Patient-Derived Tumoroids Services

Multiplexed plate-reader based drug screening of 3D-tumoroid models

Xiaoyu (Jenny) Yang[1], Andrew Tsao[1], Garrett Wong[1], Chris Yankaskas[2], Colin Paul[2], Brittany Balhouse[2], Amber Bullock[2], Anthony Chatman[2], Shyanne Salen[2], Jason Sharp[1], Matt Dallas[2], Erik Willems[1] and David Kuninger[2], Thermo Fisher Scientific, Carlsbad, CA, USA[1], Thermo Fisher Scientific, Frederick, MD, USA[2]

Introduction

- Cancer drug development is an extremely challenging and resource-consuming process. The high failure rate is partly due to:
  - Inadequacy of traditional 2D cell culture model to predict drug efficacy and toxicity
  - Inability of established cancer cell lines to reflect drug sensitivity and behavior of patient tumors
- Patient-derived 3D tumoroids are better models at predicting tumor response to anti-cancer agents as they:
  - Recapitulate physiological architecture of *in vivo* tumor
  - Retain tumor heterogeneity and clinically relevant genetic alterations
  - Have been shown to reflect patient clinical outcome [1]
  - Expedite drug discovery process towards personalized medicine
- 3D suspension culture models are not limited by extracellular matrix encapsulation and allow easy scale-up and quantitative high-throughput drug screening

Materials and methods

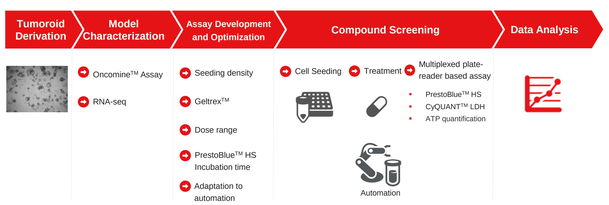


Figure 1. Overall workflow of multiplexed plate-reader based drug screening in tumoroid models

Patient-derived tumoroid lines

- Dissociated tumor cells used in this study were provided by Discovery Life Sciences and cultured in Gibco™ OncoPro™ Tumoroid Culture Medium. Characteristics of patient-derived tumoroid lines are listed in Table 1.

Table 1. Clinical information of patient-derived tumoroids lines

Tumoroid Line	Diagnosis	Stage	Sex	Age	Race	Tobacco History
HuCo3209	Colorectal Adenocarcinoma	I	Female	59	White	Never Used
HuCo1044	Colorectal Adenocarcinoma	III-B	Female	80	White	Never Used
HuCo021320	Colorectal Adenocarcinoma	IV	Female	58	White	Never Used

Model Characterization

- Patient-derived tumoroids were sequenced for cancer relevant mutations and altered gene expression profile using Oncomine™ Comprehensive Assay v3 and Ion AmpliSeq™ Transcriptome Human Gene Expression Panel.

Assay Development and Optimization

- Tumoroids were dissociated at Day 1 and seeded at 20k or 40k per well in Ultra-Low Attachment 96-well plates. Geltrex™ matrix at concentrations of 0%, 2% and 4% was added in cell suspension by directly dropping-in or pipet up-and-down to mix. Tumoroids were fed at Day 4 by media change or media addition. 22µl/well PrestoBlue™ HS reagent was added at Day 7 and incubated for 8h or overnight. Fluorescence was read at 560/590 nm using Thermo Fisher Scientific Varioskan™ LUX Multimode Microplate Reader.

Compound Screening

- Tumoroids were dissociated at Day 1 and seeded at 15k/well with 4% Geltrex™ mixed in. Cells were treated with increasing concentrations of drug compound at Day 4. Drug response readout was multiplexed using three different plate reader-based assays: Invitrogen™ PrestoBlue™ HS, Invitrogen™ CyQUANT™ LDH assay and quantification of ATP production.

Data Analysis

- Data was analysis using GraphPad Prism 9

Results

Model Characterization

A

	Mutations
HuCo3209	TP53 Truncating Mutation
HuCo1044	KRAS G12S
HuCo021320	TP53 R273C
HCT-116[2]	KRAS C13D, PIK3CA H1047R

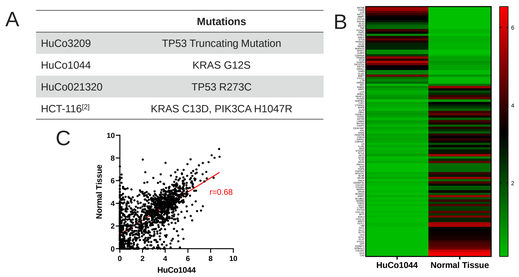


Figure 2. Tumoroid Model Characterization. (A) Mutations of patient-derived tumoroid lines were called using Oncomine™ Comprehensive Assay v3. **(B)** Heatmap and **(C)** correlation matrix of differential expressed genes between HuCo1044 line and normal colorectal tissue. RNAseq data were generated using Ion AmpliSeq™ Transcriptome Human Gene Expression Panel.

Assay Development and Optimization

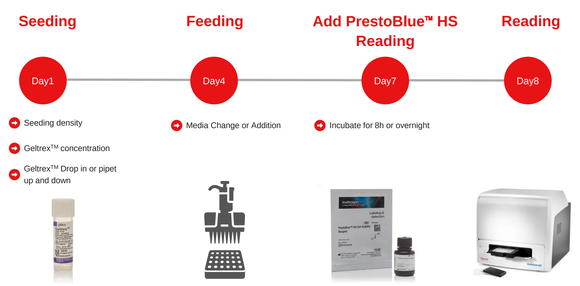


Figure 3. Overall workflow of compound screening assay development and optimization. Diagram illustrates steps required and parameters tested, including seeding density, Geltrex™ matrix concentration and addition manner, feeding by media change or media addition and PrestoBlue™ HS reagent incubation time.

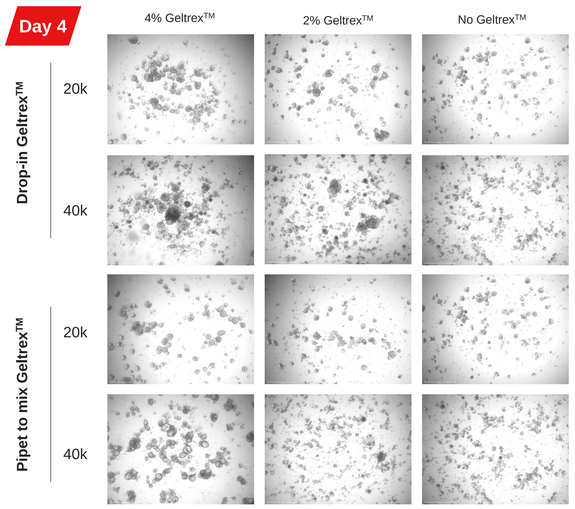


Figure 4. Tumoroid formation of HuCo021320 line on Day 4 of Assay Optimization. Tumoroids were dissociated and seeded at 20k or 40k per well. 0%, 2% or 4% Geltrex™ matrix was added to cell culture by simply dropping in or pipetting to mix. Images were taken on Day 4 using the Invitrogen™ EVOS™ M7000 Imaging System. Scale bar = 650 µm.

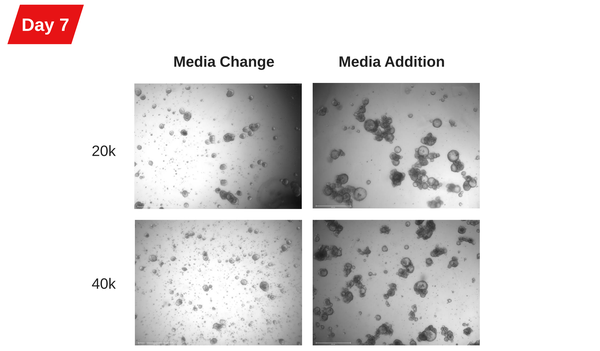


Figure 5. Tumoroid formation of HuCo021320 line on Day 7 of Assay Optimization. Tumoroids were dissociated on Day 1 and seeded at 20k or 40k per well with 2% Geltrex™ added to cell culture by pipetting up and down. Cells were fed on Day 4 by Media addition (add 100uL media with 2% Geltrex™) or Media change (replace 50uL media with 2% Geltrex™). Images were taken at Day 7 using the Invitrogen™ EVOS™ M7000 Imaging System. Scale bar = 650 µm.

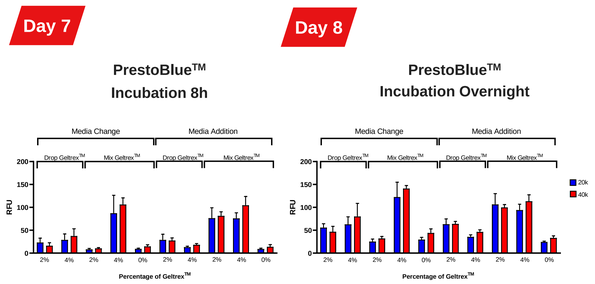
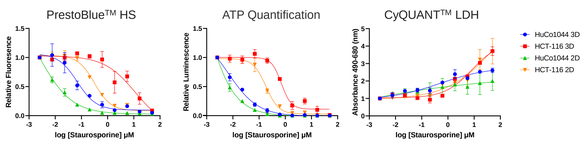


Figure 6. PrestoBlue™ HS fluorescent reading of HuCo021320 line on Day 7 and Day 8 of Assay Optimization. 22µl/well PrestoBlue™ HS reagent was added on Day 7 of Assay Optimization to all seeding density, Geltrex™ or feeding conditions and incubated for 8h or overnight. Fluorescence was read at 560/590 nm using Thermo Fisher Scientific Varioskan™ LUX Multimode Microplate Reader.

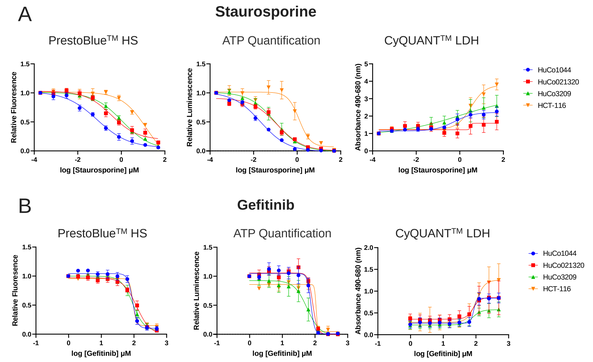
2D vs 3D



IC50s	PrestoBlue™ HS		ATP Quantification	
	2D	3D	2D	3D
HuCo1044	0.0025	0.0697	0.00039	0.0065
HCT-116	0.3143	11.61	0.1829	0.7082

Figure 7. Dose response comparison of patient-derived tumoroid line HuCo1044 and cancer cell line HCT-116 in 2D vs 3D culture. Dissociated tumoroids were seeded in either Ultra-Low Attachment 96-well plates for 3D culture or Collagen I-coated 96-well plates for 2D culture on Day 1. Tumoroids were treated with increasing concentrations of Staurosporine on Day 4. Drug response readout was multiplexed using Invitrogen™ PrestoBlue™ HS reagent, Invitrogen™ CyQUANT™ LDH assay and ATP quantification and read on Varioskan™ LUX Multimode Microplate Reader. IC50s were calculated using GraphPad Prism 9. Data shown are representative of two independent repeats.

Compound Screening



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IC50s	Staurosporine (µM)		Gefitinib (µM)	
	PB	ATP	PB	ATP
HuCo3209	1.10	0.10	91.46	57.16
HuCo1044	0.08	0.02	90.75	74.73
HuCo021320	0.36	0.12	120.50	85.35
HCT-116	14.42	1.23	92.96	108.40

Figure 8. Multiplexed 10-point drug screening result in 3D patient-derived tumoroids and colorectal cancer cell line. Dissociated tumoroids were seeded at 15k/well on Day 1 and treated with increasing concentrations of **(A)** Staurosporine and **(B)** Gefitinib at Day 4. Drug response readout was multiplexed using Invitrogen™ PrestoBlue™ HS, Invitrogen™ CyQUANT™ LDH and ATP Quantification and read on Varioskan™ LUX Multimode Microplate Reader. **(C)** IC50s were calculated using GraphPad Prism 9. Data shown are representative of three independent repeats.

Conclusions

- Developed, optimized and established workflow for multiplexed plate-reader based drug screening in patient-derived tumoroid models
- Demonstrated potential advantages of 3D patient-derived tumoroid models over 2D culture and cancer cell lines for accurate prediction of drug response
- Through targeted NGS and RNA-seq, patient-specific drug targets could be identified for personalized drug screening
- 3D suspension culture does not reply on time-consuming and labor-intensive extracellular matrix encapsulation, allowing scalable workflow and adaptation to automation for high-throughput screening

References

[1] Berg, H.F., Hjelmeland, M.E., Lien, H. *et al.* Patient-derived organoids reflect the genetic profile of endometrial tumors and predict patient prognosis. *Commun Med* 1, 20 (2021).
[2] Ahmed, D., Eide, P., Eilertsen, I. *et al.* Epigenetic and genetic features of 24 colon cancer cell lines. *Oncogenesis* 2, e71 (2013).

Acknowledgements

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