Use of conditional reprogramming to develop and characterize cell cultures from patient-derived xenograft (PDX) models of human lung and ovarian cancer

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Abstract

Patient-derived xenografts (PDX) are widely recognized as a more physiologically relevant preclinical model to standard cell line xenografts. PDX models faithfully recapitulate the original patient genetic profile, gene expression patterns and tissue histology. Despite their benefits, PDX models are limited by their inherent variability, lower throughput and lack of growth in vitro. The ability to generate cell lines from PDX models would enable high throughput chemosensitivity screens, ex vivo genetic manipulation and the development of novel orthotopic models. Development of stable PDX cell lines remains a challenge due to murine stromal outgrowth, lineage commitment and limited differentiation potential. Conditional reprogramming (CR) cell technology is a novel cell culture system facilitating the generation of stable cultures without genetic manipulation. The success of CR cell technology is dependent upon the combination of feeder cell-derived factors and Rho Kinase (ROCK) inhibitor. CR cells, therefore, represent a new class of progenitor-like cells, distinct from the phenotype of embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. The purpose of this study was to identify the advantages, limitations and potential applications of CR technology for derivation of PDX explant cell lines. Early passage human lung and ovarian PDX tumors were cultured in CR conditions to create stable explant cell lines. Cell lines were established from 5/8 (63%) PDX tumors and were expanded over 6 months in culture with varying morphologies and growth kinetics. Due to normal outgrowth of murine stromal cells, early CR cultures contained mixed populations and required murine depletion to enrich for human cells. Key oncogenic mutations in a model of ovarian papillary serous adenocarcinoma were preserved in the enriched tumor cell population. While purified CR PDX cell lines were amenable to high throughput chemosensitivity screens, in vitro chemosensitivity did not consistently predict response in in vivo murine models. The CR PDX cell lines were additionally assessed for genetic manipulation and ability to form tumors in vivo. Collectively, these results demonstrate the applications of CR technology for the generation of stable explant cell lines from PDX models for preclinical studies.

Introduction

- Development of stable PDX cell lines remains a challenge due to murine stromal outgrowth, lineage commitment and limited differentiation potential.
- CR technology is a novel cell culture system facilitating the generation of stable cultures without genetic manipulation.
- CR cell technology is dependent upon the combination of feeder cell-derived factors and Rho Kinase (ROCK) inhibitor[2].
- The purpose of this study was to identify the potential applications of CR technology for derivation of PDX cell lines.

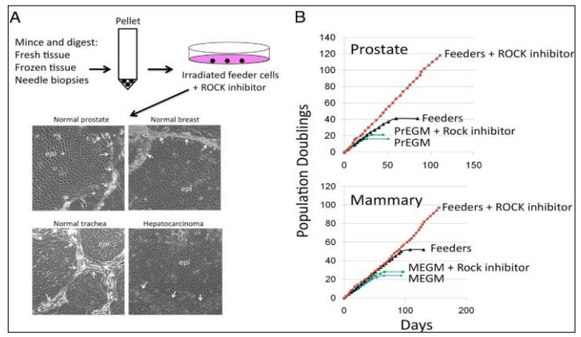

Figure 1. Propagation and immortalization of human adult epithelial cells[3]

Results

Table 1. PDX model characteristics

Model	Indication	Pathology	Parental Mutations	CR-PDX mutations	Average doubling time/day
OV0857F	Ovarian	Serous Carcinoma	TP53 (R248Q)	TP53 (R248Q)	0.27
LG0567F	Lung	Adenocarcinoma	KRAS (G12C) TP53 (R273C)	KRAS (G12C) TP53 (R273C	0.37
HLXF-036LN	Lung	Adenocarcinoma	TP53 (P152L)	TP53 (P152L)	0.54
HLXF-056	Lung	Adenocarcinoma	KRAS (G12R) TP53 (K292*)	KRAS (G12R) TP53 (K292*)	0.16

Conditional reprogramming expands PDX cells and maintains key mutations

- Conditional reprogramming expands lung and ovarian PDX cells
- Human cells were enriched by through magnetic murine cell depletion.
- Sequencing analysis identified that the CR-PDX cell lines maintained mutations of the parental PDX.

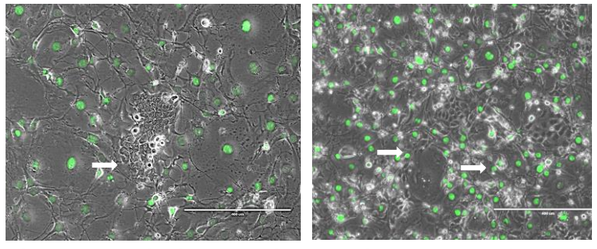

Figure 2. CR technology expands PDX explants. Representative images of PDX cells growing in co-culture with GFP-expressing irradiated fibroblast cells (arrows). Left: HLXF-036LN, P1 10X, right: OV0857F, P4, 10X.

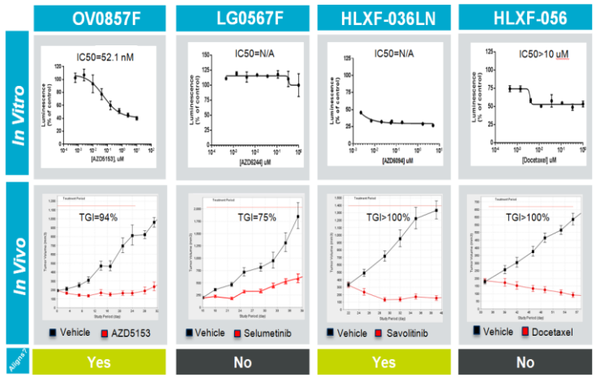

Figure 3. CR-PDX cell lines are amenable to *in vitro* chemosensitivity screens. CR-PDX cells were treated with indicated inhibitors and *in vitro* drug sensitivity was compared to *in vivo* response.

CR-PDX models are amenable to chemosensitivity screens

- Compounds with known *in vivo* drug response were selected for chemosensitivity screening, including the BRD4 inhibitor AZD5153, selumetinib (AZD6244, ARRY-142886), savolitinib (AZD6094, HMP-504, Volitinib) and docetaxel.
- In some cases, *in vitro/in vivo* discrepancy can exist, similar to what is observed in conventional cell line xenografts models.

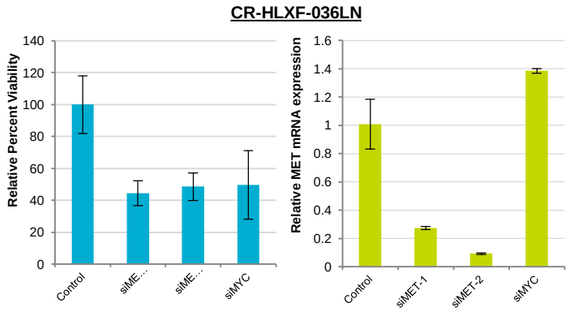

Figure 4. CR-PDX cells can be utilized for gene knockdown studies. CR-HLXF-036LN cells were treated with siRNAs for MET MYC (positive control) and non-targeting control (left). Knockdown efficiency was confirmed by qPCR (right).

CR-PDX cells can be utilized for gene knockdown studies

- To determine if CR-PDX models can be utilized for genetic manipulation studies and confirm sensitivity to MET signaling, siRNA transfection was used to knockdown MET expression in CR-HLXF-036LN cells. Knockdown efficiency was confirmed by qPCR.
- MET knockdown significantly reduced viability of CR-HLXF-036LN cells *in vitro,* as compared to controls.

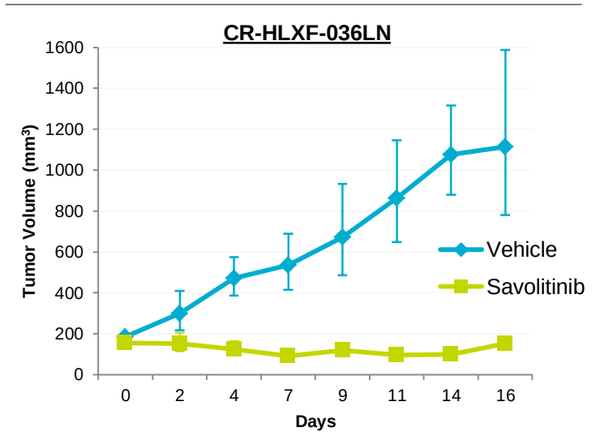

Figure 5. CR-PDX cells form tumors and retain drug sensitivity *in vivo*

CR-PDX cells form tumors *in vivo*, retain drug sensitivity

- Approximately 3 weeks following implantation, CR-HLXF-036LN tumors formed and had similar growth kinetics to the parental PDX model.
- AZD6094 induced regressions in CR-HLXF-036LN tumors, similar to the parental PDX response, resulting in >100% tumor growth inhibition (Figure 3).

Conclusions

- CR technology generates stable explant cell lines from PDX models
- CR-PDX models retain parental mutations and are amenable to high throughput chemosensitivity screening and genetic manipulation
- CR-PDX models form tumors *in vivo* and maintain drug response

References

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Acknowledgements

The authors would like to Theresa Prioia, Evan Barry, Danielle Greenawalt and Kelsey Biely for their help with this work.

Supported by AstraZeneca

Presented at the AACR Annual Meeting, New Orleans, LA, 17 April, 2016