

Jarid2 Links MicroRNA and Chromatin in Th17 Cells

Escobar et al. now bring microRNAs and chromatin together by showing how activation-induced miR-155 targets the chromatin protein Jarid2 to regulate pro-inflammatory cytokine production in T helper 17 cells.

MicroRNAs are small non-coding RNAs that control gene expression by destabilising protein-coding mRNAs and reducing their translation. The microRNA miR-155 is derived from the non-protein-coding transcript of *Bic*, an oncogene that promotes the development of B cell lymphomas in mice and humans. miR-155 is of interest to immunologists because it is induced by signals that activate T cells, B cells and antigen-presenting cells, and regulates many aspects of the immune response such as the germinal center reaction and the differentiation of Treg cells and CD4⁺ T helper (Th) cells (Thai et al., 2007; O'Connell et al., 2010; Oertli et al., 2011; Lu et al., 2009; Kohlhaas et al., 2009).

In previous studies, miR-155 was required for the differentiation of inflammatory Th1 and Th17 cells in experimental autoimmune encephalomyelitis (O'Connell et al., 2010) and *Helicobacter pylori* infection (Oertli et al., 2011). Escobar et al (2014) now demonstrate that both Th1 and Th17 cells develop during *Toxoplasma gondii* infections in miR-155-deficient mice. However, miR-155-deficient CD4⁺RORγ⁺ cells are defective in the expression of IL22, IL10, IL9, IL17F and IL17A.

Although microRNAs are post-transcriptional repressors, they can facilitate the transcription of genes, for example through the post-transcriptional repression of a negative regulator of the genes in question. Escobar et al (2014) therefore searched for miR-155 targets that might repress cytokine genes. The leading candidate, Jarid2, is the founding member of the Jumonji family of chromatin modifiers. These proteins remove methyl groups from lysine residues in histone tails, and this histone demethylase activity is important for the regulation of gene expression. In contrast to other Jumonji proteins, Jarid2 has amino acid substitutions in its catalytic domain that abolish histone demethylase activity. Although catalytically inactive, Jarid2 is nevertheless important for embryonic development and for embryonic stem (ES) cell differentiation, suggesting that in relinquishing its histone demethylase activity, Jarid2 may have acquired novel and non-redundant functions. Exactly what these are

remains enigmatic, but Jarid2 associates with the polycomb repressive complex PRC2 in a range of different cell types, including ES cells and thymocytes (Landeira and Fisher, 2011). First discovered as a regulator of Hox genes in *Drosophila*, polycomb repressive complexes regulate cellular differentiation and gene expression by modifying the state of chromatin (Margueron and Reinberg, 2011) (Figure 1). In this way, polycomb proteins contribute to gene regulatory events from the poising of lineage-specific genes in pluripotent ES cells to Th cell differentiation (Landeira and Fisher, 2011; Tumes et al., 2013).

At the molecular level, Jarid2 promotes the recruitment of PRC2 to its target genes (Figure 1). There is disagreement whether Jarid2 also regulates the methyltransferase activity of PRC2 (Landeira and Fisher, 2011). However, Jarid2 deficiency is not thought to derepress PRC2 target genes. Rather, many polycomb target genes show reduced expression and impaired recruitment of RNA polymerase II in Jarid2-deficient ES cells. In contrast to PRC2-deficient ESCs, Jarid2-deficient ESCs have a severe differentiation block, and it has therefore been argued that Jarid2 may have a role independent of conventional PRC2 components (Landeira and Fisher, 2011).

The work of Escobar et al (2014) links miR-155-mediated post-transcriptional regulation of Jarid2 (and probably other miR-155 targets) to the transcriptional regulation of cytokine genes in Th17 cells (Figure 1). Although the paper does not directly demonstrate that miR-155 targets Jarid2 (e.g. by mutating putative miR-155 binding sites in the *Jarid2* 3'UTR), this assertion is supported by computational predictions and independent experimental studies (Bolisetty et al., 2009). Escobar et al (2014) find Jarid2 mRNA and protein expression elevated approximately 2-fold in miR-155-deficient Th17 and Treg cells (Figure 1). Such a moderate increase is not unexpected (microRNAs typically act as fine tuners rather than on/off switches) but raises the question how the dosage of a chromatin protein can explain the regulation of cytokine genes in Th17 cells.

To explore the link between Jarid2 and reduced *Il22* expression, Escobar et al. (2014) polarize control and miR-155-deficient Th17 cells *in vitro*. They observe that the production of IL-17, but not IL-22, by miR-155-deficient Th17 cells is restored by exogenous IL-1 β , a factor that promotes Th17 cytokine expression. ChIP experiments showed increased signal for Jarid2, the PRC2 component Suz12 and the repressive histone modification H3K27me3 at the *Il22* locus in miR-155-deficient

Th17 cells. Consistent with lower *Il22* expression in the absence of miR-155, the *Il22* promoter showed reduced occupancy by RNA Polymerase II. These observations are consistent with a scenario where the loss of miR-155 from Th17 cells results in increased Jarid2 expression, Jarid2 binding and PRC2 recruitment to the *Il22* locus, increased repressive H3K27me3 histone modifications, reduced RNA polymerase II activity and reduced *Il22* expression (Figure 1). This may be somewhat idealised and at least in ES cells the situation is more complex, as Jarid2 promotes the recruitment not only of PRC2, but also of RNA polymerase II to a set of developmental regulator genes (Landeira and Fisher, 2011).

If increased expression of Jarid2 in miR-155-deficient Th17 cells is responsible for impaired Th17 cytokine expression, then cytokine expression should be restored when one or both copies of the *Jarid2* gene are deleted. Escobar et al (2014) find that this prediction is partially upheld. Conditional deletion of *Jarid2* restores the expression of *Il17a* in miR-155-deficient Th17 cells, but does not rescue *Il22*. Since Jarid2 does not directly bind *Il17* loci, restoration of IL-17 expression is indirect and may be due to another Jarid2 target, *Atf3* (Escobar et al., 2014). The failure of Jarid2 deletion to rescue *Il22* expression in miR-155-deficient Th17 cells complicates the model that miR-155-mediated regulation of Jarid2 is key to the loss of *Il22* expression and suggests that additional, miR-155 targets contribute the regulation of *Il22*. One candidate is the *Il22* repressor c-Maf, which binds the locus in the absence of Jarid2 (Escobar et al., 2014).

One of the major challenges in understanding the action of transcriptional regulators is how they are recruited to specific genes. This is especially critical when regulation is dose-dependent, as described for Jarid2 in miR-155-deficient Th17 cells. Escobar et al. (2014) show that *Il22* and other genes important for Th17 cells, including *Il9*, *Il10*, *Eomes*, *Tbx21*, and *Atf3*, are targeted by Jarid2 even in control Th17 cells, and that binding merely increased with elevated Jarid2 dosage when miR-155 is deleted. Interestingly, *Il22*, *Il9* and *Il10* expression were increased in Jarid2-deficient Th17 cells (Figure 1). The expression of other PRC2 target genes was unaffected, suggesting additional layers of regulation.

The impact of Jarid2 on the activity of PRC2 may depend on developmental state: Jarid2 is required not for the self-renewal but for the differentiation of ES cells, even though Jarid2 itself is downregulated during differentiation (Landeira and Fisher, 2011). Is there a similar role for Jarid2 in poising genes for future expression during

Th cell differentiation? Finally, miR-155 expression is highly dynamic during T cell activation, and it would be interesting to know how this affects the regulation of miR-155 targets to facilitate changes in the transcriptional and chromatin landscape of activated T cells. The finding that microRNAs and chromatin modifiers form regulatory circuits to control cytokine gene expression is exciting and, in the longer term, may offer opportunities for therapeutic intervention. Th17 cells are important mediators of inflammation and both microRNAs and chromatin modifiers are potential drug targets.

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Figure legend. Jarid2 links post-transcriptional regulation by miR-155 to the transcriptional regulation of cytokine genes in Th17 cells. Jarid2 recruits PRC2, which cooperates with PRC1 (Margueron and Reinberg, 2011). PRC2 and PRC1 repress transcription by catalyzing the repressive histone modifications H3K27me3 and H2AK119ub1, inhibiting RNA polymerase II, and compacting chromatin. Escobar et al. report that loss of miR-155 results in elevated Jarid2 expression, increased PRC2 recruitment and reduced *Il22* transcription. In Jarid2-deficient Th17 cells PRC2 recruitment and repressive histone modifications are reduced, and the expression of a subset Jarid2 target genes is increased.

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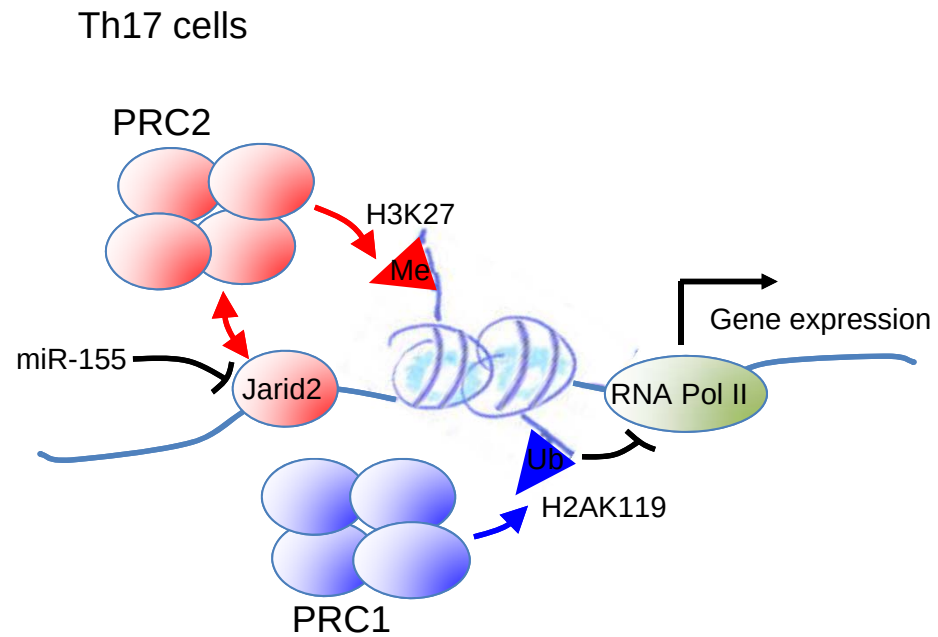
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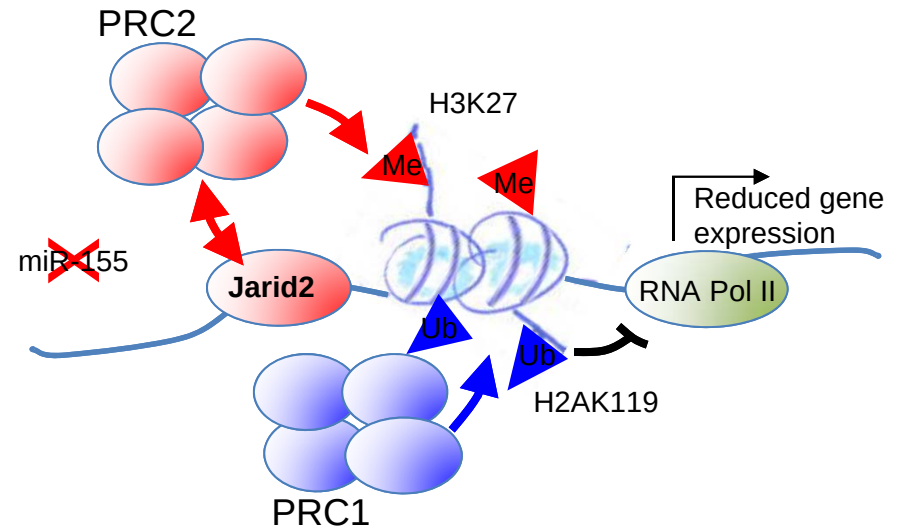
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miR-155-deficient Th17 cells



Jarid2-deficient Th17 cells

