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REVIEW

Alteration of vascular permeability in burn injury

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Massive burn trauma is characterized by hypovolemic shock induced by the loss of plasma from vessels. The elevation of vascular permeability and the ultimate formation of tissue edema are important events during the development of severe burn injury. The underlying mechanisms involved in the increased permeability include the activation of multiple endothelial signaling pathways and the changes of endothelial structure and functions. This review summarizes some of our recent discoveries in endothelial mechanisms during burn-induced vascular hyper-permeability. The emphasis is put on tight junction, adherens junction, and the contraction of endothelial cells. The effects of several protein kinases, including Rho kinase, protein kinase C, and MAPKs are also stressed.

KEYWORDS: Microcirculation; Permeability; Burn.

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INTRODUCTION

Increase of vascular permeability is the most important pathological event in the pathogenesis of burn injury. Those with burns greater than 25% of total body surface area (TBSA) are at risk of circulatory complications. Massive leakage of fluid from vascular space leads to loss of blood plasma and to a decrease in effective circulatory blood volume, resulting in the formation of severe tissue edema, hypotension or even shock in severe burn injury patients.[1,2] Due to the lack of overall and profound understanding of the mechanisms of burn-induced vascular hyper-permeability response, fluid resuscitation has been the only valid method to sustain a burn patient's blood pressure and peripheral circulation.

The burn-induced hyper-permeability response happens not only in the location of the burn insult but also in distal organs and tissues, and is attributed to the release and circulation of various permeability-increasing cytokines and inflammatory mediators, such as thrombin, bradykinin, histamine, serotonin, radical oxygen species, VEGF, IL-1β, IL-6, TNF-α and LPS, etc.[1,3-5] This mediator-induced endothelial barrier dysfunction is the major reason for high vascular permeability following a burn.

The notion of vascular permeability includes two different aspects: one is the filtration of water and hydrophilic substances through intact capillaries and microvessels under normal physiological condition; the other is the massive leakage of macro-molecules and fluid from venules under acute and chronic inflammatory situations.[6]

The endothelium controls the flux of fluid and solutes across the vessel wall, and it is highly regulated by different transport pathways, including transcellular and paracellular (or intercellular) pathways. While many researchers emphasized the importance of the pathway in which they were most interested, a generally accepted belief is that the transport of protein and liquid in quiescent endothelium occurs via the transcellular pathways, i.e. through the movements of caveolae in capillary endothelial cells and vesiculo-vacuolar organelles (VVOs) in the endothelium of venules and small veins. The capillaries fulfill the ultimate physiological exchanging function of the circulation system, whereas post-capillary venules, characterized by their high sensitivity to inflammatory mediators, play a more important role in the alteration of vascular permeability during inflammatory processes. Under inflammatory conditions the intrinsic and extrinsic stimulating mediators would force the endothelium to open up the paracellular gap by additional signaling regulation that allows transport of solutes through inter-endothelial junctions (IEJs).[5] The endothelial barrier dysfunction is accompanied by cellular morphological alteration, intercellular gap formation, and trans-endothelial permeability augmentation.[7] The underlying mechanisms involved in endothelial barrier dysfunction include the activation of multiple endothelial signaling pathways and alterations of endothelial structures and functions.

The agonist-induced hyper-permeability is usually reversible.[8] The process of recovery of barrier function could emerge with the re-annealing of previously open inter-endothelial junctions and the strengthening of adhesion of endothelial cells to the extracellular matrix, which result from the re-equilibrium of competing contractile and adhesive forces generated by the cytoskeletal proteins and

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the adhesive molecules. Some stabilizing mediators play positive roles in enhancing inter-endothelial junctional connections and preventing the increase of vascular permeability.[5,9]

As early as 1966, our lab reported that vascular permeability increased in skin and muscle injury, as well as in the injury of distant organs, such as the liver, spleen and kidney, even at very early stages of severe burn. This increase of permeability could be prevented by pretreatment with cortisone.[10] We then proceeded to explore the pathogenesis of endothelial barrier dysfunction in burn shock and other circumstances of inflammation.

This review will mainly discuss the alteration in the organization of inter-endothelial junctions, as well as the motility of actomyosin contractile force during inflammation and burn shock. It will also cover some of the important signal pathways involved in those morphological and functional modifications. Emphasis is put on tight junction, adherens junction, and contractile forces of endothelial cells. The effects of several signaling protein kinases, including Rho kinase (ROCK), mitogen activated protein kinases (MAPKs), and protein kinase C (PKC) are stressed. We shall also discuss the contributions of some stabilizing mediators of endothelial barrier function, such as sphingosine 1-phosphate (S1P).

THE CHARACTERISTICS OF VASCULAR PERMEABILITY ALTERATION AFTER BURN INJURY

Clinically, massive tissue edema usually occurs between 4-12 hours after burn or thermal insult. But *in vivo* and *in vitro* studies demonstrated that vascular permeability increased much earlier than the onset of obvious tissue edema. The early increased permeability in the thermally affected area might result from the direct effect of heating and the subsequent protein denaturation. The immediate release of pro-inflammatory mediators from injured cells, as wells as from activated neutrophils, which rapidly accumulate in the injured dermis, will affect the vascular barrier function in distal organs. In a tissue level experiment, venules were isolated from the thermal area immediately after thermal injury in dorsal skin of rats, and then perfused through cannulation. The venular permeability was measured with a fluorescence ratio technique. The result revealed a remarkable elevation in the permeability coefficient of albumin (Pa) compared with venules from control rats.[11] The *in vivo* detection of **mesenteric** venular permeability showed that the vascular permeability in this distant non-burnt tissue was also increased 15 min after dorsal thermal injury as shown in Fig. 1.[12]

When normal mesentery venules were isolated and perfused with burned plasma obtained from thermally injured rats 3-6 h after burn, the venular permeability coefficient of albumin increased 10 min after the burned plasma perfusion and was sustained for about 6 h, as shown in Fig. 2.[12] This late effect of burned plasma in vascular permeability resulted from the second phase synthesis and release of cytokines from more intensely activated inflammatory cells. The inflammatory mediators and cytokines not only disrupt the endothelial barrier and increase the outflow of macromolecules and fluid from vessels in local injured area, but also affect the vascular permeability in distant non-burnt tissues and organs through blood circulation. These factors are the major reasons for the massive tissue edema in severely burnt patients. By binding to their specific receptors, those cytokines or mediators target endothelial cells and result in morphological and functional alterations in endothelial barrier function. Therefore, it is important to elucidate the mechanisms controlling this barrier function.

THE DISRUPTION OF INTER-ENDOTHELIAL JUNCTIONAL STRUCTURES IN BURN-INDUCED VASCULAR HYPER-PERMEABILITY

The endothelium acts as a permeability barrier and an active interface between blood and the underlying tissues. The integrity of ECs helps to maintain the thrombo resistance and selective permeability to cells and proteins. Normally, endothelial cells are tightly connected through various proteins that regulate the organization of intercellular junctional complex. The junctional structures then bind to cytoskeletal proteins or cytoplasmic interaction partners that allow the transfer of intracellular signals and govern the barrier function of ECs under normal or inflammatory conditions (Fig. 3). Without exception, burn injury will damage inter-endothelial junctional structures and lead to the leakage of macromolecules and fluid from the vessels.

Tight junctions

The main structures responsible for the endothelial barrier properties are tight junctions (TJs). TJs act as a primary barrier to the diffusion of solutes through the intercellular space, and they create a boundary between the apical and the basolateral plasma membrane domains. TJs are molecularly composed of integral membrane proteins and cytoplasmic proteins. TJ's integral membrane proteins include junctional adhesion molecules (JAM1, 2), occludin, claudin-1, 2, and 5. They polymerize linearly within lipid bilayers between two corresponding endothelial membranes of adjacent cells, and then associate with cytoplasmic zonula occludins-1, 2, and 3 (ZO-1, ZO-2, ZO-3) and cingulin. By recruiting various cytoskeletal as well as signaling molecules at their cytoplasmic surface, ZOs and cingulin provide a direct link between TJ strands and the cytoskeleton, especially actin filaments, and play an essential role in developing and stabilizing TJs.

By using an immunofluorescence technique, the assembling of ZO-1 was displayed to form a smooth line alongside the EC border both in cultured HUVECs and in intact vessel, showing a tight connection between adjacent cell membranes.[13] When cells were stimulated with plasma from burn-shocked rat, ZO-1 deviated from the junctional area and internalized into the cytoplasm or serrated the edge, accompanied by the intercellular gap formation on cultured HUVECs (Fig. 4A).[13] The monolayer permeability of cultured HUVECs revealed a concomitant increase with the opening of the inter-endothelial junction (Fig. 4B).[13] Similar damage was seen in mesentery microvessels from dorsal burn injured rats, as seen in Fig. 5.[13] These data not only suggest that burned plasma affects the endothelial intercellular structures in distant tissue and organ, but also reveal the important role of TJs in maintaining the integrity of the endothelial barrier during burn injury.

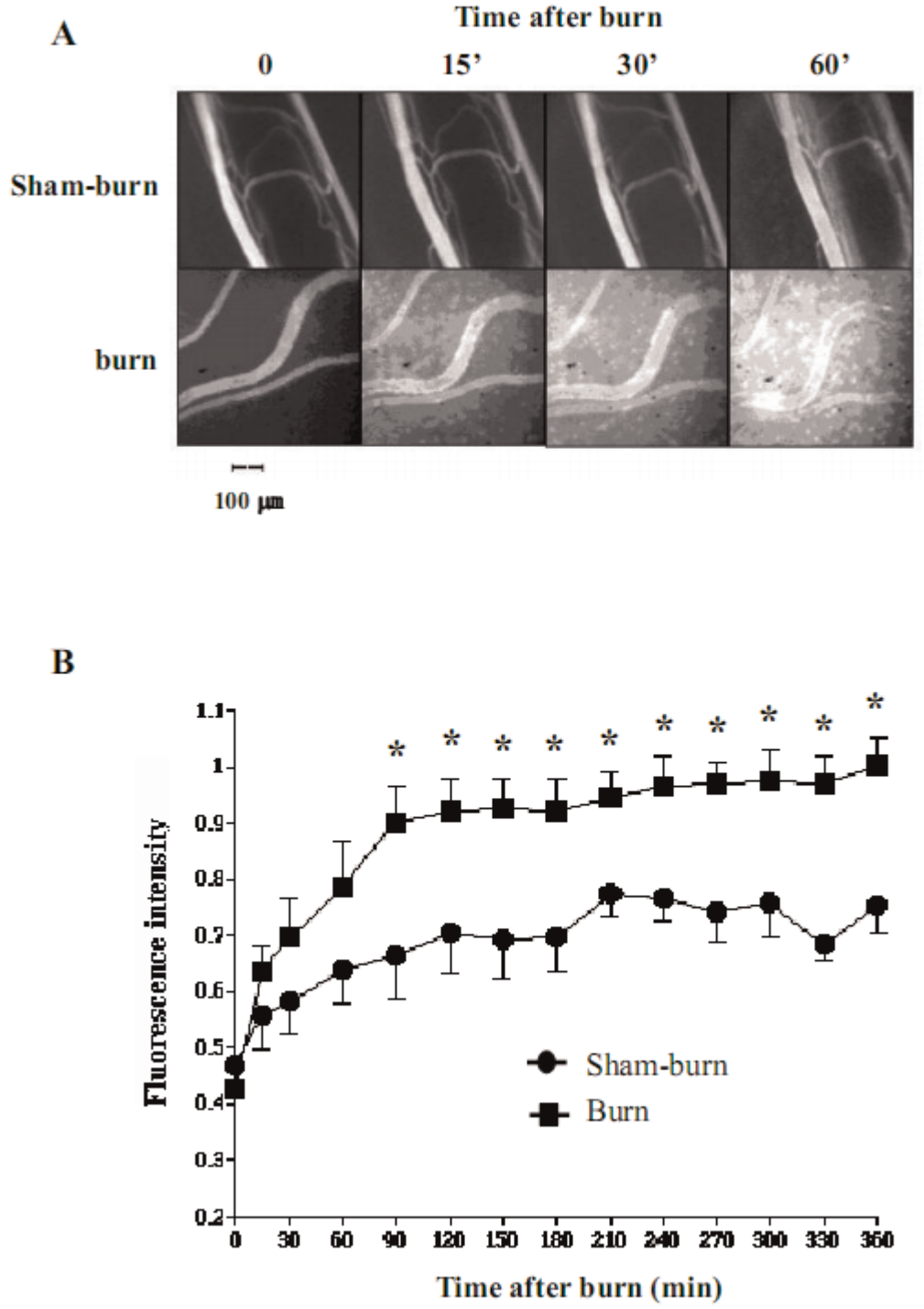

Figure 1 - Changes of vascular permeability in non-burn distant tissues after dorsal thermal injury. A: Fluorochrome leakage was observed in in vivo mesenteric venules as early as 15 min postburn. B: The sustain increase of mesenteric venular permeability after burn.

Adherens Junctions

Adherens junctions between endothelial cells are centered by transmembrane molecule vascular endothelial-cadherins (VE-cadherins), which form intercellular homodimers in the presence of Ca^{2+} and play a pivotal role in endothelium integrity and in the control of vascular permeability.[14] The ion Ca^{2+} here not only facilitates the formation of adherens junctions but also protects multicellular configurations by preventing the cadherins from hydrolysis. The cytoplasmic carboxyl portion of VE-cadherin is connected with α-catenin/β-catenin or plakoglobin/γ-catenin complexes and is then directly linked to actin, leading to strong cell–cell interaction as shown in Fig. 3.[15] Other catenins, p120 and the p120-related

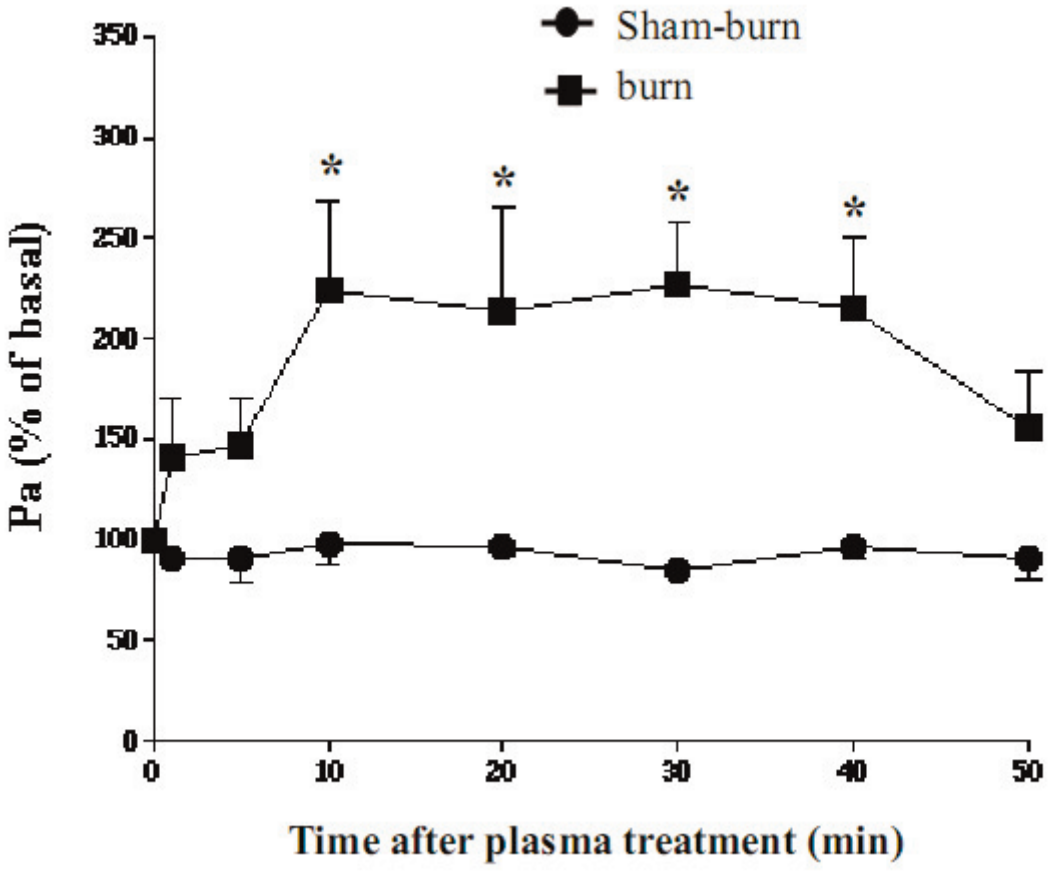

Figure 2 - Effects of burned rat plasma on the permeability of mesenteric venules.

protein p0071, bind to an identical juxtamembrane intracellular domain of VE-cadherin and exert various functions, such as the regulation of cadherin levels by controlling cadherin internalization and degradation, as well as cadherin clustering at the junction.[15]

The disruption or disassembly of VE-cadherin based adherens junction is involved in the response to pro-inflammatory mediators, and contributes to the increase in endothelial permeability. Our study also showed that the intervention of cultured EC with burn serum could cause a noticeable alteration of VE-cadherin spreading at the cellular border, displaying a blurred distribution in the location of per se sharp lining without burn serum stimulation. This change was accompanied with increased permeability in the cultured endothelial monolayer, as illustrated in Fig. 6.[16]

ACTOMYOSIN CONTRACTION IN BURN-MODULATED VASCULAR PERMEABILITY

Filamentous actin (F-actin) and myosin are, respectively, the track and motor components that comprise one of the major systems for molecular movement in the cell.[17] Actin may also be more than a simple structural component of the junctions. In fact, there are ample ultra-structural data that implicate the temporal expression, dynamic organization, and spatial distribution of the actin cytoskeleton in altering TJ and AJ complexes under various conditions.[18] Therefore, actin is likely to play a critical role in modulating the integrity of the endothelial barrier function. Under normal condition, F-actin forms a prominent peripheral actin rim (PAR) at the outer area of endothelial cells and apparently delineates the cell-to-cell borders.[11] The phosphorylation of a myosin light chain by myosin light chain kinase (MLCK) triggers the formation of the actomyosin complex and produces the contractile force in the cells that is symbolized by polymerization of actin and the emergence of stress fibers.[17,19]

The actin cytoskeleton of non-muscle cells responds to extracellular stimuli through a spatially and temporally regulated series of polymerization and depolymerization reactions. Our study[20] reveals that cells stimulated with rat burned plasma showed obvious stress fiber formation with a time-dependent enhancement, along with the disappearance of PAR structures at the cellular border. We previously reported that the inhibition of MLC phosphorylation with an MLCK inhibitor, ML-7, modulated the venular basal barrier function and significantly attenuated the increase in vascular permeability in response to permeability enhancers, such as PMA or neutrophils.[20]

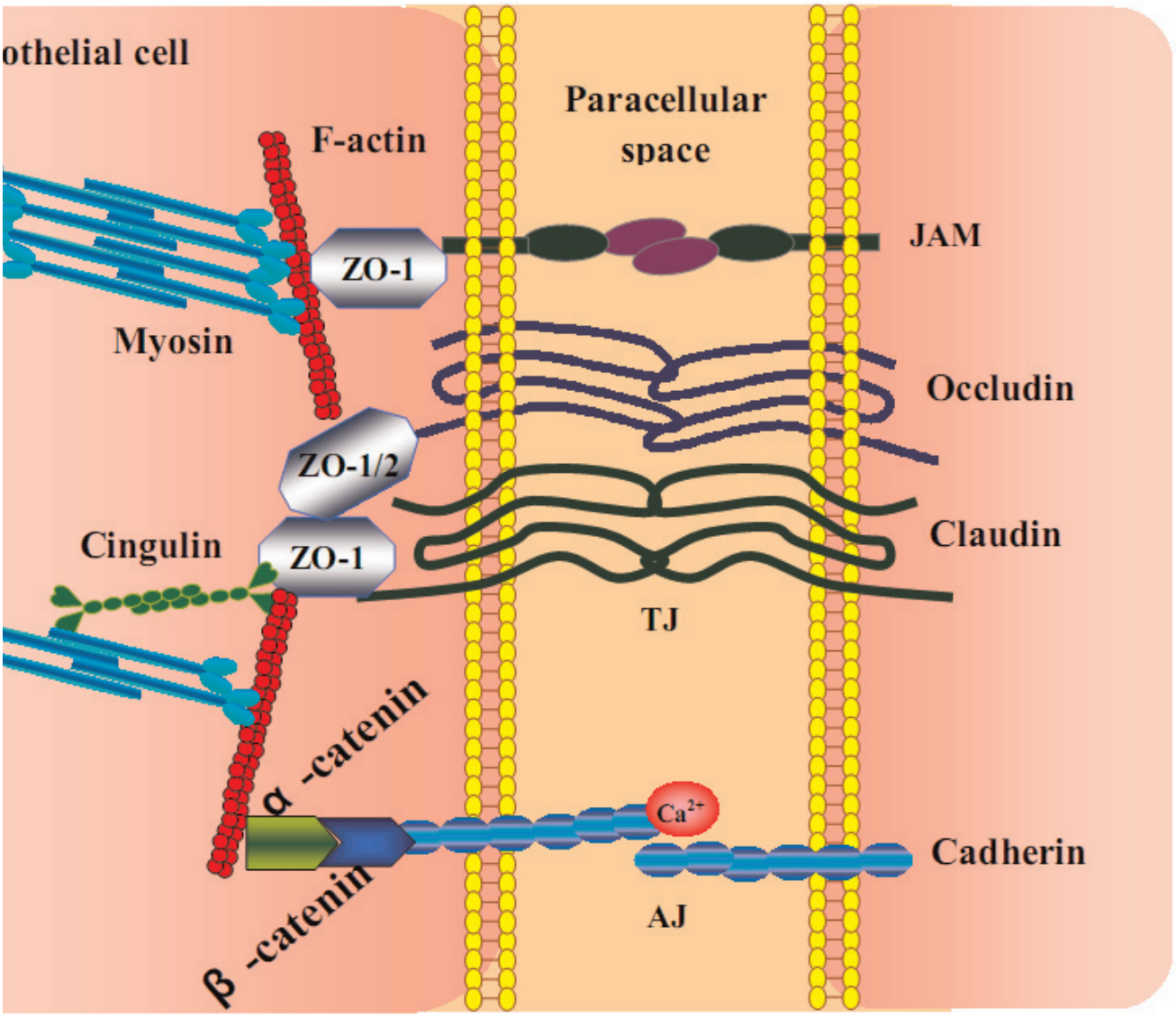

Figure 3 - Simplified schematic image of tight junction (TJ) and adherens junction (AJ).

Another study showed that the inhibition of MLCK attenuated the burn-evoked hyper-permeability in micro-vasculature, providing evidence that endothelial cell contraction induced by MLCK-mediated MLC phosphorylation is also involved in thermal injury-induced alteration of the endothelial barrier function.[13] This effect was confirmed in *in vitro* studies by pre-treating cultured HUVECs with ML-7 that prevented the formation of cellular stress fiber after burned plasma exposure. Furthermore, ML-7 could also reverse the actin reorganization in endothelial cells pretreated with burned plasma.[21] When F-actin and related proteins are double-stained with specific fluorescence probes and relative antibodies, the above mentioned junctional molecular mal-distribution is always concomitant with F-actin reorganization and stress fiber formation (Fig. 4A, Fig. 6),[13,16] indicating the intimate interaction of F-actin and junctional proteins.

Having lipopolysaccharide (LPS) as the major mediator, burn-induced gut dysfunction plays an important role in the development of sepsis and multiple organ dysfunction.[22] The translocation of LPS from intestines to the blood stream triggers a hyperpermeability response in endothelial cells as well. Our study showed that at a concentration of 400-500 μg/L, LPS induced obvious disorganization of VE-cadherin in cultured primary human umbilical vein endothelial cells (HUVECs) with formation of remarkable serrata along cellular border and enlargement of intercellular gaps, which was apparently different from the smooth lining of immunofluorescence staining of VE-cadherin in adjacent HUVECs under quiescent state.[23] LPS stimulation also causes the formation of stress fibers in cultured endothelial cells. LPS in high concentrations caused the appearance of broken F-actin dots, indicating the disruptions of F-actin.[24] This result is consistent with Chakravortty's report that described the assembly and excessive polymerization of actin filaments and, finally, their disruption after a high concentration LPS treatment.[25]

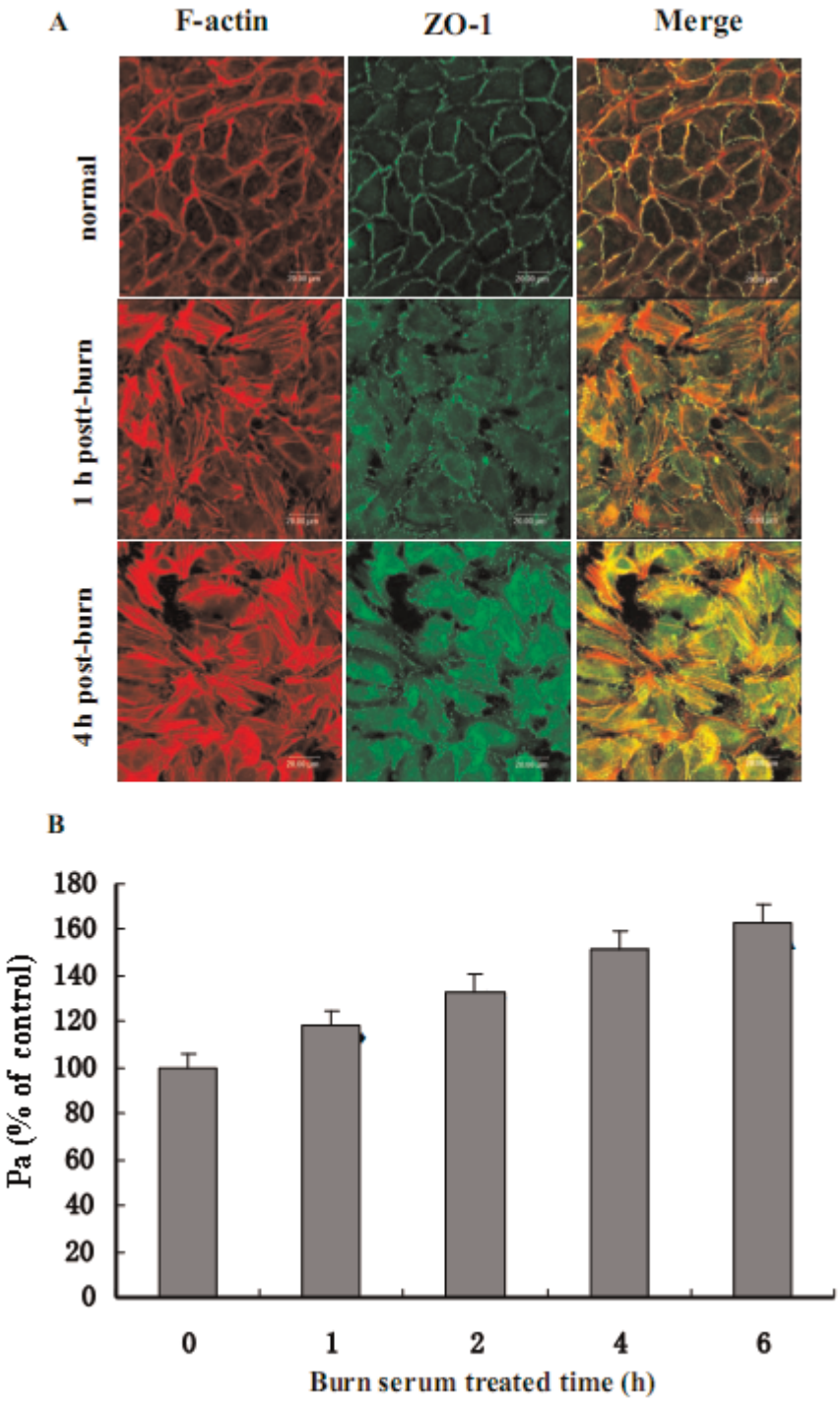

Figure 4 - Effects of burned plasma on morphological and functional changes in cultured HUVECs.

THE SIGNALING OF VASCULAR PERMEABILITY REGULATION

The tethering power produced by junctional structures and the contractile strength generated by actinomyosin cross-bridging are two major opposite forces contributing to the maintenance of endothelial barrier integrity. The imbalance of these forces evokes the increase of vascular permeability in inflammatory situations. Different intracellular signaling processes have been proposed, and considerable evidence suggests that endothelial cytosolic calcium and various protein kinases, including ROCK, MAPKs and PKC are involved in the regulation of endothelial barrier function.

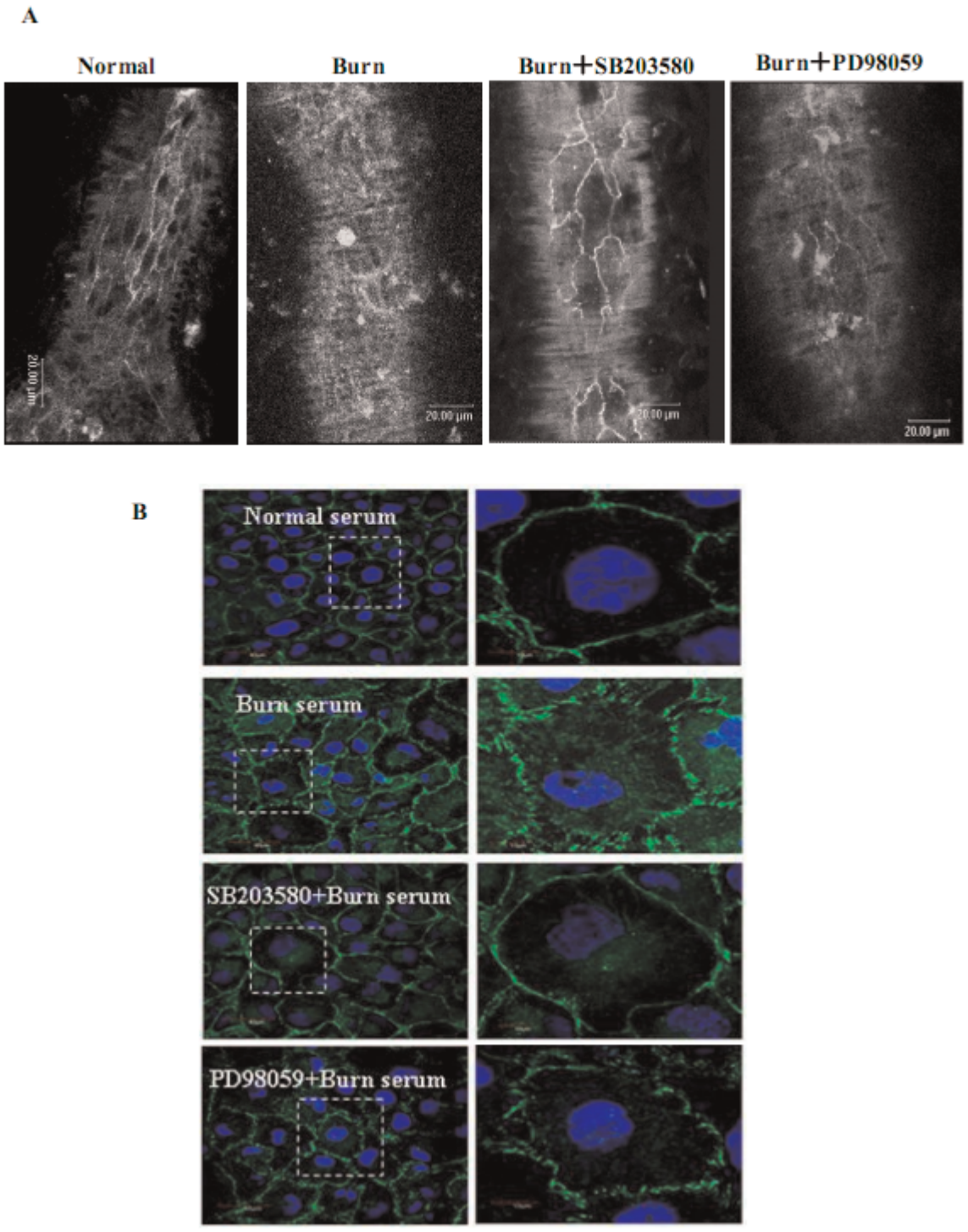

Figure 5 - Burn injury induced the ZO-1 distribution in mesenteric venular endothelia and cultured HUVECs and inhibition of p38 MAPK preserved the ZO-1 organization.

Effects of RhoA/ROCK in vascular hyper-permeability response to burn injury

The Rho family of small GTPases, with major components of RhoA, Cdc42, and Rac, has been shown to play a key role in the control of the assembly of the actin-based cytoskeleton and in regulation of cadherin-based intercellular junctions. It has been demonstrated that RhoA proteins regulate the formation of stress fibers and focal adhesions; Rac proteins manipulate the formation of lamellipodia and membrane ruffles; and Cdc42 proteins adjust the formation of filopodia.[26] It has been suggested that the activation of RhoA may increase endothelial permeability, while Rac

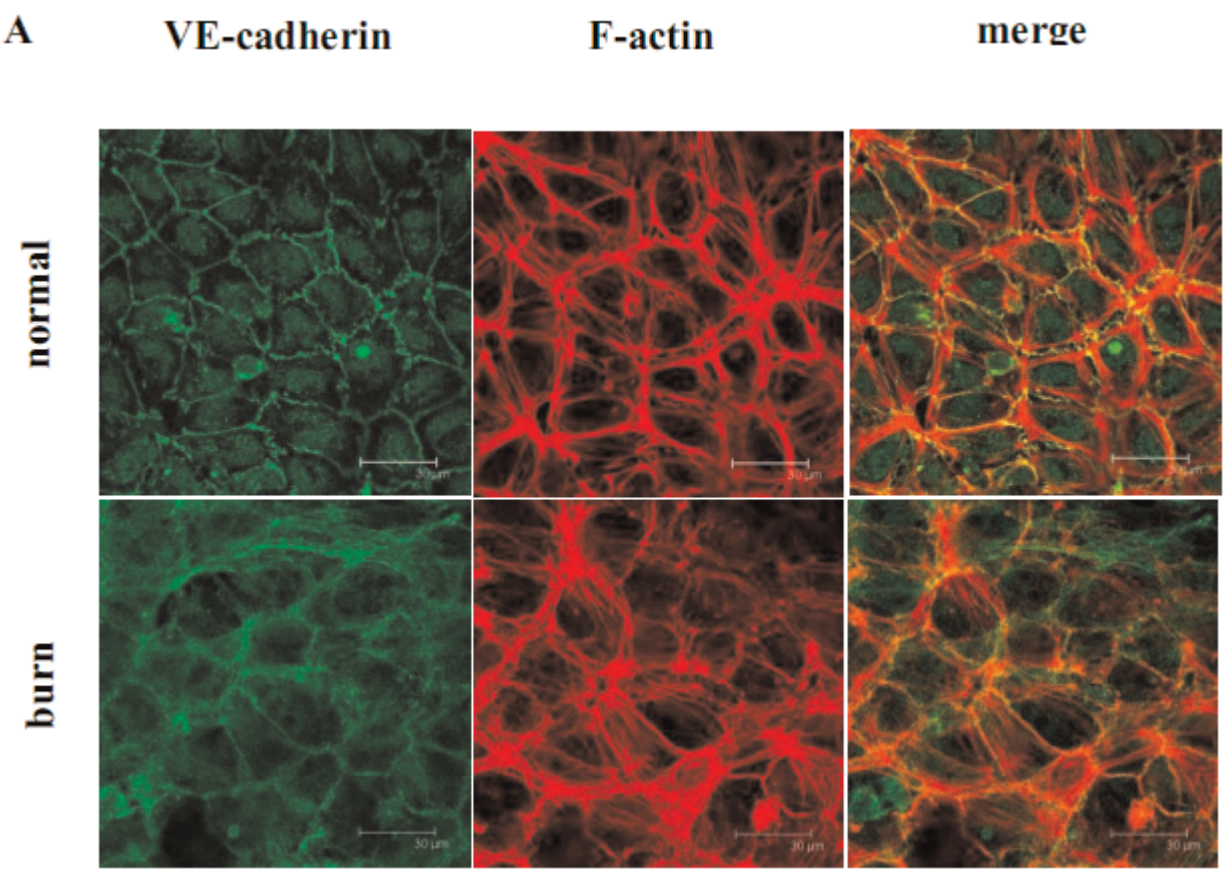

Figure 6 - The distribution of VE-cadherin in cultured endothelial cells before and after burn injury.

function is required to maintain integrity and normal barrier function,[27] and the activation of Cdc42 paralleled with the time-course of endothelial barrier recovery.[28]

RhoA, through its downstream effecter Rho kinase (ROCK), stimulates the phosphorylation of myosin light chain phosphatase (MLCP or PP1) regulatory subunit, which attenuates the phosphatase activity, hence resulting in a net increase in phosphorylated MLC and sustainment of endothelial cell contractile response. Our study has approved that isolating and cannulating scalding skin venules increased the permeability value to about threefold, compared to normal skin venules in 120 min.[11] The peripheral actin rim in burned group vascular endothelial cells showed less organized and remarkable interruption with a large amount of fluorescein isothiocyanate (FITC)-albumin leakage.[12,13] In cultured HUVECs, F-actin filaments were primarily displayed in the cortex of normal cells. The exposure to burned plasma caused a rapid assembly of prominent stress fibers in cultured cells, which could also be partially inhibited by Y-27632, as shown in Fig. 7A.[21] Inhibition of ROCK activity with Y-27632 dose-dependently attenuated the hyper-permeability responses to scalding and induced recovery of actin filament arrangement in venule wall after scalding (Fig. 7B).[11] These results indicate that burn injury leads to an increase of dermal venular permeability with endothelial cytoskeleton depolymerization and disruption. The RhoA/ROCK signal transduction pathway is involved in these responses.

Involvement of MAPK in modulation of vascular permeability

MAPKs are a major signaling system that transduce a variety of extracellular signals through a cascade of intracellular protein phosphorylation and play important roles in regulation of cell growth, differentiation, apoptosis, and cellular response to environmental stress. In mammals, four major subgroups of MAPK super-family members have been identified: the extracellular signal-regulated kinase (ERK), the c-Jun N-terminal kinase (JNK), p38 MAPK, and ERK 5.[29-31]

MAPKs have been noted to exert some regulating effects on contraction of different smooth muscle cells.[32,33] We found that three major MAPKs, i.e. p38, ERK and JNK, were all activated in thermally stressed EC, but only pharmaceutical inhibition with SB203580 for p38 and/or PD98059 for ERK MAPKs could abolish burned plasma-induced EC stress-fiber formation. To distinguish whether p38 and ERK are equally important in this respect, we then visualized a paracellular tight junction protein, ZO-1, and found that p38 MAPK inhibitor worked more significantly in preventing venular (Fig. 5A) and cultured (Fig. 5B) EC junctional damages.[34,35] Exposure of EC to constructs with dominant negative isoforms of p38 MAPKs showed that the suppression of both p38α or p38δ activation could prevent the F-actin disorganization upon burned plasma stimulation in cultured ECs, though the preventive effect of the dominant negative p38δ was greater than p38α. Using

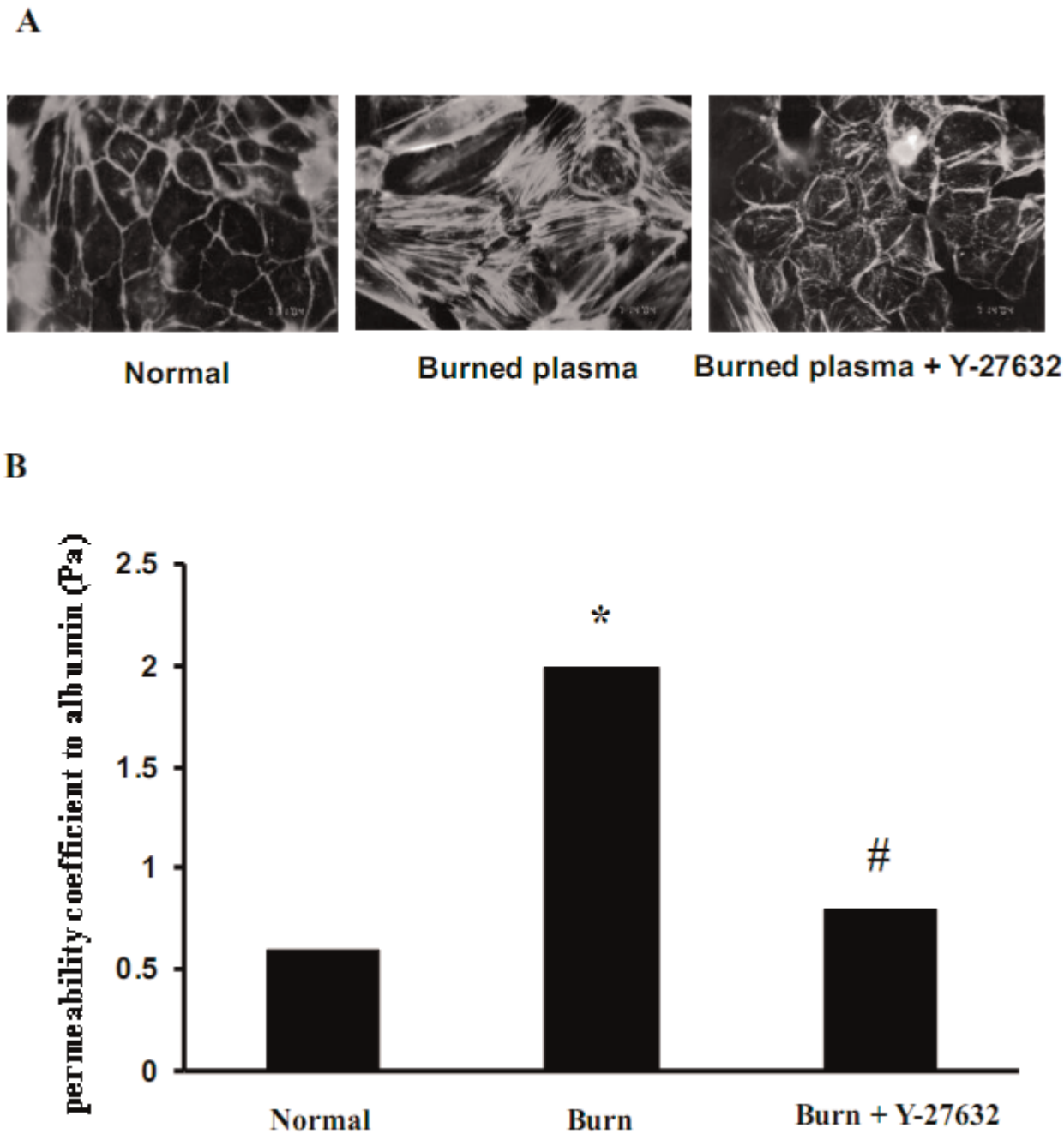

Figure 7 - ROCK inhibitor Y-27632 abolished burned plasma-induced formation of stress fiber in cultured ECs (A) and increase of vascular permeability in thermal skin (B).

adenovirus-based constructs containing interfering kinases to block p38α and δ kinases, or their upstream effecter kinases (MKK3 and MMK6), the venular hyperpermeability that resulted from exposure of isolated vessels to burned plasma was impeded. The depression of these kinases also significantly enhanced the survival of burned rats during the first critical 72 h.[34]

Some studies indicate that p38 might regulate actin re-arrangement independent of MLCK phosphorylation.[33] Borbiev et al reported that p38 MAPK inhibitor SB203580 decreased thrombin induced EC actin stress-fiber formation, while dominant negative p38 had no effect on thrombin-induced myosin light chain diphosphorylation.[36] But thrombin-induced total and site-specific caldesmon phosphorylation (Ser789), as well as dissociation of the caldesmon-myosin complex, was found to be attenuated by SB203580 pretreatment. These results suggest the involvement of p38 MAPKs activities in caldesmon phosphorylation and an MLCK-independent regulation of thrombin-induced EC permeability. These results called for further investigation of downstream substrates of p38α and δ kinases, especially those of p38δ, which is not well

studied. Currently, several p38 MAPK-regulated kinases have been identified as downstream substrates of p38, such as MSK, PRAK, and MAPKAPK-2 (MK2), MAPKAPK-32. PRAK and MAPKAPK-2 have also been reported as kinases of HSP27. The activation of HSP27 might be another mechanism (rather than phosphorylation of MLC) that regulates actin polymerization in endothelial cells and thus their barrier function.

A morphological study from our lab showed that the suppression of MK2 activity by transfecting the cells with the MK2 dominant negative form alleviated the formation of stress fiber induced by burned plasma, while constitutive activation of MK2 induced obvious rearrangement of F-actin.[35] The result of western blotting demonstrated that burned-plasma stimulation increased the phosphorylation of HSP27, while MK2 activation with transfection of its constitutively active form could also phosphorylate HSP27. The inhibition of MK2 by transfecting the cells with the MK2 dominant negative form before burned-serum administration diminished the phosphorylation of HSP27.[37] Our data indicate that burned plasma induced rearrangement of endothelial cytoskeleton F-actin through a p38/MK2/SP27 pathway.

Protein Kinase C plays a role in vascular permeability regulation in burn injury

Protein kinase C (PKC) has been known as an important second messenger in the regulation of microvascular barrier function during stimulation by phorbol esters, diacylglycerol (DAG), thrombin, bradykinin, and platelet-activating factor.[38,39] Inhibition of PKC with H7 and calphostin could abolish the increased vascular permeability induced by those pro-inflammaotry mediators.

In isolated and perfused porcine coronary venules, we showed that phorbol myristate acetate (PMA), a specific PKC activator, evoked a rapid increase of permeability coefficient (Pa), and this effect was blocked by a selective PKC inhibitor bisindolylmaleimide (BIM) (Fig. 8A). Another PKC inhibitor, GF-109203X, was able to decrease the hyperpermeability response triggered by typical inflammatory mediator histamine.[40] In cultured HUVECs, we detected an increase of phosphorylated PKC content, as well as a translocation of PKC from the cytoplasm to the inner membrane after stimulating the cells with PMA or burned serum. The attenuation of PKC activity with an inhibiting polypeptide (PKC19-36) could block this translocation and downregulate the phosphorylation of PKC.[41]

PKC has been reported to activate the endothelial contractile apparatus by inducing MLC phosphorylation, polymerization of actin and intermediate filaments, and activation of actin-binding proteins.[42,43] We then tested various PKC inhibitors for their influence on the disturbance of venular endothelium tight junctions and cytoskeleton reorganization that were induced by serum from burned rats. Indeed, inhibition of PKC by Ro-31-7549 could partially inhibit EC actin rearrangement, although it was not as potent as the inhibition by p38 MAPKs in reducing damage to the tight-junction of venular EC.[34,35] Vandenbroucke St Amant et al[44] claim that PKCα caused AJ disassembly by phosphorylation of p120-catenin at serine 879 that disassociated fromp120 of VE-cadherin. It has also been shown that some PKC isozymes, such as PKCα, could activate RhoA by inducing rapid phosphorylation of GDP dissociation inhibitor (GDI), indicating that RhoA would be one of the important substrates of PKC.[45,46] Our previous study also suggested that NOS was a potent substrate of PKC, while inhibition of nitric oxide synthase (NOS) with specific blocker NG-monomethyl-L-arginine (L-NMMA) greatly attenuated the hyperpermeability effect of PMA, indicating that PKC may alter endothelial permeability by directly acting on endothelial structural proteins and/or indirectly by modulating activity of common signaling protein NOS, as can be seen in Fig. 8B.[40]

The relationship of different signaling pathways in modulating endothelial barrier function

The above mentioned signal pathways might not work independently during the regulation of cellular functions. There have been several instances showing that there is a cross-talk between Rho/ROCK and p38 MAPK pathways, with most of the reports demonstrating that ROCK is the upstream regulator of p38 MAPK activation.[47,48] The result of our previously discussed study[37] showed that inhibition of Rho kinase with Y-27632 could attenuate the phosphorylation of p38 MAPK induced by burned plasma stimulation, suggesting the interaction of ROCK and p38 MAPK. As mentioned above, RhoA might be one of the important substrates of PKC.[44,45] These data re-stress the pivotal role of Rho/ROCK pathway in the modulation of endothelial barrier function. In contrast, while PKC also restrained the formation of stress fiber in burned-serum treated endothelial cells, the inhibition of PKC with Ro-31-7549 did not attenuate the phosphorylation of p38 after burned plasma administration, implying the p38-independent effect of PKC on endothelial morphological regulation.[37]

THE MEDIATOR-ENHANCED RECOVERY OF VASCULAR PERMEABILITY

There is an emerging concept in recent years that some so-called stabilizing mediators play positive roles in enhancing inter-endothelial junctional connections and preventing the increase of vascular permeability.[5,7,49,50] These mediators, such as cAMP, ATP, adenosine, adrenomedullin, and sphingosine 1-phosphate (S1P), may be released in response to pro-inflammatory mediators and serve to restore endothelial barrier function. Some of these stabilizing mediators are important even in quiescent states because they preserve basal vascular permeability at low levels.[7,51]

Produced by phosphorylation of sphingosine, S1P is an abundant lipid mediator in plasma that regulates numerous physiological functions of vascular and immune cells. Platelets are an important source of plasma S1P due not only to the rich presence of S1P synthesis enzyme, sphingosine kinase (SPHK), but also to the absence of S1P lyase, which is responsible for the degradation of S1P. Endothelial cells are another contributor to plasma S1P through secretion of S1P in a constitutive manner. By binding to its multiple receptors on endothelia, S1P serves as a barrier stabilizer via actin organization, strengthening intercellular and cell-matrix adherence.[51]

It has been reported that there is a significant decrease of blood platelets and a known platelet dysfunction in severely burned patients. This platelet deficiency correlates with a higher mortality after severe trauma and sepsis in humans. In platelet depletion in mice, mortality increased remarkably after thermal injury.[52] The underlying mechanisms

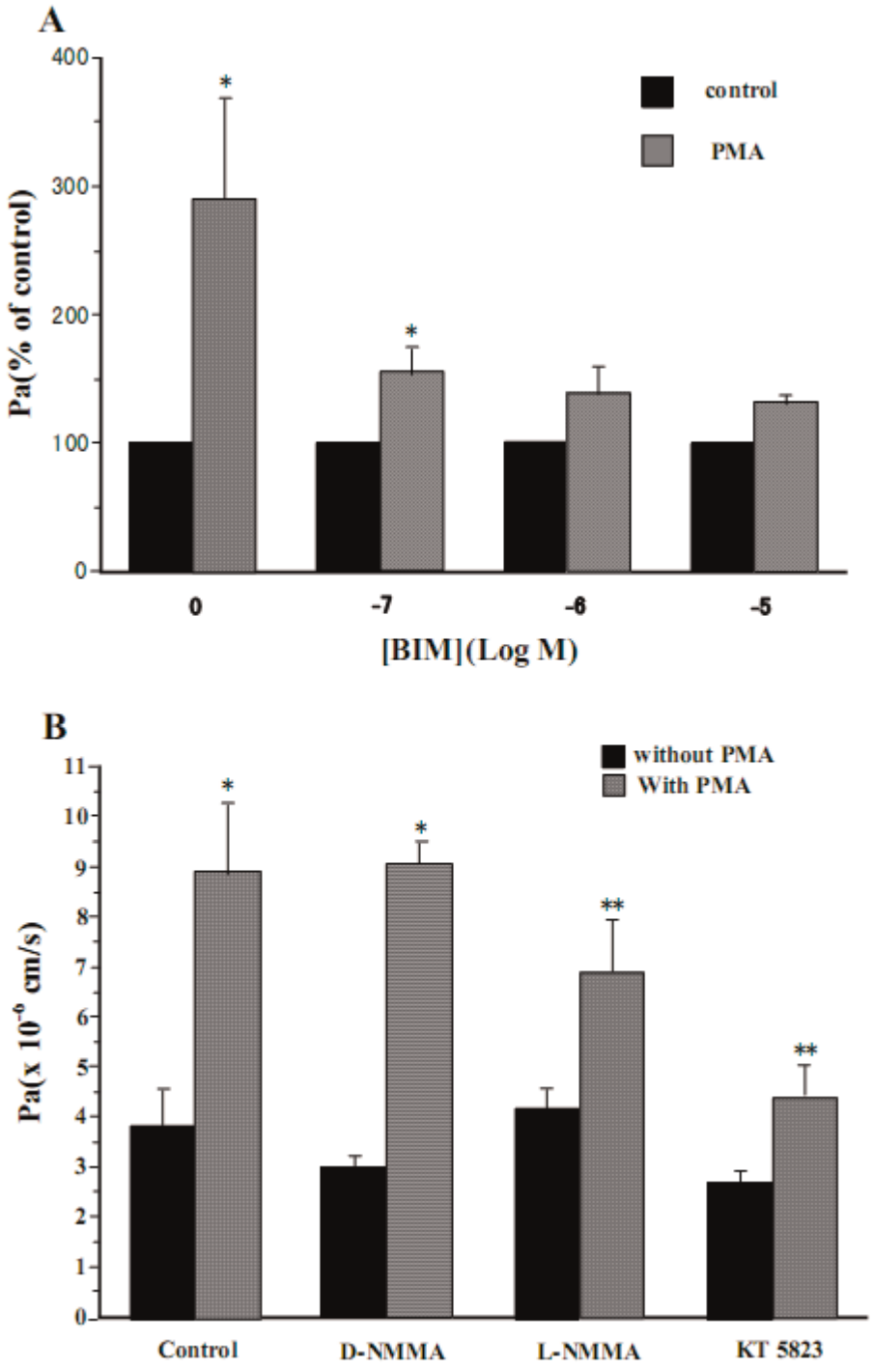

Figure 8 - Effects of PKC on microvascular permeability regulation. A: PKC activator PMA induced a remarkable increase in isolated vessels. PKC inhibitor BIM block its effect. B:The PMA-induced hyperpermeability could be attenuated by NOS inhibitor L-NMMA.

associated with these observations have not been fully understood.

We undertook a study based on the hypothesis that consumption of platelets by activation and aggregation at the early stage of thermal injury exhausts the storage of S1P, leaving the vascular endothelial cells more vulnerable to various permeability-increasing mediators released after severe burn injury, and eventually results in the exudation of fluid and protein from vascular space to interstitium. The purpose of the study was to observe the effects of S1P on distributions of a major adherens junction protein, vascular endothelial cadherins (VE-cadherin) and cytoskeletal F-actin in endothelial cells (ECs) upon the stimulation of burned plasma, and to evaluate the role of exogenous S1P on hyper-permeability response in venules isolated from thermal model of rats. In this study, cultured HUVECs with an

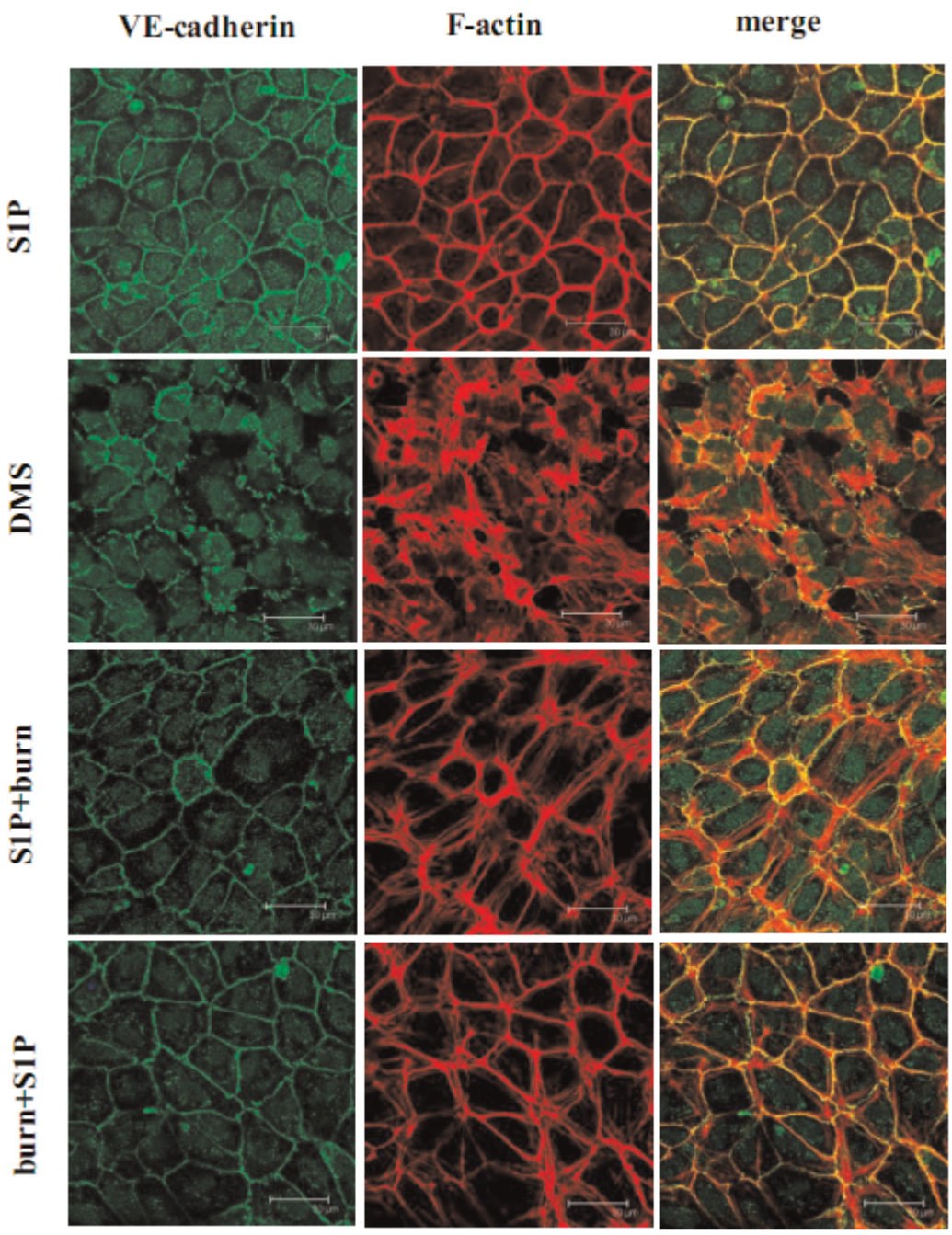

Figure 9 - Effects of S1P on VE-cadherin and F-actin organization in cultured HUVECs before or after burn.

addition of 1 μmol/L or 5 μmol/L S1P to the medium showed a clearer peripheral actin rim and a tighter intercellular junction compared with control cells. The inhibition of S1P synthesis by sphingosine kinase inhibitor DMS caused obvious disorganization of F-actin and junctional proteins in cultured HUVECs, accompanied by the increase of vascular permeability in isolated venules as seen in Fig. 9.[16] These data are consistent with the reports from Garcia JG et al that S1P-mediated enhancement of endothelial junctional integrity involved the formation of a strong cortical actin ring.[53] These results imply that under quiescent conditions, S1P might play a role in maintaining basal endothelial barrier function and its physiological levels would be sufficient for this purpose. The decline in S1P production will hamper the integrity of the vascular wall, resulting in an increase in albumin leakage from vessel space.

We also showed[16] that burned plasma stimulation also caused a time-dependent disturbed distribution of intercellular adherens junction protein VE-cadherin. This morphological alteration was attenuated by pre- or post-addition of S1P in an incubated medium as illustrated in Fig. 9. S1P stabilized and restored the cortical distribution of the F-actin ring and the continuous lining of VE-cadherin

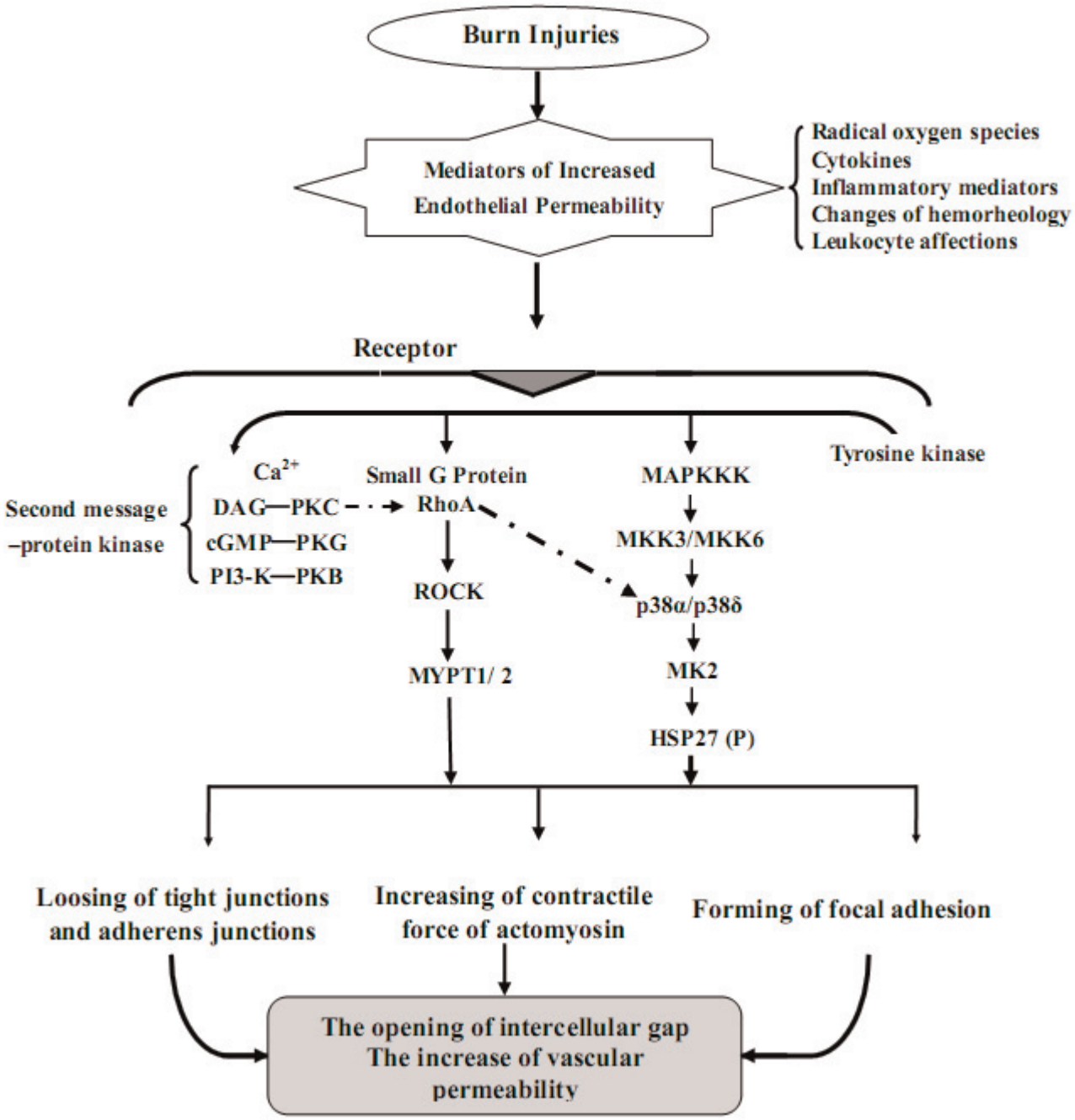

Figure 10 - The inferential signal pathways that regulate vascular permeability during burn injury and inflammation.

in the endothelial membrane area of burned plasma.[16] This is consistent with the results of Lee MJ et al[54] that S1P enhanced the assembly of adherens junctions. Parallel to this cultured cell study, the observation of isolated venules displayed similar effects of S1P on the organization of F-actin in vascular endothelial cells.[16] The hyper-permeability response of isolated venules exposed to burned plasma was also inhibited by administration of S1P, coincident with its above-mentioned morphological alteration. Our previous *in vivo* study has also demonstrated that exogenous applications of S1P attenuated the leakage of albumin in post-capillary venules within 20 min to 50 min after burn injury in mouse models.[55]

While an appropriate or physiological level of S1P acts as a stabilizer for endothelial barrier function by preventing the inflammatory hyper-permeability response, excessive S1P will evoke the activity of different receptors and cellular signaling pathways, resulting in active cytoskeleton rearrangement and barrier disruption. This diversity of S1P effects is due to the activations of different S1P receptors upon different concentrations of S1P.[56] Calcium is believed to play an important role in those controversial-ridden processes.

THE EFFECT OF CALCIUM IN ENDOTHELIAL BARRIER FUNCTIONAL REGULATION

There have been numerous reports showing that increases of cytosolic calcium (Ca^{2+}) are sufficient to initiate the cytoskeletal reorganization that increases cell tension and disrupts cell junctions, resulting in the retraction of endothelial cell borders and increased macromolecular permeability.[38] To resolve whether Ca^{2+} released from the endoplasmic reticulum (ER), or Ca^{2+} entering across the plasma membrane, is required to disrupt the endothelial cell barrier, inflammatory agonists have been used to activate the endothelium in the absence of extracellular Ca^{2+}.[57] Under these experimental conditions, most studies find no significant increase in endothelial cell permeability, illustrating that Ca^{2+} entry across the plasma membrane, and not Ca^{2+} release from the ER, is required to disrupt the endothelial cell barrier.[54] These differentiated effects of one simple Ca^{2+} ion might be due to incredible versatility of Ca^{2+} signaling, whereby Ca^{2+} can act in the various contexts of space, time and amplitude and achieve specificity and activate only a subset of those targets. While most of the Ca^{2+}-endothelium related studies only detected the global cytosolic Ca^{2+} concentration $Ca^{2+}i$ or bulk Ca^{2+}, the intracellular spatiotemporal dynamics of Ca^{2+} might play a more critical role in manipulating the endothelial response to various mediators. It is proposed that an appropriate amount or physiological level of S1P bind to S1P receptor 1 and induce the Ca^{2+} release from the ER, resulting in the phosphorylation of Rac GDP. The activation of Rac signaling will enhance the formation of lamellipodia and assembly of adhesion and tight junctions, leading to strengthening of the barrier. In contrast, excessive S1P will combine with S1P receptor 2/3 and promote the phosphorylation of RhoA GDP, accompanying the entry of Ca^{2+} across the plasma membrane. The activation of the RhoA/ROCK pathway will trigger the actin-myosin driven contraction and stress fiber formation, leading to the disruption of adhesion and tight junctions, resulting in endothelial barrier dysfunction and vascular hyperpermeability.[15] This theory needs to be confirmed by exploring the intracellular spatiotemporal dynamics of calcium in S1P stimulated endothelial cells and comparing with the alterations of the sub-cellular localizations and functional changes of protein family Rho GTPases, especially Rac and RhoA.

SUMMARY

According to research performed in this decade, this review inferentially delineated a schematic signal pathway that regulates vascular permeability during inflammation and shock (Fig. 10). Burn insult, inflammation and other traumatic injuries will trigger the release of various vascular-permeability-increasing mediators, including reactive oxygen species, cytokines (platelet activating factor, tumor necrosis factor, etc), and other inflammatory mediators (histamine, bradykinin, etc). The changes in hemorheology, such as the slowdown of blood flow, and the adhesion of leukocytes to the vascular wall will also evoke the activation of EC. By binding to their specific chemical receptors or mechanisensors, these mediators activate multiple signal pathways through second messengers and protein kinases. Specifically, PKC, RhoA and different MAPKs and their downstream substrates play very crucial roles in regulating the cell-cell contacts and the cellular contraction. The opening of tight junctions and adherens junctions and the increasing contractile force through actin-myosin interaction widen the intercellular gaps, allowing the transflux of large molecular substances and leakage of mass fluid from vascular space, resulting in the increase of permeability and tissue edema.

The semipermeable property of the endothelium is maintained through the equilibrium of competing contractile and adhesive forces generated by the cytoskeletal proteins and the adhesive molecules located at cell-cell and cell-matrix contacts. One aspect we do not discuss in this section is the alteration of cell-matrix contact that supply an anchoring site for the contracting cell, which includes the activation of various integrins and their interacting partners.[58,59]

While focusing on the paracellular junctional regulation in control of mediator-triggering of vascular hyperpermeability, we could not ignore the fundamental role of transcellular pathways under basal conditions, because the transport of albumin and liquid mostly depends on the trafficking of caveolae in capillary endothelia and vesiculo-vacuolar organelles (VVOs) in the endothelia of venules and small veins. This process of transcytosis involves a series of interactions between plasma membrane proteins and cytoplasmic signaling molecules. We would not expand further discussion in this dissertation since we have yet to set foot in this aspect. Readers could refer it to certain comprehensive reviews[5,60] in which these emerging principles have been extensively discussed.

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