Perspective

Tumor-associated macrophages (TAMs) as biomarkers for gastric cancer: A review

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Abstract

Gastric adenocarcinoma is one of the most common types of cancer worldwide, with an incidence of a million new cases annually. In addition to having a high mortality rate due to often delayed detection and its poor response to cancer therapy, it also spreads aggressively. Inflammation has been shown to play a role in carcinogenesis. Consequently, macrophages are important in phagocytosis, antigen presenting and producing cytokines and growth factors. As a response to microenvironmental signals, they may polarize into tumor resisting M1 or tumor promoting M2 macrophages. Recently, studies have indicated that M2-type macrophage resembling tumor-associated macrophages (TAMs) might be used as an independent prognostic factor for gastric cancer. This review will discuss the possible use of TAMs as prognostic tools for gastric cancer and whether they are suitable for use in clinical environment.
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Background

Worldwide, gastric cancer is one of the most commonly diagnosed types of malignancy. Even though the incidence of the disease has decreased in recent years, it is still the second most common cause of all cancer-related deaths.[1,2] Each year approximately 990,000 new cases are diagnosed and 378,000 of these patients will die because of gastric cancer.[3] Gastric cancer is approximately 50% more common among males than females and the highest incidence rates can be found in Eastern Asia, South America and Central and Eastern Europe.[4]

The classification of tumors is based on clinical and pathological findings. Due to wide morphological variation, there are several ways to classify gastric adenocarcinomas. The most common classification tumor-node-metastasis (TNM) divides them to either a superficial (T1) or an advanced type (T2−T4).[5] Classification can also be made by morphological characteristics including mucus production to the cytoplasm and glandular *vs.* tubular differentiation rate. Despite the fact that all gastric adenocarcinomas originate from

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the glandular epithelium of the stomach, the types vary morphologically depending on the location of the affected glands (pyloric, fundic, cardiac), the surface area and possible metastasized lesions of the cancer.[6] A histopathological classification method, Lauren's criteria that divide it into intestinal, diffuse and mixed types, is widely used to describe gastric adenocarcinoma.[7] It has been noted that diffuse-type adenocarcinoma is more commonly diagnosed in females and young patients while intestinal type is associated with *H. Pylori* infection and intestinal metaplasia.[8,9]

Gastric cancer may be treated with radio- and/or chemotherapy, although surgical resection forms the cornerstone of the treatment. Despite the development of modern medicine, surgical or endoscopic intervention remains the only cure for the disease since the sensitivity to the oncologic treatment varies among the patients. This creates a real challenge for physicians to determine the optimal individually tailored treatment plan for each patient.[9] Adjuvant chemo- and radiotherapy has shown no evidence of increasing the overall survival (OS) rate following resection if used alone. However, chemotherapy may enhance the quality of life when compared to best supportive care.[10]

In the microenvironment of solid tumors, macrophages are the most abundant immune cells. Their presence correlates with worse prognosis in several cancers. Tumor-associated macrophages (TAMs) promote tumor progression, invasion and angiogenesis by secreting inflammatory substrates, cytokines, growth factors and proteolytic enzymes. In addition, they suppress the host immune response and engage in the activation of growth enhancing signaling pathways in tumor cells.[11]

Macrophages and cancer

A crucial part of our immune system is maintained by tissue macrophages which arise from monocytes circulating in the blood. Connective tissue is rich in macrophages, especially the mucosal layer of the gastrointestinal tract. Macrophages have an important effect on apoptotic cell destruction, vessel formation and inflammation development.[12]

In response to environmental signals, such as interferon gamma (IFN-γ) and lipopolysaccharide (LPS), macrophages may polarize into two main types: M1 and M2 macrophages (Fig. 1). M1-type macrophages have an important role in antitumoral immunity and inflammatory response. They inhibit tumor growth by producing pro-inflammatory cytokines such as IL-6, IL-12, IL-8 and tumor necrosis factor alpha (TNF-α).[13]

In addition, M1 macrophages express major histocompatibility class one and two complexes that are required in presenting tumor-specific antigens.[14] M2-type macrophages act as anti-inflammatory agents by suppressing host immune response. They also have an impact in tumor matrix remodeling which promotes tumor proliferation and invasion. In addition, M2-type macrophages advance the tumor progression by producing anti-inflammatory agents such as IL-10, IL-4 and IL-13.[15] In contrast to M1-type macrophages, TAMs are referred to as type M2 macrophages that are maturated in tumor-tissue environment after leaving the blood circulation as monocytes. They may have an effect on the inactivation of T-cells, which crucially decreases the body's ability to resist cancer development and progression.[16] It has been shown that the patients with a large number of TAMs in cancer tissue have worse surgical outcome compared to the patients with a lower number of TAMs. TAMs also promote tumor angiogenesis, enhance metastatic spreading and contribute to invasion by producing cytokines such as IL-6, IL-17, IL-23 and inhibiting cytotoxic T lymphocyte responses.[17]

Infiltration of TAMs correlates with tumor invasion and metastasis. Tumor cells secrete the colony stimulating factor 1 (CSF-1), while TAMs secrete epithelial growth factor (EGF). They have inducing effect in co-migration and invasion of both cell types towards blood vessels.[18] *In vitro*, distant metastasis macrophages increase the invasion rate compared to non-metastatic cells. The migration rate increases significantly when macrophage-containing cell line is cultured under hypoxic conditions. Hypoxia increased the expression of disintegrin and metalloproteinase domain-containing protein 8 & 9 coding genes (*ADAM8 & ADAM9*), while it decreased expression of matrix metallopeptidase 9 (*MMP9*) and tissue inhibitor of metalloproteinase 3 (*TIMP3*). ADAMs and *TIMP3* gene together with *MMP9* may contribute to the TAMs role in gastric cancer's fast and aggressive invasion.[19]

Based on previous studies, the development of new cancer treatments targeting tumor promoting macrophages is possible. Researchers are optimistic that by therapeutic manipulation, the M2 macrophages can be reprogrammed into M1 macrophages that inhibit the tumor growth and proliferation.[20] Manipulation of M2 macrophages is done by interfering TAM specific signaling pathway that controls the shift between tumor-promoting and tumor-preventing macrophages.[21] There are also potential treatment possibilities that inhibit the TAM accumulation in tumor
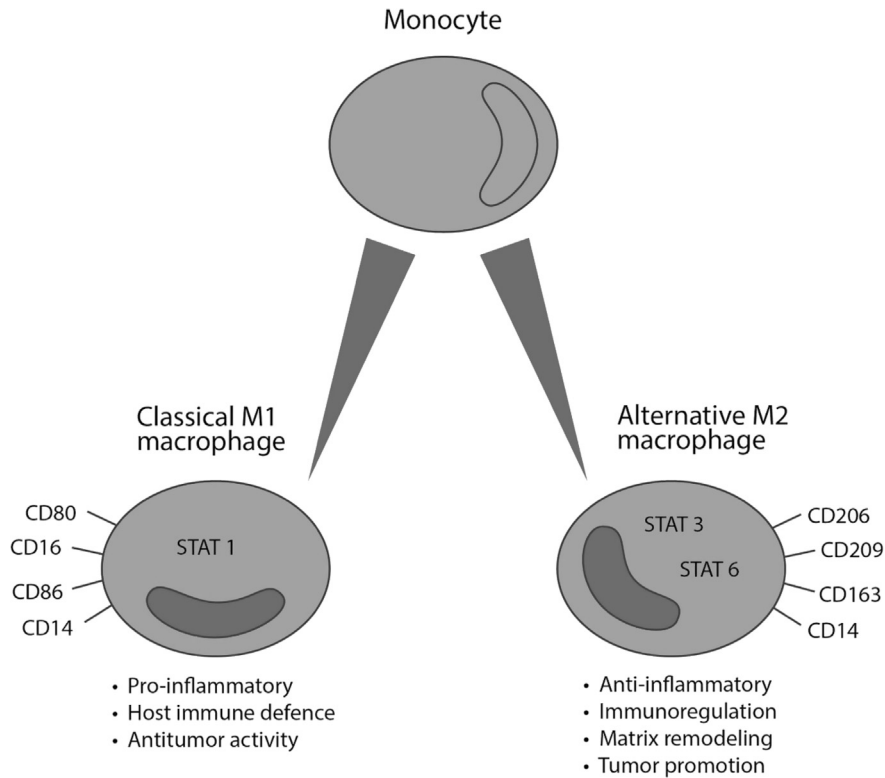
Fig. 1. Macrophage polarization. CD: cluster of differentiation; STAT: signal transducer and activator of transcription.

stroma and, thus, have an inhibiting influence in tumor progression.[22]

TAM as a potential biomarker for gastric cancer

TAMs have shown a great potential as diagnostic biomarkers in multiple myeloma, breast cancer, prostate cancer and pancreatic cancer. Further, as a prognostic biomarker, TAMs have shown potential in lung cancer, esophageal squamous cell carcinoma and bladder cancer.[23]

Leukocytes infiltrate commonly solid tumors, indicating that the tumor has triggered the host immune response. This is mediated by tumor antigens that distinguish tumor cells from normal healthy cells.[24] These leukocytes include a variety of different subsets and the activation and the complexity of these cells vary by location and by the malignancy stage of the tumor. Usually, TAMs express cluster of differentiation (CD) 163, CD204 or CD206 receptors on their surface, are nonspecific for different types of cancer and could possibly be used as a screening method for several advanced cancers in clinical practice.[25] Current understanding on TAMs as a potential biomarker for gastric cancer is summarized in Table 1.[26–44]

Tumor cell/tissue protein markers

Thymidine phosphorylase (TP) is an enzyme that promotes angiogenesis, metastasis and invasion of various tumors. TP persuades oxidative stress and inflammation signals, taking part in producing reactive oxygen species by 2-deoxy-D-ribose. They influence vascular endothelial cell growth and cytokine production.[26] Angiogenesis, invasion and metastasis of gastric cancers are correlated with production of TP in tumor stroma. Highly differentiated gastric adenocarcinomas expressed higher level of TP than poorly differentiated cancers.[27] TP is found to be expressed in both tumor cells and macrophages in the tumor stroma of the gastric cancer. Microvessel density correlated with expression of TP in infiltrating macrophages/TAMs in both intestinal and diffuse type gastric cancer. This suggests that TP might be a potential biomarker for detecting macrophage infiltration in gastric cancer as well as predicting the possible lymph node and liver metastases in both diffuse and intestinal types of gastric adenocarcinoma. There is a significant association between macrophage infiltration in patients with intestinal type of gastric cancer and the poorer OS rate. Increased tumor-infiltration of TP expressing

Table 1
Potential biomarkers for gastric cancer prognosis.

Marker	Reference	Source	Elevated amount predicts
TAM infiltration (CD163^{+})	35	Tumor tissue	Poor prognosis
TAM density in solid tumors	36,40	Tumor tissue	Poor prognosis
Macrophage M2 infiltration	40	Tumor tissue	Poor prognosis
Macrophage M1 infiltration	40	Tumor tissue	Improved prognosis
TP	26—28	Tumor stroma	Poor prognosis
Diametrically polarized tumor-associated macrophages (protumoral M2 macrophages)	38	Tumor tissue	Poor prognosis
Tumor infiltrating antigen CD11^{+}	37	Tumor tissue	Poor prognosis
Serum macrophage MIF + CEA	41—43	Serum/tumor tissue	Poor prognosis
OPN	29	Tumor stroma	Poor prognosis
KRS	34	Tumor tissue/tumor-associated inflammatory cells	Poor prognosis
MR	31	Tumor tissue	Poor prognosis
CCL5/RANTES	32,33	Tumor cells, macrophages, T cells	Increased tumor invasion Increased incidence of lymph node metastasis
NF-κB CCL2/CCR2 chemokines	44	Gene allele rs230510 and CCL2 rs4586	A allele of rs230510 associated with improved OS; T allele of rs4586 associated with poor OS
Gastric adenoma, CD204 positive TAMs	39	Tissue stroma	Risk-factor for developing gastric adenocarcinoma
Tim-3	30	Macrophages	Increased tumor invasion; increased incidence of lymph node metastasis & advanced clinical stage

TAM: tumor-associated macrophage; CD: cluster of differentiation; TP: thymidine phosphorylase; MIF: macrophage migration-inhibitory factor; CEA: carcinoembryonic antigen; OPN: osteopontin; KRS: lysyl-tRNA synthetase; MR: mannose receptor; CCL: chemokine (C—C motif) ligand; RANTES: regulated upon activation, normal T-cell expressed and presumably secreted; NF-κB: nuclear factor-kappa B; CCR: C—C chemokine receptor; OS: overall survival; Tim-3: T-cell immunoglobulin and mucin domain-containing molecule-3.

macrophages suggests increased tumor angiogenesis leading to poorer prognosis. In addition, the macrophage infiltration rate correlates jointly with TP expression and angiogenesis of the tumor.[28]

Lin et al[29] studied osteopontin (OPN) and its role in macrophage recruitment in gastric cancer. Osteopontin is an extracellular matrix protein which is involved in many normal physiological processes. It promotes cell-mediated immune responses, acts as a cytokine and controls cell migration. Information was collected from 170 gastric cancer specimens and amounts of OPNs and TAMs were measured. This study indicated that OPNs skew macrophages to M2-TAM form. Co-expression of OPNs and CD204 in TAMs correlated with disease progression and poor 5-year survival (48.90%, $P = 0.0131$). This suggests the possible use of OPN and TAM amounts as biomarkers in gastric cancer.

New data regarding gastric cancer progression have also emerged from T-cell immunoglobulin and mucin domain-containing molecule-3 (Tim-3) expressed in macrophages, and its effect on the cancer immune system. The function of Tim-3 in macrophages and monocytes remains unclear. The gastric cancer patients showed upregulation of Tim-3 in monocytes compared to healthy controls. Upregulated Tim-3 is associated with lymph node metastasis, depth of tumor invasion and advanced clinical stages which suggest that Tim-3 might have an important effect in gastric cancer progression.[30]

Mannose receptor (MR) can be primarily found on the surface of macrophages where it acts as an immune adhesion molecule. It has an important function in phagocytosis and endocytosis. Tumor microenvironment expresses noticeably high amounts of MRs. Elevated amount of MR in gastric cancer cells correlated with tumor size, T-stage and N-stage. It was also noted that an elevated number of MRs were associated with shorter survival of the patients compared to patients with a lower number of MRs.[31]

Chemokine (C—C motif) ligand 5/regulated upon activation, normal T-cell expressed and presumably secreted (CCL5/RANTES) is a protein belonging to the chemokine family and is mainly expressed in T cells, macrophages and some tumor cells. It plays an important role in recruiting other leukocytes to the site

of inflammation and together with cytokines induces proliferation and activation of natural killer cells (NK-cells) to form chemokine-activated killer (CHAK) cells.[32] Gastric cancer tissues expressed highly elevated values of CCL5. Further, high values were associated with depth of tumor invasion, lymph node metastasis, advanced TNM stage and poor tumor differentiation. Serum CCL5 values of gastric cancer patients were also elevated in comparison to healthy individuals. CCL5 secreted by TAMs may have a promoting effect in tumor invasion, proliferation and metastasis of gastric cancer cells. Correlation studies suggested that CCL5 could be an important molecular marker for gastric cancer staging and disease progression.[33]

Lysyl-tRNA synthetase (KRS) is an aminoacyl-tRNA synthetase essential for protein synthesis. An abnormal amount of KRS is associated with many types of cancer. 43.3% of studied gastric carcinomas expressed high amounts of KRSs. Tumor-associated inflammatory cells (including macrophages and monocytes) expressed high amounts of KRSs in 37.2% of cases. There is an association between expression of KRSs in tumor or tumor-associated inflammatory cells and known clinicopathological parameters for prognosis of gastric cancer and therefore KRS may be an independent prognostic marker for gastric cancer.[34]

TAM-cell infiltration

Infiltration of TAMs (CD163^{+}) differs essentially when tumor tissue is compared to normal one. In a study of 178 gastric cancer patients, the tumor tissue expressed high CD163^{+} infiltration rates in 52.8% of cases. Normal tissue expressed high amounts of CD163^{+} only in 21.3% of cases. Patients with high expression of CD163^{+} had a shorter OS time (28.00 ± 2.20 months) compared to the ones with low expression of CD163^{+} (46.00 ± 3.05 months, $P < 0.001$). Study suggests that the high expression of CD163^{+} together with transforming growth factor beta (TGF-β) is associated with aggressive features of the cancer leading to poor prognosis, and therefore, could be used as independent prognostic factors in gastric cancer.[35] A recent meta-analysis showed that the OS was significantly decreased with solid tumor patients who had a high density of TAMs ($RR = 1.64$, 95% CI: $1.24–2.16$). This suggests that TAM infiltration is associated with worsened prognosis in gastric cancer patients. It was also noted that the increased numbers of M1 macrophages may be associated with better OS among gastric cancer patients.[36]

One possible independent prognostic factor is tumor-infiltrating CD11b^{+} antigen (glycoprotein) presenting cells that include TAMs and dendritic cells. They act as important factors in antitumor immune response. Factors including a massive size of a tumor, venous invasion and lymph node metastases correlated with intratumoral infiltration of CD11b^{+} cells, where gastric cancer patients with a high amount of CD11b^{+} cells had a poor survival rate compared to the patients with a low amount of CD11b^{+} cells.[37]

Diametrically polarized TAMs in this case protumoral M2 macrophages, have been proven to have an impact on OS of surgically resected gastric cancer patients. It has been proposed that they influence tumor phenotype alteration, which supports the fact that tumor microenvironment has a crucial impact in tumor biology. Lymph node metastases correlated with infiltration rate of polarized TAMs in gastric cancer. TAMs could be combined with the TNM stage to refine a risk stratification system for prognosis in patients with gastric cancer.[38]

Recently there have been findings on the role of gastric adenomas in the development of gastric cancer. A higher amount of CD204 positive TAMs in stroma was found to be a risk factor for an adenoma developing into a gastric adenocarcinoma. This suggests that there might be a potential way to screen high-risk patients who may develop gastric adenocarcinoma from gastric adenoma.[39]

In a study of 143 cases of microsatellite instability-high (MSI-high) advanced gastric cancer, the low density of CD68^{+} and CD163^{+} TAMs suggested intestinal type tumor in Lauren's classification. CD163^{+} receptor was used to distinguish M2 type macrophages from other subsets. Infiltration of other lymphocytes also correlated with density of CD163^{+} TAMs. Additionally, the poor disease-free survival was associated with low amounts of CD163^{+} TAMs. There is an interaction between TAMs and tumor infiltrating lymphocytes (TILs). Prognosis can be assessed based on the balance of these two.[45]

Another meta-analysis about the significance of TAMs in gastric cancer was made based on twelve studies that included all together 1388 patients. Shorter OS correlated with total increase of TAM infiltration ($HR = 1.70$; 95% CI: $1.39–2.09$; $P < 0.001$). Similar effect was observed concerning M2 macrophages infiltration ($HR = 1.71$; 95% CI: $1.19–2.45$; $P = 0.004$). Moreover, an elevated amount of M1 macrophages was associated with better OS ($HR = 0.46$; 95% CI: $0.33–0.65$; $P < 0.001$). This

suggests that the total TAMs and infiltrating M2 macrophages might be a negative prognostic factor for patients with gastric cancer while increased density of M1 macrophages predicted better OS. Analysis also showed a correlation between high TAM amount and decreased risk for lymph node metastasis in gastric and ovary cancers. This indicated that patients with high density of TAMs have lower probability to have lymph node metastasis. Even so, a significant negative impact was seen in OS. To clarify this ambivalence, more studies are still needed.[40]

Serum enzyme marker

Serum macrophage migration-inhibitory factor (MIF) has shown a great potential in diagnosing gastric cancer in patients with dyspepsia. MIF is a proinflammatory cytokine that takes part in innate immune response by influencing macrophage.[41] Serum MIF level increased with advancing gastric pathologies ($P < 0.001$). High serum MIF values (above 6600 pg/ml) indicated lower 5-year survival rate among gastric cancer patients. High level of carcinoembryonic antigen (CEA) also predicted worse survival and by combining MIF and CEA it was possible to make more exact prediction of the 5-year survival.[42] Under lipopolysaccharide (LPS) stimulation, MIF, CD74 and toll-like receptor 4 (TLR4) could form a complex that significantly stimulates cell proliferation. Upregulation of these three molecules is associated with increasing clinical stage in gastric cancer.[43] Expression of MIF was higher in gastric cancer tissues compared to adjacent non-cancer normal tissues ($P < 0.001$). A high amount of MIF is significantly associated with lymph node metastasis, poor tumor differentiation, advanced tumor stage and poor survival ($P < 0.05$ for all).[41]

Gene related markers

One interesting point in gastric cancer research is the importance of genes coding for macrophage function. Nuclear factor-kappa B (NF-κB) and CCL2/C−C chemokine receptor 2 (CCR2) chemokines have a role in tumor progression, angiogenesis, invasion and migration. The nuclear factor-κB p50 (NFKB1) allele rs230510 and CCL2 rs4586 were significantly associated with clinical outcome in patients with locoregional gastric cancer. This suggests that the genetic propensity of the host has an important impact in determining the auto-immune component of the tumor for the progression of gastric cancer.[44]

Summary

Being one of the most lethal types of cancer, gastric cancer has also shown to have the capability to be very resistant to several oncologic treatments. Early-stage cancers show no symptoms and the diagnosis is usually made at an advanced stage when the tumor starts to cause symptoms. At an advanced stage, spreading of the tumor is fast and the effect of the cancer therapy is limited. Therefore, it is important to find biomarkers for early diagnosis enabling better treatment planning, decreasing the mortality and aiming physicians to make more specific prognosis for the patient.[46]

TAMs have shown great potential as biomarkers for evaluating the gastric cancer staging and progression. In addition to gastric adenocarcinoma, it may also be a useful biomarker in several other cancers, such as lung cancer, esophageal squamous cell carcinoma and bladder cancer. Some potential gastric cancer-biomarkers have already been identified including TP, OPN and MR. Studies have shown that the amount of TAMs in tumor stroma predicts the size, stage and the metastasis of the gastric tumor. This ensures more accurate prognosis and individual treatment planning for patients with gastric cancer. Patients with higher amounts of TAMs have shorter OS rate compared to those with lower amounts of TAMs. TAMs may, thus, provide a work tool for risk-patient screening, early diagnosis and prognosis formation.

Perspectives and future directions

According to some studies, the division between M1/M2 macrophages is excessively simplistic for TAM classification since it does not take into account many factors affecting TAMs function including the location of the tumor microenvironment, type of the cancer and stage of the tumor. TAMs have also been noticed to express features from both macrophage types, and because of these overlapping features the division seems old-fashioned. It is challenging to identify different subgroups of macrophages due to functional and phenotypical heterogeneity. This suggests that more precise classification is needed in order to tell apart different subtypes of TAMs.[47]

Several reviews do not recognize macrophages as clinically significant biomarkers for gastric cancer due to the fact that many recently studied biomarkers tend to show low sensitivity when tested on a large population. Many biomarkers, especially genetic markers, have been tested by very controlled parameters, and since in gastric cancer the environmental and ethnic

factors have a significant influence, it is a real challenge to find both a very specific and an unbiased marker.[48] Nonetheless, many studies have shown that the TAMs are associated with worse prognosis among gastric cancer patients.

There is still a lot to learn about the macrophage metabolism and how they react to the signals they are getting from the tumor microenvironment. TAMs have shown a great potential in formation of more individual prognosis for gastric adenocarcinoma patients. In addition, they have also shown promising results in prediction of tumor size, depth of invasion and lymph node metastasis. More studies are still needed in order to evaluate whether the TAM could be used as a biomarker for gastric cancer in clinical use.

Conflicts of interest

The authors declare no conflict of interest.

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