Active DNA Methylation Changes in Response to Ionizing Radiation*

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Introduction

Eukaryotic DNA methylation, which consists in the addition of a methyl group on cytosine bases, is an epigenetic mechanism controlling a variety of biological processes. Previous work reported that ionizing radiation (IR) can induce epigenetic aberrations, including genomic instability [1]. Moreover, several attempts to correlate radiation-induced genomic instability to changes in DNA methylation patterns have been pursued. However, in these studies DNA methylation levels were measured several population doublings after exposure to IR [2]. Whether DNA methylation changes may take place also at short times post irradiation remains controversial. In this study we aim to investigate global DNA methylation levels in several cell types before and after exposure to IR. Preliminary results on fibroblast and tumor cell lines suggest that X-rays induce changes in global DNA methylation levels, even at short times (0.5 hours) after exposure. Both increased and decreased DNA methylation levels were observed which were dependent on the cell line.

Results

We investigated different cell lines for their global DNA methylation levels before and after irradiation with 10Gy X-rays (Fig.1). We observed that DNA methylation levels do not change in MEF (mouse embryonic fibroblasts), while decreasing in AG1522 (human foreskin fibroblasts) and HeLa and increasing in U2OS (human osteosarcoma cells), NFF hTERT (immortalized human fibroblasts) and HCT116 (human colorectal carcinoma cells).

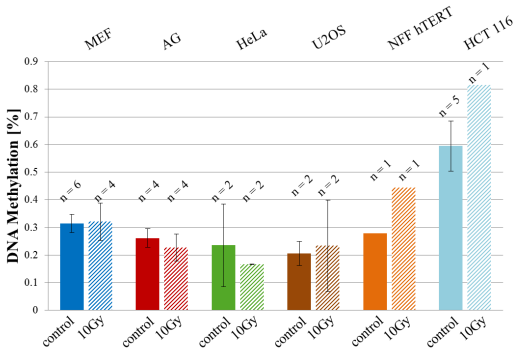

Figure 1: Fibroblast cell lines (MEF, AG1522 and NFF hTERT) or tumor cell lines (HeLa, U2OS and HCT116) were irradiated with 10Gy X-ray. Genomic DNA was isolated 30 minutes post irradiation and global DNA methylation analysis was performed using an ELISA-based technique quantifying 5-Methylcytosine colorimetrically. N indicates the number of experiments. 2 duplicates per sample were used. Error bar: standard error

Due to the different behaviour of the investigated cell lines, we thought that considering additional timepoints might reveal a common tendency of all cell lines towards DNA hypo- or hypermethylation. In a preliminary attempt we analyzed MEF and AG1522 cells. In MEF cells global DNA methylation increased 10 minutes and 3 hours after exposure to IR while 30 minutes and 24 hours after irradiation there was almost no change compared to control level. In contrast DNA methylation in AG1522 cells seemed to steadily decrease with increasing incubation time until the 3 hour timepoint. In both cell lines we observed complete (MEF) or partial (AG1522) recovery of DNA methylation after 24 hours relative to control level.

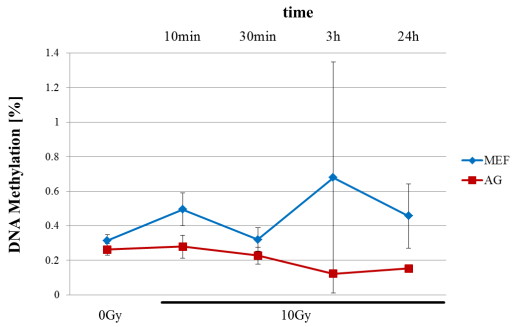

Figure 2: MEF and AG1522 cells were irradiated with 10Gy X-rays. DNA was isolated at the indicated timepoints and global DNA methylation was analyzed as described in Fig.1. Results for control, 10 min and 30 min are shown as mean for 3 experiments, results for 3h and 24h are from 2 experiments. 2 duplicates per sample were used in each experiment. Error bar: standard error

Conclusions

Our preliminary results show that global DNA methylation changes up to one duplication after irradiation are dependent on the cell line. While there is no clear tendency for methylation changes in MEF cells, a steady hypomethylation tendency is shown in AG cells. Further experiments are needed to elucidate methylation changes more closely and to characterize differences between different cell lines.

References

[1] Aypar et al. (2011) Int. J. Radiat. Biol., Vol. 87, No. 2, pp. 179–191

[2] Goetz et al. (2011) Radiation Research, Vol. 175, No. 5, p. 575-587

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