

# Epigenetic regulation of cancer growth by histone demethylases

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Cancer is traditionally viewed as a primarily genetic disorder. However, it is now increasingly apparent that epigenetic abnormalities play a fundamental role in cancer development. Aberrant expression of histone-modifying enzymes has been implicated in the course of tumor initiation and progression. The discovery of a large number of histone demethylases suggests an important role for dynamic regulation of histone methylation in biological processes. The observation that overexpression, amplification or mutations of several histone demethylases have been found in many types of tumors, raise the possibility of using these enzymes as diagnostic tools as well as pave a way for the discovery of novel therapeutic targets and treatment modalities. Here, we review the current knowledge of the potential role of H3K4, H3K9 and H3K27 histone demethylases in tumorigenesis.

Recently, it has become apparent that not only genetic mutations but also epigenetic alterations lead to the activation of oncogenes and the loss of function of tumor-suppressor genes. Since epigenetic abnormalities in DNA methylation patterns as well as histone modifications are potentially reversible in contrast to gene mutations, much effort has been directed toward understanding the mechanism of epigenetic aberration to develop epigenetic therapies. Indeed, inhibitors of histone deacetylases (HDACs) and DNA methyltransferases (DNMTs) have been approved for treatment of certain types of cancers. Recently, overexpression, amplification or mutations of several histone demethylases have been linked to many types of tumor, raising the possibility of using these enzymes as diagnostic tools and therapeutic targets. Here, we focus mainly on the potential role of some selected histone lysine demethylases in tumorigenesis.

The N-terminal tails of histones are subjected to several types of post-translational modifications, including acetylation, methylation, phosphorylation and ubiquitination. The combination of these modifications determines chromatin structure and transcriptional activation or repression of genes. In contrast to other histone modifications, the importance of histone methylations is highlighted because of their enormously specific dynamics with respect to gene regulation. Histone lysine residues on histone H3 and H4 (H3K4, H3K9,

H3K27, H3K36, H3K79 and H4K20) can become mono-, di- or trimethylated. These modifications are regulated by two classes of enzymes with opposing activities: histone methyltransferases and histone lysine demethylases. LSD1, also known as AOF2 and KDM1, is the first discovered histone lysine demethylase that belongs to the flavin adenine dinucleotide-dependent enzyme family.<sup>1</sup> Subsequently, another family of histone lysine demethylases structurally different from LSD1 was described, all of which sharing the conserved jumonji C (JmjC) domain (Table 1).

## H3K4 Demethylases in Cancer

### LSD1/KDM1A

Tri- and dimethylated H3K4 (H3K4me3/2) are often found at actively transcribed genes. Although H3K4me3 is highly enriched around transcriptional start sites, H3K4me2 seems to be present throughout the coding region of transcribed genes (Table 2). By removing active histone marks, H3K4 histone demethylases function primarily as transcriptional repressors. There are two families of H3K4 histone demethylases. LSD1 is highly specific for mono- and dimethylated H3K4, whereas members of the JARID1 subfamily are able to demethylase tri- and dimethylated H3K4. Apparently, the demethylation activity for the JARID1 family overlaps with the specificity observed for LSD1 suggesting possible redundant or cooperative functions of LSD1 and the JARID1 proteins.

Aberrant overexpression of LSD1 has been observed in many types of cancers. High expression of LSD1 in prostate cancer was a predictive marker for aggressive tumor biology and tumor recurrence during therapy.<sup>6,7</sup> Recently, our group could also show that LSD1 expression was highly upregulated in poorly differentiated neuroblastoma and estrogen receptor (ER)-negative breast cancer, correlating LSD1 with adverse clinical outcome.<sup>8,9</sup> In addition, pharmacological inhibition of LSD1 reduced the growth of neuroblastoma xenograft in nude mice, indicating that LSD1 may provide not only a

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**Table 1.** Histone lysine demethylases: specificity, transcriptional role and association to cancer

	Names	Enzyme activity	Transcriptional role	Interacting partner	Link to cancer
KDM1	LSD1; AOF2	H3K4me2/1	Repression		Overexpression in prostate cancer, breast cancer, neuroblastoma and bladder cancer
		H3K9me2/1 in the presence of AR and ER	Activation	AR, ER	
	LSD2; AOF1	H3K4me2/1	Repression		N.R.
KDM2	JHDM1A; FBXL11	H3K36me2/1	Repression		N.R.
	JHDM1B; FBXL10	H3K36me2/1	Repression		Downregulation; mutation in hematopoietic cancer
		H3K4me3	Repression		
KDM3	JMJD1A; JHDM2A; TSGA	H3K9me2/1	Activation	AR	N.R.
	JMJD1B; JHDM2B; 5qNCA	H3K9me2/1	Activation	AR	5q31 (JMJD1B) frequent deletion in cancer
	JMJD1C; JHDM2C; TRIP8	N.R.	N.R.	AR	Expression in diffuse-type gastric cancer
	s-JMJD1C	N.R.	Activation	AR	Downregulation in breast cancer
KDM4	JMJD2A; JHDM3A	H3K9me3/2	Activation	AR	Overexpression in prostate cancer
		H3K36me3/2	Repression		
	JMJD2B; JHDM3B	H3K9me3/2	Activation	N.R.	Overexpression in prostate cancer
		H3K36me3/2	Repression		
JMJD2C; JHDM3C; GASC1	H3K9me3/2	Activation	AR	Overexpression/amplification in prostate cancer, esophageal squamous cell carcinoma, desmoplastic medulloblastoma, metastatic lung sarcomatoid carcinoma and breast cancer	
		H3K36me3/2	Repression		
KDM5	JARID1A; RBP2	H3K4me3/2	Repression		Translocation: NUP98-JARID1A fusion protein
		Not active in the presence of pRB and c-Myc	Activation	pRB, c-Myc	
	JARID1B; PLU-1	H3K4me3/2	Repression		Overexpression in breast, testis, esophageal and prostate cancer
		Not active in the presence of c-Myc	Activation	c-Myc	
	JARID1C; SMCX	H3K4me3/2	Repression		N.R.
	JARID1D; SMCY	H3K4me3/2	Repression		N.R.
KDM6	UTX	H3K27me3/2	Activation		Downregulation; inactivating somatic mutations in cancer
	JMJD3	H3K27me3/2	Activation		Downregulation; 17p13.1 (JMJD3) frequent deletion in cancer

Because of the size limitation, the primary references were omitted, but information about most of the histone demethylase has been reviewed.<sup>2,3</sup> N.R., not reported.

predictive marker for aggressive biology but also a novel therapeutic target for the treatment of aggressive cancer. At the cellular level, RNA interference inhibition of LSD1 markedly inhibited proliferation of cancer cells and led to a broad change in proliferation-related gene expression profile.<sup>6,9</sup>

LSD1 was originally identified as a component of transcriptional repressor complexes comprising transcriptional corepressor protein (CoREST) and HDAC1/2. The LSD1-CoREST-

HDAC core is functionally conserved and associated with various tissue-specific factors, involving LSD1 in diverse cellular processes including cellular growth, proliferation, apoptosis and fate specification. LSD1 has also been implicated as an essential player in nuclear receptor signaling. Interestingly, the interaction of LSD1 with nuclear receptors appears to change its substrate specificity from H3K4me2/1 to H3K9me2/1 and LSD1 functions as a transcriptional coactivator of nuclear

**Table 2.** Genome wide distribution of histone modifications from a transcriptional perspective<sup>4,5</sup>

Lysine	Methylation	Genomic region	Gene localization	Functions in transcription
H3K4	me3	Euchromatin	Promoters, enhancers	Activation
	me2	Euchromatin	ORFs, promoters, enhancers	Activation
H3K9	me3/2	Heterochromatin	Promoters	Long-term silencing
		Euchromatin	Promoters	Repression
		Euchromatin	ORFs	Activation
H3K27	me3/2	Heterochromatin	Promoters	Long-term silencing
		Euchromatin	Promoters, ORFs	Repression

**Table 3.** The potential role of the histone lysine demethylases in cancer

	Demethylase activity	Possible mechanisms	Role in cancer
LSD1	Euchromatic H3K4me2/1	Silencing tumor suppressor genes through collaboration with CoREST/HDACs or DNMTs ( <i>e.g.</i> , <i>INK4B</i> (p15), <i>SFRP</i> , <i>GATA</i> )	Putative oncogene
	Euchromatic H3K9me2/1	Transcriptional activation of AR- or ER target genes	
	DNMT	Increasing the protein stability	
	p53 (K370me2/1)	Antagonizing p53 signaling	
PLU-1	Euchromatic H3K4me3/2	Silencing tumor suppressor genes ( <i>e.g.</i> , <i>BRCA1</i> )	Putative oncogene
	Not active	Acting as a coactivator of c-Myc	
GASC1	Heterochromatic H3K9me3/2	Increasing genomic instability	
		Antagonizing the formation of SAHFs transactivating growth-promoting genes	Putative oncogene
	Euchromatic H3K9me3/2	Transcriptional activation of oncogenes ( <i>e.g.</i> , <i>NOTCH1</i> , <i>MDM2</i> )	
UTX	Euchromatic H3K27me3/2	Transcriptional activation of AR target genes	
		Transcriptional activation of pRB binding proteins contributing to the pRB-mediated cell cycle arrest	Candidate tumor suppressor
JMJD3	Euchromatic H3K27me3/2	Transcriptional activation of <i>INK4A</i> /ARF region inducing senescence	Candidate tumor suppressor

receptors (the androgen receptor (AR) and ER).<sup>6,10</sup> Recently, Metzger *et al.* showed that phosphorylation of histone H3 at threonine 6 (H3T6) is the key event that prevents LSD1 from demethylating H3K4 during AR-dependent gene activation.<sup>11</sup> In response to AR signaling, protein kinase C beta 1 (PKC $\beta$ <sub>1</sub>) is recruited to AR target gene promoters and leads to H3T6 phosphorylation, which blocks the LSD1-mediated H3K4 demethylation. Several demethylases were shown to collaborate with LSD1 for transcriptional activation of AR target genes; the H3K9me3/2 specific demethylase GASC1/JMJD2C and H3K9me2/1 specific demethylases JMJD1A and a short variant JMJD1C (s-JMJD1C).<sup>6,12–15</sup> It is noteworthy that LSD1, GASC1/JMJD2C and PKC $\beta$ <sub>1</sub> are aberrantly upregulated in prostate cancer, suggesting that these enzymes might function together in pathological settings.<sup>7,9,14,16</sup>

The underlying mechanism by which overexpression of LSD1 leads or contributes to tumor formation is not fully elucidated, possibly because of its widespread role in biological processes. However, it is possible that specific tumorigenic effects of LSD1 are linked to its capacity to silence tumor suppressor genes as a transcriptional corepressor (Table 3). The frequent physical association of LSD1 with HDACs implies that LSD1 and HDACs collaborate to repress the transcription of common sets of genes. Indeed, the ZNF217 oncoprotein interacts with a CoREST/HDACs/LSD1 core and the ZNF217 complex acts as a negative regulator of the tumor suppressor gene p15<sup>INK4B</sup>.<sup>17</sup> Likewise, a transcriptional repressor Snail1, which is a known interacting partner of HDACs, was shown to recruit LSD1 to its target gene

promoter and lead to the suppression of E-cadherin expression increasing cell migration.<sup>18</sup>

Moreover, LSD1 is functionally linked to DNA methylation and subsequent gene silencing. DNMT3L, a noncatalytic paralog of *de novo* DNMT3A and -3B, specifically interacts with the histone H3 tail, only when H3K4 is unmethylated.<sup>19</sup> Given that LSD1 demethylates methylated H3K4 to the unmethylated state, LSD1 seems to play an important role in *de novo* DNA methylation by generating H3K4me0 and its binding to the DNMT3L-DNMT3A protein complex. An alternative mechanism could be that LSD1 can directly demethylate the lysine residues of a maintenance DNMT1 and stabilize it from protein degrading.<sup>20</sup> Interestingly, DNMT1 is frequently upregulated in many types of cancer. Whether LSD1, which is often upregulated in cancer as well, might be partly responsible for overexpression of DNMT1 in cancer remains to be determined. Further evidences on collaborative actions of LSD1 and DNMTs in epigenetic silencing of tumor suppressor genes were provided by Huang *et al.* They showed that a combination of LSD1 inhibitors and a DNMT inhibitor has proven to be highly efficient in reactivating aberrantly silenced genes in cancer and led to profound inhibition of the growth of human colon cancer xenografts in nude mice.<sup>21</sup>

The oncogenic potential of LSD1 could also be the result of the p53 antagonizing action of LSD1. LSD1 can control the tumor suppressor activity of p53 by directly demethylating a specific p53 lysine (Lys370me2/1), which is required for efficient binding to the transcriptional coactivator p53-binding protein-1. Through demethylation of Lys370 in p53, LSD1 blocks p53 proapoptotic activity while knockdown of LSD1 increases p21<sup>CIP1</sup> and MDM2, well known p53 target genes.<sup>22</sup>

However, the potential roles of LSD1 are not always oncogenic. Wang *et al.* showed that the LSD1 and Mi-2/nucleosome remodeling and deacetylase (NuRD) complexes are involved in repression of TGF $\beta$ -1 signaling pathway that is critically involved in epithelial mesenchymal transition and tumor invasion. In this way, LSD1 could suppress breast cancer metastatic potential.<sup>23</sup> The tumor suppressive versus tumor promoting effect of LSD1 appears to depend on many factors and cellular context. Therefore, a careful systemic analysis on the different biological effects of LSD1 inhibition is needed.

#### **RBP2/JARID1A/KDM5A**

The JARID1 subfamily, specific for the demethylation of H3K4me3/2, encompasses four members: RBP2/JARID1A, PLU-1/JARID1B, SMCX/JARID1C and SMCY/JARID1D. Despite the fact that other members of JARID1 family catalyze the demethylation of the same histone mark, they appear to have unique functional properties probably because of their divergent expression profiles and presence in distinct protein complexes. RBP2 is broadly expressed in diverse tissues and involved in differentiation and hematopoiesis. The role of

RBP2 in cancer is conflicting. RBP2 has been shown to be a key effector of retinoblastoma protein (pRB) mediated cell cycle withdrawal and differentiation by interacting with the tumor suppressor pRB.<sup>24</sup> Recently, RBP2 protein was shown to be an integral part of the core Notch-RBP-J repressor complex, contributing to switch off the Notch signaling.<sup>25</sup> In contrast to its tumor suppressor function, Sharma *et al.* have shown the link between RBP2 and drug resistance.<sup>26</sup> RBP2 was elevated in drug-tolerant subpopulation of cancer cells, which were treated with a receptor tyrosine kinase inhibitor and RBP2 knockdown significantly reduced the number of drug tolerant population. This indicates that acquired drug resistances are accompanied by epigenetic changes and combining a chromatin-modifying agent with a single rationally targeted agent would prevent the development of drug resistance.

#### **PLU-1/JARID1B/KDM5B**

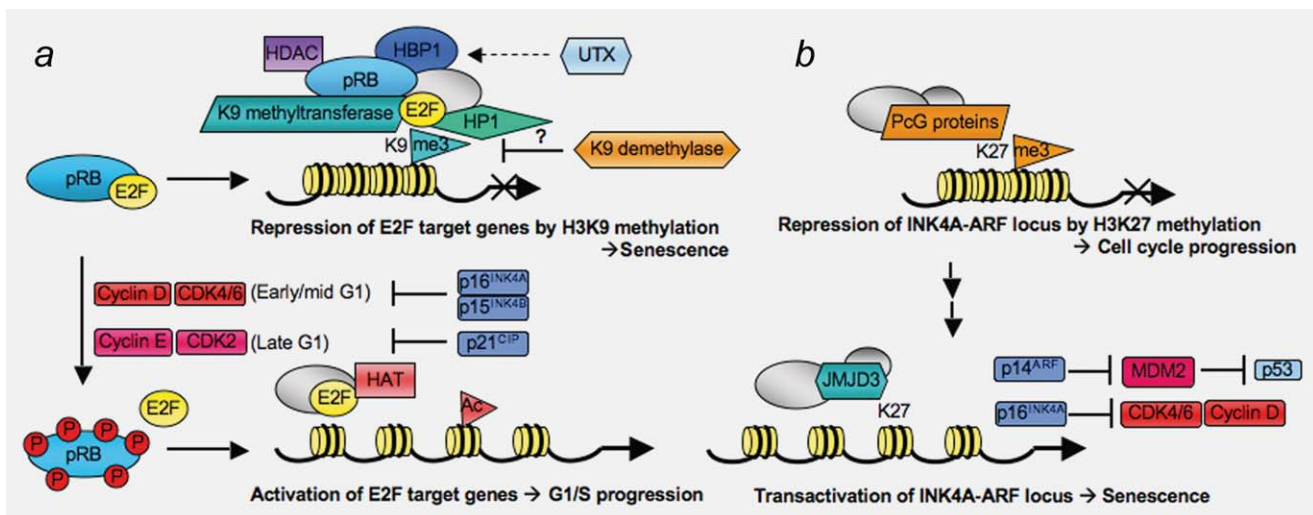
Among all the JARID1 family members, the implication of PLU-1 in cancer has already been reported. Although significant expression of PLU-1 is restricted to testis and only low levels of PLU-1 are seen in other normal adult tissues, its expression increases markedly in pathological conditions such as malignant breast cancer, metastatic prostate cancer and testis cancer (Table 1).<sup>27,28</sup> Based on its overexpression in cancer, PLU-1 was suggested as a testis cancer antigen. Yamane *et al.* have shown the oncogenic potential of PLU-1 at the cellular level. PLU-1 knockdown slowed down the proliferation of breast cancer cells and tumor growth of mammary carcinoma cells in a syngeneic mouse mammary tumor model.<sup>29</sup> Like LSD1, PLU-1 was shown to be involved in silencing of a series of tumor suppressor genes previously linked to breast cancer including *SFN* (14-3-3- $\sigma$ ), *BRCA1*, *CAVI* and *HOXA5*.

PLU-1 can also act as a transcriptional coactivator by recruiting distinct proteins such as transcription factor c-Myc. Secombe *et al.* have shown that the *Drosophila* homologue of JARID1 protein, little imaginal disc (Lid) binds to *Drosophila* Myc and behaves as a dMyc coactivator, contributing to dMyc-induced cell growth.<sup>30</sup> The interaction of dMyc abrogates Lid's enzymatic activity resulting in the maintenance of H3K4 trimethylation, which is important for the effective binding of dMyc to the promoters. The Lid-dMyc interaction was shown to be conserved, as endogenous c-Myc and JARID1 complexes were also detected in mammalian system. Although the significance of the interaction between c-Myc and PLU-1 for tumorigenesis remains to be revealed, PLU-1 could be a promising alternative drug target for c-Myc-induced tumors.

#### **H3K9 Demethylases in Cancer**

##### **JMJ1/KDM3**

In general, H3K9 methylation marks have been associated with transcriptional repression and formation of heterochromatin (Table 2). Global loss of H3K9 methylation was



**Figure 1.** H3K9 and H3K27 demethylases in cellular senescence. (a) RB family proteins are corepressors of the E2F transcription factors. As cells approach S phase, cyclin D and E-dependent kinases phosphorylate pRB and free E2F, allowing it to act with histone acetyltransferases (HATs) to open chromatin structure and transactivate E2F-responsive genes important for G1 to S phase transition. During senescence, RB family members are thought to contribute to the senescence associated heterochromatin foci (SAHF) formation and long-term silencing of E2F target genes by recruiting HDACs and certain K9 methyltransferases to E2F-dependant promoters. Growth-promoting S-phase genes are stably repressed by H3K9 methylation and heterochromatin formation. K9 demethylases may possibly dissolve senescence associated heterochromatin foci and prevent senescence induction. UTX positively regulates transcription of many pRB binding proteins including HBP1, contributing to the RB-mediated cell cycle arrest. (b) PcG proteins mediate the silencing of tumor suppressive INK4A-ARF locus through methylation of H3K27, while JMJD3 is recruited to the locus during induction of stress-induced senescence and demethylates H3K27me3 leading to the transcriptional activation of p16<sup>INK4A</sup> and p14<sup>ARF</sup>, which are key regulators of cellular senescence. p16<sup>INK4A</sup> causes cell arrest by working as a CDK4/CDK6 inhibitor acting upstream of the pRB-E2F pathway, whereas p14<sup>ARF</sup> controls the level of p53 by inhibiting the p53-specific ubiquitin ligase MDM2.

observed in several cancers.<sup>31</sup> Moreover, the loss of H3K9 methyltransferase activity is likely associated with many types of tumors. Two families of histone demethylases are involved in reversing H3K9 methylation. Although JMJD1 family proteins are specific for H3K9 mono- and dimethylation,<sup>15</sup> the members of JMJD2 family demethylate tri- and dimethylated H3K9 and H3K36 (Table 1).<sup>2,14,32,33</sup> Nevertheless, little is known about the implication of JMJD1 in tumorigenesis. Frequent deletion of 5q31 locating *JMJD1B* gene or reduced expression of JMJD1C in various malignancies suggests possible roles of JMJD1 proteins in tumor suppression.<sup>13,34</sup> Recent studies have shown that JMJD1A is the target of hypoxia-inducible transcription factors and under hypoxia JMJD1A increases a subset of hypoxia-inducible genes enhancing tumor growth.<sup>35</sup>

#### GASC1/JMJD2C/KDM4C

Although the potential role of JMJD1 in cancer remains controversial, the JMJD2 family members are mainly regarded as oncogenes. The oncogenic potential of the JMJD2 family could result from demethylating heterochromatic H3K9me3/2. This mark is important for formation and maintenance of heterochromatin by recruiting heterochromatin protein 1 (HP1), which specifically binds to H3K9me3. Since disruption of H3K9 methyltransferase Suv39h1/h2 leads to loss of

H3K9 trimethylation at pericentric chromatin and impairment of heterochromatin structures,<sup>36</sup> one would expect that an antagonistic role of JMJD2 to heterochromatin might be linked to the genomic instability often observed in cancer cells.

Moreover, recent studies suggest a key role for trimethylation of H3K9 in the establishment of senescence-associated heterochromatic foci (SAHF) and induction of the senescent state (Fig. 1). Cellular senescence is a specific form for irreversible growth arrest, which is an important process in suppression of tumorigenesis. Rb family members are thought to contribute to the SAHF formation and long-term silencing of E2F target genes by recruiting HDACs and certain K9 methyltransferases to E2F-dependant promoters.<sup>37</sup> Demethylases removing the repressive H3K9me3 marks may possibly dissolve SAHFs and prevent senescence induction. Indeed, ectopic expression of JMJD2A, -B and -C significantly decreased H3K9me3/2 levels, and delocalized HP1.<sup>14,38</sup> Thus, cancer cells with high expression of JMJD2 might evade this important tumor suppressor mechanism.<sup>14</sup>

In agreement with the oncogenic potential of JMJD2 to tumor development, overexpression of JMJD2 proteins was observed in prostate cancer.<sup>14</sup> Among them, JMJD2C, also known as GASC1 and KDM4C, has already been reported as a gene amplified in various malignancies (Table 1).<sup>16</sup>

Depletion of GASC1 from cancer cells, expressing high levels of GASC1, resulted in inhibition of cell growth,<sup>14</sup> whereas overexpression of GASC1 in human nontransformed mammary epithelial cells resulted in phenotypic alterations that are hallmarks of neoplastic transformation, including growth factor-independent proliferation and anchorage-independent growth in soft agar. Introduction of GASC1 gene in normal breast MCF10A cells could increase higher capacity to generate mammospheres, a phenotype of cancer stem cells, suggesting that GASC1 acts as a transforming gene.<sup>16</sup> GASC1 can function as a transcriptional activator by removing the euchromatic H3K9me3, which is generally associated with transcriptional repression. Analysis of genes altered by overexpression of GASC1 in MCF10A-GASC1 cells revealed *NOTCH1* as a target gene of GASC1.<sup>16</sup> Induction of NOTCH signaling promotes self-renewal of normal human mammary stem cells. This may support the finding that the phenotype of cancer stem cell was increased in MCF10A-GASC1 cells.

The involvement of GASC1 in tumorigenesis has been supported further by a recent report demonstrating the functional interaction between GASC1 and AR in prostate carcinoma.<sup>12</sup> It was known that LSD1 and JMJD1A are also required for transcriptional activation of AR responsive genes, and for proliferation of prostate cancer cells. Although LSD1 and JMJD1A only demethylate mono- and dimethylated H3K9, GASC1 is especially capable of efficiently demethylating trimethylated H3K9, inducing the robust cooperative stimulation of AR transcriptional activity. Thus, specific modulation of GASC1 activity alone, or in combination with LSD1, may be a promising therapeutic strategy to control AR activity in tissues where AR has a pivotal physiological role.

### H3K27 Demethylases in Cancer

#### JMJD3/KDM6B

Other important repressive histone marks are tri- and dimethylation of H3K27, which are catalyzed by the polycomb group (PcG) proteins (Table 2). The PcG genes have been characterized as oncogenes and are frequently overexpressed or amplified in cancer. Their oncogenic potential is mainly mediated through PcG-mediated H3K27 methylation and epigenetic inactivation of the INK4A-ARF locus.<sup>39,40</sup> The INK4A-ARF locus encodes tumor suppressor genes p16<sup>INK4A</sup> and p14<sup>ARF</sup>, which are key regulators of cellular senescence.

H3K27 demethylases were suggested as tumor suppressor genes by functioning antagonistically to the oncogenic PcG proteins. There are two known histone demethylases specific for H3K27me3/2, UTX (KDM6A) and JMJD3 (KDM6B) (Table 1). Although JMJD3 and UTX have the same catalytic activity, they most likely have, as the JARID1 proteins, different biological functions. It was shown that JMJD3, but not UTX, contributes to the activation of INK4A-ARF by removing H3K27me3 during induction of oncogene- or stress-induced senescence.<sup>41,42</sup>

In this context, it is noteworthy that H3K27 demethylases are significantly decreased in various malignancies as ana-

lyzed in the Oncomine database.<sup>42</sup> Moreover, it appears that a large part of the genetic lesions leading to p53 loss likewise causes the deletion of JMJD3. The JMJD3 gene is located on chromosome 17 in close proximity to the p53 tumor suppressor gene. The allelic loss at 17p13.1 including both p53 and JMJD3 was also significantly correlated with more aggressive tumor behavior.<sup>43</sup> Taken together, genetic lesions and/or decreased expression of JMJD3 might contribute to the development of some human cancers, most likely by epigenetic silencing of the INK4A-ARF tumor suppressor locus.

#### UTX/KDM6A

Biallelic somatic mutations of *UTX* was described in multiple tumor types and low *UTX* activity correlated with poor patient prognosis, suggesting *UTX* as a tumor suppressor gene.<sup>44</sup> Recently, Wang *et al.* found using a genome-wide chromatin occupancy analysis that many pRB-binding proteins including HMG-box protein 1 (HBP1) are *UTX* target genes (Fig. 1).<sup>45</sup> *UTX* prevents H3K27 methylation and silencing HBP1, thereby enforcing cell cycle arrest by pRB. Consistently, *UTX* depletion results in elevated levels of proliferation. In addition to the *UTX*-pRB pathway connections, *UTX* was also shown to be a Notch antagonist for cell cycle control in *Drosophila*.<sup>46</sup>

### Concluding Remarks

Dysregulation of demethylases has been associated with cancer aggressiveness proposing them as molecular tumor markers. In addition, targeting specific demethylases can be an alternative option for cancer treatment. However, one of the biggest concerns with current epigenetic regulators is their nonspecific effects, since some epigenetic enzymes are involved in various biological processes such as hematopoiesis or immune responses.<sup>47,48</sup> It is also possible that interfering with H3K4 demethylases activities, for example, has the potential to activate other oncogenes as well as induce additional genomic instability. To minimize these side effects and maximize the therapeutic effects, it might prove effective to combine epigenetic modifiers with other conventional therapies or other epi-drugs. Combination of epi-therapy with chemotherapy may erase the histone marks associated with the chemotherapy-resistant phenotype sensitizing tumor cells to chemotherapy. Importantly, several histone demethylases involved in tumorigenesis have been shown to be essential players in AR- or ER-mediated transcription and proliferation hinting that they can be attractive drug targets in cancers where hormonal signaling play an important role. Combination of the H3K4 demethylase inhibitor with DNMT/HDAC inhibitors would be also promising to synergistically reactivate aberrantly silenced tumor suppressor genes.

One area of significant interest is the role of histone demethylases in cancer stem cells. Interestingly, epigenetic modifiers that maintain the pluripotent state in stem- or progenitor cells, such as PLU-1, GASC1 or PcG proteins, are tightly linked to tumorigenesis. It is tempting to speculate

that dysregulation of epigenetic modifiers is implicated in disruption of the normal stem- or progenitor-cell program, leading to the neoplastic transformation of these cells. It will be of interest to assess the contribution of these epigenetic modifiers to transformation of stem- or progenitor cells. Further studies are needed to fully understand the molecular mechanism by which these specific demethylases contribute to the course of tumor initiation and progression. Additional animal and human genetic studies are necessary to further clarify the role of specific demethylases in tumorigenesis *in*

*vivo*. The relevance of other lysine demethylases to tumor formation and/or metastasis awaits further studies. Readers are referred to references<sup>3,49</sup> for details of the enzymology and molecular mechanisms of histone lysine demethylases.

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