MINREVIEW

Signaling mechanisms for regulation of chemotaxis

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ABSTRACT

Chemotaxis is a fascinating biological process, through which a cell migrates along a shallow chemoattractant gradient that is less than 5% difference between the anterior and posterior of the cell. Chemotaxis is composed of two independent, but interrelated processes-motility and directionality, both of which are regulated by extracellular stimuli, chemoattractants. In this mini-review, recent progresses in the understanding of the regulation of leukocyte chemotaxis by chemoattractant signaling are reviewed.

Keywords: chemotaxis, leukocyte, signaling, chemoattractant.

INTRODUCTION

Leukocyte chemotaxis is regulated by a number of chemoattractants include bacterial by-product fMLP, complement proteolytic fragment C5a, and the superfamily (~50) of small (8-10 kDa), inducible, secreted, pro-inflammatory cytokines called chemokines [1, 2]. These chemoattractants act as immediate mediators of inflammatory responses by regulating leukocyte recruitment, infiltration, homing, and trafficking as well as their development and function [3]. While inflammation plays an important role in host defense, uncontrolled inflammatory reactions are responsible for initiation and progression of many human diseases, including atherosclerosis, ischemia-reperfusion injury, virus-induced myocarditis, rheumatoid arthritis, allergic reactions, psoriasis and other inflammatory skin conditions, and even tumorigenesis and tissue-targeted metastasis [4-7]. However, the lack of specific therapeutic agents has impeded effective treatment of these inflammatory conditions. Thus, a better understanding of the regulation of leukocyte chemotaxis by chemoattractants may provide novel therapeutic targets or strategies to treat these diseases.

CHEMOATTRACTANT SIGNALING PATHWAYS

Chemoattractants bind to their specific cell surface receptors and mainly activate the Gi proteins in leukocytes [8]. The Gi proteins belong to the family of heterotrimeric G proteins, which consist of three subunits: α, β, and γ. The a subunits contain GTPase activity. Ligand-bound receptors promote the exchange of GDP with GTP on the a subunits, and the GTP-bound forms of Gα subunits are subsequently dissociated from the $\beta\gamma$ subunits. While the a subunits of the Gi proteins inhibit adenylyl cyclases, the G$\beta\gamma$ subunits can regulate a number of effectors, including phospholipase (PLC) β2/3, phosphatidylinositide 3 (PI3K)γ, ion channels, G protein-coupled receptor kinases, and some subtypes of adenylyl cyclases [9]. We recently added PAK1 to the list by demonstrating the interaction between G$\beta\gamma$ and PAK1, which is involved in chemoattractant-mediated activation of Cdc42 and PAK1 [10]. Chemoattractants can also couple to G16 [11] and probably G12/13 [12], which have been shown to activate small GTPase RhoA via a guanine nucleotide exchange factor (GEF) 115 [13-15]. In the past ten years, many of the chemoattractant signaling pathways were comprehensively characterizing using a combination of biochemical, molecular and cell biological, and transgenic approaches. These pathways are summarized in Fig. 1A.

SIGNALING MECHANISMS IN THE REGULATION OF DIRECTIONALITY

Chemotaxis is a fundamental biological process in which a cell migrates following the direction of a spatial cue. This spatial cue is provided in a form of a gradient of chemoattractants. Cells ranging from prokaryotic bacteria to eukaryotic mammalian cells possess, to varying degrees, an ability to interpret the gradient, but through diverse mechanisms. For a bacterial cell, it is mainly a trial-and-error process in which the cell tumbles around and senses

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the surrounding periodically. If it tumbles into a location that contains a higher concentration of a chemoattractant, it continues in that direction for a while longer. If it senses that it has moved to a location that contains a lower level of a chemoattractant, it will change direction. By this trial-and-error approach, the bacterial cell would eventually move up the gradient. The cells of higher organisms develop more sophisticated machineries to sense and process the information conveyed by the chemical gradient. These machineries allow a cell to tell small differences in chemoattractant concentration between its two ends (usually only a few percent) and translate this small difference into a much greater intracellular biochemical gradient. Neutrophils and *Dictyostelium* cells are two fine model systems for studying chemotaxis, both of which can efficiently interpret and chemotax under a shallow gradient of chemoattractants to allow observing, recording and analyzing their migration quantitatively using videomicroscopy. These analyses provide the means to discern the roles of signaling mechanisms in the regulation of directionality from those in regulating motility.

MECHANISM OF CELL POLARIZATION IN REGULATION OF CHEMOTAXIS

For a neutrophil to move even in a random migration, the cell has to polarize by forming F-actin-rich lamellipodia at the direction of the migration, because continuous formation of F-actin at the leading edge drives cell locomotion. Chemoattractants augment and stabilize cell polarization by stimulating F actin polymerization particularly in the lamellipodia; thus, the cell becomes highly polarized (Fig. 1B). When a chemoattractant gradient is present, the cell can sense the gradient and align its polarity with the gradient to migrate directionally up the gradient. Therefore, cell polarization provides a link between the two basic components of chemotaxis-motility and directionality. One of the characteristics of a highly polarized cell is its elongated shape with a more blunted leading edge and narrower posterior, resulting from the formation of F actin-rich lamellipodia at the leading edge and actomyosin structure at the posterior (Fig. 1B). In addition, in a polarized cell many intracellular proteins, including F actin and lipids show polarized distribution (Fig. 1B). Among all these, the polarized distribution of a phospholipid, PIP_3, which was initially observed in *Dictyostelium* [16] and later in neutrophils [17] and other cells [18], appears to occur prior to other polarization events. PIP3 is converted from PIP_2 by PI3K [19]. Some of the Pleckstrin homology (PH) domains specifically recognize PIP_3, thus leading to the translocation of the PH domain-containing proteins to the plasma membrane. Such translocation often leads to the regulation of the activity of these proteins that include many

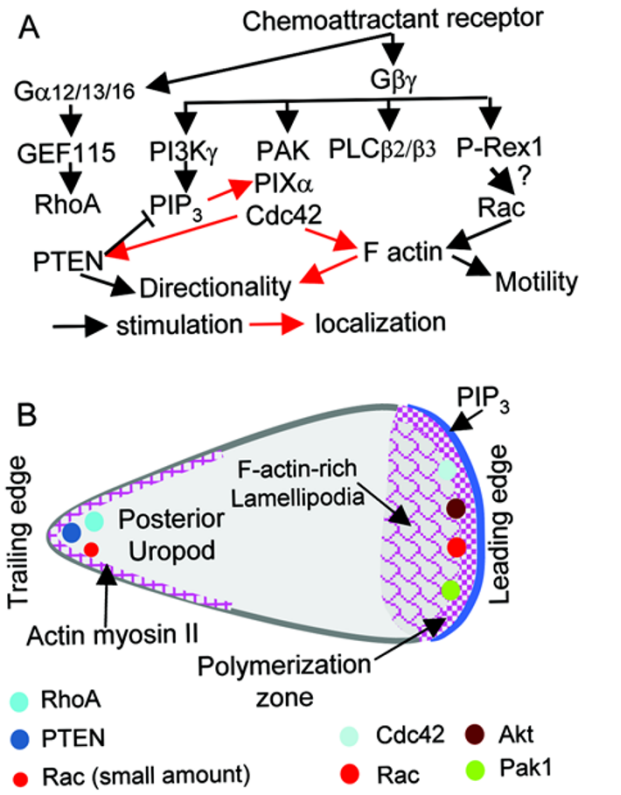

Fig. 1 Chemoattractant signaling pathways and neutrophil cell polarization. (**A**) Summary of chemoattractant signaling pathways and their actions in leukocyte chemotaxis. (**B**) Highly motile neutrophils develop high polarity upon stimulation. Only some of the proteins that show polarized distribution are shown.

protein kinases, GEFs, and structural proteins [19-22]. Chemoattractants have been shown to stimulate PIP_3 production in leukocytes, presumably by activating a PI3K. The identification of PI3Kγ, a G protein-regulated PI3K isoform [23, 24], led to the hypothesis that chemoattractants may stimulate PIP_3 production via PI3Kγ. To test the hypothesis and determine the significance of PIP_3 in chemoattractant-induced chemotaxis, we and others generated PI3Kγ-null mice [25-27]. PI3Kγ-deficiency completely abrogated chemoattractant-induced PIP_3 production in neutrophils, thus demonstrating that PI3Kγ is required for chemoattractant-induced PIP_3 production in mouse neutrophils. In addition, PI3Kγ-deficiency resulted in impaired neutrophil and macrophage chemotaxis in response to a number of chemoattractants [25-27]. To better understand the mechanism by which PI3Kγ regulates chemotaxis, we went on examining migratory and polarization characteristics of PI3Kγ-deficient neutrophils in a shallow chemoattractant gradient. This study revealed that PI3Kγ or PIP_3, although not required for neutrophils to polarize, is required for the cells to align their polarity with

the chemoattractant gradient [28]. In other words, PIP_3 is required for the cells to sense and interpret the spatial cue provided by the gradient and to orientate their polarity in accordance to the cue. Because the location of F actin or lamellipodia marks the direction of cell migration, the failure of preferential localization of F actin in accordance to the chemoattractant gradient explains the loss of directionality for PI3Kγ-deficient cells. The study of PI3K-null *Dictyostelium* cells also yielded similar results [29]. More recently, we have gained further insights into how the PIP_3 polarization regulates the orientation of cell polarity-by localizing the formation of F actin-rich lamellipodia at the side of the cell facing the source of chemoattractants. PIP_3 does this by localizing the activation of Cdc42 [10].

Cdc42 is a member of the Rho family of small GTPases [30-33]. Cdc42 is a key regulator of cell polarity in many cell types [34, 35] and directionality in macrophages [36]. Although it has been shown to activated by chemoattractants in leukocytes, the mechanism by which it is regulated by heterotrimeric G proteins remained elusive. We recently found that Gβγ could directly interact with PAK1 and activate PAK1 in the presence of Cdc42 and a small GTPase exchange factor PIXα. PIXα is constitutively associated with PAK1 and can activate both Cdc42 and Rac in *in vitro* and overexpression assays [37, 38]. In addition, we observed that Gβγ activated Cdc42 in the presence of PIXα and PAK1. Putting all these results together, we hypothesized that Gβγ may activate Cdc42 by recruiting the PAK-PIXα complex to the plasma membranes, where PIXα activates Cdc42. Activated Cdc42 in turn activates its downstream effectors that include PAK1. This hypothesis was validated by the observation that chemoattractant-induced activation of Cdc42 required both PAK1 and PIXα in leukocytes as siRNA-mediated suppression of PAK expression or inactivation of PIXα by gene targeting abrogated Cdc42 activation [10]. The fact that neither PAK1 suppression or PIXα inactivation affected chemoattractant-induced Rac1 activation suggest that PIXα functions as a specific Cdc42 exchange factor even though PIXα can activate both Rac1 and Cdc42 in *in vitro* or overexpression assay. In addition, our findings indicate that PAK1 is not only one of the effectors for Cdc42, but also functions as a scaffold protein that is required for Cdc42 activation. A recent study using *in vitro* reconstitution assay has provided a mechanistic basis for the specific regulation of Cdc42 by PIXα upon chemoattractant stimulation. This study showed that Gβγ, via interacting with PAK, activates the nucleotide exchange activity of PIXα toward Cdc42 by stimulating the dissociation of the PIXα dimer. PIXα, when it is in the dimer form, shows little activity on Cdc42 [39].

It is interesting to note that this chemoattractant and Gβγ-mediated activation of Cdc42 shares a close resemblance with a mechanism by which pheromone activates Cdc42 in yeast. In the yeast mechanism, a scaffold protein called Far1p bridges Gβγ to the guanine nucleotide exchange factor Cdc24 [40-42]. Mammalian homolog of Far1p has not been found; thus, we suspect that PAK1 may be the functional equivalent of Far1p. Consistent with the role of yeast Cdc42 in regulating cell polarity, the mammalian Cdc42 pathway also plays an important role in regulation of directionality during chemotaxis, i.e., aligning cell polarity with the chemoattractant gradient. Neutrophils that lack PIXα could no longer migrate following the gradient. Instead, these cells, like the PI3Kγ-nullcells, wandered around with little sense of direction in a chemoattractant gradient [10]. As occurred to PI3Kγ-null neutrophils, cells lacking PIXα also failed to localize F actin formation in accordance to the chemoattractant gradient despite that PIXα-deficiency did not affect the formation of F actin in response to chemoattractants [10].

Role of PI3Kγ-linked pathway and the PIXα-Cdc42 pathway in directionality

Because both PI3Kγ-linked pathway and the PIXα-Cdc42 pathway regulate directionality by regulating the localization of F actin, the relationship between these two signaling pathways was investigated. It seems that the PI3Kγ pathway may acts upstream of PIXα-Cdc42 pathway, as PI3Kγ is required for directional localization of active Cdc42. However, active Cdc42 and F actin are still co-localized in PI3Kγ-null neutrophils despite the defective localization of active Cdc42. These observations indicate that Gβγ-mediated activation of PI3Kγ determines the localization of active Cdc42, probably by regulating the localization of PIXα through its PH domain, and that active Cdc42 in turn determines where F actin is formed. Putting all these into the context of a cell, the polarization distribution of PIP_3, whose production requires PI3Kγ, leads to polarized activation of Cdc42 and eventually to polarized distribution of F actin.

Knowing that the PIP_3 gradient determines the localization of lamellipodia and thus the direction of cell locomotion, the ensuing questions are how the polarized distribution of PIP_3 is established and orientated. Although we do not have a definitive answer to the question, one hypothesis suggests that this may be a result of amplification of a small receptor activation gradient corresponding to the ligand gradient through a cascade of positive feedbacks and negative regulations [43, 44]. Studies from *Dictyostelium* suggest that PTEN is one of the negative regulators that helps establishing and maintaining the PIP_3 gradient [45, 46]. PTEN is a protein and phospholipid phosphatase that can dephosphorylate PIP_3 [19, 47-51]. In chemotaxing

Dictyostelium cells, PTEN was found to be translocated to the posterior membranes of the cells [45, 46]. In chemotaxing neutrophils, we found that PTEN was also localized at the posterior and that this localization was dependent on PIXα [10]. Work is under the way for determining the mechanisms by which chemoattractants regulate PTEN via small GTPases. Because PTEN is a major tumor suppressor that is frequently mutated in a wide range of human tumor [52], elucidation of its regulation mechanisms, which still remain unclear, is clearly of great importance not only for better understanding chemotaxis, but also for its role in tumor suppression.

While PIP_3 and Cdc42 localize F actin formation, they are not required for F actin formation because chemoattractants could still stimulate F actin formation in PI3Kγ- or PIXα-deficient neutrophils [10, 26, 53]. Thus, the next important question is how the actin polymerization is regulated by chemoattractants. Work using Rac-deficient neutrophils [54] and Rac dominant-negative mutants [55] has demonstrated the requirement of Rac for F-actin formation. What remains unresolved is how Rac is regulated by chemoattractants. A few years back, P-Rex1 was identified as a Gβγ and PIP_3-regulated GEF that could activate Rac in *in vitro* and overexpression assays [56]. P-Rex1 has since been believed to be the primary Rac activator in leukocytes, particularly in neutrophils. It is difficult to unambiguously determine the specificity of GEFs relying solely upon *in vitro* and overexpression assays because these GEF molecules, particularly their catalytic domains, share close amino acid sequence homology. Our study of PIXα provides an excellent example; PIXα was shown to activate both Rac and Cdc42 *in vitro* [37], but it only activates Cdc42 in neutrophils [10]. Thus, to unambiguously determine if P-Rex1 is the major Rac regulator *in vivo*, study of P-Rex1-null mice will be required.

Chemotaxis is a complex biological process. What we have learned now may be only the tip of an iceberg. There are still many important questions that remain unanswered. In addition, chemotaxis is a process that is not only used by leukocytes. Neuronal and embryonic cells migrate during development. During angiogenesis, endothelial cells undergo chemotaxis to form blood vessels, while epithelial cells and fibroblasts chemotax during wound healing. Chemotaxis also has an important role in tumorigenesis, particularly tissue-targeted metastasis. Many cancer cells such as breast cancer cells are known to preferentially metastasize into certain tissues and organs. This preference has now been correlated with the production of chemoattractants by the target tissues and organs and upregulation of chemoattractant receptors in the cancer cells. Although findings on the regulation of chemotaxis are often from studies using neutrophils and Dictyostelium cells as model systems, many of the fundamental principles should be applied to other cell types, including those aforementioned.

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