No formation of γH2AX foci or micronuclei in bystander cells

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Introduction

In previous studies we have observed a transient cell cycle arrest as well as an altered expression and activation of cell cycle regulating proteins in bystander cells after exposure to heavy ions. In general, damage to the DNA in bystander cells is considered as a possible mechanism triggering the observed effects.

Here we address whether heavy-ion induced DNA and cytogenetic damage is detectable in bystander cells. For this purpose, we evaluate both the formation of γH2AX foci and micronuclei as markers for damaged DNA. In the course of this study, and to our knowledge for the first time worldwide, parallel experiments at two heavy-ion microbeams (GSI, JAEA) using parallel cultures of the same cell strain, duplicate conditions, and irradiation protocols have been conducted [1].

Experiment

Normal human fibroblasts (AG1522) were thawed and cultivated to confluence at GSI. One part of this culture was flown to JAEA, the other remained at GSI. Both parts were kept at identical culture conditions until the respective irradiation. Microbeam irradiation was performed at GSI (targeted exposure, UV excited nuclear dye, 1%, 3% proliferating cells hit) and JAEA (geometrical micro pattern of exposure, no UV, 1% confluent cells hit). The quantitative assessment of γH2AX foci (only GSI) was performed by immunofluorescence staining and semi-automatic detection and quantification. The formation of micronuclei (GSI and JAEA) was investigated using two different protocols: According to protocol 1, the proliferating cells were incubated for 72h in medium supplemented with 1µg/ml Cytochalasin B directly after microbeam irradiation. The treatment corresponding to protocol 2, confluent cells were incubated in conditioned medium for 24h after irradiation, and then reseeded at low density in fresh medium with 1µg/ml Cytochalasin B and incubated for 24h. In both cases, bi-nucleated cells containing micronuclei were identified by microscopy using a set of morphological criteria (blinded samples, three independent researchers).

Results

Regarding γH2AX analysis in bystander populations, Fig. 1 depicts the percentage of cells exceeding the average number of foci per control cell. The kinetics of the foci formation shown in Fig. 2 does not give any hint to an effect at earlier time points. No significant elevation of the number of foci in bystanders was observed. The formation of micronuclei is depicted for the different protocols and facilities in Fig. 3. Although a clear dependence of the frequency of cells with micronuclei on the protocol was observed, no significant difference was detected after exposure.

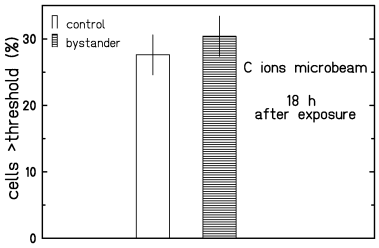

Fig.1: Percentage of cells containing more γH2AX foci than control mean plus 1.5 standard deviations. More than 1000 cells scored.

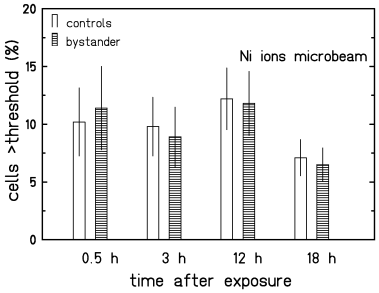

Fig.2: Same criterion as in Fig 1. No effect at earlier time points (one experiment).

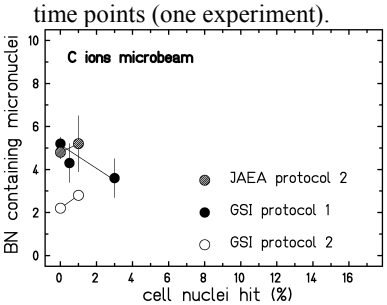

Fig.3: Percentage of bi-nucleated cells containing micronuclei for two protocols and at two microbeam facilities. No bystander effect at this endpoint visible.

In conclusion no significantly elevated formation of γH2AX foci or micronuclei was observable in bystander cells [1].

References

[1] C. Fournier, P. Barberet, T. Pouthier, S. Ritter, B. Fischer, K. O. Voss, T. Funayama, N. Hamada, Y. Kobayashi, and G. Taucher-Scholz, Radiat Res accepted for publication