Integrin ß4, keratinocytes and papillomavirus infection (Review)

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Abstract. Integrin ß4 is a transmembrane protein expressed predominantly on epithelial cells. In human epidermis integrin ß4 associates with integrin ß6. Integrin α6ß4 is concentrated at the basement membrane zone, where it localizes to specialized adhesion structures called hemidesmosomes. In addition to its adhesive functions, keratinocyte integrin ß4 has been identified as an important regulator of epidermal homeostasis. This review summarizes the current knowledge regarding the role of integrin ß4 in keratinocyte adhesion, migration, as well as growth and differentiation. Changes in integrin ß4 expression in pathological conditions in skin and mucosa, especially those associated with human papillomavirus infection, are described.

Contents

1. Integrins - general features

Integrins are a family of glycosylated heterodimeric transmembrane adhesion receptors that consist of noncovalently linked α- and ß-subunits. Most integrins bind to the components of the extracellular matrix, e.g. laminins, collagens, fibronectin, while others bind to counter-receptors of the immunoglobulin-like superfamily (1). The name integrin refers to their function of integrating the cells' exterior, extracellular matrix to the cells' interior, cytoskeleton. Sequencing of the human genome has revealed as many as 24 α- and 9 ß-subunits (2). Among them are the newly identified 6 α- and 1 ß-subunits, however, their existence has not yet been firmly established. Currently, 24 functional heterodimers of different composition are known to be generated in humans and expressed on a variety of cell types, i.e. epithelial cells, endothelial cells, fibroblasts, hematopoietic cells, neurons and muscle cells (1,3). In these tissues integrins serve to modulate many aspects of cell behavior, including adhesion, cell shape, motility, survival, proliferation and differentiation. Signals transduced via integrins are essential for embryonic development, tissue regeneration, immune defense and tumor progression.

The combination of the α- and ß-subunits determines the ligand specificity of the integrin. Despite the fact that many integrins have binding specificities for the same ligands, the loss of almost any integrin subunit leads to biological defects in knockout mice. These defects vary from subtle imperfections, as in the α1-knockout mouse to severe abnormalities in several α- or ß-subunit knockout mouse strains, resulting in lethality at embryonic stages or shortly after birth (4-7).

The integrin-stimulated signaling pathways are similar to those triggered by growth factor receptors and are intimately coupled with them (reviewed in ref. 8). Many cellular responses to soluble growth factors, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) are dependent on the cells being adherent to a substrate via integrins (4). Additionally to the classical 'outside-in signaling', integrins function as bidirectional signaling receptors. Their activity can also be modulated through a yet incompletely understood 'inside-out signaling' mechanism, which involves the propagation of conformational changes from the cytoplasmic tails across the membrane towards the ligand-binding region (9). Integrins have been described by Hynes (4) as signal transduction receptors, which are at least as significant to cells as more traditional growth factor receptors.

In human epidermis, integrin expression is restricted to the basal layer. It is down-regulated as keratinocytes move through

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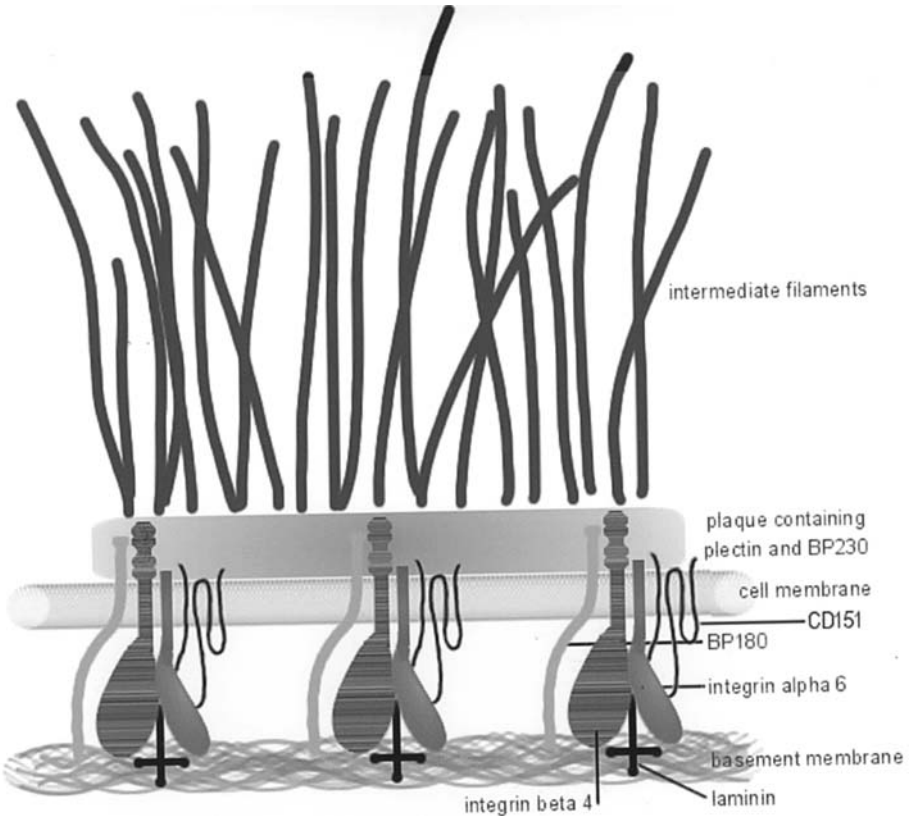

Figure 1. Hemidesmosomes are dense cytoplasmic plaques that mediate the attachment of epidermal cells to the underlying dermis by connecting the extracellular anchoring filaments (laminin) of the basement membrane with the cytoskeletal intermediate filaments. Clusters of integrin α6ß4 form the core of hemidesmosome along with BP180 and tetraspan CD151 protein. Plectin and BP230 belong to the plakin family of proteins and contain intermediate filament-binding domains.

the suprabasal layers and undergo terminal differentiation. The most abundant constitutive integrins in the epidermis are α2ß1, a receptor for collagen, and α3ß1 and α6ß4, receptors for laminins. The ß1 containing integrins localize to focal contacts and are distributed over the basal, lateral and apical surfaces of basal cells. The α6ß4 integrin is primarily concentrated at the basement membrane zone. It localizes to hemidesmosomes and appears as large patches organized in ring-like structures. In addition to its adhesive functions, integrins in the skin have been identified as important regulators of epidermal homeostasis, influencing the balance between keratinocyte proliferation and differentiation (10-12).

2. Integrin ß4 - genomic and structural organization

According to the HUGO Gene Nomenclature Committee (www.gene.ucl.ac.uk/nomenclature/) 'integrin ß4' is the official name of the protein. Less frequently used alternate names are CD104 antigen or GP150. The human ITGB4 gene is located on chromosome 17q25. It spans about 28 kb and contains 41 exons (Genebank acc. no. Y11107) (13,14).

Untranslated DNA sequences in the first exon precede the translated sequences in 5' region. The initiation codon and the amino acid residues of the signal peptide are entirely contained within exon 2. Fifteen exons ranging in size from 24 to 265 bp encode the NH2-terminal extracellular region of the polypeptide composed of 710 amino acids and containing a 4-fold of a cysteine-rich motif similar to those of other integrin ß subunits and displaying high sequence homology with the epidermal growth factor-calcium-binding motif (EGF-CB) (14).

The short 23-amino-acid transmembrane region is encoded by the 18th exon and the long cytoplasmic tail of the COOH-terminus, approximately 1000 amino acid long, is distributed within the last 23 exons (14-16). The uniquely long intracellular domain of integrin ß4 bears no homology with cytoplasmic tail of other ß subunits (15). It contains binding sites for plectin and bullous pemphigoid antigen 180 (BP180) and two pairs of fibronectin type III-like repeats. Each repeat is encoded by two distinct exons, as in the fibronectin gene.

Most of the known functions of the cytoplasmic tail, such as the localization of ß4 in the cell membrane and recruitment of plectin, reside in the first pair of fibronectin III domain and the first 36 amino acids of the connecting segment (17,18). The cytoplasmic tail of integrin ß4 contains also a calx-ß motif, which is commonly present in the cytoplasmic domain of Na-Ca exchangers, where it overlaps domains used for calcium-binding and regulation. Its functional role for integrin ß4 remains unclear (19).

The molecular weight of integrin ß4 subunit is about 202 kDa. In addition to the most common form of integrin ß4, a number of integrin ß4 variants have been described. Some are generated by proteolytic processing of the mature form of the ß4 polypeptide, while others are translated from an alternatively spliced pre-mRNA. For the cytoplasmic

domain of integrin ß4 five (A-E) splicing variants have been described, with ß4A being the most abundant variant. The ß4B and ß4C contain insertion in the connecting segment of 53 and 70 amino acids, respectively. The ß4D variant carries a 21-bp deletion within intron 38 and ß4E has a cytoplasmic domain of only 232 amino acids (20-23).

Integrins ß4A and ß4B demonstrated similar ability to associate with hemidesmosomal components. The splice variants ß4B and ß4C were detected at a constant ratio in a subset of analyzed human tissues (23). Undifferentiated human intestinal crypt cells in contrary to differentiated cells, which express a full length integrin ß4A, express a novel integrin ß4A subunit lacking the intracellular COOH-terminal segment. This new variant associates with integrin α6 but is not functional for adhesion to laminin-5 (24). Function and detailed characteristics of these minor ß4 variants remain to be elucidated.

3. Integrin ß4 - expression and hemidesmosome formation

Integrin ß4 transcription is regulated from a promoter region characterized by a high G/C content and lack of TATA and CAAT boxes (25). Data base analysis of the 5'-flanking sequence of the integrin ß4 gene revealed the presence of several putative-binding sites for transcription factors, including AP1, Ets, MyoD, NFκB, and Sp1. Functional assays indicated that AP1 and Ets cooperate and activate integrin ß4 expression. Among the transcription factors comprising AP1, to integrin ß4 promoter bound c-Jun, JunB, JunD and a fos family protein, Fra-2 (25). Consistent with the differentiation-specific appearance of various AP1 transcription factors, JunB, JunD and Fra-2 are all expressed in proliferating basal keratinocytes, where integrin ß4 is also detected (26).

Additionally, integrin ß4 expression was shown to be up-regulated by EGF or re-expression of integrin ß1 and down-regulated by c-Myc activation and high calcium concentration (27-30). Expression of integrin ß4 was also down-regulated by human chorionic gonadotropin (hCG) in human endometrial adenocarcinoma cell line or acute hyperglycaemia in human endothelial cells (31,32). However, the intracellular signaling pathways involved in the regulation of integrin ß4 expression remain poorly understood.

All cells that express ß4 also express integrin α6 subunit. Integrin ß4 associates solely with integrin α6 to form a receptor for most of the known basement membrane laminins, binding to laminin 5 with the highest affinity (33). Integrin α6ß4 is expressed on epithelial cells, such as keratinocytes, endothelial cells and epithelial cells lining the gastrointestinal, respiratory and genitourinary tracts, but also on thymocytes, where its density parallels thymocyte maturation. Expression of integrin α6ß4 was also detected on Schwann cells and fibroblast in the peripheral nervous system, where the α6ß4 is involved in ensheathment and myelination of axons (34-38). Integrin α6ß4 has also been found on human first-trimester trophoblast and term placenta (39). During embryonic development and differentiation, integrin α6ß4 plays an important role in tissue and organ morphogenesis (40-43).

In epithelia, α6ß4 is typically concentrated at the ventral surface of the cells opposed to the basal membrane zone in specialized adhesion structures, called hemidesmosomes (Fig. 1). They are implicated in the stable attachment of the basal cells to the underlying basement membrane by connecting the intermediate keratin filaments with the extracellular matrix. Based on structural constituents, two subtypes of hemidesmosomes are distinguished. Type I or classical hemidesmosomes are present in basal keratinocytes of stratified squamous epithelia. At the core of the hemidesmosome is α6ß4 integrin, accompanied by cytoplasmic proteins, such as plectin, bullous pemphigoid antigen 180 (BP 180), transmembrane bullous pemphigoid antigen 230 (BP 230) and tetraspanin CD151 (44-46). Type II hemidesmosomes also termed hemidesmosome-like structures contain only α6ß4 and plectin and are found in simple epithelia and cultured epithelial cells (47,48).

Intracellularly, integrin ß4 interacts with the intermediate filament system, i.e. keratin filaments in epidermal cells or vimentin filaments in endothelial cells (49,50). *In vitro*, α6ß4 is also found on the leading lamellae of a migrating cell in association with filamentous actin (51).

4. Integrin ß4 - role in keratinocyte adhesion and migration

Generation of mice lacking ß4 integrin underscored its importance in cell adhesion to the underlying basement membrane. Mice with targeted deletion of the ß4 subunit died shortly after birth and displayed extensive detachment of the epidermis and other epithelia normally expressing α6ß4. The effect was most pronounced in the gastrointestinal tract (40,41). When integrin ß4 was missing, α6 integrin was barely detectable in mouse skin, suggesting that in the absence of ß4, α6 is unstable. Hemidesmosomes were completely absent in the ß4 null mouse keratinocytes and despite the presence of other components of hemidesmosomes such as BP180 and BP230, the keratin filaments failed to attach to the basal membrane (40,41).

Humans who have mutations in ß4 integrin gene develop junctional epidermolysis bullosa with pyloric atresia (JEB-PA), an autosomal recessive disorder with a high mortality rate. In addition to severe blistering at the dermo-epidermal junction in epidermolysis bullosa with pyloric atresia, recurrent erosions occur in the gastrointestinal and genitourinary tracts as well as in the cornea and the respiratory tract (52).

Patients with the lethal variant of the disorder usually have mutations leading to premature termination of integrin ß4, whereas missense mutations rather lead to nonlethal phenotypes (53). Immunofluorescent studies of affected skin revealed negative staining for integrin ß4 in lethal cases and positive but attenuated staining in nonlethal cases (53). The phenotype is strongly influenced by the position of the mutation in the protein functional domains. Lethal variant of JEB-PA has been described in a patient with homozygous in-frame 33-bp deletion in the ITGB4 gene. It has been hypothesized that the deletion interferes with folding of the mutated protein leading to its rapid degradation (54).

Recently, a 2 bp-deletion encompassing the third fibronectin III repeat in the cytoplasmic domain of integrin ß4 was shown to be clinically pathogenic and manifested by the predominant features of epidermolysis bullosa simplex (EBS) (55).

Analyses of the human and mice tissues lacking integrin ß4 indicated that other epidermal integrins seem unable to

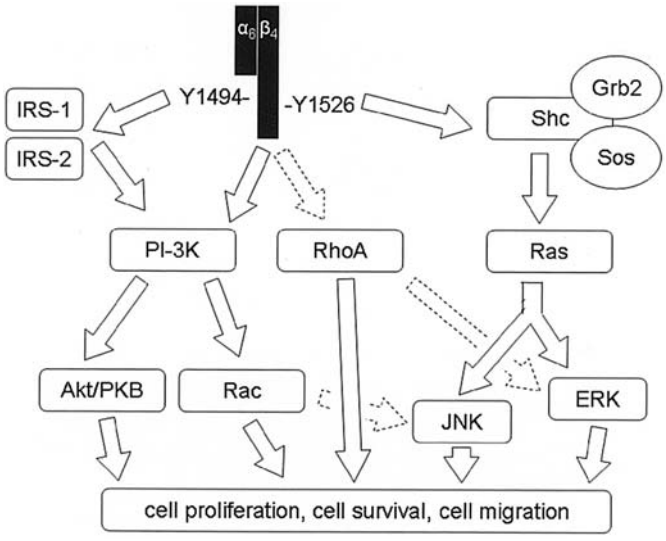

Figure 2. Integrin α6ß4 signaling. Details in text.

compensate for the loss of α6ß4 in providing the strong cell-substratum adhesion necessary to anchor the epidermis to the skin. The weak adhesion that does occur in the absence of α6ß4 is most likely attributable to integrin heterodimers containing integrin ß1 subunit, but this is not sufficient to withstand the extensive traumas to which the skin is routinely subjected.

Recent studies have demonstrated a novel and apparently contrasting function for integrin α6ß4 in the migration of epithelial cells. These findings revealed that in response to migratory stimuli, hemidesmosomes are disassembled and integrin α6ß4 associates with filamentous actin and localizes at the leading edge of a migrating cell in actin protrusions. It has been shown that integrin ß4 has the ability to promote formation and stabilization of the membrane protrusions associated with migration, as antibody-specific for α6ß4 inhibited the formation of filopodia and lamellipodia and inhibited cell migration (51). The observation that α6ß4 interacts with actin filaments suggested that it could transmit forces to the substrate generated by the acto-myosin system. This hypothesis was confirmed by the results of traction-force detection assays, indicating that the traction forces were exerted directly through α6ß4 in cells plated on laminin or on anti α6ß4-antibody, without the need to engage other integrins. The α6ß4-dependent forces were organized into the compression machine localized at the base of lamellae. Thus, in this way integrin α6ß4 may remodel the basement membrane components and this ability of α6ß4 could have important implications for the mechanism of cell migration and invasion (56).

Recently, it has become clear that self-association of the ß4 cytoplasmic domains is capable of ligand-independent signal transduction influencing migration of carcinoma cells. This possibility implies that ß4 signaling is not strictly limited to a specific matrix environment and might also occur on non-laminin substrates. In these cases, integrin ß4 might be phosphorylated after activation of the cells by growth factors (51).

The central molecule regulated by ß4 and implicated in epithelial motility is phosphatidylinositol 3'-kinase (PI-3K). In breast carcinoma cell lines, activation of PI-3K by integrin α6ß4 took place in cells expressing also erbB2, a receptor of the EGFR family. This finding indicated that cooperation of integrin α6ß4 with a specific growth factor receptor is required for PI-3K activation. Alternatively, activation of PI-3K was shown to involve the insulin receptor substrates (IRS-1 and IRS-2) and the tyrosine residue (Y1494) located at the third fibronectin type III repeat of integrin ß4 cytoplasmic tail (Fig. 2) (51,57). Activation of PI-3K by α6ß4 may stimulate the function of other integrins, especially α3ß1, that are important for epithelial migration. Additional signaling pathways by which α6ß4 stimulates cell migration are mitogen-activated protein kinase (MAPK) and Rac and RhoA GTPases (51,57).

Integrin ß4 was initially identified as a tumor-associated antigen (TS180) associated with metastasis. However, given its widely known function of mediating adhesive contacts in epithelia, this role of integrin ß4 was long not anticipated (58). Expression of integrin ß4 is maintained or even increased in several types of invasive and metastatic carcinoma and the expression level often correlates with tumor aggressiveness (57). In the skin, an association between enhanced ß4 integrin expression and tumor progression has been demonstrated for squamous cell carcinoma (59,60). In a usually non-metastatic basal cell, carcinoma of the skin ß4 integrin expression is reduced (61,62).

5. Integrin ß4, role in keratinocyte growth and differentiation

Several observations indicate that α6ß4 integrin is implicated in transducing signals from the extracellular matrix that do not only control the cytoskeleton organization and assembly of hemidesmosomes but also influence cell proliferation, survival and differentiation (63). In normal undamaged epidermis the expression of α6ß4 is restricted to the basal cell layer, which contains cells endowed with proliferative capacity (10,34). As cells leave the basal layer, hemidesmosomes disappear and no integrins are expressed (12). It is also known that epidermal stem cells are more adhesive to the extracellular matrix than their transit-amplifying daughter cells, committed to differentiation (64). Thus, the restricted integrin ß4 expression pattern implies its involvement in the regulation of cell proliferation.

Keratinocytes exit the cell cycle and begin their differentiation program when they detach from the basement membrane to migrate to the upper epidermal layers (65). This process could be replicated *in vitro* by depriving cultured keratinocytes of anchorage to their endogenously produced matrix, which is rich in laminin-5, a ligand for α6ß4 integrin (66).

A proposed α6ß4-mediated signaling cascade affecting keratinocyte proliferation seems to lead via the activation of Ras-MAPK pathways. Activation of α6ß4 in response to ligation and adhesion leads to recruitment of Shc, Grb2 and Sos, activation of Ras and stimulation of the MAPK, Jnk and Erk signaling cascades (67). The tyrosine residue (Y1526) located in the third fibronectin type III repeat of integrin ß4 was reported to be the binding site for Shc (Fig. 2) (51).

Signaling through α6ß4 has also been proposed to be independent of its adhesive functions due to its ability to

interact with activated receptor tyrosine kinases, i.e. EGF receptor or Met. In this model the cytoplasmic domain of integrin ß4 becomes phosphorylated by the activated receptors and serves as an amplifier of their mitogenic and motogenic signals (reviewed in ref. 68).

The primary effect of complete ß4 integrin deletion is massive epidermal blistering associated with degeneration. The epidermal regions that remain attached to the basement membrane also contained cells presenting signs of degeneration similar to those described when keratinocytes could not adhere to their substratum (40). These data implied that survival of mitotically active cells of stratified squamous epithelia is dependent upon ß4 integrin, whereas its lack makes the cells susceptible to degeneration.

Furthermore, Murgia *et al* (69) found that keratinocytes from mouse embryos carrying a targeted deletion of the cytoplasmic tail of the ß4 subunit display a significant proliferative defect. However, according to Di Persio *et al* (70) decrease of keratinocyte proliferation was not observed in ß4-null embryos.

In addition, Raymond *et al* (71) have recently shown using a conditional knockout mouse strain, in which the integrin ß4 gene was inactivated only in small stretches of skin, that epidermal differentiation and proliferation was normal in the absence of integrin α6ß4 provided that cell adhesion was not compromised. They concluded that there is no evidence for a role of α6ß4 integrin in controlling cell proliferation and survival which is independent of its adhesive function (71).

In human hyperproliferative skin pathologies, for example during wound healing, in psoriatic lesions or in squamous cell carcinoma, integrins have an aberrant expression pattern and beside the basal cell layer appear also in suprabasal differentiating cells (11). Recently, it has been demonstrated that the suprabasal α6ß4 expression has positive influence on the susceptibility of keratinocytes to chemical carcinogenesis. In response to treatment with a phorbol ester tumor promoter, the suprabasal α6ß4 integrin enhanced the proliferative response of basal cells leading to the formation of papillomas and carcinomas. The effect of suprabasal α6ß4 on cell proliferation involved disruption of TGF-ß-mediated growth inhibition by suppressing nuclear translocation of activated Smad2/3 proteins. It has been shown that for the inhibition of TGF-ß signaling suprabasal α6ß4 integrin required PI-3K activity and E-cadherin-mediated cell-cell adhesion (72).

In case of carcinoma cells, α6ß4-mediated signals appear to have different effects on cell behavior depending on the cell type and their differentiation status. For example, in a keratinocyte-derived A431 human epidermoid carcinoma cell line activation of α6ß4 with a monoclonal antibody directed against ß4 protected the cells from apoptosis through activation of PI-3K and Akt kinase signaling pathway (73). In squamous cell carcinoma-derived HPV-18 positive HeLa cells ligation of α6ß4 by laminin 5 promoted transcription from the fos serum response element and cell proliferation (67). Similarly, expression of α6ß4 on epithelial cells lines derived from colon, breast and thyroid carcinomas positively correlated with proliferation. On the contrary, expression of the ß4 subunit in the rectal or gastric carcinoma cells was associated with apoptosis induction (reviewed in ref. 74).

6. Integrin ß4 and human papillomaviruses

Human papillomaviruses (HPVs) induce a wide spectrum of epithelial proliferative lesions ranging from benign warts to invasive carcinoma (75). The targets of HPV infection are stratified epithelia at different anatomical sites. According to the preferred site of tropism, two categories of HPV can be distinguished, the cutaneous HPVs and the mucosal HPVs. The latter primarily infect epithelia of anogenital region, oral cavity or upper respiratory tract (76).

Infection by HPVs is believed to occur through micro-traumas in the stratified epithelium, exposing the basal cells to entry for viruses. The receptor for HPV entry has not been functionally identified. However, the α6 integrin complexed with either ß1 or ß4 integrin has been proposed to be the HPV receptor (77). During wound healing, expression of integrins is up-regulated in epithelial cells, which makes them good candidates. Nevertheless, it should be underlined that no functional studies have yet shown the α6ß1 or α6ß4 integrins to mediate HPV entry.

Most lesions induced by HPVs in immunocompetent individuals are benign warts, which display self-limited growth and usually regress, spontaneously or after treatment (78). On the contrary, infection with cutaneous HPV types, i.e. HPV5 and HPV8 is associated with the development of skin carcinoma in patients suffering from an inherited skin disease, epidermodysplasia verruciformis (EV) (79,80).

Furthermore, specific mucosal HPV types play a central role in anogenital carcinogenesis, especially in carcinoma of the uterine cervix, the second leading cause of cancer-related deaths in women worldwide (81). Approximately 80% of cervical cancers are associated with four HPV types, i.e. HPV16, 18, 31 and 45 (82). Oncogenic genital HPV types, especially HPV type 16, are also causally associated with a fraction of vaginal, vulvar, penile, and anal cancers (83).

In vitro study of HPV16 oncogene E6/E7-immortalized esophageal keratinocytes revealed a lower level of integrin ß4 in these cells. Reduced expression of ß4 integrin led to more rapid proliferation and anchorage-independent growth potential, which was interpreted by the authors as a critical step in malignant progression (84).

In situ studies of vulvar warts and neoplasia of the uterine cervix gave different results. In vulvar warts associated with HPV infection up-regulation of integrin ß4 expression was detected. In these lesions, expression of ß4 integrin was found at the periphery of basal cells, in epibasal and spinous layers of warts (85).

Immunohistochemical analysis of integrin ß4 expression pattern in cervical intraepithelial neoplasia revealed its presence in the upper cell layers of cervical epithelium. The extent of extrabasal staining for integrin ß4 corresponded with the grade of cervical intraepithelial neoplasia (CIN) (86,87). Similar results were obtained in HPV-associated cervical intraepithelial neoplastic lesions. In this study, high expression of integrin ß4 was found in HPV16-associated CIN III and invasive tumors as compared with low integrin ß4 levels in CIN I and II (88). In the analyzed lesions, expression of integrin ß4 reversely correlated with the presence of viral E2 transcripts.

The papillomavirus E2 protein is a transcription factor involved in regulation of viral replication and transcription.

E2 functions predominantly as a repressor of viral E6, E7 oncogene expression in the mucosal high risk HPVs. The E2 open reading frame is disrupted in HPV-induced cervical carcinoma as a result of viral genome integration into the cellular genome. Loss of E2 leads to up-regulation of viral oncogene E6 and E7 transcription, an event considered to play a key role in cellular transformation (89).

Recently, we have found that the E2 protein from cutaneous HPV8 and mucosal high risk HPV18 is an important regulator of human integrin ß4 expression. Expression of HPV8 or HPV18 E2 protein in human keratinocytes led to a dose-dependent reduction of integrin ß4 expression. In case of HPV8 E2, the suppression at least partially resulted from direct interactions between E2 and the human integrin ß4 promoter (90).

We hypothesized that in papillomavirus infection, down-regulation of integrin ß4 may provide a signal inducing differentiation and enabling the virus to begin its productive life cycle, which takes place exclusively in the suprabasal layers of stratified epithelia. Loss of E2 expression as a result of progression of HPV-induced lesions towards malignancy may lead to deregulation of integrin ß4 expression and its appearance in the suprabasal layers. The mechanism involved in the aberrant expression pattern of integrin ß4 as shown for high grade CIN lesions (88) still has to be defined.

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