

## **No Evidence for DNA and Early Cytogenetic Damage in Bystander Cells after Heavy-Ion Microirradiation at Two Facilities**

Fournier C, Barberet P, Pouthier T, Ritter S, Fischer B, Voss K-O, Funayama O, Hamada N, Kobayashi Y, Taucher-Scholz G  
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The occurrence of bystander effects has challenged the evaluation of risk for heavy ions, mainly in the context of space exploration and the increasing application of carbon ions in radiotherapy. In the present study, we addressed whether heavy-ion-induced DNA and cytogenetic damage is detectable in bystander cells. The formation of  $\gamma$ -H2AX foci, sister chromatid exchanges and micronuclei were used as markers of damage to DNA. Normal human fibroblasts were exposed to low fluences of carbon and uranium ions, and alternatively single cells were targeted with heavy ions using the GSI microbeam. We did not observe a significant increase in the bystander formation of  $\gamma$ -H2AX foci, sister chromatid exchanges or micronuclei. In addition, we performed for the first time parallel experiments at two microbeam facilities (GSI, JAEA) using the same cell line, culture conditions and irradiation protocols. No significant enhancement of the micronucleus frequencies in bystander cells was detected after targeted carbon-ion irradiation, confirming the results. Details regarding the history, culture conditions or support of the cells might be affecting the detection of bystander effects. On the other hand, the potential X-ray- and heavy-ion-induced bystander effects investigated herein clearly do not exceed the experimental error and thus are either lacking or are less pronounced than the effects reported in the literature for similar end points after  $\alpha$ -particle and X-ray exposure.