miRNA Might Mediate Intergenerational Inheritance of Enhanced Synaptic Plasticity by Alterations of Gene Expression

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ABSTRACT

In a previous paper, intergenerational inheritance of changes in synaptic plasticity, which was related to the increased level of microRNA212/132, was found in mice. A hypothesis about a specific mechanism is made that the increased miRNA212/132 can transfer from adult male mice's hippocampus to their sperms through the circulatory system. Then the miRNA changes the gene's expression level related to mouse embryos' neurodevelopment to alter synaptic plasticity. This hypothesis indicates the connection between epigenetics and neuroscience. This work comes up with several experiments to verify the hypothesis made and other possible precise mechanisms for intergenerational inheritance of enhanced synaptic plasticity in mice.

Keywords: microRNA212/132, sperm, enhanced synaptic plasticity

1. INTRODUCTION

Universally, animals' phenotype will change after exposure to certain environmental stimuli, usually aversive stimuli, such as chronic diseases or early life stress, that can give rise to depressive-like behavior (Gapp et al.,2018)[1]. Besides, some of these alterations can be passed down to their offspring and even more following generations through non-genetic mechanisms (Bale, 2017[2]), which means the genotypes and DNA sequences are not changed, known as epigenetics.

This kind of transgenerational inheritance, especially transgenerational memory, has been detected in several animals. It involves several aspects, including but not limited to olfactory imprinting and fear memory in mice, heat stress effect, and avoidance behavior to bacterial pathogens in C. elegans (Zhang et al., 2021[3]; Moore et al.,2021[4]; Dias and Ressler, 2014[5]).

Noticeably, in a research paper, the enhanced mice synaptic plasticity after environmental enrichment (EE) that depends on RNA was just intergenerational inheritance instead of transgenerational inheritance expected before experimenting.

According to this paper, environmental enrichment combines physical and mental exercise, increasing cognitive abilities in mice and humans. Particularly in that experiment, toys like tunnels, housing, and differently shaped objects were placed in the cage of the EE group, and there was daily replacement and rearrangement of the toys. The offspring of male mice subjected to EE also show a similar increase. This effect is dependent on the altered expression of sperm RNA and especially microRNA212/132 (Benito et al., 2018[6]).

Although the research paper mentioned above has already discovered the overall process of RNA-dependent intergenerational inheritance of EE-induced enhanced synaptic plasticity, its precise mechanisms remain to be identified. Hence, this paper will make assumptions of precise mechanisms and give proposals of corresponding experiments that can be used to verify these assumptions based on the previous research that mainly focused on transgenerational inheritance through sperm.

2. POSSIBLE RESULTS

Although increased miR212/312 due to EE has been detected in both neurons in the hippocampus and sperm of mice, how the environmental enrichment can impact sperm RNA remains unidentified, as theoretically, EE will not increase miRNA level in sperm directly. The previous study hypothesized that increased miR212/132 expression in sperm might be the result of the increased miR212/132 expression in other tissues, such as the brain (Benito et al., 2018[6]), since brain-derived exosomes that carry miRNAs were detected in the circulation of mice before (Shi et al., 2014[7]). The relevant data also shows that increased miR212/312 expression first appeared in the hippocampus of mice after two weeks of EE, but it takes ten weeks for sperm miR212/312 to increase (Benito et al., 2018[6]).

Along this line, the reason why environmental enrichment alters miR212/132 expression level in sperm is likely to be the transfer of miR212/132 through the circulatory system in mice. Radioisotope labeling will be used to verify this hypothesis to trace miR212/132 in the hippocampus. First, adult male mice in the EE group will undergo ten weeks of EE training, while no extra conditions will be

added in the home-caged (HC) group. The EE training process is the same as in the previous study. Second, a large amount of miR212/132 will be replicated by PCR, the ribonucleotide ingredients, which will include four types of ribonucleotide labeled by 32P. Then, the labeled miRNA will be injected into the hippocampus of the EE hippocampus group and HC hippocampus group mice. At the same time, the labeled miRNA will also be injected into the veins of the EE and HC vein group mice. Both hippocampus slices, sperm, and blood extract of four groups of mice will be observed and analyzed ten weeks after injection. During this period, mice from both groups will be kept in the same conditions.

The purpose of tests on the hippocampus is to ensure the effectiveness of injection, whereas the observation of blood extract and sperm is to discover the transfer of miR212/132. The HC control group is set to avoid the effect of the potential negative feedback mechanism on experimental results. If the hypothesis is correct, for the hippocampus injection group, miR212/132 labeled by 32P will show up in the circulation system and sperm for both EE and HC groups. For the veins injection group, miR212/132 labeled by 32P will be detected in sperm in both EE and HC groups.

Besides, the subsequent function of sperm RNA is also not identified. In the previous study, there was a hypothesis about the role sperm RNA played in transmitting EE-induced intergenerational brain enhancement from F0 to their adult offspring. It said that the sperm miRNAs changed the mice's brain plasticity by altering gene expression levels when the embryos were still developing (Benito et al., 2018[6]).

To confirm this hypothesis, further observation of the brains of mouse embryos is suggested. First, the F0 mice were treated the same way as the previous study (EE adult male mice will undergo ten weeks of EE training, while no extra conditions will be added in the HC group). Next, the male mice from both groups will mate with home-caged females kept in other cages. Then, when the embryos develop into E9.5-E,13.5 stage, at which the nervous system of mouse embryos is relatively mature, for both groups, some of them will be picked out randomly and tested for synaptic plasticity in brain neurons and the field EPSP(Excitatory postsynaptic potential), as well as detect the miR212/132 level. All the data will be recorded. The same test will also be done on the remaining F1 mice when they grow into adults. If the hypothesis is correct, increased synaptic plasticity and higher miR212/132 levels will be found in the F1-EE group compared with the F1-HC group.

Since paternal behavior might have an impact on their offspring to some extent due to the social interaction of mice if all of them are caged together, to exclude the possibility of potential social behavior influences between F0 and F1 (Dias et al., 2014[5]), all the F0 male mice should not see their offspring during the process of experiments. At the same time, to prevent other factors from interfering with experiments, all the F0 males will also be separated from F0 females after mating in this experiment.

3. DISCUSSION

If no phenomenon mentioned above shows in the experiments about the transfer of miRNA212/132, then the mechanism behind it might be a more complex one; for instance, there can be some other factors that can transfer information between the somatic cells and sperm, leading to the increased miRNA212/132 level in sperm by activating the transcription of miRNA212/132 in sperm.

Precise mechanisms behind enhanced synaptic plasticity of the treated mice offspring will be different; for example, the alteration brought by sperm RNA may be made after birth instead of during the embryonic development period in conception. Hence, similar experiments can be performed to verify the new hypothesis, like doing the same test on mice a few days after birth or when they just grow into juveniles. To further study this mechanism, the expression levels of genes related to brain neurodevelopment in mouse embryos must also be tested to determine the expression levels of which genes are increased or decreased by miRNA212/132.

Moreover, what is unique in the previous paper is that the inheritance of enhanced synaptic plasticity is only intergenerational. The research team gave two explanations for it. One is that, from an evolutionary point of view, non-genetic inheritance can bring certain benefits in demanded situations but vanish when the environmental settings rechange so that the animals can better adapt to the new environment (Benito et al., 2018[6]). Another one is that overhigh plasticity can lead to aberrant neuronal activity that is correlated with neurodegenerative diseases (Fischer et al., 2005[8]), which means that synaptic plasticity is risky itself, making it a burden when this benefit is not so significant, and the environment is not that demanding. Therefore, the phenotype of enhanced synaptic plasticity is likely to vanish itself. As it is not easy to breed mice with EE on a large scale and keep tracking the neurogenerative rate of F0, F1, and F2, the validity of these explanations remains to be verified.

4. Conclusion

In conclusion, EE training in adulthood provides a cognitive benefit to the individual mouse undergoing this

procedure and its offspring. Possible mechanisms of the intergenerational inheritance are as follows:

There is an increase in the level of miRNA212/132 in adult male mice's hippocampus after environmental enrichment training. Then, the increased miRNA212/132 might transfer from the hippocampus to sperms through the circulatory system. Then the miRNA may change the gene expression relevant to mice's brain neurodevelopment in embryos after fertilization. Therefore, the synaptic plasticity of the offspring, whose fathers undergo environmental enrichment training, is enhanced.

However, it is not clear if the same mechanisms exist in humans (Benito et al., 2018[6]). If environmental enrichment training can also make a significant difference in human synaptic plasticity intergenerationally or even transgenerationally, people's quality of life may improve by taking advantage of this mechanism. Apart from that, there will be more scientific evidence to back up various proposals of exercise, increasing their validity; it can eugenically guide couples who decide to have a child as well, since it reveals the significance of male's preparation for reproduction, while traditionally female's importance of pregnancy was focused more. Therefore, relevant experiments and investigations can be conducted to determine whether EE training has intergenerational or transgenerational effects on humans.

However, there seems to be little research about humans' transgenerational memory like the one mentioned above. A reason for this may be that it is impossible to treat humans in the same way that we treat the mice, suggesting that we are not able to force someone to live a designed life and see what will happen next to this person and his or her children, which is unethical and immoral. However, relevant experiments are still required to learn more about humans' transgenerational memory, which can play a crucial role in future studies of the mechanisms of some genetic diseases. A possible solution to the dilemma is required. It can be indirect research, which might be acceptable and doable, like doing a social investigation on a large scale or performing experiments on primates.

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