Original article

Glucose starvation impairs DNA repair in tumour cells selectively by blocking histone acetylation

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ABSTRACT

Background and purpose: Tumour cells are characterized by aerobic glycolysis and thus have high glucose consumption. Because repairing radiation-induced DNA damage is an energy-demanding process, we hypothesized that glucose starvation combined with radiotherapy could be an effective strategy to selectively target tumour cells.

Material and methods: We glucose-starved tumour cells (A549, FaDu) in vitro and analysed their radiation-induced cell responses compared to normal fibroblasts (HSF7).

Results: Irradiation depleted intracellular ATP levels preferentially in cancer cells. Consequently, glucose starvation impaired DNA double-strand break (DSB) repair and radiosensitized confluent tumour cells but not normal fibroblasts. In proliferating tumour cells glucose starvation resulted in a reduction of proliferation, but failed to radiosensitize cells. Glucose supply was indispensable during the late DSB repair in confluent tumour cells starting approximately 13 h after irradiation, and glucose starvation inhibited radiation-induced histone acetylation, which is essential for chromatin relaxation. Sirtinol – an inhibitor of histone deacetylases – reverted the effects of glucose depletion on histone acetylation and DNA DSB repair in tumour cells. Furthermore, a glucose concentration of 2.8 mmol/L was sufficient to impair DSB repair in tumour cells and reduced their clonogenic survival under a fractionated irradiation regimen.

Conclusions: In resting tumour cells, glucose starvation combined with irradiation resulted in the impairment of late DSB repair and the reduction of clonogenic survival, which was associated with disrupted radiation-induced histone acetylation. However, in normal cells, DNA repair and radiosensitivity were not affected by glucose depletion.

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Although radiotherapy is a highly effective treatment modality for cancer, the use of high doses is limited by the surrounding normal tissue. Consequently, to improve the outcome, it is necessary to establish treatment regimens that target tumour cells selectively and spare normal tissues.

As early as the 1920s, Otto Warburg discovered that tumour cells are characterized by their increased glucose uptake and lactate production – a phenomenon called aerobic glycolysis [21] – which contributes to treatment resistance in tumour cells [22].

Ionizing radiation (IR) induces DNA double-strand breaks (DSBs), which will induce cell death if left unrepaired [13].

However, repairing DNA DSBs is a highly energy-demanding process [4] because it requires the opening of chromatin to facilitate access for repair machinery [17].

Because aerobic glycolysis makes tumour cells highly dependent on glucose to fulfil their energy requirements, we suggest the combination of glucose starvation and radiotherapy as an effective strategy to target tumour cells selectively. It has already been reported that glucose restriction sensitizes cancer cells to chemotherapeutic agents in vitro and in vivo [14,11]. In addition, fasting, caloric restriction and a ketogenic diet have been shown to improve the effectiveness of radiotherapy in vivo [14,15,1,2]. However, the underlying molecular mechanism is poorly understood. Consequently, herein we analysed the impact of glucose starvation on tumour cells compared to normal fibroblasts after exposure to radiation in vitro.

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Materials and methods

Cell culture and irradiation

Experiments were performed with the human non-small cell lung cancer cell line A549 (ATCC CCL-185), the human head and neck squamous cell carcinoma cell line FaDu (ATCC HTB-43) and normal human skin fibroblasts HSF7 [6]. Unless otherwise indicated, confluent cells were used for the experiments. Irradiation was performed using the X-ray cabinet of X-Strahl RS225 at a dose rate of 1.0 Gy/min.

Glucose consumption

The glucose concentration of the culture medium was quantified according to the manufacturer's instructions (BioAssay Systems, EnzyChrom Glucose Assay Kit).

ATP assay

The ATP assay was performed according to the manufacturer's instructions (Abcam, ATP-colorimetric Assay).

Lactate assay

Lactate quantification was performed with a lactate assay kit (MAK064) from Sigma–Aldrich according to the instructions of the manufacturer.

γH2AX-foci assay

Cells were grown to confluence on CultureSlides (Becton Dickinson). After the cells were glucose starved for 24 h, they were irradiated with 2 Gy. Glucose starvation was continued, unless otherwise noted, and the number of γH2AX-foci was determined at the indicated times after irradiation. Cells were fixed with 70% ethanol and permeabilized with 0.1% Triton X-100/PBS. Unspecific binding was blocked with 3% BSA/PBS. γH2AX-foci were visualized by incubation with γH2AX antibody (1:500, BioLegend, clone 2F3) for 1 h, followed by incubation with Alexa Fluor 488 labelled goat anti-mouse serum (1:500, Life Technologies) for 1 h. Coverslips were mounted with Vectashield mounting medium with DAPI (Vector Laboratories). γH2AX-foci were scored manually by the same operator using a Zeiss Axiovert 135 fluorescence microscope. Foci amounts were counterchecked by a second operator. The average number of foci per nucleus was calculated from a minimum of 300 cells per data point.

Colony formation assay

Confluent cells were glucose-starved for 24 h or 72 h and irradiated. Then, 24 h after irradiation, the cells were plated in DMEM with 25 mmol/L glucose containing 20% FCS and 1% penicillin/streptomycin. The assay was performed according to standard procedures [9].

Western blotting

At the indicated times after irradiation, cell lysates were prepared and proteins were resolved by SDS-PAGE. Western blotting was performed according to standard procedures. The blots were incubated with the specific primary antibodies anti-histone H3 (Cell Signaling, 96C10), anti-histone H3 acetyl K9 (Cell Signaling, C5B11), anti-histone H4 (Cell Signaling, L64C1), anti-histone H4 acetyl K12 (Cell Signaling) and anti-actin (Sigma–Aldrich). Quantification was performed with the LI-COR detection system (LI-COR, Odyssey Fc). All primary antibodies were diluted as 1:1000.

SRB assay

Cells were seeded at a low density in 96-well plates (500 cells per well). After 24 h, glucose starvation was performed for 48 h, followed by irradiation with 2 Gy. After incubation for an additional 7 days under starvation conditions, the cells were fixed, and the cell numbers were quantified using the SRB assay [16].

Statistics

All data presented show the mean ± SEM of three independent experiments, unless otherwise indicated. When comparing two means, an unpaired Student's t-test was calculated. One-way analysis of variance (ANOVA) was used to study the differences among three or more means. Two-way ANOVA was performed to test the interaction and main effects of two factors. The results were considered statistically significant if the p-value was less than 0.05 ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). All post hoc comparisons were conducted with Bonferroni-corrected alpha levels. All statistics were calculated using SPSS 23 software.

Results

Irradiation increased the glucose consumption of confluent tumour cells and normal fibroblasts

Measurements of the glucose consumption of the confluent cells over 48 h after irradiation revealed a significant increase for the A549, FaDu and HSF7 cells (Fig. 1A). The glucose consumption of the normal fibroblasts was, in general, significantly smaller compared to that of the tumour cells. Bromodeoxyuridine (BrdU) staining, which was used to determine the percentage of proliferating cells, showed that under confluent conditions, 4.8% of the A549 cells and 6.8% of the FaDu cells were positive for BrdU incorporation (data now shown).

Irradiation reduced the intracellular ATP content and increased the lactate production preferentially in the tumour cells

For both tumour cell lines in confluency, we observed a significant decrease in the intracellular ATP content after irradiation (Fig. 1B). The ATP content of the HSF7 cells was much higher than that in the tumour cells, and despite a significant decrease after irradiation, the intracellular ATP levels remained high. In both tumour cell lines, lactate production increased in response to irradiation (Fig. 1C). In contrast to the tumour cells, the normal fibroblasts HSF7 produced only low levels of lactate, and only a small increase in the intracellular lactate content could be detected after irradiation with 4 Gy.

Glucose starvation impaired the DNA DSB repair and clonogenic survival selectively in the tumour cells

To determine if DNA DSB repair was dependent on the presence of glucose, we quantified the γH2AX-foci after glucose starvation and irradiation (Fig. 2A, Suppl. Fig. 1). We observed significantly more residual γH2AX-foci in the starved cells compared to the non-starved cells 24 h after irradiation in both tumour cell lines. In contrast, starvation did not have a significant effect on DSB repair in the normal fibroblasts. In general, the residual DNA damage was highest in the FaDu cells.

In agreement with the increased residual DNA damage, glucose starvation radiosensitized both tumour cell lines A549 and FaDu as measured with the clonogenic survival assay, whereas the radiosensitivity of the HSF7 normal fibroblasts was not affected by the glucose starvation (Fig. 2B). In addition, glucose starvation