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Importance of mitophagy in acute myeloid leukemia cells

Význam mitofagie pro buňky akutní myeloidní leukémie

Bachelor´s thesis

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Poděkování patří především mé školitelce Kateřině Kuželové za mnoho užitečných rad a postřehů, skvělou komunikaci a také za nadstandardní trpělivost, se kterou přistupovala k vedení mé práce. Dále bych ráda poděkovala své rodině a přátelům za veškerou podporu nejen při psaní této práce, ale i po celou dobu studia.

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Prague. April 28, 2025 Adéla Pavleová

Abstract

Acute myeloid leukemia is a highly heterogeneous hematological malignancy characterized by clonal expansion of immature myeloid cells. The disease originates from leukemic stem cells, which are pivotal in the persistence and relapse of the disease. They predominantly rely on oxidative phosphorylation for energy production, making them dependent on mitochondrial function. This metabolic dependency makes leukemic stem cells highly vulnerable to mitochondrial dysfunction. This has to be balanced by the process of mitophagy, which is a selective degradation of damaged, dysfunctional, or excess mitochondria. Increased mitophagy supports leukemic stem cell self-renewal and therapy resistance by clearing damaged mitochondria and allowing them to evade apoptosis during chemotherapy.

Key mitophagy-related proteins, such as FIS1, p62, OPTN, and MFN2, regulate mitochondrial dynamics and quality control in leukemic stem cells. Upregulation of mitophagy protein expression can contribute to disease progression and resistance to treatment, making mitophagy a promising therapeutic target. Inhibiting mitophagy or modulating its key components could sensitize leukemic stem cells to conventional therapies or even targeted treatments, such as BCL-2 inhibitors.

Key words: acute myeloid leukemia, mitophagy, apoptosis, leukemic stem cells, targeted therapy, reactive oxygen species

Abstrakt

Akutní myeloidní leukémie je velmi různorodým hematologickým onemocněním charakterizovaným klonální expanzí nezralých myeloidních buněk. Původcem nemoci jsou leukemické kmenové buňky, které hrají klíčovou roli při přetrvání onemocnění a při případném relapsu. Tyto buňky se ve svém energetickém metabolismu spoléhají převážně na oxidativní fosforylaci, což je činí závislé na správné funkci mitochondrií. Tato metabolická závislost zvyšuje zranitelnost leukemických kmenových buněk při nesprávném fungování mitochondrií a je nutné ji kompenzovat pomocí mitofagie, což je selektivní degradace poškozených, nefunkčních či přebytečných mitochondrií. Zvýšená míra mitofagie napomáhá zachování schopnosti sebeobnovy leukemických kmenových buněk a jejich rezistenci vůči terapii, jelikož jim umožňuje zbavovat se mitochondrií poškozených vlivem léčiv a tím uniknout apoptóze.

Klíčové proteiny spojené s mitofagií, jako například FIS1, p62, OPTN a MFN2, regulují dynamiku mitochondrií a zajišťují udržování jejich kvality v leukemických kmenových buňkách. Zvýšená exprese mitofagických proteinů může napomáhat progresi onemocnění a odolnosti proti léčbě, což činí mitofagii slibným cílem pro nově se vyvíjející terapeutické postupy. Inhibice mitofagie nebo ovlivnění jejích klíčových komponentů by totiž mohly zvýšit citlivost leukemických kmenových buněk nejen ke konvenčním způsobům terapie, ale také k modernější cílené léčbě, jako jsou inhibitory BCL-2 proteinů.

Klíčová slova: akutní myeloidní leukémie, mitofagie, apoptóza, leukemické kmenové buňky, cílená terapie, kyslíkové radikály

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Abbreviations

AML – Acute Myeloid Leukemia

AMPK – AMP-Activated Protein Kinase

APAF1 – Apoptotic Protease Activating Factor 1

BAX – BCL-2 Associated X Protein

BAK – BCL-2 Antagonist Killer

BCL-2 – B-Cell Lymphoma 2

CAR – Chimeric Antigen Receptor

CLL-1 – C-Type Lectin-Like Molecule-1

CQ – Chloroquine

ETC – Electron Transport Chain

FMN – Flavin Mononucleotide

FIS1 – Mitochondrial Fission 1

FLT3 – FMS-Like Tyrosine Kinase 3

GAC – Glutaminase C

GSK3 – Glycogen Synthase Kinase 3

HSCs – Hematopoietic Stem Cells

IAP – Inhibitor of Apoptosis Protein

IDH1/2 – Isocitrate Dehydrogenase 1/2

IMM – Inner Mitochondrial Membrane

LIR – LC3-Interacting Region

LSCs – Leukemic Stem Cells

MFN1/MFN2 – Mitofusin 1/2

NPM1 – Nucleophosmin 1

OPTN – Optineurin

OXPHOS – Oxidative Phosphorylation

PINK1 – PTEN-Induced Putative Kinase 1

ROS – Reactive Oxygen Species

SOD2 – Superoxide Dismutase 2

SQSTM1 – Sequestosome 1 (p62)

TRX2 – Thioredoxin 2

VDAC1 – Voltage-Dependent Anion Channel

1 Introduction

Acute myeloid leukemia (AML) is an aggressive clonal hematologic malignancy arising from clonal expansion of myeloid precursors in the bone marrow, leading to impaired hematopoiesis and rapid disease progression. A defining feature of AML is its substantial heterogeneity at both the cytogenetic and molecular levels, reflected in variable clinical presentations and prognostic outcomes. Advances in sequencing and molecular profiling have enabled stratification of patients based on distinct genetic mutations, including aberrations in *FLT3*, *NPM1*, *TP53*, and epigenetic regulators such as *DNMT3A*, *TET2*, and *IDH1/2*, all of which contribute to disease pathogenesis and therapeutic response (Döhner et al., 2015). The tumor consists of multiple cellular subtypes, from which the leukemic stem cells (LSCs) represent a therapeutically challenging subpopulation due to their self-renewal capacity, metabolic adaptability, and resistance to conventional therapies. Unlike bulk tumor cells or healthy hematopoietic stem cells (HSCs), LSCs rely predominantly on oxidative phosphorylation (OXPHOS) for energy production and survival (Lagadinou et al., 2013).

This metabolic dependency creates a critical vulnerability: LSCs are highly sensitive to mitochondrial stress. Consequently, mitochondrial quality control mechanisms, particularly mitophagy, the selective autophagic degradation of damaged or excess mitochondria (Kim et al., 2007), emerge as essential for maintaining LSC viability and function. Mitophagy prevents the accumulation of dysfunctional mitochondria and reactive oxygen species (ROS), maintaining the mitochondrial network required for OXPHOS and LSC survival. Furthermore, mitophagy intersects with apoptotic signaling, forming a tightly regulated balance between survival and cell death under stress conditions, including cytotoxic therapy.

Mitophagy is orchestrated through two primary molecular mechanisms: receptor-mediated and ubiquitin-dependent (adaptor-mediated) pathways. Both systems converge on the recruitment of autophagic machinery to mitochondria via LC3-interacting receptors and adaptor proteins such as BNIP3, NIX, p62, OPTN, and NDP52. In recent years, mitophagy has gained attention not only as a metabolic safeguard but also as a contributor to therapy resistance in AML. Elevated mitophagic activity has been associated with resistance to BH3 mimetics like venetoclax, and inhibition of mitophagy-related pathways has been shown to sensitize AML cells to a variety of treatments (Glytsou et al., 2023; Lin et al., 2022). This thesis is a literary recherche exploring the mechanisms by which mitophagy supports leukemic progression and therapeutic resistance, evaluates the role of specific mitophagy-related proteins in AML

biology, and discusses current efforts to exploit mitophagy as a therapeutic target in overcoming LSC-mediated relapse.

2 Acute myeloid leukemia

Acute myeloid leukemia (AML) is a type of malignant disease affecting hematopoietic stem cells, specifically through malignancy of the precursors from the myeloid lineage, which under normal circumstances differentiate into monocytes, granulocytes, erythrocytes, and platelets. AML is highly heterogeneous at the cytogenetic and molecular levels which contributes to significant variability in its pathogenesis, clinical presentation and prognosis. In some cases, the cause can be directly ascribed to prolonged exposures to various chemicals like petrochemicals, benzenes or cytotoxic agents used in chemotherapy. However, the disease can also arise without an identifiable external cause (Chelghoum et al., 2002; Godley and Larson, 2008).

The prognosis of AML is influenced by several key factors, with patient age, karyotype, and specific genetic markers being the most critical determinants of disease progression and treatment outcomes. The European LeukemiaNet (ELN) classifies patients into three primary risk categories: favorable, intermediate and adverse, with regard to their cytogenetic and molecular profiles. Some examples of key cytogenetic abnormalities are t(8;21)(q22;q22), t(15;17)(q22;q12) and inv(l6) (p13q22)/t(l6;16)(p13;q22), these are usually associated with favorable prognosis. In contrast, complex karyotypes, monosomies of chromosomes 5, 7 as well as structural abnormalities involving the long arms of chromosomes 3 and 5 are linked to poor prognosis (Grimwade et al., 2010).

2.1 Somatic mutations and their impact on prognosis

A link has been established between somatically acquired mutations in various specific genes and the development of AML. One of the most recurrent mutations is internal tandem duplication in the FMS-like tyrosine kinase 3 gene (*FLT3*-ITD), which results in ligand-independent activation of the receptor. This constitutive signaling leads to aberrant activation of the RAS-RAF, JAK–STAT, and PI3K–AKT signaling pathways. The FLT3-ITD mutation is generally associated with negative prognosis, as the sustained activation of those pathways provides the leukemic cells with a proliferative advantage and contributes to disease progression (Rombouts et al., 2000). Mutations in nucleophosmin 1 (NPM1), a protein normally shuttling between the nucleus and cytoplasm, helping with ribosome biogenesis, DNA repair, regulation of apoptosis etc., lead to a change in localization of NPM1 and NPM1-interacting proteins into the cytosol. While *NPM1* mutations alone tend to have a favorable effect on the

prognosis, it often occurs alongside other mutations. For example, in combination with *FLT3*-ITD the prognostic outcome shifts into the intermediate risk group (Döhner et al., 2005). *TP53*, a tumor suppressor gene encoding the p53 transcription factor, is very frequently mutated in many types of cancer, and AML is no exception (Stengel et al., 2016). Another commonly mutated group of genes is involved in DNA methylation and includes *IDH1*, *IDH2*, *TET2*, and *DNMT3A*. *IDH1* and *IDH2* genes encode NADP-dependent isocitrate dehydrogenases, which catalyze the conversion of isocitrate into alpha-ketoglutarate. This metabolite is essential for the function of TET proteins, because they utilize it during histone demethylation (Paschka et al., 2010). *DNMT3A* is directly involved in the process of DNA methylation as it encodes the DNA-methyltransferase 3A, an enzyme responsible for adding methyl groups to CpG dinucleotides (Ley et al., 2010). Abnormalities in function of these genes can alter the epigenetic landscape, thus influencing gene expression and contributing to leukemogenesis (Döhner et al., 2015).

2.2 Treatment

The treatment of AML consists of two main phases: an induction therapy and a post-remission therapy. Before initiating the induction therapy, the physical condition of the patient has to be taken into consideration, since the standard treatment may pose significant risks for older individuals or those with comorbidities. The standard regimen for fit patients includes chemotherapy with a combination of cytarabine and anthracycline (Wiernik et al., 1992). For patients who are unfit for intensive chemotherapy, the alternative approaches include cytarabine in lower doses or hypomethylating agents, which help counteract the aberrant hypermethylation frequently observed in AML due to mutations in key regulatory genes (Kantarjian et al., 2012). The majority of patients achieve disease remission, i.e. normalized blood counts and significant reduction of leukemia cells in the bone marrow. The main purpose of the post-remission therapy is to prevent relapse. This can be typically achieved through consolidation therapy which employs chemotherapy and hematopoietic stem cell transplantation to eliminate possibly remaining leukemic cells (Visani et al., 2006).

With advances in our understanding of AML and its molecular characteristics, more targeted treatments are being developed. These strategies primarily focus on inhibiting mutated proteins commonly associated with AML, such as FLT3 (Pratz and Levis, 2008) and IDH1/2, with varying degrees of clinical success. Another potential therapeutic target that has been identified is the B-cell lymphoma 2 protein (BCL-2), an antiapoptotic protein that plays a crucial role in promoting leukemic cell survival by inhibiting the intrinsic apoptotic pathway

(Brunelle and Letai, 2009). One of the most widely studied BCL-2 inhibitors is venetoclax, a BH3 mimetic that competitively binds to antiapoptotic BCL-2, thereby enabling the activation of BAX and BAK and initiating mitochondrial-mediated apoptosis (Souers et al., 2013).

In addition to molecular inhibitors, immunotherapeutic strategies are being actively explored. These include CAR T-cell therapies, where T cells are engineered to express chimeric antigen receptors (CARs) targeting AML-associated antigens such as CD33, CD123, C-type lectin-like molecule-1 (CLL-1), and FLT3. Upon antigen recognition, CAR T cells undergo activation, leading to proliferation, cytokine release, and the induction of cytotoxic mechanisms, enhancing the immune response against the tumor (Zarychta et al., 2023). Other options in immunotherapeutic approach include monoclonal antibodies targeting CD33 and CD123, bispecific antibodies connecting CD3+ T cells to AML cells, facilitating immune-mediated destruction, or adoptive transfer of T-cells with modified T-cell receptors targeting AML-associated antigens. (Döhner et al., 2015).

However, these methods face significant challenges, as the antigens utilized to target AML cells are also expressed on healthy myeloid lineage cells. Therefore, it is crucial to develop strategies that can selectively target leukemic cells while sparing normal cells to minimize off-target cytotoxicity and preserve healthy hematopoiesis. Autologous NK cells that have been pre-activated *ex vivo* by cytokines and turned into memory-like NK cells are another therapeutic option currently being explored for AML. Furthermore, the NK cells can also be modified to express CARs (Berrien-Elliot et al., 2022). Immune checkpoint inhibitors represent another promising therapeutic approach by targeting regulatory proteins that cancer cells use to evade immune surveillance. Inhibiting these checkpoint proteins restores T-cell activity, enhancing antitumor immune responses and offering another potential treatment strategy for AML (Abaza and Zeidan, 2022).

3 Mitochondria

Although mitochondria are primarily known for their role in cellular respiration, where they produce adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS), they fulfill a wide range of additional functions within the cell. Mitochondria play a critical role in maintaining redox homeostasis, synthesizing key metabolic intermediates, regulating cellular signaling pathways, and orchestrating apoptosis.

3.1 Oxidative phosphorylation

The process of oxidative phosphorylation (OXPHOS) takes place along the inner mitochondrial membrane (IMM) and is responsible for generating the majority of adenosine triphosphate (ATP) in aerobic eukaryotic cells. It involves a coordinated series of redox reactions carried out by the electron transport chain (ETC), which transfers electrons derived from reduced cofactors to molecular oxygen. The flow of electrons drives the active pumping of protons across the inner membrane, creating an electrochemical gradient that powers ATP synthesis via ATP synthase. The ETC is composed of four main protein complexes: Complex I, Complex II, Complex III, and Complex IV (see Figure 1).

Complex I contains NADH dehydrogenase, multiple iron–sulfur (Fe-S) clusters, and flavin mononucleotide (FMN). NADH, originating from glycolysis or the Krebs cycle, is oxidized by NADH dehydrogenase, releasing two electrons. These electrons are transferred via FMN and Fe-S clusters to coenzyme Q. The conformational changes associated with electron transfer enable the translocation of four protons into the intermembrane space (Walker et al., 1992). Another entry point into the electron transport chain is Complex II, where succinate from the Krebs cycle is oxidized to fumarate by succinate dehydrogenase. This reaction generates two electrons, which are carried by flavin adenine dinucleotide (FAD) and Fe-S clusters and subsequently passed to coenzyme Q (Cecchini, 2003).

Coenzyme Q shuttles the electrons to Complex III, which consists of cytochrome b, cytochrome c_1, and the Rieske subunits. Cytochrome c contains heme groups that can switch between Fe^{2+} and Fe^{3+} states, enabling it to accept and transfer single electrons. Electron transfer at Complex III is organized through a process known as the Q cycle. Initially, one electron from ubiquinol is transferred to cytochrome c through the Rieske Fe-S subunit, while the second electron is passed back to a second coenzyme Q molecule via cytochrome b, generating a semiquinone intermediate. In the second half of the cycle, another ubiquinol molecule undergoes oxidation, donating one electron to cytochrome c and another that fully reduces the

semiquinone to regenerate ubiquinol. Throughout the complete Q cycle, four protons are translocated into the intermembrane space (Trumpower, 1990).

At Complex IV, known as cytochrome c oxidase, electrons from cytochrome c are transferred to molecular oxygen, the final electron acceptor of the respiratory chain. Complex IV accepts one electron at a time from four molecules of cytochrome c. These electrons are sequentially transferred through redox centers, including cytochrome a, cytochrome a_3, and two copper centers (CuA and CuB). The complete reduction of oxygen to water requires four electrons and the uptake of four protons from the mitochondrial matrix. Simultaneously, four additional protons are pumped into the intermembrane space per oxygen molecule reduced (Capaldi, 1990). This cascade results in the creation of a proton gradient across the IMM, which is harnessed by ATP synthase to drive ATP production. As protons flow back into the mitochondrial matrix through ATP synthase, the energy released drives the rotation of its catalytic subunits, initiating the conversion of adenosine diphosphate (ADP) and phosphate into ATP (Junge et al., 1997).

While essential for energy production, the activity of the ETC can inadvertently contribute to the generation of reactive oxygen species (ROS), primarily through the premature leaking of electrons that react with molecular oxygen to form superoxide radicals or hydrogen peroxide (Turrens, 2003). To mitigate oxidative stress, mitochondria possess several antioxidant defense mechanisms. One of the primary defenses is superoxide dismutase 2 (SOD2), a manganese-dependent enzyme that catalyzes the dismutation of superoxide radicals into hydrogen peroxide. Hydrogen peroxide can be further detoxified into water by enzymes such as catalase and glutathione peroxidase. Additionally, the thioredoxin system, including mitochondrial thioredoxin 2 (TRX2) and associated peroxiredoxins, contributes to hydrogen peroxide reduction as well (Andreyev et al., 2005).

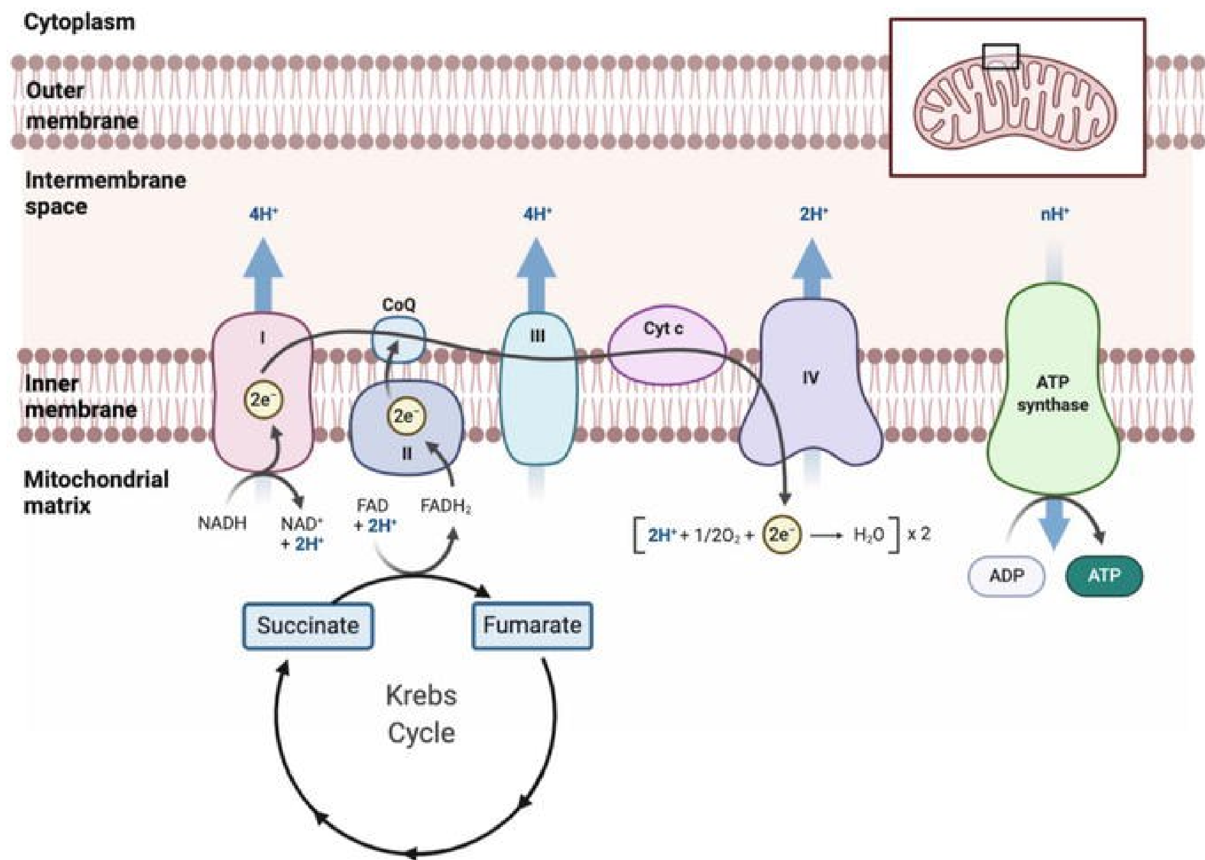

Figure 1: *Overview of oxidative phosphorylation.* The diagram illustrates the flow of electrons through Complexes I–IV and the associated proton pumping that generates a proton gradient used by ATP synthase for ATP production. Adapted from Chiara et al., 2021

3.2 Mitochondrial apoptotic pathway

In response to cellular stress, the intrinsic apoptotic pathway gets activated (see Figure 2). This process is mediated by pro-apoptotic BCL-2 family proteins, specifically the BCL-2 associated X protein (BAX) and BCL-2 antagonist killer (BAK). Their activity is inhibited by pro-survival BCL-2 proteins which retrotranslocate mitochondrial BAX and BAK back into the cytosol. Additionally, pro-survival BCL-2 proteins can protect cells from apoptosis through sequestering BH3-only proteins, which otherways play a key role in promoting apoptotic signaling (Brunelle and Letai, 2009). Upon activation, BAX and BAK undergo conformational changes, oligomerize, and translocate to the outer mitochondrial membrane (OMM), where they aggregate and form pores. This permeabilization of the OMM allows the release of apoptotic factors, such as cytochrome c, apoptosis-inducing factor, and second mitochondria-derived activator of caspases (Smac) from mitochondria into the cytosol (Du et al., 2000). As a consequence of losing cytochrome c, mitochondria start producing more reactive oxygen species (ROS) and cytochrome c activates apoptotic protease activating factor 1 – APAF1.

Upon activation, APAF1 changes its conformation and undergoes oligomerization, forming a complex, which then recruits caspase 9 zymogen, which consequently becomes activated. Caspase 9, in turn, triggers the activation of effector caspases 7 and 3, initiating the process of apoptosis (Wu and Bratton, 2013). Meanwhile, Smac promotes apoptosis by binding to and neutralizing inhibitor of apoptosis proteins (IAPs), which normally suppress caspase activity. By inhibiting IAPs, Smac facilitates full activation of the caspase cascade, further reinforcing the commitment to programmed cell death (Du et al., 2000).

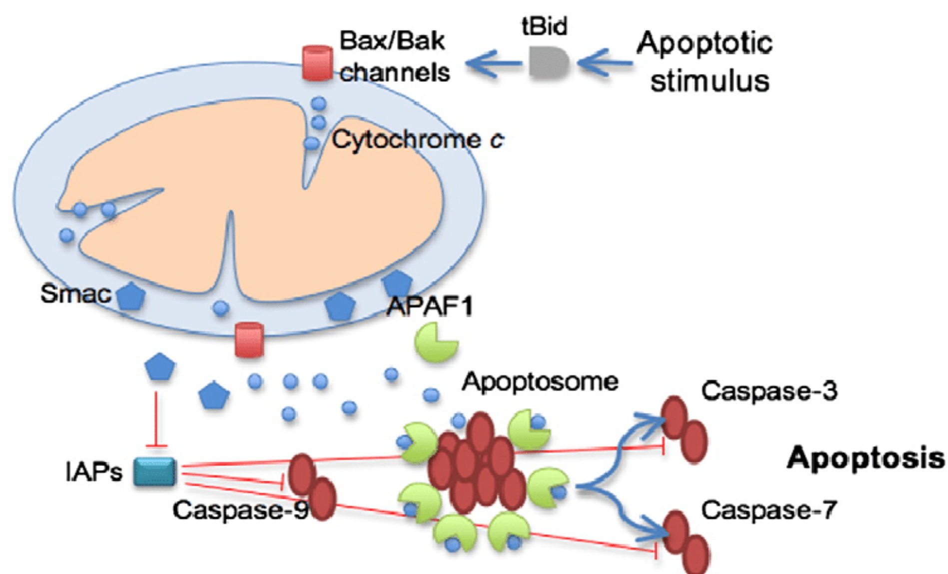

Figure 2: *Schematic of the intrinsic (mitochondrial) apoptotic pathway.* In response to cellular stress or mitochondrial damage, pro-apoptotic BCL-2 family proteins BAX and BAK are activated and permeabilize the OMM. This leads to the release of apoptogenic factors such as cytochrome c, which activates APAF1, and Smac. The apoptosome then activates caspase-9, which in turn triggers effector caspases 3 and 7, ultimately leading to apoptosis. Created by Bolaños et al., 2009.

4 Mitophagy

Autophagy is an essential catabolic process through which cells degrade and recycle cytoplasmic components, including damaged organelles and misfolded proteins, to maintain cellular homeostasis. This process involves the formation of double-membraned vesicles called autophagosomes, which sequester cargo and subsequently fuse with lysosomes where the contents are degraded. Autophagy is tightly regulated by a set of conserved autophagy-related (ATG) proteins, which coordinate cargo recognition, autophagosome formation, and degradation (Klionsky et al., 2003). Mitophagy is a specialized form of autophagy that provides cells with the ability to remove old, damaged, or unnecessary mitochondria, playing a crucial role in mitochondrial quality control and cellular homeostasis. It can be triggered either in response to mitochondrial damage or as a part of metabolic adaptation to cellular energy demands (Kim et al., 2007). Given its significance, dysregulation of mitophagy has been implicated in various pathologies including neurodegenerative diseases, cancer, as well as hematological malignancies like AML (Gustafsson and Dorn, 2019).

There are two major possible pathways through which mitophagy proceeds (see Figure 3). While these pathways are often described separately, they are not entirely independent and there are multiple crosstalk points between them. This interplay allows for a more flexible and efficient regulation of mitochondrial clearance, depending on cellular conditions. The first pathway is the receptor-mediated mitophagy, which relies on outer mitochondrial membrane receptors that interact directly with the autophagic machinery. Two key receptors involved in this process are BNIP3 and NIX (Chen et al., 1999). BNIP3, a proapoptotic protein, is initially expressed as an inactive monomer in the cytosol. Upon activation, it forms stable homodimers anchored to the outer mitochondrial membrane (OMM) via its C-terminal transmembrane domain. The protein contains an LC3-interacting region (LIR) in its N-terminus. This motif is flanked by two phosphorylated serine residues (Ser17 and Ser24), which facilitate its interaction with autophagy-related proteins from the ATG8 family, like LC3, residing in the autophagosomal membrane (Hanna et al., 2012). NIX shares structural and functional similarities with BNIP3, forming homodimers and possessing an N-terminal LIR motif (Matsushima et al., 1998). Another important mitophagy receptor is FUNDC1, which plays a key role in activating mitophagy under hypoxic conditions. Under normal conditions, FUNDC1 is inhibited by phosphorylation, whereas during hypoxia it becomes dephosphorylated, enabling its interaction with LC3 and the initiation of mitophagy (Liu et al., 2012).

The second possibility is the adaptor-mediated mitophagy, which involves ubiquitination of damaged mitochondria. Ubiquitinated mitochondrial proteins are subsequently recognized by the adaptor proteins linking them to the rest of autophagy machinery. The most common adaptor proteins involved in this process include p62 (sequestosome 1 - SQSTM1), TAX1BP1, optineurin (OPTN), NDP52, and NBR1, all of which contain ubiquitin-binding domains that enable them to recognize ubiquitinated mitochondria and facilitate their sequestration into autophagosomes via interaction with LC3 (Palikaras et al., 2018; Yoo and Jung, 2018). This process requires the activity of ubiquitin ligases, with one of the most well-characterized mechanisms involving the PTEN-induced putative kinase 1 (PINK1) and the ubiquitin ligase Parkin. Under normal circumstances, PINK1 uses a mitochondrial targeting sequence which is identified by translocases and leads to its import into mitochondria via TOM40 and TIM23. Subsequently, PINK1 gets cleaved by the mitochondrial processing peptidases, causing its degradation. However, in damaged mitochondria, the translocation through TIM23 is inhibited due to the adenine nucleotide translocator TIM44. Therefore, PINK1 accumulates on the OMM, where it binds to TOM7 which in turn stabilizes its localization (Vives-Bauza et al., 2009). After that, PINK1 undergoes autophosphorylation, leading to its activation and destabilization of its dimeric form. Activated PINK1 phosphorylates Parkin, increasing its ubiquitin ligase activity. The accumulation of ubiquitinated mitochondrial proteins further amplifies the PINK1 kinase activity establishing a positive feedback loop that promotes the recruitment of adaptor proteins and consequential mitophagy initiation (Okatsu et al., 2012).

OPTN and NDP52 belong to the key adaptor proteins that are recruited to PINK1/Parkin-ubiquitinated mitochondria. They also play a role in amplification of mitophagy signals through their interaction with ATG8 family proteins. OPTN helps with the sequestration of damaged mitochondria and directs autophagosome formation as well (Lazarou et al., 2015). Alternative options independent of PINK1/Parkin ubiquitination exist as well, for example the p62 adaptor protein utilizes KEAP1 and RBX1 ubiquitin ligases instead (Yamada et al., 2019). Certain mitophagy receptors can influence the adaptor-mediated pathway and vice versa. For instance, BNIP3 plays a protective role by preventing the proteolytic cleavage of PINK1, thereby promoting its accumulation on the OMM (Zhang et al., 2016). Another example is NIX serving as a substrate for Parkin. Upon ubiquitination, NIX facilitates the recruitment of mitophagy-related proteins to the mitochondria. (Gao et al., 2015).

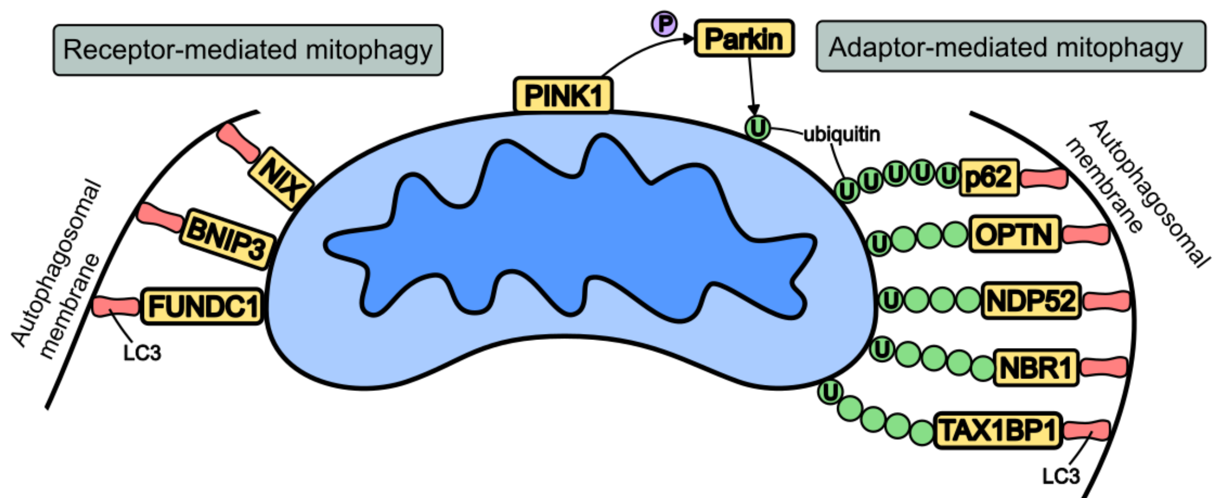

Figure 3: *Schematic representation of mitophagy pathways.* Mitochondria are cleared via two major mechanisms: receptor-mediated mitophagy, which involves direct interaction of receptor proteins with LC3, and adaptor-mediated mitophagy, in which damaged mitochondria are labeled for degradation through ubiquitin signaling and recruitment of adaptor proteins that also bind LC3. This interaction facilitates the formation of the autophagosome.

4.1 Interaction between mitophagy and apoptosis

While mitophagy primarily functions to maintain mitochondrial quality by removing damaged organelles, its regulation is closely linked to the mitochondrial apoptotic pathway. Interestingly, mitophagy receptors and intrinsic apoptotic pathway receptors share notable similarities and some of them participate in both processes. This crosstalk allows certain proteins involved in mitophagy to help either promote or prevent cell death while also enabling the BCL-2 family proteins involved in apoptosis to influence Parkin translocation. Beyond its role in phosphorylating the ubiquitin ligase Parkin, PINK1 also phosphorylates other substrates, including BCL-X_L, a member of the BCL-2 family. When phosphorylated by PINK1, BCL-X_L is protected from getting cleaved, thereby preventing apoptosis (Wanderoy et al., 2020). Parkin ubiquitinates the BH3-binding site of BAK which can affect its interactions with regulatory BH3-only proteins as well as BCL-2 regulatory proteins, meaning it can work both in favor of mitophagy and apoptosis (Bernardini et al., 2019).

Moreover, Parkin can induce either mitophagy or apoptosis via monoubiquitination or polyubiquitination of voltage-dependent anion channel (VDAC1). When VDAC1 gets monoubiquitinated, it increases mitochondrial calcium intake to the point of mitochondrial membrane permeabilization and subsequently triggers apoptosis. In contrast, polyubiquitination of VDAC1 starts the mitophagy pathway (Ham et al., 2020). Surprisingly,

pro-survival BCL-2 family proteins suppress the recruitment and translocation of Parkin in damaged mitochondria, setting a higher threshold for starting mitophagy and potentially leading to accumulation of damaged mitochondria, which could lead to cellular death. This is critical when removing cells with acute mitochondrial damage, but the heightened threshold for beginning mitophagy is also suggested to help with preventing healthy mitochondria from removal (Hollville et al., 2014). Additionally, the p53 protein, which promotes apoptosis in response to cellular damage, also plays a role in suppressing PINK1/Parkin mitophagy either by binding to and directly inhibiting Parkin, or by downregulating the transcription of *PINK1*. In cells harboring *TP53* mutations, the regulation of mitophagy is disrupted, often leading to an increased mitophagic flux (Hoshino et al., 2013; Goiran et al., 2018). This close relationship between mitophagy and apoptosis demonstrates that mitophagy is a crucial process for cell survival.

5 Mitophagy in acute myeloid leukemia

Due to the highly heterogeneous nature of AML, identifying universal traits shared across all cases remains challenging. The cytogenetic landscape varies significantly among patients, although certain mutations occur more frequently than others. In addition, AML consists of distinct subpopulations of leukemic cells, consisting of AML blasts, including leukemic stem cells (LSCs), and more differentiated bulk tumor cells, contributing to the biological variability. The bulk tumor cells lack self-renewal and proliferation abilities which makes them an easier target for general chemotherapy but the LSCs persist and are one of the major causes for therapy resistance or relapse (Shlush et al., 2017). LSCs exhibit unique metabolic characteristics that distinguish them from other leukemic and normal hematopoietic stem cells (HSCs). The LSCs depend mainly on OXPHOS for energy production and survival (Lagadinou et al., 2013). This strong dependence on mitochondrial respiration makes the maintenance of mitochondrial integrity, particularly through mitophagy, critical for LSC persistence. One of the key distinctions between LSCs and HSCs lies in their energy metabolism. While HSCs primarily rely on glycolysis and can shift their metabolic profile in response to stress, LSCs are metabolically more rigid and remain dependent on OXPHOS. Even some of the more differentiated AML cells are capable of compensating for impaired mitochondrial function by upregulating glycolysis, an ability that LSCs seem to be lacking. This level of reliance on oxidative phosphorylation presents a promising therapeutic target, as selectively disrupting oxidative phosphorylation could eradicate LSCs while sparing normal HSCs (Jones et al., 2018).

Moreover, oxidative phosphorylation in LSCs is closely interconnected with amino acid metabolism, making this pathway an additional point of vulnerability. Therefore, therapies targeting amino acid metabolism, particularly glutamine and arginine dependency, have shown potential in disrupting mitochondrial function and sensitizing leukemic cells to apoptosis. A key step in glutamine metabolism is glutaminolysis, which is catalyzed by glutaminases, especially the glutaminase C (GAC) isoform in AML. This process can be inhibited by CB-839, a glutaminase inhibitor that has shown therapeutic potential in combination with other agents such as BCL-2 or FLT3 inhibitors (Jacque et al., 2015; Gregory et al., 2018). Another therapeutic strategy exploits the dependence of certain AML subtypes on extracellular arginine. Agents such as BCT-100, a recombinant human arginase, are able to deplete systemic arginine levels and effectively starve the AML blasts (Mussai et al., 2018).

Another defining feature of LSCs, as well as HSCs, is their requirement for a low-reactive oxygen species (ROS) environment to maintain their undifferentiated state. Otherwise, elevated oxidative stress promotes differentiation and impairs self-renewal capacity of the stem cells (Cipolleschi et al., 1993; Zhou et al., 2014). In this context, mitophagy serves as a crucial quality control mechanism by clearing old or damaged mitochondria, which are a major source of intracellular ROS. Therefore, the ability to maintain a healthy mitochondrial network through mitophagy plays a crucial role in preserving LSC function and, by extension, their capacity for disease persistence.

Mitophagy also plays a crucial role in maintaining the function of healthy hematopoietic stem cells, although its purpose differs from that in leukemic cells. Rather than serving mainly as a response to cellular stress, mitophagy in HSCs is essential for preserving cellular quiescence and metabolic balance in hypoxic conditions. To achieve this, HSCs actively eliminate excess, functional mitochondria to maintain a low level of oxidative metabolism and prevent unwanted activation. Disruption of mitophagy in HSCs can lead to a loss of stemness and, in some cases, contribute to leukemic transformation. For example, genetic knockout of the *Atg7* autophagy gene in mice impairs mitophagy and triggers aberrant activation of the NOTCH signaling pathway, which interferes with normal HSC differentiation and results in pathological myeloid expansion (Cao et al., 2015). Moreover, the process of oncogenic transformation itself can induce cellular stress and mitochondrial damage. This intrinsic stress may necessitate mitophagy as an early adaptive response, enabling leukemic cells to remove dysfunctional mitochondria, limit ROS accumulation, and sustain proliferation (Pei et al., 2018).

Although hematopoietic stem cells (HSCs) also depend on mitophagy to maintain their function, this does not necessarily preclude mitophagy-related processes from being viable therapeutic targets in acute myeloid leukemia (AML). One concern is that inhibiting mitophagy could disrupt normal hematopoiesis. However, HSCs and leukemic stem cells (LSCs) preferentially utilize different mitophagy pathways depending on the context. In HSCs, mitochondrial removal under hypoxic conditions predominantly occurs via receptor-mediated mitophagy, for example through FUNDC1. In contrast, LSCs, particularly under conditions of mitochondrial damage, rely more heavily on adaptor-mediated mitophagy pathways involving ubiquitin-dependent mechanisms. Therefore, targeting specific components of the adaptor-mediated mitophagy machinery may selectively impair LSC survival while sparing normal HSCs.

The importance of mitophagy in AML cells is further highlighted by the overexpression of several proteins involved in mitochondrial quality control. One such protein is the mitochondrial dynamics regulator FIS1 (mitochondrial fission 1). FIS1 promotes mitochondrial division by separating specific regions of the organelle, such as those with accumulated damage, so they can be efficiently removed through mitophagy. FIS1 expression is upregulated by AMP-activated protein kinase (AMPK), a metabolic stress sensor that is constitutively active in leukemic stem cells (LSCs). Notably, the loss of FIS1 has been shown to disrupt mitochondrial dynamics in LSCs and lead to the inactivation of glycogen synthase kinase 3 (GSK3), a kinase involved in cell cycle regulation and maintenance of stemness. Inactivation of GSK3 can trigger differentiation of LSCs (Pei et al., 2018). Another mitophagy-related protein that has been shown to play a significant role in AML is p62. High expression of this autophagy adaptor has been associated with poor prognosis in AML patients. Experimental studies have demonstrated that inhibition of p62, or the use of p62-deficient cell lines, results in a marked reduction in leukemic cell survival and disease progression *in vitro* (Nguyen et al., 2019; Li et al., 2021). Additionally, the loss of p62 has been shown to sensitize AML cells to mitochondria-targeting agents such as rotenone and FCCP, suggesting that p62 contributes to mitochondrial stress tolerance in leukemic cells. In the same study, NIX knockdown has also been tested, yielding similar results (Rodrigo et al., 2019). Similarly, depletion of another mitophagy adaptor protein, OPTN, also impaired leukemia progression and enhanced the sensitivity of AML cells to mitochondrial stress-inducing compounds (Meyer et al., 2023).

One of the major clinical challenges in AML treatment is therapy resistance and disease relapse, primarily driven by the persistence of leukemic stem cells (LSCs), as discussed above. While standard therapies such as chemotherapy and BCL-2 inhibitors effectively eliminate the bulk of leukemic cells, they often fail to eradicate the more resilient LSC population. A key mechanism facilitating this resistance is the ability of LSCs to survive therapy-induced mitochondrial damage. Many chemotherapeutic agents, as well as BH3 mimetics like venetoclax, exert their cytotoxic effects by inducing mitochondrial stress and promoting apoptosis. However, LSCs with elevated mitophagic activity can selectively eliminate damaged mitochondria, thereby reducing ROS accumulation and evading apoptosis.

Providing further evidence, AML cells that have developed resistance to BCL-2 inhibitors such as venetoclax exhibit enhanced mitophagic flux. Inhibition of mitophagy, whether genetically or pharmacologically, has been shown to sensitize these resistant cells to treatment. One of the proteins that have been connected to this resistance mechanism is mitofusin 2 (MFN2), a mitochondrial dynamics regulator that, among other functions, facilitates the

recruitment of Parkin to damaged mitochondria, thereby enhancing mitophagic clearance. Another important protein that has been studied in this context is the E3 ubiquitin ligase MARCH5, which has also been implicated in maintaining mitochondrial homeostasis through the regulation of mitochondrial dynamics machinery. Deletion of either MFN2 or MARCH5 has been shown to increase the sensitivity of AML cells to BH3 mimetics, indicating that inhibitors targeting these proteins could provide a promising addition to venetoclax treatment (Glytsou et al., 2023; Lin et al., 2022). Another approach to sensitizing AML cells to treatment involves blocking mitophagy at the level of autophagosome degradation. Chloroquine (CQ) inhibits the final step of autophagy by preventing lysosomal fusion and degradation (Janku et al., 2011). While effective at impairing mitophagy, its clinical use is limited by high systemic toxicity and lack of specificity for AML cells. As an alternative, more targeted strategies are being explored, one of them being ULK1 kinase inhibitors, which disrupt autophagosome formation and may offer a more selective therapeutic window with reduced off-target effects. Altogether, these findings highlight that mitophagy is a critical survival mechanism in therapy-resistant AML and should be considered as a potential target in synergistic treatment regimens (Yang et al., 2021).

While it is generally observed that AML cells exploit mitophagy for survival, in the context of C6-ceramide-tamoxifen treatment, mitophagy has been shown to have cytotoxic effect on leukemic cells, leading to cell death in an ATG5-dependent manner. This suggests that overactivation of the mitophagic machinery can result in excessive mitochondrial clearance, culminating in bioenergetic failure and apoptosis (Morad et al., 2019).

6 Conclusion

Mitophagy was demonstrated to be a central mechanism by which LSCs maintain their mitochondrial integrity, suppress apoptotic signaling, and resist cytotoxic therapies. This selective degradation pathway allows LSCs to tolerate mitochondrial damage induced by both intrinsic metabolic stress and extrinsic therapeutic pressure. Through the removal of dysfunctional mitochondria and suppression of reactive oxygen species, mitophagy preserves the function of the mitochondrial network essential for OXPHOS, which LSCs depend on for energy production and survival.

Therapeutic strategies that target mitophagy have demonstrated promising preclinical potential. Inhibiting regulators such as ULK1, disrupting mitochondrial dynamics through MFN2 or MARCH5 suppression, or blocking mitophagy adaptor or receptor proteins (for example p62, OPTN or NIX) has been shown to sensitize AML cells to agents like venetoclax and mitochondrial toxins. Moreover, pharmacological agents that impair autophagosome formation or lysosomal degradation (e.g., chloroquine) may further enhance treatment responses when combined with conventional or targeted therapies. However, these approaches must be pursued with caution, as mitophagy is also important for the maintenance of normal hematopoietic stem cells, particularly for preserving quiescence and preventing premature differentiation. To maximize therapeutic efficacy while minimizing toxicity, future research should be focused on identifying AML-specific mitophagy regulators, understanding the differential pathway usage in leukemic versus healthy cells, and developing selective inhibitors that disrupt mitophagy only in leukemic cells.

7 References

Abaza, Y. & Zeidan, A. M. Immune Checkpoint Inhibition in Acute Myeloid Leukemia and Myelodysplastic Syndromes. Cells 11, 2249 (2022).

* Andreyev, A. Yu., Kushnareva, Yu. E. & Starkov, A. A. Mitochondrial metabolism of reactive oxygen species. Biochemistry (Moscow) 70, 200–214 (2005).

Bernardini, J. P. et al. Parkin inhibits BAK and BAX apoptotic function by distinct mechanisms during mitophagy. The EMBO Journal 38, e99916 (2019).

Berrien-Elliott, M. M. et al. Hematopoietic cell transplantation donor-derived memory-like NK cells functionally persist after transfer into patients with leukemia. Sci. Transl. Med. 14, 1375 (2022).

Bolaños, J. P., Moro, M. A., Lizasoain, I. & Almeida, A. Mitochondria and reactive oxygen and nitrogen species in neurological disorders and stroke: Therapeutic implications☆. Advanced Drug Delivery Reviews 61, 1299–1315 (2009).

Brunelle, J. K. & Letai, A. Control of mitochondrial apoptosis by the BCL-2 family. Journal of Cell Science 122, 437–441 (2009).

Cao, Y. et al. Autophagy Sustains Hematopoiesis Through Targeting Notch. Stem Cells and Development 24, 2660–2673 (2015).

* Capaldi, R. A. Structure and Function of Cytochrome c Oxidase. Annu. Rev. Biochem. 59, 569–596 (1990).

* Cecchini, G. Function and Structure of Complex II of the Respiratory Chain. Annu. Rev. Biochem. 72, 77–109 (2003).

Cipolleschi, M., Dello Sbarba, P. & Olivotto, M. The role of hypoxia in the maintenance of hematopoietic stem cells. Blood 82, 2031–2037 (1993).

Chelghoum, Y. et al. Influence of cigarette smoking on the presentation and course of acute myeloid leukemia. Annals of Oncology 13, 1621–1627 (2002).

Chen, G. et al. NIX and NIP3 Form a Subfamily of Pro-apoptotic Mitochondrial Proteins. Journal of Biological Chemistry 274, 7–10 (1999).

Chiara, M.-D. et al. Metastatic Paragangliomas and Pheochromocytomas: An Epigenetic View. in Pheochromocytoma, Paraganglioma and Neuroblastoma (eds. Cianci, P., Restini, E. & Agrawal, A.) (IntechOpen, 2021). doi:10.5772/intechopen.96126.

Döhner, H., Weisdorf, D. J. & Bloomfield, C. D. Acute Myeloid Leukemia. N Engl J Med 373, 1136–1152 (2015).

Döhner, K. et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood 106, 3740–3746 (2005).

Du, C., Fang, M., Li, Y., Li, L. & Wang, X. Smac, a Mitochondrial Protein that Promotes Cytochrome c–Dependent Caspase Activation by Eliminating IAP Inhibition. Cell 102, 33–42 (2000).

Gao, F. et al. The mitochondrial protein BNIP3L is the substrate of PARK2 and mediates mitophagy in PINK1/PARK2 pathway. Human Molecular Genetics 24, 2528–2538 (2015).

Glytsou, C. et al. Mitophagy Promotes Resistance to BH3 Mimetics in Acute Myeloid Leukemia. Cancer Discovery 13, 1656–1677 (2023).

Godley, L. A. & Larson, R. A. Therapy-Related Myeloid Leukemia. Seminars in Oncology 35, 418–429 (2008).

Goiran, T. et al. Nuclear p53-mediated repression of autophagy involves *PINK1* transcriptional down-regulation. Cell Death Differ (2018) doi:10.1038/s41418-017-0016-0.

Gregory, M. A. et al. Glutaminase inhibition improves FLT3 inhibitor therapy for acute myeloid leukemia. Experimental Hematology 58, 52–58 (2018).

Grimwade, D. et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood 116, 354–365 (2010).

Gustafsson, Å. B. & Dorn, G. W. Evolving and Expanding the Roles of Mitophagy as a Homeostatic and Pathogenic Process. Physiological Reviews 99, 853–892 (2019).

Ham, S. J. et al. Decision between mitophagy and apoptosis by Parkin via VDAC1 ubiquitination. Proc. Natl. Acad. Sci. U.S.A. 117, 4281–4291 (2020).

Hanna, R. A. et al. Microtubule-associated Protein 1 Light Chain 3 (LC3) Interacts with BNIP3 Protein to Selectively Remove Endoplasmic Reticulum and Mitochondria via Autophagy. Journal of Biological Chemistry 287, 19094–19104 (2012).

Hollville, E., Carroll, R. G., Cullen, S. P. & Martin, S. J. BCL-2 Family Proteins Participate in Mitochondrial Quality Control by Regulating Parkin/PINK1-Dependent Mitophagy. Molecular Cell 55, 451–466 (2014).

Hoshino, A. et al. Cytosolic p53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction in the mouse heart. Nat Commun 4, 2308 (2013).

Jacque, N. et al. Targeting glutaminolysis has antileukemic activity in acute myeloid leukemia and synergizes with BCL-2 inhibition. Blood 126, 1346–1356 (2015).

Janku, F., McConkey, D. J., Hong, D. S. & Kurzrock, R. Autophagy as a target for anticancer therapy. Nat Rev Clin Oncol 8, 528–539 (2011).

Junge, W., Lill, H. & Engelbrecht, S. ATP synthase: an electrochemical transducer with rotatory mechanics. Trends in Biochemical Sciences 22, 420–423 (1997).

Kantarjian, H. M. et al. Multicenter, Randomized, Open-Label, Phase III Trial of Decitabine Versus Patient Choice, With Physician Advice, of Either Supportive Care or Low-Dose Cytarabine for the Treatment of Older Patients With Newly Diagnosed Acute Myeloid Leukemia. JCO 30, 2670–2677 (2012).

Kim, I., Rodriguez-Enriquez, S. & Lemasters, J. J. Selective degradation of mitochondria by mitophagy. Arch. Biochem. Biophys. 462, 245–253 (2007).

Lagadinou, E. D. et al. BCL-2 Inhibition Targets Oxidative Phosphorylation and Selectively Eradicates Quiescent Human Leukemia Stem Cells. Cell Stem Cell 12, 329–341 (2013).

Lazarou, M. et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature 524, 309–314 (2015).

Ley, T. J. et al. *DNMT3A* Mutations in Acute Myeloid Leukemia. N Engl J Med 363, 2424–2433 (2010).

Li, Y. et al. A mitophagy inhibitor targeting p62 attenuates the leukemia-initiation potential of acute myeloid leukemia cells. Cancer Letters 510, 24–36 (2021).

Lin, S. et al. An In Vivo CRISPR Screening Platform for Prioritizing Therapeutic Targets in AML. Cancer Discovery 12, 432–449 (2022).

Liu, L. et al. Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. Nat Cell Biol 14, 177–185 (2012).

Meyer, L. M. et al. Deciphering the mitophagy receptor network identifies a crucial role for OPTN (optineurin) in acute myeloid leukemia. Autophagy 19, 2982–2996 (2023).

Matsushima, M. et al. Isolation, mapping, and functional analysis of a novel human cDNA (BNIP3L) encoding a protein homologous to human NIP3. Genes Chromosom. Cancer 21, 230–235 (1998).

Mussai, F. et al. Arginine dependence of acute myeloid leukemia blast proliferation: a novel therapeutic target. Blood 125, 2386–2396 (2015).

Nguyen, T. D. et al. Loss of the selective autophagy receptor p62 impairs murine myeloid leukemia progression and mitophagy. Blood 133, 168–179 (2019).

Okatsu, K. et al. PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria. Nat Commun 3, 1016 (2012).

Onishi, M., Yamano, K., Sato, M., Matsuda, N. & Okamoto, K. Molecular mechanisms and physiological functions of mitophagy. The EMBO Journal 40, e104705 (2021).

Palikaras, K., Lionaki, E. & Tavernarakis, N. Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. Nat Cell Biol 20, 1013–1022 (2018).

Paschka, P. et al. *IDH1* and *IDH2* Mutations Are Frequent Genetic Alterations in Acute Myeloid Leukemia and Confer Adverse Prognosis in Cytogenetically Normal Acute Myeloid Leukemia With *NPM1* Mutation Without *FLT3* Internal Tandem Duplication. JCO 28, 3636–3643 (2010).

Pei, S. et al. AMPK/FIS1-Mediated Mitophagy Is Required for Self-Renewal of Human AML Stem Cells. Cell Stem Cell 23, 86-100.e6 (2018).

Pratz, K. & Levis, M. Incorporating FLT3 inhibitors into acute myeloid leukemia treatment regimens. Leukemia & Lymphoma 49, 852–863 (2008).

Rodrigo, R. et al. Knockdown of BNIP3L or SQSTM1 alters cellular response to mitochondria target drugs. Autophagy 15, 900–907 (2019).

Rombouts, W., Blokland, I., Löwenberg, B. & Ploemacher, R. Biological characteristics and prognosis of adult acute myeloid leukemia with internal tandem duplications in the *FLT3* gene. Leukemia 14, 675–683 (2000).

Shlush, L. I. et al. Tracing the origins of relapse in acute myeloid leukaemia to stem cells. Nature 547, 104–108 (2017).

Souers, A. J. et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med 19, 202–208 (2013).

Stengel, A. et al. The impact of *TP53* mutations and *TP53* deletions on survival varies between AML, ALL, MDS and CLL: an analysis of 3307 cases. Leukemia 31, 705–711 (2017).

Trumpower, B. L. The protonmotive Q cycle. Energy transduction by coupling of proton translocation to electron transfer by the cytochrome bc1 complex. Journal of Biological Chemistry 265, 11409–11412 (1990).

Turrens, J. F. Mitochondrial formation of reactive oxygen species. The Journal of Physiology 552, 335–344 (2003).

Visani, G. et al. Consolidation therapy for adult acute myeloid leukemia: A systematic analysis according to evidence based medicine. Leukemia & Lymphoma 47, 1091–1102 (2006).

Vives-Bauza, C. et al. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. Proc. Natl. Acad. Sci. U.S.A. 107, 378–383 (2010).

Walker, J. E. The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains. Quart. Rev. Biophys. 25, 253–324 (1992).

Wanderoy, S., Hees, J. T., Klesse, R., Edlich, F. & Harbauer, A. B. Kill one or kill the many: interplay between mitophagy and apoptosis. Biological Chemistry 402, 73–88 (2020).

Wiernik, P. et al. Cytarabine plus idarubicin or daunorubicin as induction and consolidation therapy for previously untreated adult patients with acute myeloid leukemia. Blood 79, 313–319 (1992).

Wu, C.-C. & Bratton, S. B. Regulation of the Intrinsic Apoptosis Pathway by Reactive Oxygen Species. Antioxidants & Redox Signaling 19, 546–558 (2013).

Yamada, T., Dawson, T. M., Yanagawa, T., Iijima, M. & Sesaki, H. SQSTM1/p62 promotes mitochondrial ubiquitination independently of PINK1 and PRKN/parkin in mitophagy. Autophagy 15, 2012–2018 (2019).

Yang, W. et al. Inhibition of ULK1 promotes the death of leukemia cell in an autophagy irrelevant manner and exerts the antileukemia effect. Clinical & Translational Med 11, e282 (2021).

Zarychta, J., Kowalczyk, A., Krawczyk, M., Lejman, M. & Zawitkowska, J. CAR-T Cells Immunotherapies for the Treatment of Acute Myeloid Leukemia—Recent Advances. Cancers 15, 2944 (2023).

Zhang, T. et al. BNIP3 Protein Suppresses PINK1 Kinase Proteolytic Cleavage to Promote Mitophagy. Journal of Biological Chemistry 291, 21616–21629 (2016).

Zhou, D., Shao, L. & Spitz, D. R. Reactive Oxygen Species in Normal and Tumor Stem Cells. in Advances in Cancer Research vol. 122 1–67 (Elsevier, 2014).