Bruceine F in Brucea Javanica Regulates Cellular STK3 and CK2A2 Targets to Correct Huntington Protein Misfolding in Mammalian Huntington's Disease

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Abstract

Huntington's disease is an autosomal dominant disorder characterized by progressive central nervous system degeneration characterized by motor, cognitive, and psychiatric disorders. This disease occurs frequently and is not easy to cure. We set out to investigate the pathogenesis of The Hippo pathway mediated by Brucea javanica in Huntington's disease (HD) by studying the critical role of Brucea javanica in the pathogenesis of Huntington's disease (HD) to provide a basis for the study of targeted drugs for this disease. Methods: Western blotting was used to rapidly determine YAP/TAZ activity and the expression level of HSF1 in tissues. YAP/TAZ activity and the expression level of HSF1 in tissues will show us the correct transcription level in the nucleus to measure efficacy. Hypothesis: Bruceine F in Brucea Javanica could effectively act on STK3 and CK2A2 targets to increase nuclear YAP activity and HSF1 expression, thus effectively treating Huntington's disease. Possible Results: 1: Bruceine F does not act effectively on SYK3 and CK2A2 sites. There were no significant changes in YAP activity in either the nucleus or cytoplasm, as well as in STK3 and CK2A2 for all samples,the expression of HSF1 in tissues also did not change significantly. 2: Bruceine F successfully inhibited STK3, but activated CK2A2, and YAP activity was decreased in the cytoplasm and elevated in the nucleus. , HSF1 expression levels were decreased. 3:Bruceine F activated STK3 but inhibited CK2A2, and YAP activity was elevated in the cytoplasm and decreased in the nucleus. , HSF1 expression levels were elevated. 4: Bruceine F activated STK3 and CK2A2, and YAP activity was elevated in the cytoplasm and decreased in the nucleus. , HSF1 expression levels were reduced. 5:Bruceine F inhibited STK3 and CK2A2, YAP activity was decreased in the cytoplasm and increased in the nucleus, and HSF1 expression levels were increased. 6:Bruceine F activated CK2A2 in knockout STK3 mice, and YAP activity was unchanged in the cytoplasmic nucleus with reduced HSF1 expression. 7: Bruceine F inhibited CK2A2 in knockout STK3 mice, YAP activity was unchanged in the cytoplasmic nucleus, and HSF1 expression levels were elevated. 8: Bruceine F inhibited STK3 in knockout CK2A2 mice, and YAP activity was reduced in the cytoplasm and increased in the nucleus, while HSF1 expression levels remained unchanged. 9: Bruceine F activated STK3 in knockout CK2A2 mice, and YAP activity was increased in the cytoplasm and decreased in the nucleus, while HSF1 expression levels remained unchanged..

Keywords: Brucea Javanica,Bruceine F,Hippo Signaling Pathway,Brain and Neuronal Stem Cells, Huntington's Disease,heat shock transcription factor 1

1. Introduction

Huntington's disease is an autosomal dominant disorder that causes degeneration or destruction of nerve cells in the brain. It often leads to motor, thinking, and mental disorders, and is associated with bipolar disorder, mania, and other psychiatric disorders. The causative gene of Huntington's disease, also known as Huntington's gene, is in the upper part of chromosome 4 at position 4P16.3 on the short arm, and the metabolite of Huntington's gene is known as Huntington's protein.[1] Patients with Huntington's disease have many repeats of the abnormal Huntington protein with glutamine, leading to adhesions and aggregation of the abnormal Huntington protein, which ultimately causes the death of nerve cells. In people with Huntington's disease, the three-letter Codon sequence C-A-G is repeated many times at one end of the Huntington's gene. [1] So the detection of Huntington's disease is to calculate the size of the CAG repeats by PCR amplification and gel electrophoresis after the DNA in the patient is separated from the blood. The number of CAG repeats will determine whether an individual will develop Huntington's disease.

Pharmacological treatments for Huntington's disease are often focused on suppressing the onset of symptoms, so there is a serious lack of pharmacological treatments for the pathogenesis of Huntington's disease today. Based on the idea of modulation of peripheral targets and multi-target therapy as a therapeutic approach, this article focuses on two important targets, Human serine/threonine-protein kinase 3 (STK3) and Casein kinase II subunit

alpha' (CK2A2). The former target can be inhibited to close the Hippo pathway to normalize Htt gene transcription, while the latter target is inhibited to protect heat shock transcription factor 1 from phosphorylation, and the non-phosphorylated HSF1 protein can ensure proper intracellular protein folding and aggregation by regulating the HSR pathway. [2][3] Previous studies have identified Brucea Javanica as a potential herbal treatment for Huntington's disease.[4] Brucea Javanica is a herbal medicine with a bitter taste and mild toxicity, it is the dried and ripe fruit of Brucea javanica (L.) Merr., a plant in the family Siberaceae. It has the effect of clearing away heat and detoxifying, and can also treat malaria, stop dysentery, and corrode warts.Accelrys Discovery Studio(DS) software was used to connect All Compounds from TCM Database@Taiwan to STK3 and CK2A2, and Bruceine F, which is one of the extracts of Brucea javanica, was screened by calculating the ligand-protein docking fraction. Bruceine F is believed to act as an ATP competition inhibitor at STK3 and CK2A2 sites.[4]

2. Hippo pathway

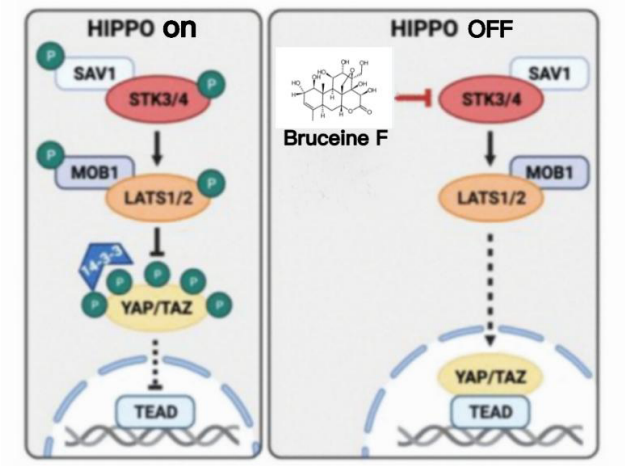

Figure 1.Bruceine F inhibits STK3 phosphorylation to shut down the Hippo pathway

When Bruceine F is docked with STK3, the formation of the homologous dimer of MST2 kinase and the process of phosphorylation or autophosphorylation are inhibited, the Hippo signalling pathway is closed (Figure 1), and the phosphorylation of STK3 in promoting plasma membrane binding of LATS is lost, and LATs1/2 cannot be phosphorylated and activated. Inactivated LATs1/2 also does not mediate YAP/TAZ phosphorylation, and YAP/TAZ not phosphorylated eventually accumulates in the nucleus, YAP/TAZ interacts with members of the TEA structural domain family (TEAD) transcription factors in the nucleus to form a protein complex for normal transcription of the Htt gene.[2]

3. HSR pathway

In the normal state of the cell, molecular chaperones bind to HSF-1 to form a complex and maintain it in an inactive state. When misfolded htt proteins appear in the cytoplasm, conformational changes in HSF1 release molecular chaperones, which interact with misfolded htt proteins to fold, maintain and restore their correct three-dimensional structure, or direct their degradation. However, CK2A2 expression causes phosphorylation of HSF1, which ultimately leads to inhibition of HSF1 activity. bruceine F docks with CK2A2 and then inhibits CK2A2 expression to protect HSF1.[3][5][6]

We hypothesized that Bruceine F in Brucea Javanica could effectively act on STK3 and CK2A2 targets to increase nuclear YAP activity, and HSF1 expression thus effectively treating Huntington's disease. To confirm this hypothesis, we investigated the actual regulatory effects of the target drug in mice in vivo, using Western blotting to rapidly determine YAP/TAZ as well as HSF1 activity.

4. Methods and materials

4.1 Mouse strains

For this study we used a full-length knock-in mouse model of HD known as zQ175 on the C57BL/6J background (Stock No. 027410). ZQ175 mice with mouse Htt exon 1 replaced by the human HTT exon 1 sequence with a ~190 CAG repeat tract and wild type mice (C57Bl6). All mice were housed under standard SPF conditions. The body weight of mice was 20±0.5g.Mouse samples can be purchased online.

4.2 Traditional Chinese medicine (TCM)

a. Brucea javanica

Medicinal material base source: for the bitter wood family plant crow brucea fruit, winnow net impurities, wash, dry, peel off the shell, take the whole kernel.

Then the herbs were prepared into 10ml 10% brucea brucea vein emulsion.

b. Sample processing

We operated six groups of mice according to different treatment protocols, each group of 5 mice, all experimental samples were reared in the same nutritional environment for 12 months. Every five patients in the group receiving traditional Chinese medicine were intraperitoneally injected with brucea javanica vein emulsion 2.5g/ (kg). Days) ×7 days, 5.0g/ (kg. Days) ×7 days,The negative control group 1 was wild-type mice, the negative control group 2 was wild-type mice treated with drugs, and the positive control group was zq175 HD mice

treated with Dichroa febrifuga at the same dose as Brucea javanica. The first group was STK3 knockout zq175 HD mice were treated with Brucea Javanica, and the second group was CK2A2 knockout zq175 HD mice treated with Brucea Javanica. The third group consisted of zq175 HD mice that received no treatment. Group 4 used zq175 HD mice treated with Brucea Javanica.

4.3 Nucleus/cytoplasm separation

The extracted mouse cells were homogenized with hypotonic lysis buffer, the supernatant was discarded after centrifugation, then the remaining samples were sonicated, and the supernatant was retained as cytoplasmic samples after centrifugation. The remaining samples were sonicated again and stirred at 4°C for 30 min.2 The supernatant was taken as the nucleus sample after centrifugation.

4.4 Western blotting

The extracted mouse cell nuclei and cytoplasmic samples were processed and loaded onto 4-20% glycine gels (Invitrogen), fractionated at 110v for 90 min, and the results were transferred to PVDF membranes after treatment with 5% milk in tris-buffered saline with Tween 20 (TBST), followed by incubation with YAP1 Antibody (13584-1-AP) (thermofisher), diluted and processed overnight, followed by washing and incubation with Goat Anti-Rabbit IgG-HRP Secondary Antibody (product # 31460) (thermofisher) for at least 1 hour, washed and developed.

Western blot analysis of HSF1 was performed by loading the sample onto a 4-20% Tris-HCl polyacrylamide gel. The proteins were then transferred to PVDF membranes and blocked with 5% BSA/TBST for at least 1 hour. The rabbit polyclonal antibody to HSF1 (product # PA3-017) (thermofisher) was diluted 1:1000 overnight on a 4°C shaker, washed with TBS-0.1% Tween 20, then diluted 1:2000 for at least 1 h with goat anti-rabbit IgG-HRP secondary antibody (product # 31460) (thermofisher). Finally, the machine is used to develop the image.

5. Possible result

Table 1 "/" represents no change in expression level compared to the negative control, "+" represents an increase in expression level compared to the negative control, "-" represents a decrease in expression level compared to the negative control.

	Method of measure	Possible result 1	Possible result 2	Possible result 3	Possible result 4	Possible result 5	Possible result 6	Possible result7	Possible result8	Possible result9
YAP in the cytoplasm	Western blotting	/	-	+	+	-	/	/	-	+
MST2(STK3)	Western blotting	/	-	+	+	-	/	/	-	+
CK2A2	Western blotting	/	+	-	+	-	+	-	/	/
YAP in the nucleus	Western blotting	/	+	-	-	+	/	/	+	-
HSF1	Western blotting	/	-	+	-	+	-	+	/	/

Possible result 1:Bruceine F does not act effectively on SYK3 and CK2A2 sites

There were no significant changes in YAP activity in either the nucleus or cytoplasm, as well as in STK3 and CK2A2 for all samples,the expression of HSF1 in tissues also did not change significantly

Possible result 2:Bruceine F successfully inhibited STK3, but activated CK2A2, and YAP activity was decreased in the cytoplasm and elevated in the nucleus. , HSF1 expression levels were decreased.

Possible result 3:Bruceine F activated STK3 but inhibited CK2A2, and YAP activity was elevated in the cytoplasm and decreased in the nucleus. , HSF1 expression levels were elevated

Possible result 4: Bruceine F activated STK3 and CK2A2, and YAP activity was elevated in the cytoplasm and decreased in the nucleus., HSF1 expression levels were reduced.
Possible result 5:Bruceine F inhibited STK3 and CK2A2, YAP activity was decreased in the cytoplasm and increased in the nucleus, and HSF1 expression levels were increased.
Possible result 6:Bruceine F activated CK2A2 in knockout STK3 mice, and YAP activity was unchanged in the cytoplasmic nucleus with reduced HSF1 expression.
Possible result 7:Bruceine F inhibited CK2A2 in knockout STK3 mice, YAP activity was unchanged in the cytoplasmic nucleus, and HSF1 expression levels were elevated.
Possible result 8:Bruceine F inhibited STK3 in knockout CK2A2 mice, and YAP activity was reduced in the cytoplasm and increased in the nucleus, while HSF1 expression levels remained unchanged.
Possible result 9:Bruceine F activated STK3 in knockout CK2A2 mice, and YAP activity was increased in the cytoplasm and decreased in the nucleus, while HSF1 expression levels remained unchanged.

6. Discussion

Previous studies through Molecular Dynamics Simulation, Pharmacophore Analysis and Cross-Validation, Quantitative Structure-Activity Relationship (QSAR) Bioinformatic research methods such as Models and Predictive Work predict that STK3 and CK2A2 can be potentially effective targets for the treatment of Huntington's disease, and regulate the transcription of Htt protein by reducing the level of YAP in the nucleus to achieve therapeutic purposes. In this study, an animal model of mice was used to verify this prediction, and different genetic conditions were set for different experimental groups. Finally, the content of YAP in the nucleus was measured by western blotting, following strict laboratory regulations to reduce the risk of contamination to the lowest.
For possible result 1, Bruceine F could not achieve its role as an inhibitor of target binding and did not help the diseased samples, contrary to the hypothesis. For possible results 2 and 3, Bruceine F only inhibits one of the two targets and activates the other, and although B can correct Htt protein misfolding by inhibiting the action of a single target, activation of the other target can affect the extent of the disease. pathway. For possible results 4, 5, Bruceine F activates or inhibits both STK3 and CK2A2 sites, and the simultaneous activation of both targets indicates that B is not effective in treating Huntington's disease and may even lead to worsening of the disease, whereas the simultaneous inhibition of both targets is the most desirable level, and B can be used to develop drugs targeting both targets to modulate HTT proteins for Huntington's disease. For the possible results 6, 7, 8, 9, which give the changes in the expression of YAP and HSF1 in the tissues of the treated group with the STK3 gene knocked out and the mice with the CK2A2 gene cut out, by comparing them with each other, it can be basically determined that the two targets correct the misfolding of Htt with their own pathways of regulating proteins, respectively, and the two pathways do not affect each other.
Based on literature support from previous studies, we found that STK3 and CK2A2 reduce YAP levels in the nucleus differently, CK2A2 is regulated differently than STK3, CK2A2 was validated as a direct target of the miR-29 family to negatively regulate Wnt signalling, while The Wnt pathway regulates the Hippo pathway by relocating YAP to the nucleus, and Wnt-induced nuclear localization of YAP/TAZ parallels transcriptional activation of a synthetic TEAD reporter (8xGTIIC-Lux) and direct YAP/TAZ target genes Cyr61 and Ankrd1, perhaps This way will be faster.[7] If a targeted drug is developed at the CK2A2 target, the effect of the drug will be greater, and more research is needed for drug development.
The assessment of neurological recovery in mice can also be added to the experiment as an auxiliary criterion for the effectiveness of Huntington's disease treatment.
1. Recovery of neurological function
Neurological Severity Scores (NSS), Open Field TestOFT, Inclined Plane Test, and Novel Objective Recognition Test (NORT) were used to assess neurological recovery and learning memory in mice. Test (NORT) to assess the neurological recovery and learning memory of mice.
2. Assessment of the degree of nerve cell damage
a. Laser scatter blood flow imager was used to record the overall blood perfusion in the brain tissue of mice
b. Using Longa score to record the degree of improvement of neurological function score in mice
c. TTC staining was used to detect the volume of ischemic injury and assess the degree of ischemic injury in mice
d. The extent of damage and reactive proliferation of neuronal cells in the cerebral cortex and hippocampus on the ischemic side was assessed using Nissler staining, GFAP antibody and Iba-1 specific antibody staining
e. The morphology of neurons in the cerebral cortex and hippocampus on the ischemic side was evaluated using NeuN and MAP2 specific antibody staining.

7. Conclusion

In conclusion, this study investigated the therapeutic effect of Brucea javanica on Huntington's disease in transplanted mice. Our results suggest that the inhibition of both STK3 and CK2A2 targets by Brucea javanica may be an effective treatment for Huntington's disease and lay the foundation for human clinical trials. As for the possible existence of the CK2A2 target, it is necessary to study whether the real effect of this target in the treatment of Huntington's disease is mainly the regulation of YAP level or the protection of HSF1, which should be discussed in future studies on the regulation of CK2A2 target. Adding western blot markers (such as neurons, striatum.) will increase the likelihood of success. The ingredients in Brucea javanica and how they work need to be better understood by scientists because of the lack of research into TCM treatments.

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