78a Elucidation of the Spatial Pdgf Gradient Sensing Mechanism in Fibroblast Chemotaxis

Ian Schneider and Jason Haugh

During wound healing, fibroblast migration is biased by extracellular gradients of platelet-derived growth factor (PDGF). As in other chemotactic cells, such as neutrophils and *Dictyostelium discoideum*, spatial gradient sensing in fibroblasts is mediated by receptor activation of phosphoinositide (PI) 3-kinases and the localized production of 3' PI lipids in the plasma membrane. However, the responses of these cell types to uniform stimulation suggest fundamental differences in their respective spatial sensing mechanisms. Indeed, while shallow chemoattractant gradients yield robust, all-or-none polarization of 3' PI signaling and actin polymerization in *Dicty* and neutrophils, we postulated that the sensitivity of PDGF gradient sensing in fibroblasts depends to a much greater extent on the average PDGF concentration. Using total internal reflection fluorescence microscopy to monitor 3' PI levels in fibroblasts expressing the GFP-AktPH probe, in conjunction with kinetic and spatial modeling, we found that fibroblasts possess three distinct regimes of PDGF gradient sensitivity. In response to PDGF gradients with low midpoint concentrations, spatial sensing is *absolute*, in that the magnitude of the 3' PI gradient is roughly proportional to the change in receptor occupancy across the cell. PDGF gradients with intermediate midpoint concentrations, sufficient to recruit all of the intracellular PI 3-kinase without saturating receptor occupancy, elicit maximal 3' PI gradients sensitive to the *relative* change in receptor occupancy. At still higher PDGF concentrations, receptor occupancy is saturated, and the response is indistinguishable from that seen after uniform stimulation. Our analysis also revealed that the morphological polarity of the cell imposes an additional, intrinsic bias to the 3' PI pattern. Peripheral, leading-edge regions of the contact area were consistently found to be hot spots of 3' PI lipid concentration, which enhance or oppose the response depending on the cell orientation relative to the external gradient.