ISSN: 1813-4890

Tremella Polysaccharides Induce Autophagy in HaCaT Cells Starts

Honghong Qiu [1, a], Kaiye Yang [2, b], Guangrong Liu[1, c], Zhong Liu[1], Biao Che[2, *]

[1] College of Life Science and Technology, Jinan University, Guangzhou 510632, China

[2] Infinitus (China) Company Limited, Guangzhou 510632, China

[a]897516927@qq.com, [b]kyle.yang@infinitus-int.com, [c]Jim.Liu@infinitus-int.com, [*]Bill.Che@infinitus-int.com

Abstract

Tremella polysaccharide is the most important functional component in Tremella, which has physiological functions such as anti-inflammatory, anti-oxidant, anti-tumor, and immune regulation. Autophagy plays an important role in cell growth, differentiation, cell defense, cellular immunity, tumor occurrence, tissue remodeling, and the regulation of functions to adapt to the external environment. By using Tremella polysaccharide to act on HaCaT cells, using cell biology and molecular biology methods to detect autophagy-related genes, autophagosomes, autophagic flux and autophagy lysosomes respectively, the results show that Tremella polysaccharides promote autophagy-related gene expression, autophagy-related protein expression, and autophagy lysosomes increase with increasing concentration, which proves that Tremella polysaccharide can induce autophagy in HaCaT cells.

Keywords

Tremella polysaccharide, autophagy, autophagy lysosome.

1. Introduction

Tremella belongs to the genus tremella of the basidiomycete family tremella, and it is a widely distributed fungus. Tremella polysaccharides extracted from tremella is the most important functional component in tremella fruiting bodies, accounting for 70% to 75% of the dry weight of Tremella. Studies have confirmed that Tremella polysaccharide is a heteropolysaccharide, and its main chain is a mannan composed of α-(1-3)-glycosidic bonds. The 2, 4, and 6 positions of the main chain are connected with the side chains composed of glucose, xylose, fucose, uronic acid and other residues, and its active center is α-(1-3)-mannan. In recent decades, studies have found that Tremella polysaccharide has anti-inflammatory[1], anti-oxidation and anti-aging [2], anti-tumor[3], immune regulation[4], lowering blood lipids and blood sugar[5, 6] and other physiological functions. This makes tremella polysaccharides have huge market potential in food, medicine, cosmetics and other fields.

Autophagy is that cells form autophagic vesicles through monolayer or double-layer membrane wrapping long-lived proteins or senescent organelles, etc., and are transported to lysosomes to form autophagy lysosomes, which are digested and degraded by a variety of enzymes. Realize the needs of cell metabolism and the renewal of certain organelles. During the formation of autophagosomes, the expression of autophagy-related genes plays an important role in regulating the maturation of autophagosomes[7]. The immaturity of autophagosomes and the incomplete function of autophagosomes often lead to the inhibition of autophagy[8]. For a long time, autophagy of cells has been regarded as the self-rescue behavior of cells, which is of great significance for maintaining the homeostasis of cells. It is currently believed that autophagy is a new form of apoptosis (also known as type II programmed cell death), it plays an important role in cell growth, differentiation, cell defense, cellular immunity, tumor occurrence, tissue

ISSN: 1813-4890

remodeling, and regulation of functions to adapt to the external environment. Normal physiological levels and stress-induced autophagy are important factors that promote the health of mammals. During the scar formation and repair phase, autophagy removes excessive cells (such as fibroblasts, etc.) and deposited extracellular matrix (ECM) components (such as fibrin, collagen, etc.) to achieve the purpose of scar reconstruction . In addition, autophagy can also be used as a regulatory mechanism for cell growth, regulating cell growth[9]. Therefore, this study proved that tremella polysaccharides induced autophagy in HaCaT cells by detecting autophagy-related indicators, and provided a scientific basis for further research on its effect on wound healing and scar repair.

2. Method

2.1. Cell Counting Kit-8 (CCK8)

Take the HaCaT cells in the logarithmic growth phase and evenly spread them into a 96-well plate at a density of 5×104 cells/mL, with 100 μL per well. After the cells adhere to the wall, add medium containing different concentrations of tremella polysaccharide (complete medium) with 3 multiple wells for each concentration. After 24 hours of incubation, add 10 μL of CCK8 to each well. After 2 hours of incubation, culture 96-well cells place the plate in the microplate reader, measure the absorbance at 450 nm with 630 nm as the reference wavelength, and record the measurement results.

2.2. Quantitative Polymerase Chain Reaction (qPCR)

The tremella polysaccharide is formulated into the test solution of the corresponding concentration, and each concentration is set with 3 multiple wells and 3 background control wells. Take the HaCaT cells in logarithmic growth phase and spread them into a 6-well plate at a density of 5×105 cells/mL, 2 mL per well. After the cells adhere to the wall, add medium (complete medium) containing different concentrations of tremella polysaccharide and incubate for 24 h. The total RNA was extracted with TRNzol, and the cDNA chain was obtained by cDNA synthesis reagent for Real-Time PCR to quantitatively detect the expression level of Beclin 1, p62, Atg5 mRNA.

2.3. Western Blot

Take the HaCaT cells in logarithmic growth phase and spread them into a 6-well plate at a density of 5×105 cells/mL, 2 mL per well. After the cells adhere to the wall, add medium (complete medium) containing different concentrations of tremella polysaccharide. After incubating for 24 hours, extract the cell protein with RIPA lysate, and use BCA kit to detect the concentration of each cell protein sample. Adjust the protein concentration of other samples according to the concentration of the lowest protein sample to make the concentration of each protein sample consistent. Dilute with 5×Loading Buffer to 30 μg of the loaded protein. Separate the protein by SDS-PAGE electrophoresis, and then transfer the protein to the PVDF membrane. After blocking with 5% skimmed milk powder for 1 h, add primary antibody (anti-LC3II, anti-SQSTM1) and incubate overnight at 4°C, wash with TBST 3 times for 10 min each time, add secondary antibody and incubate for 1 h, then wash with TBST 3 times for 10 min each time, and then image with a chemiluminescence instrument.

2.4. Acridine Orange Fluorescene

Take the HaCaT cells in the logarithmic growth phase and spread them into a 12-well plate at a density of 5×104 cells/mL, 1 mL per well. After the cells adhere to the wall, add medium (complete medium) containing different concentrations of tremella polysaccharide and incubate for 6 h. The culture medium was discarded and washed 3 times with PBS for 5 min each time. Acridine orange staining solution with a final concentration of 10 μg/mL was added

ISSN: 1813-4890

to each well, stained at room temperature and protected from light for 5 min, and washed with PBS for 3 times after staining. Observed under a fluorescence microscope, the excitation filter has a wavelength of 488 nm, and the blocking filter has a wavelength of 515 nm. The control group and the tested sample group are taken in a field of view with similar cell numbers to take pictures.

3. Results

3.1. Effect of Tremella Polysaccharide on HaCaT Cell Activity

Tremella polysaccharide has no obvious toxic effect on HaCaT cells. When the concentration is greater than 1 mg/mL, it promotes cell proliferation, see Fig. 1.

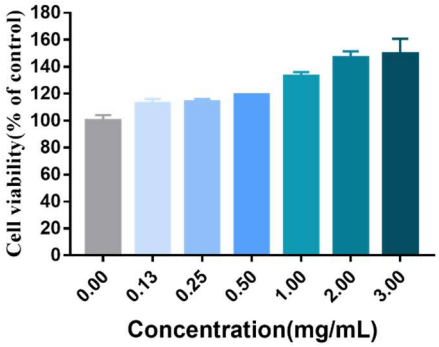

Fig. 1 CCK8 detects the effect of Tremella polysaccharide on HaCaT cell activity

3.2. Effect of Tremella Polysaccharide on Autophagy Related Gene Expression In HaCaT Cells

After Tremella polysaccharides treat HaCaT cells, the expression levels of autophagy-related genes p62, Beclin 1 and Atg5 are up-regulated as their concentrations increase, see Fig.2.

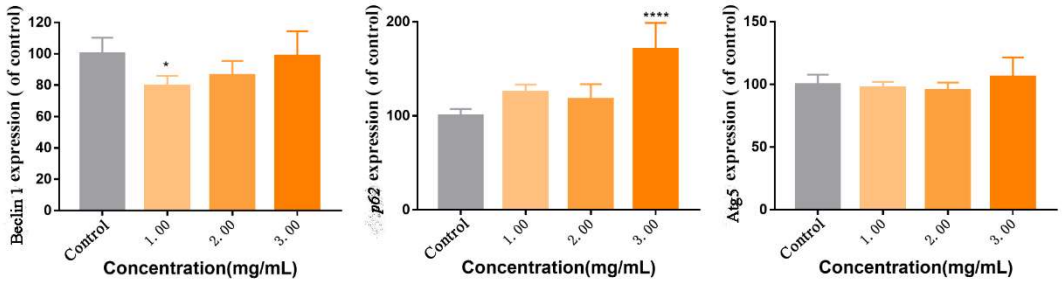

Fig. 2 qPCR Detection of the Effect of Tremella Polysaccharides on the Expression of Autophagy Related Genes in HaCaT Cells

3.3. Effect of Tremella Polysaccharides on the Expression of Autophagy Related Proteins in HaCaT Cells

Tremella polysaccharides can induce autophagy at 1 mg/mL. Compared with the control group, the synthesis of LC3-II increases by 45.5%, and the effect of high concentration (3 mg/mL) is more obvious. The synthesis amount of LC3-II is the control group 2.4 times, the change trend of SQSTM1 protein is opposite to that of LC3-II, see Fig. 3.

ISSN: 1813-4890

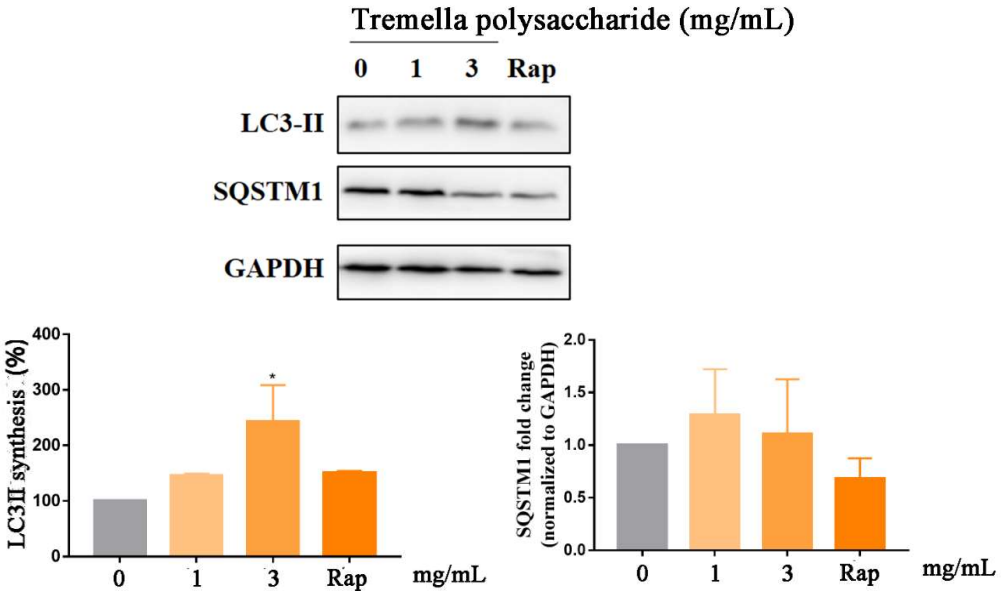

Fig. 3 Western blot detection of the effect of tremella polysaccharides on the expression of autophagy-related proteins in HaCaT cells

3.4. Effects of Tremella Polysaccharides on Autophagy Lysosomes in HaCaT Cells

Compared with the control group, after 6 hours of treatment of HaCaT cells with tremella polysaccharide, the red-yellow fluorescence intensity was enhanced by acridine orange staining, indicating that the acid autophagy lysosomes in HaCaT cells increased significantly, see Fig. 4.

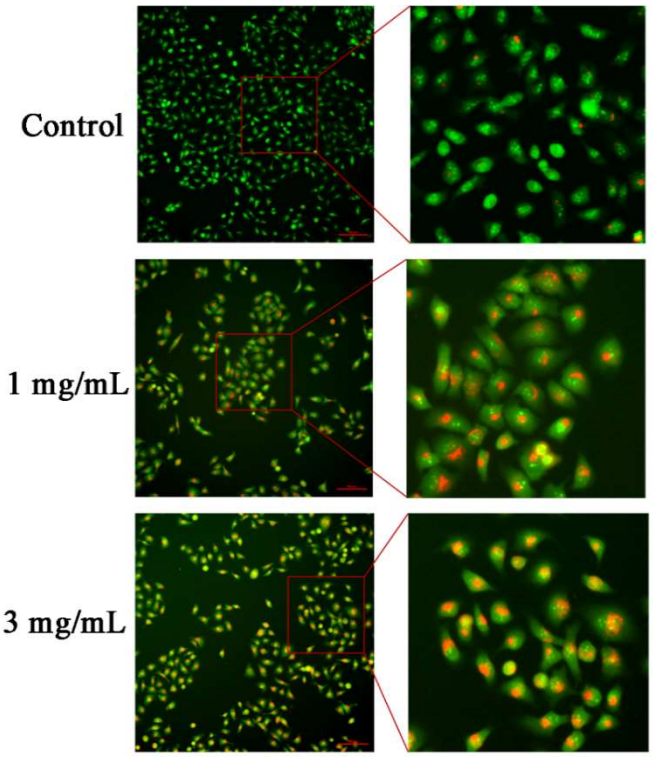

Fig. 4 Fluorescence microscope observation of the effect of tremella polysaccharides on autophagy lysosomes in HaCaT cells

ISSN: 1813-4890

4. Discussion

Autophagy is a universal and important life phenomenon, which can realize the recycling and renewal of some long half-life proteins and some organelles in the cell, and meet the metabolic needs of the cell itself [10, 11]. Epithelial cells can eliminate pathogens through phagocytosis, present antigens and secrete a variety of cytokines to regulate the body's inflammatory response and immune response, and play an important role in the body's anti-infection, promotion of wound healing, and scar formation, remodeling, and repair. Therefore, it can be used as an important cell model in autophagy research[12, 13]. HaCaT cells are human immortalized epithelial cells. LC3 is a homolog of the yeast Atg8 gene in mammalian cells. It is located on the surface of autophagosome and autophagosome membrane. It is a universal marker of autophagosome membrane. The LC3 synthesized by the cell is processed to become LC3-I. This cytosolic soluble LC3-I undergoes a ubiquitin-like processing and modification process and combines with phosphatidylethanolamine (PE) on the surface of the autophagic bubble membrane to become LC3-II. LC3- content is proportional to the number of autophagosomes [14, 15]. Detecting the conversion of LC3-I to LC3-II by Western blot has become the basis for quantitative analysis of autophagy activity. The chemical dye acridine orange (AO) is a metachromatic lysogen-based basic fluorescent dye, which can penetrate into acidic organelles, such as autophagic lysosomes. Autophagy cells are stained with acridine orange, Red-yellow dots can be observed under a fluorescence microscope, so the occurrence of autophagy can be judged according to the fluorescence intensity. Therefore, after treating HaCaT cells with tremella polysaccharide, the effect of tremella polysaccharide on the autophagy of HaCaT cells was proved by detecting the synthesis of LC3Ⅱ and the content of autophagy lysosome.

5. Conclusion

In summary, it can be seen that the formation, repair and remodeling of wound healing scars are closely related to changes in autophagy. In this process, epithelial cells, as an important cell, play an important role in promoting wound healing, scar formation, and remodeling and repair. Therefore, Tremella polysaccharide can induce autophagy in human epidermal keratinocytes for further research on wound healing and scar formation. And the remodeling and repairing process provides a scientific basis.

References

[1] Shi, X., W. Wei and N. Wang, Tremella Polysaccharides Inhibit Cellular Apoptosis and Autophagy Induced by Pseudomonas Aeruginosa Lipopolysaccharide in A549 Cells through Sirtuin 1 Activation. Oncol Lett, Vol. 15 (2018) NO. 6, p. 9609-9616.

[2] Xu, X., J. Yang, Z. Ning and X. Zhang, Lentinula Edodes-Derived Polysaccharide Rejuvenates Mice in Terms of Immune Responses and Gut Microbiota. Food Funct, Vol. 6 (2015) NO. 8, p. 2653-63.

[3] Chen, B., Optimization of Extraction of Tremella Fuciformis Polysaccharides and Its Antioxidant and Antitumour Activities in Vitro. Carbohydrate Polymers, Vol. 81 (2010) NO. 2, p. 420-424.

[4] Wang, Yufeng, Tian, Youqiu, Shao, Jiangjuan, Shu, Xu, Jia and Jinxia, Macrophage Immunomodulatory Activity of the Polysaccharide Isolated from Collybia Radicata Mushroom. International Journal of Biological Macromolecules Structure Function & Interactions, Vol. (2018).

[5] Perera, P.K. and Y.M. Li, Mushrooms as a Functional Food Mediator in Preventing and Ameliorating Diabetes. (2011).

[6] Wang, X., Z. Zhang and M. Zhao, Carboxymethylation of Polysaccharides from Tremella Fuciformis for Antioxidant and Moisture-Preserving Activities. International Journal of Biological Macromolecules, Vol. 72 (2015), p. 526-530.

ISSN: 1813-4890

[7] Anderson, J.O., J.G. Thundiyil and A. Stolbach, Clearing the Air: A Review of the Effects of Particulate Matter Air Pollution on Human Health. J Med Toxicol, Vol. 8 (2012) NO. 2, p. 166-75.

[8] Kim, K.E., D. Cho and H.J. Park, Air Pollution and Skin Diseases: Adverse Effects of Airborne Particulate Matter on Various Skin Diseases. Life Sci, Vol. 152 (2016), p. 126-34.

[9] Zhang, D., C. Lu, Z. Yu, X. Wang, L. Yan, J. Zhang, H. Li, J. Wang and A. Wen, Echinacoside Alleviates Uvb Irradiation-Mediated Skin Damage Via Inhibition of Oxidative Stress, DNA Damage, and Apoptosis. Oxid Med Cell Longev, Vol. 2017 (2017), p. 6851464.

[10] Shintani, T. and D.J. Klionsky, Autophagy in Health and Disease: A Double-Edged Sword. Science, Vol. 306 (2004) NO. 5698, p. 990-5.

[11] Rubinsztein, D.C., J.E. Gestwicki, L.O. Murphy and D.J. Klionsky, Potential Therapeutic Applications of Autophagy. Nat Rev Drug Discov, Vol. 6 (2007) NO. 4, p. 304-12.

[12] Todde, V., M. Veenhuis and I.J. van der Klei, Autophagy: Principles and Significance in Health and Disease. Biochim Biophys Acta, Vol. 1792 (2009) NO. 1, p. 3-13.

[13] Shi, C.S. and J.H. Kehrl, Myd88 and Trif Target Beclin 1 to Trigger Autophagy in Macrophages. J Biol Chem, Vol. 283 (2008) NO. 48, p. 33175-82.

[14] Kabeya, Y., N. Mizushima, T. Ueno, A. Yamamoto, T. Kirisako, T. Noda, E. Kominami, Y. Ohsumi and T. Yoshimori, Lc3, a Mammalian Homologue of Yeast Apg8p, Is Localized in Autophagosome Membranes after Processing. Embo j, Vol. 19 (2000) NO. 21, p. 5720-8.

[15] Kouno, T., M. Mizuguchi, I. Tanida, T. Ueno, T. Kanematsu, Y. Mori, H. Shinoda, M. Hirata, E. Kominami and K. Kawano, Solution Structure of Microtubule-Associated Protein Light Chain 3 and Identification of Its Functional Subdomains. J Biol Chem, Vol. 280 (2005) NO. 26, p. 24610-7.