Intermediate Filaments in the Endothelial Cell

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Contributor: Anton Shakhov, Irina Alieva

Vimentin, the main protein of endothelial intermediate filaments, is one of the most well-studied of these and belongs to type-III intermediate filaments, commonly found in cells of mesenchymal origin. Vimentin filaments are linked mechanically or by signaling molecules to microfilaments and microtubules by which coordinated cell polarisation and migration are carried out, as well as control over several endotheliocyte functions. Moreover, the soluble vimentin acts as an indicator of the state of the cardiovascular system, and the involvement of vimentin in the development and course of atherosclerosis has been demonstrated.

Keywords: intermediate filaments ; vimentin filaments ; soluble vimentin ; nestin ; neirofilaments

1. Introduction

The main function of cell cytoskeleton intermediate filaments has long been considered to be the maintenance of cellular and tissue integrity due to their mechanical properties. In addition, intermediate filaments play an important role in the distribution of proteins and organelles throughout the cell. There has been significant recent progress in understanding the cellular function of intermediate filaments, including their role within the endothelial cell. It has turned out that intermediate filaments are not static structures in their mechanical support (as previously thought), but they interact functionally with other components of the cytoskeleton, and other cellular processes. In many experimental works, the involvement of vimentin in a plenty of cellular processes has been demonstrated. Vimentin is also involved in the processes of intercellular exchange due to its connection with the extracellular matrix. Recent research has demonstrated evidence for the functional involvement of vimentin intermediate filaments in the physiological functions of endotheliocyte and the development of inflammatory processes and atherosclerosis.

2. Features of Intermediate Filaments—The Third Component of the Cytoskeleton

Historically, during the last 50 years, when the emergence of new molecular-cell approaches and microscopic techniques, marked by the explosive growth of studies on the dynamics and functionality of microtubules and actin filaments, intermediate filaments remained the least studied component of the cell cytoskeleton. The classical concept of the third component of the cytoskeleton [1][2] is as follows: (1) intermediate filaments are located in the form of three-dimensional networks in different parts of the cell cytoplasm, surround the nucleus, participate in the formation of intercellular contacts, and maintain the shape of processes; (2) the main function of intermediate filaments, based on their mechanical properties and self-assembly ability, is to maintain cellular and tissue integrity; (3) intermediate filaments consisting of different proteins are expressed in different types of cells. The latter property distinguishes intermediate filaments from microtubules (polymerized from tubulin) and microfilaments (composed of actin). Indeed, intermediate filaments have a different protein composition, both in different tissues and at different stages of embryonic development and at different stages of differentiation. Moreover, some types of cells simultaneously contain several different intermediate filaments. In total, about 70 genes have been found in the human genome, encoding various proteins of intermediate filaments, which form one of the most numerous protein families. However, since the beginning of the nineties of the last century, interest in the study of intermediate filaments began to actively grow since it turned out that mutations in these proteins are associated with severe skin (keratins), nervous (neurofilaments) human pathologies, including muscular dystrophies (desmin) and cardiomyopathies (desmin and vimentin) [3][4][5][6].

3. Classification of Intermediate Filaments and Its Types Found in Endothelial Cells

The homology of protein sequences of intermediate filaments is sometimes no more than 20%; nevertheless, based on biochemical, immunological, and structural similarities, six different types of intermediate filaments are distinguished.

According to the modern classification [7], intermediate filaments are divided into keratins type-I (acidic keratins) and keratins type-II (basic keratins) proteins. For the assembly of keratin intermediate filaments, proteins of both types are required, which form heteropolymers. Type-III intermediate filament proteins are comprised of desmin, vimentin, peripherin, and glial acidic proteins. These proteins can form both homopolymers and also heteropolymers with other type-III protein members as well as with the neurofilament light (NF-L) protein. Type-IV intermediate filament proteins are expressed predominantly in nerve cells. This type represents α-internexin and a triplet of neurofilament proteins: NF-L, NF-M, and NF-H (neurofilament light, medium, and heavy proteins, respectively). The protein nestin, first discovered in the precursors of nerve cells, is sometimes referred to as a special type of intermediate filament protein. However, based on its structural features, nestin can be classified as type IV. Type-V intermediate filament proteins include nuclear lamins, and type-VI proteins include two proteins found in the lens of the eye (filensin (CP115) and phakinin (CP49)). Keratin intermediate filaments (53–55 kDa) are characteristic of epithelial cells. Desmin intermediate filaments of muscle tissue (with the exception of vascular myocytes) are composed of desmin protein (53–55 kDa). Vimentin filaments, characteristic of various cells of mesenchymal origin (fibroblasts, macrophages, osteoblasts, endothelium, and vascular smooth myocytes), consist of the protein vimentin (54–58 kDa). Peripherin (57 kDa) is present in peripheral neurons, participating in the assembly of intermediate filaments in place with neurofilament proteins. Neurofilaments are intermediate filaments of neurons that play an important role in maintaining the shape of the processes of nerve cells. They consist of at least three high molecular weight polypeptides (68, 140, and 210 kDa). Glial filaments contain glial fibrillar acidic protein (56–58 kDa) and are found only in glial cells (astrocytes, oligodendrocytes).

Proteins, which are also referred to as proteins of intermediate filaments, are present not only in the cytoplasm (as listed above) but also in the cell nucleus. The dense envelope located under the nuclear membrane, imparting rigidity to the nucleus (lamina), consists of lamina proteins. Lamins are intermediate filaments of nuclei of various types of cells that form an intranuclear skeleton (karyoskeleton). Unlike other intermediate filament proteins, laminae do not form filaments but rather a reticular structure. Even with the destruction of cell membranes (for example, treatment with detergents), the nucleus retains its integrity due to the lamina.

There are several types of intermediate filaments present in endothelial cells. The main protein of intermediate filaments in the endothelium is vimentin [8][9]. The structure of vimentin is conserved in mammals and shows dynamic expression profiles in various cell types and different developmental stages. Additionally, nestin was found in endotheliocytes [10][11][12] as well as neurofilaments [13]. Nestin has been found in cardiac endothelial cells and is generally considered a marker of revascularization [10][11][12]. In addition, nestin expression is specific not only for proliferating endothelial cells (bovine aortic endothelial cells (BAEC) in vitro), but in the vascular endothelium of brain tumors in the stage of rapid growth [14]. Furthermore, the rate of nestin expression is one of the important criteria for prognosis in breast cancer [15]. After studying nestin-positive microvascular density in breast cancer patients, Nowak and her colleagues [15] concluded that a high level of nestin expression is characteristic of newly formed tumor vessels. In addition, a high level of nestin expression may be related to an aggressive course of the disease and a poorer prognosis. In contrast, it is necessary to mention another nestin-related research work [16], which included body-wide transcriptome and protein-profiling analysis. Dusart and colleagues demonstrated, that nestin is constitutively expressed in human endothelial cells; its expression does not depend on cell proliferative status and is not specific to tumor endothelium. Nestin co-localizes with vimentin in different types of endothelia in vitro (umbilical vein (HUVEC), dermal microvessels (HDMEC), the coronary artery (HCAEC), and the pulmonary artery (HPAEC) under static conditions and laminar shear stress [16]. Especially interesting is that nestin expression is lower in regions of atherosclerotic plaques than in normal vessels [16].

As noted earlier, neurofilaments are the type-IV family of intermediate filaments and are usually associated with neural tissues. Nestin (member of type-VI intermediate filament), is a well-known marker of endothelial cells in newly formed blood vessels and is developmentally and structurally related to type-IV intermediate filaments. Based on this similarity, Rusu and colleagues [13] noted the neurofilament positive labeling of endothelial cells may be due to interactions of nestin and neurofilaments within cadaver samples (sinoatrial nodes/right atrial walls) of both normal and diabetic donors [11]. Positive labeling of endothelial cells leads to the question if neurofilaments may qualify as markers of angiogenesis [13].

4. Molecular Structure of Vimentin Filaments as the Basis of Their Remarkable Mechanical Properties

The most conservative representation of type-III filaments is with vimentin. The outstanding mechanical properties of vimentin filaments are explained by the subunit packing geometry making up the filament. The vimentin monomer is subdivided into three domains: N-terminal (“head”), C-terminal (“tail”), and a highly conserved central domain, which includes four coiled domains and three linker non-coiled regions [17]. The central domain contains tandem repeats of amino acids (heptade repeats), which are capable of forming a supercoiled dimer. The vimentin dimer, the basic structural

element of the vimentin filament, consists of 466 amino acid residues [18]. The two dimers bind anti-parallel and stepwise, forming a tetramer, the fundamental unit of intermediate filaments (ULF) [16].

As a result, intermediate filaments have unique stability against applied forces and mechanical rupture. This property depends on the formation of many simultaneous bonds along the filament diameter, many of which are electrostatic or hydrogen bonds. In actin (microfilaments) or tubulin polymers (microtubules), the binding sites between subunits are largely hydrophobic. In contrast, the bonds between vimentin dimers in the tetramer (and amongst tetramer filaments) involve the overlap of local regions of opposing charge. These interactions occur not only in regions within the filament core, but the alpha helices, and around the N-terminus of the proteins [19][20].

5. Vimentin Filaments: The Role of Post-Translational Modifications in the Regulation of Depolymerization and Stabilization of Vimentin Filaments

After synthesis, intermediate filaments can undergo various chemical modifications [21]. Regulation of the vimentin network is highly complex and is driven by post-translational modifications, such as phosphorylation and cleavage by intracellular proteases. The reorganization of the network of vimentin filaments occurs through phosphorylation, a post-translational modification, due to which the assembly and disassembly of filaments occur during the cell cycle [22]. The first evidence of the dependence of vimentin organization on phosphorylation was obtained in in vitro experiments on complete disassembly of vimentin filaments using purified protein kinase C or cAMP-dependent protein kinase [23]. Analysis of vimentin mutants with deletions at the N-terminus (“head”) revealed that it is the N-terminal domain that is critical for the assembly of vimentin filaments, as well as for the formation of the network [24]. Of the many found vimentin phosphorylation sites (serine residues), those responsible for filament disassembly are located precisely on the vimentin head domain and are phosphorylated by a number of protein kinases. Phosphorylation of the N-terminal domain increases the distance between the two head domains of the dimer, which makes it impossible to form a complete filament from vimentin tetramers [7]. Vimentin also undergoes other post-translational modifications, such as sumoylation, citrullination, and glycosylation by O-linked N-acetylglucosamine (O-GlcNAcylation) [7]. In the process of apoptosis, arginine deamination (citrullination) of vimentin occurs [25], and citrullination sites are located in the “head” domain [22]. This modification occurs in the presence of calcium ions and causes depolymerization of vimentin filaments. O-N-acetylglucosamine glycosylation (O-GlcNAcylation) of vimentin in neurons can prevent excessive phosphorylation of filaments and their depolymerization [26]. Vimentin sumoylation is triggered by STAT inhibition in glioblastoma cells [27]. All this indicates the importance of post-translational modifications in the regulation of depolymerization and stabilization of vimentin filaments.

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