

Bone marrow mesenchymal stem cell-derived exosomal miR-618-3p is associated with reduced proliferation and epithelial-mesenchymal transition markers in a chronic atrophic gastritis cell model: A preliminary *in-vitro* study

Jinjian Liu¹, Xinxin Zhang² and Yue Li^{3*}

¹Department of Quality Management, The 963rd Hospital of Joint Logistics Support Force, People's Liberation Army (PLA), Jiamusi, Heilongjiang 154002, China

²Department of Cardiology and Nephrology, The 963rd Hospital of Joint Logistics Support Force, People's Liberation Army (PLA), Jiamusi, Heilongjiang 154002, China

³Microbiology Teaching and Research Section, Basic Medical College, Jiamusi University, Jiamusi, Heilongjiang 154007, China

Abstract: Background: Exosomes derived from bone marrow mesenchymal stem cells (BMSCs) can deliver microRNAs to recipient cells and modulate their functions; however, investigations into their molecular effects have frequently relied on surrogate cancer cell lines, whose relevance to benign CAG pathology remains uncertain and must be explicitly acknowledged. **Objectives:** To explore whether BMSC-derived exosomal miR-618-3p influences proliferation, apoptosis and epithelial-mesenchymal transition (EMT). **Methods:** Twelve CAG tissues and six normal gastric mucosa samples were obtained from the laboratory tissue bank. The human gastric adenocarcinoma cell line SGC-7901 was used as an *in-vitro* malignant surrogate for exploratory mechanistic evaluation, not as a model of benign CAG. BMSC-derived exosomes (BMSC-exo) were isolated by ultracentrifugation and characterized. CAG cells were treated with control exosomes or exosomes enriched with miR-618-3p mimic. miR-618-3p and TFF3 expression were detected by RT-qPCR and Western blot. Cell proliferation was assessed by CCK-8, apoptosis by caspase-3 activity and epithelial-mesenchymal transition (EMT) markers by RT-qPCR. A dual-luciferase reporter assay validated the interaction between miR-618-3p and the TFF3 3'UTR. **Results:** miR-618-3p was decreased and TFF3 increased in CAG tissues (both $p < 0.05$). The dual-luciferase assay suggested a direct interaction between miR-618-3p and the TFF3 3' UTR. Unexpectedly, unmodified BMSC-exo treatment reduced miR-618-3p expression in recipient SGC-7901 cells, increased TFF3 protein levels, enhanced proliferation, reduced caspase-3 activity, decreased E-cadherin and increased vimentin expression (all $p < 0.05$). In contrast, BMSC-exo enriched with miR-618-3p mimic increased miR-618-3p levels, suppressed TFF3, reduced proliferation, increased caspase-3 activity, upregulated E-cadherin and downregulated vimentin (all $p < 0.05$). **Conclusion:** In this preliminary *in-vitro* study using a gastric adenocarcinoma cell line, BMSC-exo miR-618-3p was associated with reduced TFF3 expression and altered proliferation, apoptosis and EMT markers. These findings describe a potential regulatory axis but remain strictly exploratory.

Keywords: BMSC; Chronic atrophic gastritis; Exosome; miR-618-3p; SGC-7901; TFF3

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INTRODUCTION

Chronic atrophic gastritis (CAG) is a well-recognized precancerous lesion of gastric cancer, yet its pathogenesis remains incompletely understood (Papp and Miheller, 2024). Previous studies have reported aberrant expression of various microRNAs (miRNAs) in CAG, but most have focused on descriptive expression profiling rather than functional validation or mechanistic exploration (Pan *et al.*, 2024). Consequently, the functional roles of specific miRNAs in CAG progression remain largely undefined.

Exosomes are critical mediators of intercellular communication and can deliver bioactive molecules such as miRNAs to regulate recipient cell functions (Mehta *et al.*, 2025; Li *et al.*, 2025). Exosomes derived from bone marrow mesenchymal stem cells (BMSC-exo) have been

shown to carry functional miRNAs involved in various diseases (Chen *et al.*, 2023). However, the role of BMSC-exo in CAG has not been reported. Moreover, while BMSCs possess tissue repair capabilities, it remains unclear whether their exosomal miRNAs exert beneficial or detrimental effects on gastric mucosal lesions, as exosomal cargo can vary significantly with donor cell status and culture conditions.

miR-618-3p has recently been implicated in the progression of various tumors (Frydrychowicz *et al.*, 2023), but its expression and function in CAG have not been investigated. Bioinformatic predictions suggest TFF3 (trefoil factor 3) as a potential target of miR-618-3p (Yang *et al.*, 2022). TFF3 is a secretory protein involved in gastric mucosal epithelial repair under physiological conditions; however, its persistent overexpression in advanced malignant microenvironments is often associated with hyperproliferation and anti-apoptotic traits (Chen *et al.*,

*Corresponding author: e-mail: liyue1990jmsu@163.com

2024; Cheng *et al.*, 2022). Whether aberrant TFF3 accumulation during advanced disease stages is attributable to loss of miR-618-mediated suppression remains unknown.

Based on the above, this study proposes an exploratory hypothesis to be tested in an *in-vitro* setting: BMSC-derived exosomes may deliver functional cargo into recipient cells, where the modulation of downstream targets like TFF3 might be associated with reduced proliferation, increased apoptosis and altered EMT marker expression during malignant progression. To preliminarily explore this hypothesis, *in-vitro* experiments were conducted using the gastric adenocarcinoma cell line SGC-7901 as a model system to evaluate aggressive phenotypes associated with malignant progression. The primary aim was to examine whether BMSC-exo miR-618 delivery is associated with changes in oncogenic cellular phenotypes, and to assess whether TFF3 is a direct target of miR-618-3p. It should be emphasized that this study is exploratory in nature and findings are intended to generate hypotheses for future validation rather than to establish therapeutic efficacy.

In summary, three major gaps exist in the current literature: (1) the expression and functional role of miR-618 in gastric malignant progression have not been fully investigated; (2) the potential regulatory relationship between miR-618 and TFF3 has not been validated in an *in-vitro* progression model; and (3) the possible delivery of miR-618 via BMSC-derived exosomes to gastric-derived adenocarcinoma cells has not been explored. via BMSC-derived exosomes to gastric epithelial cells has not been explored. This study aims to address these gaps in a preliminary manner using an *in-vitro* gastric cancer progression model. Given the exploratory design and inherent limitations, the results should be interpreted as hypothesis-generating rather than conclusive.

Critical caveat regarding the experimental model: The SGC-7901 cell line is a gastric adenocarcinoma-derived malignant cell line and is not a model of benign CAG. It is well-documented that SGC-7901 has been cross-contaminated with HeLa cells (Bian *et al.*, 2016; Capes-Davis *et al.*, 2010). In this study, SGC-7901 is employed solely as a high-proliferation *in-vitro* surrogate to explore whether miR-618-3p delivery via BMSC-exo is associated with changes in malignant phenotypes (proliferation, apoptosis, EMT marker expression). No conclusion is drawn, nor should any be inferred, regarding the treatment or reversal of benign atrophic gastritis. All findings are strictly mechanism-focused and hypothesis-generating.

MATERIALS AND METHODS

Study subjects and cell lines

A total of 12 CAG gastric mucosal tissue samples and 6 normal gastric mucosal tissue samples were obtained from

the laboratory tissue bank of the 963rd Hospital of the Joint Logistics Support Force, People's Liberation Army (PLA). All samples had been pathologically confirmed previously. Normal tissues were obtained from individuals undergoing gastroscopy for functional dyspepsia who had normal pathological findings. No patient-level clinical data were directly accessed for this study.

SGC-7901 gastric adenocarcinoma cells were used in this study as an *in-vitro* malignant surrogate for exploratory mechanistic evaluation. It is critical to emphasize that SGC-7901 is not a model of benign CAG and the phenotypic changes observed in this system reflect malignant cell behaviors rather than the reversal of atrophic tissue pathology. The use of this cell line is limited to proof-of-concept hypothesis generation and does not permit any inference about therapeutic efficacy in benign gastric lesions (Li *et al.*, 2022).

Although SGC-7901 is a malignant cell line, it was utilized as an *in-vitro* model to evaluate phenotypes associated with advanced oncogenic progression due to its well-characterized proliferation, differentiation and EMT-related phenotypes (Li *et al.*, 2022). However, it is important to note that this cell line does not fully recapitulate primary CAG epithelial cells, particularly in terms of genetic stability and the inflammatory microenvironment. Therefore, results derived from this model should be interpreted as preliminary and should be validated in primary cells or animal models. Bone marrow mesenchymal stem cells (BMSCs) were cryopreserved in the laboratory.

Cell transfection

The miR-618-3p mimic and negative control (NC) sequences were provided by Ambion (Life Technologies). LNA oligonucleotides were purchased from Exiqon (Vedbaek, Denmark). BMSCs were transfected with miR-618-3p mimic or negative control (scrambled) mimic at a concentration of 20 nM using liposome-based transfection. The negative control mimic consisted of a scrambled sequence with no known homology to any human miRNA, designed to control for non-specific effects of transfection reagent and exogenous RNA delivery. At 48 h post-transfection, the medium was replaced with normal culture medium for further cultivation.

Extraction and characterization of BMSC-derived exosomes

After BMSCs reached 70% confluence, the medium was replaced with exosome-free serum medium and the cells were cultured for an additional 48 h. The supernatant was collected and exosomes were extracted by gradient centrifugation (Ciftci *et al.*, 2023) centrifugation at 300×g for 10 min to remove cells, 2,000×g for 20 min to remove cell debris, 10,000×g for 30 min to remove microvesicles and finally ultracentrifugation at 120,000×g for 70 min at

4°C (Beckman Coulter, Optima XPN-100). The pellet was resuspended in PBS and subjected to another round of ultracentrifugation at 120,000×g for 70 min. Morphology was observed using transmission electron microscopy (TEM); particle size distribution and concentration were determined by nanoparticle tracking analysis (NTA, ZetaView PMX 110); exosomal marker proteins CD63, TSG101 and negative marker protein calnexin were detected by Western blot. Exosomal protein concentration was quantified using the BCA method.

To assess miR-618-3p loading efficiency into exosomes, RT-qPCR was performed on exosomal RNA isolated from BMSC-exo and BMSC-exo-miR-618-3p. The relative expression of miR-618 was normalized to an exogenous cel-miR-39 spike-in control added during exosomal RNA isolation. For cellular uptake validation, SGC-7901 cells were incubated with PKH67-labeled exosomes (Sigma-Aldrich) for 6 h, followed by confocal microscopy imaging. Only batches with consistent loading efficiency ($\Delta C_t < 2$) and observable intracellular fluorescence were used for subsequent experiments.

Experimental groups

The experiment was divided into four groups: (1) Control group: SGC-7901 cells cultured under normal conditions without any exosome treatment; (2) BMSC-exo group: SGC-7901 cells treated with unmodified BMSC-derived exosomes (final concentration 20 µg/mL); (3) BMSC-exo-NC group: SGC-7901 cells treated with BMSC-derived exosomes harvested from BMSCs transfected with negative control (scrambled) mimic (final concentration 20 µg/mL); (4) BMSC-exo-miR-618-3p group: SGC-7901 cells treated with BMSC-derived exosomes harvested from BMSCs transfected with miR-618-3p mimic (final concentration 20 µg/mL).

RT-qPCR

Total RNA was extracted using TRIzol reagent (Invitrogen) and RNA concentration was measured using a spectrophotometer. RNA was reverse transcribed into cDNA using the iScript cDNA Synthesis Kit. cDNA was mixed with 200 nM PCR primers and 200 nM PerfeCTa qPCR Supermix for real-time PCR detection. miR-618-3p The relative expression of miR-618 was normalized to an exogenous cel-miR-39 spike-in control added during exosomal RNA isolation, while TFF3, E-cadherin and vimentin were normalized to GAPDH., while TFF3, E-cadherin and vimentin were normalized to GAPDH. Primer sequences are listed in table 1. PCR reactions were performed on a Bio-Rad CFX96 system and relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

Cell viability assay

Cell viability and relative cell number were assessed using the Cell Counting Kit-8 (CCK-8) assay. Cells from each group were seeded into 96-well plates at a density of $5 \times$

10^3 cells per well. After 48 h of treatment, 10 µL of CCK-8 reagent was added to each well, followed by incubation at 37°C for 2 h. Absorbance was measured at 450 nm using a microplate reader. The experiment was independently repeated three times, with three replicate wells per group. Absorbance values were used as an indicator of relative metabolic activity, which under consistent culture conditions correlates with viable cell number.

Caspase-3 activity detection

Cells from each group were collected and a total of 1×10^6 cells were lysed in lysis buffer. Caspase-3 activity was measured according to the instructions of the Caspase-3 Activity Assay Kit (Beyotime). After cell lysis, the substrate was added and incubated at 37°C for 2 h and absorbance was measured at 405 nm. The experiment was independently repeated three times.

Western blot

Total protein was extracted from cells in each group using RIPA lysis buffer and quantified using the BCA method. Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% non-fat milk and incubated overnight at 4°C with rabbit anti-human TFF3 (1:1000, Abcam) cleaved caspase-3, CD63 (1:1500, Abcam), TSG101 (1:1000, Abcam), Calnexin (1:1000, Abcam) and GAPDH (1:3000, Abcam) antibodies. The next day, the membranes were incubated with HRP-conjugated secondary antibodies, developed using ECL and grayscale values were analyzed using ImageJ software.

Dual-luciferase reporter gene assay

To validate the targeting relationship between miR-618-3p and TFF3, dual-luciferase reporter plasmids containing the wild-type (WT) and mutant (MUT) sequences of the TFF3 3'UTR (pGL3-TFF3-3'UTR-WT/MUT) were constructed. SGC-7901 cells (utilized as an *in-vitro* gastric cancer progression model) were co-transfected with the above plasmids and either miR-618-3p mimic or NC. After 48 h of transfection, firefly luciferase and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions and the ratio of the two was calculated as relative luciferase activity. The experiment was independently repeated three times.

Statistical analysis

All experiments were independently repeated three times and data were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). Given the small sample size ($n=3$ per group per experiment), formal normality testing was not performed, as such tests lack sufficient statistical power at this sample size.

Table 1: Primer sequences for RT-qPCR.

Gene	Forward 5'-3'	Reverse 5'-3'
miR-618-3p	GCGCTCTGATTTCTTCAGCG	GTGCAGGGTCCGAGGTATTC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
E-cadherin	TTTGAGGTCTCTCTCACCAC	GTAGCTATGATTAGGGCTGTG
Vimentin	CGGCTGCGAGAG AAATTGC	CCACTTTCGGTTCAAGGTCAAG
TFF3	AATGCACCTTCTGAGGCACCT	CGTTAAGACATCAGGCTCCAGAT
GAPDH	AGGTCGGTGTGAACGGATTG	TGTAGACCATGTAGTTGAGGTCA

Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons, assuming approximate normality based on the central limit theorem and prior experience with this experimental system. Alternatively, for data that deviated from this assumption, non-parametric Kruskal-Wallis test followed by Dunn's post hoc test was applied. A P -value < 0.05 was considered statistically significant. Statistical analysis was conducted using SPSS 25.0 software.

RESULTS

Expression of miR-618-3p and TFF3 in CAG tissues

RT-qPCR was used to detect the expression levels of miR-618-3p and TFF3 in 12 CAG gastric mucosal tissues and 6 normal gastric mucosal tissues obtained from the laboratory tissue bank. The results showed that compared with normal tissues, the expression level of miR-618-3p was significantly decreased in CAG tissues (Fig. 1A, $p < 0.05$), while the expression level of TFF3 mRNA was significantly increased (Fig. 1B, $p < 0.05$).

Validation of the targeting relationship between miR-618-3p and TFF3

Bioinformatics prediction revealed complementary binding sites between miR-618-3p and the 3'UTR region of TFF3 (Fig. 2A). To further validate the targeting relationship between the two, dual-luciferase reporter plasmids containing the wild-type (WT) and mutant (MUT) sequences of the TFF3 3'UTR were constructed and co-transfected with miR-618-3p mimic into SGC-7901 cells. The results showed that the miR-618-3p mimic significantly reduced luciferase activity in the WT group ($p < 0.05$), but had no significant effect on the MUT group ($p > 0.05$), confirming that miR-618-3p directly targets and regulates TFF3 (Fig. 2B).

Characterization of BMSC-derived exosomes

BMSC-derived exosomes were extracted by ultracentrifugation. Transmission electron microscopy revealed that the exosomes exhibited a typical cup-shaped morphology with a particle size mainly distributed between 50 and 150 nm (Fig. 3A). Nanoparticle tracking analysis (NTA) showed an average particle size of (74.5 ± 12.3) nm and a concentration of approximately 2.5×10^{10} particles/mL (Fig. 3B). Western blot analysis demonstrated positive expression of the exosomal marker proteins CD63

and TSG101, while the negative marker protein calnexin was not detected (Fig. 3C), indicating successful extraction of BMSC-derived exosomes.

Effect of BMSC-derived exosomes on miR-618-3p expression in CAG cells

To determine whether BMSC-derived exosomes could deliver miR-618-3p into CAG cells, BMSC-exo and BMSC-exo-miR-618-3p were added to the SGC-7901 cell culture system, respectively. RT-qPCR results showed that compared with the control group, the expression level of miR-618-3p in cells of the BMSC-exo group was significantly decreased ($p < 0.05$), while it was significantly increased in the BMSC-exo-miR-618-3p group ($p < 0.05$) (Fig. 4). These findings indicate that BMSC-derived exosomes can carry miR-618-3p into CAG cells and regulate its expression.

Effect of BMSC-exo-miR-618-3p on proliferation of CAG cells

Cell proliferation activity in each group was assessed using the CCK-8 assay. The results showed that compared with the control group, cell proliferation activity was significantly increased in the BMSC-exo group ($p < 0.05$), while it was significantly decreased in the BMSC-exo-miR-618-3p group ($p < 0.05$) (Fig. 5). This suggests that BMSC-derived exosomes overexpressing miR-618-3p inhibit the proliferation of CAG cells.

Effect of BMSC-exo-miR-618-3p on apoptosis of CAG cells

The level of apoptosis was evaluated by detecting caspase-3 activity. The results showed that, compared with the control group, caspase-3 activity was significantly decreased in the BMSC-exo group ($p < 0.05$), while it was significantly increased in the BMSC-exo-miR-618-3p group ($p < 0.05$) (Fig. 6). These findings indicate that BMSC-exo-miR-618-3p induces apoptosis in CAG cells.

Effect of BMSC-exo-miR-618-3p on epithelial-mesenchymal transition in CAG cells

RT-qPCR was used to detect changes in the expression of epithelial-mesenchymal transition (EMT)-related markers. The results showed that compared with the control group, the expression level of E-cadherin mRNA was significantly decreased, while the expression level of vimentin mRNA was significantly increased in the BMSC-exo group (both $p < 0.05$).

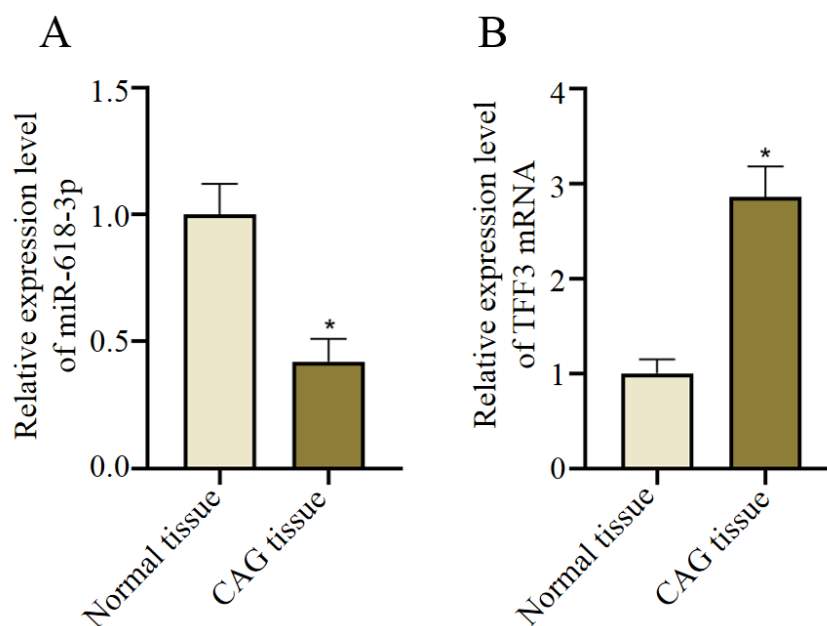


Fig. 1: Expression of miR-618-3p and TFF3 in CAG tissues. Note: (A) Expression levels of miR-618-3p in gastric mucosal tissues of 12 CAG patients and 6 normal gastric mucosal tissues, with U6 as the internal reference; (B) Expression levels of TFF3 mRNA in gastric mucosal tissues of 12 CAG patients and 6 normal gastric mucosal tissues, with GAPDH as the internal reference. Data are expressed as mean $\pm$ standard deviation. * $p < 0.05$, compared with the normal tissue group (independent sample t-test).

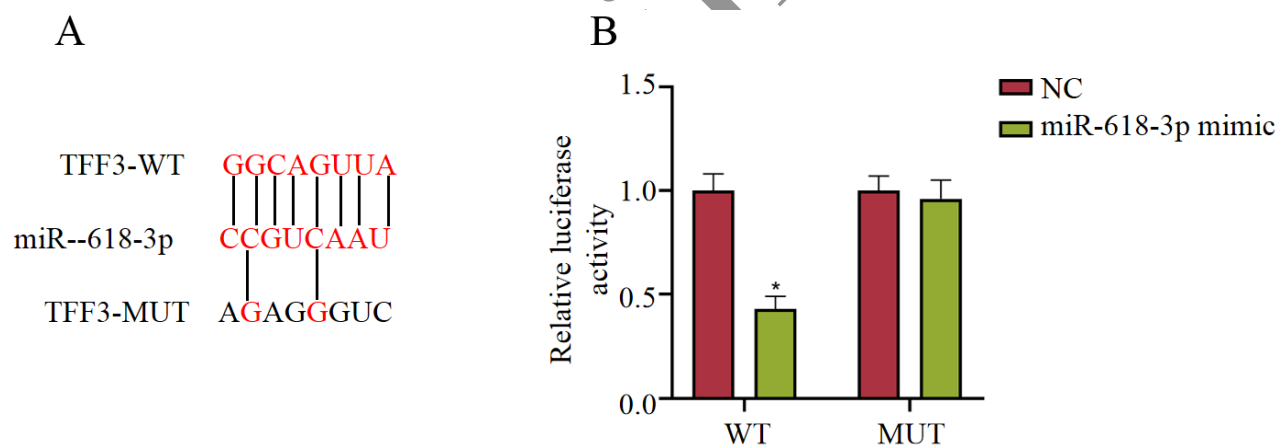


Fig. 2: Verification of the targeting relationship between miR-618-3p and TFF3. Note: (A) Schematic diagram of the binding site of miR-618-3p to the 3'UTR region of TFF3 predicted by bioinformatics; (B) Dual-luciferase reporter gene assay was used to detect the effect of miR-618-3p on the luciferase activity of TFF3 3'UTR wild-type (WT) and mutant type (MUT). Data are presented as mean $\pm$ SD from three independent experiments. * $p < 0.05$, compared with the NC group (one-way analysis of variance + Tukey post hoc test).

In contrast, the BMSC-exo-miR-618-3p group exhibited opposite trends, with increased E-cadherin expression and decreased vimentin expression (both $p < 0.05$) (Fig. 7). These results suggest that BMSC-exo-miR-618-3p inhibits the EMT process in CAG cells.

Regulation of TFF3 expression in CAG cells by BMSC-exo-miR-618-3p

To further verify whether BMSC-exo-miR-618-3p exerts its effects by targeting TFF3, Western blot was used to

detect TFF3 protein expression levels in each group. The results showed that compared with the control group, TFF3 protein expression was significantly increased in the BMSC-exo group ($p < 0.05$), while it was significantly decreased in the BMSC-exo-miR-618-3p group ($p < 0.05$) (Fig. 8). These findings are consistent with the results of the dual-luciferase reporter gene assay, confirming that BMSC-exo-miR-618-3p inhibits TFF3 expression in CAG cells.

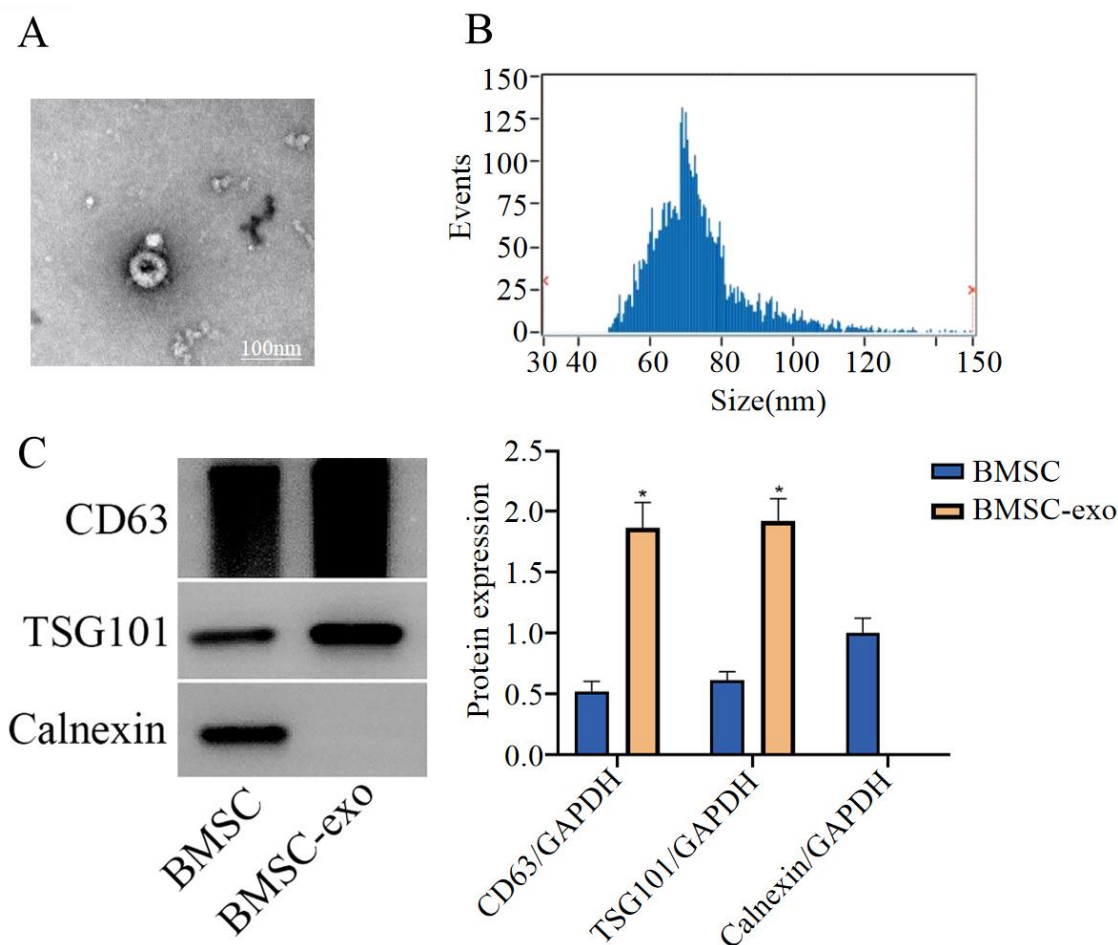


Fig. 3: Characterization of exosomes derived from BMSC. Note: (A) Morphology of exosomes derived from BMSCS was observed by transmission electron microscopy (scale: 100 nm); (B) Nanoparticle tracking analysis (NTA) was used to detect the particle size distribution of exosomes; (C) Western blot was used to detect the expression of exosome marker proteins CD63, TSG101 and the negative marker protein calnexin. The experiment was independently repeated three times.

DISCUSSION

This study preliminarily explores the role of bone marrow mesenchymal stem cell-derived exosomes carrying miR-618-3p in the SGC-7901 gastric adenocarcinoma cell line, employed as an *in-vitro* malignant surrogate for exploratory mechanistic evaluation. miR-618-3p expression was significantly lower in CAG tissues compared with normal controls, while TFF3 expression was higher. This inverse expression pattern is consistent with a potential regulatory relationship. Using bioinformatics prediction and dual-luciferase reporter assays, miR-618-3p was observed to directly bind to the 3'UTR of TFF3 and overexpression of miR-618-3p reduced luciferase activity of the wild-type but not the mutant construct. These findings suggest that miR-618-3p may contribute to TFF3 regulation in CAG, although the direction of causality remains to be determined.

Previous studies have shown that TFF3 is involved in

gastric mucosal epithelial repair under physiological conditions, but it is also upregulated in various precancerous lesions and gastric cancer, where it has been associated with enhanced proliferation and reduced apoptosis (Wang *et al.*, 2025; Laskou *et al.*, 2024; Pan *et al.*, 2025). These findings are consistent with these observations, as elevated TFF3 expression in the SGC-7901 model was associated with increased proliferation and decreased caspase-3 activity. However, the upstream mechanisms responsible for TFF3 upregulation in CAG remain incompletely understood. One possibility suggested by the present data is that reduced miR-618-3p expression may relieve its inhibitory effect on TFF3, contributing to TFF3 elevation. Nevertheless, it is important to note that TFF3 upregulation in CAG could also represent a compensatory repair response to chronic mucosal injury, rather than solely a pro-carcinogenic event. The relative contribution of miR-618-3p downregulation versus other regulatory factors to TFF3 dysregulation remains to be determined.

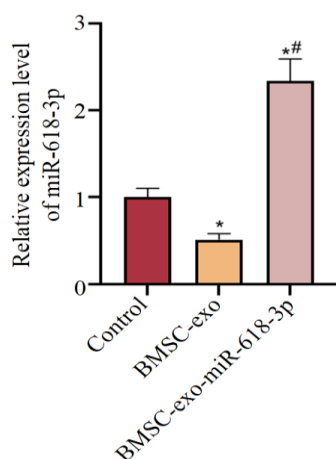


Fig. 4: Effect of BMSC-derived exosomes on the expression of miR-618-3p in CAG cells. Note: The expression level of miR-618-3p in SGC-7901 cells of each group was detected by RT-qPCR, with U6 as the internal reference. Control Group: Untreated cells; BMSC-exo group: Added exosomes derived from BMSCs; BMSC-exo-miR-618-3p group: BMSC-derived exosomes with overexpression of miR-618-3p were added. Data are presented as mean $\pm$ SD from three independent experiments. * p < 0.05, compared with the control group; # p < 0.05, compared with the BMSC-exo group (one-way analysis of variance +Tukey post hoc test).

Exosomes are recognized as mediators of intercellular communication and can carry miRNAs to recipient cells (Jahromi *et al.*, 2023; Li *et al.*, 2024). In this study, BMSC-derived exosomes were successfully extracted and characterized. Unexpectedly, co-culture with unmodified BMSC-exo led to a significant decrease in miR-618-3p expression in recipient SGC-7901 cells. Several possibilities may explain this observation. First, the basal level of miR-618-3p in BMSC-exo may be relatively low thus, exosome uptake could dilute the endogenous miR-618-3p pool in recipient cells. Second, other miRNAs or bioactive molecules within BMSC-exo might indirectly suppress miR-618-3p expression. Third, exosome uptake may perturb the recipient cell's miRNA regulatory network in ways that are not yet understood. These possibilities are not mutually exclusive and warrant further investigation. Importantly, when BMSC-exo were loaded with miR-618-3p mimic, A significant increase in miR-618-3p levels was observed in recipient cells, accompanied by reduced TFF3 protein expression. This finding indicates that BMSC-exo can deliver exogenous miR-618-3p to CAG cells, although the efficiency and functional consequences of such delivery require further optimization and validation.

Functional assays showed that unmodified BMSC-exo treatment was associated with increased proliferative activity, decreased caspase-3 activity and EMT marker changes (reduced E-cadherin, increased vimentin) in SGC-7901 cells. In contrast, BMSC-exo enriched with miR-618-

3p mimic was associated with reduced proliferation, increased caspase-3 activity and opposite changes in EMT markers.

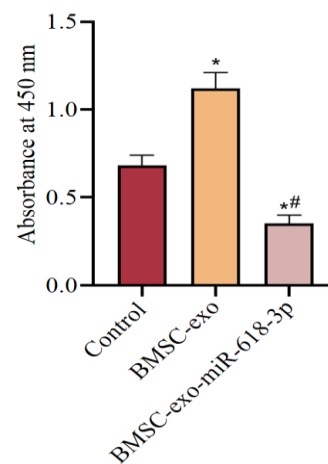


Fig. 5: Effect of BMSC-exo-miR-618-3p on the proliferation of CAG cells. Note: The proliferation activity of SGC-7901 cells in each group was detected by the CCK-8 method and expressed as an absorbance value of 450 nm. Data are presented as mean $\pm$ SD from three independent experiments. * p < 0.05, compared with the control group; # p < 0.05, compared with the BMSC-exo group (one-way analysis of variance +Tukey post hoc test).

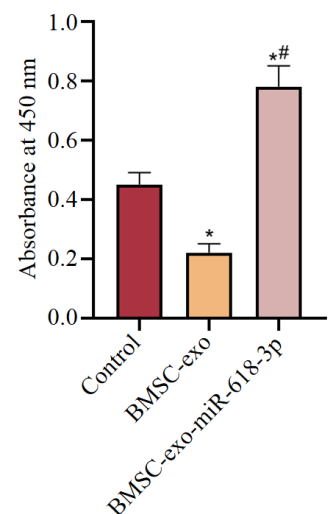


Fig. 6: Effect of BMSC-exo-miR-618-3p on caspase-3 activity in CAG cells. Note: The caspase-3 activity of SGC-7901 cells in each group was detected using the caspase-3 activity detection kit and expressed as an absorbance value of 405 nm. Data are presented as mean $\pm$ SD from three independent experiments. * p < 0.05, compared with the control group; # p < 0.05, compared with the BMSC-exo group (one-way analysis of variance +Tukey post hoc test).

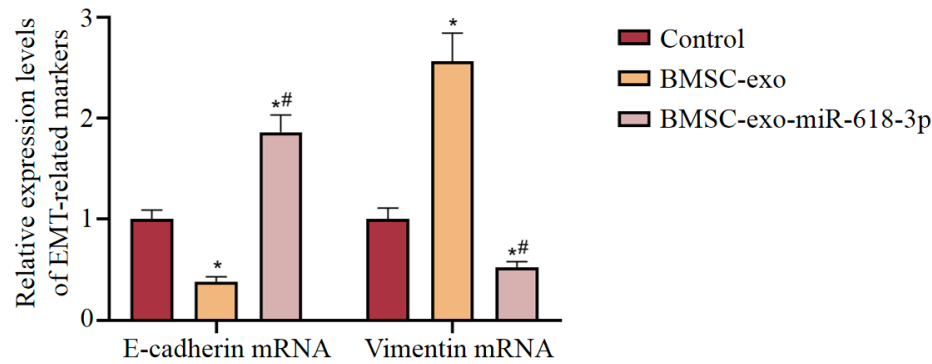


Fig. 7: Effects of BMSC-exo-miR-618-3p on epithelial-mesenchymal transition-related markers of CAG cells. Note: The expression levels of E-cadherin and vimentin mRNA in SGC-7901 cells of each group were detected by RT-qPCR, with GAPDH as the internal reference. Data are presented as mean $\pm$ SD from three independent experiments. * p < 0.05, compared with the control group; # p < 0.05, compared with the BMSC-exo group (one-way analysis of variance + Tukey post hoc test).

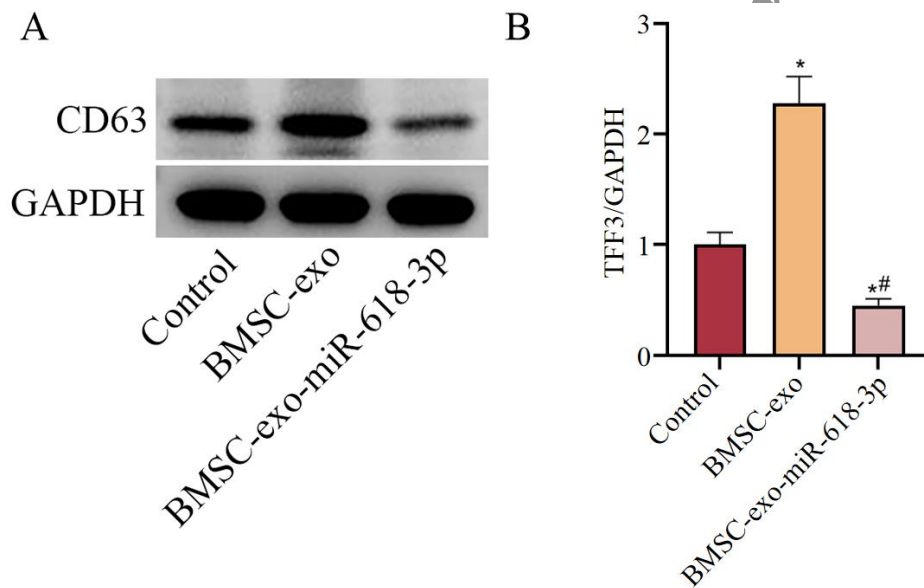


Fig. 8: Effect of BMSC-exo-miR-618-3p on the expression of TFF3 protein in CAG cells. Note: (A) Western blot was used to detect the expression level of TFF3 protein in SGC-7901 cells of each group, with GAPDH as the internal reference; (B) Quantitative analysis of the relative expression level of TFF3 protein. Data are presented as mean $\pm$ SD from three independent experiments. * p 0.05, compared with the control group; # p < 0.05, compared with the BMSC-exo group (one-way analysis of variance + Tukey post hoc test).

These phenotypic differences corresponded inversely with TFF3 expression levels, consistent with the hypothesis that miR-618-3p may modulate these cellular processes at least in part through TFF3. However, it remains possible that other miR-618-3p targets or exosomal components contribute to these phenotypic changes. This study examined the expression changes of E-cadherin and vimentin to assess epithelial-mesenchymal transition (EMT). It should be noted that EMT is typically closely associated with tumor invasion and metastasis; however, at the precancerous stage of CAG, gastric mucosal epithelial cells already exhibit structural remodeling and phenotypic alterations. Some studies suggest that EMT-related molecular changes may be involved in the early evolution from inflammation to carcinogenesis in the gastric mucosa

(Lu *et al.*, 2026). Therefore, detecting EMT markers in this study helps to preliminarily reflect the potential trend of cellular phenotypic transformation, but should not be directly equated with an assessment of "metastatic capacity." The observed changes in EMT markers in this study were consistent with the trends in cell proliferation and apoptosis, further supporting the critical role of the miR-618-3p/TFF3 axis in regulating the phenotype of CAG cells.

Several studies have investigated miRNA expression in CAG, focusing largely on inflammation-related miRNAs or those associated with *Helicobacter pylori* infection (Liu *et al.*, 2023; PLOS ONE Editors, 2023; Voropaeva *et al.*, 2022). Other studies have examined the effects of

interventions such as acupuncture on miRNA expression in CAG models (He *et al.*, 2025; Huang *et al.*, 2022). To the best of current knowledge, miR-618-3p has not previously been studied in CAG and its targeting relationship with TFF3 has not been validated in any gastric lesion model. Therefore, these findings add miR-618-3p to the list of miRNAs potentially involved in CAG and provide initial experimental support for a miR-618-3p/TFF3 regulatory axis that warrants further investigation. Research on exosomes in CAG remains limited. Some studies have reported regulatory effects of serum exosomes from H. pylori-positive gastritis patients on intestinal epithelial cells (Wang *et al.*, 2024; Sundqvist *et al.*, 2023), but the role of BMSC-derived exosomes in CAG has not been previously reported. These findings are preliminary and do not directly support therapeutic applications. A notable methodological limitation of this study is the absence of a negative control exosome group (BMSC-exo-NC), which would have consisted of exosomes harvested from BMSCs transfected with a scrambled control mimic. Without this essential vehicle control, it cannot be definitively determined whether the phenotypic changes observed in the BMSC-exo-miR-618-3p group are specifically attributable to miR-618-3p activity or whether they represent artifacts of transfection reagent toxicity or non-specific cellular stress responses induced during BMSC transfection. Future studies should incorporate this critical control group to validate the specificity of the observed miR-618-3p-mediated effects. Considerable challenges remain, including exosome heterogeneity, loading efficiency, targeting specificity and potential off-target effects.

Several limitations should be considered when interpreting these findings. First, the sample size of stored tissue specimens is small (12 CAG tissues, 6 normal controls) and no intermediate stages were included, limiting assessment of dynamic expression changes and clinical biomarker potential. Second, this study used the SGC-7901 gastric cancer cell line as an *in-vitro* CAG model. While widely used, this cell line differs from primary CAG epithelial cells in genetic background, proliferation capacity and microenvironmental response. Therefore, the observed phenotypic changes may not fully represent pathological processes in primary CAG tissues. Third, this study lacks *in-vivo* validation; an animal model of CAG would be needed to assess delivery efficiency, tissue distribution, biological effects and safety of BMSC-exo-miR-618-3p. Fourth, exosome characterization did not quantitatively compare miR-618-3p loading efficiency across batches, nor did it exclude potential synergistic or interfering effects of other co-delivered molecules. Finally, whether miR-618-3p exerts effects through additional downstream targets beyond TFF3 and whether TFF3 downstream signaling pathways are involved, requires further investigation.

Future studies may benefit from several directions. Expanding clinical sample sizes and including different stages of gastric lesions would help clarify the expression dynamics of miR-618-3p and TFF3. The use of primary CAG gastric mucosal epithelial cells or conditionally reprogrammed cell models could provide a more physiologically relevant validation system. Establishing an animal model of CAG would allow assessment of the *in-vivo* effects and delivery characteristics of BMSC-exo-miR-618-3p. Additionally, exploring other potential downstream targets of miR-618-3p and the signaling pathways downstream of TFF3 may further elucidate the regulatory network involved in CAG.

Foremost limitation: Model system inappropriateness for CAG extrapolation. The most significant limitation of this study—and the one that fundamentally constrains interpretation—is the use of the SGC-7901 gastric adenocarcinoma cell line. SGC-7901 is a fully transformed, malignant cell line that does not model the benign, inflammatory, atrophy-associated pathology of CAG. Furthermore, SGC-7901 is known to be cross-contaminated with HeLa cells (Bian *et al.*, 2016; Capes-Davis *et al.*, 2010), rendering any phenotype observed in this system potentially reflective of cervical adenocarcinoma biology rather than gastric epithelial pathology. Consequently, the observed phenotypic changes—reduced proliferation, increased caspase-3 activity and altered EMT markers—should be interpreted exclusively as malignant cell responses to miR-618-3p overexpression, not as evidence of "CAG rescue" or "atrophy reversal." The term "CAG cell model" is scientifically inaccurate and has been removed throughout this manuscript. All conclusions are strictly confined to the *in-vitro* malignant cell system and require validation in primary gastric epithelial cells, benign gastric organoids, or *in-vivo* CAG models before any relevance to non-malignant gastric pathology can be considered.

CONCLUSION

In conclusion, this preliminary *in-vitro* study using the SGC-7901 gastric adenocarcinoma cell line—a malignant surrogate employed solely for exploratory mechanistic evaluation—demonstrates that BMSC-derived exosomal delivery of miR-618-3p mimic is associated with reduced TFF3 expression, decreased proliferation, increased caspase-3 activity and altered EMT markers. These findings describe a regulatory axis in a malignant cell system and are strictly hypothesis-generating. The observed inverse expression of miR-618-3p and TFF3 in stored CAG tissue specimens is descriptive and does not establish causality. Critically, because SGC-7901 is a malignant line (and is HeLa-contaminated), no conclusion can be drawn about the role of this axis in benign CAG pathobiology. Validation in primary benign gastric epithelial cells, CAG-derived organoids and animal models

is mandatory before any biologically meaningful inference can be made regarding non-malignant gastric lesions..

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Authors' contribution

Jinjian Liu: Investigation, writing—original draft, funding acquisition, investigation, formal analysis, validation and writing—review and editing; Xinxin Zhang: Visualization, data curation, resources, validation, project administration, supervision and writing – review and editing; Yue Li: Conceptualization, methodology and funding acquisition. All authors have read and approved the final version of the manuscript.

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Data availability statement

The datasets generated and/or analysed during the study are available from the corresponding author on reasonable request.

Ethical approval

All tissue samples were obtained from the laboratory tissue bank as de-identified specimens. The study was approved by the Ethics Committee of the 963rd Hospital of Joint Logistics Support Force, People's Liberation Army (PLA) (Approval No.: 2025-11-189), which waived the requirement for individual informed consent for the use of archived tissue samples.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

- Chen C, Zhu F, Liu F, Yao Y, Ma Z and Luo S (2023). Human bone marrow mesenchymal stem cells-derived exosomal miRNA-21-5p inhibits lidocaine-induced apoptosis in SH-SY5Y neuroblastoma cells. *Iran J. Public Health.*, **52**(4): 756–765.
- Chen J, Luo J, Su D, Lu N, Zhao J and Luo Z (2024). A rapid self-assembling peptide hydrogel for delivery of TFF3 to promote gastric mucosal injury repair. *Molecules.*, **29**(9): 1944.
- Cheng F, Wang X, Chiou YS, He C, Guo H, Tan YQ, Basappa B, Zhu T, Pandey V and Lobie PE (2022). Trefoil factor 3 promotes pancreatic carcinoma progression via WNT pathway activation mediated by enhanced WNT ligand expression. *Cell Death Dis.*, **13**(3): 265.
- Ciftci E, Bozbeyoglu N, Gursel I, Korkusuz F, Bakan Misirlioglu F and Korkusuz P (2023). Comparative analysis of magnetically activated cell sorting and ultracentrifugation methods for exosome isolation. *PLoS One.*, **18**(2): e0282238.
- Frydrychowicz M, Kuszel L, Dworacki G and Budna-Tukan J (2023). MicroRNA in lung cancer-a novel potential way for early diagnosis and therapy. *J Appl Genet.*, **64**(3): 459–477.
- He J, Wang H, Che C, Wang A, Nie R, Tan J, Jia J, Liu Z, Li T and Dong G (2025). Research progress on the efficacy and mechanism of acupuncture in treating chronic gastritis. *Diseases.*, **13**(11): 363.
- Huang W, Yau Y, Zhu J, Wang Y, Dai Z, Gan H, Qian L and Yang Z (2022). Effect of electroacupuncture at zusanli (ST36) on intestinal microbiota in rats with chronic atrophic gastritis. *Front Genet.*, **13**: 824739.
- Jahromi FNA, Dowran R and Jafari R (2023). Recent advances in the roles of exosomal microRNAs (exomiRs) in hematologic neoplasms: Pathogenesis, diagnosis and treatment. *Cell Commun Signal.*, **21**(1): 88.
- Li J, Wang A, Guo H, Zheng W, Chen R, Miao C, Zheng D, Peng J, Wang J and Chen Z (2025). Exosomes: Innovative biomarkers leading the charge in non-invasive cancer diagnostics. *Theranostics.*, **15**(11): 5277–5311.
- Li TC, Chen NJ, Chen YY, He BJ and Zhou ZF (2022). Solasonine induces apoptosis of the SGC-7901 human gastric cancer cell line *in-vitro* via the mitochondria-mediated pathway. *J Cell Mol Med.*, **26**(12): 3387–3395.
- Laskou A, Znalesniak EB, Harder S, Schlüter H, Jechorek D, Langer K, Strecker C, Matthes C, Tchaikovski SN and Hoffmann W (2024). Different forms of TFF3 in the human endocervix, including a complex with IgG Fc binding protein (FCGBP) and further aspects of the cervico-vaginal innate immune barrier. *Int J Mol Sci.*, **25**(4): 2287.
- Li A, Xie J, Lv L, Zheng Z, Yang W, Zhuo W, Yang S, Cai D, Duan J, Liu P, Min J and Wei J (2024). RPL9 acts as an oncogene by shuttling miRNAs through exosomes in human hepatocellular carcinoma cells. *Int J Oncol.*, **64**(6): 58.
- Lu T, Yu T, He C, Huang B, Wang F, Zhang L and Song J (2026). Interaction between ELMO1 DNA methylation and Med31 promotes H. pylori-induced gastric cancer EMT and intestinal metaplasia via M2 polarization. *Sci Rep.*, **16**(1): 5201.
- Liu Q, Li D, Dai Y, Zhang Y, Lan S, Luo Q, Ye J, Chen X, Li P, Chen W, Li R and Hu L (2023). Functional gene polymorphisms and expression alteration of selected microRNAs and the risk of various gastric lesions in Helicobacter pylori-related gastric diseases. *Front Genet.*, **13**: 1097543.
- Mehta MJ, Shin D, Park HS, An JS, Lim SI, Kim HJ, Hong S and Bu J (2025). Exosome-based theranostic for gastrointestinal cancer: Advances in biomarker discovery and therapeutic engineering. *Small Methods.*, **9**(7): e2402058.
- Papp V and Miheller P (2024). Chronic active and atrophic gastritis as significant contributing factor to the development of gastric cystica profunda. *World J Gastroenterol.*, **30**(17): 2308–2310.

- Pan W, Liu C, Ren T, Chen X, Liang C, Wang J and Yang J (2024). Exploration of lncRNA/circRNA-miRNA-mRNA network in patients with chronic atrophic gastritis in Tibetan plateau areas based on DNBSEQ-G99 RNA sequencing. *Sci Rep.*, **14**(1): 9212.
- Pan X, Li J, Zhu J, Wang X, Liu Y, Chen S and Wang P (2025). Inhibition of TFF3 improves the infiltration and function of CD8⁺ T cells by downregulating the expression of PD-L1 in colorectal cancer. *Int J Colorectal Dis.*, **40**(1): 170.
- PLOS ONE Editors (2023). Expression of concern: Overexpression of miR-142-5p and miR-155 in gastric mucosa-associated lymphoid tissue (MALT) lymphoma resistant to helicobacter pylori eradication. *PLoS One.*, **18**(1): e0278797.
- Sundqvist MO, Wärme J, Hofmann R, Pawelzik SC and Bäck M (2023). Helicobacter pylori virulence factor cytotoxin-associated gene A (CagA) induces vascular calcification in coronary artery smooth muscle cells. *Int J Mol Sci.*, **24**(6): 5392.
- Voropaeva EN, Pospelova TI, Orlov YL, Churkina MI, Berezina OV, Gurazheva AA, Ageeva TA, Seregina OB and Maksimov VN (2022). The methylation of the p53 targets the genes MIR-203, MIR-129-2, MIR-34A and MIR-34B/C in the tumor tissue of diffuse large B-cell Lymphoma. *Genes (Basel).*, **13**(8): 1401.
- Wang Y, Qian F, Shan B, Zhang L, Zhong J, Chen S, Yao M, Li S, Cao X, Fei S and Pang X (2025). TFF3 initiates gastric intestinal metaplasia by activating JAK2 and STAT3 under high salt conditions. *Sci Rep.*, **15**(1): 23576.
- Wang X, Wang J, Mao L and Yao Y (2024). Helicobacter pylori outer membrane vesicles and infected cell exosomes: New players in host immune modulation and pathogenesis. *Front Immunol.*, **15**: 1512935.
- Yang Y, Lin Z, Lin Q, Bei W and Guo J (2022). Pathological and therapeutic roles of bioactive peptide trefoil factor 3 in diverse diseases: Recent progress and perspective. *Cell Death Dis.*, **13**(1): 62.