Mdm2 after DNA damage, it begins to provide a molecular explanation for how Mdm2 may act either as an oncogene or a tumor suppressor, depending upon the particular context (Manfredi, 2010). This latter notion has important implications for the prognosis and treatment of tumors with aberrant Mdm2 expression.

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Polycomb Regulates NF-κB Signaling in Cancer through miRNA

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The mechanisms leading to the constitutive activation of NF-κB in cancers and the pathways upstream and downstream of this activation are not fully understood. In this issue of *Cancer Cell*, Yamagishi et al. demonstrate that Polycomb-mediated silencing of miR-31 is implicated in the aberrant activation of NF-κB signaling in tumors.

Differential gene expression distinguishes one cell type from another and enables cells to build specialized tissues. Once a cell fate decision is made, the cell must be able to silence the transcriptional programs that could potentially lead to other lineages, because the DNA content of all cells is the same. Epigenetic factors play a crucial role in this type of gene expression regulation. The Polycomb group of proteins plays a pivotal role in silencing and in the long-term repression of genes implicated in cell fate decisions (Richly et al., 2010). Polycomb proteins belong either to Polycomb repressive complex 1 (PRC1) or PRC2. The PRC2 component EZH2 methylates lysine 27 of histone H3, which attracts the PRC1 complex; the presence of both PRC1 and PRC2 at promoter regions leads to transcriptional silencing (Richly et al., 2011).

It is now clear that, in addition to epigenetic complexes, microRNAs (miRNAs) also contribute greatly to posttranscriptional gene regulation. miRNAs are endogenous, short (~23 nt) RNAs that suppress gene expression via sequence-specific interactions with the 3′ untranslated regions of related mRNA targets. miRNAs affect gene silencing via both translational inhibition and mRNA degradation. Several miRNAs have been reported to have a direct role in oncogenesis, and indeed, abnormal miRNA expression is a common feature of diverse types of cancers, suggesting potential diagnostic or prognostic biomarker uses.

The NF-κB transcription factor family regulates the expression of diverse genes involved in development, cell growth, immune responses, apoptosis, and neoplastic transformation. Activation of NF-κB is a tightly regulated event. In non-malignant cells, NF-κB is activated only after appropriate stimulation, after which it transiently upregulates the transcription of its target genes. In tumor cells, different types of molecular alterations may result in an impaired regulation of NF-κB activation and deregulated expression of target genes due to constitutively active NF-κB. Recent studies have also demonstrated that miRNAs modulate NF-κB signaling in both normal and pathological scenarios (Lu et al., 2011; Ma et al., 2011).

Adult T cell leukemia/lymphoma (ATL) is an aggressive neoplasm of mature $CD4^+$ T lymphocytes caused by the human T cell leukemia/lymphoma virus type 1 (HTLV-1) infection. Aberrant activation of NF-κB stimulates cell growth and anti-apoptotic responses in ATL cells and thus directly participates in ATL pathogenesis. Recently, correlations between the epigenetic machinery, NF-κB activation, and ATL pathology have been suggested (Sasaki et al., 2011). However, mechanistic insights are lacking. Tax, an

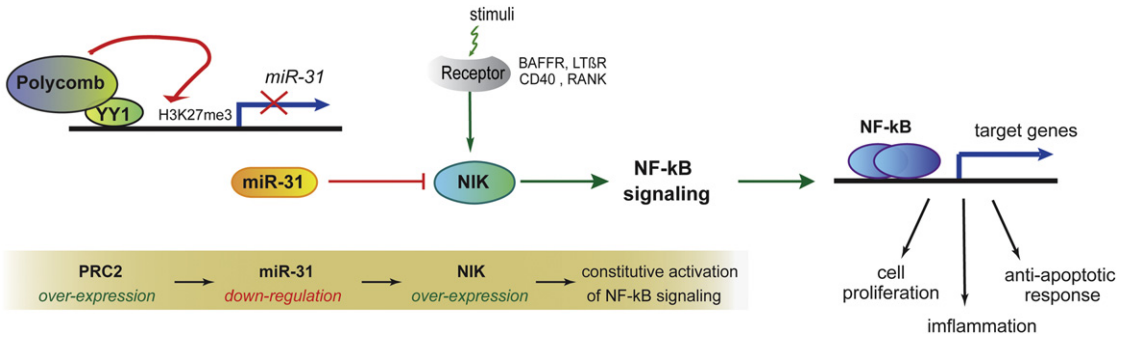

Figure 1. Polycomb-Mediated Silencing of miR-31 Activates the NF-κB Pathway
miR-31 negatively regulates the non-canonical NF-κB pathway by targeting the NIK. Polycomb can modulate NF-κB signaling via miR-31 regulation. PRC2 is recruited by YY1 to a region upstream of the miR-31 locus and epigenetically silences miR-31 expression. In ATL cells, overexpression of PCR2 components leads to miR-31 repression, NIK upregulation, and constitutive activation of NF-κB signaling.

HTLV-1 regulatory protein, has also been reported to activate the NF-κB pathway. Tax expression is required at the early stages of cellular transformation, but Tax is not expressed in the later states. In this issue of *Cancer Cell*, Yamagishi et al. (2012) describe a Tax-independent mechanism by which NF-κB is aberrantly activated in ATL cells through a PRC2-dependent miR-31 epigenetic silencing.

Given the poor understanding of the molecular basis of ATL development, Yamagishi et al. (2012) initially characterized the genetic and epigenetic background of the ATL disease. The authors demonstrated by miRNA expression microarray analyses that miR-31 is downregulated in all primary ATL samples; this suggests that miR-31 loss is a prerequisite for ATL development. Using computational algorithms and gene expression microarray analyses of ATL primary samples, the authors revealed that the NF-κB-inducing kinase (NIK) is a downstream target of miR-31. Intriguingly, NIK plays a central role in activating non-canonical NF-κB signaling and has been implicated in ATL pathogenesis (Saitoh et al., 2008).

The Weinberg lab previously demonstrated that miR-31 plays a suppressive role in breast cancer metastasis (Valastyan et al., 2009). Although miR-31 can concomitantly repress multiple prometastatic targets in breast tumors, miR-31's impact on metastasis was shown to be mediated through a small cohort of these targets. Accordingly, Yamagishi et al. (2012) now provide evidence for a high specificity of NIK regulation by miR-31 in ATL. First, NIK knockdown and miR-31 ectopic expression produced similar effects in ATL cells, such as triggering an apoptotic response and attenuating cell proliferation. Second, expression of a miR-31-resistant NIK mutant into miR-31-expressing ATL cells impaired their ability to enter apoptosis. These results emphasize the ability of miRNAs to exert their effect by modulating a precise and modest number of downstream targets in several cell types and broaden the anti-carcinogenic role of miR-31 in different types of cancers.

Computational analysis identified YY1 binding motifs upstream of the miR-31 region in both human and mouse. As the YY1 motifs have been previously shown to be involved in PRC2 recruitment, the authors propose that YY1 targets PRC2 to the miR-31 locus and thus induces its epigenetic silencing. This in turn leads to increased intracellular levels of NIK and triggers NF-κB activation (Figure 1). Interestingly, high levels of several Polycomb proteins and YY1 have been reported together in primary ATL samples (Sasaki et al., 2011). These results complement the data from Yamagishi et al. (2012) and highlight a novel pathway in ATL that is triggered by an aberrant overexpression of PRC2 complex, leading to an epigenetic-directed activation of the NF-κB pathway. It is now relevant to understand how the PRC2 complex is deregulated in ATL.

Understanding the regulatory mechanisms involving Polycomb proteins is highly relevant considering their important roles in both normal and cancer cells. In this respect, the Yamagishi study emphasizes two interesting mechanistic features of PRC2: first, its ability to regulate miRNA expression in human lymphocytes, as previously showed in *Drosophila* and in mouse ES cells (Enderle et al., 2011; Marson et al., 2008); and second, its ability to be recruited by YY1. Indeed, as Polycomb response elements have not been identified in mammals, several studies have identified a handful of PRC2 recruiters that could be cell type-specific. It would be of interest to study if YY1 binding sites are frequently present in miRNAs that are silenced by PRC2 and whether this is cell-specific.

Strikingly, 12.5% of the ATL cases studied by Yamagishi et al. (2012) had genomic deletions of the region where the human gene that encodes miR-31, *hsa-miR-31*, is located. Correspondingly, several ATL cases with no genetic deletion at *hsa-miR-31* presented a severe downregulation of miR-31 expression. Thus, this study also opens the possibility to monitor the expression of miR-31 as clinical biomarkers for ATL. Furthermore, the miRNA expression signature in ATL cells presented in this study revealed 59 other miRNAs to be downregulated, some of which have known tumor-suppressive functions. These might also be relevant for ATL pathogenesis and warrant further investigation.

The findings by Yamagishi et al. (2012) further our understanding of the highly complex transformation phenotype and oncogenic synergism in ATL. Recent data highlighted the relevance of NF-κB activation in Notch1-induced T cell acute lymphoblastic leukemia and its potential as a therapeutic target (Espinosa et al., 2010). The crosstalk between NF-κB and Polycomb-mediated silencing of miRNAs

described here opens new therapeutic possibilities for ATL treatment. Targeting Polycomb activity, restoring the tumor suppressor miR-31, or inhibiting NIK are all attractive potential strategies for eliminating ATL tumor cells. Furthermore, the described involvement of miR-31 in breast cancer cells (Valastyan et al., 2009) raises the question of whether miR-31 silencing through PRC2 occurs in other type of tumors and whether these pathways could be also be targets for molecular therapies in those malignancies. Finally, from a basic biology viewpoint, the novel mechanism described by Yamagishi et al. (2012) might have a wider role in normal cells, given the ubiquitous roles of NF-κB, Polycomb, and miRNAs in several tissues.

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How to Fool a Wonder Drug: Truncate and Dimerize

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In a recent paper, Poulikakos et al. describe a new and potentially common mechanism whereby melanomas develop resistance to the BRAF inhibitor vemurafenib by expressing truncated forms of BRAF(V600E) that can dimerize in the absence of activated RAS. Will it be possible to block this with improved BRAF inhibitor design?

Metastatic melanoma has long been renowned for being extremely difficult to treat effectively. However, the last few years have witnessed dramatic changes to the landscape of this disease. In 2002, it was discovered that over 50% of melanomas harbor activating mutations, most commonly V600E, in the gene encoding the protein kinase BRAF, which lead to constitutive activation of the RAF/MEK/ERK pro-proliferative signaling pathway (Davies et al., 2002). Within a few years, the first selective BRAF inhibitor was in clinical trials producing highly encouraging results. In a phase I clinical trial, the BRAF(V600E) selective inhibitor vemurafenib (PLX4032) resulted in complete or partial regression in the majority of melanoma patients harboring the BRAF(V600E) mutation (Flaherty et al., 2010). However, the excitement from this spectacular result was soon tempered as resistance to the therapy quickly developed, resulting in response durations of only 2 to 18 months.

Vemurafenib is only effective in BRAF mutant cells. In normal tissues and in cells where the RAF/MEK/ERK pathway is activated by mutation of the upstream RAS signaling proteins, vemurafenib actually enhances signaling. Key to understanding this surprising result is the fact that RAF isoforms BRAF and CRAF normally homo- or heterodimerize following activation of RAS proteins. RAF inhibitor binding appears to cause a conformational change that promotes the formation of BRAF-CRAF or CRAF-CRAF dimers in which the drug-inactivated molecule is able to induce activation of its drug-free partner within the dimer. On the other hand, in cells harboring BRAF(V600E), the levels of activated RAS (GTP bound) are insufficient to induce dimer formation, so BRAF(V600E) signals only as a monomer and the inhibitor can completely block its kinase activity (Hatzivassiliou et al., 2010; Heidorn et al., 2010; Poulikakos et al., 2010) (Figure 1).

This model suggests that molecular lesions that enhance RAF dimerization in tumor cells will increase RAF activity upon drug treatment and promote tumor resistance. Poulikakos et al. 2011 have now found evidence for the operation of just such a mechanism in vemurafenib-resistant, BRAF(V600E) mutant melanoma cell lines, and patient samples. The authors generated resistant cell lines by exposing a BRAF(V600E) melanoma