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# Linking miRNA Regulation to BCR-ABL Expression: The Next Dimension

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**The introduction of tyrosine kinase inhibitors in the treatment of *BCR-ABL1*-rearranged malignancies has revolutionized therapy, but the prognosis for acute leukemias remains suboptimal. In this issue of *Cancer Cell*, Bueno et al. (2008) add a new dimension to the regulation of ABL1 expression. The authors demonstrate that ABL1 is a direct target of miR-203, miR-203 is silenced by genetic and epigenetic mechanisms in hematopoietic malignancies expressing either ABL1 or BCR-ABL1, and restoration of miR-203 expression reduces ABL1 and BCR-ABL1 levels and inhibits cell proliferation. These findings may have broad implications for mechanisms underlying malignant transformation in hematopoietic and other malignancies.**

MicroRNAs (miRNAs) are noncoding RNAs that regulate many cellular functions including cell proliferation, differentiation, and apoptosis by silencing specific target genes through translational repression or direct mRNA degradation (Ambros, 2004). Although the detailed functions of the growing number of miRNAs identified in the mammalian genome are far from being completely characterized, recent studies have indicated that deregulated expression of specific miRNAs that modulate expression of oncogenes and tumor suppressors is associated with the development of malignancies, and specific miRNA expression signatures can be used to effectively classify human tumors (Lu et al., 2005). Although genome copy-number changes are associated with altered levels of miRNA expression in various human malignancies (Zhang et al., 2006), recent data suggest that miRNA inactivation by epigenetic mechanisms plays an important role as well, and re-expression of certain miRNAs by drugs that modulate epigenetic changes can lead to downregulation of target oncogenes (Fazi et al., 2007).

More than 40 years ago, the Philadelphia (Ph) chromosome was identified by Nowell and Hungerford. The Ph chromosome is a product of the t(9;22), which fuses the Abelson kinase gene (*ABL1*) from chromosome 9 with the breakpoint cluster region (BCR) from chromosome 22 that expresses the BCR-ABL1 fusion protein: a constitutively active tyrosine kinase. The BCR-ABL1 fusion oncoprotein is a hallmark of chronic myelogenous leukemia (CML) and is also present in a fraction of B progenitor acute lymphoblastic leukemia (ALL) cases that have a particularly poor prognosis. Aberrant expression of the wild-type *ABL1* oncogene may also be associated with the development of hematopoietic malignancies including T cell lymphomas (Ren, 2005). While the development of tyrosine kinase inhibitors (TKIs) like imatinib mesylate have revolutionized treatment of *BCR-ABL1*-rearranged leukemias, it has become increasingly clear in recent years that TKI treatment alone will not be curative in many cases, particularly in acute leukemias with *BCR-ABL1* rearrangement. Thus, further dissection of the regulatory networks that drive BCR-ABL1-induced malignant transformation may help to identify other novel therapeutic approaches that complement TKI treatment.

In this issue of *Cancer Cell*, a study by Bueno et al. (2008) begins to elucidate the role of silenced miRNA expression in the regulation of *BCR-ABL1*-rearranged leukemias and T cell lymphomas expressing ABL1. Using comparative genomic hybridization (CGH) analysis of murine γ radiation-induced lymphomas, the authors identify loss of heterozygosity of a fragile 7 Mb chromosomal region on murine chromosome 12, a region coding for approximately 12% (52 miRNAs) of the mammalian miRNAome known to date. miRNA profiling revealed decreased expression of one of the region's miRNAs: miR-203. Analysis of the miR-203 promoter demonstrated that miR-203 is silenced not only by genetic loss of one allele but also epigenetically by promoter CpG hypermethylation in the remaining DNA copy (Figure 1). Next, the authors assessed putative miR-203 targets and identified the *ABL1* tyrosine kinase

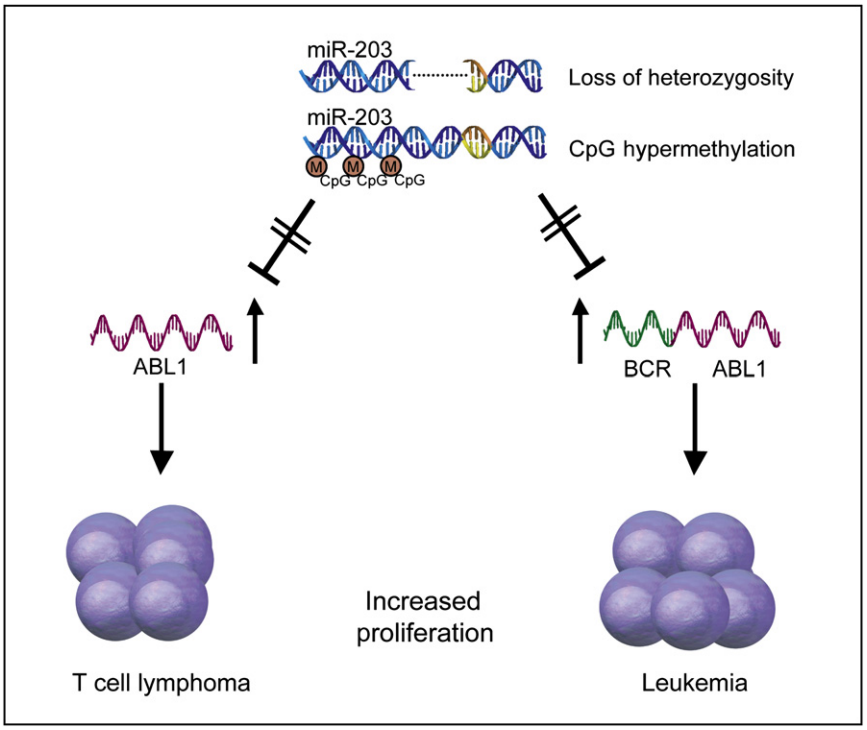


**Figure 1. Model of ABL1 and BCR-ABL1 Regulation by miR-203**
miR-203 is silenced by both genetic and epigenetic mechanisms in lymphomas expressing high levels of *ABL1* and in *BCR-ABL1*-rearranged leukemias. The loss of MiR-203 leads to elevated expression of its direct targets *ABL1* and *BCR-ABL1*, which enhances cell proliferation.

oncogene as being directly regulated by miR-203. Re-expression of miR-203 leads to decreased aberrant ABL1 expression in human T cell tumor cell lines, which was accompanied by an antiproliferative effect in these cells. Of significant interest, the authors demonstrate epigenetic silencing of miR-203 by CpG hypermethylation in human *BCR-ABL1*-rearranged leukemia cells, but not in leukemia cells without the *BCR-ABL1* rearrangement, suggesting a specific pressure to downregulate miR-203 expression in Ph-positive malignancies. Furthermore, the authors find that re-expression of miR-203 leads to a decrease of BCR-ABL1 expression and a decrease in the proliferation rate of Ph-positive human CML cell lines. A similar effect was observed after treatment with drugs that inhibit DNA methylation, including 5′-azacytidine and 4-phenylbutyrate. Treatment of Ph-positive CML cell lines resulted in demethylation of the miR-203 promoter region, restoration of miR-203 expression, and a reduction of both ABL1 and BCR-ABL1 expression. These data demonstrate that genetic and epigenetic mechanisms participate in transcriptional silencing of miR-203, which is associated with elevated expression of ABL1 or BCR-ABL1 in hematopoietic malignancies. In this context, miR-203 seems to modify leukemia cell biology by directly regulating a leukemogenic fusion oncoprotein, and restoration of miR-203 expression suppresses proliferation of these tumor cells. Interestingly, miR-203 has recently been shown to be involved in regulating the balance between stem cell proliferation and terminal differentiation in the skin (Yi et al., 2008). The antiproliferative effect of miR-203 in epidermal stem cells is mediated through the direct repression of p63 expression. Thus, miR-203 may be a general inhibitor of progenitor and stem cell proliferation in normal development, and genetic and epigenetic silencing of miR-203 contributes to uncontrolled proliferation in certain cancers.

This study has several important implications that may impact both therapeutic approaches and oncogenesis. First, it demonstrates a new mechanism regulating aberrant ABL1 and BCR-ABL1 expression. This may be particularly important for therapies incorporating TKIs, as BCR-ABL1 expression levels may influence drug sensitivity (Shah and Sawyers, 2003). Restoration of silenced miR-203 expression—potentially either directly or through the application of demethylating epigenetic drugs such as decitabine—might represent a novel therapeutic approach in ALL and CML cases carrying the BCR-ABL1 fusion and T cell lymphoma/leukemias with high-level ABL1 expression. As loss of the miR-203-containing gene region is observed during disease progression in CML blast crisis (Sercan et al., 2000), it is possible that disease progression may be accompanied by inactivation of tumor suppressor genes in this region including miR-203. However, it is likely that other mechanisms may also be involved in CML progression to blast crisis, such as loss of the transcription factor Ikaros found in many *BCR-ABL1*-rearranged ALLs as well as in a significant fraction of CML blast crisis cases (Mullighan et al., 2008). Also, activation of the Wnt pathway in myeloid progenitor cells accompanies CML progression (Jamieson et al., 2004). A critical next step in the biology of CML blast crisis will be to further dissect the significance, frequency, and potential interdependence of each of these mechanisms.

The findings of Bueno et al. (2008) also have potential implications more broadly for the regulation of fusion oncogenes in leukemia development. The demonstration that miRNAs can be lost through genetic and epigenetic mechanisms and that these miRNAs can directly regulate expression of fusion oncoproteins suggests another layer of complexity that may modify tumor biology. Future studies will continue to shed light on the extent to which miRNA-mediated regulation of fusion or other oncogene expression is a central part of the oncogenic process and whether this new dimension of regulation plays a critical role in tumor progression or drug resistance. These newly defined regulatory mechanisms and added layer of complexity will hopefully provide another avenue for therapeutic intervention.

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# The Ups and Downs of Src Regulation: Tumor Suppression by Cbp

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**Tight control of the tyrosine kinase activity of c-Src is critical for regulating its oncogenic potential. In a recent issue of *Molecular Cell*, Oneyama et al. (2008a) report that the membrane-bound adaptor protein Cbp (also known as PAG) can suppress c-Src-mediated cell transformation and tumorigenesis by binding and sequestering c-Src within lipid rafts. Cbp is also a raft-associated binding partner for Csk, a negative regulator of c-Src. However, the authors show that Cbp-mediated Src suppression is Csk independent. These findings suggest that Cbp is a tumor suppressor whose expression is downregulated during Src-driven cancer progression.**

Src family kinases (SFKs) are membrane-bound tyrosine protein kinases that share a common domain structure. The founding member of the family, Src, was originally identified as a retroviral oncoprotein that induces transformation of avian cells and sarcoma formation in chickens. It is now well established that the cellular proto-oncoprotein c-Src plays an influential role in human cancers. Increased levels of c-Src protein and/or tyrosine kinase activity have been detected in multiple tumor types, including breast, colon, lung, head and neck, and pancreatic cancers (Ishizawar and Parsons, 2004). Src activation promotes tumor progression, metastasis, and angiogenesis, while blockade of Src kinase activity results in decreased tumor cell proliferation, migration, and invasion. These findings have served as an impetus for the development of Src kinase inhibitors, several of which are currently in clinical trials for therapeutic treatment of a variety of human cancers.

The kinase activity of SFKs is tightly regulated. A critical negative regulatory tyrosine resides in the C-terminal tail of all SFKs (Tyr527 in c-Src). When Tyr527 is phosphorylated, c-Src is inactive. Dephosphorylation of Tyr527, or mutation of Tyr527 to Phe, activates c-Src and induces cellular transformation. Crystallographic studies have revealed that the structural basis for c-Src regulation involves intramolecular interactions between phospho-Tyr527 and the SH2 domain, and between the SH3 domain and a polyproline-rich region. As a result, the kinase domain is maintained in a closed, inactive conformation in resting cells.

The kinase that phosphorylates Tyr527 is Csk, C-terminal Src kinase. Csk contains an SH3, SH2, and kinase domain but lacks the N-terminal membrane-binding motif (SH4) found in SFKs. Thus, a mechanism must exist to allow cytosolic Csk to gain access to its membrane-bound substrate. In 2000, two groups identified a transmembrane protein, Cbp (also called PAG), that binds Csk (Brdicka et al., 2000; Kawabuchi et al., 2000). Phosphorylation of Cbp by SFKs serves to recruit Csk to membrane-bound Cbp. Csk then phosphorylates and consequently inactivates membrane-bound SFKs.

Given this neatly intertwined set of reactions, one might assume that the ability of Cbp to regulate SFKs is dependent on Csk. However, a recent paper in *Molecular Cell* (Oneyama et al., 2008a) provides several new twists to this scenario. First, Oneyama et al. show that Cbp can function independently of Csk. The authors used mouse embryonic fibroblasts derived from $Csk^{-/-}$ mice. When c-Src is expressed in these cells, it is activated and promotes transformation (Oneyama et al., 2008b). The authors first noted that levels of endogenous Cbp mRNA and protein were reduced when activated c-Src was expressed. They then made the seminal observation that overexpression of exogenous Cbp reversed the