Modulation of Cell Adhesion During Epithelial Restitution in the Gastrointestinal Tract

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Epithelial migration, which is a fundamental component of the ulcer healing process, is characterized by complex alterations in adhesion between cells and the extracellular matrix. Growth and motility factors involved in mucosal repair of the gastrointestinal tract seem to modulate these interactions in a coordinated fashion in order to reestablish functional and structural integrity of the mucosa. These findings may have important clinical implications for the treatment of ulcerative conditions of the gastrointestinal tract and lead to the development of specific drugs that promote mucosal healing by exploiting natural mechanisms of cell migration.

INTRODUCTION

Epithelial restitution is a fundamental protective mechanism that allows the gastrointestinal mucosa to re-establish functional and structural integrity following superficial injury [1]. This repairing mechanism is characterized by rapid migration of surviving epithelial cells around the ulcer margins over the denuded basement membrane. During this phase, there appears to be no cell division [2]. Subsequently, cell proliferation and differentiation occur to reestablish normal function and tissue architecture. In animal studies, following superficial damage of the gastric mucosa, this process may occur within a very short time span (within one hour) [3].

It is now apparent that changes in cell-cell and cell-matrix interactions occur spatially and temporally during migration [4]. Several families of adhesion receptors, which mediate the interaction between adjacent epithelial cells or between cells and the surrounding extracellular matrix (ECM)\textsuperscript{b}, have been shown to regulate migration [5]. A detailed description of the genetic, biochemical structure and function of adhesion molecules is beyond the scope of this paper. Here I describe data on the part played by soluble factors known to promote epithelial restitution and mucosal healing in the modulation of cell adhesion.

THE ROLE OF ADHESION MOLECULES IN EPITHELIAL CELL MIGRATION

Cell motility requires dynamic interactions between cells, their extracellular environment and the cytoskeletal network. The initiation and progression of epithelial cell migration require the release of the adhesive tractions between epithelial cells and establishment of stable cell-substratum adhesion to generate traction and movement forward. It is likely that the transition from a "stationary" to a "motile" cell requires perturbation of cell junctional proteins like E-cadherin/catenin complex and the modulation of

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\textsuperscript{b}Abbreviations: ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, EGF receptor; TGF, tumor growth factor; HGF/SF, hepatocyte growth factor/scatter factor.
expression, affinity and binding specificity of extracellular matrix adhesion receptors (e.g., integrins).

E-cadherin

Epithelial cadherin is the prime mediator of cell-cell adhesion in epithelial cells [6]. It is a 120 kDa polypeptide and belongs to the cadherin superfamily, a group of over 16 molecules that all have common structural and functional characteristics [7]. All family members are transmembrane glycoproteins, which bind a single calcium ion at their extracellular amino terminal end. Loss of this ion prevents cell-cell adhesion. Typically a single cadherin molecule binds homotypically to another cadherin molecule of the same type on an adjacent cell to form a homodimer [7]. The carboxy terminal cytoplasmic tail interacts with various cytoplasmic proteins (termed catenin complex), which in turn binds to the actin cytoskeleton [8].

The catenin complex was originally dissected by immunoprecipitating E-cadherin from metabolically labeled cell extracts and analyzing the coprecipitating molecules whose genes were subsequently cloned [9]. The catenin complex consists of α-catenin (102 kDa), β-catenin (92 kDa) and γ-catenin (88 kDa). There is evidence that E-cadherin binds to either β-catenin or γ-catenin [8], whereas α-catenin links β-catenin to the actin cytoskeleton via direct association with α-actin [10]. The catenins also bind to other molecules including the epidermal growth factor receptor (EGFR) [11] and the adenomatous polyposis coli tumor suppressor gene product [12, 13] and may be involved in growth regulatory signalling as well as cell adhesion [14]. Mutations or deletion of any of the E-cadherin/catenin complex components renders the cell unable to form calcium-dependent adhesions, leads to loss of cell polarity and increased cell motility in vitro [6]. Catenins also contain tyrosine residues known to be phosphorylated by oncogene products (e.g., src, ras, fos and met) and growth factors. Under these conditions, there is destabilization of the adhesion junctions with perturbation of intercellular adhesion [15, 16, 17, 18, 19].

Integrins

These are a family of transmembrane glycoproteins composed of an α and a β chain, which are associated to the actin cytoskeleton through linker proteins such as talin,vinculin and α-actin [20]. The distribution of integrins on normal and neoplastic cells has been mapped using monoclonal antibodies to the sub-units and shows a characteristic cell-lineage-dependent pattern. Most of the integrins function as cell surface receptors for extracellular matrix components including collagen, laminin and fibronectin. However, the picture is complex as the gastrointestinal epithelium expresses multiple integrin receptors (i.e., α2β1, α3β1, α6β1, αvβ1, αvβ5) with overlapping specificity [21, 22]. Integrins have been implicated in the regulation of cell migration in a variety of systems [22]. For example, we have recently shown that α2β1 integrin receptor mediates migration of colonic carcinoma cell lines on collagen [24]. This has been also demonstrated in other cell types [23]. Recently, knock-out of β1 integrin gene in embryonic stem cells has shown to inhibit cell migration and adhesion [25].

THE ROLE OF GROWTH-MOTILITY FACTORS IN CELL ADHESION DURING EPITHELIAL RESTITUTION

We have recently shown that perturbation of E-cadherin/catenin mediated adhesion is associated with epithelial migration and restitution following ulceration of the gastrointestinal tract [26]. Using immunohistochemical and in situ hybridization techniques, we have shown that in Crohn's disease and gastric ulcer, the regenerative epithelium over the ulcer bases shows loss of the normal membrane E-cadherin immunoreactivity with an abnormal cytoplasmic localization. Using a wounding monolayer in vitro
system, migratory cells also showed reduced surface E-cadherin expression with abnormal cytoplasmic immunoreactivity. Cytoplasmic E-cadherin is likely to reflect a non-functional molecule since E-cadherin needs to be expressed on the membrane to mediate cell-cell adhesion. These results, therefore, indicate that changes in the E-cadherin subcellular localization and level of expression occur in the regenerative epithelium in a variety of disease states and may promote the transition from a "stationary" to a "motile" phenotype. How are these dynamic interactions controlled during epithelial restitution?

Several growth factors may be involved in gastric mucosal repair. Epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) are two homologous peptides that bind to the same receptor (EGFR) and have been implicated in gastric ulcer healing [27]. For example, animal studies have shown that recombinant EGF can protect against the development of gastric ulcerations following indomethacin treatment [28]. In vitro, both EGF and TGF-α promote cell migration and proliferation through the regulation of adhesion receptor function. We and other groups have shown that EGF and TGF-α stimulate adhesion and migration of colonic epithelial cells on laminin and collagen [29, 30]. Both growth factors up-regulate the functional activity of at least two integrin molecules (α2β1 and α3β1), which are receptors for laminin and collagen. The cell attachment and migration induced by TGF-α and EGF can be inhibited by monoclonal antibodies to the α2 and β1 integrin chains [29, 30]. EGF has been shown to induce rapid tyrosine phosphorylation of β-catenin and γ-catenin. This is associated with scattering and dispersion of epithelial cells and loss of E-cadherin from the cell-cell junctions [18, 19]. Growth factor signalling through EGFR is, therefore, an example of how the transition from a "stationery" to a "motile" cell is mediated by reduction of cell-cell adhesion and increased binding to the extracellular matrix.

Trefoil peptides are a highly conserved family of molecules that are locally produced at sites of gastrointestinal injury, promote mucosal regeneration and healing [31]. They are small, secretory, stable peptides, bearing one or more trefoil structural motifs. They are expressed in specific regions of the gut in association with mucins [32]. To date, three trefoil peptides have been identified in humans. These are the human spasmolytic polypeptide, intestinal trefoil factor and pS2 [31]. In vitro, they have all been shown to promote cell motility, but they do not stimulate cell division in contrast to other motogenic factors such as EGF and TGF-α [28, 33, 34, 35]. Intestinal trefoil factor and human spasmolytic polypeptide have also been shown to initiate cell motility by perturbing the E-cadherin/catenin adhesion complex.

Hepatocyte growth factor/Scatter factor (HGF/SF) is also a growth and motility factor, which is primarily produced by cells of mesenchymal origin but stimulates the motility and scattering of epithelial cells [36]. HGF/SF triggers cell migration on various extracellular matrix substrates. Matsumoto et al. [36] have shown that HGF/SF stimulates motility in a two-step process: initially cells spread rapidly and form focal adhesions, and then these are rapidly disassembled, followed by increased cell motility [37]. Migration and proliferation of gastric epithelial cells in vitro has been shown to be facilitated by exogenous HGF/SF. In vivo, HGF/SF mRNA and protein have been localized in the fibroblasts around gastric ulcers but not in gastric epithelial cells, suggesting that it acts in a paracrine fashion [38]. As shown for EGF, phosphorylation of β-catenin by HGF/SF perturbs the E-cadherin/catenin adhesion complex and stimulates cell migration [18]. β-catenin phosphorylation, therefore, seems to be a common mechanism by which soluble factors involved in epithelial restitution may induce migration and regeneration.
CONCLUSIONS

Epithelial migration, which is a fundamental component of the ulcer healing process, is characterized by complex alterations in adhesion between cells and the extracellular matrix. Growth and motility factors involved in mucosal repair of the gastrointestinal tract seem to modulate these interactions in a coordinated fashion in order to reestablish functional and structural integrity of the mucosa. These findings may have important clinical implications for the treatment of ulcerative conditions of the gastrointestinal tract and lead to the development of specific drugs that promote mucosal healing by exploiting natural mechanisms of cell migration.

REFERENCES

1. Helpap, B., Hattori, T., and Gedigk, P. Repair of gastric ulcer: a cell kinetic study. Virchows Arch. Pathol. Anat. 392:159-170, 1981.
2. Silen, W. and Ito, S. Mechanisms for rapid re-epithelialization of the gastric mucosal surface. Annu. Rev. Physiol. 47:217-212, 1985.
3. Ito, S., Lacy, E.R., Rutten, M.J., Critchlow, J., and Silen, W. Rapid repair of injured gastric mucosa. Scand. J. Gastroenterol. 101(Suppl.):87-95, 1984.
4. Huttenlocher, A., Sandborg, R.R., and Horwitz, A. Adhesion in cell migration. Curr. Op. Cell Biol. 7:697-706, 1995.
5. Hynes, R.O. and Lander, A.D. Contact and adhesive specificities in the associations, migrations, and targeting of cells and axons. Cell 68:303-322, 1992.
6. Birchmeier, W., Hülsken, J., and Behrens, J. Adherens junction proteins in tumor progression. In: Hart, I. and Hogg, N., eds. Tumor cell adhesion. Cancer Surveys New York: Cold Spring Harbor Laboratory Press; 1995, pp. 113-127.
7. Takeichi, M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251:1451-1455, 1991.
8. Gumbiner, B.M. and McCrea, P.D. Catenins as mediators of the cytoplasmic functions of cadherins. J. Cell Sci. 17(Suppl.): 155-158, 1993.
9. Ozawa, M. and Kemler, R. Molecular organization of the uvomorulin-catenin complex. J. Cell Biol. 116:989-996, 1992.
10. Knudsen, K.A., Soler, A.P., Johnson, K.R., and Wheelock, M.J. Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alphacatenin. J. Cell Biol. 130:67-77, 1995.
11. Hoschuetzky, H., Aberle, H., and Kemler, R. β-catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. J. Cell Biol. 127:1375-1380, 1994.
12. Rubinfeld, B., Souza, B., Albert, I., Muller, O., Chamberlain, S.H., Masiarz, F.R., Munemitsu, S., and Polakis, P. Association of the APC gene product with beta-catenin. Science 262:1731-1734, 1993.
13. Su, L.K., Vogelstein, B., and Kinzler, K.W. Association of the APC tumor suppressor protein with catenins. Science 262:1734-1737, 1993.
14. Gumbiner, B. Signal transduction by β-catenin. Curr. Op. Cell Biol. 7:634-640, 1995.
15. Hamaguchi, M., Matsuyoshi, N., Ohnishi, Y., Gotoh, B., Takeichi, M., and Nagai, Y. p60v-src causes tyrosine phosphorylation and inactivation of the N-cadherin-catenin cell adhesion system. Embo. J. 12:307-314, 1993.
16. Sommers, C.L., Gelmann, E.P., Kemler, R., Cowin, P., and Byers, S.W. Alterations in beta-catenin phosphorylation and plakoglobin expression in human breast cancer cells. Cancer Res. 54:3544-3552, 1994.
17. Matsuyoshi, N., Hamaguchi, M., Taniguchi, S., Nagafuchi, A., Tsukita, S., and Takeichi, M. Cadherin-mediated cell-cell adhesion is perturbed by v-src tyrosine phosphorylation in metastatic fibroblasts. J. Cell Biol. 118:703-714, 1992.
18. Shibamoto, S., Hayakawa, M., Takeuchi, K., Hori, T., Oku, N., Miyazawa, K., Kitamura, N., Takeuchi, M., and Ito, F. Tyrosine phosphorylation of betacatenin and plakoglobin enhanced by hepatocyte growth factor and epidermal growth factor in human carcinoma cells. Cell Adhes. Commun. 1:295-305, 1994.
19. Shiozaki, H., Kadowaki, T., Doki, Y., Inoue, M., Tamura, S., Oka, H., Iwazawa, T., Matsui, S., Shimaya, K., and Takeichi, M. Effect of epidermal growth factor on cadherin-mediated adhesion in a human oesophageal cancer cell line. Br. J. Cancer 71:250-819, 1995.
20. Hynes, R.O. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69: 11-25, 1992.
21. Ramkisson, Y.D., Del Buono, R., Filipe, M.I., Hall, P.A., and Pignatelli, M. Expression of integrins and their extracellular matrix ligands in gastric carcinoma. Int. J. Oncol. 5:689-695, 1994.

22. Nigam, A.K., Savage, F.J., Boulos, P.B., Stamp, G.W.H., Liu, D., and Pignatelli, M. Loss of cell-cell and cell-matrix adhesion molecules in colorectal cancer. Br. J. Cancer 68:507-514, 1993.

23. Pignatelli, M. and Stamp, G.W.H. Integrins in tumor development and spread. In: Hart, I. and Hogg N., eds. Tumor cell adhesion. Cancer Surveys New York: Cold Spring Harbor Laboratory Press; 1995, pp. 113-127.

24. Kirkland, S.C., Henderson, K., Liu, D., and Pignatelli, M. Organisation and gel contraction by human colonic carcinoma (HCA-7) sublines grown in 3 dimensional collagen gel. Int. J. Cancer 60:877-882, 1995.

25. Fassler, R., Pfaff, M., Murphy, J., Noegel, A.A., Johansson, S., Timpl, R., and Albrecht, R. Lack of β1 integrin gene in embryonic stem cells affects morphology, adhesion, and migration but not integration into the inner cell mass of blastocysts. J. Cell Biol. 128:979-988, 1995.

26. Hanby, A.M., Chinery, R., Poulsom, R., and Pignatelli, M. Down-regulation of E-cadherin in the reductive epithelium of the gastrointestinal tract. Am. J. Pathol. 148:723-729,1996.

27. Tarnawski, A., Stachura, J., Krause, W.J., Douglass, T.G., and Gergely, H. Quality of gastric ulcer healing: a new, emerging concept. J. Clin. Gastroenterol. 13:S42-47, 1991.

28. Chinery, R. and Playford, R.J. Combined intestinal trefoil factor and epidermal growth factor is prophylactic against indomethacin-induced gastric damage in the rat. Clin. Sci. 88:401-403, 1995.

29. Basson, M.D., Modlin, I.M., and Madri, J.A. Human enterocytes (Caco-2) migration is modulated in vitro by extracellular matrix composition and epidermal growth factor. J. Clin. Invest. 90:15-23, 1992.

30. Liu, D., Gagliardi, G., Nasim, M.M., Allison, M.R., Oates, T., Lalani, E.-N., Stamp, G.W.H., and Pignatelli, M. Transforming growth factor α can act as morphogen and/or mitogen in a colorectal carcinoma cell line. Int. J. Cancer 56:603-608, 1994.

31. Poulsom, R. and Wright, N.A. Trefoil peptides: a newly recognized family of epithelial mucin-associated molecules. Am. J. Physiol. 265:G205-G213, 1993.

32. Poulsom, R. Trefoil peptides. In: Cytokine and Growth factors in Gastroenterology. R. Goodlad and N.A. Wright, eds. Bailliere Tindall Press; 1996, pp. 113-149.

33. Playford, R.J., Marchbank, T., Chinery, R., Evison, R., Pignatelli, M., Boulton, R.A., Thim, L., and Hanby, A.M. Human spasmylytic polypeptide is a cytoprotective agent that stimulates cell migration. Gastroenterol. 108:108-116, 1995.

34. Williams, R., Stamp, G.W.H., Pignatelli, M., Gilbert, C., and Lalani, E.-N. pS2 transfection of murine adenocarcinoma cell line (410.4) enhances dispersed growth pattern in 3-D collagen gel. J. Cell Sci. (in press), 1996.

35. Dignass, A., Lynch-Devaney, K., Kindon, H., Thim, L., and Podolsky, D.K. Trefoil peptides promote epithelial migration through a transforming growth factor beta-independent pathway. J. Clin. Invest. 94:376-383, 1994.

36. Zarnegar, R. and Michalopoulos, G.K. The many faces of hepatocyte growth factor: from hepatopoiesis to hematopoiesis. J. Cell Biol. 129:1177-1180, 1995.

37. Matsumoto, K., Nakamura, T., and Kramer, R.H. Hepatocyte growth factor/scatter factor induces tyrosine phosphorylation of focal adhesion kinase (p125FAK) and promotes migration and invasion by oral squamous cell carcinoma cells. J. Biol. Chem. 269:31807-31813, 1994.

38. Takahashi, M., Ota, S., Shimada, T., Hamada, E., Kawabe, T., Okudaira, T., Matsumura, M., Kaneko, N., Terano, A., and Nakamura, T. Hepatocyte growth factor is the most potent endogenous stimulant of rabbit gastric epithelial cell proliferation and migration in primary culture. J. Clin. Invest. 95:1994-2003, 1995.