Meta-vinculin distribution in adult human tissues and cultured cells

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Meta-vinculin distribution in adult human tissue was studied by immunoblotting technique. Meta-vinculin was found in smooth (aorta wall and myometrium) and cardiac muscle, rather than in skeletal muscle, liver, kidney and cultured cells – macrophages, foreskin fibroblasts, peripheral blood lymphocytes and vascular endothelial cells. In the primary culture of smooth muscle cells from human aorta the meta-vinculin/vinculin ratio was reduced, and on the onset of cell division meta-vinculin could hardly be detected. Subcultured smooth muscle cells from human aorta did not contain meta-vinculin. The data show that the presence of meta-vinculin is characteristic of 'contractile' smooth muscle cells rather than of proliferating in vitro.

Vinculin meta-Vinculin (Smooth muscle) Phenotypic modulation

1. INTRODUCTION

Vinculin, a protein of relative molecular mass 130 kDa found in muscle and non-muscle tissues was shown to be localized at the termini of actin microfilament bundles – in focal contacts of cultivated cells [1,2], in dense plaques regions of smooth muscle [3], in intercalated disks of cardiac muscle [4,5]. During the course of fractionating chicken gizzard smooth muscle Feramisco et al. [6] detected a protein of 152 kDa which was recognized by anti-vinculin antibodies. A study by Siliciano and Craig [7] also revealed a similar protein in chicken gizzard which was termed meta-vinculin. Unlike vinculin meta-vinculin in avian tissues appeared to be restricted to muscles – only smooth muscle according to [6] or both smooth and striated muscle according to [8].

20 kDa difference between the molecular mass of vinculin and meta-vinculin enables one to distinguish two polypeptides by means of the immunoblotting technique. Therefore we used immunoblotting to study the distribution of meta-vinculin in adult human tissues. We demonstrate here that a significant amount of meta-vinculin is found in smooth and cardiac muscles, rather than in adult human skeletal muscle, liver, kidney and cultivated cells – macrophages, lymphocytes, foreskin fibroblasts and endothelial cells. We have also shown that during cultivation smooth muscle cells from human adult aorta lose meta-vinculin.

2. MATERIALS AND METHODS

Vinculin and filamin for immunization were isolated from human uterus according to [9]. Antibodies raised in rabbits were monospecific as revealed by immunoblotting performed with uterus extract.

Immunoblotting was performed according to [10]. Secondary antibodies were either peroxidase-conjugated or ^{125}I-labeled. As substrate for peroxidase α-chloronaphthol was used. To estimate the meta-vinculin/vinculin ratio strips of nitrocellulose paper corresponding to meta-vinculin and vinculin bands revealed by autoradiography were cut out and counted.

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Tissue samples were obtained at autopsies taken within 2–3 h of death. Cells from tunica media and intima of human aorta were isolated by collagenase-elastase and collagenase digestion, respectively [11,12]. Human umbilical cord endothelial cells were a gift of Dr S. Danilov (Institute of Experimental Cardiology, Cardiology Research Center of the USSR). Macrophages were isolated from ascites fluids obtained from a patient with heart failure. Viable human peripheral blood lymphocytes were ordered from the Cardiology Center blood bank. Foreskin fibroblasts (7th passage) were isolated and cultivated as described in [13].

3. RESULTS

To determine the distribution of meta-vinculin in various human tissue and cell types the samples were examined by immunoblotting technique using anti-vinculin antibodies. The results of the experiments are shown in fig.1. Meta-vinculin appeared to be present only in smooth (subendothelial intima and media of the aorta and myometrium) and cardiac muscle; skeletal muscle (sartorius muscle), liver, kidney, as well as cultivated cells – peritoneal macrophages, lymphocytes, vascular endothelial cells and foreskin fibroblasts contained trace amounts of meta-vinculin, if any. In the samples containing meta-vinculin in addition to vinculin, the meta-vinculin/vinculin ratio differed depending on the tissue type. We estimated that the ratio was about 1 in the myometrium, about 0.6–0.7 in aorta smooth muscle and about 0.4 in cardiac muscle. It should be mentioned that at least some amount of meta-vinculin found in cardiac muscle might originate from the blood vessels present in the heart samples analysed.

To determine whether the meta-vinculin content was altered during the cultivation of smooth muscle cells, we measured the meta-vinculin/vinculin ratio in tissue samples, primary culture and subcultures of the cells from aorta by quantitative immunoblotting technique. We have analyzed the samples of tunica media and tunica intima of 20 donors (ages from 40 to 60). The meta-vinculin/vinculin ratio was about 0.65 in intima and about 0.7 in media. In the subcultures of intimal and medial cells meta-vinculin was not detected. In the primary culture the meta-vinculin/vinculin ratio was reduced during cultivation (fig.2). Vinculin content in the cells was reported to differ depending on the conditions of cultivation (cell density) [14]. Thus, the possibility should be taken into account that the meta-vinculin/vinculin ratio was reduced as a result of increasing vinculin synthesis, but it does not seem

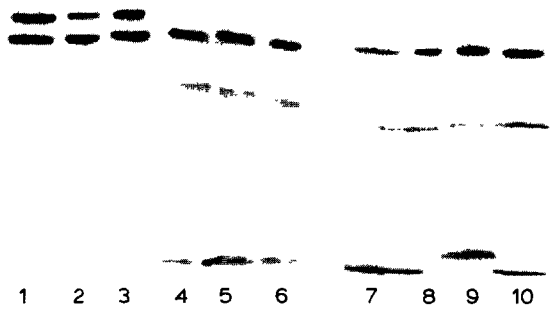

Fig.1. Immunochemical identification of meta-vinculin in different tissues and cells. Lanes: 1, uterus; 2, heart; 3, tunica media of aorta; 4, kidney; 5, liver; 6, skeletal muscle; 7, macrophages; 8, lymphocytes; 9, foreskin fibroblasts; 10, endothelial cells. Tissue samples or cells were dissolved in 20 mM Tris-HCl, pH 8.0, containing 4% SDS, 20 mM dithiothreitol, 5 mM EDTA, 2 mM phenylmethylsulfonyl fluoride and run in the SDS-polyacrylamide gel (5–15%). Blots were stained by indirect immunoperoxidase technique.

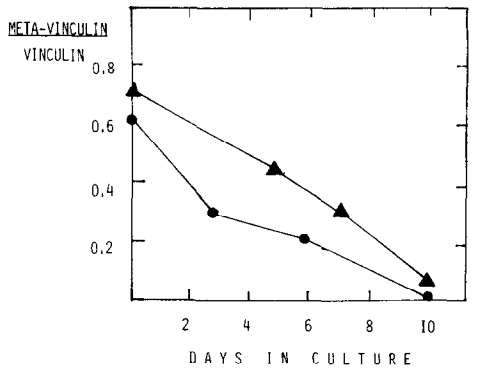

Fig.2. The meta-vinculin/vinculin ratio in smooth muscle cells from human aorta. (▲) Medial cells; (●) intimal cells.

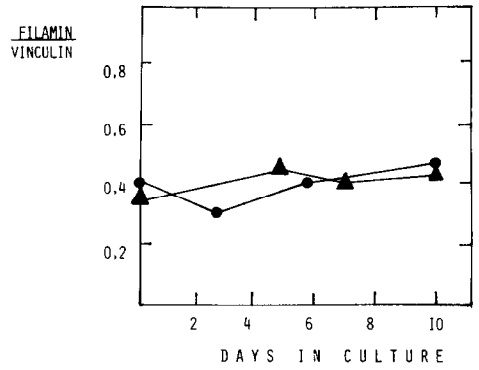

Fig.3. The filamin/vinculin ratio in smooth muscle cells from human aorta. (▲) Medial cells; (●) intimal cells.

to be the case, because the filamin/vinculin ratio in the primary culture of intimal and medial cells remained constant and very close to that found in normal tissue and isolated cells (fig.3). Also, after 10–12 days of cultivation an absolute amount of meta-vinculin appeared to be undoubtedly reduced – the cells contained only traces of the protein.

4. DISCUSSION

We demonstrate here that in adult human tissues meta-vinculin is found only in muscles – the protein was detected in smooth and cardiac muscles, although we did not reveal it in skeletal muscle. We have also found that the relative amount of meta-vinculin (i.e. the meta-vinculin/vinculin ratio) is lower in the cells from aorta if compared to myometrium. Thus, the amount of meta-vinculin per smooth muscle cell may be different in different tissues. The lower ratio could also result from the heterogeneity of the cell population of intimal and medial layers of the aorta wall.

The cells isolated from human aorta wall by enzyme digestion do not show more or less significant mitotic activity up to 6–8 days of cultivation. We were able to register a decrease in the meta-vinculin/vinculin ratio on the 3rd day and at the onset of cell division the amount of meta-vinculin in cultivated cells appeared to be almost negligible. Cultivated smooth muscle cells are considered to be 'modulated' according to certain criteria [15,16]. We conclude that meta-vinculin could serve as a marker of 'contractile' smooth muscle cells, and modulation, following the isolation of cells from tissue and exposure to growth factors is characterized by the loss of meta-vinculin.

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