DEVELOPMENT OF A RADIORESISTANT CANINE GLIOMA CELL LINE

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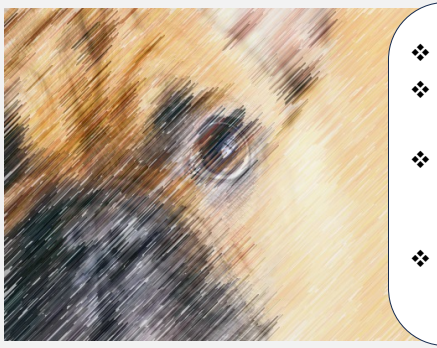
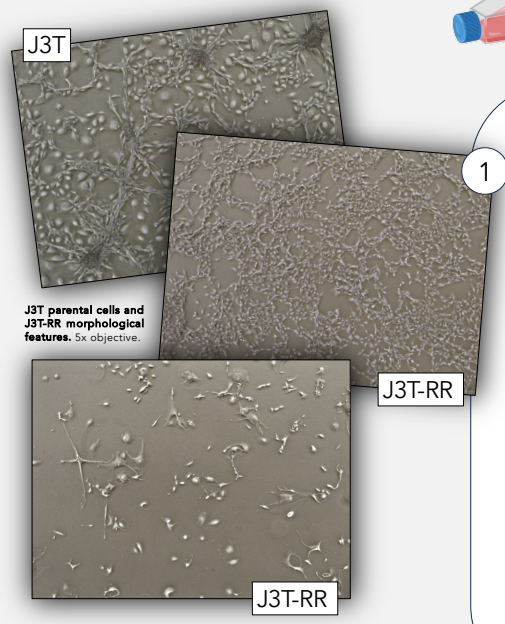


INTRODUCTION

- Malignant gliomas are aggressive brain tumours that affect humans and dogs.
- The prognosis for this disease is poor in both species, despite surgical, chemo and radiotherapeutic treatments.
- Radiotherapy is an important treatment modality for brain tumours, but the efficacy is mostly limited by radioresistance, which is believed to be mediated by tumour-initiating cells that are able to recapitulate the tumour.
- The objective of this research was to establish a canine radioresistant glioma cell line for future studies on radioresistance.

MATERIALS AND METHODS

- Canine glioma cells (J3T) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and 1% penicillin/streptomycin.
- After receiving an initial dose of 7 Gy, the cells were treated with incremental doses of 1 Gy over 21 weeks (total = 112Gy).
- Colony formation, cell viability and migration assays were used to compare the irradiated cells (J3T-RR) with the parental cell line (J3T) to confirm radioresistance.



J3T parental cells and J3T-RR morphological features. 5x objective.

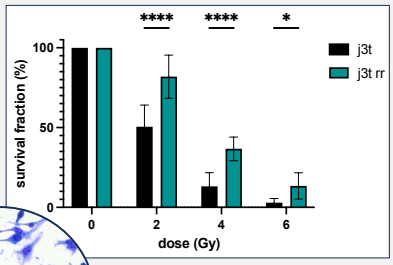
RESULTS

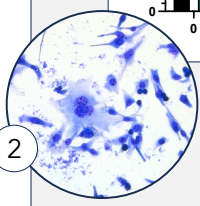
1. Cell morphology gradually changed from star-shaped cell aggregates to more spindle-shaped and anaplastic cells.

2. J3T-RR cell line showed higher resistance in colony formation assays after exposure to a single radiation dose of up to 6 Gy.

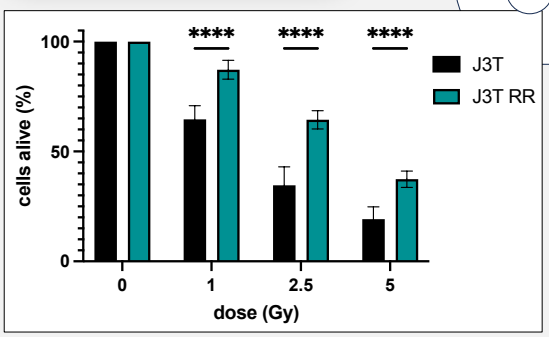
3. J3T-RR migrate faster than non-RR cells.

4. J3T-RR cell viability was higher than the parental J3T cell line when exposed to radiation doses up to 5 Gy.

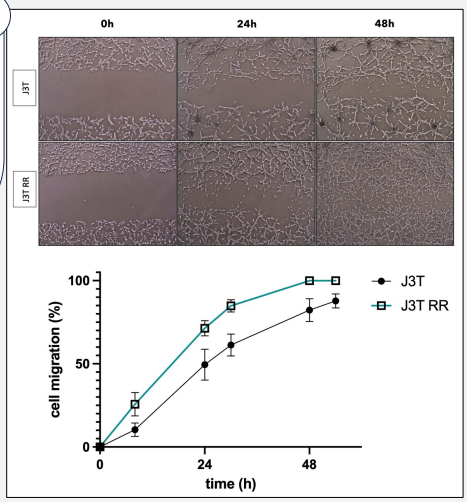




Increased colony formation (CF) for J3T-RR. CF assay results showing higher survival fraction for J3T-RR cells when compared with its parental cell line (Two-way ANOVA, p<0.0001, followed by Šídák's multiple comparisons test; p<0.0001 for 2 Gy and 4 Gy, p=0.0418 for 6 Gy; results of 3 independent experiments, data expressed as mean ± SEM).



Increased cell viability for J3T-RR. Cell viability assay comparing J3T-RR with its parental cell line. Cell viability was measured after 96h of a single dose of radiation (0, 1, 2.5 and 5 Gy) (Two-way ANOVA, p<0.0001, followed by Šídák multiple comparisons test: ****p<0.0001. Data expressed as mean ± SEM).



Increased migration for J3T-RR. Two-dimensional migration assays comparing J3T-RR with its parental cell line. Cell migration was calculated at each time point and expressed as the percentage of the scratch area occupied by migrating cells in comparison to the initial scratch area, at 0 h (Two-way ANOVA, p<0.0001, followed by Šídák multiple comparisons test: p<0.0001 at 8h, 24h, 30h, 48h and p=0.0002 at 54h; results of 2 independent experiments, data expressed as mean ± SEM).

CONCLUSIONS

- We have successfully generated a radioresistant canine glioma cell line that will be useful for mechanistic studies and the development of rational treatments against radiation resistance in cancer.

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