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Environmental Stress Drives Transgenerational Epigenetic Inheritance Through miR-212/132 Expression Upregulation

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Abstract:

This is a research proposal on environmental induced transgenerational epigenetic inheritance. This work focus on pressure on food supply lead to a modification in miR-212/132 expression that result in an increased feeding of mice, and is inherited by the transfer of the miRNAs from hippocampus to sperm. Experiments including behavior tests, miRNA differential expression, gene knock-out, and nucleotide modification are proposed to prove the hypothesis. Once carried out, the research is going to help expand the understanding of transgenerational epigenetic inheritance, explore the cause of inherited obesity susceptibility, and contribute to public health.

Keywords: transgenerational epigenetic inheritance, environmental pressure, miRNA, obesity susceptibility.

1. Introduction

Research on transgenerational epigenetic inheritance (TEI) shows the statement that we are decided by what our parents eat might be true. According to Horsthemke [1], TEI is defined as a transgenerational propagation of epigenetic information through germline. Study shows the offspring of people who have been through the Dutch famine tend to have higher risk of obesity [2]. This is an indication of environmental stress induced transgenerational behavior that thrust TEI into the public spotlight, compelling people to take notice of this issue. However, current understanding on the matter remains incomplete, and the mechanism underlying this inheritance is unclear [3]. The proposed research is necessary since it is going to contribute to the knowledge of TEI in the scientific field, and improve public health through possible prevention of inherited obesity and other related health problems.

According to Zhang et al. [4], environmental stress, specifically food supply, is going to cause transgenerational inheritance through various animals including mice. The offspring of starved mice also show an increased rate of obesity and other metabolic disorders. On the other hand, miRNAs are small non coding RNA sequence that function through binding with target gene sequences and resulting in the silence of the gene [5]. And it is known that changes in miRNA212/132 expressions are responsible for a transgenerational cognitive enhancement in mice, and their concentration are increased both in brain hippocampus and sperm in the mice having the inheritance [6].

Based on discoveries mentioned above, this passage shed light on how the inheritance increase obesity risk, whether starvation induces a transgenerational inheritance on change in feeding behavior, whether miRNA212/132 are also involved in this inheritance, and the pathway of how miRNA transfer into sperm and to the next generation. The passage hypothesized that the raised rate of obesity is induced by a tendency of overfeeding, miRNA212/132 are responsible for the starvation induced transgenerational overfeeding behavior of mice; they are made in hippocampus and transferred into sperm to the hippocampus of the next generation. This paper first describes the experimental process and method, including the process and method for behavioral assay experiment, miR212/132 effect verification experiment, and hippocampus-sperm communication mechanism experiment. The paper then illustrate the expected results gained from the experiments, followed by the discussion section.

2. Research Design and Methods

2.1 Behavioral Assay

To verify whether stress in the parental generation's food supply results in a transgenerational inheritance of an increased tendency to store energy compared to normal parents, a behavioral assay evaluating overfeeding and food hoarding behavior will be conducted.

Thirty C57BL adult male mice weighing 30g(±10) and thirty adult female mice weighing 25g(±10) are selected [7] . They are then divided randomly into two groups, with

each group containing 15 males and 15 females. Each mouse is housed individually in its own cage. The control group receives a normal food provision of 6g per mouse per day. The experimental group undergoes a deficit food provision regimen of 2 days of fasting followed by 1 day of 6g food provision in a repeating cycle. The two groups are prohibited from interacting between groups.

A month later, the mice within each group are allowed to mate. To minimize potential social effects, the F1 generation offspring are cross-fostered [8], with the experimental group's descendants being nourished by the control group in individual cages, and the descendants of the control group being nourished by parents from the experimental group. The F1 mice will be put into the cage of parents from another group as soon as they were born, and they will be prohibited to attach to their biological parents. (fig. 1).

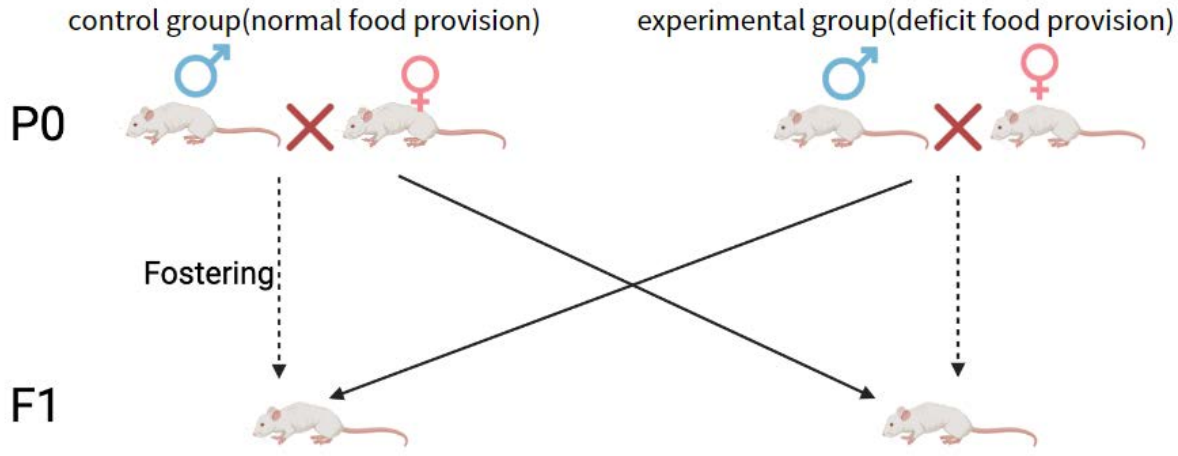

Figure 1. In group mating and cross fostering of P0 mice

Both two groups of descendants will be fed with normal food provision. After the F1 generation mature, they will undergo the behavioral test.

Two groups of F1 mice are fed with 6g of food, which makes sure they are full and store enough energy before the test. After that, 20g of food is offered in front of each cage, and the food extra intake will be measured in grams. After a 3-day period of normal food access, the mice from the control and experimental groups are placed separately in two shared arena. A 20g food source is positioned at one side. The times of mice trying to attack the other for the food source will be recorded.

2.2 Determining miRNA212/132 function

2.2.1 miRNA Gene Differential Expression

In order to specify the target miRNA whose expression is affected by starvation and determine whether miRNA212/132 are involved, the extent of miRNA expression is measured and the difference between P0 and F1 control and experimental group will be tested through differential expression experiment [9].

The total RNA will be extracted from mouse testis using the TRI reagent (T9424, Sigma). miRNA expression will then be measured using the UPARSE method, which involves generating miRNA clusters (operational taxonomic units, OTUs) from the next-generation sequencing reads of small RNAs with identical sequences. The sequences were assigned to these clusters, and the expression levels were summed up based on the clusters. Afterwards, miRNA genes with a LogMean RPM (reads per million) less than 1 were filtered out. Finally, log2 fold change calculations were performed, and the results were visualized using a heatmap. The two heatmap of experimental group and control group will be compared and the difference will be marked out to find the target miRNAs.

The experiment is able to verify whether there is an increased expression in the parental hippocampus and sperm of experimental P0 and the hippocampus of F1.

2.2.2 CRISPER-Cas9 experiment

CRISPER method is used to knock out the miRNA212/132 to prevent them from normal functioning. After that, the same behavioral assay will be carried out and determine whether the inheritance still exist on the mutated mice. This experiment aim to prove that miRNA212/132 is essential for the inheritance.

In CRISPER-Cas9 technique, specific sequences are used to delete the target gene. The gRNA sequences targeting miR-212 and miR-132 need to be designed. For miR-212, the target sequence is 5'-AAGGTGCCGGCGCGCTGCCC-3' with the PAM sequence GGG. For miR-132, the

target sequence is 5'-CCGCGTCTCCAGGGCAACCG-3' with the PAM sequence TGG. After that, a CRISPR-Cas9 plasmid construct containing these gRNAs will be assembled. This CRISPR-Cas9 system is then transfected into spermatogonial stem cells, which will introduce a double-strand break in the miR-212/132 gene sequence, resulting in its deletion. The modified stem cells are then injected back into the testes of P0 mice, and will integrate into the testicular tissue and produce sperm lacking the miR-212/132 gene.

Finally, the P0 mice are bred to generate the F1 generation of miR-212/132 knockout mice. Behavioral assays can then be performed on these F1 mice to study the effects of miR-212/132 deletion.

2.3 Hippocampus-sperm communication mechanism

To verify the pathway of the transgenerational miRNA transport, the qRT-PCR technique will be used to determine the relative expression and therefore the upregulated sites. It is expected to find the upregulated miRNA212/132 expression in the hippocampus and the sperm. After that, a modification in a nucleotide of the miRNAs will be used as an identifier to determine whether the increased amount of miRNA are made in hippocampus and transported into sperm and the next generation. For miR-212, the sequence is designed to be edited from 3'-UAACAGUCUCCAGUCACGGCCA-5' [10] to 3'-UAACAGUCACCAGUCACGGCCA-5'. And for miR-132, the sequence is modified from 3'-UAACAGUCUACAGCCAUGGUCG-5' [11] to 3'-UAACAGUCAACAGCCAUGGUCG-5' to make sure the miRNA can be notified through RNA sequencing without losing its normal function, since the section it used to bind with its target is preserved. The modified sequence is injected into the zygotes, which will grow into mutant mice.

To make sure that the modified miR sequence will only be expressed in hippocampus, the genetic pathway is designed as fig.2. After the same behavioral training, the sperm of the experimental P0 group mice and the hippocampus of the F1 descendants of them will undergo RNA sequencing analysis to determine whether the modified miR-212/132 are present in the sperm and hippocampus of descendants, and thus whether these miRNA originate from the hippocampus and is transferred into hippocampus of the next generation.

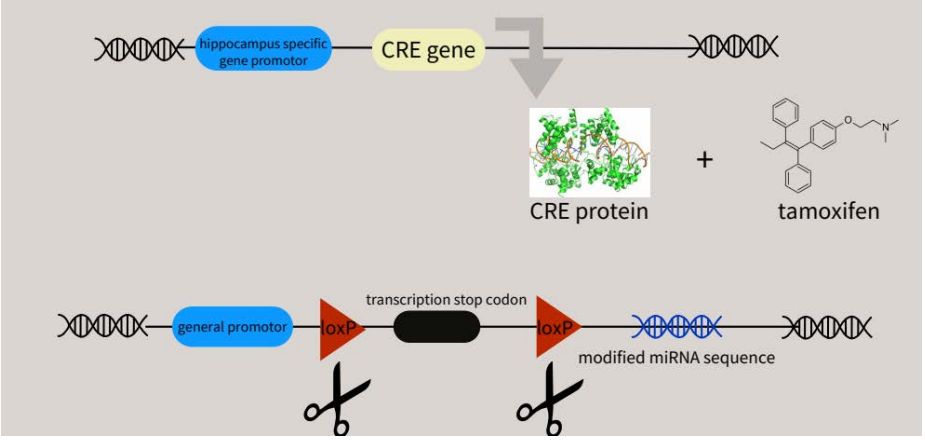

Figure 2. Genetic Pathway of Modified miRNA sequence

3. Expected Results

There are few results expected to get from the research procedure illustrated above.

The behavioral assay is expected to find that the descendants of starved parents show more food intake and more times of aggressive behavior compared to the control group (fig. 3). This indicates an inherited tendency towards excessive feeding and aggressive behavior on preserving the food, triggered by environmental stress due to inadequate food supply.

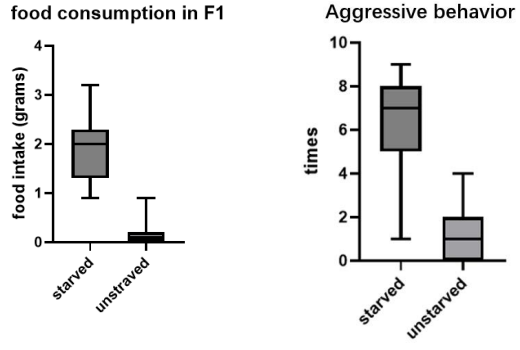

Figure 3. F1 generation of experimental

group show excessive feeding and aggressive behavior

On determining miRNA212/132 function, the differential expression test is expected to find an upregulated expression of miR-212 and miR-132 in hippocampus and sperm cell of the starved parents and in hippocampus of F1. This will prove that these two miRNAs are affected by the starvation. On the other hand, the CRISPER-Cas9 knock out of miR-212 and miR-132 is thought to lead to a disappearance of the inherited overfeeding and attacking behavior(fig. 4). Specifically, as the unmutated starved F1 mice would show increased food intake and times of conducting aggressive behavior compared to control group, the mutated descendants of starved mice is expected not to show the difference in the two indexes, which is going to prove that miRNA212/132 are involved in the inheritance.

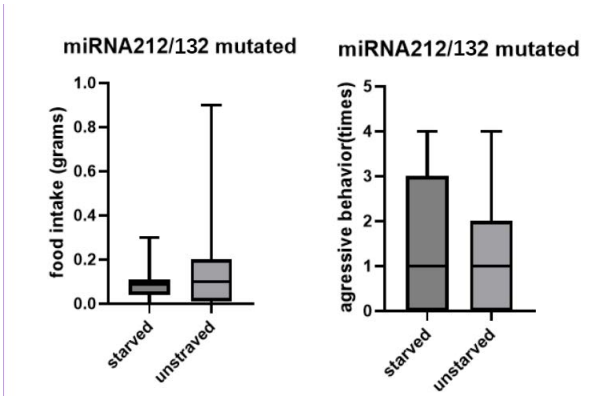

Figure 4. mutated F1 are expected not to have a behavioral difference between two groups

In addition, the modified miRNA is expected to be found both in the sperm of starved P0 males and the hippocampus of their descendants through RNA sequencing.

These results will back up the hypothesis that starvation-induced changes in miRNA-212 and miRNA-132 expression in the hippocampus can be epigenetically inherited through transferring into sperm, leading to transgenerational overfeeding behavior in mice.

4. Discussion

The research proposed is expected to prove that starvation-induced changes in miRNA-212 and 132 expression will lead to excessive feeding in mice descendants, which is inherited through the transfer of these miRNAs from hippocampus to sperm. The result will contribute to the field of environmentally induced transgenerational epigenetic inheritance, expand the understanding of miRNA function, and explore the cause and prevention of inherited obesity susceptibility, and improve the general public health [12]. The proposed hypothesis is based on solid research findings and background knowledge, yet it has several limitations. The difference between individual mice is an unavoidable factor for error to exist, and the environment is unlikely to be held strictly the same [13], resulting in a possibility of deviation among the experiment results. Aside from that, the off-target effect of CRISPER-Cas9 which cause errors in knocking out the nontarget gene is another cofounder that may interfere the experimental effect of miR-212/132 mutation [14]. Therefore, it is arguable that the results might not be so accurate to prove the hypothesis. In addition, The research proposed will not explain the pathway and mechanism of how the miRNA transfer from hippocampus to sperm. The concrete function of how the miRNA212/132 in hippocampus lead to the overeating behavior is not clarified as well. It is presumed that the miRNAs, though in a little amount, is going to modify the gene expression of the next generation and lead to an inherited raise in miRNA concentration in hippocampus [15], but the theory is also not proven. Therefore, though the research proposed is going to illustrate how the miR-212 and miR-132 upregulation and hippocampus-sperm transportation lead to the inheritance of obesity susceptibility, it has several aspects to improve and further research is still needed for the specific underlying mechanism. Study on the unsolved questions mentioned above is recommended.

5. Conclusion

In conclusion, the study wishes to explore the cause of inherited obesity susceptibility and the function of miR-212/132 in the hypothesized cause of transgenerational epigenetic inheritance of feeding behavior induced by starvation as an environmental stress. In addition, the study also want to acknowledge the transmission of the miRs from hippocampus to sperm. If the expected results are found, they will prove that the increase in obesity rates is linked to a tendency to overeat, and miRNA212/132 plays a role in starvation-induced transgenerational overfeeding behavior in mice. It will also back up the hypothesis that these molecules are produced in the hippocampus and transferred to the sperm, affecting the hippocampus of the next generation. Whilst the study has also flaws and vague, as we do not hypothesize causes of obesity susceptibility otherwise. Also, ambiguity and potential affecting factors involved in experimental techniques also cause possibilities of errors. Future studies should address these gaps to develop a clearer understanding of the pathways involved. Ultimately, further studies on these unresolved questions will deepen insights into inherited obesity risks and inform strategies for improving public health.

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