Regulation of B- and T-cell differentiation by a single microRNA

Martin Turner1 and Elena Vigorito1
Laboratory of Lymphocyte Signalling and Development, Babraham Institute, Cambridge CB22 3AT, U.K.

Abstract
miRs (microRNAs) post-transcriptionally regulate gene expression mainly by repressing translation or by inducing mRNA degradation. Dicer, an enzyme responsible for miR biogenesis, is required for T-cell function, suggesting regulatory roles for miRs in lymphocytes. However, specific roles for individual miRs are only just beginning to emerge. miR-155 is encoded within an exon of the non-coding RNA known as bic (B-cell integration cluster) and high levels of bic expression are induced upon antigen receptor stimulation of B- and T-cells, as well as TLR (Toll-like receptor) stimulation of macrophages and dendritic cells. High levels of bic/miR-155 are found in B-cell lymphomas and solid tumours, indicating that this locus may also be linked to cancer. Indeed, transgenic mice overexpressing miR-155 develop B-cell malignancies. To define the in vivo role of bic/miR-155 (bic), we have studied bic-deficient mice. These mice are immunodeficient and fail to generate high levels of class-switched antibody upon immunization with thymus-dependent and thymus-independent antigens. This defect is intrinsic to B-cells and manifested at the level of differentiation of switched plasmablasts into mature antibody secreting plasma cells. In addition, bic-deficient T-cells show skewed differentiation into the Th2 lineage under a variety of in vitro culture conditions. Microarray analysis of bic-deficient B- and T-cells under different conditions has revealed a wide spectrum of targets regulated by an miR-155 and suggested mechanisms for the regulation of lymphocyte differentiation by a single miR.

The importance of post-transcriptional mechanisms for the regulation of the homoeostasis of the immune system and the response to challenge by micro-organisms is becoming increasingly appreciated. Post-transcriptional regulation offers the capacity to integrate signal transduction events with very rapid changes in translation of mRNA or the expression of regulatory non-coding RNA including miRs (microRNAs).

miRs control gene expression by sequence-specific base-pairing with mRNA, causing inhibition of translation or degradation of the target mRNA. They may also promote mRNA translation under particular conditions such as cell cycle arrest [1]. In mammals, miRs are transcribed as primary miRs; these are then processed in the nucleus to ~70 nt pre-miRs (precursor miRs), which are subsequently transported into the cytoplasm where the RNase III Dicer mediates further processing to generate mature miRs. These are incorporated into the RISC (RNA-induced silencing complex), a multiprotein complex that induces translational repression and/or degradation of the target RNA (reviewed in [2]).

Genetic analysis using the mouse as a model organism offers a well-characterized system in which to define the function of the miR machinery and individual miRs in physiological processes such as the development and function of the immune system. In particular, the use of conditional mutagenesis can reveal tissue-specific functions for genes. Deletion of Dicer in the T-cell lineage has revealed a role for this enzyme in thymic development and the differentiation of T-helper and T-regulatory lymphocytes [3–6]. These observations suggest that miRs may be important in lymphocyte development.

Many miRs are encoded by multigene families, which makes a conventional genetic approach by gene targeting laborious; however, miR-155 resides within the spliced and polyadenylated non-coding gene bic (B-cell integration cluster), which was frequently targeted for retroviral insertion in chickens [7]. bic and miR-155 expression are low in B- and T-lymphocytes as well as myeloid cells, but greatly enhanced by stimulation through the antigen receptor, in the case of lymphocytes, or by TLR (Toll-like receptor) agonists in B-cells and macrophages. These results suggest that bic/miR-155 may regulate the function of lymphocytes and myeloid cells. Transgenic mice expressing miR-155 in B-cells developed pre-B-cell lymphomas [8], raising the possibility that this miR may function as an oncogene. In addition, Hodgkin’s lymphoma, primary mediastinal B-cell lymphoma and diffuse large B-cell lymphoma express high levels of bic and miR-155. By contrast, low levels have been found in Burkitt lymphoma [9–11]. Moreover, miR-K12-11 miR encoded by Kaposi’s-sarcoma-associated herpesvirus is homologous with miR-155 and regulates common mRNA targets, suggesting that it may have evolved to hijack regulatory processes in B-cells to the advantage of the virus [12,13]. However, despite this progress, the
functional significance of the expression of bic and miR-155 in cancer remains uncertain.

Work published by our group and others has shown that miR-155-deficient mice cannot generate protective immunity and display defective lymphocyte and antigen-presenting cell function [14,15]. There is a requirement for bic/miR-155 in B-cell responses to antigens that are thymus-independent. These antigens include bacterial LPS (lipopolysaccharide) (type I) and the repeating polymer Ficoll (type II). In addition, immunization of miR-155-knockout mice with thymus-dependent antigens resulted in impaired humoral responses with a reduced number of germinal centre B-cells, while overexpression of miR-155 led to increased number of these cells [15]. To assess the contribution of B-cells to this phenotype we generated chimaeric mice that contained bic/miR-155-deficient B-cells in combination with wild-type T-cells and a wild-type microenvironment. We found the defects in humoral immunity following primary and secondary immunization are intrinsic to B-lymphocytes, as miR-155-deficient B-cells fail to produce normal numbers of antigen-specific switched antibody-producing cells [16].

We found that somatic hypermutation and immunoglobulin class-switch recombination operate normally in the absence of bic/miR-155. Thus our results suggested that it was the differentiation of class-switched B-cells into plasma cells that was particularly sensitive to the deficiency of miR-155. The current model of plasma cell differentiation is based on the interactions of transcription factors within a network (reviewed in [17]). In this model, the transcription factor BLIMP1 (B-lymphocyte-induced maturation protein 1) is required for the generation of plasma cells and represses the expression of the transcription factors Bcl-6 and Pax5. Bcl-6, in turn, is required for germinal centre formation and acts to inhibit plasma cell differentiation by inhibiting the transcription of BLIMP1. Another transcription factor, Pax5 suppresses the expression of XBP1 (X box-binding protein 1), which mediates the unfolded protein response necessary to protect plasma cells from the unfolded protein response that arises as a consequence of the enormous amounts of antibody they produce. We found that the in vitro proliferation of mutant B-cells was normal but they failed to yield high levels of class-switched antibodies. Microarray analysis of in vitro stimulated B-cells identified 60% of up-regulated mRNAs as predicted miR-155 target mRNAs [16]. Among these target genes, we showed that miR-155 directly regulated the levels of the transcription factor Pu.1. We also showed that overexpression of Pu.1 impairs the emergence of IgG1-positive cells in vitro. As Pu.1 is expressed at high levels in germinal centre B-cells [18], its expression may need to be down-regulated to permit class-switched B-cells to undergo differentiation into plasma cells.

In vitro analysis of T-cell proliferation revealed no differences between miR-155-deficient T-cells and wild-type T-cells; however, we and others identified a tendency of miR-155-deficient T-cells to differentiate into Th2 cells that produce much IL-4 (interleukin-4) and little IFN-γ (interferon-γ). To try to identify genes that were dysregulated in miR-155-deficient Th2 T-cells, we performed a transcriptome analysis that identified approx. 100 overexpressed genes. Analysis of microarray data from miR-155-deficient Th2 cells showed increased expression of c-maf and Itk, which have both been implicated as positive regulators of Th2 differentiation. c-Maf regulates IL-4 gene transcription and overexpression of c-Maf leads to increased Th2 responses. In addition, the Tec family tyrosine kinase, Itk, has been shown to be required in vivo for Th2 cytokine production.

At the molecular level, microarray analysis of B- and T-lymphocytes showed that deletion of a single miR in primary cells causes deregulation of a large number of genes. Moreover, among the genes that showed increased expression, more than half of them were computationally predicted to be direct targets for miR-155. An open question in the miR field is how many of the genes regulated by a particular miR are causative of a particular phenotype. In this regard, in the case of miR-150 regulation of B-cell development, it appears that mby is the sole causative target [15], whereas regulation of T-cell signalling by miR-181 involves several targets [19]. Considering the complexity of the miR-155 phenotype in vivo, it is likely that several targets are involved. Further work will be required to identify miR-155 target genes that are causative of the phenotype observed in vivo.

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References


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