Heat Shock Protein 47 Is Expressed in Fibrous Regions of Human Atheroma and Is Regulated by Growth Factors and Oxidized Low-Density Lipoprotein

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Background—Heat shock protein 47 (Hsp47) is a stress protein that may act as a chaperone for procollagen. Its involvement in atherosclerosis is unknown.

Methods and Results—Hsp47 expression in human coronary arteries was assessed by immunostaining. Strong focal expression was evident in atherosclerotic, but not normal, arteries and was prevalent in the collagenous regions. Double immunostaining revealed that all cells expressing type I procollagen also expressed Hsp47. Moreover, parallel regulation of proα1(I)collagen and Hsp47 mRNA expression occurred with cultured human smooth muscle cells stimulated with transforming growth factor-β1 or fibroblast growth factor-2. However, a proportion of Hsp47-expressing cells in plaque did not express type I procollagen, and this pattern could be reproduced in culture. Heat shock and oxidized LDL stimulated the expression of Hsp47 mRNA by smooth muscle cells, without a concomitant rise in proα1(I)collagen expression.

Conclusions—These findings identify Hsp47 as a novel constituent of human coronary atheroma. Its localization to the fibrous cap, regulation by growth factors in parallel with type I procollagen, and selective upregulation by stress raise the possibility that Hsp47 is a determinant of plaque stability.

Key Words: atherosclerosis • muscle, smooth • collagen • stress
Immunohistochemistry

Deparaffinized tissue sections were subjected to microwave-based antigen retrieval; they were then incubated with primary antibodies overnight and then incubated with biotinylated horse anti-mouse IgG. Bound antibody was detected using the ABC Elite Kit (Vector Laboratories Inc) and visualized with 3,3'-diaminobenzidine (Sigma). Sections were counterstained with Harris’ hematoxylin. Human skin served as the control tissue. For both Hsp47 and procollagen, there was cytoplasmic staining of fibroblast-like cells in the dermis and no signal from epithelial cells. Expression in coronary artery sections was quantified by counting all positive cells in contiguous fields (×400). The entire section was evaluated.

For double immunolabeling, sections were immunostained for type I procollagen using SP1.D8 and developed using diaminobenzidine. They were then quenched with biotin solution, immunostained for Hsp47, and visualized using Vector SG peroxidase substrate, which yields a blue/gray color. Double-immunolabeled sections were not counterstained.

Cell Culture and Northern Blot Analysis

Primary cultures of human vascular smooth muscle cells (SMCs) were established from segments of the internal thoracic artery retrieved at the time of coronary artery bypass surgery. SMCs were incubated in M199 containing 1% fetal bovine serum for 48 hours and then stimulated with TGF-β1, FGF-2, or oxidized LDL. SMCs were also subjected to heat shock (42°C for 4 hours), which was followed by the restoration of physiological temperature (37°C) for up to 6 hours. Total RNA was isolated, and Northern blot analysis was performed as previously described.

Results

Eight of the 25 arterial segments were normal or showed diffuse intimal thickening, and they were classified as non-atherosclerotic. Seventeen samples contained atherosclerotic plaque, which was subclassified as fibrous (n=12) or lipid-rich (n=5).

Nonatherosclerotic artery samples showed no or minimal Hsp47 expression (Figure 1, A and B). In contrast, Hsp47 was readily detectable in 11 of the 17 atherosclerotic artery samples (Figure 1, C and D). The strongest and most prevalent staining was in SMC-like cells within the dense fibrous plaques and within the fibrous cap of lipid-rich lesions (Figure 1E). Cells within the necrotic lipid core did not express Hsp47.

Type I procollagen coexisted with Hsp47 in the same artery, and the 2 proteins localized to the same regions of the artery wall (Figure 1E). Interestingly, however, Hsp47-positive cells were more prevalent than procollagen-expressing cells (Figure 1, E through G). To further characterize this, selected sections were double-immunostained. Hsp47 and type I procollagen typically colocalized in a given cell, as evidenced by a mixture of brown and blue/gray color in the cytoplasm (arrows in Figure 1H). Moreover, all cells that expressed type I procollagen also expressed Hsp47. However, a proportion of cells showed immunoreactivity only to Hsp47, as indicated by the blue/color alone (arrowhead in Figure 1H).

To determine a basis for the generally close relationship between Hsp47 and type I procollagen expression, we studied cultured human SMCs. SMCs incubated with TGF-β1 displayed a dose-dependent increase in Hsp47 mRNA abundance and a parallel increase in procollagen mRNA levels (Figure 2A). Stimulation with FGF-2 yielded a dose-dependent decline in Hsp47 mRNA abundance and a parallel decline in procollagen mRNA (Figure 2B).

We also sought out conditions that might divergently regulate the expression of these 2 genes, given that some cells selectively expressed Hsp47. As shown in Figure 2C, 4 hours of heat stress (42°C) stimulated a 5.4-fold increase in Hsp47 mRNA expression but no significant change in procollagen mRNA level. In addition, the incubation of SMCs with copper-oxidized LDL (150 μg/mL) yielded a 2-fold increase in Hsp47 mRNA abundance after 12 hours. In contrast, procollagen mRNA expression declined.

Discussion

We showed that the expression of the stress protein Hsp47 was increased in human atherosclerotic coronary arteries compared with normal coronary arteries and arteries with diffuse intimal thickening. Hsp47 was especially prominent in the fibrous/collagenous regions of atheromata, including the cap that overlies a lipid-rich core. This pattern is unique among the stress shock proteins that have been associated with atherosclerosis to date. Hsp70, for example, is concentrated around areas of necrosis and lipid accumulation. The relationship between Hsp47 and collagen was further strengthened by the regional colocalization of Hsp47 and type I procollagen and by double immunostaining, which established that all type I procollagen–producing cells expressed Hsp47.

The close association between Hsp47 and type I procollagen in atherosclerotic plaque and previous in vitro data suggesting a role for Hsp47 in collagen production imply that a mechanism must exist to ensure that Hsp47 is present within the cell when collagen is being produced. It must be recognized, however, that type I collagen and Hsp47 are distinctly different proteins encoded by dissimilar genes. The current study suggests that coordinate regulation in vascular disease may be based on parallel responsiveness to growth factors. TGF-β1 increased the expression of Hsp47 in human coronary arteries, A, Movat’s pentachrome–stained section of a normal coronary artery. The box indicates the area corresponding to that shown in B, which is an adjacent section showing no immunodetectable Hsp47. C, Section of an atherosclerotic artery (Movat’s pentachrome) containing a lipid-rich plaque core and fibrous cap. The box within the fibrous cap corresponds to the region shown in D, which is an adjacent section immunostained for Hsp47 that shows numerous cells with cyttoplasmic signals. E, Prevalence of cells expressing Hsp47 and type I procollagen in normal and atherosclerotic arteries. F, Fibrous cap of a lipid-rich plaque showing numerous cells expressing Hsp47. G, Near-adjacent section immunostained for type I procollagen showing a lower prevalence of positive cells. H, Section double-immunolabeled for Hsp47 and type I procollagen (no counterstain). Bound anti-Hsp47 antibody was identified using Vector SG substrate (blue/gray color), and bound SP1.D8 was visualized using diaminobenzidine (brown color). Cells expressing both Hsp47 and type I procollagen are evident (arrows), as is a cell expressing only Hsp47 (arrowhead).
expression of both Hsp47 and proα1(I)collagen mRNA in human SMCs, whereas FGF-2 decreased the expression of both genes.

A surprising finding was that, notwithstanding the evidence for coordinate regulation of Hsp47 and type I procollagen, evidence also existed for divergent regulation. Hsp47 was more prevalent in atheroma than type I procollagen, and double immunostaining established the existence of cells in which Hsp47, but not type I procollagen, was detectable. It is possible that a procollagen type other than type I was expressed by the Hsp47 single-positive cells; however, Hsp47 seems to be a very selective chaperone. It does not, for example, bind type III collagen.8 It is also possible that the anti-Hsp47 antibody had a higher affinity to Hsp47 than SP1.D8 did to type I procollagen or that the immunoreactivity of type I procollagen to SP1.D8 was masked by the interaction of procollagen with Hsp47. However, divergent regulation of Hsp47 and type I procollagen was supported by culture data. Heat shock stimulated Hsp47 mRNA expression by human SMCs, yet proα1(I)collagen mRNA abundance did not increase. Similarly, and perhaps of greater relevance to atherosclerosis, oxidized LDL selectively increased the expression of Hsp47 mRNA.

We speculate that the presence of Hsp47 in SMCs not producing immunodetectable type I procollagen may reflect a potential role of Hsp47 in trafficking abnormal procollagen. Up to 20% of newly synthesized procollagen is destined for intracellular degradation, and this fraction may increase under cellular stress.9 If Hsp47 is required to chaperone non-native procollagen to a degradation site, then stress conditions that yield aberrant procollagen might selectively stimulate Hsp47. In this regard, it is noteworthy that Hsp47 was expressed in parallel with procollagen in response to physiological stimuli (growth factors) but that it selectively increased in response to pathophysiological stressors (heat or oxidized LDL).

In summary, the current findings identify Hsp47 as a novel constituent of coronary atheroma and link this unique protein to the fibrous cap, to growth factor-mediated collagen production, and to atherogenic stress. The extent to which Hsp47 impacts the course of atherosclerosis, including plaque stabilization, seems to be a worthwhile avenue for study.
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