



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

604. MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

The ATPase Domain of LONP1 Is Necessary for Mitochondrial Protein Solubility and the Viability of Acute Myeloid Leukemia (AML) Cells

Matthew Tcheng, PhD¹, Aaron D. Schimmer, MD PhD², Marcela Gronda³, Rose Hurren³, Shahbaz Khan, PhD⁴, Lan Xin Zhang¹, Chaitra Sarathy, PhD³, Yongran Yan², Andrea Arruda⁵, Thomas Kislinger, PhD⁴, Mark D. Minden⁴

¹University Health Network, Princess Margaret Cancer Center, Toronto, Canada

²University Health Network, Princess Margaret Cancer Centre, Toronto, Canada

³UHN, Princess Margaret Cancer Centre, Toronto, Canada

⁴Princess Margaret Cancer Centre, University Health Network, Toronto, Canada

⁵Princess Margaret Cancer Centre, University Health Network, Toronto, Canada

Compared to normal hematopoietic cells, we and others have shown that AML cells have a heightened reliance on oxidative phosphorylation and mitochondrial metabolism for survival and proliferation. To support this unique metabolic phenotype, we previously demonstrated that AML cells have increased import of nuclear-encoded mitochondrial proteins. These newly imported proteins must be properly folded by mitochondrial chaperones, proteases, and heat shock proteins, and failure to properly process and fold these precursors leads to protein aggregation, mitochondrial dysfunction, and cell death.

To evaluate the reliance of AML cells on this family of mitochondrial chaperones, proteases, and heat shock proteins, we assessed their dependencies using the gene dependency datasets (eg: depmap.org). From this analysis, we identified the mitochondrial protease, LONP1, as the top hit, and among the top 10% of all essential genes for AML. Localized to the mitochondrial matrix, LONP1 is a nuclear encoded AAA+ serine protease. Proteins are unfolded by its ATPase domain and degraded by its serine-catalyzed proteolytic domain.

Compared to normal hematopoietic cells, LONP1 mRNA was overexpressed in AML across three publicly available datasets. Compared to normal hematopoietic cells (n=8) and CD34+ cells (n=3), LONP1 protein was increased in 16/30 primary AML samples by immunoblotting.

Using shRNA and CRISPR, LONP1 knockdown and knockout reduced the growth and viability of OCI-AML2, OCI-M2, NB4, and TEX cells. Genetic knockdown or knockout of LONP1 increased levels of insoluble, aggregated mitochondrial proteins as measured by mass spectrometry, proteostat fluorescence and confocal microscopy, and immunoblotting of soluble and insoluble protein fractions. LONP1 knockdown/knockout also reduced mitochondrial respiration, depolarized the mitochondria, and induced AML cell death.

We next determined the domain of LONP1 that was necessary for mitochondrial protein solubility, mitochondrial function, and AML survival. We over-expressed wild type, ATPase dead (E591A), or proteolytically dead (S855A) LONP1 cDNA in OCI-AML2 cells and knocked down endogenous LONP1 with shRNA targeting the 3'UTR of the endogenous gene. We then measured mitochondrial protein solubility/aggregation, mitochondrial respiration and cell viability. Wild type LONP1 and the proteolytically dead (S855A) mutant, but not the ATPase mutant (E591A), rescued mitochondrial protein solubility, mitochondrial respiration and cell viability, thus demonstrating that the ATPase domain of LONP1 is necessary for these functions.

Bardoxolone methyl (CDDO-Me) is a synthetic triterpenoid that inhibits the ATPase activity of LONP1 by binding to an allosteric site near the ATPase domain of the enzyme. CDDO-Me killed OCI-AML2 and NB4 cells with IC₅₀ values of 178.5±29.7 and 156.5±39.7 nM, respectively. CDDO-Me (200nM) also killed >50% of cells in 3 out of 4 high-LONP1 expressing primary AML patient samples, but 10 of 10 tested primary AML samples with low levels of LONP1 were insensitive to the drug. Likewise, CDDO-Me induced mitochondrial protein aggregation in OCI-AML2 cells and in a primary AML patient sample with high LONP1 expression, while a primary AML patient sample with undetectable LONP1 expression showed no protein aggregation.

In summary, the mitochondrial serine AAA+ protease LONP1 is over-expressed in a subset of AML cells and primary samples. The ATPase domain is necessary for LONP1's function in maintaining mitochondrial protein solubility. Selective inhibition of this domain leads to mitochondrial protein aggregation, impaired mitochondrial respiration and AML cell death. Thus, inhibiting the ATPase domain of LONP1 may be a novel therapeutic strategy for AML.

Disclosures Schimmer: *Jazz*: Consultancy, Honoraria; *Otsuka Pharmaceuticals*: Consultancy, Honoraria; *Novartis*: Consultancy, Honoraria; *UHN*: Patents & Royalties: the use of DNT cells to treat AML; *Medical and Scientific Advisory Board of the Leukemia and Lymphoma Society of Canada*: Membership on an entity's Board of Directors or advisory committees; *Medivir AB*: Research Funding; *Takeda Pharmaceuticals*: Consultancy, Honoraria, Research Funding; *BMS*: Research Funding.

<https://doi.org/10.1182/blood-2023-178399>