other low-quality reads. Hisat2 (version: 2.0.4) was used to map the cleaned reads to the human hg38 reference genome with 2 mismatches. Then, expression level of gene was calculated by RSEM (version: 1.2.12). Differentially expressed genes were normalized using the DESeq2 (version: 1.4.5) R package. The p-value <0.05 and Fold Change ≥ 1.5 or ≤ 0.6 were set as the threshold for screening DEGs.

Gene set enrichment analysis

The GSEA software was downloaded from http://software.broadinstitute.org/gsea/index.jsp. GSEA analysis was conducted with transcript expression of transfected cells Hut78 compared with control and the chosen hallmark sub-collection downloaded from the Molecular Signatures Database (http://software.broadinstitute.org/gsea/msigdb/index.jsp) as the reference gene sets.

Real-time qRT-PCR

RNA samples were extracted from tumour stage MF lesions, as well as Hut78, Sz4 and Myla cells. Typically, 1 µg total RNA was used to generate cDNA by using SuperScript III RT (Takara, Dalian, China) with random-dT primer. Quantitative RT-PCR (qRT-PCR) was performed using the Power SYBR Green PCR Master Mix as recommended by the manufacturer (Invitrogen, Carlsbad, CA, USA). GAPDH was used as the internal control. SDS 2.2.1 software (Applied Biosystems, Foster City, CA, USA) was used to perform relative quantification of target genes using the comparative CT (ΔCT) method. The primer sequences were described in STable I.

Western blot assay

Whole cell lysates were prepared and separated by 4–20% gradient SDS-polyacrylamide electrophoresis and transferred to PVDF membrane (Millipore, Billerica, MA, USA). The membrane was blocked in 5% non-fat milk for 1 h at room temperature and incubated with the following antibodies at 4°C overnight: IKZF2, β-actin, and caspase 3, caspase8, caspase9 (Cell Signaling Technology, Boston, MA, USA). The membrane was washed in Tris-Buffered Saline and Tween 20 (TBST) (3×5 min), incubated with horse radish peroxidase (HRP)-conjugated secondary antibody, incubated with Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific, Waltham, MA, USA) and developed.

STable I. Sequences used in this study. Primers for RT-PCR and IKZF2 genotyping, oligonucleotides encoding shRNA targeting IKZF2

Gene symbol	Forward primer	Reverse primer
GAPDH	AAGATCATCAGCAATGCCTCC	TGGACTGTGGTCATGAGTCCTT
IKZF2	ACCATGTCATGTACACCATTCACA	TCATAACGGTCCTGGCTTCTGT
Oligonucleotides encoding shRNA		
shIKZF2	5′-CCGGGCTTATTCTCAGGTCTAT CATCTCGAGATGATAGACCTGAGAATAAGCTTTTT-3′	
Scramble shRNA	TTCTCCGAACGTGTCACGT	
Overexpression sequence		
IKZF2-210-p1	AGGTCGACTCTAGAGGATCCCGCCACCATGACAAGCACAAATTCAGTAAAG	
IKZF2-210-p2	CGCAGGAAAGAACAGCTAGCCTAGTGGAATGTGTGCTCCCCTC	