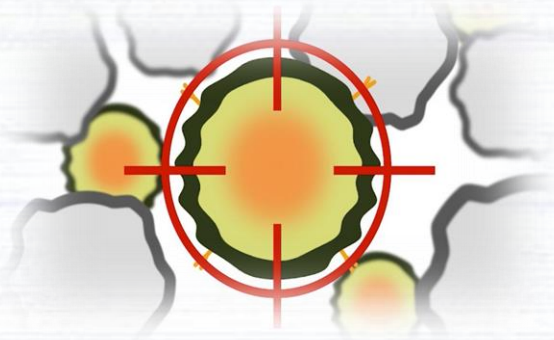
In the Name of God



A Novel Targeted Therapy System for Cervical Cancer

Co-Delivery System of Antisense LncRNA of MDC1 and Oxaliplatin

Magnetic Thermosensitive Cationic Liposome Drug Carrier

- **Cervical cancer** is one of the most common malignant tumors in women.
- The incidence and mortality of Cervical cancer in women rank **second** in developing countries.

- **Oxaliplatin (OXA)** exhibits broad-spectrum in vitro **cytotoxicity** and in vivo **antitumor activity** in multiple tumor model systems.
- This drug causes neurotoxicity, gastrointestinal abnormal reaction, hemorrhage, allergic reaction, and severe adverse reactions.

Targeted Therapy System

&

Multiagent Codelivery Systems

Targeted Therapy System

- Delivery platforms in cancer therapy

- LPs
- Microencapsulation
- Micelles
- Stimulus-sensitive polymers
- Layer-by-layer assembly technology

- Types of LP

- Magnetic LP (ML)
- Thermosensitive LP (TL)
- Cationic LP (CL)

“ MTCL “

(magnetic thermosensitive cationic liposome)

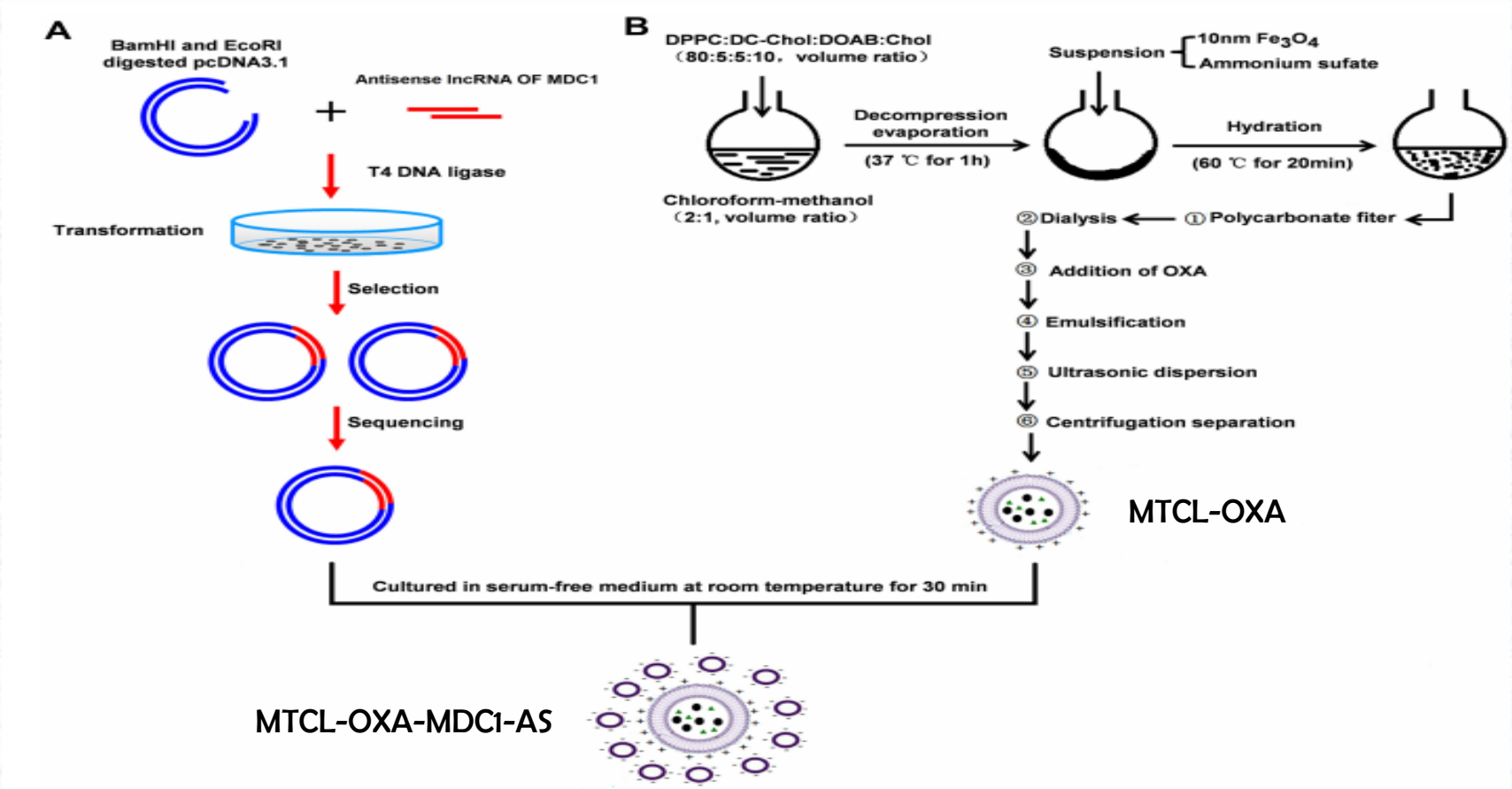
Multiagent Codelivery Systems

- **Oxaliplatin (OXA)**
  - Inhibition of DNA replication and transcription by forming inter- and intra-strand platinum-DNA adducts

- **Antisense lncRNA of Mediator of DNA damage Checkpoint 1 (MDC1-AS)**
  - The expression levels of the (MDC1) and its antisense lncRNA (MDC1-AS) were downregulated in **bladder cancer**.
  - The expression levels of MDC1-AS and MDC1 were significantly downregulated in **glioma** tissues compared with normal brain tissues, and MDC1-AS expression was positively correlated with MDC1 .

MDC1-AS is a potential therapeutic target for the treatment of Cervical cancer.

Preparation of MTCL-OXA-MDC1-AS



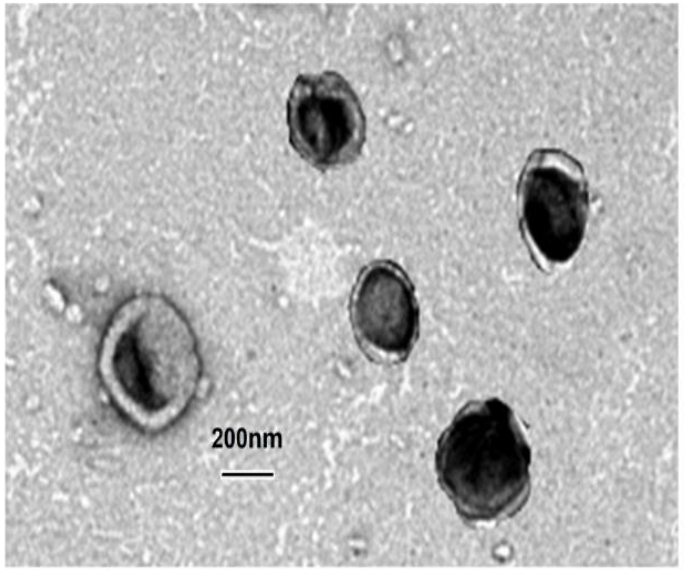
TCL-OXA (thermosensitive cationic liposome as the carrier to deliver OXA), **TCL-MDC1-AS** (thermosensitive cationic liposome as the carrier to deliver MDC1-AS), **TCL-OXA-MDC1-AS** (TCL as the carrier to codeliver OXA and MDC1-AS)

Morphological and Biophysical Characteristics

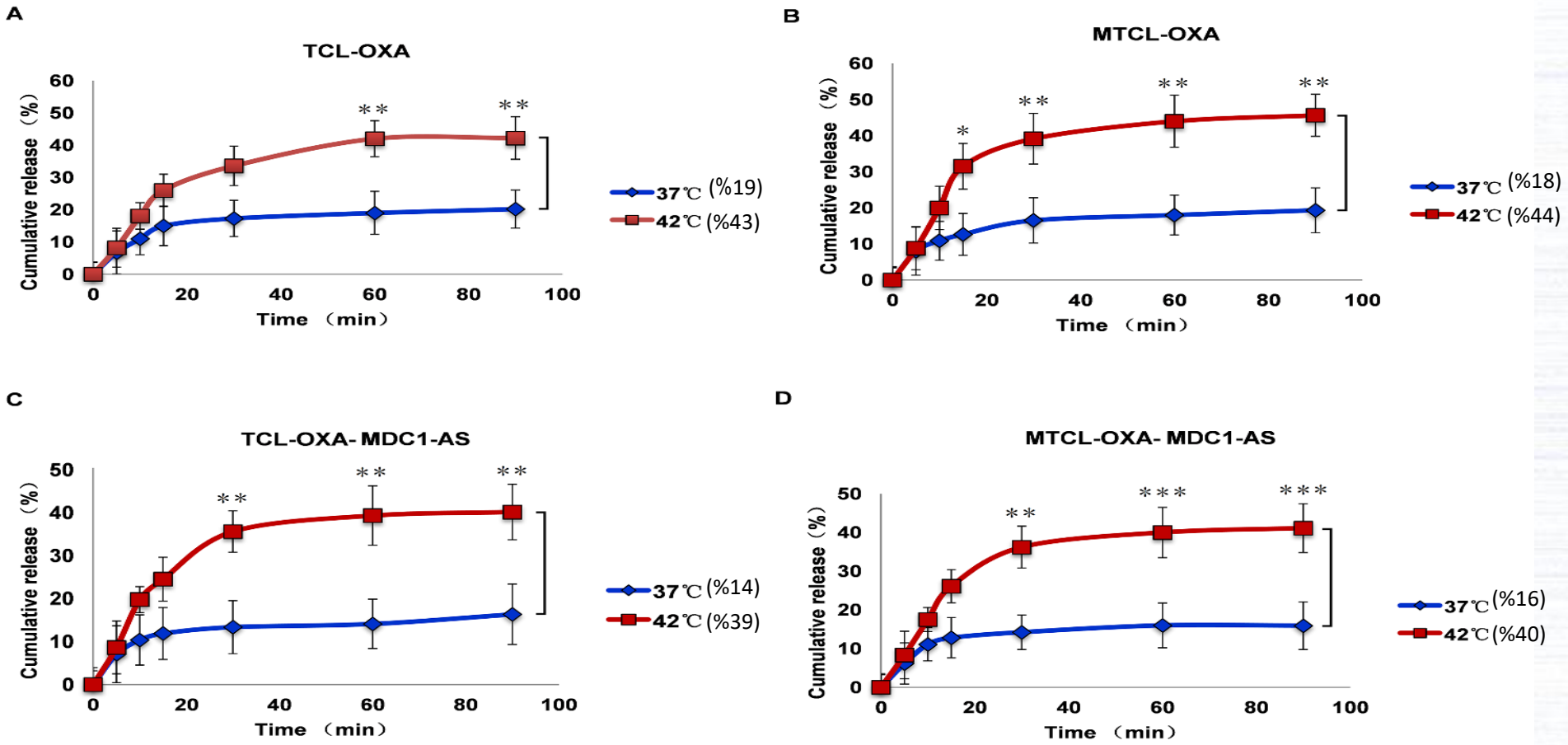
Table 2 Particle Size, Polydispersion, and Zeta Potential of Liposomes (n=3)

Groups	Particle Size (nm)	Polydispersion	Zeta Potential (mV)
TCL	86.5±9.3	0.183±0.032	+59.3±6.2
TCL-OXA	138.3±8.1	0.227±0.025	+56.0±4.1
TCL-MDC1-AS	176.2±12.5*	0.205±0.019	+41.4±5.9*
TCL-OXA-MDC1-AS	262.7±17.3*	0.301±0.051	+30.2±5.0*
MTCL	140.6±15.9#	0.246±0.034	+50.5±6.4
MTCL-OXA	178.3±16.1#	0.238±0.028	+53.2±3.7
MTCL-MDC1-AS	243.0±13.4*,#	0.262±0.021	+29.8±5.4*
MTCL-OXA-MDC1-AS	350.5±21.7*,#	0.299±0.046	+21.5±4.3*

Notes: *P<0.05, TCL–MDC1-AS vs TCL; TCL–OXA–MDC1-AS vs TCL–OXA; MTCL–MDC1-AS vs MTCL; MTCL–OXA–MDC1-AS vs MTCL–OXA. #P<0.05, MTCL vs TCL; MTCL–OXA vs TCL–OXA; MTCL–MDC1-AS vs TCL–MDC1-AS, and MTCL–OXA–MDC1-AS vs TCL–OXA–MDC1-AS.

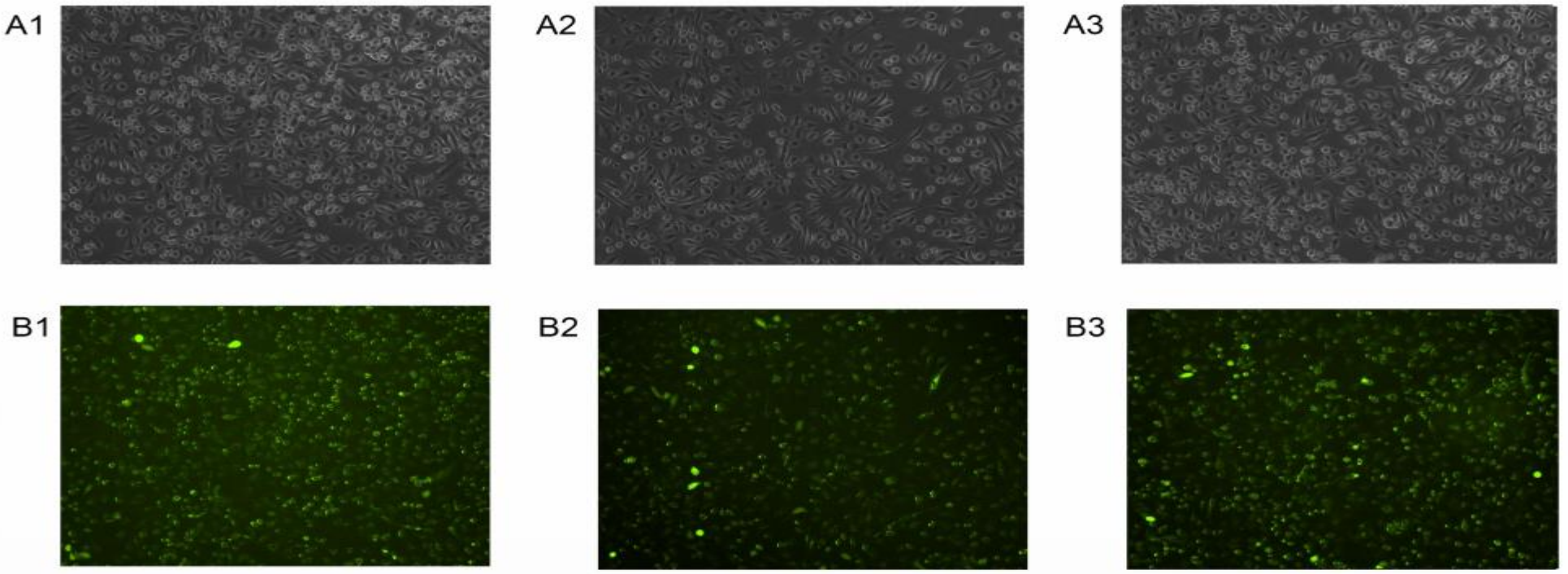


Determination of OXA Thermosensitive Release Rate



LP based on TCL exhibited efficient OXA thermosensitive release and could be used to induce OXA thermosensitive-controlled release triggered by AMF in vitro.

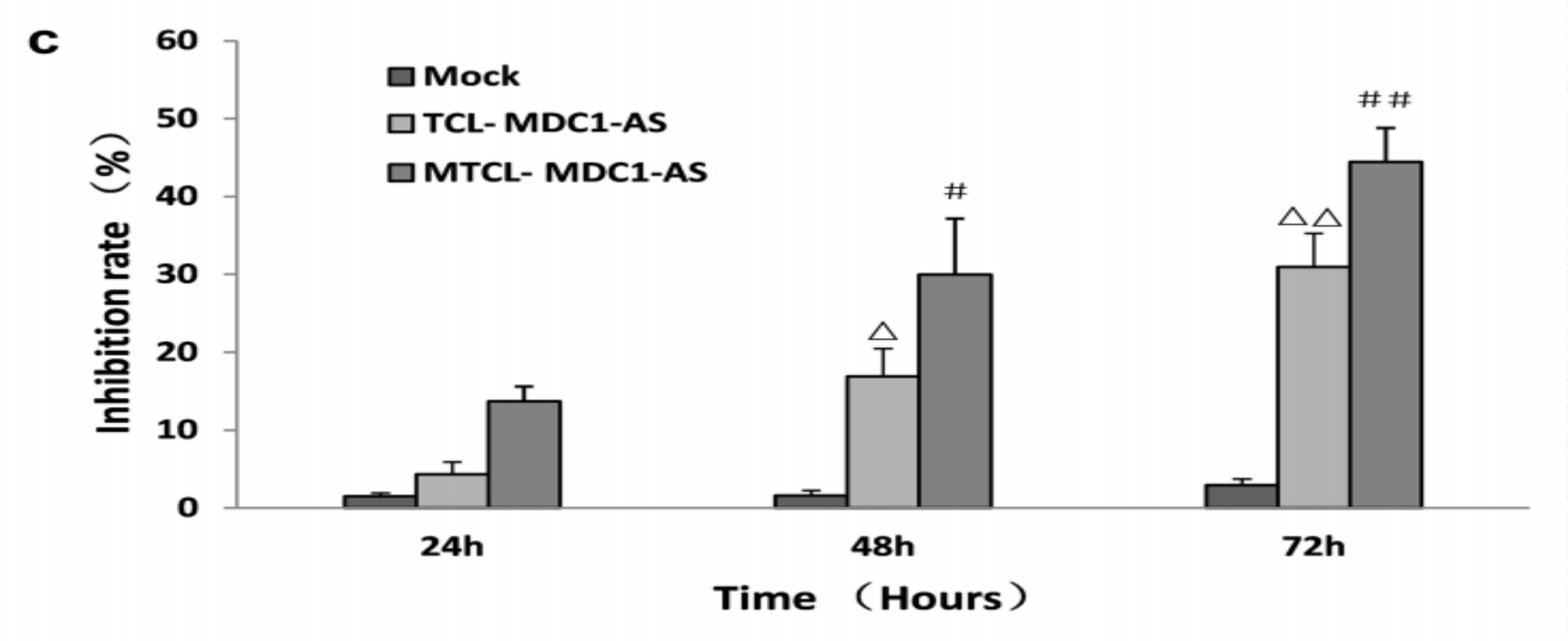
The transfection efficiency of the MDC1-AS gene



Morphology of SiHa cells in each group before transfection: A1, A2, and A3 represent the morphology of SiHa cells before transfection with **mock**, **TCL–MDC1-AS**, and **MTCL–MDC1-AS**, respectively

The combined effect of MTCL-MDC1-AS and in vitro directed magnetic field could significantly improve the gene transfection efficiency ($P<0.01$)

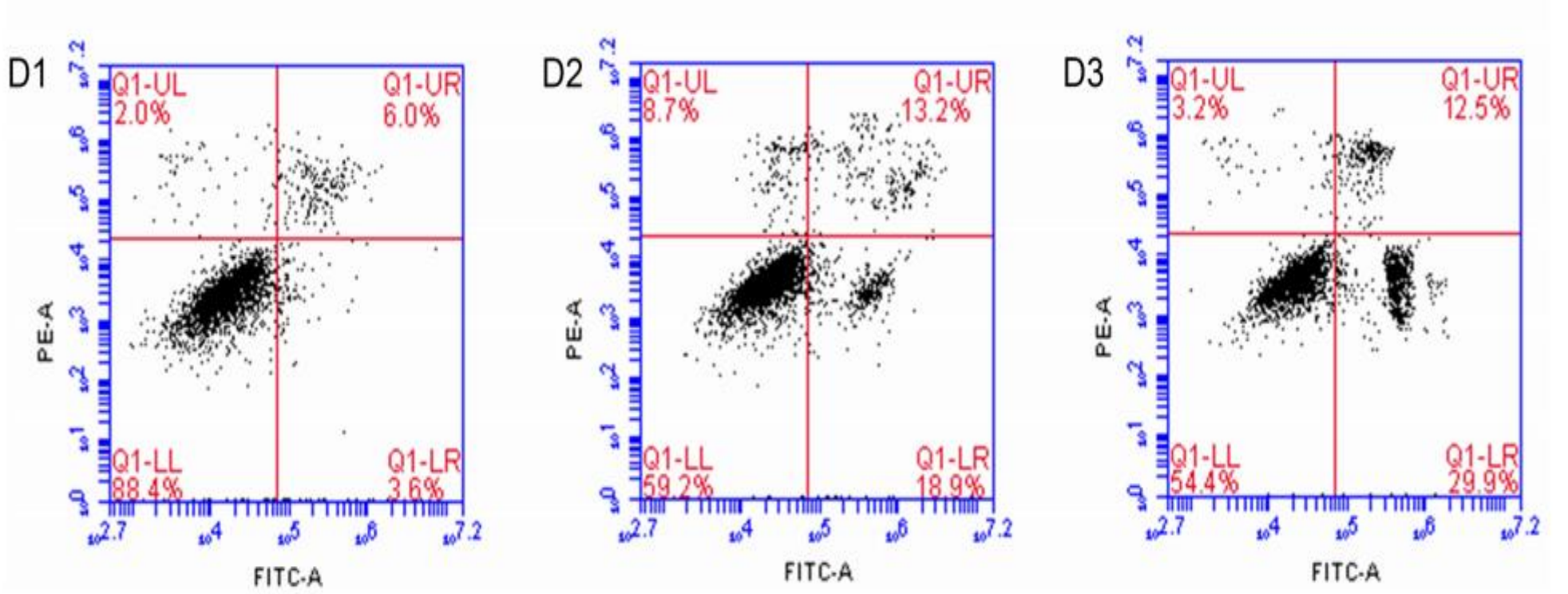
Effect on Cell Proliferation



Cell inhibition rate= [1−(experiment group A value/ mock group A value)] × 100

The inhibition rates of MTCL MDC1-AS were significantly higher than those of the TCL-MDC1-AS($P<0.0001$) groups, and the highest inhibition rates were observed after 72 h

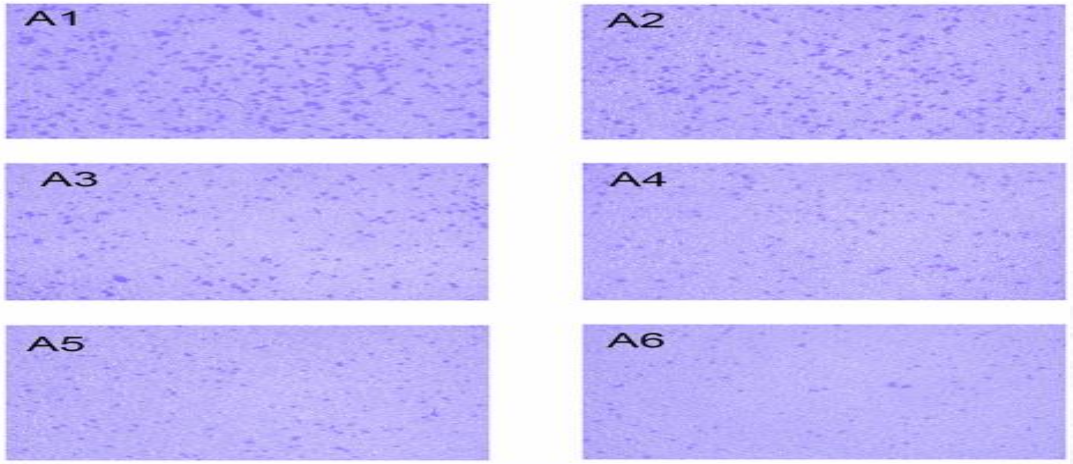
Effect on Cell Apoptosis

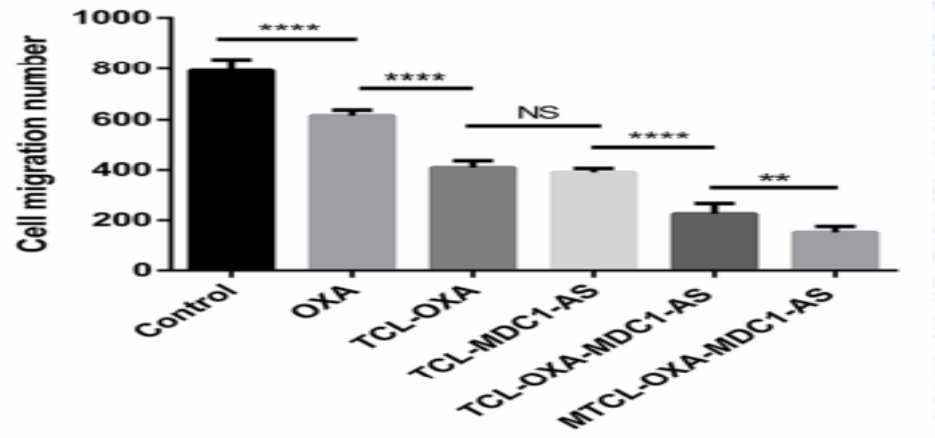


The apoptosis rates of SiHa cells transfected using TCL–MDC1-AS and MTCL–MDC1-AS: The apoptotic rates of SiHa cells in the control group and experimental groups are 3.6% ± 1.5% (D1: **Mock** group), 18.9% ± 2.6% (D2: **TCL–OXA–MDC1-AS** group), and 29.9% ± 2.5% (D3: **MTCL–OXA–MDC1-AS** group).

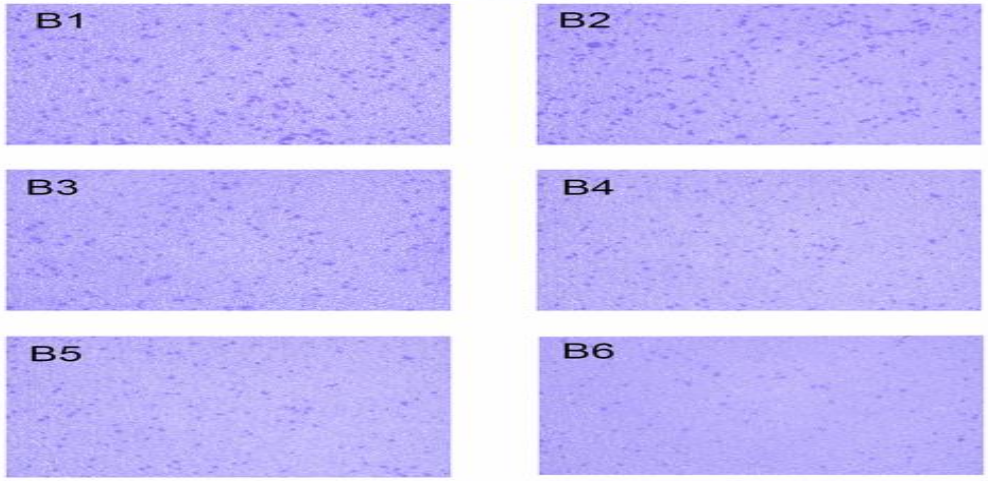
The apoptotic rates of MTCL-MDC1-AS were significantly higher than those of TCL-MDC1-AS ($P<0.01$) and mock ($P<0.001$) groups

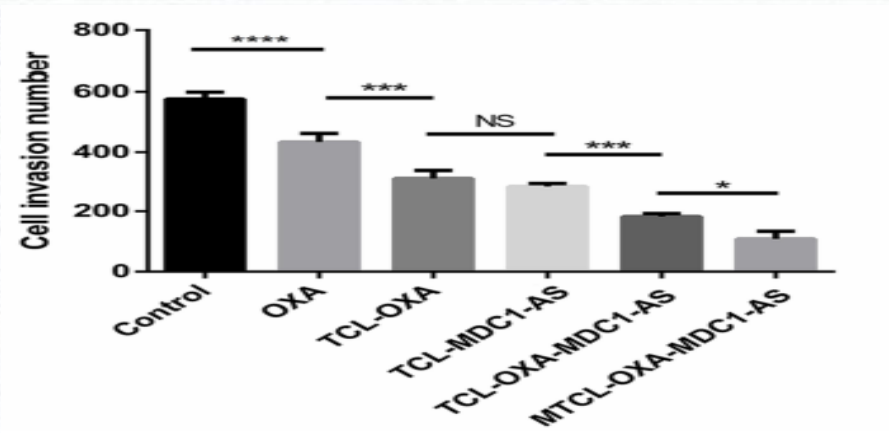
Cell Migration Assay





Cell Invasion Assay

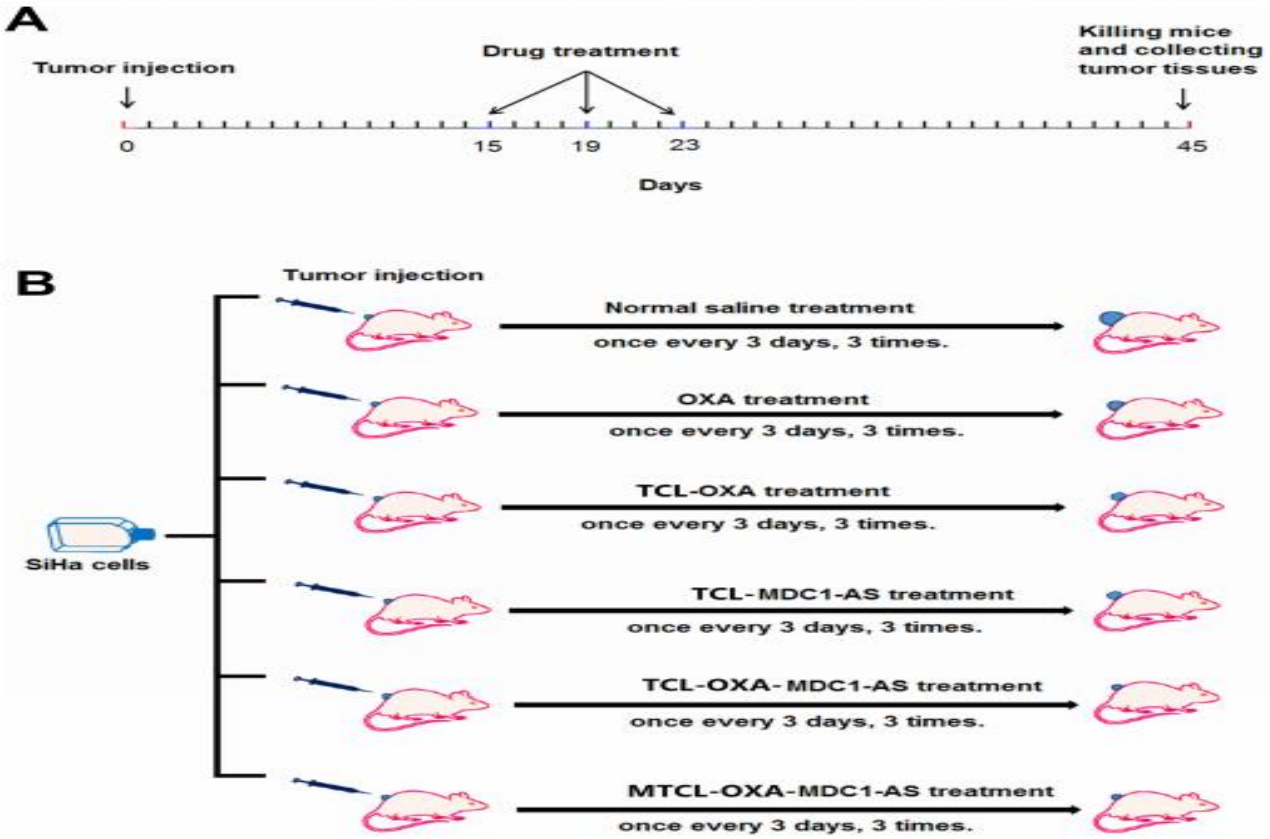


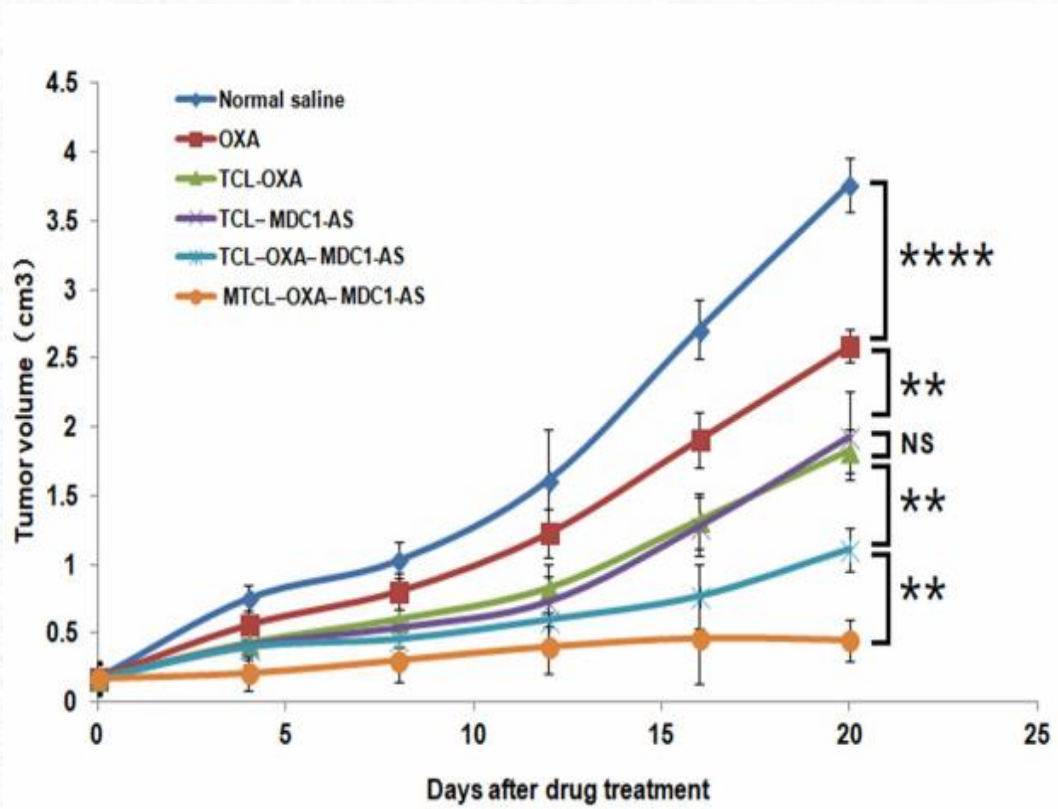


A1-A6 respectively represent the control, OXA, TCL-OXA, TCL-MDC1-AS, TCL-OXA-MDC1-AS, and MTCL-OXA-MDC1-AS groups. In the invasion state of SiHa cells, B1-B6 respectively represent the control, OXA, TCL-OXA, TCL-MDC1-AS, TCL-OXA-MDC1-AS, and MTCL-OXA-MDC1-AS groups. *P< 0.001, ****P<0.0001.

MTCL-OXA-MDC1–AS could significantly inhibit the migration and invasion activity of the SiHa cells.

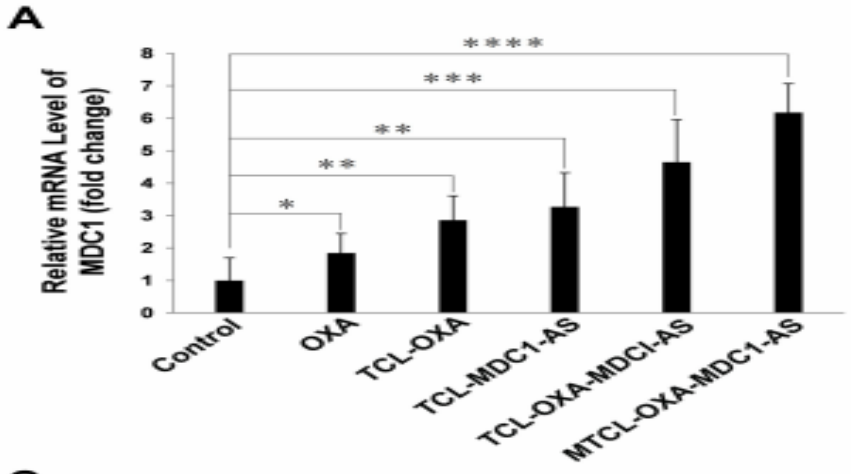
Establishment of the Animal Model and in vivo Tumor Inhibition Test

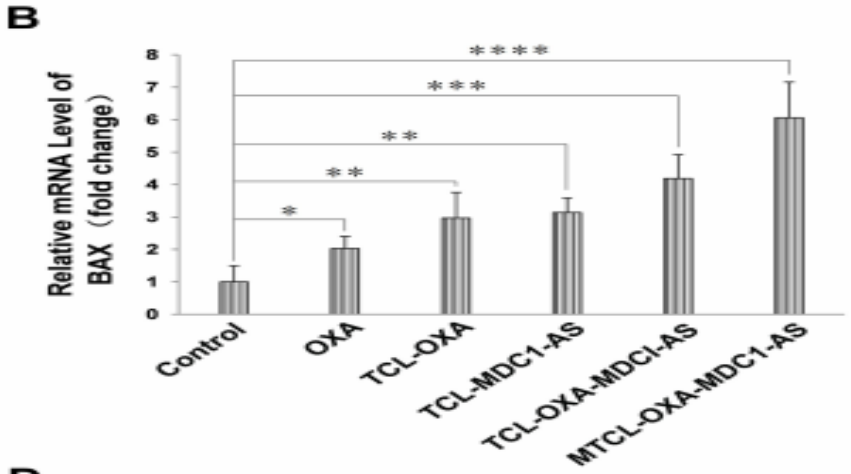


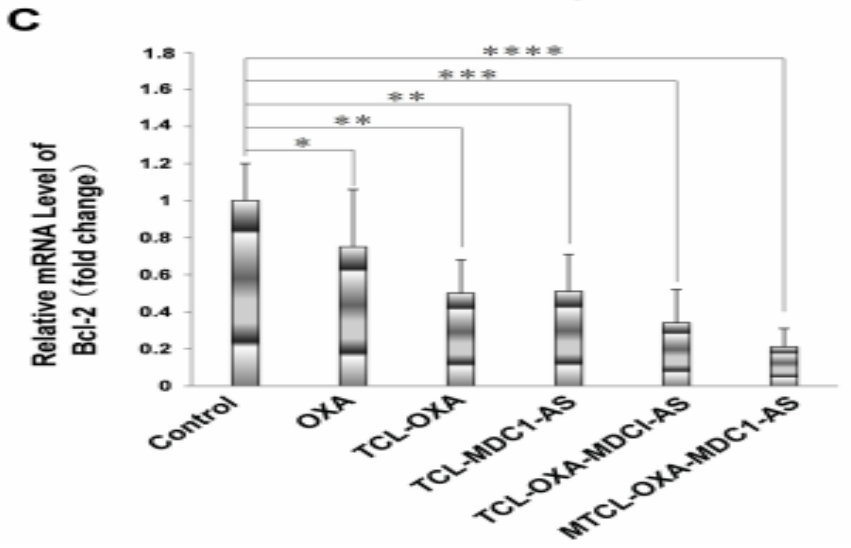


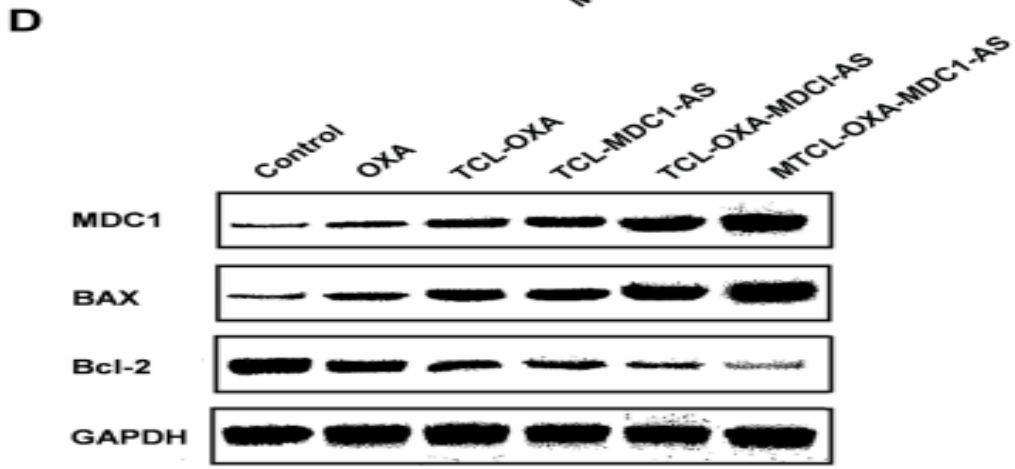
In vivo antitumor effect produced from the combined therapy was significantly stronger than that of a single application of OXA or MDC1- AS, and magnetic targeting could enhance the antitumor effect

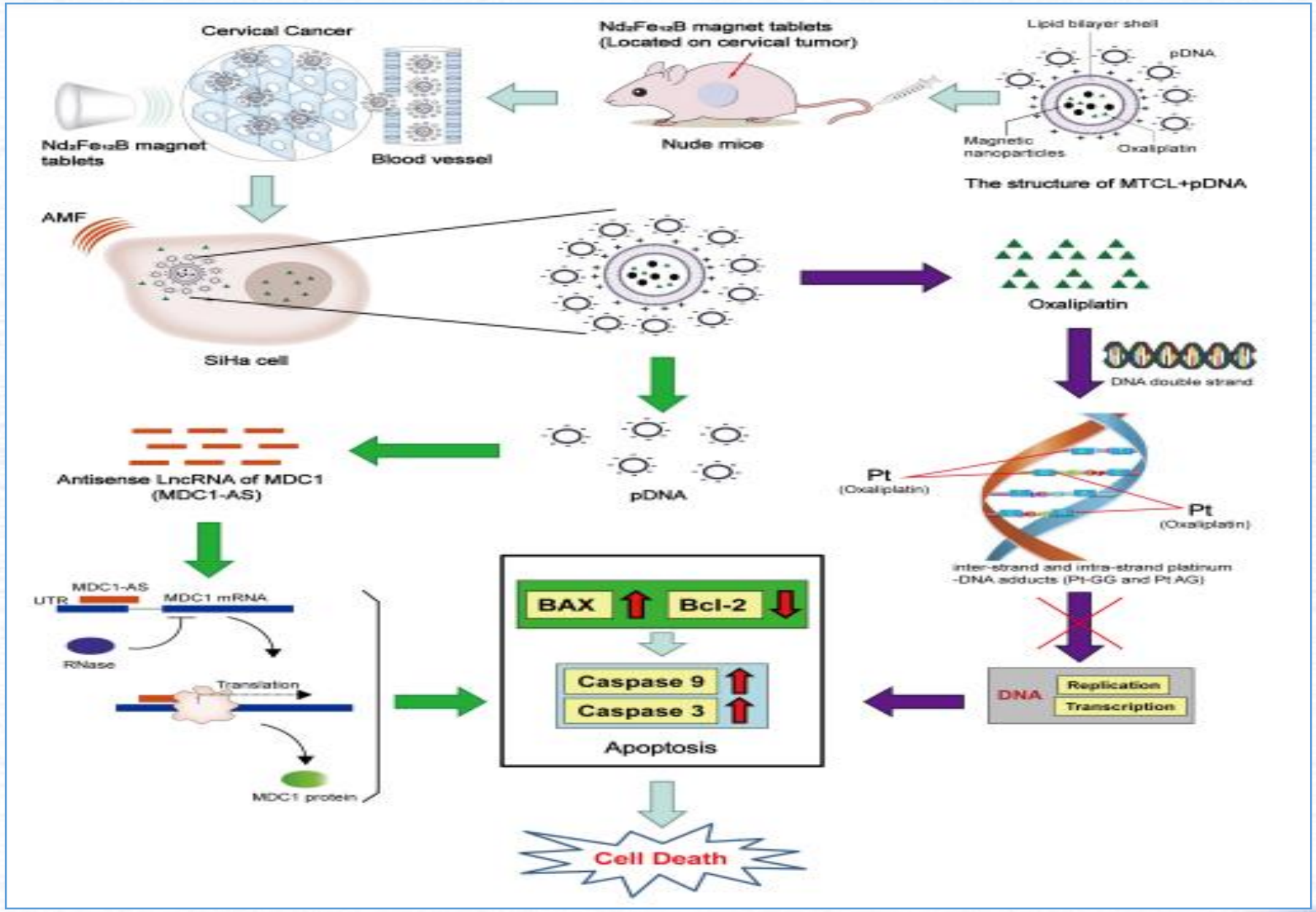
Detection of Apoptosis-Related Genes in the Cervical Cancer Through RT-QPCR Analyses and Western Blot Analysis










Cervical Cancer
Nd2Fe14B magnet tablets
Blood vessel
Nd2Fe14B magnet tablets (Located on cervical tumor)
Nude mice
Lipid bilayer shell
pDNA
Magnetic nanoparticles
Oxaliplatin
The structure of MTCL+pDNA
AMF
SiHa cell
Oxaliplatin
DNA double strand
Antisense LncRNA of MDC1 (MDC1-AS)
pDNA
Pt (Oxaliplatin)
Pt (Oxaliplatin)
inter-strand and intra-strand platinum -DNA adducts (Pt-GG and Pt AG)
MDC1-AS
UTR
MDC1 mRNA
RNase
Translation
MDC1 protein
BAX
Bcl-2
Caspase 9
Caspase 3
Apoptosis
DNA
Replication
Transcription
Cell Death

- The novel codelivery system of drug and gene (MTCLs) possessed satisfactory combined characteristics of magnetic targeting under a directed magnetic field, thermosensitive controlled release triggered by AMF, and **synergistic antitumor effect** in vitro and in vivo.

- MTCL-MDC1-AS exhibited higher cytotoxicity for Cervical cancer cells, significantly enhanced apoptosis, and inhibited the migration and invasion activities of SiHa cells.

- Transplanted tumor test of nude mice with Cervical cancer in vivo showed that codelivery of OXA and MDC1-AS carrier presented strong inhibitory effect on the tumor growth under a magnetic field.

The proposed Codelivery System offers potential applications in the Chemotherapy and Gene therapy for Cervical cancer

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