

Abstract

Acute myeloid leukemia (AML) is a clonal malignancy of hematopoietic stem cells (HSCs) characterized by expansion of myeloid blasts in the bone marrow. It has been shown that autophagy is a degradative process, which delivers cytoplasmic components to lysosomes to prevent malignant transformation by maintaining HSC integrity. Besides its function as a bulk degradation machinery to recycle cytoplasmic components during limited energy supply, autophagy also serves as an intracellular quality control mechanism. Selective autophagy requires autophagy receptors such as p62 to specifically bridge the targeted cargos into autophagosomes. p62 is known as a central signaling hub involved in pro-oncogenic signaling pathways and autophagic degradation pathways. However, little is known about the role of p62 as a selective autophagy receptor in AML. This study aims to elucidate the precise function of p62 as an autophagy receptor in leukemia development and maintenance.

In silico analysis revealed that high p62 expression was significantly associated with poor overall survival of adult patients with *de novo* AML, suggesting that p62 may promote leukemia maintenance. To address the functional role of p62 in leukemia, genome editing by CRISPR/Cas9 was used to knockout p62 in four human AML cell lines. Importantly, p62 loss reduced cell proliferation in all four cell lines. This observation could be transferred to a murine leukemia cell model in which leukemic transformation of lineage-depleted bone marrow (IdMBM) cells was induced by overexpression of the human transcriptional coactivator MN1. Knockdown of p62 by shRNA in MN1-driven leukemia cells impaired proliferation and decreased colony forming ability without altering apoptosis. This indicates that p62 is crucial for leukemia proliferation *in vitro*. To further characterize the role of p62 in leukemia development and maintenance a murine AML transplantation model was established. Therefore, IdMBM cells isolated from WT and p62^{-/-} mice were transduced with MN1 and transplanted into lethally irradiated mice. As expected, all mice developed fatal myeloid proliferation. Notably, p62 loss in MN1-driven leukemia significantly prolonged survival in mice and caused a more immature phenotype. Consistent with the *in vitro* results, *ex vivo* analysis of p62^{-/-} leukemic cells displayed decreased colony-forming ability, although p62 loss did not affect composition and function of HSCs. Moreover, re-transplantation of primary MN1-driven leukemia cells attenuated

leukemia progression upon p62 loss. These findings support a decisive role of p62 in leukemia development and maintenance.

To gain molecular insight into the function of p62 during myeloid transformation an interactome analysis of murine MN1-driven leukemia cells was performed. This revealed first that p62 predominantly interacts with mitochondrial proteins and second that inhibition of autophagic degradation causes accumulation of p62-bound mitochondria. This leads to the first assumption that loss of p62 may provoke mitochondrial accumulation with increasing mitochondrial damage and second that p62 may mediate degradation of mitochondria by mitophagy. Indeed, in the absence of p62, accumulation of dysfunctional mitochondria was detected by morphological changes of the mitochondria, increased mitochondrial ROS and impaired mitochondrial respiration capacity. Furthermore, induction of PINK1/Parkin-independent mitophagy revealed that loss of p62 caused impaired degradation of mitochondrial proteins and reduced translocation of damaged mitochondria into autophagosomes. Taken together, p62 is required for effective degradation of dysfunctional mitochondria by mitophagy in AML.

Due to the fact that p62 is a multifunctional protein, rescue experiments with different mutants of p62 were performed to clarify if p62-mediated mitophagy contributes to leukemia proliferation. Notably, the autophagy-deficient mutant (disabled to bind autophagosomes) reduced cell growth and colony-forming ability to the same extent as knockdown of p62, as the clustering-deficient mutant (disabled to form aggregates) displayed an intermediate phenotype. Strikingly, only the autophagy-deficient mutant failed to rescue mitophagy.

In conclusion, this study demonstrates the prominent role of p62 as a selective autophagy receptor for mitochondrial quality control which contributes to leukemia development and maintenance. Therefore, targeting selective autophagy opens new venues in the treatment of AML.