Cancer Microenvironment (2010) 3:177–185
DOI 10.1007/s12307-010-0053-4

REVIEW PAPER

The Extracellular Matrix in Digestive Cancer

Daniel L. Worthley · Andrew S. Giraud · Timothy C. Wang

Received: 6 June 2010 / Accepted: 6 September 2010 / Published online: 17 September 2010

Abstract The extracellular components of the cancer microenvironment play a critical role in tumor initiation, progression and invasion. In this review we examine the normal formation and function of the basement membrane and extracellular matrix. We characterize the interactions between the matrix and the epithelium and explore the causes and consequences of the extracellular remodeling that accompanies carcinogenesis. Finally, we address the therapeutic possibilities of incorporating matrix as well as epithelial strategies in the management of digestive cancer.

Sources of support The Royal Australasian College of Physicians Cottrell Fellowship, the Queensland State Government Smart State PhD award and the NHMRC/RG Menzies fellowship (D.L.W).

D. L. Worthley
Royal Brisbane and Women's Hospital Research Foundation
Clinical Research Centre,
Brisbane, Queensland, Australia

D. L. Worthley
Conjoint Gastroenterology Laboratory,
Queensland Institute of Medical Research,
Brisbane, Queensland, Australia

A. S. Giraud
Murdoch Children's Research Institute,
Royal Children's Hospital,
Melbourne, Victoria, Australia

T. C. Wang (✉)
Division of Liver and Digestive Diseases,
Department of Medicine,
Columbia University Medical Center,
1130 St. Nicholas Avenue, Room 923,
New York, NY 10032, USA
e-mail: tcw21@columbia.edu

Keywords Extracellular matrix · Neoplasms · Digestive system neoplasms · Cancer stroma · Bone-marrow derived cells · Cancer · Gastrointestinal cancer

Introduction

In our first review, "Stromal fibroblasts in digestive cancers", we characterized the cancer microenvironment from the perspective of the important stromal cells that infiltrate and influence digestive tract carcinogenesis [1]. The peritumoral stroma, however, also consists of a dynamic and integrated network of extracellular proteins stitched and woven together to support and sustain the overlying epithelium [2–5]. The gastrointestinal stroma provides more than just a structural base. It also provides context to the overlying epithelium in a process termed "dynamic reciprocity" [6, 7]. The embryological importance of the stromal–epithelial interaction was dramatically demonstrated in a series of experiments combining ectoderm and mesoderm from different species and from different tissue sites. In these studies it was shown that the stroma modified the epithelial phenotype, for instance corneal epithelium from a chick sprouted feathers when combined with mesoderm from a mouse [8]. In another example mammary gland mesenchyme induced mammary gland development in overlying skin epithelium [9].

The regulatory relationship between epithelium and the underlying mesenchymal elements is interrupted in digestive carcinogenesis. The stromal changes in carcinogenesis such as the recruitment of inflammatory cells, remodelling of the extracellular matrix (ECM), activation of fibroblasts and promotion of angiogenesis co-evolve with the progressive epithelial transformation [10].

In this review we outline the normal formation and function of the basement membrane (BM) and ECM. We examine the interactions between the matrix and the epithelium and the extracellular remodelling that accompanies carcinogenesis. Finally, we explore the therapeutic possibilities of incorporating stromal as well as epithelial strategies in the management of digestive cancer [11–14].

The Normal Extracellular Matrix: Form and Function

Firstly, we shall place the BM and ECM in the context of the adjacent epithelium. The interactions between epithelial cells inform the physical and functional interface between the epithelium and matrix. Normal epithelial cells are polarized. They have an apical pole, which in most of the alimentary tract is the luminal surface with important barrier, absorption and secretion functions. There are lateral surfaces that form important intercellular connections to communicate and provide tensile strength along the epithelial sheet. Epithelial cells also have a basal surface that provides epithelial anchoring to the underlying BM through cell–matrix interactions. The key epithelial intercellular junctions include tight junctions, adherens junctions, desmosomes and a cell–matrix junction, the hemidesmosome. These junctions are comprised of cellular adhesion molecules such as cadherins, immunoglobulin (Ig) superfamily cellular adhesion molecules (CAMs), integrins and selectins [15]. Cadherins and Ig-CAMs interact with like molecules on the opposite cell membrane. Integrins and selectins interact with important matrix proteins or cell surface sugar motifs, respectively [15]. These CAMs are also important in cell–cell and matrix–cell signaling conveyed via conformational changes in their cytoplasmic domains, as discussed below.

The typical adherens junction involves an interaction between paired E-cadherin molecules across adjacent cell membranes. The cytoplasmic domains associate with α- and β-catenin and indirectly with F-actin. Within this complex there are at least two important mediators of gastrointestinal cancer, E-cadherin and β-catenin [16, 17]. The tight junction, another important intercellular interaction, contributes to the barrier function of epithelium, particularly relevant for the gut. Tight junctions contain members of the claudin family, occludin and junction adhesion molecule [15]. Again, claudin proteins are implicated in several digestive cancer models, including esophageal, colon, gastric and hepatocellular cancers [18–21]. Finally, the desmosomes and hemidesmosomes, which anchor epithelial cells to adjacent cells and the BM, respectively, are associated with an intracellular network of intermediate fibers [15]. The hemidesmosome utilizes the integrin α6β4 to secure the epithelium to laminin within the BM [22].

The Basement Membrane (BM)

The BM is the immediate subepithelial extracellular layer (Fig. 1). The BM is a sheet-like network of extra-cellular proteins, compiled into a layer approximately 100–300 nm thick [23].The BM is comprised of three basic types of matrix molecules: type IV collagen, proteoglycans such as

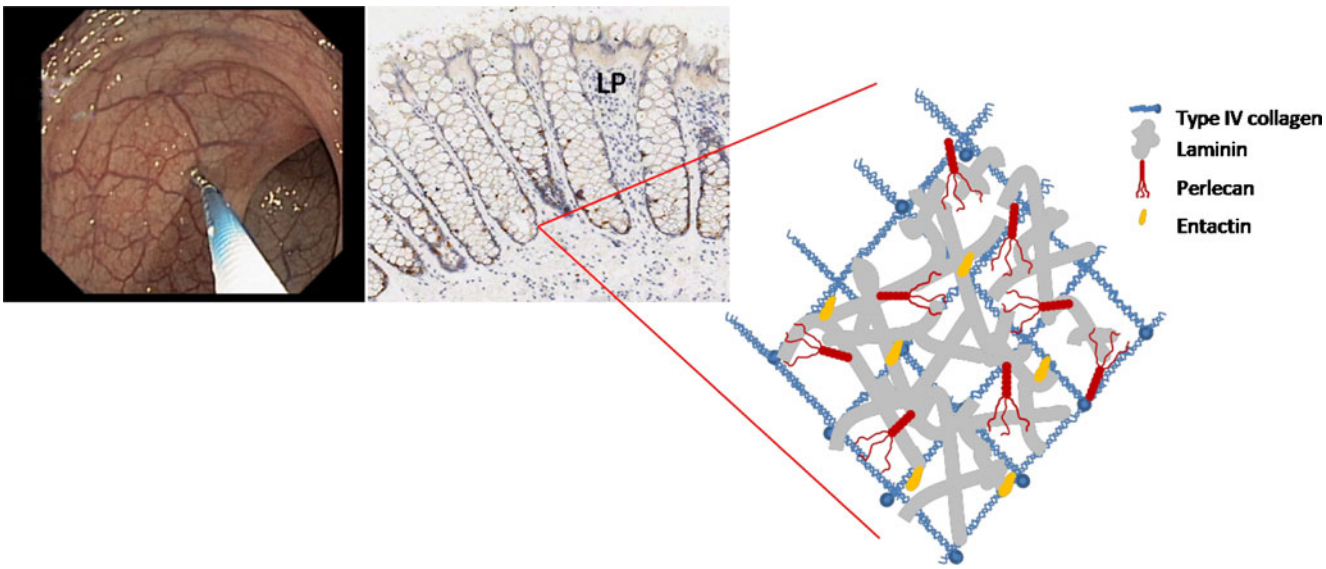

Fig. 1 Biopsy from the normal human rectum revealing normal crypts and lamina propria (LP, labeled). The BM is immediately beneath the rectal epithelium, with a schematic representation of its organization and chief components

perlecan and multiadhesive matrix molecules including laminin, fibronectin and entactin (nidogen). The BM provides a structural and functional foundation to the overlying epithelium. BMs are not exclusive to the epithelium and also surround myocytes, endotheliocytes, adipocytes as well as serving in a specialized capacity in the glomerulus [15].

Components of the Basement Membrane

Type IV collagen is exclusively expressed within, and is the chief structural protein of, the BM [15]. Type IV collagens assemble into very fine unstriated fibers, which associate to form a sheet-like structure. There are 6 type IV collagen genes (*COL4A1* (α1), *COL4A2* (α2), *COL4A3* (α3), *COL4A4* (α4), *COL4A5* (α5), *COL4A6* (α6)), but in the digestive tract the main type IV collagen is comprised of the heterotrimer α1α1α2 [24]. Whilst most mutations in *COL4A1* (α1(IV)) and *COL4A2* (α2(IV)) are embryologically lethal, other chains are mainly expressed with maturation particularly within the developing kidney [24]. *COL4A1* and *COL4A2* are located in a head-to-head orientation on chromosome 13q34. The type IV collagen network is strengthened by disulfide and aldimine bridges, as well as through its interactions with other molecules, as discussed below.

Perlecan is the major proteoglycan within the BM [15]. Perlecan consists of repeating domains, which include laminin-like and Ig-like domains. Long glycosaminoglycan (GAG) molecules are attached to the core protein backbone. This combination of core protein and GAG branches provides perlecan with its vital binding capacity, linking scores of matrix proteins including entactin and laminin, cell surface receptors and epithelial growth factors [15].

Laminin is the primary multiadhesive matrix molecule in the BM. Laminin is a large, cruciform molecule with calcium dependent globular domains that bind to cell surface receptors, such as integrins [15]. Entactin, another important matrix molecule, is a sulfated multidomain glycoprotein, which helps to cross-link BM components, including type IV collagen, perlecan and laminin [15].

Synthesis of the Basement Membrane

Does the intestinal BM represent an extension of the epithelial or of the stromal compartment? Given that the BM exists as an interface between tissues of endodermal and mesodermal origin it is not surprising that the intestinal BM itself has a dual origin, from both intestinal epithelial and mesenchymal cells [13, 25, 26]. Mesenchymal intestinal subepithelial myofibroblasts produce the majority of the BM's type IV collagen and entactin [13, 27]. Laminin is produced commensurately by epithelial and mesenchymal cells and perlecan primarily from the epithelial compartment [13, 27].

The Extracellular Matrix (ECM)

Beneath the BM is the lamina propria, which contains ECM interspersed with fibroblasts and other important stromal cells, such as recruited inflammatory cells. This is the next barrier that an invasive carcinoma must negotiate. The ECM shares many components with the BM, albeit that the matrix molecules in the ECM promote a 3-dimensional matrix rather than the sheet-like network of the BM [15, 23]. This difference results from the fibrillar collagens, particularly type I collagen, which replace type IV collagen as the defining structural glycoprotein of the ECM [15]. Like type IV collagen, type I collagen is also a heterotrimer consisting of two α1(I) chains and one α2(I) chain. Type I collagen is chiefly produced by the resident stromal fibroblasts, although in the activated stroma recruited fibroblasts may also contribute [3]. Once again, proteoglycans and the attached GAGs, such as heparin sulfate, play an important role in cell and matrix interactions. Fibronectin is an important polypeptide dimer within the ECM [28]. Fibroblasts and endothelial cells are the major producers of local fibronectin, albeit that some epithelial cells including intestinal and mammary epithelium can produce smaller amounts [28]. Fibronectin is an essential adhesive molecule that facilitates cellular-ECM attachments and can influence the morphology and motility of the associated cells. Fibronectins are also important for wound healing through their activation of clotting and chemotaxis of inflammatory cells into the activated stroma [28].

Cell–Matrix Signaling

This section examines the specific mechanisms of matrix–cell signaling and thus the means by which the ECM influences epithelial biology. These mechanisms include ECM-cellular signaling through adhesion receptor intermediaries, such as integrins, ECM as a repository for epithelial growth factors and the contribution of matrix stiffness and deformity on adjacent cell behavior.

Integrin Signaling

All cells have, or have had, a direct connection to the ECM during their development [29]. The connection to the matrix helps to regulate progression through the cell cycle and cellular differentiation [30, 31]. Integrins, which recognize fibronectin, laminin and collagens, are critical in cell signaling and regulation of cellular growth (Fig. 2) [31]. Integrins consist of an α and β-subunit, with different combinations of αβ providing binding and signaling specificity [31]. The binding of integrins to the ECM promotes the reorganization of actin filaments into larger

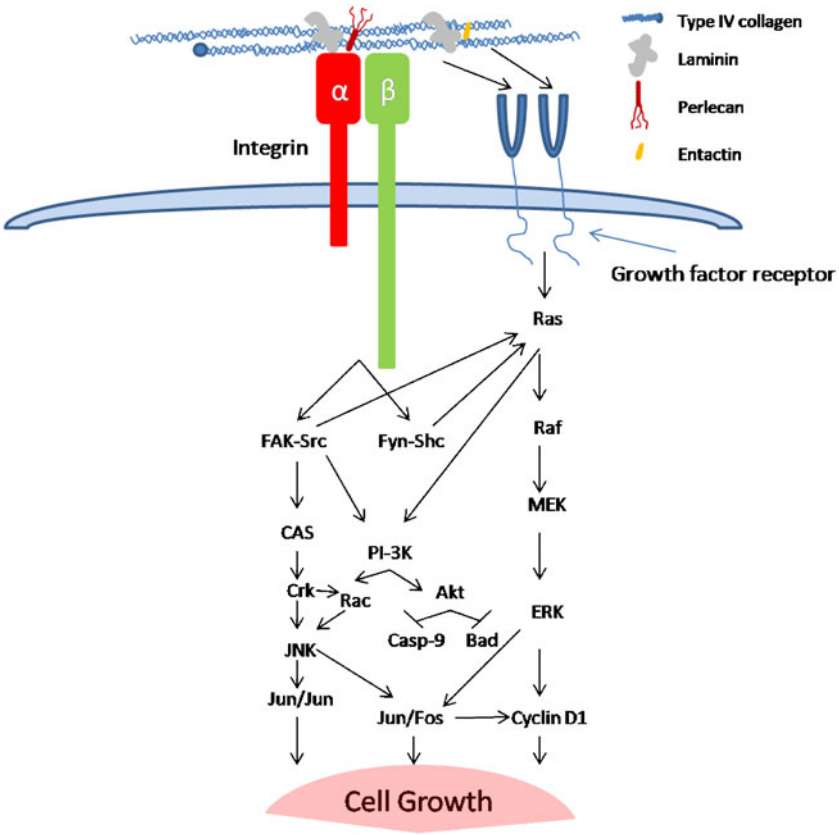

Fig. 2 Integration of the major signaling pathways regulated by integrin and growth factor receptors that promote cell cycle progression [31]

fibers and further aggregation of integrins, in a positive feedback loop [31]. Integrin-associated cytoplasmic signaling proteins are recruited to the site to promote signaling [32]. Integrins can activate a number of tyrosine kinases, including focal adhesion kinase (FAK), Src-family kinases, Abl, and integrin-linked kinase (ILK) [31]. FAK interacts with the cytoplasmic tail of the β-subunit of integrin [31]. Once activated, FAK undergoes autophosphorylation to produce a binding site for Src or Fyn. The Src kinase leads to further phosphorylation of key targets and activation of the Ras-Raf-MEK-ERK cascade, which leads to expression of several important proteins including cyclin D1 (Fig. 2) [31, 33]. As well as potentiating growth factor signaling through the Ras-Raf-MEK-ERK pathway integrins may also potentiate cellular proliferation by cycling ERK into the nucleus, a process that may depend on cellular adhesion [34]. In addition, adhesion-related increases in phosphoinoside 3-kinase are important for the expression and stability of cyclin D1 [35, 36]. Integrins vary in their mitogenic capacity. Integrin αvβ3 signaling seems to be particularly important and can result in prolonged activation of ERK and activation of the NF-κB pathway [33]. In several cell types expression of αvβ3 is associated with increased growth and even tumorigenesis [33].

Anoikis is a perfect illustration of the dependence of the normal epithelium on the matrix. *Anoikis* is a special example of apoptosis that results from the loss of cell–matrix interactions [37]. *Anoikis* is a normal daily occurrence along the gut as senescent alimentary tract cells are shed into the lumen. In addition to the loss of the mitogenic pathways, outlined above, *anoikis* also results from the inactivation of the pro-survival protein Bcl-2, due to the release of normally cytoskeleton-bound Bmf [38]. Failure of *anoikis* is an important process in carcinogenesis. In digestive cancers there are many examples of cancer mutations to circumvent anoikis, such as activating *KRAS* and *BRAF* mutations in colorectal cancers [39, 40].

Integrins connect the intracellular actin cytoskeleton to the ECM and thus mediate a rich network for detecting and responding to mechanical forces [41]. Epithelial cells exert forces on their BM through adhesive, integrin-rich structures known as focal adhesions [41]. These focal adhesions are likely to be the critical site of mechanotransduction [41]. Fibrosis and carcinogenesis can increase matrix rigidity and, via integrin signaling, can cause the activation of Rho and other typical integrin-related signaling pathways as outlined above, including Ras-Raf-MEK-ERK [42, 43]. An interesting feature of fibroblast migration is that they tend to migrate towards more rigid matrix [44]. There is a difference in tissue rigidity between the relatively stiff tumor and peritumoral stroma versus the comparatively less rigid surrounding normal tissue. It follows that this

migratory mechanism, referred to as durotaxis, may be an important factor in fibroblast recruitment into the activated stroma [43].

ECM and Growth Factors

In addition to the downstream synergy between growth factor and ECM signaling (Fig. 2), the ECM also regulates growth factors more directly [29]. Many growth factors, such as VEGF and HGF, bind to important matrix molecules including heparin sulfate and fibronectin, within the ECM [29]. The structured ECM reservoir of growth factors facilitates interstitial gradients, establishes a tissue-specific growth factor repository for release in case of tissue injury and ECM proteins also serve as important co-factors in the presentation of growth factors to their specific receptors, as is the case with FGF and TGF-β [29]. Given the important role of TGF-β in the development of stromal fibroblasts it is worthwhile to consider the interaction between the ECM and this growth factor in more detail [1]. TGF-β is secreted as a propeptide and following cleavage by furin protease, it associates with latency-associated peptide and latent TGF-β binding proteins [29]. The latent TGF-β binding proteins also bind to ECM molecules, including fibrillins and fibronectin. Active TGF-β is released following degradation of the ECM or displacement or cleavage of latency-associated peptide [29]. In addition, certain integrins such as αvβ6 and αvβ8 may activate ECM-bound latent TGF-β to facilitate localized activity of TGF-β [45]. Finally, there is increasing evidence to suggest that the ECM itself, via specific growth factor-like domains, may be able to directly activate growth factor receptors [29].

Remodeling of the BM and ECM in Cancer

Carcinomas are defined by the interruption of normal cellular compartments. Thus, "successful" neoplasia must acquire cells with the capacity to dissociate from their original tissue as well as invade adjacent and distant cellular communities [46]. Invasion involves breaching several important barriers, including the 2-dimensional BM, the 3-dimensional ECM and ultimately the series of barriers posed by both proximate and distant endothelium and by the destination tissues [46]. This involves neoplastic cellular changes but also changes in the surrounding matrix. As mentioned above, the type IV collagen network of the normal BM presents a resilient barrier. The BM has a regular pore size of about 50 nm, which limits cellular transmigration [23]. Thus, the normal BM must undergo considerable remodeling in order to permit the migration of cells [23]. In the lamina propria the 3-dimensional network, established by type I collagen, results in a structural barrier with effective pores of approximately 150 nm. Therefore, the ECM must also undergo considerable structural changes to permit invasion [23]. These ECM compartments reflect a contribution from neoplastic cells, inflammatory cells and the resident and recruited stromal fibroblasts. All have important roles in ECM remodeling.

The integrity of the BM is determined by both the synthesis of new components by the contributing epithelial and stromal cells and the degradation of the BM by proteolytic factors. The cellular production side of this equation is important and has been alluded to above and in the context of our other review [1]. The production and assembly of the BM is undoubtedly impaired in digestive cancers. Breaches in the BM are often most marked at the invasive front associated with epithelial–mesenchymal transition [47, 48]. The increased expression and activity of ECM-degrading enzymes, however, also promotes migration and metastasis [23, 49, 50]. These molecules are able to directly digest ECM proteins and are also capable of liberating growth factors and other signaling factors bound to ECM components [51].

Matrix metalloproteinases (MMPs) are one group of very important ECM proteases that have been implicated in carcinogenesis and metastasis [23, 51]. There are 25 MMPs that may be classified by their location of action. Eighteen MMPs are soluble enzymes secreted into the ECM, six are membrane-type MMPs (MT-MMPs) that are attached to a cell surface and one, MMP-23, is initially a membrane-bound MMP but can also be cleaved to form a soluble factor [23]. Expression of important MMPs and MT-MMPs occurs within, but is not restricted to, the neoplastic cells [52]. Stromal fibroblasts and endothelial cells also express these molecules to promote their own migration into the activated stroma and thus contribute to the microenvironment in terms of remodeling and angiogenesis [53–55].

MMPs are frequently over-expressed and over-activated in malignant cancers, particularly surrounding their advancing margin [53]. Considerable MMP research has focused on MMP-2 and MMP-9. These enzymes are associated with tumorigenesis [56, 57], but are not sufficient to stimulate invasion through the BM [58]. Furthermore, mice doubly deficient in these enzymes still achieve normal embryonic BM transmigration events such as pancreatic islet cell formation, suggesting that MMP-2 and MMP-9 are not essential for cellular invasion and migration [23, 59]. The roles of MMP-2 and MMP-9, as well as the other MMPs, within the cancer microenvironment are still being fully characterized [23, 60–62].

There has been greater evidence for a direct contribution from membrane-type-1 MMP (MT1-MMP), membrane-type-2 MMP (MT2-MMP) and membrane-type-3 MMP (MT3-MMP) in BM remodeling and migration [58]. COS cells that were transfected with MT1-MMP, MT2-MMP, MT3-MMP

but not MMP-2,-3,-7,-9,-11 or -13 led to BM effacement and migration in an ex vivo tumor invasion model [58].

ECM-proteases do not just break down physical barriers, they are integral biological mediators in both late and early carcinogenesis [51]. For instance, in transgenic mice and cell culture experiments it was shown that ectopic MMP-3 expression in normal mammary epithelium induced carcinogenesis, primarily through upregulation of Rac1b, reactive oxygen species and resultant genomic instability [63, 64]. The concept of matrix proteases inducing cancer was supported by a transgenic mouse study in which increased matriptase activity in the epidermis caused cutaneous squamous cell carcinomas [65].

The contribution of invadopodia to digestive cancer is an evolving area of research [66]. Invadopodia are cell–matrix contacts, similar to focal adhesions, established in the context of "invading" cellular protusions [66]. Invadopodia can promote ECM proteolysis through their associated proteases, particularly MT1-MMP [67], and thus have been implicated in cancer invasion, both neoplastic cells moving out and cancer-associated fibroblasts moving in [66]. The cause and consequences of invadopodia unites many of the key themes from the discussion above, including ECM remodeling, matrix proteases, cell motility and mechanical forces [66]. Whilst, invadosomes have been examined primarily in 2-dimensional cell culture, there is now evidence for their role in 3-dimensional cell culture studies [68]. Their in vivo contribution to the digestive cancer microenvironment, however, remains to be established [66].

In Vitro Models of the Extracellular Matrix in Digestive Cancer

2-dimensional cell culture experiments have been the in vitro cornerstone of much basic cancer research. Tumor biology, especially the interface between neoplasia and the ECM, however, extends to 3-dimensions [69]. Therefore, 3-dimensional (3D) cell culture (i.e. organotypic) model systems have been developed. 3D cell culture systems can incorporate epithelial and stromal cell populations, such as cancer-associated fibroblasts, to better approximate the in vivo cancer microenvironment (Fig. 3). Whilst, 3D cell culture represents a great advance in cancer research it still cannot examine the recruitment of important cells, such as fibroblasts, endotheliocytes and leukocytes, into the cancer stroma. Furthermore, it is still unclear as to which ECM product best represents the in vivo situation, obviously a very important issue when trying to develop a biologically faithful model. Available options for ECM include collagen gels, ECM produced by stromal cells in vitro, or even a BM-type matrix known as Matrigel™ (also known as EHS matrix) [69, 70]. Matrigel™ is extracted from the EHS tumor, which is a mouse tumor derived from parietal endoderm (named after those that discovered and characterized the tumor, Engelbreth-Holm and Swarm) [71].

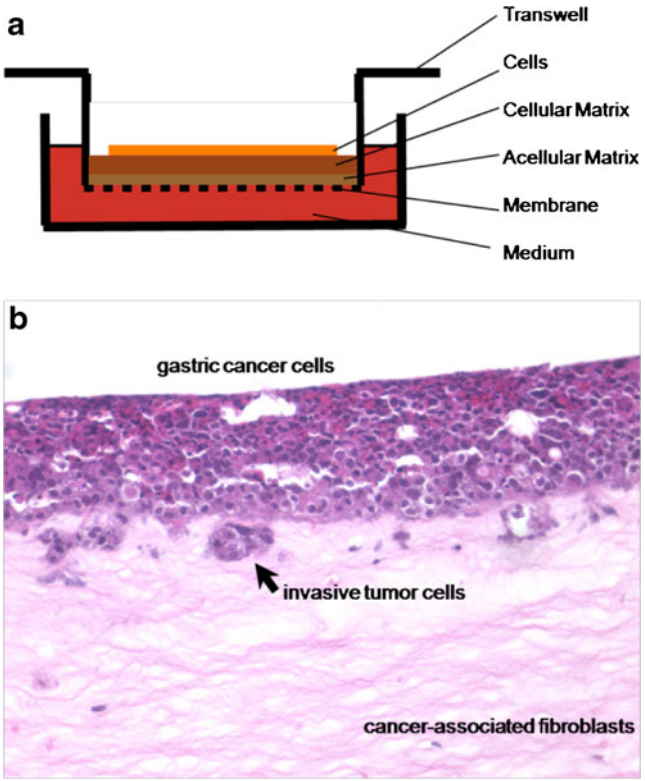

Fig. 3 **a** Schematic and **b** histological images of a 3D organotypic model examining the contribution of cancer-associated fibroblasts to gastric carcinogenesis

As in any in vitro model all of these ECM options represent a compromise between practical and biological considerations. For instance, collagen gels can mimic either loose or dense ECM depending on the concentrations used, but lack other important ECM components that influence cancer migration and growth, as outlined above. ECM that is produced by stromal cells in vitro may contain many of the important ECM components, but is limited by lower collagen concentrations and thus larger internal spaces than the true cancer stroma [69]. And finally, whilst Matrigel™ has proven to be an important product in cancer research, it is a BM-type matrix containing BM components such as type IV collagen. Type IV collagen is physiologically-organized into and specific for the 2-dimensional BM rather than the 3D ECM, thus Matrigel™ may be best considered as a BM rather than as an ECM product, per se. In addition, Matrigel™ contains several angiogenic- and growth-promoting factors, which may in part explain the enhanced tumor growth when Matrigel™ is used as a vehicle in nude mice tumorigenicity studies [72, 73].

Organotypic cell culture has undoubtedly advanced our understanding of the ECM contribution to digestive

carcinogenesis. Future in vitro systems, however, should incorporate physiological stromata that are dynamic and responsive to better reflect the changing ECM composition and cellular populations that accompany the initiation, progression and local invasion of digestive cancers.

Clinical Potential of Extracellular Matrix Research in Digestive Cancer

Despite the fact that the BM and ECM contribute to tumor initiation, progression and invasion [6, 23], our approach to cancer prevention, diagnosis and management still focuses almost exclusively on the epithelium.

As discussed in our review on stromal fibroblasts, there are developing opportunities for incorporating stromal profiling in defining the pathological progression, and perhaps even the pathological potential, of carcinomas [1, 74]. Epithelial molecular analysis of CRCs is now mainstream and advanced CRCs are routinely tested for *KRAS* mutation prior to starting monoclonal antibody treatments targeting the epidermal growth factor receptor (EGFR) to ensure that only those most likely to respond are administered these therapies [75]. Given the interaction between integrin signaling and the Ras-Raf-MEK-ERK pathway (Fig. 2), it is possible that integrin specific signaling pathways that feed into this pathway may also be relevant in predicting response to the EGFR-monoclonal antibodies cetuximab and panitumumab.

As discussed in our stromal fibroblast review, the tumor stroma could be manipulated as an adjunct to other therapy. For example, an inhibitor of hedgehog signaling was recently used in an animal model of pancreatic cancer to inhibit stromal desmoplasia and thus improve drug delivery to the tumor [76]. As discussed above, agents that effect desmoplasia may also influence the mechanical properties of the tissue, perhaps with implications for integrin related signaling and epithelial growth [41]. Targeting specific integrins or other specific molecules at the integrin-BM interface offers promise as a new approach in biological cancer treatment [74, 77–81].

The MMPs are being investigated as potential biomarkers and therapeutic targets in digestive cancer [82]. MMP-2, -7 and -9 levels in blood, pancreatic juice and tissue have been used to distinguish malignant from benign pancreatic pathologies, as well as for prognostication [83–86]. In CRC, levels of MMP-1, -7, and -13 within the malignant epithelium and stroma have been associated with decreased survival [87]. In gastric cancer, the presence of high MMP-7 staining on surgical specimens was associated with shorter survival [88]. Finally, a study of plasma MMP-2 and MMP-9 suggested that these markers could even help detect patients with colorectal neoplasia [89]. In the future, these stromal factors may serve as potential screening tests to help direct investigations or as a surveillance tool following colonoscopy or colectomy.

There has been a great deal of interest in developing and trialing MMP inhibitors in advanced cancers, including advanced CRC [82]. AE-941 (Neovastat) is derived from shark cartilage and inhibits MMP-2, -9 and -12 [90, 91]. Dalteparin is a low-molecular weight heparin and inhibitor of MMP-9 [92]. Other agents include chemically modified tetracyclines, as well as synthetic drugs structurally designed to block or reconfigure the activated MMP into its proenzyme state [82]. Whilst, there was some initial promise in a phase II trial of Neovastat in advanced renal cell cancer, overall the therapeutic effectiveness of this approach has been disappointing [82, 93]. Nevertheless, with the development of more sophisticated drugs to selectively target the most critical MMPs, this area of research still presents exciting opportunities for the discovery of new chemotherapeutics and chemopreventatives.

Summary

The ECM and BM actively contribute to the normal development and maintenance of the gastrointestinal tract and undergo extensive remodeling during digestive carcinogenesis. These extracellular compartments contain important structural and regulatory molecules that actively signal to the overlying stroma to help regulate growth and differentiation. Important ECM proteases, such as MT1-MMP, influence neoplastic growth and dissemination. Along with the peritumoral stromal cells [1], the ECM is a critical and dynamic contributor to the epithelial–stromal sequence that characterizes digestive carcinogenesis [10]. Cancer researchers are actively engaged in characterizing and translating these stromal events into better diagnostic, prognostic and therapeutic approaches to help reduce the burden of digestive cancer in our community.

Acknowledgements The authors wish to thank Dr Michael Quante for supplying Fig. 3.

References

1. Worthley D, Giraud A, Wang T (2010) Stromal fibroblasts in digestive cancer. Cancer Microenvironment. In press.
2. Bissell MJ, Radisky D (2001) Putting tumours in context. Nat Rev Cancer 1:46–54
3. Kalluri R, Zeisberg M (2006) Fibroblasts in cancer. Nat Rev Cancer 6:392–401
4. Gaggioli C, Hooper S, Hidalgo-Carcedo C et al (2007) Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol 9:1392–1400

5. Liotta LA, Kohn EC (2001) The microenvironment of the tumour–host interface. Nature 411:375–379
6. Nelson CM, Bissell MJ (2006) Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. Annu Rev Cell Dev Biol 22:287–309
7. Bissell MJ, Hall HG, Parry G (1982) How does the extracellular matrix direct gene expression? J Theor Biol 99:31–68
8. Coulombre JL, Coulombre AJ (1971) Metaplastic induction of scales and feathers in the corneal anterior epithelium of the chick embryo. Dev Biol 25:464–478
9. Cunha GR, Young P, Christov K et al (1995) Mammary phenotypic expression induced in epidermal cells by embryonic mammary mesenchyme. Acta Anat (Basel) 152:195–204
10. Littlepage LE, Egeblad M, Werb Z (2005) Coevolution of cancer and stromal cellular responses. Cancer Cell 7:499–500
11. Radisky D, Muschler J, Bissell MJ (2002) Order and disorder: the role of extracellular matrix in epithelial cancer. Cancer Investig 20:139–153
12. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB (1999) Myofibroblasts. I. Paracrine cells important in health and disease. Am J Physiol 277:C1–C9
13. Simon-Assmann P, Kedinger M, De Arcangelis A, Rousseau V, Simo P (1995) Extracellular matrix components in intestinal development. Experientia 51:883–900
14. Schuppan D, Schmid M, Somasundaram R et al (1998) Collagens in the liver extracellular matrix bind hepatocyte growth factor. Gastroenterology 114:139–152
15. Lodish H, Berk A, Kaiser C et al (2008) Integrating cells into tissues. In: Molecular cell biology, 6th edn. New York: Freeman
16. Huntsman DG, Carneiro F, Lewis FR et al (2001) Early gastric cancer in young, asymptomatic carriers of germ-line E-cadherin mutations. N Engl J Med 344:1904–1909
17. Potter JD (1999) Colorectal cancer: molecules and populations. J Natl Cancer Inst 91:916–932
18. Chang TL, Ito K, Ko TK et al (2010) Claudin-1 has tumor suppressive activity and is a direct target of RUNX3 in gastric epithelial cells. Gastroenterology 138:255–265 e251–253
19. Krishnan M, Singh AB, Smith JJ et al (2010) HDAC inhibitors regulate claudin-1 expression in colon cancer cells through modulation of mRNA stability. Oncogene 29:305–312
20. Yoon CH, Kim MJ, Park MJ et al (2010) Claudin-1 acts through c-Abl-protein kinase Cdelta (PKCdelta) signaling and has a causal role in the acquisition of invasive capacity in human liver cells. J Biol Chem 285:226–233
21. Lioni M, Brafford P, Andl C et al (2007) Dysregulation of claudin-7 leads to loss of E-cadherin expression and the increased invasion of esophageal squamous cell carcinoma cells. Am J Pathol 170:709–721
22. Dowling J, Yu QC, Fuchs E (1996) Beta4 integrin is required for hemidesmosome formation, cell adhesion and cell survival. J Cell Biol 134:559–572
23. Rowe RG, Weiss SJ (2009) Navigating ECM barriers at the invasive front: the cancer cell–stroma interface. Annu Rev Cell Dev Biol 25:567–595
24. Khoshnoodi J, Pedchenko V, Hudson BG (2008) Mammalian collagen IV. Microsc Res Tech 71:357–370
25. Simon-Assmann P, Simo P, Bouziges F, Haffen K, Kedinger M (1990) Synthesis of basement membrane proteins in the small intestine. Digestion 46(Suppl 2):12–21
26. McLin VA, Henning SJ, Jamrich M (2009) The role of the visceral mesoderm in the development of the gastrointestinal tract. Gastroenterology 136:2074–2091
27. Powell DW, Adegboyega PA, Di Mari JF, Mifflin RC (2005) Epithelial cells and their neighbors I. Role of intestinal myofibroblasts in development, repair, and cancer. Am J Physiol Gastrointest Liver Physiol 289:G2–G7
28. Hynes RO, Yamada KM (1982) Fibronectins: multifunctional modular glycoproteins. J Cell Biol 95:369–377
29. Hynes RO (2009) The extracellular matrix: not just pretty fibrils. Science 326:1216–1219
30. Xu R, Boudreau A, Bissell MJ (2009) Tissue architecture and function: dynamic reciprocity via extra- and intra-cellular matrices. Cancer Metastasis Rev 28:167–176
31. Giancotti FG, Ruoslahti E (1999) Integrin signaling. Science 285:1028–1032
32. Bray D, Levin MD, Morton-Firth CJ (1998) Receptor clustering as a cellular mechanism to control sensitivity. Nature 393:85–88
33. Schwartz MA, Assoian RK (2001) Integrins and cell proliferation: regulation of cyclin-dependent kinases via cytoplasmic signaling pathways. J Cell Sci 114:2553–2560
34. Danilkovitch A, Donley S, Skeel A, Leonard EJ (2000) Two independent signaling pathways mediate the antiapoptotic action of macrophage-stimulating protein on epithelial cells. Mol Cell Biol 20:2218–2227
35. Gille H, Downward J (1999) Multiple ras effector pathways contribute to G(1) cell cycle progression. J Biol Chem 274:22033–22040
36. Takuwa N, Fukui Y, Takuwa Y (1999) Cyclin D1 expression mediated by phosphatidylinositol 3-kinase through mTOR-p70 (S6K)-independent signaling in growth factor-stimulated NIH 3T3 fibroblasts. Mol Cell Biol 19:1346–1358
37. Frisch SM, Screaton RA (2001) Anoikis mechanisms. Curr Opin Cell Biol 13:555–562
38. Puthalakath H, Villunger A, O'Reilly LA et al (2001) Bmf: a proapoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis. Science 293:1829–1832
39. Liu Z, Li H, Derouet M et al (2006) Oncogenic ras inhibits anoikis of intestinal epithelial cells by preventing the release of a mitochondrial pro-apoptotic protein Omi/HtrA2 into the cytoplasm. J Biol Chem 281:14738–14747
40. Boisvert-Adamo K, Aplin AE (2008) Mutant B-RAF mediates resistance to anoikis via Bad and Bim. Oncogene 27:3301–3312
41. Katsumi A, Orr AW, Tzima E, Schwartz MA (2004) Integrins in mechanotransduction. J Biol Chem 279:12001–12004
42. Paszek MJ, Zahir N, Johnson KR et al (2005) Tensional homeostasis and the malignant phenotype. Cancer Cell 8:241–254
43. Berrier AL, Yamada KM (2007) Cell–matrix adhesion. J Cell Physiol 213:565–573
44. Lo CM, Wang HB, Dembo M, Wang YL (2000) Cell movement is guided by the rigidity of the substrate. Biophys J 79:144–152
45. Wipff PJ, Hinz B (2008) Integrins and the activation of latent transforming growth factor beta1—an intimate relationship. Eur J Cell Biol 87:601–615
46. Chiang AC, Massague J (2008) Molecular basis of metastasis. N Engl J Med 359:2814–2823
47. Ikeda K, Iyama K, Ishikawa N et al (2006) Loss of expression of type IV collagen alpha5 and alpha6 chains in colorectal cancer associated with the hypermethylation of their promoter region. Am J Pathol 168:856–865
48. Spaderna S, Schmalhofer O, Hlubek F et al (2006) A transient, EMT-linked loss of basement membranes indicates metastasis and poor survival in colorectal cancer. Gastroenterology 131:830–840
49. Bechetoille N, Haftek M, Staquet MJ, Cochran AJ, Schmitt D, Berthier-Vergnes O (2000) Penetration of human metastatic melanoma cells through an authentic dermal-epidermal junction is associated with dissolution of native collagen types IV and VII. Melanoma Res 10:427–434
50. Franz M, Richter P, Geyer C et al (2007) Mesenchymal cells contribute to the synthesis and deposition of the laminin-5 gamma2 chain in the invasive front of oral squamous cell carcinoma. J Mol Histol 38:183–190
51. Comoglio PM, Trusolino L (2005) Cancer: the matrix is now in control. Nat Med 11:1156–1159

52. McKerrow JH, Bhargava V, Hansell E et al (2000) A functional proteomics screen of proteases in colorectal carcinoma. Mol Med 6:450–460
53. Sternlicht MD, Werb Z (2001) How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 17:463–516
54. Hotary KB, Yana I, Sabeh F et al (2002) Matrix metalloproteinases (MMPs) regulate fibrin-invasive activity via MT1-MMP-dependent and -independent processes. J Exp Med 195:295–308
55. Sabeh F, Ota I, Holmbeck K et al (2004) Tumor cell traffic through the extracellular matrix is controlled by the membrane-anchored collagenase MT1-MMP. J Cell Biol 167:769–781
56. Morini M, Mottolese M, Ferrari N et al (2000) The alpha 3 beta 1 integrin is associated with mammary carcinoma cell metastasis, invasion, and gelatinase B (MMP-9) activity. Int J Cancer 87:336–342
57. Brooks PC, Stromblad S, Sanders LC et al (1996) Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. Cell 85:683–693
58. Hotary K, Li XY, Allen E, Stevens SL, Weiss SJ (2006) A cancer cell metalloprotease triad regulates the basement membrane transmigration program. Genes Dev 20:2673–2686
59. Perez SE, Cano DA, Dao-Pick T, Rougier JP, Werb Z, Hebrok M (2005) Matrix metalloproteinases 2 and 9 are dispensable for pancreatic islet formation and function in vivo. Diabetes 54:694–701
60. Bendrik C, Robertson J, Gauldie J, Dabrosin C (2008) Gene transfer of matrix metalloproteinase-9 induces tumor regression of breast cancer in vivo. Cancer Res 68:3405–3412
61. Cheng S, Pollock AS, Mahimkar R, Olson JL, Lovett DH (2006) Matrix metalloproteinase 2 and basement membrane integrity: a unifying mechanism for progressive renal injury. FASEB J 20:1898–1900
62. Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H, Itohara S (1998) Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res 58:1048–1051
63. Sternlicht MD, Lochter A, Sympson CJ et al (1999) The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. Cell 98:137–146
64. Radisky DC, Levy DD, Littlepage LE et al (2005) Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 436:123–127
65. List K, Szabo R, Molinolo A et al (2005) Deregulated matriptase causes ras-independent multistage carcinogenesis and promotes ras-mediated malignant transformation. Genes Dev 19:1934–1950
66. Linder S (2009) Invadosomes at a glance. J Cell Sci 122:3009–3013
67. Poincloux R, Lizarraga F, Chavrier P (2009) Matrix invasion by tumour cells: a focus on MT1-MMP trafficking to invadopodia. J Cell Sci 122:3015–3024
68. Wolf K, Friedl P (2009) Mapping proteolytic cancer cell–extracellular matrix interfaces. Clin Exp Metastasis 26:289–298
69. Yamada K, Cukierman E (2007) Modeling tissue morphogenesis and cancer in 3D. Cell 130:601–610
70. Kleinman HK, Martin GR (2005) Matrigel: basement membrane matrix with biological activity. Semin Cancer Biol 15:378–386
71. Futaki S, Hayashi Y, Yamashita M et al (2003) Molecular basis of constitutive production of basement membrane components. Gene expression profiles of Engelbreth-Holm-Swarm tumor and F9 embryonal carcinoma cells. J Biol Chem 278:50691–50701
72. Fridman R, Kibbey MC, Royce LS et al (1991) Enhanced tumor growth of both primary and established human and murine tumor cells in athymic mice after coinjection with Matrigel. J Natl Cancer Inst 83:769–774
73. Vukicevic S, Kleinman HK, Luyten FP, Roberts AB, Roche NS, Reddi AH (1992) Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. Exp Cell Res 202:1–8
74. Reuter JA, Ortiz-Urda S, Kretz M et al (2009) Modeling inducible human tissue neoplasia identifies an extracellular matrix interaction network involved in cancer progression. Cancer Cell 15:477–488
75. Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A (2009) Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. J Natl Cancer Inst 101:1308–1324
76. Olive KP, Jacobetz MA, Davidson CJ et al (2009) Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 324:1457–1461
77. Yang XH, Flores LM, Li Q et al (2010) Disruption of Laminin-Integrin-CD151-Focal Adhesion Kinase Axis Sensitizes Breast Cancer Cells to ErbB2 Antagonists. Cancer Res 70:2256–2263
78. Ning S, Nemeth JA, Hanson RL, Forsythe K, Knox SJ (2008) Anti-integrin monoclonal antibody CNTO 95 enhances the therapeutic efficacy of fractionated radiation therapy in vivo. Mol Cancer Ther 7:1569–1578
79. Hsu AR, Veeravagu A, Cai W, Hou LC, Tse V, Chen X (2007) Integrin alpha v beta 3 antagonists for anti-angiogenic cancer treatment. Recent Pat Anticancer Drug Discov 2:143–158
80. Nemeth JA, Nakada MT, Trikha M et al (2007) Alpha-v integrins as therapeutic targets in oncology. Cancer Investig 25:632–646
81. Lu X, Lu D, Scully M, Kakkar V (2008) The role of integrins in cancer and the development of anti-integrin therapeutic agents for cancer therapy. Perspect Med Chem 2:57–73
82. Roy R, Yang J, Moses MA (2009) Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol 27:5287–5297
83. Tian M, Cui YZ, Song GH et al (2008) Proteomic analysis identifies MMP-9, DJ-1 and A1BG as overexpressed proteins in pancreatic juice from pancreatic ductal adenocarcinoma patients. BMC Cancer 8:241
84. Yokoyama M, Ochi K, Ichimura M et al (2002) Matrix metalloproteinase-2 in pancreatic juice for diagnosis of pancreatic cancer. Pancreas 24:344–347
85. Kuhlmann KF, van Till JW, Boermeester MA et al (2007) Evaluation of matrix metalloproteinase 7 in plasma and pancreatic juice as a biomarker for pancreatic cancer. Cancer Epidemiol Biomark Prev 16:886–891
86. Jones LE, Humphreys MJ, Campbell F, Neoptolemos JP, Boyd MT (2004) Comprehensive analysis of matrix metalloproteinase and tissue inhibitor expression in pancreatic cancer: increased expression of matrix metalloproteinase-7 predicts poor survival. Clin Cancer Res 10:2832–2845
87. Hilska M, Roberts PJ, Collan YU et al (2007) Prognostic significance of matrix metalloproteinases-1, -2, -7 and -13 and tissue inhibitors of metalloproteinases-1, -2, -3 and -4 in colorectal cancer. Int J Cancer 121:714–723
88. Koskensalo S, Mrena J, Wiksten JP et al (2010) MMP-7 overexpression is an independent prognostic marker in gastric cancer. Tumour Biol 31:149–155
89. Tutton MG, George ML, Eccles SA, Burton S, Swift RI, Abulafi AM (2003) Use of plasma MMP-2 and MMP-9 levels as a surrogate for tumour expression in colorectal cancer patients. Int J Cancer 107:541–550
90. Latreille J, Batist G, Laberge F et al (2003) Phase I/II trial of the safety and efficacy of AE-941 (Neovastat) in the treatment of non-small-cell lung cancer. Clin Lung Cancer 4:231–236
91. Batist G, Patenaude F, Champagne P et al (2002) Neovastat (AE-941) in refractory renal cell carcinoma patients: report of a phase II trial with two dose levels. Ann Oncol 13:1259–1263
92. Sideras K, Schaefer PL, Okuno SH et al (2006) Low-molecular-weight heparin in patients with advanced cancer: a phase 3 clinical trial. Mayo Clin Proc 81:758–767
93. Finkelstein JB (2005) Sharks do get cancer: few surprises in cartilage research. J Natl Cancer Inst 97:1562–1563