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Fidelity of a PDX-CR Model for Bladder Cancer

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Abstract

Patient-derived xenografts (PDXs) are widely recognised as a more physiologically relevant preclinical model than standard cell lines, but are expensive and low throughput, have low engraftment rate and take a long time to develop. Our newly developed conditional reprogramming (CR) technology addresses many PDX drawbacks, but lacks many in vivo factors. Here we determined whether PDXs and CRCs of the same cancer origin maintain the biological fidelity and complement each for translational research and drug development. Four CRC lines were generated from bladder cancer PDXs. Short tandem repeat (STR) analyses revealed that CRCs and their corresponding parental PDXs shared the same STRs, suggesting common cancer origins. CRCs and their corresponding parental PDXs contained the same genetic alterations. Importantly, CRCs retained the same drug sensitivity with the corresponding downstream signalling activity as their corresponding parental PDXs. This suggests that CRCs and PDXs can complement each other, and

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Authors' contributions

This study was conceived and designed by AMM, AM, XL, CP. AMM, AM, GL, RY, EK and JL performed the experiments. AMM, AM, GL, LS, KJK, GKP analyzed the data. AMM, CP, RS and XL wrote the paper. All authors have read and approved the final manuscript.

[*]These authors equally contributed this work.

Disclosures

Six patents for conditional reprogramming technology has been award to Georgetown University by the United States Patent Office. The license for this technology has been given to Propagenix for commercialization. The inventors (R.S. and X.L.) and Georgetown University receive potential royalties and payments from Propagenix.

that CRCs can be used for in vitro fast, high throughput and low cost screening while PDXs can be used for in vivo validation and study of the in vivo factors during translational research and drug development.

Keywords

Conditional reprogramming; Patient derived xenograft; Cell line models; Drug discovery; Bladder cancer

Introduction

Cell lines, their derived animal models and genetically engineered mouse models (GEMMs) have been commonly used in drug development, basic and translational cancer research. Cell lines and their derived models have been selected and cultured in vitro for a long time, acquired additional genetic and epigenetic alterations, and can behave dramatically differently from cancers in patients. After a few generations, there was a great irreversible genetic divergence between primary tumor and the cell line derived from that tumor [1]. GEMMs usually harbour one or a few genetic alterations, and cancers usually develop within a few weeks to months with uniform genetic alterations in contrary to cancers in human patients which usually develop after years of exposure of carcinogens, and harbour dozens to hundreds of genetic alterations [2]. Hence, it is not surprising that findings from these models are often not translated into clinical applications. For example, models to predict chemoresistance based on studies in cell lines are rarely translated into clinical practice [3–5] while only approximately 5% of oncology drugs that have gone through all preclinical studies and entered clinical trials are finally approved by the Food and Drug Administration [6].

To overcome the shortcomings of these models, patient-derived models of cancer (PDMCs) have recently been proposed to better replicate human cancers because they are directly derived from clinical patient cancer specimens with little and short out-of-patient intervention [7]. Not surprisingly, as we previously reported, patient-derived xenografts (PDXs) retained 92–97% of genetic alterations of their parental patient cancers [8]. Both positive and negative clinical outcomes of patient cancers to treatment can be reproduced in PDXs and retained over time [9].

There are several types of PDMCs currently being actively pursued for translational research and drug development. Patient-derived organoids (PDOs) and patient-derived xenografts (PDXs) are two robust preclinical models [10, 11]. Organoid cultures fairly maintain genetic stability, can be grown for a long period of time and are not clonal in selection, but rather capture partial heterogeneity of the original tumor [12]. Additionally, organoid cultures are more suitable for low-throughput rather than high throughput drug screening. On the other hand, PDXs are biologically stable when passaged in mice in terms of global gene-expression patterns, mutational status, metastatic potential, drug responsiveness and tumor architecture [13, 14]. Although preclinical PDX models closely recapitulate the human tumor heterogeneity that is needed for more-efficient oncology drug development, the major challenges include the immense resources needed to establish and maintain such living

biobanks; high cost; low engraftment rate; interference of mouse stroma in the model-associated data generated from PDX tumor; low stringency in ‘response’ criteria that may not translate to benefit cancer patients and the lack of high throughput drug screening [14]. In addition, considering that it takes a long time (months) to establish the first (Passage 0) PDXs and more time to expand in order to generate sufficient numbers of PDXs for drug testing, individual patients with a rapidly progressing disease may not benefit from PDX studies. Clearly, PDX models need to be viewed as complementary to other preclinical models.

Recently, our group established CR technology that enables normal and tumor primary epithelial cells to be propagated indefinitely *in vitro* while maintaining their original karyotype, genetic integrity and differentiation ability [15, 16]. Unlike other cell models using viral [17–21] or cellular oncogenes [22], the CR protocol uses irradiated mouse fibroblasts and a ROCK inhibitor (Y-27632) to propagate epithelial cells indefinitely without any genetic manipulation. Interestingly, the induction of CR is rapid (within 2 days) and results from reprogramming of the whole cell population rather than clonal selection, as is the case with conventional cell lines, and thus tumor CRCs retain morphological features and intra-tumor heterogeneity [23]. Unlike embryonic stem cells and induced pluripotent stem (iPS) cells, CRCs from normal tissue do not express high levels of Sox2, Oct4, Nanog or Klf4 [24] and do not form teratomas in mice [16]. In tumor CRCs, the phenotypic and genotypic features of primary tumors are maintained and have recently been used to identify appropriate therapy for various cancers [25].

Even with multiple PDMCs being actively pursued, there is little direct comparison between these PDMCs on their genetic alterations and response to therapeutic intervention. In this study, we conducted biological comparisons of PDXs and CRCs, and determined whether CR technology can overcome the drawbacks associated with PDXs. We used our PDXs in bladder cancer for this comparison study as we have conducted extensive research in bladder cancer PDXs [8, 26–30], and are the only NCI-funded center (U54) to develop bladder cancer PDXs for health disparity, translational research and drug development.

Methods

Development of patient-derived bladder cancer xenografts and CR cell culture

The protocol for collecting cancer specimens and clinical information was approved by the Institutional Review Board of the University of California (UC) Davis (Protocol No. 218204). Written informed consents from all the participants were taken before any clinical information or specimens were collected. The protocol for animal work was approved by the UC Davis Institutional Animal Care and Use Committee (Protocol No. 17794). The PDX tumors were developed in 4–5 weeks old NOD. Cg-Prkdcscid Il2rgtm1Wjl/SzJ (aka, NSG) mice by subcutaneous implantation of fresh bladder cancer specimens (3–5mm^3) as described previously [8]. Four CRC lines were generated from the above bladder PDX tumors according to an established protocol as described previously [15, 16]. CRCs were passaged at 1:8 when reached 80–90% confluent. The viability of the cells was measured using trypan blue staining before every passaging.

Differential trypsinization to separate epithelial cells from feeders

The epithelial cells were harvested from co-culture with irradiated feeder 3T3-J2 fibroblasts using two-step trypsinization [15, 16].

Genomic DNA isolation and PCR amplification

Total genomic DNA was isolated from the harvested cell pellets of CRCs using the DNeasy Blood and Tissue Kit (Qiagen). PCR amplifications of the genomic DNA samples from CRCs were performed using PCR master mix from Promega (Cat. no. M7502) using the primers for specific targets as shown in supplemental Table.

STR Profiling

Genomic authentication of the bladder PDX CRCs was conducted to ensure donor identity. This analysis was performed by short tandem repeat (STR) profiling using Genetica PowerPlex 16HS Cell line PCR kit (Genetica DNA Laboratories, a LabCorp brand).

DNA-sequencing

PCR products that showed single-band amplification were purified using the QIAquick PCR Purification Kit (Qiagen, Cat. No. 28104). Sanger sequencing of the purified PCR products were performed in GENEWIZ according to manufacturer's instructions. Sequencing for each PCR samples were performed in both the directions using forward and reverse primers (Table 1). Sanger sequencing results were compared with whole-exome sequencing (WES) of the parental tumor PDXs [8].

Drug sensitivity assay

CRCs were cultured at 25% density taking same number of cells that examined. Irradiated 3T3-J2 feeders were taken at 10% in the CRC cultures. Low density of feeders compared to CRCs (approx. 25%) were used to reduce drug interference by the feeders. Next day, culture media were replaced with fresh media containing PI3K inhibitor GDC-0941 at 10 nM, 100 nM and 1000 nM. DMSO only was used as a control without adding drug. To quantify cell proliferation, cultures were monitored using the IncuCyte live-cell analysis system with IncuCyte ZOOM software (Essen BioScience).

Immunoblot analysis

Epithelial cells were harvested as discussed above and performed immunoblotting as described previously [31, 32]. The primary antibodies Phospho-AKT (S473, Cat. no. 9271), total-AKT (Cat. no. 2920), Phospho-ERK (Thr202/Tyr204, Cat. no. 9101), total-ERK (Cat. no. 9107) and GAPDH (Cat. no. 5174) were purchased from the Cell Signaling Technology (Danvers, MA). The secondary antibodies used were goat anti-rabbit-HRP (Bio-Rad Cat. no. 1706515) and goat anti-mouse-HRP (Bio-Rad Cat. no. 1706516).

Results

Establishment of CRCs from bladder PDX tumors

The PDX tumors used in this study were originally developed through subcutaneous implantation of patient tumors into immunocompromised NSG mice and consecutively passaged *in vivo*. None of the patients received any prior neoadjuvant chemotherapy (Fig. 1a). Among the four PDX tumors, two (BL0269 and BL0269_GDC-R) were from the same patient and two others were from two different patient donors (Fig. 1a). PDX tumor BL0269_GDC-R was developed from the parental BL0269 PDX after mice were treated with a PI3K inhibitor GDC-0941, and developed resistance to this drug. We have generated 4 CRC lines using the CR technology from the parental PDXs. Although the CRCs showed different cellular morphologies and varying doubling times, they all were growing efficiently and maintained steady rates of proliferation in the CRC conditions (Fig. 1b). CRC lines were tested and authenticated by STR profiling from Genetica DNA laboratories (Fig. 1c). As the CRC269 and CRC269-R were originated from the same donor's PDXs, they showed the same allelic distribution of the STR markers.

CRC lines maintain genetic mutations of the parental PDX tumors

The cancer cell lines do not accurately represent the genetics and heterogeneity of primary tumors and are therefore limited to their applicability in translational medicine [24]. Whole exome sequencing of the PDX tumors revealed mutations of known functionally active and significantly mutated genes previously identified in bladder cancer [7, 33, 34] and many of them are involved in transcriptional regulation, signal transduction and tumor suppression [35]. To determine if CRCs maintain the parental mutations identified in PDX tumors [8], targeted sequence analysis were performed for 20 genes (FAT4, KMT2C, KMT2D, PIK3CA, FOXA1, SYNE1, SYNE2, ZFR2, ADCY2, ARID1A, TP53, EP300, CDKN2A, MTOR, ATM, KLF5, MERTK, MYH10, NUP98 AND SETD2) in the cell lines of CRCs after 3 passages in CR culture conditions (Table 2). The genes considered in this study exhibit at least one single nucleotide variation leading to missense or nonsense mutations. All the CRCs consistently maintained genetic mutations, as demonstrated in the parental PDX tumors [8], in all the genes analysed in this study (Table 2).

CRCs retain drug resistance as the parental PDX tumors

Next, we sought to determine if the CRCs could be used for *in vitro* drug sensitivity screening and if so, whether the cells maintain sensitivity to the targeted inhibitor *in vitro* as observed by the parental PDX tumors *in vivo*. PIK3CA, which encodes the p110a catalytic isoform of class I PI3K, is one of the most commonly mutated or amplified kinases in a variety of tumors [36]. The frequency of mutation and amplification of PIK3CA in bladder cancer ranged from 15.2% to 26.0% (http://www.cbioportal.org/). We previously reported that GDC-0941, a PI3K inhibitor, showed *in vivo* antitumor activity in bladder cancer PDXs [29]. We examined CRC269 and CRC269-R cells that were established from the PDX tumor BL0269 and BL0269_GDC-R, respectively. Although, these two PDXs were originated from the same patient donor, but BL0269_GDC-R showed *in vivo* resistance to the PI3K inhibitor GDC-0941 [29]. In this study, when the CRC lines were treated at 10–1000 nM of GDC-0941, we observed a dose dependent sensitivity of the CRC269 cells, whereas

CRC269-R cells were mostly resistant to GDC-0941 (Fig. 2a and 2b). These results suggest that the drug sensitivity of the tumor PDX *in vivo* is retained in the CRCs *in vitro*.

Increased phosphorylation of AKT and ERK in the GDC-0941 resistant CRCs

It is well evident that patients with solid tumors show marked increase of AKT and ERK activation [37–40]. In line with this mechanism, we previously [29] reported that the expression of phospho-AKT and phospho-ERK were upregulated when tumors become resistant to GDC-0941 treatment in the PDX BL0269 model. To explore the mechanism potentially underlying the development of drug resistance to the PI3K inhibitor in bladder cancer, and whether that is maintained in the CRC lines, we investigated the CRC269 and CRC269-R cells for AKT and ERK protein expression. CRC269-R cells showed increased expression of both the phosphorylated form of AKT and ERK as compared with the CRC269 cells, when the total levels were unchanged (Fig. 3a and 3b). These data suggest that alternative oncogenic pathways became activated to compensate for the PI3K/AKT pathway in the GDC-0941 resistant CRCs, similar to the previous report for the treatment resistant PDX tumors *in vivo* [29]. These findings implicate the importance of rational combinations of therapeutic agents to overcome drug resistance that was induced by compensatory activation of other pathways.

Discussion

This is the first study showing that two patient-derived models of cancer retained the genetic alterations, response to targeted therapy matched to the underlying genetic alterations and the underlying signalling activities. Therefore, CRCs can potentially complement the *in vivo* PDX models, and be used to translational research and development of targeted therapies, but can be achieved at a much faster pace, and much lower cost.

Several *in vitro* patient-derived models of cancer (PDMC), such as the CR technology we developed [15], organoid [41–46], and induced pluripotent cells (iPS) [47, 48], have been widely employed in cancer research. Because of the *in vitro* culture system and lack of tumor microenvironment, these models are considered physiologically distinct from patient cancers *in vivo*. Hence, the *in vivo* PDXs are considered more physiologically relevant to cancers in clinical patients. We showed that the *in vivo* PDXs not only retained the pathohistological features, but also 92–97% of genetic alterations of parental patient cancers [8]. However, some intrinsic factors associated with PDXs, such as long engraftment time, low engraftment rate, and high cost, preclude its widespread use in translational research.

In this study we established four cell lines from the PDX tumors using the CR technology and assessed the genomic compositions and compared with their parental tumors. It has been always a challenge to establish a single model system that is rapid, simple to perform, and has a high rate of success. Conventional cell culture allows the least differential cells to thrive, resulting in distinct and irreversible losses of important biological properties, such as tumor heterogeneity and gene expression [1]. The CR technology meets all these needs and the CRCs retain cell lineage commitment with sustained expansion and maintain the heterogeneity of the cells present in a biopsy. This method offers the ability to expand PDX

cells *in vitro* for subsequent high throughput drug screening assays, *ex vivo* genetic manipulation as well as for use *in vivo* to reduce animal usage, variability and study costs.

Overall, CRCs, like PDO and PDX, are able to capture the heterogeneity of the tumor of origin, but it still remains to be seen the level and similarity of heterogeneity captured by all these methods. Each of these above models has both merits and flaws with regard to their utility and in faithful representation of tumor architecture, microenvironment, cellular composition and heterogeneity, stem-differentiation states, growth patterns and responses to perturbagens, with respect to the patient specimen from which the model was initially derived. Combination of these models, for example, PDX-derived CRCs and CRC-derived xenografts (CDX,) will enable rapid, low cost expansion of the tumor cells, genetic manipulation, high-throughput analysis of drug sensitivity and comparisons with the genetic/cellular heterogeneity of these model systems (Fig. 3c).

Even though we studied only four PDX-derived CRCs, we previously showed that the success rate of CRC from primary patient cancer specimens is very high. For example, CRCs were established in 37/37 (100%) of primary prostate cancer specimens [15], and 18/18 (100%) with high-grade bladder cancer (unpublished data), compared to less than 5% with prostate cancer PDXs. Our results of retention of genetic alteration and drug response fidelity of PDXs by CRCs suggest CRCs can serve as a fast and low-cost alternative of the *in vivo* PDX model for translational research and precision medicine. One drawback of CR technology is lack of the in vivo factors. Hence drug absorption, distribution, metabolism and elimination or excretion (**ADME**), vascularization and local drug delivery can be assessed with the PDX platform, but CR. Combination of these two platforms can accelerate translational research and drug development, and readily translate patient-derived models of cancer for clinical applications.

In summary, we demonstrated that CRCs retained the fidelity of genetic alteration, drug response and signalling activity of the parental *in vivo* PDXs, and can potentially function as an alternative of the more expensive PDXs in the research of precision medicine, drug development and translational research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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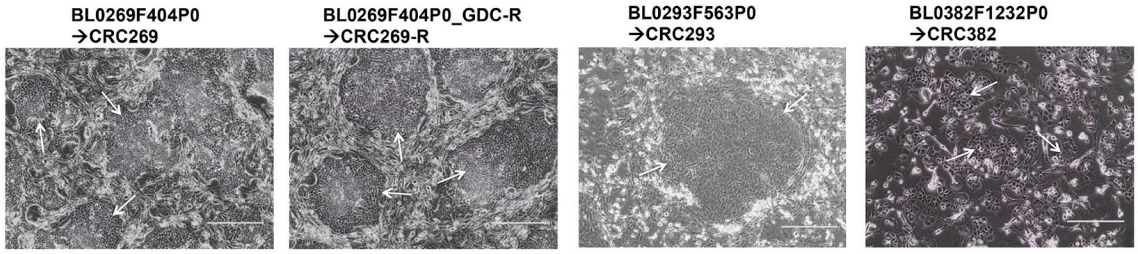
Highlights:

1. Conditional Reprogramming Cells (CRCs) were established from PDX bladder tumors.
2. CRCs maintained same genetic alterations as those in the original PDX tumors.
3. CRCs retained the same drug sensitivity with the corresponding downstream signalling activity as their corresponding parental PDXs.

a

Tumor ID	Age (yrs)	Stage	Surgery	Prior chemo	Bladder PDX ID
BL0269F	58	pT4 N0 Mx	Cystectomy	No	BL0269F404P0
					BL0269F404P0_GDC-R
BL0293F	77	pT2a N2 Mx	Cystectomy	No	BL0293F563P0
BL0382F	82	pT2 Nx Mx	TURBT	No	BL0382F1232P0

b



c

PDX CRC ID	D3S1358	TH01	D21S11	D18S51	Penta_E	D5S818	D13S317	D7S820	D16S539	CSF1PO	Penta_D	AMEL	vWA	D8S1179	TPOX	FGA
CRC269	15,17	7,9	31,32.2	12,14	16	11	8	9,10	12	10,13	9	X,Y	17,18	11,13	11	19,21
CRC269-R	15,17	7,9	31,32.2	12,14	16	11	8	9,10	12	10,13	9	X,Y	17,18	11,13	11	19,21
CRC293	16,17	6	30	17	12	10	9,11	8	9,12	11,12	10,12,14	X	17,18	12,14	8,11	21
CRC382	16	9.3	29,30	12	17	12	11,13	8,11	11,12	12	12,13	X	17	13,16	8,10	22

Fig. 1.

Establishment of CRCs from 4 bladder PDX tumors. **a** Clinical characteristics of the 3 donor patients. Four PDXs were developed from the 3 advanced bladder cancer patients. BL0269_GDC-R was developed from a BL0269 PDX after with the host mouse was treated with a PI3K inhibitor GDC-0941 and developed resistant PDXs. **b** Representative images of CRCs grown (arrow) in co-culture with irradiated 3T3-J2 mouse fibroblasts. **c** STR analysis of the PDX CRCs after 3 passages in CR culture. Electropherograms are shown in Additional files 1–4: Figures S1–S4.

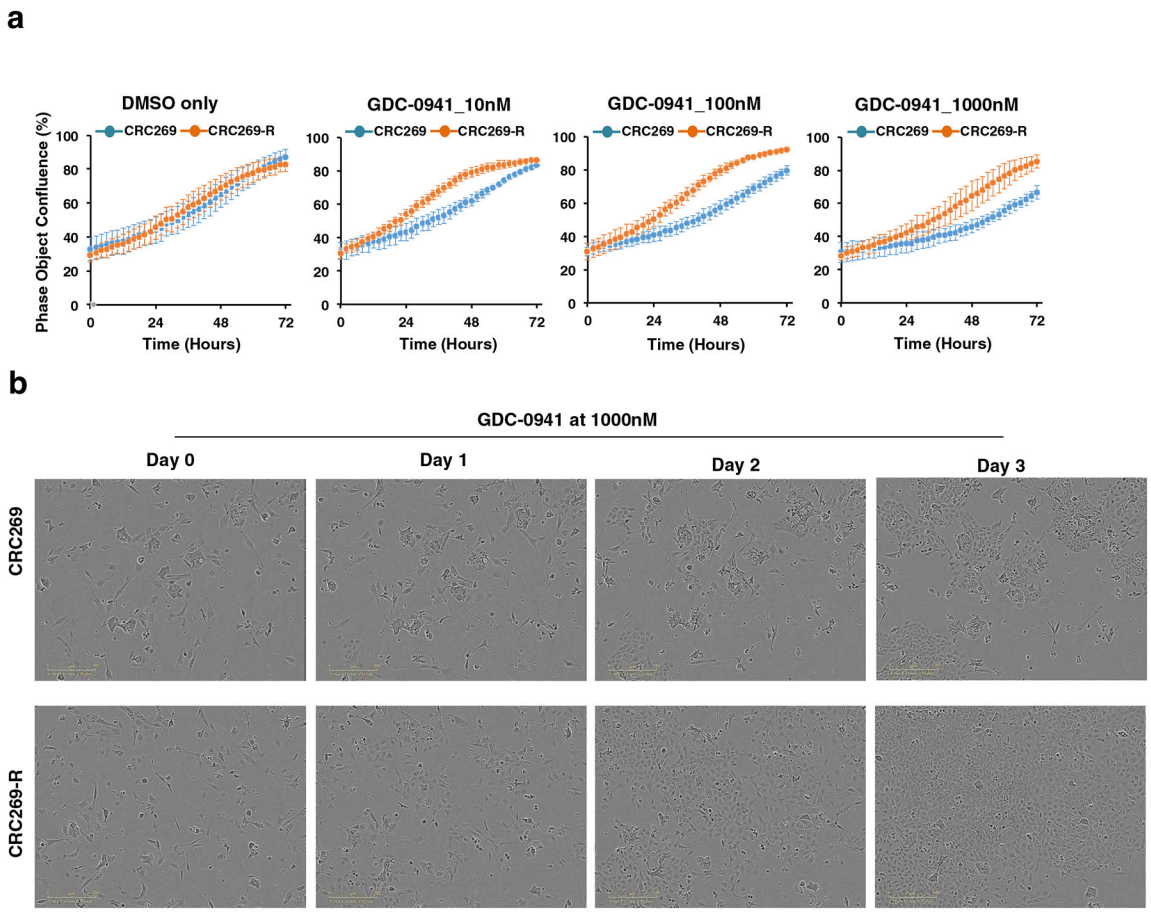


Fig. 2.

In vitro drug sensitivity of the CRC lines in response to a PI3K inhibitor GDC-0941. **a** CRC269 and CRC269-R cells were treated with GDC-0941 at 10 nM, 100 nM and 1000 nM final concentration (n = 3, mean ± SD shown). DMSO only was the control cells. **b** Representative cell images of the CRC269 and CRC269-R cells treated with 1000 nM of GDC-0941 at Day 0, Day 1, Day 2 and Day 3.

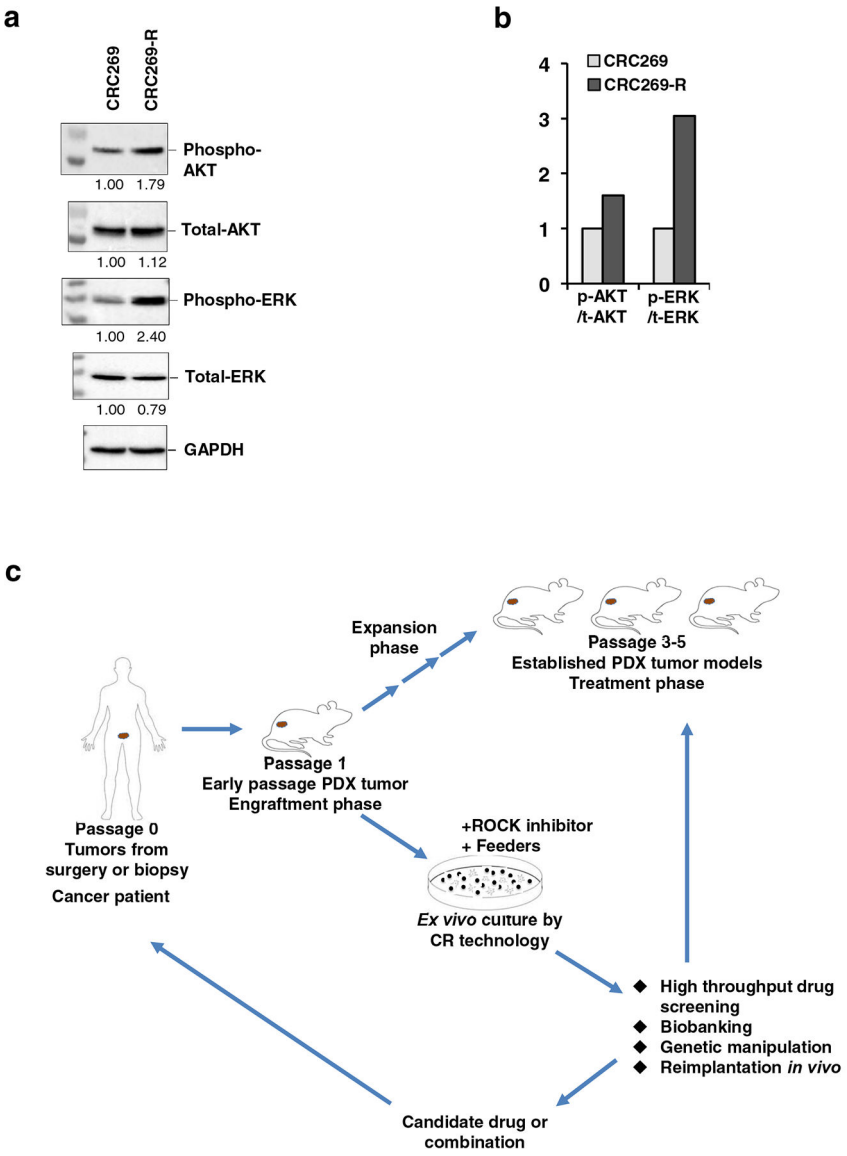


Fig. 3.

Increased phosphorylation of AKT and ERK in the GDC-0941 resistant CRCs and workflow. **a** Immunoblot analysis of phospho-AKT, total-AKT, phospho-ERK and total-ERK in the CRC269 and CRC-269-R cells. GAPDH was used as a loading control for quantification. Normalized densitometric values for expression levels are indicated below each lane relative to CRC269 (defined as 1.0). Uncropped blot images of the blots are shown in Additional file 5: Figure S5. **b** Bar graph of phospho-AKT and phospho-ERK levels compared with their total-AKT and total-ERK, respectively, in the cells shown in **a.** 'p' represents phospho, 't' represents total. **c** Workflow for the establishment of CRCs from bladder PDXs and comparative molecular analysis of the CRCs.

Table 1.

Primers used for PCR amplification and sequencing.

PDX CRC ID	Gene Symbol	Primer	Sequence 5'−−3'
CRC269 and CRC269 R	FAT4	Forward	GTGTCTGTGGTTGAGAATGC
		Reverse	TGGACCGGGTAGAAGACAGG
	KMT2C	Forward	TGAAGAGTGGCTCCAGGAGA
		Reverse	CCTGTTCTAGCTGTTTCTGAACC
	PIK3CA (for c.2176)	Forward	ACAGTTAGCCAGAGGTTTGG
		Reverse	AAACAACTCTGCCCCACTGC
	PIK3CA (for c.3140)	Forward	AGCTATTCGACAGCATGCCA
		Reverse	GGTCTTTGCCTGCTGAGAGT
	KMT2D (for c.2438)	Forward	CTATGCGCTGTGCCTGAGG
		Reverse	CTTCTCAAGCTCAGGGGAC
	KMT2D (for c.7411)	Forward	GTTACCCCTCGCTTCCAGTC
		Reverse	CTTGGGACCTTGGCATGGAG
	FOXA1	Forward	GGCTTCTTCACTCGCTGTCT
		Reverse	CTACTGCGCCGGGACTCAG
	SYNE1	Forward	TGTTGTGGGGTTTCATTTCGT
		Reverse	TGCATTTTCCCTGGCTCACA
	ZFR2	Forward	GGGGCTTTGGTGTGTGTTTG
		Reverse	GTAGGTAGCCATGGTGGTGG
	ADCY2	Forward	GGAGGCTCTTAGAAACCAGAA
		Reverse	CCTGGTGGGATGTGGAAAGT
CRC293	ARID1A	Forward	GCCCTGAACAATAACCTCACG
		Reverse	GGTTGCCCGAAGCCGTAG
	TP53 (for c.626)	Forward	GCTGGGGCTGGAGAGACG
		Reverse	GCACCACCACACTATGTCGA
	TP53 (for c.743)	Forward	TGGCTCTGACTGTACCACCA
		Reverse	CTG GAG TCT TCC AGT GTG ATG
	SYNE2	Forward	TTGGCGTCTCTCAGAACAGC
		Reverse	CGATGTTTCACAGCTGGAACA
	EP300	Forward	TGGTGATTCCAGTCTGAATGAGT
		Reverse	ACAAATCCGGAGCTAGCCAC
	CDKN2A	Forward	GCTTCCTTTCCGTCATGCC
		Reverse	TGGAAGCTCTCAGGGTACAA
	MTOR	Forward	TGATGAACTTCGAAGCTGTGC
		Reverse	CTCAGTGACCTTCTTCTGCA
CRC382	ATM	Forward	CTCAAACTATTGGGTGGATTTGT
		Reverse	TCGTTTGCGAGAAGTGTCGA
	TP53 (for c.610)	Forward	GCTGGGGCTGGAGAGACG

PDX CRC ID	Gene Symbol	Primer	Sequence 5'−−3'
		Reverse	GCACCACCACACTATGTCGA
	TP53 (for c.25)	Forward	TGCTGGATCCCCACTTTTCC
		Reverse	AGACAAGAGCAGAAAGTCAGTC
	KLF5	Forward	CGTTGTCACAGGTGAAAAGCC
		Reverse	GTGGTCAGAGCGCGAGAAG
	MERTK	Forward	TGTGTGTGTATGTGTGTGTGT
		Reverse	ATGCTGCAATTCCTGAACGG
	MYH10	Forward	CTGTGTACGTATGTAATAGGGGCA
		Reverse	CCACTTGAAAATCCAAAATATGCTTCT
	NUP98	Forward	GCCTAGTCCCTCGTGAAAAGTC
		Reverse	GCATCAAGAAATGTGACTCACGG
	SETD2	Forward	GCCTATGTGGATCCCAGCAA
		Reverse	GCACTGGCAAGACAGCAAC

Table 2.

Mutation statuses of 20 genes in the CRC lines in comparison with PDX tumors.

PDX CRC ID	Bladder PDX ID	PDX CRC ID	Gene Symbol	Transcript ID	CDS mutation (in PDX)	AA Mutation (in PDX)	CDS mutation (in PDX CRCs)
CRC958-1	BL0269F404P0	CRC269	FAT4	NM_001291303.1	c.1855C>T	p.R619C	c.1855C>T
			KMT2C	NM_170606.2	c.9694G>T	p.E3232★	c.9694G>T
			PIK3CA	NM_006218.2	c.2176G>A	p.E726K	c.2176G>A
					c.3140A>G	p.H1047R	c.3140A>G
			KMT2D	NM_003482.3	c.2438C>T	p.P813L	c.2438C>T
					c.7411C>T	p.R2471★	c.7411C>T
			FOXA1	NM_004496.3	c.150G>A	p.M50I	c.150G>A
			SYNE1	NM_182961.3	c.2653T>G	p.L885V	c.2653T>G
			ZFR2	NM_015174.1	c.172G>A	p.G58S	c.172G>A
			ADCY2	NM_020546.2	c.439G>T	p.V147L	c.439G>T
CRC958-2	BL0269F404P0_GDC-R	CRC269-R	FAT4	NM_001291303.1	c.1855C>T	p.R619C	c.1855C>T
			KMT2C	NM_170606.2	c.9694G>T	p.E3232★	c.9694G>T
			PIK3CA	NM_006218.2	c.2176G>A	p.E726K	c.2176G>A
					c.3140A>G	p.H1047R	c.3140A>G
			KMT2D	NM_003482.3	c.2438C>T	p.P813L	c.2438C>T
					c.7411C>T	p.R2471 ★	c.7411C>T
			FOXA1	NM_004496.3	c.150G>A	p.M50I	c.150G>A
			SYNE1	NM_182961.3	c.2653T>G	p.L885V	c.2653T>G
			ZFR2	NM_015174.1	c.172G>A	p.G58S	c.172G>A
			ADCY2	NM_020546.2	c.439G>T	p.V147L	c.439G>T
CRC958-3	BL0293F563P0	CRC293	ARID1A	NM_006015.4	c.413C>G	p.S138★	c.413C>G
			TP53	NM_001126112.2	c.626G>A	p.R209Q	c.626G #
					c.743G>A	p.R248Q	c.743G>A
			SYNE2	NM_015180.4	c.4177C>T	p.R1393W	c.4177C>T
			EP300	NM_001429.3	c.4040G>T	p.G1347V	c.4040G>T
			CDKN2A	NM_000077.4	c.442G>A	p.A148T	c.442G>A

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PDX CRC ID	Bladder PDX ID	PDX CRC ID	Gene Symbol	Transcript ID	CDS mutation (in PDX)	AA Mutation (in PDX)	CDS mutation (in PDX CRCs)
			MTOR	NM_004958.3	c.5533G>A	p.E1845K	c.5533G>A
CRC958-4	BL0382F1232P0	CRC382	ATM	NM 000051.3	c.5557G>A	p.D1853N	c.5557G>A
			TP53	NM_001126112.2	c.610G>T	p.E204★	c.610G>T
					c.25G>T	Sense mutation	c.25G>T
			KLF5	NM_001730.4	c.1255G>C	p.E419Q	c.1255G>C
			MERTK	NM_006343.2	c.878G>A	p.R293H	c.878G>A
			MYH10	NM_001256012.1	c.2879C>G	p.S960C	c.2879C>G
			NUP98	NM_016320.4	c.3424C>G	p.Q1142E	c.3424C>G
			SETD2	NM_014159.6	c.6694C>T	p.P2232S	c.6694C>T

★STOP codon

#no mutataion observed

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