Editorial

Placental miR-340 expression: a key to gestational programming of activity-based anorexia?

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Comment on: Schroeder M, Jakovcevski M, Polacheck T, *et al.* Placental miR-340 mediates vulnerability to activity based anorexia in mice. Nat Commun 2018;9:1596.

Received: 30 August 2018; Accepted: 15 September 2018; Published: 17 September 2018.

doi: 10.21037/ncri.2018.09.01

View this article at: http://dx.doi.org/10.21037/ncri.2018.09.01

Anorexia nervosa (AN) is a severe eating disorder associated with the highest mortality rate among all mental disorders (1). However, despite its severity, the etiology of the disease is still to be better understood. Case-control studies indicate that women who experienced abuse in their childhood had a higher risk of developing an eating disorder later in life compared to controls without this life event (2). This association between early-life stress or trauma and psychiatric disorders is supported by a large cohort study (n=2,110,756) where prenatal and early-postnatal severe stress induced by the death of a close relative caused a higher risk of developing an eating disorder (3). It has been hypothesized that the placenta as key organ of maternal-fetal communication—besides its role to exchange nutrients and gases—mediates stress effects thereby influencing fetal neuronal development (4). Since trophoblast cells of the placenta originate from the embryo and therefore carry the sex of the fetus (XX or XY) (5), the placenta might contribute to sex-specific differences early on (6), possibly also in the development of AN known to affect females with a higher prevalence than males (1).

Further investigations have shown that numerous small non-coding RNAs, so called microRNAs (miRNA), are continuously released by trophoblasts of the placenta into the maternal blood (7). These miRNAs were originally found in the nematode *Caenorhabditis elegans* in 1993 (8) and regulate gene expression by inhibition of mRNA translation (9). A correlation between altered miRNA expression and anorexia has already been indicated in an anorexia mouse model showing that miRNA target genes were significantly upregulated in the hypothalamus and decreased in the cortex of anorectic mice (10).

Following up on these findings, Schroeder and colleagues further studied the etiology of AN investigating the origin of susceptibility to AN *in utero* by focusing on the placental miRNA expression using the activity-based anorexia (ABA) model (11).

Prenatal stress and ABA

ABA was established early on (12) and combines two core features of AN, namely food restriction and increased physical activity (13). Despite its limitations ABA is a well-established and so far best suited model to mimic AN in rodents.

When applying ABA to adolescent female mice, only 40% of the animals developed the ABA phenotype, while 60% were resistant to the protocol (11). In line with previous studies on hypothalamic dysregulation in AN (14,15), female ABA mice showed significant upregulation in gene expression for serotonin receptor (HTR) 1a (*HTR1a*) and hypocretin/orexin (*Hcrt*) and downregulation of agouti-related peptide (*AgRP*) and arginine vasopressin (*AVP*) compared to ABA resistant mice (11) giving rise to an involvement of these genes/gene products in the susceptibility to develop ABA.

Interestingly, prenatal stress caused ABA resistance in female mice as indicated by the fact that all males and

prenatally stressed females scored significantly lower regarding ABA parameters (food intake, total activity, body weight change, circadian disruption, days until collapse, recovery of food intake) compared to female controls (11). Furthermore, prenatal stress induced elevated maternal corticosterone (CORT) levels in ABA mice, likely associated with downregulated placental *11ßhsd*$_2$ gene expression, an enzyme that inactivates CORT (11). These results extend clinical data that indicated an association between elevated maternal cortisol levels during the late second and third trimesters and an increased cortisol response after a painful heel-stick blood draw (16).

The role of placental miR-340 in gene regulation

To further explore the effects of prenatal stress, several placental miRNAs were identified showing a pronounced downregulation of miR-340 following prenatal stress, where pregnant females were exposed to two stressors during the dark phase (e.g., multiple cage changes, immobilization for 30 min, swim stress for 15 min) and one further manipulation during the light phase (11). Located in the junctional zone of the placenta, miR-340 as part of the second intron of the *Rnf130* gene is regulated by gene body methylation. Since gene body methylation normalizes gene overexpression, this mechanism might be used in the treatment of cancer (17).

Next, a higher methylation on the CpG_2 sites of miR-340 was shown in the placenta of female prenatally stressed mice compared to placentas of non-stressed control females (11). Also, global DNA methylation was higher in females compared to males (11), indicating an impact of sex on the regulation of miR-340. This finding is in line with results of a previous genome study investigating the differences in methylation profiles in women with anorexia (n=29) and healthy controls (n=15) that showed elevated and less variable global DNA methylation patterns in women with AN (18).

Sex-dependent differences in ABA predisposition

The analysis of target genes for placental functioning in female mice that underwent prenatal stress indicated a significant upregulation of the glucocorticoid receptor (*GR*), cryptochrome *2* (*Cyr2*) and histone 3, family 3b (*H3f3b*) genes as well as their corresponding protein levels compared to controls (11). Interestingly, these gene and protein levels were inversely correlated with miR-340 expression levels (11). Glucocorticoids have been implicated in fetal development, especially in intrauterine growth or the risk of metabolic or neuroendocrine diseases later in life (19). It is to note that the fetal response to elevated glucocorticoid levels is sex dependent, resulting in hypertensive changes in females, while in males a glucocorticoid resistance has been observed (19).

When a female fetus shared space with only male *in utero*, miR-340 levels in the female fetus were significantly decreased compared to females accompanied by other females or females and males (11). This finding is likely associated with testosterone production shown to be produced by 18 days old male fetuses at high concentrations and released into the amniotic fluid (20). Taken together, this might indicate that prenatal androgen exposure and hence lower miR-340 levels on the one hand contribute to a decreased vulnerability towards ABA, and on the other hand give an explanation for the sex dependent differences in the incidence of AN.

The placenta as a gate to fetal developmental programming

One of the primary tasks of the placenta is to supply the fetus with nutrients, thereby allowing the offspring to grow and develop. Schroeder and colleagues investigated a possible correlation between placental miR-340 levels and the expression of placental nutrient transporters, focusing on the solute carrier (SLC) group, cholesterol transporters and the insulin-like growth factor (IGF) family. *Igf*$_2$ is a fetal signal regulating placental growth and function whose deletion resulted in reduced placental growth, decreased passive permeability and ultimately fetal growth restriction (21). Schroeder and colleagues detected an inverse correlation of nutrient transporters and expression levels of miR-340 which was shown in the junctional zone of the placenta, where high miR-340 levels lead to a downregulation of *Igf*$_2$ thereby reducing placental nutrient transport (11). This finding was further investigated in human placentas where also an inverse correlation of miR-340 levels and selective nutrient transporters was found (11). These data give rise to an impact of miR-340 on placental functioning in mouse as well as in human indicating that fetal nutrient acquisition is partly miR-340-dependent and can influence fetal development.

MiR-340 levels and vulnerability to ABA

These findings led to the assumption that overexpression of

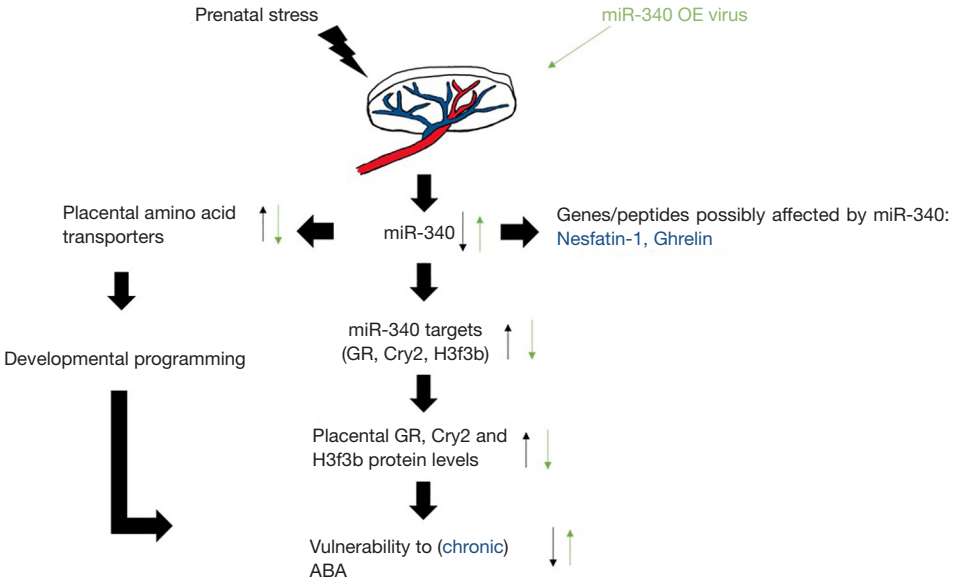

Figure 1 Effects of miR-340 on vulnerability to activity-based anorexia and gaps in knowledge. Black: prenatal stress causes a downregulation in placental miR-340 which is regulated by gene body methylation. MiR-340 target genes (*GR*, *Cry2*, *H3f3b*) and their corresponding placental protein levels are inversely correlated with miR-340 levels. However, not only prenatal stress but also male sex of an adjacent fetus lead to a downregulation in miR-340 that decreases ABA vulnerability. Hereby, developmental programming, caused by an inverse correlation of miR-340 levels and placental amino acid transporters, also influences the susceptibility to ABA. Green: conversely, an upregulation of miR-340 caused by a placental lentivirus overexpressing miR-340 results in increased vulnerability to ABA. Blue: gaps in knowledge. Further studies should focus on candidate genes/peptides involved in the regulation of food intake and body weight known to be altered in anorexia nervosa (e.g., nesfatin-1 and ghrelin) and investigate whether these are affected by miR-340 levels, and check alterations of miR-340 under conditions of chronic ABA. ABA, activity-based anorexia.

miR-340 might be associated with increased susceptibility to ABA. Transgenic mice of both sexes placenta-specifically overexpressing miR-340 induced by a lentivirus infecting the blastocysts' trophoblast cells underwent the ABA protocol by the age of 30 days. Indeed, in female mice threefold overexpressing miR-340 73.3% developed ABA compared to only 36.4% mice developing ABA in the control virus group (11). Interestingly, mi-R340 overexpression also increased the susceptibility to ABA in males increasing the number of ABA-prone animals from 12.5% (control) to 50% (miR-340 overexpression) (11). Therefore, elevated miR-340 levels lead to an increase in ABA vulnerability in both sexes.

In summary, Schroeder and coworkers provide key experiments highlighting the role of miR-340 as a target substance in the prenatal programming of ABA (*Figure 1*). Since these data were generated under rather acute conditions, it will be interesting to investigate whether miR-340 is also altered in a more chronic model of ABA which was recently established in rats (22). In addition, it will be important to study whether these changes also impact on major food intake-regulatory hormones such as ghrelin (23) or nesfatin-1 (24) known to be affected under conditions of ABA. Lastly, the regulation of miR-340 will have to be investigated in patients with AN along with the characterization of the exact mechanisms how maternal stress leads to a downregulation of miR-340.

Acknowledgments

Funding: This work was supported by funding of the German Research Foundation (STE 1765/3-2) and Charité University Funding (UFF 89/441-176, A Stengel).

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Dr. Jin Li (Cardiac Regeneration and Ageing Lab, School of Life Sciences, Shanghai University, Shanghai, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/ncri.2018.09.01). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/ncri.2018.09.01
Cite this article as: Kühne SG, Stengel A. Placental miR-340 expression: a key to gestational programming of activity-based anorexia? Non-coding RNA Investig 2018;2:53.

