Review

Protease-Activated Receptor 2 and Its Role in the Progression from Metabolic-Disfunction Associated Steatotic Liver Disease to Hepatocellular Carcinoma

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Review

Protease-Activated Receptor 2 and Its Role in the Progression from Metabolic-Disfunction Associated Steatotic Liver Disease to Hepatocellular Carcinoma

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Abstract: Metabolic dysfunction-associated steatotic liver disease (MASLD) has recently emerged as the predominant aetiology of chronic liver disease worldwide. This condition can progress to hepatocellular carcinoma (HCC) through various pathogenetic mechanisms. Briefly, metabolic dysfunction, which may occur in genetically susceptible individuals, disrupts lipid metabolism homeostasis. This imbalance leads to increased oxidative stress and DNA damage. Concurrently, chronic inflammation intensifies, impairing immune surveillance and facilitating HCC progression. Recent research has shed light on the significant role of Protease-activated receptor 2 (PAR2) in metabolic regulation. PAR2 is not only pivotal in inflammatory and fibrotic process but has also been identified as a key metabolic regulator. Given its multifaceted functions, PAR2 has become a focal point in studies exploring obesity, MASLD progression and HCC development. This review aims to synthesize the major findings from this growing field of research, offering insights into the intricate relationship between PAR2, metabolic dysfunction, and liver disease progression.

Keywords: metabolic dysfunction; liver cancer; protease-activated receptor

Metabolic Dysfunction-Associated Liver Disease and Liver Cancer

Metabolic dysfunction-associated steatotic liver disease (MASLD) has recently emerged as the predominant aetiology of chronic liver disease. It comprises a continuum of different stages, beginning with steatotic liver disease (SLD), defined as intra-hepatocyte lipid accumulation, and progressing through inflammation and metabolic dysfunction-associated steatohepatitis (MASH), to fibrogenesis, cirrhosis, and ultimately hepatocellular carcinoma (HCC) [1]. Given its significant role in the pathogenesis of liver cirrhosis, MASLD has been extensively studied as a cause of liver-related events. However, research has also been established between MASLD and cardiovascular disease, as well as the development of extrahepatic cancer [2]. Notably, these conditions represent the leading causes of mortality in among MASLD patients [3,4].

MASLD progression to HCC does not depend on cirrhotic evolution, as it is a recognized risk factor even in the absence of liver cirrhosis [5]. It has been estimated that in Italy nearly 50 % of patients with HCC in the context of metabolic liver disease, are not cirrhotic [6]. There are different risk factors for HCC in MASLD, including obesity, metabolic dysfunction, type 2 diabetes, metabolic syndrome and insulin resistance [7]. MASLD can progress to HCC through different pathogenetic mechanisms.

In brief, metabolic dysfunction, which may occur in genetically predisposed individuals, leads to an imbalance in lipid metabolism, resulting in increased oxidative stress and DNA damage. This process is accompanied by chronic inflammation which impairs immune surveillance and promotes HCC progression [8,9]. The connection between obesity and MASLD progression to HCC lies in the fact that obesity induces systemic inflammation and insulin resistance. These factors lead to a deterioration in the regulation of surveillance and lipid metabolism in the liver. Obesity alone

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doubles the risk of developing HCC, and this risk quadruples in individuals with a BMI exceeding 35 kg/m² [5].

A growing body of evidence indicates the significant role of the Protease-activated receptor 2 (PAR2) in regulating metabolic processes. PAR2 is a G protein-coupled receptor (GPCR) that is activated upon proteolytic cleavage by various enzymes, including trypsin, tryptase, and coagulation factors VIIa and Xa, among others [10]. PAR2 plays a crucial role in inflammation and fibrosis and has recently been identified as a key regulator of metabolism. Consequently, it has been extensively studied in the context of obesity, MASLD progression and HCC development (Figure 1).

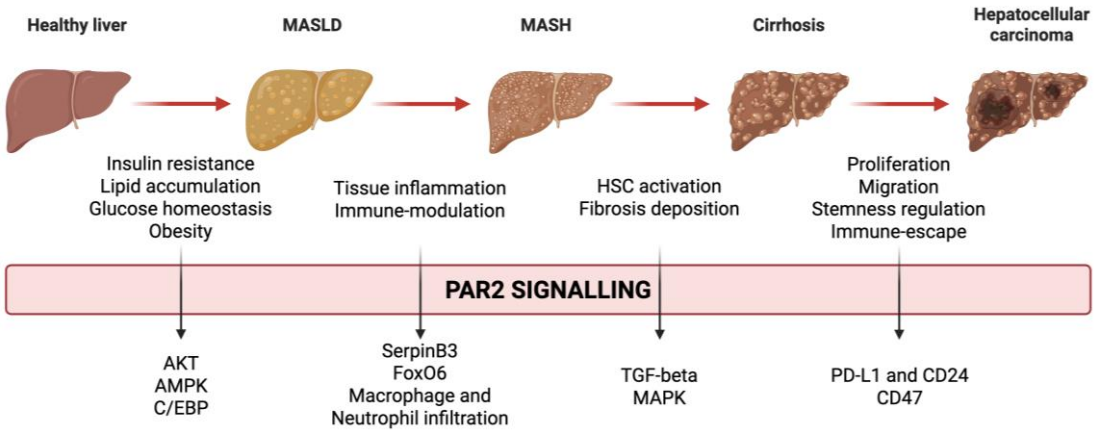

Figure 1. Interplay between Protease-activated receptor 2 (PAR2) and liver disease progression in metabolic dysfunction-associated liver disease (MASLD). The figure illustrates the primary PAR2 signalling pathways activated at various stages of liver disease progression The diagram encompasses the spectrum from early MASLD to advanced stages, including Metabolic Dysfunction-Associated Steatohepatitis (MASH) and progression to more severe conditions, including cirrhosis and hepatocellular carcinoma.

PAR2 and Metabolism

Insulin resistance, a hallmark of type 2 diabetes mellitus and obesity, is a central pathogenic mechanism in the development of metabolic dysfunction and hepatic steatosis. Impaired insulin signalling leads to a reduction in protein kinase B (Akt) activation, exacerbating dysregulation of glucose and lipid metabolism. A recent study investigated the role of PAR2 in promoting insulin resistance [11]. The authors observed an increased expression of PAR2 in hepatocytes from patients with concurrent diabetes and hepatic steatosis. Using a murine model of steatosis and diabetes, they also demonstrated that genetic deletion of PAR2 (PAR2-KO) improved histological markers of steatosis activity and led to a reduction in plasma glucose and insulin levels. These effects were primarily mediated by an increased expression of the glucose transporter GLUT2, which permits glucose uptake and glycogen storage in the hepatocytes. Mechanistically, they showed that PAR2 activation inhibited insulin-Akt signalling, promoting insulin resistance, whereas pharmacological inhibition or genetic silencing of PAR2 restored insulin sensitivity.

The contribution of PAR2 to insulin resistance was also reported in another study where the authors found elevated levels of forkhead transcription factor (FoxO) 6 in insulin-resistant rats [12]. FoxO6 upregulation was accompanied by increased PAR2 expression. Deletion of FoxO6 improved insulin sensitivity and reduced PAR2 expression. The mechanistic link between FoxO6 and PAR2 was found in IL-1β, a cytokine, induced by FoxO6 signalling, which in turn stimulated PAR2 expression and inhibited insulin signalling.

Insulin resistance promotes lipid uptake, de novo lipogenesis, and lipid storage, ultimately leading to hepatic steatosis. Thus, lipid metabolism represents another critical pathway in the pathogenesis of MASLD. In this context, the role of PAR2 was also evaluated in lipid homeostasis. A recent study observed that hepatic PAR2 expression was elevated in steatotic livers and that patients

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with high PAR2 levels in the liver had significantly increased plasma LDL cholesterol [13]. In PAR2-KO mice fed a high-fat diet (HFD), PAR2 deficiency was associated with lower hepatic and plasma cholesterol levels. This effect was mediated by three mechanisms: a) reduced cholesterol synthesis, b) increased hepatic cholesterol uptake and c) enhanced biliary cholesterol excretion, as demonstrated by PCR analysis of gene transcription. Despite unchanged plasma triglyceride levels, PAR2-KO mice exhibited reduced hepatic accumulation of triglyceride and fatty acid. This was accompanied by downregulation of key lipogenic enzymes and upregulation of genes associated with β-oxidation. Mechanistically, the authors proved that PAR2-induced activation of JNK1/2 promoted sterol regulatory element binding protein 1 (SREBP1c) activation and inhibited AMP-activated protein kinase (AMPK), a key regulator of lipid catabolism.

Further evidence supporting the link between PAR2 and AMPK came from Kim et al., who demonstrated that PAR2-KO mice were protected from developing hepatic steatosis when fed on HFD. PAR2-KO mice showed higher AMPK activation, compared to wild-type controls. Conversely, in vitro, overexpression of PAR2 was associated with AMPK inhibition. The downregulation of AMPK by PAR2 resulted in an impairment of autophagy, contributing to hepatic lipid accumulation [14].

Badeanlou et al. reported that genetic deletion of PAR2 or tissue factor (TF), which mediates coagulation factor VIIa-induced PAR2 activation, protected mice from developing obesity and insulin resistance when subjected to an HFD [15]. Furthermore, PAR2 deletion was associated with a reduction in macrophage infiltration in the adipose tissue, supporting the role of PAR2 in the development of tissue inflammation. Interestingly, the researchers uncovered a dual role for PAR2. Specific deletion of PAR2 or TF in non-hematopoietic cells led to a reduction in weight gain. Conversely, deletion of these targets in immune cells did not affect obesity development but led to decreased markers of insulin resistance and inflammation.

Some years later, the same group explored the role of TF–PAR2 signalling in the liver [16]. Genetic deletion or pharmacological inhibition of TF, PAR2, or both of them, led to reductions in gluconeogenesis, lipogenesis, and liver inflammation in a murine model of diet-induced obesity. Notably, these beneficial effects were also observed when TF or PAR2 deletion was restricted to hematopoietic cells, further supporting the role of this receptor in the crosstalk between the immune system and target tissue. Interestingly, wild-type (WT) mice fed an HFD exhibited increased hepatic infiltration of $CD11b^{+}CD11c^{+}$ macrophages and $CD8^{+}$ lymphocytes, compared to mice fed a low-fat diet (LFD). Accordingly, TF deletion decreased $CD11b^{+}CD11c^{+}$ macrophage infiltration, while PAR2 deletion reduced the $CD8^{+}$ T cell population.

A novel mechanism involving PAR2 in liver disease was recently identified by Villano et al. who demonstrated an interaction between PAR2 and SerpinB3 [17]. PAR2 activation induced the expression of the transcription factor CCAAT/enhancer-binding protein β (C/EBPβ), a known regulator of SerpinB3. In turn, SerpinB3, a member of the serine protease inhibitor family, was shown to be essential for PAR2 activation. Importantly, the authors found that PAR2 activity could be effectively inhibited using a small molecule called 1-Piperidine Propionic Acid (1-PPA): treatment with 1-PPA prevented lipid accumulation, inflammation, and liver fibrosis in mice overexpressing SerpinB3. Additionally, it suppressed C/EBPβ expression in both THP-1 and HepG2 cells.

SerpinB3 has been previously implicated in the pathogenesis of steatotic liver disease. In a recent study, due to its involvement in lipid accumulation and inflammation, it has been proposed as a new *hepatokine* [18]. In this study, two mouse models were used: one overexpressing SerpinB3 (TG/SB3), and the other expressing an isoform lacking its antiprotease activity (KO/SB3). Mice were fed on two different steatogenic diets: methionine–choline-deficient (MCD) or choline-deficient, L-amino acid-defined (CDAA). Overexpression of SerpinB3 resulted in increased hepatic lipid accumulation and inflammation, while loss of its activity conferred protection compared to WT mice. Moreover, TG/SB3 mice exhibited increased hepatic accumulation of crown-like structures formed by macrophages, a characteristic histological feature of steatotic liver disease, mirroring the findings of Wang et al. regarding TF–PAR2 signalling and $CD11b^{+}CD11c^{+}$ macrophage recruitment [16].

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PAR2 and Inflammation

The role of PAR2 in regulating inflammation has been investigated in various clinical contexts. Recent evidence has highlighted its involvement in promoting antigen responses during allergic reactions. A recent study demonstrated that inhibition of PAR2 reduces the production of inflammatory cytokines in a model of hypersensitivity [19]. It has also been shown that PAR2 antagonism effectively reduces airway hyperresponsiveness and inflammation in a murine model of allergic reaction [20]. Similar results were obtained in a mouse model of allergen-induced asthma [21], as well as in vitro [22].

In line with these findings, our research team recently published a study evaluating the efficacy of PAR2 antagonism using 1-PPA in a murine model of sepsis induced by intraperitoneal injection of lipopolysaccharide [23]. Treatment with 1-PPA significantly attenuated the inflammatory response and vasodilation, consequently enhancing cardiac function, reducing organ damage and alleviating clinical symptoms, which ultimately led to improved survival rates. The role of PAR2 in the response to microorganisms was also highlighted by Chu et al. who described a novel PAR2-dependent mechanism of neutrophil activation, driving inflammation and giving the rationale for inhibiting this pathway in the treatment of neutrophil-mediated inflammatory disease [24]. Other evidence demonstrated that PAR2 inhibition can prevent infection by *Candida albicans* [25], and periodontal inflammation induced by *Porphyromonas gingivalis* [26].

Our research team recently published findings on the effects of PAR2 inhibition using 1-PPA in neuroinflammation, demonstrating its efficacy in reducing amyloid deposition and neuroglial inflammation in fibroblasts in patients with Parkinson's disease [27]. Consistently, in another study, it has been shown that inhibition of mast cell tryptase effectively reduced PAR2-driven neuroinflammation in a murine model of cardiac arrest [28].

Several studies have also explored the role of PAR2 in intestinal diseases. It has been reported that a microbiome with high proteolytic activity can trigger colitis via PAR2 activation in mice [29]. Bacterial proteolytic activation of PAR2 has also been identified as a potential therapeutic target in inflammatory bowel disease [30] and in mediating both inflammation and pain in colitis [31]. However, Ke et al. showed that PAR2 deficiency in myeloid-derived suppressor cells enhanced their immunosuppressive activity, suggesting a dual role for this receptor in this context [32].

Of particular relevance to this review is the link between PAR2, inflammation and metabolism, an interplay that involves not only the liver but also other organs. Ha et al. showed that PAR2-deficient mice were protected from HFD-induced kidney inflammation and injury [33]. In a subsequent study, the same group demonstrated that PAR2-KO mice were also protected from age-related kidney inflammation and senescence [34].

Two different studies further demonstrated the efficacy of PAR2 inhibition in preventing LDL-induced vascular damage in models of atherosclerosis, reinforcing the role of this receptor in the crosstalk between metabolism and inflammation [35,36].

More recent studies have provided further insights into the complex interaction between the immune system and target organs. Reches et al. addressed this issue by investigating the role of PAR2 in two distinct murine models of liver injury: one immune-mediated (using concanavalin A) and one toxin-induced (with carbon tetrachloride CCL_4). The authors compared wild-type and PAR2 knockout mice and repeated the experiments after bone marrow transplantation between the two strains. Their results demonstrated that PAR2 expression in liver tissue was essential for hepatocyte regeneration after toxic injury, whereas PAR2 expression in immune cells enhanced inflammation and worsened immune-mediated damage [37].

This dual role of PAR2 was later confirmed by the same group in a model of autoimmune diabetes. In this context, PAR2 expression in pancreatic β-cells was protective against immune damage, while its expression in lymphocytes promoted β-cell destruction and diabetes onset [38].

This dichotomous role of PAR2 in immune and target tissues warrants further investigation, particularly given the emerging importance in tumour development of this receptor.

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PAR2 and Fibrosis

The importance of PAR2 in regulating the immune response is particularly relevant in liver disease, as chronic inflammation increases the risk of liver fibrosis, eventually leading to cirrhosis. It has been reported that inhibition of PAR2 using the pepducin PZ-235 was effective in preventing liver fibrosis. PZ-235 directly reduced hepatic stellate cell (HSC) activation and decreased hepatocyte death, thereby limiting the main stimulus for chronic inflammation [39].

Other studies have confirmed the involvement of PAR2 in modulating HSC function. Knight et al. showed that PAR2-KO mice were protected from fibrosis induced by CCl_4, a protection associated with reduced expression of TGF-β, the main mediator of fibrogenesis [40]. In a later study, the same authors investigated the relationship between TF activation and liver fibrosis using three different genetically modified murine models lacking PAR2, the cytoplasmic domain of TF, or both. Deletion of either PAR2 or TF conferred protection against fibrosis, and simultaneous deletion of both did not produce any additive benefit, suggesting that TF-PAR2 signalling is a shared pathway driving fibrosis [41].

In vitro studies also support this evidence. HSCs upregulated both PAR1 and PAR2 expression when cultured on plastic to promote myofibroblastic phenotype transformation. Stimulation of either receptor increased proliferation and activation of mitogen-activated protein kinase (MAPK), while their inhibition had the opposite effect [42]. Another in vitro study explored the role of PAR2 in apoptosis. Activation-induced cell death, triggered by phorbol myristate acetate, was suppressed by concurrent PAR2 stimulation with tryptase, suggesting a protective anti-apoptotic role of PAR2 in activated HSCs [43].

The profibrotic activity of PAR2 is not limited to the liver. Tisch et al. recently reported that genetic deletion of PAR2 reduced airway fibrosis following allergen exposure [44]. Similar protective effects were observed in the kidney, where PAR2KO mice were protected from renal inflammation and fibrosis [45]. Along the same lines, both PAR2 and PAR1 contribute to kidney injury and fibrosis in a diabetic mouse model [46].

Additional studies have examined the effects of PAR2 on intestinal fibrosis. Mast cell-derived tryptase activated PAR2 on intestinal fibroblasts, promoting their transition to myofibroblasts and mirroring the findings in hepatic HSCs [47]. Two additional studies confirmed that PAR2 inhibition protected against intestinal fibrosis [48,49].

Collectively, these findings underscore the role of PAR2 in orchestrating immune responses and promoting fibrosis across multiple organ systems [50]. This has important implications, as sustained inflammation and fibrotic signalling may contribute to tumour development via activation of oncogenic pathways.

PAR2 and Cancer

The role of PAR2 in oncogenesis has been extensively studied. In the liver, PAR2 expression in HSCs promotes HCC growth and angiogenesis in a murine xenograft model, whereas genetic deletion of PAR2 suppresses these effects. Furthermore, PAR2-deficient HSCs exhibited reduced activation following TGF-β stimulation and Hep3B-conditioned medium, indicating that PAR2 expression in HSCs contributes not only to fibrogenesis but also to HCC progression [51].

Moreover, a recent study showed that high PAR2 expression in HCC tissue samples after radical resection was associated with more aggressive clinical features. Patients with elevated PAR2 levels had larger tumours, more advanced stages, and a higher incidence of microvascular invasion. Building on these findings, the authors demonstrated in two in vitro hepatic cancer cell models that PAR2 overexpression enhanced both proliferation and metastatic potential, while silencing of PAR2 reversed these effects. In vivo, PAR2 silencing reduced the extent of metastatic spread in a mouse model of liver metastasis [52].

In another study, the authors showed that secreted cathepsin S interacts with PAR2 to regulate the transition of cancer stem cells in HCC. Cathepsin S was secreted by $CD47^+$ cells displaying

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stemness characteristics. Suppression of CD47 reduced HCC cell proliferation, and disruption of the cathepsin S/PAR2 axis increased chemosensitivity, suggesting a role for this pathway in therapy resistance [53].

Immune checkpoint inhibitors (ICIs) have become the first-line therapy for various cancers. Given its role in modulating immune responses, PAR2 has been investigated in this context.

In a recent study, a novel gene signature predictive of response to immunotherapy across multiple cancer types was identified [54]. Interestingly, among the genes analysed, PAR2, together with RNA-binding motif protein 9, was associated with poorer response to ICI therapy.

The role of PAR2 in regulating anti-tumour immune responses was further explored in a study on metastatic colorectal cancer [55]. The authors found that PAR2 expression correlated with reduced macrophage-mediated phagocytosis of malignant cells. This effect was driven by increased expression of the "don't eat me" signal, CD24. Conversely, neutrophil elastase, which cleaves and inhibits PAR2, downregulated CD24 expression, thereby enhancing phagocytosis. Similar findings were reported also in breast cancer, where activation of PAR2 signalling via coagulation factor VIIa led to reduced phagocytosis in an in vitro model [56]. Moreover, genetic deletion of PAR2 in vivo resulted in a smaller tumour size. Both studies highlighted a synergistic effect between PAR2 inhibition (whether pharmacological or genetic) and ICI treatment.

Apart from phagocytosis, other authors have focused on the effects of PAR2 in regulating T-cell response. A recent study reported that activation of PAR2 by coagulation factor Xa (FXa) conferred resistance to anoikis in HCC cells, thereby promoting metastasis. FXa-mediated PAR2 activation also increased PD-L1 expression while simultaneously inhibiting $CD8^+$ T-cell infiltration into tumours. Inhibition of FXa in vivo resulted in decreased metastasis and downregulation of PD-L1. These effects were further enhanced by concomitant administration of anti–PD-L1 antibodies [57]. Similar results were reported in the context of breast cancer, where PAR2 activation leads to increased production and stabilization of PD-L1 and a reduction in $CD8^+$ T cell activity [58].

These findings suggest that PAR2 expression in cancer cells not only promotes tumour progression and invasion but also suppresses the immune response. On the other hand, as previously reported, PAR2 expression in the immune system may activate immune response (Figure 2). However, to the best of our knowledge, no study to date has directly investigated the impact of PAR2 activation on immune cells within the tumour microenvironment.

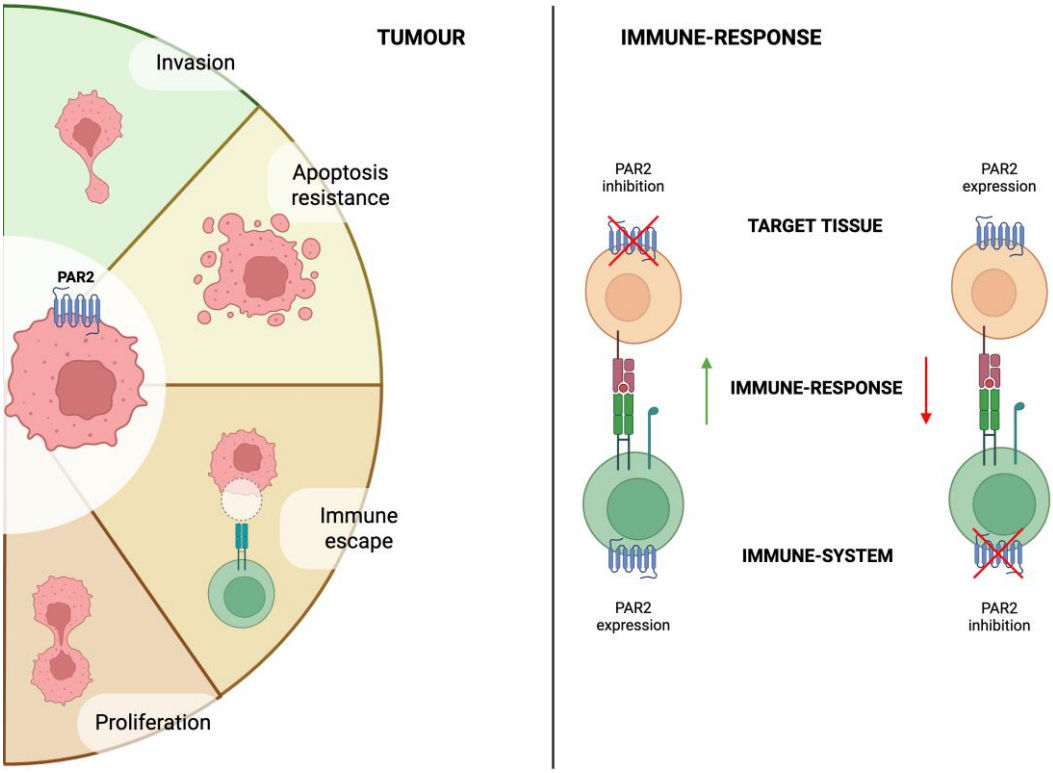

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Figure 2. The dichotomous functions of PAR2 in cancer biology. PAR2 expression in cancer cells promotes tumour progression and invasion, while simultaneously compromising immune surveillance. On the other hand, Conversely, PAR2 expression in the immune cells can stimulate and enhance immune response.

Conclusion

In conclusion, PAR2's pivotal role in the development and progression of the MASLD spectrum has been extensively investigated given its dual role in regulating metabolism and inflammation, ultimately contributing to tumorigenesis. Current research is increasingly focused on PAR2's role in modulating the interplay between the immune system and target tissues. This emerging field of study holds particular promise, considering the growing significance of immunotherapy in oncology.

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Abbreviations

The following abbreviations are used in this manuscript:

MASLD	Metabolic dysfunction-associated steatotic liver disease
HCC	hepatocellular carcinoma
PAR2	Protease-activated receptor 2
MASH	metabolic dysfunction-associated steatohepatitis
HFD	high-fat diet

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