Cell Adhesion-Mediated Radioresistance (CAM-RR)

Extracellular Matrix-Dependent Improvement of Cell Survival in Human Tumor and Normal Cells in Vitro

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Background: Cell-extracellular matrix (ECM) contact is thought to have great impact on cellular mechanisms resulting in increased cell survival upon exposure to ionizing radiation. Several human tumor cell lines and normal human fibroblastic cell strains of different origin, all of them expressing the wide-spread and important integrin subunit β1, were irradiated, and clonogenic cell survival, β1-integrin cell surface expression, and adhesive functionality were investigated.
Material and Methods: Human tumor cell lines A172 (glioblastoma), PATU8902 (pancreas carcinoma), SKMES1 (lung carcinoma), A549 (lung carcinoma), and IPC298 (melanoma) as well as normal human skin (HSF1) and lung fibroblasts (CCD32) and human keratinocytes (HaCaT) were irradiated with 0–8 Gy. Besides colony formation assays, β1-integrin cell surface expression by flow cytometry and adhesive functionality by adhesion assays were analyzed.
Results: All cell lines showed improved clonogenic survival after irradiation in the presence of fibronectin as compared to plastic. Irradiated cells exhibited a significant, dose-dependent increase in β1-integrin cell surface expression following irradiation. As a parameter of the adhesive functionality of the β1-integrin, a radiation-dependent elevation of cell adhesion to fibronectin in comparison with adhesion to plastic was demonstrated.
Conclusion: The in vitro cellular radiosensitivity is highly influenced by fibronectin according to the phenomenon of cell adhesion-mediated radioresistance. Additionally, our emerging data question the results of former and current in vitro cytotoxicity studies performed in the absence of an ECM. These findings might also be important for the understanding of malignant transformation, anchorage-independent cell growth, optimization of radiotherapeutic regimes and the prevention of normal tissue side effects on the basis of experimental radiobiological data.

Key Words: Radioresistance · β1-integrin · Extracellular matrix · Tumor cells · Ionizing radiation

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Zelladhäsionsbedingte Radioresistenz (CAM-RR). Verbesserung des Zellüberlebens von Tumor- und Normalzellen in Abhängigkeit von der extrazellulären Matrix in vitro

Hintergrund: Es wird angenommen, dass Zellkontakt zu einer extrazellulären Matrix zelluläre Mechanismen stark beeinflusst, die u.a. zu einem verbesserten Zellüberleben nach Bestrahlung führen können. Humane Tumorzelllinien und Fibroblastenzellstämme, von denen alle die weit verbreitete und wichtige Integrin-Untereinheit β1 exprimieren, wurden bestrahlt und das klonogene Überleben, die β1-Integrin-Oberflächenexpression und die adhäsive Funktion des Rezeptors untersucht.
Material und Methodik: Die humanen Tumorzelllinien A172 (Glioblastom), PATU8902 (Pankreaskarzinom), SKMES1 (Bronchialkarzinom), A549 (Bronchialkarzinom) und IPC298 (Melanom) sowie die normalen Haut- (HSF1) und Lungenfibroblasten (CCD32) sowie humane Keratinozyten (HaCaT) wurden mit 0–8 Gy bestrahlt. Neben Koloniebildungsassays wurden die β1-Integrin-Oberflächenexpression mittels Flusszytometrie und die Rezeptorfunktionalität in Adhäsionsassays analysiert.
Ergebnisse: Fibronektin führte im Vergleich zu Plastik in allen getesteten Zellen zu einem gesteigerten Überleben nach Bestrahlung. In bestrahlten Zellen konnte ein signifikanter und dosisabhängiger Anstieg der β1-Integrin-Oberflächenexpression beobachtet werden. Im Adhäsionstest zeigte sich parallel dazu eine bestrahlungsbedingte Zunahme der Zelladhäsion an Fibronektin.
Schlussfolgerung: Die zelluläre Radiosensibilität in vitro wird stark durch Fibronektin im Sinne einer zelladhäsionsbedingten Radioresistenz beeinflusst. Des Weiteren stellen unsere Daten früher und heute in Abwesenheit einer extrazellulären Matrix durchgeführte In-vitro-Zytotoxizitätsstudien in Frage. Die hier gewonnenen Erkenntnisse könnten darüber hinaus für das Verständnis der malignen Transformation, des adhäsionsunabhängigen Zellwachstums, der Optimierung radiotherapeutischer Konzepte und der Prävention von Normalgewebsreaktionen auf der Basis experimenteller radiobiologischer Daten von Interesse sein.

Key Words: Strahlenresistenz · β1-Integrin · Extrazelluläre Matrix · Tumorzellen · Ionisierende Strahlung

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Introduction

It is thought that the presence of an extracellular matrix (ECM) confers an improved cellular status of resistance to cell-damaging agents such as ionizing radiation or drugs in vitro [9, 21, 41]. This phenomenon has been shown for several different types of cells and agents [13, 18, 21, 37, 44].

Cell adhesion to the ECM or isolated matrix components, e.g., fibronectin (FN), is mediated particularly, among other cellular adhesion molecules such as intercellular adhesion molecules, vascular adhesion molecule-1 [6] or proteins of the CD44 family [5], by the integrin receptor family [24]. Integrin clustering initiated subsequent to cell attachment to the ECM activates cytoplasmic effectors that are capable of regulating survival, proliferation, migration, adhesion, and differentiation, and many more [1, 8, 19, 23, 24, 33, 34]. Specific integrin signaling in convergence with growth factor signaling modulates these critical cellular functions [3, 31, 39, 40].

Ionizing radiation highly affects the wide-spread β1-integrin signaling pathway on different levels [9, 30]. The integrin subunit β1 is upregulated by irradiation on the cell surface providing an improved adhesion of cells to ECM proteins such as FN or laminin. The downstream located protein kinases integrin-linked kinase [20], protein kinase B/Akt [15] and glycogen synthase kinase-3β [43] are stimulated by irradiation in a

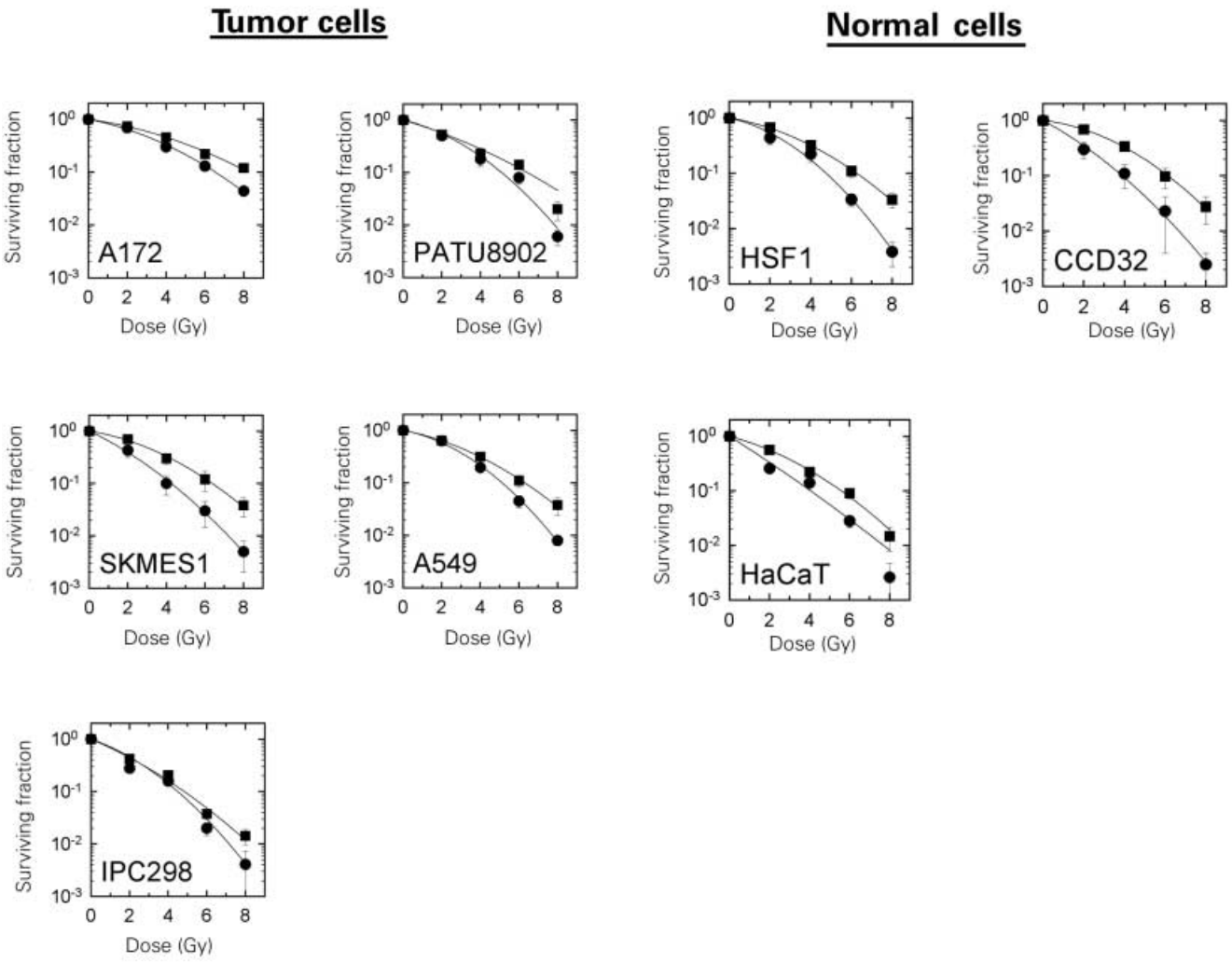

Figure 1. Comparison of the clonogenic survival of cells grown on plastic (●) or fibronectin (FN; ■) showed an increase in survival of cells attached to the extracellular matrix (ECM) protein FN. Doses ≥ 4 Gy for A172, SKMES1 and A549 cells, ≥ 6 Gy for PATU8902 and IPC298 cells, and ≥ 2 Gy for normal fibroblasts and keratinocytes grown on FN resulted in a $p < 0.05$ compared to cells grown on plastic. Each data point represents the mean ± SD of three independent experiments (n = 18).

Abbildung 1. Der Vergleich des klonogenen Zellüberlebens von auf Plastik (●) oder Fibronektin (FN; ■) wachsenden Zellen zeigte einen Überlebensvorteil für Zellen, die auf dem Matrixprotein FN wuchsen. Ein $p < 0,05$ wurde für Dosen ≥ 4 Gy bei A172, SKMES1 und A549 Tumorzellen, ≥ 6 Gy für PATU8902 und IPC298 Tumorzellen und ≥ 2 Gy für Fibroblasten und Keratinozyten, die FN-Kontakt hatten, im Vergleich zu Plastik errechnet. Jeder Datenpunkt zeigt den Mittelwert ± Standardabweichung von drei unabhängigen Experimenten (n = 18).

matrix-dependent manner clearly indicating their participation in the modulation of signaling cascades determining the individual cellular radiosensitivity.

In this study, we provide molecular data of six human tumor cell lines and five normal human fibroblastic cell strains with a focus on ECM-dependent cell survival, β1-integrin cell surface presentation, and the cellular adhesion of theses cells to the ECM protein FN as a function of radiation exposure. These data strongly support the important role of the integrin subunit β1 for radiation-modulated cell adhesion via a functional receptor and for ECM-dependent changes in cellular radiosensitivity.

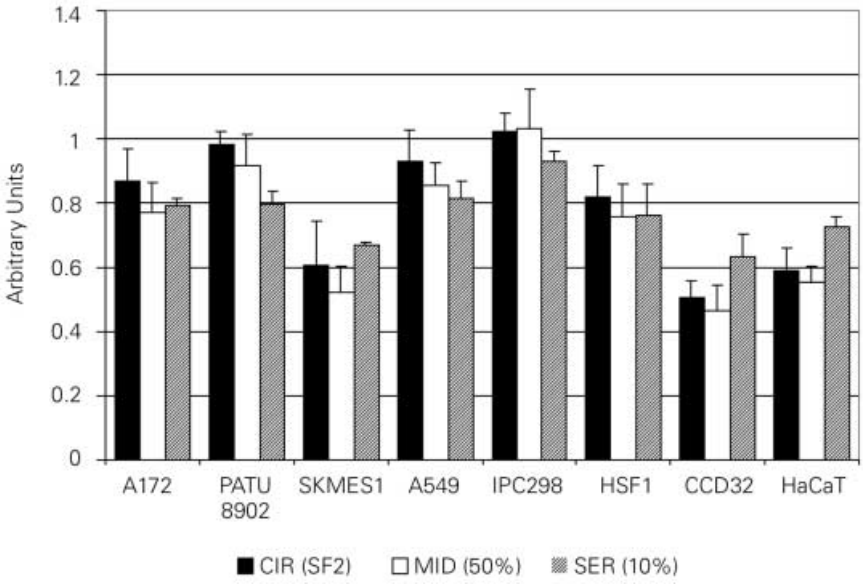

Figure 2. Calculation of the clonogenic inactivation ratio (CIR), mean inactivation dose (MID), and sensitizer enhancement ratio (SER) of presented dose-effect curves (see Figure 1). The CIR was calculated according to the equation $SF(2)_{plastic}/SF(2)_{FN}$. The MID (at 50% cell survival) was calculated using the equation $MID_{plastic}/MID_{FN}$. The SER was calculated by dividing the survival fraction of cells grown on plastic by the survival fraction of cells grown on FN at 10% cell survival. Values > 1.0 indicate enhanced FN irradiation interaction, and values < 1.0 show protection (value = 1: no interaction). Each data point represents the mean ± SD of three independent experiments (n = 18).

Abbildung 2. Berechnung der klonogenen Inaktivierungsratio (CIR), der mittleren Inaktivierungsdosis (MID) und des sensibilisierenden Verstärkungsfaktors (SER) aus den gezeigten Dosis-Effekt-Kurven (s. Abbildung 1). Die klonogenen Inaktivierungsratio wurde mit Hilfe der Gleichung $SF(2)_{Plastik}/SF(2)_{FN}$ berechnet. Die MID (Dosis bei 50% Zellüberleben) ergibt sich aus der Formel $MID_{Plastik}/MID_{FN}$. Zur Berechnung des SER wurde der Wert 10% Zellüberleben auf Plastik durch den Wert 10% Zellüberleben auf FN geteilt. Werte > 1,0 zeigen eine verstärkte FN-Bestrahlungsinteraktion, Werte < 1,0 eine Protektion an (Wert = 1: keine Interaktion). Jeder Datenpunkt zeigt den Mittelwert ± Standardabweichung von drei unabhängigen Experimenten (n = 18).

Material and Methods

Cells

A172 (glioblastoma), A549 (lung carcinoma) and CCD32 (normal lung fibroblastic cell strain) cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). PATU8902 (pancreas carcinoma), SKMES1 (lung carcinoma) and IPC298 (melanoma) were obtained from the DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). The normal human skin fibroblastic cell strain (HSF1) was a generous gift from Prof. H.P. Rodemann (Section of Radiobiology and Molecular Environmental Research, University Tübingen, Germany). The human keratinocytes HaCaT were a kind gift from Prof. N.E. Fusenig (Deutsches Krebsforschungszentrum [German Cancer Research Center], Heidelberg, Germany). Dulbecco's modified Eagle's medium (PAA, Linz, Austria) or RPMI-1040 medium (Sigma-Aldrich GmbH, Taufkirchen Germany; for IPC298 cells), supplemented with 10% fetal bovine serum (PAA) and 1% nonessential amino acids (GIBCO, Karlsruhe, Germany), was applied to cultivate the cells. Routinely, cells were incubated at 37 °C in a humidified atmosphere containing 10% or 5% (for RPMI-1040 medium) CO_2 buffered at pH 7.35.

Radiation Exposure

Irradiation was delivered at room temperature using single doses of 240-kV X-rays (Isovolt 320/10; Seifert, Ahrensburg, Germany) filtered with 3 mm Be. The absorbed dose was measured using a Duplex dosimeter (PTW, Freiburg, Germany). The dose rate was approximately 1 Gy/min at 13 mA. Applied doses ranged from 0 to 8 Gy.

Colony Formation Assay

The colony formation assay was applied for measurement of clonogenic cell survival. Exponentially growing cells were plated onto noncoated or FN-precoated (1 μg/cm²; Becton Dickinson, Heidelberg, Germany) six-well dishes (Becton Dickinson) 24 h prior to irradiation. 8–10 days after irradiation, grown colonies were stained with Coomassie blue. Colonies > 50 cells were counted. All experiments were repeated three times (n = 18).

Flow Cytometric Analysis of β1-Integrin Expression

Analysis of b1-integrin cell surface expression was performed as described previously [9]. In brief, cells were irradiated with different doses (2, 2.5, 5, or 6 Gy), and measured at defined time points (0, 12, 24, 48 h). Nonirradiated controls were prepared in parallel at each time point. Staining with fluorescein isothiocyanate-(FITC-)conjugated anti-β1-integrin IgG antibodies (Dako, Hamburg, Germany) was performed for 1 h at room temperature. Isotype control IgG was from Serotec Ech-

ing, Germany). Finally, prepared cells were resuspended in FACSFlow™ (Becton Dickinson) and measured using a Fluorescence-Activated Cell Sorter (FACScan, Becton Dickinson), equipped with a CELLQuest software. Experiments were repeated three times.

Adhesion Assay

Determination of the adhesive functionality of the β1-integrin was employed according to a previously described method [9]. Shortly, 96-well plates (Nunc, Wiesbaden, Germany) were coated with 10 µg/ml FN in serum-free medium for 1 h at room temperature. Subsequently, blocking of plates for 30 min at room temperature using 1 mg/ml BSA in PBS was performed. 48 h after radiation exposure, 5×10^4 nonirradiated or irradiated cells were washed with serum-free medium and plated onto prepared wells in the absence or presence of function-blocking β1-integrin antibodies (mAb 13, 100 µg/ml, Pharmingen, Heidelberg, Germany). Nonadherent cells were withdrawn by gentle washing after 45-min incubation at 37 °C. Adherent cells were fixed and stained for 1 h using methylene blue. After washing, 100 µl of 0.1 M HCl solution were added to each well, followed by measurement of absorbance of the resulting solution at 630 nm by means of a microplate spectrophotometer (Spectra max® 190, molecular devices, Krefeld, Germany). All experiments were performed in triplicate and repeated three times.

Data Analysis

Means ± standard deviations (SD) of surviving fractions, clonogenic inactivation ratios, mean inactivation doses, sensitizer enhancement ratios, flow cytometric analysis of β1-integrin cell surface expression, and cell adhesion to substrates were calculated with reference to untreated controls defined as 1.0 or in a percentage scale. To test statistical significance, analysis of variance was performed by means of ANOVA with a software package (Microsoft, Excel 2000) on IBM computer systems. Results were considered statistically significant if a p-value of < 0.05 was reached. Clonogenic inactivation ratios (CIR) were calculated according to the equation $SF(2)_{plastic/}SF(2)_{FN}$, mean inactivation doses (MID, at 50% cell survival) according to the equation $MID_{plastic}/MID_{FN}$, and sensitizer enhancement ratios (SER) by dividing the survival fraction of cells grown on plastic by the survival fraction of cells grown on FN at 10% cell survival. The fit of the dose-effect curves was calculated by means of the linear-quadratic model ($\log S = -\alpha D - \beta D^2$) indicating, additionally, means ± standard errors (SE) for α- and β-values.

Results

Colony Formation Assay

Cells irradiated in the presence of FN showed a significantly higher cell survival than cells plated on plastic. This effect was independent of the cell line. There was a significantly higher clonogenic cell survival at doses ≥ 4 Gy for A172, SKMES1 and A549 cells, at ≥ 6 Gy for PATU8902 and IPC298 cells, and at ≥ 2 Gy for the normal fibroblasts and keratinocytes adhered to FN (Figure 1). As delineated in Figure 2, calculations of the clonogenic inactivation ratio, mean inactivation dose, and sensitizer enhancement ratio indicated a reduced radiosensitivity in all cell lines grown on FN except IPC298 cells compared to cells grown on plastic. The α- and β-values ± SE were calculated by means of the linear-quadratic model ($\log S = -\alpha D - \beta D^2$) and are listed in Table 1. Calculated α/β-ratios show a great diversity between the cell lines. FN as compared to plastic does not necessarily lead to an increase in the ratio, although, the dose-effect curves of all tumor and normal cells tested demonstrated a reduced radiosensitivity when the cells were attached to FN.

Flow Cytometric Analysis of β1-Integrin Expression

Within a 48-h time interval after irradiation (2, 2.5, 5, 6 Gy), a pronounced, significant dose-dependent rise of β1-integrin

Table 1. Analysis of the α- and β-values ± SE was performed by means of the linear-quadratic model describing the dose dependence of cell inactivation according to the formula $\log SF = -\alpha D - \beta D^2$. α- and β-values as well as α/β-ratios are given for all cells tested in dependence on the substrate (plastic, FN) to which cells were adhered to at the time of radiation exposure.

Tabelle 1. Die Analyse der α- und β-Werte ± SE erfolgte mit Hilfe des linear-quadratischen Modells, welches die Dosisabhängigkeit der Zellinaktivierung beschreibt, entsprechend der Formel $\ln SF = -\alpha D - \beta D^2$. Die α- und β-Werte sowie die α/β-Ratios sind für alle getesteten Zellen entsprechend des Substrats (Plastik, FN), an das sie zum Zeitpunkt der Bestrahlung adhäriert waren, aufgelistet.

Experiment	$-\alpha$ (Gy^{-1})	$-\beta$ (Gy^{-2})	α/β
A172			
• Plastic	1.61e-1 ± 2.41e-2	2.92e-2 ± 3.56e-3	5.5
• FN	1.15e-1 ± 1.39e-2	1.99e-2 ± 2.5e-3	5.8
PATU8902			
• Plastic	1.99e-1 ± 1.02e-1	4.93e-2 ± 1.63e-2	4
• FN	2.49e-1 ± 9.32e-2	1.69e-2 ± 1.59e-2	14.7
SKMES1			
• Plastic	4.01e-1 ± 4.6e-2	3.29e-2 ± 7.38e-3	12.2
• FN	1.35e-1 ± 2.76e-2	3.52e-2 ± 4.63e-3	3.8
A549			
• Plastic	1.58e-1 ± 3.5e-2	5.66e-2 ± 5.22e-3	2.8
• FN	1.63e-1 ± 1.38e-2	3.19e-2 ± 2.46e-3	5.1
IPC298			
• Plastic	2.93e-1 ± 1.48e-1	4.88e-2 ± 3.28e-2	6
• FN	3.57e-1 ± 7.58e-2	2.46e-2 ± 1.25e-2	14.5
HSF1			
• Plastic	1.83e-1 ± 7.78e-2	6.30e-2 ± 1.26e-2	2.9
• FN	1.37e-1 ± 1.51e-2	3.68e-2 ± 2.48e-3	3.7
CCD32			
• Plastic	4.53e-1 ± 6.43e-2	3.54e-2 ± 9.84e-3	12.8
• FN	9.56e-2 ± 1.69e-2	4.54e-2 ± 3.13e-3	2.1
HaCaT			
• Plastic	5.34e-1 ± 1.39e-1	8.45e-2 ± 2.88e-2	6.3
• FN	2.23e-1 ± 2.84e-2	3.37e-2 ± 5.75e-3	6.6

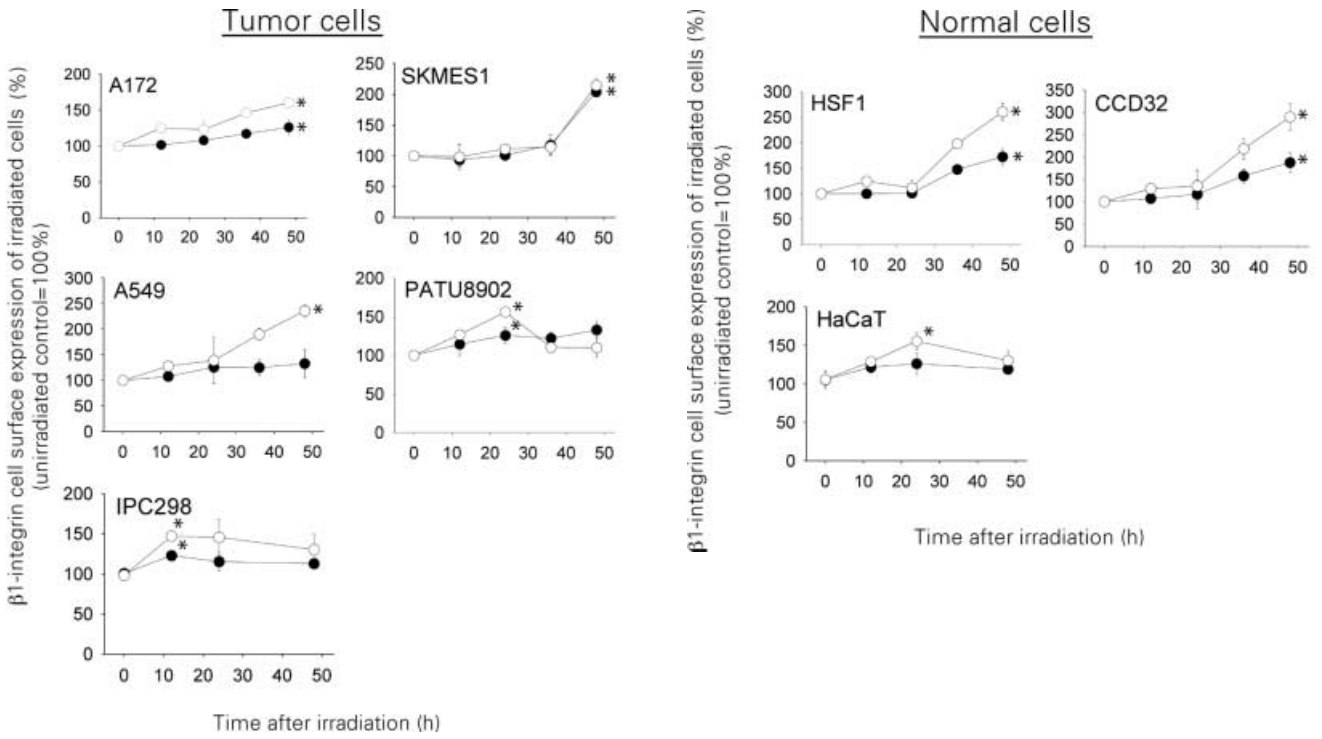

Figure 3. Cells grown on plastic were irradiated with 2 Gy (●; A172, PATU8902, SKMES1, A549, HSF1, CCD32), 2.5 Gy (●; IPC298, HaCaT), 5 Gy (❍; IPC298, HaCaT), or 6 Gy (❍; A172, PATU8902, SKMES1, A549, HSF1, CCD32). Subsequently, a 48-h time interval was measured using flow cytometry to detect radiation-dependent changes of β1-integrin cell surface expression. Each data point shown represents the mean ± SD of three independent experiments of the β1-integrin cell surface expression of irradiated cells in relation to untreated control cells (= 100%) at the same time point. *p > 0.01.

Abbildung 3. Auf Plastik wachsende Zellen wurden mit 2 Gy (●; A172, PATU8902, SKMES1, A549, HSF1, CCD32), 2,5 Gy (●; IPC298, HaCaT), 5 Gy (❍; IPC298, HaCaT) oder 6 Gy (❍; A172, PATU8902, SKMES1, A549, HSF1, CCD32) bestrahlt. Bestrahlungsbedingte Veränderungen der Oberflächenexpression des β1-Integrins wurden während eines 48-h-Intervalls mittels Flusszytometrie gemessen. Jeder Datenpunkt zeigt den Mittelwert ± Standardabweichung der Oberflächenexpression des β1-Integrins bestrahlter Zellen in Beziehung zur unbehandelten Kontrolle (= 100%) von drei unabhängigen Experimenten. *p > 0.01.

cell surface expression was detectable in all cells examined (Figure 3). After delivery of 2 or 2.5 Gy, the amount of β1-integrin was raised in a range from 110 to 200% at 48 h compared to untreated controls. After delivery of 5 or 6 Gy, the amount of β1-integrin was raised in a range from 115 to 270% at 48 h compared to untreated controls which resulted both in $p < 0.05$ cell line-dependently. By contrast, PATU8902, IPC and HaCaT cells demonstrated a faster upregulation of the β1-integrin presentation within 12–24 h after irradiation.

Adhesion Assay

Attachment of cells to FN was found to be five- to tenfold higher compared to attachment to uncoated plastic or BSA-blocked culture plastic throughout the experiments (Figure 4). This radiation-dependent improvement of cell attachment to FN was indicative of being dose-dependent for normal skin (HSF1) and lung (CCD32) fibroblasts. The tumor cells only showed an increased adhesion at the higher doses (6 Gy) applied. Incubation of nonirradiated and irradiated cells using the β1-integrin function-blocking antibody mAb 13 inhibited adhesion to FN effectively and significantly (Figure 4). At all time points measured adhesion to plastic or BSA of irradiated cells showed similar results compared to nonirradiated controls (Figure 4).

Discussion

Cellular radiosensitivity is a product of the convergence of multiple cellular signaling networks. In this study, we present molecular data of several human tumor cells and normal human fibroblasts and keratinocytes of different origin giving strong evidence of the involvement of the β1-integrin cell surface expression and functionality of this receptor in radioresistance-mediating mechanisms in vitro. The findings of this study are a significant improvement of cell survival on FN after irradiation in a cell line-independent manner compared to irradiation on plastic. A second result is a dose-dependent radiation-induced upregulation of the integrin subunit β1 in all cells tested within a time interval of 48 h. The third effect shows a confirmation of the radiation-induced, upregulated receptor's adhesive functionality toward the ECM protein FN.

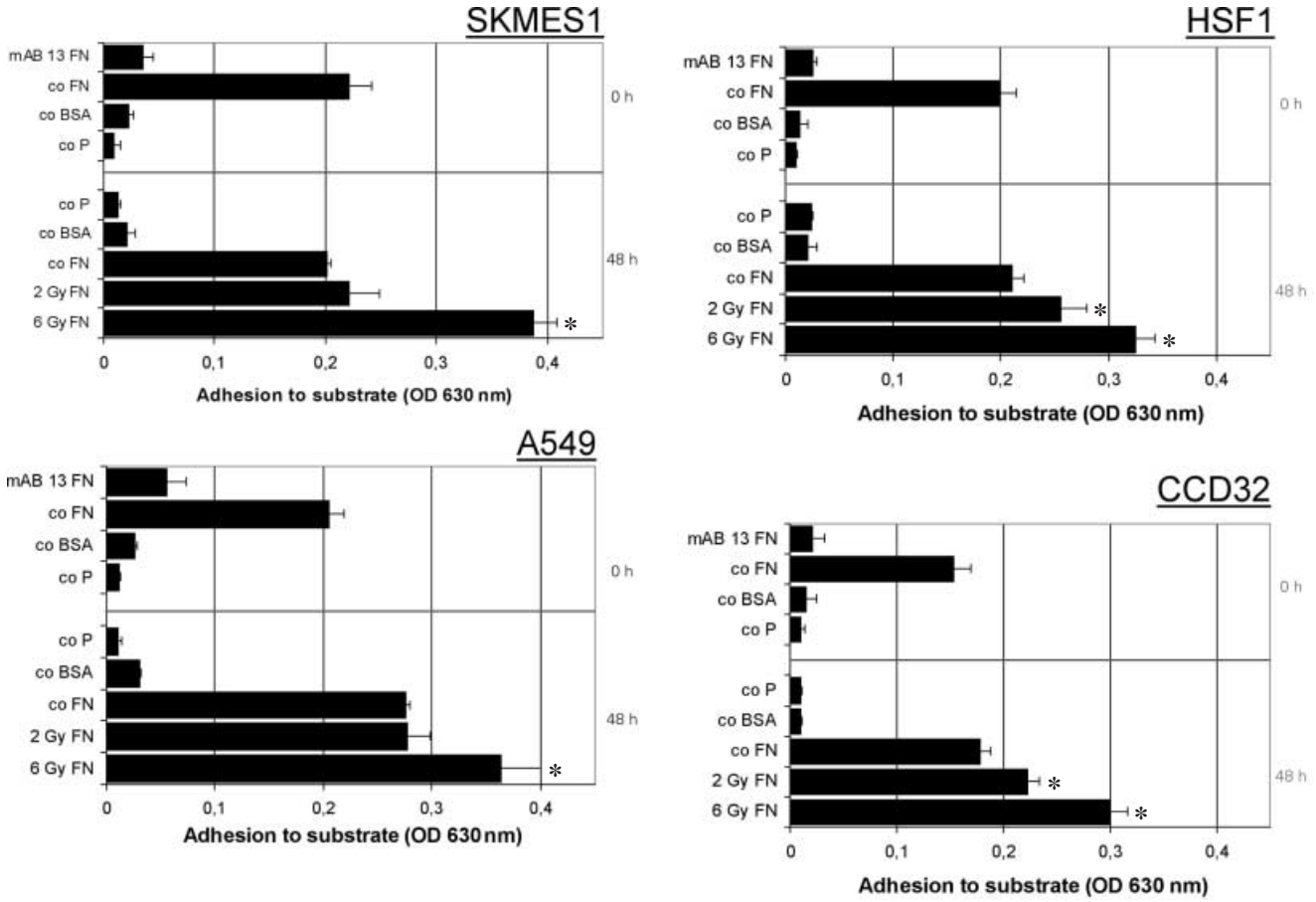

Figure 4. The functionality of the β1-integrin subunit was tested by plating nonirradiated and irradiated (2 or 6 Gy) SKMES1, A549, HSF1 and CCD32 cells onto plastic, FN, or BSA for 45 min. Specific inhibition of adhesion through the β1-integrin was provided by incubation of nonirradiated controls or irradiated cells using function-blocking mAb 13 antibodies (100 µg/ml). Columns show the mean ± SD of the optical densities (OD) at 630 nm representing the cellular capability of adhesion to the different substrates of three independent experiments. Statistical significance was calculated by comparing adhesion on FN of irradiated cells to adhesion on FN of nonirradiated cells. *$p < 0.05$.

Abbildung 4. Die Adhäsionsfähigkeit unbestrahlter und bestrahlter SKMES1-, A549-, HSF1- und CCD32-Zellen via β1-Integrin an Plastik, FN oder BSA wurde 48 h nach Bestrahlung (2 oder 6 Gy) überprüft. Zur Blockierung der ECM-Bindungsstelle des β1-Integrins wurden unbestrahlte und bestrahlte Zellen mit 100 µg/ml eines spezifischen Antikörpers (mAb 13) inkubiert. Die Säulen zeigen die Mittelwerte ± Standardabweichung der optischen Dichte (OD) bei 630 nm von drei unabhängigen Experimenten, die die zelluläre Adhäsionsfähigkeit an das entsprechende Substrat repräsentiert. Die statistische Signifikanz wurde für die Adhäsion von bestrahlten zu unbestrahlten Zellen an FN berechnet. *$p < 0,05$.

Determination of clonogenic survival is a common method of assessing the cytotoxic potential of a specific agent such as ionizing radiation or drugs [4, 10–12, 17, 27, 28, 35]. To date, only a few groups have tested the modulating impact of the ECM or isolated components on clonogenic survival [9, 13, 18, 37, 41]. In this study, we strongly support these findings with data generated in several human malignant and nonmalignant cell lines of different origin. Presence of FN clearly showed that the reduced cellular radiotoxic effects are independent of the cell's origin, differentiation status or genotype. The phenomenon of cell adhesion-mediated radioresistance (CAM-RR) is defined here as reduction of cellular sensitivity toward ionizing radiation which is due to a modulation of specific critical cellular functions as a result of cell adhesion to either single ECM proteins or a physiologic composition of an ECM. Attachment of cells via integrins, especially β1-integrins, serves as survival factor [32] controlling apoptotic pathway events via PKB phosphorylation of, e.g., Bad or caspase-9 [14, 26] as well as regulating the cell cycle by cyclin D1 proteolysis inhibition [16] and retinoblastoma protein hyperphosphorylation [21]. Additionally, differentiation, motility-related cellular events, i.e., adhesion or migration [40], immunologic [2, 29, 36] and ECM-remodeling events [42] are being controlled. Recent findings, furthermore, indicated the participation of β1-integrin signaling upon exposure of cells to DNA-damaging agents in DNA repair mechanisms [25], prevention of double-strand break occurrence [22], and upregulation of the p-glycoprotein responsible for a multidrug resistance phenotype

[38]. Thus, regarding all these specific resistance-transducing processes, the importance of the ECM for physiologic cellular homeostasis and behavior is strongly supported. Exposure of cells to ionizing radiation at ECM presence might not only modulate these mechanisms on a cell membrane level but also seems to affect cytoplasmic and nuclear events. By describing the multiple regulatory functions of β1-integrin pathway signaling, the radiation-dependent alterations of integrin cell surface patterns can be interpreted more deeply. An increased cell membrane density of β1-integrin is likely to positively influence cellular resistance to cytotoxic agents (such as ionizing radiation) due to a recruitment of an increasing number of β1-integrin downstream mediators and/or a stimulation of specific β1-integrin signaling protein kinases leading to an optimized, cell death-reducing cell physiology.

One of the most important functions of the β1-integrin is cell adhesion specific to ECM components such as FN. This adhesion process was shown here to be highly improved after irradiation and corresponds to previous findings [9, 30]. The significant elevation in adhesion of cells to FN in a dose-dependent manner via an upregulation and/or switching-on of a functional integrin receptor is likely to result in an enhanced anchorage of cells in their corresponding microenvironment. In case of tumor cells, migration and metastatic spread could possibly be disturbed or even prevented this way. By specifically blocking the adhesive capability of β1-integrins using specific monoclonal antibodies, we could support that this receptor plays a highly substantial role in the adhesion processes of the tested nonirradiated and irradiated cells.

In summary, the presented data of several human tumor and normal cells give evidence of the potential and radiosensitivity-reducing impact of the ECM component FN. Furthermore, by correlating the induction of functional β1-integrin receptors on the cell surface to elevated cell adhesion to FN, irradiation demonstrated its possible impact on the impairment of cell motility and, thereby, participation in the inhibition of the first step of metastasis formation. These findings provide further insights into the cellular regulating processes concerning in vitro radiosensitivity and might also lead to a better molecular understanding of tumorigenesis, normal tissue side effects as well as possible molecular determinants for the failure of radiotherapeutic regimens.

Acknowledgments

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