RESEARCH ARTICLE

Tumor necrosis factor alpha upregulates exosomal matrix metalloproteinase-9 secretion and aggravates metastatic phenotype in SKOV-3 cell line and patients with ovarian cancer

Susmita Saha[1$], Sraddhya Roy[2$], Nabanita Chatterjee[2*], Snehasikta Swarnakar[1*]

ABSTRACT

Background: Ovarian cancer is one of the most aggressive gynecologic malignancies, marked by high metastatic potential and poor prognosis. Exosomes, small extracellular vesicles secreted by tumor cells, have emerged as critical mediators of cancer progression by facilitating intercellular communication within the tumor microenvironment. It is hypothesized that TNFα promotes the release of MMP-9-rich exosomes, contributing to ovarian cancer progression via EMT and immune modulation. *Methods:* We used the TNF-α-resistant, p53-mutated SKOV-3 ovarian cancer cell line to examine the effects of TNF-α stimulation on exosome secretion, MMP-9 content, and EMT-related changes. Exosome isolation was performed from cell culture supernatants and patient serum samples. MMP-9 content and activity were analyzed via zymography and immunoblotting. EMT markers and cytoskeletal morphology were assessed by immunofluorescence staining. Functional validation of patient-derived exosomes was carried out using THP-1 monocyte co-culture assays. *Results:* TNFα treatment significantly increased the secretion of exosomes from SKOV-3 cells and elevated the MMP-9 content in the conditioned media. Treated cells exhibited reduced E-cadherin expression and acquired a mesenchymal-like morphology, confirmed by phalloidin staining of the actin cytoskeleton. Exosomes isolated from ovarian cancer patient serum also showed elevated MMP-9 activity, supporting the *in-vivo* relevance of our *in-vitro* findings. Additionally, patient-derived exosomes were biologically active and capable of modulating immune responses in THP-1 monocytes. *Conclusion:* TNF-α may drive ovarian cancer progression by enhancing MMP-9-enriched exosome secretion, promoting EMT and immune modulation. Targeting this pathway could offer new therapeutic strategies.

Keywords: Ovarian cancer, exosomal content, MMP-9, TNF-α treatment, EMT markers

Indian Journal of Physiology and Allied Sciences (2025); DOI: 10.55184/ijpas.v77i03.536 **ISSN:** 0367-8350 (Print)

INTRODUCTION

The prevalence of ovarian cancer (OC) is on the rise, with a high mortality rate. The Global Cancer Observatory reported 324,603 new OC cases in 2022 globally. With a cumulative risk of incidence of 0.73, OC treatment is the most challenging.[1] In India, according to GLOBOCAN 2022, 47,333 (6.6%) new OC cases were reported, making it the third most common type of cancer among females. The National Cancer Registry Programme reported that OC rates are increasing in various geographical areas, including the North Eastern region of India, with 29% of cases being both localized and distant metastasis, and 41.9% being locally advanced/locoregional cases.[2] Shih *et al.* determined two main categories of OC based on molecular pathways leading to OC. Firstly, the type I category comprised low-grade serous-papillary, endometrioid, and borderline tumors with lesser malignant potential, and secondly, the high-grade serous carcinomas (HGSOC) with high malignant potential.[3] The most frequent genetic change in HGSOC involves p53 mutations, involving 50 to 80% of this form of cancer.[4] Thus, this alarming situation demands an absolute necessity for early-stage diagnosis with a proper treatment regimen for patients to improve their survival rate.

Genetic and epigenetic changes drive OC cell transformation, indicating that the disease may originate from three main sites: the ovarian surface, fallopian tube, and mesothelium-lined peritoneal cavity.[5] The early stages of metastasis involve an epithelial-to-mesenchymal transition (EMT) of cancer cells, caused by alterations in cadherin and integrin expression levels and enhanced proteolytic pathways.[5] Here, the crucial roles of matrix metalloproteinase (MMP) enzymes become evident, as they extensively remodel the extracellular matrix (ECM) by degrading components like collagen and gelatin, thus facilitating the migration of cancer cells to distant sites.[6] Gelatinases, specifically MMP-2 and MMP-9, are heavily involved in solid tumor invasion, angiogenesis, and

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How to cite this article: Saha, S., Roy, S., Chatterjee, N,. Swarnakar, S. Tumor necrosis factor alpha upregulates exosomal matrix metalloproteinase-9 secretion and aggravates metastatic phenotype in SKOV-3 cell line and patients with ovarian cancer. *Indian J Physiol Allied Sci* 2025;77(3):25-36.

Conflict of interest: None

Submitted: 30/07/2025 **Accepted:** 02/09/2025 **Published:** 17/09/2025

metastasis, with MMP-2 linked to angiogenesis and MMP-9 aiding in ECM breakdown, promoting tumor spread.[7] Tumor necrosis factor alpha (TNF-α), a pro-inflammatory cytokine, is recognized as a key mediator in promoting both tumor growth and metastasis.[8] However, resistance to TNF-α in OC complicates treatment options.[9] Notably, TNF-α-resistant cell lines such as SKOV-3, often used as models for studying drug resistance, exhibit reduced sensitivity to TNF-α-induced apoptosis, which further supports tumor survival and progression.[10] This resistance is worsened by mutations in the tumor suppressor gene p53, which is common in OC cells. p53 mutations hinder the cells' ability to respond to DNA damage and stress signals, thereby intensifying resistance to TNF-α and resulting in a more aggressive tumor phenotype.[11] In this context, cell-to-cell communication within the tumor microenvironment (TME) is also vital for the metastatic process, where a specific type of extracellular vesicle, namely exosomes, plays a crucial role alongside other regulatory factors like cytokines and chemokines. These exosomes, which are bioactive nano biomolecules, can travel to distant sites, creating a suitable TME for cancer cell proliferation by reprogramming various cells, such as transforming fibroblasts into cancer-associated fibroblasts, and evading immune surveillance.[12] The release of exosomal cargoes like miRNAs, including non-coding miRNAs, upon internalization into recipient cells, alters the fate of the host cells, thereby facilitating the homing of migrating cancer cells.[13]

TNF-α is a vital cytokine involved in both survival and death signaling in cells.[14,15] In SKOV-3 cells, resistance to TNF-α-induced death poses a problem because it enables cancer cells to avoid the body's normal mechanisms for eliminating damaged or abnormal cells, thereby supporting tumor progression, immune evasion, and treatment resistance. The presence of a p53 mutation and the activation of pro-survival pathways like NF-κB are essential factors that prevent SKOV-3 cells from undergoing apoptosis in response to TNF-α, which ultimately benefits the cancer cells but impairs the body's ability to control tumor growth.[16]

Exosomes released by chemoresistant cancer cells significantly influence the TME by transforming immune cells into an anti-inflammatory, immunosuppressive phenotype and establishing a tumor-supportive environment. This leads to increased immune evasion, tumor survival, metastasis, and therapy resistance.[17,18] Using cargo such as immune checkpoint proteins, cytokines, miRNAs, and growth factors, these exosomes encourage the polarization of immune cells (e.g., M2 macrophages, Tregs) and support the tumor's structural, metabolic, and immune-suppressive requirements.[17,18] Understanding these exosomal roles could offer new insights into therapeutic strategies to overcome chemoresistance and improve cancer treatment outcomes.

In this research, we aimed to investigate the expression of TNF-α and MMP-9 in patients from Eastern India, focusing on their role in driving metastasis in OC under the influence of exosomes. Our clinical findings suggest a possible association of MMP-9 in the exosomes of late-stage OC patients and its role in intercellular communication. Additionally, *in-vitro* studies showed TNF-α-mediated upregulation of MMP-9 in cell culture media and exosome content, as well as EMT in the TNF-α-resistant SKOV-3 cell line. This research could help illuminate the mechanisms by which these exosomes modulate MMP activity and promote metastasis, which can be targeted in future studies. Understanding the complex interactions between TNF-α, MMP-9, exosomes, and p53 mutations is crucial for uncovering the mechanisms behind OC metastasis and therapy resistance. Targeting these pathways may provide promising therapeutic options to limit cancer spread, overcome TNF-α resistance, and improve patient outcomes.

MATERIALS AND METHODS

Clinical Data and Sample Collection

OC patients at later stages who attended Chittaranjan National Institute, Regional Cancer Centre, Kolkata, India, for their treatment were considered in the study. The study has been approved by the Institute Ethics Committee (CNCI-IEC-40104). The data set comprises clinical characteristics, including age, CA-125, ascites malignancy, metastasis, tumor stage, laterality of tumor, and chemotherapy. The clinicopathological information related to the patients was collected from the clinical database of the hospital and was kept confidential. The tissue samples from advanced-stage OC patients (N = 44) were collected during surgery at the Chittaranjan National Cancer Institute. Adjacent normal tissues from the respective patients were also collected. Patients' details were anonymized, and samples were collected with informed consent forms from the patients.

Cell Culture and TNF-α Treatment

Human ovarian carcinoma SKOV-3 cells were obtained from ATCC and cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 2mM L-glutamine. Cells were maintained at 37°C in a 5% CO_2 humidified incubator. For TNF-α treatment, SKOV-3 cells were seeded at a density of 2×10^5 cells/well in 6-well plates and allowed to grow to approximately 70 to 80% confluence. After 24 hrs of incubation, cells were treated with recombinant human TNF-α (10, 30, 50 ng/mL) for 12 and 24 hours, depending on the experiment. Control cells were treated with an equivalent volume of vehicle (PBS).

Gelatin Zymography for Matrix Metalloproteinase Activity

To assess the activity of MMPs, gelatin zymography was performed as described previously.[19] Briefly, conditioned media from TNF-α-treated and control SKOV-3 cells were collected after the respective incubation periods. The conditioned media were clarified by centrifugation at 2000 rpm for 10 min at 4°C.

For zymography, equal volumes of conditioned media (20 μL) were mixed with non-reducing sample buffer (without SDS) and subjected to electrophoresis on a 10% SDS-PAGE gel copolymerized with 1 mg/mL gelatin. Gels were electrophoresed at 90 V for 2 hours and then washed twice in 2.5% Triton X-100 for 30 min to remove SDS. The gels were incubated in zymography buffer (50 mM Tris-HCl, pH 7.5, 10 mM $CaCl_2$, 0.02% NaN_3) at 37°C for 24 hours. After incubation, the gels were stained with 0.1% Coomassie Brilliant Blue R-250 and destained to visualize the clear bands corresponding to gelatinase activity.

Protease activity was analyzed by measuring the intensity of the clear bands on the zymogram using ImageJ software. The size and intensity of the bands were compared between the TNF-α-treated and control groups.

Cell Lysate, Tissue Lysate and Exosomal Lysate Preparation and Western Blot

The preparation of cell lysate, tissue lysate, and exosomal lysate, followed by western blot analysis, was performed according to the protocols described previously.[19] Briefly, the tissues were suspended in PBS containing protease inhibitors and minced at 4°C. The suspension was centrifuged at 12,000 g for 15 minutes, and the supernatant was collected as PBS extracts. The pellet was further extracted in lysis buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100, and protease inhibitors) and centrifuged at 12,000 g for 15 minutes to obtain Triton X-100 (Tx) extracts. *In-vitro* cells were directly homogenized in lysis buffer containing a protease inhibitor cocktail and centrifuged at 12,000 g for 15 minutes to obtain the whole cell extract. Proteins were estimated either by the Lowry method or the Bradford assay. Equal amounts of protein (70 µg) were separated by SDS-PAGE (10% gel) and transferred to PVDF membranes (Millipore), and in some cases, NCM membranes. The membranes were blocked with 5% non-fat milk in Tris-buffered saline with 0.1% Tween-20 (TBST) for 1-hour at room temperature (RT) and then incubated overnight at 4°C with primary antibodies: anti-MMP-9, Anti-TNF-α, anti-CD-63, and anti-TSG-101. After washing with TBST, membranes were incubated with appropriate HRP-conjugated secondary antibodies for 1 hr at RT. Protein bands were visualized using an ECL detection system (Bio-Rad), and densitometric analysis was performed using ImageJ software. A list of antibodies has been given in Supplementary Table S2.

EV Preparations and Treatments

Extracellular vesicles (EVs) were isolated and characterized based on established protocols and modifications specific to the SKOV-3 cell line and serum sample used in the study.[22] Exosomes were isolated from the conditioned media of the SKOV-3 OC cell line following a differential ultracentrifugation method. Briefly, the media was clarified by centrifugation at 400 × g for 5 min to remove cellular debris, followed by a subsequent spin at 2,000 × g for 10 minutes, and at 10,000 × g for 30 minutes to remove large vesicles. The supernatant was then ultracentrifuged at 120,000 × g for 2 hours in a fixed-angle rotor (Beckman Coulter, Type 70.1 Ti Rotor). The resulting exosome pellets were resuspended in an appropriate buffer for further experiments.

Exosomes from serum samples of patients with OC and control individuals were isolated using the miRCURY Exosome Serum/Plasma Kit protocol. The isolation was performed according to the manufacturer's instructions, with appropriate modifications to ensure the purity and yield of exosomes suitable for downstream applications such as immunoblot analysis.

The characterization of isolated EVs was performed using several methods such as atomic force microscopy (AFM), to assess the size and surface morphology of exosomes; dynamic light scattering (DLS) to determine the size distribution of exosomes; Immunoblot (Western Blot), to confirm the presence of exosomal markers, such as CD63 and TSG101, using standard protocol for immunoblotting.

Serum samples were also isolated using ultracentrifugation for the treatment of macrophages, and serum samples were first centrifuged at 6,000 × g for 5 min to remove platelets and large cellular debris. The supernatant was then centrifuged at 10,000 × g for 30 min to remove any further smaller cellular debris. Finally, exosomes were isolated by ultracentrifugation at 120,000 × g for 3 hours. The exosome pellet was resuspended in incomplete media for subsequent treatment or experimentation, and the number of exosomes was calculated using NTA (Nano Tracker Analysis).

EV Treatments to PMA-induced THP-1 Cell-derived Macrophage Model

The isolated EVs from control individuals and patients with OC were resuspended in appropriate media and used for PMA-induced THP-1 cell-derived macrophage treatments at 0 and 5 hours to assess whether it is biologically active and have the potential to internalize into the recipient cells.

Immunohistochemistry

IHC analysis was performed on the slides, both adjacent normal and tumor tissues, where deparaffinization using xylene was done, followed by rehydration in alcohol gradient (100, 90, 70, 50%) for 5 minutes in each gradient. Antigen expression was enhanced upon treating the slides in 10 Mm citrate buffer solution (pH 6.0). 3% hydrogen peroxide diluted in 100% methanol was added to the slides for 15 min at RT in the dark. To stop the endogenous peroxidase activity, the slides were washed with 1x PBS for 5 min, followed by the addition of primary antibodies of TNF-α and MMP9 (1:500) overnight at 4°C. The next day, anti-rabbit HRP secondary antibody (1:1000) was added and incubated for 2 hrs at RT. The reaction was visualized by adding 3,3'-diaminobenzidine tetrahydrochloride (DAB) for 10 min, and finally, the slides were counterstained using Mayer's haematoxylin. The slides were then dehydrated using gradient alcohol (50, 70, 90, 100%) and covered with coverslips after DPX mounting. The images were captured under a brightfield compound microscope (Leica Microsystems: #Model DM1000) and analyzed using GraphPad Prism[23]

Immunofluorescence

Immunofluorescence was performed as described previously. Briefly, 5000 SKOV-3 cells were seeded on a coverslip in a 35 mm cell culture dish. The treatment of 50 ng/mL of rhTNF-α was given for 24 hours; then the media was discarded, and the cells were washed with PBS once. Cells were fixed with 4% paraformaldehyde for 15 minutes at RT, followed by washing with PBS three times for 5 minutes each. Cells were blocked using blocking buffer (1 × PBS, 5% serum, 0.1% Triton X-100) for one hour. Cells were then incubated with antibody dilution buffer (1 × PBS, 1% BSA, 0.1% Triton X-100) containing MMP-9, N-cadherin, and E-cadherin antibody (1:100) for 1.5 hours at RT. Subsequently, cells were incubated with TRITC-conjugated anti-rabbit (1:100) secondary antibodies in antibody dilution buffer for 1.5 hours at RT in the dark. Cells were then washed with 1 × PBS (3 times) for 5 min each. For phalloidin staining, after permeabilisation, cells were incubated with phalloidin. Cells were washed with 1 × PBS (3 times) for 5 minutes, then nuclei were stained using Hoechst (2 μg/mL) for 15 minutes in the dark. Cells were then washed with 1 × PBS 6 times for 5 minutes each. Imaging was done at a 63x Leica confocal microscope.

Bioinformatics Analysis

Pan-cancer analysis of TNF expression was performed using UALCAN, a web-based tool that facilitates in-depth analysis of the TCGA database.

Statistical Analysis

All experiments were performed independently at least three times. Protein band intensities were quantified using densitometric analysis with Lab Image software (version 2.7.1, Kapelan GmbH, Germany). All data have been represented as mean ± standard deviation (SD). The statistical analysis used GraphPad Prism (version 5.01, San Diego, California, USA) software. An unpaired t-test with Welch's correction was used to compare two experimental groups to determine statistical significance. A one-way ANOVA followed by Bonferroni's multiple-comparison test was performed for comparisons involving more than two groups. A calculated probability of <0.05 indicates a statistically significant difference.

The statistical analysis of the clinical records was performed using IBM SPSS 27 Statistics software. The frequency table was prepared using this software. The image analysis was performed using Fiji-ImageJ software (https://imagej.net/Fiji). GraphPad Prism software (version 5.01) was used to prepare the graphs. A probability value <0.05 was considered statistically significant.[24]

RESULTS

A. IHC analysis of MMP-9; Graphical representation of the area of expression of MMP-9 in normal and tumor tissues. B. IHC analysis of TNF-α; Graphical representation of the area of expression of TNF-α in normal and tumor tissues C. MMP-9 activity were assessed by gelatin zymography using OC patient derived tissue (n=9) compared with non-malignant ovary (n=6) (100 μg/lane) D. Representative western blots of TNF-α in OC patient derived tissue (n=7) compared with non-malignant ovary (n=6) (100 μg/lane) E**.** MMP-9 activity were assessed by gelatin zymography using OC patient derived serum (100 μg/lane) compared with individuals without cancer related complications (n=24 samples/group). F. Representative images from the western blot analysis and quantification of the protein signal of TNF-α and MMP-9 in serum of patients with OC (n=8) compared with individuals without cancer (n=8). Means ± SD. p <0.05 was accepted as the level of significance (Figure 1).

MMP-9 Expression is Influenced by TNF-α Expression, as Indicated by their Higher Expression in Tumor Tissues and Serum as Compared to the Normal Tissues

TILs are generally higher in tumor tissues compared to normal tissues. We were motivated to investigate MMP-9 expression and activity in OC patients from the Eastern zone of India, where we observed that MMP-9 levels are higher in tissues than in normal tissues (Figure 1A). Analysis of gelatinolytic activity revealed that MMP-9 activity was increased in both tumor tissue lysates (Figure 1C) and patients' serum (Figure 1E) compared to adjacent normal tissue lysates and serum from healthy volunteers. Since the inflammatory TME influences MMP activity due to elevated cytokine levels, we also observed that the pro-inflammatory cytokine TNF-α was elevated in tumor tissues compared to adjacent normal tissues (Figure 1B). Similar findings were seen in the protein expression of TNF-α, with higher levels in tumor tissues (Figure 1D) and patients' serum (Figure 1F). These results suggest that the upregulated expression of MMP-9 is modulated by TNF-α, which promotes ECM degradation, facilitating cancer cell migration by evading surrounding tissues at the primary site, thereby initiating EMT. Interestingly, Figure 1G shows pan-cancer TNF-α expression from the TCGA database, where it is higher in OC. The induction of EMT was supported by our clinicopathological findings, which showed metastasis in 95.5% of cases, with 43.2% of patients in stage III and 34.1% in stage IV, as detailed in Supplementary Table S1.

MMP-9 Activity and Expression Levels Increased in Exosomes Isolated from the Serum of Patients with OC

To assess whether MMP-9 is selectively enriched in circulating exosomes during OC, we examined its enzymatic activity and protein expression in serum-derived exosomes from OC patients and healthy controls. Successful isolation of exosomes was confirmed through NTA, showing a size range within the expected exosomal diameter (30–250 nm) (Figure 2A). AFM and DLS were used to evaluate the morphology and hydrodynamic size of the exosomes, respectively. Western blotting verified the presence of exosomal markers (e.g., CD63, TSG-101), indicating successful enrichment of serum exosomes (Figure 2B). Gelatin zymography was performed on serum-derived exosomal lysates (100 μg/lane) from 13 OC patients and 9 healthy individuals. A prominent gelatinolytic band corresponding to pro-MMP-9 (~92

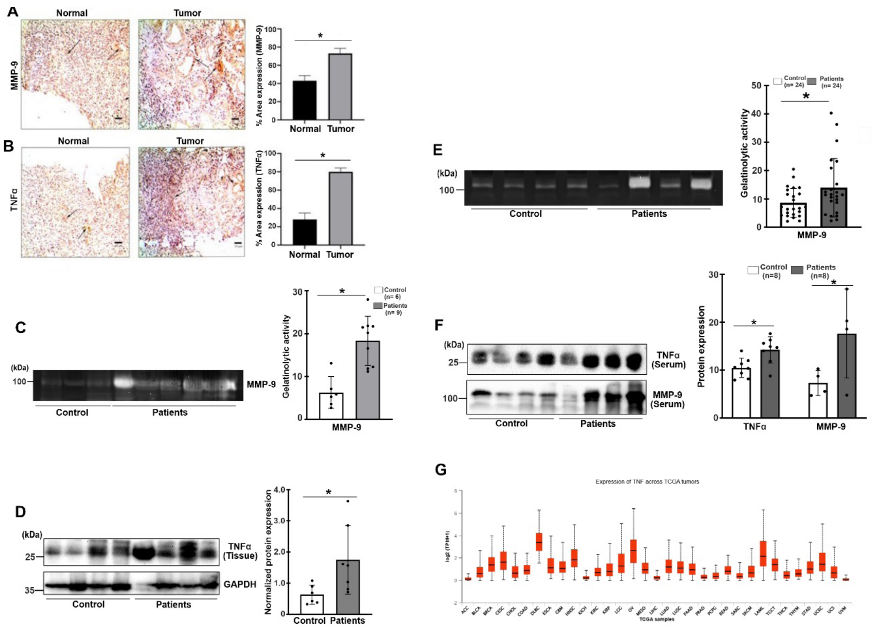


Figure 1: Increased expression of TNF-α and MMP-9 activity in OC patient-derived serum and tissue.

kDa) was observed in exosomes from OC patients, with markedly higher intensity compared to controls. These findings suggest significantly increased gelatinase activity, especially of MMP-9, in exosomal cargo from OC patients (Figure 2C). Western blot analysis further supported this, showing elevated expression of MMP-9 in exosomes from OC patient serum (n = 4) compared to controls (n = 4), using 50 µg of protein per lane. Densitometric analysis confirmed a statistically significant increase in MMP-9 expression ($p < 0.05$), as shown in Figure 2D.

A. NTA of patients with OC and healthy individuals- derived exosomes (n = 2/sample). B. AFM, DLS, and western blot analysis of exosomal markers of serum-derived exosomes. C. MMP-2 and MMP-9 activity of patients (n = 13) and controls (n = 9) individuals in exosomal lysate (100ug/lane). D. Western blot analysis of MMP-2, MMP-9 expression normalized against TSG101 in exosomal lysate from control (n = 4) and patients with OC (n=4) (50µg/lane). Means ± SD. p<0.05 was accepted as the level of significance (Figure 2).

Recombinant human TNF-α is required for MMP-9 upregulation in a time and dose-dependent manner in the SKOV-3 Cell line

To assess the effects of recombinant human TNF-α (rhTNF-α) on EMT induction and MMP activity, SKOV-3 cells were treated with varying doses of rhTNF-α (10–50 ng/mL) for 12 hrs and 24 hrs (Figure 3A). Gelatin zymography revealed a dose- and time-dependent increase in MMP-9 enzymatic activity in the conditioned media of treated cells. The strongest induction was observed at 50 ng/mL after 24 hours of treatment, indicating TNF-α-mediated upregulation of secreted MMP-9. Phase-contrast microscopy showed a marked morphological shift in SKOV-3 cells treated with 50 ng/mL rhTNF-α for 24 hours, transitioning from a cobblestone epithelial appearance to an elongated, spindle-shaped mesenchymal phenotype, consistent with EMT (Figure 3B). Immunofluorescence analysis further confirmed the induction of EMT markers following 24 hours treatment with 50 ng/mL rhTNF-α (Figures 3C-G). Phalloidin staining highlighted reorganization of the actin cytoskeleton with stress fiber formation (Figure 3C). MMP-9 expression was strongly upregulated and localized in the cytoplasm (Figure 3D). E-cadherin, an epithelial marker, showed a significant decrease in membrane staining, while mesenchymal markers (Figure 3E). N-cadherin and vimentin exhibited increased expression and cytoplasmic distribution. Merged images (third panels) confirmed co-localization patterns and changes in protein distribution (Figures 3F and G).

These findings demonstrate that TNF-α promotes EMT-like morphological changes and enhances the proteolytic activity of MMP-9 in SKOV-3 cells, supporting a potential role for TNF-α in driving tumor invasiveness and extracellular matrix degradation in OC.

TNF-α upregulates exosomal MMP-9 secretion and enhances metastatic phenotype in SKOV-3 cells and OC patient-derived exosomes

To assess whether TNF-α modulates exosomal MMP-9 secretion and contributes to metastatic behavior, we analyzed exosomes derived from SKOV-3 cell-conditioned media after TNF-α stimulation. Exosomes isolated from rhTNF-α-treated SKOV-3 cells were characterized using dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). DLS revealed a homogenous population of vesicles with an average diameter consistent with the expected size of exosomes (~100 nm). NTA confirmed a significant increase in exosome concentration upon TNF-α treatment, indicating enhanced vesicle secretion (Figures 4A and B). To assess the

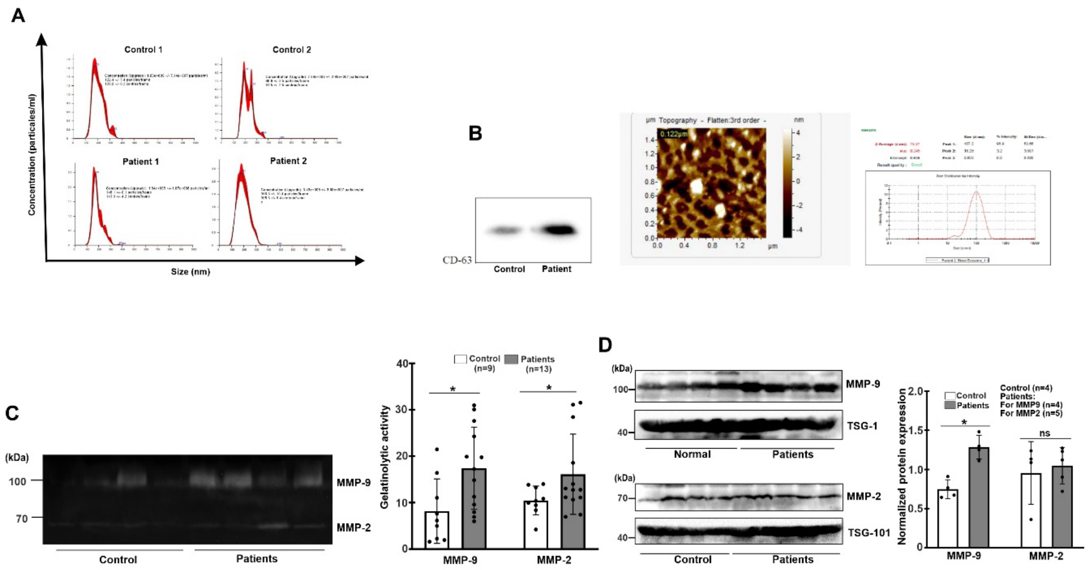


Figure 2: Characterization of serum-derived exosomes and status of MMP-9 expression and activity in exosomal cargo. Compared between patients with OC and healthy individuals.

uptake and potential functional relevance of these exosomes, confocal microscopy was performed on PMA-activated THP-1 macrophages treated with PKH-26-labeled exosomes at 0 and 5 hours. Time-course imaging demonstrated a robust internalization of exosomes by macrophages within 5 hours, suggesting active intercellular communication. Enhanced uptake of TNF-α-induced exosomes may contribute to the pro-metastatic microenvironment through modulation of recipient immune cells (Figure 4C).

Collectively, these findings indicate that TNF-α promotes the release of MMP-9-enriched exosomes from SKOV-3 cells, which may potentiate tumor-stroma interactions and metastatic behavior in the OC microenvironment.

DISCUSSION

OC remains a highly deadly gynecological cancer, mainly because it is often detected late, in over 70% of patients.[25] The current gold-standard biomarkers, CA125 and HE4, are useful for diagnosis and monitoring but are inadequate for early detection and do not offer detailed insights into tumor progression.[26,27] This study explores the interaction between TNF-α, MMP-9, and EMT, with a particular focus on the exosomal pathway of MMP-9 transport and activity, especially in late-stage, TNFα-resistant ovarian tumors.

The TME of advanced OC is known to be rich in inflammatory cytokines and proteolytic enzymes, forming a niche that promotes tumor growth and metastasis.[28,29] TNF-α is a central component in this inflammatory pathway, aiding not only immune cell infiltration but also encouraging EMT and matrix breakdown.[10] Our immunohistochemical and serum-based analyses show increased TNF-α levels in late-stage OC, supporting previous research.[29] Additionally, Kaplan-Meier plot analyses indicate that high levels of TNF-α, TNFR2, and MMP-9 are linked to poorer overall survival (OS) and progression-free survival (PFS), emphasizing their clinical significance.[29]

MMP-9, a type IV collagenase, promotes ECM remodeling and plays a role in various stages of tumor invasion and metastasis.[31] Notably, MMP-9 is not only secreted in its soluble form but also transported via exosomes—small extracellular vesicles involved in intercellular communication within the TME.[32] Our study shows for the first time that exosomal MMP-9 expression and activity are significantly higher in the serum of late-stage OC patients and in patient-derived tumor tissues.

Using the SKOV-3 cell line, a TNFα-resistant, p53-mutant epithelial OC model, we mimicked the in vivo late-stage tumor condition. The p53 mutation in SKOV-3 disrupts apoptosis downstream of TNFα signaling, a phenomenon known to contribute to chemoresistance and tumor aggressiveness.[16] Interestingly, upon treatment with recombinant human TNF-α (rhTNFα), SKOV-3 cells displayed classical EMT features: morphological changes, downregulation of E-cadherin, and upregulation of N-cadherin and vimentin. These effects were abrogated by inhibition of the NF-κB pathway, confirming its role as a downstream effector of TNF-α-induced MMP-9 expression. The TNF-α, NF-κB, MMP-9 axis has been reported in other tumor types, including colorectal and breast cancer,[16] but its mechanistic significance in exosome-mediated signaling in OC is novel.

Exosomes are small extracellular vesicles (30–150 nm) secreted by most cell types, including tumor cells, and play a crucial role in intercellular communication within the TME. While significant focus has been placed on exosomal miRNAs and mRNAs, the protein cargo of exosomes is increasingly recognized as an essential component influencing disease progression, especially in OC. Tumor-derived exosomes carry a unique set of proteins, including enzymes, adhesion

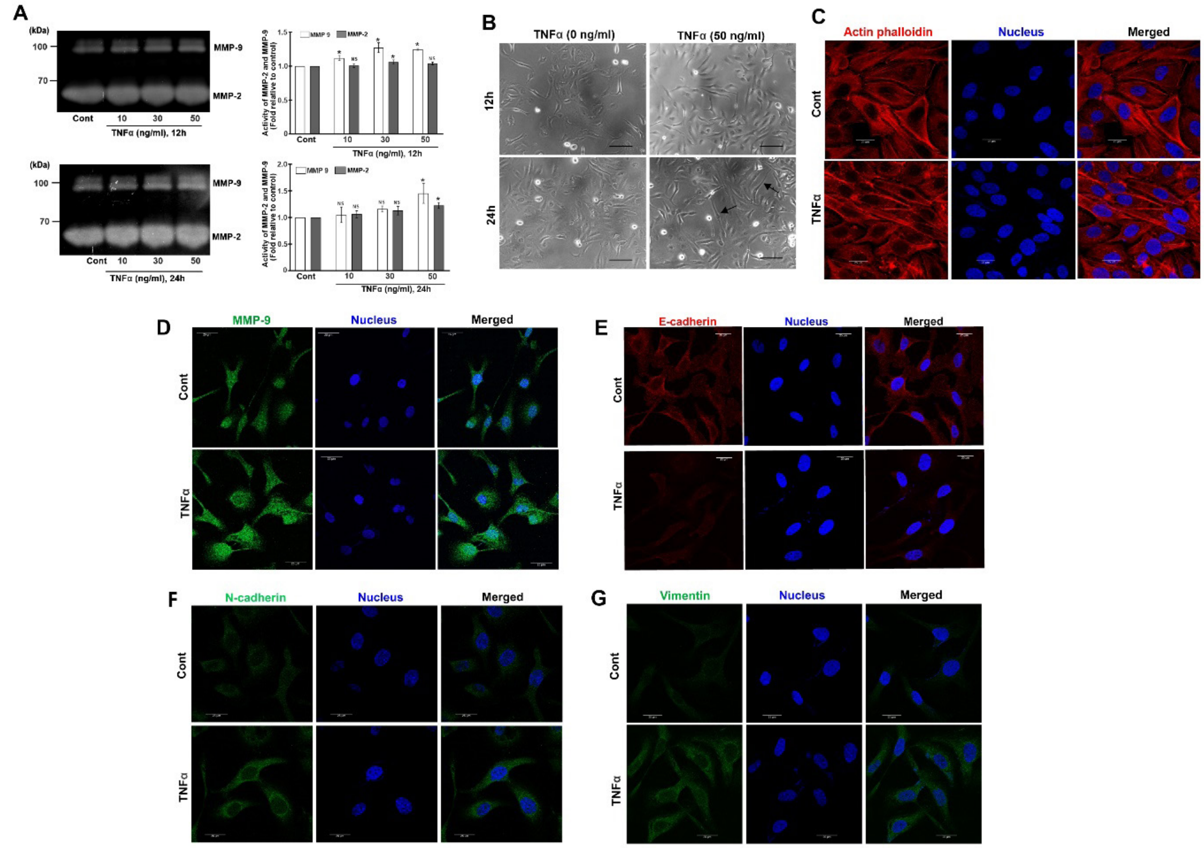


Figure 3: Recombinant human TNF-α induces EMT and expression of MMP-9 in SKOV-3 cell line A. Zelatin zymography of MMP-9 activity from rhTNF-α-treated cell conditioned media for 12h and 24h at 10 ng, 30 ng, and 50 ng. B. Phase contrast images for cell morphology at 50 ng of rhTNF-α treatment compared with control. C. Immunofluorescence study for Phalloidin, D. MMP-9, E. E-Cadherin, F. N-cadherin, G. Vimentin for rhTNF-α treatment (50 ng/ml) for 24 hrs in OC epithelial cells. The third panel shows the merged images. Means ± SD. $p<0.05$ was accepted as the level of significance

molecules, growth factors, and matrix metalloproteinases like MMP-9, which modify the extracellular environment and affect recipient cell behavior. Proteomic analyses of exosomes isolated from the serum, ascitic fluid, and tumor cells of OC patients have shown a consistent enrichment of proteins involved in EMT, immune evasion, angiogenesis, and ECM remodeling. For instance, exosomal proteins such as transforming growth factor beta (TGF-β1), CD44, fibronectin, integrins, and MMP family members have been demonstrated to promote tumor invasiveness and the formation of metastatic niches.[33,34] Tumor-derived exosomes carry TGF-β, which enhances Tregs activity and inhibits NK cell cytotoxicity, thereby contributing to an immunosuppressive microenvironment.[35] In this context, the regulatory roles of exosomes are evident in the TME, as they carry cytokines that can induce inflammation, which in turn supports the metastatic process of cancer cells. Proteomic analyses have shown that exosomes transport TGF-β, TNF-α, interleukin (IL)-6, β-catenin, and even MMPs to promote EMT in cancer.[36,37] The presence of tumor-promoting proteins in exosomes enables the functional reprogramming of surrounding non-cancerous cells, including fibroblasts, mesothelial cells, and immune cells, to establish a pro-tumorigenic microenvironment.[38] Additionally, exosomal protein signatures have been proposed as non-invasive biomarkers for disease staging, chemoresistance, and recurrence in OC. This protein-rich exosomal cargo reflects the dynamic state of the tumor and offers insight into intracellular signaling and pathological remodeling, highlighting the functional importance of exosome-mediated protein transfer beyond the traditional role of nucleic acid transport.[39,40]

Exosomal MMP-9 is increasingly recognized as a multifunctional mediator of pathological remodeling across diverse disease contexts, reinforcing its emerging role in OC progression. In cancers such as breast, prostate, and glioma, MMP-9-enriched exosomes facilitate metastatic spread by degrading the ECM and enabling recipient cells to acquire invasive traits. These exosomes not only mediate direct ECM degradation but also induce a pro-inflammatory phenotype in stromal cells, enhancing tumor-stroma communication.[38] Beyond oncology, MMP-9-containing exosomes have been implicated in neuroinflammatory and neurodegenerative diseases. For instance, in multiple sclerosis (MS), activated microglia release MMP-9 rich exosomes

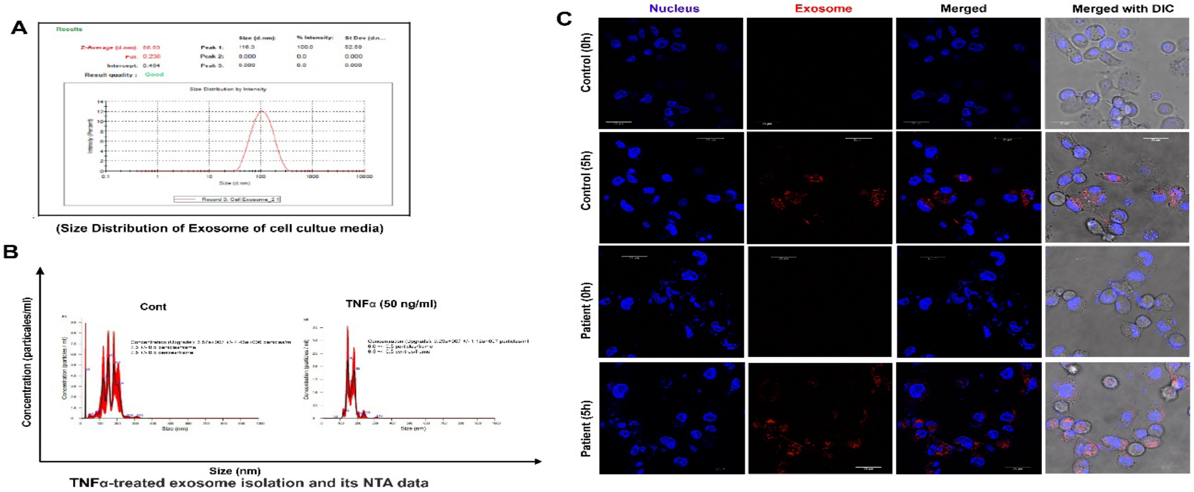


Figure 4: TNF-alpha upregulates exosomal MMP-9 secretion and aggravates metastatic phenotype in SKOV-3 and patients with OC A, B. Exosome characterisation by DLS and NTA for SKOV-3 cell line treated cell conditioned media derived exosome. C. Confocal microscopy for exosomes stained with PKH-26 and treatment at 0 and 5 hours in PMA-activated THP-1 cell line.

that contribute to blood-brain barrier disruption and local inflammation. Similarly, elevated exosomal MMP-9 levels in the cerebrospinal fluid of Alzheimer's patients are associated with synaptic remodeling and neuronal degeneration.[41,42] In cardiovascular conditions, exosomes from ischemic cardiomyocytes and macrophages in atherosclerotic plaques carry MMP-9, promoting adverse remodeling and plaque instability, respectively.[43] In inflammatory diseases such as rheumatoid arthritis, synovial fibroblast-derived exosomes loaded with MMP-9 exacerbate cartilage degradation and joint inflammation.[44,45] Environmental exposures also modulate MMP-9 exosomal content; bronchial epithelial cells exposed to cigarette smoke secrete exosomes enriched in MMP-9, contributing to ECM breakdown in chronic pulmonary conditions.[46] Collectively, these findings underscore that exosomal MMP-9 is a common effector in tissue remodeling and inflammation, supporting its relevance in the altered TME of late-stage, TNF-α rich OC.

Our findings demonstrate that TNF-α stimulation enhances MMP-9 activity in the culture supernatant and increases exosome production in SKOV-3 OC cells. In parallel, OC patient samples revealed elevated TNF-α levels in both serum and tumor tissue, along with increased exosomal MMP-9 content. These observations suggest that chronic inflammatory signaling in the TME may contribute to sustained exosomal MMP-9 release *in- vivo*.

While we did not directly measure MMP-9 activity within exosomes derived from TNF-α treated SKOV-3 cells, the overall increase in MMP-9 secretion and exosome number under these conditions raises the possibility that TNF-α could also modulate exosomal MMP-9 content and/or activity. It is conceivable that prolonged exposure to TNF-α, as seen in the TME, facilitates both the production and selective packaging of MMP-9 into exosomes. This remains a testable hypothesis for future studies.

These findings have several implications. First, exosomal MMP-9 may contribute to tumor progression not only through its classical role in ECM degradation but also by acting as a signaling molecule that promotes EMT and primes pre-metastatic niches. Second, the observed TNF-α driven changes in exosome biogenesis and MMP-9 secretion in p53-deficient SKOV-3 cells may reflect a tumor-specific adaptation to inflammatory stress, potentially exploitable for biomarker development or therapeutic targeting.

In summary, our study supports a model in which TNF-α enhances MMP-9 secretion and exosome production in OC, with potential implications for exosome-mediated tumor-stroma communication and metastasis. Future investigations are needed to determine whether TNF-α directly influences the activity and functional impact of exosomal MMP-9 in this context.

Overall, our study underscores the critical role of TNFα-induced MMP-9 expression and its exosomal trafficking in driving EMT and poor prognosis in late-stage, p53-mutant OC. The levels of TNF-α and MMP-9 were also higher in the serum and tissues, indicating that the exosomes in late-stage OC patients are oncogenic, influencing the inflammatory atmosphere and fueling EMT and angiogenesis by degrading connective tissue through MMP-9 actions. Targeting this pathway may offer a dual strategy to halt tumor progression and modulate the immunosuppressive TME.

CONCLUSION

TNFα increases MMP-9 secretion and exosome production in OC cells, with patient data indicating a role for exosomal MMP-9 in disease progression. Although exosomal MMP-9 activity after TNFα treatment has not yet been directly tested, our findings support a model in which TNFα-driven inflammation may promote exosome-mediated EMT and immune modulation. This emphasizes the potential of targeting exosomal pathways in OC therapy.

AUTHOR'S CONTRIBUTION

Susmita Saha - performed conceptualization (supporting), formal analysis, investigation, methodology, validation, visualization, statistical analysis, writing original draft, reviewing, and editing;
Sraddhya Roy - formal analysis, investigation, methodology, validation, visualization, statistical analysis, writing original draft, reviewing, and editing.
Dr. Nabanita Chatterjee- performed conceptualization (supporting), formal analysis, investigation, methodology, validation, visualization, statistical analysis, writing original draft, reviewing, and editing
Dr. Snehasikta Swarnakar conceptualised (lead), supervised (lead), reviewed, and edited (supporting) the paper. All the authors read and approved the final version.

ACKNOWLEDGMENT

This study was supported by the Institutional grant No. P-07 from CSIR-Indian Institute of Chemical Biology, Kolkata, India. Susmita Saha is thankful to the University Grants Commission (UGC), India, for providing financial support. We acknowledge the Director, CSIR-IICB, for his kind support.

DATA AVAILABILITY STATEMENT

All data presented in the article is available to the author and can be provided upon reasonable request.

REFERENCES

1. 1. Filho AM, Laversanne M, Ferlay J, *et al*. The GLOBOCAN 2022 cancer estimates: Data sources, methods, and a snapshot of the cancer burden worldwide. *Int J Cancer*. 2025;156(7):1336-46. DOI: 10.1002/ijc.35278.
2. 2. Chaturvedi M, Krishnan S, Das P, *et al*. Descriptive epidemiology of ovarian cancers in India: A report from the National Cancer Registry Programme. 2023;21(1):25. Available at: https://link.springer.com/article/10.1007/s40944-022-00694-1.
3. 3. Shih Ie M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. *Am J Pathol*. 2004;164(5):1511-8. DOI: 10.1016/s0002-9440(10)63708-x.
4. 4. Singer G, Stohr R, Cope L, *et al*. Patterns of p53 mutations separate ovarian serous borderline tumors and low- and high-grade carcinomas and provide support for a new model of ovarian carcinogenesis: a mutational analysis with immunohistochemical correlation. *Am J Surg Pathol*. 2005;29(2):218-24. DOI: 10.1097/01.pas.0000146025.91953.8d.
5. 5. Lengyel E. Ovarian cancer development and metastasis. *Am J Pathol*. 2010;177(3):1053-64. DOI: 10.2353/ajpath.2010.100105.
6. 6. Quintero-Fabian S, Arreola R, Becerril-Villanueva E, *et al*. Role of Matrix Metalloproteinases in Angiogenesis and Cancer. *Front Oncol*. 2019;9:1370. DOI: 10.3389/fonc.2019.01370.
7. 7. Jiang H, Li H. Prognostic values of tumoral MMP2 and MMP9 overexpression in breast cancer: a systematic review and meta-analysis. *BMC Cancer*. 2021;21(1):149. DOI: 10.1186/s12885-021-07860-2.
8. 8. Balkwill F. Tumour necrosis factor and cancer. *Nat Rev Cancer*. 2009;9(5):361-71. DOI: 10.1038/nrc2628.
9. 9. Charles KA, Kulbe H, Soper R, *et al*. The tumor-promoting actions of TNF-alpha involve TNFR1 and IL-17 in ovarian cancer in mice and humans. *J Clin Invest*. 2009;119(10):3011-23. DOI: 10.1172/JCI39065 .
10. 10. Kulbe H, Thompson R, Wilson JL, *et al*. The inflammatory cytokine tumor necrosis factor-alpha generates an autocrine tumor-promoting network in epithelial ovarian cancer cells. *Cancer Res*. 2007;67(2):585-92. DOI: 10.1158/0008-5472.CAN-06-2941.
11. 11. Kern SE, Pietenpol JA, Thiagalingam S, *et al*. Oncogenic forms of p53 inhibit p53-regulated gene expression. *Science*. 1992;256(5058):827-30. DOI: 10.1126/science.1589764.
12. 12. Zhu Y, Wang C, Ma Z, *et al*. Role of Exosomes in the Invasion and Metastasis of Ovarian Cancer and Application Potential of Clinical Diagnosis and Treatment. *J Cancer*. 2023;14(7):1141-50. DOI: 10.7150/jca.83663.
13. 13. Jeppesen DK, Fenix AM, Franklin JL, *et al*. Reassessment of Exosome Composition. *Cell*. 2019;177(2):428-45 e18. DOI: 10.1016/j.cell.2019.02.029.
14. 14. Aggarwal BB, Gupta SC, Kim JH. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood*. 2012;119(3):651-65. DOI: 10.1182/blood-2011-04-325225.
15. 15. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death Differ*. 2003;10(1):45-65. DOI: 10.1038/sj.cdd.4401189.
16. 16. Wu Y, Zhou BP. TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. *Br J Cancer*. 2010;102(4):639-44. DOI: 10.1038/sj.bjc.6605530.
17. 17. Pritchard A, Tousif S, Wang Y, *et al*. Lung Tumor Cell-Derived Exosomes Promote M2 Macrophage Polarization. *Cells*. 2020;9(5) DOI: 10.3390/cells9051303.
18. 18. Xu C, Chen J, Tan M, *et al*. The role of macrophage polarization in ovarian cancer: from molecular mechanism to therapeutic potentials. *Front Immunol*. 2025;16:1543096. DOI: 10.3389/fimmu.2025.1543096.
19. 19. Jana S, Chatterjee K, Ray AK, *et al*. Regulation of matrix metalloproteinase-2 activity by COX-2-PGE2-pAKT axis promotes angiogenesis in endometriosis. *PLoS One*. 2016;11(10):e0163540. DOI: 10.1371/journal.pone.0163540 .
20. 20. Chatterjee A, Roy T, Kumar Mishra V, *et al*. Shatavarin-IV, a steroidal saponin from Asparagus racemosus, inhibits cell cycle progression and epithelial-to-mesenchymal transition in AGS cells under hyperglycemic conditions. *Steroids*. 2024;210:109487. DOI: 10.1016/j.steroids.2024.109487.
21. 21. Chatterjee A, Roy T, Swarnakar S. Transcriptional upregulation of MMP-9 gene under hyperglycemic conditions in AGS cells: Role of AP-1 transcription factor. *Cell Signal*. 2024;124:111435. DOI: 10.1016/j.cellsig.2024.111435.
22. 22. Ghosh S, Hom Choudhury S, Mukherjee K, *et al*. HuR-miRNA complex activates RAS GTPase RalA to facilitate endosome targeting and extracellular export of miRNAs. *J Biol Chem*. 2024;300(3):105750. DOI: 10.1016/j.jbc.2024.105750.
23. 23. Khushman M, Bhardwaj A, Patel GK, *et al*. Exosomal markers (CD63 and CD9) expression pattern using immunohistochemistry in resected malignant and non-malignant pancreatic specimens. *Pancreas*. 2017;46(6):782-88. DOI: 10.1097/MPA.0000000000000847.
24. 24. Roy S, Das A, Vernekar M, *et al*. Understanding the correlation between metabolic regulator SIRT1 and exosomes with CA-125

in ovarian cancer: A clinicopathological study. *Biomed Res Int*. 2022;2022:5346091. DOI: 10.1155/2022/5346091.

25. 25. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: A review. *Cancer Biol Med*. 2017;14(1):9-32. DOI: 10.20892/j.issn.2095-3941.2016.0084.
26. 26. Moore RG, MacLaughlan S, Bast RC, Jr. Current state of biomarker development for clinical application in epithelial ovarian cancer. *Gynecol Oncol*. 2010;116(2):240-5. DOI: 10.1016/j.ygyno.2009.09.041.
27. 27. Felder M, Kapur A, Gonzalez-Bosquet J, *et al.* MUC16 (CA125): tumor biomarker to cancer therapy, a work in progress. *Mol Cancer*. 2014;13:129. DOI: 10.1186/1476-4598-13-129.
28. 28. Kulbe H, Chakravarty P, Leinster DA, *et al*. A dynamic inflammatory cytokine network in the human ovarian cancer microenvironment. *Cancer Res*. 2012;72(1):66-75. DOI: 10.1158/0008-5472.CAN-11-2178.
29. 29. Naylor MS, Stamp GW, Foulkes WD, *et al.* Tumor necrosis factor and its receptors in human ovarian cancer. Potential role in disease progression. *J Clin Invest*. 1993;91(5):2194-206. DOI: 10.1172/JCI116446.
30. 30. Szlosarek PW, Grimshaw MJ, Kulbe H, *et al*. Expression and regulation of tumor necrosis factor alpha in normal and malignant ovarian epithelium. *Mol Cancer Ther*. 2006;5(2):382-90. DOI: 10.1158/1535-7163.MCT-05-0303.
31. 31. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell*. 2010;141(1):52-67. DOI: 10.1016/j.cell.2010.03.015.
32. 32. Li X, Liu Y, Zheng S, *et al.* Role of exosomes in the immune microenvironment of ovarian cancer. *Oncol Lett*. 2021;21(5):377. DOI: 10.3892/ol.2021.12638 .
33. 33. Peinado H, Zhang H, Matei IR, *et al*. Pre-metastatic niches: Organ-specific homes for metastases. *Nat Rev Cancer*. 2017;17(5):302-17. DOI: 10.1038/nrc.2017.6.
34. 34. Becker A, Thakur BK, Weiss JM, *et al*. Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. *Cancer Cell*. 2016;30(6):836-48. DOI: 10.1016/j.ccell.2016.10.009.
35. 35. Berchem G, Noman MZ, Bosseler M, *et al*. Hypoxic tumor-derived microvesicles negatively regulate NK cell function by a mechanism involving TGF-beta and miR23a transfer. *Oncoimmunology*. 2016;5(4):e1062968. DOI: 10.1080/2162402X.2015.1062968.
36. 36. Vella LJ. The emerging role of exosomes in epithelial-mesenchymal-transition in cancer. *Front Oncol*. 2014;4:361. DOI: 10.3389/fonc.2014.00361.
37. 37. Harris DA, Patel SH, Gucek M, *et al.* Exosomes released from breast cancer carcinomas stimulate cell movement. *PLoS One*. 2015;10(3):e0117495. DOI: 10.1371/journal.pone.0117495.
38. 38. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367(6478) DOI: 10.1126/science.aau6977.
39. 39. Li X, Wang X. The emerging roles and therapeutic potential of exosomes in epithelial ovarian cancer. *Mol Cancer*. 2017;16(1):92. DOI: 10.1186/s12943-017-0659-y.
40. 40. Szajnik M, Derbis M, Lach M, *et al*. Exosomes in plasma of patients with ovarian carcinoma: Potential biomarkers of tumor progression and response to therapy. *Gynecol Obstet*. (Sunnyvale) 2013;Suppl 4:3. DOI: 10.4172/2161-0932.S4-003.
41. 41. Behl T, Kaur G, Sehgal A, *et al*. Multifaceted role of matrix metalloproteinases in neurodegenerative diseases: Pathophysiological and therapeutic perspectives. *Int J Mol Sci*. 2021;22(3) DOI: 10.3390/ijms22031413.
42. 42. Cabrera-Pastor A. Extracellular vesicles as mediators of neuroinflammation in intercellular and inter-organ crosstalk. *Int J Mol Sci*. 2024;25(13) DOI: 10.3390/ijms25137041.
43. 43. Barile L, Vassalli G. Exosomes: Therapy delivery tools and biomarkers of diseases. *Pharmacol Ther*. 2017;174:63-78. DOI: 10.1016/j.pharmthera.2017.02.020.
44. 44. Zhang HG, Liu C, Su K, *et al*. A membrane form of TNF-alpha presented by exosomes delays T cell activation-induced cell death. *J Immunol*. 2006;176(12):7385-93. DOI: 10.4049/jimmunol.176.12.7385.
45. 45. Zhang S, Duan Z, Liu F, *et al*. The impact of exosomes derived from distinct sources on rheumatoid arthritis. *Front Immunol*. 2023;14:1240747. DOI: 10.3389/fimmu.2023.1240747.
46. 46. Obernolte H, Niehof M, Braubach P, *et al*. Cigarette smoke alters inflammatory genes and the extracellular matrix - investigations on viable sections of peripheral human lungs. *Cell Tissue Res*. 2022;387(2):249-60. DOI: 10.1007/s00441-021-03553-1.

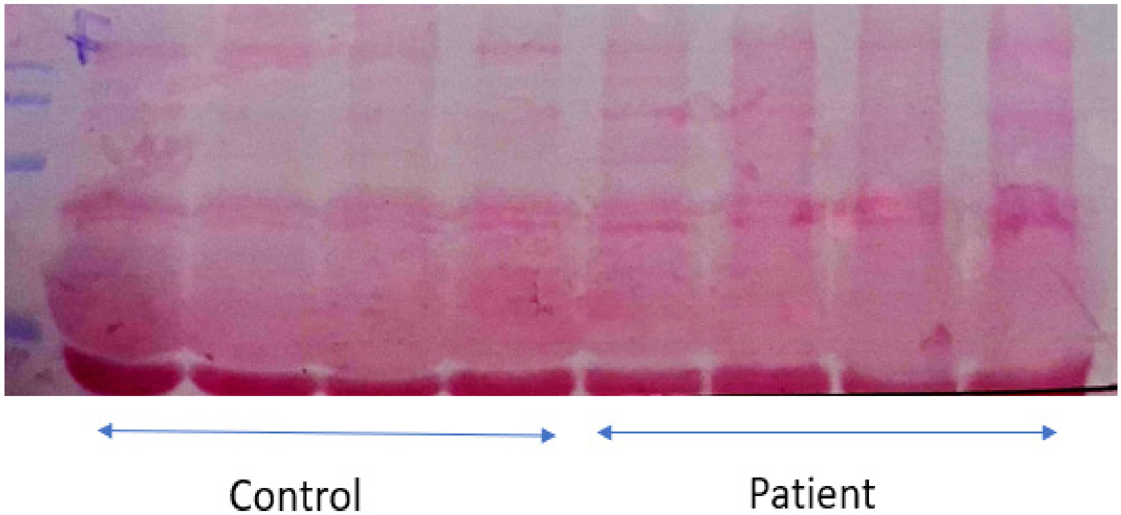
SUPPLEMENTARY

Supplementary Table S1: Frequency distribution of clinicopathological parameters

Clinicopathological parameters		*Number (%)*	*Clinicopathological parameters*		*Number (%)*
Age	20-40	6 (13.6)	Metastasis	Yes	42 (95.5)
	41-60	22 (50.0)		No	2 (4.5)
	>60	16 (36.4)	Stage	Stage I	0 (0.0)
CA-125 (U/mL)	<=35	2 (4.5)		Stage II	10 (22.7)
	35.1-200	13 (29.5)		Stage III	19 (43.2)
	200.1-500	20 (45.5)		Stage IV	15 (34,1)
	>=500	9 (20.5)	Laterality	Unilateral	42 (95.5)
Ascites malignancy	Yes	42 (95.5)		Bilateral	2 (4.5)
			Chemotherapy	Yes	0 (0.0)
	No	2 (4.5)		No	44 (100.0)

Supplementary Table S2: List of antibodies used in this study

Antibody	Catalogue No.	Company
Anti-MMP-9	Sc-393859	Santa Cruz Biotechnology
Anti-GAPDH	sc-47724	Santa Cruz Biotechnology
Anti-CD-63	NBP2-32830	Novus Biologicals
Anti-TNF-α	CST #3707	Cell Signalling Technology
Anti-E-cadherin	CST#3195	Cell Signalling Technology
Anti-N-cadherin	CST#13116	Cell Signalling Technology
Anti- Vimentin	CST#5741	Cell Signalling Technology
Anti- TSG101	A2216	ABclonal
Anti- actin Phalloidin	Ab176757	Abcam
Alexa-fluor 647	A-21235	Ivitrogen
Alexa Fluor 488	A11034	Invitrogen
Anti-Mouse IgG, HRP-linked Antibody	CST#7076	Cell Signalling Technology
Anti-Rabbit IgG, HRP-linked Antibody	CST#7074	Cell Signalling Technology



Supplementary Figure 1: Ponceau S staining of control and patient with ovarian cancer

PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.