Clinical and Translational Radiation Oncology 7 (2017) 1–8

Contents lists available at ScienceDirect

Clinical and Translational Radiation Oncology

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

Original Research Article

The impact of the IGF-1 system of cancer cells on radiation response – An *in vitro* study

Senthiladipan Venkatachalam [a], Esther Mettler [a,*], Christian Fottner [a], Matthias Miederer [b], Bernd Kaina [c], Matthias M. Weber [a]

[a] Department of Endocrinology and Metabolic Diseases, University Medical Center, Mainz, Germany
[b] Department of Nuclear Medicine, University Medical Center, Mainz, Germany
[c] Institute of Toxicology, University Medical Center, Mainz, Germany

ARTICLE INFO

Article history:
Received 4 May 2017
Revised 29 August 2017
Accepted 18 September 2017
Available online 5 October 2017

Keywords:
Radiosensitivity
Colorectal carcinmoma
Insulin-like growth factor-1 receptor
Dominant negative mutant
RAD 51
BRCA2

ABSTRACT

Background: Overexpression of the insulin-like growth factor-1 receptor (IGF-1R) is associated with increased cell proliferation, differentiation, transformation, and tumorigenicity. Additionally, signaling involved in the resistance of cancer cells to radiotherapy originates from IGF-1R. The purpose of this study was to investigate the role of the IGF-1 system in the radiation response and further evaluate its effect on the expression of DNA repair pathway genes.

Methods: To inhibit the IGF-1 system, we stably transfected the Caco-2 cell line to express a kinase-deficient IGF-1R mutant. We then studied the effects of this mutation on cell growth, the response to radiation, and clonogenic survival, as well as using a cell viability assay to examine DNA damage and repair. Finally, we performed immunofluorescence for γ-H2AX to examine double-strand DNA breaks and evaluated the expression of 84 key genes involved in DNA repair with a real-time PCR array.

Results: Mutant IGF-1R cells exhibited significantly blunted cell growth and viability, compared to wild-type cells, as well as reduced clonogenic survival after γ-irradiation. However, mutant IGF-1R cells did not show any significant delays in the repair of radiation-induced DNA double-strand breaks. Furthermore, expression of mutant IGF-1R significantly down-regulated the mRNA levels of BRCA2, a major protein involved in homologous recombination DNA repair.

Conclusion: These results indicate that blocking the IGF-1R-mediated signaling cascade, through the expression of a kinase-deficient IGF-1R mutant, reduces cell growth and sensitizes cancer cells to ionizing radiation. Therefore, the IGF-1R system could be a potential target to enhance radio-sensitivity and the efficacy of cancer treatments.

Introduction

The insulin-like growth factor (IGF) system is involved in a variety of cellular processes such as cell growth, differentiation, and transformation. Most, if not all, mitogenic effects of IGF-1 and IGF-II are mediated through the IGF-1 receptor (IGF-1R) [1–3]. Activation of the receptor and/or increased levels of IGF-1R expression have been reported in many human malignancies including breast, ovarian, colorectal, and adrenocortical cancer, as well as pancreatic carcinomas and pheochromocytoma [4–9].

At present, radiotherapy remains one of the most important cancer treatment modalities. Although radiotherapy improves both the survival of patients and local disease control, the development of resistance limits the overall therapeutic efficacy [10]. Furthermore, ionizing radiation can damage DNA, either by oxidizing DNA bases or causing single-strand (SSBs) and double-strand breaks (DSBs). Too much damage can result in radiation-induced cell death, which is a complex process that can occur via multiple cell death mechanisms, such as apoptosis, mitotic catastrophe, necrosis, senescence, and autophagy [11]. Additionally, ionizing radiation activates several tyrosine kinase receptors involved in

Abbreviations: IGF-1R, insulin-like growth factor 1 receptor; SF, surviving fractions; IGF-1R/KR, kinase-deficient IGF-1R; Caco-2-KR4, IGF-1R/KR clone number 4; IRS-1, insulin receptor substrate 1; HRR, homologous recombination repair; NHEJ, non-homologous end joining; PTEN, phosphatase and tensin homolog; MVP, major vault protein; BCL-2, B-cell lymphoma 2; BAX, BCL-2-associated X.

* Corresponding author.
E-mail address: esther.mettler@unimedizin-mainz.de (E. Mettler).

https://doi.org/10.1016/j.ctro.2017.09.006

the DNA damage response, including IGF-1R [12]. Overexpression of IGF-1R is associated with increased cell proliferation, clonogenic cell survival [13,14], tumor recurrence, poor prognosis, and resistance to therapy [15–18]. Moreover, the clinical outcomes of patients who have tumors with a high expression of IGF-1R have been shown to be poor after radiotherapy [16,17]. However, another study reported that tumor recurrence was higher in a subset of patients with low levels of active IGF-1R [19]. Understanding the relationship between IGF-1R and the cellular response to radiotherapy could lead to the development of treatments to improve the efficacy of radiotherapy. Therefore, we investigated the effects of blocking IGF-1R signaling on cancer cells exposed to ionizing radiation.

To accomplish this, we utilized a dominant negative mutant, as these mutants have been applied successfully in many *in vitro* and *in vivo* experiments to target IGF-1R [20–25] Specifically, we used a kinase-deficient IGF-1R (KR mutant) in which the lysine residue at the 1003rd position of the IGF-1R ATP binding site was replaced with an arginine [26]. Expression of this mutant receptor forms a hybrid with the endogenous wild type IGF-1R. While both the mutant and hybrid receptors can still bind to IGF ligands, they are incapable of transducing the downstream signaling cascade, resulting in dominant inhibition of IGF-1R functioning [21].

The aim of the current study was to investigate the role of the IGF-1 system in the cellular response to radiation and to evaluate its effect on the expression of DNA repair genes. To this end, we first blocked IGF-1R-mediated signaling by expressing a kinase-deficient IGF-1R mutant in Caco-2 cells. Then, we compared our mutants to control cells with respect to cell growth, survival, and the repair of DSBs induced by γ-irradiation.

Methods

Cell culture

Human colorectal adenocarcinoma cell line Caco-2 was obtained from ATCC (LGC Standards GmbH, Germany) and cultured in Eagle's MEM (high glucose (4.5 g/l), Gibco, UK) supplemented with 10% heat inactivated fetal calf serum (FCS), 1 mM sodium pyruvate, and 100 U/ml penicillin–streptomycin. The cells were cultured at 37 °C with 5% CO_2 in a CO_2 incubator (Heraeus, Germany).

Plasmids and transfection

pBPV-IGF-I-KR plasmid was provided by Prof. Renato Baserga, Thomas Jefferson University, Philadelphia. This plasmid encodes for kinase deficient IGF-I receptor, in which a lysine residue at the 1003rd position of ATP binding site is replaced with an arginine. pBPV-IGF-I-KR plasmid was co-transfected with a GFP containing phrGFP II-1 plasmid (Stratagene Inc, La Jolla, CA) using X-tremeGENE HP DNA transfection reagent as per the manufacturer's instruction (Roche AG, Germany). After 48hrs, Caco-2 cells transfected with IGF-I-KR and control vector were split into 6 well plates with 550 μg/ml of geneticin. Culture medium containing geneticin was changed every 3 days until cell colonies were formed.

Isolation of Caco-2 cell clones expressing kinase deficient IGF-1R

Two weeks after selection, geneticin resistant IGF-I-KR expressing colonies were formed. Pooled colonies were subjected to clonal selection by serial dilution in a 96 well plate (with 550 μg/ml of geneticin). Over a period of 1–2 weeks, 4 clones (KR3, KR4, KR6, and KR10) were isolated. Caco2-KR4 clone was found to show highest level of IGF-I-KR expression. Caco-2 clones (KR4 and vector control) were routinely maintained in media containing 450 μg/ml geneticin.

Flow cytometry analysis

Caco2-control and KR4 cell clones were screened by flow cytometry for its surface expression of IGF-1R. Single cell suspensions (1 × 106 cells) in 100μl of ice-cold FACS buffer (2% FCS in DPBS) were incubated either with 7.5 μl of PE labelled mouse anti-human IGF1R-α antibody (BD Pharmingen # 555999) or with isotype control (7.5 μl of PE labelled mouse IgG1-K, BD Pharmingen # 555749) for 1 h in the dark at 4 °C. Cells were washed twice and the cell pellets were re-suspended in 1 ml of ice-cold FACS buffer. Cells were analyzed using BD FACSCanto II cell analyzer.

Western blot analysis

Equal amount of whole cell lysates were resolved using 10% SDS polyacrylamide gel and transferred onto PVDF membrane (Merck Millipore, Germany). The unbound sites in the membrane were blocked with 5% blotto, non-fat dry milk for 1 h (Santa Cruz Biotechnology, Santa Cruz, CA). Then the membrane was washed, incubated with rabbit polyclonal IGF-1R β antibody (1:1000 dilutions, C-20, SCBT, Santa Cruz, CA) for overnight at 4 °C and then with mouse anti-rabbit IgG-HRP (1:10,000 dilutions, SC-2357, SCBT, Santa Cruz) for 1 h. After 3 washes, the membrane was doused for 2 min in super signal pico ECL reagent (Thermo scientific, Germany), exposed to Ultracruz autoradiography film (SCBT, Santa Cruz) and the film was developed in Fujifilm developing machine. The membrane was stripped and re-probed with β-Actin antibody (1:1000, SC-47778, SCBT, Santa cruz).

In vitro cell growth assay

Caco2-control and KR4 cells were seeded in 3.5 cm dishes. After 24hrs media was replaced with fresh EMEM with 10% FCS. Cell growths were measured at different time points by trypan blue vital cell counting. Unstained cells were considered as viable.

IGF-I ligand mediated cell growth

Caco2-control and KR4 cells were plated in 3.5 cm dishes. 24 h later, media was replaced with serum free media (EMEM, 50 μg/ml transferrin and 0.5 mg/ml BSA). For dose–response study, cells were stimulated with indicated concentrations of IGF-I. Cells were counted after 72 h of incubation. For time course study, 50 ng/ml IGF-I was used, trypan blue viable cell counting was performed at different time points. Unstimulated cells were used as control.

Cell viability assay after irradiation

Caco2-control and KR4 cells were plated in 3.5 cm dishes and 24 h later cells were treated with 2, 4, 6, 8, and 10 Gy of γ-radiation. Sham (0 Gy) irradiated cells were used as control. After 96 h of incubation, cells were trypsinized and collected together with floating cells by centrifugation. Cells were mixed with 0.4% trypan blue solution and the viable cells were counted manually. For time course study, Caco2-control and KR4 cells were irradiated with 5 Gy of γ-radiation after 24 h of plating. Viable cells were counted at the indicated time points after irradiation.

Clonogenic survival assay

Caco2 cell clones (control and KR4) were grown in 75 cm^2 flask to reach 80% confluence. Then cells were trypsinized, counted and

irradiated at different doses of γ-radiation in a Cs-137 irradiator. Cells were plated in 6 well plates in triplicates (EMEM with 10% FCS). After two weeks of incubation, colonies were fixed with 3:1 ratio of methanol-acetic acid and stained with 0.5% crystal violet solution. Colony images were taken on Gel doc-2000 imaging system and counted using Quantity One software (Bio-Rad laboratories). The surviving fraction was normalized to the corresponding un-irradiated control. For Rad51 inhibitor study, cells were treated with 27 μM of BO2 (Rad51 inhibitor) or DMSO (vehicle 1.35%) for 1 h and then the clonogenic assay was performed.

Immunofluorescence to measure γ-H2AX phosphorylation

Caco2-control and KR4 cells were seeded on sterile cover slips and allowed to attach. Then cells were treated with different doses of γ-irradiation and fixed one hour post irradiation. For time course study, cells irradiated at a dose of 4 Gy were fixed at the indicated time points. Cells were fixed with 4% formaldehyde solution and ice cold methanol. 10% goat serum was used for blocking before staining with anti-phospho–Histone H2A.X (Ser139) antibody (1:1000, Millipore) for overnight at 4 °C and Cy3-affinipure goat anti-mouse IgG F (ab′) 2 fragment (1:700, Jackson immune research labs) for one hour at room temperature in the dark. Coverslips were mounted using VECTASHIELD mounting medium with DAPI. To record γ-H2AX foci, a Zeiss Imager.M1 microscope (Carl Zeiss AG, Oberkochen, Germany) attached with Metafer automated slide scanning platform was used. 100 cell nuclei were recorded per sample to calculate the average number of foci per cell.

Human DNA repair pathway PCR array

Caco2-control and KR4 cells seeded in 25 cm^2 flasks were serum starved for overnight and then treated with 50 ng/ml of IGF-I for 20 min. Then RNA isolation was performed using RNeasy mini kit (Qiagen, Germany) as per the manufacturer's instruction. 1.5 μg of RNA from each sample was used for cDNA synthesis using RT2 first strand kit (SA Biosciences). Real-time PCR was performed using the human DNA repair RT2 profiler PCR array (SA Biosciences-Qiagen) as per the manufacturer's instruction in MyiQ2 cycler (Bio-Rad laboratories). ACTB and RPLP0 genes were used for normalization. Validation of PCR array results was done using individual PrimePCR assay (three genes and ACTB) as per the manufacturer's instruction (Bio-Rad laboratories Inc.).

Statistical analysis

Graph plotting and data analysis were performed using Graphpad Prism version 6 (San Diego, California, USA). Significant differences were identified using Levene's test for equality of variances and independent samples *t*-test. In all tests, the significance level was set at 5% ($P < 0.05$).

Results

Characterization of the Caco-2 cell line expressing a kinase-deficient IGF-1R (KR mutant)

After clonal selection, stable cell clones were screened by flow cytometry for IGF-1R expression. Since the IGF-1R/KR mutant is the same size as wild type IGF-1R, an overall increase in the level of IGF-1R is indicative of stable integration and expression of the mutant IGF-1R cDNA. We obtained several KR mutant-expressing clones, of which clone number four (KR4) showed the highest level of expression. Specifically, 86.6% of KR4 cells were positive for IGF-1R expression compared to 31.6% of Caco-2-control cells (Fig. 1A).

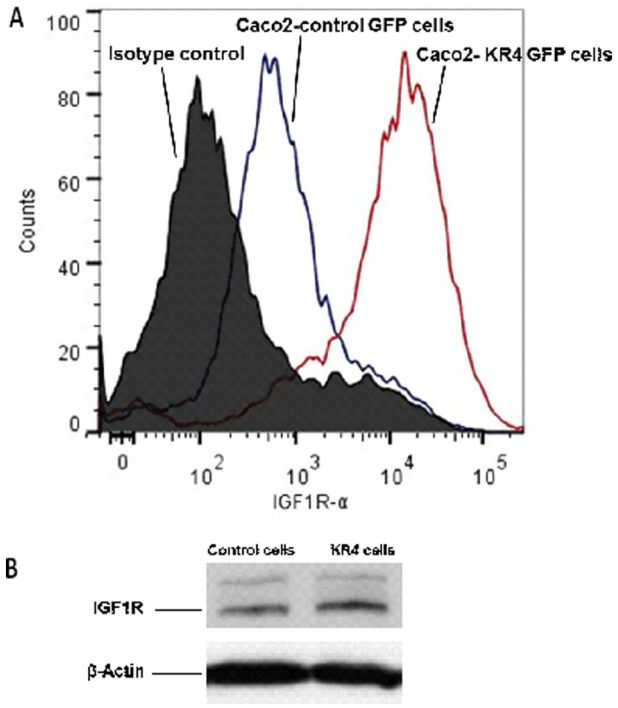

Fig. 1. Expression of kinase-deficient IGF-1R in the Caco-2 cell line. (A) Caco-2 stable clones were analysed with flow cytometry. A total of 86.6% of Caco-2-KR4 cells and 31.6% of Caco-2 control cells were positive for IGF-1R expression. (B) Western blot analysis of IGF-1R expression in Caco-2 stable clones. The KR mutant protein (lysine-arginine substitution in the ATP binding domain of the β subunit) is the same size as the wild type IGF-1R and overlaps with the wild type IGF-1R signal.

In addition, Western blot analyses were performed to confirm the expression of IGF-1R (Fig. 1B). Both the flow cytometry and western blot results revealed an increased level of IGF-1R in KR4 cells compared to Caco-2 control cells. In the following sections, IGF-1R/KR mutant cDNA and mock-transfected cells are referred to as Caco-2-KR4 and Caco-2-control, respectively.

Expression of kinase-deficient IGF-1R reduces cell proliferation of Caco-2 cells

Expression of the IGF-1R/KR mutant significantly reduced the growth of KR4 cells, when grown as a monolayer in 10% FCS containing medium (Fig. 2A). In serum-free media, KR4 cells showed little or no response to exogenous IGF-1 stimulation in both dose–response (Fig. 2B) and time course studies (Fig. 2C).

The IGF-1R/KR mutant enhances the radio-sensitivity of the Caco-2 cell line

A dose-dependent reduction in cell viability of both KR4 and control cells was observed after 96 h of radiation treatment; however, 50% of KR4 cells were killed (EC50) at a dose of 4 Gray (Gy), whereas the EC50 wasn't reached in control cells until 6 Gy (Fig. 3A). Viable KR4 cells were significantly reduced after 5 Gy of irradiation and reached 50% at ~72 h. In contrast, 75% of control cells remained viable at 72 h (Fig. 3B). These results show that kinase-deficient IGF-1R expression reduced cell viability after γ-irradiation in a dose- and time-dependent manner. In addition, less colonies formed after radiation treatment in Caco-2-KR4 cells. The surviving fractions (SF) were calculated by normalizing the plating efficiency of irradiated cells to that of corresponding sham-irradiated cells (0 Gy). Compared to control cells, KR4 cells exhibited a reduction in the SF of 27.3%, 54.5%, and 63.6% after

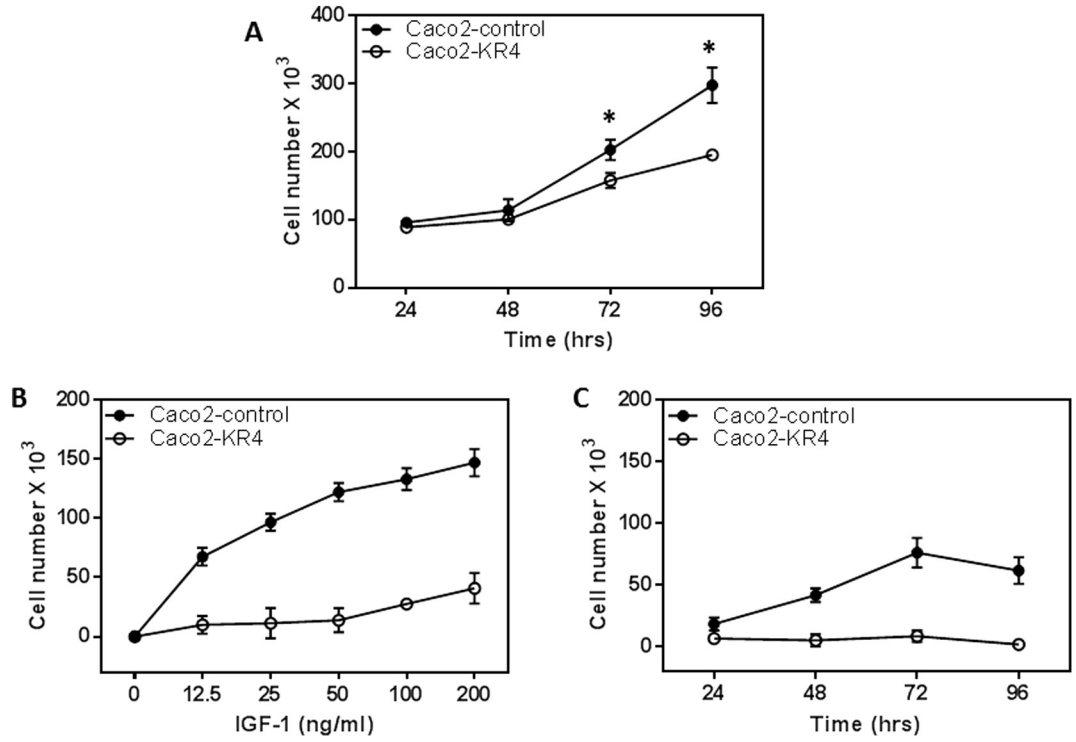

Fig. 2. *In vitro* growth assay of Caco-2 control and KR4 cells. (A) The *in vitro* cell growth of Caco-2 stable clones in complete media (10% FCS) is shown. The expression of kinase deficient IGF-1R in Caco-2-KR4 cells inhibits growth. (B) The *in vitro* cell growth of Caco-2 stable clones in serum free media (SFM), with the indicated concentrations of IGF-1, is shown. Viable cells were counted after 72 h of IGF-1 stimulation and the results were normalized to unstimulated cells (0 ng/mL). C) Caco-2 clones were incubated with and without IGF-1 (50 ng/mL) in SFM, and viable cells were counted at the indicated time points. Results were normalized to unstimulated cells at the corresponding time points. Results are represented as the mean values ± SEM ($n = 3$). $^{*}p < 0.05$.

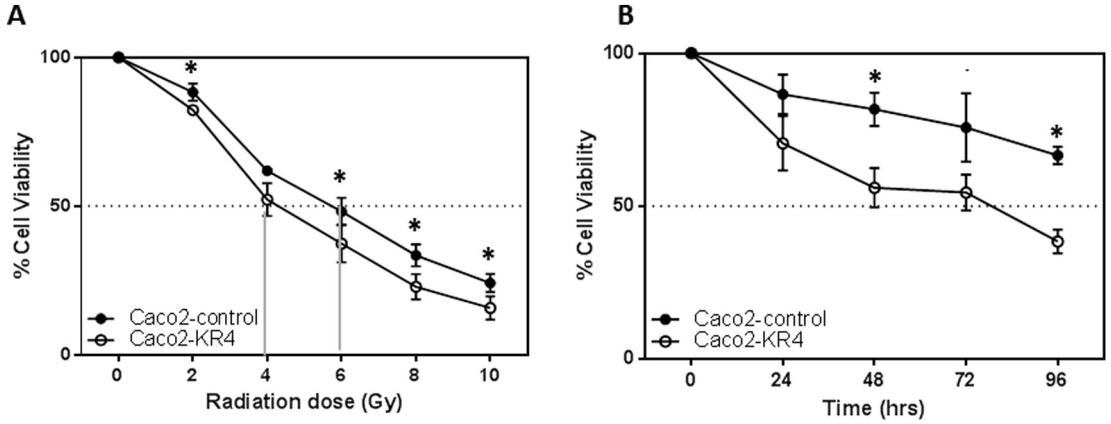

Fig. 3. Cell viability assay after γ-irradiation. (A) The dose-response curve after irradiation is shown. Cells were irradiated with different doses of γ-radiation and viable cells were counted after 96 h of incubation. Fifty-percent of Caco-2 control cells were killed after 6 Gy, whereas the same effect was seen in Caco-2-KR4 cells after only a dose of 4 Gy. (B) A time course for cell viability after 5 Gy of irradiation was performed. The viability of KR4 cells was significantly reduced after irradiation, compared to control cells, and reached 50% at approximately 78 h post-irradiation. The results are represented as the mean values ± SEM ($n = 3$). $^{*}p < 0.05$.

irradiations of 2, 4, and 6 Gy respectively (Fig. 4A). Taken together, our results indicate that expression of the kinase-deficient IGF-1R mutant enhanced the response to γ-radiation by reducing cell viability and clonogenic cell survival.

Effect on radiation-induced DNA double-strand breaks (DSBs)

A known DSB marker, γ-H2AX, was used to study the effects of the KR mutant on DSB repair. Compared to controls, Caco-2-KR4 cells showed only a minor but no significant increase in the number of γ-H2AX foci after 1 h of irradiation (Fig. 5A). In time-course experiments, little or no delay in γ-H2AX foci resolution was observed up to 48 h after 4 Gy irradiation (Fig. 5B), indicating that KR4 cells repair DSBs similar to control cells.

The effect of kinase-deficient IGF-1R on the expression of DNA repair genes

We investigated the effect of mutant IGF-1R on the expression of 84 key genes involved in DNA repair with a real-time PCR array. The results are represented in Table 1.

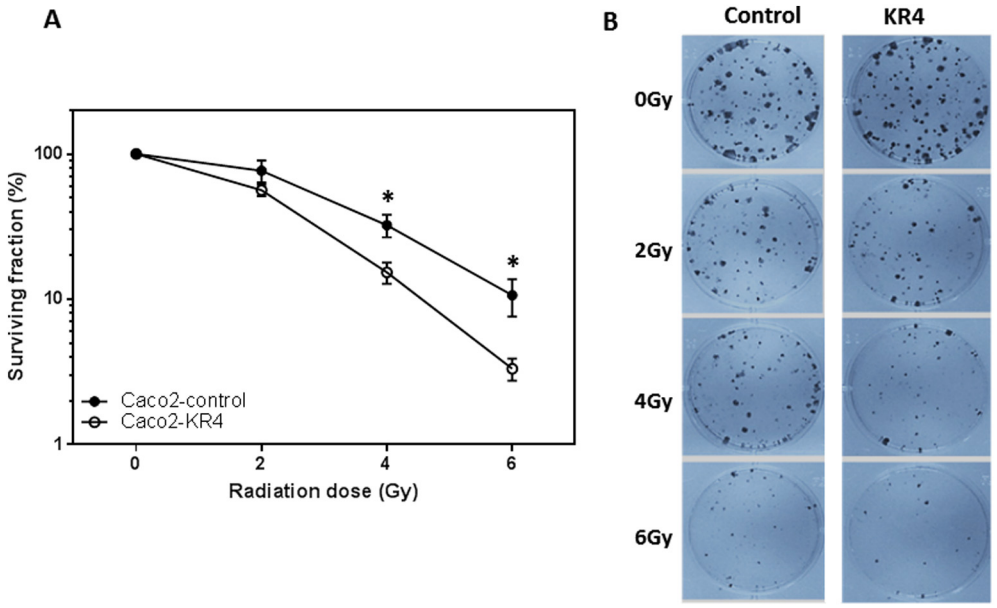

Fig. 4. A clonogenic cell survival assay after γ-irradiation. (A) Single cell suspensions of Caco-2 control and KR4 cells were irradiated with different dose of γ-rays. Sham-irradiated cells (0 Gy) were used as a control. Cells were plated, incubated, and the number of colonies was counted after 14 days. The surviving fractions (SF) were calculated after normalizing to the plating efficiency of the corresponding sham-irradiated cells. The expression of kinase-deficient IGF-1R significantly reduced clonogenic survival after radiation. The results are represented as the mean % SF ± SEM of three independent experiments, with each performed in triplicates ($n = 9$), $^*p < .05$. B) Representative images of Caco-2 control and KR4 colonies.

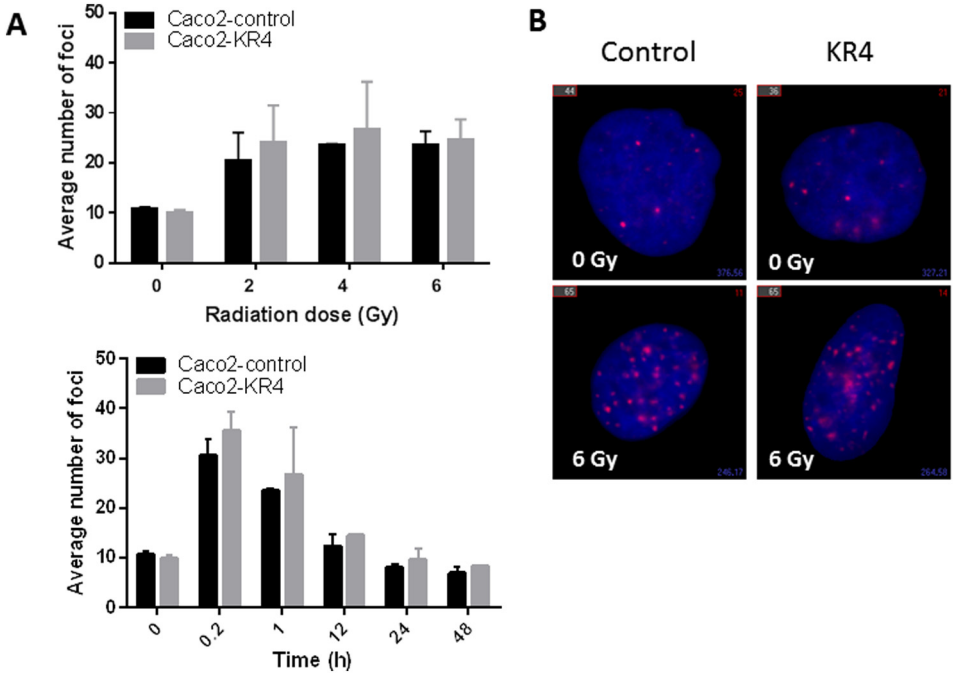

Fig. 5. The effect of kinase-deficient IGF-1R on the repair of radiation-induced DSBs. (A) Cells seeded on coverslips were irradiated with the indicated dose of γ-rays and incubated. Sham-irradiated cells (0 Gy) were used as controls. The γ-H2AX foci were stained and 100 nuclei per sample were counted using ImageJ software. (B) A time course was performed following 4 Gy irradiation. A '0' indicates sham irradiated cells. Data are presented as the mean values ± SEM of two independent experiments. C) Representative images of γ-H2AX foci with and without irradiation. The γ-H2AX foci were stained with anti-phospho-histone H2AX (pink) and cell nuclei were stained with DAPI (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Compared to controls, 11 genes were significantly up-regulated and eight were significantly down-regulated in KR4 cells. We further validated two genes that were upregulated (MSH4, RAD51) and one gene that was downregulated (BRCA2) in the IGF-1R mutant, as the fold changes observed for the other genes were small and may not be biologically relevant. We confirmed our findings by PrimePCR assay (Bio-Rad) with predesigned primers for the three validated genes (Fig. 6).

Table 1
Gene expression changes of KR4 cells.

Gene	Fold up regulation	p-Value
BRCA1	1.3	0.001
ERCC4	1.49	0.008
ERCC5	1.48	0.012
FEN1	1.47	0.045
MSH4	2.75	0.006
NEIL3	1.43	0.04
PARP3	1.32	0.033
RPA3	1.43	0.024
RAD51	1.61	0.209
XRCC4	1.19	0.018
XRCC6BP1	1.33	0.03
Gene	Fold down regulation	p-Value
BRCA2	−2.05	0.032
ERCC2	−1.66	0.003
LIG3	−1.27	0.033
LIG4	−1.48	0.002
MLH1	−1.17	0.036
MLH3	−1.47	0.014
PARP1	−1.33	0.036
RAD51B	−1.25	0.038

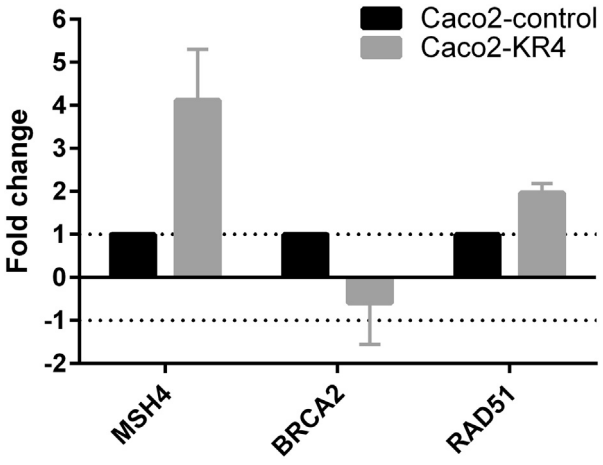

Fig. 6. Human DNA repair gene expression profiling in Caco-2-KR4 cells. Gene Expression changes of DNA repaire genes in kinase-deficient IGF-1R Caco-2-KR4 cells. PrimePCR assay was used to verify detected expression changes by PCR array. The upregulation of two genes: MSH4 and RAD51 and downregulation of BRCA2 when compared to control cells could be detected. Data is presented as the mean values ± SEM of three independent experiments.

A RAD51 inhibitor sensitizes cells expressing the kinase-deficient IGF-1R to irradiation

Caco-2-KR4 cells showed an increased level of Rad51 mRNA, when compared to control cells. Therefore, we studied the effect of RAD51 inhibition on the radio-sensitivity of Caco-2-KR4 cells using a clonogenic assay. A reduction in the SF was only observed after 6 Gy of irradiation (Fig. 7), indicating that Caco-2-KR4 cells respond only marginally to radiation treatment after RAD51 inhibition. No effect of RAD51 inhibitor to control cells in comparison to DMSO treated control cells could be detected.

Discussion

Many previous studies have demonstrated the involvement of IGF-1R in tumor growth and its role as a prognostic factor in different types of cancer [27–30]. Specifically, a high expression of IGF-1R in tumor cells has been associated with a poor clinical outcome

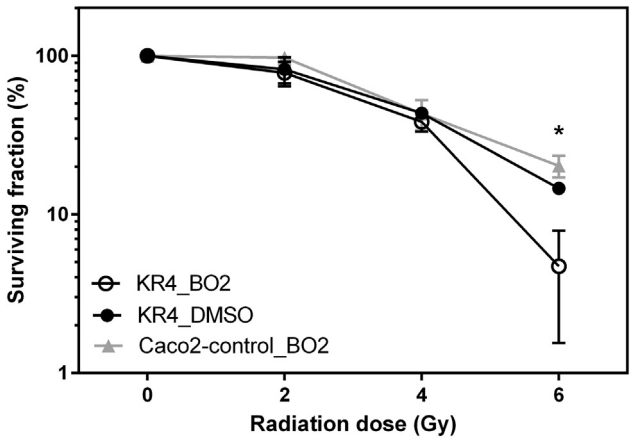

Fig. 7. Clonogenic radiation survival assay in combination of RAD51 inhibitor (BO2). Caco-2-KR4 cells were pre-treated for one hour with a RAD51 inhibitor (BO2) before cells were irradiated with different dose of γ-rays. Cells treated with DMSO were used as a control. Cells were plated, incubated, and colonies were counted after 14 days. The surviving fractions (SF) were calculated after normalizing to the plating efficiency of the corresponding sham-irradiated cells (0 Gy). Inhibition of RAD51 reduced clonogenic survival, when compared to control cells. The results are represented as the mean % SF ± SEM of three independent experiments, with each performed in triplicate ($n = 3$). $^{*}p < .05$.

in patients with breast cancer, cervical cancer, and oral cavity carcinomas [16,31–33].

Additionally, *in vitro* studies have indicated that an increased expression of IGF-1R confers radio-resistance to cells [16,34,35]. Accordingly our results utilizing a kinase-deficient IGF-1R mutant, reduced cell viability and clonogenic survival in Caco-2 cells after irradiation. Although an IGF-1R signaling blockade was shown to enhance the radiation response, little is known about the underlying mechanism(s).

We investigated the effect of IGF-1R targeting on DNA damage and repair, using γ-H2AX foci as a biomarker for DSBs [36]. By counting γ-H2AX foci, we observed a detectable level of DSBs even in un-irradiated cells, which was likely due to the genomic instability and replication stress common to cancer cells [37,38]. The number of γ-H2AX foci was not increased significantly in Caco-2-KR4 cells after γ-irradiation than in control cells. Previous studies have reported that depletion of IGF-1R delays γ-H2AX foci resolution, indicating an interruption in the DSB repair process [39]. However, we did not observe a similar delay in our study, which included measurements up to 48 h after radiation exposure.

The mechanism by which IGF-1R influences DNA repair mechanisms is not fully understood; however, a number of potential hypotheses have been raised. Previous work by Trojanek et al. postulated that IGF-1R may affect DNA repair through a mechanism involving a RAD51 and insulin receptor substrate 1 (IRS-1) interaction. Hypo-phosphorylated IRS-1 is known to interact with RAD51, thereby restricting its availability for DSB repair. However, IRS-1 becomes phosphorylated following IGF-1R activation, which decreases the affinity of IRS-1 for Rad51 [40]. It is possible that mutant IGF-1R interferes with the phosphorylation of IRS-1, thereby impairing the recruitment of RAD51 to damaged DNA. Conversely, the genomic instability arising from defects in HRR in tumor cells has been shown to be compensated for by overexpression of RAD51 [41].

In agreement with this hypothesis, we demonstrated that the inhibition of RAD51 in Caco-2-KR4 cells further reduced survival after irradiation. The inhibition of IGF-1R signaling has been shown to radio-sensitize cells through suppression of both non-homologous end joining (NHEJ) and HRR. However, blockade of IGF-1R activity does not radio-sensitize glioblastoma cells deficient

in DNA-dependent protein kinase (DNA-PK), which is required for the NHEJ pathway of DNA repair, suggesting an epistatic relationship between IGF-1R and DNA-PK [42]. However, it is unclear whether Rad51 overexpression can complement the tumor suppression function of BRCA2 or other possible functions unrelated to radiation sensitivity or DNA repair [43].

Following our investigation of DNA damage caused by irradiation, we measured the expression of key DNA repair genes. Compared to control cells, we found that mutant IGF-1R cells had an up-regulation in MSH4 and RAD51 expression, and a down-regulation of BRCA2. BRCA2 is a key mediator of repair by homologous recombination (HRR) and recruits RAD51 to the DNA damage site: cells that express defective BRCA2 are unable to form RAD51 foci after irradiation [44]. Previous reports have demonstrated that depletion of BRCA2 sensitizes cells to IGF-1R inhibition and that RAD51 foci formation is reduced, indicating defective HRR [45]. BRCA2 undergoes epistatic interactions with four RAD51 paralogs, and its loss alone can affect the HRR [46]. Defective homologous recombination caused siRNA mediated depletion of BRCA2 which was rescued by over expression of RAD51. BRCA2 defective cell line Capan-1for example utilize BRCA2 independent mechanism of Rad51 nuclear transport during S-phase, and BRCA2 dependent nuclear transport after irradiation (Exogenous damage) [44,47]. For further investigations of molecular understanding of radioresistance of kinase deficient IGF-1R cells the protein expression could also be determined. In view of the literature, we hypothesize that the down-regulation of BRCA2 observed in Caco-2-KR4 cells in our study may play a role in suppression of HRR.

The limitation of this study is that the effects were shown only in one cancer cell line. But similar studies were reported earlier that targeting IGF-1 receptor enhances the radiosensitivity of different cancer cell lines such as prostate cancer [39,48], non–small cell lung cancer [49,50], osteosarcoma [51], head and neck carcinoma [52].

Conclusions

In summary, we show that blocking IGF-1R-mediated signaling reduces cell proliferation and sensitizes cells to the destructive effects of ionizing radiation. The resulting radio-sensitization could be mediated through down-regulation of BRCA2, a mediator of HRR. Taken together, our data suggests that IGF-1R might be a potential target for enhancing tumor sensitivity to radiotherapy.

Declarations

Competing interests: The authors hereby declare no conflict of interest in this manuscript.

Funding: Research funding was provided by grants from the Bundesministerium für Bildung und Forschung (BMBF), Germany given to MMW (02 NUK 016A).

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