The effect of alpha particle radiation on human derived melanoma cell variants and the enhancement of the cytotoxic effect by manipulating DNA repair pathways

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Introduction

Intra-tumoral Diffusing alpha-emitters radiation therapy (DaRT) is a unique radiotherapy tumor ablation method, which demonstrated efficient destruction of solid tumors and induces systemic antitumor immunity using alpha-particle radiation. Malignant melanoma is a type of cancer that is relatively resistant to conventional radiotherapy. Thus, in our study, we examined the response of human melanoma cell lines to alpha radiation.

Melanoma cell variants before and after alpha radiation

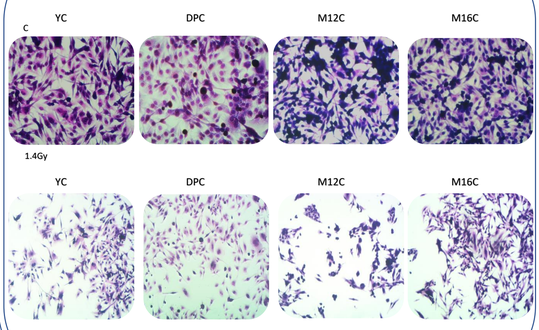


Fig.4. Melanoma cutaneous variants after radiation with 1.4 Gy of alpha particles.

The distribution of radioactive atoms inside the tumor in comparison with the necrotic areas they cause

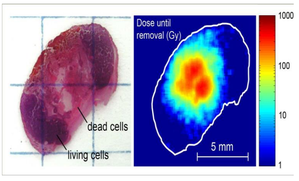


Figure 1. (Left) Hematoxylin-eosin (H&E) stained section taken from a SCC tumor treated with a Ra-224 DaRT source. Darker (purple) regions in (A) are composed of viable cells, lighter (pink) regions are necrotic. (Right) The radiation pattern of the same section.

In vitro irradiation with alpha particles using 241Americium (Am-241) irradiator

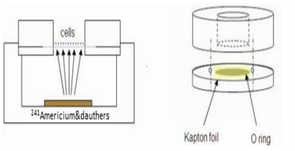


Fig 2: Schematic drawing of the alpha particle in vitro irradiation apparatus. The setup consists of two elements; **A**. a stainless-steel cylinder in which cells are seeded on a thin Kapton foil (irradiation cell holder) (right), **B**. The cell holder is positioned on top of an alpha particle irradiator composed of a well containing an Am-241 sealed source (left). Alpha particles emitted from the Am-241 source pass through the Kapton foil, irradiating the seeded cells.

Treatment of a patient with squamous cell carcinoma by DaRT achieved destruction of the tumor lesion

Fig3. Popovtzer, A., Rosenfeld, E., Mizrachi, A., Bellia, SR., Ben-Hur, R., Feliciani, G., Sarnelli, MA., Arazi, L., Deutch, L., Kelson, I., Keisari, Y. ***Initial Safety and Tumor Control Results from a "First-in-Human" Multicenter Prospective Trial Evaluating a Novel Alpha-Emitting Radionuclide for the Treatment of Locally Advanced Recurrent Squamous Cell Carcinomas of the Skin and Head and Neck***. *Int. J. Rad. Oncol. Biol. & Phys.* Int J Radiat Oncol Biol Phys. 2019 Nov 20. pii: S0360-3016(19)34040-4.

Aims

- Investigate the direct effect of alpha particle radiation on four cutaneous variants of human derived melanoma. .
- compare cell variants sensitivity to alpha radiation and photon radiation
- Investigate DNA double stranded breaks as a result from alpha radiation

Methods

- Four human derived skin melanoma cell lines: YDFR, DPC, M12, M16
- Irradiation of cell variants, *In-Vitro*, with americium-241 irradiator with increasing doses of alpha radiation
- Immunofluorescent staining of cell nuclei and analysis of nuclear morphology.
- Immunofluorescent staining of double stranded breaks (DSB) after irradiation and analysis of DNA DSB using anti gamma-H2AX abs.
- Irradiation of cell variants with gamma radiation for cell viability comparison

Results

A. Alpha Radiation D50 measurements

Variant	N	D50(Gy)	SD(Gy)
YC	4	1.17	0.52
YCB3	5	1.35	0.53
DPC	3	1.18	0.74
DPCB2	3	1.2	0.18
M12C	3	2.13	0.75
M12CB3	3	2.41	0.44
M16C	3	1.94	0.28
M16CB3	4	2.04	0.12

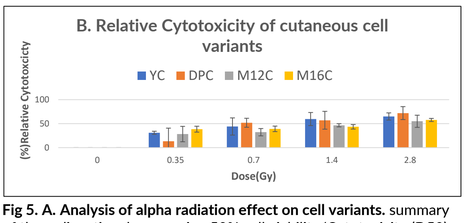


Fig 5. A. Analysis of alpha radiation effect on cell variants. summary of the radioactive dose causing 50% cell viability/Cytotoxicity (D50). D50 significant variance among cutaneous variants (P=0.0126) . **B.** relative cytotoxicity percentages comparison of cutaneous cell variants for each radiation dose

Alpha radiation inflicted higher cytotoxicity compared to gamma radiation

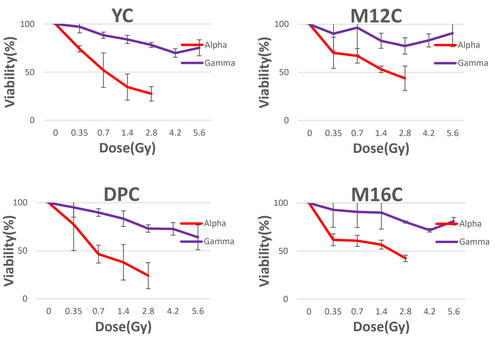
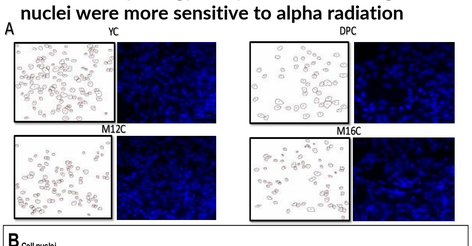


Fig 6. Comparison of cell viability percentage of four cutaneous cell variants after Gamma or alpha radiation

Nuclear morphology analysis: cells with larger nuclei were more sensitive to alpha radiation

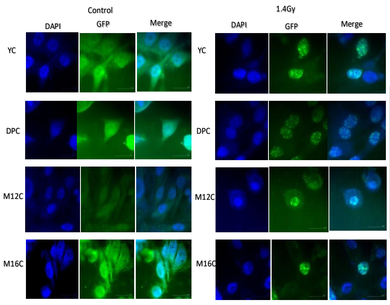


B Cell nuclei morphology

Variant	N	Perimeter(μM)	SD(μM)	Radius(μM)	SD(μM)	Diameter(μM)	SD(μM)	Area(μM^2)	SD(μM^2)
YC	122	67.66	16.86	10.77	2.68	21.54	5.37	364.49	22.64
DPC	50	71.65	15.73	11.41	2.50	22.82	5.01	408.82	19.70
M12C	96	49.66	9.02	7.90	1.43	15.81	2.87	196.34	6.47
M16C	70	51.41	13.18	8.68	1.87	17.37	3.74	236.99	10.98

Fig 7. cell nuclei morphology analysis. A. Immunofluorescence staining of cell nucleus(DAPI) of four cutaneous variants and analysis of nuclear morphology. **B.** table of nuclear parameters of cell variants.

DNA Double strand breaks (DSB) analysis after alpha radiation: More sensitive cells had more DSB



γ-H2AX foci

Variant	N	Foci/nucleus	SD
YC	28	7.67	2.12
DPC	33	9.6	2.29
M12C	29	6.17	2.22
M16C	31	6.9	1.65

Fig 8. Phosphorylated foci of four cell variants. Immunofluorescence staining of the phosphorylated histone γ-H2AX after irradiation of cells with a dose of 1.4 Gy of alpha radiation. (right) table of average number of foci per nucleus of four cutaneous cell variants. [4-5 DSB are sufficient to kill the cell]

Conclusions

- Cutaneous cell variants of human derived melanoma are sensitive to the cytotoxic effect of alpha radiation. had inter- variant differences
- Differences in response to alpha radiation were in direct correlation with the size of the nucleus and the amount of DNA double stranded breaks inside the nucleus.
- Compared to gamma radiation, alpha radiation had a stronger cytotoxic effect on cell viability of the cutaneous cell variants.