MICRORNA-223 DECREASES CELL PROLIFERATION, MIGRATION, INVASION, AND ENHANCES CELL APOPTOSIS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA VIA TARGETING FORKHEAD BOX O 1

PRESENTER: SEYED MOHAMMAD HOSSEINI

Bioscience Reports (2020) **40** BSR20200485
https://doi.org/10.1042/BSR20200485

PORTLAND PRESS

Research Article

MicroRNA-223 decreases cell proliferation, migration, invasion, and enhances cell apoptosis in childhood acute lymphoblastic leukemia via targeting Forkhead box O 1

Chunyu Li[1,*], Tana Zhao[1,*], Lei Nie[1], Yanhong Zou[1] and Quan Zhang[2]

[1]Department of Pediatrics, The First Affiliated Hospital of Jiamusi University, No. 348 dexiang Street, Jiamusi City, Heilongjiang Province 154002, China; [2]Department of Gastroenterology, Jiamusi Central Hospital, No. 256, Zhongshan Street, Xiangyang District, Jiamusi City, Heilongjiang Province 154002, China

Correspondence: Yanhong Zou (zoiyanhong218@163.com)

Introduction

miRNA

- definition → MicroRNA (miRNA) is a small non-coding RNA molecule, typically 20 to 25 nucleotides in length
- Role → binding to complementary sequences on target mRNAs, leading to either degradation of the mRNA or inhibition of its translation into protein .
- Functions
  - •Development and differentiation
  - •Cell cycle regulation
  - •Apoptosis
  - •Responses to stress and environmental changes
  - •tumorogenesis, metastasis, and resistance to therapy.
- Biogenesis → Pri- miRNA —(Drosha, In nucleuse)→ Pre- miRNA —(Dicer, In Cytoplasm)→ miRNA
- Methods of Analysis
  - Quantitative (qRT-PCR) → Expression
  - Micro-RNA array profiling → Expression of several mi-RNA, across different conditions
  - Next-generation sequencing (NGS) → identifying novel miRNAs and understanding their functions

Tumor cell lines

Defenition
- derived from human tumors
- researche without some ethical and practical limitations

Application
- **Drug Screening**: test the efficacy and toxicity of new anticancer drugs.
- **Mechanistic Studies**: study specific cancer pathways and how they are affected by various treatments
- **Biomarker Discovery**: identify potential biomarkers for prognosis etc.

Examples
- **MCF-7**: breast cancer cell line.
- **A549**: lung cancer cell line.
- **SKBR3**: breast cancer cell line.
- **U87 MG**: Glioblastoma cell line.
- **CCRF-CEM**: T-ALL cell line
- **MOLT-4**: T-ALL cell line
- **REH**: B-ALL cell line
- **NALM-6**: B-ALL cell line
- **JURKAT**:T-ALL cell line

Methods and results

1- Real-Time PCR

The purpose

Evaluation of gene expression quantitatively

The basis of the method in general

1- RNA extraction
* Cell lysis → Trisol
* Separate RNA of component → Chloroform
* RNA deposition → Isopropanol alcohol

2- convert RNA to cDNA

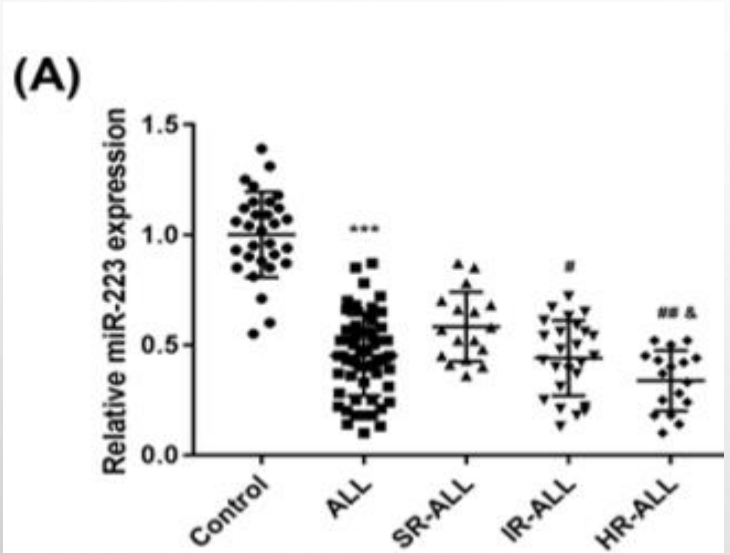
3- Real-Time PCR

Real-time PCR in this article

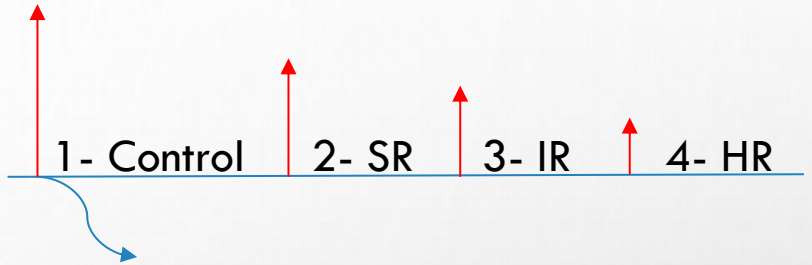
59 ALL children and 30 control children

- 17 Standard risk(SR),
- 25 intermediate risk(IR)
- 17 high risk(HR).

Results

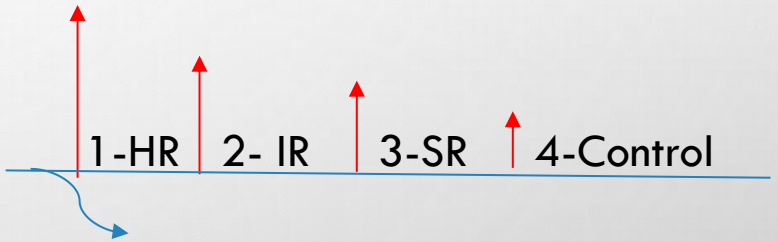


A. miR-223 expression

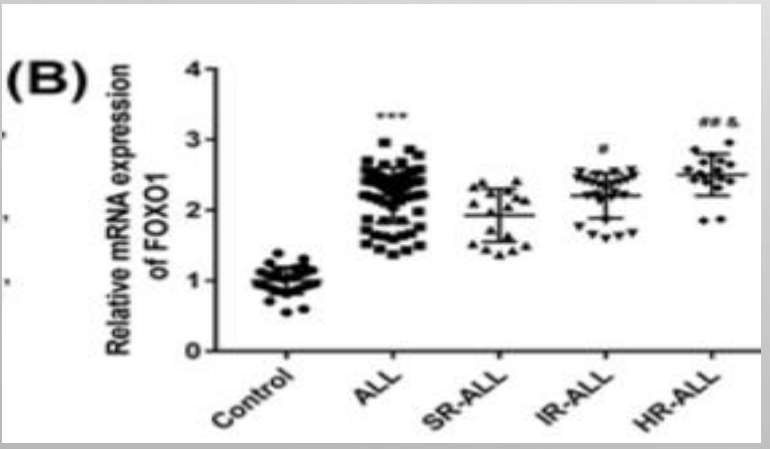


Expression of miR-223 was down-regulated in ALL children

B. FOXO1 expression



FOXO1expression was increased in ALL children



MiR-223 inhibited the protein expression of FOXO1 in ALL cells

2- Cell transfection

The purpose

introduce foreign nucleic acids (like DNA or RNA) into cells

The basis of the method in general

1.Preparation of Nucleic Acids: Isolate and purify the DNA or RNA to ensure high quality for transfection.

2.Selection of Transfection Method: Choose the appropriate transfection method based on the cell type (Chemical, Physical ,viral)

3.Transfection Procedure: Follow the protocol specific to the method chosen, including mixing nucleic acids with transfection reagents, adding to the target cells, and providing appropriate incubation time.

4.Post-Transfection Treatment: After transfection, cells may need to be cultured under specific conditions to allow for expression of the transfected genes.

5.Validation: Assess transfection efficiency through techniques such as RT-PCR, Western blotting, or fluorescence microscopy (if using fluorescent reporters).

Cell transfection assay in this study

Used chemical method : Lipofectamine (Invitrogen)

_NALM-6 cells → Transfected with miRNA-223 inhibitor

_CCRF-CEM → transfected with miRNA-223

validation

RT-PCR →

The expression of miRNA-223 in NALM-6 ↓

The expression of miRNA-223 in CCRF-CEM ↑

3- Colony formation assay (CFA)

purpose

- Assess the long-term survival of cells
- Assess the ability of single cell to grow into visible colonies.

The basis of the method in general

- **Agarose or Methocel**: To create a semi-solid medium for colony growth.
- **Suspension cell lines**
- **Incubator (14 d)** : Set to the appropriate temperature and CO2 level for cell growth.
- **Solvent for treatment**: If testing drug effects, use the appropriate solvent.
- **Staining**

CFA in this study

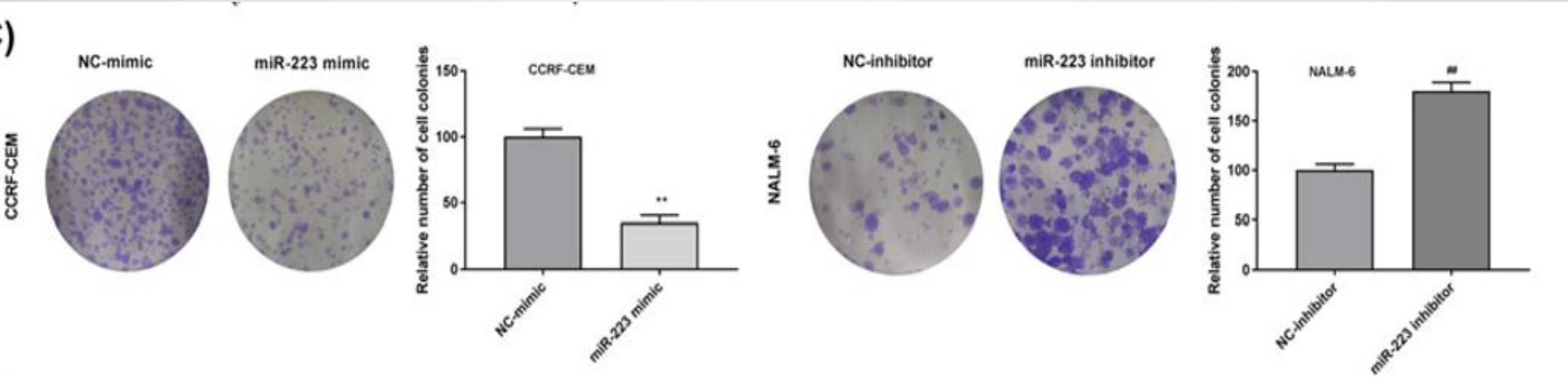
Stain :Crystal Violet

Results

miR-223 → Decreased the number of CCRF-CEM colonies

miR-223 inhibitor → increased the number of NALM-6 colonies

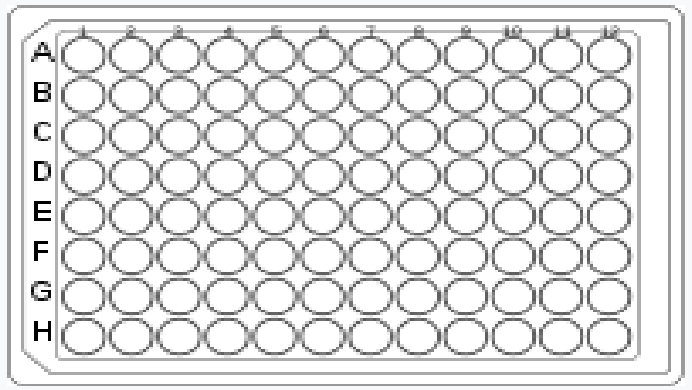
MiR-223 inhibited colony formation ability in ALL cells



4- MTT assay

Purpose

- assess Cytotoxicity
- assess viability of cell



The basis of the method in general

1. Cell Preparation: Cells are typically seeded in 96-well plate.

2. Drug Treatment: compounds or drugs are added to the wells. The cells are incubated for a specified duration (commonly 48 hours) under controlled conditions (e.g., 37°C with 5% CO_2).

3. MTT Addition: Following incubation, MTT solution is added to each well and further incubated for several hours to allow formazan formation

4. Measurement: The optical density (OD) of each well measured . The OD values correlate with the number of viable cells, as higher OD indicates greater metabolic activity and cell viability.

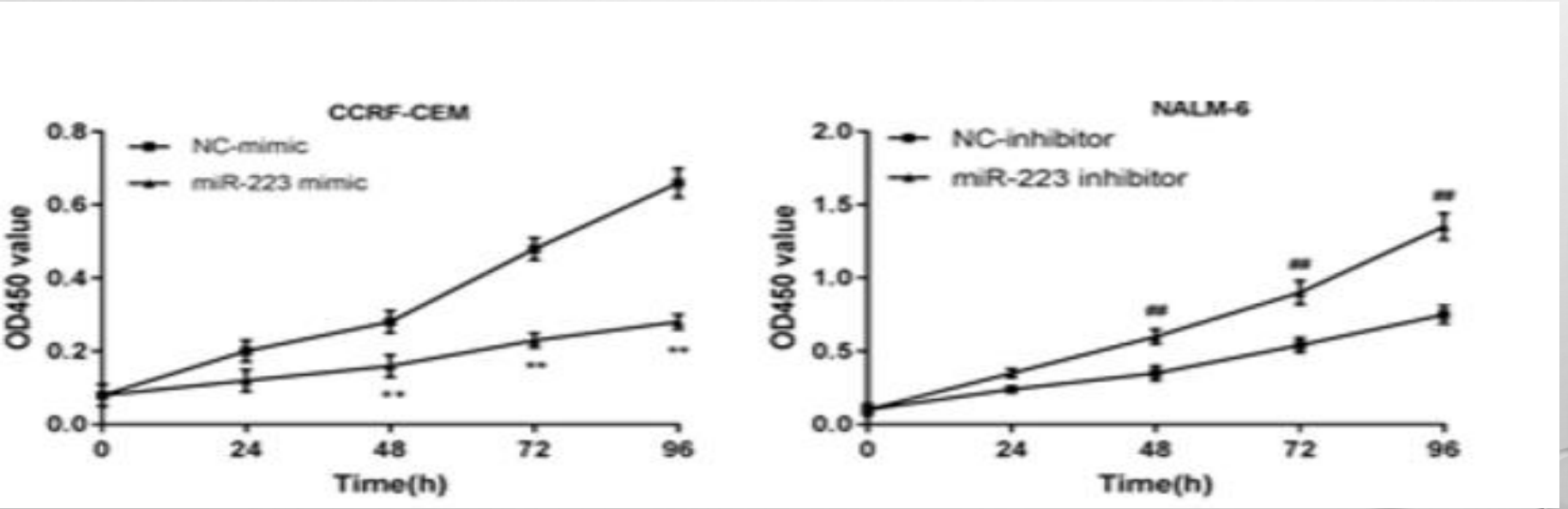
MTT in this study

Result

OD450nm of CCRF-CEM cells with the miR223 → markedly lower than negative controle group

OD450nm of NALM-6 cells with the miR-223 inhibitor → markedly higher than negative controle group

MiR-223 inhibited the proliferation of ALL cells



5-Transwell assay

purpose

Assess the invasion and migration ability of cells

The basis of the method in general
For migration

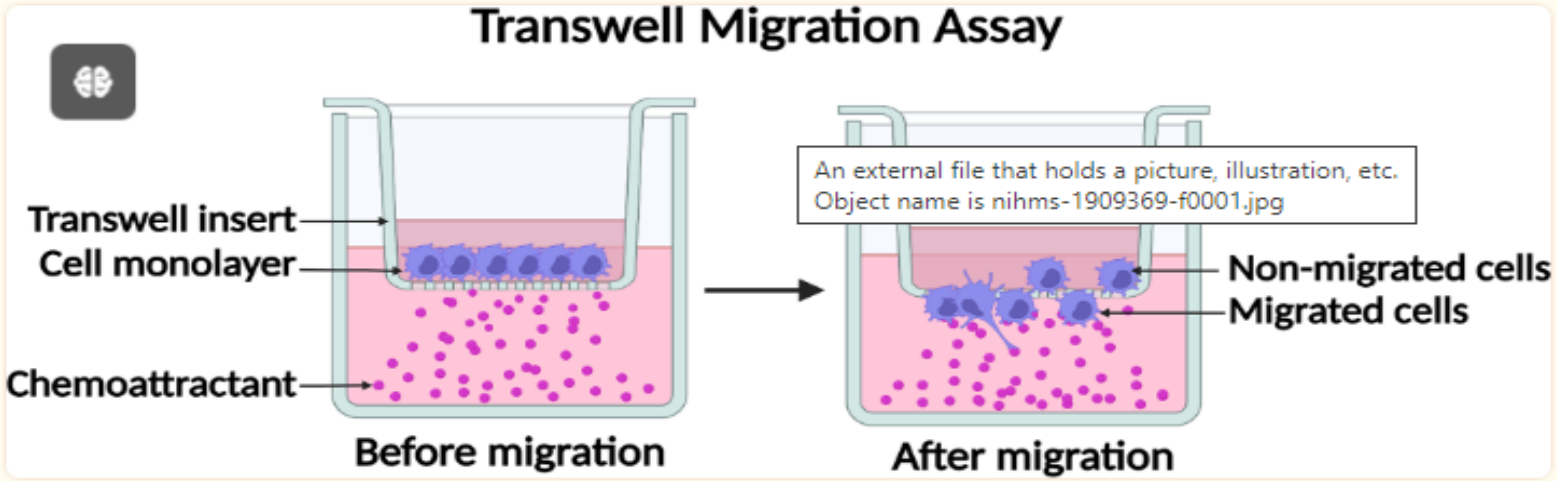


Figure 1.

Diagram of the transwell cell migration assay. Cells in migration buffer are added into a transwell insert with a porous membrane. A chemoattractant is added into the bottom chamber to form a chemotactic gradient. Cells sense the chemotactic gradient and migrate through the pores of the transwell membrane. Created with BioRender.com.

The basis of the method in general
For invasion

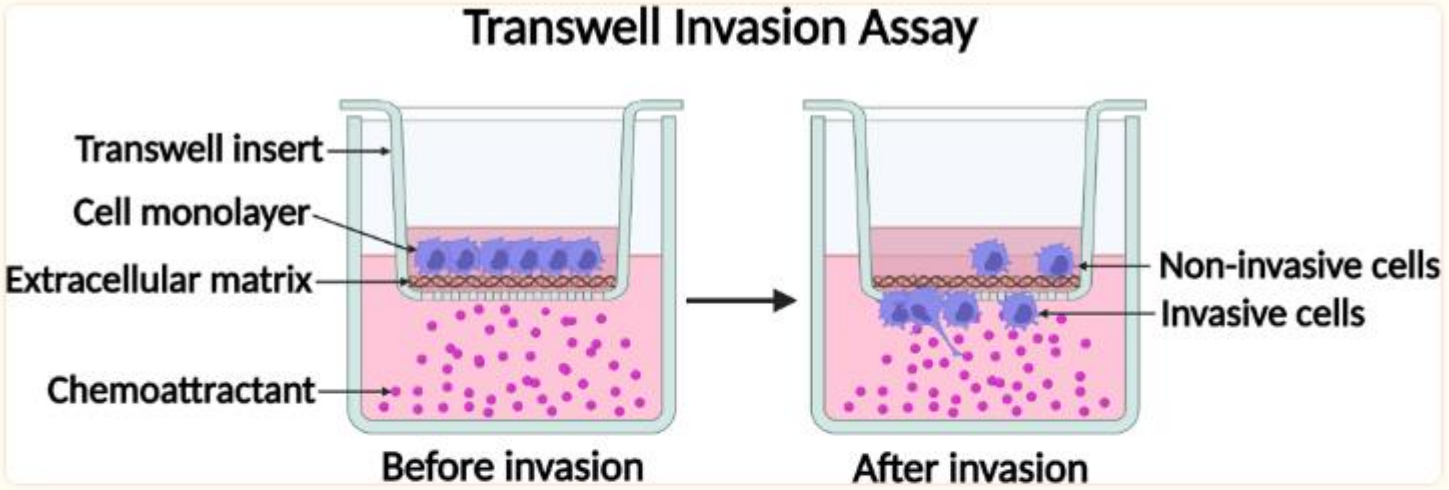


Figure 2.

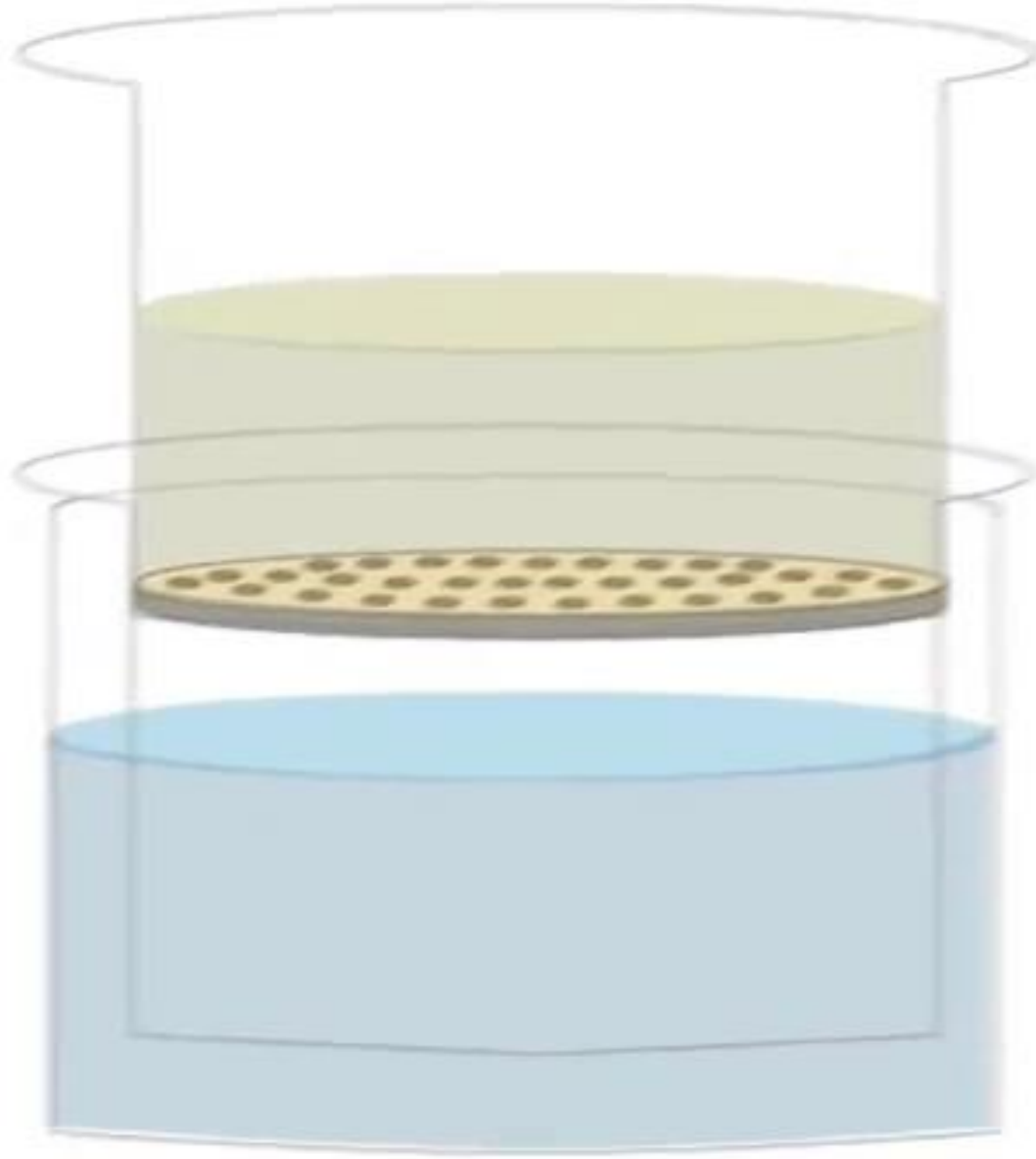
Diagram of the transwell cell invasion assay. An extracellular matrix such as Matrigel is added to the apical side of the transwell membrane to form a uniform thin layer of gel. Cells in migration buffer are added into the transwell insert. A chemoattractant is added into the bottom chamber to form a chemotactic gradient. Cells with invasive capability can sense the chemotactic gradient, invade through the extracellular matrix, and migrate through the pores of the transwell membrane. Created with BioRender.com.

* Quantification of Migrated Non-Adherent Cells

1.Non-adherent cells migrating through the pores of the transwell membrane will drop into the medium in the bottom chamber. The 600 µL of cell-containing medium in the bottom chamber can be collected for quantification.

2.Migrated cells in the medium can be directly counted using hemocytometer, flow cytometer or cell counting instrument (e.g., automated cell counter).

* If the cell concentration is low, the samples may be centrifuged to concentrate the cells, then resuspended in a smaller volume of medium and counted using a hemocytometer.



Transwell assay in this study

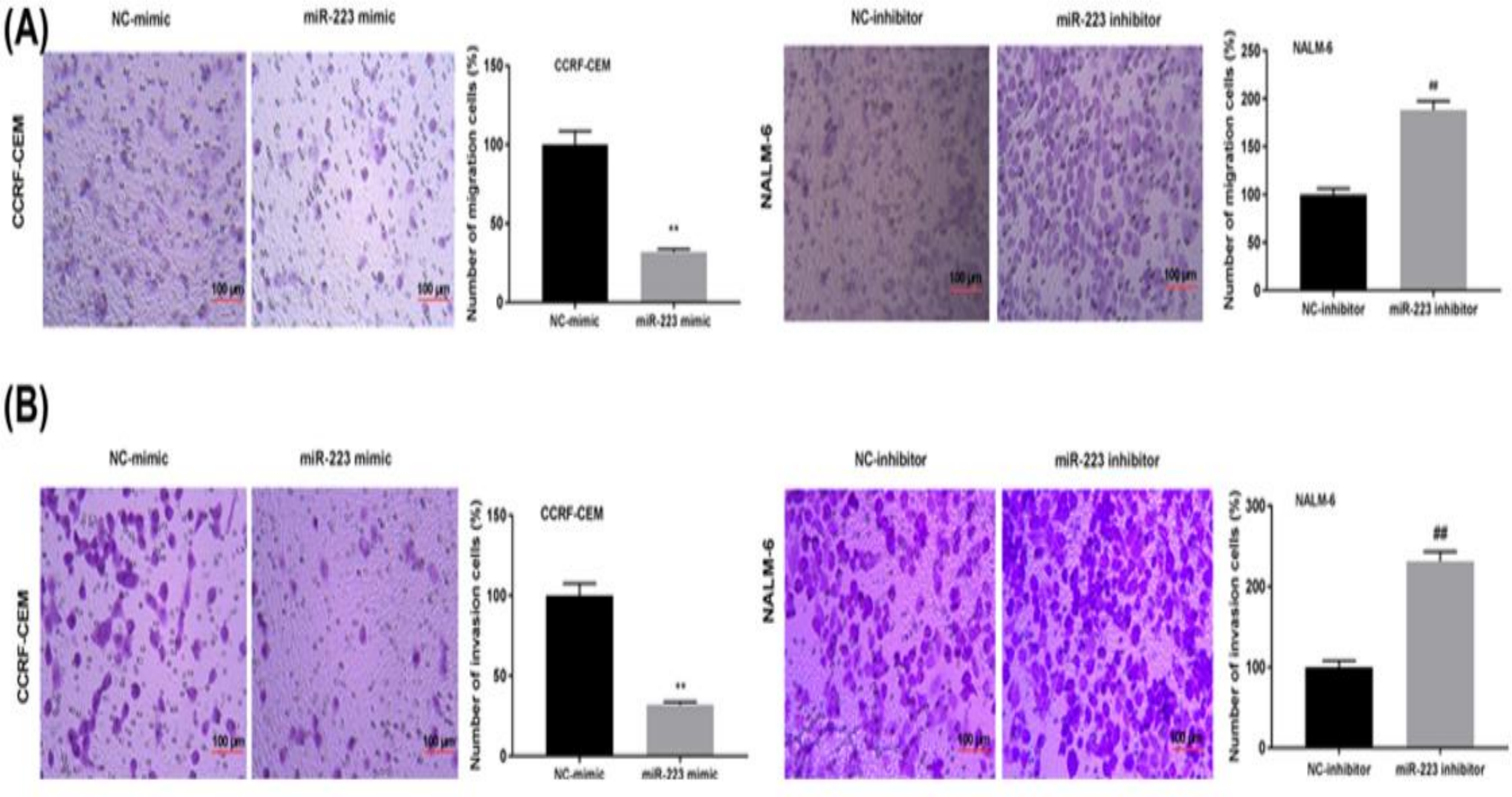
Counting: hemocytometer

Results

Invasion and mitigation of CCRF-CEM cell → decreased by transfect miR-223

Invasion and mitigation of NALM-6 cell → Increased by transfect mir223 inhibitor

MiR-223 inhibited the cell invasion and migration in ALL cells


(A)
NC-mimic
miR-223 mimic
CCRF-CEM
100 μm
Number of migration cells (%)
CCRF-CEM
**
NC-mimic
miR-223 mimic
NC-inhibitor
miR-223 inhibitor
NALM-6
NALM-6
#
NC-inhibitor
miR-223 inhibitor
(B)
NC-mimic
miR-223 mimic
CCRF-CEM
Number of invasion cells (%)
CCRF-CEM
**
NC-mimic
miR-223 mimic
NC-inhibitor
miR-223 inhibitor
NALM-6
NALM-6
##
NC-inhibitor
miR-223 inhibitor

6-Western blot

Purpose

Measuring the amount of protein in different conditions

The basis of the method in general

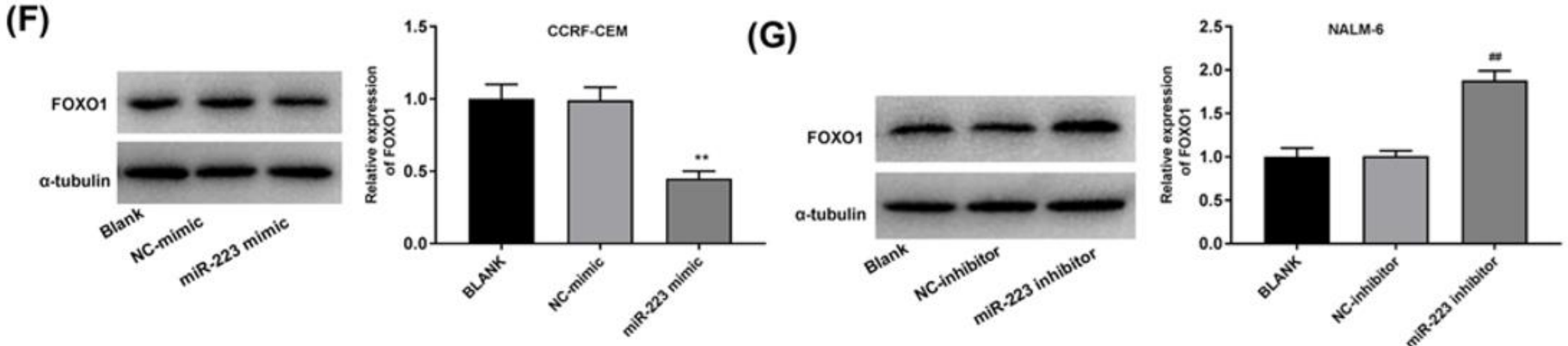
1. **Sample preparation** derised eht gniniatnoc elpmas a ,tsriF : ro lamina eb nac elpmas sihT .deraperp eb dluohs nietorp tcab ,sllec derutluc ,seussit tnalperi.
2. **Protein extraction:** by chemicals such as protein extraction buffers, lysis buffers, aqueous buffers, urea, Triton X-100,SDS.
3. **Protein Electrophoresis:** separated in the agarose gel according to their size and molecular weight and are placed in the gel in the form of distinct bands.
4. **Transfer of proteins to the membrane :** polyvinylidene fluoride (PVDF).
5. **Interaction of proteins with antibodies**
6. **Washing**
7. **Detection :** Chemiluminescence or radioactivity

Western blot in this study

FOXO1 protein expression in CCRF-CEM cells → ↓

FOXO1 protein expression in NALM-6 cells → ↑

MiR-223 inhibited the protein expression of FOXO1 in ALL cells



7-RNA pull down assay

Application

- Identification of RNA-Binding Proteins
- Understanding Post-Transcriptional Regulation
- Analyzing miRNA Function
- Studying RNA Modifications
- Investigating Disease Mechanisms

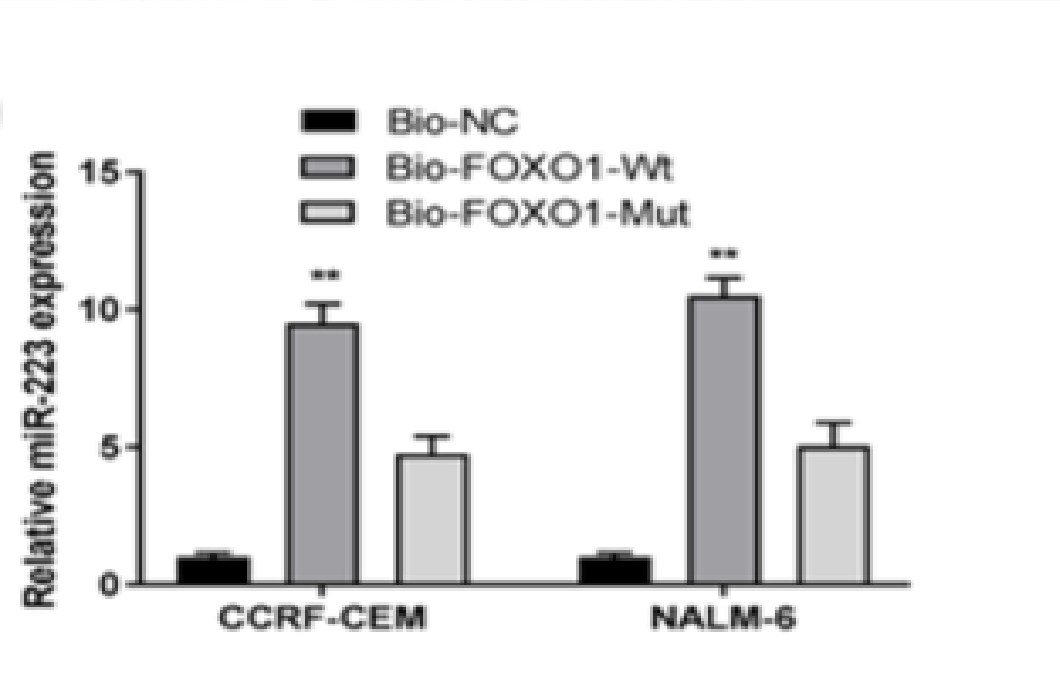
Steps to Analyze miRNA Function AND Understanding Post-Transcriptional Regulation by Using RNA Pull-Down Assays:

1. **Biotinylating**: Synthesize the RNA of interest (mRNA or non-coding RNA) and label it with a biotin tag either during synthesis or by post-synthetic modification.
2. **Transfection**
3. **Incubation:** the biotinylated RNA with the cells, allowing it to bind to any associated proteins or other RNA species.
4. **Cell lysis**
5. **Affinity chromatography:** streptavidin
6. **Analysis of Bound RNAs:** qPCR, Northern blot, RNA seq

RNA pull down assay in this study

Bio-FOXO1 WT/MUT mRNA → transfect → into NALM-6 and CCRF-CEM cells

Results



MiR-223 inhibited the protein expression of FOXO1 in ALL cells

8- dual-luciferase reporter assay

Purpose

-to study the interaction between microRNAs (miRNAs) and their target mRNAs

_ determine the binding target of miRNA on mRNA

The basis of the method in general

MUT/WT of mRNA co-transfected in cell lines with miRNA

Detected luciferase activity by dual-luciferase reporter gene assay system

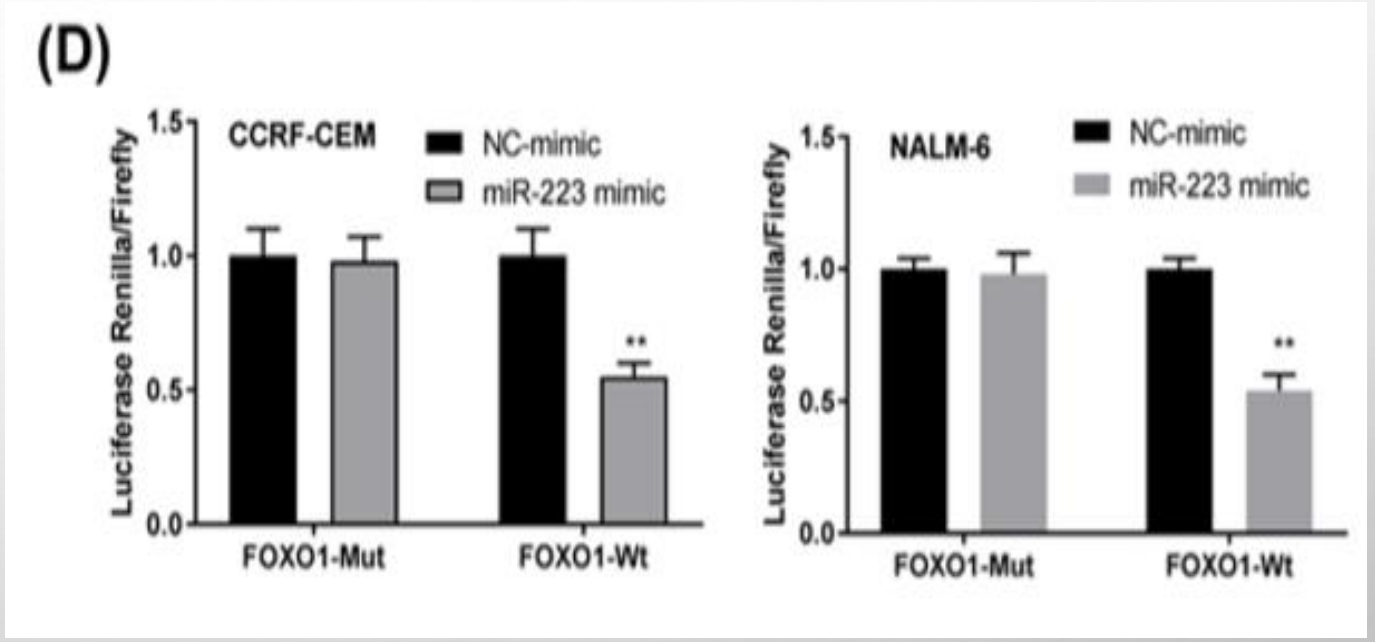
Decreased Luciferase Activity: If the miRNA suppresses the expression of the target mRNA, the luciferase activity will be reduced. This is indicative of effective binding of the miRNA to its target within the luciferase construct.

Increased Luciferase Activity: Conversely

dual-luciferase reporter assay in this study

FOXO1.MUT/FOXO1.WT mRNA + miRNA223 —Co-transfected→ Into CCRF-CEM and NALM-6 cells with Lipofectamine

Results



FOXO1 Target: 5'accuGUUUUCUGCGGAACUGACg 3'

miR-233-3p miRNA: 3'acccCAUAA---ACUGUUUGACUGu 5'

FOXO1 Mut: 5' accaCGACUUACUAUUAACUGGu 3'

MiR-223 inhibited the protein expression of FOXO1 in ALL cells

Conclusion

_miR-223 expression was down-regulated in ALL cells

_miR-223 could inhibit cell proliferation and promote apoptosis in ALL cells via regulating FOXO1

_miR-223 might act as a potential therapeutic target for ALL patient


Thank you!