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Histone Demethylase JHDM2A Is Involved in Male Infertility and Obesity

Running title: JHDM2A and male infertility/obesity

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Abstract

Recent studies indicate that histone lysine methylation is subject to enzyme-catalysed reversion, and jumonji C (JmjC)-domain-containing proteins have been identified as one of the members of histone demethylases. Although an increasing number of histone demethylases have been identified and biochemically characterized, their biological functions are poorly characterized. In order to elucidate the physiological functions, we generated the knockout mouse model of H3K9me2/1-specific demethylase JHDM2A (JmjC-domain-containing histone demethylase 2A, a.k.a. JMJD1A and KDM3A) and showed that JHDM2A is essential for spermatogenesis. Jhdm2a-deficient mice exhibited impaired post-meiotic chromatin condensation, that caused infertility although the hormonal levels were maintained. Further molecular and biochemical analysis revealed that JHDM2A directly bound to the core promoter regions of transition nuclear protein 1 (*Tnp1*) and protamine 1 (*Prm1*) genes, and induced the transcriptional activation of these genes by removing H3K9 methylation, that is known as a silencing marker of gene transcription. This work uncovered a role for JHDM2A in spermatogenesis and identified two downstream genes, which are critical for sperm nuclear condensation. In addition, we also showed that JHDM2A plays a role in regulating fat metabolic gene expression in muscle and brown fat tissue, and the knockout mice exhibited obesity and hyperlipidemia. Thus, JHDM2A possesses organ/tissue-specific target genes, and impairment of this molecule cannot be compensated by other JmjC-containing histone demethylases, suggesting the importance of this molecule *in vivo*.

Introduction

Despite its relatively short history, histone methylation has become one of the best studied research areas in epigenetics mainly due to its importance in transcriptional regulation of gene expression in various organisms. In mammals, in particular, many histone methyltransferases are known to be involved in human diseases and cancers through controlling transcription of downstream target genes, and studies using knockout mice have demonstrated that many histone methyltransferases possess indispensable function *in vivo*.

Histone methylation is denominated as “cellular memory”, since the modification is sometimes maintained over cell division (Peters and Schubeler, 2005). Therefore, the existence of histone demethylase(s) has been questioned until the first discovery of histone demethylase, LSD1, in 2004 (Shi, et al., 2004). The next identified histone demethylase, Jhdm1a (JmjC-containing histone demethylase)/Fbxl11, consisted of a large family called JmjC-domain containing proteins (Tsukada, et al., 2006). Because the JmjC domain is responsible for the catalytic activity, it was easy to predict that other JmjC-domain containing proteins are also histone demethylases. At present, nearly 20 JmjC family

members have been identified as demethylases for distinct methylated lysine residues in H3 (Klose, et al., 2006), and now the discovery of histone demethylases raises a new question; what is the physiological output to cancel "cellular memories"?

H3K9 methylation and spermatogenesis

During mammalian spermatogenesis, unique and dynamic genetic/epigenetic changes are observed such as establishment of imprinting information in primordial germ cells (PGCs), meiotic chromosomal recombination and segregation, and histone removal followed by chromatin
condensation in spermiogenesis. In these events, it has been elucidated that alteration of chromatin structure by histone modifications plays an important role (Rousseaux, et al., 2005) (Godmann, et al., 2009), and among these modifications, dynamics of histone 3 lysine 9 (H3K9) methylation is one of the best-characterized modifications in the study of germ cell development
For instance, genome-wide methylation of H3K9 catalyzed by GLP/Eu-HMTase1 occurs at the early-middle stages of PGC development (Seki, et al., 2007). Mice carrying knockouts of G9a and Suv39h, other H3K9 methyltransferases, exhibit impaired spermatogenesis due to meiotic defects, suggesting that H3K9 methylation is indispensable for meiosis (Tachibana, et al., 2007) (Peters, et al., 2001). After meiosis, H3K9 methylation is accumulated in nuclei of round spermatids in a region corresponding to the chromocenter, and binding of Cdyl (chromodomain protein, Y-Like) to methylated H3K9 is reported to be important for chromatin condensation in elongated spermatids (Lahn, et al., 2002). Thus, the genome-wide level of H3K9 methylation is precisely regulated and maintained, implying the importance of H3K9 methylation at multiple stages of spermatogenesis (summarized in Fig 1).

Spermiogenic defect in the Jhdm2a knockout mouse

Before JHDM2A (JmjC-containing histone demethylase 2a), also known as JMJD1A or KDM3A, was identified as an H3K9 demethylase (for mono- and di-methylation) in 2005, it was originally cloned as a testis-specific gene transcript (Yamane, et al., 2006) (Hoog, et al., 1991). Consistent with this previous report, immunohistochemical analysis using anti-JHDM2A antibody revealed an intense nuclear expression in round spermatids, and a subnuclear distribution that was merged with the expression of RNA polymerase II, indicating that JHDM2A may contribute to transcriptional activation (Okada, et al., 2007) (Fig. 2).

However, as described above, the genome-wide H3K9 methylation level is continuously maintained during spermatogeneis. So how does the demethylase play a role? To further elucidate the importance of JHDM2A during spermatogenesis, Jhdm2a deficient mice were generated (Okada, Scott, Ray, Mishina and Zhang, 2007). Although the Jhdm2a knockout mice were viable, males exhibited

smaller testes, and they were functionally infertile. Histologically, spermatids of the knockout mice failed to elongate due to impaired chromatin condensation (Fig. 3). Unexpectedly, genome-wide H3K9 methylation was unaltered. In addition, levels of LH, FSH, and testosterone were also maintained in the knockout mice, although

JHMD2A was reported to function as a transcriptional co-activator of the androgen receptor in a human prostate cancer cell line (Yamane, Toumazou, Tsukada, Erdjument-Bromage, Tempst, Wong and Zhang, 2006). If neither genome-wide hypomethylation of H3K9 nor impaired hormonal regulation is the cause, what is the underlying molecular mechanism of the infertility? According to the subnuclear distribution that was similar to that of RNA polymerase II, it was speculated that JHDM2A was involved in transcriptional activation of gene(s), that must be essential for sperm chromatin condensation. In fact, RT-qPCR analysis revealed decreased expression of two testis-specific basic proteins, transition protein 1 (*Tnp1*) and protamine 1 (*Prm1*) in round spermatids of the knockout mice. Chromatin immunoprecipitation (ChIP) assays further demonstrated that JHDM2A was recruited to the core promoter regions of both *Tnp1* and *Prm1* in round spermatids, whereas the recruitment of JHDM2A was not observed in the knockout mice. In addition, the methylation levels of H3K9 in these promoter regions were significantly higher in round spermatids of Jhdm2a knockout mice compared with that of the wildtype control. Thus, we propose a model in which JHDM2A contributes to spermatogenesis by directly controlling expression of *Tnp1* and *Prm1*, which are both essential for sperm chromatin condensation. One of the questions that remains is how Jhdm2a is specifically recruited to the *Tnp1* and *Prm1* promoters, but not to other genes. JHDM2A itself does not contain a defined DNA binding motif, and it appears no consensus DNA sequence consistently exists in the promoter regions of the target genes including not only *Prm1* and *Tnp1* but also *Ppara* and *Ucp1* described in the next paragraph. Therefore, it can be argued that other JHDM2A binding proteins are responsible for the DNA targeting.

Obesity in Jhdm2a knockout mouse

Other than male infertility, Jhdm2a knockout mice also exhibit an obese phenotype such as increased body fat deposition and higher serum lipid content as they age without affecting food intake (Tateishi, et al., 2009) (Fig. 4). Microarray analysis revealed that the Jhdm2a deficiency affects the expression of metabolic genes, which caused impaired β-oxidation and glycerol release in skeletal muscle. Among these affected genes, Jhdm2a directly targeted the PPRE of the *Ppara* enhancer, and resulted in demethylation of H3K9 at the enhancer region followed by the transactivation of *Ppara*. In addition, defective adaptive thermogenesis of the Jhdm2a knockout mice also pinpointed a potential role of Jhdm2a in β-adrenergic signaling in brown adipose tissue (BAT). In fact, expression of several genes involved in mitochondrial functions including *Ppara* was decreased in the Jhdm2a knockout. In addition, analysis of *UCP1*, a key

gene involved in β-adrenergic signaling-mediated thermogenesis in BAT, demonstrated that cold-induced *Ucp1* upregulation was completely abolished in the knockout mice. ChIP analysis further demonstrated that *Ucp1* was also one of the downstream target genes of JHDM2A, and JHDM2A induced transactivation through removing H3K9 methylation from the promoter. Taken together, we proposed that JHDM2A is involved in regulating systemic metabolic control including *Ppara* and β-adrenergic signaling pathways, and its deficiency induces an obese phenotype in mice.

Is there a link between infertility and obesity in Jhdm2a knockout mice?

A potential link between male infertility and obesity has been proposed in humans, and several causative factors have been suggested such as hormonal abnormality and genetic mutation (Kasturi, et al., 2008) (Hammoud, et al., 2006). However, genetic factors that contribute to obesity are often complicated and depend on multiple other genes and factors (Kasturi, Tannir and Brannigan, 2008) (Hammoud, Gibson, Peterson, Hamilton and Carrell, 2006). Similarly, ablation of several genes leads to male infertility when they are disrupted in mice, but very few of them are mutated in human patients showing male infertility (O'Bryan and de Kretser, 2006). How about in the Jhdm2a deficient mice? Unlike the majority of human patients who show both male infertility and obesity, no abnormal hormonal changes were observed in the knockout mice. Despite the previous report that JHDM2A interacts with the androgen receptor in prostate cancer cells, both male hormones (androstenedione and testosterone) and female hormones (LH, FSH, and estradiol) in the knockout mice were maintained within a normal range, and so were other hormones related to fat metabolism (norepinephrin, epinephrin, T3, adiponectine, and corticosterone) (Okada, Scott, Ray, Mishina and Zhang, 2007) (Tateishi, Okada, Kallin and Zhang, 2009). However, LSD1, a histone demethylase which also catalyzes H3K9 demethylation, contributes to transactivation of estrogen receptor α (ERα)-target genes pS2, suggesting that there might be a possibility that JHDM2A plays some role in the ERα pathway (Garcia-Bassets, et al., 2007). Another possible factor(s) that can link these two phenotypes is expression of glucose metabolism-related genes expressed in testis, especially because the Jhdm2a knockout mice are diabetic. Furthermore, although the possibility might be low, searching for genetic mutations in the Jhdm2a gene has been under investigation in human patients.

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Figure legends

Fig. 1. Dynamics change of genome-wide H3K9 dimethylation (H3K9me2) during male germ cell development. Timing of dimethyl H3K9 methyltransferases (GLP and G9a) and a demethylase (JHDM2A) expression is also shown. PGC; primordial germ cell, SG; spermatogonium, SC; spermatocyte, RS; round spermatid, ES; elongated spermatid.

Fig. 2. Immunohistochemical anaylsis of JHDM2A in mouse testis. JHDM2A (green) is highly expressed in round spermatids (RS), and not quite merged with γH2AX-positive (red) spermatogonia (SG) and spermatocytes (SC). JHDM2A-positive signals disappear in elongated spermatids (ES). Cytoplasmic staining of JHDM2A is observed in Sertoli cells (S). DAPI staining is shown as blue.

Fig. 3. Impaired spermiogenesis in Jhdm2a knockout mice. (A) Abnormal spermatids at step 15 of spermiogenesis. Smaller, round-shaped permatids are observed in the KO mice. (B) Ultrastructurally, chromatin condensation is incomplete in the knockout spermatids. Bar = 1 μm.

Fig. 4. Jhdm2a knockout mouse (KO; left) exhibits obese phenotype.

Fig. 5. Summary of molecular events occur in Jhdm2a knockout mice.

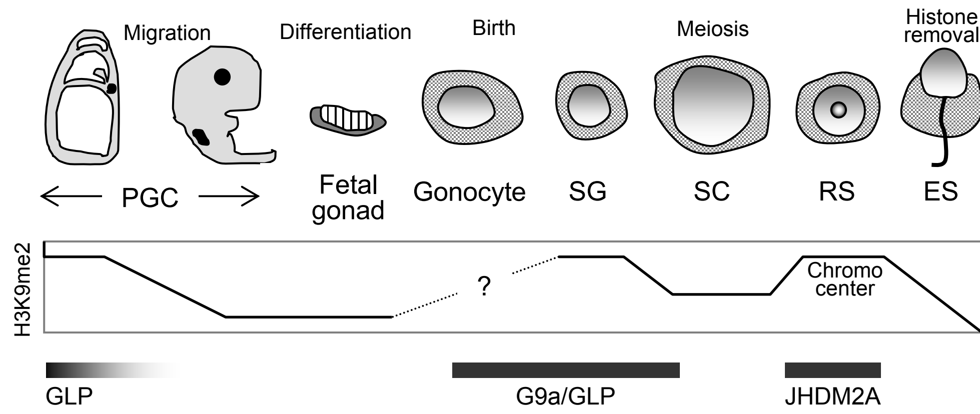

Fig.1 (Okada et al.)

JHDM2A + γH2AX + Hoechst

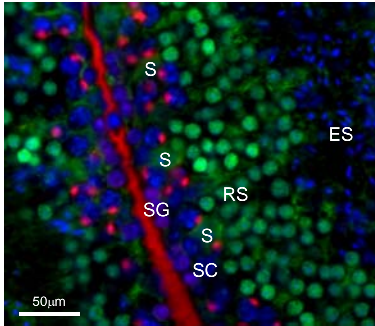

Fig.2 (Okada et al.)

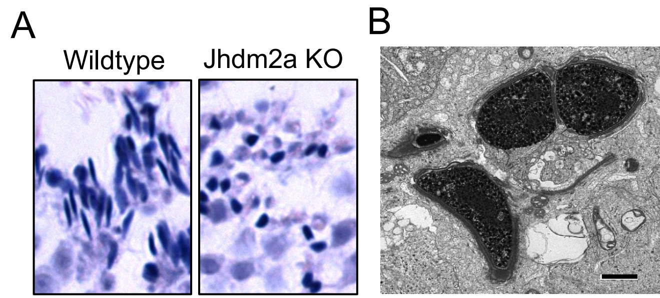

Fig.3 (Okada et al.)

Fig.4 (Okada et al.)

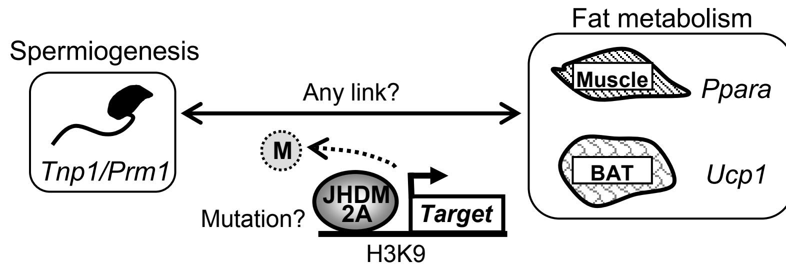

Fig.5 (Okada et al.)