Response of MCF-7 breast cancer cells to proton and carbon ion irradiations

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BACKGROUND

Between various DNA damages induced by ionising radiation, the most lethal forms are DNA double strand brakes (DSB). Phosphorylated H2AX histone (γH2AX) is one of the key proteins involved in DNA DSB repair. It appears close to the DSB and initiates DNA repair. Ataxia-telangiectasia mutated and Rad3-related kinase (ATR) acts as γH2AX activator and regulator of different DNA repair mechanisms. It is involved in homologous recombination and along with Rad51 stimulates repair of residual DSB and cell survival.

The aim of this study is to analyse effects of protons and carbon ions on MCF-7 breast cancer cells.

RESULTS

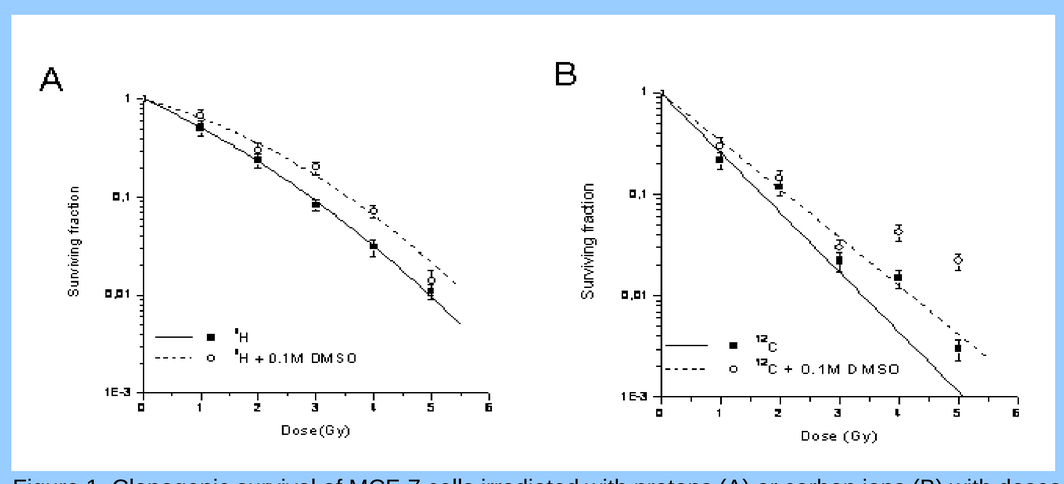

Figure 1. Clonogenic survival of MCF-7 cells irradiated with protons (A) or carbon ions (B) with doses 1, 2, 3, 4 and 5 Gy. Results are shown as mean±SEM.

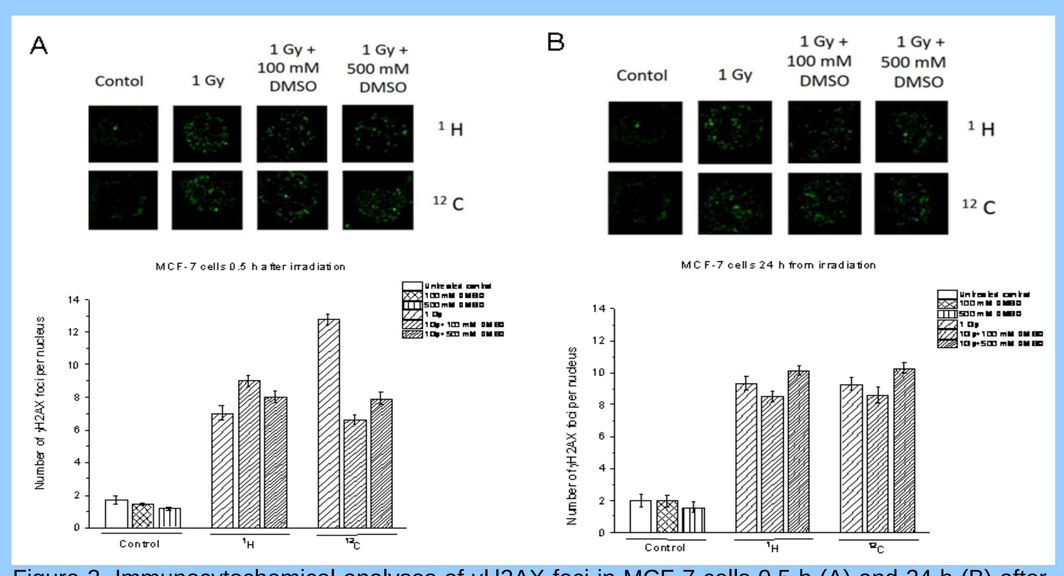

Figure 2. Immunocytochemical analyses of γH2AX foci in MCF-7 cells 0.5 h (A) and 24 h (B) after irradiation with protons and carbon ions. Results are presented as mean number of γH2AX foci±SEM.

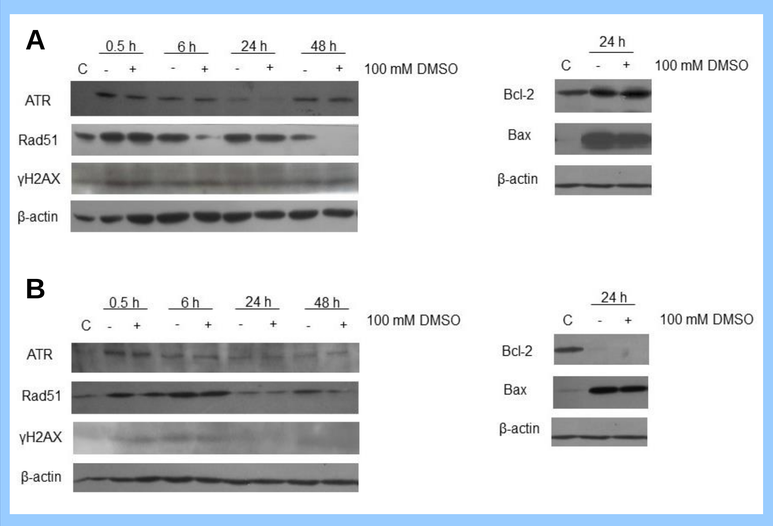

Figure 3. Expression of ATR, Rad51, γH2AX, Bcl-2 and Bax proteins after irradiation with protons (A) and carbon ions (B) at 0.5, 6, 24 and 48 h time points. C – untreated control.

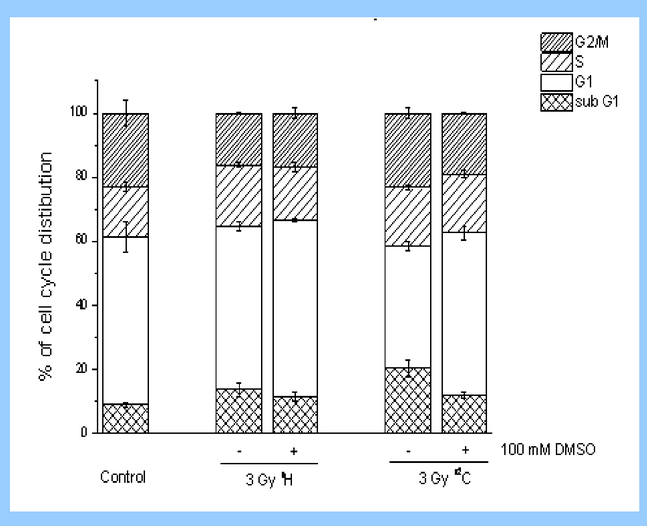

Figure 4. Cell cycle analysis of MCF-7 cells 24 h after irradiation with protons and carbon ions. Results are presented as mean±SEM.

Materials and methods

MCF-7 breast adenocarcinoma cells were obtained from the ATCC (Manassas, Va, USA) and cultured in standard conditions in humidified atmosphere at 5% CO2 and 37°C (Heraeus, Hanau, Germany). To identify direct action of radiation, DMSO was applied as free radical scavenger.

Irradiation position for protons was in the middle of the therapeutic 62 MeV/u spread-out Bragg peak, while the position within somewhat widened Bragg peak of the 62 MeV/u carbon ion beam was chosen as to obtain LET values with the highest biological effectiveness. Cells were irradiated with doses ranging from 1 to 5 Gy.

Conclusions

- Clonogenic assay reveals higher cell inactivation with carbon ions than protons, while protective effect of DMSO is observed after both applied irradiations.
- Elevated number of γH2AX foci is generated by both irradiation types 0.5 h post-irradiation and is more pronounced with carbon ions. In both cases, residual foci are detected after 24 h, indicating unrepaired DNA DSB.
- γH2AX expression increases 0.5 and 24 h after proton irradiation, while after 0.5 h ATR rises. Levels of Rad51 are not significantly changed.
- Carbon ions increase γH2AX 0.5 h after irradiation, with the maximum at 6 h, while the level of ATR remains elevated up to 48 h post-irradiation. The drop of γH2AX and Rad51 24 h from irradiations could be due to high cell inactivation, while residual DNA damages induce ATR accumulation.
- Cellular arrest in G1/S phase, 24 h after irradiations, indicates operating DNA repair processes. Increased sub G1 phase and elevated Bax/Bcl2 ratio, particularly after carbon ion irradiations, are associated with apoptosis.