Involvement of $\alpha_6\beta_4$ integrin in the mechanisms that regulate breast cancer progression

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Abstract
Integrin $\alpha_6\beta_4$ is mostly expressed in epithelial tissues and endo- 
thelial and Schwann cells. Expression of $\alpha_6\beta_4$ is increased in many 
epithelial tumours, implicating its involvement in tumour malig-nancy. Moreover, this integrin activates several key signalling 
molecules in carcinoma cells, but its ability to activate the 
phosphatidylinositol 3-kinase/Akt pathway is among the 
mechanisms by which $\alpha_6\beta_4$ integrin regulates tumour behaviour. In 
this review we discuss the biological and clinical features of $\alpha_6\beta_4$ 
integrin that allow it to promote tumour survival and progression of 
mammary tumours.

Introduction
Integrins belong to a family of heterodimer transmembrane 
receptors whose major function is to mediate adhesion and 
migration through extracellular matrix components [1,2]. They 
also regulate other processes such as cell proliferation, 
growth and differentiation [3]. Studies conducted during the 
past 10 years have provided evidence that integrins are 
involved in mechanisms that influence tumour progression by 
activating various intracellular signalling pathways [4].

Among the integrin family of receptors, $\alpha_6\beta_4$ integrin was 
initially identified as tumour-specific antigen-180 [5]. A 
subsequent study [6] demonstrated the true identity of 
tumour-specific antigen-180 to be $\alpha_6\beta_4$ integrin. In most 
epithelial tissues, including mammary epithelium, the 
distribution of $\alpha_6\beta_4$ integrin is restricted to the basal layer 
[7,8], where it participates in the formation and stabilization of 
hemidesmosomes [9]. Most epithelial tumours lack 
hemidesmosomes, and $\alpha_6\beta_4$ integrin is distributed diffusely 
over the cell surface; the markedly increased expression of 
$\alpha_6\beta_4$ integrin in this setting suggests that it is involved in 
tumour progression [10,11]. Direct involvement of this integrin 
in tumour progression was first demonstrated by the finding 
that de novo expression of the $\beta_4$ integrin subunit increases 
the invasive capacity of $\beta_4$-negative colon carcinoma cells 
[12]. In mammary tumour cells it has been demonstrated that 
$\alpha_6\beta_4$ integrin, cooperating with Erb family members, plays an 
important role in regulating cancer behaviour [10].

Breast cancer is one of the most heterogeneous carcinomas 
in terms of metastatic capacity, expression of hormone 
receptors and responsiveness to treatment. In women it is the 
most common form of cancer and it is the second leading 
cause of cancer mortality (after lung cancer). It has been 
demonstrated that in many breast cancers the effect of 
therapy can be abrogated by high levels of ErbB-2 and 
vigorous activation of the phosphatidylinositol 3-kinase (PI3K) 
pathway [13]. Many breast cancer cell lines also exhibit high 
levels of expression of $\alpha_6\beta_4$ integrin; in these cell lines it has 
been observed that overexpression of this integrin results in 
potent activation of specific signalling pathways, especially 
the PI3K/Akt pathway [14]. These studies have revealed a 
specific function of $\alpha_6\beta_4$ integrin in tumours and have 
elucidated the mechanisms by which this integrin promotes 
the survival and progression of mammary tumours.

Role of $\alpha_6\beta_4$ integrin in cell migration
The altered expression of $\alpha_6\beta_4$ integrin in tumour cell lines of 
epithelial origin suggests that this integrin promotes tumour 
progression and spread [14]. Only during the past few years 
has it become clear how $\alpha_6\beta_4$ integrin contributes to these 
tumour behaviours. The large cytoplasmic tail of the $\beta_4$ 
subunit, which comprises more than 1,000 amino acids, is 
quintessential among the integrin family members [15].

Much progress has been made in identifying specific 
domains that mediate functions such as adhesion and 
migration in normal and tumour cells [16,17]. Chemotactic 
migration of carcinoma cells on laminin-1 requires not only 
the formation of F-actin-rich cell protrusions, which mediate 
$\alpha_6\beta_4$-dependent cell migration, but also disruption of
hemidesmosomes by protein kinase C [18]. Moreover, stimulation of epidermal growth factor causes mobilization of α6β4 integrin from the hemidesmosomes and increases formation of lamellipodia and membrane ruffles that contain α6β4 integrin [18,19]. It has also been shown in keratinocytes that spreading on laminin-5 activates Ron, which results in a protein kinase C dependent translocation of α6β4 integrin from hemidesmosome to lamellipodia [20]; this corroborates previous work in suggesting that α6β4 integrin, by helping to stabilize dynamic structures, promotes migration. In addition, the ability of α6β4 integrin to regulate the expression of genes such as the transcription factor NFAT [21] and the mitogen autotaxin/ENPP2 factor [22] could be among the mechanism through which the integrin can promote breast cancer cell motility.

Another mechanism by which α6β4 integrin could be involved in breast cancer cell invasion is dependent on hypoxia [23]. Hypoxia causes a marked increase in surface expression of α6β4 integrin in mammary tumour cells, which modulates their motility and ability to invade.

Role of α6β4 integrin in PI3K-dependent tumour survival

As in keratinocytes, overexpression of α6β4 integrin in breast cancer cells promotes motility by specifically activating PI3K [24]. In particular, regulation of PI3K by α6β4 stimulation is required for the formation of motility structures and for activation of Rac to promote invasion. It has also been reported that α6β4 integrin promotes survival by activating the PI3K/Akt pathway and that this function may be dependent on p53 status [25]. Specifically, it has been demonstrated that α6β4 integrin promotes PI3K/Akt-dependent survival of p53-deficient mammary tumour cells. Moreover, in response to α6β4 ligation for the recruitment of PI3K on the plasma membrane are implicated two insulin receptor substrate (IRS) members, namely IRS-1 and IRS-2, as adaptor proteins, that mediate the α6β4-dependent PI3K activation [26].

A study conducted in our laboratory [27] provided the first evidence that in mammary tumour cells α6β4 associates with ErbB-2, the orphan receptor of the epidermal growth factor receptor (EGFR) family, which is frequently found to be highly expressed in mammary tumours. A subsequent study conducted in a NIH3T3 cell model system [28] demonstrated that the β4 cytoplasmic tail is required in this interaction and that both α6β4 and ErbB-2 molecules are required for PI3K-dependent invasion. Both β4 integrin subunit and ErbB-2 lack the consensus sequence to bind p85, the regulatory subunit of PI3K; this suggests that involvement of another EGFR family member is required to activate PI3K. Among EGFR family members, the ErbB-2/ErbB-3 heterodimer is the most potent activator of PI3K. α6β4 Integrin and ErbB-2/ErbB-3 have been implicated in breast cancer progression and metastasis [29,30], findings that suggest a role for the ErbB-3 molecule in α6β4/ErbB-2 cooperation. Indeed, α6β4 regulates the translation of ErbB-3 protein in mammary cells, which results in significant induction of ErbB-2/ErbB-3 heterodimerization and consequent activation of the PI3K/Akt survival pathway [31]. Moreover, activation of PI3K/Akt pathway mediated by α6β4 ligation is inhibited upon ErbB-3 depletion, indicating that the survival function of α6β4 integrin is dependent on ErbB-3.

These data are apparently in contrast with the finding that clustering of α6β4 under adherent conditions does not result in significantly increased PI3K activity [32]. However, these latter findings were obtained in a cell line that expresses high levels of α6β4 integrin and that does not express ErbB-3 receptor. A probable explanation for the discrepant observations is that the cells used by Gilcrease and coworkers [32] express the Nrdp1 ubiquitin ligase, which promotes ErbB-3 degradation [33].

Previous work [34] demonstrated that α6β4, through the PI3K/Akt pathway, phosphorylates and inactivates 4E-BP1 (a translational repressor), which in turn stimulates production of vascular endothelial growth factor (VEGF) and promotes survival of breast cancer cells. The VEGF is an autocrine survival factor for breast carcinoma cells [34], and because α6β4 cooperates with EGFR members to promote survival of breast cancer cells [31] we cannot exclude the possibility that α6β4-mediated ErbB-2/ErbB-3 signalling promotes breast carcinoma survival by elevating VEGF expression. It is interesting to note that the cross-talk between α6β4 integrin and EGFR family members is strong. For example, it was recently shown that α6β4 integrin also regulates the translation of ErbB-2 protein and that expression of α6β4 integrin is necessary to regulate Ras activation [35]. Moreover, H-Ras promotes carcinoma invasion by E2F-dependent regulation of α6β4 integrin [36]. Overexpression of E2F family members increases levels of the β4 subunit both at transcriptional and protein levels, resulting in markedly increased invasion.

Role of α6β4 integrin in breast cancer progression

A specific role for α6β4 integrin in breast cancer progression and tumourigenesis was recently demonstrated by the finding that depletion of α6β4 integrin, by RNA interference, strongly reduces in vitro and in vivo tumourigenicity [37,38]. Interestingly, loss of α6β4 integrin reduces significantly the tumourigenicity of mammary tumour cells, reducing the production of VEGF in vivo. This finding is in agreement with previous data indicating that α6β4 integrin regulates VEGF translation [34], supporting the important role played by α6β4 integrin in sustaining the survival of mammary tumour cells [25]. Loss of α6β4 integrin also results in significant downregulation of PI3K/Akt activity [38].

It has been found that in the absence of hormone β4-depleted cells exhibit a 17% rate of apoptosis, which increases to
45% with to hormone therapy [38]. This finding indicates that α6β4 integrin plays a role in promoting survival in mammary tumour cells in the absence of hormone. Recently, it has been described that mice carrying a deletion of the c-terminal region of the β4 tail evidenced that α6β4 integrin promotes mammary tumourigenesis [39]. In vivo, cooperation between α6β4 integrin and ErbB-2 enhances activation of the transcription factors STAT3 and c-Jun, which results in disrupted epithelial polarity and hyperproliferation, respectively [39].

The α6β4 integrin cooperates with other tyrosine kinases such as c-met (a hepatocyte growth factor receptor) to promote invasion [40]. Stimulation with hepatocyte growth factor (c-met kinase) transphosphorylates β4, which associates with Shc and PI3K, potentiating hepatocyte growth factor-triggered signalling on both Ras and PI3K dependent pathways. The same group [41] recently found that overexpression of β4 subunit is sufficient to transform rodent fibroblasts, increases the in vitro growth of mammary tumour cells, and induces tumourigenicity in nude mice, whereas depletion of α6β4 integrin abrogates the transformed phenotype.

Altogether, these studies demonstrate that the crosstalk between α6β4 integrin and receptor tyrosine kinase signalling is strong and contributes to increased ability of mammary tumour cells to grow and invade.

In vivo expression and localization of α6β4 integrin in breast cancer

Although expression of α6β4 integrin in vivo has not been evaluated extensively, the in vitro data obtained in the following studies are in agreement with in vitro findings.

The first study to analyze the distribution of α6β4 integrin in human carcinomas [42] identified high levels of expression in various epithelial tumours. Similar findings were reported from a study that analyzed the β4 subunit in normal, hyperplastic and neoplastic breast cancers [43]. Among the tumours analyzed, immunohistochemical study found that mucinous carcinomas are highly positive for β4 expression, whereas luminal cells of normal ducts and acini as well as fibroadenocystic tissue exhibit rare, weak reactivity.

In contrast to previous findings, an analysis conducted in a limited number of breast cancers [44] demonstrated loss of α6β4 integrin in grade III ductal carcinoma (7/11 cases) whereas most of grade I and II carcinomas analyzed were positive (6/8 cases). Even though this study is restricted to a small number of mammary tumours and the results obtained did not achieve statistical significance, it revealed marked deregulation of integrin expression that correlated with loss of polarization, which is a characteristic feature of epithelial tumours. Similar results were obtained in an analysis of the expression of α6 and β4 subunits in primary and metastatic breast cancers [45]. Interestingly, the findings obtained indicate that, as expected, in normal mammary gland both subunits colocalize at the basolateral aspect of the epithelium, which remains unchanged in benign breast lesions; in contrast, in primary carcinomas expression of both chains is reduced and restricted over the cell surface. The same study revealed that α6β4 integrin is present at lymphonodal foci, but that the level of expression is less in metastases to the pleural cavity and parenchymal tissues. Moreover, lack of laminin and collagen type IV correlates in these tumours with reduced α6β4 expression [45].

More recently, a study conducted in 119 tumours from patients with invasive breast carcinoma [46] demonstrated that low levels of expression of α6 subunit correlate with survival, whereas high levels of expression correlate with significantly reduced survival. Indeed, elevated expression of α6 was present in 90% of cases with distant metastases, indicating that this integrin plays a role in tumour progression. In agreement with the findings of that study, an analysis of α6β4 integrin in 80 patients with breast carcinoma [47] revealed that 36 of these tumours expressed high levels of β4 subunit, with a significant association between expression of α6β4 subunit and production of laminin by tumour cells. That study demonstrated, for the first time, that concomitant expression of α6β4 integrin and laminin production has clear prognostic value, suggesting that these molecules mediate signalling events that are important for tumour progression.

An in situ hybridization study was recently conducted in which β4 mRNA expression was evaluated in paraffin-embedded sections of tissue from 25 patients with invasive breast carcinoma, and compared with immunohistochemical findings from frozen sections derived from same tumours [48]. It revealed that all cases positive for β4 protein also expressed β4 mRNA; three cases in which β4 mRNA was detected did not express β4 at the protein level. Using in situ hybridization, the same group analyzed β4 subunit mRNA expression in early breast cancer and correlated their findings with both tumour size and nuclear grade [49]. They also analyzed the expression of laminin-5 (the major substrate for α6β4 integrin in epithelial tissue) in these tumours. They observed a strong correlation between β4 mRNA expression and tumour size and nuclear grade, but correlation between β4 and laminin-5 expression was not found to have prognostic significance [50].

All of these studies, in agreement with literature published during the past 2 years, strongly support a role for α6β4 expression in promoting tumour progression. These studies also indicate that the biological role played by α6β4 integrin in mammary tumours is complex; studies should be extended to other molecules that may cooperate with α6β4 integrin to render tumours more aggressive. These studies could help us to determine whether α6β4 integrin should be considered an early marker of tumourigenicity and to evaluate tumour prognosis better.
Conclusion

Both experimental data and in vivo studies discussed in this review indicate that $\alpha_6\beta_4$ integrin plays a major role in mediating the aggressiveness of mammary tumours. Metastasis is a complex process that involves many molecules and many biochemical alterations in tumour cells. Integrin $\alpha_6\beta_4$ is a key molecule in this process, but much more needs to be learned about the contribution of this integrin to tumour progression. There is much cooperation between $\alpha_6\beta_4$ integrin and EGFR family members; furthermore, this integrin can modulate the expression of growth factors and growth factor receptors that are involved in tumour progression. Acknowledgement of the intimate relationships between these molecules could represent an aid in determining prognosis, could guide management decisions, and could facilitate development of drugs with novel targets.

Competing interests

The authors declare that they have no competing interests.

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