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Original Research

Downregulation of Hsp27 inhibits proliferation, migration and invasion in human choriocarcinoma cell line JAR

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Summary

Purpose of Investigation: Heat shock protein 27 (Hsp27), a member of the heat shock protein (Hsp) family, is critical in the stress response. However, the role of Hsp27 in the pathogenesis of preeclampsia remains unknown. The aim of the present study was to examine effect of downregulation of Hsp27 in proliferation, migration, and invasion in human JAR cells. *Materials and Methods:* To determine their effect, siRNA interference was used to knock down Hsp27 expression in JAR lines. The effects of transfected cells were screened for HSP27 expression by Western blotting analysis. Cell proliferation determined by CCK-8 assay and cell clonogenic assay. Wound healing assay measured the migration ability. Transwell assay measured migration and invasion ability. *Results:* Downregulation of Hsp27 inhibits proliferation, migration, and invasion in human choriocarcinoma cell line JAR. *Conclusion:* The present data suggested that Hsp27 may play an important role in preeclampsia.

Key words: Hsp27; siRNA; Proliferation; Migration; Invasion.

Introduction

Preeclampsia is a major cause of maternal morbidity and mortality, but the pathophysiology of this disorder remains unknown. Recent studies have implicated placental oxidative stress as having a key role in the generation of this disease [1-3]. Heat shock protein 27 (Hsp27) is a well-known stress response protein [4]. Like other gestational disease, preeclampsia is a chronic and a multiple-step process in which accumulation of genetic and epigenetic alternations are involved. To achieve a more effective treatment of human preeclampsia, understanding the mechanisms that drive preeclampsia progression is essential.

The heat shock proteins (Hsps) are divided into several families according to their molecular weight and the similarity of their amino acid sequences. Many studies have demonstrated that Hsps act as molecular chaperones by stabilizing intracellular proteins when they suffer a pathological insult, such as hyperthermia, ischemia, and oxidative injury [5]. At present, Hsp27 is expressed in mammary tumors and may play a role in tumor growth and response to anti-neoplastic drug therapy. Hsp27 may protect tumor cells from chemotherapy and result in aggressively-growing and therapy-resistant tumors [6]. Knockdown of Hsp27 expression induces apoptosis via Bax activation in a PI3K dependent mechanism in renal epithelial cells and decreases clonogenic survival in HCT116 human colon cancer cells [7]. Although numerous studies regarding Hsp27 have been performed on malignant cells from a solid tumor, little is known about the role of Hsp27 in preeclampsia [8]. It has also been reported that Hsp27 expression is higher in patients with preeclampsia as compared to patients with normal term gestations [9-12]. However, up to date, these have been little reported attempts to study the relationship between Hsp27 and preeclampsia.

To study the function of Hsp27 in the mechanism of preeclampsia, further investigations are required. Therefore, in this study, down-regulation of Hsp27 through siRNA tested the hypothesis weather Hsp27 may have a direct participation in human JAR cell proliferation, migration, and invasion. The present results may provide a new insight into the mechanism of preeclampsia.

Materials and Methods

The human JAR cell lines was originally obtained and maintained regularly in RPMI 1640 medium supplemented with FBS and incubated in a humidified atmosphere at 37°C in 5% CO_2. When they reached ~70-80% confluency, cells were trypsinized and resuspended in an antibiotic-free media.

Small interference RNA (siRNA) treatment

JAR was seeded into six-well plates for 24 hours before transfection in the complete medium. Transient transfection was performed with lipofectamine 2000 according to the manufacturer's protocol. Human JAR cells were transfected with 5 nM control siRNA or human Hsp27 siRNA oligos (5'- ATGAGACTGC-CGCCAAGTA -3'). The transfected cells were cultured in complete medium and incubated at 37 °C for 24 and 48 hours.

For Western blot analysis, four protein samples of human placental tissue were prepared from control groups and four samples

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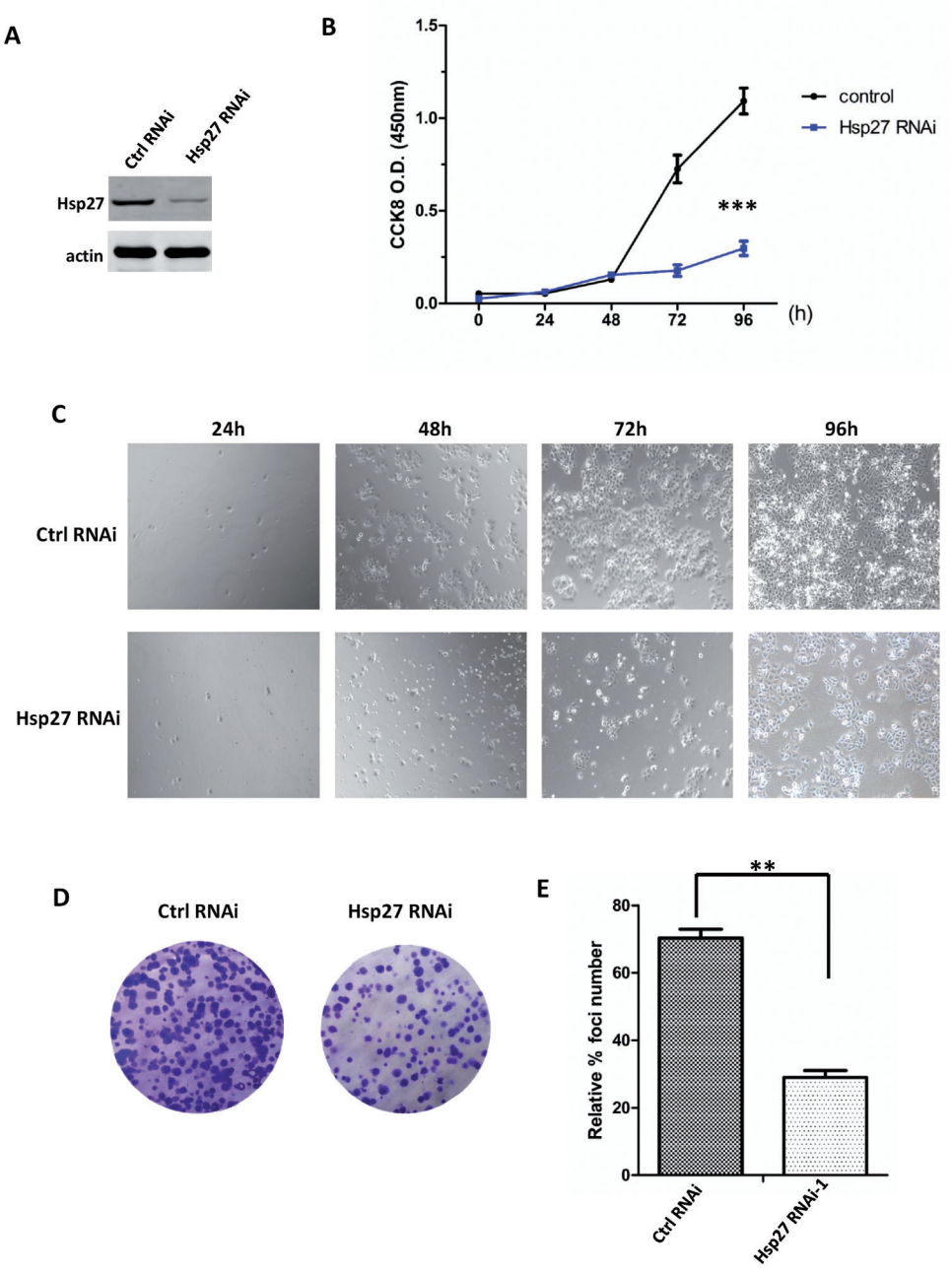

Figure 1. — Silencing of Hsp27 significantly inhibits proliferation in human JAR cell lines. (A) Western blot analysis of Hsp27 expression in siCtrl and siHsp27/JAR cells. β-actin is used as a loading control. (B-C) Silencing of Hsp27 significantly inhibits the proliferation of JAR cells at 72 and at 96 hours compared with the control, as determined by CCK-8 test ($p < 0.05$). (D) Silencing of Hsp27 significantly inhibits the colony formation of JAR cells. The percentage of proliferative cells or colony formation were normalized to that of the control group. All the data are presented as mean±SEM in three repeats (*$p<0.05$).

from PE groups, separately. Human placental tissue was homogenized in 500 μL 1×RIPA buffer containing protease inhibitors (1 μg/mL leupeptin and 1 μg/mL phenylmethylsulfonyl fluoride). Samples were loaded at 30 μg/ lane and separated in a 10% SDS gel. The separated samples were transferred to a nitrocellulose transfer membrane. After incubating for one hour with blocking buffer, the membrane was incubated overnight at 4°C with primary antibodies against Hsp27 (1:1000) and β-Actin (1:1000) at 4°C overnight. After three washes with 1×TBST, pH 7.4, each membrane was incubated with the appropriate secondary antibody (1: 1000) at room temperature for one hour. After additional three washes, protein intensities were visualized with the enhanced ECL detection system.

The confluent cultured JAR were trypsinized and suspended in RPMI 1640 medium with 10% FBS. The cells were plated in 96-well plates at a density of 1×10^4 cells per well with fresh medium and/or siRNA. After treatment for 24, 48 or 72 hours, the cell proliferation was determined by a cell counting kit-8 . The medium of each well was removed and a mixture of 10 μL CCK-8 and 90 μL of 10% FBS RPMI-1640 was added. The plates were incubated for a further three hours, and the absorbance at 450 nm was measured by a microplate reader . All of the groups at each time-point had five wells. The relative cell survival (%) was determined by the following formula: $(OD_{siRNA}/OD_{scramble})\times100\%$.

JAR cells were seeded in six-well plates (500 cells/well) and treated with 0.1 % DMSO (vehicle) or Hsp27 siRNA for 24 hours. After treatment, the medium was removed and fresh medium was

added to the wells. Medium was changed every four days for 10–14 days to allow for colony formation. Then, the cells were fixed with 4 % formaldehyde and stained with 0.1 % crystal violet solution. Finally, positive colony formations were manually counted.

Human JAR cells were starved in 1% FBS culture medium for 24 hours and a wound was made with an even trace using a standard 200-μL pipette tip. Cells were incubated in 5% CO_2 at 37°C for 24 hours, and images of the wound were captured at intervals. Wound healing was quantified by measuring the migratory distance of cells under a light microscope.

Cells (5×10^4 or 1×10^5) were harvested and resuspended in serum-free medium, and then added into the upper chamber of Transwell chambers with polycarbonate membranes (8-μm-pore-size, Corning) coated without or with Matrigel for migration or invasion assay after transfection. RPMI-1640 medium supplemented with 10% FBS were added into the lower chamber. After incubation for 24 hours, the migrated or invaded cells were fixed, stained, and counted by averaging ten fields with an inverted microscope.

Statistical analysis was carried out using Prizm 6 from GraphPad Software. Statistical significance for comparison between groups was determined by using Student's *t*-test. All samples were tested in triplicate, and the data are expressed as means ± SD.

Results

First, the authors sought to down-regulate the mRNA expression of HSP27 in JAR cells using siRNA techniques. First, Hsp27 siRNA sequences were transfected into JAR cells which were indicated as siHsp27/JAR cells. A scrambled siRNA sequence was also transfected to the JAR cells and appointed as Ctrl/JAR cells. The effects of transfected cells were screened for Hsp27 expression by Western blotting analysis. The expression of Hsp27 protein was significantly reduced after silencing with siRNAs (Figure 1 A)

The results of the cell proliferation tested by CCK-8 showed that Hsp27-siRNA-transfected JAR cells exhibiting decreased cell growth ability ($p < 0.05$) compared with the control group (Figures 1 B-C, $p < 0.05$). The CCK-8 experiment confirmed that JAR cells were sensitive to down-regulation of HSP27 displayed an inhibitory effect on the proliferation of human JAR cells. The clonogenic assay also indicated that down-regulation of HSP27 alter colony forming efficiency (Figures 1 D-E).

Migration is a fundamental property of cells that occurs during many physiological and pathological processes including organogenesis in the embryo, repair of damaged tissue after injury and the spread of cancer [13]. The authors examined the potential role of HSP27 in cell migration according to the hypothesis of preeclampsia associated with migration. As shown in Figure 2 A, the authors found that the time required for wound closure of Hsp27-knockdown JAR cells was significantly longer than the time required for the corresponding control cells. A Transwell migration assay further revealed that far fewer siHsp27 JAR cells migrated to the lower chamber, compared with siCtrl JAR cells (Figure 2 B). The present results obviously demonstrated that knockdown HSP27 greatly impaired the migratory ability of JAR cells.

The reduction of the cell migratory ability usually leads to a reduction of the invasive ability [14]. Hsp27 was found to regulate the migration of JAR cells cells in the previous study. In the following study, the authors assessed the invasiveness of the cells with Hsp27 downregulation in Transwell assays to determine whether Hsp27 affects cell invasion. The invasion assay showed significantly differences in invasive ability between the siHsp27 cells and siCtrl cells ($p < 0.01$; Figure 3). The quantitative analysis of cell numbers revealed that the siCtrl cells had a 2.8-fold higher rate of invasion than that of siHsp27/JAR cells. Collectively, these results indicate that Hsp27 plays an important role in the invasion of JAR cells.

Discussion

Hsps have been shown to have pleiotropic biological functions. Hsp60, Hsp70, and Hsp90 are dysregulated in maternal circulation in both mild and severe preeclampsia [15]. Hsp70 also has been proven to play an important role in preeclamptic stress [16]. Recent studies indicated a correlation of Hsp27 with preeclampsia. Pro-

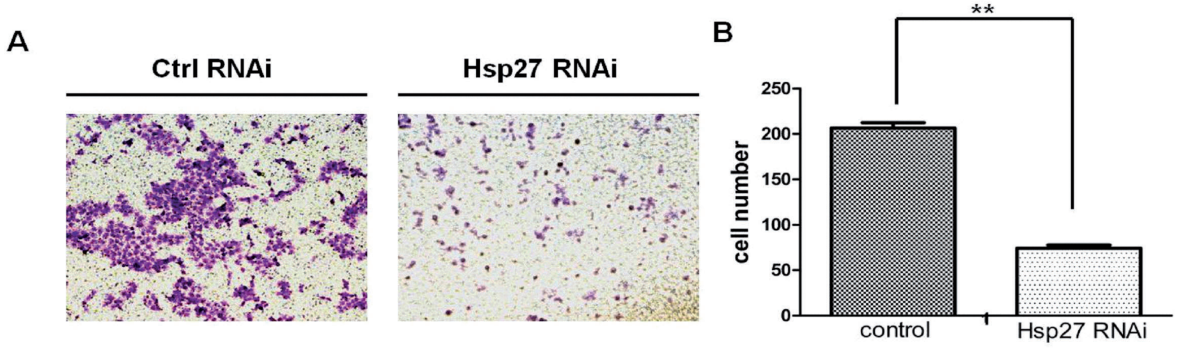

Figure 3. — Reduction of Hsp27 by siRNA impairs invasive ability in vitro. siCtrl and siHsp27 cells invading through Matrigel-coated 8-μm pore size Transwell inserts are evaluated as described in manufacturer's instructions. The number of invading cells is quantified by counting the stained cells in random fields of the membrane.

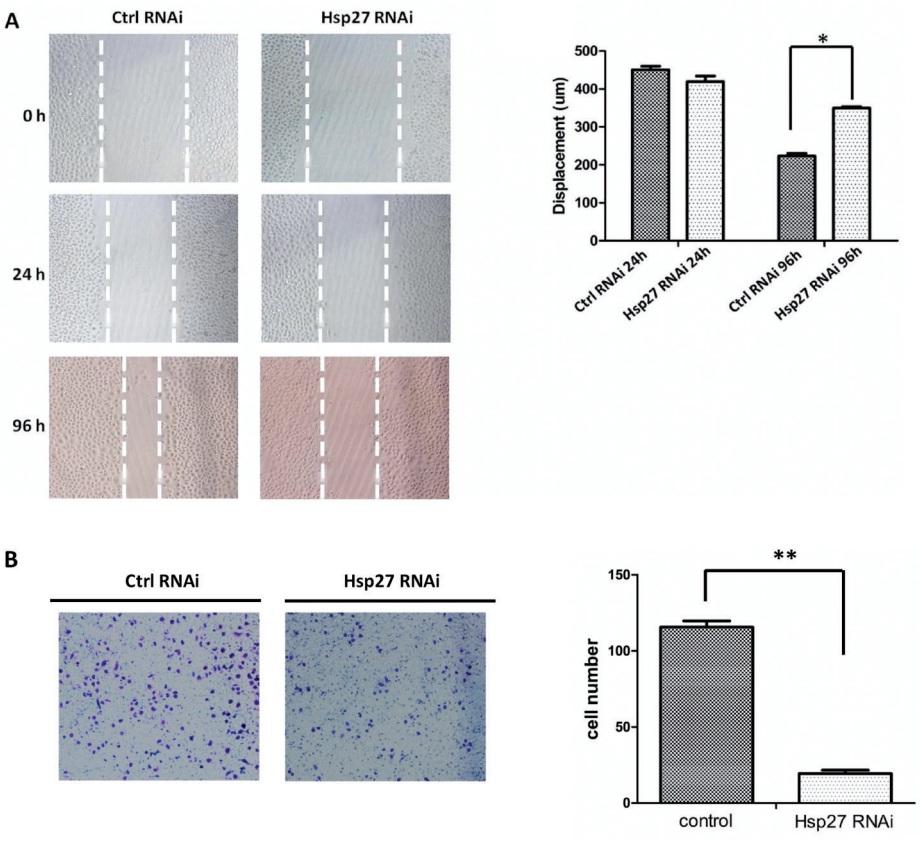

Figure 2. — Reduction of Hsp27 by siRNA impairs migration ability in vitro. (A) Scratch assay in JAR cells. siCtrl and siHsp27 cells are grown to confluence on a 35-mm tissue dish, and the monolayer is wounded with a 200-µL pipette tip. Images are captured at 0, 24 and 96 hours. (B) Representative photographs (left) and quantification (right) of the Transwell migration assay with JAR cells transfected with siCtrl or siHsp27 control (*$p < 0.05$; Student's *t*-test).

teomic analysis reveals an elevated expression of Hsp27 in preeclamptic placentas [10, 11]. Yet the biological significance of Hsp27 in preeclampsia needs further investigation. Thus, the aim of the study was to study the relationship between Hsp27 and the pathogenesis of preeclampsia.

In the present study, the authors decreased the expression level of Hsp27 in JAR cells. Its efficiency and specificity were confirmed by Western blot analysis. The results demonstrated that downregulation of Hsp27 in JAR cells could significantly inhibit cell proliferation. Hsp27 probably functions as a positive regulator in preeclampsia development. Reduction of Hsp27 can impair JAR cells' migration and invasion. Cell migration is a multistep process involving numerous soluble growth factors, cytokines, proteases, and extracellular matrix proteins [17]. The authors found that Hsp27 reduction slows JAR cell migration in cell chemotaxis and wound healing assays suggesting that Hsp27 is involved in JAR cells' migration. Similar to migration, invasion through the extracellular matrix is an important step in preeclampsia [18]. It is also strongly associated with the infiltration and invasion of tumors [19]. The present Matrigel invasion assay showed that reduction of Hsp27 led to a decrease of invasiveness in the JAR cells.

Although various achievements have been obtained in preeclampsia, the detailed understanding of the mechanism remained to be elucidated. Invasion of trophoblasts into the endometrial stroma is a strictly regulated process. Understanding the molecular mechanisms of human JAR is critical for the development of novel therapeutic strategies or treatments. In the present study, the authors discovered the function of Hsp27 in human JAR cells. Hopefully, improved chemotherapy targeting Hsp27 may be available to patients with JAR in the next few years.

Conclusion

In this study, the authors provided evidence for a new role of Hsp27 in JAR cells. Their results emphasize that decreased Hsp27 expression inhibits proliferation, migration, and invasion in human JAR cells. In conclusion, the present data suggested that Hsp27 may play an important role in JAR.

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