Recent advances in research regarding to natural compounds that target pro-tumor macrophages

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Recent findings have demonstrated that tumor-associated macrophages (TAMs) are closely linked with tumor progression via the induction of tumor cell activation, angiogenesis, tumor cell resistance to chemo/radiotherapy, and immune suppression. More recently, various companies have developed compounds or antibodies that target TAMs, and some of these agents are in clinical trials. We identified several natural compounds that inhibit pro-tumor activation of TAMs (but do not kill TAMs), such as onionin A and various triterpenoid compounds, including corosolic acid. These compounds also suppress myeloid-derived suppressor cells and abrogate tumor progression by boosting anti-tumor immune responses in animal models. In this article, we review our current studies of natural compounds that target TAMs.

Keywords: corosolic acid; onionin A; triterpenoid compounds; macrophages; cancer; CD163; CD204

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Introduction

The significance of tumor-associated macrophages (TAMs) in tumor progression

A number of non-tumor stromal cells, such as macrophages, fibroblasts, neutrophils, endothelial cells, and lymphocytes, can be found in tumor tissues [1, 2]. Macrophages, neutrophils, and fibroblasts detected in tumor stroma tend to have pro-tumor functions and are referred to as TAMs, tumor-associated neutrophils (TANs), and cancer-associated fibroblasts (CAFs), respectively [1-6]. Interactions between these stromal cells are considered to be involved in tumor progression and chemo/radio-resistance [7-9]. The roles of stromal cells in tumor development differ according to organ or histologic subtype. For example, few TANs and CAFs are found in brain tumors, but CAFs are critical stromal cells in pancreatic tumors [8-10]. TAMs can be detected in almost all malignant tumors, and compared with other stromal cells, activated TAMs secrete high amounts of soluble factors that influence the tumor microenvironment.

TAMs are generally derived from circulating monocytes rather than resident macrophages [11, 12]. Various chemokines, including chemokine (C-C motif) ligand (CCL)2, as well as cytokines such as macrophage colony-stimulating factor (M-CSF, also known as colony stimulating factor-1, CSF-1) and granulocyte-macrophage colony-stimulating factor...
Macrophages are difficult to classify due to their heterogeneity. Since the late 1990s, activated macrophages have been broadly divided into two categories based on differences in surface markers or nitric oxide/ornithine production: classically activated and alternatively activated macrophages. Although the concept of “M1” or “M2” macrophages is a convenient and simple means of dividing TAMs into two populations, it is now known that TAM populations can be quite heterogeneous, with cells exhibiting a wide range in polarization status stimulated by complex signals in the tumor microenvironment. Recently, researchers have suggested that TAMs should be classified into six groups based on their location and function: angiogenic, immunosuppressive, invasive, metastasis-associated, perivascular, or activated macrophages. Human studies using resected cancer specimens demonstrated that a higher density of CD163- or CD204-positive TAMs (rather than CD68 [pan-macrophage marker]-positive TAMs) is closely associated with higher rates of histologic malignancy, resistance to chemo/radiotherapy, and worse clinical prognosis in many cancers, indicating that pro-tumor TAM subpopulations play important roles in cancer progression. Although detailed classification of TAMs in human samples has proven to be difficult, it is thought that CD163- or CD204-expressing pro-tumor TAMs secrete a variety of soluble factors involved in cancer cell activation, invasion, metastasis, angiogenesis, and immunosuppression (Figure 1). Therefore, targeting pro-tumor TAMs is considered a novel approach for cancer therapy.

Various compounds or neutralizing antibodies against CSF-1 receptor (CSF-1R) have been identified as potential inhibitors of the pro-tumor function of TAMs or as
molecules for use in depleting TAMs in cancer tissues. Clinical trials of combination therapies with anti-cancer drugs or immune-checkpoint inhibitors are now underway [20, 21]. Various natural compounds with a potential to target pro-tumor TAMs have also been identified in recent years, and in this review, we summarize our recent studies pertaining to anti-cancer natural compounds that target myeloid cells, including TAMs.

**Onionin A**

Onionin A (3,4-dimethyl-5-[1E-propenyl]-tetrahydrothiophen-2-sulfoxide-S-oxide; ONA) is a natural sulfur-containing compound recently isolated from acetone extracts of Allium cepa (onion) [22, 23]. A total of 7 natural compounds similar to ONA have been isolated from onion by Nohara and colleagues, though the unique structures of these compounds might be associated with the acetone extraction method used for their isolation.

ONA inhibits CD163 expression on human macrophages stimulated with IL-10 [22] and decreases PD-L1 and increases interleukin (IL)-12 production by macrophages [24]. The cytokines IL-10 and IL-12 are associated with immunosuppression and -activation, respectively. PD-L1 is a ligand of PD-1, which suppresses T-cell activation, and studies have shown that blocking PD-L1/PD-1 signaling strongly activates anti-tumor immune responses in various malignant tumor types, including melanomas and cancers of the lung and kidney [25, 26]. Macrophage expression of CD163 and PD-L1 is regulated by Stat3 signal activation, which is known to play roles in angiogenesis and immunosuppression [27], and in vitro studies have shown that ONA inhibits Stat3 activation in activated human macrophages [24]. Based on these data, which suggest that ONA inhibits the immunosuppressive function of TAMs, in vivo studies using sarcoma and ovarian cancer mouse models were performed [24, 28]. In an LM8 sarcoma model, oral administration of ONA (20 mg/kg, twice weekly) significantly abrogated tumor development and lung metastasis, prolonging survival [28]. Immunohistochemical analyses of tumor tissues revealed increased infiltration of CD8- and CD4-positive lymphocytes and decreased Stat3 activation in ONA-treated mice as compared with control mice. The observed suppression of the anti-tumor effect of ONA in a sarcoma model using immune-deficient nude mice also indicates that the anti-tumor effect of ONA is dependent upon anti-tumor immune responses. ONA was also found to abrogate the immunosuppressive function of myeloid-derived suppressor cells (MDSCs) in a sarcoma model. In addition to its effect on anti-tumor immunity, ONA suppresses cell-cell interactions between tumor cells and TAMs.

Many soluble factors derived from TAMs stimulate tumor cell proliferation and migration. ONA suppresses TAM-related tumor cell proliferation and migration by inhibiting CCL5 production in TAMs. The anti-tumor effect of ONA was also investigated in an ovarian tumor model. ONA abrogated ovarian tumor progression via inhibition of cell-cell interactions between tumor cells and TAMs, prolonging survival. Notably, ONA also increased the sensitivity to anti-tumor drugs such as cisplatin (CDDP) and paclitaxel (PTX) and improved the anti-tumor effect of CDDP in an ovarian tumor model. As Stat3 activation also affects the chemo-resistance of tumor cells, these additional effects are thought to be the result of ONA-associated Stat3 inhibition. Thus, ONA is a promising new anti-macrophage agent for use in anti-tumor therapies.

**Triterpenoid compounds**

An in vitro study using human macrophages revealed that corosolic acid (CA) and oleanolic acid (OA) suppress pro-tumor activation of TAMs via inhibition of Stat3 signaling [29,30]. CA and OA also exhibit direct anti-tumor effects against tumor cells and increase the sensitivity of tumors to chemotherapeutic agents via Stat3 inhibition [29-31]. Similar to ONA, CA was shown to inhibit M2-polarization of macrophages and the immunosuppressive functions of MDSCs and abrogate LM8 sarcoma development and metastasis via activation of anti-tumor immune responses [32]. CA is also reportedly effective against a variety of malignant tumors [33-35]. Ursolic acid (UA) is a natural compound that is structurally similar to CA and OA, and collectively, these compounds are classified as triterpenoids. Both CA and UA are reportedly effective in suppressing the development of skin cancers via suppression of inflammatory responses, and this anti-inflammatory effect is mediated by inhibition of IGF-1R, STAT3, and Src signaling [36].

In a previous study of vegetable and fruit extracts, we found that soybean extract significantly inhibited M2 polarization of human macrophages [37]. Soyasapogenol A and B, the aglycan forms of natural compounds derived from soybeans, significantly suppressed both M2 polarization and sarcoma development and progression in a murine sarcoma model involving treatment with soyasapogenol B, with a mechanism similar to that of CA and ONA. Other studies have shown that soyasapogenols exhibit tumoricidal activity against several types of malignant tumors [38].

The expression of CD169 by macrophages has become a topic of recent research interest in the field of tumor immunity [39]. Antigen-presenting cells such as dendritic cells and macrophages in the lymph nodes are known to play important roles in the induction of anti-tumor immune
Recent findings indicated that CD169-positive sinus macrophages are closely involved in the induction of anti-tumor immune responses, and upregulated CD169 expression by sinus macrophages is associated with a better clinical course for several malignant tumor types \[44-46\]. The observation that CD169 expression is upregulated by interferons indicates that CD169 is a marker for M1-like macrophages or macrophages exhibiting a higher potential for antigen presentation \[39\]. The significant increase in CD169 expression on human macrophages in response to co-culture with CA (unpublished data) suggests that CA induces a phenotypic change in lymph node macrophages and plays a role in the induction of anti-tumor immune responses.

**Conclusion**

Here, we described the results of recent research involving natural compounds that target TAMs. ONA and triterpenoid compounds inhibit the polarization of TAMs to M2 or pro-tumor phenotypes and potential conversion to an M1-like phenotype. Targeting of macrophages using Yondelis (Trabectedin) or CSF-1R inhibitors is expected to deplete TAMs, however, it can deplete macrophages with an M1-like phenotype including lymph node macrophages. Therefore, therapy that promotes conversion of macrophage phenotype, rather than targeting of macrophages, could enhance the success rate of anti-tumor therapies. Mills et al. recently described a therapeutic strategy for changing macrophage phenotype and designated it ‘macrophage-innate or macrophage-adaptive conversion (MIC/MAC) therapy’ or ‘macrophage-directed cancer immunotherapy’ \[47\]. Thus, macrophage conversion approaches are expected to emerge as novel immunotherapies that will enhance the conventional anti-tumor effects of chemo/radiotherapy or molecular-targeting therapies (Figure 2).

**Conflicting interests**

The authors have declared that no conflict of interests exist.

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