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Sudden coronary death in the young: Evidence of contractile phenotype of smooth muscle cells in the culprit atherosclerotic plaque☆

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ABSTRACT

Background: Culprit coronary atherosclerotic plaques (APs) from young sudden cardiac death (SCD) victims are mostly non-atheromatous, i.e., consisting of proliferative smooth muscle cells (SMCs). Coronary vasospasm has been advocated to explain plaque instability in the absence of thrombosis. Our aim was to characterize the SMC phenotype in the intima and media of coronary arteries from young SCD victims.
Methods and Results: A total of 38 coronary artery segments were studied: (a) 18 APs from young (≤40 years old) SCD patients, (b) 9 APs from old (>40 years old) SCD patients, (c) 11 non-atherosclerotic coronary arteries from young patients (≤40 years old). Markers of differentiated SMCs such as α-smooth muscle actin (α-SMA), smooth muscle myosin heavy chains (SMMHCs), and heavy-caldesmon (h-CaD), were assessed in intima and media by immunohistochemistry and quantified morphometrically. In the intima, their expression was higher in non-atherosclerotic arteries (44.37 ± 3.03% for α-SMA, 14.21 ± 2.01% for SMMHCs, 8.90 ± 1.33% for h-CaD) and APs from young SCD victims (38.95 ± 2.29% for α-SMA, 11.92 ± 1.92% for SMMHCs, 8.93 ± 1.12% for h-CaD) compared with old patients (22.01 ± 3.56% for α-SMA, 6.39 ± 0.7% for SMMHCs, 3.00 ± 0.57% for h-CaD; all P statistically significant). The media of non-atherosclerotic arteries and APs from young SCD victims exhibited strong positivity for the differentiation markers unlike that of old patients.
Conclusions: SMCs of coronary APs as well as from the underlying media from young SCD victims exhibit strong contractile phenotype. In the setting of critical stenosis, both intima and media SMC contractility might contribute to transient coronary spasm leading to myocardial ischemia and SCD.

1. Introduction

Although much less frequent than in adults, atherosclerotic coronary artery disease (CAD) is a major cause of sudden cardiac death (SCD) in young people (≤40 years old), accounting for nearly 20% of all the fatal events [1–3]. In the young, culprit atherosclerotic CAD exhibits distinctive features in terms of extent, site and morphology of the atherosclerotic plaque (AP). It is often a single vessel disease, mostly affecting the proximal segment of left anterior descending coronary artery and is rarely complicated by thrombosis [1,4]. With regards to the histopathological findings, APs in the young are often non-atheromatous, also known as pathological intimal thickening [5],consisting predominantly of smooth muscle cells (SMCs) with a variable amount of interstitial matrix, and minimal or absent lipid core [1–5]. SCD triggered by the so-called non-atheromatous atherosclerosis (NAA) cannot be ascribed to the rupture of a thin fibrous cap [1,6]. Other causes are invoked, such as thrombotic occlusion due to endothelial erosion or coronary vasospasm [1,7–9].

The aim of this study was to characterize the phenotype of SMCs in coronary APs of young SCD patients and to investigate whether they exhibit a differentiated phenotype compatible with a strong contractile capacity. For this purpose, cytoskeletal proteins that are well-accepted markers for SMC differentiation, i.e., α-smooth muscle actin (α-SMA)

Abbreviations: AA, atheromatous atherosclerosis; AP, atherosclerotic plaque; α-SMA, α-smooth muscle actin; CAD, coronary artery disease; γ-SMA, γ-smooth muscle actin; h-CaD, heavy-caldesmon; IEL, internal elastic lamina; NAA, non-atheromatous atherosclerosis; SCD, sudden cardiac death; SMC, smooth muscle cell; SMMHCs, smooth muscle myosin heavy chains; VV, vasa vasorum.

☆ The authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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[10], smooth muscle myosin heavy chains (SMMHCs) [11] and heavy-caldesmon (h-CaD) have been used [12]. The expression of γ-smooth muscle actin (γ-SMA), which is well represented in vascular SMCs even though to a lower extent than α-SMA [13], and S100A4, a calcium-binding protein described as a suitable marker of dedifferentiated intimal SMCs in APs and restenotic lesions [14], were also investigated.

2. Methods

In the Registry on Juvenile Sudden Death of the Veneto Region, Northeast Italy, among 690 consecutive cases of SCD in the young (≤40 years old), 125 (18%) were due to atherosclerotic CAD (mean age 32.3 ± 5.3, M/F = 111/14) [1,2]. Among the latter, 76 (61%) had critical lumen stenosis in the absence of thrombosis. Only recent cases in whom the paraffin embedded culprit coronary APs were still available for further investigation were included in the study.

The samples consisted of: (a) 18 coronary APs from 16 young coronary SCD patients (≤40 years old); (b) 9 coronary APs from 8 old coronary SCD patients (>40 years old); (c) 11 non-atherosclerotic coronary arteries from 5 young non coronary SCD patients (≤40 years old).

Methods are available in detail in the online-only Data Supplement.

3. Results

3.1. General features

Main clinical and pathological data from the 3 study groups are described in the Supplemental Table.

Immunostaining for α-SMA at low magnification (Fig. 1) showed that non-atherosclerotic coronary arteries from young patients exhibited a mild intimal thickening composed of SMCs. NAA lesions from young SCD victims consisted predominantly of SMCs in the absence of a lipid/necrotic core. In contrast, AA lesions from young SCD victims and from old patients denoted a plaque with a fibrous cap overlying a lipid/necrotic core.

The cross-sectional area of the media was significantly smaller in old patients (0.972 ± 0.109 mm^2) compared with that of non-atherosclerotic coronary arteries (1.366 ± 0.206 mm^2, $p = 0.048$) and that of young SCD victims (1.740 ± 0.160 mm^2, $p = 0.00024$). No difference was present between the last two situations. Thus, media atrophy is present in APs from the old patients.

As expected the cross-sectional area of the intima was significantly smaller in non-atherosclerotic coronary arteries (2.213 ± 0.380 mm^2) compared with that of APs from the old patients (5.337 ± 0.815 mm^2, $p = 0.0017$) and that of APs from the young SCD victims (5.208 ± 0.535 mm^2, $p = 0.00005$). No difference was present between the last two situations.

3.2. SMC differentiation marker expression in the media

The area of the media expressing α-SMA was markedly higher in non-atherosclerotic coronary arteries (72.05 ± 1.94%) and underlying APs from the young SCD victims (75.22 ± 2.66%) compared with that of APs from the old patients (64.64 ± 1.97%, $p = 0.0047$ and $p = 0.0012$, respectively; Figs. 1 and 2). Evaluation of SMMHCs and h-CaD showed that the highest degree of differentiation was observed in the media of non-atherosclerotic coronary arteries (39.19 ± 2.72% for SMMHCs and 28.44 ± 1.69% for h-CaD) and in that of APs from the young SCD victims (38.84 ± 3.30% for SMMHCs and 32.48 ± 1.48% for h-CaD) whereas the media underlying the APs of old patients exhibited a lower degree of differentiation (30.40 ± 1.55% for SMMHCs and 21.93 ± 2.39% for h-CaD, $p = 0.0077$ and $p = 0.0056$ respectively vs non-atherosclerotic coronary arteries and $p = 0.018$ and $p = 0.0002$ respectively vs APs from the young SCD victims) (Fig. 1). The expression of γ-SMA in the

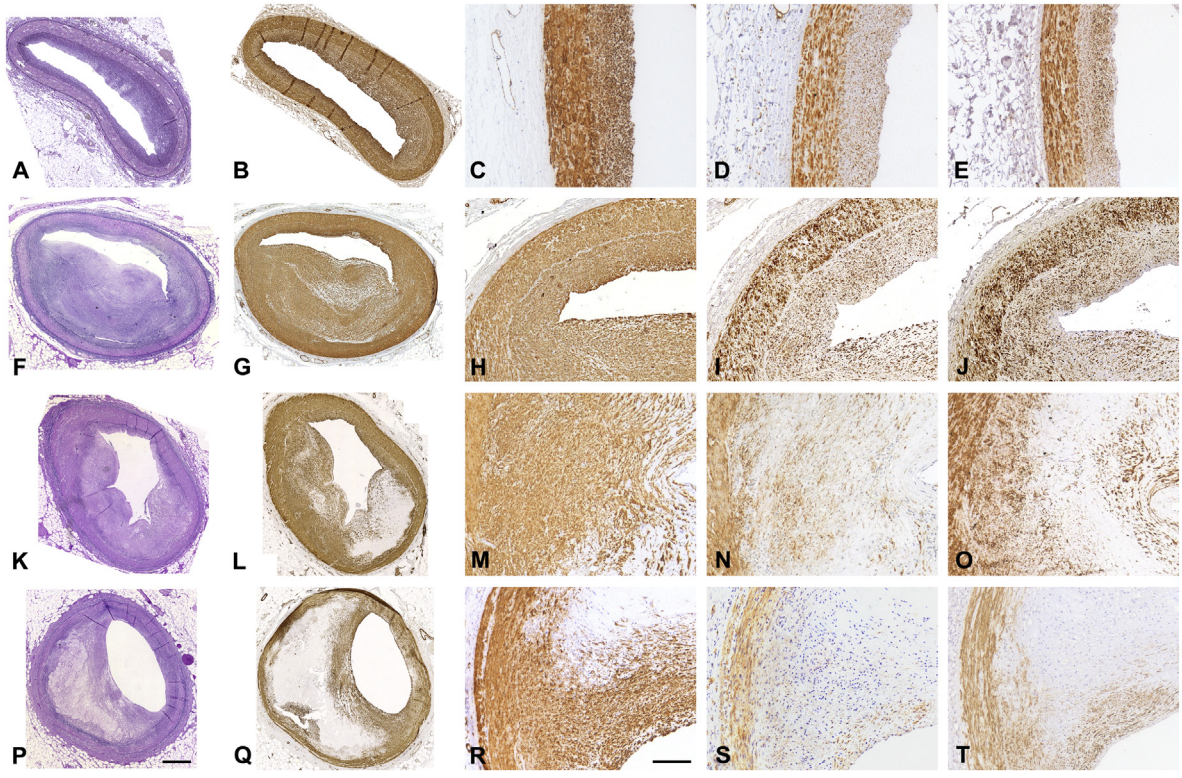

Fig. 1. Representative cases for the study groups. Miller staining (A, F, K, P) and immunostaining for α-SMA at low magnification (B, G, L, Q) and high magnification (C, H, M, R), SMMHCs at high magnification (D, I, N, S) and h-CaD at high magnification (E, J, O, T) in non-atherosclerotic coronary arteries (A–E), NAA lesions from young SCD victims (F–J), AA lesions from young SCD victims (K–O) and in APs from old patients (P–T). Scale bar = 1 mm (P) for all pictures taken at low magnification and 100 μm in (R) for all pictures taken at low magnification. α-SMA, SMMHCs and h-CaD are strongly expressed in SMCs of NAA (G–J) and AA (L–O) lesions from young SCD victims similarly to SMCs present in non-atherosclerotic coronary arteries (B–E). In contrast, α-SMA, SMMHCs and h-CaD are poorly expressed in SMCs of APs from old patients (Q–T). Note the absence of lipid/necrotic core in NAA lesions from young SCD victims (F, G).