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Review

Histone lysine demethylases - a new set of regulators of epigenetic plasticity

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ABSTRACT

Histone lysine methylation marks are epigenetic regulators that have important functions in controlling cell-specific identity. Distinct combinations of lysine methylation marks, all of which make up the chromatin signature of a particular cell type index the network system that controls which genes are activated and which are kept repressed. For a long time this type of marking by histone lysine methylation was thought to be robust and irreversible and that it is required for long term inheritance or locking a cell into its specific epigenotype. However in recent years, with the discovery of lysine specific demethylases, particularly the jmjC domain containing proteins, that target the tri-methylated lysines, it has not only been demonstrated that these marks are dynamic, but also revealed a whole new set of regulators of epigenetic plasticity. In this review we discuss the different histone lysine demethylases, their targets, biological function and potential role in cellular reprogramming and pluripotency.

KEYWORDS: epigenetics, reprogramming, histone lysine methylation, lysine demethylases, differentiation, cellular fate, pluripotency

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INTRODUCTION

Epigenetic modifications form the regulatory networks that control gene expression and hence maintain cell specific identity. DNA in the cell nucleus is compacted with histones and non-histone proteins and packaged into a structure called chromatin [1, 2]. The basic repetitive unit of chromatin is the nucleosome which consists of 147 bp of DNA wrapped around an octamer of core histones H2A, H2B, H3 and H4 [1, 2]. Epigenetic regulation occurs at all levels of compaction with DNA methylation and post-translation modification of histone residues being the best studied. Numerous post-translation modifications like acetylation, phosphorylation, ubiquitination and methylation have been identified to occur mostly on the N-termini and rarely at some residues of the globular portion of histones [1, 2]. Depending on how and which amino acid of which specific histone is modified, it can open or close the chromatin and thereby regulate gene transcription which is important for the selective usage of genetic information during development and differentiation [3]. While acetylation, phosphorylation and ubiquitination were long recognized as reversible marks, histone methylation marks were regarded as thermodynamically stable modifications that reflect long term epigenetic memory. Histones can be methylated at lysine and arginine residues; however, lysine methylation is increasingly becoming recognized as an important epigenetic mark implicated in determining cellular state [4, 5, 6, 7, 8]. Five well characterized histone

lysine methylations are positions 4, 9, 27 and 36 on histone H3 and position 20 on histone H4 [7]. Histone lysine methylation has also been identified within the core of histone H3 at lysine 79 [1, 7, 9]. Each of these lysine residues can either be mono- (me1), di- (me2) or tri-methylated (me3) [1, 7, 9]. The distinct methylation states add an additional regulatory layer. The effect of the modification increases with the number of methyl groups present through strengthening the binding affinity or providing binding sites for different chromatin remodeling factors [3, 7, 9, 10]. As a result, the chromatin structure is modified according to which and how the lysine residue is methylated and can result in an activating or repressive effect on gene transcription.

Histone lysine modifications are not equally distributed throughout the genome but preferentially occupy specific chromatin domains. The repressive and very stable histone H3 lysine 9 me3 (H3K9me3) and H4K20me3 marks are enriched in transcriptionally inactive pericentric heterochromatic regions of the genome while less stable marks like H3K9me1/me2 and H4K20me1/me2 are associated with euchromatic gene silencing [8, 10, 11, 12, 13]. Chromosome and gene-specific localizations showed the implication of histone lysine methylation marks in epigenetic phenomena like genomic imprinting and X-chromosome inactivation, with the me3 form of H3K27 being the key player in the latter [4, 14, 15, 16, 17, 18]. However, a number of studies have also identified the presence of repressive marks such as H3K9me3 and H4K20me3 at transcriptionally active sites thus reflecting the complex nature of histone lysine methylation [19].

The histone methyltransferases that catalyze the introduction of different histone lysine methylation marks are well characterized. All these enzymes, except for Dot1, belong to the SET domain protein family [1, 7, 20]. In some cases the same histone lysine residue can be targeted by multiple histone methyltransferases which may then lead to different outcomes depending on the methylation status [1, 7, 20]. However, the recent discovery of histone lysine demethylases has proven to be exciting as it has demonstrated the reversible, dynamic nature of histone lysine methylation marks. Thus, much of the attention has been focused on the study of the demethylation of histone lysine methylation marks. Many of these demethylases have since been implicated in regulating states of cellular differentiation in normal development and diseases; thereby emerging as important components of the epigenetic network that control cell fate [2, 21, 22, 23, 24].

Reversing the stable methylation of histone lysine residues

Histone modifications like acetylation, phosphorylation and ubiquitination are transient marks and several deacetylases, phosphatases and deubiquitinases have been identified [1]. In the absence of any known histone demethylases, histone methylation was initially regarded as stable and irreversible modification [25]. The reasoning for irreversibility of particularly lysine methylation came from several lines of evidence. Firstly, it was shown that the turn-over rate of the methyl-lysine groups were identical to that of the histones themselves [26, 27]. Secondly, since methyl-lysine marks like H3K9me3 are required for heterochromatin formation, which represents a stable part of chromatin, and are important for cell lineage establishment it was assumed that these marks needed to be thermodynamically very stable to ensure reliable inheritance of these silenced states [9, 25]. Thirdly, attempts of direct cleavage of the crucial amino-methyl bond to remove the methylation were unsuccessful and this emphasized the extraordinary thermodynamic stability [9, 25]. However, it became increasingly evident that epigenetic reprogramming does occur during differentiation and development and included methylated histone modifications as evidenced by nuclear transfer studies in mammals [28]. Several molecular studies provided the first direct evidence that histone lysine methylation could be dynamic. The investigation of H3K9 methylation associated with certain inducible inflammatory genes showed that the modification was erased following activation of these genes and restored during post-induction transcriptional repression [29]. Another illustration is the repression of the cyclin E promoter by H3K9 methylation in the G1 phase of the cell cycle which is later activated in the G1/S transition phase suggesting

that a reversal in H3K9 methylation has occurred to mediate this activation [30]. Similar dynamic changes were also observed for other histone methylation marks. For example, H3K27 methylation on the inactive X-chromosome in trophoblast cells has been observed to disappear during differentiation [15]. To explain this turnover of methyl groups in histones it was initially proposed that removal of methylation may occur either via clipping of histone tails or by replacement of the methylated histones with variant histones [31]. This hypothesis was dismissed with the discovery of the first histone lysine demethylase LSD1, an H3K4me1/2 demethylase. Although LSD1 can only mediate removal of mono- and di-lysine methylations, the discovery of these reactions shows that the introduction of histone methylation marks is reversible [25, 32, 33, 34]. This was shortly followed by the discovery of jmjC domain containing histone demethylases that can target all states of histone methylation, including me3 states [2]. These enzymes mediate demethylation by catalyzing hydroxylation of the methylated histones with most members of this family of demethylases targeting lysine methylation [2].

The flexibility of the histone lysine methylation system is a key component of the mechanism that regulates cellular state. In mouse embryonic stem (ES) cells the balance between H3K27me3 and H3K4me3 has been shown to determine whether the cells remain pluripotent or move towards differentiation. ES cells were found to contain regions with both repressive H3K27me3 and activating H3K4me3 marks [35, 36]. These regions have been termed bivalent domains and were found to occur at promoters of genes associated with developmental and signaling pathways [35, 36]. It has been proposed that these bivalent domains serve the function of keeping developmental genes repressed while ensuring that they remain poised for activation. This allows for the necessary responsiveness to ES cell differentiation and the demand for lineage-specific changes to the gene expression profiles [35, 36]. Later a study by Bilodeau *et al.* also identified the presence of H3K9me3 marks and the associated histone methyltransferase Setdb1 at some of these bivalent regions where they, in association with H3K27me3, function to keep developmental genes in a repressed state [37]. The induction of the appropriate histone demethylases during differentiation would be essential for this type of conversion from the bivalent to the active or repressed state. Changes in histone lysine methylation pattern have also been shown to be a prime occurrence in cellular differentiation during normal embryonic development or in cases of diseases [21, 22, 23]. As development proceeds from pluripotency to a differentiated, specialized organism considerable changes are seen in histone lysine methylation patterns [28, 38, 39]. Certain histone lysine methylation marks are initially lost and reintroduced at later stages as cells become more specialized, indicating the presence of active methylases as well as demethylases that regulate this precise patterning of histone lysine methylation marks. Similarly, during nuclear transfer the established histone lysine methylation pattern in the donor nuclei needs to be reprogrammed to the extent that it will be capable of supporting development. This process involves the activity of histone demethylases and the initial removal of lysine methylation marks. In accordance with this requirement, stage specific appearance of the jmjC domain containing demethylases has been identified during embryonic development [40, 41]. Erasure of stable marks like H3K9me3 and H3K27me3 and the presence of the associated jmjC domain containing demethylases have also been detected in several other cellular processes such as inflammation, induced trans-differentiation and cancer [23].

In the following review, we summarize our present knowledge about the biological function of the main histone lysine demethylases that has emerged from intensive research efforts since the discovery of the first histone lysine demethylase in 2004. The main focus is placed on the role these enzymes play in cellular reprogramming and pluripotency. Table 1 provides a list of the lysine demethylases described in this review with a brief synopsis of their substrate specificity, effects on gene transcription and cellular function.

KDM1/LSD1 family

LSD1 (KDM1A/Aof2) was the first histone lysine specific demethylase to be identified [34]. With the rapid discovery of more demethylases a new nomenclature was proposed which refers to lysine

Table 1. Histone lysine demethylases substrate specificity and known cellular functions.

Family	Demethylase	Substrate	Transcriptional effect	Function
KDM1/ LSD	KDM1A	H3K4me1/2 H3K9me1/2	Repression Activation	Mouse embryonic development [58] Cellular differentiation [46, 48, 50, 51, 52] Maintenance of human ES cell state [53] AR and ER-α signaling [55, 56] Negative or positive regulation of cancer growth [47, 54, 56] Epigenetic reprogramming of epithelial to mesenchymal transition [57]
	KDM1B	H3K4me1/2	Repression or Activation[1]	Establishment of maternal imprints [61] Transcriptional elongation [60]
KDM2/ JHDM1	KDM2A	H3K36me1/2	Repression or Activation[1]	Context dependent negative or positive regulator of cell growth [70, 71] Reprogramming to induced pluripotency [71]
	KDM2B	H3K36me1/2 H3K4me3	Repression or Activation[1]	rRNA repression [65] Repression at CpG island [68] Context dependent negative or positive regulator of cell growth [66, 67, 69, 70, 71] Reprogramming to induced pluripotency [71]
KDM3/ JHDM2/ JMJD1	KDM3A	H3K9me1/2	Activation	Self-renewal of mouse ES cells [75] AR signaling [73] Gene transcription during spermatogenesis [77] TGF-β-mediated smooth muscle cell differentiation [78] HIF-1α signaling [79]
	KDM3B	H3K9me1/2	Repression	Putative tumor suppressor [80]
	KDM3C	H3K9me1/2	Activation	Expressed in undifferentiated ES cells [76] THR and AR signaling [81, 82] Testicular steriodogenesis [74]
KDM4/ JHDM3/ JMJD2	KDM4A	H3K9me2/3 H3K36me2/3	Activation Repression	Both negative and positive regulation of cell cycle progression [88, 89] AR signaling [91] Promoter of cancer growth [90, 91, 92] Skeletal muscle differentiation [93]
	KDM4B	H3K9me3 H3K36me2	Activation Repression	De-repression of heterochromatin-associated transcripts [83] ER-α signaling [95, 96, 97] HIF-1α signaling [79, 97] Growth promotion in ER-α responsive cancers [95, 96, 97]

Table 1 continued..

	KDM4C	H3K9me2/3 H3K36me2/3	Activation Repression	Negative regulator of heterochromatin formation [98] Maintenance of pluripotency in ES cells [75] Reprogramming of genome during embryonic development [40] AR signaling [101] Activation of oncogene [102]
	KDM4D	H3K9me1/2/3 H1.4K26me2/3	Activation Activation	De-repression at heterochromatic sites [103, 104] AR-signaling [91] Promoter of colon cancer cell proliferation [105] Co-activator of p53 [105] Regulation of spermatogenesis [104]
KDM5/ JARID1	KDM5A	H3K4me2/3	Repression	RB binding [107, 108] Hox gene silencing [107] Regulation of ES cell pluripotency and differentiation [107, 111]
	KDM5B	H3K4me1/2/3	Repression or Activation[1]	ES cell renewal [112] Embryonic development [114] Neuronal differentiation [113] Growth promoter in ER-α positive breast cancer and prostate tumors [114, 115]
	KDM5C	H3K4me2/3	Repression	Repression at the X chromosome [118] X –linked mental disorders [118, 119] REST-mediated neuronal differentiation [120]
	KDM5D	H3K4me2/3	Repression	Association with Y chromosome [118] Association with polycomb-like protein Ring6 and suppression of transcription [121]
KDM6	KDM6A	H3K27me2/3	Activation	Activation of Hox gene expression [124, 125] Reprogramming to iPS cells [126] Gonadal development [126]
	KDM6B	H3K27me2/3	Activation	Activation of Hox gene expression [125] Regulation of gonadal development [125] ES cell differentiation to neuronal lineage and neurogenesis [127, 128, 129] Reprogramming of genome during embryonic development [130] Inflammation induced cellular differentiation [131] Cancer dynamics [132]

Table 1 continued..

KDM7	KDM7A	H3K9me1/2 H3K27me1/2	Activation Activation	Follistatin gene transcription in neuronal cells [134] Brain development in zebrafish [134] ES to neuronal state transition [135]
	KDM7B	H3K9me1/2 H4K20me1 H3K27me3	Activation Activation Activation	rRNA transcriptional activation [136] Cell cycle progression [138, 139] Brain development in zebrafish [138] X-linked mental retardation syndrome and craniofacial anomalies [140]
	KDM7C	H3K9me1/2	Activation	rRNA transcriptional activation [137] PKA dependent transcriptional activation [141]
JmjC domain only	MAPJD	H3K4me1/2/3 H3K36me2/3	Activation or Repression[1]	Repressor of osteoblast differentiation [142] Growth promoting effects [143]
	KDM8	H3K36me2	Activation or Repression[1]	Embryonic development [144, 145] Regulation of cell cycle associated gene expression [145, 146] Growth promoting effects in cancer cells [144, 146]

AR, Androgen receptor; ER-α, Estrogen receptor-α; TGF-β, Transforming growth factor-β; rRNA, ribosomal RNA; THR, Thyroid hormone receptor; HIF-1α; Hypoxia-inducible factor-1α; RB, Rentinoblastoma protein; PKA, Protein kinase A; iPS, Induced pluripotent cells.

[1]KDM-mediated demethylation of the same histone lysine methylation result in transcriptional activation or repression depending on the chromatin environment and recruitment.

demethylases as KDMs, which stands for K (lysine) demethylase [42]. Because the original names are still in frequent use today we will provide both old and new names for the enzymes we describe in detail below. KDM1A, previously known as LSD1, has been characterized as a flavin-dependent amine oxidase due to extensive sequence homology with these enzymes but it also contains a nuclear localization signal and a SWIRM domain that is often found in chromatin-associated proteins [34, 43]. Demethylation by KDM1A does not involve direct cleavage of the amino-methyl bond but rather an amine oxidation of the methylated histone lysine. This oxidation results in the formation of an imine intermediate with concomitant reduction in flavin adenine dinucleotide (FAD) to $FADH_2$ which is then reoxidized to hydrogen peroxide [25, 34, 44]. Subsequently, the imine is hydrolyzed by a non-enzymatic reaction to a carbinol amine which in the following reaction step is degraded to formaldehyde, all of which ultimately results in an unmethylated lysine. Since the formation of the imine requires a protonated lysine, only mono- or di-methylated but not tri-methylated lysines are suitable substrates for KDM1A [25, 34, 44]. The enzyme targets me1 and me2 H3K4 marks with the demethylase activity resulting in an effect of transcriptional repression [34].

The association of KDM1A with co-repressor complexes such as Co-REST and NuRD further supports its functional role as a repressor of gene transcription [43, 45, 46, 47, 48]. Knockout studies in mice have demonstrated that KDM1A deletion results in embryonic lethality and its presence is essential for lineage commitment. Consistent with this notion, KDM1A complexed with NuRD and Co-REST co-repressors has been identified as a key regulator that controls the changes of the transcriptional programme during ES cell differentiation [46, 48]. In mouse ES cells, KDM1A was identified in complexes with NuRD at bivalent control regions and the enhancers and promoters of actively transcribed genes occupied by the ES specific transcription factors Oct4, Sox2, Nanog and the Mediator co-activator complex [46]. Its presence was not essential to maintain ES cell state but was required for demethylation of H3K4me1 at these regions and associated silencing of ES cell-specific gene expression patterns; thereby facilitating successful differentiation into new cell states [46]. In association with Co-REST complexes that possess histone deacetylase activity, KDM1A has been demonstrated to be required for maintaining the precise expression levels of lineage commitment genes like brachyury, homobox genes and retinoic acid receptor γ during ES cell differentiation and embryonic development [48]. This suggests that the function of KDM1A in facilitating ES cell differentiation is intricately regulated by pluripotency factors and other post-translation histone modifications [46, 49]. Further support comes from findings that the presence of histone acetyltransferases and acetylated histones can negatively regulate KDM1A demethylase activity [49]. Based on these observations it has been proposed that recruitment of histone acetyltransferases to Oct4-controlled enhancers keeps the acetylation of histones sufficiently high to suppress KDM1A demethylation. However, when ES cells are stimulated to differentiate and the levels of Oct4 and associated histone acetyltransferases decrease, the resulting lower histone acetylation levels then allow KDM1A-mediated H3K4me1 demethylation and silencing of these enhancers [46]. The function of KDM1A as a key player in normal differentiation is not restricted to mouse ES cells but has also been demonstrated in a number of other cell types such as pre-adiopocytes, plasma cells and skeletal muscle cells [50, 51, 52]. However, KDM1A may have a different role in human ES cells where it was shown to be essential for maintaining pluripotency by balancing the level of H3K4me2/H3K4me3 marks at the promoters of target developmental genes [53]. Further support for this functional correlation comes from the finding that KDM1A is upregulated in pluripotent cancer cells such as teratocarcinoma, embryonic carcinoma and seminoma that express the Oct4 or Sox2 where it promotes cell growth but not in non-pluripotent cancer cells [54].

In addition to its role as transcriptional repressor in association with the demethylation of H3K4me1/me2 marks, KDM1A has activator function when bound to androgen (AR) or estrogen receptors (ER) due to a change in substrate preference for the demethylation of me1 and me2 H3K9

marks [55, 56]. In MCF-7 breast cancer cells, the H3K9me1/me2 demethylation activity of ER-α-associated KDM1A was shown to be required for transcription of the receptor's target genes and suggests a role for KDM1A in the epigenetic regulation of ER-α responsive breast tumor cells [56]. Contrary to this activating role, KDM1A was found to suppress the metastatic potential of ER-α-negative breast cancer MDA-MB-231 cells [47]. Yet another study has linked the dual roles of KDM1A in repressing and activating gene loci with histone remodeling and cellular state during epithelial to mesenchymal transitions of development [57].

Besides histones as a substrate target, KDM1A has subsequently been found to have specificity for some non-histone substrates such as p53 and the DNA methyltransferase DNMT1 [58, 59]. KDM1A-mediated demethylation of DNMT1 results in the stabilization of the methyltransferase and was shown to be required for maintaining global DNA methylation levels in mouse ES cells [58].

Together these findings demonstrate an important role for KDM1A in maintaining or influencing cellular epigenotype.

KDM1B also known as Aof1 or LSD2 is another member in the KDM1 demethylase family [60]. Like KDM1A, it is an amine oxidase, possesses the flavin-dependent amine oxidase and SWRIM domains, demethylates H3K4me1/me2 and functions as a transcriptional repressor. Despite these common features it has less than 31% sequence identity with KDM1A [60]. Deletion of KDM1B in mouse oocytes increased H3K4me1 methylation and prevented *de novo* DNA methylation at a subset of imprinted genes during oogenesis which resulted in biallelic expression of these genes in embryos derived from these oocytes causing embryonic lethality at day 10.5 d.p.c. [61]. These findings suggest a key role for KDM1B in establishing epigenetic state of oocytes and maternal imprints required for successful embryonic development. Uniquely, KDM1B was found to associate with gene bodies of active genes particularly at the 3' end, regions enriched in the activating H3K36me3 mark [60]. Demethylation by KDM1B was shown to reduce H3K4 methylation and, due to the association with the H3K9me2 methyltransferase G9a, maintain H3K9me2 levels within these regions. This generates a repressive environment that appears to be required for optimal transcriptional elongation and gene expression [60]. The incorporation of repressive marks into transcriptional active regions to ensure correct gene expression displays the complex nature of epigenetic regulation and possibly represents the intricate mechanistic systems employed by cells to prevent non-specific or unwanted transcription [60].

JmjC domain containing histone lysine demethylases

In the quest of identifying other potential histone lysine demethylases and in particular a tri-methyl-specific one, it was proposed that more potent mechanisms such as oxidative methylation or hydroxylation catalyzed by enzymes containing 2-oxoglutarate dependent dioxygenases could be involved [62]. One candidate for such demethylases is the class of 2-oxoglutarate (OG) Fe II dependent dioxygenases that use Fe II in their catalytic site to activate a molecule of dioxygen to form highly reactive oxo-ferryl species which are capable of hydroxylating the specific substrate [62]. The reaction of histone demethylation catalyzed by these enzymes was proposed to be similar to the mechanisms used by the DNA repair demethylase AlkB, a 2-OG Fe II dependent dioxygenase, to hydroxylate the methyl groups implicated in certain forms of DNA methylation damage [62].

Based on structural similarities with a jmjC domain containing protein hydroxylase that belongs to the family of 2-OG Fe II dependent dioxygenases, the yeast protein EpeI, known to regulate heterochromatin integrity and to contain a conserved jmjC domain, was predicted to be a histone demethylase [62]. Subsequently, sequence alignment analyses confirmed that several other jmjC domain containing proteins shared distinct features with 2-OG Fe II dependent dioxygenases suggesting that many of them could be capable of catalyzing demethylation [62].

The jmjC domain originates from the jmj or jumoji gene that was initially identified in mouse as a key gene important for development [63]. It contains a DNA binding ARID domain and two

conserved jmj domains, jmjN and jmjC [63]. The jmjC domain is highly conserved between species and approximately 30 jmjC domain containing proteins have been identified in the mouse genome [63]. Based on sequence homology, the jmjC domain containing proteins with histone demethylase activity have been classified into seven families; KDM2/JHDM1, KDM3/JHDM2/JMJD1, KDM4/JHDM3/JMJD2, KDM5/JARID1, KDM6, KDM7 and MAPJD/KDM8 families [2, 23]. Although the jmjC domain is present in all seven families, additional domains also appear to be essential for their catalytic activity [2].

Structural analyses have shown that the jmjC domain folds into eight β-sheets to form the enzymatically active site that co-ordinates Fe II and α-ketoglutarate. Consistent with their important function, the three amino acids that co-ordinate Fe II binding at the active site, are fully conserved in all members [44]. In the first step, the reaction generates a carbinol amine intermediate and succinate. Carbinol amine is then hydrolyzed by non-enzymatic reactions to formaldehyde which produces an unmethylated lysine [44]. While many more members of the various jmjC demethylase families have been identified, the detailed description below has been limited to those with histone lysine demethylase activity.

KDM2/JHDM1 family

The KDM2 enzymes were the first jmjC domain containing demethylases to be identified. This family consists of many related proteins but key ones that have been shown to possess demethylase activity include KDM2A/JHDM1A/FBXL11, KDM2B/JHDM1B/FBXL10 and the yeast homologue EpeI [64]. Apart from the crucial jmjC domain, members of this family contain four additional domains: the F-box, CXXC zinc finger, leucine-rich repeat and PHD domains [2]. Demethylase activity of KDM2 enzymes mainly depends on the jmjC domain although deletions of the CXXC zinc finger, PHD domain and leucine-rich repeats were found to partially impair the enzymatic activity [64].

The demethylases in this family target mono- and di-methyl H3K36. Both are known to be marks of actively transcribed genes [64]. However, KDM2B has also been shown to target H3K4me3 marks, another mark associated with transcriptional activation [65]. H3K36me2 and H3K4me3 marks are primarily enriched at the 3' regions of transcribed genes and may play an important role in transcriptional elongation. Hence, the major function of KDM2-mediated demethylation is transcriptional repression [7]. Consistent with this, KDM2B has been shown to be recruited to CpG islands, suppress transcription of ribosomal RNA genes, inhibit the oncogenic protein C-jun and thereby negatively regulate cellular proliferation [65, 66, 67, 68]. Based on these findings KDM2B was suggested to be a negative regulator of cellular transformation in tumor progression. In accordance with this notion, retroviral insertional mutagenesis of the KDM2B encoding gene resulted in the development of lymphomas in BLM mice and very low levels of KDM2B were detected in aggressive brain tumors [65, 69]. In contrast to these studies, Pfau *et al*. reported that human lymphomas and mammary adenocarcinomas were associated with increased levels of KDM2B. Furthermore, overexpression of KDM2B as well as KDM2A resulted in the immortalization of mouse embryonic fibroblasts and identified these demethylases as oncoproteins [70]. Further support for KDM2B's role as a positive regulator of cellular proliferation came from a study investigating the Ink4/Arf locus, a region known to function as a roadblock for cell cycle progression. KDM2B-mediated demethylation of H3K36me2 marks at this locus resulted in the silencing of this region and promoted cellular proliferation [67]. Apart from tumor biology, KDM2 demethylases have been studied in the context of cellular reprogramming to pluripotency. Wang *et al*. observed that the reprogramming of fibroblasts into induced pluripotent stem cells following the transduction with the four reprogramming factors Sox2/Klf4/Oct4/c-Myc in the presence of the known reprogramming enhancer vitamin C, correlated with increased levels of KDM2 demethylases and reduced H3K36me2/me3 levels [71]. Subsequent knockdown of KDM2 demethylases resulted in impaired reprogramming and demonstrated their role as key effectors of vitamin C assisted reprogramming of somatic cells to pluripotency. Further characterization of the cells showed that KDM2B promotes Oct4 reprogramming by repressing the Ink4/Arf locus

and also co-operates with Oct4 to activate the pluripotency associated microRNA 302/387 cluster [71]. Both functions were dependent on its demethylation activity, since a significant reduction in H3K36 methylation was observed at the Ink4/Arf locus and at the promoter of micro RNA 302/387 cluster [71]. An independent study that investigated KDM2B-dependent demethylation of HK36me2 marks at the promoters of genes involved in early reprogramming confirmed the ability of KDM2B to facilitate reprogramming and improve the generation of induced pluripotent stem cells [72].

The effects of KDM2 histone demethylases, with their ability to remove the activating histone methylation marks H3K36 me2/3 and H3K4me3 that can result in both gene activation and suppression functions, illustrate the complexity and context dependency of the epigenetic regulation of histone modifications.

KDM3/JHDM2/JMJD1 demethylase family

KDM3A/JHDM2A/JMJD1A, KDM3B/JHDM2B/JMJD1B and KDM3C/JHDM2C/JMJD1C are the three proteins in this family with demethylase activity; displaying substrate specificity for mono- and di-methyl H3K9 marks [2, 73, 74]. Structurally, members of this family contain the jmjC domain and a zinc finger domain which were found to be essential for their catalytic activity [2].

KDM3A was found to be involved in transcriptional activation of the pluripotency genes Tcl1, Tcl1, Tcfcp2l1 and Zfp57 by mediating demethylation of H3K9me2 at their promoter regions [75]. In addition, KDM3A and even KDM3C were identified as direct downstream targets of Oct4, an important regulator of pluripotency in ES cells [75, 76]. The RNAi-mediated knockdown of KDM3A resulted in decreased Oct4 expression, which was associated with the stimulation of differentiation of ES cells and supports a functional role for KDM3A in maintaining the self-renewal potential of ES cells.

Besides the involvement in determining ES cell function, KDM3A has been implicated in a number of other biological processes. It associates with AR in a hormone-dependent manner and can activate AR associated gene transcription by demethylating H3K9me1/me2 marks [73]. Deletion of KDM3A in mice suggested a role in spermatogenesis. Knockout mice displayed post-meiotic condensation defects in sperm cells which led to low sperm count, aberrant differentiation into mature sperm with majority of them showing morphological defects and low motility [77]. Moreover, KDM3A-mediated demethylation of H3K9me1/me2 resulted in transcriptional activation of Tnp1 and Prm1, two proteins involved in histone replacement during sperm packaging and condensation [77]. Its interaction with smooth muscle cell differentiation associated myocardin related transcription factor A and upregulation of smooth muscle cell differentiation associated gene transcription, as a result of H3K9 methylation-dependent activation of TGF-β, suggests a role for KDM3A in regulating smooth muscle cell differentiation [78]. KDM3A has also been implicated in the signaling pathway of hypoxia-inducible factor-1α which is a regulator of cellular adaptability to low oxygen. Association with KDM3A induces its expression in hypoxic conditions. These findings suggest possible roles for KDM3A in regulating epigenetic reprogramming in hypoxic conditions as prevalent in tumor growth [79]. Despite a H3K9me1/me2 demethylase activity very similar to KDM3A, a tumor suppressor function has been designated for the closely related enzyme KDM3B [80]. Evidence for this comes from the findings that the 5q31 chromosomal region which carries the KDM3A gene is deleted in malignant myeloid disorders like acute myeloid leukemia and myelodysplasias. Furthermore forced expression of KDM3B in 5q31 deleted cell line resulted in inhibition of clonogenic growth [80].

Like KDM3A, KDM3C has also been implicated in hormone-dependent signaling. The demethylase was first identified as a binding partner of thyroid hormone receptor and later was shown to associate with AR in a hormone-dependent manner [81, 82]. A role for KDM3C has been demonstrated in gene transcription during testicular steroidogenesis [74]. KDM3C was found to interact with an H3K4 and H3K27 methyltransferase, WHISTLE and regulate steroidogenic factor-1 (SF-1) mediated transcription of the steroidgeneic marker p450c17. SF-1 activated gene transcription of p450c17 is kept repressed due to its interaction with WHISTLE and associated methylation of H3K4

and H3K9. However, upon induction of testicular steroidogenesis, SF-1 switches to KDM3C as its preferred binding partner to remove the H3K9 methyl marks and thereby activate p450c17 transcription. Thus, these findings suggest role for KDM3C in epigenetic regulation of transcription in the testis during development.

KDM4/JHDM3/JMJD2 demethylase family

The KDM4/JHDM3/JMJD2/family consists of four histone demethylases: KDM4A/JMJD2A, KDM4B/JMJD2B, KDM4C/JMJD2C and KDM4D/JMJD2D [45]. All four members contain the jmjC domain, the jmjN domain and the conserved amino acids associated with Fe II binding and α-ketoglutarate at the active site [2]. KDM4D is an exception in this family because it lacks the two PHD and two tudor domains present in the other three demethylases [45, 83]. Unlike KDM2A and KDM3 enzymes, members of the KDM4 family are able to catalyze demethylation of tri-methylated histones and this catalytic activity depends on the presence of the jmjC and jmjN domains [83]. The substrate specificity for this family of demethylases includes H3K9 and H3K36 methylation marks that are associated with repressed and actively transcribed regions, respectively [84]. Thus, via their dual specificity for contrasting histone modifications these demethylases have the potential to regulate both gene suppression and activation. H3K9me3 modifications are an essential part of the epigenetic regulatory system that maintains repression and stability of heterochromatin [8, 85]. The current view of the underlying mechanism assumes that the introduction of H3K9me3 modifications at gene promoters results in the creation of a heterochromatin-like environment where gene transcription is repressed [23]. Due to the great stability of H3K9me3 marks, these marks represented ideal candidates for sustaining lineage commitment and establishing long-term epigenetic memory. However, the discovery of the KDM4 demethylases capable of removing these repressive marks revealed that even these “stable” marks are dynamic. The discovery of a regulatory mechanism for tri-methyl histone modifications provided new opportunities to gain insights into chromatin dynamics and to modulate cellular fate.

KDM4A shows substrate specificity for the me2 and me3 states of H3K9 and H3K36 [84, 86]. Consistent with its dual role as a repressor or activator, the tudor domain of KDM4A binds to the activating mark H3K4me3 and the repressive marks H4K20me2/me3, although the physiological significance of this binding is not known [87]. KDM4A mediates its transcriptional repressive role through interacting with the tumour suppressor protein retinoblastoma (pRB) and inducing pRB-mediated repression of E2F transcription factors; proteins that activate genes involved in cell cycle progression [88]. This demethylase also associates with the co-repressor N-CoR to repress N-CoR target genes like ASCL [86]. In contrast to this repressive function, Black and co-workers showed that overexpression of KDM4A enhanced S-phase progression and chromatin accessibility [89]. The finding was further supported by a recent study that provided evidence for the role of KDM4A as a positive regulator of cell proliferation in colon cancer [90]. KDM4A demethylase has also been shown to be involved in hormone-dependent signaling [91]. It can interact with AR in a ligand-dependent manner and higher levels of KDM4A have been detected in prostate tumors, suggesting a role in regulating cellular transformation in pathological conditions [91]. Consistent with such a function, knockdown of KDM4A by RNA interference in breast cancer cell lines resulted in the inhibition of proliferation and tumor invasion [92]. Another functional aspect was revealed in a study with an isoform of KDM4A. Mediated by its H3K9me2/me3 demethylase activity, KDM4A lifted the repression of the muscle differentiation-specific gene Myog and initiated skeletal muscle differentiation [93].

An elegant study by Jeong *et al.* used fusion proteins of KDM4A with heterochromatin protein 1 (HP1) or Oct4 to induce site-specific demethylation of H3K9me3 at HP1 binding sites in heterochromatin and regulatory regions of Oct4 target genes, respectively [94]. Tagging with HP1 proteins enhanced the efficiency of H3K9me3 demethylation due to greater accessibility to the repressive pericentric regions. KDM4A fused to Oct4 resulted in demethylation of H3K9me3 at the promoters of Oct4 target genes like Sox2 and Nanog. Surprisingly, KDM4A-Oct4-mediated demethylation of H3K9me3 also turned on Sox2

and stem cell related microRNA expression in somatic cells, where they are normally repressed [94]. These findings suggest that demethylation of robust repressive marks like H3K9me3 by lysine demethylases allows epigenetic memory to be reprogrammed and influences transformation of somatic cells to a pluripotent state.

KDM4B catalyzes demethylation of both H3K9me3 and H3K36me2 but there appears to be a greater preference for the me3 state of H3K9 [83]. Overexpression of KDM4B mainly resulted in removal of H3K9me3 from the pericentric heterochromatin regions, accompanied by only a small decrease in H3K36me2 [83]. As a consequence of H3K9me3 removal, enhanced transcription was observed at the target regions of the H3K9me3-specific methyltransferase Suv39h1, such as the major satellite repeats which are embedded in regions of pericentric heterochromatin and other repetitive elements like LINE L1 and IAPLTR1 [83]. In addition, KDM4B has been identified as a component of the MLL2 complex that catalyzes H3K4 methylation, and has been shown to be implicated in ER-α associated transcription [95]. ER-α activated stimulation of transcription was found to require demethylation of H3K9 as a prerequisite for methylation of H3K4 by MLL2 methyltransferase to occur [95]. KDM4B itself was found to be a target of ER-α activity, indicating that the interaction between the two proteins forms a positive feedback loop in the estrogen-dependent signaling pathway [95]. Consistent with this close relationship, high levels of KDM4B have been detected in ER-α positive breast tumors where they function as mediators of estrogen-induced growth [95, 96, 97]. KDM4B is also a target of hypoxia inducible factor and has been implicated in regulating hypoxic gene expression pattern [79, 97].

KDM4C, also known as GASCI, can demethylate di- as well as tri-methyl H3K9 and tri-methyl H3K36 [98]. KDM4C-mediated removal of H3K9me3 was found to adversely affect heterochromatin formation by delocalizing HP1 [98]. In ES cells, KDM4C has an essential role for maintaining pluripotency [75]. Deletion of KDM4C in these cells resulted in a decrease in Oct4, Nanog and other pluripotency associated genes and subsequent differentiation [75]. KDM4C activity was essential for removing H3K9me3 marks from the Nanog promoter which in turn removes the binding sites of repressive proteins such as HP1 and KAP1 and positively regulates Nanog expression [75]. The demethylase has also been shown to be essential for embryonic development and was found to be stage-specifically expressed in mouse embryos [40]. Mirroring the consecutive waves of DNA demethylation and methylation during early embryonic development [28, 38, 39, 99, 100], KDM4C was found to increase from the two-cell to the eight-cell stage and thereafter decrease with very low levels being detected at morula and blastocyst stages [40]. The knockdown of KDM4C in metaphase II arrested oocytes with subsequent parthogenetic activation showed decreased expression of pluripotency- and proliferation-associated genes in early embryos and resulted in developmental arrest prior to the blastocyst stage [40]. These findings indicate that KDM4C is essential for the correct reprogramming of the embryonic genome and plays a role in regulating cellular state or fate and differentiation. In addition, KDM4C has been implicated in AR-dependent transcriptional activation and has been shown to promote transcription of the oncogene Mdm2 [101, 102].

Unlike other KDM4 enzymes, KDM4D lacks the PHD and tudor domains [2, 84]. The enzyme can catalyze demethylation of H3K9me1/me2 and me3 and also H1.4K26me2/me3 [84, 103]. H1.4K26 methylation marks are enriched in heterochromatin and maintain repression by recruiting HP1 [103, 104]. In contrast to the other members of the demethylase family, KDM4D does not appear to have any enzymatic activity towards H3K36 methylation [105]. Thus, KDM4D functions as a transcriptional activator by removing repressive histone methylation marks. Consistent with a role as transcriptional activator due to the removal of repressive H3K9 marks, KDM4D has been shown to be a promoter of proliferation in colon cancer cells [105]. However, in contrast to the function as promoter of cancer growth, the demethylase was also found to operate like a co-activator of p53, a tumor suppressor [105]. Moreover, the H3K9me3-specific demethylase activity of KDM4D has been shown to be essential

for spermatogenesis [104] and similar to the activity of other KDM4 demethylases, is involved in hormone-dependent signaling in association with AR [91].

From the accumulated experimental details of these studies, KDM4 demethylases with their variable substrate specificity emerge as key determinants in regulating cellular state, differentiation and embryonic development.

KDM5/JARID1 family

The mammalian KDM5 family consists of four demethylases: KDM5A/JARID1A, KDM5B/ JARID1B, KDM5C/JARID1C/SMCX and KDM5D/JARID1D/SMCY [2]. Structurally these demethylases possess jmjC, jmjN, AT-rich interactive Arid, C5HC2 zinc finger and PHD finger domains [2]. All four demethylases target H3K4 methylation and so function as transcriptional repressors [106]. KDM5B can remove all three methylation states of H3K4 while KDM5A, KDM5C and KDM5D can demethylate only the me3 and me2 forms [106, 107, 108, 109].

KDM5A also known as retinoblastoma binding protein 2, was first characterized as a binding partner of pRB [107, 108]. The association with pRB was shown to transform KDM5A from a repressor to an activator of genes involved in differentiation [110]. Consistent with this role as a cellular state regulator, KDM5A has been implicated in Hox gene silencing in Drosophila and in mouse ES cells [107, 111]. KDM5A is recruited to target sites by Polycomb Repressive Complex 2 (PRC2); which is a complex with H3K27me3 methyltransferase activity that represses developmental genes. PRC2 is displaced from these regions during ES differentiation, which is consistent with its recruitment function for KDM5A and correlated with an increase in H3K4me3 levels [107, 111]. Coordinated regulation by PRC2 and KDM5A was also reported in association with ES cell differentiation, which involved specific repression of genes not required for the induction of the differentiated cell types [107, 111].

KDM5B has been linked to multiple biological processes, including cellular differentiation, embryonic development, ES cell self-renewal and regulation of transcriptional programs of ES cells during differentiation into neuronal lineages [112, 113, 114]. In mouse ES cells KDM5B was found to function as a transcriptional activator of genes involved in proliferation and DNA synthesis and thereby facilitate self-renewal [112]. The demethylase was demonstrated to be a target of Oct4 and Nanog transcription and, like KDM1B, is recruited to intragenic sites of its target genes by H3K36me3 to create a repressive environment for optimal elongation by preventing unwanted transcription. In addition, KDM5B has been implicated in the regulation of the cancer cell state and was found to be upregulated in ER-α positive breast cancer cells, ductal breast carcinomas and prostate cancer tissues [114, 115, 116].

KDM5C co-localizes with the X chromosome [117]. Binding to the X-chromosome is accomplished through the interaction of its N-terminal PHD domain with H3K9me3 marks located on the X-chromosome and helps to coordinate demethylation of H3K4me3 with H3K9 methylation to maintain transcriptional repression [118]. Point mutations in KDM5C are associated with X-linked mental disorders which are characterized by a lower binding affinity for H3K9me3 and reduced H3K4me3 demethylation [118, 119]. KDM5C has also been shown to associate with other chromatin regulators such as the co-repressor REST and via its demethylation has been implicated in REST-mediated neuronal gene transcription [120].

Similar to KDM5C, KDM5D is specifically associated with a sex-chromosome, in this case with the Y chromosome [118]. The demethylase was found to associate with polycomb-like protein Ring6 and suppress transcription of specific genes [121].

Taken together, findings from these different studies demonstrate that KDM5 demethylases are important regulators of cellular state during normal development and pathological conditions.

KDM6/UTX/UTY family

KDM6A/UTX (ubiquitously transcribed tetracopeptide) is located on the X chromosome and escapes X inactivation, KDM6C/UTY is located on the Y chromosome [122]. Structurally

both KDM6A and KDM6C possess the jmjC domain and tetracopeptide domains, while the other member of the protein family, KDM6B (also known as JMJD3), only contains the jmjC domain and lacks the tetracopeptide domains [2]. Both KDM6A and KDM6B have been shown to possess H3K27me3/me2 demethylase activity and therefore function as transcriptional activators. H3K27 methylations are repressive marks that are implicated in a variety of important biological process such as X chromosome inactivation, germline development, ES cell state, inflammation and cancer [123]. Given their role in a wide range of functions, the discovery and study of H3K27 demethylases that regulate these repressive marks has provided new insights into the chromatin dynamics associated with these cellular processes.

Consistent with this role, KDM6A has been shown to positively regulate transcription of Hox genes [124, 125]. Upon retinoic acid induced differentiation of human pluripotent carcinoma NT2/D1 cells to neuronal lineages and the differentiation of mouse ES cells, KDM6A was found to be recruited to the promoters of Hox genes [124, 125]. This targeting of KDM6A coincided with the disappearance of H3K27me3 and components of the PRC2 complex and resulted in the transcriptional activation of Hox genes. Conversely, knockdown of KDM6A inhibited both H3K27me3 demethylation and the activation of Hox genes. Furthermore, KDM6A-mediated reduction of H3K27me3 marks at Hox gene promoters is associated with an increase in H3K4 methylation because KDM6A interacts with and can recruit the H3K4-specific methyltransferase complexes MLL2/3 to these target sites [124, 125].

Another aspect of the biological role of KDM6A is its involvement in cellular reprogramming. KDM6A was found to associate with the key reprogramming factors Oct4, Sox2 and Klf4 and, mediated through its H3K27me3 demethylase activity, facilitated efficient induction of somatic into iPS cells [126]. During embryonic development, KDM6A is required for the reprogramming of primordial germ cells and removes their H3K27me3 marks between embryonic day 10.5 and 11 in mouse to allow for correct germ cell development [126]. Similar to the localization described for KDM6A, KDM6B associated with Hox gene promoters which play an essential function in mediating normal gonadal development, was demonstrated for the C. elegans orthologue of KDM6B [125]. The demethylase has also been implicated in neurogenesis and was shown to be required for ES cell differentiation to the neural lineage [127, 128, 129]. Consistent with this function as a developmental and cellular differentiation regulator, the demethylase regulates H3K27me3 levels in pre-implantation bovine embryos which enables correct embryonic genome activation and facilitates development to blastocysts [130]. RNA interference-mediated knockdown of KDM6B in metaphase II arrested oocytes followed by parthogenetic activation, inhibited the wave of H3K27me3 demethylation, reduced the number of blastocysts generated and blastocyst cell number. The regulation of H3K7me3 methylation marks in pre-implantation embryos appears to be specific for KDM6B and not shared by KDM6A because, while the expression of KDM6B coincides with the decrease in H3K27me3, this is not the case for KDM6A.

Additional functional aspects that have been associated with KDM6B-mediated H3K27me3 demethylase activity include the control of macrophage differentiation during inflammation [131] and correlation of enhanced expression levels with prostate cancer [132]. Thus, these findings suggest that KDM6 demethylases are important regulators of cellular differentiation, and KDM6B has a more specialized role in pre-implantation development. However, this does not include KDM6C for which no histone demethylase activity has been described so far [125, 133].

KDM7/PHF family

Three demethylases: KDMF7A/KIAA1718, KDMF7B/PHF8 and KDM7C/PHF2 have been identified in this family [2, 134]. Members of this family share an identical jmjC domain with KDM2 demethylases, except for KDM7C which has a histidine to tyrosine substitution in the third Fe-binding residue of its jmjC domain [2, 134]. Apart from the jmjC domain, members of this family also contain a PHD finger domain [2]. KDM7A has dual substrate specificity and targets

H3K9me1/me2 and H3K27me1/me2 marks [134]. In mouse neuronal cells, KDM7A-mediated removal of H3K9me1/me2 and H3K27me1/me2 was shown to be associated with activation of follistatin gene transcription whilst deletion of KDM7A in zebra fish was found to result in abnormal brain development [134]. Furthermore, in support of its role in facilitating a neural state, KDM7A-mediated H3K9/27 demethylation was demonstrated to be a key mediator of mouse ES to neural cell state transition via the regulation of fibroblast growth factor-ERK1/2 signaling [135].

Both KDM7B and KDM7C are recruited to the promoters of ribosomal RNA genes through their interaction with H3K4me3 marks, where they function as H3K9me1/me2-specific demethylases [136, 137]. However, KDM7B also has substrate specificity for H4K20me1 and at least *in vitro*, can use H3K27me2 as a substrate for its demethylation activity [138, 139]. The presence of KDM7B was found to be essential for brain development in zebra fish [138]. Moreover, mutations in KDM7B have been detected in patients with X-linked mental retardation syndrome and craniofacial anomalies, implicating imbalances in histone modifications as a potential underlying cause of the diseased states [138, 140]. In mammalian cells KDM7B is detected at transcriptionally active sites, and by binding to H3K4me3 has been shown to associate with cell cycle regulating factors E2F1, HCF-1 and Set1A and was detected at promoters of E2F target genes [139]. The binding of KDM7B to H3K4me3 recruits its demethylase activity to these sites which promotes cell cycle progression and proliferation [138, 139]. Of particular importance is the KDM7B-mediated removal of H4K20me1 that is required for timely G1/S phase progression [139]. However, as cells enter mitosis KDM7B is phosphorylated by cyclin-dependent kinase1 and disassociates from the chromatin. This disassociation correlates with the appearance of H4K20me1 marks. The modification allows for the loading of condensin II during prophase, which is essential for correct chromatin condensation [139]. Together, these findings support a function for KDM7B as an essential component of the cell cycle regulatory network.

In its native state, KDM7C is enzymatically inactive but it can be activated following protein kinase A (PKA)-mediated phosphorylation [141]. This enables the phosphorylated KDM7C to interact with the DNA binding protein Arid. The resulting complex is recruited to promoter regions where it can demethylate H3K9me2 marks [141]. Knockdown of KDM7C in hepatocytes was found to impair PKA-dependent transcription of Pepck and G6Pase genes [141]. Furthermore, upon activation of the PKA signaling pathway KDM7C-Arid2 was shown to function as a co-activator of hepatocyte nuclear factor in the livers of fasted mice [141].

Thus, important functions for this family of demethylases are the regulation of cellular state in normal physiological processes such as gluconeogenesis, when PKA signaling is activated, and during development. Given the roles of KDM7A and KDM7B in brain development, it appears that these enzymes via their demethylase activity are key regulators of neuronal cell fate and differentiation.

MAPJD and KDM8 demethylases

MAPJD (Myc associated protein with JmjC domain, also known as nucleolar protein 66 or NO66) and KDM8/JMJD5 are two histone lysine demethylases that possess only the jmjC domain. MAPJD exhibits demethylase activity towards H3K4me1/me2/me3 and H3K36me2/me3 marks [142]. The enzyme regulates cellular differentiation and growth by functioning as both a suppressor and activator of gene expression. Removal of H3K4 and H3K36 methylation by MAPJD was shown to repress osterix, a factor that promotes osteoblast differentiation and thereby prevent expression of osterix target genes [142]. In contrast to its gene suppression function, MAPJD via its association with c-Myc was shown to have growth promoting and gene activation effects in normal small lung cancer cells [143]. The gene activation effects of MAPJD were demonstrated to be dependent on the recruitment of c-Myc-related histone acetyltransferase complexes to the promoters of its target genes and subsequent increase in histone 4 acetylation [143]. Considering the implication in transcriptional activation it is possible that MAPJD has additional, yet undiscovered substrates or that its growth promoting functions are independent of its demethylase activity. However, removal of

H3K36 methylation has been associated with both gene activation and suppression depending on the context in the genome [71].

KDM8 shows a slight structural difference when compared to other jmjC demethylases, and has a serine instead of the theronine at the first α-ketoglutarate binding site [2]. KDM8 has substrate specificity for H3K36me2 [144, 145, 146] and knockout studies in mice demonstrated that the demethylase activity of KDM8 is essential for successful embryonic development. Severe deficiency in KDM8 was found to result in stunted growth and mid-gestation embryonic lethality [144, 145]. Thus, KDM8 has growth promoting effects which were found to be due to epigenetic regulation of cell cycle associated proteins. Demethylation of H3K36me2 by KDM8 enhanced expression of some cyclins and cyclin-dependent kinases that promote cell cycle progression and repressed expression of cyclin-dependent kinase inhibitors [145, 146]. Consistent with such a role, KDM8 was found to repress p53 and promote cellular proliferation in human cancer cells [144, 146].

Taken together, these studies demonstrate that MAPJD and KDM8 are important epigenetic regulators of cellular differentiation and reprogramming during normal physiological process and transformation to cancerous states.

CONCLUSION

The recent discovery of histone lysine demethylases has allowed new insights into the complex world of dynamic chromatin regulation which has broad implications in various physiological processes. Taken together, the studies described in this review highlight histone lysine demethylases as important epigenetic regulators of cellular growth, state and fate during embryonic development and lineage commitment, differentiation and maintenance of pluripotency as well as stress-induced cellular transformation. The main functional aspects in maintaining self-renewal and differentiation of ES cells and induction from somatic to pluripotency are summarized in Figure 1.

The regulation involves a sophisticated recruitment system, which is influenced by local changes in chromatin environment, to attract specific demethylases to the appropriate target regions. A further level of complexity provides the context dependency, which in the case of H3K36 demethylation can result in either gene activation or repression as the transcriptional outcome of demethylating the same lysine methylation mark. This is evident during vitamin C induced reprogramming to pluripotency and cell cycle progression. Vitamin C induction of pluripotency is associated with KDM2B-mediated removal of H3K36 methylation at the Ink4/Arf locus and microRNA 302/387 cluster and results in opposite outcomes of gene repression and activation respectively, but both biological changes facilitate transformation to pluripotency [71]. Similarly, KDM8-mediated demethylation of H3K36me2 results in activation of cyclins and inhibition of cyclin-dependent kinase inhibitors, which together promote cell cycle progression [145, 146]. Further studies investigating the recruitment mechanisms would not only provide greater understanding of lysine demethylase-mediated regulation but will also enhance our knowledge of the complex and diverse functions of histone lysine methylation.

The role of lysine demethylases in regulating epigenetic reprogramming highlights the possibility of using these enzymes to manipulate epigenetic memory and enhance reprogramming to induced pluripotent cells and during somatic cell nuclear transfer (Figure 1). Histone lysine hypomethylation is known to be associated with enhanced epigenetic plasticity [100, 147]. Hence, the introduction of lysine demethylases in somatic cells could make them more amenable for reprogramming during nuclear transfer or transformation to induced pluripotency.

Another emerging aspect of histone lysine demethylases is their role in diseased states such as cancer- and inflammation-induced transformation of cellular states. Thus, KDMs have potential as effector molecules or represent pharmaceutical drug targets for developing treatment options that could re-transform cells to their normal state or guide them onto an apoptotic pathway. Consistent with this idea, KDM1A inhibitors have been shown to enhance H3K4 methylation and thereby activate tumor suppressive proteins and inhibit proliferation of cancer cells [54, 59, 87].

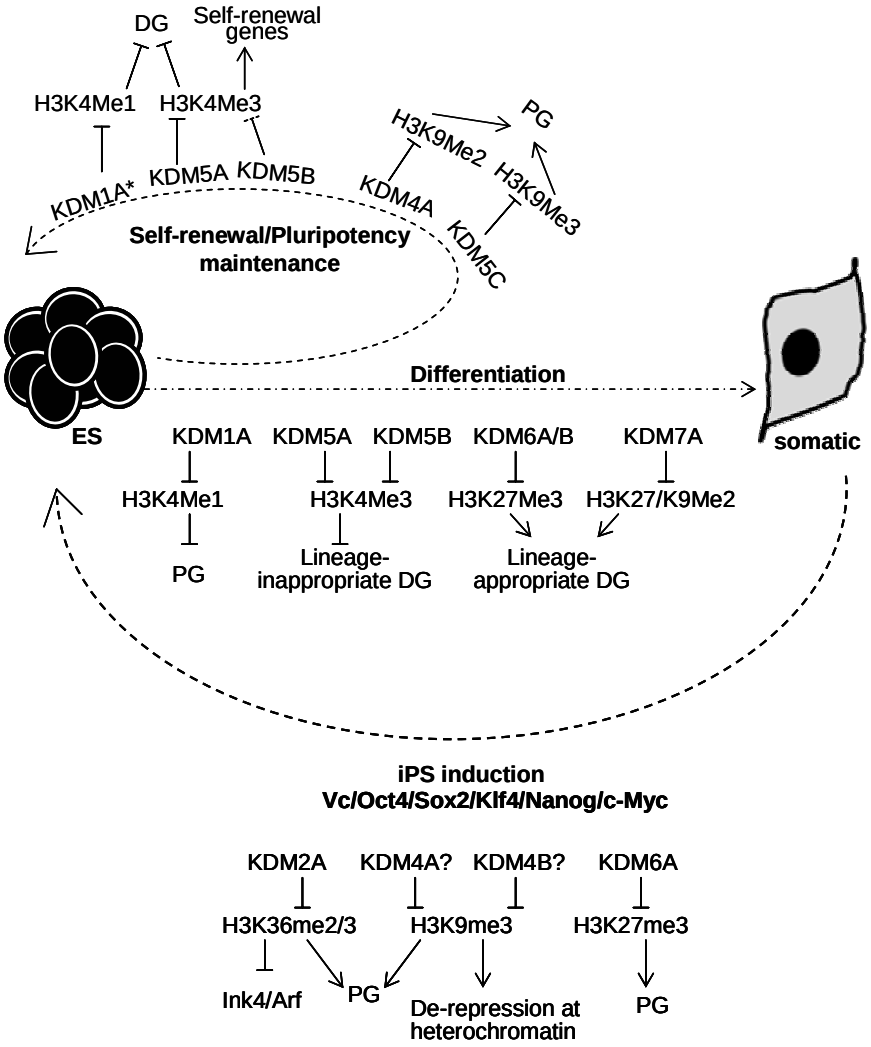

Figure 1. KDMs involved in regulating self-renewal and differentiation of ES cells and induction of pluripotency. Specific histone lysine demethylases are induced to mediate H3K4/K9 demethylation and repress developmental genes and activate self-renewal and pluripotency genes to maintain ES cell state. Upon differentiation, depending on the context, appropriate lysine demethylases are recruited to repress pluripotency, lineage-inappropriate developmental genes and activate lineage-appropriate specific genes to lock the cells into the required somatic epigenotype. Somatic cells can be reprogrammed back to pluripotency via transduction of pluripotency factors Oct4/Sox2/Klf4/c-Myc along with Vitamin C treatment. Epigenetic reprogramming from the somatic cell state to pluripotency triggers the induction of specific KDM demethylase activity to suppress expression of lineage commitment genes and activate the pluripotency gene transcription programme.

Lines with a flat head indicate inhibition; continuous straight lines with arrows indicate activation; DG, Developmental genes; PG, Pluripotency genes; Vc, Vitamin C; *KDM1A is involved in maintaining self-renewal only in human ES cells.

In conclusion, the discovery of lysine demethylases has provided the confirmation that even the most stable lysine methylation marks such as tri-methylation of H3K9 and H3K27 are dynamic and purposefully regulated. This flexibility of histone lysine methylation marks may provide the basis for a complex mechanism that allows cells to switch between cellular states. However, the function of demethylases to regulate cellular transformation or fate involves the coordinated

action of a large number of other regulatory proteins and ultimately depends on environmental cues. The discovery of the histone lysine demethylases as crucial determinants in these processes will provide new, exciting opportunities to experimentally probe and unravel the complex and intricate mechanism underlying cell pluripotency and cell fate over the coming years.

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