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## Research Article

**Keywords:** esophageal cancer, epithelial-mesenchymal transition, invasion, migration, Qigesan, TGF- $\beta$ 1 pathway

**Posted Date:** April 29th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-424745/v1>

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# Qigesan reduced the migration and invasion of esophageal cancer cells via inhibiting TGF- $\beta$ 1 pathway

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**Abstract Background:** The present study aim to investigate the mechanism of Qigesan (QGS) on migration and invasion of esophageal cancer cells. **Methods:** First, we detected the cells in the normal group and the QGS group using microarray, and analyzed the ontology function and signaling pathway of target genes, and screened out TGF- $\beta$  as one of the important targets of QGS. The subsequent verification experiments were divided into four groups: control group, model group (TGF- $\beta$ 1 stimulation group), QGS group (TGF- $\beta$ 1+QGS group) and positive control group (TGF- $\beta$ 1+TGF- $\beta$ 1 receptor inhibitor group). The migration and invasion abilities, as well as the expressions of related proteins and mRNA of each experimental group were detected. The migration and invasion ability of cells were detected by cell scratch test, and the Gene expression levels of E-cadherin, Vimentin, Smad2 and Smad7 mRNA in TGF- $\beta$ 1/Smad pathway were detected by RT-qPCR. The expression of E-cadherin, Vimentin, p-Smad2/3, Smad2/3 and Smad7 were detected by WB. **Results:** The results showed that TE-1 cells were reversed from fibroblast induced by TGF- $\beta$ 1 to epithelial cells after being treated QGS with the concentration of 20ug/mL for 36 h. Besides, QGS inhibited the migration and invasion of TE-1 cells and down-regulated the Gene and protein expression of Vimentin, Smad2 mRNA and Vimentin, p-Smad2/3 and Smad2/3. Meanwhile, The gene and protein overexpression E-cadherin and Smad7 was observed in QGS-treated cells. **Conclusions:** Together, these data indicated that QGS interfered with the epithelial-mesenchymal transition (EMT) process of TE-1 cells and reduced the migration and invasion of TE-1 cells via regulating TGF- $\beta$  pathway, which maybe provided idea for the treatment of esophageal cancer.

**Keywords:** esophageal cancer, epithelial-mesenchymal transition, invasion, migration, Qigesan, TGF- $\beta$ 1 pathway

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## Introduction

Esophageal cancer is a kind of digestive tract tumor with high morbidity and mortality, and it is the eighth most common malignant tumor in the world with mortality ranking sixth in the global cancer<sup>[1]</sup>. In 2018, there were about 572,000 new patients with esophageal cancer, and 508,600 people died of esophageal cancer<sup>[2]</sup>. China is one of the areas with high incidence of esophageal cancer, among which Lin County is a high-risk area, and its incidence type is mostly squamous cell carcinoma<sup>[3]</sup>. When patients are diagnosed with Esophageal cancer, most of them are in the middle or advanced stage with metastasizing, resulting in a low 5-year survival rate<sup>[4-5]</sup>. It is reported that the migration and invasion of esophageal cancer is an important factor affecting the survival rate of patients with esophageal cancer, and epithelial mesenchymal transition is the premise of tumor migration and invasion<sup>[6]</sup>. Epithelial-mesenchymal transition (EMT) plays an important role in the occurrence and development of tumors, and mediates tumor energy metabolism, heterogeneity, drug resistance, invasiveness, stem cells and other processes<sup>[7,8]</sup>. It was reported that EMT changed the polarity of tumor cells, rearranged the cytoskeleton, degraded extracellular matrix, lose intercellular connections, and promoted tumor cells to metastasize with the blood and lymphatic system<sup>[9]</sup>. Once they reached a new suitable location, they went through the reverse EMT process. Mesenchymal-epithelial transition (MET) allowed cells to regain epithelial polarity and invade the tissues of the colonization site<sup>[10-11]</sup>. EMT of esophageal squamous cell carcinoma avoided immune surveillance by inhibiting the activity of natural killer cells (NK) and reducing the release of effective molecules<sup>[12]</sup>. TGF- $\beta$  is an important factor affecting EMT of tumor cells, and the process of inducing EMT of tumor cells mainly involving Smad-dependent and Smad-independent signaling pathway<sup>[13]</sup>. Qigesan (QGS) is a classical Chinese Herbal Formula for esophageal cancer treatment<sup>[14-15]</sup>, which contains Shashen, Danshen, Fuling, Chuanbei, Yujin, Sharen. Jiawei Qigesan improved the toxic side effects caused by radiotherapy and chemotherapy in patients undergoing radical esophagectomy, improved the quality of life of patients, reduced the recurrence rate and prolong the disease-free survival time<sup>[16]</sup>. Jiawei Qigesan inhibited lung metastasis of esophageal cancer in nude mice in

animal experiment <sup>[17]</sup>. Our previous studies showed that the combination of Qigesan's drug-containing serum and cisplatin enhanced the inhibitory effect of chemotherapeutic drugs on esophageal cancer EC9706 cells under hypoxia environment, increased the expression of phosphatase, tensin analogue (PTEN) mRNA and programmed death protein 4 (PDCD4), and decreased the expression of miR-21 <sup>[18]</sup>. The QGS combined with cisplatin enhanced the sensitivity of EC9706 cells to cisplatin and achieve the effect of enhancing the curative effect <sup>[19]</sup>. Qigesan was proved to improve the quality of life of esophageal cancer model mice by protecting organs and tissues, and improving the suppression and imbalance of immune mechanism <sup>[20]</sup>. The above reports indicated that QGS played key roles in treating esophageal cancer by inhibiting the metastasis of esophageal cancer. However, there were few reports on the intervention and mechanism of QGS on cancer cells migration and invasion. In this study, the intervention ways of QGS on migration and invasion of esophageal cancer were screened by gene expression microarray technology, and verified by experiments as follows.

## **Materials and methods**

### **Preparation of QGS**

The Chinese materia medica of QGS come from Tong Ren Tang Group (Beijing, China). See Table 1 for QGS composition. Beat the medicine into coarse powder, reflux that medicine with 70% ethanol in a ratio of m (medicine weight, unit: g): v (volume of 70% ethanol, unit: ml) = 1: 8, filtering, concentrate, uniformly mixing the concentrated solution and ethyl acetate in a volume ratio of 2:1 in a separatory funnel for extraction. The ethyl acetate extract was dried and weighed in vacuum at low temperature to obtain the extract quality, and the final paste yield (mass ratio of the finally obtained paste medicine to the original medicinal materials) was 0.82%. 1.00 g drugs was dissolved in 50mL dimethyl sulfoxide (DMSO), mixed well to fully dissolved, and then stored at -20°C for later use.

Table 1. The composition of Qigesan

Scientific name	Chinese name	Weight	%
<i>Adenophora stricta</i> Miq.	Nan-sha-shen	9g	32
<i>Salvia miltiorrhiza</i> Bge.	Dan-shen	9g	32
<i>Poria cocos</i> (Schw. ) Wolf.	Fu-ling	3g	11
<i>Fritillaria cirrhosa</i> D. Don	Chuan-bei-mu	4.5g	16
<i>Curcuma wenyujin</i> Y. H. Chen et C. Ling	Yu-jin	1.5g	5
<i>Amomum villosum</i> Lour.	Sha-ren	1.2g	4

## Reagents

The recombinant human TGF- $\beta$ 1 (PEPROTECH company, USA), SB431542 (MCE company, USA), Phosphate-buffered saline (PBS)( Solarbio, Beijing, China), Trypsin-EDTA Solution(Solarbio, Beijing, China), RPMI 1640 cell culture medium( Solarbio, Beijing, China), MTT(Solarbio, Beijing, China),DMSO(Solarbio, Beijing, China), fetal bovine serum(FBS)(GIBCO Company, USA), Matrigel matrix adhesive(BD Company, USA), bovine serum albumin(BSA)(AMRESCO Company, USA).

## Cell lines and cell culture

Experimental esophageal cancer cell line TE-1 purchased from National Collection of Authenticated Cell Cultures. Cell culture (10% FBS-RPMI-1640) and passage according to protocol, Incubator condition 5% CO<sub>2</sub>, 37°C.

## Screening of intervention pathways of QGS on TE-1 by gene chip of expression profile

The cells were divided into control group and QGS group. After incubating 36 h, the cells were washed twice with cold PBS. Total RNA was extracted by TRIZOL (Thermo fisher company, USA) method, and then purified by electrophoresis. The experimental sample RNA was amplified, labeled and purified by Affymetrix expression profile chip kit, and the biotin labeled cRNA was obtained. According to the hybridization standard flow and kit provided by Affymetrix expression profile chip (Affymetrix, Human Genome U219 array Strip), the hybridization was carried out in a rolling hybridization furnace(Affymetrix, USA, 645 p/n 00-0331,220 V) at 45°C for 16 hours, and the chip was washed in a Fluidics Station (Affymetrix, USA,

450 p/n 00-0079) after hybridization. Chip results were scanned by Gene Chip® Scanner 3000 (Affymetrix, USA, 3000 p/n 00-00212), and the original data were read by Command Console Software 3.1. The qualified data were normalized by genesprings software 11.0 (Agilent Technologies, Santa Clara, CA, US), and the algorithm used was MAS 5.0. After processing with BRB-ArrayTools 4.2, the genes of blank control group and Qigesan group were tested by multiple, and the genes with geometric mean of intensity (blank control group/Qigesan group) < 0.33 were up-regulated genes. The geometric mean of intensity (blank control group/qigesan group) > 3.0 is the down-regulated gene. Using the online DAVID tool <http://david.abcc.ncifcrf.gov/>, the target gene ontology function and signal pathway were analyzed.

#### **MTT assay was used to detect the effect of QGS on TE-1 cell activity**

The TE-1 cells were cultured in 96-well plate at the density of  $8 \times 10^3$  cells/well. Serum starved for 12 hours after cell adhesion, and then incubated with different concentrations of QGS (0, 20, 30, 40, 60, 80  $\mu\text{g}/\text{mL}$ ) for 12, 24, 36, 48 and 60 hours. The cells viability were detected with MTT (Solarbio company, China) assay.

#### **Experimental grouping**

The cells were divided into control group, model group (adding TGF- $\beta$ 1 with the concentration of 15 ng/mL), QGS group (adding QGS on the basis of model group) and SB431542 group (adding TGF- $\beta$ 1 receptor inhibitor SB431542 on the basis of model group). The drug concentrations of TGF- $\beta$ 1 and SB431542 were determined by previous experiments.

#### **The effect of QGS on migration and invasion of TE-1 cells was detected by cell scratch test**

TE-1 cells were cultured in 24-well plate at a density of  $2 \times 10^5$  cells/well, and serum starved for 12 hours after cell adhesion. Drawing a crossing line in the center of the well with a 200  $\mu\text{L}$  tips. The previous culture medium was sucked out, and the cell surface was washed with sterile PBS for 2-3 times to remove the cell debris in the scratch hole. Add different concentrations of QGS solution (0  $\mu\text{g}/\text{mL}$ , 20  $\mu\text{g}/\text{mL}$ , 30  $\mu\text{g}/\text{mL}$ , 40  $\mu\text{g}/\text{mL}$ ), 1000  $\mu\text{L}$ /well, put the cell culture plate into Cytation5 cell microplate imager (Bio Tek Instruments, USA), determine the photographing position

of scratch area, and continue to culture at 37°C and 5%CO<sub>2</sub>. After scratching, automatic photos were taken once at 0h, 12h, 24h and 36h, so as to record the changes of cell scar area in each cell hole of each group.

When testing the cell invasion ability, it was necessary to add the step of spreading glue after scribing, the volume ratio of medium to Matrigel matrix glue was found in the pre-experiment.

#### **Detection of gene expression with RT-qPCR method.**

The cells with the density of 1.5×10<sup>5</sup> cells/well were cultured in 24-well plate. According to control group, model group, QGS group and SB431542 group, after 36 hours of culture, total RNA was extracted and the purity and concentration of RNA products were determined. After reverse transcription, RT-qPCR was performed according to the instructions, and the relative expression of target gene mRNA was obtained by the ratio of target gene(hSmad2 F-CGTCCATCTTGCCATTCACG,R-CTCAAGCT CATCTAATCGTCCTG;hSmad7 F-TTCCTCCGCTGAAACAGGG,R-CCTCCCAGTA TGCCACCAC;hE-cadherin F-CGAGAGCTACACGTTACGG,R-GGGTGTCGAG GGAAAATAGG;hVimentin F-GACGCCATCAACACCGAGTT,R-CTTTGTCGTT GGTTAGCTGGT, Nanjing jinweizhi biology) mRNA expression to internal reference gene(hGAPDH F-GGAGCGAGATCCCTCCAAAAT,R-GGCTGTTGTCAT ACTTCTCATGG, Nanjing jinweizhi biology) mRNA expression.

#### **Western blot assay**

Cell lysate was prepared from blank control group, model group, Qigesan group and SB431542 group. The lysate was centrifuged in a centrifuge for 4°C, 12000× g, 10 min. Extracting supernatant and protein quantification using the bicinchoninic acid (BCA) kit from BOSTER, Wuhan. SDS-PAGE electrophoresis was performed, then transferred to the membrane, polyvinylidene fluoride (PVDF) membrane (Millipore, U.S.A.). PVDF membranes were pre-blotted with 5% Skim milk (Solarbio, Beijing). Incubating PVDF membranes with primary antibody overnight: Vimentin rabbit anti-human polyclonal antibody (Wuhan Cloud-CloneCorp), E-cadherin rabbit anti-human polyclonal antibody (Wuhan Cloud-CloneCorp), p-Smad2/3 rabbit anti-human polyclonal antibody (CST Company), Smad2/3 rabbit anti-human polyclonal antibody (CST Company), Smad7 rabbit anti-

human polyclonal antibody (Wuhan Proteintech Company), GAPDH mouse anti-human monoclonal antibody (Wuhan Cloud-CloneCorp Company)). The next day labeling fluorescent secondary antibody: horseradish enzyme labeled goat anti-mouse IgG secondary antibody (Beijing Zhongshan Jinqiao Company). Imaging PVDF membrane with gel imaging analysis system (Bio-rad, USA). The result is expressed by the ratio of protein/ GAPDH.

### **Statistical analysis**

Differences between groups were determined by one-way analysis of variance with the LSD and Dunnett's T3 post-hoc tests using the SPSS 25.0 software. All results were presented as a mean  $\pm$  standard error. Differences were considered to be significant when  $P < 0.05$ .

### **Results**

***Gene Expression Changes.*** There are 1487 differential genes between QGS and control group, of which 1080 are down-regulated and 407 are up-regulated, and the down-regulated genes account for 72.63% of the differential genes. ***Changed genes function.*** The main biological processes involved in down-regulated genes are cytoskeletal protein binding, ATP binding, adenyly nucleotide binding, adenyly ribonucleotide binding and so on (Figure 1a); Up-regulated genes mainly participate in biological processes such as RNA binding, DNA binding, transcription regulator activity, transcription activator activity, nucleotide binding, etc (Figure 1b). ***Signal pathways involved in changed genes.*** KEGG PATHWAY involved by down-regulated genes mainly includes TGF- $\beta$  signaling pathway, cell cycle, ECM-receptor interaction, pathways in cancer, oocyte meiosis, etc (Figure 1c). The KEGG PATHWAY involved by up-regulated genes mainly includes MAPK signaling pathway, bladder cancer, renal cell carcinoma, pathways in cancer, P53 signaling pathway, etc (Figure 1d).

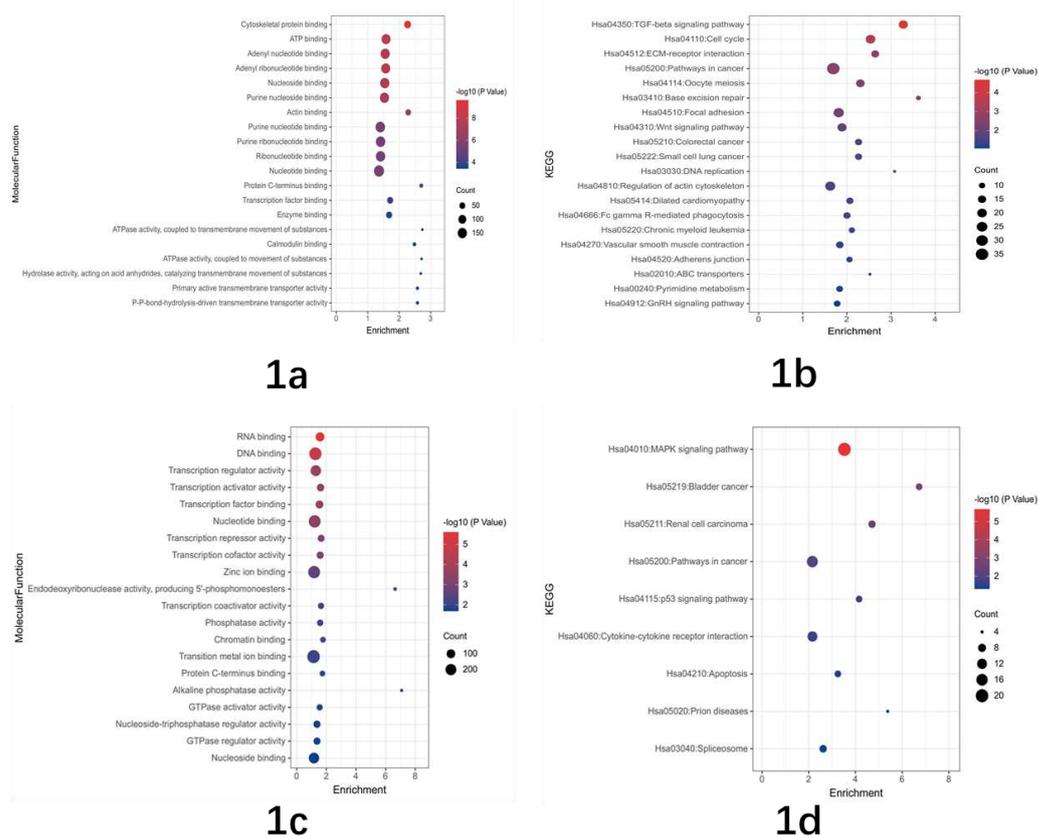


Fig 1. Main functions and signal pathways of changed genes. 1a and 1c: Down-regulated genes; 1b and 1d: Up-regulated genes.

### The effects of Qigesan on TE-1 cells viability and the morphological changes of cells in each experimental group

After treating cells for 12h, 24h, 36h, 48h and 60h, there were significant differences between different concentrations of QGS group ( $P < 0.05$ ). After different concentrations of QGS acted on TE-1 cells, the inhibition of cell activity in each group increased with the increase of drug concentration at the same time; When the concentration of QGS is constant, the inhibition rate of cell activity increases with the extension of drug action time within 12 h-36 h (Figure 2a). After 36 hours of treatment, the inhibition rate of cell activity in QGS 20 $\mu\text{g}/\text{mL}$  group has reached 35%. Therefore, in order to explore the effect of QGS on the migration and invasion ability of TE-1 cells without obviously inhibiting the activity of TE-1 cells, the concentration of QGS was 20 $\mu\text{g}/\text{mL}$  and the action time was 36 hours.

After each experimental group acted on TE-1 cells for 36 hours, the cells in the control

group grew well, the cytoplasm was uniform and transparent, the cells were closely connected, and they were oval or paving stone-like, which was a typical epithelial cell phenotype. The cells in the model group were elongated and showed fibroblast phenotype, and the intercellular connections were reduced. In QGS group, the number of cells decreased, showing paving stone-like, cell connection was tight, showing epithelial cell phenotype; The cell morphology of SB431542 group was similar to that of QGS group (Figure 2b).

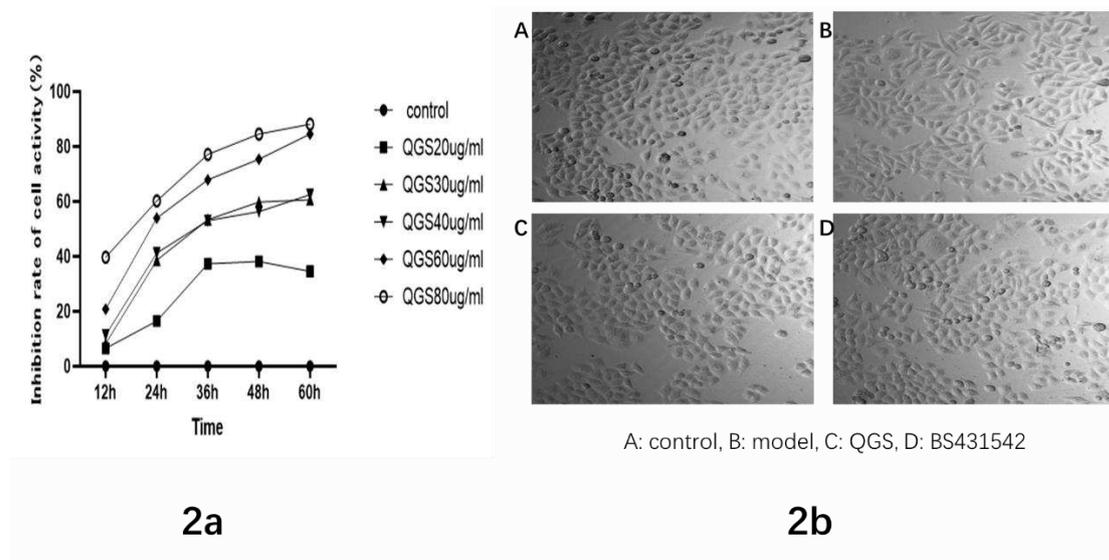


Fig 2. Inhibition of QGS on TE-1 Cells viability and Morphology of TE-1 cells in each group under microscope (36h,  $\times 100$ )

### Effects of QGS on migration and invasion of TE-1 cells

On the whole, the change of cell area in each group increased with the increase of time. Compared with the control group, the migration and invasion abilities of the model group were enhanced ( $P < 0.05$ ). Compared with the model, the ability of cell migration and invasion in QGS group and SB431542 group was inhibited and weakened ( $P < 0.05$ ). However, there was no significant difference in migration and invasion ability between QGS group and the SB431542 group ( $P > 0.05$ ) (Figure 3,4).

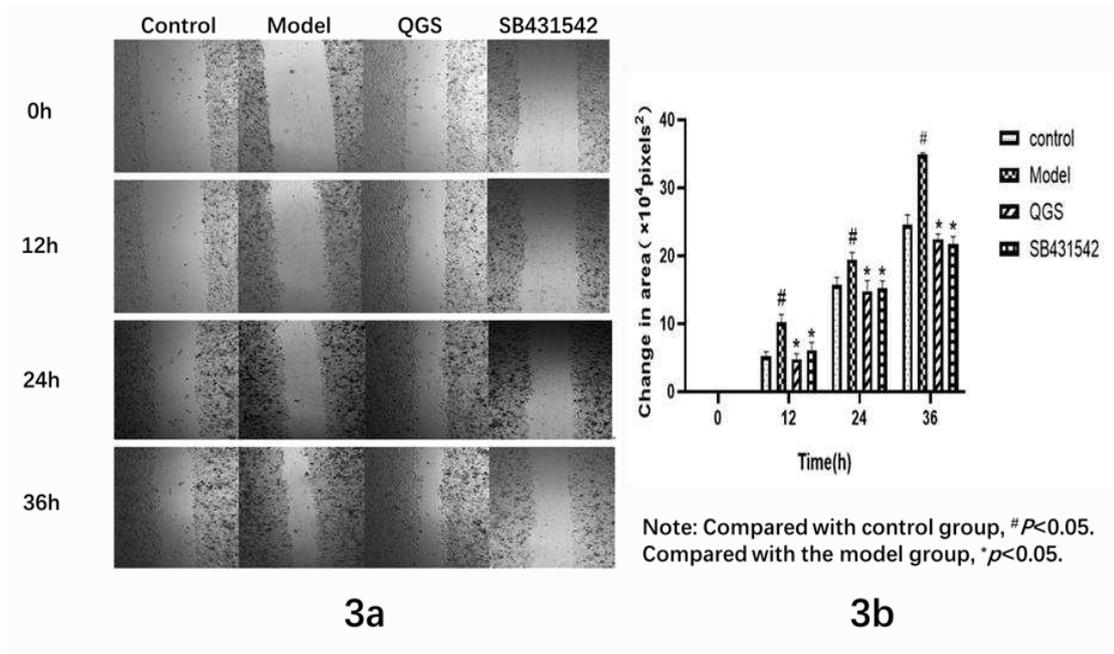


Fig 3. Migration ability of TE-1 cells in each experimental group(×100)

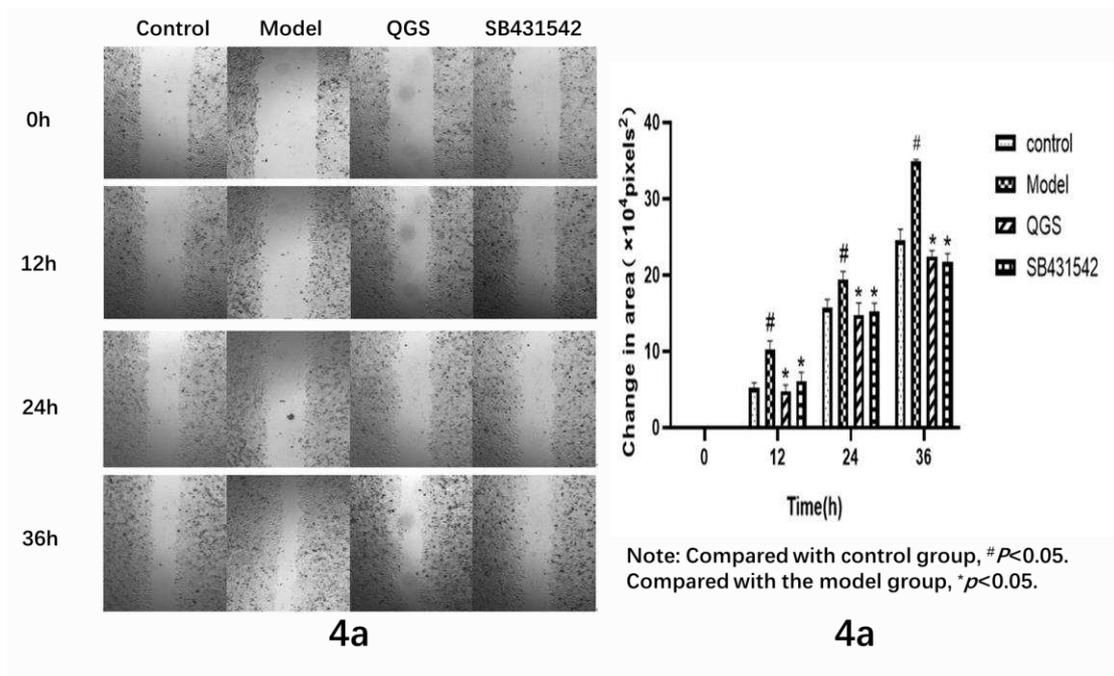


Fig 4. Invasion ability of TE-1 cells in each experimental group(×100)

### Effect of QGS on mRNA expression of TGF-β1 pathway related genes

Compared with the control group, the expression levels of Vimentin and Smad2 mRNA of the model group were significantly increased ( $P < 0.05$ ) (Figure 5a,5b), while the mRNA expression levels of E-cadherin and smad7 were significantly decreased ( $P < 0.05$ ) (Figure 5c,5d). Compared with the model group, the expression levels of

Vimentin and Smad2 mRNA in QGS group and SB431542 group were significantly decreased ( $P < 0.05$ ) (Figure 5a,5b), while the expression levels of E-cadherin and Smad7 mRNA were significantly increased ( $P < 0.05$ ) (Figure 5c,5d). There was no significant difference in mRNA expression between QGS group and SB431542 group ( $P > 0.05$ ).

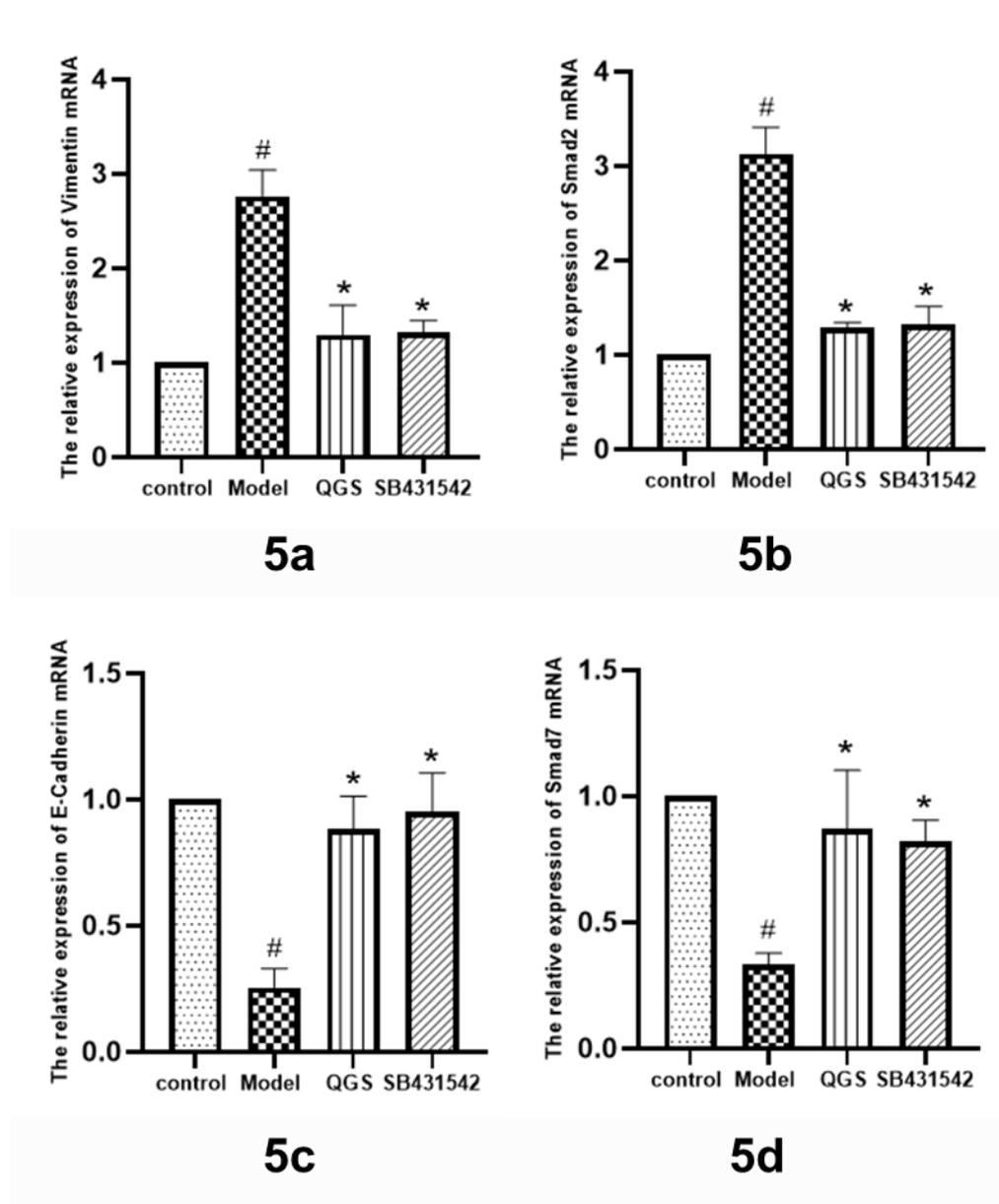


Fig 5.Effects of each experimental group on related genes of TE-1 cells

Note: Compared with control group, # $P < 0.05$ . Compared with the model group, \* $P < 0.05$ .

## Effect of QGS on expression of TGF-β1 pathway related proteins

Compared with the control group, the protein expression levels of Vimentin, p-Smad2/3 and Smad2/3 in the model group were significantly increased ( $P < 0.05$ ) (Figure 6a,6b,6c), while the protein expression levels of E-cadherin and Smad7 were significantly decreased ( $P < 0.05$ ) (Figure 6d,6e). Compared with the model group, the protein expression levels of Vimentin, p-Smad2/3 and Smad2/3 in the QGS group were significantly decreased ( $P < 0.05$ ) (Figure 6a,6b,6c), while the protein expression levels of E-cadherin and Smad7 were significantly increased ( $P < 0.05$ ) (Figure 6d,6e). There was no significant difference in mRNA expression level between QGS group and SB431542 group ( $P > 0.05$ ).

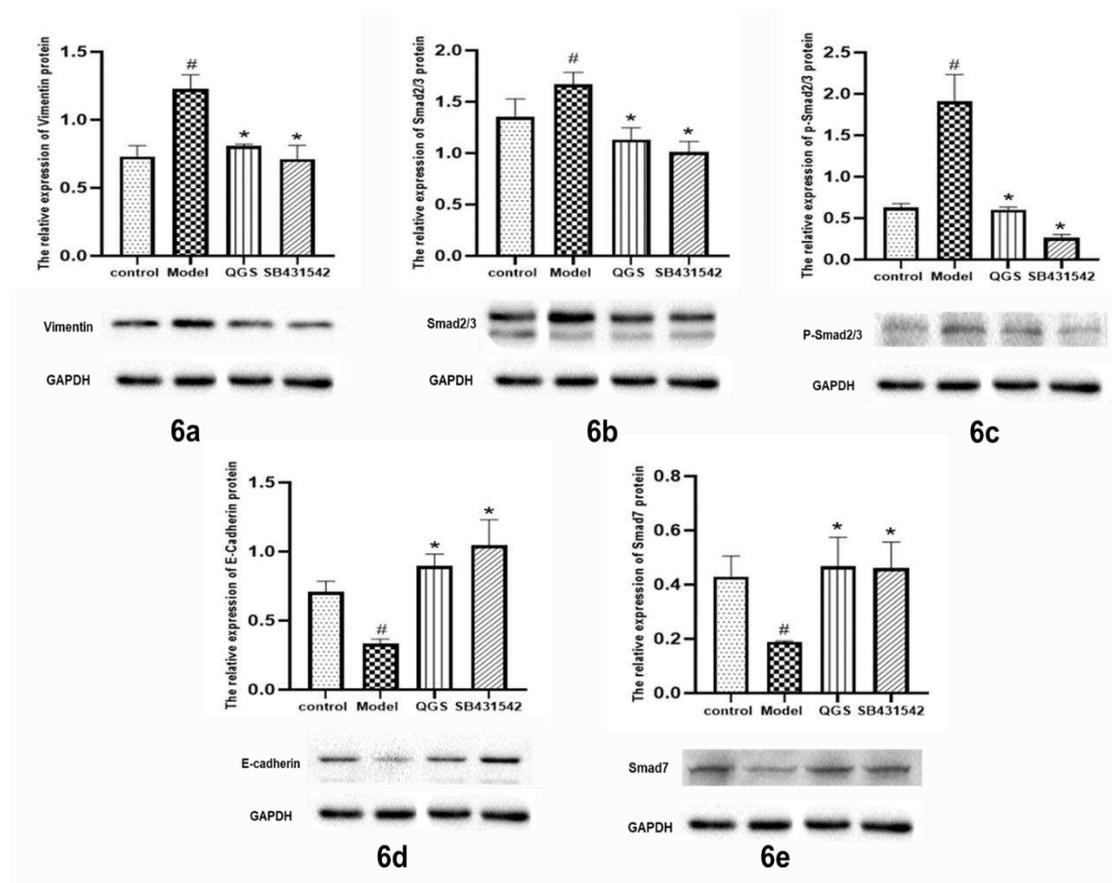


Fig 6. Effect of each experimental group on TE-1 cell pathway related proteins  
Note: Compared with control group, # $P < 0.05$ . Compared with the model group, \* $P < 0.05$ .

## Discussion

Esophageal cancer (EC) is one of the most aggressive diseases worldwide and deadliest types of cancer. The early symptoms are not obvious, which makes it difficult to diagnose them in time. Esophagectomy is still the main treatment, and some methods are being tried, such as endoscopic treatment for early EC, prophylactic chemoradiation, Perioperative treatment, targeted therapy and immunotherapy, etc. However, the choice of these treatments is affected by the type and stage of the disease, tumor location, individual differences of patients, and there is no global consensus on the optimal regimen<sup>[21]</sup>.

Metastasis and invasion of esophageal cancer is an important reason affecting the therapeutic effect, and EMT is the main factor causing metastasis and invasion. The EMT program is often activated reversibly, It will lead to the appearance of intermediate cells, which may promote the proliferation and spread of cancer cells at different stages of tumor development<sup>[22]</sup>. It was reported that EMT program in melanoma cells potentiates migration rate and development of lung colonies into immunodeficient host of cells grown in standard pH<sup>[23]</sup>. Another study showed that EMT also upregulated the expression of FHOD1, may contribute to tumour progression<sup>[24]</sup>. Yan<sup>[25]</sup> found that overexpression of integrin-linked kinase (ILK) promotes migration and invasion of colorectal cancer cells by inducing epithelial–mesenchymal transition via NF- $\kappa$ B signaling. These studies have proved that EMT is directly related to tumor migration and invasion. TGF- $\beta$  signaling pathway is an important way to participate in the regulation of EMT, and TGF- $\beta$ 1 is considered as an effective inducer of epithelial–mesenchymal transition in tumor microenvironment. Research has found that TGF- $\beta$ 1 induces epigenetic silence of TIP30 to promote tumor metastasis in esophageal carcinoma<sup>[26]</sup>. A clinical study on esophageal cancer patients in Xinjiang, China showed that: TGF- $\beta$ 1/Smad signaling pathway regulates Epithelial-to-Mesenchymal Transition in esophageal squamous cell carcinoma<sup>[27]</sup>.

Traditional Chinese medicine also plays a certain role in the treatment of esophageal cancer, it was reported that Anti-metastatic effects of Aidi on human esophageal squamous cell carcinoma by inhibiting epithelial–mesenchymal transition and

angiogenesis<sup>[28]</sup>. A study on esophageal cancer found that Chinese herb medicine matrine induce apoptosis in human esophageal squamous cancer KYSE-150 cells through increasing reactive oxygen species and inhibiting mitochondrial function<sup>[29]</sup>. Berberine inhibits the metastatic ability of prostate cancer cells by suppressing Epithelial-to-Mesenchymal Transition (EMT)-associated genes with predictive and prognostic relevance<sup>[30]</sup>. Other Chinese herbal medicine components or extracts have also been proved to be related to the drug resistance<sup>[31]</sup> and cycle<sup>[32]</sup> of esophageal cancer.

At the beginning of this study, we analyzed TE-1 cells in the control group and QGS group by gene chip technology, and found that the main biological processes involved in changed genes are cytoskeletal protein binding, ATP binding, adenyly nucleotide binding, adenyly ribonucleotide binding and so on; the KEGG PATHWAY involved by changed genes are TGF- $\beta$  signaling pathway, cell cycle, pathways in cancer, etc. Cytoskeletal protein binding and TGF- $\beta$  signaling pathway are both related to cell migration and invasion, therefore, we conducted a follow-up experiment to verify the effect of QGS on the migration and invasion of TE-1 cells.

Firstly, the suitable concentration and action time of QGS were screened out by MTT experiment, that is, 20  $\mu\text{g/ml}$ , 36h. In the validation experiment, we used the recombinant human TGF- $\beta$ 1 to stimulate TE-1 cells to induce epithelial mesenchymal transition as the experimental model group, and used the intervention of TGF- $\beta$ 1 receptor blocker SB431542 as the positive control group. The intervention model group of QGS will be compared with the SB431542 group to measure the influence of QGS on the EMT of TE-1 cells. First of all, on the morphology of TE-1 cells, QGS alleviated the epithelial cell phenotype induced by recombinant human TGF- $\beta$ 1, which is similar to SB431542. Secondly, in terms of cell migration and invasion ability, QGS also showed the effect similar to SB431542, which reduced the migration and invasion ability of TE-1 cells. There was a report that Qigesan inhibits migration and invasion of esophageal cancer cells via inducing connexin expression and enhancing gap junction function<sup>[33]</sup>, Our experimental results support this view. In the following experiments, we detected related molecules Vimentin, p-Smad2/3, Smad2/3, E-cadherin, Smad7. Smad is a TGF- $\beta$ /Smad pathway protein, Vimentin is one of the

interstitial molecular markers, and E-cadherin is the epithelial molecular marker [34-36]. It has been reported [37-41] that TGF $\beta$ 1, Smad3 and Smad4 are highly expressed and Smad7 is low expressed in esophageal carcinoma. TGF- $\beta$ 1 leads to epithelial mesenchymal transition of esophageal cancer cells, and the expression of E-cadherin decreases, while the expression of p-Smad2/3, Smad2 and Smad3 increases, while T $\beta$ R inhibitor can inhibit the expression of related proteins. Our research is consistent with the existing literature research. Compared with the blank control group, the expression of E-cadherin in the model group decreased and the expression of Vimentin increased in both mRNA and protein levels, suggested that the epithelial-mesenchymal transition occurred in TE-1 cells of esophageal cancer. At the same time, the expression of TGF- $\beta$ 1/Smad signaling pathway related indicators p-Smad2/3 and Sma2/3(Smad2 mRNA) increased, while Smad7 decreased. Therefore, the epithelial-mesenchymal transition of TE-1 cells is related to TGF- $\beta$ 1/Smad signaling pathway. Compared with the model group, the expressions of E-cadherin and Smad7, which are related to epithelial stroma and TGF- $\beta$ 1/Smad signaling pathway, increased significantly in QGS group and SB431542 group, while the expressions of p-Smad2/3, Smad2/3(Smad2mRNA) and Vimentin decreased significantly ( $P<0.05$ ), suggested that QGS inhibited EMT and its migration in TE-1 cells.

In conclusion, this study suggests that TGF- $\beta$  signaling pathway is involved in EMT, and Qigesan inhibits the migration and invasion of TE-1 cells by regulating TGF- $\beta$  signaling pathway.

#### Abbreviations

QGS: Qigesan; RT-qPCR: Real-time Quantitative PCR; WB: Western Blot; EMT: epithelial-mesenchymal transition; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; TGF- $\beta$ : transforming growth factor- $\beta$ ; p-Smad2/3: phospho-Smad2/3.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

#### Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

#### Competing interests

The authors declare that they have no competing interests.

#### Funding

This work was supported by National Natural Science Foundation of China (Nos. 81873285 and 82074313). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

#### Authors' contributions

YLC and LHY designed the study and modified the manuscript. YWS and RZ performed the main experiments. YWS wrote the manuscript. DHM and YSW revised the manuscript. XC and ZXZ performed the extracts preparation. YSW analyzed the data. SSR drafted all figures and tables. All authors read and approved the final version of the manuscript.

#### Acknowledgements

The authors thank Dr. Yan-qin Qin for her advice in writing.

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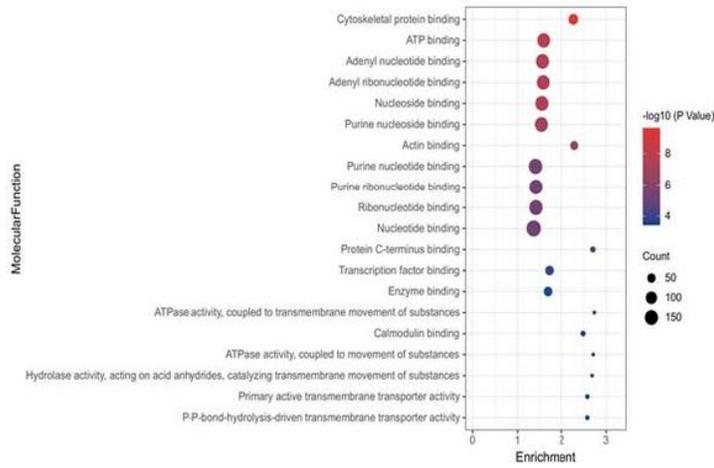
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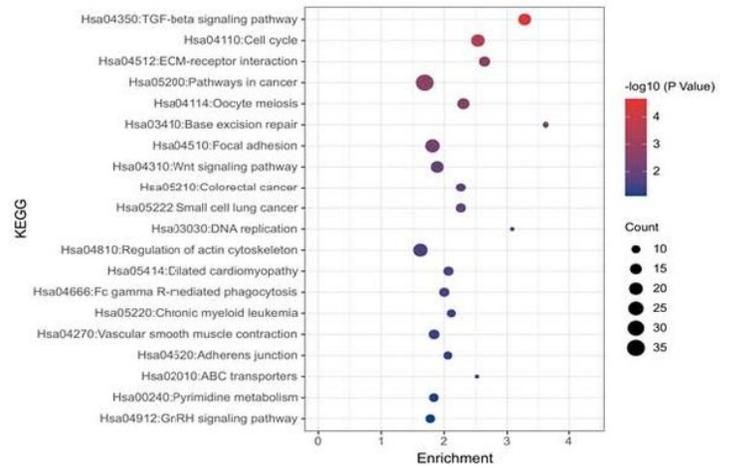
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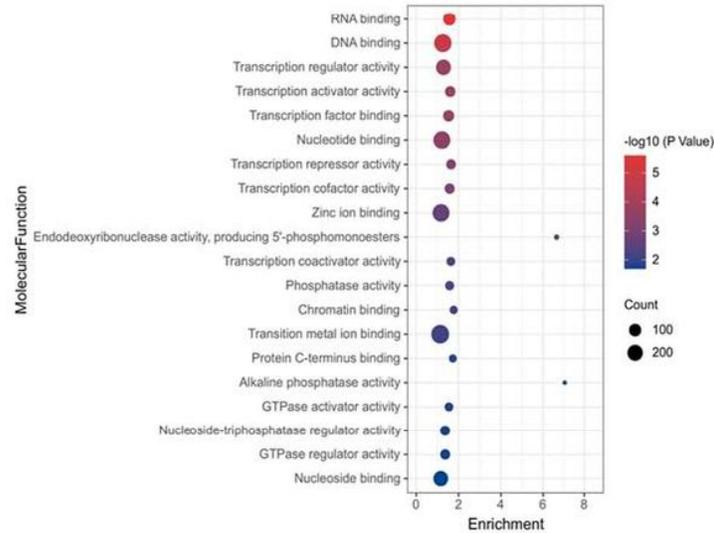
# Figures



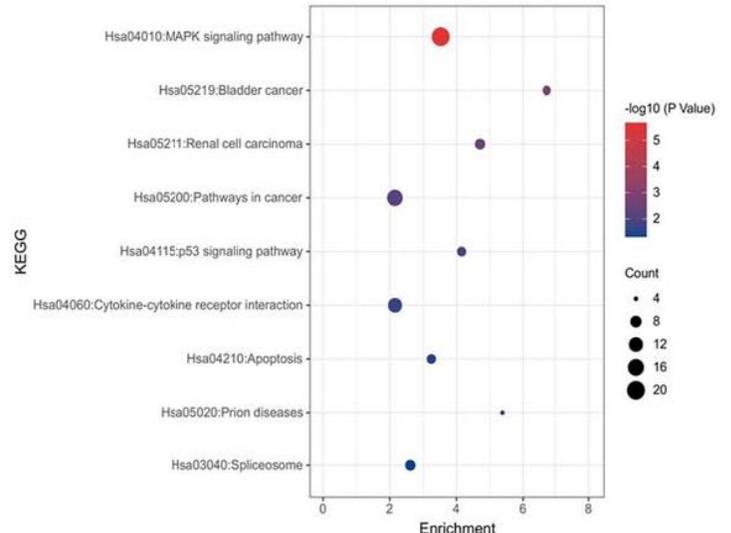
**1a**



**1b**



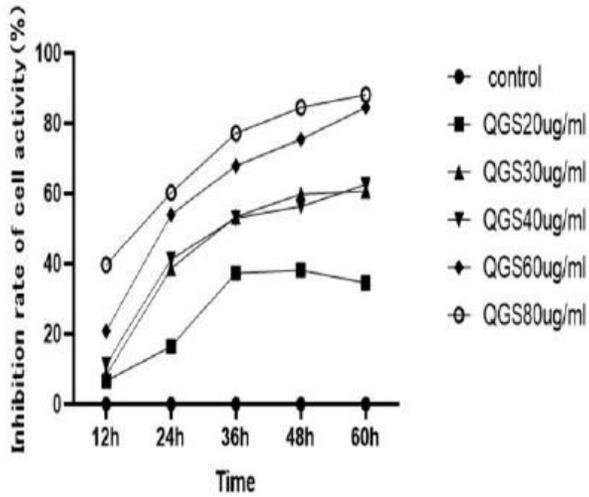
**1c**



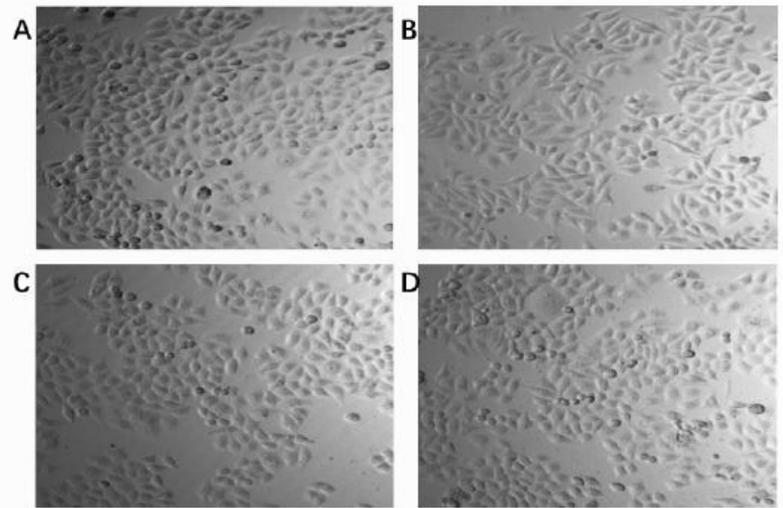
**1d**

**Figure 1**

Main functions and signal pathways of changed genes. 1a and 1c: Down-regulated genes; 1b and 1d: Up-regulated genes.



2a

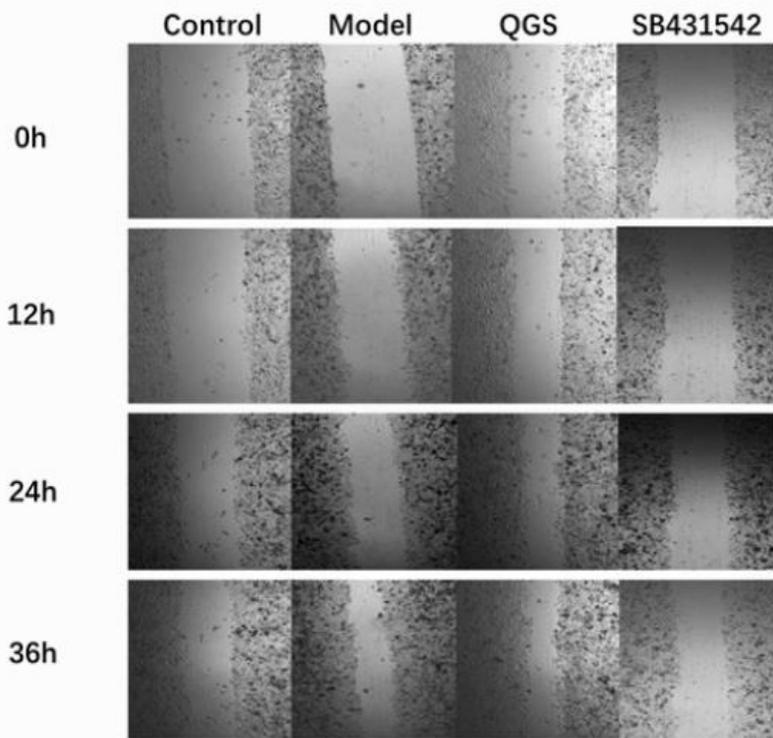


A: control, B: model, C: QGS, D: BS431542

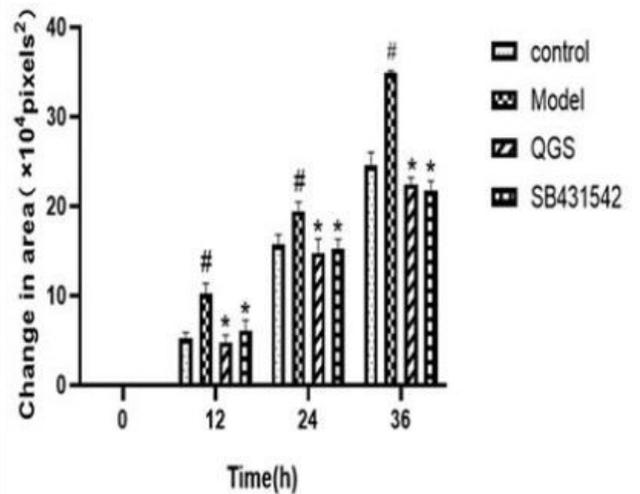
2b

Figure 2

Inhibition of QGS on TE-1 Cells viability and Morphology of TE-1 cells in each group under microscope (36h,  $\times 100$ )



3a

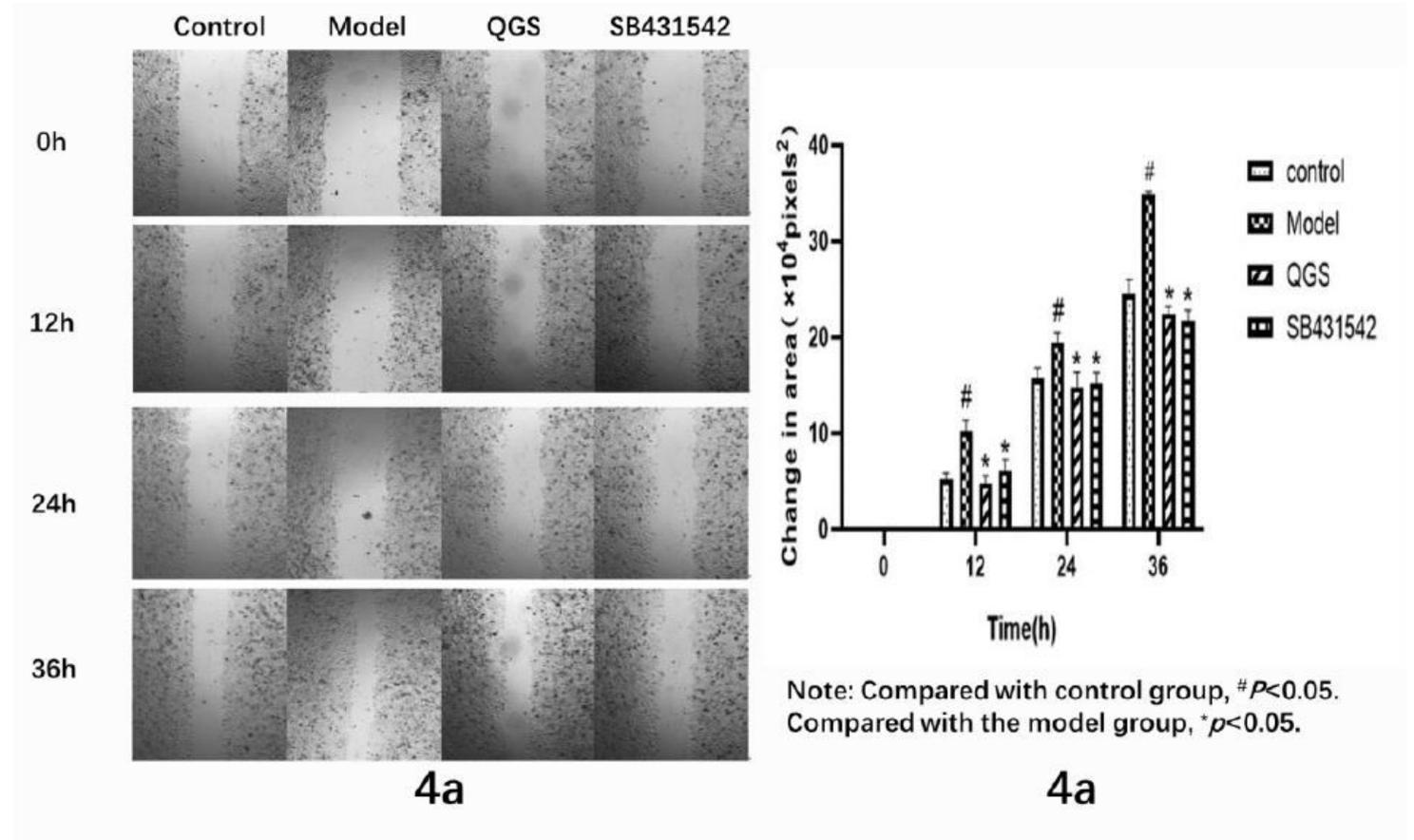


Note: Compared with control group, #  $P < 0.05$ .  
Compared with the model group, \*  $p < 0.05$ .

3b

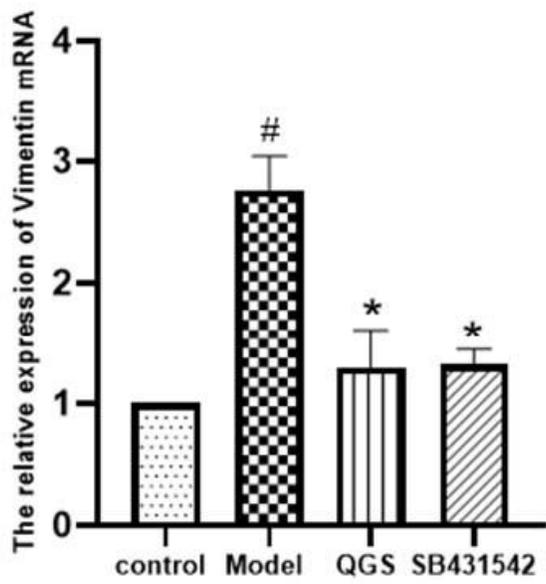
**Figure 3**

Migration ability of TE-1 cells in each experimental group( $\times 100$ )

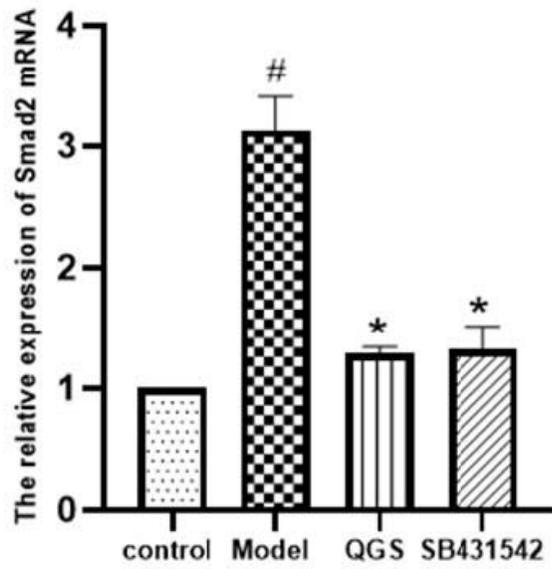


**Figure 4**

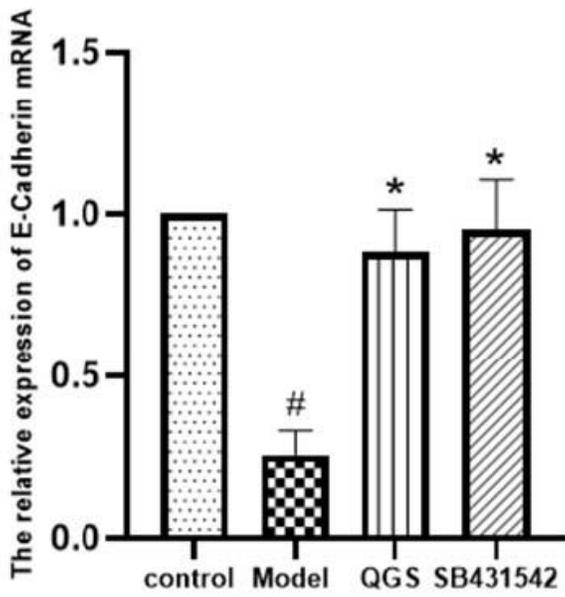
Invasion ability of TE-1 cells in each experimental group( $\times 100$ )



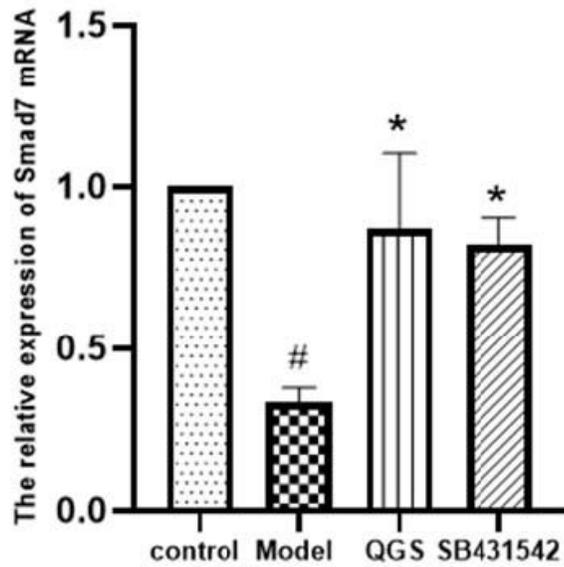
**5a**



**5b**



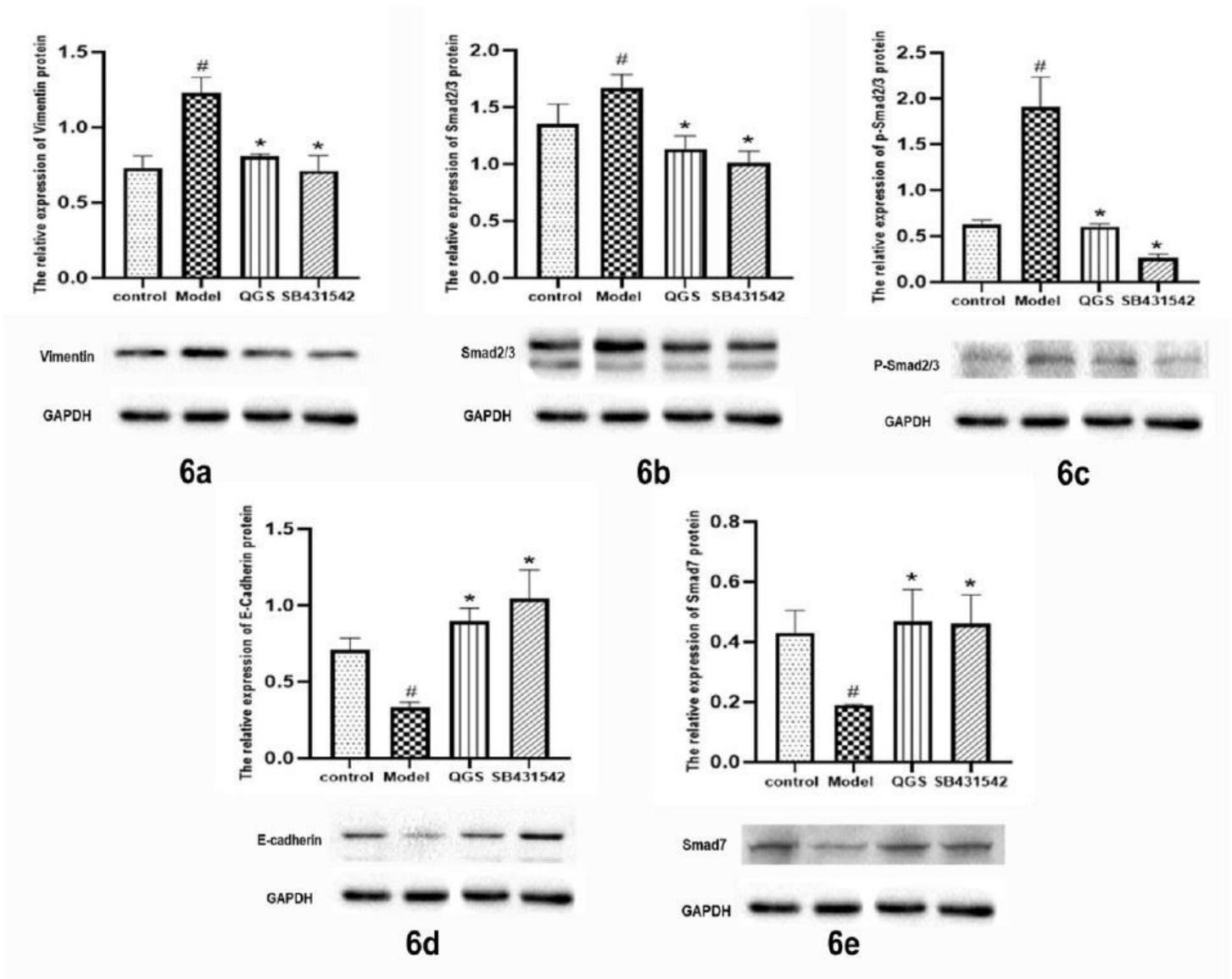
**5c**



**5d**

**Figure 5**

Effects of each experimental group on related genes of TE-1 cells Note: Compared with control group, #P < 0.05. Compared with the model group, \*P < 0.05.



**Figure 6**

Effect of each experimental group on TE-1 cell pathway related proteins Note: Compared with control group, #P < 0.05. Compared with the model group, \*P < 0.05.