

Cytoplasmic Actins in Endothelial Cell

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Definition

The primary function of the endothelial cells (EC) lining the inner surface of all vessels is to regulate permeability of vascular walls and to control exchange between circulating blood and tissue fluids of organs. The EC actin cytoskeleton plays a crucial role in maintaining endothelial barrier function. Actin cytoskeleton reorganization result in EC contraction and provides a structural basis for the increase in vascular permeability, which is typical for many diseases. Actin cytoskeleton in non-muscle cells presented two actin isoforms: non-muscle β -cytoplasmic and γ -cytoplasmic actins (β -actins and γ -actins), which are encoded by ACTB and ACTG1 genes, respectively. They are ubiquitously expressed in the different cells in vivo and in vitro and the β/γ -actin ratio depends on the cell type. Both cytoplasmic actins are essential for cell survival, but they perform various functions in the interphase and cell division and play different roles in neoplastic transformation.

1. Introduction

The vascular endothelium is formed by the monolayer of specialized tightly adjoining and connecting to each other endothelial cells (EC). EC form a selective semi-permeable barrier between the interior space of blood vessels and the underlying tissues. The endothelial barrier regulates liquid and macromolecule transport between the blood and the interstitial space and is highly susceptible to regulation by various stimuli of physiological and pathological origins. EC barrier integrity is critically dependent upon intact cytoskeletal structure and cell junctions. Reorganization of the endothelial cytoskeleton, especially and the actin system, results in alteration in cell shape and its compression and provides a structural basis for an increase in vascular permeability, which has been implicated in the pathogenesis of many diseases including asthma, sepsis, acute lung injury ischemia, and diabetes.

Actin is one of the most abundant proteins in eukaryotic cells. The ability of actin to polymerize and interact with enormous number of other proteins enables it to perform many different functions. All the cytoskeletal proteins are capable of polymerization, however, the exchange between monomeric and polymerized actin in the cytoplasm occurs rapidly and the exchange time at the edge of the cell is several seconds. The high dynamics of the actin network provides it the ability to play multiple roles in cell mobility, maintaining cell shape, signal transduction, cell adhesion, transcription and muscle contraction, and the formation of a contractile ring during cytokinesis ^{[1][2]}.

Six actin isoforms are present in the cells of vertebrates—four predominantly tissue-specific muscle isoforms (skeletal, cardiac, and smooth muscles) and two non-muscle isoforms (named cytoplasmic β -actins and γ -actins)—that are essential for almost all the cells ^{[2][3]}. The ratio and subcellular distribution of actin isoforms are variable and depend on the cell type ^{[4][5][6]}.

2. Cytoplasmic Actin Isoforms: Structures and Functions in the Interphase Non-Muscle Cells

The family of actin proteins is highly conserved. The greatest difference is observed in the amino acid sequence of muscle and non-muscle isoforms ^[2]. Two cytoplasmic actins and many actin-binding proteins, which provide the organization of various structures, form the microfilament cytoskeleton of non-muscle cells. The amino acid sequences of cytoplasmic β -actin and γ -actin (hereafter β -actin and γ -actin) differ only in four residues located at the N-terminus of the polypeptide chain ^[3].

β -Actin gene is essential for survival during embryonic development of mammals. Embryos of mice lacking β -actin are much smaller in size and die at the early stages of development ^[7]. Embryos without γ -actin pass the prenatal period of development with some delay and they experience an increased

mortality rate after birth [8]. Mouse embryonic fibroblasts with β -actin knockout show reduced motility compared to normal cells [7]. There is a pronounced compensatory expression of α -smooth muscle actin and activation of the Rho signaling pathway in these fibroblasts. ROCK inhibitors restored motility of cells without β -actin. γ -Actin is an important structural element and positive regulator of cell migration. Knockdown of γ -actin results in excessive phosphorylation of cofilin and myosin light chain, which indicates ROCK activation, increased contractility, and inhibition of the cell motility [9][10][11][12]. Morphological studies of the structures that are formed by non-muscle actins became possible due to highly specific monoclonal antibodies against β -actin and γ -actin and the method of confocal microscopy [9]. In the non-muscle cells of different origin (epithelial, endothelial, and fibroblasts) β -actin and γ -actin organize different cytoskeletal structures that are diversely located within the cell, and can perform distinct functions [9][10][13]. In fibroblasts, β -actin is predominantly located in the stress fibers and in the focal contacts area; cortical and lamellar branched actin network consists of γ -actin (**Figure 1A,B**). In the lamellipodia, the colocalization of β - and γ -actin is observed (**Figure 1B**). β -Actin filaments are involved in the processes of cell contraction. In the epithelial cells, β -actin forms basal microfilament bundles and participates in the adhesion junctions; γ -actin organizes the cortical (dorsal) network of actin filaments (**Figure 1D**) and some stress fibers [9].

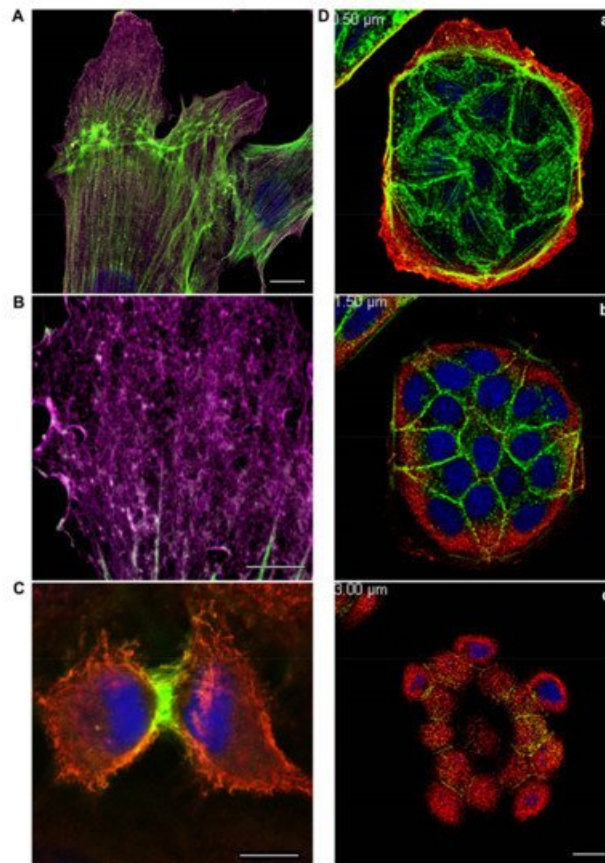


Figure 1. (A,B): β -actin and γ -actin display distinct distribution at the leading edge of fibroblasts moving towards the wound. An experimental wound was performed by scratching a monolayer of normal human subcutaneous fibroblasts. The cells were fixed and stained for cytoplasmic actins three hours later after scratching. β -actin bundles (green) and γ -actin network (purple) are pronounced in the motile cells. Laser scanning microscopy (LSM). Scale bars: (A) 10 μm ; (B) 5 μm . (C): Segregation of cytoplasmic actin isoforms in cytokinesis of normal mitotic epithelial cells. β -actin and γ -actin are spatially segregated in cytokinesis of epithelial HaCaT cells. β -actin (green), γ -actin (red), and DNA (blue). LSM. Scale bar: 5 μm . (D): β -actin and γ -actin are localized in different structures and cell compartments. MDCK epithelial cells were cultivated for 3 days, fixed, and stained for cytoplasmic actins. β -actin (green) is present in basal bundles (a) and cell-cell contacts (b). γ -actin (red) is present in lamellar (a) and dorsal/cortical (b,c) networks. LSM. Scale bar: 10 μm .

The distribution of β -actin and γ -actin in the endothelial cells is similar to epithelial cells [14]. In endothelial cells, the actin cytoskeleton is represented by actin stress fibers, which include a cortical network of actin fibers and a membrane skeleton. Moreover, in the cells of other tissues, β -actins and γ -actins are segregated in the cytoplasm of endothelial cells in vitro (**Figure 2**) and in vivo (**Figure 3**). They form different types of intracellular structures in endotheliocytes. In endothelial cells of the human pulmonary artery (HPAEC) and cells of the human umbilical vein (EAhy926), in vitro β -actin mainly forms stress fibers and rounded microparticles in the cytoplasm of cells, while the cells consisted of γ -actin in the branched actin network in the cortical regions [15][16].

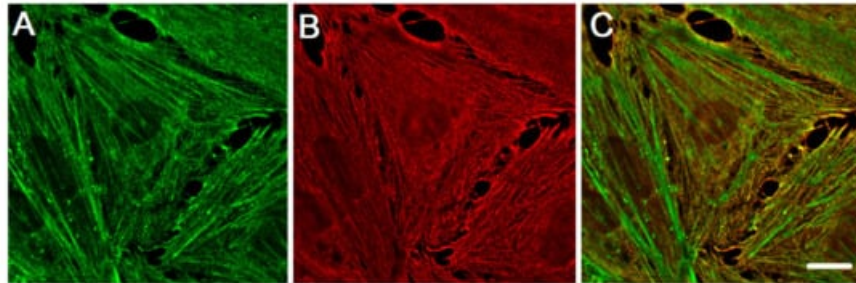


Figure 2. The distribution of cytoplasmic actin isoforms in the cytoplasm of endothelia cell in vitro. β -actin and γ -actin are spatially segregated in human pulmonary aorta endothelial cells. (A) β -actin (green), (B) γ -actin (red), and a merge of β -actin (green) and γ -actin (red) images (C). Structured Illumination Microscopy (SIM). Scale bars: 5 μ m.

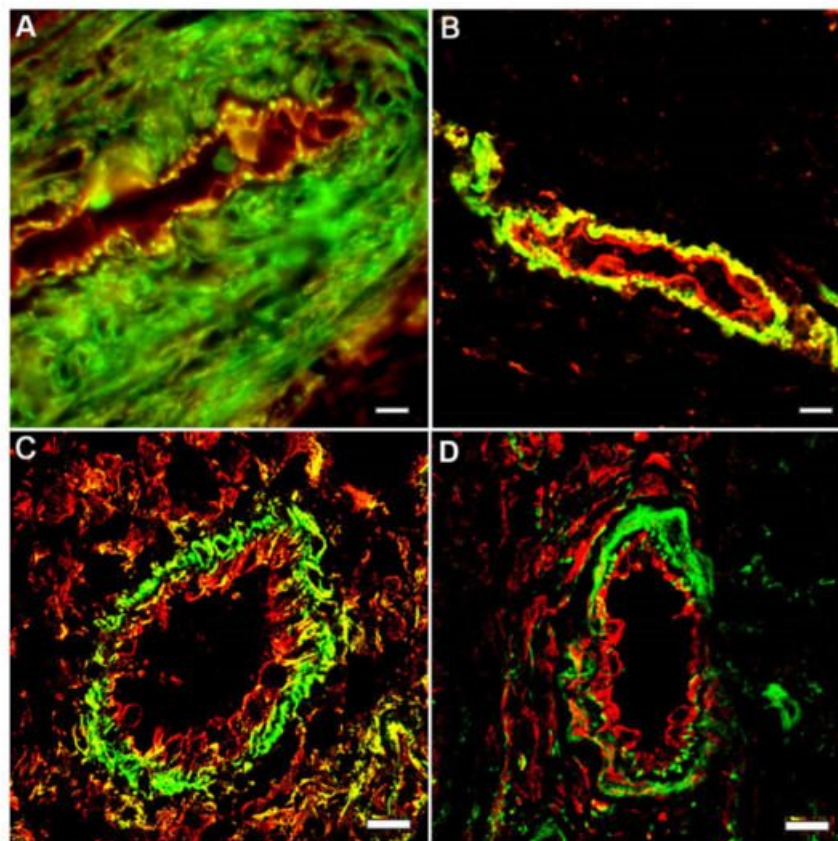


Figure 3. Segregation of cytoplasmic actin isoforms in the endothelia cell in vivo. Capillaries in tissue sections: longitudinal (A,B) and cross (C,D) tissue sections of the wound healing areas of stratified epithelium of human skin. Immunofluorescence staining of β -actin (green) and γ -actin (red): A-C; β -actin (green) and vimentin (red): D. LSM. Scale bars: 5 μ m.

In the endothelial cells lining the vessel walls in a living organism, β -actin and γ -actin structures are also spatially separated. It was shown that endothelial cells of large vessels have very similar cytoskeleton organization, in contrast to microvascular endothelium [15][16][17]. The distribution (but not the density) of

β -actin and γ -actin structures in the endotheliocytes of human artery and veins does not differ.

β -Actin is important for the structure and functional regulation of the adhesion junctions in epithelial cells and determines an apical-basal cell polarity. γ -Actin is associated with tight junctions in the epithelial cells. Suppression of β -actin causes the loss of intercellular contacts in the epithelial cells, while a downregulation of γ -actin induces an epithelial-myofibroblast transition (EMyT) that is accompanied by an increase in stress fibers and enhanced expression of α -smooth muscle actin [10][13].

Cytoplasmic β -actins and γ -actins are also involved in the endothelial barrier function [17]. β -Actin is found mainly in the stress fibers, while γ -actin forms a branched cortical network in the interphase endothelial cells of large vessels [15]. Coordinated rearrangements of β -actin and γ -actin filaments contribute significantly to the development of endothelial microparticles [14]. Endothelial microparticles are membrane vesicular structures released upon endothelial cell activation or the induction of apoptosis [18][19]. Both actin isoforms are responsible for the endothelial barrier function and the dynamics of cell contacts. However, β -actin is vital for endothelial cells: β -actin knockout results in the death of almost all endothelial cells [20]. The connection of the actin structures with microtubules (MT) is important for the functional activity of endothelial cells. Microfilament–MT interaction provides compression and relaxation of the cell during the endothelial barrier function. Thrombin or nocodazole treatment impairs the barrier function, induces MT disassembly, and results in the formation of large stress fibers [21]. MT affects the actin filament organization via local changes of actomyosin contractility at the end of stress fibers [22]. Actin filaments interact with the dynamic MT [21][23], which are the majority in endothelial cells [21]. The dynamics of MT is γ -actin-dependent, which suggests the presence of a mechanical joint between γ -actin and MT [24]. This joint is possibly established through the intermediates, which may be isoform-specific. For example, MT plus-end binding protein EB1 is known to interact mainly with γ -actin, but not β -actin [25].

3. Different Impact of Actin Isoforms on the Process of Cell Division

Cytoplasmic actins are segregated in anaphase-telophase of normal mitotic epithelial cells [9][26]. The organization and functions of β -actins and γ -actins at different phases of mitosis of non-tumor epithelial cells were studied using laser scanning microscopy (LSM) [26]. It was shown that β -actins and γ -actins are spatially separated in early prophase, anaphase, telophase, and during cytokinesis (**Figure 1C**). A decrease in β - or γ -actin expression via small interference RNAs (siRNAs) results in a significant reduction in cell population. A decrease in β -actin causes the generation of multinucleated cells, which indicates a possible cytokinesis failure in these cells. Suppression of γ -actin expression diminishes the number of mitoses. The interdependence between actin isoforms and the MT system during mitosis is observed: The reduction in γ -actin induces the disorganization of the mitotic spindle, and suppression of tubulin polymerization influences β -actin arrangement. The role of actin isoforms is fundamentally different in the process of daughter cells separation: The contemporaneous production of a β -actin contractile ring at the cell equator and the loss of γ -actin from the poles are required to generate a stable cytokinetic furrow and for the completion of cell division [27]. Thus, both actin isoforms are required for normal cell division, but each isoform has its specific contribution to this process.

Actin cytoskeleton is reorganized during tumor transformation. This reorganization provides motility, invasion, and metastasis of tumor cells. Cytoplasmic actins play different roles in neoplastic transformation. The predominance of β -actin by exogenous expression induces epithelial differentiation and suppresses cell growth in culture, experimental invasion, and tumor xenografts growth of colon, lung, and mammary gland carcinoma cells. Thereby, β -actin acts as a tumor suppressor in the epithelial tumor cells of different tissue origin. On the contrary, γ -actin enhances malignant features of epithelial tumor cells [28][29]. The depletion of each cytoplasmic actin results in impaired proliferation/cell cycle in carcinoma cells [28][29].

The cytoplasmic actins play distinct roles in cell cycle regulation of breast cancer cells. Downregulation of each cytoplasmic actin isoform inhibits the proliferation of breast cancer cells, but only suppression of β -actin stimulates the expression of cyclins A2, B1, and D3, whereas suppression of γ -actin reduces

expression of these cyclins. γ -Actin is co-localized with extracellular signal-regulated kinases 1/2 (ERK1/2) in breast cancer MCF7 cells. The reduction in β -actin induces ERK1/2 activation, while γ -actin downregulation inhibits phosphorylation of ERK1/2. ERK1/2, γ -actin, and cyclin A2 directly interact in the same protein complex. The reduction in γ -actin results in a decrease in cyclin A2, inhibits ERK1/2 signaling, and inhibits cell proliferation [28]. Nevertheless, quantitative differences in severity are possible even in carcinomas of the same tissue origin. Perhaps this is due to the initial ratio of isoforms and different levels of ERK activation, which results in different sensitivity toward RNA interference or the effect of low molecular weight inhibitors of signaling pathways.

4. Crucial Involvement of Cytoplasmic Actins in the Endothelial Cell Motility and Angiogenesis

Both non-muscle actin isoforms are involved in the implementation of the barrier function, but they also play distinct roles in the other functions of endothelial cells. The migration of endothelial cells is essential for the blood vessels formation and Rho family GTPases participate in this process. The activation of Rac1 GTPase mediates the migration of endothelial cells by the actin cytoskeleton reorganization [30]. Cytoplasmic γ -actin plays a key role in endothelial cell motility and chemotaxis. The addition of fetal calf serum (FCS), fibroblast growth factor β (FGF β), or endothelial cell growth factor (ECGF) significantly increases the endothelial cell motility [20]. This effect is not observed in the cells transfected with siRNA to γ -actin. Knocking down γ -actin expression had no significant effect on adhesion but strongly decreased endothelial cell motility and migration abilities. This effect was associated with an accumulation of thick actin stress fibers, large focal adhesions, and increased phosphorylation of myosin regulatory light chain, which suggests activation of the ROCK signaling pathway [20]. Remarkably, γ -actin knockdown cells were able to initiate morphological differentiation into capillary-like tubes but these structures were not formed completely and rapidly regressed. Incubation with H-1152 and Y-27632 and ROCK inhibitors, completely rescued the γ -actin knockdown-induced motility phenotype but not the angiogenic potential of endothelial cells [20]. These observations suggest that γ -actin plays a crucial role in angiogenesis through both ROCK-dependent and ROCK-independent mechanisms [20].

The participation of γ -actin in the both Rho-dependent and Rho-independent angiogenesis was demonstrated by Pasquier and colleagues [20] and the fact that the knockdown of γ -actin gene does not affect the ability of endothelial cells to attach to various substrates (fibronectin, laminin, and collagen-1, for example) means that γ -actin is not involved in interaction with focal contacts.

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Keywords

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