Matrix Biology 30 (2011) 118–125

Contents lists available at ScienceDirect

Matrix Biology

journal homepage: www.elsevier.com/locate/matbio

# Induction of type XVI collagen expression facilitates proliferation of oral cancer cells

Sabine Ratzinger [c,d], Susanne Grässel [b,c], Albert Dowejko [a,c], Torsten E. Reichert [a], Richard J. Bauer [a,c,*]

[a] Dept. of Oral and Maxillofacial Surgery, University Hospital Regensburg, Germany
[b] Dept. of Orthopaedic Surgery, Experimental Orthopaedics, University Hospital Regensburg, Germany
[c] Centre for Medical Biotechnology, BioPark I, Regensburg, Germany
[d] Dept. of Othorhinolaryngology, University Hospital Regensburg, Germany

ARTICLE INFO

*Article history:*
Received 11 October 2010
Received in revised form 10 January 2011
Accepted 11 January 2011

*Keywords:*
Cell-matrix adhesion
Cell cycle
Invasion
Oral oncology
Collagen XVI

ABSTRACT

Type XVI collagen belongs to the family of fibril-associated collagens with interrupted triple helices (FACIT). Recently, high affinity to integrin alpha1beta1 has been shown allowing cells expressing those integrins to attach and spread on recombinant type XVI collagen. Here, we show that type XVI collagen is overexpressed in dysplastic areas of mucosal epithelium from oral squamous cell carcinoma (OSCC) patients. Induction of its expression in OSCC cell lines (COLXVI cells) leads to an increased expression of Kindlin-1. Moreover, we demonstrate a significantly increased Kindlin-1/beta1-integrin interaction. Additionally, we detected a higher number of activated beta1-integrins in COLXVI cells and found a neo-expression of alpha1 integrin subunit on these cells. FACS analysis revealed a significantly higher amount of COLXVI cells in S-phase and G2/M-phase 6 h after synchronisation leading to a markedly higher proliferation activity. Blocking beta1-integrins with a specific antibody resulted in reduced proliferation of COLXVI cells. In summary, we demonstrate that overexpression of type XVI collagen in aberrant oral keratinocytes leads to Kindlin-1 induction, increased Kindlin-1/beta1-integrin interaction, integrin activation and subsequently to a proliferative cellular phenotype.



## 1. Introduction

Head and neck cancer is one of the 10 most frequent cancers worldwide and affects more than 500,000 people each year. OSCC represents >95% of all head and neck cancer forms and within the past ten years its incidence has increased more than 50% (Bray et al., 2002; Parkin et al., 2005). Despite multimodal therapies including excision of malignant tissue and a combination of radio- and chemotherapy the 5-year survival rate is still only 53% (Parkin et al., 2005). A high rate of patients remains with a poor response to therapy and high frequencies of relapses (Bettendorf et al., 2004). Moreover, frequent lymph node metastasis involving migration and invasion of aberrant cells from the primary neoplasm to distant sites results in poor prognosis (Bray et al., 2002). Our aim was to find molecular cues which are responsible for the relatively high invasive, migratory and proliferative activity of OSCC cells. In this study we found type XVI collagen to have a proliferative effect on OSCC cells. Type XVI collagen belongs to the fibril-associated collagens with interrupted triple helices (FACIT) family of collagens (Pan et al., 1992). It constitutes a minor component of the extracellular matrices (ECM) of skin, intestinum and cartilage. The protein is incorporated into structurally and functionally discrete matrix aggregates in skin where it is localized in the dermal epidermal junction zone of the papillary dermis (Kassner et al., 2003; Grassel et al., 1999). Its location suggests that type XVI collagen plays an active role in anchoring microfibrils to basement membranes as type XVI collagen is not only produced by fibroblasts but also by keratinocytes similar to type VII collagen. Although many matrix proteins self-assemble and thus contribute to the organization of supramolecular networks, cells also affect the architecture of the ECM networks by grasping and moving ECM proteins. These interactions are modified by integrins. Type XVI collagen has been shown to interact with integrins alpha1beta1 (Eble et al., 2006). In the present study we have demonstrated that the observed proliferative effect in OSCC clones was conferred via integrins. As cell surface proteins that mainly bind extracellular matrix (Hynes, 2002), integrins are heterodimeric transmembrane glycoproteins which connect the actin cytoskeleton to the extracellular matrix. Depending on signals from inside or outside the cell the integrin binding pocket can adopt states from low to high affinity. Besides the mechanical anchorage of the cell, integrins transduce signals from the ECM into the cell and vice versa (Guo and Giancotti, 2004). By utilizing integrins as receptors, the components of the ECM not only induce cellular reactions such as cell adhesion, migration, proliferation and gene activation in an outside-in fashion, but can also be remodelled by the cells in an inside-out manner (Yamada et al., 1982).

Kindlin-1 belongs to a novel family of cytoplasmic adaptor proteins consisting of three members (Kindlin-1,2,3) (Ussar et al., 2006). They localize to cell-matrix adhesion sites where they regulate

* Corresponding author. University Hospital Regensburg, Dept. of Oral and Maxillofacial Surgery, Josef-Engert-Strasse 9, ZMB/Biopark I, 93053 Regensburg, Germany. Tel.: +49 941 943 1627; fax: +49 941 943 1631.
*E-mail address:* richard.bauer@klinik.uni-regensburg.de (R.J. Bauer).

0945-053X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.matbio.2011.01.001

integrin function through modulation of integrin signalling (Has et al., 2009; Petricca et al., 2009). Recently, it has been demonstrated that Kindlin-1 seems to mediate important signalling cues in keratinocytes where it is involved in the regulation of polarity, proliferation and motility of epidermal keratinocytes (Herz et al., 2006). Progressive malignancy disturbs physiological interactions between cells and ECM. Very recent data suggested that type XVI collagen plays a role in tumour progression of glioblastoma (Senner et al., 2008). Our data showed abundant type XVI collagen in dysplastic areas from primary OSCC tumours. Therefore, the data implied that aberrantly expressed type XVI collagen is a candidate responsible for facilitating the emergence of abnormal cellular behaviour in terms of proliferation. To address this issue and to investigate what functional and molecular influence abundantly expressed type XVI collagen had on OSCC cells we overexpressed it in the type XVI collagen deficient OSCC cell line PCI13.

For the first time, we demonstrated an impact of excessive expression of type XVI collagen on proliferation of abnormal keratinocytes presumably via Kindlin-1 and beta1-integrin interaction which was correlated with enhanced integrin activation.

## 2. Results

### 2.1. Type XVI collagen is overexpressed in epithelium from OSCC patients

The poor prognosis of oral squamous cell carcinoma is mostly due to lack of prognostic tools available. It would be of great benefit to find biomarkers which allow a timely identification of the initial stages of cancer progression. Because type XVI collagen lately emerged as a molecule which plays a role in carcinogenesis we analyzed its expression in oral tissue from OSCC patients. Therefore, we performed an immunohistochemical analysis of paraffin sections from OSCC patients and healthy controls. Representative images taken from dysplastic areas of OSCC tumour sections showed that type XVI collagen was highly expressed in the superficial epithelial layers (Fig. 1a, b). The staining was clearly visible in the extracellular matrix with an additional diffuse intracellular staining (Fig. 1b). Additionally, we observed a strong decrease of type XVI collagen staining in the basement membrane in all observed tissues of OSCC patients (Fig. 1a, white arrow). In normal mucosa, type XVI collagen was not detected around keratinocytes of superficial epithelial layers instead there was a strong staining for type XVI collagen restricted to the dermal–epidermal junction (Fig. 1c, d, white arrows). Furthermore, we performed immunoblot analysis with lysates from frozen tissues of OSCC patients and healthy controls. The 180 kDa form (lacks the NC 11 domain) of type XVI collagen was clearly detected in OSCC patient material (Fig. 1e, lanes 1–3) whereas there was only a weak signal in the lysates from healthy controls.

### 2.2. Overexpression of type XVI collagen in OSCC cell line PCI13

Our observation that type XVI collagen was highly expressed in dysplastic areas of OSCC patients raised the question whether these abundant amounts had a functional impact on the cells. To examine what impact type XVI collagen overexpression had on the cellular behaviour of aberrant oral keratinocytes, we used a model system where we stably overexpressed the molecule in the type XVI collagen deficient OSCC cell line PCI13. A collagen XVI overexpressing cell culture model was employed because it enabled us to detect well defined specific functional effects, which is more difficult in knockdown experiments with a cell line bearing endogenous collagen XVI expression. Fig. 2a shows an immunoblot analysis of three type XVI collagen overexpressing cell clones, denominated as COLXVI C1–3 and two mock controls, denominated as mock1 and mock2. To demonstrate that type XVI collagen is released from the cells we also analyzed the supernatant of our clones where we detected the 220 kDa full-length and the 180 kDa truncated forms of type XVI collagen (exemplified with COLXVI C1). In summary, these results

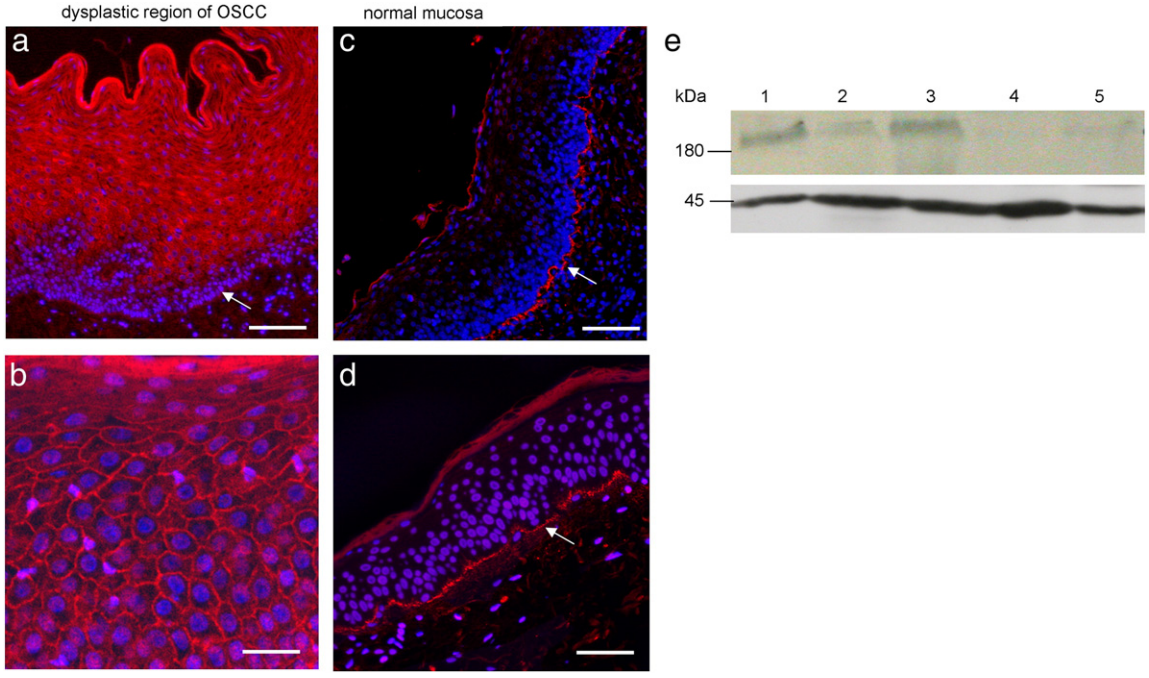


**Fig. 1.** Immunofluorescent stainings revealed a profound overexpression of type XVI collagen in dysplastic areas taken from oral tissue sections of OSCC patients (a). Type XVI collagen staining signals are detected in the extracellular matrix and intracellular (b, bar: 200 μm, magnification 40×). On the contrary, there is only weak type XVI collagen expression in normal superficial oral epithelium (c,d). A decrease of type XVI collagen basement membrane staining was observed compared to normal mucosa (see white arrows a–d, bar: 600 μm, magnification 10×). The strong red staining in the upper lining of image (a) is due to auto-immunofluorescence of the superficial layer of dead cells. Images are representative images from 10 OSCC patients. e: Immunoblot analysis shows upregulated type XVI collagen expression in tissue lysates from three OSCC patients (upper panel, lanes 1–3) in contrast to two healthy controls with little expression (upper panel, lanes 4–5). The lower panel shows beta-actin as loading control.