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VIEWPOINT

Protease-activated receptor signaling in intestinal permeability regulation

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Protease-activated receptors (PARs) are a unique class of G-protein-coupled transmembrane receptors, which revolutionized the perception of proteases from degradative enzymes to context-specific signaling factors. Although PARs are traditionally known to affect several vascular responses, recent investigations have started to pinpoint the functional role of PAR signaling in the gastrointestinal (GI) tract. This organ is exposed to the highest number of proteases, either from the gut lumen or from the mucosa. Luminal proteases include the host's digestive enzymes and the proteases released by the commensal microbiota, while mucosal proteases entail extravascular clotting factors and the enzymes released from resident and infiltrating immune cells. Active proteases and, in case of a disrupted gut barrier, even entire microorganisms are capable to translocate the intestinal epithelium, particularly under inflammatory conditions. Especially PAR-1 and PAR-2, expressed throughout the GI tract, impact gut permeability regulation, a major factor affecting intestinal physiology and metabolic inflammation. In addition, PARs are critically involved in the onset of inflammatory bowel diseases, irritable bowel syndrome, and tumor progression. Due to the number of proteases involved and the multiple cell types affected, selective regulation of intestinal PARs represents an interesting therapeutic strategy. The analysis of tissue/cell-specific knockout animal models will be of crucial importance to unravel the intrinsic complexity of this signaling network. Here, we provide an overview on the implication of PARs in intestinal permeability regulation under physiologic and disease conditions.

Abbreviations
APC, activated protein C; ATIII, antithrombin-III; CD, Crohn's disease; CG, cathepsin G; EPCR, endothelial protein C receptor; FIX(a), activated coagulation factor IX(a); FV(a), activated coagulation factor V(a); FVII(a), activated coagulation factor VII(a); FVIII(a), activated coagulation factor VIII(a); FX(a), activated coagulation factor X(a); FXIII(a), activated coagulation factor XIII(a); GI, gastrointestinal; GVB, gut-vascular barrier; IBD, inflammatory bowel diseases; IBS, irritable bowel syndrome; IECs, intestinal epithelial cells; ISCs, intestinal stem cells; LPS, lipopolysaccharide; MAMPs, microbial-associated molecular patterns; MLCK, myosin light chain kinase; NE, neutrophil elastase; NOD, nucleotide-binding oligomerization domain; PARs, protease-activated receptors; PGA2/E2, prostaglandin A2/E2; PR3, proteinase 3; RgpA, arginine-specific gingipain-A; RgpB, arginine-specific gingipain-B; TF, tissue factor; TJs, tight junctions; TLRs, Toll-like receptors; UC, ulcerative colitis.

Introduction

Protease-activated receptors (PARs) are a unique class of G-protein-coupled transmembrane receptors affecting several biochemical responses, such as hemostasis, vascular biology and various inflammatory phenotypes. Since the cloning of PAR-1 in 1991 [1], three additional receptors have been described: PAR-2 [2,3], PAR-3 [4] and PAR-4 [5,6]. The first pathway ascribed to PAR signaling was thrombin-induced platelet aggregation. Since then, the observation that PARs are expressed by multiple cell types and responsive to a steadily increasing number of proteases placed these receptors at center stage in vascular biology. The mechanism of PAR activation revolutionized the perception of proteases from being simply degradative enzymes to pivotal context-specific signaling factors. After protease-induced cleavage at the extracellular N-terminal domain, the newly generated N-terminus acts as a tethered ligand by interacting with the second loop of the receptor [7]. To complicate the picture, PARs are subjected to a 'biased agonist' activation, depending on the position of the N-terminal peptide bond hydrolyzed [8–10] (Fig. 1A). Due to its remarkable complexity, PAR signaling still represents a challenging therapeutic target. Vorapaxar, for instance, is a novel PAR-1 antagonist used as an antiplatelet agent for secondary prevention of cardiovascular events [11].

The epithelium of the gastrointestinal (GI) tract has become a new hot spot of PAR research. The GI tract is indeed the body compartment exposed to the highest number of proteases, either luminal or from the mucosa. Several investigations demonstrated that all PARs are expressed throughout the human GI tract, from the salivary glands, to the stomach, along the length of the gut, in the pancreas and in the liver [12–17]. In the intestine, PAR signaling has been reported both to mediate physiological functions, e.g. ion transport, regulation of intestinal barrier function, gut motility and sensory functions [18,19], and to exacerbate pathological conditions, like tumor development and progression of inflammatory bowel diseases (IBD) [20–22]. In conclusion, systematic investigation of PAR signaling is required for a precise understanding of GI tract pathophysiology.

PAR expression in the intestine and their role in intestinal stem cell differentiation

The GI epithelium is the largest body surface (approximately 400 m^2) separating the external surrounding from the internal milieu. To fulfill barrier function while enabling selective assimilation of nutrients and electrolytes, the intestinal epithelial cells (IECs) are sealed by junctional complexes controlling paracellular transport [23]. Notably, all members of the PAR family were detected in the small intestine epithelium, and in particular PAR-1 [24,25] and PAR-2 [26,27] are expressed by human enterocytes and cells of the lamina propria (Fig. 1B). PAR-2 is expressed both in the apical and basolateral membrane of enterocytes, thus regulating GI permeability through myosin light chain kinase (MLCK) and β-arrestin [28]. In addition, PAR-1, PAR-2 and PAR-4 are present in endothelial cells [29], submucosal/myenteric neurons and immune cells [30], while PAR-1 and PAR-2 were also detected in fibroblasts, smooth muscle cells, mast cells and human colon cancer epithelium. Surprisingly, the PAR-3 expression profile still remains unexplored [18].

The intestinal epithelium is the tissue with the most vigorous renewal rate (every 4–5 days) in the human body [31], fueled by rapid cell replacement in the villus structures through differentiation of multipotent intestinal stem cells (ISCs) localized in the bottom of the Lieberkühn crypts [32]. Intriguingly, trypsin-mediated PAR-2 activation is thought to promote intestinal regeneration through stabilization of YAP protein in colonic epithelial cells [33]. A villus/crypt structure, the surrounding subepithelial fibroblasts and the underlying mesenchymal tissue form an anatomical unit [34], able to generate four terminally differentiated IEC types, enterocytes (90% of the epithelial lineage), goblet cells, enteroendocrine cells and Paneth cells. Each crypt contains 4–6 independent ISCs [35], which may either self-renew or differentiate to transit amplifying daughter cells, which undergo cycles of cell division and differentiation while migrating along the crypt-villus axis [34,36,37], except for mature Paneth cells, which escape upwards migration to localize at the base of the crypts [38,39]. In this scenario, PAR-1 and PAR-2 have been detected in ISCs, and are thought to affect epithelial cell proliferation and upwards migration. In particular, PAR-2 (but not PAR-1), being essential for glycogen synthase kinase-3β activation, plays a critical role in the regulation of ISC survival and proliferation in normal crypts, but also in colon cancer [40]. Clearly, further research is needed to unravel the role of PARs in IEC proliferation and differentiation.

Activation of epithelial PARs by host proteases

Both from the luminal and from the mucosal side, the GI barrier is exposed to a myriad of proteases

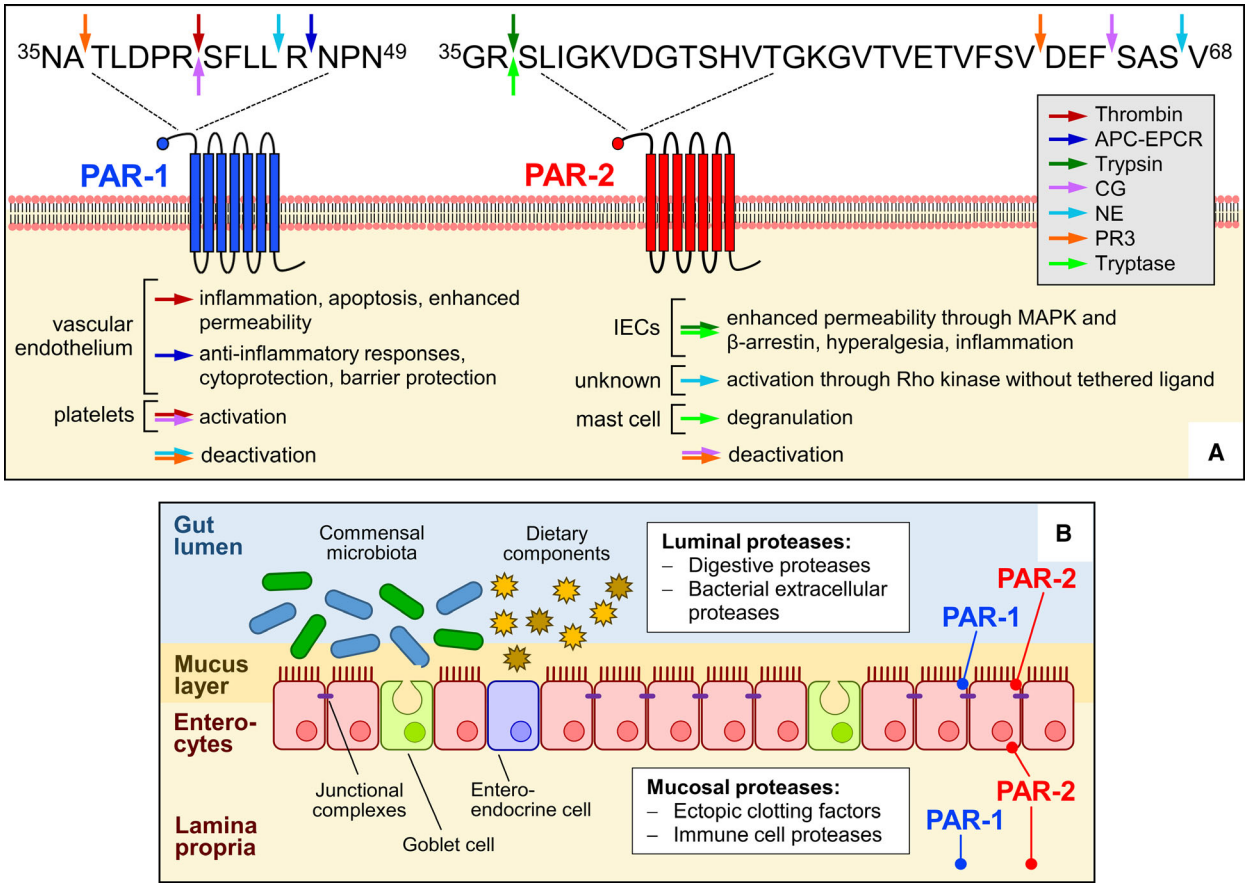

Fig. 1. (A) Cleavage and activation of PAR-1 and PAR-2; (B) Activation of intestinal PARs by host proteases. (A) Schematic representation of transmembrane human PAR-1 (in blue) and PAR-2 (in red) and their mechanism of proteolytic activation. For both receptors, the various cleavage sites are represented by color-coded arrows along the amino acidic sequence. After proteolytic cleavage at the N-terminal extracellular domain, the new N-terminus acts as a tethered ligand, transducing intracellular signalling by interacting with the second extracellular loop of the receptor. An important exception is represented by biased activation of PAR-2 by NE, which does not require the tethered ligand. Only representative proteases are shown: clotting factors (thrombin and the complex APC-EPCR), digestive trypsin, neutrophil proteases (CG, NE, PR3) and tryptase from mast cells. (B) Representative drawing of the small intestinal epithelial lining. Mucin-secreting goblet cells (in green) and enteroendocrine cells (in blue) are intermingled into the monolayer of simple absorptive enterocytes (in pink), which are sealed by junctional complexes and represent approximately 90% of the epithelial lineage. At the base of Lieberkühn crypts (not shown) ISCs self-renew and differentiate also into Paneth cells, which escape upwards migration. While all members of PAR family are expressed throughout the GI tract, PAR-1 and PAR-2 were detected on small intestine enterocytes (for PAR-2, both in the apical and in the basolateral side), and lamina propria cells, being thus susceptible to activation by either luminal or mucosal proteases, which are classified in a simplified framework.

that have the capacity to activate PARs (Table 1). In a conceptual view, luminal proteases include the host's digestive enzymes and the extracellular proteases from the commensal gut microbiota, while mucosal proteases are represented by the ectopically expressed (extravascular) clotting factors and by the enzymes released from gut resident (mast cells, macrophages) or infiltrating immune cells (neutrophils) (Fig. 1B) [41]. It has been reported that luminal proteases can cross the gut-vascular barrier (GVB) into the bloodstream and vice versa, especially under inflammatory conditions. Orally administered proteases may translocate the GVB in an intact and catalytically competent form by a self-enhanced paracellular transport, through disruption of tight junctions (TJs). Protease formulations in enteric-coated tablets are currently used for the treatment of digestive and pancreatic disorders, leading to increased serum proteolytic activity [42]. Conversely, prothrombin and active thrombin were detected in the intestinal lumen and in the mucosa during inflammation [43].

Table 1. Proteases acting on intestinal PARs. Proteases are classified into luminal (digestive and microbial enzymes) and mucosal (extravascular clotting factors, proteases from resident and infiltrating immune cells). For each protease listed, the targeted PAR(s) and the tissue/cell type involved are detailed. Square brackets indicate an inactivating effect on PAR signaling; round brackets indicate a less relevant signaling.

Protease	Targeted PAR	Cells and tissues	Ref
Luminal proteases			
Digestive proteases			
Trypsin	PAR-2 (PAR-1, PAR-4)	Luminal trypsin: intestinal enterocytes; pancreatic trypsin: (inflamed) pancreas	[26]
Extra-pancreatic trypsin IV	PAR-2, PAR-4	Extra-pancreatic tissues, e.g. healthy and tumor-affected colon	[44]
Microbial proteases (resident gut microbiota; invading pathogens)			
Gelatinase (*E. faecalis)*	PAR-2	Gastrointestinal tract, enterocytes	[72,73]
Gingipains *(P. gingivalis)*	PAR-1, PAR-2	Human gingival epithelial cells (RgpB), fibroblasts (RgpB), oral epithelial cells, oral keratinocytes	[76–80]
Serralysin (*S. marcescens)*	PAR-2	HeLa cells, human intestinal carcinoma	[81]
Protease (*H. pylori*)	PAR-2	Gastric epithelial cells	[82]
LasB (*P. aeruginosa*)	PAR-2	Small intestine, duodenum	[88]
Mucosal proteases			
Clotting factors (extravascular)			
Thrombin	PAR-1, PAR-3, PAR-4	Intestinal mucosa and lamina propria	[43]
FXa	PAR-2 (PAR-1, PAR-3, PAR-4)	Sites of FX extravascular expression	[18]
TF-FVIIa	PAR-2 (PAR-1)	Hepatic, non-hepatic cancer cell lines	[52]
TF-FVIIa-FXa	PAR-2	Cells in which co-expression of PARs and TF (high levels) occurs, like monocytes, keratinocytes and endothelial cells	[46,48–51]
TF (non proteolytic)	[PAR-2]	Cancer cells, endothelial cells	[53]
Immune cell proteases (resident or infiltrating)			
Cathepsin G	PAR-4	From neutrophils: activates PAR-4 on platelets surface and endothelial cells	[89,126]
Neutrophil elastase	PAR-2	From neutrophils, biased agonism	[91]
Cathepsin G, elastase, proteinase 3	[PAR-1] [PAR-2] [PAR-4]	From neutrophils, disarming of PARs	[90]
Tryptase	PAR-2	From mast cells; relevant action on colonic nerve fibers in IBD	[92]

Digestive proteases

Digestive proteases, such as trypsin and chymotrypsin, are normally synthetized in the liver as inactive precursors. Pancreatic acinar cells express multiple isoforms for trypsinogen: trypsinogen I, II, mesotrypsinogen and its splice variant trypsinogen IV, which is resistant to trypsin inhibitors and co-expressed with enteropeptidase. After secretion from the pancreatic ducts, trypsinogen is converted to trypsin on the brush border of enterocytes by enteropeptidase. Active trypsin, in turn, promotes its own activation, attacks chymotrypsinogen, activates PAR-2 thus enhancing paracellular permeability, and, to a less extent, PAR-1 and PAR-4 [26]. Under inflammatory conditions, trypsinogen I-II are prematurely activated in the pancreas, yielding pancreatic trypsin that strongly stimulates PAR2 signaling. Activation of trypsinogen IV in extra-pancreatic epithelial tissues, including healthy and tumor-affected colon, induces a strong and prolonged stimulation of PAR-2 and PAR-4 [44].

Blood coagulation proteases

The effect of coagulation factors on platelet and endothelial PAR signaling has been extensively studied. Besides thrombin, activated coagulation factor VIIa (FVIIa) and FXa, in complex with tissue factor (TF) or endothelial protein C receptor (EPCR), are also able to activate PARs [45,46]. On the vascular endothelium and on monocytes, co-expression of PARs and TF drives PAR-2 activation by the TF-FVIIa-FXa complex [46,47], triggering intracellular calcium release, von Willebrand factor release and exposure of P-selectin. Notably, also human keratinocytes express PAR-2 and TF [46,48–51]. Although it is generally known that the TF-FVIIa complex is present on the surface of hepatic

Table 2. Ectopically expressed coagulation factors. List of coagulation factors detected in extravascular compartments and cells, except the traditional sites of clotting factor synthesis: liver, platelets and endothelial cells.

Clotting factor	Cell type, tissue, body district of detection	Ref
Prothrombin	Luminal and mucosal site of inflamed gut	[43]
Thrombin	Luminal and mucosal site of inflamed gut, synovial fluid, sputum of asthmatic patients, prostate cancer	[59,60,127,128]
Fibrinogen	Sputum of asthmatic patients	[59,60]
FXa	Human macrophages, human epithelial cells (nose, bronchus, duodenum), human fibroblasts; human (pre)adipocytes; murine lungs epithelium (also in asthmatic and fibrotic conditions)	[55–58,62]
FVa	Human monocytes	[62]
FVIIa	Hepatic and non-hepatic cancer cells lines	[52]
FVIIIa	Human fibroblasts, human macrophages, human monocytes, human (pre)adipocytes	[62]
FIXa	Murine enterocytes	[63]
FXIIIa	Human macrophages, human monocytes	[62]
TF	Human monocytes, human keratinocytes	[47,48]

[a] FIX(a), activated coagulation factor IX(a); FV(a), activated coagulation factor V(a); FVIII(a), activated coagulation factor VIII(a); FXIII(a), activated coagulation factor XIII(a).

and non-hepatic cancer cell lines [52], the origin and transport of extravascular FVII is obscure. It has been proposed that FVII expression, inducible by hypoxic conditions, is sufficient to promote pro-invasive FXa-mediated PAR-1 and PAR-2 signaling. Notably, PAR-2 signaling may also be downregulated by a direct non-proteolytic effect of the TF cytoplasmic tail [53].

In the last years, increasing attention is arising on PAR activation by coagulation factors that are expressed on extravascular sites (Table 2). According to the prevailing view, the synthesis of coagulation factors is restricted to the liver and the vascular endothelium (e.g. FVIII) [54]. This paradigm was challenged in 1996, when Yamada and Nagai first detected FX expression in macrophages and in human epithelial cells from nose, bronchus and duodenum [55]. Notably, in contrast to blood circulating FXa, surface-expressed FXa is protected from inhibition by antithrombin III (ATIII) and tissue factor pathway inhibitor [46]. Extravascular clotting factors have indeed been often detected in selected body districts, e.g. in the lungs, or under pathological conditions, e.g. in the atheroma [56–60]. Surprisingly, albeit devoid of a nucleus, also platelets were reported to produce significant amounts of clotting factors [61]. Extravascular expression of clotting factors was systematically analyzed by Dashty and co-workers in eight human primary cell types (Table 2) [62]. To complete the picture, a recent investigation demonstrated the presence of ectopic FIX in mouse small intestine, where expression was upregulated by stimulation with Toll-like receptor (TLR) agonists, such as lipopolysaccharide (LPS) from *Escherichia coli* [63]. So far, the expression and proteolytic activity of extravascular clotting factors is largely unexplored and deserves further investigation.

Microbial proteases

The gut microbiota, harboring over 10^{14} commensal microorganisms, influence several aspects of the intestinal (patho)physiology. It is known that epithelial PARs respond to microbial-associated molecular patterns (MAMPs), as they communicate with nucleotide-binding oligomerization domain (NOD)-like receptors and TLRs in the orchestration of the immune response [64]. However, the molecular mechanisms underlying PAR signaling pathways are still under debate (Fig. 2). Microbial colonization [65] and antibiotic treatment [66] dramatically affect transcriptomes of IECs, by up- or down-regulating the expression of hundreds of genes [67]. Vascular remodeling of villus structures of colonized mice, with respect to germ-free counterparts [68], seems to be controlled by the microbiota-dependent upregulation of PAR-1, triggering TF glycosylation. The activation of the PAR-1/TF loop, in turn, results in increased transcript levels of the proangiogenic angiopoietin-1 in IECs and phosphorylation of its receptor Tie-2 [69]. In other body compartments, such as human corneal epithelial cells, PAR-2 expression is upregulated by a bacterial serine protease (i.e. *Acanthamoeba* plasminogen activator) [70].

In this context, the proteases secreted by commensal bacteria represent a weapon arsenal through which the microorganisms shape the GI habitat. Through their proteolytic activity, bacterial proteases may weaken the intestinal barrier by directly attacking TJs, or via PAR signaling [71]. Recently, the observation that fecal samples from IBD patients present an increased gelatinolytic activity prompted researches to investigate gelatinase from *Enterococcus faecalis*, which disrupts the GI barrier through

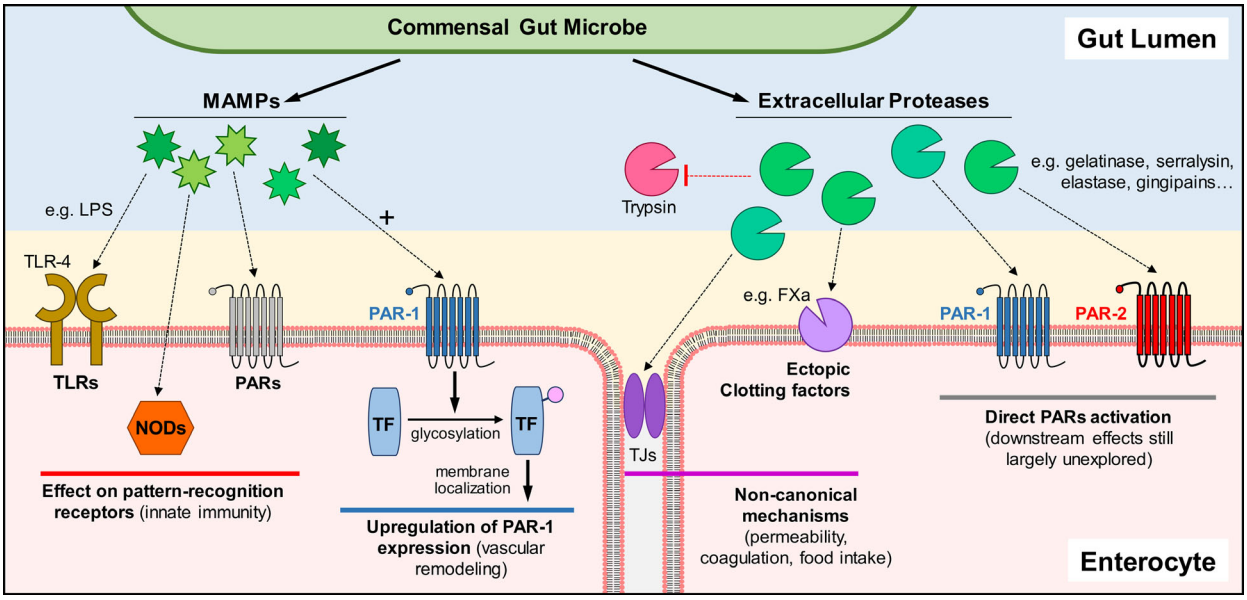

Fig. 2. Impact of gut microbiota on intestinal PAR signaling. Cartoon representing the apical side of two adjacent enterocytes connected by TJs. Gut resident microorganisms continuously secrete MAMPs, which may either stimulate TLRs (e.g. LPS), NODs (e.g. peptidoglycan) or PARs, thus triggering innate immune responses. Moreover, it has been reported that intestinal microbial colonization upregulates PAR-1 expression, promoting TF glycosylation and membrane migration. On the other hand, bacterial proteases, secreted by the resident microbes, may either directly activate PARs (most importantly, PAR-1 and PAR-2), or promote proteolytic-mediated non-canonical pathways, thus altering gut permeability.

proteolysis of E-cadherin [72] and, notably, via PAR-2 activation [73]. As a matter of fact, activation of epithelial PARs may be virtually promoted by all the arginine-specific luminal bacterial proteases [74]. The most striking example is probably provided by gingipains, responsible for the virulence of *Porphyromonas gingivalis* in the onset of periodontal diseases [75]. In particular, arginine-specific gingipain-A (RgpA) and arginine-specific gingipain -B (RgpB) activate PARs on several cell types, including human platelets [76] neutrophils [77], gingival fibroblasts [78,79] and human oral epithelial cells [80]. Proteolysis of intestinal PAR-2, involved in the generation of inflammatory stimuli and in IBD, was also reported for *Serratia marcescens* and *Helicobacter pylori*. While serralysin from *S. marcescens* has been suggested to activate PAR-2 in human carcinoma and HeLa cells [81], the protease secreted from *H. pylori* that activates PAR-2 in gastric epithelial cells is still under investigation [82] (Table 1).

Beyond direct proteolysis, bacterial proteases may activate PARs through alternative pathways. Non-canonical activation of blood clotting factors, as in the case of prothrombin [83], FX [84] and FIX [85] by gingipains, could play a relevant role in case of extravascular localization. It was recently reported that subtilisin, a serine protease from the gut commensal *Bacillus subtilis*, is able to activate human prothrombin [86]. Moreover, bacterial enzymes were shown to interfere with digestive proteases and food uptake in health and disease. From small to large intestine, trypsin activity is proteolytically inactivated in a microbiota dependent fashion (e.g. by *Bacteroides distasonis*) [87]. On the other hand, duodenal biopsies from celiac disease patients revealed a higher glutenasic activity with respect to healthy controls, ascribed to LasB elastase from *Pseudomonas aeruginosa*. In these genetically predisposed subjects, LasB establishes a pro-inflammatory phenotype through proteolysis of intestinal PAR-2 [88]. It is therefore likely that many unresolved microbiota-host interactions exist on the basis of microbial proteases that interfere with epithelial PARs.

Proteases of immune cell origin

In the small intestine, the proteases released from inflammatory cells represent a relevant mucosal source of proteolytic activities. They are derived from (a) mast cells, secreting e.g. tryptase, chymase, cathepsin G (CG) and granzyme B, (b) resident macrophages, producing matrix metalloproteases (MMPs), caspase, cathepsin L, cathepsin D and, (c) under inflammatory conditions, from infiltrating neutrophils, releasing

neutrophil elastase (NE), CG and proteinase 3 (PR3) (Table 1) [41]. To give a few examples, while CG activates PAR-4 on the platelet surface [89], all neutrophil proteases deactivate PAR-1 and PAR-2 by attacking peptide bonds beyond the functional cleavage site [90]. Intriguingly, NE mediates a biased PAR-2 activation through Rho kinase, in contrast to the traditional trypsin-induced MAPK-mediated signaling [91] (Fig. 1B). Whether this pathway is relevant in PAR-2 expressing immune cells or in colonocytes in IBD is still under investigation. Notably, tryptase from degranulated mast cells induces a prolonged PAR-2 activation which, if localized in proximity to colonic nerves, correlates with pain and gut dysfunction in IBD [92].

Conversely, PARs are widely expressed by immune cells like lymphocytes, macrophages, monocytes, mast cells and neutrophils [18,93]. Recruitment of innate immune cells is of particular interest in the onset of a pro-inflammatory state in IBD: a role of PAR-1 in the regulation of chemical-induced colitis in mouse models was indicated, although its precise effect has been reported to be variable, with a still unresolved molecular mechanism [94]. On the other hand, PAR-2 was implicated in the onset of a pro-inflammatory phenotype in the small intestine of mice infected with *Toxoplasma gondii*. More specifically, mediators of innate immunity like interleukin-6, KC/chemokine (C-X-C motif) ligand 1 as well as PGE2 and 8-isoPGA2 are under the control of PAR-2 [95]. Moreover, PAR-2 agonists are capable of activating mast cells in human subjects [96]. In turn, increased mast cell numbers and tryptase levels have been reported in ulcerative colitis (UC), and an up-regulated histamine metabolism was reported in UC [97], Crohn's disease (CD) [97], and food allergy [98]. The effects of these proteases on PAR activation is extremely complex, and, although extensively studied in humans and animal models, the general picture is still incomplete. Variable results have been presented depending upon the species, genetic background, and disease model used.

Even though a detailed understanding of the action of PAR-activating proteases could offer a strategic target for the development of therapies, the clinical outcomes of digestive, microbiota or innate immune cell-derived proteases has not been pinpointed so far.

PAR-mediated intestinal permeability regulation in inflammatory bowel diseases

Regulation of trans- and para-cellular permeability of gut epithelium warrants the host's immune balance between the luminal and extra-luminal environment. In this context, imbalance of the protease/antiprotease activity and bacterial dysbiosis may lead to epithelial damage and increased intestinal permeability through direct TJ cleavage or via PAR activation [71]. Actually, plasma and colonic tissue from CD and UC patients exhibit enhanced levels of the PAR-1 activating proteases thrombin and matrix metalloprotease-1, which correlate with the disease severity index [99–101]. Gut pathologies are frequently associated with alterations of paracellular epithelial permeability, e.g. UC [102], CD [103], celiac disease [104,105] and irritable bowel syndrome (IBS) [106], and this factor is indeed correlated to the disease activity index [106–109]. Furthermore, stress has been shown to increase colonic permeability through activation of mast cells and stress-related stimulation of exocrine pancreatic secretion, particularly trypsin [110].

In this context, PAR1-induced apoptosis contributes to the epithelial barrier function in a caspase-3 dependent manner both *in vitro* and *in vivo* [25]. On the other hand, PAR-2 was found overexpressed in biopsies from IBD patients, thus hinting towards a pathophysiological role in the etiology of colonic inflammation [111], as well as in a mouse model of spontaneous chronic colitis [112]. Indeed, luminal activation of PAR-2 by trypsin, bacterial proteases or infusion of a PAR-2 agonist in mice is known to increase intestinal paracellular permeability by contraction of the cytoskeleton [113]. This has been supported by the fact that in contrast to PAR-2 deficient mice, trypsin administration into the colon lumen of wild type mice induced a fast and short inflammatory reaction, with an increase in paracellular permeability and bacterial translocation [27].

Similar to what was previously observed in UC patients, a recent study showed the presence of high levels of serine proteases in the feces of IBD and IBS patients [114], and interestingly, incubation of the IBS supernatants with PAR-1 lead to the activation of the receptor, which was implicated in nerve activation [115]. Strikingly, application of diluted fecal supernatants from diarrhea-predominant IBS patients on the mucosal side of murine colonic epithelium lead to enhanced paracellular permeability in Ussing chamber experiments. Pre-incubation of the supernatant with a cocktail of serine protease inhibitors suppressed this effect, which was not observed in PAR-2 deficient mice [116]. Finally, oral antibiotic treatment in animal models was associated with a downregulation of PAR-2 receptors on epithelial cells, reduced permeability and the response to luminal activating factors [113].

Involvement of PARs in gastrointestinal tumor progression

Although IBD patients are considered to be at high risk of developing colonic cancer, due to the effect of bacterial dysbiosis and chronic gut inflammation, the impact of PAR signaling in the onset and progression of intestinal cancer is less explored [45,117]. Beyond being expressed in the healthy epithelium, PAR-1 and PAR-2 are highly expressed in human intestinal adenocarcinoma cell lines and in stromal cells, and could possibly enhance their proliferation and metastatic capacity [45]. In this respect, stimulation of human intestinal adenocarcinoma cell lines *in vitro* with either thrombin or trypsin yielded an increase in proliferation and invasiveness [118–120]. A pivotal role of PAR-1 in colonic adenocarcinoma growth was also found in C57BL/6 mice *in vivo*, as PAR-1 deficient mice showed reduced tumor growth when inoculated with the murine MC-38 or the human HCT116 intestinal adenocarcinoma cell line [121]. This study demonstrated that tumor growth was dependent on the hepatic prothrombin synthesis, thus leading to thrombin formation and subsequent PAR stimulation. In humans, the prognosis of patients with colorectal cancer is rather poor if the tumor has the ability to express trypsin [122], thus stressing the clinical importance of PAR signaling. In a different study, PAR-1 deletion led to tumor progression in the colon and favored the apoptosis of transformed epithelial cells. Here, PAR-1 deficient mice were crossed with adenomatous polyposis coli Min mice [activated protein C (APC)Min/+], resulting in spontaneous development of intestinal adenomas. In C57BL/6 wild type mice, fewer and significantly smaller adenomas were detected *in vivo*, in comparison to PAR-1-deficient mice. Similarly, after PAR-1 deletion, transformed epithelial cells presented less apoptotic events [123]. These studies demonstrate that the role of PARs signaling in intestinal tumor progression is by no means clear and depends on various factors (i.e. model selection, influence of different proteases). Conversely, other studies suggested that colon cancer cells can induce cancer-associated thrombosis by thrombin via PAR-4 activation on platelets and subsequent amplification [124]. Not only PAR-1 and PAR-2 are expressed by colon cancer cells, but also overexpression of PAR-4 was detected in carcinogenic tissue, associated with increased proliferation and migration of cancer cells. PAR-4 is involved in extracellular signal-regulated kinase (ERK) 1/2 phosphorylation and in the progression of colorectal cancer [125]. Further mechanistic insights on the role of PARs from murine colon cancer models and mounting clinical evidence are needed to understand the role of PAR signaling in intestinal carcinogenesis.

Conclusions

The variety of functions exerted by PARs in the intestine and their widespread presence in different cell types brought essential information about their effects in the context of IBD and intestinal cancer. However, the high complexity of PAR signaling is a big challenge for future therapies, based on targeted modulation of PAR function. Basic research, performed on transgenic animal models, for example with tissue-specific deletion of PARs restricted to specific cell types, will be instrumental to uncover the role of these receptors in various intestinal pathologies. In particular, the functional role of the gut microbiota and its contribution to PAR signaling through various microbial proteases or by the stimulation of mucosal host protease activities, but also by the differential regulation of PAR expression profiles in the intestine, will be an interesting area of research in the near future.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

GP, AM, IB, FM, MS and CR wrote the manuscript.

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