miR-155-SOCS1 as a Functional Axis: Satisfying the Burden of Proof

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The importance of individual target genes for miRNA activity has been difficult to establish. In this issue of Immunity, Lu et al. (2015) disrupt the miR-155 binding site in the SOCS1 3’ UTR in the mouse germline and show that this axis is important for T and NK cell function.

For more than a decade now, the study of microRNAs (miRNAs) within the immune system has revealed exciting roles for these small non-coding RNAs in modulating immune responses. Several miRNAs are clearly expressed by a wide range of immune cells, including cells that comprise both the innate and adaptive immune systems, and in some cases, expression is dependent upon cellular activation or lineage skewing (O’Connell et al., 2012). Among the immunologically relevant miRNAs is miR-155, which was originally identified as an oncogene in chicken lymphocytes (Tam et al., 1997) and subsequently was shown to be induced in activated immune cells, making it an inflammatory response gene (O’Connell et al., 2007). Further, through the use of gain- and loss-of-function approaches, it has become clear that miR-155, like a handful of other miRNAs, plays indispensable roles in a variety of cell types during responses to infection, during immunization, in the context of autoimmune reactions, and during tumor immunity (Vigorito et al., 2013).

As a field, we have made great strides in our understanding of miRNA biogenesis, their expression patterns in distinct cell types, and the functional importance of miRNAs in the immune system (O’Connell et al., 2012). However, our understanding of the molecular mechanisms by which miRNAs mediate their functional effects remains incomplete, especially in terms of deciphering which direct miRNA targets are truly responsible for downstream functional outcomes. miRNAs appear to function by repressing dosage-sensitive target genes, which occurs as miRNAs recognize cognate binding sites in the 3’ UTRs of their target mRNAs. This process also involves recruitment of the RISC complex, which mediates target gene repression. Based upon bioinformatic predictions, assaying de-repression of predicted targets in miRNA knockout cells, conducting 3’ UTR luciferase assays, and performing biochemical assays such as HITS-Clip, it appears that individual miRNAs might have many targets in a given immune cell type. This complexity, along with the relatively small amount by which miRNAs repress their targets, has challenged our capacity to firmly grasp which targets are truly important in specific circumstances.

However, although the methods used to identify miRNA targets have indicated that individual miRNAs can target a wide range of genes, they do not directly test whether a given target gene is functionally relevant. In fact, other experimental evidence has indicated that the opposite might be true. Some reports have shown that certain miRNA-dependent phenotypes can be largely, or at least partially, explained by single target genes. Such conclusions are based upon showing that reducing the levels of specific miRNA targets that become de-repressed in miRNA knockout cells can partially or completely rescue the miRNA phenotype. This is achieved by using shRNAs (Simpson et al., 2014) or by crossing miRNA-deficient mice to mice heterozygous for a given target gene (Escobar et al., 2014) to partially reduce the miRNA target in question. Although these approaches have yielded strong evidence that individual as opposed to large sets of miRNA targets are responsible for immunological phenotypes, they have certain caveats that must be taken into consideration. For instance, removal of one allele of a gene could impact expression of that gene in cells where the miRNA under study is not expressed or during a cellular developmental stage where the miRNA has not yet been produced. This haploinsufficiency could trigger a miRNA-independent phenotype that confounds the study of the miRNA in question.

Although much has been learned from the approaches mentioned above, the gold standard for studying the relevance of individual miRNA target genes during cognate miRNA-dependent functions is the genetic disruption of the miRNA binding site in the 3’ UTR of an individual putative target gene. Such an approach can be used to directly assess the importance of a miRNA interacting with a specific target gene during a cellular process.

In the current study, Lu et al. (2015) clearly demonstrate that mice with a mutant SOCS1 3’ UTR that is unresponsive to miR-155 are able to phenocopy several, but not all, T and NK cell phenotypes that are observed when mice lack miR-155 (Figure 1). This interaction between miR-155 and SOCS1 plays an important role in promoting T regulatory (Treg) cell fitness in competitive reconstitution assays. Additionally, this connection is essential for the survival and function of CD8+ T cells during chronic viral infection with mouse LCMV clone 13. Finally, miR-155-mediated repression of SOCS1 is necessary for the expansion of virus-specific natural killer (NK) cells in response to MCMV infection. Taken together, these results find that a single miRNA-mRNA interaction is required for a range of immunological functions by multiple immune cell types.

Such an elegant approach has been reported in the literature only a few times to date, including the disruption of the miR-155 target site in AID (Dorsett et al., 2008; Teng et al., 2009) and PU.1 (Lu et al., 2015).
These mutant mouse strains were used to show that these miR-155 targets regulate B cell phenotypes. In the case of disrupting miR-155 targeting of AID, this led to a high degree of Myc-Igh translocations and consequences for class-switch recombination (CSR) and affinity maturation of antibody genes. Alternatively, preventing miR-155 inhibition of PU.1 resulted in impaired T-cell-dependent B cell responses. Taken together, this approach has provided unequivocal evidence that each of these targets, AID, PU.1, and now SOCS1, is required for certain aspects of miR-155 biology in the immune system. However, it must be kept in mind that just because a single target is required for a phenotype does not imply that additional targets are not also involved. Thus, although prevention of SOCS1 targeting by miR-155 does hinder the above-mentioned phenotypes, it is formally possible that one or many additional targets also play obligatory roles.

Although these studies have been truly insightful, this approach to studying miRNA targets has not been used very often. Clearly this is a high-risk endeavor because of the significant costs, in terms of both financial and time investments, and the potential for a putative target to not be functionally relevant. With the recent explosion of the genome-editing field, as a result of the emergence of Crispr-Cas9 technology, perhaps more innovative, high-throughput approaches to disrupting 3’ UTR miRNA binding sites can be developed and used to rapidly screen for functionally important miRNA targets in the context of specific immunological phenotypes.

In addition to clearly demonstrating the requirement for miR-155 targeting of SOCS1 during some miR-155-dependent immune responses, the current study also points to the existence of additional miR-155 targets that regulate SOCS1-independent miR-155 phenotypes. These include CD8+ T cell responses to acute viral infection, DC production of inflammatory cytokines, and CD4+ T cell differentiation into Th17 cells (Figure 1). Such an understanding of how miRNAs selectively use different targets to mediate distinct functional effects is critical both for our basic understanding of miRNA biology and for our future capacity to manipulate immune responses in the clinic where it would be advantageous to selectively inhibit or promote specific immune responses without interfering with others.

**REFERENCES**


