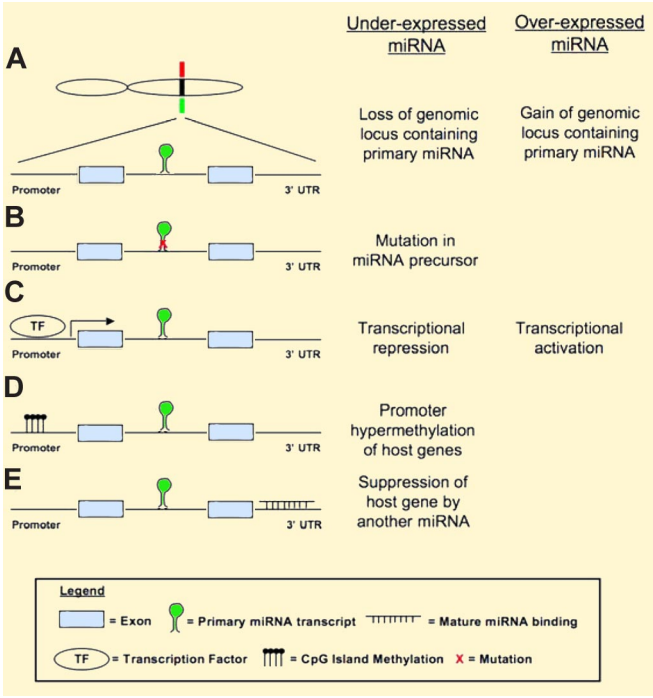

Mechanisms of miRNA dysregulation in cancer. The example shown here to illustrate the different mechanisms has the miRNA located in the intron of a host gene containing 2 exons. (A) A miRNA may be underexpressed or overexpressed due to deletion (green bar) or gain (red bar) of the genomic locus containing the miRNA. (B) Mutation in the miRNA precursor that affects the processing of miRNA may lead to underexpression of the miRNA. (C) Transcriptional repression (eg, by MYC) or activation (eg, by p53) by transcription factors may lead to underexpression or overexpression of miRNA. (D) miRNA expression may be suppressed by promoter methylation. (E) A novel mechanism identified by Ballabio and colleagues where miRNA expression is suppressed by other miRNA inhibiting the expression of the host gene.

miRNA to just one suggests there are inconsistencies across different platforms (microarray vs reverse transcriptase–polymerase chain reaction) and datasets. It would be important to independently validate this result to determine the wider applicability of this result.

How are miRNA dysregulated in SzS? Ballabio et al extrapolated data from their previous studies using different datasets showing that many of the dysregulated miRNAs are located in loci of gains and losses previously identified; in addition, many of the predicted targets have abnormal gene expression suggesting miRNA dysregulation in SzS has functional consequences. Although compelling, the DNA copy-miRNA-mRNA correlation should be performed on the same samples before one can confirm a cause-and-effect relationship.

In my opinion, the most important contribution of the current study is the identification of a novel mechanism by which miRNA expression is regulated. Various mechanisms that may lead to abnormal miRNA expression in cancer have been identified[4] (Figure 1). The current finding that miRNA expression can be regulated by other miRNAs through modulation of host gene expression expands the repertoire. This was robustly verified experimentally for miR-342, which was also shown to have tumor suppressor properties in SzS. Future studies should be directed at identifying addition regulator miRNAs. This will help define the true prevalence of this novel mechanism of miRNA regulation.

The relationship between miRNAs and genes is becoming complex, but it is important to rationally dissect this maze. miRNA may affect gene expression, yet certain genes—for example, transcription factors and genes involved in DNA methylation who are themselves targets of miRNA—may then affect miRNA expression. Analogous to identifying the driving mutations from the passenger mutations in the large number of somatic mutations identified in sequencing studies in cancer, we need a comprehensive view of what constitutes primary events and secondary events in dysregulated miRNA expression in cancer. This will become increasingly important in the future as miRNA-based gene therapy becomes reality. We will need to identify the critical nodes in this complex network for therapeutic targeting. To this end, the work of Ballabio and colleagues will have wider implication in our understanding of miRNA dysregulation in cancers.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Ballabio E, Mitchell T, van Kester MS, et al. MicroRNA expression in Sézary syndrome: identification, function, and diagnostic potential. *Blood*. 2010;116(7):1105-1113.
2. Hwang ST, Janik JE, Jaffe ES, Wilson WH. Mycosis fungoides and Sezary syndrome. *Lancet*. 2008;371(9616):945-957.
3. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol*. 2009;10(2):127-139.
4. Croce CM. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Cancer*. 2009;10(10):704-714.
5. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nat Genet*. 2007;39(5):673-677.
6. Kumar MS, Pester RE, Chen CY, et al. Dicer1 functions as a haploinsufficient tumor suppressor. *Genes Dev*. 2009;23(23):2700-2704.
7. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A*. 2004;101(9):2999-3004.

● ● ● PHAGOCYTES & GRANULOCYTES

Comment on O'Connell et al, page 1136

S100A10: a complex inflammatory role

Lindsey A. Miles and Robert J. Parmer THE SCRIPPS RESEARCH INSTITUTE; UNIVERSITY OF CALIFORNIA SAN DIEGO AND VETERANS ADMINISTRATION SAN DIEGO HEALTHCARE SYSTEM

In this issue of *Blood*, O'Connell and colleagues demonstrate that macrophage recruitment in response to an inflammatory stimulus is markedly decreased in S100A10-deficient mice compared with wild-type mice, establishing the plasminogen receptor, S100A10, as a major mediator of this key plasmin-dependent component of the inflammatory response.[1]

Activation of plasminogen to the broad-spectrum serine proteinase, plasmin, is markedly enhanced when plasminogen is bound to the cell surface.[2] The plasmin formed remains associated with the cell where plasmin is protected from inactivation by its primary inhibitor, α_2-antiplasmin.[2] This results in arming cells with the broad-spectrum proteolytic activity of plasmin. Because macrophages have a high capacity for plasminogen, no single molecule can account for the entire plasminogen-binding capacity of these cells. However, a specific subset of plasminogen receptors, exposing C-terminal lysines on the cell surface, is responsible for promoting plasminogen activation on macrophages and other eukaryotic cells.[3]

In addition to its well-known role in thrombolysis, plasmin(ogen) is required for optimal recruitment of macrophages in the response to inflammatory stimuli.[4,5] During macrophage recruitment, cell-surface plasmin activity functions to directly degrade components of the extracellular matrix as well as to activate pro–matrix metalloproteinase-9 for degradation of collagen, thus allowing passage of cells through tissues.[5] Plasminogen-dependent macrophage recruitment is regulated by plasma carboxypeptidase B[6] (whose active form removes C-terminal basic arginyl and lysyl residues) consistent with a requirement for interaction of plasminogen with the C-terminal lysine residues of plasminogen receptors on the cell surface.

S100A10 belongs to the S100 family of calcium-binding proteins and is usually found in cells bound to its ligand, annexin A2, in the heterotetrameric complex $(S100A10)_2$–$(annexin\ A2)_2$, AIIt.[1] S100A10 is synthesized with a C-terminal lysine and binds plasminogen.[1]

O'Connell and colleagues show that the number of macrophages recruited to the peritoneal cavity in response to thioglycollate is 53% less in S100A10 $^{-/-}$ mice, compared with wild-type mice. The functions of other macrophage plasminogen receptors that are synthesized with C-terminal lysines have been evaluated, previously, in the thioglycollate-induced peritonitis model after intravenous injection of specific antibodies. These receptors include histone H2B (48% less macrophage recruitment compared with injection of nonimmune control),[7] α-enolase (24% less recruitment),[7] and, recently, Plg-R_{KT} (58% less recruitment).[8] In another model of inflammation, monocytoid cells overexpressing α-enolase exhibit increased recruitment to the acutely inflamed lung compared with cells overexpressing a mutant α-enolase that does not bind plasminogen (lacking the 30 C-terminal amino acids).[9] The study by O'Connell is the first examination of macrophage recruitment in a specific plasminogen receptor knockout mouse model.

In concordance with the foregoing studies, the O'Connell study emphasizes that multiple plasminogen receptors with C-terminal basic residues participate in macrophage invasion. S100A10$^{-/-}$ macrophages exhibited impaired plasmin-dependent invasion through Matrigel (an extract of murine tumor-derived basement membrane, commonly used to assess the role of proteinases in cell migration). When macrophages from either wild-type or S100A10$^{-/-}$ mice were treated with carboxypeptidase B, a major reduction in Matrigel invasion was observed. O'Connell and colleagues interpreted the effect of carboxypeptidase B on S100A10$^{-/-}$ macrophages to indicate that other plasminogen receptors with C-terminal basic residues also participate in macrophage invasion.

It is apparent that the sum of the effects of functional blockade of specific plasminogen receptors, analyzed to date, is greater than a 100% reduction in plasminogen-dependent macrophage recruitment. Thus, it is possible that each specific plasminogen receptor may be required at different steps in the inflammatory response; for example, crossing different layers of peritoneal tissue at which different contributions of direct plasmic cleavage of the extracellular matrix or activation of matrix metalloproteinase-9 for collagen degradation[5] may predominate.

In the O'Connell study, S100A10$^{-/-}$ mice also showed a marked decrease in neutrophil recruitment in response to thioglycollate. However, neutrophil recruitment in response to thioglycollate is not diminished in plasminogen$^{-/-}$ mice compared with wild-type mice.[4,5] O'Connell and colleagues are currently investigating the possibility that S100A10 may regulate proteases other than plasmin on the neutrophil surface. Thus, it is likely that the contribution of S100A10 to cellular inflammation is both complex and multifaceted.

S100A10 is present on most cells in a heterotetrameric complex with its ligand, annexin A2: AIIt composed of 2 molecules of S100A10 and 2 molecules of annexin A2.[1] Although annexin A2 is not synthesized with a C-terminal lysine, it has been proposed that annexin A2 may directly bind plasminogen after processing at a specific site to reveal a C-terminal lysine.[10] Distinguishing between the contributions of S100A10 and annexin A2 to plasminogen binding has been very difficult because annexin A2$^{-/-}$ mice also exhibit markedly reduced S100A10 protein expression.[11] Surprisingly, in the O'Connell study, knockout of S100A10 also reduced cell-surface expression of annexin A2.[1] O'Connell and coworkers used 2 approaches to sort out the respective contributions of S100A10 and annexin A2 to functions dependent on plasminogen binding. First, cell-surface annexin A2 on thioglycollate-stimulated peritoneal macrophages was analyzed in Western blotting. Processing of annexin A2 was not detectable, suggesting that annexin A2 was not directly binding plasminogen on the recruited peritoneal macrophages. Second, when S100A10 was added back to S100A10$^{-/-}$ macrophages (that exhibited reduced invasion across a Matrigel barrier), invasiveness was increased. However, addition of annexin A2 to S100A10$^{-/-}$ macrophages did not significantly increase invasion. These data suggest that annexin A2 most likely serves as a cell-surface binding partner of the plasminogen receptor, S100A10, but does not directly bind plasminogen under these experimental conditions.

The study by O'Connell provides a strong rationale for future studies to address the interplay between the contributions of multiple plasminogen receptors to macrophage recruitment in the inflammatory response. In addition, the distinct contributions of these plasminogen receptors should also be investigated in other physiologic and pathologic processes requiring cell migration.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. O'Connell PA, Surette AP, Liwski RS, Svenningsson P, Waisman DM. S100A10 regulates plasminogen-dependent macrophage invasion. *Blood.* 2010;116(7):1136-1146.

2. Miles LA, Hawley SB, Baik N, et al. Plasminogen receptors: the sine qua non of cell surface plasminogen activation. *Front Biosci.* 2005;10:1754-1762.

3. Felez J, Miles LA, Fabregas P, et al. Characterization of cellular binding sites and interactive regions within reactants required for enhancement of plasminogen activation

by tPA on the surface of leukocytic cells. *Thromb Haemost*. 1996;76(4):577-584.

4. Ploplis VA, French EL, Carmeliet P, Collen D, Plow EF. Plasminogen deficiency differentially affects recruitment of inflammatory cell populations in mice. *Blood*. 1998;91(6):2005-2009.

5. Gong Y, Hart E, Shchurin A, Hoover-Plow J. Inflammatory macrophage migration requires MMP-9 activation by plasminogen in mice. *J Clin Invest*. 2008;118(9):3012-3024.

6. Swaisgood CM, Schmitt D, Eaton D, Plow EF. In vivo regulation of plasminogen function by plasma carboxypeptidase B. *J Clin Invest*. 2002;110(9):1275-1282.

7. Das R, Burke T, Plow EF. Histone H2B as a functionally important plasminogen receptor on macrophages. *Blood*. 2007;110(10):3763-3772.

8. Lighvani S, Baik N, Diggs JE, Parmer RJ, Miles LA. The novel plasminogen receptor, Plg-RKT, facilitates plasminogen-dependent macrophage migration and recruitment [abstract]. *FASEB J*. 2010;24:lb419.

9. Wygrecka M, Marsh LM, Morty RE, et al. Enolase-1 promotes plasminogen-mediated recruitment of monocytes to the acutely inflamed lung. *Blood*. 2009;113(22):5588-5598.

10. Hajjar KA, Jacovina AT, Chacko J. An endothelial cell receptor for plasminogen/tissue plasminogen activator. I. Identity with annexin II. *J Biol Chem*. 1994;269(33):21191-21197.

11. He KL, Deora AB, Xiong H, et al. Endothelial cell annexin A2 regulates polyubiquitination and degradation of its binding partner S100A10/p11. *J Biol Chem*. 2008;283(28):19192-19200.