

Mitigating inflammatory response in breast cancer patients: the inhibitory effect of acupuncture and emotional nursing on the ST8SIA6-AS1/LINC00504/p38 pathway

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Abstract

Introduction: Breast cancer patients often suffer from psychological distress such as anxiety and depression, which can exacerbate inflammation and potentially hinder treatment outcomes. This research investigates the effects of emotional nursing combined with acupuncture on inflammation in breast cancer patients diagnosed with liver depression and stagnation.

Material and methods: A total of 40 breast cancer patients with liver depression and stagnation were divided into 2 groups: a control group ($n = 20$) and an observational group ($n = 20$). The control group was subjected to standard nursing care, whereas the observational group received a synergised regimen of emotional nursing and acupuncture alongside the regular nursing care. Depression and anxiety levels were assessed using the Self-rating Depression Scale (SDS) and Self-rated Anxiety Scale (SAS). Inflammatory cytokine levels were analysed using ELISA and Western blot, while cell viability and apoptosis rate in breast cancer cells were assessed through specific assays.

Results: Emotional nursing and acupuncture significantly reduced psychological distress and inflammation. Moreover, a significant reduction in ST8SIA6-AS1 and LINC00504 expression levels in tumour tissues was observed following the emotional nursing and acupuncture intervention – 2 entities known to be elevated in breast cancer scenarios and associated with patient survival. Additionally, this therapy restrained the activation of p38 signalling in breast cancer tumour tissues. Furthermore, the silencing of ST8SIA6-AS1 and LINC00504 dampened IL-6-mediated inflammation in breast cancer cells through the p38 pathway.

Conclusions: Emotional nursing and acupuncture potentially reduce inflammation in breast cancer patients with liver depression and stagnation by modulating specific factors and deactivating the p38 pathway.

Key words: emotional nursing, acupuncture, breast cancer, liver depression and stagnation.

Introduction

Globally, breast cancer has now overtaken lung cancer as the most prevalent form of cancer, with an estimated 2.3 million new cases and nearly 690,000 fatalities recorded in 2020 [1]. Although advancements in breast cancer therapy have led to significant improvements in diagnosis and survival rates [2], numerous patients continue to experience emotional distress due to a complex interplay of psychosocial, medical, and hormonal factors. This distress is linked to inflammatory responses that may compromise treatment efficacy and exacerbate physical symptoms [3–7]. Consequently, enhancing depression management strategies is pivotal for augmenting treatment results and enhancing the quality of life for individuals with breast cancer.

In the realm of traditional Chinese medicine (TCM), liver depression and stagnation are often indicated with symptoms of dysphoria, vexation, numbness of the limbs, anger, forgetfulness, and depression, manifesting symptoms such as firm breast lumps, pale tongue, tidal fever, numbness of the limbs, irritability, tense pulse, and frustration [8, 9]. Therefore, new treatments for liver depression and stagnation in breast cancer patients still need to be sought. Current research emphasises the potential benefits of psychological nursing interventions in offering emotional support to breast cancer patients, thereby reducing anxiety, stress, and depression, and fostering an improved quality of life [10, 11]. In China, the approach of emotional nursing, which centralises on ameliorating mental attitudes, is a common practice in medical treatments. Evidence suggests that this form of nursing not only enhances self-efficacy but also boosts the immune responses of patients undergoing treatment [12, 13]. Additionally, it has been noted to diminish somatic pain related to liver depression and stagnation, decreasing adverse emotional responses and effectively improving treatment outcomes [14]. However, the fundamental mechanisms of emotional nursing in breast cancer remain relatively unexplored and warrant further investigation.

For over two and a half millennia, acupuncture – a cornerstone of TCM – has exhibited promising capabilities in managing symptoms associated with cancer [15]. This treatment modality is reputed for its ability to clear primary and auxiliary channels, harmonise Yin and Yang, and bolster the body's resilience to pathogenic elements [16]. Acupuncture has proven to be an effective non-pharmaceutical intervention against depressive symptoms, often employed successfully in treating depression seen in breast cancer patients [17–19]. Furthermore, acupuncture has shown promise in mitigating inflammation, potentially

aiding in the alleviation of depression [17]. Notably, electroacupuncture has been highlighted for its role in modulating inflammatory responses through vagus nerve stimulation, thereby promoting anti-cancer immunity in animal models of breast cancer [20].

Long noncoding RNAs (lncRNAs), noncoding RNA molecules exceeding 200 nucleotides in length with no protein-coding capacity, have recently garnered attention [21]. A plethora of studies underscore their role in the tumourigenesis of various cancers, influencing cellular activities like proliferation and apoptosis [22]. Moreover, several lncRNAs have emerged as potential biomarkers and therapeutic targets in depression [23]. A recent analysis based on the GSE217811 dataset from the GEO database and overexpressed genes in the GEPIA database pinpointed an upregulation of the plasma exosomal-derived lncRNAs ST8SIA6-AS1 and LINCO0504 in individuals with both breast cancer and major depressive disorder. These lncRNAs have been identified as promoters of breast cancer progression, orchestrating various aspects of cellular dynamics, including proliferation and invasion, via pathways like p38 MAPK [24–26]. Intriguingly, this pathway is not only crucial in modulating inflammatory responses but has also been linked with depressive disorders in the context of cancer, accentuating the malignancy of breast cancer in rodent depressive disorder models [27, 28].

In light of this, our study seeks to address critical research questions concerning the potential impact of emotional nursing interventions on the inflammatory responses in patients with breast cancer and liver depression and stagnation, and the modulation of these responses through acupuncture therapy. Additionally, we aimed to uncover the mechanism of the intersection of breast cancer and major depressive disorder, and how these might be influenced by emotional nursing or acupuncture therapies. To elucidate these areas, we hypothesise that emotional nursing interventions will significantly alleviate symptoms of depression and improve the quality of life in patients. Simultaneously, we anticipate that acupuncture therapy will effectively modulate inflammatory responses and enhance anticancer immunity in this demographic. Furthermore, we propose that the lncRNAs ST8SIA6-AS1 and LINCO0504 are up-regulated in patients experiencing both conditions, potentially serving as markers for disease progression and treatment response. By addressing these aspects, our study intends to shed new light on the therapeutic management of breast cancer patients experiencing liver depression and stagnation, potentially paving the way for novel, integrated treatment approaches.

Material and methods

Participants and recruitment

A total of 40 breast cancer patients with liver depression and stagnation were included in this study from the Hai'an Hospital of Traditional Chinese Medicine from January 2022 until December 2022. The inclusion criteria were as follows: 1) female patients aged 18–65 years; 2) pathologically diagnosed with breast cancer; 3) identified with liver depression and stagnation based on syndrome differentiation of traditional Chinese medicine, characterised by symptoms such as breast pain, hypochondriac pain, fatigue, bitterness in the mouth, irritability, reduced appetite, reddish and dark tongue, white tongue coating, and a stringy pulse [29]; and 4) signed informed consent prior to the study. The exclusion criteria were as follows: 1) patients with cognitive impairment or psychiatric disorder; 2) patients receiving anti-anxiety or antidepressant medication; and 3) concurrent diagnosis of other tumour types.

To ensure the credibility of the randomisation process, eligible participants were assigned to either the control or the observation group using a computer-generated random number table. This random allocation was performed by an independent statistician who was not involved in the recruitment process. The randomisation was stratified by age and stage of breast cancer to ensure balanced distribution of these variables across both groups.

Participants were informed of detailed information about the study, including its purpose, duration, process, and potential risks and benefits. Participants could withdraw their consent at any time and for any reason without consequences. Following randomisation, 20 patients were allocated to the control group receiving standard care, and 20 to the observation group receiving special care. Serum and tissue samples (tumour, $n = 20$; adjacent normal, $n = 20$) were collected from both groups before and after treatment for further analysis.

Intervention

Patients in the control group received standard nursing care, meticulously designed to cater to the needs of breast cancer patients. This care was guided by established protocols and best practices as outlined in the nursing literature related to breast cancer [11, 30], including adherence to the 5th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 5) [31]. The specifics of the care included the following:

(1) Preoperative preparation: This involved educating patients about the surgical procedure, addressing any questions or concerns they might

have, ensuring psychological readiness, and preparing them physically for surgery. Patients were instructed on fasting guidelines, hygiene practices, and were provided with information on what to expect post surgery.

- (2) Postoperative wound care: This entailed regular monitoring and dressing of surgical wounds, management of drains, if any, and instruction on wound care at home. Nurses also assessed the wound for signs of infection or complications and provided appropriate interventions when necessary.
- (3) Observation of patient's condition: Continuous monitoring of vital signs, pain levels, and overall physical condition was conducted. Nurses were vigilant for any signs of complications such as infection, bleeding, or thrombosis, and ensured timely medical intervention when required.
- (4) Management of post-surgery adverse reactions: This included managing common post-operative symptoms such as nausea, vomiting, pain, and fatigue. Pain management strategies were individualised based on patient feedback and doctor's orders.
- (5) Dietary guidance: Patients were provided with dietary recommendations to promote healing and overall health. This included guidance on nutrient-rich foods, hydration, and dietary adjustments to manage side effects of treatment.
- (6) Exercise guidance: To aid in recovery and improve physical function, patients were advised on appropriate physical activities. This included gentle exercises to improve mobility and prevent lymphoedema, progressing to more active exercises as the patient's condition allowed.

Patients in the observational group received both emotional nursing and acupuncture in addition to routine nursing. The emotional nursing methodology was as follows:

- (1) Enlightenment: We clarified basic medical knowledge to the patients and corrected their negative perceptions about their disease conditions. We prioritised active communication with patients, sought to understand their concerns, and provided mental encouragement.
- (2) Emotional venting: We guided patients to express their feelings, share their worries, and release emotions, which sometimes included crying.
- (3) Diversion of attention from negative emotions: We selected suitable music, books, and physical exercises for patients based on their personality, habits, and educational background, aiming to distract them from dwelling on negative emotions.

- (4) Enhancement of positive emotions: We assisted patients in recalling happy memories from their past and cultivated a hopeful outlook towards their future, which served to bolster their confidence in their fight against breast cancer.
- (5) Suggestion: Patients intuitively absorbed the thoughts and suggestions of the medical staff through behavioural or linguistic cues, thereby improving their collaboration and confidence in the treatment process.

The acupuncture procedures were performed by qualified acupuncturists with over 5 years of experience in oncology. The acupuncturists sterilised the skin covering the acupuncture points LR3 (located on the dorsal side of the feet and in the depression before the junction of the first and second metatarsal bones) and LA14 (located below the nipple, the sixth intercostal space, 4 cun [4×3.33 cm] from the anterior median line) before inserting needles (0.3 × 40 mm; Hwato, China) at LR3 at the depth of 0.8 cun via perpendicular insertion and LA14 at the depth of 0.8 cun via horizontal insertion. All needles were manually manipulated to achieve de qi, a state characterised by soreness, numbness, or distension. The needles were left in place for 30 min, and this process was repeated 5 times a week over a period of 6 weeks. The patient's physical and emotional responses during treatment were observed and terminated if there was any abnormality.

Observational index

We evaluated the changes in the self-reported depression scale (SDS) and self-assessment anxiety scale (SAS) scores before and after treatment in both the control and observational groups. The primary indicator for both scales is the total score.

For the SDS, a benchmark of 50 points serves as the demarcation for diagnosing depression. The higher the score, the more severe the depression.

Similarly, for the SAS, which comprises 20 items in total, a score of 40 or above indicates the presence of anxiety in patients. A higher score signifies a more pronounced tendency towards anxiety.

Haematoxylin-eosin staining

Breast cancer samples and adjacent normal samples were fixed using 3% paraformaldehyde embedded in paraffin and sectioned into 5- μ m-thick slices. Then, the samples were stained with haematoxylin-eosin staining (HE, Beyotime, Shanghai, China), and the histological changes were observed under a microscope.

Immunohistochemistry (IHC)

The tissue sections were deparaffinised with xylene (Sigma-Aldrich) and rehydrated in an alco-

hol gradient. The antigen was retrieved by citric acid buffer, and then sections were washed with PBS, followed by treatment with 3% hydrogen peroxide solution. Then, the sections were cultured with anti-p-p38 (ab4822, 1 : 100, Abcam) overnight at 4°C, followed by 3 PBS washes. Then, the sections were incubated with the secondary antibody at ambient temperature for 60 min. A DAB Horseradish Peroxidase Colour Development Kit (Beyotime) was used for colour development. An Olympus microscope was used to capture the images in each group. Positive stained cells ranged from 0–5%, 6–25%, 26–50%, 51–75%, and 76–100%, respectively. Staining intensity was scored on a scale of 0 to 3, corresponding to negative, weakly positive, moderately positive, and strongly positive. The Immune Reactivity Score (IRS) was defined as the product of the degree score and the intensity score. An IRS of ≤ 3 was defined as negative and > 3 was scored as positive. Two experts evaluated all specimens in a blinded manner [32].

Cell culture and treatment

Human breast cancer cell lines (MDA-MB-231, SKBR-3) were provided by Procell Life Science & Technology Co., Ltd. (Wuhan, China). MDA-MB-231 cells were incubated in DMEM (Thermo Fisher) with 10% FBS and 1/100 penicillin–streptomycin. SKBR-3 cells were incubated in McCoy's 5A medium (Thermo Fisher) with 10% FBS and 1/100 penicillin–streptomycin. All cells were maintained in humidified incubators at 37°C with 5% CO₂. For IL-6 treatment, cells were stimulated with 10 ng/ml (MedChemExpress) for 24 h [33]. For anisomycin treatment, cells were stimulated with 20 μ g/ml anisomycin (Santa Cruz Biotechnology) for 6 min to activate p38 signalling [34].

Cell transfection

For ST8SIA6-AS1 and LINC00504 knockdown, sh-ST8SIA6-AS1#1/#2 and sh-LINC00504#1/#2 were obtained from GeneChem (Shanghai, China). Plasmid transfection into MDA-MB-231 and SKBR-3 cells was conducted by Lipofectamine 3000 (Invitrogen, USA) in line with the manufacturer's protocol for 48 h.

RT-qPCR

A TRIzol Kit (Invitrogen) was used for total RNA isolation from breast cancer tissue samples and cell lines. The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) was applied for cDNA synthesis. Then, qPCR was conducted using LightCycler FastStart DNA MasterPLUS SYBR Green I mix (Roche) on the ABI-7500 platform. Relative RNA levels were evaluated by the 2^{- $\Delta\Delta$ C_q} method with GAPDH as the internal con-

trol. The sequences of the primers are presented below:

ST8SIA6-AS1
F: 5'-ACCTCCCTCTTCTGATCTC-3',
R: 5'-TGTCAGTTCCAATAGGTTCTG-3';
LINC00504
F: 5'-ATGACTCTGAGGCCTACAC-3',
R: 5'-TTCCACCACACTGAAACTG-3';
GAPDH
F: 5'-TCATTTCTGGTATGACAACGA-3',
R: 5'-GTCTTACTCCTTGGAGGCC-3';

Western blot

Total protein isolation from breast cancer tissue samples and cells was conducted using RIPA lysis buffer (Thermo Fisher). A BCA Detection Kit (CW Biotech, Beijing, China) was applied for protein concentration evaluation. Next, protein was loaded on SDS-PAGE gels and subsequently electrotransferred to PVDF membranes (Bioered, China). Next, anti-IL-6 (ab233706, 1 : 1000, Abcam), anti-IL-1 β (ab254360, 1 : 1000, Abcam), anti-TNF- α (ab183218, 1 : 1000, Abcam), p-p38 (ab4822, 1 : 1000, Abcam), and p38 (ab170099, 1 : 1000, Abcam) were co-incubated with the membranes at 4°C overnight with GAPDH as a loading control. After culturing with the secondary antibodies for 60 min at ambient temperature, Super Signal enhanced chemiluminescence (Pierce) was applied for visualisation, and ImageJ software was used for quantification.

Enzyme-linked immunosorbent assay (ELISA) assay

The concentrations of inflammatory factors (IL-6, TNF- α , IL-1 β) in breast cancer patient serum were measured using Human IL-6 ELISA Kit (PI330, Beyotime), Human IL-1 β ELISA Kit (PI305, Beyotime), and Human TNF- α ELISA Kit (PT518, Beyotime) following the manufacturer's protocol.

Cell viability

The breast cancer cell viability after the indicated treatment was measured with Cell Counting Kit-8 assay kits (C0037, Beyotime). Cells were seeded into 96-well plates at 2000 cells/well. Then, each well was supplemented with 10 μ l CCK-8 solution and maintained for another 2 h. Infinite M200 (Tecan) was applied to examine the OD value at 450 nm.

TUNEL assay

The treated breast cancer cells were fixed using 4% formaldehyde at 4°C for 25 min and then subjected to 0.2% Triton X-100 for 5 min. A TUNEL Apoptosis Detection Kit I POD (Boster, USA) was applied to evaluate breast cancer cell apoptosis

following the manufacturer's protocol. DAPI was applied to stain the nucleus. The images were captured using fluorescence microscopy.

Statistical analysis

GraphPad Prism 8 software was used for result analysis. The results are presented as the mean \pm standard deviation. The one-way ANOVA was performed to compare the differences among the groups. Student's *t*-test was performed to compare the differences between 2 groups. A *p*-value less than 0.05 indicated statistical significance.

Results

Interventional approaches mitigate inflammatory response in breast cancer patients suffering from liver depression and stagnation

We utilised the SDS and SAS scales to gauge the levels of anxiety and depression in breast cancer patients undergoing liver depression and stagnation. After administration of emotional nursing and acupuncture interventions, it was observed that patients in the study group exhibited a noteworthy reduction in SDS and SAS scores compared to the control group (Figure 1 A). Further analysis of serum samples from these patients highlighted a notable decline in the concentrations of inflammatory markers such as IL-6, TNF- α , and IL-1 β after treatment (Figure 1 B). This trend was mirrored in tumour samples, which also displayed heightened levels of these proteins, indicating a severe inflammatory response compared to adjacent normal tissues (Figures 1 C, D).

ST8SIA6-AS1 and LINC00504: significant players in breast cancer patient survival

Subsequently, we scrutinised the gene expression variations in individuals experiencing depression and breast cancer. Analysis of the GSE217811 dataset revealed 7183 elevated RNAs in the plasma exosomes of patients with adolescent major depressive disorder (logFC < 0). Further filtering through the GEPIA database identified 85 highly expressed RNAs, with 4 shared RNAs, including lncRNAs ST8SIA6-AS1 and LINC00504, being chosen for deeper analysis (Figure 2 A). In-depth study of these lncRNAs revealed their increased presence in breast cancer tissues compared to normal samples, with a significant decrease observed in the study group, indicating the positive impact of emotional nursing and acupuncture on their expression levels (Figures 2 B–D). Notably, variations in ST8SIA6-AS1 and LINC00504 levels had implications on disease-free and overall survival rates in breast cancer patients (Figures 2 E, F).

The p38 pathway: a crucial participant in breast cancer progression

Further, the effectiveness of emotional nursing and acupuncture at a molecular level was investigated. It was discerned that the levels of phosphorylated p38 were markedly higher in breast cancer tissues when compared to healthy tissues across both study and control groups (Figures 3 A, B). The results of immunohistochemistry showed

that the expression of P-P38 was significantly increased in breast cancer tissues when compared to control groups. The suppression of p38 pathway activation observed in the study group was noteworthy, indicating the inhibitory role of the applied interventions on this pathway in breast cancer (Figure 3 C). Additional data suggested a modest positive correlation between p38 α and the lncRNAs LINC00504 or ST8SIA6-AS1 in breast cancer specimens (Figure 3 D).

A

Parameters	Groups	Before treatment	After treatment	P-value
SAS	Control	65.32 \pm 9.53	53.25 \pm 8.87	< 0.001
	Observational	66.29 \pm 9.29	46.38 \pm 7.83	
SDS	Control	61.32 \pm 10.42	54.39 \pm 9.46	< 0.001
	Observational	63.47 \pm 9.51	51.43 \pm 8.58	

B

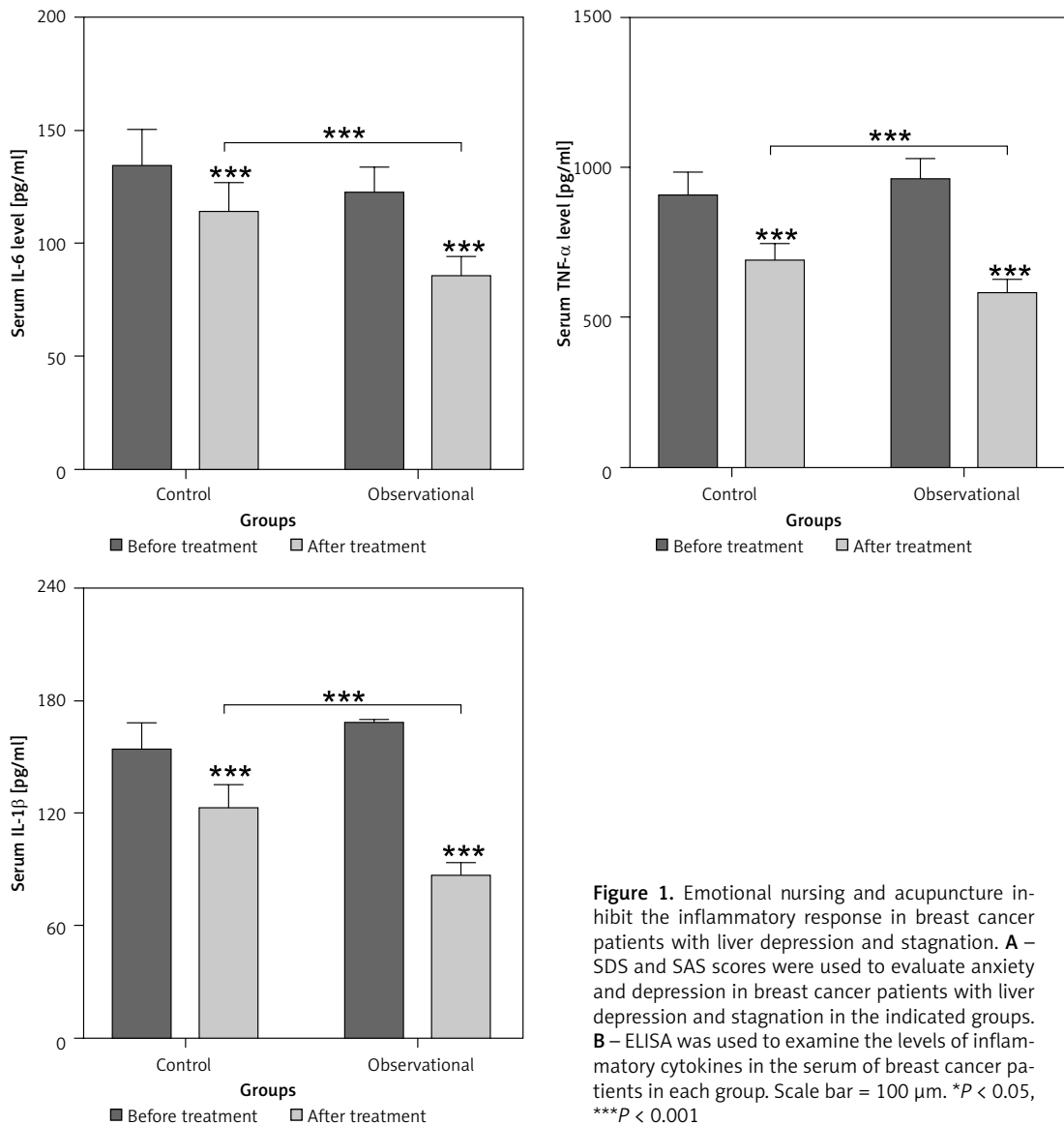
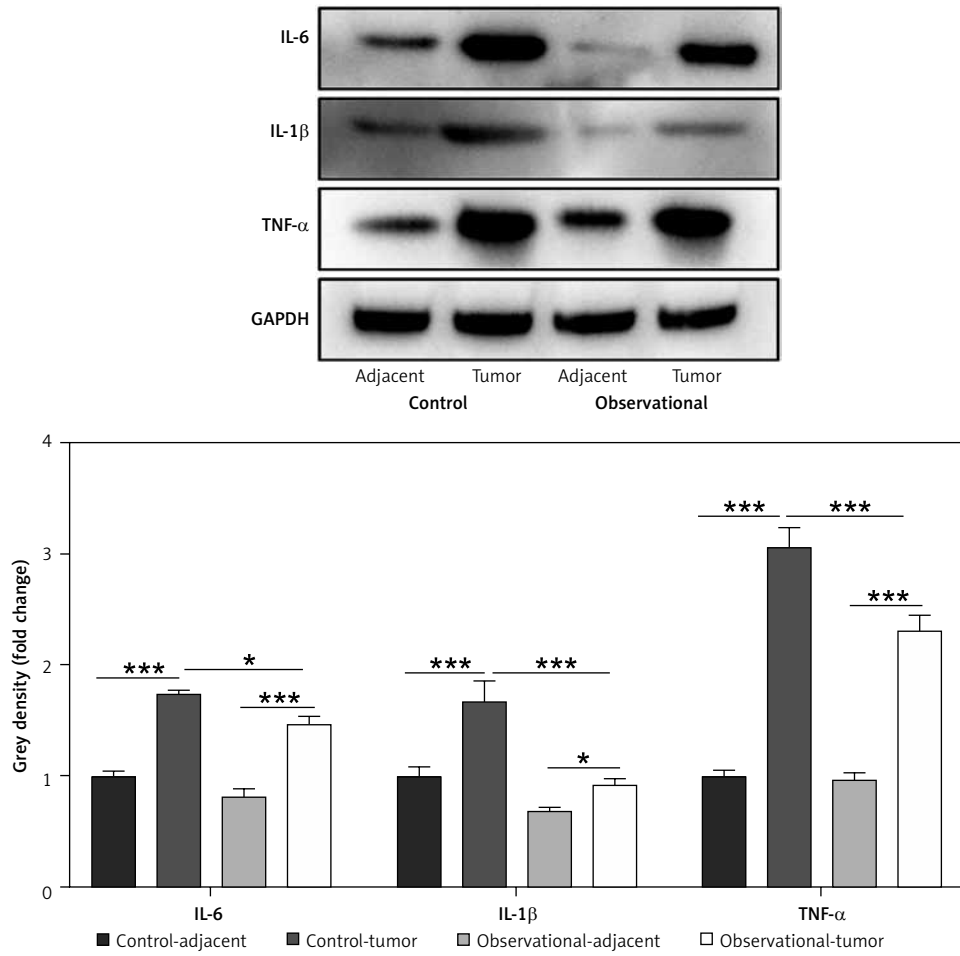


Figure 1. Emotional nursing and acupuncture inhibit the inflammatory response in breast cancer patients with liver depression and stagnation. **A** – SDS and SAS scores were used to evaluate anxiety and depression in breast cancer patients with liver depression and stagnation in the indicated groups. **B** – ELISA was used to examine the levels of inflammatory cytokines in the serum of breast cancer patients in each group. Scale bar = 100 μ m. *P < 0.05, ***P < 0.001

C



D

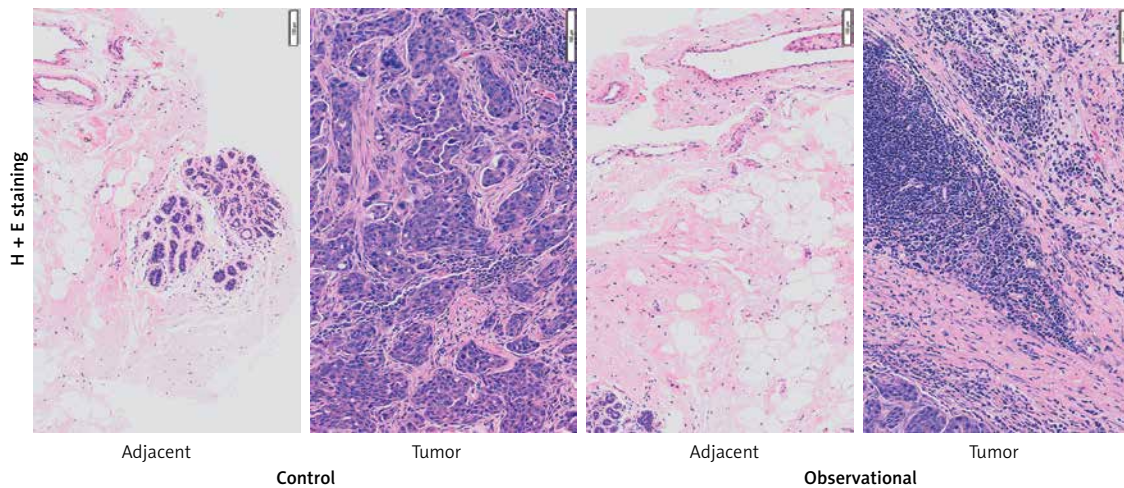
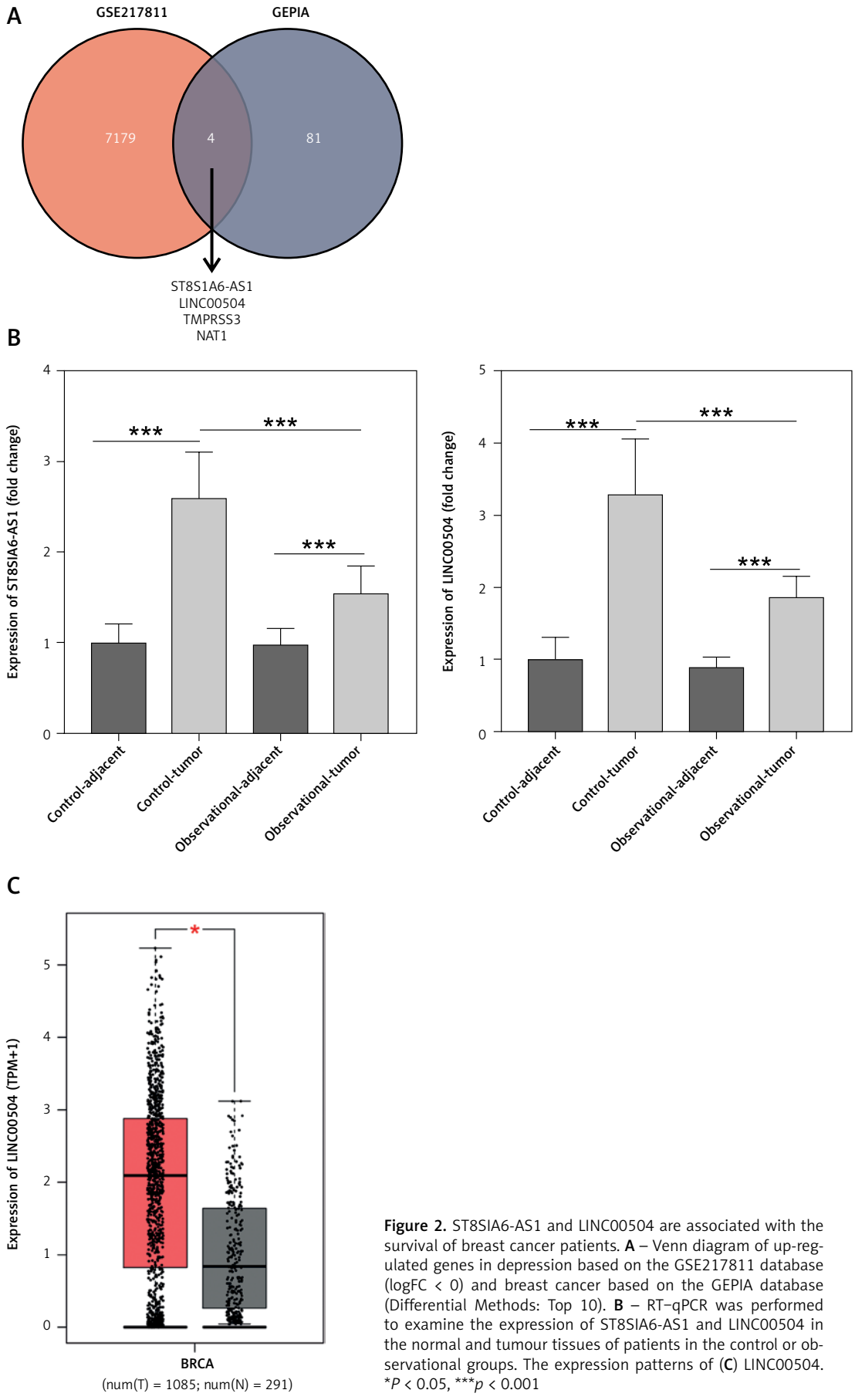
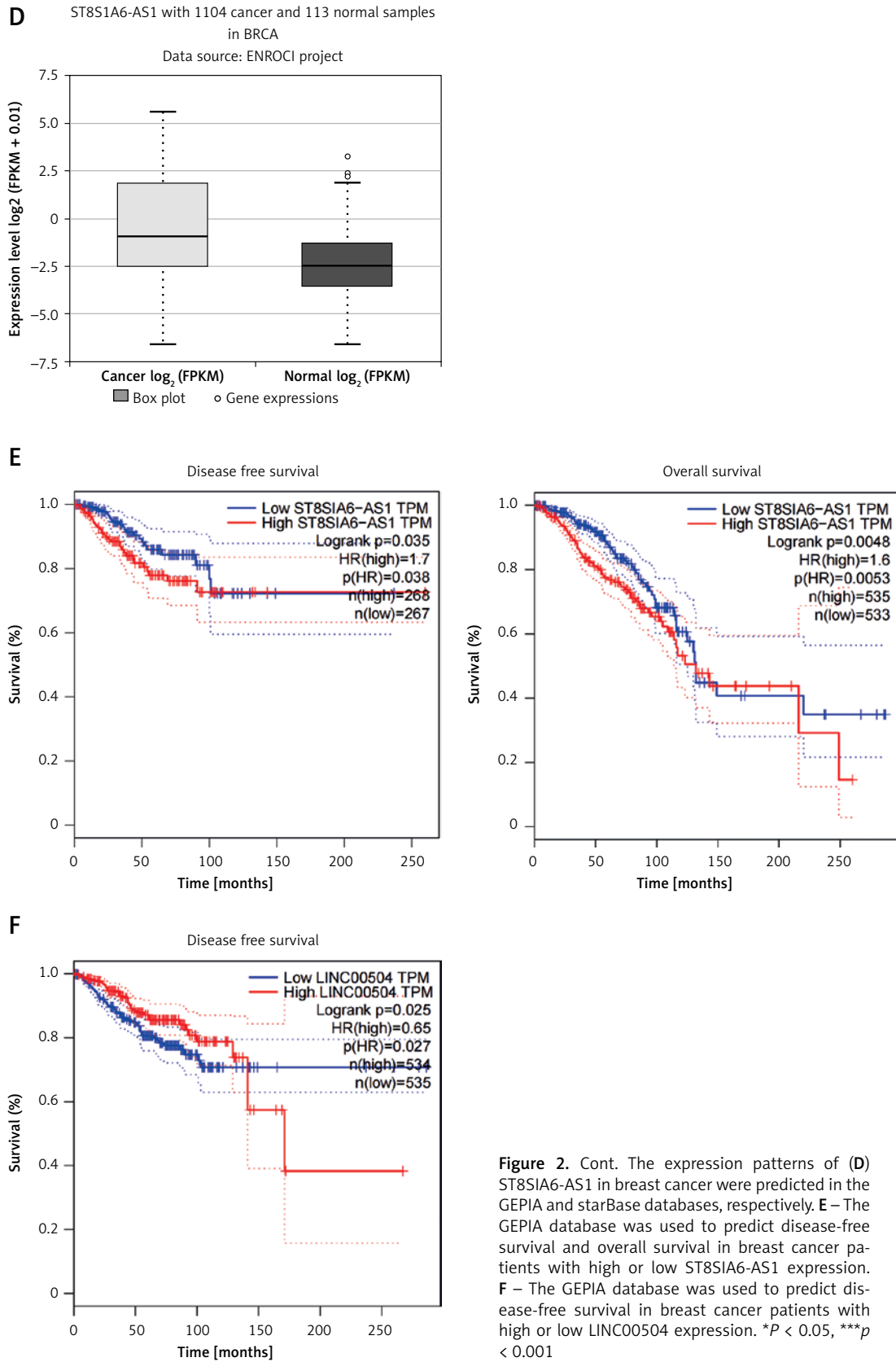


Figure 1. Cont. C – Western blotting was used to detect the protein levels of IL-6, TNF- α , and IL-1 β in each group. D – HE staining was performed to examine the histological changes in tumour tissues and adjacent normal tissues after the indicated treatments. Scale bar = 100 μ m. * P < 0.05, *** P < 0.001





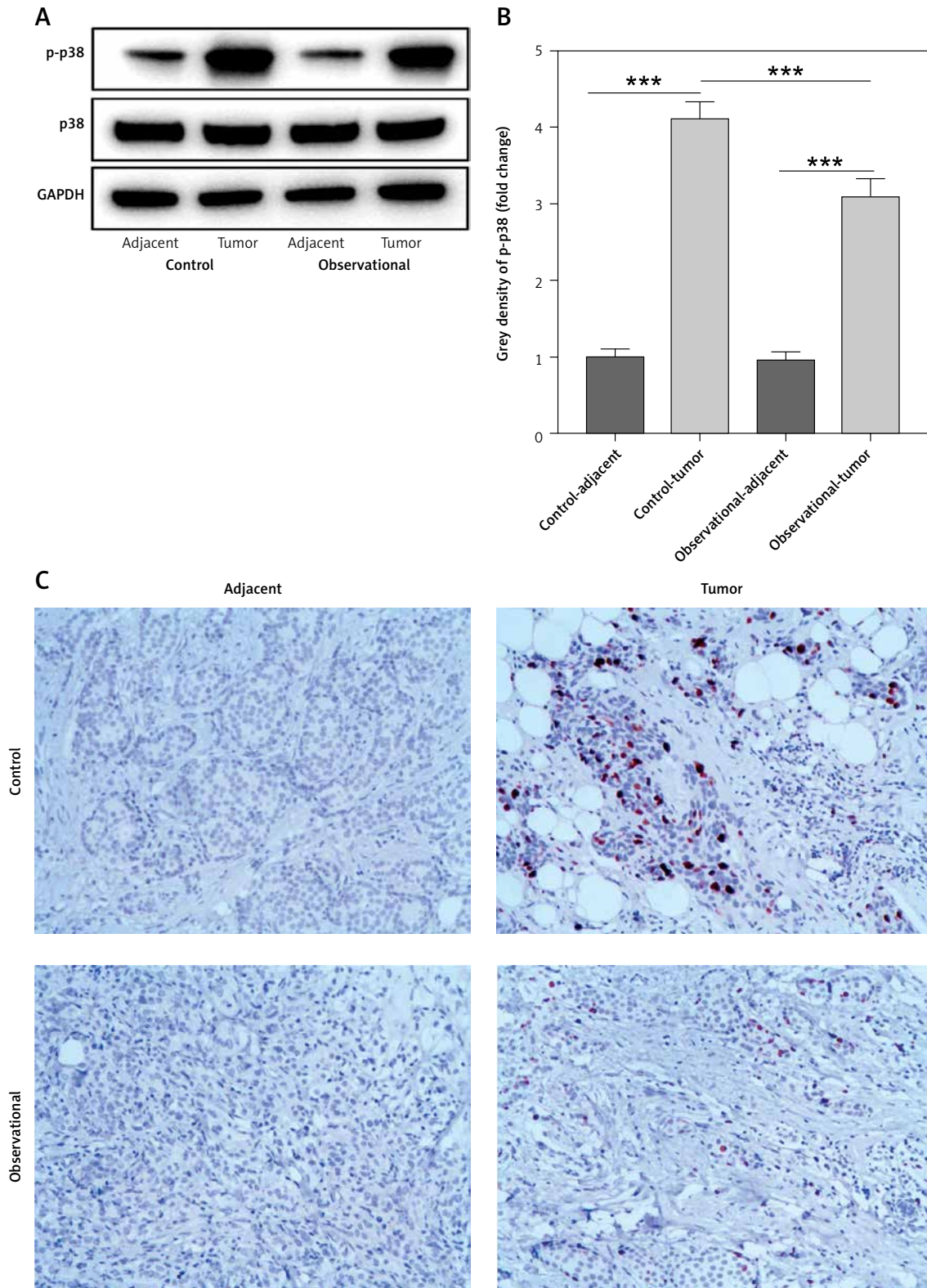


Figure 3. The p38 pathway is involved in the progression of breast cancer. **A, B** – Western blotting was performed to detect the protein levels of p-p38 and p38 in the tumour tissues and adjacent normal tissues in the control and observational groups. **C** – Immunohistochemistry was used to examine p-p38 protein expression in breast cancer tumour tissues and adjacent normal tissues of the indicated groups. Scale bar = 50 μm. *** $P < 0.001$

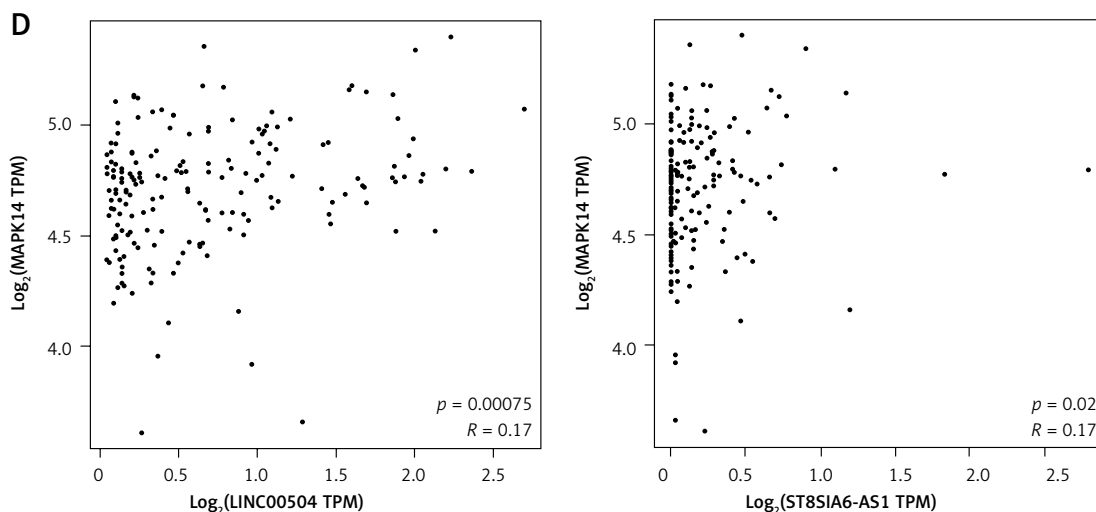


Figure 3. Cont. **D** – The GEPIA database was used to explore the correlation between the expression of MAPK14 (p38 α) and LINC00504/ST8SIA6-AS1 in breast cancer tissues. Scale bar = 50 μm . *** $P < 0.001$

Knockdown of ST8SIA6-AS1 and LINC00504 modulates IL-6-induced inflammatory responses in breast cancer cells

To further analyse the role of ST8SIA6-AS1 and LINC00504, their expression was silenced in MDA-MB-231 and SKBR-3 cells, which led to a significant decrease in cell viability (Figures 4 A, B). Moreover, this silencing counteracted IL-6-induced inflammatory responses, as shown by reduced levels of inflammatory proteins (Figures 4 C, D).

Silencing of ST8SIA6-AS1 and LINC00504 hinders the activation of the p38 pathway

Further investigations into the effects of ST8SIA6-AS1 or LINC00504 silencing revealed a substantial suppression of p-p38 protein levels without significantly affecting p38 levels, indicating a potential dampening of p38 pathway activation in the breast cancer cells (Figures 5 A, B).

ST8SIA6-AS1 and LINC00504 modulate IL-6-driven inflammatory response through the p38 pathway

Finally, we examined the regulatory effects of ST8SIA6-AS1 and LINC00504 on IL-6-induced inflammatory reactions at the pathway level. The use of anisomycin, a known activator of the p38 pathway, showed a rescuing effect against the apoptosis induced by ST8SIA6-AS1 or LINC00504 silencing, indicating that these lncRNAs might be mediating the IL-6-driven inflammatory reactions in breast cancer cells via the p38 pathway (Figures 6 A, B).

Discussion

Our research reveals a notable enhancement in the mental well-being and a decrease in the

inflammatory response in breast cancer patients who underwent emotional nursing and acupuncture therapies, as observed in the study group. This therapy modality appears to hinder the activation of the p38 signalling pathway in breast cancer cells. Additionally, the lncRNAs ST8SIA6-AS1 and LINC00504 were identified as facilitators of the IL-6-mediated inflammatory reactions in breast cancer cells, working through the p38 pathway.

An increasing body of research is highlighting the critical function of lncRNAs in a range of human ailments, encompassing both depression and cancer [23, 35, 36]. Numerous studies have spotlighted the role of ST8SIA6-AS1 in aiding the proliferation, migration, and invasion capabilities of cancer cells in a variety of malignancies including but not limited to hepatocellular carcinoma, cholangiocarcinoma, and lung adenocarcinoma [37, 38]. Similarly, LINC00504 has been found to influence tumour metabolism in colorectal cancer, encouraging the growth and initiation of cancer cells [39]. Furthermore, it has been noted that the knockdown of LINC00504 suppresses the proliferative, migrative, and invasive potentials in both breast and lung cancers [26, 40]. Yet, the interaction between these 2 lncRNAs and depression during cancer development remains scarcely explored. By utilising the GEO database (GSE217811) to analyse up-regulated genes in adolescents with major depressive disorder (MDD) and cross-referencing with the elevated gene expression in breast cancer from the GEPIA database, we isolated lncRNAs ST8SIA6-AS1 and LINC00504 for our study. We determined that both these lncRNAs exhibit higher levels of expression in breast cancer tissues and have a correlation with patient survival rates. Importantly, the deployment of emotional nursing and acupuncture therapies

showcased a decrease in the expression levels of ST8SIA6-AS1 and LINC00504 in breast cancer tissues. The silencing of these lncRNAs appeared to neutralise the repercussions of IL-6 treatment on cell survival, apoptosis, and inflammatory reactions in MDA-MB-231 and SKBR-3 cells, aligning with prior research outcomes.

The p38 MAPK pathway, which often finds itself disrupted during the progression of cancer, plays a central roles in stress responses and inflammatory processes, being a prominent player in the onset, growth, and metastasis of various cancer types [41, 42]. Existing literature suggests that acupuncture can potentially mitigate inflamma-

tion through the attenuation of p38 MAPK, ERK, and JNK signalling pathways, and by altering the release patterns of inflammatory mediators [43]. Separate clinical trials in China have also highlighted the efficacy of emotional nursing interventions in reducing inflammatory reactions in individuals with ailments like sepsis, plasma cell mastitis, and ulcerative colitis [44–46]. Our investigation corroborates the potential of emotional nursing and acupuncture in reducing inflammatory reactions and curbing p38 signalling pathway activation in breast cancer tissues. Moreover, the inactivation of the p38 pathway was noted upon the silencing of ST8SIA6-AS1 and LINC00504, while the admin-

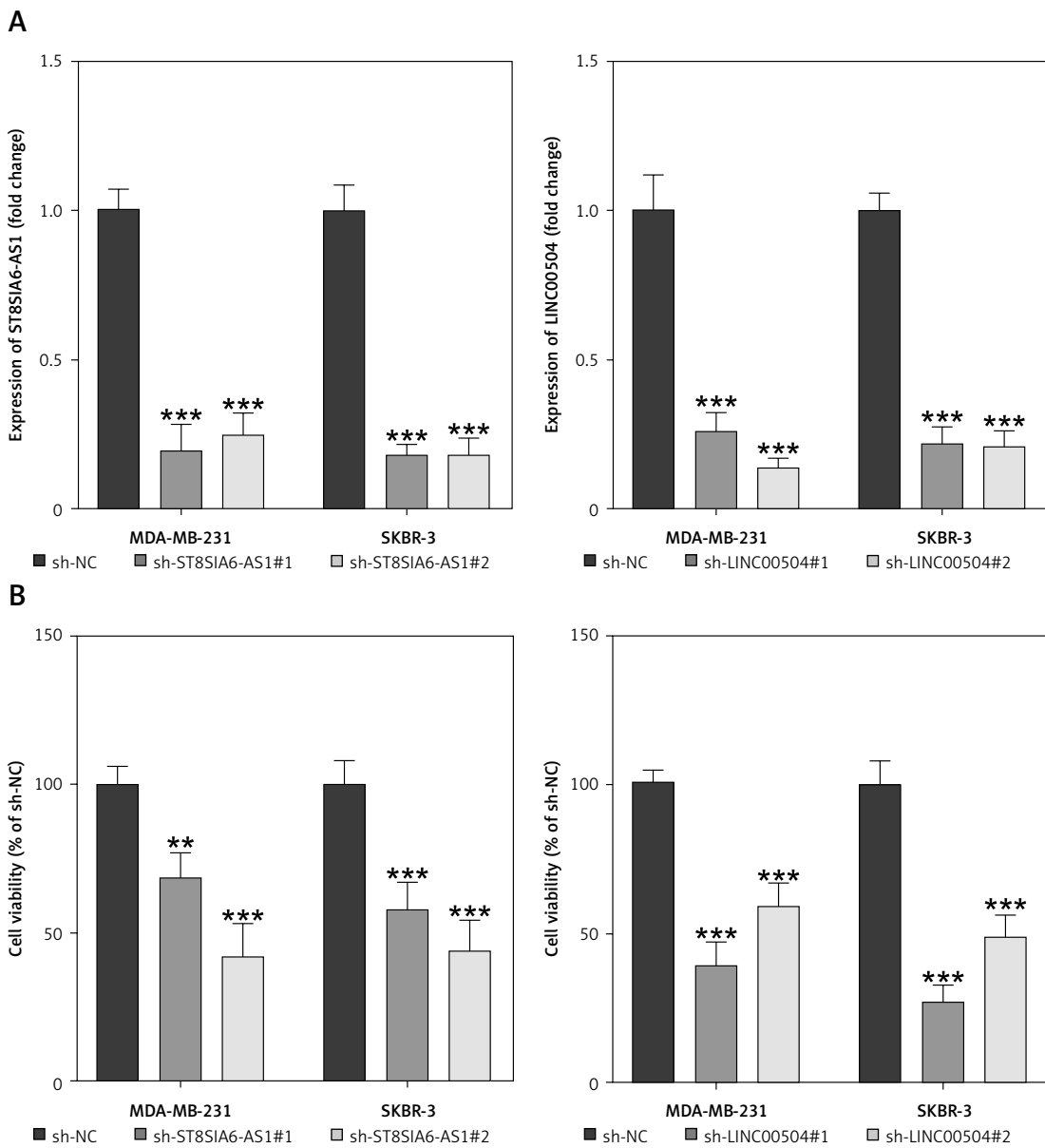
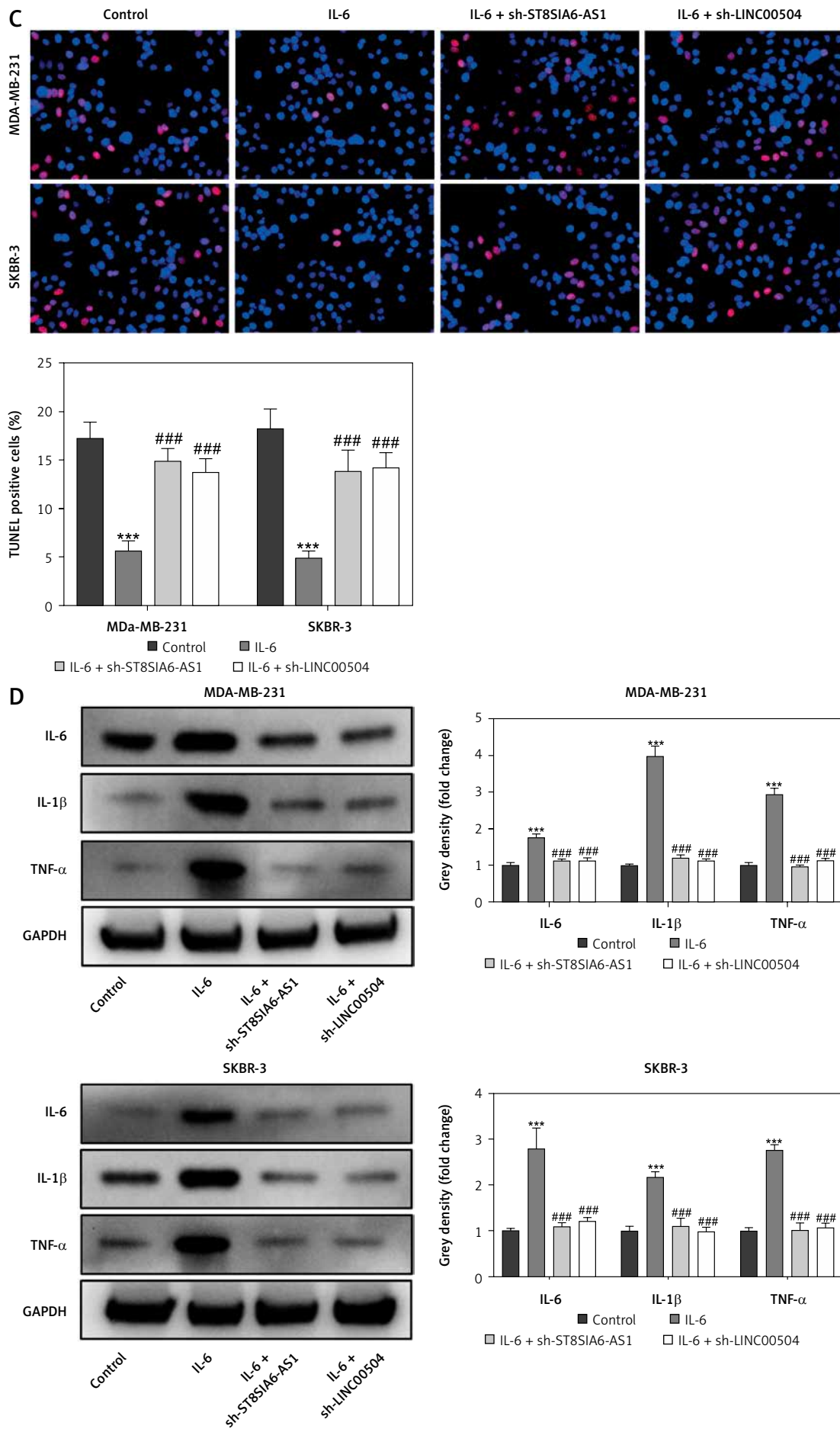


Figure 4. Silencing of ST8SIA6-AS1 and LINC00504 rescued the IL-6-induced inflammatory response of breast cancer cells. **A** – RT-qPCR analysis was conducted to examine the knockdown efficacy of ST8SIA6-AS1 and LINC00504 in breast cancer cells. **B** – CCK-8 assays were used to detect the viability of breast cancer cells after ST8SIA6-AS1 or LINC00504 silencing. ** $P < 0.01$, *** $p < 0.001$ compared with the control group; ### $P < 0.001$ compared with the IL-6 group



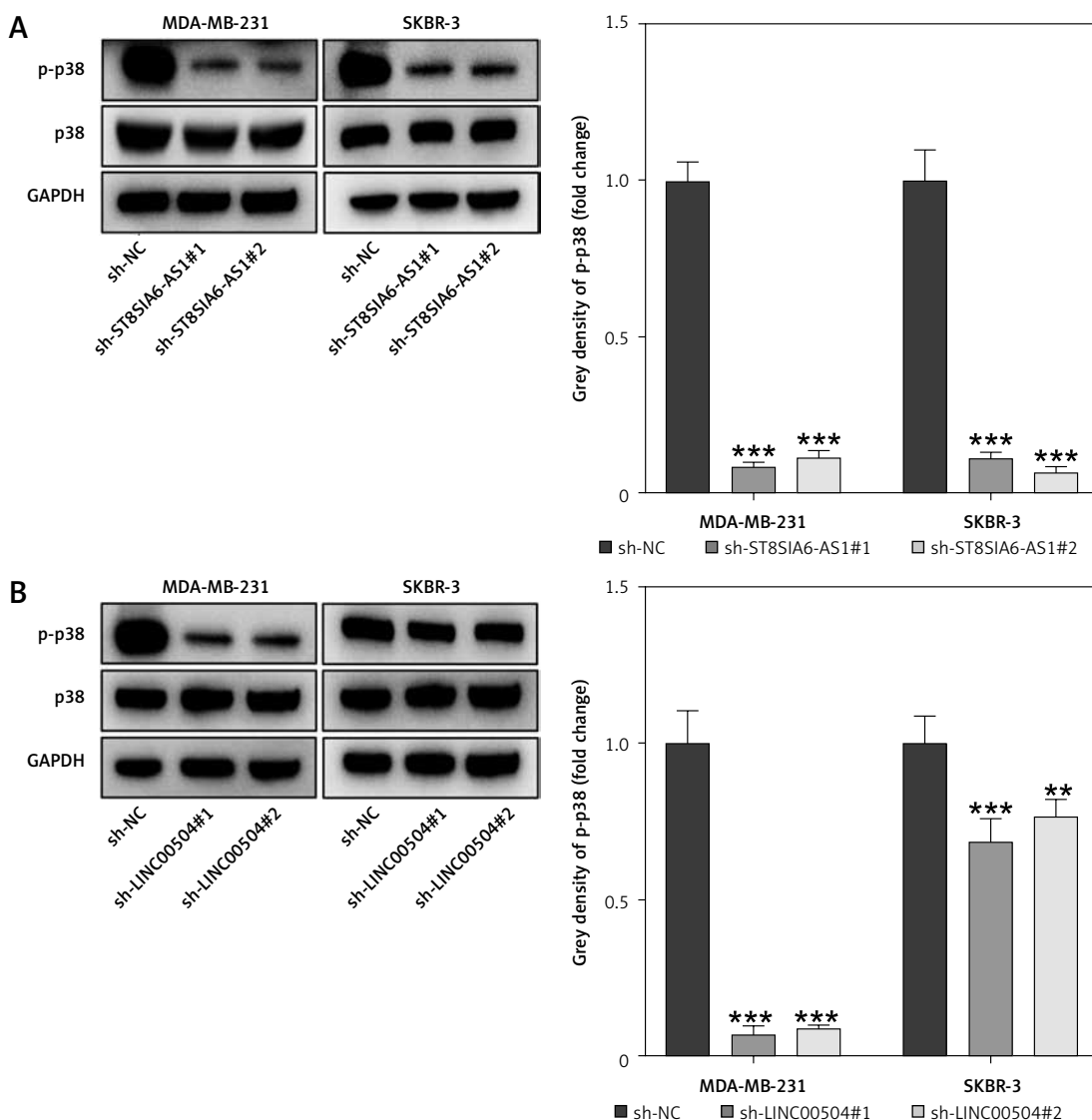


Figure 5. Silencing of ST8SIA6-AS1 and LINC00504 inactivates the p38 pathway. Western blotting was conducted to examine the protein levels of p-p38 and p38 after (A) ST8SIA6-AS1 or (B) LINC00504 knockdown in breast cancer cells. ** $P < 0.01$, *** $p < 0.001$

istration of anisomycin, a potent stimulator of the p38 pathway, reversed the escalated cell apoptosis and diminished inflammatory marker expression induced by the silencing of these lncRNAs. However, there are some limitations to this study. Factors such as breast cancer type, patient age, clinical characteristics, sample size, etc., increase the likelihood of bias. We found that emotional care and acupuncture can alleviate symptoms of hepatic depression and stagnation in breast cancer patients, and briefly elucidated the underlying mechanisms. In subsequent studies, we will use animal models and expand the study sample to delve into the details of their mechanisms.

In conclusion, the study illustrates that emotional nursing and acupuncture could serve as viable strategies in mitigating inflammation in breast cancer patients experiencing liver depression and

stagnation, chiefly through the downregulation of ST8SIA6-AS1 and LINC00504, which in turn inactivates the p38 pathway. This research potentially amplifies our comprehension of the mechanistic basis and potential impact of emotional nursing and acupuncture in the treatment regimen for breast cancer patients. It also has the advantages of high safety, few side effects, and strong patient adaptability, which provides an evidence-based foundation for clinical treatment of traditional Chinese medicine and is worthy of clinical promotion.

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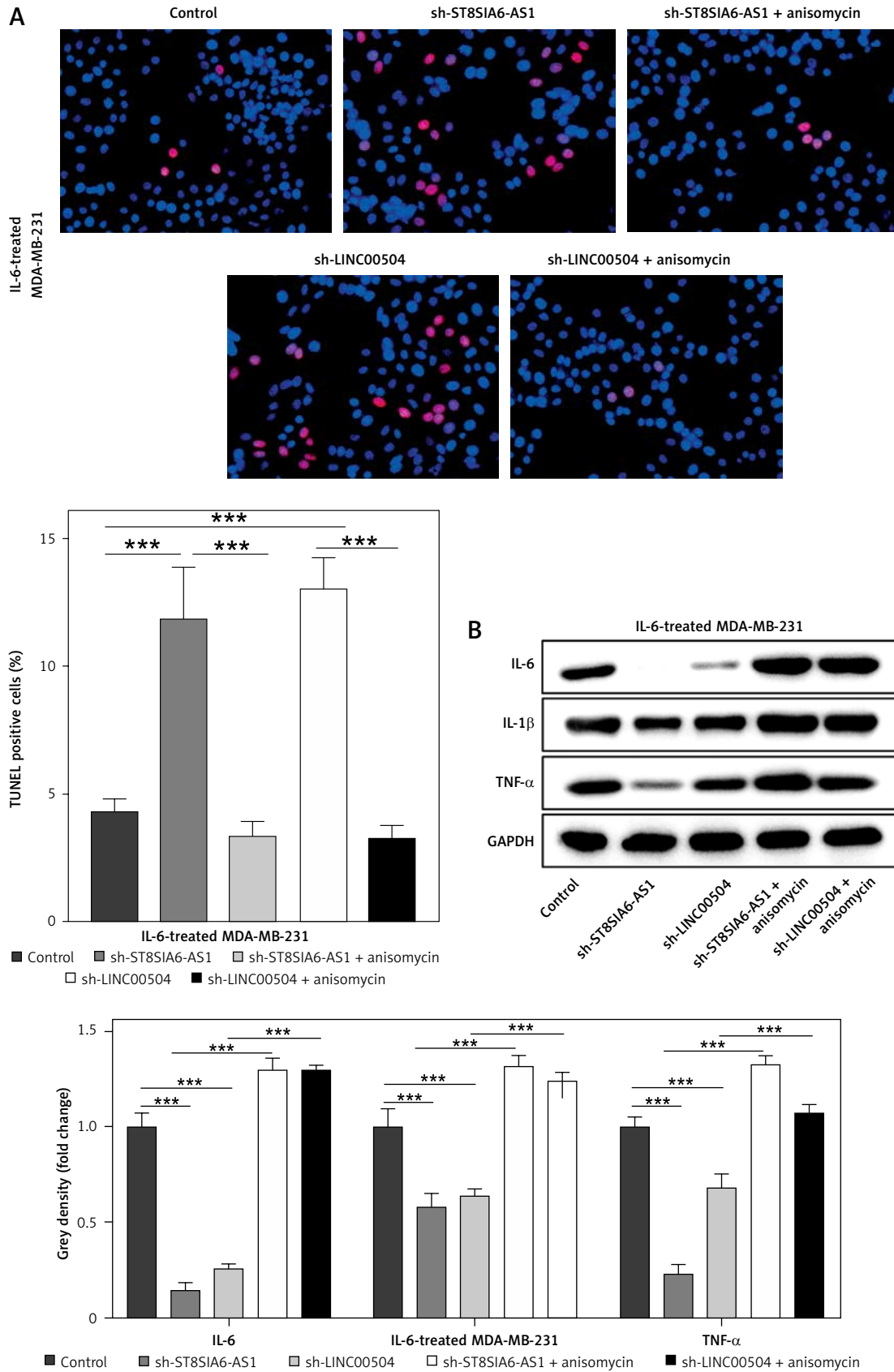


Figure 6. ST8SIA6-AS1 and LINC00504 regulate the IL-6-induced inflammatory response of MDA-MB-231 cells by the p38 pathway. **A** – TUNEL assays were conducted to examine the apoptosis of MDA-MB-231 cells after ST8SIA6-AS1 and LINC00504 knockdown and anisomycin treatment. **B** – Western blotting was used to detect the protein levels of inflammatory cytokines (IL-6, IL-1β, and TNF-α) in each group. *** $P < 0.001$

Ethics approval

GS[L]2020.

Conflict of interest

The authors declare no conflict of interest.

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