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Current Opinion in Genetics & Development

Organoid cultures for the analysis of cancer phenotypes

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Preclinical models of cancer are essential for a basic understanding of cancer biology and its translation into efficient treatment options for affected patients. Cancer cell lines and xenografts derived directly from primary human tumors have proven very valuable in fundamental oncology research and anticancer drug discovery. Both models inherently comprise advantages and caveats that have to be accounted for. We will outline in these and discuss primary patient derived organoids as third preclinical cancer model. We propose that cancer organoids could potentially fill the gap between simple cancer cell lines suitable for high-throughput screens and complicated, but physiologically relevant xenografts. The resulting applications for cancer organoids range from basic research to drug screens and patient stratification.

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Current Opinion in Genetics & Development 2014, **24**:68–73

This review comes from a themed issue on **Cancer genomics**

Edited by **David J Adams** and **Ultan McDermott**

For a complete overview see the Issue and the Editorial

Available online 31st December 2013

0959-437X/$ – see front matter,

http://dx.doi.org/10.1016/j.gde.2013.11.012

Introduction

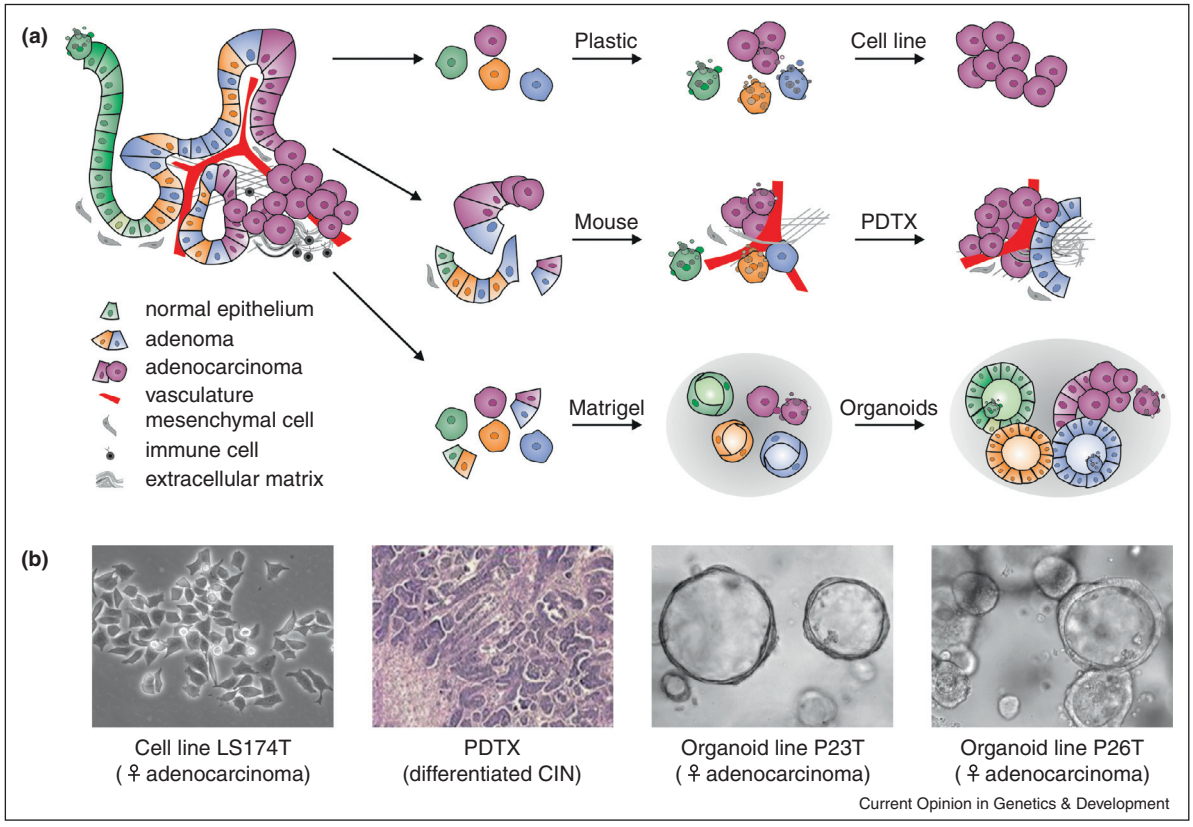
Despite decreasing mortality rates, cancer still represents a major public health problem in many parts of the world [1]. Apart from improving health choices and diagnostics, it is therefore essential to advance cancer therapeutics. In order to study cancer biology and translate this knowledge into health benefits, preclinical tumor models are necessary that resemble real malignancies and predict *in vivo* drug responses. However, cancer models too rarely fulfill these requirements due to limitations in power or simple inaccuracy [2]. As a consequence, many drug candidates that perform well in preclinical models fail to deliver in clinical trials, resulting in suboptimal patient treatment and wasted resources [3]. Current cancer models can be divided into animal models, where cancer is induced experimentally, and human-derived models, where primary human tumors are studied outside their host. Mouse cancer models have tremendously contributed to the basic understanding of cancer and have been extensively reviewed elsewhere [4,5]. Human-derived models currently include cancer cell lines and primary patient-derived tumor xenografts (PDTX). While reviewing benefits and drawbacks of these two models, we will focus on potential (dis)advantages of a third human-derived cancer model: primary tumor organoids.

Cancer cell lines

The first ever-growing human cancer cell line was established from the cervical carcinoma of Henrietta Lacks in 1951 [6]. Since then, scores of cancer cell lines have been generated which have proven invaluable for cancer research and drug development. For example, the discovery that human breast cancer cell lines MCF-7 and ZR75-1 grow estrogen dependently [7] was pivotal to the development of the estrogen receptor antagonist fulvestrant (Faslodex, AstraZeneca) [8]. Drug screens across large panels of cancer cell lines yielded additional findings, such as the identification of drug targets and gene signatures that predict drug responses [9,10].

There are several practical advantages of working with cell lines: they are homogenous, easy to propagate, grow almost infinitely in simple media, and allow extensive experimentation including high-throughput drug screens. Disadvantages such as genotypic drift and cross-contamination can usually be prevented by rigorous quality control and freezing well-characterized, low passage stocks [11]. More difficult to overcome is the poor efficiency with which permanent cell lines can be established from solid tumors: for primary breast cancers the success rate is between 1 and 10% [12] while prostate cancer is represented by less than 10 cell lines [13••]. This inefficiency is mainly due to a challenging *in vitro* adaptation of primary tumor cells which usually lose growth potential after few passages and go into crisis. Clonal cells only rarely emerge from the dying culture. As a result, the available cancer cell lines fall short of faithfully representing the clinical cancer spectrum. Since many cancer cell lines have been generated from metastatic and fast growing tumors, primary and slowly growing tumors are severely underrepresented. Control cell lines from normal tissue of the same patient are also scarce. Current cancer cell lines can therefore not adequately serve as models for tumor progression [11] (Figure 1). Additional problems arise from the loss of tumor heterogeneity and adaptation to *in vitro* growth. Consequently, gene expression profiles of tumors are regularly closer to corresponding normal tissues rather than cancer cell lines [14]. To reestablish a physiological environment and counteract genotypic divergence, cell lines have been transplanted into mouse models. Although these xenografts offer improvements over traditional cell culture, more success has been

Figure 1



Patient-derived tumor cell lines, xenografts, and organoids. **(a)** Schematic representation of establishing cell lines, xenografts, and organoids from different stages of human colon cancer. Cancer cell lines (top row) undergo crisis, *in vitro* adaptation, and selection favoring the growth of advanced clones. Following injection into immunocompromised mice, PDTX (middle row) preserve tumor heterogeneity and tumor–host interactions. Advanced tumor subclones generally grow best. Organoids (bottom row) form under permissive growth conditions in matrigel and can be established from all tumor stages as well as normal tissue. **(b)** Microscopic examples of preclinical models of colorectal adenocarcinomas with different degrees of heterogeneity. Cell line LS174T and organoid lines P23T and P26T (phase contrast) are shown next to PDTX P6X2 (H&E stain, reprinted from [18]).

achieved by avoiding *in vitro* culture altogether and directly engrafting human cancers [15] (Table 1).

Patient-derived tumor xenografts

PDTX are obtained by directly implanting freshly resected tumor pieces subcutaneously or orthotopically into immuno-compromised mice [16,17]. Following tumor take, PDTX grow progressively and can be serially engrafted into increasing numbers of animals. Since the physiological *in vivo* environment, although from a different species, mimics the original tumor conditions much better than a plastic dish, success rates of establishing PDTX are higher than for cell lines and genetic divergence is less common [15]. Importantly, biological stability of PDTX from a variety of primary tumors including colon, lung, breast, pancreas, prostate, and ovarian cancer has been established [16,17]. Xenografted colon tumors, for example, preserve their original genetic and histological profiles for up to 14 passages [18]. In addition, several subclones grow in parallel and partially conserve parental tumor heterogeneity (Figure 1). These benefits make PDTX a valid preclinical model and allow meaningful biological assays including drug efficacy and predictive biomarker development studies [17]. To this end, PDTX have been used to functionally verify rationally predicted drug response scores [19], develop predictive biomarkers for standard and novel anticancer drugs [17], and identify effective treatment regimens for patients [20••].

Even though PDTX bear great promise as preclinical model for human cancer, there are several caveats. First, tumor take is unsatisfactory with aggressive tumors engrafting best. In some instances, the ability to xenograft even serves as a negative predictor of the patients' disease free survival [21]. Second, although similarities between PDTX and parental tumors are common, they cannot be assumed and must be rigorously tested [17]. Third, tumor-host interactions are

Table 1

Characteristics of the three described preclinical cancer models. We judged the representation of the respective feature as best (+++), suitable (++), possible (+), and unsuitable (−)

Feature	Cell lines	Xenografts	Organoids
Ease of maintenance	+++	−	+
Success rate of initiation	+	++	+++
Expansion	+++	+	+++
Biological stability	+	++	++
Representation of cancer spectrum	+	++	++
Genetic manipulation	+++	−	++
Normal control	−	−	++
3D growth	+/−	+++	++
Heterogeneity	−	++	+
Dose-limiting organ toxicity	−	++	−
Tumor–stroma interaction	−	++	−
Immune system	−	−	−
Testable drug classes[a]	2	3	2
Low throughput drug screens	+++	+	+++
High throughput drug screens	+++	−	++
Conferment of drug resistance	−	++	+

[a] The following three general classes of anti-cancer drugs have been established for judging the use of preclinical models: agents targeting tumor-specific proteins, agents targeting host-tumor interactions, and agents targeting tumor cells empirically [3].

not always conserved across species (e.g. HGF-MET) and tumor immunity is entirely absent [3]. Fourth, the use of animals is labor intense, time consuming, and ethically problematic. Consequently, PDTX are no substitute for *in vitro* cultures with respect to initial high throughput drug screens. This is particularly relevant since altered signaling pathways often cross-talk to others which requires combinatorial therapy of many drug candidates for optimal treatment [22]. Recently established organoid cultures from primary tumors [23••] may expand the repertoire of available preclinical tumor models by bridging this gap between cancer cell lines and xenografts.

Organoids

The past years have seen unprecedented developments in the use of human tissue surrogates *in vitro*. Adult stem cells are embedded in a three-dimensional matrix and allowed to self-organize into epithelia of the respective organ of origin. The resulting organoids represent the physiology of native epithelia much better than traditional cell lines. Mini-guts, for example, reproduce the epithelial architecture of small intestine and colon [23••,24•].

The base for growing human intestinal organoids was laid by discovering the culture conditions of mouse intestinal organoids [25•]: by mimicking the environment of intestinal stem cells, Sato and colleagues succeeded in establishing the minimal requirements for sustainable growth of crypt-villus structures without mesenchyme. In short, R-spondin-1 (enhances Wnt signaling), EGF (mitogen), Noggin (inhibits BMP signaling), and Matrigel (basement membrane substitute) are indispensable stem cell maintenance factors for small intestinal cultures with supplementary Wnt being necessary for colonic organoid growth. Human intestinal organoids additionally require nicotinamide, A83-01 (Alk inhibitor), SB202190 (p38 inhibitor), and prostaglandin E_2 (PGE_2, mitogen) for long-term expansion (human intestinal stem cell culture (HISC) condition). Differentiation can be achieved by withdrawing growth factors while simultaneously blocking Notch signaling (dibenzazepine, γ-secretase inhibitor) [23••,24•]. Intestinal organoids are currently unique, because they efficiently form, self-renew, and expand long-term while remaining genetically stable [23••]. These features allow many applications ranging from basic to translational research [26,27]. Importantly, patient derived intestinal organoids emulate human disease as has recently been demonstrated for cystic fibrosis [28•]. Currently, organoids are being established from a variety of tumors with colorectal cancer (CRC) leading the way.

Cancer organoids

Cancer occurs through a chain of cellular alterations allowing uncontrolled proliferation and gradual loss of differentiation [29,30]. Most CRCs progress sequentially from adenomatous polyps to advanced adenomas, carcinomas *in situ*, and adenocarcinomas. There are strong indications that successive genetic changes are causal to cancer progression [31,32]. Mutations in the tumor suppressor gene *APC* (adenomatous polyposis coli) or other Wnt pathway components (*AXIN2*, *CTNNB1*) can be found in most microscopic lesions and are therefore considered initiating and rate-limiting mutations for the majority of CRCs [31,32]. Additional mutations associated with CRC affect DNA repair (*MLH1*, *MSH2*, and *MSH6*), cell-cycle regulation (*TP53*), and growth factor signaling (*TGFBR2*, *SMAD4*, *KRAS*, *BRAF*, and *PTEN*)

[31,32]. Recent evidence furthermore suggests that cancer stem cells rather than random cells fuel tumor growth in several tissues including the intestine [33–35]. It is therefore plausible to attempt culturing epithelial-derived cancers using the HISC protocol described earlier.

Organoids are indeed readily established from surgically resected intestinal tissue and endoscopic biopsies of patients suffering from adenomas and adenocarcinomas [23••]. These CRC organoids grow as irregular compact structures and can be expanded seemingly indefinitely. Apart from Goblet and enteroendocrine cells, they mostly contain proliferating cells [23••]. The presence of differentiated cells within CRC organoids potentially allows conferment of drug resistance to cancer stem cells [36]. Additional heterogeneity can be introduced by co-culturing patient-matched healthy control organoids established from tumor-adjacent normal tissue (Figure 1). However, in order to avoid cross-contamination, it is essential to initiate the tumor organoid culture from a pure tumor population and/or use selective culture conditions. CRC lesions are generally well defined which allows the pathologist to exclude potentially contaminating normal epithelium. Theoretically, selective culture conditions can be applied for the majority of CRCs given the high penetrance of activating Wnt pathway mutations [31,32]. Indeed mouse intestinal organoids with genetically inactivated *Apc* grow in the absence of Wnt or R-spondin-1, whereas wild-type organoids do not [23••,37,38]. Likewise, this selection pressure can be applied to most CRC organoids by withdrawing R-spondin-1 [23••] or Wnt. Since EGF is dispensable for growing a different subset of CRC organoids (presumably with *KRAS* or *BRAF* mutations) [23••], withdrawal of this growth factor or addition of EGFR inhibitors can enforce the necessary selection pressure. However, standard HISC conditions have to be used in order to grow organoids from adeno(carcino)mas without Wnt or EGFR pathway mutations. In that case, the differentiation between normal and CRC organoids relies on sample purity and organoid characterization. It is therefore not trivial to generate organoid lines that fully represent the spectrum of CRCs. Given the high success rate of establishing CRC organoids, their unlimited proliferative potential, biological stability, and cryostorage ability it seems, however, to be merely a question of effort to do so. If combined with genetic information and pharmacological profiles, such an organoid collection could aid in identifying CRC specifics that predict a patient's drug response similar to the Cancer Cell Line Encyclopedia [13••].

Advanced cancers display genomic instability which drives tumor progression by accumulating additional mutations [30]. Assuming random mutability, it is therefore unlikely that tumor organoids *ex vivo* undergo the same genetic alterations as their parental tumor *in vivo* (unless the same selection pressures apply). On the other hand, targeted therapeutic treatment is known to evoke resistance and favor the selection of subclones, potentially also *in vitro*. To directly compare tumor progression and drug induced selection *in vitro* and *in vivo*, multiple organoid lines from the same patient could be established (e.g. early, progressed, and metastasized cancers; pre-treatment and post-treatment) and treated in parallel.

A possible disadvantage of organoid culture may be that organoids from progressed cancers counterintuitively grow worse than those from early tumors or normal tissue due to culture conditions (optimized for normal culture) and potential loss of epithelial integrity (epithelial-mesenchymal transition). On the other hand, organoids from early tumor stages can be established at much higher success rates than cancer cell lines or PDTX allowing a better representation of the respective cancer spectrum. Organoids as pure epithelial cultures lack tumor stroma and vasculature. In that respect, PDTX models are more physiologically relevant and allow drug tests that target host–tumor interactions. Regarding tumor heterogeneity, organoids therefore fall in between purely clonal cancer cell lines and PDTX. Ambivalent is the requirement of matrigel which makes organoid culture more labor intense than culturing cell lines in 2D and adds a complicating parameter to potential drug screens. Then again, the laminin-rich and collagen IV-rich matrigel functions as a basement membrane substitute which, given its tumor origin [39], may be physiologically relevant. Also, organoid culture is considerably easier than maintaining PDTX. Currently available human (cancer) organoid lines are limited to the intestine. However, given recent advances in organoid cultures of several mouse tissues (stomach, liver, pancreas, and others [40–42]) it seems merely a question of time and effort before equivalent human (cancer) organoids can be cultivated as well. A future collection of organoids that is representative of the respective cancer group, could help patient stratification as well as oncogenic therapeutics.

Conflict of interest

HC is inventor on several patent applications related to organoid culture.

Acknowledgements

We thank Dr. M. van de Wetering for providing organoid pictures. Funding was provided by KWF/PF-Hubr 2007-3956.

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