Establishing Long-Term Circulating Tumor Cells (CTCs) in a 3D Culture System In Vitro

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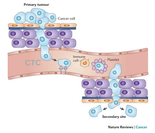
Abstract

Circulating tumor cells (CTCs) are cells that have detached from the primary tumor and entered the bloodstream with the potential to seed metastatic tumors in distal sites. High CTC numbers correlate with aggressive disease, increased metastasis, and decreased time to relapse. It has also been shown that cancer stem cells (CSCs) represent a proportion of the CTCs present in patients. Given that CSCs are resistant to chemotherapy and are responsible for tumor initiation, it is posited that the CSCs are the seeds of metastasis. However, direct evidence for this hypothesis is limited due to the fact that there are few methods for culturing and studying these rare cells. We used a 3D culture chamber system (RealBio D4 ™) to establish long-term cultures of human-derived pancreatic tumors and to assess the effect of gemcitabine treatment on the CSC population of these cultures. We observed that the system's 3D matrix supported culture development and incorporation of tissue organization and microenvironment. The chamber design allowed CTCs generated within the cultures to migrate out of the cell mass into the circulating nutrient medium where they were collected for characterization. Further, tumor tissue grown on the matrix showed an enrichment of CSCs after gemcitabine treatment analogous to the enrichment observed in vivo. Future studies will focus on characterizing isolated CTCs and circulating CSCs from pancreas and breast cancer models, determine their response to therapy, and elucidate the relationship of different CTC populations on tumor spread. Ultimately, these studies may support the use of CTCs and circulating CSCs as biomarkers in clinical trials.

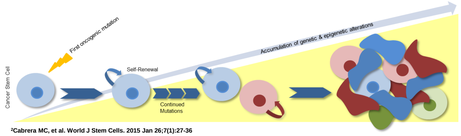
Background

Circulating Tumor Cells (CTCs)

- CTCs are cells that have detached from a primary tumor and entered the bloodstream with the potential to seed metastatic tumors in distal sites
- High CTC numbers in blood correlate with decreased survival in patients
- The presence of CTCs is a significant predictor of outcome
- CTC numbers can predict response to systemic therapy in multiple cancers.



[1]Schroeder A, et al. Nature Reviews Cancer 2012;12:39-50



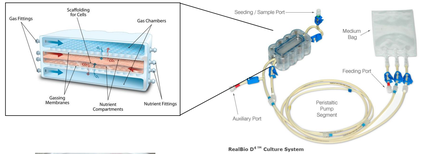
[2]Cabrera MC, et al. World J Stem Cells. 2015 Jan 26;7(1):27-36

- Cancer Stem Cells (CSCs) are a rare subpopulation of tumor cells that have the highest potential for tumor formation
- They have the ability to renew limitlessly and can "differentiate" into the multiple cell types present in tumors
- They share many of the same properties and active pathways as normal stem cells
- Are generally quiescent, divide infrequently, and spend most of their time in G0

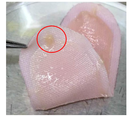
Techniques and Skills

Long-term "D4" Cell Culture

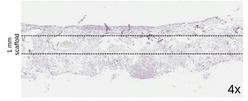
- Support biologically-relevant three-dimensional structures that mimic the natural composition and architecture of normal or tumor tissue



RealBio D4™ Culture System



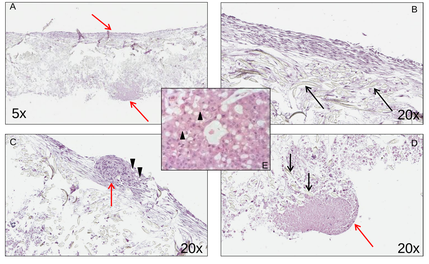
Tumor mass grown through the 3-D Scaffold. PA0143 Tumor 100 days in culture



H&E of 3D scaffold displaying a multilayered tissue mass with tumor cells migrated through the scaffold. PA0143 tumor 85 days in culture

Results

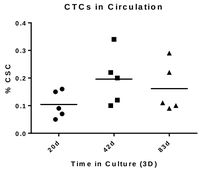
Figure 1. Long term "D4" in vitro culture of PA0143 pancreas PDX tumor model shows histological similarities to in vivo cohort.



Representative H&E sections illustrate morphological structures in D4 cultures of PA0143 pancreas tumor xenograft model in culture for 85 days. A. Cross section of 3D matrix scaffold seeded with PA0143 tumor cells. B-D Multilayered cell structures growing throughout scaffold with areas of fibrosis displaying multiple cell types. E. H&E section from in vivo tumor of PA0143 PDX. Red arrows point to structural components; black arrows point to polyester matrix fibers; black arrow heads point to adipocytes.

Results

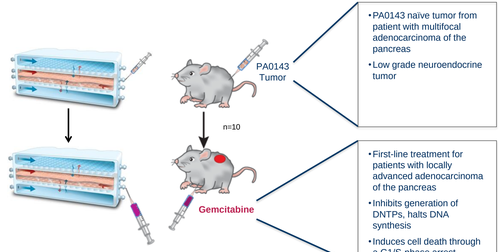
Figure 1. Circulating tumor cells from in vitro "D4" PA0143 model displayed cancer stem cell markers



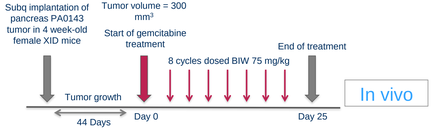
CTCs generated from tumor tissue grown on the 3D matrix migrated out of the cell mass and into the circulating nutrient medium. CTCs were collected at days 20, 42, 83 and assessed using flow cytometry for CSC markers Epcam+, CD24+, CD44+. At each timepoint, a percentage of CTCs displayed CSC characteristics.

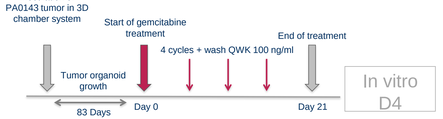
Study Design

Study design to determine if in vitro "D4" system models in vivo drug results.



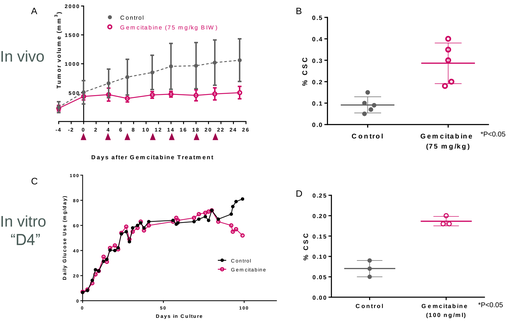
Dosing Regimen: Pancreas PDX Model + Gemcitabine in vivo vs. in vitro ('D4')





Results

Figure 3. Gemcitabine Treatment of PA0143 Model in vitro "D4" enriches CSC population as seen in vivo



In vivo and D4 in vitro culture of PA0143 showed an enrichment of CSCs. A,B (In vivo) A. Tumors treated with Gemcitabine (75mg/kg BIW x8) showed a significant decrease in tumor volume post treatment. B. Gemcitabine treatment of PA0143 tumors caused an enrichment in the percentage of CSCs. C-D (D4 In vitro). C. D4 tumors treated with gemcitabine showed a decrease in glucose consumption. D. D4 tumors treated with Gemcitabine (100 ng/ml QWK x4) showed a significant enrichment in the percentage of CSCs

Conclusions

- Human pancreas adenocarcinoma (PA0143) model was successfully cultured long-term in 3D culture chamber perfusion system (Realbio D4)
- Circulating tumor cells migrated out of the chamber and were collected in the nutrient medium bag. A percentage of these CTCs displayed CSC characteristics.
- In vitro PA0143 culture had histological similarities to human PDAC tumors
- Cancer stem cells were a percentage of the population of tumor mass grown in vitro in 3D culture
- In vivo cancer stem cell population in PA0143 was enriched ~3-fold after treatment with gemcitabine
- In vitro PA0143 cultures treated with gemcitabine showed a ~3-fold enrichment of cancer stem cells similar to the observations made in vivo

Future Directions

- Establish the circulating tumor cell model of breast cancer in vitro 3D in order to look at the relationship between cancer stem cells and circulating tumor cells.
- Characterize circulating tumor cells gene expression in vitro
- Determine the effects of therapeutics on in vitro 3D breast cancer model and compared to in vivo data

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[1]Schroeder A, et al. Treating metastatic cancer with nanotechnology. Nature Reviews Cancer 2012;12:39-50. Figure 1.
[2]Cabrera MC, Hollingsworth RE, Hurt EM. Cancer stem cell plasticity and tumor hierarchy. World J Stem Cells. 2015 Jan 26;7(1):27-36. Figure 1.