Translational Radio-Biology

Generation of an experimental model for CRISPR screening in muscle invasive bladder cancer

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INTRODUCTION

- Bladder cancer is the 10th most common cancer worldwide
- Chemotherapy resistance is a problem in muscle invasive bladder cancer (MIBC) due to intra-tumoural hypoxia [1]
- Whole genome CRISPR screening (WGCS) is a powerful tool for discovering novel genes involved in therapy resistance

Research aim: characterise and generate a cell line model to use in WGCS experiments to study hypoxia-induced chemotherapy resistance in MIBC

WORKFLOW

1. MIBC cell lines used for model characterisation: T24, J82 and UMUC3.
2. Baseline cell death rates: assessed by Annexin V/7-AAD staining using flow cytometry.
3. Cisplatin dose curves: cells were pre-exposed to normoxic (21% O2) or hypoxic (1% & 0.1% O2) conditions for 24 h and then treated with varying concentrations of cisplatin for 72h.
4. Cas9 lentiviral transduction: T24 cells were transduced with lentiviral Cas9 expression vector and transduced cells were selected for by blasticidin antibiotic selection.
5. Cas9 transduced vs. wild type (WT) growth curves: T24 WT & T24 cells transduced Cas9 (T24 Cas9) were grown under normoxic (21% O2) and hypoxic (1% & 0.1% O2) conditions for 5 days.

Whitley H35 Hypoxystation

RESULTS

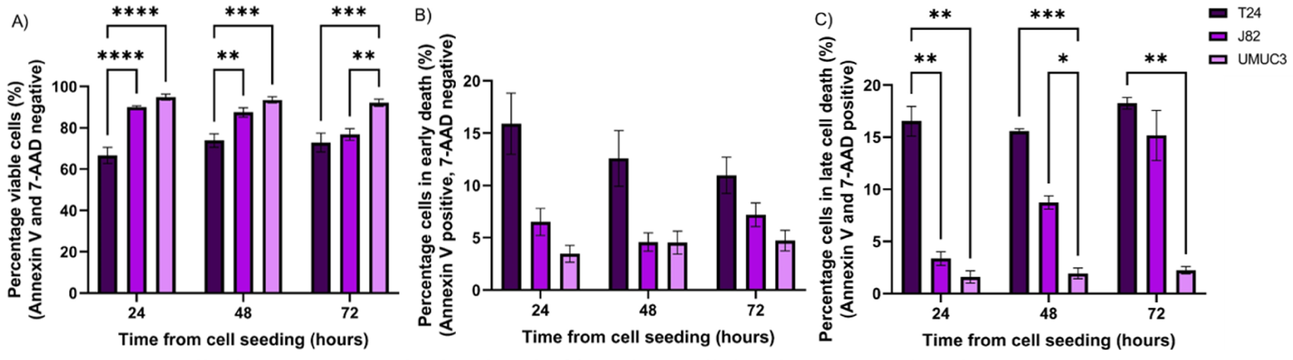

Figure 1. T24 MIBC cells demonstrate a higher level of baseline cell death compared with UMUC3 and J82: T24, J82 & UMUC3 MIBC cells baseline cell death rates were determined using Annexin V/7AAD flow cytometric staining in at 24, 48 and 72h (n=3). T24 cells show decreased viability (A) and increased early stage (B) and late stage (C) cell death compared to J82 and UMUC3 cells at all time points. Results analysed by 2way ANOVA/ Tukey's multiple comparisons test.

RESULTS

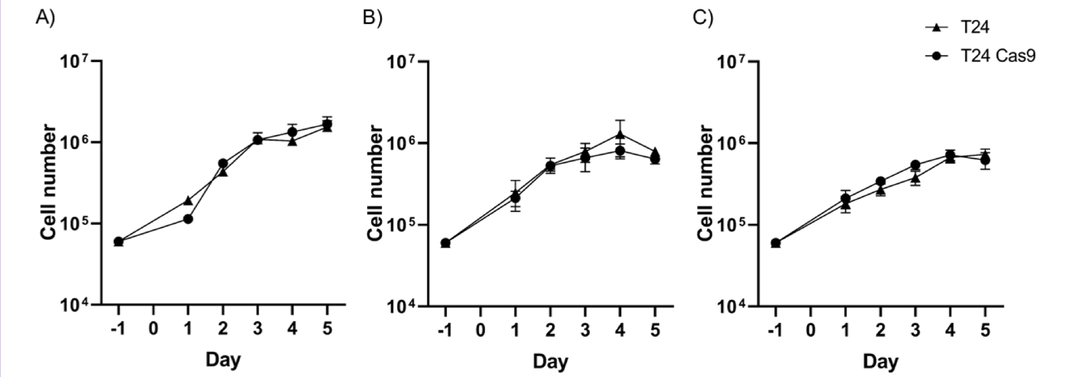

Figure 3. Cas9 expression does not affect T24 cell growth rates under normoxic and hypoxic conditions: T24 WT and T24 Cas9 cells were incubated under normoxic (21% O2)(A) and hypoxic conditions of 1% (B) and 0.1% (C) O2 for 5 days (n=3). Cell counts were taken daily. Results determined by 2way ANOVA.

RESULTS

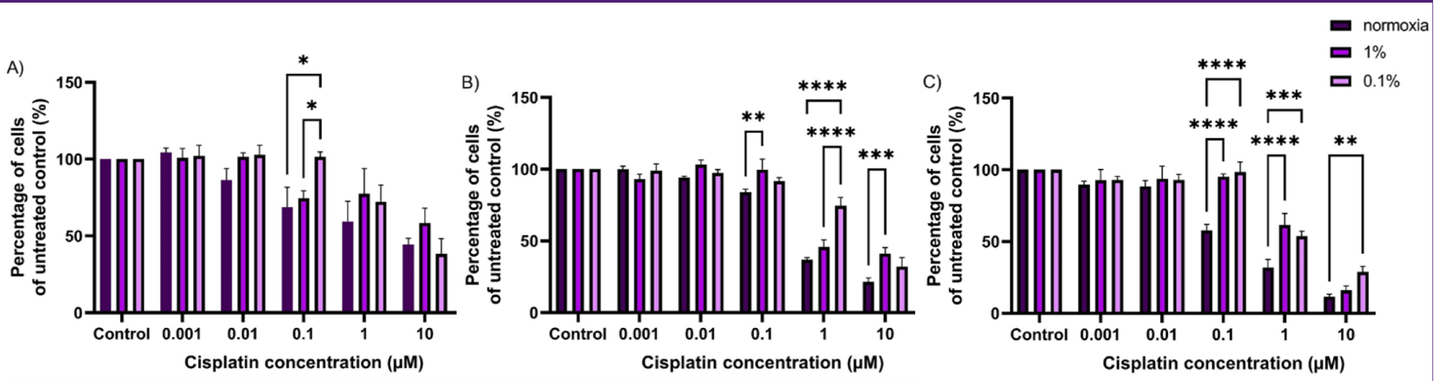

Figure 2. T24 cells demonstrate increased resistance to cisplatin under hypoxic conditions: T24 were treated with varying doses of cisplatin (0.001μM–100μM) (n=4). Cell viability was determined at 24 (A), 48 (B) and 72h (C) under normoxic (21% O2) and hypoxic conditions (0.1%, 1% O2). Results analysed by 2way ANOVA/ Tukey's multiple comparisons test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

CONCLUSIONS & FUTURE WORK

- We have developed a workflow to characterise the suitability of MIBC models for future WGCS experiments to study cisplatin resistance in MIBC
- Rapidly dividing cancer cells can have high baseline cell death rates which could affect multiplicity of infection ratios and seeding densities for WGCS experiments
- Hypoxia induces cisplatin resistance in T24 cells and this resistance was observed at concentrations corresponding to peak plasma concentrations in patients [2]
- Cas9 expression in T24 cells has no effect on growth rates in normoxic and hypoxic conditions.
- At present, T24 cells show suitability for our study
- Ongoing studies will also include generating a monoclonal T24 Cas9 population and assessment of the cutting efficiency of Cas9 endonuclease in these cells

REFERENCES

1. Devarajan N et al. Critical Reviews in Oncology/Hematology (2021); 162, p.103327.
2. Rajkumar P et al. Journal of Clinical and Diagnostic Research (2016): JCDR, 10(7), p.XC01

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