RNA sequencing analysis

To elucidate the transcriptional profile altered by CLEC3B overexpression in cholangiocarcinoma, RNA sequencing (RNA-seq) was performed on QBC939 cells. Cells were transfected with either the CLEC3B overexpression plasmid or an empty vector control (NC), with three biological replicates per group. Total RNA was extracted using TRIzol reagent (Invitrogen, USA) following the manufacturer's instructions. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), and RNA integrity was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). All samples had an RNA Integrity Number (RIN) > 8.0.

RNA-seq libraries were constructed using the NEBNext® Ultra™ II RNA Library Prep Kit (New England Biolabs, USA). Briefly, mRNA was enriched using oligo(dT) magnetic beads, fragmented, and subjected to first-strand and second-strand cDNA synthesis. After end repair, adenylation, and adapter ligation, the libraries were amplified by PCR and purified. Library quality and size distribution were assessed using an Agilent 2100 Bioanalyzer. Sequencing was performed on an Illumina NovaSeq 6000 platform (Illumina, USA) with 150-bp paired-end reads, generating approximately 6 Gb of raw data per sample.

Raw sequencing data were processed as follows: quality control was performed using FastQC (v0.11.9). Adapter sequences, low-quality reads (quality score < Q20), and ribosomal RNA reads were removed using fastp (Chen et al., 2018), and reads shorter than 25 bp were discarded. Clean reads were aligned to the human reference genome GRCh38 (Ensembl release 91) using HISAT2 (v2.1.0) with default parameters for spliced alignment. Gene expression levels were quantified using StringTie (v1.3.3b) and normalized as Fragments Per Kilobase of transcript per Million mapped reads (FPKM).

Differential expression analysis was conducted using the edgeR package (v3.30.3) in R (v3.6.1). Genes with an absolute log2 fold change > 1 and an adjusted p-value (False Discovery Rate, FDR) < 0.05 were considered significantly differentially expressed. Functional enrichment analysis of differentially expressed genes was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database via the clusterProfiler package in R. Significantly enriched pathways were identified using Fisher's exact test, with a q-value < 0.05 considered statistically significant.

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