Chapter

Targeting Tumor-Associated Macrophages by Plant Compounds

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Abstract

Macrophages play an important role in cancer development, as they represent almost half of the cells forming the tumor microenvironment. They are called tumor-associated macrophages (TAMs) and most of them are alternative activated macrophages (M2 polarized), promoting cancer progression, angiogenesis and local immunosuppression. Blocking the macrophages recruitment, preventing their activation or turning M2 cells toward M1 phenotype (classic activated macrophage promoting an efficient immune response) is a modern immunotherapeutic approach for fighting cancer. Several studies showed that plant compounds (phenolics, triterpenes, coumarins, etc.) exert antitumor properties, not only by a direct toxical effect to malignant cells but also by influencing macrophage phenotypic differentiation.

Keywords: macrophage polarization, phenolic compounds, saponins, polysaccharides, coumarins, anthraquinones, alkaloids, tumor microenvironment

1. Introduction

Macrophages represent up to 50% of the cells infiltrating into the tumor microenvironment (TME) and modulation of macrophage polarization is an interesting and novel therapeutic approach in preclinical or clinical cancer research.

An increasing number of studies have also shown that tumor-associated macrophages (TAMs) can antagonize, augment or mediate the antitumor effects of cytotoxic agents, tumor irradiation, anti-angiogenic/vascular damaging agents and checkpoint inhibitors [1].

In the tumor microenvironment, TAMs are one of the major contributors in *angiogenesis* by secreting pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), adrenomedullin (ADM), platelet-derived growth factor (PDGF), tumor growth factor-beta (TGF-β) and matrix metalloproteinases (MMPs). Also, TAMs promote tumor cell *invasion and metastasis* by modifying the composition of extracellular matrix and cell-cell junctions and promoting basal membrane disruption. It was demonstrated that macrophages *facilitate the metastasis* by enhancing the ability of cancer cells to enter a local blood vessel and also are involved in *immunosuppression* by inhibiting the T-cell response or by secreting immunosuppressive cytokines and proteases such as IL-10, TGF-β, arginase-1 and prostaglandins, which inhibit T-cell activation and proliferation [2].

TAMs often exhibit an array of activation states. In general, they are skewed away from the "classically" activated, tumoricidal phenotype (sometimes referred to as M1) toward an "alternatively" activated tumor-promoting one (M2) [1]. The classically activated M1 macrophages are stimulated by microbial substrates such as lipopolysaccharide, Toll-like receptor ligands and cytokines such as IFN-γ. They are characterized by secretion of pro-inflammatory cytokines such as interleukins IL-6, IL-12, IL-23 and TNF-α and express high levels of major histocompatibility complex class II (MHC-II), CD68, and CD80 and CD86 costimulatory molecules. The alternatively activated M2 macrophages are stimulated by IL-4 and IL-13, secrete IL-10 and TGF-β and express low levels of MHC-II and feature expression of CD163 and CD206 [3].

Unfortunately, M2 cells are the most representative cells of the TAM population within the tumor promoting genetic instability, local immunosuppression and stem cell nurturing [4] and providing essential support for a malignant phenotype [5].

In the early stages of cancers of the lung, colon and stomach, the macrophages in the normoxic milieu display an M1 phenotype and are associated with good prognosis, but within avascular areas of the tumor, TAMs alter the gene expression profile, favoring a protumor M2 phenotype, correlated with a bad prognosis [6]. In **Table 1** are showed recent conclusions concerning the correlation between TAMs and clinical prognostics in several tumor types. In human breast carcinomas, high TAM density is also associated with poor prognosis [7]. TAMs in renal cell carcinoma show a mixed M1/M2 phenotype. CD68 alone has a poor predictive value, while low CD11+ and high CD206+ as single variables correlated with reduced survival [8]. There is strong evidence for an inverse relationship between TAM density and clinical prognosis in solid tumors of the breast, prostate, ovary and cervix. Type I and II endometrial carcinomas had significantly higher macrophage density in both epithelial and stromal compartments than benign endometrium [9]. Type II cancers have nearly twice the TAM density of type 1 cancers and this difference may be due to M1 macrophage predominance in the stroma of type II cancers [10].

TAMs' distribution pattern could be an independent prognostic factor for the overall survival of gastric cancer patients, invasive front-/stroma-dominant pattern having worse outcomes [11]. Studies have shown that the amount of TAMs in tumor stroma predicts the size, stage and metastasis of the gastric tumor [12]. In lung cancer, M2 subset and TAMs in tumor stroma were associated with worse survival, while M1 subset and TAMs in tumor islet were associated with favorable survival of lung cancer [13].

While most cancer research has focused upon these changes and most therapeutics are directed against these tumor cells, it is now apparent that the non-malignant cells in the microenvironment evolve along with the tumor and provide essential support for their malignant phenotype [5]. The knowledge of TAM activation status may allow the therapeutic targeting of TAMs, once TAMs' targeting/modulating agents pass clinical trials and become widely available [6, 14]. The role of macrophages in tumor progression remains to be fully elucidated, in part due to the contrasting roles they play depending on their polarization [15]. Both the systemic and local environments play a tumor-initiating role through the generation of persistent inflammatory responses to a variety of stimuli [16]. To support this correlative data between macrophage-mediated inflammation and cancer induction, genetic ablation of the anti-inflammatory transcription factor STAT3 in macrophages results in a chronic inflammatory response in the colon that is sufficient to induce invasive adenocarcinoma. However, it is unclear whether macrophages in some inflammatory situations can kill aberrant cells before they become tumorigenic and thus be antitumoral [17].

Cancer type	TAMs as prognostic factors	Reference
Breast	CD68 as a biomarker for TAMs to evaluate the risk is better than CD163 or CD206 alone; high infiltration of TAMs was significantly associated with negative hormone receptor status and malignant phenotype	[18]
Gastric	The amount of TAMs in tumor stroma predicts the size, stage and metastasis of the gastric tumor Invasive front-/stroma-dominant pattern having worse outcomes Although CD68+ TAMs infiltration has the neutral prognostic effects on OS, the M1/M2 polarization of TAMs are predicative factors of prognosis in gastric cancer patients	[11, 12, 19]
Lung	The prognostic value of tumor-infiltrating TAMs in lung cancer is still controversial. M2 subset and TAMs in tumor stroma were associated with worse survival, while M1 subset and TAMs in tumor islet were associated with favorable survival of lung cancer. CD204-positive TAMs are the preferable marker for prognostic prediction in NSCLC Although the density of total CD68+ TAMs is not associated with overall survival, the localization and M1/M2 polarization of TAMs are potential prognostic predictors of NSCLC	[13, 20, 21]
Cervix	Tumor-infiltrating CD204+ M2 macrophages may predict poor prognosis in patients with cervical adenocarcinoma	[22]
Ovarian	CD163+ TAM infiltration was associated with poor prognosis of ovarian cancer and high M1/M2 macrophage ratio in tumor tissues predicted better prognosis	[23]
Pancreatic	Although TAM populations in tumor stroma are high, marking them as a probable prognostic factor, the multiple roles that TAMs play in pancreatic cancer progression have not yet been delineated. Additional mechanistic insight into the pathways that regulate the differentiation of TAMs from monocytes is required The density of TAMs has an impact on the overall survival of pancreatic cancer patients. M2-TAMs can be recognized as a prognostic indicator in pancreatic cancer	[24, 25]
Renal	CD68 alone has a poor predictive value, while low CD11+ and high CD206+ as single variables correlated with reduced survival	[8]
Glioblastoma	TAM, accounting for approximately 30% of the GBM bulk cell population, may explain, at least in part, the immunosuppressive features of GBMs	[26]
Hepatocellular carcinoma	The prognostic value of TAMs in patients with hepatocellular carcinoma (HCC) is still controversial. TAMs could serve as independent predictive indicators and therapeutic targets for HCC. Further trials are needed to elucidate the exact relationship and the underlying mechanism	[27]
Melanoma	Independent of their intratumoral distribution, the prevalent accumulation of M2 TAMs in MM is statistically confirmed to be a poor indicator of patients' outcome	[28]
Non-Hodgkin's lymphoma	High-density CD68+ and CD163+ TAMs, and also high CD163+/CD68+ TAMs ratio is significantly correlated with poor overall survival	[29]
Hodgkin's lymphoma	High density of either CD68+ or CD163+ TAMs is a robust predictor of adverse outcomes in adult cHL	[30]
Colorectal (CRC)	The role of tumor-associated macrophages (TAMs) in predicting the prognosis of CRC remains controversial. Still, high-density CD68+ macrophage infiltration can be a good prognostic marker	[31]
Squamous cell carcinoma of the head and neck (SCCHN)	CD68+ marker has no prognostic utility in patients with SCCHN; the M2-like marker CD163+ predicts poor prognosis	[32]

Table 1.
TAMs as potential predictive indicators in several tumor types.

Targeting a single signaling axis that promotes the immunosuppressive and protumoral functions of macrophages is inadequate as there are multiple signals involved in the communication between tumor cells and TAMs. Identifying and inhibiting key driver pathways, which are critical for both cancer cell survival and TAM activation, may offer therapeutic advantages as they disrupt the vicious positive feedback loop between tumor and TAMs [33]. Prevention of TAM accumulation and reduction of TAM presence by depleting existing TAMs represent novel strategies for an indirect cancer therapy specifically aimed at tumor-promoting cells within the microenvironment, but the challenge with this approach is to find ways for local administration of such drugs to the tumor [15]. Targeting TAM polarity toward an M1 phenotype also became a real immunotherapeutical approach in cancer, recalling responses from both innate and adaptive immune systems, leading to tumor regression [4].

Triple combination of anti-CTLA-4, anti-PD-1 and G47Δ-mIL12 was associated with macrophage influx and M1-like polarization in two glioma models [34]. A combination of a bivalent ganglioside and β-glucan, a yeast-derived polysaccharide, able to differentiate TAMs into an M1 phenotype is currently under investigation in a phase I clinical trial of patients with neuroblastoma [35]. Vadimezan, a fused tricyclic analog of flavone acetic acid, was found to repolarize macrophages in M1 phenotype, and it has been the subject of numerous preclinical studies and clinical trials [36]. Zoledronic acid, a clinical drug for cancer therapy, has been found to inhibit spontaneous mammary carcinogenesis by reverting macrophages from the M2 phenotype to the M1 phenotype [37].

2. Herbal compounds in TAM modulation

Research to date suggests that, despite the potency of cytotoxic anticancer agents and the high specificity that can be achieved by immunotherapy, neither of these two types of treatment is sufficient to eradicate the disease. Moreover, even in standard chemotherapy, there has been efficiency through the introduction into current practice of treatments with combinations of drugs [38]. In general, literature data show that the combination of conventional treatment with natural compounds exerts an additive effect caused by the alternative activation of signaling pathways that induce cell death or increase the activity of the chemotherapeutic agent. The involvement of these natural compounds (alone or in combination therapy) in the immunobiology of cancer is a branch that has not yet been studied but offers major therapeutic opportunities. Herbal compounds have many regulatory effects on macrophage polarization, but the specific mechanisms, signaling pathways and target genes involved remain incompletely understood [39]. Their effects, according to recent research studies, are summarized in **Figure 1**.

Although natural products have historically been a critical source for therapeutic drugs, sometimes natural molecules may suffer from insufficient efficacy, unacceptable pharmacokinetic properties, undesirable toxicity or reduced availability, which impedes their direct therapeutic application. Poor availability of some natural compounds, despite their pharmacological effects, limits their clinical application. In recent years, there has been an increased interest in developing nanoformulations with increased bioavailability and fewer side effects. For instance, TAM-rich tumors, due to their enhanced permeability, demonstrated an elevated retention (>700%) of the nanotherapeutic (poly(D,L-lactic-*co*-glycolic acid)-*b*-poly(ethylene glycol) (PLGA-PEG)), as compared to TAM-deficient tumors [14].

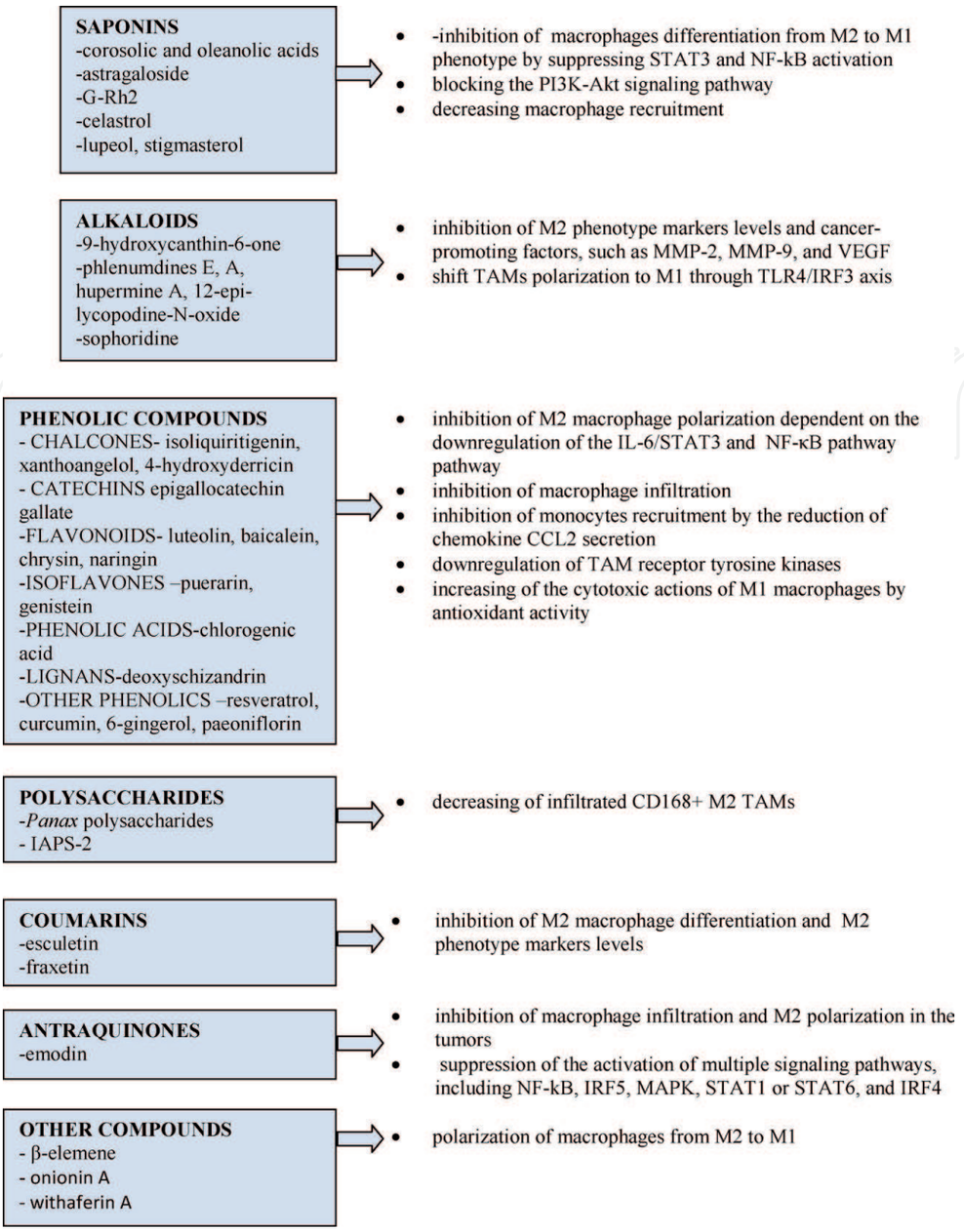

Figure 1.
Herbal compounds and their main actions on TAMs in cancer progression.

2.1 Saponins

Triterpenic compounds, including corosolic acid, tigogenin, timosaponin AIII, neoaspidistrin and oleanolic acid, suppress the CD163 expression. Corosolic and oleanolic acids change M2 polarization to M1 polarization in human monocyte-derived macrophages (HMDMs) by suppressing STAT3 and NF-kB activation. The effects of these two compounds were exerted not only on macrophages but also on glioblastoma cells, suppressing tumor cell proliferation and sensitizing tumor cells to anticancer drugs [40, 41].

M2 polarization was switched also by astragaloside IV (AS-IV, 3-O-β-D-xylopyranosyl-6-O-β-D-glucopyranosyl cycloastragenol), a natural saponin extracted from *Astragali* radix, by modulating the AMPK signaling pathway. In the intravenous lung cancer model, AS-IV treatment did not alter the percentage of macrophages but did significantly reduce the number of M2 macrophages [42]. In another study, G-Rh2, a monomeric compound extracted from *Panax ginseng* C. A.

Mey (ginseng), converts the differentiation of macrophages from M2 to M1 phenotype resulting in the decreased levels of MMPs and VEGF. By blocking the PI3K-Akt signaling pathway, the compound prevented the metastasis of lung cancer (NSCLC) cells [43]. Recently, a novel EV-liked ginseng-derived nanoparticle (GDNP) was tested in melanoma, and it altered M2 polarization both *in vitro* and *in vivo*, depending on TLR4 and MyD88 signaling and contributing to an antitumor response [44].

A potential role of celastrol, a pentacyclic triterpenoid in antimetastasis treatment, was suggested by Yang et al. [45], which found that this compound suppresses M2-like polarization by interfering with STAT6 signaling pathway after stimulation with IL-13. An active role in decreasing macrophage recruitment and tumor angiogenesis was showed for lupeol and stigmasterol in an *in vivo* model [46].

2.2 Alkaloids

Treatment with 9-hydroxycanthin-6-one, a β-carboline alkaloid isolated from the *Ailanthus altissima* stem bark, inhibited the levels of M2 phenotype markers and some cancer-promoting factors, such as MMP-2, MMP-9 and VEGF, in macrophages educated in ovarian cancer–conditioned medium. The compound also decreased the expressions of MCP-1 and RANTES, major determinants of macrophage recruitment at tumor sites, in ovarian cancer cells [47].

A regulatory effect on macrophage differentiation during tumor development exerts phlenumdines E, A, hupermine A and 12-epi-lycopodine-N-oxide isolated from the club moss *Phlegmariurus nummulariifolius* (Blume) Ching, which exhibited an inhibitory effect on IL-10–induced expression of CD163, an M2 phenotype marker, in HMDMs [48].

Sophoridine, a bioactive alkaloid extracted from the seeds of *Sophora alopecuroides* L, was able to reshape gastric cancer immune microenvironment by shifting TAM polarization to M1 and suppressing M2-TAM polarization through TLR4/IRF3 axis [49].

2.3 Phenolic compounds

2.3.1 Chalcones

In a model of azoxymethane (AOM)/dextran sodium sulfate (DSS)-induced colitis-associated tumorigenesis, it was showed that isoliquiritigenin (6′-deoxychalcone) inhibits M2 macrophage polarization depending on the downregulation of the IL-6/STAT3 pathway [50]. The same mechanism was proposed by Sumiyoshi et al. [51], for xanthoangelol and 4-hydroxyderricin, chalcones isolated from *Angelica keiskei* roots. In the *in vivo* study, the antitumor action of xanthoangelol was higher than that of 4-hydroxyderricin and it was proposed that the presence of a 4-free phenolic OH and/or the presence of a longer isoprene moiety in C-3 could be the cause of better activity of xanthoangelol. Reducing breast cancer cells' migration with the aid of M2 macrophages was achieved *in vitro* by the total flavonoid from *Glycyrrhizae Radix et Rhizoma* and isoliquiritigenin. These compounds inhibited gene and protein expression of Arg-1, upregulated gene of HO-1 and protein expression of iNOS, and enhanced the expression of microRNA 155 and its target gene SHIP1 [52].

2.3.2 Catechins

Macrophage infiltration and differentiation of macrophages into tumor-promoting M2 macrophage were decreased by epigallocatechin gallate (EGCG) treatment in murine tumor models and the molecular mechanism proposed was

the downregulation of NF-κB pathway [53, 54]. EGCG can be rapidly degraded *in vivo* limiting its clinical application. A peracetate-protected EGCG (Pro-EGCG) synthesized by modification of the reactive hydroxyl groups with peracetate groups proved six times more stability than EGCG and showed greater efficacy in induction of cell death in leukemic cells. Treatment with Pro-EGCG inhibits differentiation of macrophages toward TAMs through decreasing CXCL12 expression in endometrial stromal cells with no influence on the expression level of CD163 and CD206 [55].

2.3.3 Flavonoids

Luteolin, 3 0,4 0,5,7-tetrahydroxyflavone, is a common flavonoid derived from various plants and inhibits IL-4–induced phosphorylation of STAT6 and the TAM phenotype, ameliorating the recruitment of monocytes and the migration of lung cancer cells by the reduction of chemokine CCL2 secretion from macrophages [56]. The antitumor mechanism of luteolin in non-small cell lung carcinoma (NSCLC) was mediated by downregulation of TAM receptor tyrosine kinases (RTKs), and it was found to decrease the protein levels of all three TAM RTKs in the A549 and A549/CisR cells in a dose-dependent manner [57]. In an *in vitro* tumor model, cobalt chloride (CoCl2) was used to simulate hypoxia and it was showed that luteolin decreased the expression of VEGF and MMP-9, which promote angiogenesis. In addition, luteolin also suppressed the activation of HIF-1 and phosphorylated-signal transducer and activator of STAT3 signaling, particularly within the M2-like TAMs [58].

The regulation of M2 macrophage repolarization through inhibiting PI3K/Akt signal pathway is the mechanism proposed for baicalein (5,6,7-trihydroxyflavone), a widely used Chinese herbal medicine derived from the root of *Scutellaria baicalensis*. Changing the phenotype of macrophages from M2 to M1 was supported by decreasing of M2-specific marker CD206 correlated to the increased M1-specific marker CD86. Still, the authors of the study suggested that the cytotoxic effect of baicalein on breast cancer cells directly is more pronounced than on TAMs (IC 50 of baicalein for MDA-MB-231 at 24 h, 48 h and 72 h was 79.12/50.10/34.77 μmol/L, for MCF-7 at 24 h, 48 h and 72 h was 49.76/43.73/39.44 μmol/L, for TAM at 24 h, 48 h and 72 h was 191.5/107.1/41.78 μmol/L, respectively) [59].

It has been reported that a novel chrysin (5,7-dihydroxyflavone) analog 8-bromo-7-methoxychrysin has anticancer activities with more potent bioactivity than the lead compound [60]. It also has the capacity to regulate the tumor microenvironment by inhibition of NF-κB activation, suppressing significantly the expression of the M2 macrophage marker CD163 and modulating the secretion profile of TAM cytokines [61].

According to traditional Chinese medicine (TCM) theory, herbs with Qi-tonifying character are involved in improving the defense capacity of immune system. Total flavonoids from *Glycyrrhizae Radix et Rhizoma* significantly inhibited the expression of Arg-1 (above 90% at 100 μg/mL), one of the phenotype markers of M2 macrophages, and suppressed M2 polarization of macrophages partly by inactivating STAT6 pathway. The regulation of M1 and M2 markers' expressions was partly due to the enhancement of miR-155 levels [62].

Naringin (4′,5,7 trihydroxyflavanone-7-rhamnoglucoside) exert a potential inhibitory effect on tumor progression by inducing CD169-positive and M1-like macrophages, potentially correlating with cytotoxic T-cell activation [63].

2.3.4 Isoflavones

Puerarin [4H-1-benzopyran-4-one, 8-β-D-glucopyranosyl-7-hydroxy-3-(4-hydroxyphenyl)] is the major bioactive ingredient isolated from the root of

traditional Chinese medicine Ge-gen (*Radix Puerariae*) able to suppress the cell invasion and migration probably through inactivating MEK/ERK 1/2 pathway in a model of NSCLC. Also, it was showed that puerarin acts directly on macrophages by increasing M1 macrophage markers (CD197+, iNOS+ and CD40+) and reducing the expression of M2 markers (CD206+, Arg-1+ and CD163+) [64].

Another isoflavone, genistein, can inhibit the increased M2 polarization of macrophages and stemness of ovarian cancer cells by co-culture of macrophages with ovarian cancer stem-like cells through disrupting IL-8/STAT3 signaling axis [65].

2.3.5 Phenolic acids

Chlorogenic acid (5-caffeoylquinic acid, CA), the ester of caffeic acid, is a phenolic compound widely found in plants. It was showed that this compound inhibits growth of G422 glioma *in vivo*, an effect associated with a decrease of M2-like TAMs and recruitment of M1-like TAMs into tumor tissue. Low dose (1 μM) of CA could significantly inhibit the M2 macrophage-induced proliferation of glioma and breast cancer cells, mainly via STAT1 and STAT6 signaling pathways [66]. Oršolić et al. [67] concluded that the antitumor activity of CA is the result of the synergistic activities of different mechanisms by which CA acts on proliferation, angiogenesis, immunomodulation and survival. Mice with Ehrlich ascites tumor (EAT) and treated for 10 days with CA in a dose of 40 and/or 80 mg kg^{-1} showed an increase of the cytotoxic actions of M1 macrophages and inhibition of the tumor growth, probably mediated through its antioxidative activity.

2.3.6 Lignans

Deoxyschizandrin, a major dibenzocyclooctadiene lignan present in *Schisandra chinensis* berries, significantly suppressed CD163 and CD209 expression, inhibiting protumor mediator production as well as M2 polarization in TAM macrophages stimulated by the conditioned medium of A2780 cells [68].

2.3.7 Other phenolic compounds

Several studies focused on a stilbene derivative, resveratrol (3,4′,5-trihydroxystilbene), a widely studied compound that exhibits potent preventive effects on lifestyle-related disorders such as hyperlipidemia, obesity, coronary heart disease and cancer, as well as on aging. In lung cancer tumors, resveratrol induced their sluggish growth by decreasing F4/80 positive expressing cells and M2 polarization (lower expression of M2 markers-IL-10, Arg-1 and CD206), probably by STAT3 suppression [69]. Antitumor and antimetastatic effects of resveratrol (25 and 50 μM) based on the regulation of M2 macrophage activation and differentiation were confirmed by Kimura and Sumiyoshi [70], which also conducted a study for correlation of stilbene structure with biological activity. Among the nine stilbenes examined, 2,3-,3,4-, and 4,4′-dihydroxystilbene inhibited the production of MCP-1 in M2-polarized THP-1 macrophages at a concentration of 50 μM, demonstrating that the inhibitory effects of stilbenes with dihydroxy groups on the production of MCP-1 were greater than those with mono-hydroxyl groups. Dihydroxystilbene at 25 and 50 μM, 3,4-dihydroxystilbene at 50 μM, and 4,4′-dihydroxystilbene at 10, 25 and 50 μM significantly inhibited the production of IL-10 by M2 THP-1 macrophages. The three dihydroxystilbenes, 2,3-, 3,4-, and 4,4′-dihydroxystilbenes, at concentrations of 10–50 μM inhibited p-STAT3 increase during M2 THP-1 macrophage differentiation induced by IL-4 plus IL-13 [71].

The resveratrol analogue, HS-1793 (4-(6-hydroxy-2-naphthyl)-1,3-benzenediol), was also shown to elevate the level of IFN-γ production conducting reprograming of TAMs M2 phenotype [72].

Curcumin ((1*E*,6*E*)-1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione), a natural phenol and the main active ingredient in turmeric, acts in several ways as a suppressor of macrophage functions. Even though curcumin has previously received considerable attention from researchers as an anti-inflammatory agent, it has a promising future in the area of immunomodulation [73]. Most of the studies on curcumin focused on the anti-inflammatory effect, promoting the conversion of macrophages from M1 to an anti-inflammatory and protective M2 phenotype [73]. Gao et al. [74] demonstrated that curcumin plays a key role in M2 polarization in two ways: (1) via the inhibition of DNA methyltransferase3b (DNMT3b), overexpression of which can promote increased M1 polarization, and (2) via increased phosphorylation of signal transducer and activator of transcription STAT-6, an important transcription factor activated by IL-4 and IL-10. Other studies showed that curcumin also induces TAMs re-polarization from tumor-promoting M2 phenotype toward the more antitumor M1 phenotype in tumor-bearing hosts, mediated by inhibition of STAT3 activity [75]. Curcumin administration and delivery to glioblastoma brain tumors (GBM) caused a dramatic re-polarization of TAMs from an M2 to M1 phenotype and tumor remission in 50–60% of GBM-bearing mice [76]. Hydrazinocurcumin, a synthetic analog of curcumin encapsulated within nanoparticles, reeducates TAMs to an M1-like phenotype IL-10 low IL-12 high TGF-β low [54].

It was showed that TriCurin, a synergistic formulation of curcumin, resveratrol, and epicatechin gallate (molar ratio C:E:R: 4:1:12.5) can shift TAM polarity in HPV-positive HNSCC by silencing the M2 TAM and activating/recruiting a discrete population of M1 TAM while maintaining a constant number of overall intra-tumor Iba1+ TAM, along with expression of activated STAT3 and induction of activated STAT1 and NF-kB (p65) [77]. Moreover, a liposomal formulation of TriCurin with increased bioavailability (TrLp) was able to cause repolarization of M2-like tumor (GBM)-associated microglia/macrophages to the tumoricidal M1-like phenotype and intra-GBM recruitment of activated natural killer cells [78].

In a urethane-induced lung carcinogenic model, lung carcinogenesis was ameliorated with increased M1 macrophages and decreased M2 macrophages in the lung interstitial by administration of 6-gingerol ((*S*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone), the main bioactive component in ginger (*Zingiber officinale* Roscoe). M2 macrophage-resetting efficacy of 6-gingerol was confirmed in a Lewis lung cancer allograft model and the mechanism proposed was the reduction of Arg-1 and ROS levels and elevation of L-arginine and NO levels [79].

Also, it was showed that paeoniflorin, one of the major active constituents of *Paeonia lactiflora* Pallas, inhibits the alternative activation of macrophages in subcutaneous xenograft tumors of the C57BL/6 J mice at doses of 40 and 20 mg·kg^{-1} [80].

2.4 Polysaccharides

It was suggested that modulation of TAM polarization was implicated in the antitumor immunostimulatory activity of polysaccharides from *Panax japonicus* (ginseng). The transcription and production of TGF-β and IL-10, two well-known immunosuppressive cytokines secreted by TAMs, were reduced in response to *Panax* polysaccharides and also the number of infiltrated CD168+ M2 TAMs was substantially declined although the number of CD68+ total macrophages in transplanted tumor tissues remained almost unchanged [81]. A significant inhibition of Arg-1 expression (above 90% at 100 μg/mL), one of the phenotype markers of

M2 macrophages, was also observed for the ethanol extract of Ginseng *Radix et Rhizoma* [62]. Recently, Chen et al. [82], showed that water extract of Ginseng and Astragalus could be a novel option for integrative cancer therapies due mainly to their ability to regulate macrophage polarization.

In a murine model of sarcoma, immunotherapy with IAPS-2 (acidic polysaccharide, namely IAPS-2, from the root of *Ilex asprella*) demonstrated that it could significantly inhibit the growth of tumors via modulating the function of TAMs and increase the animal survival rate [83]. Similar results were obtained with an aqueous extract of *Trametes robiniophila* Murr (Huaier), a sandy beige mushroom found on the truck of trees and has been widely used in TCM for approximately 1600 years for its antitumor, antiangiogenic and immunomodulatory effects. Huaier not only modulates the macrophage polarization but also could inhibit the macrophage-induced angiogenesis by decreasing the expression of VEGF, MMP2 and MMP9, thus inhibiting the formation of new blood vessels in tumor [84].

2.5 Coumarins

Esculetin (6,7-dihydroxycoumarin) and fraxetin (6-methoxy-7,8-dihydroxycoumarin) (50, 75 and 100 μM) inhibited the production of IL-10, MCP-1 and TGF-β-1 in macrophages and the phosphorylation of STAT 3 without affecting its expression during the differentiation of M2 macrophages. Esculetin also suppressed the increased production of these cytokines during M2 macrophage differentiation at 10–100 μM. On the other hand, daphentin (7,8-dihydroxycoumarin) had no such effects, revealing that coumarins with two hydroxyl groups at the 6 and 7 positions (esculetin) or coumarins with a methoxy group at the 6 and two hydroxyl groups at the 7 and 8 positions (fraxetin) are more active, exhibiting antitumor and antimetastatic actions in osteosarcoma LM8 cells [85]. The antitumor and antimetastatic actions of esculetin may be due to the dual actions at tumor and TAM sites: inhibition of the expression of cyclin D 1 and CDK4 in osteosarcoma LM8 cells, and also decreasing the STAT 3 phosphorylation in macrophages. In the case of fraxetin, the effects are partly attributed to the inhibition of M2 macrophage differentiation [85].

A classical formula of traditional Chinese medicine (TCM) to alleviate lung cancer–related symptoms is Bu-Fei decoction (BFD), consisting of six herbal Chinese medicines-*Codonopsis pilosula*, *Schisandra chinensis*, *Rehmannia glutinosa*, *Astragalus* sp., *Aster* sp. and *Morus* sp.-but it has not been established whether it induces an antitumor effect or it modulates the tumor microenvironment. The result of an *in vivo* study revealed that BFD successfully interrupted the interaction between tumor cells and TAMs by inhibiting the expression of two important markers: IL-10 (correlated with late stage (stage II, III and IV), lymph node metastases, pleural invasion, lymphovascular invasion and poor differentiation in NSCLC patients) and PD-L1 (correlated with poor prognosis in a number of human cancers, including breast cancer, kidney cancer and NSCLCs) [86].

2.6 Anthraquinones

It has been shown that emodin (6-methyl-1,3,8-trihydroxyanthraquinone), the active ingredient of several Chinese herbs including Rhubarb (*Rheum palmatum*), inhibits the growth of a variety of tumors and enhances the responsiveness of tumors to chemotherapy agents. In breast cancer, emodin directly inhibited macrophage infiltration and M2 polarization in the tumors, independent of tumor size [87]. Previously, Jia et al. [88], showed that emodin is not cytotoxic to breast cancer cells

at concentration achieved *in vivo* (up to 30 μM) and it failed to affect macrophage infiltration in primary tumors. In contrast to its lack of effects on primary tumors, emodin dramatically suppressed lung metastasis by diminishing phosphorylation of STAT6 and C/EBPβ signaling upon IL-4 stimulation [88]. Further, it was showed that emodin suppresses the activation of multiple signaling pathways, including NF-kB, IRF5, MAPK, STAT1 or STAT6, and IRF4, depending on the environmental settings. It acts mostly on M2 polarization, suggesting that emodin could be most beneficial for patients with M2 macrophage-driven diseases [89].

2.7 Other herbal compounds/preparations

In oral squamous cell carcinoma (OSCC) animal models, highly pure super critical CO_2 leaf extract of *Azadirachta indica* (Neem) induces an M1 phenotype in TAMs *in vivo*, and the primary active component, nimbolide (a limonoid tetranortriterpenoid with an α,β-unsaturated ketone system and a δ-lactone ring) has significant anticancer activity in established OSCC xenografts [90]. β-Elemene, a widely known sesquiterpene, regulated the polarization of macrophages from M2 to M1, inhibiting the proliferation, migration and invasion of lung cancer cells and enhancing its radiosensitivity [91].

Onionin A (ONA), a natural low molecular weight compound containing sulfur isolated from onions, inhibited the EOC cell-induced M2 polarization of HMDMs, and STAT3 activation was significantly inhibited by ONA treatment in all cell lines [92].

Adjunctive treatment with Withaferin A, the most abundant constituent of *Withania somnifera* (Ashwagandha) root extract, reduced myeloid cell-mediated immune suppression and polarized immunity toward a tumor-rejecting type 1 phenotype, facilitating the development of antitumor immunity [93].

Traditional Chinese medicine provides pharmacologically efficient preparates such as KSG-002, a hydroalcoholic extract of radices *Astragalus membranaceus* and *Angelica gigas* at 3: 1 ratio that suppresses breast cancer growth and metastasis through targeting NF-κB–mediated TNF α production in macrophages [94] and SH003, mixed extract from *Astragalus membranaceus*, *Angelica gigas* and *Trichosanthes kirilowii* Maximowicz that suppresses highly metastatic breast cancer growth and metastasis by inhibiting STAT3-IL-6 signaling path [95].

Traditional Chinese medicine Jianpi Yangzheng Decoction (JPYZ) used for improving the quality of life and prolonging the survival of gastric cancer patients was more effective compared with Jianpi Yangzheng Xiaozheng Decoction (JPYZXZ) for inducing the phenotypic change in macrophages from M2 to M1. JPYZXZ inhibits the gastric cancer EMT more effectively than JPYZ, but JPYZ primarily works to regulate the phenotypic change in macrophages from M2 to M1 [96].

CXCL-1 was also found to be a cytokine secreted by tumor-associated macrophage, which recruits myeloid-derived suppressor cells to form pre-metastatic niche and led to liver metastasis from colorectal cancer. The current study demonstrated that after administration of XIAOPI formula (consisting of 10 herbs including *Epimedium brevicornum*, *Cistanche deserticola*, *Leonurus heterophyllus*, *Salvia miltiorrhiza*, *Curcuma aromatica*, *Rhizoma Curcumae*, *Ligustrum lucidum*, *Radix Polygoni Multiflori preparata*, *Crassostrea gigas* and *Carapax trionycis*), the density of TAMs decreased significantly and the level of CXCL-1 was also inhibited in both mouse plasma and cellular supernatants. When CXCL-1 cytokine was co-administrated with XIAOPI formula, the antimetastatic property of XIAOPI formula was blocked, indicating that CXCL-1 might be the principal gene involved in the network regulating the action of XIAOPI formula [97].

3. Conclusions

Macrophages, as key players in the tumor microenvironment, play essential roles in maintenance and progression of malignant state. Due to their plasticity, these cells balance between pro- and antitumoral effects in close correlation to specific factors. Recent immunotherapeutic strategies focus on tumor-associated macrophages in two main directions: to inhibit protumor macrophages and their suppressive effects (CCL2 inhibitors, trabectedin, zoledronic acid, JAK/STAT inhibitors, etc.) and to activate TAMs to an antitumor phenotype (TLR and CD40 agonists, PI3kδ inhibitor, VEGF and Ang2 inhibitors, etc.).

Several natural compounds/herbal extracts were studied as therapeutic/supportive agents for macrophage modulation in different types of cancers, most of them being able to change M2 polarization (protumoral) to M1 polarization (antitumoral). They belong to various classes of herbal compounds: saponins (corosolic and oleanolic acids, astragaloside, ginsenosides, celastrol, etc.), alkaloids (9-hydroxycanthin-6-one, phlenumdines E, A, hupermine A and 12-epilycopodine-N-oxide, sophoridine, etc.), flavonoids and polyphenolcarboxylic acids (isoliquiritigenin, xanthoangelol and 4-hydroxyderricin, baicalein, naringin, genistein, deoxyschizandrin, chlorogenic acid, curcumin, 6-gingerol and paeoniflorin), polysaccharides (isolated from various vegetal sources), coumarins (esculetin, fraxetin, etc.), and anthraquinones (emodin). This action is most probably achieved by downregulation of the STAT3, STAT 6 and NF-kB pathways with consecutive modulation of the secretory profile of TAM cytokines.

TCM supports the dual approach of cancer therapy, to destroy cancer cells on one hand and to improve patients' immunological status on the other hand. For several preparations such as Jianpi Yangzheng Decoction, Bu-Fei decoction and XIAOPI formula, research studies proved the correlation between cancer cells and tumor microenvironment and the effective intervention of these herbal products in delaying/breaking the tumorigenic process.

Low solubility of some herbal compounds limits their clinical application and it conducted to designing of new analogs with improved bioavailability-ginseng-derived nanoparticles, peracetate-protected EGCG, chrysin and resveratrol analogs.

By now, many herbal compounds have been shown to exhibit antitumor effects in various cancer types. Further, more researches need to be focused on the influence of these valuable compounds/preparations on modulation of the tumor microenvironment, as key element in the relation of tumor-host.

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