An integrated approach using cell lines and plasma from patients suggest miR-210 as promising non-invasive biomarker for astrocytoma

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Introduction and Objetives

Astrocytomas are the most frequent malignant brain tumors, among them glioblastoma (GB) is the most common and one of the most lethal human malignancy. Patients present a median survival of 14 months and a five-year survival rate of only 9%. Surgery followed by radiotherapy and chemotherapy with temozolomide is the first line treatment. Recurrence and tumor progression are related to treatment resistance and occurs in the majority of cases. In this context, microRNAs are regulators of gene expression and play an important role in tumorigenesis and treatment resistance. miR-210 has a controversial role in apoptosis and proliferation, then understanding its role in GB treatment response is clinically relevant.

Objetive: Understanding miR-210 role in response to treatment with ionizing radiation, *in vitro* and its impact in astrocytomas' classification.

Methods and results

U251-MG, A172 and T98G radiation resistance profile

U251, T98G and A172 GB cell lines were exposed to ionizing radiation to evaluate DNA fragmentation by flow cytometry. Since U251 was the most sensitive and A172 the most resistant, miR-210 expression was modulated to evaluate cells response to radiotherapy.

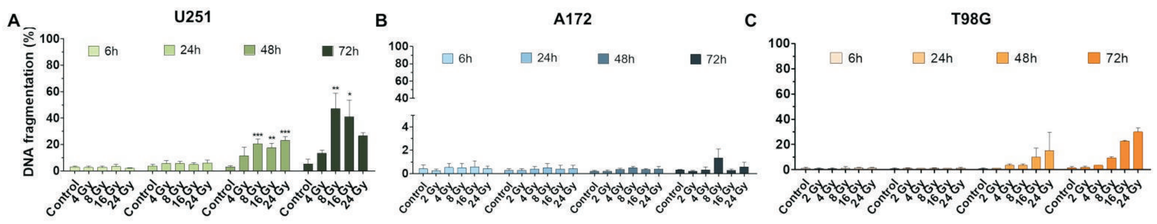

Figure 1. DNA fragmentation induced by ionizing radiation in three glioblastoma cell lines. (A) U251, (B) A172 and (C) T98G cells were exposed to different doses of ionizing radiation and DNA fragmentation was evaluated by flow cytometry after 6h, 24h, 48h and 72h. Mean of three independent experiments ±SD for U251 and two independents experiments for A172 and T98G. * p<0.05 ** p<0.01 ***p<0.001

miR-210 involvement in radiation resistance *in vitro*

Inhibition of miR-210, respectively by antagomiRs did not induce changes in A172 and U251 cells' susceptibility to ionizing radiation by annexin V/PI and DNA fragmentation. However, ionizing radiation reduced cell viability in A172 cells by tripan blue exclusion assay. Considering that A172 cells are p53 wild-type and this reduction can be associated with senescence, further experiments are ongoing to evaluate this hypothesis.

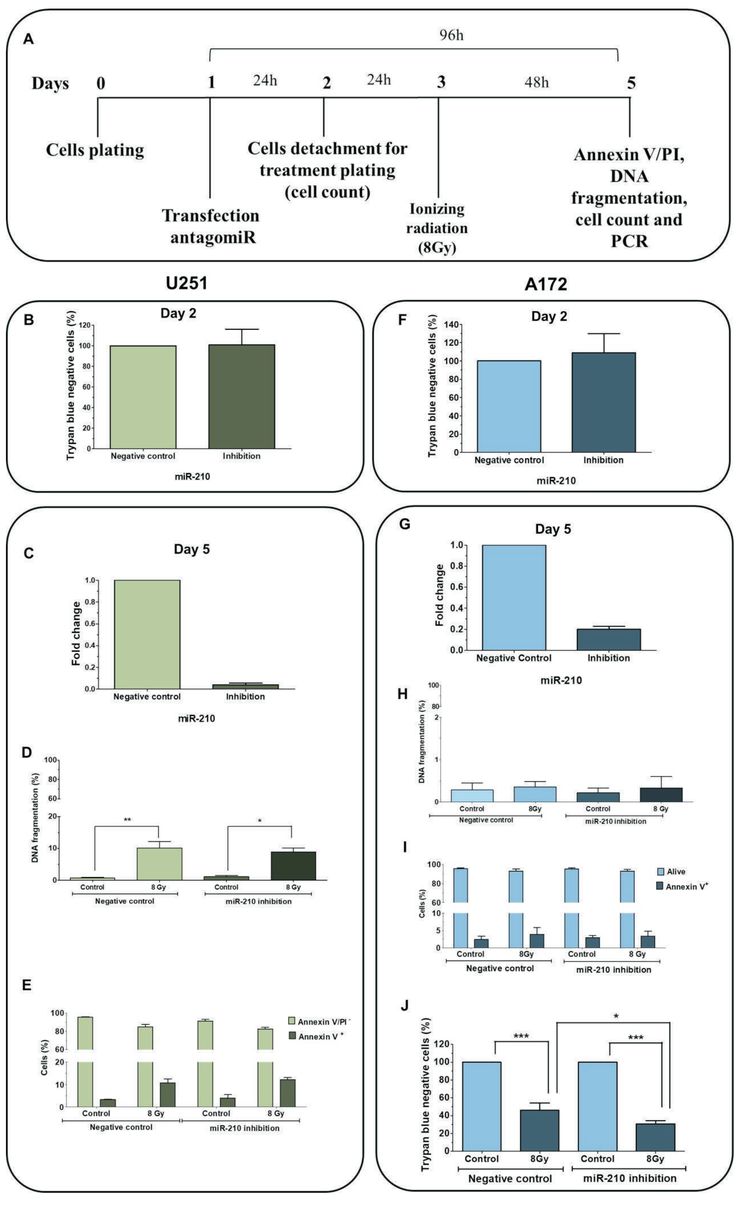

Figure 2. Effect of miR-210 inhibition followed by ionizing radiation treatment on cell death and viability. miR-210 expression levels after inhibition with antagomiRs evaluated by PCR in (B-E) U251-MG cells (TP53 mutated) and (F, J) A172 cells (TP53 wild type). (A) Experimental design. (B, F) Percentage of trypan blue negative cells in relation to control after inhibition of miR-210. (C, G) miR-210 expression fold change 96h after inhibition. (D, H) DNA fragmentation and (E, I) annexin V/PI labeling (annexin V+ = annexin V+/PI- + annexin V+/PI+) evaluated after miR-210 inhibition followed by ionizing radiation. (J) Percentage of trypan blue negative cells in relation to control after inhibition of miR-210 followed by ionizing radiation in A172 for 24h. Mean of three independent experiments ±SD. * p<0.05 ***p<0.001

miR-210 impact in astrocytoma's classification

Moreover, fourteen peripheral blood samples were obtained from patients with astrocytoma for miR-210 expression. GB patients' plasma samples had higher miR-210 levels in comparison to lower astrocytomas grades. Among the cases studied, five patients died. Besides the small sample size, this group expressed higher levels of miR-210.

Table 1. Neurosurgery service patients' samples from Instituto Nacional de Câncer (INCA)

Pacient code	Diagnostic	Age (year)	Gender	Deceased	Moment of sample collection
P2	Anaplastic astrocytoma (OMS grade III)	79	Female	Yes	DIAGNOSTIC
P3	Glioblastoma (OMS grade IV)	48	Female	No	DIAGNOSTIC
P4	Pilocytic astrocytoma (OMS grade I)	43	Male	No	DIAGNOSTIC
P7	Glioblastoma (OMS grade IV)	60	Male	Yes	Post surgery, RT + TMZ
P10	Anaplastic astrocytoma (OMS grade III)	29	Female	No	Second surgery, no previous RT or CT
P14	Glioblastoma (OMS grade IV)	45	Male	Yes	Post surgery, RT + TMZ
P21	Diffuse astrocytoma (OMS grade II)	53	Male	Yes	DIAGNOSTIC
P26	Glioblastoma (OMS grade IV)	63	Female	Yes	Post surgery, RT + TMZ
P28	Diffuse astrocytoma (OMS grade II)	39	Female	No	Second surgery, no previous RT or CT
P29	Glioblastoma (OMS grade IV)	53	Male	No	Post surgery, RT + TMZ
P30	Glioblastoma (OMS grade IV)	66	Male	Yes	DIAGNOSTIC
P31	Anaplastic astrocytoma (OMS grade III)	38	Female	No	After surgery and CT
P32	Diffuse astrocytoma (OMS grade II)	49	Female	No	DIAGNOSTIC
P34	Pilocytic astrocytoma (OMS grade I)	38	Male	No	DIAGNOSTIC
P36	Anaplastic astrocytoma (OMS grade III)	32	Male	Yes	After surgery and RT
P38	Glioblastoma (OMS grade IV)	51	Male	No	After surgery and CT (TMZ)
P45	Anaplastic astrocytoma (OMS grade III)	70	Male	Yes	After surgery and RT
P48	Anaplastic astrocytoma (OMS grade III)	53	Male	No	DIAGNOSTIC
P50	Glioblastoma (OMS grade IV)	43	Female	Yes	After surgery
P53	Glioblastoma (OMS grade IV)	59	Male	No	DIAGNOSTIC
P54	Diffuse astrocytoma (OMS grade II)	41	Female	No	DIAGNOSTIC
P58	Glioblastoma (OMS grade IV)	57	Female	No	After surgery
P61	Glioblastoma (OMS grade IV)	77	Female	No	DIAGNOSTIC
P63	Glioblastoma (OMS grade IV)	76	Male	No	DIAGNOSTIC
P65	Diffuse astrocytoma (OMS grade II)	62	Male	No	DIAGNOSTIC
P66	Glioblastoma (OMS grade IV)	43	Female	Yes	Após cirurgia
P67	Glioblastoma (OMS grade IV)	76	Male	Segment loss	DIAGNOSTIC
P68	Glioblastoma (OMS grade IV)	36	Male	No	DIAGNOSTIC
P71	Glioblastoma (OMS grade IV)	57	Male	Yes	After surgery

CT = chemotherapy/ RT = radiotherapy / TMZ = temozolomide

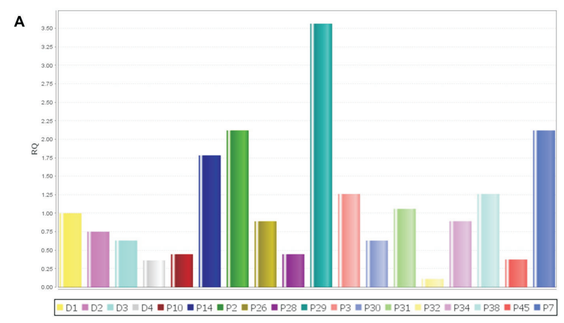

Figure 3. miR-210 expression in astrocytic tumor samples obtained from patients' blood. miR-210 expression levels were evaluated by qRT-PCR in patients' plasma samples. (A) miR-210 expression levels in each sample individually. (B) miR-210 expression levels in patients' samples grouped together (all astrocytoma grades) in relation to donors (healthy individuals. (C) miR-210 expression levels among different astrocytic tumors grade. (D) miR-210 expression levels compared between alive versus deceases patients. miR-210 expression was normalized by miR-16 and cel-miR-39 expression. Dotted red line = donors normalization.

Conclusions

Our results demonstrate that although miR-210 was not responsible for radioresistance in GB cells, its inhibition contributed to reduction of cell proliferation in A172 cells. In addition, miR-210 expression was higher in GB patients' samples and in samples of patients who evolved to death, pointing out miR-210 as promising non-invasive biomarker for astrocytoma.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

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