The role of granulocyte colony-stimulating factor in breast cancer development: A review

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Abstract. Granulocyte-colony-stimulating factor (G-CSF) is a member of the hematopoietic growth factor family that primarily affects the neutrophil lineage. G-CSF serves as a powerful mobilizer of peripheral blood stem cells and recombinant human G-CSF (rhG-CSF) has been used to treat granulocytopenia and neutropenia after chemotherapy for cancer patients. However, recent studies have found that G-CSF plays an important role in cancer progression. G-CSF expression is increased in different types of cancer cells, such as lung cancer, gastric cancer, colorectal cancer, invasive bladder carcinoma, glioma and breast cancer. However, it is unclear whether treatment with G-CSF has an adverse effect. The current review provides an overview of G-CSF in malignant breast cancer development and the data presented in this review are expected to provide new ideas for cancer therapy.

Contents

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1. Introduction

In the 1960s, two independent groups discovered several soluble factors when measuring mouse lymphoid leukemia cell growth (1,2) and these soluble factors were named 'colony-stimulating factors' (CSFs). After isolation and purification, four CSFs were identified, including macrophage CSF (M-CSF, CSF1) (3), granulocyte-macrophage CSF (GM-CSF, CSF2) (4), granulocyte CSF (G-CSF, CSF3) (5) and multipotential CSF (also known as interleukin-3) (6). All these factors are essential stimulators of blood cell development and play a crucial role in hematopoietic stem cell proliferation and differentiation at different stages (7).

The biological effects of G-CSF are mediated by the specific G-CSF receptor (G-CSFR) (8). G-CSF is a critical regulator of neutrophil production and activity. It promotes proliferation and differentiation of the neutrophil lineage, and enhances the transition of immature metamyelocytes into mature neutrophils. G-CSF not only prolongs the survival of neutrophils and their precursors, but also promotes the functions of mature neutrophils, such as superoxide production, phagocytosis and pathogen killing (9).

G-CSF can act as a mobilizer of hematopoietic progenitor stem cells in blood donors or cancer patients (10). Therefore, recombinant human G-CSF (rhG-CSF) is commonly used to prevent and treat febrile neutropenia and mucositis after chemotherapy and radiotherapy for cancer patients (11). However, recent studies have found that G-CSF plays a crucial role in tumorigenesis. G-CSF promotes tumor growth, metastasis and chemotherapy resistance (12), inhibits tumor cell apoptosis, induces angiogenesis (13,14), participates in cancer-associated thrombosis (15,16), and is associated with a poor clinical prognosis (17).

2. Structure of the G-CSF gene

The human G-CSF gene is localized on chromosome 17q21-17q22 and spans ~2.5 kb (18). As shown in Fig. 1, the G-CSF gene consists of five exons and four introns. The promoter has 80% sequence similarity and the coding region shows 69% similarity between the human and murine G-CSF gene (19,20). The TATA box is located -29 bp upstream of

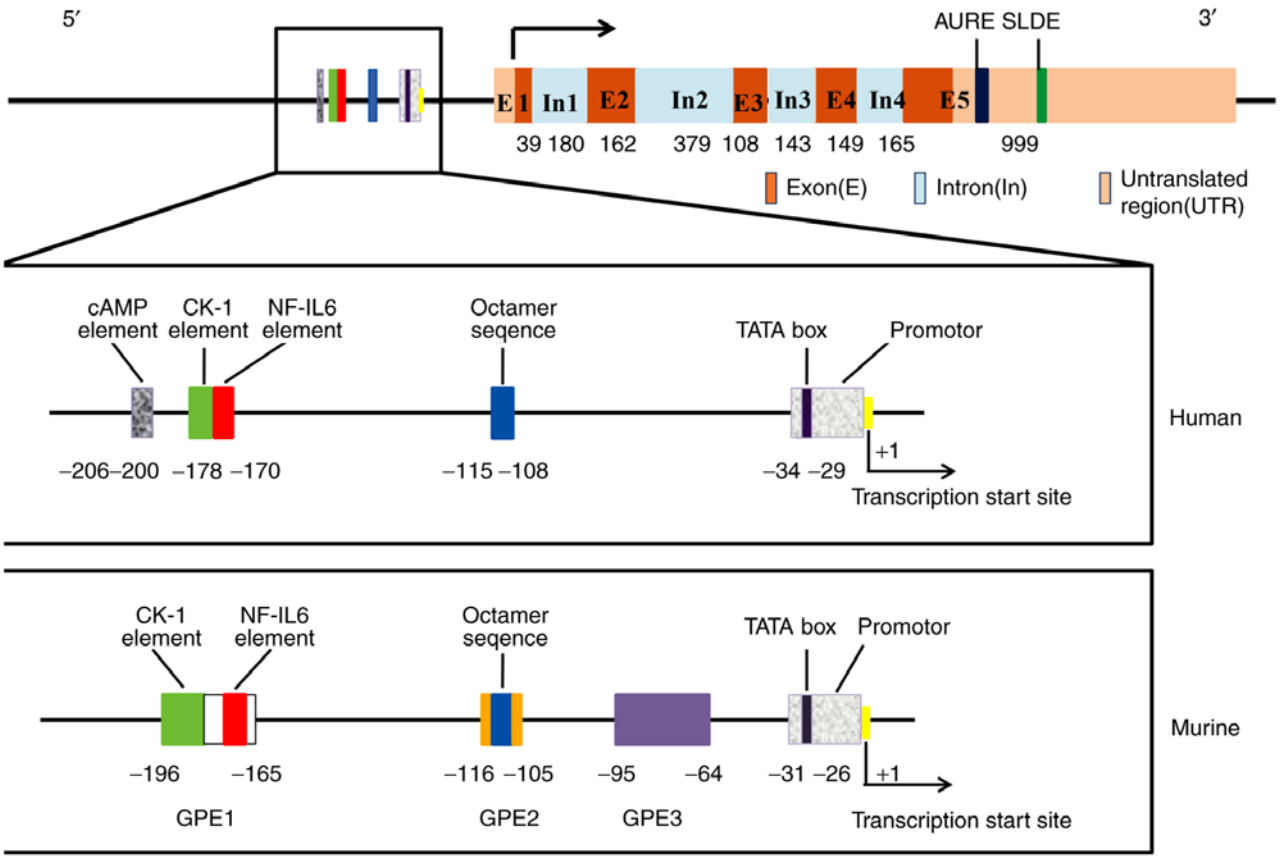

Figure 1. Structure of the G-CSF gene. The rectangular box below shows a detailed enlargement of the upstream transcriptional regulatory elements in the human and murine G-CSF gene promoter. The lengths of exons and introns are expressed in base pairs. G-CSF, granulocyte-colony stimulating factor; IL, interleukin; GPE, G-CSF promoter elements.

the transcription start site (21) and four specific regulatory elements have been identified within the promoter of the human G-CSF gene. Serving as the binding site of nuclear factor NF-GMa (20) and nuclear factor (NF)-κB (22,23), the CK-1 element (GAGATTCCA/CC) located ~200 bp upstream of the transcription start site (24) is a highly conserved sequence that is found not only in the G-CSF gene but also in the GM-CSF and interleukin (IL)-3 genes (25). The NF-IL6 consensus element (ATTNNGNAAT) at a position ranging from -178 to -170 is the binding site of the transcription factor NF-IL6, which is involved in regulating the genes activated by lipopolysaccharide (LPS) (26).The octamer (OCT) sequence (ATTTGCAT) at -115 to -108 upstream of the transcription start site is the typical OCT transcription factor (OTF) binding site (27). Shannon *et al* (28), found that the above three elements in the G-CSF promoter are essential for tumor necrosis factor (TNF)-α and IL-1β responses. The cyclic AMP-responsive element at 11 bp upstream of CK-1 is the response element of cAMP-induced G-CSF gene transcription (29). A total of three regulatory regions within the murine G-CSF gene promoter known as G-CSF promoter elements (GPEs) 1-3 are required for G-CSF gene expression (30); of these three elements, CK-1 and NF-IL6 are both in GPE1. GPE3 is a G-CSF-specific sequence and mutations in its corresponding region cause a 6- to 50-fold reduction in its activity (31). In addition, there are two destabilizing elements in the 3' untranslated region of G-CSF mRNA, including adenylate uridylate-rich element and stem-loop destabilizing element (32).

It has been acknowledged that there are two different G-CSF mRNA isoforms in humans: G-CSFa and G-CSFb. Compared with G-CSFa, G-CSFb lacks 9 base pairs (GTGAGTGAG) in the second exon (21). G-CSFa and G-CSFb mRNAs encode polypeptides that consist of 207 and 204 amino acids, respectively. After cleavage of the 30-amino acid signal peptide, mature proteins containing 177 and 174 amino acids are secreted. Arakawa *et al* (33), found that the activity of the 174-amino acid form is 50-fold higher than that of the 177-amino acid form. The secreted form of the protein was found to be O-glycosylated and to have a molecular weight of 19,600 Da (34). One O-linked glycosyl group at Thr 133 in G-CSF isolated from human blood protects the molecule from aggregation (35).

The G-CSF protein contains five cysteines and two pairs of disulfide bonds are formed between residues Cys36 and Cys42 and residues Cys74 and Cys64. The disulfide bonds play an important role in maintaining the biological functions of G-CSF. Within the G-CSF protein, 104 of the 175 residues form a total of four α-helix bundles that are designated helix A (residues 11-39), B (71-91), C (100-123) and D (143-172) (36). A study of the three-dimensional crystal structure of recombinant interferon (IFN)-β suggested that the receptor binding region of G-CSF is located on the loop connecting helix A and B and on the outer surface of helix D (37).

3. Regulation of G-CSF gene expression

Under physiological conditions, the G-CSF concentration in plasma is almost undetectable, but when an infection occurs, the G-CSF concentration is significantly increased. The number of neutrophils is dependent on the G-CSF concentration, especially during the infection process or chemotherapy use (38). G-CSF can be secreted by numerous cells, including monocytes, macrophages, endothelial cells, epithelial cells and fibroblasts, when they are stimulated by inflammatory mediators such as LPS (39), IL-17 (40), TNF-α and IFN-β (41). Moreover, some malignant cells, such as triple-negative breast cancer (17), lung carcinoma (42,43), bladder cancer (44) and squamous cell carcinoma (45), can constitutively express and secrete G-CSF.

G-CSF expression in breast cancer is under the control of various signaling pathways. It has been reported that carbonic anhydrase IX (CAIX) stimulates G-CSF production by activating NF-κB signaling in hypoxic conditions (46). Extracellular signal-regulated kinase (ERK) 2 is responsible for the transcriptional regulation of G-CSF and ERK2 knockdown by short hairpin RNA significantly inhibits the expression of tumor-derived G-CSF (47). H-Ras upregulates G-CSF expression and promotes breast epithelial MCF10A cell invasiveness (48). Protease-activated receptor (PAR) 2 stimulates G-CSF expression in breast cancer and PAR2 gene knockdown or PAR2 antagonist use can reduce G-CSF secretion (49). Carcinoembryonic antigen-related cell adhesion molecule (CEACAM) 1 expression in breast cancer MCF-7 cells inhibits G-CSF secretion by M1 macrophages (50). In addition, G-CSF is the main downstream mediator of the mammalian target of rapamycin (mTOR) pathway during the induction of myeloid-derived suppressor cell (MDSC) formation in breast cancer and Welte *et al* (51), suggested that the regulation of G-CSF by mTOR may occur at the transcriptional level. In other diseases, some factors have been shown to regulate G-CSF expression, all of which are shown in Table I.

4. The G-CSF receptor

The G-CSFR gene located on chromosome 1p35-34.3 is a member of the class I cytokine receptor superfamily (52). G-CSFR is a single transmembrane protein consisting of 813 amino acid residues, which is composed of extracellular, transmembrane and intracellular regions. Its extracellular region includes immunoglobulin-like (Ig-like) domains and cytokine receptor homology (CRH) domains, as well as three fibronectin type III domains. The intracellular region of G-CSFR protein includes two motifs called box 1 and box 2, and cytoplasmic region of G-CSFR contains four conserved tyrosine residues which function as docking sites for the phosphorylation of multiple SH2-containing signaling proteins (53). G-CSF binds to the extracellular Ig-like and CRH domains of G-CSFR, which triggers receptor homodimerization (54) and activates Janus tyrosine kinases (JAKs), leading to a cross-phosphorylation. Activated JAKs proteins can phosphorylate G-CSFR by binding to its Box 1 and 2 domains and generate potential docking sites of signal transducer and activator of transcription (STAT) protein in cytoplasm. The inactive STAT protein binds to the phosphorylated G-CSFR through its SH2 domain and phosphorylates it under the cooperation with JAKs (55). Activated STATs then form a homodimer/heterodimer and translocate into the nucleus to activate the transcription of target genes, which promote the proliferation and metastasis of cancer cells (56). Although the G-CSF-induced JAK2/STAT3 pathway has been well-established (57), previous studies also show that G-CSF can activate other downstream signaling pathways, including mitogen-activated protein kinase (MAPK)/ERK and phosphatidylinositol 3 kinase/protein kinase B (AKT) (54,58).

5. G-CSF expression in breast cancer

Some studies have reported that serum G-CSF levels are significantly higher in breast cancer patients compared with healthy controls (48,59-63). Lawicki *et al* (60), demonstrated that the plasma levels of G-CSF and M-CSF were significantly increased in 54 breast cancer patients compared with in control group patients. The authors of the present review were surprised to learn that, after surgical resection, the level of G-CSF decreased significantly, but the level of M-CSF increased, suggesting that measuring G-CSF may be useful in the diagnosis of breast cancer. Compared with 20 healthy controls, the mean level of serum G-CSF in 20 breast cancer patients was significantly increased (48). In a total of 190 samples, plasma G-CSF levels were significantly increased in samples from 110 patients with ductal breast cancer and 40 patients with benign breast cancer compared with samples from untreated healthy patients. Moreover, the serum levels of G-CSF were significantly increased in patients with clinical stage III and IV tumors compared with in healthy controls or patients with benign breast cancer (61). In addition, in a total of 196 samples, serum G-CSF levels were increased in patients with advanced breast cancer compared with in patients with early-stage cancer and the highest G-CSF levels were observed in patients with N3 tumors (62). By analyzing the wound healing fluid of breast cancer surgery patients, it was found that G-CSF, together with IL-6 and monocyte chemotactic protein (MCP)-1/CCL2, was more abundant in invasive and high-grade breast cancer than in situ breast cancer (63).

Wojtukiewicz *et al* (64), also reported that G-CSFR was expressed in only breast cancer tissues, not in normal breast tissues and its expression level was as high as 71% in the collected clinical specimens. Notably, high G-CSFR expression was observed in 21% of cases, especially in cases involving small focal invasive breast cancer. In addition, other studies have confirmed the expression of G-CSF in breast cancer tissues and its association with breast cancer invasion (17,46,65,66). Within a larger group of human breast cancer samples (n=548), triple-negative breast cancer was shown to exhibit higher G-CSF expression, which was related to cluster of differentiation (CD)163+ macrophages and associated with a poorer overall survival rate, than other types of breast cancer (17). Compared with noninvasive breast cancer T47D and MCF-7 cells, invasive MDA-MB-231 cells exhibit higher G-CSF expression (47), which suggests that the association between G-CSF expression and malignant disease should be explored.

Through The Cancer Genome Atlas data analysis, Guo *et al* (67) discovered a high-risk luminal A dominant breast

Table I. Regulation of G-CSF gene expression.

Author, year	Cell type	Producer cell and contexts	Inducer (+) / inhibitor (-)	(Refs.)
Park et al, 2011	Tumor cells	Human MCF10A cells	+ H-Ras	(48)
Lee et al, 2013		Human MDA-MB-231 breast cancer cells	+ ERK2	(47)
Chafe et al, 2015		Hypoxic breast cancer cells and tumors in an orthotopic model	+ CAIX	(46)
Carvalho *et al*, 2018		Mouse 4T1 and human MDA-MB-231 cells	+ PAR2	(49)
Welte *et al*, 2016		Mouse P53N-A and 4T1 breast cancer cells	+ mTOR	(51)
Cao *et al*, 2014		Mouse mammary tumors	- BMP4	(65)
Uemura *et al*, 2005		Human lung cancer OKa-C-1 and MI-4 cells	+ PKC inhibitor	(43)
Nakata *et al*, 2003		Human lung cancer cells	- NS-398	(100)
Cui *et al*, 2015		Human non-small-cell lung cancer cells	+ Radiation, β-catenin	(101)
Pickup *et al*, 2017		Human pancreatic ductal adenocarcinomas	- TGF-β signaling	(81)
Ramakrishna *et al*, 2018	Immune cells	Human CD11b+ macrophages and neutrophils	- IFN-γ	(102)
Chang *et al*, 2016		Human macrophages	+ SB203580	(103)
Samineni *et al*, 2013		Human breast tumor-associated macrophages	- CEACAM1	(50)
Fujimoto *et al*, 2011;		Human macrophage RAW 264 cells	- SOCS1	(104)
Kamio *et al*, 2008;			+ Adiponectin	(105)
Zhang et al, 2011			- HSF1	(106)
He *et al*, 2009;		Human monocytes and macrophages	+ SAA	(22)
Hareng *et al*, 2003			+ cAMP	(29)
Aoki *et al*, 1998;		Mouse macrophage cell line	+ Fibronectin, vitronectin	(107)
Chou *et al*, 2011			+ LTA, - rapamycin	(108)
Sallerfors *et al*, 1992;		Human monocytes	+ LPS, +IL-1, +GM-CSF,	(109)
Vellenga *et al,* 1988;			+TNF	(39)
Motz *et al*, 2013;			+ LPS	(77)
Almand *et al*, 2001			+ CSF-HU, + IL-4	(78)
Tajuddin *et al*, 2010		Human peripheral blood mononuclear cells	+ TLR7/8 agonist (CL097), - IFN-α	(110)
Ichinose *et al*, 1990		Human neutrophils	+ LPS	(111)
Lindemann *et al*, 1989		Human polymorphonuclear leukocytes	+ GM-CSF	(112)
Lu *et al,* 1988		Human T lymphocytes	+ IFN-γ, IL-1β	(113)
Meixner *et al*, 2008;	Epithelial cells	Human epidermal cells	- JunB	(91)
Lennard *et al*, 2016;			+ Fli-1	(114)
Rajavashisth *et al*, 1990;			+ Modified low-density lipids	(115)
Seelentag *et al*, 1987			+ IL-1 and TNF-α	(116)
Saba *et al*, 2002;		Human airway epithelial cells	+ Bacterial (*P. aeruginosa* and *S. aureus*)	(117)
Jones *et al*, 2002;			+ IL-17, TNF-α	(40)
Suzukawa *et al*, 2015			+ Leptin	(118)
Numasaki *et al*, 2004		Human lung microvascular endothelial cells	+ IL-17, TNF-α, IL-1β – IL-17F	(119)
Witowski *et al*, 2007	Mesothelial cells	Human peritoneal mesothelial cells	+ IL-17, TNF-α	(120)
Demetri *et al*, 1989		Human mesothelial cells	+ EGF, LPS	(121)
Carr *et al*, 2017;	Fibroblasts	Human dermal fibroblasts	+ IL-1	(122)
Ramachandran *et al*, 2006		Human bronchial fibroblasts	+ PAR2 agonists	(123)
Himes *et al*, 1993;		Human fibroblasts	+ Tax	(23)
Koeffler *et al*, 1987;			+ TNF-α	(124)
Seelentag *et al*, 1989			+ IL-1β	(125)
Zgheib *et al*, 2013	Stem cells	Human mesenchymal stromal cells	+ ConA, MT1-MMP inducer	(126)
Fibbe *et al*, 1988		Human marrow stromal cells	+ IL1	(127)
Tesio *et al*, 2013	Bone marrow -	Human myeloid cells	- PTEN	(128)
Grace *et al*, 2012	derived cells	Human hematopoietic progenitor cells	+ 5-AED	(129)
Kimura *et al*, 2004		Mouse bone marrow	- SOCS3/CIS3	(130)
Smith *et al,* 2017	Others	Human first-trimester trophoblast cells (Sw.71)	- Cortisol	(131)
Ordelheide *et al*, 2016		Human myoblasts	+ Palmitate, stearate	(132)

Table I. Continued.

Author, year	Cell type	Producer cell and contexts	Inducer (+) / inhibitor (-)	(Refs.)
Hudock *et al*, 2012;		Mouse intraplantar tissue	+ LPS, IL-1, IL-17A	(133)
Soria-Castro *et al*, 2010			+ Cot/tpl2	(134)
Janelle *et al*, 2006		Mouse lung tissue	+ Pre-elafin	(135)
Bohannon *et al*, 2016		-	+ Monophosphoryl lipid A	(136)
Ellis *et al*, 2005		-	+ FRH	(137)

TNF-α, tumor necrosis factor-α; CAIX, carbonic anhydrase IX; ConA, concanavalin A; SOCS1/CIS3, suppressor of cytokine signaling-1; HSF1, heat shock factor 1; IFN-α, interferon-α; SAA, serum amyloid A; FRH, febrile-range hyperthermia; SOCS3/CIS3, cytokine signaling-3; 5-AED, 5-Androstenediol; PAR2, protease-activated receptor 2; Fli-1, friend leukemia insertion site 1; Tax, transactivator protein; LTA, lipoteichoic acid; CSF-HU, human urinary colony-stimulating factor; EGF, epidermal growth factor; LPS, lipopolysaccharide; IL, interleukin.

cancer subtype with increased motility (C3) that exhibited high G-CSF expression with neutrophil aggregation. Cancer cells that produced high G-CSF levels could stimulate neutrophils to form neutrophilic extracellular traps (NETs) and thereby promote cancer cell migration. G-CSF secreted by 4T1 cells can stimulate neutrophils to form NETs and the anti-G-CSF antibody reduces cancer cell-induced NET formation (68). In addition, anti-G-CSF treatment results in the hypercitrullination of histone H3 in neutrophils from cancer-free mice, which increases the susceptibility to NETosis and thrombolysis (69).

Cancer-related thrombosis is the second leading cause of death and is usually associated with poor prognosis in cancer patients. NET formation is crucial for thrombosis formation in tumor-bearing mice. 4T1 cell-derived exosomes induce NET formation in neutrophils from G-CSF-treated mice, which can promote thrombus formation in tumor-free neutrophilic mice. The results suggested that tumor-derived exosomes and neutrophils play a synergistic role in the formation of cancer-associated thrombosis (70). Demers *et al* (15), discovered that cancer-associated G-CSF exacerbates the innate immune response of the host which leads to thrombosis. NET formation induces a pro-thrombotic state which may result in the consumption of platelets, clotting factors and microthrombosis in rhG-CSF-treated 4T1 mice. IL-1β modulates the expression of G-CSF and the levels of G-CSF and IL-1β are elevated in 4T1 mice which exhibit a NET-dependent prothrombotic state. Blocking IL-1R reduces the G-CSF level, NET formation and abolishes the pre-thrombotic state in 4T1 tumor-bearing mice (16).

6. Direct effects of G-CSF on breast cancer

rhG-CSF was shown to promote the proliferation of MCF-7 and SKBR-3 breast cancer cells, but it had little effect on normal breast epithelial cells. Chronic exposure to low doses of rhG-CSF (0.125 μg) promotes tumorigenesis in estrogen receptor-positive breast cancer by promoting the proliferation of normal and precancerous tissues in MMTV-erbB2 mice (71). Waight *et al* (72), showed that tumor-derived G-CSF can directly promote tumor growth and G-CSF knockdown slows tumor growth in mouse breast tumor models. G-CSF, in combination with other proinflammatory cytokines such as GM-CSF, IL-8 and MCP-1 that are secreted by highly aggressive tumor cells, induces an epithelial mesenchymal transition/stemness-like invasive phenotype in nonaggressive breast cancer cells (73). Higher G-CSF expression increases the invasiveness of breast and lung cancer cells, and ERK2 inhibition is necessary to reduce the expression of TNF-α-induced G-CSF in aggressive cancer cells (47). These results indicate that G-CSF is a critical factor that promotes breast tumorigenesis and specific ERK2 inhibitors may be used to treat G-CSF-producing tumors.

G-CSF-induced invasiveness in breast epithelial MCF10A cells is closely related to H-Ras oncogene upregulation. Stable expression of G-CSF induced by H-Ras upregulates matrix metalloproteinase (MMP)-2 expression by activating Rac 1 and promotes MCF10A cell migration/invasion. MMP-2-mediated degradation of extracellular matrix components is a key step in the development of invasiveness. Overexpression of G-CSF in MCF10A cells also activates other signaling pathways, including MKK3/6, p38 MAPK, ERK1/2 and AKT (48). The results of a serological *in vivo* analysis of breast cancer patients were also consistent with observations made *in vitro*, suggesting that G-CSF may be used as a serum indicator in the treatment of breast cancer (48).

G-CSF induces ErbB2 expression in breast cancer cell lines. The present review was surprised that the binding of both trastuzumab and G-CSF inhibits tumor colony formation and simultaneously induces apoptosis in these cells. This inhibition is more pronounced after pretreatment with G-CSF. A total of five of the nine breast cancer patients showed an increase in their Herceptest scores, which were used to detect ErbB2 expression after G-CSF administration (74). The ErbB2 (HER2) proto-oncogene encodes a tyrosine kinase receptor that is overexpressed in 15-20% of human breast cancer cases with aggressive clinical behavior (75).

7. Role of tumor microenvironment in the effect of G-CSF on breast cancer

Breast cancer is the most common form of cancer in women worldwide (76). The interaction between breast cancer cells and the tumor microenvironment is crucial for the dynamic development of tumors. The malignant progression of tumors depends mainly on evading and inhibiting host immune

responses, which can be achieved through stimulation of the immunosuppressive activity of MDSCs (77,78). MDSCs are a heterogeneous population defined as CD11b+Gr1+ cells, which are divided into monocytic and granulocytic subsets using the markers Ly6G and Ly6C, respectively. MDSCs can mobilize and infiltrate into tumors during tumorigenesis, where they promote tumor angiogenesis and induce premetastatic niche formation. They also interrupt mechanisms of immune surveillance, including antigenic presentation by dendritic cells (DCs), T cell activation, M1 macrophage polarization and NK cell cytotoxicity inhibition (79), in multiple tumor types, such as glioma (80) and pancreatic ductal adenocarcinoma (81). It has been reported that MDSCs accumulate around tumors and the level of circulating MDSCs is correlated with the clinical grade, metastasis and therapy response in solid tumors (82,83).

MDSCs can be induced by G-CSF treatment or by G-CSF secretion from tumors (65). Tumor-derived G-CSF facilitates the generation of granulocytic MDSCs in breast cancer, which effectively inhibit T cell activation and proliferation, leading to metastatic enhancement (72). BMP4, a member of the transforming growth factor (TGF)-β growth factor family, suppresses G-CSF secretion by inhibiting the activity of NF-κB in tumor lines, resulting in decreases in MDSCs in human and mouse breast cancer (65).

Studies have found that G-CSF promotes MDSC accumulation in breast cancer via the mTOR signaling pathway. Reverse-phase protein array analysis in mammary tumor models revealed that MDSC accumulation is accompanied by increased AKT-mTOR signaling pathway activity and induces G-CSF expression in cancer cells. Surprisingly, the expression of G-CSF in tumor-initiating cells (TICs) is high and MDSCs facilitate the expression of stemness-related genes in cancer cells, including Nanog, LGR5 and MSI-1. Moreover, MDSCs stimulate improved TIC performance via the Notch signaling pathway and TICs promote G-CSF enhancement and thus increase MDSC accumulation, which therefore establishes a feed-forward loop between TICs and MDSCs. In addition, mice treated with the mTOR inhibitor rapamycin showed significant tumor growth delay. These data demonstrate that the mTOR/G-CSF/MDSC signaling pathway regulates the malignant progression of breast tumors (51). Tumor-secreted G-CSF can increase the number of Ly6G+Ly6C+ granulocytes, which are a subset of CD11b+Gr1+ cells, in organ-specific transfer sites and further promote the production of the proangiogenic factor Bv8 protein to enhance breast tumor metastasis. Anti-G-CSF treatment can significantly reduce lung metastasis in mammary carcinoma models (66).

The hypoxic tumor microenvironment is conducive to driving metastatic niche development (84). Breast tumor cell exposure to a hypoxic microenvironment results in the activation of hypoxia-inducible factor (HIF)-1/2-mediated transcriptional programs that mediate adaptive responses in cells. Chafe *et al* (46), first revealed the relationship between the CAIX-NF-κB-G-CSF cell signaling axis and breast cancer lung metastasis. In the absence of oxygen, the expression of CAIX in breast cancer is significantly upregulated due to HIF-1 activation. NF-κB activation in the microenvironment is critical for the expression of CAIX, which is required for the G-CSF-driven mobilization of granulocytic MDSCs to the breast cancer-derived lung metastatic niche. Constitutive NF-κB activation can normalize the secretion of G-CSF, even if CAIX is completely consumed. Mobilized G-CSF-dependent granulocytic MDSCs can enhance the growth and proliferation of disseminated tumor cells to promote the formation of lung metastasis via immunosuppression (46).

Tumor-associated macrophages (TAMs) play an extremely important role in the tumor microenvironment. Macrophages in the peripheral circulation are recruited to the tumor area as a result of the action of chemokines and cytokines, such as MCP-1, M-CSF, CCL8 and vascular epithelial growth factor (VEGF), which are secreted by tumor cells or the tumor stroma. M-CSF (CSF-1) is a major contributor to TAM infiltration and promotes tumor growth (85). Blockade of the M-CSF/CSF-1R signaling pathway suppresses tumor growth in mammary carcinoma models (86) and targeting TAMs with a CSF-1R antibody is a viable strategy for cancer therapy (87).

According to Hollmén *et al* (17), high G-CSF expression in heterogeneous triple-negative breast cancer is closely associated with seeding metastasis and low overall survival. G-CSF, similar to M-CSF, regulates the differentiation of monocytes into TAMs that resemble alternatively activated (M2) macrophages. M2-polarized macrophages are characterized by a tolerant phenotype that promotes tissue repair and vasculogenesis and supports tumor growth (88). By acting on the G-CSFR on the surface of TAMs, G-CSF increases TGF-α secretion to promote breast tumor cell migration. Interestingly, in the 4T1 mammary tumor model, which is known to secrete high levels of G-CSF, an anti-G-CSF antibody significantly reduced tumor growth and lung metastasis incidence and burden. However, anti-CSF-1R promoted tumor growth and enhanced lymph node and lung metastasis. Blockade of both G-CSF and CSF-1R partly reduced lung metastasis but significantly increased lymph node metastasis, which suggests that different mechanisms may underlie lung metastasis seeding and lymph node metastasis. A possible explanation for this is that CD169+ macrophages are important gatekeeper cells in the subcapsular sinus, where they prevent the systemic dissemination of pathogens. Depletion of CD169+ macrophages in the subcapsular sinus via treatment with anti-CSF-1R or a combination of anti G-CSF and anti-CSF-1R increased lymph node metastasis, mainly because CSF-1R is the major growth factor for CD169+ macrophages in the subcapsular sinus. This important discovery suggests that M-CSF/CSF-1R-targeted inhibitors should be used with caution in the presence of high G-CSF levels (17). Similarly, the inhibition of M-CSF/CSF-1R signaling increases spontaneous lung and bone metastasis without altering tumor growth in mouse 4T1 mammary tumors, which is associated with increased serum G-CSF levels and increased neutrophil numbers at multiple sites. However, targeting G-CSF receptors with neutralizing antibodies reversed this effect, indicating that the facilitation of metastasis is driven by G-CSF in the 4T1 mammary tumor model (89).

CEACAM1 is a cell adhesion molecule that is downregulated in numerous cancers that originate from the epithelium (90). CEACAM1 plays a role in inhibiting inflammation, partly by inhibiting G-CSF production by myeloid cells. The lack of CEACAM1 expression in breast tumors promoted the secretion of high levels of G-CSF by TAMs, which in turn promoted tumor angiogenesis and initial tumor

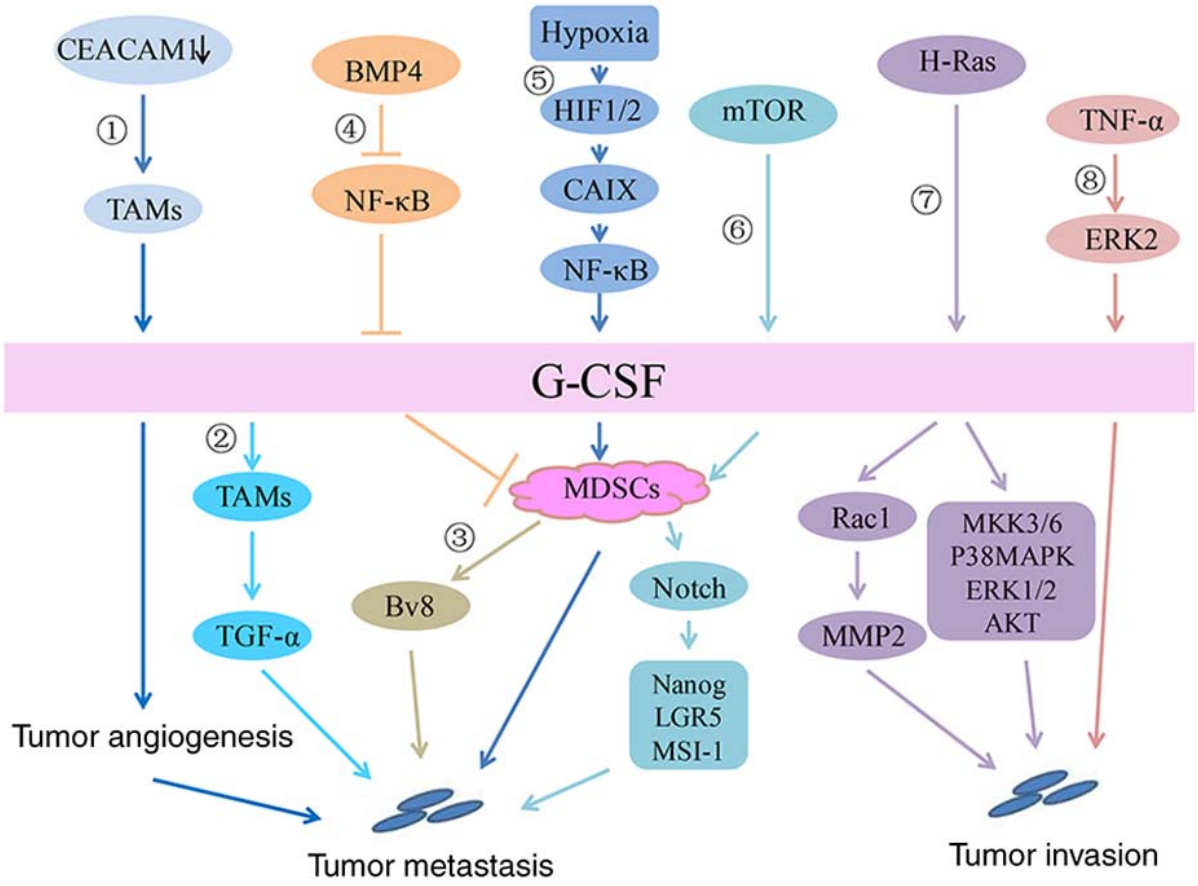

Figure 2. Signaling pathway of G-CSF in breast cancer. In the breast cancer microenvironment: 1) CEACAM1 downregulation promotes G-CSF secretion by TAMs, thereby promoting tumor angiogenesis and initial tumor establishment. 2) By acting on G-CSFR on TAMs, G-CSF increases transforming growth factor-α secretion to promote tumor cell migration. 3) G-CSF increases Ly6G+Ly6C+ granulocytes, which are a type of MDSC and further promotes the production of the proangiogenic factor Bv8 to enhance breast tumor metastasis. 4) BMP4 inhibits the expression and secretion of G-CSF by inhibiting NF-κB, resulting in decreases in the number and activity of MDSCs. 5) In a hypoxic environment, HIF1/2 upregulates CAIX and increases G-CSF expression by activating the NF-κB signaling pathway, which then promotes the mobilization of MDSCs and eventually leads to the lung metastasis of breast cancer. 6) Activation of the AKT-mTOR signaling pathway increases G-CSF expression in tumor cells, thereby promoting the accumulation of MDSCs. MDSCs promote the expression of stem-associated genes, including Nanog, LGR5 and MSI-1, in cancer cells via Notch signaling to promote tumor progression. Direct effect of G-CSF on breast cancer: 7) Stable expression of G-CSF induced by H-Ras upregulates the expression of MMP-2 by activating Rac 1 and promotes the migration/invasion of breast epithelial cells. In addition, overexpression of G-CSF activates other signaling pathways, including MKK3/6, p38 MAPK, ERK1/2 and AKT, thus promoting an invasive phenotype in breast epithelial cells. 8) TNF-α promotes the expression of G-CSF by activating the ERK2 signaling pathway to promote tumor invasion. TNF, tumor necrosis factor; AKT, protein kinase B; G-CSF, granulocyte-colony stimulating factor; HIF, hypoxia inducible factor; MAPK, mitogen associated protein kinase; NF, nuclear factor; mTOR, mammalian target of rapamycin; MDSC, myeloid-derived suppressor cell; CAIX, carbonic anhydrase IX; MMP, matrix metalloproteinase; ERK, extracellular signal regulated kinase; TAM, tumor-associated macrophages; CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1.

establishment. It has been suggested that G-CSF plays an important role in tumor promotion induced by CEACAM1 downregulation (50). Generally, as shown in Fig. 2, G-CSF plays a crucial role in breast cancer malignant progression.

8. Conclusions

G-CSF stimulates the proliferation and survival of hematopoietic stem progenitor cells and their differentiation into neutrophils by acting on their specific receptor G-CSFR. Under physiological conditions, G-CSF affects the mobilization of hematopoietic stem cells, progenitor cells and mature cells, especially neutrophils, to the blood circulation. When the body is infected, the serum G-CSF level is significantly increased to promote neutrophil mobilization to the peripheral circulation. Therefore, rhG-CSF can be used to treat neutropenia induced by chemotherapy and radiation therapy. As an adjunct to cancer therapy, G-CSF induces ErbB2 proto-oncogene expression in breast cancer patients, making it an effective drug for improving the sensitivity of breast cancer patients to trastuzumab (91). Currently, an increasing number of studies have found that tumors with high G-CSF expression show significant proliferative and metastatic properties and lead to poor prognosis (17,47,48,67). Therefore, the safety of G-CSF as an adjunct to cancer treatment should be addressed.

Some basic studies have shown that G-CSF is a promoter of tumor growth, which plays a role in immunosuppression by increasing tumor angiogenesis and mobilizing MDSCs (13,14,46,51,72). Kim *et al* (92), confirmed that G-CSF treatment in mice with precise focused radiation promoted tumor growth by stimulating angiogenesis in tumor-bearing mice and reduced the antitumor effect of radiotherapy. Coincidentally, in cervical cancer patients treated with platinum-based chemotherapy drugs, G-CSF expression in tumors

is an indicator of poor prognosis in patients. Secreted G-CSF not only has an antiapoptotic effect but also promotes the formation of tumors by mobilizing MDSCs to inhibit T cell activity and Bv8 secretion (12). In some clinical case reports, the use of safe therapeutic doses of G-CSF may cause unpredictable side effects such as bone pain, local skin reactions at the injection site and even spleen rupture or infarction (93-96). This evidence also raised concerns for clinical work. Effective methods are needed to evaluate the G-CSF usage window.

The JAK/STAT signal transduction pathway has been shown to be an important downstream pathway for G-CSF regulation in cancer models such as colorectal cancer and is inseparable from cancer proliferation and migration (57,97-99). Studying the relationship between the G-CSF-JAK/STAT signaling pathway and breast cancer can provide new insights for targeted breast cancer therapy and its prognostic strategies.

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Authors' contributions

LL and YL wrote the manuscript, prepared the figures and repeatedly revised the paper. CZ collected the articles regarding G-CSF expression. XX and XY reviewed drafts of the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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