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AUTHOR'S VIEWS

Targeting neurolysin in acute myeloid leukemia

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ABSTRACT

We recently identified the mitochondrial peptidase, neurolysin (NLN), as a top hit in an acute myeloid leukemia (AML) viability screen. Using chemical and genetic approaches, we demonstrated that loss of NLN disrupted respiratory chain supercomplex assembly and impaired oxidative metabolism in AML. Moreover, inhibition of NLN *in vitro* and *in vivo* reduced the growth of AML cells.

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Acute myeloid leukemia (AML) is an aggressive hematological malignancy that is defined by the uncontrolled proliferation of immature blast cells. The rapid proliferation of these immature blasts, called myeloblasts, impairs normal hematopoiesis, resulting in infection, anemia, and hemorrhage. Despite recent advances and new therapies for this disease, the prognosis for most patients with AML remains poor. Patients with relapsed and refractory disease have a particularly dismal outcome. Likewise, patients who are unfit for chemotherapy have a median survival of only 6–9 months.[1]

Recently, we and others demonstrated that AML cells and stem cells have unique mitochondrial properties that can be therapeutically targeted.[2,3] Among these properties is overexpression of mitochondrial proteases and dysregulated mitochondrial protein quality control pathways.[4,5] In a genetic screen to identify members of the mitochondrial proteome that are essential for AML viability, we identified the mitochondrial peptidase, neurolysin (NLN), as a top hit.[4] NLN's mitochondrial function is not well characterized and its role in AML has not been previously studied.

NLN is a zinc metallopeptidase that was originally identified in the nervous system where it was shown to cleave vasoactive peptides such as bradykinin and neurotensin.[6] However, this function appears redundant as *NLN* knockout mice do not have abnormalities with blood pressure regulation. Instead, *NLN* knockout mice are viable with mild metabolic defects, such as fewer oxidative muscle fibers and poor performance in endurance exercise tests.[7] These results suggest that loss of NLN impairs metabolism.

We began our study by investigating *NLN*'s expression in AML using a publicly available dataset of 536 AML and 73 normal control bone marrow samples. *NLN* mRNA was overexpressed in a subgroup of AML patient samples and was equally expressed in bulk and progenitor populations. To confirm the results of our shRNA screen and to investigate NLN's importance in AML growth, we knocked down *NLN* using shRNA in OCI-AML2, NB4, TEX, and MV4-11 leukemia cell lines. *NLN* knockdown reduced the growth of all tested cell lines and also reduced the colony-forming potential of OCI-AML2 and NB4 cells, suggesting that *NLN* knockdown targets both bulk and progenitor AML cells. In line with this finding, knockdown of *NLN* reduced the engraftment of TEX cells into mouse marrow.[5]

NLN's mitochondrial function has not been well defined. To understand NLN's role in the mitochondria, we identified its mitochondrial interactors via proximity-dependent biotin labeling coupled with mass spectrometry (BioID-MS). NLN interacted with several proteins involved in respiratory electron transport and assembly. Based on these findings, we investigated NLN's role in oxidative metabolism and found that *NLN* knockdown reduced oxygen consumption rates (OCR) in leukemic cells. Moreover, *NLN* knockdown impaired the formation of large quaternary structures called respiratory chain supercomplexes (RCS).[5] RCS consist of complexes I, III, and IV and increase the efficiency of electron transport.[8]

RCS have not been previously studied in leukemia. We found that RCS were increased in a subset of AML patient samples, compared to normal hematopoietic cells. Moreover, RCS expression correlated with NLN levels. To understand how NLN affects RCS, we returned to our BioID-MS results and found that one of NLN's top interactors was LETM1 (leucine zipper-EF-hand containing transmembrane protein 1).[5] LETM1 has previously been shown to regulate RCS assembly and forms two complexes, the minor and major.[9] *NLN* knockdown reduced the formation of both the LETM1 minor and major complexes. Of note, knockdown of *LETM1* also reduced AML growth and oxidative metabolism.[5]

We assessed the effects of pharmacologically inhibiting NLN in AML using the previously reported NLN inhibitor, R2 (3-[(2 S)-1-[(3 R)-3-(2-Chlorophenyl)-2-(2-fluorophenyl) pyrazolidin-1-yl]-1-oxopropan-2-yl]-1-(adamantan-2-yl) urea).[10] Treatment of AML cells *in vitro* with R2 impaired growth of AML cells and primary AML culture models. Moreover, R2 treatment reduced RCS levels, LETM1 complex formation, and oxidative metabolism. *In vivo*, R2 reduced the leukemic burden in mice engrafted with primary AML patient

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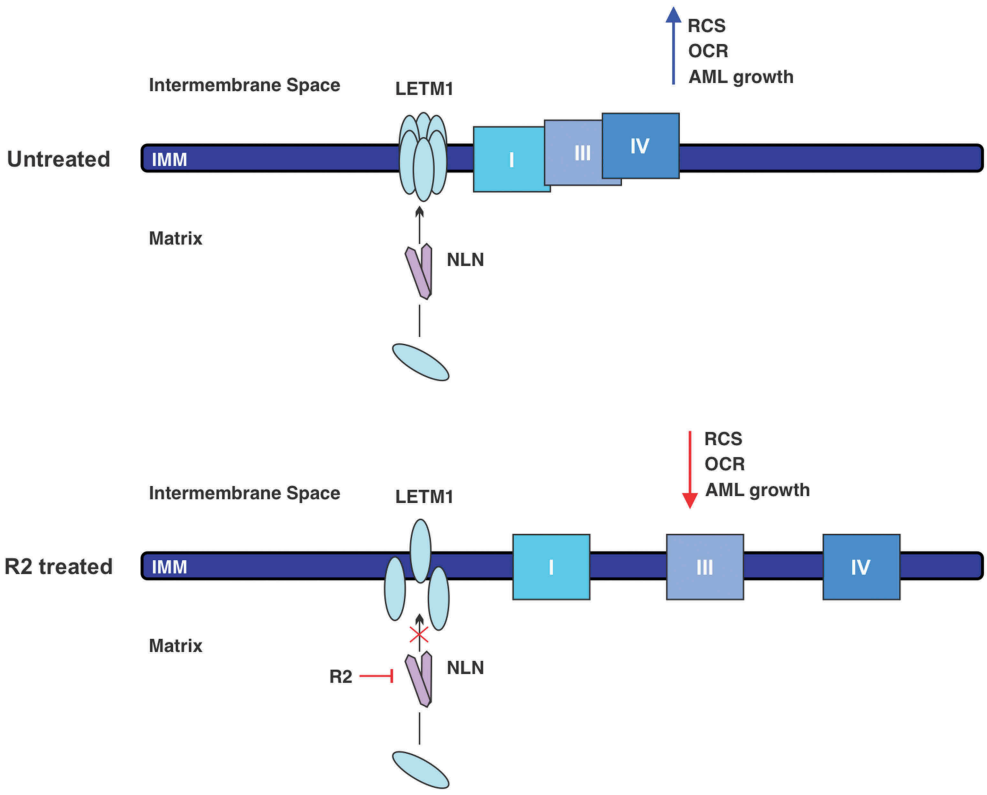

Figure 1. Chemical inhibition of NLN disrupts RCS and growth in AML cells. **(Top)** NLN (neurolysin) promotes the formation of LETM1 (leucine zipper-EF-hand containing transmembrane protein 1) complexes through an unknown mechanism. LETM1 complexes stabilize RCS (respiratory chain supercomplexes) and promote efficient oxidative metabolism and AML (acute myeloid leukemia) growth. **(Bottom)** Inhibition of NLN by R2 disrupts the assembly of LETM1 complexes and destabilizes RCS. Consequently, OCR (oxygen consumption rate) is impaired and AML growth is reduced.

samples and also reduced engraftment in secondary transplantations. Importantly, R2 treatment did not affect the engraftment of normal human cord blood cells. Collectively, these data suggest that pharmacological inhibition of NLN selectively targets bulk and progenitor AML cells without affecting normal hematopoietic cells.[5] Although an effective tool compound, additional work will be required to develop more potent NLN inhibitors and identify potential leads for therapeutic candidates. Moreover, R2 is predicted to cross the blood-brain barrier.[10] NLN inhibitors that do not cross the blood brain barrier may have a superior therapeutic index.

AML cells and stem cells depend on oxidative metabolism for their survival. NLN promotes the assembly of RCS, which drives efficient mitochondrial energy production. We showed that genetic and chemical inhibition of NLN targeted AML cells by disrupting RCS formation and OCR (Figure 1). However important biological questions remain unanswered and will form the basis for future studies. Future work will explore the precise mechanism by which NLN mediates LETM1 complex and RCS formation and whether this is related to its peptidase activity. In addition to uncovering important new biology, determining whether the effects of NLN on LETM1 and RCS formation are dependent or independent of its peptidase function will help guide the development of NLN inhibitors. Moreover, our findings do not exclude the possibility that NLN regulates RCS directly and future mechanistic studies will aim to address this. Finally, little is known about the composition of the LETM1 minor and major complexes. Characterizing these complexes will provide new insight into how LETM1 promotes RCS and potentially new ways to target RCS formation. In summary, our work demonstrates the importance of RCS in AML and supports inhibition of NLN as a novel therapeutic approach.

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Disclosure of potential conflicts of interest

A.D.S. has received honorariums or consulting fees from Novartis, Jazz, Otsuka, and Takeda Pharmaceuticals and research support from Medivir AB and Takeda. A.D.S. owns stock in Abbvie Pharmaceuticals and is named on a patent application for the use of DNT cells for the treatment of leukemia.

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