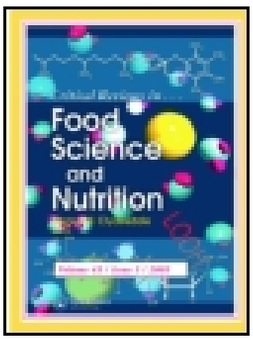

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Tumor microenvironment as a new target for tumor immunotherapy of polysaccharides

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Abstract

Many researches show that polysaccharides derived from fungi and plants have strong pharmacological activities such as enhancing the organism immune and anti-tumor function, and have few toxic and side effects. So the polysaccharides show a wide application prospect in the prevention and therapy of tumor. The tumor microenvironment consists of tumor cells and tumor cells' surrounding environment. The tumor microenvironment not only plays a key role in the development of tumor, but also is a potential treasure for searching new ways to treat tumor. In this review, we summarized polysaccharides' regulation effects on tumor microenvironment progression and tried to give a new theoretical basis for the exploitation of polysaccharides with anti-tumor activity.

Key words

Polysaccharide, Tumor microenvironment, Immunological therapy

1.Introduction

The tumor microenvironment is born with the cellular environment in which the tumor exists, at the same time, the microenvironment is dominated by tumor-induced. They are interacting with each other. The tumor microenvironment not only plays a key role in the development of tumor, but also is a potential treasure for searching new ways to treat tumor (Tähtinen *et al.*, 2015, Zhou *et al.*, 2014). The new ways to treat tumor can turn to the non-tumorous compositions or the interaction between the non-tumorous compositions and tumor cells.

Polysaccharides are polymeric carbohydrate macromolecules composed of long chains of monosaccharide units joined by glycosidic linkages. It is a kind of important biomacromolecule in the organisms besides protein and nucleic acid (Lowe and Marth, 2003). It has been recently shown that some fungal polysaccharides and polysaccharides from higher plants enhanced the organism immune function and exhibited anti-tumor activity (Xie *et al.*, 2013). Polysaccharide can use various ways to motivate or mobilize the immune system of organism, enhance the anti-tumor immunity in the tumor microenvironment and then control the development of tumor cells (Kim *et al.*, 2013, Sunila *et al.*, 2011, Zhan *et al.*, 2014). In addition, polysaccharide shows antiviral, anti-aging, antioxidant activities and hypoglycemic effects (Cheng *et al.*, 2015, Li, H. *et al.*, 2014, Xie *et al.*, 2015). Besides it has few toxic and side effects. So the research on the anti-tumor effect of polysaccharide has become a hotspot in the fields of modern biological science and medicine. Polysaccharide shows a wide application prospect in the prevention and therapy of tumor (King,

2006). It was put forward as a new target spot of polysaccharides anti-tumor therapy and gave a new train of thought for researching the bioactive polysaccharides.

This review includes the mechanism by which the polysaccharides regulate the tumor microenvironment. We will summarize the roles of tumor associated cells and secreted proteins in tumor microenvironment, and the mechanism of action which may be used when the polysaccharide controls the tumor microenvironment. And we try to give a new theoretical basis for the exploitation of polysaccharides with anti-tumor activities.

2. Tumor Microenvironment

The tumor microenvironment is highly heterogeneous, consists of tumor cells and tumor cells' surrounding environment. And it is also a tiny ecosystem which is evolving while the tumor is developing. This ecosystem sustaining proliferative, resisting cell death and enabling replicative immortality (Hanahan and Weinberg, 2011). Although the expansion of malignant cells originate from the initial instigateing to the creation of the tumor niche, non-tumorous compositions within the tiny ecosystem co-evolve with the tumor cells, so that both of them continuously participate in the process of tumorigenesis (Junttila and de Sauvage, 2013). The tiny ecosystem mainly contains tumor cells, tumor-associated fibroblasts, immune cells, endothelial cells, various growth factors, extracellular matrix and its degrading enzyme (Gould and Courtneidge, 2014, Lorger, 2012). Besides, the microenvironment exhibits some special physicochemical properties such as hypoxia and low-pH environment. Here, we discuss how signals from the tumor environment, including

immune cells, inflammatory factors, extracellular matrix, vasculature and chemokine, affect the formation and function of the tiny ecosystem to regulate tumor cell invasion and metastasis.

2.1 Immune cells

From the clinical treatment perspective, the strategy of removing local immune cells has more advantages than the systemic therapy: 1) Systematically dislodging $CD4^+$T cells may lead a defect in protective immune response, while the local treatment can avoid this side effect; 2) Local treatment will not affect the maturity of $CD8^+$ T cells in lymph nodes; 3) Selective inhibition of the regulatory T cells in tumor will provide a attractive prospect on local treatment. So infiltration of immune cells in tumor is one of the targets of the tumor immunotherapy.

2.1.1 Mastocyte

Mastocyte always infiltrates in the tumor stroma, especially in breast cancer, prostate cancer and melanoma. Many molecules secreted by the mastocyte take part in the growth of tumor cells in at least four ways: 1) Producing growth factors that are involved in the formation of hemangioma, such as heparin and vascular endothelial growth factor (VEGF); 2) Paracrining proteases decomposing the cellular matrix around it and promoting the tumor metastasis; 3) Secreting several growth factors that promote the development of tumor, such as epidermal growth factor, nerve growth factor, platelet-derived growth factor and stem cell factor; 4) Generating immunosuppressive agents such as histamine, IL-10 and transforming growth factor-β together with some activated dendritic cells inducing immune tolerance. So mastocyte may become a new target for adjuvent therapy by

selective inhibition of tumor promoting molecules' expression (Rigoni *et al.*, 2014). The possible strategies include the use of flavonoids. Flavonoids are polyphenolic compounds which naturally exist in the green plants and seeds. They have anti-oxidation, anti-inflammation and tumor inhibition activities. An epidemiology study has found that the intake of flavonoids compounds was inversely proportional to the possibilities of suffering from pancreatic cancer (Nothlings *et al.*, 2007). Gallocatechin, quercetin and curcumin have been found to have anti-tumor effects. These compounds were found to inhibit the secretion of pro-tumor molecules by mastocyte, and promote the secretion of antitumor molecules (Saif *et al.*, 2009).

2.1.2 Macrophage

Macrophage is one of the inflammatory cells which have the potential to promote the formation of tumor (Whiteside, 2008). More and more clinical evidences have shown the correlation between the infiltration of tumor associated macrophages and adverse outcome. In many types of tumor, macrophage is essential in the processes of tumor cells' invasion and metastasis. Macrophage may have different polarization under different activation of the environment. When macrophage is activated by irritants such as lipopolysaccharide and interferon-γ (IFN-γ) in a classical way, macrophage shows M1 phenotype. Macrophage of M1 type shows the features as the enhancement of antigen presenting ability and cytotoxic effect. On the other hand, when the immune system responses to parasite and allergen, Th2 type cells always produce cell factors like interleukin (IL)-4 and IL-13. And these cell factors can make macrophage mature into M2 type macrophage, which

control the inflammatory response by down-regulation M1-mediated functions and adaptive immunity. (Solinas *et al.*, 2009). These evidences that macrophage takes part in the tumor's growth and development include immunosuppression, promotion of tumor angiogenesis and metastasis. In the tumor, macrophage can raise the level of $CD4^{+}CD25^{+}$ regulatory T cells by producing factors like IL-10 and so on. So TAMs and Tregs can have a continuous interaction, TAMs may trigger a rise of Tregs population. (Zhou *et al.*, 2009). Besides, the promotion of sustained inflammation in tumor microenvironment is another important aspect for the TAM's effect on promoting tumor. Epidemiological studies have found that chronic infection and inflammation are important risk factors for cancer. The connection between cancer and inflammation can be found in the exogenous or the endogenous signaling pathways. The exogenous pathways mean that inflammation or infection can lead to malignant transformation. And the endogenous pathways mean that the tumor gene mutation leads to tumor which has the characteristics of inflammation in the tumor microenvironment. Then it can promote the tumor's growth and development (Mantovani *et al.*, 2008).

2.1.3 Tumor infiltrating lymphocyte (TIL)

Tumor microenvironment is famous for its large number of various lymphocytes. These lymphocytes are named after tumor infiltrating lymphocytes (TIL). Recent studies have shown that the TIL is composed of a variety of lymphocytes subpopulations instead of single composition. Therefore, TIL can't be used in immunophenotyping. Further studies found that most functions of

TIL are obviously defective for the reason of the gathering of immunosuppression cells in the tumor microenvironment and the immune escape mechanism of the tumor cells. So, in order to clarify relationship between TIL and clinical prognosis, we must systematically look into the compositions of TIL and their diversified functions.

$CD8^+$T cells

$CD8^+$ T cells' cytotoxic T cells (CTL) are quite important in many lymphocyte subsets. Because most tumor cells only express MHC class I molecules instead of MHC class II molecules, CTL may play a lethal effect after accepting the antigen which was present by tumor cell MHC class I molecules and being activated. As such, the anti-tumor immune response was mainly focused on $CD8^+$T cells for a long time. $CD8^+$T cells are indeed the key effect cells for anti-tumor immune response and are recognized as dominant T cells subgroup in anti-tumor immune response.

$CD4^+$T cells

$CD4^+$T cells are compositions of adaptive immunity, but their effects on strengthening anti-tumor immune response are under debate. $CD4^+$T cells' different subgroups include helper T cells and regulatory T cells. We speculate that in tumor initiation stage $CD4^+$T cells mainly play anti-tumor effect by presenting the antigen. And as soon as a tumor forms and develops continuously, a large number of agminated regulatory T cells will restrain $CD8^+$T cells, and tumor surface antigens also will be covered. $CD4^+$T cells are similar to $CD8^+$T cells, the tumor specific $CD4^+$T cells can recognize the existence of tumor antigen. And the fact that they can migrate to tumor was confirmed

in a mouse model and human tumor tissue (Beck-Engeser *et al.*, 2001, Pardoll and Topalian, 1998). However, in tumor's development process, as the continuously gathering of $CD4^+$ T cells in tumor microenvironment, they seemingly impede $CD8^+$ T cells' functions. $CD4^+$ T cells can be divided into different subtypes according to their cytokine profiles. T help cell 1 (Th1) has the feature which can produce IFN-γ and the others cytokines. And T help cell 2 (Th2) produces IL-4, IL-5 and other cytokines. The balance between Th1 and Th2 has a significant impact on various kinds of immune responses. For example, Th1 induces cellular immunity at first, Th2 induces humoral immunity. And the cytokine IFN-γ has a positive effect on presenting antigen. Because the expression levels of MHCI, MHCII and other molecules are all controlled by IFN-γ (York *et al.*, 1999). So the responses of Th1 are always related to positive cell reactions and responses of CTL. It is considered that responses of Th1 are beneficial to anti-tumor immune response. The study also found that Th2 lowered the anti-tumor immune response (Kacha *et al.*, 2000). Immune deviation is the transition from Th1 to Th2. There are already signs which are major factors for the failure of T cell-mediated anti-tumor immunity. Actually, the deviation of Th2 cytokines array was found in cancer patients at the progressive stage (Pellegrini *et al.*, 1996). In addition, Th2 cytokines were found to promote tumor's growth in animal models (Hu *et al.*, 1998). For this reason, to learn the effective anti-tumor immunity, we need to focus on the relationship between Th1 and Th2.

$CD4^+$ T regulatory cells

It was found that $CD4^+$ T regulatory cells (Tregs) had an inhibiting effect in TIL in the development

of tumor (Hindley *et al.*, 2011), and tumor microenvironment has become an important to show this balance. And the most important one is the balance between effective immune response and regulative immune response (Jones *et al.*, 2002, Sutmuller *et al.*, 2001). Recent studies have shown that $CD4^+$ Tregs can restrain the differentiation of $CD8^+$ T cell (McNally *et al.*, 2011). And this restraining effect mainly comes into playing in CTL's activation phase. It was proved that removing these Tregs, maybe through motivating their autoimmune response, can obviously enhance immune response of lung carcinoma, ovarian carcinoma, pancreatic carcinoma, breast carcinoma and gastroenteric tumor and lymphoma (Marshall *et al.*, 2004, Woo *et al.*, 2001).

Th17 cell

Th17 cells are newly defined $CD4^+$ T helper cell subgroup (Dong, 2008). Though the specific function of Th17 cells is still unclear, Th17 cells can be detected in tumors from animal models and patients. Inflammatory microenvironment plays an important role in the development of tumor. The production of tumor is closely linked with infection, chemical irritant and pro-inflammatory state, which is guided by autoimmunity. IL-23 in inflammatory conditions was closely related to the formation of tumor (Langowski *et al.*, 2006). IL-6 and IL-23 promote the development of tumor by means of activating STAT3 signaling pathway (Kortylewski *et al.*, 2005).

2.1.4 Natural killer (NK) cells

Natural killer cells have an effect on in-vitro dissolution of a variety of tumor cells, eliminating transplantation tumor and taking part in immune surveillance of spontaneous tumors in mice. These

cells have attracted extensive attention from tumor immunology experts. The functions of NK cells are modulated by suppressing and activating receptor-dependent signals which are combined and produced by their cell surface and a variety of specific ligands.

2.1.5 Myeloid-derived suppressor cells (MDSCs)

MDSCs are important cells which link inflammation with tumor. They mainly exist in the cell populations with functions of immunosuppression in the inflammatory or tumor patients' bodies. They are composed of undifferentiated mature and heterogeneous cells from the source of bone marrow, and dendritic cells, macrophage, granulocyte and other cells. Tumor and inflammatory cells secrete a variety of cytokines and chemotactic factors. And these factors can guide the production, migration and functional activation of MDSCs. The ways that MDSCs restrain body immune function and non-immune function are multifactorial and complex, including overproduction of iNOS, ROS, arginase and IL-10. MDSCs can lower the level of arginase in tumor microenvironment to effect on T cells' activation. MDSCs also can decrease the expression of T cell adhesion molecule CD62L. CD62L plays a key role in interaction between leucocyte and endotheliocyte. This molecule can induce the initial lymphocytes to peripheral lymph nodes effectively and guide T cells migrating to inflammatory site. MDSCs also can induce $Foxp^{3+}$ Tregs by producing TGF-p, IL-10 or arginase. And Tregs may promote the tumor's occurrence and development by restraining the production of body's anti-tumor immune cells.

2.2 Inflammatory factors

More and more clinical researches indicate that there are many types of inflammatory mediators and effectors, which take effects on the malignant transformation of epithelial cells. Inflammatory microenvironment may already exist before tumor genesis. And carcinomatous degeneration may lead to the change of inflammatory microenvironment. In the other side, it promotes the development of tumor. Inflammation can cause the genome instability and epigenetic changes, followed by abnormal gene expression and abnormal angiogenesis which promotes cell proliferation and resists apoptosis and then leads to the formation of tumor. With the beginning of epithelial cell's malignancy, the host's anti-tumor immune reaction is lost in the microenvironment and chronic inflammation is further increased, and finally causes the formation of tumor. So inflammation mediates tumor's development and spread. Tumor-related inflammation seems to have both proinflammatory and anti-inflammatory functions. When the balance is inclined to the proinflammatory environment, the vicious transformational epithelial cells can escape from immune surveillance. With the growth of their own blood vessels, these tumor cells gradually develop and become mature, and finally break through the basement membrane and diffuse and transfer.

Studies have found that NF-kappaB and STAT3 signal pathways play an important role in maintaining chronic inflammation by the means of regulation of proinflammatory cytokines. They are important mediators which promote the growth and deterioration of epithelial cells in proinflammatory environment (Karin, 2006, Karin and Greten, 2005, Marino *et al.*, 2014, Rius *et al.*,

2008). In proinflammatory environment, there are a large number of molecules which promote the development of tumor, including transcription factors (such as NF-kappaB), signal transduction transcription activating factors (such as STAT3) and main inflammatory cytokines (such as IL-1β, IL-6, IL-23 and TNF-α). Among them, STAT3 was reported to play a very important role in maintaining several oncogene signaling pathways (Lee *et al.*, 2009, Yu *et al.*, 2007). A study on colon cancer found that STAT3 was an important regulatory factor for cell proliferation and survival. It regulated the expression levels of c-Myc, Mcl-1, CyclinD and Bcl-2 (Becker *et al.*, 2004). The study also indicates that inflammatory cytokines link the chronic inflammation with tumor. These cytokines are activated in inflammatory cells and tumor cells. And they play an important role in the processes of maintaining chronic inflammation, promotion of malignant epithelial cells' translation and inhibition of tumor immune surveillance. Cytokines can be divided into proinflammatory factors such as IL-1, IL-6, IL-8, IL-11, IL-12, IL-18, IL-23, IFN-γ, TNF-α and MIF, and anti-inflammatory factors such as IL-4, IL-10, IFN-α, IFN-β and TGF-β. By activating STAT1 and STAT3, IL-6 was found to be related to the development of chronic inflammation, intestinal cancer and gastric cancer (Bromberg and Wang, 2009). The study also found that IL-$6^{-/-}$ DSS/AOM mice model cannot generate colitis associated carcinoma. This effect is controlled by activation of STAT3 in enterocytes (Bollrath *et al.*, 2009, Grivennikov *et al.*, 2009). Morever, not only to enterocyte, IL-6 is also very important to the other components in tumor microenvironment, such as activable DCs and Th cells. The dynamic overall outcome of the interaction among IL-6, malignant epithelial cells, and

other elements in microenvironment lead to the continuous production and secretion of cytokines and a further extension of the chronic inflammatory state which is aggravated by tumor-promoting factors. Chemotactic factors (CXXL5, CXCL12) also take part in MDSCs' recruitment. MDSCs are important effectors in the production of tumor vessels like TAM. They may mediate the generation of blood vessels within the tumor and then form a resistance to treatment (Shojaei *et al.*, 2007).

2.3 Extracellular matrix

The extracellular matrix (ECM) is a network of molecules supporting tissues, and is also able to influence and interact with cells in the environment. It consists of collagen, elastin, fibronectin, laminin, proteoglycan and other macromolecular substances (Klein *et al.*, 2004). Basement membrane (BM) is a specialized structure which contains ECM constituent. It can provide stable matrix for normal epithelial cells' adhesive growth. The destruction of BM's integrity is considered to be a symbol of malignant tumor invasion. Matrix metalloproteinases (MMPs) are a class of zinc ions dependent protein family and endopeptidases. They can hydrolyze a variety of constituents of ECM. MMPs' tissue inhibitor contains 4 homologous family molecules, TIMP-1, TIMP-2, TIMP-3 and TIMP-4. MMPs play an important role in the tumor's evolutional process. MMPs are produced by macrophage, neutrophil, mastocyte, adipocyte, blood vessel and cells around blood vessel in the tumor tissue carry great weight (Noel *et al.*, 2008). And the MMPs which are secreted by these cells may break the balance of ECM's degradation. Then they make the tumor cells to have the capacity to break through the BM and histochemical barrier composed by ECM. At last, they provide

necessary conditions for tumor's invasion and transfer.

2.4 Vasculature

2.4.1 Endothelial progenitor cells (EPCs)

The formation of tumor vessels not only comes from the new endothelial progenitor cells which are formed by adult vessels' sprout differentiation, but also comes from bone marrow derived circulating endothelial progenitor cells (EPCs). The latter is considered to be the main source, which decides the location where the tumor and the tumor vessel forms. It is still controversial that EPCs decide the growth of the tumor. Though many studies have found that EPCs cells played a role in tumor biology, some researches reported opposite results.

2.4.2 Formation of lymphatic

Lymphatic is necessary for maintaining the balance of normal body fluid and immune response. And it also takes part in some important pathological processes like lymphedema and tumor metastasis. In the forming process of lymphatic, the dynamic interaction between lymphatic endothelial cell and the extracellular matrix around it is necessary to lymphatic endothelial cell's invasion, migration and subsistence. Lymphatic system collects exudates to balance the normal tissue fluid. On the other side, lymphatic absorbs and transports the lipids released by enterocyte. Lymphatic also acts an important part of immune surveillance in carrying immune cell and antigen. Many researches have proved that there are proliferative lymphatics both in tumors and around tumors, Lymphatic vessels are also necessary parts of the body's immune defenses (Alitalo *et al.*,

2005). The formation of tumor lymphatic is related to tumor's transfer (Mumprecht and Detmar, 2009). The development and remodeling of lymphatic system require the complex interactions between lymphatic endothelial cell and extracellular matrix. These interactions are mostly regulated by cellular matrix receptor family--integrin. Integrin combines with specific extracellular matrix molecules, and initiates intracellular signaling cascade.

2.5 Chemokine

Chemokine is a kind of low molecular weight cytokine. They are able to mediate the migration of leukocyte in the reaction of stimulus of inflammation and pathogens. Chemokine and its receptors have a variety of functions including regulation of cell growth, survival, angiogenesis, movement and metastasis, control of leukocyte infiltration to affect tumor immune activity, and influence on the incidence, growth, angiogenesis, invasion and metastasis of tumor cells. The amount and type of leukocyte infiltration depend on the kind of chemokine and the specific receptor which is expressed by infiltrating cells in the tumor microenvironment.

In various kinds of human malignant tumors, the expression of CC-chemokine is an important decision factor for macrophage and lymphocyte infiltration (Zlotnik, 2006). CCL2 was considered as monocyte specific chemotactic agent until in vitro study proved that CCL2 was a main chemotactic agent. It worked in the procedure of which plant lectins (PHA) stimulate T cell chemotaxis in white blood cells. In addition, the differences between the expression and reaction of chemokine mainly depend on migrating and homing of Th1 and Th2 to a large degree. Macrophages

and lymphocytes gathered in tumors have been proved to be mediated by CC-chemokine from the tumor, so the expression of chemokine receptor in tumor specimens has the potential for becoming biomarkers of relative tumor aggressiveness (Zlotnik, 2006). With the targeted cell surface receptors, CC-chemokine is able to recruite macrophages, T lymphocytes, NK cells and dendritic cells into tumors (Ruffini *et al.*, 2007). The anomalies of chemokine in the tumor microenvironment may affect the immune inhibiting type 2 macrophages (M2), which can release immunosuppressive cytokines IL10 and TGF-β. M2 macrophages can also produce a large number of CCL2. In tumor microenvironment, the chronic expression of CCL2 can promote the occurrence and metastasis of tumor (Lu *et al.*, 2007). In all of the chemokine, CXCL8 has been widely studied as an effective angiogenesis-promoting molecule. In vitro studies also found that CXCL8 dose-dependently stimulated the proliferation of endothelial cells and the formation of capillary lumen (Petreaca *et al.*, 2007). Moreover, these effects can be inhibited by CXCL8 antibody. Some other members of chemokine's family have been confirmed to play important parts in angiogenesis. IL-17 was found to selectively secrete an array of angiogenic CXC chemokines in human lung cancer, including CXCL5, CXCL6 and CXCL8 (Numasaki *et al.*, 2005). In renal cell carcinoma, the expression levels of CXCL1, CXCL3, CXCL5 and CXCL8 were increased in tumor tissue. The expression of CXCL9 and CXCL10 in the tumor may help reduce the tumor size (Kondo *et al.*, 2004).

3 Polysaccharides and tumor

Tumor immune microenvironment is the place in which tumor infiltrating immune cells

participate in an complicated and dynamic crosstalk with tumor cells. Immune system restrains the development of tumor by recognizing and killing tumor cells. Tumor weakens immune cells' anti-tumor activities by inhibitory molecules on cell surface, cytokines secretion, and degradation of polypeptide chain. Researchers used to consider that body's immune system may play a role in killing tumor and protecting itself in the process of tumor's development. However, recent studies have found that tumor infiltrating immune cells did not have an anti-tumor effect. On the contrary, they may promote the growth, invasion and transfer of tumor. This is mainly because tumor cells can secrete various immunosuppressive factors and chemotactic agents, therefore the domesticated immune cells become the accomplice which plays a role in the process of tumor's evolution and immune escape. So the attempt of improving the prognosis of tumor by changing immune microenvironment which is by the means of immunotherapy is born at the right time.

3.1 Immunoregulatory activity of polysaccharide

The immunoregulatory activity of polysaccharide has been widely accepted in recent years. King's study have provided clear evidence for the anti-tumor properties of polysaccharides in clinical trials might be due to the biological activities such as immunoregulatory activity of polysaccharide (King, 2006). A large number of researches have demonstrated that many kinds of natural polysaccharides from different plants, animals and microorganisms have anti-tumor effects by improving body's immunity. For example, our laboratory has reported that the *ganoderma atrum* polysaccharide (PSG-1) significantly increased the spleen index of mice, improved the proliferation

of T lymphocyte and B lymphocyte, increased the levels of superoxide dismutase (SOD), TNF-α and IL-2 and decrease the content of MDA (Li, W.J. *et al.*, 2012, Li *et al.*, 2011). And our team also found that PSG-1 can activate macrophage RAW264.7 through NF-kappaB pathway (Yu *et al.*, 2013). We further found that in CT26 and S180 tumor-burdened mouse models, PSG-1 activated the immunocompetence of macrophage and restrained the growth of tumor by inhibiting MAPK signal transduction which was induced by TLR4 (Zhang *et al.*, 2013a, Zhang *et al.*, 2013b).

Another polysaccharide *Lentinan* exhibited anti-inflammatory activity through inhibition of IL-8 mRNA expression, and this was associated with NF-kappaB pathway (Nishitani et al., 2013). *Lentinan* was also found to reduce the expression of tumor necrosis factor receptor 1 (TNFR1) and then influence cell's endocytosis (Nishitani *et al.*, 2013). *Anji white tea* polysaccharides was found to significantly increase spleen index, thymus index and phagocytosis ability of peritoneal macrophages in tumor-bearing mice, and inhibite the proliferation of S180 cell in a dose-dependent manner (Xia et al. , 2013). *Agaricus blazei* polysaccharide promoted the differentiation and maturation of dendritic cells and strengthened the anti-tumor effects in dendritic cells (Qin et al., 2014). Our laboratory found that, *Plantago asiatica* polysaccharide (PLP-2) increased expression of maturation markers on DCs. PLP-2 was also found to decrease DCs endocytosis and increase IL-12 levels (Huang *et al.*, 2014). Furthermore, *aloe* polysaccharide, *ginseng* polysaccharide and *achyranthes* polysaccharide were also reported to improve the activity of tumor-bearing mice's immune cells. In order to restrain the growth of tumor, they produce many kinds of cytokines to

activate natural killer cells and T lymphocyte.

3.2 Immunomodulatory effects of polysaccharide in tumor microenvironment

The bioactivity presented by natural polysaccharide makes it has a large exploration potentiality in reversing immunosuppression of tumor microenvironment. And researches in recent years have made some progress. Polysaccharide does no harm to body's immune response, at the same time, the natural polysaccharide owns the immune regulating function to activate body's immunity. The immunoregulation effects of polysaccharide on tumor microenvironment were summarized as followed.

3.2.1 Chemotactic factors and cytokines

The overexpression of chemotactic factors on cytomembrane seems to be the signs of tumor cells. An in vitro study by Li et al (2012) showed that through recovering the balance of cytokines in tumor microenvironment, *astraglus* polysaccharides (APS) inhibited the migration of regulatory T cells by reducing the expression of stromal cell-dericed factor-1 (SDF-1) through the CXCR4/CXCL12 pathway, and then restrained the growth and proliferation of $CD4^{+}CD25^{+}$Treg cells in a dosage and time dependent manner. APS can renovate the cytokine balance and restrain Treg cells' immune suppressive function. These results suggest that APS may effectively increase the survival of patients with human hepatocellular carcinoma (Li, Q. *et al.*, 2012). It was also found the *Thuja* polysaccharide can reduce the increase in the levels of pro-inflammatory cytokines in the serum of metastatic tumor-bearing animals. Treatment of *Thuja* polysaccharide also increased the

levels of anticancer molecules such as IL-2 and TIMP in the serum, and restrained the metastasis of tumor cells (Sunila *et al.*, 2011). Wang's present study showed that oral *mushroom* beta-glucan treatment significantly increased the expression of IL-12 and IFN-γ , but significantly reduced the expression of COX-2 , IL-6, IL-10 and TGF-β (Wang *et al.*, 2015). *Maitake* beta-glucan possibly helping to relieve the immunosuppression which results from chemotherapies. Ito et al demonstrated *Maitake* beta-glucan that enhances the granulopoiesis and mobilization of granulocytes and their progenitors by stimulating G-CSF production (Ito *et al.*, 2009).

3.2.2 Th1 /Th2 balance

The balance of Th1/Th2 plays a very important role in the regulation of body's immune activation and inhibition. The decreased level of Th1 and the increased level of Th2 in the tumor microenvironment may weaken the activities of many immunocytes. These activities include the increase of immature DC content, differentiation from TAM to M2 type macrophage, decrease of CTL's killing capability. The current approach is using cytokines and its antagonists to reverse the shift state of Th1/Th2. Th1 type cytokines can change Th1/Th2 into Th1, and restrain the preferential expression of Th2. Th2 type cytokines have an opposite effect. Gene therapy can produce a series of phenotypic change and induce the shift state of Th1 /Th2 by an intentional priming or inhibition of the expression of one or several genes. For example, transfection of the exogenous IL-12 and B-7 genes can promote floating of Th0 subpopulation to the direction of Th1. In addition, vaccine also can lead the body to achieve active immunity. But the above methods have

limitations including serious adverse reaction, short action time and high cost. Therefore, researchers turn their eyes to safe and natural compounds including polysaccharide.

It was reported that the anti-tumor function of polysaccharide fraction extracted from *Agaricus brasilie* (ATF) may be due to the selective induction of the immunocompetent cells from spleen to the tumor site(Pinto *et al.*, 2009). And the switch of this selectivity is controlled by cytokines. ATF was found to promote spleen of tumor-bearing mice to secrete IFN γ and restrain the secretion of IL-10. Yang et al (2006) found that *Angelica sinensis* polysaccharides exhibited its immunomodulatory activity by regulating the expression of Th1 and Th2 related cytokines. It promoted spleen cells to secrete Th1 type cytokines IFN γ and IL-2, but restrain Th2 type cytokines to secrete IL-4. The polysaccharide firstly activated nonspecific immunity including macrophage and NK cells. And then it activated helper T lymphocyte. The proportion of $CD4^+$ T cells was dramatically elevated by the polysaccharide, while the proportion of $CD8^+$ T cells was slightly reduced (Yang *et al.*, 2006). He et al (2011) found *glycyrrhizae* polysaccharide can decreased the percentage of Tregs in tumor-bearing mice's lymph node and spleen and lowered the expression of $Foxp3^+$ in Tregs. At the same time, the serological test found the decrease of IL-10 and TGF- β levels and the increase of IL-2 and IL-12p70 levels. So *glycyrrhizae* polysaccharide restrained the development of tumor by regulating the proportion of Th1/Th2 in the tumor-bearing mice (He *et al.*, 2011). Zhao et al (2012) found polysaccharide-protein complex from *Scolopendra subspinipes mutilans L Koch* (SPPC) by

enhancing the ratio of Th1/Th2 cytokines inhibits tumor growth in vivo (Zhao *et al.*, 2012).

3.2.3 Dendritic cells

Promoting the maturity and improving ability of dendritic cells' (DC's) antigen presentation is another important mechanism by which polysaccharides reverse the immunosuppression. The polysaccharides purified from *Medlar, Astragalus, Antrodia camphorate, Ganoderma lucidum* and *Angelica gigas* have all been proven to promote the maturation and differentiation of DC. *Mori fructus* polysaccharide was found to promote the maturation of DC through increasing phosphorylation of MAPKs and NF-kappaB p65 subunit, significant semaphore molecules downstream from TLR4. And it likewise could be used as a supplement in DC-based tumor immunotherapy (Shin *et al.*, 2013). *Pueraria lobata* polysaccharide also promoted the maturation of DCs' phenotype and functioned through MAPK pathway which is induced by TLR4(Kim *et al.*, 2013) . In addition, Chen et al (2012) found that *Lycium barbarum* polysaccharide promoted the molecular phenotype maturation of DCs and stimulated allogeneic lymphocyte proliferation, and increased the expression of IL-12p70 and IFN-γ. And the expression of NF-kappaB in the DCs was also elevated by the polysaccharide. These results imply *Lycium barbarum* polysaccharide may play an anti-tumor role in the virus-related environment. In addition, *Gekko* sulfated polysaccharide-protein complex was found to be helpful to restore the defective biological feature of DCs by changing the tumor microenvironment and reducing the secretion of IL-10 in DCs (Chen, D. *et al.*, 2012). *Lycium barbarum* polysaccharide (LBP) could increase the expression of the

phenotype of DCs by secretion of IL-12p70 plays the anti-tumor role (Chen, J. R. *et al.*, 2012).

3.2.4 MMPs inhibitor

Taxus mairei polysaccharides effectively inhibited melanoma cell line's migration and invasion ability, and reduced the expression of MMP-2 and MMP-9 (Zheng *et al.*, 2014). *Cordyceps sinensis* polysaccharide was found to influence hepatic stellate cell's activity by restraining the expression of MMP2, MMP9 and TIMP2. Treatment of the polysaccharide down-regulated expression of hepatic α smooth muscle actin, TGF-β1, 1 receptor, TGF-βR-Ⅱ, p-Smad2, p-Smad3 and TIMP2 proteins, and inhibited the activities of MMP2 and MMP9 in liver. These results indicated that the anti-liver fibrosis effect of the polysaccharide may be associated with the suppression on hepatic stellate cell's activation by TGF-β1/Smads signaling pathway (Peng *et al.*, 2013).

3.2.5 Tumor-associated Macrophage (TAM)

Tumor-associated macrophages (TAM) play essential roles in the multiple stages of tumor development and progression. TAM can secrete a plethora of cytokines that induce cell migration, mediate immunosuppression and promote angiogenesis. Eliminating TAMs can be a promising strategy. Zhan et al (2014), it was found that the polysaccharide from *Bletilla striata* (BSP)-alendronate (ALN) conjugate accumulated in macrophages and promoted them into apoptosis. In the S180 tumor-bearing mouse model, BSP-ALN could effectively remove TAMs, significantly inhibited angiogenesis, strengthened local immune surveillance, eventually inhibited tumor progression without causing any side effects such as systematic immune response(Zhan *et al.*, 2014).

3.2.6 Formation of tumor vessel

The suppression of angiogenesis by polysaccharide may be one of the mechanisms of the inhibition of tumor cell's metatasis. Qu et al (2014) reported that 48 hours treatment of *Schisandra chinensis* polysaccharides restrained the proliferative effect of tumor cells. In vivo experiment also showed that 4 weeks intervent of *Schisandra chinensis* polysaccharides significantly suppressed transplanted tumor growth. The polysaccharides also significantly decreased the expression levels of vascular endothelial growth factor (VEGF), CD31 and CD34 in renal carcinoma tissues. A further analysis found that *Schisandra chinensis* polysaccharides could accelerate tumor cell's apoptosis, restrain tumor growth and angiogenesis by up-regulating apoptosis genes Bax and p53 and down-regulating anti-apoptosis gene Bcl-2 (Qu *et al.*, 2014). Polysaccharide K was found to inhibit hepatic metastasis and suppress the growth of RCN-H4 cells (Tamagawa *et al.*, 2012). In addition, a low molecular weight polysaccharide from *agaricus blazei* was found to be a potential treatment for tumor therapy, and the anticancer and antiangiogenic effects of the polysaccharide may be related to the reduction of VEGF expression (Niu *et al.*, 2009).

4 Conclusions

At present, the chemotherapy agents and radiation therapy which are directly targeted at tumor cells not only have a serious adverse reaction but also result in an unsatisfactory therapeutic effect. And they may have a risk of tumor metastasis and recidivation. The important reason may be that these treatments ignore important functions of tumor microenvironment in the process of tumor

progression. Polysaccharides are a class of natural substances with anti-tumor activity. Though the studies on anti-tumor activities of polysaccharides have made some advances, few polysaccharides have been used for cancer treatment. The main reason may be that most researches on polysaccharides anti-tumor mechanism are limited to the immunoregulation, which may not show the novelty and effectiveness of therapeutic target for polysaccharides.

Recent years, presenting the tumor microenvironment as a new target for polysaccharide anti-tumor therapy undoubtedly provides a new field for the research and development of such drugs (Pinto *et al.*, 2009, Ren *et al.*, 2012). Natural polysaccharides possess a wide range of immune activations and so have a good application prospect in adjusting the treatments in tumor microenvironment. Polysaccharides have some advantages such as plentiful source and less adverse reaction. The common chemicals are difficult to compare with them. With a further research, polysaccharides which take tumor microenvironment as a target will become a new field of anti-tumor therapy(Wang *et al.*, 2015, Li, S. *et al.*, 2014). They, in combination with the traditional anti-tumor therapies which focus on tumor cells, may provide a safer and effective strategy for clinical intervention of tumor.

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