Brief Review

Cellular signaling in rapid intestinal epithelial restitution: implication of polyamines and K^+ channels

Jian-Ying WANG*

Departments of Surgery and Pathology, University of Maryland School of Medicine and Baltimore Veterans Affairs Medical Center, Baltimore, Maryland, USA

Abstract: Epithelial cells line the gastrointestinal (GI) mucosa and form an important barrier that protects the subepithelial tissue against a wide array of noxious substances, allergens, viruses, and luminal microbial pathogens. Restoration of mucosal integrity following injury requires epithelial cell decisions that regulate signaling networks controlling gene expression, survival, migration, and proliferation. Over the past few years, polyamines have been shown to play a critical role in GI mucosal repair, and the control of cellular polyamines is a central convergence point for the multiple signaling pathways. Both the function of polyamines in rapid intestinal mucosal epithelial restitution and the underlying mechanism, especially the implication of K^+ channel activity, are the subject of this mini-review article.

Key words: intestinal epithelium; intracellular Ca^{2+}; mucosal injury; membrane potential; cell migration, K^+ channels

肠上皮快速复原过程中的细胞信号传递：多胺和 K^+ 通道的影响

汪建英

马里兰大学医学院外科学和病理学系，美国

摘　要： 胃肠道粘膜上皮细胞具有重要的屏障作用，可以保护次上皮组织抵御一系列的有害物质，包括过敏原、病毒以及微生物病原体。粘膜损伤后的修复有赖于上皮细胞对信号网络的调节，而这一网络系统控制着基因的表达、细胞的存活、迁移及增殖。近几年的研究结果显示，在胃肠道粘膜的修复中，多胺起到关键作用；且细胞多胺的调控是众多信号传递路径的焦点。本文简要综述了多胺在肠粘膜上皮快速复原中的功能和机制，特别是对 K^+ 通道活性的影响。

关键词：肠上皮；细胞内钙离子；粘膜损伤；膜电位；细胞迁移；钾通道

中图分类号：Q257

Maintenance of mammalian gastrointestinal epithelial integrity is determined by a balance between damaging factors and protective factors, which include rapid restitution. Mucosal injury occurs commonly from mild physical trauma during digestion to localized damage from the ingestion of alcohol, aspirin, and/or non-steroidal anti-inflammatory compounds, or from *Helicobacter pylori* infection. Restoration of normal intestinal mucosal integrity-successful repair of wounds and ulcers-requires epithelial cell decisions that regulate signaling networks controlling gene expression, survival, migration, and proliferation. In the acute response to injury, damaged cells are sloughed, and the remaining viable cells from areas adjacent to or just beneath the injured surface migrate to cover the denuded area. This early restitution refers to resealing of superficial wounds as a consequence of epithelial cell migration into the defect, a process independent of epithelial cell proliferation[1,2]. In contrast to this rapid repair process, deeper damage and chronic ulcers manifest long-term com-

Received 2003-02-11 Accepted 2003-03-17

This work was supported by the National Institutes of Health (NIH) Grants DK-57819 and DK-61972 and a Merit Review Grant from US Department of Veterans Affairs (VA).

* Corresponding author. Surgical Service, Baltimore Veterans Affairs Medical Center, 10 North Greene Street, Baltimore, MD 21201, USA; Tel: 410-605-7000 Ext. 5678; Fax: 410-605-7919; E-mail: jwang@ smail. umaryland. edu

The speaker of first Chih-Chun Wang Memorial Distinguished Lectureship, Chinese Association of Physiological Sciences, Wuyi-Shan, RP China, October 14, 2002

万方数据

plex responses that require *de novo* mRNA and protein synthesis and cell replication.

Rapid epithelial restitution is a complex process that is regulated by numerous factors including cellular polyamines. The natural polyamines, including spermidine, spermine and their precursor putrescine, are organic cations found in all eukaryotic cells. It has been recognized for some time that the control of cellular polyamines is a central convergence point for the multiple signaling pathways driving different epithelial cell functions. Cellular polyamine levels are dramatically increased during the process of early mucosal restitution in both *in vivo* and *in vitro* systems, and polyamine depletion through inhibition of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis, inhibits cell migration and delays mucosal restitution[3,27-29]. Over the past several years, our group and others have demonstrated that polyamines regulate voltage-gated K^+ (Kv) channel expression and cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$), which plays a critical role in the stimulation of intestinal epithelial cell migration after wounding. In this article, we will provide an overview of Kv channels and $[Ca^{2+}]_{cyt}$ in polyamine-dependent cell migration, and then analyze in some details of the possible downstream signaling pathways following polyamine-induced $[Ca^{2+}]_{cyt}$ in migrating epithelial cells.

Polyamines and Kv channel expression

The polyamines are intimately involved in, and required for, distinct epithelial cell functions including migration, proliferation, and apoptosis[3,4]. Cellular polyamine levels are highly regulated by biosynthesis, degradation, and transport system. Polyamines synthesis depends on the activation or inhibition of ODC, which catalyzes the first rate-limiting step in polyamine biosynthesis. ODC decarboxylates the amino acid ornithine to form putrescine (1,4,-diaminobutane); propylamine groups are then added to one or both amino groups of putrescine to form spermidine and spermine, respectively (Fig. 1). An other enzyme involved in this is S-adenosylmethionine decarboxylase (SAMDC), and it may also be rate-limiting. Instead of conversion into spermidine, putrescine can be degraded by diamine oxidase or monoamine oxidase. It has been demonstrated that spermidine/spermine-N-acetyltransferase (SSTA) is the rate-limiting factor in the polyamine degradation[4].

Kv channels are a group of membrane proteins and

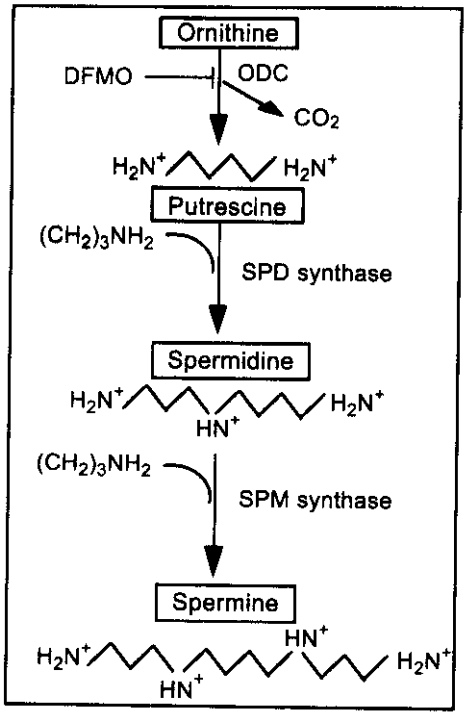

Fig. 1. The biosynthesis of the major physiological polyamines and their precursor putrescine in mammalian cells. Putrescine has 4 CH groups, spermidine (SPD) 7, and spermine (SPM) 10. They carry 2, 3, and 4 positive charges that reflect the strength of their binding ability. ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine.

play an important role in regulating resting membrane potential (E_m) in many types of cells[5]. At the molecular level, Kv channels are composed of the pore-forming α subunits and the regulatory β subunits[6,7]. Activation of Kv channels causes membrane hyperpolarization, whereas inhibition of Kv channels predisposes to membrane depolarization. A series of studies from our group have recently demonstrated that polyamines are required for expression of Kv channels in intestinal epithelial cells[8-11]. Depletion of cellular polyamines inhibits expression of Kv channel α subunits (Kv1.1), reduced whole cell K^+ currents ($I_{K(v)}$), and depolarized E_m (Fig. 2). Polyamine depletion does not alter expression of Kv channel β subunit. In contrast, increased cellular polyamines by addition of exogenous spermidine or by overexpression of the ODC gene stimulate Kv channel activity and results in membrane hyperpolarization. These results clearly indicate that polyamines are major stimulators for expression of the Kv channel genes and are involved in the control of E_m in intestinal epithelial cells.

万方数据

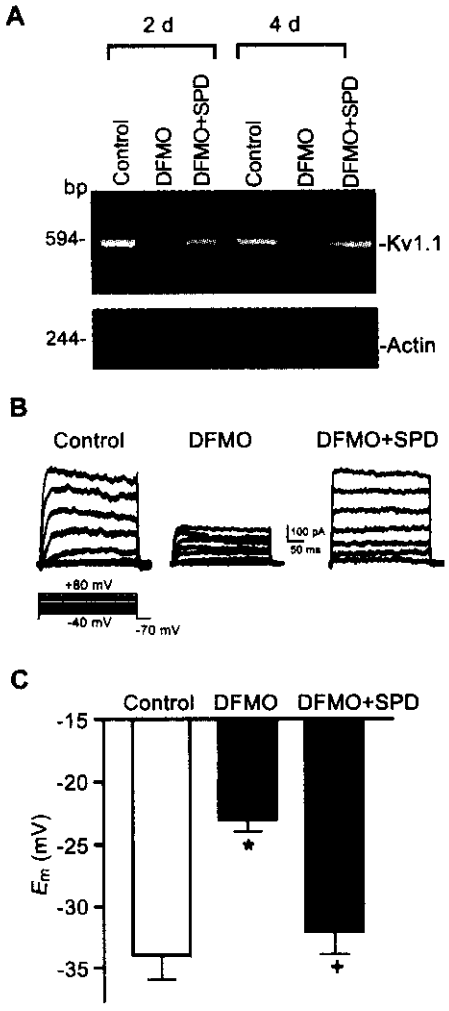

Fig. 2. Effect of depletion of cellular polyamines by α-difluoromethylornithine (DFMO) on mRNA expression of voltage-gated K^+ (Kv) 1.1 channel (*A*), whole cell Kv currents (*B*), and resting membrane potential (*C*) in intestinal epithelial cells (IEC-6 line). Cells were grown in the DMEM containing 5% dialyzed FBS and 5 mmol/L DFMO with or without 5 μmol/L spermidine (SPD) for 4 d. Note that polyamine depletion inhibited Kv channel expression, decreased whole cell Kv currents, and resulted in membrane depolarization. $^{*,+}P < 0.05$ compared with control and DFMO alone, respectively.

Kv expression and Ca^{2+} homeostasis

Increasing evidence shows that activity of Kv channels controls E_m that regulates $[Ca^{2+}]_{cyt}$ concentration by governing the driving force for Ca^{2+} influx[6,8]. At the cellular level, cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$) is derived from two sources — external and internalad[12]. Ca^{2+} can enter from outside the cell by passing through channels that span the external barrier, plasma membrane and also be released from internal Ca^{2+} stores (endoplasmic and/or sarcoplasmic reticulums). Ca^{2+} influx depends on the Ca^{2+} driving force, or the electrochemical gradient across the plasma membrane. While the chemical gradient, the ratio of extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$) to $[Ca^{2+}]_{cyt}$, and the Ca^{2+} equilibrium potential, E_{Ca} {$E_{Ca} = 12.5 \ln([Ca^{2+}]_o/[Ca^{2+}]_{cyt}) = 117 - 131$ mV at 25℃} are constant, the Ca^{2+} driving force is mainly determined by the electrical gradient, the difference between E_m and E_{Ca} ($E_m - E_{Ca}$). In other words, E_m is a major determinant of the driving force for Ca^{2+} influx.

By controlling the Ca^{2+} driving force, E_m is an important regulator of $[Ca^{2+}]_{cyt}$ in non-excitable cells including epithelial cells and lymphocytes. Membrane depolarization decreases the Ca^{2+} driving force and inhibits its Ca^{2+} influx. In contrast, membrane hyperpolarization increases the Ca^{2+} driving force and enhances Ca^{2+} influx. Therefore, in the cells that do not express L-type voltage-dependent Ca^{2+} channels (VDCC), Ca^{2+} influx is decreased by membrane depolarization but increased by membrane hyperpolarization. Nonetheless, in excitable cells such as neurons, cardiomyocytes and muscle cells, VDCC that are opened by membrane depolarization are the major pathway for Ca^{2+} influx[5,7]. In contrast to the voltage-independent pathway for Ca^{2+} influx in non-excitable cells, membrane depolarization opens VDCC and thus increases $[Ca^{2+}]_{cyt}$ in excitable cells.

E_m is primarily determined by the K^+ permeability and K^+ gradient across the plasma membrane[5]. Since K^+ gradient is maintained by Na^+-K^+ ATPase, the K^+ permeability is directly related to the activity and number of membrane K^+ channels. Kv channels are a major determinant of resting E_m in many types of cells. When K^+ channels close or the total numbers of total K^+ channels decrease, E_m becomes less negative (i. e., depolarization). When K^+ channels open or the numbers of total K^+ channels rise, E_m becomes more negative (i. e., hyperpolarization)[5,6]. Therefore, inhibition of K^+ channel gene expression would decrease the number of K^+ channels and attenuate K^+ channel activity. The subsequent membrane depolarization decreases the Ca^{2+} driving force, and thus inhibits Ca^{2+} influx. Since Ca^{2+} entry is a major source for $[Ca^{2+}]_{cyt}$, inhibition of Ca^{2+} influx would reduce $[Ca^{2+}]_{cyt}$ in cells lacking VDCC[5].

Kv channels and $[Ca^{2+}]_{cyt}$ in polyamine-dependent cell migration

Our studies have shown that polyamines regulate intestinal epithelial cell migration by altering K^+ channel activity, E_m, and $[Ca^{2+}]_{cyt}$ and that the resultant increase in $[Ca^{2+}]_{cyt}$ exerts its regulatory effects on cell motility through interaction with specific targets during

万方数据

restitution[8,10]. Because intestinal epithelial cells do not express VDCC, the depolarized E_m by polyamine depletion decreases $[Ca^{2+}]_{cyt}$ through the reduced driving force for Ca^{2+} influx through capacitative Ca^{2+} entry

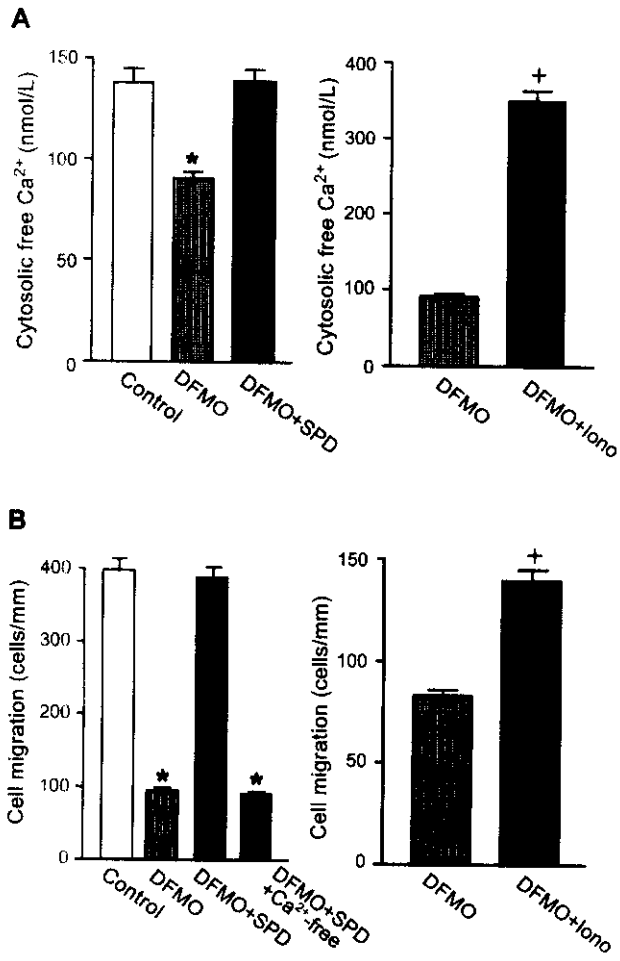

Fig. 3. Effects of polyamine depletion and the Ca^{2+} ionophore ionomycin (Iono) on cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) and cell migration after wounding. *A*: Summarized data showing $[Ca^{2+}]_{cyt}$ measured in peripheral areas of cells that were grown in control cultures and in cultures containing 5 mmol/L DFMO with or without 5 μmol/L SPD for 4 d, and cells treated with DFMO for 4 d and then exposed to Iono (1 μmol/L). *B*: Summarized data showing cell migration after wounding in cells described in *A*. Polyamine depletion reduced $[Ca^{2+}]_{cyt}$ and inhibited cell migration (life), while increased $[Ca^{2+}]_{cyt}$ by Iono stimulated cell migration in polyamine-deficient cells (right). $^{*,+}P < 0.05$ compared with controls and DFMO alone.

(Fig. 3). Migration is reduced by 80% in the polyamine-deficient cells[8,9]. Decreased $[Ca^{2+}]_{cyt}$ by depolarization of E_m by 4-aminopyridine also inhibits normal cell migration and prevents the restoration of cell migration by exogenous spermidine in polyamine-deficient cells. In contrast, increased $[Ca^{2+}]_{cyt}$ by the treatment with Ca^{2+} ionophore ionomycin stimulates cell migration in the absence of cellular polyamines. These results indicate that polyamine-mediated intestinal epithelial cell migration is partially due to increase of Kv channel expression. The subsequent membrane hyperpolarization raises $[Ca^{2+}]_{cyt}$ by increasing the driving force for Ca^{2+} influx and thus stimulates cell migration.

In another set of experiments, we have found that differentiated intestinal epithelial cells (IEC-*Cdx*2L1 line) induced by forced expression of the *Cdx*2 gene, which encodes a transcription factor controlling intestinal epithelial cell differentiation, migrate over the wounded edge much faster than undifferentiated parental IEC-6 cells (IEC-6 line). We have also demonstrated that increased migration of differentiated IEC-*Cdx*2L1 cells after wounding results, at least partially, from the K^+ channel activation and the increase in the driving force for Ca^{2+} influx during restitution[10,12]. IEC-*Cdx*2L1 cells with highly differentiated phenotype express higher basal levels of Kv1.1 and Kv1.5 mRNAs and proteins than those observed in undifferentiated parental IEC-6 cells. Neither IEC-*Cdx*2L1 cells nor parental IEC-6 cells express VDCC. The increased expression of Kv channels in differentiated IEC-*Cdx*2L1 cells is associated with an increase in whole cell K^+ currents ($I_{K(v)}$), membrane hyperpolarization, and a rise in resting $[Ca^{2+}]_{cyt}$. The migration rates in differentiated IEC-*Cdx*2L1 cells are ~4 times of parental IEC-6 cells. Inhibition of Kv channel expression by depletion of cellular polyamines reduced $[Ca^{2+}]_{cyt}$, and resulted in cellular reorganization of cytoskeletal proteins, along with a marked reduction in actomyosin stress fiber formation, and the inhibition of epithelial cell migration. In contrast, elevation of $[Ca^{2+}]_{cyt}$ by the Ca^{2+} ionophore, ionomycin, promoted formation of actomyosin stress fibers, and increased epithelial cell migration after wounding.

Regulation of $[Ca^{2+}]_{cyt}$ and transient receptor potential channels (TRPC)

As pointed out above, the Ca^{2+} driving force in intestinal epithelial cells is the major determinant of transmembrane influx of Ca^{2+}, but the involvement of Ca^{2+} channels in this process is still unknown. In endothelial and epithelial cells, passive Ca^{2+} leakage, receptor-operated Ca^{2+} channels (ROC), nonselective cation channels (NSOC), and store-operated channels (SOC), all contribute to Ca^{2+} influx[13,14]. Non-excitable cells such as intestinal epithelial cells lack VDCC but have developed the Ca^{2+} entry mechanism that is coupled with the depletion of intracellular Ca^{2+} stores to activate transient receptor potential channels (TRPC). Thus the capacitative Ca^{2+} entry via TRPC channels

万方数据

may be a major source of intracellular Ca^{2+} in mammalian cells[15]. TRPC-1 and TRPC-5 that encode Ca^{2+} permeable channels are detected in the intestinal epithelial cells[8,10]. These results indicate that the capacitative Ca^{2+} entry through TRPC-1 and TRPC-5 channels may serve as an important source of the agonist-mediated rise in $[Ca^{2+}]_{cyt}$ during restitution in intestinal epithelial cells. In support of this possibility, we have recently demonstrated that mRNA and protein levels of TRPC-1 in differentiated intestinal epithelial cells are significantly higher than those observed in undifferentiated parental cells. The higher levels of TRPC-1 in differentiated epithelial cells are associated with a significant increase in basal $[Ca^{2+}]_{cyt}$ concentration[10,12]. Clearly, studies to elucidate the exact role of TRPC in the regulation of Ca^{2+} influx in gastrointestinal epithelial cells are urgently needed.

Downstream targets of Ca^{2+}

Although Ca^{2+} regulates almost everything that we do, different cell types and cellular functions select combinations of Ca^{2+} signals with the precise parameters to fit their processes. A specific role of Ca^{2+} in the regulation of various biological functions completely depends on downstream targets of elevated $[Ca^{2+}]_{cyt}$. The research has been reviewed elsewhere[7,13], and the following discussion will focus on just a few points of current interest. The coordinated movement of intestinal epithelial cells during restitution is a complex process that is controlled by the cytoskeleton. Changes in both the distribution and formation of the cytoskeleton alter the adhesion, spreading, and motility of cells. There is increasing evidence, indicating that elevated $[Ca^{2+}]_{cyt}$ activates Rho guanine nucleotide triphosphate (GTP)-binding proteins that are key regulators of the cytoskeletal reorganization[16,17].

Rho Proteins. The Rho family, including Rho, Rac, and Cdc42, is a member of the Ras superfamily of small guanosine triphosphatases (GTPases) and functions as molecular switches by cycling between an active GTP-bound state and an inactive GDP-bound state[16,18]. Activation of Rho proteins, through GDP-GTP exchange, is stimulated by guanine nucleotide exchange factors (GEFs), whereas inactivation of the proteins is promoted by GTPase-activating proteins (GAPs). Activated Rho proteins interact with cellular target proteins or effectors to regulate a signal transduction pathway linking surface receptors to the formation of actomyosin stress fibers and focal adhesions. The transformation of RhoA from its inactive GDP-bound form to its active GTP-bound form activates Rho kinase that results in the formation of actomyosin stress fibers by initiating myosin light chain phosphorylation[18]. On the other hand, activation of Rac promotes *de novo* actin polymerization at the cell periphery to form lamellipodial extensions and membrane ruffles, and activation of Cdc42 results in actin polymerization to form filopodia or microspikes.

It has been recently reported that Ca^{2+}-activated RhoA activity plays a critical role in regulation of cell migration after wounding in intestinal epithelial cells[9,19]. Decreased $[Ca^{2+}]_{cyt}$ concentration, either by reducing the Ca^{2+} driving force for Ca^{2+} influx via membrane depolarization by polyamine depletion or removal of $[Ca^{2+}]_o$ from the culture medium inhibited RhoA expression and activity. Increasing $[Ca^{2+}]_{cyt}$ through treatment with the Ca^{2+} ionophore ionomycin stimulated RhoA activity in intestinal epithelial cells (Fig. 4). Exposure to 1 μmol/L ionomycin for 4 and 6 h not only increased RhoA protein level in normal cells, but also significantly overcame the inhibitory effect of polyamine depletion on RhoA expression. RhoA protein levels in response to ionomycin were correlated to the elevation of $[Ca^{2+}]_{cyt}$, and the responses in polyamine-deficient cells were smaller than those observed in normal cells because of reduced driving force for Ca^{2+} influx. Elevation of $[Ca^{2+}]_{cyt}$ increased RhoA activity partially through alteration of RhoA protein synthesis and stability in intestinal epithelial cells[9]. The rate of newly synthesized RhoA protein was stimulated by increasing $[Ca^{2+}]_{cyt}$, but inhibited after exposure to the Ca^{2+}-free medium. On the other hand, the stability of RhoA protein also was regulated by $[Ca^{2+}]_{cyt}$ in intestinal epithelial cells. Although elevation of $[Ca^{2+}]_{cyt}$ slightly increased the stability of RhoA protein, removal of $[Ca^{2+}]_o$ dramatically destabilized RhoA protein and accelerated its degradation.

Furthermore, decreased Rho activity by treatment with Clostridium botulinum exoenzyme C_3 transferase (C_3) blocks formation of actin-myosin stress fibers and inhibits intestinal epithelial cell migration after wounding[9,19]. At present it is not clear whether other members of the mammalian Rho family, including RhoB, RhoC, RhoD, RhoE, RhoG, Rac1, Rac2, and Cdc42, are regulated by $[Ca^{2+}]_{cyt}$ alterations or are involved in the regulation of intestinal epithelial cell migration. These results indicate that increasing

万方数据

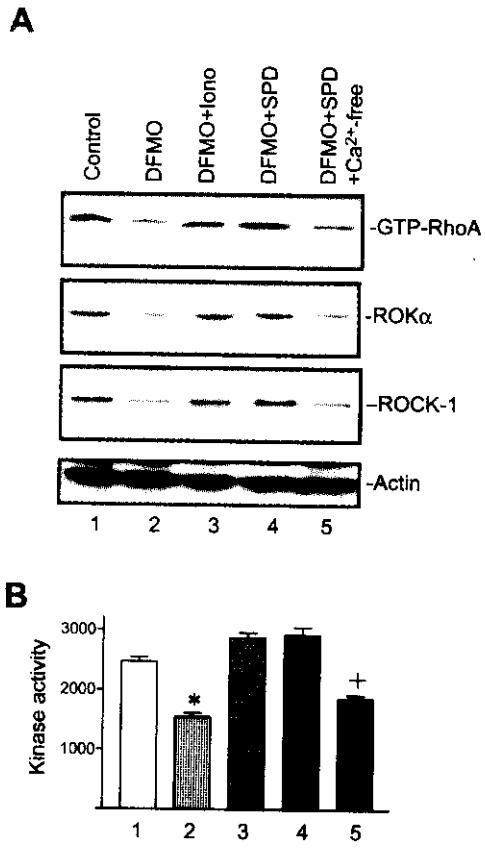

Fig. 4. Changes in GTP-bound RhoA (GTP-RhoA), ROKα and ROCK-1 proteins, and the Rho-kinase enzyme activity in control and polyamine-deficient cells. *A*: Western blots for GTP-RhoA and ROKα and ROCK-1. In the experiments dealing with manipulation of $[Ca^{2+}]_{cyt}$, DFMO-treated cells and cells treated with DFMO plus SPD were exposed to the Ca^{2+}-free medium or 1 μmol/L ionomycin (iono) for 6 h. *B*: Summarized data showing the Rho-kinase enzyme activity in cells described in *A*. Note that decreased $[Ca^{2+}]_{cyt}$ by polyamine depletion or exposure to the Ca^{2+}-free medium inhibited GTP-RhoA and inactivated Rho-kinase, while induction of $[Ca^{2+}]_{cyt}$ by exogenous SPD or Iono increased GTP-RhoA and activated Rho-kinase in polyamine-deficient cells. $^{*,+}P < 0.05$ compared with controls and DFMO + SPD, respectively.

$[Ca^{2+}]_{cyt}$ activates RhoA activity and increases the formation of actomyosin stress fibers and stimulates intestinal epithelial migration during the early phase of mucosal restitution.

Effector of Rho. To understand the exact mechanisms through which Rho GTPases regulate the cytoskeletal rearrangement and other associated activities, an array of possible cellular effectors (targets) has been reported[20,21]. Using yeast two-hybrid selection and affinity purification techniques, more than 20 candidate targets have been identified so far, which represent a wide variety of enzymatic activities and protein-protein interaction domains.

It has been shown that the Ser-Thr kinase p^{160}ROCK interacts with Rho in a GTP-dependent manner[22,23]. This kinase is an excellent candidate for mediating Rho-induced changes to actin-myosin cytoskeleton because it mimics the function of Rho when overexpressed or constitutively activated. Both myosin light chain (MLC) and MLC phosphatase, which are known to regulate the assembly of actin-myosin filament bundle[18,24], are substrates of the Ser-Thr kinase p^{160}ROCK. Whether p^{160}ROCK is the only downstream target of Rho required for inducing the formation of stress fibers remains to be demonstrated. Treatment with cytochalasin D blocks assembly of stress fibers, suggesting that some actin polymerization might be required. However, there are some results indicating that the actin-myosin filaments induced by p^{160}ROCK are not correctly organized nor are they contractile as they are when induced by Rho[21].

Although no direct targets of Rho are identified yet, the ERM proteins, including ezrin, radixin, and moesin, are emerging as key regulators of the actin-myosin cytoskeleton. The interaction of ERM with a transmembrane protein, CD44, through their NH-2-termini has been shown to be regulated by Rho, and their COOH-terminal ends interact with filamentous actin[25]. Moreover, ERM proteins are essential for both Rho- and Rac-induced cytoskeletal organization. It is possible that ERM proteins behave as regulatable scaffold proteins that anchor actin-myosin filaments to the membrane and that this is a prerequisite for Rho and Rac to induce stress fibers and lamellipodia, respectively.

We have recently examined whether increased RhoA following elevated $[Ca^{2+}]_{cyt}$ activates Rho-kinase (ROK/ROCK) resulting in MLC phosphorylation[26]. Reduced $[Ca^{2+}]_{cyt}$, by either polyamine depletion or exposure to the Ca^{2+}-free medium, decreased RhoA protein expression, which was paralleled with significant decreases in GTP-bound RhoA, ROCK-1 and ROKα proteins, Rho-kinase enzyme activity (Fig. 4), and MLC phosphorylation. The reduction of $[Ca^{2+}]_{cyt}$ also inhibited cell migration after wounding. Elevation of $[Ca^{2+}]_{cyt}$ induced by ionomycin increased GTP-bound RhoA, ROCK-1 and ROKα proteins, Rho-kinase enzyme activity, and stimulated MLC phosphorylation. Inhibition of RhoA function by a dominant negative mutant RhoA decreased ROKα protein expression and the Rho-kinase enzyme activity, and resulted in cytoskeletal reorganization. Inhibition of ROK/ROCK activity by the specific inhibitor Y27632 not only decreased MLC phosphorylation but also suppressed cell migration. These results indicate that increase in GTP-bound RhoA by polyamines via $[Ca^{2+}]_{cyt}$ can interact

万方数据

with and activate Rho-kinase during intestinal epithelial restitution. The activated Rho-kinase subsequently phosphorylates MLC, leading to the stimulation of myosin stress fiber formation and cell migration.

Summary

Based on our findings, we propose a model delineating the role of K^+ channel activation following increased polyamines in the process of intestinal epithelial cell migration after wounding (Fig. 5). In this model, increased polyamines enhance K^+ channel expression, cause membrane hyperpolarization, raise $[Ca^{2+}]_{cyt}$ concentration through enhancing the driving force for Ca^{2+} influx, and increase RhoA activity, leading to ROK/ROCK activation. The resultant activation of ROK/ROCK increases MLC phosphorylation resulting in stimulation of stress fiber formation and cell migration during restitution. In contrast, depletion of cellular polyamines inactivates RhoA/Rho-kinase signaling pathway by reducing $[Ca^{2+}]_{cyt}$ through down-regulation of K^+ channel activity, decreases MLC phosphorylation, and inhibits stress fiber formation, thus leading to inhibition of cell migration.

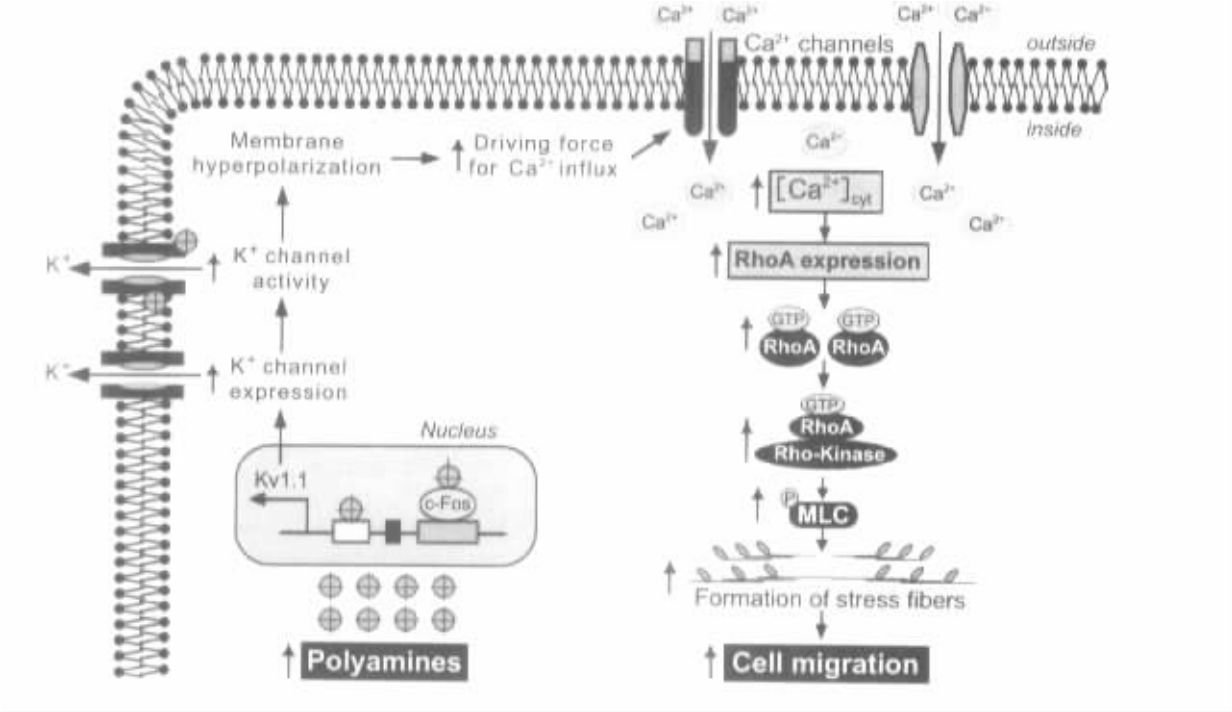

Fig. 5. Schematic diagram depicting the proposed roles of K^+ channels and Ca^{2+}-RhoA signaling in the stimulation of intestinal epithelial cell migration by polyamines after wounding. Polyamines stimulate K^+ channel activity, cause membrane hyperpolarization, and raise $[Ca^{2+}]_{cyt}$ by increasing the driving force for Ca^{2+} influx, leading to activation of RhoA. Increased GTP-bound RhoA can interact with ROK/ROCK and activate it. Activated ROK/ROCK increases myosin light chain (MLC) phosphorylation and actomyosin stress fiber formation, leading to stimulation of cell migration during restitution.

REFERENCES

[1] Rutten MJ, Ito S. Morphology and electrophysiology of guinea pig gastric mucosal repair *in vitro*. Am J Physiol 1983;244:G171 – G182.

[2] Silen W, Ito S. Mechanisms for rapid re-epithelialization of the gastric mucosal surface. Ann Rev Physiol 1985; 47:217 – 219.

[3] McCormack SA, Johnson LR. Role of polyamines in gastrointestinal mucosal growth. Am J Physiol 1991;260: G795 – G806.

[4] Tabor CW, Tabor H. Polyamines. Annu Rev Biochem 1984;53:749 – 790.

[5] Fleischmann BK, Washabau RJ, Kotlikoff MI. Control of resting membrane potential by delayed rectifier potassium currents in ferret airway smooth muscle cells. J Physiol 1993;469:625 – 638.

[6] Isom LL, DeJongh KS, Catterall WA. Auxiliary subunits of voltage-gated ion channels. Neuron 1994;12:1183 – 1194.

[7] Brown EM, Vassilev PM, Hebert SC. Calcium as extracellular messengers. Cell 1995;83:679 – 682.

[8] Wang JY, Wang J, Golovina VA, Li L, Platoshyn O, Yuan JX. Role of K^+ channel expression in polyamine-dependent intestinal epithelial cell migration. Am J Physiol 2000;278:C303 – C314.

[9] Rao JN, Li L, Golovina VA, Platoshyn O, Strauch ED, Yuan JX, Wang JY. Ca^{2+}-RhoA signaling pathway required for polyamine-dependent intestinal epithelial cell migration. Am J Physiol 2001;280:C993 – C1007.

[10] Rao JN, Platoshyn O, Li L, Guo X, Golovina VA, Yuan J-XJ, Wang JY. Activation of K^+ channels and increased migration of differentiated intestinal epithelial cells after wounding. Am J Physiol 2002;282:C885 – C898.

[11] Rao JN, Wang JY. Ca^{2+} signaling in epithelial restitu-

万方数据

tion. In: Cho CH, Wang JY ed. Gastrointestinal Mucosal Repair and Experimental Therapeutics. Switzerland: S. KARGER AG, Frontiers of Gastrointestinal Research. 2002, 25: 29 – 42.

[12] Rao JN, Li L, Li J, Bass BL, Wang JY. Differentiated intestinal epithelial cells exhibit increased migration through polyamines and myosin II. Am J Physiol 1999; 277: G1149 – G1158.

[13] Parekh AB, Penner R. Store depletion and calcium influx. Physiol Rev 1997, 77: 901 – 930.

[14] Nilius, B, Viana F, Droogmans G. Ion channels in vascular endothelium. Annu Rev Physiol 1997, 59: 145 – 170.

[15] Zhu X, Jiang M, Peyton M, Boulay G, Hurst R, Stefani E, Birnbaumer L. Trp, a novel mammalian gene family essential for agonist-activated capacitative Ca^{2+} entry. Cell 1996, 85: 661 – 671.

[16] Nobes CD, Hall A. Rho, Rac, Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, filopodia. Cell 1995, 81: 53 – 62.

[17] Kiosses WB, Daniels RH, Otey C, Bokoch GM, Schwartz MA. A role for P21-activated kinase in endothelial cell migration. J Cell Biol 1999, 147: 831 – 843.

[18] Amano M, Chihara K, Kimura K, Fukata Y, Nakamura N, Matsuura Y, Kaibuchi K. Formation of actin stress fibers and focal adhesions enhanced by Rho-kinase. Science 1997, 275: 1308 – 1311.

[19] Santos MF, McCormack SA, Guo Z, Okolicany J, Zheng Y, Johnson LR. Rho proteins play a critical role in cell migration during early phase of mucosal restitution. J Clin Invest 1997, 100: 216 – 225.

[20] Hall A. Rho GTPase and the actin cytoskeleton. Science 1998, 279: 509 – 514.

[21] Aelst LV, D'Souza-Schorey C. Rho GTPases and signaling networks. Gene Dev 1997, 11: 2295 – 2322.

[22] Leung T, Chen X-Q, Manser E, Lim L. The P^{160} RhoA-binding kinase ROKα is a member of a kinase family and is involved in the reorganization of the cytoskeleton. Mol Cell Biol 1996, 16: 5313 – 5327.

[23] Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K, Iwamatsu A, Kaibuchi K. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science 1996, 273: 245 – 248.

[24] De Lanerolle P, Paur RJ. Myosin phosphorylation/dephosphorylation and regulation of airway smooth muscle contractility. Am J Physiol 1991, 261: L1 – L14.

[25] Pestonjamasp K, Amieva MR, Strassel CP, Nauseef WM, Furthmayr H, Luna EJ. Moesin, ezrin, P^{205} are actin-binding proteins associated with neutrophil plasma membranes. Mol Biol Cell 1995, 6: 247 – 259.

[26] Rao JN, Guo X, Liu L, Zou TT, Murthy KS, Yuan JX-J, Wang JY. Polyamines regulate Rho-kinase and myosin phosphorylation during intestinal epithelial restitution. Am J Physiol-Cell Physiol 2003; 284: C848 – C859.

[27] Wang JY, McCormack SA, Johnson LR. Role of non-muscle myosin II in polyamine-dependent intestinal epithelial cell migration. Am J Physiol 1996, 270: G355 – G362.

[28] Wang JY, Johnson LR. Luminal polyamines stimulate repair of gastric mucosal stress ulcers. Am J Physiol 1990; 259: G584 – G592.

[29] Banan A, McCormack SA, Johnson LR. Polyamines are required for microtubule formation during gastric mucosal healing. Am J Physiol 1998, 274: G879 – G885.

万方数据