MicroRNAs and the Regulation of Biological Function

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Regulation of B-cell differentiation by microRNAs and RNA-binding proteins

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Abstract
Post-transcriptional control of gene expression is an important mechanism for maintaining cellular homeostasis and regulating the immune response to infection. It allows control of mRNA abundance, translation and localization. Mechanisms for post-transcriptional control involve RNA-binding proteins and miRNAs (microRNAs). TTP (tristetraprolin) family of proteins recognize and bind AU-rich elements. Deletion of TTP led to a systemic autoimmune syndrome with excess circulating TNFα (tumour necrosis factor α) and GM-CSF (granulocyte/macrophage colony-stimulating factor) due to aberrantly stabilized mRNA. The family may also have a role in control of lymphocyte development and function. miRNAs regulate gene expression by promoting decay or inhibiting translation of transcripts with base pair complementarity. The importance of miRNAs in lymphocytes is highlighted by the T-cell-specific deletion of Dicer, an enzyme required for miRNA-mediated processing and from the phenotype of bic (B-cell integration cluster)/miR-155 (miRNA 155)-deficient mice.

Introduction
Post-transcriptional regulation controls the abundance, turnover and translation of mRNA and offers the capacity to integrate signal transduction events with very rapid changes in gene expression during cellular differentiation. These processes dictate the fate of a given transcript from active translation, decay/degradation or translation inhibition. The importance of post-transcriptional regulation in maintaining cellular homeostasis and regulating the immune system in response to infection is now increasingly appreciated.

There are a number of different pathways that regulate mRNA abundance. Some of these are regulated by RBPs (RNA-binding proteins) such as the TTP (tristetraprolin) family which include the BRF (butyrate-response factor)-1 and -2, whereas others involve the expression of small non-coding RNAs called miRNAs (microRNAs). Both of these mechanisms have been implicated in the regulation of transcripts involved in the immune response. RBPs may interact with miRNAs through mechanisms that are not fully understood, and there is evidence that both mechanisms can target the same mRNA [1].

The TTP family of RBPs
The TTP family of proteins are defined by tandem RNA-binding CCCH zinc fingers. They recognize targets containing AREs (AU-rich elements) in the 3′-UTR (untranslated region). Binding promotes mRNA decay [2] or inhibition of translation [3]. Analysis of TTP-deficient mice showed a phenotype characterized by aggressive autoimmunity. Molecular analysis identified elevated levels of TNFα (tumour necrosis factor α) [4] and GM-CSF (granulocyte/macrophage colony-stimulating factor) [5] and showed that TNFα was the cause of pathology. Work in our laboratory and in others generated the germline deletion of BRF-1, also called TIS11b, which was lethal at embryonic day 10.5 [3,6]. Analysis of
mutant embryonic fibroblast identified VEGFA (vascular endothelial growth factor A) mRNA as a target of BRF-1 that was regulated at the level of translation rather than alterations in mRNA decay [3]. This finding indicates that BRF-1 may control targets by affecting translation, although the mechanism remains unidentified. A germline deletion of BRF-2 has yet to be reported, although work on a truncated version indicates a role in control of fertility [7].

Post-transcriptional control of lymphocyte function

There is evidence for the function of the TTP family in lymphocytes. TTP is expressed in both B- and T-cells. In T-cells, TTP is induced in response to anti-CD3 and CD28 stimulation [8], and in B-cells by both LPS (lipopolysaccharide) and BCR (B-cell receptor) ligation [9]. Targets for TTP include the T-cell cytokine IL (interleukin) 2 [8] and other cytokines with AREs such as IL-10. Both TTP and BRF-1 have been shown to be induced rapidly in B-cells responding to antigen, and BRF-1 is induced following elevation of intracellular calcium in B-cells [10]. Other RBPs have been implicated in regulation of lymphocyte development. The HuR (Hu antigen R) and AUF-1 (AU-rich-binding factor 1) have been identified in thymic and splenic lymphocytes [11]. HuR is thought to promote mRNA stability, whereas AUF-1, which can be alternatively spliced into four isoforms, can promote both mRNA decay and stability depending on the levels of different isoforms. There is evidence that HuR can compete with BRF-1 for target mRNAs [12]. This may allow a scenario where the fate of an ARE mRNA is determined by the relative abundance of particular RBPs. The only other characterized RBP with the CCCH zinc finger found in TTP proteins is Roquin [13]. It is expressed in T-cells and localizes with stress granule components, suggesting that it has a role in mRNA processing. It has been characterized as a repressor of ICOS (inducible T-cell co-stimulator) mRNA and mutations in Roquin lead to increased ICOS levels in follicular T-cells, large germinal centres and lupus-like autoimmunity [14].

miRNAs are 20–22-nt-long non-coding single-stranded RNAs that post-transcriptionally regulate gene expression by binding to target sites and inhibiting RNA translation or promoting mRNA degradation. There is also evidence that they may promote translation under certain conditions. The process of miRNA-mediated repression involves certain key enzymes including the RNase III enzyme Dicer. T-cell-specific deletion of Dicer revealed a role for miRNAs in control of T-cell development, in particular development of T-helper and regulatory T-cells [15–17].

Regulation of B-cell differentiation by miR-155 (miRNA 155)

To understand further the role a single miRNA plays in regulation of gene expression, we and others have taken an approach of targeted deletion of individual miRNAs. This work involved the deletion of the non-coding bic (B-cell integration cluster), which contains miR-155. bic is expressed by B- and T-cells, as well as by myeloid cells, and is induced in lymphocytes by stimulation through the antigen receptor or with other mitogens. Stimulation of B-cells and macrophages by Toll-like receptor agonists also induces bic. bic-deficient mice display defective lymphocyte and antigen-presenting cell function [18,19]. In particular, there were reduced germinal centre B-cells in response to a Td (thymus-dependent) antigen [19] and impaired TI (thymus-independent) immune responses [20]. These defects were not due to defective somatic hypermutation) or CSR (class-switch recombination) as they were unaffected by the deletion of bic. Reduced CD138+ plasma cells were observed in miR-155-deficient B-cells using in vitro plasma cell differentiation cultures [20]. Together with the normal SHM and CSR, this indicated that differentiation into plasma cells required miR-155. We showed using chimaeric mice that the plasma cell phenotype was B-cell intrinsic. Microarray analysis of stimulated B-cells identified 60% of up-regulated genes to be predicted mir-155 targets. Molecular analysis identified the transcription factor PU.1, which is normally expressed in the germinal centre, as a direct target for miR-155 and we showed that overexpression of PU.1 in in vitro B-cell cultures mimicked aspects of the bic-deficient phenotype under the same conditions [20]. As PU.1 is expressed at high levels in germinal centre B-cells [21], its down-regulation may be necessary to permit class-switched B-cells to undergo differentiation into plasma cells. In addition, although no difference in proliferation between the miR-155-deficient T-cells and wild-type T-cells was observed, we and others have noticed a propensity to differentiate into Th2-cells which produce IL-4 and little IFNγ (interferon γ). Microarray analysis of miR-155-deficient Th2-cells identified over 100 genes that were overexpressed, including genes involved in driving Th2 differentiation.

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References


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