Evidence of K⁺ channel function in epithelial cell migration, proliferation, and repair

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Girault A, Brochiero E. Evidence of K⁺ channel function in epithelial cell migration, proliferation, and repair. Am J Physiol Cell Physiol 306: C307–C319, 2014. First published November 6, 2013; doi:10.1152/ajpcell.00226.2013.—Efficient repair of epithelial tissue, which is frequently exposed to insults, is necessary to maintain its functional integrity. It is therefore necessary to better understand the biological and molecular determinants of tissue regeneration and to develop new strategies to promote epithelial repair. Interestingly, a growing body of evidence indicates that many members of the large and widely expressed family of K⁺ channels are involved in regulation of cell migration and proliferation, key processes of epithelial repair. First, we briefly summarize the complex mechanisms, including cell migration, proliferation, and differentiation, engaged after epithelial injury. We then present evidence implicating K⁺ channels in the regulation of these key repair processes. We also describe the mechanisms whereby K⁺ channels may control epithelial repair processes. In particular, changes in membrane potential, K⁺ concentration, cell volume, intracellular Ca²⁺, and signaling pathways following modulation of K⁺ channel activity, as well as physical interaction of K⁺ channels with the cytoskeleton or integrins are presented. Finally, we discuss the challenges to efficient, specific, and safe targeting of K⁺ channels for therapeutic applications to improve epithelial repair in vivo.

epithelia; injury and repair; K⁺ channels; cell migration; cell proliferation

EPITHELIAL TISSUE acts as a protective barrier and plays a major role in ion and liquid homeostasis. This tissue is exposed to frequent aggressions, which may cause acute or chronic injuries and also trigger tissue remodeling. Epithelial regeneration, necessary to restore epithelial integrity and function, depends on several processes that are engaged sequentially after injury, including cell migration, proliferation, and differentiation, to restore a polarized and functional epithelium (Fig. 1) (16, 32, 61). These complex processes integrate many proteins and mechanisms, which are regulated by various components, including growth factors and downstream signaling effectors (32, 61). Another class of proteins, i.e., K⁺ channels, that exert their main recognized function in epithelium as the control of membrane potential and the maintenance of driving force for transepithelial ion/liquid transport (11, 14, 53) seems also to regulate cell migration and proliferation processes of various cell types, including epithelial cells.

The purpose of our review is, first, to briefly summarize the major mechanisms and proteins responsible for epithelial repair. We then report evidence demonstrating that K⁺ channels are involved in the regulation of repair processes, especially cell migration and proliferation. We also present an overview of the mechanisms whereby K⁺ channels may control these processes. Finally, we report some data indicating a protective role of K⁺ channels after injury in vivo and discuss the challenges to efficient, specific, and safe targeting of K⁺ channels for therapeutic applications to improve epithelial regeneration.

Mechanisms of Epithelial Injury and Repair

Covering epithelial tissue, lining the outside surface of the body (skin and cornea) and the lumen of internal organs (gastrointestinal, urinary, and respiratory tracts), is frequently exposed to insults from external (e.g., pathogens, noxious chemicals, mechanical stress, and trauma) and internal (e.g., sepsis, ischemia-reperfusion, and exacerbated/chronic immune and inflammatory responses) sources. These aggressions trigger cascades of cellular events, culminating in epithelial damage and/or remodeling. The nature and severity of morphological/histological alterations may vary, depending on the source, duration (acute vs. chronic), and intensity of the injury and epithelium localization/type. However, despite this diversity, epithelial tissue will usually respond to damage by engaging multiple cellular events, including cell adhesion, migration, proliferation, and differentiation processes, in an attempt to restore its structural and functional integrity (Fig. 1).

Cell migration is one of the first mechanisms of epithelial repair. This complex phenomenon integrates well-established processes, including cytoskeleton reorganization, membrane protrusion formation, and focal adhesion to the extracellular matrix (ECM) at the front edge and release of adhesion sites at
the rear edge of migrating cells (Fig. 1) (74). Cell movement on provisional ECM and on denuded basement membranes depends on a fine equilibrium via concerted actions of adhesion and de-adhesion proteins (e.g., cadherin, integrins, and proteases) (4, 52, 56, 71, 75, 139). Indeed, numerous matrix metalloproteinases (MMPs) and a disintegrin and metalloprotease domain (ADAM) are involved in ECM remodeling, as well as in release of growth factors, such as EGF. Moreover, integrin and EGF receptor (EGFR) trigger intracellular signaling, which is crucial for cell migration and proliferation.

Cell migration, proliferation, and differentiation processes depend on many cellular events (Ca$^{2+}$/H$^{+}$ signaling, changes in cell volume/shape, and membrane potential), proteins (e.g., integrins and cyclins), and signaling pathways (22, 44, 73, 100, 106, 127, 135, 165). Growth factors [e.g., EGF, transforming growth factor-α, and hepatocyte growth factor (HGF)] play a prominent role by acting via paracrine and/or autocrine pathways and downstream signaling cascades, inducing mitogenic, as well as motogenic and morphogenic, cellular responses (48, 90, 144, 145, 151, 165).

Thus a large number of studies have contributed to knowledge of the mechanisms responsible for epithelial repair and have identified major proteins, factors, and signaling pathways regulating them. However, in addition to these well-defined components, other regulators of epithelial repair have emerged during the last two decades. Among them, ion channels and transporters have been shown to modulate the migration and proliferation of many cells. In the following sections, we present proof of the role of K$^{+}$ channels in the control of repair processes, focusing particularly on epithelial cells.

### Control of Repair Processes by K$^{+}$ Channels

Molecular diversity, regulatory mechanisms, and most recognized functions of epithelial K$^{+}$ channels. K$^{+}$ channels, coded by ~90 different genes, belong to the largest ion channel family and are widely expressed in all organs. An impressive number (~60) of different K$^{+}$ channel subtypes have been detected in epithelial cells (Fig. 2). Only pore-forming α-subunits are represented on phylogenetic trees in Fig. 2; however, numerous auxiliary β-subunits are involved in the formation of
Fig. 2. Molecular diversity of epithelial K⁺ channels. Phylogenetic trees have been generated (see http://www.phylogeny.fr, “treeviewers,” Drawtree) after alignment (with ClustalW software) of amino acid sequences of pore-forming α-subunits of K⁺ channels reported in epithelial cells. Different classes of K⁺ channels are distinguished as voltage-dependent (Kᵥ; A) and Ca²⁺-activated [small-conductance (SK), intermediate-conductance (IK), and Slo; B and B’] 6-transmembrane domain (TMD) channels, 2-pore 4-TMD (K₂P) channels (C), and inwardly rectified, 2-TMD [inwardly rectified (Kir)] channels (D). The exception, KCNMA1 [large-conductance Ca²⁺-activated K⁺ (BKCa), or Slo1], is formed from 7 TMDs. Although KCNT1 and KCNT2 are classified in the Slo Ca²⁺-activated K⁺ channel subfamily, these channels are atypical because of their activation by Na⁺, instead of Ca²⁺. K⁺ channel members are identified by their HUGO Gene Nomenclature Committee designations and International Union of Pharmacology names.
K^+ channels, further increasing their molecular diversity. These channels are classified into different subfamilies on the basis of their structural and functional properties (Fig. 2). Their molecular identity, regulatory mechanisms, and major functions, particularly in the control of membrane potential and maintenance of driving force for transepithelial ion/liquid transport, have been reported in several reviews (11, 14, 53, 55, 93, 96, 157) and are briefly summarized below.

Voltage-dependent K^+ channels. A large number of voltage-dependent (Kv) channels, each formed from four subunits of six transmembrane domains (TMDs), have been detected in epithelial cells (Fig. 2A). However, the functional role of most of these channels in epithelial tissue remains undefined. KvLQT1 (Kv7.1, KCNQ1) channels have probably been the most characterized in epithelial cells, and their functions have been well established. These channels have been associated with control of Cl^- secretion in airway, as well as colon and intestinal, epithelial cells (31, 39, 50, 72, 83, 149), while they have been shown to participate in Na^+ and fluid absorption through alveolar epithelial cells (10, 77). Moreover, pharmacological KvLQT1 channel inhibition in vivo or KCNQ1 gene knockout (KO) in mice has been shown to impair gastric acid secretion (49, 102, 149).

The electrophysiological properties of the KvLQT1 channel are determined by the regulatory subunits (KCNE members) involved in its formation. In epithelial tissues, several lines of evidence indicate that the KCNE3 subunit could be associated with KCNQ1, leading to constitutively activated KvLQT1 channels (17, 50, 125). This channel is inhibited by chromanol 293B and clofilium and is activated by cAMP. Moreover, it has been shown in epithelial cells that the KvLQT1 channel is downregulated by AMP-activated protein kinase (3) or estrogen (97) and upregulated through the EGF-EGFR pathway (144, 145). Growth factors also regulate other Kv channels, for example, in the corneal epithelium, where the 4-aminopyridine (4-AP)-sensitive Kv channel has been shown to be stimulated by EGF and FBS (120).

Ca^{2+}-activated K^+ channels. Ca^{2+}-activated K^+ (KCa) channels, formed from six TMDs (small- and intermediate-conductance channels; Fig. 2B) or seven TMDs (large-conductance Slo1 channels; Fig. 2B’), are expressed in epithelial cells. Many studies have focused on intermediate-conductance KCa3.1 (KCNN4) channels, which are involved in transepithelial ion transport. Indeed, KCa3.1 channel inhibition has been shown to impair Ca^{2+}-activated Cl^- secretion through airway epithelia (13, 31, 82). Conversely, KCa3.1 channel activation has been found to stimulate CFTR- and Ca^{2+}-activated Cl^- channel-mediated Cl^- transport in airway and jejunum epithelial cells (39, 54, 82, 138). Large-conductance KCa (also called maxi-KCa, BKCa, Slo1, and KCa1.1) channels, identified in epithelial cells, play a role in K^+ secretion in colonic and airway epithelia (84, 123, 172), where they have been demonstrated to secondarily favor Cl^- secretion and maintain adequate airway surface liquid volume (84).

As indicated by their name, members of the KCa family are activated by Ca^{2+}, except for the atypical KCa4.1 and KCa4.2 (or Slo2.1, Slo2.2) channels, which are activated by Na^+ and Cl^- (107). Changes in cell volume and secondary Ca^{2+} influx, as well as modifications of pH, regulate BKCa channels (41, 160). Moreover, KCa3.1 and BKCa channels are regulated by many growth factors [e.g., HGF/scatter factor (SF), fibroblast growth factor 2 (FGF-2), EGF, and platelet-derived growth factor], as well as signaling proteins (MEK and Ras), also identified as mitogenic and motogenic regulators (51, 59, 64, 68, 80, 116).

Two-pore domain K^+ channels. Another class of K^+ channels, the two-pore domain K^+ (K2P) channel, is characterized by a two-dimensional structure with four TMDs and two pores. Although several members of this subfamily are represented in epithelia (Fig. 2C), their function has been studied less extensively. For example, it has been reported that these channels modulate Cl^- secretion and Na^+ absorption in airway epithelial cells (174). Furthermore, metabolic acidosis due to deficient renal bicarbonate reabsorption has been observed in TASK-2 (KCNK5) KO mice (158), while impaired regulation of phosphate and water transport in the kidneys has been reported in TWIK-1 (KCNK1) KO mice (91).

K2P channel function is regulated by a wide range of parameters, including intra- and extracellular pH, membrane stretch, temperature, oxygen, and signaling pathways (42, 92, 134).

Inwardly rectified K^+ channels. Finally, several epithelial K^+ channels belong to the inwardly rectified, two-TMD K^+ (Kir) channel subfamily (Fig. 2D). Although different K^+ channels are expressed in the kidneys, renal outer medullary K^+ (ROMK, KCa1.1) channels are probably the best characterized renal K^+ channels, and their role in NaCl absorption has been well established (156). ATP-sensitive K^+ (KATP) channels, encoded by the KCNJ8 gene, are also important in epithelial function. In alveolar cells, these channels control Na^+ and fluid absorption in vitro (10, 76, 77), and KATP channel activation has been shown to favor lung liquid clearance in an ex vivo model (121).

Epithelial KATP channels are sensitive not only to intracellular ATP, but also to pH (12, 60, 85, 146), as well as intracellular tuarine, involved in volume regulatory decrease (19, 94). Conversely, we have reported stimulation of the alveolar and bronchial epithelial KATP channel through an autocrine EGF-EGFR loop (144, 145). Activities of members of the Kir channel subfamily are also controlled via phosphorylation by different protein kinases [i.e., PKA, PKC, and tyrosine kinase] (85, 166, 170).

In summary, epithelial K^+ channels are controlled by a wide range of parameters, which are, in large part, also involved in the regulation of repair processes. We have described some examples of major epithelial K^+ channel functions, especially in transepithelial ion and fluid transport. In subsequent sections, we review the main evidence implicating K^+ channels in epithelial repair processes, especially cell migration and proliferation.

Regulation of cell migration by K^+ channels. Although the function of K^- channels has been less studied in nontumoral cell migration than in cancer cells, several reports (see below) indicate that different types of K^- channels, including Kv, KCa, and Kir subfamilies, could control epithelial cell motility.

In intestinal epithelial (IEC-6) cells, derived from rat intestinal crypts, Kv current inhibition with 4-AP or decreased Kv1.1 expression after polyamine depletion reduced cell migration after wounding (154). Wang et al. (154) also demonstrated that a highly differentiated phenotype in stably Cdx2-transfected IEC-6 cells (IEC-Cdx2L1) was associated with heightened Kv1, (Kv1.1, Kv1.5) channel expression and enhanced cell migration (117). Furthermore, polyamine depletion...
was followed by decreased K⁺ channel expression and reduced IEC-Cdx2L1 cell migration (117).

It appears that growth factor-induced cell migration could be controlled by K⁺ channels. Indeed, in an epithelial tubular cell line (HK2 cells), application of 4-AP or dendrotoxin prevented HGF-induced cell migration (115). Similarly, an effect of 4-AP on basal and EGF-stimulated cell migration was observed in gastric epithelial RGM-1 cells during wound healing (163). In the same cell model, nicotine exposure downregulated Kᵢ.1.1 expression and slowed cell migration (137). Our group reported previously that another Kᵢ. channel, Kᵢ.LQT1 (K.7.1, KCNQ1), regulates the migration of alveolar and bronchial epithelial cells under control and EGF-stimulated conditions (144, 145). Interestingly, upregulation of Kᵢ. channels, especially Kᵢ.2.1, has been found to promote bone marrow stem cell migration (58), which enhances therapeutic interest in targeting Kᵢ. channels, considering the importance of stem cells in epithelial repair.

A role of Kᵢ.C3.1 (SK4, intermediate-conductance Ca²⁺-activated Kᵢ, and KCNN4) channels in Madin-Darby canine kidney (MDCK) cells has been well established (64, 68, 128, 132, 133). It was first demonstrated that Ba²⁺, tetraethylammonium, and charybdotoxin (a Kᵢ.Ca channel inhibitor) inhibited MDCK-F cell migration (133). A relationship between Ca²⁺ oscillation and Kᵢ.C3.1 channel function was then reported in migrating MDCK-F cells (128). Time-lapse videomicroscopy experiments also highlighted a link between FGF-2 signaling and Kᵢ.C3.1 channel function for the stimulation of MDCK-F cell migration (68). Moreover, Jin et al. (64) determined that HGF-stimulated migration of transformed MDCK-II cells was inhibited by charybdotoxin, clotrimazole, and iberiotoxin, indicating the involvement of Kᵢ.C3.1 and BKCa channels. Furthermore, we observed reduced EGF-stimulated migration of normal and cystic fibrosis bronchial cells after Kᵢ.C3.1 channel inhibition (145). Other smaller-conductance Ca²⁺-activated Kᵢ.Ca₂.3 (or SK3, KCNN3) channels seem to participate in normal colon cell migration after wounding (112).

Few studies have examined the function of Kᵢᵦ channels in epithelial cell migration. In alveolar epithelial cells, we noted that Kᵢ.ATP channel activation with pinacidil significantly enhanced cell migration, while glibenclamide reduced it (144). The impact of Kᵢ. channel activation on epithelial cell proliferation has been assessed as well. Interestingly, pinacidil treatment to activate Kᵢ.ATP channels significantly stimulated alveolar cell proliferation (144). Similarly, Braun et al. (18) demonstrated that Kᵢ.ATP channel activation favors the growth of ureteric bud/nephron culture in vivo.

Of interest for tissue regeneration, Kᵢ. channels seem likewise to regulate stem cell growth, as indicated by data showing that human ether-a`-go-go (hEAG) and BKCa channel inhibition or silencing significantly decreased the proliferation rates of mesenchymal stem cells (171, 173).

Cell proliferation and differentiation are interrelated, and the balance between these two processes is finely regulated. Accordingly, it would be interesting to determine if there is a relationship between Kᵢ. channel activity/expression and epithelial cell differentiation/maturation. Kᵢ. channel inhibition with 4-AP and dendrotoxin prevented the morphogenic response to HGF (differentiation and formation of tubular structures) in kidney epithelial cells (115). In addition, modification of Kᵢ.ATP and ROMK channel expression profiles has been observed during kidney development. More precisely, Kᵢ.6.1 (Kᵢ.ATP) mRNA levels are downregulated through the embryonic to postnatal periods, whereas Kᵢ.1.1b (ROMK2) expression is markedly stimulated during cortical collecting duct maturaton (18). Similarly, Nüssing et al. (95) described heightened ROMK protein expression during kidney maturation. These two studies thus indicated differentiation-dependent ROMK expression. Furthermore, it has been shown that highly differentiated intestinal epithelial cells (stably Cdx2-transfected IEC-6 cells) express higher basal levels of Kᵢ.1.1 and Kᵢ.1.5 mRNA and protein than parental IEC-6 cells (117). However, it is not always clear if modifications of Kᵢ. channel expression/function occur subsequent to changes in cell phenotype or, conversely, if Kᵢ. channel modulation could participate in cell differentiation and tissue development processes. In fact, the relationship between Kᵢ. channels and differentiation states has also been studied in nonepithelial cells. Köhler et al. (70) showed that a proliferating phenotype in vascular smooth muscle cells is accompanied by a switch of KCa channels expression profiles from BKCa to Kᵢ.Ca₃.1. Similarly, an upregulation of Kᵢ.6.2 expression has been detected upon osteogenic differentiation of mesenchymal stem cells (40). Hofmann et al. (57) reported data indicating that activation of hEAG-related (hERG) K⁺ currents (Kᵢ.11.1, KCNH2) is a determinant signal for osteoclastic differentiation of leukemic FLG 29.1 cells after adhesion on fibronectin (FN) and integrin upregulation. Similarly, hyperpolarization of human cardiomyocyte progenitor cells after low K⁺ exposure or coculture with Kᵢ.2.1-overexpressing cells permitted cardiogenic differentiation (150). This accumulation of evidence indicates a link between Kᵢ. channel function and cell differentiation; how-
ever, further studies are necessary to clearly define the role of these channels in epithelial cell differentiation and maturation.

Mechanisms responsible for control of cell migration/proliferation by K⁺ channels. Blockade of K⁺ channels by pharmacological inhibitors and/or molecular silencing has demonstrated the crucial function of these channels in epithelial repair processes. Because of the many different K⁺ channels detected in the same cell type, it may have been expected that inhibition of one particular subtype of K⁺ channel should be compensated by another. However, this paradox may be explained by the specific electrophysiological and functional characteristics of each type of K⁺ channel, in particular, its spatial, temporal, and intrinsic regulatory mechanisms (see below). In addition, an impairment of cell migration/proliferation after K⁺ channel inhibition/silencing implies that these channels are already active and/or that they can be recruited/activated following early mitogenic or motogenic signals induced after injury.

In an intact, polarized epithelium, membrane K⁺ channels are localized on the apical or basolateral side, where they elicit distinct functions. After injury, a change in cell polarity in healthy migrating and proliferating cells is accompanied by relocalization of many membrane proteins at the front and rear edges of the cell, thus creating functionally distinct cellular domains. Kᵥ1.4 channels stably expressed in migrating MDCK-F cells are preferentially distributed at the leading edge (119). On the other hand, local applications of charybdotoxin allowed Schwab and colleagues (129, 131) to demonstrate that KCa3.1 channels localized at the rear edge of MDCK-F cells are involved in their movement.

In addition to such spatial distribution, K⁺ channel expression/function is also temporally regulated, including during the cell cycle. For example, the electrophysiological properties of EAG channels in heterologous expression systems (Xenopus oocytes and Chinese hamster ovary cells) are modulated during the cell cycle (25, 105). Similarly, changes in EAG and KCa3.1 currents have been observed among cell cycle phases of tumoral epithelial breast cancer cells, and their inhibition interfered with cell cycle progression and proliferation (101). There is proof that K⁺ channel inhibition impaired epithelial cell proliferation (115, 120, 144, 145), but, to the best of our knowledge, extended evidence of K⁺ channel modulation during the epithelial cell cycle is still lacking.

There are many reasons to believe that K⁺ channels are modulated by factors, also described as mitogenic, motogenic, and morphogenic signals, regulating epithelial repair. Indeed, cell migration, proliferation, and differentiation processes are under the control of many proteins, including receptor protein tyrosine kinases (such as growth factor receptors, e.g., EGFR) and nonreceptor kinases (e.g., FAK, Src, and MEK/ERK) (4, 90, 100, 106, 139, 144, 145, 147, 165). An autocrine EGFR-EGFR loop, subsequently triggering downstream signaling pathways, has been identified during epithelial wound repair (144, 145, 147, 165). We have shown that EGFR stimulation was able to upregulate KᵥLQT1 and KᵥATP channels in alveolar and bronchial cells (144, 145). It has also been reported that EGF and FGF-2 stimulate 4-AP-sensitive Kᵥ currents in rabbit corneal epithelial cells (120) and KCa3.1 channels in MDCK cells (68), respectively. In addition to these signaling pathways, each K⁺ channel subfamily is also controlled by specific signals, such as Ca²⁺ or pH (see Molecular diversity, regulatory mechanisms, and most recognized functions of epithelial K⁺ channels), which are also involved in the regulation of cell migration and proliferation. Regardless of the signals stimulating K⁺ channels during healing, one could question how their function could then trigger epithelial repair processes.

Multiple mechanisms could participate in the regulation of cell migration and proliferation processes by K⁺ channels. One of the first consequences of K⁺ channel modulation is a change in membrane potential (Fig. 3A). It has been proposed that

Fig. 3. Schematic models of possible mechanisms by which K⁺ channels could control cell migration and proliferation processes. A: changes in membrane potential (∆Vm), cell volume/shape, pH (∆pH), Ca²⁺ signal (intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ)), or other signaling pathways could trigger the effect of K⁺ channel modulation on cell migration/proliferation. B: bidirectional autocrine loop linking K⁺ channels to growth factor receptors, such EGFR, may be another control mechanism of epithelial repair by K⁺ channels. C: direct interaction between K⁺ channels and proteins of the migratory machinery such as integrins and cytoskeleton may also occur during wound repair.
transient hyperpolarization after K⁺ channel activation could be essential for G1 phase progression (162). Thus, cell cycle control by K⁺ channels could be one of the mechanisms by which they are involved in cell growth regulation. As reviewed in detail by Felipe et al. (43), K⁺ channel activity is not strictly related to G1 phase progression, since some members may be implicated in the subsequent S and G2 phases. The coupling between K⁺ channel function and proteins regulating the cell cycle has been further evaluated in mesenchymal stem cells. More precisely, it has been observed that BKCa and EAG channel downregulation affected cell proliferation and cycle progression, probably through inhibition of cyclin D1, cyclin E, ERK, and Akt signaling (173). Cellular assays where membrane potential or K⁺ gradient has been modified (by application of a K⁺ ionophore or by decreasing/increasing external K⁺) have demonstrated a link between K⁺ and the proliferation/migration of different cells, including epithelial cells (27, 30, 79, 111, 152, 155). It has been proposed that K⁺ could modulate renal epithelial cell proliferation in another way, i.e., through growth factor release, thus engaging an autocrine stimulation loop (141). In addition, it has been proposed that K⁺, per se, could be considered a second messenger (99), involved in, for example, protein synthesis (23). Changes in extracellular K⁺ concentration, membrane potential, and K⁺ channel activity will also induce pH variations (8, 62), which could, for example, affect integrin function (103). Another consequence of K⁺ flux modulation could be Ca²⁺ fluctuations, another key event for cell migration, proliferation, and differentiation (Fig. 3A). Drawing from their data on intestinal epithelial cells, Wang et al. (154) proposed a model of Kv channel upregulation by polynamines, inducing membrane hyperpolarization, which increases the driving force for Ca²⁺ influx and, consequently, enhances cell migration capacity after wounding. A relationship between cell migration dynamics, Ca²⁺ signaling, and Kv3.1 channel activity has also been established (for review see Refs. 126 and 127). However, experiments on human embryonic kidney (HEK-293) cells expressing a nonconducting Kv3.1 mutant have indicated that Kv3.1 protein, per se, may also directly regulate cell proliferation through ERK1/2 and JNK signaling (88).

Cell shape and volume changes induced, at least in part, by K⁺ channels are crucial for cell movement and division as well (104, 126). It has been shown that Kv1.3 channels are implicated in cell volume regulation and cytoskeleton rearrangement (132, 153). In migrating cells, K⁺ efflux through Kv3.1 channel activation has been proposed to cause shrinkage at the pole of migrating cells, with reorganization of the actin cytoskeleton, promoting their movement (126). Coordinated action of different ion/water channels and transporters is necessary for integrated cell volume control at the front and rear edges of migrating cells (for review see Refs. 127 and 130). The relationship between K⁺ channels and cytoskeleton components seems to be physical and functional. Tian et al. (140) demonstrated interactions between the COOH-terminal pore-forming α-subunit of BKCa channels and cortactin, which created bridges with the cortical actin cytoskeleton. Moreover, the actin-cortactin-BKCa channel complex was shown to regulate BKCa channel activity (140). Similarly, cortactin was found to interact with Kv1.2 channels and to modulate their function and trafficking in HEK-293 cells (161). EAG K⁺ (K10.1, KCNH1) channels established relationships with cytoskeleton elements during the cell cycle. More precisely, microtubule disruption was demonstrated to modify channel rectification during the G2/M phase, while actin cytoskeleton disturbance was accompanied by heightened current density in expression systems (25). In corneal epithelial cells, FN coating enhanced cell adhesion/migration and favored focal adhesion formation at the leading edge. In parallel, FN coating was associated with increased K想办法2.1 channels at the leading edge, compared with BSA coating (69).

Activation of growth factor receptors and downstream signaling pathways may be involved in the control of repair processes by K⁺ channels (Fig. 3B). A relationship between K⁺ channels and growth factor signaling has been seen in many cell types. It has been reported that EGF potentiates acetylcholine-induced K⁺ currents in mucous cells from freshly isolated porcine tracheal glands (63). In our laboratory, we have shown that EGF upregulates KATP and KvLQT1 function in alveolar and bronchial cells (144, 145). Moreover, EGF/EGFR autostimulation of cell migration, proliferation, and wound healing depends on KATP and KvLQT1 channel activity (144, 145). Similarly, EGF-stimulated wound healing of rat gastric epithelial cell monolayers is reduced after Kv1 channel blockade with 4-AP (163). Proliferation of rabbit corneal epithelial cells is regulated by 4-AP-sensitive Kv channels, stimulated by FBS or EGF (120). Similarly, a relationship between HGF/SF and K⁺ channels has been described. Indeed, HGF/SF has been found to kindle Ca²⁺-activated K⁺ currents, necessary for HGF/SF-stimulated MDCK-F cell migration (64). The signaling cascade between Kv1 channels and IGF-I has been studied in HEK-293 cells. Phosphatidylinositol 3-kinase 3-kinase appears to be involved in Kv1 channel activity and upregulation of mRNA expression, a prerequisite for stimulation of cell proliferation (45). Other kinases, such as ERK and JNK, were also reported to participate in coupling between K⁺ channels and mitogenic responses (88).

Several hypotheses have been proposed to explain how K⁺ channels regulate cell migration and proliferation, for example, through modulation of membrane potential, cell volume, intracellular Ca²⁺, and signaling pathways (Fig. 3, A and B). However, because these mechanisms are interlinked, it is difficult to isolate the direct consequence of K⁺ channel modulation on a particular mechanism regulating epithelial repair processes. Further increasing this complexity, it appears that K⁺ channels may also control cell motility by coupling with migratory machinery proteins, such as integrins and associated signaling molecules (Fig. 3C). Indeed, different types of K⁺ channels, for example, BKCa, K1.1, hERG, GIRK, and Kv4.2, interact with integrins (28, 29, 37, 57, 67, 78, 86, 164, 167). Several studies, especially with expression systems, indicate that coupling between K⁺ channels and integrins is functional and bidirectional (for review see Refs. 5, 20, 35, and 109). First, K⁺ channel-integrin complex assembly seems to be modulated by their respective activities. Indeed, it has been shown that interaction between the Kv1.3 channel and β₁-integrin, estimated by resonance energy transfer, is promoted by cell adhesion on FN and inhibited by Kv1 channel blockers (6). In addition, the association between hERG and β₁-integrin is strengthened after β₁-integrin activation (28). Furthermore, β₁- and/or β₃-integrin activation has been found to potentiate, for example, hERG and BKCa currents (28, 57, 67, 164, 167). Integrin might regulate K⁺ channels through...
Ca\textsuperscript{2+} signaling, tyrosine (FAK, Src, EGFR, protein tyrosine kinase-2, and JAK2) or serine/threonine (PKC) phosphorylation, phosphatidylinositol 3-kinase, or G proteins (5, 34, 35, 57, 67, 167). On the other hand, K\textsuperscript{+} channels have been discerned to modulate integrin expression/activation, and it has been postulated that channel opening could be directly transmitted to integrin via conformational coupling or through kinases (FAK and Rac), forming macromolecular complexes with integrin and K\textsuperscript{+} channels (5, 28, 159). Cherubini et al. (28) reported that hERG inhibition prevented FAK phosphorylation and Rac1 activation after \( \beta_1 \)-integrin-mediated adhesion on FN. K\textsubscript{2.1}-FAK complex formation, promoted by FN/integrin, has been identified (159). Interestingly, K\textsubscript{2.1} channel silencing (or mutations in the NH\textsubscript{2}-terminal domain interacting with FAK) reduces FAK phosphorylation, cell migration, and wound closure in vitro, as well as in a mouse model of corneal repair (159). Such functional coupling between K\textsuperscript{+} channels and migration machinery proteins has not been explored extensively in epithelial cells.

**K\textsuperscript{+} channels as a therapeutic target in epithelial repair?** As described above, data from the literature clearly indicate that K\textsuperscript{+} channels are regulators of migration/proliferation of epithelial, as well as progenitor, cells in vitro. These channels could act directly via physical interaction, for example, with integrins, or indirectly through cascades of cellular events, including changes in membrane potential, cell shape/volume, intracellular Ca\textsuperscript{2+}, and other signaling pathways. On the basis of this evidence, it may be postulated that K\textsuperscript{+} channels play a pivotal role in epithelial regeneration for normal renewal, as well as repair after injury. K\textsuperscript{+} channels may thus be identified as potential therapeutic targets, with K\textsuperscript{+} channel activators as promoters of epithelial regeneration. To confirm this hypothesis, good cell and animal models of epithelial repair are necessary. The wound-healing assay, with mechanical or chemical injury, is the most frequently used in vitro model (7, 15, 143, 147, 169). This approach has confirmed that K\textsuperscript{+} channel activities regulate the wound-healing rates of different epithelial cell types (21, 112, 117, 144, 145, 163). The disadvantage of the assay, performed on epithelial cell monolayers grown on plastic supports, is the absence of cell polarity and differentiation. An alternative procedure consists of epithelial cells cultured on permeant filters for better differentiation and follow-up of epithelial regeneration over a longer time period (15). To the best of our knowledge, direct evaluation of the role of K\textsuperscript{+} channels in healing of differentiated epithelial cultures has not been reported. Nevertheless, a recent study by Buchanan et al. (21) showed that lipoxin A\textsubscript{4} increases the wound-healing rate, as well as K\textsubscript{ATP} currents, in polarized bronchial cells cultured at the air-liquid interface. Further evaluation in nonpolarized cultures on plastic supports indicated involvement of K\textsubscript{ATP} channels in the lipoxin A\textsubscript{4}-induced stimulation of wound healing and cell migration and proliferation (21).

Thus, direct or indirect in vitro evidence has provided a proof-of-concept that K\textsuperscript{+} channels could be part of a complex network of regulatory factors influencing wound repair mechanisms. What about their actual involvement in vivo? Several animal models have been developed to study epithelial regeneration after injury, but, to the best of our knowledge, there are no data in the literature proving the role of K\textsuperscript{+} channels in epithelial tissue regeneration using K\textsuperscript{+} channel KO mice. Moreover, regarding the huge diversity of K\textsuperscript{+} channels expressed in epithelia, it is conceivable that alternative K\textsuperscript{+} channels could compensate for the extinction of one K\textsuperscript{+} channel type in vivo. However, Wei et al. (159) showed that treatment of mice with K\textsubscript{2.1} shRNA markedly reduces healing of cornea epithelium after damage. There are also some reports that pharmacological modulation of one class of K\textsuperscript{+} channels has an impact on epithelial injury/repair parameters in vivo. In a model of murine skin injury, it has been shown that openers of K\textsubscript{ATP} and K\textsubscript{Ca} channels or exposure to the K\textsuperscript{+} ionophore valinomycin increased the barrier recovery rate, whereas K\textsuperscript{+} channel blockers slowed it (38). The impact of K\textsubscript{ATP} channel modulation could be mediated, at least in part, through tight junction regulation. Indeed, analysis of the subcellular distribution of K\textsubscript{ATP} channel subunits revealed a colocalization with tight junction proteins in gastric, intestinal, and kidney epithelial tissues (65). The same study also showed that the paracellular intestinal permeability of the intestinal epithelial barrier was altered after K\textsubscript{ATP} channel inhibition, whereas K\textsubscript{ATP} channel activation improved it. In fact, K\textsubscript{ATP} channel function has been quite extensively studied in several gastrointestinal models. The K\textsubscript{ATP} channel opener diazoxide has been shown to attenuate the damage and/or accelerate the restitution of indomethacin- and ethanol-induced intestinal and gastric injury in rats and mice (2, 87, 98, 113, 114, 122, 142), whereas K\textsubscript{ATP} channel inhibition worsened damage parameters. Moreover, the gastroprotective effect of different compounds, such as the steroid saponin hecogenin (122), the antidepressant drug citalopram (124), or prostaglandins (108), seems dependent on K\textsubscript{ATP} channel function. Even if a protective role of the K\textsubscript{ATP} channel has been well established, it is difficult to clearly establish which mechanisms are involved in the phenomenon. Because of the wide distribution of K\textsubscript{ATP} channels, in an animal model, it is indeed complex to clearly define if this beneficial effect is due to the function of K\textsubscript{ATP} channels located at the plasma membrane or in mitochondria, as well as the relative contribution of the K\textsubscript{ATP} channel, for example, on epithelial tissue, the cardiovascular system, or immune cell function. Moreover, this ubiquitous expression of some members of the K\textsuperscript{+} channel family could further increase the possibility of side effects. Thus the development of new approaches for the tissue-specific administration of K\textsuperscript{+} modulators would represent significant progress, with a therapeutic strategy targeting K\textsuperscript{+} channels. Because of the diversity of K\textsuperscript{+} channels in epithelial cells, it will also be important to define the precise role and relative contribution of each K\textsuperscript{+} channel to target the best candidates in each tissue. Finally, before K\textsuperscript{+} channel activators can be administered therapeutically to improve epithelial repair, much work is needed to bypass the lack of specificity of some of these drugs. Indeed, further studies of K\textsuperscript{+} channel three-dimensional structure will allow a better understanding of their binding sites and development of drugs that will be more efficient and specific to each K\textsuperscript{+} channel subtype. Because of the large number of publications and patents involving the use of K\textsuperscript{+} channels as targets in several different diseases (9, 33, 47, 66, 118), there is no doubt that improved tools targeting K\textsuperscript{+} channels will progressively be developed. In parallel, knowledge of K\textsuperscript{+} channel function in epithelial repair and tissue regeneration will probably increase exponentially within the next decade.
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Author Contributions

A.G. and E.B. prepared the figures; A.G. and E.B. drafted the manuscript; A.G. and E.B. edited and revised the manuscript; A.G. and E.B. approved the final version of the manuscript.

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K+ CHANNEL FUNCTION IN EPITHELIAL REPAIR


