Editorial

Epigenomics

The Agouti mouse: a biosensor for environmental epigenomics studies investigating the developmental origins of health and disease

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Keywords: A^{vy} mouse • DNA methylation • diet • environmental epigenomics • epigenetics • parity

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The field of epigenetics continues to grow exponentially, doubling approximately every 2 to 3 years [1]. Concomitant with this growth is an increased interest in using the agouti viable yellow (A^{vy}) isogenic mouse for environmental epigenomic studies investigating the developmental origins of health and disease. The *Agouti* gene in this mouse model is metastable because of a retroviral intracisternal A particle (IAP) insertion upstream of the *Agouti* transcription start site [1,2]. Metastable genes are highly variable in their expression. This results from stochastic allelic changes in the epigenome rather than mutations in the genome.

The degree of IAP methylation at this alternative promoter in the proximal end of the IAP varies dramatically among individual animals, causing a wide distribution in coat color, ranging from brown (i.e., methylated IAP) to yellow (i.e., unmethylated IAP); mottled A^{vy} mice are epigenetically mosaic (Figure 1) [3,4]. Furthermore, mice with any yellow fur become obese because of ectopic production of the agouti protein. They are also more prone to developing diabetes and cancer than the brown pseudo agouti mice that are of normal weight [1,5,6].

This mouse model has been successfully used as a biosensor for detecting the effect of maternal diet [7,8], chemicals [9–11] and physical agents [12] on the epigenome (Figure 2). Because of its exquisite sensitivity to environmental exposures, careful attention must be given to experimental design for its optimum use. Environmental conditions as subtle as diet [7,8,10,12] and parity [13] can change the epigenome, thereby altering the epigenetic effects of test compounds. Thus, to appropriately interpret the results of environmental epigenomics studies utilizing the A^{vy} mouse, and to maximize its sensitivity, careful attention must be given to the experimental conditions employed.

A^{vy} mouse model

The A^{vy} mutation initially arose spontaneously in C3H/HeJ mice in 1962 [14]. Animals carrying this mutation were backcrossed with C57BL/6J mice, followed by more than 220 generations of sibling mating. This has resulted in generation of A^{vy} mice with a genetically invariant background that is 93% C57BL/6J [15].

Coat color classification

Coat color assessment should always be done by the same individual, and performed when the offspring are weaned at 22 days of age. A five coat color classification system is required to accurately assess the effect of environmental factors on the epigenome (Figure 1). Collapsing the five coat color classes into three, as some investigators have done [13], fails to make biological sense since the lean pseudoagouti mice are inappropriately grouped with the obese heavily mottled animals (Figure 1). It also reduces the sensitivity of the assay since the coat color shifts, in response to environmental exposures, are seen most significantly

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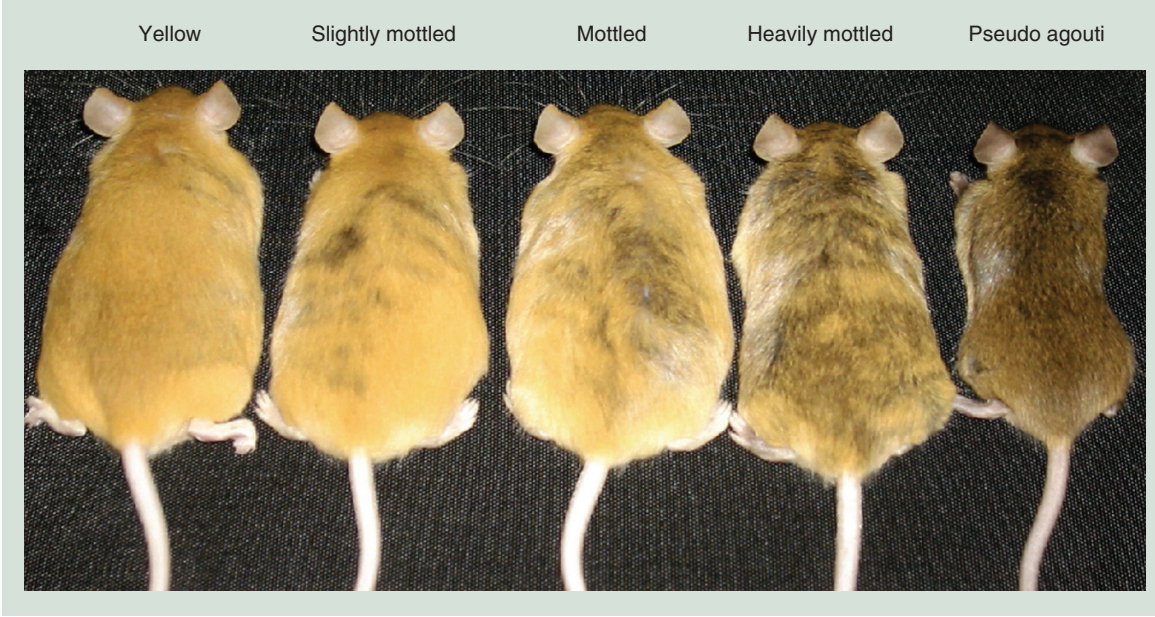

Figure 1. A^{vy} mouse coat color classification. Mouse coat color is grouped into one of five categories based on the proportion of brown-to-yellow fur: yellow (<5% brown), slightly mottled (between 5 and 50% brown), mottled (50% brown), heavily mottled (between 50 and 95% brown) and pseudoagouti (>95% brown). The isogeneic A^{vy} mice shown are of the same age and sex.
Reproduced from [8] with permission from Environmental Health Perspectives.

in the tails of the coat color distribution (i.e., the pure yellow and pure pseudoagouti mice). Thus, contracting the coat color classification system to three groups markedly reduces the ability to assess effects of the environment on coat color distribution.

Epigenetic measurements

DNA methylation [7,8,10,12] and/or histone modifications [16] in the promoter region of the IAP upstream of the transcription start site for the *Agouti* gene should always be assessed concomitantly with coat color classification. For example, the measurement of DNA methylation in A^{vy}/a mice quantifies the percentage of cells methylated, and provides an independent and unbiased corroboration of observed coat color shifts [7,8]. Furthermore, unlike coat color data, which are limited to categorical statistical analysis, calculating percent methylation allows for more sophisticated statistical tests to be performed due to the continuous percent methylation variable [7,8,10,12].

Animal breeding

Male A^{vy}/a mice of varying coat colors always need to be mated with female virgin *a/a* mice 8 to 10 weeks of age. The A^{vy} allele is passed through the paternal lineage since the epigenotype is effectively reset with paternal, but not with maternal transmission of the A^{vy} locus [17].

The female *a/a* mice should be bred only once! There are significant parity effects on the epigenome in *a/a* mice, and multiple pregnancies increase the incidence of brown offspring [13]. Importantly, this parity effect would be expected to reduce the ability to detect the hypomethylating effect of test compounds, as seen with bisphenol A (BPA) [13]. There are even parity or birth order effects in people with autism [18,19] and schizophrenia [20,21] that could also result from epigenetic dysregulation of genes involved in their etiology.

For optimal statistical analyses, 10 to 15 pregnant dams are required for each experimental dose. This results in 40 to 60 A^{vy}/a offspring per group since half of the mice have an *a/a* genotype. To utilize all the mice produced following perinatal exposures, the *a/a* offspring can be used for genome-wide methylation studies [22] or followed for life course health effects [23,24].

Matings also need to be performed within a short time span to minimize potential seasonal effects on the epigenome, and changes in the diets that could occur due to batch effects and/or food aging. If this is not possible, appropriate controls must be performed throughout the complete time span for the experimental studies. Quality control measures such as ensuring that caging, bedding and water are free of contamination from the test compounds are also imperative.

Animal exposure

This *in vivo* epigenetic mouse bioassay is sensitive to many environmental factors, including diet composition [7,8]. For example, we demonstrated that

the phytoestrogenic compound, genistein, found in soya products results in a significant increase in DNA methylation at the A^{vy} locus, with a concomitant increase in the frequency of brown offspring [7,8]. Thus, to reduce the exposure to genistein, *a/a* females serving as controls receive a modified AIN-93G diet (i.e., diet 95092 with 7% corn oil substituted for 7% soybean oil, Harlan Teklad, WI, USA) rather than standard mouse chow. In contrast, dams exposed to chemical compounds receive a modified AIN-93G diet supplemented with the test compound. All diets are provided to the *a/a* females 2 weeks before mating with A^{vy}/a males, and throughout pregnancy and lactation. If a single dose is to be given to the *a/a* females, as in our low-dose radiation study [12], the optimal time for exposure is at the blastocyst stage of development (i.e., 4.5 days after fertilization) because the repertoire of epigenetic marks in the embryonic stem cells are being reset at this time during gestation.

Tissue collection

When the mice are weaned at 22 days after birth, all offspring should be weighed, digitally photographed and rated for coat color phenotype prior to sacrifice. Tissue from the three germ layers (e.g., brain, liver and kidney) and tail tissue are collected, flash frozen in liquid nitrogen and stored at -80°C until DNA methylation and histone modifications are assessed.

Conclusion

It is important to be meticulous when using the A^{vy} mouse to investigate the effects of the maternal environment on the epigenome in the offspring, otherwise sensitivity and interpretability will be lost. If the following suggestions are adhered to, this epigenetic biosensor will be useful in determining the effects of maternal diet [7,8], chemicals [9–11] and physical agents [12] on the epigenome of embryonic stem cells *in vivo*:

- Use a five coat color classification system;
- Determine DNA methylation and/or histone modifications at the A^{vy} locus;
- Use only virgin *a/a* female mice to avoid parity effects on the epigenome;
- Introduce the A^{vy} allele through the male mouse to eliminate epigenetic transgenerational inheritance at this locus;

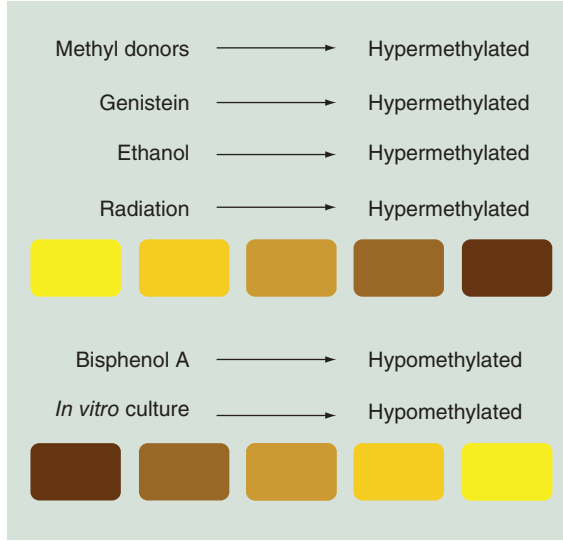

Figure 2. A^{vy} mouse as a biosensor for environmentally induced alterations in the epigenome. Maternal exposure to methyl donors [7], genistein [8], ethanol [9], low-dose ionizing radiation [12], bisphenol A (BPA) [10] and *in vitro* culturing [11] cause coat-color changes in A^{vy} offspring by hypermethylating or hypomethylating an intracisternal A particle inserted into the *Agouti* locus. Boxes: coat color.
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- Use 10–15 dams (i.e., 40–60 A^{vy} offspring) per experimental dose;
- Use a single cohort of animals for controls and exposure groups, or match controls and exposure groups across all cohorts to avoid seasonal effects;
- Use the modified AIN-93G diet to eliminate the epigenetic effect of genistein;
- Ensure caging, bedding and water are free of contamination from the experimental agent or compounds known to alter the epigenome (e.g., BPA).

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