Hematopoietic colony-stimulating factors: new players in tumor–nerve interactions

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Abstract A variety of cancers are accompanied by debilitating pain, which constitutes the primary reason for poor quality of life in cancer patients. There is an urgent demand for the development of specific mechanism-based therapies against cancer pain. Recently, important advances have been made in mechanisms contributing to cancer pain. A notable finding was that the tumor-derived hematopoietic growth factors, granulocyte- and granulocyte-macrophage-colony-stimulating factors (G-CSF/GM-CSF), subserve important functions in the generation of pain hypersensitivity in tumor-affected regions. In this context, their receptors were unexpectedly found on pain-sensing nerves and were observed to be functionally linked to nociceptive sensitization and tumor-induced pain. Here, we review evidence supporting a role for G-/GM-CSF in sensitization of pain-sensing nerves, the underlying signaling pathways and the cross-talk with other pronociceptive cytokines, peptides and modulators derived from immune cells, osteoclasts and tumor cells. These findings hold implications in the therapy of pain in disease states, such as cancer and rheumatoid arthritis.

Keywords Cancer pain · G-CSF · GM-CSF · Hyperalgesia · Peripheral sensitization

Introduction

Approximately one third of adults who are actively receiving treatment for cancer and two thirds of those with advanced malignant disease experience pain [1]. Children with cancer have similar experiences, which is why the International Association for the Study of Pain (IASP) defined the year 2008–2009 as a global year against cancer pain. In a large number of clinical cases, cancer-associated pain, particularly the neuropathic component thereof, is resistant to conventional therapeutics or their application is severely limited owing to the widespread side effects [1, 2]. In order to develop novel, mechanism-based therapeutic strategies, it is imperative to delineate the cellular and molecular mechanisms underlying cancer-induced pain. Unlike pain of neuropathic or inflammatory origin, cancer pain has not been widely studied [2, 3]. Well-characterized animal models, which merge pain research and cancer research, have only recently become available and now provide a platform for interdisciplinary research [2–4]. These studies have revealed that although tumor-induced pain shares features of inflammatory as well as neuropathic pain, it is clearly distinguished by distinct pathophysiological and mechanistic aspects [3, 4].

Various types of carcinomas and sarcomas metastasize to skeletal bones and cause spontaneous bone pain, hyperalgesia (exaggerated pain) and allodynia (pain in response to a normally innocuous stimulus), which is accompanied by bone degradation and remodelling of peripheral nerves [3, 4]. A cardinal feature of cancer pain is the involvement of mediators secreted by tumor cells (tumor-associated mediators) [2, 3]. These include growth factors, cytokines and peptides, which have the potential of either directly activating nociceptive nerves or sensitizing them towards sensory stimuli. The identity of tumor-associated mediators and precisely how they affect sensory nerve function is an
area of research which carries immense promise in understanding and treating cancer pain. For example, a variety of tumors of myeloid and non-myeloid origin secrete large quantities of the cytokines, granulocyte-colony-stimulating factor (G-CSF) and granulocyte/macrophage-colony-stimulating factor (GM-CSF) [5]. Here, we will review evidence supporting a novel role for G-/GM-CSF in pain associated with cancer and inflammation and we will discuss potential underlying mechanisms.

A novel role for G-CSFR and GM-CSFRα in cancer pain

A recent study has, for the first time, functionally linked G-/GM-CSF secreted by tumor cells in bone metastases to sensitization of pain-sensing nerves (nociceptors) and tumor-evoked pain [6]. Although G-/GM-CSF receptors (G-/GM-CSFR) and G-/GM-CSF signaling are classically associated with modulation of hematopoietic and tumor cells, three independent lines of evidence extend the range of G-/GM-CSF signaling to sensory nerves: (1) protein and mRNA analyses reveal expression of G-CSFR and the α-subunit of GM-CSFR (GM-CSFRα) in sensory nerves in peripheral tissues, including cancerous pancreas and bone matrix/periosteum, as well as in their somata lying in the dorsal root ganglia (DRG). (2) Exposure to G-/GM-CSF activates their cognate receptors and their typical signaling pathways in DRG neurons and leads to potentiation of neuropeptide release from nociceptive neurons. (3) Exposure to G-/GM-CSF sensitizes sensory nerves to nociceptive stimuli, which can be observed at the level of activity of single nerves in electrophysiological analyses ex vivo as well as the level of behavioral responses to noxious stimuli in vivo. These observations are highly suggestive of activation of receptors on sensory nerves by tumor-derived G-/GM-CSF. Indeed, blocking G-/GM-CSFR signaling by receptor neutralising antibodies or via signaling inhibitors leads to an abrogation of bone tumor-induced pain hypersensitivity; however, these treatments also partially affect tumor growth, raising the question as to whether pain reduction is only secondary to reduced tumor growth. A key finding was that specific downregulation of GM-CSFRα in DRG neurons specifically reduces tumor-induced pain hypersensitivity without affecting tumor growth. The finding was that specific downregulation of GM-CSFRα in DRG neurons specifically reduces tumor-induced pain hypersensitivity without affecting tumor growth, indicating thereby that GM-CSFRα signaling in sensory nerves is causally linked to cancer pain.

Signaling mechanisms linking G-/GM-CSFR to nociceptor sensitization

Receptors for G-CSF and GM-CSF can signal via multiple pathways and diverse signaling mediators may be employed in different cell types [5, 7, 8] (see Figs. 1 and 2). The GM-CSF receptor is a heterodimer consisting of the ligand-binding α-subunit, which is specific for GM-CSFR, and the signal transducing β-subunit, which is shared with the receptors for IL-3 and IL-5 [5]. G-CSFR is a typical class I cytokine receptor [5].

The three main signaling pathways, which are activated by G-/GM-CSF and mediate their functions in hematopoietic cells, include the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, the mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinase (PI3K) pathway [5, 8], all of which have been directly or indirectly implicated in pain modulation (see below). Furthermore, G- and GM-CSF are also able to signal via other mechanisms like activation of phospholipases or changes in cyclic nucleotide levels [7], which seem to be of marginal importance for G-/GM-CSF functions in hematopoietic cells, but could be essential for signaling in nociceptors. Possible links between signaling pathways stimulated by G-/GM-CSF and sensitization of nociceptors are highlighted below and represented schematically in Figs. 1 and 2.
Sensitization processes in nociceptors often involve potentiation of nociceptive transducers, such as the ion channels of the transient receptor potential (TRP) family. For example, the vanilloid receptor TRPV1, a sensor of noxious heat, protons and lipid algogens is potentiated via phosphorylation by diverse protein kinases, including the protein kinase C (PKC) and the non-receptor tyrosine kinase, Src, amongst others [9–11] (Fig. 1). Increased excitability can also come about by enhanced membrane expression of nociceptive transducers or transcriptional upregulation of key modulators. Prominent amongst the latter are tetrodotoxin-resistant sodium channels, such as Na\textsubscript{v}1.8, which specifically impart nociceptors with their characteristic activation properties [9, 11] (Fig. 1). The activation properties of nociceptors may also be changed by shifts in the membrane potential, e.g. via modulation of potassium channels. Long-lasting sensitization of nociceptors can also be accompanied by structural changes, e.g. sprouting of peripheral terminals.

Activation of the JAK-STAT pathway by G-/GM-CSF leads to the activation of the STAT family transcription factors, which dimerize and translocate to the nucleus upon activation and modulate gene expression [5, 8, 12]. In cultured DRG neurons, STAT3 is rapidly phosphorylated and translocates to the cell nucleus upon exposure to G-/GM-CSF [6] (Fig. 2). However, the targets of STAT3 in pain-sensing neurons remain unknown. So far, G-CSF treatment has been reported to enhance the expression of nociceptive transducers such as TRPV1, Na\textsubscript{v}1.8 and potassium channels which are involved in regulating excitability of sensory nerves, such as K\textsubscript{v}4.2 in cultured DRG neurons [6] (Fig. 2). G-/GM-CSF signaling in nociceptors was associated with hyperalgesia to thermal and mechanical stimuli. However, it has not been worked out which of the above targets of G-/GM-CSF signaling mediate modulation of which modality of nociception and further studies are required to address this important question. The modulation of TRPV1 expression by CSF signaling in sensory neurons is particularly interesting since TRPV1 is an important mediator of pain evoked by tissue acidosis, which is frequently observed in tumor-affected tissues. Although TRPV1 is primarily associated with thermal hyperalgesia, some studies have also linked it to mechanical hyperalgesia under certain pain conditions [13–16]. Blocking TRPV1 has been reported to alleviate tumor pain [14]. Unfortunately, therapy with TRPV1 antagonists appears to be problematic on account of hyperthermia, which has been reported in clinical trials [17]. Another interesting aspect is that STAT3 signaling in sensory nerves has been implicated in structural changes, such as enhancement of neurite outgrowth in cultured DRG neurons [18] and regeneration [19]. This raises the possibility that STAT3 may mediate G-/GM-CSF-induced nerve remodelling which was observed in cultured DRG neurons as well as in tumor-affected mouse paw in vivo [6].

The two other main signaling mechanisms of G-/GM-CSF, the MAPK pathway and the PI3K pathway, are also promising candidates for mediating G-/GM-CSF-induced hyperalgesia. PI3K and MAP kinases, such as the extracellular signal regulated kinases 1 and 2 (ERK1/2) and p38, have both been strongly implicated in sensitization of peripheral sensory neurons [9, 20–22]. In cultured DRG neurons, application of G-/GM-CSF leads to a rapid activation and nuclear translocation of ERK1/2 in a PI3K-dependent manner, which is highly interesting in the light of a study showing that the PI3K-ERK pathway induces heat hyperalgesia by sensitizing TRPV1 [21].

G-/GM-CSF also activate signaling pathways less known for their functions in hematopoietic cells, but strongly involved in peripheral sensitization. Colony-stimulating factors are able to stimulate guanine nucleotide-binding proteins (G proteins) [7]. Most notably in this context is a study showing that GM-CSF stimulates G-protein activity in a cholera toxin-sensitive manner and increases levels of cyclic adenosine monophosphate (cAMP) in T lymphocytes [23]. This suggests an involvement of Gs proteins with subsequent increase of cAMP possibly followed by activation of protein kinase A (PKA). This pathway plays a predominant role in sensitization of nociceptors and mediates the action of well-known algesic agents, such as prostaglandins and bradykinin [24–26], which cause phosphorylation of key ion channels such as TRPV1 and Na\textsubscript{v}1.8 via PKA [27, 28] (Fig. 1). However, whether a
cAMP-PKA pathway contributes to G-/GM-CSF-induced hyperalgesia has yet to be addressed.

Another well-investigated mechanism of nociceptor sensitization is the activation of phospholipase C downstream of G-protein-coupled receptors or receptor tyrosine kinases [9, 11]. This leads to the breakdown of phosphatidylinositol 4,5-bisphosphate, which subsequently increases membrane levels of diacylglycerol and cytosolic levels of inositol 1,4,5-trisphosphate followed by activation of PKC. All of these signaling components are involved in sensitization of pain-related ion channels, such as TRPV1 [29–31]. Moreover, PKC can increase membrane levels of TRPV1 by stimulating membrane insertion [32] (Fig. 1). In hematopoietic cells, the PLC-PKC pathway is activated by G-/GM-CSF [33–36], suggesting that G-/GM-CSF may also stimulate this pathway in nociceptive neurons. In particular, the isoform PKC-ε seems to be solely essential for function in human bone marrow cells [37]. Interestingly, the same isoform, PKC-ε, is critical for sensitization of TRPV1 and Na⁺,1.8 in nociceptors [29, 38, 39]. However, the role of PLC-PKC signaling in G-/GM-CSF-induced hyperalgesia still remains to be investigated. The cAMP binding protein Epac has been reported to link the cAMP/PKA and PLC/PKC pathways [40, 41]. Whether this important form of cross-talk between these two key pathways in nociceptor sensitization comes about following G-/GM-CSF exposure in chronic pain states is not known. Furthermore, G-CSF is able to activate kinases of the Src family [42, 43]. Src kinases have been shown to sensitize TRPV1 [10]. This creates another likely link between colony-stimulating factor signaling and sensitization of nociceptors.

Addressing precise mechanisms via which G-/GM-CSF signaling potentiates pain and nerve remodeling may be important in understanding the mechanisms of cancer pain and their therapeutic modulation. For example, it will be interesting to address which nociceptive mediators and ion-channel transducers are modulated by G-/GM-CSF signaling in sensory neurons and whether the repertoire of activated signaling components is different in sensory neurons versus hematopoietic cells. Indeed, this could represent one way to enable targeting G-/GM-CSF receptors on pain-sensing nerves devoid of side effects on the hematopoietic system, such as neutropenia.

**The role of G-/GM-CSF in bone morphology and bone pain**

G-CSF and GM-CSF are widely used in clinical practice for their stimulating properties on proliferation and release of myeloid lineage cells into the blood stream [8]. Clinical indications for therapy with these colony-stimulating factors include prevention and treatment of neutropenia associated with myelosuppressive chemotherapy, reconstitution after bone marrow transplantation and mobilization of hematopoietic progenitor cells for transplantation of peripheral blood progenitor cells. Numerous clinical studies have documented adverse drug reactions upon clinical usage of G-CSF and GM-CSF, the most frequent and acutely harmful adverse event being intense bone pain. Reported frequencies reach up to 90% [44–46], making bone pain a serious problem of a therapy with G-/GM-CSF. The findings of a role for G-/GM-CSF receptors expressed on sensory neurons in nociceptor sensitization can directly account for this poorly understood clinical observation [6]. Additional potential mechanisms arise from studies focusing on the effect of G-CSF on bone metabolism. While long-term application of G-CSF may lead to osteoporosis [47], short-term effects on bone metabolism are less clear. One study demonstrated decreased serum levels of osteocalcin and increased levels of bone-specific alkaline phosphatase following short-term (5 days) G-CSF application in healthy blood progenitor cell donors [48]. This particular regulatory pattern of bone metabolism parameters has been described in patients with osteolytic bone metastases [49]. Osteolytic bone metastases are well known to cause pain partly by activation of osteoclasts and local acidosis [50]. Furthermore, bisphosphonates, a class of drugs inhibiting osteoclast activity, can ease bone metastases-induced cancer pain [51]. Thus, changes in bone metabolism induced by G-CSF may represent a bone-specific mechanism by which G-CSF causes bone pain, in addition to direct effects on sensory neurons as described recently [6]. This concept is in line with the findings that tumor-derived G-CSF plays a crucial role in a mouse model of cancer pain induced by osteolytic bone metastases [6].

**Implications for G-/GM-CSF signaling in inflammatory pain states**

In addition to a role in cancer biology, G-/GM-CSF signaling has been implicated in the modulation of immune function. G-CSF and GM-CSF are locally released following tissue injury [52] or inflammation [53] and diverse functions have been ascribed to them in modulating local and systemic inflammatory responses [5]. Furthermore, G-/GM-CSF may locally contribute to inflammatory pain by directly sensitizing peripheral nerves. Indeed, G-CSF and GM-CSF levels are elevated in wound exudates of women having undergone caesarean delivery [52]. Interestingly, analgesic consumption during the first 24 h after surgery was found to be inversely proportional to G-CSF levels in the wound exudate [52], suggesting that CSF signaling is indeed functionally linked to exaggerated pain in humans.
G-CSF and GM-CSF may also contribute to opioid-induced hyperalgesia, i.e. heightened pain sensitivity after chronic use of opioids. One study demonstrated that cytokine skin levels, including G-CSF, and pain sensitivity are elevated in a mouse model of post-incisional pain following long-term application of morphine [54]. These results may be explained on a mechanistic basis by sensitization of peripheral nerves by colony-stimulating factors. In rheumatoid arthritis, a systemic inflammatory disorder characterized by joint pain, G-CSF and GM-CSF levels are also highly elevated [55–57]. G-CSF and GM-CSF are implicated in the pathogenesis of rheumatoid arthritis [58–60] and are considered as promising therapeutic targets [61].

Cross-talk between G-/GM-CSF and other immune modulators of pain

A very interesting and relevant aspect in the context of tumor–nerve interactions constitutes the potential cross-talk between hematopoietic growth factors and several of the previously identified paracrine mediators of cancer pain and immune disorders. These include cytokines, such as tumor necrosis factor α (TNF-α) and interleukins [62], as well as peptides such as endothelin. For example, TNF-α, a prominent mediator of cancer pain [63–65], is known to strongly stimulate the release of several hematopoietic growth factors, including G-CSF and GM-CSF [66]. In turn, GM-CSF is known to synergistically enhance interferon-induced secretion of TNF-α from monocytes. Interestingly, GM-CSF and TNF-α synergize in enhancing the secretion of other cytokines, such as interleukin 1 (IL-1) [67]. That these interactions are clinically relevant is suggested by the observation that GM-CSF, interferons as well as diverse interleukins have been co-detected in synovial effusions of patients with rheumatoid arthritis [68]. The production of IL-1α, IL-1β as well as TNF-α by human mononuclear cells is stimulated by GM-CSF [69] and in turn, IL-1 and TNF-α additively increase the production of G-CSF and GM-CSF at the mRNA level in human fibroblasts [70].

Endothelin 1 has gained a very prominent position in the pathophysiology of cancer pain and is amongst the few targets which have found its way from the bench to the bedside in the context of the treatment of cancer pain [71]. It is interesting to note that GM-CSF potentiates the production and release of endothelin 1 from human monocytes, suggesting that this might be another mechanism via which cancer pain can be potentiated [72]. Interestingly, a chemokine called the CC chemokine L2 (CCL2), also known by the name MCP1, is known to be upregulated in splenic T lymphocytes of tumor-bearing animals by the concurrent production of GM-CSF by tumor cells.
cells [73]. CCL2 immunoreactivity is present in tumors and high levels of CCL2 peptide have been reported in microperfusates from tumors. Interestingly, CCL2 directly sensitizes sensory nerves and leads to the upregulation of voltage-gated calcium channels, which may contribute to tumor-induced pain in the fibrosarcoma cancer model [74]. A summary of potential roles of G-CSF and GM-CSF in the cross-talk between tumor cells, inflammatory cells and sensory nerves in the context of bone cancer pain is given in Fig. 3.

Therapeutic implications

In the light of the cross-talk described above, it is very likely that synergistic associations between multiple paracrine mediators, which are present in the microenvironment of tumors and nerves, can contribute to tumor–nerve-immune interactions and to the pathogenesis of cancer-induced pain. Combination therapies targeting hematopoietic growth factor receptors and the above-mentioned peptides and chemokines therefore hold a high level of promise in the clinical management of cancer pain. In this regard, it is interesting to note that clinical trials are currently under way with various ligands and antagonists and chemokine receptors; furthermore, the elucidation of the crystal structure of the GM-CSFR complex holds promise for developing small molecule drugs which inhibit GM-CSFR-mediated functions in context of tumor–nerve interactions [75]. One antagonist of the GM-CSF receptor, E21R [76], has already been tested in clinical trials for the treatment of solid tumors [77]. The effects of E21R on cancer pain, however, were not investigated. In the light of evidence from the studies discussed above, it seems worthwhile including pain as another parameter to be tested in future clinical studies.

Conclusions

Recent evidence indicates that hematopoietic growth factors, which are secreted at high levels in the microenvironment of tumors, are capable of sensitizing pain-sensing nerves in the vicinity of tumor cells and thereby evoking exaggerated sensitivity to tactile stimuli in tumor-bearing mice. Complex interactions occur between hematopoietic growth factors and other known mediators of nerve sensitization, such as interleukins, TNF-α and endothelins, which promote pain hypersensitivity in diseased tissue. Taken together with reports on high levels of G-/GM-CSF in patients with rheumatoid arthritis, this indicates that intervention of G-/GM-CSF signaling may hold promise in the treatment of debilitating pain associated with cancer and rheumatoid arthritis.

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Disclosure of potential conflicts of interest

The authors declare no conflicts of interest related to this work.

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