

Use of conditional reprogramming cell, patient derived xenograft and organoid for drug screening for individualized prostate cancer therapy: Current and future perspectives (Review)

JESSICA CAO¹, WING C. CHAN² and MOSES S.S. CHOW³

¹College of Osteopathic Medicine of The Pacific, Western University of Health Sciences, Pomona, CA 91766-1854;

²City of Hope Comprehensive Cancer Center, City of Hope Medical Center, Duarte, CA 91010-3012;

³College of Pharmacy, Western University of Health Sciences, Pomona, CA 91766-1854, USA

Received August 20, 2021; Accepted January 14, 2022

DOI: 10.3892/ijo.2022.5342

Abstract. Prostate cancer mortality is ranked second among all cancer mortalities in men worldwide. There is a great need for a method of efficient drug screening for precision therapy, especially for patients with existing drug-resistant prostate cancer. Based on the concept of bacterial cell culture and drug sensitivity testing, the traditional approach of cancer drug screening is inadequate. The current and more innovative use of cancer cell culture and *in vivo* tumor models in drug screening for potential individualization of anti-cancer therapy is reviewed and discussed in the present review. An ideal screening model would have the ability to identify drug activity for the targeted cells resembling what would have occurred in the *in vivo* environment. Based on this principle, three available cell culture/tumor screening models for prostate cancer are reviewed and considered. The culture conditions, advantages and disadvantages for each model together with ideas to best utilize these models are discussed. The first screening model uses conditional reprogrammed cells derived from patient cancer cells. Although these cells are convenient to grow and use, they are likely to have different markers and characteristics from original tumor cells and

thus not likely to be informative. The second model employs patient derived xenograft (PDX) which resembles an *in vivo* approach, but its main disadvantages are that it cannot be easily genetically modified and it is not suitable for high-throughput drug screening. Finally, high-throughput screening is more feasible with tumor organoids grown from patient cancer cells. The last system still needs a large number of tumor cells. It lacks *in situ* blood vessels, immune cells and the extracellular matrix. Based on these current models, future establishment of an organoid data bank would allow the selection of a specific organoid resembling that of an individual's prostate cancer and used for screening of suitable anticancer drugs. This can be further confirmed using the PDX model. Thus, this combined organoid-PDX approach is expected to be able to provide the drug sensitivity testing approach for individualization of prostate cancer therapy in the near future.

Contents

1. Introduction
2. Anticancer chemotherapy screening for individualization of therapy
3. Conditional reprogramming (CR)
4. Patient derived xenograft (PDX) model
5. Organoid model
6. Summary and future perspective

Correspondence to: Dr Moses S.S. Chow, College of Pharmacy, Western University of Health Sciences, 309 E. Second St., Pomona, CA 91766-1854, USA
E-mail: msschow@westernu.edu

Abbreviations: ADT, androgen deprivation therapy; AR, androgen receptor; CR, conditional reprogramming; CTC, circulating tumor cell; ECM, extracellular matrix; mCRPC, metastatic castrate resistant prostate cancer; NOD, non-obese diabetic; OS, overall survival; PCa, prostate cancer; PDX, patient derived xenograft; PFS, progression-free survival; SCID, severe compromised immunodeficient

Key words: prostate cancer, drug testing, cell culture, organoid, individualization of therapy

1. Introduction

Prostate cancer (PCa) is one of the most common cancers in men worldwide and there is a great need for a method of accurately identifying drugs for individualized therapy, especially for those with existing drug-resistant prostate cancer. The American Cancer Society estimates >190,000 new cases and >33,000 mortalities from PCa in 2020 (1). Of newly diagnosed PCa, ~81% will be localized (2). The primary recommended treatment for newly diagnosed localized cancer is radiation, radical prostatectomy, brachytherapy, or active monitoring for those with low risk (3). Patients with localized but high-risk disease

are recommended to have surgery with lymph node dissection or androgen deprivation therapy (ADT) with radiation. Those with metastatic disease are recommended ADT and possibly radiation (3). Although treatment with ADT has high initial response rate, resistance will most likely occur within a year (4). Subsequently, these metastatic castrate resistant prostate cancers (mCRPC) are usually treated with androgen receptor targeted therapy, chemotherapy, immunotherapy or bone targeted therapy such as bisphosphonates, RANKL antibody, alpha-emitting calcium mimetic for osseous metastases (3-5).

Newly diagnosed mCRPC has a low 5-year survival rate of only ~30% (6). Although there are 6 chemotherapeutic agents approved for mCRPC, the average improvement in overall survival is only ~4.8 months (Table I) (7,8). In view of the poor survival outcomes of patients of mCRPC, more effective treatment modalities are needed.

One approach of improving treatment can be a personalized medicine treatment plan based on specific genetic lesions. In the past few decades, genotypic screening for mutated genes that lead to inherited predisposition to cancer have been developed for several conditions (9-11). For PCa, germline genetic testing is currently recommended if there is personal and family history, Ashkenazi Jewish ancestry and BRCA 1/2 and Lynch syndrome mutation (3). However, genetic testing in the absence of family history will provide a low yield for PCa (3). Furthermore, patients may not benefit from screening due to variants with uncertain significance, missed mutations, or lack of mutation-specific intervention (7). Testing for somatic mutation in tumors is used in the hope of identifying possible targets for therapy. However, this approach is limited by the uncertainty in predicting response based on mutations and the availability of interventions (8).

Another approach for personalized or individualized therapy is explored in the current review: Phenotypic or empiric drug screening. This is similar to the concept of antimicrobial susceptibility testing, in which specific antimicrobial agents that will be most effective for individual patients are identified (12). In cancer therapy, a living organoid biobank that matched the wide varieties of breast cancer phenotypes, including histopathology, hormone receptor status and oncogene activation, such as HER2, was described by Sachs *et al.* (13). Such an organoid biobank may allow *in vitro* phenotypic screening for cancer treatment. In addition, an organoid biobank can be a promising model for drug discovery, biological insights and translational and clinical research (14,15). Thus, a PCa model that can accurately represent an individual's tumor and its microenvironment will be useful for screening for effective therapies. Such a model is especially applicable for PCa clinical therapeutic strategy since it is a relatively slowly growing cancer.

In the current review, three potential screening models for prostate cancer will be reviewed and the ideal model that can apply to the treatment of mCRPC will be discussed: Conditional reprogramming cells, patient derived xenografts and organoids. Their experimental conditions, advantages and disadvantages and what could be improved to achieve the most effective screening for individualized PCa therapy are discussed below.

2. Anticancer chemotherapy screening for individualization of therapy

Model requirements. Individualizing anticancer chemotherapy is best facilitated via a model that can accurately represent the tumor of the patient and its progression, such as from being localized to exhibiting metastases. Such cancer screening models need to take into consideration the complexity of the tumor and its microenvironment to best replicate the *in vivo* environment *in vitro*. It is impossible with the current *in vitro* technology to completely mimic the *in vivo* conditions. However, the following features are considered essential and should be included in a representative *in vitro* model.

Heterogeneity of expression. PCa tumors are heterogeneous with variations in genetic abnormalities, gene expression, epigenetic regulation and responses to therapeutics (7). Prostate cells are also normally dependent on testosterone and have androgen receptors (AR) (16-19). Normal development as well as cancer growth and survival is effected by AR (20). While including all these variations in a dynamic manner is impossible with current *in vitro* technology, a good *in vitro* model should incorporate as a number of these characteristics as possible.

Cell types. The prostate gland is made of epithelial and stromal cell types. Epithelial cells include luminal and basal cells (21,22). Secretory products are released into the gland lumen by luminal cells. Luminal cells and ductal structure are derived from basal cells (23). Studies have suggested that luminal and basal cells are sporadically bipotent and have stem cell-like properties (24-26). Basal cells are more efficient at forming organoids and providing self-renewal, whereas luminal cells are more ready to differentiate into ducts and acini. PCa can be derived from both cell types (7,27,28) and also, rarely, from neuroendocrine cells (29-34). While each model may not need all of these cells, it is important that the type of cells that correspond to each individual's unique cancer is exhibited in a model.

Stromal interactions. There is a fibromuscular stroma consisting of smooth muscle, fibroblasts and elastic fibers. In addition, there are blood vessels, peripheral nerves, macrophages and white blood cells (29,34). A co-evolution is undergone by tumor cells and the associated stroma. Growth factors and proteases are released by cancer cells, causing angiogenesis and inflammation, activating the surrounding stroma, which in turn secretes additional growth factors, proteases and pro-migratory extracellular matrix (ECM) components (Table II) (35,36). The stromal cells are influenced by cancer cells to become more supportive of tumor progression, which can in turn, increase the malignancy of cancer cells or progression to becoming drug- and castration-resistant (35-37). Cancer cells are highly heterogeneous due to genomic instability and selection pressure from the microenvironment (37). Another phenomenon based in interaction with the immune system and the microenvironment is immune escape. It is the failure of the immune system to eliminate cancer, allowing it to continue developing into metastatic cancer (38). Tumor cells may lose their antigenicity through immune selection, lose

Table I. Efficacy of drugs for mCRPC.

Study title	Test substance	Compare substance	Primary endpoint	Indication	Increase in OS; Hazard ratio; P-value	(Refs.)
COU-AA301	Abiraterone/ Prednisolone	Placebo/ Prednisolone	OS	mCRPC after Docetaxel	3.9 months; 0.65; <0.0001	(113)
COU-AA302	Abiraterone/ Prednisolone	Placebo/ Prednisolone	OS, PFS	mCRPC before Docetaxel	Not reached (PFS 8.2 months; 0.75; 0.01)	(113)
TROPIC	Cabazitaxel	Mitoxantrone	OS	mCRPC after Docetaxel	2.4 months; 0.7; <0.0001	(113)
TAX327	Docetaxel	Mitoxantrone	OS	mCRPC	2.4 months; 0.76; P=0.009	(113)
AFFIRM	Enzalutamide	Placebo	OS	mCRPC after Docetaxel	4.8 months; 0.63; <0.0001	(113)
PREVAIL	Enzalutamide	Placebo	OS	mCRPC before Docetaxel	2.2 months; 0.7; <0.0001	(113)
ALSYMPCA	Radium-223	'Best supportive care'	OS	mCRPC before and after Docetaxel	3.6 months; 0.69; <0.0018	(113)
IMPACT	Sipuleucel-T	Placebo/ Prednisone	OS	mCRPC before Docetaxel	4.1 months; 0.78; 0.03	(114)

mCRPC, metastatic castrate resistant prostate cancer; OS, overall survival; PFS, progression-free survival.

Table II. Common components regulating prostate growth.

Author(s), year	Component	Function	(Refs.)
van Moorselaar and Voest, 2002	Vascular endothelial growth factor	Angiogenic factor	(35,130)
van Moorselaar and Voest, 2002	Basic fibroblast growth factor (FGF-2)	Angiogenic factor; stimulates endothelial cell division; pro-inflammatory	(35,130,131)
Zittermann <i>et al</i> , 2006	Hepatocyte growth factor/scatter factor	Stimulates cell growth	(35,132)
Lail-Trecker <i>et al</i> , 1998	Transforming growth factor (TGF- β)	Increases angiogenesis, stimulates stromal growth, inhibits epithelial cell growth, induces apoptosis	(35,133)
Blanchère <i>et al</i> , 2002	Insulin-like growth factor	Stimulates cell growth, blocks apoptosis	(35)
Chung <i>et al</i> , 2005	Interleukin-6	Promotes differentiation, apoptosis inhibition; pro-inflammatory; activating STAT3	(35,134)
Royuela <i>et al</i> , 2004	Keratinocyte growth factor (FGF-7)	Stimulates cell growth	(35,135)

their immunogenicity through expressing immunoinhibitory molecules, or by creating a suppressive microenvironment for immune cells (38,39). While it may not be currently possible to replicate all the stromal interactions with cancer cells, the components representing key signals and relevant factors released by the stromal cells should be added to the growth medium.

3. Conditional reprogramming (CR)

Primary cells in culture usually undergo senescence, a cessation of proliferation and changes in the metabolism and phenotype of the cells after a few passages (40). Thus, unlike microorganisms, direct culture and sensitivity approach

utilized for identifying specific antimicrobial agents may not work for cancer therapeutic screening. Although numerous immortal cancer cell lines, including PCa cell lines such as PC3, LnCap, DU145 have been developed and have been routinely used for initial drug development purposes, they may not be representative of the primary tumor and lack 3D structure and broad representation of different tumor types and subtypes (7,16-18,41-49).

In the past decade, long term proliferation without changing most of the fundamental genetic makeup and expression of cells has been made possible by a newly developed approach called conditional reprogramming (CR) (44,48,50). Establishment of cultures of almost all types of epithelial cells is enabled by CR. Considerable interest has been generated

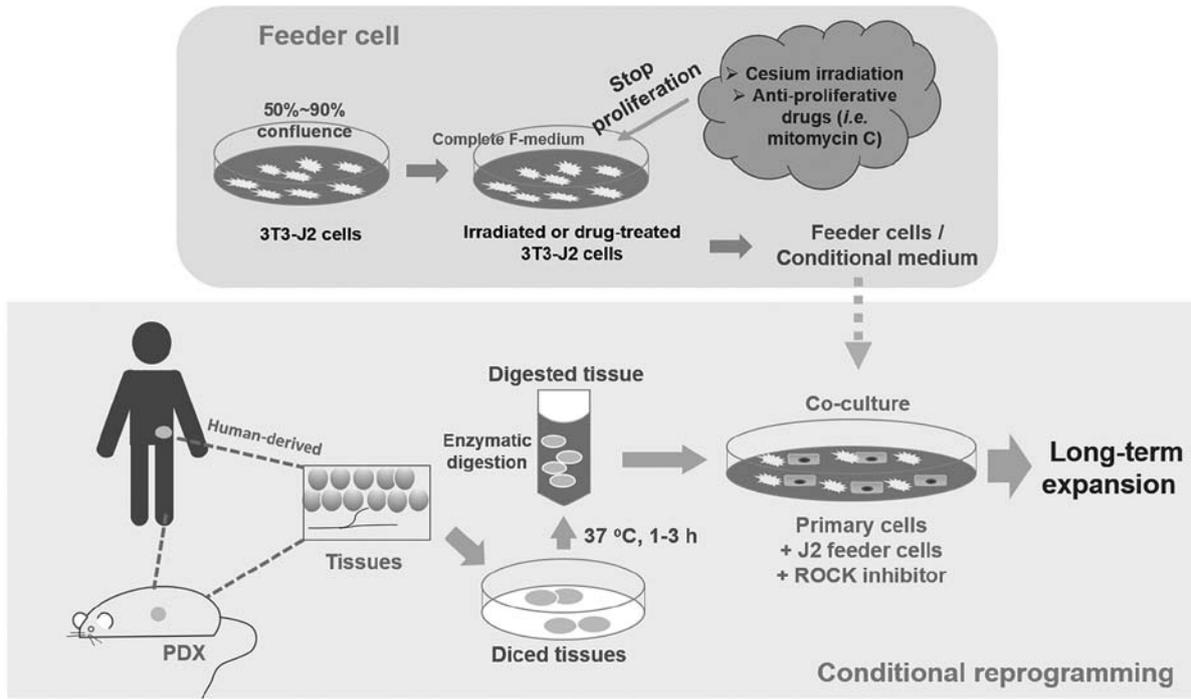


Figure 1. Overview of conditional reprogramming culture (48).

in its possible applications such as establishment of disease models, therapeutic drug assessment and new platforms for basic and translational research (48).

Culture condition. CR cells, which can be disaggregated human or patient derived xenograft (PDX) primary cells (48), are co-cultured with inactivated mouse 3T3-J2 fibroblasts as feeder cells and RHO-related protein kinase (ROCK) inhibitor Y-27632 (Fig. 1). [The digested cells will also need pathological evaluation to distinguish cancer cells from normal cells (48)]. J2 fibroblasts are given a dose of irradiation or treated with mitomycin C (2-4 $\mu\text{g/ml}$) to stop their proliferation. These cells are used as a feeder cell layer for physically contact with primary cells (51). Alternatively, a conditional medium with J2 feeder cell secreted factors can be used (44). Apoptosis and differentiation is inhibited by ROCK inhibitor Y-27632 (48). Stem-like characteristics and the ability to fully differentiate can be observed from this co-culture (40). The establishment of tissue cultures with this method appears to be highly efficient (40,44).

Advantages. The advantages of CR is that it is simple, rapid and has a high rate of success (88-100% success rate for some cells) (40,44,52-54). For example, the primary tumor or normal epithelial cells obtained from prostate surgery biopsy and undergoing CR culture can generate 2×10^6 cells or reach confluence in ~ 5 days (40). Cells can also proliferate for long periods while still maintaining much of the genetic makeup, gene expression and heterogeneity of the primary cells from the biopsy (40,55-57). Thus CR cells can allow for drug sensitivity screening and testing after 5 days (57-59). Culturing cells of carcinoma of the lung in a patient using conditional reprogramming and then determination of drug sensitivity, leading to the selection of an effective therapeutic agent, was reported

by Yuan *et al* (60). Such an approach can be an important advance for individualized therapy (48).

CR can be used to create 2D cultures, spheroids and organoids (40,48,52,53,55,61) and can be implanted into an animal to create PDX models (56,62). In reverse, cell cultures can also be propagated from PDX and organoid cultures (44). CR can also be used to create biobanks due to its ability to generate inexhaustible cell populations (40,44). The cells' ability to differentiate are restored by removal of the CR conditions (40,52,55,63), which allows the possibilities for regenerative medicine investigations (53).

Disadvantages and possible solutions. A drawback is that CR cells, when in a stem-like state, do not have the same characteristics as the primary cancers. Normally, prostate tissue has basal cell marker P63 and AR, but these are not expressed in CR primary human prostate cells (41,64). Some of the irradiated 3T3-J2 feeder cells are not arrested in cell proliferation, as they should be, and can transform to become malignant and gain cancer-like characteristics *in vivo* (65-67). ROCK inhibitor Y-27632 can also alter the actin cytoskeleton, which is involved in migration and invasion of tumor cells (55). Instead of irradiation, some studies inactivate 3T3-J2 feeder cells using mitomycin C, so there may be biological differences in CR (51,68,69). Thus, at present, CR cells are not suitable for modeling PCa due to the culture components having unwanted influences. More research could be done to optimize take rates as well.

In the future, several improvements will need to be made. To improve the take rate *in vitro*, combining CR with 3D culture to provide the best conditions for improved differentiation and recognition of normal cells was suggested by Liu *et al* (70). In addition, prevention of normal epithelial cell over proliferation, which could outcompete cancer cell proliferation, can be

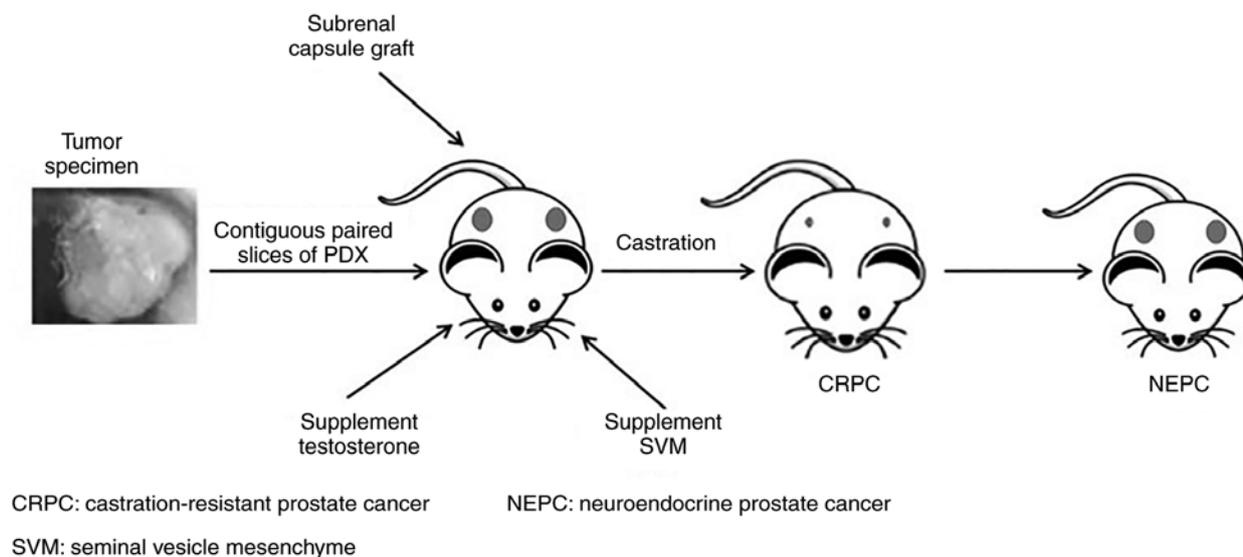


Figure 2. Overview of PDX development (16). PDX, patient derived xenograft; CRPC, castrate resistant prostate cancer; NEPC, Neuroendocrine prostate cancer.

improved by using human fibroblasts instead of mouse fibroblasts in CR. Using human fibroblasts does not support long term *in vitro* proliferation of non-cancer cells, such as normal airway and lung epithelial cells (48), so further research on this technique for PCa may improve PCa cell take rates.

A phenotypic evaluation and selection of tumor cells was suggested by Wu *et al* (48). Fluorescence-activated cell sorting can be used to identify certain cell markers specific to the tumor. Selecting for the tumor cells would prevent the overgrowth of normal cells, supporting improved proliferation and take rate of tumor cells. DNA sequencing and tumor-specific antibodies can also be used to select for tumor cells (48). Use of differentially expressed surface molecules could identify and help isolate specific cells, although more research is needed on this method and to describe the phenotypic profiles of specific cell types (29).

4. Patient derived xenograft model (PDX model)

A PDX model is established by transplanting patient tissue into immunocompromised mice, which can be athymic nude mice, severe compromised immunodeficient (SCID) mice, non-obese diabetic (NOD)-SCID mice and recombination-activating gene 2 (Rag2)-knockout mice (16,71). Recently, NSG (NOD. *Cg-Prkdcscid Il2rgtm1Wjl/SzJ*) mice are preferred because there is also a deficiency in innate immunity (72).

Culture or growth condition. Prostate tissue samples are usually transplanted sub-renal capsule and supplemented with testosterone and mouse seminal vesicle mesenchyme (16) (Fig. 2). Mouse seminal vesicle mesenchyme implanted along with PCa tissue is able to mimic the stromal micro-environment and androgen secretion to support prostate cell differentiation and proliferation (73,74). The ideal transplantation site is one similar to the origin of the tissue, but mice have limited capacity in the pelvic region (16). Instead, the tissue is transplanted under the renal capsule due to its rich vasculature (37,75,76).

Advantages. Similar tissue histology, heterogeneity, major markers and genetic profile and expression to individual human PCa are shown in PDX models. Due to these similar features, PDX can also predict metastatic potential and drug response of the human tumor, deeming it suitable for drug screening and validation (16,44,77-79). Addition of testosterone can increase the establishment and help growth of PCa (80); although establishment rates (10%) are still low (80,81). With testosterone supplementation and transplantation with seminal vesicle mesenchyme, increases of graft establishment to >90% have been reported (77,82). In addition, aggressiveness and growth of grafted tumors has been found to correlate with worse clinical outcomes (77).

Genome changes and the phenotypic markers similar to the primary cancers obtained from clinical PCa patients can be shown in PDX models. Loss of PTEN and RB1, amplification of AR and TMPRSS2-ERG fusion gene are often seen in PDX models (16). In addition, similar to the original tumor, AR, prostate specific antigen, prostate-specific membrane antigen and alpha-methylacyl-CoA-racemase are expressed in these models (16). Stromal and vascular components (7) and some interactions within the tumor microenvironment (16) between stromal components and epithelial tumor cells are also displayed in PDX models (44). Hormone dependence or independence can be partly simulated and the transition from hormone dependence to independence can be simulated (16,77,83,84). Most importantly, similar treatment responses in patients have been shown in PDX models (16,78,85,86). For example, the LuCaP PDX model series, which has 21 successfully established PDXs, is shown to display similar responses to the corresponding clinical patients (81). While there are a number of articles finding that PDX models correlate with clinical responses (86-88), there are few that describe PDX screening followed by a clinical trial, which is a co-clinical trial. Reviews by Gao and Chen (85) describe a good correlation in treatment responses between initial PDX screening and subsequently individual patients for a wide variety of cancers, but not prostate cancer. Some studies also include integration of genomic

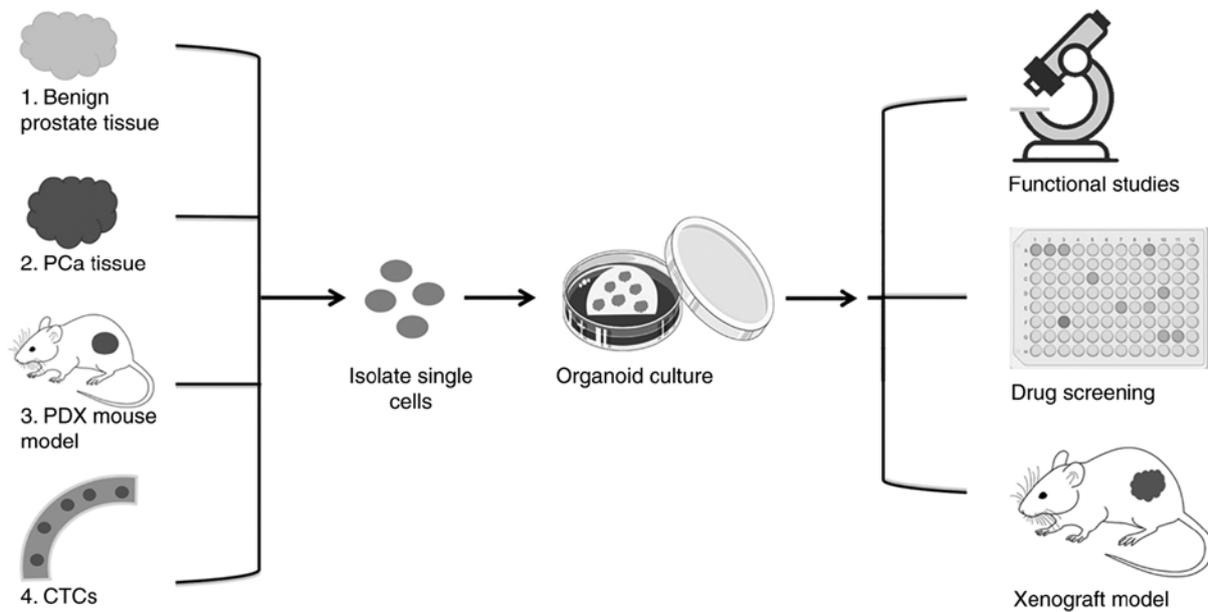


Figure 3. Different modalities of organoid establishment and applications. Organoids can be established from biopsies, PDX models and CTCs. Organoids can be used for genetic manipulation, gene function studies, drug screenings and establishment of xenografts (7). PDX, patient derived xenograft; CTC, circulating tumor cell; PCa, prostate cancer.

data with PDX model to identify precision treatment (89,90). For example, xenografts from patients with BRAF wild-type metastatic melanoma recapitulate the treatment response to digoxin plus trametinib in such patients (125).

Due to its similarities to clinical PCa, PDX can be a useful model for studying the biology and progression of PCa, validation of screening for effective therapies for individual cancers and drug development. PDX can be grown and passaged for long periods, allowing tumor progression and stages to be observed and used for drug and other therapy testing (16,81).

Disadvantages and possible solutions. Despite the advantages described above, one important disadvantage of the PDX model is the expense due to the high maintenance cost of mice and long (2-5 month) ‘incubation’ period (16,44).

The second disadvantage is that PDX models are not easily genetically modified or used for high-throughput drug screening assays (85). Development of cell lines from PDX models would allow easier high-throughput drugs screenings and genetic modification, but this is challenging due to overgrowth of stroma and limited differentiation potential, especially if it is under CR conditions (44,57). There is also a replacement of human stromal components with those of the mice (91) with time. Since PDX lacks human immune cells, the model is not suitable for immunotherapy studies.

To address the problem of immunotherapy study in the PDX models, human hematopoietic stem cells have been transplanted into the immunodeficient mice to create a human-like immune system (16,92,93). There are various approaches to improve the generation of the human hematopoietic/immune system and the reduction of graft vs. host disease (94). The technology is still evolving and the cost of producing humanized mice is substantial.

The third disadvantage is that fresh surgical or biopsy material is needed to establish a PDX (16,95). In the future,

the circulating tumor cells (CTCs) from the tumors that are released into the vasculature may be utilized. Collecting CTCs is like a ‘liquid biopsy’ that is safe and does not require an invasive procedure (96). However, there are a number of concerns which will need to be resolved. For example, CTCs often have undergone epithelial-to-mesenchymal transition, which has a downregulation of epithelial markers. In addition, epithelial cell adhesion molecule, which is used to isolate CTCs, may not be expressed in subtypes of cancer (16,97,98). Last, it is often difficult to isolate sufficient number of CTC for PDX and the lack of associated stromal cells may reduce engraftment potential.

The final disadvantage is that although PDXs can predict metastatic potential, the occurrence and development of PCa metastases cannot be simulated. Most models do not spontaneously metastasize (16). It is also well recognized that some primary tumors only have a minority of cells with metastatic ability (77). Shi *et al* and Nguyen *et al* further suggested that PDX models should match different stages of a patient's disease and that they be treated in parallel (16,81). Careful evaluation of histological cell types, signalers and receptors to match PDX and patient disease stage can be explored in future research.

In summary, despite the a number of suitable features with the PDX model, its long growth time, costly upkeep, biopsy sample requirement and the absence of a human stroma and a human immune system (unless humanized) are disadvantages. The model can be used for individualized evaluation of drug treatment but not for high throughput screening for therapeutic discovering and development.

5. Organoid model

Culture condition. An organoid is a 3D model of tissues *in vitro* that mimics the *in vivo* organ from which the cells originated.

Table III. Components of generic serum-free organoid media.

Author(s), year	Component	Function	(Refs.)
Gleave <i>et al</i> , 2020 Francis <i>et al</i> , 2013	R-spondin	Enhances Wnt signaling, which is for development and stem cell growth and differentiation	(7,136)
Gleave <i>et al</i> , 2020 Cook <i>et al</i> , 2007	Noggin	Bone morphogenic protein inhibitor that regulates and promotes patterned growth, budding and proliferation	(7,137)
Gleave <i>et al</i> , 2020 Jarrard <i>et al</i> , 1994 Sastry <i>et al</i> , 2006 Watanabe <i>et al</i> , 2007	Epithelial growth factor	Stimulates organoid growth and invasive nature and protects from apoptosis	(7,138-140)
Watanabe <i>et al</i> , 2007	Rho Kinase inhibitor Y-27632	Reduces dissociation-induced apoptosis	(140)
Gleave <i>et al</i> , 2020 Tojo <i>et al</i> , 2005	ALK5 inhibitor (A83-01)	Blocks transforming growth factor beta signaling so there is sustained proliferation	(7,141)
Gleave <i>et al</i> , 2020 Zhang <i>et al</i> , 2011	N-acetylcysteine	Protects cells from reactive oxygen species and nitrogen species	(7,142)
Gleave <i>et al</i> , 2020	Nicotinamide	Coenzyme in metabolism	(7)
Karthaus <i>et al</i> , 2014	Fibroblast growth factors (FGF 10 and FGF2)	Promote proliferation	(27)
Gu <i>et al</i> , 2011	B27 supplement	Promotes proliferation and growth	(143)

The cells can be from benign and cancerous enzymatically digested prostate tissue, circulating tumor cells (CTCs) or stem cells and from PDXs (7,17,85,96,99-101) (Fig. 3). Although CTCs are generally difficult to obtain, they are more abundant in metastatic cancers (102). PDXs can be a source for cells when there is limited patient cancer tissue.

Organoids are grown in Matrigel, a 3D gel made of basement membrane proteins, to support cells growth and differentiation. Generic serum-free media for all organoids with prostate-specific growth factors are also used (7) (Tables III, IV and V) (103-114). The complex matrix environment is set to be similar to that *in vivo*, to allow easy propagation of cells. Such conditions should provide more reliable drug testing responses (16,115).

Advantages. The utility of organoids in PCa research and therapy assessment not only allow high throughput drug testing and screening (95,116), but also can be useful to study basic biology in early stage cancers, identify drug targets and study drug resistance (44). Additionally, further research options can be explored in cryopreservation of organoids (19).

The key advantage is that the diverse histological and genetic features in primary noncancer or PCa tissues can be recapitulated in organoids. Specifically, the 3D culture system allows the self-organization of organoids and luminal and basal cell architecture and AR signaling can be retained (27). This model also can be genetically modified using several genome editing techniques (27,41,117). It can be used for *in vitro* studies and can be transplanted *in vivo* for xenografts studies (27,100,118). Notably, organoids can produce similar responses to therapies as those in patients (99).

Organoids derived from PDX tissues can be conveniently transplanted into mice for long term growth study (119,120). One specific advantage of PDX-derived organoids is improved cost efficiency, especially for high throughput drug screens, while PDX models are not practical (7,99). However, PDX tissue can be an additional source of tissue for organoid establishment when the primary tissue, is scarce. Lastly, the PDX-derived organoids are easier to genetically modify when compared to PDX (85).

Another practical advantage is that organoids can be detected within 2-3 days of plating while small cystic organoids are observed from luminal cells after 5-7 days (118). Organoids can have a high culture take rate for certain types of cells, e.g., more advanced or aggressive PCa, which allows side by side comparison and evaluation between organoids and human primary tumor (119).

Disadvantages and possible solutions. Despite the studies showing that organoids and patient cancers have similar responses to therapies (121-123), the translation organoid studies to clinical cases needs to be further verified (16). The first disadvantage of organoids is its lack of blood vessels, immune cells and its need for ECM substitutes (16). Since cancer cell growth is effected by the microenvironment, lack of such microenvironment could affect cell polarity, organization, migration and invasion (120).

To improve the microenvironment, there have been developments to include immune cells in the microenvironment with organoids. In addition, Richards *et al* co-cultured PCa with prostate fibromuscular stroma, increases organoid formation and directs organoid growth into branched acinar structure, similar to that seen *in vivo* (112). In addition, light-mediated

Table IV. Prostate-specific growth factors used in addition to serum-free organoid media.

Author(s), year	Component	Function	(Refs.)
Karthaus <i>et al.</i> , 2014 Chua <i>et al.</i> , 2014	Dihydrotestosterone (DHT)	Increases prostate luminal cell size and increases organoid expansion rate	(27,100)
Gleave <i>et al.</i> , 2020 Karthaus <i>et al.</i> , 2014	Prostaglandin E2 (PGE2)	Inflammatory mediator that also supports proliferation of prostate cell lines	(7,27)

Table V. Customized media additions for organoid culture.

Author(s), year	Component	Function	(Refs.)
Karthaus <i>et al.</i> , 2014	P38 MAPK inhibitor SB202190	Essential for human small intestinal cultures, was also included in prostate organoid media	(27,99,144)
Beshiri <i>et al.</i> , 2018 Sato <i>et al.</i> , 2011		A study showed that p38 inhibition decreased growth and survival, indicating that it might not be beneficial and needs further investigation	
Gleave <i>et al.</i> , 2020 Gstraunthaler <i>et al.</i> , 2013	Fetal bovine serum	Supplement for cell culture media, ill-defined, variable in each batch, may contain adverse factors and it may effect experimental consistency and outcomes Culture would not be serum free culture once fetal bovine serum is added	(7,106)

These may improve organoid growth but may need further exploration for the PCa model.

patterning technologies are used to create gradients of these biochemical cues to imitate the spatio-temporal patterns seen *in vivo* (124). It is hoped that these will be applicable to prostate organoids in the future (103-105).

A possible second disadvantage is having foreign factors such as the ECM substitute (16) and not having elements similar to the human microenvironment which may affect drug screening results. Although media components are able to effect organoid growth and allow recapitulation of important features of the original tumor, there may be an underrepresentation of biochemical signals, such as growth factors. Due to this, organoids may not completely reflect real-life growth. There is variability of components between fetal bovine batches (106). The role and differences of components between batches of Matrigel (107,108) is still unknown. Matrigel matrix and FBS also may have components that effect experimental outcomes. Steps are being made to more closely imitate the microenvironment and to improve the matrix. There is development of Matrigel matrix alternatives (109,110) and medium alternatives (111).

The third disadvantage is that although organoid cultures from mouse and human prostate tissue can be established with >95% efficiency (19). Due to having the small amount of starting tissue and normal epithelial overgrowth, the establishment rates are only 15-20% for advanced PCa (19,118). Puca *et al.* (95) also established 3/30 organoids. Among the prostate basal cell and luminal cell cancers, the establishment rates are ~70% from basal cell and 1-2% from luminal cell PCa (27). Long term propagation may be difficult for certain organoids, e.g., those basal cell-derived (41,99,100,118). To

improve and maintain long term growth, fresh medium <2 weeks old with well tested and stored growth factors and chemicals should be used. Histological examinations may also help detect the overgrowth of normal epithelial cells.

The fourth disadvantage at present is the problem with high heterogeneity. Growth rate and morphology of organoids from advanced PCa can vary between tumors of different patients as well as tumors from the same patient (118). There is a need to match patient, tumor and model, such as by using genomic analysis. A larger organoid biobank (7) can be useful in the future to provide genomic analysis improve the stratification of the organoids and correlate them with patient samples and data.

6. Summary and future perspective

Although the ideal drug-testing model for individualized cancer therapy screening is difficult to construct or establish, of the current 3 models, the PDX model and organoid model are more suited for use in culture and drug sensitivity testing for individualized PCa therapy (Table VI). There is continuing research to mimic the microenvironment more closely, which includes immune cells and fibromuscular stroma. Using light-mediated patterning technologies to mimic patterns of biochemical cues can further improve these models (112,124). Cell cultures could be the first step to explore if the immune cells are able to react to the target cells (by killing and/or elaboration of cytokines) that are supposed to express the appropriate antigen.

Despite the existing shortcomings associated with the PDX and organoid models, further development of a biobank

Table VI. Key advantages and disadvantages of cancer models.

Model	Advantages	Disadvantages
CR	<ul style="list-style-type: none"> - Simple, rapid - Maintains most characteristics of primary cells - Used for drug and therapy screening, testing, discovery - Used in 2D cultures, spheroids, organoids, PDXs - Can be propagated from primary cells, PDX and organoid cultures - Can create biobanks - Removal of conditions of CR restores cell's ability to differentiate 	<ul style="list-style-type: none"> - Difficulty in culturing primary cells. Normal cells outgrew cancer cells (There have been some successes in developing methods for selection of cancer cells) - Cells in stem-like state do not have same characteristics as some cancers (lack P63 and androgen receptor) - Feeder cells can become malignant - RHO-related protein kinase inhibitor can alter the actin backbone - Different methods in inactivating feeder cells may effect results
PDX	<ul style="list-style-type: none"> - Similar characteristics to PCa - Can predict metastatic potential and response of human tumor - Displays architecture and interactions - Simulates hormone dependence or independence - Used to study biology, progression of PCa, screen for therapies and drug development 	<ul style="list-style-type: none"> - High cost and long incubation - Cannot simulate occurrence and development of PCa metastases - Not easily genetically modified - Low-throughput drug screenings - Low graft establishment. Growth of PDX reflected the aggressiveness of the clinical tumor - Replacement of human stromal components with those of mice. (There is research to address this) - The circulating tumor cell method of attaining cells may result in obtaining cells that no longer resemble the primary tumor
Organoid	<ul style="list-style-type: none"> - Recapitulate characteristics, architecture and signaling in PCa tissues - Genetically modifiable - Can be derived from PDX tissues and can also establish PDXs - High-throughput drug screens - Rapid establishment - Used for basic biology, high-throughput drug screenings and biobanks 	<ul style="list-style-type: none"> - Lack of blood vessels, immune cells and extracellular matrix substitutes (there is research to address this) - There is an underrepresentation of biochemical signals (there is research to address this) - Culture components still need to be confirmed for their effects - Low efficiencies of establishment and difficulties in long term propagation - Underrepresentation in biobanks

CR, conditional reprogramming; PDX, patient derived xenograft; PCa, prostate cancer.

with cryopreserved organoids can be an important step to overcome the shortcomings for practical application. Together with careful histological examination, DNA sequencing and tumor immunophenotyping can be used to characterize the tumor sample of a given patient and match it with a specific organoid in the biobank. Once the organoid is matched and selected, screening of several potentially effective compounds can be performed to identify the best therapeutic candidate. Afterwards, further verification of *in vivo* efficacy can be conducted with the PDX models. This approach can be applied to existing approved drugs or to investigational agents. This combined organoid-PDX approach should be especially applicable for PCa, which is usually a more slowly progressing

cancer that can benefit from such culture, drug sensitivity and verification testing before selecting the drug or combination for patient treatment. Such culture/drug sensitivity testing may be especially useful for drug resistant cancer. While there is no published success story of the proposed approach from organoid to PDX and subsequent confirmation with a clinical trial, there are successes from PDX to patient efficacy as well as potential use of genetic mouse for drug screening (13,86-88,125-129). In view of the lack of practicality of using PDX or other mouse models for rapid drug screening, incorporation of the initial step using organoids can be a distinct advantage. Future exploration with pre-clinical trials using combined organoid and PDX models may pave the way toward discovering novel

agents, repurposing FDA approved drugs or specific combinations or new sequencing of agents for precision treatment of resistant prostate cancer.

Acknowledgements

The authors thank Ms. Yuna Kwon, College of Pharmacy, Western University of Health Sciences for her assistance on manuscript submission.

Funding

This work was supported by the Western University of Health Sciences Summer Research Fellowship (grant no. 20/SSR/011), 2020.

Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

JC gathered articles regarding tumor models and evaluated their possible use as a cancer model for improving individualized therapy. WCC read, suggested edits and approved the final manuscript. MSSC provided the concept of the topic for review and edit each draft. Data authentication is not applicable. All authors reviewed and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Siegel RL, Miller KD and Jemal A: Cancer statistics, 2020. *CA Cancer J Clin* 70: 7-30, 2020.
- Stanford JL, Feng Z, Hamilton AS, Gilliland FD, Stephenson RA, Eley JW, Albertsen PC, Harlan LC and Potosky AL: Urinary and sexual function after radical prostatectomy for clinically localized prostate cancer: The prostate cancer outcomes study. *JAMA* 283: 354-360, 2000.
- Mohler JL, Antonarakis ES, Armstrong AJ, D'Amico AV, Davis BJ, Dorff T, Eastham JA, Enke CA, Farrington TA, Higano CS, *et al*: Prostate cancer, version 2.2019, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* 17: 479-505, 2019.
- Nuhn P, De Bono JS, Fizazi K, Freedland SJ, Grilli M, Kantoff PW, Sonpavde G, Sternberg CN, Yegnasubramanian S and Antonarakis ES: Update on systemic prostate cancer therapies: Management of metastatic castration-resistant prostate cancer in the era of precision oncology. *Eur Urol* 75: 88-99, 2019.
- Dorff TB and Agarwal N: Bone-targeted therapies to reduce skeletal morbidity in prostate cancer. *Asian J Androl* 20: 215-220, 2018.
- Kelly SP, Anderson WF, Rosenberg PS and Cook MB: Past, current, and future incidence rates and burden of metastatic prostate cancer in the United States. *Eur Urol Focus* 4: 121-127, 2018.
- Gleave AM, Ci X, Lin D and Wang Y: A synopsis of prostate organoid methodologies, applications, and limitations. *Prostate* 80: 518-526, 2020.
- Cheng HH, Sokolova AO, Schaeffer EM, Small EJ and Higano CS: Germline and somatic mutations in prostate cancer for the clinician. *J Natl Compr Canc Netw* 17: 515-521, 2019.
- Lucas AL, Frado LE, Hwang C, Kumar S, Khanna LG, Levinson EJ, Chabot JA, Chung WK and Frucht H: BRCA1 and BRCA2 germline mutations are frequently demonstrated in both high-risk pancreatic cancer screening and pancreatic cancer cohorts. *Cancer* 120: 1960-1967, 2014.
- Couch FJ, Nathanson KL and Offit K: Two decades after BRCA: Setting paradigms in personalized cancer care and prevention. *Science* 343: 1466-1470, 2014.
- Nishikawa T, Maemura K, Hirata I, Matsuse R, Morikawa H, Toshina K, Murano M, Hashimoto K, Nakagawa Y, Saitoh O, *et al*: A simple method of detecting K-ras point mutations in stool samples for colorectal cancer screening using one-step polymerase chain reaction/restriction fragment length polymorphism analysis. *Clin Chim Acta* 318: 107-112, 2002.
- Bayot ML and Bragg BN: Antimicrobial Susceptibility Testing. In: *StatPearls* [Internet]. Treasure Island, FL: StatPearls Publishing; 2020 [cited Jun 26, 2020]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK539714/>.
- Sachs N, de Ligt J, Kopper O, Gogola E, Bounova G, Weeber F, Balgobind AV, Wind K, Gracanin A, Begthel H, *et al*: A living biobank of breast cancer organoids captures disease heterogeneity. *Cell* 172: 373-386.e10, 2018.
- Weeber F, Ooft SN, Dijkstra KK and Voest EE: Tumor organoids as a pre-clinical cancer model for drug discovery. *Cell Chem Biol* 24: 1092-1100, 2017.
- Muthuswamy SK: Organoid models of cancer explode with possibilities. *Cell Stem Cell* 22: 290-291, 2018.
- Shi C, Chen X and Tan D: Development of patient-derived xenograft models of prostate cancer for maintaining tumor heterogeneity. *Transl Androl Urol* 8: 519-528, 2019.
- Namekawa T, Ikeda K, Horie-Inoue K and Inoue S: Application of prostate cancer models for preclinical study: Advantages and limitations of cell lines, patient-derived xenografts, and three-dimensional culture of patient-derived cells. *Cells* 8: 74, 2019.
- Cunha GR, Donjacour AA, Cooke PS, Mee S, Bigsby RM, Higgins SJ and Sugimura Y: The endocrinology and developmental biology of the prostate. *Endocr Rev* 8: 338-362, 1987.
- Drost J, Karthaus WR, Gao D, Driehuis E, Sawyers CL, Chen Y and Clevers H: Organoid culture systems for prostate epithelial tissue and prostate cancer tissue. *Nat Protoc* 11: 347-358, 2016.
- Heinlein CA and Chang C: Androgen receptor in prostate cancer. *Endocr Rev* 25: 276-308, 2004.
- Robinson EJ, Neal DE and Collins AT: Basal cells are progenitors of luminal cells in primary cultures of differentiating human prostatic epithelium. *Prostate* 37: 149-160, 1998.
- Barclay WW, Woodruff RD, Hall MC and Cramer SD: A system for studying epithelial-stromal interactions reveals distinct inductive abilities of stromal cells from benign prostatic hyperplasia and prostate cancer. *Endocrinology* 146: 13-18, 2005.
- Kurita T, Medina RT, Mills AA and Cunha GR: Role of p63 and basal cells in the prostate. *Development* 131: 4955-4964, 2004.
- Kwon OJ, Zhang L and Xin L: Stem Cell Antigen-1 identifies a distinct androgen-independent murine prostatic luminal cell lineage with bipotent potential. *Stem Cells* 34: 191-202, 2016.
- Shibata M, Epsi NJ, Xuan S, Mitrofanova A and Shen MM: Bipotent progenitors do not require androgen receptor for luminal specification during prostate organogenesis. *Stem Cell Reports* 15: 1026-1036, 2020.
- Ousset M, Van Keymeulen A, Bouvencourt G, Sharma N, Achouri Y, Simons BD and Blanpain C: Multipotent and unipotent progenitors contribute to prostate postnatal development. *Nat Cell Biol* 14: 1131-1138, 2012.
- Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, van Boxtel R, Wongvipat J, Dowling CM, Gao D, Begthel H, Sachs N, *et al*: Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* 159: 163-175, 2014.
- Choi N, Zhang B, Zhang L, Ittmann M and Xin L: Adult murine prostate basal and luminal cells are self-sustained lineages that can both serve as targets for prostate cancer initiation. *Cancer Cell* 21: 253-265, 2012.

29. Liu AY and True LD: Characterization of prostate cell types by CD cell surface molecules. *Am J Pathol* 160: 37-43, 2002.
30. Hudson DL: Epithelial stem cells in human prostate growth and disease. *Prostate Cancer Prostatic Dis* 7: 188-194, 2004.
31. Zenzmaier C, Untergasser G and Berger P: Aging of the prostate epithelial stem/progenitor cell. *Exp Gerontol* 43: 981-985, 2008.
32. Di Sant'Agnese PA: Neuroendocrine cells of the prostate and neuroendocrine differentiation in prostatic carcinoma: A review of morphologic aspects. *Urology* 51 (5A Suppl): S121-S124, 1998.
33. Abrahamsson PA: Neuroendocrine differentiation in prostatic carcinoma. *Prostate* 39: 135-148, 1999.
34. Prostate gland [Internet]. Kenhub. [cited Sep 7, 2020]. Available from: <https://www.kenhub.com/en/library/anatomy/the-prostate-gland>.
35. Chung LW, Baseman A, Assikis V and Zhau HE: Molecular insights into prostate cancer progression: The missing link of tumor microenvironment. *J Urol* 173: 10-20, 2005.
36. Mueller MM and Fusenig NE: Friends or foes-bipolar effects of the tumour stroma in cancer. *Nat Rev Cancer* 4: 839-849, 2004.
37. Wang R, Xu J, Juliette L, Castilleja A, Love J, Sung SY, Zhau HE, Goodwin TJ and Chung LW: Three-dimensional co-culture models to study prostate cancer growth, progression, and metastasis to bone. *Semin Cancer Biol* 15: 353-364, 2005.
38. Igney FH and Kramer PH: Immune escape of tumors: Apoptosis resistance and tumor counterattack. *J Leukoc Biol* 71: 907-920, 2002.
39. Beatty GL and Gladney WL: Immune escape mechanisms as a guide for cancer immunotherapy. *Clin Cancer Res* 21: 687-692, 2015.
40. Liu X, Ory V, Chapman S, Yuan H, Albanese C, Kallakury B, Timofeeva OA, Nealon C, Dakic A, Simic V, *et al*: ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. *Am J Pathol* 180: 599-607, 2012.
41. Drost J, van Jaarsveld RH, Ponsioen B, Zimmerlin C, van Boxtel R, Buijs A, Sachs N, Overmeer RM, Offerhaus GJ, Begthel H, *et al*: Sequential cancer mutations in cultured human intestinal stem cells. *Nature* 521: 43-47, 2015.
42. Toivanen R, Taylor RA, Pook DW, Ellem SJ and Risbridger GP: Breaking through a roadblock in prostate cancer research: An update on human model systems. *J Steroid Biochem Mol Biol* 131: 122-131, 2012.
43. Nupponen NN, Hyytinen ER, Kallioniemi AH and Visakorpi T: Genetic alterations in prostate cancer cell lines detected by comparative genomic hybridization. *Cancer Genet Cytogenet* 101: 53-57, 1998.
44. Palechor-Ceron N, Krawczyk E, Dakic A, Simic V, Yuan H, Blancato J, Wang W, Hubbard F, Zheng YL, Dan H, *et al*: Conditional reprogramming for patient-derived cancer models and next-generation living biobanks. *Cells* 8: 1327, 2019.
45. Dasgupta P, Baade PD, Aitken JF, Ralph N, Chambers SK and Dunn J: Geographical variations in prostate cancer outcomes: A systematic review of International evidence. *Front Oncol* 9: 238, 2019.
46. Parrinello S, Samper E, Krtolica A, Goldstein J, Melov S and Campisi J: Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. *Nat Cell Biol* 5: 741-747, 2003.
47. Panchision DM: The role of oxygen in regulating neural stem cells in development and disease. *J Cell Physiol* 220: 562-568, 2009.
48. Wu X, Wang S, Li M, Li J, Shen J, Zhao Y, Pang J, Wen Q, Chen M, Wei B, *et al*: Conditional reprogramming: Next generation cell culture. *Acta Pharm Sin B* 10: 1360-1381, 2020.
49. Sharpless NE and DePinho RA: The mighty mouse: Genetically engineered mouse models in cancer drug development. *Nat Rev Drug Discov* 5: 741-754, 2006.
50. Chapman S, Liu X, Meyers C, Schlegel R and McBride AA: Human keratinocytes are efficiently immortalized by a Rho kinase inhibitor. *J Clin Invest* 120: 2619-2626, 2010.
51. Hynds RE, Ben Aissa A, Gowers KHC, Watkins TBK, Bosshard-Carter L, Rowan AJ, Veeriah S, Wilson GA, Quezada SA, Swanton C, *et al*: Expansion of airway basal epithelial cells from primary human non-small cell lung cancer tumors. *Int J Cancer* 143: 160-166, 2018.
52. Suprynowicz FA, Upadhyay G, Krawczyk E, Kramer SC, Hebert JD, Liu X, Yuan H, Cheluvvaraju C, Clapp PW, Boucher RC Jr, *et al*: Conditionally reprogrammed cells represent a stem-like state of adult epithelial cells. *Proc Natl Acad Sci USA* 109: 20035-20040, 2012.
53. Suprynowicz FA, Kamonjoh CM, Krawczyk E, Agarwal S, Wellstein A, Agboke FA, Choudhury S, Liu X and Schlegel R: Conditional cell reprogramming involves non-canonical β -catenin activation and mTOR-mediated inactivation of Akt. *PLoS One* 12: e0180897, 2017.
54. Sugaya M, Takenoyama M, Osaki T, Yasuda M, Nagashima A, Sugio K and Yasumoto K: Establishment of 15 cancer cell lines from patients with lung cancer and the potential tools for immunotherapy. *Chest* 122: 282-288, 2002.
55. Liu X, Krawczyk E, Suprynowicz FA, Palechor-Ceron N, Yuan H, Dakic A, Simic V, Zheng YL, Sripathan P, Chen C, *et al*: Conditional reprogramming and long-term expansion of normal and tumor cells from human biospecimens. *Nat Protoc* 12: 439-451, 2017.
56. Timofeeva OA, Palechor-Ceron N, Li G, Yuan H, Krawczyk E, Zhong X, Liu G, Upadhyay G, Dakic A, Yu S, *et al*: Conditionally reprogrammed normal and primary tumor prostate epithelial cells: A novel patient-derived cell model for studies of human prostate cancer. *Oncotarget* 8: 22741-22758, 2017.
57. Borodovsky A, McQuiston TJ, Stetson D, Ahmed A, Whitston D, Zhang J, Grondine M, Lawson D, Challberg SS, Zinda M, *et al*: Generation of stable PDX derived cell lines using conditional reprogramming. *Mol Cancer* 16: 177, 2017.
58. Saeed K, Rahkama V, Eldfors S, Bychkov D, Mpindi JP, Yadav B, Paavolainen L, Aittokallio T, Heckman C, Wennerberg K, *et al*: Comprehensive drug testing of patient-derived conditionally reprogrammed cells from castration-resistant prostate cancer. *Eur Urol* 71: 319-327, 2017.
59. Vondálová Blanařová O, Šafaříková B, Herůdková J, Krkoška M, Tománková S, Kahounová Z, Anděra L, Bouchal J, Kharaishvili G, Král M, *et al*: Cisplatin or LA-12 enhance killing effects of TRAIL in prostate cancer cells through Bid-dependent stimulation of mitochondrial apoptotic pathway but not caspase-10. *PLoS One* 12: e0188584, 2017.
60. Yuan H, Myers S, Wang J, Zhou D, Woo JA, Kallakury B, Ju A, Bazylewicz M, Carter YM, Albanese C, *et al*: Use of reprogrammed cells to identify therapy for respiratory papillomatosis. *N Engl J Med* 367: 1220-1227, 2012.
61. Brown DD, Dabbs DJ, Lee AV, McGuire KP, Ahrendt GM, Bhargava R, Davidson NE, Brufsky AM, Johnson RR, Oesterreich S and McAuliffe PF: Developing in vitro models of human ductal carcinoma in situ from primary tissue explants. *Breast Cancer Res Treat* 153: 311-321, 2015.
62. Ellis L, Ku S, Li Q, Azabdaftari G, Seliski J, Olson B, Netherby CS, Tang DG, Abrams SI, Goodrich DW and Pili R: Generation of a C57BL/6 MYC-Driven Mouse Model and Cell Line of Prostate Cancer. *Prostate* 76: 1192-1202, 2016.
63. Jensen TJ, Foster C, Sayej W and Finck CM: Conditional reprogramming of pediatric human esophageal epithelial cells for use in tissue engineering and disease investigation. *J Vis Exp* 121: e55243, 2017.
64. Tricoli L, Naem A, Parasido E, Mikhael JP, Choudhry MU, Berry DL, Abdelgawad IA, Lee RJ, Feldman AS, Ihemelandu C, *et al*: Characterization of the effects of defined, multidimensional culture conditions on conditionally reprogrammed primary human prostate cells. *Oncotarget* 9: 2193-2207, 2017.
65. Serrano-Heras G, Domínguez-Berzosa C, Collantes E, Guadalajara H, García-Olmo D and García-Olmo DC: NIH-3T3 fibroblasts cultured with plasma from colorectal cancer patients generate poorly differentiated carcinomas in mice. *Cancer Lett* 316: 85-90, 2012.
66. Yu F, Lu Y, Tao L, Jiang YY, Lin DC, Wang L, Petersson F, Yoshiyama H, Koeffler PH, Goh BC and Loh KS: Non-malignant epithelial cells preferentially proliferate from nasopharyngeal carcinoma biopsy cultured under conditionally reprogrammed conditions. *Sci Rep* 7: 17359, 2017.
67. Yu F, Hsieh W, Petersson F, Yang H, Li Y, Li C, Low SW, Liu J, Yan Y, Wang DY and Loh KS: Malignant cells derived from 3T3 fibroblast feeder layer in cell culture for nasopharyngeal carcinoma. *Exp Cell Res* 322: 193-201, 2014.
68. Zhao W, Liu K, Sun Z, Wang L, Liu B, Liu L, Qu X, Cao Z, Sun J and Chai J: Application research of individualized conditional reprogramming system to guide treatment of gastric cancer. *Front Oncol* 11: 709511, 2021.
69. Dong Y, Wang J, Ji W, Zheng M, Wang P, Liu L and Li S: Establishment and preclinical application of conditional reprogramming culture system for laryngeal and hypopharyngeal carcinoma. *Front Cell Dev Biol* 9: 744969, 2021.

70. Liu X, Krawczyk E, Timofeeva O, Palechor-Ceron N, Dakic A, Simic V, Kallakury B, Dritschilo A and Schlegel R: Functional analysis for cancer precision medicine using patient-derived 2D and 3D cell models. *Cancer Res* 76 (Suppl 14): S4256, 2016.
71. Morton CL and Houghton PJ: Establishment of human tumor xenografts in immunodeficient mice. *Nat Protoc* 2: 247-250, 2007.
72. Cao X, Shores EW, Hu-Li J, Anver MR, Kelsail BL, Russell SM, Drago J, Noguchi M, Grinberg A, Bloom ET, *et al*: Defective lymphoid development in mice lacking expression of the common cytokine receptor γ chain. *Immunity* 2: 223-238, 1995.
73. Govindaraj V, Arya SV and Rao AJ: Differential action of glyco-protein hormones: Significance in cancer progression. *Horm Cancer* 5: 1-10, 2014.
74. Lawrence MG, Taylor RA, Toivanen R, Pedersen J, Norden S, Pook DW, Frydenberg M; Australian Prostate Cancer BioResource, Papargiris MM, Niranjana B, *et al*: A preclinical xenograft model of prostate cancer using human tumors. *Nat Protoc* 8: 836-848, 2013.
75. McLean DT, Strand DW and Ricke WA: Prostate cancer xenografts and hormone induced prostate carcinogenesis. *Differentiation* 97: 23-32, 2017.
76. Lam HM, Nguyen HM and Corey E: Generation of prostate cancer patient-derived xenografts to investigate mechanisms of novel treatments and treatment resistance. *Methods Mol Biol* 1786: 1-27, 2018.
77. Lin D, Wyatt AW, Xue H, Wang Y, Dong X, Haegert A, Wu R, Brahmabhatt S, Mo F, Jong L, *et al*: High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. *Cancer Res* 74: 1272-1283, 2014.
78. Williams SA, Anderson WC, Santaguida MT and Dylla SJ: Patient-derived xenografts, the cancer stem cell paradigm, and cancer pathobiology in the 21st century. *Lab Invest* 93: 970-982, 2013.
79. Wu CH, Yang CY, Wang L, Gao HX, Rakhshandehroo T, Afghani S, Pincus L, Balassanian R, Rubenstein J, Gill R, *et al*: Cutaneous T-cell lymphoma PDX drug screening platform identifies cooperation between inhibitions of PI3K α/δ and HDAC. *J Invest Dermatol* 141: 364-373, 2021.
80. Russell PJ, Russell P, Rudduck C, Tse BW, Williams ED and Raghavan D: Establishing prostate cancer patient derived xenografts: Lessons learned from older studies. *Prostate* 75: 628-636, 2015.
81. Nguyen HM, Vessella RL, Morrissey C, Brown LG, Coleman IM, Higano CS, Mostaghel EA, Zhang X, True LD, Lam HM, *et al*: LuCaP prostate cancer patient-derived xenografts reflect the molecular heterogeneity of advanced disease and serve as models for evaluating cancer therapeutics. *Prostate* 77: 654-671, 2017.
82. Wang Y, Revelo MP, Sudilovsky D, Cao M, Chen WG, Goetz L, Xue H, Sadar M, Shappell SB, Cunha GR and Hayward SW: Development and characterization of efficient xenograft models for benign and malignant human prostate tissue. *Prostate* 64: 149-159, 2005.
83. Yoshikawa T, Kobori G, Goto T, Akamatsu S, Terada N, Kobayashi T, Tanaka Y, Jung G, Kamba T, Ogawa O and Inoue T: An original patient-derived xenograft of prostate cancer with cyst formation. *Prostate* 76: 994-1003, 2016.
84. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, Marotz C, Giannopoulou E, Chakravarthi BV, Varambally S, *et al*: Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat Med* 22: 298-305, 2016.
85. Gao D and Chen Y: Organoid development in cancer genome discovery. *Curr Opin Genet Dev* 30: 42-48, 2015.
86. Owonikoko TK, Zhang G, Kim HS, Stinson RM, Bechara R, Zhang C, Chen Z, Saba NF, Pakkala S, Pillai R, *et al*: Patient-derived xenografts faithfully replicated clinical outcome in a phase II co-clinical trial of arsenic trioxide in relapsed small cell lung cancer. *J Transl Med* 14: 111, 2016.
87. Williams JA: Using PDX for preclinical cancer drug discovery: The evolving field. *J Clin Med* 7: 41, 2018.
88. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, Zhang C, Schnell C, Yang G, Zhang Y, *et al*: High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nat Med* 21: 1318-1325, 2015.
89. Ni J, Ramkissoon SH, Xie S, Goel S, Stover DG, Guo H, Luu V, Marco E, Ramkissoon LA, Kang YJ, *et al*: Combination inhibition of PI3K and mTORC1 yields durable remissions in orthotopic patient-derived xenografts of HER2-positive breast cancer brain metastases. *Nat Med* 22: 723-726, 2016.
90. Corcoran RB, Atreya CE, Falchook GS, Kwak EL, Ryan DP, Bendell JC, Hamid O, Messersmith WA, Daud A, Kurzrock R, *et al*: Combined BRAF and MEK inhibition with dabrafenib and trametinib in BRAF V600-Mutant colorectal cancer. *J Clin Oncol* 33: 4023-4031, 2015.
91. Lai Y, Wei X, Lin S, Qin L, Cheng L and Li P: Current status and perspectives of patient-derived xenograft models in cancer research. *J Hematol Oncol* 10: 106, 2017.
92. Bartucci M, Ferrari AC, Kim IY, Ploss A, Yarmush M and Sabaawy HE: Personalized medicine approaches in prostate cancer employing patient derived 3D organoids and humanized mice. *Front Cell Dev Biol* 4: 64, 2016.
93. Lamouille S, Xu J and Derynck R: Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* 15: 178-196, 2014.
94. Ito R, Takahashi T and Ito M: Humanized mouse models: Application to human diseases. *J Cell Physiol* 233: 3723-3728, 2018.
95. Puca L, Bareja R, Prandi D, Shaw R, Benelli M, Karthaus WR, Hess J, Sigouros M, Donoghue A, Kossai M, *et al*: Patient derived organoids to model rare prostate cancer phenotypes. *Nat Commun* 9: 2404, 2018.
96. Praharaj PP, Bhutia SK, Nagrath S, Bitting RL and Deep G: Circulating tumor cell-derived organoids: Current challenges and promises in medical research and precision medicine. *Biochim Biophys Acta Rev Cancer* 1869: 117-127, 2018.
97. Gorges TM, Tinhofer I, Drosch M, Röse L, Zollner TM, Krahn T and von Ahsen O: Circulating tumour cells escape from EpCAM-based detection due to epithelial-to-mesenchymal transition. *BMC Cancer* 12: 178, 2012.
98. Armstrong AJ, Marengo MS, Oltean S, Kemeny G, Bitting RL, Turnbull JD, Herold CI, Marcom PK, George DJ and Garcia-Blanco MA: Circulating tumor cells from patients with advanced prostate and breast cancer display both epithelial and mesenchymal markers. *Mol Cancer Res* 9: 997-1007, 2011.
99. Beshiri ML, Tice CM, Tran C, Nguyen HM, Sowalsky AG, Agarwal S, Jansson KH, Yang Q, McGowan KM, Yin J, *et al*: A PDX/organoid biobank of advanced prostate cancers captures genomic and phenotypic heterogeneity for disease modeling and therapeutic screening. *Clin Cancer Res* 24: 4332-4345, 2018.
100. Chua CW, Shibata M, Lei M, Toivanen R, Barlow LJ, Bergren SK, Badani KK, McKiernan JM, Benson MC, Hibshoosh H and Shen MM: Single luminal epithelial progenitors can generate prostate organoids in culture. *Nat Cell Biol* 16: 951-961, 1-4, 2014.
101. Clevers H: Modeling development and disease with organoids. *Cell* 165: 1586-1597, 2016.
102. Allard WJ: Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res* 10: 6897-6904, 2004.
103. Tsai S, McOlash L, Palen K, Johnson B, Duris C, Yang Q, Dwinell MB, Hunt B, Evans DB, Gershan J and James MA: Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models. *BMC Cancer* 18: 335, 2018.
104. Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, Liu IH, Chiou SH, Salahudeen AA, Smith AR, *et al*: Organoid modeling of the tumor immune microenvironment. *Cell* 175: 1972-1988.e16, 2018.
105. Dijkstra KK, Cattaneo CM, Weeber F, Chalabi M, van de Haar J, Fanchi LF, Slagter M, van der Velden DL, Kaing S, Kelderman S, *et al*: Generation of Tumor-Reactive T Cells by Co-culture of peripheral blood lymphocytes and tumor organoids. *Cell* 174: 1586-1598.e12, 2018.
106. Gstraunthaler G, Lindl T and van der Valk J: A plea to reduce or replace fetal bovine serum in cell culture media. *Cytotechnology* 65: 791-793, 2013.
107. Hughes CS, Postovit LM and Lajoie GA: Matrigel: A complex protein mixture required for optimal growth of cell culture. *Proteomics* 10: 1886-1890, 2010.
108. Patel R and Alahmad AJ: Growth-factor reduced Matrigel source influences stem cell derived brain microvascular endothelial cell barrier properties. *Fluids Barriers CNS* 13: 6, 2016.
109. Nguyen EH, Daly WT, Le NNT, Farnoodian M, Belair DG, Schwartz MP, Lebakken CS, Ananiev GE, Saghir MA, Knudsen TB, *et al*: Versatile synthetic alternatives to Matrigel for vascular toxicity screening and stem cell expansion. *Nat Biomed Eng* 1: 0096, 2017.

110. Zhang S, Gelain F and Zhao X: Designer self-assembling peptide nanofiber scaffolds for 3D tissue cell cultures. *Semin Cancer Biol* 15: 413-420, 2005.
111. Stingl J, Rowbotham D, Thomas TE, Eaves AC and Louis SA: Expansion of mouse prostate epithelial stem cells in serum-free ProstaCult Organoid Growth Medium. *Cancer Res* 78 (13 Suppl): S3111, 2018.
112. Richards Z, McCray T, Marsili J, Zenner ML, Manlucu JT, Garcia J, Kajdacsy-Balla A, Murray M, Voisine C, Murphy AB, *et al*: Prostate stroma increases the viability and maintains the branching phenotype of human prostate organoids. *iScience* 12: 304-317, 2019.
113. von Amsberg G and Merseburger AS: Treatment of metastatic, castration-resistant prostate cancer. *Urologe A* 59: 673-679, 2020 (In German).
114. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, *et al*: Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 363: 411-422, 2010.
115. Kleinman HK and Martin GR: Matrigel: Basement membrane matrix with biological activity. *Semin Cancer Biol* 15: 378-386, 2005.
116. Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Sboner A, Sailer V, Augello M, Puca L, Rosati R, *et al*: Personalized in vitro and in vivo cancer models to guide precision medicine. *Cancer Discov* 7: 462-477, 2017.
117. Koo BK, Stange DE, Sato T, Karthaus W, Farin HF, Huch M, van Es JH and Clevers H: Controlled gene expression in primary Lgr5 organoid cultures. *Nat Methods* 9: 81-83, 2012.
118. Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, Dowling C, Wanjala JN, Undvall EA, Arora VK, *et al*: Organoid cultures derived from patients with advanced prostate cancer. *Cell* 159: 176-187, 2014.
119. Risbridger GP, Toivanen R and Taylor RA: Preclinical models of prostate cancer: Patient-derived xenografts, organoids, and other explant models. *Cold Spring Harb Perspect Med* 8: a030536, 2018.
120. Lawrence MG, Obinata D, Sandhu S, Selth LA, Wong SQ, Porter LH, Lister N, Pook D, Pezaro CJ, Goode DL, *et al*: Patient-derived models of abiraterone- and enzalutamide-resistant prostate cancer reveal sensitivity to ribosome-directed therapy. *Eur Urol* 74: 562-572, 2018.
121. Ooft SN, Weeber F, Dijkstra KK, McLean CM, Kaing S, van Werkhoven E, Schipper L, Hoes L, Vis DJ, van de Haar J, *et al*: Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. *Sci Transl Med* 11: eaay2574, 2019.
122. Tiriach H, Belleau P, Engle DD, Plenker D, Deschênes A, Somerville TDD, Froeling FEM, Burkhart RA, Denroche RE, Jang GH, *et al*: Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. *Cancer Discov* 8: 1112-1129, 2018.
123. Ganesh K, Wu C, O'Rourke KP, Szeglin BC, Zheng Y, Sauvé CG, Adileh M, Wasserman I, Marco MR, Kim AS, *et al*: A rectal cancer organoid platform to study individual responses to chemoradiation. *Nat Med* 25: 1607-1614, 2019.
124. Sawicki LA and Kloxin AM: Light-mediated formation and patterning of hydrogels for cell culture applications. *J Vis Exp* 115: e54462, 2016.
125. Koga Y and Ochiai A: Systematic review of Patient-Derived xenograft models for preclinical studies of anti-cancer drugs in solid tumors. *Cells* 8: 418, 2019.
126. Nardella C, Lunardi A, Patnaik A, Cantley LC and Pandolfi PP: The APL paradigm and the 'co-clinical trial' project. *Cancer Discov* 1: 108-116, 2011.
127. Clohessy JG and Pandolfi PP: Mouse hospital and co-clinical trial project-from bench to bedside. *Nat Rev Clin Oncol* 12: 491-498, 2015.
128. Chen M and Pandolfi PP: Preclinical and coclinical studies in prostate cancer. *Cold Spring Harb Perspect Med* 8: a030544, 2018.
129. Lunardi A, Ala U, Epping MT, Salmena L, Clohessy JG, Webster KA, Wang G, Mazzucchelli R, Bianconi M, Stack EC, *et al*: A co-clinical approach identifies mechanisms and potential therapies for androgen deprivation resistance in prostate cancer. *Nat Genet* 45: 747-755, 2013.
130. van Moorselaar RJA and Voest EE: Angiogenesis in prostate cancer: its role in disease progression and possible therapeutic approaches. *Mol Cell Endocrinol* 197: 239-250, 2002.
131. Zittermann SI and Issekutz AC: Basic fibroblast growth factor (bFGF, FGF-2) potentiates leukocyte recruitment to inflammation by enhancing endothelial adhesion molecule expression. *Am J Pathol* 168: 835-846, 2006.
132. Lail-Trecker M, Gulati R and Peluso JJ: A role for hepatocyte growth factor/scatter factor in regulating normal and neoplastic cells of reproductive tissues. *J Soc Gynecol Invest* 5: 114-121, 1998.
133. Blanchère M, Saunier E, Mestayer C, Broshuis M and Mowszowicz I: Alterations of expression and regulation of transforming growth factor beta in human cancer prostate cell lines. *J Steroid Biochem Mol Biol* 82: 297-304, 2002.
134. Royuela M, Ricote M, Parsons MS, García-Tuñón I, Paniagua R and de Miguel MP: Immunohistochemical analysis of the IL-6 family of cytokines and their receptors in benign, hyperplastic, and malignant human prostate. *J Pathol* 202: 41-49, 2004.
135. Planz B, Wang Q, Kirley SD, Marberger M and McDougal WS: Regulation of keratinocyte growth factor receptor and androgen receptor in epithelial cells of the human prostate. *J Urol* 166: 678-683, 2001.
136. Francis JC, Thomsen MK, Taketo MM and Swain A: β -catenin is required for prostate development and cooperates with pten loss to drive invasive carcinoma. *PLoS Genet* 9: e1003180, 2013.
137. Cook C, Vezina CM, Allgeier SH, Shaw A, Yu M, Peterson RE and Bushman W: Noggin is required for normal lobe patterning and ductal budding in the mouse prostate. *Dev Biol* 312: 217-230, 2007.
138. Jarrard DF, Blitz BF, Smith RC, Patai BL and Rukstalis DB: Effect of epidermal growth factor on prostate cancer cell line PC3 growth and invasion. *Prostate* 24: 46-53, 1994.
139. Sastry KS, Karpova Y and Kulik G: Epidermal growth factor protects prostate cancer cells from apoptosis by inducing BAD phosphorylation via redundant signaling pathways. *J Biol Chem* 281: 27367-27377, 2006.
140. Watanabe K, Ueno M, Kamiya D, Nishiyama A, Matsumura M, Wataya T, Takahashi JB, Nishikawa S, Nishikawa S, Muguruma K and Sasai Y: A ROCK inhibitor permits survival of dissociated human embryonic stem cells. *Nat Biotechnol* 25: 681-686, 2007.
141. Tojo M, Hamashima Y, Hanyu A, Kajimoto T, Saitoh M, Miyazono K, Node M and Imamura T: The ALK-5 inhibitor A-83-01 inhibits Smad signaling and epithelial-to-mesenchymal transition by transforming growth factor-beta. *Cancer Sci* 96: 791-800, 2005.
142. Zhang F, Lau SS and Monks TJ: The Cytoprotective Effect of N-acetyl-L-cysteine against ROS-induced cytotoxicity is independent of its ability to enhance glutathione synthesis. *Toxicol Sci* 120: 87-97, 2011.
143. Gu Y, Fu J, Lo PK, Wang S, Wang Q and Chen H: The Effect of B27 Supplement on Promoting In Vitro Propagation of Her2/neu-Transformed mammary tumorspheres. *J Biotech Res* 3: 7-18, 2011.
144. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD and Clevers H: Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's Epithelium. *Gastroenterology* 141: 1762-1772, 2011.