

Putting the brakes on leukocyte chemotaxis: an interview with Dr. Ramesh K. Ganju

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The manuscript, “Slit-2/Robo-1 Modulates the CXCL12/CXCR4-induced Chemotaxis of T cells” was selected as a Pivotal Advance because it is the first to propose a molecular mechanism via which a Slit/Robo (Roundabout receptor) complex inhibits leukocyte chemotaxis. Specifically, in this manuscript, Dr. Ganju and colleagues demonstrate that the Slit/Robo complex inhibits Akt phosphorylation and Rac activation that result from the interaction of the chemokine SDF-1 (CXCL12) with its seven-transmembrane receptor, CXCR4. The Slit ligands and Robo receptors, originally identified as modulators of neuronal migration, have more recently been characterized as inhibitors of specific chemokine-mediated inflammatory responses [1].

Dr. Ganju, before we begin to discuss the manuscript, can you tell us a bit about yourself, where you did your medical training, why you decided to pursue a career in research?

RKG: I have been deeply interested in the biological sciences since high school. I started my research career at the Indian Institute of Science in Bangalore, India, where I performed my graduate studies on thermophilic cellulases. However, due to my desire and fascination with exploring intracellular signaling mechanisms, I moved from India to the United States where I had the opportunity to work on T cell receptor-mediated signaling mechanisms at Dana Farber Cancer Institute. I later joined the Division of Experimental Medicine at Beth Israel Deaconess Medical Center to work on chemokine receptor-mediated signaling. This opportunity opened up a new avenue of research which had a major influence on my career. Here, I further explored signaling pathways mediated by CXCR4 and CCR5, as these receptors had recently been identified as co-receptors for the HIV virus. Soon, the signaling axis of CXCR4 and its ligand CXCL12 was shown to play an important role in metastasis and we started exploring CXCR4-mediated mechanisms that regulate chemotaxis and chemoinvasion. Currently, our major focus is to understand how Slit/Robo and chemokine receptor interactions modulate chemotactic/chemoinvasive signaling pathways and HIV pathogenesis. Undoubtedly, I would say that exploring the role of Slit/Robo in T-cells and breast cancer cells has been one of the most fascinating and fruitful experiences in my research career.

The Slit ligands and Robo receptors are not as familiar to inflammation biologists as they are to those working in developmental neuroscience.



Dr. Ramesh K. Ganju received his Ph.D. degree from Indian Institute of Science in Bangalore, India, and currently holds positions of Associate Professor, Division of Experimental Medicine, Beth Israel Deaconess Medical Center and Associate Professor, Department of Medicine, Harvard Medical School.

Can you give the readers of *JLB* a bit of insight into the nature of Slit ligands and Robo receptors, some background on the important features, and specifically how they became of particular interest to leukocyte biologists?

RKG: As stated, most of our current knowledge about these proteins comes from the field of neuroscience. There are three Slit genes (Slit-1, Slit-2, Slit-3) which encode ~200 kDa secretory proteins that interact with the Roundabout or Robo receptors, which are members of the immunoglobulin superfamily. Interactions between various Slits and Robos direct axon branching and neuronal migration in the developing nervous system. Slit/Robo has also been shown to play a role in mesodermal and endothelial cell migration, lung and liver development, inflammation, tumor angiogenesis, and tumor

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metastasis. Wu and colleagues [2] were the first to report on the role of Slit-2 and Robo interactions in inhibiting chemokine-mediated leukocyte trafficking in vitro. Specifically, these authors showed that rat lymphocyte migration to the chemoattractant SDF-1 was inhibited by the addition of exogenous Slit-2; neutrophils derived from the human HL-60 promyelocyte cell line were similarly inhibited by Slit-2 in chemotaxis assays with fMLP. Given the other roles played by the seven-transmembrane chemokine receptors, including their involvement in inflammation, tumor angiogenesis, cancer metastasis, and HIV pathogenesis, the larger impact of these findings remains to be explored.

To date, the roles of Slit and Robo have been explored in only a few chemokine/receptor interactions. Do you think that Slit/Robo modulation is likely to be more universal, or in contrast, limited to a discrete group or family of chemokine ligand-receptor interactions?

RKG: It is not easy to predict the answer to this question with the information available at the moment. In the neuronal system, the Slit/Robo receptor has been shown to interact with netrin/DCC receptor. We know that Slit-2 can inhibit leukocyte chemotaxis promoted by fMLP and SDF-1 (CXCL12). In addition, we also have unpublished data suggesting that Slit/Robo might also block chemotaxis by other chemokines. The interaction between Slit/Robo and chemokine receptors does not change the chemoattractant receptors in any way, nor are there any other characterized functional effects besides chemotaxis that might help us to determine a pattern or make a generalization about the scope of their roles. Therefore, more work needs to be done before any substantial conclusions can be drawn.

What evidence exists suggesting that Slit/Robo has an impact on leukocyte migration in vivo?

RKG: There are only a few papers that address this issue directly. One is by Kanellis and colleagues [3], in which the authors used a rat model of induced glomerulonephritis to demonstrate that the glomerular levels of Slit-2 fall during this disease process, and that exogenous administration of Slit-2 resulted in reduced levels of inflammation and of leukocyte chemotaxis. Another manuscript is by Guan et al. [4], where the authors have shown that Slit-2 is up-regulated in the skin by allergen sensitization. Furthermore, they have shown that Slit-2 inhibits delayed-type hypersensitivity response induced by contact allergens by inhibiting the migration of dendritic cells and Langerhans cells. Progress in studies performed in vivo has been hampered by the fact that various gene-deletions are lethal to the developing progeny.

In the literature, you and others discuss the possibility that the interactions of Slit with CXCR4 might have an impact on HIV pathogenesis [1, 2]. Is there any direct evidence available to support this hypothesis?

RKG: Certainly, this is a subject of major interest in our laboratory. At this point, these studies are too preliminary to

suggest conclusively whether Slit-Robo interactions with CXCR4 play an important role in the regulation of HIV pathogenesis.

Can you give us some sense of where you'd like to take this work in the immediate and/or long term future?

RKG: We are currently working on a number of studies in our laboratory focusing on the tumor suppressor activity of the Slit ligands and Robo receptors. We have previously explored the inhibition of CXCL12-induced/CXCR4-mediated chemotaxis and chemoinvasion signaling in breast cancer cells [5]. We are particularly interested in how the Slit ligands might interact with chemotactic and chemoinvasive signaling pathways that regulate metastasis.

On a more personal note, would you be able to tell the readers of *JLB* a bit about yourself—perhaps what you like to do other than scientific pursuits? Is there anything else you might like to add to this interview?

RKG: In all honesty, managing a research laboratory occupies most of my time—particularly nowadays because of the tight situation with grants. I hardly have any time to do anything other than science. However, I love to spend my free time with my family and travel and explore various parts of the world. On another note, I would like to specifically thank two colleagues. First, I must mention the contribution of Dr. Anil Prasad, the first author on this manuscript who provided most of the experimental findings underlying this work. I would also like to thank Dr. Jerome Groopman, the chairman of our division, who provided invaluable support as I established my laboratory here at the Beth Israel Deaconess Medical Center/ Harvard Medical School.

REFERENCES

1. Fernandis, A. Z., Ganju, R. K. (2001) Slit: a roadblock for chemotaxis. *Sci. STKE* **91**, PE1.
2. Wu, J. Y., Feng, L., Park, H. T., Havlioglu, N., Wen, L., Tang, H., Bacon, K. B., Jiang, Z. H., Zhang, X. C., Rao, Y. (2001) The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors. *Nature* **410**, 948–952.
3. Kanellis, J., Garcia, G. E., Li, P., Parra, G., Wilson, C. B., Rao, Y., Han, S., Smith, C. W., Johnson, R. J., Wu, J. Y., et al. (2004) Modulation of inflammation by slit protein in vivo in experimental crescentic glomerulonephritis. *Am. J. Pathol.* **165**, 341–352.
4. Guan, H., Zu, G., Xie, Y., Tang, H., Johnson, M., Xu, X., Kevil, C., Xiong, W. C., Elmets, C., Rao, Y., et al. (2003) Neuronal repellent Slit2 inhibits dendritic cell migration and the development of immune response. *J. Immunol.* **171**, 6519–6526.
5. Prasad, A., Fernandis, A. Z., Rao, Y., Ganju, R. K. (2004) Slit protein-mediated inhibition of CXCR4-induced chemotactic and chemoinvasive signaling pathways in breast cancer cells. *J. Biol. Chem.* **279**, 9115–9124.