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Activating p53 and Inhibiting Superenhancers to Cure Leukemia

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Abstract

In a recent study, Minzel and colleagues identified a novel series of molecules that inhibit casein kinase 1 α (CK1 α), CDK7, and CDK9, resulting in p53 activation and preferential inhibition of superenhancer (SE)-driven transcription. This study demonstrates a highly effective therapeutic strategy combining p53 activation with suppression of SEs to promote the cooperative killing of leukemic cells.

Casein kinases are serine/threonine kinases that phosphorylate proteins to mediate normal biological functions and malignant transformation [1]. CK1 α functions as a tumor suppressor in several cancers through negative regulation of Wnt/ β -catenin signaling [1,2]. CK1 α phosphorylates β -catenin at Serine 45 (Ser45) leading to β -catenin ubiquitination and degradation [1,2] (Figure 1). CK1 α also negatively regulates p53 by phosphorylating murine double minute X (MDMX) at Ser289, resulting in enhanced binding of MDMX to p53 [3]. Moreover, a complex of CK1 α and MDM2 negatively regulates p53 [2]. Thus, pharmacological inhibition of CK1 α with subsequent p53 activation has the potential to be effective in an array of cancers.

In addition, CK1 α is encoded by a commonly deleted region in deletion 5q [del (5q)] myelodysplastic syndrome, and CK1 α haploinsufficiency promotes aberrant hematopoietic stem cell self-renewal [4]. At the same time, complete loss of CK1 α results in complete failure of hematopoiesis [5]. Lenalidomide and related molecules (thalidomide and pomalidomide) result in proteasomal degradation of CK1 α [4]. Thus, selective inhibitors of CK1 α that are more potent may provide a greater therapeutic window compared with lenalidomide.

In recent work, Minzel and colleagues performed a massive cell-based small-molecule screen to identify a class of pyrazole-pyrimidine scaffold molecules (termed ‘A-series’) that are potent pan-CK1 inhibitors [6]. A-series inhibitors reduced Ser45 β -catenin phosphorylation and increased p53 and β -catenin protein expression in a dose-dependent manner. Across two distinct genetically engineered mouse models of acute myeloid leukemia (AML), a single dose of the A-series inhibitor A51 led to rapid induction of

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apoptosis and reduced leukemia burden while sparing normal tissues. Long-term treatment with the inhibitors A51 or A14 prolonged the survival of these mice. Moreover, serial transplantation studies from treated mice demonstrated normal hematopoiesis and no evidence of leukemia in recipients, indicating selective targeting and elimination of the leukemic stem cell population. The antileukemic activity of A51 was also reproduced in human AML cell lines, and patient-derived xenograft models.

To further elucidate the mechanism for selective killing of leukemia cells with A-series compounds, Minzel and colleagues measured gene expression in AML cells treated with A-series inhibitors versus a thalidomide analog, BTX 161. In contrast to BTX 161, treatment with A51 and A86 reduced protein and mRNA expression of the proto-oncogene c-MYC. To understand how these compounds might influence c-MYC expression, the investigators measured the binding of A-series inhibitors to major transcriptional kinases and identified high-affinity binding of these molecules to both cyclin-dependent kinase 7 (CDK7) and CDK9. CDK7 phosphorylates the carboxy-terminal domain (CTD) of RNA polymerase II (Pol II) at Ser5 and Ser7, enabling transcriptional initiation[7]. CDK9 phosphorylates RNA Pol II CTD at Ser2 to facilitate transcription elongation [7]. Active A-series inhibitors (A51 and A86), but not the inactive compound A64, led to decreased phosphorylation of RNA Pol II CTD at Ser2 and Ser5 as well as decreased c-MYC protein. Inhibition of CDK7 and CDK9 by A51 in AML cells led to a reduction in SE histone acetylation (Figure 1). SEs are large clusters of enhancers bound by transcription factors that drive expression of genes involved in cell identity and are associated with key oncogenes, such as c-MYC [8]. Treatment with A51 also led to a preferential decrease in RNA Pol II CTD pSer2 binding to SE compared with typical enhancers. Hence, A-series compounds have additional inhibitory activities against of CDK7 and CDK9, which preferentially block SE-driven transcription.

In their study, Minzel *et al.* demonstrated cooperative induction of apoptosis by inhibiting CK1 α , CDK7, and CDK9. Treatment of an AML cell line with combinations of BTX 161, a CDK9 inhibitor, and/or a CDK7 inhibitor demonstrated that inhibiting all three kinases led to maximal induction of apoptosis compared with treatment with any single agent alone. By selectively inducing apoptosis in leukemia-initiating cells in AML, the development of a combined CK1 α , CDK7, and CDK9 inhibitor represents a remarkable achievement.

However, this study also raises several questions. For example, are A-series inhibitors selectively killing AML due to the effects on CK1 α , CDK7, and/or CDK9? Here, combined inhibition of CK1 α , CDK7, and CDK9 led to higher expression of p53 and maximal caspase 3 activation compared with CK1 α inhibition alone or inhibiting CK1 α with CDK7 or CDK9. Thus, future studies are needed to determine whether inhibition of all three targets is needed for selective leukemia killing.

Given the number of proteins targeted by these inhibitors, it will be important to understand what additional proteins are inhibited by these compounds. For example, dual-specificity tyrosine phosphorylation-regulated kinase 1a (DYRK1A) is another kinase that promotes RNA Pol II CTD Ser 2/5 phosphorylation and has high affinity to A-series inhibitors [9]. In addition, A-series inhibitors also demonstrated a ‘rapid hit’ mechanism of action with near-complete elimination of RNA Pol II Ser2/Ser5 CTD phosphorylation and MDM2 expression

after only 5 min of treatment in a mouse model of AML. It will be important to learn whether the ‘rapid hit’ pharmacodynamics/pharmacokinetics of A series compounds will be reproduced in humans. Finally, identifying genetic subsets of AML most sensitive to A-series inhibitors will be helpful. Given the haploinsufficient expression of CK1 α and sensitivity to lenalidomide in patients with del(5q), one would expect this population to be most responsive to A-series inhibitors. Conversely, it will be important to determine whether the effects of these inhibitors are dependent on the presence of wild-type p53.

Overall, this study highlights a novel approach for cancer therapy, combining p53 activation with inhibition of transcriptional kinases that regulate SE-driven transcription. Ultimately, such a therapeutic may have far-reaching implications for the treatment of AML as well as other cancers.

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Figure 1. Simultaneous Inhibition of Casein Kinase α (CK1 α), CDK7, and CDK9 Results in Preferential Killing of Acute Myeloid Leukemia (AML) Cells over Normal Blood Cells.

CK1 α inhibits p53 by phosphorylating murine double minute X (MDMX) to enhance MDMX/p53 binding and by forming a complex with MDM2 to inhibit p53. In addition, the phosphorylation of β -catenin by CK1 α results in proteasomal degradation of β -catenin. Recently, Minzel et al. identified a series of compounds that inhibit CK1 α as well as the transcriptional kinases CDK7 and CDK9. In so doing, these molecules induce p53-mediated cell cycle arrest as well as block [87_TD\$DIFF][85_TD\$DIFF]CDK7/9-mediated Serine 2 and 5 phosphorylation (Ser2P and Ser5P, respectively) of RNA polymerase II (RNA Pol II), resulting in reduced transcription from leukemogenic superenhancers. Abbreviations: GTFs, general transcription factors; TBP, TATA-binding protein.