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Blood 142 (2023) 2785–2786

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

The UBE2J2/UBE2K-MARCH5 Ubiquitination Machinery Regulates Apoptosis in Response to Venetoclax in Acute Myeloid Leukemia

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Evasion of apoptosis is crucial for the growth, survival and chemoresistance of many cancer types, including acute myeloid leukemia (AML); thus, the reactivation of apoptosis can be exploited as a therapeutic approach. Apoptosis induction is mainly controlled by the balance between anti-apoptotic and pro-apoptotic BCL2 family proteins on the mitochondrial membrane. Venetoclax, a selective inhibitor antagonizing the anti-apoptotic protein BCL2, has emerged as a promising therapy in AML. Despite high response rates in combination with hypomethylating agents, however, some patients display upfront resistance, and most patients will ultimately relapse. Therefore, identification of synergistic targets for combination therapies with venetoclax is important for improving the clinical application of this drug.

To systematically identify key genes that can modulate the venetoclax effect, we performed a genome-scale CRISPR-Cas9 screen in the AML cell line MV4-11. Consistent with previous reports, loss of the apoptosis effector *BAX* or the pro-apoptotic gene *NOXA* (*PMAIP1*) caused venetoclax resistance, while sgRNAs targeting the anti-apoptotic genes *BCLXL* (*BCL2L1*), *BCL2L2* and *BCL2A1* were significantly more depleted in venetoclax-treated cells. Furthermore, depletion of the E2 ubiquitin-conjugating enzymes, UBE2J2 and UBE2K, ranked highly among the venetoclax sensitizers. We validated that deletion of either *UBE2J2* or *UBE2K* increased induction of apoptosis in multiple AML cell lines and patient-derived xenograft (PDX) cells upon venetoclax treatment and was deleterious as single gene perturbation in some models.

Exploiting the Broad Institute Cancer DepMap dataset, which includes genome-scale CRISPR-Cas9 screens in over 1000 cancer cell lines, revealed that dependency on UBE2J2 or UBE2K significantly correlated with a dependency on the E3 ligase MARCH5. We previously identified the repression of MARCH5 as a strong inducer of apoptosis in AML. Since E2s coordinate with ubiquitin E3 ligases to execute ubiquitination, this DepMap result suggested that UBE2J2 and UBE2K serve as MARCH5 E2 partners. To test this hypothesis, we utilized NanoBiT technology, a structural complementation reporter system, to detect protein interactions between MARCH5 and the E2 candidates. LgBIT and SmBIT, two split subunits of luciferase, were fused with MARCH5 and the E2 proteins, respectively. The luminescent signal was activated upon the co-expression of LgBIT-MARCH5 with either of the SmBIT-tagged E2 proteins but not an empty vector, suggesting that MARCH5 and UBE2J2/UBE2K constitute ubiquitination machinery that regulates apoptosis in AML. We next tested the possible redundancy between the two E2s and showed that the double knockout of *UBE2J2*/ *UBE2K* further enhanced the venetoclax sensitivity.

MARCH5 depletion results in increased NOXA expression, an important node in dictating venetoclax response. Several reports indicated that NOXA is also a critical downstream mediator of MARCH5. In contrast, we previously showed that MARCH5 can regulate apoptosis independently of NOXA in AML. To reassess the role of NOXA and other pro-apoptotic proteins in MARCH5-mediated apoptosis, we conducted an unbiased CRISPR rescue screen in a MARCH5-dTAG degradation system derived from PDX17-14, a complex karyotype, MLL-AF10 PDX model. BAX was the top rescuing target, emphasizing that apoptosis induction is the main mechanism accounting for the growth inhibition of MARCH5-depleted cells. However, depletion of other pro-apoptotic BCL2 members, including NOXA and BIM, did not rescue MARCH5 depletion in this screen consistent with our previously published data. Here, we confirmed that *NOXA* KO does not rescue MARCH5 depletion in additional AML models. Similarly, KO of *UBE2J2* or *UBE2K* can repress AML cell growth and increase venetoclax sensitivity even in the context of *NOXA* KO.

Our study highlights that UBE2J2 and UBE2K are two important functional partners of MARCH5 in regulating apoptosis in AML cells and can serve as additional targets for enhancing venetoclax efficacy. Unbiased screening and low-throughput target validation further emphasize that the MARCH5 ubiquitination machinery regulates apoptosis in AML cells largely in a NOXA-independent manner. Additional studies are needed to dissect the MARCH5/UBE2J2/UBE2K complex-mediated apoptosis regulation in AML.

Disclosures Stegmaier: *Auron Therapeutics:* Current holder of *stock options* in a privately-held company; *Novartis:* Research Funding; *Kronos Bio:* Research Funding.

https://doi.org/10.1182/blood-2023-182312