Genetics

Histone acetyltransferase KAT8 and oocyte development

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Oocyte development is necessary for female fertility and is characterized by dramatic changes in gene expression and chromatin structure. Histone acetylation is an important epigenetic mechanism for gene regulation as it changes the chromatin structure and affects the binding ability of transcription factors to DNAs. Histone acetylation level is regulated antagonistically by two classes of enzyme, histone acetyltransferases (HATs) and histone deacetylases (HDACs). In this review, we briefly summarize the current knowledge about an important HAT, K (Lysine) Acetyltransferase 8 (KAT8), and introduce the role of this enzyme in oocyte and follicle development, which gives new insight about epigenetic and female reproduction.

Kat8 | histone acetylation | oocyte | follicle | reproduction

Histone acetylation and oocyte development

Oogenesis plays an irreplaceable role in female fertility as it determines the production of healthy eggs (1). To ensure the normal development of oocytes, accurate gene expression and appropriate chromosome configuration are needed (2, 3). Among the various post-translational modifications, histone acetylation is one of the most well-studied modifications. Acetylation regulates the chromatin conformation between open and closed state by changing the electrostatic affinity between histones and DNA, thus affecting the binding ability of transcription factors to DNAs (4). Histone acetylation level is regulated by two classes of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs) (5-7). HATs are responsible for adding acetyl groups to lysines to neutralize the positive charge on histones. As a result, the electrostatic affinity between histone and DNA is weakened and chromatin maintains an open structure for factors that promote transcription (4). In contrast, HDACs are mainly required for the removal of acetyl groups. This removal causes excess positive charges on histone, which contributes to strong electrostatic affinity between histones and negatively charged DNAs, then compacts the chromatin structure and represses gene expression (8).

Several HDACs have been reported necessary for oocyte and follicle development. For example, knockdown *Sirt6* in germinal vesicle (GV) stage mouse oocytes by RNA interference results in abnormal spindle morphology and chromosome alignment, as well as elevated incidence of aneuploidy. Consequently, oocyte maturation is disrupted with fewer first polar bodies being released (9). Conditional knockout of another HDAC-encoding gene, *Hdac2*, in mouse oocyte causes similar oocyte maturation defects as *Sirt6* knockdown in oocytes. In addition, the knockout mice contain fewer antral follicles compared with that of control (10). Disrupted follicle development is also observed in oocytes of *Hdac1* and *Hdac2* double conditional knockout mice. The mutant mice are interfile with small ovarian size and disrupted oogenesis (5). It is worth mentioning that oocytes lack *Sirt6* or *Hdac2* display specific increase in H4 Lys 16 acetylation (H4K16ac) levels (9, 10), suggesting a potential role of H4K16ac in oogenesis.

Members of the HATs, family comprised of the MYST, Gcn5/PCAF and p300/CBP subfamilies function as gene co-factors (4). However, compared with the well-studied roles of HDACs in oogenesis, how HATs regulate oogenesis is poorly understood. Here, based on our recent report, we briefly introduce a HAT, K (Lysine) Acetyltransferase 8 (KAT8, also known as MOF or MYST1) (11-13), and summarize the experiments results of this conditional gene knockout mice to elaborate the roles of KAT8 in oocytes and follicle development.

Histone acetyltransferase KAT8

K (Lysine) Acetyltransferase 8 (KAT8, also known as MOF or MYST1) is a a HAT protein that is conserved among multiple species and expressed widely in different tissues (11-13). It is a histone acetyltransferase belonging to the MYST family (Moz-Ybf2/Sas3-Sas2-Tip60) (14) that contains a conserved acetyl-CoA-binding motif, a zinc finger domain (15-17) and a chromo domain (18). This acetyltransferase acetylates histone substrates via two complexes, male-specific lethal (MSL) and non-specific-lethal (NSL) (19-21). Though the NSL complex can acetylate histone H4 at lysines 5 and 8 (22), the major histone substrate for KAT8 is H4K16 (23, 24). KAT8 also acetylates non-histone substrate, such as p53 at lysine 150. Acetylated p53 promotes the expression of several pro-apoptotic factors, such as PUMA and BAX (25). In addition, KAT8 can regulate its own activity by acetylating itself at lysine 274 (26).

KAT8 is involved in multiple biological process, such as transcription, DNA damage repair, apoptosis and metabolism. In *Drosophila,* MOF (KAT8) is a key component of the X chromosome dosage compensation complex (27), which is essential for balancing the expression of X-linked gene between male and female (14, 28, 29). Microarray analysis showed that several key genes, such as human leukocyte antigen complex P5 (*HCP5*), are significantly down-regulated in *KAT8* knockdown HeLa cells (30). Similarly, in *Kat8* knockout mouse embryonic stem cells, levels of several critical transcription factors, such as *Oct4*, *Nanog* and *Sox2* are significantly decreased and these genes are directly targeted by KAT8 (31). Once DNA damage occurs in cells, ataxia-telangiectasia-mutated (ATM) can phosphorylate KAT8 at the T392 site, then the phosphorylated KAT8 could colocalize with γ-H2AX, ATM and p53BP1 foci to recruit BRCA1 and MDC1 (32, 33). The role of *Kat8* in apoptosis is contradictory. For example, in H1299 cells, KAT8 can acetylate p53 to activate the apoptotic pathway (25), while *Kat8* may play an anti-apoptotic role as in *Kat8* knockout mouse embryos, many cells show features of apoptosis including Caspase3 activation and DNA fragmentation (34). These results suggest that *Kat8* may play diverse roles in apoptosis via different pathways. *Kat8* is also involved in metabolism by binding to mitochondrial DNAs and regulating their expression. Serious mitochondrial degeneration, high-energy consumption, as well as defective oxidative phosphorylation could be observed after *Kat8* deletion in mouse cardiomyocytes, which indicates that *Kat8* plays an important role in connecting epigenetics with metabolism (35).

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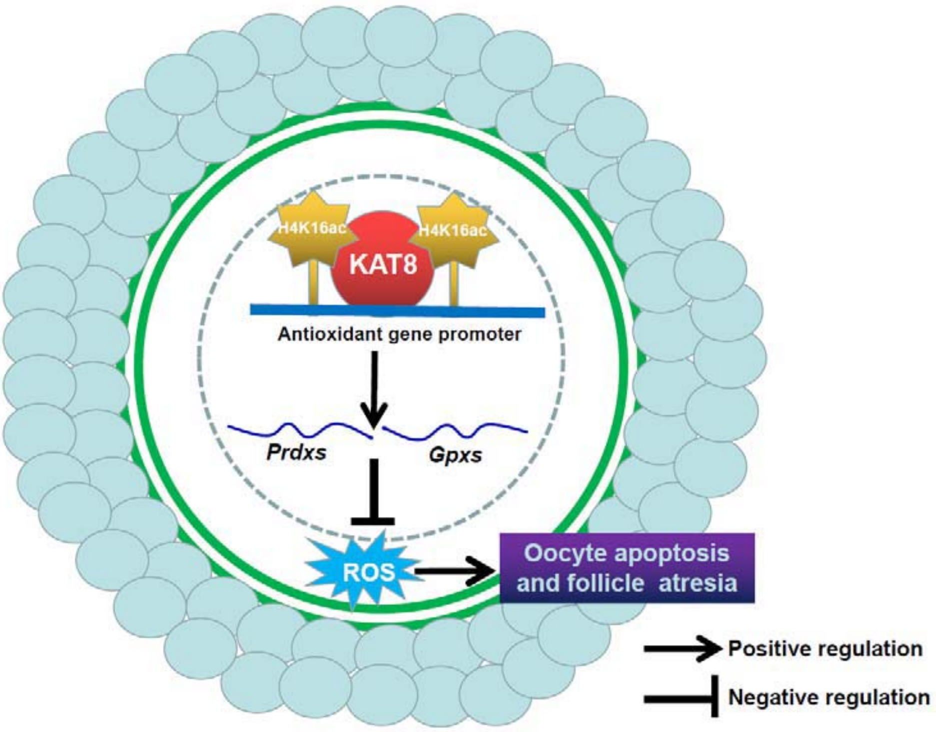

Figure 1. Working model of how oocyte and follicle development is regulated by KAT8. KAT8 and H4K16ac bind to promoters of several antioxidant genes and promote their expression. ROS levels are subsequently decreased, which leads to the prevention of oocyte apoptosis and follicle atresia.

Expression of KAT8 during mouse oocyte development

During mouse folliculogenesis, *Kat8* expression in oocytes exhibits temporal and spatial patterns. Real-time PCR showed that the level of *Kat8* transcript increases slightly from 5 days postpartum (5d) to 14d, subsequently reaches the highest level at full-grown GV stage, then decreases rapidly during metaphase I and metaphase II. Immunocytochemical analysis proved that the protein localizes in nuclei from 5d to full-grown GV stage and is uniformly dispersed throughout the entire upon germinal vesicle breakdown (GVBD) (36). Interestingly, H4K16ac, the major histone substrate for KAT8, also displays a similar expression profile to KAT8 as the nuclear staining of H4K16ac increased from 5d to GV stage and decreased significantly upon GVBD (36).

KAT8 is essential for mouse oocyte and follicle survival

Based on the fact that global ablation of KAT8 causes embryonic lethality (37), specific CRE recombinase (*Gdf9-Cre*) mediated deletion was performed to reveal the function of this acetyltransferase in mouse oocyte. Mice lacking KAT8 in oocytes are infertile with smaller ovarian size. Moreover, the *Kat8* conditional knockout (cKO) mice contain fewer full-grown GV stage oocytes with small sizes and defective morphologies. Follicle development is also disrupted as much fewer secondary, preantral and antral follicles are observed in the cKO mice compared to control (36). The proliferation of granulosa cells is not affected based on BrdU incorporation and PCNA immunostaining, however, higher incidences of apoptosis and DNA damage in oocytes of secondary or later stage follicles are observed after *Kat8* deletion (36). All these results indicate that KAT8 is necessary for oocyte and follicle development.

KAT8 regulates ROS levels by mediating antioxidant genes expression in oocytes

Oocyte development is characterized by dramatic changes in gene expression (38). Whole transcriptome sequencing analysis elaborates that expressions of 858 transcripts are altered after *Kat8* deletion in oocytes, including 500 up-regulated and 358 down-regulated genes (36). Interestingly, several antioxidant genes, including peroxiredoxin 1 (*Prdx1*), *Prdx2*, glutathione peroxidase 1 (*Gpx1*), *Gpx4* and *Gpx6* (39, 40) are significantly down-regulated in *Kat8*-defecient oocytes. Consistent with this, increased reactive oxygen species (ROS) levels are observed in *Kat8* knockout oocytes. Chromatin immunoprecipitation (ChIP) analysis shows that some of these genes are directly regulated by KAT8 (36), which indicates the down-regulation of antioxidant genes is directly caused by *Kat8* deletion. Generally, to ensure the normal oocyte development, excessive ROS levels should be removed by endogenous antioxidant enzymes (41). Thus, we hypothesize that when *Kat8* is deleted in the oocytes, accompanied with decreased expression of antioxidant genes, the evaluated ROS levels could disrupt oocyte development. Indeed, ROS levels are down-regulated significantly in *Kat8*-defecient oocyte after intraperitoneal injection of N-acetylcysteine (NAC), a widely used antioxidant (42-44) to the cKO mice. The ovarian size is larger in cKO mice injected with NAC compared to those injected with PBS. Oogenesis is also improved in NAC-treated group. Moreover, more secondary, preantral and antral follicles are observed in NAC-treated cKO mice. Consistently, both the incidences of apoptosis and DNA damage in *Kat8* knockout oocyte are decreased after NAC administration (36). Thus, histone acetyltransferase KAT8 is essential for mouse oocyte development by regulating ROS levels.

Conclusion

Here we summary the recent studies of histone acetylation in relation to oogenesis and focus on an important acetyltransferase KAT8. This conserved enzyme functions widely in multiple biological process and is highly expressed in full-grown GV stage oocytes. KAT8 in oocytes is specially responsible for H4K16ac and it can directly bind to the promoter of several key antioxidant genes to mediate their expression. The cellular ROS levels are consequently decreased, thus maintaining the environment favorable for the normal oocyte and follicle development (Fig.1). Of note, as a gene co-factor, various pathways are affected upon *Kat8* deletion, whether and how these pathways affect oocyte development need to be further confirmed. In conclusion, the study of *Kat8* on oocyte development supplies new information about how epigenetic factors regulate female reproduction and may provide new solutions to human oogenesis or folliculogenesis problems.

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