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Immunoreactive forms of caldesmon in cultivated human vascular smooth muscle cells

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150 kDa caldesmon was shown to be characteristic of vascular smooth muscle cells in normal tissue rather than in subculture. Subcultured smooth muscle cells from human aorta contained only the 70 kDa immunoreactive form of caldesmon. During the course of primary culture the amount of 150 kDa caldesmon as well as metavinculin decreased significantly whilst 70 kDa caldesmon became the predominant form, and by the onset of cell division the 150 kDa form was practically substituted by 70 kDa caldesmon. The data show that the predominance of 150 kDa caldesmon is characteristic of contractile smooth muscle cells, while in proliferating cells 70 kDa caldesmon is expressed.

Caldesmon; Phenotypic modulation; (Smooth muscle)

1. INTRODUCTION

During the course of primary culture, smooth muscle cells undergo 'phenotypic modulation' [1], which includes an increase in proliferative, synthetic and secretory activity, and rearrangement of the cytoskeleton and contractile apparatus. A definite shift to the expression of non-muscle forms and isoforms of contractile and cytoskeletal proteins during cultivation has been demonstrated: desmin is replaced by vimentin; the amount of smooth muscle α-actin is decreased significantly whilst β-actin becomes the major actin isoform [2]; the amount of meta-vinculin, a vinculin-related protein, becomes negligible by the onset of cell division in primary culture [3]. These in vitro changes are believed to be relevant to the process of phenotypic modulation which occurs in vivo during the development of certain diseases involving the vascular wall (atherosclerosis, hypertension).

Caldesmon is an F-actin- and calmodulin-binding protein found in all smooth muscle and non-muscle cells examined so far [4–6]. It is considered to be involved in the regulation of actin-myosin interactions. Two immunoreactive forms of caldesmon are found in tissues and cells, namely those of 120–150 and 71–77 kDa [5]. Both forms are capable of interacting with Ca^{2+}-calmodulin and F-actin. In smooth muscle cells 150 kDa caldesmon rather than the 70 kDa form was revealed [7].

Here, we identify immunoreactive forms of caldesmon in smooth muscle cells from human aorta in primary culture and subculture, and show that the 150 kDa form, found in smooth muscle cells isolated from normal tissue by enzyme digestion, by the onset of cell division in primary culture is practically substituted by the 70 kDa form characteristic of subcultured cells.

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2. MATERIALS AND METHODS

Cells from tunica intima of human aorta were isolated by collagenase-elastase digestion [8,9] and cultivated as in [10]. Tissue samples were obtained at autopsies taken within 3 h of death. Foreskin fibroblasts (10th passage) were isolated and cultivated according to [11]. Human peritoneal macrophages were isolated from ascites fluid obtained from a patient suffering from heart failure.

Vinculin and filamin for immunization were purified from human uterus according to [12] and caldesmon as described in [13]. Antibodies raised in rabbits were shown to be monospecific as judged by immunoblotting performed with uterus extract. IgG fractions were obtained by $(NH_4)_2SO_4$ precipitation followed by DEAE chromatography.

Cells or tissue samples were lysed using 40 mM Tris-HCl buffer, pH 8.0, containing 5% β-mercaptoethanol, 4% SDS, 10 mM EDTA, 20% glycerol, 2 mM PMSF, 20 μg/ml each of leupeptin, pepstatin and aprotinin and 130 μg/ml of benzamidine. Extracts were run in 5–15% SDS-polyacrylamide slab gels. Immunoblotting was performed as described [14]. Each sample contained an extract of 5–7 $\times$ 10^5 cells. Anti-caldesmon IgG (4 mg/ml) were diluted down to 150 μg/ml, anti-vinculin (10 mg/ml) to 70 μg/ml and anti-filamin (2 mg/ml) to 40 μg/ml. ^{125}I-labeled goat anti-rabbit IgG (10^6 cpm/μg, 10 μg/ml) were used as secondary antibody. Immunoblots were exposed to X-ray film (Tasma, USSR) overnight. The method was reproducible with repeated measurements on the same sample and was linear over the range of protein loadings used.

3. RESULTS AND DISCUSSION

To determine the amount of filamin, meta-vinculin, vinculin and the two immunoreactive forms of caldesmon (150 and 70 kDa) in cultivated smooth muscle cells we used a quantitative immunoblotting technique. Since the high-molecular-mass forms of caldesmon and meta-vinculin have very similar electrophoretic mobility, two aliquots of the same cell extract were analyzed separately to reveal both proteins. After the samples underwent electrophoresis and the proteins transferred onto nitrocellulose, the blots were cut into two parts, the upper ones being further treated with anti-

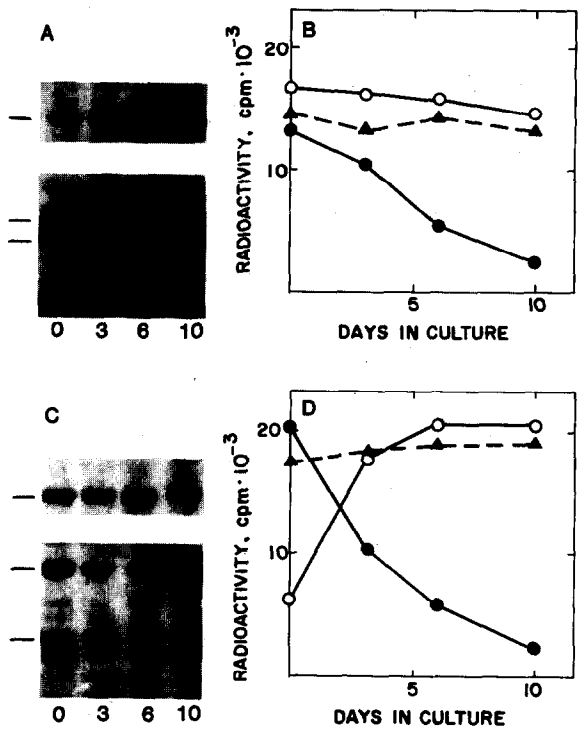

Fig.1. Relative quantitative determination of filamin, meta-vinculin, vinculin and the two immunoreactive forms of caldesmon in cultivated smooth muscle cells from tunica intima of human aorta. (A) Autoradiograph of an immunoblot revealing filamin, meta-vinculin and vinculin. The upper part of the nitrocellulose strip was treated with anti-filamin primary antibody, the lower part with anti-vinculin antibody to reveal meta-vinculin and vinculin. Both parts of the blot were then treated with ^{125}I-secondary antibody and exposed to X-ray film. The positions of purified proteins in the gel are indicated by the lines (from top to bottom): filamin, 250 kDa; meta-vinculin, 150 kDa; vinculin, 130 kDa. Values beneath the autoradiograph correspond to the cultivation time in days. (B) The strips of nitrocellulose, corresponding to filamin, meta-vinculin and vinculin were cut out and counted: (▲---▲) filamin, (●—●) meta-vinculin, (○—○) vinculin. (C) Autoradiograph of immunoblot revealing filamin, and the 150 kDa and 70 kDa immunoreactive forms of caldesmon. The same as in A, but the lower part of the blot was treated with anti-caldesmon primary antibody. The positions of purified proteins in the gel are indicated by the lines (from top to bottom): filamin, 250 kDa; caldesmon, 150 kDa; caldesmon, 70 kDa. (D) The strips of nitrocellulose, corresponding to filamin, and the 150 kDa and 70 kDa forms of caldesmon were cut out and counted: (▲---▲) filamin, (●—●) 150 kDa caldesmon, (○—○) 70 kDa caldesmon.

filamin antibodies (fig.1A,C) and the lower with anti-vinculin (fig.1A) or with anti-caldesmon antibodies (fig.1C). The data presented in fig.1A and B show that the absolute amounts of filamin as well as vinculin in different samples remained practically constant whereas, as demonstrated earlier [3], the amount of meta-vinculin decreased during cultivation and by the 10th day (onset of intensive cell division) the protein could hardly be detected.

In cells isolated from human aorta tunica media and tunica intima 150 kDa caldesmon was predominant, if not the only immunoreactive form of the protein revealed. 70 kDa caldesmon was detected only in some samples and, if at all present, could account for only 10–25% of the total (150 kDa + 70 kDa) immunoreactive caldesmon (fig.1C,D). On the 3rd day of cultivation, i.e. when the cells had just finished spreading, they already contained even more 70 kDa than 150 kDa caldesmon, and by the 10th day the amount of 150 kDa caldesmon decreased about 10-fold. The results suggest that the predominance of the 150 kDa form is characteristic of contractile smooth muscle cells, while the 70 kDa form is expressed in proliferating cells. Thus, the loss of meta-vinculin and the switch in the immunoreactive caldesmon pattern indicate rearrangement of the cytoskeleton and contractile apparatus, accompanying phenotypic modulation of vascular smooth muscle cells in culture.

Subcultured vascular smooth muscle cells (fig.2) as well as other cultivated cells, foreskin fibroblasts and macrophages, contained only trace amounts of 150 kDa caldesmon, if any.

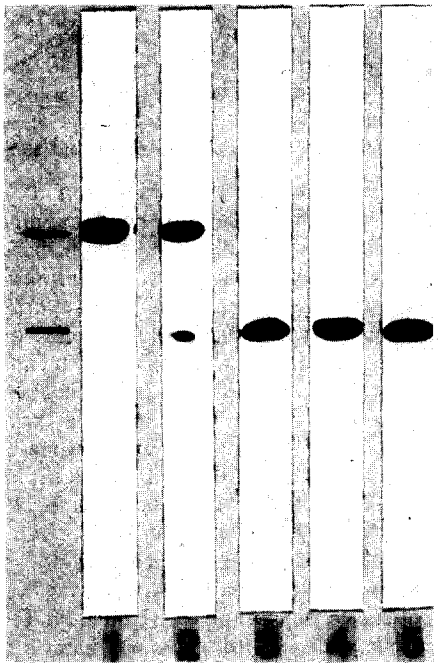

Fig.2. Identification of immunoreactive forms of caldesmon in tunica media and tunica intima of human aorta and cultivated cells. Lanes: 1, tunica intima of human aorta; 2, tunica media of human aorta; 3, smooth muscle cells from tunica media of human aorta, 5th passage; 4, human foreskin fibroblasts, 10th passage; 5, human peritoneal macrophages. Secondary antibodies were peroxidase-conjugated. α-Chloronaphthol was used as a substrate for peroxidase. Positions of purified caldesmon (150 kDa and 70 kDa) are indicated by the lines.

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