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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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Introduction

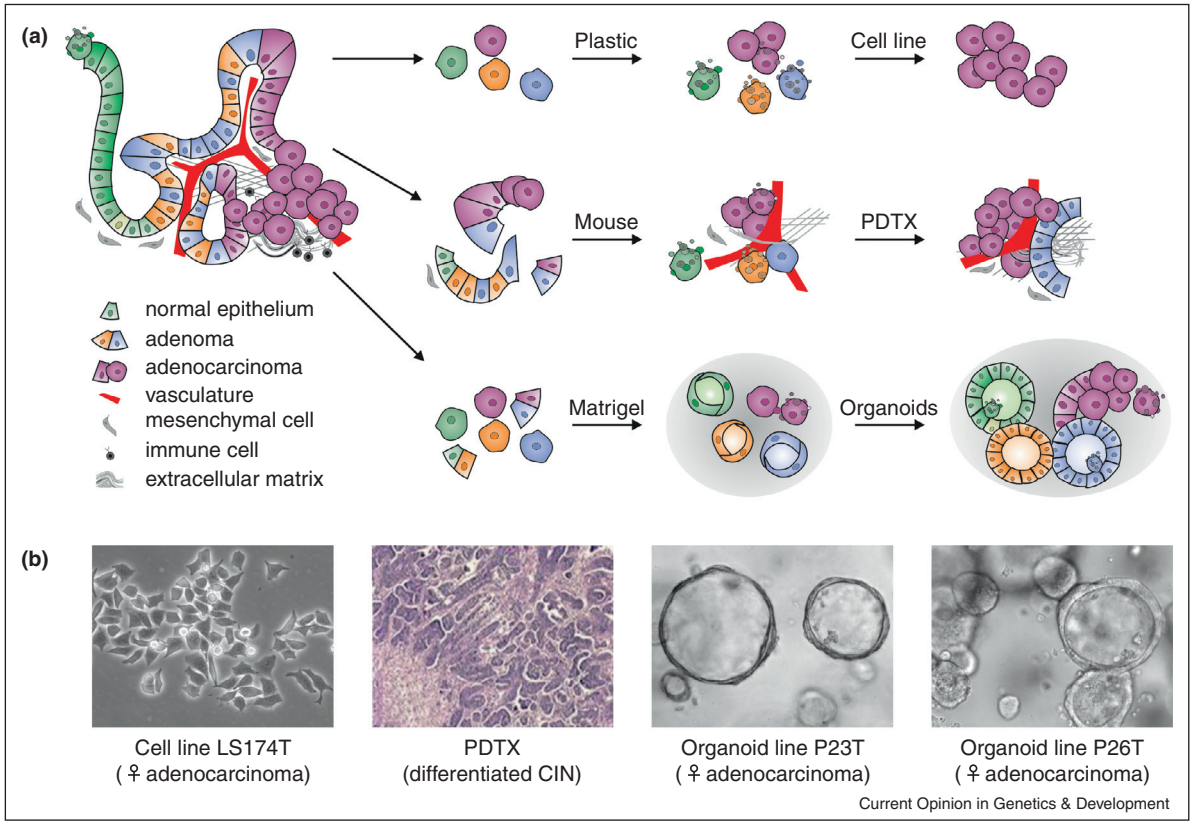
Despite decreasing mortality rates, cancer still represents a major public health problem in many parts of the world [1]. Apart from improving health choices and diagnostics, it is therefore essential to advance cancer therapeutics. In order to study cancer biology and translate this knowledge into health 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