**ILAR J**

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***Epigenetics***

**Dolinoy and Faulk. Introduction: The Use of Animal Models to Advance Epigenetic Science, pp. 227-231**

Domain 3: Research; T1. Facilitate or provide research support; T2. Advise and consult with investigators on matters related to their research; T3. Design and conduct research; K6. Characterization of animal models

SUMMARY:Modifications to the epigenome induced by the environment have been documented in multiple phyla and include cromatine remodeling, histone tail modifications, DNA methylation, and non-coding and microRNA gene regulation. Exposure to chemical, nutritional, behavioral, and physical factors has been recognized to alter gene expression and health via mutation and modification of the epigenome. Epigenetic changes differ from mutation in that they can be reversible. Epigenetic mutations can be inherited both mitotically and transgenerationally. Epigenetic patterns are prone to changes throughout life course and there is important time point in the life of one individual when the epigenome is more likely be changed. Increased susceptibility to disease after early life experiences Time points and susceptible periods need to be evaluated in animal models across the entire life span. IACUC, AV and other animal care officials will be facing this challenge of animals kept long term. Integration of animal models with human approaches is the key to really understand epigenetic mechanisms underlying disease susceptibility.

QUESTIONS

1. Cromatine remodeling, histone tail, DNA methylation, noncoding RNA and microRNA are the most common epigenetic modification

a. True

b. False

2. Unlike genetic mutations, epigenetic changes are potentially reversable

a. True

b. False

3. The epigenome is particularly dynamic at specific time points like for examples embryogenesis

a. True

b. False

ANSWERS

1. a

2. a

3. a

**Kim and Kim. Recruitment and Biological Consequences of Histone Modification of H3K27me3 and H3K9me3, pp. 232-239**

Domain 3: Research

SUMMARY: Two histone marks, H3K27me3 and H3K9me3 are known for their roles related to repression in the genic and nongenic regions of metazoan genomes. H3K27m3 is preferentially detected in gene-rich regions, primary with genes which control embryonic development at stem cell stage. On the other hand, H3K9me3 is detected in gene-poor regions, in several families of retrotransposons that have been amplified through RNA-mediated mechanisms. Thus, H3K9me marks are overall closely associated with tandem repeat sequences, whereas H3K27me3 marks are associated with CpG-rich sequences. The relevant of this mark lies in the significant of repression and the developmental stage. Both marks are prevalent during early embryonic stages. Genomic regions related with H3K27me3 are protected for DNA-methylation which is regarded a temporary repression signal for controlling a set of development regulators. On the other side, H3K9me3 are methylated in somatic cell, so this signal is designed for a permanent repression involved in the heterochromatin formation of chromosomal regions with tandem repeat structures.

The modification of these marks is highly complex. H3K27me3 mark is established by a protein complex called polycomb repression complex 2 (PRC2). Several studies have defined the proteins of this complex: EZH2 (enzyme), EED, SUZ12, RbAp46/48, and two DNA-binding proteins AEBP2 & JARID2. In the case of H3K9me, several enzymes generate this mark: SETDB1, SUV39H1, SUV39H2, EHMT1 and EHMT2.

Defects in histone-modifying complexes involve serious consequences in the embryonic development. PRC2 plays a role in maintaining the pluripotency and self-renewal properties of ES cells, so any defect of the core components will cause unscheduled differentiation of them. Depletion of SETDB1 results in differentiation into trophectoderm cell and de-repression of a large number of endogenous retroviruses in the mouse genome. In the case of double deletion of SUV39H1 and SUV39H2, this induces lethality in mouse. All of these exposed data describe clearly the importance and relevancy of this mark as the major repression signal in the development processes of human and other mammals.

QUESTIONS

1. Fill the blanks,

a. H3K27me3 mark, \_\_\_\_\_\_\_\_\_\_\_ on Lis \_\_ of histone 3

b. H3K\_me3 mark, trimethylation on \_\_\_ 9 of histone \_\_

c. H3\_4me\_ mark, trimethylation on Lis 4 of \_\_\_\_\_\_\_ 3

2. T/F. H3K27me3 is detected in genes traditionally defined as R-banding regions by Giemsa staining; while regions with the H3K9me3 mark are defined as G-banding regions by the same staining technique.

3. H3K9me3 mark is related with several families of retrotransposons including long terminals repeats (LTRs) and long interspersed DNA elements.

4. H3K27me3 and H3K9me3 histone modification marks are also part of the main repression mechanisms for imprinting and X chromosome inactivation

5. Deletion of Suv39h1 or Suv39h2 induce embryonic lethality on mice

6. Defects in the machinery for establishing H3K27me3 signals induce embryonic lethality of development modification at early stages than in proteins for H3K9me3 signals

ANSWERS

1. Trimethylation, 27 // 9, Lis, 3 // K, 3, histone

2. True. R-banding regions correspond to gene-rich regions and G-banding  with gene-poor regions.

3. True

4. True

5. False, mice with a single deletion are viable

6. True

**Seelan et al. Developmental Epigenetics of the Murine Secondary Palate. ILAR J 53(3-4):240-252**

Primary Species:Mouse (*Mus musculus)*

Domain 3, T3

SUMMARY: The secondary palate separates the oral cavity from the nasal cavity.  Cleft palate is caused by the secondary palatal processes failure to fuse.  In mice this occurs at gestational days 12-14 and the 7th week in humans.  Failure to fuse has a complex etiology caused by both gene mutations and environmental effects.  Any process affecting the size of the palatal processes, orientation, or fusion can cause cleft palate.  Developmental processes include cell proliferation, extracellular matrix metabolism, epithelial mesenchymal transition, apoptosis, cell migration and activity of signal transduction pathways.   Epigenetics refers to changes caused by processes other than changes to the underlying DNA sequence and is thought to play a part in cleft palate.  The most actively studied epigenetic mechanisms are DNA methylation of C in CG dinucleotides, action of miRNAs, and chromatin remodeling involving histone modifications.  Methylation-induced gene silencing can occur by decreasing binding of transcription factors or the methyl group serving as substrate for methyl-CpG binding proteins.  High frequencies of cleft palate occur when DNA demethylating agent 5-aza-2’-deoxycytidine is given to pregnant female mice.  DNA methylation patterns are precisely programmed during embryogenesis and failure of these patterns can lead to craniofacial malformations.  Sox4 regulates many aspects of neural crest cell development.  Its role in palatal development is unknown, but has been implicated in human cleft palate.  Upstream sequence of Sox4 shows dramatic change in DNA methylation between gestational day 12 and 13, CpG islands are predominantly unmethylated and exhibit no differential methylation during palatogenesis.  MicroRNAs have been established as regulators of proliferation, differentiation, and apoptosis in embryonic development.  MicroRNA 140 has been found to inhibit PDGF receptor alpha mediated attraction of cranial neural crest cells to the oral ectoderm in zebrafish.  Another function of miRNAs is as effectors of signaling mediators which govern cellular events essential for orofacial development.  Another study found that of 588 murine miRNA genes, 68, 72, and 66 miRNAs were expressed in developing orofacial region on gestation day 12, 13, and 14.  Extensive epigenetic studies are lacking for understanding cleft palate, but evidence suggests that epigenetic processes contribute to the etiology.

QUESTIONS

1. In mice, when does the secondary palatal processes fuse?
   1. Gestational Day 7
   2. Gestational Days 7-10
   3. Gestational Days 14-16
   4. Gestational Days 12-14
2. What developmental processes can affect palate fusion?
3. MicroRNAs have been established as regulators of what processes during embryonic development?
   1. Size of palatal processes, orientation, and fusion
   2. regulators of proliferation, differentiation, and apoptosis
   3. regulators of proliferation, orientation, and fusion
   4. size of palatal processes, differentiation, and apoptosis

ANSWERS

1. d. Gestational Days 12-14
2. Developmental processes include cell proliferation, extracellular matrix metabolism, epithelial mesenchymal transition, apoptosis, cell migration and activity of signal transduction pathways
3. b. regulators of proliferation, differentiation, and apoptosis

**Jasarevic. Sexually Selected Traits: A Fundamental Framework for Studies on Behavioral Epigenetics, pp. 253-269**

Domain 3 TT3

SUMMARY: Most behavioral differences, in the past were attributed to polymorphism in select groups of genes or physicochemical differences of unknown etiology.  More recently, behavior has also been attributed to epigenetic changes.  This paper defined epigenetic change to be a mitotically, postmitotically, or meiotically heritable change in gene expression that occurs independent of alterations in DNA sequence.  This paper also proposed that sexually selected traits are vulnerable to environmentally induced epigenetic alteration.  Prenatal exposure to gonadal sex steroid hormones can shape expression of developmental genes in brain and other organs by epigenetic control mechanisms.  Postnatal exposures to sex steroid hormones program an animal for later reproductive competition, mate choice, and parenting.  Changes in these sex steroid hormones at either time can influence later manifestation of traits.  Ornament structures and behavioral patterns in mate choice are strongly affected by current and prior developmental conditions.  The choosing mate’s choice reflects the ability to interpret these species-specific traits.  In intrasexual competition, differences in competition components may be governed by prenatal or perinatal exposure to sex hormones.  Sex hormones may trigger epigenetic changes during early and postnatal development and may transmit information that leads to vital reproductive behaviors at maturity.

Exposure to endocrine disrupting compounds (EDC), such as chemicals like bisphenol A (BPA) and vinclozolin during development may change epigenetic processes.  BPA exposure can induce epigenetic changes in DNA methylation.  It has been found that BPA can disrupt other adult traits such as learning, memory, anxiety behaviors, and parental behaviors.  A study in deer mice found that BPA exposed females preferred control males to BPA exposed males.  DNA methylation of CpG islands is recognized as controlling gene expression in cell types including neuron cells of the brain, and most likely contributes to sexual behaviors. Sex hormones may cause masculinization and feminization in the rodent brain through DNA methylation of genes encoding estrogen receptors.  It was seen that an agonist for one of these receptors increased cell death in the anteroventral periventricular nucleus with enhanced cell survival in sexually dimorphic nucleus of the preoptic area leading to loss of female sex receptivity.  Another way readout status of genes can be programmed in a heritable manner is with modification of histone proteins that bind DNA.  Three best-characterized modifications to histone proteins are acetylation, deacetylation, and methylation.  Sex steroids can directly or indirectly alter histone proteins.  Treatment of neonatal mice to increase histone protein 3 acetylation in the brain, caused bed nucleus of the stria terminalis to have cell numbers comparable to females.  This paper proposed that sexually selected traits could be useful in examining the evolution of sex steroid-induced epigenetic mechanisms since traits are dependent on pre and postnatal sex steroid hormone exposure.

QUESTIONS

1. How does this paper define epigenetic change?

2. Bisphenol A exposure can induce epigenetic changes in

a. RNA methylation

b. DNA methylation

c. Histone proteins

d. Bed nucleus of the stria terminalis

3. What are the three best-characterized modifications to histone proteins?

ANSWERS

1. This paper defined epigenetic change to be a mitotically, postmitotically, or meiotically heritable change in gene expression that occurs independent of alterations in DNA sequence.

2. b. DNA methylation

3. The three best-characterized modifications to histone proteins are acetylation, deacetylation, and methylation

**Niculescu. Nutritional Epigenetics, pp. 270-278**

SUMMARY: Within the last two decades, significant progress has been made in understanding the importance of epigenetic mechanisms in the regulation of gene expression as a consequence of gene-environment interactions. Nutrition, among many other environmental factors, is a key player that can induce epigenetic changes not only in the directly exposed organisms but also in the subsequent generations through the transgenerational inheritance of epigenetic traits. This article aims to provide insights into the usefulness of the mouse model for epigenetic studies involving nutrition as well as the inherent limitations when compared with epigenetic phenomena in humans. Mice are one of the most versatile models for nutrition and epigenetic studies because of several features, such a short life-span, relative low cost for generating samples, the existence of well-characterized genetically engineered lines, the detailed sequencing of genomes, and the relative similarity of their metabolic process to human metabolism. However, several limitations have to be acknowledged, such as the different location of genes on the chromosomes (and hence possibly different consequences of some epigenetic alterations), differences in the epigenetic patterns established during late embryogenesis, and possible epigenetic differences associated with cellular senescence caused by the different structure of telomeres when compared with humans. All these aspects have to be carefully analyzed when deciding whether a mouse model should be considered for a study in nutrition and epigenetics. Consequently, the results obtained from mouse studies should be carefully interpreted regarding their relevance to humans.

QUESTIONS

1. Epigenetics refers to molecular events, other than changes in the DNA sequences. Which of the following are epigenetic mechanisms?

a. DNA methylation

b. Histone modifications

c. Nucleosome positioning along DNA

d. Modulation of gene expression by non-coding RNA

e. All of the above

2. DNA methylation is a biologic process that consists of the covalent addition of methyl groups to DNA. In eukaryotes, DNA methylation occurs mainly at the 5 position of the cytosine ring (5-methylcytosine) that is followed by a guanine nucleotide (CpG sites). T or F.

3. Specific DNA methylation patterns contribute decisively to the establishment of specific cellular phenotypes, which become stable in differentiated cells. T or F.

4. DNA methylation is always a/an \_\_\_\_\_\_\_\_\_\_\_\_chemical process, loss of methylation (DNA demethylation) can occurs\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

a. Active/passively or actively

b. Passive/passively or actively

5. Posttranslational modifications of histones occurring especially at their flexible tail regions. T or F.

6. MicroRNAs are coding RNAs up to 25 nucleotides in length that regulate gene expression through RNA interference. T or F.

7. The main effect of microRNAs on gene expression is gene silencing by means of posttranscriptional repression (small interfering RNA). T or F.

8. The versatility of nutritional epigenetic studies in mice is related to:

a. Short life span

b. Sample size

c. Genetics

d. Cost

e. Diet

f. Assay methods

g. All of the above

9. Genetic background plays an important role when deciding which mouse strains is best to use. Exposure to the following contaminant (1,3-butadiene) for two weeks identified major (DNA methylation increased) epigenetic differences in which of the following strains:

a. CAST/EiJ, A/J

b. NOD/LtJ, WSB/EiJ

c. PWK/PhJ, 1291/SvImJ, C57BL/6J

10. Epigenetic involvement in obesity and metabolic syndrome has become of great interest because of the hypothesized role of early life nutrition in the outset of chronic disease later in life and in aging. The most used diets were the \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ diets administered either before and during gestation or postnatally.

a. High protein

b. High carbohydrate

c. High fat

d. High fat and high carbohydrate

11. The\_\_\_\_\_\_\_\_\_\_ model, as opposed to the \_\_\_\_\_\_\_\_\_\_\_\_ model, benefits from better support in the availability of assay platforms and from numerous transgenic lines.

a. Mouse/rat

b. Rat/mouse

c. Mouse/guinea pig

d. Rat/guinea pig

12. Epigenetic phenomena are dynamic during the life cycle of mammals, the selection of the proper exposure period to nutritional cues is essential for the successful accomplishment of epigenetic studies. T or F.

13. The use of same mouse model in two different studies in which opposite epigenetic outcomes were reported for the same gene according to the exposure period. The opposite results point toward the importance of different development periods for the establishment of nutrient-driven epigenetic patterns. T or F.

14. Author statement. The following questions should be first addressed when designing a nutrition experiment with an epigenetic component:

a. How relevant is mouse model in comparison with humans for that particular metabolic, molecular, or developmental process?

b. Which tissue(s) is (are) the most relevant in regard to hypothesized outcome?

c. Which mouse strain or transgenic model (if available) would be best suited?

d. What should be the period exposure?

e. If relevant which parental lineage (maternal vs. paternal) should be exposed to the nutritional cue?

ANSWERS

1. e

2. T

3. T

4. a

5. T

6. F [non-coding]

7. T

8. g

9. c

10. c

11. a

12. T

13. T

**Gudsnuk and Champagne. Epigenetic Influence of Stress and the Social Environment, pp. 279-288**

**Primary Species:** Mouse (Mus musculus) and Rat (Rattus norvegicus)

**Domain 3:** Research; T3: Design and conduct research; K6: Characterization of animal models (e.g., phenotyping, behavioral assessment)

**SUMMARY:** Rodent models demonstrating the effects of Prenatal stress, Maternal separation and/or deprivation, Maternal care, Infant abuse, Juvenile social environment, and Adult social stress have significantly advanced our understanding of the mechanistic pathways through which environmental experiences induce long-term biobehavioral consequences. In particular,these studies provide evidence for the role of epigenetic mechanisms, such as DNA methylation and histone modifications, in mediating the effects of early- and later-life experiences.

The plasticity in these mechanisms, which was once thought to be limited but has been revealed to be highly responsive to physiological and neurobiological experiences may account for the dynamic changes in neuroendocrine function that accompany variation in the quality of the social environment.

In addition to being sensitive to these cues, it appears that epigenetic mechanisms can also confer the stability of environmentally mediated effects on various outcome measures—a finding that raises challenging questions about the specific molecular pathways that mediate plasticity versus stability. Although the HPA response to stress has been the focus of these studies, stress and social experiences may have a profound impact on a diverse range of phenotypic outcomes, including cognitive ability and reproductive behavior. Moreover, there is increasing evidence for the transgenerational impact of early-life experiences and the involvement of epigenetic pathways in these effects. The development of laboratory rodent models to study epigenetic effects has been instrumental to the study of epigenetic pathways in humans and will continue to serve as a critical tool for developing hypotheses about the timing, targeting, and persistence of environmentally induced effects. These studies also highlight the importance of contextual variables to the health and welfare of laboratory animals and illustrate the molecular mechanisms through which the quality of social experiences can shape development and impact variation in behavior.

Plasticity in neurobiological pathways regulating stress responsivity, cognition, and reproductive behavior is apparent during the prenatal period and continues into adulthood, suggesting a lifelong sensitivity to environmental cues. Recent evidence suggests that dynamic epigenetic changes—molecular modifications that alter gene expression without altering the underlying DNA sequence account for this plasticity.

QUESTIONS

1. T/F. Consideration of the sensitivity of laboratory animals to environmental cues may be an important factor in predicting long-term health and welfare.

2. T/F. The quality of social interactions or the experience of stress cannot induce neuroendocrine effects but it will have influence social behavior, reproductive success, cognitive ability, and stress responses.

3. T/F. Although it is clear that during prenatal and early postnatal development there is a period of enhanced sensitivity to these environmentally induced effects, there may not be plasticity beyond infancy that extends into adolescence and adulthood.

4. T/F. Although plasticity is certainly evident during prenatal and postnatal periods of development, the impact of later-life experiences occurring during juvenile development or in adulthood is increasingly apparent.

5. T/F. Although plasticity in epigenetic pathways was initially thought to be limited to the early stages of embryogenesis, it is becoming increasingly evident that experiences occurring across the lifespan are capable of inducing epigenetic variation.

6. T/F. In the context of studies on the influence of social interactions and stressors, epigenetic variation, likewise, has been associated with changes in gene expression and phenotype during the infant stages of development and not in later stages of development.

7. T/F. Thus, housing conditions within the laboratory and manipulations of those conditions during experimental protocols may induce molecular changes followed by a long-term impact on social and anxiety-like behaviors.

ANSWERS

1. True

2. False (can induce neuroendocrine effects)

3. False ( may be plasticity ...)

4. True

5. True

6. False (both stages of development)

7. True

**Ho et al. Environmental Epigenetics and Its Implication on Disease Risk and Health Outcomes, pp. 289-305**

Domain 3: Research; T1. Facilitate or provide research support; T2. Advise and consult with investigators on matters related to their research; T3. Design and conduct research

Primary Species: Mouse (Mus musculus) and Rat (Rattus norvegicus)

SUMMARY

Abstract: This review focuses on how environmental factors through epigenetics modify disease risk and health outcomes. Major epigenetic events, such as histone modifications, DNA methylation, and microRNA expression, are described. The function of dose, duration, composition, and window of exposure in remodeling the individual’s epigenetic terrain and disease susceptibility are addressed. The ideas of lifelong editing of early-life epigenetic memories, transgenerational effects through germline transmission, and the potential role of hydroxylmethylation of cytosine in developmental reprogramming are discussed. Finally, the epigenetic effects of several major classes of environmental factors are reviewed in the context of pathogenesis of disease. These include endocrine disruptors, tobacco smoke, polycyclic aromatic hydrocarbons, infectious pathogens, particulate matter, diesel exhaust particles, dust mites, fungi, heavy metals, and other indoor and outdoor pollutants. We conclude that the summation of epigenetic modifications induced by multiple environmental exposures, accumulated over time, represented as broad or narrow, acute or chronic, developmental or lifelong, may provide a more precise assessment of risk and consequences. Future investigations may focus on their use as readouts or biomarkers of the totality of past exposure for the prediction of future disease risk and the prescription of effective countermeasures.

Key Words:developmental basis of disease; environmental epigenetics; epigenetic memories; exposome; histone modification; low-dose effects; susceptible windows; ten-eleven translocations; 5’-hydroxylmethylcytosine

Summary definitions, ideas, etc.

* Environmental epigenetics: How epigenetics explains the variability in the risk and severity of environmental disease.
* Environmental diseases: diseases that are caused by factors that exist in our external environment. (To reduce the complexity of this review, the authors avoid “those caused by lifestyle factors linked to stress, abuse, addiction, alcoholism, and metabolic changes.”
* Three major (“best-studied”) epigenetic events that shape the epigenome:
  + DNA methylation
  + Histone modifications
  + Feedback and feed-forward circuitry of microRNAs (miRNA)
* Cytosine methylation and demethylation: oxidative stress caused by environmental factors has been proposed to regulate the degree of DNA hydroxylmethylation at promoters of specific genes.
* Histones: are the major proteins that facilitate the assembly of DNA into nucleosomes, the basic units of chromatin. The association variation between histones and DNA that is wrapped around it leads to differences in DNA transcription, replication, recombination and “higher-order” chromosome organization.
* MicroRNAs: are a class of small, noncoding RNAs transcribed from their cognate genes or derived from introns or exons of other genes. They bind to the 3’ end of gene transcripts and initiate messenger RNA degradation or suppression of protein translation. This action find-tunes the level of gene transcription and translation, creating checks and balances within and across gene networks, and serving as regulatory “hubs” for phenotype expression. They also can influence the expression of other epigenetic regulators, including DNA methylation and histone-modification enzymes.
* Environmental Epigenetic Changes are dependent on dose and duration of exposures, as well as age/life stage/sex of the exposed individual
  + Acute, low-dose exposure
  + Low-dose, chronic exposures
* Environmental Factors Shown to Trigger Epigenetic Events and Affect Disease States
  + Some of the epigenetic marks may serve as biomarkers of exposure or prognostic markers of disease risk and progression
  + Examples:
    - Endocrine disruptors: diethylstilbesterol (DES); bisphenol A (BPA); phytoextrogens; cinclozolin (a fungicide)
    - Tobacco smoke
    - Polycyclic aromatic hydrocarbons (PAHs): present in coal, crude oil, tar deposits, and from burning of fossil fuels, forest fires and volcanic eruptions.
    - Infectious pathogens: *Helicobacter pylori*, human papillomavirus, hepatitis B virus, Epstein-Barr virus.
    - Outdoor pollutants: particulate matter, diesel exhaust particles, zoon and other outdoor pollutants
    - Indoor pollutants: dust mites, pet dander, insects, fungi and other indoor allergens
    - Heavy metals: cadmium, chromium, arsenic, nickel, methyl mercury, lead, organotin.
  + Important points:
    - Most environmental exposures involve mixtures – very different from the typical classical toxicology approach to studying health effects of environmental agents.
    - The environmental exposure to various elements over the course of an individual’s life is referred to as their “exposome”. The composition and temporal sequence of these exposures are complex, with the interactions having potential to be synergistic, antagonistic, combinatorial, attenuating, summating, subtractive, opposite and more. The consequences can only be viewed in the entirety of the exposome; with no single component of the exposome (a single exposure, a window of susceptibility, a dose or a route, the exposure frequency or a single target) able to predict the disease or health outcome.

QUESTIONS

1. Define environmental epigenetics
2. Define environmental disease
3. List three major (“best-studied”) epigenetic events that shape the epigenome
4. What are some general goals for the study of environmental epigenetics with regard to the usefulness of being able to identify epigenetic biomarkers?
5. List some general types of environmental factors (and when you can, a specific example or two) that are known to trigger epigenetic events and affect disease states.

ANSWERS

1. Environmental epigenetics: How epigenetics explains the variability in the risk and severity of environmental disease.
2. Environmental diseases: diseases that are caused by factors that exist in our external environment.
3. Three major (“best-studied”) epigenetic events that shape the epigenome:
   1. DNA methylation
   2. Histone modifications
   3. Feedback and feed-forward circuitry of microRNAs (miRNA)
4. An awareness of the presence of specific epigenetic biomarkers may provide evidence for exposure to certain epigenetic factors, or they may serve as prognostic markers of disease risk and progression
5. Some general types of environmental factors (with a few examples) that are known to trigger epigenetic events and affect disease states include:
   1. Endocrine disruptors (e.g. – DES, BPA, cinclozolin)
   2. Tobacco smoke
   3. Polycyclic aromatic hydrocarbons (PAHs) (e.g. - products of fossil fuel burning, volcanic eruptions)
   4. Infectious pathogens (e.g. – *Helicobacter pylori*, human papillomavirus, HBV, EBV)
   5. Outdoor pollutants ( e.g. – diesel exhaust particles, ozone)
   6. Indoor pollutants (e.g. allergens such as dust mites, pet dander, fungi)
   7. Heavy metals (e.g. cadmium, chromium, arsenic, nickel, methyl mercury, lead, oranganotin)

**Ganu et al. Early Origins of Adult Disease: Approaches for Investigating the Programmable Epigenome in Humans, Nonhuman Primates, and Rodents, pp. 306-321**

Domain 3: Research; T1. Facilitate or provide research support; T2. Advise and consult with investigators on matters related to their research

SUMMARY: The in utero environment can influence metabolic disease risk later in life without changing the DNA sequence by modulating gene expression through epigenetic mechanisms.  Historical and ongoing population based human studies have shown a link between maternal nutrition and a variety of health factors in their adult offspring including adiposity, glucose tolerance, and risk of coronary heart disease. Mechanisms for epigenetic changes include histone modification via acetylation, methylation, ubiquitylation, and sumoylation. These histone modifications are thought to act as guides for DNA methylation, which in turn affects gene expression. A number of process can lead to histone modifications and differential methylation patterns that are clinical significant. Genomic imprinting is an epigenetic phenomenon that silences expression of paternally or maternally derived alleles. Prader-Willi syndrome and familial pseudohypoparathyroidism type 1b are caused by disruptions in genomic imprinting.  The authors promote nonhuman primates as model organisms for the epigenetic research due to their similarities to humans in regards to metabolic and placental physiology. In addition, human mental health disorders have been associated with histone modifications (schizophrenia) and dysregulation of methylation (depression).

Factors that can shape the fetal epigenome:

* Maternal nutrition - “one carbon metabolism,” protein restriction, high fat diet exposure.
  + One carbon metabolism = Methionine is an essential amino acid that is converted to S-adenosyl methionine, which acts as a single-carbon donor and gets converted into S-adenosyl homocysteine.
* Environmental exposures: tobacco, alcohol
* Maternal behavior such as grooming behavior in rodents and gestational depression in humans
* Proposed role for maternal microbiome given current knowledge of gut microbiota and metabolism

This review paper summarizes important studies regarding in utero factors affecting the epigenetic regulation and programming for adult diseases with an emphasis on the importance of nonhuman primate models in this field of research.

QUESTIONS

1. All of the following are histone modifications EXCEPT:
   1. Acetylation
   2. Alpacylation
   3. Methylation
   4. Ubiquitylation
2. T/F: One carbon metabolism is associated with the essential amino acid leucine.
3. T/F: Genomic imprinting is an epigenetic phenomenon that silences expression of paternally or maternally derived alleles.
4. Development of which of the following diseases is influenced by epigenetic mechanisms?
   1. Prader-WIlli syndrome
   2. Schizophrenia
   3. Familial pseudohypoparathyroidism type 1b
   4. Depression
   5. All of the above

ANSWERS

1. B

2. F, methionine

3. T

4. E

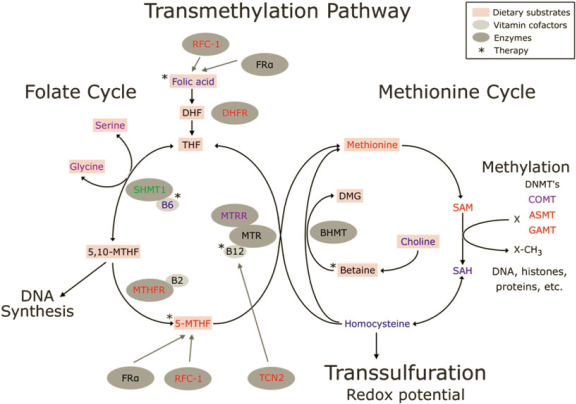
**Schaevitz and Berger-Sweeney. Gene-Environment Interactions and Epigenetic Pathways in Autism: The Importance of One-Carbon Metabolism, pp. 322-340**

Primary Species: Mouse (Mus musculus) and Rat (Rattus norvegicus)

Domain (Task): D1 (T 2), D2, (T1-3)

SUMMARY: This review focuses on DNA methylation as a key epigenetic mechanism in Autism and Autism Spectrum Disorders (ASDs). Global hypomethylation of DNA has been reported in both parents of autistic children and the children themselves. However despite global hypomethylation, it has been found that genes implicated in ASDs were hypermethylated, leading to down regulation of gene expression. One-carbon (C1) metabolism is comprised of three interconnected pathways, two of which form the transmethylation pathway (Figure 1). The C1 pathway plays a critical role in establishing and maintaining DNA methylation patterns and this makes it a likely candidate pathway to regulate epigenetic processes in ASDs. This review focuses on how altering the C1 metabolic function through genetic and environmental factors (diet) may lead to aberrant DNA methylation and increase susceptibility to ASDs. Findings are however that no single dietary or genetic factor within the C1 metabolic pathway was found to reliably contribute to susceptibility across studies but a number of different metabolic and genetic abnormalities were apparent. Our understanding of how C1 metabolic dysfunction may affect susceptibility to ASD’s is still limited. Many researchers hypothesize that recent changes in nutrition intake and in the frequency of genetic polymorphisms associated with C1 metabolism may account for the recent explosion in the incidence of ASDs. Specifically, an increase in the diagnosis of ASDs occurred concurrently with mandated fortification of food with folic acid in the United States, while at the same time pregnant women are advised to take folic acid. Studies in rodents have shown that excess dietary folic acid is associated with altered DNA methylation patterns and phenotypic behavior reminiscent of ASDs. Knockout mice have also been demonstrated to recapitulate the C1 Metabolic abnormalities reported in peripheral tissues in humans. Studies in mouse models of ASD’s support the potential benefits of nutraceutical interventions in prevention of development of ASDs.

Given the central position of C1 metabolism in regulating the epigenome and its ability to be modulated by a large number of genetic and environmental factors, it is an ideal candidate pathway in development disorders of heterogeneous etiology such as ASD. Thus, by understanding the gene-diet risk factors and the critical time windows during which C1 metabolic function affects neuron development, there is the significant potential to prevent, not just treat ASDs.

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**Figure 1:** Folate and methionine cycles in one-carbon (C1) metabolism. Shown is a diagram depicting many of the important substrates, vitamin cofactors, and enzymes that participate in the C1 metabolic pathway. Abnormalities in a number of the metabolites and enzymes within the transmethylation pathway have been reported in children with autism spectrum disorders (ASDs). An overview of the abnormalities reviewed herein are indicated by the color of the letters. Black letters indicate that no significant abnormalities have been noted in that metabolite or enzyme in ASDs. The concentrations of metabolites written in red are frequently low in children with ASDs. The concentrations of metabolites in blue are frequently elevated, whereas the concentrations of metabolites in purple are lower in some studies and elevated in others. For the enzymes, color represents risk for ASDs in individuals carrying polymorphisms in the gene that encodes the enzyme. Specifically, enzymes written in red are associated with increased susceptibility for ASDs. Enzymes in green are protective, whereas risk for ASDs is inconsistent across studies for enzymes in purple. Folate receptor \_ (FR\_), reduced folate carrier (RFC-1), and transcobalamin II (TCN2) are transport proteins. Gray arrows point to the vitamin transported into the cell by each protein. Abbreviations: 5-MTHF, 5-methyl tetrahydrofolate; 5,10-MTHF, 5,10-methylene tetrahydrofolate; ASMT, acetylserotonin-*O-*methyltransferase; *COMT*, catechol-*O*-methyltransferase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; DNMTs, DNA methyltransferases; GAMT, guanidinoacetate methyltransferase; MeCP2, methyl-CpG binding protein 2; MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; SAH, *S*-adenosyl-homocysteine; SAM, *S*-adenosyl-methionine; SHMT-1, serine hydroxylmethyltransferase; THF, tetrahydrofolate.

QUESTIONS

* + - 1. Choose the correct completion of this statement. DNA methylation involves:

a. The addition of methyl groups to DNA nucleotides and generally impedes transcription of genes

b. The addition of a methyl or acyl group to nucleotides and is not essential for normal development

c. Is NOT considered to be an epigenetic mechanism

d. Is not considered to be influenced by environmental factors

1. One-Carbon (C1) metabolism is considered to be a key candidate pathway in the regulation of epigenetic processes that lead to the development of ASDs because:
2. C1 metabolism plays a critical role in establishing and maintaining DNA methylation patterns
3. C1 metabolism may be modulated by a large number of genetic and environmental factors, making it an ideal candidate pathway in development disorders of heterogeneous etiology such as ASD
4. A and B

ANSWERS

1. a
2. c

**Skaaer et al. The Human Imprintome: Regulatory Mechanisms, Methods of Ascertainment, and Roles of Disease Susceptibility, pp. 341-358**

Primary Species: Mouse (Mus musculus)

Domain (Task): D1 (T 2), D2, (T1-3)

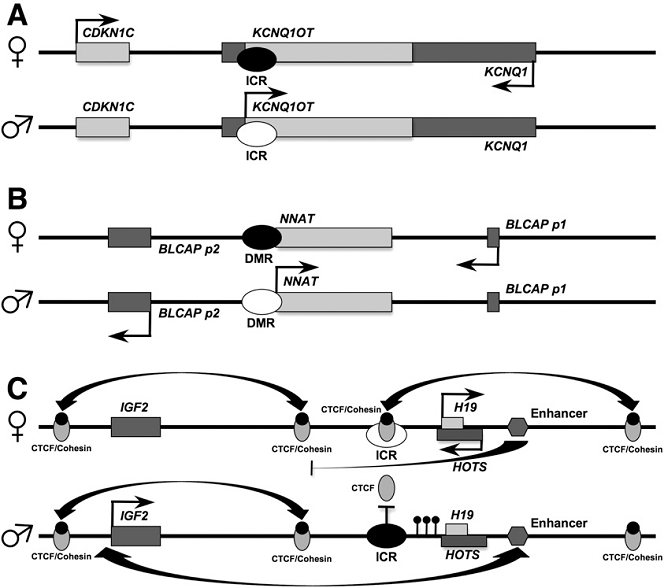
SUMMARY:  Imprinted genes are a subset of the genome that exhibits expression in a parent of origin dependent fashion.  This is controlled by epigenetic markers which are established in gametogenesis and early embryonic development and persist in all somatic cells throughout life.  These cis-acting epigenetic elements are defined as imprintomes.  The imprintomes contain DNA methylation and histone modifications that regulate expression affecting promoter accessibility, chromatin structure, and chromatin configuration.  A significant number of human imprinted genes are implicated in complex diseases such as cancer and psychiatric disorders.

Genomic Imprinting:  Parent-of-origin–specific transcriptional control has only been seen in flowering plants and placental mammals, marsupials and eutherians

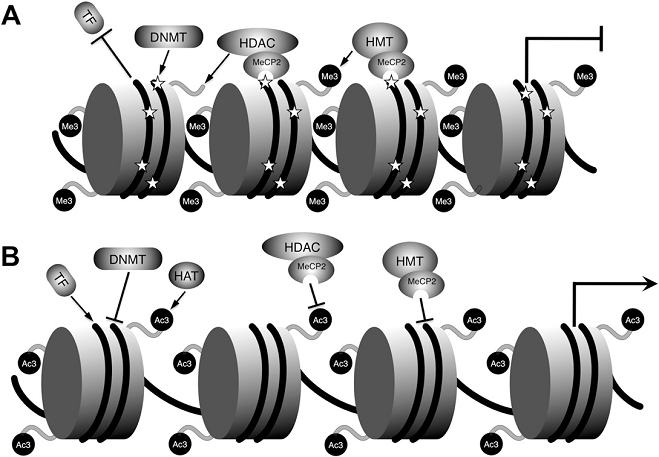
both undergo X chromosome inactivation in females. There is even some degree of X-linked gene inactivation in the platypus, a nonplacental monotreme (egg-laying mammal).  The evolution of imprintomes is still under debate but it is thought that it is a competition between mother and fetus with the paternal germline trying to maximize fetal growth and the maternal strategy to restrict growth to give equal chance of survival to all offspring.

Species-Specific Imprintomes: Understanding the human imprintome has become a greater necessity because of the demonstration of species-specific differences in imprinting establishment and regulation. There is evidence that mouse and human imprinted gene repertoires show only about 30% overlap so we can’t rely on animal models to distinguish all of these associations.

Imprintome Establishment: Imprintome creation involves both DNA methylation and histone modifications, with methylation being more conducive for study for a variety of reasons the best reason for studying DNA methylation is that it may be the most consistent marking of the imprint control regions (ICRs1) and the genes they regulate.



**Figure 1:** Mechanisms of monoallelic gene expression controlled by parent-of-origin imprintome elements. (A) Differential germline methylation controls expression of an antisense noncoding RNA that represses neighboring genes in an imprinted cluster. Example shown: *CDKN1C*/*KCNQ1*. Light oval, unmethylated imprint control region (ICR); dark oval, methylated ICR. (B) Differential methylation controls expression, and transcriptional interference affects promoter usage of oppositely transcribed genes. Example shown: *NNAT*/*BLCAP*. Light oval, unmethylated ICR; dark oval, methylated ICR; p1, efficient promoter, used in maternal monoallelic expression; p2, inefficient promoter, used in paternal monoallelic expression. (C) Parent-of-origin–specific methylation prevents CTCF binding and cohesion-mediated chromatin remodeling. Differently arranged chromatin loops allow or prevent promoter–enhancer interaction. Example shown: *IGF2/H19/HOTS*. Light oval, unmethylated ICR; dark oval, methylated ICR; filled circles, *H19* promoter methylation.



**Figure 2:** Interactions of DNA methylation and histone modifications. (A) Closed chromatin has CpG methylation (star) generated by DNA methyltransferase (DNMT) and histone methylation at H3K9, H4K20, and H2A/H4R3, with recruitment of histone deacetylase (HDAC) and methyltransferase (HMT) by methylated CpG by means of MeCP2. Transcription factors (TFs) are inhibited from binding, and gene expression is silenced. (B) Open, unmethylated chromatin does not recruit HDAC and HMT, and H4K3 methylation blocks DNMT binding. TFs bind unmethylated chromatin, recruiting histone acetyltransferase (HAT), which promotes transcriptional activation.

Imprintome Dysregulation in Diseases and Neurologic Disorders

Developmental Disorders:  Beckwith-Wiedemann syndrome (BWS1) is characterized by fetal overgrowth and childhood tumors, showing similarities to the overgrowth of mice expressing both copies of *Igf2* due to *H19* deletion.

The Imprintome and Cancer:  Imprintome dysregulation of *IGF2* associated with Wilms’ tumors has already been mentioned, and at least 24 other known imprinted

human genes show altered expression or methylation in tumors. Significantly, multiple imprinted genes have tumor-suppressive functions, such as *ZAC*/*PLAGL1*, *BLCAP*, *RB1*, and *TP73*. *IGF2* is the imprinted gene with the most extensively documented cancer involvement, with loss of imprinting observed in most cancer types examined.

The Imprintome and Psychiatric Disorders:  Connections have been found linking increased relative effects of maternally expressed genes in psychotic spectrum conditions, such as schizophrenia, bipolar disorder, depression, PWS, and Klinefelter syndrome.  Conversely, autism spectrum conditions, including autism, Asperger syndrome, Rett syndrome, Turner syndrome, AS, and BWS, often show increased effects from paternally expressed genes. Psychotic spectrum conditions are associated with undergrowth in brain development, whereas autism spectrum conditions can involve brain and body overgrowth.

Nothing in human development and disease makes sense except in the light of the imprintome because it plays such a fundamental role in normal fetal growth and development.  It is highly important to get a proper human imprintome map to assist with diagnosis and prevention of human disease.

QUESTIONS (T/F)

1. The major impact of imprintomes is the establishment of paternal specific influence.
2. Methylation is not important in the imprintome theory of altering gene expression.
3. Imprintome dysregulation has been implicated in developmental disorders, cancer and psychiatric disorders.

ANSWERS

1. T
2. F
3. T

**Virani et al. Cancer Epigenetics: A Brief Review, pp. 359-369**

SUMMARY: Cancer is a disease that results from the successive accumulation of genetic and epigenetic alterations. Despite intense study, many unanswered questions about the nature of the contribution of epigenetic changes to carcinogenesis remain. In this review, we describe principles of epigenetics as they relate to our current understanding of carcinogenesis. There are a number of in vivo models of specific pathways of carcinogenesis that are very useful for the characterization of epigenetic mechanisms that link environmental exposures or genetic susceptibility and cancer progression. Because epigenetic alterations are thought to be reversible, they offer great promise for treatment of cancer. The use of animal models to evaluate the effects of decitabine and zebularine has elucidated the mechanisms of action and indicated the potential for these types of treatment. Ultimately, the greatest challenge lies in the integration of laboratory and epidemiologic data to best prevent and treat this deadly disease.

QUESTIONS

1. CpG sites are unevenly distributed throughout the genome, concentrating in repetitive sequences such as tandem and interspersed repeats, distal gene regulatory regions, and CpG islands. T or F.

2. DNA methylation is highly regulated in cancer. T or F.

3. A major epigenetic mechanism is the methylation of the fifth carbon of a cytosine nucleotide to create 5-methylcytosine (5mC1). The methyl group of 5mC lies in the major groove of the double helix and can interfere with transcription factor binding to prevent gene expression. T or F.

4. The whole genome enzymatic digests with high-performance liquid chromatography were used to show that overall 5mC content is directly associated with tumor progression. T or F.

5. Alu and LINE-1 elements are retrotransposons. Which are?

a.    RNA intermediates elements

b.    Genetic elements with the ability to amplify themselves

c.    Genetic elements without the ability to amplify themselves

d.    RNA completes elements

6. In normal tissues, LINE-1 and Alu elements are silenced through DNA methylation; these elements are hypomethylated in cancer. T or F.

7. Hypermethylation of promoter regions leads to activation of silenced genes, promoting aberrant gene expression, disruption of normal cellular processes, and overall genomic instability. T or F.

8. DNA methylation is regulated by DNA methyltranferases (DNMTs) that act as the methyl donors to the cytosine residue. Five members of the DNMT family have been discovered and only DNMT1, DNMT3a, and DNMT3b are known to contribute to the global pattern of cytosine methylation. DNMT overexpression seems to be a common characteristic of tumors, although only DNMT1 and DNMT3a/b are implicated in tumorigenesis. T or F.

9. A CpG island is generally defined as a 1000-kb stretch of DNA with GC content grater that 50%. T or F.

10. CpG islands occupy approx. 20% of human genome promoters, most of which are constitutively expressed genes. T or F.

11. The genomic regions that are targeted for hypermethylation tend to be CpG islands. Contrary to individual CpG sites throughout the genome in intergenic regions, CpG islands are usually unmethylated in normal cells, regardless of gene expression. However, there are very specific instances in which CpG islands are methylated in normal cells, such as in imprinted genes and X-chromosome inactivation. T or F.

12. The cancer cell genome is characterized by hypermethylation of CpG islands in promoter regions. The hypermethylation of CpG islands promotes the progression of tumorigenesis by silencing tumor suppressor genes. T or F.

13. Promoter hypermethylation is often an early event in tumorigenesis. T or F.

14. The basic chromatin unit is the nucleosome, which consist of a protein octamer containing pairs of each of the four core histone protein (H2A, H2B, H3, H4). T or F.

15. There are two common higher levels of nucleosome organization that are defined by the level of compaction of the nucleosome structures the euchromatin and the heterochromatin. Euchromatin is densely packed and typically represents transcriptionally active genic regions due to the increased accessibility of the DNA in the nucleosome structure. Heterochromatin is loosely packed, with intense cytological nuclear staining due to the high density of nuclear proteins. T or F.

16. Histone acetylation has been widely shown to regulate transcription and the methylation at specific histone tail residues is associated with both transcriptional activation and repression. T of F.

17. Histone methylation can be associated with transcriptional activation or repression based on the specific residue methylated, histone acetylation is strongly associated with transcriptional activation. T or F.

18. The dynamic states of chromatin conformation are controlled by histone acetyltransferases (HATs), also known as K-acetyltransferases, and histone deacetylases (HDACs). The normal in vivo role of HATs and HDACs is often obfuscated in cancer, leading to an abnormal chromatin phenotype. T or F.

19. Exposure to 3-methylcholanthrene and diethylnitrosamine has been known to induce lung tumors in animal models. T or F.

20. A mouse model of acute lymphoblastic leukemia, *Il15* transgenic FVB/NJ mice, is used to identify epigenetic alterations specific to leukemia progression. T or F.

21. Methylation of tumor suppressor genes is a common characteristic of tumorigenesis then demethylating agents such as 5-azacytidine, decitabine, and zebularine have been studied in various animal models. Mice induced to develop oral cavity carcinogenesis were treated with 5-azacytidine and exhibited reduced lesions compared with untreated mice. T or F.

22. The acute lymphoblastic leukemia xenograft in SCID mouse model treated with vorinostat, an HDAC inhibitor, reinstated gene expression of *BIM*, a tumor suppressor, which is silenced in lymphoid malignancies. T or F.

Authors Conclusions: The greatest challenge to cancer researchers is the integration of human and animal data to realize the translational potential of findings. Although epidemiologic studies have identified dietary and environmental factors as associated with risk of cancer, animal models can identify the mechanisms and temporal relationship between these factors and carcinogenesis. Translational research that integrates results from human and animal studies will provide insight into the temporal nature of epigenetic dysregulation during tumor initiation and progression and into how environmental and dietary exposures influence the epigenetic phenotype of a tumor. Additionally, these studies will determine which cancer subtypes are susceptible to chemotherapy that influences epigenetic state, including DNA demethylating agents, HDAC inhibitors, or a number of the other promising epigenetic therapies currently in preclinical and clinical trials. Molecular characterization of tumor progression, including genetic and epigenetic profiles, is a key step in the development of individualized treatment modalities and personalized cancer therapy.

ANSWERS

1. T

2. F (is highly dysregulated)

3. T

4. F (5mC content is inversely associated with tumor progression and almost every type of cancer has been shown to have an overall deficiency of 5mC compared with normal tissue

5. b

6. T

7. F (hypomethylation)

8. T

9. T

10. F 60%

11. T

12. T

13. T

14. T

15. F (euchromatin is loosely packed and heterochromatin is densely packed)

16. F (histone methylation)

17. T

18. T

19. T

20. T

21. T

22. T

**Harris. Animal Models in Epigenetic Research: Institutional Animal Care and Use Committee Considerations across the Lifespan, pp. 370-376**

Domain (Task): D1 (T 2), D2, (T1-3)

SUMMARY:Epigenetics is defined by heritable changes in gene expression that are not caused by primary DNA sequence. Examples of such modifications are [DNA methylation](http://en.wikipedia.org/wiki/DNA_methylation) and [histone modification](http://en.wikipedia.org/wiki/Histone_modification), both of which serve to regulate gene expression without altering the underlying [DNA sequence](http://en.wikipedia.org/wiki/DNA_sequence). The epigenetic mechanisms are involved in stem cell differentiation, embryogenesis, developmental biology, cancer, metabolic disorders, neurodegenerative diseases and many others. The epigenetic is not limited to any single phase of an organism’s life course and can have acute, chronic and multigenerational significance. The environmental, chemical, toxic, therapeutic and psychological stressors used in animal studies to elicit epigenetic changes. Generally, regulatory regions that are methylated are silenced. One of the best characterized and most useful experimental animal models for the study of epigenetic regulation is Avy viable yellow agouti mouse. In this model DNA methylation and histone modification determine the expression of hair follicle pigment (pheomelanin and eumelanin), the characteristic yellow coat color. Hypomethylation results in constitutive ectopic agouti expression and yellow coat color. Increasing level of DNA methylation results in progressive molting until mice appear completely brown. The Avy phenotype include obesity (yellow obese syndrome), prone to cancers and diabetics. Another mouse model for epigenetic study is Axin1-fused (Axin1fu) which exhibit a range of tail abnormality from minor wavy to kinking of the tail. Complete hypomethylation results in the most severe kinked tail morphology. In pregnant mouse diet rich in foliate, methionine, choline and vitamin B12 can rescue this abnormal trait. Institutional Animal Care and Use Committee (IACUC) should exercise due diligence in making sure that animals and their offspring are adequately monitored for possible pain and disabilities.

QUESTIONS

1. T/F. Epigenetics is defined by heritable changes in gene expression that are caused by primary DNA sequence
2. The epigenetic mechanisms are involved in which of the following:
   1. Stem cell differentiation
   2. Embryogenesis
   3. Cancer
   4. Metabolic disorders
   5. All of the above
3. Which of the following mouse strain is the best characterized and most useful experimental animal model for the study of epigenetic regulation?
   1. MDX black mouse
   2. Avy viable yellow agouti
   3. BM4mu
   4. None of the above
4. T/F. Generally, regulatory regions that are methylated are silenced.
5. The epigenetic mechanism usually have which of the following manifestation
   1. Acute
   2. Chronic
   3. Multigenerational
   4. All of the above
   5. None of the above

ANSWERS

1. F
2. e
3. b
4. T
5. d