Mouse models in epigenetics: insights in development and disease

Jesús Espada and Manel Esteller

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Abstract

Epigenetics is devoted to the study of molecular mechanisms that can modify the structure of the chromatin fiber and, in that way, regulate large-scale patterns of gene transcription. In mammals, most molecular mechanisms that are considered ‘epigenetic’ have key roles during development and in adult cells and tissues, and have been implicated in a number of human diseases, including cancer. Here, we outline a brief overview on the contribution of the mouse model system to the emergence of epigenetics as a research field on its own.

Keywords: epigenetic; cancer; mouse model

INTRODUCTION

Regulation of gene transcription can be defined as the process by which it is established what genes in the genomic landscape are to be expressed or repressed in a cell in order to obtain the most adequate output of cellular activity at a given moment and under a given set of external stimuli. Since this process supports the replication and maintenance of the whole genome, it can be consider a key essential process for all biological systems, ranging from metazoan to viral particles.

In higher metazoan eukaryotes, all cells and tissues in the adult organism are derived from a single fertilized oocyte, implying the existence of a very well-defined strategy that dictates gene transcription patterns in each different step of development and in the routine homeostatic maintenance of adult tissues. All the information required for this wide-range strategy is self-contained in the species genome and is activated after the fertilization of the oocyte and completed after the onset of each new fertilization event.

In eukaryotic cells, regulation of gene transcription is achieved at the most basic level by a subset of DNA-binding proteins, the so-called transcription factors. These proteins recognize specific motifs in the linear sequence of the DNA molecule, comprising core promoter and regulatory domains associated to most genes. As a rule, these sequences can be recognized by transcription factor complexes even in naked, non-nucleosome containing, DNA molecules. In this sense, the basal transcription machinery has been defined as ‘the factors, including RNA polymerase II, that are minimally essential for transcription “in vitro” from an isolated core promoter’ [1]. Upon binding to target DNA sequences, transcription factor complexes can activate or repress gene transcription by promoting or blocking the recruitment of RNA polymerases [1]. Every binding event of a transcription factor to a specific gene target sequence is the result of a complex but finely tuned dynamic network of protein–protein and protein–DNA interactions and associated biochemical activities that ultimately depend on what the cell is sensing inside and outside.

Beyond this first level of gene expression regulation exerted by transcription factors, which basically depends on the linear sequence of the DNA molecule, a second level of genomic regulation has emerged in the last decades. This additional mode
of gene transcription regulation is now widely defined as ‘epigenetic’ and mainly depends on the higher order structure of the chromatin (Figure 1).

**THE BIOLOGICAL MEANING OF EPIGENETICS**

The term ‘epigenetics’ was first used by Waddington in 1942 trying to conceptualize from a developmental perspective ‘the causal interactions between genes and their products, which bring the phenotype into being’ [2]. This rather diffuse definition was further qualified by the author to describe ‘genetic cellular events that could not be explained by genetic principles’ [3]. By that time, DNA methylation was emerging as a major epigenetic mechanism and, in parallel, the term ‘epigenetics’ evolved to a refined form embracing ‘heritable changes in gene expression that are not based on changes in the fundamental sequence of base-pairs of the DNA molecule’ [4]. In subsequent decades, the field of ‘epigenetic’ research witnessed an impressive emergence and it is now widely accepted that most molecular mechanisms actually accepted as ‘epigenetic’ are indeed key cellular processes. At present, these mechanisms include DNA methylation and hydroxymethylation, covalent modifications of histone tails, binding of architectural proteins (linker histones and HMG proteins), ATP-dependent remodeling of the nucleosome core and transcribed non-coding RNAs (ncRNAs).

Taking into account the molecular mechanistic underlying all these ‘epigenetically’ annotated molecular processes, the question of the heritability of epigenetic macromolecular states is a devious concept. At present, the only epigenetic mechanism for which a continuous and stable heritability has been causally demonstrated is DNA methylation. For most other epigenetic processes, the heritability of the associated epigenetic states is far from conclusive. Thus, it is widely assumed that the global state of acetylation, methylation and phosphorylation of histone tails in a particular genomic region determines transcriptional activity [5]. However, although potential mechanisms have been proposed for a histone modification-dependent nucleosome state transmission through the mitotic cycle [6], no causal demonstration for a stable heritability ‘in vivo’ in mammals of histone patterns has been reported to date.

In the same context, high-throughput next-generation sequencing approaches have revealed that although less than 1.5% of the genome is translated to amino acidic sequences and more than 98% of the genomic DNA is transcribed to RNA [7]. This astounding observation suggests the existence of previously unknown functions for ncRNAs. Actually, in the last few years, key studies have reported a plethora of new cellular functions for ncRNAs that have been quickly cataloged as ‘epigenetic’, many of them acting in conjunction with previously known ‘epigenetic’ mechanisms such as histone modification or DNA methylation. However, as with histone...
modifications, no chromatin state regulated by ncRNA functions has been demonstrated to date to be heritable in mammals ‘in vivo’.

It is clear that an essential characteristic that all the epigenetic mechanisms share in common is the ability to modulate the three-dimensional structure of the chromatin fiber, facilitating the transition from open to close conformations or vice versa, and/or regulating in some way DNA transcription (Figure 1). Thus, the definition of epigenetic mechanisms has flexibly shifted to a more functional nuance, comprising ‘any molecular mechanism able to alter the outcome of a genomic locus or a whole chromosome without changing the underlying DNA sequence’ [8] or, in a broad context, ‘chromatin-based events that regulate DNA-templated processes’ [9].

Many of the studies that have contributed to the emergence of epigenetics as a research field on its own are based on cultured cell systems, simple organismal models or systematic analysis of gene expression or repression in cell or tissue samples. Up to now, the use of complex mammalian models in epigenetics, particularly transgenic or knockout mice, has been limited, but has undoubtedly provided invaluable information to understand the role of epigenetic mechanisms in normal development and disease. Thus, the use of these models to test epigenetics is expected to steadily increase in the near future.

EPIGENETICS IN MAMMAL GAMETOGENESIS AND EMBRYO DEVELOPMENT

The use of mouse models has been essential to establish key roles for epigenetic mechanisms during mammalian gametogenesis and embryo development. Gametes are highly specialized haploid cells responsible for the generation of a diploid embryo during sexual reproduction. Both paternal and maternal gametes, and the genomes they contain, are biologically non-equivalent, and, therefore, strictly required for the proper development of reproduction and embryogenesis programs in sexual organisms. The functional differences between maternal and paternal gametes are due to differential expression of a particular set of alleles that arise from a differential modification of the chromatin or genomic imprinting in each type of gamete.

A correct DNA methylation pattern in differentially methylated regions in each type of gamete is essential for genomic imprinting during gametogenesis and in the maintenance of the allelic imprinted patterns during embryogenesis and in adult tissues. Global DNA methylation patterns in the genome are established and maintained by DNA methyltransferases (Dnmt). Dnmt1 is responsible for the maintenance of previously established DNA methylation marks through successive rounds of DNA replication while Dnmt3a and 3b are required for the ‘de novo’ methylation events. A third important enzyme, Dnmt3-like (Dnmt3l), shares the PHD zinc-finger domain with Dnmt3a and 3b, but lacks the catalytic Dnmt domain and, therefore, has no enzymatic activity.

Interestingly, mouse females lacking the Dnmt3l gene produce mature oocytes that have lost maternal-specific methylation in several imprinted genes [10]. These defects result in the loss of monoallelic expression of maternally imprinted genes and lethality during mid-gestation. Spermatogenesis is also arrested in mice lacking Dnmt3l, resulting in a complete impediment to produce mature sperm [10, 11]. Dnmt3l seems to make up for its complete lack of DNMT enzymatic activity by interaction and cooperation with DNMT3a and 3b, acting as a specific recruiting factor [10, 11]. In fact, it has been reported an essential role for Dnmt3a and 3b during mouse paternal and maternal imprinting [12] and mouse spermatogenesis [13].

A correct H3 methylation pattern is also required during gametogenesis. Male mice lacking the histone methyltransferases Suv39h1 and h2 are sterile, showing gross alterations of the H3-K9 methylation pattern associated to homologous chromosome impairment in spermatogonia and arrest at the pachytene state [14]. In parallel, it has been shown that core histones are hyperacetylated in spermatogonia and pre-leptotene spermatids but not in post-meiotic spermatocytes [15]. On the other side, it has been demonstrated that the histone H3 methylation status is also required for maternal imprinting during oogenesis [16]. These observations suggest that a coordinated action of DNA methylation and histone modification activities on imprinted regions is necessary during gametogenesis to establish adequate imprinting patterns. In this sense, it has been reported that Dnmt3l can indeed act as a hinge between DNA and histone H3 methylation [17], while the methylation pattern of histone H3 can alter the DNA methylation status of imprinted genes [16]. In addition, it has been also reported
that a KRAB zinc finger transcription factor of maternal-zygotic effect, Zfp57, is required to maintain both post-fertilization maternal and paternal imprints [18]. In the same way, a large ncRNA can regulate imprinting [19] and the piRNA pathway is required to establish parental germline methylation in imprinted genes [20]. These observations suggest the existence of multiple layers of cooperation between epigenetic mechanisms to establish and maintain germline imprints.

Just after fertilization, an extensive reprogramming of genome transcription patterns is activated in the fertilized egg. This reprogramming event requires extensive DNA demethylation of both maternal and parental genomes, which is actively bypassed in previously imprinted genes, and involves the conversion of 5-methylcytosine to hydroxymethylcytosine [21–23]. Interestingly, it has been recently reported that this conversion cycle can be regulated by the methylation status of histone H3 [24]. A first DNA demethylation round occurs in the male pronucleus [25, 26] and, once the zygote is formed, is followed by a passive and progressive demethylation process lasting to the pre-implantation blastocyst stage [27–29]. Establishment of ‘de novo’ DNA-methylation patterns in the embryo is activated just after implantation and requires the activity of ‘de novo’ Dnmt 3a and 3b [30]. In a finely tuned lineage-dependent process, ‘de novo’ DNA methylation begins in the inner cell mass of the blastocyst [27, 28, 31], is rapidly extended to the primitive endoderm, which gives rise to all embryonic tissues, and is excluded from the trophoblast and endoderm lineages, which give rise to all non-embryonic tissues, including the placenta and yolk sac membrane [32, 33]. Consistent with the essential role of DNA methylation during embryogenesis, it has been shown that maintenance of either newly established DNA methylation patterns [34] or inherited DNA methylation patterns controlling mono-allelic expression of imprinted genes [35], processes that require the catalytic activity of Dnmt1, is essential for proper embryo development. Reinforcing this view, it has been also reported that proteins of the methyl CpG-binding family (Mbd), important for the regulation of chromatin transitions in DNA methylated regions, are required during mouse embryogenesis [36].

Other important components of the epigenetic machinery have also key roles during embryogenesis. Thus, mice lacking the histone deacetylase Hdac1 [37] or the histone H3-K9 methylase G9a [38] present severe embryonic growth impairment and lethality. In the same way, mice lacking members of the ATP-dependent chromatin remodeling complexes of the SWI2/SNF2 family, Brg1 [39] and Snf5 [40], and of the Polycomb family, Eed [41], Ezh2 [42] and Yy1 [43], show defective gastrulation or embryo peri-implantation lethality. Interestingly, mice lacking the Lsh component of the SWI/SNF chromatin remodeling complex show postnatal lethality associated to global DNA demethylation. On the other side, mice showing overexpression of the Atrx protein, a component of the SWI/SNF complex, display a severe neurogenesis impairment and a high incidence of embryonic death [44]. More recently, it has been reported that the deletion of the ARID DNA-binding domain-containing subunit of the SWI/SNF complex also results in embryo lethality [45], indicating that an ATP-dependent chromatin activity is required during embryogenesis. As a whole, these observations suggest that, as with germline imprinting, different epigenetic mechanisms act coordinately to finely regulate a precise program of gene transcription during mouse embryo development.

**EPIGENETIC CONTROL OF X CHROMOSOME INACTIVATION**

The evolutionary advantage of sexual reproduction in mammals is intrinsically linked to the necessity of allelic dosage compensation of the X chromosome in females. This goal is achieved in placental mammals by a random inactivation of one of the two copies of the X chromosome during early embryo development. The inactivation event involves a large-scale transition of vast regions of the X chromosome from euchromatin to highly packaged heterochromatin. This chromatin state transition is regulated basically by epigenetic mechanisms, including ncRNA activity, DNA methylation and histone modifications, and does result in the transcriptional inactivation of most, but not all, genes or locus in the inactivated chromosome.

In the mouse, the X inactivation process begins just after implantation of female embryos and, in sharp contrast to what occurs with most other X-linked loci, is associated to a strong transcriptional activation of the ncRNA Xist locus in the X chromosome that is to be inactivated and to a complete repression of this locus in the future active
chromosome. After massive transcription, ncRNA Xist copies rapidly coat the whole target chromosome spreading from the X inactivation center (XIC), promoting chromatin transition and transcriptional repression [46]. Targeted deletion of the Xist locus completely blocks X chromosome inactivation [47, 48]. A central regulator of Xist activity is Tsix, a ncRNA antisense to Xist [49] that acts by blocking Xist expression in cis [50–52]. Inhibition of Tsix transcription results in the inactivation of both maternal and paternal chromosomes in the trophoblast, promoting embryonic lethality and, interestingly, also a non-random X-chromosome inactivation in embryonic tissues [51, 53].

It has been reported that, when compared with the active X chromosome, the inactive X shows high levels of DNA methylation and histone modification marks specifically associated to transcriptional repression [54]. Differential methylation of distinct histone H3 lysine residues is an essential event during X-chromosome inactivation, being methylation of histone H3-K9 associated with the inactive X chromosome and methylation of histone H3-K4 associated with the active X [55, 56]. Coating of the inactive X chromosome by Xist can act as a molecular mark to recruit the epigenetic machinery that regulates H3-K9 methylation associated to chromatin compaction [56]. On the other hand, genetic studies in the mouse have supported the notion that DNA methylation is a late event during X inactivation probably required for long-term repression [57–59]. DNA methylation of X chromosome requires ‘de novo’ Dnmt3b activity [58], Dnmt1 for stable repression maintenance in embryonic tissues [59], and is associated to the recruitment of Dnmts by structural components of the chromosome [57, 58]. These observations again indicate that X chromosome inactivation is a global epigenetic process involving the cooperation of different mechanisms.

EPIGENETICS AND DISEASE

There are several indications pointing out a central role of epigenetic mechanisms in several diseases, particularly cancer [60, 61], consistent with potential key roles of these mechanisms in the homeostasis of adult tissues. This view has been undoubtedly underpinned by the use of mouse models, which are otherwise a central step in the development of new pharmacological drugs. Two experimental approaches are currently used in this context. The first is the generation of mouse models targeting components of the epigenetic machinery, analyzing its impact on disease. The second is the use of mouse models of human diseases to evaluate dysfunctions in the epigenetic machinery.

A major drawback inherent in the first experimental approach is the embryonic lethality that, as mentioned above, is caused by the targeted deletion of many of the components of the epigenetic machinery. This disadvantage can be bypassed through alternative experimental designs like the use of haploinsufficient mice or tissue-specific targeting. One the first and more powerful set of examples in this respect came from research in cancer. It is well known that a major hallmark of cancer is a global DNA demethylation of the genome and hypermethylation of certain CpG islands [62]. However, it is not clear if these aberrant DNA methylation patterns are a cause or a consequence of the carcinogenic process.

In an attempt to fix this question, haploinsufficient mice were generated expressing a hypomorphic allele of Dnmt1. These mice showed a global DNA demethylation in most tissues associated to the developing of aggressive T-cell lymphomas [63], suggesting that, actually, DNA hypomethylation plays a causal role in tumorogenesis. On the other side, the combination of ApcMin/wt mice, a model for intestinal cancer, with either a Dnmt3b hypomorph model, defective in the ability to establish new DNA methylation patterns, or an Mbd2 hypomorph model, defective in transcriptional repression of genes contained in DNA methylated regions offered a complementary scenario. Double mutant mice of both genotypes showed a strong blocking in the progression of intestinal adenomas to adenocarcinomas [64–67], suggesting that defective ‘de novo’ DNA methylation, probably of CpG islands, is also an important event during carcinogenesis. A direct role for Dnmt3b in carcinogenesis has been also demonstrated in a mouse model of MYC-induced lymphomagenesis. In this model, conditional deletion of Dnmt3b activates cell proliferation and accelerates the formation of lymphomas in association with promoter CpG demethylation and transcriptional activation of the tumor modifier Ment [68]. These results identify the activity of Dnmt3b as an important target for anticancer therapies.

Using the ApcMin/wt background in combination with homozygous deletions of other members of the methyl-binding protein family have confirmed the importance of the DNA methylation machinery in
intestinal carcinogenesis, although adding additional layers of complexity to the scenario. Thus, Mbd4-deficient mice showed increase tumorigenesis rate and formation of intestinal polyps, associated to enhanced mutability in CpG islands [69], while Kaiso-deficient mice showed a strongly delayed onset of intestinal tumorigenesis and slower growth of polyps [70]. These results indicate that the way in which DNA methylation patterns are maintained in adult tissues and the way in which these patterns are read and interpreted by the DNA methylation machinery are key events during tumorigenesis, probably playing an essential role in the homeostatic maintenance of normal tissues.

In this sense, non-lethal embryonic mutations of components of the DNA methylation machinery can cause severe diseases in the adult mouse. Thus, different mutations in the catalytic domain of Dnmt3b cause low body weight, facial anomalies and T-cell death associated to hypomethylation of repetitive sequences, a phenotype that closely resembles the Immunodeficiency, Centromeric instability and Facial anomalies syndrome in humans [71]. A critical role for DNA methylation in the central nervous system and in the genesis of neuropsychiatric disorders has been also proposed [72, 73]. Mice deficient in the methyl-binding protein MeCP2 present impaired bone formation and premature death associated to a phenotype resembling the human neurodevelopmental Rett syndrome [74]. Induction of DNA hypomethylation specifically in the forebrain by a conditional deletion of Dnmt1 results in severe cortical degeneration and impairment of postnatal neuronal maturation [75]. Conditional DKO in the forebrain excitatory neurons showed that Dnmt1 and Dnmt3a are required to maintain DNA methylation patterns that are essential for synaptic plasticity, learning and memory [76]. Interestingly, haploinsufficiency of Snf5, a core component of the SWI/SNF chromatin remodeling complex, promotes the formation of malignant rhabdoid in the mouse, a highly lethal pediatric cancer in humans [40].

There are now several studies reporting the use of mouse models of human diseases, particularly cancer, to identify genes whose expression is epigenetically modified, in an attempt to characterize new pharmacological targets. Here, we briefly outline some relevant cases. One of the first examples was the use of Restriction Landmark Genomic Scanning for methylation (RLGS-m) to characterize differences in the DNA methylation pattern between normal and tumoral livers in SV40 T/t antigen transgenic mice, a model for the development of liver tumors [77]. In this model, the promoter of Mlt1, a member of Snail/Gf1-1 family of transcriptional repressors, is differentially hypermethylated in liver tumors and the expression of the gene is silenced. In a similar way, it was shown that global genomic DNA hypomethylation and CpG island hypermethylation of key gene promoter regions, such as E-cadherin, consistently occurs associated to tumor progression and aggressiveness in a mouse model of skin multistage carcinogenesis [78]. Interestingly, hypermethylation of CpG islands is associated in most cases to a loss of histone H4 acetylation and to dimethylation of histone H3-K4 [78]. In this context, it has been also reported that 5-hydroxymethylcytosine can play an important role in the global DNA demethylation process that occurs in a mouse model of liver cancer promoted by non-genotoxic exposure to carcinogens [79].

Using similar approaches, DNA hypermethylation and silencing of genes that have key roles in oncogenic signaling pathways, including the InK4a/Arf network and Dapk, have been detected in mouse models of lung, prostate, pancreatic and colorectal cancer [80–85]. Jumping from DNA methylation to chromatin remodeling, a shRNA screening to identify chromatin regulators specifically required in a mouse model of acute myeloid leukemia has recently uncovered a critical role of the Polycomb repressive complex 2 in the maintenance of the disease state [86]. As a whole, these observations indicate that the use of high-throughput approaches and similar large-scale techniques to analyze epigenetic changes in mouse models of human disease can help to understand the molecular basis of these diseases and to identify new molecular targets or pathways of potential therapeutic interest.

CONCLUDING REMARKS

Despite some criticism, the use of animal models, particularly the mouse, is an essential and irreplaceable tool in biomedical research. The eclosion in last years of new generation high-throughput sequencing approaches is yielding a vast amount of data on previously unknown aspects of different molecular mechanisms, including epigenetics [9], of indisputable therapeutic importance. However, experimental designs with mouse models following adequate guidelines [87, 88] can provide invaluable functional information ‘in vivo’ in a whole organism...
that is difficult to obtain using these new approaches. Such information will help not only to understand the basic functioning of different biological mechanisms ‘in vivo’ in an organism that closely resembles humans in many aspects but also acts as a key translational bridge from the bench to the bedside, constituting a unique battlefield to test different therapeutically approaches to treat human diseases.

Here, we have annotated several cases in which the use of mouse models has significantly contributed to our knowledge on how different epigenetic mechanisms can regulate embryo development and promote different diseases when altered in adult organisms. These examples highlight the importance of epigenetics in placental mammals and also reinforce the view of using mouse models to get a deeper insight on the importance of epigenetic mechanisms for biomedical research. As with mouse models in other research areas, a conceptual question of special relevance is to ascertain whether the phenotype displayed by each targeted disruption of each component of the epigenetic machinery is the result of either a local distortion of gene expression, a global alteration of the genomic architecture or a combination of both factors. In this sense, a step forward in the field will be an extended use of mouse models for a tissue-specific conditional deletion of different components of the epigenetic machinery. This strategy has rendered breaking results analyzing the importance of DNA methylation in the central nervous system [75, 76]. More recently, a similar approach has been used trying to elucidate the role of Dnmt1 in the regulation of hair follicle growth and skin homeostasis [89]. This kind of studies will undoubtedly foster our basic knowledge of the epigenetic landscape in the near future.

Key points

- Epigenetic mechanisms regulate gene transcription by a fine control of chromatin conformational state transitions.
- Mouse models have been an irreplaceable tool to demonstrate the key role ‘in vivo’ of different epigenetic mechanisms during mammal embryogenesis.
- Mouse models have also essentially contributed to establish the importance of different components of the epigenetic machinery in the genesis of different diseases, including cancer.

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