UNIVERSITY OF NAPLES
FEDERICO II

PHD IN MOLECULAR MEDICINE
AND **MEDICAL BIOTECHNOLOGIES**

XXXVI CYCLE

TARGETING GROUP3 MEDULLOBLASTOMA BY THE ANTI-PRUNE-1 AND ANTI-LSD1/KDM1A EPIGENETIC MOLECULES

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2020-2023

CHAPTER I

BACKGROUND

1. BACKGROUND

1.1.1 PRUNE-1

Human PRUNE-1 is the human homologue of the Drosophila Prune gene that was originally characterized in Drosophila mutant phenotypes and involved in eye development in a Drosophila model (Banfi et al., 1996). The Prune-1 protein (Fig. 1.1) belongs to the DHH protein superfamily, named after the characteristic Asp-His-His motif in the N-terminal region of member proteins. In the Prune-1 protein, the DHH domain is followed by a DHH2 domain. The C-terminal region of Prune-1 is involved in its homodimerization and in the interaction with several protein partners (Zollo et al., 2005; Marino and Zollo, 2007; Kobayashi et al., 2006). Moreover, the Prune-1 amino-acid sequence contains at least three putative domains and among these an inorganic pyrophosphatase/exopolyphosphatase domain, which is involved in energy production and conversion (Tammenkoski et al., 2008).

Figure 1.1. Prune-1 protein domains.

1.1.2 Prune-1 phosphodiesterase

Prune-1 has been shown to possess two activities: a nucleotide phosphodiesterase (PDE) activity (D'Angelo et al., 2004), and the exopolyphosphatase activity (Tammenkoski et al., 2008). Prune-1 has a PDE activity, with a preferential affinity for cAMP over cGMP as substrates, with Km values of 0.9 ± 0.03 and 2.3 ± 0.11 M, respectively (D'Angelo et al., 2004). It catalyzes the hydrolysis of 3',5'-cyclic nucleotides to their corresponding nucleoside 5'-monophosphates. Cyclic nucleotide second messengers have central roles in signal transduction and in the regulation of physiological responses, thus PDEs have pivotal roles in maintaining cellular homeostasis (Essayan, 2001).

1.1.3 Prune-1 exopolyphosphatase

The second known activity of Prune-1 is as an exopolyphosphatase (polyphosphate-phosphohydrolases). The exopolyphosphatases are proteins that hydrolyze inorganic

polyphosphate into inorganic phosphate (Pi) (Kulakovskaya and Kulaev, 2013), and are among the major enzymes of polyphosphate metabolism, an important source of the energy for the cell. Prune-1 exopolyphosphatase activity was shown to be greater than its PDE activity by three orders of magnitude. Prune-1 is the exopolypolyphosphatase in animals and is evolutionarily close to eukaryotic PPXs (Tammekoski et al. 2008). Specific reduction of mitochondrial polyP, by S. cervevisiae polyphosphatase scPPX1 expression, significantly modulates mitochondrial bioenergetics (Pavlov et al., 2010). Nakamura et al. (2018) explored the role of polyP in metabolism by producing transgenic (TG) mice widely expressing exopolyphosphatase scPPX1. In female TG mice, ATP storage in liver and brain tissues was reduced significantly (Nakamura et al., 2018).

Prune-1 is highly expressed in the central nervous system during fetal development and plays a crucial role in cell migration and proliferation, with proteins involved in cytoskeletal rearrangement (Wu et al., 2023; Bibbò et al., 2021; Zollo et al., 2017). Patients with loss of phosphatase activity of Prune-1 showed delayed myelination, thin corpus callosum, white matter abnormalities mild frontal cerebral atrophy and prominent cerebellar atrophy (Scorrano et al., 2023), demonstrating the importance of its correct dosage for brain pathophysiology.

1.1.4 Prune-1 protein interaction with binding partners

Prune-1 is a naturally unfolded multi-domain adaptor protein. The three-dimensional structure of the carboxyl- terminus (C-terminus) region of Prune-1 was obtained through nuclear magnetic resonance (NMR) studies and molecular dynamics techniques (Carotenuto et al., 2013). Two globular regions responsible for its enzymatic activities (DHH and DHHA2 domains) were found within the amino-terminus (N-terminus) domain of Prune-1 (Carotenuto et al., 2013). The DHHA2 domain also contained a conserved DXK motif at its N-terminus. An intrinsically disordered domain (starting at 371 amino acid residue) containing two stretches with a tendency for helix structures was also identified. The C-terminal domain of Prune-1 is characterized by residues (postulated to reside between 393 and 420) that are involved in its homodimerization and a small globular region responsible for its interactions with other proteins (Middelhaufe et al., 2007; Marino and Zollo, 2007). In fact, Prune-1 was found with the ability to bind several intracellular protein partners mainly involved in cytoskeletal rearrangement, including α- and β-tubulins,

glycogen synthase kinase 3β (GSK-3β) (Kobayashi et al., 2006), and Nm23-H1/H2 (or NDPK-A/B) (D'Angelo et. al, 2004). Through these interactions, Prune-1 can modulate different intracellular pathways that might be then also translated into extracellular signaling, including the canonical WNT via GSK-3β (Carotenuto et al., 2014) and TGF-β via Nm23-H1 binding (Ferrucci et al., 2018 I).

1.1.5 Prune-1 interaction with GSK-β

The region of Prune-1 responsible for the interaction with GSK- 3β starts from 330 to 394 amino acid residues within the C- terminus domain (Fig. 1.2) (Carotenuto et al., 2014; Kobayashi et al., 2006). Because of the co-localization between Prune-1 and the focal adhesion proteins (F-actin, paxillin, and vinculin) in several cell types, this Prune-1–GSK-3β interaction suggests that Prune-1 can also modulate the microtubular architecture and dynamics (Kobayashi et al., 2006; Garzia et al., 2006; Garzia et al., 2008). Importantly, GSK-3β is considered a negative modulator of the canonical WNT/β-catenin pathway through phosphorylation at its amino acid residues S9 and S21 (Carotenuto et al., 2014; Diana et al., 2013). Of importance is that, through GSK-3β interaction, Prune-1 was found to activate β-catenin signaling cascade and to further promote Wnt3a secretion in non-small cell lung cancer (NSCLC) cells (Carotenuto et al., 2014); thus, a connectome related to the WNT signaling activation was identified (Freeman et al., 2015).

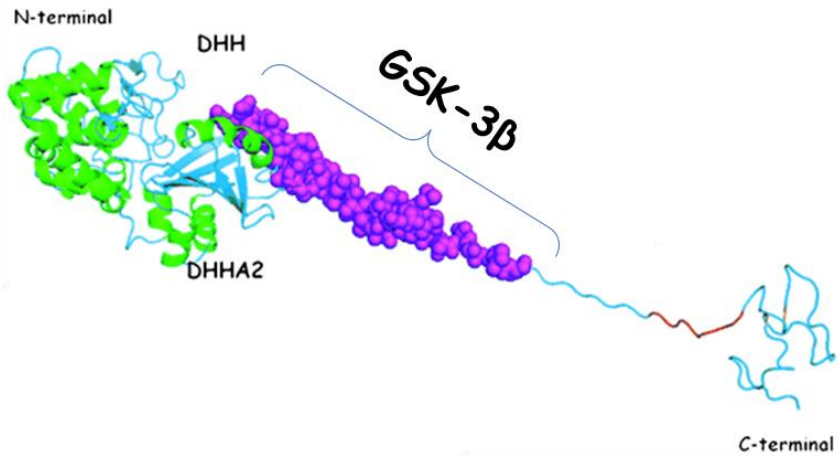


Figure 1.2. Prune-1 interaction with GSK-3β (Diana D. et al., 2013).

1.1.6 Prune-1 interaction with NME-1 (Nm23-H1)

Prune-1 was also shown to physically interact with the C- terminus domain of Nm23-

H1 through its D388 and D422 residues (Fig. 1.3) (Carotenuto et al., 2013). Nm23-H1 is described as the first anti-metastatic gene with different functions: a nucleoside diphosphate kinase (NDPK) activity that catalyzes the transfer of a phosphoryl group from a nucleoside triphosphate (NTP) to a nucleotide diphosphate (NDP), a histidine kinase function, and a 3′−5′ exonuclease activity (Ferrucci et al., 2018 II). In detail, in vitro studies demonstrated that the complex formation involved the dimer of Prune-1 and the hexameric form of Nm23-H1 (Middelhaufe et al., 2007). Interestingly, Nm23-H1 and GSK-3β could bind Prune-1 at the same time by using non-identical regions of its C-terminus domain for their binding, thus suggesting two independent interaction sites for signaling complex assembly (Middelhaufe et al., 2007). Of importance the overexpression of both Prune-1 and Nm23-H1 was found in different tumors, such as MB (Ferrucci et al., 2018 I). In this regard, the data indicate that the Prune-1/Nm23-H1 complex could affect the anti-metastatic activity of Nm23-H1 and increase the cell motility properties (Zollo et al., 2005; Galasso et al., 2009).

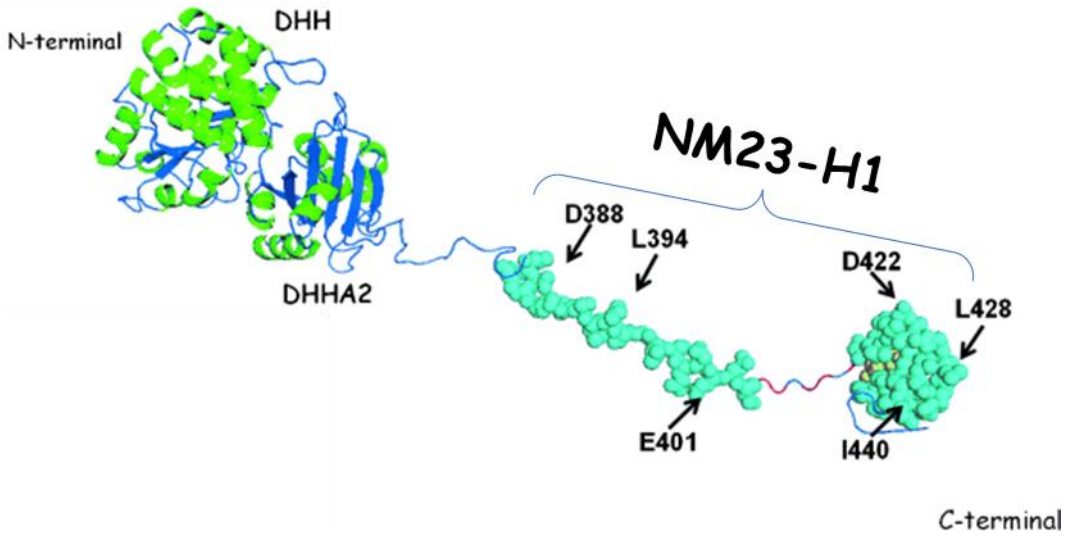


Figure 1.3. Prune-1 interaction with NM23-H1 (Diana D. et al., 2013).

1.1.7 Prune-1 interaction with tubulins

Other potential Prune-1 interactors were identified. Among these, the interaction between Prune-1 and β-tubulin was proposed because of the crucial functions played by tubulins in neural development (Garzia et al., 2006; Bahi-Buisson et al., 2014; Siskos et al., 2021).

1.1.8 Prune-1 in tumorigenesis

To date, high expressions of Prune-1 have been found in several metastatic solid tumors: Group3 (Gr3) and Group4 (Gr4) Medulloblastoma (MB) (Ferrucci et al., 2018 I), gastric cancer (GC) (Oue et al., 2007), esophageal squamous cell carcinoma (ESCC) (Noguchi et al., 2009), NSCLC (Carotenuto et al., 2014), thyroid cancer (TC) (Nambu et al., 2016), colorectal cancer (CRC) (Hashimoto et al., 2016), neuroblastoma (NBL) (Carotenuto et al., 2013), breast cancer (BC) and metastatic triple-negative breast cancer (TNBC) (D'Angelo et al., 2004; Zollo et al. 2005; Ferrucci et al., 2021). Prune-1 overexpression occurs via the amplification and/or gain of chromosome 1q (as identified in tumors of epithelial origin), where PRUNE-1 is located (1q21.3) (D'Angelo et al., 2004). For instance, Gr3 MB (γ-subtype), in which Prune-1 was found overexpressed, has a trend for gain of chromosome 1q (Cavalli et al., 2017).

1.2.1 Medulloblastoma (MB)

MB is an embryonal tumor occurring in the developing cerebellum and represents ∼20% of all primary childhood CNS tumors (Gajjar et al., 2004). Recent integrative genomics, RNA expression, and methylation profiling have allowed MB to be stratified into different molecular subgroups: Wingless (WNT), Sonic Hedgehog (SHH), Group3 (Gr3), and Group4 (Gr4) (Cavalli et al., 2017). Heterogeneity within these subgroups has been recognized, and it has been suggested that MB may consist of up 12 subtypes [WNT (α and β), SHH (α, β, γ, and δ), Group3 (α, β, and γ) and Group4 (α, β, and γ)] (Cavalli et al., 2017). Distinctive transcriptional, mutational, and epigenetic profiles were reported for each MB subtype, with different clinical features (Cavalli et al., 2017). The SHH subgroup is characterized by an aberrant activation of the SHH pathway and arises from cerebellar granule neuron progenitors (GNPs) mainly mutated in PTCH1 or SMO receptors (Cavalli et al., 2017). Although ∼60% of MB belongs to Gr3 and Gr4, future studies are needed to clarify the developmental origins and the biological mechanisms of these latest subgroups (Cavalli et al., 2017). Gr3 MB is considered the most aggressive subgroup because of the high metastatic potential and the poor survival rate. The amplification of c-MYC is a common genetic feature in Gr3 MB patients that was found to be inversely correlated with the clinical outcomes (Nortchott et al., 2011). One-third of Gr4 MB patients are diagnosed with

metastasis and show recurrent alterations in the genes involved in chromatin modification (Kool et al., 2012). Furthermore, amplifications of the MYCN and cyclin-dependent kinase 6 (CDK6) genes are alterations more commonly found in Gr4 MB (Sharma et al., 2019). Of interest genomic profiling studies have recently reported a strong heterogeneity in Gr3 and Gr4 MB in terms of molecular and clinical features, thus showing a subset of tumors with overlapping signatures (Hoverstadt et al., 2019). The impact of this heterogeneity on therapy has been limited to trials testing Smoothened (SMO) antagonists for patients with SHH MB (Robinson et al., 2015) and efforts to reduce therapy for those children affected by the WNT MB (Goschzik et al., 2018), who have relatively good outcomes. Tailored therapies for Gr3 and Gr4 MB are currently lacking, and the combination of surgery, craniospinal radiotherapy (except in young children for whom radiotherapy has devastating neurocognitive side effects), and multi-agent chemotherapy is used (Gajjar et al., 2014). Although recently Michalski et al. carried out a phase III trial of reduced-dose and reduced-volume radiotherapy with chemotherapy for newly diagnosed average-risk MB (Michalski et al., 2021), the molecular drivers of metastatic dissemination in MB remain elusive. Recently, we highlighted a new metastatic axis (independent of c-MYC amplification) in Gr3 MB. Here we found that Prune-1 enhances the canonical TGF-β pathway followed by OTX2 up-regulation and PTEN down-regulation (Ferrucci et al., 2018). OTX2 maintains rhombic lip (RL) identity by inhibiting the CBFA complex until cells exit the RL and differentiate (Hendrikse et al., 2022). Overexpression of OTX2 results in failed RL differentiation. The resulting ball of RL progenitor cells are retained with the RL in the nascent nodulus of the developing cerebellum, where ongoing mitotic activity eventually results in a mass lesion diagnosed as Gr3 or Gr4 MB (Hendrikse et al., 2022). Recently, high expressions of Prune-1 were found in these metastatic MB subgroups (Gr3 and Gr4 MB), and a new metastatic axis (independent of c-MYC amplification) was dissected in Gr3 MB with the poorest prognosis. In this regard, Prune-1 protein, due to the binding to Nm23-H1, enhances the canonical TGF-β pathway, thus leading to the increase of OTX2 expression and the reduction of PTEN (Ferrucci et al., 2018 I). Furthermore, gene expression and gene ontology analyses allowed other genes (OTX2, CYFIP1, and GLI2) involved in neurogenesis to be correlated to Prune-1 (Ferrucci et al., 2018 I). Interestingly, a cell competitive permeable peptide (cell-penetrating peptide, CPP) that impairs Prune-1/Nm23-H1 complex formation was found to impair both tumorigenesis and metastatic spread in

orthotopic models implanted with metastatic Gr3 MB cells overexpressing c-MYC and mutated for TP53 (D425-Med cells), thus representing the most aggressive type of MB with the poorest prognosis (Hill et al., 2015). Furthermore, an anti-Prune-1 molecule (pyrimido-pyrimidine derivative, AA7.1) (Virgilio et al., 2012) was also found with the ability to bind Prune-1 protein, to enhance its intracellular degradation and to increase the PTEN protein level, thus inhibiting the Prune-1-mediated metastatic network both in vitro in primary human medullospheres obtained from patients and also in vivo in orthotopic xenograft models of metastatic Gr3 MB (Ferrucci et al., 2018 I).

1.2.2 Epigenetics in MB: DNA methylation and histone modifications

MB tumorigenesis has been reported with predominant epigenetic alterations consisting of large hypomethylated chromosomal regions that cause increased gene expression (Pugh et al., 2012; Hovestadt et al., 2014). It is estimated that more than 30% of MB samples, depending on the subgroup, are mutated in those genes encoding for epigenetic regulators, thus suggesting that epigenetic alterations are a very important part of MB progression. Each MB subtype may require different sets of chromatin remodelers and histone modifiers driving different transcription programs (Yi and Wu, 2018). The epigenetic regulators that mostly affect MB are DNA methylation and histone modifications (Fig. 1.4) and in the following chapter we will discuss further.

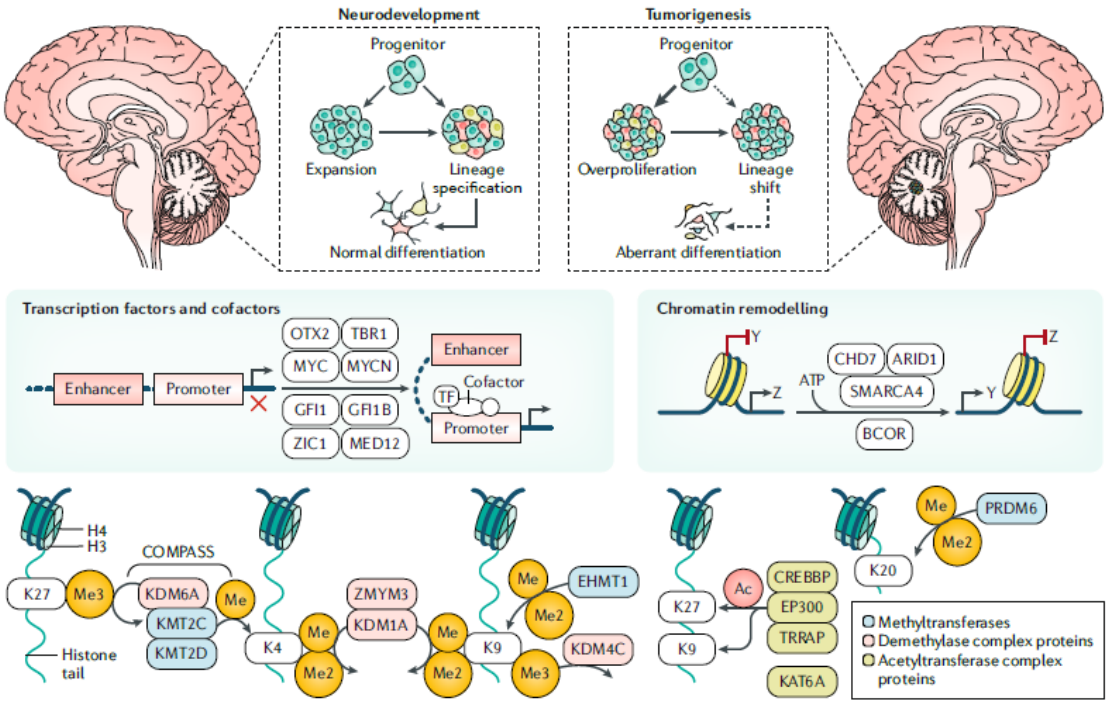


Figure 1.4. Epigenetic regulators in MB tumorigenesis (Northcott et al., 2019).

1.2.3 Histone modifications

The complex network of histone modifications including acetylation, methylation, phosphorylation, and ubiquitination alters histone chromatin structure locally into the genome by recruiting protein effectors which are known to control the genetic transcription machinery. Numerous studies have found alteration of the genes that code for the enzymes responsible for the epigenetic modification of histone proteins in the various MB subgroups (Northcott et al., 2009). In particular, Yi et al. identified homozygous deletions and recurrent focal amplifications in genes responsible for the methylation/demethylation of lysine at position 9 of histone 3 (H3K9): L3MBTL3, L3MBTL2 and SCML2 (Polycomb proteins), EHMT1 and SMYD4 (methyltransferase), and JMJD2B and JMJD2C (demethylases). Confirming this finding, the 40% of MB samples show lower global H3K9me3 levels than normal (Yi and Wu, 2018). Other important sites for chromatin regulation involve the methylation/demethylation of H3K4 and H3K27. In this regard, MLL2/KMT2D and MLL3/KMT2C complexes are required to maintain H3K4me1 levels in enhancers (Rao and Dou, 2015; Sze and Shilatifard, 2016), thereby modulating enhancer activities during development and in cancer. In 16% of MB with recurrences in SHH and Gr4 subgroups, inactivating mutations in MLL2/KMT2D and MLL3/KMT2C

(two lysine methyltransferases that enhance H3K4me2/3) were found, thus suggesting these genes could act as tumor suppressors in MB (Roussel and Stripay, 2018). Histone/protein lysine demethylases (KDMs), which take away the methyl group (s) from a methylated lysine side chain, dynamically regulates histone methylation.

The UTX/KDM6A protein is a specific demethylase of the repression marker H3K27me3 which interacts with the MLL2/ 3 complexes (specific H3K4) (Cho et al., 2007; Lee et al., 2007). In fact, the two subunits MLL2/3 and UTX can destabilize the epigenetic structure by methylating H3K4 and demethylating H3K27me3 mutually exclusive in MB (Dubuc et al., 2013). It is additionally known that 4% of MBs have homozygous mutations or deletions of UTX/KDM6A usually in male patients but are also present in female patients in case they have lost an X chromosome (Xp11.3), with a greater enrichment in Gr4 tumors (Dubuc et al., 2013; Jones et al., 2013).

Currently, numerous irreversible LSD1/KDM1A inhibitors have been identified and among them tranylcypromine (TCP), ORY-1001 and SP-2509 are now evaluated in clinical trials as potential cancer treatments (Fang et al., 2019; Liang et al., 2020). Recently, LSD1 has shown to have a crucial role in the GFI1-mediated transformation of MB by binding to GFI1. Pharmacological inhibition of LSD1/KDM1A with ORY-1001 successfully inhibited the growth of GFI1-driven tumors, suggesting therapeutic potentials of LSD1 inhibitors in GFI1-driven MB (Lee et al., 2019). Moreover, it has been demonstrated that SP-2509 hindered LSD1/KDM1A enzymatic activity rather than disabling the CoREST- LSD1 complex's protein-protein interactions. By directly inhibiting LSD1/KDM1A, SP-2509 was able to stop the growth of several human MB cell lines (DAOY, D283-Med, and ONS-76) (Inui et al., 2017).

1.3.1 LSD1/KDM1A

LSD1/KDM1A was the first histone demethylase discovered. It specifically demethylates specific lysine residues of histone H3, H3K4me1/2 and H3K9me1/2, using FAD as the cofactor (Shi et al., 2004), behaving as either a repressor or activator of gene expression, respectively (Rotili and Mai, 2011). When methyl groups are removed from mono and di-methylated H3K4, LSD1/KDM1A is known to repress gene expression (Shi et al., 2004). Conversely, it has been reported to activate gene transcription by demethylating H3K9 in complex with nuclear hormone receptors (Metzger et al., 2005).

1.3.2 Structure of LSD1/KDM1A

The human LSD1/KDM1A protein is 852 aa long and consists of several domains (Fig. 1.5) (Shi et al, 2004; Forneris et al., 2005; Forneris et al., 2006; Forneris et al., 2009):

- A N terminal domain, composed of the SWIRM domain consisting mainly of α helices and of an unstructured region made of linear motifs that could represent functional sites responsible for the association LSD1/KDM1A with different transcriptional complexes;
- A central tower domain consists of two long anti-parallel α helix with a typical coiled coil conformation, which offers a surface platform for interaction with partners. This domain is essential both for demethylase activity and for interaction with the CoREST co-repressor;
- A C terminal domain includes the amino oxidase domain (AO), which shares a homology with the FAD dependent AO domains.

Close interactions between the SWIRM domain and amine oxidase domain through an extensive hydrophobic interface, forming a highly conserved cleft in the vicinity of the active site may serve as an additional histone tail-binding site. The tower domain, inserted into the AOL domain with a long helix-turn-helix structure, comprises binding spots for interacting proteins such as CoREST (co-repressor for RE-1 silencing transcription factor), CtBP1 (carboxyl-terminal binding protein 1), HDAC1/2, and Snai1. The corepressor CoREST-binding domain is indispensable for the histone demethylase activity of LSD1/KDM1A, where a deletion mutant (LSD1ΔTower) replaced by a pentaglycine loop elicits an inability to reduce methylation of H3K4 (Hosseini A. and Minucci S., 2017).

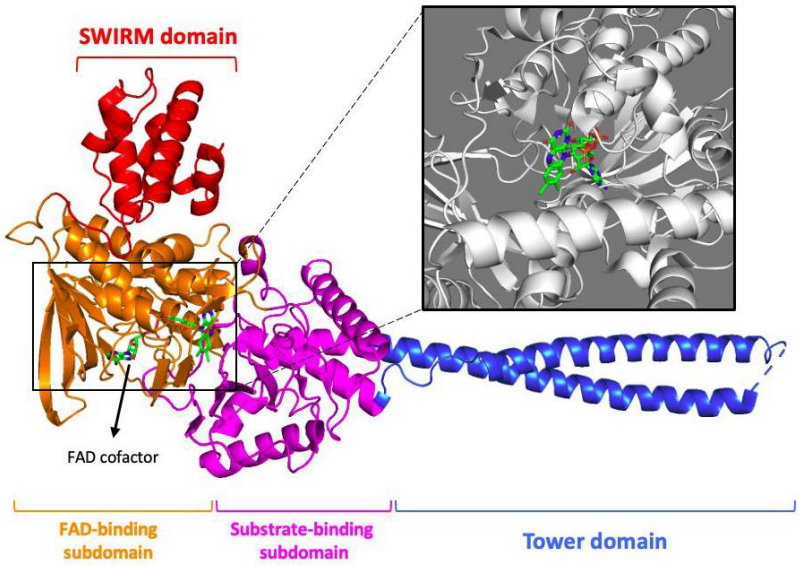


Figure 1.5. Lysine specific demethylase 1 (LSD1/KDM1A) protein domains and structure (Lee at al., 2023).

The aspartic acid 375 and the glutamic acid 379 of the AO domain build an interaction with Arg8 of the histone H3, while the glutamic acids in position 553, 555, and 556 interact with Arg2 of histone H3. Mutation of these critical residues abolish LSD1 catalytic activity on demethylation in H3K4 (Lin et al., 2010).

1.3.3 LSD1/KDM1A reaction mechanism

LSD1/KDM1A is classified a flavin-dependent monoamine oxidase (MAO) using the flavin dinucleotide (FAD) cofactor during demethylation catalysis (Forneris et al., 2006). During the reaction, single-electron oxidation of the mono- or di-methylated amine at the ε-position of lysine gives an iminium cation with simultaneous reduction of FAD to $FADH_2$. After $FADH_2$ reoxidation to FAD by O_2, H_2O_2 is generated. The iminium cation is unstable and after easy hydrolysis furnish the amine with one methyl less and formaldehyde as by-product (Fig. 1.6) (Dai et al., 2021).

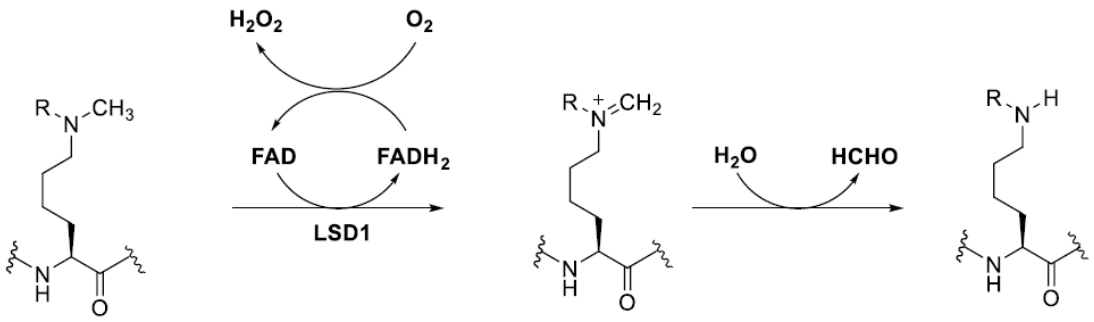
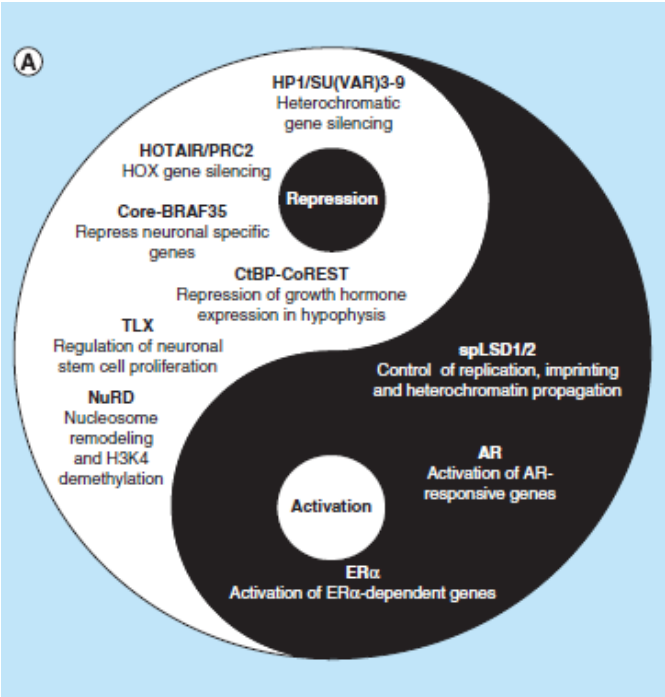


Figure 1.6. Catalytic mechanism of histone demethylation by LSD1/KDM1A (Noce et al., 2023).

1.3.4 Biological and pathological functions of LSD1/KDM1A

LSD1/KDM1A mediates many cellular signaling pathways (Bennesch et al., 2016; Shao et al., 2015; Huang et al., 2013) and plays critical roles in regulating fundamental cellular processes (Fig. 1.7A) (Maiques-Diaz et al., 2013; Amente et al., 2013; Wu et al., 2014). The diverse biological roles of LSD1/KDM1A may explain why its dysfunction is associated with initiation and development of several diseases such as stem cell differentiation, neurodegenerative diseases, cardiovascular diseases, viral infections, inflammation and cancers (Fig. 1.7B) (Stazi et al., 2016). Aberrant overexpression of LSD1/KDM1A has been observed in various human cancer cells (Fig. 1.7C) and is closely associated with differentiation, proliferation, migration, invasion and poor prognosis (Ly et al., 2012; Hojfeldt et al., 2013; Przespolewski et al., 2016; Ding et al., 2013).



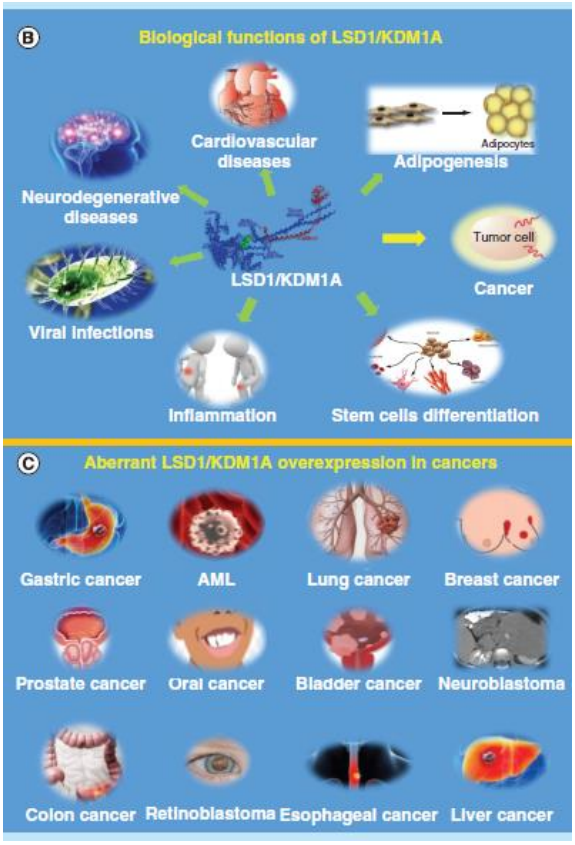


Figure 1.7. (A) LSD1/KDM1A-mediated biological processes; (B) Types of disease where LSD1/KDM1A is involved; (C) Cancer types where LSD1/KDM1A is aberrantly overexpressed (Fu et al., 2017).

1.3.5 Mechanism of transcriptional regulation of target genes

LSD1/KDM1A mainly functions as a transcription regulator protein that can either repress or activate the transcription process depending upon its association with different complexes and protein partners. The binding of LSD1/KDM1A with different multi-component complexes and proteins determines the specificity of LSD1/KDM1A towards its target histones. Methylated H3K4 residues act as transcription activator markers, and LSD1/KDM1A as a component of CoREST and NuRD complex actively demethylates H3K4 residues and serves the role of a transcription co-repressor (Fig. 1.8A). Similarly, in the case of methylated H3K9 residues that act as transcription repressor markers, LSD1/KDM1A in association with androgen (AR) and estrogen (ER) receptor actively demethylates H3K9 residues and act as transcription co-activator (Fig. 1.8B) (Metzger et al., 2005).

Different roles and specificities of the tissues have been attributed to the splicing variant of LSD1/KDM1A (LSD1+8a), within the domain AO, containing four

additional amino acids (exon 8a) (Hwang et al., 2018). The presence of exon 8a generates a docking site for supervillain (SVIL) that converts LSD1/KDM1A into an H3K9demethylase in neuronal differentiation (Zibetti et al, 2010; Laurent et al., 2015). Furthermore, LSD1+8a has also been described to promote transcription of genes regulated by neurons removing H4K20me2 (Fig. 1.8C) (Wang et al., 2015).

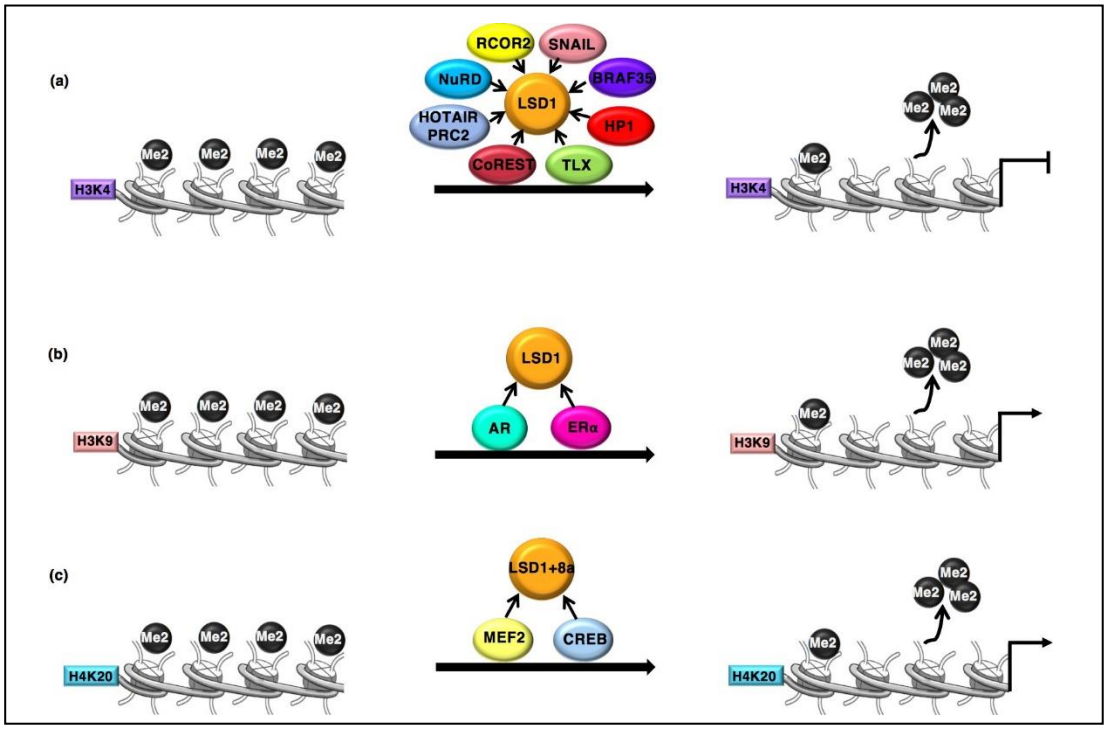


Figure 1.8. (A) LSD1/KDM1A is recruited at the target gene by a wide number of transcription factors (indicated in figure). (B) LSD1/KDM1A demethylates as coactivator with the androgen or estrogen receptor. (C) LSD1+8a interact with CREB and MEF2, catalyzes the demethylation of the repressive mark H4K20me2 (Majello et al., 2019).

1.3.6 LSD1/KDM1A in cancer

The role of LSD1/KDM1A either as a transcription co-repressor or co-activator has its significance in promoting cancer progression. LSD1/KDM1A utilizes different binding partners in different cancer types to promote its expression. In particular, the role of LSD1/KDM1A as an epigenetic modulator plays a crucial role in controlling the fate of epithelial-mesenchymal transition (EMT) in various human cancer cells. LSD1/KDM1A in association with Snail represses the expression of epithelial marker CDH1 and contributes to the progression of cancer cells by promoting EMT (Fig. 1.9A) (Lin et al., 2010). LSD1/KDM1A, along with ERRα, promotes EMT by active demethylation of H3K9 residues of pro-invasive genes (Fig. 1.9B) (Ambrosio et al., 2017 I; Carnesecchi et al., 2017). LSD1/KDM1A promotes

EMT by repressing metastatic suppressor gene NGRG1 by forming a complex with MYCN (Fig. 1.9C) (Ambrosio et al., 2017 II). LSD1/KDM1A, in association with the NuRD complex, inhibits EMT by repressing TGF-β genes (Fig. 1.9D) (Li et al., 2011). LSD1/KDM1A in complex with UTX and HDAC1 represses the expression of transcription factors (Zeb1, Zeb2) that promote EMT (Fig. 1.9E) (Choi et al., 2015).

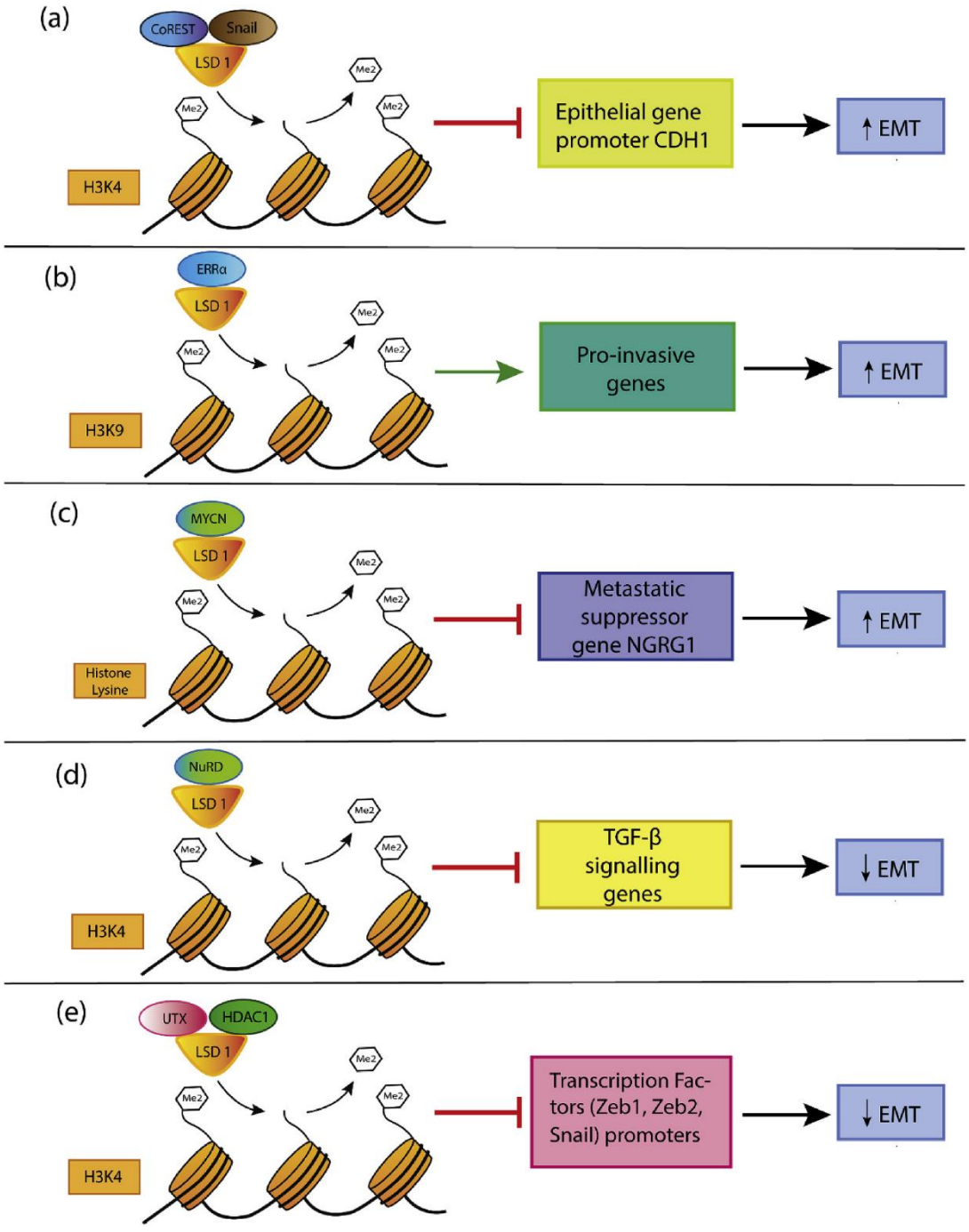


Figure 1.9. (A) LSD1/KDM1A in association with Snail contributes to EMT. (B) LSD1/KDM1A with ERRα actives transcription of pro-invasive genes. (C) LSD1/KDM1A promotes EMT by repressing metastatic suppressor gene NGRG1. (D) LSD1/KDM1A, in association with the NuRD complex,

inhibits EMT. (E) LSD1/KDM1A in complex with UTX and HDAC1 represses the expression of transcription factors that promote EMT.

1.3.7 LSD1/KDM1A in the crossroads of cancer epigenetics and immune checkpoint

LSD1/KDM1A was found negatively correlated with the expression of CD8+ on T cells and positively correlated with that of PD-L1 (Shen et al., 2022). Shen et al. (2022) observed also that LSD1 inhibited the response of T cells in the TME of gastric cancer by inducing the accumulation of PD-L1 in exosomes (Shen et al., 2022). Recently, it has been shown that LSD1/KDM1A inhibition in triple negative breast cancer cell lines induces expression of CD8+ T cell-attracting chemokines, including CCL5, CXCL9, and CXCL10 (Qin et al., 2019). Soldi et al. investigated the ability of LSD/KDM1A inhibitor SP-2577 to promote anti-tumor immunity and T-cell infiltration in small cell carcinoma of the ovary hypercalcemic type (SCCOHT) and ovarian clear cell carcinomas (OCCC) cell lines. They found that SP-2577 stimulated IFN-dependent anti-tumor immunity in SCCOHT and promoted the expression of PDL1 in both SCCOHT and OCCC (Soldi et al., 2020).

A critical role for epigenetic changes is emerging in regulating T-cell exhaustion (Fig. 1.10), with critical implications for immuno-oncology. T-cell exhaustion is a phenotypic adaptation of T-cells thought to arise as consequence of chronic antigen exposure and T-cell receptor signaling (Moskophidis et al., 1993). Elevated LSD1/KDM1A levels are reported to be a major contributor to the exhausted T cell phenotype (Tu et al., 2020), where targeting LSD1/KDM1A in exhausted T cells of immunotherapy-resistant mice noticed increased T cell effector functionality (corresponding to elevated IFN levels and greater T cell infiltration) (Lee et al., 2023).

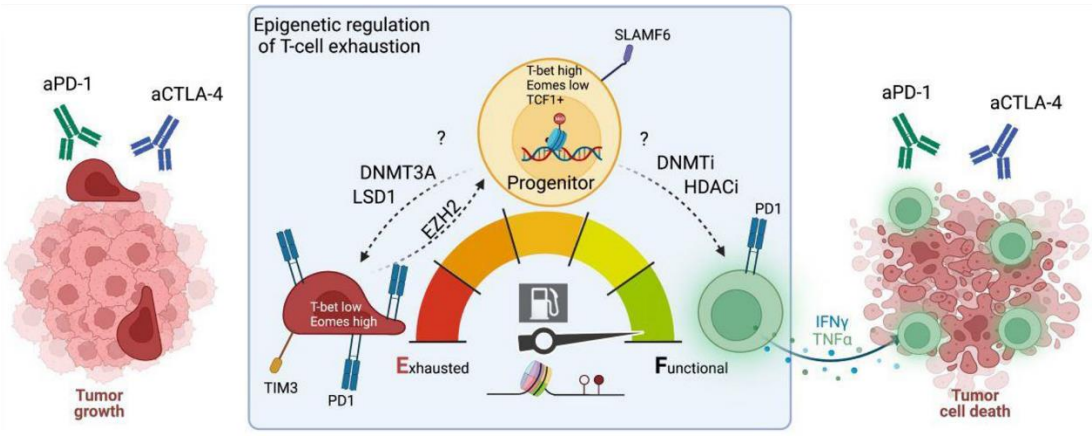


Figure 1.10. Exhausted T-cells, marked by TIM3, have an epigenetic landscape that limits their ability to respond to immune checkpoint blockade. The main epigenetic drivers are DNMT3A, TET2, LSD1 (Micevic et al., 2023).

1.3.8 LSD1/KDM1A inhibitors

There are several pharmaceutical LSD1/KDM1A inhibitors. They can be divided into synthetic, metal complexed and natural (Fig. 1.11).

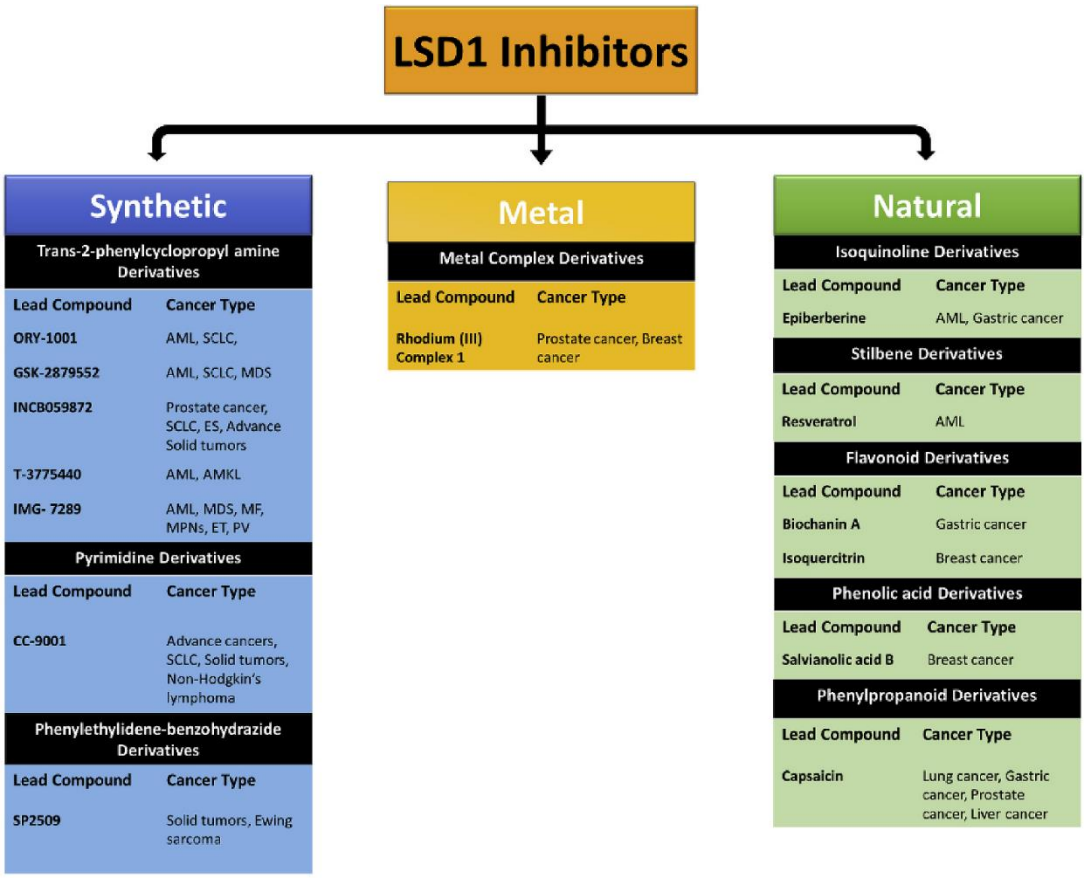


Figure 1.11. Schematic representation of classification of LSD1 inhibitors (Dong et al., 2022).

A host of classical amine oxidase inhibitors such as TCP, pargyline, and phenelzine together with LSD1/KDM1A tool compounds such as SP-2509 and GSK-LSD1 have been extensively utilized in LSD1 mechanistic cancer studies. Several optimized new chemical entities have reached clinical trials in oncology such as ORY-1001 (Iadademstat), GSK2879552, SP-2577 (Seclidemstat), IMG-7289 (Bomedemstat), INCB059872, and CC-90011(Pulrodemstat) (Sacilotto et al., 2021). To date, six TCP-based LSD1/KDM1A inhibitors, including TCP, GSK2879552, IMG-7289, ORY-1001, INCB059872, and ORY-2001 have entered clinical trials for the treatment of

AML and SCLC (Fang et al., 2020, Dai et al., 2020). Also, the reversible LSD1/KDM1A inhibitors CC-90011 and SP-2577 have recently entered the clinical trials (Mould et al., 2015, Dai et al., 2021).

Seclidemstat (SP-2577) is a selective and reversible inhibitor LSD-1/KDM1A currently being evaluated in combination with AZA for MDS/CMML following HMA-failure in phase 1/2 study (NCT04734990) (Sacilotto et al., 2021).