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Immunotherapy: Isolation and Detection of Exosomes of Natural Killer Cells

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Abstract: Natural killer (NK) cell-based immunotherapies show increasing research popularity in cancer treatment. With the ability to kill target cells in a non-MHC-restricted manner (Major Histocompatibility Complex), NK cells have drawn remarkable attention for their effectiveness and significance. In particular, exosome isolation and detection are crucial in clinical use because exosomes can serve as valuable biomarkers for examining diseases. Their ability to carry and transfer biomolecules makes them an effective tool that can provide the world with a deeper understanding of disease mechanisms and develop new therapies by harnessing the potential of exosomes. Moreover, NK cell-derived exosomes (NK-Exo) also have a significant effect on cancer treatment. They direct the immune system to generate effective antitumor immune responses, making them an adaptive therapy in treating cancer. In these cases, this research aims to suggest an optimal method to isolate and detect exosomes of NK cells, which are the critical first steps in employing NK-Exos into clinical applications. Isolating them by ultracentrifugation, observing them under fluorescence microscopy, and detecting protein markers in exosomes will surely allow us to understand the significance of exosomes in nuanced detail.

1. Introduction

Immunotherapy is crucial in cancer treatment due to its significance in harnessing the human immune system to fight cancer cells, offering various benefits in clinical applications, including fewer side effects compared to traditional cancer treatments such as chemotherapy. Nevertheless, while being an effective application for curing cancer, the limitations should also be taken into consideration, such as unpredictable efficacy, difficulties in identifying biomarkers, and the development of resistance. Alternatively, Natural killer (NK) cells, a vital component of the innate immune system, are frequently utilized in cancer immunotherapy. NK cells, T-cells, and B-cells utilize distinct types of antigen receptors to recognize and respond to potential threats.

NK cell-derived exosomes (NK-Exo), extracellular vesicles secreted by NK cells, could enhance the ability of NK cells to kill cancerous cells in cell-to-cell communication. Exosomes are tiny vesicles released by cells, playing a complex and important role in cancer treatment. NK-Exo, by acting as a messenger in cells, carries molecules that can either activate other immune cells or directly

target and kill cancer cells. For instance, NK-Exos deliver cytotoxic molecules such as perforin and granzymes [1], targeting tumor cells carrying homologous antigens and inducing apoptosis in the target cells. Therefore, the induction of apoptosis as a result of DNA damage in precancerous lesions can remove potentially harmful cells, thereby blocking tumor growth. NK-Exos show promise as a cancer treatment, overcoming the limitations of NK cell therapy and their potential as a drug delivery vehicle in the future in clinical applications.

Although NK cells and NK-Exos depict great potential in cancer immunotherapy, several limitations hinder their clinical use[2] . A significant example would be, in Chimeric Antigen Receptor NK-cell (CAR-NK) cell therapy, challenges include maintaining NK cell persistence and overcoming the immunosuppressive tumor microenvironment (TME). This can be concluded that even though CAR-NK plays an important role in cancer therapy, it still presents safety concerns and requires further improvements. Yet, CAR-NK therapy could overcome its limitations by employing gene editing technology, such as CRISPR-Cas9 is used to enhance the effectiveness of CAR-NK by allowing for precise gene editing in NK cells, enabling the modification of genes. In this case, CAR genes are inserted and inhibitory genes are knocked out, improving efficacy [3]. Also, CAR-NK cell therapy demonstrated greater efficacy in treating blood cancers than employing CAR-T cells. While CAR-T cells exhibited treatment success, their clinical application faces challenges such as high production costs and life-threatening toxicities such as cytokine release syndrome. To overcome these limitations, genetically engineered CAR-NK cells are introduced to maximize safety with lower adverse effects [4].

On top of that, researchers are actively addressing alternative strategies such as designing multi-specific CARs, modifying NK cells to express chemokine receptors, and combining CAR-NK cells with immune checkpoint inhibitors[5] . As a result, this sufficiently proved that NK cells and NK-Exos are worth to obtain further observation and exploration with more advanced knowledge and methods.

Finally, this research aims to develop a reliable and optimal method to isolate and detect exosomes of NK cells. Ultracentrifugation would be mainly used for the isolation of NK cells [6]. In addition, NK cells would be labelled with fluorescent probes specific for their contents. This study mainly focuses on further improving existing detection methods or combining them with ultra-high-speed centrifugation to obtain a large amount of exosome proteins and detect them.

2. Materials and methods

2.1 NK Cell Subculture

(1) Experimental equipment

In Table 1, we have listed all key reagents, with their brands, catalog numbers, and batch numbers specified for reagent identification and quality control.

Table 1 Reagents used, their brands, catalog numbers, and batch numbers.

Reagent	Brand	Product umber	Batch number
DMEM culture medium	Gibco	C11995500BT	6124394
RPMI Medium 1640 Culture medium	Gibco	C11875500BT	SA231114
10% Fetal Bovine Serum (FBS)	Procell	164210-500	20241002
75% medical alcohol disinfectant solution	Ausbebo		

(2) Materials

In Table 2, we have summarized disposable consumables, with their brands, catalog numbers, and batch numbers indicated to ensure consistency in material usage.

Table 2 Consumables used, including brand name, product number, and batch number

Reagent	Brand	Product umber	Batch number
15mL EP tube	KIRGEN	KG2614	166231215J
50mL EP tube	KIRGEN		
90mm X 15mm round Petri dish	NEST	704001	

(3) Instrument

In Table 3, key equipment is documented, with their brands and models noted to guarantee the reproducibility of the experiment.

Table 3 Instruments used and their brands and models

Instrument	Brand	Model
Carbon dioxide incubator	Thermo Scientific	FORMA STERI-CYCLE i160
Biology safety cabinet – ultra-clean workbench	Airstream	AC2-5S1
Inverted differential phase contrast microscope	OLYMPUS	BWS-10
Centrifuge	Cence	TDZ5-WS
Waste liquid suction pump	Kylin-Bell	GL-80

(4) Experimental Procedures

Cell Passaging

1) Remove RPMI 1640 medium in the 6cm ²culture dish

2) Transfer the suspension cells into a 15 mL tube

3) Centrifugated at 900 xg for 5 minutes

4) Disinfect the equipment with 75% alcohol and place it in a laminar flow hood

5) Discard the supernatant and collect the cell pellet

6) Add 5 mL of RPMI 1640 complete medium and transfer the cell pellet to a clean 6 cm ²culture dish

7) The 6 cm ²culture dish is placed in a humidified incubator at 37 ℃ overnight, maintaining cell growth and expansion in a static environment

2.2 Isolation of NK-derived exosomes

(1) Experimental equipment

The reagents used, along with their brands, catalog numbers, and batch numbers, can be seen in Table 4.

Table 4 Reagents used, their brands, catalog numbers, and batch numbers.

Reagent	Brand	Product number	Batch number
RPMI Medium 1640 Culture medium	Gibco	C11875500BT	
1×PBS buffer (cell culture)	Biosharp	BL302A	28224968CJ

(2) Materials

The consumables used, including their brand names, product numbers, and batch numbers, can be seen in Table 5.

Table 5 Consumables used, including brand name, product number, and batch number.

Reagent	Brand	Product number	Batch number
90 mm ×15 mm round culture dish	NEST	704001	
1.5 mL microcentrifuge tube	Biosharp	BS-15-M	
100-1000μL pipette	ThermoScientific		QH38264
0.5-10μL pipette	Eppendorf		H50805H
5mL single-use sterile syringe with needle	Hongda		20230308
High-speed centrifuge tube	BECKMAN	355618	A50715

(3) Instrument

Instruments used, along with their brands and models, can be seen in Table 6.

Table 6 Instruments used and their brands and models

Instrument	Brand	Model
High-speed desktop refrigerated centrifuge	Cence	H1850R
Microbalance	METTLER TOLEDO	ME204
Ultra-high speed centrifuge	BECKMAN COULTER	Optima XE-100
Small desktop high-speed centrifuge	SCILOGEX	D1008
Dynamic light scattering (DLS) instrument	Malvern	Zetasizer Nano Zen

(4) Experimental equipment

The reagents used, their brands, catalog numbers, and batch numbers can be seen in Table 7.

Table 7 Reagents used, their brands, catalog numbers, and batch numbers.

Reagent	Brand	Product umber	Batch number
4% polyformaldehyde general purpose tissue fixative	biosharp	BL539A	24005654
1×PBS buffer (cell culture)	Biosharp	BL302A	28224968CJ
Anti-fluorescence quenching mounting medium (containing DAPI)	Beyotime	P0131-25ml	
Actin-Tracker Green-488			
SIZILIAN Nail Polish	Beyotime SIZILIAN		

(5) Materials

The consumables used, including their brand names, product numbers, and batch numbers, can be seen in Table 8.

Table 8 Consumables used, including brand name, product number, and batch number.

Reagent	Brand	Product number	Batch number
0.5-10.0μL pipette	Thermo Scientific		TO52435 PZ27320
100-1000μL pipette	Thermo Scientific		
10μL boxed tips	KIRGEN		
200μL boxed tips	KIRGEN		
1000μL extended filter cartridge Boxed tips	KIRGEN	KG5333	
Adhesive glass slide	CITOTEST	188105	
Standard microscope coverslips	CITOTEST	10212424C	

(6) Instrument

The instruments used, along with their brands and models, can be seen in Table 9.

Table 9 Instruments used and their brands and models

Instrument	Brand	Model
High-speed desktop refrigerated centrifuge	cence	H1850R
Small desktop high-speed centrifuge	Eppendorf	5425
Research-grade inverted microscope	Nikon	

(7) Experimental Procedures

1) Isolation

a) Dilute the exosome precipitate by using 1 ml Phosphate Buffered Saline (PBS)

b) Filter out any unwanted components by using a 0.8 μm sterile filter five times and a 0.2 μm sterile filter eight times, and collect the filtrate

c) Ultracentrifuged the filtrate at 10000xg for 70 minutes

d) The size and Zeta potential of NK-Exos are detected with Dynamic Light Scattering (DLS) and Zeta Potential Analyzers, respectively

2) Immunofluorescence (IF)

a) Place glass coverslips into each cell culture insert of the 12-well plate

b) Cell adhesion reagents are diluted with PBS to prepare for IF staining of suspension cells

c) 16 μL of cell solution is added to each cell culture insert. 200 μL of Alexa Fluor 488 (1 μL of Alexa Fluor 488 is diluted with 200 μL of PBS) and 500 μL of 4% Paraformaldehyde (PFA) are also added to each insert

d) Leave the samples for 10 minutes

e) Wash the samples with PBS 5min x3 on a shaker

f) Add 8μL of DAPI-containing fluorophore quencher onto a microscope slide

g) Use the forceps to gently pry up the glass coverslip and transfer it to the microscope slide. Put it under the fluorescence microscope

2.3 Detection of NK-derived exosomes by Western Blot

(1) Experimental equipment

Reagents used, their brands, catalog numbers, and batch numbers. can be seen in Table 10.

Table 10 Reagents used, their brands, catalog numbers, and batch numbers.

Reagent	Brand	Product number	Batch number
One-step gel preparation reagent (concentrated gel)	Fdbio science	FD346	20230130
10% One-Step Gel Preparation Reagent	Fdbio science	FD341	20241128
Tween-20	Fdbio science	FD0020	20221024
Tetramethylethylenediamine (TEMED)	Aladdin		D2428533
10% ammonium persulfate			
Electrophoresis solution			
Transfer solution			
PageRuler Prestained Protein Ladder	Thermo Scientific	26616	2895071
TBST			
Methanol	XIHUA		
Imprint sealing reagent	BIO-RAD		B2305261
Pure water		1706404	
TBS			
CD63 Antibody	Abmart		
β-Actin Antibody	Abmart	M051014 S	
Na，K-ATPase α1 rabbit RIPA Lysis Buffer (Strong)	Cell Signaling	P30002L	
HRP-labeled goat anti-mouse IgG (H+L)	Beyotime	3010S	
HRP Marked goat anti-rabbit IgG (H+L)	Beyotime	P0013B	122123240314
ECL Enhanced Plus Kit P	Beyotime	A0216	
		A0208	
	ABclonal		
		RM00021	0000010132

(2) Materials

The consumables used, including their brand names, product numbers, and batch numbers, can be seen in Table 11.

Table 11 Consumables used, including brand name, product number, and batch number.

Reagent	Brand	Product number	Batch number
1.0mm electrophoresis comb	Tamon		
Transfer clamp	DFRL	1703931	
Glass plate (with 1 mm spacing)	BIO-RAD	1653311	
T cell CTLL-2 protein sample			
NK cell NK92 protein sample			
PVDF transfer film	Millipore	ISEQ00010	0000204510

(3) Instrument

The instruments used, along with their brands and models, can be seen in Table 12.

Table 12 Instruments used and their brands and models

Instrument	Brand	Model
Chemiluminescence analyzer	BIO-RAD	

(4) Experimental Procedures

1) Electrophoresis

a) Put the gel into the electroporator

b) Pour the running buffer into the electroporator and make sure it covers the gel completely, and remove the comb

c) Add 3 μL of marker for identifying exosome proteins, and 10 μL of exosome sample in each well

d) Connect the electroporator to the voltage operator (red to red, black to black)

e) Run the gel with 90V for 30min and 120V for 1 hour

2) Electrotransfer

a) Cut 6 pieces of filter paper and 1 polyvinylidene fluoride (PDVF) to fit the measurement of the gel

b) Wet a sponge and the filter papers in transfer buffer, and wet the PVDF membrane in methanol

c) Retrieve the gel from the glass plates. Create an electrotransfer sandwich as such: 1 sponge, 3 filter papers, gel PDVF (ensure there are no air bubbles), and 3 filter papers

d) Transfer the sandwich to the transfer apparatus and cover the apparatus with transfer buffer

e) Place the apparatus in ice and keep the temperature at 4 ℃

f) Run the gel PDVF with 300 mA current for 2 hours

3) Blocking and antibody incubation

a) Block the membrane with skim milk in TBST on a shaker for 1 hour

b) Add primary antibody *in 5% bovine serum albumin (BSA) and incubate overnight at 4 ℃ on a shaker

c) Wash the membrane with TBST 5 minutes X3

d) Add secondary antibody in skim milk in TBST, and incubate for 1 hour

e) Wash the membrane with TBST 5 minutes X3

f) Prepare ECL mix

g) Place the membrane onto the chemiluminescence analyzer

h) Add dye to fully cover the membrane

i) Visualize the result in the scanner

2.4 Detection of Exosome-Cancer Cell Interaction

(1) Experimental equipment

The reagents used, their brands, catalog numbers, and batch numbers can be seen in Table 13.

Table 13 Reagents used, their brands, catalog numbers, and batch numbers.

Reagent	Brand	Product number	Batch number
75% medical alcohol disinfectant solution	Ausbebo		20241002
Actin-Tracker Green-48Beyotime	Beyotime		

(2) Materials

The consumables used, including their brand names, product numbers, and batch numbers, can be seen in Table 14.

Table 14 Consumables used, including brand name, product number, and batch number.

Reagent	Brand	Product number	Batch number
CTLL-2 cell vesicles 1.5mL microcentrifuge tube 20-200 microliter pipette	Biosharp	BS-15-M	L12982H
0.1-2.5μL single-channel pipette	Eppendorf	Research plus 0.1	
200μL boxed tips 10uL boxed tips	KIRGEN	KG1232	1906611
Human colorectal cancer cells HT29			

(3) Instrument

The instruments used, along with their brands and models, can be seen in Table 15.

Table 15 Instruments used and their brands and models Table

Instrument	Brand	Model
Stopwatc	DAKEWE	
Inverted microscope	Nikon	Eclipse Ti2-E
Carbon dioxide incubator	Thermo Scientific	FORMA STERI-CYCLE i160

(4) Experimental Procedures

1) HT 29 colorectal cancer cells are plated in a confocal well for live-cell imaging
2) Cells were allowed to adhere for 12 hours
3) Dye HT29 cells with 1mL of Alexa Fluor 488 for 10 minutes (1:200)
4) Centrifugate at 21100xg for 10 minutes to pellet exosomes
5) Resuspension of the exosome pellet in 1 mL PBS
6) Co-incubation of exosomes with HT29 cells for 5-10 minutes
7) Discard supernatant
8) Fixation using 4% PFA to preserve cell interaction for imaging
9) 200 μL PBS 5 minutes X3 to wash away residual PFA or unbound exosomes
10) Observation under fluorescence microscopy

2.5 Detection of Exosomes Killing Rate

1) Experimental equipment

The reagents used, their brands, catalog numbers, and batch numbers can be seen in Table 16.

Table 16 Reagents used, their brands, catalog numbers, and batch numbers.

Reagent	Brand	Product number	Batch number
0.25% Trypsin-EDTA(1X)	Gibco	25200-072	2462017
DMEM Culture Medium (1X)	Gibco	C11995500BT	6124394
CCK-8	Selleckchem	B34302	

2) Materials

The consumables used, including their brand names, product numbers, and batch numbers, can be seen in Table 17.

Table 17 Consumables used, including brand name, product number, and batch number.

Reagent	Brand	Product number	Batch number
Cell culture plate 90mm × 15mm round culture dish	NEST		08223BL010829B
Cell counting chamber	NEST	704001	
Adhesive slid	CITOTEST	188105	

3) Instrument

The instruments used, along with their brands and models, can be seen in Table 18.

Table 18 Instruments used and their brands and models Table

Instrument	Brand	Model
Biological safety cabinet	Thermo Scientific	1374
Centrifuge	Cence	TDZ5-WS
Cell counter		
Inverted phase contrast microscope	OLYMPUS	CKX53
Multifunctional enzyme marker	BioTek	Synergy H1

4) Experimental Procedures

a) Add 100 µL of HT29 cancer cell suspension into each cell culture insert of a 96-well plate

b) Incubate cell suspension in the incubator overnight

c) Create the following concentrations: control (no cell, adding PBS solution only), control (negative, adding PBS solution and HT29 cancer cells), 10 µg, 50 µg, and 100 µg of exosomes concentration

d) Add 10 uL of CCK-8 solution into each insert and ensure no air bubbles are added

e) Incubate cell suspension in the 96-well plate for 4 hours

f) Measure the absorbance of cancer cells at 450 nm using a microplate reader, detecting the activity of HT29 cancer cells, and infer the killing rate of NK-Exos

2.6 In Vivo Mouse Experiments

Experimental equipment

Curved forceps, Straight forceps, Dissecting scissors, Mouse restraint device, 1mL syringe.

Experimental Procedures

1) Tail vein injection

Figure 1: Tail vein injection of a mouse being inserted into the tail vein restrainer

a) Once the mouse enters the restrainer, gently tilt it and close your hands over the restrainer, as shown in Figure 1.

b) Inject medium through the tail vein

2) Intraperitoneal injection

a) Insert the needle bevel up in a position below the bend of the mouse's knees, either to the left or right of the midline, as shown in Figure 2.

b) Angle the needle approximately 35 °-45 °to the body and aspirate prior to ensure there are no air bubbles. Ensure no blood is seen in the needle hub

Figure 2: Intraperitoneal injection through the mouse's knees to the left of the midline

3) Subcutaneous injection

a) Firmly scruff the mouse and create a pocket of loose skin between the shoulder blades, as shown in Figure 3.

b) Insert the needle into the pocket and aspirate prior to ensure there are no air bubbles

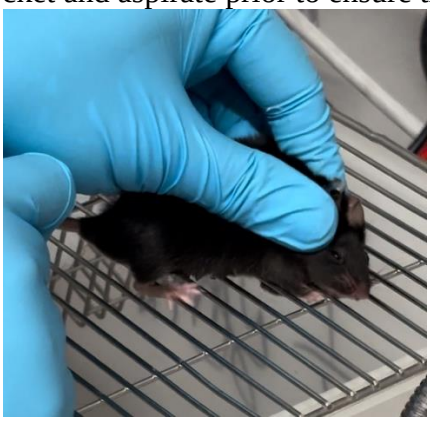

Figure 3: Subcutaneous injection in which the conductor is creating a pocket of loose skin between the shoulder blades of a mouse

3. Experimental Results and Analysis

3.1 NK Cell Subculture

Two image representations of the preparation for cell passaging are shown in Figure 4A, presenting the appearance and condition of the NK cells before adding fresh medium. It is clear to see that the cells, after adding fresh medium, the cells became more separated and were suspended. On top of that, the cell condition after overnight incubation is shown below in Figure 4B, which clearly shows

that the attached cells are recovering from the subculturing process and begin dividing.

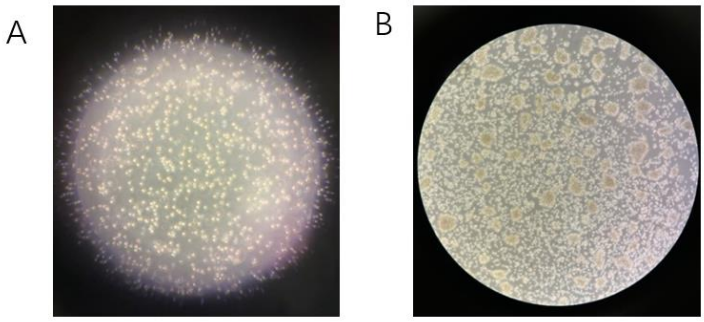

Figure 4: Cell passaging process of NK cells. A: cell sample after adding medium during cell subculture, B: cell condition after overnight incubation

3.2 Isolation of NK-derived exosomes

From the result obtained, ultracentrifugation has been demonstrated as an effective method for isolating NK-Exos. From the data on the size measurement of NK-Exos in DLS (Figure 5), the exosomes detected were in the range of 85nm to 150nm. This size range aligns with the expected size of exosomes, which is from 30nm to 150nm. This result implies a relatively narrow size distribution, implying that the sample is mostly composed of exosomes and is not significantly contaminated by other out-of-size particles.

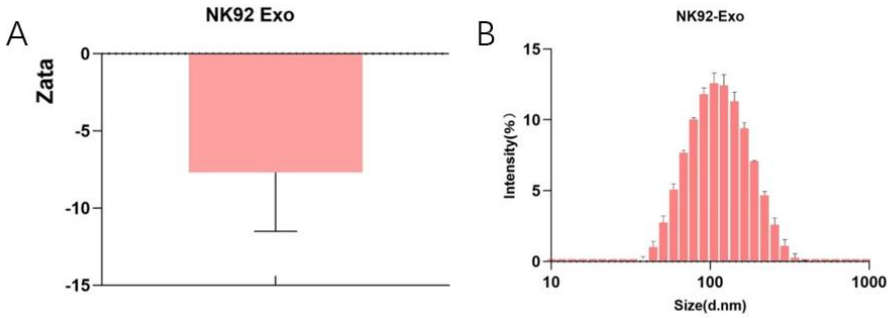

Figure 5: DLS data of NK-Exos. A: Zeta potential distribution of NK-Exos, B: Size distribution profile of NK-Exos detected by DLS

As shown in Figure 6A and Figure 6B, where the detailed Zeta potential data of NK - Exos (including measurement conditions like temperature 25.0 ℃, count rate 563.7 kcps, etc.) further support this conclusion. Moreover, the Zeta potential value of -11mV, which is a negative value, is within the range of typical Zeta potential values for exosomes (-6mV to -30mV, best at -10mV to -15mV) . This indicates that the exosomes are dispersed and have a low aggregation propensity.

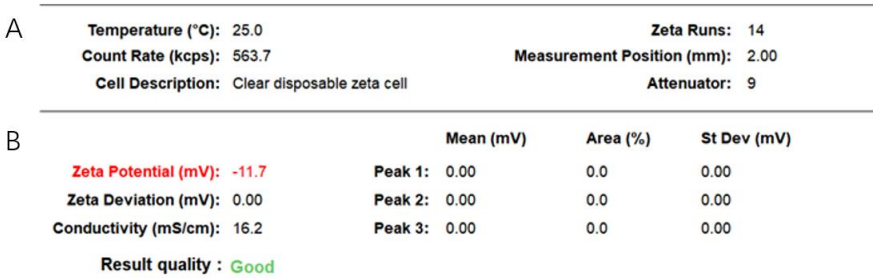

Figure 6: Zeta potential data of NK-Exos

3.3 IF Staining

The Figure 7 is an image illustrating both membrane and nuclear proteins. This presents the identification of the staining distribution in the NK cell, providing a clear visualization of protein movements out and into the membrane and the nucleus.

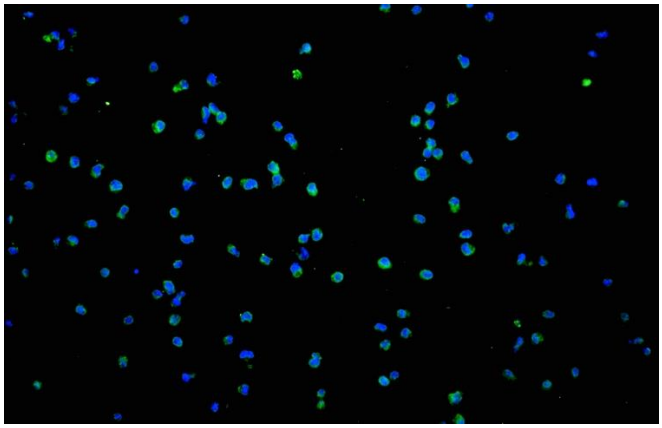

Figure 7: Visualization of exosome membrane and nucleus under fluorescence microscopy

The Figure 8 presents a clear immunofluorescence staining result with strong fluorescent signals, which indicates a successful staining process. Therefore, this implies that the protocol was optimized and effective. In particular, the protocol of IF staining included two different approaches towards the dye staining process. Respectively, half of the NK cells are added with a cell adhesion reagent before adding Alexa Fluor 488 dye; another half are added with dye first before the cell adhesion reagent. From the result, we can see that the portion of NK cells added with the cell adhesion reagent first is better stained compared to the other portion.

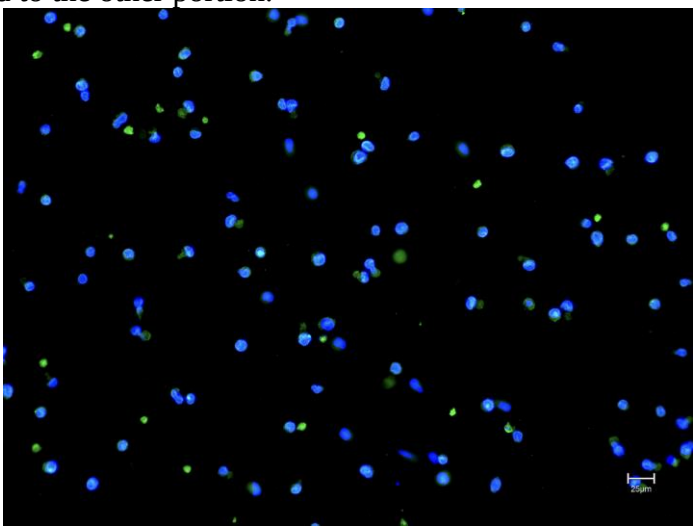

Figure 8: Visualization of exosome membrane and nucleus under fluorescence microscopy with the less preferred protocol

3.4 Western Blot

From the results, we can see that both the loading control Na, K-ATPase and the targeted protein TSG101 are detected by using Western blotting, as visualized in Figure 9. Respectively, the molecular weight (in kDa) of the protein Na, K-ATPase is within the typical range of 80 kDa to 100 kDa, and TSG101 is 40 kDa to 55 kDa. Moreover, the target protein shows a thicker band compared to the

loading control. In this case, we can conclude that there is a higher abundance of the protein TSG101 in the sample. This is because the band intensity on a Western blot is directly proportional to the amount of protein present. However, other factors like post-translational modifications or antibody concentration can also influence band thickness.

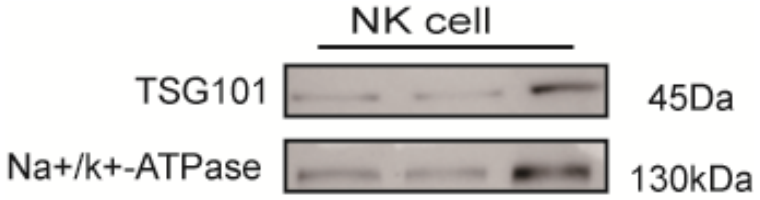

Figure 9: Western blotting showing the gene expression of the loading control Na, K-ATPase; Western blotting showing the gene expression target protein TSG101

3.5 The bingding experiment of exsomes to tumor cells

As shown in Figure10, Fluorescence microscopy is used here to visualize how NK-Exos bind to HT29 cancer cells. NK-Exos are stained with Alexa Fluor 488 dye, and appear to be green under fluorescence microscopy. HT29 cancer cells can be stained with DAPI to visualize a blue color in their nucleus. In the image below, it is shown very clearly that NK-Exos are bound to the cancer cell membrane. The successful outcome could be explained by the cancer cell preparation and binding conditions.

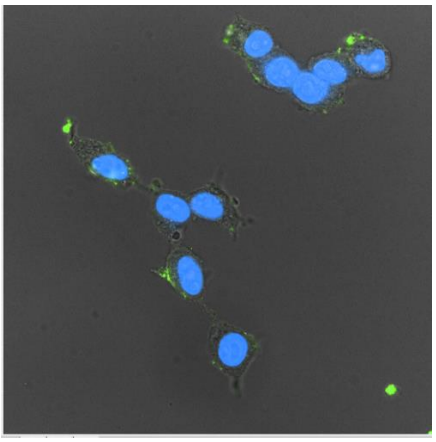

Figure 10: Visualization of NK-Exos binding to cancer cells under fluorescence microscopy

3.6 Tumor cell viability assay using CCK8

The tables 19 illustrate the data on different concentrations of exosomes inhibiting cancer cell activity.

Table 19: Different concentrations of exosomes inhibiting cancer cell activity

Exosome Concentration	Cell Activity Detection				
Control (no cell)	0.195	0.196	0.195	0.192	0.189
Control (negative)	0.51	0.461	0.478	0.45	0.457
10 ug	0.426	0.464	0.46	0.446	0.429
50 ug	0.397	0.457	0.382	0.405	0.43
100 ug	0.278	0.287	0.268	0.279	0.232

Table 20: Survival rate of cancer cells in percentage (%)

Exosome Concentration	Survival Rate (%)				
10 ug	73.3333333	101.132075	93.639576	98.4496124	89.5522388
50 ug	64.1269841	98.490566	66.0777385	82.5581395	89.9253731
100 ug	26.3492063	34.3396226	25.795053	33.7209302	16.0447761

The tables 20 illustrate that as exosome concentration increased from 10 μg to 100 μg, cancer cell activity decreased due to NK-Exos inhibition. For example, the survival rate of cancer cells in 10 μg exosome concentration is 89.6%; however, it degraded to only 16.0% survival rate in 100 μg exosome concentration. The observation of this reduction in activity conveys the cytotoxic effect of exosomes, programming cell death pathway. As a result, we can conclude that NK-Exos has a remarkable effect on inhibiting cancer cells, inhibiting more than 50% of cancer cell activity when the concentration increases from 10ug to 100ug, which presents its significance in being widely used as a clinical treatment in the future.

3.7 Live mouse experiments

The mouse tail vein (IV) injection is a common experimental technique for delivering substances like drugs, cells, or genetic material directly into the bloodstream. Also, the intramuscular (IM) injection is used for delivering substances directly into muscle tissue, used for administering vaccines and drugs. Moreover, the subcutaneous (SC) injection model in mice involves administering substances beneath the skin, commonly used for drug delivery, tumor implantation, or studying immune responses.

4. Conclusion

The significance of NK-Exos has been greatly demonstrated by its efficacy in inhibiting cancer cells' activity in this research. It is highlighted again that they show promise in cancer immunotherapy, due to their ability to inhibit cancer cell activity and potential for future clinical use. However, while NK-Exos show promise as a cancer therapy due to their capabilities as drug vehicles and their inherent anti-tumor property, challenges such as efficiency and large-scale production remain. More importantly, whether the patients would accept and employ this new therapy. In fact, patients may prefer NK-Exos as treatment more than surgery, chemotherapy, radiation therapy, targeted therapy, and other types of immunotherapies, because of its safety and having fewer side effects.

References

[1] A. S. Alfawaz Altamimi et al., "Exosomes derived from natural killer cells: transforming immunotherapy for aggressive breast cancer," Med. Oncol. Northwood Lond. Engl., vol. 42, no. 4, p. 114, Mar. 2025, doi: 10.1007/s12032-025-02647-y.

[2] S. Kundu, L. Durkan, M. O'Dwyer, and E. Szegezdi, "Protocol for isolation and expansion of natural killer cells from human peripheral blood scalable for clinical applications," Biol. Methods Protoc., vol. 10, no. 1, p. bpaf015, 2025, doi: 10.1093/biomethods/bpaf015.

[3] Y. Deng et al., "Prospects and limitations of NK cell adoptive therapy in clinical applications," Cancer Metastasis Rev., vol. 44, no. 3, p. 57, Jun. 2025, doi: 10.1007/s10555-025-10273-3.

[4] J. Chu et al., "Natural killer cells: a promising immunotherapy for cancer," J. Transl. Med., vol. 20, no. 1, p. 240, May 2022, doi: 10.1186/s12967-022-03437-0.

[5] Y. Zhong and J. Liu, "Emerging roles of CAR-NK cell therapies in tumor immunotherapy: current status and future directions," Cell Death Discov., vol. 10, no. 1, p. 318, Jul. 2024, doi: 10.1038/s41420-024-02077-1.

[6] N. Dilsiz, "A comprehensive review on recent advances in exosome isolation and characterization: Toward clinical applications," Transl. Oncol., vol. 50, p. 102121, Dec. 2024, doi: 10.1016/j.tranon.2024.102121.