Peer Review File

Article information: http://dx.doi.org/10.21037/atm-20-4023

Reviewer comments

Salvia miltiorrhiza (Danshen), commonly known as a routine traditional herbal medicine, is widely used for preventing and treating osteoarthritis (OA), coronary artery disease, liver fibrosis in clinics in China for thousands of years. Tanshinone IIA (TIIA) is a class of abietane diterpene compound isolated from Salvia miltiorrhiza, which has widespread pharmacologic effects including anti-inflammation, anti-oxidation, anticancer, and anti-atherosclerosis. In the manuscript "A Combined Molecular Biology and Network Pharmacology Approaches to Investigate the Protective Effect of Tanshinone IIA against chondrocyte dedifferentiation", authors revealed a systematical network pharmacology approach and provided a basis for the future study of TIIA as the active ingredient in the protection of cartilage.

Comment 1: ***There was a similar report (Mol Med Rep. 2017 Nov;16(5):6285-6289) in the PubMed. What is the novel idea in the paper? Please elaborate in the introduction.***

Reply 1: Many thanks for the helpful comments. The authors read the aforementioned paper carefully. It is mainly about articular cartilage degradation prevented by inhibiting apoptosis and expression of inflammatory cytokines with Tanshinone IIA (TIIA). However, we discuss this issue form another perspective. Our

study focused on the connection between cartilage degradation and chondrocyte dedifferentiation. We try to evaluate the anti-dedifferentiated effects of TIIA and investigate the underlying mechanism through network pharmacology-based prediction and experimental verification. Moreover, we also fabricated a tissue-engineered cartilage culture system with tanshinone to study its effects, which was also different from traditional intragastric administration method.

Changes in the text: We have added relevant information in revision. (see Page 5-6, Line 85-91, 101-104, 119-121). Those description highlighted the novelty of this study.

Comment 2: ***The background of the abstract was too long. Please shorten it.***

Reply 2: The suggestion is appreciated. The authors have shortened the background of abstract.

Changes in the text: We have shortened the corresponding part. (see Page 3, Line 46-50).

Comment 3: ***Please supplement the methods of abstract.***

Reply 3: The comment is highly appreciated. The authors have supplemented the information according to suggestion. The supplemented information was as following:

Methods: The targets of TIIA were explored from public databases using various methods. The related targets of OA were obtained from the GeneCards database and the OMIM database. The potential targets and signaling pathways were determined

using protein-protein interaction (PPI), Gene Ontology (GO), and the Kyoto Encyclopedia of Genes and Genomes (KEGG). Cell viability, proliferation, and metabolic activity were analyzed in vitro. The effects of TIIA on chondrocyte dedifferentiation were evaluated by assessing morphological changes, glycosaminoglycan (GAG) production, and messenger RNA (mRNA) levels of cartilage-related genes. After 48 hours of culture in medium with 100 μg/ml TIIA, chondrocytes/hydrogel spheres were implanted to repair cartilage defects in a rat model. The harvested specimens were examined with hematoxylin and eosin (H&E) staining and immunohistochemistry to evaluate cartilage regeneration.

Changes in the text: Page 3, Line 51-61

Comment 4: ***In the introduction, please enrich the progress of the treatment for OA. How about the etiology of OA? Please supplement in the introduction.***

Reply 4: The comment is highly appreciated. The related information was supplemented according to suggestions.

Changes in the text: see Page 5, Line 85-91, 101-104

Comment 5: ***In the paper, all histogram should be changed to scatter plots.***

Reply 5: Many thanks for suggestion. All histogram has been changed to scatter plots in revision.

Changes in the text: see new Fig 5, Fig 6 and Fig 7

Comment 6: ***Why to choose female SD rats? Please check the body weight and the week age of the used rats.***

Reply 6: Female rats were chosen because they tend to be more docile than male rats and can even be trained to remain still, in order to decreases the risk for additional injury of attacking each other after modeling. The authors apologized for any ambiguous description and confusion caused. The authors have gone through the manuscript carefully to avoid any ambiguation.

Changes in the text: (see Page 9, Line 187-189)

All Sprague Dawley (SD) rats were purchased from the Experimental Animal Center of Air Force Military Medical University (Xian, China). For in vitro assays, chondrocytes were isolated from the knee joint cartilage of rats (weight: 40–50 g; 2 weeks old). For the in vivo experiments in this study, female SD rats (weight: 180–200 g; 6 weeks old) were used.

Comment 7: ***How to determine the dosage of TⅡA? How to identify the chondrocyte dedifferentiation?***

Reply 7: Many thanks for the reviewer's question. The dosage of TIIA was important to the activity of chondrocyte. During the in vitro experiment, the proliferation and viability of chondrocytes were promoted by TIIA with the concentration of 100-200μg/ml, but inhibited with 400μg/ml. A higher amount of GAG and better phonotype were observed in chondrocytes cultured in medium with TIIA at concentration of 100μg/ml. Therefore, the concentration of 100μg/ml was found to be suitable to inhibit dedifferentiation of chondrocyte in current study.

Chondrocyte dedifferentiation is identified by morphological change and ECM synthesis. Morphology of dedifferentiated chondrocyte usually changes from polygon into a spindle shape. ECM component is mainly characterized by decreased contents of GAG, Col II, ACAN and increased contents of Col I, Col VI, Col IX, Col X.

The authors have supplemented the related information in revision.

Changes in the text: see Page 5-6, Line 97-105; Page 12, Line 237-239; Page 16, Line 334-339