

# Gene Chip Detection of Gene Expression Profile Changes after Treatment of Molt-4 Cells by Smo-Sirna

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**ABSTRACT.** *The gene expression profile of Molt-4 cells treated by Smo-siRNA was detected by gene chip, and the potential target genes regulated by Smo-siRNA were screened. The transfection group Molt- was constructed by lentiviral transfection technique. 4 cell lines. Bioinformatics analysis showed that these genes are mainly involved in cell growth and cycle, signal transduction, cell communication, cell adhesion, cell metastasis and invasion, and the signaling pathways involved antigen processing and presentation, cytokine and receptor interaction, Cell adhesion molecules, complement system, etc. Conclusions The study of potential target genes or signaling pathways regulated by Smo-siRNA is important for exploring the mechanism of Mot-4 cell development after Smo-siRNA treatment.*

**KEYWORDS:** *Gene expression profile, Smo-sirna, Real-time pcr*

## 1. Introduction

Smo-siRNA is one of the p63 gene transcription products, which is related to the degree of tissue differentiation and prognosis of Molt-4 cells. Patients with low expression of Smo-siRNA have a higher risk of tumor metastasis and recurrence, and the prognosis is worse [1-2]. In this study, the cell line of Molt-4 cells treated with Smo-siRNA was established by in vitro gene transfection technology, and the gene expression profile was detected by gene chip technology. The related genes and signal pathways were analyzed by bioinformatics technology. The potential target genes directly or indirectly regulated by Smo-siRNA were predicted, and the role and regulation mechanism of Molt-4 cells in the development and progression of Smo-siRNA treatment were analyzed [3-6].

Molt-4 cells were purchased from the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences [7-8]; DMEM medium and fetal bovine serum were purchased from Gibco, USA; TRIzol Reagent and Li-pofectamine 2000 were purchased from Invitrogen, USA; gene chip For the Agilent human 4×44K chip genome-wide expression profile, Smo-siRNA cDNA was purchased from ORIGENE, USA; plasmid pHelper1.0, plasmid pHelper 2.0 was purchased from Guangzhou Ledell Biotechnology Co., Ltd.; FastQuant RTKit was purchased from TIANGEN, Beijing; SYBR Premix Ex Taq™ was purchased from TaKaRa, Japan; Plasma Midi Kit was purchased from QIAGEN, Germany [9-10].

## 2. Gene Chip Detection Method

### (1) Cell culture

DMEM culture medium containing 10% fetal calf serum, 0.1% cyan/streptomycin was placed in an adherent culture at 37 °C in a 5% CO<sub>2</sub> incubator, and the cells were passaged by trypsin digestion in the logarithmic growth phase.

### (2) Establishment of Molt-4 cell line after Smo-siRNA treatment

The full-length Smo-siRNA cDNA was amplified and ligated into the pLVX-IRES-ZsGreen1 lentiviral vector digested with Xho I and BamH I to construct the recombinant lentiviral plasmid pLVX-IRES-ZsGreen1+Smo-siRNA, which was sent to Gene Company. Sequencing and identification. The Plasmid Midi Kit was used to purify the recombinant virus plasmid (or empty vector) with no endotoxin and its two auxiliary packaging plasmids. The human embryonic kidney (HEK) 293T cells were transfected with Lipofectamine 2000. The medium was changed 8 h after dyeing, and after 48 h of culture, the supernatant of the cell containing the lentivirus particles was collected and concentrated to obtain a high titer of the lentivirus concentrate. After the virus titer was measured and calibrated in 293T cells, the infected cells were constructed and transferred. The cell line Molt-4 and its control group were stained.

### (3) Gene chip detection

The total RNA of the transfected group and the control group was extracted and purified by TRIzol kit. The RNA sample was sent to Shanghai Kangcheng Biotechnology Co., Ltd. for RNA quality detection and human genome expression profiling. The chip was scanned with Axon GenePix. 4000 B), image and data processing using the American Axon GenePix Pro 6.0 software.

### (4) Gene chip data analysis

GO (Gene Ontology) ontology functional analysis was performed using the PANTHERFR (<http://www.pantherdb.org/>) online tool. Pathway analysis uses DAVID (<http://david.abcc.ncifcrf.gov/>) online software. Using STRING ( The

<http://string.embl.de/>) online tool further investigates the interaction between proteins encoded by differential genes in Path-way analysis results.

(5) Real-time quantitative PCR (qRT-PCR) to verify differentially expressed genes

Total RNA from Smo-siRNA and Molt-4 cells was extracted by TRIzol, reverse transcribed into cDNA using FastQuant RT Kit, and detected by qRT-PCR using SYBR Premix Ex Taq™. Primers were designed using Primer5.0 software and sent to Huada Gene Technology. Co., Ltd. Synthetic. Bone morphogenetic protein 7, BMP7 F: 5'-AGTC-TATCAGGTGCTCCAGG-3', R: 5'-TTGCTGGTGGCT- GTGATG-3'; Leukocyte differentiation antigen 74 (CD74 F: 5'-CGG-GAAGATCAGAAGCCAGTC-3', R :5'-GCGAGGAG- CAGAGTACCAG-3'; Smo-siRNAF: 5'- GACTCAATT- TAGTGAGCCACAGT-3', R: 5'-GCAATTGGCAG-TAGAGTTT-3' GAPDHF: 5'-GGCACAGTCAAGGCT- GAGAATG-3', R: 5'-ATGGTGGTGAAGACGCCAGTA-3'. qRT-PCR amplification was performed on a RocheLightCycler 480 instrument using the following conditions: 95 °C, 10 s, 95 °C, 5 s, 60 °C, 30 s, melting curve temperature range: 65 ~ 95 °C, 40 cycles, each sample was repeated 3 times, the Ct value was recorded, and analyzed by 2- $\Delta\Delta$ Ct method.

(6) Statistical processing

The data were analyzed by SPSS 21.0 software, and the data were represented by  $\bar{x} \pm s$ . Two independent samples were used for the measurement data between the groups. P<0.05 was considered statistically significant.

### 3. Analysis of Results after Detection of Molt-4 Cells by Smo-Sirna by Gene Chip

According to the chip data, differential genes with P<0.05, difference multiples>1.5 or <1:1.5 were screened for analysis. There were 1405 differentially expressed genes, of which 843 were up-regulated and 562 were down-regulated.

(1) Differential gene GO analysis

The PANTHERFR online analysis tool was used to analyze the above genes into GO. It showed that the differentially expressed genes were mainly related to cell growth, cell cycle regulation, signal transduction, cell metastasis invasion, cell adhesion, cell communication and other functions. Its function classification, the results shown in Figure 1, in the biological process classification, cell physiology accounted for 19.9%, metabolic process accounted for 22.7%; in the molecular function classification, the binding gene accounted for 32.3%, catalytically active genes accounted for 27.1%; In the intracellular localization classification, organelle protein accounted for 22.6% and membrane protein accounted for 13.9%.

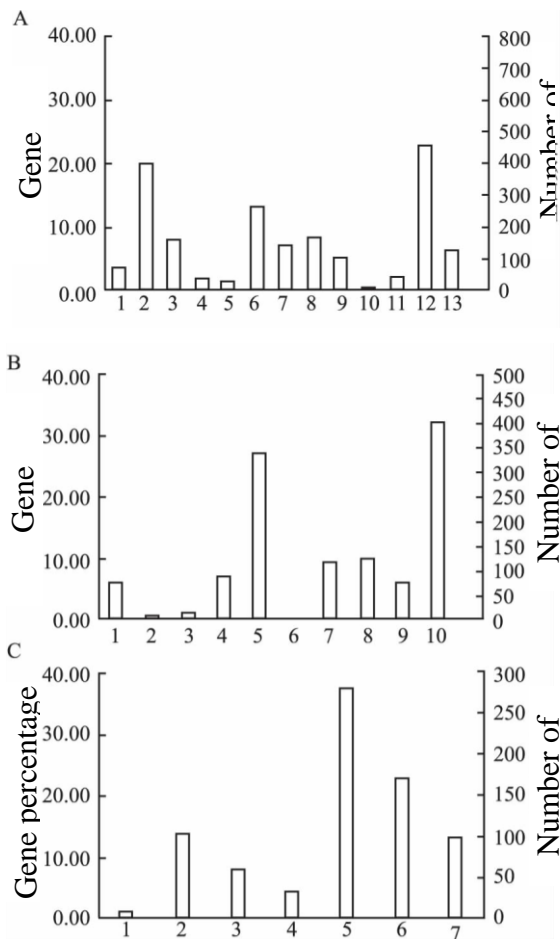


Fig.1 Results of Differential Analysis of Go Genes

A: biological process (1: biosynthesis process; 2: cell physiology; 3: localization process; 4: apoptosis process; 5: cell proliferation; 6: biological regulation process; 7: stress; 8: development process; 9: Multicellular organism process; 10: transport; 11: adhesion process; 12: metabolic process; 13: immune system process); B: molecular function (1: transcription factor activity; 2: regulatory factor activity; 3: protein binding transcription factor Activity; 4: enzyme regulator activity; 5: catalytic activity; 6: signal transduction activity; 7: receptor activity; 8: nucleic acid binding transcription factor activity; 9: molecular structure activity; 10: binding); C: intracellular Positioning (1: cell

junction region; 2: membrane component; 3: macromolecular complex; 4: extracellular matrix component; 5: cytoskeleton; 6: organelle; 7: extracellular region)

## (2) KEGG pathway analysis

KEGG pathway analysis was performed on 1405 differentially expressed genes in the microarray results using DAVID online analysis software. A total of 17 pathways of  $P < 0.05$  were selected for study. The main pathways involved were: antigen processing and processing, phagocytosis, viruses and bacteria. Infection and autoimmune diseases and cell adhesion are shown in Table 1

*Table 1 Kegg Pathway Analysis Results*

Signal path	Number	P value	Gene name
Antigen processing and presentation	16	0.00	B2M CD74 CTSL HLA-A HLA-B HLA-C HLA-DPB1 HLA-DRA HLA-DRB5 HLA-E HLA-F HLA-G CIITA TAP1 TAP2 TAPBP
Viral myocarditis	14	0.00	CAV1 CD55 HLA-A HLA-B HLA-C HLA-DPB1 HLA-DRA HLA-DRB5 HLA-E HLA-F HLA-G ITGB2 EIF4G3
Rheumatoid arthritis	16	0.00	ANGPT1 CTSL CXCL1 HLA-DPB1 HLA-DRA HLA-DRB5 IL6 IL11 IL15 IL18 ITGB2 JUN LTB CCL20 TLR2 VEGFA
Phagosome	22	0.00	CD36 CTSL HLA-A HLA-B HLA-C HLA-DPB1 HLA-DRA HLA-DRB5 HLA-E HLA-F HLA-G ITGAM IT-GB2 NCF2 OLR1 TAP1 TAP2 TLR2 EEA1 PLA2R1 PIKIFYVE TUBB8
Graft-versus-host disease	10	0.00	HLA-A HLA-B HLA-C HLA-DPB1 HLA-DRA HLA-DRB5 HLA-E HLA-F HLA-G IL6
Allograft rejection	9	0.00	HLA-A HLA-B HLA-C HLA-DPB1 HLA-DRA HLA-DRB5 HLA-E HLA-F HLA-G
Type I diabetes mellitus	9	0.00	HLA-A HLA-B HLA-C HLA-DPB1 HLA-DRA HLA-DRB5 HLA-E HLA-F HLA-G
Staphylococcus aureus infection	10	0.00	CFB C1QB C1S C4B HLA-DPB1 HLA-DRA HLA-DRB5 CFI ITGAM ITGB2
Hematopoietic cell line-age	13	0.00	CD1A CD7 CD36 CSF3 CD55 HLA-DRA HLA-DRB5 IL4R IL6 IL11 ITGAM KITLG CD24
Cell adhesion	17	0.00	ALCAM CNTN1 HLA-A HLA-B HLA-C

molecules (CAMs)			HLA-DPB1 HLA-DRA HLA-DRB5 HLA-E HLA-F HLA-G ITGAM ITGB2 CLDN11 SDC4 NFASC JAM2
Complement and coagulation cascades	11	0.00	A2M CFB C1QB C1S C4B C6 CD55 F3 CFI PLAT THBD
Cytokine-cytokine receptor interaction	27	0.00	AMHR2 BMP7 CCR1 CSF3 EDA CXCL1 CXCL2 CXCL3 HGF IL4R IL6 IL11 IL15 IL15RA TNFRSF9 IL18 INHBB LIF LTB KITLG TNFRSF11B CCL20 CXCL11 VEGFA IL18R1 IL20 CXCL16
Autoimmune thyroid disease	9	0.00	HLA-A HLA-B HLA-C HLA-DPB1 HLA-DRA HLA-DRB5 HLA-E HLA-F HLA-G
Focal adhesion	21	0.00	BIRC3 CAV1 CAV3 COL4A6 COL5A1 HGF TNC JUN LAMA3 LAMA4 MYL5 MYLK PPP1R12A PPP1CB VEGFA VTN PIK3R3 ITGA10 ROCK2 AKT3
Amoebiasis	13	0.00	COL4A6 COL5A1 CXCL1 IL6 ITGAM ITGB2 LAMA3 LAMA4 SERPINB2 SERPINB3 SERPINB4 TLR2 PIK3R3
Leishmaniasis	10	0.00	HLA-DPB1 HLA-DRA HLA-DRB5 ITGAM ITGB2 JUN NCF2 NFKBIB PTGS2 TLR2
Malaria	8	0.00	CD36 CSF3 HGF IL6 IL18 ITGB2 SDC4 TLR2

(3) Construction analysis of the interaction network between proteins encoding differentially expressed genes in KEGG pathway results

The 98 genes and TP63 in the KEGG pathway analysis results were uploaded to the STRING online tool to analyze the interaction between the proteins encoded by the above genes. The protein interactions were mainly concentrated in JUN, IL6, IL11, B2M, ITGAM, VEGFA. Between CD74 and BMP7, the literature revealed that most of the genes involved in immune-related mechanisms, and the proteins that interact directly with the protein encoded by tp63 are CD74 and BMP7.

(4) qRT-PCR verification

For the above-mentioned genes encoding proteins (CD74 and BMP7) that interact directly with the tp63-encoded protein, GAPDH was used as an internal control and qRT-PCR was used to verify the difference in expression. In the transfected cells, compared with the control group. Smo-siRNA expression was up-regulated ( $5.73 \pm 0.23$ ) times, BMP7 expression was down-regulated ( $1.59 \pm 0.06$ ) times, and CD74 expression was down-regulated ( $2.65 \pm 0.06$ ) times, the difference was statistically significant ( $t=24.633, -6.89, -8.534, P < 0.05$ ).

#### 4. Conclusion

The nuclear transcription factor p63 is an important member of the p53 family and plays a key role in epithelial cell proliferation, differentiation, and tissue growth and development. In Molt-4 cells, p63 mainly expresses Smo-siRNA subtypes and is concentrated in basal cells. In the middle, the parabasal cells also expressed a small amount; with the epithelial differentiation and stratification, the expression of Smo-siRNA gradually decreased until disappeared. Previous studies in our group showed that the expression level of Smo-siRNA in the Molt-4 cells was correlated with epithelial- The epithelial-mesenchymal transition (EMT) was significantly correlated. The expression level of Smo-siRNA was also different in Molt-4 cell line. Among them, Smo-siRNA was highly expressed in ME-180 cells, and it was in Molt-4 cells. Low expression. Therefore, in this study, Smo-siRNA was overexpressed in Molt-4 cells, and its expression profile was detected by gene chip. A total of 1405 genes with differential folds  $>1.5$  and  $P<0.05$  were screened (843 up-regulated genes, down-regulated) Gene 562). GO clustering analysis of the above genes showed that it mainly involved in cell growth and cycle, signal transduction, cell transfer and invasion. Further analysis of KEGG pathway showed that the differential genes were mainly Involved in the immune response-related signaling pathway. The correlation between the proteins encoded by the 98 genes in the KEGG pathway analysis results showed that the genes encoding the genes directly related to Smo-siRNA were BMP7 and CD74, and qRT-PCR was performed. Verification, the results are consistent with the chip results.

BMP7 is a member of the transforming growth factor (TGF)-beta superfamily and is prominently expressed in a variety of epithelial tumors. This study showed that Mot-4 cells can down-regulate BMP7 expression after Smo-siRNA treatment, and the EMT process and invasion and metastasis ability of the cells are inhibited. Moreover, in the previous miRNA expression profile data, Smo-siRNA treatment directly caused Up-regulation of miR-22 expression in BMP7 was regulated (Fold change=2.32,  $P<0.05$ ) [4,9]. Therefore, whether miR-22/BMP7 mediates Smo-siRNA regulation of Molt-4 cell EMT is worthy of further study.

CD74, the MHC-II molecular-associated invariant chain, acts as a high-affinity receptor for macrophage migration inhibitory factor (MIF) and mediates MIF-regulated cell survival signals. CD74 can form complexes with MIF and CD44. Activation of NF- $\kappa$ B signaling pathway regulates cell development in Molt-4 cells. In expression profiles, NF- $\kappa$ B-related factors such as IL6 and TLR2 were down-regulated by 1.72-fold and 1.61-fold, respectively, after Smo-siRNA was up-regulated. The regulatory relationship between Smo-siRNA and CD74 deserves further investigation.

In this study, we used gene chip to detect the difference of gene expression profile of Mot-4 cells treated by Smo-siRNA, and screened CD74 and BMP7 as candidate genes for Smo-siRNA. It is concluded that these two genes may be combined with Smo-siRNA.

Inducing immune function-related signaling pathways in Molt-4 cells, which affects tumor development. Therefore, the next step will be to detect the expression of Smo-siRNA, CD74 and BMP7 in the transfected and control cells at the protein level. Overexpression or silencing of CD74 and BMP7 in two cells, comparing changes in Smo-siRNA and other immune-related factors, and performing functional experiments such as cell proliferation, invasion and apoptosis, and exploring the role of CD74 and BMP7 in cell function of Molt-4 cells. The above studies will provide important ideas and clues for further exploration of the mechanism of action of Smo-siRNA in Molt-4 cells.

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