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Angiogenesis and lymphangiogenesis and expression of lymphangiogenic factors in the atherosclerotic intima of human coronary arteries☆

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Summary Little information regarding the development of lymphangiogenesis in coronary atherosclerosis is available. We immunohistochemically investigated the correlation among intimal neovascularization (CD34 for angiogenesis and lymphatic vessel endothelial hyaluronan receptor-1 [LYVE-1] and podoplanin for lymphangiogenesis), the expression of lymphangiogenic factors (vascular endothelial growth factor [VEGF]-C and VEGF-D), and the progression of atherosclerosis using 169 sections of human coronary arteries from 23 autopsy cases. The more the atherosclerosis advanced, the more often the neointimas contained newly formed blood vessels ($P < .0001$). Vascular endothelial growth factor-C was expressed mostly in foamy macrophages and in some smooth muscle cells, whereas VEGF-D was abundantly expressed in both. The number of VEGF-C–expressing cells, but not that of VEGF-D–expressing cells, was increased as the lesion advanced and the number of intimal blood vessels increased ($P < .01$). Lymphatic vessels were rare in the atherosclerotic intima (LYVE-1 vs CD34 = 13 vs 3955 vessels) compared with the number seen in the adventitia (LYVE-1 vs CD34 = 360 vs 6921 vessels). The current study suggests that VEGF-C, but not VEGF-D, may contribute to plaque progression and be a regulator for angiogenesis rather than lymphangiogenesis in coronary atherosclerotic intimas. Imbalance of angiogenesis and lymphangiogenesis may be a factor contributing to sustained inflammatory reaction during human coronary atherogenesis.

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Abbreviations: VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; AHA, American Heart Association; SMC, smooth muscle cell; LYVE-1, lymphatic vessel endothelial hyaluronan receptor-1; DIT, diffuse intimal thickening.

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1. Introduction

Emerging evidence supports the hypothesis that atherosclerosis is an inflammatory disease [1], and sustained inflammatory reaction of coronary atherosclerosis is suggested to be a cause of plaque instability, resulting in its rupture [2]. Therefore, investigation regarding the factors affecting the acceleration and/or maintenance of the inflammatory reaction in atherosclerotic plaque is required to understand the pathophysiology of coronary atherosclerosis and to develop an efficient strategy to prevent plaque rupture.

Angiogenesis is a common event occurring in the inflammatory foci, and newly formed blood vessels that originated mainly from the adventitia and rarely from the lumen have also been observed in the atherosclerotic plaques of human coronary arteries [3-5]. We previously demonstrated that the number of neovessels in the intimas of human coronary arteries was correlated with the degree of the inflammatory infiltration and the severity of the atherosclerosis [4], suggesting the essential contribution of plaque angiogenesis to the inflammatory reaction in, and progression of, atherosclerotic lesions. This was also supported by an experimental study indicating that systemic administration of angiogenic inhibitors, endostatin and TNP-470, resulted in suppression of the plaque progression in apolipoprotein E–deficient mice [6].

Recent studies suggest that vascular endothelial growth factor (VEGF; namely, VEGF-A) is a key regulator of angiogenesis in atherosclerotic lesions. We [7] and others [8] demonstrated that VEGF-expressing cells were distributed around the inflammatory foci of coronary atherosclerosis in human subjects, and experimental studies indicated that acceleration of intimal hyperplasia was associated with angiomatoid proliferation of leaky capillaries by arterial gene transfer of VEGF-A [9] and that VEGF-A accelerated the rate of lesion development in animal models of atherosclerosis [10-13], suggesting the essential contribution of VEGF-A to the progression of atherosclerosis.

In turn, a network of lymphatic vessels drains the extravasated bloodless fluid, protein, and inflammatory cells from the tissues [14]. Lymphatic vessels also serve an immune function by antigen-presenting cells that patrol the tissues to the various lymphoid organs. Congenital lymphedema (Milroy's disease) and lymphedema distichiasis are caused by impaired lymphatic drainage and have mutations in the gene of VEGF receptor (VEGFR)-3 (Flt-4) and FOXC2, respectively [15]. The lymphatic system may be implicated in numerous diseases including lymphedema, inflammation, autoimmune diseases, and malignancy [14]; however, very few studies have been done on lymphangiogenesis than those on angiogenesis.

Recent studies demonstrated that the relatively new members of the VEGF family, namely VEGF-C and VEGF-D, are ligands for both VEGFR-2 (Flk-1/KDR) and VEGFR-3 and promote lymphangiogenesis [16-19] as well as angiogenesis [20-22]. Very little information, however, is available regarding the expression and distribution of producing cells of these angiogenic and lymphangiogenic factors, which suggest the possible contribution of angiogenesis and lymphangiogenesis in the progression of human atheroma.

In this study, we immunohistochemically examined human coronary arteries obtained from autopsy cases to define the relationship among the coronary atherosclerotic lesion types on the basis of the American Heart Association (AHA) classification, intimal neovascularization (angiogenesis and lymphangiogenesis), and expression of VEGF-C and VEGF-D. We found that lymphangiogenesis was a relatively rare event compared with angiogenesis, although both VEGF-C and VEGF-D were abundantly expressed, suggesting the imbalance of angiogenesis and lymphangiogenesis in the atherosclerotic lesions of human coronary arteries.

2. Materials and methods

2.1. Sample processing

Within 16 hours of the death of 23 Japanese patients (14 men and 9 women), ranging in age from 57 to 93 years (mean ± SD, 76 ± 11 years), hearts were obtained at autopsy at the Kyushu University Hospital (Table 1). All patients were selected by randomized prospective sampling, and their deaths were not caused by acute coronary disease. Eight patients were clinically and microscopically revealed to undergo old and focal myocardial infarction. The coronary arteries were cannulated, washed with 0.1 mol/L phosphate-buffered saline (pH 7.4), and perfused with 1 L of freshly prepared 4% (wt/vol) paraformaldehyde in 0.1 mol/L sodium phosphate (pH 7.4) at 100 mm Hg. Then, each heart was immersed in 4% paraformaldehyde for at least 24 hours at 4°C. The right coronary artery and left anterior descending coronary artery were dissected free from the surface of the heart, cut perpendicular to the long axis at 3-mm intervals, processed according to standard methods, and then embedded in paraffin. One hundred sixty-nine blocks were obtained and cut into 3-μm-thick serial sections at once. Sections from each block were serially subjected to hematoxylin and eosin, elastica-van Gieson's and Masson trichrome stainings, as well as immunohistochemistry. In accordance with the definitions proposed by the Committee on Vascular Lesions of the Council on Arteriosclerosis (AHA) [23], the atherosclerotic lesion type of each section was carefully classified by 3 independent pathologists (TN, YN, and SS).

2.2. Antibodies

The following antibodies were used for immunohistochemical studies: rabbit polyclonal antihuman VEGF-C (2.1 μg/mL, Zymed Laboratories, Inc, South San Francisco, Calif); rabbit polyclonal antihuman VEGF-D (2.0 μg/mL, H-144, Santa Cruz Biotechnology, Santa Cruz, Calif); mouse monoclonal antihuman CD68 (1:100, KP-1, isotype