The Effects of Curcumin in a Machado-Joseph Disease Cellular Model : The Modulation Pathway of p53 and Carbonic Anhydrase 8

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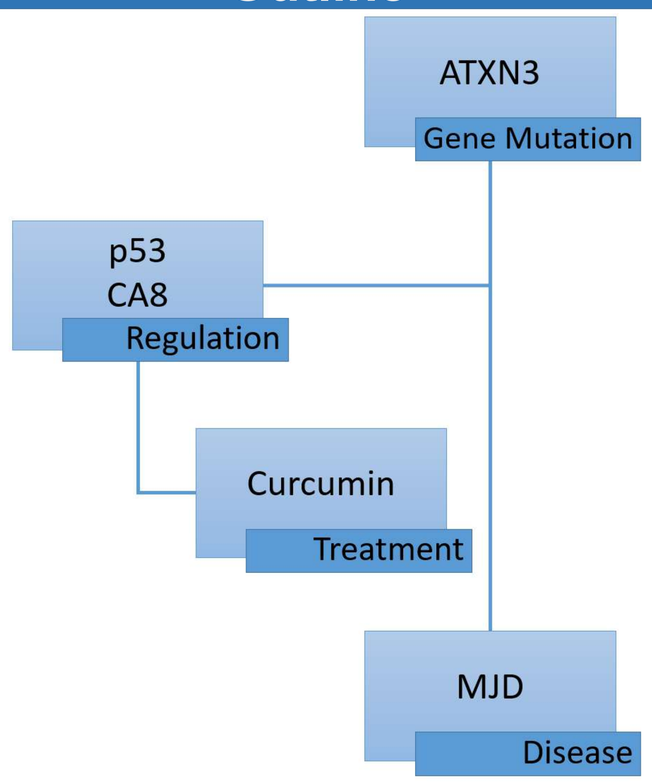
Introduction

MJD is an autosomal dominant genetic disease, caused by the mutation of CAG triplet repeat in MJD/ataxin-3 (ATXN3). The expansion of ATXN3 contains a polyglutamine (polyQ) repeat and aggregates in cells, leads to pathological onset such as cerebellar atrophy, motor neuron disorder and mental retardation.

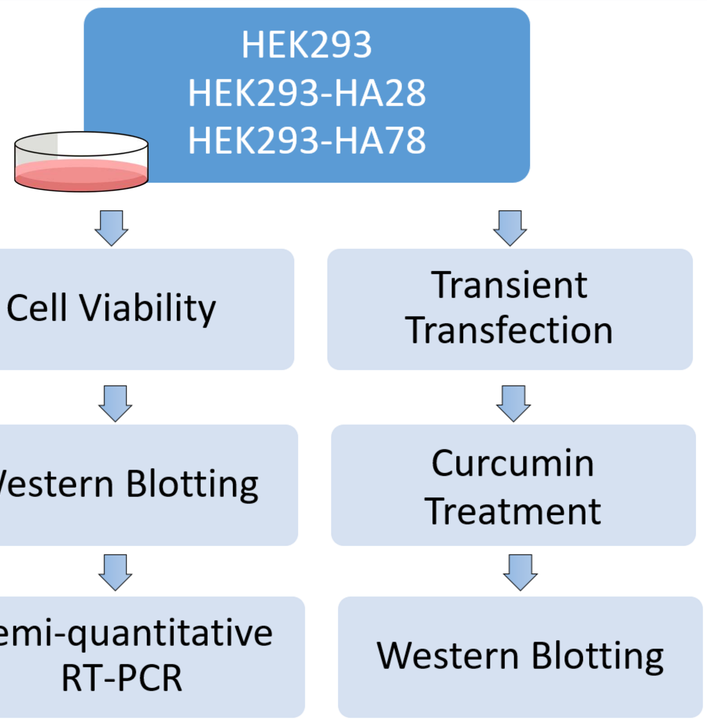
Studies have found that p53 and carbonic anhydrase 8 (CA8) have critical effects on the disease. CA8 is an isozyme of α-carbonic anhydrase family which lacks of reversible hydration activity due to the loss of a histidine residue. p53 is a tumor suppressor and able to trigger cell growth arrest or apoptosis.

This project emphasizes whether curcumin plays any protective role in MJD and the possible mechanisms underlying through p53 and CA8. Researches showed curcumin which has significant effects as a strong anti-oxidant and anti-inflammatory has the potential to become the prevention of neurodegenerative diseases.

Outline



Materials and Methods



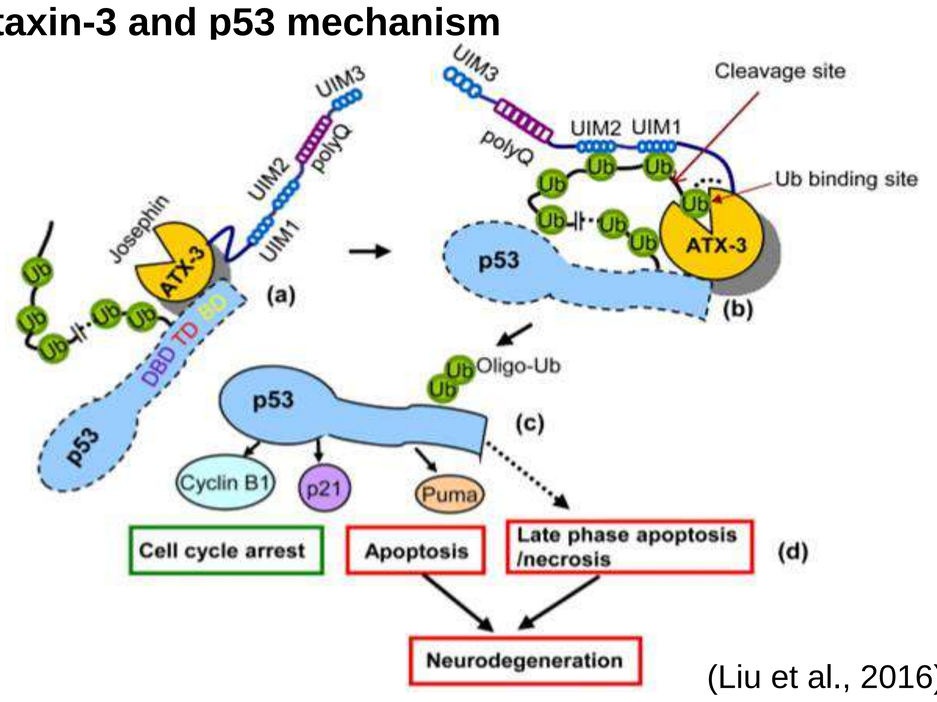
In this project, HEK293 cell line will be used as the control group, and HEK293 stably expressing MJD gene containing 28 and 78 CAG repeats, HEK293-HAMJD28 and HEK293-HAMJD78 cell lines, will be used as the experimental groups. By conducting semi-quantitative RT-PCR and protein analysis, we expect to detect the expression of p53 and CA8 in curcumin-treated cells. In addition, we will alter the expression of p53 and CA8 in the MJD disease models for the study of the relationship among curcumin, p53, CA8 and ATXN3.

Conclusion

It is preliminarily expected curcumin has similar effects on MJD as in HD or AD, that HEK293, HEK293-HAMJD28 and HEK293-HAMJD78 treated with curcumin will up-regulate CA8 and down-regulate p53, indicating that cell growth is affected under curcumin treatment. I will continue the experiments to observe p53 and CA8 in the MJD disease models under the treatment of curcumin to obtain the final conclusion.

Results

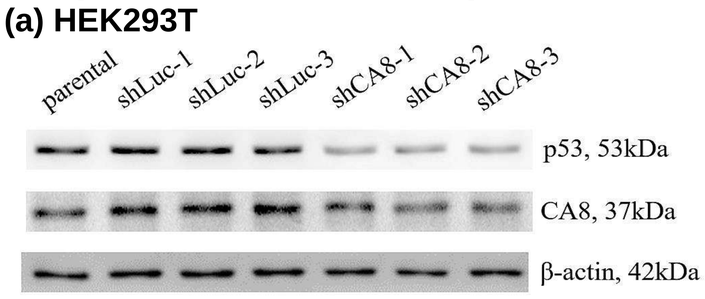
Figure 1.
A. Ataxin-3 and p53 mechanism



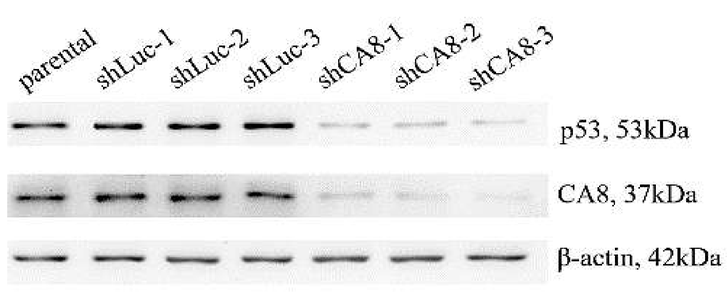
(Liu et al., 2016)

Figure 1. The mechanism between ATXN3 and p53
A. A diagram for ATXN3 interaction and regulation of p53. (a) ATXN3 binds to the C-terminal regulatory domain of p53 via Josephin. (b) During the DUB process, ATXN3 primarily binds to ubiquitinated p53 through UIM1 and UIM2. (c) The deubiquitination of p53 by ATXN3 in physiological conditions leads to the stabilization of p53. (d) Result in the activation of p53-responsive genes involving cell cycle arrest and apoptosis.

Figure 2.
A. Expression of CA8 and p53 in HEK293T and SW620
(a) HEK293T



(b) SW620



(c)

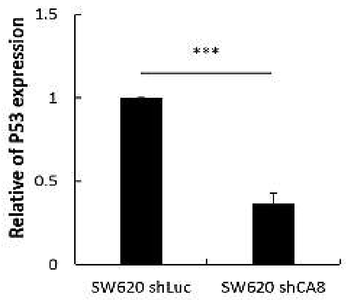


Figure 2. Protein level change of CA8 and p53 in HEK293T and SW620 (Wang., unpublished data)
A. Expression of CA8 and p53 in HEK293T and SW620 by lentivirus transfection. (a) Western blotting result of protein extract from HEK293T, human embryonic kidney cells, using β-actin as internal control. The expression of CA8 was temporarily down-regulated by lentivirus transfection. The data showed that the expression of p53 was also down-regulated. n=1. (b) Western blotting result of protein extract from SW620, human colon cancer cells, using β-actin as internal control. The expression of CA8 was down-regulated by lentivirus transfection and the expression of p53 was also down-regulated. n=3. (c) The statistic data was presented according to the expression levels of shLuc and shCA8 obtained from SW620 cell lines.