

Akt signaling is pleiotropic but nonetheless key to tumor cell homeostasis. This figure has been adapted with permission from Vivanco and Sawyers.⁶

in cancer cells.⁹ These include inhibitors (kinase inhibitors and monoclonal antibodies) that target upstream regulators of PI3K/AKT, such as PI3K and growth factor receptors, and also downstream effectors, such as the components of the mammalian target of rapamycin/AKT pathway, and finally those that target AKT itself, principally through its adenosine triphosphate (ATP) binding site of PI3K and phosphorylation sites.

As a consequence, several inhibitors of proteins that are involved in PI3K/AKT signaling pathways have been under development for some time for myeloma, and some have even entered phase 3 clinical trials.¹⁰ However, despite compelling evidence of the key role of this pathway in tumor cells, particularly in the context of the feeding bone marrow milieu and microenvironment for myeloma tumor cells, no drug used alone or in combination with existing therapies developed to exploit activation of the PI3K/AKT pathway within myeloma cells and/or enhance the efficacy of existing chemotherapeutic agents has been approved to date for myeloma—a marker of truth on whether a drug has been proved worthy of achieving better therapy for multiple myeloma.

This first-time-in-human study (PKB112835) was designed to evaluate the safety, pharmacokinetics, and initial clinical efficacy of afuresertib in patients with relapsed and/or refractory hematologic malignancies.

Afuresertib is a reversible, ATP-competitive, oral pan-AKT kinase inhibitor. Once-daily oral administration to mice delayed the growth of various human tumor xenografts in a dose-dependent manner. The frequency of sensitivity was particularly high in T-cell acute lymphoblastic leukemia (ALL), B-cell ALL, chronic lymphocytic leukemia, and non-Hodgkin lymphoma cell lines, with the 50% effective dose <1 μM. The maximum-tolerated dose was established at 125 mg per day. Clinical activity of single-agent afuresertib was observed in patients with myeloma, with an overall response rate (partial response or better) of 8.8% and a clinical benefit rate (≥ minor response) of 17.6% although the majority of patients had very advanced and/or end-stage myeloma. Interestingly, it appeared that single-agent afuresertib had a favorable safety

profile, whereas previous PI3K/AKT inhibitors were often hampered by toxicities, including myelosuppression (mostly thrombopenia) and also nonhematologic gastrointestinal toxicities, metabolic disturbances (hyperlipidemia and hyperglycemia) and, to a lesser extent, skin issues, dyspnea, and dry cough, among others.

Therefore, the promise is great that a PI3K/AKT pathway inhibitor might finally complete the armamentarium for treating myeloma that will enable several choices of combinations to enhance treatment and limit development of mechanisms of resistance, given the favorable safety profile.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

- Spencer A, Yoon S-S, Harrison SJ, et al. The novel AKT inhibitor afuresertib shows favorable safety, pharmacokinetics, and clinical activity in multiple myeloma. *Blood*. 2014;124(14):2190-2195.
- Palumbo A, Anderson K. Multiple myeloma. *N Engl J Med*. 2011;364(11):1046-1060.
- Bahlis NJ. Darwinian evolution and tiding clones in multiple myeloma. *Blood*. 2012;120(5):927-928.
- Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. *Nat Rev Cancer*. 2012;12(5):335-348.
- Staal SP. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. *Proc Natl Acad Sci USA*. 1987;84(14):5034-5037.
- Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer*. 2002;2(7):489-501.
- Bellacosa A, Testa JR, Staal SP, Tsichlis PN. A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. *Science*. 1991;254(5029):274-277.
- Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature*. 2001;411(6835):355-365.
- Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer*. 2009;9(8):550-562.
- Stewart AK. Novel therapeutics in multiple myeloma. *Hematology*. 2012;17(Suppl 1):S105-S108.

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● ● ● LYMPHOID NEOPLASIA

Comment on Low et al, page 2223

PP2A inactivation by ROS accumulation

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In this issue of *Blood*, Low and colleagues show that reactive oxygen species (ROS)-mediated phosphorylation of Bcl-2 overcomes chemoresistance in hematopoietic malignancies and other cancers and that this function is mediated

by selective nitration of Y289 on the Bcl-2-bound B56δ subunit of protein phosphatase 2A (PP2A), which interferes with the interaction of Bcl-2 with the PP2A catalytic core, leading to increased Bcl-2 S70 phosphorylation.¹

ROS are involved in many vital physiological processes such as host defense and biosynthesis at low to moderate concentrations; however, high levels of ROS can cause biological damage termed oxidative stress and are thereby implicated in many pathophysiological conditions, including cardiovascular diseases, neurological disorders, and cancers.² Under oxidative stress conditions, excessive ROS can damage cellular proteins, lipids, and DNA, leading to genomic instability, genetic mutation, and modulation of gene expression that may contribute to tumorigenesis.³ Because cancer cells often display increased ROS generation and disturbed redox regulation, the increase of ROS in cancer cells often induces redox adaptation in response to the sustained oxidative stress, leading to upregulation of antioxidant molecules, such as reduced glutathione, which may confer resistance toward anticancer agents. One of the most important ways that ROS function in cancer is by activating various intracellular signaling pathways. Hydrogen peroxide (H₂O₂) can also function as a second messenger, activating intracellular pathways such as mitogen-activated protein kinases (MAPKs), phosphoinositide

3-kinase/serine-threonine kinase, and nuclear factor-κB pathways. H₂O₂ can activate protein kinase and protein phosphatase through oxidative modification of key cysteine residues.⁴ Tyrosine nitration is also one of the important posttranscriptional modifications of proteins that may interfere with signaling pathways. Peroxynitrite (ONOO⁻) reacts with tyrosine residues in proteins to form nitrotyrosine and often changes protein structure and function. Aberrant protein tyrosine nitration is associated with different diseases, including inflammatory diseases and neurodegenerative diseases.⁵ In cancers, there are only a few reports identifying molecules that are targeted for tyrosine nitration. These include tumor suppressor p53,⁶ the xenobiotic-metabolizing enzyme, arylamine N-acetyltransferase 1,⁷ cytochrome c, and procaspase 3⁸; however, the functional significance of these nitration events remains unknown. Besides protein tyrosine nitration being an important biomarker for oxidative stress, ONOO⁻ is now being recognized as an important modulator of various cell signaling pathways. Therefore, of particular interest is the discovery of specific protein targets for nitration in various signal transduction pathways.

In a previous study, the Pervaiz group demonstrated that the antiapoptotic activity of Bcl-2, first discovered in t(14:18) follicular lymphomas, is related to intracellular superoxide (O₂⁻) levels in human acute lymphoblastic leukemia CEM cells.⁹ Because Bcl-2 overexpression has been implicated in the growth and chemoresistance of tumor cells, Low et al evaluated the mechanisms by which cellular ROS can strengthen the antiapoptotic function of Bcl-2 and whether the mechanisms are clinically relevant. A mild increase in intracellular O₂⁻ concentration by pharmacological inhibition (diethyldithiocarbamate [DDC]) or knockdown of superoxide dismutase 1 [SOD1] augmented S70 phosphorylation of Bcl-2, and this phosphorylation was essential for O₂⁻-mediated chemoresistance in Jurkat T-cell leukemia and other cancer cells. Because the phosphorylation status of Bcl-2 is regulated by MAPKs (extracellular signal-regulated

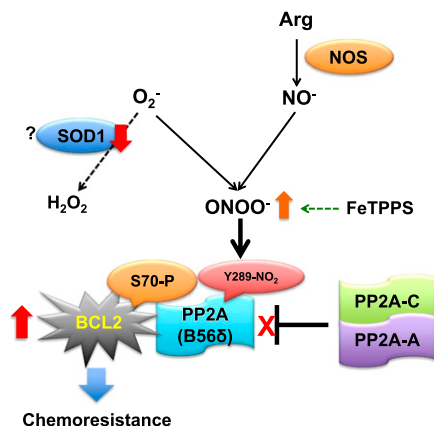
kinase, c-Jun N-terminal kinase, and p38) and/or heterotrimers of PP2A, the effects of DDC on the functions of these signaling proteins were evaluated. The results show that inactivation of PP2A phosphatase is the main cause of the upregulation of Bcl-2 S70 phosphorylation. The elevated O₂⁻ concentration inhibited the recruitment of PP2A to mitochondrial Bcl-2 by preventing the holoenzyme assembly of PP2A. By coimmunoprecipitation experiments, B56δ was identified as an interacting partner of Bcl-2 and the binding of Bcl-2 to PP2A-AC heterodimer was significantly inhibited by increased intracellular O₂⁻. Furthermore, Low et al show that reduction of SOD1 expression in Jurkat cells enhances the production of ONOO⁻ by unknown mechanisms, and that ONOO⁻ nitrates Y289 of B56δ and inhibits PP2A holoenzyme assembly, leading to enhanced S70 phosphorylation of Bcl-2 and apoptotic resistance to anticancer drug (see figure). Importantly, elevated phosphorylation of Bcl-2 S70 was shown to be associated with O₂⁻-induced nitration of B56δ Y289 in primary cells derived from clinical human lymphomas.

PP2A is a well-known tumor suppressor that is frequently mutated in a variety of human malignancies.¹⁰ Posttranslational modifications, including phosphorylation, nitration, and methylation, can also affect the function of PP2A. This is the first report of the association between ROS and promotion of chemoresistance via nitration of PP2A. This study also suggests that nitration of PP2A-B56δ and phosphorylation of S70 Bcl-2 could serve as biomarkers for diagnosis or molecular targets for therapeutic intervention, particularly in ROS-driven neoplastic processes.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Low ICC, Loh T, Huang Y, Virshup DM, Pervaiz S. Ser70 phosphorylation of Bcl-2 by selective tyrosine nitration of PP2A-B56δ stabilizes its antiapoptotic activity. *Blood*. 2014;124(14):2223-2234.
2. Brieger K, Schiavone S, Miller FJ Jr, Krause KH. Reactive oxygen species: from health to disease. *Swiss Med Wkly*. 2012;142:w13659.
3. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. *Toxicol Pathol*. 2010;38(1):96-109.
4. Truong TH, Carroll KS. Redox regulation of protein kinases. *Crit Rev Biochem Mol Biol*. 2013;48(4):332-356.



ROS generation begins with the formation of O₂⁻, which is produced by 1-electron reduction of molecular oxygen (O₂). The first line of cellular defense against high O₂⁻ levels is dismutation of O₂⁻ into O₂ and H₂O₂ by SOD. In the presence of nitric oxide (NO), O₂⁻ can more rapidly react with NO than with SOD to form ONOO⁻, a highly reactive ROS species. In lymphoma cells, downregulation of SOD1 expression increases intracellular ONOO⁻ formation by reacting with NO, which is formed from arginine by NOSs. ONOO⁻ can nitrate Y289 of B56δ and leads to the dissociation of the PP2A-AC heterodimer from the Bcl-2-bound B56δ subunit of PP2A, resulting in the enhanced phosphorylation of Bcl-2 S70. This may confer resistance to drug-induced cell death. NOS, NO synthase.

5. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev*. 2007;87(1):315-424.

6. Cobbs CS, Whisenhunt TR, Wesemann DR, Harkins LE, Van Meir EG, Samanta M. Inactivation of wild-type p53 protein function by reactive oxygen and nitrogen species in malignant glioma cells. *Cancer Res*. 2003;63(24):8670-8673.

7. Dairou J, Atmane N, Rodrigues-Lima F, Dupret JM. Peroxynitrite irreversibly inactivates the human xenobiotic-metabolizing enzyme arylamine N-acetyltransferase 1 (NAT1) in human breast cancer cells: a cellular and mechanistic study. *J Biol Chem*. 2004;279(9):7708-7714.

8. Ueta E, Kamatani T, Yamamoto T, Osaki T. Tyrosine-nitration of caspase 3 and cytochrome c does not suppress apoptosis induction in squamous cell carcinoma cells. *Int J Cancer*. 2003;103(6):717-722.

9. Clément MV, Hirpara JL, Pervaiz S. Decrease in intracellular superoxide sensitizes Bcl-2-overexpressing tumor cells to receptor and drug-induced apoptosis independent of the mitochondria. *Cell Death Differ*. 2003;10(11):1273-1285.

10. Westermarck J, Hahn WC. Multiple pathways regulated by the tumor suppressor PP2A in transformation. *Trends Mol Med*. 2008;14(4):152-160.

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understand the biology behind the expression of SOX11 in MCL. Earlier work by the same group demonstrated a role for SOX11 in MCL by upregulating *PAX5* and blocking B-cell maturation by maintaining *PRDM1/BLIMP1* repression.⁷

In the current work, tools developed from these earlier studies, such as cell lines with *SOX11* knockdown, enabled further examination of the role of SOX11 in MCL pathogenesis. Gene expression studies comparing SOX11 knockdown and SOX11-positive xenografts showed enrichment of genes involved in angiogenesis in the SOX11-positive tumors. This was confirmed at the protein level with antibody arrays and supported by increased microvessel density in the SOX11-positive xenograft tumors. Functional evidence of a secreted factor was shown in conditioned media from SOX11-positive cell lines, which promoted angiogenesis in tube formation, endothelial proliferation, and migration assays.

The authors identified platelet-derived growth factor alpha polypeptide (PDGFA) as the responsible factor in a series of elegant experiments in which a limited set of candidate proangiogenic factors was identified by comparing the gene expression profiles of SOX11-positive and SOX11-silenced cell lines. They then interrogated conditioned media for evidence of secretion of the corresponding proteins by antibody arrays, and PDGFA was the only one of these candidates found to be present in the medium. PDGFA was further validated based on prior genome-wide chromatin immunoprecipitation-on-chip studies that identified blood vessel development as an important process that was overrepresented by SOX11-bound genes. Interestingly, of the candidate genes identified in the aforementioned gene expression studies, *PDGFA* was the only SOX11-bound gene found in these chromatin immunoprecipitation studies. As further confirmation, the authors show that SOX11 was capable of upregulating *PDGFA* expression by luciferase reporter assays.

Furthering the functional and potential clinical importance of these findings, the authors demonstrate that conditioned media from SOX11-positive cell lines resulted in phosphorylation of PDGFR α but not PDGFR β in human vascular endothelial cells. Importantly, inhibition of PDGFA signaling with imatinib or PDGFA-neutralizing

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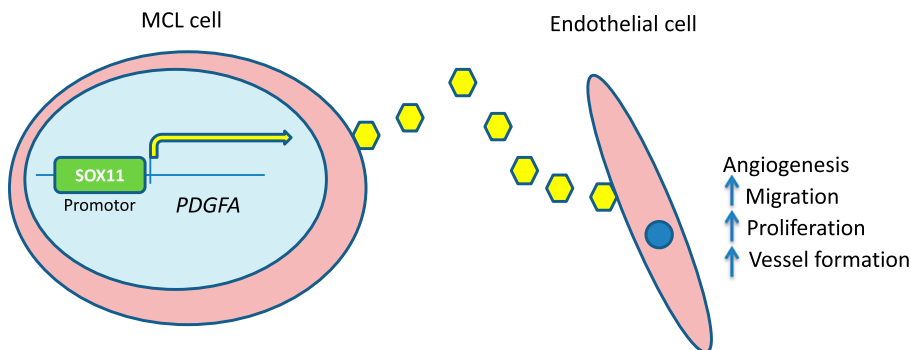
The SOX11-PDGFA axis in mantle cell lymphoma

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In this issue of *Blood*, Palomero et al elucidate a mechanism whereby SOX11 (*SRY [Sex determining region-Y]-box 11*) promotes angiogenesis in mantle cell lymphoma (MCL).¹

MCL is a mature CD5-positive B-cell lymphoma, thought to be derived from a pregerminal center antigen naive B cell, although a subset of cases appear to show evidence (immunoglobulin heavy chain somatic hypermutation) of germinal center passage.² The genetic hallmark is the *IGH/CCND1* fusion that leads to overexpression of cyclin D1.² At first thought to be an aggressive lymphoma with poor prognosis, an indolent nonnodal form has been recognized with disease mostly limited to blood, bone marrow, and spleen.^{3,4} SOX11, a member of the C subgroup of the SOX

family of genes, has recently been shown to be expressed in virtually all cases of aggressive mantle cell lymphoma (MCL) whereas indolent forms appear to have decreased expression.^{2,4} In contrast, it is not expressed in other mature small B-cell lymphomas but can be seen in a subset of aggressive B-cell lymphomas such as Burkitt lymphoma and B-lymphoblastic lymphoma.⁵ SOX11 is not expressed in normal lymphoid progenitors or mature B cells. As a diagnostic marker, SOX11 has been used to recognize cyclin D1-negative MCL.⁶ Although we have learned how to use SOX11 diagnostically, we are only beginning to



SOX11-PDGFA paracrine axis. SOX11 serves to transcriptionally regulate PDGFA. Secretion of PDGFA promotes angiogenesis to support lymphomagenesis. Inhibition of this pathway by agents such as imatinib mesylate may be an avenue for clinical trial investigation.