

Review

# Lysine-Specific Histone Demethylases Contribute to Cellular Differentiation and Carcinogenesis

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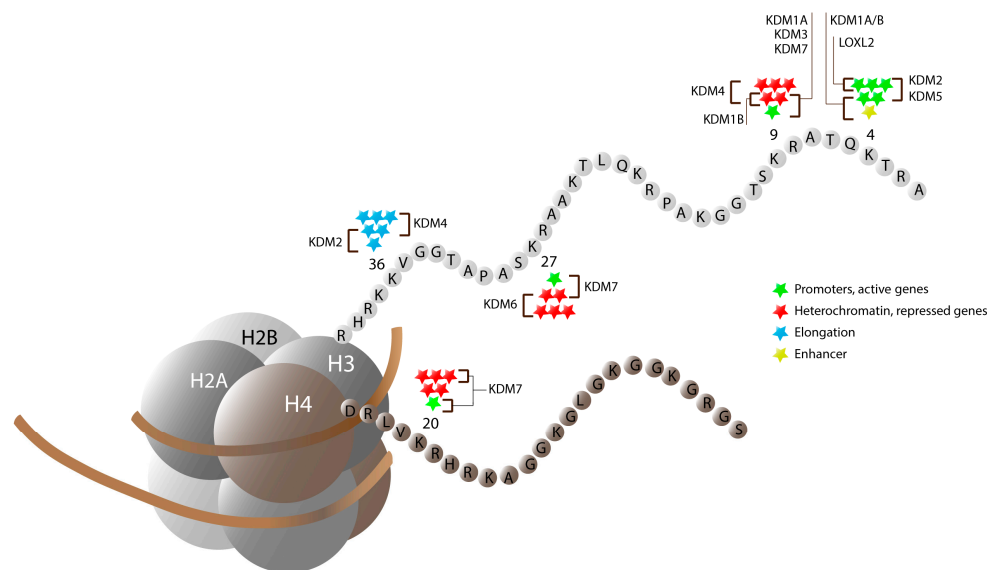
**Abstract:** Histone modifications regulate chromatin structure, gene transcription, and other nuclear processes. Among the histone modifications, methylation has been considered to be a stable, irreversible process due to the slow turnover of methyl groups in chromatin. However, the discovery of three different classes of lysine-specific demethylases—KDM1, Jumonji domain-containing demethylases, and lysyl oxidase-like 2 protein—has drastically changed this view, suggesting a role for dynamic histone methylation in different biological processes. In this review, we describe the different mechanisms that these enzymes use to remove lysine histone methylation and discuss their role during physiological (cell differentiation) and pathological (carcinogenesis) processes.

**Keywords:** epigenetics; histone demethylases; cellular differentiation; cancer

## 1. Introduction

Histone N-terminal tails can be post-translationally modified with different chemical groups, including methylation. Histone modifications play key roles in chromatin structure and can influence different DNA processes, such as gene transcription, DNA repair, replication, and recombination. Histones can be methylated on both lysine (K) and arginine (R) residues; however, methylation is most frequently observed on lysine residues of the H3 and H4 histone tails [1]. In contrast to other histone modifications, how histone lysine methylation affects gene transcription is strongly dependent upon which amino acid of the peptide is modified. Thus, methylation of lysine 27 (H3K27) or lysine 9 (H3K9) on histone H3, or of lysine 20 on histone 4 (H4K20), is associated with silenced chromatin [2,3]. In contrast, methylation of lysine 4 on histone 4 (H3K4) is primarily associated with gene activation [4], and methylation of lysine 36 on histone H3 (H3K36) generally regulates transcriptional elongation [5]. Moreover, so-called bivalent promoters contain both active (H3K4me3) and repressive (H3K27me3) histone methylation [5]. Genes controlled by bivalent promoters, like the *Hox* genes, are in a poised intermediate state in stem cells. They can be activated or repressed during stem cell differentiation by removing H3K27me3 or H3K4me3, respectively [5]. It was long believed that methylation is an irreversible modification, since the half-lives of histones and the methyl-lysine residues within them are the same [6,7]—in other words, if it is an irreversible modification, the methyl group would be “removed” by natural histone turnover or by dilution after DNA replication. However, during the last decade, different active lysine demethylation mechanisms have been identified. Three different classes of lysine-specific demethylases catalyze these mechanisms: the lysine (K)-specific demethylase

1 (KDM1A-B, also known as LSD1-2), the Jumonji C domain-containing demethylases (KDM2–7; also known as JHDM), and lysyl oxidase-like 2 (LOXL2). Each of these enzyme groups specifically removes one or more lysine histone methylation groups and plays a key role in gene transcription (Figure 1).



**Figure 1.** Lysine-specific histone methylation and the activity of histone demethylases. Schematic representation of H3 and H4 histone tails, showing the most frequent lysine residues involved in mono-, di-, and trimethylation (stars). The lysine-histone demethylases KDM1A/B, JHDM clusters (KDM2–KDM7), and LOXL2, involved in demethylation of specific methyl markers, are shown.

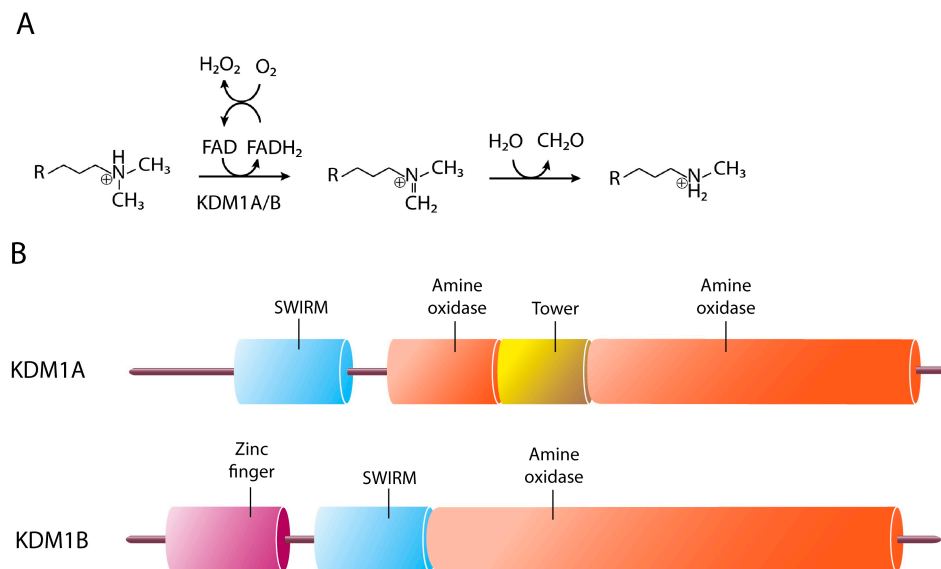
In this review, we describe the mechanisms that these enzymes use to remove the lysine histone methylation and discuss their roles during both cell differentiation and carcinogenesis.

## 2. Lysine-Specific Demethylase 1

### 2.1. KDM1A

The first histone demethylase to be discovered was KDM1A (also known as LSD1), which removes methylation from mono- and dimethylated lysine 4 (H3K4me1/me2) and lysine 9 of histone 3 (H3K9me1/me2) [8,9]. Based on its sequence analysis, KDM1A is classified as a flavin-dependent monoamine oxidase (MAO) homolog, and it uses the cofactor flavin adenine dinucleotide (FAD) during demethylation catalysis [8,10]. The chemistry of the reaction follows the classical amine oxidase scheme: molecular oxygen is consumed during methyl removal to produce formaldehyde and hydrogen peroxide. Two electrons are transferred from the methyl carbon of the dimethylated residue of the histone to the flavin in the form of a hydride anion. Molecular oxygen then reacts with the reduced FAD, generating hydrogen peroxide. The resulting imine-containing peptide is then hydrolyzed, releasing formaldehyde and the demethylated histone 3 [11,12] (Figure 2A). Structurally, KDM1A comprises four domains: an N-terminal disordered segment, a SWIRM (SWI3p, Rsc8p, and Moira) domain, an amine oxidase domain (AOD), and a Tower domain (Figure 2B). The N-terminal disordered segment contains about 150 amino acids and is subjected to post-translational modifications [13–16]. This domain is required for KDM1A nuclear localization and protein–protein interactions [17,18]. The SWIRM domain, comprising six  $\alpha$  helices and a  $3_{10}$  helix, contributes to the structural stability of KDM1A and, compressed against AOD, forms a groove that allows a larger interaction region with the histone 3 tail [11,19]. AOD contains the catalytic activity for the demethylation reaction and is divided into two different halves by the Tower domain. The two halves of AOD form a unique globular domain consisting of two lobes. The first lobe, comprising a six-stranded  $\beta$ -sheet and five  $\alpha$

helices, enables KDM1A to bind to its substrates [20]. The second lobe allows KDM1A to bind to the FAD cofactor and contains an expanded Rossmann fold, commonly found in dinucleotide binding modules [10,19]. Finally, the Tower domain, comprising two helices packed against each other in an antiparallel orientation, protrudes from the catalytic core of the protein. This domain is generally affected by chemical post-translational modifications and is required to allow KDM1A constitutive binding to the co-repressor CoREST1 [21]. The interaction between KDM1A and CoREST1 is essential for KDM1A-dependent demethylation activity [20].



**Figure 2.** Catalytic mechanism (A) and protein structures (B) of the KDM1 A/B. (A) The amine oxidation reaction used by KDM1A/B depends on flavin adenine dinucleotide (FAD) to demethylate mono- and dimethylated lysine. (B) Schematic representation of KDM1A/B protein structures. SWIRM: SWI3p, Rsc8p, and Moira domain.

## 2.2. KDM1A and Long Non-Coding RNA

Long non-coding RNAs (lncRNAs) are a heterogeneous group of RNAs ranging in length between 200 nucleotides to over 100 kb [22,23]. During the last decade, a high number of lncRNAs have been identified, many of which are the key regulators of gene transcription and therefore are required for the establishment of cell fate [24,25]. It was recently reported that at least four lncRNAs (Kcnq1ot1, Airn, Xist, and HOTAIR) regulate gene transcription by modifying the histone marks [26–28]. Consistent with these data, many chromatin modifiers contain RNA-binding domains rather than DNA-binding domains [29,30]. KDM1A can associate with different lncRNAs (TERRA, HOTAIR, and steroid receptor-RNA activator, SRA), suggesting a regulatory role for RNA in KDM1A function [28,31]. Interestingly, some lncRNA are able to simultaneously associate with more than one histone modifiers. The lncRNA HOTAIR, for example, has two different domains that bind to the KDM1A/CoREST complex and to the polycomb repressive complex 2 (PRC2), which trimethylates H3K27 [28]. This interaction may be required for coordinating the removal of activating marks by KDM1A/CoREST and the addition of repressive marks by PRC2 on histone. Genome-wide binding analysis reveal that KDM1A and PRC2 co-localize at specific sets of genes, although these proteins do not directly interact with each other, suggesting that both molecular complexes could be recruited to shared target gene by HOTAIR lncRNA [28]. In breast cancer cells, the lncRNA SRA acts as complex scaffold for KDM1A/CoREST. This interaction contributes to repressing key progesterone-inducible genes that must be kept silent in the absence of hormones [31]. Interestingly, biochemical analysis showed that unstructured single-stranded RNA is not able to associate with KDM1A/CoREST. In contrast, when the lncRNA TERRA forms a G-quadruplex (GQ) structure,

it specifically associates with the functional KDM1A/CoREST complex. Moreover, DNA with a GQ structure is not able to interact with the same molecular complex, suggesting that KDM1A is able to distinguish RNA from DNA [32], although it is still not clear how KDM1A would be able to distinguish between the two nucleic acids.

### 2.3. KDM1A and Cellular Differentiation

KDM1A has different roles in maintaining the cellular phenotype. For instance, it regulates the stem cell state and plays a key role during cellular differentiation processes. Indeed, its depletion from mouse embryonic stem cells (ESCs) leads to increased apoptosis and differentiation impairment [1]. In ESCs, KDM1A is generally bound at enhancer regions of actively transcribed genes, although its demethylase activity is inactivated by the presence of acetylated nucleosomes. During cellular differentiation, in contrast, histone deacetylation by histone deacetylases (HDACs) activates the KDM1A enzymatic activity, leading to demethylation of the genomic regions to which it is bound. Histone hypomethylation, consequently, contributes to inactivating the enhancer genomic regions that control the expression of pluripotency genes. This epigenetic mechanism, known as “enhancer decommissioning”, allows cellular differentiation [33].

KDM1A is required for the differentiation of the hematopoietic cell lineage. Comparing the phenotype of KDM1A knock-out mice revealed that KDM1A depletion reduces the levels of terminally differentiated granulocytes and erythrocytes, whereas the levels of their precursors are increased. In this context, KDM1A represses the expression of stem- and progenitor-specific genes by removing the methylation from lysine 4 of histone 3 (H3K4) at gene promoters and/or enhancer genomic regions that regulate this class of genes. Moreover, KDM1A dually controls erythrocyte differentiation by modulating the expression of the two key transcription factors (GATA1 and GATA2) of this process. In hematopoietic progenitor cells, KDM1A associates with GATA2, thereby allowing *GATA1* gene repression. In erythrocyte differentiated cells, KDM1A forms a complex with TAL1 (T-cell acute lymphocytic leukemia 1) in order to repress *GATA2* expression [34]. KDM1A contributes to cerebral cortex development. During this process, the complex KDM1A/CoREST interacts with the RBPJ-k protein to negatively regulate the Notch pathway, thereby controlling the transition from neuronal stem cells to differentiated neurons. Indeed, KDM1A depletion in mice impairs neuronal migration [35]. Consistent with these data, KDM1A interacts during *Drosophila* development with the histone deacetylase SIRT1 to negatively regulate Notch target gene expression by modifying histone modifications [36].

Interestingly, KDM1A has recently been found to be required for brown adipocyte differentiation, as its inhibition or depletion represses brown adipocyte tissue differentiation. Mechanistically, KDM1A demethylates H3K4me1/me2 at promoter regions of Wnt-pathway target genes, activating their expression and promoting brown adipogenesis [37].

Finally, KDM1A is indispensable for the epithelial-to-mesenchymal transition (EMT). EMT refers to the trans-differentiation of epithelial cells into motile mesenchymal cells, which is integral during cancer progression. During this process, epithelial cells lose their junctions and apical-basal polarity, reorganize their cytoskeleton, and undergo a change in their signaling programs that defines cells and enables the development of an invasive phenotype. In this process, KDM1A interacts with the zinc-finger protein SNAIL1 and demethylates H3K4me1/me2 at epithelial-specific gene promoters, including that of *E-cadherin*. The loss of H3K4 methylation represses epithelial-specific gene expression, with a consequent transition to the mesenchymal state [13,15,38,39].

### 2.4. KDM1A and Cancer

Deregulation of KDM1A activity contributes to carcinogenesis of many different cancers, including prostate, bladder, breast, non-small cell lung, colorectal, oral cancers, and neuroblastoma. High levels of this protein often correlate with cancer recurrence and progression [9,40–50]. In hormone-dependent tumors, such as prostate, bladder, and breast cancer, KDM1A associates with steroid hormone

receptors (HR) to regulate HR-mediated gene transcription and cell proliferation [9,42–44]. In estrogen receptor (ER)-positive breast cancer cells, for example, KDM1A is recruited by ER $\alpha$  to regulate ER $\alpha$ -target gene expression [40]. In this context, deubiquitination of KDM1A by the USP28 protein stabilizes KDM1A structure and confers cancer stem cell-like properties to breast cancer cells, thereby increasing their tumorigenicity [46].

Deregulation of KDM1A expression in non-small cell lung cancer (NSCLC) profoundly alters cell proliferation, migration, and invasion, thereby affecting the EMT process. Indeed, KDM1A overexpression in adenocarcinomic human alveolar basal epithelial cells downregulates epithelial-specific gene expression (e.g., *E-cadherin*), activates mesenchymal-specific gene expression (e.g., *Twist1* and *N-cadherin*), and increases cell proliferation as well as migration rates [45]. In colorectal and head and neck squamous cancer cells, KDM1A can contribute to tumorigenesis via two different molecular mechanisms: (a) In HCT116 colon cancer cells, it activates *Dickkopf-1* (*DKK1*) gene expression, and the corresponding DKK1 protein negatively regulates the Wnt/ $\beta$ -catenin pathway. Repression of the Wnt/ $\beta$ -catenin pathway reduces cell differentiation and increases cell proliferation [41]. (b) In colon carcinoma cells, as well as in head and neck squamous cell carcinoma lines, KDM1A demethylates H3K4me1/me2 at the *E-cadherin* gene promoter, repressing its expression [49]. The repression of the *E-cadherin* gene expression induces the EMT, affecting cell migration and invasion [50,51].

In human neuroblastoma cells, KDM1A is recruited to promoter regions of *CDKN1A/p21* and *Clusterin* (*CLU*) tumor suppressor genes by the MYC-N protein, where it demethylates H3K4me1/me2 demethylation and thereby reduces *CDKN1A/p21* and *CLU* gene expression, leading to increased cell proliferation [48]. Consistent with these data, the micro-RNA 137 (miR-137) in neuroblastoma cells was found to directly target KDM1A mRNA and activate cell properties consistent with tumor suppression [47].

## 2.5. KDM1B

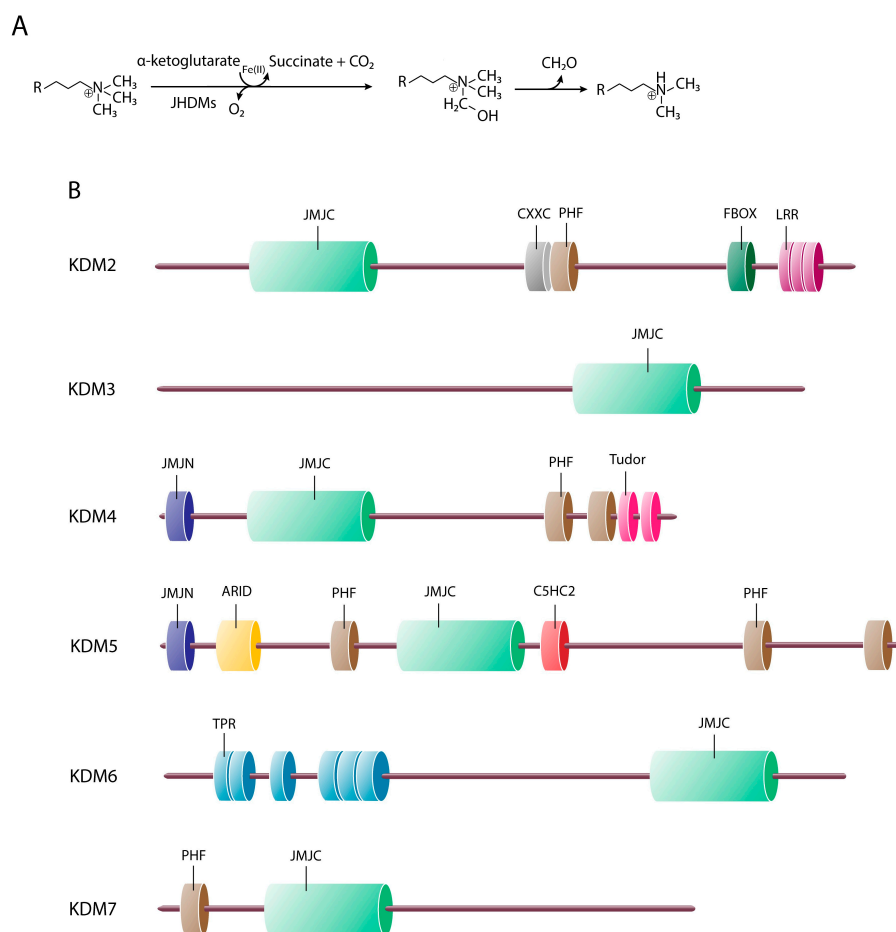
The second protein of the KDM1 histone demethylase cluster is KDM1B (also known as LSD2). KDM1B is classified as a homolog of KDM1A based on its DNA sequence. Although KDM1B shares less than 31% sequence similarity with KDM1A, it demethylates H3K4me1, H3K4me2, and H3K9me2 through a FAD-dependent amine oxidation reaction (Figure 2A) [8,52]. In contrast to KDM1A, KDM1B does not contain the Tower domain but rather contains a zinc-finger area at its N-terminal region (Figure 2B). This zinc-finger domain or SWIRM motif is indispensable for its demethylation activity [53,54]. Genome-wide studies have demonstrated that KDM1B is bound mainly within gene bodies of highly transcribed genes and is almost completely absent from gene promoters [55]. It promotes transcription elongation by interacting with the RNA polymerase II elongating complex and demethylating H3K4me2. The same molecular complex includes the methyltransferases G9A, which monomethylate H3K9, and the nuclear SET domain-containing protein 3 (NSD3), which trimethylate H3K36 [55]. Finally, the NPAC/GLYR1 protein, a putative H3K36me3 reader, can interact with KDM1B to enhance its H3K4 demethylation activity [56]. Interestingly, in addition to its role in transcriptional elongation, KDM1B was found to induce gene derepression in mouse dendritic cells by removing the methyl group from H3K9me2 at NF- $\kappa$ B target gene promoters [57].

KDM1B plays key roles during embryo development, and embryos derived from KDM1B-depleted oocytes die before mid-gastrulation. This maternal-lethal effect is most probably due to the role of KDM1B in the establishment of genomic imprinting [58]. Indeed, this enzyme is mainly expressed in oocytes, where, through its H3K4 demethylation activity, it enables de novo DNA methylation in several imprinted genes (genes expressed in a parent-of-origin-specific manner) [58]. Finally, KDM1B inhibits lung cancer cell growth independently from its demethylase activity [54]. The zinc finger domain of KDM1B catalyzes the ubiquitylation of O-GlcNAc transferase (OGT) and induces its degradation. OGT degradation, in turn, reduces oncogene expression and promotes tumor cell growth [54]. The finding that the histone demethylases KDM1B has E3 ligase activity raises the question of a possible connection between histone demethylase and ubiquitin-dependent pathway.



### 3. Jumonji C Domain-Containing Proteins

The second and largest class of demethylase enzymes contains a Jumonji C (JmjC) domain and catalyzes lysine demethylation of histones through an oxidative reaction that requires iron Fe(II) and  $\alpha$ -ketoglutarate ( $\alpha$ KG) as cofactors [59]. Unlike KDM1 demethylases, which can only remove mono- and/or dimethyl lysine modifications, the JmjC domain-containing histone demethylases (JHDMs) can remove all three histone lysine methylation states. Various studies of  $\alpha$ KG-dependent oxygenases suggest the following reaction mechanism: First, the enzyme binds iron (Fe) through its metal-binding motif HXD/EXnH, the so-called facial triad. Next, the Fe(II) enzyme complex binds the cofactor  $\alpha$ KG, and subsequently the substrate and oxygen. Oxygen binding is followed by the oxidative decarboxylation of  $\alpha$ KG to produce succinate, carbon dioxide, and ferryl. The latter is a highly reactive group and can potentially oxidize a C–N bond in a lysine-methyl group, forming an unstable carbinolamine that will rapidly break down, leading to the release of formaldehyde and loss of a methyl group from lysine (Figure 3A).



**Figure 3.** Catalytic mechanism (A) and protein structures (B) of the JHDM (JmjC domain-containing histone demethylase; KDM2–KDM7) enzymes. (A) The JHDM family (KDM2–KDM7) with the two cofactors (iron ion, Fe(II), and  $\alpha$ -ketoglutarate,  $\alpha$ KG) uses a dioxygenase reaction to demethylate lysine histones. (B) Schematic protein structure representation of the different clusters (KDM2–KDM7) of JHDM proteins. JmjC, Jumonji C domain; PHF, PHD finger; LRR, leucine-rich repeat; TPR, tetratricopeptide.

Structurally, these enzymes contain different domains: in addition to the JmjC domain, they can have PHD finger, Bright/Arid, C5HC2, CXXC, Tudor, F-box, leucine-rich repeat (LRR), and tetratricopeptide-like (TPR) domains (Figure 3B).

The JmjC domain is structurally conserved and has a similar structure in all of the JHDMs [60]. It folds into eight  $\beta$ -sheets to form an enzymatically active pocket that coordinates Fe(II) and  $\alpha$ KG binding, essential for the demethylase activity [61]. The PHD finger domain is required to bind both methylated and unmethylated histones; the Bright/Arid and the C5HC2 domains enable JHDMs' binding to DNA, and the Tudor domain allows the binding to the H3K4me3 mark [62–64]. The F-box, LRR, and TPR domains mediate protein–protein interactions and are required to form multiprotein complexes [65–67]. The substrate specificity of each JHDM, finally, seems to rely on both the JmjC domain and the specific additional domains found within each protein [61]. How the JmjC domain cooperates with other domains to regulate histone modifications is still unclear [68].

There are 27 different JmjC-containing proteins in the human genome that can be grouped into different subfamilies depending on their structure and function. Here, we describe the specific roles of the six JmjC clusters (KDM2–KDM7) during cellular differentiation and carcinogenesis (Table 1).

### 3.1. JmjC-Containing Proteins and Cellular Differentiation

#### 3.1.1. KDM2 Cluster (FBXL Subfamily)

In addition to their roles in different signaling pathways, histone modifications are essential during mesenchymal stem cell (MSC) differentiation [69]. Two proteins belong to this cluster: KDM2A (FBXL11) and KDM2B (FBXL10). KDM2A was the first discovered JmjC domain-containing protein able to erase mono- and di-methyl marks from lysine 36 of histone 3 (H3K36me1/me2) [70]. KDM2B, in contrast, is responsible not only for demethylating H3K36me1/me2 but also for removing the methyl mark from H3K4me3 [71]. Both demethylases regulate MSC differentiation through their association with the BCL-6 co-repressor (BCOR) complex [72]. KDM2A interacts with BCOR and inhibits odontogenic differentiation of human stem cells from apical papilla (SCAPs), a type of dental MSC [72]. In addition, the KDM2A/BCOR complex inhibits osteogenesis by reducing the epiregulin (*EREG*) gene transcription through the demethylation of H3K4me3 or H3K36me1/me2 from its promoter. Epiregulin is a key transcription factor required for the expression of osteogenic-related genes, such as osterix (*OSX*) and distal-less homeobox 5 (*DLX5*) [73]. Consistent with these data, KDM2A depletion in SCAPs enhances adipogenic differentiation in vitro [74]. Similarly, in MSCs, the KDM2B/BCOR complex contributes to osteogenesis differentiation by repressing *AP-2 $\alpha$*  (activating enhancer-binding protein 2 $\alpha$ ) gene expression through the demethylation of H3K4me3 and H3K36me2 from its promoter [72].

Finally, KDM2B impairs adipogenesis by recruiting the polycomb repressive complex 1 (PRC1) to repress key genes (e.g., *Cdk1*, *Uhrf1*, *Pparg1*, and *Pparg2*) required for the conversion of pluripotent cells into the adipogenic lineage. Interestingly, KDM2B-dependent adipogenesis inhibition is independent from the demethylase activity of the protein; however, its F-box and leucine-rich repeat domains are required for recruiting a noncanonical PRC1 containing the RING1B, SKP1, PCGF1, and BCOR proteins [75].

#### 3.1.2. KDM3 Cluster (JMJD1 Subfamily)

Methylation of lysine 9 of histone 3 (H3K9me1/me2/me3) is an epigenetic mark associated with gene repression, and the level of this mark strongly correlates with cell state differentiation. The neural progenitors cells (NPCs), for instance, have lower H3K9 methylation levels than the undifferentiated ESCs [76]. The loss of this epigenetic mark by the KDM3A member JMJD1A is crucial for maintaining ESC self-renewal and for various cell differentiation processes, including spermiogenesis and vascular smooth muscle cell differentiation.

In ESCs, KDM3A contributes to self-renewal maintenance by preventing the H3K9 dimethylation at pluripotency-associated gene promoters (e.g., the *Tcl1* gene) [77–80]. During spermiogenesis, KDM3A binds to and positively regulates the expression of two genes, transition nuclear protein (*Tnp1*) and protamine 1 (*Prm1*), by removing the repressive H3K9 marks from their promoters. During sperm

chromatin maturation, the Tnp1 and Prm1 proteins play key roles in the histone replacement process. Indeed, sperm cells from *Kdm3a* gene-trap mice exhibit post-meiotic chromatin condensation defects, with a low number of mature sperm cells, suggesting that KDM3A could be a good candidate gene for azoospermia and globozoospermia (two clinical syndromes characterized by spermatogenesis defects) [81]. Similar to KDM3A, KDM3C (JMJD1C) is expressed in a sex- and stage-specific manner in the germline when changes in chromatin architecture take place [81]. A series of knockout studies have demonstrated that both proteins play essential roles in male gametogenesis. Indeed, their depletion severely affects the formation of functional gametes and fertility [81].

KDM3A also contributes to smooth muscle cell (SMC) differentiation. The myocardin transcription factor interacts with KDM3A and recruits it to SMC-specific gene promoters. KDM3A subsequently demethylates H3K9me3 from these genomic regions and regulates their TGF $\beta$ -mediated activation [82].

KDM3B (JMJD1B) displays histone H3K9me1/me2 demethylase activity, and different pieces of evidence suggest that it plays a role in leukemogenesis, via activation of *lmo2* through interdependent actions with the histone acetyltransferase (HAT) complex containing CBP. Consistent with these data, KDM3B expression induces leukemic transformation by inhibiting leukemia cell differentiation and is upregulated in blood cells obtained from certain types of leukemia patients [82].

KDM3C (JMJD1C), finally, is required for the correct formation of dendritic cells. Mutations of this enzyme have been reported in patients affected by intellectual disability, such as Rett syndrome. Further, mutations of this enzyme in HEK 293 affects the KDM3C demethylation activity, impairing the loss of methylation of MDC1 protein required to repair DNA damage. Consequently, the mutated KDM3C protein affects the cell response to DNA damage [83].

### 3.1.3. KDM4 Cluster (JMJD2 Subfamily)

The KDM4 cluster catalyzes the removal of di- and tri-methyl marks of H3K9 (H3K9me2/me3) and H3K36 (H3K36me2/me3) [69]. The members of this cluster that contribute to embryonic development and cell differentiation are KDM4A, -4B, and -4C. KDM4A (JMJD2A) is crucial for muscle differentiation, inducing *Myog* gene expression by demethylating the H3K9me2 and H3K9me3 on its promoter [84]. In human MSCs, in contrast, KDM4B (JMJD2B) removes the H3K9me3 mark at the *DLX5* gene promoter, allowing osteoblast differentiation [69]. Different studies have suggested that KDM4B is required during adipogenesis; however, its role in this process is still controversial. Ye et al. (2012) found that KDM4B reduces adipogenic lineage specification from MSCs [85]. Guo et al. (2012), on the other hand, demonstrated that KDM4B can promote preadipocyte differentiation, acting as a cofactor of the C/EBP $\beta$  protein [86]. Further studies are required to clarify the role of KDM4B during adipogenesis.

In contrast to KDM4B, KDM4C (JMJD2C) is induced during adipocyte differentiation, and depletion of KDM4C is sufficient to block this kind of cellular differentiation [87]. However, the molecular mechanism by which KDM4C contributes to this process is still not clear. Finally, in ESCs, KDM4C is required to maintain the euchromatic status of *Nanog* gene promoter by removing H3K9me3 mark. Further, H3K9 demethylation prevents binding of transcription co-repressors, including heterochromatin protein 1 (HP1) and KRAB-associated protein 1 (KAP1), to *Nanog* gene promoter [88].

Finally, while KDM4D (JMJD2D) is absent in different tissues, it is highly expressed in spermatocytes and spermatids; however, its role during spermatogenesis is still unknown [89]. Mutant *KDM4D* male mice have globally higher levels of H3K9me3 than control mice, yet they are as fertile as control mice [89]. In contrast, this demethylase is required for the inflammatory response of macrophages and dendritic cells. In this context, KDM4D demethylates the H3K9me3 levels at the enhancer regions that control the expression of *Mdc* and *Il12b* genes, enabling their expression during the inflammatory response [90]. Interestingly, it was recently reported that KDM4D is able to bind RNA through two distinct RNA-binding domains, one of which is the JmjC domain. The KDM4D binding to RNA is independent of KDM4D demethylase activity and is required for its association with



chromatin [91]. Whether all the JmjC-containing proteins require RNA for their enzymatic activity as well as for their association with chromatin is still unknown and should be clarified by further studies.

### 3.1.4. The KDM5 Cluster (JARID1 Subfamily)

The members of this cluster—KDM5A (JARID1A), KDM5B (JARID1B), and KDM5C (JARID1C)—demethylate H3K4me2/me3, thereby acting as transcriptional repressors [92]. As for the other JHDMs proteins, the KDM5 cluster is also implicated in eukaryotic cellular differentiation and development [93]. KDM5A binds to *Hox* genes and represses their transcription in undifferentiated ESCs [92]. During retinoic acid differentiation of ESCs, however, it is displaced from *Hox* genes, leading to an increase of H3K4me3 levels at their promoters. The increased levels of this epigenetic mark induce *Hox* gene expression in the differentiated cells [92]. On the other hand, KDM5A affects osteogenic differentiation of human adipose-derived stem cells, occupying the promoters of the osteogenesis-associated genes *OSX* and *OC* to maintain the levels of H3K4me3 [94].

KDM5B acts as a co-repressor of specific transcription factors required during embryonic development [95]. It binds to and modulates the transcriptional activity of BF-1 (brain factor) and Pax9 (paired box 9), two transcription factors that play key roles during neural cell differentiation and craniofacial development, respectively [96].

Finally, evidence suggests that KDM5C is required for brain development. Indeed, the *KDM5C* gene is expressed specifically in the brain during development and is frequently mutated in X-linked mental retardation [97]. However, whether KDM5C is essential for brain development, and how it could play this role, are still unknown.

### 3.1.5. KDM6 Cluster (UTX/JMJD3 Subfamily)

*KDM6A* (UTX) is a X-linked chromosome gene encoding an ubiquitously expressed protein that controls the basal levels of H3K27me3 and the induction of ectoderm differentiation, and it is essential for somatic cell reprogramming [98,99]. *KDM6B* (JMJD3), in contrast, is induced upon inflammation or exposure to viral or oncogenic stimuli [100,101]. It controls neuronal and epidermal differentiation and inhibits reprogramming [102]. In addition, both *KDM6A* and *KDM6B* have key roles in MSC lineage specification. Interestingly, in contrast to *KDM5A*, *KDM6A* induces the expression of three key genes (*RUNX2*, *Osteopontin*, and *Osteocalcin*) that are required for osteoblast differentiation, by removing the H3K27me3 mark from their promoters [103]. Similarly, *KDM6B* (together with *KDM4B*) demethylates H3K27me3 from the *BMP* (bone morphogenetic protein) and *HOX* gene promoters, inducing osteogenic differentiation of MSCs [85,104]. Finally, these proteins are involved in the inhibition of adipogenic differentiation of MSCs: depletion of *KDM6B* or *KDM4B* in human bone marrow-derived MSCs enhances adipogenesis and decreases osteogenic differentiation [85]. However, the mechanism(s) underlying their role in this process are still unknown.

*KDM6C* (UTY) is Y-linked chromosome gene that belongs to H3K27me3 demethylase family, although its demethylase activity is still debated [105]. Surprisingly, *KDM6C* and *KDM6A* activate gene expression during embryonic development by associating with RBBP5, a subunit of the H3K4 methyltransferase complex. Thus, in contrast to the other JmjC containing proteins, these enzymes participate in H3K4 methyltransferase activity rather than in histone demethylation [105].

### 3.1.6. The KDM7 Cluster

The recently identified KDM7 cluster includes *KDM7A* (PHF8), *KDM7B* (KIAA1718), and *KDM7C* (PHF2), which activate gene transcription by demethylating H3K9me2/me1, H3K27me2/me1, and H4K20me1 [106]. During zebrafish neuronal differentiation and craniofacial development, *KDM7A* induces *MSX* gene expression. The *MSX* gene family encodes homeodomain transcription factors that act downstream of the transforming growth factor beta (TGFβ), BMP, and Wnt-signaling pathways essential in craniofacial and neural development [107].

In mouse ESCs, KDM7B is required for neural differentiation, as it removes the H3K9me2 and H3K27me2 from the *FGF4* gene promoter. FGF4 (fibroblast growth factor 4), in turn, phosphorylates the extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2, respectively), thereby allowing neural fate determination [108]. Additionally, KDM7C is required for osteoblast differentiation and bone development: its overexpression in mice enhances bone development, whereas its depletion in rats protracts calvarial bone regeneration [109]. Moreover, KDM7C modulates the function of Runx2 (Runt-related transcription factor 2), a key protein required for osteoblast differentiation during bone development. Interestingly, through its JmjC domain, KDM7C interacts with Runx2 and reduces its lysyl methylation level. The loss of Runx2's post-translational modifications enhances its DNA binding capability, thereby modifying the Runx2-mediated gene transcription [109].

### 3.2. JmjC Proteins and Cancer

The JmjC-containing proteins regulate different processes that contribute to tumor development, including genome stability and cell senescence. Several genetic alterations of Jumonji family genes have been described in human cancers [95,106]. However, the specific role of these proteins in cancer is largely debated in literature. Depending on the tumor type, the great majority of JmjC-containing proteins could act either as tumor suppressors or as oncogenes.

#### 3.2.1. The KDM2 Cluster (FBXL Subfamily)

KDM2A (FBXL11) has tumor suppressor properties in transformed mouse embryonic fibroblasts, fibrosarcoma, and prostate and colon cancer cells [110–112]. However, the same gene is overexpressed in breast, gastric, and lung cancers, promoting tumorigenesis both in cell lines and mouse xenografts [113–115]. Interestingly, KDM2A represses migration and invasion of breast cancer cells, and inhibits angiogenic tubule formation by endothelial cells [116]. The same authors also found that KDM2A can bind to Rb and E2F1 proteins and repress the transcriptional activity of E2F1. KDM2B (FBXL10) was first proposed to have tumor suppressor properties, as its expression is reduced in different cancers, such as in aggressive brain tumors, and its presence is required to maintain genome stability in these cancer types [71,117]. However, in human pancreatic ductal adenocarcinoma, KDM2B is overexpressed and promotes tumorigenesis. In this type of cancer, KDM2B interacts with the Polycomb proteins, c-Myc, and KDM5A, and regulates the expression of developmental and metabolic genes [118]. In addition, KDM2B could act as a critical epigenetic factor for leukemogenesis. The *KDM2B* gene is overexpressed in leukemias, and ectopic expression of this protein contributes to hematopoietic progenitor cell proliferation allowing leukemic transformation. Indeed, in leukemia stem cells, KDM2B contributes to repressing the expression of the tumor suppressor *p15<sup>Ink4</sup>* gene by removing the methylation mark H3K36me2 from its promoter [119].

#### 3.2.2. The KDM3 Cluster (JMJD1 Subfamily)

KDM3A (JMJD1A) is expressed at significantly higher levels in breast, colon, and renal cancers than in normal tissue [120–122]. Under hypoxia conditions, KDM3A increases renal and colon tumor growth by inducing the expression of a subset of hypoxia-inducible genes (including *adrenodemullin* and *GDF15* [growth and differentiation factor 15]). The induction of hypoxia-inducible gene expression is due to the KDM3A-mediated reduction of the methylation marks H3K9me1/me2 [123]. Frequent deletion of 5q31, which contains the *KDM3B* (*JMJD1B*) gene, or reduced expression of *KDM3C* (*JMJD1C*), in various malignancies suggests possible roles of these proteins in tumor suppression [123]. KDM3C has been proposed to be an important driver of MLL-AF9 and HOXA9-driven leukemias. In this context, KDM3C directly interacts with HOXA9 and modulates a HOXA9-controlled gene expression program. Depletion of KDM3C in mice reduces the frequency of leukemia stem cells and causes differentiation of MLL-AF9- and HOXA9-driven leukemias [124]. In addition to its roles in leukemias, KDM3C levels are decreased in breast tumors, where it specifically coactivates androgen receptor and responds to alterations in systemic androgen levels [125].

### 3.2.3. The KDM4 Cluster (JMJD2 Subfamily)

Tri- and dimethylation of lysine 9 on histone H3 (H3K9me3/me2) enable heterochromatin protein 1 (HP1) binding and are generally associated with gene repression. The members of the KDM4 cluster are overexpressed in prostate cancer, where they widely remove the methylation marks from H3K9me3/me2. The loss of H3K9me3 enables HP1a delocalization, with a consequent reduction in heterochromatin [126]. Moreover, KDM4C (JMJD2C) is amplified or upregulated in several cell lines derived from oesophageal squamous carcinomas, medulloblastoma, and breast cancer [126–130]. In addition, in agreement with a contribution of KDM4C to tumor development, inhibition of *KDM4C* expression reduces cell proliferation [126].

KDM4A (JMJD2A) regulates gastric cancer growth and serves as an independent prognostic factor. The depletion of this protein induces apoptosis of gastric cancer cells by upregulating the expression of pro-apoptotic proteins and microRNA (miR-34a) and by downregulating anti-apoptotic proteins. Importantly, KDM4A expression is associated with tumor stage and nodal status, and high levels of this protein predict poor overall and disease-free survival [131].

### 3.2.4. The KDM5 Cluster (JARID1 Subfamily)

KDM5A (JARID1A) promotes gastric cancer cell growth and is enriched in drug-resistant lung cancer cells. The depletion of this protein from lung cancer tissues, indeed, drastically affects cell proliferation, motility, migration, invasion, and metastasis [132]. In this context, KDM5A binds to *integrin-b1* (*ITGB1*) promoters and induces its expression. Integrin-b1, in turn, plays key roles in lung cancer metastasis. Simultaneously, KDM5A induces the expression of cyclins D1 and E1 while suppressing the expression of cyclin-dependent kinase inhibitor p27 (CDKN1B), each contributing to KDM5A-mediated cell proliferation [132,133]. Besides its roles in gastric and lung cancer, KDM5A has been suggested to contribute, through its interaction with retinoblastoma protein (pRb), to uncontrolled growth in malignant melanomas. Indeed, KDM5A expression is downregulated in advanced and metastatic melanomas. Further, the great majority of benign melanocytic nevi are KDM5A positive, whereas 90% of primary malignant melanomas and 70% of melanoma metastases were found to lack this protein [134]. In agreement with in vivo data, melanoma cell lines exhibit low and heterogeneous levels of KDM5A expression [134].

KDM5B (JARID1B) has been described as a putative oncogene in several cancers, and its level of expression is correlated with tumor malignancy [135–138]. The KDM5B-mediated H3K4 demethylase activity plays an important role in the proliferative capacity of breast cancer cells through repression of tumor suppressor genes, including BRCA1 [135–138]. Similar to breast cancer, KDM5B is upregulated also in prostate cancer tissues as compared to benign prostate samples. In this context, KDM5B associates with androgen receptor and regulates its transcriptional activity [138]. On the other hand, knockdown of KDM5B in hepatocellular carcinoma cells (HCCs) inhibits cell proliferation via cell cycle arrest at the G1/S phase. In this case, KDM5B induces the expression of key cell regulatory genes (e.g., p15 and p27) by removing the methylation mark of histone H3K4me3 from their promoters [139]. Consistent with these data, KDM5B overexpression in HCCs increases proliferation, EMT, migration, and invasion in vitro, and enhances tumorigenic and metastatic capacities in vivo [137].

Finally, KDM5C (JARID1C) is upregulated in prostate cancers, where it regulates proliferation-associated genes [140]. In vitro, KDM5C depletion enhances the expression of several proliferation-associated genes and impairs prostate cancer cell growth [140]. KDM5C localizes in heterochromatin, leading to the demethylation of H3K4me3 and enhancing the deposition of H3K9me3 on chromocenters by SUV39H1. When KDM5C is mutated, an increased expression of heterochromatic noncoding RNAs (ncRNAs) triggers genomic instability, leading to clear cell renal cell carcinoma (ccRCC) [141].

### 3.2.5. The KDM6 Cluster (UTX/JMJD3 Subfamily)

KDM6A (UTX) functions as a tumor suppressor and is frequently genetically inactivated in many tumors, including lung, liver, renal, bladder, colorectal, and T-cell acute lymphoblastic leukemia (T-ALL) [142–145]. Interestingly, KDM6A and KDM6B (JMJD3) have opposing roles in the context of the same tumor. KDM6B is required for the initiation and maintenance of T-ALL by regulating key oncogenic gene targets by demethylating H3K27me3 [144]. KDM6A, in contrast, is frequently inactivated in T-ALL and has a tumor suppressor role [144]. Beside its role in T-ALL, KDM6B has different functions depending on the tumor types: it is downregulated in lymphomas, leukemias, lung, liver, colon, colorectal, pancreatic cancers, and gliomas [100,146–148], whereas it is upregulated in some melanomas, gliomas, lymphomas, myelodysplastic syndrome, and prostate cancers [149–155]. In addition, it was recently demonstrated that the expression of KDM6B is significantly higher in ccRCC tissue as compared with non-tumor tissue, and increased KDM6B expression positively correlates with cancer stage, tumor size, and lymph node metastasis [156]. Furthermore, high levels of KDM6B expression positively correlate with poor prognosis. Finally, KDM6B can cause EMT by inducing the expression of the master transcription factor SLUG [156].

### 3.2.6. The KDM7 Cluster

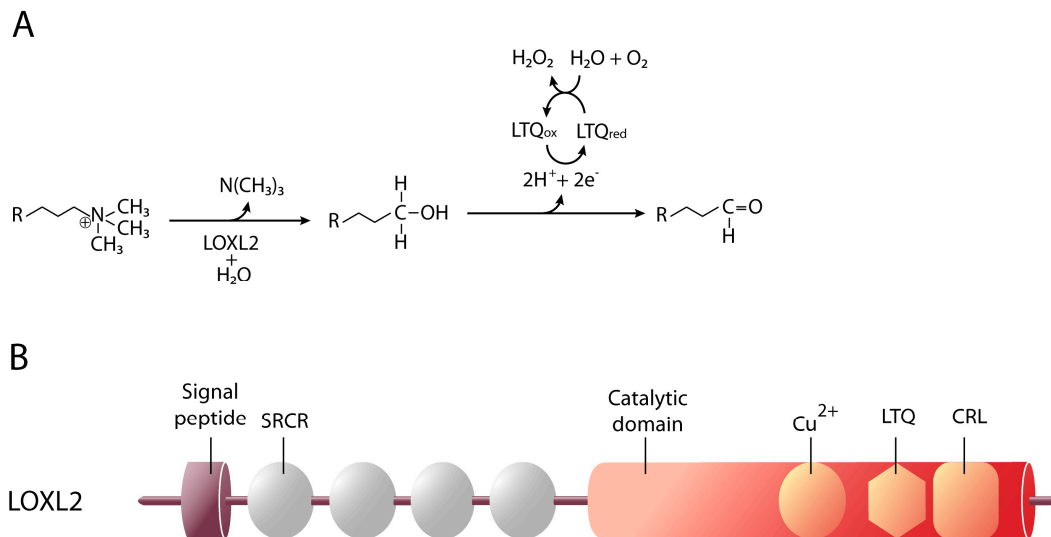
The histone demethylase KDM7A (PHF8) is overexpressed and works as an oncoprotein in many cancers, including prostate and non-small cell lung cancer, leukemia, and esophageal squamous cell carcinoma [157]. In many of these tumors, high KDM7A expression is significantly associated with shorter overall survival and disease-free survival. KDM7A depletion from prostate cancer cell lines decreases cell proliferation and migration with a simultaneously significant increase of apoptosis, demonstrating that KDM7A is indispensable for prostate cancer proliferation and metastasis [158]. Consistent with these data, substantial evidence indicates that modulation of KDM7A expression regulates the transcription of genes related to cell cycle and cytoskeletal dynamics [159–162]. Among these, KDM7A induces microRNA miR-21 gene expression and, interestingly, miR-21 depletion impedes the KDM7A effects on proliferation and apoptosis of lung cancer cells, demonstrating that the role of KDM7A during these processes is mediated by miR-21 microRNA [158]. Moreover, KDM7A is required for the retinoic acid response of acute promyelocytic leukemia. It was demonstrated, indeed, that all-trans retinoic acid (ATRA) sensitivity depends on the histone demethylation activity of KDM7A, suggesting that the pharmacological regulation of PHF8 activity can be useful to restore the ATRA sensitivity to resistant cells [163]. In contrast to the oncogenic role of KDM7A, KDM7C (PHF2) could have tumor suppressor role during cancer development. It interacts with p53 protein in cancer cells and demethylates H3K9me2 at p53 target genes, allowing p53-dependent apoptosis under genotoxic stress [164].

## 4. Lysyl Oxidase-Like 2

The lysyl oxidase-like 2 (LOXL2) enzyme belongs to the lysyl oxidase family, comprising the lysyl oxidase (LOX) and four lysyl oxidase-like proteins (LOXL1–4). These enzymes are amine oxidases able to remove the amino group located in the  $\epsilon$ -position of lysine residues through oxidation, leaving an aldehyde group as a product of the reaction. They require two cofactors to catalyze the reaction, copper and quinone [165] (Figure 4A).

Structurally, the C-terminal regions of LOX proteins contain all the elements required for their enzymatic activity: a copper-binding motif, residues for lysine tyrosylquinone cofactor formation, and a cytokine receptor-like domain. The N-terminal domain, in contrast, gives sequence variability to each member of the family protein and, except for LOXL1, the other LOXL members (LOXL2/3/4) also contain four scavenger receptor cysteine-rich (SRCR) domains that are commonly found in cell surface receptors and adhesion molecules [166] (Figure 4B). Although this family was first known to crosslink collagen and elastin in the extracellular matrix, nuclear-related functions have been described for some

of the members [167,168]. LOXL2 specifically removes the amino group from trimethylated lysine 4 in histone H3 (H3K4me3). The proposed model for this reaction involves the nucleophilic attack by the water-derived hydroxide ion to lysine C $\epsilon$ . The reaction produces triethylamine and alcohol [169]. The alcohol is then oxidized twice by the active site of LOXL2 (lysine tyrosylquinone; LTQ) [170], releasing hydrogen peroxide and an aldehyde group in the product lysine (allysine) (Figure 4A). This new histone modification is referred as H3K4ox [169]. Since LOXL2 removes an active epigenetic mark, such as H3K4me3, it generally acts as a transcriptional repressor.



**Figure 4.** (A) Proposed chemical reaction model for H3K4me3 deamination by LOXL2. LTQox, oxidized lysine tyrosylquinone; LTQred, reduced lysine tyrosylquinone. (B) Schematic representation of LOXL2 protein structure. SRCR, scavenger receptor cysteine-rich domain; Cu<sup>2+</sup>, copper-binding domain; LTQ, lysine tyrosylquinone domain; CRL, cytokine receptor-like.

#### 4.1. LOXL2 and Cellular Differentiation

Different studies have linked LOXL2 expression to cell differentiation. LOXL2 transcription, for instance, is downregulated during keratinocyte differentiation [171]. Concomitantly, the progression of skin squamous carcinoma cells (cells characterized by aberrant differentiation) is associated with enhanced LOXL2 expression [172]. In addition, while LOX, LOXL1, LOXL3, and LOXL4 levels are increased in adult human dental pulp stem cells (hDPSCs) that differentiate to odontoblast-like cells, LOXL2 is reduced at both mRNA and protein levels during the same process. Consistently, the inhibition of LOXL2 activity promotes hDPSC differentiation to odontoblasts [173]. In contrast, LOXL2 expression increases significantly during chondrocyte differentiation, and its depletion abolishes chondrocyte differentiation [174].

Recently, a new role for LOXL2 during ESC differentiation has been described [175]. When ESCs exit the pluripotency state upon retinoic acid treatment, LOXL2 oxidizes the TAF10 transcription factor (a subunit of either the TFIID general transcription factor complex and of the SAGA histone acetyltransferase complex) and represses the pluripotency core network. In addition, LOXL2 depletion in zebrafish affects embryo development, leading to overexpression of the *Oct4* pluripotency factor and a failed neural commitment development [175].

Finally, EMT inducers, such as hypoxia and TGF $\beta$ , promote *Loxl2* gene expression [176,177], and the LOXL2 protein, in turn, interacts and stabilizes the SNAIL1 transcription factor to repress epithelial-specific gene promoters [176,177].



#### 4.2. LOXL2 and Cancer

LOXL2 has been proposed to regulate tumor cell survival, chemoresistance, cell adhesion, motility, and invasion, and to remodel the tumor microenvironment [166]. Different human cancers show an LOXL2 up-regulation [178,179] that correlates with tumor grade, poor prognosis, and decreased survival [168]. In fact, a possible role for LOXL2 in pre-metastatic niche formation has been suggested [180]. Further, LOXL2 can modify the extracellular matrix components in the tumor microenvironment and metastatic niche of HCCs and promote intrahepatic metastasis by increasing tissue stiffness, thereby enhancing the cytoskeletal reorganization of HCC cells [180]. Additionally, LOXL2 facilitates extrahepatic metastasis by enhancing recruitment of bone marrow-derived cells to the metastatic site [180]. Finally, since LOXL2 plays a key role during EMT (as described above), alterations of LOXL2 could increase the metastatic progression of cancer cells by affecting this process. Further studies are required to investigate this possibility.

### 5. Beyond the Histone Demethylase Function

Besides their role as histone demethylases, lysine-specific demethylases can remove methyl groups from non-histone proteins in order to regulate their function and/or stability. Moreover, some of them can have others enzymatic activities independent of their demethylation function.

During carcinogenesis, KDM1A interacts with the tumor suppressor protein p53 and demethylates its lysine 370, preventing p53 from interacting with the coactivator binding protein 1 (53BP1) [181]. The absence of a p53-53BP1 molecular complex inhibits the p53 transcriptional activity and enhances the tumor cell growth [181]. The same histone demethylase is required for gastrulation during mouse embryogenesis by removing methyl groups from lysine 1096 on DNA methyltransferases 1 (DNMT1) [182]. The KDM1A-mediated demethylation of DNMT1 increases its stability and provides a mechanistic link between the histone and DNA methylation systems [182]. Similar to KDM1A, KDM6B is able to demethylate p53 but, in this case, the p53 demethylation stabilizes the protein, allowing p53 to accumulate in the nucleus [183]. KDM1A can also demethylate the transcription factor E2F1 and the myosin phosphatase target subunit 1 (MYPT1) [184,185]. E2F1 demethylation by KDM1A in p53-deficient cells is crucial for the DNA damage-induced cell death [184], whereas demethylation of MYPT1 at lysine 442 induces MYPT1 degradation, such that the retinoblastoma protein (Rb) is persistently phosphorylated and supports cell progression [185]. KDM2A negatively regulates the nuclear factor NF- $\kappa$ B through the demethylation of lysine 218 and 221 on the p65 subunit [112]. Besides the role of NF- $\kappa$ B in inflammation and innate immunity, a role for it in cancer initiation and progression has also been reported [186]. In addition, as mentioned above, LOXL2 plays a key role in the balance between pluripotency and differentiation of ESCs by demethylating non-histone proteins. For instance, it oxidizes methylated transcription factor TAF10 and regulates TFIID-dependent genes expression during neural progenitor differentiation [175].

In contrast to the other histone demethylases, KDM1B has an E3 ubiquitin ligase activity that is independent of its demethylase function. Through its polyubiquitylation of the O-GlcNAc transferase OGT, KDM1B induces the OGT proteasomal degradation. OGT is often upregulated in cancer, and loss of KDM1B E3 ligase activity leads to activation of different classes of oncogenes, demonstrating that KDM1B may act as a suppressor of tumorigenesis through its E3 ligase activity and its effects on OGT stability [54].

Finally, besides their enzymatic activities, some of the lysine-specific demethylases can act as molecular scaffolds to target chromatin modifiers to specific genomic regions. KDM2B, for instance, is able to bind to CpG island regions (DNA regions in which the frequency of the CG sequence is higher than in other regions) through its Zn-finger CxxC DNA-binding domain, where it can recruit PRC1 to these genomic regions [187,188]. PRC1, in turn, can ubiquitinate lysine 119 of histone H2A, leading to a closing of the chromatin structure [46,189]. Similarly, KDM3A and KDM6 demethylases play a role in chromatin remodeling by linking transcription factors with the SWI/SNF chromatin remodeling complex [190,191]. Since these proteins regulate the chromatin compaction associated with

genes involved in carcinogenesis and cell proliferation, mutation or depletion of histone demethylases could interfere with these biological processes.

## 6. Conclusions

Several mechanistic classes of lysine-specific histone demethylases have been identified in the last decade and have been found to have activities crucial for the regulation of gene expression. These enzymes are involved in important processes, such as cell cycle and cell differentiation, and misregulation of these enzymes can have an enormous impact on cell behavior. Due to the link between this enzymatic activity and cancer, and to the latest discoveries about the structural and biochemical properties of these enzymes, they have become potential drug targets for cancer treatment. However, it is essential to keep in mind that these enzymes are not only involved in cancers but also have critical physiological roles in gene regulation and genome organization, such that altering their activity might have far-reaching negative (and yet unknown) consequences.

**Table 1.** Lysine-specific histone demethylases and their related cellular differentiation processes and putative roles in cancer.

Names	Cellular Differentiation	Ref.	Putative Cancer Role	Ref.
KDM1A	Hematopoiesis, cerebral cortex development, brown adipogenesis, EMT	[24–30]	Oncogene	[31–40]
KDM1B	Embryo development	[58]	Tumor suppressor	[54]
KDM2A	Osteo/dentinogenic differentiation, adipogenesis	[88–90]	Pro- and anti-oncogenic functions	[126–132]
KDM2B	Osteogenesis; adipogenesis	[88,91]	Pro- and anti-oncogenic functions	[87,133–135]
KDM3A	Spermatogenesis; smooth muscle cell differentiation	[97,98]	Oncogene	[136–139]
KDM3B	Leukemogenesis	[98,140]	Tumor suppressor	[139]
KDM3C	Neurogenesis?	[99]	Tumor suppressor	[139–141]
KDM4A	Skeletal muscle cell differentiation	[100]	Oncogene	[142,147]
KDM4B	Osteoblast differentiation; adipogenesis	[85,101,102]	Oncogene	[142]
KDM4C	Adipogenesis	[103]	Oncogene	[142–146]
KDM4D	Spermatogenesis?	[105]	NR	
KDM5A	Osteogenesis	[110]	Pro- and anti-oncogenic functions	[136,148–150]
KDM5B	Neural cell differentiation, craniofacial and development	[111,112]	Oncogene	[151–154]
KDM5C	Brain development	[113]	Oncogene	[156,157]
KDM6A	Ectoderm differentiation; cell reprogramming; osteoblast differentiation	[114,115,119]	Tumor suppressor	[158–161]
KDM6B	Neuronal and epidermal differentiation; cell reprogramming; osteogenic and adipogenic differentiation	[101,118,120]	Pro- and anti-oncogenic functions	[116,160,162–172]
KDM7A	Neuronal differentiation, craniofacial development	[123]	Oncogene	[173–178]
KDM7B	Neuronal differentiation	[124]	NR	
KDM7C	Osteoblast differentiation; bone formation	[125]	Tumor suppressor	[180]
LOXL2	Keratinogenesis; odontoblast genesis; chondrogenesis; zebrafish development; EMT	[171,173–177]	Oncogene	[166,178–180]

NR, not reported. EMT, epithelial-mesenchymal transition.

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