J Oral Pathol Med (2006) 35: 1–10
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REVIEW ARTICLE

αvβ6 integrin in wound healing and cancer of the oral cavity

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Integrins are a family of heterodimeric cell surface receptors, which are expressed on most cells where they mediate cell-cell and cell-extracellular matrix (ECM) interactions. The αvβ6 integrin is epithelial-specific and binds to the ECM proteins fibronectin, vitronectin and tenascin, and also to the latency associated peptide of TGF-β. Unlike most epithelial integrins, αvβ6 is not expressed constitutively by healthy oral epithelia, but is upregulated during tissue remodelling, including that accompanying wound healing and carcinogenesis. Although, the data at present have been generated principally from *in vitro* studies, there is increasing evidence to suggest that αvβ6 may promote carcinoma progression: αvβ6 has been shown to modulate invasion, inhibit apoptosis, regulate protease expression and activate TGF-β1. This review examines the current literature, and discusses the possible role of αvβ6 in wound healing, and in the development and progression of oral squamous cell carcinoma.
J Oral Pathol Med (2006) **35**: 1–10

Keywords: integrin; keratinocyte; squamous carcinoma; wound healing

Introduction

Integrins are the principal cell surface receptors that enable both normal and transformed cells to attach to and respond to their extra-cellular environment. Integrins mediate cell-to-cell or cell-to-extracellular matrix (ECM) adhesion, providing adhesion for stationary cells, traction during cell movement and, importantly, the promotion of many signalling pathways that regulate diverse processes including proliferation, migration, cell survival, differentiation, tumour invasion and metastasis (1). Structurally, integrins are heterodimers composed of two different, non-covalently associated, α and β subunits. They appear by electron microscopy to have a large extra-cellular domain composed of a membrane-distal, globular head (that contains the ligand binding site), on two long stalks [reviewed in (2)]. The carboxy-(C-) termini of the α and β subunits traverse the cell membrane and extend a short distance (usually <60 amino-acid residues) into the cytoplasm (1). The exception to this is the intracellular portion of α6β4 which is much larger than that of all other known β subunits and bears no apparent homology with them (3–5).

To date 18 α and eight β subunits have been identified that form 24 different integrins (Fig. 1). Subsequent to ligand binding integrins aggregate together, bringing into juxta-position many signalling and structural molecules that are associated with their cytoplasmic tails, allowing them to interact [reviewed in (6)]; in this way integrins serve as the major mechanism by which extracellular matrix cues are translated into intracellular signal transduction pathways [reviewed in (7); Fig. 2]. Owing to space restrictions it will not be possible to provide a detailed description of integrin biology in general and thus the reader is referred to several excellent reviews (1, 2). In this review, we will concentrate on the accumulating data supporting the role of one particular integrin, αvβ6, in the processes of wound healing and carcinogenesis, emphasizing the development and progression of oral squamous cell carcinoma (OSCC).

Over the last 10 years many different functions have been associated with an increased expression of αvβ6 including promotion of cell migration, control of cell proliferation, activation of TGFβs, suppression of apoptosis, modulation of protease activity and mediating invasion of carcinoma cells. It remains to be determined which of these functions are active when αvβ6 is upregulated *in vivo* but in this section we shall review those functions attributed to αvβ6 from *in vitro* analyses (Table 1).

αvβ6 promotes migration and invasion

As with all other αv integrins, αvβ6 binds to a tripeptide recognition sequence arginine-glycine-aspartic acid (RGD) in its ligands which include fibronectin (8), tenascin-C (9), vitronectin (10) and the latency-associated peptide (LAP) of TGFβ1 (11) and TGFβ3 (12)

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Accepted for publication August 23, 2005

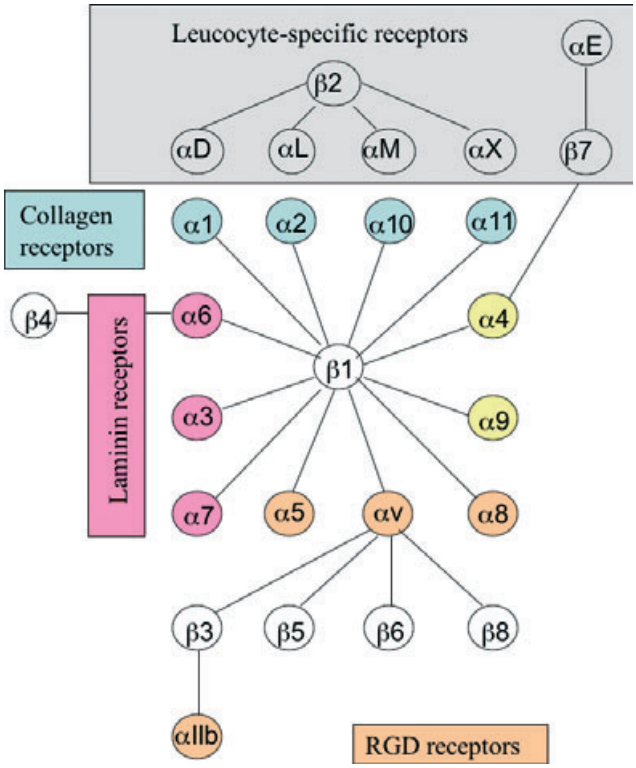

Figure 1 The integrin superfamily: 18 α and eight β subunits have been identified combining to form at least 24 heterodimeric integrins which can be considered in several subfamilies based on ligand specificity and, in the case of β2 and β7 integrins, restricted expression on leucocytes. The different subunits show selectivity in their binding partners; for example the αv subunit can pair with multiple β subunits (β1, β3, β5, β6 and β8) but the β6 subunit can only bind with αv. Adapted from Hynes, 2002.

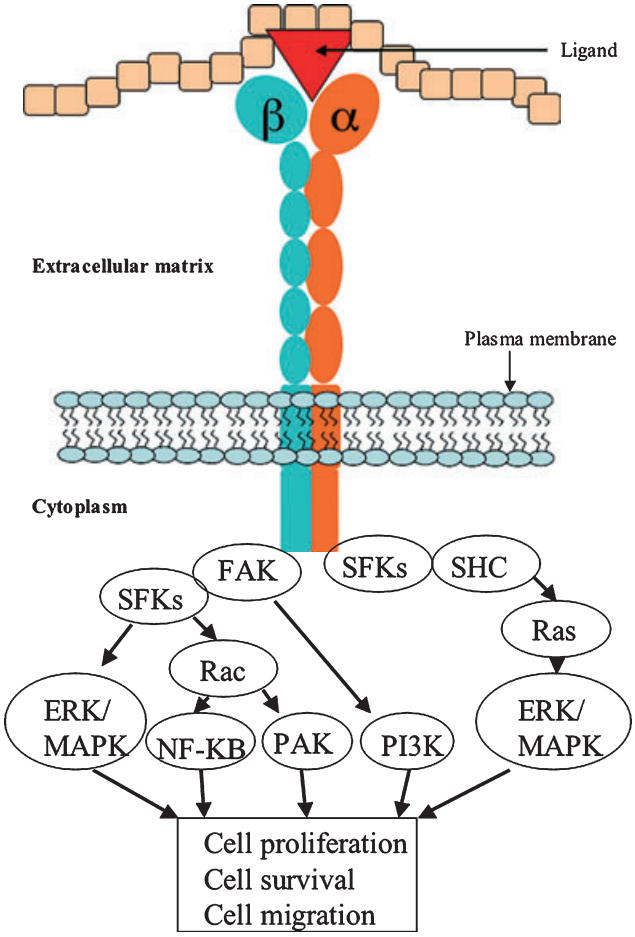

Figure 2 Integrin structure and signalling. Integrins are composed of α and β subunits that span the cell membrane and have a short cytpolasmic tail on the inner side of the plasma membrane and a long extracellular domain extending into the extracellular space. The short cytoplasmic domains of both α and β subunits of integrins interact with a cohort of intracellular proteins, activating several signalling pathways. In this manner integrin signalling controls critical cell processes such as proliferation, survival and migration.

(Table 2). Several studies have found that αvβ6 expression promotes keratinocyte migration. Huang et al. (10) used mouse β6-/- keratinocytes to show that αvβ6 has a critical role in migration over fibronectin and vitronectin and that this process could be increased by hepatocyte growth factor (HGF) and was modulated through a pathway involving protein kinase C. We also demonstrated that αvβ6 promotes migration of human primary oral keratinocytes on fibronectin, and showed that binding of αvβ6 to this ligand upregulated secretion of the pro-enzyme form of type IV collagenase, matrix metalloproteinase-9 (MMP-9) (13). This protease localized to the tips of αv-positive filopodia, cell membrane structures thought to be involved in cell movement [reviewed in (14)]. Exogenous activation of MMP-9 further increased keratinocyte migration (13). Similarly, using keratinocytes derived from TNFα-knockout mice Scott et al. (15) described TNFα-dependent upregulation of αvβ6 which was associated with increased migration and MMP-9 secretion, both processes being dependent on αvβ6 expression.

αvβ6 activates TGFβ

The TGFβ cytokine family is composed of TGFβ1, TGFβ2 and TGFβ3 [reviewed in (16)]. TGFβ is secreted into the ECM as a latent complex and is found *in vivo* primarily in this latent form associated with its LAP from which it must be released to become biologically active [reviewed in (17)]. The presence of RGD motifs in the pro-peptide of TGFβs led to them being investigated as possible integrin ligands. Munger and colleagues reported that LAP is a high affinity ligand for αvβ6 and that the interaction of LAP with αvβ6 provides a mechanism for the activation of the cytokine (11). TGFβ activation results from a conformational change in the latent TGFβ molecule rather than via cleavage of the peptide and is dependent on the ability of αvβ6 to connect with the actin cytoskeleton of the cell. Cells expressing mutated β6 subunits, that were unable to interact with actin, could still bind LAP but not activate TGFβ. Although this study primarily used colon adenocarcinoma cells, keratinocytes were also shown to activate TGFβ1 in a similar fashion (11). The LAP of TGFβ3 (LAP-3) also contains an RGD sequence, and is similarly activated (12), whereas TGFβ2 does not contain an RGD sequence and is not activated by αvβ6.

Table 1 Tumour-promoting effects of αvβ6

Function	*Comment*	*Cell type*	*Reference*
Promotion of migration and invasion	Promotion of migration over FN, VN, LAP	Keratinocytes, OSCC	Huang et al. (10) Thomas et al. (13) Thomas et al. (66)
	Promotion of invasion modulated through upregulation of MMP-9	OSCC	Thomas et al. (54, 55)
	Promotion of invasion modulated through upregulation of MMP-3	OSCC	Ramos et al. (53) Li et al. (58)
	Upregulation of uPA	Ovarian carcinoma	Ahmed et al. (46)
	TNFα-dependent upregulation of αvβ6 associated with increased migration and upregulation of MMP-9	Keratinocytes	Scott et al. (15)
Activation of TGF-β	Binds and activates latent TGF-β1	Colon carcinoma, Keratinocytes	Munger et al. (11)
	Binds and activates latent TGF-β3	Colon carcinoma	Annes et al. (12)
	Promotes TGF-β-dependent EMT, risk factor for early stage disease, poor prognostic marker	Colon carcinoma	Bates et al. (48)
Promotion of proliferation & generation of survival signals	Upregulation of αvβ6 protects cells from anoikis by activating an AKt-dependent survival signal	OSCC	Janes & Watt (24)
	αvβ6 promotes proliferation through C-terminal 11 amino acids of β6 subunit	Colon carcinoma	Agrez et al. (59)

Table 2 Ligands of αvβ6

Ligand	*Type of protine*	*Reference*
Fibronectin	ECM protien	Busk et al. (8)
Tenascin-C	ECM protien	Prieto et al. (9)
Vitronectin	ECM protien	Huang et al. (10)
LAP of TGFβ-1	Cytokine	Munger et al. (11)
LAP of TGFβ-3	Cytokine	Annes et al. (12)
Foot-and-mouth disease virus (FMDV)	Viral capsid	Miller et al. (95)
Coxsackievirus 9 (CAV-9)	Viral capsid	Williams et al. (96)

The TGFβs modulate numerous processes including cell growth, inflammation, matrix synthesis and apoptosis (18). Defects in TGFβ function are associated with a number of pathological conditions including autoimmune disease, and tumour cell growth (19). Additionally, TGFβ expression is increased in numerous fibrotic conditions (20), which appear to be modulated, in part, through the TGFβ-driven trans-differentiation of fibroblasts into myofibroblasts. These contractile, secretory cells lead to a net accumulation of ECM with consequent scarring (21, 22). Consistent with this, αvβ6 dependent activation of TGFβ has been shown to be pivotal in mouse models of pulmonary and renal fibrosis, suggesting that this mechanism may be of general importance in fibrosis in multiple epithelial organs (23).

αvβ6 generates survival signals

When tumour cells migrate away from their origin the physiological ECM cues (which provide survival signals necessary for all normal cells) are likely to differ from those of the original tissue. Thus, tumour cells must develop survival strategies in these foreign tissue sites. Janes and Watt studied the switch from αvβ5 to αvβ6 that occurs naturally when keratinocytes transform from normal to malignant (24). These authors reported that *de novo* expression of αvβ6 protects OSCC from anoikis (i.e. apoptosis caused by lack of appropriate ligand binding) by upregulating Akt, and proposed that upregulation of αvβ6 in SCCs allows growth of tumour cells in the absence of a basement membrane, representing a novel way in which αvβ6-upregulation contributes to cancer progression (24). It has not yet been established that non-transformed keratinocytes also are protected from anoikis when they transiently upregulate αvβ6 during wound healing but intuitively, this seems a likely response.

Thus, αvβ6 promotes adhesion and migration on several different ECM ligands, promotes increased MMP secretion, can activate TGFβ1 and TGFβ3 and promotes the survival of OSCC cells. Let us now review when and where αvβ6 is expressed *in vivo* and suggest which functional processes are important.

Expression of αvβ6 in normal and wound keratinocytes

In skin epidermis, integrin expression is confined to the basal layer of keratinocytes [reviewed in (25)]. In contrast, integrin expression in oral mucosa often is also found in the suprabasal epithelial layers, sometimes as high as the prickle cell layer, possibly reflecting the increased turnover of oral epithelium (26, 27). Oral stratified squamous epithelium shows strong expression of the integrins α6β4, α2β1, α3β1, all of which probably contribute to the maintenance of a stable tissue structure (26–29). In addition, a weaker, more variable expression of α5β1, α9β1 and αvβ5, is also present (26–29); however the fact that these integrins may be upregulated during wound healing may implicate them in this process.

αvβ6 is interesting in that it is not expressed constitutively in healthy epithelia, but is upregulated during tissue remodelling, including wound healing and carcinogenesis (30). Several studies on human and animal wounds have shown that that β6 mRNA is detectable in keratinocytes at the wound edge (30–32). Although αvβ6 was expressed by migrating keratinocytes in early wounds, maximal expression was seen relatively late

during mucosal and dermal wound healing, when migrating edges of the wound epithelium have joined (32). *In vitro* studies have shown that αvβ6 facilitates keratinocyte adhesion and migration on fibronectin, tenascin and vitronectin, all of which are components of the early wound matrix (8, 33, 34). Additionally, αvβ6-dependent upregulation of the type IV collagenase MMP-9 would facilitate cell movement by allowing detachment from the basement membrane (13). These data suggest that αvβ6 has the potential to promote migration of epithelium across a wound surface. However, several studies reported that expression of the αvβ6 protein occurs at a later stage, and is maximal when epithelial integrity has been restored (31–33). Recently, Hakkinen (35) used immunohistochemistry to show that expression of αvβ6 in murine skin was strong and relatively uniform on most basal keratinocytes close to the wound edge 3 days after insult and still strongly expressed, although less uniform, at initial wound closure.

Although the *de novo*, but transient, expression of αvβ6 by wound keratinocytes is well documented, the molecular mechanisms leading to its expression and eventual disappearance are still unclear (31, 32). The correlation of coincident increase in αvβ6 and tenascin-C (TN-C) expression has led to the suggestion that the principal functions of αvβ6 in wound healing may be modulated through an interaction with this matrix protein (31–33). TN-C is also a ligand for α9β1, which is upregulated in the early stages of wound healing (33) but down-regulated in the later stages, coinciding with the induced expression of αvβ6. The finding that α9β1 expression in cells plated on TN-C induces proliferation whereas expression of αvβ6 has the opposite effect (36) suggests that the switch between α9β1 and αvβ6 expression in wound epithelium may be a mechanism for regulating cell responses during epithelial regeneration. Thus data from human studies suggest that the principal role of αvβ6 in wound-healing may not be related to initial cell migration of keratinocytes but to late events associated with wound-resolution (31, 32). It is possible that the differences in the detection of αvβ6 in mouse vs. human wound tissues is because of species differences, the difference between oral vs. skin wounding, or possibly the use of different anti-αvβ6 antibodies. At the present time, it is not known whether either or both mechanisms (i.e. αvβ6-dependent migration and αvβ6-dependent resolution) are involved in human wound healing in the oral mucosa.

It is possible that the principal role of αvβ6 in oral and skin wound healing is to control temporally the activation of TGFβ which has an important role in wound repair by regulating re-epithelialization, suppression of inflammation and by promoting connective tissue regeneration and scar formation (37). To investigate the role of αvβ6 in cutaneous wounds, transgenic mice were generated which either lack, or constitutively express β6. The β6-/- mice do not have a reduced healing rate nor do they show altered wound morphology (38), possibly because the keratinocytes may express other receptors for fibronectin and tenascin (such as α5β1 and α9β1, respectively). However, due to the lack of the immunosuppressive effect of αvβ6-activated TGFβ,β6-/- mice show exaggerated inflammation of the skin in response to injury (38). Interestingly, these inflammatory infiltrates were mainly composed of macrophages resembling those found in the TGFβ1 null transgenic mouse (39).

As maximal expression of αvβ6 in humans occurs at a late stage of wound healing it is possible that expression at an earlier stage may alter the rate of repair or morphology of the wound. To investigate this possibility, Hakkinen et al. (35) created a transgenic mouse model with constitutive αvβ6 expression under the control of a cytokeratin 14 promoter. Similar to the β6-/- mouse, the rate of wound closure was unaltered and the mice healed without significant scarring (38). Levels of TGFβ in healthy skin were similar between control and over-expressing animals (35). However, during breeding, the β6 transgenic mice developed spontaneous, fibrotic chronic ulcers of the skin. These lesions contained numerous activated fibroblasts and macrophages and expressed higher levels of TGFβ than corresponding normal skin from the same animals or from control wild-type mice (35). It appears that only in a compromised healing situation, involving chronic inflammation or perhaps immunosuppression, does β6-modulated fibrosis and ulceration occur.

It is worth noting that wound healing and carcinogenesis have many biological processes in common, such that carcinogenesis has been described as a mis-regulated form of wound healing (40). Thus the advancing edge of a wound and the invasive edge of a carcinoma require co-ordinated adhesive and de-adhesive processes to promote motility of a migrating front. Many of the ECM ligands for αvβ6 are usually modulated and often upregulated during both tissue remodelling and cancer (41). Specific proteases are required for both processes [reviewed in (42, 43)]. Furthermore, as discussed below, most OSCC cells express high levels of αvβ6 (30, 44, 45). However, unlike wound healing, carcinoma αvβ6 appears to be permanently 'switched on' and may be responsible for promoting tumour progression (discussed below).

Expression of αvβ6 in carcinomas

Although expression of αvβ6 is restricted to carcinomas, it is not restricted to oral and skin SCC. To date αvβ6 expression has been reported in carcinomas of the lung, breast, pancreas, stomach, colon, ovary, salivary gland as well as oral and skin squamous cell carcinoma (27, 30, 46–51; Table 3).

Ahmed et al. (46), immunostained 45 ovarian carcinomas for αvβ6 and found 100% positivity. Staining intensity correlated with tumour grade suggesting that a gradual increase in the expression may be a correlative index of the progression of this disease. In a small study Kawashima (49) showed that 18 of 38 (47%) gastric carcinomas expressed αvβ6 at the mRNA level and, interestingly, expression correlated with metastasis to locoregional lymph nodes. More recently, Bates et al. (48)

Table 3 αvβ6 expression in carcinomas

Carcinoma	*Reference*	*Number of carcinomas*	*% of positive tumours*	*Evidence*	*Comment*
Oral SCC	Breuss et al. (30)	30	90	ISH	Absent αvβ6 expression in normal oral mucosa from same patients
	Jones et al. (27)	17	100	IHC	
	Hamidi et al. (44)	5	80	IHC	41% expression in oral leukoplakia peri-tumoural dysplasia also αvβ6 +ve
	Impola et al. (97)	11	100	ISH	Expression maintained in LN mets
	Regezi et al. (45)	40	100	IHC	αvβ6 expression colocalized with TN-C peri-tumoural dysplasia also αvβ6 +ve
Colon	Bates et al. (48)	488	37	IHC	Poor prognostic marker, αvβ6 expression maintained in mets
Pancreas	Sipos et al. (50)	34	100	IHC	Well differentiated tumours expressed more than poorly differentiated
Gastric	Kawashima et al. (49)	38	47	IHC, RT-PCR	94% αvβ6 +ve carcinomas had LN mets
NSCLC	Smythe et al. (98)	51	50	IHC	αvβ6 +ve carcinomas well-differentiate and node negative; ? Good prognostic marker
Breast	Arihiro et al. (47)	90	18	IHC, WB	No grade 1 tumours αvβ6 +ve
Ovary	Ahmed et al. (46, 61)	45	100	IHC	Staining correlated with grade; Benign mucinous tumours also αvβ6 +ve

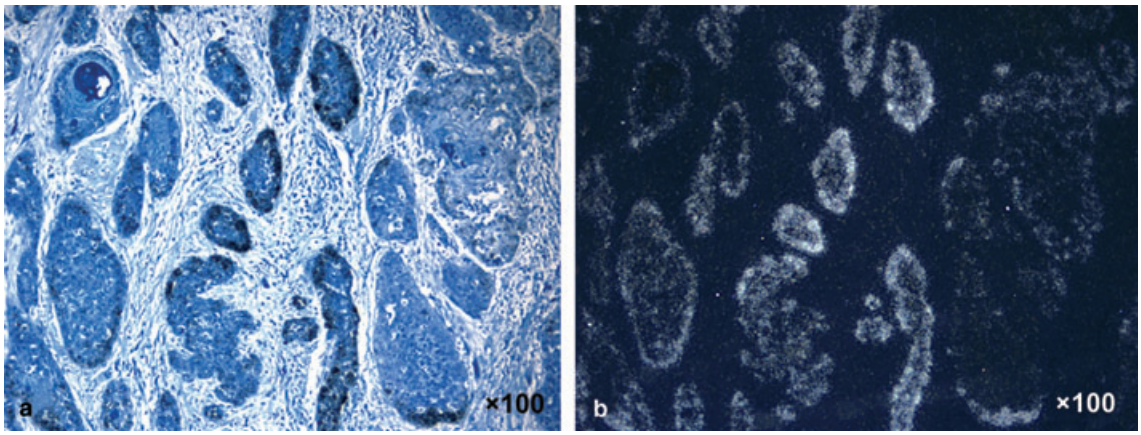

Figure 3 β6 expression in oral squamous cell carcinoma. *In situ* hybridization showing β6 RNA expression in oral squamous cell carcinoma. The figure shows a bright field image (a) and a dark field image (b). β6 RNA was detected throughout the tumour but was often most concentrated at the periphery of tumour islands.

examined 488 clinical samples and reported that strong expression of αvβ6 is a prognostic indicator in colorectal carcinoma. Furthermore, αvβ6 has also been detected in approximately 18% of breast carcinomas (47).

A high level of αvβ6 expression has been described most consistently in OSCC (Fig. 3), which has been shown in a number of studies. The first study examined 30 OSCC samples for β6 expression using *in situ* hybridization (30). Although undetectable in normal oral mucosa, strong β6 expression was found in 27 of 30 tumours where it often was concentrated at the invading edge of tumour cells. More recently, Impola et al. (52), also examined αvβ6 expression in 11 OSCC samples using *in situ* hybridization and found that β6 mRNA was detectable in 100% of tumours although expression did not relate to tumour differentiation, but was maintained in lymph node metastases. In another study, αvβ6 was strongly expressed in 90% of OSCC samples but was undetectable in normal oral mucosa (44). Interestingly, these authors demonstrated αvβ6 positivity in 41% of leukoplakia specimens where expression correlated with progression to malignant disease (44). These data suggest that αvβ6 expression may be useful in predicting malignant transformation and also that αvβ6 expression may play an active role in this process. Indeed, Bates et al. (48) showed that epithelial-to-mesenchymal transition of colon cells was associated with increased expression of αvβ6. However, epithelial cells in samples of lichen planus (a chronic mucocutaneous disease) have also been shown to express αvβ6 suggesting that αvβ6 expression *per se* is insufficient to drive malignant progression (44). Regezi et al. (45) examined floor of mouth carcinomas and found that β6 was expressed by keratinocytes in all *in situ* and invasive lesions; moreover, expression was often most intense at the invasive front of the tumour. As in previous studies, αvβ6 was not detectable in normal epithelium, but was shown to colocalize with tenascin (45). Ramos and colleagues demonstrated that αvβ6 was also expressed in dysplastic epithelium prior to invasive change and concluded that transgression of the basement membrane required additional molecular changes (53).

αvβ6 promotes oral cancer

The consistent observation that αvβ6 is undetectable in normal oral tissues but is upregulated in transformed lesions, particularly at the invasive front, is consistent with the proposal that αvβ6 promotes invasion of oral carcinoma. We examined the role of increased αvβ6 in OSCC cells by using retroviral transduction of β6 cDNA to create the OSCC cell line, VB6, expressing high levels of αvβ6 (54, 55). This cell line was significantly more invasive through Matrigel® in Transwell® invasion assays than the control line C1 (transfected with the empty vector alone and expressing low levels of αvβ6). The mechanism for the increased invasion was, in part, through αvβ6-dependent upregulation of the type IV collagenase MMP-9 (and to a lesser extent MMP-2). This activity was modulated through the unique terminal 11 amino acids of the β6 tail; thus if these amino acids were removed both invasion and MMP-9 levels were reduced to control levels (56). In a similar study in colon carcinoma cells αvβ6 has been shown to regulate MMP-9 by a process modulated through the extracellular-regulated kinase (ERK) binding to the β6 cytoplasmic tail (57).

Using a similar approach, Ramos et al. (53) retrovirally infected the poorly invasive SCC9 cell line with β6 cDNA and showed that αvβ6 expression correlated with OSCC invasion and tumour growth *in vivo* and *in vitro*. However, in this study the increased invasion was modulated through MMP-3 rather than MMP-9. Additionally, over-expression of αvβ6 promoted cell growth and altered deposition of fibronectin matrix. A more recent study, using the same cell line, suggested that the signalling pathway regulating these processes involved the β6-dependent activation of the tyrosine kinase Fyn (58). The integrin αvβ6 also has been described as promoting the growth of colon adenocarcinoma cells grown in 3-D culture *in vitro* and as xenografts *in vivo*, an effect that in both situations required the presence of the C-terminal 11 amino-acids of β6 (59).

Serine proteases are also modulated by αvβ6 expression. Dalvi et al. (60) showed that overexpression of αvβ6 in OSCC cells was associated with a transcriptional reduction of the uPAR receptor, which was modulated through the C-terminal 11 amino acids of β6. In a different model, high αvβ6 expression correlated with elevated uPA, uPAR and MMP-9 in ovarian carcinoma cell lines (61).

The activation of TGFβ through association of αvβ6 with LAP may initially seem to contradict the hypothesis that αvβ6 promotes tumour progression as the growth inhibitory effects of this cytokine are well described [reviewed in (62)]. However, the role of TGFβ in tumour biology is complex, involving several signalling pathways, and a number of studies have demonstrated that TGFβ1 may be pro-oncogenic, driving malignant progression, invasion and metastasis (63, 64). It is now suggested that TGFβ has biphasic effects during tumourigenesis, initially acting as a tumour suppressor, but later stimulating cancer progression (63, 65). We have shown previously that binding of αvβ6 to the TGFβ1 latency associated peptide (LAP) promotes cell migration and MMP-9 expression, and also that cells will invade towards soluble LAP in an αvβ6-dependent manner (66). The interaction between αvβ6 and TGFβ in SCC therefore appears complex. On the one hand, αvβ6 may activate TGFβ1, with a growth inhibitory effect, while on the other hand, LAP is capable of modulating cell movement and protease production if present in suitable form. The net effect on tumour behaviour is likely to depend on the stage of tumour development. Growth inhibition may be dominant in the earlier stages of carcinogenesis with αvβ6-activated TGFβ1 acting as a tumour suppressor. However, in the later stages of tumour development, as cells become refractory to growth inhibition, then the role of LAP in promoting both αvβ6-dependent cell movement and MMP-9 expression may become prominent, culminating in a pro-oncogenic effect of TGFβ. Another potential pro-tumourigenic effect of TGFβ is by activating the surrounding stroma. We have shown that tumour-derived activated TGFβ is sufficient to trans-differentiate fibroblasts to myofibroblasts which in turn promote OSCC invasion although secretion of HGF (67).

The integrin αvβ6 as a target for imaging and therapy

A molecule that is suitable for targeting with therapeutic agents should be (i) expressed on the cell surface, (ii) have little or no expression on normal tissues and (iii) be expressed at levels significantly higher than surrounding normal tissues. The integrin αvβ6 fulfils all these criteria and should be considered a novel target for the imaging of oral cancer. As αvβ6 also is implicated as a major contributor to oral cancer progression, this integrin should also be considered as a potential therapeutic target.

Targeting αv integrins for imaging cancer has been reported previously by Haubner et al. (68) who used [^{18}F]-labelled peptides to target αvβ3 on melanoma cells using positron emission tomography (PET) for imaging in small animals. They concluded that [^{18}F]-labelled peptides were suitable compounds for the non-invasive determination of αvβ3 integrin status and could be used for monitoring of therapy (68). These results are encouraging, and serve as a proof-of-principle for targeting of integrins on cancers. Moreover, αvβ6 upregulation is seen commonly in many carcinomas, unlike expression of αvβ3 which is largely restricted to melanoma (69) and glioblastoma (70); thus successfully developing imaging strategies for αvβ6 is likely to have a much greater clinical impact.

αvβ6-specific probes are required for effective targeting of αvβ6-positive tumours and potential biopharmaceuticals include monoclonal antibodies, single chain Fv antibodies (scFv), peptides or peptide mimetics and viral vectors; each has advantages and disadvantages.

Monoclonal antibodies have inherent specificity, thus are ideal targeting agents and can be used to deliver radionuclides, cytotoxic drugs or biological toxins to cells expressing the antigen of interest. However they are relatively large in size (approximately 150 kDa) with

slow clearance from the circulation, resulting in significant exposure to normal organs whilst poor penetration of tumour vasculature limits delivery to the tumour (71). Monoclonal antibodies are also immunogenic, promoting human anti-mouse antibody (HAMA) responses, limiting their potential clinical application [reviewed in (71–73)]. This problem can be minimized by 'humanizing' the antibody in order to reduce the immunogenic components (72) and such humanized monoclonal antibodies have become widely used in cancer therapeutics (74).

Single-chain Fv recombinant proteins are prepared by connecting genes encoding heavy chain and light chain variable regions of immunoglobulins at the DNA level using an oligonucleotide linker. These fragments are approximately 25 kDa in size and have better tumour penetration and faster clearance than whole immunogobulins but, being monovalent, have lower affinity. Thus affinity maturation and oligomerization are often required to generate multivalent forms of scFv molecules, optimizing their affinity [reviewed in (75, 76)]. A significant advantage of scFv is that they can be cloned easily into a human IgG 'backbone' to create a humanized antibody, ready for therapy (71) or, indeed, some scFv libraries have been constructed that are entirely of human immunoglobulin sequences (77).

Peptides represent another approach to biopharmaceutical targeting and have the advantage that they have low immunogenicity owing to their usually small size, but are cleared rapidly from the circulation (78). For targeting, the affinity of the peptide would have to be high enough to generate a rapid and significant (i.e. easily measurable) signal:noise ratio in order to discriminate the target tissue. Such peptides do exist (e.g. octreotide targets somatostatin receptors) and allow imaging to occur within hours of injection (79). However, the high excretion rate of peptides would preclude their use for integrin 'blocking' therapy as it would require continuous administration. In addition, peptides may require modification (e.g. cyclisation) to protect them from serum peptidases (80). Another modification of peptides that can improve their pharmacokinetic profile is pegylation [the technology of polyethylene glycol (PEG) conjugation] which prolongs the circulating half-life, increases solubility and masks antigenic sites on the peptide from immune detection [reviewed in (81)].

A therapeutic precedent exists for using αvβ6 as a therapeutic target, as two αv-binding antagonists already have found a place in the oncologist's armamentarium. Vitaxin, a humanized monoclonal antibody to αvβ3, and cilengitide, a cyclic peptide mimicking the RGD ligand recognition peptidic domain common to αv integrins, are in phase II clinical trials [(74, 82); reviewed in (83)]. Thus, unsurprisingly, pharmaceutical companies are now developing reagents specific to αvβ6. For example, Merck has developed small molecule inhibitors for αvβ6 (84) and BiogenIdec has developed αvβ6 inhibitory monoclonal antibodies (85). Although limited data exist, there is experimental support for the idea of αvβ6-directed therapy. Xue et al. (86) co-injected OSCC HSC-3 cells and anti-αvβ6 antibody into the floor of the mouth of nude mice. Ten days after injection 100% of control animals (18 of 18) had formed tumours but only 40% of the αvβ6-treated mice (eight of 20). These same authors also investigated systemic administration of an αv inhibitory antibody (which inhibited all αv integrins and was not specific to αvβ6) and found that although the antibody was inefficient at inhibiting early tumour growth at 10 days, after 38 days the tumours in the treated animals were 40% smaller than in the control group (86). Importantly, systemic administration of a drug that inhibited αvβ6 had no detrimental effect on the animals, giving increased hope that such an approach may be useful in humans. To date there have been no published studies that have targeted αvβ6 for tumour imaging but this is likely to change in the foreseeable future.

Another promising approach is the use of viral vectors for therapy of cancer [reviewed in (87)] and perhaps the most advanced programmes utilize adenoviral vectors for gene therapy (88). Adenoviruses enter cells by a combination of initial binding to cell-surface Coxsackie-Adenovirus-Receptors (CAR), followed by integrin-mediated internalization, usually via αvβ3 and αvβ5 (89). Using molecular modifications, adenoviruses have been developed which target specific integrins (90) and thus a similar strategy may be possible to modify such vectors to recognize αvβ6 specifically and deliver therapeutic genes to oral cancers.

Regulation of αvβ6 expression

In addition to using αvβ6 as cancer-specific 'beacon' to direct imaging or targeting agents, a clearer understanding of how expression of this integrin is regulated may also provide novel targeting strategies. The exact mechanisms by which αvβ6 expression is induced in wound keratinocytes, or the mechanisms that result in its disappearance once the wound is healed, are unknown. However, if such information were available it may be possible to design tissue-specific strategies to down-regulate αvβ6 in cancers and thereby reduce the invasive activity of tumour cells.

Keratinocytes are known to be 'activated' when isolated from epidermis and placed in culture [reviewed in (91)] where they are believed to resemble wound keratinocytes. Freshly isolated epidermal keratinocytes do not express αvβ6 until subcultured; however, αvβ6 is detectable from the first passage (32). We have found that cultured oral primary keratinocytes similarly express relatively high levels of the integrin (13). The ready expression of αvβ6 in cell culture makes the study of its induction difficult. Niu et al. (92) reported that high cell density selectively enhanced αvβ6 expression in colon carcinoma cells, and suggested that this effect was mediated through protein kinase C signalling. The strong expression of αvβ6 in wounds only after epithelial integrity is restored, is consistent with the possibility that cell–cell contact may be important for upregulating the integrin. Interestingly, mutating the β6 cytoplasmic domain by removing the terminal 11 amino acids

prevents the density-dependent upregulation of αvβ6 and replaces it by upregulating αvβ5, further suggesting that the expression of these integrins is linked and expression may be switched from one to the other (57).

It has been reported that TGFβ1 induces *de novo* expression of αvβ6 in normal human keratinocytes (93) and on the non-transformed keratinocyte cell line, HaCaT (34). More recently, Scott et al.(15) used TNFα-deficient mice to demonstrate that αvβ6 is upregulated by TNFα. TNFα-/- keratinocytes expressed significantly less αvβ6 than wild-type keratinocytes, but both upregulated αvβ6 when treated with exogenous TNFα. In support of this, oligonucleotide microarray analysis of skin keratinocytes treated with TNFα showed approximately more than sevenfold increase in αvβ6 expression 48 h after treatment (94). The molecular basis of this cytokine-mediated regulation of αvβ6 is beginning to be resolved. Recently, Bates et al. (48) reported that TGFβ worked synergistically with TNFα to upregulate αvβ6 expression in colon cells by upregulating the Ets-1 transcription factor which bound to a site within 1 kb upstream of the β6 transcription start site. As both TGFβ and TNFα are commonly detected in cutaneous and oral wounds, this may be part of the mechanism controlling αvβ6 induction. However, additional research is necessary to confirm the generality of the TGFβ/TNFα/Ets-1 relationship to αvβ6 induction.

In summary, an increasing number of studies have described expression of αvβ6 in a high percentage of OSCC. Although, the data at present have been generated principally from *in vitro* studies, there is strong evidence to suggest that αvβ6 promotes OSCC progression. At the present time, surgery remains the treatment of choice for most OSCC. Neither radiotherapy nor chemotherapy is tumour cell specific and both cause damage to normal tissues. As the ideal cancer treatment would be based on molecular differences between normal and tumour cells, permitting greater targeting specificity, it is possible that αvβ6 may represent such a therapeutic target.

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Acknowledgements

We thank R Poulson (CRUK, Lincolns Inn Fields, London) for the beta 6 *in situ* hybridisation. Research has been supported by Cancer Research UK, the Health Foundation, UK Medical Research Council and by grants from DebRA and AICR. MN is an MRC Clinical Training Fellow and GJT is a Health Foundation Senior Clinical Fellow.

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