Targeting Histone Demethylase LSD1/KDM1a in Neurodegenerative Diseases

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ABSTRACT: The autophagy-lysosome pathway sustains cellular homeostasis and is a protective mechanism against neurodegenerative diseases. Recent findings highlight the role of the histone demethylases LSD1/LDM1A as a pivotal regulator of autophagy process, by controlling the mTORC1 cascade, in neuroblastoma cells. LSD1 binds to the promoter region of the *SESN2* gene, where LSD1-mediated demethylation leads to the accumulation of repressive histone marks that maintain SESN2 expression at low levels. LSD1 depletion results in enhanced SESN2 expression and consequently mTORC1 inhibition, thereby triggering the induction of autophagy. Our study provides important insight into neuroepigenetic mechanisms regulating the autophagic process, offering additional opportunities for the development of novel therapeutic strategies in diseases associated with dysfunctional autophagy-lysosomal pathway.

KEYWORDS: LSD1, Sestrin2, autophagy

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Macroautophagy (described herein as "autophagy") is an evolutionary conserved, lysosomal-degradative process which allows the restrained degradation of damaged organelles and protein aggregates for recycling of cellular components. As a major cytoprotective mechanism in response to environmental adversity, autophagy plays an essential role in survival, organismal development, and maintenance of cellular homeostasis.[1] Aberration in the autophagic process contributes to the pathogenesis of several human diseases, including cancer and neurodegenerative disorders. Autophagy is emerging as a vital pathway in brain, protecting postmitotic neurons from the rigors of a long-duration mission; dysfunction or dysregulation in this process has been strongly linked to neurodegenerative diseases in humans. Accordingly, increasing autophagy activity has been shown to exert a beneficial effect on the clearance of mutant α-synuclein protecting cells against apoptosis in Alzheimer disease mouse models.[2]

Accumulating evidence argue in favor of the key role of transcriptional and epigenetic factors in regulating autophagy[3]; as autophagy dysfunction is implicated in several diseases, there is great interest in unraveling the nuclear programs underlying the autophagic machinery and understanding how these programs are dysregulated in pathological contexts could be beneficial to improve autophagy-based clinical treatments.

In a recent work, we have shown that lysine-specific demethylase 1, LSD1, is a critical regulator of autophagy in neuroblastoma (NB).[4] We found that LSD1 inactivation, by depletion or pharmacological inhibition, blocks the mechanistic target of rapamycin complex 1, mTORC1, cascade in different NB cell lines in a dose-dependent manner. mTORC1 is a negative regulator of autophagy and acts by inhibiting the cytoplasm-to-nucleus shuttling of transcription factor EB, TFEB, a master transcriptional regulator of lysosomal and autophagy genes.[5] We demonstrated that LSD1 inhibition triggers a strong nuclear localization of TFEB, suggesting that reduction in LSD1 activity leads to the mTOR-dependent activation of the autophagic pathway in NB cells. Consistently, LSD1 depletion results in a significant increase in the conversion of microtubule-associated protein 1A/1B-light chain, LC3, from LC3-I to LC3-II and formation of mature autophagolysosomes.

RNA-Seq data set identified SESN2/Sestrin2 as an LSD1-negative target gene involved in the mTORC1 pathway regulation. mTORC1 activity is under the control of the Rag GTPases that promote its localization to the lysosomal surface. Rags are inhibited by GATOR1 complex, which in turn is negatively regulated by GATOR2 complex.[6] SESN2 directly interacts with GATOR2, suppressing the Rag-dependent recruitment of mTORC1 to the lysosomal membrane thereby blocking mTORC1 signaling.[7] Thus, LSD1 depletion leads to the transcriptional activation of SESN2 expression, which in turn inhibits mTORC1 activity, lastly activating autophagy. Consistently, SESN2 overexpression promotes autophagy in NB cells, whereas SESN2 knockdown partially abolishes the effects of LSD1 inhibition on mTORC1 activity and autophagy.[4]

Chromatin immunoprecipitation assay demonstrated that LSD1 is directly bound to the transcriptional start site (TSS) in the SESN2 promoter. Moreover, genetics depletion or pharmacological inhibition of LSD1 triggers an epigenetic active state by accumulation of the activating histone mark H3 acetylation and decreasing of repressive histone mark H3K27me3, according to enhanced SESN2 expression.

LSD1 acts as corepressor or coactivator in a target-specific manner, by mediating demethylation of mono- and dimethylated

lysines 4 and 9 of histone H3, respectively.[8] Consistently with the repressive function, LSD1 inhibition increases H3K4me2 level at TSS of *SESN2* gene, whereas H3K9Me2 signature appears to be not affected.

LSD1 ability to selectively demethylate K9 or K4 residues depends on its interactions with distinct DNA-binding transcription factors. However, the mechanism by which LSD1 is recruited to the *SESN2* gene promoter to establish a repressive chromatin state still is not known.

SESN2 is a member of the PA26-related protein family, involved in cellular response to different stress conditions. TP53/p53 is a major regulator of SESN2 expression under genotoxic insults and oxidative stress.[9] We demonstrate that treatment with LSD1 enzymatic inhibitors increases SESN2 expression and induces autophagy also in the SK-N-BE NB cell line, carrying a mutation which inactivates p53, indicating that SESN2 transcriptional regulation by LSD1 is achieved in a p53-independent manner.

SESN2 plays important roles in tumor suppression, regulating stress response, cell proliferation, and survival and appears often downregulated in cancer cells.[10] Accordingly, we found that high SESN2 expression correlates with a better survival probability in patients with NB. Furthermore, SESN2 expression is inversely correlated with the expression of LSD1 in NB.

Growing body of evidence highlights the defensive role of SESN2 against neurodegeneration and age-related diseases. In in vitro model of Parkinson disease, SESN2 overexpression induces autophagy and prevents α-synuclein accumulation. SESN2 knockdown in primary cortical neurons has been shown to abrogate the autophagic response and aggravate amyloid-β peptide–induced neurotoxicity.[10] Moreover, SESN2 exhibits an oxidoreductase activity in vitro and protects cells from oxidative stress inhibiting accumulation of reactive oxygen species (ROS).[11] Thus, SESN2 could exert a dual protective role against neurodegeneration by simultaneously preventing ROS accumulation and activating autophagy.

Accumulating evidence reveal that the histone demethylase LSD1 is an essential autophagy epigenetic regulator and that LSD1 depletion may induce autophagy in a range of different human cell lines.[12–14] Noteworthy, it has been shown that LSD1 and the ubiquitination factor E4 B (UBE4B) synergistically improve clearance of misfolded proteins, through the control of the p53-dependent transcription program. Knockdown of both UBE4B and LSD1 activates proteasomal degradation and autophagy and suppresses neurotoxicity associated with protein aggregates in *Caenorhabditis elegans*.[15] Furthermore, in NB cells, LSD1 negatively controls the expression of the molecular chaperone clusterin,[16] which is involved in the protein quality control system and reduction in misfolded proteins and aggregates under physiological conditions.[17] These additional layers of regulation suggest that LSD1 is a central hub in regulation of proteasomal and autophagic degradation machineries and that LSD1 inhibitors could represent a suitable pharmacological strategy against proteotoxicity.

An additional function has emerged for LSD1 in mammalian brain development and synaptic maturation. A neurospecific splice variant of LSD1, which contains the additional exon 8a, is expressed specifically during mammalian neuronal development.[18] This neuronal isoform does not have the intrinsic capability to demethylate H3K4me2 but demethylates the repressive mark H3K9me2, with its partner SVIL/supervillain, activating transcription of neuronal-specific genes during differentiation.[19] Knocking down specifically the LSD1+8a isoform compromises neuronal differentiation in vitro and, accordingly, LSD1+8a-deficient mice show cognitive deficits and impaired spatial learning. The alternative splicing mechanism regulating inclusion of exon E8a is fundamental for the regulation of specific expression programs that promote neuronal differentiation and is strictly regulated during mammalian brain development. Intriguingly, the coordinated regulation of both LSD1 and LSD1+8a splice variants could offer the opportunity to derepress autophagy-related genes and activate neuronal genes at the same time, providing a suitable therapeutic tool in neurodegenerative diseases.

More recently, Christopher et al[20] showed that an LSD1 total gene deletion in adult mice caused widespread hippocampus and cortex neurodegeneration. The mutant mice exhibited severe motor deficit, paralysis, and memory impairment along with increased stem cell gene (*Klf4*, *Myc*, and *Foxo1*) expression in the hippocampus. Based on gene ontology and gene set enrichment analysis of the RNA-Seq data, the authors found that loss of LSD1 induces common neurodegeneration pathways. Moreover, they demonstrated that LSD1 protein was aberrantly localized in the cytoplasm and associated with pathological protein aggregates in brains of patients with Alzheimer disease and frontotemporal dementia.

Collectively, the neuroepigenetic role of LSD1 is emerging and given its important role in neural homeostasis and plasticity, further understanding of LSD1 histone modifications function on its targets could be instrumental to develop novel therapeutic strategies to reprogram cell phenotype in brain cancers and neurodegenerative disorders.

Author Contributions

Both authors contributed equally to this work.

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