Epigenetic reprogramming in mammalian cell differentiation, transdifferentiation and dedifferentiation.

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Abstract
Epigenetic and chromatin modifications have important roles in governing gene activity and nuclear architecture. They are also necessary for normal embryonic development and cell differentiation. Early epigenetic programming events during mouse embryogenesis are believed to be essential for normal growth and development. Aberrant epigenetic profiles are associated
with the conversion of normal cell phenotypes into cancer cells. Because epigenetic alterations are potentially reversible, experimental progress in this area may offer great promise for new cancer therapy. Nuclear epigenetic profiles can be manipulated using techniques such as somatic cell reprogramming, genetic engineering and small molecules, which can reprogramme the cell towards dedifferentiation and transdifferentiation. Advances into the mechanisms will improve the potential for regenerative medicine. In this review, we describe the principles of epigenetics and its relation to cell reprogramming, differentiation, dedifferentiation and transdifferentiation.

**Keywords:** Epigenetics, Reprogramming, Differentiation, Dedifferentiation, Transdifferentiation, Cancer

**Review methodology**

Literature was sourced from PubMed, Google Scholar and Web of Science; journal publishers; meeting reports and communications from colleagues. In addition to these articles, we checked references cited by the authors for additional relevant material.

**Epigenetic definitions**

Epigenetics describes the phenomena between genotype and phenotype that can alter the phenotypic outcome associated with genomic loci in the absence of changes to the underlying DNA sequence [1]. For example, the majority of cells in our body share an identical genotype originally derived from the first single cell after fertilisation, the zygote, yet these cells can have different morphologies and functions. During development, cells eventually generate a diversity
of disparate and stable cell types in a process called cellular differentiation. These transitions were first envisioned by Waddington as being governed by changes in trajectory across a conceptual ‘epigenetic landscape’, rather than by alterations in genetic inheritance [2]. In molecular terms, epigenetics is defined as the study of any potentially stable and heritable change in gene expression or cellular phenotype that occurs without altering the DNA sequence of the cell lineage [1]. The mechanisms can involve covalent modification marks on histones and DNA or non-coding RNAs (ncRNAs) [3-5]. ncRNAs are transcribed from DNA but are not translated into proteins; those ncRNAs that appear to be involved in epigenetic processes [6] can mediate non-mendelian inheritance of an epigenetic change [7].

Epigenetic modifications include the methylation states of cytosine residues of DNA and posttranslational methylation groups on the histone proteins associated with the DNA. Co-regulator proteins with binding domains for these chemical groups may associate with these epigenetic marks and affect the activity of nearby genes [8, 9]. The specific combination of epigenetic modifications may furthermore determine the conformation of the chromatin fibre into which the DNA and histones are packaged, and can thereby regulate the transcriptional potential of the underlying genes [4, 10]. This is based on the notion that repression of gene expression is caused by a lack of accessibility to the gene by the RNA polymerase [11], as well as its recruitment [12].

During development as well as in adult life, an interplay exists between the environment and genome; however, the currently known framework of gene–environment interactions is not sufficient to fully explain the risks of common diseases, some of which appear modulated by epigenetic mechanisms [13]. Many environmental factors have been implicated in aberrant epigenetic changes both in experimental and epidemiological studies [14]. These environmental epigenetic modulators include nutrients, oxygen, temperature, radiation, pollution, chemicals and
toxins [13-17]. Specific epigenetic modifications and transcriptional profiles have been shown to have a dynamic potential for rapidly adapting to culture conditions [17, 18]

**DNA methylation**

DNA methylation involves the addition of a methyl group to CpG sequences at the 5’ position of the cytosine ring (5-methylcytosine; 5mC). This modification, which occurs in the DNA of most but not all eukaryotes, is catalysed by DNA methyltransferases (Dnmt) and is generally associated with gene repression [3, 9, 11]. The repression mechanisms can act through direct interference by the methyl group or involve a family of methyl binding proteins and associated complexes in vertebrates [9]. DNA methylation can occur in two ways: de novo methylation relies on Dnmt3a and Dnmt3b enzymes that add new methyl groups to CpG sequences; whereas methylation maintenance requires Dnmt1 to restore methyl groups to hemi-methylated CpG sequences following DNA replication [3, 12]. DNA methylation can be further modified, as part of a presumed active demethylation mechanism, by the Tet family of enzymes converting 5mC to 5-hydroxymethylcytosine (5hmC) and its higher oxidative products 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [19-21].

**Histone methylation**

Post-translational modifications can be found on the histone protein globular core regions, around which DNA is wrapped [22]. However, the majority of histone modifications occur on the lysine-rich N-terminal amino acid “tails” extending from the nucleosome structure [8]. These include: acetylation, phosphorylation, sumoylation, ubiquitination and methylation [23].

Methylation of histones is an epigenetically heritable histone modification found on lysine (K) or arginine (R) residues, produced by a family of histone methyltransferases (HMTs). Conversely, histone demethylases work to remove methyl groups from these residues [8]. Histone lysine residues can be mono- (me), di- (me2), or tri-methylated (me3); each of these posttranslational
modifications can have a decisive influence on gene and chromatin functions [24]. For instance, H3K9me3 represses gene transcription and assists in the formation of constitutive heterochromatin, while H3K9me2 represses genes in euchromatin as well as forming facultative heterochromatin [25]. Modifications of histone lysine can act as either repressive or active marks. For example, methylations of H3K4, H3K36 and H3K79 have been highly correlated with transcriptional activation, whereas methylations of H3K9, H3K27 and H4K20 are associated with repressive chromatin states [26, 27]. The repression mechanisms typically involve protein complexes with binding modules for these methyl groups [8]. This has led to a model describing the proteins involved in histone modification management as ‘writers, erasers and readers’, in an analogy with other signalling pathways [28].

Methylation of histone arginine residues by arginine methyltransferases (PRMTs) is less extensively studied but it has been found to play significant roles in gene regulation, development and cancer [29]. Histone arginine methylation can be transcriptionally activating [30] or repressive [31] depending on the target residue, and on whether the methylation is symmetric or asymmetric. In mammals, PRMT1- and CARM1-catalysed histone asymmetric histone dimethylation at arginine is involved in gene activation [30] while PRMT5-catalyzed symmetric histone dimethylated arginine is associated with gene repression [31].

**Epigenetics and differentiation.**

In developmental biology, cellular differentiation is the process by which a less specialized cell (stem cell or progenitor) becomes a more specialized cell type. Differentiation can dramatically change a cell's morphology and function, as a result of alterations in gene expression with the involvement of epigenetic modifications [32, 33]. Differentiation occurs numerous times during development and continues into adulthood, as adult stem cells divide and create fully differentiated daughter cells during tissue repair and during normal cell turnover [34]. In cancer,
the differentiation state (sometimes referred as redifferentiation) is used in grading tumours to assess cancer progression, by comparing the genotype and phenotype of cancer tissue to normal tissue. Well-differentiated cancer cells are comparable to normal cells, in that they grow and spread more slowly than poorly differentiated or undifferentiated cancer cells [35, 36].

Each cell population is thought to have its own characteristic epigenetic signature, which correlates with its differentiation potential. Mammalian development is a unidirectional process during which there is a progressive loss of developmental potential. It begins with the formation of a unicellular zygote and ends with the establishment of more than 200 specialized cell types of the mammalian body [37]. According to this diminishing differentiation potential, specific terms have been assigned to the individual cell populations that arise during development, such as: totipotency (ability to differentiate into intraembryonic tissue and extraembryonic tissue), pluripotency (ability to differentiate into intraembryonic tissue; ectoderm, mesoderm and endoderm), multipotency (ability to differentiate into two or more lineages) and unipotency (ability to differentiate to one lineage).

It is thought that at specific stages in development and differentiation, biologically important differences in the ‘openness’ of chromatin occur. For example, chromatin in preimplantation embryos is more open than in postimplantation cells, while stem cell chromatin is less compact and more transcription-permissive than that of differentiated cells [18, 38]. Open chromatin structure, characterized by relatively few condensed heterochromatin areas, has a higher proportion of active epigenetic marks (i.e., H3K4me3, H3K39me and H3K79me) compared to repressive epigenetic marks (i.e., H3K9me, H4K20me and DNA methylation) [39]. In addition, developmental genes can be ‘bivalent’, marked by both the active epigenetic mark H3K4me3 and the repressive epigenetic mark H3K27me3, which signifies genes that are silent but transcriptionally poised for activation [40, 41]. The bivalent histone modification patterns
disappear after cell differentiation, when most stemness genes are repressed in association with repressive epigenetic marks while specific differentiated genes become activated [40, 42]. At the same time, heterochromatin spreading takes place and chromatin plasticity is diminished [38, 43].

**Reprogramming, dedifferentiation and transdifferentiation.**

Unlike in lower vertebrates, reprogramming, dedifferentiation and transdifferentiation rarely occur naturally in mammals. However, under certain experimental conditions, differentiated cells can revert into a less differentiated state, in a dedifferentiation process induced by nuclear/cell reprogramming. Examples include the generation of induced pluripotent stem cells (iPSC) [44], or the creation of a totipotent embryo derived from somatic cell nuclear transfer (SCNT) [45]. Reprogramming also describes the conversion of one differentiated cell type into another, for instance of a B lymphocyte into a macrophage [46], or a fibroblast into a cardiac muscle cell [47], following the induced expression of defined transcription factors. Because these two examples of cell fate change may not involve a gain in differentiation potential, the term `lineage conversion' or `transdifferentiation' is currently used to describe these processes. Moreover, cellular dedifferentiation has also been implicated in cancer. As cancer can only be established from cells that have the potential to divide, and not terminally differentiated cells, one theory suggests that tumours may arise from the unrestrained growth of dedifferentiated cells that resemble embryonic or stem cells [48].

In molecular terms, cell reprogramming describes the molecular changes that cells undergo as their fate changes. Epigenetic reprogramming has been used to describe certain nuclear epigenetic changes that occur irrespective of changes to the differentiation state of cells, such as the DNA and histone methylation changes after fertilisation [49, 50], during germ cell maturation [51], dedifferentiation [37] and transdifferentiation [52].
Epigenetic patterns can be inherited within cell lineages, as well as being dynamic during early development. Most somatic DNA and histone methylation modifications are erased during germ cell development [53, 54] and preimplantation stages [55], and are subsequently reinstated during pre and postimplantation [18, 32, 50]. It is thought that the erasure of ‘epigenetic memory’ is required for proper development, while incomplete and aberrant epigenetic reprogramming may cause developmental arrest and abnormalities [56-58].

**Manipulation of epigenetic profiles.**

Mechanisms of endogenous origin, as well as exogenous factors can be used to manipulate nuclear epigenetic profiles, and can in this way alter cell fate. Techniques include nuclear transfer [59], cell fusion [60], cell treatment with other cell extracts [61] or small molecules [62], and over expression of specific genes [63]. Such epigenetic reprogramming strategies may be useful for future therapies, for example in the treatment of cancers and mental retardation which have epigenetic abnormalities [64, 65]. Cell fate reprogramming strategies are already employed in disease modelling and have potential for regenerative medicine approaches aimed at tissue renewal [66]. Veterinary applications extend further into transgenic animal generation, drug development, and the preservation of biological diversity [67].

Somatic cell nuclear transfer (SCNT) experiments in amphibians, and subsequently in sheep and other mammals, first demonstrated that it is possible to generate an adult cloned animal from a differentiated cell, albeit at low efficiency. [45, 68, 69]. Studies in different species using the nuclear transfer technique have shown that eggs or oocytes have the ability to erase somatic epigenetic patterns (epigenetic memory) of the donor nucleus and replace these with embryonic marks, leading to the development of pluripotent stem cells that are functionally equivalent to those derived from fertilized embryos. However, incomplete reprogramming by nuclear transfer may result in failure of full term development [58, 69-71].
In a major advance demonstrating that cell reprogramming capability is not restricted to oocytes, ectopic expression or introduction of recombinant proteins of the pluripotency transcription factors (Oct4, Sox2, Klf4, Myc, Nanog, Lin28) was shown to be sufficient for reprogramming of a small proportion of somatic cells into a pluripotent state in mouse [44] and human cells [68]. These iPSCs are also in many respects similar to natural pluripotent embryonic stem cells (ESCs), such as the expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, and viable chimera formation, as well as potency and differentiability. Genomic mapping studies show that iPSC techniques can induce global epigenetic reprogramming of differentiated cells (fibroblasts) towards a pluripotent cell epigenome [63, 68]. The iPSC methodology permits the derivation of either patient-specific or disease-specific pluripotent cells. These cells can be used for drug screening, and as a model for the pathogenesis of degenerative diseases such as Alzheimer’s, Parkinson’s or multiple sclerosis, as well as having potential for cell therapy [72]. The study of iPS cells that are corrected for a gene mutation to rescue sickle cell anaemia and thalassemia in mouse models has demonstrated ‘proof of principle’ for the use of iPSC combined with gene therapy for disease treatment [73, 74]. Nevertheless, analysis of human iPSC lines suggests that variability in differentiation potential and the epigenetic control of cancer dedifferentiation during cell reprogramming need to be better understood prior to application in regenerative medicine [75, 76].

Cell reprogramming in veterinary pre-clinical models and agriculture.

Mouse models suggest treatment of a number of common degenerative diseases is possible using transplanted induced pluripotent stem cells. Mice lack physiological similarity with humans, however; while targeted gene mutation in mouse often fails to reproduce human phenotypes [77].
Interest in the use of iPSCs in large animal pre-clinical models for disease modelling has ranged from rhesus monkey (pancreatic insulin-producing cells for diabetes), pig (rod photoreceptor cells for retinal disease; endothelial cells for cardiovascular disease), dog (endothelial cells), to macaque (dopaminergic neurons for Parkinsons disease). By filling the gaps between laboratory findings in the mouse and clinical trials in humans, domestic animal models are therefore invaluable for testing the safety and potential of iPSCs [67]. Interestingly, while somatic cell reprogramming to iPSCs in many species has relied on over-expression of the conserved Yamanaka set of transcription factors, additional steps are likely needed here to achieve a true pluripotent state with competence for germ-line transmission [67, 78]. Epigenetic modifications are evolutionary conserved but some variation exists between species in the modifying enzymes and binding protein homologues involved [9]. Overall, iPSC and ES cell technology have been mainly limited to rodents and humans. Instead, SCNT cell reprogramming technology has continued to deliver live births in a range of species including transgenic production in farm animals for potential agricultural applications [77].

**Epigenetic inhibitors.**

In the last decade, a variety of small molecules have been created and discovered, some of which have the potential to alter epigenetic marks resulting in cell differentiation, transdifferentiation and dedifferentiation. Early examples include DNA demethylating agents such as 5-azacytidine (5-AzaC), which is a cytosine analogue that can cause extensive global DNA demethylation and reduce DNA methyltransferase activity in the cells [79, 80]. It was originally developed as an antitumor agent, and has been useful in the treatment of leukaemia and myelodysplastic syndrome [79, 81]. The effect of 5-AzaC is unpredictable, as it may cause dedifferentiation [82], differentiation [83] or transdifferentiation [84]. Other types of modifiers include histone deacetylase (HDAC) inhibitors such as valproic acid (VPA) and trichostatin (TSA). VPA has
been utilised for decades as a treatment for epilepsy, as a mood stabiliser and in migraine therapy, while TSA is currently applied as an anticancer medicine [85, 86]. It has been shown that both VPA and TSA can inhibit HDACs and then trigger active global demethylation of the mammalian epigenome, causing reprogramming of gene expression [87]. VPA and TSA induce dedifferentiation by enhancing epigenetic reprogramming in iPSC and SCNT technologies [88-90]. Moreover, VPA and TSA can induce redifferentiation in cancer, causing growth inhibition and apoptosis [91, 92].

In addition, histone methylation inhibitors such as BIX-01294 (a diazepin-quinazolin-amine derivative) have been demonstrated to selectively impair G9a (Ehmt2) HMTs and levels of H3K9me2 [93]. A combination of BIX-01294 with defined factors (chromatin remodellers) could increase the cellular and epigenetic reprogramming rate of iPSC methodology [94].

**Epigenetic modifier enzyme roles in differentiation in development and disease.**

Gene knockdown and knockout technologies in both in vivo and in vitro studies have revealed that mammalian development and differentiation requires both DNA/histone methylation (see table 1) and demethylation (see table 2). Most of the DNA and histone methyltransferases are important for normal embryonic development and differentiation. Conversely, most demethylase knockout embryos can survive until birth but cell differentiation is affected.

Epigenetic modifiers are therefore essential and their defects are strongly linked to dedifferentiation towards stem cells or cancer (see table 1 and 2). The tables show that modifiers related to formation of heterochromatin, such as Suv39h, Suv420h, Dmnt1 and Dnmt3l, tend to act as repressors for dedifferentiation (table 1), whereas histone demethylases Jmjd1 and Jmjd2 act as activators (table 2). H3K4 histone methyl transferases tend to activate cell reprogramming towards stem cells, but repress cancer (table 1). The important roles played in various
differentiation aspects identify these enzymes as potential drug targets for treating disease phenotypes and in regenerative reprogramming.

Conclusion/Summary.

In this review, we conclude that epigenetic marks are important for development, differentiation and that their dysregulation can cause dedifferentiation. Each cell phenotype has a unique epigenetic signature, which undergoes alteration when the cells are differentiated, transdifferentiated or dedifferentiated. Manipulation of epigenetic mechanisms can help control cell phenotypic outcomes in disease, and in this capacity can be useful for medicine and veterinary medicine, while animal production from reprogrammed somatic cells can find use in agriculture and animal conservation. The detailed information that is now available on the epigenomic maps (http://www.roadmapepigenomics.org/) from different adult and embryonic tissues, cross-referenced with disease states will provide the roadmap to these goals, as well as advancing our understanding of underlying epigenetic processes [95-97].

References


associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7


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cogovern histone H3K27 trimethylation and are essential for hair follicle homeostasis and wound

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**Table 1 – 2. Legend**

Reported activating roles in cancer include: the protein was observed to be overexpressed in cancer tissues and/or loss-of-function experiments reduced cancer phenotypes, or overexpression enhanced cancer phenotypes. Reported repressive roles in cancer include: the protein was observed to be underexpressed in cancer tissues and/or loss-of-function was observed to result in cancer phenotypes, or overexpression reduced cancer phenotypes.
Table 1 The importance of histone lysine methyltransferase and DNA methyltransferase for development, differentiation, cell reprogramming (SCNT and iPS) and cancer.

<table>
<thead>
<tr>
<th>Site</th>
<th>Enzyme</th>
<th>Embryonic Lethality</th>
<th>Abnormal differentiation</th>
<th>Cell reprogramming</th>
<th>Cancer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3K4</td>
<td>Setd1a/ Kmt2f</td>
<td>Yes (E7.5)</td>
<td>No</td>
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<td>Activating (leukemia)</td>
<td>[98, 99]</td>
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<td></td>
<td>Setd1b/ Kmt2g</td>
<td>Yes (E11.5)</td>
<td>No</td>
<td>-</td>
<td>Repressive (squamous cell carcinoma)</td>
<td>[98, 100]</td>
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<td></td>
<td>Mll/ Kmt2a</td>
<td>Yes (E11.5)</td>
<td>Yes (blood)</td>
<td>Enhance</td>
<td>Repressive (prostate carcinoma)</td>
<td>[101-105]</td>
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<td></td>
<td>Kmt2b/ Mll4</td>
<td>Yes (E10.5)</td>
<td>Yes (heart)</td>
<td>Enhance</td>
<td>Repressive (squamous cell carcinoma)</td>
<td>[103, 106-109]</td>
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<td></td>
<td>Mll3/ Kmt2c</td>
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<td>Yes (blood)</td>
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<td>Repressive (myeloid leukemia)</td>
<td>[103, 110-112]</td>
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<td></td>
<td>Kmt2d/ Mll2</td>
<td>No</td>
<td>Yes (heart)</td>
<td>Enhance</td>
<td>Repressive (lung cancer)</td>
<td>[108, 109, 113, 114]</td>
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<tr>
<td></td>
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<td>-</td>
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<td>[103, 115, 116]</td>
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<td>Smyd3/ Kmt3e</td>
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<td>Yes (muscle)</td>
<td>Enhance</td>
<td>Activating (hepatocellular carcinomas)</td>
<td>[121-123]</td>
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<td>H3K9</td>
<td>Ehh1/ Kmt1d</td>
<td>Yes (E9.5)</td>
<td>Yes (fat)</td>
<td>Enhance</td>
<td>Activating (parotid gland tumour)</td>
<td>[124-127]</td>
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<td></td>
<td>Ehh2/ Kmt1c</td>
<td>Yes (E9.5)</td>
<td>Yes (blood)</td>
<td>Enhance</td>
<td>Activating (lung cancer)</td>
<td>[124, 126, 128-130]</td>
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<td></td>
<td>Eset/ Kmt1e</td>
<td>Yes (E8.5)</td>
<td>Yes (bone)</td>
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<td>Activating (parotid gland tumour)</td>
<td>[126, 127, 131, 132]</td>
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<td></td>
<td>Suv39h1.2/ Kmt1a,b</td>
<td>Yes (E14.5)</td>
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<td>[126, 133-136]</td>
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<td>Prdm2/ Kmt8c</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<td>Ezh1/ Kmt6b</td>
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<td>Yes (skin)</td>
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<td>-</td>
<td>[142, 143]</td>
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<tr>
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<td>Inhibit</td>
<td>Repressive (skin cancer)</td>
<td>[144-147]</td>
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<td>Setd8/ Kmt5a</td>
<td>Yes (E2)</td>
<td>Yes (skin)</td>
<td>-</td>
<td>Activating (bladder cancer)</td>
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<td>-</td>
<td>Activating (breast cancer)</td>
<td>[155-157]</td>
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<td></td>
<td>Whsc1/ Nsd2</td>
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<td>Yes (bone)</td>
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<tr>
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<td>[161-163]</td>
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<td>Setmar/ Metnase</td>
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<td>Ash1l/ Kmt2h</td>
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<td>Dnmt3b</td>
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<td>Yes (neuron)</td>
<td>-</td>
<td>Repressive (colorectal carcinoma)</td>
<td>[172, 177, 180]</td>
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</table>
### Table 2. The importance of histone histone (lysine) demethylase and DNA demethylases for development, differentiation, cell reprogramming (SCNT and iPSC), and cancer.

<table>
<thead>
<tr>
<th>Site</th>
<th>Enzyme</th>
<th>Embryonic lethality</th>
<th>Abnormal differentiation</th>
<th>Cell reprogramming</th>
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