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REVIEW **Open Access**

The prognostic role of M2 tumor-associated macrophages in non-small-cell lung cancer

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Summary. Lung cancer is a high-risk tumor and is a main cause of death worldwide. The tumor aggressiveness and degree of malignancy depend not only on the tumor itself, but also on the microenvironment. The inflammatory microenvironment is one of the key factors in promoting the progression of lung cancer. It has been found that macrophages are the most abundant immune cells in the tumor microenvironment, with strong plasticity and heterogeneity. Tumor-Associated Macrophages (TAMs) are important components of the tumor immune microenvironment. TAMs are thought to be polarized into two main phenotypes: inflammatory or classically activated (M1) and antiinflammatory or alternatively activated (M2) macrophages. Their phenotype and function change according to environment and the appearance of tumor cells. M2 macrophages have been reported to be protumorigenic, because they can promote the formation of blood vessels in the tumor microenvironment, helping tumor cells escape the body's immune defense and promote their growth, by releasing a variety of cytokines, including chemokines, inflammatory factors and growth factor. However, the prognostic impact of TAMs and their phenotypes in non-small-cell lung cancer (NSCLC) remains to be fully elucidated. Some reports of the association between the characteristics of macrophages in lung tumor and patients' survival outcomes show contradicting results. In order to explore the prognostic role of TAMs in NSCLS, the association between the phenotype, density and distribution of macrophages and the prognosis of human NSCLC, as well as the potential mechanisms of M2 macrophages leading to poor prognosis in NSCLC, are reviewed in this study.

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Introduction

Macrophages belong to the body's innate immune system and play an important role in clearing pathogens and maintaining host homeostasis. Macrophages are an ancient evolutionary cell type, which maintains tissue homeostasis and immune defense against pathogens. Macrophages have been rediscovered as regulators of several diseases, including lung cancer (Liu and Cao, 2014; Franklin et al., 2014). Lung cancer is a high-risk tumor and is a major cause of death worldwide (Wang et al., 2017; Bray et al., 2018). The mechanism of lung cancer remains unclear and the 5-year survival rate is still lower in comparison to other cancers (Mery et al., 2015). However, NSCLC accounts for >85% of lung cancer, and squamous cell carcinoma and adenocarcinoma account for approximately 70% of NSCLC. Due to the high incidence and mortality of lung cancer and the lack of effective early diagnosis and screening techniques, in many cases, a lesion has metastasized and is in an advanced stage when a patient visits a doctor. Thus, there are difficulties that urgently need to be overcome. Exploration of the mechanisms of NSCLC, diagnostic approaches that allow its diagnosis at an early stage, and improvement of the survival rate are all expected.

Tumor aggressiveness and the degree of malignancy depend not only on the tumor itself, but also on the microenvironment. The tumor microenvironment, which includes not only tumor cells, but also surrounding cellular components, such as stromal cells, infiltrating inflammatory cells, cancer stem cells, and non-cellular components (e.g., cytokines and growth factors, which promote the growth and migration of tumor cells) (Lin et al., 2018). In turn, these cells provide the signals and metabolites required by the tumor cells to provide a suitable growth microenvironment for the tumor (Yuan

et al., 2016). Macrophages, which are the most abundant immune cells in the tumor microenvironment, show strong plasticity and heterogeneity (Sedighzadeh et al., 2021). In the tumor microenvironment, tumor cells recruit macrophages to the tumor microenvironment and "re-educate" macrophages to alter their phenotype and function (Bosco, 2019). Studies have shown that macrophages have a certain correlation with the prognosis of tumors. In this review, we mainly focus on the correlation between macrophage differentiation and a poor prognosis in NSCLC patients.

Macrophages

Macrophages belong to the body's innate immune system and play an important role with pathogens and body homeostasis. Macrophages with strong plasticity and heterogeneity are the most abundant immune cells in the microenvironment of many solid tumors. When the microenvironment changes, the phenotype and function of macrophages change accordingly (Sedighzadeh et al., 2021). Macrophages are mainly divided into the M1 and M2 types (Biswas et al., 2012) (Fig. 1). M1 type macrophages are activated by interferon gamma (IFN-γ), agonists like lipopolysaccharide (LPS), and tumor necrosis factor-alpha (TNF-α), which can express high inducible nitric oxide synthase-1 (iNOS) and release pro-inflammatory factors, such as tumor necrosis factor (TNF)-α, IL-1 and IL-6, which can inhibit tumor activity. CD80, CD86, and CD64 are some common biomarkers of M1 macrophages. Meanwhile, M2 macrophage polarization can be induced by different stimulating factors, such as IL-4, IL-13 and IL-10. Common characteristic proteins of M2 macrophages include CD206, CD163, and CD204 (Stout and Suttles, 2004; Mantovani et al., 2004; Martinez et al., 2008; Sica and Mantovani, 2012; Liu et al., 2014). M2 macrophages can inhibit the occurrence of inflammatory reactions, and regulate tissue remodeling and angiogenesis. In the tumor microenvironment, tumor cells recruit macrophages to the tumor microenvironment and "re-educate" macrophages to alter their phenotype and function (Bosco, 2019). Therefore, clinical studies usually identify different macrophages in the microenvironment through these specific markers (Li et al., 2018) (Fig. 2).

Macrophages and cancer

The occurrence and development of cancer is a

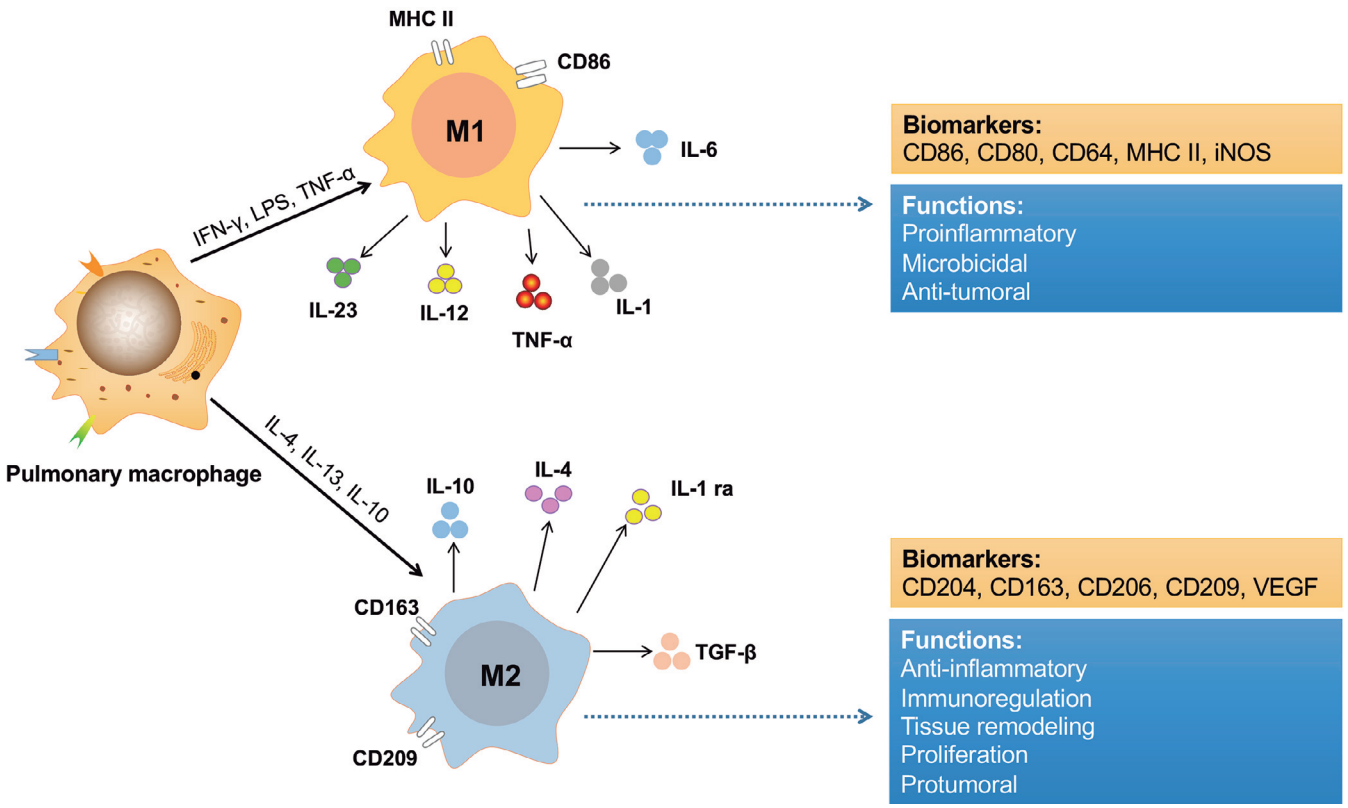

Fig. 1. Pulmonary macrophages polarize into the M1 or M2 phenotype. IFN-γ, interferon gamma; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; MHC, major histocompatibility complex; iNOS, inducible nitric oxide synthase; IL, interleukin; TGF-β, tissue growth factor-beta; VEGF, vascular endothelial growth factor.

complex cascade process (Yuan et al., 2016). Most tumors have the characteristics of maintenance of proliferation signals, resistance to cell death, infinite replication, induction of angiogenesis, escape from immune surveillance and invasiveness (Hanahan and Weinberg, 2000). The macrophage is a critical immune cell type in the tumor microenvironment and is derived from circulating monocytes. Macrophages are associated with tumor growth and invasion (Noy and Pollard, 2014), which can promote tumor transformation and immune escape (Williams et al., 2016). In *in vivo* and *in vitro* experiments, M2 macrophages have been demonstrated to promote the invasion and migration of tumor cells (Chen et al., 2017a,b). The polarization state of macrophages is related to the stage of cancer (Hung et al., 2018). In a mouse model of breast cancer, M2 macrophages were demonstrated to be the most abundant cells in the stroma, which can help tumor cells escape immune surveillance and promote cancer development (Quail and Joyce, 2013; Lin et al., 2015), and M2 macrophages were reported to be associated with a poor prognosis in breast cancer patients (Quail and Joyce, 2013). The density of M2 macrophages in the tumor microenvironment is often associated with a poor prognosis and promotes metastasis of solid tumors by releasing a variety of cytokines, including chemokines, inflammatory factors, and growth factors (Cho et al., 2012; Kawata et al., 2012). CD163-positive macrophages can promote the expression of PD-L1 on pancreatic ductal carcinoma cells, which is associated with a poor prognosis in patients with pancreatic ductal carcinoma (Tsukamoto et al., 2019). The density of M2 macrophages infiltrating into the stroma can be used as an independent predictor for patients with pancreatic ductal carcinoma (Hu et al., 2016). The distribution and density of macrophages in tumors are independent risk factors for recurrence in patients with surgically-treated colon cancer (Kim et al., 2018). Accordingly, researchers are increasingly paying attention to the relationship between the tumor prognosis and macrophages.

Macrophages and NSCLC

An increasing number of studies are attempting to explore the correlation between macrophages and the prognosis of NSCLC. The phenotype, density and distribution of macrophages were found to affect the prognosis of NSCLC patients (Table 1).

Several reports on the association between macrophage density in lung tumors and patient outcomes in terms of survival have produced contradictory results. Kawai et al. indicated that evaluation of the numbers of macrophages in cancer nests and the stroma are useful biomarkers for predicting the prognosis of stage IV NSCLC patients treated with chemotherapy, because patients with more tumor-infiltrating macrophages in cancer nests than in cancer stroma showed a significantly better survival time (Kawai et al., 2008). Similarly, studies showed that the infiltration of large

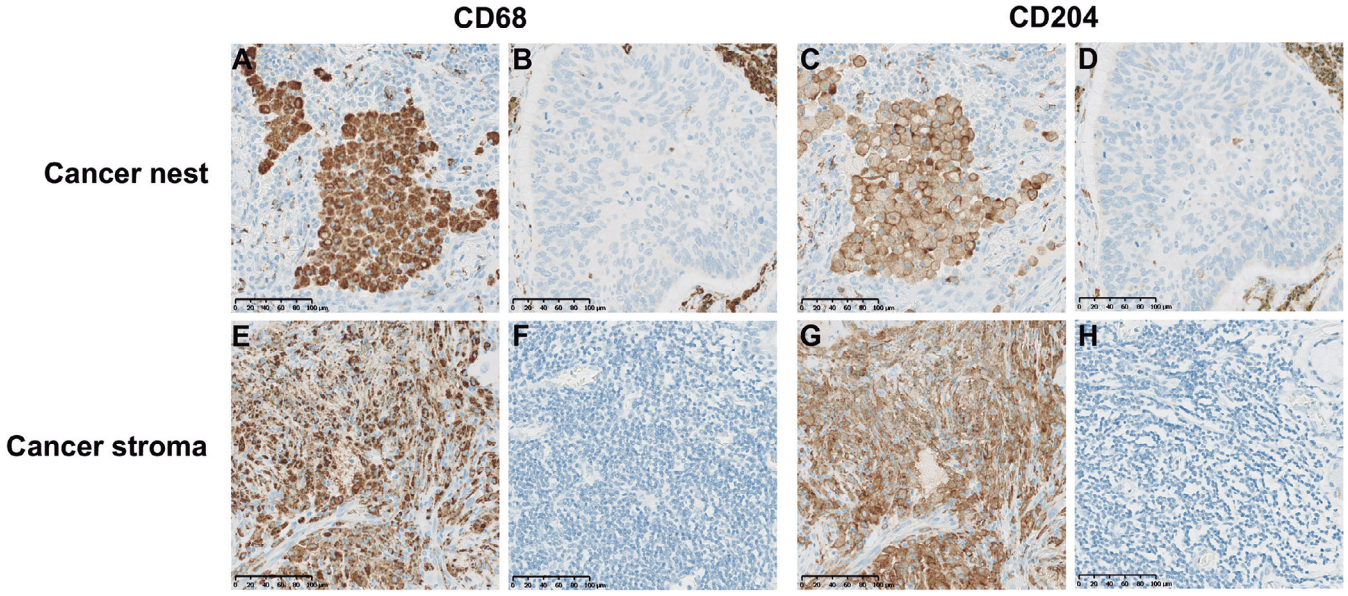

Fig. 2. Distribution of macrophages in cancer nests and cancer stroma of NSCLC (Li et al., 2018). Immunostaining of NSCLC with CD68 or CD204 antibodies for macrophages. **A.** Cases with a high number of CD68$^+$ TAMs in cancer nests. **B.** Cases with a low number of CD68$^+$ TAMs in cancer nests. **C**. Cases with a high number of CD204$^+$ TAMs in cancer nests. **D.** Cases with a low number of CD204$^+$ TAMs in cancer nests. **E.** Cases with a high number of CD68$^+$ TAMs in cancer stroma. **F.** Cases with a low number of CD68$^+$ TAMs in cancer stroma. **G**. Cases with a high number of CD204$^+$ TAMs in cancer stroma; **H.** Cases with a low number of CD204$^+$ TAMs in cancer stroma.

Table 1. Tumor associated macrophages and lung cancer prognosis.

Region	Tumor type	Study period	Methods	Markers	Cases	Macrophage distribution and prognosis	Article
UK	NSCLC	1991-1994, 1999.1-1999.12	IHC	CD68	175	a high nest macrophage density: better survival a high stromal macrophage density: poor survival	Welsh et al., 2005
Japan	NSCLC Stage IV	1996.1 - 2004.12	IHC	CD68	199	macrophages in nests > macrophages in stroma: better survival; macrophages in nests < macrophages in stroma: poor survival	Kawai et al., 2008
Korea	NSCLC	1997.1-1998.12	IHC	CD68	144	a high nest macrophage density: better survival stromal macrophage counts not associated with survival	Kim et al., 2008
UK	NSCLC (stage I, II, IIIa, IV)	1991-1994, 1999.1-1999.12	IHC	M1: iNOS, HLA-DR, MRP 8/14, TNF-α M2: CD163, VEGF	40	M1 macrophages in nests > M2 macrophages in nests: better survival	Ohri et al., 2009
China	NSCLC	1999.8-2001.8	IHC	CD68	99	a high nest macrophage density: better survival a high stromal macrophage density: poor survival high nest macrophage /stromal macrophage ratio: better survival	Dai et al., 2010
China	NSCLC	1999.6-2001.8	IHC	M1: CD68, HLA-DR M2: CD68, CD163	100	higher M1 macrophage densities in the tumor stroma and nests: better survival; The M2 macrophage densities were not associated with patient's survival	Ma et al., 2010
Japan	lung adenocarcinoma	1996.1-1998.3	IHC	M2: CD68, CD204	170	CD204-positive macrophages: tumor-promoting phenotype	Ohtaki et al., 2010
China	lung adenocarcinoma stage I, II, III, IV	2003-2006	IFC	M1: CD68, iNOS M2: CD68, CD206	65	M2-polarized macrophage: poor prognoses	Zhang et al., 2011
Japan	lung adenocarcinoma stage I	1995.1-2007.12	IHC	M2：CD204	106	intravascular CD204(+) macrophages: higher in patients with recurrences	Kaseda et al., 2013
Japan	Lung Squamous Cell Carcinoma	2000.1-2006.12	IHC	M2：CD204	208	CD14 (+)CD204(+): metastasis of cancer cells	Maeda et al., 2014
Japan	Lung Squamous Cell Carcinoma	2000.1-2006.12	IHC	M2：CD204	208	higher numbers of CD204 (+) TAMs in the stroma: poor clinical outcome	Hirayama et al., 2012
China	Lung Adenocarcinoma Stage I	2004-2011	IHC	M2: CD204	182	5-year disease-free survival (DFS) rateCD204 of high-density group: worse; 5-year disease-free survival (DFS) rate of CD204 low-density group: better	Sun and Xu, 2018
Lithuania	NSCLC	2012.9-2015.4	IHC	M1: CD68, iNOS M2: CD68, CD163	80	high infiltration of M1 macrophages in tumor nests: better survival; high infiltration of total M2 macrophages in tumor (stroma and nests): poor survival	Jackute et al., 2018
Japan	NSCLC	2005-2013	IHC	M2: CD68, CD204	297	higher numbers of CD204-positive TAMs in the tumor stroma: an independent worse prognostic predictor	Li et al., 2018
Sweden	NSCLC	2006-2010	IFC	M2: CD68, CD163	352	a low proportion of CD163-positive macrophages: a high survival rate	La Fleur et al., 2018
Norway	NSCLC stage I to III	1990-2010	IHC	M1: CD68, HLA-DR; M2: CD68, CD204; CD68, CD163	696	M1 macrophages in metastatic lymph nodes: positive prognostic marker	Rakaee et al., 2019
China	NSCLC stage I to III	2012-2014	IFC	M2: CD68, CD163	137	high infiltration of M2 macrophages in nests: poor prognosis	Cao et al., 2019
Japan	NSCLC	2011.11-2014.10	IHC	M2: CD163	160	high infiltration of M2 macrophages in stromal: poor prognosis; high infiltration of M2 macrophages in alveolar: poor prognosis	Sumitomo et al., 2019a
Germany	NSCLC (lung squamous cell carcinoma)	/	RNA sequencing qRT-PCR; IFC	CD68, IL12, CCR7, CD163, ALOX15	104	higher spatial density of M1 macrophages: longer overall survival; higher spatial density of M2 macrophages: reduced overall survival	Zheng et al., 2020
USA	NSCLC	2010.1-2012.12(n=102), 1993-2004(n=247)	IHC	CD68, CD163, VEGF-A, VEGF-C	349	elevated M2 ratio (CD163+/CD68+) was significantly associated with poor overall survival	Hwang et al., 2020

numbers of macrophages in cancer nests can be a favorable independent prognostic factor for NSCLC (Kim et al., 2008). Welsh et al. indicated that the density of CD68-positive macrophages in cancer nests was a powerful independent predictor of survival in NSCLC patients (Welsh et al., 2005). The results showed that patients with high-density macrophages in the cancer nest have a significantly higher survival time. Dai et al. also found that the number of macrophages in the cancer nests was positively associated with patient survival time (Dai et al., 2010). In contrast, the number of macrophages in the cancer stroma was negatively associated with patient survival time. Therefore, the number of macrophages in cancer nests or stroma is an independent predictor of survival time in NSCLC patients.

NSCLC is thought to be associated with gene mutations (e.g., epidermal growth factor receptor [EGFR]-mutation, Kirsten rat sarcoma [KRAS]-mutation, etc.), which are important factors for clinical treatment. However, studies have shown that high cancer nest macrophage infiltration is correlated with improved patient survival but not with gene mutation or the gene copy number in resected non-small cell lung cancer (Kim et al., 2008).

It can be concluded from the above-mentioned findings that the number of macrophages in cancer nests or stroma might be an independent predictor of survival time in NSCLC patients. The number of macrophages in cancer nests may be positively associated with patient survival time, whereas the number of macrophages in the cancer stroma may be negatively associated with patient survival time. During this period, there has been little research on why macrophages at different locations in the tumor microenvironment contribute to different patient outcomes.

Macrophage differentiation in NSCLC

To explore why the different ratio of macrophages in cancer nests to cancer stroma are associated with differences in the prognosis of NSCLC patients, researchers used specific markers to study the status of macrophages in different locations in the microenvironment of NSCLC. The evidence showed that the body's immune response plays a critical role in the development, inhibition, or promotion of cancer, and that the activity and type of immune cells can affect the balance between its tumor-promoting and anti-tumor effects (Murata, 2018; Nagahashi et al., 2018). Further research has found that the phenotype and function of macrophages also change when the microenvironment changes, and that M1 and M2 macrophages play different roles in the body's immune response. M2 macrophages can promote the invasion and growth of NSCLC cells A549. M1 macrophages can enhance the sensitivity of A549 cells to cisplatin and reduce the angiogenesis ability in the tumor microenvironment by inducing apoptosis (Yuan et al., 2015). In the immunohistochemical analysis of 20 pathological tissue specimens from patients with extended survival (median 92.7 months) and 20 pathological tissue specimens from patients with poor survival (median 7.7 months), the density of M1 macrophages in cancer nests was significantly higher than that of M2 macrophages in patients with extended survival. The longer patient survival time may be related to the cytotoxicity of M1 macrophages in cancer nests, and it can be seen that cellular immune responses play an important role in inhibiting tumor cell growth (Ohri et al., 2009). Then, HLA-DR and CD163 were used to label M1 and M2 macrophages, respectively. The M1 macrophage density in the cancer nests and stroma of the long survival group was significantly higher in comparison to patients with a short survival time. However, they found no significant difference in M2 macrophage density between the long survival and short survival groups (Ma et al., 2010).

What's more, in lung adenocarcinoma, M2-polarized subtype macrophages are detected in the tumor microenvironment of patients with a poor prognosis. This phenomenon may be caused by M2 macrophages accelerating lymphangiogenesis and the promotion of lymph node metastasis (Zhang et al., 2011). Ohtaki et al. found that CD204-positive macrophages (M2 macrophages) clearly reflected the tumor-promoting phenotype of TAMs in lung adenocarcinoma (Ohtaki et al., 2010). Then, Kaseda et al. found that large numbers of intravascular CD204-positive macrophages were a significant predictor of recurrence in patients with pathological stage I lung adenocarcinoma (Kaseda et al., 2013). Hirayama et al. found a similar phenomenon in lung squamous cell carcinoma, the CD204-positive macrophages and other tumor-promoting stromal cells may create tumor-promoting microenvironments in lung squamous cell carcinoma (Hirayama et al., 2012). Futhermore, Maeda et al. found that circulating CD14 CD204-positive macrophages contribute to metastasis of cancer cells (Maeda et al., 2014). They also showed that blocking of circulating CD14 CD204-positive macrophages activity may prevent postoperative recurrence in patients with resected NSCLC. Sun et al. found that the expression of a high density of CD204 macrophages is associated with the aggressiveness of lung adenocarcinoma (Sun and Xu, 2018). In summary, macrophages are distributed in various tissue compartments in lung cancer, including the stroma and cancer nests. CD204-positive macrophages create tumor-promoting microenvironments in lung adenocarcinoma and lung squamous cell carcinoma.

Based on this, researchers further studied the different characteristics of macrophages in NSCLC. A study using immunohistochemical analysis to investigate the tumor tissues of 80 patients with NSCLC found that M2 macrophages were mainly in the tumor stroma. The high infiltration of M1 macrophages in cancer nests was associated with increased overall survival in NSCLC, while the high infiltration of total M2 macrophages in the stroma and nests was associated with poor survival

in NSCLC (Jackute et al., 2018). In a previous study, our team collected tumor tissues from 297 patients with NSCLC, and found that CD204-positive M2 TAMs were a preferable marker for prognostic prediction in NSCLC, especially in lung adenocarcinoma (Li et al., 2018), which is consistent with the findings of the above study. La Fleur et al. calculated the survival rates of patients with adenocarcinoma and found that patients with a low proportion of CD163-positive macrophages had a three-year survival rate of 62.3%, while those with a high proportion had a three-year survival rate of 49.1% (La Fleur et al., 2018). Furthermore, stromal infiltration of the M1 TAM level was found to be significantly decreased from pathological stage I to III NSCLC (Rakaee et al., 2019). The M1 macrophages in nests were shown to gradually transform into M2 macrophages and the high infiltration of M2 macrophages was associated with a poor prognosis in NSCLC (Cao et al., 2019). During the progression of NSCLC, M2 macrophages may induce tumor cell aggressiveness and proliferation and increase metastatic potential, leading to a poor prognosis in patients (Sumitomo et al., 2019a). Patients with higher M1 densities and lower M2 densities showed significantly better overall survival rates in NSCLC, and the spatial density and distribution of macrophages predict the patient prognosis (Zheng et al., 2020). Another recent study suggests that TAMs are significantly associated with angiogenesis and lymphangiogenesis, contributing to the progression of NSCLC. Furthermore, an elevated M2 ratio ($CD163^+/CD68^+$) is a strong indicator of poor prognosis in patients with NSCLC (Hwang et al., 2020).

In summary, macrophage density has a certain correlation with patient prognosis. A higher proportion of M1 macrophages and a lower proportion of M2 macrophages in tissues is beneficial to patient prognosis. This may be due to the cytotoxicity of M1 macrophages, which can promote the body's inflammatory response, while M2 macrophages have the effect of inhibiting the body's inflammatory response and promoting tumor growth. Therefore, the location and M1/M2 polarization of TAMs may potentially be independent predictors of the prognosis of NSCLC.

Mechanisms through which M2 macrophages lead to a poor prognosis in NSCLC

In the early stage of NSCLC tumor formation, macrophages induce an epithelial mesenchymal transition that promotes tumor cell invasiveness, while also protecting tumor cells from being recognized by T cells (Casanova-Acebes et al., 2021). TAMs are the most abundant population of inflammatory cells and play an important role in tumors. TAMs can promote metastasis and growth of NSCLC through an NF-κB/PP2Ac-positive feedback loop (Liang et al., 2021). Another study found that TAMs are related with angiogenesis and lymphangiogenesis, contributing to the progression of NSCLC. Further research found that a high proportion of TAMs and high expression of VEGF-C in the tumor stroma are associated with poor overall survival (Hwang et al., 2020) .

Studies showed that M2 macrophages are associated with poor prognosis in NSCLC, due to the promotion of tumor malignant progression. However, the mechanisms underlying the promotion of tumor cell proliferation, migration and invasion by M2 macrophages are largely unclear. In resected tissue from NSCLC patients, the expression of PD-L1 was significantly higher on tumor cells with more M2 macrophages. The tumor promoting M2 macrophages might affect the PD-L1 expression both on tumor cells and immune cells, while the PD-L1 expression on tumor cells was associated with aggressive malignant behavior in tumor cells (Sumitomo et al., 2019b). M2 macrophages can facilitate the tumor expression of PD-L1 through TGF-β in early lung adenocarcinoma (Shima et al., 2020). The interaction of PD-1 with PD-L1 expressed in antigen-presenting cells promotes immune suppression and helps tumor cells evade host immune surveillance. In another study, M2 macrophages were demonstrated to secrete IL-10, which promotes cancer stem cell properties and tumor growth in NSCLC via JAK1/STAT1/NF-κB/Notch1 signaling (Yang et al., 2019).

In recent years, the exosomes of the tumor microenvironment have been reported to play a critical role in the communication of information between macrophages and tumor cells. Exosomes alter the phenotype and function of recipient cells through the transmission of various proteins, DNA and RNAs (El et al., 2013; Record et al., 2014). The study found that miR-155 and miR-196a-5p were abundant in the M2 macrophages and exosomes secreted by M2 TAMs. Exosomes derived from M2 TAMs were able to promote cell viability, migration, and invasion in NSCLC (Li et al., 2021). Tumor-derived exosomal circFARSA mediates M2 macrophage polarization via the PTEN/PI3K/AKT signaling pathway in NSCLC (Chen et al., 2021).

Conclusion

There are many immune cells in the complex lung cancer microenvironment, including, but not limited to, T cells, B cells, natural killer (NK) cells, mast cells, dendritic cells, and macrophages. These components play different roles in the development and prognosis of NSCLC (Soo et al., 2018; Tuminello et al., 2019). In this review, we mainly focused on the effects of different phenotypes of macrophage on the prognosis of NSCLC.

Macrophages decide the progress of NSCLC, and have different characteristics in different parts of the tumor. Tumor cells can recruit macrophages to the microenvironment and induce macrophage polarization. The M1 and M2 macrophage densities in different parts of the tumor microenvironment differently influence the prognosis of patients with NSCLC. M2 macrophages in the stroma are associated with poor prognosis in NSCLC

patients and may be an independent predictor of poor prognosis. However, more patient testing, standardized platforms of detection and, multi-institutional studies are needed before M2 macrophages can be considered as a prognostic biomarker in NSCLC (Altorki et al., 2019). In the tumor microenvironment, M2 macrophages can promote tumor cell progression and metastasis. The mechanisms through which M2 macrophages promote cancer metastasis and the communication between macrophages and tumor cells should be explored. Some studies found that M2 macrophages regulate immunosuppression through their facilitation of the expression of PD-L1 by tumor cells in NSCLC (Sumitomo et al., 2019a; Shima et al., 2020). Importantly, besides being a useful predictor of clinical outcomes, the reprogramming of TAMs from the M2 phenotype to the M1 phenotype may be a potential antitumor immunotherapy for NSCLC (Yin et al., 2020). In the clinical setting, chemotherapy is the standard treatment for NSCLC. Unfortunately, in addition to inhibiting the growth of tumor cells, commonly used drugs cause many serious adverse reactions, including nausea, vomiting and diarrhea. Furthermore, patients can develop drug resistance if they take such drugs for a long time. Therefore, cellular immunotherapy may be a new approach and the M2 macrophage is a promising target for the treatment of NSCLC (Sedighzadeh et al., 2021b). For example, reprogramming M2 to M1 macrophages may be one way to improve the prognosis and survival of NSCLC.

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References

Altorki N.K., Markowitz G.J., Gao D., Port J.L., Saxena A., Stiles B., Mcgraw T. and Mittal V. (2019). The lung microenvironment: an important regulator of tumour growth and metastasis. Nat. Rev. Cancer. 19, 9-31.

Biswas S.K., Chittezhath M., Shalova I.N. and Lim J.Y. (2012). Macrophage polarization and plasticity in health and disease. Immunol. Res. 53, 11-24.

Bosco M.C. (2019). Macrophage polarization: reaching across the aisle? J. Allergy Clin. Immunol. 143, 1348-1350.

Bray F., Ferlay J., Soerjomataram I., Siegel R.L., Torre L.A. and Jemal A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 68, 394-424.

Cao L., Che X., Qiu X., Li Z., Yang B., Wang S., Hou K., Fan Y., Qu X. and Liu Y. (2019). M2 macrophage infiltration into tumor islets leads to poor prognosis in non-small-cell lung cancer. Cancer Manag. Res. 11, 6125-6138.

Casanova-Acebes M., Dalla E., Leader A.M., Leberichel J., Nikolic J., Morales B.M., Brown M., Chang C., Troncoso L., Chen S.T., Sastre-Perona A., Park M.D., Tabachnikova A., Dhainaut M., Hamon P., Maier B., Sawai C.M., Agullo-Pascual E., Schober M., Brown B.D., Reizis B., Marron T., Kenigsberg E., Moussion C., Benaroch P., Aguirre-Ghiso J.A. and Merad M. (2021). Tissue-resident macrophages provide a pro-tumorigenic niche to early NSCLC cells. Nature 595, 578-584.

Chen P., Zuo H., Xiong H., Kolar M.J., Chu Q., Saghatelian A., Siegwart D.J. and Wan Y. (2017a). Gpr132 sensing of lactate mediates tumor-macrophage interplay to promote breast cancer metastasis. Proc. Natl. Acad. Sci. USA 114, 580-585.

Chen T., Liu Y., Li C., Xu C., Ding C., Chen J. and Zhao J. (2021). Tumor-derived exosomal circFARSA mediates M2 macrophage polarization via the PTEN/PI3K/AKT pathway to promote non-small cell lung cancer metastasis. Cancer Treat Res. Commun. 28, 100412.

Chen Y., Zhang S., Wang Q. and Zhang X. (2017b). Tumor-recruited M2 macrophages promote gastric and breast cancer metastasis via M2 macrophage-secreted CHI3L1 protein. J. Hematol. Oncol. 10, 36.

Cho H.J., Jung J.I., Lim D.Y., Kwon G.T., Her S., Park J.H. and Park J.H. (2012). Bone marrow-derived, alternatively activated macrophages enhance solid tumor growth and lung metastasis of mammary carcinoma cells in a Balb/C mouse orthotopic model. Breast Cancer Res. 14, R81.

Dai F., Liu L., Che G., Yu N., Pu Q., Zhang S., Ma J., Ma L. and You Z. (2010). The number and microlocalization of tumor-associated immune cells are associated with patient's survival time in non-small cell lung cancer. BMC Cancer 10, 220.

Ei A.S., Mager I., Breakefield X.O. and Wood M.J. (2013). Extracellular vesicles: biology and emerging therapeutic opportunities. Nat. Rev. Drug Discov. 12, 347-357.

Franklin R.A., Liao W., Sarkar A., Kim M.V., Bivona M.R., Liu K., Pamer E.G. and Li M.O. (2014). The cellular and molecular origin of tumor-associated macrophages. Science 344, 921-925.

Hanahan D. and Weinberg R.A. (2000). The hallmarks of cancer. Cell 100, 57-70.

Hirayama S., Ishii G., Nagai K., Ono S., Kojima M., Yamauchi C., Aokage K., Hishida T., Yoshida J., Suzuki K. and Ochiai A. (2012). Prognostic impact of CD204-positive macrophages in lung squamous cell carcinoma: possible contribution of CD204-positive macrophages to the tumor-promoting microenvironment. J. Thorac. Oncol. 7, 1790-1797.

Hu H., Hang J.J., Han T., Zhuo M., Jiao F. and Wang L.W. (2016). The M2 phenotype of tumor-associated macrophages in the stroma confers a poor prognosis in pancreatic cancer. Tumour Biol. 37, 8657-8664.

Hung C.H., Chen F.M., Lin Y.C., Tsai M.L., Wang S.L., Chen Y.C., Chen Y.T. and Hou M.F. (2018). Altered monocyte differentiation and macrophage polarization patterns in patients with breast cancer. BMC Cancer 18, 366.

Hwang I., Kim J.W., Ylaya K., Chung E.J., Kitano H., Perry C., Hanaoka J., Fukuoka J., Chung J.Y. and Hewitt S.M. (2020). Tumor-associated macrophage, angiogenesis and lymphangiogenesis markers predict prognosis of non-small cell lung cancer patients. J. Transl. Med. 18, 443.

Jackute J., Zemaitis M., Pranys D., Sitkauskiene B., Miliauskas S., Vaitkiene S. and Sakalauskas R. (2018). Distribution of M1 and M2

macrophages in tumor islets and stroma in relation to prognosis of non-small cell lung cancer. BMC Immunol. 19, 3.

Kaseda K., Ishii G., Aokage K., Takahashi A., Kuwata T., Hishida T., Yoshida J., Kohno M., Nagai K. and Ochiai A. (2013). Identification of intravascular tumor microenvironment features predicting the recurrence of pathological stage I lung adenocarcinoma. Cancer Sci. 104, 1262-1269.

Kawai O., Ishii G., Kubota K., Murata Y., Naito Y., Mizuno T., Aokage K., Saijo N., Nishiwaki Y., Gemma A., Kudoh S. and Ochiai A. (2008). Predominant infiltration of macrophages and CD8(+) T Cells in cancer nests is a significant predictor of survival in stage IV nonsmall cell lung cancer. Cancer 113, 1387-95.

Kawata M., Koinuma D., Ogami T., Umezawa K., Iwata C., Watabe T. and Miyazono K. (2012). TGF-beta-induced epithelial-mesenchymal transition of A549 lung adenocarcinoma cells is enhanced by pro-inflammatory cytokines derived from RAW 264.7 macrophage cells. J. Biochem. 151, 205-216.

Kim D.W., Min H.S., Lee K.H., Kim Y.J., Oh D.Y., Jeon Y.K., Lee S.H., Im S.A., Chung D.H., Kim Y.T., Kim T.Y., Bang Y.J., Sung S.W., Kim J.H. and Heo D.S. (2008). High tumour islet macrophage infiltration correlates with improved patient survival but not with EGFR mutations, gene copy number or protein expression in resected non-small cell lung cancer. Br. J. Cancer 98, 1118-1124.

Kim Y., Wen X., Bae J.M., Kim J.H., Cho N.Y. and Kang G.H. (2018). The distribution of intratumoral macrophages correlates with molecular phenotypes and impacts prognosis in colorectal carcinoma. Histopathology 73, 663-671.

La Fleur L., Boura V.F., Alexeyenko A., Berglund A., Ponten V., Mattsson J., Djureinovic D., Persson J., Brunnstrom H., Isaksson J., Branden E., Koyi H., Micke P., Karlsson M. and Botling J. (2018). Expression of scavenger receptor MARCO defines a targetable tumor-associated macrophage subset in non-small cell lung cancer. Int. J. Cancer 143, 1741-1752.

Li Z., Maeda D., Yoshida M., Umakoshi M., Nanjo H., Shiraishi K., Saito M., Kohno T., Konno H., Saito H., Minamiya Y. and Goto A. (2018). The intratumoral distribution influences the prognostic impact of CD68- and CD204-positive macrophages in non-small cell lung cancer. Lung Cancer 123, 127-135.

Li X., Chen Z., Ni Y., Bian C., Huang J., Chen L., Xie X. and Wang J. (2021). Tumor-associated macrophages secret exosomal miR-155 and miR-196a-5p to promote metastasis of non-small-cell lung cancer. Transl. Lung Cancer Res. 10, 1338-1354.

Liang Z.W., Ge X.X., Xu M.D., Qin H., Wu M.Y., Shen M., Zhang Y., Liu X.M., Chen K., Li W., Duan W. and Qin S. (2021). Tumor-associated macrophages promote the metastasis and growth of non-small-cell lung cancer cells through NF-kappaB/PP2Ac-positive feedback loop. Cancer Sci. 112, 2140-2157.

Lin L., Chen Y.S., Yao Y.D., Chen J.Q., Chen J.N., Huang S.Y., Zeng Y.J., Yao H.R., Zeng S.H., Fu Y.S. and Song E.W. (2015). CCL18 from tumor-associated macrophages promotes angiogenesis in breast cancer. Oncotarget 6, 34758-34773.

Lin Y.H., Wu M.H., Yeh C.T. and Lin K.H. (2018). Long non-coding RNAs as mediators of tumor microenvironment and liver cancer cell communication. Int. J. Mol. Sci. 19, 3742

Liu Y. and Cao X. (2014). The origin and function of tumor-associated macrophages. Cell. Mol. Immunol. 12, 1-4.

Liu Y.C., Zou X.B., Chai Y.F. and Yao Y.M. (2014). Macrophage polarization in inflammatory diseases. Int. J. Biol. Sci. 10, 520-529.

Ma J., Liu L., Che G., Yu N., Dai F. and You Z. (2010). The M1 form of tumor-associated macrophages in non-small cell lung cancer is positively associated with survival time. BMC Cancer 10, 112.

Maeda R., Ishii G., Neri S., Aoyagi K., Haga H., Sasaki H., Nagai K. and Ochiai A. (2014). Circulating CD14+CD204+ cells predict postoperative recurrence in non-small-cell lung cancer patients. J. Thorac. Oncol. 9, 179-188.

Mantovani A., Sica A., Sozzani S., Allavena P., Vecchi A. and Locati M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 25, 677-686.

Martinez F.O., Sica A., Mantovani A. and Locati M. (2008). Macrophage activation and polarization. Front. Biosci. 13, 453-461.

Mery B., Guy J.B., Swalduz A., Vallard A., Guibert C., Almokhles H., Ben M.M., Rivoirard R., Falk A.T., Fournel P. and Magne N. (2015). The evolving locally-advanced non-small cell lung cancer landscape: Building on past evidence and experience. Crit. Rev. Oncol. Hematol. 96, 319-327.

Murata M. (2018). Inflammation and cancer. Environ Health Prev. Med. 23, 50.

Nagahashi M., Abe M., Sakimura K., Takabe K. and Wakai T. (2018). The role of sphingosine-1-phosphate in inflammation and cancer progression. Cancer Sci. 109, 3671-3678.

Noy R. and Pollard J.W. (2014). Tumor-associated macrophages: from mechanisms to therapy. Immunity 41, 49-61.

Ohri C.M., Shikotra A., Green R.H., Waller D.A. and Bradding P. (2009). Macrophages within NSCLC tumour islets are predominantly of a cytotoxic M1 phenotype associated with extended survival. Eur. Respir. J. 33, 118-126.

Ohtaki Y., Ishii G., Nagai K., Ashimine S., Kuwata T., Hishida T., Nishimura M., Yoshida J., Takeyoshi I. and Ochiai A. (2010). Stromal macrophage expressing CD204 is associated with tumor aggressiveness in lung adenocarcinoma. J. Thorac. Oncol. 5, 1507-1515.

Quail D.F. and Joyce J.A. (2013). Microenvironmental regulation of tumor progression and metastasis. Nat. Med. 19, 1423-1437.

Rakaee M., Busund L.R., Jamaly S., Paulsen E.E., Richardsen E., Andersen S., Al-Saad S., Bremnes R.M., Donnem T. and Kilvaer T.K. (2019). Prognostic value of macrophage phenotypes in resectable non-small cell lung cancer assessed by multiplex immunohistochemistry. Neoplasia 21, 282-293.

Record M., Carayon K., Poirot M. and Silvente-Poirot S. (2014). Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. Biochim. Biophys. Acta 1841, 108-120.

Sedighzadeh S.S., Khoshbin A.P., Razi S., Keshavarz-Fathi M. and Rezaei N. (2021). A narrative review of tumor-associated macrophages in lung cancer: regulation of macrophage polarization and therapeutic implications. Transl. Lung Cancer Res. 10, 1889-1916.

Shima T., Shimoda M., Shigenobu T., Ohtsuka T., Nishimura T., Emoto K., Hayashi Y., Iwasaki T., Abe T., Asamura H. and Kanai Y. (2020). Infiltration of tumor-associated macrophages is involved in tumor programmed death-ligand 1 expression in early lung adenocarcinoma. Cancer Sci. 111, 727-738.

Sica A. and Mantovani A. (2012). Macrophage plasticity and polarization: *in vivo* veritas. J. Clin. Invest. 122, 787-795.

Soo R.A., Chen Z., Yan T.R., Tan H.L., Iacopetta B., Tai B.C. and Soong R. (2018). Prognostic significance of immune cells in non-small cell lung cancer: meta-analysis. Oncotarget 9, 24801-24820.

Stout R.D. and Suttles J. (2004). Functional plasticity of macrophages:

reversible adaptation to changing microenvironments. J. Leukoc Biol. 76, 509-513.

Sumitomo R., Hirai T., Fujita M., Murakami H., Otake Y. and Huang C.L. (2019a). M2 tumor-associated macrophages promote tumor progression in non-small-cell lung cancer. Exp. Ther. Med. 18, 4490-4498.

Sumitomo R., Hirai T., Fujita M., Murakami H., Otake Y. and Huang C.L. (2019b). PD-L1 expression on tumor-infiltrating immune cells is highly associated with M2 TAM and aggressive malignant potential in patients with resected non-small cell lung cancer. Lung Cancer. 136, 136-144.

Sun Y. and Xu S. (2018). Tumor-associated CD204-positive macrophage is a prognostic marker in clinical stage I lung adenocarcinoma. Biomed. Res. Int. 2018, 8459193.

Tsukamoto M., Imai K., Ishimoto T., Komohara Y., Yamashita Y.I., Nakagawa S., Umezaki N., Yamao T., Kitano Y., Miyata T., Arima K., Okabe H., Baba Y., Chikamoto A., Ishiko T., Hirota M. and Baba H. (2019). PD-L1 expression enhancement by infiltrating macrophage-derived tumor necrosis factor-alpha leads to poor pancreatic cancer prognosis. Cancer Sci. 110, 310-320.

Tuminello S., Veluswamy R., Lieberman-Cribbin W., Gnjatic S., Petralia F., Wang P., Flores R. and Taioli E. (2019). Prognostic value of immune cells in the tumor microenvironment of early-stage lung cancer: a meta-analysis. Oncotarget 10, 7142-7155.

Wang Q., Wang Q., Wang S.F., Jiao L.J., Zhang R.X., Zhong Y., Zhang J. and Xu L. (2017). Oral Chinese herbal medicine as maintenance treatment after chemotherapy for advanced non-small-cell lung cancer: a systematic review and meta-analysis. Curr. Oncol. 24, e269-e276.

Welsh T.J., Green R.H., Richardson D., Waller D.A., O'Byrne K.J. and Bradding P. (2005). Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. J. Clin. Oncol. 23, 8959-8967.

Williams C.B., Yeh E.S. and Soloff A.C. (2016). Tumor-associated macrophages: unwitting accomplices in breast cancer malignancy. NPJ Breast Cancer 2, 15025.

Yang L., Dong Y., Li Y., Wang D., Liu S., Wang D., Gao Q., Ji S., Chen X., Lei Q., Jiang W., Wang L., Zhang B., Yu J.J. and Zhang Y. (2019). IL-10 derived from M2 macrophage promotes cancer stemness via JAK1/STAT1/NF-kappaB/Notch1 pathway in non-small cell lung cancer. Int. J. Cancer 145, 1099-1110.

Yin W., Zhao Y., Kang X., Zhao P., Fu X., Mo X., Wang Y. and Huang Y. (2020). BBB-penetrating codelivery liposomes treat brain metastasis of non-small cell lung cancer with EGFR(T790M) mutation. Theranostics 10, 6122-6135.

Yuan A., Hsiao Y.J., Chen H.Y., Chen H.W., Ho C.C., Chen Y.Y., Liu Y.C., Hong T.H., Yu S.L., Chen J.J. and Yang P.C. (2015). Opposite effects of M1 and M2 macrophage subtypes on lung cancer progression. Sci. Rep. 5, 14273.

Yuan Y., Jiang Y.C., Sun C.K. and Chen Q.M. (2016). Role of the tumor microenvironment in tumor progression and the clinical applications (Review). Oncol. Rep. 35, 2499-515.

Zhang B., Yao G., Zhang Y., Gao J., Yang B., Rao Z. and Gao J. (2011). M2-polarized tumor-associated macrophages are associated with poor prognoses resulting from accelerated lymphangiogenesis in lung adenocarcinoma. Clinics (Sao Paulo) 66, 1879-1886.

Zheng X., Weigert A., Reu S., Guenther S., Mansouri S., Bassaly B., Gattenlohner S., Grimminger F., Pullamsetti S., Seeger W., Winter H. and Savai R. (2020). Spatial density and distribution of tumor-associated macrophages predict purvival in non-small cell lung carcinoma. Cancer Res. 80, 4414-4425.

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