Advances in Life Science for Adolescents

Research VOL. 1 No.2 | Sep 2025

Effects of MPSSS on the Differentiation of Tumor-Associated Fibroblasts

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Abstract:

Tumor development is closely related to the tumor microenvironment(TME),with cancer-associated fibroblasts(CAFs)being a key component.Lentinan,a polysaccharide fromLentinus edodes,inhibits tumor cell proliferation and induces apoptosis,but its effects on CAFs are unclear.This study investigates the role of MPSSS,a key component of lentinan,in the activation of prostate cancer-associated fibroblasts(CAFs)through in vitro experiments.A co-culture model using PC-3 cell supernatant and LX-2 cells was established to simulate CAF activation in the TME.MPSSS cytotoxicity was assessed using the MTT assay,while fibroblast activation protein α(FAP)mRNA expression was measured by qPCR.Results show that PC-3 supernatant reduced LX-2 cell viability,but MPSSS restored it in a concentration-dependent manner.FAP mRNA expression was upregulated after 96 hours of induction,correlating positively with MPSSS concentration.This indicates that MPSSS regulates CAF activation,providing a basis for targeted CAF therapy.

Keywords: Lentinan, Cancer-associated fibroblasts(CAFs) , Prostate cancer, Fibroblast activation protein

1. Background:

The formation of tumor cells is related to genetic mutations in normal cells. In addition to the impact of genomic abnormalities, tumor formation is closely associated with the tumor microenvironment (TME) [1]. Studies have shown that CAFs can promote the formation of an inflammatory environment in the TME, enhancing the proliferation and metastasis of tumors[2]. CAFs are one of the main stromal cells in the TME, regulating the TME through cell-cell direct contact and secretion of specific cytokines[3]. Therefore, targeting CAFs is likely to become an important direction for tumor therapy.

In the formation of CAFs, normal fibroblasts (NFs) activated by various factors and recruited into tumor tissues are an important source. CAFs can promote tumor angiogenesis, regulate immune responses, or directly act on tumor cells to promote their proliferation and invasion, playing a significant role in the occur-rence, development, and metastasis of tumors[4]. Current therapies targeting CAFs mainly include inducing apoptosis to reduce or eliminate cells, inhibiting cell function to further reduce tumor activity, and promoting cell differentiation or blocking the activation pathways of CAFs.

Some active components of traditional Chinese medicine have significant effects on inhibiting tumor growth and regulating immunity. MPSSS, a novel polysaccharide isolated fromLentinus edodes, has been shown to inhibit cancer development through immune regulation[5]and inhibition of cancer cell proliferation[6]. However, previous studies on the anti-tumor effects of lentinan have mainly focused on immune regulation and inhibition of tumor cell growth, with few studies on the effects on the activation process of tumor-associated fibroblasts. This study aims to investigate the role of MPSSS in the activation process of prostate cancer-associated fibroblasts (CAFs) and the possible molecular mechanisms involved through in vitro experiments.

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Received: Aug. 3, 2025.
Accepted: Aug. 18, 2025.

2. Methods

2.1 Cell Lines

The cell lines used in this study were obtained from the Cell Bank of the Institute of Biophysics, Chinese Academy of Sciences. The human prostate cancer cell line PC-3 was provided by Professor Gao Weiqiang from the School of Life Science and Engineering, Shanghai Jiao Tong University. The LX-2 cell line, established by Professor Xu Lieming from Shanghai University of Traditional Chinese Medicine, was derived from hepatic stellate cells isolated from healthy adult livers and selected through transfection with the SV40T gene and long-term low-serum culture. Other materials included 10%fetal bovine serum (Pan, Germany) , 1%antibiotics (streptomycin and penicillin) , high-glucose DMEM culture medium (CM15019, Macgene Biotech) , and MPSSS (lentinan purchased from Johncan Company and purified according to previous studies) .

2.2 Cell Culture

Human prostate cancer PC-3 cells and human hepatic stellate LX-2 cells were cultured in high-glucose DMEM medium containing 10%fetal bovine serum and 1%antibiotics (streptomycin and penicillin) at 37°C in a 5%CO_2 incubator until the logarithmic growth phase. Trypsin was used to digest the cells and prepare cell suspensions, with medium changes every 2-3 days and passages for 3-4 generations. During cell culture, PC-3 supernatant was collected and filtered using a 0. 22 μm cell filter. To ensure adequate nutrition, the supernatant was mixed with DMEM culture medium in a 7:3 ratio to prepare the induction medium for culturing LX-2 cells, with medium changes every 48 hours for a total induction period of 96 hours. Pipette retarder (Beijing EnnovateJoy Co., Ltd) were used to mitigate the damage to cells caused by fluid shear stress.

2.3 MTT Assay for Proliferation Inhibition of LX-2 and PC-3 Cells

Normal and induced LX-2 cells were divided into two groups and seeded in 96-well plates at a density of 3000-4000 cells per well. MPSSS solutions at concentrations of 0 mg/ml and 0. 5 mg/ml were added to each group, with four replicates per group. The cells were cultured at 37°C in a 5%CO_2 incubator for 36 hours. MTT solution (10 μl per well) was added and reacted at 37°C for 4 hours. A lysis solution (10 g SDS, 5 ml isobutanol, 0. 1 ml 10 M HCl, and distilled water to 100 ml) was added overnight to dissolve the formazan crystals completely. The absorbance was measured at 570 nm to reflect the relative cell viability.

2.4 Real-Time Quantitative PCR (qPCR) for Detection of FAP mRNA Levels

Total RNA was extracted from cells using TRIzol reagent and chloroform-ethanol precipitation and washed. Pipette retarder (Beijing EnnovateJoy Co., Ltd) were used to reduse the mechnical damage to the RNA. Then the RNA was reverse-transcribed into cDNA using the M-MLV reverse transcriptase system. FAP primers were added and qPCR was performed using a real-time quantitative PCR system, with fluorescence from PCR-coupled fluorescent dyes reflecting the qPCR process. GAPDH was used as the internal reference gene, and the relative FAP mRNA levels were calculated using the Rotor Gene 5 software and the ΔΔCt method. The primer sequences were sourced from PrimerBank and synthesized by Invitrogen Shanghai Trading Co. , Ltd.

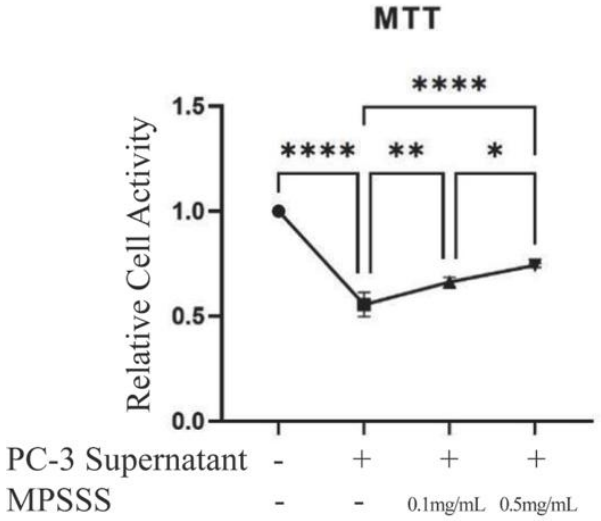
2.5 Statistical Analysis and Graphing

Data were analyzed using IBM SPSS 22 software, and statistical graphs were created using GraphPad Prism 9. 0. 0 software. Differences were considered statistically significant at P<0. 05.

3. Results

3.1 MPSSS Inhibits the Apoptotic Effect of Cancer Cell Supernatant on LX-2 Cells

LX-2 cells were induced with PC-3 cell supernatant for 36 hours, and different concentrations of MPSSS were applied to the induced cells. The results showed that the viability of LX-2 cells induced by supernatant was significantly lower than that of the control group (P<0. 0001), indicating that the cancer cell supernatant inhibited the viability of LX-2 cells. Compared with the supernatant-induced group, the relative viability of LX-2 cells increased significantly after the application of different concentrations (0. 1 mg/ml and 0. 5 mg/ml) of MPSSS (P<0. 01), and this increase was concentration-dependent (P<0. 05). This suggests that MPSSS can effectively inhibit the apoptotic effect of cancer cell supernatant on LX-2 cells.

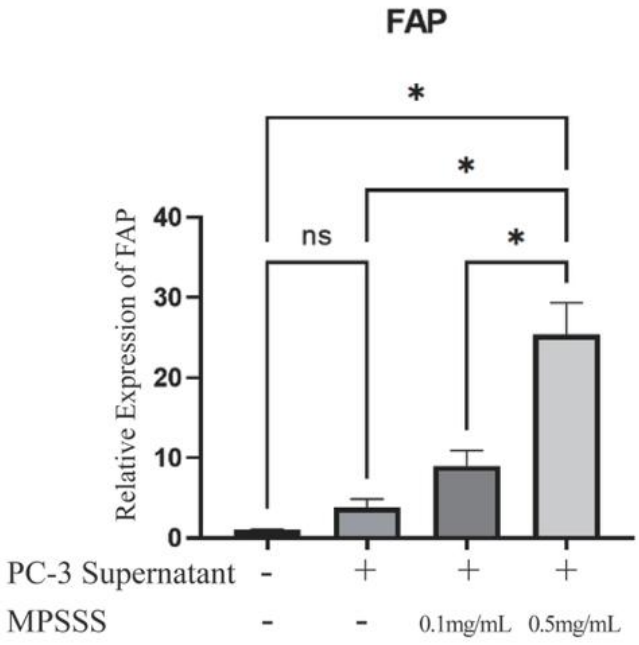


(Note: "*" indicates P<0.05, "**" indicates P<0.01, and"****"indicates P<0. 0001)

Figure 1: Effects of Different Concentrations of MPSSS on the Relative Viability of LX-2 Cells Induced by Tumor Supernatant for 36 Hours

3.2 MPSSS Upregulates FAP mRNA Expression in LX-2 Cells Induced by PC-3 Supernatant

The effects of MPSSS on FAP mRNA expression in supernatant-induced cells are shown in Figure 2. Compared with the control group, FAP mRNA expression in supernatant-induced LX-2 cells showed no significant change (P>0. 05). However, after treatment with 0. 1 mg/ml and 0. 5 mg/ml MPSSS, FAP mRNA expression increased significantly (P<0. 05), and this increase was positively correlated with the concentration of MPSSS used (P<0. 05).



(Note:"*"indicates P<0. 05, "ns"indicates P>0. 05)

Figure 2: Effects of Different Concentrations of MPSSS on FAP mRNA Expression in Induced LX-2 Cells after 96 Hours

4. Discussion

MPSSS, a novel polysaccharide isolated fromLentinus edodes, has been shown in recent studies to inhibit tumor development through immune regulation[4, 6]. To investigate the role of MPSSS in the activation process of CAFs, we used PC-3 cell supernatant to induce LX-2 cells. MTT assays were performed to determine cell viability. The results indicated that the viability of hepatic stellate cells (LX-2) induced by cancer cell supernatant was significantly reduced, suggesting that cancer cells can induce apoptosis in normal fibroblasts (NFs) through the secretion of cytokines. However, when different concentrations of MPSSS were applied to LX-2 cells in combination with cancer cell supernatant, cell viability increased, indicating that MPSSS can effectively inhibit the apoptotic effect induced by cytokines secreted by cancer cells, thereby slowing the formation of the tumor microenvironment (TME) .

Fibroblast activation protein α (FAP) is selectively expressed in various types of human epithelial cancer-associated CAFs[7]and is one of the most widely used markers for identifying CAFs, with significantly higher expression levels in CAFs than in NFs. Real-time quantitative PCR (qPCR) results revealed that FAP expression was upregulated in LX-2 cells treated with MPSSS and cancer cell supernatant. We hypothesize that this may be related to the positive effects of FAP in the TME. The same cytokine can have opposite effects in different cells, as has been demonstrated multiple times. For example, with the in-depth study of transforming growth factor-β (TGF-β) , its dual role in the TME during different stages of tumor formation has been increasingly validated. Studies have shown[8]that in pancreatic ductal adenocarcinoma (PDA) , typical TGF-β signaling can inhibit the proliferation of epithelial pancreatic cancer cells. However, in CAFs, TGF-β can promote the production of interleukin-6 (IL-6) , thereby promoting the continuous proliferation of pancreatic tumor cells. This line of thinking may also apply to explain the results of our study. Although the presence of FAP in malignant tissues is undeniable, its physiological role in tissues is not consistent in the literature. One study[9]has shown that in infiltrating ductal carcinoma with high FAP expression in stromal fibroblasts near the tumor cell nest, the invasiveness is relatively weaker, and elevated FAP expression is associated with improved patient survival rates and extended survival time. Another study[10]found that the activation of pancreatic stellate cells can inhibit tumor cell proliferation. The overexpression of FAP observed in our study may potentially inhibit the formation of the TME and have potential benefits, which could be synergistic with MPSSS-targeted tumor therapy.

These results suggest the impact of MPSSS on the transformation of normal fibroblasts into CAFs, but the specific mechanisms still need to be further explored and validated.

5. Conclusion and Future work

MPSSS, a novel polysaccharide extracted fromLentinus edodes, has been proven to directly inhibit tumor cell proliferation and regulate immunity. This study initially focused on the effects of MPSSS on the activation process of CAFs. Using PC-3 cell supernatant induction, we constructed a model of CAF activation in the TME and detected changes in related cytokines during the activation process. This study proposed the hypothesis that FAP may play a positive regulatory role in the formation of CAFs and investigated the effects of MPSSS on FAP expression during CAF activation. As research on FAP function continues to advance, targeting the tumor microenvironment with MPSSS may provide new insights for inhibiting tumor growth and offer new pathways for cancer prevention and treatment.

Due to the limitations of the author's academic and knowledge levels, this study was unable to fully verify the effects of MPSSS on the formation of CAFs and its molecular mechanisms. It is hoped that future research in the direction of CAF activation will be perfected and developed to explore the preventive

and early therapeutic effects of MPSSS on tumors.

It is hoped that in the future, the author will continue to pursue their scientific research ideals, acquire solid research skills, and obtain resource support to conduct in-depth research in this direction, making a modest contribution to the common goal of curing cancer and addressing the current shortcomings.

6. References

[1] Cheung K J, Ewald A J. A collective route to metastasis:seeding by tumor cell clusters[J]. Science, 2016, 352 (6282) :167-169.

[2] Denk PO, Hoppe J, Hoppe V, Knorr M. Effect of growth factors on the activation of human Tenon's capsule fibroblasts. Curr Eye Res. 2003 Jul; 27 (1) :35-44.

[3] Shi Wenting, Sun Li, Yuan Shengtao. Cancer-associated fibroblasts and tumor microenvironment[J]. Modern Biomedical Progress, 2011, 11 (18) :3573-3576.

[4] Wu H, Tao N, Liu X, Li X, Tang J, Ma C, Xu X, Shao H, Hou B, Wang H, Qin Z. Polysaccharide fromLentinus edodesinhibits the immunosuppressive function of myeloid-derived suppressor cells. PLoS One. 2012; 7 (12) :e51751.

[5] Zhang T, Chen X, Sun L, Guo X, Cai T, Wang J, Zeng Y, Ma J, Ding X, Xie Z, Niu L, Zhang M, Tao N, Yang F. Proteomics reveals the function reverse of MPSSS-treated prostate cancer-associated fibroblasts to suppress PC-3 cell viability via the FoxO pathway. Cancer Med. 2021 Apr; 10 (7) :2509-2522.

[6] Xu Y, Ma J, Zheng Q, Wang Y, Hu M, Ma F, Qin Z, Lei N, Tao N. MPSSS impairs the immunosuppressive function of cancer-associated fibroblasts via the TLR4-NF-κB pathway. Biosci Rep. 2019 May 10; 39 (5) :BSR20182171.

[7] Park J E, Lenter M C, ZimmermannR N, et al. Fibroblast activation protein, a dual specificity serine protease expressed in reactive human tumor stromal fibroblasts[J]. The Journal of Biological Chemistry, 1999, 274 (51) :36505-36512.

[8] Huang H, Zhang Y, Gallegos V, Sorrelle N, Zaid MM, Toombs J, Du W, Wright S, Hagopian M, Wang Z, Hosein AN, Sathe AA, Xing C, Koay EJ, Driscoll KE, Brekken RA. Targeting TGFβR2-mutant tumors exposes vulnerabilities to stromal TGFβ blockade in pancreatic cancer. EMBO Mol Med. 2019 Nov 7; 11 (11) :e10515.

[9] Ariga N, Sato E, Ohuchi N, Nagura H, Ohtani H. Stromal expression of fibroblast activation protein/seprase, a cell membrane serine proteinase and gelatinase, is associated with longer survival in patients with invasive ductal carcinoma of breast. Int J Cancer. 2001 Jan 20; 95 (1) :67-72.

[10] Xiao Y, Zhang H, Ma Q, Huang R, Lu J, Liang X, Liu X, Zhang Z, Yu L, Pang J, Zhou L, Liu T, Wu H, Liang Z. YAP1-mediated pancreatic stellate cell activation inhibits pancreatic cancer cell proliferation. Cancer Lett. 2019 Oct 10; 462:51-60.