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Original Article

Immunohistochemical expression of prosaposin in normal arteries and atherosclerosis

ChuHee Lee[1], Jae-Ryong Kim[1], Joon Hyuk Choi[2]

Departments of [1]Biochemistry and Molecular Biology, Smart-Aging Convergence Research Center, [2]Pathology, Yeungnam University College of Medicine, Daegu, Korea

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Abstract: Prosaposin is a glycoprotein encoded by the *PSAP* gene located on human chromosome 10 and is involved in various biological processes. To investigate the role of prosaposin in vascular aging and atherosclerosis, we analyzed its expression in normal young, normal old arteries, and atherosclerotic arteries by immunohistochemistry. Eighteen samples each of normal young arteries (from individuals aged 1 to 38 years), normal old arteries (from individuals aged 47 to 70 years), and atherosclerotic arteries were selected from surgically resected specimens, and prosaposin expression was detected by immunohistochemistry. All normal young arteries showed no expression of prosaposin (0 of 18 samples, 0%), whereas normal old arteries showed increased prosaposin expression (6 of 18 samples, 33%). Prosaposin expression was higher in normal old arteries than in normal young arteries. Prosaposin was expressed in the cytoplasm of endothelial cells and smooth muscle cells and in the vascular wall in normal old arteries. All atherosclerotic samples showed markedly increased expression of prosaposin (18 of 18 samples, 100%). Prosaposin was expressed in the cytoplasm of smooth muscle cells and macrophages, and in the atheromatous plaque in the atherosclerotic samples. The expression of prosaposin was significantly increased in the atherosclerotic arteries than in normal young and old arteries. Our findings suggest that prosaposin may play a role in vascular aging and development of atherosclerosis.

Keywords: Prosaposin, blood vessels, aging, atherosclerosis, immunohistochemistry

Introduction

Prosaposin is a 517-amino acid glycoprotein and the precursor of saposin A, B, C, and D which degrade sphingolipids via activation of sphingolipid hydrolases in the lysosome [1]. In addition to this role, prosaposin is found in secretory body fluids including human milk, cerebrospinal fluid, and seminal fluid, and in the surface membrane of neuronal cells [2, 3].

Prosaposin is as an essential factor in the development and maintenance of the nervous system and other systems [4]. It stimulates neurite outgrowth, enhances choline acetyltransferase activity, and prevents neural cell death [5, 6]. Prosaposin overexpression or amplification has been found in breast and prostate cancer [7, 8]. These findings suggest that prosaposin has multiple functions.

Atherosclerosis and the subsequent cardiovascular diseases such as myocardial and cerebral infarction, are a major cause of death in the Western world [9, 10]. Increasing evidence indicates that aging is an important risk factor for atherosclerosis, and aging persists as an independent contributor when all other known factors are controlled [10]. Aging processes contribute to the pathogenesis of atherosclerosis.

Kim et al. [11] found that prosaposin mRNA was elevated in senescent human dermal fibroblasts (HDFs) and human umbilical vein endothelial cells (HUVECs) *in vitro.* It seems that prosaposin could act as a growth factor, and its expression might be altered by aging. However, little is currently known about the functions of prosaposin in vascular aging and atherosclerosis *in vivo*.

The aim of this study was to investigate the role of prosaposin in vascular aging and atherosclerosis. We analyzed the expression of prosaposin in normal young, normal old, and atherosclerotic arteries by immunohistochemistry.

Table 1. Expression of prosaposin in normal young, normal old, and atherosclerotic arteries

	Cases	Prosaposin (-) (%)	Prosaposin (+) (%)	Scores positive for prosaposin				*P* value
				3	4	5	6	
Normal young arteries	18	18 (100)	0 (0)	0	0	0	0	
Normal old arteries	18	12 (66.7)	6 (33.3)	4	1	1	0	
Atherosclerotic arteries	18	0 (0)	18 (100)	2	4	5	7	*

*Bonferroni-corrected $P < 0.05/3$ versus normal young arteries and normal old arteries.

Materials and methods

Tissue specimens

Eighteen samples each of normal young arteries (from individuals aged 1 to 38 years), normal old arteries (from individuals aged 47 to 70 years), and atherosclerotic arteries were selected. Normal young and normal old arteries were obtained from specimens resected owing to benign lesions or tumors of the gastrointestinal tract, kidney, testis, breast, and uterus at the Yeungnam University Hospital from 2002 to 2009. Atherosclerotic samples were obtained from the abdominal aorta (13 samples), carotid artery (3 samples), and femoral artery (2 samples). All resected specimens were fixed in 10% formalin. The histology was examined by using hematoxylin-eosin stain. The study was approved by the Institutional Review Board of Yeungnam University Hospital (YUH-2017-03-029).

Immunohistochemistry of prosaposin

Representative paraffin blocks were selected for immunohistochemistry. Four micron-thick sections were deparaffinized, rehydrated in a series of alcohols, and processed with a Dako-EnVision Detection Kit (DakoCytomation, Carpinteria, CA, USA). Prosaposin monoclonal antibody (ProteinTech group, Rosemont, IL, USA) was applied at 1:500 dilution. Diaminobenzidine was used as a chromogen, and the sections were counterstained with Mayer's hematoxylin. For negative controls, the primary antibody was omitted.

Assessment of prosaposin expression

Expression of prosaposin was scored semi-quantatively by the staining intensity and proportion of stained cells. The staining intensity was classified as follows: no stain (0), weak (1), moderate (2), and strong (3). The proportion of stained cells was quantified as a percentage of the total number of cells and assigned as follows: 0 (no positive staining of cells), 1 (positive staining in < 10% cells), 2 (positive staining in 10-50% cells), or 3 (positive staining in > 50% cells). The staining intensity and proportion were then summed to produce total scores. A total scores of three or higher was considered positive for prosaposin expression.

Statistical analysis

IBM SPSS 20.0 for Windows (IBM Co., Armonk, NY, USA) was used for statistical analysis. Chi-square tests and Fisher's exact test were used to compare the expression of prosaposin in normal young, normal old arteries, and atherosclerotic arteries. The Bonferroni correction was used for statistical adjustment for multiple comparisons. Difference was considered significant when Bonferroni-corrected *P* value was less than 0.05/3 (0.0166).

Results

Sample characteristics

Of the 18 normal young arteries samples, 14 were obtained from males and four from females, with ages ranging from 1 to 38 years (median age, 24 years) and a mean age of 22.22 ± 11.30 years. Of the 18 normal old arteries samples, 13 were obtained from males and five from females. Their age ranged from 46 to 70 years (median age, 62 years), with a mean age of 60.33 ± 7.10 years. Of the 18 atherosclerotic samples, 14 were obtained from males and four from females, with ages range from 55 to 75 years (median age, 65.5 years) and a mean age of 66.33 ± 5.97 years.

Immunohistochemical results

All normal young arteries showed no expression of prosaposin (0 of 18 samples, 0%; **Table 1**; **Figure 1**). The expression of prosaposin was

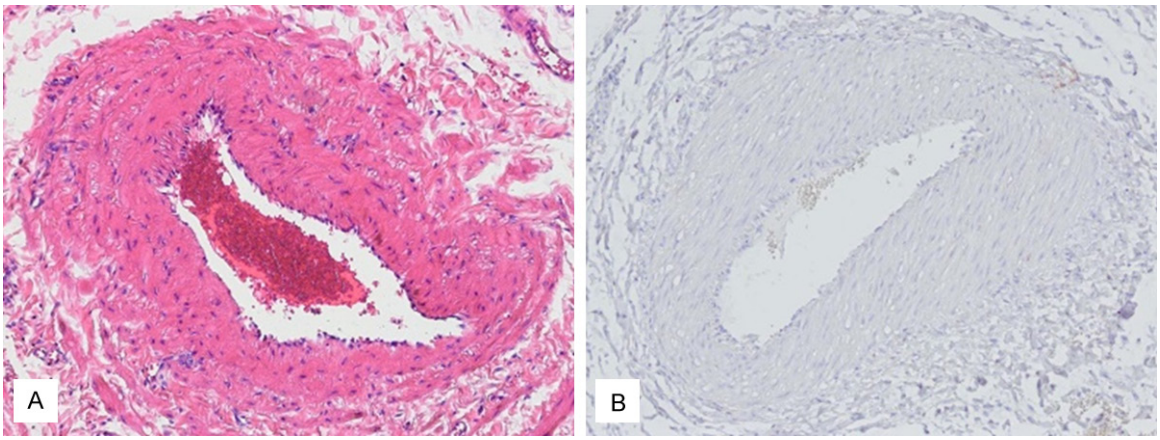

Figure 1. Normal young artery. A. Normal young artery is present. B. No expression of prosaposin is present.

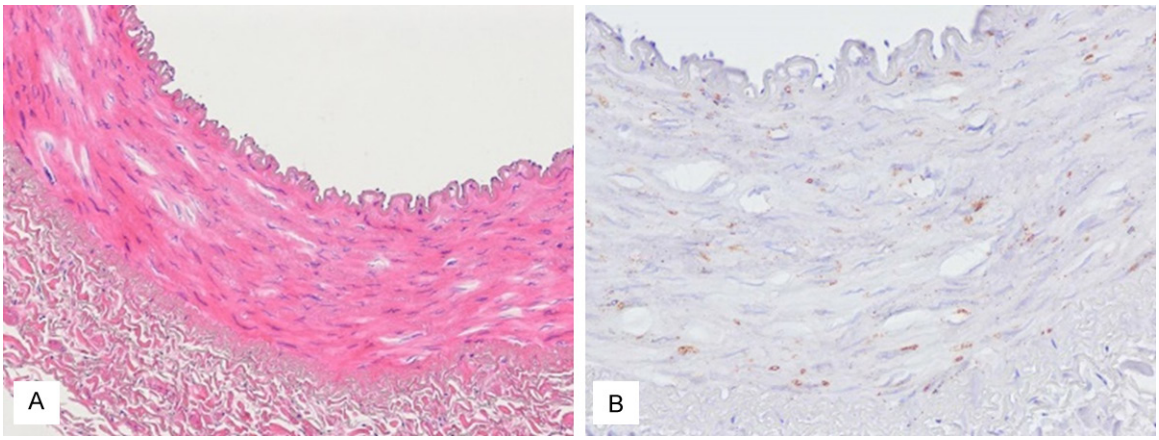

Figure 2. Normal old artery. A. Normal old artery is present. B. Prosaposin is moderately expressed in the cytoplasm of smooth muscle cells, endothelial cells, and the vascular wall.

present in 33% (6/18) of the normal old arteries (**Figure 2**). The expression of prosaposin was higher in normal old arteries than in normal young arteries, but this difference was not statistically significant (Bonferroni-corrected $P = 0.019$). Prosaposin was expressed in the cytoplasm of endothelial cells and smooth muscle cells, and in the vascular wall in normal old arteries. Prosaposin was expressed in all atherosclerotic samples (18/18; **Figures 3** and **4**) and was present in the cytoplasm of smooth muscle cells and macrophages, and the atheromatous plaque. Predominantly expression was seen in the cytoplasmic granules. Expression of prosaposin was significantly higher in atherosclerosis than in normal young arteries (Bonferroni-corrected $P < 0.0001$) and normal old arteries (Bonferroni-corrected $P < 0.0001$).

Discussion

Cardiovascular disease resulting from atherosclerosis is the most common cause of death in the world, and aging is a major risk factor for cardiovascular disease [10]. The pattern of prosaposin expression in normal young, normal old arteries, and atherosclerotic arteries was examined by immunohistochemistry, to clarify the role of prosaposin in vascular aging and atherosclerosis.

Prosaposin was absent in normal young arteries and present in 33% of normal old arteries. The expression of prosaposin was higher in normal old arteries than in normal young arteries. The increased expression of prosaposin in normal old arteries is consistent with the finding that prosaposin expression is up-regulated in

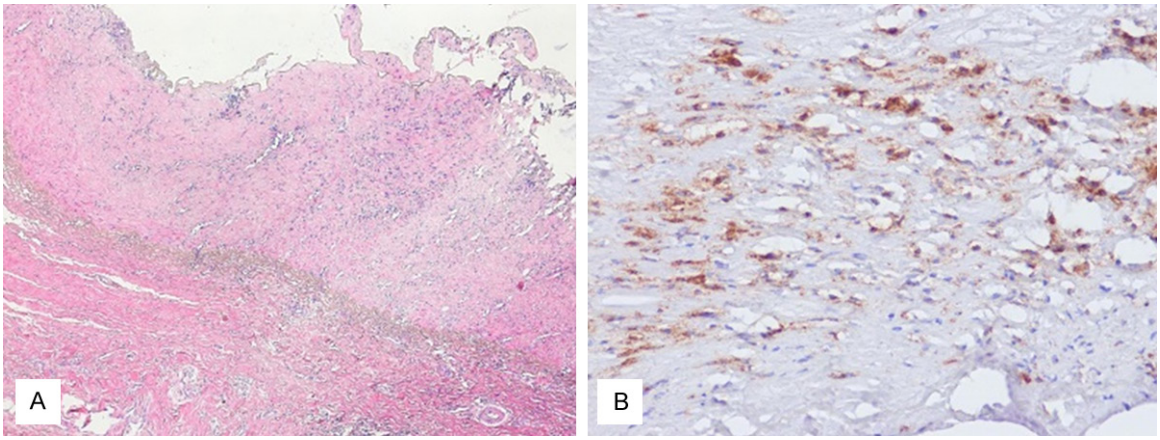

Figure 3. Atherosclerotic artery. A. Atherosclerotic artery is present. B. Prosaposin is strongly expressed in the cytoplasm of smooth muscle cells.

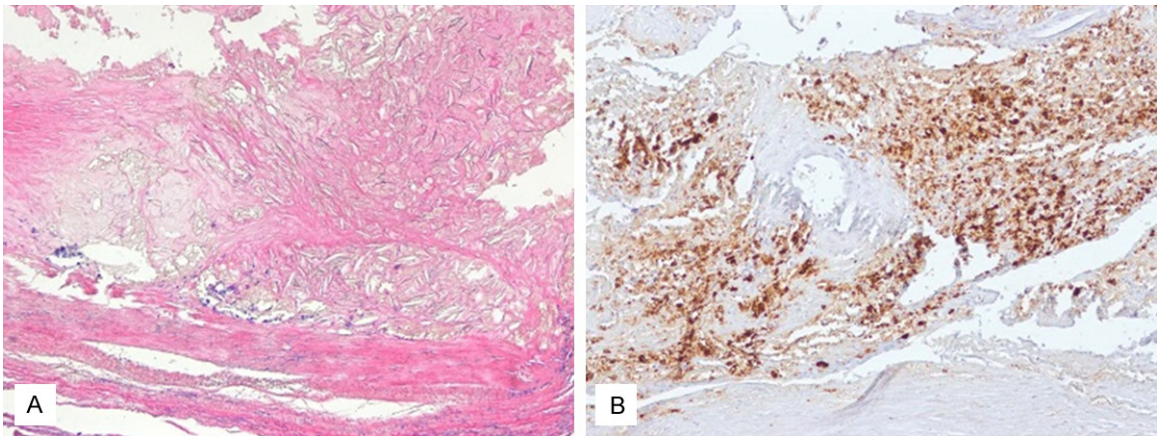

Figure 4. Atheromatous plague. A. Atheromatous plaque is composed of fibrous tissue, cholesterol crystals, and macrophages. B. Prosaposin is strongly expressed in the cytoplasm of macrophages and in the atheromatous plague.

prematurely senescent HDFs and HUVECs in response to hydrogen peroxide or interferon-γ treatment [11]. Kim et al. [11] suggested that prosaposin might be a novel senescence-associated gene and may be involved in vascular aging.

In the present study, prosaposin expression was significantly higher in atherosclerotic arteries than in normal young arteries and old arteries. All atherosclerotic samples exhibited markedly increased prosaposin expression in the cytoplasm of smooth muscle cells and macrophages, and in the atheromatous plaque. The expression of prosaposin may contribute to oxidative stress, inflammation, foam cell formation, nitric oxide production, and autophagy, thereby playing a role in the development of atherosclerosis [9].

Prosaposin is an intriguing, ubiquitous, and highly conserved protein believed to be involved in a various biological processes [12]. Hiraiwa et al. [13] demonstrated that prosaposin is a naturally occurring injury-repair protein, which acts to prevent degeneration and to promote the regeneration of peripheral nerves. Prosaposin is involved in signal transduction pathways and plays significant roles in the regulation of cell proliferation and apoptosis [14, 15].

Prosaposin may be expressed in various subcellular locations including the plasma membrane, lysosome, and extracellular matrix [16].

Like certain other lysosomal proteins, prosaposin can be secreted into the extracellular spaces, with this secretory process being enhanced under conditions of cellular stress [17]. In this study, prosaposin was found in the vascular wall of normal old arteries and in the atheromatous plaque. These findings suggest that prosaposin plays a role in the process of vascular aging and development of atherosclerosis.

Two main prosaposin staining patterns were observed in the cytoplasm of positive cells [12]. First, a granular pattern was observed in the supranuclear and basal regions of the majority of epithelial cells, and a granular perinuclear pattern was present in the neurons. The size and distribution of the granules were reminiscent of lysosomes. Second, a homogeneous cytoplasmic pattern was observed in the Sertoli cells of the seminiferous epithelium, type II pneumocytes, and in epithelial cells lining the choroid plexus. In the present, the main cytoplasmic immunostaining pattern observed was granular.

A limitations of this study is that we did not observe prosaposin mRNA levels in normal and atherosclerotic arteries. The functional significance of prosaposin in the vascular aging process, atherosclerosis, and carcinogenesis is mostly unknown. Further studies of the mechanisms underlying cellular senescence will provide new insights into the pathogenesis of age-associated vascular disorders, and the potential of anti-senescence therapy for vascular aging [18, 19].

In summary, our study is the first to measure prosaposin expression in normal young, normal old, and atherosclerotic arteries by immunohistochemistry. Prosaposin was expressed in normal old and atherosclerotic arteries, which suggests that it may play a role in the process of vascular aging and development of atherosclerosis.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Joon Hyuk Choi, Department of Pathology, Yeungnam University College of Medicine, 170 Hyeonchung-ro, Namgu, Daegu 42415, Korea. Tel: +82-53-640-6754; Fax: +82-53-656-1429; E-mail: joonhyukchoi@ynu.ac.kr

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