Sperm tsRNAs and acquired metabolic disorders

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Abstract

Many findings support the hypothesis that metabolic changes associated with environmental factors can be transmitted from father to offspring. The molecular mechanisms underlying the intergenerational transmission of metabolic changes remain to be fully explored. These acquired metabolic disorders in offspring may be partially explained by some potential epigenetic information carriers such as DNA methylation, histone modification and small non-coding RNAs. Recent evidence shows that sperm tRNA-derived small RNAs (tsRNAs) as a type of paternal epigenetic information carrier may mediate intergenerational inheritance. In this review, we provide current knowledge of a father's influence on metabolic disorders in subsequent generations and discuss the roles of sperm tsRNAs and their modifications in paternal epigenetic information transmission.

Introduction

A number of studies have shown that the effects of environmental factors such as nutrition, stress, toxins and infections in parents can be transmitted to their offspring. These findings support the theory of Lamarck, who proposed that if an organism changes its phenotype to adapt to its environment, those changes would be passed on to its offspring (Weigmann 2014). The early experimental evidence was provided by the Dutch famine of 1944–1945. Children, born to women who were pregnant at the time of this famine, were more susceptible to some health problems such as diabetes and obesity (Ravelli *et al.* 1976). In addition, female mice with diabetes or who are fed a high-fat diet increase their offspring's risk of developing insulin resistance, diabetes or obesity (Aerts & Van Assche 2006, Huypens *et al.* 2016). These reports show that intergenerational transmission of metabolic phenotypes will occur through the maternal lineage.

Meanwhile, a growing amount of evidence shows that a father's exposure to a variety of environmental factors can also trigger changes in acquired characteristics, such as metabolic disorders, in subsequent generations (Fig. 1; Weigmann 2014, Rando 2016). Epigenetic factors including DNA methylation, histone modification and small non-coding RNAs (sncRNAs) are potentially involved in the inheritance of paternal-environment-induced metabolic changes (Daxinger & Whitelaw 2012, Heard & Martienssen 2014, Rando 2016). However, the molecular mechanisms for inheritance of acquired metabolic disorders from father to offspring are elusive. Recent studies show that the amounts of some sperm tRNA-derived small RNAs (tsRNAs), in mice fed a low-protein diet or a high-fat diet (HFD), were significantly increased (Chen *et al.* 2016, Sharma *et al.* 2016). Moreover, offspring from zygotes injected with sperm head, sperm total RNA or even only sperm tsRNAs
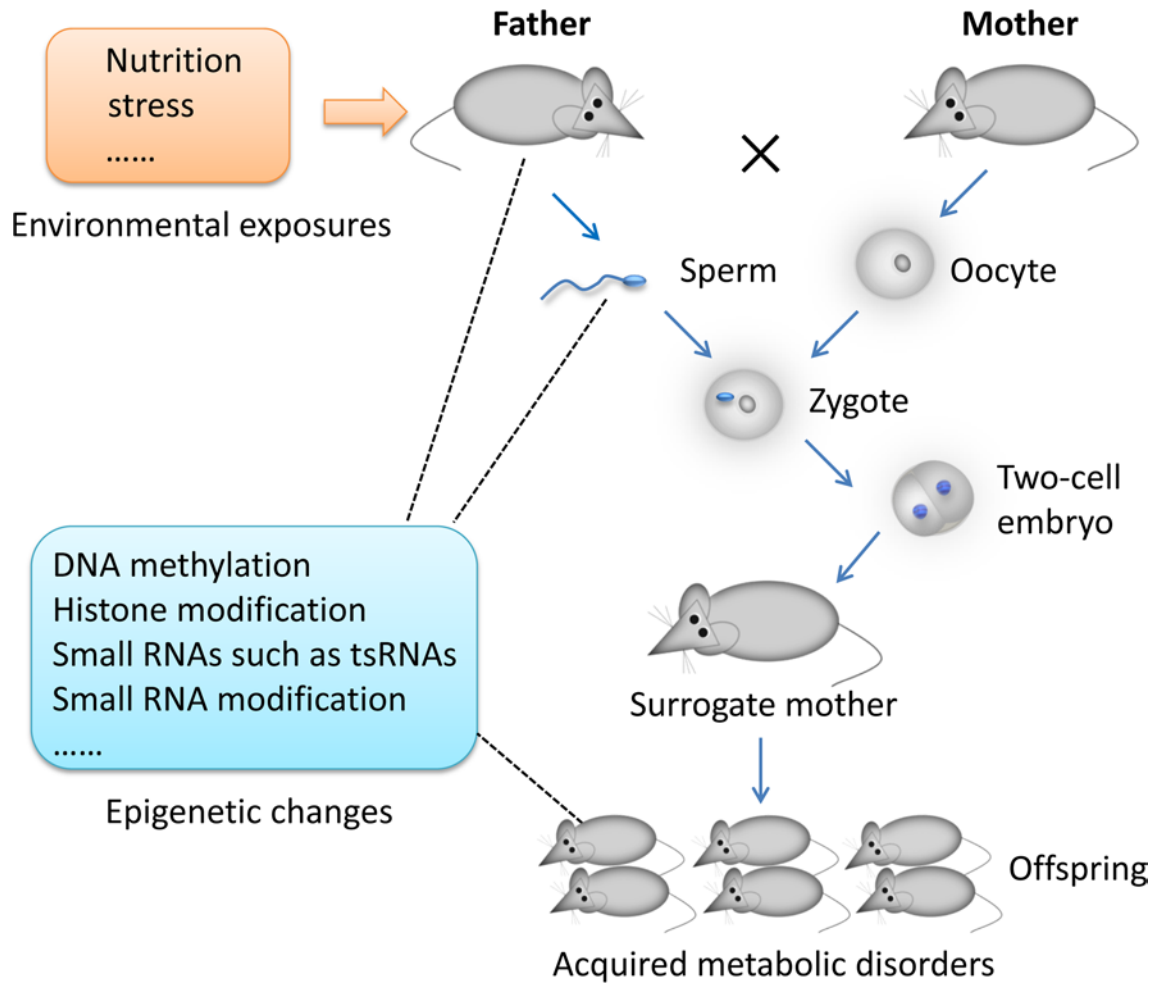
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Figure 1
Transmission of acquired metabolic disorders from father to offspring through sperm. Environmental factors such as diet and stress lead to metabolic changes in the germ line of a father that can be transmitted to the offspring. Epigenetic mechanisms including DNA methylation, histone modification, small non-coding RNAs such as tsRNAs, piRNAs and miRNAs, and small RNA modification have been reported. Abbreviations: tsRNAs, tRNA-derived small RNAs; piRNAs, Piwi-interacting RNAs; miRNAs, microRNAs.

from mice fed a high-fat diet showed impaired glucose tolerance and insulin secretion (Chen *et al.* 2016). These findings provide evidence that sperm tsRNAs are involved in the intergenerational transmission of metabolic disorders (Fig. 1; Chen *et al.* 2016, Sharma *et al.* 2016).

In this review, we describe the current understanding of the paternal intergenerational transmission of metabolic disorders to their offspring, the underlying molecular mechanisms, and pay particular attention to the involvement and modification of sperm tsRNAs.

Paternal dietary conditions and acquired metabolic disorders

Many researchers have examined a father's contribution to intergenerational inheritance (Daxinger & Whitelaw 2012, Rando 2016). An interesting study reported that a father's diet affected his daughters' health (Ng *et al.* 2010). Male rats fed a high-fat diet showed increased body weight, glucose intolerance and insulin resistance. Interestingly, impaired glucose tolerance and insulin secretion were observed in female offspring fed a normal diet (ND) (Ng *et al.* 2010). Similarly, the offspring of males which were fed a low-protein diet exhibited elevated hepatic expression of many genes involved in lipid and cholesterol biosynthesis, and decreased levels of cholesterol esters (Carone *et al.* 2010). These studies demonstrate that fathers fed a high-fat or low-protein diet may bring some metabolic changes to their offspring.

Two recent studies used *in vitro* fertilization to eliminate the potential influence of male-female contact and semen factors during natural mating; these studies

further demonstrate that paternal metabolic disorders can be transmitted to their offspring (Chen *et al.* 2016, Huypens *et al.* 2016). Sperm from ND and HFD males were isolated and used for *in vitro* fertilization, and the two-cell embryos subsequently obtained were transferred into healthy foster mothers on a normal diet to generate offspring (Huypens *et al.* 2016). The offspring of HFD parents obtained by *in vitro* fertilization are more susceptible to developing glucose intolerance and insulin resistance when fed a HFD (Huypens *et al.* 2016). Interestingly, injection of sperm heads from HFD mice into normal mouse oocytes also resulted in offspring with impaired glucose tolerance and insulin resistance (Chen *et al.* 2016). These results demonstrate that the sperm head contains information which transmits the acquired metabolic disorder susceptibility to offspring.

These findings from various research groups show that paternal dietary conditions can affect offspring metabolism.

Paternal stress and acquired metabolic disorders

The effects of other factors such as paternal stress on offspring metabolism have also been investigated (Rando 2016). It was reported that paternal traumatic stress in early life altered the behavioral and metabolic responses in their progeny (Gapp *et al.* 2014). Male mice (F_1) that experienced unpredictable maternal separation combined with unpredictable maternal stress in childhood, suffered from depression, loss of behavioral control, impaired cognitive functions, and impaired social skills in adulthood. Their subsequent progeny (F_2) have behavioral symptoms similar to those of the F_1 mice, and also show hypermetabolism and insulin hypersensitivity (Gapp *et al.* 2014). Likewise, the offspring whose fathers experienced psychological stress show elevated blood glucose and increased hepatic gluconeogenesis (Wu *et al.* 2016).

These findings demonstrate that paternal stress may lead to metabolic disorders in offspring.

The correlation of DNA methylation and histone modification with acquired metabolic disorders

Paternal behavioral and metabolic changes induced by environmental factors can be transmitted to the next generation through sperm. However, the factors transmitted in the father's sperm which may affect

the development of the embryo and ultimately result in metabolic disorders in the offspring, are still largely unknown. The inheritance of metabolic disorders induced by environmental exposures is thought to occur mainly via some epigenetic factors, which include non-coding RNAs, DNA methylation and histone modification (Daxinger & Whitelaw 2012, Heard & Martienssen 2014, Rando 2016).

Altered cytosine methylation at a putative enhancer of *Ppara*, a key lipid transcription factor, has been observed in the livers of offspring from male mice that were fed a low-protein diet (Carone *et al.* 2010). Deep sequencing of sperm from lean and obese men reveals that DNA methylation patterns are markedly different, and the methylation pattern is dynamically remodeled after bariatric surgery (Donkin *et al.* 2016), indicating that human gametic epigenetic variation can be related to nutritional status. Changes in histone modification by mutation of histone methyltransferase SET-2 also correlate with the transgenerational effects of extended life span in worms (Rando 2016). Histone acetylation is altered in paternal sperm and the seminiferous tubules of the testes in mice in response to chronic cocaine exposure; this modification can be transmitted to the offspring (Vassoler *et al.* 2013).

These reports show that DNA methylation and histone modification are correlated with the inheritance of metabolic disorders. However, whether DNA methylation and histone modification directly participate in epigenetic information transmission and cause the alteration of phenotypes in progeny still needs further evidence.

Sperm RNA and acquired metabolic disorders

To investigate whether sperm RNA participates in the inheritance of metabolic traits induced by environmental factors, some studies have used the approach of RNA isolation and injection into zygotes. Interestingly, after the injection of total RNA from the sperm of traumatized male mice into fertilized eggs, the offspring demonstrate similar behavioral alterations as their fathers (Gapp *et al.* 2014). Moreover, the injection of sperm total RNA from male mice fed a Western-like diet into normal fertilized eggs results in the establishment of the Western-like diet-induced metabolic phenotype in the progeny (Grandjean *et al.* 2015). Similarly, after the injection of sperm total RNA from HFD mice into normal zygotes, the offspring develop impaired glucose tolerance and insulin secretion (Chen *et al.* 2016).

Taken together, these studies provide compelling evidence that an RNA-dependent mechanism mediates the paternal transmission of metabolic traits in mammals. However, the kind of RNA that is responsible for delivering this paternal information to the offspring is yet to be elucidated.

There is evidence that small non-coding RNAs are involved in some epigenetic inheritance paradigms (Gapp *et al.* 2014, Weigmann 2014, Rando 2016). The expression level of sncRNAs such as microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), tRNA fragments and small nuclear RNA fragments was reported to be altered in the sperm of obese men (Donkin *et al.* 2016). Altered piRNA expression might coordinately modulate the expression of genes involved in behavior and food intake and could participate in predisposing the offspring to obesity (Donkin *et al.* 2016). In addition, deep sequencing analysis revealed that several miRNAs were upregulated and piRNAs were downregulated in the sperm of the traumatized mice (F_1), whereas these changes were not observed in F_2 mouse sperm (Gapp *et al.* 2014). miR-19b was found to be upregulated in the sperm of mice fed a Western-like diet, and the injection of synthetic miR-19b into normal fertilized eggs induced impaired glucose tolerance and insulin resistance in adulthood, which is similar to the diet-induced phenotype (Grandjean *et al.* 2015). This phenotype could also be inherited by the offspring after crosses with healthy partners (Grandjean *et al.* 2015), suggesting that injection of an exogenous miRNA may lead to alterations of metabolic traits. However, it is still unclear whether endogenous small RNAs are directly involved in the transmission of metabolic disorders.

Sperm tsRNAs and acquired metabolic disorders

Recent evidence has demonstrated that tsRNAs are biologically functional and are present in mature mammalian sperm (Peng *et al.* 2012). Analysis by deep sequencing reveals that tsRNAs are mainly 30–34 nt and are predominantly derived from the 5′ ends of tRNAs in mature mouse sperm (Peng *et al.* 2012, Sharma *et al.* 2016). Similarly, analysis of the sncRNA content in the spermatozoa of lean and obese men reveals that tRNA fragments are one of the most abundant sncRNA subtypes (Donkin *et al.* 2016). Therefore, it is possible that tsRNA is associated with metabolic alterations.

More recently, two research groups published exciting data on sperm tsRNAs. In one study, deep sequencing data showed that a large proportion of tsRNAs (11.53%) exhibited significant differences in sperm small RNAs (18–40 nt) between HFD and ND mice (Chen *et al.* 2016). Moreover, three types of RNA fragments with different lengths, 15–25 nt, 30–40 nt and >40 nt RNAs, from HFD and ND mouse sperm were separated, purified and then injected separately into normal zygotes to explore their potential effects. Interestingly, only offspring injected with 30–40 nt RNAs developed similar metabolic effects as were produced by the injection of sperm total RNA (Chen *et al.* 2016). These results demonstrate that sperm tsRNAs are necessary to reproduce the effect of sperm total RNA in inducing acquired metabolic disorder in offspring.

In the other study, researchers found that protein restriction in mice affects the levels of miRNAs and tsRNAs in mature sperm, which included decreased let-7 and increased amounts of glycine tRNA 5′ fragments (Sharma *et al.* 2016). tRF–Gly–GCC is one of several abundant tRNA fragments regulated by a low-protein diet. When RNA fractions (<40 nt) were purified from low-protein sperm and injected into control zygotes, it was found that RNAs from low-protein sperm could inhibit tRF–Gly–GCC targets in two-cell embryos, which indicates that paternal diet can affect preimplantation gene regulation through sperm tsRNAs. In addition, tRNA–glycine–GCC fragments repress genes associated with the endogenous retroelement MERVL, in both embryonic stem cells and embryos (Sharma *et al.* 2016).

Together, this data all suggests that tsRNAs contribute to the intergenerational inheritance of an acquired metabolic disorder. These studies not only provide concrete evidence for tsRNAs being responsible for the transmission of epigenetic information from one generation to the next in mammals, but also provide important insights into the relationship between sperm tsRNAs and epigenetic mechanisms.

RNA modifications and acquired metabolic disorders

Interestingly, injection with a synthetic combination of the most highly expressed sperm tsRNAs had no effects on offspring metabolism. The synthetic tsRNAs showed faster degradation rates in zygote lysates than sperm-derived tsRNAs did, which might be due to the modification of sperm tsRNAs (Chen *et al.* 2016). RNA modification in sperm tsRNAs were detected and quantified by LC–MS/MS; two RNA modifications, 5-methylcytidine (m^5C) and

N^2-methylguanosine, were significantly upregulated in HFD-group sperm compared with ND-group sperm (Chen *et al.* 2016). The modified nucleoside, 5-methylcytidine, has been reported to protect tRNA Asp^GTC, Val^AAC and Gly^GCC from endonucleolytic cleavage in *Drosophila* to maintain tRNA secondary structure (Schaefer *et al.* 2010). RNA methyltransferases, DNA methyltransferase-2 and NSun2 can methylate cytosine to form m^5C in tRNAs (Goll *et al.* 2006, Hussain *et al.* 2013). DNA methyltransferase-2 is required for the establishment and hereditary maintenance of *Kit* and *Sox9* paramutation phenotypes (Kiani *et al.* 2013). However, whether the sperm tsRNA modification or the enzymes which participate in tsRNA modification contribute to acquired metabolic disorders in offspring needs further investigation.

Future perspective

A series of studies have demonstrated that metabolic changes in fathers can affect the phenotypes of the offspring, and the process of intergenerational transmission is associated with alterations in DNA methylation, through histone modification and by the involvement of RNA populations (Rando 2016). However, the exact mechanisms remain to be further elucidated.

Finding elevated levels of RNA modification in HFD mouse sperm provides a clue to explaining the function of tsRNAs in mediating paternally acquired traits (Chen *et al.* 2016). RNA modifications may be the mediators of paternal intergenerational inheritance. It remains to be explored whether sperm tsRNA modifications participate in the transmission of meaningful information into the zygote. Injections of carefully designed tsRNAs with specific modifications into zygotes will be an important strategy. However, modification of tsRNAs, especially the type and position of modification in each tsRNA, is yet to be investigated. Whether and which RNA modifications in germ cells are responsible for altering behavioral and metabolic phenotypes in offspring is still an open question.

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