Protein chip analysis of cytokines reveals a key mechanism of the antitumor and immunostimulatory activities of *Tricholoma matsutake* polysaccharide

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Abstract: Polysaccharide has been widely used in medical and health field because of its function of immune regulation. The aim of present study was to use protein chip to test the 200 cytokines secreted by macrophages which were induced by the polysaccharides of *Tricholoma matsutake* (TMP-A) to study the role of TMP-A acting on macrophages and its mechanism, further understanding the mechanism of the TMP-A effect on immune activity. The results of the analysis indicated that among all of these cytokines, including IL-1β, IL-10, IL-23, TNF-α, CD40L, G-CSF, etc. there are 73 up-regulated and 43 down-regulated cytokines. The KEGG analysis indicated that *T. matsutake* polysaccharide can influence the immune response of macrophages through a series of signaling pathways, and the three major signaling pathways are Jak-STAT signaling pathway, PI3K-Akt signaling pathway and NF-kappa B signaling pathway. Those three signaling pathway are closely related to the pathogenesis of many diseases. The results showed that TMP-A can activate immune cells to regulate the immunity.

Keywords: *Tricholoma matsutake,* polysaccharide, protein chip, macrophages.

INTRODUCTION

Matsutake is an ectomycorrhizal, an important member of subgenus mushroom fungi (Hou *et al*., 2013b), widely distributed in China, Japan and South Korea and the other Asian countries(Gevelt, 2014). In China, it has been regarded as a well-known food and a kind of biomedical materials (Takakura, 2015). The fruiting bodies of *T. matsutake* mushroom is very important (Yin *et al*., 2014), not only delicious, but also nutritious. The polysaccharides, as an important part of *T. matsutake* (Vaario *et al*., 2011), has antineoplasmic activity, multiple immunostimulatory activities (Hou *et al*., 2013a), antioxidation, antimutation and hematopoietic activities (Yin *et al*., 2014). Polysaccharides activities are closely related to increasing the immune system functional activation (Tong *et al*., 2013), for instance, production of cytokines, modulation TGF-β and NK cell activity, lymphocyte proliferation, and the innate immune responses activated by macrophages-mediated (Byeon *et al*., 2009; Gevelt, 2014).

Macrophages, as the first line of immune defense (Zhang *et al*., 2015), perform the phagocytic clearance in simple multicellular organisms evolved in development and adult life's dying cells (Behmoaras *et al*., 2015). In the host response, macrophage is a key cell (Vasconcelos *et al*., 2013) to protect the host by innate immunity, both as resident macrophage and recruited cell for monocyte-derived during inflammation. They respond to bacterial components and a number of cytokines, which requires a rigorous revision of gene expression allowing an appropriate adjustment of immune response (Cassetta *et al*., 2011; Date *et al*., 2014). The acquired immunity developed with reciprocal interactions between activated T and B lymphocytes and macrophages provided regulation and acquisition for antimicrobial resistance and enhanced novel levels. Recently, many studies demonstrated that the organs and tissues of all human are under perpetual and strictly control of the immune system, which, conversely, is regulated by its inherent component, including macrophages.

Macrophages, phagocytosis pathogens, recognition the pathogen-associated molecular patterns (PAMPs), activate the other immune cells by secretion proteins, and to present the lymphocytes with antigens to participate in the innate immune responses. According to the character of the stimulating agent (e.g. PAMP or cytokines), they develop into two categories activated macrophages, classically (M1) or alternatively (M2), typically characterized by the secretion of pro- and anti-inflammatory cytokines, respectively (Martinez and Gordon, 2014).

Polysaccharide used as immunomodulator currently, can activate immune cells, secretion cytokine interleukin and tumor necrosis factor to regulate the anti-tumor immune system. Mediated by specific receptors, the polysaccharides can cause the macrophage activation. The specific receptors, called pattern recognition receptors, can be identified in the initial stage for the foreign ligand

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immune response. Macrophages and the glycan recognition receptors are mainly connected with Toll-like receptors (toll like receptors, TLRs), complement receptor (complement receptor 3, CR3), lipopolysaccharide receptor CD14 (cluster of differentiation 14), scavenger receptor (scavenger receptor, SR), dendritic cell-associated C-type lectin 1 (dendritic cell associated C-type lectin-1, Dectin-1) and mannose receptor (mannose receptor, MR) etc.. In addition, the within endocytosis by macrophages mediated can also be activated by polysaccharides. For this reason, the receptor-mediated process in activated macrophages is very important.

Protein chips, well known as protein micro arrays, are miniaturized and parallel detection systems, contains small quantities of purified proteins in a high-density format (Chen and Zhu, 2006; Jin *et al*., 2014). Used all sorts of proteins (such as an antigen, anti-Thereof), as well as receptor polypeptide, ligand, etc. fixed to a carrier ordered (e.g., gels, Membranes , slides, microplate above and nano-beads), to analyze the test sample which can interact with the specific component (Hui *et al*., 2007; Lehr, 2010). Be similar as a gene chip, it is simple, fast, high-throughput, etc., basically satisfied the analysis of proteomics research stage currently. Investigators can use CHIP to find out in its natural environment which proteins bind to a specific site. Experimental manipulations can display how cellular conditions change the protein's binding behavior. In addition, CHIP can also be used to study histone modifications. In view of these advantages for the protein chip, we use the protein chip to detect the cytokines secreted by the macrophages, which are stimulated by TMP-A, for quantitative analysis, further to understand the effect of TMP-A acted on macrophages.

MATERIALS AND METHODS

Chemicals

The polysaccharides TMP-A of *T. matsutake* was prepared and preserved in Key Laboratory for Biological Resource and Ecological Environment of Education Ministry, College of Life Sciences, Sichuan University. DEAE-Cellulose 52 purchased from Sigma–Aldrich (Mainland, China). Monosaccharide standards were purchased from Beijing Biodee Biotechnology Co., Ltd. (Beijing, China). The other reagents used were of analytical grade.

Cell experiment

Macrophage (RAW264.7 cell, bought from Chengdu golden Kay Biological Technology Co., Ltd.) recovered, use 1640 basic culture medium (Phenol red free) for suspension culture, and replace the culture medium on time. The culture medium formula is as follows: the final concentration of solution was 90% in RPMI-1640 culture medium, 10% fetal bovine serum and 1% double antibody (anti 200mmL glutamine (Gln), 10mg/ml streptomycin and 10000U penicillin). 1640 basic culture medium (Phenol red free) 180ml; fetal bovine serum 20ml; double antibody 2ml. Two days later, when the cells in the logarithmic growth phase, replace the culture medium, and dilute the culture solution to 2×10^5/ml. Use the serum free medium to formulate the polysaccharides of *Tricholoma matsutake* to 10ug/ml. Around sample hole, each hole join sterile water 200ul; blank control group: cell dilution100ul; TMP-A: each hole cell dilution 100ul. At 37C, 5% of the carbon dioxide incubator culture for 72h, add 100ul cell culture solution to each hole. When add these drugs, mix the drug and cell with a gun for several times. Take the cultured cells supernatant and send to the company to carry out protein chip experiment.

Protein chip

The QAM-CAA-4000 reagent kit was bought from the Ray Biotech, Inc., Guangzhou the protein array pioneer company. The operation of this experiment is carried out according to the operation of the kit.

Differential expression and quantification analysis of the cytokines

After the end of the chip experiment, use the micro array analysis software, Array Vision, to analyze the experimental data. Use Quantibody Q-Analyzer software to do the quantitative data analysis.

KEGG enrichment analyses

All the cytokines were introduced into KEGG for cell signaling pathway analysis. Use software to draw the map of the identified signal pathways. Use NCBI to analyze the biological function of the related cytokines and the mechanism of the expression of macrophage protein.

STATISTICAL ANALYSIS

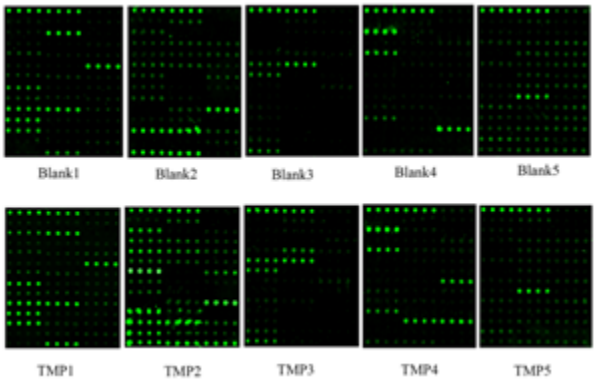
In the light of the larger difference between this experimental data, we have used logarithm of the original data when taking the histogram on the graph in order to express more clearly. All data were presented as mean ± standard deviation (SD) of three replications. Statistical analyses were performed using student's t-test and one-way analysis of variance. Values of $P<0.05$were considered to be a statistically significant finding.

RESULTS

Protein micro array experiment

The cells were cultured to take the supernatant to complete the protein chip experiment. Through the protein chip, we did the original diagram of the chip, including QAH-CYT-4, QAM-CYT-5, QAM-CYT-6, QAM-CYT-7 and QAM-CYT-8, and the results are shown in fig. 1. In this experiment, we made a standard curve also, as shown in fig. 2, so each cytokine has an standard curve, for example TNF-α, was shown in fig. 3.

Through this five protein chip test, we tested 200 cell factors, the results showed that there are 73 up-regulated and 43 down-regulated cytokines (fig. 4). The diagrammatic sketch was shown as fig. 5.



(QAH-CYT-4:blank1-TMP-A1; QAM-CYT-5:blank2-TMP-A2; QAM-CYT6: blank-TMP-A3; QAM-CYT-7:blank4-TMP-A4; QAM-CYT-8: blank-TMP-A5).

Fig. 1: Original diagram of protein microarray experiment

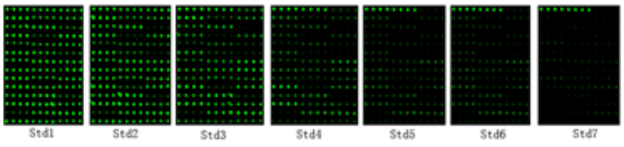


Fig. 2: Sketch map of cell factor standard

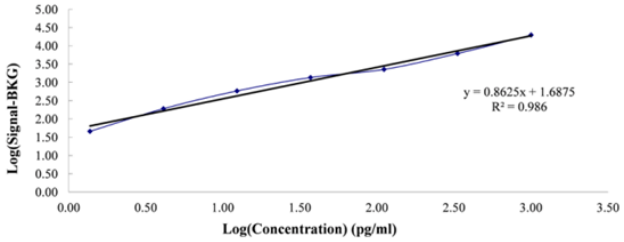


Fig. 3: The standard curve line of TNF-α

The up regulation of protein

This protein micro array experiments, measured two hundred cytokines, indicated that 74 cytokines were up regulated, such as AR, CD27L, CD30T, CD40, Fractalkine etc. (table1). The diagrammatic sketch was shown as fig. 6. The multiple of up regulation in the drug group compared to the blank group was shown as table2, and the diagrammatic sketch was shown as fig. 7.

The down regulation of protein

In this protein micro array experiment, the protein expression down regulation of 43cytokines, such as CXCL16, EGF, IGF1 and IL21 etc. are shown in table3.

Analysis of KEGG pathway

The KEGG pathway analysis indicated *T. matsu take* polysaccharide through a series of signaling pathways to induce the immune response of macrophages, such as Jak-STAT signaling pathway, TNF signaling pathway, NF-kappa B signaling pathway, PI3K-Akt signaling pathway, T cell receptor signaling pathway, pathways in cancer, TGF-beta signaling pathway and MAPK signaling pathway. This study focus on these three major signaling pathways, which are Jak-STAT signaling pathway (fig. 8), PI3K-Akt signaling pathway (fig. 9) and NF-kappa B signaling pathway (fig. 10).

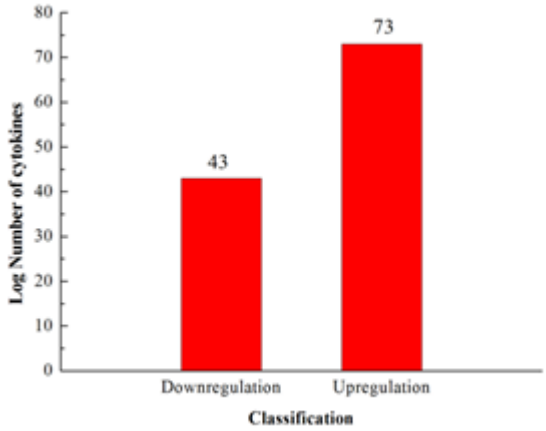
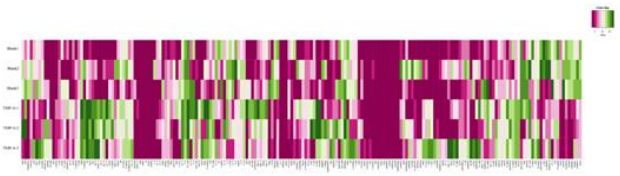
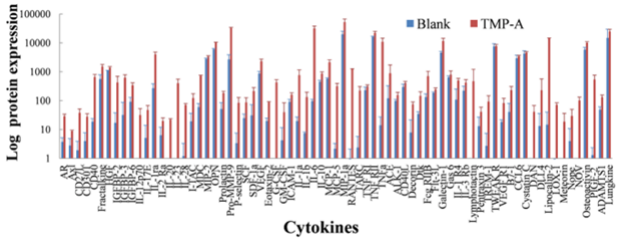


Fig. 4: Upregulation and downregulating of cytokines



Note: Logarithm of the original value and use the R-reject software to plotting

Fig. 5: The heatmap of QAH-CYT-4,5,6,7,8.



Note: The value of the vertical coordinate is the logarithm of the expression of the corresponding cytokines.

Fig. 6: Contrast diagram of the drug group and the blank group of the cytokines upregulation of protein

As shown in fig. 8, the ligands, such as IL6, IL10, IL23, CSF3 and PRL, combined with special receptors to leads receptor dimerization then the receptor associated JAK kinase activation, followed by phosphorylation on tyrosine residues of the receptor cytoplasmic tail. The phosphorylation of tyrosine residues will serve as docking sites for stat. Phosphorylation of STAT by Src homology 2 (SH2) in the domain of a tyrosine phosphate interact to dimerization and transfer to the nucleus, in the nucleus as a transcription activator induced gene expression.

Table 1: Comparison of the TMP-A and the Blank group in the upregulation of protein expression

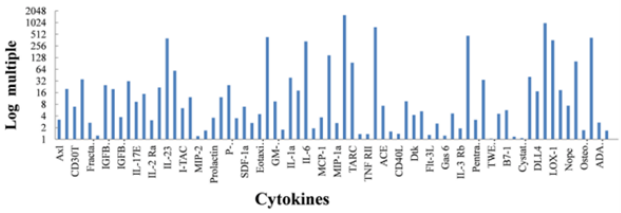
Cytokines	Blank	TMP-A	Cytokines	Blank	TMP-A
AR	3.72	28.76	IL-1β	7.36	132.79
Axl	2.77	8.91	IL-6	95.49	32048.36
CD27L	1.91	38.16	IL-10	450.25	863.65
CD30T	3.97	27.53	MCP-1	579.63	2137.03
CD40	18.68	658.67	MCP-5	2.15	317.67
Fractakine	562.00	1507.06	MIP-1α	20041.37	52500.56
HGF	1108.69	1380.30	RANTES	0.77	1234.96
IGFBP-2	16.94	422.88	TARC	2.36	223.15
IGFBP-3	31.81	626.76	TNF RI	228.30	312.44
IGFBP-6	91.23	341.38	TNF RII	16214.61	22119.17
IL-12p70	0	31.55	TNFα	13.99	10924.55
IL-17E	5.11	47.38	ACE	120.03	879.70
IL-1rα	270.53	4001.71	ALK-1	97.59	155.92
IL-2Rα	6.36	19.75	CD40L	297.73	411.38
IL-20	0	21.77	Decorin	7.83	75.29
IL-23	0	399.71	Dtk	34.45	146.11
IL-28	1.21	71.12	Fcg RIIB	132.53	702. 30
I-TAC	19.30	122.44	Flt-3L	186.28	242.33
MDC	59.64	726.81	Galectin-1	4576.92	11689.49
MIP-2	2742.07	3337.89	Gas-6	638.56	796.62
OPN	6019.70	10158.46	IL-1 R4	107.60	500.28
Prolactin	50.89	182.88	IL-3Rβ	220.75	421.60
Pro-MMP-9	2685.23	32762.70	Lymphotactin	0	469.68
P-selectin	3.35	83.48	Pentraxin 3	12.94	41.17
SCF	24.80	86.96	TREM-1	2.70	92.64
SDF-1α	30.70	213.13	TWEAK R	7514.40	7796.00
VEGF	878.73	2319.97	VEGF R1	18.10	82.43
Eotaxin-2	19.68	88.19	B7-1	40.49	229.21
G-CSF	0	433.52	CCL6	2934.83	3436.21
GM-CSF	4.22	39.81	Cystatin C	4301.28	4638.44
ICAM-1	90.52	160.05	DAN	0	40.89
IL-1α	19.62	765.95	DLL4	13.18	227.54
LOX-1	0.20	71.33	Osteoactivin	5831.09	10021.23
Meteroin	0	18.63	PIGF-2	1.30	541.66
Nope	3.95	29.25	ADAMTS1	49.11	132.76
NOV	0	101.75	Lungkine	14921.21	25070.03

Note: Each factor we did three sets of duplicate, including three repeat sets of blank group and three repeat sets of TMP-A group.

Fig. 9 showed the PI3K-Akt signaling pathway in macrophages induced by *T. matsutake* polysaccharide. Phosphatidylinositol 3-kinase (PI3K) has a kind of catalytic activity for the phosphorylation of D3 of the lipid kinase (Cail, *et al*., 2014; Shi-da, *et al*., 2010). With various growth factors acting on the membrane receptor and activation, the PI3K signaling pathway is also activated. PI3K through two ways to activate: a kind is interaction with growth factor receptor of phosphorylated tyrosine residues or connection protein to cause the conformational change of the two polymers and to be activated; the other is the activation of PI3K by direct binding of P110 and Ras (not shown in the fig). PIP3 binds to PDK1 and PH in the Akt domain to makes them to translocate to the plasma membrane, and the Thr308 site of the Akt catalytic domain is phosphorylated by PDK1, which enables to activate the Akt. In this pathway, the up-regulated cytokines induced include VEGF, LTA etc.

It is worth noting that in NF-kappa B signaling pathway, there are even more up-regulated cytokines (fig. 10). There are two kinds of NF-kappa B signal pathway, one is the MyD88 dependent signal transduction pathway. IL-1β activated IL-1R, and then the signal will be transmitted across the cell membrane to the cell interior. MyD88 combined with serine/threonine kinase Irak can further activate TRAF-6, activated of TRAF-6 can induced NF kappa B inhibitor kinase I kappa B of phosphorylation and degradation, the NF kappa B is

released from the resting state of I kappa B/NF- kappa B trimer and from the cytosol to the nucleus, combined with specific gene kappa B sequence promoter gene transcription, and promote the expression of some cytokines. The other is the MyD88 un-dependent signal transduction pathway. CD40L activated CD40 and TNF-α activated TNF-R1, the signal will be transmitted across the cell membrane to the cell interior. The way CD40 activated of TRAF-6 is similar with the MyD88 dependent signal transduction pathway. The way CD40 activated TRAF2/3, to stimulate the NFkappaB-inducible kinase (NIK), and promote the expression of some cytokines. In this pathway, the up-regulated cytokines induced include IL-1β, TNF-α, etc.



Note: The value of the vertical coordinate is the logarithm of the expression of the corresponding cytokines.

Fig 7: Multiple of up regulation in the drug group compared to the blank group

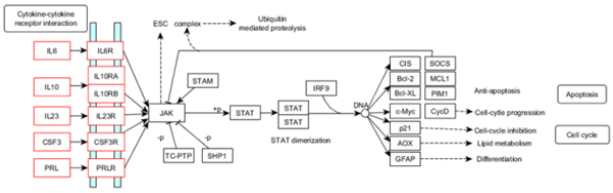


Fig. 8: Jak-STAT signaling pathway

DISCUSSION

Many scholars have studied on polysaccharides of *T. matsutake* on human disease, but the mechanism of polysaccharide of how to act on the immune system through the signaling pathway and the experimental study of a certain signaling pathway are still scarce. In view of this, we conducted the biological information analysis of polysaccharides of *T. matsutake* on macrophage signal transduction pathway. Use the DEAE-Sepharose fast flow column to extract and purify the polysaccharides of *T. matsutake* (TMP-A), and use TMP-A to stimulate the macrophages to detect the cytokines from macrophages. After that we used protein micro array experiments to test the macrophages cytokines (200), including IL-1β, IL-10, IL-23, TNF-α, CD40L, G-CSF, etc. Among these cytokines, there are 73 up-regulated and 43 down-regulated cytokines, while cytokines without significant changes are 84. The results also indicated that TMP-A can activate immune cells to secrete cytokines interleukin. To further study the mechanism of the TMP-A acting on the human immune system, we put all the factors into the KEGG pathway software to do the signal pathway analysis. The KEGG pathway analysis showed that TMP-A can through a series of signaling pathways to induce the immune response of macrophages, such as Jak-STAT signaling pathway, TNF signaling pathway, NF-kappa B signaling pathway, PI3K-Akt signaling pathway, T cell receptor signaling pathway, pathways in cancer, TGF-beta signaling pathway and MAPK signaling pathway. But some signaling pathways are not significant, so we focus on three major signaling pathways, which are Jak-STAT signaling pathway, PI3K-Akt signaling pathway and NF-kappa B signaling pathway. These three signaling pathways are associated with a variety of diseases, for instance TNF-α, IL-6 and IL-1 are closely related to the pathogenesis of Rheumatoid Arthritis (RA) and joint destruction. Therefore, studies of these pathways can make us know more about the pathogenesis of the diseases which are not very clear currently and formulate the treatment plan.

Firstly, JAK (Janus kinase) belongs to a protein tyrosine kinase (PTK), which can mediate the activation of cell factors and its receptor in the signal protein cascade (Mukthavaram *et al*., 2015; Springuel *et al*., 2015). JAK / STAT signaling pathway, an important signal transduction pathway found in recent years (Song *et al*., 2015), is one of the common signal pathway in the body. TMP-A can promote cytokine secretion by JAK / STAT signaling pathway to effect on the macrophage functions to regulate the immune system, which might involved in cell proliferation(de Freitas and da Costa Maranduba, 2015), differentiation, survival, apoptosis, immune dysfunction and tumor formation(Duzagac *et al*., 2015).

Phosphoinositide 3 Kinase, PI3K/Akt, is a kind of promoting cell survival signal transduction pathway (Carmona *et al*., 2015; Dimitrova and Arcaro, 2015; Wang *et al*., 2015), involved in the pathological process of cerebral ischemia, closely related to the survival of neurons. PI3K/Akt is well known as the most important cell survival signal transduction pathway currently (Brotelle and Bay, 2015; Yang *et al*., 2014). *T. matsutake* polysaccharide can induce the immune response of macrophages through PI3K-Akt signaling pathway.

At the same time, NF kappa B is a pleiotropic transcription factor of the regulation of immune responses (Diel *et al*., 2010), and the main functions are the regulation of cell proliferation and apoptosis, immune and inflammatory reaction, which play important roles in macrophages activation (Kleniewska *et al*., 2013). Meanwhile, IL-1β, the protein encoded by this gene is a member of the interleukin 1 cytokine family and produced by activated macrophages as a proprotein. The protein plays a role in thymocyte proliferation and is involved in the inflammatory response (Kosour *et al*., 2015; Ve *et al*., 2015). CD40, This gene is a member of the TNF-receptor

Table 2: Multiple of up regulation in the TMP-A group compared to the blank group

Cytokines	Multiple of up regulation	Cytokines	Multiple of up regulation	Cytokines	Multiple of up regulation
AR	7.73	MIP-1α	2.62	ICAM-1	1.77
Axl	3.22	RANTES	1594.74	IL-1α	39.03
CD27L	20	TARC	94.47	IL-1β	18.03
CD30T	6.94	TNF RI	1.37	IL-6	335.62
CD40	35.27	TNF RII	1.36	IL-10	1.92
Fractakine	2.68	TNFα	781.16	MCP-1	3.69
HGF	1.24	ACE	7.33	MCP-5	147.45
IGFBP-2	24.97	ALK-1	1.60	Nope	7.40
IGFBP-3	19.70	CD40L	1.38	NOV	101.75
IGFBP-6	3.74	Decorin	9.61	Osteoactivin	1.72
IL-12p70	31.55	Dtk	4.24	PIGF-2	417.66
IL-17E	9.27	Fcg RIIB	5.30	ADAMTS1	2.70
IL-1rα	14.79	Flt-3L	1.30	Lungkine	1.68
IL-2Rα	3.11	Galectin-1	2.55	Eotaxin-2	4.48
IL-20	21.77	Gas-6	1.25	G-CSF	433.52
IL-23	399.71	IL-1 R4	4.65	GM-CSF	9.44
IL-28	58.61	IL-3Rβ	1.91	DLL4	17.26
I-TAC	6.35	Lymphotactin	469.68	Lipocalin-2	996.38
MDC	12.19	Pentraxin 3	3.18	LOX-1	358.72
MIP-2	1.22	TREM-1	34.27	Meteroin	18.63
OPN	1.69	TWEAK R	1.037	SDF-1α	6.94
Prolactin	3.59	VEGF R1	4.56	DAN	40.88
Pro-MMP-9	12.20	B7-1	5.66	VEGF	2.64
P-selectin	24.93	CCL6	1.17	Cystatin C	1.08
SCF	3.51				

Note: The table above is the average data of the three groups in the TMP-A group compared to the control group

Table 3: Comparison of the TMP-A and the blank group in the down regulation of protein expression

Cytokines	Blank1	TMP-A-1	Cytokines	Blank1	TMP-A-1
CXCL16	512.89	312.11	IL-17B R	1010.74	249.94
EGF	18.52	5.10	MMP-2	208.15	69.64
IGF-I	156414.73	126236.60	MMP-3	170.95	69.81
IFNg	177.69	134.33	Persephin	66.69	8.13
IL-2	89.86	67.62	sFRP-3	128.63	57.32
IL-21	20.90	12.49	Shh-N	122.86	25.20
LIX	127.25	50.86	SLAM	2400.27	353.48
M-CSF	21.77	0.74	TACI	278.95	36.69
Galectin-3	2429.93	2318.91	TCK-1	88.44	22.09
MFG-E8	689.33	517.57	TECK	523.22	234.67
Chemerin	404.70	345.89	TRANCE	468.35	120.60
Endocan	199.74	75.63	TremL1	224.33	26.36
Fetuin A	400.60	84.84	TWEAK	491.08	58.99
IL-7 Rα	137.92	3.24	VEGF-B	416.03	84.51
Progranulin	29872.53	25549.21	VEGF-R2	78.29	4.53
6Ckine	175.79	34.35	E-Cadherin	86.11	32.01
Activin A	50.62	35.78	Epigen	85.22	7.84
ANG-3	170.29	85.95	Epiregulin	2121.17	170.65
ANGPTL3	51.48	18.27	Galectin-7	931.27	234.77
CCL28	254.74	74.69	Granzyme B	132.20	30.44
CD36	2656.43	1428.08	Chordin	216.46	26.71
IL-17B	1662.77	201.44			

Note: Each factor we did three sets of duplicate, including three repeat sets of blank group and three repeat sets of TMP-A group.

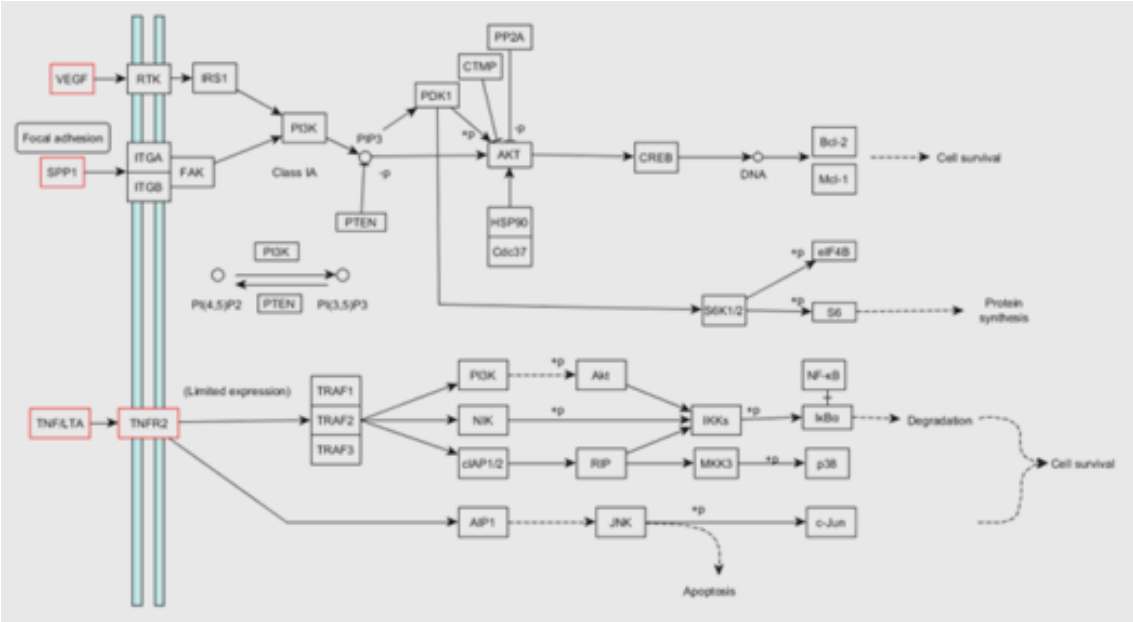


Fig. 9: PI3K-Akt signaling pathway

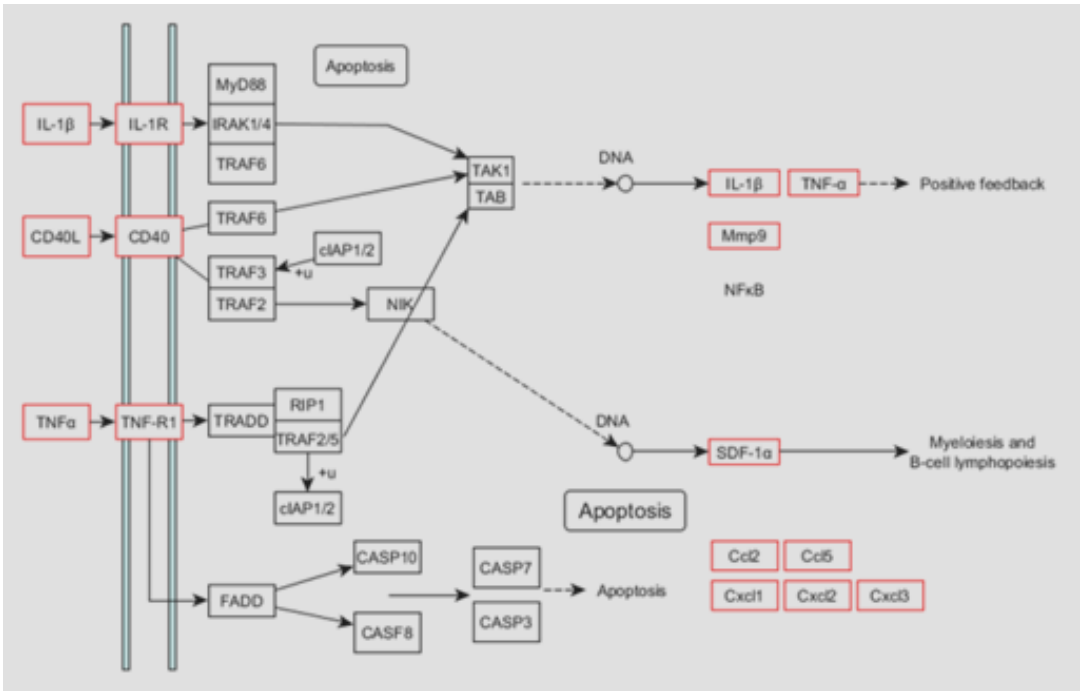


Fig. 10: NF-kappa B signaling pathway

Super family: It is a receptor on antigen-presenting cells in the immune system (Haller *et al*., 2013) and is essential for mediating a variety of inflammatory and immune responses, including T cell-dependent immunoglobulin class switching, germinal center formation and memory B cell development (Borcherding *et al*., 2010). As shown in fig. 6, TMP-A can positively promote the secretion of immune factors, activate macrophages by NF-κB which has positive effect on the cytokine secretion of macrophages, such as IL-1*β*, CD40 and TNF-*α* etc.

In addition, TNF-*α*, encodes a multifunctional cytokine of proinflammatory, a member of the tumor necrosis factor (TNF) super family, is mainly secreted by macrophages. It participated in the regulation of a wide range of biological processes (Zhenzhong, *et al.*, 2011), including cell proliferation, lipid metabolism, differentiation, apoptosis, and coagulation (Zabrodskii *et al*., 2015; Harel *et al*., 2015). Except for interacting with the receptor to activate macrophages, TMP-A can also be internalized by endocytosis. Different from the starch and glycogen, *T.*

matsutake polysaccharide molecules were swallowed up by the cells is not an easy solution, incomplete degradation of *T. matsutake* polysaccharide molecules in macrophages by unknown pathways may activate NF-κB pathway in macrophages, which plays a role in immune regulation. The activated macrophages can produce a number of biological active molecules, such as Oxide Nitric (NO), interleukin (IL), tumor necrosis factor (TNF) etc. which are related to immune response and inflammation.

CONCLUSION

In conclusion, TMP-A can activate immune cells to secrete cytokines interleukin and tumor necrosis factor to regulate the anti-tumor immunity and other related aspects. This study showed that Polysaccharides of *T. matsutake* (TMP-A) stimulated the macrophages to secrete inflammatory cytokines through three major signaling pathways, including Jak-STAT signaling pathway, PI3K-Akt signaling pathway and NF-kappa B signaling pathway, which are closely related to many diseases. The next, in our further study, we can analyze the effect of TMP-A on cytokines in the cell, and utilize Western Blot and ELISA to verify.

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