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Preascitic Sodium Retention in Cirrhosis: A Role for Disregulated Proteolysis by Proprotein Convertases?

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ABSTRACT

Loss of effective arterial blood volume, secondary hyperaldosteronism, adrenergic activation and nonosmotic hypersecretion of vasopressin induce sodium and water retention in cirrhotic patients with ascites. The mechanisms of sodium retention that precede ascites formation remain elusive. In patients who are at the preascites stage of cirrhosis, no sign of reduced effective volaemia is found; nonetheless, tubular sodium retention is already present. Maturation and full functionality of epithelial sodium channels (ENaC) in distal segments of the nephron and, therefore, final control of sodium excretion are dependent on regulated proteolysis by proprotein convertases. Evidence of abnormal or incomplete maturation of ENaCs in preascitic cirrhosis exists, but the complex mechanisms of regulated proteolysis leading to ENaC maturation through sequential action of serine endopeptidases (i.e., furin, site-1 protease, prostasin, plasmin) have never been studied in liver cirrhosis. Also, the mechanisms of cirrhosis-associated immune dysfunction, which are characterised by systemic sterile inflammation and release of proinflammatory cytokines that profoundly influence renal function, remain largely unknown. Release of proinflammatory cytokines and functions of respective receptors are controlled through regulated proteolysis by cell membrane metallopeptidases (mainly ADAM-10 and -17). Once again, little is known in preascitic cirrhosis about potential disregulated proteolysis of proinflammatory cytokines that may trigger systemic inflammation and renal dysfunction. We advance a new hypothesis that (a) may link proprotein convertases to disregulated proteolysis of tubular sodium channels, renin-angiotensin system receptors and inflammatory mediators, and that (b) may shed light on the mechanisms of sodium retention before any systemic neurohormonal activation in liver cirrhosis.

Abbreviations: AAT, alpha-1 antitrypsin; ACLF, acute-on-chronic liver failure; ADAM-10, a disintegrin and metalloproteinase domain-10; ADAM-17, a disintegrin and metalloproteinase domain-17; AKI, acute kidney injury; Ang II, angiotensin II; AT1R, angiotensin type 1 receptor; AVP, arginine vasopressin; CAID, cirrhosis-associated immune dysfunction; CaRs, calcium-sensing receptors; CD, collecting duct; EGF, epidermal growth factor; EGF-R, epidermal growth factor receptor; ENaC, epithelial sodium channel; HRS, hepatorenal syndrome; IFNγ, interferon γ; IL-5, interleukin-5; IL-6, interleukin-6; IL-6R, interleukin-6 receptor; IL-7, interleukin-7; IL-8, interleukin-8; IL-12, interleukin-12; MCP-1, monocyte chemoattractant protein-1; mPRR, membrane-bound prorenin receptor; mTNF-α, transmembrane tumour necrosis factor α; NCC, thiazide-sensitive sodium chloride cotransporter; NF-κB, nuclear factor-κB; PRR, prorenin receptor; RAAS, renin-angiotensin-aldosterone system; ROS, reactive oxygen species; RP, regulated proteolysis; S1P, site-1 protease; SNS, sympathetic nervous system; sPRR, soluble prorenin receptor; sTNF-α, soluble tumour necrosis factor α; TACE, tumour necrosis factor α converting enzyme; TGF-α, tumour growth factor alpha; TNF-α, tumour necrosis factor α; $TNFR_I$, tumour necrosis factor α type I receptor; $TNFR_{II}$, tumour necrosis factor α type II receptor.

This work is dedicated to Prof. Federico Manenti (1935-2016), former secretary-general of the Italian Association for the Study of the Liver.

Summary

- Pharmacological modulation of non-classical RAAS functioning experimentally and clinically is showing promise in the setting of liver cirrhosis and its early or late complications.
- Enzymes and peptide receptors of the renin-angiotensin aldosterone system (RAAS), renal sodium and water channels, and proinflammatory and profibrogenic peptides must undergo specific maturation through regulated proteolysis by proprotein convertases both inside cells and on the cell surface.
- It can be hypothesised that early (preascitic) renal sodium retention and critical mechanisms of decompensation of liver cirrhosis may be influenced by disregulated proprotein convertases function.

1 | Introduction: Sodium Retention in the Ascitic Patient

Ascites formation in patients with liver cirrhosis is the hallmark of clinical decompensation, and this indicates a worsening of prognosis for the patient [1]. It is related to progressive renal sodium and water retention, which leads to hemodynamically ineffective expansion of the extracellular fluid volume and preferential translocation of excess fluid into the peritoneal cavity as ascites and into the tissues as peripheral oedema.

This loss of effective arterial blood volume in decompensated cirrhosis is related to splanchnic and systemic arterial vasodilatation that leads to compensatory overactivity of the sympathetic nervous and renin-angiotensin-aldosterone systems (respectively, SNS and RAAS) and nonosmotic hypersecretion of vasopressin [2]. In turn, the activated SNS and RAAS mediate constriction of the efferent glomerular arteriole more than the afferent one, resulting in a reduction of renal plasma flow and a decrease in peritubular capillary hydrostatic pressure. The latter favours retention of sodium and water along the tubular nephron [3].

In addition, angiotensin II (Ang II) causes sodium reabsorption in the proximal convoluted tubule through direct stimulation of tubular angiotensin type 1 receptors (AT1Rs) and activation of the renal cortical Na^+/H^+ exchanger [4].

Enhanced release of Ang II and increase in oxidative stress (through AT1R-dependent activation of NADPH-oxidase and RhoA/Rock 1 kinase pathways) are also keys to further renal sodium retention via increased activity of thiazide-sensitive sodium chloride cotransporters (NCC) in the distal convoluted tubule [5].

Increased systemic levels of Ang II and secondary aldosteronism lead to expected aldosterone-dependent Na^+/K^+-ATPase and epithelial sodium channels (ENaC) upregulation in the collecting duct, but also to stimulation of arginine vasopressin (AVP) secretion.

In turn, increased plasma AVP and increased reactive oxygen species (ROS) (due to stimulation of kidney AT1Rs) enhance the activity of $Na^+–K^+–2Cl^–$ cotransporters in the thick ascending limb of Henle's loop [6].

Finally, it is to be remembered that, well before nonosmotic AVP hypersecretion, early retention of electrolytes occurs in the thick ascending limb of Henle's loop in liver cirrhosis, as shown by increased expression of $Na^+–K^+–2Cl^–$ cotransporters, decreased expression of calcium-sensing receptors (CaRs) in this tubular segment, and remarkable natriuretic and

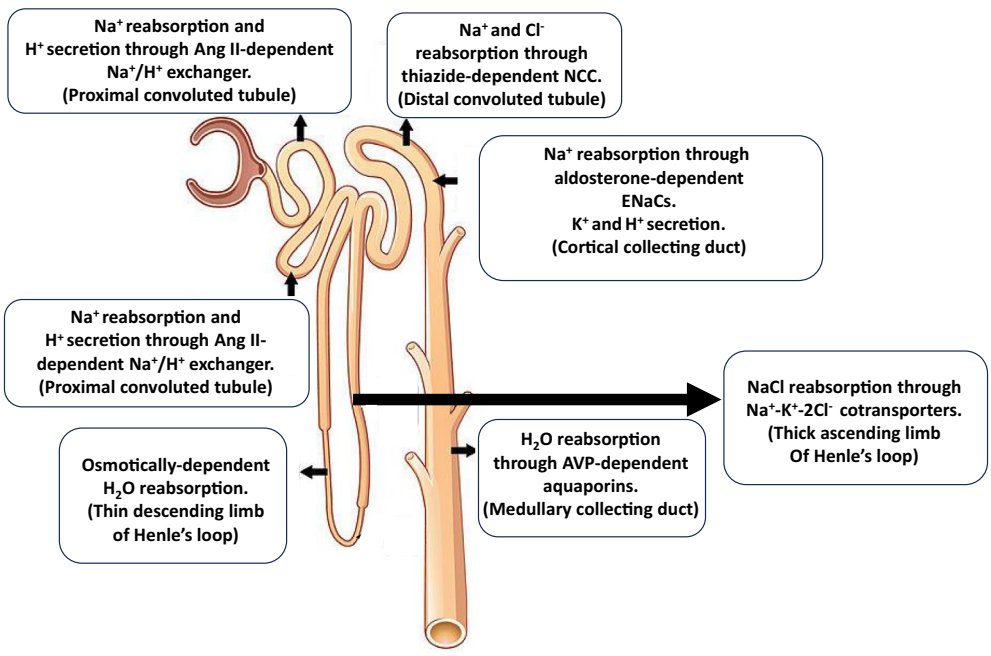

FIGURE 1 | Ascitic cirrhosis. Schematic depiction of tubular sites of sodium and water retention and respective mechanisms. AVP, arginine vasopressin; EnaC, epithelial sodium channel; NCC, thiazide-sensitive sodium chloride cotransporter.

aquaretic effects after administration of i.v. calcium or calcimimetic agents [6, 7].

In other words, increased systemic release of Ang II, secondary aldosteronism and adrenergic activation lead to sodium retention along all segments of the tubular nephron in ascitic cirrhosis (Figure 1).

2 | Uncertain Explanations for Preascitic Sodium Retention

Whilst we understand very well the neurohormonal changes that lead to renal tubular sodium retention in ascitic (decompensated) cirrhosis, there are some aspects of the pathophysiological events that lead to clinical decompensation that are still unclear. In other words, what causes the renal sodium retention in pre-ascitic cirrhosis that precedes the accumulation of ascites? In this state of preascites, the patient shows the combination of normal or minimally reduced systemic vascular resistance, normal or elevated glomerular filtration rate and normal or suppressed RAAS, features that exclude the possibility of an inadequately filled effective arterial blood volume as a cause of subtle sodium retention. Therefore, it is conceivable that the critical loss of effective arterial blood volume must be a later event in the natural history of the preascites state [8].

Several explanations for preascitic sodium retention have been proposed.

First, the intrarenal renin-angiotensin system seems to be activated earlier than its systemic counterpart because the application of lower-body negative pressure, which reduces central blood volume, enhances renal venous spillover of renin and Ang II in preascitic cirrhotics more than in healthy controls [9]. Moreover, in these patients, despite baseline suppression of the systemic RAAS, sodium overload induced by a high sodium diet can be reversed by the AT1R antagonist losartan administered at a dose not perturbing systemic hemodynamics [10, 11]. Unfortunately, clinical evidence that RAAS-blocking agents could prevent ascites formation remains elusive or impractical because they also tend to drop systemic blood pressure.

Along similar lines of investigation, the hypothesis that exclusive intrarenal activation of the SNS might occur in preascites was put forward but was never confirmed [8, 9].

Finally, it has repeatedly been suggested that, in preascites, an acquired defective activity of 11β-hydroxysteroid dehydrogenase (a glucocorticoid-clearing enzymatic activity embedded in mineralocorticoid receptors) could favour a pathological stimulation of the mineralocorticoid receptor itself by normal circulating levels of cortisol [12, 13].

3 | Serine Proteases and Preascitic Sodium Retention

Proteolytic processing mediates the transition of proteins from inactive to biologically active state. If a protein undergoes specific proteolytic cleavage, such proteolytic processing is referred to as regulated proteolysis (RP) and may lead to a highly active cleaved, i.e., mature, form of many enzymes, ion channels, bioactive peptides, and peptide receptors, either circulating in blood or bound to cell membranes [14]. Among hundreds of such processes, some examples of protein activation through RP, if malfunctioning, might be critically involved in the pathogenesis of preascitic sodium retention of liver cirrhosis.

Site-1 protease (S1P) is a serine protease that belongs to the family of proprotein convertase subtilisin/kexins. To become fully active, the S1P proprotein is first cleaved by a signal peptidase in the endoplasmic reticulum and then undergoes autocatalytic processing at multiple sites before being displayed as a mature enzyme bound to cell membranes. Mature S1P processes the prorenin receptor (PRR) to generate its soluble form (sPRR), which is a biomarker of a number of diseases including renal dysfunction and arterial hypertension [15]. It is known that binding of plasma renin and its circulating secreted precursor (prorenin) [together known as (pro)renin] to sparse membrane-bound PRR (mPRR) induces conformational changes in the ligands that fully activate local renin function and, therefore, final synthesis of Ang II. In turn, this tissue Ang II production leads to local, not systemic, effects, which may be, depending on the organ involved, profibrogenic, proinflammatory or, in the case of the kidney, sodium retentive [16].

Recently, a significant role of sPRR (whose production is dependent on S1P expression and activity) has been demonstrated in edematous states. This pathway involves S1P and ENaCs of the distal nephron. In practice, in renal tubular cells, binding of locally produced Ang II to the basolateral AT1R stimulates S1P-dependent conversion of mPRR to sPRR and its apical release into the tubular fluid. In turn, the sPRR or the sPRR bound to (pro)renin have direct effects to stimulate ENaC expression in cortical and medullary collecting duct (CD) [15]. Noticeably, ENaCs appear to be overexpressed in distal convoluted tubules, connecting tubules and CDs of experimental models of preascitic cirrhosis, which leads to sodium retention despite normal or suppressed systemic levels of Ang II and aldosterone [13]. This finding might be explained by increased conversion of mPRR to sPRR through disregulated proprotein convertase (i.e., S1P) function. For the time being, little is known about S1P function in experimental or human preascitic cirrhosis. Nonetheless, experimental evidence of PRR up-regulation in human and mouse fibrotic livers and in activated hepatic stellate cells already exists. Not unexpectedly, PRR down-regulation attenuates liver fibrosis through inactivation of the ERK/TGF-β1/Smad3 pathway [17]. Moreover, increased renal expression of full-length PRR and urinary excretion of sPRR, obtained through high K^+ diet in Sprague–Dawley rats, was followed by upregulation of β subunits of EnaCs in the collecting duct, and this was blunted by means of specific PRR inhibitors [18].

4 | Serine Convertases and ENaCs Maturation in Preascites

Even more intriguing is the possibility that, in preascitic cirrhosis, the maturation and function of ENaCs themselves might be affected by acquired disorders of regulated proteolysis. The probability of cellular apical ENaCs to convert to the open

configuration, and therefore their capacity to reabsorb sodium from the tubular fluid in the CD, is dependent on finely regulated proteolysis of the α, β and γ subunits of these sodium channels. In fact, following proteolytic processing by another serine proprotein convertase (furin) [19] in the Golgi complex, ENaC α, β and γ subunits are transferred to the apical plasma membrane of CD cells, where they are assembled and submitted to further regulated proteolysis mostly by membrane serine protease prostasin before acquiring a high probability of open configuration [20] (Figure 2).

Four aspects of this pathway are worth mentioning in relation to chronic liver disease.

First: abnormally cleaved γ subunits of ENaC have been identified in CD principal cells of rats with CCl_4-induced experimental liver cirrhosis and sodium retention [12, 13].

Second: the above-mentioned intracellular serine protease furin may well be expressed as bound to cell membranes or floating in plasma [19, 21], and may compete with prostasin and alter the normal maturation of ENaC subunits anchored to the apical membranes of CD cells [20]; moreover, plasmin, a furin-like serine protease whose levels are high in patients with cirrhosis due to increased tissue-type plasminogen-activator activity and decreased alpha 2-antiplasmin [16, 22, 23], shares the same furin cleavage site in ENaC subunits [20], once again competing with physiological prostasin-dependent activation of ENaCs.

Third: prostasin itself, the physiological activator of ENaC subunits, is activated through regulated proteolysis by a further membrane serine protease, matriptase, in the so-called endopeptidase cascade [20]. Therefore, the endopeptidase cascade itself might be dysregulated in preascitic cirrhosis.

Fourth: not unexpectedly, systemic plasma of healthy subjects contains serine protease inhibitors (e.g., serpins, alpha-1 antitrypsin) to limit this and other endopeptidase cascades [24]. It is well known that alpha-1 antitrypsin (AAT) deficiency due to the homozygous carriage of the Pi*Z gene variant increases the risk for developing cirrhosis. Likewise, the heterozygous carriage of the Pi*Z variant is a strong single nucleotide polymorphism-based risk factor for cirrhosis development in non-alcoholic fatty liver disease and alcohol misuse [25].

5 | Cirrhotic Immune Dysfunction and Sodium Retention

Recently, cirrhosis-associated immune dysfunction (CAID) has been described [26]. It encompasses the wide array of abnormalities observed in the immune system of cirrhotic patients. This syndrome consists of excessive systemic inflammation as an initial phenotype in compensated cirrhosis, followed, in later stages of decompensated disease, by eventual exhaustion of the immune response system, which switches to an immunodeficient phenotype [27]. In other words, CAID is characterised by early but lasting systemic inflammation even in the absence of infection (so-called sterile inflammation) and, on top of this, by later immunodeficiency, represented by lack of adequate immune response to infectious agents in advanced stages of liver cirrhosis, such as acute-on-chronic liver failure (ACLF). The immunologic mechanisms of cirrhosis development in chronic liver disease, albeit extremely interesting, are far from the purpose of this review.

Up until now, excessive systemic inflammation has been attributed somewhat generically to ongoing hepatocyte necrosis from an untreated underlying aetiology or to translocation of bacterial antigens or DNA from the gut to the systemic circulation [28]. For sure, cirrhosis is associated with small intestine bacterial overgrowth, perturbation within the intestinal microbiota and altered integrity of intestinal mucosa, that is, dilatation of extracellular space between adjacent enterocytes, infiltration of inflammatory cells into the gut lamina propria, reduced intestinal immunoglobulin A secretion, persistent activation of

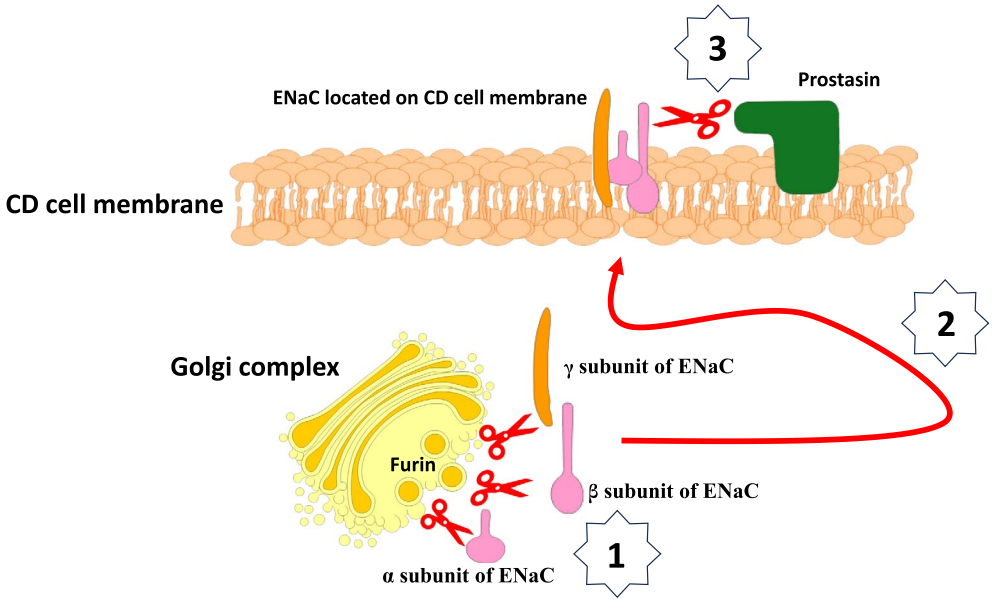

FIGURE 2 | Diagram depicting pathways of synthesis and activation of epithelial sodium channels (EnaCs) in collecting duct (CD) cells.

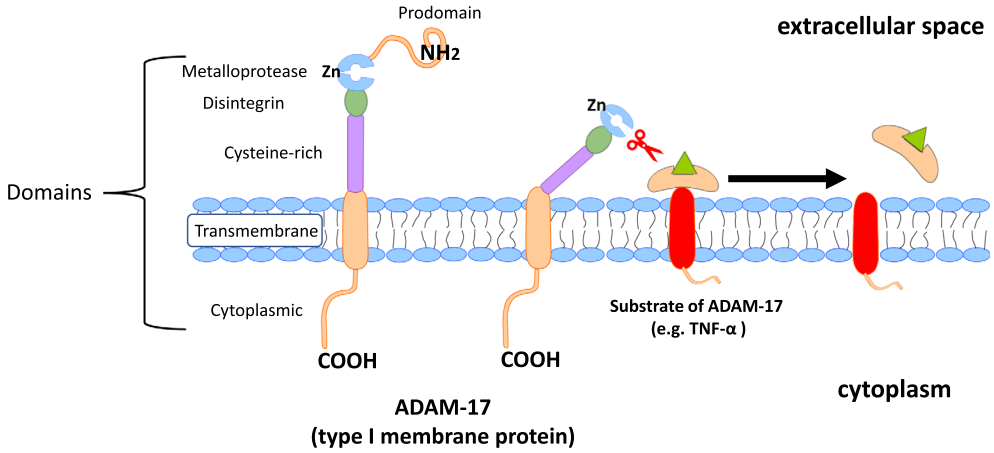

FIGURE 3 | Schematic depiction of Type I transmembrane protein ADAM-17, its common domains, and its protease activity on main substrate (plasma membrane TNF-α).

toll-like receptors [29]. The latter phenomenon, in turn, is associated with activation of cell-intrinsic inflammasomes and nuclear factor-κB (NF-κB), leading to the release of proinflammatory cytokines [30]. All of this may result in the progression of cirrhosis to acute decompensation or acute on chronic liver failure with later immune exhaustion [29].

CAID is dynamic and occurs across the spectrum of all aetiologies of cirrhosis [31, 32]. Patients with compensated or stable ascitic cirrhosis exhibit systemic inflammation with greater production of proinflammatory cytokines [33], whilst patients with ACLF display 'sepsis-like' immune paralysis due to markedly reduced expression of HLA-DR molecules on monocytes (celluar immune depression), since the antigen-presenting HLA-DR receptor complex is important for a fully functional innate immune response upon contact with different pathogens [32].

Compared with healthy controls, patients with compensated, preascitic liver cirrhosis display significantly higher levels of proinflammatory cytokines (interleukin-6 [IL-6], interleukin-7 [IL-7], interleukin-5 [IL-5], interleukin-12 [IL-12], monocyte chemoattractant protein-1 (MCP-1) and, most importantly, tumour necrosis factor-α [TNF-α]), without any evidence of overt infection [27]. In turn, increasing evidence indicates that proinflammatory cytokines mediate renal microvascular dysfunction and glomerular filtration rate reduction [34], whereas the oxidative stress that results from the interaction of Ang II and TNF-α (whose release into systemic circulation is in part Ang II-dependent) [35], with the tubular epithelial cell can hurt tubular function and cause AKI in the absence of overt signs of renal hypoperfusion [31]. As expected, in preascitic cirrhotic patients, the presence of renal dysfunction (i.e., subtle tubular sodium retention) is strictly associated with high TNF-α levels [33].

Among ascitic patients, Clària et al. demonstrated that patients with ACLF have even higher plasma levels of TNF-α, IL-6, interleukin-8 (IL-8) and MCP-1 than patients with decompensated cirrhosis without ACLF [34]. Among patients with ACLF, those with acutely decompensated disease with isolated renal failure or with hepatorenal syndrome (HRS) have maximally increased plasma levels of proinflammatory cytokines such as IL-6, TNF-α, vascular cell adhesion molecule-1 and augmented urinary excretion of MCP-1 [33], confirming a definite role at least for TNF-α—thought to be the key mediator of the systemic inflammatory response syndrome [35]—in the occurrence of progressive cirrhotic renal dysfunction. On the whole, sterile inflammation, along with systemic haemodynamic alterations, seems to contribute to the development of renal dysfunction ranging from preascitic sodium retention up until acute kidney injury (AKI) and HRS [36]. The nature of liver–kidney crosstalk in liver cirrhosis may be influenced also indirectly through a state of relative adrenal insufficiency, which leads to failure to suppress the transcription of inflammatory cytokines and further aggravation of immunological incompetence [37].

Circulating Ang II and TNF-α can gain access to the renal tubules mostly by proximity of the tubular capillaries and elicit a local inflammatory response [31]. Interestingly, binding of TNF-α to TNF-α type II receptors ($TNFR_{II}$) leads to mobilisation of NF-κB [38], the same proinflammatory intracellular pathway directly activated by Ang II binding to AT1Rs [16].

Based on current knowledge of the above messengers of inflammation, of their production pathways and, at times, unexpected mechanisms of signal transduction, we hypothesise that early sterile inflammation, high systemic levels of some proinflammatory cytokines and subtle renal dysfunction in preascitic cirrhosis might be influenced by possible abnormal functioning of proprotein convertases, as explained in the next paragraph.

6 | 'Sheddase' ADAM-17, Cirrhotic Sterile Inflammation and Altered Renal Function

In addition to the serine proteases mentioned above, there are also zinc metallo endopeptidases belonging to the family of a disintegrin and metalloproteinase domain-10 and -17 (respectively, ADAM-10 and ADAM-17) that are involved in regulated proteolysis. ADAMs are particularly interesting in relation to renal dysfunction in liver cirrhosis [24].

ADAM-17 is a Type I cell membrane protein with an N-terminal pro-domain attached to the extracellular zinc-metalloprotease domain. This is followed by a disintegrin domain, a cysteine-rich

domain, a membrane-spanning proximal domain ending with a C-terminal cytoplasmic tail (Figure 3) [39, 40]. ADAM-17 is synthesised in the cell through removal of an inhibitory pro-domain in the Golgi complex by means of classic furin-mediated cleavage [40–43]. Moreover, trafficking through the endoplasmic reticulum and Golgi and maturation of ADAM-17 strictly require the presence of iRhom proteins, which are inactive members of the rhomboid protease family [44, 45]. Phosphorylation of the cytoplasmic tail of ADAM-17 by MAP kinase is also necessary to lead to activation of ADAM-17 once anchored to the cell membrane [46].

ADAM-17 mRNA is expressed in most tissues, and mature ADAM-17 is found in both central and peripheral organs including the heart, arterial blood vessels, kidney, liver, brain, testicle, lungs, spleen, and muscles [43]. Moreover, it has been demonstrated that, in clinical settings characterised by enhanced systemic production of Ang II (e.g., in decompensated cirrhosis), AT_1Rs- and ADAM-17-enriched exosomes may transfer such proteins to peripheral target cells. Exosomes are extracellular nanovesicles of 30–100 nm in size that are released into the extracellular space by many cell types through reverse budding of multivesicular intracellular bodies [16, 47, 48].

Among substrates of regulated proteolysis by ADAM-17 are many pro-inflammatory messengers and their receptors. Noticeably, binding of Ang II to AT1Rs is a crucial mechanism leading to activation of ADAM-17 and other cell membrane proprotein convertases [49]. For this reason, the main vasoconstrictor peptide of the RAAS, Ang II, has been described as a vigorous inducer of inflammatory effects through the release of pro-inflammatory mediators: TNF-α but also epidermal growth factor (EGF), tumour growth factor alpha (TGF-α), MCP-1, IL-6 and interferon γ (IFNγ) [24, 50].

Among the substrates of the regulated proteolysis by Ang II-inducible ADAM-17, there is TNF-α, whose increased plasma concentrations are correlated with worsening of renal function in liver cirrhosis [33]. TNF-α is synthesised as a 26 kDa protein, anchors to plasma membranes as type II transmembrane TNF-α (mTNF-α), and is subjected to proteolysis by ADAM-17, which, for this reason, is also named TNF-α converting enzyme (TACE). The final effect is release of the 15 kDa form of soluble TNF-α (sTNF-α) [43], the active systemic messenger of inflammation and key cytokine found increased in plasma of cirrhotic patients with sterile inflammation. Interestingly, both TNF-α type I receptor ($TNFR_I$) and $TNFR_{II}$ are subjected to regulated proteolysis by ADAM-17 itself, leading to truncated soluble forms of both receptors [43].

Another important ADAM-17 substrate is the IL-6 receptor (IL-6R). IL-6R is a type I membrane protein, which binds the cytokine IL-6 [51]. The complex of IL-6 and IL-6R (being the latter either bound to cell membranes or previously made soluble through proteolysis by ADAM-17) binds to the cell membrane signalling receptor gp130, which thereupon initiates intracellular signal transduction, mainly via the JAK/STAT and PI3K signalling pathways [52], leading to systemic sterile inflammation.

Finally, ADAM-17 was recognised to cleave also epiregulin, EGF, epigen, amphiregulin and heparin-binding EGF-like growth factor [43]. These peptides, once released from cell membranes through ADAM-17-dependent proteolysis, are EGF receptor (EGF-R) ligands and lead to EGF-R activation. This signalling pathway is called EGF-R transactivation. However determined, activation of EGF-R by amphiregulin, which is made soluble by Ang II-stimulated 'sheddase' ADAM-17, was shown to bring about renal dysfunction and kidney peritubular fibrosis [43].

There could be a role for disregulated proteolysis by ADAM-17 also in sodium retention in human or experimental liver cirrhosis? This is conceivable due to the finding that amphiregulin and its sheddase (ADAM-17) promote the process of hepatic stellate cell proliferation and fibrogenic activity in vitro [53]. Moreover, liver protective effects of ursodeoxycholic acid appear to be mediated, at least in part, through inhibition of ADAM-17 and decreased release of pro-inflammatory TNF-α, both inside the liver and systemically [54]. Finally, ADAM17 has been shown to be upregulated in the kidneys of diabetic mice, contributing to renal inflammation and fibrosis [55].

7 | Conclusions

To sum up, we propose that possible dysregulated proteolysis by specific serine or metallo proteases (also named sheddases), based on the seminal studies and mechanisms of disease mentioned above, should be a brand new topic of scientific investigation to solve the problem of sodium retention in cirrhosis before systemic neurohormonal activation. This, perhaps, would lead to a better understanding of the subtle mechanisms of clinical decompensation in this serious disease.

Author Contributions

Drafting of the article, critical revision of the article for important intellectual content, and final approval of the article: G.S., M.A., F.W.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

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