# UNIVERSITY OF SALAMANCA

# MEDICAL SCHOOL

## DOCTORAL THESIS

# Influences of Umbilical Cord Mesenchymal Stem Cells and Their Exosomes on Tumor Cell Phenotype


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## HEREBY CERTIFY:

That the thesis entitled **"Influences of Umbilical Cord Mesenchymal Stem Cells and Their Exosomes on Tumor Cell Phenotype"**, by Liu Guoqing, who is seeking the degree of Doctor of Medicine from this University, has been carried out under our supervision in the Department of Surgery of the Faculty of Medicine of the University of Salamanca, and fulfils all the requirements for its presentation and defence in a court of law.

In witness whereof, we have hereunto set our hands, at Salamanca, this 7 day of March, two thousand and twenty-four.

Signed: PhD. Mª Begoña García Cenador
Thesis director

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# Abstract

Backgrounds: Umbilical cord mesenchymal stem cells (UCMSCs) are derived from umbilical cord tissue. Due to their low immunogenicity, easy availability, mature culture technology, and lack of ethical controversy, they have gradually emerged as a focal point in stem cell research. Exosomes are cell-derived vesicles with a structure consistent with the cell membrane, primarily functioning as carriers to transport substrates for intercellular communication and regulation. Presently, both stem cells and their exosomes have emerged as highly promising biopharmaceuticals widely utilized in various clinical studies, including wound healing, spinal cord injury, post-operative rehabilitation, neurological disorders, and autoimmune diseases. However, the safety of these biologics remains uncertain, with some studies suggesting the potential tumorigenicity of stem cells and their exosomes. The objective of this study was to investigate the effects of UCMSCs and their exosomes on four types of tumor cells.

Methods: In this study, first of all, UCMSCs and their exosomes were isolated and characterized. Subsequently, the study was performed in cell experiments. The effects of UCMSCs and their exosomes on four types of tumor cells (blast cancer cell line, gastric cancer cell line, glioma cell line, lung cancer cell line) were observed through co-culture experiments. The observables included the effects on in vitro phenotype and in vivo growth characteristics. Changes in gene expression were analyzed using high-throughput sequencing technology to elucidate the biological functions and roles of UCMSCs and their exosomes on tumor cells.

Results: UCMSCs were successfully isolated, cultured, and identified. They demonstrated the capacity for differentiation into adipocytes, osteoblasts, chondrocytes, and neuron-like cells, indicating their multi-lineage differentiation potential. The UCMSCs-exos exhibited a diameter ranging from 30 to 150 nm, with an average size of 126.62 ± 1.64 nm, and showed increased expression of Tsg101, CD9, and CD63 proteins ($P < 0.05$). In cell experiments, the intervention of UCMSCs and UCMSCs-exos led to increased invasion and migration of MDA-MB-231 and BGC-823 cells ($P < 0.05$). A549 cells displayed enhanced migratory capacity ($P < 0.05$) with minimal change in invasiveness, while LN-229 cells exhibited suppressed activity ($P < 0.05$). Molecular experiments revealed no significant difference in ROS, MDA, and SOD assays between the primary cells, UCMSCs group, and UCMSCs-exos group ($P > 0.05$). However, in LN-229 cells, ROS and MDA levels were reduced, and SOD levels were increased in the UCMSCs group compared to the UCMSCs-exos group ($P < 0.05$). Furthermore, UCMSCs and UCMSCs-exos did not affect the expression of NF-κB and p53 proteins in MDA-MB-231, BGC-823, and A549 cells ($P > 0.05$). In contrast, they

inhibited the expression of NF-κB and p53 proteins in LN-229, with UCMSCs-exos demonstrating a more pronounced inhibitory effect ($P < 0.05$).

Conclusions: In conclusion, this study successfully isolated UCMSCs and their exosomes. They promote the proliferation, migration, and invasion of four types of tumor cells involves altering the cell cycle. Additionally, UCMSCs and their extracellular vesicles are influenced by oxidative stress, and the NF-κB/P53 signaling pathway is implicated. These findings provide preliminary evidence that stem cells and their exosomes may not be suitable for patients with tumor diseases, offering an experimental basis for the potential clinical application safety of this technology in treatment.

**Keywords:** UCMSCs; exosomes of UCMSCs; tumor phenotype; glioma; gastric carcinoma; lung carcinoma

# Resumen

Antecedentes: Las células madre mesenquimales del cordón umbilical (UCMSCs) se derivan del tejido del cordón umbilical. Debido a su baja inmunogenicidad, fácil disponibilidad, tecnología de cultivo madura y falta de controversia ética, se han convertido gradualmente en un punto focal en la investigación con células madre. Los exosomas son vesículas derivadas de células con una estructura consistente con la membrana celular, que funcionan principalmente como portadores de sustratos de transporte para la comunicación y regulación intercelular. En la actualidad, tanto las células madre como sus exosomas se han convertido en productos biofarmacéuticos muy prometedores ampliamente utilizados en diversos estudios clínicos, como la cicatrización de heridas, las lesiones de la médula espinal, la rehabilitación postoperatoria, los trastornos neurológicos y las enfermedades autoinmunes. Sin embargo, la seguridad de estos productos biológicos sigue siendo incierta, y algunos estudios sugieren la posible tumorigénesis de las células madre y sus exosomas. El objetivo de este estudio fue investigar los efectos de las UCMSCs y sus exosomas en cuatro tipos de células tumorales.

Métodos: En este estudio, en primer lugar, se aislaron y caracterizaron las UCMSC y sus exosomas. Posteriormente, el estudio se realizó en experimentos celulares. Los efectos de las UCMSC y sus exosomas en cuatro tipos de células tumorales (línea celular de cáncer de blasto, línea celular de cáncer gástrico, línea celular de glioma y línea celular de cáncer de pulmón) se observaron a través de experimentos de cocultivo. Los observables incluyeron los efectos sobre el fenotipo in vitro y las características de crecimiento in vivo. Los cambios en la expresión génica se analizaron utilizando tecnología de secuenciación de alto rendimiento para dilucidar las funciones biológicas y los roles de las UCMSC y sus exosomas en las células tumorales.

Resultados: Las UCMSC fueron exitosamente aisladas, cultivadas e identificadas. Demostraron la capacidad de diferenciación en adipocitos, osteoblastos, condrocitos y células similares a neuronas, lo que indica su potencial de diferenciación multilinaje. Los UCMSCs-exos exhibieron un diámetro que varió de 30 a 150 nm, con un tamaño promedio de 126.62 ± 1.64 nm, y mostraron una mayor expresión de las proteínas Tsg101, CD9 y CD63 ($P < 0.05$). En experimentos celulares, la intervención de UCMSCs y UCMSCs-exos condujo a un aumento de la invasión y migración de células MDA-MB-231 y BGC-823 ($P < 0,05$). Las células A549 mostraron una mayor capacidad migratoria ($P < 0,05$) con un cambio mínimo en la invasividad, mientras que las células LN-229 mostraron una actividad suprimida ($P < 0,05$). Los experimentos moleculares no revelaron diferencias significativas en los ensayos de ROS, MDA y SOD entre las células primarias, el grupo de UCMSCs y el grupo de UCMSCs-exos ($P > 0,05$). Sin embargo, en las células LN-229, los niveles de ROS y MDA se redujeron, y los niveles de SOD aumentaron en el grupo de UCMSCs en comparación con el grupo de UCMSCs-exos (P

< 0,05). Además, las UCMSCs y las UCMSCs-exos no afectaron la expresión de las proteínas NF-κB y p53 en las células MDA-MB-231, BGC-823 y A549 (P > 0,05). Por el contrario, inhibieron la expresión de las proteínas NF-κB y p53 en LN-229, y las UCMSCs-exos demostraron un efecto inhibidor más pronunciado (P < 0,05).

Conclusiones: En conclusión, este estudio aisló con éxito las UCMSC y sus exosomas. Promueven la proliferación, migración e invasión de cuatro tipos de células tumorales que implican la alteración del ciclo celular. Además, las UCMSC y sus vesículas extracelulares están influenciadas por el estrés oxidativo, y la vía de señalización NF-κB/P53 está implicada. Estos hallazgos proporcionan evidencia preliminar de que las células madre y sus exosomas pueden no ser adecuados para pacientes con enfermedades tumorales, ofreciendo una base experimental para la posible seguridad de la aplicación clínica de esta tecnología en el tratamiento.

Palabras clave: UCMSCs; exosomas derivados de UCMSCs; fenotipo tumoral; glioma; carcinoma gástrico; carcinoma de pulmón

# Abbreviations

| | |
|---|---|
| NA | North America |
| US | United States |
| MSCs | Mesenchymal stem cells |
| COVID-19 | Corona Virus Disease 2019 |
| ucmscs | UCMSCs |
| MSCs-UC | UCMSCs |
| PD | Parkinson's disease |
| RNA | Ribonucleic Acid |
| DNA | deoxyribonucleic acid |
| Exos | Exosomes |
| FBS | fetal bovine serum |
| DAC | 5-Aza-2'Deoxycytidine |
| NTA | Nanoparticle tracking analysis |
| ELISA | enzyme-linked immunosorbent assay |
| TEM | Transmission Electron Microscopy |
| DLS | Dynamic light scattering |
| AFM | atomic force microscopy |
| PS | phosphatidylserine |
| FITC | fluorescein isothiocyanate |
| PI | propidium iodide |
| BC | breast carcinoma |
| GC | gastric carcinoma |
| LC | lung carcinoma |
| ROS | reactive oxygens |
| MDA | malondialdehyde |
| SOD | Superoxide Dismutase |
| O2 | superoxide ions |
| H2O2 | chlorine peroxide |
| - OH | hydroxyl radicals |
| CAT | catalase |
| GSH-Px | glutathione peroxidase |
| MPTs | membrane permeability transport pores |
| FADD | Fas related death domain |
| DISC | death inducing signaling complex |
| PARP-1 | polyadenyldiphosphate ribose polymerase 1 |
| TTFA | thiophenyltrifluoroacetone |
| 2-ME | 2-methoxyestradiol |
| TGF- β 1 | transforming growth factor- β 1 |

| | |
|---|---|
| VEGF | vascular endothelial growth factor |
| IL | interleukin |
| CXCR | corresponding chemokine receptor |
| EVs | Extracellular vesicles |
| miRNA | MicroRNA |
| CSCs | cancer stem cells |
| L-PGDS | Lipocalin type prostaglandin D2 synthase |

# 1. Introduction

According to the latest global cancer burden data released by the International Agency for Cancer Research of the World Health Organization in 2020, China ranks first in terms of new cancer cases and cancer deaths worldwide[1]. Research has found that the tumor microenvironment has a significant impact on the occurrence and development of tumors. Mesenchymal stem cells (MSCs) have been widely used in the repair and regeneration therapy of various tissues and organs due to their excellent multi-directional differentiation, self-renewal potential, and immune regulatory characteristics[2]. Research shows that MSCs can produce extracelular vesicles, a bioactive substance, through paracrine action. Extracelular vesicles affect the proliferation, migration, and angiogenesis of tumor cells by transmitting multiple signaling pathways. At present, inconsistent conclusions have emerged in reports on whether MSCs and their extracelular vesicles inhibit or promote tumors. This study will explore the impact of UCMSCs and their exosomes on the occurrence and development of tumors and their potential mechanisms of action[3].

## 1.1 Stem cells

Stem cells are undifferentiated or partially differentiated cells originating from the Inner cell mass. They can differentiate into various types of cells in life, and maintain the activities of life through continuous differentiation and proliferation. In mammals, stem cells can differentiate into various cells in the Ectoderm, Mesoderm and Endoderm. Stem cells can be divided into two categories according to the sequence of their appearance during development[3, 4]: embryonic stem cells and Adult stem cell. Embryonic stem cells are a kind of stem cells derived from the Inner cell mass of blastocyst stage embryos, which are isolated and cultured in vitro and remain in the primitive stem cell stage. According to the differentiation characteristics of stem cells, they can be further divided into[5, 6]: pluripotent stem cells, pluripotent stem cells, and single or specialized stem cells. In mammals, pluripotent stem cells are stem cells that can differentiate into a specific type of cell, such as hematopoietic stem cells used to supplement blood and immune cells, and basal cells used to maintain skin epithelial renewal. Pluripotent stem cells refer to a type of stem cell that can differentiate into several cell types, including mesenchymal stem cells, induced pluripotent stem cells, and embryonic stem cells cultured in vitro[7]. Mesenchymal stem cells are a type of cell that exists in the interstitium of tissues and organs and has multiple differentiation potentials. Induced pluripotent stem cells are stem cells that conversely transform mature somatic cell, which were first successfully studied by the Japanese team led by the team[8, 9]. The experiment was initially completed on

mouse cells, and subsequently successfully induced human fibroblasts into pluripotent stem cells.

Stem cells have two main characteristics: self-renewal and multi-directional differentiation. On the one hand, they are self-sustaining and regenerative, that is, they undergo continuous cell growth and division while maintaining the undifferentiated state of cells, maintaining a certain number of cells; On the other hand, it is the characteristic of differentiation into special cell types, that is, the ability to differentiate into mature Somatic cell cells in multiple directions or in one direction under the regulation of positive feedback or negative feedback in the special microenvironment[10]. The characteristics of self-renewal and multi-directional differentiation mentioned above are mainly achieved through two regulatory mechanisms. One mechanism is asymmetric cell division, where when a stem cell divides into two cells, one cell is identical to the original stem cell and remains pluripotent in an undifferentiated state, while the other cell differentiates into a special form of cell. Therefore, as the stem cell continues to divide, it can maintain its original characteristics, and the stem cell will adjust the cell cycle based on the feedback information received, control the directional differentiation of stem cells[11]. The other mechanism is the random differentiation mechanism. When two stem cells are in the state of division, one of them will grow and divide into two differentiated daughter cells. At the same time, the other stem cell will generate two cell stem cells identical to the original cell through Mitosis to protect DNA by maintaining telomerase, so as to extend the cell division limit and maintain the characteristics of proliferation[3, 12]. When the self-renewal mechanism of stem cells is defective, it can lead to developmental defects, premature aging phenotype, and cancer.

Because of its special biological characteristics, stem cells are widely used in biomedical research and clinical treatment. Therefore, stem cells have many benefits for human health and disease treatment, and research on their mechanisms will continue to deepen. Their applications will also become increasingly widespread, and they will maximize the benefits of human society.

## 1.2 Mesenchymal stem cell

MSCs derived from the early developmental mesoderm and ectoderm have good multi-directional differentiation potential, self-renewal ability, and immune regulatory performance. They have been isolated and cultured from various tissues such as bone marrow, fat, umbilical cord, placenta, skin, peripheral blood, teeth, and so on[13, 14]. The Interstitial and Tissue Stem Cell Committee of the International Society for Cell Therapy has proposed a minimum standard for defining human MSCs. Firstly, MSCs must adhere to the wall when cultured under standard culture conditions, secondly, MSCs express CD105, CD73, and CD90 positively, and do not express CD45, CD34, CD14, or CD11b，CD79a or CD19 and HLA-DR surface molecules, and finally

MSCs differentiate into osteoblasts, adipocytes, and chondrocytes under standard induction conditions in vitro. Due to its excellent biological properties, MSCs have been applied in basic and clinical research of various diseases, and have good clinical application prospects in diseases such as heart disease and autoimmune diseases[9]. The biological role of MSCs is mainly achieved through the following mechanisms[15, 16]: firstly, the migration and homing characteristics of MSCs, which enable them to homing to corresponding lesion area. The second is paracrine characteristics, where MSCs promote tissue repair and regeneration by secreting paracrine factors. The third characteristic is immune regulation, where MSCs participate in immune regulation by inhibiting the activation and function of immune cells such as T cells, B cells, macrophages, etc.

MSCs are widely distributed in the human body, therefore, they can be associated with swelling in the body. Contact with various cells such as tumor cells can recognize tumor signals and generate corresponding reactions, while MSCs will be recruited and become part of the tumor microenvironment part. Studies have shown that MSCs are involved in the occurrence, development, metastasis, and prognosis of tumors, and the latter plays an important role[17, 18].

## 1.3 Umbilical cord mesenchymal stem cells (UCMSCs)

Umbilical cord mesenchymal stem cells (UCMSCs) are pluripotent stem cells derived from umbilical cord matrix Wharton's jelly. After removing umbilical vein and umbilical artery from umbilical cord epidermis, the remaining colloid is Wharton's jelly, which contains a large number of UCMSCs[19, 20]. UCMSCs undergo a lengthy process of discovery, isolation, and identification. In addition to the matrix containing mesenchymal stem cells, the umbilical cord also includes two umbilical arteries and one umbilical vein[21]. Through observation of the structure of the umbilical artery, it was found that the outer layer of the two-layer structure is responsible for contraction, while the inner layer is prone to axial movement. At this point, researchers have conducted a comprehensive exploration of the vascular and stromal structures of the umbilical cord, and successfully isolated and cultured fibroblast like stromal cells from human umbilical cord through tissue block culture[22, 23]. Subsequent research found that these cells possess the characteristics of smooth muscle and fibroblasts, and have secretory effects. Fibroblast like cells from umbilical cord stroma are officially identified as mesenchymal stem cells. They do not contain endothelial cell or leukocyte specific antigens, but can express smooth muscle actin and some mesenchymal cell markers. Compared with mesenchymal stem cells from other sources, these cells have similar surface phenotype, adhesion and pluripotency. These characteristics and properties of UCMSCs have been confirmed in various subsequent studies, such as transplantation experiments in mouse models and clinical studies on inhibiting Parkinson's disease（PD）[24, 25].

In the treatment of malignant tumors, the application of mesenchymal stem cell extracellular vesicles for targeted drug delivery exhibits targeted targeting to the tumor site and is often used as a therapeutic target or drug delivery tool. In vitro experiments, it was found that exosomes combined with other anti-tumor drugs can effectively inhibit the proliferation of non small-cell carcinoma A549 and H1299. In addition, in vivo and in vitro experiments, studies have confirmed that mesenchymal stem cell exosomes can carry paclitaxel to inhibit the cell viability of breast cancer cells, indicating that exosomes can be used for the treatment of breast cancer[26, 27]. Similarly, Gomari et al found that mesenchymal stem cell exosomes can not only effectively load doxorubicin, but also significantly reduce the tumor growth rate in the mouse breast cancer model. The researchers not only conducted research on the possibility of drug loading into extracellular vesicles, but also conducted a detailed exploration of the therapeutic effects after drug loading. Research has shown that mesenchymal stem cell extracellular vesicles can not only load paclitaxel, but also reduce its cytotoxicity by more than 7 times[28, 29]. Intravenous injection can reduce subcutaneous primary tumors by more than 60%, and the concentration of paclitaxel in the extracellular vesicles is reduced by about 1000 times. However, the effect is similar to that of directly using high concentrations of paclitaxel, 50% reduction in metastatic lesions can still be observed in the distal organs of the lungs, liver, spleen, and kidneys. These studies have confirmed that mesenchymal stem cell extracellular vesicles can effectively load drugs to assist in the treatment of cancer[30].

The biological characteristics of UCMSCs. In addition to meeting the identification criteria for mesenchymal stem cells, UCMSCs have also shown many advantages in application[31, 32]: they have a wide range of raw material sources, do not cause harm to the human body when obtained, have no ethical disputes, and have a large amount of cells obtained from the median tissue, making it easy to expand. At the same time, UCMSCs also have better stemness than the gold standard for mesenchymal stem cells known as bone marrow-derived mesenchymal stem cells. Therefore, UCMSCs are more valuable than other adult-derived stem cells in the translation of applications.

Numerous studies have shown that UCMSCs have the following characteristics[33, 34]: strong stemness, ability to produce immune suppression, strong homing ability, and ability to enhance co transplant cell activity. Specifically, UCMSCs have better stemness, mainly because they form the connective tissue matrix of Wharton's jelly, which is derived from the extracellular mesoderm or embryonic mesoderm of embryonic development, and their stemness is better than that of stem cells developed from the proximal ectoderm. UCMSCs can balance immunity, which refers to the absence of immune response after transplantation to the host, and can effectively reduce the incidence of rejection after transplantation. For example, in the treatment of neurological diseases such as Parkinson's, immune rejection can be effectively controlled. The homing ability of UCMSCs refers to the tendency of UCMSCs towards damaged or rapidly growing tissues, which quickly aggregates

towards the damaged site, similar to bone marrow mesenchymal stem cells[35]. UCMSCs can enhance the activity of co transplanted cells, which refers to the role of UCMSCs as supporting cells. Cotransplantation with other cells can promote cell proliferation, such as co transplantation with hematopoietic stem cells, which can enhance the activity of hematopoietic thousand cell cells. Meanwhile, UCMSCs can also serve as trophoblast cells, supporting the growth of embryonic stem cells. In addition, there are two important problems in cord blood transplantation, insufficient transplantation cells and slow cell transplantation[36, 37]. Because of the characteristics of rapid proliferation and low immunogenicity, umbilical cord mesenchymal thousand cells can solve the above two problems.

Due to their special biological properties, stem cells are widely used in biomedical research and clinical treatment. A large number of studies have also confirmed their efficacy. Therefore, stem cells have many benefits for human health and disease treatment and are considered to be the next generation of biopharmaceuticals.

## 1.4 Exosomes (Exos)

Extracellular vesicles (EVs) are nanoscale extracellular vesicles actively released by various mammalian cells. Based on their biological origin, size, and biophysical characteristics, they can be divided into exosomes, microbubbles, and apoptotic bodies. Exosomes were first discovered in sheep reticulocytes in 1983 and were initially considered cell fragments or cell waste. Their role has been underestimated, and studies have found that exosomes are important carriers of intercellular communication[38]. Exosomes are membranous vesicles wrapped in lipid bilayers, with a diameter of 30 nm-200 nm. They mainly participate in material exchange and signal transduction between cells. The exosomes contain abundant bioactive molecules, including proteins, nucleic acids, and lipids, and regulate the biological activity of receptor cells through these bioactive molecules[39, 40]. MicroRNA (miRNA) is considered an important component of exosomes. Extracellular vesicles derived from MSCs have similar biological functions to mother cells, but have the following advantages compared to MSCs[41]: ① Extracellular vesicles are stable, can be stored for a long time at -80 ℃, and the content is not easily degraded; ② Exosomes are safer and there is no possibility of aneuploidy; ③ Exosomes can transport across membranes and directly target the lesion site; ④ Cellular free therapy reduces the risk of immune rejection, reduces tumorigenicity, and solves the problem of low survival rate of MSCs in vivo.

Exosomes play important roles in multiple biological processes. Firstly, exosomes serve as mediators of intercellular communication, transmitting signals between cells and tissues, and participating in regulating biological processes such as cell proliferation, differentiation, and apoptosis[42]. Secondly, exosomes can regulate the gene expression and biological activity of target cells by carrying bioactive molecules

such as nucleic acids, proteins, and lipids, thereby affecting cell function. In addition, exosomes are also involved in biological processes such as immune regulation, inflammatory transmission, tumor metastasis, and tissue repair.In recent years, exosomes, as a new type of biological medium, have shown great potential in biomedical applications[43]. Firstly, exosomes, as biomarkers, have potential applications in clinical diagnosis and monitoring. Extracellular vesicles carry abundant biological molecules, such as proteins, nucleic acids, and metabolites, which can serve as biomarkers of diseases for early diagnosis, prognostic evaluation, and therapeutic monitoring[44]. For example, miRNAs in exosomes have been widely studied for early diagnosis and prognostic evaluation of tumors. Secondly, exosomes have also shown potential application value in drug delivery and gene therapy. Exosomes can serve as a natural nano drug delivery system, achieving targeted drug delivery by modifying exosomes' surface proteins or directly loading drugs, thereby improving drug bioavailability and reducing toxic side effects[45]. In addition, exosomes can also serve as an effective gene delivery tool, allowing the carried nucleic acid to enter target cells and exert biological effects, thereby achieving the goal of gene therapy. Finally, exosomes have potential applications in tissue repair and regeneration. Research has shown that extracellular vesicles can promote cell proliferation and migration, regulate cell differentiation and repair, and thus play a positive role in tissue repair and regeneration[46].

Various cells and bodily fluids in the human body can secrete extracellular vesicles, including endothelial cells, immune cells, platelets, smooth muscle cells, etc. When it is secreted from the host cell into the receptor cell, the exosomes can regulate the biological activity of the receptor cell by carrying proteins, nucleic acids, lipids, etc[47]. Extracellular communication mediated by extracellular vesicles is mainly achieved through three ways[48]: firstly, extracellular vesicle membrane proteins can bind to target cell membrane proteins, thereby activating signaling pathways within target cells. Secondly, in the extracellular matrix, extracellular membrane proteins can be cleaved by proteases, and the cleaved fragments can act as ligands to bind to receptors on the cell membrane, thereby activating intracellular signaling pathways. There are reports that some extracellular membrane proteins have not been detected on their source cell membranes. The third is that the exosomes membrane can directly fuse with the target cell membrane, non selectively releasing the proteins, mRNA, and microRNAs it contains. When exosomes were first discovered in 1983, they were considered a way for cells to excrete waste. Nowadays, with extensive research on their biological sources, material composition and transportation, intercellular signal transduction, and distribution in body fluids, exosomes have been found to have various functions. The function of exosomes depends on the type of cell they originate from, and they can participate in various aspects of the body's immune response, antigen presentation, cell migration, cell differentiation, and tumor invasion. Studies have shown that exosomes derived from tumors participate in the exchange of genetic

information between tumor cells and basal cells, leading to the generation of a large number of new blood vessels and promoting tumor growth and invasion[49, 50].

For the morphological characteristics of exosomes, transmission electron microscopy can be used to identify them. Firstly, uranyl acetate is used to dehydrate and fix the sample, increase the contrast of the analyzed sample, and finally, the morphology of the small cup cap sample is obtained through scanning, which is internationally recognized.The extraction of extracellular vesicles mainly includes the following methods[51, 52]: first, ultracentrifugation, which is the most commonly used method for extracellular vesicle extraction. This method produces a large amount of extracellular vesicles, but its purity is insufficient. During electron microscopy identification, it was found that extracellular vesicles aggregate into blocks. Due to the lack of a very unified identification standard for microbubbles and extracellular vesicles, some studies also believe that this method produces microbubbles rather than extracellular vesicles. The second is filtration centrifugation, which is a simple and time-saving operation that does not affect the biological activity of extracellular vesicles, but also has the problem of insufficient purity. The third method is density gradient centrifugation. The exosomes separated by this method have high purity, but the preparation work in the early stage is complex, time-consuming, and small in quantity. The fourth method is the immunomagnetic bead method, which can ensure the integrity of the extracellular morphology, high specificity, simple operation, and does not require expensive instruments and equipment[53, 54]. However, non neutral pH and non physiological salt concentrations can affect the biological activity of the extracellular vesicles, making it inconvenient for further experiments. The fifth is the phosphatidylserine (PS) affinity method, which combines PS with magnetic beads and uses the affinity principle to capture PS outside the extracellular vesicles. This method is similar to the immunomagnetic bead method, and the obtained extracellular vesicles have complete morphology and the highest purity. Due to the absence of denaturants and the fact that they do not affect the biological activity of extracellular vesicles, extracellular vesicles can be used for cell co culture and in vivo injection. The sixth method is chromatography, which separates extracellular vesicles of uniform size under electron microscopy, but requires special equipment and is not widely used. The seventh method is microfluidic separation, which uses mechanical principles such as negative pressure and oscillation to separate extracellular vesicles. The yield and purity have been significantly improved, but specific equipment is required to cooperate.

In addition, exosomes serve as drug carriers to achieve targeted drug delivery. Exosomes have a load of "goods The ability to transmit it to target cells can be used for intercellular communication, especially long-distance communication. Exosomes, as drug transport carriers, have unique advantages such as low immunogenicity, high transport efficiency, good stability, strong targeting, and ability to cross the blood-

brain barrier. Exosomes are also suitable for delivering various chemicals, proteins, nucleic acids, etc. Buller B and others inject exosomes loaded with miR-146 into mice with keratinoma at the tumor site, miRNAs were found to effectively inhibit tumor growth[53]. Exosomes can also serve as molecular markers for the diagnosis of certain diseases. Different types of cells from different diseases can secrete extracellular vesicles containing different miRNAs and RNAs, making it possible to separate extracellular vesicles from patient fluids for disease diagnosis, especially in tumor diagnosis. In addition, exosomes can also be used for the diagnosis of non tumor diseases. Welker M W et al[55]. found that serum extracellular vesicle CD81 may be a potential protein marker for the diagnosis of chronic hepatitis C.Recent studies have found that stem cell derived exosomes are an important way of paracrine activity in stem cells, which can mediate their promotion of tissue regeneration[56]. Chen et al. found that the combination therapy of ADSCs exo can reduce the infarct size of acute ischemic stroke in rats and promote neurological function recovery[57]. ADSCs exo can avoid the drawbacks of immune rejection and ethical issues in stem cell therapy, and has advantages such as high stability, easy storage, no proliferation, and easy quantitative use[58]. Compared with a single cytokine, ADSCs exo has higher safety and greater tissue regeneration potential.

The protein molecules or lipid ligands on the surface of exosomes directly activate receptors on the surface of target cells, generate signal complexes, and activate intracellular signaling pathways. In addition, exosomes can also fuse with the plasma membrane of target cells or directly enter the cells through endocytosis, bringing their own active molecules such as proteins, nucleic acids, lipids, etc. into the cells, thereby regulating their function and biological behavior.

Research has found that MSCs play a role mainly due to their paracrine mechanism, and exosomes, as important factors in the paracrine mechanism, have received increasing attention in tumor occurrence, development, and treatment[59]. Studies have shown that exosomes play a double-edged sword role in tumor immunity. In the tumor microenvironment, exosomes can transmit growth between tumor cells, immune cells, and other cells[39, 41, 42]. Bioactive molecules help cancer cells evade immune surveillance and induce immune tolerance, while extracellular vesicles from immune cells can play a role in inhibiting tumor growth, proliferation, and metastasis.

## 1.5 Tumor

The term "tumor" refers to the new organism formed by local tissue cell proliferation under the influence of various tumor-causing factors. Tumors originate from normal cells and lose control due to various internal and external factors, leading to the formation of solid or diffusely distributed tumor tissue[60, 61]. According to the Statistical Classification of International Diseases and Related Health Problems, tumors can be categorized into malignant tumors, benign tumors, in situ tumors, and

tumors of unknown origin, with malignant tumors being the primary focus of oncology research[62].

In clinical practice, it is possible to differentiate between benign and malignant tumors and assess the degree of malignancy of malignant tumors based on their phenotype[63]. This assessment allows for the prediction of the therapeutic effect and prognosis of tumors. Malignant tumors are a group of malignant diseases characterized by abnormal cell growth that may invade or spread to other parts of the body[64]. They can produce multiple primary and secondary symptoms, including overall pain, weight loss, extreme fatigue, cognitive impairment, severe anxiety, and depression[65]. Many of these symptoms can occur synergistically, causing significant distress to patients. Almost all malignant tumors exhibit the following typical characteristics: sudden and asymptomatic continuous growth and division of cells, avoidance of programmed cell death, promotion of cell division and angiogenesis, continuous tissue invasion, metastasis, and spread to other parts of the body.

In clinical practice, conventional cancer treatment methods mainly include surgical resection, radiotherapy, chemotherapy, targeted therapy, and a combination of multiple approaches[66, 67]. (1) Surgical resection involves the removal of the tumor and surrounding tissue. It is often used to remove localized tumors and prevent the spread of cancer cells to other parts of the body. (2) Radiation treatment uses high-energy radiation to kill cancer cells or shrink tumors. It can be used as a standalone treatment or in combination with surgery or chemotherapy. (3) Chemotherapy involves the use of drugs to kill cancer cells or stop them from growing and dividing. It can be administered orally or intravenously and may be used in combination with other treatments. (4) Targeted therapy specifically targets the changes in cancer cells that help them grow, divide, and spread. It can be used alongside other treatments and is often less harmful to normal cells than traditional chemotherapy. (5) Immunotherapy uses the body's immune system to help fight cancer. It can either boost the body's natural defenses or train the immune system to attack cancer cells specifically. These conventional treatments are often used in combination or with other supportive therapies, depending on the type and stage of cancer.

Stem cells and exosomes have multiple effects on tumors. Firstly, mesenchymal stem cells and exosomes exhibit anti-inflammatory effects, enhance systemic immune levels, and can effectively contribute to tumor treatment. Secondly, the interaction between mesenchymal stem cells and exosomes, as well as the exosomes secreted by tumor cells themselves, can create a microenvironment that promotes tumor growth, thus facilitating tumor development. Targeting and blocking these key factors can indirectly achieve therapeutic goals. Finally, the exosomes of mesenchymal stem cells serve as targeted drug carriers, delivering drugs to specific sites to provide adjuvant therapy[68, 69]. Due to the different effects and modes of action of mesenchymal stem cells and their exosomes on various tumors, targeted use of mesenchymal stem

cells and their exosomes is necessary for the treatment of different cancers[70]. The exosomes of mesenchymal stem cells can directly impact the treatment of cancer. In other words, it is controversial whether stem cells and exosomes therapy can be used for tumors.

1.5.1 Lung cancer

Lung cancer is the main cause of cancer death for both men and women worldwide, and it is also the most common cancer among men. Researchers co cultured human bone marrow MSCs with lung cancer cell line A549, and the results showed that MSCs can inhibit the migration and proliferation of A549 cells, block the cell cycle in G1 phase, and induce cell apoptosis[71]. MSCs conditioned medium achieved the same effect. However, MSCs combined with A549 cells were injected subcutaneously into the back of BALB/c mice to detect tumor growth, and the results showed that 80% -90% of the MSCs group formed visible tumors to the naked eye[71, 72]. Moreover, the volume was significantly larger than that of the control group, and abundant blood vessels were distributed on the surface of the tumor in the MSCs group, indicating that in the tumor microenvironment, MSCs promote angiogenesis, which is beneficial for tumor growth. The combination of MSCs and Lewis lung cancer LL3 cells was injected subcutaneously into the left back of C57BL/6 mice, and the results showed that the combination of MSCs and LL3 cells significantly promoted tumor growth[73, 74]. MSCs can inhibit the inflammatory response induced by adenovirus Fas ligands in primary tumors, eliminate rejection reactions, and reduce the expansion of tumor specific T cells. After co culturing umbilical cord MSCs with lung cancer cell line H1299, their proliferation, apoptosis, invasion, and cell cycle were detected. The results showed that umbilical cord MSCs could significantly inhibit H1299 cell invasion and induce H1299 cell apoptosis, but had no significant effect on H1299 cell proliferation and cell cycle[75, 76]. This indicates that umbilical cord MSCs have anti-tumor effects on lung cancer cells. Further research suggests that it may be AKT/PI3K/STAT3 signaling pathway roads play a role in the regulation process.

1.5.2 Liver cancer

According to the global ranking of cancer related deaths, liver cancer has risen to the second place, and if not diagnosed and treated in a timely manner, the probability of survival for patients is very low[77]. Researchers cultured bone marrow MSCs and human liver cancer cell lines through a Transwell co culture system to detect the invasion and proliferation ability of liver cancer cells[64, 78]. The results showed that the invasion ability of liver cancer cells co cultured with MSCs decreased, while the proliferation ability increased. MSCs and human liver cancer cell H7402 were injected into SCID mice. Compared with the control group, the MSCs treatment group showed a delayed tumor formation time and significantly reduced tumor volume. There were extensive necrotic areas in the tumor tissue, with fibroblast like cells visible, and in situ calcification visible in the tumor area, indicating that MSCs can inhibit tumor growth[79]. The nude mouse model of liver cancer was injected with MSCs through

the tail vein, and then the angiogenesis ability was evaluated[80]. Compared with the control group, the MSCs treatment group showed an increase in microvascular density and promoted angiogenesis, which may be related to the mechanism of transforming growth factor- β 1 (TGF- β 1) /Smad signaling pathway.

1.5.3 Gastric cancer

Gastric cancer is a common malignant tumor of digestive tract, and its incidence rate and mortality rate rank third in malignant tumors in China[81]. Human foreskin MSCs were co cultured with gastric cancer cell line SGC-7901, and both in vitro and in vivo results showed inhibition of gastric cancer cell proliferation. It was found that human foreskin MSCs conditioned medium could inhibit the expression of bcl-2 in SGC-7901 cells, upregulate the expression of bax and caspase-3, indicating that human foreskin MSCs can inhibit tumor cell growth by inhibiting gastric cancer cell proliferation and promoting gastric cancer cell apoptosis. Co cultured gastric cancer derived MSCs with gastric cancer cell lines BGC-823 and MKN-28, the results showed that compared with bone marrow MSCs and adjacent non cancer tissue derived MSCs, gastric cancer derived MSCs promoted the proliferation and migration of gastric cancer cells, and proangiogenic factors such as vascular endothelial growth factor (VEGF), macrophage inflammatory protein-2, and TGF- β 1[82]. The expression level of interleukin (IL) -6 was significantly increased, and further research suggests that IL-8 secreted by gastric cancer derived MSCs may be involved in tumor formation. By neutralizing antibodies to block IL-8 secretion, the results showed that the tumorigenic effect of MSCs was weakened, and AKT and ER K1/2 signaling pathways were involved in this process. Research shows that MSCs can activate the Wnt5a/Ror2 signaling axis to produce CXCL16, thereby activating the corresponding chemokine receptor (CXCR) 6, by activating STAT3, they increase the expression of Ror1, ultimately promoting the proliferation and migration of gastric cancer cells, thereby promoting tumor progression[83].

1.5.4 Colorectal cancer

Colorectal cancer is one of the most serious threats to human health, and its incidence rate and mortality rate rank second and fifth among all tumors. Injecting MSCs overexpressing CXCR4 (MSCs CXCR4) into a colon cancer mouse model, the results showed that the weight loss of MSCs CXCR4 injected mice was alleviated, the colon length was prolonged, the number of tumors was reduced, the tumor load was reduced, and the levels of pro-inflammatory cytokines and STAT3 phosphorylation in colon tissue were reduced, indicating that MSC-CXCR4 has effective anti-tumor effects[84]. Other studies have shown that injecting MSCs can block the cell cycle of colorectal cancer cells in G1 phase, inducing cell apoptosis, leading to Wnt and TGF- β in vivo. The Smad signaling pathway is dysregulated, which in turn interferes with the occurrence of tumors. Subcutaneous injection of colon cancer HCT116 targeted tumor stem cells into nude mice to form xenograft tumors of MSCs[85].The results after treatment showed that MSCs promoted tumor growth, and in vitro experiments found

that rat MSCs increased the invasion and migration of HCT116 targeted tumor stem cells.

1.5.5 Breast cancer

In 2020, female breast cancer will surpass lung cancer for the first time and become the most common cancer in the world. Among Chinese female cancer patients, the main type of incidence is breast cancer[86]. MSCs conditioned medium co cultured with breast cancer cell MDA-MB-231 inhibits the migration, proliferation and angiogenesis of MDA-MB-231 cells by inhibiting the activation of STAT3. The umbilical cord MSCs were co cultured with breast cancer cells. Compared with the pure breast cancer cell group, MSCs promoted the proliferation of tumor cells and the expression of mesenchymal cell markers in co cultured tumor cells indicate that the interaction between MSCs and tumor cells promotes the growth of tumor cells[87]. The co culture of mouse bone marrow MSCs and mouse breast cancer cell 4T1 can promote the proliferation of 4T1 cells. MSCs and 4T1 are injected into nude mice. The results show that MSCs and 4T1 can promote the proliferation of tumor cells, increase the tumor volume, increase the tumor vascular area, and reduce the necrosis in the center of the tumor.

1.5.6 pancreatic cancer

Pancreatic cancer is one of the most malignant tumors in the digestive system. its clinical manifestations are: occult, rapid progress, poor prognosis. The MSCs expressing NK4 were co cultured with pancreatic cancer cell line SW1990 and found to significantly inhibit the proliferation and migration of SW1990 cells[88]. Tumor necrosis factor related apoptosis inducing ligand engineered pancreatic derived MSCs can significantly inhibit the growth of pancreatic cancer cells. MSCs can promote the local invasion of pancreatic cancer cells by up regulating bimodulin, and specific small interfering RNA blocks the production of bimodulin and eliminates the local invasion of pancreatic cancer cells, indicating that bimodulin may participate in this process[89]. Muscle fibroblast like cells derived from MSCs can regulate the state of cancer cell endoderm, improve the expression level of various stem cell related genes, enhance the ability of pancreatic cancer cells to form spheres, promote the formation of mouse pancreatic cancer lumps, increase the resistance to anticancer drugs, and promote the progress of pancreatic cancer.

The above research results indicate that the interaction between MSCs and tumor cells can regulate the tumor microenvironment[90]. On the one hand, MSCs, due to their homing characteristics, can effectively enter the tumor microenvironment and inhibit tumor growth by activating multiple signaling pathways, overcoming the limitations of low drug delivery efficiency and tumor microenvironment infiltration[91]. Therefore, transplantation of pre edited and modified MSCs has become a new method for treating tumors; On the other hand, MSCs promote tumor progression by reshaping the tumor microenvironment to generate tumor cell niches that support tissue neovascularization, tumor invasion, and metastasis. Based on the

above factors, a deeper understanding of the crosstalk between MSCs and tumor cells will become a new strategy for future cancer treatment[92].

1.6 Oxidative stress

The maintenance of redox homeostasis is the foundation for ensuring normal cell function and survival. The characteristics of tumor cells include increased aerobic glycolysis (Warburg effect) and high levels of oxidative stress[93]. Oxidative stress is produced by reactive oxygen species (ROS), which accumulate due to an imbalance in their production and clearance. High levels of ROS in tumor cells are the result of changes in multiple signaling pathways that affect cell metabolism. Tumor cells rely on improving antioxidant capacity to eliminate the accumulation of ROS.

ROS refers to a class of reactive oxidants, mainly including superoxide anions (O2), hydroxyl radicals (-HO), and free radical molecules such as hydrogen peroxide (H2O2)[94]. The main fields that produce ROS internally include mitochondria, peroxisomes, and endoplasmic reticulum, among which mitochondria are considered the main source of intracellular ROS. Mitochondria are the main sites for oxygen consumption within cells, and approximately 2% of the oxygen consumed by mitochondria is reduced to superoxide through electron transfer chains. Peroxisomes are another organelle involved in redox regulation[95]. In peroxisomes, B. oxidation of fatty acids and flavin oxidase produce ROS, which then relies on its unique catalase to decompose H2O2, thereby maintaining the balance of redox in the peroxidase body. The endoplasmic reticulum is the site of protein folding, which involves the formation of disulfide bonds. Therefore, the endoplasmic reticulum is often in an oxidative environment. There are two types of intracellular ROS production reactions: enzymatic and non enzymatic. Enzymatic response mainly. It is completed by a series of oxidoreductases such as NADPH oxidase and xanthine oxidase, while non enzymatic sources are mainly leakage of mitochondrial electron transfer chains.

As early as the 1980s, the relationship between ROS and cell transformation had already been established. At that time, it was found that insulin increased intracellular H202 levels and promoted the proliferation ability of tumor cells. More than thirty years have passed, and due to the complexity of ROS, there is still no consensus on its exact function in cancer[96]. In the process of tumor occurrence and development, it is not only necessary to face the accumulation of ROS caused by the harsh external environment (hypoxia, nutrient deficiency), but also to face the ROS height caused by the activation of one's own oncogenes and the inactivation of tumor suppressor genes, which can lead to oxidative stress in cells[97]. At this point, the tumor will upregulate the expression of various antioxidant enzymes to clear ROS and maintain its own redox homeostasis. The best example is the frequent occurrence of high expression of NRF2 or loss of its negative regulatory protein KEAPI during the occurrence and development of tumors. Research has evaluated the production and levels of ROS under different conditions to determine when ROS is carcinogenic and when it has

inhibitory effects. Obviously, the levels and functions of ROS within tumor cells vary at different stages of tumor development.

At low to moderate levels, ROS is believed to promote the occurrence and development of tumors mainly through two ways. One is to act as signaling molecules within cells to function, and the other is to promote mutations in genomic DNA. Due to the presence of cysteine residues sensitive to redox in the active centers of many proteins, the function of ROS as a signaling molecule mainly relies on its oxidative modification of cysteine on the target protein[98]. There are many types of oxidative modification, and the level of ROS determines the type of cysteine ultimately oxidized. Specifically, at low levels of ROS, the thiol groups of cysteine residues on the protein are first oxidized to sulfonic acid. As ROS increases, sulfinic acid is further oxidized into reversible disulfide bonds (RS-SRorRS-SR'), glutathioneization (RS-SG), sulfite, and irreversible sulfonic acid (R-S03H). These modifications can cause changes in protein conformation, thereby affecting the function of the protein. On the one hand, ROS can directly enhance the activity of kinases, such as promoting cell survival and proliferation by activating MAPK and ERK. On the other hand, ROS can also reversibly inactivate tumor suppressors such as phosphatase PTEN and tyrosine phosphatase PTP to maintain downstream signaling. Conversely, PTP can regulate signal transduction, thereby increasing the expression of antioxidant proteins and reducing ROS levels.

In addition, ROS has been found to play an important role in the renewal and differentiation of normal stem cells, but its mechanism of action in tumor stem cells is still poorly understood. Known tumor stem cells are the most malignant group of cells in tumors, playing an important role in the initiation, resistance, and recurrence of tumors[99]. At present, it is known that breast cancer and liver cancer stem cells often have low ROS levels. Therefore, although chemotherapy and radiation therapy that induce ROS production are available.

In order to eliminate most cancer cells, but due to the high ROS clearance system of tumor stem cells, these therapies find it difficult to eliminate tumor stem cells, and instead give this group of cells a selective advantage. Moreover, the additional ROS brought about by these therapies can cause DNA damage and mutations, leading to the development of drug resistance in tumor cells.

At high levels, ROS can cause severe cell damage and promote cell death. Tumor cells need to resist high levels of ROS, especially in the early stages of tumor development. Recent studies have shown that oxidative pressure can increase the selection pressure of tumor initiating cells to promote their antioxidant capacity enhancement. At the same time, the detachment of tumor cells from the cell matrix can also induce the production of high-level ROS. This poses a challenge for the survival of tumor cells that metastasize to distant organs. Recent research reports have confirmed that tumors enhance their antioxidant capacity during metastasis, ensuring their survival and completion during the process. However, it is worth noting

that a recent article published in Cancer Cell has reached the opposite conclusion. Researchers found that in pancreatic cancer, ROS with high water content is more conducive to cell metastasis.Therefore, one of the characteristics of tumor cells is their high level of antioxidant capacity, which, although able to regulate ROS to meet the normal biological functions of cells, is still higher than the level in normal cells. So some scientists believe that these enhanced antioxidant defense mechanisms may represent a strategy for treating tumors, which can specifically kill tumor cells, including tumor stem cells, but has little impact on normal cells[100].

Given the complexity of ROS in tumors, there are currently two strategies for using ROS as a tumor treatment. One is to eliminate ROS as an anti-tumor treatment, and the other is to enhance ROS as an anti-tumor treatment.The strategy of using ROS clearance as an anti-tumor treatment is mainly based on the function of ROS as a proto-oncogene[101]. It is believed that ROS can promote gene mutations and serve as a second messenger during cell proliferation, so the use of antioxidants can prevent the occurrence of tumors. As early as 1993, the Linxian trial was one of the first large-scale, randomized, double-blind, primary prevention studies aimed at studying the prevention methods for specific cancers through the intake of selenium, vitamin E, and B. Hulotsu supplements. The research results show that supplementing these antioxidants can significantly reduce overall mortality, total cancer mortality, and gastric cancer mortality[102]. Interestingly, after stopping supplementing antioxidants for 10 years, the research results showed that these antioxidants still have a significant protective effect. Meanwhile, another large-scale epidemiological study conducted in China has shown that supplementing or dietary intake of vitamin E can reduce the risk of liver cancer. However, in large-scale studies involving supplementation with other antioxidants such as B. carotene and vitamin A or vitamin E, the opposite results were found, with an increase in the incidence of lung cancer rather than a decrease. In addition, a large-scale "selenium and vitamin E cancer prevention experiment" abroad has sounded the alarm for using antioxidants to prevent tumors. Because long-term follow-up not only did not find a decrease in the risk of prostate cancer in healthy individuals taking selenium or vitamin E, but also a significant increase in the risk of prostate cancer in healthy men. The latest article also suggests that blindly supplementing antioxidants not only does not achieve the goal of preventing tumors, but also promotes tumor metastasis. The above results indicate that the role of antioxidants in cancer prevention is very complex, and suggest not to blindly use antioxidants to prevent tumors. More careful evaluation is needed regarding the effectiveness of antioxidants in cancer prevention.

The strategy of enhancing ROS as an anti-tumor treatment is mainly based on the fact that high levels of ROS are harmful to cells, and oxidative stress has a tumor inhibitory effect, which should be a mainstream approach at present. Although tumor cells enhance their antioxidant system, their levels of ROS are still higher than normal cells, providing an interesting therapeutic window. Tumor cells are more sensitive to

drugs that can cause ROS accumulation than normal cells. Currently, many drugs with direct or indirect effects on ROS have been used for effective cancer treatment, mainly involving the following aspects. Firstly, chemotherapy is still an important means of treating tumors. Many tumor chemotherapy drugs used in clinical practice can induce high levels of ROS[103]. For example, taxanes represented by paclitaxel and docetaxel drugs, vincristine and vinblastine represented by vincristine alkaloids, as well as anti metabolites such as folic acid drugs, can produce superoxide free radicals through mitochondrial apoptosis pathways. Next is radiotherapy, which is another commonly used method for treating tumors in addition to surgery and chemotherapy. On the one hand, radiation therapy can cause an increase in ROS levels by causing abnormalities in mitochondrial function. On the other hand, in addition to causing mitochondrial abnormalities, radiation therapy can also directly activate NADPH oxidase, causing sustained ROS production. Inhibition of ubiquitin and protease, and the drugs involved in the systemic pathway and the inhibition of proteasomes lead to the accumulation of apoptotic proteins, oxidative damage, and mitochondrial dysfunction. For example, bortezomib is a proteasome inhibitor that can cause oxidative stress. Pharmacologically, drugs that induce endoplasmic reticulum pressure can promote ROS accumulation and effectively kill tumor cells by increasing endoplasmic reticulum pressure. Currently, many enhancers of endoplasmic reticulum pressure have begun preclinical trials. Drugs that affect GSH, GSH is one of the main reducing substances in cells. Tumor cells have higher levels of GSH than normal cells, and inhibiting GSH synthesis is more sensitive to tumor cells. GCL is a limiting enzyme for GSH synthesis, and has been studied as an anticancer target for over 30 years. The inhibitor of GCL, butylamine sulfoxide imine (BSO), is currently the only clinically used inhibitor that inhibits de novo synthesis of GSH. There are also drugs that affect the metabolism of thioredoxin, similar to GSH, which is also a central member of the redox system in animal cells. Jinnufen, a thioredoxin inhibitor, is a gold compound used clinically as an anti rheumatic drug. In head and neck squamous cell carcinoma cell lines, genistein and BSO can synergistically enhance the sensitivity of tumor cells to EGFR inhibitors and promote cell death. The key point is that antioxidant N. acetylcysteine can reverse this synergistic effect, indicating that it can be caused by interfering with thioredoxin.

In summary, since ROS plays an important role in the occurrence and development of tumors, and its level is significantly higher than that of normal cells, it is feasible to use ROS as a target for anti-tumor therapy. However, due to its complexity in tumors, we need more research to reveal its mode of action in tumors, in order to better utilize it for the treatment of tumors.

## 1.7 NF-κB/p53 signaling Pathway

With the development of molecular biology, the research on tumor related signal transduction factors is becoming more and more in-depth, such as factors that promote cell proliferation, anti apoptotic factors, and factors related to metastasis and angiogenesis. The tumor related factors P53 and NF studied in this experiment- κ B not only has the effects of promoting proliferation and anti apoptosis, but also has factors that promote metastasis[104]. P53 gene is the gene that has been found to have the highest correlation with human tumors. Under physiological conditions, P53 plays a regulatory role as a tumor suppressor gene. P53 inhibits the occurrence of tumors, and its regulatory mechanism is to detect whether the G1 phase DNA of the cell cycle is damaged, such as guiding DNA repair or promoting cell apoptosis to prevent the occurrence of tumors; In addition, P53 also regulates normal life activities through signal transduction, with over 160 genes involved in its regulation.

The occurrence of tumors is closely related to P53 gene mutations, and mutant P53 has an anti apoptotic effect, which causes damaged or mutated cells to continuously proliferate and become tumors. Mutant P53 has a long half-life and participates in cell transcription regulation directly or through other protein interactions, leading to malignant transformation of cells into tumors. Due to its long half-life, the P53 protein detected in tumor cells is often mutated[105]. At present, P53 protein has been detected in liver cancer, breast cancer, stomach cancer, bladder cancer cancer, colon cancer, prostate cancer, ovarian cancer, esophageal cancer, lung cancer, lymphocyte tumor and other malignant tumors[106]. It can be seen that there is a correlation between P53 and tumors. Mutant P53 promotes tumor cell proliferation through the expression of anti apoptotic genes, and can also regulate the expression of anti apoptotic genes by mediating related signal transduction.

NF- κ B (Nuclear factor κ Appa B) is a widely distributed set of cellular transcription factors that become important transcription factors due to their involvement in the body's defense response, tissue damage and stress, cell differentiation, apoptosis, and tumor growth inhibition processes. Overexpression of NF- κB is related to many diseases as it is a convergence point of multiple signaling pathways and plays a regulatory role in the occurrence of diseases. In the process of tumor development, it plays an important role in tumor development by promoting the transcription of target genes[107]. NF- κ as an important regulatory factor in the body, B participates in the regulation of processes such as inflammation, immunity, cell proliferation, and apoptosis. NF- κ B during tumor development can protect cells from apoptosis caused by tumor necrosis factor and ionizing radiation; At the same time, it can also promote the expression of oncogenes and the inactivation of anticancer genes through cell signal transduction function, therefore NF- κ B is closely related to the occurrence of tumors.

Current NF- κ B has significant cell growth promoting and anti apoptotic effects, closely related to cell malignant transformation. NF- κ By binding to specific gene sequences, B achieves regulation of the gene and affects the biological effects of cells, such as apoptosis or anti apoptosis. Research has found that NF is present in various human tumor cell lines- κ B is in a continuously activated state, and NF-KB regulates various target genes, such as TGF- α, COX-2, VEGF, MMPS, uPA, Bcl-2, Ras, c-myc, and other genes all contain sites that bind to NF-KB, thereby promoting the occurrence, development, and metastasis of tumors. By analyzing NF- κ It is of great significance to further understand the molecular mechanisms underlying the occurrence and development of gastric cancer by how B affects the expression of the above tumor related factors[108].

P53 and NF- κ B both factors exist and play a regulatory role in normal cells, regulating normal cellular life activities. However, they are highly expressed in tumor cells, indicating a correlation between these two factors and the occurrence of tumors[109]. Tumor cells have biological characteristics such as unlimited proliferation, evasion of apoptosis, promotion of neovascularization, and invasion and metastasis, which make them exhibit malignant characteristics. The proliferation and death of cells in normal tissues are balanced, which relies on cell apoptosis to maintain balance. The avoidance of apoptosis by tumor cells is the basis for their occurrence and proliferation; Mutant P53 and NF- κB can regulate the escape of cancer cells from apoptosis in tumor cells, and can regulate the expression of specific proteins within cancer cells to promote tumor cell invasion and metastasis[109, 110]. The disorder of cell apoptosis is closely related to the formation of tumors. Apoptosis not only maintains a stable number of cells in the body, but also maintains the accuracy of genes. Therefore, abnormal apoptosis has become an important link in carcinogenesis.

The above content explains P53 and NF- κ B plays an important regulatory role in the occurrence and development of tumors, promoting tumor formation and development by inhibiting cell apoptosis. Some scholars believe that NF- κ B can regulate the expression of P53, NF- κ B enters the nucleus and binds to a specific sequence on the corresponding gene promoter to initiate target gene transcription. By inhibiting P53 dependent apoptosis transcription, it upregulates the expression of Bcl-2 family anti apoptotic members and caspase inhibitors, mediating anti apoptosis[111]; P53, NF- κB is related to the regulation of the apoptosis inhibitory gene Bcl-2. Therefore, NF- κB, P53 may become a molecular marker for gastric cancer and achieve clinical targeted therapy.

NF- κB is widely present in eukaryotes and is an important nuclear transcription factor. It is an immunoglobulin discovered by Sen and Baltimore in mature B cells and plasma cells in 1986 κA protein that binds to a specific sequence of enhancers within a light chain, hence it is called NF- κB[112]. NF- κB is a member of the Rel protein family and exists in the form of homologous or heterodimeric dimers. Five have been found in mammalian cells NF- κ BRel family member[113]: NF- κ B1 (P50/P105), NF- κ B2

(P52/P100), RelA (P65), RelB, C-Rel. These proteins have a conserved region composed of approximately 300 amino acids at the amino terminus, called the Rel homologous region, which contains DNA binding domains, dimer binding domains, and nuclear localization signals. In the resting state of cells, NF- κ B1 binds to its inhibitor IKB and exists in an inactive state in the cytoplasm. If the amino terminal serine residue of IKB is phosphorylated by IKK under the action of relevant stimulating factors such as cytokines (TNF, IL-l), ultraviolet radiation, and radiation, the phosphorylated IKB is ubiquitinated and further degraded by protease, resulting in NF- κ B from NF- κ The active P50/P65 transcription factors are dissociated from the B complex and transferred to the nucleus, binding to corresponding target genes in the nucleus to regulate biological effects.

In recent years, due to the discovery of NF- κB protein is highly concerned for its involvement in important pathological and physiological processes such as immunity, inflammatory response, cell proliferation, and apoptosis in the body. NF- κ B exists in various cells and is an important signaling pathway with multidirectional regulatory effects. It can regulate the transcription of protein molecule genes involved in cell kinases, chemokines, growth factors, cell adsorption factors, and early responses[114]. NF- κB binding to specific gene sequences, achieves the regulation of that gene and affects the biological effects of cells, such as regulating cell apoptosis or anti apoptosis. The mechanism may be to promote the expression of some cytokines such as IL-1, VEGF, MMP, and uPA. NF- κ B can activate IL-1, IL-2, c-myc, and TNF- α byThe anti apoptotic effect of promoting cell apoptosis may be through upregulation of IL-1, IL-2, IL-6, macrophage colony stimulating factor, and peroxidase degrading enzymes such as superoxide dismutase, as shown in NF- κ The characteristic of multi-directional regulation of B[115]. NF- κ B is closely related to tumors, and is found in various malignant tumors. It is expressed and plays a very important role in inhibiting tumor apoptosis, promoting tumor cell proliferation, and tumor metastasis.

# 2. Hypothesis and objectives

## 2.1 Hypothesis

Our scientific hypothesis is that UCMSCs and their exosomes, although proven to be safe and reliable in healthy populations, have the potential to accelerate tumor progression if used in tumor patients.

## 2.2 Objectives

The main objective: To provide basic data for the clinical safety application of UCMSCs and their extracellular vesicles by studying the effects of UCMSCs and their extracellular vesicles on tumor cell phenotype.

The first part: This part mainly involves the isolation and cultivation of UCMSCs, including tissue block culture methods and enzyme digestion methods. To induce differentiation of obtained mesenchymal stem cells through three embryonic layers, that is, to detect their multidirectional differentiation potential, including adipogenic, osteogenic, chondrogenic, and neurogenic cell induction differentiation.

The second part: Explore the potential application of cogrowth with polylactic acid membranes in tissue engineering. Through the above experiments, human UCMSCs that meet the standard definition were obtained, laying an experimental foundation for studying the effects of UCMSCs and its exosomes on different types of tumor cells.

The third part: To study the effects and mechanisms of UCMSCs and their exosomes on four types of tumor cells.

# 3. Methods and materials

## 3.1 Reagents and instruments

Table 1. Reagents

| Name | Brand |
|---|---|
| MEM medium | Hyclone |
| DMEM | Hyclone |
| fetal bovine serum(FBS) | Gibico |
| Penicillin Streptomycin | Hyclone |
| Serum substitute | Hyclone |
| MSC—BM culture medium | Helios |
| Trypsin | Gibico |
| Osteogenic induction kit | Cyagen |
| Lipid induction kit | Cyagen |
| Chondrogenic induction kit | Cyagen |
| collagenaseII | Gibico |
| Oil Red Staining Kit | Gibico |
| Alizarin Red Staining Kit | Gibico |
| Standard Alixin Blue Kit | Gibico |
| Neutral Formaldehyde#Forms | Helios |
| Toluidine blue dye | Helios |

Table 2. Instruments

| Instrument name | Brand |
|---|---|
| Inverted fluorescence microscope | Olympus Bx71 |
| Low temperature centrifuge | Thermo Scientific |
| Nucleic acid concentration detector | Aosheng |
| Ultra Clean Workbench | Suzhou Purification |
| Ultra low temperature refrigerator | Haier |
| Flow cytometry | BD in the United States |
| Field emission electron scanning microscope | Hitachi in Japan |
| CO2 constant temperature incubator | Panasonic |
| Biosafety cabinet | Suzhou Antai |
| Thermal cycling instrument | Eppendorf |
| Gel imager | Shanghai Peiqing |
| Constant temperature shaker | Jintan Ronghua |
| Electrophoresis apparatus | Bio-Rad |

## 3.2 Isolation and Identification of UCMSCs

In this experiment, UCMSCs were isolated and cultured, and their biological characteristics were identified and studied. The main experimental process includes the isolation and acquisition of UCMSCs, surface labeling identification, and characteristic exploration.

Pregnant women with normal full-term pregnancy delivered between January 2021 and October 2021 in our hospital were taken as research subjects. After obtaining the informed consent of the puerperae, the umbilical cord tissues and blood after childbirth were examined for pathogenic microorganisms and virus infection, and the follow-up research was carried out after confirming that there was no pollution.

UCMSCs are separated and cultured using two methods: tissue block culture and pancreatic enzyme digestion. Umbilical cord was disinfected, fresh cord blood and tissue block were collected for detection to confirm that there was no pathogenic microorganism and virus infection. The band comes from medical waste meets ethical requirements.

The disinfection treatment method for fresh umbilical cord is as follows: Prepare three 250mL glass beakers after high-pressure washing, and add 1% double antibody to beaker 1， Soak and wash about 100mL of PBS once. In beaker 2, wipe the entire umbilical cord with iodine once. Add 1% to beaker 3,The PBS of the double antibody is about 150mL, soaked for 1min, and then taken out and placed at 150mm spare in a flat dish.

Separation and acquisition of Huatong glue: Cut off the umbilical cord at approximately 20-30 Cm, with knots at both ends, and use tweezers to squeeze out blood as much as possible. Use a syringe to draw 1% PBS of double antibody to flush the umbilical cord blood vessel, and be sure to flush the blood. Cut the washed umbilical cord into small segments, each approximately 3-4cm in length, for easy subsequent separation operations. Carefully follow the direction of the blood vessels with a deterrent, remove 2 arteries and 1 vein, and then peel off the epidermis to obtain pure Huatong glue. Tear the Huatong glue into strips, and wash twice with filtered PBS for later use. Steps of tissue block culture method: the tissue treatment is to gently crush the Huatong glue tissue block into the required granules in a glass beaker through surgical scissors, with the length and width of about 3mm, without damaging the cells. Spread the tissue blocks evenly on a flat plate and place them in an incubator to adhere to the wall for 30 minutes. Culture steps of enzyme digestion method: obtain Huatong gum, add 0.05% non animal derived Collagenase at a ratio of 1:1, and treat at 37 ° C for 20min, so that cells can be separated from tissues, but will not damage the structure of cell surface facial mask. After the Interstitial fluid is treated with comminution enzyme, add 10 times of the volume of PBS to mix, centrifugate for 10min at 1000r/min, and discard the supernatant. Resuspension the

sediment to a constant volume of 45mL. Place the treated tissue inside the dish and place it in a cell culture incubator at 37 ° C for 30 minutes. After it adheres to the wall, add preheated culture medium.

At least 8 replications of each experiment were done.

## 3.3 Culture and preservation of UCMSCs

Cell culture system and environment: Serum-free complete culture medium is obtained by mixing synthetic serum substitutes with serum-free basic culture medium in a ratio of 1:19. Use an electric Pipette to add the mixed culture medium into the above flat dish that has been attached with tissue to ensure that the tissue is completely immersed, and act gently to prevent the tissue block from floating; After adding the liquid, place the plate at 37 °C. Cultivate in a saturated humidity environment with a concentration of 5% C02.

Cell passage method: Cells are passaged until the logarithmic growth phase, grown to 80% -90%, harvested cells, culture medium is aspirated, and washed twice with DPBS; Add recombinant pancreatic enzyme and react at 37 ° C for 3-5 minutes; Add 6 times the volume of DPBS to terminate the reaction, blow cell clusters until all cells are digested, and prepare a cell suspension; Place the cell suspension in a centrifuge and centrifuge at 1500rpm for 5 minutes. Discard the supernatant to collect cells, and pass them in a 1:3 ratio or freeze them.

Cell cryopreservation method: Use serum-free cryopreservation solution, label the cell information in detail, and first place it in a programmed cooling box, overnight at 80 ° C for programmed cooling; Transfer and freeze in liquid nitrogen on the second day.

Cell morphology detection: During the process of cell culture and separation, it is necessary to promptly observe the changes in cell morphology through a microscope, regularly inspect cell surface markers, and verify their pluripotency and proliferation characteristics.

## 3.4 Inducing osteogenic differentiation of UCMSCs

The osteogenic differentiation process is induced by preparing reagents and gelatin according to the requirements of the kit, and inducing as follows:

Prepare board gelatin with an appropriate amount of 0.1 added: Gelatin can cover the entire bottom of the culture vessel when added to it. Shake the liquid evenly to cover the bottom of the entire culture vessel, and apply 0.1% gelatin to the culture vessel. Place the culture vessel on the ultra clean table, discard the gelatin at least 30 minutes later, and let the culture vessel dry to form a thin film. Afterwards, it can be used to inoculate cells;The induction experiment was conducted with a six hole plate.

When the Cell fusion degree reached 80-90%, use 0.25% Trypsin , 0.04% EDTA for digestion;Digested human UCMSCs were divided into 2*10 4,Cell density vaccination of cells/cm2 in progress. Add 2mL of complete culture medium to each well in a six well plate coated with 0.1% gelatin;Place cells at 37° C and 5% C02 incubator ;When the Cell fusion degree reaches 60%,at 70%, carefully remove the complete culture medium from the well and move it towards the six well plate;Add 2mL of complete culture medium for osteogenic induction and differentiation of human UCMSCs;Replace with fresh OriCell every 3 days;Complete Culture of Osteogenic Induction and Differentiation of Human UCMSCs; Base needs to be preheated to 37 ° C to induction , After 2~4 weeks, depending on the morphological changes and growth status of the cells, stain them with alizarin red.

Osteogenesis induced differentiation detection using alizarin red staining method: After the completion of osteogenic induction differentiation, remove the complete osteogenic induction differentiation medium from the six well plate and rinse 1-2 times with IxPBS. Add 2mL of 4% neutral formaldehyde solution to each well and fix for 30 minutes. Absorb the neutral formaldehyde solution and rinse twice with lxPBS. Add 1mL of alizarin red dye solution to each well and dye for 3-5 minutes. Absorb the alizarin red dye solution and rinse 2-3 times with lxPBS. Place the culture plate under a microscope to observe the osteogenic staining effect and obtain images.

At least 8 replications of each experiment were done.

## 3.5 Inducing adipogenic differentiation of UCMSCs

After preparing induction reagents A and B as per the kit requirements, the induction process should be carried out as follows: Cultivate human UCMSCs in a 37°C, 5% CO2 incubator. When the cell fusion degree reaches 80%-90%, perform digestion using 0.25% Trypsin and 0.04% EDTA. Divide the digested mesenchymal stem cells into 2x106 cells and inoculate them at a density of 4 cells/cm2 into a six-well plate, adding 2mL of complete culture medium per well. Place the cells in a 37°C, 5% CO2 incubator for cultivation. Change the medium every 3 days until the cell fusion degree reaches 100% or over fusion. Carefully remove the complete medium of mesenchymal stem cells and add 2mL of OriCell human umbilical cord mesenchymal stem cell adipogenic differentiation medium A to the six-well plate. After 3 days of induction, extract liquid A from the six-well plate and add 2mL of RiCell human umbilical cord mesenchymal stem cell adipogenic differentiation medium B. After 24 hours, aspirate liquid B and switch back to liquid A for induction. After inducing alternating action of liquid A and liquid B for 3-5 times, approximately 12-20 days, continue to culture with liquid B for 4-7 days until the lipid droplets become sufficiently large and round. During the maintenance culture period of solution B, fresh B solution needs to be used every 2-3 days.

The method for detecting fat induction differentiation, using the oil red staining method, is as follows: Upon completion of adipogenic differentiation induction, aspirate the adipogenic differentiation medium of mesenchymal stem cells in the six-well plate and rinse 1-2 times with 1xPBS. Add 2mL of 4% neutral formaldehyde solution to each well and fix for 30 minutes. Remove the neutral formaldehyde solution and rinse twice with 1xPBS. Apply 1mL of oil red O dye working solution to each well for staining for 30 minutes. To prepare the oil red O working solution: mix distilled water and oil red O storage solution in a 3:2 ratio, ensuring thorough mixing and filtration using neutral filter paper. Remove the oil red O dye solution and rinse 2-3 times with 1xPBS. Place the culture plate under a microscope to observe the lipid staining effect and capture images.

At least 8 replications of each experiment were done.

## 3.6 Inducing chondrogenic differentiation of UCMSCs

After preparing the induction reagents according to the kit requirements, the process of inducing chondrogenic differentiation is as follows: Prior to conducting the chondrogenic differentiation induction experiments, it is necessary to perform cell counting after routine digestion. Transfer 3-4 × 10^5 cells to a 15mL centrifuge tube and centrifuge at 250g for 4 minutes. Remove the supernatant, add 0.5mL of premix, and resuspend the pellet obtained by centrifugation to clean human umbilical cord mesenchymal stem cells. Centrifuge at room temperature at 150g for 5 minutes, repeating the cell cleaning process. Resuspend the pellet obtained from the previous step with 0.5mL of human UCMSCs to induce chondrogenic differentiation complete culture medium. Centrifuge at room temperature at 150g for 5 minutes. Loosen the centrifuge tube cover to facilitate gas exchange, and place it in a 37°C, 5% CO2 incubator. Note that this step does not require removing the supernatant and resuspending the cells. Do not shake the centrifuge tube within 24 hours. When cell clusters gather, typically after 24 or 48 hours, depending on cell growth, gently flick the bottom of the centrifuge tube to detach the chondrosphere from the tube and suspend it in the liquid. Starting from inoculation, replace the cells with fresh chondrogenic-induced differentiation complete culture medium every 2-3 days, using approximately 0.5mL per tube. Be careful not to aspirate the cartilage ball. After changing the fluid, lightly flick the bottom of the centrifuge tube to detach the chondrosphere from the tube and suspend it in the liquid. Slightly loosen the centrifuge tube cover and place it in a 37°C, 5% CO2 incubator to continue the induction culture. Generally, after 21-28 days of continuous induction, the cartilage ball can be fixed in formaldehyde, embedded in paraffin, and finally stained with Alcian blue.

Chondrogenic induction differentiation detection using Alcian blue staining method. The cartilage ball was embedded in paraffin and sliced with a thickness of 7-

10 μm. The staining process involves dewaxing and dehydrating the sample. Subsequently, the sample is treated with Alcian blue staining solution and stained for 30 minutes. The sample is then rinsed with tap water for 2 minutes and distilled water once to remove residual dyes. Upon observing the effect of Alcian blue staining under a microscope, the Alcian blue staining section reveals the presence of intracellular acidic mucopolysaccharides in the cartilage tissue, as depicted in the accompanying image.

At least 8 replications of each experiment were done.

## 3.7 Inducing neurogenic differentiation of UCMSCs

The method for inducing neurogenic cells using 3-mercaptoethanol is as follows: In Induction Scheme A, cells were continuously induced with 1mmol/L of 3-mercaptoethanol for 24 hours, followed by staining detection. The induction protocol involved continuous induction with 1mmol/L for 24 hours, followed by 25mmol/L induction for 6 hours, and subsequent staining detection. The Toluidine blue detection method is as follows: After removing the cell supernatant from the six-well plate, wash twice with PBS, immediately fix in 95% ethanol for 15 seconds, and drain. Then, the toluidine blue staining solution was added and cells were stained for 5 minutes. At least 8 replications of each experiment were done.

## 3.8 Identification of surface markers

The labeling of CD11b, CD34, CD45, and HLA-DR with FITC was performed on the negative surface, along with corresponding FITC-labeled control type antibodies. The positive surface was marked with PE-labeled CD90, CD73, CD105, and corresponding PE-labeled control antibodies, while a blank control group was established. The flow cytometry detection process is outlined as follows: Cells were digested with trypsin at 1500 rpm, centrifuged for 5 minutes, supernatant discarded, and then washed with 1% FBS DPBS. After resuspending in DPBS containing 1% FBS, cells were counted using a cell counter and the cell suspension concentration was adjusted to 5-10 x 10^5/mL. Antibodies were added to each tube, ensuring the system was controlled at 4°C, and allowed to react on ice for approximately 30 minutes. The cells were washed with DPBS containing 1% FBS to remove unbound antibodies, centrifuged at 1500rpm for 5 minutes, and the process was repeated twice. After resuspension in 500 mL of DPBS containing 1% FBS, the cell suspension concentration was adjusted to approximately 1 x 10^6/mL in preparation for machine testing. It is important to note that on the same day of testing, fixation with polyformaldehyde is not necessary, unless for storage purposes. An equal amount of distilled water was added dropwise onto the smear, mixed well, and then stained for 15 minutes. The samples were washed, air-

dried in the six-well plate, and subjected to microscopic examination to capture images. At least 8 replications of each experiment were done.

## 3.9 Growth of UCMSCs on polylactic acid membrane

The cell culture method on the surface of the polylactic acid membrane is as follows: Sterilize the polylactic acid membrane under ultraviolet irradiation. Cut it into small pieces and place it on a 6-well plate, cover it with basic culture medium for 4 hours, and incubate it in a 5% CO2, 37°C incubator for later use. Inoculate 1x10^5 UCMSCs onto each membrane at a dose of 5 and place them in a 5% CO2, 37°C incubator, allowing them to stand for 1 hour. After the cells are attached to the membrane, add 2mL of complete culture medium along the walls of the 6-well plate for each well.

The specific operational method for the S4800II field emission electron scanning microscope detection is as follows: Remove the polylactic acid membrane with cells growing on the surface from the incubator and wash it with PBS once, followed by sterile water. For front fixation, mix 2.5% paraformaldehyde with pentene: acid in Minding solution and let it sit overnight at 4-16°C. Wash with 0.1M PBS or double-distilled water 3-4 times. Perform gradient dehydration, with ethanol ranging from 30% to 50% to 70% overnight, followed by 80%, 90%, 95%, and 100% ethanol, and secondary addition of anhydrous Na2S04. Dry in the CPD300 critical point dryer. Affix the sample, with the observed surface facing upwards, onto the sample stage. Conduct conductive spraying on the surface of the sample inside the SCD500 ion sputtering instrument. Scan and observe on the computer, and take photos for analysis. Precautions: Do not touch the sample when replacing the liquid, and do so quickly, especially when replacing high concentration dehydrating agents. Stay for 10 minutes per step, and for 15 minutes with 70% ethanol dehydrator. Dehydration from 80% ethanol should start at room temperature.

## 3.10 DAC and TSA treatment of squeezed UCMSCs

The drug concentration screening involved setting up 5 concentration gradients for dexcitabine 5-Aza-2'Deoxycytidine (DAC). The gradient treatment concentrations for DAC were 5, 2.5, 1.25, 0.625, and 0 (pM). For Trichostatin A (TSA), the gradient treatment concentrations were 100, 50, 25, 12.5, and 0 (ng/mL), and the time gradient treatment was conducted over 3, 6, 12, and 24 hours. Following the treatment, the reagent was removed, and cultivation continued. In the growth experiment, the passage ratio was maintained at 1:3, with passage occurring approximately 90% of the time, initiating fusion down passage, and counting every generation.

## 3.11 Isolation and identification of exosome from UCMSCs

Inoculate UCMSCs into a cell culture bottle and subculture them when they grow to 80-90% fusion. Collect fresh culture supernatant and extract extracellular vesicles of UCMSCs using differential centrifugation and reagent kit extraction methods.

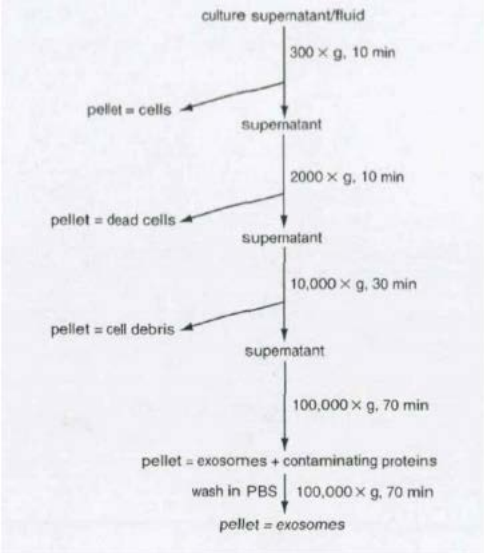



Figure 1. Separation and purification of exosomes from UCMSCs using differential centrifugation method

The differential centrifugation method is employed to isolate extracellular vesicles using centrifuges at varying speeds. This involves initially eliminating impurities such as cells and cell fragments using a standard centrifuge, followed by the extraction of extracellular vesicles through ultracentrifugation. The process is illustrated in Figure 1, and the specific operations are as follows: Remove suspended cells and cell fragments through centrifugation at 300xg, 4°C for 10 minutes. Transfer the supernatant to a new centrifuge tube, discarding the sedimented cells at the tube's bottom. Centrifuge the new tube at 2000xg, 4°C for 10 minutes. Collect the supernatant and transfer it to a new tube, discarding the precipitated dead cells at the bottom. Centrifuge the new tube at 10000xg, 4°C for 30 minutes. Collect the supernatant and transfer it to a new tube, discarding the precipitated cell fragments at the bottom. Transfer the upper clear portion to a new ultra-speed centrifuge tube, mark the direction, and centrifuge at 100000xg, 4°C for 70 minutes. Discard the supernatant, precipitating proteins and extracellular vesicles, and carefully resuspend the precipitate with filtered PBS. Mark the direction, and centrifuge at 100000xg, 4°C for 70 minutes. The obtained precipitate comprises the extracellular vesicles, which are dissolved in PBS for subsequent use.

The kit method uses commercially available kits to extract exosomes, which are mainly divided into three parts: removing cell fragments, precipitating exosomes, extracting exosomes, and purifying them. The process is shown in Figure 2.

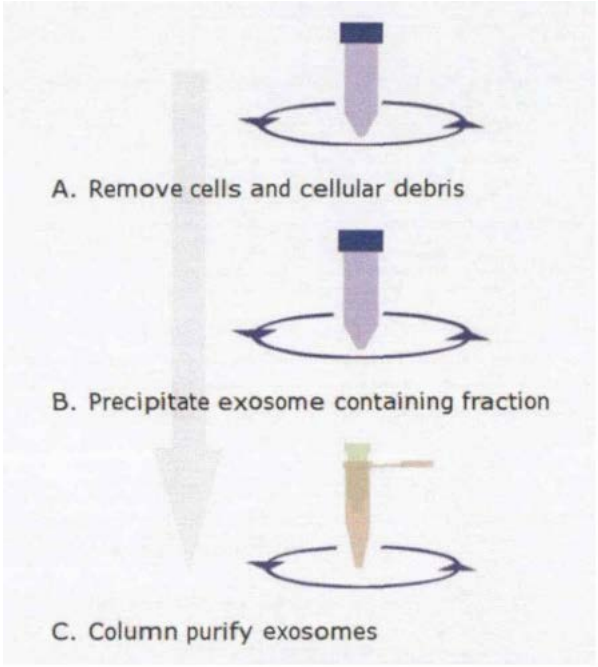



Figure 2. Method for extracting extracellular vesicles from UCMSCs using a kit

The specific steps for extracting the reagent kit are as follows: Remove suspended cells and cell fragments, add about 50mL of cell culture supernatant, and centrifuge to 50mL，In the tube, at 300x at a speed of g, centrifuge for 10min to remove cells. Transfer the supernatant to one, in a new centrifuge tube, at a speed of 16000x, 4℃ centrifuge at for 30 minutes, enter one，Step by step to remove cell fragments. Sediment exosomes, transfer the previous transfer the obtained supernatant to a new centrifuge tube in a ratio of 1:2, for example, add 50 mL of supernatant and 25 mL of extracellular precipitate reagent. Mix the two thoroughly until no viscous liquid is visible, and as for 4 ℃, incubate for 1 hour until overnight. The next day, mark the direction of placing the precipitated mixture in the tube and place it at a rotational speed of 16000xg 4° C，centrifuge for 60 minutes. Carefully remove the centrifuge tube from the centrifuge and remove the supernatant in the marked direction, taking care not to sink it Dianqian stemness. Dissolve and precipitate again with 100pL of DPBS. To purify the exosomes, first pre-treat the exosomes purification column, remove the upper cover, and use a test to dissolve the exosomes agent balance filter column, placed at 50x, 4°C centrifuge for 10 seconds, repeat one second, if on the pillar residual liquid, 50xat a speed of g, 4°C centrifugation for 5 seconds. Using a micropipette, carefully transfer the product obtained in step 7 above to the upper layer of the filter column for purification at a speed of xg, centrifuge at 4° C for 30 seconds and re elute with DPBS to obtain purified extracellular vesicles

## 3.12 Determination of exosomes concentration

The concentration of exosomes is measured through three methods: BCA protein quantification, Nanodrop assay, and Qubit measurement.

BCA protein quantification method is currently one of the widely used protein quantification methods, which can achieve rapid and efficient analysis of proteins, the specific steps for stable and sensitive concentration determination are as follows: Diluted BSA standard: used with the protein sample to be tested one. The diluent used to dilute BSA standards is shown in the table below Product. Prepare BCA working solution and make 7 gradient dilution tubes everyone next from previous. Take from a tube one half dilute the liquid and use it as a standard, with a total of 6 effective points and 1 zero point; Calculate the total amount of BCA working fluid, BCA working fluid total=(Number of BAS standard samples+unknown samples). This number x the number of pores x the volume of BCA working solution for each sample. (3) Based on the calculated total amount of BCA working fluid required, convert BCA-ASoMon and BCA_B Solution: Prepare BCA working solution in a 50:1 volume ratio and mix thoroughly. (4) Quantitative testing: Add diluted BSA standards and protein samples to the sample at 25 mL each in the well marked 96 well plate, it is recommended to do 2 for each sample to be tested. Three parallel reactions.(Based on the sample),the concentration can be diluted 5 or 10 times in advance; (5) Add 200pLBCA working solution to each well, mix well, cover with a 96 well plate cover, and incubate at 37 ° C for 30 minutes, cool to room temperature, at 3 complete the test within 5 minutes; (6) Use an enzyme-linked immunosorbent assay (ELISA) to measure the absorbance values of each sample and BSA standard at 562nm, while also preparing Record; (7) Draw a standard curve and calculate the protein concentration in the sample.

Qubit quantification is a method of using fluorescent labeled proteins for measurement: (1) Firstly, dilute the standard reagents from the reagent kit into three standard samples for plotting, and add them proportionally. Add fluorescent reagents, standards, and diluents, let stand for 15 minutes, and draw a 3-point standard curve; (2) Dilute the sample to be tested and make 3 Fengxing samples. After standing for 15 minutes, measure it with an instrument; After zero calibration, add the sample to be tested for measurement and take the mean.

Nanodrop measurement method: (1) Clean the measuring head and use solvent from the exosomes one Blank correction; (2) Measure the protein concentration of the sample; (3) If the value is large, it is necessary to dilute the sample and continue measuring, taking the average of multiple measurements.

At least 8 replications of each experiment were done.

## 3.13 Identification of surface antigens of exosomes

Collect fresh cell and extracellular samples for the following identification process, with the specific steps as follows: Adhesive making, SDS - PAGE gel preparation, SDS produced by Biotech is used in this study- PAGE Pre Gluing. Sample preparation, adding 5X SDS -PAGE protein loading buffer, 100°C or boiling water bath heating 3-5 minutes to fully denature the protein. Add sample, cool the sample to room temperature, mix the protein sample with approximately 20pL/sample, and directly add the sample， Simply add the SDS-PAGE adhesive into the sample well and add the pre dye marker known as L. Electrophoresis, it is usually recommended to use low voltage 80V constant voltage electrophoresis on the upper layer of gel, while in bromophenol when blue enters the lower layer of gel, use high voltage ll 0v constant voltage electrophoresis to place the entire electrophoresis system in an ice box. The electrophoresis duration is about l00min, and when bromophenol blue approaches the bottom line, the electrophoresis can be stopped. Cut the film, this study used PVDF film, compared with the marker on the adhesive, estimated the size of the film used, and cut it off Specific size film, marked with a pencil, gently picked up with tweezers for film rotation and placed in methanol, medium activation membrane. Transfer the membrane in the following order: anode, sponge, multi-layer filter paper, PVDF membrane, SDS adhesive, multi-layer filter Paper, sponge, cathode Sandwich Structure, followed by the entire Sandwich insert the structure into the slot and place it in the rotating film liquid, the current of the rotating film is 300mA, about 40min, and the entire system is placed in ice to cool down. Pre dye, transfer the film to the predetermined time, and then dye it with spring red staining solution to determine whether the transfer has ended. Seal and immediately place the protein film in the pre prepared washing solution after membrane transfer, and place it on a shaking table Rinse 1-2 minutes, rinse off the transfer solution on the membrane, and follow all steps after the transfer is completed, one definitely necessary Pay attention to the moisturizing of the membrane and avoid drying it, otherwise it is easy to produce a high background. Slowly shake the shaking table at a speed of 45r/min at closed overnight at 4° C, lateral swing speed comparison, slow and easy to allow the solution to cover the protein film. Anti incubation, absorb the sealing solution thoroughly, wash with the washing solution, and immediately add the diluted solution according to the instructions oneAnti, Slowly shake and incubate overnight on a side rocking bed at 4° C. Film washing and recycling one. After resistance, use film washing solution and repeatedly wash the film 3 times, washing 5 times each time 10min. Incubate the second antibody, absorb all the washing solution, and remove the membrane. Place it on the side on absorbent paper and use it in an appropriate proportion, dilution of horseradish peroxidase with secondary antibody diluent HRP labeled secondary antibody, at room temperature on a side sway shaker slow shaking incubation l~2 hours. At least 8 replications of each experiment were done.

## 3.14 Transmission electron microscopy

The electron microscopy detection method of Exsomes is as follows: Take freshly extracted Exsomes and dilute them with different gradients using DPBS. Sample preparation of exosomes: Place the copper mesh containing the exosomes sample on a plastic film, and then place the film on top of it. On the filter paper, then drop the sample onto the copper mesh of the sample carrier, so that the copper mesh of the sample carrier is located inside the droplet and precipitated. After about 2 minutes of sedimentation, use filter paper to absorb excess samples, and then apply copper mesh one, dry the surface on filter paper for 5 minutes. Negative staining sample: Place the loaded copper mesh back on the thin film, add 1% uranyl acetate dropwise for negative staining for 2 minutes, and then aspirate.Remove excess dye and dry for 40 minutes before use. Observe the sample under a transmission electron microscope and collect images. Wash the film, recover the secondary antibody, use the film washing solution, repeatedly wash the film 3 times, each time washing 5-10min Protein detection, using ECL reagents to detect proteins and collecting images. At least 3 replications of each experiment were done.

## 3.15 Particle size analysis of exosomes

The principle of NTA detection is to track the Brownian motion of each particle.By analyzing and combining the Stokes Einstein equation to calculate the hydrodynamic diameter and concentration of nanoparticles, this technology has been recognized as one of the characterization methods for exosomes in the field of exosomes research. Compared to other characterization methods, NTA technology has simpler sample processing, better assurance of the original state of extracellular vesicles, and faster detection speed.

This study used NTA technology to detect the obtained extracellular vesicles. Dissolve the extracellular vesicles obtained during the above experimental process with DPBS, classify and label them, and perform NTA particle size analysis to obtain data on particle size and distribution. Among them, C1 represents the extracellular vesicles obtained by differential centrifugation, S2 represents the purified extracellular vesicles extracted by the kit, and S3 represents the unpurified extracellular vesicles extracted by the kit.

At least 8 replications of each experiment were done.

## 3.16 Cell culture and preservation

The human breast carcinoma cell line MDA-MB-231, gastric carcinoma cell line BGC-823, lung carcinoma cell line A549, and glioma cell line LN-229 were all procured from ATCC. In vitro cell phenotypes, including cell proliferation, cell scratch healing, cell cycle detection, and cell invasion, were investigated to assess the impact of UCMSCs and their extracellular vesicles on the proliferation, migration, and invasion of various tumor cells.

The cell culture medium comprised DMEM culture medium supplemented with 10% FBS and 1% penicillin-streptomycin. The following steps were followed for cell handling: Thaw MDA-MB-231, BGC-823, A549, and LN-229 cells from liquid nitrogen and incubate them in a 37°C water bath. Gently agitate the cryopreservation tube to dissolve the cryopreservation solution. Upon dissolution of the cryopreservation solution, transfer the cells to a centrifuge tube containing 5ml of culture medium, and pellet them by centrifugation at 1000 rpm for 5 minutes, followed by discarding the supernatant. The suspended MDA-MB-231, BGC-823, A549, and LN-229 cells were cultured in a 10% fetal bovine serum medium. Subsequently, these cells were inoculated into a culture flask, gently agitated, and evenly mixed before being maintained at 37°C and 5% CO2 in a humidified atmosphere.

When the density of MDA-MB-231, BGC-823, A549, and LN-229 cells reaches 80%, subculture begins: Discard the culture medium and wash it once with PBS. Add 2ml of 0.25% trypsin to digest MDA-MB-231, BGC-823, A549, and LN-229 cells, and observe under a microscope. After digestion for 2 minutes, it can be observed that MDA-MB-231, BGC-823, A549, and LN-229 cells are separated from each other and become round, indicating that digestion has been completed. Discard trypsin and add it to the culture medium. Blow MDA-MB-231, BGC-823, A549, and LN-229 cells to prepare MDA-MB-231, BGC-823, A549, and LN-229 single cell suspensions. Incubate in a 1:3 ratio and continue to expand the culture at 37 ℃ and 5% CO2 saturated humidity.

When the cells fill about 80% of the culture bottle, discard the cell culture medium and wash twice with 2ml of PBS. Add lml of pancreatic enzyme to digest cells. Place the cell suspension in a 1.5ml Ep tube at 800-1000rpm and centrifuge for 5 minutes. Simultaneously prepare cell cryopreservation solution: 9001xl fetal bovine serum+100I-tl DMSO. Suck off the supernatant, suspend the precipitate with cell cryopreservation solution, and transfer it to a cryopreservation tube for even blowing. Mark the time and cell type, then freeze the cells gradient (4 ° C for 40 minutes, 20 ° C for 40 minutes, and 80 ° C overnight), and finally store them in a liquid nitrogen tank.

## 3.17 Co-culture

The co-cultivation process uses Transwell plugins, with UCMSCs used in the upper plugins and tumor cells laid in the lower well plates for easy observation in the experiment. The plug-in in the middle can isolate the upper and lower pore cells, but products such as exosomes secreted by the upper mesenchymal stem cells can affect the lower tumor cells. The diameter and pore size of the pore plates used in different experiments are different: 48 well plate inserts were used in cell proliferation experiments, 6 well plate inserts were used in wound healing and cell cycle detection experiments, and 24 well plate inserts were used in cell invasion experiments. The pore sizes of the membrane were 0.4, 3 nm, and 8 nm, respectively. At least 10 replications of each experiment were done. At least 10 replications of each experiment were done.

## 3.18 Adding exosomes

Use the exosomes of UCMSCs obtained in the previous step and store them in a -80 ° C ultra-low temperature refrigerator. Add a concentration of 200 μ g/mL to the cell culture medium after the experiment begins, and replace it at the specific experimental design time point in fresh cell culture medium.

## 3.19 Cell proliferation

Cell Counting Kit was used for cell proliferation in this study by CCK-8, abbreviated as CCK-8 reagent kit, is a WST based kit in a rapid and highly sensitive detection kit widely used for cell proliferation and cytotoxicity. According to the time requirements, lay the board to cultivate cells, and the initial cell volume for the cell proliferation experiment is to add about 2000 cells per well at a rate of 100 μ m. When testing, after cleaning cells with PBS, add CCK-8 and cell culture medium premix in a ratio of 1:10, that is, 1 % 10uL of culture medium added to 10 teams of CCK-8 solution. Place the cell culture plate in a 5% CO2, 37 ° C incubator and incubate for 3-4 hours. Transfer the incubated solution to a new 96 well plate, avoid light for detection, and continue to culture the cells in a 5% C02, 37 ° C incubator after treatment. Use an enzyme-linked immunosorbent assay to measure absorbance at 450nm, while using 650nm as the reference wavelength for dual wavelength measurement. Draw a cell growth curve using the 0D value as the ordinate and time as the abscissa of the results. At least 8 replications of each experiment were done.

## 3.20 Scratch experiment

The cell scratch experiment is a method used to detect the lateral migration ability of cells, which is widely used in experiments due to its simplicity and ease of operation. The principle is that when cells grow and fuse to a monolayer state, a mark is manually made on the monolayer cells, and the cells continue to be cultured. The cells at the edge of the scratch will gradually migrate until they fuse at the scratch. In order to achieve consistency in the experiment, the Ibidi plugin was used in this study, which can make cells form uniform initial scratches of about 500pm. The main steps of this method are as follows: (1) Take logarithmic growth stage tumor cells and inoculate them into a 6-well plate, with approximately 5x105 cells per well. Incubate them in a 5% C02, 37 ° C incubator for later use. At the same time, culture mesenchymal stem cells in a Transwell plugin; (2) Disinfect and soak the Ibidi plug-in with alcohol, repeatedly clean both sides with PBS, place it in a biosafety cabinet, turn on the fan and UV, and air dry after about 30 minutes; (3) When the cells grow to 80% convergence, digest the cells and count them; (4) Place the plug-in evenly in a 6-hole plate, and after approximately 5 minutes, the plug-in should adhere firmly to the bottom wall of the 6-hole plate; (5) Add a specific number of cells to the plugin according to the proliferation characteristics of different cells, approximately 2-4xl104/well; (6) Incubate for about 12 hours, and observe that the cells reach about 80%. Remove the plug, soak with PBS, wash away free cells, and add experimental treatment of transwell nesting on the cells. Pay attention to the cell's adhesion ability and move gently; (7) Add 2mL serum-free medium, culture in a 5% C02, 37 ° C incubator; (8) The time points for collecting images in this study are: Oh, 24h, 48h; (9)The images were analyzed using Image plus software, and the results were evaluated using a transfer index. The migration index of point B relative to point A: point B migration index=(scratch area A/scratch height A - scratch area B/scratch height B), where A is the initial timing point. At least 8 replications of each experiment were done.

## 3.21 Cell cycle

The cell cycle detection experiment was completed using a cell cycle and apoptosis detection kit. This kit is a classic propidium staining method, which uses n-dye method to detect cell cycle and apoptosis analysis. After treating cells with this kit, The main steps for further detection by flow cytometry are as follows: (1) Cell digestion: Collect the supernatant, clean the cells twice with DPBS, add 0.25% trypsin for digestion, wait for the tumor cells to become round and the gap to become larger, add the supernatant of the above cell culture medium to terminate digestion, recover all cells into a centrifuge tube, and gently disperse the cells to form a single cell state;

(2) Cell collection: Centrifuge at room temperature at a speed of 1000g for 3-5 minutes. Due to the different sedimentation rates and degrees of tumor cells, the supernatant can be carefully discarded after centrifugation by adjusting the centrifugation time and force; (3) Sample pretreatment, add about lmL of pre cooled DPBS, resuspend cells, and transfer them to a 1.5mL centrifuge tube. Centrifuge and precipitate cells again, carefully discard the supernatant, and gently tap the bottom of the centrifuge tube to disperse the cells; (4) Fixation of cells: Add 1mL of pre cooled 70% ethanol, gently blow and mix, fix at 4 ° C for 2 hours, depending on the situation. Fixation for 12-24 hours may be more effective. Centrifuge and discard the supernatant; (5) After sample post-processing, approximately lmL of pre cooled PBS resuspended cells were added to the fixed sample, and the cells were centrifuged and precipitated again. All cells were collected into a centrifuge tube and gently dispersed to form a single cell state; (6) Preparation of propidium iodide staining solution, prepared propidium iodide staining solution: 0.5mL staining buffer, 25 porphyrin propidium iodide staining solution（20x), l0MlRNaseA (50x), final volume 535M1, can be stored at 4 ° C in a short period of time; (7) Cell sample staining: Add 0.5mL of propidium iodide staining solution to each tube of cell sample, slowly and fully resuspend cell precipitation, and take a dark and warm bath at 37 ° C for 30 minutes. Subsequently, it can be stored at 4 ° C or in a dark place with ice. It is recommended to complete flow cytometry testing within 24 hours after dyeing is completed. Cell flow cytometry is used to detect red fluorescence excited by dyes at the excitation wavelength of 488nm using a flow cytometry; Cell flow cytometry analysis uses Modfit software for cell cycle analysis and light scattering analysis of cell DNA content. At least 8 replications of each experiment were done.

## 3.22 Cell invasion

The invasion experiment is commonly used to study the invasiveness of tumor cells in vitro, the matrix components in EHS sarcoma mainly include collagen, laminin, and proteoglycan. This colloid is cultured in cells.The membrane structure can be reconstructed in the nutrient medium, which is very similar to the matrix membrane structure of natural tissue cells, so it can be used for detection.

The procedure for assessing tumor invasion ability involves the following steps: (1) Allow the Matrigel Matrix to melt overnight at 4℃ and then dilute it with pre-cooled serum-free medium (Mxtrix) to a concentration of 10mg/mL, yielding a final concentration of 2mg. This process should be conducted on ice; (2) Add 100 µl of the diluted Matrigel Matrix to the upper chamber of a 24-well transwell; (3) Incubate the transwell at 37°C in a 5% CO2 environment for approximately 6 hours to allow the Matrigel Matrix to coagulate into a gel. (4) Prepare logarithmically growing tumor cells, wash and digest them, centrifuge at 1000g for 5 minutes, and suspend the cells in serum-free culture medium. Count the cells using a cell counter and adjust the cell

concentration to 10^6 cells/mL. Preheat the serum-free culture medium in a 37°C incubator with 5% CO2, and then add the cell suspension to the upper compartment of the transwell containing the Matrigel Matrix in the middle and lower pore plates. The experimental group should include UCMSCs or extracellular vesicles, while the control group serves as a reference. (7) At the specified time, remove the culture medium from the upper chamber, fix the cells with anhydrous methanol for 30 minutes, remove the methanol, and allow it to dry in a fume hood; (8) Add approximately 500 µl of crystal violet staining solution to each well and check for staining after 15 minutes. If staining is incomplete, continue the process; (9) Remove the crystal violet dye solution and wash with pre-prepared PBS three times to eliminate any residual crystal violet dye solution; (10) Carefully remove the Matrigel Matrix from the transwell membrane using a cotton swab, ensuring not to apply excessive force to prevent wrinkling of the membrane or dislodging of cells beneath it; (11) Invert the microscope to observe and capture images, and analyze the images using appropriate software such as Kagawa Image-Analyze or Proflus software. At least 8 replications of each experiment were done.

## 3.23 HLEC apoptosis

There is phosphatidylserine (PS) present on the inner side of the cell membrane. In the early stages of apoptosis, PS turns outward to the cell surface and can highly selectively bind to Annexin V. After labeling Annexin V with fluorescein isothiocyanate (FITC), an Annexin V-FITC probe with green fluorescence is formed, which binds to the everted PS to present green fluorescence in the cells. The nucleic acid dye propidium iodide (PI) can bind to DNA or RNA in the nucleus to make the nucleus appear red. The membrane permeability of cells increases during the late stage of necrosis, apoptosis, or apoptosis, and PI passes through the cell membrane, causing the cells to exhibit red fluorescence. Living cells will not experience the above situation. Therefore, after annexin V-FITC/PI double staining, flow cytometry can be used to distinguish between apoptotic and necrotic cells at different stages.

0.25% trypsin solution 500 µ L Digestive cells. Collect cells and count them, adjust the final concentration to 1 × 105 pieces/ml, thoroughly blow and inoculate onto a 6-well plate. 2ml per well, i.e. 2 × 105 pieces/hole. Shake the 6-well plate horizontally to evenly distribute the cells. Incubate with 5% CO2 in a 37 ℃ cell incubator. Cells fuse to 80% and continue cultivation. Discard the culture medium and add 500 to each well µ Digest cells with L-trypsin, gently blow and collect. Centrifuge at 800rpm for 4 minutes. Abandon Shangqing. Wash cells three times with pre cooled PBS. 195 µ L Annexin V-FITC binds to resuspended cells and filters the cell suspension through a 200 mesh filter into a flow cytometry tube. Avoid light and add 5 µ L Annexin V-FITC and 10 µ Gently mix L PI well. Incubate at room temperature in dark for 10 minutes. After the reaction is completed, it should be detected by flow cytometry within 1 hour.

Detection of cell apoptosis by flow cytometry. After loading the probe, the cells were digested with thawed trypsin at a rate of 500 per well μ L. After moderate cell digestion, gently blow the bottom of the dish and collect it into the EP tube. Centrifuge at 800rpm for 4 minutes. Abandon Shangqing, 500 μ L sterile PBS resuspended cells and centrifuged again. After cleaning, use 500 more μ L sterilized PBS resuspended cells and filtered the cell suspension onto a flow cytometry tube using a 200 mesh filter. Use flow cytometry to detect cell apoptosis. Set excitation light at 488nm and emission light at 525nm, and record the Median values of each group. Perform data analysis using Flowjo 7.6 software. At least 8 replications of each experiment were done.

## 3.24 Oxidative stress

Seed cells in the logarithmic growth phase onto a 6-well plate at an appropriate density. After overnight cell culture, subject the cells to the corresponding experimental conditions. Wash the cells twice with 1 mL of 1x PBS buffer. Use pancreatin to digest the cells until they detach. Halt the pancreatic enzyme digestion with 1 mL of DMEM, pipette the cells evenly, and transfer them to a 15 mL centrifuge tube. Centrifuge at 1200 rpm for 3 minutes and remove the supernatant. Add 700 μL of 1x PBS containing 5% FBS, wash once, transfer to a 1.5 mL centrifuge tube, and centrifuge at 3000 rpm for 3 minutes, then discard the supernatant. Repeat this step. Resuspend the cells in 200 μL of 1x PBS (without serum), add ROS, MDA, and SOD probes (CM.H2DCFDA) to a final concentration of 10.20 nmol/L, and incubate in the dark at 37°C for 20-30 minutes. Centrifuge at 3000 rpm for 5 minutes and wash twice with 700 μL of 1x PBS containing no more than 5% FBS. Resuspend the final cell pellet in 500 μL of 1x PBS containing 5% FBS. Filter the cell suspension through a 200-mesh gauze into a flow cytometry tube, place it on ice, shielded from light, and wait for machine detection. Employ ELISA to measure intracellular ROS, MDA, and SOD levels. At least 8 replications of each experiment were done.

## 3.25 Western blot

Preparation of protein samples: Extraction of total protein from monolayer adherent cells. Pour out the culture medium and invert the six well plate onto the absorbent paper to allow it to dry. Add 1ml of 4 ℃ pre cooled PBS to each well cell. Gently shake flat for 1 minute, wash the cells, and then discard the washing solution. Repeat the above operation twice, washing the cells three times in total to remove the culture medium. Discard the PBS and place the culture bottle on ice. Add 10 to 1ml of cracking solution μ L PMSF (100mM), shake well and place on ice (PMSF should be shaken until there is no crystallization before mixing with the cracking solution).

Add 150 cells per well μ The lysate containing PMSF should be lysed on ice for 30 minutes, and the culture plate should be frequently shaken back and forth to fully lyse the cells. After lysis, use a clean scraper to scrape the cells on one side of the well (quickly), and then use a gun to transfer the cell fragments and lysis solution into a 1.5ml centrifuge tube (the entire operation should be carried out on ice as much as possible). Centrifuge at 12000 rpm at 4 ℃ for 5 minutes (pre cooling with centrifuge). Transfer the centrifuged supernatant into a 0.5min centrifuge tube and store at -20 ℃.

To determine protein concentration, appropriately dilute the protein sample (mix 2 μL of the sample with 18 μL of PBS) to achieve a 10-fold dilution. Prepare standard proteins by diluting BSA standard to concentrations of 1, 0.8, 0.6, 0.4, and 0.2. Add the protein sample diluted with PBS and the standard protein sample diluted with PBS into a 96-well plate, with 2 parallel wells for each standard sample and 3 parallel wells for the sample to be tested, adding 20 μL to each well. Include two parallel wells of PBS as blank controls. Prepare the BCA reagent by mixing solution A and solution B in a 50:1 ratio, adding 100 μL of the mixture to each well in the 96-well plate, taking care to avoid bubble formation that could affect the reaction. Incubate in the dark at 37°C for 30 minutes. Measure the OD568 using an enzyme-linked immunosorbent assay. Calculate the linear regression equation based on the standard protein concentration and corresponding OD value, and use the regression equation to calculate the sample protein concentration based on the OD value of the protein sample. Finally, place the extracted protein supernatant and 5x protein sample buffer in boiling water for 10 minutes.

At least 8 replications of each experiment were done.

## 3.26 Electrophoresis

Preparation of electrophoretic gel (10% separation gel and 5% separation gel) (as mentioned earlier). Electrophoresis separation: Fix the prepared gel onto the electrophoresis tank, and pour the electrophoresis solution into the storage tank. Use a micro sampler to add the prepared protein sample and Marker to the sample well, and the total protein content of each sample is 40 μ G. After adding samples, first conduct electrophoresis at constant pressure of 80V until bromophenol blue indicator is linear at the junction of concentrated gel and separation gel, and then conduct electrophoresis at constant pressure of 120V until bromophenol blue reaches the bottom of gel. This process takes about 1.5h.

## 3.27 Electric transfer

Take out the gel, cut the target strip according to Marker, wash it with distilled water, cut the PVDF membrane and filter paper of the same size as the PAGE gel, soak the PVDF membrane with methanol for a few seconds, and then soak it with the filter paper in the electrokinetic buffer solution. Place the black plate - fiber pad - filter paper - gel - PVDF membrane - filter paper - fiber pad - white plate in order, clamp the plate and put it into the membrane rotator. One side of the black plate is compared with the black negative electrode. Fill the membrane transfer tank with electric liquid and start the membrane transfer.

## 3.28 Immunoblotting staining: ECL staining system

Soak the PVDF membrane in 5% skim milk sealing solution and seal it on a shaking table at room temperature for 2 hours. Dilute the first antibody, immerse the PVDF membrane in the first antibody incubation solution, and incubate at 4 ℃ overnight. Wash off excess primary antibody: TBST thoroughly washes PVDF membrane 3 times, 5 minutes per time. Secondary antibody: Dilute the corresponding HRP labeled secondary antibody with blocking solution, immerse the PVDF membrane in the secondary antibody for incubation in a shaker at 37 ℃ for 2 hours in liquid. Wash off excess secondary antibodies: TBST thoroughly washes PVDF membrane 3 times, 5 minutes per time. Color exposure: Mix the enhancement solution in ECL reagent with a stable peroxidase solution in a 1:1 ratio, add the working solution dropwise onto the PVDF film, and react for a few minutes until the fluorescence band is obvious. Use filter paper to absorb excess substrate solution, cover it with a cling film, and then develop and fix it sequentially after X-ray film compression. Develop the film. Dry the film, scan the film, and use BandScan to analyze the grayscale value of the film.

## 3.29 Detection of cell apoptosis by flow cytometry

There is PS present on the inner side of the cell membrane. In the early stages of apoptosis, PS turns outward to the cell surface and can highly selectively bind to Annexin V. After labeling Annexin V with FITC, an Annexin V-FITC probe with green fluorescence is formed, which binds to the everted PS to present green fluorescence in the cells. The nucleic acid dye propidium iodide (PI) can bind to DNA or RNA in the nucleus to make the nucleus appear red. The membrane permeability of cells increases during the late stage of necrosis, apoptosis, or apoptosis, and PI passes through the cell membrane, causing the cells to exhibit red fluorescence. Living cells will not experience the above situation. Therefore, through Annexin V-FITC/PI. After

double staining, flow cytometry can be used to distinguish between apoptotic and necrotic cells at different stages.

0.25% trypsin solution 500 μ L Digestive cells. Collect cells and count them, adjust the final concentration to 1 × 105 pieces/ml, thoroughly blow and inoculate onto a 6-well plate. 2ml per well, i.e. 2 × 105 pieces/hole. Shake the 6-well plate horizontally to evenly distribute the cells, incubate with 5% CO2 in a 37 ℃ cell incubator. Cells fused to 80% and continued to be cultured for 12 hours. Discard the culture medium and add 500 to each well μ Digest cells with L-trypsin, gently blow and collect. Centrifuge at 800rpm for 4 minutes. Abandon the supernatant. Wash cells three times with pre cooled PBS. 195 μ L Annexin V-FITC binds to resuspended cells and filters the cell suspension through a 200-mesh filter into a flow cytometry tube. Avoid light and add 5 μ L Annexin V-FITC and 10 μ Gently mix L PI well. Incubate at room temperature in dark for 10 minutes. After the reaction is completed, it should be detected by flow cytometry within 1 hour. Collect at least 10000 cells per sample. After loading the probe, the cells were digested with thawed trypsin at a rate of 500 per well μ L. After moderate cell digestion, gently blow the bottom of the dish and collect it into the EP tube. Centrifuge at 800rpm for 4 minutes. Abandon supernatant, 500 μ L sterile PBS resuspended cells and centrifuged again. After cleaning, use 500 more μ L sterilized PBS resuspended cells and filtered the cell suspension onto a flow cytometry tube using a 200-mesh filter. Use flow cytometry to detect cell apoptosis. Set excitation light at 488nm and emission light at 525nm, and record the Median values of each group. Perform data analysis using Flowjo 7.6 software.

At least 8 replications of each experiment were done.

# 4. Results

## 4.1 Isolation and cultivation of UCMSCs

Successfully isolated UCMSCs through tissue block culture and enzyme digestion methods. As shown in Figure 3 B1 and C1 points. The UCMSCs obtained during the initial separation stage by tissue block culture and enzyme digestion methods are shown in Figures 2-2B2 and C2. To cultivate P3 generation UCMSCs, the cells showed vortex like growth and obvious growth characteristics. Meanwhile, the tissue block culture method has a higher amount of cell acquisition than the enzyme digestion method.

As shown in Figures 1 D1 and D2, there was a significant difference in the growth performance of the P3 generation on the 6th day of comparison ($p<0.01$), there was a significant difference in the growth performance of the P7 generation on the 7th day of comparison ($p<0.05$). Therefore, the organizational block the proliferation characteristics of UCMSCs obtained by culture method are superior to those obtained by enzyme digestion method. In summary, the later experiments will obtain UCMSCs using tissue block culture method.

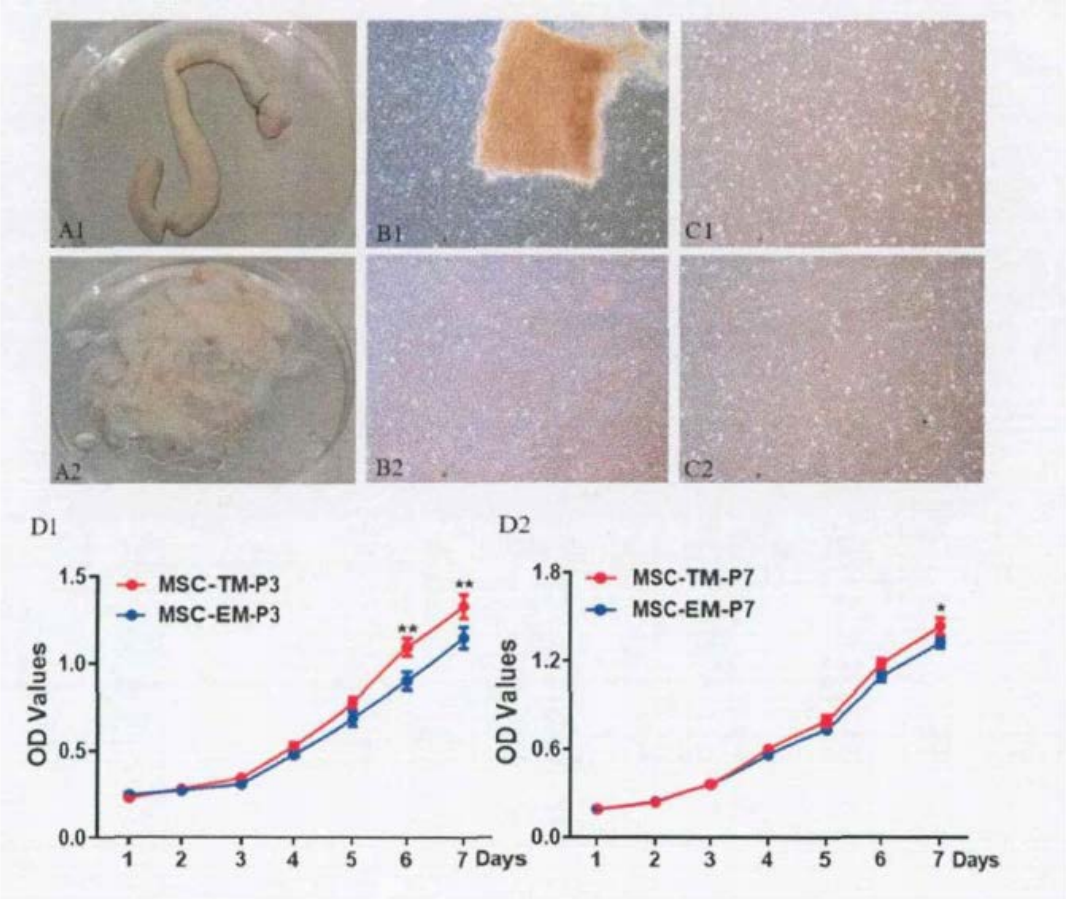



Figure 3. Isolation and Culture of UCMSCs. A1 represents disinfected and washed umbilical cord tissue, A2 represents isolated Huatong glue tissue. B1, B2 represents umbilical cord tissue cultured into UCMSCs. Cl, C2 were isolated using enzymatic digestion to obtain UCMSCs. D1 and D2 were the P3 and P7 passages of the isolated cells, respectively comparison of secondary growth performance, MSC-TM is tissue block culture method, MSC-EM is an enzyme digestion method, and the result is an average OD soil SEM, * $p$<0.05, Water * $p$<0.01, * * * p<0.001. n=8.

## 4.2 Identification of surface antigens of UCMSCs

Detection of surface markers of UCMSCs using flow cytometry. As shown in Figure 4, the positive result of PE labeling, the surface antigen is highly expressed, and the expression rate is as follows: CD90 is 92.24%, CD73 is 87.14%, CD105 is 82.25%, CD11b is 4.12%, CD45 is 3.14%, CD34 is 1.25%. In summary, the results of surface antigen identification are consistent with the standard for mesenchymal stem cells.

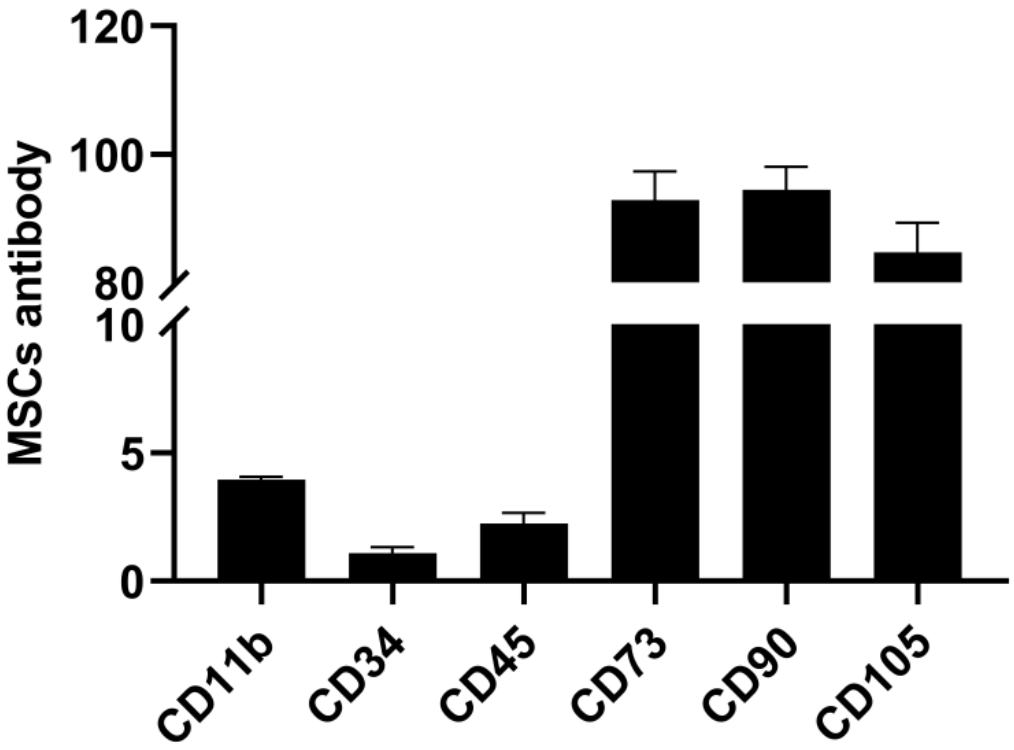



Figure 4. The results of UCMSCs marker detection. The surface antigen is highly expressed, CD90 is 92.24%, CD73 is 87.14%, CD105 is 82.25%, CD11b is 4.12%, CD45 is 3.14%, CD34 is 1.25% , $p$<0.05. n=8.

## 4.3 Multidirectional differentiation potential analysis of UCMSCs

The multidirectional differentiation potential of isolated human UCMSCs was verified through osteogenesis, adipogenesis, and softening. Figure 5 demonstrates the result of lipid induction, with oil staining red in 5A, and oil droplets resembling grape clusters being clearly observed. In Figure 5B, the results of chondrogenic induction show the entire slice stained blue, indicating the presence of acidic mucopolysaccharides in cartilage tissue stained with Alcian blue.

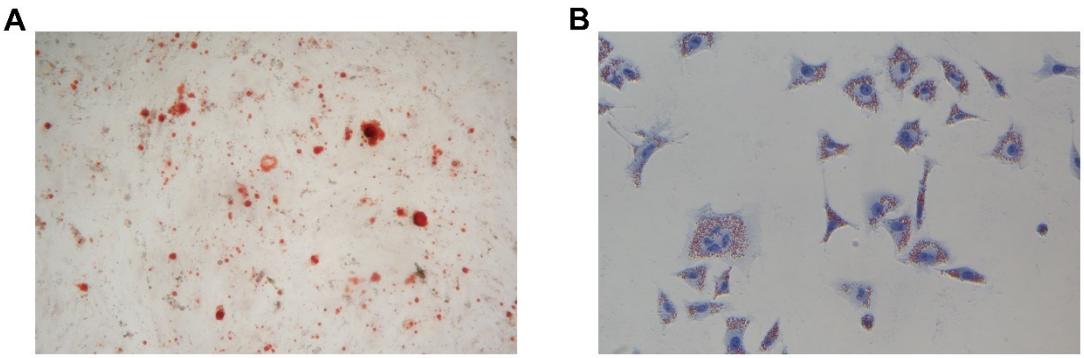



Figure 5. Differentiation potential of UCMSCs. (A) The representative photos of Alizarin red S staining. (B) The representative photos of Oil red o staining.

## 4.4 Induction of UCMSCs into neuronal cells

The experiment of inducing UCMSCs into neuronal cells was conducted using the method of P-mercaptoethanol induction. As shown in Figure 6, in this experiment, compared with the control group, both induction methods can obtain results of UCMSCs and their differentiation into neuronal cells. The number of live cells in treatment A was significantly higher than that in treatment B, and compared with the control group, the cell volume induced by treatment A decreased, with neurons exhibiting axonal like structures. Treatment B exhibits a lower cell viability compared to treatment A due to the higher drug concentration during induction, but the induction effect is better. In the enlarged image, it can be clearly seen that the induced cells exhibit phenotypic characteristics similar to those of primitive cells, with axons and protruding structures, and obvious cell connections between cells.

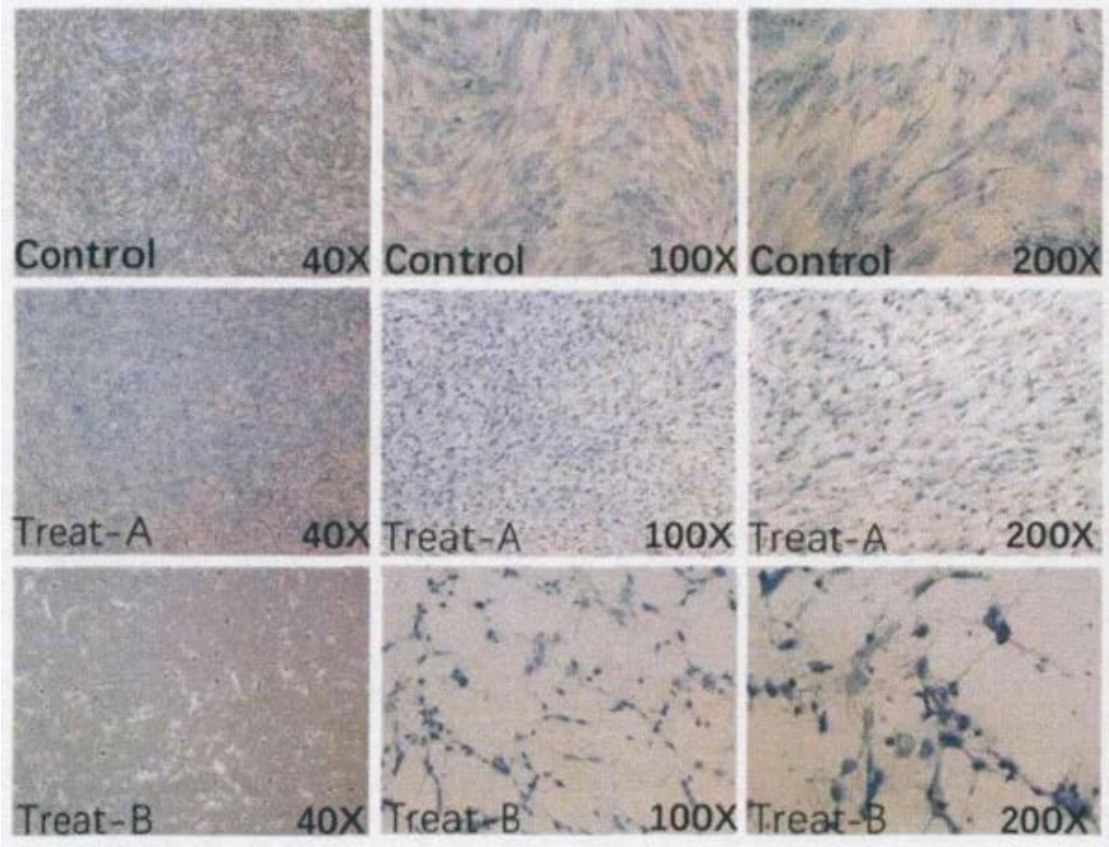



Figure 6. Umbilical cord mesenchymal induction into neuronal cells. Compared to the treatment group, both have varying degrees of induction efficiency, with treatment A being a low concentration treatment group and treatment B being a high concentration group. n=8.

## 4.5 Growth of UCMSCs on polylactic acid membrane

To test the potential application of human UCMSCs in tissue engineering, UCMSCs were used Laying on a polylactic acid membrane for growth, the results are shown in Figure 7. Among them, Figure A shows cell growth observed under an inverted microscope, normal growth of cells at the bottom indicates that cells and polylactic acid membranes can grow normally in the same system, as shown in the red box.It can be observed that cells can aggregate and stack. Figure B shows the scanning electron microscopy results, showing mesenchymal stem cell nodules. The structure is normal and has good adhesion with the material, indicating that it can grow normally on the membrane.

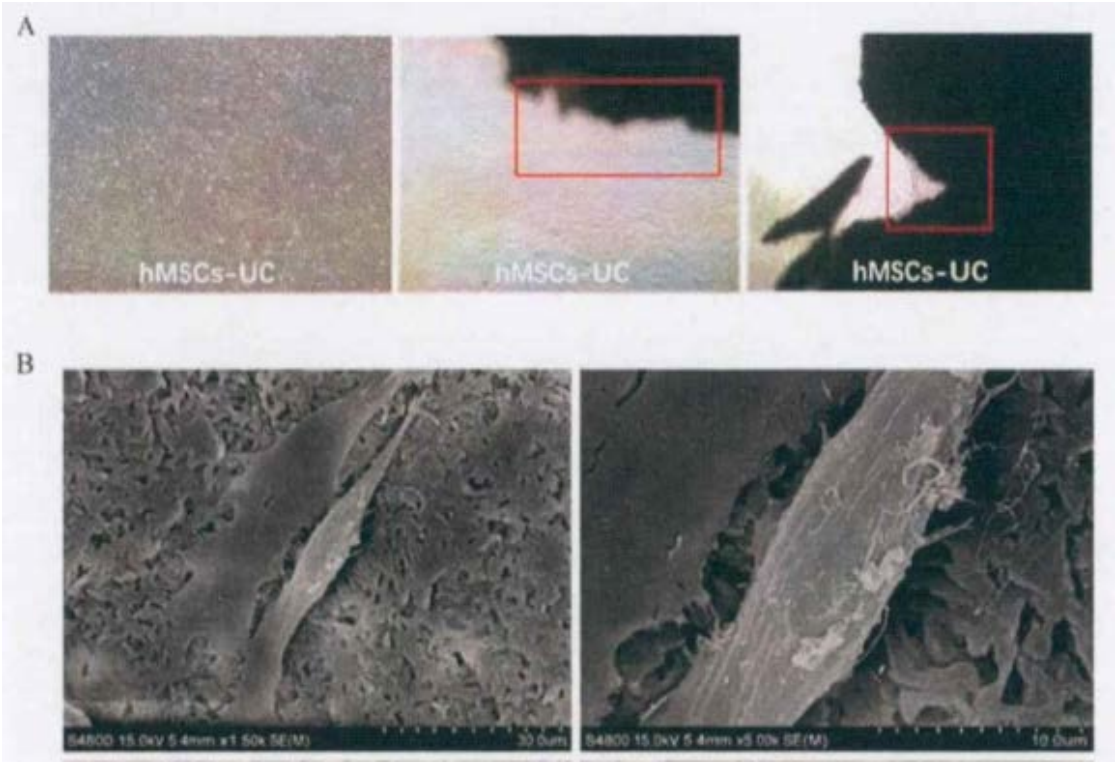



Figure 7. Figure A shows the cell growth diagram, with the growth status of the planking cells on the far left and the two images on the right showing the collected images during the growth process, as shown in the red box. Normal cell growth can be clustered and stacked, as shown in the electron microscope scanning image in Figure B. The left image shows a large field of view, and the right image shows the corresponding local magnification. n=8.

## 4.6 Isolation and identification of exosomes

Next, this study will employ serum-free medium to culture UCMSCs, acquire cell culture supernatant, and extract extracellular vesicles using differential centrifugation and reagent kit methods. Subsequently, the obtained exosomes will be examined using transmission electron microscopy to observe their morphological characteristics. Following the determination of morphological features, Nanoparticle tracking analysis (NTA) will be conducted to analyze the size and concentration of exosomes in a large sample. Finally, for exosome samples meeting the criteria for identifying their morphological characteristics, Western blotting will be utilized to qualitatively detect proteins specifically expressed in exosomes. Through these experiments, the extracellular vesicles of UCMSCs obtained in this study will be comprehensively evaluated, and qualitative and quantitative testing will be performed to obtain extracellular vesicles meeting the standards for further research.

## 4.7 Electron microscopic examination of exosomes

In this experiment, two methods were used to obtain extracellular secretion of UCMSCs for scanning electron microscopy detection. As shown in Figures 8, small vesicles of extracellular vesicles with a diameter of 30-150nm and a cup shaped shape can be observed. Among them, A1-A3 is the exosomes extracted by the reagent kit method, and many small particles, possibly protein particles, can be seen in the background. B1-B3 is obtained by differential centrifugation method, and the background is clean and the structure of the exosomes is clear.

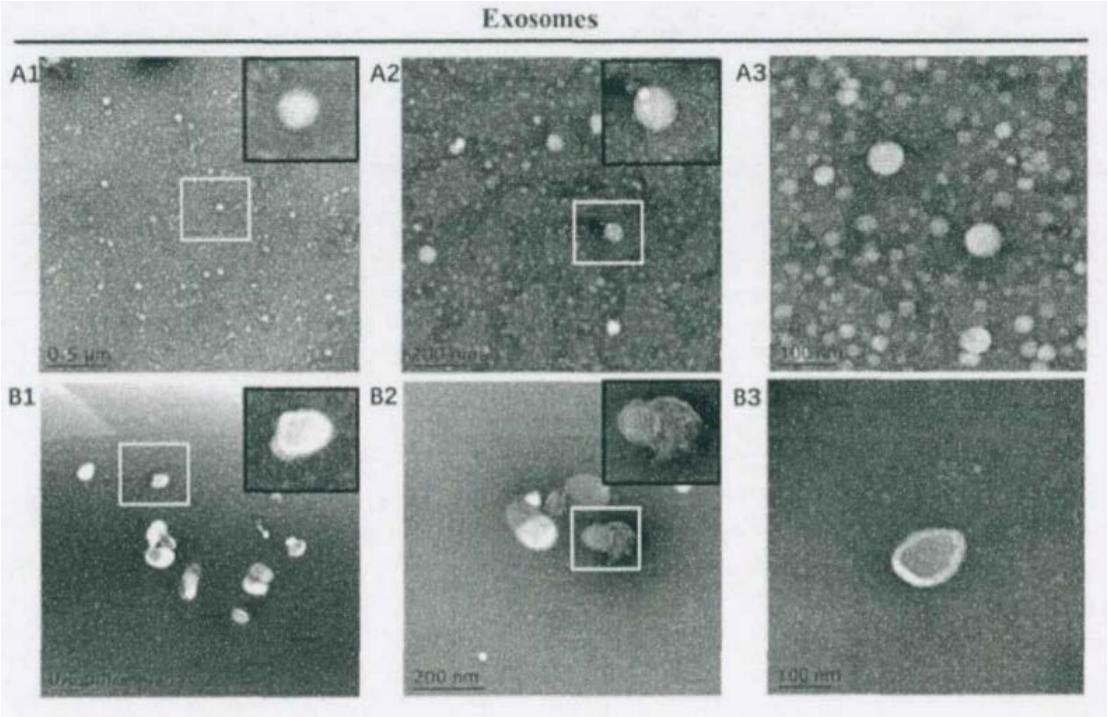
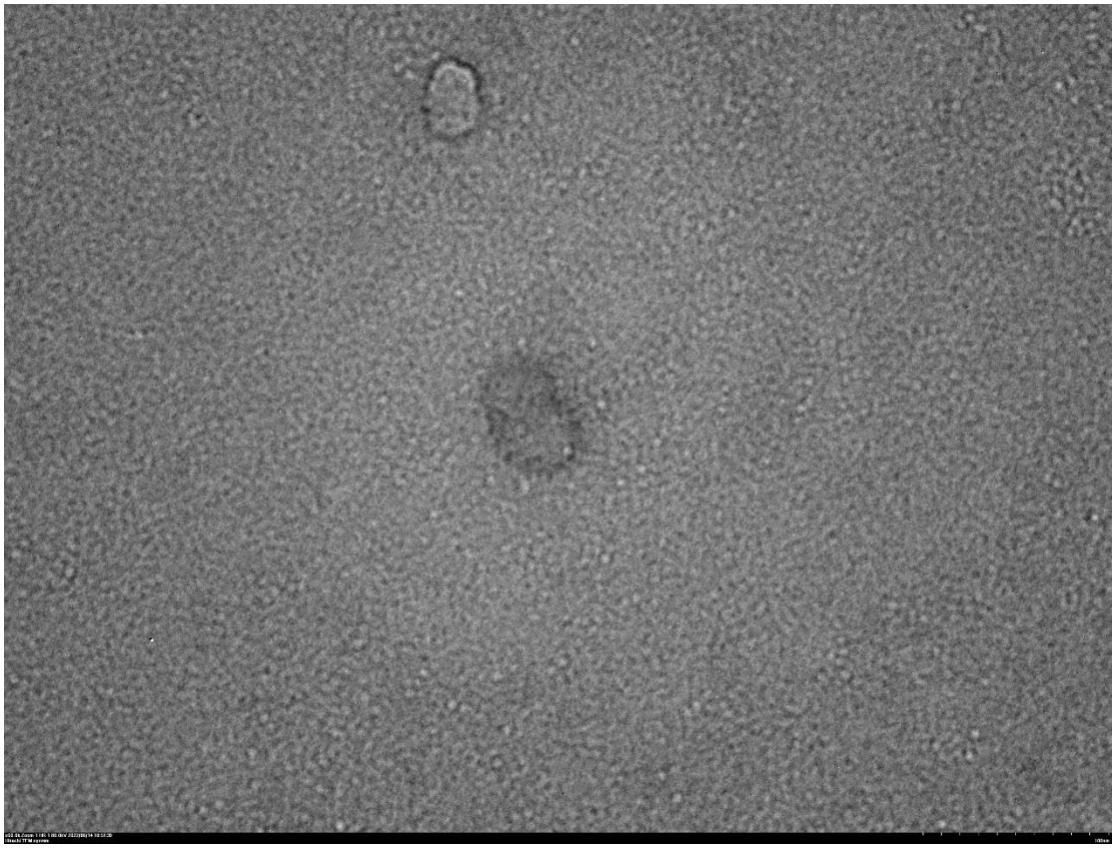



Figure 8. Isolation and identification of extracellular vesicles from UCMSCs using electron microscopy scanning. A1-A3 is the extracellular vesicle of mesenchymal stem cells obtained by the kit method; As shown in Figure B1. As shown in B3, it is the dry and fine interstitial material obtained by differential centrifugation method. n=8.

## 4.8 Identification of exosome proteins

Under the electron microscope, UCMSCs-exos were cup-shaped vesicles with about 30-150 nm in diameter; the background was clean and the exosomes' structure was clear; the particle size was about 126.62±1.64 nm, which accorded with the size distribution of exos. Finally, the detection results of exosomes' marker proteins showed higher Tsg101, CD9, CD63, CD81, HSP70 and HSP90 mRNA levels in UCMSCs-exos compared with UCMSCs ($p$<0.05), which confirmed the successful separation of UCMSCs-exos (Figure 9).

A



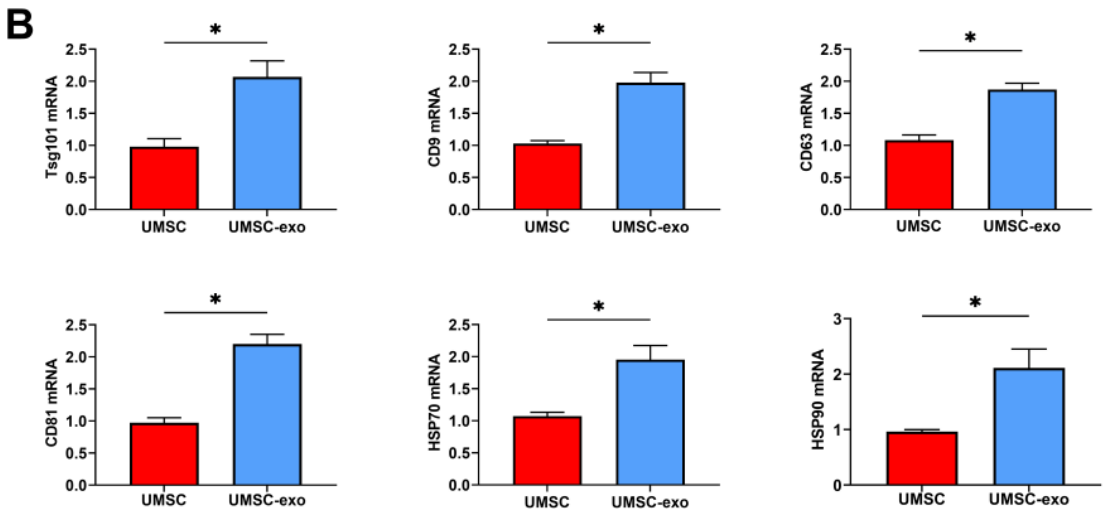



Figure 9. UCMSCs-exos isolation results. (A) TEM of UCMSCs-exos. (B) The relative mRNA expression levels of exosomes biomarkers. $^{*}p<0.05$. n=8.

## 4.9 Influences of UCMSCs and UCMSCs-exos on cell proliferation

Next, we will further explore the effects of UCMSCs and their extracellular vesicles on tumor cells. In order to achieve this goal, this study selected breast cancer cell line, gastric cancer cell line, glioma cell line, lung cancer cell line and other major tumor cell lines. In terms of the treatment of tumor cells, this study divided the experiment into three groups: extracellular vesicle treatment group, mesenchymal stem cell co culture group, and control group. For the co culture group of mesenchymal thousand cells and tumor cells, the transwell plugin co culture method was used. The two types of cells can only transmit information through pore size, and the cells do not mix; For the tumor cell group treated with exosomes, the exosomes were directly added to the tumor cell culture medium; At the same time, a control group was set up. To evaluate the impact of UCMSCs and their extracellular vesicles on the biological phenotype of tumor cells through in vitro cell phenotype experiments such as cell proliferation, apoptosis, oxidative stress testing, and cell invasion and migration. Through in vitro experiments, the therapeutic effects of UCMSCs and their extracellular vesicles on different tumor cells can be obtained, providing a fundamental theoretical reference for the safe clinical application of UCMSCs.

First, the cell proliferation ability of A549, MDA-MB-231 and BGC-823 was not significantly changed compared with the original cells by MTT assay ($P>0.05$). Further, the comparison revealed reduced proliferation capacity of UCMSCs-LN-229 and UCMSCs-exos-LN-229 compared with LN-229, with the lowest proliferation capacity found in UCMSCs-exos-LN-229 ($p<0.05$) (Figure 10).

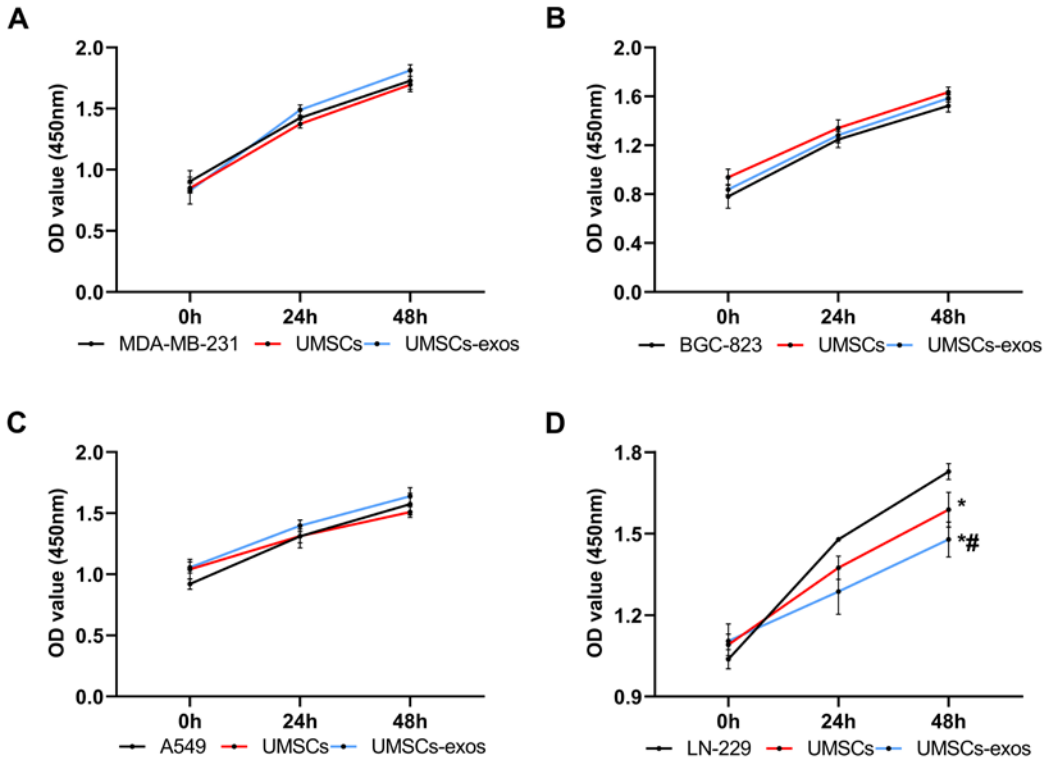
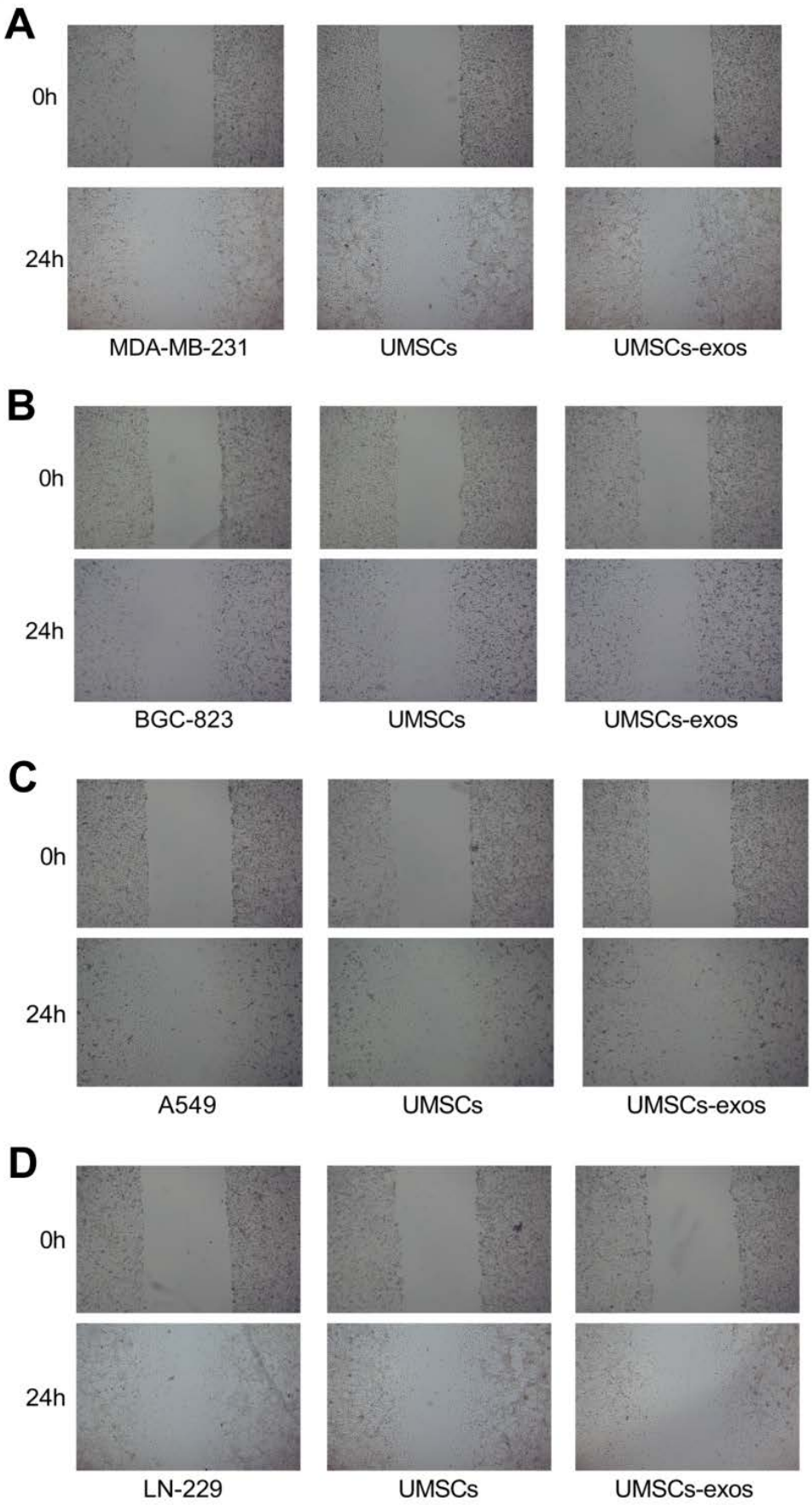



Figure 10. Influences of UCMSCs and UCMSCs-exos on cell proliferation. (A) The growth curve of MDA-MB-231. (B) The growth curve of BGC-823. (C) The growth curve of A549. (D) The growth curve of LN-229. Compared with normal tumor cells, $^{*}p<0.05$, compared with Tumor cells intervened by UCMSCs, $^{\#}p<0.05$. n=8.

## 4.10 Influences of UCMSCs and UCMSCs-exos on cell migration capacity

Both UCMSCs and UCMSCs-exos interventions resulted in altered cell migration ability in the cell wound scratch assay. Significantly enhanced migration of MDA-MB-231, BGC-823 and A549 and decreased migration of LN-229 were observed after the intervention ($p<0.05$). In addition, comparing the effects of UCMSCs and UCMSCs-exos intervention, it can be seen that UCMSCs-exos intervention led to more significant changes in cell migration of breast carcinoma (BC), gastric carcinoma (GC), lung carcinoma (LC) and glioma ($p<0.05$) (Figure 11).


A
0h
24h
MDA-MB-231
UMSCs
UMSCs-exos
B
0h
24h
BGC-823
UMSCs
UMSCs-exos
C
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D
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LN-229
UMSCs
UMSCs-exos

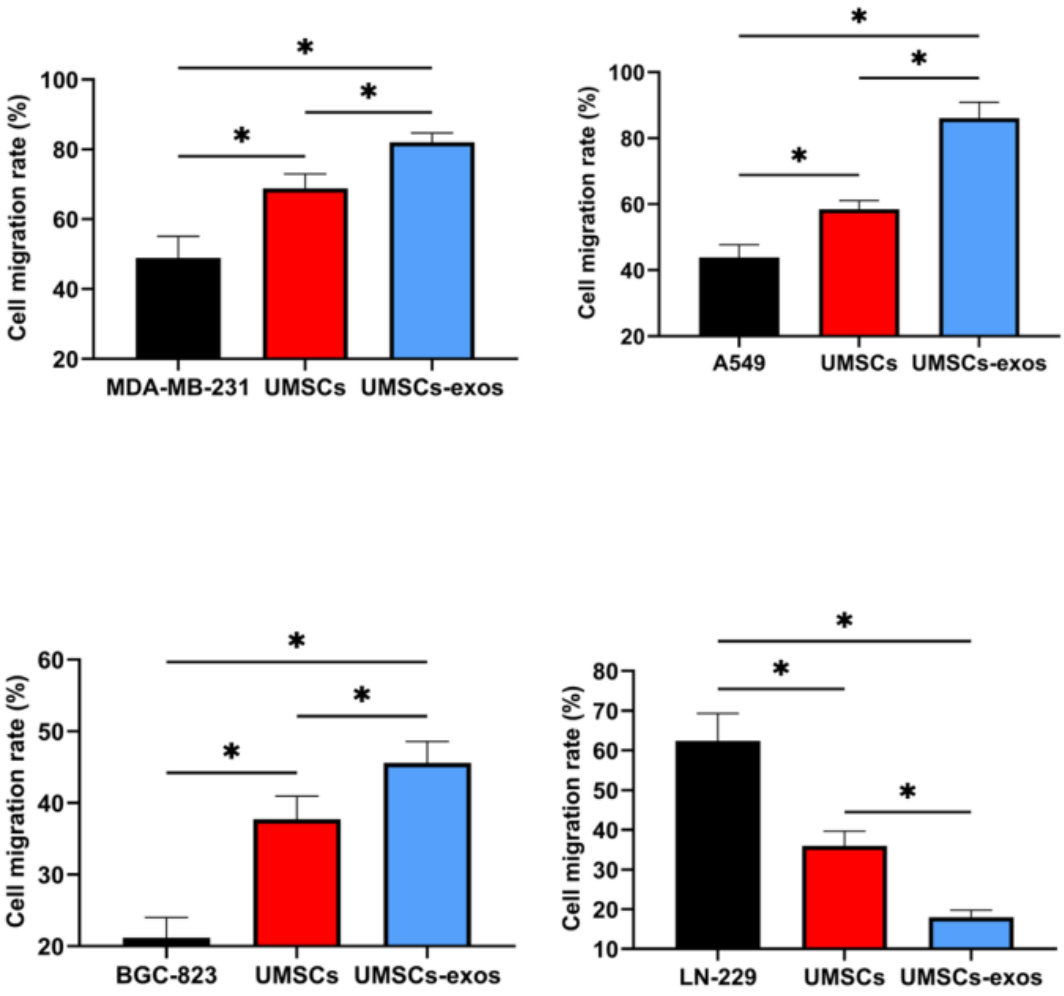
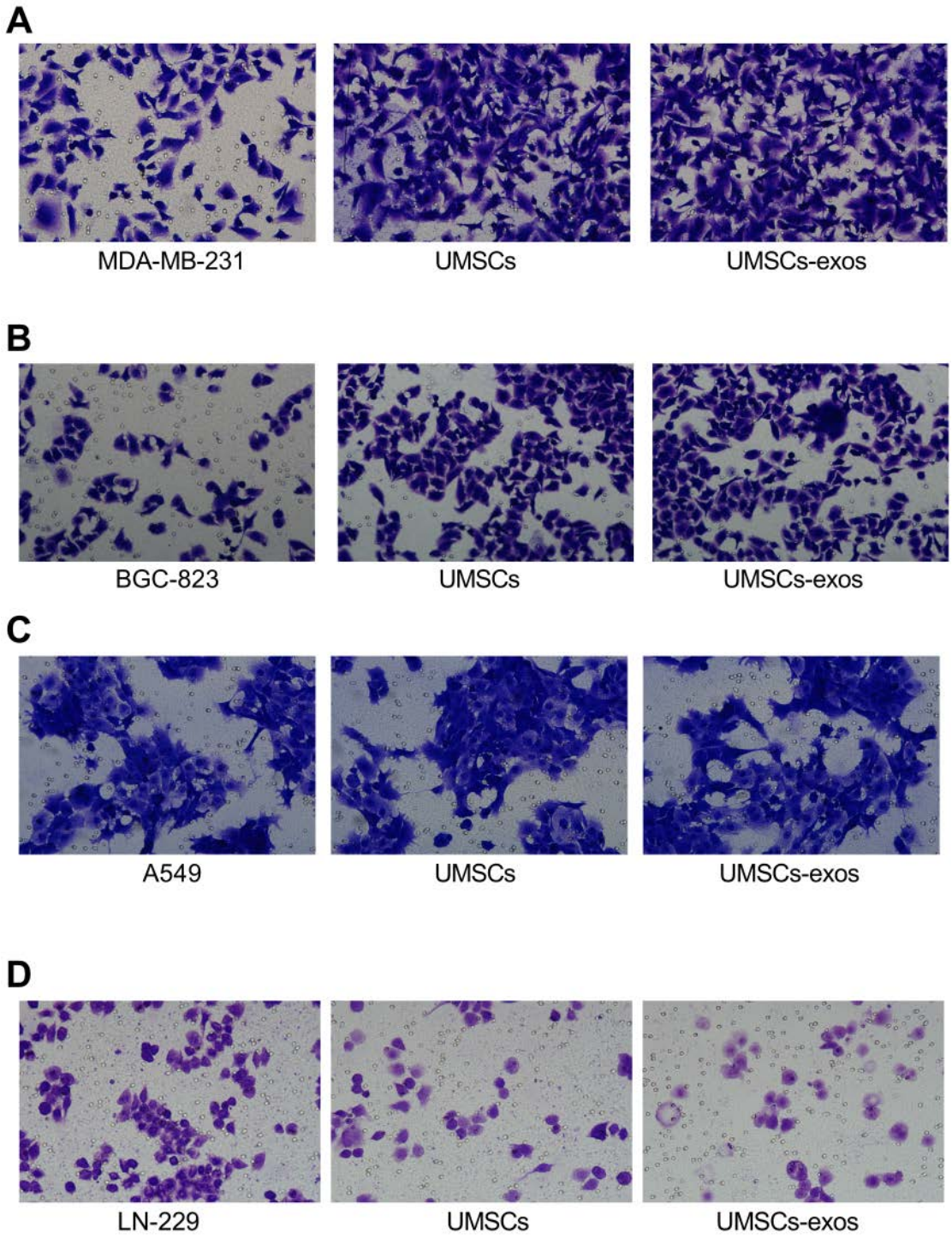



Figure 11. Influences of UCMSCs and UCMSCs-exos on cell migration capacity. (A) The effect of UCMSCs and UCMSCs-exos on the migration of MDA-MB-231. (MDA-MB-231 vs. UMSCs vs. UMSCs-exos, 48.23±8.38 vs. 67.83±5.84 vs. 81.27±3.61, %, n=8) (B) The effect of UCMSCs and UCMSCs-exos on the migration of BGC-823. (BGC-823 vs. UMSCs vs. UMSCs-exos, 3.47±5.46 vs. 37.21±6.12 vs. 48.68±5.89, %, n=8) (C) The effect of UCMSCs and UCMSCs-exos on the migration of A549. (A549 vs. UMSCs vs. UMSCs-exos, 44.25±3.27 vs. 58.51±3.89 vs. 84.33±6.52, %, n=8) (D) The effect of UCMSCs and UCMSCs-exos on the migration of LN-229. (LN-229 vs. UMSCs vs. UMSCs-exos, 64.47±12.61 vs. 38.82±6.24 vs. 18.22±3.82, %, n=8). $^{*}p<0.05$.

## 4.11 Impacts of UCMSCs and UCMSCs-exos on cell invasiveness

Both UCMSCs and UCMSCs-exos interventions contributed to little altered invasive ability of A549 (P>0.05), but obviously increased BC and GC cell migration ($p<0.05$); LN-229 showed a decreased invasion rate after the intervention ($p<0.05$).

However, for various cells, their invasive capacity after UCMSCs intervention was not statistically different from that after UCMSCs-exos intervention ($p$>0.05) (Figure 12).



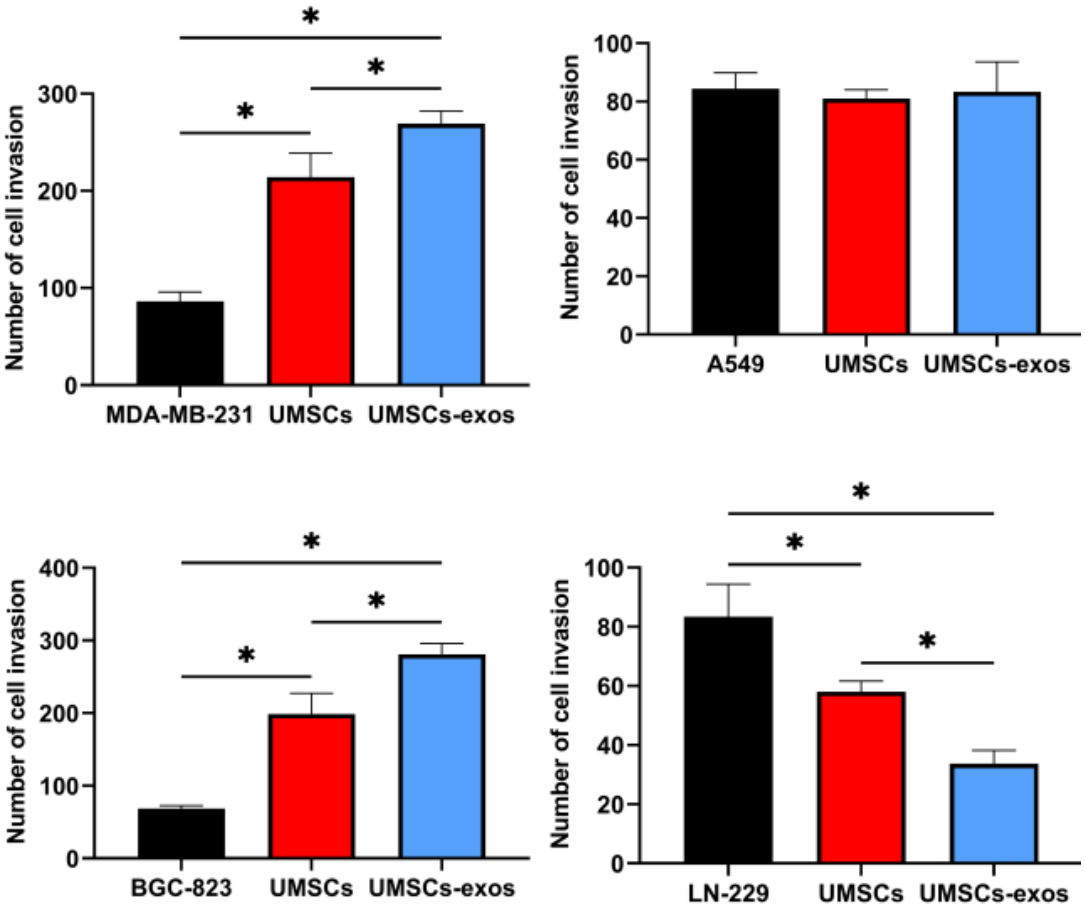
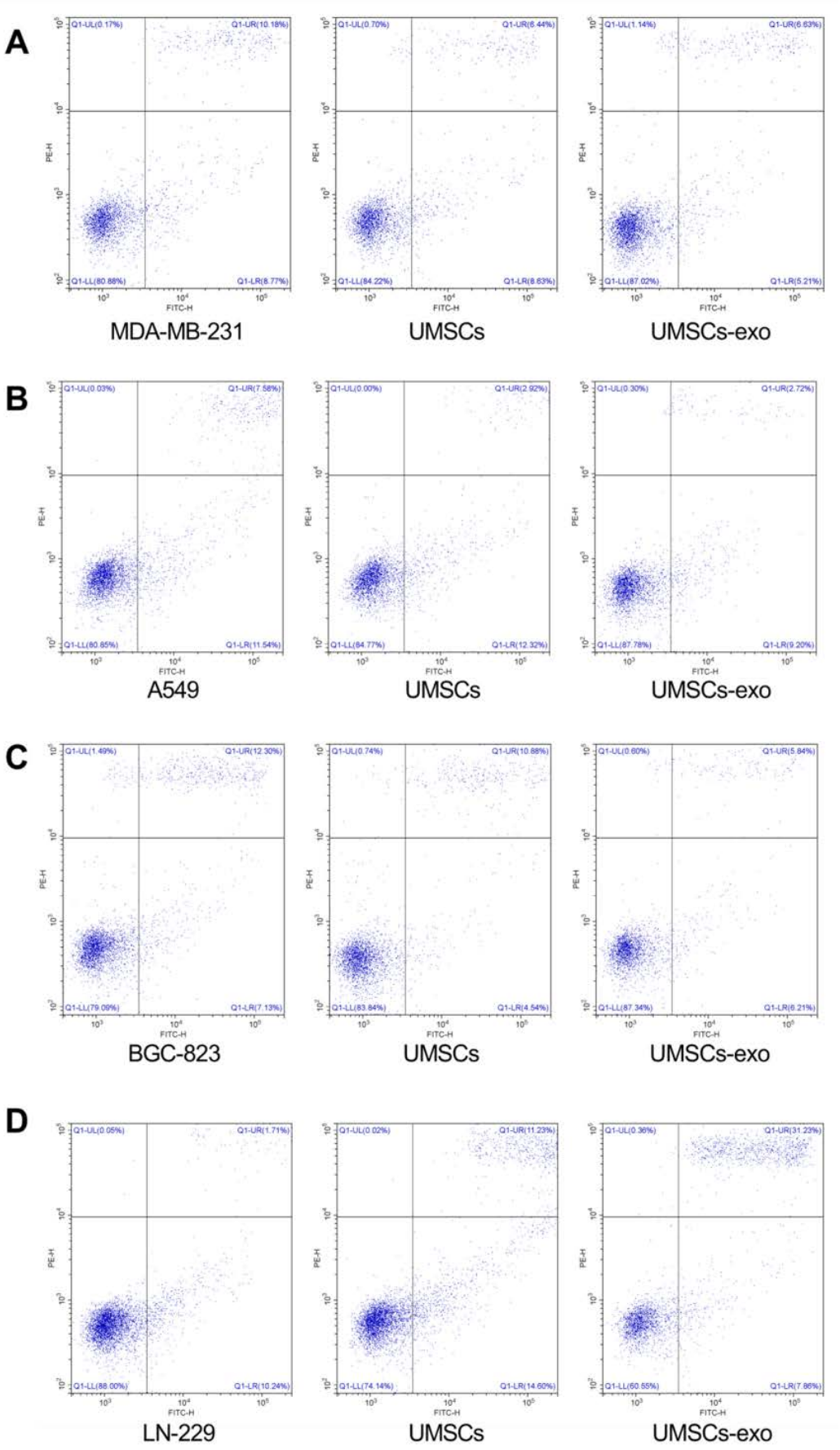



Figure 12. Impacts of UCMSCs and UCMSCs-exos on cell invasiveness. (A) The effect of UCMSCs and UCMSCs-exos on the invasiveness of MDA-MB-231. (MDA-MB-231 vs. UMSCs vs. UMSCs-exos, 81.25±8.37 vs. 223.8±33.32 vs. 267.9±13.52, n=8) (B) The effect of UCMSCs and UCMSCs-exos on the invasiveness of BGC-823. (BGC-823 vs. UMSCs vs. UMSCs-exos, 66.35±5.33 vs. 183.29±22.89 vs. 284.89±18.54, n=8) (C) The effect of UCMSCs and UCMSCs-exos on the invasiveness of A549. (A549 vs. UMSCs vs. UMSCs-exos, 83.15±7.34 vs. 79.11±6.21 vs. 85.93±10.12, n=8) (D) The effect of UCMSCs and UCMSCs-exos on the invasiveness of LN-229. (LN-229 vs. UMSCs vs. UMSCs-exos, 82.11±16.67 vs. 58.55±4.78 vs. 36.64±3.22, n=8). $^{*}p<0.05$.

## 4.12 Impacts of UCMSCs and UCMSCs-exos on cell apoptosis

In MDA-MB-231, BGC-823, and A549 cells, there was no significant difference in apoptosis rates between the groups ($p>0.05$). In contrast, in LN-229, the highest apoptosis rate was observed in the UCMSCs-exos group, and the UCMSCs group also had a higher apoptosis rate than the primary cells ($p<0.05$) (Figure 13).


A
Q1-UL(0.17%)
Q1-UR(10.18%)
Q1-LL(80.88%)
Q1-LR(8.77%)
MDA-MB-231
Q1-UL(0.70%)
Q1-UR(6.44%)
Q1-LL(84.22%)
Q1-LR(8.63%)
UMSCs
Q1-UL(1.14%)
Q1-UR(6.63%)
Q1-LL(87.02%)
Q1-LR(5.21%)
UMSCs-exo
B
Q1-UL(0.03%)
Q1-UR(7.58%)
Q1-LL(80.65%)
Q1-LR(11.54%)
A549
Q1-UL(0.00%)
Q1-UR(2.92%)
Q1-LL(84.77%)
Q1-LR(12.32%)
UMSCs
Q1-UL(0.30%)
Q1-UR(2.72%)
Q1-LL(87.78%)
Q1-LR(9.20%)
UMSCs-exo
C
Q1-UL(1.49%)
Q1-UR(12.30%)
Q1-LL(79.09%)
Q1-LR(7.13%)
BGC-823
Q1-UL(0.74%)
Q1-UR(10.88%)
Q1-LL(83.84%)
Q1-LR(4.54%)
UMSCs
Q1-UL(0.60%)
Q1-UR(5.84%)
Q1-LL(87.34%)
Q1-LR(6.21%)
UMSCs-exo
D
Q1-UL(0.05%)
Q1-UR(1.71%)
Q1-LL(88.00%)
Q1-LR(10.24%)
LN-229
Q1-UL(0.02%)
Q1-UR(11.23%)
Q1-LL(74.14%)
Q1-LR(14.60%)
UMSCs
Q1-UL(0.36%)
Q1-UR(31.23%)
Q1-LL(60.55%)
Q1-LR(7.86%)
UMSCs-exo
PE-H
FITC-H

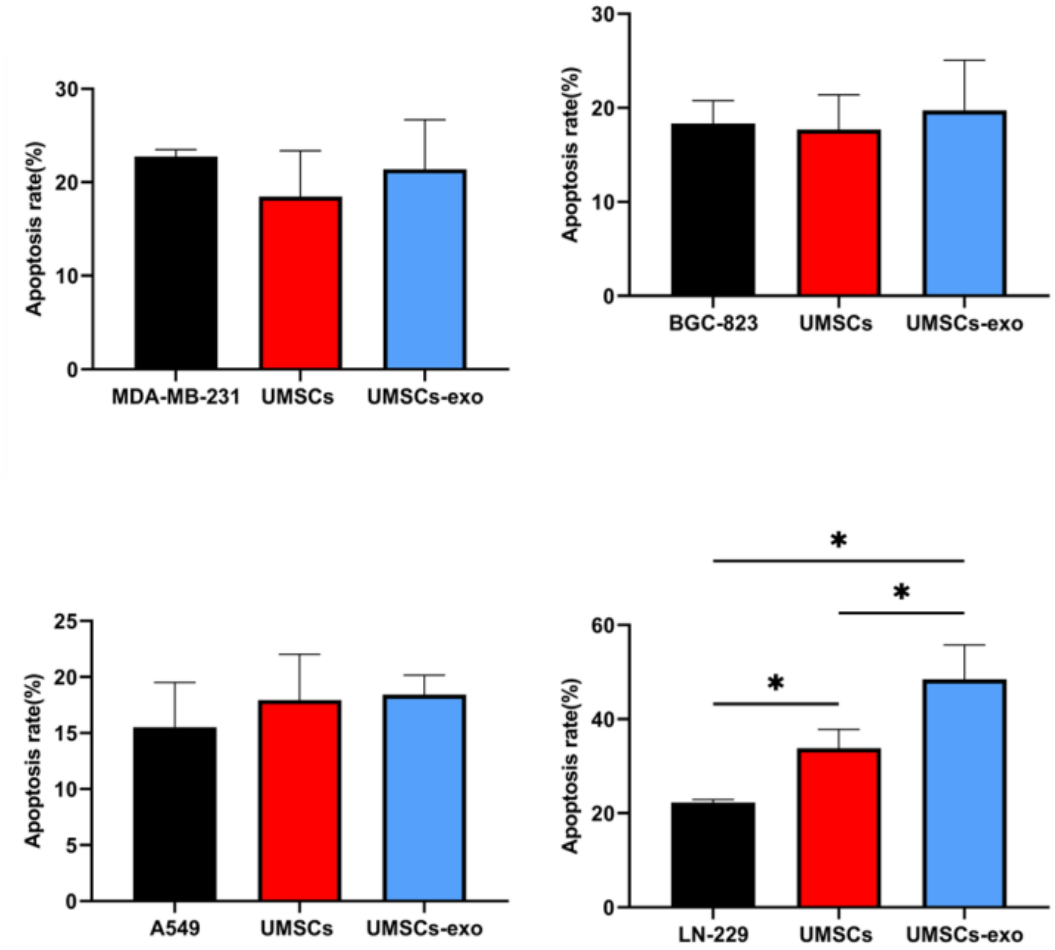



Figure 13. Impacts of UCMSCs and UCMSCs-exos on cell apoptosis. (A) The effect of UCMSCs and UCMSCs-exos on the apoptosis of MDA-MB-231. (MDA-MB-231 vs. UMSCs vs. UMSCs-exos, 23.31±1.56 vs. 17.61±10.57 vs. 21.37±9.25, %, n=8) (B) The effect of UCMSCs and UCMSCs-exos on the apoptosis of BGC-823. (BGC-823 vs. UMSCs vs. UMSCs-exos, 18.61±5.52 vs. 17.19±6.39 vs. 19.59±8.33, %, n=8) (C) The effect of UCMSCs and UCMSCs-exos on the apoptosis of A549. (A549 vs. UMSCs vs. UMSCs-exos, 15.57±4.98 vs. 17.57±4.28 vs. 19.16±2.59, %, n=8) (D) The effect of UCMSCs and UCMSCs-exos on the apoptosis of LN-229. (LN-229 vs. UMSCs vs. UMSCs-exos, 23.19±3.76 vs. 34.19±4.51 vs. 43.91±7.19, %, n=8). $^{*}p<0.05$.

Similarly, in the cell cycle assay results, MDA-MB-231, BGC-823, and A549 cells were not significantly altered after UCMSCs versus UCMSCs-exos intervention. However, in LN-229, the G0-G1 phase was shortened and the G2-M phase was prolonged in UCMSCs group versus UCMSCs-exos group (Figure 14).

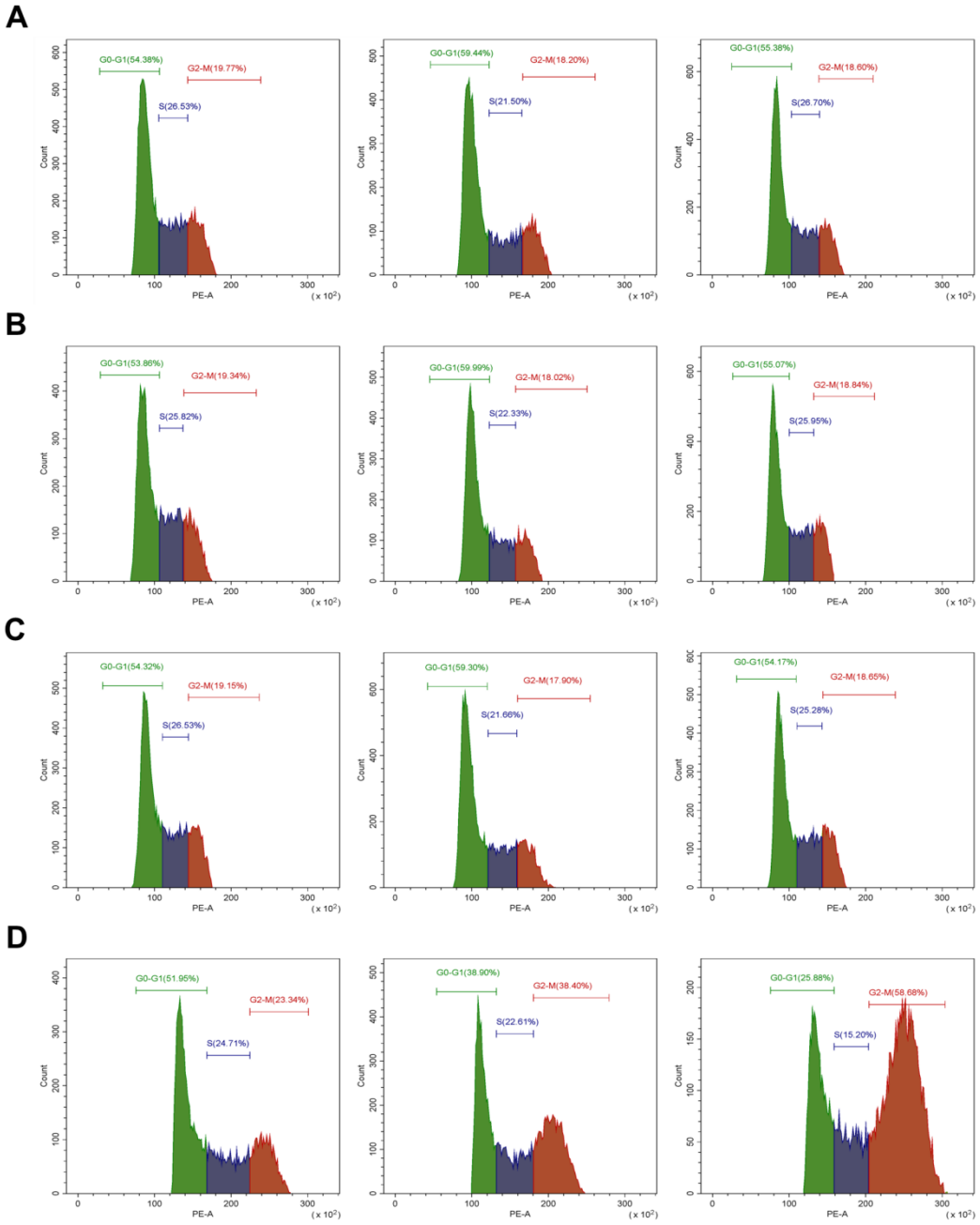



Figure 14. Impacts of UCMSCs and UCMSCs-exos on cell cycle. (A) The effect of UCMSCs and UCMSCs-exos on the cell cycle of MDA-MB-231. (B) The effect of UCMSCs and UCMSCs-exos on the cell cycle of BGC-823. (C) The effect of UCMSCs and UCMSCs-exos on the cell cycle of A549. (D) The effect of UCMSCs and UCMSCs-exos on the cell cycle of LN-229.

## 4.13 Impacts of UCMSCs and UCMSCs-exos on cell oxidative stress

The previous sections found that UCMSCs and their extracellular vesicles can significantly promote the proliferation and invasion ability of tumor cells, while inhibiting migration ability and affecting the cell cycle distribution of tumors. In addition, in vitro experiments, this study found that both treatments can promote tumor growth, with extracellular vesicle treatment having a more significant promoting effect. However, how UCMSCs and their extracellular vesicles are involved in regulation, leading to the biological transformation of these phenotypes，but about the process and regulatory mechanism are still unclear. To investigate the potential molecular mechanisms underlying phenotypic changes in tumor cells treated with UCMSCs and their extracellular vesicles. This study will further clarify the regulatory mechanism of UCMSCs and their extracellular vesicles, and screen out key genes and signaling pathways involved in gene regulation.

In the results of oxidative stress response markers, it was seen that UCMSCs and UCMSCs-exos had no significant effect on MDA-MB-231, BGC-823, and A549, because in these cells, there was no difference in ROS, MDA, and SOD assays between the primary cells, UCMSCs group and UCMSCs-exos group ($P>0.05$). However, in LN-229 cells, ROS and MDA were decreased and SOD was increased in the UCMSCs group versus UCMSCs-exos group ($p<0.05$), indicating that UCMSCs and UCMSCs-exos could improve the oxidative stress response in LN-229 cells (Figure 15, 16, and 17).

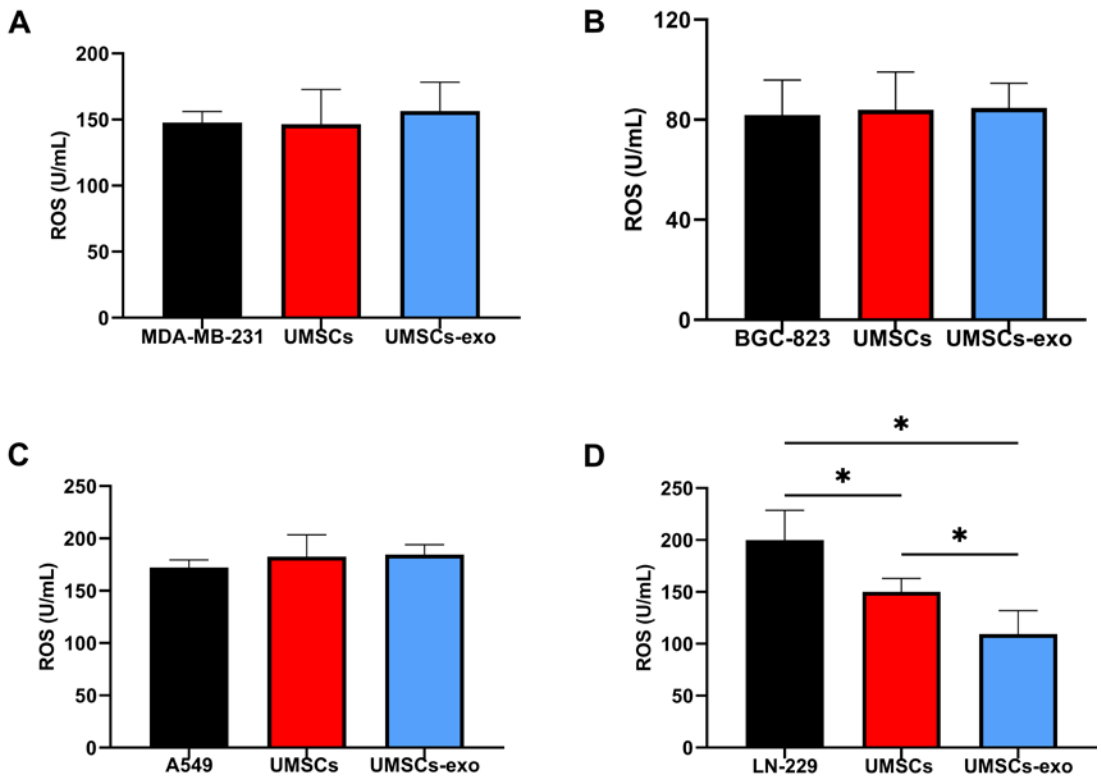



Figure 15. Impact of UCMSCs and UCMSCs-exos on ROS. (A) The effect of UCMSCs and UCMSCs-exos on the ROS of MDA-MB-231. (MDA-MB-231 vs. UMSCs vs. UMSCs-exos, 144.77±9.82 vs. 142.32±26.49 vs. 153.19±15.39, U/mL, n=8) (B) The effect of UCMSCs and UCMSCs-exos on the ROS of BGC-823. (BGC-823 vs. UMSCs vs. UMSCs-exos, 80.55±13.17 vs. 81.27±14.12 vs. 82.01±9.48, U/mL, n=8) (C) The effect of UCMSCs and UCMSCs-exos on the ROS of A549. (A549 vs. UMSCs vs. UMSCs-exos, 170.92±11.64 vs. 172.82±24.31 vs. 182.32±9.19, U/mL, n=8) (D) The effect of UCMSCs and UCMSCs-exos on the ROS of LN-229. (LN-229 vs. UMSCs vs. UMSCs-exos, 198.26±27.92 vs. 144.21±12.15 vs. 105.66±20.18, U/mL, n=8) $^{*}p<0.05$.

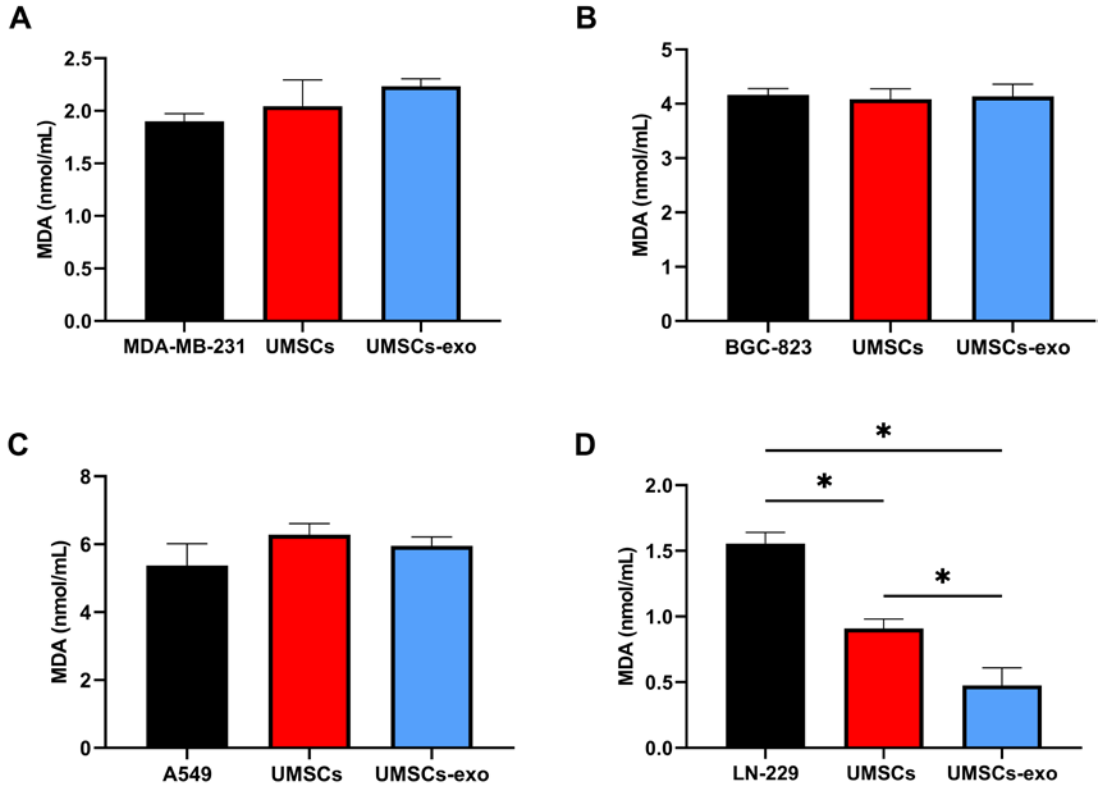



Figure 16. Impact of UCMSCs and UCMSCs-exos on MDA. (A) The effect of UCMSCs and UCMSCs-exos on the MDA of MDA-MB-231. (MDA-MB-231 vs. UMSCs vs. UMSCs-exos, 1.881±0.098 vs. 2.097±0.352 vs. 2.231±0.091, nmol/mL, n=8) (B) The effect of UCMSCs and UCMSCs-exos on the MDA of BGC-823. (BGC-823 vs. UMSCs vs. UMSCs-exos, 4.045±0.103 vs. 4.121±0.151 vs. 4.335±0.261, nmol/mL, n=8) (C) The effect of UCMSCs and UCMSCs-exos on the MDA of A549. (A549 vs. UMSCs vs. UMSCs-exos, 5.301±0.853 vs. 6.304±0.361 vs. 5.893±0.283, nmol/mL, n=8) (D) The effect of UCMSCs and UCMSCs-exos on the MDA of LN-229. (LN-229 vs. UMSCs vs. UMSCs-exos, 1.525±0.229 vs. 0.839±0.201 vs. 0.459±0.491, nmol/mL, n=8) $^{*}p<0.05$.

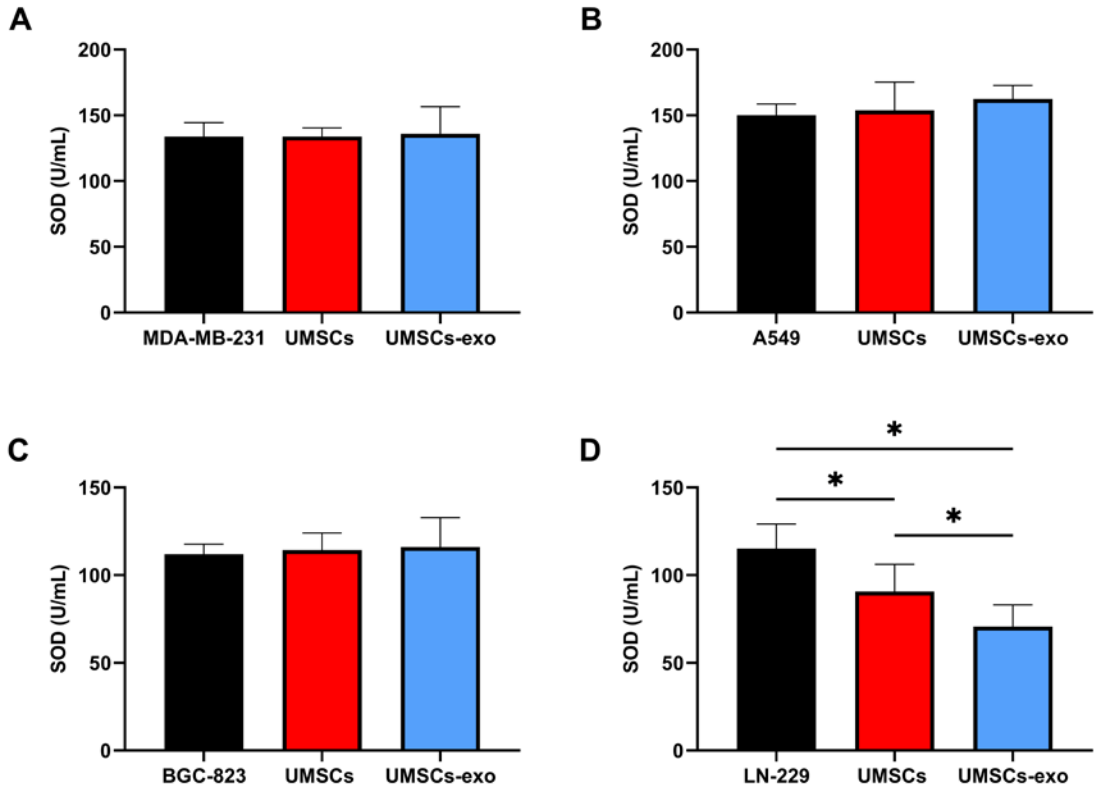
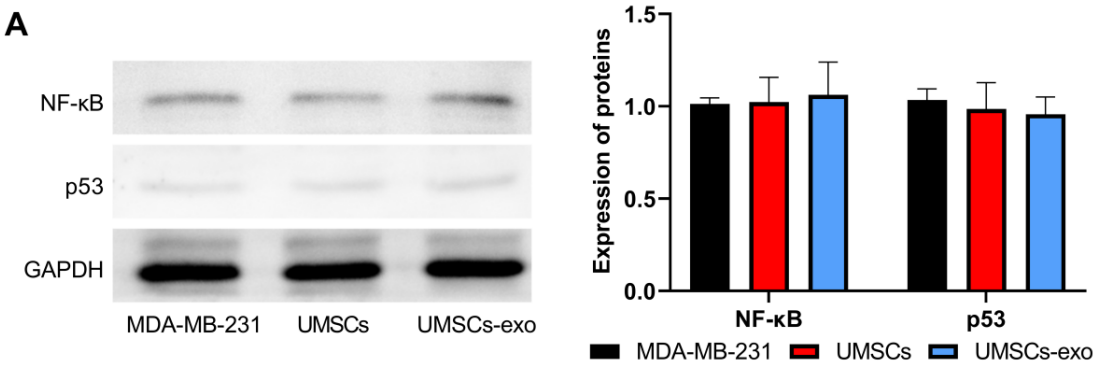
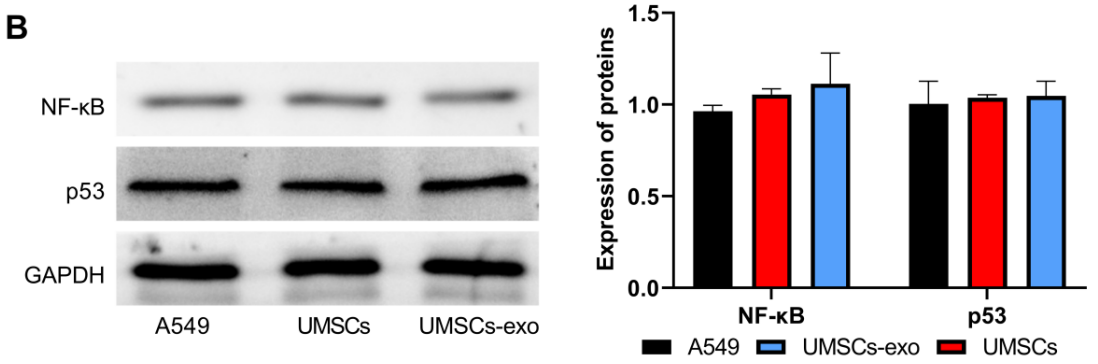


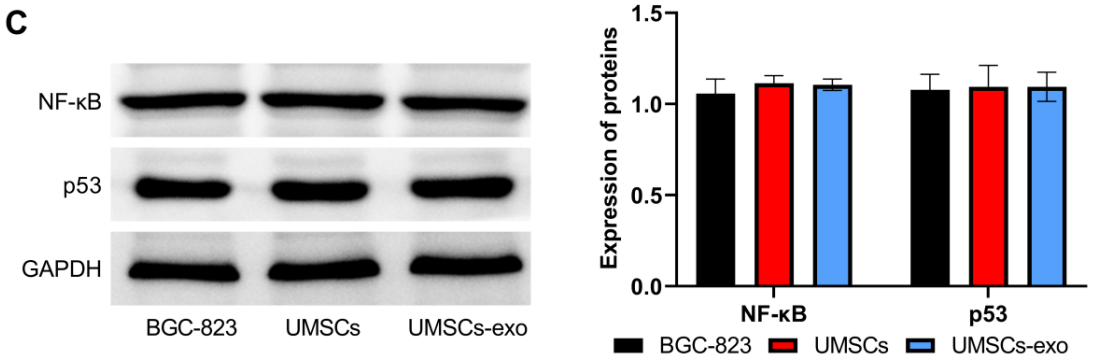

Figure 17. Impact of UCMSCs and UCMSCs-exos on SOD. (A) The effect of UCMSCs and UCMSCs-exos on the SOD of MDA-MB-231. (MDA-MB-231 vs. UMSCs vs. UMSCs-exos, 136.34±9.52 vs. 135.39±5.11 vs. 138.43±12.91, U/mL, n=8) (B) The effect of UCMSCs and UCMSCs-exos on the SOD of BGC-823. (BGC-823 vs. UMSCs vs. UMSCs-exos, 149.48±6.93 vs. 151.23±20.23 vs. 161.23±15.33, U/mL, n=8) (C) The effect of UCMSCs and UCMSCs-exos on the SOD of A549. (A549 vs. UMSCs vs. UMSCs-exos, 112.54±5.39 vs. 116.82±7.82 vs. 117.23±15.66, U/mL, n=8) (D) The effect of UCMSCs and UCMSCs-exos on the SOD of LN-229. (LN-229 vs. UMSCs vs. UMSCs-exos, 115.39±13.48 vs. 82.76±15.64 vs. 67.33±13.86, U/mL, n=8). $^{*}p<0.05$.

## 4.14 Impacts of UCMSCs and UCMSCs-exos on cell NF-κB/p53

Finally, we found no effect of UCMSCs and UCMSCs-exos on the expression of NF-κB, p53 protein in MDA-MB-231, BGC-823, and A549 cells as well (P>0.05). However, they could inhibit the expression of NF-κB, p53 proteins in LN-229, and the inhibitory effect of UCMSCs-exos was more obvious among them ($p<0.05$) (Figure 18).


A
NF-κB
p53
GAPDH
MDA-MB-231
UMSCs
UMSCs-exo
Expression of proteins
1.5
1.0
0.5
0.0
NF-κB
p53
MDA-MB-231
UMSCs
UMSCs-exo


B
NF-κB
p53
GAPDH
A549
UMSCs
UMSCs-exo
Expression of proteins
1.5
1.0
0.5
0.0
NF-κB
p53
A549
UMSCs-exo
UMSCs


C
NF-κB
p53
GAPDH
BGC-823
UMSCs
UMSCs-exo
Expression of proteins
1.5
1.0
0.5
0.0
NF-κB
p53
BGC-823
UMSCs
UMSCs-exo

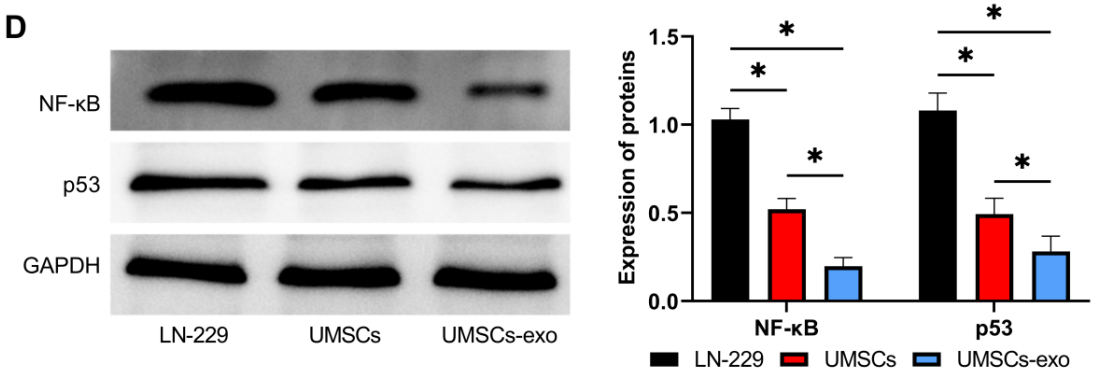

D
NF-κB
p53
GAPDH
LN-229
UMSCs
UMSCs-exo
Expression of proteins
1.5
1.0
0.5
0.0
*
*
*
*
*
*
NF-κB
p53
LN-229
UMSCs
UMSCs-exo

Figure 18. Effect of UCMSCs and UCMSCs-exos on NF-κB and p53 protein expression. (A) The effect of UCMSCs and UCMSCs-exos on the NF-κB, p53 proteins of MDA-MB-231. (B) The effect of UCMSCs and UCMSCs-exos on the NF-κB, p53 proteins of BGC-823. (C) The effect of UCMSCs and UCMSCs-exos on the NF-κB, p53 proteins of A549. (D) The effect of UCMSCs and UCMSCs-exos on the NF-κB, p53 proteins of LN-229. $^{*}p<0.05$. n=8.

# 5. Discussion

## 5.1 Successful isolation and clinical application potential of UCMSCs

In this study, UCMSCs were successfully isolated and cultured through tissue block culture, and their biological characteristics were explored. The surface markers of UCMSCs obtained in this study were detected by flow cytometry, which showed high expression of PE labeled positive markers CD73, CD90, and CD105, while almost no expression of FITC labeled negative markers CDllb, CD34, CD45, and HLA-DR, It indicates that it conforms to the expression characteristics of surface markers of mesenchymal stem cells[116]. The exploration of the differentiation potential of mesenchymal stem cells into three embryonic layers can be directed to induce differentiation into adipocytes, osteoblasts, and chondrocytes. Meanwhile, neuron like growth and differentiation of cells can be obtained through two methods of P-mercaptoethanol induction. The above induced differentiation experiments indicate that the UCMSCs isolated in this study have multidirectional differentiation potential[117]. In the exploration of tissue engineering, it can not only co cultivate with polylactic acid membrane, but also adhere and grow normally on polylactic acid membrane, confirming its potential for tissue engineering.

The principle of treating diseases with UCMSCs is to replace and repair dead and damaged cells, activate dormant and inhibited cells, exert paracrine effects (such as secreting neurotrophic factors and anti apoptotic factors), promote the recovery of intercellular electrical capacity and conduction (such as mesenchymal stem cells secreting junction proteins to help intercellular connections and promote the opening of ion channels)[118]. At present, the advantages of stem cell therapy include: broad treatment range and no toxicity; To avoid rejection, there is no need to delve into the pathogenesis of the disease; Sufficient sources of treatment materials. The application of stem cell technology has broad prospects. Stem cell technology can treat major and difficult diseases that are difficult to treat with traditional drugs or surgical methods, relieving patients from suffering greatly[119]. It has been successfully applied in regenerative medicine aesthetics and has a broader application prospect in future biological development.

In clinical practice, UCMSCs have multiple uses. Firstly, mesenchymal stem cells can have strong immune regulatory effects and can be used to treat autoimmune diseases such as lupus erythematosus and scleroderma, reduce immune rejection reactions after cell or organ transplantation, and improve the success rate of cell or organ transplantation; Secondly, mesenchymal stem cells can promote hematopoietic

recovery function[119, 120]. Compared with single hematopoietic stem cell transplantation, co transplantation of mesenchymal stem cells and hematopoietic stem cells can significantly improve the therapeutic effect of diseases such as leukemia and refractory anemia; In addition, mesenchymal stem cells can repair damaged or diseased tissues and organs, and are used to treat bone and muscle degenerative diseases, cardiovascular and cerebrovascular diseases, liver diseases, brain and spinal cord nerve injuries, and elderly dementia[121]. And mesenchymal stem cells have achieved good results in these fields.

## 5.2 Successful isolation and characterization of UCMSCs-derived exosomes

In this study, UCMSCs were cultured in serum free medium, and extracellular vesicles were isolated and identified. The products obtained by transmission electron microscopy scanning differential centrifugation and kit extraction methods both detected exosomes with cup shaped structures[116]. NTA particle size analysis shows that both methods can obtain extracellular vesicles that match the 30-150nm, exosomes with a reasonable threshold size of 150nm particle size. Western blot detection of proteins expressed in exosomes showed that exosomes specifically expressed Tsg101, CD9, CD63, CD81, HSP70 and HSP90 proteins and mRNA, which were consistent with the characteristics of exosomes[118]. In summary, this study successfully obtained extracellular vesicles of UCMSCs cultured in serum-free medium, which were identified to meet the criteria for extracellular vesicle identification and can be used for further studies.

Exosomes are nanoscale vesicle structures released by cells in vitro and in vivo, used for intercellular communication. They are decorated with phospholipid membranes and proteins, and contain a large number of cell-derived molecules. In previous studies, bovine serum culture medium was often used to cultivate mesenchymal stem cells, which introduced extracellular vesicles derived from bovine serum, making the experimental results unreliable. In order to reduce the impact of bovine serum, some studies have also conducted ultracentrifugation of serum to remove serum extracellular vesicles[117]. Although this method can to some extent reduce the exosomes derived from bovine serum, ultracentrifugation can lead to the loss of many nutrients in the serum. In order to avoid the drawbacks of the above methods, this study used serum free culture medium to culture UCMSCs, and then collected the cultured supernatant to extract extracellular vesicles for further research[122]. This method not only effectively avoids the introduction of bovine serum extracellular vesicles, but also does not lead to the loss of nutrients that affect cell growth.

So far, many methods for separating extracellular vesicles have been developed, mainly including differential centrifugation, density gradient centrifugation, ultrafiltration, and immunoaffinity separation. Among all methods for extracting extracellular vesicles from cell culture media and body fluids, differential centrifugation and density gradient centrifugation are the most widely used invits[123]. In addition, in order to save extraction time and expand the sample size, many companies have developed fast, simple, and reliable separation test boxes, such as Invitrogen, Norgen, Cellguidancesystem, etc. In this study, mesenchymal stem cell extracellular vesicles were used, and exosomes was also obtained from cell culture supernatant using the above method[124].

For the morphological characteristics of exosomes, transmission electron microscopy can be used to identify them. Firstly, uranyl acetate is used to dehydrate and fix the sample, increase the contrast of the analyzed sample, and finally, the morphology of the small cup cap sample is obtained through scanning, which is internationally recognized[125]. The characteristic standards of Xibubi. In this study, two methods of scanning with transmission electron microscopy were used to obtain extracellular vesicles, both of which obtained extracellular vesicles with a small cup shaped structure. If research requires higher requirements for the structural analysis of exosomes, cryoelectron microscopy technology is needed[126]. This technology fixes the exosomes sample in liquid nitrogen. Due to the extremely low temperature during this process, the water is in a glassy state and does not form destructive ice crystals, thus keeping the exosomes structure basically intact[127, 128]. However, the experimental equipment and cost are also relatively high. This study only needs to identify the external characteristics of exosomes, so transmission electron microscopy scanning can fully meet the requirements. Through transmission electron microscopy, the diameter and morphology of exosomes in a small number of samples can be clearly observed, but it is necessary to measure the particle size and concentration of exosomes in a large number of samples, which requires NTA Particle size analysis[53]. This method records the Brownian motion of particles and uses algorithms to calculate the size of each individually tracked particle, thereby detecting the information of all particles of 10-1000nm size in the sample and determining the average size distribution and concentration of particles in the given sample[54, 129]. Since NTA was first reported as providing a suitable detection method for the characterization of extracellular vesicles, it has become one of the standard techniques for characterizing extracellular vesicles. In this study, NTA particle size analysis was performed on all extracted exosomes samples, and the results showed that both extraction methods could obtain exosomes with particle sizes of around 126.62±1.64 nm.

The identification of extracellular vesicle proteins commonly involves extracellular tetramer transmembrane proteins and intrinsic proteins. Among them, CD9, CD63, and CD81 are common transmembrane proteins[53]. They are a type of tetramer in the outer vesicle that contains four transmembrane domains and features,

commonly used for the identification of exosomes. In addition, the protein encoded by the Tsgl01 gene belongs to the homology of ubiquitin coupling enzymes and is also commonly used for the identification of exosomes. In this study, Tsg101, CD9, CD63, CD81, HSP70 and HSP90 proteins and mRNA were selected for expression to confirm that the extracted vesicles were exosomes, with Actin gene protein as the internal reference[130]. The Western blotting test results showed that the exosomes obtained in the experiment specifically expressed Tsg101, CD9, CD63, CD81, HSP70 and HSP90 proteins, which were consistent with the characteristics of exosome[43-45]. In summary, this study successfully obtained extracellular vesicles of UCMSCs, which were identified to conform to the characteristics of extracellular vesicles and can be used for further research.

## 5.3 UCMSCs and their exosomes in tumor therapy

The interaction between exosomes and exosomes secreted by tumor cells themselves can provide a microenvironment for tumor growth, but instead promote tumor development[46]. Targeting and blocking these key factors can indirectly achieve therapeutic goals; Finally, the extracellular vesicle of mesenchymal stem cells is a targeted drug carrier that can deliver drugs to designated sites for adjuvant therapy. Due to the different effects and modes of action of mesenchymal stem cells and their extracellular vesicles on different tumors, targeted use of mesenchymal stem cells and their extracellular vesicles is necessary for different cancer treatments[48, 49]. The extracellular vesicles of mesenchymal stem cells can directly affect the treatment of cancer. For example, in breast cancer, the exosomes of mesenchymal stem cells can inhibit tumor angiogenesis by downregulating VEGF, thereby inhibiting tumor growth. Similarly, extracellular vesicles from bone marrow mesenchymal stem cells carrying microRNA-16-5p can inhibit the proliferation, migration, and invasion of colorectal cancer cells, and promote cell apoptosis by downregulating ITGA2, achieving the goal of inhibiting the progression of colon cancer[131]. Some researchers have used hamster cheek pocket cancer, As a clinical model of human oral squamous cell carcinoma, it has been demonstrated that injecting extracellular vesicles of mesenchymal stem cells into the tumor can lead to the disappearance of tumor blood vessels and exhibit significant anti-tumor effects[132, 133]. In addition, the extracellular vesicles of mesenchymal stem cells can also be used to assist traditional chemotherapy and radiation therapy, or targeted drug delivery, to achieve the goal of adjuvant cancer treatment.

Mesenchymal stem cell extracellular vesicles exhibit targeting properties towards tumor sites and are often used as therapeutic targets or drug delivery tools. In vitro experiments, it was found that the exosomes combined with celadol can effectively inhibit the proliferation of non-small cell carcinoma A549 and H1299[128, 134]. Research has found that extracellular vesicles can serve as carriers to transport

the anticancer drug Dox to assist in the treatment of lung cancer. In addition, in vivo and in vitro experiments, it was confirmed that the extracellular secretion of mesenchymal stem cells can carry paclitaxel to inhibit the cell viability of breast cancer cells, indicating that it can be used for the treatment of breast cancer[109, 135]. And it can effectively load doxorubicin, and can significantly reduce the tumor growth rate in the mouse breast cancer model. The researchers not only conducted research on the possibility of drug loading into extracellular vesicles, but also conducted a detailed exploration of the therapeutic effects after drug loading[136]. Research has shown that extracellular vesicles of mesenchymal stem cells can not only load paclitaxel, but also reduce its cytotoxicity by 7.6 times[137]. Intravenous injection can reduce subcutaneous primary tumors by more than 60%, and the concentration of paclitaxel in extracellular vesicles is reduced by about 1000 times. However, the effect is similar to that of directly using high concentrations of paclitaxel, A 50% reduction in metastatic lesions can still be observed in the distal organs of the lungs, liver, spleen, and kidneys[125]. These studies have confirmed that mesenchymal stem cell extracellular vesicles can effectively load drugs to assist in the treatment of cancer.

Numerous studies have shown that mesenchymal stem cells and their extracellular vesicles can enhance the effectiveness of tumor treatment. Research has found that the extracellular vesicles of mesenchymal stem cells enhance the radiotherapy effect of melanoma, promote cell death, and inhibit metastasis and diffusion[138]. In the study of breast cancer, bone marrow mesenchymal stem cells can inhibit the proliferation and invasion of breast cancer cells, reduce the abundance of stem cell like surface markers, reduce its sensitivity to the chemotherapy drug Docetaxel, and promote the production of cancer cell dormancy phenotype after tumor resection[139, 140]. Similarly, mesenchymal stem cells and their exosomes can also promote the dormancy of early breast cancer. In the treatment of colon cancer, miR-128-3p is transmitted by extracellular vesicles through negative regulation of Bmil and MRP5, improving the chemotherapy sensitivity of colon cancer cells, thereby promoting the chemotherapy effect of oxaliplatin. In the treatment of gastric cancer, mesenchymal stem cells and their extracellular vesicles may affect the drug sensitivity of gastric cancer cells through the CaM-Ks/Raf/MEK/ERK pathway, which can be targeted to cut off and improve the efficacy of chemotherapy[141]. Studies have found that extracellular miRNA-221 derived from bone marrow mesenchymal stem cells promotes the development of gastric cancer, which can be controlled by targeting miRNA-221. Knocking down miR-25 regulates PTEN/PI3K/Akt/Bad[119, 142, 143].

Although the aforementioned studies generally concur that stem cells and their exosomes can impede tumor development, there are several pertinent objections in practice. For instance, many of the cited studies illustrate the bioengineering of stem cells and their exosomes to yield biologically tailored products with specific effects. If

unaltered UCMSCs and their exosomes are utilized, they may not exhibit tumor growth inhibition. This aspect will be the subject of our subsequent discussion.

## 5.4 The role of UCMSCs and their exosomes in suppressing tumor development is related to tumor type

Malignant tumors are what people call cancer, which is a collective term for over 100 related diseases. When cells in the body undergo mutations, they will continue to divide beyond the control of the body, ultimately forming cancer. All organs in people's bodies are composed of cells. Cell growth and differentiation can meet the needs of the body, and this orderly process can maintain people's physical health[120, 144]. However, if the cells continue to divide, these additional large numbers of cells form tumors. Malignant tumor cells can invade and destroy adjacent tissues and organs. Moreover, cancer cells can penetrate from the tumor and enter the bloodstream or lymphatic system, which is how cancer forms new tumors from the original site to other organs[122, 145]. This process is called cancer metastasis. There are many types of malignant tumors, with different types of properties, affected tissues and organs, different stages of disease, and different reactions to various treatments. Therefore, most patients need comprehensive treatment. The so-called comprehensive treatment refers to the comprehensive use of surgery, chemotherapy, radiotherapy, immunotherapy, traditional Chinese medicine treatment, interventional therapy, microwave therapy, and other methods based on the patient's physical condition, pathological type of tumor, and extent of invasion, in order to significantly increase the cure rate and improve the patient's quality of life[146].

For some early-stage tumors, patients can still get a good prognosis after comprehensive clinical treatment; but for some middle-stage and late-stage tumors that generally present serious infiltration, there is no effective clinical treatment plan[147]. The effects of UCMSCs and UCMSCs-exos on tumors are multi-faceted[148]. On the one hand, they have anti-inflammatory effects that can improve the immune level of patients. On the other hand, UCMSCs and UCMSCs-exos can bind to exos produced by tumor cells themselves to change tumor cell growth. They can be used in the treatment of tumors when they are in the state of activity inhibition. And when a promoting effect is presented, the key factors of the combination of the two can be further found out for targeted blocking, which can also achieve the purpose of indirect therapy. In addition, UCMSCs-exos are also an excellent targeted drug carrier, which can reduce the side effects of drugs while prolonging the half-life, with great potential significance for most tumor chemotherapeutics[149]. Therefore, a thorough understanding of the effects of UCMSCs and UCMSCs-exos on tumors may provide important references for cancer control in the future.

This study used two methods to treat tumor cells, namely co culture with UCMSCs and UCMSCs. Extracellular vesicle processing, among them, the co cultivation treatment adopted the Transwell plugin method, while the Xibulin body treatment adopted the method of directly adding exosomes[150]. Unlike other studies that directly mixed culture cells after labeling, this study used the Transwell plugin co culture method. The two can only transmit cell information through the pore size on the membrane, and the two types of cells do not cross grow and mix. This method can better evaluate the effect of UCMSCs on tumor cells under normal growth conditions. The treatment method for exosomes is to directly add a specific amount of exosomes to the fresh culture medium.

Proliferation, invasion, migration, and apoptosis are important biological behaviors of malignant tumors. The higher the degree of malignancy of a tumor, the stronger its ability to proliferate, invade, and migrate, and the less apoptosis. Mix the culture medium and then use this medium to treat tumor cells. In the cytological experiment, this study selected human breast, gastric, lung carcinoma cells and glioma cells. Detect the effects of UCMSCs and their extracellular vesicles on the growth, migration, and invasion ability of tumor cells.In the early stages of multi-stage tumor progression, tumor cells proliferate infinitely in the primary lesion, often taking several years to form a clear primary tumor lesion. When these tumors grow to a certain size, they can affect the function of their organs and produce varying degrees of clinical symptoms. Although primary tumors are very dangerous, they ultimately only cause about 10% of tumor patients to die, while about 90% of tumor patients ultimately die from the growth of metastatic tumors outside the primary lesion. The formation of this metastasis is the result of tumor cells leaving the primary lesion, searching for new parts of the body along the "highway" of blood vessels and lymphatic vessels, and regrowing to form cell clones[151]. The dissemination of tumor cells is the most dangerous process in the development of tumors. When tumor cells form clones in distant organs, they often cause serious damage to the body. The invasiveness of tumor development mainly depends on the complex biochemical and biological changes in the tumor cells themselves and related matrices.

Epithelial derived malignant tumors and other tissues, such as connective tissue and neural tissue tumors, have similar invasion and metastasis patterns. Even some hematopoietic system tumors, especially lymphoma, also have a localized early stage tumor and a late stage tumor stage that spreads to distant tissues and organs. This series of replication steps is called the "invasion metastasis cascade reaction"[152]. The overall process overview of the invasion transfer cascade reaction consists of 7 independent steps. At the initial stage, local invasion causes in situ cancer cells to damage and break through the basement membrane, invading the lymphatic capillaries or capillaries. Subsequently, these tumor cells follow the systemic circulation and reach the anatomical distal site. The tumor cells remain and penetrate the walls of the lymphatic capillaries or capillaries, forming occult small metastases.

Finally, some small metastatic lesions gain the ability to "clone" at the attachment site, forming visible metastatic tumors. The final step, cloning, is the least efficient of all steps. The likelihood of successfully completing all the cascade steps is very low, which is why it is difficult for a single tumor cell to successfully form a distant visible metastasis after leaving the primary tumor. Recent molecular and cellular biology studies have described this process using another two-stage invasion metastasis cascade reaction theory[153]: the first stage is the physical dissemination of tumor cells from the primary tumor to distant tissues and organs; The second stage of "cloning" relies on the adaptation of disseminated tumor cells to the distant tissue microenvironment.Once metastatic cancer cells reach the parenchymal tissue, they can proliferate and form small clusters of disseminated cancer cell clusters, known as micrometastases. Some of these small metastases can eventually grow into clinically detectable tumor masses, a process known as clonal formation that progresses from small metastases to visible metastases[154]. The probability of a single cancer cell successfully completing all steps of the invasion metastasis cascade is very low. The low success rate of transfer formation is sometimes referred to as transfer incompetence. This is the final result of a series of invalid step sequences in the invasion transfer cascade process. Experiments have shown that metastatic cancer cells have a high completion efficiency in the early stages of the invasion metastasis cascade reaction, but the success rate in the final step, involving clone formation, is very low, which is the speed limiting step in the entire cascade[155]. Therefore, before metastatic cancer cells and their descendants ultimately form clinically detectable masses, there have been a relatively large number of small metastatic lesions spread throughout various tissues and organs throughout the body for a period of time.

Gastric cancer is a malignant tumor originating from gastric mucosal epithelium, mostly adenocarcinoma, with obvious regional distribution differences in China[81]. At present, the incidence rate of gastric cancer ranks second among various malignant tumors. In this study, UCMSCs and UCMSCs-exos showed a tendency to promote proliferation, migration, and invasion in both gastric cancer cell lines, and could inhibit gastric cancer cell apoptosis. Research has found that the extracellular vesicles of bone marrow mesenchymal stem cells promote the growth of osteosarcoma (MG63) and gastric cancer (SGC7901) cells through the Hedgehog signaling pathway[156]. This experiment also showed that both co culture and extracellular vesicle treatment can promote tumor growth at specific time periods. Mesenchymal stem cells and their extracellular vesicles, although they can promote the proliferation and migration of gastric cancer cells, also have the characteristic of targeting gastric cancer cells. Research results have shown that mesenchymal stem cells derived from extracellular vesicles have a raking regulatory effect on drug resistance in gastric cancer cells[157]. Targeted treatment of mesenchymal stem cell extracellular vesicles can improve the efficacy of gastric cancer chemotherapy. Therefore, in the postoperative treatment of

gastric cancer, targeted control of exosomes secretion can effectively improve the therapeutic effect of prognosis.

Lung cancer is the most common malignant tumor in China and even the world, and it is the cancer with the highest mortality rate among male tumors in China. Affected by air pollution and other factors, lung cancer poses an increasingly serious threat to people. In this study, UCMSCs and UCMSCs-exos promoted proliferation and migration, altered cell cycle, and had no significant impact on invasion in human lung cancer cell lines[158, 159]. This study also found that the treated A549 exhibited a phenotype that promotes invasion and migration. Studies have found that co cultured lung cancer cell A549 and adipose mesenchymal stem cells can promote the secretion of IL-6 and VEGF, and resveratrol can effectively reduce the secretion of these inflammatory factors[160]. Similarly, some researchers have shown that Astragalus polysaccharides can reduce the proliferation of bone marrow mesenchymal stem cells when co cultured with A549 lung cancer cells and the influence of morphology[71]. In summary, in the treatment of lung cancer, the environment and external factors of mesenchymal stem cells have a significant impact on the tumor. By using drugs to block adverse factors and utilizing their secretory anti-inflammatory effects, effective treatment can be achieved.

Glioma, also known as glioblastoma, is the most common primary central nervous system tumor, accounting for about half of all intracranial primary tumors[161]. It broadly refers to all neuroepithelial tumors and narrowly refers to tumors originating from various types of glial cells. The treatment of glioma is mainly surgical, but due to the infiltrative growth of the tumor and the lack of clear boundaries with brain tissue, it is difficult to achieve complete resection except for early cases where the tumor is small and located in an appropriate location[162]. Generally, comprehensive treatment is advocated, including postoperative radiotherapy, chemotherapy, etc., which can delay recurrence and prolong survival. The results of this study showed that when UCMSCs and UCMSCs-exos were co-cultured with glioma cells, the growth, proliferation rate, invasion and metastasis ability of glioma cells were reduced, and the apoptotic ability was significantly higher than the control group[163]. This indicates that both UCMSCs and UCMSCs-exos can inhibit the malignant biological phenotype of glioma.

Finally, in order to confirm the influence of UCMSCs and UCMSCs-exos on tumor phenotype, we selected several of the most common tumors as the research targets. BC is currently a malignancy with the highest incidence in the world. Previous studies have found that lung UCMSCs can trigger lipid storage, promote BC metastasis to the lungs, and activate the Hippo signal pathway to promote BC cell growth. Our experiment results identified enhanced BC cell increased migration and invasion capacities under the intervention of UCMSCs and UCMSCs-exos, which is consistent with previous research results, indicating that MDA-MB-231 may be regulated by both autologous tumor cell exos and UCMSCs-exos. And similar to previous research results,

UCMSCs and UCMSCs-exos also accelerated the growth phenotype of GC cells. In addition, LC was the most common tumor worldwide before 2020, so we also included LC cells for analysis. However, the experimental results showed that A549 treated by UCMSCs and UCMSCs-exos exhibited a phenotype that promoted migration, while no significant changes were found in cell invasion ability and apoptosis, suggesting that the microenvironment of UCMSCs and the induced autophagy signal might be potential targets for blocking the proliferation and migration of LC cells. Contrary to the results of this trial, we found in previous studies that bone marrow UCMSCs can promote the invasion ability of A549, which we believe may also be due to the different sources of UCMSCs. For this, we will conduct verification as soon as possible in the future. Finally, when exploring their impacts on glioma cells, we found that both UCMSCs and UCMSCs-exos effectively suppressed the activity, invasiveness and metastasis of glioma cells, promote apoptosis, which indicates that both of them are expected to become new treatment methods for glioma in the future.

This study obtained phenotypic results of the impact of UCMSCs and their exosomes on tumor cells. UCMSCs and their exosomes exhibit an inhibitory migration phenotype, but promote proliferation and invasion, altering the phenotype of the cell cycle. In order to explore its molecular mechanism, high-throughput sequencing will be conducted on tumor cells treated with UCMSCs and their exosomes in the next section of the experiment, further studying their gene expression profile and involved gene regulatory pathways.

## 5.5 UCMSCs and UCMSCs-exos regulate tumor cells by modulating NF-κB/p53 signaling-mediated oxidative stress

Here, we found that both UCMSCs and UCMSCs-exos can affect the biological phenotype of malignant tumors, affecting tumor cell proliferation, invasion, migration, apoptosis, and other factors. Through further exploration of their mechanisms of action, we believe that UCMSCs and UCMSCs-exos may be involved through NF-κB/p53 signaling pathway regulates the formation of oxidative stress.

The biological phenotype of tumors originates from abnormalities in cell transduction mechanisms. To explore the signal transduction mechanisms of cell proliferation, apoptosis, adhesion, movement, and invasion dysfunction during cancer transformation, the laboratory has focused on the activation and regulation of ROS in different tumors and tumor stages, as well as downstream signaling molecular pathways[93]. The aim is to explore the regulatory mechanisms of signaling molecules during cellular carcinogenesis, as well as the changes in signal transduction molecules within and between cells or between cells and extracellular matrix, and to analyze the molecular basis for generating tumor cell specific biological phenotypes.

In the Bcl-2 regulatory protein family (Bcl-2 regulatory protein is an anti apoptotic protein), the pro Bcl-2 regulatory protein and anti apoptotic protein that play a balancing role are responsible for controlling the "apoptotic trigger" that transmits signals between regulatory and effector factors. As prototypes, Bcl-2 and its homologues (Bcl-xl, Bcl-w, Mcl-1, A1) are apoptosis inhibitors that bind and suppress two types of apoptotic proteins (Bax and Bak). These two are embedded in the outer membrane of mitochondria. When the inhibitory effect of anti apoptotic homologues is lifted, they will disrupt the integrity of the mitochondrial outer membrane and release apoptotic signaling proteins, the most important of which is cytochrome C. The release of cytochrome C triggers a cascade reaction of cysteine esterases, which ultimately leads to multiple cellular changes related to apoptosis. Bax and Bak share the protein-protein interaction region, also known as the BH3 domain, and Bcl-2 like proteins with anti apoptotic functions mediate their various physiological effects. The subfamily of activation related proteins, each of which contains a separate BH3 region, is coupled to various receptors that sense abnormal states in cells. These BH3 specific proteins take action to interfere with the action of the anti apoptotic protein Bcl-2, or directly activate pre apoptotic factors.

Although the cell conditions that trigger apoptosis have not yet been fully listed, the expected role of some abnormal signal receptors in tumor development has been confirmed. The most noteworthy is the DNA damage receptors that function through TP53. TP53 induces apoptosis by downregulating the expression of Noxa and Puma BH3 specific proteins, in response to numerous DNA breaks and chromosomal abnormalities. Another possibility is that insufficient survival factor signaling can induce apoptosis through a BH3 specific protein called Bim. However, another condition that triggers cell death is the high level of signal expression activated by certain cancer proteins. For example, MYC can induce cell apoptosis (partially through Bim and other BH3 specific proteins), unless there are anti apoptotic factors to counteract this result.

Tumor cells have various strategies for limiting and avoiding apoptosis. The most common way is to disable TP53, which can clear the most important destructive receptors in the apoptosis inducing pathway. Tumor cells can also achieve the same results by increasing the expression of anti apoptotic factors (Bcl-2, Bcl-xL) or by increasing the expression of survival signals (such as IGF1/2). These survival signals can downregulate pre apoptotic factors or disrupt external ligand induced death signaling pathways[17, 164]. The diversity of anti apoptotic mechanisms can fully reflect the diversity of apoptosis inducing signals encountered by tumor cells during their malignant transformation process.

The malignant biological behaviors of tumor cells, such as proliferation, invasion, migration, and apoptosis, are all mediated by signaling pathways. Therefore, the activation and deactivation of signaling pathways are crucial for tumor growth and metastasis. Nuclear factor-κB (nuclear factor-NF-κB), it is a fast-acting transcription

factor commonly present in eukaryotic cells, playing a central regulatory role in the complex cytokine network during inflammation and playing an important role in cell proliferation, differentiation, apoptosis, and autophagy[165]. NF-κB is a DNA binding protein, especially NF- κ.The NF-KB of the dimer of two members of the B family proteins interacts with the strengthening region of the target gene. NF-κMembers of the B family include P65 (RelA), cRel, RelB, and P50 (NF-κB1) and P52 (NF-κB2), a typical dimer is a combination of P50 and P65. The transcriptional activation domain of P65 interacts with the basal transcriptional site to induce transcription. In normal cells, NF- κB is subjected to NF in the cytoplasm- κ. The inhibitory protein effect of B exists in a free form, and when the cell is stimulated externally, IrB is stimulated from NF dissociation on κB. NF- κB is activated and the nuclear localization sequence is exposed, thus NF-κB transcribes from the cytoplasm into the nucleus and binds to the curved regulatory garden.

NF-κB regulates genes related to cell transformation, proliferation, and vascular regeneration. Due to NF- κ B plays an important role in cell differentiation, inflammation, and cell growth, the activation of NF- κB is closely related to the carcinogenic process. Gall causing substance tranhswelling promoter can activate NF-KB through external stimulation. This carcinogen and tumor promoter mainly include toxic metals, phorbol esters, asbestos, alcohol, ultraviolet radiation, and benzopyrene[166]. On the other hand, it has been proven that cell proliferation can induce the expression of NF-KB, while on the other hand, inhibiting NF- κB activation of can block cell sulfhydrylation. There are reports that tumors such as hematological tumors, colon cancer, breast tumors, and pancreatic cell tumors can increase NF-κB[167]. Reactive oxygen species are believed to act as second messengers to activate NF through TNF and IL-1- κ B. Research has shown that reactive oxygen species can activate NF-κB through the blocking effect of antioxidants such as N-acetylcysteine (NAC), L-cysteine, green tea, vitamin E (Vr), and mercaptan[168]. Research suggests that damage factors activate NF- κ After B, it can induce neural cells to produce proapoptotic factors such as p53, c-Myc, cyclin D1, etc., leading to neuronal apoptosis[19]. The tumor suppressor factor p53 is a key mediator of cell apoptosis. P53 is NF- κ B target gene, the nuclear translocation of NF- κB upregulates the expression of p53 and inhibits NF-κB nuclear translocation can downregulate the expression of p53, thereby exerting an anti excitotoxicity effect. P53 participates in cell apoptosis in two ways, firstly, it regulates the transcription of apoptotic signaling molecules through its transcriptional activity. P53 can regulate multiple genes, including some that affect mitochondria, thereby affecting the occurrence of apoptosis. P53 induces the expression of proapoptotic genes such as Bax and PUMA, as well as inhibits the expression of anti apoptotic gene Bcl-2, which can directly or indirectly lead to depolarization of mitochondrial membranes. Secondly, p53 can also participate in cell apoptosis through non transcriptional functions. P53 can transfer to

mitochondria, affect the depolarization of mitochondrial membranes, and release proapoptotic factors, inducing apoptosis[20].

P53 is a protein first discovered by humans in 1987, with 393 amino acid residues and 53 KD. It is located at number 17 on the human chromosome and can bind specific DNA double strands to control gene transcription. P53 eggs are mainly present in the form of inactive proteins under normal conditions, namely wtP53, and are highly susceptible to degradation. When cells are in conditions such as hypoxia, oxidative damage, and DNA damage, the level of P53 eggshells increases, transitioning from inactive to active, from unstable to stable, and at the same time, their binding ability to DNA is enhanced. Research has found that ROS can affect P53 protein, and an antioxidant can inhibit P53 protein and induce apoptosis[169]. Later, it was proven that these antioxidants can clear ROS. It has also been found that there is a certain connection between the P53 gene and oxidative stress genes, and there are reports that the P53 gene can induce oxidative stress genes. Meanwhile, P53 induced apoptosis also plays an important role in proteins such as Fas/FasL and Myc. P53 egg self-renewal is related to Bcl-2 and Bax on the mitochondrial membrane. Bcl-2 protein can promote cell survival, while Bax protein can cause cell death. They are homologous proteins. Bcl-2 and Bax can form heterodimers within cells, while Bax itself can form homodimers. The Bax/BaX and Bel-2/Bax values are key factors in controlling cell survival, with the former having more offspring and the latter promoting cell apoptosis. When the latter has multiple offspring, the former inhibits cell apoptosis. There are bases on the Bax promoter that can be bound by P53, which can cause transcription. Studies have shown that the transcription of the Bcl-2 gene can be blocked by P53, leading to apoptosis[170]. The tumor suppressor factor p53 is a key mediator of cell apoptosis. P53 participates in cell apoptosis in two ways: firstly, it regulates the transcription of apoptotic signaling molecules through its transcriptional activity. P53 can regulate multiple genes, including some that affect mitochondria, thereby affecting the occurrence of apoptosis. P53 induces the expression of proapoptotic genes such as Bax and PUMA, as well as inhibits the expression of anti apoptotic gene Bcl-2, which can directly or indirectly lead to depolarization of mitochondrial membranes. Secondly, p53 can also participate in cell apoptosis through non transcriptional functions. P53 can transfer to mitochondria, affect the depolarization of mitochondrial membranes, and release proapoptotic factors, inducing apoptosis.

Oxidative stress is an imbalance between oxidative and antioxidant effects in the body, which tends to be in an oxidative state and can lead to damage to the body[171]. Under normal circumstances, in order to maintain the body's aerobic metabolism, the body produces normal levels of antioxidants, but under pathological conditions, antioxidants are produced in large quantities. Oxidative stress can participate in different signaling pathways in cells and regulate a series of biological processes including cell growth, differentiation, proliferation, apoptosis, and even intercellular

communication[172]. Excessive oxidative stress can cause damage to important components of cells, such as proteins, DNA, and membranes, which can lead to cell death. ROS exist in aerobic organisms, with stability and diverse forms, including superoxide ions (O2), chlorine peroxide (H2O2), hydroxyl radicals (- OH), and other free radicals. Each form has different levels at different positions within the cell. Reactive oxygen species are chemical substances such as peroxides and superoxides, which are byproducts of essential human functions, such as the energy generated by utilizing oxygen and cellular dynamics, known as mitochondria. Zhou said that one reason why the ROS levels of cancer cells are naturally high is that they have a high demand for energy and constantly strive to grow and spread. Some levels of ROS are also beneficial for our healthy cells, including cell proliferation and differentiation.

However, too many are also deadly normal cells, and even damage DNA.ROS in cells may attack DNA, causing oxidative damage and mutations. All cells of eukaryotes contain relatively powerful antioxidant enzymes. These three main types of antioxidant enzymes mainly include SOD, catalase (CAT), and glutathione peroxidase (GSH-Px). In addition, there are also many specialized antioxidant enzymes at work, which can generally be said to be mixtures of oxidants that can detoxify. Indirect antioxidant function is achieved through these enzymes: one is to utilize backup functions, such as flavoprotein GSSG reductase, to reduce GSSG to GSH and supply; the other is to eliminate the transport of reactive compounds. Transport systems such as glutathione S transferase and glutathione S conjugate. Different distance cell sites and cell types can include various antioxidant enzymes. At the same time, class cells have various tNs to counteract the toxic effects of ROS. For example, antioxidant enzymes CAT, SOD, and GSH-Px can relieve the toxicity of ROS.Malondialdehyde (MDA) is a product of intracellular lipid peroxidation and an important indicator of oxidative stress. In cells, lipid peroxidation reactions generate MDA, which is a highly reactive metabolic product that can covalently bind to proteins, nucleic acids, and other biological molecules within the cell, leading to cellular functional damage and inflammatory reactions. Therefore, the content of MDA can reflect the degree of intracellular oxidative stress and the risk of related diseases.

Research has shown that a decrease in SOD activity can cause excessive ROS production, leading to changes in the expression of P53 and other related proteins[173]. Some studies also suggest that P53 has an inhibitory effect on NF-KB, and a decrease in NF-KB activity can cause a decrease in the activity of the E3 ubiquitin ligase MDM2 in the P53 protein, leading to an increase in the activity of the P53 protein[40]. Bax is the main protein in the Bcl-2 family that regulates cell apoptosis. Bax exists in the cytoplasm and P53 can bind to the shoulder effector of the Bax gene, causing changes in the conformation of the Bax egg itself. This leads to the formation of Bax/Bax homologous dimers, increases the permeability of the mitochondrial membrane, and leads to a Bax/Bax ratio greater than Bcl-2/Bax. The level of Bcl-2 protein decreases, which is insufficient to stabilize Cyt-clt, leading to the release of

Cvt-c and binding to Caspases-9, resulting in Caspases-7 Activation of Caspases-3 leads to cell apoptosis.

The results of this study indicate that UCMSCs and UCMSCs-exos had no significant effect on MDA-MB-231, BGC-823, and A549, because in these cells, there was no difference in ROS, MDA, and SOD assays between the primary cells, UCMSCs group and UCMSCs-exos group (P>0.05). However, in LN-229 cells, ROS and MDA were decreased and SOD was increased in the UCMSCs group versus UCMSCs-exos group ($p$<0.05), indicating that UCMSCs and UCMSCs-exos could improve the oxidative stress response in LN-229 cells. In addition, we found no effect of UCMSCs and UCMSCs-exos on the expression of NF-κB, p53 protein in MDA-MB-231, BGC-823, and A549 cells as well (P>0.05). However, they could inhibit the expression of NF-κB, p53 proteins in LN-229, and the inhibitory effect of UCMSCs-exos was more obvious among them ($p$<0.05). UCMSCs and UCMSCs-exos also had a significant ameliorating effect on LN-229 cells, but no significant effect on other cells. In addition, the expression of NF-κB and p53 protein was reduced in both UCMSCs and UCMSCs-exos groups, which confirmed the therapeutic effect of UCMSCs and UCMSCs-exos on glioma. In previous studies, some studies mentioned that UCMSCs and their exos can accelerate the apoptosis and inhibit the growth of glioma cells, which can also testify to the results of this experiment, further demonstrating the clinical application value of UCMSCs and UCMSCs-exos. Not only that, in further assays of cellular oxidative stress, UCMSCs and UCMSCs-exos also had a significant ameliorating effect on LN-229 cells, but no significant effect on other cells. In addition, the expression of NF-κB and p53 protein was reduced in both UCMSCs and UCMSCs-exos groups, which confirmed the therapeutic effect of UCMSCs and UCMSCs-exos on glioma.

Basic scientific research has shown that almost all tumor cells have an imbalance in the intracellular redox system. High levels of long-term oxidative stress, on the one hand, directly cause tissue damage by causing an imbalance in the intracellular redox system, and on the other hand, can also lead to oxidative modification of amino acid residues, leading to DNA damage and mutations, as well as changes in the spatial structure of esters and mutated proteins, the result will mediate the activation of oncogenes or the inactivation of tumor suppressor genes, resulting in abnormal cell proliferation and the formation of tumors[174]. High levels of oxidative stress have cytotoxicity and can induce cell apoptosis or necrosis. But if low levels of oxidative stress persist, cell division and tumor growth are prone to occur. Basic scientific research has shown that almost all tumor cells have an imbalance in the intracellular redox system. High levels of long-term oxidative stress, on the one hand, directly cause tissue damage by causing an imbalance in the intracellular redox system, and on the other hand, can also lead to oxidative modification of amino acid residues, leading to DNA damage and mutations, as well as changes in the spatial structure of esters and mutated proteins[175]. The result will mediate the activation of oncogenes or the inactivation of tumor suppressor genes, resulting in abnormal cell proliferation and

the formation of tumors. High levels of oxidative stress have cytotoxicity and can induce cell apoptosis or necrosis. But if low levels of oxidative stress persist, cell division and tumor growth are prone to occur.

The death receptor pathway of cell apoptosis, Fas-FasL, and mitochondrial pathway, are largely dependent on ROS. And these two pathways are interrelated and also related to ROS levels. FasL induces the production of ROS in cells. Studies have shown that FasL activates the expression of Racl protein, which is regulated by Ras signaling. Racl protein can transfer electrons from nicotinamide adenine dinucleotide phosphate to molecular oxygen (02) to form O2-, and the generated ROS downregulates the expression of caspase-8 like inhibitory protein, which regulates apoptosis through ubiquitination. The reduction of caspase-8 like inhibitory protein can activate downstream molecule caspase-8, leading to cell apoptosis. In addition, ROS acts on phospholipase A2 on the cell membrane, causing intracellular Ca2+release or influx. The increase in Ca2+in turn promotes ROS production. ROS can activate the p38 mitogen activated protein kinase signaling pathway and participate in Fas FasL mediated cell apoptosis, which is also regulated by the downstream molecule c-Jun of p38 mitogen activated protein kinase. Cox et al. found that H2O2 induces cell apoptosis by increasing the expression of Fas and FasL mRNA, which is regulated by cAMP.

Mitochondria are the main site for producing reactive oxygen species. The increase in ROS can lead to the opening of mitochondrial membrane permeability transport pores (MPTs), which cause mitochondrial transmembrane potential (△) ψ m) Decreased release of cytochrome C, followed by activation of a series of caspase enzymes, induces the occurrence of cell apoptosis. On the other hand, in the process of cell apoptosis, the increase of ROS is also closely related to the increase of Ca2+concentration. Cao et al found that in the apoptosis pathway of breast cancer cell MCF-7 induced by surfactant, the production of ROS causes the release of Ca2+in the endoplasmic reticulum and mitochondria, and the increase of both leads to the opening of MPT and △ ψ M reduction, MPT opening, and △ ψ The decrease in m in turn leads to an increase in cytoplasmic Ca2+, which in turn increases Ca2+in mitochondria, forming a cycle that promotes cell apoptosis through the mitochondrial pathway. Previous studies have shown that overexpression of the anti apoptotic gene Bcl-2 reduces the production of oxygen free radicals. However, existing studies have shown that ROS induced by certain chemotherapy drugs can downregulate Bcl-2 and upregulate Bax expression, leading to an increase in Bax/Bcl-2 ratio, thereby participating in mitochondrial pathway cell apoptosis.

The exogenous and endogenous pathways of cell apoptosis are interrelated. Fas binds to FasL, causing receptor trimerization and activation. The trimeric death receptor recruits adapter proteins such as Fas related death domain (FADD) through the death domain. The adapter protein forms a complex with the precursor of caspase-8 through the death effector domain, known as the death inducing signaling

complex (DISC). Activated caspase-8 can break the bid in the cytoplasm into tbid, which is transferred to mitochondria, inducing the release of cytochrome C from mitochondria into the cytoplasm, thereby connecting the death receptor pathway with the mitochondrial pathway. The latest research shows that caveolin-1, an intrinsic membrane protein located on the cell surface, regulates the Fas signaling pathway and mediates the interconnection between the two pathways of cell apoptosis. The main mechanism is that Cav-1 phosphorylation regulates cell apoptosis by interacting with Fas and Bid, and the two pathways are interconnected. This interaction is achieved by ROS regulating the phosphorylation of Cav-1 Y14, but there are also studies indicating that, Cav-1 plays a dual role in cell apoptosis, and Rungtab napa et al. found that Cav-1 inhibits tumor cell apoptosis in a ROS dependent mechanism in human lung cancer H460 cell line.

Meanwhile, the p53 gene also plays an important role in the ROS induced cell apoptosis pathway. Research has shown that p53 affects ROS levels by regulating metabolic pathways or the expression of pro oxidative and antioxidant genes. ROS promotes p53 activation by inducing processes such as DNA oxidative damage. Sabrina et al.'s experimental studies have shown that under oxidative stress conditions, p53 enhances the expression of proapoptotic genes PIG3, Bax, and PUMA, which further increase the level of ROS. Moreover, in cell apoptosis induced by oxidative stress, the proapoptotic function of p53 is concentration dependent. On the other hand, p53 induces downregulation of the expression of antioxidant enzymes manganese superoxide dismutase and glutathione peroxidase 1, causing an imbalance in the body's redox state and further promoting the increase of ROS. The increase of ROS can further enhance oxidative stress and induce cell apoptosis. However, there are also many views that p53 plays an important role in the antioxidant system. Therefore, further research is needed on the specific mechanism of the interaction between p53 and ROS.Cell necrosis and apoptosis are two ways of cell death. Previous studies have shown that excessive intracellular ROS induces cell apoptosis, and a large amount of ROS can cause cell necrosis. Of course, necrosis and apoptosis can also occur simultaneously in the same tissue. HYD1 is a D-amino acid peptide, and studies have shown that HYDI causes necrosis of multiple myeloma cells in an ROS dependent manner. Due to the higher sensitivity of tumor cells to ROS compared to normal cells, ROS can selectively kill tumor cells. In order to elucidate the molecular mechanism of cell apoptosis or necrosis induced by the p53 gene in response to oxidative stress, Gogan et al. used gold nanoparticles Au-NP10 and Au-NP80 to act on human colorectal cancer HCT (p53+/+) cells. They observed the effects and molecular mechanisms of two different particle sizes of gold nanoparticles on cell apoptosis or necrosis, and found that different levels of ROS were activated by different gold nanoparticles, ROS activates different protein kinases and acetyltransferases, leading to different post-translational modifications of p53 genes, thereby mediating cell apoptosis or necrosis. Therefore, p53 dependent tumor cell apoptosis or necrosis is determined by

intracellular ROS levels. The transition from apoptosis to cell necrosis is determined by the content of ROS in tumor cells. Recent studies have found that mitochondria also play an important role in ROS induced cell necrosis. The necrosis of neuroblastoma caused by H2O2 depends on changes in mitochondrial membrane potential and ROS production, and this necrosis is regulated by c-Jun phosphorylation and polyadenyldiphosphate ribose polymerase 1 (PARP-1).

Autophagy is a programmed cell death mode, and oxidative stress can induce autophagy in cells under certain specific conditions. In recent years, research has found that ROS produced in mitochondria plays an important role as a signaling molecule in autophagy. The autophagy induced by ROS has a dual effect on the cells themselves. On the one hand, it can alleviate oxidative stress and provide self-protection, and on the other hand, it can have a destructive effect on cells by stimulating autophagic cell death. Chen et al. demonstrated through experiments that the inhibitors of electron transfer chain complexes I and II, rotenone and thiophenyltrifluoroacetone (TTFA), induce autophagy in glioma cell line U87, and this process is regulated by ROS; Chen et al. also found that H2O2 and 2-methoxyestradiol (2-ME) also induce autophagic cell death by increasing ROS levels in tumor cells. Mitochondria are the main source of intracellular ROS, so some people believe that mitochondria are indispensable during autophagy and that the increase in intracellular ROS leads to $\triangle \psi$ The changes in m subsequently trigger autophagy in cells. Khan et al. treated human lung cancer A549 cell line with iron oxide nanoparticles and found that nanoparticles induced a significant increase in intracellular ROS levels, $\triangle \psi$ M decreases, leading to mitochondrial damage[176]. The possible mechanism is that mitochondrial depolarization can rapidly reduce intracellular ATP levels. The consumption of ATP activates adenylate activated protein kinase, causing its phosphorylation. The phosphorylation of adenylate activated protein kinase can induce autophagy by inhibiting the expression of rapamycin target protein. Essick et al. found that ROS induces autophagic cell death in a dependent manner on the autophagy related protein Beclin-1[177]. The possible mechanism is that the increased ROS activates the ubiquitination protease system, which in turn degrades the anti apoptotic protein Bcl-2, leading to the activation of Beclin-1 and triggering autophagy in cells. In addition, oxidized low-density lipoprotein can also upregulate the expression of Beclin-1. Similarly, the occurrence of cellular autophagy in turn affects ROS, which may inhibit ROS levels and further increase oxidative stress levels. However, some people believe that ROS derived from mitochondria is not necessary for cellular autophagy. Therefore, further research is needed on the role of ROS in autophagic cell death.

## 5.6 Outlook

In recent years, research on the effects of MSCs and extracellular vesicles derived from MSCs on tumors has been a hot topic. On the one hand, MSCs and their extracellular vesicles can inhibit tumor growth and progression by inhibiting the expression of genes related to cell proliferation, cycle, and angiogenesis; On the other hand, the miRNA and tumor promoting molecules produced by MSCs through paracrine action alter signaling pathways related to tumor growth, angiogenesis, and drug resistance, promoting tumor progression. The dual effects of MSCs and their extracellular vesicles on tumors may be regulated by various factors, such as cell source, culture conditions, and mutual interference between MSCs and tumor cells. Although there is controversy over the use of MSCs and their extracellular vesicles in tumor treatment, scientists have overcome some obstacles by using pre editing or modifying MSCs for targeted treatment of tumors before transplantation, and using extracellular vesicles in combination with anti-tumor drugs as ideal carriers for tumor targeted treatment. In the future, in the process of using MSCs and their extracellular vesicles for targeted tumor treatment, attention should be paid to the injection timing, drug delivery pathways, cell types and characteristics of MSCs and their extracellular vesicles. At the same time, the gold standard for relevant treatment plans should also be determined to provide new strategies for targeted tumor treatment.

## 5.7 Limitations

This study preliminarily obtained the effects of UCMSCs and their extracellular vesicles on tumor cell phenotype through a large number of cell and individual phenotype experiments. Further sequencing and data analysis linked phenotype changes to relevant pathways and genes. However, further functional verification is needed for these results. In addition, the key genes that affect these phenotypic changes have been identified in the study, but there are many factors that affect gene expression and phenotype in the organism, such as signaling pathways, oxidative stress, epigenetic regulation, and complex extracellular components. In addition to the regulatory pathways found in this study, There may be other pathways. Subsequent research will combine advanced sequencing techniques to further explore individual spatial transcriptome and full transcriptome expression profiles through in vivo animal experiments, as well as the modification of post transcriptional products and their impact on the formation of these differential phenotypes. The results of this study provide important reference value for the treatment methods of tumors.

# 6. Conclusions

In conclusion, this study successfully isolated UCMSCs and their exosomes, which were found to promote the proliferation, migration, and invasion of four types of tumor cells. Additionally, UCMSCs and their exosomes were observed to be influenced by oxidative stress, with implications for the NF-κB/P53 signaling pathway. These findings provide preliminary evidence that raises concerns regarding the suitability of stem cells and their exosomes for patients with tumor diseases, and offers an experimental basis for assessing the potential clinical application safety of this technology in treatment. In clinical practice, UCMSCs are utilized for treating aging, injury, and other conditions. However, it is imperative to exclude patients who have already developed cancer and tumors when considering the application of this treatment.

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