Review

Understanding the role of the cytoskeleton in the complex regulation of the endothelial repair

J.S.Y. Lee and A.I. Gotlieb

Toronto General Research Institute, Department of Pathology, University Health Network, Toronto, ON, Canada and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

Summary. Actin microfilaments and microtubules are important cytoskeletal proteins that regulate endothelial repair through alterations in cell shape and through regulation of cell migration following wounding of the endothelium. Upstream pathways have been identified in the regulation of actin and microtubule organization, especially small GTPases. Recently, there have been numerous proteins suggested to be capable of regulating interaction between microtubules and microfilaments to mediate microtubule regulation of endothelial repair, an important process in limiting injury to the artery wall and in reducing the extent of arterial disease. If disrupted, a rapid repair mechanism is important in reestablishing the integrity of the endothelium in order to reestablish its function as a macromolecular barrier, a thromboresistant surface, and a biologically active tissue. Strategies to improve repair should alter the pathobiology of the atherosclerotic plaque and thus improve the prognosis of patients with atherosclerosis.

Key words: Atherosclerosis, Endothelial repair, Cytoskeleton, Actin, Microtubules

Introduction

An important objective in vascular biology is to understand those cellular processes that protect against and those that promote atherosclerosis, a serious vascular disease. The lesion, fibro-inflammatory lipid atheroma (Gotlieb and Silver, 2001), begins in the intima, become larger over time and involves the lumen, and the media. The pathogenesis of the lesions is very closely associated with dysfunction of the surface endothelium of the vessel wall. The progressive accumulation of inflammatory and smooth muscle cells, matrix, and lipids, the formation of a central necrotic

core separated from the lumen by a fibrous cap, and the formation of new blood vessels within the atheroma (angiogenesis) characterize its important features (Stary et al., 1995). The loss of structural and functional integrity of the intact endothelial monolayer is an important aspect of atheroma initiation and growth. Loss of integrity occurs in frank denudation of endothelial cells and also when cell-cell junctions are disrupted and small gaps form between cells. Frank denudation of endothelium, although unlikely to be the cause of the initial lesions, is seen on the surface of lesions leading to ulceration and fissure formation which may promote thrombosis in the vessel lumen and rupture of the atheroma through the fibrous cap. This often results in acute thrombotic occlusion and myocardial infarction (Virmani et al., 2001). Unfortunately, endothelial denudation also occurs following common interventional treatments of coronary artery atherosclerosis such as angioplasty, stenting, atherectomy, and saphenous vein by-pass grafting and is thus implicated in the serious ensuing clinical conditions of acute thrombosis, restenosis and vein graft atherosclerosis (Gotlieb and Silver, 2001). Thus understanding how the vascular endothelium repairs itself is important in limiting vascular disease and improving responses to treatment.

It has now been well established by work from our laboratory and that of others, that dynamic actin microfilaments and microtubules and associated endothelial cell-cell and cell-substratum adhesion complexes, are essential for maintaining integrity and once lost, for regulating efficient repair. In the event of loss of monolayer integrity, cytoskeletal systems are activated to regulate endothelial cell spreading, establish cell polarity, regulate migration and along with subsequent cell proliferation, function to efficiently and rapidly reestablish endothelial integrity. These two systems have been studied independently in both in vitro and in vivo models of endothelial injury and repair (Wong and Gotlieb, 1986, 1988; Ettenson and Gotlieb, 1992, 1993, 1995; Vyalov et al., 1996; Lee et al., 1996; Lee and Gotlieb, 1999, 2002). In the past few years, new knowledge has shown that cytoskeletal networks

Offprint requests to: Avrum I. Gotlieb, MD, CM, FRCPC, Department of Laboratory Medicine & Pathobiology, University of Toronto, Banting Institute, 100 College Street, Room 110, Toronto, ON M5G 1L5. Fax: 416-971-2130. e-mail: avrum.gotlieb@utoronto.ca

cooperate to perform many functions, especially intracellular trafficking and cell spreading and migration, cellular processes that are important in endothelial repair. It is likely that both direct physical connections between the systems and indirect functional interactions whereby activities of one system indirectly affects the other are important in maintaining endothelial integrity and repairing it when it is disrupted.

Actin microfilaments and endothelial integrity and repair

Actin microfilaments play a role in force-generation in migration during repair and are essential in maintaining the structural integrity of the endothelium (Wong and Gotlieb, 1988) by association with cell-substratum and cell-cell adhesion complexes. Endothelial cells in confluent monolayers contain a dense peripheral band of actin microfilament bundles and central microfilament or 'stress fibers' (Wong and Gotlieb, 1986; Ettenson and Gotlieb, 1992). Dense peripheral bands are thought to be important in cell-cell adhesion. Stress fibers are contractile and serve to develop strong substrate anchorage in association with both classical focal adhesion complexes and motile fibrillar adhesions (Pankov et al., 2000). Microfilaments are also present in the lamellipodia and filopodia at the cell periphery and are required for spreading and motility.

F-actin is continuously being polymerized and depolymerized and in association with actin binding proteins is very dynamic within the cell (Condeelis, 2001). The actin microfilaments are composed of two chains of spherical G-actin monomers, which wind around each other to form a 7 nm helix of filamentous actin or F-actin. Each actin subunit has defined polarity and one end (plus end) assembles faster than the other (minus end). ATP-dependent formation of these actin filament arrays is regulated by members of the Ras-homologous (Rho) family of small GTPases. Three important regulatory proteins that act on the Rho family are guanine nucleotide dissociation inhibitors (GDIs), guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). GDIs slow the rate of GDP dissociation from the GTPase, GEFs activate Rho GTPases by enhancing the exchange of GDP for GTP, and GAPs stimulate GTPase activity. GEF-H1 has been shown to mediate cross-talk between microtubules and actin microfilaments in HeLa cells (Krendel et al., 2002) and may be responsible for regulating Rho activity in response to microtubule depolymerization. The classical function of Rho (3 isoforms Rho A, B, C) regulates stress fiber formation whereas lamellipodia and filopodia formation are regulated by Rac and Cdc42 respectively. However more diverse functions exist (Braga et al., 1999). These three proteins also regulate the assembly of integrin-containing focal adhesion complexes which promotes cell-matrix interactions. Rho proteins also enhance endothelial barrier-dysfunction by inhibition of

myosin phosphatase thus disrupting actin myosin interactions at the endothelial cell periphery via Ca^{2+}/calmodulin-dependent activation of myosin light chain kinase (MLCK) (Van Hinsbergh, 1997) and by regulation of adherens junctions. RhoA/Rho kinase is also important in vascular smooth muscle cell migration and intimal thickening (Eto et al., 2000).

There are numerous actin binding proteins which regulate the structure and function of F-actin. For example, myosins are a large family of molecular motor proteins that are the only known type of actin-based motor. An individual myosin converts energy derived from ATP hydrolysis into unidirectional movement towards one specified end of the actin microfilament, either the plus or the minus end. Arp 2/3 is important in actin nucleation. The Arp 2/3 complex consists of seven polypeptides and is associated with regions of actin motility and dynamic assembly. Although the mechanism of action is still controversial, it is thought that significant differences in action may occur in different cell types and signaling pathways (Condeelis, 2001). Other actin binding proteins include vinculin, α-actinin, filamin, cofilin, severin, to name a few, which function in a variety of ways as bundling or severing proteins during polymerization and depolymerization of microfilaments. In directed cell migration, p120 Ras-GAP helps in polarizing cells by regulating the reorientation of actin stress fibers and focal adhesion sites (Kulkarni et al., 2000).

In the first few hours following wounding, prior to migration, quiescent endothelial cells undergo three sequential stages of actin microfilament remodeling to become activated migrating cells (Lee et al., 1996). Following injury, endothelial cells at the wound edge enter stage I (0-2 h) of early repair (Fig. 1A,B). This is characterized by the breakdown of the dense peripheral band, partial detachment of cells from their neighbors at the wound edge and spreading by lamellipodia extension into the wound. It is known that lamellipodia protrusion in wound edge cells is driven by a Rac-regulated dendritic array of microfilaments (Waterman-Storer et al., 1999). This has not yet been studied in endothelial cells. The cells then enter stage II (2-4 h) (Fig. 1B,C), which is characterized by the presence of central microfilaments organized in a parallel orientation relative to the wound edge associated with spreading of the cell laterally. The cells then enter stage III (6-8 h) (Fig. 1D), which is characterized by the organization of central microfilaments perpendicular to the wound edge and by the initiation of cell migration.

The monolayer becomes reestablished over time by continued migration and proliferation of the endothelial cells. We have shown that FGF-2 is an important factor in promoting effective rapid repair. It is not known how the microfilaments are reorganized during these early stages of repair. Although different patterns of organization of actin microfilaments appear, the dynamics of actin polymerization and depolymerization that regulate the reorganization of the spatial structure of

the endothelial cell microfilaments have yet to be defined. New methods are available to visualize cytoskeletal dynamics in living cells that can answer some of these questions. Molecular candidates to study include changes in the Rho GTPases, integrins and associated matrix binding proteins, actin binding proteins, focal adhesion complexes, and the influence of the other major cytoskeletal system involved in repair,
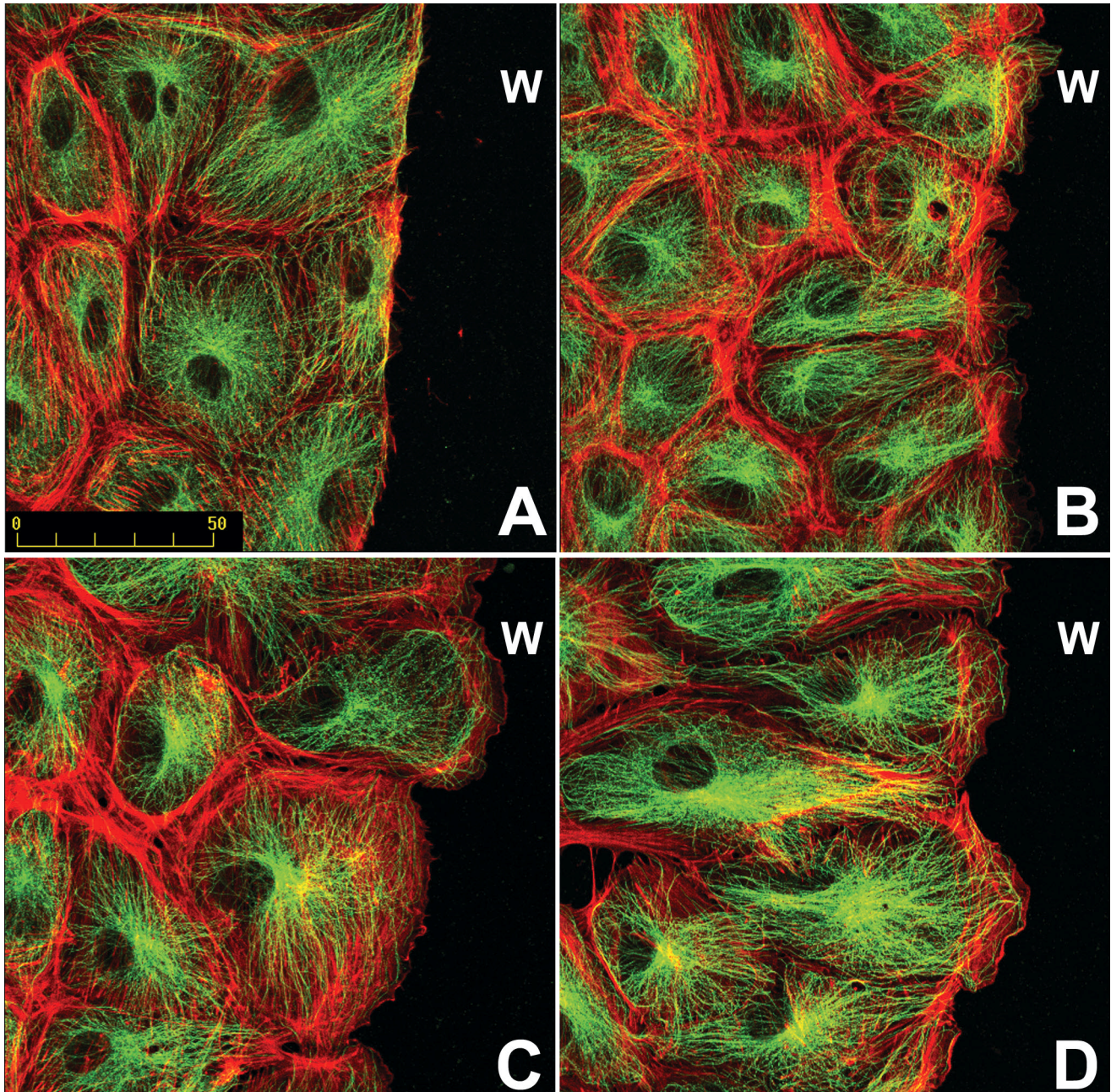

Fig. 1. Porcine aortic endothelial cells were grown to confluency and 1 mm wound created using a scraper. Cells were fixed and double-stained for actin (red)/tubulin (green) at 0 **(A)**, 2 **(B)**, 4 **(C)**, and 6 **(D)** hours after wounding. **A.** Immediately after wounding (w), microtubules emanate from the centrosomes to the cell periphery in a random manner. **B.** By 2 hours, there is formation of lamellipodia and stress fibers rearrange to become perpendicular to the wound (w). Centrosomes migrate around the nucleus towards the wound edge and the microtubules begin rearranging toward the wound edge. **C.** By 4 hours, the changes in microtubules and microfilaments become more prominent and closely associated microtubules and microfilaments spread parallel to the wound edge (w) as the cells begin to spread. **D.** 6 hours after wounding, the microtubules and microfilaments rearrange to become perpendicular to the wound edge. Cells are now elongated and begin moving into the wound (w). This wound will close at approximately 24-48 hours. x 600

the microtubule cytoskeleton. Tyrosine phosphorylation of adhesion proteins, such as paxillin, may be important in regulating actin and focal adhesion reorganization during repair (Lee and Gotlieb, 1999)

Microtubules and endothelial integrity and repair

Microtubules play a role in maintaining cell shape and directional migration of endothelial cells (Wong and Gotlieb, 1988; Ettenson and Gotlieb, 1993). Microtubules formed by self-assembly of alpha-beta (αß) tubulin heterodimers grow and shorten by addition and removal of tubulin subunits from their ends. The faster growing end is known as the plus end and has a ß-tubulin exposed. The slower end is known as the minus end and has an α tubulin exposed (Downing and Nogales, 1998; Pous et al., 1998). The plus ends of microtubules are crucial in that they exhibit dynamic instability, alternating between phases of growing and shrinking (Waterman-Storer and Salmon, 1999). Minus ends are frequently located at a single site in the cell called the microtubule organizing center (Cassimeris, 1999). Many proteins bind to tubulin and regulate microtubule dynamics (Walczak, 2000; Kinoshita et al., 2002).

Microtubules are essential for regulating endothelial cell migration. We showed that endothelial cells at the edge of a wounded monolayer reposition their centrosome to a position between the cell nucleus and the leading edge of the cell (Wong and Gotlieb, 1988; Ettenson and Gotlieb, 1992, 1993). Failure to reposition their centrosome leads to impairment of endothelial cell migration at wound edges and markedly delays repair of the monolayer. Thus, when microtubules were disrupted with colchicine, either at the onset of wounding or during repair, neither centrosome reorientation nor cell migration occurred and wounds remained open. However, when microfilaments were disrupted with cytochalasin B but microtubules left intact, cell migration still occurred but it took four times longer (Ettenson and Gotlieb, 1993). We have shown that basic fibroblast growth factors (FGF-2) released from endothelial cells promote centrosome redistribution and enhance endothelial cell migration (Ettenson and Gotlieb, 1995). FGF-2 also promotes smooth muscle cell migration by altering ß1-integrin-mediated interactions with the extracellular matrix (Pickering et al., 1997). Centrosome redistribution in migrating astrocytes is due, in part at least, to microtubule end-associated protein complex, cytoplasmic dynein implicating motor proteins (Etienne-Manneville and Hall, 2001). Gundersen's group has reported that Cdc 42, dynein and dynactin regulate microtubule organizing center reorientation independent of Rho-regulated microtubule stabilization (Palazzo et al., 2001). Disruption of actin microfilaments resulted in a long delay in the reorientation of centrosomes in cells at the wound edge (Ettenson and Gotlieb, 1993). Thus cooperation between cytoskeletal networks is important in regulating cell function.

Focal adhesion proteins

Focal contacts or adhesions are protein complexes at the cell membrane (Fig. 2) which regulate both cell attachment to the extracellular matrix and cytoskeletal anchoring to the plasma membrane (Aplin et al., 1998;
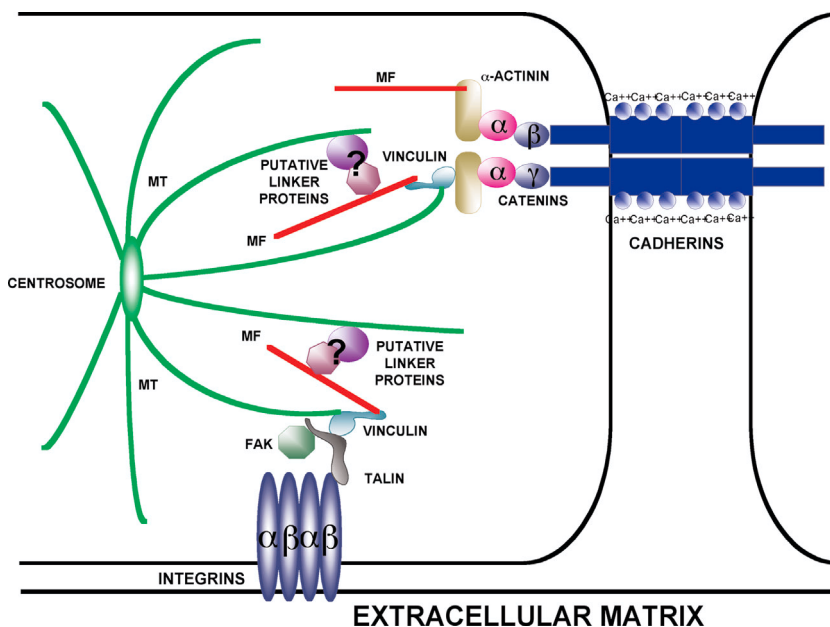

Fig. 2. Integrin αß heterodimers cluster at the endothelial cell membrane and bind both cytoskeletal elements and catalytic signaling proteins to nucleate formation of focal adhesion complexes at the cell-substratum adhesions. Talin connects the actin cytoskeleton to focal adhesions by binding with vinculin and focal adhesion kinase (FAK). Vinculin is composed of a globular head essential for binding to talin and a rod-like tail region which binds to actin. Focal contact proteins form a complex, which serves as the link between integrins and actin cytoskeleton. The vascular endothelial (VE)-cadherin, the major cadherin in endothelial cells, interact with the catenins (α-, ß-, and γ-catenins) at the cell-cell junctions. ß-catenin binds directly to the cadherin cytoplasmic domain, which in turn binds α-catenin. α-catenin either binds directly to actin or to α-actinin to link the cadherin/catenin complex with actin. α-catenin has been observed to have substantial homology to vinculin, which binds α-actinin as well as talin. In some cases, plakoglobin or γ-catenin replaces ß-catenin in mediating the cadherin-cytoskeletal complexes. Microtubules (MT) have been shown to interact with the actin microfilaments (MF) via putative linker proteins in regulating cell-substratum and cell-cell adhesions as well as interacting directly with the vinculin (Kaverina et al., 1998, 1999).

Luttrell et al., 1999). These can then act as a signaling device (Yasuda et al., 1999). Integrins, transmembrane-domain receptors that lack intrinsic enzymatic activity, form the backbone of focal adhesions (Schoenwaelder and Burridge, 1999). Integrin heterodimers form the transmembrane links between the extracellular matrix and the actin cytoskeleton (Schoenwaelder and Burridge, 1999). Assembly of integrin αß heterodimers then nucleate the formation of focal adhesion complexes by binding both cytoskeletal elements and catalytic signaling proteins such as p125FAK. p125FAK binding to the intracellular tail of ß integrin then leads to tyrosine autophosphorylation (Kadi et al., 1998; Luttrell et al., 1999). Focal adhesion kinase (FAK) has several functions within the focal adhesion such as integrin signaling, formation of large protein complexes, and activation of signal transduction pathways. Talin is a heterodimer of two 270 kDa polypeptides, localized in focal adhesions and binds to actin, vinculin and FAK (Aplin et al., 1998). Vinculin, a 116 kDa polypeptide found enriched in focal contacts, binds to both actin and talin. Thus, focal contact proteins form an elaborate multiprotein complex which serves as a structural and functional link between the actin microfilaments and integrins (Aplin et al., 1998; Steimle et al., 1999). Microtubules have also been shown to play a role in regulating focal adhesion complexes (Kaverina et al., 1998; 1999; Small et al., 1999). Focal contact sites are targeted by microtubules and the microtubules may then deliver relaxing signals to those sites to delay or reverse development of the focal contact.

Cell-cell adhesion proteins

Cadherins are Ca^{++}-dependent cell-cell adhesion molecules (Fig. 2) which interact in a homophilic manner (Behrens, 1999) and localize in adherens junctions (Aplin et al., 1998). They consist of an extracellular adhesive domain, a transmembrane segment, and a cytoplasmic domain, the latter being responsible for the adhesive properties of cadherins (Vasioukhin et al., 2000). A highly conserved cytoplasmic domain interacts with catenins, which link cadherins to the actin cytoskeleton. In endothelial cells, the major cadherin is vascular endothelial (VE)-cadherin (Dejana, 1996; Noria et al., 1999). Cadherins regulate migration (Vasioukhin et al., 2000), invasiveness and differentiation of cells, changes in the organization of the cytoskeleton, expression of proteases and expression of several cell adhesion receptors (Behrens, 1999). Cadherin function has been shown to be regulated by Rho and Rac (Braga et al., 1999).

There are three isoforms of catenins that interact with cadherins, the α-, ß-, and γ- catenins (also known as plakoglobin) (El-Bahrawy and Pignatelli, 1998). The 88 kDα ß-catenin binds directly to the cadherin cytoplasmic domain, which in turn binds the 102 kDa α-catenin (Dejana, 1996). While the ß-catenin plays a regulatory role in adhesion, α-catenin links the actin microfilament

to the adherens junction (Vasioukhin et al., 2000). α-catenin links the cadherin/catenin complex by direct interaction with actin and α-actinin, an actin bundling protein. The α-catenin has substantial homology to the structure of vinculin which binds α-actinin as well as talin. In some cases, the plakoglobin or 82 kDa γ-catenin replaces the ß-catenin in mediating the cadherin-cytoskeletal complexes (Aplin et al., 1998).

Adherens junction remodeling is affected by hemodynamic influences which are known to promote endothelial dysfunction and atherosclerotic fibroinflammatory plaque development. Partial disassembly of the adherens junction complex is induced by shear stress, followed by reassembly that is associated with shear-induced shape change and reorganization of F-actin distribution (Noria et al., 1999). Cells in vitro have poorly developed junctions in the early stages of confluency or when they are detaching and migrating from a monolayer. At these times VE-cadherin is heavily phosphorylated in tyrosine and mostly associated with ß-catenin. At confluency, much of VE-cadherin loses its tyrosine phosphorylation and binds with plakoglobin and actin (Dejana, 1996). Thus in the endothelium, assembly and disassembly of junctional proteins are regulated by several cellular processes associated with wound repair including shape change, cytoskeletal reorientation, migration and proliferation (Noria et al., 1999).

Microtubule-actin microfilament interaction

Microtubules fill the cell cytoplasm and usually span the distance from plasma membrane to nucleus allowing signal transmission originating from receptor occupancy (Gundersen and Cook, 1999; Rogers and Gelfand, 2000). Microtubule growth and shortening have been shown to activate Rac1 and RhoA signaling, respectively, which then regulate actin microfilament structure and function (Van Hinsbergh, 1997). RhoA activity itself may in turn regulate the microtubule dynamics by inducing the formation of a subset of stabilized microtubules (Gundersen and Cook, 1999; Schoenwaelder and Burridge, 1999; Waterman-Storer and Salmon, 1999). Specific inhibitors of RhoA have recently been demonstrated to block microtubule-depolymerization-induced formation of stress fibers, focal adhesions, and clustering of integrins by fibronectin (Waterman-Storer and Salmon, 1999). Furthermore, microtubule breakdown has been shown to stimulate stress fiber formation accompanied by the assembly of focal adhesions and FAK tyrosine phosphorylation (Gundersen and Cook, 1999; Waterman-Storer and Salmon, 1999). We have recently shown that Rho regulates centrosome redistribution in addition to central microfilament remodeling during the early stages of repair. We also showed that FGF-2 regulates centrosome distribution, at least in part, by a Rho-independent pathway while FGF-2 promotes actin microfilament remodeling through a downstream Rho-dependent

pathway (Lee and Gotlieb, 2002). Actin microfilaments may also affect microtubule structure and function. Actin microfilaments organize the distribution and function of microtubule in the neural growth cone. Actin microfilaments in filopodia appear to guide the advance of microtubules and regulate retrograde microtubule transport (Schaefer et al., 2002).

Microtubule-actin microfilament crosslinking:

There has been increasing evidence of coordination and physical interaction between actin and microtubule cytoskeleton systems (Sider et al., 1999) either through bifunctional linker proteins or through a series of protein-protein interactions that form a physical bridge between microtubules and microfilaments (Table 1). This is important since it allows for efficient and effective coordination and integration of cell processes that are regulated by both systems in endothelial repair, especially cell spreading and cell migration.

For example, (Mip)-90, isolated from HeLa cells (Gonzalez et al., 1998), showed colocalization with both microtubules and microfilaments as demonstrated by western blots in fibroblast extracts.

Coronin1p (Crn1p), a novel component of the yeast cortical actin cytoskeleton, shares strong sequence homology with a highly conserved mammalian actin-associated protein, coronin (Goode et al., 1999). It was demonstrated using actin microfilament cosedimentation assays that Crn1p promotes rapid barbed-end assembly of actin microfilaments, cross-linking them into bundles and networks. Microtubule cosedimentation assay also showed Crn1p binding to microtubules in vitro via its unique region. This shows Crn1p to be capable of potentially cross-linking actin microfilaments and microtubules thus linking microfilaments and microtubule cytoskeleton functions in yeast.

D-CLIP-190, a 195 kDa protein that co-immunoprecipitates with a class VI myosin (Drosophila 95F unconventional myosin) has been identified (Lantz and Miller, 1998). It is the first homologue of the cytoplasmic linker protein (CLIP)-170 to be identified, whose NH2-terminal domain localizes to the plus ends of microtubules in vivo. To determine if D-CLIP-190 associates with microtubules, co-sedimentation assay using D-CLIP-190 and microtubules was performed. Microtubules were polymerized using endogenous tubulin by adding GTP and taxol to early Drosophila embryo extracts. This resulted in D-CLIP-190 cosedimentation with microtubules. Disruption of actin using cytochalasin D showed loss of D-CLIP-190 localization as assessed by immunofluorescence staining of the posterior pole. The CLIP-associated proteins (CLASPs) were shown to be able to bind both CLIPs and microtubules, co-localize with CLIPs at the microtubule distal ends, and have microtubule-stabilizing effects in transfected cells (Akhmanova et al., 2001).

Plakins may be associated with actin and microtubule networks. A mammalian plakin similar to the Drosophila kakapo isoform 2 of ACF7 (Karakesisoglou et al., 2000) has been shown to bind both actin and microtubules in vitro. In keratinocytes, anti-ACF7 labeling was found in regions of the cytoskeleton where microtubules and actin microfilaments intersect, including at focal adhesion sites. ACF 7 has also been shown to facilitate microtubule-microfilament interactions at the cell periphery and to couple microtubules to cell junctions (Karakesisoglou et al., 2000).

A receptor for hyaluronic acid mediated motility/ intracellular hyaluronic acid binding protein (RHAMM/IHABP) interacts with both microtubules and microfilaments (Assmann et al., 1999). RHAMM is part of a membrane-bound, multimeric hyaluronic acid receptor complex. Highly purified wild type and mutant

Table 1. Linker proteins shown to interact with both micrtutubules and actin miofilaments.

PROTEIN	SYSTEM	SIZE	FUNCTION	REFERENCE
Mip-90 (microtubule interacting protein)	Human fibroblasts	90 kDa	Associates with microtubules	Gonzalez et al., 1998
Crn1p (Coronin 1p)	Yeast	85 kDa	Actin-associated protein	Goode et al., 1999
D-CLIP-190 (homologue to cytoplasmic linker protein-170)	Embryos and embryonic cultured cells	189 kDa	Localize to plus end of microtubules	Lantz and Miller, 1998
CLASPs (-1 and -2) (CLIP-associated proteins	COS-1 cells and Swiss 3T3 fibroblasts	170 kDa (α), 140 kDa (ß/γ)	Binds both CLIP and microtubules	Akhmanova et al., 2001
ACF7/kakapo (Plakin)	SW13 human adrenal carcinoma cells	600 kDa	Cytoskeletal linker protein, interact with intermediate filaments	Kakaesisoglou et al., 2000
RHAMM/IHABP (Types A-D) (receptor for hyaluronic acid mediated motility/ intracellular hyaluronic acid binding protein)	HeLa cells and human cervical and mammary cancer cells	66, 75, 85-90 kDa	Part of hyaluronic acid receptor complex (membrane-bound, multimeric protein complex)	Assmann et al., 1999
X-PAK5 (p21-activated kinase)	Xenopus cell lines	75 kDa	Rac and Cdc42 effector	Cau et al., 2001
MAP2c (microtubule-associated protein)	melanoma cell lines	280 kDa	microtubule-associated protein	Cunningham et al., 1997
mDia (mDiaphemous)	HeLa cells	140 kDa	Rho effector	Ishizaki et al., 2001

RHAMM/IHABP plasmids, tagged with GFP when transfected into HeLa cells showed colocalization with microtubules and microfilaments. This interaction between the receptor RHAMM/IHABP and microtubules and between it and microfilaments was also shown with a microtubule-binding assay and with microfilament cosedimentation analysis, respectively.

The p21-activated kinase (PAK), a well-characterized effector of Rac and Cdc42, was observed to colocalize and bind to both the actin and microtubule networks in transfected XTC and XL-2 Xenopus cell lines. X-PAK5 promoted formation of stabilized microtubules, interfering with the microtubule dynamics and affecting their morphology. X-PAK5 bound microtubules were thick and curly, mostly forming whorls around the nucleus. Similarly, over-expression of X-PAK5 colocalized with F-actin and caused actin reorganization cortically with a decrease in stress fibers (Cau et al., 2001).

A microtubule-associated protein-2c (MAP2c) is thought to mediate interaction between microtubules and actin microfilaments (Cunningham et al., 1997). Furthermore, specific phosphorylation states of MAP2c has been shown to impair MAP2c's ability to colocalize with microtubules but enhance the interaction of MAP2c with the actin cytoskeleton and may even promote lamellipodial extension (Ozer and Halpain, 2000; Alexa et al., 2002).

Finally, mDia (mDiaphenous), a Rho effector, has been shown to coordinate microtubule and F-actin through its FH1 and FH2 regions respectively (Ishizaki et al., 2001). Active mDia1 induces longitudinal parallel alignment of microfilaments in elongated cells and also orientates microtubules parallel to these actin bundles. Aligned microtubules and actin microfilaments have also been observed to terminate in mDia1-enriched, foot-like processes that contain focal adhesions. When the FH2 region of mDia was interfered with, microtubule alignment was inhibited and microfilaments became disorganized, followed by cell shortening.

Furthermore, mutations in Diaphenous or inhibition of mDia in cultured cells interferes with cytokinese. Mutations in human mDia results in premature ovarian failure or non-syndromic deafness (DFNA1) which may reflect effects of Dia on microtubules in cytokinese. Each of these processes also require actin and as mDia stimulates the assembly of fine actin fibres in serum-starved cells, most Dia phenotypes have been interpreted as effects of Dia on the actin cytoskeleton (Palazzo et al., 2001). G.G. Gundersen's group has identified mDia as the first Rho effector to be implicated in regulating microtubule stability in cells. They showed that mDia induces stable microtubules and that mDia may promote this microtubule capping by directly binding to microtubules. mDia has not been observed where actin stress fibres are formed without formation of stable microtubules which indicates that microtubules may have a role in formation of actin stress fibres (Palazzo et al., 2001).

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