*Proc. Natl. Acad. Sci. USA*
Vol. 85, pp. 9542–9546, December 1988
Cell Biology

# Modulation of human aorta smooth muscle cell phenotype: A study of muscle-specific variants of vinculin, caldesmon, and actin expression

(cytoskeleton/atherosclerosis)

MARINA A. GLUKHOVA*, ALEXANDER E. KABAKOV, MARIA G. FRID, OLGA I. ORNATSKY, ALEXEY M. BELKIN, DMITRY N. MUKHIN, ALEXANDER N. OREKHOV, VICTOR E. KOTELIANSKY, AND VLADIMIR N. SMIRNOV

Institute of Experimental Cardiology, U.S.S.R. Cardiology Research Center, Academy of Medical Sciences, 3rd Cherepkovskaya Street 15A, 121552, Moscow, U.S.S.R.

*Communicated by I. M. Gelfand, August 16, 1988 (received for review May 31, 1988)*

ABSTRACT **Vinculin- and caldesmon-immunoreactive forms and actin isoform patterns were studied in samples of normal and atherosclerotic human aorta. After removal of adventitia and endothelium, the remaining tissue was divided into three layers: media, muscular-elastic (adjacent to media) intima, and subendothelial (juxtaluminal) intima. In media of normal aorta, meta-vinculin accounted for 41.0 ± 0.9% (mean ± SEM) of total immunoreactive vinculin (meta-vinculin + vinculin); 150-kDa caldesmon accounted for 78.2 ± 5.1% of immunoreactive caldesmon (150-kDa + 70-kDa); the fractional contents of α-smooth muscle actin, β-nonmuscle, and γ-isoactins were 49.0 ± 0.6%, 30.4 ± 0.6%, and 20.8 ± 0.8%, respectively. Muscular-elastic intima was very similar to media by these criteria. In subendothelial intima, the fractional content of meta-vinculin and 150-kDa caldesmon was significantly lower (6.9 ± 1.5% and 32.7 ± 7.0%, respectively) than in muscular-elastic intima and media, whereas the isoactin pattern was identical to that in adjacent layers, demonstrating the smooth muscle origin of subendothelial intima cells. In atherosclerotic fibrous plaque, the fractional content of α-actin was decreased in subendothelial intima, rather than in media and muscular-elastic intima. Additionally, the proportion of subendothelial intima cells [i.e., the cells that express low amounts of smooth muscle phenotype markers (meta-vinculin, 150-kDa caldesmon, and α-actin)] in the total intima cell population increased dramatically in atherosclerotic fibrous plaque. The results suggest that changes in the relative content of meta-vinculin and 150-kDa caldesmon as well as α-actin in human aortic intima are associated with atherosclerosis although, in subendothelial intima of normal aorta, a certain smooth muscle cell population exists that expresses reduced amounts of "contractile" phenotype markers, even in the absence of the disease.**

Smooth muscle cells exist both *in situ* and in culture as differentiated, contractile cells and as synthetic cells. Contractile smooth muscle cells have a well-developed contractile apparatus, and their cytoplasm is packed with thin and thick filaments. Synthetic smooth muscle cells have large amounts of free ribosomes, rough endoplasmic reticulum, and mitochondria, but few myofilaments. According to a concept of Chamley-Campbell *et al.* (1–3), these two distinct phenotypes are the opposite points on a continuous spectrum of intermediate states of differentiation available to the smooth muscle cell.

The transition from contractile to synthetic state [i.e., "phenotype modulation" (2)] can be correlated with changes of cytoskeleton and contractile apparatus. One of the most characteristic features of smooth muscle cells is their content of smooth muscle-specific contractile and cytoskeletal proteins. Three different actin isoforms were detected by isoelectrofocusing in vascular smooth muscle cells (4–8): α-smooth muscle, β-nonmuscle, and γ-isoactin, consisting of both γ-smooth muscle and γ-nonmuscle variants. In the normal adult aorta, medial smooth muscle cells show predominance of α-actin. In the course of primary culture of cells obtained by enzyme digestion of aorta tissue, however, the β-nonmuscle isoform becomes predominant (5, 7). Also, in human atheromatous plaques as well as in experimental intimal thickening in rats, β-nonmuscle actin rather than the α-smooth muscle type prevails (6, 9, 10). Recently, the shift in myosin expression during the adaptation of smooth muscle cells to culture conditions was demonstrated (11, 12); smooth muscle-specific myosin was decreased while nonmuscle variant was shown to be synthesized. Unlike other muscle cells that contain only desmin as a major intermediate filament protein, vascular smooth muscle cells express various ratios of desmin and vimentin that depend on both vessel type and biological species (9, 13–17). During cultivation, smooth muscle cells lose desmin, which is substituted by vimentin. In fibrous plaques of human arteries and in experimental intimal thickening in rats, the amount of desmin-positive cells dramatically decreases (8, 9). The expression of actin isoforms, desmin, and vimentin has also been shown to change during development (18–21). Thus cytoskeletal and contractile proteins can serve as reliable markers of phenotype modulation as it occurs in culture, during development, and in pathological situations.

For our study, we have chosen three proteins whose variants are expressed differently in smooth muscle and nonmuscle cells: actin, a major contractile protein; vinculin, a cytoskeletal protein; and caldesmon, a protein acknowledged to be involved in the regulation of smooth muscle contraction.

Vinculin is a protein of 130 kDa, and it is localized at a variety of cellular adhesive structures in all nonmuscle and muscle cells so far examined. In studying smooth muscle, two groups of investigators (22, 23) have found a protein of 150 kDa that was immunologically and structurally related to vinculin. The protein, termed meta-vinculin, appeared to be restricted to muscle tissue (22, 24, 25). In smooth muscle cells from human aorta, meta-vinculin could account for up to 40% of the total protein recognized by anti-vinculin antibody (25).

Caldesmon is a major calmodulin- and actin-binding protein of smooth muscle and nonmuscle cells (26). It is believed to be involved in a $Ca^{2+}$-dependent control mechanism

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*To whom reprint requests should be addressed.

modulating actin–myosin interaction and thereby contraction (26–28). Immunochemical analysis reveals a significant heterogeneity in caldesmon species in different tissues and cells: anti-smooth muscle caldesmon antibodies recognize polypeptides in the molecular mass range of 120–150 kDa and those in the range of 71–77 kDa; some tissues and cultured cells contain both classes of immunoreactive polypeptides (29, 30). In vascular smooth muscle, 150-kDa caldesmon was shown to be a predominant if not the only form of the protein (27, 31, 32). Cultured fibroblasts and smooth muscle cells (long-term culture) as well as peritoneal macrophages, blood lymphocytes, and platelets contain the lower molecular mass form, that of about 70 kDa (ref. 33; A.E.K., unpublished data).

The wall of human aorta consists of three layers: tunica intima, tunica media, and tunica adventitia. Two sublayers can be distinguished in intima of human adult aorta—juxtaluminal, further referred to as subendothelial, and muscular-elastic, which is adjacent to the media. The predominant cell type in tunica media and tunica intima of the aorta is smooth muscle (34, 35). After removal of endothelium, media and two intimal sublayers were separated mechanically and assayed for vinculin, caldesmon, and actin variants content.

The aim of this study was to determine whether changes in the fractional content of smooth muscle characteristic variants of vinculin, caldesmon, and actin in different layers of the human aorta are associated with atherosclerosis.

## MATERIALS AND METHODS

**Tissue Samples.** Thoracic aortas were obtained at autopsies taken within 3–7 hr of death. The age of donors ranged from 35 to 60 years. Adventitia was discarded, the aorta was opened, and endothelium was removed either mechanically or by a short trypsin treatment. Normal segments and plaques were separated and mechanically divided into two (medial and total intimal) or three (36) [medial and two intimal: subendothelial (juxtaluminal) and muscular-elastic (adjacent to media)] layers. Then tissue samples were immediately dispersed in the buffer for PAGE or isoelectric focusing.

**Antigens and Antibodies.** Vinculin and caldesmon were purified for immunization from human uterus according to refs. 37 and 31, respectively. Antisera raised in rabbits were shown to be monospecific as judged by immunoblotting performed with the uterus extract. The IgG fraction was obtained by $(NH_4)_2SO_4$ precipitation, followed by DEAE-chromatography.

**PAGE and Immunoblotting.** Cells or tissue samples were lysed in 40 mM Tris·HCl buffer at pH 8.0 containing 5% (vol/vol) 2-mercaptoethanol, 4% (wt/vol) $NaDodSO_4$, 10 mM EDTA, 20% (vol/vol) glycerol, 2 mM phenylmethylsulfonyl fluoride, leupeptin (20 μg/ml), pepstatin (20 μg/ml), aprotinin (20 μg/ml), and benzamidine (130 μg/ml). Extracts (100–200 μg of protein per sample) were run in $NaDodSO_4$/polyacrylamide (5–15%) slab gels. Immunoblotting was performed according to ref. 38. Anti-caldesmon and anti-vinculin IgG were used at concentrations of 150 μg/ml and 70 μg/ml, respectively. Secondary antibodies were $^{125}I$-labeled goat anti-rabbit IgG ($10^6$ cpm/μg; working dilution, 1 μg/ml). Immunoblots were exposed to x-ray film (Tasma, Shostka, U.S.S.R.) overnight to determine the positions of the proteins. Then the strips of nitrocellulose corresponding to vinculin, meta-vinculin, and 150- and 70-kDa caldesmons were cut out, and radioactivity was measured in a γ counter. Radioactivity levels for each variant of caldesmon and vinculin were converted into a percent of total vinculin-(meta-vinculin + vinculin) or caldesmon- (150-kDa + 70-kDa) associated radioactivity in a sample. The method was reproducible with repeated measurements on the same sample (i.e., the ratio of the SD to the mean was less than 5% of the mean) and was linear over the range of protein loadings used.

**Two-Dimensional Isoelectric Focusing/PAGE.** A modification of O'Farrell's method was used for resolving isoactins (39) in an ampholyte mixture restricted to the pH 4–6.5 interval; prefocusing was done for 20 min at 200 V, 30 min at 300 V, and 45 min at 400 V. Tissue samples or cells were lysed in 9 M urea/2% (vol/vol) Nonidet P-40/5% 2-mercaptoethanol/0.5 mM phenylmethylsulfonyl fluoride. The samples (50–100 μg of protein) were loaded on a prefocused gel, focused at 400 V for 14 hr, and then focused at 1000 V for 1 hr. For two-dimensional electrophoresis, the concentrating gel was 4% acrylamide, and the running gel was 10% acrylamide. After completion of electrophoresis, gels were fixed in 25% (vol/vol) isopropanol/10% (vol/vol) acetic acid and stained with Coomassie brilliant blue (R-250). Gel segments containing resolved isoactins were separated and extracted overnight at room temperature into 25% (vol/vol) pyridine, and the optical density was measured at 605 nm (40). Optical density values for each isoform were converted into a percent of total optical density for all isoactins (α + β + γ) in a sample. The method was reproducible with repeated measurements on the same sample (i.e., the ratio of the SD to the mean was less than 5% of the mean) and linear over the range of protein loadings used.

**Cell Culture.** Smooth muscle cells from tunica media of human aorta were isolated by collagenase (0.3%)/elastase (0.08%) digestion (41) and cultivated in Dulbecco's modified Eagle's medium (Flow Laboratories) supplemented with 10 mM Hepes, sodium pyruvate (100 μg/ml), ascorbic acid (50 μg/ml), L-glutamine (0.6 mg/ml), gentamicin sulfate (50 μg/ml), and 10% (vol/vol) heat inactivated human serum.

## RESULTS

**Variants of Vinculin, Caldesmon, and Actin in Different Layers of Normal Human Aorta.** Three actin isoforms were revealed by two-dimensional isoelectric focusing/PAGE in the samples from aorta wall: α, consisting of α-smooth muscle; β, identical to β-nonmuscle actin; and γ, consisting of γ-smooth muscle and γ-nonmuscle isoactins (4–8). Actin isoform patterns appeared to be identical in all aorta wall layers studied (Fig. 1*A* and Table 1): media, muscular-elastic, and subendothelial intima. α-Smooth muscle actin was a predominant isoform and accounted for about 48.3–49.0% of

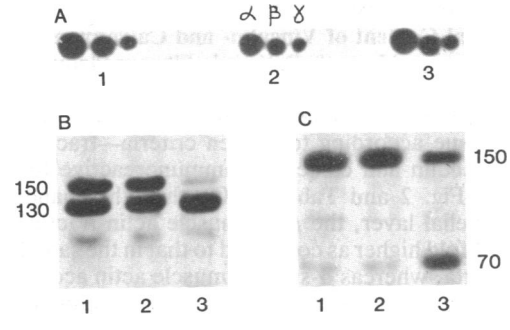


FIG. 1. Actin isoforms and vinculin- and caldesmon-immunoreactive forms in normal adult human aorta. Samples: 1, media; 2, muscular-elastic intima; 3, subendothelial intima. (*A*) Coomassie blue-stained two-dimensional isoelectric focusing/PAGE gels showing actin isoforms in different layers of the aorta wall. (*B*) Autoradiograph of immunoblot performed with anti-vinculin antibodies. Molecular mass markers (in kDa) are shown at left (150 kDa, meta-vinculin; 130 kDa, vinculin). (*C*) Autoradiograph of immunoblot performed with anti-caldesmon antibodies. The positions of the 150- and 70-kDa caldesmon are indicated.

Table 1. Fractional content of isoactins, meta-vinculin, and 150-kDa caldesmon in different layers of normal adult human aorta

| Aorta wall layer | n | Fractional isoactin content, % | | | Fractional meta-vinculin content, % | Fractional 150-kDa caldesmon content, % |
|---|---|---|---|---|---|---|
| | | α | β | γ | | |
| Media | 7 | 49.0 ± 0.6 | 30.4 ± 0.6 | 20.8 ± 0.8 | 41.0 ± 0.9 | 78.2 ± 5.1 |
| Total intima | 10 | ND | ND | ND | 39.3 ± 0.7 | 75.0 ± 0.7 |
| Muscular-elastic intima | 7 | 48.3 ± 0.8 | 31.5 ± 0.5 | 20.1 ± 0.8 | 41.6 ± 0.9 | 77.4 ± 3.2 |
| Subendothelial intima | 7 | 48.5 ± 0.1 | 30.4 ± 0.8 | 21.1 ± 0.6 | 6.9 ± 1.5* | 32.7 ± 7.0* |

Values represent means ± SEM. n, Number of samples analyzed; ND, not determined.
*Fractional content of the protein variant is significantly different from that in adjacent layers ($P < 0.0001$).

total actin; the fractional content of β-nonmuscle actin was 30.4–31.5%.

To study immunoreactive vinculin and caldesmon patterns, we used a quantitative immunoblotting technique. In media and in the two intimal layers, both meta-vinculin and vinculin were detected although the ratio of the proteins differed depending on the layer (Fig. 1*B* and Table 1). In media and muscular-elastic intima, the share of meta-vinculin was 41.0 ± 0.9% (mean ± SEM) and 41.6 ± 0.9%, respectively, whereas in subendothelial intima, meta-vinculin accounted for only 6.9 ± 1.6% of total vinculin (meta-vinculin + vinculin).

The 150- and 70-kDa caldesmons were revealed in all the samples obtained from media, muscular-elastic, and subendothelial intima (Fig. 1*C* and Table 1). The 150-kDa caldesmon appeared to be a predominant form of the protein in media and muscular-elastic intima, whereas its subendothelial intima it could account for only 32.7 ± 7% of total caldesmon (150-kDa + 70-kDa).

Significantly, the isoactin pattern of subendothelial intima was shown to be identical to that of media and muscular-elastic intima, thus confirming the smooth muscle origin of subendothelial intima cells. On the other hand, expression of smooth muscle-specific variants of vinculin and caldesmon (i.e., meta-vinculin and 150-kDa caldesmon) was significantly decreased in cells of the subendothelial layer as compared to that in the adjacent muscular-elastic intima and media.

In additional experiments, the samples of total intima (endothelium was removed, but sublayers were not separated) were analyzed (Table 1). Fractional content of meta-vinculin and 150-kDa caldesmon in these samples appeared to be very close to that in muscular-elastic intima and media. The data suggest that the share of subendothelial intima cells (i.e., the cells with a decreased meta-vinculin and 150-kDa caldesmon fractional content) in total intima cell population in normal aorta is rather low.

**Fractional Content of Vinculin- and Caldesmon-Immunoreactive Forms and Isoactin Pattern in Fibrous Plaque.** Samples of media and muscular-elastic intima taken from aorta segments with fibrous plaques were identical to those taken from normal tissue according to chosen criteria—fractional content of vinculin and caldesmon immunoreactive forms and isoactins (Fig. 2 and Table 2). On the other hand, in the subendothelial layer, the β-nonmuscle actin fractional content was 2-fold higher as compared to that in the samples from normal aorta, whereas α-smooth muscle actin accounted for only 20.2 ± 0.7% of total actin (Fig. 2 and Table 2). Meta-vinculin and 150-kDa caldesmon fractional contents were 6.9 ± 1.7% and 38.8 ± 4.1%, respectively. Therefore, the expression of three proteins characteristic of smooth muscle, meta-vinculin, 150-kDa caldesmon, and α-actin was decreased in subendothelial intima of fibrous plaque as compared to media and muscular-elastic intima.

In total intima of aorta segment containing fibrous plaque, the share of meta-vinculin was 17.9 ± 1.3% and that of 150-kDa caldesmon was 50.5 ± 5.4% (Table 2), whereas in its muscular-elastic layer, the shares were 38.5 ± 1.1% and 72.0 ± 1.8%, respectively. Moreover, in several plaques, meta-vinculin content in total intima was very close to the level characteristic of the isolated subendothelial layer (Fig. 3). Thus, we conclude that in fibrous plaque the proportion of two intimal layers is altered if compared to normal aorta. In the plaque, the cells of subendothelial intima (i.e., those with low meta-vinculin, 150-kDa caldesmon and, α-actin fractional content) represent a significant part of intimal cell population, whereas in normal aorta the proportion of subendothelial intima cells is very low (see Table 1).

**Vinculin- and Caldesmon-Immunoreactive Forms in Cultivated Smooth Muscle Cells from Human Aorta.** To examine whether modulation of smooth muscle cell phenotype during cultivation is accompanied by changes in the immunoreactive vinculin and caldesmon pattern, we have isolated cells from media of human aorta by enzyme digestion. The proportion of smooth muscle characteristic and nonmuscle variants of vinculin and caldesmon was determined by quantitative immunoblotting technique in the course of primary culture. By the onset of intensive cell division (10–12th day in culture), meta-vinculin could hardly be detected, while 150-kDa caldesmon was almost entirely substituted by the 70-kDa form (Fig. 4). A share of α-actin in the total actin pool, as was previously reported by others (5, 7), also decreased dramatically during cultivation (Fig. 4). Thus meta-vinculin and 150-kDa caldesmon, as well as actin, can serve as markers of "contractile" smooth muscle cell and are reduced (or even lost) prior to onset of cell proliferation in culture.

## DISCUSSION

In this study, the expression of smooth muscle cell phenotypes in normal and atherosclerotic human aorta was analyzed. We have estimated fractional content of actin isoforms, vinculin, and caldesmon immunoreactive forms in the

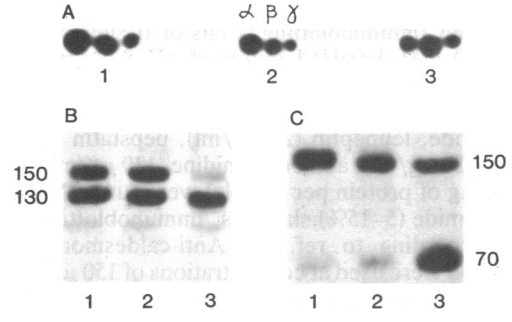


FIG. 2. Actin isoforms and vinculin- and caldesmon-immunoreactive forms in fibrous plaque of human aorta. Samples: 1, media; 2, muscular-elastic intima; 3, subendothelial intima. (*A*) Coomassie blue-stained two-dimensional isoelectric focusing/PAGE gels showing actin isoforms in different layers of the aorta wall. (*B*) Autoradiograph of immunoblot performed with anti-vinculin antibodies. Molecular mass markers (in kDa) are shown at left (150 kDa, meta-vinculin; 130 kDa, vinculin). (*C*) Autoradiograph of immunoblot performed with anti-caldesmon antibodies. The positions of the 150- and 70-kDa caldesmon are indicated.

Table 2. Fractional content of isoactins, meta-vinculin, and 150-kDa caldesmon in atherosclerotic plaque

| Aorta wall layer | *n* | Fractional isoactin content, % | | | Fractional meta-vinculin content, % | Fractional 150-kDa caldesmon content, % |
|---|---|---|---|---|---|---|
| | | α | β | γ | | |
| Media | 8 | 48.9 ± 0.5 | 30.4 ± 0.2 | 20.7 ± 0.3 | 39.1 ± 0.5 | 79.0 ± 6.4 |
| Total intima | 10 | ND | ND | ND | 17.9 ± 1.3* | 50.5 ± 5.4* |
| Muscular-elastic intima | 8 | 48.3 ± 0.5 | 30.7 ± 0.4 | 20.0 ± 0.3 | 38.5 ± 1.1 | 72.0 ± 1.8 |
| Subendothelial intima | 9 | 20.2 ± 0.7* | 60.5 ± 0.9* | 19.4 ± 0.8 | 6.9 ± 1.7* | 38.8 ± 4.1* |

Values represent means ± SEM. *n*, Number of samples analyzed; ND, not determined.
*Fractional content of the protein variant is significantly different from that in adjacent layers ($P < 0.001$).

three layers of aorta wall: media, muscular-elastic intima, and subendothelial intima. In the media of normal aorta, meta-vinculin accounted for 41.0 ± 0.9% of total vinculin (meta-vinculin + vinculin); 150-kDa caldesmon accounted for 78.2 ± 5.1% of total caldesmon; and the fractional content of α-, β- and γ-isoactins was 49.0 ± 0.6%, 30.4 ± 0.6%, and 20.8 ± 0.8%, respectively. Muscular-elastic intima was very similar to media, whereas subendothelial intima appeared to differ significantly from subjacent layers. The isoactin pattern in the cells of subendothelial intima was identical to that in media and muscular-elastic intima, thus indicating the smooth muscle origin of the majority of the cells inhabiting this layer. However, meta-vinculin and 150-kDa caldesmon fractional content was rather low, 6.9 ± 1.6% and 32.7 ± 7.0%, respectively. Therefore we assume that in subendothelial intima a population of smooth muscle cells exists that expresses reduced amounts of certain smooth muscle phenotype markers, even in the absence of atherosclerosis. It is not clear whether these cells have lost some smooth muscle characteristic features, and should, therefore, be referred to as "modulated" or whether they never expressed those features, thus representing a partly immature smooth muscle cell population. We cannot, however, completely disregard a possibility that these cells are not of smooth muscle origin because pericytes, the cells that are associated with capillaries and postcapillary venules, express some smooth muscle characteristic contractile proteins too (42, 43). In the course of primary culture, meta-vinculin, 150-kDa caldesmon, and α-actin are reduced simultaneously as the cells finish spreading. Thus cultivated smooth muscle cells differ from the cells of subendothelial intima, although their phenotype is also not "contractile."

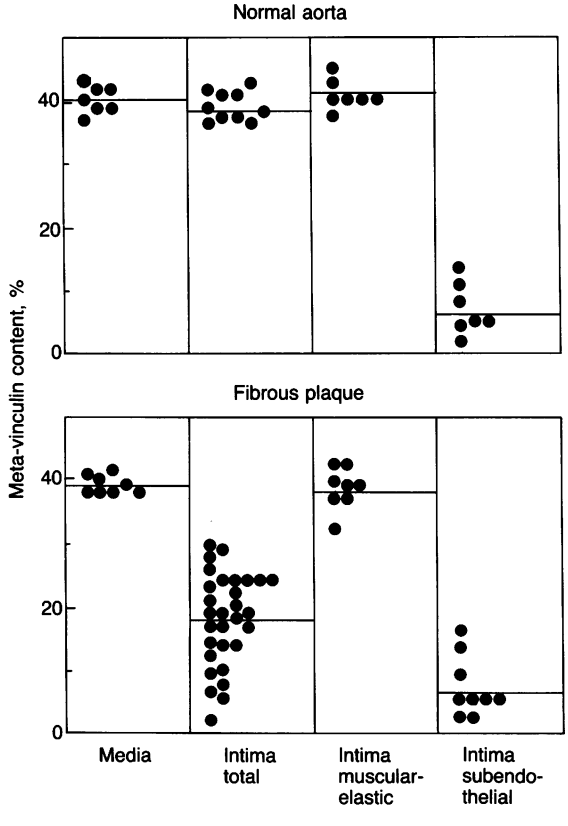


FIG. 3. Fractional content of meta-vinculin in different layers of normal (*Upper*) and atherosclerotic (*Lower*) human aorta. The fractional content of meta-vinculin was determined by the quantitative immunoblotting technique. Circles show the values obtained for individual samples; the horizontal lines represent the means.

In subendothelial intima of fibrous plaque, meta-vinculin and 150-kDa caldesmon fractional content was low; β-nonmuscle actin appeared to be a predominant isoform, whereas media and muscular-elastic intima remained intact. Theoretically such changes could be caused both by the alteration of smooth muscle cell phenotype and by the presence of blood-borne cells in the atherosclerotic aorta. However, Gown *et al.* have recently demonstrated that smooth muscle is a predominant cell type present in the fibrous plaque (44). Therefore, we assume that our results suggest the existence of different smooth muscle cell populations in normal and atherosclerotic human aorta: (*i*) cells of media and muscular-elastic intima with high α-actin, meta-vinculin, and 150-kDa caldesmon content; (*ii*) cells of normal aorta subendothelial intima with high α-actin content but low meta-vinculin and 150-kDa caldesmon content; (*iii*) cells of fibrous plaque with low α-actin, meta-vinculin, and 150-kDa caldesmon content. Long-term cultured smooth muscle cells are very close to the third category. Variability of vascular smooth muscle cell population as judged by the expression of cytoskeletal and contractile proteins has also been demonstrated by Skalli *et al.* (20).

In the total (not divided into sublayers) intima of fibrous plaque, meta-vinculin and 150-kDa caldesmon content differed from that in subjacent media; in about 25% of the fibrous plaques analyzed, the meta-vinculin fractional content in total intima was very similar to that in the subendothelial layer, 6–10% (Fig. 3). The data suggest that in atherosclerosis the share of subendothelial intima cells expressing decreased amounts of smooth muscle characteristic variants of vinculin, caldesmon, and actin in the total intimal cell population increases as compared to normal aorta. How does the cell population of subendothelial intima grow in atherogenesis? It could increase as a result of focal proliferation of the cells inhabiting this layer in normal aorta or migration and proliferation of the cells from adjacent and subjacent layers as was proposed and discussed earlier (for review, see ref. 45). Nevertheless, the data presented in this report suggest that in subendothelial intima of normal aorta cells that can provide a background for the development of the pathological process are present.

In conclusion, we have reported here the results of experiments showing that, in human aorta, smooth muscle cells of subendothelial intima differ from smooth muscle cells of adjacent layers as judged by the expression of cytoskeletal and contractile proteins. In addition, the proportion of tunica intima sublayers in atherosclerosis changes dramatically: in

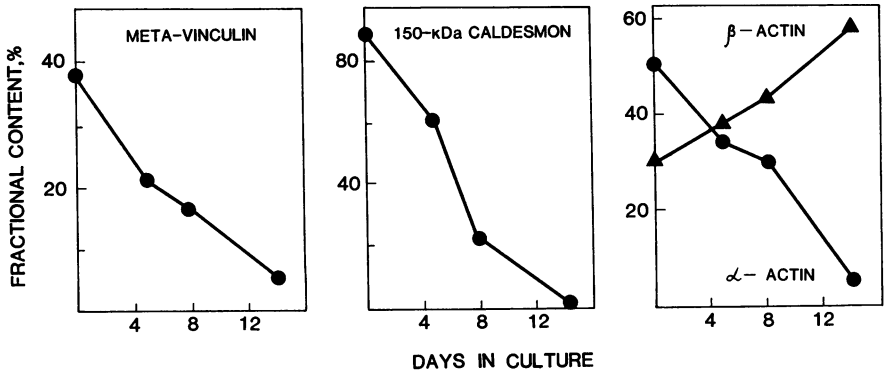


FIG. 4. Meta-vinculin (*Left*), 150-kDa caldesmon (*Center*), and isoactins (*Right*) fractional content in smooth muscle cells from media of human aorta in primary culture.

fibrous plaque the share of subendothelial intima cells (i.e., the cells that express reduced amounts of smooth muscle phenotype markers) is significantly higher than in normal aorta. These findings may allow the better understanding of cellular and molecular mechanisms of atherogenesis and vessel-wall functioning.


1. Chamley-Campbell, J., Campbell, G. R. & Ross, R. (1979) *Physiol. Rev.* **59,** 1–61.
2. Chamley-Campbell, J. & Campbell, G. R. (1981) *Atherosclerosis* **40,** 347–357.
3. Chamley-Campbell, J., Campbell, G. R. & Ross, R. (1981) *J. Cell Biol.* **89,** 379–383.
4. Vandekerckhove, J. & Weber, K. (1979) *Differentiation* **14,** 123–133.
5. Fatigati, V. & Murphy, R. A. (1984) *J. Biol. Chem.* **259,** 14383–14388.
6. Gabbiani, G., Kocher, O., Bloom, W. S., Vandekerckhove, J. & Weber, K. (1984) *J. Clin. Invest.* **73,** 148–152.
7. Owens, G. K., Loeb, A., Gordon, D. & Thompson, M. M. (1986) *J. Cell Biol.* **102,** 343–352.
8. Kocher, O. & Gabbiani, G. (1986) *Hum. Pathol.* **17,** 875–880.
9. Kocher, O., Skalli, O., Bloom, W. S. & Gabbiani, G. (1984) *Lab. Invest.* **50,** 645–652.
10. Barja, F., Coughlin, C., Belin, D. & Gabbiani, G. (1986) *Lab. Invest.* **55,** 226–233.
11. Rovner, A., Murphy, R. A. & Owens, G. K. (1986) *J. Biol. Chem.* **261,** 14740–14745.
12. Kawamoto, S. & Adelstein, R. S. (1987) *J. Biol. Chem.* **262,** 7282–7288.
13. Bermer, P. F., Frank, E., Holtzer, H. & Somlyo, A. P. (1981) *J. Muscle Res. Cell Motil.* **2,** 439–452.
14. Frank, E. & Warren, L. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3020–3024.
15. Gabbiani, G., Schmid, E., Winter, S., Chaponnier, C., De Chastonay, C., Vandekerckhove, J., Weber, K. & Franke, W. W. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 298–302.
16. Osborn, M., Caselitz, J. & Weber, K. (1981) *Differentiation* **20,** 196–202.
17. Schmid, E., Osborn, M., Rungger-Brandle, E., Gabbiani, G., Weber, K. & Franke, W. W. (1982) *Exp. Cell Res.* **137,** 329–340.
18. Kocher, O., Skalli, O., Cerutti, D., Gabbiani, F. & Gabbiani, G. (1985) *Circ. Res.* **56,** 829–838.
19. Owens, G. & Thompson, M. (1986) *J. Biol. Chem.* **261,** 13373–13380.
20. Skalli, O., Ropraz, P., Trzeciak, A., Benzonana, G., Gillessen, D. & Gabbiani, G. (1986) *J. Cell Biol.* **103,** 2787–2796.
21. Kuroda, M. (1985) *Biochim. Biophys. Acta* **843,** 208–213.
22. Feramisco, J. R., Smart, J. E., Burridge, K., Helfman, D. M. & Thomas, G. P. (1982) *J. Biol. Chem.* **257,** 11024–11031.
23. Siliciano, J. D. & Craig, S. W. (1982) *Nature (London)* **300,** 533–535.
24. Saga, S., Hamaguchi, M., Hoshino, M. & Kojima, K. (1985) *Exp. Cell Res.* **156,** 45–56.
25. Glukhova, M. A., Kabakov, A. E., Belkin, A. M., Frid, M. G., Ornatsky, O. I., Zhidkova, N. I. & Koteliansky, V. E. (1986) *FEBS Lett.* **207,** 139–141.
26. Bretcher, A. (1986) *Nature (London)* **321,** 726–727.
27. Furst, D. O., Cross, R. A., De May, J. & Small, J. V. (1986) *EMBO J.* **5,** 251–257.
28. Smith, C. W. J., Pritchard, K. & Marston, S. B. (1987) *J. Biol. Chem.* **262,** 116–122.
29. Bretcher, A. & Lynch, W. J. (1985) *J. Cell Biol.* **100,** 1656–1663.
30. Ngai, P. K. & Walsh, M. P. (1985) *Biochem. Biophys. Res. Commun.* **127,** 533–539.
31. Bretcher, A. (1984) *J. Biol. Chem.* **259,** 12873–12880.
32. Clark, T., Ngai, P. K., Sutherland, C., Groschel-Steward, U. & Walsh, M. P. (1986) *J. Biol. Chem.* **261,** 8028–8035.
33. Owada, M. K., Hakura, A., Iida, K., Yahara, I., Sobue, K. & Kakiuchi, S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3133–3137.
34. Rhodin, J. A. G. (1980) in *Handbook of Physiology: The Cardiovascular System,* eds. Bohr, D. F., Somlyo, A. P. & Sparks, H. V. (Am. Physiol. Soc., Washington, DC), Vol. 2, Sect. 2, pp. 1–31.
35. Geer, J. C. (1965) *Lab. Invest.* **14,** 1764–1783.
36. Orekhov, A. N., Andreeva, E. R., Tertov, V. V. & Krushinsky, A. V. (1984) *Acta Anat.* **119,** 99–105.
37. Ferramisco, J. R. & Burridge, K. (1980) *J. Biol. Chem.* **255,** 1194–1199.
38. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
39. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
40. Fenner, C., Traut, R. R., Mason, D. T. & Wikman-Coffelt, J. (1975) *Anal. Biochem.* **63,** 595–602.
41. Orekhov, A. N., Kosykh, V. A., Repin, V. S. & Smirnov, V. N. (1983) *Lab. Invest.* **48,** 749–754.
42. Joyce, N. C., Haire, M. F. & Palade, G. E. (1985) *J. Cell Biol.* **100,** 1387–1395.
43. Herman, I. M. & D'Amore, P. A. (1985) *J. Cell Biol.* **101,** 43–52.
44. Gown, A. M., Tsukada, T. & Ross, R. (1986) *Am. J. Pathol.* **125,** 191–207.
45. Schwartz, S. M., Campbell, G. R. & Campbell, J. H. (1986) *Circ. Res.* **58,** 427–444.