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COMMENTARY

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EnABLing microprocessor for apoptosis

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ABSTRACT

The Microprocessor complex consisting of DROSHA (a type III ribonuclease) and DGCR8 (DiGeorge syndrome critical region gene 8-encoded RNA binding protein) recognizes and cleaves the precursor microRNA hairpin (pre-miRNA) from the primary microRNA transcript (pri-miRNA). The Abelson tyrosine kinase 1 (ABL) phosphorylates DGCR8 to stimulate the cleavage of a subset of pro-apoptotic pri-miRNAs, thus expanding the nuclear functions of ABL to include regulation of RNA processing.

Abbreviations: ABL, Abelson tyrosine kinase 1; BCR, breakpoint clustered region gene; DGCR8, DiGeorge syndrome critical region gene 8; DROSHA, a type III ribonuclease; FS, flanking sequence surrounding a pre-miRNA hairpin in the pri-miRNA; KAT5, lysine (K) acetyltransferase 5, also known as TIP60; miRNA, microRNA; pri-miRNA, primary microRNA; pre-miRNA, precursor microRNA; RNA-CLIP, RNA crosslinking and immunoprecipitation; TP53, p53 tumor suppressor; TP73,, tumor protein 73, a member of the p53 family of transcription factors; WRN, Werner syndrome RecQ helicase

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The Microprocessor complex consisting of DROSHA, a type III ribonuclease (RNase-III), and a RNA-binding protein DGCR8 (DiGeorge syndrome critical region gene 8), is so named because a DROSHA/DGCR8 complex is necessary and sufficient to process a primary microRNA (pri-miRNA) into a precursor microRNA (pre-miRNA) hairpin *in vitro*.[1,2] The RNase-III DICER then cleaves the pre-miRNA hairpin at the loop to generate the mature microRNA (miRNA).[2] Fidelity of DROSHA cleavage in the pri-miRNA is critical for the production of a functional microRNA.[2] How the Microprocessor recognizes the pre-miRNA hairpin and selects the cleavage sites is under active investigation and not yet fully understood. Structural and biochemical studies have led to some consensus views but not to a unifying model for the *in vitro* processing reactions.[3,4] *In vivo*, the processing of pri-miRNA is further regulated by local RNA sequence motifs and other RNA binding proteins.[2,5] For this commentary, it is sufficient to say that regulation of pri-miRNA processing requires both protein–protein and protein–RNA interactions. Our recent finding that phosphorylation of DGCR8 at tyrosine-267 by the Abelson tyrosine kinase 1 (ABL) is required for the processing of selective pri-miRNAs[6] adds a new layer to Microprocessor regulation.

The ubiquitously expressed ABL protein is a signal transducer with a wide range of cell context- and subcellular localization-dependent functional capabilities.[7] The ABL protein enters the nucleus through its 3 nuclear localization signals (NLS). In response to DNA damage, nuclear ABL phosphorylates transcription factors (e.g., tumor protein TP73), chromatin modifying enzymes (e.g., lysine acetyltransferase 5 [KAT5]), DNA repair proteins (e.g., Werner syndrome RecQ-helicase, [WRN]), and RNA polymerase II, indicating that this kinase regulates DNA repair and transcription.[7] It is indisputable that the BCR-ABL fusion protein of chronic myelogenous leukemia has oncogenic activity. In the creation of this oncoprotein, BCR fusion not only activates the ABL kinase but also inactivates its 3 NLSs, because enforced nuclear accumulation of BCR-ABL induces apoptosis.[8] We have mutated the 3 NLSs in the mouse *Abl*1 gene to create the *Abl-μNLS* allele. *Abl-μNLS* homozygous mice express Abl only in the cytoplasm and show resistance to cisplatin-induced renal epithelial apoptosis, providing *in vivo* evidence for the pro-apoptotic function of nuclear Abl kinase.[9]

In mice, the *Abl-μNLS* and *Tp53-null* mutations are epistatic to each other in stimulating the renal epithelial apoptosis response to cisplatin, suggesting that nuclear Abl and Tp53 (tumor protein 53, best known as p53) function in the same pathway.[9] Because cisplatin induces p53 and p53-dependent transcription in *Abl-μNLS* mouse renal epithelial cells, the essential pro-apoptotic function of nuclear Abl operates downstream of transcription in the p53 pathway. The p53-activated pro-apoptotic pathway consists of transcriptional activation of mRNAs that encode pro-apoptotic proteins as well as pro-apoptotic miRNAs such as miRNA-34a and miRNA-34c. Interestingly, we found that ABL kinase is required for the expression of miRNA-34c, but not miRNA-34a, in p53-positive and p53-negative cell lines and in the mouse kidney.[6] Through miRNA sequencing and subsequent validation experiments, we revealed that miRNA-34c and several other pro-apoptotic miRNAs are dependent on ABL kinase for expression.[6]

Using miRNA mini-genes driven by the CMV promoter, we showed that ABL stimulated DROSHA-dependent processing of minigene-derived pri-miRNA-34c but did not stimulate the processing of pri-miRNA-34a. To determine the molecular

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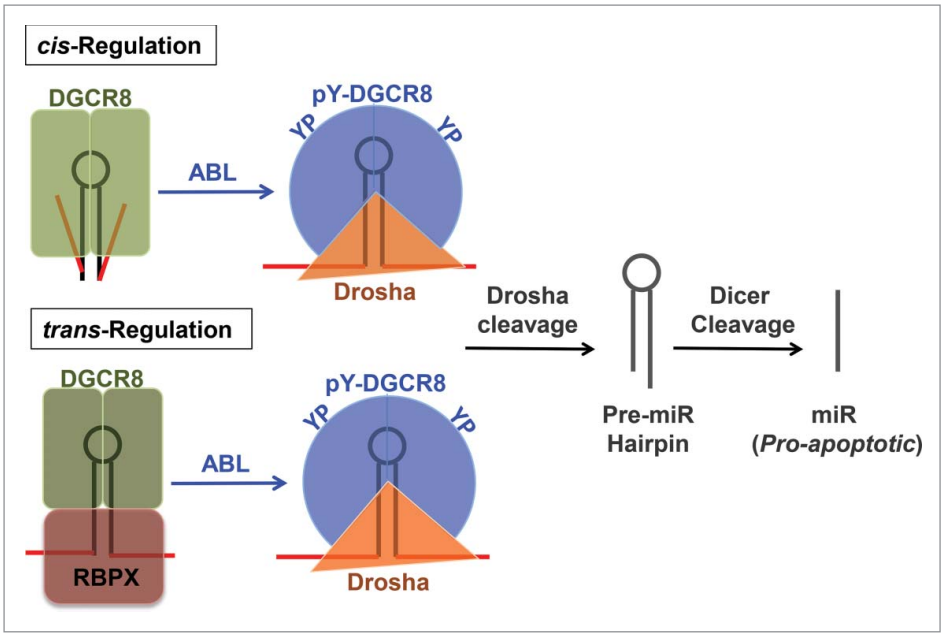

Figure 1. ABL phosphorylates DGCR8 to stimulate production of pro-apoptotic microRNAs. Sequences flanking the precursor microRNA hairpin of primary microRNA-34c (depicted as red lines) are inhibitory for processing. In the *cis*-regulation model, these flanking sequences interact with DGCR8 (DiGeorge syndrome critical region gene 8-encoded RNA binding protein, a subunit of microprocessor) to generate a non-productive complex that cannot interact with DROSHA (a type III ribonuclease, also a subunit of Microprocessor). In the *trans*-regulation model, these flanking sequences recruit another RNA binding protein (RBPX) that binds DGCR8 in place of DROSHA. The Abelson tyrosine kinase 1 (ABL) overrides the inhibitory effects of these flanking sequences by phosphorylating DGCR8 on tyrosine-267. In the *cis*-regulation model, tyrosine phosphorylation converts the non-productive complex into a productive DGCR8-DROSHA complex. In the *trans*-regulation model, tyrosine phosphorylation disrupts the DGCR8 interaction with RBPX to make room for DROSHA. ABL is required for processing of a subset of primary microRNAs that have been shown to have pro-apoptotic functions.

basis of this pri-miRNA-specific effect of ABL, we constructed hybrid minigenes by swapping the hairpins and the flanking sequences (the minigenes each contain 100 nucleotides of flanking sequences on either end of the hairpin) and found that the pri-miRNA-34c flanking sequence (34c-FS) is an important determinant for the ABL requirement. In other words, DROSHA cleavage of a hybrid pri-miRNA containing the pre-miR-34a hairpin and the 34c-FS becomes dependent on ABL.[6]

Using RNA-CLIP (RNA-crosslinking immunoprecipitation), we found that DGCR8 strongly associated with pri-miRNAs containing the 34c-FS and that this association was reduced upon stimulation of pri-miRNA processing by ABL or DROSHA. These findings suggest that 34c-FS can cause a more stable but non-productive interaction of DGCR8 with the pri-miRNA (Fig. 1). The 34c-FS may directly interact with DGCR8 to form a non-productive RNA–protein complex (*cis*-regulation) or it may interact with another RNA binding protein (RBPX) that also interacts with DGCR8 to generate a non-productive RNA–protein complex (*trans*-regulation) (Fig. 1). Because ABL destabilizes the DGCR8 interaction with the 34c-FS-containing pri-miRNAs, ABL must be able to disrupt this non-productive RNA–protein complex.[6] As several independent phosphoproteomics analyses have previously identified tyrosine-267 (Y267) as phosphorylated in DGCR8 (PhosphoSitePlus.org; [10]), we mutated that tyrosine to phenylalanine to generate Y267F-DGCR8.[6] We showed that ABL phosphorylated DGCR8 but not Y267F-DGCR8, and that ABL could not disrupt the interaction of Y267F-DGCR8 with pri-miRNA containing the 34c-FS.[6] We also showed that Y267F-DGCR8 inhibited the processing of pri-miRNA-34c and the interaction of DROSHA with pri-miRNA-34c.[6] Taken together, these results suggest that tyrosine phosphorylation of Y267 by ABL is required for the stimulation of pri-miRNA-34c processing. If the 34c-FS acts in *cis* (Fig. 1), Y267 phosphorylation would inhibit DGCR8 interaction with 34c-FS to disrupt the *cis*-inhibition. If the 34-FS recruits a *trans*-inhibitor, the Y267 phosphorylation might disrupt DGCR8 interaction with that inhibitor (e.g., RBPX in Fig. 1). As a result, only pY267-DGCR8 can productively interact with DROSHA and allow cleavage of pri-miRNAs containing 34c-FS, and possibly other related flanking sequences (Fig. 1).

The finding that ABL-mediated Y267 phosphorylation of DGCR8 can stimulate the processing of a subset of pri-miRNAs opens several new avenues of investigation into the regulation of Microprocessor and of apoptosis. ABL is not likely to be the only kinase capable of phosphorylating DGCR8 on Y267. Finding other Y267-kinases will provide insight into how this modification of DGCR8 links the Microprocessor to other kinase-regulated signaling pathways. Delineating the sequence and/or secondary structure in the 34c-FS that causes a non-productive interaction of DGCR8 with pri-miRNA will provide clues on the *cis* versus *trans* regulatory mechanisms depicted in Fig. 1. Defining the structure and/or the sequence motifs that can inhibit the function of DGCR8 may lead to the identification of other pri-miRNAs whose processing requires ABL. MAP kinase-mediated serine/threonine phosphorylation of DGCR8 has been linked to the production of miRNAs that promote cell proliferation.[10] On the other hand, ABL-mediated tyrosine phosphorylation of DGCR8 may contribute to the production of pro-apoptotic miRNAs. The current data suggest that the

phosphorylation status of DGCR8 may determine which selective subsets of pri-miRNAs are processed and provide a mechanism for regulation of the Microprocessor by kinase pathways.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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