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Microtubules regulate aortic endothelial cell actin microfilament reorganization in intact and repairing monolayers

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

Department of Pathology, University Health Network, Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

Summary. To understand the role of microtubules and microfilaments in regulating endothelial monolayer integrity and repair, and since microtubules and microfilaments show some co-alignment in endothelial cells, we tested the hypothesis that microtubules organize microfilament distribution. Disruption of microtubules with colchicine in resting confluent aortic endothelial monolayers resulted in disruption of microfilament distribution with a loss of dense peripheral bands, an increase in actin microfilament bundles, and an associated increase of focal adhesion proteins at the periphery of the cells. However, when microfilaments were disrupted with cytochalasin B, microtubule distribution did not change. During the early stages of wound repair of aortic endothelial monolayers, microtubules and microfilaments undergo a sequential series of changes in distribution prior to cell migration. They are initially distributed randomly relative to the wound edge, then align parallel to the wound edge and then elongate perpendicular to the wound edge. When microtubules in wounded cultures were disrupted, dense peripheral bands and lamellipodia formation were lost with increases in central stress fibers. However, following microfilament disruption, microtubule redistribution was not disrupted and the microtubules elongated perpendicular to the wound edge similar to non-treated cultures. Microtubules may organize independently of microfilaments while microfilaments require microtubules to maintain normal organization in confluent and repairing aortic endothelial monolayers.

Key words: Microtubules, Actin, Endothelium, Wounding, Repair

Offprint requests to: Dr. Avrum I. Gotlieb, Department of Laboratory Medicine and Pathobiology, University of Toronto, Banting Institute, 100 College Street, Rm 110, Toronto, Ontario, M5G 1L5, Canada. e-mail: avrum.gotlieb@utoronto.ca

Introduction

An intact endothelial monolayer is required for normal blood vessel function (Ettenson et al., 1993, 1995). Disruption of this monolayer leads to the initiation and progression of atherosclerosis. In addition, treatments such as atherectomy, angioplasty, and saphenous by-pass grafting result in endothelial denudation and require that the endothelial monolayer be repaired and re-established. Actin microfilaments (Wong and Gotlieb, 1986, 1988; Ettenson et al., 1992; Ettenson and Gotlieb, 1993; Colangelo et al., 1998; Small et al., 1999) and microtubules (Wong and Gotlieb, 1988; Ettenson and Gotlieb, 1993; Downing and Nogale, 1998; Pous et al., 1998; Waterman-Storer, 1998; Cassimeris, 1999) are important for regulating endothelial cell shape and cell migration which are essential processes in maintaining an intact endothelial monolayer at rest and re-establishing it following disruption (Wong and Gotlieb, 1984, 1988; Ettenson and Gotlieb, 1992, 1993, 1995).

Since microtubules are essential in regulating endothelial cell migration during repair, we tested the hypothesis that microtubules regulate the distribution of microfilaments to establish and maintain the integrity of the endothelial monolayer. We used two models to test our hypothesis, the confluent aortic endothelial monolayer and the repairing wound model where small wounds were created in the aortic endothelial monolayers and subsequent events leading to wound closure studied. We also studied the focal adhesion distribution in confluent monolayers in which one of the two cytoskeletal systems was disrupted. These form elaborate multi-protein complexes and serve as cell-matrix links associated with the cytoskeleton. In addition, the remodeling of microtubules and microfilaments was observed in migrating aortic endothelial cells and their distribution assessed when either of the cytoskeletal systems was disrupted. Microtubules are able to regulate microfilament

organization in the confluent monolayer and are essential in the redistribution of focal contact proteins. In wound repair, microtubules are able to remodel in the absence of microfilaments and regulate cell elongation and migration, whereas microfilaments require the presence of microtubules to reorganize during the repair of the aortic endothelial monolayers.

Materials and methods

Cell culture

Porcine aortic endothelial cells were harvested from slaughterhouse porcine aortas by the collagenase enzyme dispersion method and cultured in M199 supplemented with 5% fetal bovine serum (FBS), 50 U/ml of penicillin, 50 mg/ml streptomycin and 0.25 mg/ml Fungizone at 37 °C, 5% CO_2, and 100% humidity as previously described (Lee and Gotlieb, 1999). Confluent cells were harvested with trypsin/ ethylenediamine-tetraacetic acid (EDTA) and re-plated onto 22x22 mm sterile glass coverslips (Corning, New York, NY) in 35 mm tissue culture dishes for immunofluorescence and 60 mm tissue culture dishes for western blots (Nunc, Burlington, ON). Tissue culture reagents were purchased from GibcoLife Technologies (Burlington, ON).

Actin microfilament and microtubule disruption

Stock solutions of colchicine and cytochalasin B (Sigma Chemical Co.) were prepared at 1mg/ml in phosphate buffered saline (PBS) and dimethylsulfoxide (DMSO) respectively and diluted in supplemented media immediately before use (Ettenson and Gotlieb, 1992).

Antibodies

Monoclonal primary antibodies were purchased from Sigma Chemical Co. (St. Louis, MO) (anti-α-tubulin; anti-actin; anti-vinculin; anti-talin monoclonal antibodies) and from Upstate Pharmaceuticals (Chicago, IL) (anti-focal adhesion kinase [FAK] monoclonal antibody). Rhodamine Phalloidin and Alexa 488 conjugated goat-anti-mouse IgG were purchased from Molecular Probes (Burlington, ON) and HRP conjugated goat-anti-mouse IgG (Fc) was purchased from Jackson Immunoresearch Labs (West Grove, PA).

Localization of focal adhesion proteins

To assess the distribution of focal adhesion proteins following microtubule and microfilament disruption, immunofluorescence was performed on aortic endothelial cells grown to confluency (Lee and Gotlieb, 1999). Two days after reaching confluency, cells were incubated in either 10^{-1} mg/ml colchicine or 1.25 mg/ml cytochalasin B for 0, 6 and 24 hours. The cells were double-stained for actin and a-tubulin, vinculin, talin, or FAK. Briefly, cells were fixed for 15 minutes with 4% paraformaldehyde, permeabilized in 0.1% Triton X-100 for 3.5 minutes, then washed and incubated with the antibodies for 1 hour. The cells were then incubated with a mixture of Alexa 488 goat-anti-mouse IgG and Rhodamine Phalloidin and mounted using Prolong Antifade mounting agent (Molecular Probes). Cells were examined with a BioRad MRC 1024ES scanning confocal laser imaging system (BioRad MRC 600, BioRad, Toronto, ON). Images of the aortic endothelial monolayers were obtained by serial optical sections at 0.5 µm intervals and merged. Photomicrographs of the merged images at 600x magnification were randomly selected in order to quantify peripheral focal adhesion proteins. The periphery of the cells was determined by Rhodamine Phalloidin staining of the actin microfilaments. Quantitation of peripheral focal adhesion proteins was carried out by identifying and counting all focal adhesion plaques at the periphery of 30 to 40 cells per condition (n=3).

Western Blot analysis

Western blots were carried out to assess changes in the levels of actin, tubulin, and focal adhesion proteins following microtubule and microfilament disruption. Briefly, aortic endothelial cells were cultured to confluency in 60 mm plates and exposed to 10^{-1} µg/ml colchicine or 1.25µ g/ml cytochalasin B for 0, 6 and 24 hours. Total cell lysates were extracted using an SDS lysis buffer. Briefly, 1ml of ice-cold PBS was added, the cells scraped with a plastic cell scraper and collected. Cells were centrifuged, supernatant removed and 250 µl of lysis buffer added. The cells were incubated for 15 minutes on ice with agitation every 5 minutes and centrifuged. Protein concentration was determined using the Lowry Assay (BioRad). Ten micrograms of sample was subjected to 10% SDS-PAGE and transferred onto PVDF membrane (BioRad). Membranes were blocked with 5% Bio-Rad blocking milk for 1 hour and incubated at 4 °C overnight with the same antibodies used for immunofluorescence, anti-α-tubulin, anti-vinculin, anti-talin, or anti-FAK monoclonal antibodies. Actin was detected using anti-actin monoclonal antibody. Membranes were then incubated with HRP conjugated goat-anti-mouse IgG (Fc) antibody for 1 hour at room temperature. Bands were visualized using the ECL detection system (Amersham, Arlington Heights, IL). Images were scanned and densitometry performed using BioRad "Quantity One". Experiments were repeated in triplicate.

Wounding

To assess the distribution of microtubules and microfilaments under dynamic conditions, wounds were created in confluent monolayers of aortic endothelial cells using a 1mm plastic scraper (Lee and Gotlieb, 1999). The cells were allowed to repair for 0, 2, 4, 6, and 8 hours. To assess the effects of microtubule and

microfilament disruption during wound repair, confluent monolayers were wounded, and 30 minutes later, treated with either 5^{-1} µg/ml colchicine or 0.625 µg/ml cytochalasin B for 0, 2, 4, 6, and 8 hours. The cells were stained for actin and α-tubulin and the pattern of microtubule orientation quantified in cells along the wound edge. Cells with 75% of their microtubules in a parallel orientation relative to wound edge were classified as 'parallel'. Those with 75% of their microtubules in a perpendicular orientation relative to the wound edge were classified as 'perpendicular'. Aortic endothelial cells with a mixture of both parallel and perpendicular microtubules were classified as 'mixed'. Approximately 70 cells at the wound edge were examined per slide and a total of 5-9 slides were quantified per time point. The numbers were expressed as means ± standard error of means.

Statistical analysis

One way analysis of variance (ANOVA) was used to test the significance of adhesion protein redistribution and changes in the total protein levels in cells incubated with colchicine or cytochalasin B for 0, 6, and 24 hours. ANOVA was also used in determining significance of changes in microtubule orientation. ANOVA was followed by the Bonferroni test to compare all pairs of columns. A p value of less than 0.05 was considered significant. Statistical analysis was performed with GraphPad Prism for windows (version 3.02) (GraphPad Software, San Diego, CA).

Results

The optimal concentration and duration of incubation was determined for colchicine and cytochalasin B. Aortic endothelial cells in confluent monolayers were incubated with varying concentrations of colchicine (10^{-3}-10^{-1} µg/ml) or cytochalasin B (0.5-1.25 µg/ml) for different lengths of time (0, 1, 3, 4, and 6 hours). The extent of disruption and the adhesion of the cells to the culture dish were determined (data not shown). The maximum microtubule and microfilament disruption without loss of cells from the confluent monolayer occurred at 10^{-1}µg/ml for colchicine and at 1.25 µg/ml for cytochalasin B following incubation for 6 hours. Therefore, subsequent studies were performed using these concentrations in the confluent monolayers. Disruption of the cytoskeleton was reversible once the colchicine or cytochalasin B was washed out of the aortic endothelial cultures.

When normal confluent monolayers were assessed by immunofluorescence, the aortic endothelial cells showed a dense peripheral band of actin microfilaments with some central stress fibers (Fig. 1a,g). Microtubules spanned the entire cell, radiating out to the cell periphery (Fig. 1d,j) from the centrosome which was randomly distributed around the nucleus as previously described (Lee et al., 1996). When the cells were exposed to 10^{-1} µg/ml colchicine, there was a complete loss of microtubules with α-tubulin staining diffusely throughout the cytoplasm at 6 and 24 hours (Fig. 1e,f) and a loss of dense peripheral bands with an increase of microfilament bundles at 6 and 24 hours (Fig. 1b,c). When cells were exposed to 1.25 µg/ml cytochalasin B for 6 and 24 hours, there was disruption of dense peripheral bands, formation of dense aggregates of F-actin, and stress fibers were distinctive and short in length at 6 and 24 hours (Fig. 1h,i), especially at 6 hours. However, there was no observable disruption of microtubule structures (Fig. 1k,l).

Effect of actin and microtubule disruption on focal adhesion proteins

The disruption of actin microfilaments and microtubules was associated with changes in vinculin. Double staining with actin (Fig. 2a-c,g-i) and vinculin (Fig. 2d-f,j-l) showed that aortic endothelial cells in a normal confluent monolayer expressed punctate vinculin plaques at the ends of stress fibers and at the cell periphery (Fig. 2d,j). When the cells were exposed to colchicine for 6 hours, there was an increase in the number of vinculin plaques at the periphery of the cells (Fig. 2e) followed by a decrease at 24 hours (Fig. 2f). The distribution of two other focal adhesion proteins, talin and FAK (figure not shown) were similar to that of vinculin.

When the cells were exposed to cytochalasin B, there was a visible decrease in the peripheral vinculin (Fig. 2k,l), talin, and FAK (figure not shown) plaques at 6 and 24 hours which were expected with the marked changes in stress fibers. Because it was difficult to identify the cell periphery, quantitation of peripheral staining of focal adhesion proteins was not carried out.

The mean number of peripheral vinculin staining per cell following microtubule disruption with colchicine was increased from 31.0±4.2 at 0 hours to 44.8±3.4 at 6 hours, and decreased to 29.5±2.5 at 24 hours. The changes in the number of peripheral vinculin staining per cell were statistically significant by ANOVA ($p \leq 0.037$) (Fig. 3). There was an increase in the number of peripheral talin plaques from 19.3±3.1 to 33.0±4.9 per cell at 6 hours with a decrease to 26.0±5.9 at 24 hours (Fig. 3). Similarly, the mean number of FAK peripheral staining per cell was increased at 6 hours from 18.6±0.7 to 31.03±1.9 per cell and decreased to 22.3±5.8 at 24 hours (Fig. 3). Although only the vinculin redistribution was statistically significant by ANOVA, talin and FAK both demonstrated similar trends with an increase at 6 hours followed by a decrease at 24 hours. The changes were not statistically significant by Bonferroni test.

Western blots showed no detectable significant changes in the protein levels of α-tubulin and actin following colchicine and cytochalasin B treatment. Similarly, vinculin, talin, and FAK protein levels remained unchanged in the presence of colchicine and cytochalasin B (figure not shown). Therefore,

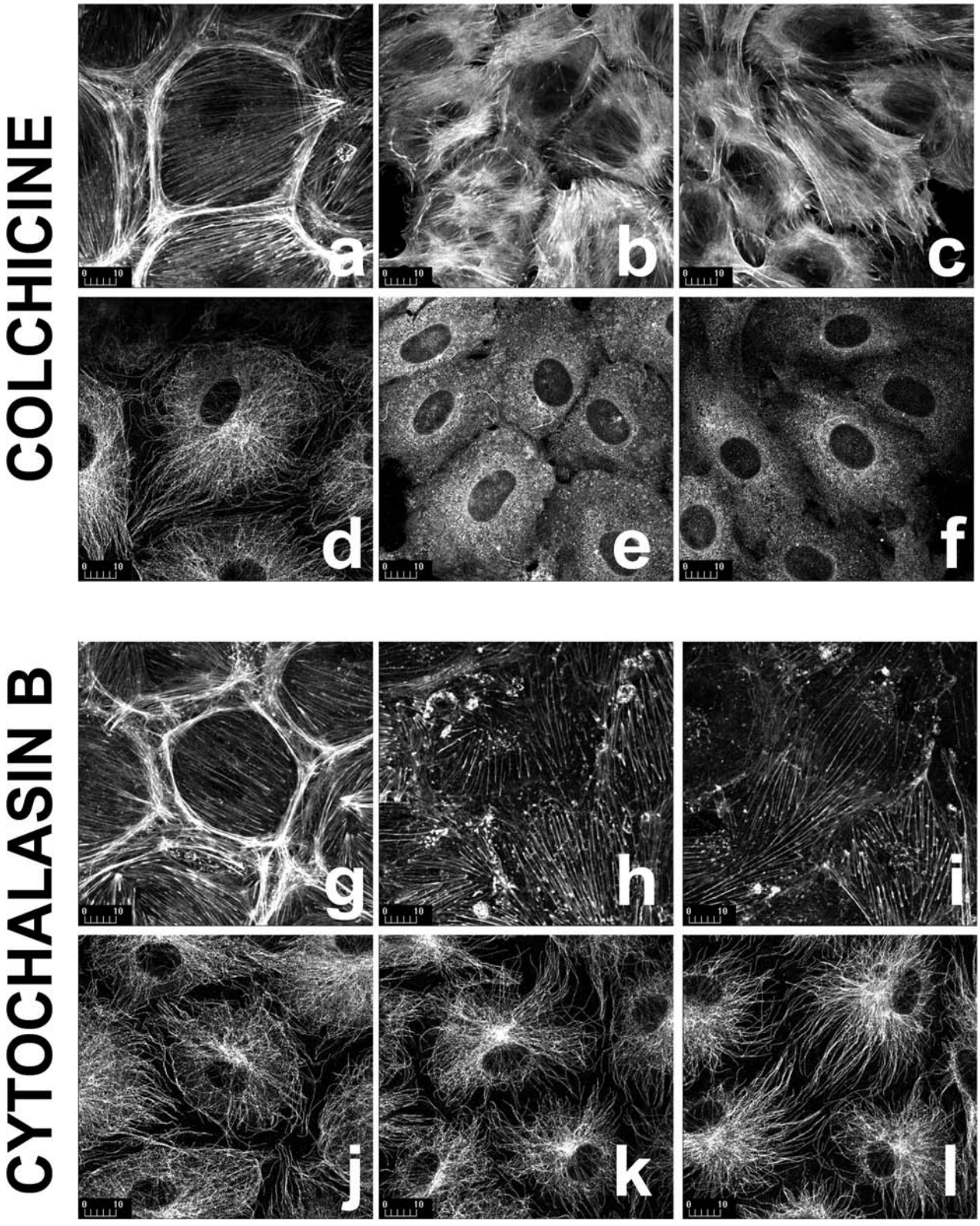

Fig. 1. Microtubule and actin microfilament disruption in aortic endothelial cells. Aortic endothelial cells were incubated with 10^{-1} µg/ml colchicine for 0 **(a, d)**, 6 **(b, e)**, and 24 **(c, f)** hours and 1.25 µg/ml cytochalasin B for 0 **(g, j)**, 6 **(h, k)**, and 24 **(i, l)** hours and double-stained for actin **(a-c, g-i)** and α-tubulin **(d-f, j-l)**. x 900

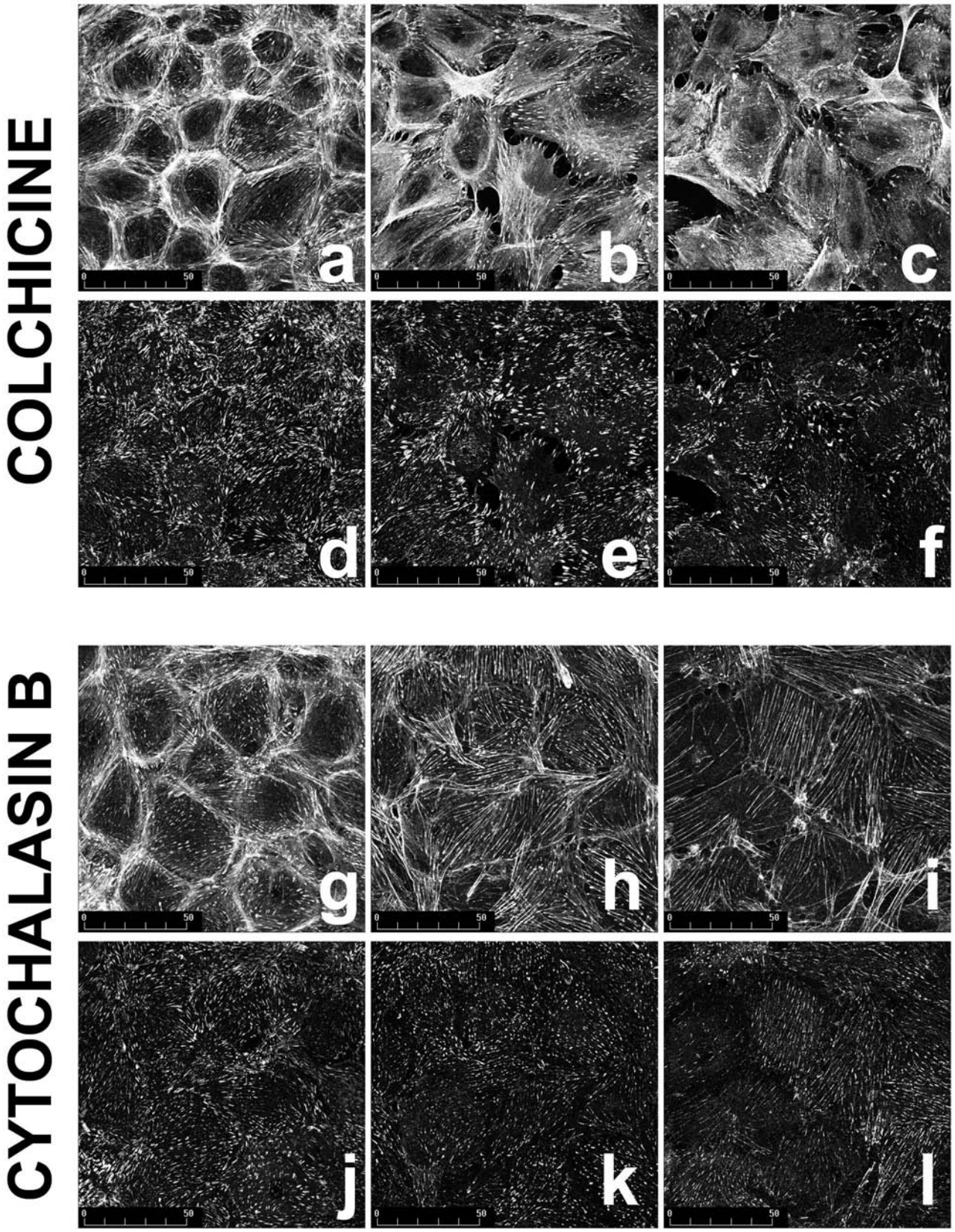

Fig. 2. Effects of colchicine and cytochalasin B on vinculin. Aortic endothelial cells were incubated with 10^{-1} µg/ml colchicine for 0 **(a, d)**, 6 **(b, e)**, and 24 (**c, f**) hours and 1.25 µg/ml cytochalasin B for 0 (**g, j**), 6 (**h, k**), and 24 **(i, l**) hours and double-stained for actin **(a-c, g-i)** and vinculin **(d-f, j-l).** x 800

microtubules appear to play a crucial role in the distribution and organization of the proteins but not in the total expression levels of these proteins.

Microtubule reorientation in the wound

Following wounding, microfilaments undergo redistribution which has been previously described (Lee et al., 1996). In this study, we found that immediately following wounding, microtubules were still randomly distributed, emanating from the centrosome to the periphery of the cells (Fig. 4b). Two hours after wounding, microtubules were oriented parallel relative to the wound edge (Fig. 4e) as the lamellipodia became prominent (Fig. 4d). By 4 hours, microtubules were still parallel to the wound edge, similar to the distribution of microfilaments as the cells spread out. At 6 hours, some aortic endothelial cells had microtubules re-aligned perpendicular to the wound edge in close association with the microfilaments. Both the microtubules (Fig. 4n) and stress fibers (Fig. 4m) were primarily perpendicular to the wound edge by 8 hours as the cells began elongating and migrating into the wound. The aortic endothelial cells from either edge of the wound met by 24 hours but did not yet become confluent or quiescent (figure not shown).

Wound repair in the absence of actin or microtubules

The importance of microtubules on microfilament distribution was also evident in wound repair. When microtubules were disrupted with 5^{-1} µg/ml colchicine 30 minutes after wounding, there was a loss of dense peripheral bands, an increase in microfilament bundles, and an impairment of lamellipodia formation as early as 2 hours (Fig. 4g). The microfilaments did not undergo the series of changes from parallel orientation to perpendicular relative to the wound edge. The bundling of actin microfilaments was more evident at 8 hours (Fig. 4p) and the cells were somewhat rounded, unable to elongate into the wound.

When microfilaments were disrupted with 0.625 µg/ml cytochalasin B following wounding, there was no lamellipodia formation and there were multiple leading edges with F-actin aggregates (Fig. 4j). Some stress fibers remained but these microfilaments did not undergo the three specific stages of microfilament remodeling. However, microtubules remained intact and were able to realign from random to parallel and then to perpendicular orientation relative to the wound edge during the various stages of wound repair as shown at 2 (Fig. 4k) and 8 (Fig. 4t) hours.

The alignment of microtubules was quantified to distinguish between the 'parallel', 'perpendicular', and 'mixed' orientations in approximately 70 cells per wound in both the untreated wound (n=7 to 9 per time point) (Fig. 5A) and in wound incubated with 0.625 µg/ml cytochalasin B (n=5 to 6 per time point) (Fig. 5B). At 0 hours in the untreated cells, 13.1±1.4% of the cells along the wound edge had microtubules that were parallel to the wound, 16.4±2.5% cells were perpendicular, while 70.9±2.7% of the cells had a mixture of both orientations. At each time point of 2, 4, 6, and 8 hours, there was no difference in the orientation of microtubules between untreated or treated cells (Fig. 5). For example, the percentage of cells with parallel microtubules in the untreated cells was 41.3±3.6% at 2 hours, 30.8±2.3% at 4 hours, 30.0±2.4% at 6 hours, and 21.4±2.4% at 8 hours. Similarly, in cells treated with cytochalasin B, there were 44.0±0.6% cells at 2 hours, 33.3±0.9% at 4 hours, 32.0±3.0% at 6 hours, and 23.3±3.2% at 8 hours. There was a progressive decrease in the number of cells with parallel microtubules from 2 to 8 hours.

The percentage of cells with perpendicular microtubules in the untreated cells was 21.0±2.7% at 2 hours, 31.0±3.5% at 4 hours, 31.1±2.0% at 6 hours, and 39.7±3.3% at 8 hours (Fig. 5A). The treated cells showed similar percentage of cells with perpendicular microtubules; 20.7±1.2% cells at 2 hours, 33.0±1.5% at 4 hours, 32.3±1.3% at 6 hours, and 50.0±2.1% at 8 hours (Fig. 5B). There was a progressive increase in the number of cells with perpendicular microtubules from 2 to 8 hours. Following the initial decrease of cells with a mixture of microtubules in both orientations from 0 to 2 hours, there was no significant change at the subsequent time points in either the untreated or treated cells. The change in the microtubules from parallel at 2 hours to the perpendicular orientation at 8 hours was statistically significant by ANOVA ($p \leq 0.0001$) and by Bonferroni test ($p \leq 0.001$) under both conditions, with and without cytochalasin B. Therefore, the microtubules are able to redistribute independently of microfilaments to facilitate aortic endothelial cell elongation and cell migration during wound repair.

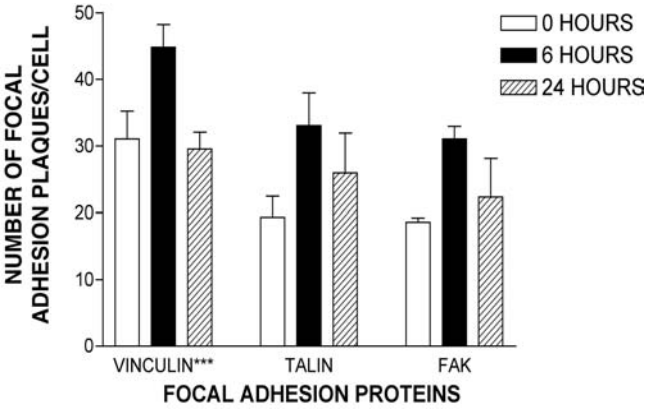

Fig. 3. Quantitation of peripheral focal adhesion plaques following microtubule disruption. Peripheral plaques of vinculin, talin and FAK were quantified in immunostained aortic endothelial cells. Changes in vinculin plaques (***) were statistically significant by ANOVA (p=0.037).

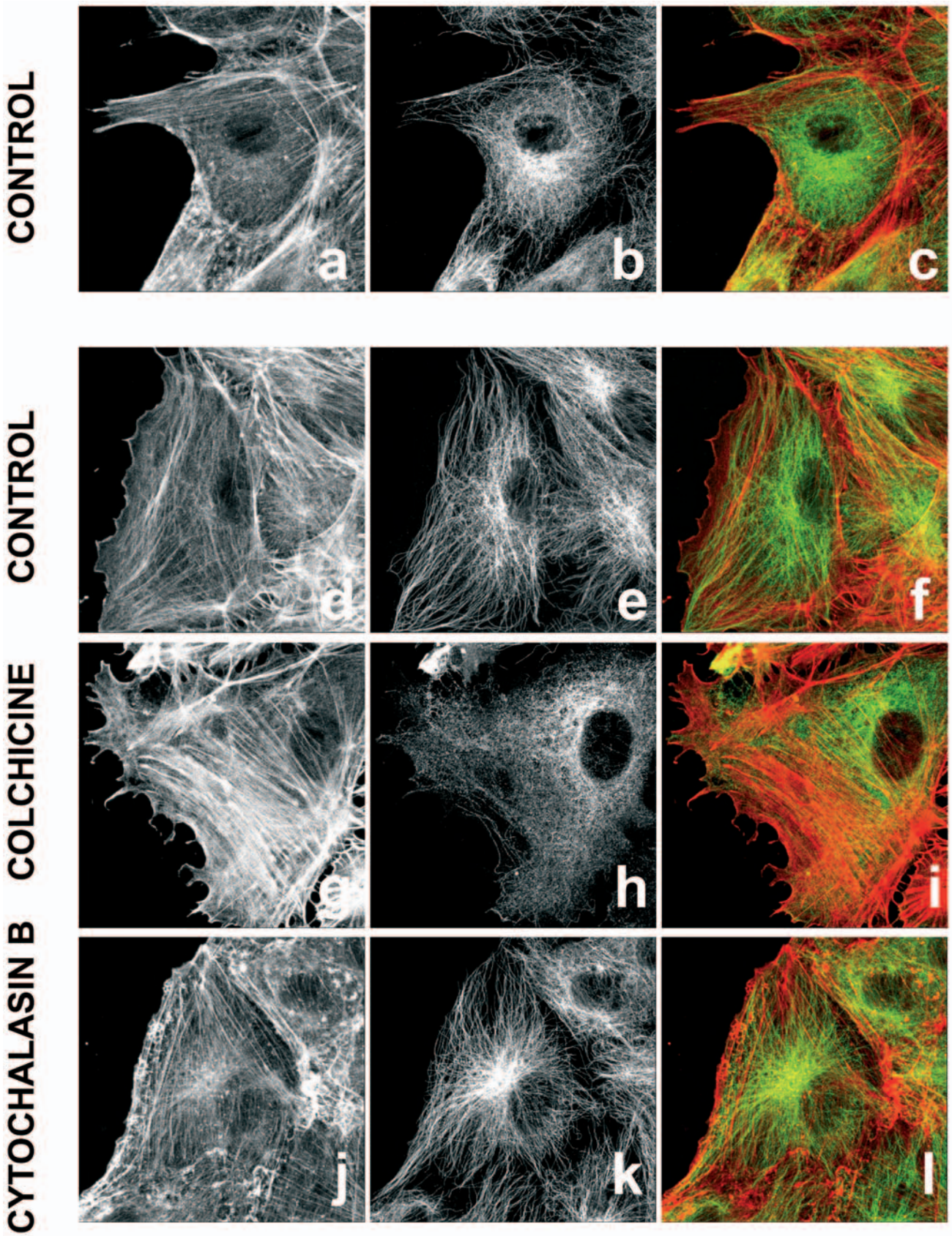

Fig. 4. Redistribution of microtubules and actin microfilaments during aortic endothelial cell migration in wound repair in the presence or absence of actin or microtubules. Aortic endothelial cells were grown to confluency, 1 mm wound created using a scraper (wound edge on the left of each panel) and double-stained for actin (**a, d, g, j, m, p, s**) and α-tubulin (**b, e, h, k, n, q, t**) at 0 **(a-c)**, 2 **(d-l)**, and 8 hours **(m-u)** after wounding [also shown merged **(c, f, i, l, o, r, u)**]. Thirty minutes after wounding, either colchicine **(g-i, p-r)** or cytochalasin B **(j-l, s-u)** was added and cells were double-stained for actin and α-tubulin. x 1200

Discussion

Our studies show that the loss of microtubules inhibits the ability of aortic endothelial cells to maintain and to re-establish an intact aortic endothelial monolayer. When aortic endothelial cells were wounded and allowed to repair, the cells elongated and migrated into the wound. We found that microtubules went through the different stages of remodeling as previously shown for actin microfilaments (Lee et al., 1996). Initially, cells spread out as microtubules re-arrange to become parallel to the wound edge. This may be an attempt by the cells to spread out as much as possible in order to cover as much of the wound as they can. Once it becomes evident that the wound cannot be repaired by spreading, the microtubules shift from parallel to perpendicular orientation and cells begin elongating and migrating into the wound. Microtubule disruption with colchicine in the in vitro wound model showed a bundling of microfilaments in the aortic endothelial cells limiting their ability to remodel. In addition, the cells became rounded and were unable to elongate. The

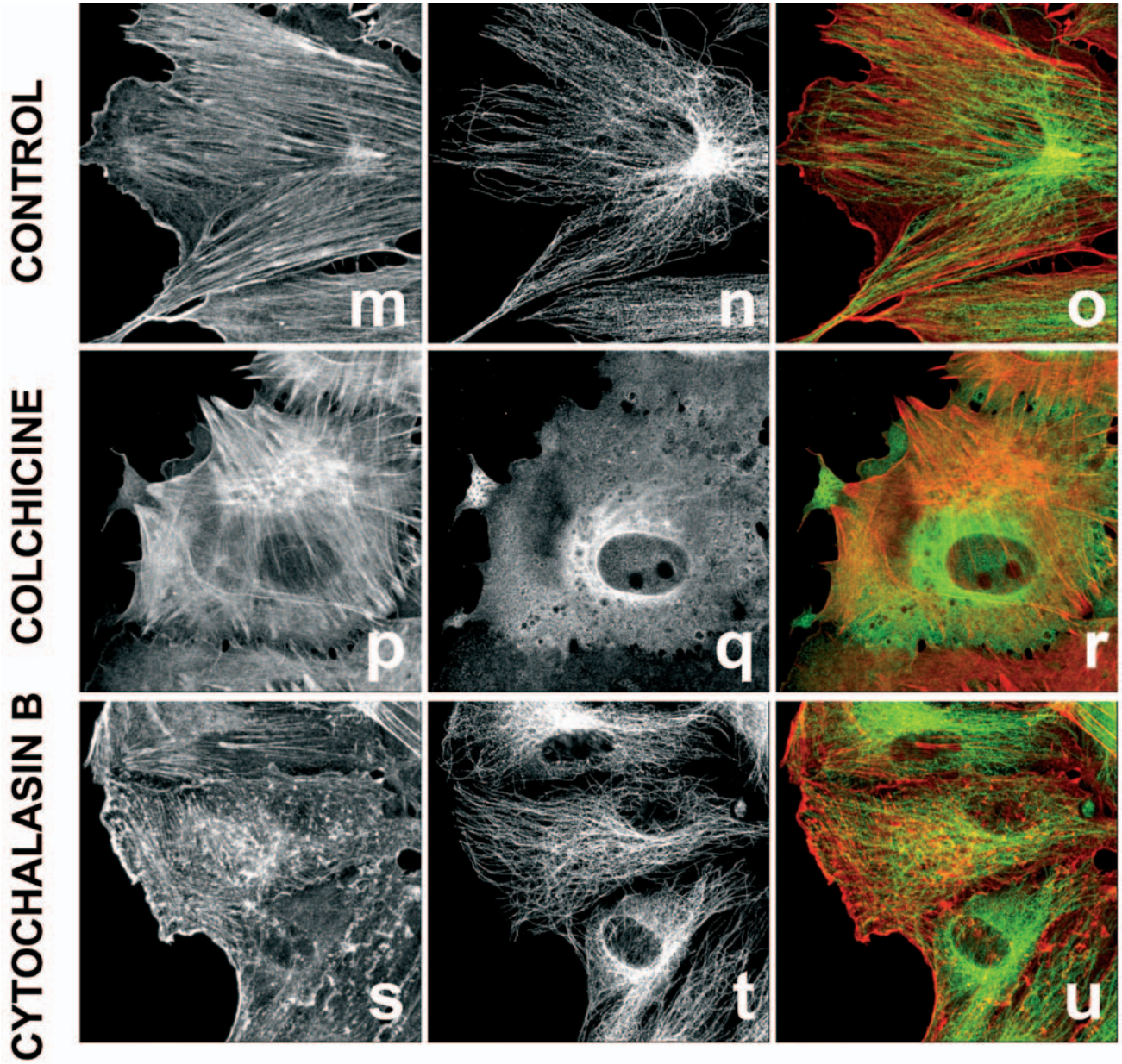

Fig. 4 (continued).

reversible increase in microfilament bundling following microtubule disruption has been shown in several cell types including human epidermal keratinocytes (Kee et al., 2002) and BalbC/3T3 cells (Enomoto, 1996).

When microfilaments were disrupted with cytochalasin B, dense peripheral bands were disrupted. Although some stress fibers were present, they were unable to undergo the three stages of remodeling (Lee et al., 1996). Microtubules however were intact and able to remodel, promoting cell elongation and migration. This suggests that the remodeling of microtubules at the wound edge is independent of microfilaments while microfilaments appear to be regulated, at least in part, by microtubules. One of the possible molecules responsible for the regulation of microfilament by microtubules may be the RhoA family of GTPases. It has been shown that disruption of microtubules leads to activation of RhoA, which in turn regulates stress fiber formation, and that polymerization of microtubules leads to an increase of Rac1, which regulates lamellipodial formation. Microtubule disruption was shown to result in an increase in stress fibers and focal adhesion assembly in serum starved BalbC/3T3 cells (Enomoto, 1996). Furthermore, when Rho was inhibited with microinjection of C3, ADP-ribosyltransferase, a specific inhibitor of rho GTPase, stress fiber and focal adhesion assembly induced by microtubule disruption were blocked (Enomoto, 1996). An increase in RhoA has also been shown in an in vitro shear model to cause an increase in stress fibers and cell contraction allowing the aortic endothelial cells to assume a non-polar morphology. This was followed by a decrease in RhoA and an increase in Rac1 and Cdc42 in the cells at the leading edge allowing cells to elongate and move efficiently in the direction of flow (Wojciak-Stothard and Ridley, 2003). Cdc42 was not required to establish shear induced polarity although they contributed to optimal migration speed (Wojciak-Stothard and Ridley, 2003).

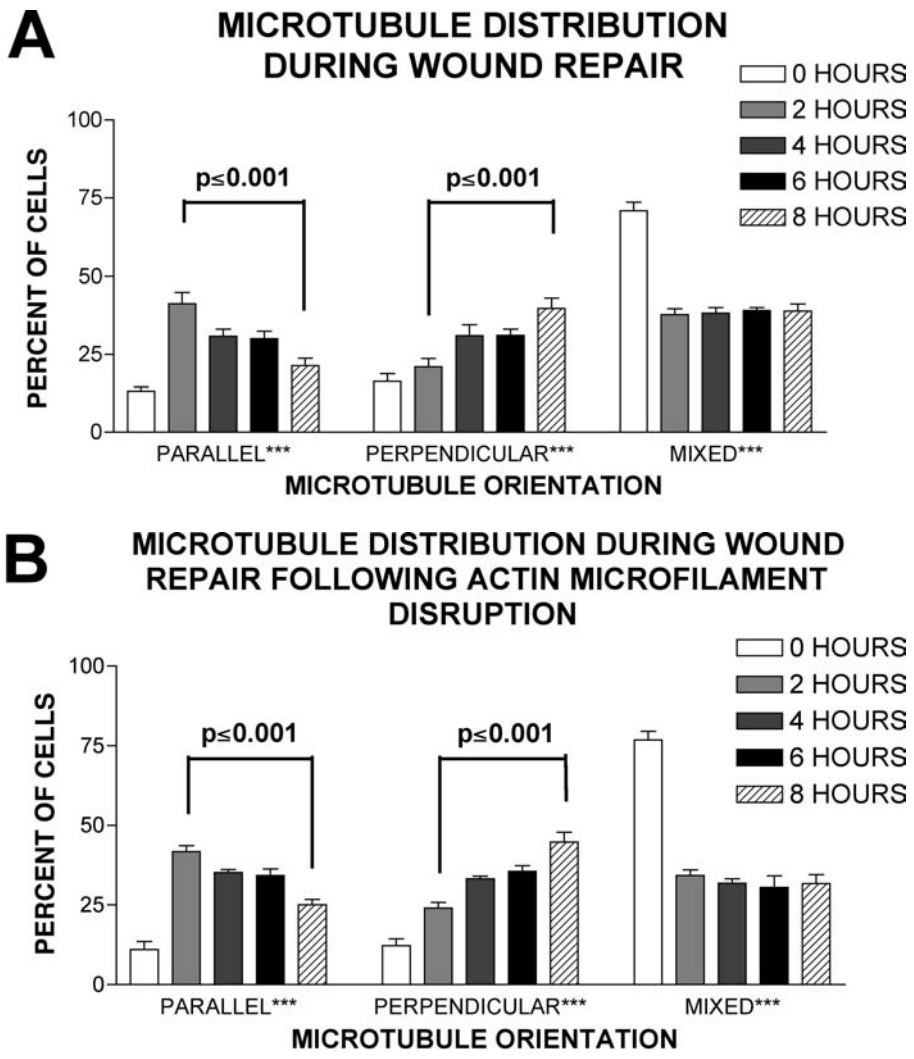

Fig. 5. Quantitation of microtubule orientation during various stages of wound repair with and without microfilament disruption. Aortic endothelial cells with 75% of microtubules in parallel orientation relative to wound edge were classified as 'parallel', those with 75% in perpendicular orientation quantified as 'perpendicular', and the rest, with mixture of both parallel and perpendicular microtubules, were classified as 'mixed'. ***: statically significant by ANOVA (≤ 0.0001).

Tzima et al also showed that Cdc42 is required for microtubule organizing center reorientation (Tzima et al., 2003). Microtubules were previously shown to be the first skeletal components to assume orientation during cell shape change and play a central role in alignment in direction of flow in an in vitro shear model (Tzima et al., 2003). This may be a mechanism by which microtubules regulate microfilament organization as well as cell elongation and migration in the in vitro wound model. Therefore, microtubule dynamics and microfilament reorganization may be linked (van Horck et al., 2001).

Our results demonstrate that microtubules may interact or align with microfilaments in aortic endothelial cells in both the resting and the repairing monolayer. This interaction may occur via linker proteins. Several linker proteins have been identified to date in non-endothelial cells including D-CLIP-190 (Lantz and Miller, 1998), microtubule interacting protein (Mip)-90 (Gonzalez et al., 1998), RHAMM/IHABP (receptor for hyaluronic acid mediated motility/ intracellular hyaluronic acid binding protein) (Assmann et al., 1999), and a mouse ACF (broadly expressed mammalian plakin) (Bernier et al., 2000; Karakesisoglou et al., 2000; Sun et al., 2000), recently renamed microtubule actin crosslinking factor (MACF) (Bernier et al., 2000). Coronin1p (Crn1p) (Goode et al., 1999), p21-activated kinase (PAK) (Cau et al., 2001), microtubule-associated protein (MAP)-2c (Cunningham et al., 1997), and mDia (mDiaphenous) (Ishizaki et al., 2001; Yarm et al., 2001; Lee et al., 2002) have also been shown to be capable of potentially mediating interaction between microtubules and microfilaments. IQGAP1, an actin binding protein and an effector of Rac1 and Cdc42, directly interacts with CLIP-170, which binds to the plus end of microtubules (Fukata et al., 2002). This IQGAP1 and CLIP-170 complex may facilitate the interaction or alignment of actin microfilaments and microtubules. More recently, an Able-related gene (Arg) was shown to be able to mediate physical contact between F-actin and microtubules at the cell periphery and that this cross-linking activity is required for Arg to regulate lamellipodial dynamics in fibroblasts (Miller et al., 2004). The possible linker proteins in endothelial cells need to be identified and characterized to further our understanding of microtubule regulation of endothelial cell migration and wound repair.

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References

Assmann V., Jenkinson D., Marshall J.F. and Hart I.R. (1999). The intracellular hyaluronan receptor RHAMM/IHABP interacts with microtubules and actin filaments. J. Cell. Sci. 112, 3943-3954.

Bernier G., Pool M., Kilcup M., Alfoldi J., de Repentigny Y. and Kothary R. (2000). Acf7 (MACF) is an actin and microtubule linker protein whose expression predominates in neural, muscle, and lung develpopment. Dev. Dyn. 219, 216-225.

Cassimeris L. (1999). Accessory protein regulation of microtubules dynamics throughout the cell cycle. Curr. Opin. Cell Biol. 11, 134-141.

Cau J., Faure S., Comps M., Delsert C. and Morin N. (2001). A novel p21-activated kinase binds the actin and microtubule networks and induces microtubule stabilization. J. Cell Biol. 155, 1029-1042.

Colangelo S., Langille B.L., Steiner G. and Gotlieb A.I. (1998). Alterations in endothelial F-actin microfilaments in rabbit aorta in hypercholesterolemia. Arterioscler. Thromb. Vasc. Biol. 18, 52-56.

Cunningham C.C., Leclerc N., Flanagan L.A., Lu M., Janmey P.A. and Kosik K.S. (1997). Microtubule-associated protein 2c reorganizes both microtubules and microfilaments into distinct cytological structures in an actin-binding protein-280-deficient melanoma cell line. J. Cell Biol. 136, 845-857.

Downing K.H. and Nogales E. (1998). Tubulin and microtubule structure. Curr. Opin. Cell Biol. 10, 16-22.

Enomoto T. (1996). Microtubule disruption induces the formation of actin stress fibers and focal adhesions in cultured cells: possible involvement of the Rho signal cascade. Cell. Struct. Funct. 21, 317-326.

Ettenson D.S. and Gotlieb A.I. (1992). Centrosomes, microtubules, and microfilaments in the reendothelialization and remodeling of double-sided *in vitro* wounds. Lab. Invest. 66, 722-733.

Ettenson D.S. and Gotlieb A.I. (1993). In vitro large-wound re-endothelialization: Inhibition of centrosome redistribution by transient inhibition of transcription after wounding prevents rapid repair. Arterioscler. Thromb. 13, 1270-1281.

Ettenson D.S. and Gotlieb A.I. (1995). Basic fibroblast growth factor is a signal for the initiation of centrosome redistribution to the front of migrating endothelial cells at the edge of an in vitro wound. Arterioscler. Thromb. Vasc. Biol. 15, 515-521.

Fukata M., Watanabe T., Noritake J., Nakagawa M., Yamaga M., Kuroda S., Matsuura Y., Iwamatsu A., Perez F. and Kaibuchi K. (2002). Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. Cell 109, 873-885.

Gonzalez M., Cambiazo V. and Maccioni R.B. (1998). The interaction of Mip-90 with microtubules and actin filaments in human fibroblasts. Exp. Cell Res. 239, 243-253.

Goode B.L., Wong J.J., Butty A.C., Peter M., McCormack A.L., Yates J.R., Drubin D.G. and Barnes G. (1999). Coronin promotes the rapid assembly and cross-linking of actin filaments and may link the actin and microtubule cytoskeletons in yeast. J. Cell Biol. 144, 83-98.

Ishizaki T., Morishima Y., Okamoto M., Furuyashiki T., Kato T. and Narumiya S. (2001). Coordination of microtubules and the actin cytoskeleton by the Rho effector mDia1. Nat. Cell Biol. 3, 8-14.

Karakesisoglou I., Yang Y. and Fuchs E. (2000). An epidermal plakin that integrates actin and microtubule networks at cellular junctions. J. Cell Biol. 149, 195-208.

Kee S.H., Jang S.I., Ahvazi B., Larsen M., Yamada K.M. and Steinert P.M. (2002). Cell-cell adhesion and RhoA-mediated actin polymerization are independent phenomena in microtubule disrupted keratinocytes. J. Invest. Dermatol. 119, 440-448.

Lantz V.A. and Miller K.G. (1998). A class VI unconventional myosin is associated with a homologue of a microtubule-binding protein, Cytoplasmic Linker Protein-170, in neurons and at the posterior pole

of Drosophila embryos. J. Cell Biol. 140, 897-910.

Lee T-Y.J. and Gotlieb A.I. (1999). Early stages of endothelial wound repair: conversion of quiescent to migrating endothelial cells involves tyrosine phosphorylation and actin microfilament reorganization. Cell. Tissue Res. 297, 435-450.

Lee J.S.Y. and Gotlieb A.I. (2002). Microtubule-actin interactions may regulate endothelial integrity and repair. Cardiovasc. Pathol. 11, 135-140.

Lee T-Y.J., Rosenthal A. and Gotlieb A.I. (1996). Transition of aortic endothelial cells from resting to migrating cells is associated with three sequential patterns of microfilament organization. J. Vasc. Res. 3, 13-24.

Miller A.L., Wang Y., Mooseker M.S. and Koleske A.J. (2004). The Abl-related gene (Arg) requires its F-actin-microtubule cross-linking activity to regulate lamellipodial dynamics during fibroblast adhesion. J. Cell Biol. 165, 407-419.

Pous C., Chabin K., Drechou A., Barbot L., Phung-Koskas T., Settegrana C., Bourguet-Kondracki M.L., Maurice M., Cassio D., Guyot M. and Durand G. (1998). Functional specialization of stable and dynamic microtubules in protein traffic in WIF-B cells. J. Cell Biol. 142, 153-165.

Small J.V., Kaverina I., Krylyshkina O. and Rottner K. (1999). Cytoskeleton cross-talk during cell motility. FEBS Lett. 452, 96-99.

Sun D., Leung C.L. and Liem R.K.H. (2000). Characterization of the microtubule binding domain of microtubule actin crosslinking factor (MACF): identification of a novel group of microtubule associated proteins. J. Cell Sci. 114, 161-172.

Tzima E., Kiosses W.B., del Pozo M.A. and Schwartz M.A. (2003). Localized Cdc42 activation, detected using a novel assay, mediates microtubule organizing center positioning in endothelial cells in response to fluid shear stress. J. Biol. Chem. 278, 31020-31023.

van Horck F.P.G., Ahmadian M.R., Haeusler L.C., Moolenaar W.H. and Kranenburg O. (2001). Characterization of p190RhoGEF, a RhoA-specific guanine nucleotide exchange factor that interacts with microtubules. J. Biol. Chem 276, 4948-4956.

Waterman-Storer C.M. (1998). Microtubules and microscopes: How the development of light microscopic imaging technologies has contributed to discoveries about microtubule dynamics in living cells. Mol. Biol. Cell. 9, 3263-3271.

Wojciak-Stothard B. and Ridley A.J. (2003). Shear stress-induced endothelial cell polarization is mediated by Rho and Rac but not Cdc42 or PI 3-kinases. J. Cell Biol. 161, 429-439.

Wong M.K.K. and Gotlieb A.I. (1984). *In vitro* reendothelialization of a single-cell wound: Role of microfilament bundles in rapid lamellipodia-mediated wound closure. Lab. Invest. 51, 75-81.

Wong M.K.K. and Gotlieb A.I. (1986). Endothelial cell monolayer integrity: Characterization of dense peripheral band of microfilaments. Arteriosclerosis 6, 212-219.

Wong M.K.K. and Gotlieb A.I. (1988). The reorganization of microfilaments, centrosomes, and microtubules during in vitro small wounds reendothelialization. J. Cell Biol. 107, 1777-1783.

Yarm F., Sagot I. and Pellman D. (2001). The social life of actin and microtubules: interaction versus cooperation. Curr. Opin. Microbiol. 4, 696-702.

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