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CFTR Involvement in Cell Migration and Epithelial Restitution

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<http://dx.doi.org/10.5772/66309>

Abstract

Over the past decade, research has shown that *cystic fibrosis transmembrane conductance regulator* (CFTR) plays an important role in epithelial cell migration and wound healing. Experiments with airway epithelium, ovarian epithelial cells, placental epithelium and epidermal keratinocytes demonstrated that CFTR function is necessary to achieve maximum migration rates during restitution and in certain cancer cells, CFTR activity contributes to tumor cell invasion. Multiple mechanisms appear to underlie the motility-promoting actions of CFTR, and although many details remain to be established, our present understanding indicates that processes such as electrotaxis (galvanotaxis), integrin-mediated cell adhesion and lamellipodia protrusion are dependent on normal CFTR function. In this chapter, the role of CFTR in epithelial cell migration and its implications in cystic fibrosis (CF) will be reviewed with emphasis on the underlying mechanisms that may explain observations made in various epithelial tissues, particularly in airways. Ultimately, a better understanding of CFTR involvement in epithelial repair may lead to new therapeutic approaches to improve barrier function and reduce airway infection and inflammation associated with CF.

Keywords: cystic fibrosis, CFTR, wound healing, collective migration, barrier function, inflammation

1. Introduction

1.1. Ion channels and cell motility

The role of ion channels and membrane transporters in cell migration has been the subject of several recent reviews [1–4], so only a few examples will be highlighted in this section to provide

the reader with an appreciation of their importance in cell motility. Ion channels and other membrane transport pathways participate in multiple housekeeping functions within cells that include regulation of membrane potential, intracellular $[Ca^{2+}]$, cytoskeletal assembly, integrin-mediated signaling, cell volume regulation, as well as the maintenance of intracellular and extracellular pH. Each of these housekeeping functions can influence cell migration. For instance, changes in ion channel activity produces changes in membrane potential that can facilitate uptake of Ca^{2+} from the extracellular media. A recent example involves the slow calcium wave that develops approximately 1 h after wounding of corneal endothelial cells [5]. The rise in intracellular $[Ca^{2+}]$ is associated with plasma membrane depolarization of cells along the margin of the wound and serves to increase the rate of cell migration. This depolarization has been attributed to increased expression and activity of epithelial Na^+ channels (ENaC) within cells that border the wound, resulting in elevated intracellular $[Na^+]$. The combined effect of depolarization and increased Na^+ load drives Na^+/Ca^{2+} exchange (NCX) activity in reverse mode to produce Ca^{2+} uptake, which propagates from the border of the wound into the epithelium. There may also be an additional role for transient receptor potential (TRP) channels in this process since inhibition of NCX activity does not completely block Ca^{2+} uptake, whereas inhibition of both NCX and TRP activity abolishes the increase in intracellular $[Ca^{2+}]$.

Membrane hyperpolarization can also stimulate Ca^{2+} uptake and enhance the rate of cell migration. Differentiated intestinal epithelial cells with increased expression of voltage-gated K^+ channels (Kv1.1/Kv1.5) exhibit membrane hyperpolarization and increased intracellular $[Ca^{2+}]$ as a result of a greater driving force for electrogenic Ca^{2+} uptake across the plasma membrane [6]. The elevation in intracellular $[Ca^{2+}]$ was shown to augment formation of myosin II containing stress fibers necessary for efficient cell migration. Similarly, ionotropic $P2X_7$ receptors have also been shown to contribute to changes in intracellular $[Ca^{2+}]$ and cell migration. During injury of corneal epithelial cells, $P2X_7$ receptors redistribute to the leading edge of cells that border the wound [7]. Adenosine triphosphate (ATP) is released from the damaged cells leading to activation of these receptors and subsequent uptake of Ca^{2+} from the extracellular solution. The increase in intracellular $[Ca^{2+}]$ induces actin cytoskeletal rearrangements that facilitate the formation of branched dendritic networks of actin within lamellipodia, promoting the dynamic regulation of focal adhesions within cells at the wound margin.

ATP release and $P2X_7$ receptor activation have also been shown to be initiated in response to ligand-activated $\alpha_v\beta_3$ integrin and syndecan-4 engagement leading to increased formation of focal adhesions and an enhanced rate of migration in astrocytes [8]. The mechanism of ATP release involved activation of PI3K, PLC γ and IP $_3$ receptors following integrin activation. This resulted in opening of Cx43/Panx-1 hemichannels in the plasma membrane, facilitating ATP release, transactivation of $P2X_7$ receptors and ultimately, an increase in intracellular $[Ca^{2+}]$. Furthermore, enhanced expression of both $\alpha_6\beta_4$ integrin and TRPV1 receptors at the leading edge of keratinocytes after wounding has also been linked to increases in intracellular $[Ca^{2+}]$. Evidence appears to support a model where TRPV1-mediated increases in intracellular $[Ca^{2+}]$ trigger the activation of transcription factors such as nuclear factor of activated T cells (NFAT) and cAMP response element binding protein (CREB) to stimulate expression of β_4 integrins in cells at the margin of the wound leading to an increase in directional migration [9]. Direct

coupling between the β_1 integrin and $K_{Ca}3.1$ channel expression has been demonstrated in alveolar type II cells grown on a fibronectin matrix and inhibition of channel activity was shown to decrease the rate of migration [10]. This inhibitory effect may be due in part to reducing Ca^{2+} uptake through TRPC4 channels which were also shown to participate in migration during wound repair. TRP channel-associated Ca^{2+} uptake has also been shown to be stimulated by mechanical stretch of the plasma membrane associated with tension and cell shape changes occurring during migration. A specific example involves activation of TRPM7 which mediates transient and highly localized increases in intracellular $[Ca^{2+}]$ known as Ca^{2+} flickers that take place within lamellipodia in response to mechanical forces linked to contraction [11]. This initial Ca^{2+} response is amplified by localized Ca^{2+} -induced Ca^{2+} release from internal stores leading to transactivation of protein kinase A (PKA) through stimulation of Ca^{2+} -sensitive adenylyl cyclases. PKA is known to have multiple cell migration-associated targets including components of the cytoskeleton and the focal adhesion proteome that can have both positive and negative effects on migration depending on intracellular localization.

An interesting example of enhanced cell migration linked to K^+ channel regulation has been reported in glioblastoma cells [12]. In astrocytes and oligodendrocytes from normal brain tissue, the $\alpha_9\beta_1$ integrin is not expressed; however, expression has been shown to increase with glioma grade and appears to be critical for sustaining increased migration rates following exposure to urokinase receptor (uPAR), agonists. A unique feature of the α_9 subunit is that its cytoplasmic domain specifically interacts with spermidine/spermine-N-acetyl transferase (SSAT), which catalyzes the breakdown of higher-order polyamines (spermidine and spermine) to putrescine. Spermidine and spermine are known to regulate the rectification properties of Kir channels by binding to negatively charged residues within the channel pore, significantly reducing K^+ efflux from the cell. In contrast, putrescine is a much less effective blocker of outward K^+ current in Kir channels. In glioma cells, the α_9 subunit colocalizes with Kir4.2 and silencing of the channel inhibits uPAR-enhanced cell migration. A proposed mechanism to explain the increase in migration rate involves activation of SSAT in response to uPAR-dependent $\alpha_9\beta_1$ integrin activation, which produces a localized decrease in the [spermidine/spermine] ratio ultimately leading to reduced rectification, increased K^+ efflux and membrane hyperpolarization.

1.2. Airway inflammation and epithelial damage

Loss of CFTR function in the airways of CF patients leads to reduced anion secretion, enhanced Na^+ absorption and a decrease in the depth of airway surface liquid that ultimately impairs mucociliary clearance and the removal of pathogens from the lungs [13–15]. Reduced pathogen clearance facilitates infection that induces neutrophilic inflammation, leading to progressive epithelial damage within the conducting airways [16–19]. Over time, a recurrent cycle of intense inflammation, epithelial injury and airway remodeling produce irrevocable damage that dramatically compromises lung function [20–22]. Mounting evidence from in vitro studies and animal models of CF indicate that CFTR malfunction appears to alter the innate immune response of the airways leading to increased release of proinflammatory mediators evoking an amplified, yet less effective inflammatory reaction that is unable to

eliminate airway pathogens [17, 18]. In some cases, elevated cytokine levels, neutrophil infiltration and neutrophil elastase (NE) concentrations within the bronchial alveolar lavage (BAL) fluid have been reported in infants without signs of infection, although other studies support the concept that infection is necessary to initiate inflammation [17, 23–25]. Neutrophils represent the major inflammatory leukocyte recruited into CF airways where they release a variety of mediators including oxidants and proteases such as neutrophil elastase (NE), which possess bacteriocidal properties [26, 27]. Moreover, NE catalytic activity is also known to damage the epithelium and reduce structural integrity of the airways leading to bronchiectasis and deteriorating lung function [28–31]. Furthermore, the airways of CF patients encounter various reactive oxygen species (ROS) derived from bacterial pathogens or from the environment [32]. ROS production can exceed the endogenous oxidative defense capacity of the airways leading to oxidative stress and additional injury. In adults, the concentration of reduced glutathione (GSH), a major ROS scavenger present in the airway surface liquid, is significantly reduced in CF patients [33, 34]. This condition may be directly related to the loss of CFTR function since the channel is known to transport GSH in addition to anions in normal airways [35].

Decreases in CFTR channel activity also result in acidification of airway surface liquid coupled to an increase in intracellular pH, which reduces antimicrobial function of the airway surface liquid, promoting bacterial infection [36–41]. Intracellular alkalinization also appears to enhance the accumulation of ceramide, a metabolite of sphingomyelin, within lysosomes [42–44]. Ceramide is thought to amplify the inflammatory response by triggering tumor necrosis factor (TNF) α signaling pathways involving mitogen-activated protein kinases (MAPK), I κ B-kinase degradation [an inhibitory regulator of necrosis factor (NF)- κ B] and NF- κ B nuclear localization [45–47]. Additionally, for class II CFTR mutations, the accumulation of misfolded CFTR protein within the endoplasmic reticulum (ER) induces stress and stimulates what is known as an unfolded protein response, which involves activation of signaling pathways that mitigate ER stress [48–51]. For the most common class II mutation, retention of misfolded Δ F508 CFTR within the ER causes an unfolded protein response that stimulates inflammation by activating NF- κ B and inducing cytokine secretion that can result in apoptosis.

1.3. Evidence for defective epithelial regeneration in CF

In an earlier investigation, a humanized airway xenograph model was created by inoculation of CF and non-CF airway epithelial cells onto epithelium-deleted rat trachea that was then subcutaneously implanted into nude mice over a period from 4 to 35 days [52]. This model was then used to investigate the process of reepithelialization following injury and to determine if remodeling of CF epithelium is a consequence of defective epithelial regeneration independent of infection. The results showed that CF epithelial cells exhibited enhanced proliferation along with continuous expression of IL-8, matrix metalloproteinases (MMP7, MMP9) and tissue inhibitor of metalloproteinase (TIMP)-1. Moreover, regeneration was delayed and final restitution resulted in a remodeled epithelium that appeared to be a product of aberrant regeneration unrelated to bacterial contamination. A relationship between

abnormal regeneration and loss of CFTR function was not identified in this study, although it was concluded that it might be a consequence of altered MMP/TIMP/IL-8 expression observed in CF epithelium. In a subsequent study, wound healing experiments using immortalized normal (NuLi-1 cells) and CF (CuFi-1 cells) human airway epithelial cells revealed that CuFi-1 cell migration was significantly delayed relative to NuLi-1 cells [53, 54]. This difference in migration activity was attributed to defective epidermal growth factor (EGF)/epidermal growth factor receptors (EGFR) signaling and reduced K^+ channel expression. Interestingly, no significant effect on migration was reported in the presence of the CFTR inhibitor, CFTR_{inh}-172 [53]. In subsequent investigations described below, loss of CFTR function was shown to directly contribute to delayed epithelial repair in CF airways and that expression of normal CFTR augments epithelial restitution.

2. Anion channels, cell migration and epithelial restitution

2.1. Volume-sensitive anion channels in cell migration and invasion

Earlier electrophysiological studies of human glioma cells showed that they express voltage-sensitive Cl^- channels that were blocked by chlorotoxin (Ctx), a peptide isolated from scorpion venom as well as tamoxifen, an estrogen receptor modulator [55–57]. Furthermore, hypotonic solutions were also shown to activate tamoxifen and 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB)-sensitive, outwardly rectifying Cl^- currents carried by channels that were shown to contribute to the resting Cl^- conductance under isotonic conditions [55]. Treatment with either Ctx or NPPB inhibited glioma cell migration and invasiveness in transwell migration assays. Similarly, osmotically activated cell swelling and regulatory volume decrease (RVD) were also blocked by Ctx and tamoxifen indicating a role in the regulation of cell volume that contributes to migration and tumor cell invasion [56]. Simultaneous time lapse imaging and patch clamp recording of glioma cells demonstrated detectable changes in cell shape and movement that was associated with activation of volume-sensitive Cl^- currents. Changes in cell shape and motility were attributed to Cl^- efflux coupled to K^+ and water movement across the plasma membrane resulting in cell shrinkage, which appeared to be localized at the leading edge of the cell. Consequently, cell flattening at the leading edge was proposed to facilitate protrusion through restricted extracellular spaces required for tumor cell invasion [58].

Experiments with murine primary microglial cells or a microglial (BV-2) cell line demonstrated that exposure to hypotonic saline or an elevated extracellular $[K^+]$ produced localized swelling and protrusion of lamellipodia at the leading edge of these cells [59]. Blockade of volume-activated Cl^- channels or inhibition of K-Cl co-transporters (KCC) effectively inhibited lamellipodia formation. The migratory response induced by localized increases in extracellular $[K^+]$ may likely result from cell death caused by injury. Ischemia for example, has been shown to increase extracellular $[K^+]$ by more than 20 fold [60]. Such increases in $[K^+]$ would provide a favorable driving force for KCl uptake by KCC leading to cell swelling and produce membrane depolarization. This would establish conditions for electrogenic Cl^- influx through

volume-activated anion channels which also contributes to localized swelling. Furthermore, signaling proteins such as the chemokine ligand CCL21 is released by damaged neurons and is known to induce a chemotaxis response in microglia which is inhibited by Cl^- channel blockers [61]. This response was not dependent on activation of the canonical CCL21 receptor CCR7, but instead was shown to stimulate CXCR3 receptors. Short-duration exposure (30 s) to CCL21 or the selective CXCR3 ligand CXCL10 in either brain slice preparations or microglial cells in culture produced a sustained increase in Cl^- channel activation that appears to represent an initial trigger for stimulating directed cell migration in response to neuronal injury.

2.2. CFTR and epithelial wound repair

The first direct evidence of a role for CFTR in cell migration was obtained from studies of airway epithelial cells [62]. Experiments using Calu-3 cells, a human airway adenocarcinoma cell line and normal human bronchial epithelial cells revealed that inhibition of CFTR channel activity with the selective CFTR blocker, CFTR_{inh}-172 or silencing CFTR expression by RNAi significantly slowed cell migration and epithelial restitution (see **Figure 1**). Moreover, CFTR channel inhibition or silencing also reduced the extent of lamellipodia protrusion during migration. These results demonstrated that the ion transport activity of CFTR was necessary for airway epithelial cells to achieve a maximum rate of migration during wound closure and that lamellipodia protrusion was at least one aspect of the migration process that was affected by the loss of CFTR function. Following publication of this initial investigation, Sun et al. (2011) showed that epithelial wound repair in a tracheal preparation from rhesus monkeys was delayed following treatment with CFTR_{inh}-172 [63]. Experiments employing the use of a noninvasive vibrating probe demonstrated that inhibition of CFTR activity inhibited the spontaneous outward current induced by wounding and that treatment with aminophylline, a phosphodiesterase inhibitor and CFTR activator, stimulated this outward current. These results suggested that CFTR activity contributes to the wound current that serves as a guidance cue for directed migration and that inhibition of CFTR activity disrupts the process of electrotaxis, thus delaying wound closure. Further support for the importance of CFTR in airway cell migration and epithelial restitution was provided by a set of rescue experiments involving (i) expression of wild-type CFTR into CF airway epithelial cell lines to restore the normal rate of wound closure and (ii) treatment with VRT-325, a CFTR corrector molecule that facilitates apical membrane localization of CFTR with the ΔF508 mutation in CFBE- ΔF508 cells and in primary bronchial epithelial cells obtained from CF patients [64].

Involvement of CFTR in cell migration has also been observed in other epithelial cell types besides airways. For example, in human trophoblast (BeWo) cells, CFTR activation with forskolin increased cell migration into the wound and subsequent addition of CFTR_{inh}-172 significantly inhibited the response to forskolin [65]. Poor trophoblast migration/invasiveness and associated spiral artery remodeling represent early recognizable pathologies that underlie preeclampsia and previous studies demonstrated that CFTR expression is reduced in preeclamptic placentas [66]. Thus, changes in CFTR function not only appear to have consequences on placental ion and fluid transport but may also contribute to altered trophoblast invasion in preeclampsia. Another example based on experiments with human ovarian carcinoma cells

showed that CFTR silencing by RNAi significantly reduced cell migration and invasion under in vitro conditions and that the tumorigenic potential of these cells in vivo was suppressed compared to controls [67]. This result was consistent with the observation that CFTR expression in ovarian cancer was higher relative to normal ovarian epithelial cells or benign ovarian tumors and that enhanced CFTR expression was associated with advanced International Federation of Gynecology and Obstetrics (FIGO) staging and poor histopathology grade. Lastly, CFTR was also shown to play a role in cutaneous wound healing, where $\Delta F508cftr^{-/-}$ mice that lack plasma membrane localization and normal CFTR channel function exhibited delayed wound closure compared to wild-type mice [68].

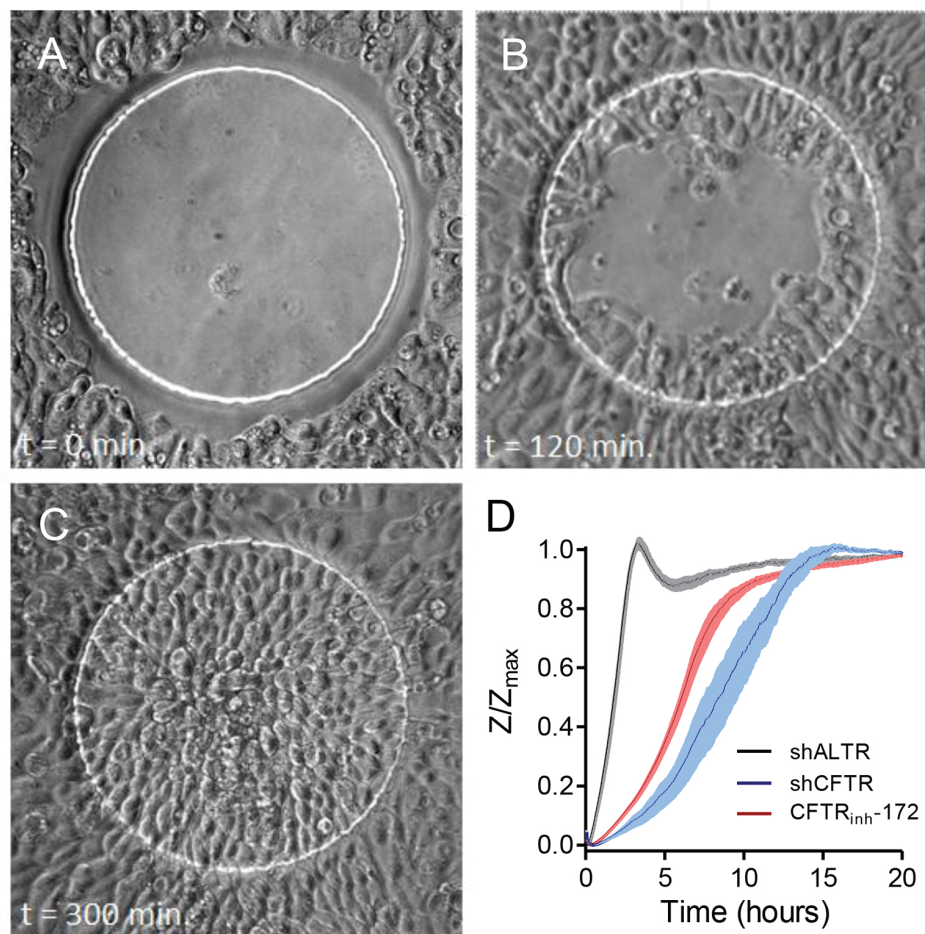


Figure 1. Inhibition of CFTR channel activity or silencing expression by RNAi delay airway epithelial restitution. (A)–(C) Impedance-sensing arrays were used to track the process of Calu-3 cell migration over the surface of a 250 μm diameter electrode following wounding by electroporation. Images show the extent of Calu-3 cell confluence at three time points (0, 120 and 300 min). (D) Normalized impedance (Z/Z_{max}) measurements as a function of time for Calu-3 cells expressing shRNAs designed to selectively target CFTR (shCFTR cells) or have an altered sequence that no longer recognizes CFTR mRNA (shALTR cells). Note that as cells reach confluence on the electrode surface, the normalized impedance value approaches 1, which indicates complete epithelial restitution. For these experiments, shALTR cells were used as controls where the black line represents the mean Z/Z_{max} values and the shaded grey area corresponds to the SEM ($n = 8$). The blue line (mean) and light-blue-shaded area (SEM) shows the effects of silencing CFTR on wound closure, where the slope provides a measure of the average rate of cell migration into the wound ($n = 8$). Finally, the red line (mean) and pink-shaded area (SEM) are the results from shALTR cells treated with 20 μM CFTR_{inh}-172, a selective inhibitor of CFTR channel activity, throughout the process of restitution ($n = 8$). Images were adapted from Ref. [114].

Exceptions to the migration-promoting actions of CFTR can be found in studies of non-small cell lung cancer (NSCLC) cells and human keratinocytes [69]. Experiments with NSCLC cells showed reduced CFTR expression which correlated with an advanced stage of the cancer, lymph node metastasis and enhanced malignant behavior which manifested as an increase in epithelial-mesenchymal transition, invasion and migration. In contrast, overexpression of CFTR reduced cancer progression and metastasis, supporting the observation that in some types of cancer, CFTR appears to function as a tumor suppressor. Similarly, CFTR silencing by RNAi in human keratinocytes was shown to promote cell migration and inhibit differentiation, whereas overexpression inhibited migration and stimulated differentiation [68]. The effects of manipulating CFTR expression on migration appeared to be related to its role in the formation of epithelial junctions since silencing the channel downregulated adhesion molecule (E-cadherin, ZO-1 and β -catenin) expression and intercellular junction formation while overexpression promoted junction formation.

2.3. ANO1, cell migration and cystic fibrosis

TMEM16A/ANO1 is one of the 10 known members of the anoctamin family (TMEM16A-K) of proteins, some of which function as anion channels. Certain members of this family, such as ANO1, ANO2 and ANO6, can be activated by increases in intracellular $[Ca^{2+}]$ and are classified as Ca^{2+} -activated chloride channels (CaCCs) [70–72]. CaCCs exhibit voltage dependence, outward rectification and are perhaps best known for their role in Ca^{2+} -dependent Cl^- secretion in various epithelial tissues. Compounds including T16Ainh-A01, CaCCinh-A01 and NS3728 block channel activity to varying degrees depending on cell type [73]. Prior to the discovery of its anion channel activity, ANO1 was regarded as either a tumor cell marker or as an oncogene in human cancers with poor prognosis [74, 75].

In prostate cancer (LNCaP and PC-3) cells, ANO1 is highly expressed and these cells exhibit large CaCC currents in response to increases in cytosolic $[Ca^{2+}]$ [76]. Silencing ANO1 by RNAi in PC-3 cells significantly inhibited cell proliferation and migration/invasion. Studies using Ehrlich Lettre ascites (ELA) cells revealed that they express both ANO1 and ANO6 [71]. Interestingly, silencing ANO1 expression was shown to alter directionality of ELA migration while knockdown of ANO6 was shown to cause a $\sim 40\%$ decrease in the overall rate of migration. Although the mechanism responsible for ANO1-dependent control of directionality is not understood, it is likely that some contribution to outward current associated with wounding may be important in electrotaxis. Various pancreatic ductal adenocarcinoma cells have also been shown to have increased expression of ANO1 and enhanced CaCC activity. Knockdown of ANO1 or inhibition by CaCC blockers including CaCCinh-A01, and NS3728 delay migration in BxPC-2 cells, however, T16Ainh-A01 exhibited no effect [77]. The authors speculated that activation of ANO1 was important for cell volume changes necessary to control cell shape and that the channel may serve as a potential target for reducing the metastatic potential of pancreatic tumor cells.

Investigations of bronchial epithelial cell repair in cystic fibrosis (CF) demonstrated that the expression of ANO1 and CaCC channel activity were significantly reduced in CF cells compared to bronchial epithelial cells from normal subjects [78]. Consequently, epithelial

restitution in wound healing assays was delayed in CF cells relative to non-CF cells. Moreover silencing ANO1 expression in non-CF cells reduced the rate of migration, whereas overexpression of ANO1 in CF cells partially restored cell motility, although complete recovery was not achieved. To establish whether ANO1 channel function was necessary for supporting cell migration, primary non-CF cells were treated with T16Ainh-A01 which produced a significant delay in wound closure. These findings indicate that reduced rates of cell migration in bronchial epithelial cells from CF patients may be attributed to an overall decrease in apical membrane Cl^- conductance resulting from loss of both CFTR and ANO1 anion channel activity.

3. Mechanisms of CFTR-dependent cell migration and epithelial repair

Although the molecular mechanisms underlying the contribution of CFTR to the processes of cell migration and epithelial restitution remain to be fully characterized, the data collected so far have identified three important aspects of migration that merit further investigation. These include the process of lamellipodia protrusion, electrotaxis and the dynamics of integrin-mediated adhesion, each of which are discussed in more detail below.

3.1. Lamellipodia protrusion

Lamellipodia are actin-containing, sheet-like structures that protrude from the leading edge of migrating cells [79]. They are capable of sensing environmental cues and are necessary for sustained directional migration. A key force contributing to the protrusion of lamellipodia is provided by the extension of actin filaments at the leading edge of the cell. Within lamellipodia, actin forms networks of branched filaments with highest density near the membrane at the leading edge, where the barbed (positive) ends of the filaments are directed toward the plasma membrane to form brush-like assemblies [80, 81]. Elongation occurs primarily at junctions formed by a multiprotein structure known as the Arp2/3 complex, which functions as a nucleation site for new actin monomers to attach to the sides of existing actin polymers to create a branched arrangement of fibers [82]. As these monomers add to the growing meshwork at the barbed end, cleavage and dissociation of monomers takes place at the pointed (minus) end of filaments located in the more proximal regions of the lamellipodium. ATPase activity associated with actin filaments facilitates accumulation of ADP-actin at the pointed ends as filament disassembly takes place. This dynamic process of simultaneous actin monomer addition to the barbed end and dissociation at the pointed end of the filament is known as treadmilling and is controlled by several actin-regulatory proteins [82, 83] as well as intracellular pH [84, 85]. Previous studies have shown that during polarization along the axis of movement, a redistribution of the $\text{Na}^+\text{-H}^+$ exchanger (NHE1) occurs, which localizes toward the leading edge of the cell. Redistribution of NHE1 results in the development of a steady-state pH gradient extending from the front of the cell, which becomes more alkaline, to the rear, developing a more acidic pH relative to the leading edge [86–88]. A key regulator of polarization is Cdc42, a small guanosine-5'-triphosphatase (GTP)ase that accumulates at the leading edge where it stimulates actin polymerization. Cdc42 activation is pH sensitive, requiring NHE1 activation and proton efflux to produce localized alkalization of the

cytoplasm to enhance its activity [86]. Moreover, alkalization also promotes F-actin cleavage by cofilin, an actin binding protein that facilitates treadmilling by causing depolymerization at the pointed ends of actin filaments [87]. Other acid extruding or base loading transport mechanisms could potentially contribute to this alkalization process, including CFTR and its ability to conduct bicarbonate ions, provided that a favorable electrochemical driving force exists.

As previously mentioned localized osmotic swelling can also contribute to the force that powers lamellipodia protrusion [59]. Solute uptake serves as a driving force for fluid uptake into the cell, often involving electroneutral transporters that couple cation uptake with Cl^- transport (e.g. KCC or NKCC cotransporters). It is also possible that if the plasma membrane is depolarized to a voltage that is more positive than the reversal potential of anion channels such as CFTR or ANO1, then the inwardly directed Cl^- concentration gradient would facilitate influx, setting up a favorable osmotic gradient for fluid uptake and lamellipodia protrusion. Whether Cl^- influx or efflux is occurring at the leading edge may not be predictable, since this would depend on the activity of multiple electrogenic transport pathways or conditions associated with injury. It is worth emphasizing that depending on the electrochemical gradient for Cl^- , CFTR could contribute instead to retraction taking place at the rear of the cell. In this case, efflux of Cl^- , perhaps coordinated with K^+ channel activity, would enable localized solute and fluid exit at the trailing edge of the cell, promoting forward movement [3].

3.2. Electrotaxis

Epithelia engaged in active electrolyte transport typically generate spontaneous transepithelial potentials (TEP) that provide an electrical driving force for paracellular ion movement across the epithelium [89]. Following wounding, the TEP at the site of the wound collapses as laterally oriented electric fields develop with the cathode (negative pole) located at the center of the wound. In dermal, corneal and airway epithelia, for example, outward current can be detected using a noninvasive technique that employs a self-referencing vibrating probe [90]. Many epithelial cell types respond to wound-induced electric fields by migrating toward the cathode although some cell types exhibit anodal migration in response to electric field stimulation [91, 92]. In fact, changing the polarity of the field will reverse the direction of migration. In experiments with primate tracheobronchial epithelial cells, an applied electric field with a threshold intensity of 23 mV/mm was effective at stimulating migration with a displacement speed that increased with field strength [63]. The displacement speed reflected greater migration efficiency and in the case of tracheobronchial epithelial cells, the increase in speed primarily resulted from improved directionality, which was quantitatively expressed using a directedness parameter for the migrating cells. Directedness was expressed as the angle (θ) that individual cells moved relative to the electric field vector, where $\cosine \theta$ was defined as the directedness value. Cells moving randomly in the absence of an electric field have an average directedness value near zero, whereas those that move entirely along the electric field lines toward the cathode have a value approaching 1. Experiments with tracheobronchial epithelial cells showed that directedness increased with increasing field strength such that when the voltage achieved 90 mV/mm, a number of the cells migrated directly toward the

cathode. The finding that inhibition of CFTR reduces the electric field evoked by wounding and that CFTR inhibition or silencing decreases lamellipodia protrusion [62], strongly suggests that CFTR activity plays an important role in sustaining directed migration in airway epithelial cells.

The cellular mechanisms underlying the increase in directed migration induced by wound-evoked electric fields are complex and cell type dependent. Studies of corneal epithelial cells and keratinocytes, for instance, revealed changes in the localization of epidermal growth factor receptors (EGFR) toward the cathode-facing borders of migrating cells, and in at least one study, EGFRs appeared to be activated by the electric field independently of ligand binding to the receptor [93–97]. Moreover, inhibition of EGFR-MAPK signaling was shown to alter the actin cytoskeleton at the leading edge and diminish directed migration of epithelial cells, demonstrating an important role for EGFR in detecting and initiating the epithelial response to electric field stimulation [96]. Additionally, electric fields can redistribute and activate PI3K/Akt signaling in a polarized manner at the cathode-oriented leading edge of the cell [98]. When PI3K is activated, membrane protrusion and lamellipodia formation is initiated at that site, facilitating directed migration toward the cathode. Pharmacological inhibition of PI3K activity or selective disruption of the PI3K- γ isoform has been shown to block electrotaxis in wound healing assays and organ cultures [98, 99]. Furthermore, in keratinocytes, deletion of phosphatase and tensin homolog (PTEN), a phosphatase that functions as a negative regulator of PI3K, resulted in increased Akt phosphorylation and enhanced electrotaxis. Other kinases linked to the control of cell motility such as extracellular signal-regulated kinase (ERK) have also been shown to be involved in electric field-evoked migration [97, 100, 101]. In experiments with glioma and fibrosarcoma cells, electric field stimulation induced NADPH oxidase activation, resulting in the production of reactive oxygen species (ROS) [100]. Intracellular accumulation of ROS stimulates ERK phosphorylation/activation which leads to reorganization of the cytoskeleton and an increase in directed migration [101]. Based on these observations, it appears likely that further studies will uncover additional molecular targets and signaling pathways involved in the detection and regulation of directionality by injury-induced electric fields.

3.3. Dynamics of integrin-mediated adhesion

A recent study examining the consequences of CFTR silencing on cell migration and epithelial repair in human airway (Calu-3) epithelial cells demonstrated a ~60% reduction in GM1 ganglioside content within the plasma membrane of CFTR deficient cells compared to controls [102, 103], which was restored following expression of wild-type CFTR. Similarly, treatment of cells with the selective CFTR blocker, CFTR_{inh}-172, also produced comparable reductions in GM1 content in cells expressing wild-type CFTR. These observations were consistent with earlier studies showing reduced levels of sialylated gangliosides in cells expressing CFTR with the Δ F508 mutation [104, 105]. Furthermore, previous investigations have also shown that gangliosides are capable of regulating integrin signaling and cell migration [106, 107]. Experiments with Calu-3 cells revealed that CFTR knockdown did not directly affect β_1 integrin surface expression; however, the level of activated β_1 integrin was

significantly lower than observed in CFTR expressing control cells [102]. β_1 -integrin activation could be completely recovered by incubating CFTR deficient cells with exogenous GM1, but not with GM3 gangliosides, confirming that integrin activation was dependent on GM1 and CFTR expression. Reduced β_1 integrin phosphorylation was associated with lower levels of focal adhesion kinase (FAK) and Crk-associated substrate (CAS) phosphorylation which was

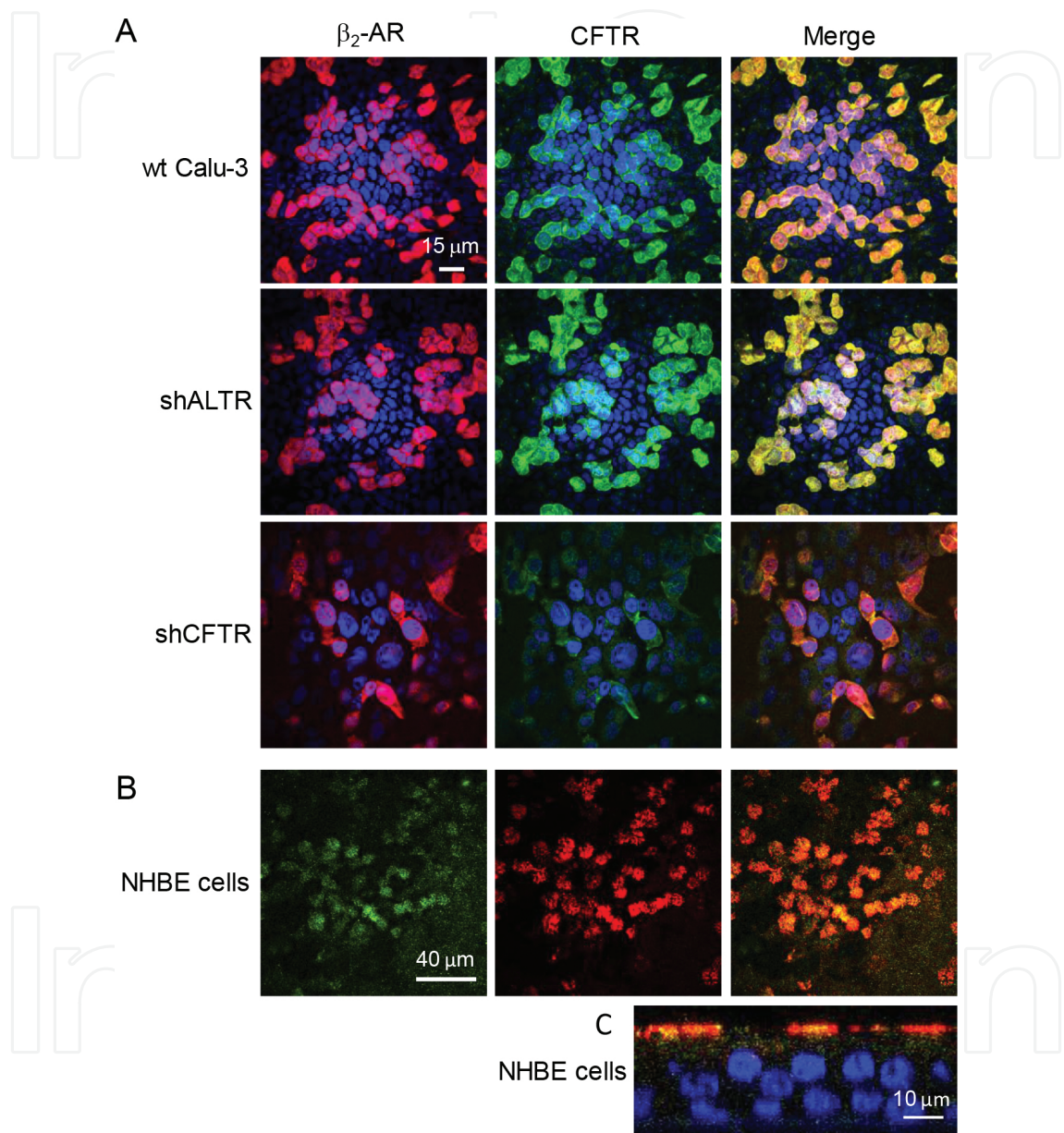


Figure 2. Colocalization of CFTR and the β_2 -adrenergic receptor (β_2 -AR) in the apical membrane of Calu-3 cells and in cilia of differentiated primary normal human bronchial epithelial (NHBE) cells grown under air-liquid interface conditions. **(A)** Antibody labeling of the β_2 -AR, CFTR and merged images (where yellow represents colocalization) collected from wild-type Calu-3 cells (wt Calu-3), Calu-3 cells expressing shRNA that does not recognize CFTR (shALTR) and CFTR-deficient cells where CFTR expression was silenced by RNAi (shCFTR). **(B)** Labeling of the β_2 -AR and CFTR within the cilia of differentiated primary NHBE cells. Yellow-orange represents colocalization of the receptor and channel. **(C)** Cross section of differentiated, pseudostratified primary NHBE cells showing colocalization of the β_2 -AR and CFTR within the cilia. Note the layering of nuclei reflecting pseudostratification. Images were adapted from Refs. [114] and [115].

also restored by incubation with exogenous GM1 ganglioside. A possible explanation for the reduction in β_1 activation may be related to loss of localization to specific membrane microdomains that function as integrin signaling platforms. This would be consistent with GM1 localization within lipid raft domains where it is known to associate with CFTR [108, 109]. Moreover, recovery of FAK and CAS phosphorylation along with β_1 integrin activation with GM1 repletion also produced partial restoration of cell migration, suggesting that reduced integrin engagement with the extracellular matrix, presumably at the leading edge of the cell, accounts for at least part of the effect that loss of CFTR expression or inhibition of channel activity has on epithelial restitution.

More recently, stimulation of β_2 -adrenergic receptors (β_2 -AR) expressed on the apical membrane of normal human bronchial epithelial cells and Calu-3 cells caused a significant delay in cell migration and wound closure. This effect could be reproduced using carvedilol, a β_2 -AR agonist that functions as a bias ligand to activate cAMP-independent, β -arrestin-dependent signaling cascades [110]. The inhibitory effects of β_2 -AR agonists could be blocked if cells were pretreated with an inhibitor of PP2A phosphatase, indicating that PP2A activation was a critical step in regulating cell migration [111, 112]. Interestingly, in airway epithelial cells, β_2 -ARs form a tightly coupled signaling complex with CFTR in the apical membrane (see **Figure 2**) such that receptor activation by endogenous ligands, such as epinephrine, or by selective β_2 -AR agonists, like salbutamol, stimulate CFTR channel activity [113]. The reduced rate of migration following β_2 -AR activation was associated with a reduction in lamellipodia protrusion, similar in magnitude to the effect produced by CFTR channel inhibition with CFTR_{inh}-172 or silencing by RNAi. Furthermore, β_2 -AR agonists, including carvedilol, decreased β_1 -integrin activation, and in CFTR-deficient Calu-3 cells, β_2 -AR activation had no effect on cell migration [114]. These findings suggested a model where exposure to β_2 -AR agonists stimulates PP2A phosphatase

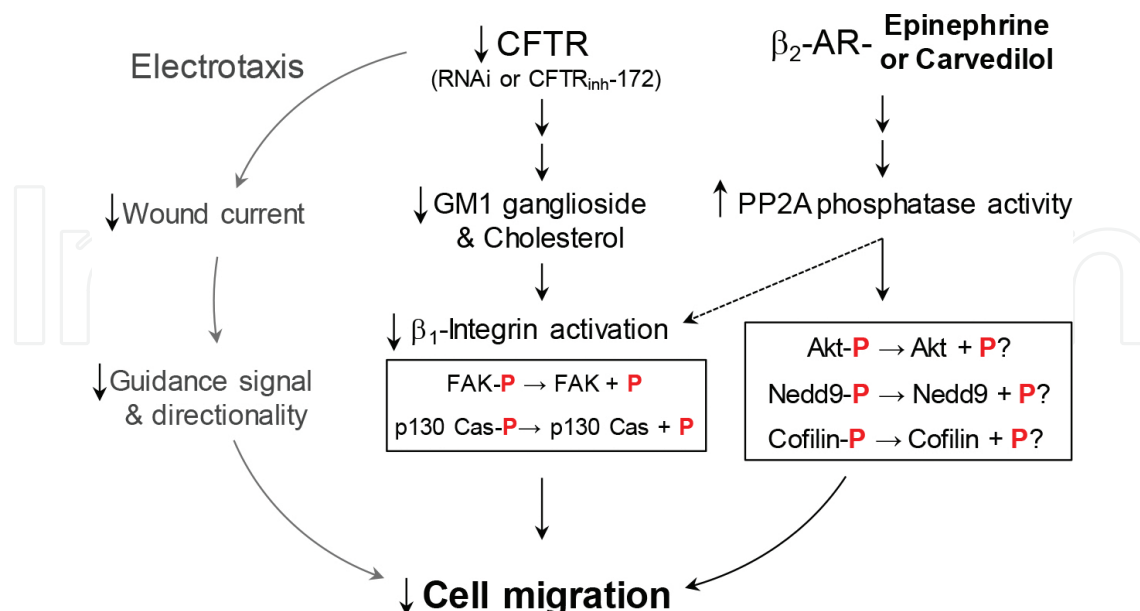


Figure 3. Summary of proposed interactions and pathways accounting for the decrease in cell migration and epithelial repair associated with loss of CFTR function and β_2 -AR activation in airway epithelial cells. This model was adapted from Ref. [114].

activity to produce dephosphorylation of multiple proteins involved in the control of cell motility (see **Figure 3**). Moreover, CFTR inhibition or silencing and β_2 -AR stimulation appear to converge on a common control point involving the activation of β_1 integrin, which is thought to be the reason why CFTR silencing and β_2 -AR activation do not produce additive effects on cell migration.

4. Conclusions

This chapter has focused on the impact of CFTR dysfunction on cell migration and epithelial repair that has direct relevance to airway barrier function. This role for CFTR constitutes an important intrinsic deficit of the CF epithelium that contributes to disease progression. Other intrinsic deficits such as those linked to altered innate immune function appear to underlie abnormal regeneration and remodeling of the CF epithelium that occurs in the absence of infection. Delayed wound repair exacerbates intrinsic inflammation by providing opportunities for pathogen access to the airway submucosa, augmenting inflammation and tissue damage. Furthermore, dysregulation of the repair process establishes a chronic cycle of injury and inadequate restitution that intensifies remodeling, ultimately leading to deterioration of lung function. Further investigation is required to more clearly understand the molecular and cellular mechanisms by which CFTR expression and function affect cell motility. Results from these studies should aid in identifying pathways that could be targeted for development of novel pharmacotherapies to reduce airway infection and inflammation in CF.

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