The G-Protein Coupled Formyl Peptide Receptors and Their Role in the Progression of Digestive Tract Cancer

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Abstract

Chronic inflammation is a causative factor of many cancers, although it originally acts as a protective host response to the loss of tissue homeostasis. Many inflammatory conditions predispose susceptible cells, most of which are of epithelial origin, to neoplastic transformation. There is a close correlation between digestive tract (DT) cancer and chronic inflammation, such as esophageal adenocarcinoma associated with Barrett's esophagus, *helicobacter pylori* infection as the cause of stomach cancer, hepatitis leading to liver cirrhosis and subsequent cancer, and colon cancer linking to inflammatory bowel diseases and *schistosomiasis*. A prominent feature of malignant transformation of DT tract epithelial cells is their adoption of somatic gene mutations resulting in abnormal expression of proteins that endow the cells with unlimited proliferation as well as increased motility and invasive capabilities. Many of these events are mediated by Gi-protein coupled chemoattractant receptors (GPCRs) including formyl peptide receptors (FPRs in human, Fprs in mice). In this article, we review the current understanding of FPRs (Fprs) and their function in DT cancer types as well as their potential as therapeutic targets.

An Overview of Formyl Peptide Receptors

Formyl peptide receptors (FPRs in human, Fprs in mice) are members of the seven transmembrane, G-protein coupled chemoattractant receptor (GPCRs) family, which includes "classical chemoattractant GPCRs" and "chemokine GPCRs," critical to immune cell trafficking during infection, inflammation, immune responses, and cancer progression,[1,2] FPRs (Fprs) belong to the "classical chemoattractant GPCRs,"[3] initially identified on neutrophils but have thus far been observed in many cell types including immune cells and cells of the non-hematopoietic origin.[4-8] There are three FPRs in human: FPR1, FPR2, and FPR3, which share about 70% identity at the amino acid level. FPR1 responds with high affinity to bacterial and

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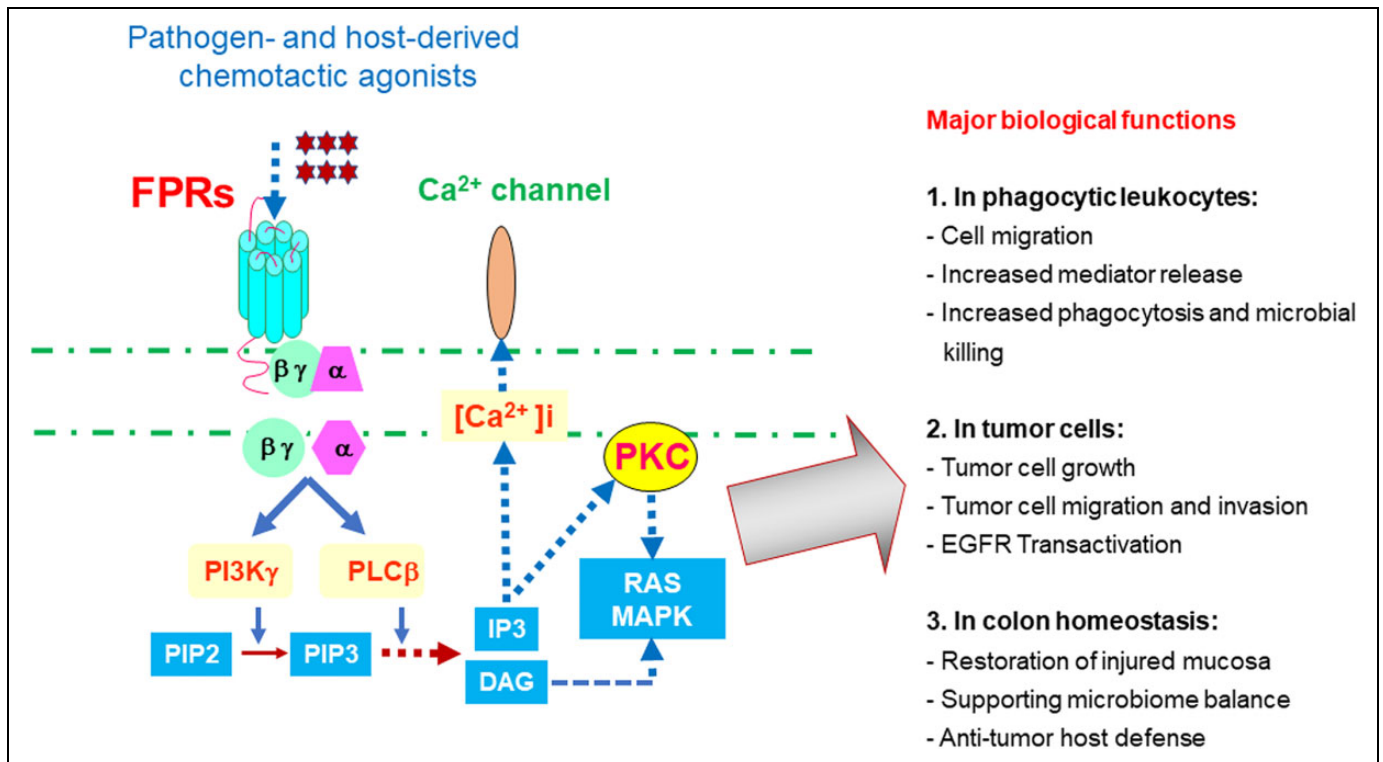
Figure 1. Signaling and function of FPRs. The binding of FPRs on leukocytes and epithelial cells by pathogen or host-derived agonists triggers Gi-protein-coupled signaling cascade resulting in increased calcium (Ca^{2+}) mobilization, cell migration, mediator release, proliferation and activation of transcription factors involved in new gene transcription. In normal myeloid cells, FPRs (Fprs) mainly mediate cell recruitment, increased phagocytosis, and microbial killing. In colon epithelial cells, FPRs (Fprs) participate in mucosal repair, balance of microbiome, and anti-cancer host defense. Activation of FPR1 in glioblastoma cells transactive the receptor for the epidermal growth factor and two receptors cooperated to exacerbate the malignant phenotype of tumor cells.

mitochondrial formylated peptides, such as formyl-methionyl-leucyl-phenylalanine (fMLF) produced by Gram- bacteria including $E.Coli$.[4] FPR2 is a more promiscuous GPCR responding to fMLF with lower affinity, but also to a variety of viral, bacterial, host-derived and synthetic peptides, many of which are not formylated and without sequence homology.[9] Activation of FPR1 and FPR2 by chemotactic agonists dissociates trimetric G-proteins coupled to the intracellular domains of the receptors, followed by a signaling cascade leading to cell activation (Figure 1).[10] Unlike FPR1 and FPR2, the expression of human FPR3 is more restricted to monocytes and probably also dendritic cells (DCs), with only one better defined host-derived, endogenous peptide agonist, F2 L,[11] presumably participating in DC recruitment *in vivo*.

The mouse FPR (mFPR or Fpr) gene family consists of at least 8 members including Fpr1 and Fpr2, as well as several genes without definitive cellular products.[9] Fpr1 is considered as an orthologue of human FPR1, whereas Fpr2 is structurally and functionally closer to human FPR2.[12,13] Although the mouse counterpart of human FPR3 is not well defined, murine Fpr2 shares the human FPR3 ligand F2 L.[14,15] and is thus suggested to act as a counterpart of both FPR2 and FPR3.[2,16] Table 1 lists major agonists of FPRs (Fprs) better known so far and their capacity to interact with one or more FPRs (Fprs).

The Major Functions of FPRS (FPRS)

One of the most important and well characterized functions of FPRs (Fprs) is their capacity to mediate myeloid cell chemotaxis in response to agonists derived from bacteria, damaged cells and other sources, including also many synthetic peptides. When activated, the receptors also enhance neutrophil phagocytosis of dead tissues and bacteria.[17] mediator (such as ROS) generation, NET (neutrophil extracellular trap) formation, and cytokine release.[18] These receptors are additionally reported to promote wound healing by recruiting inflammatory cells and maintain gut mucosal integrity (Figure 1).[19,20] Therefore, FPRs (Fprs) are critical sentinels of host innate defense. However, like many GPCRs, FPRs (Fprs) also possess the dark side of their function, i.e. promoting cancer progression by their aberrant expression in cancer cells for abnormal growth, survival and invasion.[21,22]

More recently, a fascinating aspect of the functions of FPRs, thanks to the availability of genetically engineered mice, has emerged, which represents an important conceptual advance in understanding these receptors in disease models from a temporal and spatial point of view. The first piece of evidence was obtained from a mouse model of allergic airway inflammation, in which a chemokine GPCR CCR2 mediates the mobilization of DC precursors from the circulation to the perivascular region

Table 1. Promiscuous Ligand Recognition by FPRs.

Source Diseases	Agonists	FPR1	FPR2	FPR3
		(Fpr1)	(Fpr2)	(Fpr3)
Bacteria Infection	fMLF (E. Coli)	++++	++	
	Listeria peptides	++++	+++	
Stomach cancer	Hp(2-20)	-	+++	++
Host Inflammation	Serum amyloid A	-	++++	
Alzheimer's	Aβ42, Humanin	+	++++	
Prion disease	Prp106-126	-	+++	
Angiogenesis	Annexin 1(ANXA1)	++	+++	++
Tissue damage	Mitochondria pep	+++	+++	
	F2 L (Heme)	-	++	++++
Coagulation	uPAR frag	-	+++	
Host defense	LL37 (CRAMP)	-	+++	
	Cathepsin G		+++	
Cancer	ANXA1	++	+++	

+ Denotes the relative potency of the ligands to activate a given FPR *References: 1, 2, 16).*

in an antigen stimulated, inflamed lung, where the cells lost the functional CCR2 but instead they gained the expression of high levels of Fpr2 to direct the cells across the tissue to peribronchiolar regions, where DC became mature and express a homing chemokine GPCR, CCR6, for eventual cell homing to draining lymph nodes to trigger an adaptive immune response.[23] This new paradigm thus positions Fpr2 as an essential player in a sequential DC "chemotaxis signal relay" that also involves chemokine GPCRs (CCR2-Frp2-CCR6) to complete orchestrated host immune responses. This relay of GPCR functions in a stepwise trafficking of DCs was later found to be imitated by neutrophils in at least two models of bacterial infection, namely *Listeria* and *E. coli,* both requiring rapid accumulation of neutrophils at the sites of infection for clearance of the invading pathogens.[18,24] In both models, neutrophils rapidly accumulate in the liver in response to intravenously injected pathogens in response to bacterial chemotactic peptides that activate both Fpr1 and Fpr2. This Fpr-mediated "first wave" of neutrophil recruitment proves to be vital for the host to establish in a timely manner a defense line that would be later further consolidated by subsequent waves of neutrophils accumulation in response to CXCR2 chemokines elicited by bacterial lipoproteins or LPS through TLRs expressed by liver cells. In addition, in a sterile ear skin wound, a rapid neutrophil "swarming" was initiated by leukotriene B4 that activates its classical chemoattractant GPCR, followed by Fpr2 and CxcR2, a receptor for the mouse IL-8 (CXCL8) analogue CxcL2.[25] These novel findings indicate the necessity to take consideration of the co-operation of multiple chemoattractant GPCRs for leukocyte trafficking as host responses to pathogen invasion and tissue injury, in which Fprs are indispensable participants.

The Role of FPRs (FPRS) in the Progression of DT Cancers

Chronic inflammation is a prolonged form of protective host response to tissue damage, which however has also been

recognized as a causative factor of many cancers. In fact, inflammation and neoplasia co-develop into "wounds that do not heal."[21] Leukocytes, mainly myeloid cells such as neutrophils, monocytes, macrophages, and eosinophils, produce soluble factors including metabolites of arachidonic acid, cytokines, chemokines, and free radicals, may benefit the development of inflammation-associated cancer.[26] A variety of inflammatory conditions promote susceptible cells, such as epithelial cells in the DT, to undergo neoplastic transformation. This is particularly true in segments of the DT in which chronic inflammation is associated with cancers in multiple locations, including *helicobacter pylori* infection in the development of gastric cancer, esophageal adenocarcinoma associated with reflux esophagitis (Barrett's esophagus), hepatitis promoting liver cirrhosis and subsequent cancer, and colon cancer linked to inflammatory bowel diseases (IBD, chronic ulcerative colitis and Crohn's disease) and *schistosomiasis*.[1,21] A prominent feature of malignant transformation of DT epithelial cells is their acquirement of somatic gene mutations culminating in abnormal expression of proteins that enable unlimited cell proliferation, increased motility and invasiveness, many of which are directly mediated by GPCRs, including FPRs (Fprs) (Table 2). In the following chapters, we briefly review the role of FPRs (Fprs) in the carcinogenic process, or in contrast suppression, of DT cancers, in hope to benefit the development of novel anti-cancer strategies.

Oral Squamous Cell Carcinoma (OSCC)

OSCC is an aggressive tumor type that occurs at several sites of the oral mucosa of DT. It is also the most common type of head and neck cancer.[27] Despite advances in its treatment, the 5-year survival rate of OSCC wanders at ∼50%, which is mostly due to late diagnosis.[28] LL-37 is a neutrophil-derived antimicrobial peptide that mediates chemotaxis of inflammatory cells via activation of FPRs, mainly FPR2 (Table 1).[29,30] Interestingly, LL-37 might act as a tumor suppressor in OSCC. Immunohistochemical analyses showed that lower expression of LL-37 in OSCC tissues compared to the normal mucosa tissues is correlated with poor differentiation and increased lymph node metastasis,[31] implicating a protection exerted by this antimicrobial peptide. In addition, the regulation of the gene for LL-37 in several oral cancer cell lines involves higher DNA methylation status in the promoter region, potentially the cause of the lower expression of the peptide. It had also been shown that incubation of a C-terminal domain peptide of LL-37 (aa 109-135) induces caspase-independent apoptosis in OSCC SAS-H1 cells but not in human gingival fibroblasts or HaCaT cells.[32] Thus, LL-37 and its derivatives inhibit OSCC cell growth by inducing cell death.

The anti-inflammatory protein annexin 1 (ANXA1), a 37-kDa member of the annexin superfamily, is a steroid-regulated protein implicated in certain beneficial activities of glucocorticoids.[33] ANXA1 is important in cancer progression because it was released by necrotic glioblastoma cells to stimulate live tumor cells as an autocrine/paracrine factor to stimulate FPR1

Table 2. The Role of FPRs (Fprs) in the Progression of DT Cancer.

DT cancer	FPRs	Biological function	Refs
OSCC	FPRs	The agonist LL-37 might act as a tumor suppressor, and with its derivatives inhibit OSCC cell growth by inducing cell death ANXA1 was implicated as a tumor suppressor in OSCC	31,32 33-36
EC	FPRs	ANXA 1 expression has a significant correlation to the status of ESCC differentiation	38
	FPRs	No clear information about FPRs in the pathogenesis of EAC	39,40
GC	FPR1	*In vitro*, FPR1 suppresses GC cell growth	45-47
		Higher FPR1 expression is associated with poor clinical results in patients	48
		Up-regulated ANXA1 expression is involved in cancer invasion and lymph node metastasis and were implicated in poor prognosis of patients	49,50
	FPR2	Promotes the invasion and metastasis of GC cells and predicts the poor prognosis of patients	51 44
		Hp(2-20) promotes migration and proliferation of gastric epithelial cells by interacting with FPR2 in vitro	
		LL-37 contributes to the balance between host mucosal defense and H. pylori viability that governs chronic infection	56-58
HCC	FPR1	Participates in the inflammatory courses in the liver and the progression of HCC	63-66
PC	FPRs	ANXA1 participates in many pathophysiological processes in PC cells, over-expressed in PC tissues from patients and contributes to the malignant phenotype of PC cells and their metastatic potential through FPR-independent pathways.	68-71
CRC	FPR1	A risk factor in the prognosis of CRC	74
	FPR2	Supports the malignant transformation of colon epithelial cells	74-76
	Fpr2	Protects normal colon mucosa from inflammation and carcinogenesis in mice	77
	FPR2 (Fpr2)	The agonists LL-37/CRAMP directly inhibit tumorigenesis in the colon and maintains colon microbiota balance	78-84

Abbreviations: DT: digestive tract; FPRs (Fprs in mice), formyl peptide receptors; OSCC, oral squamous cell carcinoma; EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; EAC, esophageal adenocarcinoma; GI, gastrointestinal; ANXA1, annexin 1; GC, gastric cancer; HCC, hepatocellular carcinoma; PC, pancreatic carcinoma; CRC, colorectal cancer.

for tumor progression.[34] However, ANXA1 has been reported to activate both FPR1 and 2 (Fpr1, 2) to exert an anti-inflammatory function.[8] In Tca-8113 and SCC-9 cell study, ANXA1 was also found to be a potential tumor suppressor in OSCC.[35] These un-orthodox roles of LL-37 and ANXA1 are not entirely in accordance with the conventional conception of FPRs as their receptors. Nevertheless, FPRs do mediate the chemotactic activity of these ligands for tumor cells. Therefore, it is postulated that LL-37 and ANXA1 may elicit skewed signaling cascade via FPRs in OSCC cells, or they may also activate other membrane sensors that transmit growth inhibition signals. This calls for more extensive studies of FPRs in OSCC progression.

Esophageal Cancer (EC)

Esophageal cancer remains an important cause of cancer-related death in under-developed countries, and squamous cell carcinoma (ESCC) is the predominant cancer type.[36] The lower expression of the ANXA 1 is correlated with ESCC tumorigenesis.[37] Analysis of the protein profiles of 24 pairs of ESCC and adjacent normal epithelia showed a positive correlation between ANXA 1 expression and the status of tumor differentiation. Although most of the upper esophageal cancers are of the squamous cell origin, the incidence of esophageal adenocarcinoma (EAC) of the lower esophageal section has risen rapidly over the past three decades.[38] Esophageal adenocarcinoma is a typical model of an inflammation-associated cancer.[39] Various proinflammatory states are known to be involved, including obesity and gastro-esophageal reflux disease. Release of proinflammatory mediators such as IL-6, IL-8 (CXCL8) and TGF-β by activated epithelial cells and infiltrating leukocytes promotes esophageal cell transformation, growth and invasion via activation of intracellular signaling pathways involving NF-kB and STAT3. However, there is no clear information about FPRs in the pathogenesis of EAC, which awaits clarification.

Gastric Cancer (GC)

GC or cancer of the stomach is divided into two main classes: gastric cardia cancer (cancer of the top inch of the stomach, where it meets the esophagus) and non-cardia gastric cancer (cancer in all other areas of the stomach).[40] GC is the fifth most common malignancy and the third leading cause of cancer-related deaths worldwide.[41] Although remarkable achievements in surgical and other therapeutic options have been obtained, the overall 5-year survival rate of GC patients remains low,[42] due to the advanced stage at diagnosis and the more vicious malignant nature of invasion and metastasis of the disease.

All members of FPR family, including FPR1, FPR2, and FPR3, are expressed in human GC cells,[43] but only the functions of FPR1 and FPR2 have been better understood in GC.

FPR1. The involvement of FPR1 in GC progression has been controversial. It is interesting that FPR1 is found to act as a tumor suppressor by inhibiting angiogenesis in GC xenograft experiments.[44] FPR1 silencing (shFPR1) significantly enhanced the growth of GC cell-derived xenograft tumors. This is attributed to the augmented vessel density in tumor stroma and GC cell proliferation in the absence of FPR1.[44] The genetic or pharmacologic regulation of FPR1 in GC cells is also achieved by ALOX5/15 expression and production of the SPMs Resolvin D1 (RvD1) and Lipoxin B4 (LXB4) that transduce "anti-inflammatory" signals through the receptor to reduce the rate of GC progression by inhibiting inflammatory and angiogenic processes in the tumor microenvironment.[45] To support this notion, Otani et al. reported that a specific FPR1 polymorphism, which causes loss of FPR1 function, is positively correlated with the risk of GC.[46] However, opposing results have also been reported in which FPR1 was found to be highly expressed in GC tissues, in particular in stage IV disease, in association with deeper invasion depth and poorer clinical outcome of the patients.[47] GC patients with high FPR1 expression in tumors had lower overall survival rates than those with negative/low FPR1 expression. Cox multivariate analysis confirmed that FPR1 expression was an independent prognostic marker for poorer overall survival in GC patients. Thus, FPR1 expression is suggested as a predictor of outcome of GC patients treated with gastrectomy.[47] These observations highlight the importance of taking into consideration of pro- versus anti-GC potential of FPR1 in designing treatment schemes.

FPR1 expression was significantly increased when ANXA1 was overexpressed in GC cells.[47] ANXA1 expression positively correlated with the invasiveness of human GC cells both *in vitro* and *in vivo*. *In vivo*, ANXA1 regulates GC cell invasion through FPR1-associated extracellular signal regulated kinase/integrin β-1-binding protein pathway.[48] Elevated ANXA1 expression was observed in 76 of 135 cases of GC and correlations were found between ANXA1 expression and the depth of organ wall invasion, lymphatic/venous involvement, and node metastasis. Thus, up-regulated ANXA1 expression is implicated in poor prognosis of GC patients,[49] and ANXA1, similar to FPR1, is also suggested as as a prognostic biomarker in gastric (and colon) cancer and a potential target for treatment.

FPR2. FPR2 was expressed more frequently in GC cancerous tissues than in adjacent tissues and increased expression levels in cancerous tissues were correlated with the tumorigenesis, metastasis as well as the poor survival of the patients.[50]

The ability of FPR2 to promote tumorigenesis of GC cells is demonstrated in observation in which both FPR2-knockdown SGC7901 or XN0422 GC cells formed substantially smaller xenograft tumors in nude mice as compared with control mock cells.[50]

It has also been demonstrated that chronic inflammation resulted from Helicobacter (H) pylori infection is a major step in the initiation and development of GC. H. pylori is a Gram-negative spirochete infecting more than half of the world population, particularly in lesser developed countries, likely due to water contamination and poor living conditions. The bacterium colonizes the stomach of its host, where it attaches to the mucosal epithelia. Infection often persists for the lifetime of the host without treatment that eventually induces carcinogenesis in the stomach.[51,52] Hp (2–20), an exogenous ligand of FPR2, is a cecropin-like peptide derived from *H. pylori*, as a recognized risk factor of GC.[53] Hp (2–20) induces the migration and proliferation of GC cells by activating FPR2[43] that may exacerbate a pro-invasiveness of GC cells.

The ability of FPR2 to promote the invasion and metastasis of GC cells was supported by experiments *in vitro* and *in vivo* with GC cell line SGC7901 and primary GC cell XN0422, in which FPR2 deficiency significantly impaired the migratory and invasive potentials induced by FPR2 agonist peptides.[50] FPR2 promotes the malignancy of GC cells by induction of epithelial-mesenchymal-transition (EMT) through ERK/MAPK pathways that converts tumor cells into an elongated, motile and invasive phenotype, pivotal for increased aggressiveness of tumor cells. The evidence for FPR2 to mediate EMT in GC cells was supported by the fact that treatment with FPR2 ligands decreased the expression of E-cadherin mRNA but enhanced vimentin, a process partially reversed by FPR2 knockdown, which did not entirely abolish the effect of FPR2 ligands on the expression of EMT-related molecules, possibly due to the presence of the prototype FPR1 in GC cells. Thus, FPR2 could be potentially used not only as a prognostic biomarker but also as a therapeutic target for GC patients. The potential involvement of FPR2 in GC progression is illustrated in Figure 2.

FPR2 ligand LL-37 in GC. H. pylori infection plays a predominant role in the etiology of GC.[54] The FPR2 ligand LL-37 (hCAP-18, FALL-39)[55,56] was produced by gastric epithelium as well as chief and parietal cells in the fundic glands of normal gastric mucosa. LL-37, up-regulated in *H. pylori*-infected stomach of the patients, alone or in synergy with human defensin 1, was bactericidal for several *H. pylori* strains. Thus, LL-37 in the stomach may control the balance between host mucosal defense and *H. pylori*. Bleach of such a balance constitutes the cause of bacterial overexpansion and the consequent stomach inflammation-carcinogenesis.[57]

FPR1 in Hepatocellular Carcinoma (HCC)

As a malignancy that emerges from a background of chronic liver diseases,[58] HCC is the fourth most common tumor worldwide with an extremely poor prognosis.[59] Chronic inflammation and improper healing of hepatic injury are causative factors for cancer in the liver.[60] The link between inflammation and liver cancer can be viewed from an extrinsic perspective for which viral infection and subsequent chronic inflammation drive oncogenesis.[61] Functional FPR1 is detected in hepatocytes and promotes the production of acute phase proteins by the cells in response to the prototype FPR1 ligand, the bacterial
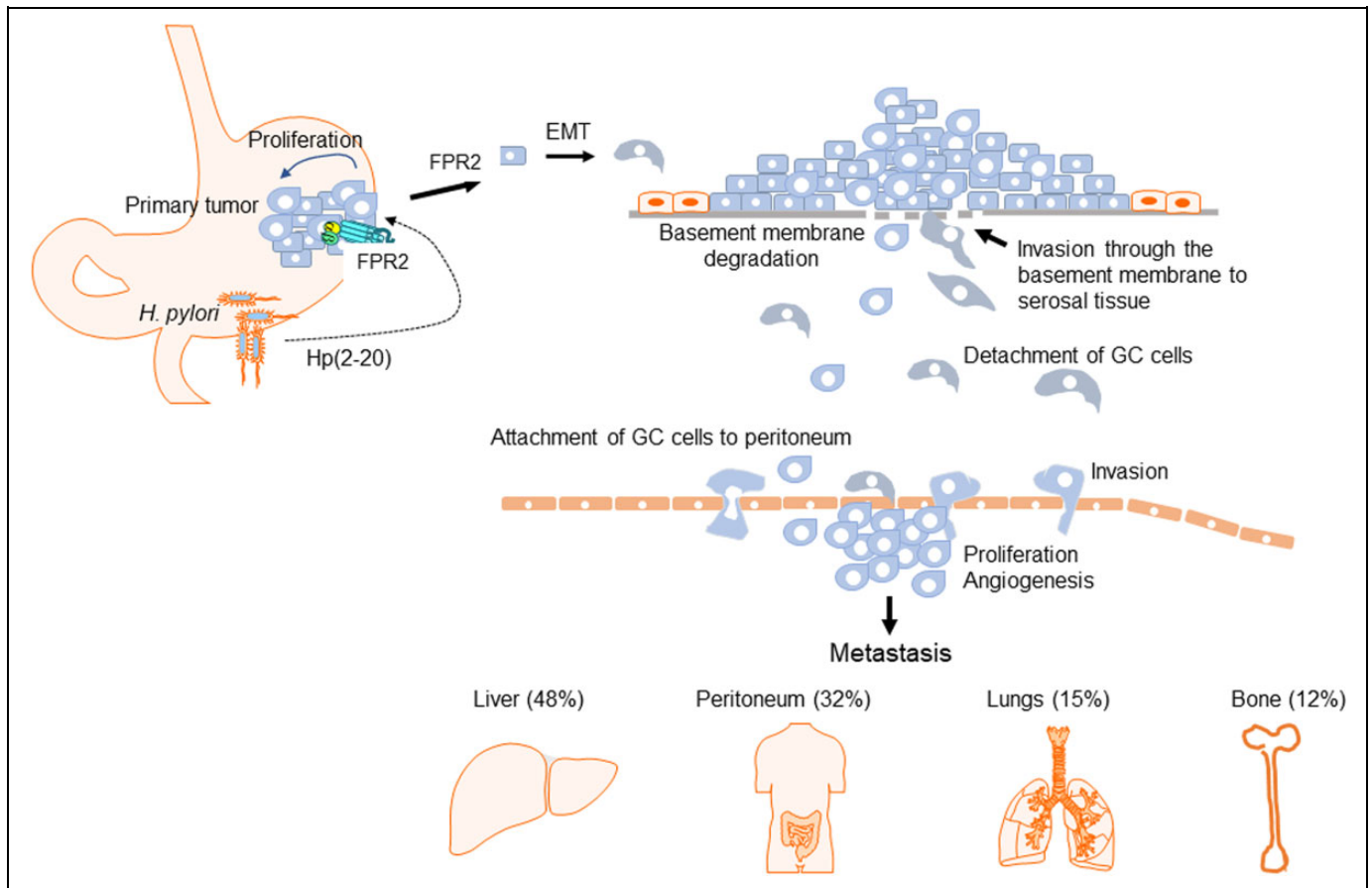
Figure 2. The role of FPR2 in the progression of GC. *H.* pylori is a major carcinogenic of factor of human GC. Its infection causes chronic inflammation with abnormal growth of epithelial cells a mechanism of mucosal repairment, which forms the basis for malignant transformation. *H. pylori* produces an FPR2 agonist peptide HP (2-20) that stimulates GC cell migration, proliferation and EMT. This process results in increased malignancy of GC cells that more rigorously invades and penetrate the organ wall, accompanied by angiogenesis. The motile tumor cells thereafter metastasize through lymphatics and blood vessels to form lethal secondary lesions.

fMLF.[62] Thus, FPR1 expressed by hepatocytes may participate in the inflammatory courses in the liver, which acts as a cleaner of toxins in human body. It has been shown that the levels of chemokines in the liver are positively correlated with the severity of hepatic inflammation in chronic hepatitis C virus (HCV) infection[63] by attracting the infiltration of leukocytes mainly myeloid cells into the liver.[64] Given that HCC is typically an example of inflammation-associated cancer and FPR1 activation promotes the migration and ERK-dependent production of the neutrophil chemokine IL-8 (CXCL8) by HCC cells, FPR1 knockdown in HCC cells substantially reduced their tumorigenicity in nude mice, as an important piece of evidence that its presence in hepatic cells may act in a concerted inflammatory cycle in the liver to exacerbate the carcinoma development and progression.[65]

FPRs in Pancreatic Carcinoma (PC)

PC is one of the most aggressive GI malignancies, with an extremely low 5-year survival rate of 8%.[66] ANXA1 participates in many pathophysiological processes in PC cells, including inhibition of cell proliferation, as well as regulation of cell migration,

differentiation and death.[67] ANXA1 is over-expressed in PC tissues from patients and despite its potential ability to transduce anti-inflammatory signals, and ANXA1 appears to be associated with malignant transformation and poor prognosis of PC.[68] The regulatory activity of extracellular ANXA1 on tumor cells is mediated by FPRs.[69] Therefore, ANXA1 may promote the metastasis of PC by favoring tumor cell invasion through its function as a cytoskeleton remodeling factor intracellularly and extracellularly through activation of FPRs on cell surface.[68] However, it has also been reported that ANXA1 contributes to the malignant phenotype of PC cells and their metastatic potential through FPR-independent pathways.[70] Therefore, ANXA1 as a multifaceted regulator of pathophysiological process in PC cells may exert its detrimental effect through various sensory molecules in the cells, FPRs among them.

FPRs in Colorectal Cancer

Colorectal cancer (CRC) is one of the most deadly cancers that affects a large world population.[71] It has been well established that chronic inflammation is a major causative factor.[72]

FPR1 as a risk factor for poor prognosis of human CRC. Investigation of the expression of FPRs in primary human CRC revealed that the mRNA of FPRs, especially FPR1, was significantly higher in CRC tissues than in distant and adjacent non-tumor tissues, suggesting an association between FPR1 and CRC progression. FPR1 mRNA expression was also correlated with the size of tumors and serosal infiltration of CRC. In addition, FPR1 is found as a risk factor that predicts the prognosis of CRC. Absence of FPR1 increases the survival rate of colitis-associated CRC.[73] These observations link FPR1 expressed in CRC cells to a more malignant nature.

FPR2 in exacerbated malignant behavior of human colon cancer cells. Compared to FPR1, FPR2 appears to be a more reliable maker in CRC that rigorously promotes the invasive phenotype of cancer cells.[73] FPR2 was preferentially expressed in colon cancer (89%) than in rectal cancer (11%).[19] The ability of FPR2 to promote the malignancy of CRC cells was demonstrated by experiments, in which human CRC cell line SW1116, its proliferation, migration, invasion, anti-apoptosis and pro-angiogenesis properties were attenuated by silencing FPR2 with shRNA, resulting in reduced tumorigenicity in xenograft models. Mechanistically, silencing FPR2 reduced the expression of proteins linked to EMT such as E-cadherin, N-cadherin, Snail, Slug and vimentin,[74] indicating the capacity of FPR2 to support the malignant transformation of colon epithelial cells.

Fpr2 protection of mouse colon from inflammation and cancer. Colon cancer is believed to be consequences of chronic inflammatory responses of the epithelial layer. Clinically, patients with chronic ulcerative colitis or Crohn's disease have a five- to seven-fold increase in the risk of developing CRC, as a result of persistent colon inflammation.[26] beginning with focal proliferation of dysplastic cells, formation of benign adenomatous polyps, and transformation to malignant adenocarcinomas. It is noteworthy that in contrast to the reported capacity of FPR2 to promote colon cancer progression in human, the murine counterpart Fpr2 demonstrates a unique role in protecting colon mucosa from inflammation and carcinogenesis. This was demonstrated by observations in mice in which Fpr2 deficiency was associated with a prolonged chronic inflammation shown by greatly shortened colon, increased foci of ulcers in damaged mucosa and exacerbated inflammatory cell infiltration in ulcerative lesions, culminating in markedly increased number of adenomas.[17] Thus, Fpr2 is essential for limiting the susceptibility of mouse colon to chronic inflammation-induced tumors. In this respect, Fpr2 (FPR2) appears to be a double-edged sword in that if the receptor was aberrantly expressed by transformed human CRC cells, its capacity to mediate normal epithelial migration, proliferation and mucosal restitution is exploited by tumor cells to exacerbate the malignant behavior.

The protective role of FPR2 agonist LL-37 in the colon. LL-37 is expressed by normal colon epithelial cells. It is interesting that unlike normal human colon epithelial cells, CRC cells do not express or only express low levels of LL-37.[75] Therefore, the absence of LL-37 is used as one of the biomarkers of human colon cancer.[76] FK-16, a fragment of LL-37, corresponding to the aa residues 17 to 32, induces the death of colon cancer cells by activating caspase-independent apoptosis and autophagy,[77] suggesting its direct anti-colon cancer activity. The colon carcinoma cell line HCT116 treated *in vitro* with FF/CAP18, a 27-residue analogue of LL-37, exhibits a marked loss in major metabolic profiles required for the proliferation of cancer cells.[78] In addition, LL-37 inhibits EMT of colon cells and fibroblast-supported colon cancer cell proliferation.[79] In mice, the LL-37analogue CRAMP reduces chemically induced colon cancer by inhibiting vimentin positive fibroblasts to produce collagen and disrupting tubulin distribution in fibroblasts.[79] These studies indicate that LL-37/CRAMP inhibits tumorigenesis in the colon. *In vivo*, the capacity of CRAMP to protect colon homeostasis and anti- tumorigenic host defense was demonstrated by observations in *CRAMP*^{-/-} mice,[80] which exhibited exacerbated chemically induced chronic colitis, associated with delayed mucosal restoration and increased carcinomas.[81] Importantly, CRAMP deficiency disrupted the balance of microbiota in mouse colon, indicating the importance of CRAMP in controlling the microbiota homeostasis, while the peptide also mediates epithelial cell migration and growth via FPRs. These observations reflect two-fold benefits of LL-37/CRAMP in the colon, i.e. by activating FPR2/Fpr2 in cancer cells to promote their apoptosis with reduced malignancy and its anti-microbial property to maintain a physiological homeostatic microbiota critical for host defense against inflammation and carcinogenesis.

Perspectives

FPRs as a subfamily of the classic chemoattractant GPCRs interact with the most diverse ligands among chemoattractant GPCRs and they are expressed by a great variety of cell types, notably cancer cells as well. The roles of FPRs in cancer progression are increasingly explored during the recent years. These receptors, in addition to mediating host defense against many pathological insults, have also been shown to be hijacked by a plethora of tumor cells including those of the human glioblastoma and breast cancer.[82] In terms of interaction with chemotherapeutic drugs, overexpression of FPR1 may contribute to drug-resistance of bladder cancer and promotes tumor deterioration.[83] On the other hand, FPR1 has been reported to increase chemotherapy-induced anticancer immune responses.[84] As reviewed in this article, FPRs are also exploited by tumor cells of the DT system to their advantage. Although advances in new diagnosis and treatment have led to declining mortality rates, GI tract cancers remain a leading cause of death among all cancers. Therefore, a better understanding of the interactions between tumors and inflammation that involve FPRs and their ligands may lead to the discovery of novel molecular targets for therapeutic intervention, as shown by the role of FPR2/Fpr2 and the ligands LL-37/CRAMP as guardians of normal colon.

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