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Original Articles

α integrin targeting for radiosensitization of three-dimensionally grown human head and neck squamous cell carcinoma cells

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ABSTRACT

Integrin cell adhesion molecules play a crucial role in tumor cell resistance to radio- and chemotherapy and are therefore considered attractive targets for cancer therapy. Here, we assessed the role of β1 integrin-interacting α integrin subunits in more physiological three-dimensional extracellular matrix grown head and neck squamous cell carcinoma (HNSCC) cell cultures for evaluating cytotoxic and radiosensitizing potential. α2, α3, α5 and α6 integrins, which are overexpressed in HNSCC according to Oncomine database analysis, were coprecipitated with β1 integrin. More potently than α2, α5 or α6 integrin inhibition, siRNA-based α3 integrin targeting resulted in reduced clonogenic cell survival, induced apoptosis and enhanced radiosensitivity. These events were associated with diminished phosphorylation of Akt, Cortactin and Paxillin. Cell line-dependently, simultaneous α3 and β1 integrin inhibition led to higher cytotoxicity and radiosensitization than α3 integrin blocking alone. Stable overexpression of wild-type and constitutively active forms of the integrin signaling mediator focal adhesion kinase (FAK) revealed FAK as a key determinant of α3 integrin depletion-mediated radiosensitization. Our findings show that α3 integrin is essentially involved in HNSCC cell radioresistance and critical for a modified cellular radiosensitivity along with β1 integrins.

Introduction

Head and neck squamous cell carcinomas (HNSCC) are a heterogeneous group including tumors of different anatomical regions [1]. About 40% of all HNSCCs are diagnosed at an advanced stage, highly limiting patient cure even after multimodal therapy concepts including surgery and radiochemotherapy. Additionally, intrinsic therapy resistance of HNSCC hampers treatment outcome demanding the identification of the underlying mechanisms. We and others have previously shown that adhesion molecules of the integrin family and their associated adaptor proteins are critically involved in mediating resistance to radio- and chemotherapy [2–12].

Integrins are heterodimeric transmembrane receptors consisting of an α and a β subunit [13]. Currently, there are 18 α and 8 β subunits known, together forming 24 different receptors with distinct specificity for extracellular matrix (ECM) proteins [13,14]. Via recruitment of signaling molecules and adaptor proteins to cytoplasmatic tails of β subunits, integrins contribute to the regulation of cell survival, proliferation, adhesion, motility as well as cancer therapy resistance [13–19].

In HNSCC development and progression, integrins seem to play major roles [20]. Recently, we showed that β1 integrin targeting with inhibitory antibodies resulted in radiosensitization of HNSCC [4]. Dephosphorylation of focal adhesion kinase (FAK) and dissociation of a FAK/Cortactin protein complex presented key mechanistic events [4]. However, only little is known about how α subunits as obligate part of an integrin heterodimer are involved in radioresistance mechanisms. For instance, inhibition of α5β1 integrin binding to fibronectin with an α5 integrin specific antibody radiosensitized breast cancer cells [6]. αV integrin as part of αVβ3 and αVβ5 integrin heterodimers can be targeted with the pentapeptide compound Cilengitide [21], an approach impairing angiogenesis. Nevertheless, studies specifically investigating the

Abbreviations: 3D, three-dimensional; 2D, two-dimensional; siRNA, small interfering RNA; HNSCC, head and neck squamous cell carcinoma; FAK, focal adhesion kinase; lrECM, laminin-rich extracellular matrix; IP, immunoprecipitation.

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role of α subunits in HNSCC radiation survival and resistance are missing.

In the present work, we addressed α integrin complexation with β1 integrin and how this contributes to radioresistance of 3D lrECM grown HNSCC cells. Here, we show that α2, α3, α5 and α6 integrins coprecipitated with β1 integrin and are overexpressed in HNSCC. Intriguingly, α3 integrin inhibition showed the highest potential to reduce cell survival and increase cellular radiosensitivity cell line-dependently. Moreover, α3 integrin depletion resulted in reduced phosphorylation of FAK, Akt, Cortactin and Paxillin and stable overexpression of wild-type (wt) and constitutively active (ca) forms of FAK were able to prevent radiosensitization from α3 integrin inhibition.

Materials and methods

Antibodies and reagents

Antibodies used for Western blotting include Akt, phospho-Akt S473, Cortactin, phospho-Cortactin Y421, FAK, phospho-FAK Y397, α6 integrin, β1 integrin (rabbit), ERK1/2, phospho-ERK1/2 T202/Y204, Paxillin, phospho-Paxillin Y118 (Cell Signaling), GFP (Abcam), α2 integrin, β1 integrin (mouse; BD), α3 integrin, α5 integrin (US Biological), β-actin (Sigma-Aldrich), horseradish peroxidase-conjugated donkey anti-rabbit and sheep anti-mouse antibodies (Amersham). Antibodies for immunofluorescence staining include α3 integrin (mouse; BD), α3 integrin (rabbit; US Biological), β1 integrin (biomol), AlexaFluor594-labeled AIIB2 (as described in [22]), AlexaFluor488 anti-rabbit and AlexaFluor488 anti-mouse antibodies (Invitrogen). For immunoprecipitation AIIB2 and rat immunoglobulin G (IgG; Santa Cruz Biotechnology Inc.) were used. Antibodies were purchased as indicated.

Cell culture

Human HNSCC cell lines UTSCC5, UTSCC14, UTSCC15, Cal33 and HSC4 were kindly provided by R. Grenman (Turku University Central Hospital, Turku, Finland). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing GlutaMAX-I supplemented with 10% fetal calf serum and 1% non-essential amino acids (NEAA; PAA) at 37 °C in a humidified atmosphere containing 7% CO_2 as published [23]. In all experiments, asynchronously and exponentially growing cells were used.

Immunoprecipitation of α integrins

Immunoprecipitation (IP) was performed as described in Ref. 4. In brief, cells were treated with AIIB2 or non-specific IgG for 1 h prior to cell lysis using Cell Lysis Buffer (Cell Signaling). Cell lysates were added to the Protein G-Agarose beads (Sigma) and incubated overnight at 4 °C, followed by IP and Western blot analysis of AIIB2-bound β1 integrin and interacting proteins.

Total protein extracts and Western blotting

Protein extracts were harvested using modified radioimmunoprecipitation assay (RIPA) buffer [50 mmol/l Tris–HCl (pH 7.4), 1% Nonidet-P40, 0.25% sodium deoxycholate, 150 mmol/l NaCl, 1 mmol/l EDTA, complete protease inhibitor cocktail (Roche), 1 mmol/l $NaVO_4$ and 2 mmol/l NaF] as published [3]. SDS-PAGE, transfer of proteins onto nitrocellulose membranes (Schleicher and Schuell), probing and detection of specific proteins with indicated antibodies and SuperSignal West Dura Extended substrate (Pierce) were carried out as published previously [24].

Oncomine database analysis

Oncomine transcriptome database (www.oncomine.org) was used to determine *ITGA2, ITGA3, ITGA5* and *ITGA6* mRNA expression in HNSCC and corresponding normal tissues. Two studies for each α integrin subunit were chosen and depicted.

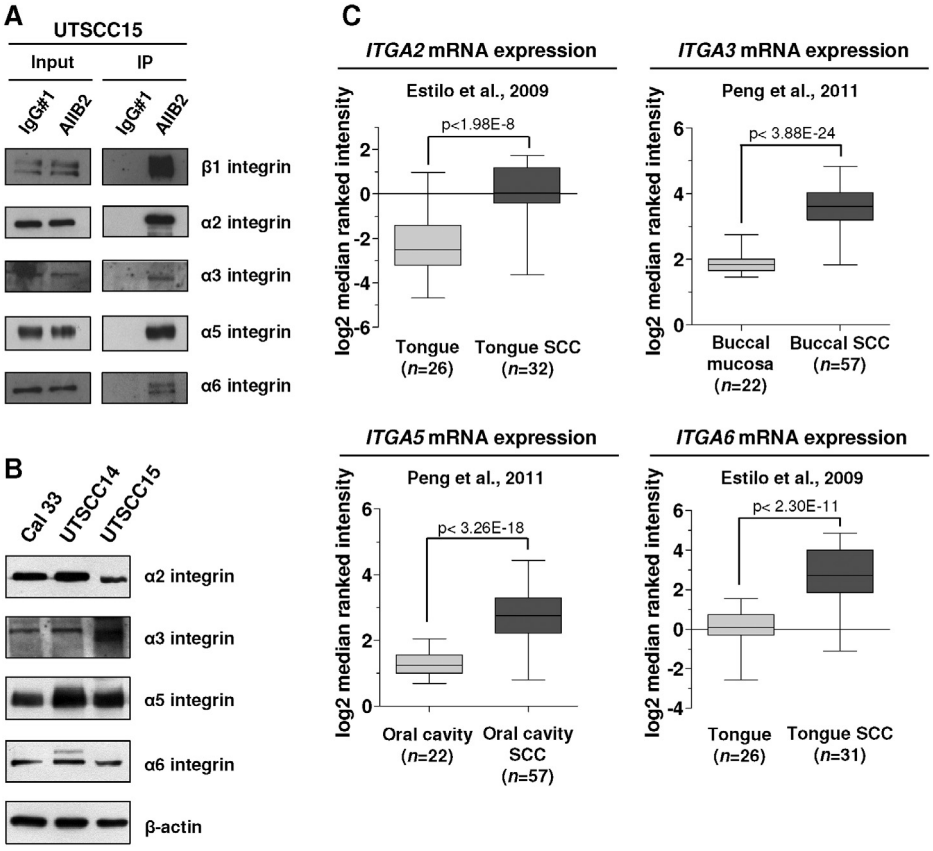

Fig. 1. Distinct α integrins interact with β1 integrin and are overexpressed in HNSCC. (A) IP of β1 integrin in UTSCC15 using AIIB2 or IgG#1 control. β1, α2, α3, α5 and α6 integrins were detected by Western blot analysis of the precipitates using specific antibodies. Input lysates are shown as control. (B) Western blot analysis of α2, α3, α5 and α6 integrin expression in the HNSCC cell lines Cal33, UTSCC14 and UTSCC15. β-actin served as loading control. (C) Oncomine database analysis of mRNA expression of α2, α3, α5 and α6 integrins in HNSCC tumor tissues compared to corresponding normal tissue (Additional data and appropriate publications are listed in the supplemental information.).