FINAL TECHNICAL REPORT

GRANT #: N00014-03-1-0594

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INSTITUTION: North Carolina State University

GRANT TITLE: Integration of Soluble and Adhesive Gradient Signals in Directed Cell Migration

AWARD PERIOD: 1 June 2003 - 30 November 2006

OBJECTIVE: Quantitatively characterize PI 3-kinase signaling and biophysical aspects of random fibroblast motility stimulated by uniform PDGF and immobilized fibronectin; relate polarized PI 3-kinase signaling and directed fibroblast motility stimulated by PDGF gradients, as a function of uniformly immobilized fibronectin; analyze polarized PI 3-kinase signaling and directed migration in response to gradients of both PDGF and fibronectin oriented in various directions.

APPROACH: Migration signaling through the PI 3-kinase pathway in mouse fibroblasts is monitored in real time using total internal reflection fluorescence (TIRF) microscopy. Quantitative analysis and mathematical models are used to parse out the relative contributions of the PDGF receptor and integrin signaling, which are presented in spatially distinct ways.

ACCOMPLISHMENTS (throughout award period): Through a quantitative analysis of mathematical models and live-cell total internal reflection fluorescence microscopy experiments, we demonstrated that PDGF detection is governed by mechanisms that are fundamentally different from those in the well-characterized chemotactic cells, *Dictyostelium discoideum* and neutrophils (Schneider and Haugh, 2005. J Cell Biol, 171:883). This analysis was extended to specifically analyze the undesirable effect of lateral diffusion in blurring the intracellular messenger profile. This effect was encapsulated in a single parameter, the effectiveness factor, akin to the analysis of reactions in porous catalysts, which allows the effect of intracellular messenger diffusion on spatial gradient sensing to be quantified for individual cells (Haugh and Schneider, 2006. Chem Eng Sci, 61:5603). To put our analysis of PDGF gradient sensing in the context of wound healing, we developed a mathematical model of wound invasion, incorporating the PDGF gradient sensing mechanism we elucidated. The key insight from this model is that, through receptor-mediated endocytosis and intracellular proteolysis of PDGF, dermal fibroblasts have a significant impact on the PDGF concentration profile in the wound as they invade (Haugh, 2006. Biophys J, 90:2297; Schneider and Haugh, 2006. Cell Cycle, 5:1130). Finally, we have conducted a comprehensive study of PI 3-kinase signaling during fibroblast spreading on adhesive surfaces, as a model system for understanding membrane protrusion. These studies have revealed that active cell spreading requires the activation of PI 3-kinase, which is triggered spontaneously even on surfaces that do not apparently engage specific adhesion receptors (Michael Weiger and Haugh, manuscript in preparation).

Methods developed during the award period include spatial modeling of PI 3-kinase signaling, by the finite element method, using the actual contact area geometry of each individual cell and conversion of the results for pixel-by-pixel comparison with experimental images (Schneider et al., 2005. Biophys J, 89:1420).

CONCLUSIONS: We showed that robust PDGF sensing requires steeper gradients and a much narrower range of absolute chemoattractant concentration, consistent with a simpler system lacking the feedback loops that yield signal amplification and adaptation in amoeboid cells. Thus, whereas other chemotactic cells seem to employ an adaptive mechanism to migrate persistently in the direction of the chemoattractant gradient, which progressively increases in concentration as the cells move, quantitative modeling of dermal wound invasion suggests that fibroblasts instead erode the PDGF gradient such that it is constantly maintained within the optimal range. Our experiments also revealed that fibroblasts have an intrinsic PI 3-kinase activation mechanism that is activated during cell spreading and, perhaps, during migration.

SIGNIFICANCE: Our research has led to a comprehensive model of fibroblasts' responsiveness to PDGF gradient stimulation at the level of PI 3-kinase signaling, and we have analyzed the implications of this mechanism for dermal wound invasion. Thus, our research has contributed to the fundamental understanding of directed fibroblast migration, a critical process in wound healing.

PATENT INFORMATION: N/A

AWARD INFORMATION: The PI received the Camille Dreyfus Teacher-Scholar Award from the Camille & Henry Dreyfus Foundation, 2005.

REFEREED PUBLICATIONS (for total award period):

1. Schneider, I.C., Parrish, E.M., and Haugh, J.M. (2005). Spatial analysis of 3' phosphoinositide signaling in living fibroblasts, III: Influence of cell morphology and morphological polarity. *Biophysical Journal* , 89: 1420-1430.

2. Schneider, I.C. and Haugh, J.M. (2005). Quantitative elucidation of a distinct spatial gradient-sensing mechanism in fibroblasts. *Journal of Cell Biology*, 171: 883-892.

3. Haugh, J.M. (2006). Deterministic model of dermal wound invasion incorporating receptor-mediated signal transduction and spatial gradient sensing. *Biophysical Journal*, 90: 2297-2308.

4. Haugh, J.M. and Schneider, I.C. (2006). Effectiveness factor for spatial gradient sensing in living cells. *Chemical Engineering Science*, 61: 5603-5611.

5. Schneider, I.C. and Haugh, J.M. (2006). Mechanisms of gradient sensing and chemotaxis: conserved pathways, diverse regulation. *Cell Cycle*, 5: 1130-1134.

BOOK CHAPTERS, SUBMISSIONS, ABSTRACTS AND OTHER PUBLICATIONS (for total award period)

Haugh, J.M. and Weiger, M.C. (2007). Modeling intracellular signal transduction processes. In *Chemical Biology: From Small Molecules to Systems Biology and Drug Design*, Vol. 3, S. Schreiber, T. Kapoor, and G. Wess, eds. Wiley-VCH (Review).

Presentation: "Dermal wound healing: from outside to inside the cell and back again." Department of Chemical Engineering, Princeton University, Princeton, NJ, 9/2004.

Presentation: "Spatial sensing in fibroblasts: distribution of 3' PI lipids in response to platelet-derived growth factor gradients." American Institute of Chemical Engineers Annual Meeting, Austin, TX, 11/2004 (delivered by Ian Schneider).

Presentation: "Mechanistic model of growth factor/fibroblast population dynamics during wound healing." American Institute of Chemical Engineers Annual Meeting, Austin, TX, 11/2004 (w/ Michael Monine and Stanislav Shvartsman [Princeton Univ.]).

Presentation: "Phosphoinositide 3-kinase-dependent migration of fibroblasts towards platelet-derived growth factor (PDGF) is governed by an absolute gradient sensing mechanism." American Society for Cell Biology Annual Meeting, Washington, DC, 12/2004 (poster, w/ Ian Schneider and Elizabeth Parrish).

Presentation: "PDGF gradient sensing in fibroblasts: the 'absolute' truth." Gordon Research Conference on Gradient Sensing & Directed Cell Migration, Ventura, CA, 2/2005.

Presentation: "Platelet-derived growth factor-mediated spatial sensing in fibroblasts is governed by three regimes of gradient sensitivity." Gordon Research Conference on Gradient Sensing and Directed Cell Migration, Ventura, CA, 2/2005 (poster, w/ Ian Schneider).

Presentation: "The ins and outs of receptor tyrosine kinase signaling." National Institute of Environmental Health Sciences, Research Triangle Park, NC, 3/2005.

Presentation: "Kinetic and spatial analyses of intracellular signal transduction." Biochemical Engineering XIV, Harrison Hot Springs, BC, Canada, 7/2005.

Presentation: "Reaction/diffusion in intracellular signal transduction: a multi-scale case study." Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, 8/2005.

Presentation: "Spatial gradient sensing in fibroblasts: a case study in quantitative imaging and modeling." Imaging: Integrating Across Disciplines, Symposium in honor of Nina S. Allen, North Carolina State University, Raleigh, NC, 9/2005.

Presentation: "A distinct mechanism of spatial gradient sensing in fibroblasts." Department of Cell & Developmental Biology, University of North Carolina-Chapel Hill, Chapel Hill, NC, 10/2005.

Presentation: "Elucidation of the spatial PDGF gradient sensing mechanism in fibroblast chemotaxis." American Institute of Chemical Engineers Annual Meeting, Cleveland, OH, 11/2005 (w/ Ian Schneider).

Presentation: "Dynamic relationships among PI 3-kinase signaling, contact area spreading, and cell polarization following the attachment of fibroblasts to surfaces." American Institute of Chemical Engineers Annual Meeting, Cleveland, OH, 11/2005 (delivered by Michael Weiger).

Presentation: "A divergent mechanism of spatial gradient sensing revealed through quantitative imaging and modeling." Symposium on Computational Modeling of Cell Migration, American Society for Cell Biology Annual Meeting, San Francisco, CA, 12/2005 (delivered by Ian Schneider).

Poster: "Quantitative elucidation of a unique spatial gradient sensing mechanism in fibroblasts." American Society for Cell Biology Annual Meeting, San Francisco, 12/2005 (delivered by Ian Schneider).

Poster: "Dynamic relationships among PI 3-kinase signaling, cell spreading, and asymmetric membrane protrusion following the attachment of fibroblasts to surfaces." American Society for Cell Biology Annual Meeting, Washington, DC, 12/2005 (delivered by Michael Weiger).

Presentation: "Quantitative analysis and modeling of signal transduction processes." Department of Environmental & Molecular Toxicology, North Carolina State University, Raleigh, NC, 1/2006.

Presentation: "Modeling and analysis of receptor-mediated signal transduction, spatial gradient sensing, and wound invasion." Workshop on Computational Approaches to Cell Motility, University of Minnesota, Minneapolis, MN, 4/2006.

Presentation: "Intracellular signal transduction networks." Workshop on Medical Sciences Research, NSF Science & Technology Center for Environmentally Responsible Solvents and Processes, Raleigh, NC 5/2006.

Presentation: "Dynamic relationships among PI 3-kinase signaling, cell spreading, and membrane protrusion following the attachment of fibroblasts to surfaces." 19th Annual Mid-Atlantic Biochemical Engineering Consortium (MABEC), Raleigh, NC, 6/2006 (delivered by Michael Weiger).

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14. ABSTRACT

Our research has led to a comprehensive model of fibroblasts' responsiveness to PDGF gradient stimulation at the level of PI 3-kinase signaling, and we have analyzed the implications of this mechanism for dermal wound invasion. In this research, we have utilized and in some cases developed advanced tools for live-cell microscopy and quantitative analysis including mathematical modeling. Thus, our research has contributed to the fundamental understanding of directed fibroblast migration, a critical process in wound healing.

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