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Feature Articles

Colorectal Cancer and the Integrin Family of Cell Adhesion Receptors: Current Status and Future Directions

M.V. Agrez and R.C. Bates

Tumour progression is thought to be determined, at least in part, by the balance between available cell surface receptors and the nature of the surrounding extracellular matrix. The integrin family of transmembrane adhesion receptors involved in tumour cell–matrix interactions mediates cell adhesion, migration, and differentiation. Certain patterns of integrin receptor expression on normal and malignant colon epithelial cells are emerging, and it is now clear that integrins can also regulate such divergent processes as cell proliferation and programmed cell death in this tumour type. This implies that integrins are involved in signal transduction events within colon carcinoma cells consequent upon their adhesive interaction with matrix molecules. A better understanding of the mechanisms involved in these events may lead to useful therapeutic strategies in the management of this disease.
Key words: Colorectal carcinoma, integrin cell adhesion receptors
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CLASSIFICATION OF INTEGRINS

CELLS ADHERE to each other and to the extracellular matrix in a highly specific manner determined by the repertoire of cell adhesion receptors present on the cell surface. One large family of receptors is called integrins because of their integration across the plasma membrane. This provides a structural and functional bridge between extracellular matrix molecules (ligands), which bind to the large extracellular domains of integrins, and cytoskeletal components (e.g. talin and α-actinin), which bind to the shorter cytoplasmic domains of the receptors within the cells. Individual integrins comprise an alpha (α), and beta (β) subunit in non-covalent association, and many receptors recognise and bind an arginine-glycine-aspartate (RGD) sequence contained within the specific matrix molecule to which they adhere [1].

There are now known to be at least 15 alpha subunits and 8 beta subunits and 21 combinations of α and β chains that can produce individual receptors. Excluding the leucocyte integrins which are designated by the β2 nomenclature, and which mediate cell–cell interactions, integrins can be divided into two major groups. The β1 subfamily defines one group sometimes referred to as VLA (very late antigens) in which the β1 chain combines with nine alpha chain members (α 1–9) [2]. The name VLA arose because certain integrins, VLA-1 and VLA-2 (α1β1 and α2β1, respectively) appear late on activated lymphocytes [3]. All except two of the alpha partners in the VLA subfamily are monogamous and are associated only with the β1 subunit (in addition to pairing with β1, α6 and α4 exist as functional receptors in association with different β partners, β4 and β7, respectively) [1]. Hence, VLA refers to the presence of the promiscuous β1 subunit and the appended number refers to the associated alpha chain which determines the specificity of the matrix–receptor interaction. Similarly, the other group of receptors, designated αv, has been so named because the promiscuous αv subunit binds to individual and different β subunits (including β1) which, thereby, determine ligand specificity for an individual receptor. A distinguishing feature of this subfamily is that all αv integrins, in contrast to only one of the VLA integrins (VLA-5), bind with high affinity to the RGD sequence [1].

It is apparent then that individual cells can simultaneously express multiple VLA and αv integrins with the capacity to bind either the same or different matrix molecules. Moreover, the same integrins on different cells may bind different ligands, and expression of an integrin on the cell surface does not necessarily guarantee function [3]. It remains to be determined how multiple integrins with overlapping specificities regulate diverse biological processes within a given tumour cell. It seems likely, however, that interactions between integrins, growth factors, matrix molecules, matrix-degrading enzymes and their inhibitors play a major role. For example, integrins can mediate signals which include metalloproteinase gene expression [4]. Growth factors can stimulate synthesis and secretion of matrix proteins, metalloproteinases and their inhibitors, and increase expression of constitutive integrins on the cell surface [5–9]. The responsiveness of cells to growth factors may also be determined by the nature of the extracellular matrix environment [10]. Alterations in integrin expression have been reported as a result of changes

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in the peri-cellular matrix milieu as shown by the increased expression of the VLA-2 collagen receptor when cells are cultured in a collagen matrix [11]. To what extent these interactions influence *in vivo* tumour growth, migration and metastasis is unknown.

VLA INTEGRIN EXPRESSION IN THE NORMAL COLON

The VLA-2 receptor binds collagen and laminin, and it has been suggested that decreased VLA-2 expression in the colonic crypt may lead to a loss of cell–matrix binding and sloughing of mature cells at the mucosal surface [12]. However, published data describing the immunostaining patterns of integrin expression along the length of the colonic crypt have not presented a consistent picture for some of the VLA integrins. For example, Choy and colleagues [13] have identified the VLA-2 receptor at both the crypt apex amd base, in contrast to other investigators who have not observed its presence at the apical aspect of colonic epithelium [14]. Moreover, the presence of the VLA-5 receptor (for fibronectin) anywhere within the crypt has been identified by only one research group [14]. It is known that the $\alpha 2$ and $\alpha 5$ subunits exist only as monogamous partners to the promiscuous $\beta 1$ chain and, therefore, immunostaining with an antibody against either of these alpha subunits infers the presence of the whole receptor, $\alpha 2\beta 1$ (VLA-2) or $\alpha 5\beta 1$ (VLA-5). The most likely reason for reported discrepancies in immunostaining patterns is the use of different antibodies which recognise different epitopes on the same receptor (some of which may be masked by tissue fixation techniques).

Alternatively, there has been consistency in the identification of both VLA-3 (laminin, fibronectin, collagen receptor) and VLA-6 (laminin receptor) throughout the length of the colonic crypt, and strong staining for VLA-6 has been observed at contact sites of epithelial cells with basement membrane [13, 14]. It has to be recognised, however, that integrin expression in the normal colon is likely to be a dynamic event with receptors being switched on and off during different stages of cell migration and replication. Studies directed at VLA integrin expression during gut morphogenesis in animal models as well as in human colonic crypts cultured *in vitro* should offer further insights into these processes.

VLA INTEGRIN EXPRESSION IN COLORECTAL CARCINOMA

Tissue distribution

A systematic study of integrin expression in normal colon, adenomas and carcinomas within the same patients has revealed progressive loss of expression of the VLA-3 and VLA-5 receptors in the transition from adenomas to carcinomas [14]. In this study, eight adenomas were examined, but in only one was VLA-5 expressed by all epithelial cells. In three adenomas, some cells lacked this receptor completely and generalised staining for VLA-5, as seen in normal tissues, was observed in only 1/19 adenocarcinomas. Moreover, 15/19 carcinomas were completely negative for VLA-5 expression. Interestingly, loss of the VLA-2 and VLA-3 receptors has been shown to parallel loss of differentiation in colon carcinoma [15]. However, no relationship has been observed between expression of these receptors for fibronectin, collagen and laminin and clinicopathological tumour stage. Whether these data reflect causal events or merely secondary phenomena is not known, but it could be argued that loss of either one or more of these receptors may benefit migration of invading tumour cells consequent upon less stringent binding to the surrounding matrix scaffold.

The pattern of tissue expression of the laminin receptors VLA-6 and $\alpha 6\beta 4$ (for which there is no VLA designation since the beta subunit differs from the promiscuous $\beta 1$ chain) appears to be less clear-cut in the adenoma-carcinoma sequence than is the case for the VLA-5 fibronectin receptor. A reduced expression of these receptors has been observed in colorectal carcinomas compared with adenomas which could facilitate detachment of colon carcinoma cells from basement membranes and their penetration into the underlying mesenchyme [14]. However, laminin receptors also appear to be essential for colon carcinoma cell invasion of basement membranes, at least *in vitro* [16, 17], suggesting co-operation between different laminin receptors in this process.

Experimental data from human colon carcinoma cell lines

It is recognised that cell lines may not always reflect the phenotype of the tissue of origin. However, integrins which have been identified on established colon carcinoma cell lines together with their respective ligands (in parentheses) include VLA-2 (collagen), VLA-5 (fibronectin), VLA-6 (laminin), and $\alpha 6\beta 4$ (laminin). Consistent with the changes observed in tissue expression of VLA-5, in the progression from normal to malignant epithelium, induced expression of this receptor in human colon carcinoma cells constitutively lacking this integrin has been shown to result in a marked reduction in tumorigenicity in immune-deficient mice [18]. Moreover, the capacity of such cells to grow *in vitro* is also reduced, possibly consequent upon failure of the transfected cells to transcribe genes which control the cell cycle. Interestingly, when the appropriate ligand for the receptor is present (in this case fibronectin), cell proliferation is restored suggesting that unoccupied integrins may provide negative growth signals to a cell [18].

If the loss of VLA-2 seen with colon cancer de-differentiation is causal [15] then a differentiating effect of collagen on colon cancer cells cultured *in vitro* might be expected. Indeed, Richman and Bodmer [19] have shown that glandular differentiation of human colon carcinoma cells could only be achieved by seeding cells within three-dimensional collagen gels. This is consistent with observations from our laboratory that the proliferative capacity of colon carcinoma cells in culture is inversely related to the concentration of collagen within a gel [20].

It appears, however, that not all cell lines use the VLA receptors expressed on their cell surface to bind to collagen. In a study of seven lines by Bodmer's group, only two adhered to a collagen substrate *in vitro* [21]. Although the pattern of VLA expression for some of these lines is still not known, the ability of the cells to bind to collagen was not related to the origin of the cells from either a primary or metastatic source. Whether differences in binding of tumour cells to collagen reflects co-operation between growth factors and integrins remains speculative. Growth factors can exert their effects indirectly by stimulating either integrin expression and/or matrix synthesis. For example, transforming growth factor-beta (TGFβ) causes an increase in expression of VLA-2, -3, and -6 integrins in colon carcinoma cells, and such cells exhibit enhanced binding to collagen in the presence of TGFβ [21].

Based on the differentiated growth patterns and enhanced collagen binding of human colon carcinoma cell lines in the presence of TGFβ, it has been proposed that tumour cells become unresponsive to this growth factor through loss of one of the main targets of TGFβ action such as an integrin-like collagen receptor [21]). Lack of such a receptor, the expression of which might be expected to enhance differentiation through

effects on extracellular matrix binding, may contribute to tumour progression. Alternatively, the primary event in tumour progression may be a change in matrix composition surrounding a tumour cell which results in loss of responsiveness of the cell to the growth-inhibitory effects of TGFβ. It is known, for example, that the inhibitory effect of TGFβ on growth of colon carcinoma cells *in vitro* is enhanced in the presence of a matrix substratum containing laminin and fibronectin amongst other proteins [10].

αV EXPRESSION IN THE NORMAL COLON

Immunostaining with antibodies directed specifically at the αv subunit has shown that this subfamily of receptors is present in abundance in normal colonic mucosa, but not in association with β3, the classical vitronectin receptor [14, 22]. The lack of specific antibodies against beta partners to αv has hampered the identification of individual receptors until recently with the discovery of a novel subunit, designated β6, which functions as a fibronectin receptor [23, 24]. In normal adult primate tissues this receptor is restricted to a variety of epithelial cell types including colonic epithelium [25] suggesting that it may play a role in regulating differentiation and migration of cells within the colonic crypt.

Furthermore, there is now strong circumstantial evidence implicating αv integrins in the process of programmed cell death (apoptosis). This distinctive form of cell death is an essential component of embryonic development, and differs markedly from necrosis on biochemical and morphological grounds [26]. Unlike simple cell degeneration, apoptosis is dependent on active biochemical processes within a cell that can potentially be suppressed. Bates and co-workers have studied the apoptotic process in a colon carcinoma cell line which grows as structural "organoids" of goblet and columnar cells arranged around a central lumen [27, 28]. These investigators have shown that an antibody directed against αv inhibited cell–cell contact, and as a consequence of this inhibition, the colon carcinoma cells rapidly underwent apoptosis. Interestingly, this process was accompanied by the rapid translocation of a tumour suppressor gene product, p53, to the cell nucleus [27]. If the "organoid" model is representative, at least in part, of normal colonic crypt cells, then it seems reasonable to propose that αv integrin-mediated cell–cell attachment may be an important factor contributing to the regulation of apoptosis in the human colon.

αV EXPRESSION IN COLORECTAL CARCINOMA

Similar to the normal colon, immunostaining of tumour specimens has revealed the presence of αv, but not in association with β3 [14, 22]. At first glance this is surprising given that αvβ3 (which can bind many matrix molecules besides vitronectin) has been shown to play a major role in tumour growth and metastasis in melanoma. For example, this receptor has been found to be restricted exclusively to cells within the vertical growth phase of primary melanomas and also to metastatic lesions [29]. In experimental models, loss of αvβ3 expression in melanoma cells leads to reduced *in vivo* proliferation which is restored upon re-expression of the receptor [30]. The identification of other beta partners which associate with αv on colon carcinoma cells such as β5 (vitronectin receptor) [24] now makes it possible to dissect out the role of the αv subfamily in this disease.

One important function of this integrin subfamily may be the regulation of cell growth. We have shown in a colon carcinoma cell line that the αvβ6 receptor (which binds fibronectin) stimulates cell proliferation both *in vitro* and *in vivo* in nude mice, and that this growth-promoting effect is mediated through a unique portion of the cytoplasmic domain of the beta-6 subunit [31]. The signal transduction pathways involved in augmentation of proliferation by this integrin are currently the subject of intense investigation. One could speculate, for example, that as in the case of integrin-mediated induction of collagenase in normal fibroblasts via the VLA-5 fibronectin receptor [4], a similar signal transduction process occurs consequent upon ligand occupancy of the αvβ6 receptor. As a result, the collagen concentration in the immediate peri-cellular environment may be lowered, thereby lifting the constraints on proliferation imposed upon colon carcinoma cells by the surrounding collagen matrix. At the very least, there appears to be some functional imbalance with respect to growth regulation by the major fibronectin receptors in colorectal carcinoma, with tumour cells capable of simultaneously expressing a potentially negative growth regulator, VLA-5 [18], in the presence of αvβ6 which stimulates tumour growth. The final outcome for a cell may be determined by the balance struck between available competing receptors and their relative affinities for the shared ligand. An absolute requirement for receptor occupancy in the case of αvβ6-mediated growth signals remains to be determined.

ACCESSORY CELL ADHESION MOLECULES AND TUMOUR SUPPRESSOR GENES

There are now data which show that integrin-mediated binding of colon carcinoma cells to collagen *in vitro* can be regulated by other molecules. For example, carcinoembryonic antigen (CEA) does not have any collagen-binding activity itself, but several antibodies against CEA have been shown to inhibit binding of a colon carcinoma cell line to collagen indicating that this glycoprotein can function as an accessory adhesion molecule [32]. Interestingly, CEA is a member of the immunoglobulin family which includes well-characterised intercellular adhesion molecules such as neural cell adhesion molecule N-CAM [33].

Recently, a novel gene called cell adhesion regulator (CAR) has been identified which enhances integrin-mediated adhesion of colon carcinoma cells to collagen [34]. The gene encodes a protein containing a tyrosine phosphorylation site essential for its activity, and appears to be located just inside the cell membrane. This suggests that CAR protein may either assist in coupling the cytoplasmic domain of integrins to the cytoskeleton and/or induce a conformational change to the extracellular domain of integrins which facilitates enhanced binding to collagen. CAR is, therefore, a candidate tumour suppressor gene and loss of CAR activity could be important in tumour invasion, causing loss of differentiation or tumour cell release from matrix attachments [34].

Current molecular techniques directed at the identification of tumour suppressor genes as mRNAs in only one of a pair of related cell populations are based on the positive selection at the mRNA level for genes expressed in normal epithelial cells, but decreased or lost in corresponding tumour cells [35]. A candidate tumour suppressor gene recovered by this method in breast carcinoma is the integrin α6 subunit (VLA-6, laminin receptor) [36], and it should come as no surprise if the VLA-5 receptor in colorectal carcinoma also proves to qualify as a tumour suppressor gene in future applications of this technique.

THERAPY BEYOND THE YEAR 2000

During the next decade, we can expect to see the development of new therapeutic strategies directed at colorectal carcinoma. The targeting of anti-integrin antibodies to tumour cells to

inhibit integrin–ligand binding and, thereby, intracellular signalling offers one approach. The disruption of cell–cell contacts by antibodies against adhesion receptors could also prove useful as a means of stimulating apoptosis. Another approach is the use of synthetic peptides to inhibit tumour invasion and metastasis. For example, RGD-containing peptides have been shown to inhibit tumour cell invasion *in vitro*, and tumour metastases from melanoma in an animal model [37, 38]. These peptides are also able to alter the ligand specificity of integrins *in vitro* and enhance collagen binding [39]. However, any potential inhibitory effect of synthetic peptides on tumour growth and metastatic spread of colon carcinoma cells will need to be addressed in experimental animal models which make use of orthotopic tumour implantation techniques so as to mimic as closely as possible the metastatic process in human colorectal carcinoma [40].

Although the treatment of cancer by gene-based therapy is still in its infancy, such therapeutic strategies in the future may utilise a "dominant negative" approach to interfere with the function of a subclass of integrins. Genes code for each of the integrin subunits which go to make up a functional αβ heterodimer expressed on the cell surface. In the case of the αv subfamily, different β subunits share the same αv partner. The introduction into cells of a gene coding for the β6 subunit has been shown to decrease the expression of another monogamous subunit, β5, by competition for the shared promiscuous αv partner [24]. As a consequence of this transfection, the ability of the genetically altered cells to bind to vitronectin, the ligand for αvβ5, was significantly reduced [24]. It is possible, therefore, that transfection of a cell with a gene coding for a mutated version of an integrin subunit could inhibit expression and subvert activity of the endogenous non-mutated form of the receptor, as well as reducing expression of other integrins in that cell which pair with the same partner. "Dominant negative" effects *in vitro*, secondary to transfection of mutated versions of a gene, have now been described for the p53 tumour suppressor gene product, growth factor receptors, and integrins [41–44]. Alternatively, the incorporation of a non-mutated gene coding for a functional integrin receptor, which is normally only weakly expressed by the cell, could result in enhanced expression of this gene product. This might be an advantage where the integrin of interest transduces negative growth signals (e.g. the unoccupied VLA-5 fibronectin receptor). Methods to achieve optimal delivery of genes targeted to tumour cells which result in stable integration of the DNA still need to be resolved [45]. However, the future for the management of colorectal carcinoma looks promising as integrin-mediated signal transduction pathways within a cell are unravelled and more potential therapeutic options become available.

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Protease Inhibitors: Role and Potential Therapeutic Use in Human Cancer

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Proteases and protease inhibitors have been increasingly recognised as important factors in the physiopathology of human diseases, and our understanding of their role in cancer has dramatically increased over the last decade. We have obtained causal evidence linking proteases to tumour invasion and metastasis, and have become aware of genuine mechanisms used by tumour cells to optimise the use of proteases in the pericellular matrix. Many synthetic and natural inhibitors of these proteases have also been characterised, and their mechanisms of interaction with their corresponding enzymes are progressively unveiled as the X-ray crystal structures of these enzymes and their inhibitors are now reported. It has also become evident that many of these inhibitors, in addition to preventing the dissemination of cancer cells, have an inhibitory effect on tumour growth. Thus protease inhibitors are emerging as potentially therapeutic tools to treat cancer. In this article, recent studies on the role of proteases and their inhibitors in cancer are reviewed, and current ideas on their potential use as therapeutic agents are discussed.

Key words: proteases, protease inhibitors, invasion, metastasis, angiogenesis
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PROTEASES AND THEIR INHIBITORS IN HUMAN DISEASES

PROTEASES PLAY a key role in many physiological processes such as blood coagulation and fibrinolysis, complement and cytokine activation, cell migration, organogenesis, trophoblastic implantation, or tissue remodelling, and have been increasingly identified as important factors in the pathophysiology of a large number of human diseases. Several pathological conditions including thrombotic disorders, hypertension, osteoarthritis, chronic degenerative diseases and cancer are caused by changes in protease activity [1–4], and many human pathogens rely on proteases to infect the host [5–7].