Review Article

RGD-Dependent Epithelial Cell-Matrix Interactions in the Human Intestinal Crypt

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Interactions between the extracellular matrix (ECM) and integrin receptors trigger structural and functional bonds between the cell microenvironment and the cytoskeleton. Such connections are essential for adhesion structure integrity and are key players in regulating transduction of specific intracellular signals, which in turn regulate the organization of the cell microenvironment and, consequently, cell function. The RGD peptide-dependent integrins represent a key subgroup of ECM receptors involved in the maintenance of epithelial homeostasis. Here we review recent findings on RGD-dependent ECM-integrin interactions and their roles in human intestinal epithelial crypt cells.

1. Introduction

Cell contacts with the extracellular matrix (ECM) provide both cohesive and functional properties in a variety of tissues, such as epithelia, nerves, muscle, and stroma, through specific interactions with cell membrane receptors [1, 2]. All ECMS are made up of collagen fibrils and/or networks, proteoglycans as well as specialized glycoproteins such as fibronectin and laminins that are archetypal of interstitial ECM and basement membrane (BM), respectively [3, 4]. Cells from multiple origins interact with ECM molecules using a variety of receptors, most of them being members of the integrin superfamily [2]. Integrins are noncovalent transmembrane α/β heterodimers. In mammals, over 24 distinct integrin heterodimers have been characterized to date, describing the association between 18α and 8β subunits [5–7]. The fact that integrin-mediated connections between the ECM and the cytoplasm regulate key cell functions such as adhesion, migration, proliferation, apoptosis, and differentiation is well recognized [8–11].

Epithelia express a wide variety of typical integrin receptors such as the α1β1, α2β1, α3β1, and α6β4 integrins that serve as collagen and/or laminin receptors [12–15]. Although less well documented in epithelia, the RGD-dependent integrins are another group of receptors that appears to be involved in epithelial cell homeostasis [15–17]. RGD-dependent integrins include α5β1-, α8β1-, and αV-containing integrins and are named as such because they specifically recognize the RGD motif, a sequence of three amino acids (Arg-Gly-Asp) found in many ECM molecules such as fibronectin and osteopontin [5, 12, 14, 15]. Collectively, these interactions are termed the “RGD-dependent adhesion system” (Figure 1). Interestingly, RGD-dependent cell interactions represent a key role in hierarchical assembly and maturation of adhesion structures including focal complexes (FXs), focal adhesions (FAs), and fibrillar adhesions (FBs) [1, 2, 18].

Therefore, RGD adhesion can be divided into three distinct components, the extracellular component (e.g., fibronectin), the membrane receptor (e.g., the α5β1 integrin), and the intracellular molecule (e.g., vinculin). Moreover, each component acts in concert with the others to organize and regulate RGD adhesion dynamics. In this paper, we will focus on the importance of the RGD-dependent adhesion system for human intestinal crypt cell homeostasis (Section 2). We chose to elaborate on recent findings from
integrins and specific intestinal cell functions such as proliferation, migration, and differentiation [23–30]. A schematic illustration of the human crypt-villus axis and the spatial expression of laminins, laminin receptors of the integrin family, and the two classic RGD components fibronectin and the αvβ1 integrin (as depicted by dark areas) is shown in Figure 2. Moreover, another example is the transient expression of the tenascin and osteopontin receptor α9β1 integrin in the lower third of the crypt of the immature small intestine as well as in proliferative epithelial crypt cells [31] and its reexpression in colon adenocarcinoma cells [32].

The RGD archetype fibronectin is another ECM component that was found strongly expressed in the epithelial BM of the crypts in both human and small laboratory animals [33–36]. Synthesis and deposition of fibronectin by proliferating intestinal epithelial cells was confirmed in vitro [26, 34]. Furthermore, expression of the fibronectin receptors, αvβ1 and αv-containing integrins, was found to be associated with intestinal cell proliferation [21, 29, 37]. Taken together, these observations suggest that fibronectin may significantly contribute to the RGD system regulating intestinal crypt cell functions.

To investigate this hypothesis, we used a strategy combining expression studies in the intact human intestine and functional studies using HIEC cells, a human intestinal epithelial crypt cell model well-characterized for the expression of typical features of intestinal crypt cells [38–41]. As summarized in the next sections, this experimental approach has led to the identification and characterization of new components of the RGD-dependent adhesion system that emphasize the importance of this adhesion system in human intestinal crypt homeostasis.

3. Integrin α8β1 as a Crucial Mediator of Crypt Cell-Matrix Interaction

3.1. Integrin α8β1 Is a Novel Regulator of Epithelial Cell Adhesion. Initially characterized in the chicken nervous system [42, 43], integrin α8β1 represents an important RGD-dependent receptor [44]. Ligand binding to integrin α8β1 was shown to be important for RhoA GTPase activation and subsequent actin stress fiber assembly in vascular smooth muscle cells [45–47]. Integrin α8β1 was also recently found to play an important role in microfilament organization which was central to RGD-dependent intestinal epithelial crypt cell adhesion [48]. α8 subunit knockdown experiments, carried out in HIEC cells, showed that this integrin is important for proper vinculin recruitment to adhesion structures [48] (Figure 3). Intestinal epithelial crypt cells in which α8 was knocked down exhibited lower numbers of vinculin-positive FAs compared to controls, while paxillin localization was not affected [48]. It is well known that RhoA/ROCK signalling enhances actin stress fiber assembly and increases cell adhesion [49–51]. RhoA activity was shown to promote scaffolding protein recruitment, including vinculin, to the developing adhesion structures [51, 52]. Thus, the increased RhoA activity displayed by α8 knockout cells leads to the absence or reduced levels of vinculin observed within these cells [48, 53].
Based on the scheme of adhesion structures hierarchical assembly, vinculin recruitment occurs at later stages of FX formation, while paxillin is recruited at early stages [54]. Thus, observations made in intestinal epithelial crypt cells suggest that integrin αβ1 is essential, at this particular stage of FX maturation into FA, via its role in RhoA activation [48] (Figure 3(a)). A similar function could also be predicted for the collagen-binding integrin α2β1, considering the expression of this receptor in undifferentiated intestinal epithelium cells and its participation in RhoA activation [44, 55, 56].

Interestingly, ectopic expression of the enterocytic differentiation associated factor GATA-4 in intestinal epithelial crypt cells caused a depletion of α8 subunit expression [39, 48]. In these same cells, reduced levels of α8β1 were associated with a decrease in cell growth, marked by Cyclin D1 inhibition and accumulation of cells in the G1 phase [48]. Similarly, decreased RhoA activity was observed in differentiated and nonproliferative HT29 cells compared to their undifferentiated and proliferative counterparts [55]. Together with the role of integrin α8β1 in RGD-dependent adhesion, these findings support the concept that cell-ECM interactions are crucial to maintaining a proliferative state in epithelial cells, which is anchorage and cell position dependent, reflecting its exclusive localization in the lower crypt of the intact intestine.

3.2. Integrin α8β1 Regulates Crypt Cell Migration. Due to the role of integrin α8β1 in RGD-dependent cell adhesion and RhoA GTPase activity, this receptor was shown to exert a critical influence on intestinal epithelial cell motility [48]. Alteration of RhoA activity was found to modulate migration in different systems [49, 57]. We recently reported that loss of RGD-α8β1 interactions in intestinal epithelial cells caused increased cell migration [48]. From a physiological perspective, proliferating intestinal epithelial cells must be restricted to the lower two-thirds of the crypt to avoid premature terminal differentiation and loss of proliferative capacity [38, 40]. Therefore, without necessarily affecting the expression of differentiation master regulator genes, RGD-dependent adhesion plays a major role in regulating cell migration, which in turn is crucial for wound healing, cell differentiation, and tissue integrity [24, 58, 59].

3.3. Integrin α8β1 RGD-Dependent Interactions Act as a Check Point in the Intestinal Crypt Epithelium. Cell survival is tightly regulated by RGD-dependent ECM-integrin interactions [11]. Indeed, integrin receptors, such as α5β1 and αV integrins, play a central role in controlling anoikis or apoptosis by loss of attachment [10, 11, 60]. Specifically, engagement of β1 integrins was found to be essential to intestinal epithelial cell survival through FAK signalling [60, 61].

As mentioned above, integrin α8β1 is involved in efficient vinculin recruitment to developing adhesion structures [48, 53]. The presence of vinculin in cell-ECM adhesion structures affects cell survival signal transduction. As previously described, HIEC cells share a number of features with intestinal epithelial stem cells, including a proliferative and undifferentiated state as well as the expression of several putative stem cell markers [39]. Interestingly, silencing of vinculin expression in F9 embryonic teratocarcinoma cells, another cell model closely related to stem cells [62], has shown increased resistance to anoikis, while ectopic reexpression of vinculin restored sensitivity to anchorage-dependent survival [63]. A similar phenomenon was observed in nonadherent α8 knockdown intestinal epithelial crypt cells [53]. In both studies, elevated levels of FAK phosphorylation...
on tyrosine 397 were noted in nonadherent cell cultures. The absence of vinculin combined with the presence of paxillin in primitive adhesion structures prior to loss of adhesion could explain such a phenomenon (Figure 3). At the molecular level, it has been shown that paxillin exhibits partially overlapping binding sites for FAK and vinculin [64]. Thus, the vinculin tail domain appears to compete with FAK for paxillin binding. In the presence of vinculin, FAK activation would depend on ECM-integrin binding. However, absence of vinculin leads to a conformational change in adhesion structures which results in constitutive activation of FAK when bound to paxillin [63] where FAK activity no longer relies on ECM-integrin interactions [53, 63]. Additionally, nonadherent α8β1-depleted intestinal epithelial cells showed increased activity of the PI3 K/Akt signalling pathway compared to nonadherent controls [53]. A summary of integrin α8β1 contribution to anoikis regulation in epithelial intestinal crypt cells is presented in Figure 3.

Considering the proliferative and highly adaptive capacities of crypt cells, such as stem and transit amplifying cells, RGD-dependent α8β1 interactions with ECM are suggested to act as a security switch that keeps the detachment of undifferentiated epithelial cells in check. It is worth noting that none of the five human colorectal cancer cell lines tested were found to express the integrin α8 subunit and that ectopic expression of this RGD-dependent receptor restored sensitivity of malignant cells to anoikis [53]. The mechanism by which colon cancer cells repress α8 expression to bypass this checkpoint remains unknown. However, in normal cells, this security step mediated by α8β1 occupancy is potentially important to support homeostasis in the human intestinal crypt. New evidence from the literature has shown that colon cancer may originate from defective crypt stem cells [65]. Therefore, in light of the expression of α8β1 in the region associated with intestinal stem cells, combined with its role in sensitizing epithelial cells to anoikis [53], it could

Figure 3: RGD-dependent adhesion influences anoikis sensitivity in undifferentiated epithelial cells. (a) Schematic representation of the proposed mechanism by which integrin α8β1 and RGD-dependent adhesion regulates anoikis sensitivity, through differential interactions between vinculin, paxillin, and FAK in intestinal epithelial crypt cells. As described in Section 3, α8β1 is essential to vinculin recruitment within maturing adhesion structures, while paxillin localization is not affected by α8 subunit silencing. (b) Following α8 subunit silencing, the absence of vinculin, combined with the presence of paxillin in the adhesion complexes, leads to an anchorage-independent activation of FAK and, consequently, anoikis resistance.
be speculated that α8 integrin silencing represents a key step in cancer initiation, in order to escape apoptosis upon modification of the malignant stem cell niche or location. In this context, α8β1 could promote defective progenitor cell elimination, and consequently prevent the onset of ecto-cryptal proliferative structures. Such specific involvement of RGD-dependent integrins is not without precedent since altered expression of other heterodimers has been reported in colon cancer. For instance, integrin expression has been found to be specifically decreased in anoikis-resistant cryptal proliferative structures. Such specific involvement of the malignant stem cell niche or location.

4. Integrin-Linked Kinase (ILK) as an Integrator of Cell-Fibronectin Interaction

The intestinal epithelial cell mediates RGD interactions through expression of specific integrin receptors, as observed with α8β1, as well as through the production and deposition of RGD ligands, such as fibronectin. The efficient deposition of fibronectin into the BM relies upon its recognition by RGD-dependent integrins, which mediate its unfolding to expose specific fibronectin structural domains, which in turn mediates the formation of insoluble fibronectin fibrils [67]. Fibronectin deposition is characterized by the formation of specialized cell-matrix contact structures containing integrins, cytosolic proteins, and actin referred to as fibrillar adhesion (FB) points [67].

The integrin-linked kinase (ILK) is a constituent of integrin containing adhesion sites where it mediates multiple cellular processes. ILK is a pseudokinase and scaffolding protein ubiquitously expressed in mammalian cells forming a trimeric complex with PINCH and parvins named the IPP complex [68–70]. ILK interacts with the cytoplasmic domain of integrin β1 and β3 subunits to create a physical link between integrins and the actin cytoskeleton [68, 71]. Interestingly, it has been suggested that ILK regulates fibronectin expression/deposition [72–74] and other studies have placed IPP complex members within FA points [75, 76]. In vivo, fibronectin expression is restricted to the BM underlying epithelial crypt cells and HIEC cells produce copious amount of fibronectin and generate numerous well-defined adhesion structures. The expression and roles of ILK were therefore investigated in human intestinal crypt cells.

We first focused on the localization of ILK-related components in the small intestine. As previously observed for fibronectin and integrin α5β1 in the intact intestine [21, 29, 33], ILK, PINCH-1 α-parvin, and β-parvin were found to be predominantly expressed by the proliferative epithelial cells of the crypts [58]. In HIEC cells, ILK, PINCH-1, α-parvin, and β-parvin were all closely associated with FA points (Figure 4(a)). A siRNA strategy was used to knock down ILK expression in HIEC cells in order to further investigate the role of ILK in intestinal crypt cells [58]. Interestingly, ILK knockdown in HIEC was accompanied by severe disruption of the IPP complex including the loss of PINCH-1 and parvins as well as major alterations in fibronectin synthesis and functional matrix deposition (Figure 4(b)). Overexpression of ILK was previously shown to increase fibronectin deposition in rat intestinal cells [76] while ILK knockdown decreases fibronectin expression in mice and human colon cancer cells. Indeed, the fibronectin gene promoter contains response elements that have been shown to be potentially regulated by ILK-mediated signalling [68, 77]. However, in HIEC cells, although a reduction of fibronectin was observed at the transcript level, ILK knockdown had no net effect on fibronectin protein amounts found in the culture medium suggesting that it was mainly the ability to process and deposit soluble fibronectin that was altered by the loss of the IPP complex [58]. The exact mechanism by which ILK knockdown impairs fibronectin deposition remains to be elucidated. Expression levels of the fibronectin integrin receptors were not altered in HIEC ILK knockdown cells suggesting that the required receptors for fibrillogenesis [67] remain available for binding. However,
and migration. HIEC phenotype with regard to cell proliferation, spreading tension necessary for fibrillogenesis. Alternatively, ILK-deficient cells may reflect a reduction in the cytoskeletal complexes, the decrease of fibronectin deposition in these functions can be stimulated by fibronectin in intestinal epithelial crypt cell models [80–82] and ILK and the IPP complexes can regulate these signalling molecules [83–85].

In addition to an alteration in fibronectin deposition, ILK knockdown severely affected basic intestinal crypt cell functions such as cell spreading, migration restitution abilities as well as cell proliferation [58]. Alterations in these functions in ILK-knockdown HIEC cells were not surprising since these functions can be stimulated by fibronectin in intestinal epithelial cells [86–89]. Interestingly, exogenously deposited fibronectin was found to fully rescue the ILK-knockdown HIEC phenotype with regard to cell proliferation, spreading and migration [58].

Taken together, as summarized in Figure 4, these data reveal that ILK and, by extension, the IPP complexes, perform crucial roles in the control of human intestinal crypt cell homeostasis, especially as key mediator of fibronectin deposition in the BM, which in turn regulates cell proliferation, migration, and restitution.

5. BM Collagen VI as a Regulator of Crypt-Cell-Fibronectin Interaction

Type VI collagen is a ubiquitously expressed ECM component [90]. In interstitial ECM, collagen VI acts as an anchoring meshwork bridging collagen fibers to the surrounding matrix [91, 92]. Collagen VI has also been shown to directly interact with the BM-specific type IV collagen [93] supporting a key role for this collagen in connecting BM to ECM [94, 95]. However, we recently identified collagen VI as a bona fide component of the basal lamina in the intestinal BM and found that it is synthesized in significant amounts by crypt epithelial cells [59].

To investigate the function of type VI collagen in the intestinal epithelial crypt cell, we used a similar knockdown strategy with HIEC cells as described in the previous sections for integrin α5β1 and ILK. Surprisingly, abolition of collagen VI expression resulted in a striking increase in cell size and spreading accompanied by a significant increase in the number of stress fibers and tensin recruitment at the FB points [59]. The observations that removal of collagen VI emphasized features normally associated with fibronectin suggested that collagen VI regulates fibronectin assembly in epithelial cells. Interactions between collagen VI and fibronectin have been previously reported [93, 96]. Further investigation in collagen-VI-depleted HIEC cells revealed that fibronectin was increased at both protein and transcript levels and was subjected to extracellular rearrangement into long, parallel fibrils. Importantly, exogenous collagen VI, but not collagen I or IV, was able to fully rescue the knockdown phenotype indicating that the effect is specific for type VI collagen [59]. Considering that exposure of fibronectin-binding sites is critical for both cell binding and fibrillogenesis [67, 97], one may hypothesize that, under normal conditions, collagen VI acts by limiting cellular accessibility to fibronectin through competition for integrin receptors (Figure 5(a)) or by a direct interaction with fibronectin in the ECM (Figure 5(b)). Consistent with this possibility, collagen VI has been reported to be recognized by the RGD-binding α5β1 and αV integrins [98–100]. Furthermore,
HIEC binding to collagen VI is integrin β1 dependent and it was the FB complexes, specifically enriched in tensin and α5β1 integrin [6, 54, 67], that were enhanced in collagen-VI-depleted HIEC [59].

To further investigate the mechanism underlying the generation of FB complexes in collagen-VI-depleted intestinal crypt cells, the regulation of actomyosin forces was analyzed. Actin contractility depends on the phosphorylation of the myosin light chain (MLC), which is mainly mediated by the kinases MLC (MLCK) and Rho (ROCK) acting on MLC and myosin phosphatase, respectively [101–104]. Interestingly, MLCK-dependent activation of MLC phosphorylation was observed in poor collagen VI-rich fibronectin ECM environments consistent with the observed generation of higher numbers of tensin-enriched FB complexes and extensive fibronectin fibrillar deposition [59] (Figure 5(c)).

As summarized in Figure 5, these data identified collagen VI as a major regulator of fibronectin synthesis and fibrillogenesis and suggest that collagen VI influences intestinal epithelial crypt cell behaviour by restraining cell-fibronectin interactions and their downstream events.

6. Conclusions

Described as a predominant epithelial BM component in the intestinal crypt more than 3 decades ago [33–36], fibronectin has been confirmed to play an important role in the RGD system regulating crypt epithelial cell functions. The recent findings summarized herein further emphasize the crucial importance of this RGD-adhesion system and its regulatory mechanisms. Indeed, intestinal epithelial cells can regulate RGD interactions through expression of specific integrin receptors, as exemplified by α8β1, which exerts major regulatory influences on key cell functions such as cell proliferation, migration, and survival [48, 53]. Regulation of RGD interactions can also be accomplished by regulating production and deposition of their ligands, such as fibronectin, as illustrated by the finding that ILK/IPP complexes are key mediators of fibronectin deposition into the BM, which in turn regulates cell proliferation, migration, and restitution [58]. Finally, regulation of RGD-dependent cell interactions can also be achieved by interaction with other ECM molecules as shown with type VI collagen, a basement membrane component that regulates epithelial cell-fibronectin interactions. Taken together, these studies define new molecular elements and shed new light on the relative complexity of specific cell-matrix interactions in a well-defined environment such as the intestinal crypt and the critical impact these interactions have on cell function.

Authors’ Contribution

Y. D. Benoit and J.-F. Groulx contributed equally to this work.

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Y. D. Benoit and J.-F. Groulx contributed equally to this work.

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