Novel 3-D In Vitro Models for Studying Pancreatic Cancer Drug Response and Resistance

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Utilization of novel 3D *in vitro* models for studying pancreatic cancer drug response and resistance

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Objectives/Key Points

- Describe the role of preclinical systems focusing on organoids in modelling pancreatic cancer.
- Present a method for the establishment of isogenic primary cell lines from patient derived organoids (PDOs) and the recapitulation to 3D cell line organoids (CLOs).
- Demonstrate that 3D CLO culture method can be used as an expandable, easy scale-up, affordable, and less time-consuming research model.
- Highlight a methodology for the development and characterization of drug resistance using pancreatic cancer organoids.
Pancreatic cancer

- Pancreatic ductal adenocarcinoma (PDAC) is the most common type
- Aggressive, poor prognosis with 5-year survival rate 13%, distant metastases 3%*.
- Diagnosed at late state
  - Silent progression
  - Nonspecific symptoms
  - More than half of patients diagnosed at an advanced stage
- Treatment
  - Surgery offers curative intent however, approximately 20% patients are operable
  - Chemotherapy and/or radiotherapy as the standard of care for most patients.
  - Inherent or acquired resistance
- 7th leading cause of cancer mortality in the World
  - Increasing trends in incidence and mortality of PDAC across the World

* SEER = Surveillance, Epidemiology, and End Results (2013-2019)
Greatest challenge in cancer treatment

- Predict response because cancer and its treatment are patient-specific

- Personalised medicine, individual prevention, and treatment strategies based on unique patient-specific variables
Landscape of PDAC

- Genomic and transcriptomic subtypes can enrich for therapeutic vulnerabilities
  - > 90% cases present with KRAS mutation
  - TP53, SMAD4 and CDKN2A inactivated in > 50%
  - Limited success in targeted therapy strategies

- Familial predisposition
  - Genetic syndromes account for 5-10% of PDAC
  - Germline mutations in DNA damage repair pathway (BRCA1/BRCA2 etc)


Established cell lines
- Widely available
- Represent common genetic features
- Cost effective
- Easy to manipulate
- High throughput screening
- Homogenous
- Genomic drift
- Low in vivo relevance

Primary cell lines
- Mirrors heterogeneity and tumor characteristics
- Personalized drug screening
- Difficult to establish
- Limited doubling
- Stromal cell contamination

Patient-derived xenografts
- Mirrors heterogeneity and tumor characteristics
- Response reflects clinical results
- Unlimited resource of tumor – generations
- Time consuming
- Lacks functional immune system
- Expensive
- Animals

GEMM
- De novo tumor development
- Retain the histopathological and molecular features of human tumors
- Expensive, time consuming and complex
- Mouse biology
- Animals

Preclinical models of pancreatic cancer
Cancer organoids as models of disease

- **Miniaturized** and **simplified** 3D structures
- Patient specific as derived from **patient tissue**
- **Self-organizing**, resembling the **original tumor architecture**
- Show **heterogeneity** of tumors
- **Long-term growth** potential - biobanking
- **Alternative to animal models**
PDOS in personalized medicine

- Personalized therapy development – providing accurate and reliable drug screening systems
- Uncover underlying mechanisms driving cancer progression – genetic mutations, signalling pathways etc.
- Can be developed into more complex models to mimic tumor microenvironment
- Platform to study early and late stages of tumor development
Developing 3D organoid systems to model pancreatic cancer
Development of 3D organoid models

Schematic: Sara Noorani (Biorender)
Nelson et al., Scientific reports, 2020;10(1), 2778

xenograft

Removal of infiltrating mouse cells

Created with BioRender.com
Development of primary 2D cell lines from organoids

Culture for 10-14 days, with addition of media every 2-3 days. Repeat establishment using confluent plate of CLOs.
Generation of organoid-derived primary cell lines and 3D cell line organoids (CLOs)

- Developed three new primary 2D cell lines derived from 3D organoids purchased from ATCC
  - HCM-CSHL-0090-C25 (ATCC® PDM-37™)
  - HCM-CSHL-0094-C25 (ATCC® PDM-41™)
  - HCM-BROD-0008-C25 (ATCC® PDM-106™)

- 2D cell lines were expanded over 2 passages and recapitulated to cell line organoids (CLOs) using 3D organoid culture conditions
CLOs maintain the phenotypic and growth characteristics of similar to organoids

Differential proliferation rate observed between the three cell lines

Comparison of proliferation between CLOs and respective isogenic matched organoid revealed similar rates of proliferation between PDM37 and PDM106 models;

PDM41-CLO proliferated faster compared to PDM41-organoid

CLOs retain therapeutic drug response comparable to derived organoids

Stem cell marker expression in 2D cell line, CLOs and organoids
Overexpression of Cancer Stem Cell markers in 3D models compared to 2D cell lines

RNA-Seq transcriptomic analysis identifies similar CLO and organoid signature

Nelson et al., Scientific reports, 2020;10(1), 2778
CLOs accurately reflect the cellular architecture and heterogeneity of organoids *in vivo*

(C) ALDH1A1, (D) CXCR4, (E) ESA/EpCAM, (F) CD44, (G) MASPIN, (H) PDX1, (I) Ki67, and (J) negative control.

Cancer Stem Cell expression altered in long-term cultured 2D cell lines

PDM37 cell line  PDM41 cell line  PDM106 cell line
Long-term 2D culture loses Cancer Stem Cell CD133 expression
Long-term 2D culture expression of CD44/CD24/ESA is cell line dependent
Stem cell plasticity is reconciled by culture conditions

**Stochastic model**
- Tumor
  - Unlimited cell division & differentiation
  - All isolated tumor cells have the capacity to differentiate indefinitely and form new tumors

**Cancer stem cell model**
- Cancer stem cell
  - No tumor formation
  - Only cancer stem cells have the ability to form tumors
  - Self-renewal

**Drug treatment**
- Crossed-out drug

**2D cell line culture**
- CSC biomarkers
  - CD44
  - CD24
  - ESA

**3D organoid/CLO culture**
- CSC biomarkers
  - CXCR4
  - CD133
  - CD24
  - ALDH1A1
  - ESA

Created with BioRender.com
CSC expression altered between tumour and normal

Cancer Stem Cell expression

CD44 associated with overall survival
Modelling drug resistance *in vitro*:

- Poor long-term survival rates of pancreatic cancer are the consequences of rapidly acquired chemoresistance and represent a major therapeutic challenge.

- Studying the emergence of resistance to therapeutics would allow us to identify key markers to guide treatment strategies.
Long-term drug selection derived organoids
Advanced *in vitro* organoid models
Establishment of 5-FU resistant PDAC organoids

PDM37 and PDM37-5FUR

PDM37 Early Passages

PDM37 Late Passages

Folirinox Combination

- PDM37 Parental
- PDM37 5-FU Resistance
Transcriptomic identification of differentially regulated genes involved in 5-FU resistance
Common and differential mediators of 5FU drug resistance
Conclusions

- Organoids can be used as an **emerging technology to advance of personalized medicine**
- Model tumorigenesis, and recapitulate critical features of original cancer tissue
- CLOs are flexible, expandable, traceable models used for high-throughput screening of sensitive drugs to provide individualized treatment options
- Tools for understanding the mechanisms of drug resistance
  - Identify markers of resistance
  - Develop novel therapeutics to overcome drug resistance
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