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REVIEW

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# Role of Microtubule Cytoskeleton in Regulation of Endothelial Barrier Function

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**Abstract**—Cytoplasmic microtubules are an obligatory component of the cytoskeleton of all types of cells. Microtubules are involved in many cellular processes including directed transport of vesicles and signaling molecules and changes in cell shape during its spreading, polarization, and movement. The intracellular organization of the system of microtubules and their dynamic properties are different in different types of cells because they play a key role in the implementation of a variety of cell and tissue functions, including the regulation of the endothelial barrier function. This review presents an overview of current studies on the properties of endothelial microtubules, their interaction with other components of the cytoskeleton and cell adhesion structures, and the role of microtubules in the regulation of the endothelial barrier function.

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## INTRACELLULAR ORGANIZATION OF THE MICROTUBULE SYSTEM – ARCHITECTURE IS DETERMINED BY FUNCTION

Cytoplasmic microtubules, which are highly dynamic polar biopolymeric structures, are an obligatory component of the cytoskeleton of all types of cells. Microtubules are multifunctional structures involved in various cellular processes, including directed cytoplasmic transport of vesicles and signaling molecules and changes in the shape of cells and their spreading, polarization, movement of individual cells, and their division. For all these processes it is crucially important to produce and maintain in the intracellular space a system of microtubules specific for the given type of cells (or the cell cycle phase).

The radial model of organization of the intracellular microtubule system is generally known due to electron microscopic works [1] and is canonical. According to this model, microtubules are derived from the centrosome located in the cell center towards the cell periphery, and minus-ends of the microtubules are fastened on the centrosome and protected against disassembly [2-5]. Such organization of the microtubule system when the centrosome is the only organizing beginning for microtubules is called “autoritaire” [6]. The described model is true for a

number of cells that have microtubules connected only with the centrosome, e.g. CHO and NRK [7].

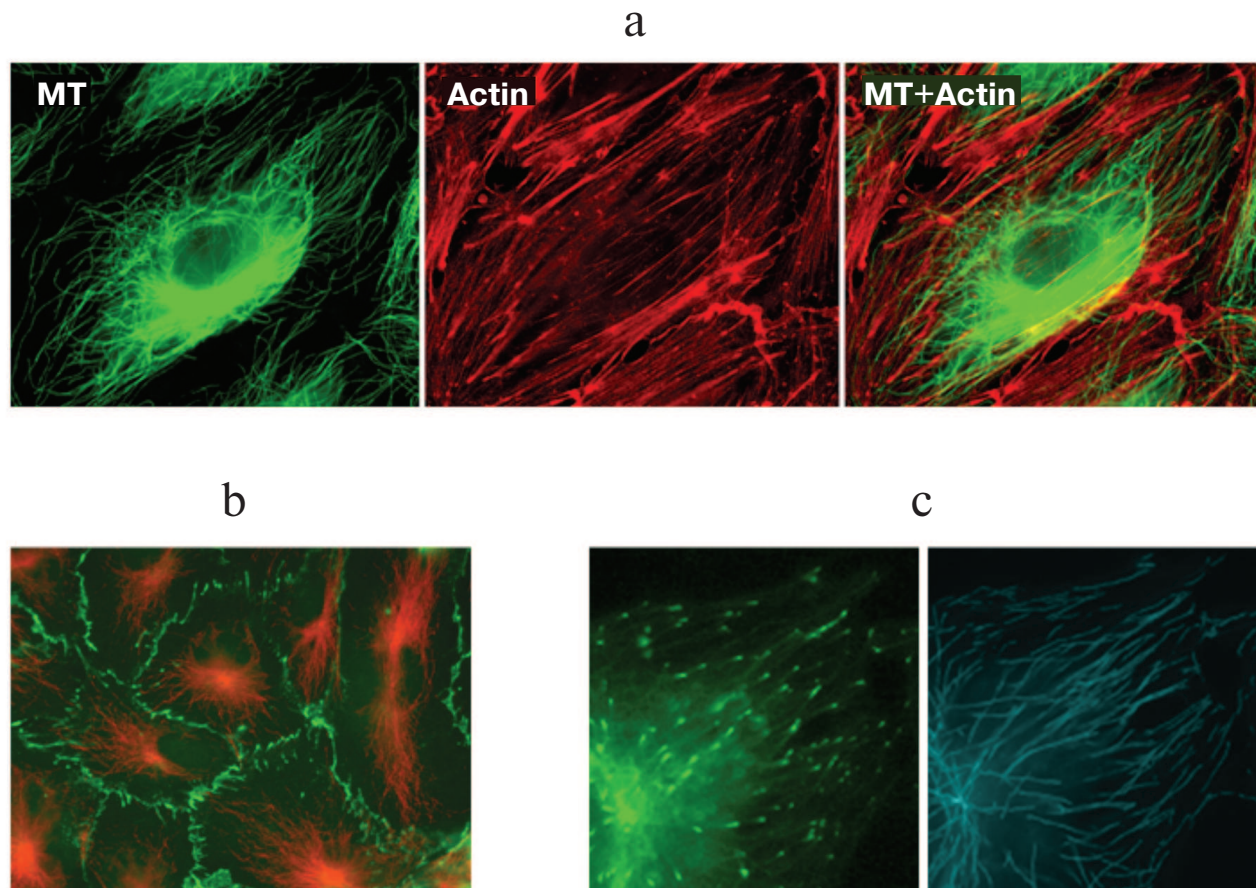
The centrosome located in the geometrical center of the cell is believed to determine the radial organization of the microtubule system by binding their minus-ends and allowing a microtubule to grow up to the cell boundary due to polymerization from the plus-end. The radial model of the microtubule system organization is highly effective from the standpoint of the intracellular transport in small or disk-shaped cells, such as keratocytes and melanophores [8, 9]. In these cells, the general organization of the microtubule system is determined only by dynamics of the plus-ends of centrosomal microtubules. However, in addition to the centrosome-attached microtubules, the majority of cells also have in the cytoplasm free microtubules with specific features of dynamics and organization [10, 11]. According to the literature, the most significant numbers of free microtubules are found in muscle cells and neurons and in epithelial cells of different organs: kidneys [12], liver [13], intestine [14], and in other highly specialized cells [15-20]. In such cells free microtubules are strictly ordered and localized along the long axis of the cell and ensure the transport of neurotransmitters in neurons, effective distribution of membrane components, transduction of mechanical vibrations by internal cells of the organ of Corti [21], and vesicle translocation in polar epithelium cells [14]. The struc-

ture of the microtubule network can fundamentally change depending on the functional activity of the cell; thus, in polar epithelium cells the radial system of microtubules connected by the minus-ends with the centrosome can convert into the basoapical system [22].

Microtubules are critically required for some processes, e.g. for mitosis and meiosis, but their presence in the cell during interphase is not obligatory because the cell can live in the absence of microtubules. In this case, intracellular processes including those specific for executing functions of the given type cells will be disturbed. Thus, the radially organized microtubule system determines the redistribution of pigment cells (melanophores) in response to the action of humoral factors [8, 9]. In the above-mentioned neurons, parallel bundles of microtubules are responsible for the effective transport of neurotransmitters, whereas a change or destruction of such a system inevitably leads to disorders in the traffic and to pathologic processes not only in the tissue or organ, but

also in the organism as a whole. Similarly, disorders in the normal organization of basoapically-oriented microtubules on the polar epithelium cells will lead to dysfunction of the organ of Corti [21].

In endothelial cells *in vitro*, the microtubule system is organized radially (figure, panel (a)), and the majority of microtubules are attached to the centrosome [23, 24]. The density of microtubules is the highest in the inner cytoplasm and gradually decreases towards the cellular boundary where VE-cadherin contacts are settled (figure, panel (b)) and plus-ends of the microtubules are preferentially localized (figure, panel (c)) [24]. This position seems to be functionally essential for endothelium cells because an effective interaction of the plus-ends of microtubules with cellular cortex structures is fundamentally important for the maintenance and even the recovery of the barrier function [25]. The population of microtubules in endothelial cells is not uniform – a certain part of it is represented by less dynamic and therefore more



a) Double immunofluorescent staining of endothelial cells of human pulmonary artery with antibodies against  $\beta$ -tubulin (green) for detecting microtubules and with antibodies against actin (red) for detecting actin stress-fibers. The scale section represents 20  $\mu$ m. b) Double immunofluorescent staining of endothelial cells of the human pulmonary artery with antibodies against  $\beta$ -tubulin (red) and VE-cadherin (green). The scale section represents 20  $\mu$ m. c) Transfection of cells with the microtubular plus-end EB1 protein fused with GFP allows identification of the position of growing plus-ends of the microtubules and study of the growth dynamics (left, EB1-GFP-labeled plus-ends of microtubules (green); right, tracks of EB1-GFP comets characterizing the length of the microtubule processive growth)

resistant to exposure, stable acetylated microtubules [24, 26].

#### CYTOSKELETON OF ENDOTHELIOCYTES – INTERACTION OF COMPONENTS PERFORMING THE BARRIER FUNCTION

Endothelial cells (endotheliocytes) are immediately involved in performing functions not only of tissues, but also even of organs. The main function of endothelial cells closely bordering one another and spreading on the inner surface of vessels regulates vascular tonus and permeability of the vascular wall, which ensures the exchange between the blood circulating inside the vessels and tissue fluid of the organs containing these vessels. Thus, the eye corneal endothelium is responsible for the normal permeability and exchange of the retina with the connective tissue stroma [27], whereas the endothelium of pulmonary vessels regulates the movement of fluids, macromolecules, and leukocytes into the interstice and aerial space of alveoli. Therefore, the integrity of the endothelial monolayer of pulmonary vessels is crucial for maintaining lung function. The endothelial barrier is dynamic and very sensitive to various physiological and pathological stimuli. Disorders in the barrier function of the endothelial cells of pulmonary vessels (endothelial dysfunction) leads to penetration of fluid from the vessels into surrounding tissues and/or into alveoli, which significantly weakens gas exchange. The endothelial barrier is disturbed during inflammatory diseases, e.g. an acute lung injury and more seriously – acute respiratory distress syndrome leading to pulmonary edema associated with 30-40% lethality [28].

The cytoskeleton of endothelial cells plays the main role in the barrier function. Changes in the normal architecture of the cytoskeleton lead to changes in the shape of endotheliocytes and, as a result, to increase in vascular permeability characteristics not only for the above-mentioned diseases, but also for various pathological conditions such as bronchial asthma or sepsis [29-34].

The cytoskeleton of endotheliocytes, similarly to cytoskeletons of other types of cells, is represented by three types of filaments. In addition to the aforementioned microtubules, the cytoskeleton of endotheliocytes includes actin filaments and intermediate filaments. The actin filaments are responsible for changes in cell shape and mobility, whereas the intermediate filaments are mainly responsible for cell shape. Reorganization of the endotheliocyte cytoskeleton consisting of actin filaments, microtubules, and intermediate filaments and the subsequent change in the cell shape creates a structural basis for increase in vascular permeability.

It historically occurred that contributions of different cytoskeletal components to changes in the shape of endotheliocytes during barrier function development

were studied differently. In addition, up to now the role of intermediate filaments has been studied extremely poorly. The obvious role of actin filaments has been studied rather actively [29-31], whereas the involvement of the microtubule system in this process was revealed significantly later [35, 36]. The main achievement of recent years is the understanding that all fibrillar components of the cell cytoskeleton are functioning with interrelations and their interactions are coordinated on different levels of the regulation. Moreover, all fibrillar components are more or less interacting with adhesive structures of the cell. Moreover, just this complicated and finely regulated interaction allows the cells to realize the great variety of cellular, tissue, and organ functions.

In the present review, we shall analyze interactions of the cytoskeletal elements during changes in the endothelial permeability and emphasize the role of microtubules in regulation of the barrier function of the endothelium.

#### ENDOTHELIAL PERMEABILITY AND INTERACTION OF ENDOTHELIOCYTE CYTOSKELETAL COMPONENTS

On analyzing interactions of the cytoskeletal structures of the cell, it should be noted at once that the contribution of intermediate elements is now studied the least not only in endotheliocytes, but also in other types of cells.

Vimentin filaments are the main intermediate filaments of endothelial cells [37, 38]. Vimentin filaments are rather stable structures, and their network remains virtually unchanged *in vitro* during monolayer formation [38]. It is supposed that vimentin filaments might be involved in cytoskeletal interactions indirectly: they might be responsible for centrosome positioning [39] and thus influence the architecture of the microtubule system.

It seems that actin and vimentin filaments can interact structurally because they are connected on the biochemical level [40, 41]. Thus, the tail domain of vimentin is shown to interact directly with actin filaments [41], but up to now no vimentin-binding domain of fibrillar actin has been found. Data on interactions of microtubules and intermediate filaments are still fragmentary; it is likely that kinesin [42] and some other proteins [43, 44] can act as connecting proteins between vimentin and microtubules. This insufficient attention given to intermediate filaments can be easily explained, because actin filaments are generally assumed to be the major player responsible for normal endothelial permeability.

Actin polymerization in endothelial cells is very dynamic. This dynamicity allows actin structures to rapidly rearrange and change the static phenotype characterized by a massive cortical actin ring and minimal number of stress-fibers to the so-called activated phenotype characterized by a poor layer of cortical actin (up to its com-



plete absence) and by numerous stress-fibers compressing the cell. Moreover, just this explains why it was initially thought that the system of actin filaments should be the main cytoskeletal component responsible for functioning of the endothelium and development of its dysfunction [33]. An important role of actin filaments in barrier dysfunction development was shown in many works. In particular, treatment of cultured cells with cytochalasin D destroying the actin cytoskeleton induced a sharp increase in the endothelial monolayer permeability [45], whereas treatment with phalloidin inhibiting actin depolymerization prevented appearance of the barrier dysfunction [46]. The interaction of actin with many binding proteins (filamin, vinculin, etc.) and adhesive structures responsible for the barrier function also seem to evidence its key role in the development of endothelial dysfunction [33].

Actin microfilaments are highly dynamic, the time of their exchange on the cellular boundary being only some seconds [47]; nevertheless, they effectively regulate the cell shape and under *in vitro* conditions maintain a prostrated state of the cell. Adhesive structures of the cell, focal [48, 49] and intercellular contacts [35, 36, 50, 51], are also able to rapidly, during a few minutes, assemble and disassemble. In fact, actin filaments are actively involved in interactions with adhesive structures of the cell [52, 53]. It is generally assumed that the formation of intercellular gaps is regulated just by balance of competing compressing forces, which curtail the cell towards its center, and forces generated by adhesive intercellular junctions and focal cell–substrate adhesions jointly regulating the cell shape [54]. The two competing forces in this model are related through actin fibers, which interact with the numerous adhesive molecules of the focal adhesions and intercellular adherent junctions of membranes.

However, recent works have shown that actin filaments are not immediately involved in development of endothelial barrier dysfunction, while microtubules are just the structure responsible for initial stages of this process [25, 55]. Thus, thrombin, which is produced on the surface of damaged cells from prothrombin circulating in blood and inducing blood coagulation, can disturb the barrier function of the endothelium both *in vitro* and *in vivo*. Thrombin is shown to increase the permeability of the endothelium that is associated with a rapid decrease in the number of microtubules on the cell periphery and reorganization of the microtubule system in the inner cytoplasm of endotheliocytes during the first minutes of exposure. Actin stress-fibers are formed step-by-step, and the maximal effect is observed only 30 min after treatment with thrombin [25, 51]. Thus, the development of microtubule response was faster than the reorganization of the actin filament system responsible for the subsequent changes in the shape of the cells during the development of barrier dysfunction. A direct depolymerization of microtubules under the influence of nocodazole also

induced an increase in the permeability of the endothelial cell monolayer and triggered a molecular cascade leading to barrier dysfunction [25, 56]. The increase in the endothelial barrier permeability under treatment with nocodazole is directly associated with the degree of depolymerization of peripheral microtubules. The normal permeability of the endothelial barrier can be disturbed even on minimal disruption of peripheral microtubules when the system of actin filaments remains intact and no morphological changes are observed [25]. The contraction of the cell body in the absence of stress-fibers is surprising, but not unique fact, as already described earlier [54]. In the great majority of cases barrier dysfunction is accompanied by formation of stress-fibers not depending on factors responsible for disturbance of the endothelial permeability, either pharmacological preparations [35, 36, 57] or neurohumoral factors [32, 35]. Thus, the disruption of peripheral microtubules was shown to be necessary and sufficient for arising of endothelial barrier dysfunction [25, 55].

Microtubules, the main focus of our review, are involved in the most active dynamic interaction with both actin filaments and adhesive structures of different types of cells. During recent decades, these interactions were actively studied initially on mobile cells, but later cells that are not normally characterized as motile also became subjects of study. The interaction of microtubules with actin filaments was found to be important for functional activities of cells of different tissues [54, 58], including endotheliocytes [59, 60], and dynamic properties of microtubules are especially important for such interactions [25, 61].

Similarly to microfilaments, microtubules are highly dynamic – they are continuously growing or shortening even in cases when no visual changes can be observed in the cytoplasm region they occupy. The ends of individual microtubules are growing or disassembling at distances of several microns [62, 63], and the whole system is continuously exchanging with the pool of tubulin dissolved in the cytoplasm with exchange time of 5–20 min [64, 65]. Such behavior was named “dynamic instability” [62, 63], because each microtubule, as discriminated from the system as a whole, is not in a stationary state. However, notwithstanding the dynamic behavior of individual microtubules, changes in the whole microtubule system occur rather slowly even in motile cells, whereas in immotile or poorly motile cells the microtubule system is maintained virtually unchanged in space and time.

The assembly of microtubules is characterized by enrichment of newly polymerized fragments of microtubules with GTP- $\beta$ -tubulin. Because  $\beta$ -tubulin gradually hydrolyzes its GTP upon incorporation into the microtubule, GDP- $\beta$ -tubulin dominates in the remaining part of microtubules. The so-called plus-end proteins specifically bind to the GTP-enriched fragments of microtubules [66, 67]. These proteins represent a large group,

and the most specific role in the polymerization of microtubules belongs to proteins EB1 and EB3 from the family of End-Binding (EB) proteins of this group, which immediately interact with tubulin. Then to the EB proteins, structural proteins CLIP 115 and CLIP 170 bind (of the Cytoplasmic Linker Proteins family), and these proteins in turn bind with structural proteins of the CLASP family (CLIP Associated Proteins). The complex of plus-end proteins plays an important role in regulation of intracellular traffics and is also directly involved in the interaction of microtubules with the cell boundary and the structures localized in this region [67-69].

At present, it is thought that just the dynamic instability of the ends of microtubules and their ability to frequently change the assembly/disassembly stages allow the microtubules to locally modulate the dynamics of cell contacts – in moving fibroblasts through a direct targeted interaction of plus-ends of microtubules with the focal cell–substrate adhesions (so-called “targeting” of cell–substrate adhesions) [48, 49, 70, 71] – and also to regulate the dynamics of intercellular junctions [49, 60, 72, 73]. Moreover, microtubules can control the organization of the cell actin skeleton inducing local changes in the actomyosin contractibility on the ends of stress-fibers [74]. Later it was found in the laboratory of I. Kaverina that changes in the dynamics of microtubules in the zone of contacts depend on the presence of paxillin [75, 76], and a model that connected the asymmetric distribution of focal adhesions and the asymmetric distribution of phases of catastrophes (i.e. transitions to the assembly) of microtubules in the region of adhesion sites was proposed [77-79].

The leading role in the interaction of microtubules and actin cytoskeleton in development of the barrier dysfunction of the pulmonary endothelium is played by Rho-dependent mechanisms [35, 36, 51, 56].

#### RHO-DEPENDENT MECHANISMS OF INTERACTION OF MICROTUBULES AND ACTIN FILAMENTS IN REGULATION OF ENDOTHELIAL CELL BARRIER FUNCTION

Two pathways of barrier dysfunction development are known: the so-called MLC-dependent pathway and the MLC-independent pathway, and at least the MLC-dependent pathway includes Rho-dependent mechanisms [80]. The phosphorylation level of MLC is regulated by two main intracellular factors, the calcium level and activity of Rho proteins, which are common regulatory proteins for microtubules and actin filaments. Plus-end proteins of microtubules and also proteins of the cell cortex bound to them are involved in the regulation of the barrier function through Rho protein-mediated mechanisms of interaction between microtubules and actin filaments.

As mentioned, the main achievement of recent years in studies on cytoskeletal structures is the comprehension that the dynamic morphology of the cell as a whole is associated with the dynamics of its cytoskeletal and adhesive structures that function with interrelations in any manifestation of cell activity. Moreover, a key problem for the modern biology is to understand the mechanism of these interactions fully and in detail, because it is of essential importance not only for fundamental biology but also for practical studies in embryology, medicine, and pharmacology.

Microtubules and actin filaments are connected on the structural [54, 67, 81-83], functional and regulatory levels [25, 35, 36, 55, 84-86]. In the works by A. Bershadsky, a general scheme of the regulation of cell mobility was proposed [87, 88]. According to this scheme, external signals, including those from adhesion receptors, do not directly influence the effectors (motor and adhesion elements) but are transmitted initially into a special integrating system consisting of small GTPases and G-proteins of the Rho family as main components [84-86]. Microtubules and actin filaments are two main effector systems controlled by them, and their functioning is coordinated on several levels. The structural role is played by cross-linker proteins responsible for a direct connection of microtubules and actin filaments [88-90]. However, to more flexibly coordinate in time and space the dynamics of cytoskeletal fibers, another system of regulation is needed, but not their simple mechanical connection. The most important is the regulation mediated by molecular cascades – Rho GTPases can regulate the dynamics and organization of both actin and microtubule system [91-97] and also of focal adhesions and intercellular junctions [97, 98]. One of the mechanisms underlying such parallel regulation seems to be associated with features of an immediate effector of the Rho protein, mDia [96, 99, 100], which was shown to mediate the effect of Rho also on adhesive structures [69, 88, 101]. Active mDia stimulates actin polymerization and concurrently has an independent influence on the dynamics of microtubules [102] due to concentrating their plus-ends closely to growing focal adhesions. Although microtubules suppress myosin II-dependent contractibility, their accumulation near the contacts prevents the growth of contacts and even induces their disassembly and promotes cell movement [103]. Thus, there is a system with negative control regulating the growth of focal adhesions. The effect of mDia on microtubules can be a result of its interaction with molecular complexes directly regulating microtubule dynamics, e.g. with proteins such as EB1, CLIP 170, and APC, which are associated with the ends of microtubules [69, 88, 96, 104-110]. Both EB1 and APC bind with mDia, which seems to indicate an involvement of Rho in capping microtubules [103]. Thus, mDia (a direct effector of Rho proteins) is a factor regulating the system of microtubules, actin filaments, and

cell contacts. The protein mDia is not the only such factor, the protein HDAC6 also participates in the interaction of fibrillar and adhesive structures of the cell [20, 111, 112].

It is also likely that mDia modulates the dynamics of microtubules not directly but through a signaling cascade, and some data suggest that mDia can activate Rac [113], in particular, through a pathway depending on the Rac effector IQGAP, which interacts with CLIP 170 [95, 114] and binds with APC [81]. Recent data have shown that CLIPs interact with IQGAP1, possibly acting as a linker between the plus-ends of microtubules and the cortical actin network and being "downstream" for Rac1 and Cdc42 [95, 115]. On the other hand, CLIP 170 can participate in the regulation of contacts due to interaction with IQGAP [74, 114]. CLIPs and APC are involved also in the interaction of microtubules with intercellular junctions [98, 116], whereas IQGAP can bind with  $\beta$ -catenin of intercellular junctions [68, 114, 116, 117] and thus present a factor participating in the regulation of all cytoskeletal and adhesive structures of the cell. Numerous cross pathways have also been described for regulation of microtubules and microfilaments, which are induced by factors related with the plasma membrane [118-123] and with some other proteins. Thus, microtubules and actin filaments are connected on both structural and functional levels.

Because GTPases of the Rho family and their regulatory proteins are associated with polymerized tubulin [59], it is not surprising that the actin skeleton status regulated by Rho directly depends on the ratio between polymerized and depolymerized tubulin. The activation of Rho and Rho-dependent destruction of the endothelial barrier occurs in response to the action of agents inducing the disassembly of microtubules [25, 57, 124]. An increased permeability of the endothelium under the influence of thrombin and TGF $\beta$  is associated with both dissociation of peripheral microtubules and activation of Rho [25, 35, 125]. The mechanism of microtubule-dependent activation of Rho is likely to include the factor GEF-H1 released on the disruption of microtubules [126]. Another small GTPase, GEF, is now believed to be associated with the regulation of the status of microtubules. Rap1 GEF Epac is a cAMP-dependent protein responsible for the PKA-independent regulation of the organization of the cytoskeleton. Epac is colocalized with microtubules; its specific activation by an analog of cAMP leads to increase in vascular permeability caused by TNF $\alpha$  and TGF $\beta$  [127].

Many studies have shown the leading role of microtubules in disorders of the endothelial barrier [25, 27, 128, 129]. Microtubules seem to be the first structural target in the chain of reactions resulting in endothelial barrier dysfunction [25]. The available data indicate that dynamic microtubules play a significant role in barrier function *in vitro*; depolymerization of peripheral micro-

tubules is the necessary and sufficient condition for development of endothelial dysfunction [25, 129]. This conclusion is extremely important for practical pharmacology because it allows researchers to determine the lines of searching for substances capable of lowering the increased permeability of vascular endothelium. The strategy must be based on inhibition of signaling pathways that depend on depolymerization of microtubules, including the stabilization of microtubules with substances decreasing their dynamic properties [25, 80].

Searches for such substances are rather intensive. Thus, a case of pulmonary edema development in pneumonia was described (as a consequence of endothelial dysfunction) resulting because of a massive ejection of pneumolysin after lysis of bacteria caused by high doses of the antibiotic [129]. This was associated with depolymerization of microtubules on the cell periphery, and not only dynamic but also stable acetylated microtubules were depolymerized. A TNP derivative, TIP-peptide, could protect the endothelial barrier integrity damaged by pneumolysin. This was associated with full recovery of the system of peripheral microtubules, and their number reached the level of the control [129]. Adenosine pronouncedly protected the endothelial permeability against damages and promoted its recovery [130]. Adenosine induced a rapid dose-dependent strengthening of the barrier function of endothelial cells and, thus, is a natural protector of the barrier function of endotheliocytes. The adenosine-induced strengthening of the endothelial barrier is accompanied by an increase in the amount of VE-cadherin on the cell surface and reorganization of the actin cytoskeleton of endotheliocytes [130].

#### ENDOTHELIOCYTES EXPRESSING EXOGENOUS MICROTUBULE PLUS-END PROTEIN FUSED WITH GFP AS A MODEL FOR STUDIES ON MICROTUBULE DYNAMICS AND ITS ROLE IN REGULATION OF ENDOTHELIUM BARRIER FUNCTION

Cultured endothelial cells expressing fluorochrome-fused exogenous microtubule plus-end protein are an excellent functional model for studies on involvement of the microtubule system in the maintenance and protection of endothelial barrier function [24, 25, 61]. In our studies, we used the plus-end protein EB1 fused with GFP (figure, panel (c)). The model allowed us not only to study the microtubule system organization in a living endotheliocyte, but we were the first to characterize the dynamics of endothelial cell microtubules [24] and analyze changes in their dynamic properties specific for barrier dysfunction [25].

Absolute values of the growth rate of microtubule plus-ends in endotheliocytes were shown to be comparable to the growth rates of microtubules in fibroblasts and

in functionally active regions of the cytoplasm of polarized and moving cells, i.e. the microtubules of endotheliocytes are highly dynamic [24]. We were the first to find that the growth rates of plus-ends of microtubules were different: in the cell center region the growth rate of plus-ends was higher than on the periphery of cytoplasm [24]. In addition to the regional differences in the growth rates of microtubules, we also found that the growth rate of the plus-ends depended on the presence or absence of intercellular junctions. In individual endotheliocytes, the growth rate was higher than in the cells contacting neighbors. Moreover, individual and contact-free endotheliocytes contained a subpopulation of rapidly growing microtubules, with the growth rates fourfold higher than normal [24]. It seems that such microtubules were analogs of "pioneer" microtubules observed on the leading edge of moving cells [131]. A subsequent careful analysis of dynamic properties of microtubules of endotheliocytes resulted in their subdivision into four classes: 1) fast growing and long-living; 2) slowly growing and long-living; 3) fast growing and short-living; and 4) slowly growing and short-living [61]. Thus, dynamics of the microtubules of endotheliocytes has specific features: the growth rate of plus-ends of the endothelial microtubules is different in different regions of the cell and decreases with formation of the endothelial monolayer. Summarizing these data, we suppose that endotheliocytes possess special mechanisms for local regulation of growth of the microtubule plus-ends.

Microtubules of endotheliocytes are mainly responsible for the cortical dynamics, similarly to other types of microtubules they can differently interact with the cellular cortex. In particular, there is an interaction of microtubules with plus-ends oriented towards the cell periphery [68, 132, 133]. The dynamic instability allows these plus-ends to grow towards the periphery and potentially explore peripheral structures including integrin focal adhesions [74, 133, 134] and also to organize vesicle transport towards the cell surface [135] and deliver regulatory molecules to the cell cortex [90, 133].

We think that the high dynamics of endotheliocytes and regional differences in the growth rate of microtubules determine the specific function of the microtubules of endotheliocytes: they rapidly deliver molecular signals to the cell cortex (into the region of VE-cadherin contacts) that is important for their effective and rapid local regulation in response to external and internal signals. The high growth rate allows a microtubule born on the centrosome to rapidly lengthen and reach the cell border within a very short time. In the endothelial cell monolayer with the produced intercellular VE-cadherin junctions, microtubules can interact with them, and this interaction can lead to stabilization of microtubules in the junction region. It was shown that the intercellular VE-cadherin junctions in the endothelial cells of lungs could stabilize the dynamic behavior of plus-ends of the micro-

tubules [73, 136-138]. Cytoplasmic microtubules were also shown to be stabilized locally on the cell border due to concentrating in this region of plus-end proteins of the CLASP and EB families [115, 139-144] and also due to interaction with the protein APC [115, 145]. We have shown that, along with other regulatory mechanisms, dynamic microtubules of endotheliocytes can also regulate the existent contacts and determine endothelial permeability [24]. Note also that the dynamics of microtubules is fundamentally important for the morphogenesis of endothelium [146, 147].

#### WHAT IS THE PHYSIOLOGICAL ROLE OF DYNAMIC INSTABILITY OF MICROTUBULES IN THE ENDOTHELIAL CELL?

Just the discovery of the dynamic instability of microtubules [62, 63] provoked a question of its biological expediency. The "Search-and-Capture" model [148] suggests that, during transitions between the growth and shortening of the plus-ends, microtubules rapidly explore the three-dimensional intracellular space and search for targets to interact with or capture. Our results and the literature data have shown that the dynamics of microtubules play a key role in the *in vitro* behavior of the endothelial cell monolayer. The majority of microtubules in the human arterial endothelium cells are highly dynamic [24, 61], and these dynamic microtubules can regulate the existent contacts and, consequently, the endothelium permeability [24]. We have found that the barrier dysfunction is accompanied by disappearance of peripheral microtubules and reorganization of the microtubule system in the inner cytoplasm – the microtubules derived from the centrosome become significantly shorter [35, 36, 149]. Such shortening can occur if microtubules do not reach the cell periphery because of a decrease in the growth rate of their plus-ends or a decrease in processive growth duration. This can also occur if the microtubules are rapidly shortening on reaching the cell periphery because of the absence of pauses and increase in the frequency of catastrophes on the cell periphery. Analysis of the growth rates revealed that at least one of these explanations was correct: for barrier function disfunction development under the influence of nanomolar concentrations of nocodazole the growth rate of the microtubule plus-end noticeably decreased. As a result, the plus-ends seemed unable to reach the cell periphery. We supposed earlier that dynamic plus-ends of individual microtubules could act as tools for regulation of endothelial permeability – to maintain normal permeability a certain number of plus-ends of microtubules had to be present near the cell cortex in the zone of VE-cadherin contacts. If the number of individual microtubules was lower a critical value, a cascade of events was triggered leading to the barrier dysfunction [25]. This con-



clusion is promising for a strategy for searching for protectors against endothelial dysfunction in clinical medicine; such substances have to stabilize the microtubules and prevent their disassembly. In fact, the effect of known protectors of barrier permeability is associated with changes in the dynamics of microtubules and recovery of the number of their plus-ends in the zone of VE-cadherin intercellular junctions [129, 150].

Thus, we conclude that regulation of the endothelial monolayer permeability might be a physiological role of the dynamic instability of microtubules in cells during interphase.

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