EnABLing microprocessor for apoptosis

Chi-Chiang Tu and Jean Y. J. Wang

Department of Medicine, Division of Hematology-Oncology, Moores Cancer Center, University of California, San Diego, School of Medicine, La Jolla, CA

**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

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-miRNAs6 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 oncprotein, 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 Abl1 gene to create the Abl-μ.NLS allele. Abl-μ.NLS 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-μ.NLS 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.3 Because cisplatin induces p53 and p53-dependent transcription in Abl-μ.NLS 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...
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-miRNA-34c 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 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.

**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.
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.

Funding

This work was supported by NIH R01 CA043054.

References

1. Gregory RI, et al. The Microprocessor complex mediates the genesis of microRNAs. Nature 2004; 432(7014):235-40; PMID:15531877; http://dx.doi.org/10.1038/nature03120
2. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15(8):509-24; PMID:25027649; http://dx.doi.org/10.1038/nrm3838
3. Nguyen TA, et al. Functional Anatomy of the Human Microprocessor Cell 2015; 161(6):1374-87; PMID:26027739; http://dx.doi.org/10.1016/j.cell.2015.05.010
4. Quick-Cleveland J, et al. The DGCR8 RNA-binding heme domain recognizes primary microRNAs by clamping the hairpin. Cell Rep 2014; 7(6):1994-2005; PMID:24910438; http://dx.doi.org/10.1016/j.celrep.2014.05.013
5. Auyeung VC, et al. Beyond secondary structure: primary-sequence determinants license pri-miRNA hairpins for processing. Cell 2013; 152 (4):844-58; PMID:23415231; http://dx.doi.org/10.1016/j.cell.2013.01.031
6. Tu CC, et al. The kinase ABL phosphorylates the microprocessor subunit DGCR8 to stimulate primary microRNA processing in response to DNA damage. Sci Signal 2015; 8(383):ra64; PMID:26126715; http://dx.doi.org/10.1126/scisignal.aaa4468
7. Wang JY. The capable ABL: what is its biological function? Mol Cell Biol 2014; 34(7):1188-97; PMID:24421390; http://dx.doi.org/10.1128/ MCB.01454-13
8. Vigneri P, Wang JY. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. Nat Med 2001; 7(2):228-34; PMID:11175855; http://dx.doi. org/10.1038/84683
9. Sridevi P, Nhiayi MK, Wang JY. Genetic disruption of Ab1 nuclear import reduces renal apoptosis in a mouse model of cisplatin-induced nephrotoxicity. Cell Death Differ 2013; 20(7):953-62; PMID:23660976; http://dx.doi.org/10.1038/cdd.2013.42
10. Herbert KM, et al. Phosphorylation of DGCR8 increases its intracellular stability and induces a progrowth miRNA profile. Cell Rep 2013; 5(4):1070-81; PMID:24239349; http://dx.doi.org/10.1016/j. ccelrep.2013.10.017