Supplementary material 3

ExperimentalmethodsFor:Pan-Cancer Multi-Omics Analysis Uncovers CHD4 Driving Tumor Progression via Epigenetic Regulation of Genomic Stability and the Immune Microenvironment

Cell lines and culture

All cell lines were obtained from Shanghai Jinyuan Biotechnology (Shanghai, China) and cultured under standard conditions (37°C, 5% CO_2) using their respective media: hFOB 1.19 cells in special osteoblast medium (CM-H042); 143B cells in special medium (JY-H056); HOS and U-2OS cells in MEM (Gibco, 11095080); Saos-2 cells in McCoy's 5A medium (JY-H032). All media were supplemented with 10% fetal bovine serum (excluding Saos-2 with 15% FBS) and 1% penicillin-streptomycin.

Lentiviral Vector Construction and CHD4 Knockdown

Three specific shRNA sequences targeting human CHD4 (NCBI Gene ID: 1108) and a non-targeting control shRNA were designed based on the NM_001273.4 transcript variant and cloned into the pLVX-shRNA1-Puro vector using BamH I and EcoR I restriction sites (1605 and 1611, Takara, Beijing, China). The targeting sequences were:

shCHD4-#1: 5'-CGAAGGTTTAAGCTCTTAGAA-3'

shCHD4-#2: 5'-GCGGGAGTTCAGTACCAATAA-3'

shCHD4-#3: 5'-CCTTACTAGAATTGGTGTTAT-3'

Vectors were verified by sequencing and transformed into Stbl3 competent cells (DLC106, Tsingke, Beijing, China). High-quality plasmids were extracted using the Plasmid Mini/Midiprep Kit (CW2581S, Cwbio, Jiangsu, China).

Lentiviral Transduction and Stable Cell Line Selection

Lentiviruses were packaged in 293T cells by co-transfection of CHD4-targeting shRNA plasmids and packaging plasmids (pLV-Helper1.0 and pLV-Helper2.0, Biomedicine Biotechnology, Chongqing, China) using Lentifusion Max transfection reagent (Biomedicine Biotechnology, Chongqing, China). Viral supernatants were harvested at 48 and 72 h post-transfection, concentrated with PEG-8000 (1546605, Sigma-Aldrich, MO, USA), and titers were quantified by RT-qPCR.

For transduction, 143B and Saos-2 cells were seeded in 24-well plates and infected with lentiviruses at a multiplicity of infection (MOI) of 20 and 10, respectively, in the presence of 8 μg/mL polybrene (C0351-1ml, Beyotime, Shanghai, China). After 48 h, stable knockdown polyclonal cells were selected using 3 μg/mL puromycin (St551-10mg, Beyotime, Shanghai, China) for 7 days.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from cells using TRIzon Reagent (CW0580S, Cwbio, Jiangsu, China). RNA concentration and purity were determined using a NanoDrop spectrophotometer

(Thermo Fisher, Waltham, MA, USA). cDNA was synthesized from 2 μg of total RNA using the Goldenstar™ RT6 cDNA Synthesis Kit (TSK302M, Tsingke, Beijing, China). Quantitative PCR was performed using 2×T5 Fast qPCR Mix (TSE202, Tsingke, Beijing, China) on a QuantStudio 5 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The primer sequences used were:

CHD4-F: 5′-CCACCCCACCCAGAAAATGA-3′

CHD4-R: 5′-CTGCCGGCATAAGAGCATAC-3′

GAPDH-F: 5′-TCATGACCACAGTCCATGCC-3′

GAPDH-R: 5′-CAGATCCACGACGGACACAT-3′

Amplification conditions were: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. Relative gene expression was calculated using the 2^(-ΔΔCt) method with GAPDH as the internal control.

Western blotting

Cells were lysed in RIPA buffer (P0013B, Beyotime, Shanghai, China) containing protease and phosphatase inhibitor cocktail (P1045, Beyotime, Shanghai, China). Protein concentrations were determined using a BCA Protein Assay Kit (23227, Thermo Fisher, Waltham, MA, USA). Equal amounts of protein were separated by 10% SDS-PAGE and transferred to PVDF membranes (10600023, Amersham, Germany). Membranes were blocked with 5% non-fat milk for 1 h at room temperature and incubated overnight at 4°C with the following primary antibodies: anti-CHD4 (YM8880, 1:2000; Immunoway, Plano, TX, USA), anti-MMP2 (YM8451, 1:2000; Immunoway, Plano, TX, USA), anti-MMP9 (YT1892, 1:1000; Immunoway, Plano, TX, USA), and anti-GAPDH (A19056, 1:50,000; Abclonal, Wuhan, China). After incubation with HRP-conjugated goat anti-rabbit secondary antibody (1:5000; AS014, Abclonal, Wuhan, China), protein bands were visualized using ECL reagent (34580, Thermo Fisher, Waltham, MA, USA) and quantified with ImageJ software.

Cell proliferation assay

The CCK-8 cell viability kit (C0038, Beyotime, Shanghai, China) was employed to assess the proliferative capacities of OS cells (143B and Saos-2) in accordance with the manufacturer's instructions. CCK-8 experiments were conducted by seeding and sustaining 5000 cells in 96-well plates for durations of 1, 2, or 3 days. Fresh medium was substituted with 100 μL, and 10 μL of CCK-8 was included. The cells were subsequently cultured for 2 hours before the OD-450 measurement(CMax Plus, Molecular Devices, San Jose, CA, USA).

Colony formation assay

Cells were seeded in 6-well plates at a density of 750 cells per well and cultivated for 14 days, with the media replaced every 4 days. The cells were then treated with methanol and stained with 0.5% crystal violet (G1062, Solarbio, Beijing, China). An inverted microscope (CKX41, Olympus, Tokyo, Japan) was used to count colonies that had more than 50 cells.

Wound-healing assay

Cells were seeded in 6-well plates and grown to full confluence. A sterile 10 μL pipette tip was used to create a linear scratch. After washing with PBS to remove detached cells, serum-free medium was added. Images of the wound were captured at 0 and 24 hours post-scratching using an inverted microscope (CKX41, Olympus, Tokyo, Japan). The migration rate was quantified by measuring the remaining wound area using ImageJ software.

Transwell invasion assays

Cell invasion was assessed utilizing 24-well Transwell chambers (8 μm pore size; 3422, Corning, NY, USA) that were pre-coated with Matrigel (354234, Corning, NY, USA). Cells (5×10^5) in serum-free medium were seeded into the upper chamber, while medium containing 20% FBS was added to the lower chamber as a chemoattractant. After 24 hours of incubation, a cotton swab was used to remove cells that had not invaded the upper surface. Cells that had invaded the lower membrane were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet (G1062, Solarbio, Beijing, China), and enumerated in five random fields under microscopic observation.