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The H3K27me3 demethylase UTX in normal development and disease

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Abbreviations: CBP, CREB-binding protein; ChIP, chromatin immunoprecipitation; CMML, chronic myelomonocytic leukemia; E, embryonic day; ESC, embryonic stem cell; H3K27me2/3, histone 3 lysine 27 di- and tri-methylation; HOX, homeobox; IPA, Ingenuity Pathway Analysis; iPSC, induced pluripotent stem cell; JmjC, Jumonji C; JMJD3, Jumonji D3; KO, knockout; lincRNA, long intergenic non-coding RNA; LSD1, Lysine-Specific Demethylase 1; MEF, mouse embryonic fibroblast; PRC2, Polycomb Repressor Complex 2; TCGA, The Cancer Genome Atlas; TPR, tetratricopeptide repeat; Trr, Trithorax related; TSS, transcriptional start site; UTX, ubiquitously transcribed tetratricopeptide repeat on chromosome X; UTY, ubiquitously transcribed tetratricopeptide repeat on chromosome Y

In 2007, the *Ubiquitously Transcribed Tetratricopeptide Repeat on chromosome X* (UTX) was identified as a histone demethylase that specifically targets di- and tri-methyl groups on lysine 27 of histone H3 (H3K27me2/3). Since then, UTX has been proven essential during normal development, as it is critically required for correct reprogramming, embryonic development and tissue-specific differentiation. UTX is a member of the MLL2 H3K4 methyltransferase complex and its catalytic activity has been linked to regulation of *HOX* and *RB* transcriptional networks. In addition, an H3K27me2/3 demethylase independent function for UTX was uncovered in promoting general chromatin remodeling in concert with the BRG1-containing SWI/SNF remodeling complex. Constitutional inactivation of *UTX* causes a specific hereditary disorder called the Kabuki syndrome, whereas somatic loss of *UTX* has been reported in a variety of human cancers. Here, we compile the breakthrough discoveries made from the first disclosure of UTX as a histone demethylase till the identification of disease-related *UTX* mutations and specific UTX inhibitors.

Introduction

A decade ago, the identification of the first histone demethylase *Lysine (K)-Specific Demethylase 1* (LSD1) served as a landmark discovery that triggered research on dynamic regulation of histone methylation.[1] In the following years, numerous additional histone demethylases, that execute the removal of methyl groups on specific lysine residues of the histone tails and of non-histone substrates, were characterized in more detail (reviewed in ref. 2). In 2007, several groups identified *Ubiquitously Transcribed Tetratricopeptide Repeat on chromosome X* (UTX) and *Jumonji D3* (JMJD3) as novel histone demethylases that catalyze the removal of di- and trimethyl groups on histone H3 lysine 27, thereby promoting target gene activation.[3-6] Notably, *Ubiquitously Transcribed Tetratricopeptide Repeat on chromosome Y* (UTY) is a closely related homolog of UTX on the Y-chromosome but, up until now, no enzymatic H3K27me2/3 demethylase activity has been reported for UTY.[3,7]

The X-linked H3K27me2/3 eraser UTX is a member of the MLL2 histone H3K4 methyltransferase complex[8,9] and contributes to animal body patterning by regulation of homeobox (*HOX)* genes.[4,5] In contrast, a histone demethylation-independent role for UTX and JMJD3 has been demonstrated in normal and malignant T-cells through interaction with the BRG1-containing SWI/SNF remodeling complex.[10] *UTX* knockout (KO) studies have unraveled important roles for UTX in many developmental processes, including cardiac development and hematopoiesis, but also suggested that UTX and UTY might have redundant functions during embryonic development.[11-17]

Upon the establishment of UTX as a histone eraser in the context of normal development, a number of studies started to report genetic defects targeting *UTX* as the underlying cause of specific diseases. In 2009, a role for the histone H3K27me2/3 demethylase UTX as tumor suppressor was initially postulated in several human tumors including multiple myeloma, esophageal and renal cancer.[18] In 2012, specific loss-of-function defects in *UTX* were identified in patients with a specific hereditary disorder named the Kabuki syndrome.[19] In this review, we summarize the current knowledge on UTX in normal development and highlight recent findings on its implication in cancer and hereditary disease.

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UTX Drives Context Dependent Transcriptional Regulation Mainly Through its H3K27 Demethylase Activity

H3K27me2/3 demethylation

Methylation of H3K27 is a critical mediator of transcriptional gene repression and contributes to important biological processes including X-inactivation, genomic imprinting, stem cell maintenance, animal body patterning, circadian rhythms and cancer.[5,20] Regulation of cellular H3K27me3 levels is mainly mediated by the H3K27 methyltransferase *Polycomb Repressor Complex 2* (PRC2) and the H3K27me2/3 demethylases UTX and JMJD3 (**Fig. 1**).[3-6,21-24] Two main classes of histone demethylases have been discovered until now including the flavin-dependent amine oxidases, such as LSD1,[1] and the iron and α-ketoglutarate-dependent dioxygenases with a Jumonji C (JmjC) catalytic domain, such as UTX and JMJD3.[3-7]

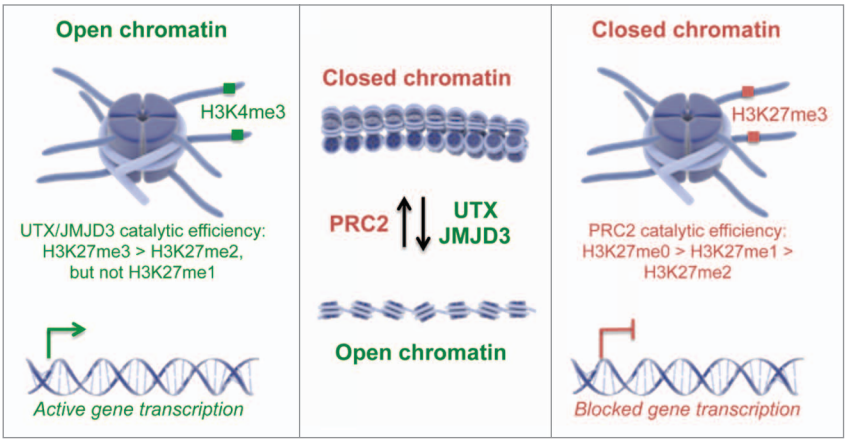

Figure 1. The UTX family mediates H3K27me2/3 demethylation. Graphical illustration of open and closed chromatin states that are mediated by the histone demethylases UTX and JMJD3 and the histone methyltransferase PRC2 hereby erasing or writing methyl groups on H3K27 enabling activation or blockage of gene transcription, respectively (graphics from www.somersault1824.com).

The H3K27me2/3 demethylases UTX and JMJD3 preferentially demethylate H3K27me3 followed by H3K27me2 in vitro and in vivo. This demethylase activity is dependent on the catalytic JmjC domain, which contains conserved residues for binding with the co-factors iron and α-ketoglutarate.[3-7] Moreover, a newly identified zinc-binding domain within these H3K27me2/3 erasers provides specificity toward the histone lysine H3K27 and excludes interaction with the near-cognate histone lysine H3K9.[25,26] Notably, the family member UTY lacks H3K27me2/3 erasing activity in vitro and in vivo despite a conserved JmjC domain and 88% sequence homology with the UTX protein.[3,7,14] Finally, UTX and UTY proteins contain tetratricopeptide repeats (TPRs) at their N-terminal regions that are important for protein-protein interactions. These TPRs are lacking in the JMJD3 protein,[3-7] which might suggest lack of redundant functions between the H3K27me2/3 demethylases UTX and JMJD3.

UTX, UTY and JMJD3 are evolutionary conserved from *Caenorhabditis elegans* (*C. elegans*) to human.[3-7] The mouse genome contains the three H3K27me2/3 demethylase family members *Utx*, *Uty* and *Jmjd3*, whereas the genome of *Drosophila melanogaster* harbors only one ortholog of the mammalian H3K27me2/3 demethylases called *dUTX*.[27] The *C. elegans* genome possesses 4 orthologs whereby *UTX-1* resembles the mammalian *UTX* and the three other orthologs are more related to *JMJD3*.[28]

UTX is a member of the MLL2 H3K4 methyltransferase complex

In 2007, an MLL2 (also called ALR and formerly called MLL4) complex was identified in different mammalian cell types containing 12 protein members including MLL2, PTIP, UTX, ASC-2, ASH2L, RBBP5, WDR5, DPY30, matrin3, MGC4606, α- and β-tubulin.[8] Furthermore, the 3 complex members MLL2, PTIP and UTX were shown to co-localize at promoter regions and first exons of MLL2 target genes marked with the transcriptional activation mark H3K4me3.[8] Another group confirmed the identification of a similar H3K4 methyltransferase complex in which MLL2, MLL3, PTIP, UTX, ASC-2, ASH2L, RBBP5, WDR5, DPY30 and PA1 were also linked to H3K4 methylation activity (**Fig. 2**).[9] Interestingly, the composition of the MLL2 complex was confirmed in the *Drosophila* ortholog Trithorax related (Trr) complex[29] and the *C. elegans* ortholog SET-16 complex.[28] Hence, this conserved MLL2 complex acts like a classical H3K4 methyltransferase complex,[8] which suggests a dynamic interplay between H3K27me2/3 demethylation and H3K4 methylation during transcriptional gene activation.

In flies, *dUTX* mutant tissues are marked by an increase in global H3K27me3 levels and surprisingly also a reduction in global H3K4me1 levels.[20,30] The effect on H3K4me1, a mark enriched at enhancer regions and at gene bodies of actively transcribed genes, seems to be independent of the demethylation capacity of dUTX.[20,30] Similarly, loss of the Trr H3K4 methyltransferase complex results in a global profound reduction of H3K4me1 levels in many tissues.[30,31] Notably, Trr and dUTX are enriched genome-wide near transcriptional start sites (TSSs) (enriched for H3K4me3 and RNAP II) but also on active enhancers (enriched for H3K4me1) indicating a promoter-proximal role as well as a promoter-distal role for the Trr-dUtx complex in transcriptional regulation.[30] Importantly, loss of Trr provoked the most profound reduction in H3K4me1 levels at the enhancer regions compared with dUTX loss[30] indicating that the Trr complex itself is the main driver of H3K4 monomethylation with support of dUTX.

UTX interacts with the SWI/SNF remodeling complex and the histone acetyltransferase CBP

Besides regulation of histone modifications like H3K27me3, ATPase-dependent remodeling complexes including the SWI/SNF family contribute to regulation of chromatin accessibility hereby enabling transcriptional activation and repression.[32] The

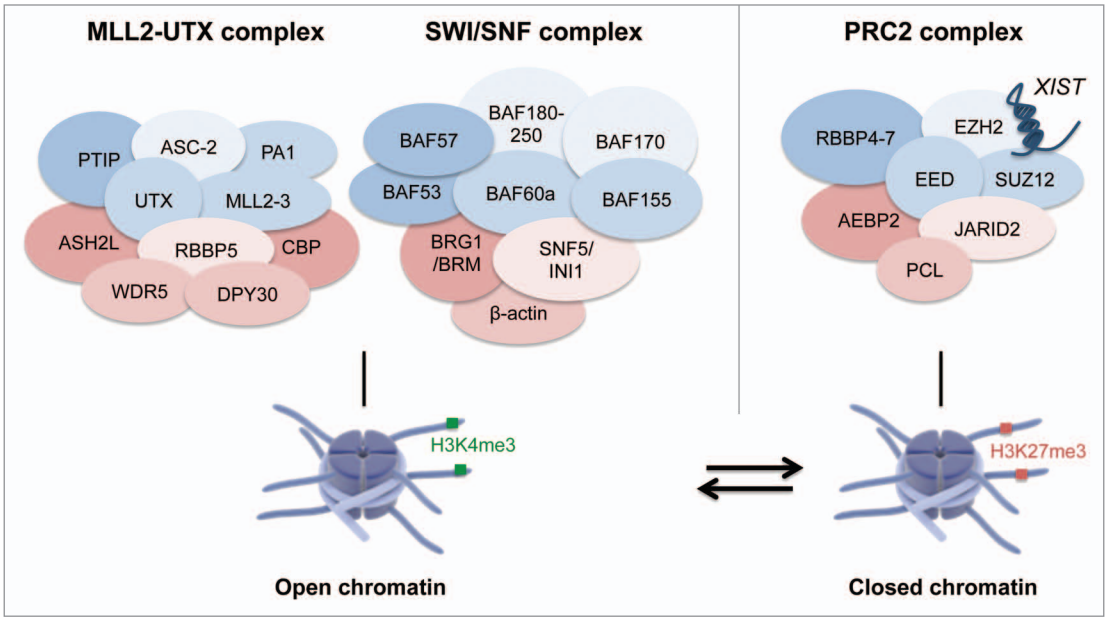

Figure 2. The chromatin complexes MLL2-UTX, SWI/SNF and PRC2 contribute to open and closed chromatin conformations. Schematic representation of the H3K4 methyltransferase complex MLL2-UTX, the SWI/SNF ATPase remodeling complex and the H3K27 methyltransferase complex PRC2 composed out of different protein-coding and non-protein-coding members. The histone eraser UTX is part of the MLL2 complex leading to a dynamic interplay between H3K4 methylation and H3K27me2/3 demethylation. Furthermore, UTX can cooperate with the BRG1-containing SWI/SNF complex where it plays a role in general chromatin remodeling independent of its H3K27 demethylase function. The MLL2-UTX and SWI/SNF complexes both contribute to an open chromatin formation. The PRC2 complex enables efficient methylation of H3K27 thereby promoting gene silencing and chromatin compaction (graphics from www.somersault1824.com).

SWI/SNF family is mainly linked to transcriptional activation whereby the ATPase complex members BRG1 and BRM interact with acetylated histone tails through their bromodomain (**Fig. 2**).[33,34] An interaction between the Brg1-containing SWI/SNF remodeling complex and the histone H3K27me2/3 erasers Utx and Jmjd3 was demonstrated promoting general chromatin remodeling that was independent of their H3K27me2/3 demethylase potential. Furthermore, UTX and JMJD3 were shown to be functionally required for transcriptional activation of lineage-defining T-box transcription factors (including T-bet, Eomes and Tbx5),[10] which represent essential transcriptional regulators in early cell-fate decisions, differentiation and organogenesis.[35]

An interaction between the histone acetyltransferase Creb-binding protein (CBP) and the SWI/SNF remodeling complex is observed in mammals[10,36] and *Drosophila*.[37] In flies, CBP and the PRC2 complex act antagonistically in the regulation of active and repressed chromatin states at Polycomb target genes by acetylation and methylation of H3K27, respectively (**Fig. 2**).[37] H3K27 acetylation is mainly detected at enhancers, promoters and gene bodies of actively transcribed genes.[30] To enable efficient H3K27 acetylation, CBP was shown to associate with dUTX and the ATPase Brm of the SWI/SNF complex.[37] The ATPase Brm in flies resembles the 2 mammalian orthologs BRM and BRG1 of the SWI/SNF complexes.[32,37] Genome-wide binding studies showed that the chromatin components dUTX, Brm and CBP co-occurred at Polycomb target genes enriched for H3K27ac, suggesting a collaborative effort antagonizing Polycomb mediated gene silencing.[37] Of note, loss of dUTX or Trr results in a reduction of global H3K27ac levels in various tissues,[30,37] so dUTX might be capable of promoting H3K27 acetylation and H3K4 monomethylation in concert with Trr, Brm and CBP.

UTX regulates dynamic *HOX* expression

The *HOX* genes are highly conserved transcriptional regulators essential during development of the anterior-posterior axis (head-tail).[38] The *HOX* family in humans consists of 39 *HOX* genes that encode 4 clusters of HOX proteins, i.e., HOXA to HOXD.[39] Aberrant expression of *HOX* genes leads to developmental defects and disease.[39] Polycomb and Trithorax proteins are implicated in the complex regulation of spatio-temporal expression of *HOX* genes thereby regulating animal body patterning and cell differentiation.[40] Evidence for direct involvement of UTX in regulation of *HOX* gene activity was demonstrated through UTX knockdown experiments in HEK293T cells in which loss of UTX induced transcriptional repression of *HOXA* and *HOXC* clusters.[5] Furthermore, UTX loss also resulted in an increase of H3K27me2/3 levels at the promoter regions of *HOXA13* and *HOXC4*, whereas no changes in MLL2 and PRC2 occupancy were observed.[5]

Chromatin immunoprecipitation (ChIP) of UTX followed by *HOX*-specific ultra-dense tiling microarrays (ChIP-chip) showed a strong UTX binding to narrow windows within 500 base pairs downstream of the TSSs of many *HOX* genes in human fibroblasts, but not in mouse embryonic stem cells (ESCs) where *HOX* genes are silenced. Importantly, H3K27 trimethylation was concomitantly diminished at UTX binding sites.[3] Hence,

HOX expression is tightly regulated by opposing activities of the H3K27me3 writer PRC2 and the H3K27me2/3 eraser UTX in order to enable context dependent transcriptional regulation.

UTX regulates a retinoblastoma gene network

Genome-wide ChIP-chip analysis of UTX, H3K4me2 and H3K27me3 in human fibroblasts identified UTX binding at 1945 promoters mostly upstream of TSS.[41] Out of these, the majority of UTX targets were enriched for H3K4me2 (62%) and showed transcriptional activity. This observation is consistent with UTX being a member of the H3K4 methyltransferase MLL2 complex.[8,9,41] Using Ingenuity Pathway analysis (IPA), a RB gene network including *RB1, HBP1* and *RBBP4, 5, 6,* and *9* was discovered as the most significant network of UTX-bound genes enriched for H3K4me2.[41] Loss of UTX in human fibroblasts and mouse embryonic fibroblasts (MEFs) confirmed increased levels of H3K27me3 at promoters of genes from the identified RB-network thereby contributing to deregulated RB-dependent cell cycle arrest leading to ectopic cell proliferation.[41,42]

UTX Escapes X-Inactivation

The female genome harbors 2 X-chromosomes of which one X-chromosome is silenced to compensate the difference in gene dosage with males.[43] X-inactivation is mediated by the long intergenic non-coding RNA (lincRNA) *XIST*, which coats the inactive X-chromosome. Next, the PRC2 complex is guided by *XIST* to the inactive X-chromosome hereby enabling H3K27 methylation leading to the formation of facultative heterochromatin and gene silencing (**Fig. 2**).[44]

Utx is one of the few genes that can escape X-inactivation in female mice and human.[45] In agreement, Utx shows higher expression in female tissue of brain, liver, neurons and sexual organs.[46,47] In line with this observation, a female-specific function was reported for Utx in the regulation of the X-linked homeobox genes *Rhox6* and *Rhox9*.[46] It was shown that Utx is expressed at higher levels in undifferentiated female ESCs as compared with male ESCs, which resulted in a stronger Utx binding in the promoter regions of *Rhox6* and *Rhox9* in females. Subsequently, Utx mediates a more profound H3K27me3 removal and transcriptional activation of these homeobox genes in female cells.[46]

The Role of UTX in Cellular Reprogramming

Pluripotency is the unique characteristic of ESCs and the early developmental stage embryo to induce the generation of the three germ layers endoderm, ectoderm and mesoderm finally constituting all tissues of the adult organism.[48] Through in vitro reprogramming of somatic cells via induction of the four transcription factors OCT4, SOX2, KLF4, and MYC; pluripotency can be re-established hereby generating induced pluripotent stem cells (iPSC).[48,49] During reprogramming, genome-wide changes in the transcriptome and the chromatin structure are induced to achieve the switch to the pluripotent state.[48]

The histone H3K27me3 demethylase Utx has a critical role in efficient induction or re-establishment of pluripotency during the generation of iPSCs in vitro, but is dispensable for maintenance of pluripotency.[50] The requirement for UTX to ensure correct reprogramming depends on its H3K27me3 demethylase activity.[50] During iPSC induction, Utx target genes that are enriched with H3K4me3 in *Utx* wild-type MEFs aberrantly accumulate H3K27me3 in *Utx* mutant MEFs. These loci, including the stem-cell maintenance genes *Sall1*, *Sall4,* and *Utf1* as well as validated *Klf4* and *Oct4* target genes, fail to reactivate during reprogramming due to loss of Utx.[50] Hence, Utx seems to play a crucial role during reprogramming through its H3K27me2/3 demethylation activity in correct re-activation of essential pluripotency genes.

A Critical Role for UTX in Embryonic Stem Cells and Embryonic Development

Embryonic stem cells

H3K27 methylation is important in the maintenance of self-renewing ESCs by repressing tissue-specific developmental genes.[51-53] During ESC differentiation, the re-activation of developmental genes is associated with loss of H3K27me3.[51] Loss of Utx does not influence ESC self-renewal and proliferation, but seems to provoke an effect on the differentiation capacity of ESCs.[11,12,50,54] This differentiation defect is reflected by loss of induction of a set of developmental genes including some ectoderm markers (*Otx2, Msi1, Sox1,* and *Pax6*),[54] endoderm markers (*Gata4*, *Gata6, Foxa2, Sox17, Gsc,* and *Ncad*)[12,55] and mesoderm markers (*Vegfr2, Wnt3,* and *Brachyury*),[12,13,54,55] presumably due to loss of Utx binding to the promoter regions of these genes.[54]

Contradicting studies report on whether the effect of UTX loss on ESC differentiation depends on its H3K27me3 demethylase activity.[12,13,54,55] Namely, male *UTX* KO ESCs that express a catalytic inactive or wild-type Utx protein both could rescue the induction of expression of the mesoderm genes *Wnt3* and *Brachyury* supporting an H3K27me3-demethylase-independent role of Utx during ESC differentiation.[54] Furthermore, Utx binds directly to the promoters of the ectoderm genes *Msi1* and *Sox1* and the mesoderm gene *Brachyury* marked with an increase in H3K4me3 levels but without a change in H3K27me3 levels.[54] In contrast, a decrease in H3K27me3 levels and an increase in H3K4me3 levels was seen at the promoter of the Utx target *Hoxb1* during differentiation of Utx wild-type ESCs confirming that Utx still plays an H3K27me3 demethylase-dependent role in transcriptional regulation of homeotic and developmental genes.[54] Therefore, UTX regulates transcriptional activation of UTX target genes during ESC differentiation in different ways dependent or independent of its H3K27me2/3 demethylation activity.

Embryonic development in mice

Several groups have shown that complete loss of Utx in $Utx^{\Delta/\Delta}$ female mice leads to embryonic lethality between embryonic day (E) 10.5 and E12.5, whereas $Utx^{\Delta/+}$ heterozygous female mice are viable and fertile.[11-15] Surprisingly, $Utx^{\Delta/Y}$ male mice are marked

by a diverse outcome ranging from embryonic lethality to tumor formation in adults.[11-15] The viable adult *Utx*$^{\Delta/Y}$ male mice are smaller in size compared with their littermates[12,14] and remain fertile indicating that Utx is not required for male fertility.[12] Interestingly, *Utx*$^{\Delta/Uty\Delta}$ male mice, in which both *Utx* and *Uty* are eliminated, phenocopy the *Utx*$^{\Delta/\Delta}$ female mice,[14] suggesting that the Y-linked *Uty* gene can partially rescue the effect of complete loss of Utx.[11] In addition, Utx and Uty can both interact with Rbbp5 (member of the Mll2 complex) regulating H3K4 methylation rather than H3K27me2/3 demethylation at Utx and Uty target genes.[14]

Embryonic development in *Drosophila melanogaster*

Homozygous *dUTX Drosophila* mutants are lethal, with only 5% of the animals that survive the pupal stage although not reaching adulthood.[20] More specifically, *dUTX*$^{\Delta}$ homozygotes that lack both maternal and zygotic dUTX protein (*dUTX*$^{\Delta mat-zyg-}$) die as larvae, whereas *dUTX*$^{\Delta}$ homozygotes that lack only zygotic dUTX protein (*dUTX*$^{\Delta mat+zyg-}$) can develop further but die quickly after the pupal stage.[56] dUTX was shown to be necessary for the controlled expression of 2 specific homeotic genes *Ubx* and *Abd-B* in very early fly development.[56] Furthermore, sex combs malformation, rough eyes, wrinkled wings and wing vein defects are observed in the *dUTX* mutants which resembles some characteristics of Trithorax mutants and is in concordance with the effect of these proteins on *Hox* gene regulation.[20,56]

Embryonic development in *Caenorhabditis elegans*

In agreement with *Drosophila*, *UTX-1* mutant worms lacking both maternal and zygotic UTX-1 protein (*UTX-1*$^{m-z-}$) die as late stage embryos or malformed L1 larvae. Mutants with loss of only the zygotic UTX-1 (*UTX-1*$^{m+z-}$) protein are viable and reach adulthood, but a reduction in fertility is observed due to defects in gonad migration and oocyte organization. The *UTX-1*$^{m-z-}$ mutants show an increase in global H3K27me2/3 levels at the embryonic stage, but introduction of a catalytic inactive mutant of UTX-1 in *UTX-1*$^{m+z-}$ mutant animals rescued the fertility indicating that the UTX-1 demethylase activity is not necessary for this developmental process. Interestingly, loss of UTX-1 complex members SET-16 (MLL2–3), PIS-1 (PTIP), WDR-5.1 (WDR5) and F21H12.1 (RBBP5) also resulted in posterior and gonadal defects further confirming that these genes are acting in the same genetic complex.[28]

A Critical Role for UTX in Tissue-Specific and Developmental Processes

UTX contributes to a variety of tissue-specific and developmental processes including cardiac development,[11-15] hematopoiesis,[13,15,57,58] myogenesis,[16,17,27,59] osteogenic differentiation,[60] wound healing,[61] and aging.[62,63] Cardiac development and hematopoiesis will be discussed in further detail.

Cardiac development

The histone eraser Utx seems to play an important role in cardiac development. *Utx*$^{\Delta/Y}$ ESCs that in vitro differentiate into mature cardiac cells show absence of effective heart-like rhythmic contractions due to a failure of inducing cardiac-specific gene expression.[11] Furthermore, *Utx*$^{\Delta/\Delta}$ female mice and *Utx*$^{\Delta/Uty\Delta}$ male mice exhibited strong cardiac developmental and neural tube closure defects (cranioschisis) at day E9-E10.5.[11-15] This cardiac malformation is presumably caused by 2 mechanisms. First, loss of Utx disables the induction of transcriptional activation of cardiac-specific genes mediated by H3K27me2/3 demethylation, whereby the core cardiac transcription factors Nkx2-5, Tbx5, Gata4 and Srf specifically guide Utx to the promoter regions of cardiac-specific genes (**Fig. 3**).[11] Second, loss of Utx disturbs the interaction between the Brg1-containing SWI/SNF chromatin remodeling complex and the core cardiac transcription factor Tbx5, an effect that is thought to be independent from the H3K27me2/3 demethylase activity of UTX (**Fig. 3**).[10,11] This dual role of UTX is further supported by an independent study showing that UTX and UTY can physically interact with BRG1, NKX2-5, SRF and TBX5 hereby inducing expression of cardiac-specific genes.[14] Hence UTX enables transcriptional activation of cardiac-specific genes through H3K27me3 demethylase dependent and independent mechanisms during cardiac development.

Hematopoiesis

Utx expression levels peak in hematopoietic stem and progenitor cells and drop during hematopoietic differentiation.[58] In *Utx*$^{\Delta/\Delta}$ female mice embryos (E10.5), a lack of red bloods cells (anemia) was observed indicating a critical role for Utx in hematopoiesis.[13] Furthermore, female adult mice in which *Utx* was homozygously eliminated at 11–14 wk of age showed an enlarged spleen, reduced hemoglobin levels, anemia, thrombocytopenia and mild leukocytopenia compared with normal controls. In addition, hematopoietic transcription factors *Tal1*, *Gata1* and *Lyl1* were downregulated in the bone marrow of Utx KO female mice.[15] Furthermore, Utx was identified as an essential regulator of stem cell migration since loss of Utx blocked the migration potential of hematopoietic progenitor cells in vitro and of primordial germ cells in vivo.[15]

Constitutional UTX Defects Cause the Kabuki Syndrome

Constitutional loss-of-function defects in *UTX* cause the so-called Kabuki syndrome. This rare congenital anomaly syndrome, also called the Kabuki make-up syndrome, was first described by Niikawa and Kuroki in 1981.[64,65] The Kabuki syndrome is characterized by moderate-to-severe mental retardation, visceral and skeletal abnormalities, postnatal growth impairment (short stature) and facial abnormalities including large protruding ears.[64-66] The Kabuki syndrome has a prevalence of about 1 in 32 000 live births.[65] Using whole-exome sequencing, nonsense and frameshift mutations in the *MLL2* gene were first identified as a cause of the Kabuki syndrome[67] in 74% of these patients.[68] Two years later, 3 focal deletions targeting the histone demethylase *UTX* were identified in 1 male and 2 female Kabuki patients.[69] Subsequently, 2 nonsense mutations and a small indel mutation were discovered in 1 female and 2 male Kabuki patients.[19]

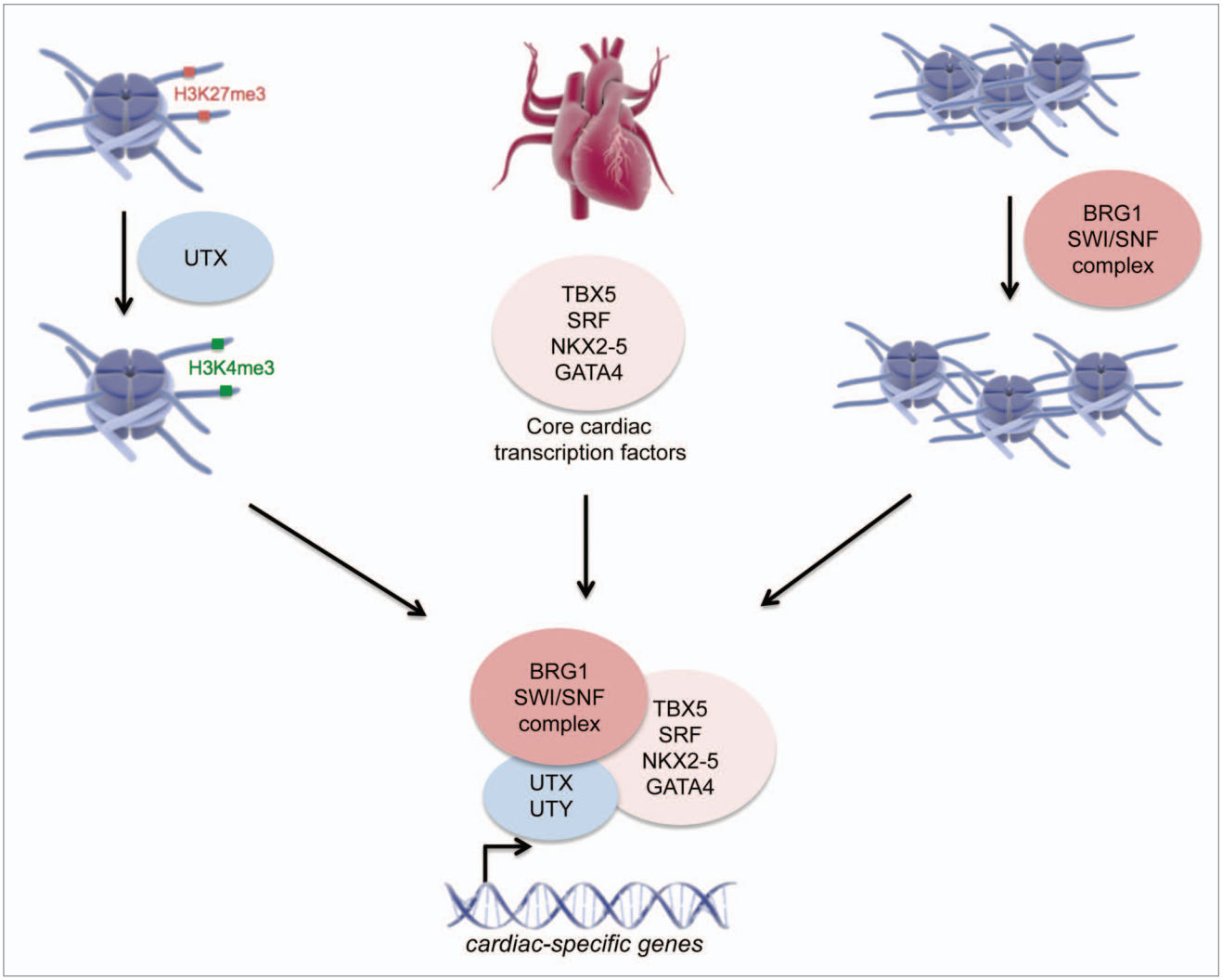

Figure 3. The involvement of UTX in genetic regulation of cardiac development. During cardiac development UTX, guided by the core cardiac transcription factors NKX2-5, TBX5, GATA4 and SRF, promotes specific gene activation of cardiac-specific genes through demethylation of H3K27me2/3 at their promoter regions. In addition, UTX and UTY can interact with the BRG1-containing SWI/SNF chromatin remodeling complex enabling general chromatin remodeling at these cardiac-specific gene loci (graphics from www.somersault1824.com).

As discussed in the next part, both the *MLL2* and the *UTX* gene have been reported as important histone modifier genes involved in cancer pathogenesis. Until now, 6 Kabuki patients have been described that developed different types of cancer including pre-B-ALL,[70] hepatoblastoma,[71] neuroblastoma,[71,72] Burkitt lymphoma,[73] and fibromyxoid sarcoma[74] marking the Kabuki syndrome as a cancer predisposition syndrome.

UTX as a Bona Fide Tumor Suppressor Gene in Cancer Biology

Over the last years, deregulated histone methylation became a major theme in cancer biology research including the balance of H3K27 methylation. In 2009, somatic loss-of-function mutations and deletions targeting the *UTX* gene were identified in multiple cancer types including multiple myeloma, esophageal and renal cancer.[18] This notion was further supported by subsequent identification of recurrent inactivating *UTX* mutations and deletions in several leukemia as well as solid tumor types (**Fig. 4**; **Fig. S1**; **Tables S1 and S2**), mainly through exome- or genome-wide sequencing strategies.[75-84] Furthermore, a recent study of The Cancer Genome Atlas (TCGA) in which whole-exome sequencing was performed on 3281 tumors derived from 12 tumor types identified 127 significantly mutated genes including the tumor suppressor gene *UTX*.[85]

Bladder cancer has one of the highest frequencies of *UTX* mutations (**Fig. 4**) typically consisting of truncating mutations in the region coding for the functional JmjC domain of UTX. Interestingly, more than 50% of bladder cancer patients harbor genetic aberrations in a bigger set of chromatin remodeling genes including *UTX, CREBBP, EP300, ARID1A, CHD6, MLL1, MLL3,* and *NCOR1*.[77] Furthermore, multiple myeloma cases harboring mutations in *UTX* or in the Trithorax complex members

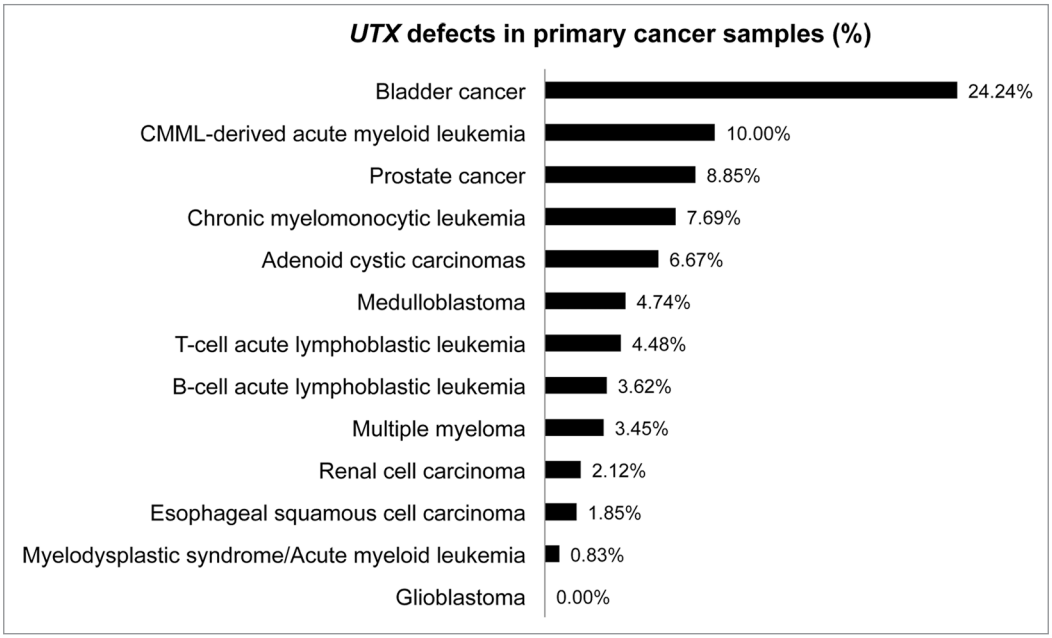

Figure 4. *UTX* defects in primary cancer samples. Schematic representation of the presence of *UTX* defects (%) in primary cancer samples of a broad range of leukemic and solid cancer types investigated in multiple genomic studies.

MLL1, MLL2, and *MLL3* were linked with high expression of *HOXA9*[76] confirming the role of UTX in *HOX* gene regulation.[3]

Interestingly, a study in chronic myelomonocytic leukemia (CMML) identified loss-of-function mutations in both *UTX* and *EZH2* despite their opposing roles on H3K27me3 regulation. Notably, these mutations occurred in a mutually exclusive manner in CMML patient samples.[86] *UTX* and *EZH2* defects in CMML were associated with *ASXL1*[87] and *TET2*[88] loss-of-function mutations, two genes also implicated in epigenetic regulation of gene expression.[86] These studies indicate that the chromatin regulatory machinery is a recurrent mutational target in a broad range of cancer types highlighting the importance of correct regulation of chromatin remodeling in the homeostasis of normal tissues.[32]

Whole-exome and whole-genome next-generation sequencing studies in medulloblastoma identified mutations in different chromatin regulators including *UTX*, *MLL2*, *MLL3*, *SMARCA4 (BRG1), ZMYM3,* and *CREBBP*.[78-80] In general, four prognostically relevant subgroups (SHH-subgroup, WNT-subgroup, subgroup 3 and subgroup 4) are defined in this malignant childhood brain tumor.[80] Of particular interest, medulloblastoma is marked by a disturbed gender distribution toward males in subgroups 3 and 4[89] and mutations targeting the X-linked *UTX* gene were exclusively identified in these 2 subgroups.[79,80,90] In line with the fact that *UTX* escapes X-inactivation,[45] female medulloblastoma patients harboring *UTX* defects showed bi-allelic *UTX* inactivation (splice-site *UTX* mutation and deletion[80] or missense mutation and loss of chromosome X[79]), suggesting that complete loss of UTX is required for malignant transformation. In addition, more than 50% of *UTX* mutant male medulloblastoma patients had a deletion of the Y-chromosome (including the UTX family member *UTY*) compared with less than 10% of *UTX* wild-type males.[80] Furthermore, gain or overexpression of the H3K27 methyltransferase *EZH2* as well as inactivating mutations in H3K4me3 regulators *CHD7* and *ZMYM3* were also present in subgroups 3 and 4 of medulloblastoma. All together, these data indicate that deregulation of H3K27 and H3K4 methylation is a core oncogenic component of medulloblastoma. Importantly, mutations targeting the X-linked gene *UTX* might partially explain the higher male prevalence in medulloblastoma subgroups 3 and 4.[80]

So far, the functional consequences of cancer related *UTX* mutations have been poorly characterized. Western blot analyses show lack of UTX protein expression in *UTX* mutant cancer cell lines.[18] Furthermore, re-expression of UTX in *UTX* mutant cancer cell lines was associated with lower H3K27me3 levels at the promoters of Polycomb target genes and concomitant inhibition of cell proliferation.[18] In adenoid cystic carcinomas, the impact of the identified *UTX* missense mutations was evaluated by introduction of these *UTX* mutants in HEK293T cells, which led to an increase in cell growth and a decrease in H3K27me3 levels.[84] Finally, a *Sleeping Beauty* based insertional mutagenesis screen using a *Kras* driven pancreatic mouse model identified 543 candidate cancer driver genes with 10% of the genes involved in chromatin regulation including the *Utx* gene. This un-biased screening approach provides an independent in vivo confirmation of the potential role of *Utx* as a bona fide tumor suppressor gene in cancer biology.[91]

Targeting UTX by Small Molecule Inhibitors

The most selective compound against JMJD3 and UTX, GSK-J1, was discovered based on the structural insights of the

human and mouse JMJD3 protein with an IC_{50} of 60nM for inhibiting JMJD3. Furthermore, GSK-J1 is active against both H3K27me3 erasers UTX and JMJD3 but inactive against a panel of demethylases of the JmjC family. Notably, the cell-penetrating derivative GSK-J4 was able to inhibit the JMJD3-induced loss of total nuclear H3K27me3 levels and to enable specific inhibition of H3K27 demethylation at promoter regions of JMJD3 and UTX target genes.[92]

Several general inhibitors of JmjC demethylases have been identified including the α-ketoglutaric acid mimics N-oxalylglycine,[93,94] methylstat[95] and 2,4-dicarboxypyridine; the iron-chelating agent deferoxamine[95]; the pyridine hydrazone JIB-04[96] and catechols.[97] Unfortunately, these inhibitors are selective against some or all JmjC enzymes but are unable to target one specific JmjC member.

Conclusions

In this review, we have summarized and discussed the identification of the histone H3K27me2/3 erasers UTX and JMJD3, and the role of UTX in normal development and disease. UTX cooperates with a set of chromatin players, including the histone methyltransferase complex MLL2, the histone acetyltransferase CBP and the BRG1-containing SWI/SNF remodeling complex,[8-10,37] to trigger active gene transcription by H3K27me2/3 demethylation or H3K4 methylation.[3-6,8-10,30,37] Up until now, the main direct target genes of UTX encompass a broad set of *HOX* genes important in animal body patterning[3,5] and a *RB* gene network implicated in cell fate control.[41] Furthermore, UTX loss can lead to the development of the Kabuki syndrome[69] and can contribute to cancer pathogenesis.[18]

The third UTX family member UTY has no clear enzymatic demethylase activity despite the presence of a conserved JmjC domain and high amino acid conservation.[3,7,14] Surprisingly, UTY can partly compensate UTX functions including the cooperation with the MLL2 and BRG1-containing SWI/SNF complexes.[14] Also, UTY can partially rescue embryonic lethality in *UTX* KO male mice.[11-15] Furthermore, *UTX* mutations are frequently found together with deletions encompassing the *UTY* gene locus in male cancer patients.[18,80] Hence, a set of overlapping functions between UTX and UTY in transcriptional regulation seems present despite the lack of H3K27me2/3 demethylase activity for UTY.

All together, the complexity of biological functions assigned to UTX is just starting to emerge and will trigger additional research in a broad range of developmental processes. Multiple studies have highlighted UTX as an important player in cancer biology, but its role as a bona fide tumor suppressor still needs further confirmation using genetically engineered in vivo models. Ultimately, these biological insights will clarify to what extend loss of UTX provides therapeutic opportunities for human disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Materials

Supplemental materials may be found here:
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References

1. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 2004; 119:941-53; PMID:15620353; http://dx.doi.org/10.1016/j.cell.2004.12.012
2. Kooistra SM, Helin K. Molecular mechanisms and potential functions of histone demethylases. Nat Rev Mol Cell Biol 2012; 13:297-311; PMID:22473470
3. Lan F, Bayliss PE, Rinn JL, Whetstine JR, Wang JK, Chen S, Iwase S, Alpatov R, Issaeva I, Canaani E, et al. A histone H3 lysine 27 demethylase regulates animal posterior development. Nature 2007; 449:689-94; PMID:17851529; http://dx.doi.org/10.1038/nature06192
4. Agger K, Cloos PA, Christensen J, Pasini D, Rose S, Rappsilber J, Issaeva I, Canaani E, Salcini AE, Helin K. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. Nature 2007; 449:731-4; PMID:17713478; http://dx.doi.org/10.1038/nature06145
5. Lee MG, Villa R, Trojer P, Norman J, Yan KP, Reinberg D, Di Croce L, Shiekhattar R. Demethylation of H3K27 regulates polycomb recruitment and H2A ubiquitination. Science 2007; 318:447-50; PMID:17761849; http://dx.doi.org/10.1126/science.1149042
6. De Santa F, Totaro MG, Prosperini E, Notarbartolo S, Testa G, Natoli G. The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. Cell 2007; 130:1083-94; PMID:17825402; http://dx.doi.org/10.1016/j.cell.2007.08.019
7. Hong S, Cho YW, Yu LR, Yu H, Veenstra TD, Ge K. Identification of JmjC domain-containing UTX and JMJD3 as histone H3 lysine 27 demethylases. Proc Natl Acad Sci U S A 2007; 104:18439-44; PMID:18003914; http://dx.doi.org/10.1073/pnas.0707292104
8. Issaeva I, Zonis Y, Rozovskaia T, Orlovsky K, Croce CM, Nakamura T, Mazo A, Eisenbach L, Canaani E. Knockdown of ALR (MLL2) reveals ALR target genes and leads to alterations in cell adhesion and growth. Mol Cell Biol 2007; 27:1889-903; PMID:17178841; http://dx.doi.org/10.1128/MCB.01506-06
9. Cho YW, Hong T, Hong S, Guo H, Yu H, Kim D, Guszczynski T, Dressler GR, Copeland TD, Kalkum M, et al. PTIP associates with MLL3- and MLL4-containing histone H3 lysine 4 methyltransferase complex. J Biol Chem 2007; 282:20395-406; PMID:17500065; http://dx.doi.org/10.1074/jbc.M701574200
10. Miller SA, Mohn SE, Weinmann AS. Jmjd3 and UTX play a demethylase-independent role in chromatin remodeling to regulate T-box family member-dependent gene expression. Mol Cell 2010; 40:594-605; PMID:21095589; http://dx.doi.org/10.1016/j.molcel.2010.10.028
11. Lee S, Lee JW, Lee SK. UTX, a histone H3-lysine 27 demethylase, acts as a critical switch to activate the cardiac developmental program. Dev Cell 2012; 22:25-37; PMID:22192413; http://dx.doi.org/10.1016/j.devcel.2011.11.009
12. Welstead GG, Creyghton MP, Bilodeau S, Cheng AW, Markoulaki S, Young RA, Jaenisch R. X-linked H3K27me3 demethylase Utx is required for embryonic development in a sex-specific manner. Proc Natl Acad Sci U S A 2012; 109:13004-9; PMID:22826230; http://dx.doi.org/10.1073/pnas.1210787109
13. Wang C, Lee JE, Cho YW, Xiao Y, Jin Q, Liu C, Ge K. UTX regulates mesoderm differentiation of embryonic stem cells independent of H3K27 demethylase activity. Proc Natl Acad Sci U S A 2012; 109:15324-9; PMID:22949634; http://dx.doi.org/10.1073/pnas.1204166109

14. Shpargel KB, Sengoku T, Yokoyama S, Magnuson T. UTX and UTY demonstrate histone demethylase-independent function in mouse embryonic development. PLoS Genet 2012; 8:e1002964; PMID:23028370; http://dx.doi.org/10.1371/journal.pgen.1002964
15. Thieme S, Gyárfás T, Richter C, Özhan G, Fu J, Alexopoulou D, Muders MH, Michalk I, Jakob C, Dahl A, et al. The histone demethylase UTX regulates stem cell migration and hematopoiesis. Blood 2013; 121:2462-73; PMID:23365460; http://dx.doi.org/10.1182/blood-2012-08-452003
16. Seenundun S, Rampalli S, Liu QC, Aziz A, Palii C, Hong S, Blais A, Brand M, Ge K, Dilworth FJ. UTX mediates demethylation of H3K27me3 at muscle-specific genes during myogenesis. EMBO J 2010; 29:1401-11; PMID:20300060; http://dx.doi.org/10.1038/emboj.2010.37
17. Wang AH, Zare H, Mousavi K, Wang C, Moravec CE, Sirotkin HI, Ge K, Gutierrez-Cruz G, Sartorelli V. The histone chaperone Spt6 coordinates histone H3K27 demethylation and myogenesis. EMBO J 2013; 32:1075-86; PMID:23503590; http://dx.doi.org/10.1038/emboj.2013.54
18. van Haaften G, Dalgliesh GL, Davies H, Chen L, Bignell G, Greenman C, Edkins S, Hardy C, O'Meara S, Teague J, et al. Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. Nat Genet 2009; 41:521-3; PMID:19330029; http://dx.doi.org/10.1038/ng.349
19. Miyake N, Mizuno S, Okamoto N, Ohashi H, Shiina M, Ogata K, Tsurusaki Y, Nakashima M, Saitsu H, Niikawa N, et al. KDM6A point mutations cause Kabuki syndrome. Hum Mutat 2013; 34:108-10; PMID:23076834; http://dx.doi.org/10.1002/humu.22229
20. Herz HM, Madden LD, Chen Z, Bolduc C, Buff E, Gupta R, Davuluri R, Shilatifard A, Hariharan IK, Bergmann A. The H3K27me3 demethylase dUTX is a suppressor of Notch- and Rb-dependent tumors in Drosophila. Mol Cell Biol 2010; 30:2485-97; PMID:20212086; http://dx.doi.org/10.1128/MCB.01633-09
21. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 2002; 298:1039-43; PMID:12351676; http://dx.doi.org/10.1126/science.1076997
22. Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, Pirrotta V. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. Cell 2002; 111:185-96; PMID:12408863; http://dx.doi.org/10.1016/S0092-8674(02)00975-3
23. Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. Genes Dev 2002; 16:2893-905; PMID:12435631; http://dx.doi.org/10.1101/gad.1035902
24. Müller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, Wild B, Miller EL, O'Connor MB, Kingston RE, Simon JA. Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. Cell 2002; 111:197-208; PMID:12408864; http://dx.doi.org/10.1016/S0092-8674(02)00976-5
25. Kim E, Song JJ. Diverse ways to be specific: a novel Zn-binding domain confers substrate specificity to UTX/KDM6A histone H3 Lys 27 demethylase. Genes Dev 2011; 25:2223-6; PMID:22056667; http://dx.doi.org/10.1101/gad.179473.111
26. Sengoku T, Yokoyama S. Structural basis for histone H3 Lys 27 demethylation by UTX/KDM6A. Genes Dev 2011; 25:2266-77; PMID:22002947; http://dx.doi.org/10.1101/gad.172296.111
27. Smith ER, Lee MG, Winter B, Droz NM, Eissenberg JC, Shiekhattar R, Shilatifard A. Drosophila UTX is a histone H3 Lys27 demethylase that colocalizes with the elongating form of RNA polymerase II. Mol Cell Biol 2008; 28:1041-6; PMID:18039863; http://dx.doi.org/10.1128/MCB.01504-07
28. Vandamme J, Lettier G, Sidoli S, Di Schiavi E, Nørregaard Jensen O, Salcini AE. The C. elegans H3K27 demethylase UTX-1 is essential for normal development, independent of its enzymatic activity. PLoS Genet 2012; 8:e1002647; PMID:22570628; http://dx.doi.org/10.1371/journal.pgen.1002647
29. Mohan M, Herz HM, Smith ER, Zhang Y, Jackson J, Washburn MP, Florens L, Eissenberg JC, Shilatifard A. The COMPASS family of H3K4 methylases in Drosophila. Mol Cell Biol 2011; 31:4310-8; PMID:21875999; http://dx.doi.org/10.1128/MCB.06092-11
30. Herz HM, Mohan M, Garruss AS, Liang K, Takahashi YH, Mickey K, Voets O, Verrijzer CP, Shilatifard A. Enhancer-associated H3K4 monomethylation by Trithorax-related, the Drosophila homolog of mammalian Mll3/Mll4. Genes Dev 2012; 26:2604-20; PMID:23166019; http://dx.doi.org/10.1101/gad.201327.112
31. Kanda H, Nguyen A, Chen L, Okano H, Hariharan IK. The Drosophila ortholog of MLL3 and MLL4, trithorax related, functions as a negative regulator of tissue growth. Mol Cell Biol 2013; 33:1702-10; PMID:23459941; http://dx.doi.org/10.1128/MCB.01585-12
32. Plass C, Pfister SM, Lindroth AM, Bogatyrova O, Claus R, Lichter P. Mutations in regulators of the epigenome and their connections to global chromatin patterns in cancer. Nat Rev Genet 2013; 14:765-80; PMID:24105274; http://dx.doi.org/10.1038/nrg3554
33. Hassan AH, Prochasson P, Neely KE, Galasinski SC, Chandy M, Carrozza MJ, Workman JL. Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. Cell 2002; 111:369-79; PMID:12419247; http://dx.doi.org/10.1016/S0092-8674(02)01005-X
34. Wang GG, Allis CD, Chi P. Chromatin remodeling and cancer, Part II: ATP-dependent chromatin remodeling. Trends Mol Med 2007; 13:373-80; PMID:17822959; http://dx.doi.org/10.1016/j.molmed.2007.07.004
35. Wilson V, Conlon FL. The T-box family. Genome Biol 2002; 3:REVIEWS3008; PMID:12093383; http://dx.doi.org/10.1186/gb-2002-3-6-reviews3008
36. Naidu SR, Love IM, Imbalzano AN, Grossman SR, Androphy EJ. The SWI/SNF chromatin remodeling subunit BRG1 is a critical regulator of p53 necessary for proliferation of malignant cells. Oncogene 2009; 28:2492-501; PMID:19448667; http://dx.doi.org/10.1038/onc.2009.121
37. Tie F, Banerjee R, Conrad PA, Scacheri PC, Harte PJ. Histone demethylase UTX and chromatin remodeler BRM bind directly to CBP and modulate acetylation of histone H3 lysine 27. Mol Cell Biol 2012; 32:2323-34; PMID:22493065; http://dx.doi.org/10.1128/MCB.06392-11
38. Mallo M, Alonso CR. The regulation of Hox gene expression during animal development. Development 2013; 140:3951-63; PMID:24046316; http://dx.doi.org/10.1242/dev.068346
39. Shah N, Sukumar S. The Hox genes and their roles in oncogenesis. Nat Rev Cancer 2010; 10:361-71; PMID:20357775; http://dx.doi.org/10.1038/nrc2826
40. Bracken AP, Dietrich N, Pasini D, Hansen KH, Helin K. Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. Genes Dev 2006; 20:1123-36; PMID:16618801; http://dx.doi.org/10.1101/gad.381706
41. Wang JK, Tsai MC, Poulin G, Adler AS, Chen S, Liu H, Shi Y, Chang HY. The histone demethylase UTX enables RB-dependent cell fate control. Genes Dev 2010; 24:327-32; PMID:20123895; http://dx.doi.org/10.1101/gad.1882610
42. Terashima M, Ishimura A, Yoshida M, Suzuki Y, Sugano S, Suzuki T. The tumor suppressor Rb and its related Rbl2 genes are regulated by Utx histone demethylase. Biochem Biophys Res Commun 2010; 399:238-44; PMID:20650264; http://dx.doi.org/10.1016/j.bbrc.2010.07.061
43. Wutz A. Gene silencing in X-chromosome inactivation: advances in understanding facultative heterochromatin formation. Nat Rev Genet 2011; 12:542-53; PMID:21765457; http://dx.doi.org/10.1038/nrg3035
44. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. Nature 2011; 469:343-9; PMID:21248841; http://dx.doi.org/10.1038/nature09784
45. Greenfield A, Carrel L, Pennisi D, Philippe C, Quaderi N, Siggers P, Steiner K, Tam PP, Monaco AP, Willard HF, et al. The UTX gene escapes X inactivation in mice and humans. Hum Mol Genet 1998; 7:737-42; PMID:9499428; http://dx.doi.org/10.1093/hmg/7.4.737
46. Berletch JB, Deng X, Nguyen DK, Disteche CM. Female bias in Rhox6 and 9 regulation by the histone demethylase KDM6A. PLoS Genet 2013; 9:e1003489; PMID:23658530; http://dx.doi.org/10.1371/journal.pgen.1003489
47. Xu J, Deng X, Watkins R, Disteche CM. Sex-specific differences in expression of histone demethylases Utx and Uty in mouse brain and neurons. J Neurosci 2008; 28:4521-7; PMID:18434530; http://dx.doi.org/10.1523/JNEUROSCI.5382-07.2008
48. Rada-Iglesias A, Wysocka J. Epigenomics of human embryonic stem cells and induced pluripotent stem cells: insights into pluripotency and implications for disease. Genome Med 2011; 3:36; PMID:21658297; http://dx.doi.org/10.1186/gm252
49. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126:663-76; PMID:16904174; http://dx.doi.org/10.1016/j.cell.2006.07.024
50. Mansour AA, Gafni O, Weinberger L, Zviran A, Ayyash M, Rais Y, Krupalnik V, Zerbib M, Amann-Zalcenstein D, Maza I, et al. The H3K27 demethylase Utx regulates somatic and germ cell epigenetic reprogramming. Nature 2012; 488:409-13; PMID:22801502; http://dx.doi.org/10.1038/nature11272
51. Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 2006; 441:349-53; PMID:16625203; http://dx.doi.org/10.1038/nature04733
52. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 2006; 125:315-26; PMID:16630819; http://dx.doi.org/10.1016/j.cell.2006.02.041
53. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, et al. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell 2006; 125:301-13; PMID:16630818; http://dx.doi.org/10.1016/j.cell.2006.02.043
54. Morales Torres C, Laugesen A, Helin K. Utx is required for proper induction of ectoderm and mesoderm during differentiation of embryonic stem cells. PLoS One 2013; 8:e60020; PMID:23573229; http://dx.doi.org/10.1371/journal.pone.0060020

55. Jiang W, Wang J, Zhang Y. Histone H3K27me3 demethylases KDM6A and KDM6B modulate definitive endoderm differentiation from human ESCs by regulating WNT signaling pathway. Cell Res 2013; 23:122-30; PMID:22907667; http://dx.doi.org/10.1038/cr.2012.119
56. Copur Ö, Müller J. The histone H3-K27 demethylase Utx regulates HOX gene expression in Drosophila in a temporally restricted manner. Development 2013; 140:3478-85; PMID:23900545; http://dx.doi.org/10.1242/dev.097204
57. Chaturvedi CP, Hosey AM, Palii C, Perez-Iratxeta C, Nakatani Y, Ranish JA, Dilworth FJ, Brand M. Dual role for the methyltransferase G9a in the maintenance of beta-globin gene transcription in adult erythroid cells. Proc Natl Acad Sci U S A 2009; 106:18303-8; PMID:19822740; http://dx.doi.org/10.1073/pnas.0906769106
58. Liu J, Mercher T, Scholl C, Brumme K, Gilliland DG, Zhu N. A functional role for the histone demethylase UTX in normal and malignant hematopoietic cells. Exp Hematol 2012; 40:487-98, e3; PMID:22306297; http://dx.doi.org/10.1016/j.exphem.2012.01.017
59. Caretti G, Di Padova M, Micales B, Lyons GE, Sartorelli V. The Polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation. Genes Dev 2004; 18:2627-38; PMID:15520282; http://dx.doi.org/10.1101/gad.1241904
60. Hemming S, Cakouros D, Isenmann S, Cooper L, Menicanin D, Zannettino A, Gronthos S. EZH2 and KDM6A act as an epigenetic switch to regulate mesenchymal stem cell lineage specification. Stem Cells 2014; 32:802-15; PMID:24123378; http://dx.doi.org/10.1002/stem.1573
61. Shaw T, Martin P. Epigenetic reprogramming during wound healing: loss of polycomb-mediated silencing may enable upregulation of repair genes. EMBO Rep 2009; 10:881-6; PMID:19575012; http://dx.doi.org/10.1038/embor.2009.102
62. Maures TJ, Greer EL, Hauswirth AG, Brunet A. The H3K27 demethylase UTX-1 regulates C. elegans lifespan in a germline-independent, insulin-dependent manner. Aging Cell 2011; 10:980-90; PMID:21834846; http://dx.doi.org/10.1111/j.1474-9726.2011.00738.x
63. Jin C, Li J, Green CD, Yu X, Tang X, Han D, Xian B, Wang D, Huang X, Cao X, et al. Histone demethylase UTX-1 regulates C. elegans life span by targeting the insulin/IGF-1 signaling pathway. Cell Metab 2011; 14:161-72; PMID:21803287; http://dx.doi.org/10.1016/j.cmet.2011.07.001
64. Niikawa N, Matsuura N, Fukushima Y, Ohsawa T, Kajii T. Kabuki make-up syndrome: a syndrome of mental retardation, unusual facies, large and protruding ears, and postnatal growth deficiency. J Pediatr 1981; 99:565-9; PMID:7277096; http://dx.doi.org/10.1016/S0022-3476(81)80255-7
65. Niikawa N, Kuroki Y, Kajii T, Matsuura N, Ishikiriyama S, Tonoki H, Ishikawa N, Yamada Y, Fujita M, Umemoto H, et al. Kabuki make-up (Niikawa-Kuroki) syndrome: a study of 62 patients. Am J Med Genet 1988; 31:565-89; PMID:3067577; http://dx.doi.org/10.1002/ajmg.1320310312
66. Miyake N, Koshimizu E, Okamoto N, Mizuno S, Ogata T, Nagai T, Kosho T, Ohashi H, Kato M, Sasaki G, et al. MLL2 and KDM6A mutations in patients with Kabuki syndrome. Am J Med Genet A 2013; 161:2234-43; PMID:23913813; http://dx.doi.org/10.1002/ajmg.a.36072
67. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, Beck AE, Tabor HK, Cooper GM, Mefford HC, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet 2010; 42:790-3; PMID:20711175; http://dx.doi.org/10.1038/ng.646
68. Hannibal MC, Buckingham KJ, Ng SB, Ming JE, Beck AE, McMillin MJ, Gildersleeve HI, Bigham AW, Tabor HK, Mefford HC, et al. Spectrum of MLL2 (ALR) mutations in 110 cases of Kabuki syndrome. Am J Med Genet A 2011; 155A:1511-6; PMID:21671394; http://dx.doi.org/10.1002/ajmg.a.34074
69. Lederer D, Grisart B, Digilio MC, Benoit V, Crespin M, Ghariani SC, Maystadt I, Dallapiccola B, Verellen-Dumoulin C. Deletion of KDM6A, a histone demethylase interacting with MLL2, in three patients with Kabuki syndrome. Am J Hum Genet 2012; 90:119-24; PMID:22197486; http://dx.doi.org/10.1016/j.ajhg.2011.11.021
70. Scherer S, Theile U, Beyer V, Ferrari R, Kreck C, Rister M. Patient with Kabuki syndrome and acute leukemia. Am J Med Genet A 2003; 122A:76-9; PMID:12949977; http://dx.doi.org/10.1002/ajmg.a.20261
71. Tumino M, Licciardello M, Sorge G, Cutrupi MC, Di Benedetto F, Amoroso L, Catania R, Pennisi M, D'Amico S, Di Cataldo A. Kabuki syndrome and cancer in two patients. Am J Med Genet A 2010; 152A:1536-9; PMID:20503331
72. Merks JH, Caron HN, Hennekam RC. High incidence of malformation syndromes in a series of 1,073 children with cancer. Am J Med Genet A 2005; 134A:132-43; PMID:15712196; http://dx.doi.org/10.1002/ajmg.a.30603
73. Ijichi O, Kawakami K, Matsuda Y, Ikarimoto N, Miyata K, Takamatsu H, Tokunaga M. A case of Kabuki make-up syndrome with EBV+Burkitt's lymphoma. Acta Paediatr Jpn 1996; 38:66-8; PMID:8992864; http://dx.doi.org/10.1111/j.1442-200X.1996.tb03439.x
74. Shahdadpuri R, O'Meara A, O'Sullivan M, Reardon W. Low-grade fibromyxoid sarcoma: yet another malignancy associated with Kabuki syndrome. Clin Dysmorphol 2008; 17:199-202; PMID:18541969; http://dx.doi.org/10.1097/MCD.0b013e3282f5f4e3
75. De Keersmaecker K, Atak ZK, Li N, Vicente C, Patchett S, Girardi T, Gianfelici V, Geerdens E, Clappier E, Porcu M, et al. Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet 2013; 45:186-90; PMID:23263491; http://dx.doi.org/10.1038/ng.2508
76. Chapman MA, Lawrence MS, Keats JJ, Cibulskis K, Sougnez C, Schinzel AC, Harview CL, Brunet JP, Ahmann GJ, Adli M, et al. Initial genome sequencing and analysis of multiple myeloma. Nature 2011; 471:467-72; PMID:21430775; http://dx.doi.org/10.1038/nature09837
77. Gui Y, Guo G, Huang Y, Hu X, Tang A, Gao S, Wu R, Chen C, Li X, Zhou L, et al. Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. Nat Genet 2011; 43:875-8; PMID:21822268; http://dx.doi.org/10.1038/ng.907
78. Jones DT, Jäger N, Kool M, Zichner T, Hutter B, Sultan M, Cho YJ, Pugh TJ, Hovestadt V, Stütz AM, et al. Dissecting the genomic complexity underlying medulloblastoma. Nature 2012; 488:100-5; PMID:22832583; http://dx.doi.org/10.1038/nature11284
79. Pugh TJ, Weeraratne SD, Archer TC, Pomeranz Krummel DA, Auclair D, Bochicchio J, Carneiro MO, Carter SL, Cibulskis K, Erlich RL, et al. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. Nature 2012; 488:106-10; PMID:22820256; http://dx.doi.org/10.1038/nature11329
80. Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, Phoenix TN, Hedlund E, Wei L, Zhu X, et al. Novel mutations target distinct subgroups of medulloblastoma. Nature 2012; 488:43-8; PMID:22722829; http://dx.doi.org/10.1038/nature11213
81. Dalgliesh GL, Furge K, Greenman C, Chen L, Bignell G, Butler A, Davies H, Edkins S, Hardy C, Latimer C, et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. Nature 2010; 463:360-3; PMID:20054297; http://dx.doi.org/10.1038/nature08672
82. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012; 487:239-43; PMID:22722839; http://dx.doi.org/10.1038/nature11125
83. Lindberg J, Mills IG, Klevebring D, Liu W, Neiman M, Xu J, Wikström P, Wiklund P, Wiklund F, Egevad L, et al. The mitochondrial and autosomal mutation landscapes of prostate cancer. Eur Urol 2013; 63:702-8; PMID:23265383; http://dx.doi.org/10.1016/j.eururo.2012.11.053
84. Ho AS, Kannan K, Roy DM, Morris LG, Ganly I, Katabi N, Ramaswami D, Walsh LA, Eng S, Huse JT, et al. The mutational landscape of adenoid cystic carcinoma. Nat Genet 2013; 45:791-8; PMID:23685749; http://dx.doi.org/10.1038/ng.2643
85. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA, et al. Mutational landscape and significance across 12 major cancer types. Nature 2013; 502:333-9; PMID:24132290; http://dx.doi.org/10.1038/nature12634
86. Jankowska AM, Makishima H, Tiu RV, Szpurka H, Huang Y, Traina F, Visconte V, Sugimoto Y, Prince C, O'Keefe C, et al. Mutational spectrum analysis of chronic myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX, EZH2, and DNMT3A. Blood 2011; 118:3932-41; PMID:21828135; http://dx.doi.org/10.1182/blood-2010-10-311019
87. Fisher CL, Berger J, Randazzo F, Brock HW. A human homolog of Additional sex combs, ADDITIONAL SEX COMBS-LIKE 1, maps to chromosome 20q11. Gene 2003; 306:115-26; PMID:12657473; http://dx.doi.org/10.1016/S0378-1119(03)00430-X
88. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 2010; 466:1129-33; PMID:20639862; http://dx.doi.org/10.1038/nature09303
89. Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol 2012; 123:465-72; PMID:22134537; http://dx.doi.org/10.1007/s00401-011-0922-z
90. Dubuc AM, Remke M, Korshunov A, Northcott PA, Zhan SH, Mendez-Lago M, Kool M, Jones DT, Unterberger A, Morrissy AS, et al. Aberrant patterns of H3K4 and H3K27 histone lysine methylation occur across subgroups in medulloblastoma. Acta Neuropathol 2013; 125:373-84; PMID:23184418; http://dx.doi.org/10.1007/s00401-012-1070-9
91. Mann KM, Ward JM, Yew CC, Kovochich A, Dawson DW, Black MA, Brett BT, Sheetz TE, Dupuy AJ, Chang DK, et al.; Australian Pancreatic Cancer Genome Initiative. Sleeping Beauty mutagenesis reveals cooperating mutations and pathways in pancreatic adenocarcinoma. Proc Natl Acad Sci U S A 2012; 109:5934-41; PMID:22421440; http://dx.doi.org/10.1073/pnas.1202490109
92. Kruidenier L, Chung CW, Cheng Z, Liddle J, Che K, Joberty G, Bantscheff M, Bountra C, Bridges A, Diallo H, et al. A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. Nature 2012; 488:404-8; PMID:22842901; http://dx.doi.org/10.1038/nature11262

93. Kristensen JB, Nielsen AL, Jørgensen L, Kristensen LH, Helgstrand C, Juknaite L, Kristensen JL, Kastrup JS, Clausen RP, Olsen L, et al. Enzyme kinetic studies of histone demethylases KDM4C and KDM6A: towards understanding selectivity of inhibitors targeting oncogenic histone demethylases. FEBS Lett 2011; 585:1951-6; PMID:21575637; http://dx.doi.org/10.1016/j.febslet.2011.05.023
94. Hamada S, Kim TD, Suzuki T, Itoh Y, Tsumoto H, Nakagawa H, Janknecht R, Miyata N. Synthesis and activity of N-oxalylglycine and its derivatives as Jumonji C-domain-containing histone lysine demethylase inhibitors. Bioorg Med Chem Lett 2009; 19:2852-5; PMID:19359167; http://dx.doi.org/10.1016/j.bmcl.2009.03.098
95. Luo X, Liu Y, Kubicek S, Myllyharju J, Tumber A, Ng S, Che KH, Podoll J, Heightman TD, Oppermann U, et al. A selective inhibitor and probe of the cellular functions of Jumonji C domain-containing histone demethylases. J Am Chem Soc 2011; 133:9451-6; PMID:21585201; http://dx.doi.org/10.1021/ja201597b
96. Wang L, Chang J, Varghese D, Dellinger M, Kumar S, Best AM, Ruiz J, Bruick R, Peña-Llopis S, Xu J, et al. A small molecule modulates Jumonji histone demethylase activity and selectively inhibits cancer growth. Nat Commun 2013; 4:2035; PMID:23792809
97. Nielsen AL, Kristensen LH, Stephansen KB, Kristensen JB, Helgstrand C, Lees M, Cloos P, Helin K, Gajhede M, Olsen L. Identification of catechols as histone-lysine demethylase inhibitors. FEBS Lett 2012; 586:1190-4; PMID:22575654; http://dx.doi.org/10.1016/j.febslet.2012.03.001