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Original Article

Ultrasound coupled RES-loaded ultrasound microbubble inhibits the proliferation of ovarian cancer cells by expression of long non-coding RNA (lncRNA) involved in apoptosis using real-time PCR

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Abstract: The primary objective of this study was to evaluate the effect of low-frequency ultrasound combined with RES-loaded ultrasound microbubble contrast agents on the transcriptional and translational activities of ovarian cancer cells. After being cultures, ovarian cancer cells (OVCAR-3) and human umbilical cord endothelial cells (HUCEC) were transfected with siRNA, which was followed by RNA extraction and real-time PCR to evaluate transcriptional activity. Translational activity was determined by western blotting, which was followed by RNA interference. Proliferative and invasive activity was measured using cell proliferation, colony formation, and immunofluorescence assays. Lastly, RNA sequencing was performed. Our findings indicated that ultrasound combined with RES microbubbles inhibited cell proliferation and invasion. The expression of ING5 was enhanced, while the expression of EMT was suppressed in ovarian cancer cells. A negative correlation was observed between of the expression of ING5 and cell proliferation/migration, which were enhanced upon inhibition of ING5, suggesting dysregulation of transcriptional and translational cellular processes which could be of diagnostic and therapeutic value in ovarian cancer. Additionally, the dysregulation of lncRNAs can alter cellular homeostasis and promote ovarian cancer progression. A combination of low-frequency and RES-loaded ultrasound microbubbles was found to effectively inhibit the proliferation of OVCAR-3 ovarian cancer cells and induce apoptosis. This approach was more effective than low-frequency ultrasound combined with RES alone.

Keywords: Resveratrol, ovarian cancer, microbubble, apoptosis

Introduction

Ovarian cancer accounts for 12% of the global cancer incidence and 11% of cancer-related deaths. Each year, more than 529,800 ovarian cancer cases are identified with an annual death toll of approximately 275,100 [1]. Certain genes are associated with carcinogenesis and progression of ovarian cancer [2]. Resveratrol (RES) is a multifunctional compound [2]. Our previous study indicated that RES could inhibit ovarian cancer cell proliferation by down-regulating the NAF-1 signaling pathway [2]. Therefore, RES could be considered a candidate for the treatment of ovarian cancer. Considering many biological activities of RES, it is presumed that RES might activate different molecular pathways. As such, the present study aimed to explore the new molecular mechanism of Low-frequency ultrasound combined with RES-loaded microbubbles on ovarian cancer.

Long non-coding RNAs (lncRNAs) are functional molecules consisting of more than 200 nucleotides with significant roles in regulating pathological mechanisms such as development and progression of cancer [3]. lncRNAs increase cellular proliferation and migration in ovarian cancer, while limiting apoptosis [3]. For example, the HOX genes were found to be either up or down-regulated in ovarian cells resulting in overexpression or reduced expression of SOCs genes, which are associated with tumorigenesis [3]. Similarly, upregulation lncRNA SPRY4-IT1 has been proposed as a suitable prognostic

factor for determining overall and progression-free survival in ovarian cancer [3].

Nikpayam *et al*. discovered that numerous lncRNAs were deregulated in patients with ovarian cancer, suggesting a significant link between the expression profiles and overall survival [4]. For instance, down-regulation of AB073614 oncogene was reported to significantly improve the overall survival of ovarian cancer patients since it inhibited tumorigenesis [4]. AB073614 exerts its function through ERK ½ and PKB-enhanced pathways [4]. Vallino *et al*. suggested that lncRNAs might become subject to regulation by RES in cancer cells, which may affect gene transcription and translation [5]. Dysregulation of lncRNAs can alter cellular homeostasis and accelerate the progression of ovarian cancer [5]. The up-regulation of lncRNAs through RES was characterized by clustered alterations in different organelle-specific processes [5]. Therefore, RES might have a significant potential for inhibiting the growth and development of ovarian cancer. Also, no toxicity or adverse side effects were reported to ensue application of RES in ovarian cancer. In clinical practice, appropriate doses of RES should be administered based on gender, microbiomes, and hormone levels [5].

According to Li *et al*. RES is a phytoalexin with negative effects on development and proliferation ovarian cancer [6]. RES increases apoptosis, endoplasmic reticulum stress, and autophagosome formation depending on the dose administered [6, 7]. The expression levels of lncRNA H19 were altered in the presence of RES, which was found to limit tumorigenesis. Additionally, they observed that other lncRNA, such as GASS, MEG3, and MALAT1, were altered in cancer cells treated with RES. H19 was identified as an oncofoetal transcript whose increased expression levels altered tumorigenesis [6].

Similarly, Giordo et al. suggested that lncRNAs might have a significant role in the pathophysiology of various cancers and chronic diseases associated with production of inflammatory cytokines and oxidative stress [8]. They found that lncRNAs significantly affected the biochemical pathways and regulated homeostasis in ovarian cancer [8]. Natural compounds such as RES may result in anti-inflammatory effects by targeting numerous lncRNAs and altering their expression levels, which could be of potential significance in the context of cancer.

In this study, we evaluated the effect of low-frequency ultrasound combined with RES-loaded ultrasound microbubble contrast agents on transcriptional and translational activity of ovarian cancer cells. The effects of low-frequency ultrasound combined with RES, along with low-frequency ultrasound or RES alone were also analyzed.

Materials and methods

Cell culture and transfection

Human umbilical cord endothelial cells (HUCEC) and OVCAR-3 ovarian cancer cell lines were provided by the Chinese Academy of Sciences Type Culture Collection Center (Shanghai, China). These cells were cultured in Dulbecco's modified eagle medium (Gibco DMEM, USA) supplemented with 10% fetal bovine serum (US HyClone), 10 mg/ml antibiotics (penicillin and streptomycin), and L-glutamine at a temperature of 37°C under an atmosphere of 5% carbon dioxide and saturated humidity.

RNA extraction and RT-qPCR

RNA extraction was performed using an RNA extraction kit (Qiagen, Hilden, Germany) following the instructions provided by the manufacturer. After DNase treatment, cDNA was synthesized using HiScript II Q RT SuperMix (R223-01, Vazyme Biotech). To determine messenger RNA (mRNA) expression, SYBR Green was used in quantitative real-time PCR (RT-PCR). The volume of the resulting RNA copies was measured using the $2^{\Delta\Delta Ct}$ method, with GAPDH as the control compound. All primers used in the study were obtained from Tsingke Biological Technology based in Beijing, China.

Western blotting

The cells were rinsed with cold PBS for three times. Afterwards, the cell lysate was placed on ice for 30 minutes and centrifuged with a velocity of 3500 rpm for 3 hours at 4°C. The resulting supernatant was extracted as total protein. Frozen tissues were first homogenized and centrifugated at 4°C for 10 minutes, which was followed by collection of the resulting supernatant. The sample protein concentration was determined using bicinchoninic acid (BCA) kit

(Sigma-Aldrich, Missouri, United States). The samples were mixed with loading buffer and boiled at 100°C for 5 minutes, then separated using SDS gel. The separated gelatin protein was transferred onto a PVD membrane, where the primary antibody was incubated at 4°C overnight. The following day, the membrane was washed with anti-skimmed milk powder for 60 min before being incubated with the labeled secondary antibody, which was then washed off after an hour. Detection and quantification of western blots were performed using the electrochemiluminescence (ECL) method and the computerized densitometry-based image analysis program (Amercontrol Biosciences, USA), respectively.

RNA interference

Small interfering RNAs (siRNA) targeting WTAP, BRCA1, and negative control RNAs (siNC) were purchased from GenePharma (Shanghai, China). Following the standard protocol, transfection was carried out using the jetPRIME kit (Polyplus Transfection, France). After 48 hours, cells were collected for qRT-PCR, while western blot analysis and functional study were conducted 72 hours later.

Cell proliferation assay

Cells undergoing logarithmic growth phase were seeded into a 96-well plate at a concentration of 5×10^4 cells per mL. After successful transfection, the cells were incubated for 0, 12, 24, 36, and 48 hours before being exposed to 20 μl of CCK-8 reagent. The readings were then detected at 450 nm using the Synergy H4 Hybrid microplate reader (BioTek, Vermont, US).

Colony formation assay

After being transfected with siRNAs, cells were seeded into a 6-well plate (200 cells per well) and allowed to be cultured for 15 days. These cells were then treated with 10% paraformaldehyde for 10 minutes and stained with 0.1% crystal violet for 15 minutes. Finally, the formed colonies was counted under a microscope.

RNA sequencing

Total RNA extraction was performed using the ESscience RNA-Quick Purification Kit (Yishan Biotech, Shanghai, China). The extracted RNA molecules were then sequenced with Illumina NovaSeq 6000 platform (Illumina, USA), with the resulting data being used for construction of an RNA library, as well as computational analysis. Differentially expressed genes were identified based on *P*-value < 0.01 and fold-change > 1.5 or < 0.5. Genes with a fold-change greater than 1.5 were considered up-regulated, while those with a fold-change below 0.5 were considered down-regulated. The RNA-sequencing data was used to analyze the expression levels of ING5 and EMT in the control and RES samples.

Immunofluorescence assay

Immunofluorescence assay involved embedding fixed tissues in paraffin, which were then washed with water and alcohol to remove the wax. Sodium citrate was used to restore antigens, after which the tissues were exposed to primary antibodies against WTAP (Abcam, 1:100) and BRCA1 (Abcam, 1:200) at 4°C, overnight. The tissues were treated with secondary antibodies at room temperature for 30 minutes, and the nuclei were stained with DAPI. Finally, confocal microscopy (Olympus, Japan) was used to capture immunofluorescence images.

Statistical analysis

Data analysis was conducted using GraphPad Prism version 9.5.1. The statistical data between the two groups were compared using an unpaired Student's t-test. Multiple comparisons were made using a one-way ANOVA with Bonferroni's correction. The in vitro experiments were carried out a minimum of three times, and the data were presented as the means ± standard deviations (SD) from at least three independent experiments. A *P*-values ≤ 0.05 was considered the threshold of significance.

Results

Ultrasound combined with resveratrol microbubbles inhibits ovarian cancer cell proliferation and invasion

To investigate whether RES attenuated proliferation of ovarian cancer cells, the cells were treated with different concentrations of RES (control, 1, 10, 20, 30, 40, 50, 60, 80, and 100 ug/ml) for 12, 24, 36, 48 h. Treatment with RES for 48 h resulted in dose-dependent suppres-

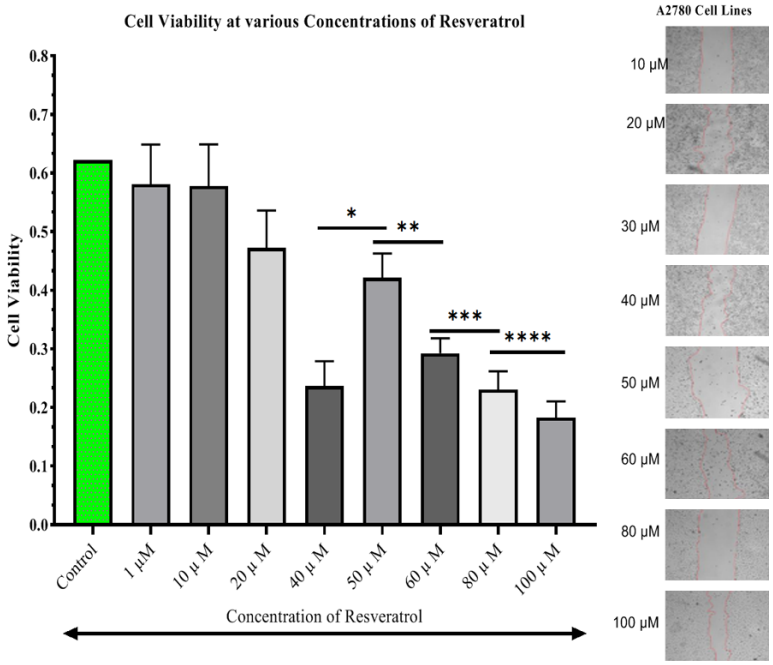

Figure 1. The concentration of resveratrol in A2780 ovarian cancer cell lines.

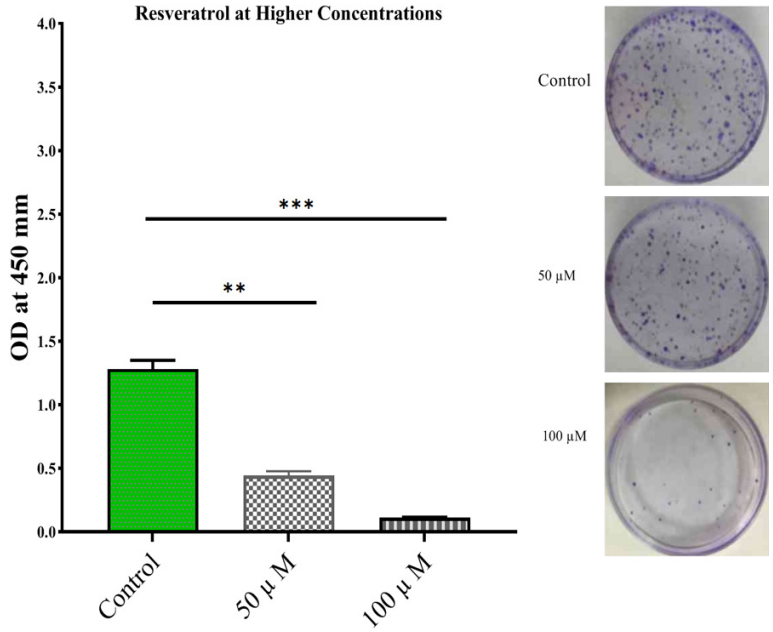

Figure 2. The action of resveratrol at higher concentrations compared to the controls.

sion of cellular proliferation, with a dose of 40 µg/ml inducing apoptosis in OVCAR-3 cells (**Figure 1**). Apoptosis was positively correlated with the concentration of RES. The viability of all ovarian cancer cell lines was significantly reduced with an increase in the dose of RES.

Ultrasound combined with resveratrol microbubbles suppresses ovarian cancer cell migration and invasion

Tumor invasion and metastasis is highly dependent on cell migration. Our results showed that RES conspicuously inhibited the migratory and invasive behavior of OVCAR-3 (**Figure 2**) at concentrations as high as 50 uM and 100 uM, rather than lower concentrations of 1 uM, 10 uM, and 20 uM. Our findings strongly suggested that RES could be an essential regulator of the invasion and migration of ovarian cancer cells.

Ultrasound combined with resveratrol microbubbles promotes the expression of ING5 and suppression of affected EMT in ovarian cancer cell lines

RNA sequencing confirmed the critical role of EMT in tumor invasion and metastasis. Tumor cells undergoing EMT exhibited enhanced viability. To determine whether the EMT-related proteins were affected by resveratrol, the levels of E-cadherin, N-cadherin, and transcription factor snail were evaluated in OVCAR-3 cells treated with or without RES. We found that E-cadherin was partially activated, and N-cadherin was partially inhibited. Immunofluorescence analysis of the cellular localization of ING5 returned a rather evenly distributed pattern in the cytosol. RES induced upregulation of cytosolic calcium ING5 in ovarian cancer cells (**Figures 3** and **4**). OVCAR-3 cells exhibited spindle-like mesenchymal morphology, while those treated with RES demonstrated an epithelial-like cobblestone appearance. This prompted us to conclude that RES changed cell

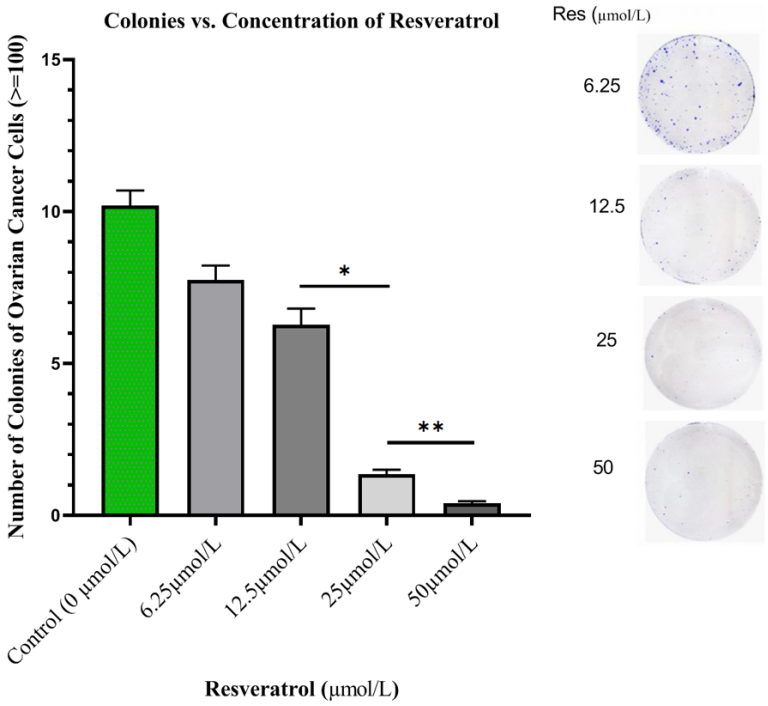

Figure 3. The number of colonies against resveratrol, control, and ING5.

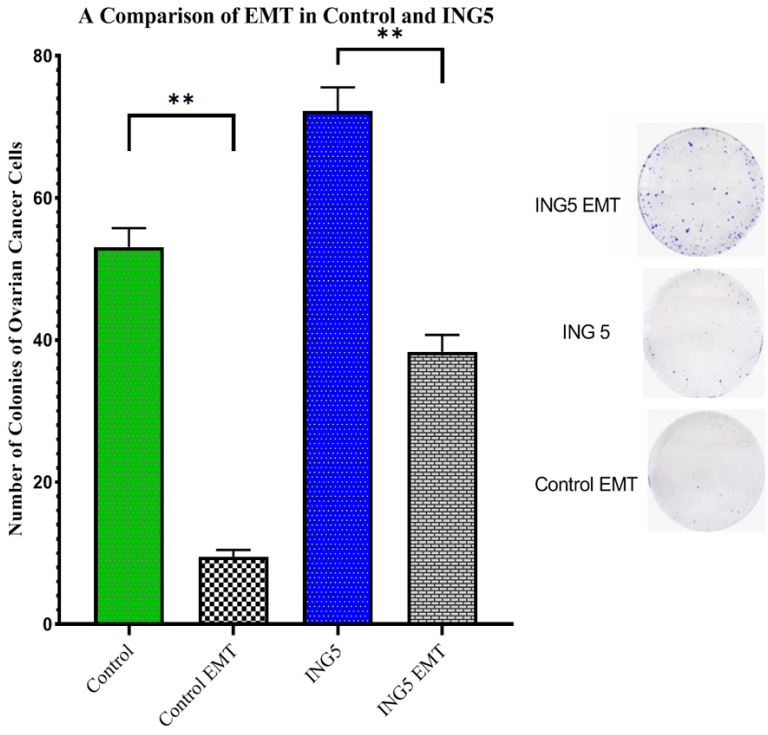

Figure 4. The number of colonies formed when EMT was introduced in the control and ING5 samples.

morphology and promoted the expression of ING5 protein.

Knockdown of ING5 promotes proliferation, migration, and invasion in ovarian cancer cells

To detect whether ING5 could regulate the proliferation, migration, and invasion in ovarian cancer cells, we used ING5 siRNA to inhibit ING5. RNA-sequencing showed that silencing of ING5 promoted cell proliferation (**Figure 5**). A scratch test was performed, confirming that ING5 knockdown increased cell migration, whereas trans-well assays verified that ING5 knockdown increased cell invasion. These observations supported the statement that knockdown of ING5 may promote the proliferation, migration, and invasion of ovarian cancer cells.

Discussion

Effects of ultrasound microbubble contrast agent on transcriptional and translational activities of ovarian cancer cells

The continuous development of microbubble contrast agents (**Figure 6**) and the application of ultrasound combined with microbubble contrast agents in malignant neoplasms have attracted significant attention [9, 10]. The development of ultrasound microbubble contrast agents has gone through three stages. The initial stage is characterized by formation of large and structurally unstable non-enveloped bubbles containing oxygen, which cannot be injected into a peripheral vein. The second stage involves formation of a layer of albumin wrapped with lipids. The air bubbles in the polymer shell show good stability in small

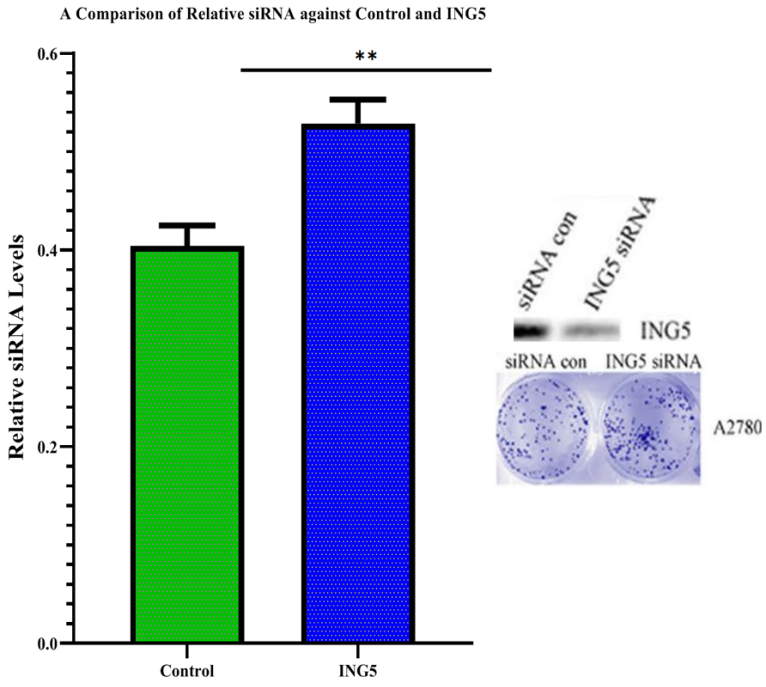

Figure 5. A comparison of the siRNA levels against control and ING5.

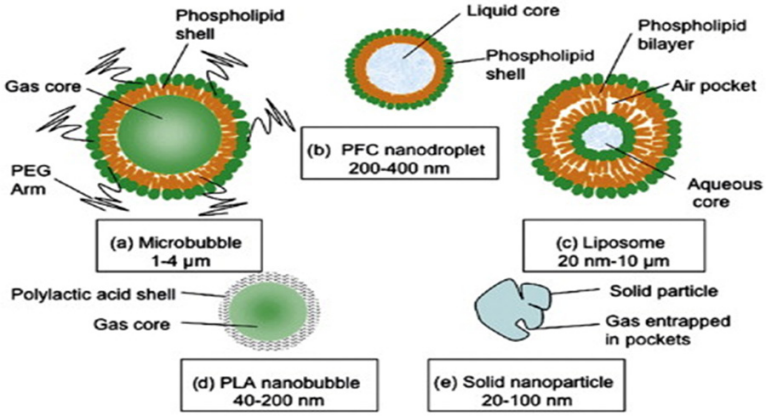

Figure 6. Illustration of micro and nanobubbles.

volumes, lasting only about 5 minutes in the blood. The third stage is characterized by formation of membrane-bound fluorocarbon-containing gas in the blood, which may last more than 15 minutes, owing to improved stability [9, 10]. The continuous innovation of microbubble contrast agents is the basis for ultrasound combined with microbubbles to be considered in adjuvant chemotherapy or targeted treatment of cancer.

Upon stimulation, microbubbles rupture and release the drug in the target tissue, which not only increases the local concentration of the drug, but also reduces the toxicity and side effects of chemotherapeutic drugs. At the same time, the mechanical energy generated by the microbubble contrast agent can further increase the permeability of the cell membrane and promote the entry of drug molecules into the cell through low-frequency ultrasound.

The therapeutic effect of combined microbubbles has been verified in various tumors. Wang *et al*. found that low-frequency ultrasound (< 1 MHz) combined with paclitaxel microbubble contrast agent-mediated drug release significantly inhibited the proliferation of cervical cancer (HeLa) cells and induced apoptosis [11]. Xu et al. found that low-frequency ultrasound irradiation of simvastatin microbubbles promoted the apoptosis of prostate cancer DU145 cells and enhanced their sensitivity to chemotherapeutic drugs by down-regulating the expression of Caveolin-1 protein [12]. It can be inferred that low-frequency ultrasound combined with microbubble contrast agents has broad applications in tumor treatment. However, it is still in the experimental stage, with numerous challenges remaining.

The results of this study confirms that low-frequency ultrasound combined with RES-loaded ultrasound microbubbles can inhibit the proliferation of OVCAR-3 cells and induce apoptosis. Its effect is stronger than low-frequency ultrasound combined with RES and low-frequency ultra-or-white cells. RES microbubble contrast agent has a diameter of 1-8 μm, containing a high-density inert gas and a single-layer film on the outside [9, 10]. It has good elasticity and

stability and has strong resistance to sound pressure. Studies have confirmed that low-frequency ultrasound can promote the periodicity of microbubble contrast agents [10].

The microbubbles intensify during the process of compression and expansion until cavitation [9], an event which may facilitate the rupture of microbubbles and formation of new microbubbles under low-frequency ultrasound. The drug being carried is released within the tissue, thereby increasing the local drug concentration. In the meantime, low-frequency ultrasound causes a transient gap in the cell membrane, which promotes the permeability of cell membrane, leading to the sonic hole effect [10]. This effect further promotes the uptake of drug molecules. Therefore, low-frequency ultrasound combined with microbubble contrast agents can increase the targeted utilization of drugs and reduce their toxicity and side effects.

The exogenous apoptotic pathway facilitates apoptosis by binding apoptosis-stimulating factors and related receptors on the cell surface, directly activating the downstream Caspase effector molecules [13, 14]. The endogenous apoptotic pathway is an apoptotic process activated by mitochondrial stress. The Bcl-2 protein family is responsible for regulating the mitochondrial-dependent apoptosis pathway. Bcl-2 apoptotic proteins can be divided into BH3-single domain and multi-domain protein groups according to their structure. BH3 single-domain proteins are inactive in the cytoplasm [14]. However, when transported to the mitochondria, they either activate the multi-domain proteins Bax and Bak or inactivate the Bcl-2 anti-apoptotic proteins Bcl-2 and Bcl-xL [13, 14]. This study showed that the expression of Caspase-3 and Bax protein in the ultrasound combined with the RES microbubble group increased, and the expression of Bcl-2 decreased. One possible explanation is that the ultrasound combined with the RES microbubble group affected the apoptotic rate of cell death.

Examination of ancestral tumor tissues based on data derived from transcriptional and translational activity of tumor cells

According to Lam et al., dysregulation of transcriptional and translational cellular processes is of significant value in diagnosing and treating ovarian cancer [15]. Pharmacological inhibitors such as cyclin-dependent kinases (CDKs) and Mnks are essential in controlling the transcription of RNA and the expression of proteins, respectively [15]. Hence, they can be targeted and used to develop therapeutic drugs. Numerous CDKs are associated with the progression of cellular cycles and the controlling of RNA transcription [15]. Therefore, they regulate homeostasis, cellular growth, and proliferation.

Moreover, inhibiting the activity of transcriptional CDKs is an efficient technique for suppression of tumorigenesis [15]. This is because ovarian cancer cells depend on the short-term production of regulators of mitotic and apoptotic processes such as Mcl-1, which increases their survival. The CDK7 derived from transcription factor II is responsible for the phosphorylation of serine-5 residues at the RNAPII C-terminal and consequently induces transcription [15]. In contrast, CDK9 derived from the catalytic subsection of P-TEFb is responsible for the phosphorylation of repressors such as DSIF and NELF and the serine 2 residue of C-terminal, which is associated with elongation of transcriptional processes [15].

Laham-Karam *et al*. observed that transcriptional and translational processes are important in regulating the synthesis of proteins [16]. Up-regulation of transcriptional and translational processes in ovarian cancer cells is critical for the maintenance of cell proliferation and metabolism. However, ovarian cancer cells are susceptible to the inhibitors of translation and transcription processes such as CDKs.

Transcription involves the synthesis of mRNA at the level of chromatin (**Figure 7**). In the epigenetic state, histone proteins are modified, and DNA methylation occurs [16]. The process is highly complex and involves numerous processes which can be classified into four steps; development of the pre-initiation complex, preceded by initiation, elongation, and termination [16].

In contrast, translation involves the production of polypeptides based on the specified RNA template (**Figure 8**). Translation involves numerous drugged protein targets and can be simplified into four stages; the initiation stage, followed by elongation, termination, and recycling

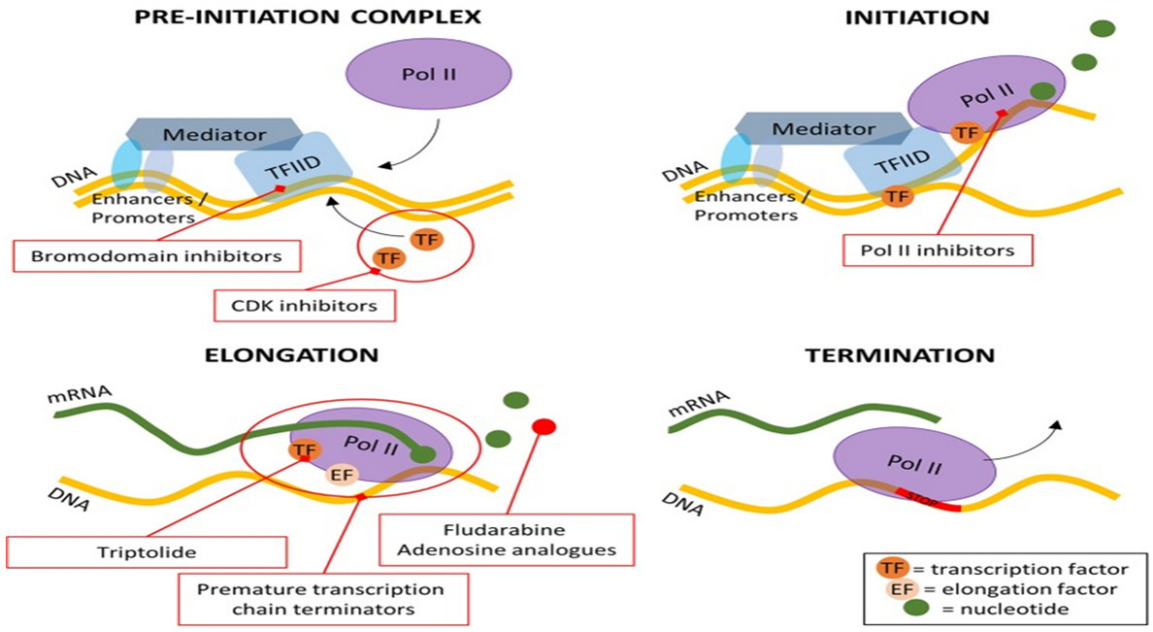

Figure 7. The process of transcription.

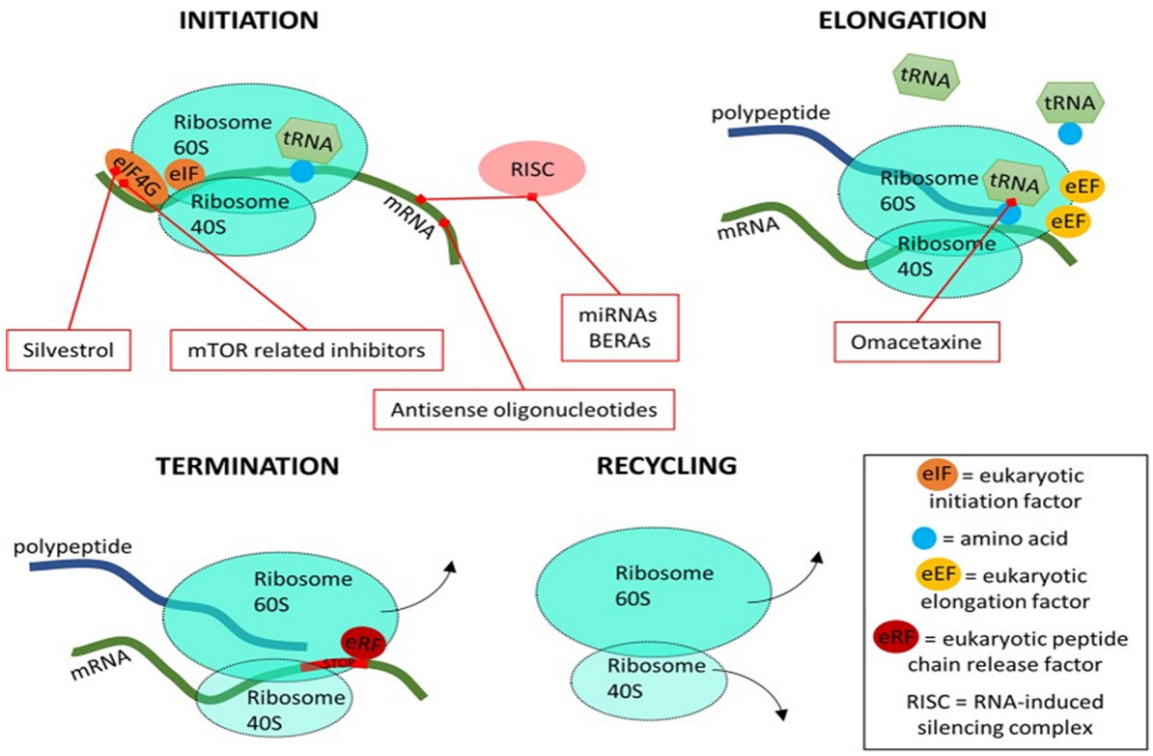

Figure 8. The process of translation.

[16]. In the initiation stage, the genes are bound to the transcription factor II D situated at the gene's promoter region. The larger pre-initiation complex is formed with numerous transcription factors, cofactors, mediators, and TFIIH [16]. The initiation is completed with a full transcription of about 30 nucleotides. In the elongation phase, the promoter is released, and the elongation factors are bound to the hyperphosphorylated Pol II C-terminal section to increase the

stability of the process. Lastly, the proteins alter the mRNA at their 3' end [16].

The initiation phase involves binding of the 60S ribosome onto the starting mRNA, after which it is transferred by the tRNA to the first codon AUG in the presence of eIFs [16]. In the elongation stage, the 60S ribosome is pushed along the template and binds with tRNA molecules and amino acids to produce the required polypeptide. The synthesis of polypeptides is enhanced by eEFs. The 60S ribosome then interacts with a termination codon and is identified by the eRFs. Lastly, the 80S ribosome complex subdivides into 40S and 60S to initiate translation [16].

According to Vaklavas *et al*., translational and transcriptional processes are regulated at the initiation stage; however, the synthesis of proteins can be controlled and enhanced during the elongation stage [17]. Translation is highly dysregulated in ovarian cancer cells and other types of tumors, which enhances the oncogenic signaling pathways, resulting in cellular transformation [17].

Computer aided techniques and drug discovery at various stages of ovarian cancer

Application of computer-aided diagnostics has significantly improved the prediction and diagnosis of ovarian cancer. According to Koch et al., computer-aided drug discovery (CADD) techniques are highly accurate predictive models that confer a low risk of bias [18]. CADD techniques can predict the stage of ovarian cancers ranging from benign to malignant [18]. Imaging modalities such as histological imaging, CT scans, MRI scans, ultrasound, or mammography are critical CADD techniques for diagnosing and treating cancer.

CADD begins with pre-processing images to eliminate the defects, noise and to resize the image with a suitable intensity [19]. Segmentation is based on discontinuity, where the image is partitioned based on sudden changes in its intensity, or similarity segmentation, where sections of an image are classified based on regions, thresholds, or clusters [19]. Feature extraction involves obtaining distinct characteristics from the malignant lesions and distinguishing between various malignancy levels. Lastly, the images are classified, and the diagnostics are obtained and evaluated [19].

According to Xu *et al*., administering RES through computer-aided techniques at various stages to ovarian cancer might increase the overall survival of patients, while improving the diagnosis and/or prognosis of ovarian cancer [20]. Additionally, incorporation of nanoparticles into CADD has led to discovery of various drugs, such as Abraxane and Doxil, which are effectove in treating early-stage ovarian cancer [20].

Development of 3D modelling platforms has allowed the simulation of the microenvironment of tumors and the molecular pathways involved in ovarian cancer [21]. 3D models are valuable in identifying cancer during early metastasis and angiogenesis [13]. Furthermore, 3D cultures are highly accurate in diagnosing and treating cancer. These models allow delivery of a personalized dose of drugs and screening to ensure the dose administered is effective based on gender, age, and cancer stage [22]. Additionally, 3D models facilitate deep analysis of cell-cell interactions or cell-ECM interactions, revealing detailed data on the drug response and cell proliferation [22].

Drug delivery systems overcome the limitations of chemotherapy, such as adverse side effects and unequal biodistribution [23]. Drug delivery at various stages of ovarian cancer, such as cisplatin, reduced tumorigenesis and increased the survival rate of patients. Furthermore, it inhibited cellular growth and proliferation which was critical in eliminating further growth of tumors [23].

Zou *et al*. proposed that epithelial ovarian cancer has a higher mortality rate and worse prognosis [24]. Therefore, the discovery of novel drugs at various stages of tumorigenesis is essential in increasing the survival rate compared to standard therapies. Differential analysis of expressed genes in epithelial ovarian cancer revealed 21 drugs, such as piperlongumine, with lower connectivity map scores and was regarded as a significant drug candidate in malignant tumors.

Network pharmacology of the natural small drug molecules as potent anti-tumor future drugs in ovarian cancer

Natural products such as RES affect mitosis and regulate cell differentiation, proliferation, growth, and development in cancer [25].

Similarly, other natural products such as taxol, Abraxane, practical, and cynology affect the microtubules by maintaining the stability of proteins. These small drug molecules inhibit the depolymerization of microtubules and cell mitosis [25]. The network pharmacology of RES starts with damaging the DNA while targeting the malignant tissues and cells without altering the normal tissues and cells. RES can be applied in other types of cancer, such as colon cancer, where it induces a DNA damage response (DDR) positively associated with the S-phase delay and apoptotic cell death. Moreover, prolonged exposure to these drug molecules increases the resistance stability of cells due to overproduction of reactive oxygen species.

Besides RES, other molecules such as sulforaphane, piperine, and camptothecin have cytotoxic activities such as the cell cycle arrest, regulation of epigenetic processes, and production of reactive oxygen species, which are critical in the destruction of DNA [25]. Therefore, these natural small drug molecules increase the expression levels of reactive oxygen species, leading to indirect damage of DNA molecules and prolonged adaptation of cancer cells to oxidation stress. In lymphoma cells, RES inhibits the S-phase by initiating DDR pathways through the up-regulation of Zta [25].

Advancements in computational methods in the diagnosis of ovarian cancer

Computational pathology increases the integration of clinical data and various tools to analyze, diagnose and control ovarian cancer empirically. For example, computational imaging facilitates rapid and precise collection of data compared to methods such as subvisual features, integrative quantitation, spatial data, and unbiased discovery [26]. Integrative quantitation is associated with computer algorithms that maintain a database of clinical outcomes associated with various grades of ovarian cancer to obtain similar phenotypes and predict possible outcomes [26]. Also, subvisual features can be analyzed using microscopes, which has led to discovery of relationships between subcellular features such as genotoxic stress, tension, or metabolic activities [26].

The adoption of artificial intelligence coupled with machine and deep learning, such as convoluted neural networks, has improved image analysis in ovarian cancer [26]. These networks can assist clinicians with decision-making and provide accurate outcomes based on cancer images. The networks use kernels to extract information from the cancer images; these kernels can be adjusted to filter data from low to high resolutions based on shapes, intensity, or location of various objects in the cancer lesions [26]. These kernels reorganize and pre-process this information to reveal new features.

Clinical applications

Our study suggests that RES, with a certain dosage, can be clinically applied for effective treatment and management of ovarian cancer. We also highlight potential clinical applications of therapeutic ultrasound combined with microbubble contrast agents. There is enormous potential in the use of low-frequency ultrasound combined with microbubble contrast agents in ovarian cancer and other tumors. Further research and experiments should be conducted to explore and optimize this approach in targeted chemotherapy or neoadjuvant chemotherapy. Additionally, the findings of the present work also provides evidence for clinical applications in analyzing transcriptional and translational mechanisms in ovarian cancer. Dysregulation of these mechanisms has novel therapeutic applications in targeting molecules such as Mnks and CDKs that regulate transcription of RNA and production of proteins, consequently increasing survival and treatment outcomes in cancer patients.

We also present evidence that suggests clinical potential of computer-aided diagnosis and the discovery of drugs. Increased adoption of computer-aided techniques such as 3D models, analysis of images, and artificial intelligence can increase the rate of predicting early ovarian cancer, diagnosis, and treatment. These approaches increase the rate of personalized medicine and care to patients; while improving drug delivery.

Further studies are warranted to assess the possibilities of optimizing ultrasound microbubble-enhanced drug delivery. These studies should highlight the exact mechanisms of drug release and uptake in the cells in the presence of microbubble contrast agents. Special attention should be focused on the specific factors

affecting drug delivery, parameters of ultrasound, and different combinations of microbubbles. It is highly recommended to perform an in-depth analysis of transcriptomes and proteomes in ovarian cancer. The transcriptomic and proteomic profiling of ovarian cancer cells could provide significant insights into various biomarkers and molecular processes. Furthermore, scientists are also encouraged to perform extensive RNA sequencing to determine various genes implicated in ovarian cancer and how their expression levels are affected by RES, as well as the regulatory networks and the KEGG signaling pathways in ovarian cancer. Prospective investigations on network pharmacology are warranted to investigate the potential mechanisms of RES in developing new anticancer treatments and their synergistic effects, as well as integrated multi-modal imaging and deep learning techniques in image analysis to detect ovarian cancer.

Conclusion

In summary, ultrasound combined with RES-loaded ultrasound microbubble contrast agents can inhibit the proliferation of ovarian cancer cells and induce their apoptosis. This study provides a theoretical basis for the clinical treatment of ovarian cancer with ultrasound combined with microbubble contrast agents. This method has broad application prospects for tumor-targeted therapy, but many problems still require further research and exploration. Therefore, we propose that by focusing on these areas of clinical applications and areas for further research, it is possible to advance the understanding and treatment of ovarian cancer, leading to improved patient outcomes and personalized therapeutic strategies.

Disclosure of conflict of interest

None.

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