# Orchestrating Airway Smooth Muscle Cell Migration: GMFγ Phosphorylation Is the Key

Smooth muscle cell (SMC) migration is a critical developmental process that occurs during the formation of hollow organs such as blood vessels and airways (1). However, cell migration has also been implicated in the pathobiology of asthma (2, 3). In asthma, airway SMC (ASMC) mass increases, contributing to airway remodeling and bronchoconstriction (2). This increase in mass is believed to result from the combined contributions of ASMC hypertrophy and hyperplasia, as well as the migration of ASMCs derived from circulating hematopoietic stem cell populations and the interstitial compartment (2). Cell migration is initiated by signals from the extracellular matrix and begins with the formation of the lamellipodia, a protrusion on the leading edge of the cell that forms through dynamic reorganization of the actin cytoskeleton (4). Next, focal adhesions are formed toward the front of the cell, strengthening attachment to the extracellular matrix (4). At the rear of the cell, the actin cytoskeleton and old focal adhesions are

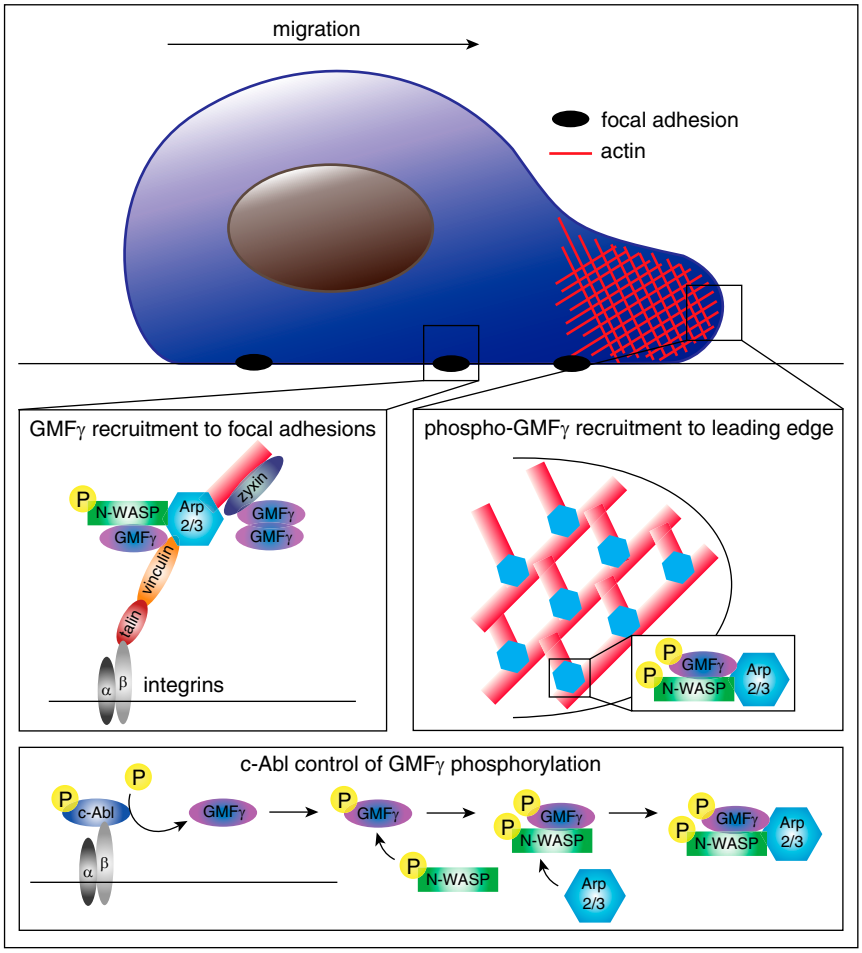


**Figure 1.** During cell migration, the actin cytoskeleton undergoes reassembly and actin branch formation at the leading edge to form the lamellipodia, and new focal adhesions form (top). Phosphorylated GMFγ (glia maturation factor γ) is recruited to the leading edge, where it is associated with the Arp2/3 (actin-related protein 2/3) complex and N-WASP (neural Wiskott-Aldrich syndrome protein; middle right). Nonphosphorylated GMFγ is recruited to focal adhesions, where it associates with N-WASP, vinculin, and zyxin to promote focal adhesion assembly (middle left). Phosphorylation of GMFγ is mediated by c-Abl (ABL proto-oncogene 1). In response to external signals, c-Abl is activated and phosphorylates GMFγ, which then recruits phosphorylated N-WASP and associates with the Arp2/3 complex to promote actin branch formation. P = phosphorylated.

disassembled, causing retraction. Together, these processes create a mechanical force to propel the cell forward (4).

In this issue of the *Journal*, Gerlach and colleagues (pp. 219–231) provide insight into the signaling mechanisms behind the regulation of lamellipodial and focal adhesion dynamics in human AMSCs (HASMCs) (5). Actin network reorganization leading to lamellipodia formation is regulated by the Arp2/3 (actin-related protein 2/3) complex, which is activated by N-WASP (neural Wiskott-Aldrich syndrome protein), a nucleation-promoting factor (3, 4). Further upstream is the nonreceptor tyrosine kinase c-Abl (ABL proto-oncogene 1), which is upregulated in asthmatic HASMCs (6–9). Previous work by this group showed that c-Abl controls actin reorganization through activation of GMFγ (glia maturation factor γ), which binds to the Arp2/3 complex to initiate actin branch disassembly (10). Specifically, they found that c-Abl phosphorylates GMFγ at Tyr-104 during contractile activation, causing GMFγ to dissociate from the Arp2/3 complex, thereby halting actin disassembly (10). With the current study, Gerlach and colleagues build on their previous work to show that phosphorylation of GMFγ at Tyr-104 by c-Abl controls normal HASMC migration through regulation of lamellipodial and focal adhesion dynamics (5). They first found that knockdown of GMFγ in HASMCs decreased the speed and distance of HASMC migration. Furthermore, they were able to rescue motility through transfection of a Y104 phosphorylation mimic mutant of GMFγ, whereas the nonphosphorylated mutant of GMFγ did not restore cell migration (10). The authors used confocal microscopy and three-dimensional reconstruction to examine the spatial distribution of GMFγ and N-WASP within HASMCs. Both GMFγ and N-WASP were localized to the leading edge of the lamellipodia and to focal adhesions within the cell. Further experiments demonstrated that GMFγ interacted with both N-WASP and vinculin, a focal adhesion marker, and that GMFγ recruited N-WASP to focal adhesions, suggesting a role for GMFγ in the regulation of focal adhesion growth. Similarly, the authors showed that GMFγ was required for N-WASP colocalization with Arp2 at the leading edge of lamellipodia. In addition, the authors used live HASMCs to examine the effect of GMFγ phosphorylation on focal adhesion dynamics by monitoring labeled paxillin, another focal adhesion–associated protein found in both nascent and mature focal adhesions, and zyxin, a protein associated only with mature focal adhesions. The nonphosphorylated form of GMFγ was associated with significantly greater stability and clustering of focal adhesions, as well as recruitment of zyxin to adhesions, promoting focal adhesion maturation. Conversely, the phosphorylated form of GMFγ was localized to nascent focal adhesions. Additionally, the authors found that actin architecture in lamellipodia was regulated by GMFγ phosphorylation, as expression of the nonphosphorylated form of GMFγ reduced actin branching, whereas phosphorylated GMFγ increased actin branching and cytoskeleton reorganization to promote lamellipodia protrusion and reduce retraction. Importantly, specific inhibitor experiments indicated that myosin activity plays a role in c-Abl and GMFγ phosphorylation and the distribution of phosphorylated or nonphosphorylated forms of GMFγ to nascent or mature focal adhesions (Figure 1). Thus, the spatial localization and phosphorylation state of GMFγ determine the direction and speed of HASMC migration by regulating a host of cellular events.

Finally, Gerlach and colleagues offer a hint that these processes may also be involved in HASMC migration in asthma. They show that expression of both total and phosphorylated GMFγ was increased in primary asthmatic HASMCs, as was migration speed and distance. Additionally, expression of nonphosphorylated GMFγ in these cells inhibited migration. These data show that GMFγ phosphorylation regulates migration in asthmatic HASMCs; however, more work is needed to definitively show that phosphorylation of GMFγ by c-Abl controls lamellipodial and focal adhesion dynamics in asthmatic cells, as normal HASMCs were used for the majority of the current study's experiments. Examining the localization of c-Abl, GMFγ, and N-WASP in asthmatic HASMCs could elucidate important differences in signaling present in asthma that influence HASMC migration and contribute to airway remodeling. Also, previous work by this group implicated contractile force as an agonist to induce c-Abl–mediated GMFγ phosphorylation (10). It is important to understand how the c-Abl/GMFγ pathway is initiated in HASMCs in the context of airway hyperresponsiveness in asthma.

In summary, the authors provide new insight into a specific cellular pathway that directs HASMC migration. Through elegant experiments using high-resolution microscopy and three-dimensional image analysis, they show that enrichment of phosphorylated GMFγ at the leading edge of migrating HASMCs promotes cell protrusion, and accumulation of nonphosphorylated GMFγ at focal adhesions increases focal adhesion maturation. With continued exploration into the role of c-Abl and GMFγ in the migration of asthmatic HASMCs, these data could provide the groundwork for new asthma therapies targeting cell migration. ■

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