Impact of microRNA miR-34a on glioblastoma radioresistance

Igor de Oliveira Carvalho[1,2], Gustavo Henrique Cardoso Guimarães[1,3], Raquel Ciuvalschi Maia[1], Paula Sabbo Bernardo[1,2]

[1]Laboratório de Hemato-Oncologia Celular e Molecular, Programa de Hemato-Oncologia Molecular, Instituto Nacional de Câncer (INCA), Rio de Janeiro – RJ, Brasil. [2]Programa de Bolsas de Formação em Pesquisa Oncológica, INCA, RJ, Brasil. [3]Programa de Pós-graduação Stricto Sensu em Oncologia, INCA, RJ, Brasil.

INTRODUCTION AND OBJECTIVE

Glioblastoma (GBM) is the most incident and aggressive subtype of central nervous system malignant tumours. GBM is known for resistance to therapy with an average survival of 15 months and 5-year survival rate of only 5%. Standard treatment is tumour resection combined with radio- and chemotherapy based on temozolomide. These treatments cause DNA damage and the TP53 is known as the genome keeper, regulating cell cycle and apoptosis in response to DNA damage. MicroRNAs (miRNA) regulate gene expression post-transcriptionally by binding to a messenger RNA (mRNA) what leads to translation inhibition. Among miRNAs, miRNA-34a is downregulated in GBM samples and directly activated by p53 in response to DNA damage. Moreover, our group already demonstrated that high expression of p53, a surrogate marker of *TP53* mutation, is correlated with better response to radiotherapy (Figure 1). In this context, our goal is investigate miR-34a role in radiation response of GBM cell lines with distinct *TP53* status. The signalling pathway studied is described in figure 2.

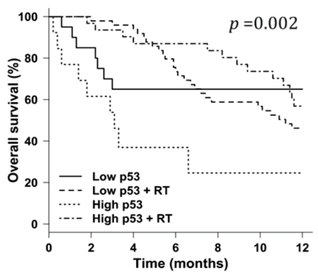

Figure 1. Kaplan-Meier survival curves displaying the impact of p53 expression in association with radiotherapy on diffuse astrocytic tumor patient survival **during 12 months**. RT: Radiotherapy. Adapted from Faccion RS, Bernardo PS, Lopes GPF et al. Cellular Oncology. 2018

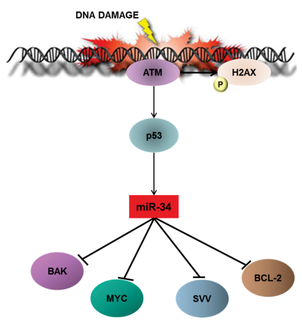

Figure 2: Representation of p53-miR34a signalling pathway in response to DNA damage.

MATERIALS AND METHODS

We used three GBM cell lines with different TP53 status. For ionizing radiation treatment, we used 2, 4, 8, 16 and 24Gy to evaluate cell cycle and DNA fragmentation by flow cytometry. Western Blot was used for investigation of p53 and target proteins of miR-34a in response to ionizing radiation. miR-34a expression was evaluate by qRT-PCR. Finally, a marker of DNA damage (γH2Ax) was evaluated by immunofluorescence. Figure 3 describes the methodology applied in the study.

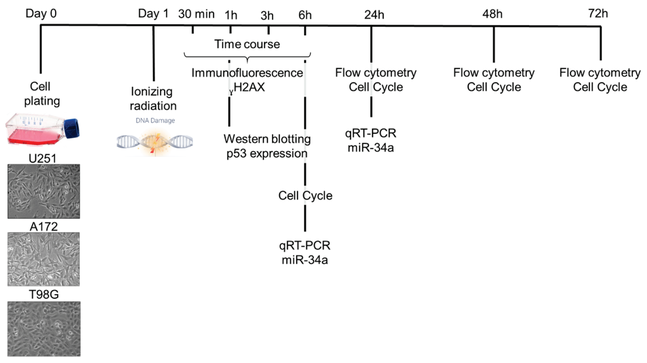

Figure 3: Schematic representation of the methodology applied and time course of experiments.

RESULTS

Our previous data demonstrated that ionizing radiation induces more DNA fragmentation in cell lines with TP53 mutation (U251 and T98G) in comparison to wild type (WT) TP53 cells (A172) (Figure 4). The doses of 2 and 4Gy did not induce DNA fragmentation after 6, 24, 48 and 72h while 8, 16 and 24Gy induced only in *TP53* mutated cells (Figure 4). Then, we selected the dose of 8Gy to evaluate the induction of DNA damage. Double strand breaks (DSB) quantified by γH2Ax focus were observed in 100% of A172 and U251 cells after 30 min as expected. However, A172 cells presented an early resolution of γH2Ax focus (Figure 5), which were reduced after 1h while U251 cells had focus maintained for 1h, 3h and 6h (Figure 6). Moreover, 48h after exposure to ionizing radiation, around 30% of cells still presented γH2Ax focus (Figure 6). Therefore, our results suggest that WT p53 cells present faster response to DNA damage than the mutated p53. These data corroborate our findings that patients with TP53 mutated benefit from radiotherapy. We also observed that the resistant cell (A172) have reduced levels of miR-34a in comparison to sensitive cell line (U251). Then, we evaluated miR-34a and p53 expression levels after treatment. Preliminary data showed that miR34a and p53 expression levels increased 6 hours after exposure to ionizing radiation in the A172 cells (Figure 7). Finally, our data points to an involvement of miR-34a in DSB response mediated by p53. Experiments are ongoing to evaluated U251 response as well as miR-34a inhibition to confirm its role in DSB response.

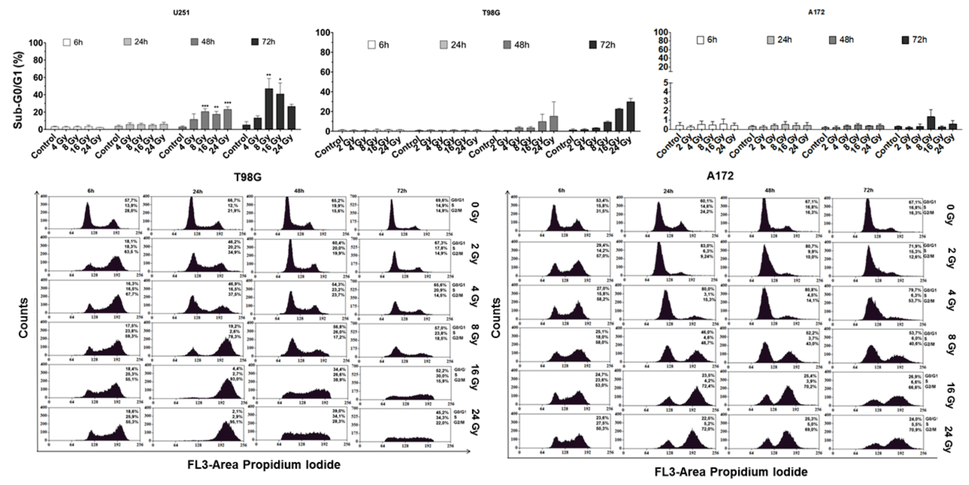

Figure 4: Flow cytometry analyses of DNA fragmentation and cell cycle after ionizing radiation. DNA fragmentation of (A) U251, (B) T98G and (C) A172 cell lines 6, 24, 48 and 72h after exposure to different doses of ionizing radiation. Cell cycle of (D) T98G and (E) A172 cell lines under the same conditions. Representative histograms of three independent experiments. *p<0,05; **p<0,01; ***p<0,001

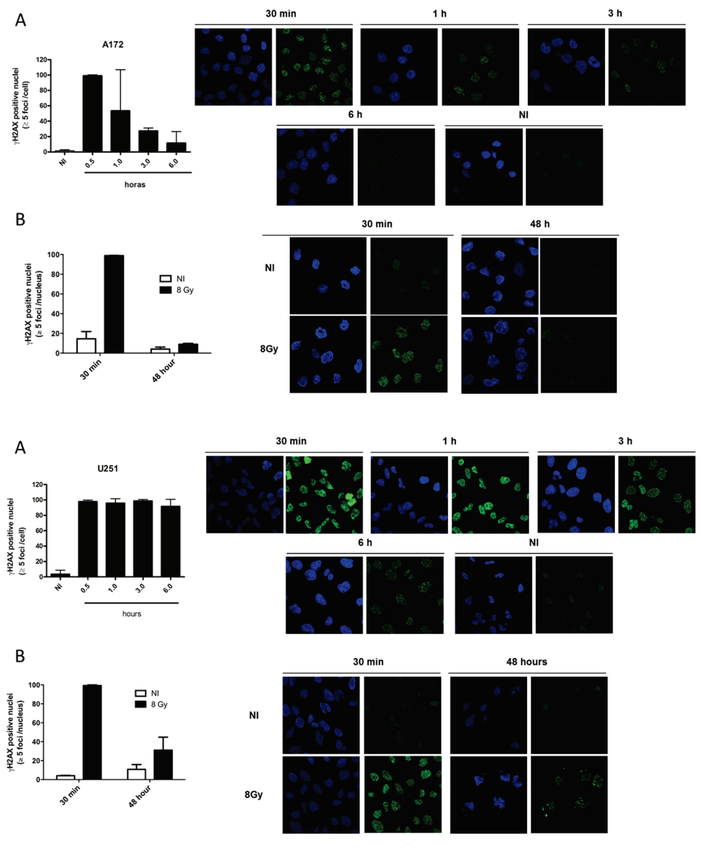

Figure 5: γH2AX phosphorylation evaluated after exposure to ionizing radiation. A172 cells were exposed to 8Gy and γH2AX foci/nucleus was evaluated by immunofluorescence 30 min, 1h, 3h and 6h after exposure (A). Also, γH2AX foci/nucleus was evaluated 48h after exposure to 8Gy (B). NI = non irradiated. Two independent experiments were performed.

Figure 6: γH2AX phosphorylation evaluated after exposure to ionizing radiation. U251 cells were exposed to 8Gy and γH2AX foci/nucleus was evaluated by immunofluorescence 30 min, 1h, 3h and 6h after exposure (A). Also, γH2AX foci/nucleus was evaluated 48h after exposure to 8Gy (B). NI = non irradiated. Two independent experiments were performed.

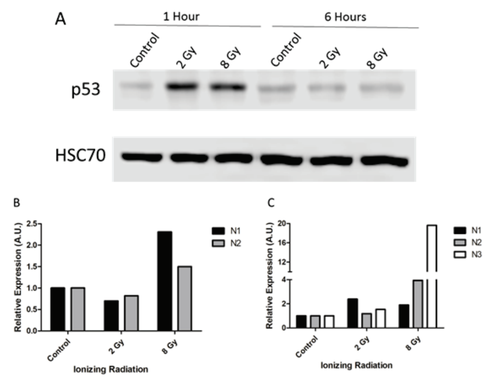

Figure 7. p53 and miR34a expression levels in A172 cell line after exposure to ionizing radiation. (A) p53 expression was evaluated by Western blotting after 1h and 6h of exposure to 2 and 8 Gy. HSC70 was used as endogenous control. (B, C) miR-34a expression was evaluated by qRT-PCR after (B) 6 h and (C) 24h of exposure to 2 and 8 Gy of ionizing radiation. RNU6B was used for normalization of miR-34a expression.

CONCLUSIONS

- A172 presents the most resistant profile while U251 is more sensitive;
- The resolution of γH2Ax foci is faster in A172 than in U251 cells, suggesting activation of repair pathways in A172;
- Ionizing radiation promotes the increase of p53 and miR34a levels in the A172 cell line.

FINANCIAL SUPPORT: Programa de Oncobiologia/ Fundação do Câncer, FAPERJ, CNPq, Ministério da Saúde/INCA

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA