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REVIEW

Gastrointestinal Mucositis: The Role of MMP-Tight Junction Interactions in Tissue Injury

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Abstract Chemotherapy for cancer causes significant gut toxicity known as mucositis. The pathogenesis of mucositis is ill defined. Recent clinical research guidelines have highlighted epithelial junctional complexes as emerging targets within mucositis research. Given the robust biological evidence linking tight junctions and matrix metalloproteinases, key mediators of mucositis, tight junction proteins have received significant attention. Despite this, the link between tight junctions, matrix metalloproteinases and mucositis development is yet to be established. This critical review therefore aims to describe the role of matrix metalloproteinases in mucositis, and how matrix metalloproteinase-dependent tight junction disruption may contribute to the pathobiology of mucositis.

Keywords Matrix metalloproteinases · Tight junctions · Gut toxicity, Mucositis, Chemotherapy

Introduction

Gastrointestinal (GI) mucositis has become an increasingly recognized and better characterised toxicity of chemotherapy and radiotherapy [1]. Different cytotoxic treatment regimens have been shown to affect different regions of the GI tract and lead to common mucositis symptoms which include, but are not limited to, pain, ulceration, nausea, vomiting, diarrhoea, proctitis and rectal bleeding [1]. Systemic effects of mucositis commonly include infection, fatigue, malnutrition, and even occasionally death [1]. Although these toxicities arise in a large proportion of patients receiving cytotoxic anti-cancer treatment, an effective intervention has yet to be introduced; this is most likely due to an incomplete understanding of the underlying pathobiological mechanisms [1].

Our current understanding of mucositis is based on early animal OM models which highlighted the multifactorial nature of the condition and implicated a cascade of events in multiple tissue regions [2, 3]. These observations gave rise to the currently accepted 5-phase model of mucositis; which describes the sequence of genetic and histopathological events following cytotoxic treatment. Subsequent studies have since implicated microvascular injury [4], pro-inflammatory cytokines [5], microbiome-host interactions [6, 7] and extracellular alterations [8, 9] in the development of mucositis. More recently, research has implicated changes in the tight junctions of the gastrointestinal epithelial barrier may also be involved in the development of mucositis [10, 11]

It is well documented that tight junctions are essential in maintaining the gastrointestinal barrier [12]. Molecularly, tight junctions are very complex, with both cytoplasmic and transmembrane proteins closely linked, thus providing barrier function [13]. Briefly, tight junctions serve as the key regulator of intestinal permeability [14] via regulation of solutes across the gut epithelial barrier [15]. There is much anecdotal clinical evidence implicating the key role of tight junctions in the development of gastrointestinal mucositis [13] with early clinical studies showing abnormalities in intestinal permeability in patients receiving high dose chemotherapy [16] and various types of myeloablative therapies [11]. These findings were supported by key morphological changes seen in tight

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junctions in patients receiving high dose chemotherapy [17]. Clinical changes in tight junctions are supported by pre-clinical animal models which have all shown significant changes in tight junctions following chemotherapy [18–20].

Matrix metalloproteinases (MMPs) are well documented in affecting many different biological functions, and in particular, are known for their proteolytic functions. Further, they have recently been identified as major mediators of mucositis [9, 21]. Research also exists linking MMPs with tight junctions; however the link between these and mucositis has not been made. Therefore the aim of this critical review paper is to describe what we know about MMPs and mucositis; tight junctions and mucositis; and then links the two with the underlying pathobiology of mucositis.

Matrix Metalloproteinases (MMPs)

Function

MMPs are a group of zinc-dependent endopeptidases; originally described as having a predominant role in extracellular matrix (ECM) homeostasis [22]. Recent research has identified a wide range of functions for these peptidases including regulation of cell growth, triggering the release of growth factors, regulating apoptosis, altering cell motility, affecting immune responses and modulating the bioactivity of cytokines and chemokines [23–25]. Originally, MMPs were divided according to their ECM substrate specificity into five classes; namely the collagenases, gelatinases, stromelysins, elastases and membrane type MMPs. However, with the discovery of additional functions of MMPs, the more commonly used nomenclature is MMP-1 to MMP-28 [26].

Regulation of MMPs

Expression of most MMPs is normally low in tissues, however, they are induced when ECM remodelling is required or following injury [27]. The levels of MMPs are tightly regulated at many stages including transcription, activation from precursor zymogens (post-translational) as well as by tissue inhibitors of metalloproteinase (TIMPs) [22, 23]. MMPs are synthesised by a range of cell types including macrophages, neutrophils, fibroblasts and epithelial cells [23, 28]. They are secreted as latent, inactive zymogens and are converted to their active form in the extracellular space [28]. A latent MMP can gain catalytic activity through the disruption of the thiol-Zn^{2+} interaction. Van Wart and Birkerdal-Hansen (1990) referred to this mechanism as the 'cysteine-switch' and proposed that the thiol-Zn^{2+} interaction can be broken by three mechanisms: (1) modification of the free thiol by physiological (oxidants, electrophiles) or non-physiological (heavy metal ions, alkylating agents) compounds; (2) cleavage of the pro-domain by pro-protein convertases such as furin; and (3) inter- or intra-molecular autolytic cleavage of the prodomain by chemical or allosteric perturbation of the zymogen [29]. Further, MMPs work co-ordinately to create a cascade of activation where an activated MMP has the capacity to catalyse the activation of other MMP zymogens [23]. MMP activity is also regulated by TIMPs. To date four family members have been identified, namely: TIMP-1, -2, -3 and -4 [23]. Whilst all members are capable of inhibiting MMPs, TIMP-1 and -2 appears to be the most active [28]. These inhibit MMP activity by forming a 1:1 complex with the catalytic site of MMPs and chelating the active-site zinc.

MMPs in Mucositis

The role of MMPs in tissue injury and remodelling has been described in a variety of conditions including cancer and mucositis [9, 21] as well as inflammatory and degenerative processes, including rheumatoid arthritis [30], periodontitis [31] and asthma [32]. MMPs are also becoming well known for their ability to modulate inflammatory processes, innate immunity and repair following injury. Parks and colleagues (2004) describe the inflammatory process as comprising of a series of cellular responses. Furthermore, inflammatory responses rely on integrating information associated with the following: 1. detection of an injury and/or the presence of microorganisms, 2. accumulation and intervention of cells that eliminate invading microorganisms and infected host cells and 3. repair of tissues damaged by the initial insult, trauma or the response of the host [33]. The host response to cytotoxic chemotherapy, specifically irinotecan, involves the initiation of an inflammatory response 48 h - 72 h following treatment. Al-Dasooqi and colleagues (2010) recently demonstrated involvement of MMP-2, −9 and −12 in the inflammatory response induced in the gut by chemotherapy 48 h following treatment. There is a close correlation of inflammatory cell infiltrate and maximal morphological disturbance with the expression of those MMPs in epithelial cells and the underlying blood vessel-rich lamina propria [9]. This is consistent with mouse studies which describe a decreased inflammatory phenotype for $MMP2^{-/-}$, $MMP9^{-/-}$ and $MMP12^{-/-}$ mice [34–36]. However, newly emerging data in the literature suggests up-regulation of MMPs in some inflammatory conditions may also aid in alleviating the inflammatory process [37–40].

MMPs have also been shown to be involved in innate immunity and repair following injury [41, 42]. In the injured mucosal epithelium, a wound repair program is rapidly recruited by cells to heal wound through cell proliferation, spreading and migration. MMPs are key downstream mediators of this wound repair program. In chemotherapy-induced mucositis studies, MMP-1 levels are significantly elevated in the healing phase of mucositis (between 72 h and 144 h following chemotherapy) in the gastrointestinal tract [9, 21]. This data supports the suggestion that MMP-1 plays a vital

role in tissue healing following mucosal injury caused by chemotherapy. The mechanism by which MMP-1 carries out this role is not clear, however, past studies have suggested MMP-1 alters the migratory substratum to drive the forward movement of repairing cells. MMPs have also been shown capable of killing bacteria. Studies by Hartzell and colleagues (1999) were able to show that MMP-12$^{-/-}$ mice develop necrotic lesions in the gut, liver and kidneys and this was associated with the presence of Gram-positive cocci in areas of tissue necrosis [40]. Furthermore, it has been suggested that degradation pathways, including MMP signalling, are initiated by colonising bacteria as well as host cell signalling (through pro-inflammatory cytokines) and are a vital initiators of the innate immune response (Birkedal-Hansen H, 1993). In regions of the gut where mucositis is present, a significant increase in pathogenic bacteria, pro-inflammatory cytokines as well as MMPs is noted in the mucosa [9, 21]. These observations suggest some level of functional linkage between these in the immune response observed following chemotherapy.

Tight Junctions

Molecular Structure and Function

Tight junctions are highly dynamic signaling complexes integral to gastrointestinal homeostasis. Located at the apico-lateral surface of adjacent epithelial cells, tight junctions regulate the passage of solutes across the intestinal epithelium [43], thus forming a barrier between the internal milieu and the external environment. This overarching function of tight junctions is termed mucosal barrier function and is responsible for determining intestinal permeability.

Tight junctions exhibit a complex arrangement of interacting cytoplasmic and transmembrane proteins [15], which form continuous adhesive strands circumscribing the apico-lateral margin of polarized epithelia [44, 45]. Peripherally located zonular occludens proteins (ZO-1, −2, −3) belong to the membrane-associated guanylate kinases family (MAGUKs) that interact to anchor transmembrane proteins (occludin and claudin) to the cytoskeleton. It is well established that ZO proteins play pivotal roles in tight junction integrity and mucosal barrier function [45, 46]. However, recent studies have confirmed the long-maintained, but previously unsupported view, that ZO proteins are also required for tight junction assembly [45, 47, 48]. Umeda and colleagues (2006) demonstrated depletion of all ZO subtypes completely abrogated tight junction formation [45]. Moreover, introduction of ZO-1 and ZO-2 rescued tight junction formation, whereas ZO-3 failed to recruit tight junction assembly, thus elucidating roles for each ZO isoform [47, 48]. ZO proteins bind to the COOH termini of claudin proteins, essential components of tight junctions and major determinants of intestinal permeability [43]. Claudins display a wide range of structural variance, with over 20 functionally unique isoforms currently documented [15]. Recently, several claudin subtypes, particularly claudin-1, have been implicated in a number of inflammatory disorders of the bowel [49, 50] characterized by mucosal barrier dysfunction. Roles for claudin-1 have also been identified in apoptosis and mucosal regeneration [51]; thus claudin proteins are considered pivotal in gastrointestinal health. Occludin is a widely documented transmembrane protein critical for tight junction integrity and mucosal barrier function [52]. In vivo studies have solidified these roles with occludin knockout ($^{-/-}$) mice displaying morphologically intact tight junctions, yet significantly altered, intestinal permeability [53, 54]. These results suggest important roles for occludin in tight junction stability as opposed to assembly.

Evidence for Tight Junction Involvement in Mucositis

Tight junctions have long been recognized for their roles in gastrointestinal health and homeostasis, however there has been renewed interest in the role tight junctions may play in the development of mucositis. Tight junctions were first hypothesised as a potential pathomechanism of mucositis in 1997, with Keefe and colleagues identifying marked increases in intestinal permeability in patients receiving high-dose, combination chemotherapy [16]. Intestinal permeability, quantified using rhamnose/lactulose permeation, was significantly increased in all patients treated with chemotherapy, peaking 7-days post-treatment and correlating with the onset and duration of gastrointestinal symptoms such as vomiting and diarrhoea [55]. Perturbed intestinal permeability has also been identified following administration of various myeloablative treatments in patients receiving haemopoietic stem cell transplantation [11]. Together these studies suggest regimen-related mucosal barrier dysfunction is not limited to a single chemotherapeutic agent, but common to numerous cytotoxic treatments.

Morphological changes in intestinal tight junctions have also been identified in patients receiving high-dose chemotherapy with Keefe and colleagues (2000) reporting a significant increase in the amount of open tight junctions, assessed using electron microscopy, within the small intestinal epithelium of patients [17]. Again, these morphological changes coincided with peak intestinal permeability and gastrointestinal symptoms [17, 55], implying a correlation between tight junctions and toxicity. However, despite this strong evidence base, very few studies have quantified the molecular changes in specific tight junction proteins following cytotoxic treatment. Hamada and colleagues (2010) were the first to establish specific tight junction changes in response to cytotoxic treatment in an animal model. Briefly, tumour-naïve rats were given consecutive doses of methotrexate (MTX) for 3–5 days. Intestinal permeability, assessed via fluorescein isothiocyanate-dextran (FD-4) permeation, was

significantly increased in MTX-treated rats suggesting poor mucosal barrier function and tight junction integrity. Although investigations were limited to a single tight junction protein, ZO-1, significant changes in its expression, distribution and phosphorylation were also reported. Reversible phosphorylation is recognized as a vital mediator of tight junction protein function [52] and has since been speculated as a potential mechanism in the development of gastrointestinal symptoms induced by chemotherapy treatment.

In line with these preclinical findings, alterations to occludin and claudin-1 protein expression have also been identified following MTX and irinotecan treatment [19, 20, 56], suggesting tight junction disruption may be common to numerous chemotherapeutic agents. Despite these promising preclinical findings, several inconsistencies remain particularly with regards to the upstream mechanisms involved. For example, Youmba et al., (2011) indicated significant decreases in the relative mRNA expression of occludin and claudin-1, yet other preclinical data [19] reports no change. As such, further research is now warranted to delineate the role tight junctions play in the pathophysiology of CIGT [56].

MMP/Tight Junction Interactions in Mucositis

Proteolytic degradation, rearrangement and disruption of tight junction proteins constitute an important regulatory feature in both physiological and pathological tissue remodeling [52]. In particular, tight junctional proteins undergo architectural and molecular changes in response to chemotherapy [19, 20], however the mechanisms responsible remain poorly understood. MMPs are recognised for their vast array functions, particularly in the inflammatory response. Recent research has shown that increased matrix metalloproteinase activity correlates with increased permeability of both endothelia and epithelial barriers, strongly implying MMP-mediated tight junction disruption [52, 57–59]. Given the integral roles of both MMPs [9] and tight junctions [13] in the development of intestinal mucositis; MMP-tight junction interactions present as a potential pathway in chemotherapy-induced mucositis pathophysiology (Fig. 1).

Endothelial MMP-Dependent Tight Junction Regulation

There is a wealth of in vitro [60, 61] and preclinical [57, 58, 62] data demonstrating the ability of MMPs to induce proteolysis of endothelial tight junction proteins, which invariably leads to increased permeability. Although endothelial cells themselves are the principal source of MMPs, astrocytes and pericytes of the blood brain barrier are also sources of these signaling proteins [52]; with these findings leading to substantial investigations of MMP-tight junction interaction in the central nervous system, particularly during blood–brain barrier pathologies. There is robust evidence for the involvement of MMP-dependent tight junction regulation within the in vitro setting. Brain-derived microvascular endothelial cells

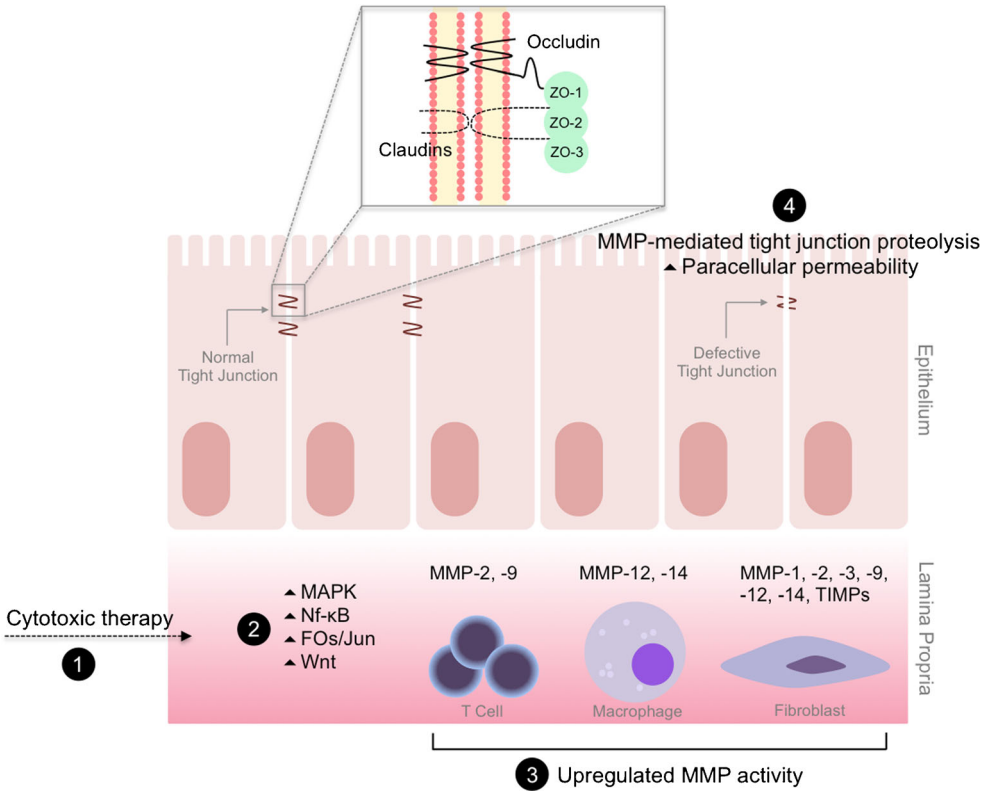

Fig. 1 Proposed MMP-tight junction interactions in chemotherapy-induced mucositis

(BMECs) exposed to oxidative stress induced significantly elevated MMP-9 activity paralleled by downregulation and redistribution of occluding [61]. Comparable correlations have been shown in murine brain endothelial cell lines [60]. Numerous preclinical studies also support a role for MMP-mediated tight junction disruption [57, 58, 62]. For example, MMP-2/-9 levels have been shown to be significantly elevated following cerebral ischemia leading to tight junction protein degradation, increased blood–brain barrier permeability and oedema [57, 63]. Furthermore, inhibition of MMP-2/-9 has been shown to reduce vascular permeability and attenuate tight junction disruption [57]; thus supporting a role for MMPs in the maintenance of tight junction integrity.

Epithelial MMP-Mediated Tight Junction Regulation

Although far more common in endothelial cells, MMP-mediated epithelial tight junction modulation has also been reported. Siu and colleagues (2003) were the first to establish MMP-tight junction interaction in the seminiferous epithelium of male Sprague Dawley rats. Authors reported that following exposed to tumour necrosis factor (TNF) tight junction integrity and transepithelial resistance declined, whilst MMP-2/-9 activity significantly increased [64]. Elevated MMP-7 has also been identified in response to oestrogen-treatment in normal human vaginal epithelial cells (hEVECs) leading to occludin proteolysis and poor epithelial barrier function [65]. Furthermore, inhibition of MMP-7 activity, by oestrogen-depletion or pharmacological intervention, abrogated occludin degradation and epithelial permeability [65]. More recently, MMP-tight junction interactions were confirmed using human airway epithelial models representative of asthma [59]. MMP-9 treatment resulted in altered localisation and expression of occludin, claudin-1 and ZO-1, whilst also increasing permeability to macromolecules. Furthermore, elevated MMP-9 activity induced severe apoptosis in epithelial cells. MMP-mediated tight junction proteolysis was most recently demonstrated using human embryonic kidney cell lines [66]. Pro-inflammatory cytokine-induced MMP-9 upregulation was shown to induce clear breaks in the tight junction continuum and reduced ZO-1 density prompting the authors to suggest that MMP-tight junction interactions are likely to drive pathological changes during inflammation.

Conclusions

This critical review has provided emerging scientific evidence supporting an interactive role between MMPs and tight junctions in the development of cytotoxic therapy-induced mucositis; a major clinical problem, having implications for both health economics and patient outcomes. To date, the underlying pathogenesis is incompletely understood. The proposed new link between MMPs and tight junctions in mucositis development now provides a novel avenue of research to explore, in order to further elucidate the underlying pathobiology of mucositis.

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