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Epigenetic Inheritance and Environmental Stress: The Role of miR-212/132 in Transgenerational Behavioral

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Abstract:

This study examines the impact of parental stress on offspring behavior, focusing on the miR212/132 gene in mice. Behavioral tests and genetic experiments, including RNA analysis and CRISPR-Cas9 editing, reveal significant behavioral changes in offspring from stressed parents. The miR212/132 gene plays a key role in these changes, offering insights into how stress affects future generations.

Keywords: Parental stress, miR212/132, mouse behavior, gene editing, inheritance.

1. Introduction

Epigenetic inheritance is an important research area that shows how the environment can influence gene expression in future generations without changing DNA sequences. Many studies have been focused on microRNAs (miRNAs) because of their role in controlling gene activity after transcription. miRNAs are small non-coding RNAs that attach to messenger RNAs (mRNAs) and either break them down or stop them from making proteins, and change gene expression in response to environmental stresses [1].

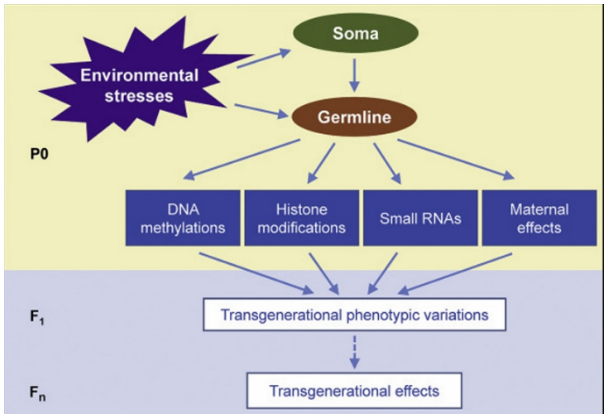

Fig.1 Environmental stresses can influence the phenotypes of offspring. Exposure to various environmental stresses can induce phenotypic changes in the parental generation that can then be transmitted to subsequent generations through the germline. [2]

More specifically, miRNA212/132 regulates synaptic plasticity, neuron growth and stress response, functions critical to the memory and learning in hippocampus. Environmental stresses that influence miRNA expression are among the many behavioural experiences that alter brain function [3]. As a result of these experiences, miRNAs also convey epigenetic information on to the next generation — experiences that parents directly affect their offspring [4]. Changes in miRNA expression represent an environmentally induced epigenetic memory mechanism between generations. DNA methylation and histone modification, likely the dominant mechanisms underlying this process, are outlined in Fig. 1. Also the role for miRNAs has yet to be established.

Specifically, our hypothesis holds that changes in hippocampal miRNA212/132 expression caused by environmental stressors would be transferred to sperm, impacting the following generation. Our hypothesis is supported by several emerging findings. It has recently been reported that miRNAs can be selectively packaged into sperm and other gametes, thereby facilitating epigenetic transgenerational inheritance. For example, specific miRNAs were found to 'co-opt' testicular germ-cell-specific spermiogenesis by being packaged into sperm and transferred to the next generation [5], a process also thought to occur with other reproductive cells. In addition, it appears that a number of miRNAs produced by the hippocampus are transferred to the sperm and other germ cells, and this involves complex cellular communication processes. For example, extracellular vesicles such as exosomes move a number of miRNAs that are packaged at the surface of these vesicles into the extracellular spaces between the cells of the reproductive organs (spermatogonia and testicular Sertoli

cells). Furthermore, because these vesicles can penetrate biological barriers and be internalized by distant cells including neurons and germ cells, their movement across biological barriers might also provide the mechanism for eventual transgenerational epigenetic inheritance [6,7].

In summary, this sequential interaction, which intergenerational environmental stress affects miRNA212/132 expression in the hippocampus and its potential escape to sperm, represents a new epigenetic inheritance mechanism. Hence, investigating this pathway might offer an opportunity to delve into how experiences affect traits in future generations and to understand the molecular basis of inheritance beyond DNA sequences.

2. Experiments and Expected Results

To test our hypothesis that changes in miRNA212/132 expression induced by environmental stress will cause transgenerational epigenetic inheritance, with miRNA being transferred from the hippocampus to sperm to the next generation, we designed a series of experiments. These experiments investigate behavioral changes, miRNA expression, and the underlying mechanisms of transgenerational inheritance.

2.1 Behavioral Assay Experiment

In the Cross Fostering Verification Group (Social Effect), P0 starvation/control groups will mate separately to produce four groups: (P0 control + F1 starvation), (P0 starvation + F1 control), (P0 male starvation + P0 female control), and (P0 male control + P0 female starvation) [8]. For P0 generation selection and training, C57BL mice will be selected with males weighing 30g (±10) and females weighing 25g (±10). These mice will be divided into two groups without interaction: the control group, receiving 6g of food per day, and the starvation group, undergoing 2 days of fasting followed by 1 day with 6g of food [9]. F1 and F2 generations will receive 6g of food per day before behavioral tests, with ethical approval obtained and adherence to ethical committee guidelines.

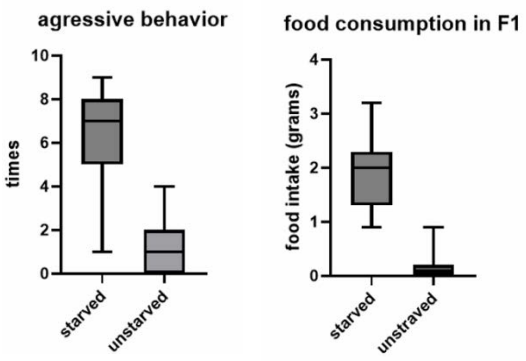

Fig.2 Expected differences of aggressive times and food consumption between starved and non-starved groups' offsprings.

In the aggressive behavioral assay, mice from different groups will be fed to full capacity with 6g of food. A food source will be placed in front of both groups, and the group with a higher propensity to attack others to obtain food will be observed [10]. In the choice index and extra food ingestion behavioral assay, after being fed 6g of food to full capacity, 20g of food will be placed in front of both groups, and extra food intake will be measured to assess the behavioral response. We expect that F1 mice from starved P0 parents will show behavioral changes compared to controls, indicating that parental environmental stress impacts offspring behavior, shown in Fig. 2. Starved mice are expected to show more aggressive behavior when competing for food and consume more additional food compared to controls.

2.2 miR212/132 and Other Potential miRNA Effect Verification Experiment

The goal is to identify target miRNAs and validate the feasibility of subsequent experiments, ensuring that behavioral changes are due to germline miRNAs [11]. RNA will be extracted from sperm of different groups to measure the extent of miRNA expression and identify up- and down-regulated miRNAs in sperm. For isolation of miRNA from testes, total RNA will be extracted from mouse testes using TRI reagent, miRNA expression measurements, clustering, and screening of miRNAs with LogMean RPM <1 will be performed using the UPARSE method [12]. Log2 fold change calculations and heatmaps will be used. We expect significant differences in miRNA212/132 expression between the control and starvation groups, confirming that environmental stress can affect miRNA levels in sperm.

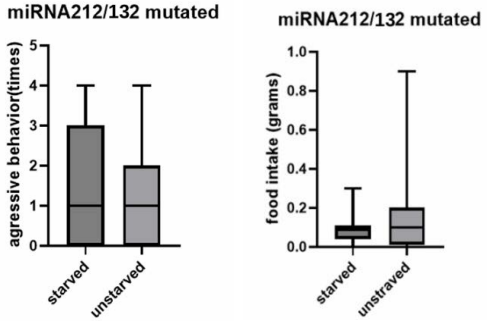

Fig.3 Expected differences of aggressive times and food consumption between starved and non-starved groups with deleted miR212/132 offsprings.

To determine the effects of miR212/132 on mouse behavior, miR212/132 and other potential miRNAs will be de-

leted using CRISPR-Cas9 in the F1 starvation group [13]. Behavioral analysis of transgenic mice will be performed. CRISPR-Cas9 will be used to delete the miR212/132 sequence and the treated sperm cells will be injected into the testes of P0 mice targeting the identified gRNA binding sites. Mice with deleted miR212/132 are expected to exhibit different behaviors than unmodified mice, as shown in Fig. 3, supporting a role for these miRNAs in mediating stress-induced behavioral changes [14].

2.3 Hippocampus-Sperm Communication Mechanism Experiment

This experiment aims to identify transgenerational epigenetic mechanisms and determine if miR212/132 is directly upregulated in sperm or hippocampus [15]. Single nucleotides in miR212/132 will be modified and injected into F1 and F2 embryos, and miRNA expression in sperm will be sequenced using rt-PCR to verify the changes. The sequences of miR-212 and miR-132 will be modified, the modified DNA will be synthesized, inserted into E. coli, then inserted into mouse fertilized eggs, and injected back into female mice [16]. Expression of miRNA in sperm will be sequenced using rt-PCR.

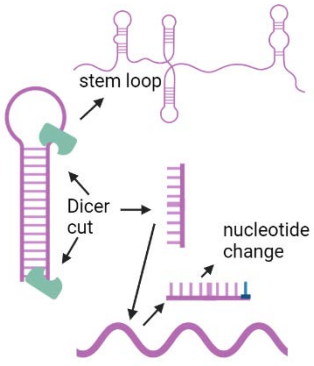

Fig.4 Process of a nucleotide change in mi212/132

cDNA synthesis will be performed using a reverse transcription kit, and miRNA levels normalized to RNU6B will be measured using miScript Primer Assays. RNA sequencing will detect altered miRNA 212/132 in sperm. We anticipate that modified miR-212/132 will be detected in sperm of later generations, indicating successful alteration and transmission, supporting the hypothesis of transgenerational inheritance. Accurate measurement of miRNA expression levels will confirm the presence of modified miRNAs. If miR212/132 modifications are detected in sperm, this would indicate direct communication between the hippocampus and sperm, supporting the mechanism of transgenerational epigenetic inheritance.

3. Discussion

Our test revealed that the offspring (F1-F2 generations) of mice from the starvation group had a greater amount of food intake under normal conditions. These findings provided evidence that epigenetic events may lead to adaptive behaviors in parts of progenies at future generations. By measuring and analyzing RNA expression through RNA-Seq and qRT-PCR, we confirmed that miR-212/132 was indeed up-regulated in the starvation group in the brains and sperm, providing additional evidence that miR-212/132 is a critical component to stress responses and behavioral changes. Through the implementation of CRISPR/Cas9 technology, we managed to mutate the miRNA sequence to further reveal the effect on miRNA function, proving miRNA can be transmitted from hippocampus to sperm. Figure 4. Graph showing the dose-dependent increase in qRT-PCR values reflecting higher level of miR-212/132 in starvation group, and the qRT-PCR result both support the data derived from RNA-Seq (Figure2C and Figure3C). These findings aligned with past research on how environmental factors can induce the hereditary epigenetic changes that affects behavior.

In future, understanding cross-generational mechanisms can also provide a rationale for personalized medicine; based on the family history and environmental background of the patient, doctors can develop personalized, disease-preventive techniques and treatments for the patient, alleviating the environmental stress of patients on their progeny, and thereby improve the effect of their treatments. Also the studying of cross-generational mechanisms can let doctors better design interventions to reduce the long-term effects of adverse environmental factors on human health. For example, after environmental stress such as natural disasters, wars, or economic crises, specialized health intervention programs can be developed to protect the health of affected populations and future generations. This knowledge can inform policies that minimize the impact of environmental stresses on public health, make early intervention and support for vulnerable populations [16,17].

4. Conclusion

This study highlights the role of miR-212/132 in transgenerational epigenetic inheritance under environmental stress. The transmission of miRNA alterations from the hippocampus to sperm underscores a novel mechanism for non-genetic inheritance. Future research should investigate the broader implications of this pathway in other stress-related conditions and its potential applications in personalized medicine.

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