REVIEW ARTICLE

How immunotherapy and targeted therapy are changing gastrointestinal cancer treatment

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Abstract

Tumor angiogenesis is a critical process that enables the progression and metastasis of solid tumors, including gastrointestinal cancer. The microenvironment of gastric cancer is characterized by hypoxia, which suppresses the ability of the immune system to fight cancer. Existing treatment regimens do not address this complication and consequently do not result in objective tumor shrinkage. Accordingly, new treatment strategies are urgently needed for gastric cancer. Targeted therapies and immunotherapy for some patients with advanced gastrointestinal cancer are new approaches to this difficult-to-treat cancer, which has not benefited from substantial therapeutic advances in recent years. We propose a new treatment strategy based on anti-angiogenic therapy for gastric cancer. Optimized anti-angiogenic therapy may relieve hypoxia and improve drug delivery, which would improve the anti-tumor immune response. In addition, we focus on the potential benefits of a combined approach using immune therapy and treatments designed for vascular normalization. This review emphasizes the potential for a new paradigm of immunotherapy aimed at modulating the tumor microenvironment to change clinical practice. Future research should identify patient populations that may benefit from this approach and quantify the synergistic effects of relevant therapies.

Keywords: Tumor blood vessel, Tumor microenvironment, Immunotherapy, Gastric cancer

1. Introduction

Gastrointestinal cancers, mainly esophageal, gastric, liver, pancreatic, and intestinal and colon cancer, are a group of aggressive malignancies with high cancer-related mortalities [1]. Despite considerable advances in the variety and effectiveness of therapeutics over the past few decades, treatment success is usually limited by resistance [2]. Cancers such as adenocarcinoma of the upper gastrointestinal tract are highly aggressive malignancies. In the United States, an estimated 16,910 new cases of esophageal cancer and 26,370 cases of gastric cancer (GC) were diagnosed in 2016 [3]. In particular, GC is the fifth most common cancer worldwide and the third leading cause of cancer-related deaths, with one million new patients diagnosed every year [4]. The vast majority of GC cases are adenocarcinomas [5]. Processes in the tumor microenvironment, such as abnormal angiogenesis, fibrosis, and chronic inflammation, are critical for the local progression and organ metastasis of solid tumors [6]. These processes can lead to a tumor microenvironment characterized by hypoxia, which suppresses the ability of the immune system to fight against malignant tumors. Consequently, no single chemotherapy agent or combination regimen consistently leads to objective tumor shrinkage, and novel treatment strategies for gastrointestinal cancers, including GC, are urgently needed [6].

The tumor vasculature in GC is an essential component of the tumor microenvironment, influencing tumor behavior and treatment responses. It can be specifically targeted by anti-angiogenic drugs [6-8]. Tumor blood vessels in GC are histopathologically different from normal blood vessels. They have an irregular shape, diameter, and branching patterns and cannot be classified as arterioles, venules, or capillaries [9]. Their endothelial cells are loosely interconnected with abnormal pericytes that are responsible for leakage. When compared to normal blood vessels, tumor vessels appear immature and incomplete. Tumor angiogenesis is not only dependent on endothelial cell invasion and proliferation, but also requires the pericyte coverage of vascular sprouts for the stabilization and maturation of vascular walls. This phenotype might be associated with structural aberrations in the basement membrane [10].

Aberrant GC tumor vasculature may be attributed to the tumor microenvironment [11]. These abnormalities may contribute to the development of tumor resistance to conventional chemo-, radio-, and immune-based therapies. Dr. Rakesh K. Jain proposed that an appropriate anti-angiogenic treatment could lead to the normalization of the tumor vasculature by reducing vascular permeability and interstitial fluid pressure, thus improving blood flow and tumor perfusion [12,13]. A normalized vasculature can reduce hypoxia and enhance the delivery of oxygen and cytotoxic agents for radiation therapy and for an improved anti-tumor immune response [14]. Preclinical and clinical studies in GC have supported the hypothesis that anti-angiogenic therapy can normalize the tumor vasculature, at least transiently [6].

Importantly, gastric tumor cells do not act

alone. Malignant tumors build a complex multifaceted relationship with the organ environment. Simply making them accessible may not be sufficient to produce a response to treatment. In particular, the immune response of the host is critical for the success of an immunotherapy regimen, such as immuno-checkpoint inhibition [6]. However, the determinants of the response are not completely understood. Tumor infiltration by immune cells, such as cytotoxic T-lymphocytes, varies widely with respect to density, composition, and clinical significance [15].

The blood vascular and lymphatic endothelial cells play important roles in immune cell trafficking, controlling the microenvironment, and modulating the immune response. Recently, Tian et al. reported that T-cells support vascular normalization, highlighting the intertwined roles of blood vessels and T-cells in cancer [16]. Improving access to malignant tumors by vascular alterations with anti-angiogenic drugs may provide an effective combinatorial strategy for immunotherapy and might be widely applicable to various tumor types, especially GC [17]. Moving forward, these insights may be useful for the development of new approaches, e.g., by combining anti-angiogenic agents with immune-checkpoint inhibitors, to improve the overall survival of patients malignant GC. In particular, we describe the roles of tumor angiogenesis and immune checkpoints in cancer, followed by a summary of recent therapeutic approaches targeting these factors and an overview of our proposed strategy focused on vascular normalization for improved immune responses.

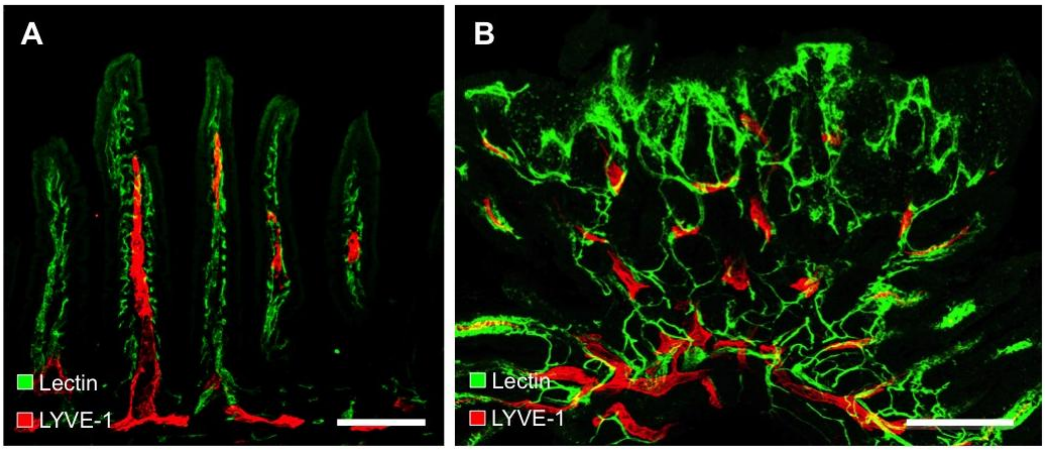

Figure 1. Relationship between tumor progression and local microvascular changes in *Apc*$^{Min/+}$ mice.

A: Double immunostaining for tomato lectin and LYVE-1 in blood vessels and lymphatic vessels in the normal small intestine (*left*). Double immunostaining for tomato lectin and LYVE-1 in tumor vessels and tumor lymphatic vessels in intestinal polyps from *Apc*$^{Min/+}$ mice (*right*). The blood vessels and lymphatic vessels in adenocarcinoma lack the vascular hierarchy (*right*). Scale bar, 150 μm.

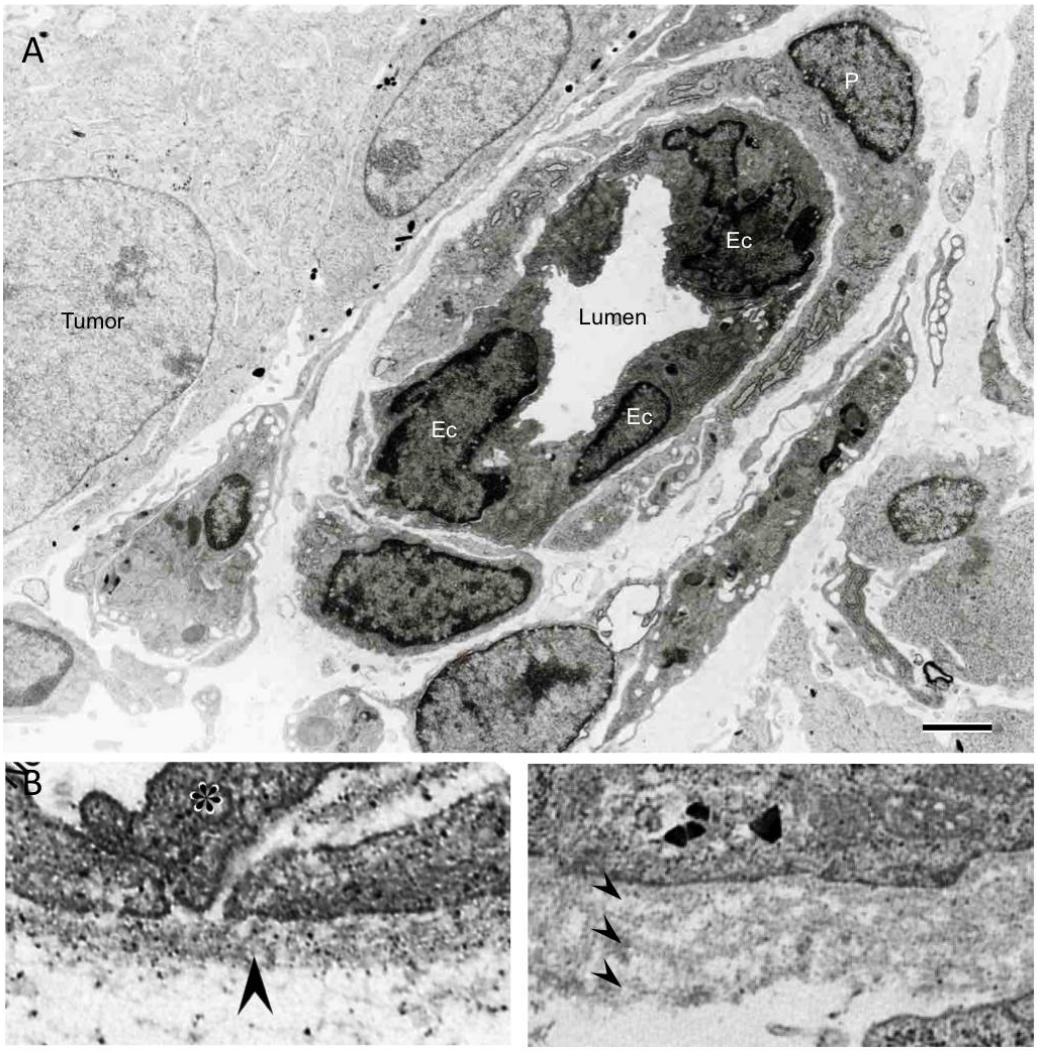

Figure 2. Electron micrograph of adenocarcinoma showing morphological changes in tumor blood vessels in gastrointestinal cancer.

A: Ultra-thin sections showing tumor blood vessel abnormalities in a mouse intestinal tumor. Tumor vessel irregularities included basement membranes. Scale bars, 1 μm.

B, C: Ultra-thin sections showing morphological changes in the basement membrane. Note the multi-layered basement membranes in adenocarcinoma. Asterisks, endothelial cells; arrowheads, a layer of the basement membrane.

2.1. Tumor blood vessels can affect the tumor microenvironment.

Angiogenesis within the tumor is a vital process in the progression and metastasis of solid benign and malignant tumors. In addition, it can lead to abnormal leaky blood vessels. Tumor vessels can create and control abnormal tumor microenvironments that are histopathologically distinct from normal vessels (Figure 1: *Apc*$^{Min/+}$ mice spontaneously develop multiple intestinal adenomas that clinically mimic

those observed in patients with familial adenomatous polyposis and undergo early transformation into adenocarcinomas) [18]. Electron microscopy revealed that most tumor vessels in adenocarcinoma (gastrointestinal cancer) have atypical diameters. In addition, tumor endothelial cells have loose interconnections, intercellular openings, and abnormal pericytes, which likely contribute to vessel leakiness. Moreover, structural abnormalities in the basement membrane of tumor blood vessels are also responsible for their relative immaturity compared to normal blood vessels. Accordingly, a tumor blood vessel has abnormal blood flow and is excessively leaky (Figure 2). Insufficient blood flow to the tumor tissue leads to hypovascular areas, severe hypoxia, and necrosis.

Although GC is a highly angiogenic cancer, it is characterized by hypoxia [6]. Hypoxia may promote GC growth and progression as well as resistance to existing therapies. Conversely, inducing vessel normalization and alleviating hypoxia might delay gastrointestinal cancer progression and metastasis. VEGF is a key factor for the abnormal structure and function of tumor vessels [18]. One of the cues driving evasive resistance is increased hypoxia induced by anti-VEGF treatment. Strong inhibition of the VEGF pathway results in pruning of the tumor vasculature, which induces hypoxia in malignant tumors [19]. Tumor hypoxia leads to HIF1α stabilization, which induces hypoxia-responsive genes, including *VEGF*. Thus, VEGF treatment leads to a harmful cycle, resulting in evasive resistance. We recently found that the standard treatment dose of an anti-VEGF agent could induce hypoxia in liver cancer, which results in infiltration by immunosuppressive leukocytes [19,20]. Based on these results, we hypothesize that dose titration of selective anti-angiogenic agents is warranted to optimize treatment and elicit anti-tumor immunity in GC.

2.2. Why target immune checkpoints in gastrointestinal cancer?

Recently, cancer immunotherapy has radically transformed clinical oncology by substantially improving outcomes in certain advanced malignant cases [1,21]. However, in most patients, the immune-suppressive microenvironment interferes with the development of an appropriate anti-tumor immune response. Co-inhibitory antigen presentation signals, called immune checkpoints, are often activated in the malignant tumor tissue, which results in the evasion of host immunity [22]. Based on the success of blocking PD-1/PD-L1 in malignant melanoma, targeting an immune-checkpoint is an emerging strategy for malignant cancer therapy [23]. Anti-immune-checkpoint therapy is ideal for patients with GC for at least three reasons. First, GC is immunogenic, but the immune response is suppressed by multiple mechanisms. This suggests that an anti-immune-checkpoint blockade could be effective [4]. Second, GC is characterized by vascular abnormalities that lead to hypoxia, fibrosis, and immune suppression. Modulating these processes by anti-angiogenic therapy could potentially shift the tumor microenvironment toward the promotion of an anti-tumor response. Finally, GC is particularly prone

to chronic inflammation, such as gastritis and chronic inflammation disease, which can create an immunosuppressive micro-environment.

Despite its wide use in clinical settings, chemotherapy has limited efficacy and high toxicity. Clearly, new systemic treatment approaches that are more efficacious and less toxic—such as low-dose anti-angiogenic therapy combined with immunotherapy—are desperately needed. Our previous data have shown that the inhibition of a tumor angiogenesis factor (Vasohibin2) could improve chronic inflammation in adenocarcinoma of intestinal cancer (Figure 3 & Table 1) [18,24]. These results indicate that the combination of anti-angiogenic therapy and immunotherapy (such as immune-checkpoint inhibition) is an effective approach for GC.

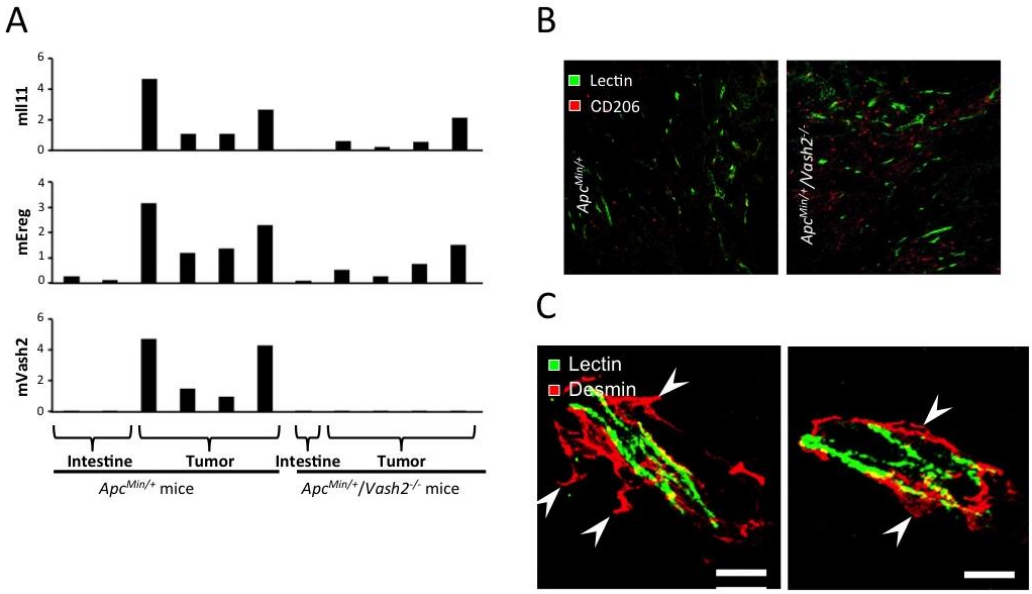

Figure 3. Inhibition of the pro-tumor angiogenesis factor vasohibin2 changes the tumor microenvironment.

A: Normal intestinal tissue and intestinal polyps were examined for *IL11*, *Ereg.* and *Vash2* expression by qRT-PCR. n = 3. A microarray analysis (Table 1) and qRT-PCR showed that the expression levels of Epiregulin and IL-6 family members (*IL-6* and *IL-11*) were down-regulated in the tumors of $Apc^{Min/+}/Vash2^{-/-}$ mice compared with $Apc^{Min/+}$ mice.

B: Double-immunostaining of tomato lectin and CD206 for M2 macrophage detection in tumors of $Apc^{Min/+}$ mice and $Apc^{Min/+}/Vash2^{-/-}$ mice. The number of M2 macrophages tended to increase in response to vasohibin2 inhibition. Scale bar, 100 μm.

C: Double-immunostaining for CD31 and Desmin of tumor vessels in gastrointestinal tumor from $Apc^{Min/+}$ mice and $Apc^{Min/+}/Vash2^{-/-}$ mice. Note that pericytes were detached from endothelia in $Apc^{Min/+}$ mice, whereas they cover vessel walls (indicating a normalization of tumor blood vessels) in $Apc^{Min/+}/Vash2^{-/-}$ mice. Scale bar, 10 μm.

Table 1

Down-regulated genes in *APC^{min}/VASAH2$^{-/-}$*	
Il6	Mus musculus interleukin 6 (Il6), mRNA [NM_031168]
Retnlg	Mus musculus resistin like gamma (Retnlg), mRNA [NM_181596]
Cd163l1	Mus musculus CD163 molecule-like 1 (Cd163l1), mRNA [NM_172909]
Xlr3b	Mus musculus X-linked lymphocyte-regulated 3B (Xlr3b), mRNA [NM_001081643]
Il11	Mus musculus interleukin 11 (Il11), mRNA [NM_008350]
S100a8	Mus musculus S100 calcium binding protein A8 (calgranulin A) (S100a8), mRNA [NM_013650]
Igh-VJ558	M.musculus VH mRNA (VH5). [X73076]
Gm5106	PREDICTED: Mus musculus predicted gene 5106 (Gm5106), misc_RNA [XR_168418]
Pappa2	Mus musculus pappalysin 2 (Pappa2), mRNA [NM_001085376]
Tmem190	Mus musculus transmembrane protein 190 (Tmem190), mRNA [NM_030028]
Il22ra2	Mus musculus interleukin 22 receptor, alpha 2 (Il22ra2), mRNA [NM_178258]
Ereg	Mus musculus epiregulin (Ereg), mRNA [NM_007950]
Padi4	Mus musculus peptidyl arginine deiminase, type IV (Padi4), mRNA [NM_011061]
Padi4	Mus musculus peptidyl arginine deiminase, type IV (Padi4), mRNA [NM_011061]
Cxcl13	Mus musculus chemokine (C-X-C motif) ligand 13 (Cxcl13), mRNA [NM_018866]
Mmp13	Mus musculus matrix metallopeptidase 13 (Mmp13), mRNA [NM_008607]

Microarray analysis showing downregulation of genes in *Apc$^{Min/+}$/Vash2$^{-/-}$* mice compared with *Apc$^{Min/+}$* mice. Microarray analysis showed that epiregulin and the IL-6 family were downregulated in the tumors of *Apc$^{Min/+}$/Vash2$^{-/-}$* mice compared with control mice.

2.3. How does gastrointestinal cancer escape from host immunity?

The majority of gastric cancers are associated with infectious agents, including *Helicobacter pylori* and Epstein–Barr virus (EBV) [25]. Recently, The Cancer Genome Atlas (TCGA) project reported that PD-L1 expression is elevated by 15% in EBV-positive GC. Additionally, an evaluation of mRNA expression revealed elevated levels of *JAK2*, *PD-L1*, and *PD-L2* [4]. Lin et al. reported that non-Asian GC is significantly enriched in signatures related to T-cell biology, including CTLA-4 signaling. Similarly, in tissue microarray cohorts, non-Asian gastric cancers show significantly higher expression of T-cell markers and lower expression of the immunosuppressive T-regulatory (Treg) cell marker FoxP3 [26]. In lymphocyte-rich gastric carcinomas, the stroma has even been termed a "tertiary lymphoid tissue" [27]. However, GCs usually evade immune surveillance. Multiple immune-sup- pressive mechanisms have been proposed. The stomach is inherently "tolerogenic," preventing aberrant immunity in response to potential antigens absorbed by the epithelium [28]. GCs are inflammation-induced malignancies; they often occur in a diseased stomach with a background of gastritis [29]. The underlying chronic inflammation and viral infection result in an immune-suppressive environment in the stomach via the production of cytokines, including interleukin (IL)-6, IL-11, tumor necrosis factor-alpha (TNF-α), and trans-

forming growth factor-beta (TGF-β) [7,30]. Our data also showed that the regulation of vasohibin2, an angiogenesis factor, could down-regulate IL-6 and IL-11 (Table 1) [24]. Another important immune evasion mechanism in gastrointestinal cancer involves the tumor infiltration of immunosuppressive leukocytes, such as Tregs and myeloid-derived suppressor cells. The exhaustion of CD4+ T cells is another mechanism underlying immune evasion in patients with advanced cancer [31,32]. While the immune response to specific antigens is recognized by major histocompatibility receptors, co-stimulatory and co-inhi- bitory molecules regulate the intensity of the response. Immune checkpoints are co-inhibitory molecules that are physiologically expressed for the maintenance of self-tolerance [33]. In the gastrointestinal cancer microenvironment, immune-checkpoint molecules, such as CTLA-4 and PD-L1, are overexpressed and broadly induce the evasive mechanism.

2.4. Can anti-vascular therapy modulate the immune response?

Reactivation of the immune response is a key to overcoming treatment-resistant GC. Growing evidence shows that combining anti-angiogenic therapy with immunotherapy in certain contexts may improve the immune response to solid cancers [14,34]. Several studies have evaluated the change in the immune response after anti-angiogenic therapy. For example, bevacizumab enhances the proportion and function of dendritic cells (DCs) in patients with solid cancer [35]. In mouse models, an anti-VEGF antibody can enhance the number and function of DCs [36]. Using a mouse model of breast cancer, Huang et al. [37] found that when an anti-VEGFR2 neutralizing antibody was administered at a low dose, the structure of the tumor vasculature was normalized and anti-tumor immunity was promoted. Conversely, a high dose of the anti-VEGFR2 neutralizing antibody induced vascular pruning and increased hypoxia and necrosis in the center of the tumor. When they looked at infiltrating leukocytes, the number of tumor-infiltrating macrophages increased, while Gr1+ cells decreased. Interestingly, the macrophages showed a decrease in the M2 phenotype, suggesting an improvement in anti-tumor immune activity. Treg activity was also lower in the low-dose anti-VEGFR2 neutralizing antibody group than in the high-dose group. Our results for the inhibition of vasohibin2 concur with the M2 phenotype macrophage data (Figure 3A, B). Recently, Chen et al. showed that therapeutic doses of sorafenib (anti-angiogenesis) in mouse liver cancer models increase hypoxia as well as Gr-1+ cells, Tregs, and macrophages [15,19]. In addition, a low-dose anti-VEGFR2 neutralizing antibody could induce the infiltration of CD8-positive lymphocytes in mouse liver cancer. These experiments suggest a potential benefit of dose titrations of anti-vascular therapy for the immune response to GC.

2.5. Immune-checkpoint targeting has the potential to overcome the immune-suppressive tumor microenvironment

To overcome the immune-suppressive microenvironment in GC, immune-checkpoint blockade, especially anti-PD-1/

PD-L1 therapy, should be a major focus. Numerous agents targeting the PD-L1/ PD-1 checkpoint are in various phases of clinical development. However, the correlation between PD-L1 expression and the prognosis of solid tumors, such as gastrointestinal cancer, is controversial [38]. Here, we propose that future studies should investigate the value of immune-checkpoint blockade and anti-angiogenic therapy for the prediction of prognosis in gastrointestinal cancer.

PD-1 is a CD28 superfamily member that conveys co-inhibitory signals for T-cell receptors [39]. PD-1 is expressed in CD8+ T cells, Tregs, and myeloid-derived suppressor cells [40,41]. PD-1 also regulates peripheral tolerance and autoimmunity. Chronic antigen exposure leads to the over-expression of PD-1 in T cells, which induces anergy or cell exhaustion [42]. Cancer cells can evade immune surveillance by hijacking PD-L1/PD-1 signaling. By expressing PD-L1 or PD-L2, PD-1 is activated in tumor-infiltrating lymphocytes, shutting down the immune response [43]. PD-1/PD-L1 expression can be detected in clinical gastrointestinal samples and is significantly correlated with the stage of human specimens (Figure 4), local recurrence rate, and poor prognosis [4]. Kim et al. observed a high correlation between PD-L1 positivity and EBV+/ MSI-H (microsatellite instability-high), suggesting that EBV-positive gastric cancer is an additional patient population with high potential to benefit from immunotherapy, on par with MSI-H patients. They proposed that EBV should therefore be routinely tested in the clinic to identify patients with GC who may benefit from immunotherapy [44]. These data support the potential benefits of anti-PD-1/PD-L1 therapy in gastrointestinal cancer, including GC, for overcoming the immune-suppressive tumor microenvironment.

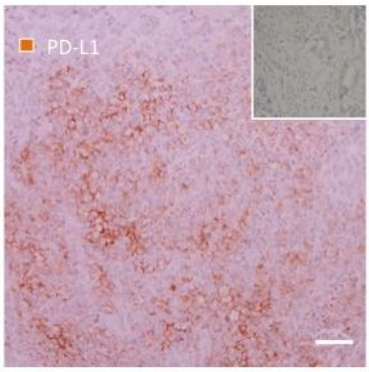

Figure 4. Tumor and immune cell expression of immune checkpoint molecules

A: Immunostaining of PD-L1 in a human pathological gastrointestinal cancer sample. A substantial number of tumor cells expressed PD-L1 in the tumor area. The inset shows a PD-L1-negative human sample. Scale bar, 100 μm.

3. Conclusion

Interactions among diverse subpopulations of malignant tumor cells create a tumor microenvironment conducive to cancer development and progression. Improving hypoxia may delay tumor progression and improve treatment outcomes. The reduction or elimination of focal hypoxia in the tumor microenvironment can minimize angiogenesis and inflammation, thereby reversing stromal heterogeneity and reducing stroma-mediated treatment resistance. With respect to cancer cells, this also reduces regional differences in selective pressures and the mutation incidence. Additionally, higher concentrations of cytotoxic therapies will reach cancer cells, further reducing the likelihood of resistance. This review has proposed vascular normalization to alleviate hypoxia with the aim of improving the outcomes of concurrent radiation, chemo-, and immune-therapy and overcoming the challenges posed by tumor heterogeneity.

Immunotherapy, which helps the immune system seek out and destroy tumor cells, has proven very effective for some patients with advanced melanoma, non-small cell lung cancer, and other cancer types. In 2017, the U.S. Food and Drug Administration approved an anti PD-1 agent for patients with certain advanced cancers of the stomach or the gastroesophageal junction, particularly for cancers that have come back or continued to grow after at least two previous treatments. The cancer cells must also test positive for the PD-L1 protein, which allows some cells to escape attack by the immune system. The FDA also approved a new lab test to detect PD-L1 and determine whether a patient is likely to benefit from immune checkpoint inhibitors. Accordingly, it is important to evaluate whether gastrointestinal cancer cells express PD-L1 or not.

Owing to the modest survival benefit of anti-angiogenic drugs, their rational combination with immunotherapy should be considered, given the potential for synergistic effects. Despite outstanding challenges, including the high costs and potential adverse effects associated with these treatment modalities, compelling evidence supports the beneficial effects of the combination of anti-angiogenic agents and immunotherapy for the modulation of both the tumor vasculature and the tumor immune microenvironment.

We therefore propose that vascular normalization can improve the immune response against GC. Research in this area will lay the groundwork for a new paradigm of immunotherapy that modulates the tumor microenvironment and could rapidly impact clinical practice [37].

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5. Conflicts of interest

The authors declare no conflicts of interest associated with this manuscript.

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