Ex vivo 3D culture of adenoid cystic carcinoma PDX models recapitulate disease biomarkers and predict drug response

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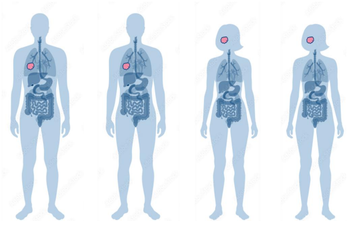
Abstract

Adenoid cystic carcinoma (ACC) is a rare, glandular cancer whose incidence rate and limited 2D cell culture capability make it difficult to study primary patient samples. Although well characterized ACC patient-derived xenografts (PDX) have proven to be a useful model to study disease mechanisms and therapeutic sensitives, animal drug studies are relatively low throughput, costly, and take months to accomplish. *Ex vivo* 3D cell culture can provide a high-throughput, less costly, and significantly faster platform for these drug studies but are hampered by the rarity of tissue. To address this unmet need for models of rare tumor types we developed 3D spheroid (3D-XPDX$_s$™) and microtumor (3D-XPDX$_{mt}$™) models of ACC using PDX as the primary tissue source. ACC PDX cells readily formed spheroids in our 3D-XPDX$_s$™ platform, remained viable for up to 14 days, and maintained disease-relevant biomarkers such as MYB and c-kit. 3D-XPDX$_{mt}$™ represent a more complex model of ACC by incorporating extracellular matrix. ACC 3D-XPDX$_{mt}$™ displayed tumor-like morphologies concordant with the parental tumors and exhibited MYB and c-kit biomarker expression for up to 56 days in culture. Drug response profiling (DRP) was performed using the 3D-XPDX$_s$™ ACC model with KIYATEC's validated KIYA-PREDICT™ DRP platform. The screening panel consisted of drugs and drug-like compounds currently in use or under investigation for use in ACC, including broad-spectrum chemotherapies and targeted agents. Individualized drug responses were noted for each model as they exhibited differential sensitivities to DNA-damaging agents, microtubule stabilizers, and c-kit targeting kinase inhibitors mirroring the diversity of clinical outcomes. KIYA-PREDICT™ also identified drugs and drug-like compounds that uniformly inhibited viability. This is significant because there is currently no standard of care drugs for ACC, as few, if any have demonstrated homogenous responses in test populations. Monensin has been shown to inhibit activity of the MYB transcription factor, making it an attractive candidate drug for the treatment of ACCs which have a high prevalence of MYB activating alterations. In our study, monensin was effective in all three models with IC_{50} concentrations in the low micromolar range. These results correlate well with immunohistochemical staining of MYB in ACC 3D-XPDX$_s$™ and 3D-XPDX$_{mt}$™. Taken together, this data represents a new *ex vivo* 3D cell culture platform for the study of ACC biology and potential therapies.

Adenoid Cystic Carcinoma (ACC) PDX Models

A.

Patient Demographics

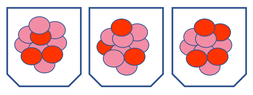


Patient	Patient #1	Patient #2	Patient #3	Patient #4
Sample ID	ACCx5M1	ACCx6	ACCx9	ACCx11
Sex	Male	Male	Female	Female
Age	57	34	77	55
Status	Metastatic	Metastatic	Primary	Primary
Anatomical Site	Lung	Lung	Parotid Gland	Sinonasal Cavity
Grade	2	2	3	3

Moskaluk et al. Lab Invest. 2011 October; 91(10): 1480-1490.

B.

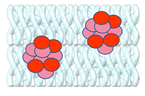
3D-XPDX$_S$™



3D spheroid cultures
- High-throughput platform
- Lower complexity model (self-assembling)
- Ideal for short-term growth

C.

3D-XPDX$_{mt}$™



3D microtumor cultures
- Medium-throughput platform
- Higher complexity model (ECM, scaffolding)
- Long-term growth capabilities

Figure 1. Overview of tissue sourcing and ex vivo model generation using ex vivo PDX and KIYATEC's 3D-XPDX™ 3D spheroid (3D-XPDX$_s$™) and 3D microtumor (3D-XPDX$_{mt}$™) platforms. (A) Characteristics of the tissue donor ACC patients. The patient cohort represented in this study included male and female donors with localized or metastatic ACC of varying grades. Tissue acquisition and animal use were carried out in accordance with the guidelines and regulations specified by each institution. Ex vivo modeling studies were performed on surgically excised, viability cryo-preserved PDX tissue fragments. Diagrammatic representations of KIYATEC's 3D-XPDX$_s$™ of 3D spheroid model **(B)** and 3D-XPDX$_{mt}$™ 3D microtumor model **(C)** platforms used in this study.

3D-XPDX$_S$™ Model Characterization

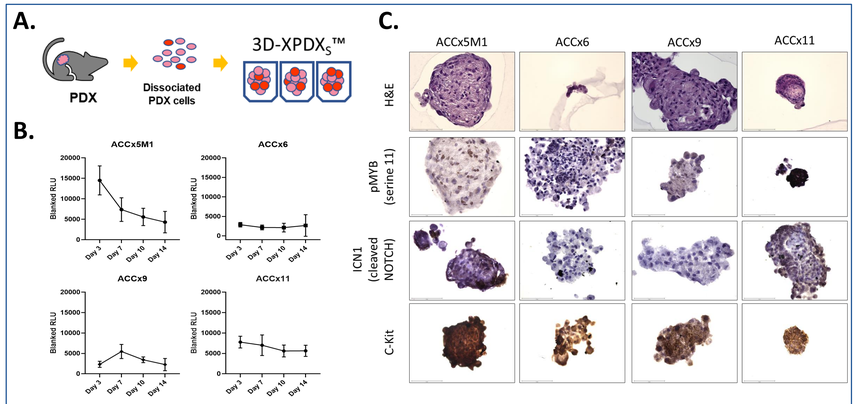


Figure 2. 3D-XPDX$_s$™ model generation and characterization. (A) ACC PDX tissues were enzymatically dissociated, and the resulting single cell suspensions were cultured as 3D spheroids using KIYATEC's 3D-XPDX$_s$™ 3D cell culture platform for 14 days. **(B)** ACC 3D-XPDX$_s$™ viability was assayed using CellTiter-Glo® 3D reagent at days 3, 7, 10 and 14. Data is expressed as the mean of background subtracted relative light units (RLUs). RLU values ≥ 1000 are indicative of healthy, viable cells. Model viability remained stable throughout the 14-day time course. **(C)** ACC 3D-XPDX$_s$™ samples were fixed and processed for histological analysis at days 3, 7, 10 and 14. ACC 3D-XPDX$_s$™ structural features were demonstrated by H&E stain (Day 14 images). Immunohistochemical staining for disease biomarkers MYB (pMYB serine 11), cleaved NOTCH 1 (ICN1) and c-Kit was observed for all samples, at each time point examined. Day 14 IHC staining is shown here.

High-throughput Ex Vivo Drug Response Profiling in ACC 3D-XPDX$_s$™

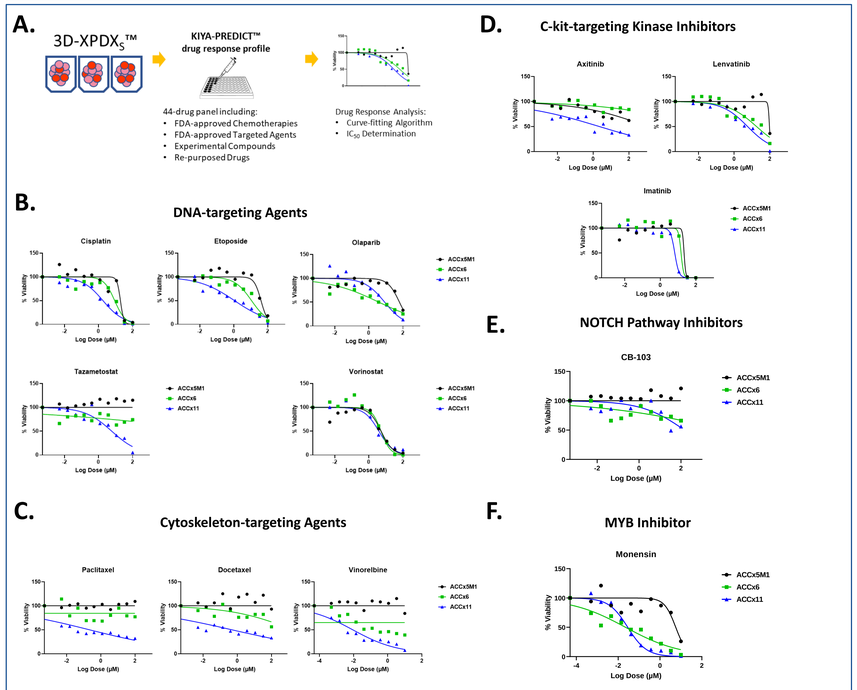
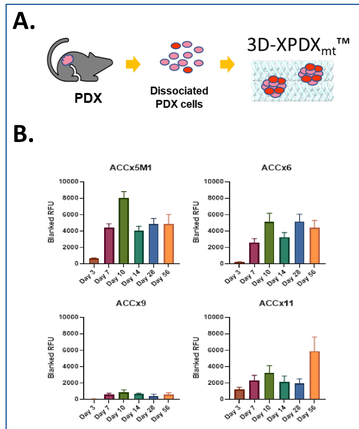


Figure 4. High-throughput ex vivo drug response profiling identifies actionable drug targets in ACC. (A) Schema demonstrating experimental workflow. ACC PDX were cultured as 3D-XPDX$_s$™ for 24 hours. Drug response profiling, using a diverse, 44-drug panel was performed using the KIYA-PREDICT™ platform. Drug treatments ranged from 72-120 h. Viability measurements were analyzed by curve fitting algorithm to determine experimental IC_{50} values for each drug. Dose response curves shown for **(B)** DNA-targeting agents: Cisplatin, Etoposide, Olaparib, Tazametostat, and Vorinostat. **(C)** Cytoskeleton-targeting agents: Paclitaxel, Docetaxel and Vinorelbine **(D)** c-kit-targeting kinase inhibitors: Axitinib, Levantinib and Imatinib. **(E)** NOTCH pathway inhibitor CB-103 **(F)** MYB-targeting agent Monensin.

3D-XPDX$_{mt}$™ Model Characterization



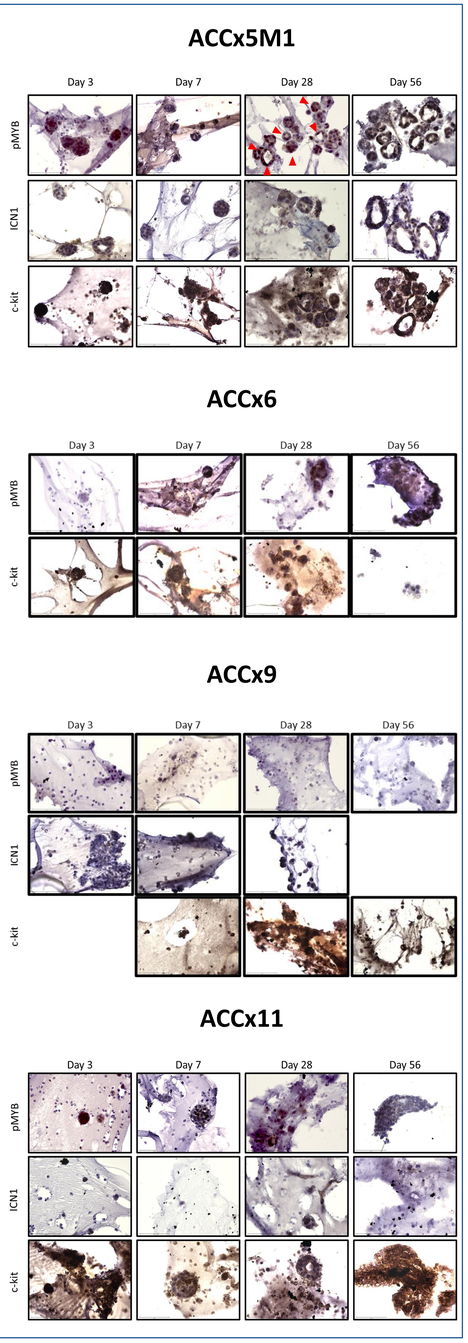


Figure 3. 3D-XPDX$_{mt}$™ model generation and characterization. (A) ACC PDX tissues were enzymatically dissociated, and the resulting single cell suspensions were cultured as 3D microtumors using KIYATEC's 3D-XPDX$_{mt}$™ 3D cell culture platform for 56 days. **(B)** ACC 3D-XPDX$_{mt}$™ viability was assayed using Presto Blue™ viability reagent at days 3, 7, 10, 14, 28, and 56. Data is expressed as background subtracted relative fluorescence units (RFUs). ACC 3D-XPDX$_{mt}$™ samples were fixed and processed for histological analysis at days 3, 7, 28, and 56. ACC 3D-XPDX$_{mt}$™ recapitulated PDX tumor morphology. **(C)** H&E stained tissue sections comparing morphology of PDX xenografts with 3D-XPDX$_{mt}$™ models at day 56. **(D)** ACC 3D-XPDX$_{mt}$™ express disease relevant biomarkers throughout 56-day cultures.

Conclusions

- ACC PDX cultured ex vivo in KIYATEC's 3D-XPDX$_s$™ platform are viable and express disease relevant biomarkers up to 14 days.
- ACC PDX cultured ex vivo in KIYATEC's 3D-XPDX$_{mt}$™ are viable long term, express disease relevant biomarkers, and recapitulate complex tissue morphology .
- ACC 3D-XPDX$_s$™ models screened against a diverse panel of drugs and drug-like compounds in KIYATEC's KIYA-PREDICT™ drug response profile platform identified actionable drug "hits" in less than 7 days.