Roles of Laminin-332 and α6β4 Integrin in Tumor Progression

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Abstract: Laminin-332 and α6β4 integrin is major hemidesmosome components in the skin. As many studies have shown that laminin-332 and α6β4 integrin play important roles in tumor progression *via* activation of phosphatidylinositol-3 kinase signaling, understanding of the molecular mechanisms of them could lead to a new drug for cancer therapy.

Key Words: Laminin-332, α6β4 integrin, basement membrane, extracellular matrix, tumor, cell migration, cell adhesion, ErbB2.

INTRODUCTION

Basement membranes are thin sheets of specialized extracellular matrix (ECM) that underlie all epithelial cell sheets and tubes such as epithelia, endothelia, muscle cells, fat cells and nerve cells. Basement membranes separate these cells and epithelia from the underlying, or surrounding, connective tissues. In addition to providing mechanical support and stability, basement membranes play an important role in cell polarity, cell metabolism, cellular activities such as cell adhesion, migration, differentiation and proliferation, and diverse developmental processes [1, 2].

Laminins are large extracellular glycoproteins that are major components of all basement membranes, and they are involved in various cellular functions such as cell adhesion, migration, proliferation and differentiation [3-5]. Laminins are heterotrimers composed of α, β and γ chain. To date, five α, three β and three γ chains have been identified in mammals and at least three of the laminin chains (α2, α3 and γ3) may exist as alternatively spliced forms, forming at least 16 distinct laminin heterotrimers [3, 6]. Each laminin chain contains a short arm and a long arm (coiled-coil domain) (Fig. (**1**)). Each short arm is subdivided into domains such as LN, LE and L4 (Fig. (**1**)). Separate chains are linked by disulfide bonds at long arms to form a cross-shaped structure. In addition to those two regions, only α chain has a large globular (G) domain at its carboxyl-terminal end (Fig. (**1**)). The G domain, which consists of five tandem modules (G1 through G5), contains binding sites for cellular receptors such as integrin, α-dystroglycan and syndecan (Fig. (**2**)) [7]. The short arms have some interaction sites with integrins and heparin. Laminin can self-polymerize through the three LN domains in the short arms (Fig. (**1**)). This laminin network can link to the type IV collagen network through nidogen and perlecan, which are able to bind to both laminin and type IV collagen, thereby forming a basement membrane structure.

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Laminin isoforms show tissue-specific expression [3, 8]. For example, laminin-211 (α2β1γ1) is primarily expressed in the developing skeletal muscle and peripheral nerve. Laminin-411 (α4β1γ1) is primarily localized in the basement membrane of blood vessels. On the other hand, laminin-111 (α1β1γ1), which was the first laminin identified and discovered in mouse Engelbreth-Holm-Swarm (EHS) sarcoma, is primarily expressed during very early embryogenesis. Thus, laminin isoforms show distinct temporal and spatial expression patterns *in vivo*.

Integrins are cell surface adhesion receptors that connect the extracellular environment to the cell interior [9, 10]. They constitute both a structural connection and a bi-directional signaling pathway that crosses the cell membrane. In mammals, 18 α and 8 β subunits are known to combine to form 24 distinct integrins. Both subunits contain a large extracellular domain, a single α-helix transmembrane domain and a cytoplasmic domain. Integrins are the major adhesive components of ECM proteins such as collagen, fibronectin and laminin. α1β1, α3β1, α6β1, α6β4, and α7β1 integrins are generally regarded as the main laminin receptors [3]. The major binding sites of integrin are located in the laminin G domain. Once laminin binds to integrin receptors, integrin undergoes conformational changes, and their cytoplasmic domains interact with the actin cytoskeleton and signaling proteins through linker molecules such as paxillin [9]. Laminin-integrin interactions induce integrin clustering and then activate intracellular signaling pathways for various cellular functions. In this review, the authors introduce one of the laminin isoforms, laminin-332 (previously known as laminin-5; α3β3γ2), and its receptor, integrin α6β4, and describe their roles in tumor progression.

ROLE OF LAMININ-332 IN CELL ADHESION

Laminin-332 is expressed in the basement membrane of skin [11, 12] and other stratified squamous epithelial tissues. Laminin-332 forms hemidesmosomes *via* its association with integrin α6β4 [13]. The hemidesmosome structure plays an essential role in maintaining stable adhesion by connecting the intracellular keratin cytoskeleton to the basement membrane. Genetic mutations of laminin-332 subunits cause the

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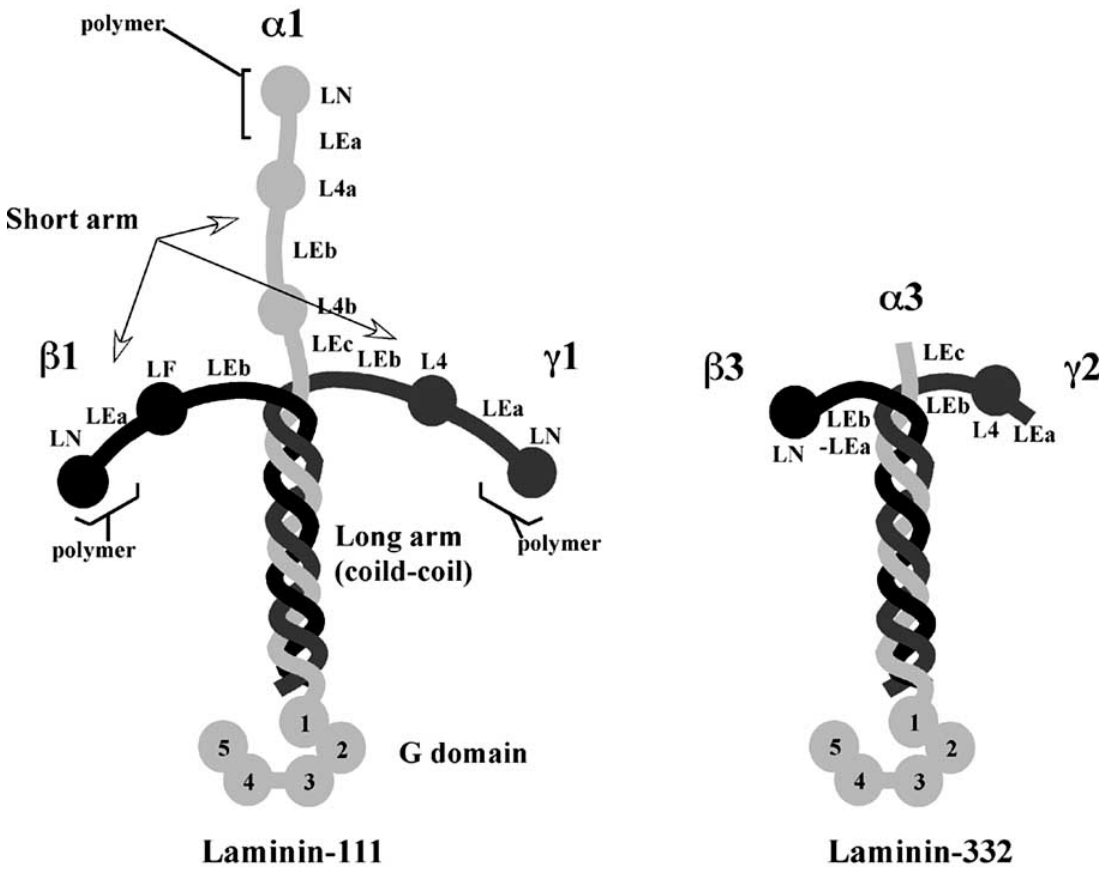

Fig. (1). Laminin-111 and laminin-332 structures.

Laminins are heterotrimers composed of α, β and γ chains, and the separate chains are linked by disulfide bonds to form a cross-shaped structure. Each laminin chain contains a short arm and a long arm (coiled-coil domain), and the α chain has a large globular (G) domain at its carboxyl-terminal end. The G domain consists of five tandem modules (G1 through G5). The amino-terminal (LN domain) and internal short arm globular (LF and L4 domains) modules are indicated by ovals. The rod-like epidermal growth factor (EGF) repeats (LE domains) are shown as lines. The coiled-coil domains are indicated as wavy lines. Laminin-111 (α1β1γ1, left), but not laminin-332 (α3β3γ2, right), can self-polymerize through the three LN domains in the short arms.

severe and lethal skin blistering disease, Herlitz's junctional epidermolysis bullosa [14, 15]. Laminin-332 is the only laminin with truncations in all three short arms (Fig. (**1**)). Therefore, it is thought to be incapable of self-assembly. However, since laminin-332 can associate with laminin-311 (α3β1γ1) and laminin-321 (α3β2γ1) [16], both of which can connect to type IV collagen *via* nidogen, laminin-332 can participate in a laminin-type IV collagen network. The G1-G3 domain of the laminin α3 chain binds to integrins α3β1, α6β1 and α6β4 [11, 17, 18] while the G4-G5 domain can associate with heparan sulfate proteoglycans such as syndecan [19, 20]. The β3 chain binds to type VII collagen [21, 22], which forms anchoring fibrils that insert into the dermis [23]. Thus, laminin-332 plays a central role in maintaining the integrity of dermal-epidermal structures.

EFFECT OF PROTEOLYTIC PROCESSING AND N-GLYCOSYLATION OF LAMININ-332 ON CELL MIGRATION

Laminin-332 was originally found as a component of the basement membrane in human skin [12] and also identified as a scattering factor, which was purified from the conditioned medium of human gastric adenocarcinoma cells [24]. *In vitro* laminin-332 promotes cell migration including keratinocytes and cancer cells through the association of the carboxyl-terminal G domain with integrins α3β1, α6β1 and α6β4 [18, 25]. Interaction of laminin-332 with integrins activates the cell migration-related signaling pathways such as phosphatidylinositol 3-kinase (PI3K), extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK), NF-κB and the small G protein Rac [26-29]. During wound healing, large amounts of laminin-332 secreted from basal keratinocytes adjacent to the leading edge of wound induces keratinocyte migration to the injured spaces [30]. Both integrins α3β1 and α6β4 localize at the leading edge and play important roles in wound healing [31, 32].

Tumor cells stimulate the surrounding stromal cells to produce matrix metalloproteinases (MMPs) or serine proteases, which degrade ECM barriers such as collagen and laminin, thus allowing cancer invasion. In contrast certain very specific proteolytic events do not lead to degradation but instead can induce functional ECM activation. In both physiological and pathological conditions, laminin-332 undergoes proteolytic processing by certain proteases after secretion from cells (Fig. (**2**)). The α3 chain is cleaved in the

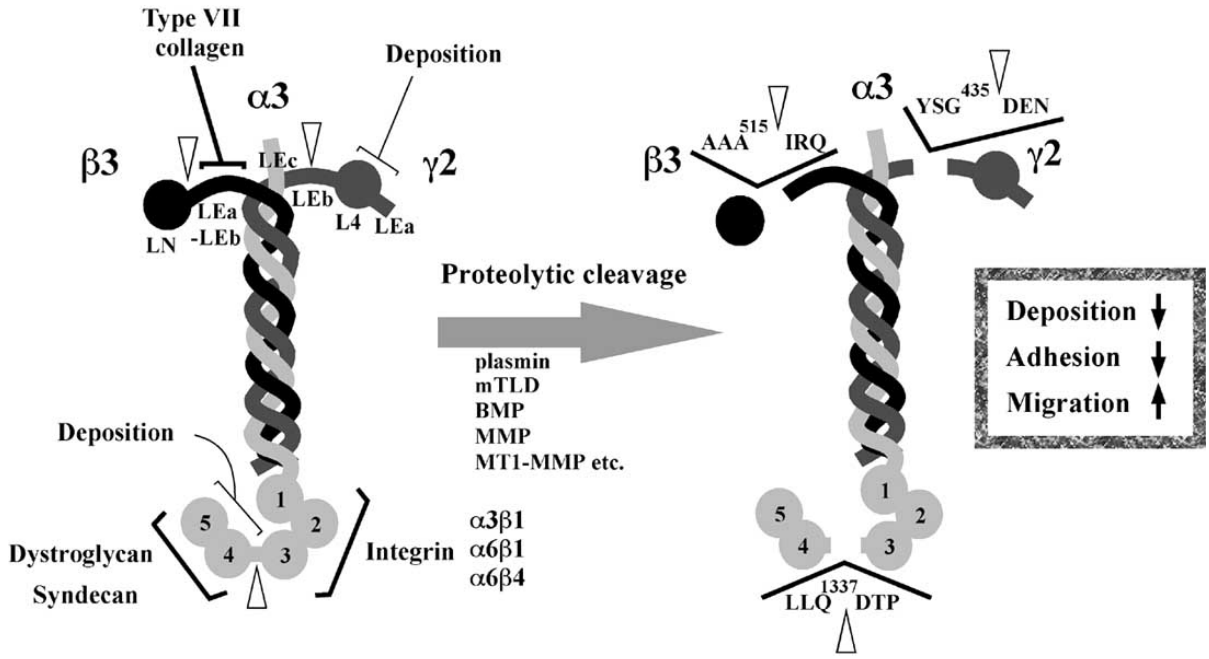

Fig. (2). Regulation of laminin-332 function by proteolytic processing.

The laminin α3 G1-G3 domain contains binding sites for integrins α3β1, α6β1 and α6β4, and the G4-G5 domain interacts with dystroglycan and syndecans. The laminin β3 domain LEb-LEa binds to type VII collagen. The laminin α3 G4-G5 domain and γ2 L4 domain play a critical role in the incorporation of laminin-332 into the extracellular matix.

Schematic representation of proteolytic cleavage sites on laminin-332. The α3 chain is cleaved in the linker region between the G3 and G4 domains. The γ2 chain and the β3 chain undergo processing in the short arm. Arrowheads indicate the major proteolytic cleavage sites.

linker region between G3 and G4 domains [33] by plasmin [34] and metalloproteinases such as mammalian tolloid (mTLD) and bone morphogenic protein-1 (BMP-1) [35, 36]. mTLD and BMP-1 [35, 36] are major processing enzymes of the human laminin γ2 short arm, although other enzymes have been shown to process this chain as well, including matrix metalloproteinase 2 (MMP2) [37], membrane type 1-matrix metalloproteinase (MT1-MMP) [38] and neutrophil elastase [39]. This proteolytic processing converts the precursor form of the α3 (190 kDa) and γ2 (150 kDa) chains to the mature forms of α3 (160 kDa and 145 kDa) and γ2 (105 kDa) chains, respectively. Recent reports indicate that the β3 chain can be processed in the short arm [22, 40] by MT1-MMP, matrilysin-1 [matrix metalloproteinase 7 (MMP7)] and hepsin [40-42]. The G4-G5 domain of the α3 chain, the short arms of the β3 and γ2 chains involves association sites with syndecans and dystroglycans, type VII collagen, and nidogen, respectively [19-22, 43]. Furthermore, the α3 chain G4-G5 domain and the γ2 short arm play important roles in laminin-332 deposition [44, 45]. Therefore, this proteolytic conversion of laminin-332 chains alter the interaction with other molecules and laminin-332 deposition, thereby affecting cellular movement [37, 38, 41, 46].

In addition to the proteolytic modification, N-glycosylation also affects the activities of laminin-332 [47]. Glycosylation is one of the most common post-transcriptional modifications and is known to be involved in various physiological and pathological events, including cell growth, migration, differentiation and tumor invasion. Laminins are heavily glycosylated molecules. It has been reported that between 13 and 30% of the total molecular weight of laminins is *N*-linked glycosylated [48]. The laminins undergo terminal glycosylation within the Golgi apparatus and then N-glycosylated laminins are secreted out of the cell. More recently, it has been reported that an increase in bisecting GlcNAc catalyzed by *N*-acetylglucosaminyltransferase III (GnT-III) on laminin-332 suppresses its cell adhesion and migration activities [47]. This effect is likely to be derived from impaired α3β1 integrin clustering and resultant focal adhesion formation by laminin-332. In some cancers, an increase of β1,6-GlcNAc catalyzed by *N*-acetylglucosaminyltransferase V (GnT-V) is related to cancer metastasis [49, 50]. In contrast, bisecting GlcNAc suppresses further processing with branching enzymes such as GnT-V [51], resulting in down-regulation of cancer metastasis. In addition, GnT-III modification of α3β1 integrin inhibits cell migration on the laminin-332 substrate [52]. Accordingly, the suppression of laminin-332 activities by the introduction of bisecting GlcNAc is probably due to the decrease in β1,6-GlcNAc on the laminin-332 molecule. Since most ECM molecules are glycoproteins, alteration of carbohydrates could change carbohydrate/carbohydrate or carbohydrate/protein interactions in the basement membrane, which presumably affect the functional activities of diverse ECM proteins.

LAMININ-332 AND α6β4 INTEGRIN IN TUMOR

The basement membrane, which consists of various ECM molecules, plays important roles in the maintenance of normal epithelial cellular homeostasis [1]. Because more than 90% of all human neoplasia arises in epithelia, the basement membrane can be a first barrier to tumor invasion into the

dermis. On the other hand, ECM and growth factors produced by cancer cells are required for tumor progression. Many studies have shown that laminin-332 is highly expressed in various types of squamous and other epithelial tumors, including cutaneous, oral, mammary, tracheal, esophageal and colon carcinoma [53, 54]. Cancer cells customize their surrounding microenvironment including ECM and growth factors to enable their growth, invasion and metastasis. Various *in vitro* activities of laminin-332, such as cell adhesion, spreading, migration and proliferation through cell surface receptors, implicate it as a key substrate for tumor progression.

The importance of laminin-332 and integrin α6β4 in tumorigenesis has been proven using an animal model of human squamous cell carcinoma (SCC) in which active H-Ras and IκBα are overexpressed in primary keratinocytes [55]. In the system, although primary kaeratinocytes transformed with Ras-IκBα form tumors within 2 weeks after subcutaneous injection into mouse skin, laminin-332-negative or β4 integrin-negative keratinocytes (derived from epidermolysis bullosa patients with null LAMB3 or ITGB4 gene mutations respectively) failed to form tumors even when cells were transformed with active Ras and IκBα. However, reintroduction of the laminin β3 and β4 integrin genes into those cells restored their tumor formation, suggesting that these molecules are required for human SCC tumorigenesis.

Recently, more extensive studies have revealed that laminin α3 G4-G5 domain [56] and laminin β3 LEb-LEa domain [57] (Fig. (**2**)) are important for SCC tumorigenesis. Analysis of laminin α3 chain negative keratinocytes expressing a wild type (with G4-G5) or a mutant lacking the G4-G5 domain (without G4-G5) laminin α3 chain vector showed that the G4-G5 domain induced laminin-332 deposition and led to increased stable cell adhesion but decreased cell migration in wound healing. Unlike the results of the wound healing assays, the G4-G5 domain promoted human SCC tumorigenesis and invasion by activating the MMP, ERK, and PI3K signaling pathways that induce proliferation and inhibit apoptosis [56]. The cells expressing the laminin β3 mutant without domain LN, which was proteolytically removed from laminin-332 molecules in some cancer cells, showed poor cell adhesion but still had tumorigenic ability. On the other hand, the cells expressing laminin β3 without domain LEb-LEa had adhesion properties similar to the cells expressing laminin β3 mutant without domain LN, but no tumor-forming ability, in a tumor model [57]. Type VII collagen NC1 domain promotes tumor cell invasion in a laminin-332 dependent manner [58]. Taken together, the association of laminin-332 β3 domain LEb-LEa with the type VII collagen NC1 domain appears to form a signaling scaffold for tumorigenesis. These studies indicate that laminin-332 might serve as a signaling inducer rather than an adhesion molecule in tumorigenesis. This concept of laminin-332 as a signaling rather than an adhesion molecule in tumor progression may also be appreciated from studies showing a soluble form of laminin-332 is able to stimulate cell migration by binding to α3β1 integrin on the cell surface [28].

α6β4 integrin, although it is now recognized as a component of hemidesmosome structure, was originally identified as a tumor-specific protein [59, 60]. Subsequent studies have reported that overexpression of α6β4 integrin was seen in several types of cancers and correlates with a poor prognosis [61]. In fact, hypoxia, which often occurs within many solid tumors, induced a significant increase in cell surface expression of α6β4 integrin but not α3β1 integrin [62]. α6β4 integrin is known to activate PKC [63], PI3K to Akt [64] and Ras to ERK [65] signaling pathways, which are linked to cell migration and proliferation (Fig. (**3**)). When binding to laminin-332, α6β4 integrin promoted EGF-dependent cell migration through the activation of Rac1, which is a key regulator of cytoskeletal dynamics and affects cell migration [31]. This Rac1 signaling regulates cofilin activation, and proper laminin-332 matrix organization, leading to keratinocyte directional migration [66-68].

α6β4 integrin combines with the receptor tyrosine kinases (RTKs) such as EGFR, ErbB2, and hepatocyte growth factor receptor Met, which bind to soluble growth factors and cytokines, and enhance the signaling function of RTK [69-71]. *The* RTKs are often mutated or amplified during tumor progression. About 20-25% of all breast cancers carry amplification of the *ErbB2* locus, which encodes ErbB2 [72, 73]. Since this amplification is common in aggressive breast tumors and correlates with a poor prognosis, ErbB2 is one of the most well known markers in breast cancer [74]. *In vitro*, constitutively active ErbB2 mutant is sufficient to transform normal mammary epithelial cells, and dimerization of ErbB2 induces proliferation and suppresses apoptosis of cells [75]. Likewise, transgenic mice expressing constitutively active ErbB2 develop invasive tumors, suggesting that ErbB2 plays a central role in mammary malignancy [76]. RTK activation induces tyrosine phosphorylation of the cytoplasmic domain of β4 integrin through activation of the Src-family kinases (SFKs), such as Fyn, causing disruption of hemidesmosomes and an increase in cell motility [70] (Fig. (**3**)). This suggests that RTKs decrease the ability of α6β4 integrin to form stable adhesion, but increase its signaling function. Conversely, α6β4 integrin promotes Src-family kinase-dependent phosphorylation of RTKs. Moreover, α6β4 integrin enhances ErbB2-mediated activation of the transcription factors, signal transducers and activators of transcription 3 (STAT3) and c-Jun, which contribute to the disruption of epithelial adhesion and polarity, and hyperproliferation, respectively [77].

The humanized anti-ErbB2 monoclonal antibody Herceptin (trastuzumab) binds to the extracellular domain of ErbB2 and results in growth inhibition and apoptosis of tumor cells expressing ErbB2. Although the mechanism of action has not been fully elucidated, Herceptin is already in clinical use and has demonstrated marked improvements in both disease-free survival and overall survival in patients with ErbB2 positive disease [78, 79]. Despite the significant benefits of Herceptin, some patients show resistance to Herceptin treatment. Guo *et al*. have demonstrated that deletion of the β4 integrin signaling domain improved the efficacy of Herceptin, suggesting that α6β4 integrin signaling promotes resistance to anti-ErbB2 therapy [77]. This study suggests that the combination of monoclonal antibodies or small molecules, which inactivate α6β4 integrin signaling, with Herceptin and chemotherapy may increase the efficiency of ErbB2-targeted therapies.

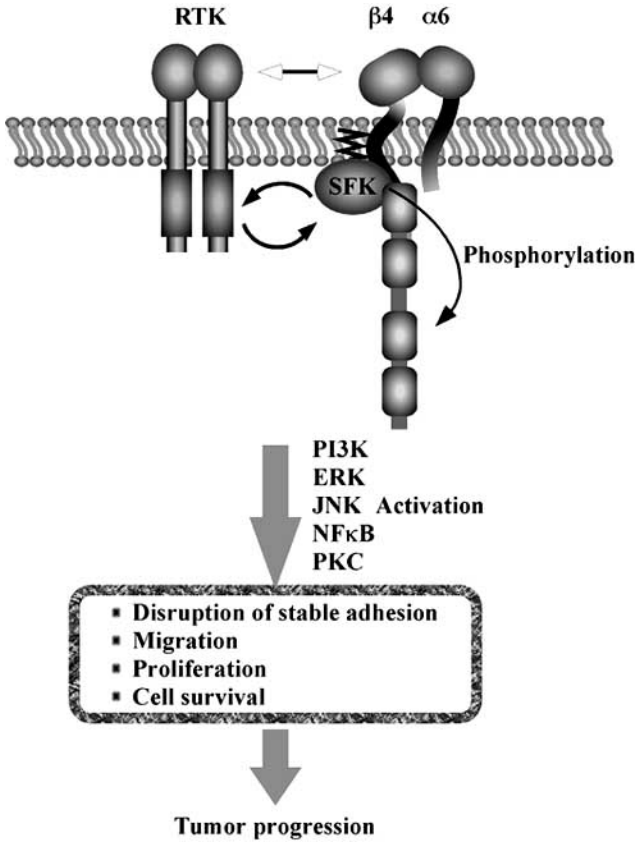

Fig. (3). Integrin α6β4 and receptor tyrosine kinase signaling.
α6β4 integrin combines with receptor tyrosine kinases (RTKs) such as EGFR, ErbB2, and hepatocyte growth factor (HGF) receptor Met and enhances the signaling function of RTKs. Activation of RTKs enhances tyrosine phosphorylation of the cytoplasmic domain of β4 integrin through activation of the Src-family kinase (SFK). Conversely, α6β4 integrin promotes SFK-dependent phosphorylation of the RTK. These cooperative signals accelerate tumor progression.

SUMMARY AND CONCLUSION

Laminin-332 and α6β4 integrin are central components of hemidesmosomes in the stratified squamous tissues and stable adhesion complexes in epithelial cells. However, in addition to providing adhesion, the association of the G1-G3 domain in laminin-332 with integrins α3β1, α6β1 and α6β4 induces cell motility through the activation of signaling pathways such as PI3K, PKC, Akt and ERK. The proteolytic processing of laminin-332 subunits alter interactions with other molecules which promote cell migration and decrease laminin-332 deposition. Laminin-332 has many N-glycosylation sites, and the modification of laminin-332 with bisecting GlcNAc catalyzed by GnT-III suppresses cell adhesion and migration induced by laminin-332. Many studies have shown that laminin-332 and α6β4 integrin play crucial roles in cancer. In an *in vivo* human xenograft model, laminin-332 α3 G4-G5 domain and β3 domain LEb-LEa, and α6β4 integrin have been proven to play essential roles for SCC tumorigenesis. About 20-25% of all breast cancers carry amplification of ErbB2 gene, which is common in aggressive breast tumors and correlates with a poor prognosis. α6β4 integrin combines with and enhances the signaling function of RTKs. The activation of RTKs induces tyrosine phosphorylation of the cytoplasmic domain of β4 integrin through activation of the Src-family kinases, causing disruption of hemidesmosomes and an increase in cell motility. Conversely, α6β4 integrin promotes Src-family kinase-dependent phosphorylation of the RTK causing the activation of STAT3 and c-Jun, which contributes to the disruption of epithelial adhesion and polarity, and hyperproliferation.

Enormous efforts using biochemical and genetic techniques have advanced our understanding of the structure and function of laminin-332 and α6β4 integrin in recent years. Although further studies are required to characterize the molecular mechanism and signal transduction in many cancers, the development of inhibitors based on an understanding of the interaction of laminin-332 and α6β4 integrin with their associated molecules, and their modification, will undoubtedly provide new specific molecular agents for cancer therapy.

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ABBREVIATIONS

ECM	=	Extracellular matrix
G domain	=	Globular domain
PI3K	=	Phosphatidylinositol 3-kinase
ERK	=	Extracellular signal-regulated kinase
JNK	=	Jun N-terminal kinase
PKC	=	Protein kinase C
MMP x	=	Matrix metalloproteinase x
MT1-MMP	=	Membrane type1-matrix metalloproteinase
mTLD	=	Mammalian tolloid
BMP-1	=	Bone morphogenic protein-1
EGF	=	Epidermal growth factor
EGFR	=	Epidermal growth factor receptor
GnT-III	=	N-acetylglucosaminyltransferase III
GnT-V	=	N-acetylglucosaminyltransferase V
SCC	=	Squamous cell carcinoma
RTKs	=	Receptor tyrosine kinases
SFK	=	Src-family kinase
STAT3	=	Signal transducers and activators of transcription 3

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