METASTATIC CANCER

Macrophages Polarization and Tumor Immunity in Metastatic Cancer: A Review

Bibi Nur Bazlini Baharun, Sabreena Safuan

ABSTRACT

Objective: **Tumour microenvironment plays an important role in the progression of cancer. This review focussed on current understanding on M1-M2 macrophage polarisation and the possible therapeutic intervention against metastatic cancer.**

Discussion: **Macrophages exhibit a variety of responses according to varying stimuli, and express different functions depending upon the microenvironment surrounding them. Macrophages can be activated by a variety of stimuli and polarized to functionally different phenotypes. Two distinct subsets of macrophages have been proposed; classically activated (M1) macrophages and alternatively activated (M2) macrophages. M1 macrophages express a series of pro-inflammatory cytokines, chemokines, and effector molecules, such as IL-6, IL-12, IL-23, TNF-α, iNOS and MHCI/II. In contrast, M2 macrophages express a wide array of anti-inflammatory molecules, such as IL-10, TGF-β, and arginase1. Tumour associated macrophages (TAM) of M2 type secrete many cytokines, chemokines, and proteases, which promote tumor angiogenesis, growth, metastasis, and immunosuppression. M2 macrophages are responsible to drive cancer cells to blood and lymphatic circulation, thus worsen cancer prognosis.**

Conclusion: **The polarization of macrophages towards an M1 response with minimal side effects may prove to be a powerful therapy against solid tumors. Thus, the development of effective strategies to tip the balance from M2 to M1 macrophages should be considered.**

KEY WORDS

cancer metastasis, macrophages polarization, tumor associated macrophages

INTRODUCTION.

Macrophages and their role in tumor progression.

The immune system plays an important role in the development and progression of cancer. In fact, immune cell infiltration to the tumor site can affect tumor malignancy and metastasis (Piao *et al*, 2018). Macrophage play a very significant role in tumor progression, this can be seen by more than 50% of the tumor mass are due to the macrophages infiltration to the tumor site (Mu *et al*., 2018). Macrophages belong to the innate immune system and its cells are derived from the myeloid lineage (Hung *et al*., 2018). The cell are derived from peripheral blood mononuclear cell (PBMC) which migrate to the tissue (Brenot *et al*., 2018). Macrophages function to phagocytose microbes and clearing the cellular debris which largely contribute to the initiation and resolution of inflammation (Mantovani *et al*., 2013). Depending on the type of surrounding stimuli which can vary from pro-inflammatory to anti-inflammatory, these cell could exhibit a different response (Murray, 2017). There are two major macrophage phenotypes that we all know which is M1 and M2 macrophages. In response to various signals, macrophages may undergo classical M1 activation or alternative M2 activation (Yin *et al*., 2018; Tariq *et al*., 2017). Figure 1 shows the signalling pathway of macrophage activation and molecules involved in driving M1-M2 macrophages polarization spectrum. In the context of cancer, classically activated or M1 macrophages function as the marker for the recognition and destruction of cancer cells, and their presence usually indicates good prognosis (Vergadi *et al*., 2017). After the cancer cell had been recognised, the M1 activation is triggered by interferon-γ (IFN-γ), bacterial lipopolysaccharide (LPS), or tumor necrosis factor α (TNFα), and is mediated by several signal transduction pathways involving signal transducer and activator of transcription (STAT), nuclear factor kappa-light-chain enhancer of activated B cells (NFκB), and mitogen-activated protein kinases (MAPK) (Brown, Recht, Strober, 2017; Piao *et al*., 2018). These flow of events enhance the production of microbicidal and tumoricidal activity using agents such as the reactive oxygen species (ROS) and nitric oxide (NO) (Moraes *et al*., 2017). Besides that, activated M1 macrophage also helps in promoting the subsequent inflammatory immune responses by increasing antigen presentation capacity (pro-inflammatory cytokines) and inducing the Th1 immunity through the production of cytokines such as IL-1β, tumor necrosis factor (TNF), IL-12, IL-18 and IL-23 (Hung *et al*., 2018; Vergadi *et al*., 2017; Ma *et al*., 2016).

Opposite to that, M2 macrophage activation is used to describe macrophages activated through other than the M1 activation (Rhee, 2016) such as IL4/IL13-stimulated macrophages (Brown *et al*., 2017; Tan *et al*., 2016), IL10-induced macrophages (Sica *et al*., 2015), and immune complex-triggered macrophages (Essandoh *et al*., 2016). M2 macrophages (alternatively activated macrophages) are anti-inflammatory in nature. M2 macrophages aid in the process of angiogenesis, tumor progression, promotion of tissue remodeling and also parasite containment. Besides that, they also express scavenger receptors apart from producing helps in large quantities of IL-10 and other anti-inflammatory cytokines (Brenot *et al*., 2018; Hung *et al*., 2018). M2 macrophages trigger the Th2 response using expression of the IL-10, and in turn Th2 cells upregulate the production of IL-3 and IL-4 (Yin *et al*., 2018; Vergadi *et al*., 2017). Apart from that, M2 macrophages possess different subsets, with each subset were induced by a different set of molecules and activated through different activation responses. M2a

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School of Health Sciences, Health Campus, Universiti Sains Malaysia
16150, Kubang Kerian, Kelantan, Malaysia
Correspondence to: Sabreena Safuan
(e-mail: sabreena@usm.my)

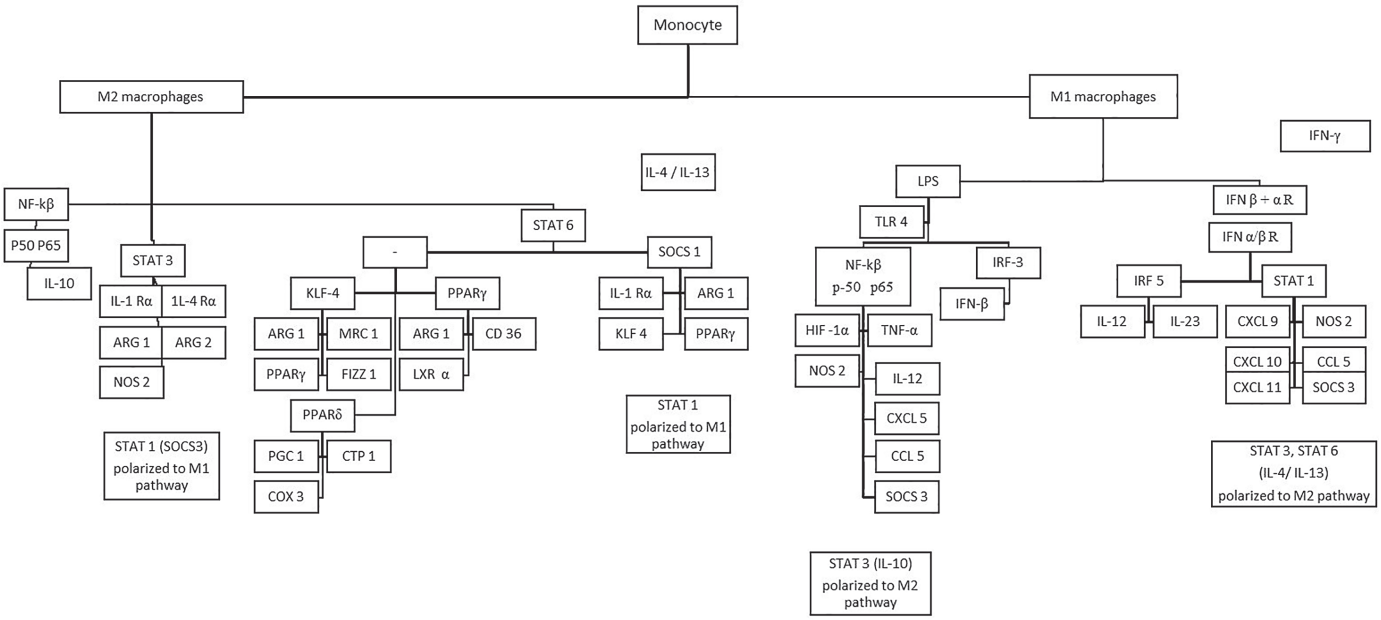

Figure 1: Shows the M1 and M2 activation pathway starting from the monocyte and divided into two subset which is M1 macrophage and M2 macrophage. After that, the cytokine and biomarker later on follow each respective activation pathway ranging from NF-kβ, STAT3 and STAT6 for M2 macrophage. For M1 macrophage the activation pathway starts through LPS or IFNβ+αR. During the polarization pathway to M1 phenotype, the biomarker generated from the M2 activation pathway could be activated through STAT1 or STAT1 (SOCS3) pathway. The same polarization process from M1 to M2 phenotype will be applied to the biomarker generated from M1 activation pathway polarize through STAT3 and STAT6 pathway.

macrophages or also called as IL-4 or IL-13 are usually referred as profibrotic. Profibrotic macrophages mainly induce Th2 response and stimulate type II responses in response to IL-4 and IL-13 (Ma *et al*., 2016; Pan *et al*., 2012). M2b or M(IC or TLR/IL1-R ligands) macrophages which are often referred as the regulators are also involved in Th2 activation and immune regulation. Meanwhile, M2c or M(IL-10 or TGF-β involved in the immune suppression, tissue repair and matrix remodeling. Another subset of the M2 macrophages called M2d or tumor associated macrophages (TAM) exhibit functions that stimulating tumor progression by promoting angiogenesis, thus feeding the malignant mass of cells (Moraes *et al*., 2017; Rhee, 2016). This macrophage subtype is currently a focus in many cancer research group.

Signaling pathway of M1-M2 polarization.

M1 macrophages could be polarized into M2 macrophages by using the tumor microenvironment or tissue-resident cell. There are *in vivo* studies on murine macrophages that proved cytokine and surface marker expression of macrophage could be utilized. In the condition where there are presence of cancer, repolarizing macrophages into M1 phenotype could stimulate the immune system to detect the tumor as foreign bodies thus letting the tumor to undergo goes apoptosis (Guiducci *et al*., 2015). M1 and M2 macrophages each have their own distinct chemokine and chemokine receptor profiles. M1 attract the Th1 cell via CXCL9 and CXCL10. Meanwhile, M2 secrete CCL17, CCL22 and CCL24. Current *in vitro* studies has recently proved that macrophages are capable of complete repolarization from M2 to M1 (Davis *et al*., 2013). Addition to that, the polarization pathway could also be reversed depending on the chemokine environment. A network of transcription factors and post-transcriptional regulators are intricate in the M1/M2 polarization (Huang *et al*., 2016; Qu *et al*., 2015). Interferon regulatory factor (IRF), signal transducers, activators of transcription (STAT) and suppressor of cytokine signaling (SOCS) proteins all take a part in skewing macrophage function towards either M1 or M2 phenotype. The IRF or STAT pathway polarize the macrophages to the M1 activation state via STAT1 by utilizing the IFN and the toll-like receptor signaling while, IL-4 and IL-13 skewing the macrophages into the M2 activation state through STAT6 (Figure 1). IRF5 are upregulated by the M1 macrophages and it is crucial for M1 polarization, also in the induction of IL-12, 1l-23, TNF, Th1 as well as Th17 response (Arnold, 2014). The LPS/TLR4 pathway also plays a role in M1 polarization by activating STAT1-α / β (Eun, 2014). In response to LPS stimulation, Bruton's tyrosine kinase (Btk) has also been implicated in the polarization of macrophages. Absence of Btk was shown to skew macrophages towards an M2 phenotype, indicating its critical role in M1 polarization (Ni, 2014). There are also other molecule incriminated in the induction of the M1 phenotype state such as G-protein coupled receptor, P2Y(2)R, which plays a role in inducing NO via *NOS2* (Eun, 2014). SOCS3 activates NF-κβ/ PI-3 kinase pathways to produce NO (Arnold, 2014) and differentiation factor Activin A, which helps in promoting M1 markers and down-regulates IL-10 (Figure 1). Arginase 1(ARG1) production is a distinct hallmark of M2 macrophages and is transcribed by STAT6 after being triggered by IL-4/IL-13 receptor signaling. M2 genes such as Arg-1, Mrc1, Fizz1 and PPARγ induced by Krüpel-like factor 4 (KLF-4) coordinated with STAT6 and inhibit M1 genes such as TNFα, Cox-2, CCL5 and NOS2 (Guo *et al*., 2016; Braun *et al*., 2013). This is mediated through sequestration of co-activators which is necessary for NF-κβ activation (Figure 1). Accordingly, the NF-κβ p50 subunit are found to be essential for M2 polarization *in vitro* and *in vivo*. Moreover, the nuclear receptor, peroxisome proliferator-activated receptor γ (PPARγ), have also been proven to regulate genes involved in oxidative metabolism and activation of the M2 phenotype (Lim *et al*., 2016; Eun, 2014) (Figure 1).

FINDINGS AND DISCUSSION

Tumor-associated macrophages (TAM) and its relation to tumor progression.

TAM is a predominant leukocyte that play critical roles in the formation of cancer. Type-1 T-cell associated with antitumor immune responses are activated by the TAM that are expressing series of pro-inflammatory cytokines such as IFN-γ, IL-1, and IL-6 (Mantovanni et al., 2015). However, TAMs are considered to be anti-inflammatory and correlated with a poor prognosis in most tumors such as breast, prostate, ovarian, cervical, lung, and cutaneous melanoma (Siegel *et al*., 2016). Epidemiological studies states that a macrophage-rich microenvironment will stimulate an aggressive tumor with a high metastatic potential (Steel *et al*., 2018). Tumor progression, angiogenesis, tumor growth, metastasis, immunosuppression, matrix deposition, and remodelling are various aspect of cancer that are orchestrated by the TAM. Besides that, tumors do not grow beyond 2-3 mm and cannot metastasize unless they are vascularized (Yin *et al*., 2018). Regulating the angiogenesis which is a vital process during vascularization is mainly associated with TAM

Table 1: List of study that had been using macrophage polarization to treat cancer.

Ref.	Journal	Target receptor/antibody	Status
[23]	Saccani *et al*., 2006	p50 NF-κβ as the factor involved in suppression of M1 macrophages, and that the inflammation reduction helped in tumor growth. When they created a p50 NF-κβ knock-out mouse, they found out that M1 aggressiveness was restored and that tumor survival was reduced.	Pre-clinical
[48]	Luo *et al*., 2006	Vaccine usage against legumain, a cysteine protease and stress protein upregulated in TAMs as a potential tumor target. When the vaccine against legumain was administered to mice, the results showed that angiogenesis genes were down-regulated and tumor growth was halted.	Pre-clinical
[2,70]	Kurahara *et al*., 2011; Huang *et al*., 2016.	TAM can acquired anti-inflammatory role in tumor shown by secreting pre-tumoral signal, recruiting other anti-inflammatory cells, de-differentiate into and from myeloid-derived suppressor cells and dampen the T cell response.	Pre-clinical
[5,75]	Mantovanni *et al*., 2015	Repolarization of TAMs into phenotypes that more closely resemble M1 macrophages has successfully produced anti-tumoral responses	Pre-clinical
[21]	Chizzolini *et al*., 2000.	Transcriptional profiling of macrophages that reside in tumors in a murine model of spontaneous breast cancer (MMTV-PyMT) shown that TAMs represent a distinct population of myeloid cells emerged in response to Notch signaling, which transduces the response to IL4 and IL13 to induce M2 macrophages.	Pre-clinical
[71]	Kim *et al*., 2015	Within tumors there is a combination of inflammatory and anti-inflammatory signals, such as TNF and IL-13, that makes the phenotypic polarization of TAMs a dynamic process	Pre-clinical
[38]	Qian and Pollard *et al*., 2010	A perivascular MRC1-expressing TAM subpopulation in patients with breast cancer has been shown to be crucial for tumor relapse after chemotherapy. Reinforcing the differences between TAMs, and macrophages polarized by anti/pro-inflammatory cues, ischemic TAMs co-express Arg1 and Nos2, which is stereotypical M2 and M1 markers help in combating cancer	crucial for

role by modulating and inducing neovascularization as well as support functions. Activated TAM could express a broad repertoire of substances including growth factors, cytokines, proteases and chemokines to promote angiogenesis. Moreover, TAMs also promote the lymphangiogenesis by vascular endothelial growth factor (VEGF) (Rhee, 2016). Lymphangiogenesis was induced by the macrophages in two different ways, either by stimulating the division of pre-existing local lymphatic endothelial cells or by transdifferentiating and directly incorporating into the endothelial layer (Huang *et al*., 2016). Apart from, promoting angiogenesis and lymphangiogenesis, TAMs also play a pivotal role in tumor growth. Studies found that proliferation of tumor cells in breast cancer is positively correlated with the TAM infiltration. Macrophages that are being co-cultured with tumor cells could secrete a series of substances which could facilitate tumor cell proliferation (Qu *et al*., 2015; Zhang *et al*., 1999). As a further matter, TAMs also limiting the cytotoxicity of the microenvironment, which assist in tumor growth. Due to TAMs characteristic are M2-like, they could secrete a large amounts of IL-10, thus suppressing the cytotoxic T-cell activity by inhibiting Th1 cells, while simultaneously inhibiting NK as well as lymphokine-activated killer cell cytotoxicity. On top of that, it was found that TAMs promote tumor metastasis (Lim *et al*., 2016). TAM infiltrates the target tissues and makes beds for the arrival of tumor cells, some TAM even change its phenotype to help extravasation, survival, and subsequent growth of tumor cells. On account of the fact that the tumor mass contains a great number of M2-like macrophages, TAMs can be used as a target for cancer treatment. Treatment through reducing the number of TAMs or polarizing them towards an M1 phenotype can help destroy cancer cells or impair tumor growth (Huang *et al*., 2016; Guiducci *et al*., 2015).

IL-1*β* and IL-6 secretion by TAM in tumour metastasis

Even though we achieved advances technology for detection and treatment of breast cancer, development of treatment for metastases cancer remains a significant problem. For instance, patients with localized breast cancer have a five-year survival rate of 98% compare to patients with metastatic breast cancer which have a five-year survival rate of only 26% (Seigel *et al*., 2016). 90% of the death that related to cancer are caused by metastases rather than the primary tumor growth. The growth of metastatic disease involve several processes which depends on both the tumor and patient characteristics (Hao *et al*, 2015). The existing of lymph node metastasis in breast cancer is related to poor overall survival. Current studies showing that lymphatic vessel invasion (LI) rather than blood vessel invasion (BI) is the predominant form of lymphovascular invasion (LVI) in initial stage invasive breast cancer (Pioa *et al*., 2018; Brenot *et al*., 2018). Higher percentage of proliferating lymphatic vessels and LN metastasis is a result of tumours with high densities of inflammatory infiltrate (Hung *et al*., 2018). The significant population of the inflammatory infiltrate represented by macrophages which also linked to breast cancer malignancy. Pro-inflammatory innate cytokines such as IL-1, TNF-α, and IL-6 are important to resolve acute inflammations. But, in chronic inflammation with high levels of innate cytokines may promote tumor development by driving sustained NF-kB activation (Moraes *et al*., 2017) and mitogen activated protein kinase (MAPK) activity (Guo *et al*., 2016). In addition, expression of pro-tumorigenic genes being promote by these cytokines which encode cell cycle, antiapoptotic and other proteins. Initialy, IL-1 is a main hematopoietic factor that triggered expression of colony stimulating factors on progenitor cells, leukocytes and stroma cells (Sica *et al*., 2015). IL-1β are capable to stimulate the differentiation of monocytes to conventional dendritic cell (Guo *et al*., 2016) and to M1-like macrophages (Rhee, 2016). In the fact of its importance as a key downstream mediator of inflammation, IL-1β also plays a key role in carcinogenesis and tumor growth. Experimental tumor models with carcinogenesis and invasiveness of the tumor are related to increase level of IL-1β in body fluids (Brown *et al*., 2017). IL-1β stimulate the tumor growth, aids driving chronic non-resolved inflammation, assist in endothelial cell activation (Tariq *et al*., 2017), promote tumor angiogenesis (Moraes *et al*., 2017) and helps in induction of immunosuppressive cells. All of these mechanism act as suppression of the adaptive immunity, tumor promotion and metastasis (Tariq *et al*., 2017). Through inflammasome activation and IL-1β production, TAM provides an inflammatory microenvironment which lead to breast cancer progression (Piao *et al*., 2018). IL-1β induces CCL2 expression in TAM and tumor cells as well as regulating myeloid cell recruitment into tumor tissue (Lim *et al*., 2016). Stimulation of cell by IL-1 or by TNF-α during these inflammatory state then, generate the production of macrophages-associated cytokines, the interleukin-6 (IL-6). Comparatively little is known about the roles of IL-6 in the regulation of LVI, and LN metastasis or even their expression in breast tumours. . IL-6 is a pleiotropic cytokine produced by a variety of normal cells including monocytes and macrophages (Zhang *et al*., 1999), which plays crucial roles in immune response, inflammation, and haematopoiesis. However, IL-6 is also expressed by multiple tumour tissue types, such as breast, prostate, colorectal and ovarian cancer (Qu *et al*., 2015). Various aspects of tumour behaviour, including apoptosis, tumour growth, cell proliferation, migration and invasion, angiogenesis and metastasis are influenced by the IL-6. The signal transducer and activator of transcription 3 (STAT3) act as a medium for IL-6 to convey it signal (Braun *et al*., 2013). IL-6 is pro-inflammatory and suppresses the expression of other cytokines by immune cells eventhough its signalling is mainly through STAT3. The occurrence of emergent event such as inflammation, immune response, and hemato-

poiesis will be notified by IL-6 which functions as a mediator. IL-6 that generated in an infectious lesion sends out a warning signal to the entire body (Qu *et al*., 2015). Pathogen associated molecular patterns which is the signature of exogenous pathogens is recognized in the infected lesion by pathogen-recognition receptors (PRRs) of immune cells. The example of the pathogen-recognition receptors (PRRs) of immune cells are monocytes and macrophages (Mantovanni *et al*., 2015). These receptors stimulate a range of signaling pathways including NF-kB and it also capable to enhance the transcription of the mRNA of inflammatory cytokines such as IL-6, tumor necrosis factor (TNF)- α, and IL-1β. TNF-α and IL-1β also are able to activate transcription factors to produce IL-6 (Vergadi **et al**., 2018). Moreover, pathological features of inflammatory lesions like angiogenesis and vascular permeability will be increased when the IL-6 induce excess production of VEGF. Recent research found that dysregulation of the IL-6 production happened in malignant cells in cancers (Brenot *et al*., 2018; Yin *et al*., 2018). Breast cancer study also consider that IL-6 reacts as a regulator of estrogen synthesis and aromatase action, conciliate a growth response related to hormone receptor status, and plays a part to pro-metastatic processes including epithelial-to-mesenchymal transition, cell invasion, cell migration, and mesenchymal stem cell recruitment (Mu *et al*., 2018). More than that, clinical studies have proven that in breast cancer patients there are increasing circulating levels of IL-6 as well as relationship between higher circulating levels and more advanced stages of disease including metastasis (Essandoh *et al*.,2016).

The way forward: Changing the dynamics of macrophages polarization

Inflammatory signals can easily polarize macrophages towards an M1 phenotype *in vitro* (Tariq *et al*. 2017), but, this has yet to be proven *in vivo* and in the clinical settings. Macrophages involving cytokines such as IL-6 that are used for treatment of breast cancer metastasis or cancer immunotherapy are found to elicit immune responses. In order to reduce tumor size and angiogenesis, recruit immune cells to the tumor site, and prevent the polarization of macrophages to an M2 phenotype these methods of approach have been study. Common transcription factors expressed by these cells are new markers that may help to discriminate M1 from M2 macrophages (Vergadi *et al*., 2017). M1 cells highly express IRF-5 which a transcription factor that is involved in stimulating the production of type 1 interferon while repressing the production of IL-10. IRF-5 in M2 macrophages can be switches to M1 macrophages by the forced expression. Genetic polymorphisms that induce overexpression of IRF-5 mRNA in human auto-immune pathologies SLE. Multiple sclerosis illustrate the link between high IRF-5 expression and type 1 inflammation in humans (Ma *et al*., 2016; Rhee, 2016). Secondly, function of immune cells are related with the signal transducer and activator of transcription (STAT) protein family. Cytokine induced phosphorylation and nuclear expression of STAT1 and is associated with a type 1 pro-inflammatory phenotype whereas STAT3 resembles an anti-inflammatory phenotype. Usually, STAT3 expression are found in tumors and tumor-infiltrating macrophages and it is induced by IL-6 and IL-10. It is known that immunotherapy requires the activation of the immune system, it is hard to find a cellular compound, or biomaterial that will not produce side effect when it is being used in the treatment. Due to the fact that macrophages belong to the innate immune system which exhibit pro-inflammatory and anti-inflammatory properties these make them the most ideal immunotherapy candidates. Current results have highlighted how adaptive immunity can set up cancer promoting inflammation and TAM through distinct molecular pathways (Hung *et al*., 2018; Yin *et al*., 2018). Table 1 highlights recent studies on changing the dynamics of macrophage polarisation in cancer treatment which using polarized M1 macrophages without p50 NF-kβ factor, repolarization of TAM into phenotypes that more closely resemble M1 macrophages are able to reduce the tumor growth and generate anti-tumoral response. TAM microenvironment also involved in contributing a dynamic polarization, differentiation into and from between the M1 and M2 macrophages.

CONCLUSION

It is obvious that macrophages play a significant role in cancer progression, and immunotherapies involving macrophage should be considered in the treatment of this disease. The polarization of macrophages towards an M1 response with minimal side effects may prove to be a powerful therapy against solid tumors. Thus, the development of effective strategies to tip the balance from M2 to M1 macrophages should be considered. Further work is also needed to identify substances and protocols that can adeptly re-educate the immune system to attack cancer cells, prevent angiogenesis and metastasis, and to protect the host from developing a damaging inflammatory responses.

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