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Defining the Role of Integrin $\alpha v \beta 6$ in Cancer

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Abstract

Integrins are a large family of heterodimeric transmembrane receptors that mediate cell-substratum adhesion. $\alpha v \beta 6$ is an epithelial-specific integrin that is a receptor for the extracellular matrix (ECM) proteins fibronectin, vitronectin, tenascin and the latency associated peptide (LAP) of TGF- β . Integrin $\alpha v \beta 6$ is not expressed in healthy adult epithelia but is upregulated during wound healing and in cancer. $\alpha v \beta 6$ has been shown to modulate invasion, inhibit apoptosis, regulate the expression of matrix metalloproteases (MMPs) and activate TGF- β 1. There is increasing evidence, primarily from *in vitro* studies, that suggest that $\alpha v \beta 6$ may actually promote carcinoma progression. In this review we summarize what has been learnt in the past few years about the role of $\alpha v \beta 6$ in cancer progression.

Keywords

Integrin $\alpha v \beta 6$; cancer; MMPs; TGF- β 1; EMT

INTRODUCTION

Integrins are heterodimeric trans-membrane receptors comprising alpha and beta subunits that function both as cell anchoring and signaling molecules [1]. There are 18 alpha and 8 beta subunits, each of which can bind to several partners giving rise to at least 24 distinct integrin heterodimers with different functions and ligand binding activities (Fig. (1)). Integrins serve as anchoring molecules by mediating the adhesion of the cellular cytoskeleton to the extracellular matrix (ECM). They also serve as bidirectional signaling molecules by mediating outside-in and inside-out signaling thereby controlling a variety of vital cell functions like adhesion, polarity, differentiation, migration and cell division [2]. These cell functions are critical during embryogenesis and help to maintain tissue homeostasis under normal physiological conditions. Under pathological states, defective integrin signaling can disrupt these functions resulting in abnormal cell division, migration and adhesion, which are hallmarks of cancer and metastasis [3,4].

The integrin $\alpha v \beta 6$ is unique in that it is expressed exclusively in epithelial cells, and $\beta 6$ partners only with αv forming a single heterodimer. During embryogenesis $\alpha v \beta 6$ is expressed at high levels in the developing lung, skin and kidney epithelia and its expression is downregulated in healthy adult epithelia [5,6]. However recent unpublished data from our laboratory suggest that $\alpha v \beta 6$ is expressed at high levels in the adult hair follicles (C. MaGraw and S. Raghavan, unpublished observations).

The KO of $\beta 6$ integrin has revealed an unexpected role for this epithelial integrin in downregulating local inflammation [7]. In the KO mice there is an increase in the number of

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macrophages in the skin that is associated with juvenile baldness. In the lungs there is a persistent accumulation of activated lymphocytes which results in an exaggerated response to acetylcholine (a bronchoconstrictor). These results suggest that $\beta 6$ may have important anti-inflammatory effects and interventions targeting this integrin could affect the course of inflammatory disorders of the lungs and skin.

The expression of $\alpha \beta 6$ is highly upregulated during wounding with an elevated expression observed at the wound edge within a few days of wounding [5,8,9]. This expression is maintained until wound closure is complete. Interestingly, Huang *et al.*, [10] showed that the $\beta 6$ KO keratinocytes could not migrate as efficiently on fibronectin or vitronectin compared to the WT keratinocytes suggesting an important role for this integrin in cell migration. Additionally integrin $\alpha \beta 6$ is highly upregulated in carcinomas of the breast, lung, oral and skin squamous cell carcinomas (SCC), colon, stomach and endometrium among others (see Table 1). Table 1 lists the different types of cancers that have been associated with $\alpha \beta 6$ and the expression of $\alpha \beta 6$ in them. In summary, $\alpha \beta 6$ functions to regulate epithelial remodeling during development, tissue repair, and neoplasia [5].

In this review we focus on the signaling pathways that have most often been associated with an increased expression of $\alpha \beta 6$ in different cancers. We anticipate that a better understanding of the signaling cascade downstream of $\alpha \beta 6$ will help us determine how elevated levels of $\alpha \beta 6$ lead to the spread and development of cancer.

Expression of $\alpha \beta 6$ in Different Cancers

It is a well-established fact now that there is a wide prevalence of $\alpha \beta 6$ -integrin expression in different kinds of cancer (table 1). The expression of $\alpha \beta 6$ is often associated with a poor prognosis [11]. Integrin $\alpha \beta 6$ seems to promote cell invasion and migration both of which are involved in metastasis. The signaling mechanisms that are operative in the presence of an increased expression of $\alpha \beta 6$ in these cancers still remain to be elucidated. There are many lines of evidence that suggest that the activation of TGF- β pathway is an important signaling pathway that is involved in the process [12]. Nevertheless more detailed studies remain to be performed as each cancer type represents a unique cellular context.

Basal Cell Carcinomas (BCCs) are one of the most prevalent forms of skin cancer. BCCs arise due to the deregulation of the Sonic hedgehog (Shh) pathway and the activation of its downstream transcriptional effectors, Gli 1 and 2 [13]. BCCs can be nodular or morphoeic, the former being slow growing and low risk, and the latter having an infiltrative growth pattern and being aggressive. Recently Marsh *et al.*, showed that while $\alpha \beta 6$ was expressed in the low-risk BCCs, its expression was markedly upregulated in the morphoeic variants [14]. Using a BCC model (generated by transfecting Gli1 & Gli2 into NTERT human keratinocytes) the authors showed that the increased expression of $\alpha \beta 6$ promoted trans-differentiation of stromal fibroblasts into myofibroblasts that was accompanied by a paracrine secretion of hepatocyte growth factor/scatter factor (HGF/SC) by the myofibroblasts. This aided the invasion of the BCC cells. Thus increased expression of $\alpha \beta 6$ promoted BCC invasion by modulation of the stroma. The TGF- $\beta 1$ pathway was also activated by $\alpha \beta 6$ during the process.

In colon cancer (the second highest cancer causing mortality), high $\alpha \beta 6$ expression is associated with a more aggressive disease outcome [15]. In colon cancer models, at the leading edge of tumors, EMT accompanied by a loss of E-Cadherin was seen [16]. The TGF- $\beta 1$ pathway was reported to be activated, which in turn promoted increased invasion of cancer cells through the stroma.

The ability of colon cancer cells to metastasize in the liver has long posed a problem in the cure and control of colon cancer itself. Investigations into the cause of such a phenomenon

have shown that the expression of $\alpha\text{v}\beta 6$ in colon cancer cells was accompanied by the elevated MMP9 expression at the invading edge of the tumor [17]. MMP9 can degrade collagen, one of the chief components of the basement membrane and thus chew through the barrier that would otherwise restrain migratory cells. Also in the liver, in the sites of metastasis, tenascin C and fibronectin which are the ligands of $\alpha\text{v}\beta 6$ are expressed [17]. Integrin $\alpha\text{v}\beta 6$ could probably bind to these ligands and trigger many downstream signals, which lead to carcinogenesis.

Metalloproteases play a pivotal role in maintaining tumor microenvironment by actively remodeling the ECM, which is required for invasion and migration. In OSCC (oral squamous cell carcinoma) it has been shown that increased $\alpha\text{v}\beta 6$ expression leads to the activation of MMP3 which is responsible for enhanced cell migration and invasion on fibronectin. A series of signaling events are orchestrated after the binding of $\alpha\text{v}\beta 6$ to its ligand, resulting in the activation of MMPs. While much remains unknown as to what those events might be, there have been some studies in this area. Li *et al.*, [18] have shown that the association of Fyn kinase with $\alpha\text{v}\beta 6$ results in its activation that in turn leads to the recruitment of FAK. The Raf-ERK-MAPK pathway is then activated that results in the MMP3 gene being transcriptionally activated. These series of events are responsible for the increased migration of OSCC cells with increased levels of $\alpha\text{v}\beta 6$ that then leads to the spread and development of the cancer. Recently Ramos *et al.*, [19] have also shown that by increasing expression of $\alpha\text{v}\beta 6$ in OSCC cells they could detect an EMT like event, which involved the expression of vimentin and down regulation of E-Cadherin.

Ahmed *et al.*, [20] reported the expression of $\alpha\text{v}\beta 6$ in ovarian cancer cell lines and ovarian cancer tissues. Integrin $\alpha\text{v}\beta 6$ was present in the cell lines in varying amounts ranging from high to moderate and low. Similarly the presence of $\alpha\text{v}\beta 6$ was also detected in ovarian cancer tissues. Integrin $\alpha\text{v}\beta 6$ was strongly expressed in Grade III tumors in comparison to Grade I tumors. Subsequently Ahmed *et al.*, [21] also showed that Grade III ovarian tumors had a strong expression of $\alpha\text{v}\beta 6$ throughout the tumor whereas in benign tumors the expression of $\alpha\text{v}\beta 6$ was restricted only to the epithelial cells at the edge of the tumor. Mucinous tumors had a stronger expression of $\alpha\text{v}\beta 6$ in comparison to the endometrioid or serous kind. In the ovarian cancer cells it was also shown that the increased expression of $\alpha\text{v}\beta 6$ was accompanied by the secretion of HMW-uPA (High molecular weight-urokinase plasminogen activator), MMP2 and MMP9 into the tumor conditioned media. In the absence of increased expression of $\alpha\text{v}\beta 6$ in the cells, there was marked reduction in the level of matrix degradation by uPA and MMPs. Thus $\alpha\text{v}\beta 6$ probably plays a role in the degradation of the ECM in concert with uPA and MMPs. Interaction of $\alpha\text{v}\beta 6$ with uPAR (urokinase plasminogen activator receptor) in ovarian cancer cells also delineates a hitherto unreported pathway that can promote cancer development. It has been shown in immunoprecipitation studies that $\alpha\text{v}\beta 6$ interacts with uPAR [22]. This interaction is responsible for the increased cell migration and ERK activation in ovarian cancer cells. It can be speculated that this interaction may have a role in localized degradation of the ECM that can promote cancer cell migration and invasion. Increased levels of TGF- $\beta 1$ have also been recorded in ovarian cancer cells and $\alpha\text{v}\beta 6$ is known to play a role in TGF- $\beta 1$ activation [23]. There is evidence to suggest that if $\alpha\text{v}\beta 6$ integrin blocking antibodies are used that block TGF- $\beta 1$ activation in these cells, then $\alpha\text{v}\beta 6$ -uPAR induced ERK activation is also abolished (as discussed by Saldhana *et al.*,) [22]. Thus it is possible that the $\alpha\text{v}\beta 6$ -uPAR interactions may have some role to play in the activation of TGF- $\beta 1$. The $\alpha\text{v}\beta 6$ -uPAR interaction is found only in ovarian cancer cells and not in normal ovarian cells, and thus depicts the specific protein interactions found only in the cancer cells as opposed to normal cells. Thus it is more likely that such interactions will demarcate specific signalosomes that lead the cell towards metastasis.

Sipos *et al.*, [24] showed that in gastroenteropancreatic adenocarcinoma, the expression of $\alpha\text{v}\beta 6$ was restricted to the tumor cells, with staining being mainly confined to the cytoplasm and very weak membrane staining. The highest expression of $\alpha\text{v}\beta 6$ was seen in the pancreatic ductal adenocarcinomas, followed by gastric carcinomas of the intestinal type and other intestinal adenocarcinomas. Additionally there was no correlation between the expression of $\alpha\text{v}\beta 6$ and its various ligands like fibronectin and tenascin C. Unlike other carcinomas like OSCC where the expression of $\alpha\text{v}\beta 6$ is at the leading edge of the tumor, in the case of gastroenteropancreatic adenocarcinoma the presence of $\alpha\text{v}\beta 6$ was poor and inconsistent at the leading edge of the tumors. Integrin $\alpha\text{v}\beta 6$ was strongly expressed in the well or moderately differentiated tumors in comparison to the poorly differentiated tumors. Thus, in contrast to its invasive role in OSCCs, in the context of the gastroenteropancreatic adenocarcinoma $\alpha\text{v}\beta 6$ seems to be involved in carcinoma differentiation.

Recently, Hetch *et al.*, [25] reported the expression of $\alpha\text{v}\beta 6$ in endometrial carcinoma. They showed that $\alpha\text{v}\beta 6$ was expressed in 42% of the endometrial carcinomas. Tumors with an infiltrative pattern of invasion expressed $\alpha\text{v}\beta 6$ at the leading edge. $\alpha\text{v}\beta 6$ expression was highly concurrent with metastasis. Thus in endometrial carcinomas, like in OSCCs, $\alpha\text{v}\beta 6$ seems to promote invasion which maybe correlated to the activation of matrix metalloproteinases (MMPs) and degradation of the ECM. Zhang *et al.*, [26] reported the expression of $\alpha\text{v}\beta 6$ in 36.7% gastric carcinoma samples and showed that expression was correlated with various stages of the cancer. More importantly they showed that the expression of $\alpha\text{v}\beta 6$ in gastric carcinoma predicted reduced survival rates. The accuracy of such predictions was very high especially with respect to early stage tumors. In studying the relationship between the expression of $\alpha\text{v}\beta 6$ and the progression of various cancers it seems that certain pathways, such as the MMP and TGF- $\beta 1$ are reiteratively activated. In this next section we discuss the activation of these pathways and others by integrin $\alpha\text{v}\beta 6$.

The Role of MMPs in $\alpha\text{v}\beta 6$ Mediated Cancer Progression

MMPs are a family of zinc-dependent metallo-endopeptidases that aid in the degradation of the components of the ECM [27]. Abnormal degradation rates often make cells more anchorage independent and lead to cancer. There have been numerous reports describing the role of $\alpha\text{v}\beta 6$ in the activation/inhibition of the MMP pathway in concert with its various ligands like fibronectin, tenascin-C, vitronectin and the latency associated peptide (LAP) of TGF $\beta 1$ or TGF $\beta 3$. Such signaling pathways have also been responsible under various conditions to enhance the progression of a malignant phenotype (Fig. (2)). Additionally abnormal degradation products often bind to the integrins and activate them resulting in altered signaling in the cells leading to cancer.

Al-Hazmi *et al.* [28] have reported a positive feedback loop for the activation of the matrix metalloproteinases, MMP2 and MMP9. MMP9 was shown to be responsible for the cleavage of fibronectin to produce the 120kDa cleaved fragment. Epithelial cells over expressing $\alpha\text{v}\beta 6$ showed enhanced migration on the 120kDa cleaved fibronectin fragment but not on the full-length fibronectin. This was accompanied by an increased expression of both MMP9 and MMP2 creating a positive feedback loop, which could play an important role in the development of cancer.

Fouchier *et al.*, [29] described the role of $\alpha\text{v}\beta 6$ in bone metastasis of cancer cells. Their studies showed the involvement of $\alpha\text{v}\beta 6$ in the adhesion of HT29-D4 adenocarcinoma cells to fibrinogen that had been processed by MMP9 in the presence of high concentration of Mn^{2+} . This process occurred via the activation of the Extracellular Signal Regulated Kinase (ERK)/Mitogen Activated Protein Kinase (MAPK) pathway and MMP9.

Morgan *et al.*, [30] demonstrated that the last 11 amino acids from the $\beta 6$ cytoplasmic tail (EKQKVDLSTDC) was able to activate MMP9 which could increase the invasiveness of OSCC cells. When this fragment of the $\beta 6$ cytoplasmic tail (EKQKVDLSTDC) was expressed in context of $\beta 3$ integrin, MMP2 was activated. Thus the 11 amino acid region of the cytoplasmic tail of $\beta 6$ integrin was able to activate different MMPs in the context of the different β subunits that it was associated with. Studies by Impola *et al.*, [31] identified MMPs 7, 9 and 12 as prognostic markers in the invasive fronts of squamous cell carcinomas along with the presence of a high levels of $\alpha v \beta 6$ integrin.

Thomas *et al.*, [32] showed for the first time that when $\alpha v \beta 6$ bound LAP, there was increased migration of OSCC cells via activation of MMP9. Interestingly, in contrast to bound (insoluble) LAP (which is a component of the ECM), soluble LAP downregulate $\alpha v \beta 6$ induced adhesion and migration of OSCC cells. Ramos *et al.*, [33] have shown that by over-expressing $\alpha v \beta 6$ in SCC9 lines they could make SCC9 lines more invasive and migrate faster on fibronectin. Fibronectin matrix assembly was down regulated and there was up regulation of MMP3. This highlighted the role of $\alpha v \beta 6$ in cancer progression by enhancing the degradation of the ECM by activating MMP3, thereby increasing the migration of cells and making tumors more invasive. Thomas *et al.*, [34] created a panel of SCC lines expressing different levels of $\alpha v \beta 6$ and showed an increased invasiveness of those cells due to the up regulation of MMP9.

Two independent groups have demonstrated that the ERK/MAPK pathway plays a role in the activation of MMPs by $\alpha v \beta 6$, either independently or in concert with the plasminogen receptor (uPAR) [20,35]. Li *et al.*, [18] demonstrated that upon binding to fibronectin the Fyn kinase was recruited to $\beta 6$ integrin which in turn recruited FAK to the focal adhesion complex activating the Raf-ERK-MAPK pathway. This leads to the transcriptional activation of MMP3 and provides a putative molecular mechanism, whereby increased expression of $\alpha v \beta 6$ enhances the metastasis in OSCC cells. Thus $\alpha v \beta 6$ seems to play a role in the activation of MMPs in various cancers. Even though detailed studies are still needed to highlight the exact molecular mechanism of such a phenomenon, to date the ERK/MAPK and TGF- $\beta 1$ pathways seems to be playing a role in the activation of MMPs by $\alpha v \beta 6$ in various cancers.

Activation of TGF- β by $\alpha v \beta 6$ Integrin

TGF- β family has 3 isoforms TGF- $\beta 1$, TGF- $\beta 2$ and TGF- $\beta 3$. Each of the TGF- β s is synthesized as homodimeric pro-TGF- β , which is comprised of TGF- β covalently linked to the latency associated protein (LAP). Pro-TGF- β is cleaved by furin-like enzymes inside the cell and remains attached to LAP in a non-covalent linkage forming the small latent complex (SLC). The TGF- β in this form is inactive and cannot bind to its receptor. It has to be released from this complex in order to be active. SLCs are found and secreted in very few instances. Mostly TGF- β is secreted as a large latent complex (LLC). The LLCs are formed by the association of latent TGF- β binding proteins (LTBPs) to the SLCs (TGF β /LAP) by disulphide covalent bonds [36]. LTBPs have 4 isoforms; LTBP1, LTBP2, LTBP3 and LTBP4. Other than LTBP3 all other LTBPs can bind to all isoforms of TGF- β . The LTBPs belong to the superfamily of fibrillin-like ECM proteins and bind other ECM proteins like fibronectin and vitronectin. Thus the LLCs provide a large pool of latent TGF- β in the ECM that is available when needed and can be activated accordingly. The activation of TGF- $\beta 1$ by $\alpha v \beta 6$ has been studied by various groups [32,37,38]. A common mechanism that has emerged from such studies is that after the binding of $\alpha v \beta 6$ to the RGD peptide present in the latency associated peptide (LAP) [that is associated with TGF- $\beta 1$], $\alpha v \beta 6$ binds to the actin cytoskeleton. This in turn triggers a conformational change in TGF- $\beta 1$ -LAP-LTBP1 complex (LLC). This releases TGF- $\beta 1$ from the LLC, which then binds to its receptor, thus activating the pathway (Fig. (3)) [36]. Integrin $\alpha v \beta 6$ cannot activate TGF- β bound to any other LTBP such as LTBP3. The different isoforms of the LTBPs have significant differences in the sequence of the hinge region, suggesting that

this region has a significant role in the specific association with and activation of TGF- β by various integrins.

TGF- β 1-LAP can bind other integrins like α v β 1 but cannot be activated by it [39]. Although both α v β 6 and α v β 1 can bind the TGF- β 1-LAP-LTBP1 complex, only α v β 6 is able to trigger the release of active TGF- β 1 from the LLC. One possible explanation for this could be that the actin cytoskeleton binding properties of α v β 6 versus α v β 1 maybe very different. It would be interesting to explore the exact mechanism of such a phenomenon and investigate the possibilities of different “signalosomes” at the cytoplasmic interface of the integrins, especially in context to ABPs (actin binding proteins), which influence the differential binding of these integrins to actin. It has also been shown that the ERK/MAPK pathways are involved in the upregulation of MMPs through the activation of the TGF- β 1 by α v β 6. Thus it is interesting that there is significant evidence in literature that actin cytoskeletal reorganization involving the ERK/MAPK pathways are implicated in the development of a malignant phenotype [40, 41]. The role of α v β 6 with respect to the development of a malignant phenotype through such means remains to be further investigated.

Role of α v β 6 Integrin in Epithelial to Mesenchymal Transition

Epithelial to mesenchymal transition (EMT) has been known to play a role in important physiological processes like embryonic development and wound healing. EMT is characterized by the loss of adherence of cells and increased motility. The downregulation of E-cadherin at the adherens junctions (AJs) is the hallmark of such a phenomenon and numerous molecular pathways involving various candidate genes have been implicated in the process. The role of EMT in the progression of cancer and in the development of metastasis by TGF- β has been explored, implicating the role of integrin α v β 6 in it [42]. There have been extensive studies conducted on the colon carcinoma model by Bates *et al.*, [15,16,42], and they have demonstrated that α v β 6 is up regulated in their EMT model. They found that β 6 mRNA was upregulated under the influence of the Ets1 transcription factor. Additionally TGF- β 1 was activated by the upregulation of α v β 6 in the EMT model and there was an increased release of soluble TGF- β 1 from the inactive TGFB1-LAP complex. They found that the increased levels of α v β 6 in this EMT model actually increased the migration of the cells on fibronectin. This demonstrated that α v β 6 was accelerating the processes that enhance metastasis. By analyzing around 500 patient samples the authors could correlate the poor prognosis of colon carcinoma patients with high expression of α v β 6. Interestingly, recently Ramos *et al.*, [19] have shown that increased expression of α v β 6 in OSCC cells triggered EMT accompanied by loss of E-cadherin and gain in vimentin expression. Importantly, the 11 amino acid cytoplasmic tail of β 6 integrin was shown to be responsible for this effect. However, the precise function of increased α v β 6 expression in the development of EMT in the various forms of OSCCs remains to be explored.

Role of Endocytosis in Cancer Progression

Recently there has been a report of the interaction of HAX-1 (HS1 associated protein) with α v β 6 [43]. In the study, the authors show that this interaction was responsible for the clathrin-mediated endocytosis of the receptor which was responsible for the promotion of the invasive behavior of the OSCCs. While there have been many reports showing the increased expression of α v β 6 in different cancers, there have been comparatively few insights into the exact mechanism by which this overexpression of α v β 6 results in progression of cancer. Recent data seem to implicate a role for endocytosis in cancer progression [44]. Evidence is emerging that endocytosis may play a pivotal role in the various forms of cancer by controlling the net signaling output of a cell at various stages. Receptors can undergo clathrin or non-clathrin mediated endocytosis whereby they may either be recycled to the membrane or sent to the lysosome for degradation. These kinds of fate determinations are often the defining points in

the cell-signaling pathways that control the development of cancers. The interaction between HAX-1 and $\alpha\beta6$ is perhaps the first evidence for endocytosis playing an important function in the genesis of cancer by a specific integrin.

Although there is increasing evidence that the overexpression of $\alpha\beta6$ in various forms of cancer is associated with a more aggressive phenotype, the signaling mechanisms underlying this phenomenon are only just starting to be elucidated. This area of research should be explored intensively as it will reveal interesting pathways causing cancer in different cells by $\alpha\beta6$. Such studies will pave the pathway towards the development of therapeutics to control and cure these cancers at an early stage.

Until recently, ERK was the only known protein directly interacting with $\alpha\beta6$ integrin, but now more candidates, like Fyn Kinase and HAX-1, have emerged. However, keeping in mind the repertoire of cancers where $\alpha\beta6$ is upregulated and the different pathways which may be linked to the cause of metastasis in each cancer, there appears to exist a goldmine of information still to be retrieved about the interacting partners of $\alpha\beta6$ integrin and their role in the cause and development of cancer.

$\alpha\beta6$ in Therapeutics of Cancer

Since $\alpha\beta6$ is not expressed in normal adult epithelia but only under special wound healing conditions and in cancers, it lends itself to be a very good molecular target for therapy. Targeting $\alpha\beta6$ should not affect adjoining normal tissues, a common problem in targeting specific molecules in the treatment of cancer. Integrin $\alpha\beta6$ is highly expressed in greater than 95% of OSCCs and thus imaging $\alpha\beta6$ in such tissues would serve as a very efficient means of imaging of the cancer tissue for the purposes of diagnosis and treatment. Hausner *et al.*, [45] have successfully designed a heavy lead peptide mimicking the normal $\alpha\beta6$ ligand. This peptide binds to $\alpha\beta6$ and could help identify the $\alpha\beta6$ tumors specifically in mice. Such peptides can be successfully used to treat OSCC during imaging in surgeries or diagnosis or chemotherapy for identifying distinct regions of treatment. US patent 7150871 claims that there was successful reduction of metastasis in lung carcinoma cells over expressing $\alpha\beta6$ when they were rendered the monoclonal antibody 10D5 that binds specifically to $\beta6$ integrin. An earlier finding showed the direct binding of ERK to the $\beta6$ subunit in colon cancer cells was responsible for the progression of the cancer [46]. The report hinted at this pathway being used in cancer therapeutics. It may be possible that the binding of 10D5 directly to $\beta6$ disrupts its binding to ERK and thus helps in the reduction of metastasis. This needs further probing by analyzing the cytosolic MAPK activity in such cells exposed to the 10D5 antibody. In fact a recent study in colon cancer cells by Zhou-Yang *et al.*, [47] revealed that apoptosis was enhanced when $\alpha\beta6$ function was blocked by the 10D5 antibody. The block in cell death was also accompanied by decreased phosphorylation of ERK. This could be an important pathway regulated by $\alpha\beta6$ integrin and could be targeted for therapy and small molecule inhibition studies. Kogelberg *et al.*, [48] have successfully designed a single chain humanized form of an antibody scFv B63 which can bind to $\alpha\beta6$ integrin and inhibit $\alpha\beta6$ mediated cell adhesion. This antibody has been designed from the viral coat protein-1, VP1 of the O1 strain of the foot and mouth disease virus. VP1 is known to be a high affinity ligand for $\alpha\beta6$. A peptide derived from VP1 was seen to exhibit $\alpha\beta6$ specific binding both *in vitro* and *in vivo*. A 17-mer peptide derived from VP1 was used to create the single chain antibody successfully which can be used to treat cancer invasion mediated by the $\alpha\beta6$ integrin.

In summary, there is very convincing evidence in literature correlating the severity of various forms of cancer with the expression of $\alpha\beta6$. These observations have been made in many cancers, including those of the lung, breast, colon, stomach and the ovary. We predict that detailed studies on the correlation of $\alpha\beta6$ expression with cancer progression and

understanding its molecular mechanism(s) will help generate molecular tools that could be used to identify specific stages of cancer in patients and in directing proper treatment.

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Fig. (1).
The 8 α and 18 β subunits form 24 distinct integrin heterodimers. Integrin $\beta 6$ can only partner with αv .

Fig. (2). Activation of different matrix metalloproteinases by $\alpha v \beta 6$ in various cancers. Activation of MMP9 has the highest prevalence.

Fig. (3).

The TGF- β 1 LLC. (A) TGF- β 1 is found at the cell membrane as a large latent complex (LLC). The LLC comprises of TGF- β 1, the latency-associated protein (LAP), and LTBP1 which belongs to the fibrillin superfamily. LTBP1 anchors the LLC to the ECM. TGF- β 1 is inactive in this form. (B) Activation of TGF- β by α v β 6. Integrin α v β 6 binds to the RGD sequence in LAP inducing a conformational change and the binding of α v β 6 to the actin cytoskeleton. The binding to α v β 6 to the actin cytoskeleton further induces a conformational change that releases TGF- β 1 from LAP. The unbound TGF- β 1 is now active and can go and bind its receptor and activate the TGF- β 1 signaling cascade.

Table 1
Expression of $\beta 6$ in Various Cancers

Method of detection: immunohistochemistry/immunoprecipitation

Cancer	Population Size	% $\alpha\beta 6$	Conclusion/Reference
Endometrial	126	42	Promotes invasion like in OSCCs. [25]
Basal Cell	15 nodular	7	Promotes invasion by myofibroblasts. [14]
	13 morphoeic	77	
Liver	63	71.4	Higher % of immunoreactivity than primary colon cancer cells. [17]
Colon	358	34	Promotes colonic cell metastasis in the liver. [17]
Colon	488	37	Poor prognosis. [42]
Gastric	300	36.7	Prognostic marker in early stage tumors. [26]
Cervical squamous	85	59	Unfavorable Prognostic marker. [49]
Oral SCC	17	100	No expression of beta6 in normal buccal mucosa. [50]
	5	80	$\alpha\beta 6$ present in oral leukoplakia. [51]
	11	100	$\alpha\beta 6$ is present at invasive front of the cancer. [31]
Pancreas	34	100	Enhanced $\alpha\beta 6$ expression in well differentiated tumors compared to poorly differentiated ones [24].
Breast	90	18	The grade 1 tumors were not positive for $\alpha\beta 6$ expression. [52]
Ovary	45	100	Staining intensity proportional to extent of malignancy. [20]