#1628 Application of Conditionally Reprogrammed Cells in Cancer Model Establishment and New Target Identification

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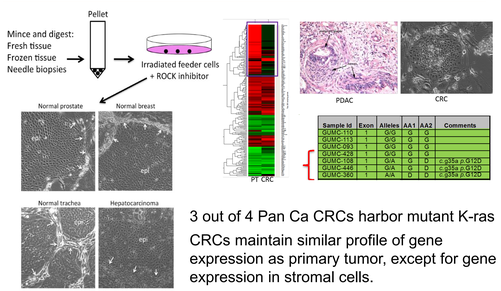
Abstract

Tumor cell immortalization through conditional reprogramming, particularly from limited biopsy specimen, is an invaluable tool to generate propagating tumor cells for cell-based drug sensitivity assays and bio-banking in vitro. We have successfully reprogrammed primary tumor cells from several tumor types, including lung carcinoma, breast cancer and glioma .etc. The conditionally reprogrammed cells exhibited typical colonized growth, which is well maintained upon cryopreservation. In some cases, the cells can be passaged for multiple times and likely become stable cell lines. We attempt to extend conditional reprogrammed tumor cells to reconstitute and grow PDX models in vivo and find the tumor formation rate is much higher than the primary tissues'. This attempt provides a new process for establishing PDX models from variant types of cancer, especially cancer types with low success rates in PDX establishment. We also establish PDX-derived cell lines by conditional reprogramming, which could be used in in-vitro drug efficacy studies and anti-cancer drug target identification. Functional library screening, such as genome-wide CRISPR/Cas9 library or specific pro-siRNA library screening, could discover new targets directly by evaluating the effect of the knockout/knockdown of specific genes. We have established several cell lines from drug resistant PDX, including Osimertinib and other targeted drugs resistant models, and found potential targets by pro-siRNA library screening. These potential targets could be validated on the matching cell line and PDX and the matching in vitro and in vivo tools will also enhance the further research of new targeted small molecules or antibodies.

Materials and Methods



3 out of 4 Pan Ca CRCs harbor mutant K-ras

CRCs maintain similar profile of gene expression as primary tumor, except for gene expression in stromal cells.

Liu, X. et al. The American Journal of Pathology. 2012 Feb;180(2):599-607.

Human specimens: All patient tumor materials used for conditional cell reprogramming, PDX model establishment and related studies have been approved by Hospital Medical Ethics Committee (HMEC).

Conditional Tumor Cell Reprogramming: Wash the tumor tissue with buffer solution and remove away non-tumor tissue and necrotic tumor tissue in biosafety cabinet. Cut the tumor into 1~3 mm^3 fragments, suspend pellet with digestion solution and incubate in 37℃ for 1-2 hours. Collect single cells through 70uM strainer and cultured the cells using OncoVee™ Conditional Reprogramming Cell Culture Kit.

PDX model from surgery tumor tissues: Fresh human tumors were received from collaborated hospitals (HMEC approval received for the projects). NOD/SCID and Balb/c nude mice were used to grow the primary tumor tissues.

PDX model from conditional reprogrammed tumor cells: 2-5 M conditional reprogrammed tumor cells and NOD/SCID and Balb/c nude mice were used to grow the primary tumor tissues.

Results

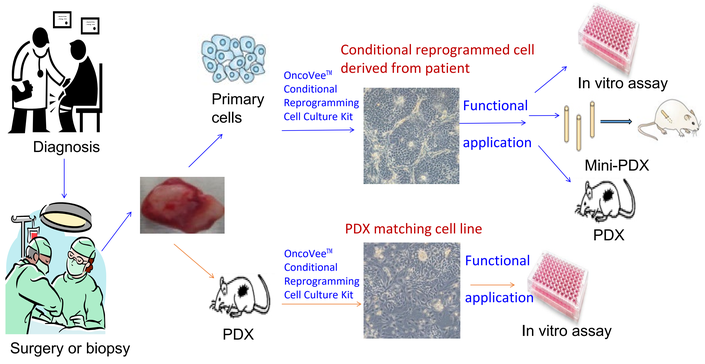


Figure 1. Flow chart of CR Cells establishment and application

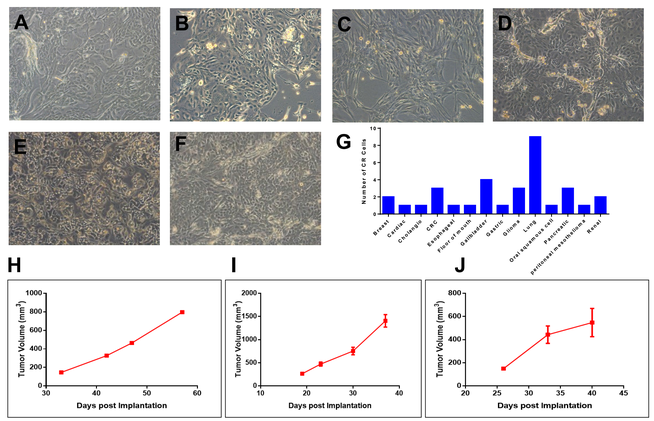


Figure 2. Conditional reprogrammed cells from various samples.

A, CR cell originated from biopsy sample of a lung cancer patient; **B**, CR cell originated from surgical sample of a gallbladder cancer patient; **C**, CR cell originated from a surgical sample of a glioblastoma patient; **D**, CR cell originated from a surgical sample of a pancreatic cancer patient; **E**, CR cell originated from a surgical sample of a ovarian cancer patient; **F**, CR cell originated from biopsy sample of a breast cancer patient ; **G**, PDX matching conditional reprogrammed cell lines in LIDE from various cancer types; **H**, growth curved of PDX derived from gallbladder cancer conditional reprogrammed cells; **I**, growth curved of PDX derived from gastric cancer CR cells; **J**, growth curved of PDX derived from esophageal cancer CR cells.

We have successfully reprogrammed primary tumor cells from fresh surgery, biopsy and pleural effusion samples of various cancer types. The conditionally reprogrammed cells exhibited typical colonized growth and morphology of cancer cells(Fig.2A-F). In some cases, the cells can be passaged for multiple times and will likely become stable cell lines. The conditional reprogrammed tumor cells could also be used to establish PDX models (Fig.2H-J). We tried 26 CR cells to establish PDX models *in vivo*. 13 CR cells succeeded in establishing PDX models and 13 failed. The tumor formation rate is 50%, much higher than the primary tissues'.

PDX model ID	Cancer type	Drug resistence	Mutations					
			EGFR	RET	ALK	ROS	KRAS	FGFR2
LD1-0025-200694	Lung cancer	--	L858R	--	--	--		
LD1-0025-215625	Lung cancer	--		--	EML4-ALK	MAP3K5-ROS1, ROS1-RILPL2		
LD1-0006-215676	Lung cancer	Erlotinib	L858R T790M	--		--		
LD1-0025-200717	Lung cancer	AZD9291	19del(746_750del)/ T790M/C797S	--	--	--		
LD1-0025-360715	Lung cancer	--	--	KIF5B-RET	--	--		
LD1-2009-361825	Breast cancer	CDK4/6 inhibitor						
LD1-2009-362263	Breast cancer	CDK4/6 inhibitor						
LD5-0024-362077	Melanoma	Imatinib, Paclitaxel						
LD1-0060-200791	Cholangiocarcinoma	-					G12C	
LD1-0025-361646	Lung cancer	-					G12C	
LD1-0060-200770	Cholangiocarcinoma	Lenvatinib sensitive Paclitaxel resistant						BICC1-FGFR2

Table 1. Partial list of PDX matching cell lines at LIDE

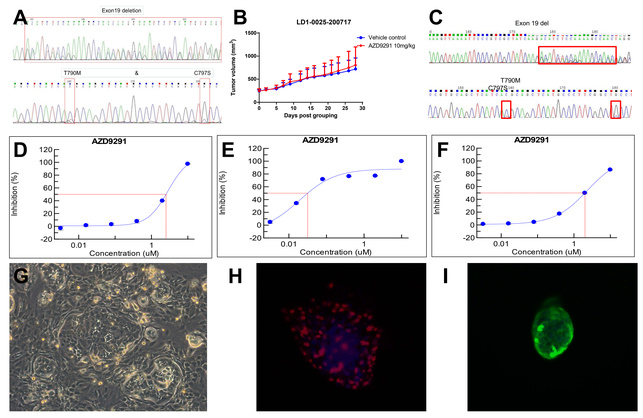


Figure 3. A case of PDX matching Conditionally Reprogrammed cell line.

A, Sanger sequencing for EGFR of LD1-0025-200717, which shows LD1-0025-200717 was an EGFR Exon 19 deletion, T790M and C797S mutant lung cancer PDX model; **B**, Efficacy study of AZD9291 in LD1-0025-200717 PDX model; **C**, Sanger sequencing for EGFR of Conditional Reprogrammed cell line derived from LD1-0025-200717 PDX; **D**, Inhibition curve of LD1-0025-200636 (EGFR WT) cell line.; **E**, Inhibition curve of LD1-0006-215676 (EGFR L858R/T790M) cell line.; **F**, Inhibition curve of LD1-0025-200717 (EGFR 19del, T790M & C797S mutant) cell line; **G**, Morphology of LD1-0025-200717 CR cell; **H**, Ki67 staining of LD1-0025-200717 CR cell derived tumor sphere; **I**, Pan-CK staining of LD1-0025-200717 CR cell derived tumor sphere

We have also established more than 70 PDX matching cell lines from various cancer types (Fig.2G). Some of the PDX matching cell lines were derived from rare PDX models(Table 1), including EGFR 19del/T790M/C797S triple mutant model(Fig.3), KIF5B-RET fusion model and CDK4/6 inhibitor resistant model .etc. These rare PDX models and matching cell lines would be powerful tools for anti-cancer drug efficacy studies and drug target identification. Functional library screening, such as genome-wide CRISPR/Cas9 library or specific pro-siRNA library screening, could discover new targets directly by evaluating the effect of the knockout/knockdown of specific genes (Fig.4). We have found potential targets by pro-siRNA library screening using PDX matching cell lines. These potential targets could be validated on the cell line and PDX and the matched *in vitro* and *in vivo* tools will also enhance the further research of new targeted small molecules or antibodies.

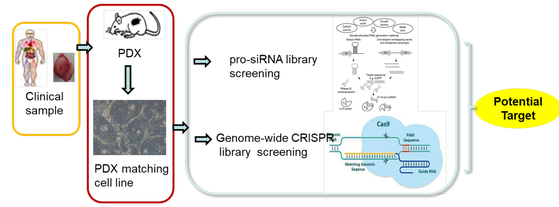


Figure 4. Target identification strategy using PDX matching cell line.

Summary and Conclusion

- Conditional reprogrammed tumor cells could be established from different kinds of clinical samples, including surgical, biopsy and pleural effusion.
- Conditional reprogrammed tumor cells could be established from multiple cancer types.
- Conditional reprogrammed tumor cells could be used in PDX establishment and the success rate of P0 is appreciably high. There are also cases in which the p0 PDX is obtained from conditional reprogrammed tumor cells, while the patient tissue failed in PDX establishment.
- Conditional reprogrammed tumor cells could be used in cell line establishment from PDX samples.
- PDX matching cell lines could be used in anti-cancer drug efficacy studies and drug target identification.

References

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