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# JMJD3 as an epigenetic regulator in development and disease☆

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## Abstract

Gene expression is epigenetically regulated through DNA methylation and covalent chromatin modifications, such as acetylation, phosphorylation, ubiquitination, sumoylation, and methylation of histones. Histone methylation state is dynamically regulated by different groups of histone methyltransferases and demethylases. The trimethylation of histone 3 (H3K4) at lysine 4 is usually associated with the activation of gene expression, whereas trimethylation of histone 3 at lysine 27 (H3K27) is associated with the repression of gene expression. The polycomb repressive complex contains the H3K27 methyltransferase Ezh2 and controls dimethylation and trimethylation of H3K27 (H3K27me2/3). The Jumonji domain containing-3 (Jmjd3, KDM6B) and ubiquitously transcribed X-chromosome tetratricopeptide repeat protein (UTX, KDM6A) have been identified as H3K27 demethylases that catalyze the demethylation of H3K27me2/3. The role and mechanisms of both JMJD3 and UTX have been extensively studied for their involvement in development, cell plasticity, immune system, neurodegenerative disease, and cancer. In this review, we will focus on recent progresses made on understanding JMJD3 in the regulation of gene expression in development and diseases. This article is part of a Directed Issue entitled: Epigenetics dynamics in development and disease.

### Keywords

Jumonji; Jmjd3; Utx; Histone demethylation; H3K27

## 1. Introduction

The most common modifications on histones are acetylation and methylation (Cloos et al., 2008), which cause steric effects on other DNA or histone modifiers, leading to either gene expression or repression. Methylation of histones occurs on arginine or lysine residues, which can be mono-, di-, or tri-methylated, differentially affecting other histone modifications, and hence, chromatin structure and gene expression. Histones are methylated by the enzymes, histone methyltransferases, and histone methylation can be antagonized

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either indirectly or directly. Peptidylarginine deiminase 4 (PADI4) is the first class of such enzymes to be identified that can indirectly reverse arginine methylation (Cuthbert et al., 2004; Wang et al., 2004). The second class identified, Lysine specific demethylase 1 (LSD1), directly reverses the methylation of the mono- and di-methyl groups on lysines 4 and 9 on histone 3 (H3K4 and H3K9) (Metzger et al., 2005; Shi et al., 2004). Conversely, enzymes of the third and largest class contain a Jumonji C (JmjC) domain and can remove all three mono-, di-, or tri-methyl groups (Klose et al., 2006; Tsukada et al., 2006). Jumonji domain-containing protein D3 (JMJD3), also called lysine-specific demethylase 6B (KDM6B) is a member of this family of JmjC histone demethylases, and along with ubiquitously transcribed X-chromosome tetratricopeptide repeat protein (UTX), specifically demethylates H3K27 (Swigut and Wysocka, 2007). JMJD3 and UTX specifically decrease H3K27 dimethylation (H3K27me2) and H3K27 trimethylation (H3K27me3) (Hong et al., 2007; Xiang et al., 2007). When H3K27 is trimethylated, it is typically associated with inactive gene promoters; whereas when H3K27 is monomethylated, it is associated with active gene promoters (Barski et al., 2007). Although JMJD3 and UTX play important roles in the epigenetic regulation of gene expression, altering cellular memory and reprogramming the fate of cells, this review will primarily focus on the role of JMJD3. Specifically, JMJD3 is involved in several cellular processes, such as differentiation, proliferation, senescence, and apoptosis. The regulation of JMJD3 is highly gene- and context-specific and is involved in several tissue responses, such as vertebrate development, cancer, inflammatory diseases, and neurodegenerative diseases.

### 1.1. JMJD3 and development

Embryonic stem cells (ESCs) repress developmental genes by utilizing H3K27 trimethylation, but ESC deficiency in *JMJD3* does not seem to affect stem cell maintenance and self-renewal capacity (Mansour et al., 2012; Ohtani et al., 2013). During differentiation, H3K27 methylation is removed in a tissue- and cell-specific manner, and the demethylases, JMJD3 and UTX, are directly involved in embryogenesis into the three germ layers, endoderm, mesoderm, and ectoderm, of a developing vertebrate.

**1.1.1. Endoderm—**JMJD3 and UTX drive the formation of the germ layer, endoderm, which gives rise to the gastrointestinal tract, respiratory tract, endocrine glands, and the auditory and urinary systems. Endoderm commitment is controlled by the WNT signaling pathway and the transforming growth factor-beta (TGF-β) superfamily member, NODAL/Activin A, which utilizes the transcription factors, SMAD2/3 (Mfopou et al., 2010; Schier, 2009). Upon treatment with NODAL/Activin A, JMJD3 recruitment to *SMAD2/3* genes is associated with decreased enrichment of H3K27me3 marks at *SMAD2/3* genes (Kim et al., 2011) (Fig. 1A). Knockdown of *JMJD3* and UTX in human ESCs, but not mouse ESCs, inhibits endoderm formation, which can be rescued by continuous activation of WNT signaling (Jiang et al., 2013), suggesting that JMJD3 and UTX are essential, at least for human, endodermal development. JMJD3 also binds to other factors essential for endoderm development. Embryonic T-box transcription factors, *Eomesodermin* (*Eomes*) and Tbx3, are essential for endoderm formation (Fig. 1B). *Eomes* is normally maintained in a transcriptionally poised state in ES cells. During early endoderm differentiation, TBX3 associates with JMJD3 at the enhancer region of *Eomes*, bringing its enhancer in close

proximity to its promoter to drive its own expression. This DNA looping of *Eomes* allows for a self-activating loop, thereby, maintaining endoderm fate (Kartikasari et al., 2013) and preventing abnormal development (Fig. 1B). Endodermal differentiation into lung tissue is also defective in mice with global knockout of *Jmjd3* (Li et al., 2014a). Mice are born smaller with thickened alveolar cell walls and inadequate air space, and expression of markers of lung differentiation is decreased. Perinatal death ensues within 30 min after birth due to respiratory failure (Li et al., 2014a; Satoh et al., 2010). However, time of death and the precise phenotype of *Jmjd3* knockout mice are dependent on the gene dosage and the gene deletion strategy used to generate the mice (Fig. 2). Jmjd3 is important for lung development in a stage-dependent manner (Fig. 3A). Jmjd3 regulates lung development via regulating SP-B expression with transcription factor Nkx2.1 and epigenetic factor Brg1 (Fig. 3B). In *Jmjd3* knockout mice, in which exons 4–5 have been deleted, the mice die before embryonic day 6.5 (E6.5) (Ohtani et al., 2013). However, deletion of exons 14–21, including the *JmjC* domain, lead to a frameshift, and the mice die perinatally (Satoh et al., 2010). Whereas knockout of *Jmjd3* in mice generated by a gene trap strategy, inserting a neo-cassette between exons 1 and 2, show postnatal lethality with normal lung development (Burgold et al., 2012). Although whether JMJD3 is absolutely essential for lung development remains contradictory, it is clear that JMJD3 is a key regulator in early endoderm specification and endoderm differentiation into lung tissue. However, whether JMJD3 mediates late endoderm commitment or differentiation into the gastrointestinal tract, endocrine glands, or auditory or urinary systems has not been investigated.

**1.1.2. Mesoderm—**JMJD3 and UTX are also involved in the formation of the germ layer, mesoderm, but JMJD3 can partially compensate for the loss of UTX during ESC differentiation into mesoderm (Morales Torres et al., 2013). Mesodermal development leads to the formation of muscle tissue, spleen, cartilage, bone, skin, kidneys, gonads, heart, blood vessels, and blood cells. *Jmjd3* deficiency in ESCs significantly increases repressive H3K27me3 marks on the promoter of the mesodermal regulator, *Brachyury* and decreases its expression, leading to impaired recruitment of β-catenin (Ohtani et al., 2013), which is a prerequisite for WNT-induced mesoderm differentiation. During late mesoderm differentiation, JMJD3 is also essential for normal organ development. *Jmjd3* knockout in ESCs reduces endothelial cell differentiation as well as cardiac progenitor cell differentiation (Ohtani et al., 2013). In addition, *Jmjd3* knockout mice at E17.5 demonstrate impaired spleen development, with smaller size and hyperemic areas (Li, 2014a). Furthermore, JMJD3 affects bone formation. Endochondral bone formation and ossification begins with multipotent mesenchymal stem cell (MSC) differentiation into chondrocytes. This cartilage maturation during endochondral bone formation is regulated by JMJD3 and its association with the transcription factor, RUNX2, promoting proliferation and hypertrophy of chondrocytes (Zhang et al., 2015). JMJD3 can also direct MSCs to differentiate preferentially into one lineage over another lineage. Specifically, depletion of *Jmjd3* leads to a decrease in osteogenic differentiation, while increasing adipogenic differentiation (Gomez-Sanchez et al., 2013). Studies implicating the involvement of microRNAs in MSC differentiation (Bengestrate et al., 2011; Inose et al., 2009; Li et al., 2008; Yang et al., 2011) has led to the discovery that the specific microRNA, *MIR146A*, interacts with the 3′ UTR of *JMJD3*, inhibiting its function and decreasing osteogenesis (Huszar and Payne, 2014).

H3K27me3 marks are notably increased in bone marrow MSCs in a mouse model of osteoporosis, suggesting that JMJD3 may contribute to the development of osteoporosis (Burgold et al., 2012). Similarly, MSCs, isolated from dental tissue, have decreased odontogenic differentiation capacity upon *Jmjd3* knockdown (Xu et al., 2013). JMJD3 is recruited to bone morphogenetic protein (BMP) 2 promoters with subsequent removal of gene silencing H3K27me3 marks on odontogenic master transcription genes (Xu et al., 2013). All of these findings provide evidence that JMJD3 is involved in early mesoderm specification and differentiation, and it is essential for cardiovascular development and bone formation.

**1.1.3. Ectoderm—**Formation of the germ layer, ectoderm, is also regulated by JMJD3 and partially by UTX (Morales Torres et al., 2013). The ectoderm can differentiate into the nervous system, including the spine, peripheral nerves, and brain. Key regulators in neurogenesis, *Pax6*, *Sox1*, and *Nestin*, harbor bivalent marks, H3K4me3/H3K27me3, which are dynamically regulated during differentiation, and *Jmjd3* can directly regulate expression of these key regulators in neurogenesis (Burgold et al., 2008). Jmjd3 is also a key regulator in Shh-dependent neural tube development (Shi et al., 2014). In fact, *Jmjd3* is essential for ESC commitment into neural lineages. In the developing spinal cord, trimethylation of H3K27 also regulates BMP activity, which, in turn, leads to JMJD3 interaction with the transcription factors, SMAD1/4, to activate the notochord-derived BMP antagonist, Noggin (Akizu et al., 2010) (Fig. 1C). This negative feedback loop ensures rigorous and anatomically defined spinal cord formation. Aberrant neural development effects of *Jmjd3* inactivation can also be observed after birth. Inactivation of *Jmjd3* in mice generated by a gene trap strategy leads to disruption of the neuronal network of the pacemaker of the respiratory rhythm generator (Burgold et al., 2012). This causes respiratory failure in mice, further emphasizing the importance of *Jmjd3* in normal vertebrate development. In the retina of the eye, H3K27 methylation and demethylation play important roles in retinal neuron proliferation and maturation, where expression of *Bhlhb4* in retinal cells is associated with decreased H3K27me3 marks (Iida et al., 2014a). In a similar study, it was shown that during late retinal development, deficiency in *Jmjd3* leads to decreased expression of *Bhlhb4*, which is associated with lower levels of H3K27me3 marks at the *Bhlhb4* gene (Iida et al., 2014b). These studies suggest that *Jmjd3* expression is required for correct maturation of retinal cells. Ectoderm can also give rise to hair, nails, sweat glands, outer layer of skin, and epithelia of various organs. However, no specific role for JMJD3 in the development of these tissues has been investigated. Overall, the important role of JMJD3 in the formation of all three germ layers suggests that JMJD3 is fundamental in cell fate and plasticity.

### 1.2. JMJD3 and cell plasticity

Not only does JMJD3 play a role in cellular differentiation in the developing embryo, it regulates cellular processes in differentiated tissues. Specifically, *Jmjd3* is intimately involved in tissue repair. Following bone injury, osteoclasts are responsible for bone resorption, and abnormal osteoclast differentiation can lead to osteoporosis. RANKL stimulation of the osteoclast cell surface receptor, RANK, leads to osteoclast differentiation. This is accompanied by JMJD3-mediated demethylation of H3K27me3 at the *Nfatc1* gene, a gene responsible for bone mass (Yasui et al., 2011). These studies suggest that JMJD3

mediates osteoclast differentiation after bone injury and may limit the onset of osteoporosis. JMJD3 has also been implicated in skin repair (Shaw and Martin, 2009). Whereas Polycomb genes, which are involved in histone methylation, are significantly downregulated during murine skin repair, the demethylases, *Jmjd3* and *Utx*, are markedly upregulated, leading to less Polycomb-mediated silencing of the wound repair genes, *Myc* and *Egfr* (Shaw and Martin, 2009). Whether JMJD3 is directly involved in other types of tissue repair is not yet known, but JMJD3 is likely involved in the cellular plasticity involved in tissue repair. In fact, JMJD3 may play a direct role in the reprogramming of adult cells into a pluripotent state. The ability to revert differentiated cells back into a pluripotent, embryonic stem cell-like state has been a breakthrough for patient-specific disease modeling and drug testing. This cellular reprogramming is induced by the exogenous addition of the transcription factors, *Sox2*, *Oct4*, *c-Myc*, and *Klf4*. OCT4 is critical for generating induced pluripotent stem cells (iPSCs) and maintaining pluripotency (Lowry et al., 2008; Pesce and Scholer, 2001). Activation of OCT4 occurs in parallel with the recruitment of JMJD3 to chromatin, suggesting that JMJD3 is involved in cellular reprogramming (Apostolou and Hochedlinger, 2013). Our own research has shown that JMJD3 is a potent negative regulator of cellular reprogramming (Zhao et al., 2013) (Fig. 4). Ablation of *Jmjd3* in mouse embryonic fibroblasts increases iPSC formation, whereas, ectopic *Jmjd3* expression inhibits reprogramming by both histone demethylase-dependent and -independent mechanisms (Zhao et al., 2013). Further understanding of the role of JMJD3 in cellular reprogramming and tissue repair may lead to therapeutic test-beds of iPSCs and enhance wound repair.

JMJD3 also modulates blood cell growth. Deletion of *JMJD3* in stem cells limits endothelial cell differentiation (Ohtani et al., 2013). In endothelial progenitor cells, $CD34^+$ stem cells, and mesioangioblasts, in which histone methylation and acetylation are inhibited, endothelial nitric oxide synthase (eNOS) gene expression is increased concomitantly with reduction of repressive H3K27me3 marks and increased expression of *JMJD3* (Ohtani et al., 2011). In addition, inhibition of JMJD3 can lead to an increase in cellular apoptosis and cellular senescence. Therefore, JMJD3 is involved, not only in cellular plasticity, but also in other cellular processes. JMJD3 may serve to prevent abnormal tissue growth associated with several pathologies by augmenting cell death and by controlling unlimited cell growth.

JMJD3 is also involved in cellular aging. As cells age, they cease to divide, and this aging process can contribute to the loss of the ability of adult stem cells to self-renew (Agger et al., 2009). When MSCs undergo cellular senescence, histone methyltransferases are downregulated and JMJD3 is upregulated, which is balanced by changes in histone acetylation status (Jung et al., 2010). Gene expression of two key regulators of cellular senescence, the tumor suppressor proteins, p16INKA and p14ARF, is silenced by H3K27me3 marks on the *INK4a/ARF*-gene locus in growing cells (Agherbi et al., 2009). Conversely, oncogenic-RAS-induced senescence leads to JMJD3 expression, which contributes to the activation of p16INK4A (Martinelli et al., 2011). Cellular senescence is also induced by the loss of the tumor repressor gene, B cell translocation gene 3 (BTG3) through increased JMJD3 expression and subsequent p16INK4A activation (Lin et al., 2012). In mouse fibroblasts, this senescence can be overcome by inhibition of JMJD3, which leads to their immortalization (Agherbi et al., 2009; Martinelli, 2011). Although it

may seem counterintuitive that JMJD3 regulates the differentiation of some cells, while regulating the senescence of other cells, cell fate decisions are likely dependent a multitude of intracellular and extracellular signals that regulate the function of JMJD3. Cellular senescence can also occur within the nervous system. Schwann cells, which support neurons, are the glia cells of the peripheral nervous system. Tumorigenic stimuli or injury can lead to uncontrolled proliferation of Schwann cells, generating neurofibromas and schwannomas. Activation of JMJD3 leads to the removal of H3K27 marks from the *Ink4a/Arf*-locus and switches Schwann cells from a proliferative state to a senescent state to prevent overproliferation after nerve injury and during regeneration (Gomez-Sanchez et al., 2013). In this setting, JMJD3 plays a beneficial role in limiting tissue transformation and progression a pathological disease state. In primary human fibroblasts, senescence-associated heterochromatin foci (SAHF) are formed to stabilize the senescence state of cells, and the non-histone protein retinoblastoma (RB) protein, which is essential for SAHF, is demethylated at its lysine 810 amino acid residue (Zhao et al., 2015). This demethylation is associated with increased senescence and SAHF formation (Zhao et al., 2015), and this senescence of fibroblasts can promote epithelial cell growth and tumorigenesis (Krtolica et al., 2001). Whether the functional role of JMJD3 in cellular senescence is beneficial or detrimental may depend on the cell and tissue type, in which JMJD3 is expressed and activated.

### 1.3. JMJD3 in neurodegenerative diseases

The causes of peripheral neuropathies are very heterogeneous and can be caused by a genetic predisposition, inflammation-mediated damage, or physical injury. Schwann cells myelinate axons in the peripheral nervous system and play a role in inherited demyelinating diseases. These cells are crucial for repair of the spinal cord and peripheral nerves, and JMJD3 limits Schwann cell overproliferation after nerve injury and during regeneration, thus protecting against neurofibroma (Gomez-Sanchez et al., 2013). Not only can JMJD3 affect the peripheral nervous system, it can also affect the central nervous system. In Parkinson's disease, an association exists in the polarization of microglia cells between a pro-inflammatory microglia M1 phenotype and an anti-inflammatory microglia M2 phenotype, and suppression of JMJD3 augments the M1 pro-inflammatory response by inhibiting M2 microglia polarization and leading to neuronal cell death (Tang et al., 2014). This was associated with increased H3K27 marks in the midbrain of aged mice, suggesting that JMJD3 plays a role in microglia polarization and may alter immune pathogenesis of Parkinson's disease (Tang et al., 2014). Furthermore, in a subtype of neurons that is associated with Parkinson's disease, the mid-brain type dopaminergic (DA) neurons, vitamin C enhances their differ entiation (He et al., 2014). Vitamin C decreases H3K27me3 marks on gene promoters in cells of the DA phenotype (He et al., 2014), suggesting that JMJD3 mediates their differentiation. The involvement of JMJD3 was confirmed using *Jmjd3* knockdown or *Jmjd3* inhibition experiments, which demonstrated that H3K27me3 changes are essential for vitamin C-mediated mid-brain type DA neuron differentiation (He et al., 2014). In addition to Parkinson's disease, JMJD3 has been implicated in Alzheimer's disease. Alzheimer's disease is thought to be associated with the accumulation of amyloid-β in neuronal cells and activation of tumor suppressor genes. A member of the p53 tumor suppressor family, p63, was shown to have dual functions. The full-length form of the tumor

suppressor p63, including its transactivation domain (TAp63γ), antagonizes neuronal apoptosis in response to amyloid-β stimulation (Fonseca et al., 2012b) as well as in neurogenesis (Fonseca et al., 2012a). TAp63γ interacts with JMJD3 to regulate neural-specific gene expression, which alters p63 methylation state, levels, and nuclear accumulation during neurogenesis (Fonseca et al., 2012a), suggesting a protective role for JMJD3 in Alzheimer's disease. Most neurodegenerative diseases are associated with neural cell death, and dead or injured neurons are not easily replaced. Neurogenesis may be key in circumventing neurodegenerative disease progression. Neurogenesis in the adult subventricular zone requires JMJD3, and JMJD3 interacts with the promoters and the enhancers of neuronal genes (Park et al., 2014). It is clear that JMJD3 is involved in differentiation of cells of the nervous system, but whether stimulating or antagonizing JMJD3 will truly prove beneficial is not yet known. Any therapeutic effects on neurodegenerative diseases may be context-dependent.

### 1.4. JMJD3 in cancer

The histone demethylase gene, *UTX*, was the first mutated histone demethylase gene to be associated with cancer (van Haaften et al., 2009), suggesting that cancer cell growth is regulated by H3K27 methylation levels at site-specific promoters or enhancers. In fact, the tumor suppressor protein p53 physically interacts with JMJD3, and these two proteins have overlapping promoter and enhancer binding sites (Williams et al., 2014). JMJD3 is also associated with several types of syndromes that can give rise to cancer. Myelodysplastic syndromes (MDS) are associated with ineffective hematopoiesis and abnormal blood cell differentiation into one or more blood cell lineages and can lead to acute lymphoblastic leukemia. Peripheral blood $CD34^+$ stem cells in patients with lower-risk MDS have increased expression of JMJD3 and an increased ability to form erythroid colonies upon inhibition of JMJD3 (Wei et al., 2013a, 2013b). This suggests that JMJD3 is intimately involved in hematopoietic lineage determination, and its inhibition may become a therapeutic option for anemia, which manifests in patients with MDS. MDS may transform into diseases such as acute lymphoblastic leukemia. Patients with T-cell acute lymphoblastic leukemia (T-ALL), harboring excess T lymphocytes, have poor prognosis with a 25% relapse rate. Whereas UTX functions as a tumor suppressor in T-ALL, JMJD3 demethylates H3K27 on oncogenes, allowing for initiation and maintenance of T-ALL (Ntziachristos et al., 2014). JMJD3 is also involved in other blood cancers. In Hodgkin's Lymphoma, during the differentiation of B cells into centroblasts, JMJD3 expression increases (Anderton et al., 2011). Furthermore, known gene targets of JMJD3 are upregulated in Hodgkin's Lymphoma and ablation of *JMJD3* restores H3K27me3 enrichment at these gene targets (Anderton et al., 2011).

The importance of JMJD3 in cancer is not limited to cancers of the blood, but is evident in solid tumors as well. In breast cancer cells, JMJD3 is recruited to estrogen receptor enhancers, leading to activation of the anti-apoptotic protein, BCL2. This epigenetic state affects ligand dependency during estrogen receptor-mediated gene expression (Svotelis et al., 2011). Moreover, transfection of the lung cancer cell line, A549, with *JMJD3* leads to increased cell proliferation and decreased expression of the cellular senescence marker, p21 (Tian et al., 2012), suggesting that increased endogenous JMJD3 expression may contribute

to lung cancer development. Histone modifications such as acetylation and methylation have been shown to predict the risk of developing prostate cancer (Seligson et al., 2005). In fact, an H3K27 methyltransferase is associated with prostate cancer progression (Varambally et al., 2002), and JMJD3 expression is upregulated in prostate cancer, with a further increase during metastasis (Xiang, 2007). These findings suggest that histone methylation status at H3K27 is highly associated with prostate cancer. JMJD3 is also upregulated in renal cell carcinoma (Shen et al., 2012), and carcinogens have been shown to increase JMJD3 expression and dimethylation of H3K27 in kidney cancer cells (Guo et al., 2014). Conversely, loss of *JMJD3* augments the aggressiveness of pancreatic cancer cells and increases liver metastasis (Yamamoto et al., 2014). Furthermore, *JMJD3* expression is markedly increased in pancreatic precancerous lesions, but its decrease is associated with malignant grade progression. Similarly, JMJD3 expression levels are lower in colorectal carcinoma tissue compared to normal tissue (Tokunaga et al., 2014), which is associated with increased expression of cell cycle related genes and protection against apoptosis. Analysis of glioma patient databases of gene expression revealed high expression levels of JMJD3 in patients with high-grade glioma (Perrigue et al., 2015). Moreover, *JMJD3* expression is induced upon glioblastoma stem cell differentiation through modulation of the *INK4a/ARF*-locus and through chromatin-independent, p53 protein stabilization (Ene et al., 2012).

Epithelial-mesenchymal transition (EMT) plays a crucial role in malignant transformation, tumor progression, and metastasis. Cells lose their polarity and adhesion properties and gain migratory and invasive properties. In mammary epithelial cells, JMJD3 allows for TGF-β-induced EMT through expression of the EMT-specific gene, *SNAI1*, leading to breast cancer invasion (Ramadoss et al., 2012). This suggests that JMJD3 is key regulator in cancer metastasis. Colon cancer cells also have the ability to undergo EMT, and vitamin D leads to EMT gene expression, including *ZEB1*, *ZEB2*, and *SNAI1*, through upregulation of *JMJD3* (Pereira et al., 2012). Regulation of *JMJD3* by vitamin D ensures precise gene activation by removing repressive marks in key genes associated with colon cancer cells (Pereira et al., 2011).

### 1.5. JMJD3 in the immune system

JMJD3 can enhance both pro-inflammatory and anti-inflammatory responses (as reviewed in Salminen et al., 2014) involved in infection and wound healing. In cytokine-stimulated human leukemia monocyte (THP-1) cells, gene expression and proteomic profiling revealed that *JMJD3* knockdown decreases expression of key inflammatory markers, heat shock protein beta-1 (HspB1), and increases tripartite motif protein (TRIM5), glutathione peroxidase (Gpx), glia maturation factor-gamma (GMFG), caspase recruitment domain family member 14 (CARMA2, and dUTP pyrophosphatase) (Das et al., 2013, 2010). Changes in these pro- and anti-inflammatory markers are involved in the NF-κB, chemokine, and CD40 signaling pathways, and knockdown of Jmjd3 inhibits several NF-κB-regulated inflammatory genes (Das et al., 2010). The bacterial toxin, lipopolysaccharide (LPS), which signals through NF-κB, decreases *JMJD3* expression in human gingival tissue-derived keratinocytes (de Camargo Pereira et al., 2013), suggesting that endogenous *JMJD3* may limit further pro-inflammatory cytokine gene expression.

Macrophages can adopt different phenotypes and are classified as M1 phenotype or M2 phenotype. Classically activated M1 macrophages are typically responsible for phagocytosing endogenous apoptotic cells and foreign bodies, such as bacteria, parasites, and viruses, as well as presenting antigens to induce an adaptive immune response. M2 macrophages promote tissue remodeling and wound healing by downregulating the pro-inflammatory response induced by M1 macrophages. Macrophages, both M1 and M2, are an integral part of both the acute and chronic phases of inflammation and have been implicated in atherosclerosis, where they engulf excess cholesterol and become foam cells. Serum amyloid A proteins (SAAs) are also associated with atherosclerosis and are primarily produced by the liver. Upon release into the circulation, SAAs associate with high-density lipoprotein (HDL) particles for cholesterol transport to the liver. SAAs can induce macrophage pro-inflammatory cytokine production and foam cell formation. SAA-stimulation of macrophages leads to increased *Jmjd3* expression, which is associated with reduced H3K27me3 marks, allowing for gene expression (Yan et al., 2014). Furthermore, *Jmjd3* silencing and depletion inhibits macrophage pro-inflammatory cytokine secretion, and *Jmjd3* is essential for SAA-induced foam cell formation (Yan et al., 2014), suggesting a possible therapeutic role in atherosclerosis. In wound healing, specifically in patients with Type 2 Diabetes (T2D), macrophages of the pro-inflammatory M1 phenotype mediate chronic inflammation. Differentiation of bone marrow stem/progenitor cells into macrophages is associated with an enrichment of H3K27me3 at the interleukin (IL)-12 gene promoter, which silences IL-12 gene expression (Gallagher et al., 2014). Under diabetic conditions, JMJD3 augments IL-12 expression, which can be regulated by *Jmjd3* inhibition, suggesting that *Jmjd3* inhibition could become a treatment option for diabetic wounds. Altogether, JMJD3 is intimately involved in modulating sterile inflammation, including atherosclerosis and wound healing, and the clinical benefits of targeting JMJD3 during these processes may depend on the disease state.

JMJD3 also plays a role in response to bacteria, parasites, or viruses. Upon macrophage administration of LPS, JMJD3 is recruited to the transcription start sites at over 70% of LPS-inducible genes (De Santa et al., 2009, 2007). However, deletion of Jmjd3 did affect the expression of most target genes except for a few hundred inflammatory genes, and most target genes had undetectable H3K27me3 marks, suggesting H3K27 demethylation-independent JMJD3-mediated gene activation (De Santa et al., 2009). JMJD3 also plays a role in the resistance of the macrophage cell line, RAW 264.7 cells, to the anthrax lethal toxin, *Bacillus anthracis* (Das et al., 2010), suggesting that the ability of JMJD3 to promote a pro-inflammatory response may prove beneficial in certain settings. During latency, the genome of the herpes simplex virus 1 (HSV-1) is associated with H3K27me3 marks, and inhibitors of histone demethylases, JMJD3 and UTX, limit the ability of HSV-1 to become reactivated in sensory neurons (Messer et al., 2015). JMJD3 is also induced by the EBV oncogene in the Epstein–Barr virus-associated malignancy, Hodgkin’s Lymphoma [63]. JMJD3 may preferentially regulate a particular subset of macrophages depending on the inciting infection. Macrophages have the ability to polarize between a pro-inflammatory M1 phenotype and an anti-inflammatory M2 phenotype and Jmjd3 plays a role in this polarization and activation (as reviewed in (Van den Bossche et al., 2014)). In one study, depletion of *Jmjd3* indicated that *Jmjd3* is not essential for M1 responses, but is imperative

for M2 polarization in response to parasitic helminth infections (Satoh et al., 2010). In another study, M2 macrophages treated with IL-4 had decreased H3K27 methylation at M2 marker gene promoters along with increased *Jmjd3* expression, which is mediated by STAT6 signaling (Ishii et al., 2009). Although compensatory mechanisms may exist for the loss of *Jmjd3*, JMJD3 may actually fine-tune cell-specific pro-inflammatory and anti-inflammatory immune responses, which may also be mediated by H3K27-independent mechanisms.

JMJD3 is also important in autoimmune diseases. Specifically, *Jmjd3*-deficient mice are resistant to experimental autoimmune encephalomyelitis (EAE) (Liu et al., 2015). In systemic lupus erythemastosus, which is characterized by overactive T cells and overstimulated B cells, $CD4^+$ T cells have decreased hematopoietic progenitor kinase 1 (HPK1) expression. The promoter of this negative regulator of T cell-mediated immune responses harbors increased H3K27me3 marks with decreased JMJD3 binding (Zhang et al., 2011). Knockdown of *Jmjd3* decreases this binding and increases H3K27me3 enrichment, suggesting that *Jmjd3* expression in $CD4^+$ T cells contributes to T cell activation and B-cell stimulation (Zhang et al., 2011). In a recent study, JMJD3 has been shown to play a role in systemic sclerosis, whereby JMJD3 overexpression is associated with decreased H3K27me3 marks in $CD4^+$ T cells (Wang et al., 2015). JMJD3 is critically important in $CD4^+$ T cell differentiation. Naïve $CD4^+$ T cell activation leads to an immediate increase in *Jmjd3* expression (Liu et al., 2015), and the absence of *Jmjd3* affects $CD4^+$ T cell differentiation. However, the mechanisms of how *Jmjd3* affects this differentiation are controversial. The T-box transcription factor T-bet, which is required for T helper (Th)1 cell differentiation and interferon (IFN)-gamma production, has the ability to recruit JMJD3 to its target genes (Miller and Weinmann, 2010). Deletion of *Jmjd3*, specifically in T cells, inhibits the differentiation of naïve T cells into Th1 helper T cell or regulatory T cells, but promotes the differentiation into Th2 helper T cells and Th17 cells (Li et al., 2014b; Liu et al., 2015). Furthermore, adoptive transfer of these *Jmjd3*-deficient T cells limits colitis in a mouse model of disease (Li et al., 2014b). Conversely, another study showed deletion of *Jmjd3* in $CD4^{\pm}$ T cells impaired Th17 cell differentiation (Liu et al., 2015). Furthermore, this study showed that ectopic expression of *Jmjd3* induced differentiation of *Jmjd3*-deficient $CD4^{\pm}$ T cells into Th17 cells, which was associated with the demethylation of H3K27 on Th17 cytokine genes, *Il17*, *Il17f*, and *Il22* as well as *Rorc*, which encodes for Th17 transcription factor Ror$\gamma$t (Liu et al., 2015). The reasons for the discrepancies in the two studies are not clear. In one study, in which *Jmjd3* deletion impaired Th17 differentiation, *Jmjd3* was deleted by targeting exons 14–20, and in the other study, in which *Jmjd3* deletion promoted Th17 differentiation, *Jmjd3* was deleted by targeting exons 15–21. These discrepancies are in agreement with the different phenotypes in *Jmjd3* knockout mice that are dependent on gene dosage and the gene deletion strategy (Burgold et al., 2012; Ohtani et al., 2013; Satoh et al., 2010). Furthermore, slight differences in the purity of naïve $CD4^{\pm}$ T cells as well as tissue type derivation of the cells may have resulted in different results. In the study by Liu et al., *Jmjd3* deletion did not change the ability of naïve T cells to differentiate into Th1 cells. However, in the study by Li et al., *Jmjd3* deletion inhibited the differentiation of naïve T cells into Th1 cells. In addition, *Jmjd3* ablation in macrophages markedly affects the expression of two genes, which mediate recruitment of T cells and Th1 cell polarization (De

Santa, 2009). The role of *Jmjd3* may be highly dependent on the specific cell type. Overall, JMJD3 is involved in modulating immune cells in response to several conditions: to sterile inflammatory mediators, to injury, to infectious agents and to self (autoimmunity). Therefore, inhibition of JMJD3 may have different affects, depending on cell type and under different conditions.

### 1.6. JMJD3 and histone demethylase independent function

JMJD3 can regulate gene expression independent of its demethylase activity (Miller et al., 2010; Zhao et al., 2013). In quiescent, differentiated cells, broad changes in gene expression are not as imperative as in cells undergoing differentiation. Demethylase proteins, such as UTX and JMJD3, which antagonize H3K27me3-mediated gene repression, are still expressed in terminally differentiated cells. This is perplexing since incorrect timing of gene activation could be detrimental to a cell. In the transformed murine thymoma cell line, EL4, the epigenetic profile is already established and set, but interaction between JMJD3 and the T-bet, the lineage-defining T-box factor for T helper 1 (Th1) cell development, still exists (Miller et al., 2008). JMJD3 mediates an interaction between a BRG-1-containing SWI/SNF remodeling complex, which is required for promoter remodeling and optimal gene expression (Miller et al., 2010). Therefore, the demethylase-independent activity of JMJD3 is still related to alterations in chromatin structure, although not directly. We also demonstrated that JMJD3 functions in a histone demethylase-independent manner (Zhao et al., 2013). Depletion of *Jmjd3* increases somatic reprogramming into a pluripotent state in a histone demethylase-independent manner. JMJD3 targets PHD finger protein 20 (PHF20) for ubiquitination and subsequent degradation through Trim26, an E3 ligase (Zhao et al., 2013). PHF20 is required for maintenance of pluripotency and cellular reprogramming. Therefore, JMJD3 may limit iPSC formation in a histone demethylase-independent manner. However, JMJD3 can also modulate iPSC formation in a histone demethylase-dependent manner by targeting dimethylation at the *INK4a/ARF* locus (Fig. 2). Besides reverting cells back into a pluripotent state, Jmjd3 can mediate or inhibit stem cell differentiation into terminally differentiated cells in a histone demethylase-independent manner (Fig. 4). During glioblastomagenesis, stem cell differentiation occurs in a chromatin-independent manner through stabilization of the p53 protein (Ene et al., 2012). During glioblastoma formation cellular terminal differentiation may become blocked and lead to malignant transformation. Dysfunction of JMJD3 may contribute to glioblastoma formation by blocking this cellular terminal differentiation (Ene et al., 2012). JMJD3 can also demethylate non-histone proteins. During cellular senescence, JMJD3 can demethylate the non-histone retinoblastoma (RB) protein at its lysine 810 residue (Zhao et al., 2015). The histone demethylase activity of JMJD3 may also be regulated by its intracellular localization (Kamikawa and Donohoe, 2014). JMJD3 contains two classical nuclear localization sequences at its N-terminus (Kamikawa and Donohoe, 2014). Forced nuclear localization of JMJD3 leads to markedly enhanced H3K27me3 demethylation, whereas cytosolic localization may led to dimethylation of non-histone proteins (Zhao et al., 2015). During SAHF formation, JMJD3 is colocalized with the Golgi protein, GM130 (Zhao et al., 2015) and may demethylate non-histone proteins undergoing posttranslational modifications within the Golgi. Both JMJD3 demethylase-dependent and -independent pathways may mediate efficient cellular

reprogramming and differentiation, but the independent pathway may play a major role in terminally differentiated cells and cancer.

### 1.7. Future prospects

Targeting JMJD3 for the development of therapeutics to treat a number of diseases may prove feasible and efficacious. Using a structure-function approach and subsequent screening of a GlaxoSmithKline corporate compound collection (approximately two million compounds), Kruidenier L et al. identified GSK-J1 as a specific JMJD3 catalytic site inhibitor (Kruidenier et al., 2012). This small molecule inhibitor was further modified by addition of cell-penetrating ethyl esters, yielding GSK-J4, which inhibited LPS-induced cytokine expression in human macrophages from healthy volunteers. This molecule was further shown to inhibit the cell proliferation of all human T-ALL cell lines tested, and GSK-J4 did not affect the growth of myeloid leukemia cells, stromal cells, or hematopoietic progenitor cells (Ntziachristos et al., 2014). GSK-J4 has been used to treat cells isolated from pediatric brainstem glioma (Hashizume et al., 2014) patients with a lysine to methionine (K27M) substitution on histones H3.1 and H3.3 (Wu et al., 2014). Preclinical studies treating K27M-mutant brainstem glioma cell lines using GSK-J4 demonstrated marked inhibition of cell growth (Wu et al., 2014). Treating cells with a JMJD3 inhibitor did not increase the levels of H3K27me3 marks on the K27M-mutant, but rather indirectly promoted the methylation of the non-mutant protein allele (Wu et al., 2014). Furthermore, Rotili et al. developed pan-histone demethylase inhibitors, which can target all Jumonji C demethylases, including JMJD3, and these inhibitors have the capacity to induce cell cycle arrest and increase apoptosis in human prostate LNCaP cells and colon HCT116 cancer cells (Rotili et al., 2014).

Alternative to using small molecule inhibitors, a new technology, (clustered regularly interspaced short palindromic repeats)/Cas9-based gene editing (Hsu Patrick et al., 2014; Qi et al., 2013), may be used to delete *JMJD3* for therapeutic applications. This gene editing can use RNA-guided DNA recognition to precisely edit the gene of interest (Qi), with minimal off-target effects. This technology can also be used as a screening approach to identify downstream effectors of JMJD3 and to identify which genes are essential for mediating JMJD3 responses.

Inhibition of JMJD3 may provide benefit in certain circumstances, but inhibition may actually produce deleterious responses. If ablation of *JMJD3* increases pancreatic cancer cell metastasis (Yamamoto et al., 2014), inhibiting JMJD3 using GSK-J4 or gene editing may be detrimental. In addition, low levels of JMJD3 expression may be associated with colorectal cancer (Tokunaga et al., 2014), and thus, inhibition of JMJD3 in certain cancers may not provide benefit. Alternatively, complete *JMJD3* ablation may have more profound effects on cells and tissues compared with a small molecule inhibitor. However, because JMJD3 is involved in a plethora of cellular processes in diverse cell types, globally administered JMJD3 inhibitors may produce off-target and side effects. Therefore, designing cell-specific agonists or antagonist would help control for any non-specific effects. Whether JMJD3 should be activated or inhibited for therapeutic efficacy may be highly dependent on the tissue or disease being targeted. The use of JMJD3 inhibitors may prove particularly

effective in treating diseases involving gene mutations, gene dosage defects, or gene imprinting, where increasing expression of the non-mutant, inactivated, or imprinted allele may alter disease state. Conversely, in diseases, such as some types of cancer, activating JMJD3 may be a more effective treatment by suppressing cancer stem cell proliferation, preventing malignant transformation, and promoting tumor suppression.

The future identification of non-histone proteins, which are demethylated or directed for ubiquitination and subsequent degradation, will provide evidence of other key molecules that are downstream and dependent on JMJD3. Also, further investigations are needed to characterize the intracellular localization of JMJD3 and to identify alternative functions of JMJD3 within these intracellular compartments. The mechanisms of how JMJD3 RNA and protein are degraded have not been determined, but may help regulate JMJD3 levels and downstream histone demethylase-dependent and -independent pathways.

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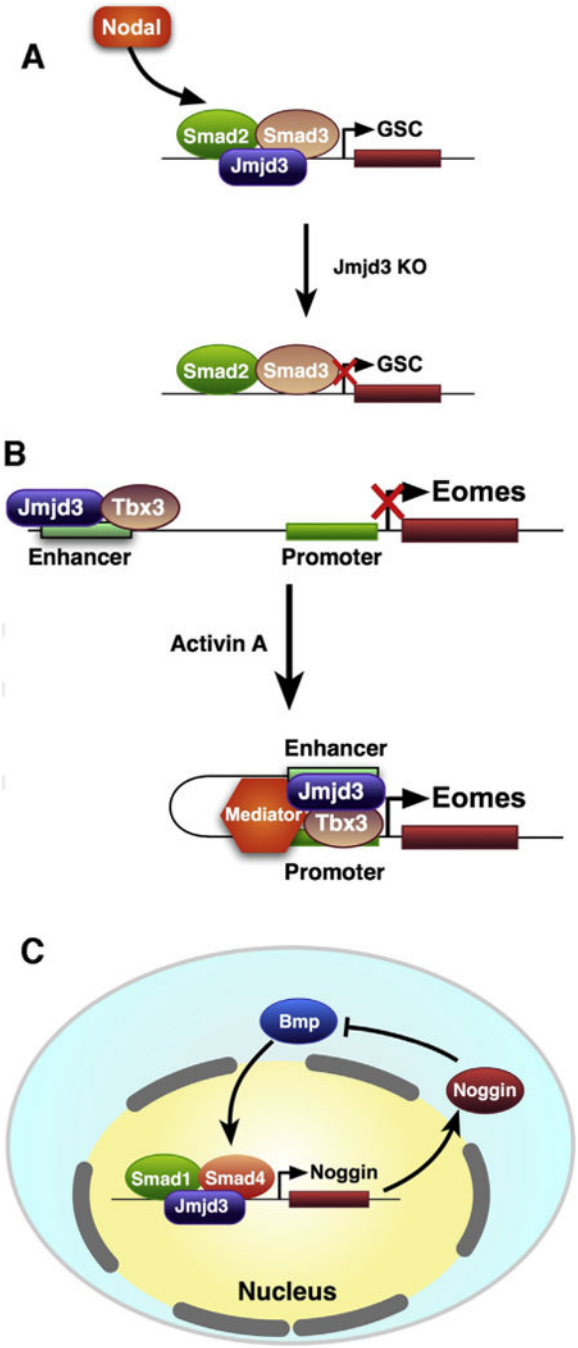


**Fig. 1.**

Role of Jmjd3 in development. (**A**) Negative feedback of Noggin to regulate Jmjd3-mediated activation of Noggin via inhibition of BMP activity. Upon treatment with Nodal, JMJD3 recruitment to *SMAD2/3* genes is associated with decreased enrichment of H3K27me3 marks at *SMAD2/3* genes (Kim et al., 2011), leading to the expression of the homeobox protein goosecoid (GSC) initiation of endoderm development. (**B**) During early endoderm differentiation, TBX3 associates with JMJD3 at the enhancer region of *Eomes*, bringing its enhancer in close proximity to its promoter to drive its own expression. This

DNA looping of *Eomes* allows for a self-activating loop, thereby, maintaining endoderm fate (Kartikasari et al., 2013) and preventing abnormal development. (**C**) Negative feedback of Noggin to BMP activity via Jmjd3 regulation.

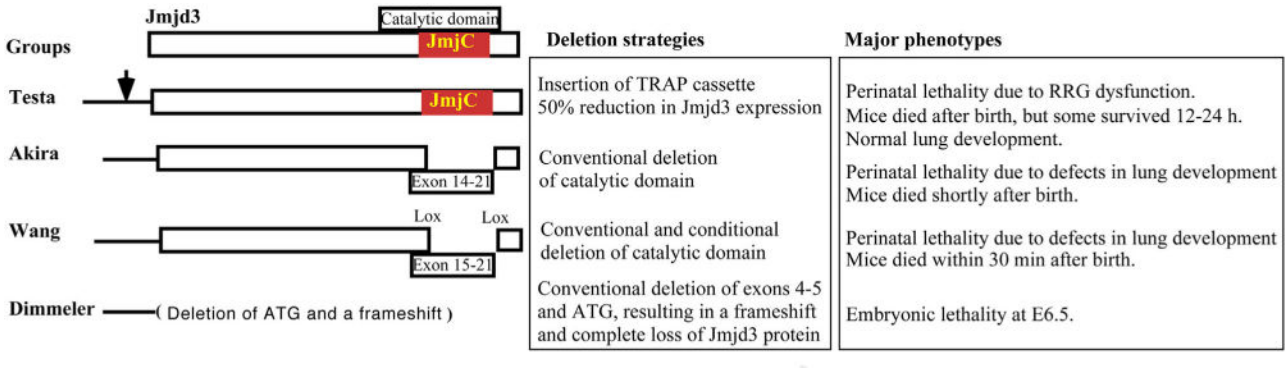


**Fig. 2.**

Deletion strategies of JMJD3 and their associated mouse phenotypes.

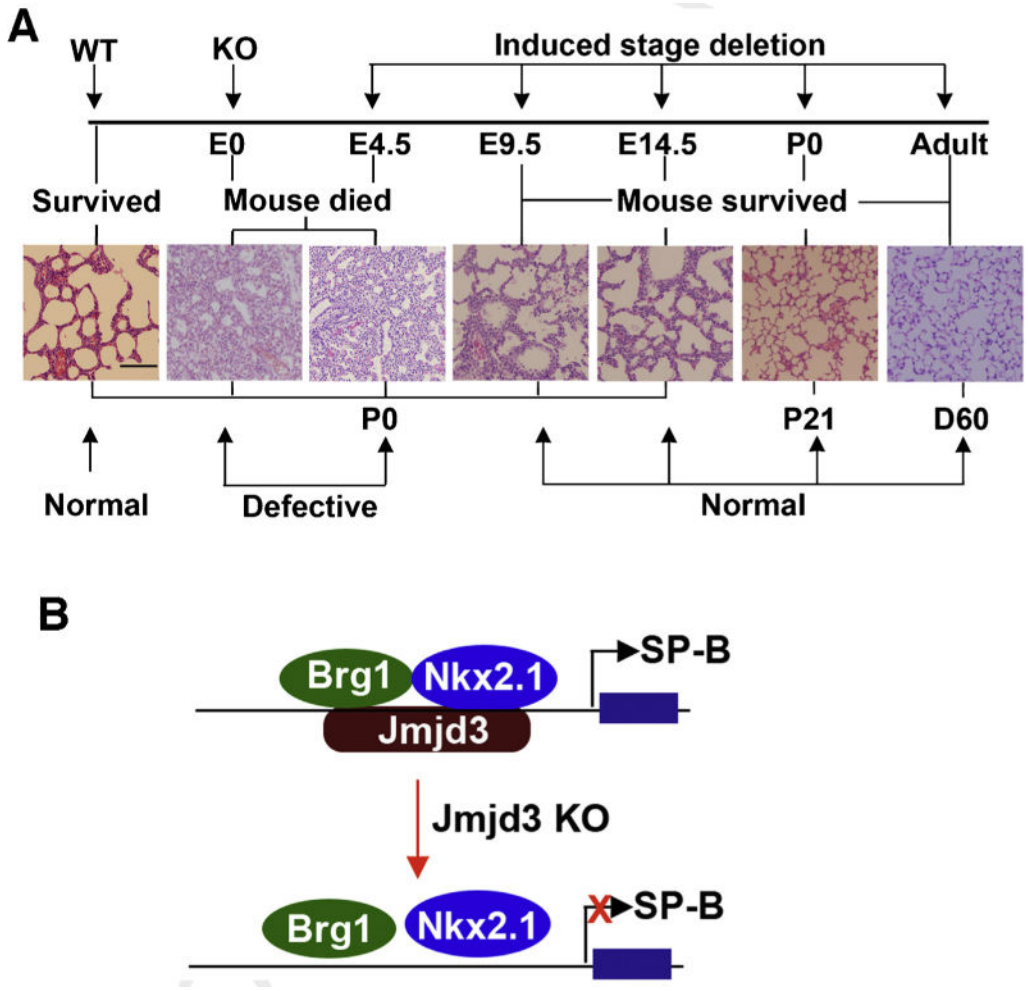


**Fig. 3.**

Stage-specific role of JMJD33 in lung development and function. (**A**) JMJD3 regulates lung development in a stage-dependent manner. H&E staining showing the stage-dependent effects of *Jmjd3* deletion on lung architecture and its correlation with embryo viability. Bar = 200 μm, (**B**). A proposed model explaining how JMJD3 specifically upregulates SP-B expression by interacting with Nkx2.1 and Brg1 at the SP-B promoter.

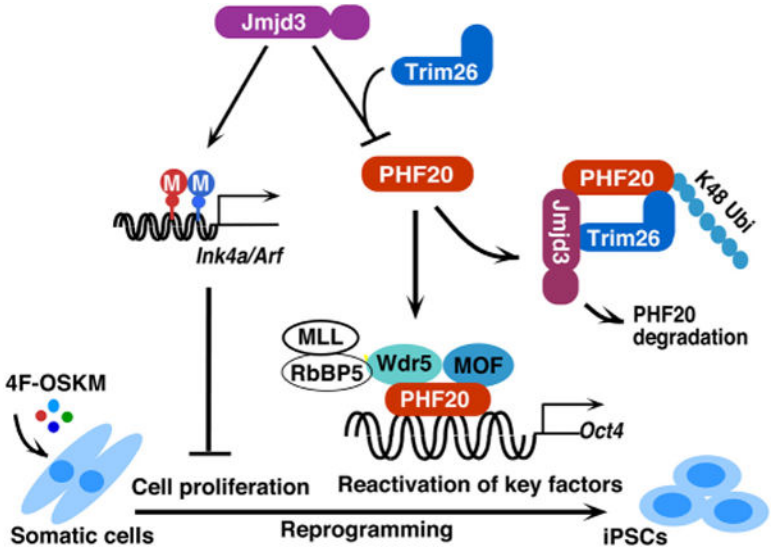


**Fig. 4.**

A working model to illustrate how JMJD3 negatively regulates cellular reprogramming through demethylase-dependent and independent pathways. JMJD3 upregulates *Ink4a/Arf* and *p21* by removal of H3K27 methylation through its H3K27me2/3 demethylase activity. Increased amounts of Ink4a and Arf induce cell senescence or apoptosis and reduce cell proliferation, which leads to decrease in efficiency and kinetics of reprogramming. Importantly, JMJD3 protein also targets PHF20 for ubiquitination and degradation by recruiting an E3 ligase Trim26 in an H3K27 demethylase activity-independent manner. PHF20 is required for the reactivation of key core reprogramming factors such as Oct4 through interaction with WDR5. Thus, downregulation of PHF20 protein by JMJD3 leads to the inhibition of reprogramming efficiency.