골모세포에서 열자극에 의한 Hsp27 발현에 대한 연구

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Abstract

HSP27 EXPRESSION IN OSTEOBLAST BY THERMAL STRESS

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Aim of the study: Thermal stress is a central determinant of osseous surgical outcomes. Interestingly, the temperatures measured during endosseous surgeries coincide with the temperatures that elicit the heat shock response of mammalian cells. The heat shock response is a coordinated biochemical response that helps to protect cells from stresses of various forms. Several protective proteins, termed heat shock proteins (hsp) are produced as part of this response. To begin to understand the role of the stress response of osteoblasts during surgical manipulation of bone, the heat shock protein response was evaluated in osteoblastic cells.

Materials & methods: With primary cell culture studies and ROS 17/2.8 osteoblastic cells transfected with hsp27 encoding vectors culture studies, the thermal stress response of mammalian osteoblastic cells was evaluated by immunohistochemistry and western blot analysis.

Results: Immunocytochemistry indicated that hsp27 was present in unstressed osteoblastic cells, but not fibroblastic cells. Primarily cultured osteoblasts and fibroblasts expressed the major hsp in response to thermal stress, however, the small Mr hsp, hsp27 was shown to be a constitutive product only in osteoblasts. Creation of stable transformed osteoblastic cells expressing abundant hsp27 protein was used to demonstrate that hsp27 confers stress resistance to osteoblastic cells.

Conclusions: The demonstrable presence and function of hsp27 in cultured bones and cells implicates this protein as a determinant of osteoblastic cell fate *in vivo*.

Key words: Osteoblasts, Heat shock response, Heat shock protein 27, Osseointegration

Ⅰ. INTRODUCTION

Bone regeneration defines the success of many clinical procedures in dentistry. Examples include grafting procedures, periodontal regeneration procedures and osseointegration of dental implants. Achieving successful bone formation following implant placement is dependent on a list of clinical factors which ultimately determine the viability of cells at the surgical interface. It is now generally accepted that surgical preparation of bone for implants should be accomplished with minimal trauma to the bone and with dimensional accuracy[1,2]. These steps represent clinical efforts to maintain the vitality of osteoblastic cells present at implant surgical margins. Early studies of tissue regeneration at titanium chambers indicated that osteoblastic cells selectively repopulated in carefully prepared wounds. The molecular detetterminants of this selective regeneration by osteoblastic cells has not been considered.

Thermal stress is a primary mediator of surgical damage to bone. Heat generation during dental implant placement has been shown to be a key determinant of bone regeneration and implant success[3]. Steps to reduce surgical cutting temperature of bone are globally advocated[1,4]. When correctly performed to limit heat generation, endosseous implant surgery results in healing with the direct apposition of vital bone against the fixture surface[1]. Failure of regeneration results in repair by fibrous scar formation.

In vivo investigations have demonstrated the negative effects of heat on subsequent bone healing[5,6]. Originally, an osseous surgical threshold temperature of 56℃ was proposed[7]. The threshold temperature beyond which bone regeneration continues was redefined using bone harvest chambers. At temperatures above 45℃, osseous regeneration was limited and above 47℃, regeneration was not observed[5]. Healing following exposure to elevated temperatures suggests that bone possesses some degree of thermal stress resistance. The precise biological impact of moderate thermal stress (37 - 45℃) on bone forming cells is still unknown. However, this range of thermal stress represents the temperature range which engenders the classical heat stress reponse of mammalian cells .

Upon exposure to heat, mammalian cells selectively express a series of proteins collectively refered to as heat shock proteins(hsp)[8]. Hsp expression protects the cell from irreversible damage associated with heat and other stresses. The hsp are characterized as families of related intracellular proteins. The hsp 90 family of proteins are molecular chaperones that interact with steroid hormone receptor proteins and assist in intracellular transduction of responses to hormones[9]. The hsp 70 family of proteins are also molecular chaperones that include a variety of proteins that assist in protein import to different organelles (e.g., Bip, HSC, hsp70[10,11] and may protect intracellular proteins from denaturation. The small Mr hsp include hsp27[12]and alpha B crystallin[13]. All of these proteins have protective attributes. A common experimental finding is that the inhibited expression of either hsp90, hsp70 or small hsp results in reduced cellular stress resistance[14-16]. Hsp expression is an important determinant of cell fate.

This study sought to define the osteoblast's biochemical responses to thermal stress. The thermal stress response of mammalian osteoblastic cells was described using immunohistochemistry and western blot analysis. With primary cell culture studies and ROS 17/2.8 osteoblastic cells transfected with hsp27 encoding vectors culture studies, we evaluated the protective role of hsp27 against thermal stress in osteoblastic cells.

Ⅱ. MATERIALS AND METHODS

1. Primary cell culture

Newborn rat calvarial cells were obtained by serial collagenase digestion[15]. At 4 days, the cells were passaged into the media (DMEM; Dulbeco's Modified Essential Media, Gibco/Life Technology, Gaithersburg, MD, USA) containing 10% Fetal Bovine Serum (FBS). The first digestion (RC I) released fibroblast-like cells and the fourth digestion released osteoblast-like cells (RC IV) which, upon culture in ascorbic acid and β - glycerophosphate, formed bone - like nodules.

2. Clonal cell culture

ROS 17/2.8 cells were cultured in DMEM/F12 media containing 5% Bovine calf serum (BCS, Hyclone, Logan, Utah, USA). Cells were passaged 1 : 3, every third day. All cultures were maintained at 37℃ in 5% CO_2 / 95% air.

3. Heat shock

For heat shock, 150,000 cells / 35 mm diameter dish were plated and grown overnight. The media was changed 12-18 hours before heat shock. Cells were approximately 80% confluent at the time of heat shock. Heat shock was accomplished by transfer of culture dishes into tissue culture incubators at the temperatures and times indicated. After heat shock, cells were allowed to recover in the same media at 37℃ for periods ranging from 4-10 hours.

4. Western blot analysis

Heat shock protein expression was evaluated by immunobloting of SDS-PAGE separated cellular proteins on nitrocellulose filters[17]. Filters were blocked

for 1 hour in 50 mM TRIS-Cl, 10mM NaCl and 0.05% Tween-20 (TBST) containing 4% bovine serum albumin. Antiserum recognizing hsp70 and hsp90, and antiserum recognizing hsp27 were added to individual filters at a dilution of 1: 1,000 (hsp27) or 1: 2,000 (hsp70 / 90) and incubated overnight at 4 ℃. After washing and incubated with secondary antibody (horseradish peroxidase conjugated goat - anti - rabbit IgG, Vector, Burlingame, CA, USA) for 1 hour at room temperature, the antigen-antibody complexes were visualized using diaminobenzidine and NiCl (Sigma-Aldrich, St. Louis, MO, USA).

5. Recombinant hsp27 production and purification

The open reading frame of murine hsp27 was subcloned by standard procedures into the Bam H1 and Pst1 sites of the prokaryotic expression vector pQE9 to generate an N - terminal 6 - histidine - hsp27 fusion protein, Expression of this construct resulted in high levels of Isopropyl b-D-thiogalactoside(IPTG) induced rhsp27.a protein expression in E. coli strain M15 (rhsp27.a). Cells were harvested from 250 mL cultures grown for 4 hours with 1 mM IPTG by centrifugation. Cells were lysed and resuspended into buffer A (6 M guanidinium HCl , 0.1 M phosphate pH 8.0). The rhsp27.a contained in the lysate was purifed using nickel chelate affinity chromatography on NTA resin[18]. The homogeniety of the purified protein was evaluated by SDS - PAGE.

6. Antibody production and purification

The rhsp27.a was used for production of polyclonal antisera in rabbits. After 8 weeks of immunization and boosting, serum was collected and antibody was subsequently purified by affinity chromatography using sepharose 4b - immobilized rhsp27.a. The affinity purified antibody was used for subsequent immunohistochemical analyses.

7. Creation and clonal selection of stable transfected osteoblastic cell lines

ROS17/2.8 cells were transfected with an hsp27.a - encoding expression vector. The hsp27.a open reading frame was cloned into the Pst1 and BamH1 sites of the pcDNA3.1 expression vector (Invitrogen, Carlsbad, CA, USA) and supercoiled plasmid DNA was prepared by an affinity matrix method (Qiagen, Valencia, CA, USA). Stable transformation of ROS 17/2.8 cells was performed using liposomes (Lipofectamine™, LifeTechnologies, Inc., Gaithersburg, MD, USA). A mixture of 4㎍ DNA, 18㎕ of Lipofectamine in 4 mL of Optimem™ (LifeTechnologies, Inc.) was placed onto a 100 mm tissue culture plate of subconfluent cells. After 5 hours of incubation, additional media and serum was added to 10 mL at 10 % BCS. 72 hours following transfection, cells were split 1 : 20 into DMEM / F12 containing 5 % BCS, penicillin / streptomycin, and 500 ㎍ / mL G418 antibiotic (LifeTechnologies, Inc.). After three weeks, 50 colonies were selected for clonal growth and these were screened for increased constitutive hsp27 expression by Western blotting. From a single 100 mm dish of transfected cells, over 100 colonies formed and of 50 selected colonies, 11 colonies demonstrated elevated hsp27 expression.

8. Metabolic labeling of cellular proteins

250,000 cells were plated in 6 well culture dishes 16-18 hours prior to preparation of heat shock. After two hour heat shock, the cells were rinsed with serum free media and during four hour recovery, the cells were cultured in the presence of leucine free media containing 0.1uCi [^{14}C] - leucine (Amersham, Arlington Heights, IL, USA) and 10 % dialysed FCS. The media was then aspirated and the cell layers were washed twice with PBS. Cells were immediately lysed and collected in 250㎕ of 1x sample solubilizing buffer and boiled for 15 minutes in preparation for SDS-PAGE.

9. Cell proliferation

DNA synthesis was evaluated by the incorporation of [^{3}H] - thymidine. 200,000 cells / 16 mm diameter well were plated and prepared for heat shock. Immediately following heat shock, 50㎕ of RPMI 1640 media containing 0.5 Ci [^{3}H] - thymidine (Amersham) and 10% dialysed FCS was added to each well and the cells were allowed to recover for 4 - 10 hours. Then media was removed, cells were washed twice with PBS and then harvested by 15 minute incubation in 0.5 mL trypsin - EDTA, which was finally diluted with 0.5 mL DMEM containing

10% FBS. Cells were recovered by centrifugation at 2,000 rpm. The cell pellets were resuspended in 0.5 mL 15 % tricholoroacetic acid (TCA), incubated at 4℃ overnight and the DNA containing precipitate was recovered by centrifugation at 10,000 rpm for 5 minutes. This precipitate was washed with 5 % TCA containing 5 mM thymidine, air dried and solubilized in 0.4 mL 0.1M NaOH. Aliquots were quantitated using a Beckman LS2801 scintillation counter.

10. Cell survival assays

ROS 17/2.8 cells and hsp27.a transfected ROS17 / 2.8 cells were plated at 50% confluence. After 18 hours growth, cells were subjected to 2 hour 43℃ heat shock. After overnight recovery, cells were passaged 1 : 20 into 6 well culture dishes and allowed to recover. Colony formation was assessed by crystal violet staining of cultures after 1 week of growth in DMEM / F12 containing 5 % BCS. In two separate experiments, the average number of colonies in 5 wells of each cell type were determined and compared by Student' s t - test.

11. Histology and immunohistochemistry

Sprague Dawley rats were used in this study. Animals were maintained in accordance with guidelines estabiashed by the Institutional Animal Care and Use Committee of Korea University Ansan Hospital. Newborn calvarial bones were cultured and the media was changed 12-18 hours before heat shock. Heat shock was accomplished by transfer of culture dishes into tissue culture incubators at 43℃ for 2 hours. After heat shock, newborn calvarial bones were allowed to recover in the same media at 37℃ for periods ranging from 4-10 hours, and were fixed in 3.7% paraformaldehyde for 2 hours and stored in 100 mM phosphate buffer (pH 7.3). The calvaria were demineralized in 10% EDTA , and 5 ㎛ paraffin embedded sections were prepared. Calvarial sections were stained using toluidine blue (pH 5.5) reveal soft tissue, cartilaginous and osseous structures. Sections were blocked with normal goat serum, rinsed and incubated for 1 - 24 hours with affinity purified anti-hsp27 antibody, normal rabbit serum or PBS. After rinsing, all sections were incubated with a horseradish peroxidase - conjugated goat anti - rabbit antibody (Vector, Burlingame, CA) at a 1: 250 dilution. Immunocomplexes were visualized using diaminobenizdine (Sigma-Aldrich, St. Louis, MO, USA) or the AEC chromagen (Zymed, South San Francisco, CA, USA).

Ⅲ. RESULTS

1. Heat shock protein expression by osteoblasts *in vivo*

Heat shock protein expression was evaluated in tissue sections of newborn calvaria. In unstressed calvaria, periosteal osteoblasts contain hsp27. Chondroblastic cells and osteocytes also contain hsp27. In heat shocked calvaria, hsp27 staining was observed in most cells, including the majority of osteocytes and fibroblastic cells of surrounding connective tissue (Fig. 1 a, b). Comparison of hsp27 abundance in calvaria to know amounts of purified hsp27 indicated that hsp27 accounted for 0.05% - 0.1% of total protein (approximately 1 - 2 ng / 20 ㎍ total protein) in the soluble calvaria protein sample(Fig. 2).

2. Hsp Expression in primary cultured osteoblasts and fibroblasts

Analysis by Western blotting indicated that primary osteoblastic cells produced the three major hsp as constitutive products and demonstrated stress-induced enhancement of hsp expression. Fibroblasts did not express hsp27 as a constitutive product of the cell (Fig. 3, lane 1) and produced much less hsp27 in response to stress. Unstressed osteoblastic cells contained hsp27 (Fig. 3, lane 3).

3. Stress resistance of primary cultured osteoblasts and fibroblasts

When primary cultured fibroblasts and osteoblasts were subjected to sub - lethal heat shock (2 hour 43 ℃), differences in their fates were noted (Table 1). Counting of cells 48 hours following heat shock revealed that the osteoblastic cell population cultured from calvaria possessed greater growth and survival following stress (45% survival) than parallel cultured fibroblastic cells (30 % survival, $p < 0.05$). The doubling times for unstressed fibroblastic cells and undif-

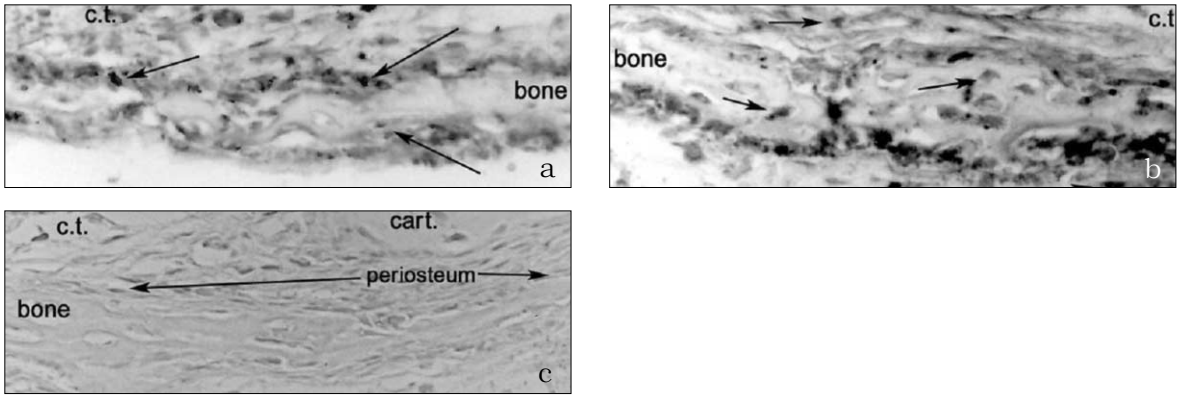

Fig. 1. Hsp27 expression in osteoblastic cells(×200). Immunohistochemical identification of hsp27 (using affinity purified anti - hsp27 antibody) in newborn rat calvaria. a) Anti hsp27 antibody identified hsp27 in periosteal osteoblasts in unstressed calcaria(arrows), b) and in osteocytes and fibrous cells in heat shocked calvaria(arrows). c) Normal rabbit serum control.

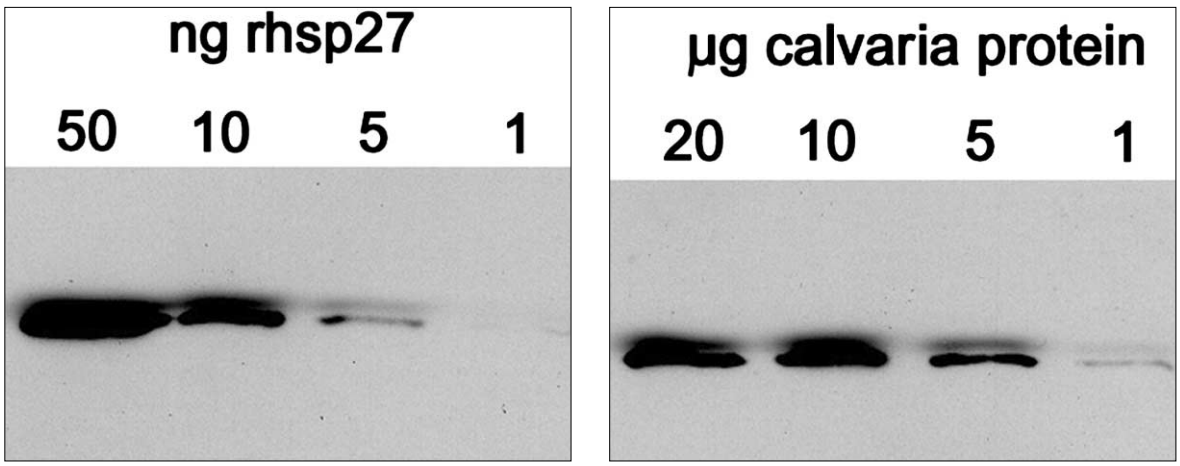

Fig. 2. Immunoblot analysis of hsp27 expression. 10 ㎍ / ㎕ (wet weight tissue / ㎕ SDS-PAGE buffer) aliqouts of lysates from calvaria were separated in 10 % SDS - PAGE, transferred to nitrocellulose, probed with anti - hsp27 antibody and identified by chemiluminescence. Estimation of hsp27 abundance in calvaria protein lysates (1 - 20 ug) by comparison to known amounts of recombinant hsp27.a (1 - 50 ng). Approximately 2 - 3 ng of hsp27 is present / ㎍ total calvaria protein .

ferentiated osteoblastic cells during 72 hours of culture was determined to be equal.

4. Hsp27 overexpression in cultured osteoblastic cells

The effect of constitutive hsp27 expression on osteoblastic cell fate was further examined by modulation of hsp27 abundance in osteoblastic cells. ROS17/2.8 cells were transfected with an hsp27.a - encoding expression vector and stable transfected cell lines were established by individual selection of clones grown in the presence of G418. Hsp27 abundance in ROS 17/2.8 cells and clone A7 cells (hsp27 +) were compared to known amounts of recombinant hsp27.a. All images were digitally evaluated using a Lumi-Imager F1 Workstation (Roche Molecular Biochemicals, Indianapolis, IN). Digital image analysis indicated that clone A7 cells possessed 12 times

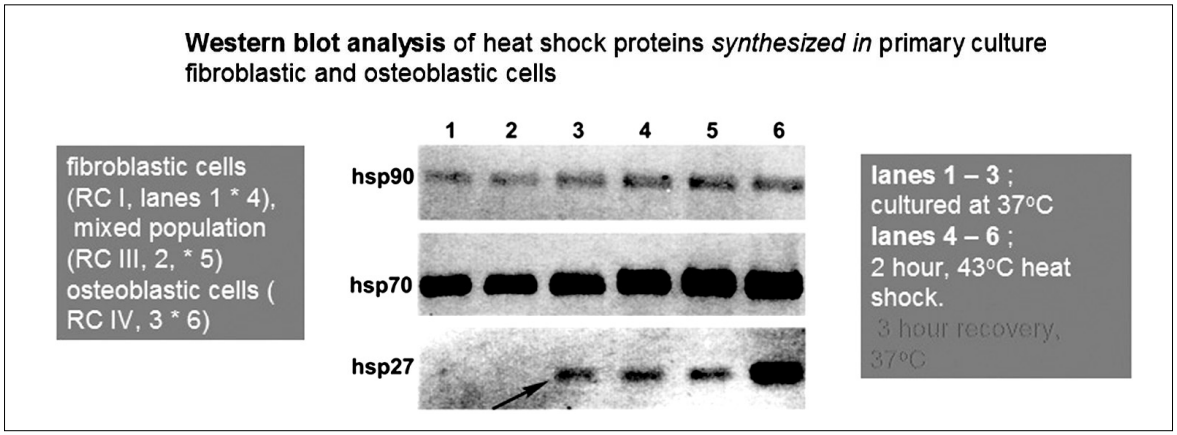

Fig. 3. Western blot analysis of heat shock proteins synthesized in primary cultured fibroblastic and osteoblastic cells - Serial digested cell populations representing fibroblastic cells (RC I, lanes 1 and 4), mixed population (RC III, lanes 2, and 5) and osteoblastic cells (RC IV, lanes 3 and 6) were cultured at 37℃(lanes 1 - 3) or subjected to 2 hour, 43 ℃ heat shock and 3 hour recovery at 37oC(lanes 4 - 6). 15 ㎍ aliquots of total protein lysates were separated by 12 % SDS - PAGE, transferred to one nitrocellulose membrane and three regions of the membrane were then probed for hsp 90 (panel a), hsp70 (panel b) or hsp27 (panel c). Note that the non-stressed osteoblastic cells contain hsp27 in the absence of stress (lane 3, panel c).

Table 1. Results of cell survival assay

Cell Type		% cells surviving 2 hour 43℃ heat shock
primary cultured fibroblasts	**	30%(+/- 6.1)
primary cultured osteoblasts		45%(+/- 4.3)
ROS 17 / 2.8 cells	*	31%(+/-2.9)
Clone A7 (hsp27.a +)		100%(+/- 10.6)

*: $p < 0.05$
**: $p < 0.0002$

The effect of heat shock on primary cultured fibroblasts (hsp27-) and primary cultured osteoblasts was compared by counting of number of vital cells present on culture dishes 48 hours following 2 hour 43℃ heat shock and calculating percent survival by comparison to the number of cells exposed to 37oC treatment. The overexpression of hsp27 in ROS17/2.8 cells was also examined. In several experiments, these cells appeared resistant to this exposure to heat shock.

more hsp27 than ROS17/2.8 cells and the level of hsp27 in clone A7 cells approached that of heat shocked ROS cells (Fig. 4).

Hsp27.a transfected cells showed enhanced resistance to heat stress by a sensitive colony forming assay (Fig. 5). Approximately twice the number of colonies resulted from the cell line expressing murine hsp27.a (99 +/-6.6, Fig. 5, lower panel) as compared to a transfected cell line that failed to express hsp27.a (57.4 +/-4.8, $p < 0.02$; Fig. 5, upper panel). DNA and protein synthetic ability measured as the incorporation of [^{3}H] - labeled thymidine and [^{35}S] - labeled cysteine / methionine into TCA - precipitable DNA and protein revealed that cell lines overexpressing hsp27.a were initially protected in their DNA and protein synthetic capacity (Fig. 6).

Ⅳ. DISCUSSION

Multiple factors may affect the process of bone formation following implant surgery[2,19]. Heat generated during the surgical manipulation of bone is regarded as one of the most influential factors affecting bone formation subsequent to surgery[1,4,5]. This study sought to define the osteoblast's biochemical responses to thermal stress. Immunohistochemical analysis using affinity purified anti - hsp27 antibody indicated that hsp27 was present in unstressed osteoblasts but not fibroblasts surrounding forming bone. While heat shock of cultured osteoblastic cells resulted in the

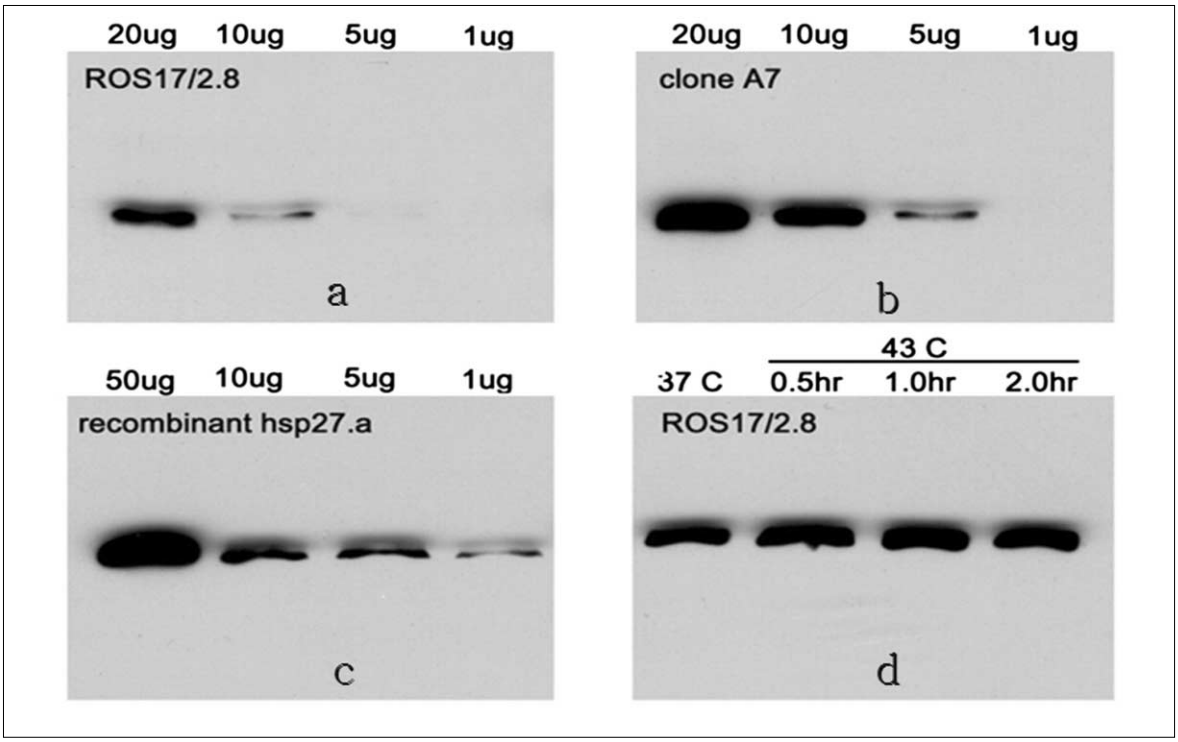

Fig. 4. Abundance of hsp27 in stable transfected osteoblastic cells. Immunoblot analysis of hsp27 in unstressed ROS 17/2.8 cells (a) and clone A7 cells (b); 20 ㎍ (lane 1) , 10 ㎍ (lane 2), 5 ㎍ (lane 3), and 1 ㎍ (lane 4) total protein extract. c) parallel blot of purified recombinant hsp27.a; 50 ng (lane 1), 10 ng (lane 2), 5 ng (lane 3), and 1 ng (lane 4). d) Induction of hsp27 by heat shock in ROS17/2.8 cells during exposure to 43℃ heat shock for 0 minutes (lane 1), 30 minutes (lane 2), 60 minutes (lane 3), and 120 minutes (lane 4).

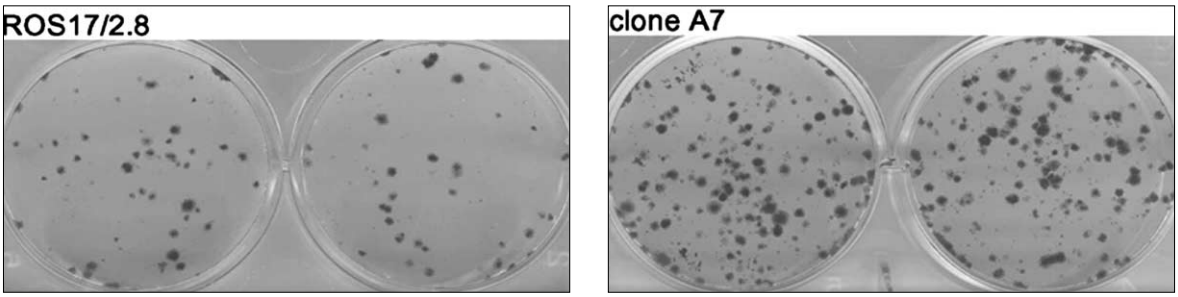

Fig. 5. Effect of murine hsp27 expression on osteoblastic cell survival. Stable transfected ROS17/2.8 - derived cell lines, which express murine hsp27.a (clone A7) or fail to express the cloned protein (clone A3) were subjected to 2 hour, 43℃ heat shock at subconfluent density. After 1 week of growth at 37℃, cultures were stained with crystal violet.

expression of the major hsp (hsp90, hsp70 and hsp27), immunoblot examination of hsp expression by calvaria derived osteoblastic cells and fibroblastic cells indicate that hsp27 was a constitutive product of the osteoblastic cells, but not fibroblastic cells. This difference was considered significant because hsp27 is regarded as a principal determinant of cellular stress resistance [20].

While the constitutive expression of hsp27 has been surveyed among tissues[22], expression in bone had not been considered. The prinicipal observation of this report was that hsp27 is present in unstressed

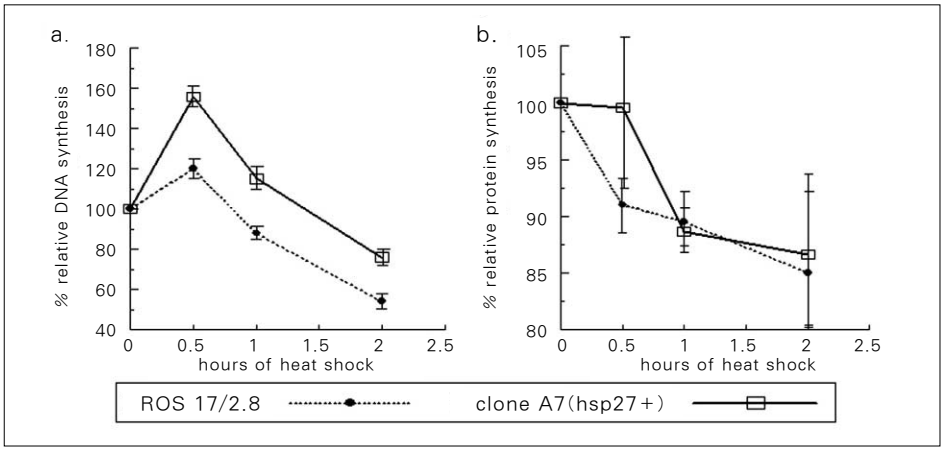

Fig. 6. Comparison of DNA and protein biosynthesis following heat shock in cell lines expressing different levels of hsp27. a) The % DNA synthesis (cpm [^{3}H] thymidine incorporation / hr at 37℃ - cpm [^{3}H] thymidine incorporation / hr at 43℃ x 100) is plotted versus duration of 43℃ heat shock of ROS17/2.8 cells and clone A7 (hsp27+). b) The % protein synthesis (cpm [^{35}S] methionine / cysteine incorporation / hr at 37℃ - cpm [35S] methionine / cysteine incorporation / hr at 43℃ × 100) is plotted (+/- sem) versus duration of 43℃ heat shock of ROS17/2.8 cells and clone A7 (hsp27+).

osteoblastic cells. Both immunohistochemical observations of bone and Western blot analysis of primary cultured osteoblastic cells indicated that hsp27 was present in unstressed osteoblastic cells. This confirmed the restricted expression of hsp27 among rat tissues, including bone, that was revealed by comparison of mRNA levels in extracts from bone and other tissues [21].

While not present in the extremely high levels noted for muscle and skin (approaching 0.2% of total protein), the estimated 0.001-0.05% level suggest that hsp27 is relatively abundant in the unstressed osteoblastic cell. By comparison, hsp27 accounts for 0.003-0.2% of total protein in other tissues[22]. Because our estimates are based on the wet weight of tissues and not cells, the relatively acellular nature of bone must be considered in these comparisons. In terms of other cellular constituents of bone, expression of hsp27 was not observed in hematapoetic or stromal marrow cells or the fibrous connective tissues associated with the immature sutures separating the calvarial bones or the calvaria - derived, primary cultured fibroblastic cells.

The calvaria derived, primary osteoblast cultures represented undifferentiated cells. Upon differentiation of primary cultured osteoblasts, hsp27 mRNA expression was reduced[23]. Other osteosarcoma cell lines have been reported to express hsp27[24]; however it is difficult to interpret the differentiation status of these transformed cell lines. The localization of hsp27 to periosteal osteoblastic cells and its limited expression in osteocytic cells of bone also suggests the preferential expression of hsp27 in relatively undifferentiated osteoblastic cells.

Evaluation of hsp27, hsp70 and hsp 90 demonstrated that the constitutive expression of hsp27 in osteoblasts represented the sole difference in the limited spectrum of hsp expression evaluated between osteoblastic and fibroblastic cells. Upon heat shock, hsp27 expression was induced in fibroblastic cells and elevated in osteoblastic cells. When the stress resistance of the calvaria derived osteoblastic and fibroblastic cells were compared, the osteoblastic cells showed greater stress resistance. Hsp27 in osteoblasts preceding stress and in greater abundance following stress was considered as a potential determinant of stress resistance. In fact, the analysis of parallel cultures of fibroblastic cells (hsp27 -) and

osteoblastic cells (hsp27 +) cultured from newborn rat calvaria indicated that the osteoblastic cells possessed greater stress resistance than the fibroblastic cells.

Based on the immunohistological data showing osteoblastic cells containing hsp27, we speculate that relative stress resistance may be afforded to osteoblastic cells *in vivo*. Controlling osteoblastic cell fate via the stress response may promote osteogenesis. However, this osteoblast culture method fails to evaluate the important population of osteoblastic progenitor cells represented by the marrow stromal cell population[25,26]. Hsp27 may be a determinant of osteoblast survival upon environmental challenge. It is essential to note that when ES cell differentiation was studied in the context of hsp27 abundance, hsp27 appeared to be a co - determinant of stem cell fate[27].

To begin to define a protective role for hsp27 in osteoblasts, experimental modulation of hsp27 was investigated in terms of cellular stress resistance. The inhibition of hsp27 expression using antisense vector technology reduced osteosarcoma stress resistance[24]. Here, the overexpression of hsp27 was used to examine a role for hsp27 in determination of stress resistance. The additional presence of murine hsp27.a in stable transfected ROS17/2.8 cells increased cell survival following heat shock. This confirms a number of reports showing that hsp27 expression confers protection to other types of cultured cells[28,29]. However, this confirmation is important. There exist instances where this conference of protection did not occur[30].

These findings have important implications to current explanations of osseous wound healing following surgical cutting of bone, particularly at endosseous implant surfaces. First, the ability of stressed osteoblasts possessing abundant hsp27 to proliferate and produce protein following stress is consistent with the *in vivo* observations that bone regeneration is maintained following heat stress at 44℃[5]. These data strongly implicate the heat shock protein response as a determinant of osteoblast survival and activity in the face of environmental change.

The osteoblast produces the structural proteins and many of the regulatory proteins of bone matrix responsible for the successful regeneration and remodeling of bone[31]. An important aspect of the physiological response to stress is the maintenance of protein synthesis to achieve such functions following injury. Several protection - related functions have been attributed to hsp27. It can function as a molecular chaperone and protects enzyme function by limiting protein denaturation and aggregation[12]. Hsp27 contributes to protect the synthetic capacity of cells involved in tissue formation.

Hsp27 is also an inhibitor of actin polymerization[32,33] and is co - localized to actin stress fibers of stressed cells[34]. Hsp27 binds *in vivo* to p84, a stress - fiber associated transglutaminase of platelets[34]. Exactly how these cytoskeleton relationships contribute to stress resistance is unclear. Recent investigations have implicated hsp27 modulation of stress fiber formation as a co - determinant of focal contact reformation following stress. The importance of hsp27 to controlling stress fiber organization in osteoblasts is not defined, but, the regulation of cell shape may directly reflect physical stress responsiveness of bone *in vivo*[35] and cell fate in general[36].

Consideration of cell fate in response to stress may ultimately require consideration of cellular selection of appropriate avenues to cell death and removal. Apoptosis is a mechanism of cell death that precludes necrosis, lysis and inflammatory sequelae leading to fibrosis[37]. Both protection of cell viability and precluding necrotic cell death by promoting apoptotic mechanisms are critical considerations in terms of cell activity leading to bone formation at alloplastic surfaces or tissue regeneration in general. Hsp27 is presently implicated in determination of apoptotic pathways in cultured cells[38,39]. Ongoing efforts must focus on the role of hsp27 in determination of osteoblastic precursor cell fate as a means of directing osteogenesis at surgical interfaces.

Ⅴ. CONCLUSION

This investigation demonstrated that osteoblasts possess the ability to respond to heat shock. Both constitutive hsp27 expression and abundant hsp expression following stress occurs in osteoblastic cells. By promoting cell survival, precluding necrosis

and supporting subsequent protein biosynthesis, the osteoblast stress response may be a significant determinant of osseous wound healing. These findings suggest molecular approaches and cellular strategies to clinically enhance osseous surgery and tissue regeneration outcomes.

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