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X- and Y-linked chromatin-modifying genes as regulators of sex-specific cancer incidence and prognosis

Rossella Tricarico[1,2,*], **Emmanuelle Nicolas**[1], **Michael J. Hall**[3], **Erica A. Golemis**[1,*]

[1]Molecular Therapeutics Program, Fox Chase Cancer Center, Philadelphia, PA, 19111, USA;

[2]Department of Biology and Biotechnology, University of Pavia, 27100 Pavia, Italy;

[3]Cancer Prevention and Control Program, Department of Clinical Genetics, Fox Chase Cancer Center, Philadelphia, PA, 19111, USA

Abstract

Biological sex profoundly conditions organismal development and physiology, imposing wide-ranging effects on cell signaling, metabolism, and immune response. These effects arise from sex-specified differences in hormonal exposure, and from intrinsic genetic and epigenetic differences associated with the presence of an XX versus XY chromosomal complement. In addition, biological sex is now recognized to be a determinant of the incidence, presentation, and therapeutic response of multiple forms of cancer, including cancers not specifically associated with male or female anatomy. While multiple factors contribute to sex-based differences in cancer, a growing body of research emphasizes a role for differential activity of X- and Y- linked tumor suppressor genes in males and females. Among these, the X-linked *KDM6A/UTX* and *KDM5C/JARID1C/SMCX*, and their Y-linked paralogs *UTY*/*KDM6C* and *KDM5D/JARID1D/SMCY* encode lysine demethylases. These epigenetic modulators profoundly influence gene expression, based on enzymatic activity in demethylating H3K27me3 and H3K4me3, and non-enzymatic scaffolding roles for large complexes that open and close chromatin for transcription. In a growing number of cases, mutations affecting these proteins have been recognized to strongly influence cancer risk, prognosis, and response to specific therapies. However, sex-specific patterns of mutation, expression, and activity of these genes, coupled with tissue-specific requirement for their function as tumor suppressors, together exemplify the complex relationship between sex and cancer vulnerabilities. In this review, we summarize and discuss the current state of the literature on the roles of these proteins in contributing to sex bias in cancer, and the status of clinical agents relevant to their function.

Introduction.

In studies of cancer risk, prognosis, and therapeutic response, sex is often underexplored as a relevant variable (1,2), even though a recent comprehensive study of 30 types of human

*Correspondence should be directed to: Erica A. Golemis, Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA 19111 USA, Erica.Golemis@fccc.edu, (215) 728-2860, or, Rossella Tricarico, Department of Biology and Biotechnology, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy, Rossella.Tricarico@fccc.edu, +39 340-2429631.

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cancer found significant sexual dimorphism of cancer incidence and presentation (3). These trends remain after controlling for epidemiologic risk factors, geographical origin and ethnicity, and excluding sex-specific cancers affecting the ovary, testis or prostate (2,3). Mechanistically, sex-based differences relevant to cancer include metabolism, immune function, exposure to mutagens, the pattern and frequency of mutations, gene dosage and expression of clinically actionable genes, and the prognostic impact of individual mutations or gene expression signatures (4–9) (Figure 1). Based on this growing recognition of the impact of sex, the National Institutes of Health and health advisory groups in the European Union have mandated sex-balanced representation of cells, biological samples and experimental animals in preclinical studies (10,11).

Typically, studies of sex-specified physiological differences focus on the roles of gonadal steroid hormones, including estrogens, androgens, and progestogens, which have profound genomic and non-genomic effects that condition cell identity and signaling (12–14). Complementing this work, recent studies have emphasized the role of genetic architecture in causing sexual dimorphism of cancer presentation (2,11), and in particular focused on contributions of the allosomes, X and Y (12,15,16). Multiple allosomally-encoded tumor suppressor genes (TSGs) have been characterized as epigenetic regulators, which function as components of large chromatin-modifying complexes that broadly influence gene expression, and modulate the efficiency of other cancer-relevant processes such as DNA repair, in a sex-biased manner (5,16). The literature defining the biological roles of allosomal TSGs in sex-specified differences in cancer is expanding rapidly; we here focus on two exemplar allosomal TSG pairs, *KDM6A*/*UTX* and *UTY/KDM6C,* and *KDM5C/JARID1C* and *KDM5D/JARID1D*.

Tumor suppressive chromatin modifiers on the X and Y chromosomes.

Epigenetic factors mediate transcriptional responses to oncogenic stimuli, and influence the propensity of DNA to become mutated, affecting cancer risk and response to therapy (17). Typically, epigenetic regulators directly modify DNA, or modify protein components of chromatin (Figure 2). Modifications such as methylation, acetylation, and mono-ubiquitination specify chromatin that is “open/permissive” or “closed/restrictive” for access by the machineries governing recombination, gene transcription, replication, and repair. Aberrant permissive and restrictive chromatin states promote cancer (17).

Some differences in chromatin regulation pertinent to sex-bias stem from the fundamental biological distinction between the mammalian sexes: the presence of two X chromosomes in females, versus one X and one Y chromosome in males (Figure 1A) (18,19). Among a limited number of X- and Y-linked genes with paralogous function (Figure 1B), *KDM6A, UTY* and *KDM5C, KDM5D* encode lysine demethylases (KDMs), a broad class of enzymes that modify histones, opposing the function of lysine methyltransferases (KMTs) (20). In addition, these KDMs have critical non-catalytic roles, including the recruitment of other epigenetic modifiers and transcription factors to specific sites on chromatin (21). *KDM6A, UTY*, *KDM5C* and *KDM5D* have significant tumor suppressor activity in several types of cancer ((22–24), and Table 1). Importantly, factors governing the expression of allosomal genes can lead to mutations in these KDMs having distinct penetrance in males and females.

Briefly (Figure 3), the most important mechanism mammals use to adjust X chromosome gene dosage between males and females is X chromosome inactivation (XCI). XCI makes females functionally haploid mosaics with respect to most X-linked genes, conferring both positive and negative features associated with genetic heterogeneity (25). In contrast, in males, mutations in X-linked genes subject to XCI have a dominant effect. However, *KDM6A* and *KDM5C* typically escape X inactivation (16,26), making them an example of EXITS (Escape from X-inactivation of tumor suppressor) genes, in which the ability to express both copies of X-linked TSGs can buffer the effect of single inherited or somatic gene-inactivating mutations in females, and increase overall gene dosage relative to males (16). Conversely, in male aging, somatic loss of the Y chromosome (LOY) (27), and the more recently defined extreme down-regulation of chromosome Y gene expression (EDY; inclusive both of LOY, and abnormal methylation of a retained Y-chromosome associated with gene silencing) can limit expression of Y-linked TSGs. LOY and EDY have emerged as signatures for cancer risk in men (15,27). Together, these mechanisms governing TSG activity contribute to sex-biased phenotypes in cancer.

The KDM5 and KDM6 protein families

KDM5 and KDM6 lysine demethylases each contain a signature catalytic motif, the Jumonji C (JmjC) domain (Figure 4A, B). These enzymes require ferrous iron Fe(II) as a cofactor, and use the TCA cycle intermediate α-ketoglutarate (α-KG, also known as 2-oxoglutarate (2OG)) and oxygen as co-factors (28,29). Because of these dependencies, their activity is influenced by cancer-associated mutations in the tricarboxylic acid (TCA) cycle enzymes isocitrate dehydrogenase 1 and 2 (IDH1 and 2), succinate dehydrogenase (SDH), and fumarase hydratase (FH), which regulate α-KG availability (30); and by tumor hypoxia, which limits oxygen levels (31).

Histone 3 mono-methylated on lysine 4 (H3K4me1) is associated with closed/poised, primed, and active enhancers; H3K4me2/3 is associated with active promoters. These methylations are introduced by the SETD1A/SET1- and KMT2C/MLL3-KMT2B/MLL4-containing COMPASS complexes. Conversely, H3K27me3 is a repressive mark found in promoters and enhancers, introduced by EZH2/KMT6A, a component of Polycomb Repressive Complex 2 (PRC2) (17). By demethylating H3K27 at enhancers, KDM6 proteins such as KDM6A and UTY contribute to gene activation. In contrast, by demethylating H3K4 at promoters, KDM5 family proteins such as KDM5C and KDM5D tip the balance toward gene repression (Figure 4C, D). In addition, these KDMs also have non-catalytic functions that promote transcription in a context-specific manner (32), pertinent to their tissue-specific actions in cancer, discussed below. For example, through interaction with other chromatin-modifying complexes (e.g. p300/CBP and the KMT2B complex), these proteins also influence other histone modifications (33).

KDM6A/UTX and *UTY/KDM6C*

KDM6A, formerly known as *UTX* (Ubiquitously transcribed Tetratricopeptide repeat (TPR) X chromosome) (Figure 4A) is located at Xp11.3; *UTY* (also known under the alias *KDM6C*), located at Yq11.221, encodes a protein that has ~84% homology with KDM6A

(34). However, UTY has markedly lower demethylase activity than KDM6A, in part due to a single amino acid difference (I1267 in KDM6A, P1214 at the comparable position in UTY) in the catalytic JmjC domain, which reduces substrate binding (35). Of note, the Km of KDM6A for α-KG is twice as high as that of UTY (35), and KDM6A is more oxygen-dependent (31,36). Based on these differences, KDM6A is a more active enzyme, and more responsive to environmental cues, than is UTY.

Studies in conditional knock-out mice indicate a sex-specific requirement for *Kdm6a* during development, and inform assessment of functional differences between *KDM6A* and *UTY* in cancer. In two different *Kdm6a* conditional mouse models, homozygous loss of *Kdm6a* is embryonic lethal in females, while heterozygous loss causes minor developmental defects. Simultaneous loss of *Kdm6a* and *Uty* is embryonic lethal in males, but in contrast to females, most *Kdm6a*-null, *Uty*-wild type male embryos also die at midgestation; the ~25% of males surviving are smaller and with reduced lifespan (37,38). Mechanistically, studies in cell lines derived from *Kdm6a*-null mice indicate Kdm6a demethylation of H3K27me3 is important for proper activation of developmental genes, and not complemented by Uty, which lacks efficient H3K27me3 demethylase activity (37,38). These data imply an important non-enzymatic function of Uty sufficient for some, but not all, aspects of development (37).

In considering *KDM6A* and *UTY* mutations in cancer, an emerging concept is that while some of their roles depend on enzymatic activities, which differ between males and females, others depend on their adaptor roles, which do not (21,33). Hence, the impact of mutations in *KDM6A* on sex-specific transcription in cancer will depend on the domain of the protein they affect; e.g., mutations in the JmjC domain, affecting enzymatic activity, would likely be associated with distinct penetrance in males and females. In addition, as *KDM6A* is an EXITS gene, the encoded protein is expressed from both alleles in females, blunting the effect of heterozygous mutations.

Multiple cancer types bear frequent somatic mutations inactivating *KDM6A*; these include multiple myeloma, esophageal squamous cell carcinoma, non-muscle-invasive bladder cancer (NMIBC) (the most common form of urothelial bladder carcinomas), renal clear cell carcinoma and T-cell acute lymphoblastic leukemia (T-ALL) (16,39,40) (Supp Figure 1). In most of these cancers, somatic mutations in *KDM6A* tend to be more common in males than in females ((16); see also an extended analysis, based on data in cBioPortal (41), in Supp Table 1). In females, when such mutations occur, they are commonly biallelic. In some male cancer cell lines, derived from acute myeloid leukemia (AML), esophageal squamous cell carcinoma and others, inactivating *KDM6A* mutations are often accompanied by the loss of its paralog *UTY* (81%) (40,42), suggesting selection pressure; in the absence of mutation of *KDM6A*, *UTY* is less frequently lost (49%) (40).

KDM6A and *UTY* are expressed in many tissues; notably, because their gene targeting and functionality is modulated by interaction with additional transcription factors, their activity is also tissue specific (e.g. (43)). Although somatic mutations of *KDM6A* have been associated with sex-biased incidence of multiple forms of cancer, how this sex bias manifests is variable between different cancer types. For instance, incidence of T-ALL

occurs at a male to female ratio of 3:1 (44). Loss of *KDM6A* tumor suppressor activity is important in T-ALL pathogenesis, and depends on intact demethylase activity, reflected in the high frequency of somatic mutations in *KDM6A*, most of which inactivate the JmjC enzymatic domain. Such mutations predominate in males, and likely reflect the fact that the less active UTY enzyme is unable to replace the catalytic activity of KDM6A.

In contrast, bladder cancer also has a male:female incidence ratio of >3:1, and the highest overall *KDM6A* mutation frequency among cancers, at 29%−41% ((45,46), Supp Table 1). However, in this case, there is an increased frequency of *KDM6A* mutations in females relative to males in NMIBC (47–49). *KDM6A* mutations have variously been reported as homozygous (48) or heterozygous in females (50), with one study suggesting that *KDM6A* may be haploinsufficient in the female urothelium (50). In this cancer type, mutations are not concentrated in the JmjC domain, but dispersed, implying dependence on both catalytic and non-catalytic roles for tumor suppression (50). In bladder and some additional cancers, the scaffold function of KDM6A may be more important than the enzymatic activity for tumor suppression, influencing sex-specific manifestation of *KDM6A* mutations. This interpretation is further supported by the fact that in some cancer settings, pro-oncogenic changes in transcription induced by *KDM6A* loss can be reversed by re-expression of enzymatically inactive KDM6A, or of UTY (51). Interestingly, in females with bladder cancer, heterozygous mutations in *KDM6A* often co-occur with mutations in other COMPASS components, such as *KMT2C* and *KMT2D*. In males, *KDM6A* and/or *UTY* alterations co-occur with *KMT2C* and *KMT2D* only in a small fraction of bladder cancer (50) (Figure 4C). Reflecting the cooperative activity of histone-modifying enzymes, mutations in *KMT2C* and *KMT2D* significantly influence KDM6A function, resulting in complex disruptions in gene expression in multiple mutated tumors (52). In addition, germline mutations in either *KMT2D* (type 1, MIM #147920) or *KDM6A* (type 2, MIM #300867) result in the developmental disorder Kabuki Syndrome, causing similar phenotypes (53), further supporting the idea of closely linked function.

In most tumor types, *KDM6A* mutations are dispersed throughout the coding exons, suggesting a predominant role in scaffolding. In these cases, KDM6A loss is associated with extensive changes in transcription that include both gene repression and activation mediated through inappropriate gene-targeting of larger chromatin modifier complexes. In human pancreatic cancer, such *KDM6A* mutations are common, and typically result in protein loss. In males, loss of *KDM6A* is frequently accompanied by EDY (whether by silencing of *UTY*, loss of the *UTY* locus at Yq11, or complete loss of the Y chromosome), and associated with a squamous phenotype with poor prognosis. In a mouse model of *Kras*-driven pancreatic cancer, total *Kdm6a* deficiency in females greatly accelerates pancreatic intraepithelial neoplasia (PanIN) and induces a squamous phenotype, while heterozygous loss of *Kdm6a* in females, or *Kdm6a* loss in males with intact *Uty*, causes less tumor acceleration (54). Notably, homozygous loss of *Kdm6a* in female mice causes premalignant changes, even in the absence of *Kras* driver mutations. In both human and murine pancreatic cancer, *Kdm6a* loss selectively activates a cluster of super-enhancers regulating oncogenes including *Tp63*, *Myc* and *Runx3*, increasing H3K4me1 modifications at these loci; while *Kdm6a* loss inactivates a suite of other genes in a non-sex biased manner (54). These findings suggest the critical sex-specific role of *Kdm6a* may be to influence lineage selection

in tumor precursor cells (43). As another example, in AML, *KDM6A* reduces tumor-suppressive GATA-dependent transcription programs while upregulatiing oncogenic ETS-dependent programs (51). These extensive transcriptional changes, accompanied by minimal changes in H3K27me3 levels, have been suggested to reflect changes in the enhancer targeting of the COMPASS complex in the absence of KDM6A and UTY scaffolding.

Although the chromosomal complement of *KDM6A* and *UTY* is clearly the major driver of their sex-biased tumor suppressive function, it is notable that sex hormones also play a role in modulating their activity (55). For example, KDM6A enhances the expression of hormone-dependent nuclear receptors such as estrogen receptor α (ERα), but is also itself transactivated by ERα, forming a feed-forward regulatory loop of hormone response (55). In addition, KDM6A is regulated by cellular metabolites (Figure 4E), which are produced in sexually dimorphic abundance (56). As a result, the expression of the KDM6A and UTY cofactor α-KG, is 2.3 fold higher in males than females (57), which would be expected to elevate the enzymatic activity of the more metabolite-responsive KDM6A protein in males. These interactions between gene complement and metabolic landscape can exacerbate or quench the effect of specific mutations.

KDM5C/JARID1C and *KDM5D/JARID1D*

KDM5C/JARID1C/SMCX localizes to Xp11.22; its paralog *KDM5D/JARID1D/SMCY*, on Yq11.223, encodes a protein with ~84% similarity (Figure 4B). KDM5C binds to the activating mark H3K9me3, present in heterochromatin, demethylates mono-, di- or tri-methylated H3K4, and associates with protein complexes regulating heterochromatin assembly; depending on the genomic element bound, KDM5C can act as context-dependent transcriptional repressor or activator. For example, KDM5C interacts with histone deacetylases (HDACs) and KMTs to restrain transcription at promoters (Figure 4D), but may also interact with distal elements to stimulate the activity of enhancers (58). Mechanistically, loss of *KDM5C* has been proposed to support tumor growth in several ways, with critical targets varying in different tumor types. For example, in *VHL*-deficient renal cancers, loss of the hypoxia-induced transcription factor *HIF2α*, *PBRM1* (the nucleosome-targeting subunit of the SWI/SNF complex), or *KDM5C* reduces an ISGF3-dependent interferon signature that is an important negative feedback mechanism for tumor growth (59). Loss of *KDM5C* in renal cancer also disrupts heterochromatin stability, causing anomalous transcription of non-coding RNAs, and triggering genomic instability (24).

As with KDM6A and UTY, genetic studies suggest similar but non-equivalent function between KDM5C and KDM5D, and both enzymatic and scaffolding activity. Specifically, inherited mutations of *KDM5C* are one of the most common sources of X-linked intellectual disability in males (Mental Retardation, X-linked, Syndromic, Claes-Jensen type (MRXSCJ, MIM #300534); inherited mutations in *KDM5D*, however, do not cause mental retardation. While most MRXSCJ-associated pathogenic variants impair the enzymatic activity of KDM5C, at least some do not, even though they significantly influence the gene expression profile (60). KDM5D, in turn, is specifically required in testicular germ cells, where it acts as part of a complex that promotes chromatin condensation prior to meiosis (61). Part of the difference in activity between KDM5C and KDM5D may also reflect differences in

expression. *KDM5C* is expressed in almost all tissues in adults, and at higher levels than *KDM5D*, which is predominantly detectable in testis, prostate, and small intestine (62).

Mutation or gene silencing of *KDM5C* is common and removes tumor suppressive activity in clear cell renal carcinomas (ccRCCs), gastric cancer, follicular thyroid carcinoma, salivary duct carcinoma, human papillomavirus (HPV)-associated cancers, and mantle cell lymphoma (63–67). Many of these cancers are more prevalent in males than females, except thyroid carcinoma and human papillomavirus (HPV)-associated cancers ((68), and Supp Table 1). Based on public data in cBioPortal (41), mutations in *KDM5C* are dispersed throughout the coding sequence for many of these cancers, emphasizing the importance of a non-catalytic (or scaffolding) role for the protein (Supp Figure 2). In addition, for some of these cancers, there is evidence of sex-biased mutation ((64) and Supp Table 1).

KDM5D also contributes to tumor suppression, with evidence for a more important TSG role than *KDM5C* in some cancers. Downregulation, loss or inactivating mutations of *KDM5D* occur in ~40% of male ccRCCs (16), while *KDM5C* is mutated in only a small fraction of tumors in males (6.2%) and females (0.5%) (64). In gastric cancer, *KDM5D* overexpression in cancer cells significantly reduces viability, suggesting a direct growth suppressive role (23). Gastric cancer occurs with an imbalanced male-to-female ratio of ~2:1, with mortality also higher in males (55). Mechanistically, gene knockdown of *KDM5D* in male gastric cancer cells inhibits the demethylation of *CUL4A*, reducing the expression of *CUL4A* target genes such as the tumor suppressor *CDKN1A*/p21 and *TP53*, and promoting metastasis (23). Loss of *KDM5D* has been shown to cause atypical patterns of H3K4me3 at promoters, targeting gene programs controlling proliferation, apoptosis, and invasion (69). In prostate cancer, loss of *KDM5D* by gene silencing or mutation is common during progression, causing defects in control of genomic stability, including DNA replication stress and ATR activation (70), and is associated with shorter overall survival (71). Interestingly, indicating non-equivalent function with KDM5D, *KDM5C* is overexpressed in prostate cancer, promotes cellular proliferation and has emerged as a predictive marker for therapy failure in patients after prostatectomy (72). Additionally, a pro-oncogenic role for *KDM5C* upregulation has been proposed in breast and hepatocellular cancers (73), implying tissue specificity in tumor-suppressive versus tumor-promoting action. The reasons for this difference are not yet well understood.

Targeting histone modifications

Aberrant chromatin states are increasingly being targeted in cancer (74–76). Drugs developed to modify chromatin include two major classes. Broad reprogramming agents significantly alter gene expression based on inhibition of widely expressed targets that include histone deacetylase inhibitors (HDACis), DNA methyltransferase inhibitors (DNMTis), and bromodomain and extra-terminal (BET)-targeting agents. Targeted agents focus more exactly on individual components of the epigenetic machinery that are mutated in specific cancers, including the H3K27 histone N-methyltransferase EZH2, and the TCA cycle components IDH1 and IDH2, which regulate α-KG availability (e.g. (77)).

Because KDM6A, UTY, KDM5C and KDM5D are components of large multimeric complexes (Figure 4), their mutation and expression can affect cellular response to clinically advanced drugs targeting complex components (Table 1). For example, in urothelial bladder cancer (78), and in multiple myeloma (79), *KDM6A* loss confers sensitivity to inhibitors of EZH2 (78), while *KDM6A* loss in pancreatic cancers (54) or knockdown in prostate cancer models (80) sensitizes to BET inhibitors. However, in aggressive prostate cancer, loss of *KDM5D* increases DNA replication stress and accelerates mitotic entry; this causes resistance to docetaxel, but sensitization to ATR inhibition, emphasizing the complex action of the KDMs (70,71). It is likely that many drugs targeting the epigenome indirectly influence KDM6A and KDM5C activity. For example, dual treatment of cells with low levels of an EZH2 inhibitor (GSK126) and a histone deacetylase inhibitor (SAHA, also known as vorinostat) caused striking defects in XCI (a process maintained by extensive H3K27me3 modification on the X chromosome). In this study, dual drug treatment resulted in loss of H3K27 methylation on the Xi chromosome, causing reprogramming of transcription. Interestingly, the effects on transcription were uneven, with these drugs selectively increasing expression of genes near the center of XCI, such as *TSPAN7* at Xp11.4, and *FOXP3* at Xp11.2; *KDM6A* (Xp11.3) and *KDM5C* (Xp11.22) are also located near this center. Such reprogramming could lead to undesirable side effects specifically in females treated with such drugs. Moreover, as noted above, EZH2 is a component of the PRC2 complex; intact KDM6A opposes PRC2 activity, and hence would function similarly as an EZH2 inhibitor; hence, administration of SAHA or other epigenetic drugs targeting PRC2 function in tumors bearing mutations inactivating KDM6A might lead to defects in maintenance of XCI, leading to greater disruption of gene expression patterns in females than in males (81).

Although *KDM6A*, *UTY*, *KDM5C* and *KDM5D* are typically tumor-suppressive, as a class, KDMs have attracted significant interest as targets for enzymatic inhibition (82). This interest was driven by the recognition that some KDMs, including *KDM1A*/*LSD1*, members of the JMJD2 subfamily, and other JmjC proteins KDM5B/JARID1B and KDM2B/FBXL10 are often overexpressed in cancers, and are thought to be pro-oncogenic. Most drugs developed for Jumonji-family KDMs target the active site of the Jumonji domain (Table 1). These agents typically bind competitively with α-KG and chelate the active site Fe(II) residue. Because the active site is similar across 17 histone demethylases in humans, this strategy has resulted in limited drug selectivity, with most compounds targeting multiple members of the KDM4, KDM5, and KDM6 families. Perhaps for this reason, use of these compounds results in significant cytotoxicity (83). Some more recently developed compounds inhibit a more restrictive subset of KDMs, with KDM6A being among the targets (84). In addition, KDM-inhibiting activity has been identified in some drugs originally developed for alternative targets. For example, metformin is currently widely used for treatment of Type 2 diabetes, and has also shown promising activity as a cancer therapeutic (85,86) through downregulation of mitochondrial complex 1 and reduced expression of enzymes mediating gluconeogenesis. Unexpectedly, metformin has also been recognized as a catalytic inhibitor of KDM6A, with activity at biologically relevant concentrations (87).

Given that mutations in *KDM6A* are sensitizing for some drug treatments, these agents may be useful in combination approaches. However, a fundamental safety issue with the use of these compounds in patients is concern that broadly specific agents will inhibit catalysis-dependent tumor-suppressive activity of KDM6A, UTY, KDM5C and KDM5D, particularly in cell types where tumor suppression depends on the catalytic activity of the protein (e.g. KDM6A in T-ALL). As most Jumonji KDMs achieve *in vivo* target specificity by residing in larger multimeric complexes that regulate gene localization and histone targeting, developing drugs that disrupt the interaction of specific oncogenic KDMs with protein partners may be one approach to increase specificity. Alternatively, use of degradation-inducing agents such as PROTACs (88), targeted to specific oncogenic KDM family members, may be a more effective approach.

Overall, there has been surprisingly little evaluation to date of sex-based differences in response to agents targeting the cellular machinery regulating the epigenome, nor of the specific roles of *KDM6A*, *UTY*, *KDM5C,* or *KDM5D* mutation or expression in mediating sex-biased response. Given the extensive evidence for sex-specific differences in their activity, such investigations would be timely. Interestingly, two recent studies evaluating metformin in colorectal cancer (CRC) in patients with concurrent diabetes identified a significantly greater reduction of CRC-specific mortality in females (89,90). Whether these results in part reflect inhibition of KDM6A, and sex-specific effects on the epigenome, is currently unknown.

Future prospects

While much of the above discussion addresses the impact of somatic mutation or silencing of *KDM6A*, *UTY*, *KDM5C*, or *KDM5D* on histone methylation, emerging topics may suggest a broader role for these proteins in influencing sexual differences in cancer incidence or treatment. Of note, a new enzymatic activity as methylarginine demethylases (RDMs) has been recently demonstrated for a subset of JmjC KDMs, including KDM5C (91). The biological significance of this class of modification is not well-understood and may reflect an important biological activity for this X-linked enzyme.

Immune checkpoint inhibitors (ICIs) have attracted considerable interest as a promising new class of anti-cancer agents. Part of the anti-tumoral activity of epigenetic inhibitors has been determined to depends in part on enhancement of innate and acquired immune responses (92), and a number of trials combining epigenetic inhibitors with ICIs are in progress (e.g., NCT02453620; see also (93)). Notably, KDM6A regulates multiple immune response genes (94–97). Further, *KDM6A* mutation status has recently been shown to influence the activity of drugs targeting the immune system in bladder cancer cells, where loss of *KDM6A* activated cytokine and chemokine pathways; these cells were more responsive to two clinically approved agents, the IL6 receptor inhibitor tocilizumab, and the CCR2 inhibitor propagermanium (98). Given the role of *KDM6A* and *KDM5C* in immune response and their ability to escape X inactivation, it is reasonable to speculate that their different gene dosage or inactivation in males and females may contribute to differential responses to immunotherapy observed in the two sexes. Moreover, these data also suggest that epigenetic therapies targeting KDM6A and KDM5C might “boost” antitumor immune response in a

sex-dependent manner. Potential sex-specific differences in the interaction of epigenetic therapies and ICIs has not been extensively investigated, but merit study.

It has long been known that many lifestyle factors, including diet, physical activity, tobacco smoking, and alcohol consumption, distinguish males and females (99). Some of these factors may plausibly influence the activity of KDM6A, UTY, KDM5C, and KDM5D (as well as other epigenetic regulators). For example, tobacco smoking is nearly five times more common in men than in women. Tobacco smoke causes overexpression of TCA cycle enzymes including fumarate hydratase (FH) and isocitrate dehydrogenase (IDH1/3) (100). In some tissues, tobacco smoke increases driver mutations in genes including *IDH1* (101). By modulating FH and IDH1/3 activity, tobacco smoke can affect levels of α-KG, or of α-KG competitive antagonists such as fumarate or the oncometabolite 2HG; this in turn would affect the activity of KDM6A and KDM5C, influencing H3K27me2/3 or H3K4me1/3 levels. As another example, caloric restriction, high fat diets, low protein diets, and other diets affect metabolic pathways and can cause significantly global epigenetic changes, affecting levels of H3K27me2/3 or H3K4me1/3 histone (102). Sex-specific differences have been reported in dietary preferences and nutrient intakes (103). Based on an extensive literature, it is reasonable to speculate that sex differences in nutrient metabolism might influence the availability of metabolic co-factors such as Fe(II) and α-KG, contributing to the selective modulation of the enzymatic activities of KDM6A, UTY, KDM5C, and KDM5D in men and women. These possibilities have received limited if any scrutiny.

Finally, in provocative work in mouse models, mutations in *KDM6A* were found to cause sex-specific intergenerational epigenetic inheritance and cancer susceptibility (104). In humans, germline variance in the lysine demethylase *KDM1A/LSD1* impact cancer risk (105). Similarly, drugs targeting EZH1/2 significantly disrupt the female germline epigenome, causing irreversible changes in H3K27 modification accompanied by growth restriction and other anomalies in offspring that resemble those observed in *Ezh2*-mutant mice (106). Little is currently known about the degree to which X- or Y- linked KDM-targeting drugs, or inherited alleles impacting activity of these proteins, might provide sex bias in hereditary cancer risk. Of note, although the topic has not attracted significant study, in at least one case the defective allele of *KDM6A* identified in a ccRCC tumor was identified as germline (40). There is much scope for future work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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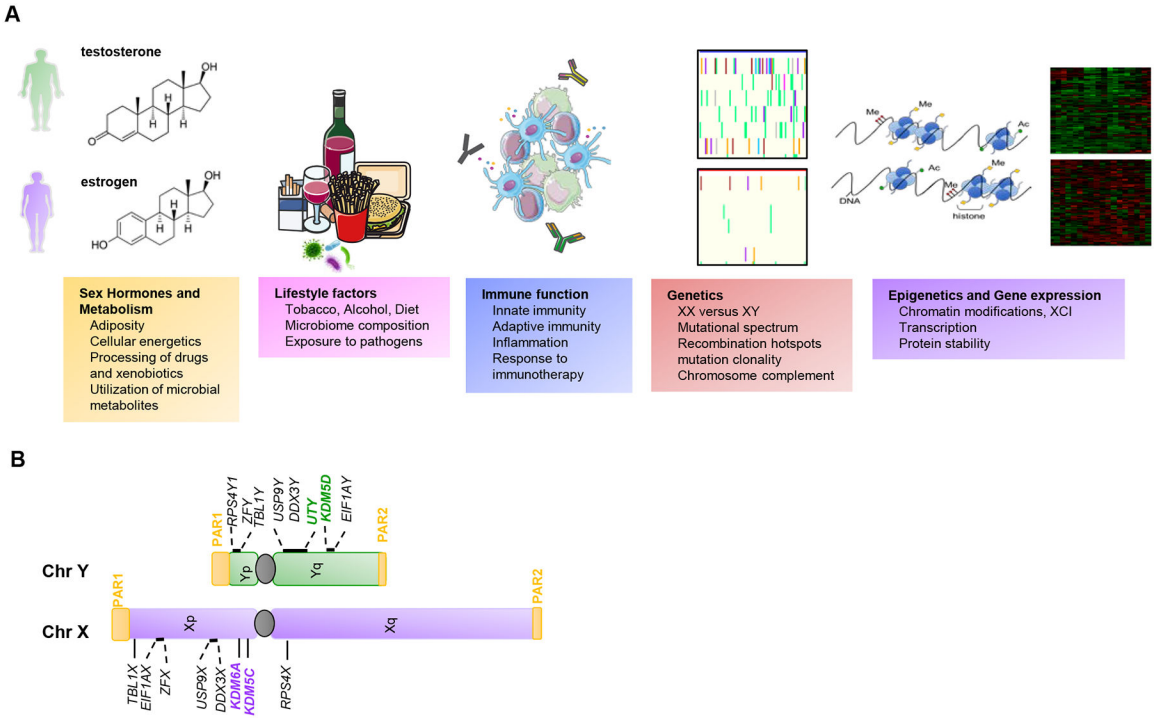

Figure 1: Sources of sex-based bias in disease.

A. Male versus female differences relevant to cancer include mutational spectrum, gene expression profile, metabolism, immune cell interactions with tumors, microbiome composition, pharmacokinetics and tissue distribution of drugs, and other factors; all now recognized as important contributors to tumor phenotypes (2,111–113) . **B**. Both X and Y evolved from an ancestral autosomal chromosome pairs; except for two small pseudo-autosomal regions (PARs) common to both chromosomes, X and Y encode distinct complements of genes. The X chromosome is a large and gene-rich, encoding over 1,000 genes and many non-coding RNAs; by contrast, the much smaller Y chromosome encodes 568 genes, with only 71 having protein-coding potential. While it has long been considered as a “genetic wasteland” undergoing rapid evolutionary deterioration except for the sex-determining region, the Y chromosome is now recognized as having biological functions beyond its role in male sex determination by bearing a small but stable group of essential genes critically important for the health and survival of males. Among the 17 surviving ancestral genes that have survived as moderately divergent paralogs on both X and Y, 8 pairs (*KDM6A, UTY*; *KDM5C, KDM5D*; *EIF1AX, EIF1AY*; *ZFX, ZFY*; *RPS4X, RPS4Y1*; *DDX3X, DDX3Y*; *USP9X, USP9Y*; *TBL1X* and *TBL1Y*) encode X- and Y-specific isoforms of global regulators of gene and protein expression (26).

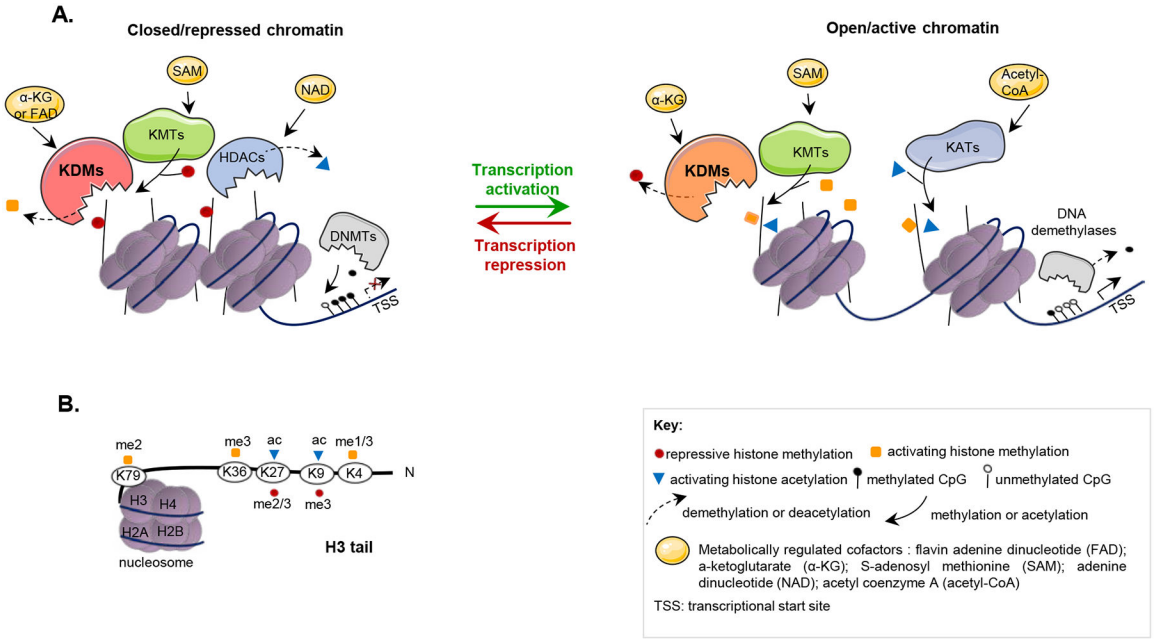

Figure 2. Epigenetic modulation of chromatin.

A. Transcription, replication, and repair of DNA are regulated by chromatin accessibility. DNA is wrapped around nucleosomes, composed of histone octamers (two molecules each of histones H2A, H2B, H3, and H4). Selective histone modification in the promoter and enhancer regions of highly regulated genes specifies whether transcription initiates. Amino-terminal sequences (or “tails”) of histones H3 and H4 are subject to extensive post-translational modifications that govern “open” versus “closed” chromosomes, with acetylation and methylation on specific lysines in the tails promoting transcription or repression. Lysine methyltransferases (KMTs) and lysine acetyltransferases (KATs) (often called “writers”) place marks on the tails; lysine demethylases (KDMs) and histone deacetylases (HDACs) (often called “erasers”) remove marks. Specific marks recruit “readers”. Repressive marks recruit DNA-modifying enzymes such as DNA methyltransferase (DNMT), which methylate CpG sites on DNA, further contributing to gene silencing. Both activating and repressive marks recruit protein complexes that can either reinforce the open or closed state of chromatin, or act to reverse these states (see also Figure 4). Pioneer transcription factors include members of the GATA, FOXA, and other protein families; these factors can target histone-modifying enzymes to specific genes, enabling switching between repressed and active states; some pioneer factors interact with transcription factors responsive to hormones including estrogen and androgens, contributing to sex-specific targeting of histone modifying complexes. In addition to having silenced gene expression, repressed chromatin is less accessible to enzyme complexes regulating DNA repair. **B**. Marks typically associated with open chromatin typically include H3K4me1/3, H3K36me3, H3K79me3, H3K27ac and H3K9ac; marks associated with closed chromatin included H3K9m3, H3K27me2/3 (114). TSS: transcriptional start site.

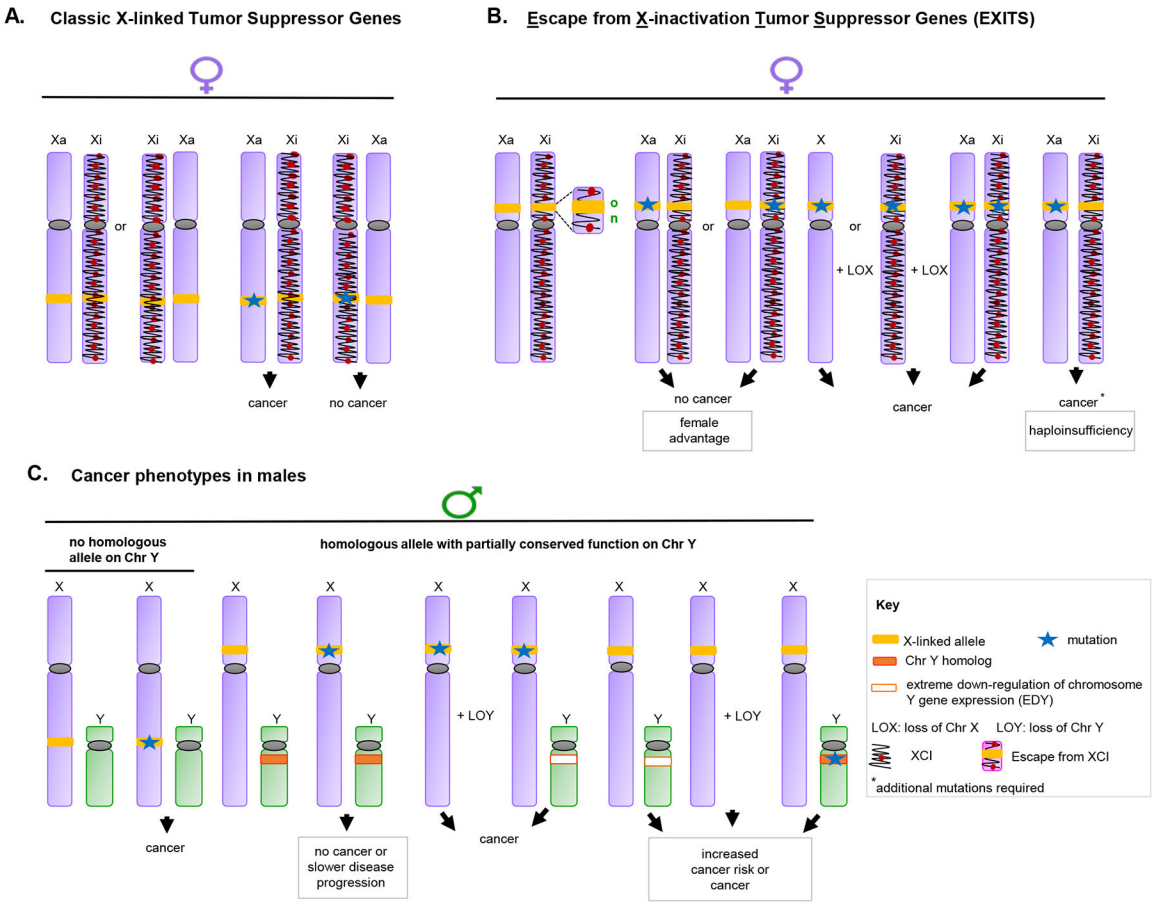

Figure 3: Mechanisms regulating the dosage of allosomal genes influence cancer risk.

A-C. In X chromosome inactivation (XCI), one of the two X chromosomes is epigenetically modified during embryogenesis to silence transcription. Chromatin features distinguishing the inactive X (Xi) from the active X (Xa) include specific histone post-translational modifications, incorporation of variant histones into nucleosomes, association with the long non-coding RNA (lncRNA) XIST, and extensive CpG DNA methylation (115,116). However, up to 23% of X-linked genes escape inactivation. X-linked genes with Y-linked homologs are more likely to escape X inactivation. These Escape from X-inactivation tumor suppressor (EXITS) genes include the examples discussed in this article. Shown, different scenarios for cancer risk in females (**A, B**) and males (**C**). **A** represents cancer risk in females with wild type or mutated tumor suppressor genes (TSGs) subject to XCI. **B** represents cancer risk in females with wild type or mutated EXITS. The two active alleles of an EXITS TSG protect females from developing cancer after mutation of a single allele. Mutations on both alleles or on a single allele with loss of the other X are required to develop cancer. In addition, concomitant mutations in KMTs (e.g. *KMT2C* and *KMT2D)* might contribute to the cancer phenotype in EXITS genes (e.g. *KDM6A*) haploinsufficient tissues in females. **C** represents cancer risks in males with or without Y-linked TSGs that moderately to significantly conserve functions with X-linked homologs. Mutation of a single allele of an EXITS gene with no Y chromosome homolog is required to develop cancer in males. Alternatively, LOY or EDY enhances cancer development in males with mutation in

an EXITS gene with a Y-chromosome homolog with partially conserved function. For some allosomal TSGs, this elevated risk occurs in a tissue-specific manner.

In addition (not shown), sporadic reactivation of X-linked genes can occur during aging, and in transformed cells. In parallel, male versus female differences in gene imprinting can cause differences in the expression of specific alleles dependent on their maternal versus paternal origin (117). In some cases, XCI occurs with skewing (the preferential inactivation of one of the two X chromosomes), amplifying or reducing the effect of inherited mutations. These factors can influence degree of sex-bias for phenotypes arising from mutations in allosomal genes, sometimes in a tissue-specific manner.

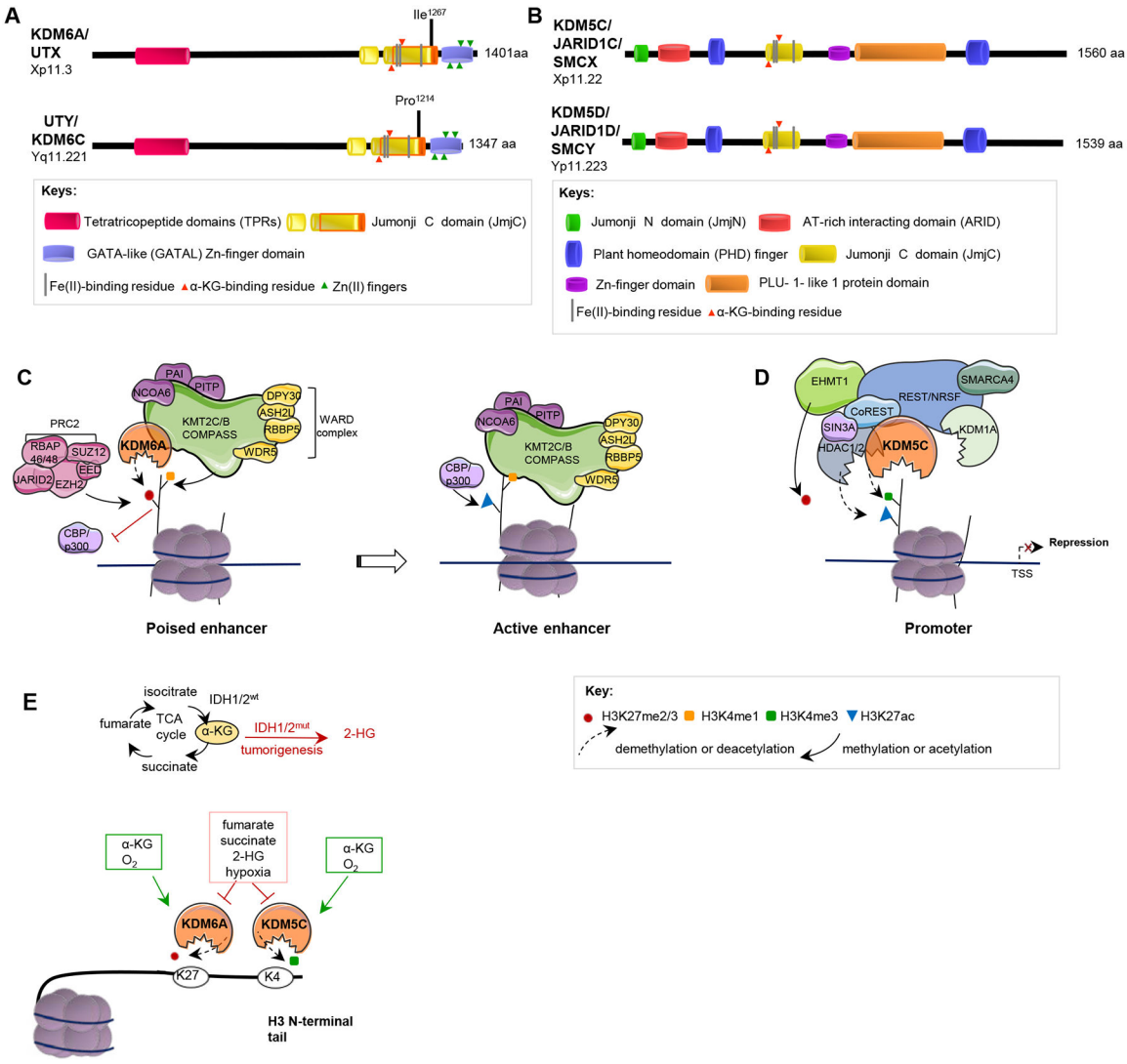

Figure 4. KDM6A, UTY, KDM5C and KDM5D: structure and function.

A, B. Domain structures of KDM6A and UTY (**A**), and for KDM5C and KDM5D (**B**) are indicated. The core catalytic domain, JmjC, demethylates histones by an oxidative mechanism requiring Fe(II) and alpha-ketoglutarate (α−KG) as cofactors; JmjN, in JARID proteins, interacts with JmjC, inducing a conformational change that promotes enzymatic activity. ARID and PLU-1 are DNA binding domains. TPR domains mediate interactions with other proteins including the KMT2C-KMT2B complex (49). The Zn-fingers mediate histone tail recognition; the PHD domain binds H3K9me3, coordinating H3K4 demethylation. Note, each protein family contains additional paralogous members (*KDM5A* (Chr 12), *KDM5B* (Chr 1) and *KDM6B* (Chr 17)), which have related but not equivalent function. See legend to Supp Fig 1 for extended comment on placement of motif boundaries. **C.** Through its activity as a histone H3K27me2/3 demethylase, KDM6A opposes activity of the repressive PRC2 complex, converting poised enhancers to transcriptionally active enhancers. KDM6A is also a component of the KMT2C-KMT2B COMPASS-like complex. As part of this complex, KDM6A contributes to protein recruitment at enhancers and

cooperates with other complex components, including the lysine methyltransferases (KMTs) KMT2C and KMT2B, which monomethylate H3K4 (H3K4me1) and the histone acetyltransferases (HATs) p300 and CBP, which acetylate H3K27. These actions result in enhancer activation (118). Loss of *KDM6A* activates PRC2-regulated transcription repression, similar to *EZH2* gain-of-function mutations, and sensitizes tumors to EZH2 inhibitors (78). *KDM6A* mutations damaging the TPR domain disrupt interaction with KMT2C-KMT2B complexes (49). **D**. KDM5C associates with Co-REST, Sin3a and other proteins to demethylate H3K4me3 at specific promoters, causing gene repression. **E**. Metabolic factors influencing the activity of KDM6A, UTY, KDM5C and KDM5D. Mutations in fumarate hydratase (*FH*), succinate dehydrogenase (*SDH*) or isocitrate dehydrogenase (*IDH1*, *IDH2*) affecting pools of the metabolites fumarate, succinate, or 2-hydroxyglutarate (2-HG), or hypoxia, repress KDM6A and KDM5C activity (119,120).

Table 1.

KDM6A, UTY, KDM5C and KDM5D in cancer therapy.

Context	Mechanistic Observation	Therapeutic opportunity	Ref.
Targeting KDM6A			
T-ALL driven by oncogenic transcription factor TAL-1	TAL-1 recruits KDM6A to aberrantly activate transcription of its target genes through H3K27me3 demethylation.	The H3K27 demethylase inhibitor GSK-J4 represses TAL-1-KDM6A target genes and kills TAL-1-positive T-ALL cells. The treatment is efficient in vivo in PDX models of *TAL-1* positive T-ALL.	(107)
Chemotherapy for CRC	Low H3K27me3 correlates with poor prognosis and oxaliplatin resistance.	GSK-J4 combined with oxaliplatin inhibits growth of oxaliplatin-resistant PDX.	(108)
Drug resistance in *ESR1*/*ERα*-positive breast cancer	*KDM1A/LSD1* and *KDM6A* are co-expressed and colocalized with *ESR1*/*ERα*.	The dual *KDM1A-KDM6A* inhibitor MC3324 down-regulates *ESR1*/*ERα* and attenuates hormone signaling.	(77)
Chemoinformatics approach to computationally predict biomolecular targets of metformin with experimental validation	Metformin inhibits the demethylation activity of purified KDM6A. Pharmacological dosage of metformin augments global H3K27me3 *in vivo*.	Low levels of H3K27me3 are a predictor of cancer aggressiveness. Metformin promotes a more resilient H3K27me3 enriched epigenome. It is currently in several clinical trials for cancer, including a phase 2 in bladder cancer NCT03379909.	(87)
Exploiting KDM6A deficiency			
Loss or inactivation of *KDM6A* in MM	*KDM6A* loss leads to abnormal *PRC2*-mediated repression.	Rebalancing KDM6A-PRC2 activities with EZH2 inhibitors (GSK343, GSK126) causes death of *KDM6A*-mutated MM cells.	(79)
Loss or inactivation of *KDM6A* in bladder cancer	*KDM6A* loss leads to abnormal PRC2-mediated repression.	The EZH2 inhibitors GSK503 and EPZ6438 * significantly attenuate the growth of *KDM6A*-null but not *KDM6A*-wt cells and engrafted tumors.	(78)
Loss of *KDM6A* in poorly differentiated and squamous-like pancreatic cancer	*KDM6A* functions in pancreatic cancer are largely non-catalytic. *Kdm6a* loss deregulates the COMPASS-like complex, activating oncogenic super-enhancers that drive squamous differentiation and metastasis.	*KDM6A* deficient pancreatic cell lines are sensitive to BET inhibitors (e.g. JQ1) that disrupt long range interactions between promoters and super-enhancers. JQ1 reverses the squamous differentiation of *KDM6A*-deficient cancers *in vivo*.	(54)
KDM6A loss blocks HSPC differentiation in mouse model for MDS and AML	H3K4 methylation is crucially involved in the differentiation block caused by *KDM6A* deficiency	Inhibition of the H3K4 KDM KDM1A by SP2509 (HCl2509) induces differentiation in *Kdm6a*-null cells.	(109)
Mouse model for *Kdm6a*-deficient bladder cancer	*Kdm6a* deficiency induces growth-promoting cytokine and chemokine signaling.	Combined inhibition of IL6 and CCL2 effectively suppresses cell growth.	(98)
Recurrent *KDM6A* mutation in AML patients relapsing after chemotherapy	Loss of *KDM6A* decreases expression of the drug influx transporter ENT1, providing a selective advantage after Cytarabine (AraC)-based chemotherapy.	Re-expression of *KDM6A* in *KDM6A*-null cells suppresses cell growth and resensitizes cells to AraC therapy.	(110)
Targeting KDM5C			
KDM5C is upregulated in CRPC	BRD4 transcriptionally induces *KDM5C*, which suppresses *PTEN* transcription to promote tumorigenesis.	Knockdown of *KDM5C* sensitizes the responses of CRPC cells to treatment with a BET inhibitor.	(80)
Exploiting KDM5D deficiency			
Loss of *KDM5D* causes DNA replication stress and poor prognosis in prostate cancer	Loss of *KDM5D* activates ATR signaling and alters histone methylation of promoter regions to increase expression of G2/M checkpoint mediators.	In *KDM5D*-deficient cells, blocking ATR activity with the ATR inhibitor VX-970 * enhances DNA damage and causes apoptosis.	(70)
KDM pan inhibitor			
In silico molecular docking to identify with drugs targeting KDMs, to support repurposing	Deferiprone (DFP, Ferriprox), approved for thalassemia, chelates the Fe^{2+} ion at	In breast cancer cell lines DFP causes a dose-dependent increase in H3K4me3 and H3K27me3 levels and cytotoxicity, with potentially greater	(84)

Context	Mechanistic Observation	Therapeutic opportunity	Ref.
	the active sites of multiple KDMs; KDM6A is the highest affinity target.	activity in triple negative breast cancer. May be useful as a combination sensitizing agent.	

* EPZ6438/Tazemetostat is in phase I/II clinical trials (NCT03854474) as is VX-970/Berzosertib (e.g. NCT03517969). CRC, colorectal cancer; ESR1/ERα, estrogen-receptor-α; MM, multiple myeloma; HSPC, hematopoietic stem and progenitor cells; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CRPC, castration-resistant prostate cancer; T-ALL, T-cell acute lymphoblastic leukemia.