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The effect of DNA polymeraseβon autism tasted by aflatoxin B1

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Abstract. Purpose: Since aflatoxins B1 can cause autism in mice by disrupting DNA repair and altering the stability of the genome, the study aims to see whether DNA pol β alleviate autism tasted by AFB1, because of its DNA repair function. What's more, the study would identify whether aflatoxins gene damage can be repaired by DNA polymerase β. **Methods:** In this study, four groups of BTBR mice were selected. The positive group was normal BTBR mice, the other group was AFB1-infected mice, and the experimental group was AFB1-infected mice treated with DNA polymerase β, the negative group was AFB1-infected mice which were treated with blank vector. RT-PCR was then used to detect changes in transcript levels as an indicator of whether DNA polymerase β could be used to treat aflatoxins autism. **Possible results:** There are three main possible results: (1) DNA polymerase β can cure autism completely. (2) DNA polymerase β can cure autism partially. (3) DNA polymerase β has no therapeutic effect on autism. **Conclusion:** The results will provide important insights into the treatment of autism with DNA polymerase β. It will increase our understanding of DNA repair function to improve people's understanding and improve human health. It also points a way to develop clinical treatments for people in autism with aflatoxins.

Keywords: DNA polymeraseβ, autism, aflatoxin, DNA repair, DNA damage.

1. Introduction

Autism, also known as autistic disorder (AD), is a representative of pervasive developmental disorder (PDD). The disease generally begins within 36 months and is mainly manifested by three core symptoms, namely: social communication disorders, communication disorders, narrow interests and stereotyped and repetitive behaviors. [1-3]. Autism was first clinically described in the 40s of the 20th century. In 1943, the American physician Kanner reported 11 cases and named them "early infantile autism." [4].

Many genetic alterations are associated with autism and non-autism, and many genetic alterations in autism are associated with DNA repair. The genes like ATRX, PTEN, if their transcription or splicing process is damaged, that will affect DNA repair function [5-7]. In many previous experiments, scientists have determined that the behavior of autism in animal models is related to the patient's associated symptoms [8].

Aflatoxin B1 (AFB1) is a type of aflatoxin, which is the most hepatotoxic and carcinogenic difuran cyclic toxoid, and aflatoxins are common in maize animal feed [9,10]. Several different deleterious effects due to fungal toxin exposure are similar to comorbidities of autism [11-13].

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DNA polymerase β (Pol β) is a monopepteptase found in eukaryotes with a length of 39 kDa. DNA Pol β has enzymatic activity suitable for base excision repair (BER) and other related DNA synthesis [14]. Normally, DNA Pol β can Identify the missing base at the AP site and insert the correct base at that site to fill in the missing base. With 5'-phosphatase activity, it can shear the phosphate backbone of AP site, remove AP site and form a broken 3'-hydroxy end [14]. It helps maintain the integrity of DNA and prevent fatal mutations in cells by inserting new bases and repairing DNA strand breaks. This makes DNA Pol β one of the indispensable enzymes in the DNA repair process.

Effects of aflatoxins or other environmental agents lead to DNA damage, resulting in an increase in apurinic/ apyrimidinic AP sites. The BER pathway of DNA polymerase β can remove simple base damage and AP sites [15], so DNA polymerase beta could, in theory, reduce autism caused by AFB1.

Bax is a member of the BCL-2 family and plays a key role in apoptosis (apoptotic process) [16]. In past studies, scientists have found abnormalities in apoptosis in brain tissue from people with autism [17], leading to research into whether Bax is related to autism. Specifically, studies have shown that Bax expression levels may be abnormal in brain tissue of people with autism. Bax is a protein that promotes apoptosis. When cells are stimulated by internal and external stimuli, Bax is upregulated, leading to apoptosis. Therefore, the researchers speculate that if Bax is abnormally expressed in the brain tissue of patients with autism, it may lead to neuronal apoptosis, thus affecting the normal development and function of the nervous system [18].

p53 is a well-known tumor suppressor protein, usually associated with cancer and apoptosis [19]. Its expression and function have also been studied in other diseases and disease models, including some neurodevelopmental and nervous system-related diseases [20]. Potential considerations for the association of p53 with autism include: development and function of the nervous system; and abnormal expression of genes regulated by the immune system [20,21].

Ogg1 is an enzyme in the BER pathway, which is responsible for repairing Base Excision Repair Eduardo Gonçalves de Oliveira (8-oxoG) damage caused by oxidative damage to DNA [22]. Ogg1's function in cells is to protect the genome from oxidative DNA damage, which is linked to a number of neurological disorder, including autism [22].

However, a study has shown that overexpression of DNA polymerase β can cause spontaneous mutations in cells, increase the genetic instability of cell lines, and decrease the sensitivity to anticancer drugs, all of which need to be noticed when using it [23].

Based on the above research introduction, I predict expression of DNA Pol β enzyme could decrease 3 measurements of autism. the negative group is vector only control and the positive group would be non-autistic mice. Introduce DNA Pol B by liposome transfection.

2. Methods

2.1. Mice

Male BTBR mice aged 7 weeks and 23±2 g were used in this study. The mice are housed in a 22±2◦C room with 50% relative humidity, a light-dark cycle of 12 h, and access to standard animal food and water.

2.2. Treatment

The positive group was a group of mice that did nothing. The experimental group was that: the wild-type pol β recombinant fluorescence vector pEGFP-C3-pol β was transfected into mouse NIH3T3 cells by liposome transfection, and a cell line stably expressing Pol β was established. At the same time, AFB1 was dissolved in corn oil and 1.25 mg/kg was administered orally every day for 28 days. Negative control mice receive AFB1 and corn oil (vector) only. Another group of mice, which were set up to infect only AFB1, was used to indicate successful modeling in the model animals. The AFB1 dose in this experiment was non-lethal (LD50 = 40.41mg/kg). Mice are sacrificed 1 day after the last AFB1 introduction and brain tissue samples are extracted, and RTPCR is performed The P values were all less than 0.05 by ANOVA test.

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2.3. RT-PCR Analysis

RT-PCR separately performed the expression level of 3 transcript: Bax, p53 and Ogg1 [17]. The P values were all less than 0.05 by ANOVA test.

Table 1. Primers used for RT-PCR.

Transcript	Forward Primer	Reverse Primer
Bax	5'-ATGGAGCTGCAGAGGATCAT-3'	5'-GATCAGCTCGGGCACTTTAG-3'
p53	5'-CACAGCGTGGTGGTACCTTA-3'	5'-TCTTCTGTACGGCGGTCTCT-3'
Ogg1	5'-GATTGGACAGTGCCGTAA-3'	5'-GGAAGTGGGAGTCTACAG-3'

2.4. Statistical Analysis

A large number of repeated experiments have shown that the method of quantitative RTPCR is statistically significant. The P values were all less than 0.05 by ANOVA test.

3. Results

Results of indicate group: The predicted indicate group results are from the literature [17]. Bax and p53 expression levels, which compared to the positive group BTBR mice, in the control mice exposed to AFB1 were significantly up-regulated, while the expression levels of and Ogg1 were slightly increased. $p < 0.01$.

Table 2. Possible Results.

Transcripts	Result 1	Result 2	Result 3	Result 4	Result 5	Result 6	Result7	Result 8
Bax decreases by PCR	+	-	+	+	-	-	+	-
p53 decreases by PCR	+	+	-	+	-	+	-	-
Ogg1 increases by PCR	+	+	+	-	+	-	-	-
Supporting Hypothesis	**YES**	**Partially**	**Partially**	**Partially**	**Partially**	**Partially**	**Partially**	**NO**

"+" Represents the Transcript Level Was Higher than Negative Group and Similar as the Positive Control;"-" Represents the Transcript Level Was the Same as the Negative Control and the Opposite of the Positive Control

Possible experimental group results compared to negative group:

Result 1: Bax and p53 expression levels were lower than the negative group, and Ogg1 expression level of was higher than that of the negative group.

Result 2: p53 expression level was lower than the negative group, and Bax and Ogg1 expression levels were higher than the negative group.

Result 3: Bax expression level was lower than the negative group, and p53 and Ogg1 expression levels were higher than the negative group.

Result 4: Bax, p53 and Ogg1 expression levels were lower than the negative group.

Result 5: Bax, p53 and Ogg1expression levels were higher than the negative group.

Result 6: Bax expression level was higher than the negative group, and p53 and Ogg1 expression levels were lower than the negative group.

Result 7: Bax and Ogg1 expression levels were lower than the negative group, and p53 expression level was higher than the negative group.

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Result 8: Bax and p53 expression levels were higher than the negative group, and Ogg1 expression level was lower than the negative group.

Possible experimental group results compared to positive group:

Other possible result:

Result 7: The results of the indicate group are not exactly the same or completely different from the expected results, then it is suggested that this study failed modeling an animal model, the results of the experimental group no longer have experimental significance, this study cannot get meaningful results.

4. Discussion

Previous studies have shown that mutations in DNA repair genes (e.g., p53) produced by AFB1, which may exacerbate the damage of genome in long-term-AFB1-exposed cells [19]. At the same time, many animal studies have linked damage to repair pathways with autism [5,24,25].

Results of indicate group: The predicted indicate group results are from the literature [17]., Bax and p53 expression levels, which compared to the positive group BTBR mice, in the control mice exposed to AFB1 were significantly up-regulated, while the expression level of Ogg1 was slightly decreased, $p < 0.01$. These results suggest that the instability of the genome of BTBR mice infected with AFB1 and the mechanism of worsening apoptosis may lead to insufficient repair of damaged DNA [17].

Result 1: This result suggests that DNA pol β can effectively attenuate the AFB1-induced changes in the level of each transcript in autistic mice, which is exactly in line with my hypothesis. This suggests that DNA pol β is the most major enzyme in the DNA damage repair in AFB1-autism. The major reason of this is that repair mechanisms may be related to the transcripts Bax, p53, Ogg1. And the specific mechanism needs to be further studied in the future. This result has great scientific implications for future treatment of aflatoxins autism.

Result 2,5,6: These results suggest that DNA pol β can repair some of the AFB1-induced changes in transcript levels in autistic mice, partially supporting my hypothesis. In the results, DNA pol β was not shown to restore the index Bax. This suggests that DNA pol β is a secondary enzyme in the DNA damage repair in AFB1-autism. DNA pol β may not be sensitive to Bax in the process of its repair, or it may fail to perform the normal function of base repair, which may be hindered by other reasons. Although research on Bax and autism is ongoing, the field is still evolving, so more research is needed to verify whether Bax can be used as a specific marker for autism in the future. These results have scientific implications for future treatment of aflatoxins autism.

Result 3,5,7: These results suggest that DNA pol β can repair some of the AFB1-induced changes in transcript levels in autistic mice, partially supporting my hypothesis. In the results, DNA pol β was not shown to restore the index p53. This suggests that DNA pol β is a secondary enzyme in the DNA damage repair in AFB1-autism. DNA pol β may not be sensitive to p53 in the process of its repair, or it may fail to perform the normal function of base repair, which may be hindered by other reasons. The use of p53 transcripts as a specific marker for autism remains controversial, and more research is needed to clarify this association. Autism is a complex disease whose etiology and biomarkers are far from fully understood. Therefore, more evidence is needed to support p53 as an indicator of autism and to determine its precise role in the pathogenesis of autism in the future. These results have scientific implications for future treatment of aflatoxins autism.

Result 4,6,7: These results suggest that DNA pol β can repair some of the AFB1-induced changes in transcript levels in autistic mice, partially supporting my hypothesis. In the results, DNA pol β was not shown to restore the index Ogg1 . This suggests that DNA pol β is a secondary enzyme in the DNA damage repair in AFB1-autism. DNA pol β may not be sensitive to Ogg1 in the process of its repair, or it may fail to perform the normal function of base repair, which may be hindered by other reasons. Autism is a complex disorder whose etiology and biomarkers are not fully understood. More research is needed to identify Ogg1 as a specific marker for autism in the future. More research, including large-scale, molecular and clinical studies, is needed to determine whether Ogg1 has the cure function in autism. Therefore, there is not enough evidence for Ogg1 as a specific indicator of autism. These results have scientific implications for future treatment of aflatoxins autism.

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Result 8: In this result, DNA polymerase β could not reverse any of the index changes included in this study. This suggests that DNA polymerase's repair function cannot repair aflatoxins damage, or that DNA polymerase's repair pathways do not cross with aflatoxins damage pathways; It is also possible that DNA polymerase β is just not able to repair all the target transcripts included in this study. Not sensitive to transcript damage, environmental factors that hinder repair both may be responsible for the inability of DNA polymerase β to repair these transcripts. The scientific community will continue to work hard to find better biomarkers to help diagnose and understand autism in the future. This result that DNA polymerase β does not treat aflatoxins autism.

Possible experimental group results compared to positive group: The expression levels of Bax and p53 were lower than those of the positive control group, and the expression levels of Ogg1 was higher than that of the positive control group. The results were reversed and more positive than in the positive group, suggesting that DNA polymerase β may not only be a therapeutic agent for autism caused by aflatoxins gene damage, it can even prevent the disease. This result fully supports my hypothesis and is more instructive than the first, pointing to a clear path for the treatment and prevention of aflatoxins autism. However, this result may also indicate that the therapeutic effects of DNA polymerase β can lead to its maintenance at high levels or overexpression, with the risk of causing side effects of DNA polymerase β. Attention should be paid during the use of other drugs to prevent the increase in cell line genome instability, cancer patients should avoid the use of it, and so on.

Other possible result: The results of the indicate group are not exactly the same or completely different from the expected results, then it is suggested that this study failed modeling an animal model, the results of the experimental group no longer have experimental significance, this study can not get meaningful results.

The other reasons of the results of this study do not support my hypothesis may be: (1) The failure of culturing mice with high expression of DNA polymerase β using liposome transfection may be due to improper materials (gene source, mouse cell line, liposome) used in establishing animal models, or other environmental factors. If the reason is true, we will use other transfection methods to culture mice, such as virus transfection, or other methods to introduce DNA polymerase β to culture mice; (2) The representative significance of index selection is not high. The markers included in this study were all transcripts, and the range of markers was narrow, or because these transcripts could not fully reflect the repair function of DNA polymerase β. If this is the case, try to include new and more representative indicators such as mouse tail activity, glutathione (GSH) in the original literature.

5. Conclusion

In conclusion, this study evaluated the effects of DNA polymerase β on AFB1-induced gene damage in mice with autism. The results will show whether DNA polymerase β can repair the genetic damage caused by AFB1. It will also hint at which specific transcripts DNA polymerase β repairs. The observed therapeutic effects will provide suitable indications for future clinical and drug development against AFB1 food poisoning.

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