Cardiovascular Pathology 24 (2015) 26–32

Contents lists available at ScienceDirect

Cardiovascular Pathology

Original Article

Immaturity of smooth muscle cells in the neointima is associated with acute coronary syndrome

Ayako Horita [a], Atsushi Kurata [b,*], Shin-Ichiro Ohno [b], Hiroaki Shimoyamada [c], Ikuo Saito [a], Hiroshi Kamma [c], Masahiko Kuroda [b]

[a] Division of Pathology, Sagamihara National Hospital, Kanagawa, Japan
[b] Department of Molecular Pathology, Tokyo Medical University, Tokyo, Japan
[c] Department of Pathology, Kyorin University School of Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form 9 September 2014
Accepted 9 September 2014

Keywords:
Acute coronary syndrome
Smooth muscle
Differentiation
Ruptured plaque

ABSTRACT

Background: Acute coronary syndrome (ACS) is mostly caused by ruptured plaques. The characteristics of rupture-prone vulnerable plaques include thin fibrous cap, large lipid core, and lower number of smooth muscle cells. Smooth muscle cells appearing in neointimal plaques are currently thought to have a uniformly synthetic phenotype, and their sub-classification has not been performed by h-caldesmon, which is supposed to be expressed in vascular smooth muscle cells that are beyond intermediately differentiated.
Methods: Stenotic coronary arteries were obtained from autopsy material of 51 adults. Cases were divided into three groups: those who died from ACS, those with a past history of ACS but died from other causes, and those without ACS history. Histological data including fibrous cap and lipid core were measured in each specimen. Immunohistochemistry for alpha-smooth muscle actin (α-SMA), h-caldesmon, and smoothelin was performed. The ratio of h-caldesmon$^+$ cells to α-SMA$^+$ cells was counted in the neointima.
Results: The positivity ratio of neointimal h-caldesmon decreased in a step-wise manner from cases without history of ACS through cases with past history of ACS to cases with ACS with statistical significance ($P<.001$). The correlation between h-caldesmon expression and progression of ACS among the different groups was more prominent than the differences in the extent of fibrous cap and lipid core. Smoothelin$^+$ cells were rarely observed in the neointima.
Conclusions: Decreased positivity of h-caldesmon in neointimal smooth muscle cells is indicative of a more immature phenotype, thus may be associated with plaque vulnerability that will promote ACS.

1. Introduction

Acute coronary syndrome (ACS) that encompasses unstable angina, acute myocardial infarction, and sudden coronary death remains a major cause of death worldwide. The great majority of ACS cases are caused by coronary thrombi, which develops on top of a ruptured atherosclerotic plaque. The histological characteristics of rupture-prone plaques, or vulnerable plaques, include large necrotic core, thin fibrous cap, and abundance of macrophages [1,2]. Although a smaller quantity of smooth muscle cells (SMCs) has been reported to be associated with vulnerable plaques [2], the quality of neointimal SMCs in association with ACS has rarely been described.

It is believed that neointimal SMCs have a uniformly "synthetic" phenotype in contrast to a "contractile" phenotype in the media [3]. Synthetic phenotype is immunohistochemically characterized by lack of staining of some differentiated SMC markers such as desmin and smoothelin [4]. Indeed, neointimal SMCs have been reported to have an almost exclusively synthetic phenotype not only in atherosclerosis but also in the neointima of Moyamoya disease [5] and of restenosis after coronary stent implantation [4]. We previously demonstrated this phenomenon also in the neointima of Buerger's disease and thromboembolism [6]. This undifferentiation or immaturity of SMCs has also been verified by molecular biology, that is, the transcription factor Krüppel-like factor 5, which is abundantly expressed in embryonic SMCs and is down-regulated with vascular development, is reinduced in the neointimal SMCs in response to vascular injury [7].

On the other hand, gradual maturation of neointimal SMCs as time goes by after coronary stent implantation has recently been reported [8]. We have recently evaluated SMC maturation in atypical polypoid adenomyoma of the uterus by immunostaining for h-caldesmon [9] and realized that this marker is useful for assessment of SMCs of beyond intermediate maturation. Although investigation of the media of coronary arteries by h-caldesmon has been reported [10], to the

Conflict of interest statement: The authors declare that they have no conflict of interest and have no funding source concerning the present study.

There are no financial disclosures to declare for any of the authors, and the authors have no conflicts of interest regarding the present study.

* Corresponding author at: Department of Molecular Pathology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan. Tel.: + 81 3 3351 6141 (393); fax: + 81 3 3352 6335.
E-mail address: akurata@tokyo-med.ac.jp (A. Kurata).

http://dx.doi.org/10.1016/j.carpath.2014.09.003
1054-8807/

best of our knowledge, evaluation of coronary neointimal SMCs by h-caldesmon has not been performed. Therefore, the present study was designed to verify whether or not immunohistochemistry for h-caldesmon in the neointima is associated with ACS.

2. Material and methods

2.1. Coronary tissue specimens and grouping

The protocol of the present study was approved by the committee of human study of Tokyo Medical University and Kyorin University School of Medicine. Specimens of coronary arteries were obtained from recently performed autopsies (2007–2012) in these two universities from 51 adults (M:F=37:14) with mean age of 73.4±11.7 years old. All autopsy tissues were fixed by immersion in formalin for 24 h and embedded in paraffin using standard techniques. In the present study, tissue specimens including most narrowed coronary artery were selected in each case. These specimens were divided into three subgroups: Group A consisted of patients who died from ACS, Group B was those who had previously suffered from ACS but died from other causes, and Group C was composed of those who had never suffered ACS and died from other causes. In Groups A and B, the infarct area of the myocardium was histologically confirmed, and the coronary arteries responsible for the infarct area were selected. Group C was established as control; therefore, cases with narrowed coronary arteries along with similar age and gender composition to Groups A and B were selected. Prior to immunostaining, selected tissue sections were re-stained with hematoxylin-eosin, elastica van Gieson, and Masson's trichrome.

2.2. Immunohistochemical staining

Immunohistochemistry was performed using paraffin-embedded sections, 4 μm thick, using the avidin-biotin-peroxidase complex according to standard methods. The monoclonal antibody to alpha-smooth muscle actin (α-SMA, clone 1A4, working dilution 1:100, Dako, Glostrup, Denmark) was used to identify all SMCs, h-caldesmon (clone h-CD, working dilution 1:100, Dako) was used to identify SMCs beyond intermediate differentiation, and smoothelin (clone R4A, working dillution 1:150, Abcam, Cambridge, UK) was used to identify fully differentiated SMCs. Deparaffinized, dehydrated sections were treated with 0.3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. To expose antigens, sections were autoclaved in 10 mmol/L sodium citrate buffer (pH 6.0) at 121 °C for 10 min for immunostaining of h-caldesmon, or in 1 mmol/L EDTA Tris buffer (pH 9.0) at 121 °C for 10 min for immunostaining of smoothelin, and were cooled for 30 min. After rinsing in 0.1 mol/L phosphate buffered saline (PBS, pH 7.4), the sections were incubated with affinity purified primary antibodies overnight at 4 °C. Thereafter, they were incubated with Envision (+) rabbit peroxidase (Dako, Carpinteria, CA, USA) for 30 min. The peroxidase reaction was performed using 0.02% 3,3'-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.1 mol/L PBS (pH 7.4). Finally, nuclear counterstaining was performed with Mayer's hematoxylin.

2.3. Assessment and statistical analyses

Light microscopy performed with a Carl Zeiss HAL 100 instrument and W-PI 10x/23 ocular lens was used to analyze and quantitate the histological data. Diameter, the shortest internal diameter, and the smallest thickness of fibrous cap of each vessel were measured using Masson's trichrome staining (Fig. 1). Further, a regular pattern of points, in the form of a square lattice, was superimposed on the Masson's trichrome staining of each specimen (Fig. 2), and the total areas of media, neointima, lipid core, and lumen were determined using a systematic point counting method [11]. The digital images of immunohistochemistry were captured by NY-D5000 super system (Microscope Network, Nikon, Tokyo, Japan) and were printed by a true-color printer (IPSiO SP C420, RICOH, Tokyo, Japan). For the assessment of α-SMA, h-caldesmon, and smoothelin, the site in the intima where positive cells were most densely observed was selected, and positive cells per mm^2 were counted in each specimen. Further, the ratio of h-caldesmon$^+$ cells to α-SMA$^+$ cells was counted in at least three sites where SMA$^+$ cells were abundant in the intima using ×200 fields of each specimen. Statistical analyses (Tukey–Kramer test) were performed using MS Excel 2000 (Microsoft Corp., Tokyo, Japan) software packages. The level of significance was set at $P<.05$.

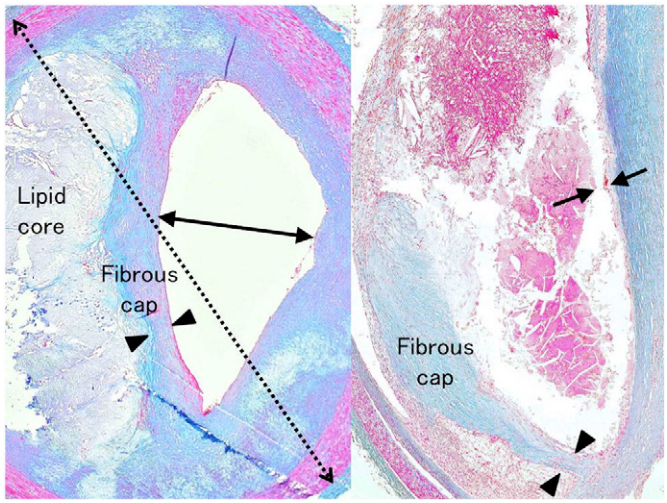

Fig. 1. Representative coronary arteries with Masson's trichrome staining. Diameter (dotted arrow), the shortest internal diameter (arrow), and the smallest thickness of fibrous cap (arrowheads) were measured (left). In arteries with ruptured plaques (right), the shortest internal diameter of the residual lumen (arrow) and the smallest thickness of the residual fibrous cap were measured (arrowheads).

3. Results

3.1. Clinical and histological findings

The number of cases in Group A, B, and C was 18 (M:F=12:6), 18 (M:F=13:5), and 15 (M:F=12:3), respectively. Representative results demonstrating average values and standard deviation in each group are shown in Figs. 3 and 4, along with statistical analysis. Although the average age of Group A (68.1±14.9 years old) was significantly lower than Group B (78.1±7.2 years old) ($P<.05$), the average age between

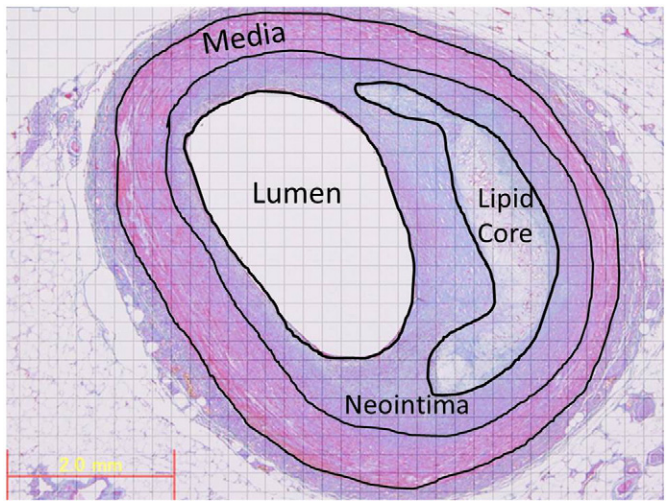

Fig. 2. A regular pattern of points (a square lattice) was superimposed on Masson's trichrome staining for systematic point counting.