Investigating the Effects of Cancer Stem Cells on Cancer Treatment Induced Cardiotoxicity

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Abstract

This study investigated how cancer stem cells (CSCs) contribute to cardiotoxicity and how modulating autophagy influences their response to the cardiotoxic drug doxorubicin. We hypothesize that enriching CSCs will increase cell resistance to doxorubicin. Using Fluorescence-Activated Cell Sorting (FACS) and cell viability assays, we found that prolonged doxorubicin exposure increased the CSC population and led to impaired autophagy, as shown by autophagy-detecting nanoparticle analysis. Contrary to our hypothesis, we also observed an increase in doxorubicin sensitivity over time. These findings suggest that chronic chemotherapy promotes CSC expansion and disrupts autophagy, possibly contributing to drug sensitivity. Targeting autophagy may therefore help re-sensitize resistant CSCs and reduce cardiotoxic effects. Future work will explore how CSCs directly affect cardiomyocyte viability in co-culture models.

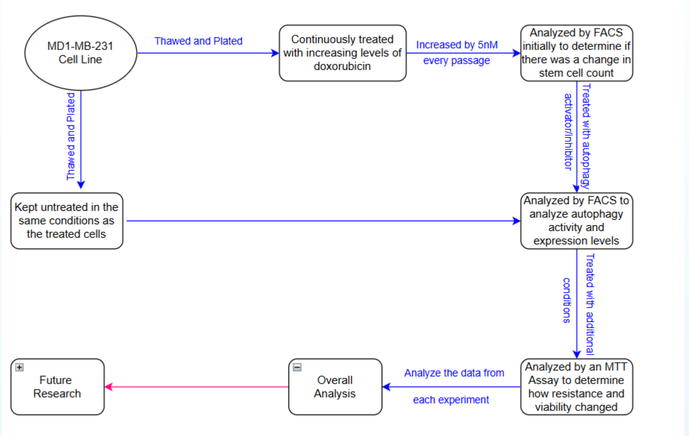
Introduction

- Cancer treatments such as doxorubicin are effective against tumors but can cause cardiotoxicity, leading to heart failure in ~4% of patients.
- CSCs are a small, drug-resistant subpopulation of tumor cells that drive recurrence, metastasis, and therapy resistance.
- Autophagy, the process by which cells recycle damaged components, helps maintain CSC survival but also protects cardiac cells from damage.
- Doxorubicin can disrupt autophagy, increasing oxidative stress, mitochondrial damage, and cardiomyocyte death.
- Prolonged exposure to doxorubicin can promote CSC expansion and drug resistance, making treatment less effective over time.
- Understanding how autophagy modulation affects CSC behavior and cardiotoxicity could lead to safer and more effective cancer therapies.

This project explores how CSCs may contribute to cardiotoxicity. The aim is to understand how modulating autophagy affects CSC behavior and cardiotoxicity, and to develop strategies that make cancer treatment safer and more effective.

Methodology

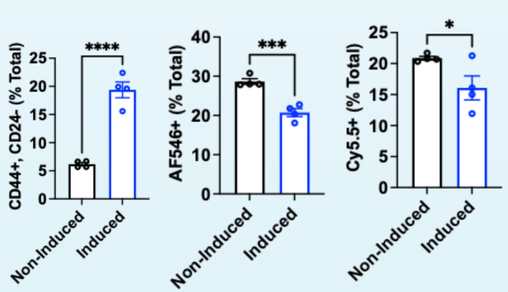
The flowchart below summarizes the general steps done throughout the research project



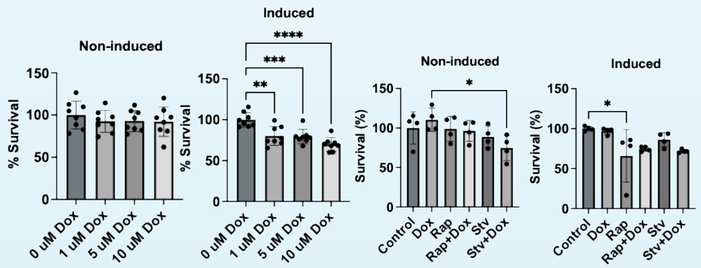
The doxorubicin induced cells were passaged 1–2 times per week, depending on their growth rate. With each passage, the doxorubicin concentration was increased by 5 nM, starting from 0 nM and reaching a final concentration of 35 nM. From this point onward, cells were maintained at 35 nM dox, as higher concentrations resulted in significant cell death.

Results & Discussion

Graphs Showing the Expression of Each Marker in Induced vs Non-Induced MDA-MB-231 Human Breast Cancer Cells



Graphs Showing the Viability of Induced vs Non-Induced MDA-MB-231 Human Breast Cancer Cells in Various Conditions



- There is a significantly higher number of CSCs in MDA-MB-231 human breast cancer cells compared to cells treated with a standard acute dose of doxorubicin.
- The MDA-MB-231 cells exposed to chronic low-dose doxorubicin for 1.5 months exhibited a marked impairment in autophagy, as detected using the second-generation autophagy-detecting nanoparticle (ADN2).
- Both ADN2 uptake (AF546 fluorescence) and autophagic activation (Cy5.5 fluorescence) were significantly reduced when compared to untreated controls.
- Cells that had been induced with doxorubicin showed a significant decrease in viability when re-exposed to doxorubicin.
- There was no significant reduction in cell viability observed in either the non-induced or induced MDA-MB-231 populations following treatment with doxorubicin for 24 hours.
- Cell survival was significantly reduced in the doxorubicin-induced population when cultured in AC16 media compared to standard culture conditions.

Conclusion

- Chronic low-dose doxorubicin exposure increased CSC populations, suggesting chemotherapy may promote therapy-sensitive cells.
- Autophagy was impaired in chronically treated cells, indicating disrupted cellular recycling and survival adaptations.
- Autophagy modulation with chloroquine (inhibitor) and rapamycin (activator) revealed its key role in drug sensitivity and CSC survival.
- MTT assay results showed that changes in autophagy and CSC enrichment directly affect cell viability and sensitivity.
- Chronic chemotherapy drives both CSC expansion and autophagy dysfunction, leading to heightened drug sensitivity.
- Targeting autophagy may help re-sensitize resistant CSCs and reduce chemotherapy-induced cardiotoxicity in future studies.

Future Work

-Co-culture studies: Co-culture CSCs with cardiomyocytes to assess how autophagy modulation influences cardiotoxicity and cardiac cell survival.
- FACS-based analysis: Isolate CSCs and non-CSC fractions to directly compare autophagy activity and drug response under doxorubicin and autophagy modulators.
- Exosome profiling: Examine whether autophagy-modulated CSCs release cardiotoxic or cardioprotective factors that affect cardiomyocyte health.
- CRISPR-based studies: Knock down autophagy-related genes to test whether autophagy dependence drives treatment resistance.

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