# Investigation of Genomic Instability in Clonal Descendants after X-ray Irradiation

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## Introduction

Exposure of normal human fibroblasts to ionizing radiation may cause a delayed instability of the genome in the descendants of the initially irradiated cells [1, 2]. This genomic instability appears as a *de novo* formation of structural chromosomal aberrations and is considered as a step towards malignant transformation and induced carcinogenesis [3]. The relevance of effects, related to genomic instability, observed in different experimental setups, cell strains and the dependence on radiation quality are still under debate [4]. In recent (or previous) experiments we have shown the occurrence of genomic instability in the progeny of mixed cell populations of fibroblasts initially irradiated with high doses of X-rays [2]. To circumvent a selective pressure and population dynamics on the yield and types of chromosomal aberrations in bulk cultures and to investigate the fate of single cells, colonies formed by single cells after irradiation with X-rays were isolated and analysed for cytogenetic changes in the present study.

## Materials and Methods

Density inhibited monolayers of AG1522D fibroblasts were exposed to a single dose of 2 Gy or a fractionated daily dose of 8x2 Gy X-rays (250 kV, 16 mA). At 24 hours after irradiation the cells were seeded at a very low density to obtain colonies from single surviving cells. After isolation of the colonies, the populations were expanded. Structural chromosomal aberrations were scored regularly using solid Giemsa staining and the mFISH-technique, when cells were in close proximity to the end of their replicative life span. Transmissible chromosomal aberrations were termed "clonal" if they were present in at least two metaphases at the same time point of analysis.

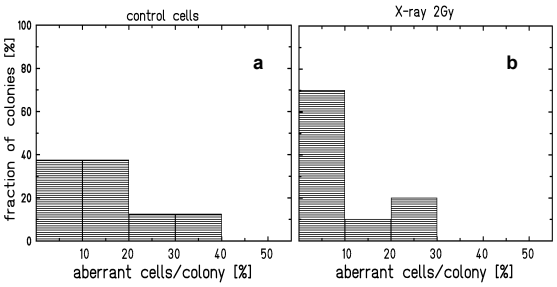


Figure1: Fractions of colonies from Giemsa analysis grouped in proportions of aberrant cells detected per colony scored in (a) 8 non-irradiated control colonies and (b) 10 colonies formed by cells initially irradiated with 2Gy X-rays.

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## Results and Conclusion

In single cell derived control populations a mean frequency of 14 ± 10 % (mean±SD) aberrant metaphases, were measured. As depicted in figure 1a, most colonies showed mild levels of instability with up to 20% aberrant metaphases while a few colonies had elevated levels up to 40% aberrant metaphases. After irradiation with 2 Gy X-rays, the mean level of aberrant metaphases was not elevated compared to control cells (11 ± 7%, SD). A high fraction of colonies (70%) showed levels below 10% aberrant cells (fig.1b). Irradiation of cells with a high dose of 8x2Gy X-rays, given in daily fractions, resulted in a similar response (mean 11% ± 8, SD, data not shown). The main types of structural chromosomal changes were dicentric chromosomes without acentric fragments as a result of telomeric fusions and chromosome and chromatid breaks. At the time point of analysis all populations were in close proximity to the end of their replicative life span, as assessed from counting population doublings. Preliminary results of mFISH analysis showed a dose dependent increase of radiation induced transmissible clonal aberrations (fig.2), but spontaneous, clonally expanding transmissible aberrations were also present in control as well as irradiated populations.

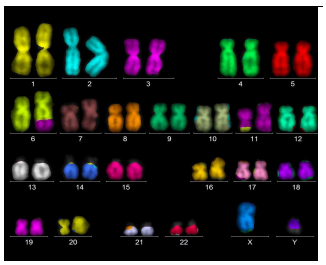

Figure2: mFISH stained karyotype of a single cell derived population initially irradiated with 2Gy X-rays showing the clonal translocation (6'-11) (11'-6) in 100% of the analyzed metaphases.

## Conclusion

So far, in contrast to our previous results obtained for mixed populations [2], we could not detect any influence of X-ray exposure on genomic stability in the clonal descendants of irradiated cells. The frequency of chromosomal instability and the observed aberration types were not different comparing controls and the progeny of irradiated cells. Thus, in the absence of a selective pressure of the bulk population, the observed overall mild increase in the proportion of aberrant cells is related to *in-vitro* aging of the cells. In further experiments the effects of high LET exposure on genomic stability will be investigated in clonal descendants of irradiated cells.

References

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