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Harnessing the Hidden Antitumor Power of the MLL-AF4 Oncogene to Fight Leukemia

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It is unclear whether the antiproliferative/proapoptotic activity of oncogenes can be pharmacologically reactivated in cancer cells. In this issue of *Cancer Cell*, Liu and colleagues report that a proteasome inhibitor reactivates an MLL-AF4 controlled antitumor program to kill leukemia cells in an oncogene dose- and cell type-dependent manner.

Oncogenes, such as constitutively activated Ras, can drive tumorigenesis; therefore, it was initially unexpected that oncogenes also possess contextual antitumor activities like causing apoptosis and cell senescence (Lowe et al., 2004; Serrano et al., 1997). It was later found that while activated oncogenes initially trigger antitumor processes in cells, they eventually lose their antitumor activity and drive tumorigenesis in a temporal and contextual manner (Lowe et al., 2004). However, it remains unclear whether the often hidden antitumor action of a particular oncogene can be reactivated pharmacologically and utilized to specifically target cancer cells. A definitive answer to this question would unravel the mechanisms choreographing the ‘‘Jekyll and Hyde’’ actions of oncogenes, i.e., antitumor versus oncogenic activity, and shed light on how to improve cancer therapy. In this regard, Liu et al. (2014) report in this issue of *Cancer Cell* that when pro-B *m*ixed *l*ineage *l*eukemia (MLL) cells are treated with a proteasome inhibitor, the MLL-AF4 protein level increases and subsequently triggers an antitumor program in concert with the pro-B cell-specific transcription factor PAX5. This antitumor program induces both the antiproliferative p27kip, encoded by *CDKN1B* and proapoptotic caspase-8, and ultimately leads to specific suppression of pro-B MLL-AF4 leukemia.

MLL is classified as a group of acute leukemias, often refractory to chemotherapy and with poor overall prognosis, that expresses one of a number of different leukemogenic fusion genes consisting of the 5′ part of *MLL* fused to one of many genes from other chromosomes (Krivtsov and Armstrong, 2007). MLL fusion proteins (MLL-FPs), in concert with the wild-type MLL protein (Thiel et al., 2010), drive leukemogenesis mainly through inducing the expression of HOX genes (Ayton and Cleary, 2003; Milne et al., 2002). Expression of MLL-AF4 tends to cause B cell lymphoblastic leukemia (Krivtsov et al., 2008).

The authors previously reported that MLL-FPs are regulated in leukemia cells via proteolysis by the proteasome (Liu et al., 2007), a molecular machine specialized in degrading proteins. Unlike many oncogenes that are highly expressed in cancer cells, MLL-AF4 tends to be expressed at low levels in leukemia cells. To address this distinct feature of MLL-AF4, Liu and colleagues investigated whether increased levels of MLL-AF4 leads to suppression of leukemia cells. They treated various human leukemia cell lines with the proteasome inhibitor bortezomib, which is approved for the treatment of multiple myeloma, to inhibit MLL-AF4 degradation. Many key proteins controlling cell survival and proliferation are regulated by proteasome-mediated proteolysis, and their levels are often increased by treatment with bortezomib (Frankland-Searby and Bhaumik, 2012). Bortezomib increased levels of wild-type MLL as well as MLL fusion proteins in all tested leukemia cell lines. Interestingly, pro-B MLL leukemia cell lines were more sensitive to bortezomib-induced G2/M cell cycle arrest and apoptosis when compared to non-MLL pro-B leukemia cell lines, whereas all of the cell lines showed similar sensitivity to other chemotherapeutic agents. Based on these findings, the authors suspected that MLL-AF4 participates in bortezomib-induced cytotoxicity in the pro-B MLL leukemia cells.

To explore this possibility, they demonstrated that selective knockdown of

MLL-AF4 led to a reduction in bortezomib-induced apoptosis in the pro-B MLL leukemia cells. Consistently, ectopic expression of MLL-AF4 cDNA in non-MLL pro-B leukemia cells enhanced their sensitivity to bortezomib-induced cytotoxicity, while ectopic expression of N-terminal MLL alone without a fusion partner failed to enhance the sensitivity to bortezomib. Collectively, these findings uncover a crucial role for MLL-AF4 in mediating bortezomib-induced cytotoxicity in pro-B MLL leukemia cells, but not in MLL-FP acute myeloid leukemia (AML) cells.

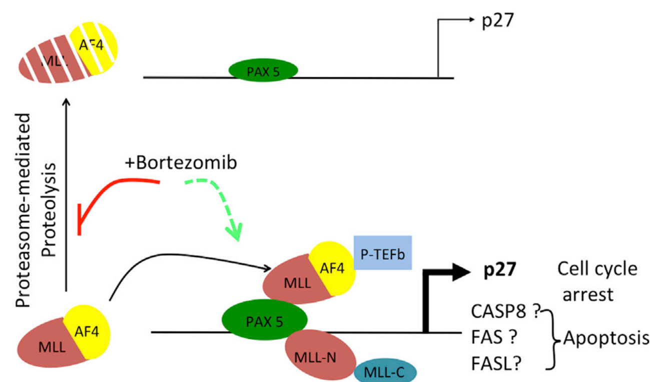

Figure 1. A Model for Proteasome Inhibitor-Induced Increase of the MLL-AF4 Level and Induction of PAX5-Dependent Transcription of p27 in Pro-B MLL-AF4 Leukemia Cells.
The proteins are not drawn to scale, nor is the protein complex precisely stoichiometric as shown.

with pro-B MLL-AF4 leukemia, but not those with non-MLL pro-B leukemia. Furthermore, they also showed the effectiveness of bortezomib for certain pro-B MLL leukemia patients in a small number of patients. Given these results, it is also interesting to speculate whether other FDA-approved chromatin modifying drugs, such as deacetylase inhibitors or DNA methyltransferase inhibitors, can also synergize with bortezomib to raise MLL-AF4 levels and increase MLL-AF4 antitumor target genes to suppress pro-B MLL-AF4 leukemia.

Liu et al. (2014) further explored how bortezomib induces apoptosis in pro-B MLL-AF4 leukemia cells. They found that bortezomib induced expression of FAS, FAS ligand, and caspase-8, all important components of an apoptotic cascade, but did not affect the classic targets of MLL-FPs such as HOXA9 and MEIS1. This suggests that the increased level of MLL-AF4 induced by bortezomib is important for inducing expression of these apoptotic genes, whereas other classic MLL-FP targets such as HOXA9 and MEIS1 might already be expressed at a maximal level, thus preventing their expression from being further augmented by additional MLL-AF4. However, whether MLL-AF4 is directly involved in upregulating transcription of these proapoptotic genes remains unclear.

Next, the authors investigated the mechanism of bortezomib-induced cell cycle arrest in the pro-B MLL leukemia cells. They demonstrated that bortezomib treatment substantially upregulated p27 at both the mRNA and protein levels, while levels of other cell cycle proteins remained unchanged. Upregulation of p27 was dependent on the MLL-AF4 level as MLL-AF4 knockdown attenuated bortezomib-induced p27 expression. Wild-type MLL may play a role in the upregulation of p27, because concurrent knockdown of both MLL-AF4 and MLL impaired the induction of p27 to a greater degree than knocking down MLL-AF4 alone. Using a chromatin immunoprecipitation (ChIP) assay, Liu et al. (2014) found that bortezomib increased recruitment of MLL and MLL-AF4 at the *CDKN1B* promoter along with P-TEFb, resulting in enhanced p27 expression (Figure 1).

However, these results still beg the question of why MLL-AF4 is only critical for bortezomib-induced cytotoxicity in pro-B MLL leukemia cells, but not in MLL-FP AML cells. To address this issue, the authors explored the possibility that pro-B cell-specific transcription factors PAX5 and EBF1 may crosstalk with MLL-AF4 to enhance transcription of target genes and found that indeed PAX5 interacted with MLL-AF4. Moreover, PAX5 was essential for bortezomib-mediated induction of p27 as PAX5 knockdown blocked the increase in p27 levels. Furthermore, ChIP assay, coupled with PAX5 knockdown, showed that PAX5 is required for recruiting MLL/MLL-AF4 to the *CDKN1B* promoter. However, PAX5 overexpression alone was not sufficient to sensitize MLL-AF9 containing THP1 cells (AML cells) to bortezomib, indicating additional factors may also be critical in pro-B cells. Collectively, these data strongly suggest that bortezomib induces expression of p27 by PAX5-mediated recruitment of MLL-AF4 and P-TEFb (Figure 1).

Next, the authors determined whether bortezomib selectively suppresses human pro-B MLL-AF4 leukemia in vivo. They transplanted MLL-AF4 pro-B or non-MLL pro-B leukemia cells into immunodeficient NOD-*scid Il2rg*$^{-/-}$ mice, followed by treatment with or without bortezomib. Consistently, they found that bortezomib reduced the leukemia burden in mice

In summary, Liu et al. (2014) have shown that a clinically effective proteasome inhibitor switches on the hidden molecular tumor-suppressing networks mediated by the leukemogenic MLL-AF4 in an oncogene dose- and cell type-specific manner. These findings demonstrate that the hidden antitumor function of an oncogene can be reactivated pharmacologically through the use of an FDA-approved proteasome inhibitor. Practically, these studies highlight the intriguing possibility that stratification of leukemias based on both their underlying oncogenic driver mutations, such as MLL-AF4, and their cell type may ultimately guide the precise choice of therapeutic agents, which may soon include proteasome inhibitors.

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