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REVIEW ARTICLE

The effect of granulocyte and granulocyte-macrophage colony stimulating factors on tumor promotion

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Summary

GM-CSF and G-CSF are capable to regulate the maturation of undifferentiated multipotent stem cells into mature granulocytes, macrophages and T cells in the bone marrow. Thereby, clinicians correct neutropenia induced by chemotherapy or radiation routinely with recombinant G- or GM-CSFs in clinical practice. However, relevant studies found that treatment for cancer patients with adjuvant G-/GM-CSF would occasionally enhance the progression of tumors. Besides, constitutive production of G-CSF or GM-CSF by lung cancer cells could stimulate the growth or the invasion of tumor and result in protecting the tumor against unfavorable environment. These findings convinced researchers that G-/GM-CSF overexpression has a positive effect on malignant tumor progression. The purpose of this article was to explore the most recent research and the mechanisms of GM-CSF and G-CSF-induced tumor promotion and their clinical therapeutic applications.

Key words: cancer, G-CSF, GM-CSF, progression, tumorigenesis

Introduction

GM-CSF and G-CSF are factors which are capable to regulate some functional cell activities, such as the proliferation and maturation of granulocytes, macrophages and their precursors [1,2]. GM-CSF, a 22-kDa glycoprotein, was first defined by its effect on the formation of granulocyte and macrophage colonies. GM-CSF can also act on relative progenitor cells and interacts with erythropoietin to stimulate eosinophil and megakaryocyte colony formation *in vitro* [2]. G-CSF, a 30-kDa glycoprotein, was first defined as a factor controlling the differentiation of granulocytes and leukocytes [3, 4]. Thereby, clinicians correct neutropenia induced by chemotherapy or radiation routinely with recombinant G- or GM-CSFs in clinical practice. Besides, G-/GM-CSF are broadly used in acute leukemia to accelerate bone marrow recovery, as well as leukocyte proliferation and maturation in solid-organ transplantation, aplastic anemia and neutropenia caused by acquired immune deficiency syndromes [5].

GM-CSF and G-CSF were first identified in the hematopoietic system. Several other types of cells, including endothelial cells, neuronal cells and fibroblasts, are also capable to produce these two cytokines [6-8]. Besides, research has shown

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that malignant cells also participate in the GM-CSF and G-CSF production [9,10]. Lahm et al. [11] revealed that human colorectal carcinoma cells secrete biologically active GM-CSF. Young et al. [12] reported that head and neck squamous cell carcinomas produce high levels of GM-CSF which can lead to immune suppressive activity. Oshika et al. [13] reported that human non-small cell lung cancer contributes to growth stimulation of GM-CSF which may promote tumor progression. Considering these discoveries, it can be assumed that GM-CSF and G-CSF are involved in the proliferation and aggravation of malignant tumors [14,15]. Several lines of observations suggest that the effect depends on the addiction of certain solid tumors to G-/GM-CSF-dependent signaling by expressing G-/GM-CSF receptors (G-/GM-CSFR) and endogenous cytokines [16,17]. Cancer patients with G-/GM-CSF(R)-positive always present an accelerated progression and advanced metastatic phenotypes compared with negative ones, which indicates that upregulated G-CSF or GM-CSF serum levels can be considered as high-sensitivity diagnostic markers [18,19]. Besides the role involved in cancer proliferation and migration, GM-CSF and G-CSF are also found to be implicated in angiogenesis and neoplastic growth, as well as stimulants of growth of endothelial cell in malignancies [20,21]. Therefore, it is imperative to explore the roles of G-CSF and GM-CSF in cancer in order to improve treatment outcomes and to better define eligible patient cohorts for antiangiogenic therapy.

In this review, we aimed to present the most recent research and the mechanisms of GM-/G-CSF-induced tumor promotion and their clinical therapeutic applications in several cancers of non-myeloid origin.

Cancer producing G-/GM-CSF

Recent research considered extracellular signal-regulated kinase (ERK) activation as a major mechanism related with G-/GM-CSF production in tumor cells [22]. ERK2 knockdown by shRNA plays a crucial and sufficient role in the elimination of tumor-derived G-CSF expression. In addition, the inhibited ERK1/2 phosphorylation followed by G-CSF mRNA expression downregulation suggests connection between ERK1/2 activation and G-CSF expression, indicating that ERK1/2 has the possibility to control G-CSF transcriptional regulation in cancers. Nevertheless, the condition of ERK1 doesn't suit this result. Therefore, ERK2 but not ERK1 is essential in the regulation of tumor-producing G-CSF transcription. These findings could support a strategy that as a useful target, ERK2 inhibitors can be of significant therapeutic value in treating G-CSF-producing cancers. In addition, ERK1/2 activation indicates that the MAPK pathway is also implicated. Hyper-activated MAPK has been identified in several cancers, and recent research aims to investigate inhibitors which can target the RAS-RAF-MEK-ERK axis to be applied in clinical oncology strategies. Notably, MEK/ERK activation can enhance the progression and invasion of malignant tumors regardless of the downregulation of G-/GM-CSF production. The phosphatidylinositol-3 kinase (PI3K)/Akt signal pathway, which is crucial in cell proliferation, apoptosis, angiogenesis, adhesion, invasion, and migration, also plays a significant role in the survival and metastasis of cancer cells [23-25]. Current research suggests that PI3K/Akt activation might accelerate the proliferation and migration of malignant tumors via an autocrine and paracrine growth loop of G-/GM-CSF. G-CSF is capable to promote tumor cells survival and proliferation via PI3K/Akt-induced suppression of caspase-9 activation. The inhibition of the PI3K/Akt/mTOR pathway would result in downregulation of G-CSF expression [26-28]. Collectively, GM-CSF and G-CSF are reported to regulate the tumor-specific phenotypes through MEK/ERK and PI3K/Akt signaling pathways.

Mechanisms

It is confirmed that GM-CSF and G-CSF make contribution in tumor progression through different mechanisms, In addition, several of adhesion molecules and other factors are related to the process. The correlated mechanism provides a therapeutic target for anti-tumor therapy. Factors correlated with the effect of G-/GM-CSF on tumor promotion are summarized in Table 1.

G-/GM-CSF induce angiogenesis

Considering the fact that blood supply is essential for malignant tumors, angiogenesis and vascularization derived from pre-existing blood vessels play a crucial role in tumor progression and metastasis. Recent research found that G-CSF and GM-CSF participate in angiogenesis and blood vessel formation to promote tumor growth [29-32]. The relationship between upregulated G-/GM-CSF level and activated ERK2 indicates the role of MAPK involved in enhanced capillaries formation and angiogenesis in malignant tumors.

Table 1. Factors correlated with the effect of G-/GM-CSF on tumor promotion

Related factors	*Functions in G-/GM-CSF-enhanced tumor promotion*	*References*
ERK	ERK2 not ERK1 is essential in the regulation of tumor-producing G-CSF transcription.	22-25
PI3K/Akt	PI3K/Akt activation accelerates the proliferation and migration of malignant tumor via an autocrine and paracrine growth loop of G-/GM-CSF. PI3K/Akt can also suppress caspase-9 activation to promote tumor cells survival.	26,27
VEGF	VEGF in upregulating EPCs ratios which play crucial roles in G-/GM-CSF-induced angiogenesis due to its potential to differentiate into mature ECs.	22,36
G-/GM-CSFR	The presence of G-/GM-CSFR enables both GM-CSF and G-CSF to enhance tumor growth, which verifies that the receptors expressing respectively in cancer cells are correlated with high functionality and efficacy in tumor promotion.	42,43
NF-kB	NF-kB is able to mediate destruction of the osteoclastic bone and growth of highly metastatic tumors by upregulating GM-CSF, as well as IL-8 and IL-6.	45-47
MMPs	The expression of MMP-26 contributes to MMP-9 activation and participates in G-/GM-CSF-induced tumorigenesis effect via processing collagen-4 and VEGF in early-invasive areas. MMP-2 is also essential factor in G-/GM-CSF-induced mobilization and maturation of cancer stem cells.	48-54
IL-2 /-12	G-/GM-CSF inhibit the expression of CD4+, CD8+, and CD16+ cells activated by IL-2 /-12, and thus suppress the production of Th cells, suppressor /cytotoxic T cells and NK cells	55
Kras	The production of GM-CSF in responding to Kras activation suppresses cellular immune function and generates a protumorigenic inflammatory microenvironment.	57,58
STAT3	The G-CSF autocrine/paracrine growth loops contribute to activating constitutive STAT3 which is significantly correlated with several malignant phenotypes in cancers.	59
TNF-α	G-CSF is found increased in a time-dependent manner after constitutive TNF-α production, indicating the involvement of tumor-producing G-CSF in TNF-α-mediated tumor promotion.	60

GM-CSF: granulocyte-macrophage colony stimulating factor, G-CSF: granulocyte colony stimulating factor, ERK: extracellular regulated protein kinases, VEGF: vascular endothelial growth factor, NF-kB: nuclear factor kB, MMP: matrix metalloproteinases, IL: interleukin, TNF-α: tumor necrosis factor-α

These findings have been confirmed by showing the effect of G-/GM-CSF on tumor growth by activating tumor angiogenesis in cancer animal models [33]. However, there is no evident showing that G-/GM-CSF have accelerating effects on the proliferation of both malignant tumor cells and endothelial cells (EC) *in vitro*, indicating that the promoting effect of G-/GM-CSF on tumor progression and angiogenesis is not dependent on the direct action with cells *in situ*. The circulating endothelial progenitor cells (EPCs) play crucial roles in G-/GM-CSF-induced angiogenesis due to their potential to differentiate into mature ECs [34]. It has been reported that EPCs are part of tumor vessels and G-/GM-CSF are able to upregulate the circulating EPCs ratio in tumor-bearing mice [35]. Thereby, G-/GM-CSF treatment upregulates circulating EPCs and thus promote the vessel formation in malignant tumors. On the contrary, G-/GM-CSF are unable to upregulate EPC ratios in mice without tumors compared to the tumor-bearing mice. This finding emphasized that some other factors are essential for G-/GM-CSF to increase EPCs proliferation. Asahara et al. discovered that VEGF participates in the mobilization of EPCs from bone marrow [36]. SU1498, a tyrosine kinase inhibitor of VEGFR-2, is reported to partially inhibit G-CSF-induced tumorigenesis effect, which plays a significant role of a certain level of VEGF in upregulating EPCs ratios in the periphery [37].

Besides the role of the circulating EPCs in tumor angiogenesis and vascularization induced by G-/GM-CSF treatment, Gr1+CD11b+ cells upregulation may also contribute to G-CSF-induced tumor angiogenesis activation. It has been reported that Gr1+CD11b+ cells are upregulated in tumor-bearing hosts along with their immunosuppressive effect [38]. Additionally, these cells are capable to differentiate into ECs in several tumors directly and thus make a contribution to tumor progression and angiogenesis. Notably, Gr1+CD11b+ cells are different from EPCs which are identified as CD11b-negative [35,39]. These findings suggest that G-/GM-CSF participate in tumor angiogenesis representing another population of cells instead of EPCs. Notably, it is reported that relatively low-dose of G-CSF is enough to promote tumor angiogenesis. Thus, G-/GM-CSF are capable to increase circulating EPCs and Gr1+CD11b+ cells, which would induce tumor

angiogenesis and contribute to the acceleration of malignant tumors growth.

G-/GM-CSF enhance tumor progression

The autocrine or paracrine growth loop for G-/GM-CSF is also crucial in mediating increasingly malignant behavior of non-hematopoietic tumor cells [40]. It is acknowledged that several types of tumor cells are found to express functional high-affinity to G-/GM-CSF receptors [41] which are capable to affect cancer cells and increase their tendency to metastasize. The upregulated G-/GM-CSF receptor expression in cancer cells suggests the relationship between tumor malignancy and these receptors. Several findings demonstrated that over half of G-/GM-CSFR universally coexpressed with its ligand G-/GM-CSF in the same malignant epithelial cells, suggesting the existence of a potential autocrine system [42]. The coexpression of G-/GM-CSFR and its ligands is likely to be correlated exclusively with angiogenic and the most aggressive malignant tumors. The presence of G-/GM-CSFR enables both GM-CSF and G-CSF to enhance tumor growth, verifying that the receptors expressed in cancer cells are correlated with high functionality and efficacy in tumor promotion. Silencing these factors with neutralizing antibodies could inhibit tumor progression, which would demonstrate that G-/GM-CSFR and its corresponding ligands are all components of the autocrine growth regulatory mechanisms. However, another third ligand of G-CSF and GM-GSF expressed in the stroma proves the presence of a potential paracrine system in which mesenchymal cells may provide the ligand to receptors expressed by malignant epithelial cells. Recent research discovered that, when comparing cancer patients with autocrine axis, patients expressing the paracrine G-/GM-CSFR loop alone always have a worse outcome [43].

In addition, some authors demonstrated that the pro-tumor effect exhibited by G-/GM-CSF may be independent of their receptors and the autocrine/paracrine growth loop [44]. The stimulation of G-/GM-CSF on tumor cells can be inhibited when given a COX-2 inhibitor, indicating that the effect of G-/GM-CSF on tumor growth is intimately correlated with COX-2 activity. GM-CSF and G-CSF regulate the expression of COX-2 and thus contribute to increased cancer growth and progression without G-/GM-CSF receptors. The IKK/NF-kB signaling pathway is also significant in G-/GM-CSF-mediated tumor progression [45]. Further assays revealed that constitutive NF-kB is able to mediate destruction of the osteoclastic bone and growth of highly metastatic tumors by upregulating GM-CSF. This data demonstrated that GM-CSF expression is associated with NF-kB activity in bone-metastatic tumor cells [48]. Inflammation and inflammatory cells are reported to be correlated with tumor microenvironment which is crucial in promoting tumor progression and metastasis. GM-CSF could recruit neutrophils and proinflammatory cytokines, as well as promote tumor cells transmigrating through the endothelial barrier [47]. Thus, it is possible that GM-CSF collaborate with several proinflammatory cytokines including IL-8 and IL-6, which can be regulated by NF-kB to contribute to osteoclast differentiation stimulation and tumor migration regulation.

MMP is correlated with G-/GM-CSF-induced tumor promotion

Besides direct stimulation of cancer cells, G-/GM-CSF are capable to enhance invasive behavior of cancer cells via increasing the expression of matrix metalloproteinases (MMPs), including pro- and active-MMP-2 and -9 [48]. It is verified that MMP-2 and -9 overexpression, as well as the consequent enhanced proteolytic activity, can be revertible to GM-CSF blocking antibodies *in vitro*. In several tumors, MMP-2 and MMP-9 are identified as pro-tumorigenic MMPs which are correlated with lymph node metastasis and poor outcome [49]. MMP-2 and MMP-9 exist in a specific class of proteolytic enzymes which play significant roles in the degradation of the extracellular matrix molecules including native collagen-4 and -5, which represent major basement membrane components [50]. Considering that collagen-4 is one of the integral components of bone marrow, MMP-2 and MMP-9 are both considered as essential factors in G-/GM-CSF-induced mobilization and maturation of stem cells. In addition, the secretion level of proteinases is positively correlated with tumor progression and migration ability. Secreting MMPs into the surrounding microenvironment is important to promote ECM degradation and consequently, contributes to the invasion and migration of tumor cells [51,52]. MMP-26 is also capable to cleave collagen-4, fibronectin, gelatin and fibrinogen significantly, as well as activate the pro-MMP-9 to become mature [53]. Consequently, the expression of MMP-26 contributes to MMP-9 activation and participates in the G-/GM-CSF-induced tumorigenesis via processing collagen-4 and VEGF in early-invasive areas. The expression

of MMPs is usually modulated by oncogenes, cytokines and tumor promoters at transcriptional level [54]. However, the regulation of these proteins and cytokines is quite discordant owing to the discrepant structures of these enzymes and their inhibitors, especially due to striking differences of their promoters. It is possible that CSFs modulate their expression to different extents. Taken together, GM-CSF is capable to drive tumor growth and invasion via promoting the expression of MMPs including MMP-2, -9 and, -26, and shed some light on the mechanisms of G-/GM-CSF-induced tumor promotion effect in cancer patients.

Other mechanisms

It is acknowledged that antitumor immune response represents the overall activity of cytotoxic T lymphocytes (CTL), NK cells and other innate immune responses *in vivo*. IL-2 and -12 can increase the expression of almost all of the components of antitumor cellular immune response, such as CD56+ cytotoxic cells, dendritic cells, IFN-c and other cytotoxic factors [55]. The autologous tumor cell killing activities exhibited by peripheral blood mononuclear cells can also be activated and enhanced against cancer cells either. Recent findings demonstrated that G-/GM-CSF are capable to suppress the activities of NK and CTL in a dose-dependent manner, thus making a contribution in antitumor activity suppression [56]. G-/GM-CSF inhibit the expression of CD4+, CD8+, and CD16+ cells activated by IL-2 /-12, and thus suppress the production of Th cells, suppressor /cytotoxic T cells and NK cells. Considering that their receptors are expressed ubiquitously on CD16+ NK cells and CD4+/CD8+ T cells, G-/GM-CSF can generally be recognized as an immune suppressor to contaminate the innate and specific immune reactions in cancer patients. G-/GM-CSF can also suppress IFN-γ production in cells collected from normal subjects. Hence, it can be said that G-/GM-CSF are potent immune suppressors even for healthy persons and directly suppress pro-inflammatory cytokine-induced activation in T and NK cells besides the induction of immunosuppressive cytokines such as TGF-β and IL-10. Oncogenic Kras also participate in GM-CSF-induced antitumor immune response suppression and immunotherapeutic resistance of Kras-driven malignant tumors. The production of GM-CSF in cases responding to Kras activation suppresses cellular immune function and generates a pro-tumorigenic inflammatory microenvironment [57,58].

STAT activation is also found to be involved in G-CSF-induced tumorigenesis. It is acknowledged that there exist two intracellular functional domains in G-CSF receptors, one being critical for differentiation and the other for proliferation [59]. Both of these two domains play crucial roles in tyrosine phosphorylation of STAT3. The constitutively-activated form of STAT3 is significantly correlated with several malignant phenotypes in cancers. There is a growing body of evidence showing that the G-CSF autocrine/paracrine growth loops also contribute to activating constitutive STAT3 in malignant tumors. Moreover, G-CSF is found to be increased in a time-dependent manner after constitutive TNF-α production, indicating the involvement of tumor-producing G-CSF in TNF-α-mediated tumor promotion [60]. However, G-/GM-CSF have been found to exhibit a contradictory property on tumor to paradoxically inhibit its growth and development. Clinicians already utilized GM-CSF as an adjuvant therapy for irradiation or a tumor vaccine to promote the production of immune cells including dendritic cells, macrophages and inflammatory cytokines [61,62]. It will be important to determine the optimal dose to avoid the adverse effect of G-/GM-CSF overuse on malignant tumor progression.

Moreover, disruption of CD8 immunity by elements of the tumor microenvironment is thought to be a major mechanism of tumor immune escape [63]. Gr-1+ CD11b+ cells are key factors of cancer inflammation, and play a significant immunosuppressive role in downregulating T cell immune responses *in vitro* and *in vivo*, which is thought to be a barrier to immune surveillance in pancreatic ductal adenocarcinoma (PDA). Bayne et al. [64] found that in a mouse model of PDA, tumor-derived GM-CSF is necessary and sufficient for *in vitro* generation of functional, immunosuppressive Gr-1+ CD11b+ cells. Abrogation of tumor-derived GM-CSF inhibited the recruitment of Gr-1+ CD11b+ cells to the tumor microenvironment and blocked tumor development - a finding that was dependent on CD8+ T cells. In humans, PDA tumor cells prominently expressed GM-CSF *in vivo*. Thus, tumor-derived GM-CSF is an important immunosuppressive regulator within the tumor microenvironment [65-67]. These findings carry important implications for the design of novel therapies for patients with PDA and highlight the potential for disrupting the crosstalk of tumor cells with the immune system by targeting Gr-1+ CD11b+ cells or the cytokines that regulate their differentiation.

Conclusion

GM-CSF and G-CSF are factors capable to regulate the functional activities (proliferation and maturation) of granulocytes, macrophages and their precursors [1,2]. Thereby, clinicians correct neutropenia induced by chemotherapy or radiation routinely with recombinant G- or GM-CSF in clinical practice. However, relevant studies found that primary tumor irradiation and adjuvant G-/GM-CSF therapy would occasionally enhance the progression of tumors. Vemura et al. [17] indicated that constitutive production of G-CSF or GM-CSF by lung cancer cells might stimulate the growth or the invasion of tumor and result in protecting the tumor cells against unfavorable environment *in vitro* , such as serum deprivation [70]. This convinced us that G-/GM-CSF overexpression has an unwanted positive effect on malignant tumor progression. Therefore, it is imperative to explore the roles of G-CSF and GM-CSF in cancer in order to improve treatment outcomes.

The mechanisms of GM-CSF and G-CSF-induced effect on malignant tumors are various. G-/GM-CSF are capable to increase the ratio of circulating EPCs significantly via activating ERK2 and constitutively MAPK to be implicated in tumor angiogenesis, while driving tumor progression and invasion via enhancing the expression MMPs including MMP-2, -9 and, -26, as well as influencing COX-2 expression. The autocrine/paracrine loops involving G-/GM-CSF also contribute to constitutive STAT3 activation in cancer. Moreover, GM-CSF and G-CSF suppress NK and CTL activity aroused to suppress antitumor activity, as well as increase the constitutive production of TNF-α which is associated with tumorigenetic effect in various types of malignancies. Considering the upregulation of G-CSF and GM-CSF in high-grade malignant tumor cells, they can be used to diagnose malignant tumors. Notably, many findings have proved the connection between G-CSF expression and prognosis-related pathology characteristics including tumor necrosis, metastasis and reoccurrence. This was convincing enough that G-CSF is one of TNFs, as well as it can act with TNF synergistically and result in tumor necrosis, which provide the possibility for G-CSF to measure the grade of malignancy. However, G-/GM-CSF have been found to paradoxically exhibit contradictory properties on inhibiting tumor growth and development.

Radiotherapy and GM-CSF as adjuvant therapy were verified to exhibit enhanced antitumor activity on malignancies compared to radiotherapy alone, while the mechanism might consist of promoting the production of antitumor immune cells, such as dendritic cells, macrophages and inflammatory cytokines [57-60]. Thus it will be most important to determine the optimal dose of GM-CSF to avoid the adverse effect of G-/GM-CSF overuse on malignant tumor progression.

Conflict of interests

The authors declare no confict of interests.

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