

Interleukin-8 in gastrointestinal inflammation and malignancy: induction and clinical consequences

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Abstract: Owing to its immense surface area, the mucosal surface of the gastrointestinal (GI) tract is continually subject to numerous endogenous and exogenous antigens capable of inducing an inflammatory response in the appropriate setting. In certain individuals, these inflammatory responses may also become dysregulated and result in the development of chronic GI inflammation, such as inflammatory bowel disease. Furthermore, gastric and colonic malignant processes are extremely common and, to date, still have high mortality rates. As a result, continuing research has focused on elucidating potential molecular mechanisms involved in these processes in an attempt to understand the pathophysiology of these diseases and, potentially, aid in the development of novel therapeutics. Interleukin-8 (CXCL8) is a small molecular weight chemokine identified almost 30 years ago. Ongoing research has demonstrated that this factor contributes to a multitude of pathophysiological processes within the GI tract, including chronic GI inflammatory states, such as inflammatory bowel disease, and gastric and colonic carcinomas. This review highlights the role of CXCL8 in GI inflammation and malignancy. It describes molecular mechanisms involved in promoting and restraining CXCL8 production in GI cells and how CXCL8 contributes to the pathophysiology of a variety of GI inflammatory processes. Finally, this review describes a variety of mechanisms by which CXCL8–CXCL1/2 signaling may contribute to GI malignancy.

Keywords: CXCL8, inflammatory bowel disease, IBD, colon cancer, IL-8, Crohn's disease, ulcerative colitis

Introduction

The gastrointestinal (GI) tract represents the largest mucosal surface in contact with the external environment and, as such, is exposed to numerous endogenous and exogenous antigens capable of inducing an inflammatory response in the appropriate setting. In addition, many malignant processes occur within the GI tract, and continual exposure to chronic inflammation increases the likelihood of these processes.^{1,2} Inflammatory bowel disease (IBD) is subdivided into two main entities: Crohn's disease and ulcerative colitis (UC). These diseases are chronic, relapsing, and remitting disorders of the GI tract that result from a combination of genetic, environmental, and immune factors. The incidence and prevalence of Crohn's disease and UC appears to be increasing worldwide, and both represent significant economic burdens to health care systems and countries.^{3,4} Similarly, colorectal and gastric cancers are two of the most commonly identified malignancies and leading causes of mortality worldwide.^{5,6} To date, our understanding of these disease processes remains incomplete, and resistance to standard therapeutics has been reported for patients with IBD and gastric or colorectal

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cancer (CRC). The mechanisms underlying this resistance remain unclear.^{7,8} As such, ongoing research has focused on elucidating the molecular mechanisms involved in the development of GI inflammatory and malignant processes in the hopes of identifying novel therapeutic targets.

Nearly 30 years ago, three separate research groups simultaneously described a small molecular weight protein secreted by stimulated human mononuclear cells that induced polymorphonuclear cell (PMN) chemotaxis, degranulation, and superoxide and hydrogen peroxide (H₂O₂) production.^{9–11} As such, the compound plays a critical role in the body's antimicrobial defense mechanisms. This product was subsequently named interleukin-8 and now often abbreviated as IL-8 or CXCL8 with both terms being used interchangeably.^{12,13} Research has subsequently demonstrated that CXCL8 is involved in a variety of processes apart from PMN activation, and its expression has been observed in a multitude of acute and chronic inflammatory processes, including many GI disease states. Its expression has been reported in a variety of resident and invading cell types within the GI tract, including monocytes, macrophages, endothelial cells, epithelial cells, fibroblasts, and neutrophils. Indeed, CXCL8 expression occurs acutely during GI infection caused by bacteria, viruses, and parasites, while elevated levels of CXCL8 are also reported in patients with chronic GI inflammation. In addition, sustained CXCL8 expression is reported in tumorous GI epithelial cell lines and in mucosal biopsy tissues collected from patients with gastric cancer or CRC. As a result, the expression or dysregulation of CXCL8 has been postulated to contribute to the pathophysiology of various disease states, and furthermore, targeting its expression may be of therapeutic benefit. In addition, the measurement of CXCL8 has been proposed as a potential diagnostic or prognostic marker for several GI disease states. The following review provides a summary of our current understanding of how CXCL8 expression contributes to the pathophysiology of various GI disease states.

Classification as a CXCL family chemokine

The primary amino acid sequence of interleukin-8 contains two N-terminal cysteine residues separated by a single amino acid that promotes formation of two disulfide bonds in higher-level amino acid structures.^{14,15} The presence of this sequence results in its assignment to the CXCL family of chemokines, hence the abbreviation CXCL8. Furthermore, the primary amino acid sequence of CXCL8 contains a glutamate–leucine–arginine (ELR+) sequence proximal to the CXC motif that

is essential for binding to its two receptors;^{16–18} deletion or modification of this motif attenuates CXCL8 receptor binding.^{19,20} The presence of these motifs designates CXCL8 as an ELR+ CXC chemokine. The CXCL8 gene is located on chromosome 4q12–13 in close approximation to other ELR+ CXC chemokines that perform similar functions, including CXCL1 (growth-related oncogene α ; GRO α), CXCL2 (GRO β), CXCL3 (GRO γ), CXCL5 (epithelial-derived neutrophil-activating peptide 78; ENA-78), and CXCL7 (neutrophil-activating peptide 2; NAP-2).^{15,21,22} Like several genes in this cluster, the CXCL8 gene contains four exons, three introns, and single CAT- and TATA-like structures.²³ Together, these genes share ~30%–50% amino acid homology.¹⁵ As mentioned, CXCL8 primarily binds two receptors that are responsible for the majority of activated intracellular signaling events and are now designated as CXCR1 and CXCR2.^{24,25} Characterization of these receptors has also led to their identification on a variety of cell types, including endothelial cells, epithelial cells, fibroblasts, and neutrophils.^{26,27} This suggests that CXCL8 has a multitude of effects that extend beyond PMN activation and chemotaxis. Moreover, although CXCR1 and CXCR2 are highly homologous, differences in intracellular signaling cascades and biological processes activated following CXCL8 binding have been described.²⁸ Molecular studies also indicate that only CXCL6 and CXCL8 are capable of binding CXCR1, while CXCR2 is capable of binding CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8.^{29,30} Therefore, although CXCL8 may structurally resemble other proinflammatory mediators of the ELR+ CXCL family, the differences in receptor–ligand binding suggest a, potentially, nonredundant role for this factor in a variety of biological or pathological processes. See Tables 1 and 2 for chemokine family members related to CXCL8 and ELR+ CXCL members.

Molecular signaling cascades and CXCL8 expression

An abundance of research examining the molecular mechanisms of CXCL8 mRNA transcription has been performed and aided in the study of how this chemokine contributes to disease in various organ systems, including the GI tract. Initial experiments using in vitro intestinal epithelial monolayers have demonstrated that GI CXCL8 mRNA transcription is regulated via the cooperation of inducible and constitutively expressed transcription factors, whereby the constitutively expressed transcription factors activator protein-1 (AP-1) and cis-regulatory enhancer binding protein-like factor cooperate with inducible nuclear factor κ B (NF- κ B) transcription

Table 1 CXCL chemokine family related to CXCL8

CXC family (human)	Alternative names	Chromosome	Mouse ortholog	Alternative names	Receptor(s)	ELR+	References
CXCL1	GRO α	4q13.3	CXCL1	GRO/KC	CXCR1, CXCR2	Yes	303,304
CXCL2	GRO β	4q13.3	CXCL2	MIP-2	CXCR2	Yes	303,304
CXCL3	GRO γ	4q13.3	GM1960	Dcip1	CXCR2	Yes	303,304
CXCL4	PF4	4q13.3	CXCL4	PF4	CXCR3b	No	303,304
CXCL5	ENA-78	4q13.3	CXCL5	LIX	CXCR2	Yes	303,304
CXCL6	GCP-2	4q13.3			CXCR1, CXCR2	Yes	303,304
CXCL7	NAP-2	4q13.3	CXCL7	Pbbp		Yes	303,304
CXCL8	IL-8	4q13.3			CXCR1, CXCR2	Yes	303,304
CXCL9	MIG	4q21.1	CXCL9	MIG	CXCR3, CXCR3b	No	303,304
CXCL10	IP-10	4q21.1	CXCL10	IP-10	CXCR3, CXCR3b	No	303,304

factors to induce expression of CXCL8 mRNA.^{31–35} These factors bind cis-acting elements within the –94 to –71 region of the CXCL8 promoter to induce mRNA transcription.^{35,36} The NF- κ B pathway has received much attention for its role in CXCL8 gene transcription, and numerous studies have demonstrated aberrant activation of this pathway during GI disease states.

The NF- κ B pathway promotes transcription of a variety of anti- and proinflammatory mediators, including CXCL8, and is subdivided into a classical and alternative pathway based on activating stimuli and composition of transcription factor subunits. NF- κ B transcription factors are homo- or heterodimers of five different subunits: p50/p105, p52/p100, p65 (RelA), RelB, or c-Rel.³⁷ In the absence of proinflammatory stimuli, these transcription factors are bound to inhibitor of κ B (I κ B) proteins and retained within the cytosol so as to prevent their nuclear translocation and subsequent induction of gene transcription.^{37,38} Activation of the classical NF- κ B pathway occurs in response to a variety of host- or pathogen-derived proinflammatory stimuli that bind different receptor families, including toll-like receptors (TLRs),³⁹ nucleotide oligomerization domain-containing protein 1 and 2,⁴⁰ interleukin-1 receptor (IL-1R),⁴¹ and tumor necrosis factor α (TNF α) receptor.^{42,43} Activation of the NF- κ B pathway is also modulated by microtubule-associated

serine/threonine protein kinase-3 (MAST3), and alteration of this pathway has been shown to affect CXCL8 secretion.⁴⁴ Moreover, CXCL8 expression and activation of the NF- κ B pathway has been reported to occur in response to reactive oxygen species (ROS), hypoxia, and acidosis.⁴⁵ It should be noted, however, that these experiments have not all been performed within GI experimental models, and additional experiments are required to confirm the relevance of these observations in the GI tract. Upstream signaling cascades from these various receptors converge and phosphorylate residues on the I κ B kinase (IKK) complex to activate the classical NF- κ B pathway; this complex is composed of two kinase subunits, IKK α and IKK β , and a regulatory subunit IKK γ /NEMO.⁴⁶ Activation of the classical pathway results in phosphorylation of Ser177 and Ser181 residues on IKK β and downstream phosphorylation, ubiquitination, and proteasomal degradation of I κ B proteins.^{47,48} Degradation of I κ B proteins permits the nuclear translocation of p65-containing NF- κ B transcription factors that subsequently induce CXCL8 mRNA transcription.^{49,50}

In the GI tract, CXCL8 mRNA transcription occurs following activation of the classical NF- κ B pathway. The upstream signaling mechanisms involved in NF- κ B p65-induced CXCL8 mRNA transcription may be cell specific. In vitro experiments using gastric epithelial cells and

Table 2 ELR+ CXCL chemokine family members related to CXCL8

CXC family (human)	Alternative names	Chromosome	Mouse ortholog	Alternative names	Receptor(s)	References
CXCL1	GRO α	4q13.3	CXCL1	GRO/KC	CXCR1, CXCR2	303,304
CXCL2	GRO β	4q13.3	CXCL2	MIP-2	CXCR2	303,304
CXCL3	GRO γ	4q13.3	GM1960	Dcip1	CXCR2	303,304
CXCL5	ENA-78	4q13.3	CXCL5	LIX	CXCR2	303,304
CXCL6	GCP-2	4q13.3			CXCR1, CXCR2	303,304
CXCL7	NAP-2	4q13.3	CXCL7	Pbbp		303,304
CXCL8	IL-8	4q13.3			CXCR1, CXCR2	303,304

monocytic THP-1 cells exposed to *Helicobacter pylori* demonstrated that these two cell lines utilize different upstream signaling cascades for CXCL8 mRNA transcription.⁵¹ Stimulation of TLR2 is also implicated in *H. pylori*-induced E-cadherin disruptions, which have a well-established association with the development of gastric cancer. Indeed, recent findings have established that *H. pylori* infection is associated with elevated serum levels of an 80 kDa E-cadherin ectodomain in human patients. This was thought to arise via a heat-labile *H. pylori* surface component that activated the host protease calpain to cleave E-cadherin in human gastric cells, independently of the virulence factors CagA and VacA.⁵² Whether and how these pathways interact with CXCL8 signaling in gastric epithelial cells have yet to be assessed. Regardless, numerous in vitro experiments have shown that pathogen- or host-derived proinflammatory mediators promote NF- κ B p65 nuclear accumulation and subsequent transcription and translation of CXCL8 within GI cell lines.^{53–58} Moreover, inhibition of the classical NF- κ B pathway in in vitro intestinal epithelial monolayers reduces CXCL8 mRNA production.³¹ Similarly, mice infected with the attaching and effacing pathogen *Citrobacter rodentium* display elevated levels of activated NF- κ B transcription factors and a paralleled increase in the CXCL8-related chemokines CXCL1 (KC) and CXCL2 (MIP-2) that were attenuated following NF- κ B inhibition.⁵⁹ In separate experiments, NF- κ B p65 subunit inhibition ameliorated 2,4,6-trinitrobenzenesulfonic acid-induced colitis in vivo; however, these experiments did not analyze expression of CXCL8-related chemokines.⁶⁰ These in vivo and in vitro experimental observations are further corroborated with data collected from human patients. Mucosal biopsy tissues collected from patients with *H. pylori*-induced gastritis display elevated levels of nuclear NF- κ B p65 and increased levels of CXCL8 that parallel an increase in PMN tissue infiltration.^{61–66} Similarly, both activated NF- κ B p65 and elevated CXCL8 have been observed in inflamed mucosal biopsy specimens collected from IBD patients, and increased expression of activated NF- κ B p65 and CXCL8 directly correlated with indices of inflammation.^{60,67–70} Elevated levels of activated NF- κ B p65 have also been reported in gastric and CRC lesions;^{71–73} however, association studies examining increased nuclear accumulation NF- κ B p65 and elevated CXCL8 have not been performed. Collectively, these studies demonstrate the importance of the classical NF- κ B pathway in contributing to CXCL8 production within the GI tract. Furthermore, they highlight the importance of this pathway in contributing to CXCL8-mediated

GI disease. See Figure 1A and B for details on factors impacting CXCL8 and possible outcomes in inflammation and neoplasia. For details on mouse orthologs and ELR+ CXCL chemokine expression in mice see Table 1 and Figure 2 for details.

Restraining NF- κ B activation and CXCL8 production

Estimates suggest that the human GI tract contains $\sim 10^{14}$ microorganisms with an estimated collective biomass of more than 1 kg.^{74–76} As a result, cells within the GI tract are constantly exposed to a multitude of potentially noxious and/or inflammatory stimuli capable of activating the NF- κ B pathway and promoting transcription of various proinflammatory cytokines, including CXCL8. Despite this, the GI tract and its microbiota have evolved to exist in a state of equilibrium.^{77,78} A key mechanism that limits inflammation to a low level is the secretion of a mucus blanket which, in health, separates the microbiota from the host epithelium. In addition, researchers have described a variety of mechanisms employed by GI tissues aimed at attenuating activation of the NF- κ B pathway and transcription of proinflammatory genes, such as CXCL8. One of the most basic mechanisms employed by the GI tract to ensure homeostasis with the gut microbiota is via the downregulation of receptor molecules capable of activating the classical NF- κ B pathway on epithelial cells lying in close apposition to the microbiota. Experiments have demonstrated that in vitro intestinal epithelial monolayers are hyporesponsive to TLR2 and TLR4 stimulation due to downregulation of these receptors. Notwithstanding, these cells were capable of activating the classical NF- κ B pathway and inducing CXCL8 mRNA transcription following transgenic expression of TLR2 or TLR4 and administration of their respective ligands.^{79,80} Importantly, experiments have demonstrated that administration of proinflammatory interferon γ or TNF α to in vitro intestinal monolayers restores TLR4 expression and NF- κ B-mediated CXCL8 production induced via lipopolysaccharide.⁸¹ These experiments parallel observations of mucosal biopsy tissues collected from IBD patients, whereby areas of active inflammation were associated with increased TLR2 and TLR4 expression.^{82–84} Another mechanism employed by GI tissue to maintain homeostasis with resident microbiota is the preferential expression of receptors on epithelial surfaces separated from the gut microbiota. For example, the flagellin receptor TLR5 is primarily expressed on the basolateral surface of intestinal epithelial monolayers to ensure TLR5-mediated induction of the NF- κ B pathway, and subsequent CXCL8 expression only occurs following

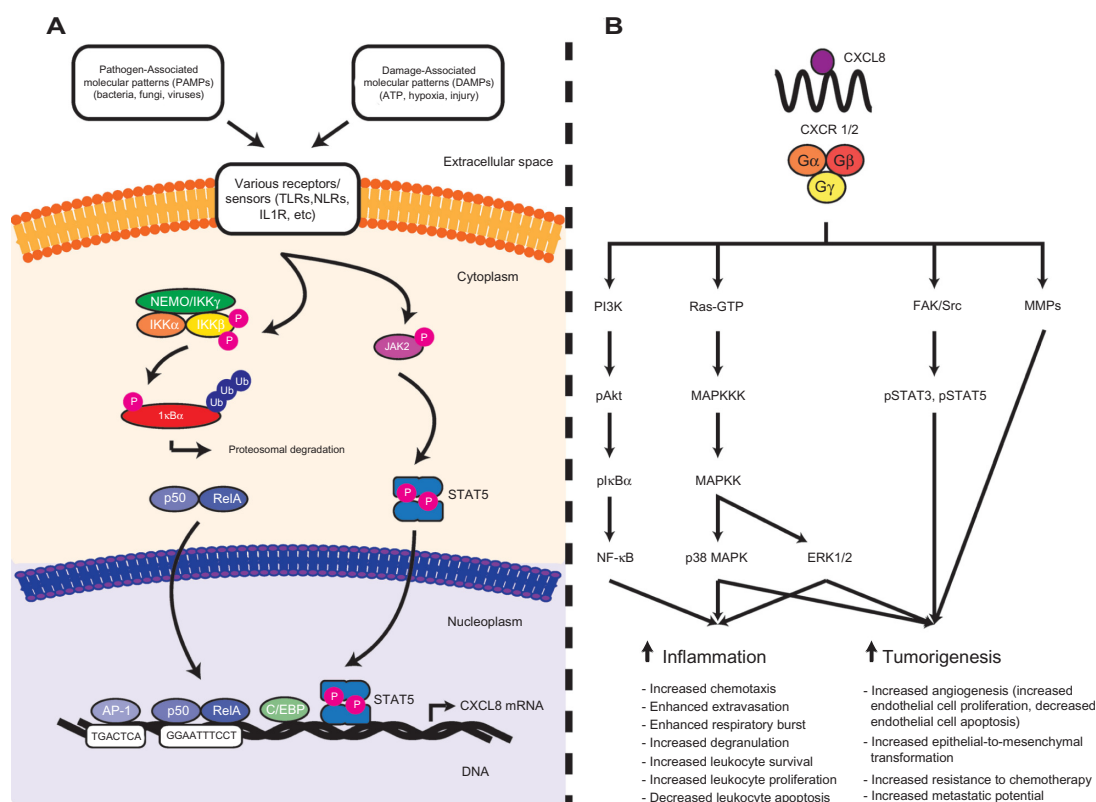


Figure 1 An overview of CXCL8 gene regulation and the signalling cascades downstream of CXCL8-mediated CXCR1/2 activation.

Notes: (A) Phosphorylation of IκBα and JAK proteins following receptor activation (eg. IL-1R, TLRs, etc) allows for the nuclear translocation of transcription factors and the subsequent expression of target genes, such as CXCL8.^{39–50} (B) The effects of CXCL8-activated signalling cascades on the pathogenesis of inflammatory bowel diseases and tumorigenesis (see Tables 3 and 4).

Abbreviations: IL-1R, interleukin-1 receptor; TLRs, toll-like receptors; PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns.

disruption of monolayer integrity, such as during infection with *Salmonella typhimurium*.^{85,86} Similarly, TLR9 ligation activates distinctive receptor-signaling cascades within intestinal epithelial cells depending on the intestinal epithelial surface, whereby only basolateral TLR9 ligation resulted in NF- κ B activation and CXCL8 secretion.⁸⁷ These processes may be actively altered by enteropathogens, as illustrated in the *Campylobacter jejuni*-induced disruption of protective apical TLR9, which in turn primes the gut for heightened levels of CXCL8 and inflammation upon exposure to proinflammatory stimuli.⁸⁸ Collectively, these results demonstrate that the GI epithelium, at least partially, regulates CXCL8 transcription via the NF- κ B pathway through reduced expression and/or localization of TLRs.

In order to restrain inflammatory responses, the GI tract also expresses molecules that negatively regulate TLR/IL-1R proinflammatory signaling cascades, such as single IgG IL-1-related receptor (SIGIRR) and Toll-interacting protein (TOLLIP).^{80,89–91} These inhibitory proteins are essential for attenuating GI CXCL8 responses and associated with a variety of clinical GI disease states. SIGIRR and TOLLIP

expressions are downregulated in mucosal biopsy tissues collected from areas of active inflammation in IBD patients.⁹² These molecules are also downregulated in immature GI tissues collected from patients with necrotizing enterocolitis and are associated with a concomitant increase in CXCL8 expression.⁹³ Decreased TOLLIP mRNA has been found in association with increased levels of TLR mRNA in colon cancer and *H. pylori*-induced gastritis.^{94,95} Interestingly, decreased TOLLIP mRNA and increased TLR mRNA are observed as progression from *H. pylori*-induced gastritis to adenocarcinoma occurred.⁹⁴ These observations may help, at least partially, explain experimental data suggesting that CXCL8 is the most upregulated gene in in vitro gastric epithelial cells exposed to *H. pylori*.⁹⁶ Clinical observations suggesting these inhibitory proteins attenuate CXCL8 production are supported experimentally. The genetic ablation of TOLLIP and SIGIRR in vitro results in enhanced NF- κ B responses and increased CXCL8 mRNA transcription, while their overexpression leads to blunted CXCL8 expression.⁹⁰ Epithelial SIGIRR deficiency in vivo increases susceptibility to dextran sulfate sodium (DSS)-induced colitis, enhances

expression of CXCL8-related chemokines, and increases tumorigenesis rates following DSS + azoxymethane treatment.⁹⁷ Similarly, genetic deletion of TOLLIP in vivo enhances susceptibility to experimental models of colitis and leads to upregulated NF- κ B activity and expression of CXCL8-related cytokines.⁹⁸ Collectively, these results demonstrate that the GI tract expresses molecules capable of dampening CXCL8 expression, and absence of these molecules can result in pathological CXCL8 expression. This pathological expression of CXCL8 may contribute to the development of chronic GI inflammatory disease states or malignancies.

CXCL8 gene polymorphisms

Although the CXCL8 promoter region is essential to mRNA transcription, gene polymorphism studies indicate that other regions within the gene may contribute to CXCL8 production and, potentially, susceptibility to certain GI disease states. For example, multiple polymorphisms within the CXCL8 gene are thought to influence susceptibility to the development of irritable bowel syndrome.⁹⁹ A collection of research also suggests that polymorphisms at position -251 of the CXCL8 gene enhance susceptibility to a variety of GI disease states. Individuals with adenosine residues at position -251 (-251A) of the CXCL8 gene are thought to be at increased risk of infection from *Clostridium difficile*, enteroaggregative *Escherichia coli*, and *H. pylori*, and following infection, have elevated inflammatory responses and/or more clinically significant disease.^{100–109} It is currently unknown whether these polymorphisms enhance susceptibility to other GI pathogens. Moreover, -251A polymorphisms are thought to increase susceptibility to the development of gastric cancer and IBD.^{110–114} It is worth noting that the reproducibility of some of the above studies has been questioned. As a result, it has been postulated that the presence of these mutations may influence the development of GI disease only within certain populations.¹¹⁵ It is unknown whether other CXCL8 gene polymorphisms contribute to the development of certain GI diseases. Research to date examining the relationship between CXCL8 gene polymorphisms and GI disease has focused on the -251 region, and additional research is required to clarify whether polymorphisms at other sites also factor into susceptibility to GI inflammation or cancer. Other CXCL8 gene polymorphisms are associated with a variety of inflammatory and malignant states, including osteoarthritis,¹¹⁶ ovarian cancer,¹¹⁷ and systemic lupus erythematosus nephritis.¹¹⁸ Additionally, it is currently unknown how -251A polymorphisms affect CXCL8 transcription. To date, experiments have only demonstrated that lipopolysaccharide stimulation

of peripheral blood cells collected from patients with -251A polymorphisms results in enhanced CXCL8 production,¹¹⁹ the mechanism via which this occurs remains obscure.

Interleukin-8 protein

CXCL8 is synthesized as a 99 amino acid precursor and secreted following proteolytic processing of leader sequences. These sequences are variable, approximately 20 amino acid residues in length, and cleaved by a variety of extracellular enzymes. The variability associated with the cleavage site results in the generation of an assortment of CXCL8 isoforms.⁵⁷ The most commonly reported active CXCL8 isoforms contain 77 (CXCL8 1-77) or 72 (CXCL8 6-77) amino acids. CXCL8 6-77 is primarily secreted by activated macrophages and monocytes and has potent effects on PMNs.^{62,105} It is compared to CXCL8 1-77 largely derived from endothelial cells and fibroblasts.^{120,121} Active CXCL8 isoforms can be further processed via a myriad of factors that further modulate CXCL8 potency. Importantly, the ELR motif must remain intact for its stimulatory effect on PMNs, while CXCL8 1-77 can be further processed by thrombin into the more potent isoform CXCL8 6-77.¹²² Moreover, PMN-derived cathepsin G, elastase, matrix metalloproteinase-9 (MMP-9; gelatinase B), and proteinase-3 are capable of proteolytically processing CXCL8 1-77 into more potent isoforms.^{123,124} Therefore, PMNs may induce a positive feedback loop that further potentiates the recruitment of additional PMNs. It has recently been proposed that modification of CXCL8 via various factors be divided into three distinct groups. The first group involves isoforms of CXCL8 generated by cleavage with aminopeptidases, which yields isoforms of 75–79 amino acids in length with intermediate activity toward PMNs. The second group includes CXCL8 isoforms of 69–72 amino acids in length that are generated by proteolytic cleavage and are highly potent PMN chemoattractants. Finally, the third group is generated via modification of arginine to citrulline residues and is a weak PMN chemoattractant.¹²⁵ Although citrullination of CXCL8 dampens PMN tissue responses, it may enhance CXCL8's ability to mobilize PMNs within the blood stream.¹²⁶ Collectively, these studies demonstrate that CXCL8 exists in a multitude of isoforms that are subject to modification by the surrounding host tissue. It remains largely unknown how proteolytic modifications to CXCL8 affect various inflammatory or malignant GI disease states. See Figure 1A and B and Tables 3 and 4 for a review of a CXCL8 signaling and in promoting inflammation and neoplasia.

Table 3 The role of CXCL8 in promoting inflammation

Function	Mechanism(s)	References
Increased tissue recruitment of PMNs	– Enhanced egression of PMNs from the bone marrow – Extravasation of PMNs from the vasculature through the promotion of firm adhesion to the endothelium via inside-out signaling to PMN integrins – Induction of PMN chemotaxis toward infectious/inflammatory sites within tissues as an intermediate chemoattractant	151–169
Enhanced respiratory burst in neutrophils	– Priming of NADPH-oxidase activity thorough the upregulation of fMLF receptors, increased intracellular calcium concentrations, and the phosphorylation of its p47^{phox} and p67^{phox} subunits	186–192
Enhanced PMN degranulation	– Induction of degranulation through the activation of CXCR1 and CXCR2 on PMNs	185,213–215
Preclinical studies and early clinical trials with CXCL8, CXCR1/2 inhibitors	– CXCR2 inhibitors used in colorectal cancer models, arthritis, IBD (DSS), and clinical trials for RA, COPD, asthma, and psoriasis	298–312

Abbreviations: PMNs, polymorphonuclear cells; NADPH, nicotinamide adenine dinucleotide phosphate; fMLF, formyl-methionyl-leucyl phenylalanine; IBD, inflammatory bowel disease; DSS, dextran sulfate sodium; RA, rheumatoid arthritis.

MMP-9-mediated proteolytic processing

Several proteases capable of processing CXCL8 have been shown to be elevated in GI disease states. MMP-9 is associated with a variety of GI disease states, and it has been shown experimentally that intestinal epithelial MMP-9 expression aggravates experimental colitis and delays wound healing.¹²⁷ Expression of this protease is also elevated in many patients with active IBD and colorectal or gastric cancer, and heightened expression is associated with poor cancer disease prognosis.^{128–138} Additionally, *H.*

pylori infection is associated with elevated MMP-9 expression in an NF- κ B-dependent manner,^{139–141} and pathogen eradication results in attenuated MMP-9 expression.¹⁴² High expression of MMP-9 and CXCL8-related chemokines has been observed in animals infected with *C. rodentium*¹⁴³ and in vivo models of colitis-associated colon cancer.¹⁴⁴ In addition, serum MMP-9 and CXCL8 are concomitantly elevated in patients with stages II–IV CRC,¹⁴⁵ and elevated MMP-9 expression is associated with increased CXCR1 and CXCR2 in gastric biopsy tissues collected from patients

Table 4 Role of CXCL8 in promoting tumorigenesis and malignancy in gastric and intestinal carcinomas

Function	Mechanism(s)	References
Increased angiogenesis	– Recruitment of inflammatory cells that express proangiogenic factors – Induction of MMP-2, MMP-9, and VEGF – Induction of endothelial cell proliferation and capillary tube rearrangement – Inhibition of endothelial cell apoptosis through the upregulation of antiapoptotic proteins (ie, Bcl-X_L, Bcl-X_S)	145,240,246, 249,254–261
Increased epithelial-to-mesenchymal transition	– Increased expression of EGFR, MMP-9, and VEGF by gastric and intestinal epithelial cells – Decreased expression of E-cadherin by gastric and intestinal epithelial cells – Enhanced migratory and invasive properties in gastric and intestinal epithelial cells	77,257
Enhanced recruitment of myeloid-derived suppressor cells to the tumor microenvironment	– Suppression of antitumor immune responses	84,278
Enhanced migration and invasion of cancer cells	– Increased angiogenesis – Possibly due to increased FAK and Src phosphorylation – Noted in prostate, melanoma, colon, and gastric cancers	78,261
Resistance to chemotherapy	– Decreased sensitivity to oxaliplatin due to enhanced CXCL8-dependent NF-κB signaling, triggering the upregulation of antiapoptotic proteins	261,267,281, 285,291
Oncogenic mutations or loss of tumor suppressor genes can increase CXCL8 signaling	– Activating mutations in Ras-GTPase or loss of functional PTEN can increase CXCL8 signaling as can Wnt/β-catenin	261,267,292, 298
Inflammation/tumor environment can increase CXCL8-CXCR1/2 signaling	– Hypoxia, acidosis, altered glucose metabolism, TNFα, and IL-1β – CXCL8-CXCR1/2 signaling can also activate numerous transcription factors, including NF-κB, AP-1, β-catenin/TCF, STAT3, and HIF	261,300
Neutralizing antibodies or other inhibitors of CXCL8-CXCR1/2 reduce tumor burden	– Numerous animal studies show efficacy of neutralizing antibodies and small molecule inhibitors (several are now in clinical trials) – Reduction in angiogenesis, cell proliferation, and cancer-associated inflammation	261

Abbreviations: MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor; GTPase, guanosine triphosphatase.

with gastric cancer.¹⁴⁶ Although both MMP-9 and CXCL8 are co-expressed in several GI disease states, it remains unknown whether proteolytic processing of CXCL8 by MMP-9 contributes to disease pathogenesis. However, in vivo studies have demonstrated that manipulation of MMP-9 alters susceptibility to experimental colitis, as inhibition of MMP-9 activity in mice is associated with decreased PMN accumulation and decreased expression of the CXCL8-related chemokine CXCL5.¹⁴⁷ Overexpression of intestinal epithelial MMP-9 in vivo enhanced animal susceptibility to DSS- or *S. typhimurium*-induced colitis, as was evidenced by increased intestinal PMN accumulation and CXCL1 expression.^{148,149} Collectively, these results demonstrate that MMP-9 is capable of modifying GI disease states, but additional research is required to examine whether MMP-9-mediated proteolytic processing of CXCL8 contributes to various GI disease states.

CXCL8 contributes to the tissue recruitment of neutrophils

Bone marrow egression and extravasation

A multitude of research findings have established that CXCL8 plays numerous roles in the recruitment of PMNs into GI tissues. CXCL8 has been shown to contribute to PMN bone marrow egression, extravasation, and tissue migration. Although not all the experimental studies have been conducted in GI tissues, the following provides a framework for describing how CXCL8 contributes to the recruitment of PMNs in GI tissues. The initial recruitment of PMNs requires their mobilization from the bone marrow into the circulation. During homeostasis, PMNs are largely retained within the bone marrow and only ~2% are found in circulation.¹⁵⁰ As in vivo experiments have shown, the retention of PMNs in the bone marrow involves antagonism between CXCR2 and CXCR4, whereby the CXCR4 ligand, CXCL12 (stroma cell-derived factor 1; SDF-1), retains PMNs within the bone marrow by enhancing receptor–ligand interactions between PMN integrins and surface receptors on bone marrow endothelial and stromal cells.^{151–153} During an acute inflammatory response, including GI inflammation, the release of granulocyte colony-stimulating factor decreases CXCL12 expression, while simultaneously increasing the expression of CXCR2 ligands, including CXCL8, by endothelial cells to promote PMN bone marrow egression.¹⁵⁴ This has been demonstrated during in vivo studies showing that intravenous administration of CXCL8 induces rapid mobilization

of PMNs and their progenitor cells from the bone marrow into the bloodstream.^{155,156}

Following this, PMNs exit the vasculature and enter tissues via postcapillary venules in a series of highly characterized steps.^{157–159} Briefly, PMN extravasation is subdivided into five overlapping steps: tethering, rolling, firm adhesion, crawling, and transmigration.¹⁵⁷ Exposure to proinflammatory stimuli induces the release of a multitude of proinflammatory mediators, such as histamines, leukotrienes, and cytokines. These mediators are released from tissue-resident immune and epithelial cells that induce expression of P- and E-selectin molecules on the luminal surfaces of endothelial cells. These molecules bind glycosylated ligands, such as P-selectin glycoprotein 1 (PSGL-1), on the surface of PMNs to induce PMN tethering and, subsequently, PMN rolling.¹⁶⁰ Firm adhesion of PMNs to the vascular endothelium occurs as rolling PMNs contact ELR+ PMN chemokines, such as CXCL8. Endothelial cells are capable of transcytosing CXCL8, produced by various cell types, to the luminal surface where it is subsequently bound to heparan sulfate,^{28,161} this ensures that secreted chemokines are immobilized and able to induce firm adhesion and migration in circulating PMNs.^{162,163} Binding of CXCL8 to its receptor triggers a signaling cascade that enhances the binding affinity and avidity of PMN integrin molecules, such as CD11a/CD18 and CD11b/CD18. This enables them to strongly bind endothelial surface ligands, such as intercellular adhesion molecules, and undergo firm adhesion.^{160,164,165} Following this, PMNs crawl across the surface of the endothelium and, subsequently, preferentially migrate into host tissues via tricellular endothelial cell corners.^{166,167} Collectively, these results demonstrate that CXCL8 participates in PMN bone marrow egression and extravasation during inflammatory responses, including those in the GI tract.

PMN tissue migration

CXCL8 is also involved in directing extravasated PMNs through GI tissues to an infectious or inflammatory site. Following extravasation, PMNs migrate through multiple and sequential chemotactic gradients within host tissues.¹⁶⁸ These gradients are classified into a hierarchy of intermediate and end-target chemoattractants. End-target chemoattractants are produced immediately at the inflammatory site and are the final chemotactic gradients PMNs will migrate through; these are host- or microbially-derived factors located in the immediate vicinity of an infectious or inflammatory site. Examples include bacterial formylated peptides and the complement fragment C5a. Intermediate

chemoattractants are host-derived factors that direct PMNs toward end-target chemoattractants and are produced at distances farther from the infectious or inflammatory site. End-target chemoattractant signaling cascades override those generated from intermediate chemoattractants. Therefore, intracellular signaling cascades from intermediate chemoattractants, such as CXCL8, via the p38 MAPK pathway are superseded by phosphatidylinositol-3-kinase (PI3K) signaling following PMN receptor binding of end-target chemoattractants.¹⁶⁹ For example, *S. typhimurium* infection of the intestinal epithelium drives expression of CXCL8 and the arachidonic acid metabolite lipoxin A₄; CXCL8 promotes PMN recruitment to the basolateral membrane, and lipoxin A₄ induces PMN transepithelial migration.^{170–172} Taken together, these results demonstrate that CXCL8 plays an important role in directing extravasated PMNs toward infectious or inflammatory sites until its signal is overridden by end-target chemoattractant signals.

CXCL8 aids in the activation of neutrophil antimicrobial machinery

Respiratory burst

Following directed chemotaxis to an inflammatory or infectious site, PMNs utilize a variety of mechanisms to clear host tissues of invading or translocated microbes, including the respiratory burst. This process is essential to host defense against a variety of infectious organisms, as has been demonstrated in patients with chronic granulomatous disease. Individuals with chronic granulomatous disease are highly susceptible to a variety of catalase-positive bacterial and fungal infections, due to mutations in genes essential for the respiratory burst.^{173,174} Similarly, genetic deletion of factors essential for the respiratory burst enhances susceptibility to in vivo infection.^{175–178} The respiratory burst involves rapid utilization of oxygen, increased glucose consumption, and the release of ROS.¹⁷⁹ In PMNs, production of ROS involves the assembly of the multicomponent enzymatic nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase (NOX2) complex. This complex is composed of several subunits distributed throughout the cytosol and cell membranes of inactive PMNs. The flavocytochrome b₅₅₈ subunit, composed of gp91^{phox}/Nox2 and p22^{phox}, is located on the plasma membrane and within specific granules, while the other components (p67^{phox}, p47^{phox}, p40^{phox}, and the small G-protein Rac2) are cytosolic proteins.¹⁸⁰ Assembly and activation of the NOX2 system on plasma and phagosomal membranes occur following PMN exposure to and phagocytosis of microbes.^{181–183} This process initially results in the production of superoxide

anion radicals (O₂^{•−}) by the NADPH-oxidase (NOX) system, whereby O₂^{•−} is subsequently converted to other oxygen radicals, such as H₂O₂.¹⁸⁴

CXCL8 itself does not induce NOX2 activity and, therefore, overt production of ROS. This has been shown experimentally in mice transgenically engineered to continually express human intestinal epithelial CXCL8; these animals show extensive PMN tissue infiltration, but lack apparent histological signs of mucosal injury that could be, potentially, induced by ROS.¹⁸⁵ Rather, CXCL8 pretreatment has been shown to enhance ROS production when PMNs are subsequently treated with formylated bacterial peptides (such as formyl-methionyl-leucyl phenylalanine [fMLF]), phorbol 12-myristate 13-acetate, or platelet activating factor.^{186,187} This appears to occur via the CXCR1 receptor.^{188,189} Importantly, this CXCL8-mediated priming of NOX2 enhances the microbicidal activity of PMNs.¹⁹⁰ Ongoing research suggests that CXCL8-mediated priming of NOX2 activity in PMNs may occur via multiple mechanisms. PMNs pretreated with CXCL8 have elevated surface expression of the fMLF receptors, which is postulated to contribute to enhanced fMLF-induced ROS production.¹⁹¹ Separately, it has been demonstrated that increased intracellular Ca²⁺ is required for activation of the NOX2 system, potentially via activation of phospholipase A₂.^{192–194} Accordingly, CXCL8 pretreatment has been shown to increase intracellular Ca²⁺.¹⁹⁵ Finally, the phosphorylation of the p47^{phox} and p67^{phox} subunits of the NOX2 system has been suggested to be essential for its activation.^{196–198} CXCL8 pretreatment has also been shown to induce the pre-assembly of the NOX2 system within PMN lipid rafts via phosphorylation of p47^{phox} and p67^{phox}.¹⁹⁹ Collectively, these results demonstrate that CXCL8 plays a critical role in the production of ROS within PMNs.

It is well known that chronic GI inflammatory states are capable of progressing to various GI cancers.²⁰⁰ Although the NOX2 system is vital in microbial elimination, the production of ROS also results in bystander damage to host tissues that can predispose to the development of cancer. PMN-derived oxidants are found in excess in mucosal biopsy tissues collected from areas of active inflammation in patients with IBD²⁰¹ and gastric biopsies collected from patients with chronic *H. pylori* infection.^{202,203} Furthermore, ROS production has been shown to contribute to intestinal epithelial damage in patients with IBD.²⁰⁴ This ongoing damage during chronic inflammatory states triggers a multitude of changes within host tissues that can result in the development of cancer. Indeed, tissues collected from patients with either *H. pylori* infection or IBD have increased rates

of DNA damage, elevated mutation levels, and defective DNA mismatch repair systems.^{205–209} Furthermore, biopsies collected from areas of active inflammation in patients with UC demonstrated higher mutation rates in the tumor suppressor p53 gene; this was attributed to an increase in ROS production.²¹⁰ Currently, it remains unknown whether CXCL8-mediated priming of the NOX2 NADPH-oxidase system is involved in promoting the progression of chronic GI inflammation to malignancy, and additional studies would be required to confirm these hypotheses.

Degranulation

PMN granules are storage organelles containing a plethora of noxious antimicrobial compounds and divided into three subsets based on their contents, order of production, and release within host tissues.¹⁸⁰ Primary granules (also known as azurophilic granules) are positive for myeloperoxidase and, therefore, have a critical role in the potentiation of the respiratory burst.²¹¹ Furthermore, these granules contain a bevy of antimicrobial compounds such as defensins, lysozyme, and bactericidal/permeability-increasing protein and three serine proteases: cathepsin G, neutrophil elastase, and proteinase 3.²¹² These are the first granules formed during PMN differentiation.²¹³ Secondary granules (also known as specific granules) contain lactoferrin and a variety of antimicrobial compounds, including neutrophil gelatinase-associated lipocalin, cathelicidin, and lysozyme.²¹² These are formed after primary granules.²¹³ Finally, tertiary granules (also referred to as gelatinase granules) contain a large number of metalloproteinases;²¹² these are the last granules formed during PMN differentiation.²¹³ The utilization of these granules by PMNs occurs in an order opposite to their production during differentiation. Degranulation of PMN tertiary granules occurs during migration through the basement membrane. It has been postulated that metalloproteinases degrade components of this structure to ease PMN migration,^{214,215} while degranulation of primary and secondary granules occurs once PMNs have arrived at the inflammatory site.

The initial identification and characterization of CXCL8 as a PMN-activating compound demonstrated its ability to induce PMN degranulation.^{10,216–218} Follow-up experiments demonstrated that CXCL8 was capable of inducing PMN degranulation of primary, secondary, and tertiary granules via CXCR1 and CXCR2.^{188,219,220} Interestingly, tertiary granule exocytosis and release of MMP-9 was reported to occur downstream of CXCR2.²²¹ Additionally, the release of antimicrobial peptides also serves as a chemoattractant

for T-cells.^{222,223} CXCL8 is subject to modification via numerous proteases elevated in several infectious, inflammatory, or cancerous states of the GI tract, where elevated CXCL8 has been concomitantly reported. In these states, PMN degranulation products represent an abundant source of proteases capable of proteolytically processing CXCL8. PMN elastase is increased in fecal samples collected from patients with CD and UC,²²⁴ while separate reports have indicated that its antagonists, elafin and secretory leukocyte protease inhibitor (SLPI), are significantly decreased.^{225–227} Elevated PMN elastase is reported in patients with CRC,²²⁸ while *H. pylori* infection results in suppression of SLPI.²²⁹ As such, the increased expression of proteases coupled with decreased antiprotease expression may lead to heightened proteolytic activity within GI tissues and, as a result, enhanced processing of CXCL8. Additional research is required to investigate this hypothesis. To date, it has been shown that exogenous administration of elafin and SLPI reduces inflammation in in vivo models of colitis, whereby a decrease in PMN tissue infiltration and expression of CXCL8-related chemokines was observed.^{226,227} However, little research has examined whether the protease:antiprotease imbalance results in modifications to CXCL8 that contribute to pathology associated with IBD, various cancers, or other GI disease states.

Interestingly, certain GI pathogens have been shown to release proteases that proteolytically process CXCL8 and alter their effects on PMNs. *Entamoeba histolytica* cysteine protease 2 cleaves CXCL8, and this product is more effective at inducing PMN chemotaxis.²³⁰ In contrast, *Giardia duodenalis* cathepsin B cysteine proteases were shown to degrade CXCL8 and attenuate CXCL8-induced PMN chemotaxis.²³¹ This research may explain how in vivo *G. duodenalis* infections were capable of attenuating granulocyte infiltration induced by intrarectal instillation of *C. difficile* TcdA/TcdB or reducing CXCL chemokine expression from ex vivo inflamed intestinal mucosal biopsy tissues.²³² It remains unknown whether other GI pathogens produce proteases or factors capable of modifying CXCL8 and whether this could, potentially, affect the pathogenesis of infection.

Involvement of CXCL8 in malignant processes

Over expression of CXCL8 and CXCR1/2 has been described in numerous inflammatory and neoplastic disease states. The link between chronic inflammation and neoplasia has been well described, and cancer-associated inflammation, due to changes in tumor microenvironment or changes within the

tumor cell, can be associated with increased tumor growth, metastasis, resistance to therapy, all of which impact prognosis and survival (see Figure 1A and B and Tables 3 and 4 for a review a CXCL8 signaling and in promoting inflammation and neoplasia).

CXCL8 induces angiogenesis

Angiogenesis, or the formation of new blood vessels, involves a sequential sequence of events beginning with basement membrane proteolysis, endothelial cell proliferation and chemotaxis, and finally, organization and maturation of tubular structures. This process has long been recognized as an integral hallmark in cancer development,²³³ and disrupting angiogenesis has been proposed as an effective therapeutic target for a variety of cancers.²³⁴ Indeed, angiogenesis is essential to providing the primary neoplasm with an adequate blood supply and contributes to cancer metastasis.^{235,236} The induction of angiogenesis occurs early during the development of multistage cancers and has been observed in histological specimens collected from premalignant, noninvasive lesions such as dysplasias and in situ carcinomas.²³⁷ It is thought that tumor progression induces activation of variety of angiogenic switches that promote surrounding vasculature to produce new vessels.²³⁸ These newly formed blood vessels tend to be heterogeneous in size and structure, are poorly formed, and have inadequate function.^{239–241} The angiogenic switches responsible for new blood vessel formation have been proposed to occur through direct and indirect mechanisms. Oncogenes within tumor cells may directly induce expression of angiogenic factors. Alternatively, these angiogenic factors may be produced indirectly by infiltrating inflammatory cells, including macrophages, PMNs, mast cells, and myeloid progenitor cells.²⁴² Indeed, individuals with chronic inflammatory disorders are at increased risk of developing cancer, which may be, at least in part, due to expression of angiogenic factors produced by infiltrating inflammatory cells.²⁴³ For example, it has been well established that chronic inflammation with *H. pylori* infection is associated with increased risk of gastric cancer.²⁴⁴ Similarly, individuals with IBD are at increased risk of colon cancer via a variety of mechanisms that remain incompletely understood.¹

CXCL8 and related chemokines have been demonstrated to have oncogenic properties capable of inducing angiogenesis;^{245–247} this occurs independently of their ability to induce PMN chemotaxis^{248–250} and results via activation of CXCR2.^{251–253} Indeed, mucosal biopsy tissues collected from patients with gastric carcinomas have increased CXCL8 levels

that parallel increased tumor vascularity.²⁵⁴ Furthermore, one study has suggested that the survival rate of patients with gastric carcinomas was significantly reduced in patients with tumors expressing high levels of CXCL8.²⁵⁵ A variety of signaling molecules produced during tumor metabolism may induce CXCL8 secretion and, resultantly, promote tumor angiogenesis. For example, the production of lactate has been shown to induce autocrine CXCL8 production and thereby promote tumor angiogenesis in an in vivo model of CRC.²⁵⁶ Experimentally, increased CXCL8 expression in vivo enhanced blood vessel formation, while genetic deletion of CXCR2 blunted this effect.²⁵⁷ Research has now demonstrated that CXCL8 contributes to angiogenesis via multiple mechanisms, including inducing expression of vascular endothelial growth factor²⁵⁸ as well as MMP-2 and MMP-9 within endothelial cells; these two factors are involved in basement degradation during angiogenesis.²⁴⁹ In patients with CRC, a concomitant increase in serum CXCL8 and MMP-9 is observed in patients with stage II disease or higher.¹⁴⁵ CXCL8 is also capable of inducing endothelial cell proliferation and capillary tube reorganization in a concentration-dependent manner, whereby lower rather than higher concentrations are more effective at inducing these effects.²⁵⁹ Finally, CXCL8 has also been shown to inhibit endothelial cell apoptosis via increasing expression of antiapoptotic proteins, such as Bcl-X_L and Bcl-X_s.^{249,260} Similarly, in in vivo models of CRC, the transgenic expression of CXCL8 in animals results in enhanced tumor burdens that were, at least partially, due to increased tumor angiogenesis.²⁶¹ CXCL8 and/or CXCL1/2 have also been shown to promote angiogenesis in several other cancers, including melanoma, pancreatic, prostate, and non-small-cell lung cancer.²⁶²

CXCL8 induces epithelial-to-mesenchymal transition, migration, and invasion

Tumor epithelial-to-mesenchymal transition (EMT) is a process involving the dedifferentiation of epithelial cells into an invasive mesenchymal phenotype. This process involves the loss of epithelial cell characteristics, including a loss in cell polarity, cell–cell contacts, epithelial surface markers, and a concomitant increase in mesenchymal properties. The properties include increased expression of mesenchymal-associated proteins, enhanced cell motility, and metastatic potential.²⁶³ Indeed, in vitro experiments have demonstrated that gastric epithelial cell lines treated with exogenous CXCL8 increased expression of the epidermal growth factor receptor, MMP-9, and vascular endothelial growth factor; at

the same time, this treatment decreased E-cadherin expression and increased the invasive potential of these cells.²⁶⁴ Clinical research has indicated that CXCL8 levels are positively associated with the extent of tissue invasion and metastasis. In patients with CRC, elevated levels of CXCL8 have been positively associated with tumor size, level of infiltration, and liver metastases.^{265,266} Heightened expression of both CXCL8 receptors in gastric mucosal biopsy tissues has been associated with gastric cancer metastasis.¹⁴⁶ CXCL8 tissue expression levels have been reported to increase as colonic adenocarcinoma cells become less differentiated, while serum levels have been shown to increase in proportion to the pathological staging of CRC tumors and distant metastases.²⁶⁷ These positive correlations between CXCL8 and tissue invasion and metastasis are further supported by in vitro experiments, whereby knockdown of CXCR1 and CXCR2 proteins inhibited the migratory capability of gastric epithelial cells.¹⁴⁶ Similarly, overexpression of CXCL8 in in vitro colonic epithelial cell lines enhanced cellular proliferation, migration, and invasive potential of these cells.²⁶⁸ Studies in prostate cancer found that CXCL8 enhances tumor cell migration and invasion via activation of Src and FAK.²⁶⁹ Moreover, in vitro experiments using a variety of colonic epithelial cell lines have demonstrated that expression of CXCL8 parallels metastatic potential, whereby increased expression of CXCL8 and its receptors is found in cell lines with higher invasive potential.²⁷⁰ Indeed, CXCL8 and its receptors are downstream targets for several EMT transcription factors.^{271,272} The overexpression of CXCL8 by in vitro CRC cell lines has also been shown to act as an autocrine growth factor.²⁷³ Furthermore, CXCL8 may also confer chemoresistance, as pretreatment of in vitro colonic and gastric epithelial cells with CXCL8 resulted in enhanced cell survival to oxaliplatin treatment.^{268,274,275} Moreover, CXCL8 treatment has been shown to reduce in vitro epithelial sensitivity to apoptosis.²⁷⁶ Importantly, inhibition of CXCR2 signaling in intestinal epithelial cells has been shown to increase sensitivity to oxaliplatin.²⁷⁷ Based on the above data, it has been proposed that targeting the CXCL8 pathway represents a potential therapeutic target for gastric cancer and CRC.²⁷⁸

Myeloid-derived suppressor cells

Immature myeloid cells are a heterogeneous group of cells and comprise numerous subpopulations, including myeloid-derived suppressor cells (MDSCs). Experimental evidence has shown that these cells are not fully differentiated and, therefore, express surface markers for both granulocytic and

monocytic cell types.²⁷⁹ Ongoing research has implicated MDSCs in the pathophysiology of numerous cancers.²⁸⁰ In patients with CRC, MDSC populations are elevated both within cancerous tissue and serum; moreover, the level of elevation positively correlates with the cancer stage and extent of metastasis.^{281,282} A complete discussion of the research surrounding MDSCs is beyond the scope of this review. However, ongoing research has demonstrated that MDSCs participate in the progression of cancer via a number of mechanisms, including via the suppression of antitumor immune responses^{280,283} and promotion of angiogenesis.²⁴² As a result, targeting MDSCs has been proposed as a potential chemotherapeutic target.²⁸⁴ Experimental research has just begun to elucidate the role that CXCL8 plays in recruiting MDSCs. In in vivo models of colon cancer, mice transgenically engineered to express human CXCL8 displayed increased infiltration of immature myeloid cells into dysplastic and surrounding tissues; this ectopic expression of CXCL8 was associated with enhanced angiogenesis and tumorigenesis.²⁶¹ Similarly, the in vivo deletion of CXCR2 resulted in decreased chronic inflammation and development of colitis-associated tumorigenesis.²⁸⁵ Collectively, these experimental results suggest that CXCL8 may play an important role in recruiting MDSCs to the tumor microenvironment. Additional research is required to elucidate the exact mechanisms involved in these early findings.

Resistance to chemotherapy

Resistance to chemotherapeutics is an ongoing concern, and research has suggested that autocrine or paracrine expression of CXCL8 may contribute to resistance to chemotherapeutic agents in a variety of GI cancers. Currently, CRC chemotherapy involves the use of 5-fluorouracil and oxaliplatin.²⁸⁶ Indeed, the efficacy of oxaliplatin treatment has been found to be dependent on genetic variations within the CXCL8 and/or CXCR2 genes,^{8,287} and CXCL8 serum levels have been reported to be elevated in CRC patients who fail to respond to chemotherapy.²⁸⁸ Moreover, induced expression of CXCL8 in in vitro colonic adenocarcinoma cell lines resulted in decreased sensitivity to oxaliplatin treatment, while silencing of CXCL8 production produced the opposite effect.²⁶⁸ The former was associated with increased activation of the NF- κ B pathway,²⁶⁸ which has been shown to induce transcription of genes that antagonize apoptosis.²⁸⁹ Similar results were observed using in vitro gastric adenocarcinoma cell lines.²⁷⁵ Additionally, blockade of CXCR2 signaling in in vitro colonic adenocarcinoma cell lines increased sensitivity to oxaliplatin and induced expression of apoptotic

proteins.²⁷⁷ In addition, CXCL8-induced resistance to oxaliplatin therapy may occur following activation of upstream signaling cascades. Exposure of *in vitro* adenocarcinoma cells to oxaliplatin has been shown to induce interleukin-22 expression that subsequently promotes CXCL8 expression and resistance to 5-fluorouracil and oxaliplatin therapy.²⁷⁴ Gemcitabine is a commonly used chemotherapeutic agent used for treatment of pancreatic ductal adenocarcinomas.²⁹⁰ However, gemcitabine resistance is often reported,²⁹¹ and experiments performed *in vivo* and *in vitro* have demonstrated that gemcitabine treatment induces CXCL8 expression in pancreatic cancer cells that subsequently results in neovascularization.²⁹² Collectively, these results demonstrate that CXCL8 is involved in resistance to chemotherapeutic drugs in GI cancers, and targeting CXCL8 or its upstream inducers enhance the efficacy of chemotherapeutic agents. See Table 4 for a review of processes by which CXCL8 can impact neoplasia.

Specific targeting of CXCL8–CXCR1/2 signaling in cancer therapy

As noted above, CXCL8 and CXCR1/2 are upregulated in several cancers, and it is felt that cancer-related inflammation results in increased migration, invasiveness, cancer cell resistance to apoptosis, and increased cancer cell proliferation.^{262,268,293} Not only can chronic inflammation increase the risk of cancer (up to 20% of all cancers may result from chronic inflammation) but also cancer cells and cancer growth often promote inflammation.^{262,294} Numerous signaling pathways can be activated downstream of CXCL8–CXCR1/2, including MAPK, PI3K/Akt, PKC, FAK, and Src, all of which have been implicated in neoplasia and inflammatory processes. CXCL8–CXCR1/2 signaling can

also activate numerous transcription factors, including NFκB, AP-1, β-catenin/Tcf, STAT3, and HIF, which can lead to upregulation of other receptor-signaling pathways, such as epidermal growth factor receptor and steroid receptors.^{262,295,296} CXCL8 signaling can be induced by numerous factors associated with both neoplasia and inflammation, including TNFα, IL-1β, ROS, death receptors (eg, Fas ligand), some steroid hormones (eg, androgens), hypoxia, acidosis, altered glucose levels, chemotherapeutic agents, and radiation therapy.^{262,297} Furthermore, the CXCL8 promoter contains consensus sites for transcription factors that are altered in inflammation and neoplasia, including NFκB, AP-1, β-catenin/Tcf, and HIF transcriptional factors.^{262,296} Oncogenic mutations and/or loss of tumor suppressor genes can alter CXCL8–CXCR1/2 signaling resulting in inflammation and cancer progression.^{262,296,298,299} See Table 4 for further details.

In view of the link between inflammation and cancer, several approaches have targeted inflammatory pathways in the fight against cancer, including the NFκB pathways and, more specifically, CXCL8–CXCR1/2 signaling. Numerous agents have been developed to block CXCL8–CXCR1/2 signaling directly or indirectly, including: 1) blocking signal transduction (inhibitors of MAPK pathways; ERK, JNK, p38, PI3K/Akt) that leads to CXCL8–CXCR1/2 expression; 2) NFκB inhibitors (downregulation of proinflammatory genes mediating, recruitment/invasion, apoptosis, proliferation, angiogenesis, and more specifically, CXCL8–CXCR1/2 expression); 3) nonsteroidal anti-inflammatory drugs (reduce inflammation and can decrease CXCL8–CXCR1/2 expression); and 4) direct targeting of CXCL8–CXCR1/2 signaling.²⁶² The main approaches to directly target CXCL8–CXCR1/2 signaling include: 1) CXCL8

Sequencing of the mouse genome has identified orthologs to numerous ELR+ CXCL chemokines on chromosome 5, with the exception of CXCL8.³⁰⁴ Moreover, *in vivo* research has demonstrated that the mouse genome contains ELR+ CXCL chemokines capable of inducing similar activities within murine PMNs when compared to their human orthologs,³⁰⁵ and it has been proposed that these chemokines have functionally replaced CXCL8.^{306,307} Initial experiments also suggested that ELR+ chemokine receptor ligation and signal transduction were mediated by CXCR2^{308,309} and, furthermore, that CXCR1 was functionally inactive.^{310,311} However, additional studies predicted the existence of another CXCR receptor capable of binding human CXCL8 with higher affinity than mCXCR2.³⁰⁸ Indeed, murine CXCR1 was subsequently identified, and its expression was found in lung, spleen, thymus, peripheral blood leukocytes, and isolated PMNs; in addition, it was shown that mCXCR1 plays an important role in several experimental models of inflammation.^{312,313} These studies demonstrate that the mouse genome has evolved in the absence of CXCL8 and that it has been functionally replaced by structurally similar chemokines that are capable of binding CXCR1 and CXCR2. Since then, CXCL8 has been ectopically expressed in several models of GI inflammation and cancer.^{185,261} This expression of CXCL8 in *in vivo* mouse models will help us to further understand the role that CXCL8 plays in a variety of GI inflammatory disorders and malignancies.

Figure 2 CXCL ELR+ chemokines in mice.

Table 5 CXCR1/2 small molecule antagonists in development for inflammation and neoplasia

Receptor	Antagonist	Preclinical	Clinical trials
CXCR1	Reparixin	Breast cancer xenographs	Breast cancer, islet transplant
CXCR1/2	DF2162	Breast cancer xenographs	Breast cancer, islet transplant
CXCR2	SCH527123	Colorectal xenographs	Ozone-induced lung inflammation, COPD, asthma
CXCR2	SB225002	Colitis	Ozone-induced lung inflammation, COPD, CF, UC
CXCR2	SB656933	Colitis	Ozone-induced lung inflammation, COPD, CF, UC
CXCR2	AZD8309		RA, COPD, asthma, bronchiectasis
CXCR2	AZD5069		RA, COPD, asthma, bronchiectasis

Note: See Campbell et al for further details.²⁶²

Abbreviations: CF, cystic fibrosis; UC, ulcerative colitis; RA, rheumatoid arthritis.

neutralizing antibodies; 2) CXCR1/2 neutralizing antibodies; and 3) small molecule CXCR1/2 antagonists and siRNA strategies. In summary, many studies have shown that CXCL8 and CXCR1/2 neutralizing antibodies, small molecule inhibitors, and siRNA can reduce inflammation and tumor growth in vitro, and some in preclinical models.²⁶² More specifically, these agents can reduce inflammation in animal models of arthritis models, IBD (DSS³⁰⁰ and 2,4,6-trinitrobenzenesulfonic acid³⁰¹ colitis), asthma, and tumor burden and metastasis in CRC models and other cancer cell types.^{262,277,302,303} Early clinical trials with primarily small molecule CXCR1/2 inhibitors are under way for IBD (UC), asthma, COPD, psoriasis, rheumatoid arthritis, solid organ transplant rejection, melanoma, CRC, breast cancer, and other malignancies²⁶² (see Table 5).

Conclusion

Since its initial discovery, our understanding of how CXCL8 contributes to various GI inflammatory and malignant states has vastly improved. Clinical research has demonstrated that CXCL8 is elevated in a variety of disease states, including GI infection, IBD exacerbations, and GI malignancy, and CXCL8 levels have been shown to correlate with predicted clinical outcomes. In addition, in vitro and in vivo experiments have suggested potential mechanisms via which these processes occur. Much research has focused on CXCL8's ability to induce various signaling events within PMNs, and indeed, experimental research has demonstrated that this factor contributes to a variety of proinflammatory PMN events, including its contribution to the oxidative burst, chemotaxis, and degranulation. However, research

has also demonstrated that CXCL8 contributes to increased angiogenesis and EMT during malignancy via its actions on endothelial and epithelial cells, respectively. In addition, CXCL8 promotes the accumulation of MDSCs in GI tissues during malignancy. As our understanding of CXCL8 has improved, it has become apparent that certain CXCL8 polymorphisms are associated with altered susceptibility to a variety of disease states, including GI infection, IBD, and gastric cancer. Despite these findings, our understanding of the role that CXCL8 plays in the pathophysiology of various GI inflammatory and malignant disorders remains incompletely understood. Further understanding of the role of CXCL8–CXCL1/2 signaling in such events may help to increase our understanding of the pathophysiology of various disease states and, potentially, contribute to the further development of novel therapeutics.

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Disclosure

The authors report no conflicts of interest in this work.

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