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IMMUNOHISTOCHEMICAL STUDY OF SOME FILAMENTOUS PROTEINS IN THE CELLS OF MATURE HUMAN UMBILICAL CORD

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ABSTRACT

Expression design of primary proteins from intermediate filaments such as cytokeratin, vimentin and desmin in any cells within the umbilical cord was immunohistochemically studied using polyclonal (PAN cytokeratin) and monoclonal (vimentin and desmin) antibodies. The results showed that the cells of the mature human umbilical cord such as amniocytes, cells of the mucilaginous connective tissue, endothelial and smooth-muscle vascular cells expressed the basic proteins of the intermediate filaments in a different way. The amniocytes reacted strongly positively towards cytokeratin while only single cells reacted towards desmin and vimentin. All the cells of the mucilaginous connective tissue reacted positively for vimentin and desmin both. The vascular endothelial cells remained vimentin-positive only while the vascular myocytes demonstrated certain peculiarities of their reaction towards vimentin and desmin related not only with their vascular belonging (arterial or venous, respectively) but also with their intramural topography. Based on these new facts the authors discussed the nature, differentiation and functions of the structures involved in this important transitory formation.

Key words: umbilical cord, desmin, vimentin, cytokeratin, endothelial cells, vascular smooth muscle cells

INTRODUCTION

Intermediate filaments represent the most important fibrillary component of the cytoskeleton that contributes essentially to cellular shape and integrity (9,17,18). During the last 10-20 years the interest in these structures increases considerably mainly because of the importance of the knowledge about their chemical nature and intracellular distributon for the understanding of the cellular differentiation and typifying as well as of the histogenesis of various neoplasms (16,17). The rapid advances of immunocytochemistry and immunohistochemistry play a crucial role in this respect, too.

Expression of proteins from intermediate filaments has not been purposefully studied in human umbilial cord while there are a lot of detailed results concerning the placenta. Data about amnial epithelium (15,20) seem of particular interest when our object of examination is concerned as the cells of same kind cover the umbilical cord. There is scanty evidence about immunohistochemical investigations and single, randomly selected umbilical cord cells during pregnancy and postpartum. Their nature is, however, not systemic, often contradictory and could hardly serve as a basis for comparison.

Taking into consideration the present situation we have undertaken a immunohistochemical study of the expression design of the primary proteins from intermediate filaments such as cytokeratin, vimentin and desmin in all the cells within the umbilical cord. We thus expect to demonstrate new facts and on that basis to contribute to the clarification of the nature, differentiation, and function of the structures involved in this important transitory formation.

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MATERIAL AND METHODS

Ten pieces of cross sections from the median third of mature human umbilical cord were used. The material was fixed in 10 per cent buffered formaline and embedded in paraffin wax, stained with HE, Azan, and after Goldner for survey observation. Use was made of some cryostat air-dried sections fixed in acetone along with paraffin ones in order to immunohistochemically outline the cytokeratin, vimentin and desmin. An avidin-biotin-immunoperoxidase method along with the following primary antihuman antibodies were applied: for vimentin: first monoclonal antibody (Zymed, USA); second monoclonal antibody (Boehringer, Germany); for desmin: monoclonal antibody (Zymed, USA), and for cytokeratin (PAN): polyclonal antibody (Zymed, USA). The secondary antibodies were from

Zymed Histostain SP kit (Zymed, USA); aminoethylcarbasol (Zymed, USA) was used as chromogen while HE after Meyer was applied to stain the nuclei. Observations were performed on Zetopan photomicroscope (Reichert, Austria). Readings were done after a five-degree scale.

RESULTS AND DISCUSSION

Our results show that the cells from the mature human umbilical cord such as amniocytes, cells of the mucilaginous connective tissue, vascular endotheliocytes and myocytes express the primary proteins of the intermediate filaments in a different way. The cells of the amnial epithelium washed by the amnial fluid and covering the umbilical cord display a positive reaction towards PAN-cytokeratin. The reaction product looks homogenous and/or finely granulated. It is located mainly in the laterobasal part of the amniocytes (Fig. 1a). This polyclonal antibody applied in our study binds to cytokeratins (4-6,8,10,13,18). Thus a relatively broad spectrum of cytokeratins is covered and presented as well.

Fig. 1. Amnial epithelium
1a – immunohistochemical reaction for PAN Cytokeratin. Magn. 10 x 10

The amniocytes show a positive reaction towards the monoclonal antibody for desmin, too. There are, however, certain quantitative peculiarities: there exist differences in the reaction intensity in single cels, i. e., they do not represent any uniform population concerning the desmin immunopositivity. In fact, the reaction varies from negative to strongly positive. Commonly, the reaction product is located in the basal region (Fig. 1b). Only single amniocytes react towards the monoclonal antibody for vimentin that is, in our opinion, an exception (Fig. 1c).
The whole cellular population of Wharton's jelly is cytokeratin-positive. In contrast, these cells demonstrate an outlined to strong vimentin immunopositivity. Their reaction towards the monoclonal antibody for desmin is the same (Fig. 1 a,b,c).

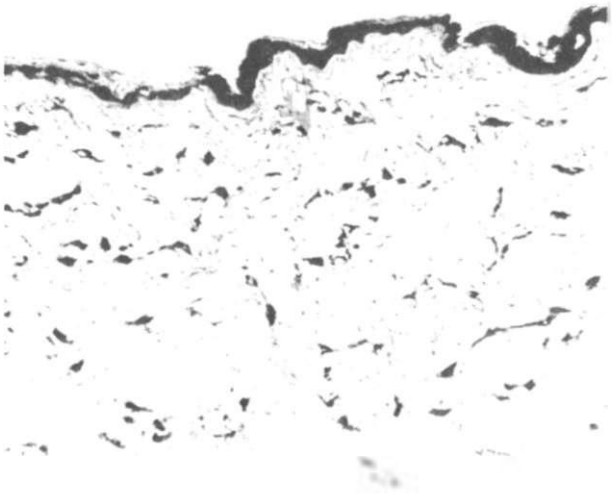


Fig. 1. Amnial epithelium
1b – immunohistochemical reaction for desmin. Magn. 20 x 10

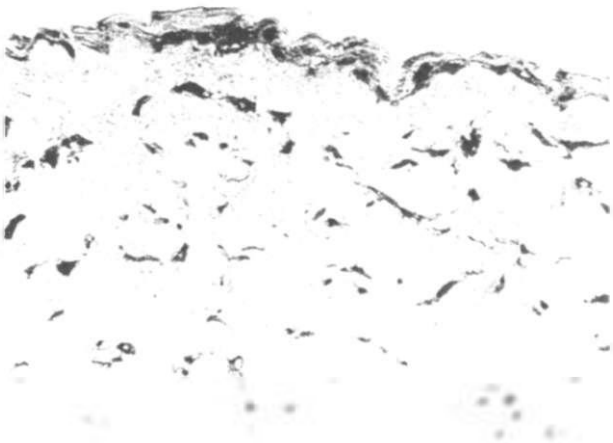


Fig. 1. Amnial epithelium
1c – immunohistochemical reaction for vimentin. Magn. 20 x 10

Both endothelial and vascular smooth muscle cells are absolutely negative for cytokeratin.
They do not represent any uniform population when their immunoreactivity towards vimentin and desmin is concerned. While the endothelial cells are characterized by vimentin positivity only (Fig. 2a,b), the vascular myocytes display a more complex picture. The qualitative and quantitative peculiarities of their reactivity depend not only on their vascular belonging (arterial or venous), but also, to a significant extent, on their intramural topography. That is

why it seems reasonable to describe in brief the features of the muscular cover of the walls of the umbilical arteries and vein.

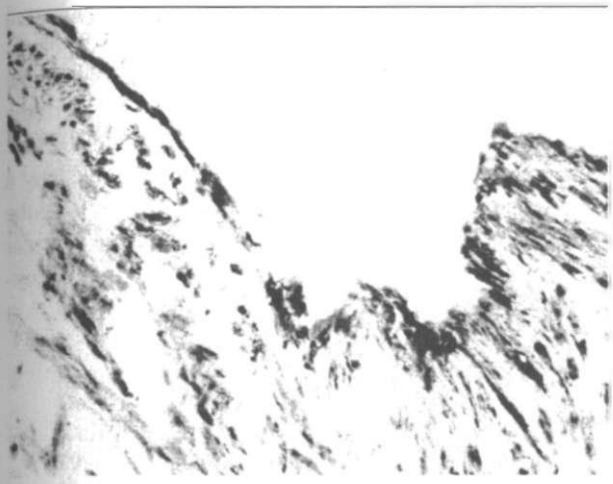
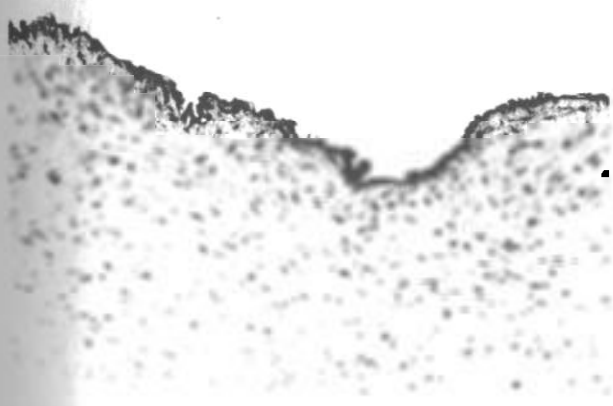


Fig. 2. Immunohistochemical reaction for vimentin
2a - Arteria umbilicalis. Magn. 20 x 10

The myocytic component of the arterial wall is very well-developed. Its internal part strongly invaginating into the lumen is built-up by mainly radially oriented smooth muscle bundles along with single circular ones. Most myocytes in the peripheral media are circular although there are some spiral ones, too, the latter being located under a different angle thus creating an impression of a longitudinal orientation (Fig. 3a,b).



Immunohistochemical reaction for vimentin
Vena umbilicalis. Magn. 10 x 10

All the arterial myocytes independently of their localization are vimentin-positive (Fig. 4). They differ with respect to their desmin expression. While the myocytes from the internal part of the media are weakly desmin-positive, the peripheral ones being circular, spiral, or longitudinal display a strongly expressed desmin positivity (Fig. 5). The myocytes in the media of the umbilical vein are mainly of circular and/or spiral orientation. All of them are vimentin-positive and of equal intensity along the whole wall depth (Fig. 2). Their reaction towards the monoclonal antibody for desmin is positive and more intensive in the peripheral parts of the wall (Fig. 6).

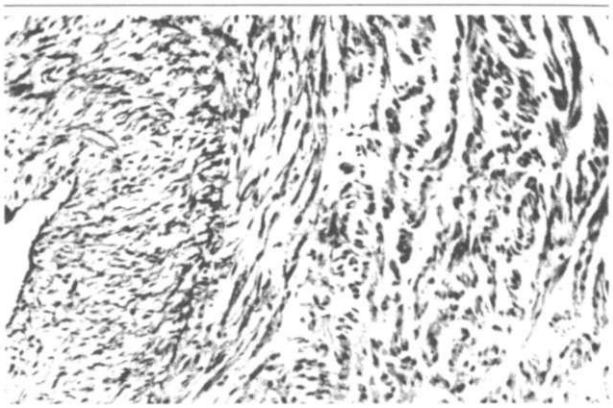


Fig. 3a. Arteria umbilicalis. Staining after Goldner. Magn. 20 x 10

Our results unambiguously indicate that the cells of the mature human umbilical cord synthesize various, in certain cases even simultaneously, several proteins of the intermediate filaments. It becomes evident that these cells are not only provided with intermediate filaments but these cytoskeletal elements also preserve their chemical integrity until the end of pregnancy. A particular interest represents the distribution of the expression of the proteins of the intermediate filaments mainly with a view to the understanding of the differentiation and thus of the cellular functions (Table 1).

Table 1. Reactivity degrees of umbilical cord cells towards the antibodies

Umbilical cord cells	Cyto-keratin	Vimen-tin	Desmin
Amniocytes	++	- (+)	+ (-)
Cells of Wharton's jelly	(-)	+++	+++
Vascular endotheliocytes	(-)	+++	(-)
Arterial myocytes (internal media)	(-)	+++	+ (-)
Arterial myocytes (external media)	(-)	+++	+++
Venous myocytes (internal media)	(-)	+++	++
Venous myocytes (external media)	(-)	+++	+++

Legend to the table:
strong reaction +++
intermediate reaction ++
weak reaction +
reaction of single cells only - + (-), - (+)
negative reaction (-)

We establish cytokeratins in the cells of the amnial epithelium only. It is known that these cytoskeletal proteins occur first during evolution (3,5,17) and this finding concerning

the engagement of the amnial epithelium through a covering and mechanical function is not surprizing. In contrast to other epithelial cells (10), the amniocytes possess a heterogenous composition (1). Cytokeratin-PAN polyclonal antibody used in this trial that binds to 7 polypeptids is capable of proving and demonstrating the cytokeratin immunopositivity as it clearly identifies the cells possessing the group of genes coding all the cytokeratins. The presence of cytokeratins and their intracellular distribution corresponds, in our opinion, more lkely to the secondary specialization for accomplishing the mechanical function within the epithelial layer rather than to the origin of the amnion from the primary ectoderm. Obviously, the cytokeratin-positive amniocytes serve first of all the stability, integrity and defense of the amnial epithelium. We fail to establish any other cytokeratin-positive cells in the mature human umbilical cord at all.

Vimentin has mainly served as a sign of mesenchymal and/or connective-tissue differentiation (17) until the discovery of other proteins of the intermediate filaments in such cells. In contrast to other authors (1,6) concerning the amnion of the chorionic plaque, we establish only single vimentin-positive amniocytes in the epithelium of the mature human umbilical cord. On the other hand, all the cells of the Wharton's jelly are vimentin-positive. Besides they are positive towards the monoclonal antibody for desmin. It argues of the fact that they undergo a myocytic differentiation, too (8,12,19) and coincides with the evidence that after the sixth month of the intrauterine development they possess the ultrastructural characteristics of the myofibroblasts (7), they have numerous microfilaments and even express alpha-smooth-muscle actin. Obviously, they possess a contractile-relaxative function and thus can be actively involved in the vascular motility taking additionally into consideration the circumstance that the umbilical vessels such as artery and vein do not possess any adventitia at all.

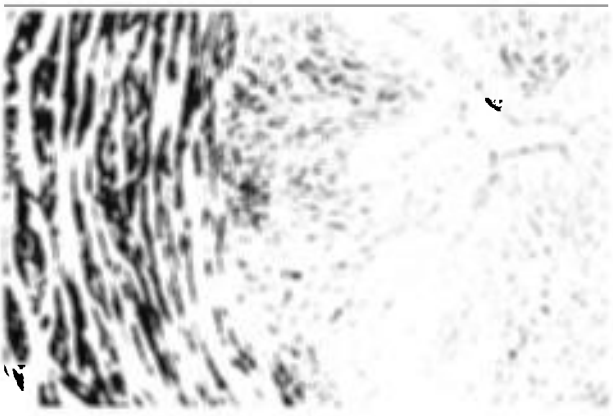


Fig. 3b. Arteria umbilicalis. Staining with Azan. Magn. 20 x 10

The cells of the vascular wall are vimentin-positive, too. The endothelial cells do not react with any other antibodies and thus do not differ from those of the mature corporal vessels at all while the myocytes are desmin-positive, too. It stresses that the reaction for desmin weakens from the peripheral areas of the media towards the lumen when the vascular myocytes are concerned (11). Probably, it means that the myocytic differentiation advances from the external media towards the lumen. In such case, the mesenchymal cells of Varton's mucus first differentiate into a myocytic direction (during the second trimester) and then follow the vascular myocytes as this process lasts until the end of the pregnancy. In our opinion, the extravasal cells of the Wharton's jelly that, obviously, are capable of accomplishing a contractile-relaxative function, too, form together with the vascular myocytes a system for regulation of the vascular tone thus achieving adaptation to the corresponding concrete situation.

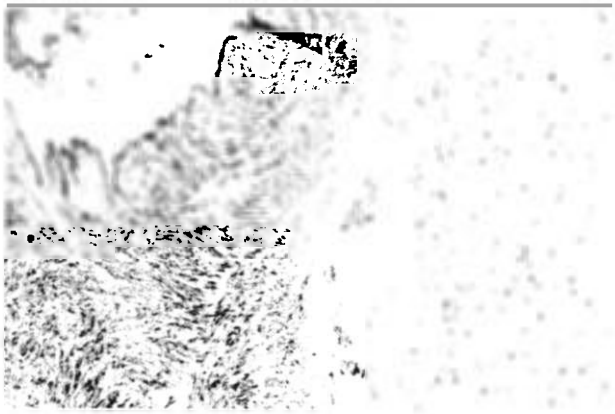


Fig. 4. Arteria umbilicalis. Immunohistochemical reaction for vimentin. Magn. 10 x 10

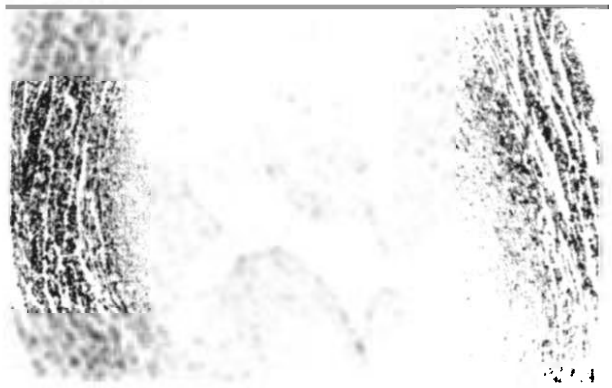


Fig. 5. Arteria umbilicalis. Immunohistochemical reaction for desmin. Magn. 4 x 10

The question emerges, however, where the concrete stimuli for contraction and dilatation, respectively, originate from, as it is known that there are no nerves in the umbilical cord (2,13). It has been proved that the endothelial cells of the umbilical vessels produce the powerful relaxing factor NO (14) and through its dilatatory action they can withstand the continuous mechanical influences leading to collapse.

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