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Epigenetic inheritance through the female germ-line: The known, the unknown, and the possible

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ABSTRACT

Although genetic mutations have long been known to influence gene expression and individual phenotype, studies emerging over the past decade indicate that such changes can also be induced in the absence of alterations in base-sequence. Epigenetically driven changes in gene expression or phenotype, when they are transmitted to succeeding generations, represent an entirely new mechanism that could generate heritable variation in a population. To understand the mechanistic basis of epigenetic inheritance, it is essential to learn how these changes may be transmitted through the germ-line to the next generation. Here, we review the process of female germ cell specification, oocyte growth, and meiotic maturation. We discuss what is known of the activity and role of three principal candidates to transmit epigenetic information – DNA methylation, histone post-translational modifications, and short non-coding RNAs – in the developing oocyte. We then consider intergenerational inheritance and true transgenerational inheritance and, in the case of the latter, compare examples in which insertional mutations have driven the heritable epigenetic phenotype with examples of environmentally induced epigenetic inheritance for which the mechanism remains to be identified.

1. Introduction

Can the foods that we eat, chemical products in our environment, or disease affect not only our health but also our unborn children and even our children's children? Can these external agents alter our phenotype, without mutating our genome, in a manner that can be transmitted to succeeding generations? Not so long ago, the possibility that such a non-genetic mechanism of inheritance exists would have – at least for mammals – been rejected out-of-hand. But over the past decade, a growing number of studies have emerged reporting just this kind of epigenetic inheritance. However, how an epigenetically based phenotype in an individual might be transmitted to her offspring remains essentially unknown. Identifying the underlying mechanisms is particularly challenging in the case of the female, as relatively few oocytes can routinely be obtained for analysis. Nonetheless, because oocytes may remain dormant for up to 50 years in a woman before entering the growth phase that will lead to ovulation and fertilization, and the growth phase itself is a protracted process that requires ~4 months in humans, they are potentially exposed for a very long time to environmental conditions that might induce heritable epigenetic modifications. Here, we review the life cycle of the female germ cell. We then discuss potential epigenetic marking mechanisms that are active in the oocyte. We conclude by considering known and proposed examples of epigenetic inheritance through the female germ line, including the difference between intergenerational and true transgenerational epigenetic inheritance.

2. Overview of oogenesis

The primordial germ cells (PGCs), whose descendants will give rise to oogonia in females, arise from cells of the embryonic epiblast and first become detectable at about embryonic day (E)6.5 in the mouse [1–3]. Over the next several days, the PGCs migrate to and colonize the gonadal primordium. During this time, and particularly once they have arrived at the future gonad [4], the PGCs proliferate extensively and generate up to seven million oogonia in humans [5]. Beginning at about E10.5 in the mouse, the gonad initiates differentiation as an ovary or a testis and the PGCs are directed down sex-specific pathways [6]. Whereas the male germ cells become mitotically arrested, female germ cells enter meiosis and progress to late diplotene where they become arrested. Near the time of birth, germ-cell cysts that have been created by incomplete cytokinesis during mitotic proliferation break down as the somatic granulosa cells invade between oocytes, and each

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oocyte becomes enclosed by a small number of granulosa cells in a structure termed a primordial follicle [3,7,8]. The ovaries of a newborn female mouse harbor about 8000 primordial follicles [9], and those of a newborn girl about 1,000,000 [10]. There is some debate whether a population of ovarian germ-line stem cells exists postnatally, but the weight of available evidence indicates that no new oocytes are generated in mammals after birth [11–14].

Prior to ovulation and fertilization, oocytes of primordial follicles undergo a prolonged and complex program of growth and differentiation (Fig. 1). The limited supply of non-renewable primordial follicles means that the entry of oocytes into this program must be tightly regulated to ensure that the pool is not prematurely depleted [15–17]. The mechanisms that regulate the rate and number of follicles entering the growth phase remain poorly understood; intriguingly, this regulation appears to be disrupted in reconstituted ovarian fragments [12,18], suggesting that these mechanisms are embedded in the physical structure of the ovary. Although the upstream signals that instruct a primordial follicle to enter the growth phase are equally mysterious, an early crucial event seems to be increased activity of the mTORC (mechanistic target of rapamycin complex) 1 pathway in the granulosa cells, which would be expected to upregulate protein synthesis [19]. This increases production of Kit ligand by the granulosa cells, which activates Kit receptor on the oocyte surface, thereby triggering oocyte growth. Other studies, however, have found that initiation of growth does not require canonical Kit signaling [20], suggesting that multiple mechanisms may be able to initiate growth. Initiation of growth also depends on translocation of the growth-inhibitory factor, FOXO3A, from the nucleus to the cytoplasm [21].

Growth is a protracted process requiring about 3 weeks in mice and 4 months in humans [10,22,23]. During growth, the oocyte increases well over 100-fold in volume as it accumulates an enormous supply of messenger RNAs, proteins and organelles that are necessary to direct the terminal stages of oogenesis and early stages of embryogenesis. Although the initiation of oocyte growth is accompanied by widespread changes in the pattern of gene transcription, there is little evidence to date of substantial qualitative changes in the transcriptome during the period of growth [24,25]. In contrast, there is extensive post-transcriptional control of gene expression [26,27]. Many messenger RNAs are not translated but are instead rapidly deadenylated at the 3′-poly(A) tail following export from the nucleus and stored, possibly in large ribonucloprotein complexes located near the cortex of the oocyte [28,29]. mRNAs stored in oocytes are remarkably stable, exhibiting a half-life of up two weeks [30,31]. Many will be translationally activated during meiotic maturation or after fertilization [32].

Transcriptional activity falls during the late stages of oocyte growth and is undetectable once oocytes reach full-size. Transcriptional arrest is accompanied by a major reorganization of the chromatin, termed the non-surrounded-nucleolus-to-surrounded-nucleolus (NSN-SN) transition, during which the chromatin condenses partially and a portion becomes arranged in a ring (as visualized in two-dimensional images) around the nucleolus [33–37]. Although temporally coincident, transcriptional arrest and the NSN-SN transition are independently regulated [38–40]. Oocytes whose chromatin is in the NSN configuration develop poorly as embryos [41,42] indicating that either the transition itself or a linked event is crucial for oocytes to acquire developmental ‘competence.’

As the oocyte grows, the follicle expands by proliferation of the granulosa cells and generation of a fluid-filled cavity termed the antrum. During late folliculogenesis, the mural granulosa cells that line the inner wall of the antral follicle express the receptor for luteinizing hormone, whose subsequent release from the pituitary triggers both ovulation and the terminal stage of oogenesis, termed meiotic maturation [43–45]. LH functions at least in part by triggering the release of peptides related to epidermal growth factor (EGF), which in turn activate EGF receptors located on both the mural granulosa cells and the cumulus granulosa cells that surround the oocyte. In contrast to the prolonged growth phase, maturation is relatively brief requiring only 12 h in mouse and ~36 h in humans. Maturing oocytes resume the cell cycle, which has been arrested since before birth, and enter metaphase, complete the first meiotic division and advance to metaphase of meiosis II where they again arrest until fertilization. Although there is no detectable transcription during maturation, translational activation or repression of specific mRNAs, often dictated by sequence motifs located in the 3′-untranslated region, drive this process [32].

3. DNA methylation during oogenesis

What processes during oogenesis might provide a vehicle for epigenetic transgenerational inheritance? An obvious candidate is the mechanism that establishes genomic imprints. Although most autosomal genes are expressed from both alleles, some are expressed preferentially or solely from either the maternally contributed or paternal contributed allele. These genes must therefore be marked, or imprinted, in a manner that identifies their parent of origin. Importantly, whereas somatic cells contain one maternally and one paternally imprinted copy of each gene, in germ cells both alleles must be maternally (in oocytes) or paternally (in sperm) imprinted. As discussed below, our current understanding is that the existing imprint is erased from each allele during early germ cell development, after which the appropriate parental imprint is established on both alleles later during gametogenesis.

DNA methylation has long been considered to be a likely component of the mechanism by which the parental origin of the imprinted genes is marked within the germ cell, and enormous effort has been invested in elucidating the timing and molecular control of DNA methylation of both imprinted and non-imprinted regions of the genome during germ cell development. Thanks to the development of high-resolution techniques that are compatible with the relatively small number of germ cells at different stages of development that can be obtained for analysis, a remarkably detailed picture of this dynamic process has begun to emerge [46–49].

Beginning shortly after they first arise, PGCs undergo extensive DNA demethylation. Two complementary mechanisms appear to drive demethylation. On one hand, reduced expression of DNMT3A, the *de novo* DNA methyltransferase, and NP95, an essential cofactor for the activity of DNMT1, the maintenance DNA methyltransferase, in the proliferating PGCs means that newly replicated DNA does not become methylated. Consequently, the existing methylated strands become gradually diluted through repeated cycles of DNA replication and mitosis [1,50–53]. This is termed passive DNA demethylation. In addition, an active process that depends on oxidation of 5-methylcytosine to 5-hydroxymethylcytosine by the TET1 and TET2 proteins [54–56] also demethylates DNA. This second mechanism notably removes DNA-methyl groups from CpG-rich regions within imprinted loci, the inactive X-chromosome and some genes expressed in the germ cell. In contrast to this widespread demethylation, however, certain genomic regions including the intracisternal A particle (IAP) family of retrotransposons and several hundred CpG islands within the genome [51–53,57] remain at least partially methylated. It is not yet known why these regions survive the wave of demethylation but they represent a potentially powerful tool that could be exploited to enable transgenerational inheritance of an epigenetic state.

The DNA remains unmethylated, with the exception of the regions noted above, throughout the time that the oocyte remains in the primordial follicle. During growth, however, the DNA

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