Intrinsic global hypomethylation and decreased *Brca1* expression are associated to DNA repair delay in irradiated thyroid cells

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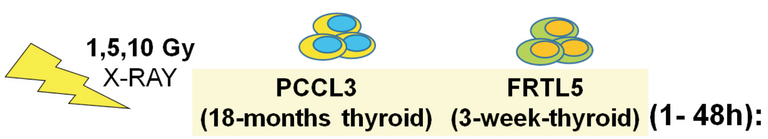
INTRODUCTION

Ionizing radiation (IR) is the most important risk factor for papillary thyroid cancer (PTC)[1], mainly due to gradual loss of DNA repair genes and DNA damage[2]. In response to double-strand breaks (DSB), homologous recombination and non-homologous end joining pathways are triggered[3]. Recent molecular characterization of PTC revealed a differential methylation of several promoters of DNA repair genes[4].

HYPOTHESIS

Aberrant methylation of DNA repair genes might be a plausible mechanism of gene expression downregulation at transcriptional level and, therefore, genomic instability in irradiated thyrocytes.

METHODOLOGY

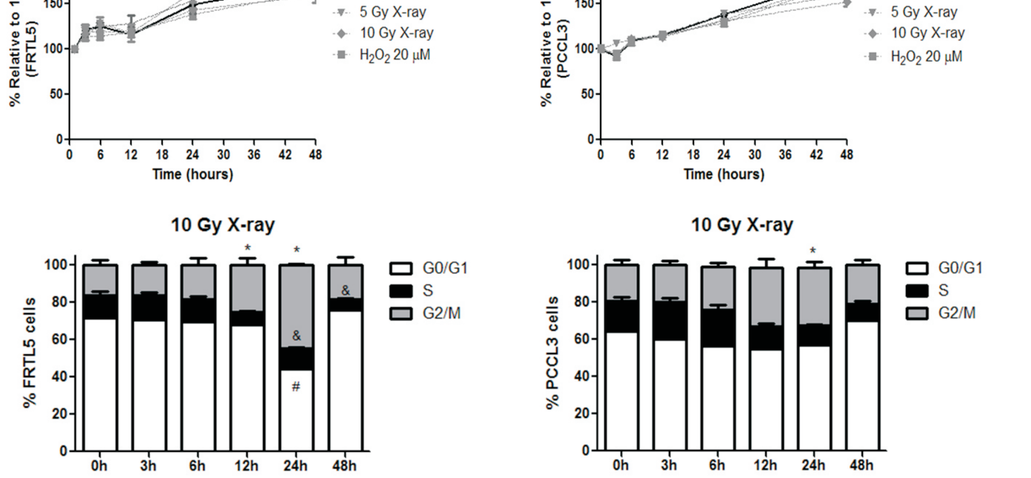


(1- 48h):

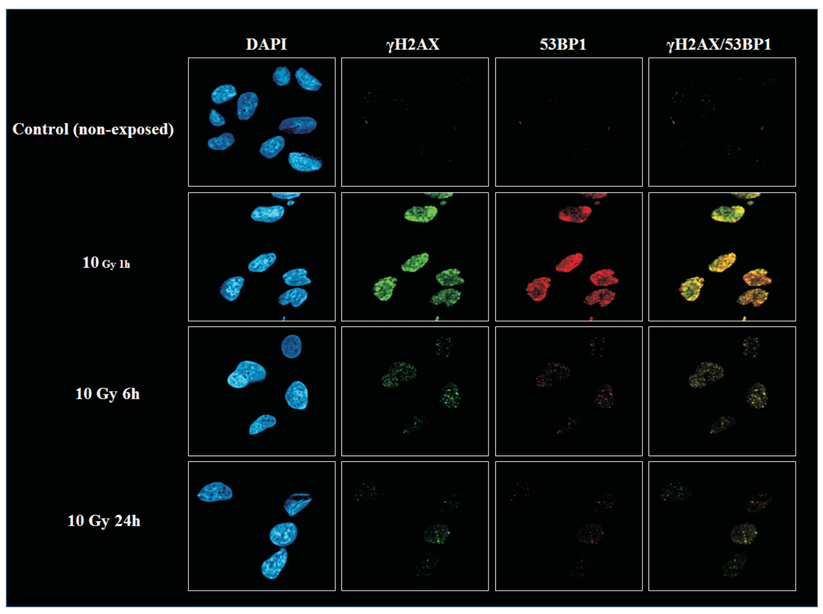
MTT (Cytotoxicity);
CYTOMETRY (Cell cycle);
IMMUNOFLUORESCENCE (γH2AX; 53BP1);
WESTERN BLOT (γH2AX, β-actina);
PYROSEQUENCING/ qPCR (*Line1*; Atm; Brca1; *Rad50; Mre11; Xrcc1, Xrcc4; Xrcc6, Lig4);* **SENESCENCE** (Morphology; β-gal activity).

RESULTS

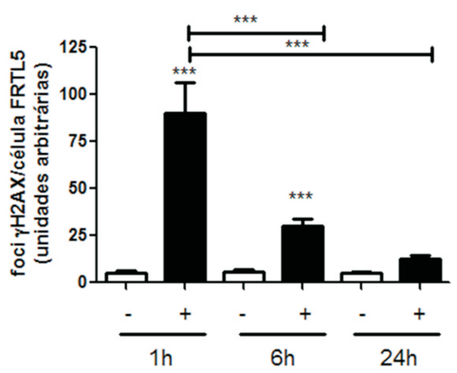
Cell viability and G2/M arrest

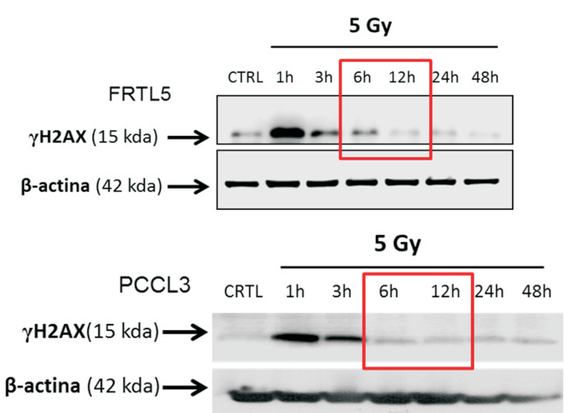


DSB markers (γH2AX/ 53BP1) in FRTL5 cell line

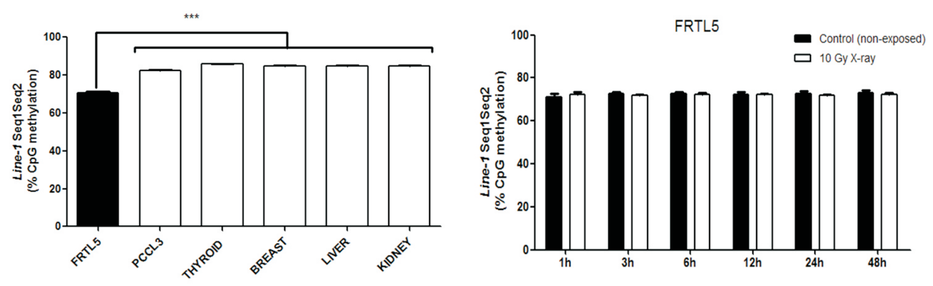


DSB repair kinetics of FRTL5 and PCCl 3 cell lines

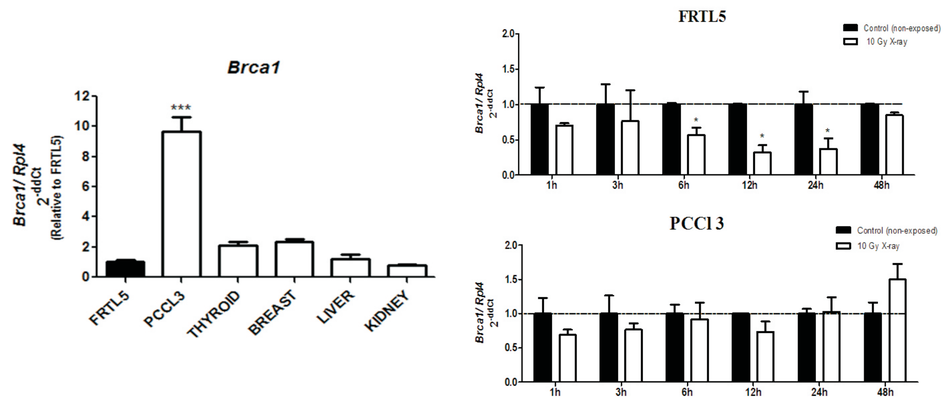




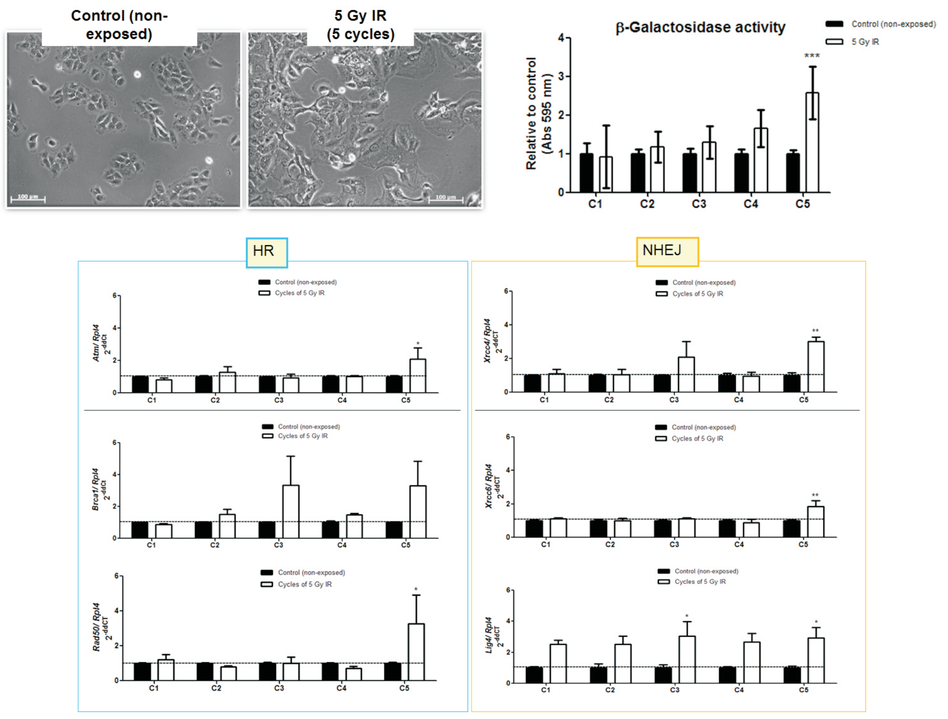
Global methylation of thyroid cell lines: basal and after X-ray exposure



Brca1 expression in thyroid cell lines: Basal and after X-ray exposure

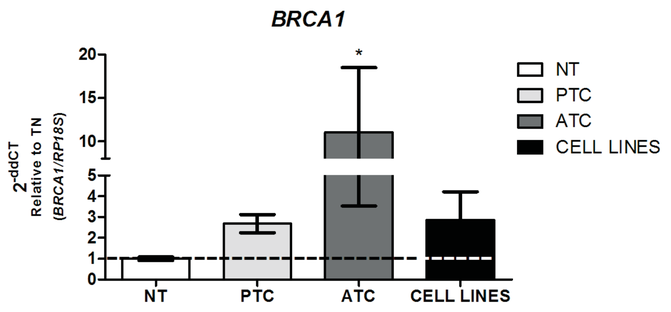


Radiation-induced senescence and HR and NHEJ gene expression in FRTL5 cell line



Brca1 expression in human thyroid tumor samples

NT (3); PTC (8); ATC (3); CELL LINES (4)



CONCLUSION

- FRTL5, derived from young rats, displays a slower kinetics of DSB repair and a lower global methylation than PCCl 3 cells, derived from 18 months-old rats;
- IR does not seem to modify the expression of genes involved in the regulation of HR and NHEJ pathways apart from the downregulation of *Brca1* in FRTL5 cells, in which Brca1 transcripts are much less abundant than in PCCl 3 cells;
- HR and NHEJ genes are induced by chronic irradiation;
- *BRCA1* is overexpressed in thyroid tumor samples.

REFERENCES

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