REVIEW

Culture and application of conditionally reprogrammed primary tumor cells

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Abstract

Cancer is still a major public-health problem that threatens human life worldwide and further study needs to be carried out in the basic and preclinical areas. Although high-throughput sequencing technology and individualized precise therapy have made breakthroughs over the years, the high failure rate of clinical translational research has limited the innovation of antitumor drugs and triggered the urgent need for optimal cancer-research models. The development of cancerous cell lines, patient-derived xenograft (PDX) models, and organoid has strongly promoted the development of tumor-biology research, but the prediction values are limited. Conditional reprogramming (CR) is a novel cell-culture method for cancer research combining feeder cells with a Rho-associated coiled-coil kinase (ROCK) inhibitor, which enables the rapid and continuous proliferation of primary epithelial cells. In this review, we summarize the methodology to establish CR model and overview recent functions and applications of CR cell-culture models in cancer research with regard to the study of cancer-biology characterization, the exploration of therapeutic targets, individualized drug screening, the illumination of mechanisms about response to antitumor drugs, and the improvement of patient-derived animal models, and finally discuss in detail the major limitations of this cell-culture system.

Key words: conditional reprogramming; primary tumor cell; cancer research; precise medicine

Introduction

Malignant tumors are expected to be the leading cause of death worldwide and the most important obstacle to increasing life expectancy in the twenty-first century, responsible for 9.6 million deaths (9.5 million excluding non-melanoma skin cancer) in 2018 [1]. For decades, scientists have been dedicated to studying the molecular and genetic mechanisms of tumorigenesis and development, and many important advances have accelerated the research of cancer treatments. However, cancer recurrence and drug resistance remain primary challenges in current cancer therapy—one of the reasons being that cancer generally becomes more heterogeneous as it progresses [2]. As

malignancies are clonal diseases with diverse subclone developments over time, the genetic and phenotypic heterogeneity of tumor cells is often found within the same patient [3, 4], which is the most important feature of all cancers and determines the response to the antitumor therapy and progression of primary disease [5].

During the current era, precision medicine is based on the recognition of individual differences in genes, environment, and lifestyle with the help of high-throughput platforms [6]. Precision medicine is at the nascent stage, with cancerous cell lines and patient-derived models including patient-derived xenograft (PDX), organoid, induced pluripotent stem cell (iPSC),

and spheroids being the major research tools, each of which has its own merits and demerits in terms of tumor heterogeneity, microenvironment, response to therapies, range of applications, and so on. Therefore, methods to propagate and study primary tumor cells that can maintain tumor heterogeneity *in vitro* and are suitable for high-throughput platforms have been the focus of scientific research. The effective isolation and culture of primary tumor cells from patients' samples under an *in vitro* environment similar to the tumor microenvironment is the first and crucial step for many types of preclinical studies to personalize cancer therapy [7].

Conditional reprogramming (CR) is a novel method of co-culturing epithelial cells with irradiated feeder cells in the presence of a Rho-associated coiled-coil kinase (ROCK) inhibitor, which achieves rapid and sustained expansion of primary cancerous and normal epithelial cells [8, 9]. These reprogrammed immortalized cells of malignant tumors [10], such as bladder cancer [11], prostate cancer [12], pancreatic cancer [13], breast carcinoma [14], and hepatocellular carcinoma [15], without genetic manipulation or chromosomal abnormalities, represent an adult stem-cell-like state but express fairly low levels of *Sox2*, *Oct4*, and *Klf4* [16], which are the pluripotent progenitor markers [17]. What is more, these non-tumorigenic cells can maintain intra-tumor heterogeneity [18] in addition to keeping their molecular features [19, 20], and are only capable of differentiating into the native tissues in which they originated [16, 21]. Therefore, CR is appropriate to effectively assess tumor biology, screen potential therapeutic targets, and preclinically evaluate the efficiency of antitumor drugs. In this review, we summarize the method for culturing conditionally reprogrammed primary cancerous cells, go over the latest advances in preclinical cancer studies in which CR has been applied, and assess the limitations of this cell-culture system.

Methods and mechanisms to establish and culture CR cells

Methodology to establish CR cells

Figure 1 shows an overview of the methodology to establish and culture CR cells. The tissue specimens from cancer patients are divided in half after being evaluated grossly and microscopically [8]. Half of the biopsies are used for histological examination to analyse the rationing of benign and malignant cells [22]. The remaining tissues are enzymatically digested into single cells and co-cultured with irradiated 3T3 J2 mouse fibroblasts in the CR medium containing a ROCK inhibitor Y-27632 [23]. The reprogrammed epithelial cells can usually reach confluence ($1 \sim 2 \times 10^6$ cells) in 5 days and continue to passage for 100 population doublings over $\geq$110 days [8, 24]. During the passage, short tandem repeat analysis, epithelial-marker examination including real-time quantitative polymerase chain reaction (RT-PCR) and immunofluorescence, comparative genomic hybridization, and karyotype analysis should be performed on both the primary tissue and the CR cells to verify the origin of the cultured cells [8, 23]. Karyotype analysis of the prostate cells at population-doubling 93 confirmed that the chromosomes of the CR cells are normal structurally and numerically as compared with the initial population [23].

It is crucial to evaluate the histology of specimen tissues for confirming the precise location of cancerous cells. Liu *et al.* [8] found that nearly one-quarter of the tumor biopsies of primary prostate carcinomas contained no tumor cells, 50% of the tumor tissues contained <10% cancerous cells, and even the tissues from the normal region contained some tumor cells. In order to improve the quality and success of CR-cell establishment, the ration of benign and malignant cells should be included in the inclusion criteria. Saeed *et al.* [22] considered that tissues with <15% contaminating benign cells could be applied for the culture of CR cells.

Growing primary epithelial cells in a co-culture system requires the irradiation and plating of feeder cells followed by the seeding of epithelial cells in the plates. Because they are postmitotic and have an inability to surviving for >3–4 days, the feeder cells need to be replaced. Irradiated Swiss 3T3 J2 fibroblasts as feeder cells are essential for improving epithelial cell growth and promoting rapid cloning efficiency as a consequence of cell growth and the merging of small colonies into larger colonies [8, 23].

p160-ROCK is a serine/threonine kinase that can mediate the actin-cytoskeleton assembly and cell contractibility by phosphorylating various downstream substrates to contribute to multiple cellular physiological activities, such as activating caspase signaling cascades and triggering cellular apoptosis [25]. It has been reported that Y-27632 as the inhibitor of the Rho/ROCK pathway is a potent inhibitor of differentiation as well as apoptosis; it could improve the *in vitro* survival of human keratinocytes [26, 27]. Consequently, the use of Y27632 in the culture medium of CR cells is capable of maintaining the immortalization of primary epithelial cells.

Mechanism to culture CR cells

Nevertheless, the mechanism for cell immortality is under investigation. At present, there are two distinct functions that can explain the promotion of long-term cell proliferation in the combination of feeder cells and Y27632: increased telomerase activity and cytoskeletal remodeling, and/or interference with the p16/Rb pathway [28], which has profound similarities with the process of cell immortalization induced by human papillomavirus [23, 26]. Possible mechanisms that fall into the two designated pathways are shown in Figure 2. E6 and E7, two oncoproteins encoded by high-risk human papillomavirus, are significant for the efficient immortalization of primary cells [29]. The major immortalizing activity of E6 is to increase cellular telomerase activity primarily by regulating c-Myc protein access to the endogenous human telomerase reverse transcriptase (hTERT) promoter and inducing the transcription of the hTERT gene [30, 31], which is essential for maintaining or elongating telomeres for continued cell replication [32]. It has been demonstrated that the predominant reasons for hTERT induction and telomerase activity increasing in a conditional reprogramming medium are diffusible factor(s) released by irradiated feeder cells [28] and the overexpression of Δ133p53α in CR cells [33], but not the inclusion of Y27632 or the direct contact between the feeder cells and keratinocytes [23, 26, 28]. Additionally, it has been proved that Δ133p53α likely inhibits p53-mediated apoptosis and senescence *in vitro* by physically interacting with wild-type p53 [34]. However, the mechanism of Δ133p53α-mediated upregulation of hTERT and the properties of diffusible factor(s) released by feeder cells are incompletely clear. The other oncoprotein E7 has recently been reported to modulate the actin-cytoskeleton structure for regulating cell proliferation [35] and inactivated Rho/ROCK [36], in addition to inactivating the Rb pathway and the p53 pathway for making cells escape from cell senescence [37]. As a ROCK inhibitor in CR medium, Y27632 enables CR cells to bypass cell crisis and proliferate indefinitely by inhibiting cell apoptosis and altering the cytoskeletal network [28] (Figure 2). It may also be possible that Y-27632 is perturbing the p16/Rb-signaling pathway to prevent cell senescence [23]. Meanwhile, the genes and molecules in these two potential pathways interact to promote
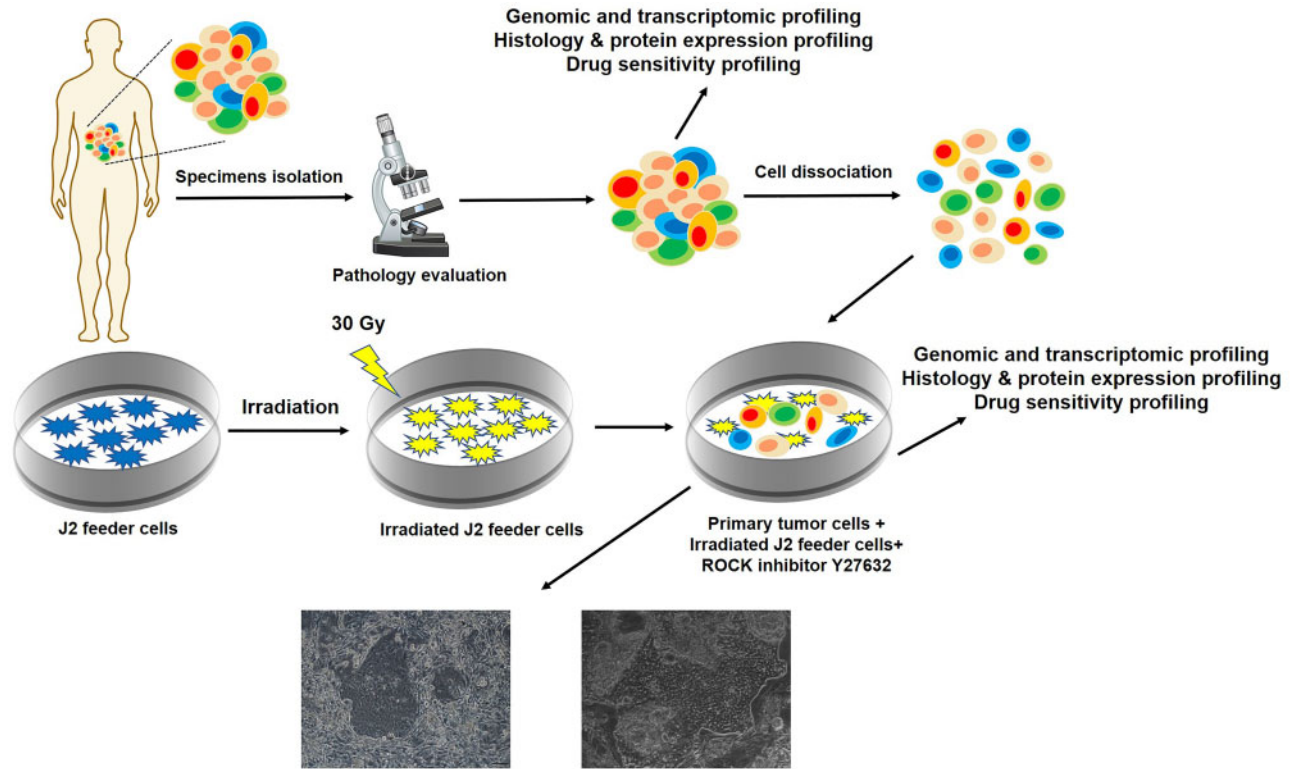
Figure 1. Overview of the establishment of conditional reprogramming (CR)-cell-culture technology. Briefly, primary tissue samples are obtained from biopsy specimens, which undergo complete pathological evaluation using immunohistochemistry (IHC) and specific biomarkers to ensure their normal/tumor status. Subsequently, these tissues are digested into single cells and co-cultured with irradiated J2 feeder cells in the presence of ROCK inhibitor. The authenticity of the CR cells should be verified by genomic and transcriptomic profiling, histology, and protein-expression profiling as well as drug-sensitivity profiling. The two pictures are primary lung-cancer cells (left) and colon-cancer cells (right) cultured with CR technology.
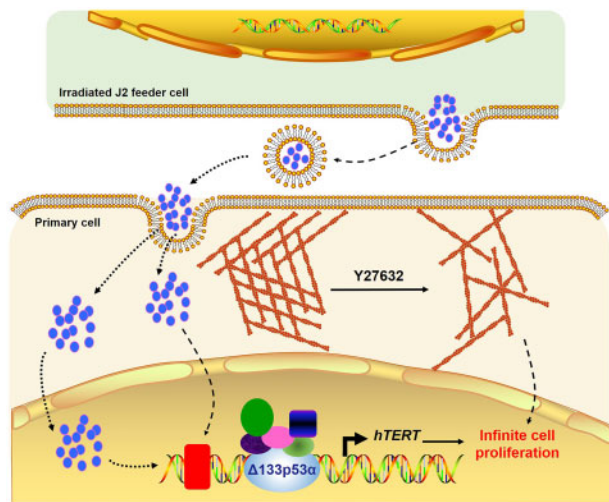

Figure 2. Potential mechanisms of cell immortalization in the CR-cell-culture model. Diffusible factor(s) released by irradiated feeder cells and the overespression of Δ133p53α contribute to human telomerase reverse transcriptase ($hTERT$) gene induction and telomerase-activity increase. Y27632 as the ROCK inhibitor can lead to the alteration of the cytoskeletal network for cell proliferation and perturb the Rb/p16 pathway to prevent cell senescence. The dotted arrows indicate that the mechanisms are unclear.

cell proliferation [23]. In addition, it was reported that the suppression of cell differentiation and upregulation of cell proliferation and adhesion including the downregulation of the TGF-β-signaling pathway as well as increased expression of

pT308Akt and pERK provided an essential basis contributing to the infinite growth of epithelial cells [38]. In all, the exact mechanism of combining feeder cells with the ROCK inhibitor in the CR to maintain cellular immortalization needs further study.

The combination of mouse fibroblast cells and Rho-kinase inhibitor Y27632 conditionally reprograms epithelial cells to an adult stem-cell-like state with the characteristic of long-term proliferation for maintaining tissue-specific lineage commitment [16]. It has been shown that Y27632 induces progenitor-clone formation by altering the expression of fundamental genes that are important for the formation of basal-cytoskeleton, cell–cell connections, and cell–extracellular matrix (ECM) interactions [39], which was verified to be regulated via $p63$ and $KLF11$ genes [40]. The latest research validated that increased β-catenin-dependent transcription, which was mediated by dephosphorylating and activating β-catenin as a result of increased interaction with PP2A but not the canonical β-catenin-activating signaling pathways, is essential for the CR of primary human epithelial cells to an undifferentiated state [41]. However, the exact mechanism by which the CR condition converts primary epithelial cells into a stem-cell-like state is still under study.

Applications of conditional reprogramming in cancer research

Both basic and preclinical cancer research is aimed at describing the biological characteristics of cancer and exploring relevant mechanisms to improve the understanding or prediction of cancer, which is dependent on model systems to generalize malignant states at the molecular and cellular levels. The

unique status of CR as a tumor-research model is closely related to the characteristics of maintaining intro-tumor heterogeneity and molecular features. CR essentially provides important *ex vivo* evidence to aid basic studies of cancer, including cancer biological characterization, the identification of antitumor targets, individualized drug screening, and elucidation of mechanisms about response to antitumor drugs (Figure 3 and Table 1).

Characterization of cancer biology

Given that the CR model faithfully mirrors primary cancerous cells, CR cells can be used to delineate the cellular, molecular, and genetic characterizations of various types of malignances. Primary nasal airway epithelial cells with gene knockout that were generated by combining a CR model with CRISPR-Cas9 technology verified that the expression of MUC18 in airway epithelial cells is conducive for airway inflammation in important
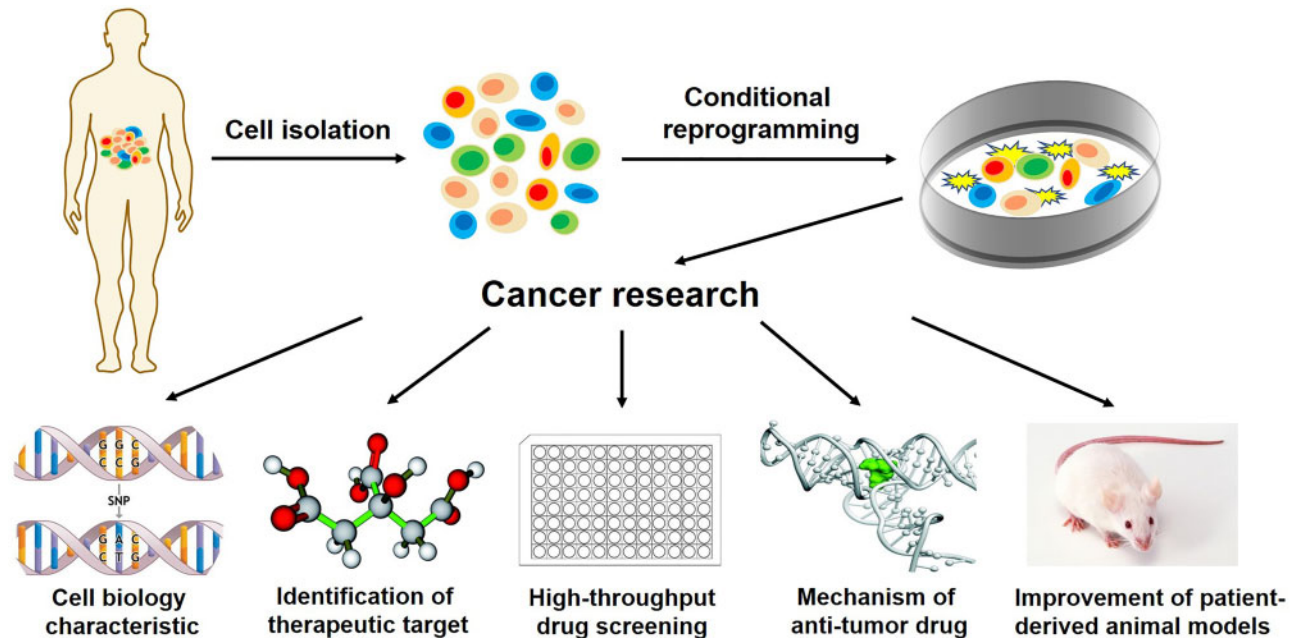

Figure 3. Applications of CR cells. The CR cells can be used for various applications, including cell-biology characterization, the identification of therapeutic targets, high-throughput drug-screening for exploring the mechanism of response to treatment, and improvement in patient-derived animal models, such as CR-cell-derived PDX models.

Table 1. Applications of conditional reprogramming cell-culture model

Tissue	Tumor types		Application	Reference
Prostate	Prostate cancer	Cell biology	Apoptosis, cell attachment, and hypoxic pathways	[42]
Prostate	Prostate cancer	Target identification	FGF23	[43]
Prostate	Prostate cancer	Drug screening	Navitoclax (Bcl-2 family inhibitor)	[22]
Prostate	Prostate cancer	Mechanism research	VMY: p53-induced apoptosis and autophagy	[44]
Bladder	Bladder cancer	Drug screening	Personalized drug-sensitivity screening	[11]
Pancreatic	PDAC	Target identification	MYC-ERCC3	[45]
Pancreatic	PDAC	Mechanism research	Enhanced activation of c-Myc induced by KRAS	[46]
Airway	RRP	Target identification	HPV-11 mutation	[47]
Airway	RRP	Drug screening	Vorinostat	[47]
Airway	RRP	Drug screening	Panobinostat, Dinaciclib, and Forskolin	[48]
Airway	NSCLC	Cell biology	NSCLC-selective signal plasticity	[49]
Airway	NSCLC	Drug screening	ALK and SRC inhibitor	[50]
Salivary gland	Mucoepidermoid carcinoma	Target identification	Fusion gene KRT14-KRT5	[51]
Salivary gland	Mucoepidermoid carcinoma	Drug screening	MK2206 (allosteric AKT inhibitor)	[51]
Salivary gland	ACC	Cell biology	SOX10$^+$Notch1$^+$FABP7$^+$CD133$^+$ cancer stem cell	[52]
Salivary gland	Salivary-gland cancer	Drug screening	Regorafenib	[53]
Breast	Breast cancer	Drug screening	ABT-263, SAL, DOX	[54]
Breast	Breast cancer	Drug screening	EGFR inhibitor and paclitaxel	[55]
Breast	Breast cancer	Mechanism research	Cell-cycle arrest, apoptosis, cellular stress	[56]
Spinal	Spinal ependymoma	Cell biology	HMGA1 and HOX genes	[57]
Skin	Melanoma	Cell biology	CD133 upregulates MMP2/MMP9, invasion, and metastasis	[58]

PDAC, pancreatic ductal adenocarcinoma; RRP, recurrent respiratory papillomatosis; NSCLC, non-small-cell lung cancer; ACC, adenoid cystic carcinoma.

microbial innate immune triggering responses and may be important in various airway diseases [59]. By making use of CR technology, Timofeeva et al. [42] suggested that a basal-like cell population might be considered the origin of prostate malignance and revealed the molecular and genetic events of prostate tumorigenesis with identification of genes involved in cellular development, growth, proliferation, and metabolism. By means of CR cells from adenoid cystic carcinoma (ACC), a population of SOX10$^+$/NOTCH1$^+$/FABP7$^+$ cells that also expressed CD133 was characterized as cancer stem-cell-like cells, and major signaling pathways that would be applied for ACC treatment in the future were delineated [52]. The CR technology was used to establish primary cultures from non-small-cell lung cancer (NSCLC) and drug–response profiling using these CR cells exposed the histopathological subtypes of NSCLC-selective signal plasticity and associated therapeutic weaknesses [49]. CR cells from pediatric spinal ependymoma were applied for revealing the significant positive correlation between highly expressed HMGA1 and HOX genes, and pathways related to HMGA1 expression included metabolic pathways, osteoclast differentiation, MAPK-signaling, and Neurotropin-signaling pathways [57]. CR of BAKP CD133$^+$ cells was applied to demonstrate that CD133 might play an essential role in invasion and metastasis by upregulating MMP2/MMP9, resulting in tumor progression and making it an attractive target for melanoma intervention [58].

Identification of cancer therapeutic targets

A tumor is an evolving disease with cellular characteristics that change and the appearance of relatively characteristic markers, including specific molecules and molecular interactions. A key role and foundation of preclinical cancer research is to aid the identification of cancer-specific targets that can be used for diagnosis, treatment, and prognosis analysis. CR cells play a leading role in the identification of tumor therapeutic targets because of the ability to maintain mutations and phenotypic heterogeneity unique to the primary tumors. Beglyarova et al. [45] demonstrated the MYC–ERCC3 interaction as a target for pancreatic ductal adenocarcinoma (PDAC) and a new mechanistic approach for the disruption of critical survival signaling in MYC-dependent cancers using the conditionally reprogrammed patient-derived pancreatic cancerous cells. The CR technique allowed Yuan et al. [47] to detect a unique and significant HPV-11 gene mutation in the viral genome due to the duplication of promoter and oncogene regions, which resulted in the observed progressive clinical features. A novel fusion gene, KRT14–KRT5, was discovered as a candidate therapeutic target for mucoepidermoid carcinoma and other salivary-gland neoplasms using CR technology [51]. Using the CR cell-culture model, Choudhary et al. [43] testified the expression of osteocytic FGF23 as an emerging drug target for prostate carcinoma and other ailments.

High-throughput drug screening and personalized agent discovery

In recent years, targeted drug therapies have been developed to inhibit abnormally expressed molecules as well as aberrant oncogenic signaling pathways and greatly improved therapeutic response in some tumor types. A major issue in cancer-drug development is the low success rate of new effective antitumor agents—one of the reasons why traditional preclinical models to screen new agents for clinical development have limited predictive value [60]. The choice of specific drug-screening models

was primarily based on the response of the models to agents already identified as being clinically effective [61], which means the models should be largely identical to the morphological and molecular characteristics of the primary tumors. In this regard, the availability of CR cells for high-throughput antitumor drugs screening is of great predictive value for personalized clinical treatment [62].

A small-scale high-throughput drug–response study with 306 emerging and clinical cancer drugs was carried out taking advantage of patient-derived conditionally reprogrammed prostate-cancer cells; it identified the Bcl-2 family inhibitor navitoclax as the most powerful malignance-specific drug for castration-resistant prostate cancer [22]. The CR-cells model from uncommon salivary-gland cancers maintaining the characteristic MYB translocation was applied to identify regorafenib as a potential anticancer drug for personalized treatment [53]. A chemosensitivity screening utilizing conditionally reprogrammed pulmonary tumor cells revealed that vorinostat is a new possible individualized therapeutic agent for HPV-11-positive recurrent respiratory papillomatosis (RRP) [47]. Recently, by means of CR and high-throughput screen platforms, Panobinostat, Dinaciclib, and Forskolin were evaluated and validated as the effective drugs for RRP therapy [48]. Alamri et al. [51] reported that MK2206 as the allosteric AKT inhibitor can inhibit the growth of mucoepidermoid carcinoma cells. By means of CR cells derived from lung-cancer patients with resistance to EGFR and ALK tyrosine kinase inhibitors, Crystal et al. [50] reported that combining ALK and MAPK kinase (MEK) inhibitors was effective in an ALK-positive resistant cancer with an activating mutation in MAP2K1, and combining EGFR and FGFR inhibitors was active in an EGFR-mutant resistant tumor with an FGFR3 mutation. The combination of ALK and SRC inhibition was also functional in ALK-driven lung cancer [50]. Drug-sensitivity screening performed on CR cells from different bladder-cancer-originating cultures showed diverse sensitivity to conventional chemotherapy, such as platinum-based drugs, taxane, topoisomerase inhibitors, proteasome inhibitors, and EGFR inhibitors [11]. Phyllodes tumor cells of the breast (PTB) obtained by CR technology were applied for evaluating the effectiveness of a range of antitumor agents; ABT-263, SAL, and DOX were found to be highly selective towards phyllodes spindle cells [54]. Based on the metaplastic breast-carcinoma (MBC) cell line with EGFR amplification from a patient using the CR method, Chung et al. [55] discovered that the combination of EGFR inhibitor and paclitaxel was a promising strategy for MBC with EGFR amplification.

Mechanism of response to antitumor drugs

Traditional chemotherapy is still the mainstay therapy modality for various types of cancers. Targeted anticancer therapy often leads to the preferential growth of drug-resistant subgroups due to selective pressure on cancerous cells, which can be inhibited through combined drug treatment. Targeting two or more onco-signaling pathways in combination is a promising strategy for malignance therapy. One of the important functions of CR is to elucidate the mechanisms associated with antitumor drugs, including the mechanisms of drug action, drug resistance, and drug-combination response. Pollock et al. [56] used CR cells to indicate that strigolactone analogs are hopeful anticancer candidates because they can specifically induce cell-cycle arrest, apoptosis, and cellular stress mediated by the downregulation of Cdc25C as well as cyclin B1, activating the stress-related MAPKs: p38 and JNK and inducing stress-related

genes in tumor cells; however, they have little effect on the survival and growth of normal cells. CR cells from prostate-cancer patients were useful for defining that p53-induced apoptotic and autophagic signaling pathways to mediate cell death in prostate-cancer cells was the possible mechanism of VMY-1–103 (VMY), which shows rapid and effective antitumor activities [44]. Using the CR-cells-culture method, Parasido et al. [46] identified that the key mechanism of primary nab-paclitaxel resistance in pancreatic ductal adenocarcinoma is the increasing stability of c-Myc and enhanced reverse activation of c-Myc target genes as a result of phosphorylation of c-Myc on serine 62 induced by the mutant oncogenic KRAS, and combining SMAP2 with nab-paclitaxel can not only significantly increase the sensitivity of nab-paclitaxel-resistant cells to nab-paclitaxel, but also reduce the level of c-MYC and lead to the redistribution of c-MYC from the nucleus to the cytoplasm.

Comparison with other tumor-research models

CR cells vs traditional cell lines

Since it is a difficult challenge to produce and maintain the long-term growth of tissue-derived primary cells in vitro as a result of anoikis, the traditionally established patient-cancer-derived cell lines have been the main model for cancer research, which have made great contributions to our current understanding of malignance molecular biology and laid an important foundation for the study of drug sensitivity and resistance [63]. However, these immortalized cell lines with properties that are different from the primary tumor at the genotypic and phenotypic levels with mRNA changes during continuous passages [64] have lost the functional and genotypic heterogeneity of primary malignances [65]. Besides, the cell–cell interactions that support the tumor survival and metastasis are lost in the absence of relevant components of the tumor microenvironment [66], which raises a key question about the extent to which cell lines generalize the biology of the tumor samples [67]. Moreover, most immortalized cancer-cell lines rely on the continued expression of strong exogenous oncogenes such as SV40 large T antigen [68], resulting in genomic instability and epigenetic modifications; however, CR cells do not undergo genetic manipulation and maintain lineage commitment as well as intra-tumor heterogeneity.

CR cells vs PDX models

The PDX models, which are immunodeficient mice engrafted with patients' tumor cells or tissues [60], faithfully conserve the genomic-expression patterns and tissue histological features of the original cancer at establishment and after serial passaging [69], making it highly translatable to the patients [70]. Unfortunately, high cost, slow growth, difficulty in being cultured long-term, and unsuitability for high-throughput platforms have been the challenges for the extensive use of PDX models as a tool for preclinical cancer research [71]. Meanwhile, because of the requirement for using immunocompromised mice to avoid xenograft rejection, current PDX models are difficult to use for evaluating the efficacy of immunotherapeutics, which is one of the powerful strategies for cancer therapy [72]. Compared with PDX models, CR cells can be used for rapid, high-throughput, and low-cost screening in vitro, but the culture system cannot fully simulate the environment and intercellular interaction necessary for tumor growth. In view of the advantages and disadvantages of CR cells and PDX models, combining these two technologies can make up for their shortcomings and has potential preclinical application value. CR PDX-derived stable explant cell lines [73] and CR-cell-derived xenografts [74] can realize rapid, low-cost expansion of cancer cells, genetic manipulation, and high-throughput chemosensitivity screens.

CR cells vs organoids

An organoid is an in vitro 3D cellular cluster completely derived from primary tissue, ESCs, or iPSCs, with the capability of self-renewal, self-organization, and displaying organ functions similar to that of primary tissue [75]. Similar to CR cells, an organoid can be reproduced for long periods of time (years) without genomic alterations [75] and it is readily applicable to high-throughput testing [76]. However, the lack of vascularization and limited nutrient supply are common growth obstacles that affect organoid maturation, and the effect of the degree of maturation of organoids on their potential for tumor-therapy research remains to be seen [77]. Palechor-Ceron et al. [78] found that CR tumor cells could form spheres and developed invasive processes by communicating with adjacent spheres, which indicated that combining CR technology and organoids may be a promising model for cancer research.

Current limitations of CR models and possible improvements

The CR-cell-culturing method, which enables the rapid and continuous growth of primary normal and cancerous epithelial cells from biological specimens without genetic manipulation and maintaining tumor heterogeneity, has generated tremendous interest in cell-biology studies and drug-sensitivity screening. Various types of conditionally reprogrammed primary tumor cells have been successfully established and applied for cancer research. However, some CR cells derived from malignant tumors were mostly non-malignant, such as CR cultures of NSCLC [79] and nasopharyngeal carcinoma (NPC) [80], with properties including lack of tumor-derived mutations, diploid copy number, mRNA-expression profiles, and morphology characteristics of non-malignant cells. It may be possible that the retention of cell–cell interaction is necessary for successful NSCLC and NPC tumor-cell cultures as reported for cultures from colorectal cancer [81] and retinoblastoma [82]. Appropriately modifying the existing protocol or developing new methodology is needed for the reproducible growth of certain kinds of tumor cells. For example, compared with the standard CR condition, a modified CR-culture method can preserve the pluripotent differentiation capacity of human bronchial epithelial basal cells including the differentiation ability to differentiate epithelial cells with cilia in air–liquid interface cultures well [83].

Another limitation of CR is that the mitotically inactivated fibroblast feeder cells would inhibit the growth of tumor-associated fibroblasts and stromal cells [8], which was observed decades ago [84] and allows the dominant propagation of epithelial carcinomas in the co-culture system, but it is difficult to evaluate the impact of stromal cells on tumor-cell growth and their effect on the tumor-cell response to treatments. During the stable passage of CR cells, small molecular substances can be developed to replace feeder cells to reduce their influence on the characteristics of cancer cells.

Besides, several important aspects of this cell-culture model have not yet been completely explored, such as the ability of a
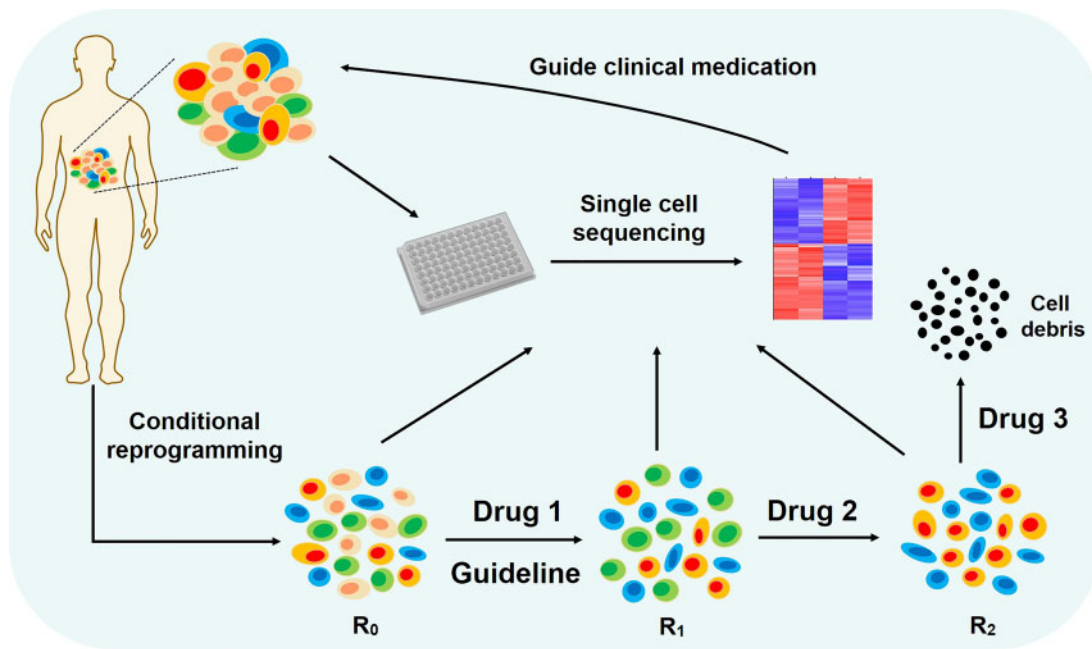
Figure 4. Precision-medicine potential of CR cells. The CR cells from primary tumor samples (R_0) are applied for drug susceptibility testing using first-line drugs recommended in clinical-practice guidelines to screen out cells that are resistant to clinical first-line medication. These resistant cells (R_1, R_2) are then used for screening other antitumor drugs until these cells are completely killed. Meanwhile, primary tumor cells, CR cells, and drug-resistant cells should be used for single-cell sequencing to identify the tumor genetic characteristics and guide clinical medication.

cell to expand, the extent of genetic and phenotypic drift during passage, and its permission for clonal growth and genetic manipulation [85].

Conclusions

A new age of precision medicine for preclinical-malignancy research is emerging, with human-based models at the center and patient-derived cells increasingly being applied as dominating discovery platforms. In this modern era of individualized oncology study and precision medicine, the CR primary tumor-cell-culture model is a promising tool for precision cancer medicine (Figure 4) and is increasingly used in translational cancer research including the study of cancer biological characteristics, the identification of therapeutic targets, personalized drug screening, and the elucidation of mechanisms about responses to antitumor drugs. There are some limitations that need to be improved in bridging primary cancerous cells with immortalized cell lines. Therefore, interdisciplinary collaboration will be essential to take full advantage of the CR-cells model as a potent tool for modern cancer research.

Authors' contributions

L.W.F. conceived of and designed the project. M.J.Z. contributed to the drafting of the manuscript and the editing of the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest

None declared.

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