Radiotherapy and Oncology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original article

Differential DNA repair pathway choice in cancer cells after proton- and photon-irradiation

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ARTICLE INFO

Article history:
Received 6 July 2015
Received in revised form 12 August 2015
Accepted 15 August 2015
Available online xxxx

Keywords:
Photon irradiation
Proton irradiation
Non-homologous end-joining
Homologous recombination
NU7026

ABSTRACT

Background and purpose: Non-homologous end-joining (NHEJ) and homologous recombination (HR) contribute to the repair of irradiation-induced DNA double-strand breaks (DSBs). We investigated the impact of the two major DSB repair machineries for cellular survival of human tumor cells in response to proton- and photon-irradiation.

Materials and methods: DNA damage repair and cell survival were analyzed in wildtype, HR- and NHEJ-repair-compromised and pharmacologically DNA-PKcs-inhibited human tumor cells in response to clinically relevant, low-linear energy transfer proton- and 200-keV photon-irradiation.

Results: Pharmacological inhibition of DNA-PKcs strongly radiosensitized lung adenocarcinoma and glioblastoma cells to photon- but to a much lower extent to proton-irradiation. Enhanced radiosensitization correlated with strongly delayed repair kinetics with elevated amounts of γH2AX foci after photon-irradiation. Interestingly, we observed reduced phosphorylation of DNA-PKcs at Ser-2056 and Thr-2609 clusters after proton-irradiation compared to photon-irradiation. In contrast, A549 cells depleted of the RAD51 recombinase were markedly hypersensitive to proton-irradiation in comparison with control cells. Likewise, human BRCA2-deficient ovarian carcinoma cells were hypersensitive toward proton- in comparison with photon-irradiation.

Conclusion: A differential DNA damage response with enhanced susceptibility of HR-deficient tumor cells to proton-irradiation and increased sensitivity of photon-irradiated tumor cells to NHEJ inhibitors were demonstrated.

Currently, a generic RBE value of 1.1 is used in the clinic for proton radiotherapy. This value can vary significantly depending on the tissue, cell line or endpoint investigated, and an increasing number of studies now investigates these differences in detail at the cellular and molecular level [1–8].

Un- and misrepaired DNA double-strand breaks (DSBs) are the major cause for IR-induced cell death [6,7]. Even though both types of ionizing radiation (IR) are regarded as low LET-irradiation, the complexity of the DNA damage in response to clinically-relevant proton- and photon-irradiation could be different [5,6]. Independent of the amount of IR-induced DSBs, the quality of DNA damage might then demand differential DNA repair capacities. We previously could not detect any significant differences in the amount of DSBs induced by the two types of irradiation, but demonstrated in a model study based on genetically-defined chinese hamster ovary cells that cells with compromised homologous recombination (HR) are more sensitive to treatment with low-LET proton-irradiation (136 MeV) compared to photon-irradiation [8].

Cells have evolved two major pathways to cope with potentially lethal IR-induced DSBs, HR and non-homologous end joining (NHEJ) [9,10]. HR is primarily active through the S/G_2-phase of the cell cycle and results in preserved sequence integrity [11]. On the other hand, NHEJ is active throughout the cell cycle and is responsible for the repair of most IR-induced DSBs in eukaryotic cells [12]. Although very efficient in a quantitative way, the quality of repair by NHEJ can steadily decrease with increasing amounts of DNA damage [13].

Here we investigated a differential treatment response to proton- versus photon-irradiation in human tumor cells on the mechanistic level and demonstrated a differential involvement of the two major DSB repair machineries also by pharmacological interference.

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http://dx.doi.org/10.1016/j.radonc.2015.08.014
0167-8140/

Materials and methods

Cell lines

A549 cells (ATCC) were cultured in RPMI cell culture media. The glioblastoma cells M059K and M059J were maintained in 1:1 MEM/F12 Ham's mixture. All media were supplemented with 10% FBS, glutamine (2 mM) and penicillin-streptomycin (100 U/ml-100 μg/ml) and cells were kept at 37 °C at 5% CO_2 atmosphere.

Drug treatment

NU7026 (10 μM, Selleckchem) and Vorinostat (2 μM, Selleckchem) were added 1 h and 24 h, respectively, to the cells prior to irradiation.

Antibodies

The following antibodies were used at the specified dilutions: rabbit monoclonal anti-H2AX-pSer139 (1:200, Abcam, Cambridge, UK); rabbit monoclonal anti-RPA32 (1:1000 Bethyl Science, Bethesda, USA); rabbit polyclonal anti-DNA-PKcs-pSer2056 (1:100, Abcam, Cambridge, UK); mouse monoclonal anti-DNA-PKcs-pThr2609 (1:200, Abcam, Cambridge, UK); mouse monoclonal anti-DNA-PKcs (1:500, Abcam, Cambridge, UK); mouse monoclonal anti-RAD51 (1:100 (IF) or 1:500 (WB) – Novus Biological, A-8 Littleton CO, USA); mouse monoclonal anti-β-actin (1:1000, Sigma-Aldrich, St. Louis MO, USA).

Irradiation procedures

Irradiation and dosimetry were essentially performed as described in [8]. Exponentially growing cultures were plated into 25 cm^2 TPP flasks 24 h prior to irradiation (IR). Photon-irradiation was performed using an Xstrahl 200 kV X-ray unit at 1 Gy/min (kVp = 200 kV; Amperage = 15 mA, HVL = 1.03 mm Cu, Filter: 1 mm Al and 0.45 mm Cu). All proton-irradiations were delivered using the spot scanning approach [14] with the cells being placed in the center of a Spread-Out-Bragg-Peak (SOBP) with a length of 5 cm and maximum proton energy of 138 MeV. The field size orthogonal to the beam was 15 × 11 cm.

Clonogenic survival

The clonogenic cell survival assay was performed in triplicates as described in detail in [8]. In order to calculate RBE and DMF

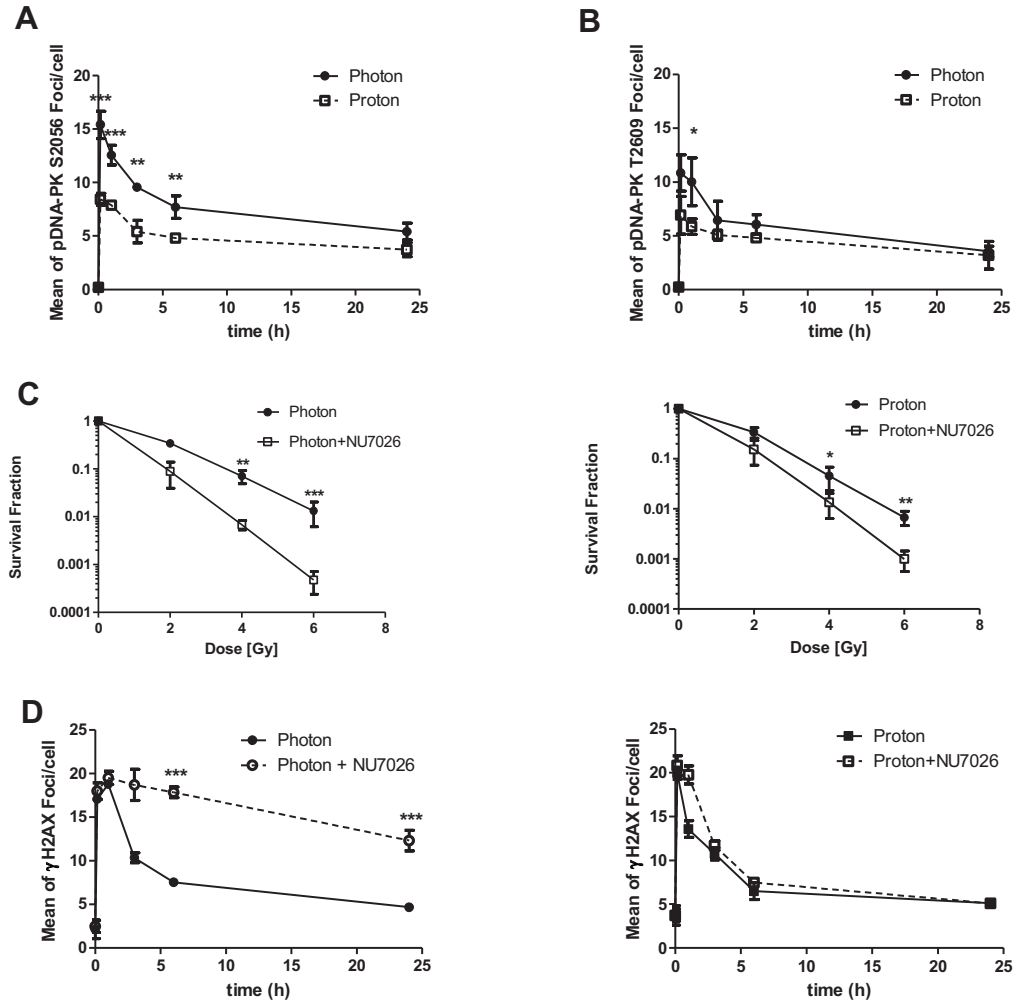

Fig. 1. Radiosensitizing effect of DNA-PKcs inhibitor NU7026 on A549 cells. Time course of DNA-PKcs-phosphorylation at the Ser-2056 (A) and Thr-2609 (B) cluster sites in response to 1 Gy of proton- and photon-irradiation. (C) Clonogenic survival of A549 cells pretreated with NU7026 followed by photon- and proton-irradiation. Clonogenic cell survival assays were performed in triplicates. Points represent mean ± SD. Data analysis was performed on pooled values from at least 3 independent experiments. Kinetics of γH2AX (D) foci removal after 1 Gy of proton- and photon-irradiation in A549 cells pretreated for 1 h with NU7026 and fixed at the indicated time points. Foci of at least 50 cells/condition were counted and means of data were pooled from at least 3 independent experiments.

Please cite this article in press as: Fontana AO et al. Differential DNA repair pathway choice in cancer cells after proton- and photon-irradiation. Radiother Oncol (2015), http://dx.doi.org/10.1016/j.radonc.2015.08.014