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Review

Patient-derived tumor organoids for prediction of cancer treatment response

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ABSTRACT

Cancer treatment, in particular radiotherapy and chemotherapy, is often hindered by an inherent resistance of cancer cells. Cancer stem cells in particular have previously been shown to be more resistant than other cells within a tumor and are thought repopulate the tumour after therapies. Therefore, it is of utmost importance to develop tools and techniques that can be used to study mechanisms of resistance of cancer stem cells as potential treatment targets. Organoids (and cancer-derived organoids), are three-dimensional tissue-resembling cellular clusters derived from tissue or tumor specific stem cells that mimic the *in vivo* (tumor) characteristics, as well as (tumor) cell heterogeneity. Cancer organoids may further enhance the *in vitro* and *in vivo* models that are currently available, improve our understanding of cancer stem cell resistance and can be used to develop novel cancer treatments by improved targeting of cancer stem cells. In this review, we compare organoids with the more traditional laboratory models, such as cell lines and xenografts, and review the literature of the current role of cancer organoids in determining treatment responses.

1. Introduction

Cancer is a major health problem, with 14.1 million new cases and 8.2 million deaths due to cancer worldwide in 2012 alone [1]. The annual number of new cases is estimated to rise to 22.2 million by the year 2030 [2]. Therefore, it is necessary to continually advance and develop cancer treatments, to better cope with increasing numbers of patients and to attain a better level of patient quality of life post-treatment. The three oldest and most common cancer treatment modalities are based on surgery [3], chemotherapy [4] and radiotherapy [5], with more modern treatments, such as immunotherapy [6–8], being developed.

While these treatment modalities are highly successful in treating many forms of cancer, unfortunately they are not successful in all cases. Even tumors within tissue of the same origin, the effect of treatment varies between patients. For example, the standard of care for esophageal cancer in the Netherlands currently consists of neoadjuvant chemoradiotherapy followed by esophageal resection, as this significantly increases disease-free and overall survival compared to surgery alone [9,10]. Furthermore, less locoregional recurrences occur in patients who undergo neoadjuvant chemoradiotherapy than surgery alone [11]. However, neoadjuvant chemoradiotherapy already results in a pathologic complete response in about 25% of these patients in whom surgery may thus be omitted [10,12]. Conversely, approximately 20% do not respond to neoadjuvant chemoradiotherapy (nCRT) and would probably benefit from early surgery [10,12] or alternative treatment. However, the current imaging tools (such as positron-emission and computed tomography (PET-CT) techniques) to identify these two patient groups prior to treatment are still unable to predict response with reliable accuracy [13–15], and as a result 25% of these patients either undergo unnecessary surgery or ineffective preoperative chemoradiotherapy (20%). Therefore, better models to predict the treatment response of esophageal cancer and other cancers with the required accuracy are essential towards a more individualized treatment of patients (Table 1).

Understanding the response of cancer stem cells to treatment may be key to a more accurate and complete response prediction to many cancer treatments. Although the existence and origin of cancer stem cells is often debated [16–19], many groups have identified populations of cells within cancers with cancer stem cell (or at the very least cancer stem-like) characteristics [20–24]. Cancer stem cells possess many similar characteristics to normal tissue stem cells, in that they are capable of dividing to symmetrically (giving rise to new stem cells) and asymmetric divisions giving rise to differentiated cells of the tumor [18,19]. However, in terms of treatment, possibly the most important property of the cancer stem cells is, in general, an increased resistance to

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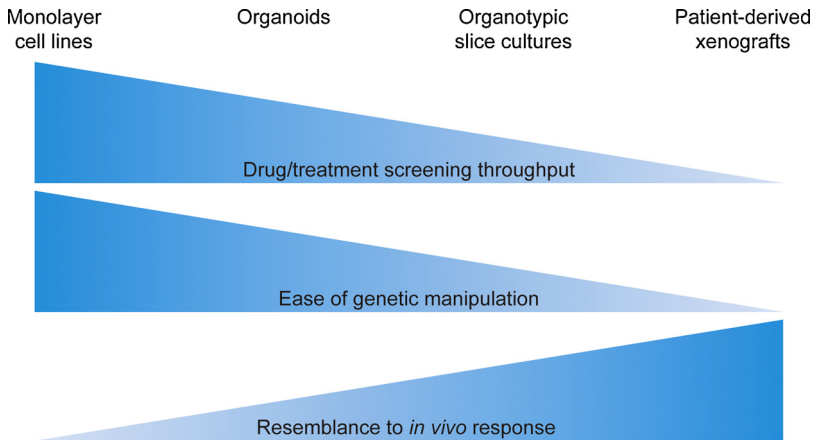

Fig. 1. Comparison of typical laboratory techniques for studying CSC responses. Organoids represent a more realistic response than cancer cell lines in terms of recapitulating the *in vivo* environment, and also allow for easier manipulation and high throughput screening than xenografts or slice cultures.

therapies, including radiotherapy [18,19]. Moreover, cancer stem cells are prone to undergo epithelial-mesenchymal transition (EMT) resulting in migration and mesenchymal-epithelial transition (MET) when they have found their new niche, thus causing metastasis [25,26]. Therefore, eliminating cancer stem cells during treatment is essential to overall long-term treatment outcome, as residual cancer stem cells have the capacity to regenerate a tumor following treatment and furthermore to metastasize.

2. Current laboratory techniques to assess treatment responses

Currently, many techniques are being used in attempts to elucidate cancer drug responses, and in particular the response of cancer stem cells. These techniques include cancer cell lines, organotypic tissue slice cultures, patient-derived xenografts and organoid culturing. Each of these techniques have their own advantages, but also their disadvantages (some advantages/disadvantages are depicted in Fig. 1). Selection of the correct tool to use in the laboratory can depend on the question on hand, but also on the resources (and knowledge) available.

2.1. Cell lines

Established cell lines are probably the most common and well-established of these techniques. There are many advantages to using established cell lines. They are easier (and cheaper) to maintain than most other *in vitro* models, they can easily be manipulated genetically, while they are also amenable to easy imaging. Techniques to determine sensitivity to drugs and/or irradiation are well-established [27,28], and thus cell lines facilitate high throughput screening of many drugs and compounds in a short period of time.

However, traditional two-dimensional (2-D) culture models lack many crucial signaling factors, such as cell-cell and cell-matrix interactions, which contribute to essential cellular functions in proliferation, differentiation and survival [29,30]. Thus, the read-outs of conventional two-dimensional models often misestimate the *in vivo* response to therapies [29]. Additionally, cell lines are generally derived from single cells and therefore do not recapitulate the complete diversity of tumors, which often consist of many different cell types [31], potentially with different treatment sensitivities and even multiple different cancer stem cells. Some of these shortcomings can be overcome by culturing as spheres/spheroids in an extracellular matrix as this introduces more 'realistic' cell-cell interactions [30,32]. However while many groups have identified populations with cancer stem-like properties in cell lines [20,33–35], true cancer stem cells (or at least the diversity of cancer stem cells in a tumor) are more difficult to identify. Furthermore, crucial factors (such as immune cells, stromal cells and blood vessels) are missing from cell culture models.

2.2. Patient-derived xenografts

Patient-derived xenografts entails the engraftment of cancerous cells or tissues into immunodeficient mice. Patient-derived xenograft models have been established for many cancer types, including colorectal [36,37], pancreatic [38] and gastric [36] cancers, and can be both subcutaneously or orthotopically transplanted. Orthotopic transplantation is thought to better resemble the true environment than subcutaneous transplantation [39]. This *in vivo* model allows for vascularization of the engrafted cells or tissue, while also enabling for the cells to assemble in a realistic tumor structure and environment including hypoxia, an important determent of radiation response [40,41]. These models allow for determination of a patient-specific response, as the engraftments can be obtained direct from biopsies. Patient-derived xenograft models also recapitulate to some extent an *in vivo* inflammatory response [42], albeit not of human origin. However, xenograft models can be both resource and time consuming, as well as costly, and require many animals in a time of more and more pressure to reduce animal experimentation. While it is possible to study the effects of different treatments on cancer stem cells using patient-derived xenografts, high throughput screening of treatments using xenografts is not possible and therefore new models that facilitate both the ease of high throughput of cell lines and both the patient specificity and a more realistic architecture of xenografts are required.

2.3. Organotypic tissue slice cultures

The relatively new *in vitro* techniques of organotypic tissue slice cultures and organoids somewhat strike a balance in many aspects between the advantages and disadvantages of both cell lines and *in vivo* patient-derived xenografts. Organotypic tissue slice cultures are tissue-derived slices of varying thickness which, under appropriate conditions, can be kept in culture for many days to weeks, even months [43,44]. Similar to patient-derived xenografts, organotypic tissue slice cultures enable treatment assessment on a more personalizes patient-specific basis while also maintaining the general histopathological structure and architecture of the tissue [44]. They are, however, far more difficult to maintain than traditional cell lines.

Organotypic tissue slice cultures can be easily maintained for short-term periods and can allow for accurate drug screening [45]. However, longer term maintenance of organotypic tissue slices is more difficult [46]. Even with culture media optimization and varying the methods of culturing (in plate culturing *versus* on a transwell insert), many organotypic tumor tissue slice cultures are only maintained for up to a

maximum of 7 days [47,48]. Thus, they do not easily facilitate high throughput screening of different treatments. However, longer organotypic tumor tissue slice culture periods (up to 35 days) have been reported [49]. While organotypic tissue slice culture techniques were originally established for normal healthy tissue, protocols have now also been established for tumor tissue of various different origins [44,45,50,51]. However, although organotypic tissue slice cultures quantify the response of a (tumor) tissue (or part of a tissue), they do not give a read-out for the (cancer) stem cell response and therefore could just be a readout of partial tumor response but not of recurrence or metastatic potential.

2.4. Organoids

Organoids, three-dimensional *in vitro* cellular structures derived from tissue specific stem cells with the capacity to self-organize into 'mini-organs' resembling the tissue of origin [52,53], may bridge the gaps left by more traditional culturing techniques in addressing the limitations of cancer stem cells in treatment response prediction. In comparison to other culturing techniques, organoids are relatively simple to maintain and expand, and thus offers many alternative means of assessing treatment responses. Similar to organotypic tissue slice cultures, the culturing techniques can vary depending on the tissue of origin (Table 1).

In general, following tissue or biopsy resection, the tissue is enzymatically and/or mechanically digested. Culturing protocols following digestion can vary from seeding directly into a Basement Membrane (BME) [54] to a floating primary culture followed by seeding in BME [55] or culture at the air-liquid interface using transwell inserts [56]. Organoids can be serially passaged every 1–2 weeks. Crucially, the media in which organoids are cultured varies, often depending on the tissue of origin. For example, EGF, R-spondin and noggin are sufficient to maintain long-term small intestinal organoids, while colon-derived organoids require the addition of nicotinamide, the p38 inhibitor SB202190, prostaglandin E2 and the Alk inhibitor A83-01 [54]. This relative ease for expansion and maintenance of organoids allows for many alternative means of assessing treatment responses. However, due to differences in criteria such as available biopsy size, biopsy site, organoid expansion rates and cell numbers required, the passage upon which analysis is performed can vary.

The alterations in cell-cell interactions and the cell-matrix interactions change the *in vitro* response to many forms of treatment, to a response which is more similar to the response seen *in vivo* than is observed using the more traditional two-dimensional cell culturing methods [29,32]. Organoids can be more easily manipulated by genetic modification than slice cultures or xenografts [57,58], while organoids are also more amenable to high throughput drug/treatment testing than these culture methods [59,60]. Furthermore, microscopy techniques to visualize organoids and protein localization within three-dimensional cultures are advancing rapidly [61]. As organoids can be derived from patient biopsies, patient-specific responses may potentially be predicted, with the results guiding medicine towards a more personalized patient approach. Organoid models have been developed for many different normal tissue types (such as gut [62], salivary glands [55,63,64], mammary glands [65] and liver [66]), as well as an increasing number of cancer types (including for example breast [59] and gastrointestinal cancers [54,67]). Despite being a relatively new technique, both normal tissue stem cell-derived organoids and cancer stem cell derived organoids have already contributed immensely to the fields of cancer biology and personalized medicine.

Table 1
Organoids offer a valuable resource to cancer biology research. Both normal tissue and tumor-derived organoids can be used as tools for studying many aspects of cancer biology including drug screening (with a more accurate and personalized outcome than traditional cell line cultures), cancer development and disease modeling. Some organoid (healthy and cancer derived) models are listed above.

Organoid models derived from normal tissue
Kidney [97,98]
Lung [99]
Liver [100,101]
Pancreas [56,66,77,101]
Salivary gland [55]
Brain [102]
Colon [54,56,68,103]
Small intestine [68,103]
Gastric epithelium [104]
Mammary [105]
Esophagus [54,86,87]
Cancer tissue-derived organoid models
Breast cancer [59]
Prostate cancer [106]
Glioblastoma [107]
Pancreatic cancer [72,80,91,101]
Colon/Colorectal cancer [54,60]
Gastrointestinal cancers [67]
Liver cancer [101]
Bladder cancer [92,93]

3. Normal tissue and cancer stem cell organoids in drug and treatment development

Currently, one of the most well-known organoid systems consist of the 'mini-gut' models developed by Clevers and his colleagues. They first identified Lgr5 as a marker for gastro-intestinal stem cells [68] and have since then been able to develop many organoid systems originating from the gut, including intestinal [62] and colon organoids [54]. Using rectal tissue-derived organoids from cystic fibrosis patients, Dekkers et al. [69] utilized this system to show the potential of organoids for in disease treatment and understanding by restoring a functional CFTR gene (a cystic fibrosis transmembrane conductance regulator). Forskolin treatment of organoids from healthy patients resulted in rapid swelling, while swelling was reduced derived from cystic fibrosis patients with a mutation in the CFTR gene [69]. Using a variety of chemical, temperature-base [69] and gene-editing techniques [70], they were capable of restoring a functional CFTR gene in organoids from cystic fibrosis patients. Furthermore, differential drug responses between organoids derived from different patients were identified [71].

In terms of cancer treatment and response prediction, organoids have been shown to accurately represent normal tissue response to common treatments, such as the chemotherapeutic drug cisplatin [72] and irradiation [73]. Below we will focus on how organoids have (and can further) contribute to cancer research, both in disease modelling and response prediction.

3.1. Organoids to model cancer development and progression

As stated above, organoid systems can be used not only for drug discovery and treatment response studies, but also in the study of disease modelling and development. Cancer modelling in particular has been possible using normal tissue derived organoids. *Li et al.* [56] demonstrated that mutation of *Kras* and/or loss of *p53* induced dysplasia and hyperproliferation in mouse-derived pancreatic and gastric organoids, and these organoids were capable of forming tumors upon transplantation into recipient mice. However, to show the oncogenic requirements of colon-derived organoids, a combination of *Apc*, *Kras*, *p53* and *Smad4* mutations were required for progression of dysplasia *in vitro* and tumor formation upon transplantation, thus showing the necessity of the multi-hit model of colorectal cancers [56].

Separate from the above study, other groups introduced mutations in *KRAS*, *TP53*, *SMAD4* and *APC*, four genes commonly mutated in colorectal cancer, into human intestinal tissue stem cells using CRISPR-Cas9 genome editing techniques [74,75]. By excluding key growth

factors from the media, *Drost et al.* [74] were able to select for mutant organoids and *in vivo* transplantation of quadruple mutants into mice formed aggressive carcinomas. Matano et al. [75] introduced one further mutation in *PIK3CA*. Mutated organoids were again capable of forming tumors upon xenotransplantation under the mouse kidney capsule [75]. In both studies, mutant organoids were capable of growth and expansion in the absence of stimulators of WNT, a key component of normal intestinal organoid culturing [74,75].

Drost et al. [76] followed up on this study by deleting crucial DNA repair genes in colon organoids, again using CRISPR-Cas9 methods. Whole genome sequencing of mismatch repair deficient organoids revealed mutation profiles similar to colorectal cancer deficient in the same pathway, while knock-out of key base excision genes resulted in a mutation signature previously associated with breast cancer [76]. More recently, it has been confirmed using pancreatic organoids that *KRAS* mutation induces macrophage phenotype changes in pancreatic ductal adenocarcinoma development [77]. Similarly, mouse-derived gastric organoids were used to demonstrate that loss of TGF-β receptor 2 induces metastatic tumor evolution and invasion upon *in vivo* transplantation [78]. Combined, these studies reveal the complexity of the cancer genome and the processes of cancer regulation, while further highlighting the capacity of organoids in furthering our understanding of cancer development and progression. These studies further contribute to our understanding of cancer development and progressions, and studies like these may enable for the discovery of specific drug targets for the early treatment, or even prevention, of cancer.

3.2. Tumor tissue-derived organoids

Since the development and optimization of normal tissue organoid culturing systems, new avenues for drug/treatment development, as well as means to investigate disease development have opened up. Furthermore, these systems have been used to show the capacity to derive organoids from many cancer types. Cancer-derived organoids offer a potential new means of investigating tumor treatment responses and could also offer an insight into personalized treatment in the future.

One of the first studies to establish organoids from cancer tissue was that of Sato et al. [54]. Initially, culture conditions were established for mouse colon crypts and subsequently adapted to allow for the culturing of organoids derived from the small intestine and colon of human [54]. Further adaption of these protocols enabled them to establish organoid cultures from colonic adenoma and adenocarcinomas, as well as from Barrett's esophagus, a metaplastic malignancy of the esophagus which is considered a precursor to esophageal adenocarcinoma [54].

Organoid technologies are developing and advancing at an extremely quick rate, and the available techniques (from survival assays, flow cytometric analysis, to fluorescent imaging) which can be performed are continually being optimized (some of which are represented in Fig. 2). Indeed, imaging methods were elegantly optimized to assess metabolism in cancer organoids derived from both murine and human tissue following anti-cancer drug treatment [79,80]. Optical metabolic imaging is based upon the fluorescent nature of nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) upon reduction, which can be performed on living tissue samples as a read-out of metabolic activity within the tissue [81]. This technique was exploited to demonstrate heterogeneous chemotherapeutic drug responses in both breast cancer organoids [79] and pancreatic cancer organoids [80]. Optical metabolic imaging combined with organoids provides a non-invasive and original means of determining patient-specific drug responses, in a potentially high throughput system.

Highlighting the strength of organoid cultures compared to two-dimensional cell cultures, Jabs et al. [82] developed an automated microscopy assay to distinguish between cell death and inhibition of proliferation induced by drugs. Ovarian cancer cells cultured as organoids were compared with ovarian cancer cells cultured in the 'classical' two-dimensional system [82]. Following treatment with clinically relevant chemotherapeutic drugs, it was found that the effects of the drugs could be linked to the patients' genome alterations in organoid cultures, which in contrast could not be found in monolayer cultures [82]. Therefore, it seems that such methods to screen relevant drugs with increased accuracy could help tailor cancer treatments in the future to a more personalized treatment plan. Furthermore, these advancements in the imaging of organoids could also be used to better test novel drugs or treatments with greater accuracy.

Excitingly, when biopsies are taken for isolation of cancer organoids, it is usually possible to obtain biopsies from normal tissue at the same time without great inconvenience for the patient. Comparing the responses of normal tissue derived organoids to those of cancer derived organoids could potentially offer the opportunity to determine a therapeutic ratio on a personalized basis. Indeed, one study [83] took advantage of this to complete sequence analysis on organoids derived from colorectal cancer compared to healthy colorectal tissue derived from the same patients. As individual organoids are derived from single cells, it is possible to perform a 'bulk' single cell sequencing on organoids [83]. Not only was it found that single cells derived from a single cancer biopsy showed a high level of mutation variation and a far greater number of mutations than normal tissue, but even cells from the same tumor with similar mutation signatures showed noticeably different responses to chemotherapeutic drugs [83]. While differences in drug responses at a clonal level exquisitely show the diversity and heterogeneity within a tumor, assessing the treatment response of organoids on larger scale (rather than single clones) may be more informative to the response of a tumor as a whole if a more personalized medicine approach is desired.

Indeed, in a highly promising study for the use of cancer organoids as a predictor of treatment response, Vlachogiannis et al. [67] cultured cancer-derived organoids from patients with gastrointestinal metastatic cancers and treated them with commonly used therapeutics. Patients were enrolled in Phase I/II clinical trials and the results of the organoid treatment were compared with the patient's own response [67]. Molecular profiling of the derived organoids revealed extremely similar profiles to those of the tumors from which they were derived [67]. Furthermore, they could identify differences between organoids derived from a patient sensitive to the chemotherapeutic drug regorafenib compared to organoids derived from a patient resistant to the same drug. Importantly, they were not only able to mimic inter-patient tumor differences using patient-derived organoids but they were also able to distinguish intra-patient tumor heterogeneity in response to chemotherapeutic drugs [67]. This study shows the strength of organoids to predict tumor-specific responses and is potentially one of the first steps towards a personalized treatment regime based on cancer stem cell organoids.

As stated earlier, for some cancers (such as esophageal cancers) we currently lack the optimal tools to accurately predict a response prior to treatment [13,14]. While great efforts are being made to enhance the prediction sensitivity of image-based methods, including PET-CT or magnetic resonance imaging (MRI) [84,85], the recent identification of a subpopulation of esophageal cancer cells with cancer stem cell-like properties [20], as well as methods to culture esophageal-derived organoids [86] and adenocarcinoma-derived organoids [54], may allow for the culturing of esophageal cancer organoids with the potential to reveal sensitivities that are not detected by these imaging techniques. The aforementioned studies show the power of organoids to predict a chemotherapeutic response, while studying the effects of radiation treatment on organoids is also possible [73]. Esophageal cancer organoids could potentially show inter-patient variability based on the cancer stem cell populations, which in turn could reveal sensitivity differences. Genomic analysis of surviving organoids following radiation and/or chemotherapeutic treatment may provide a 'cleaner' resistance signature that could be found in early passage organoids following initial biopsy collection. Furthermore, recent advances in the optimizing of normal esophageal tissue organoids culturing [87] may

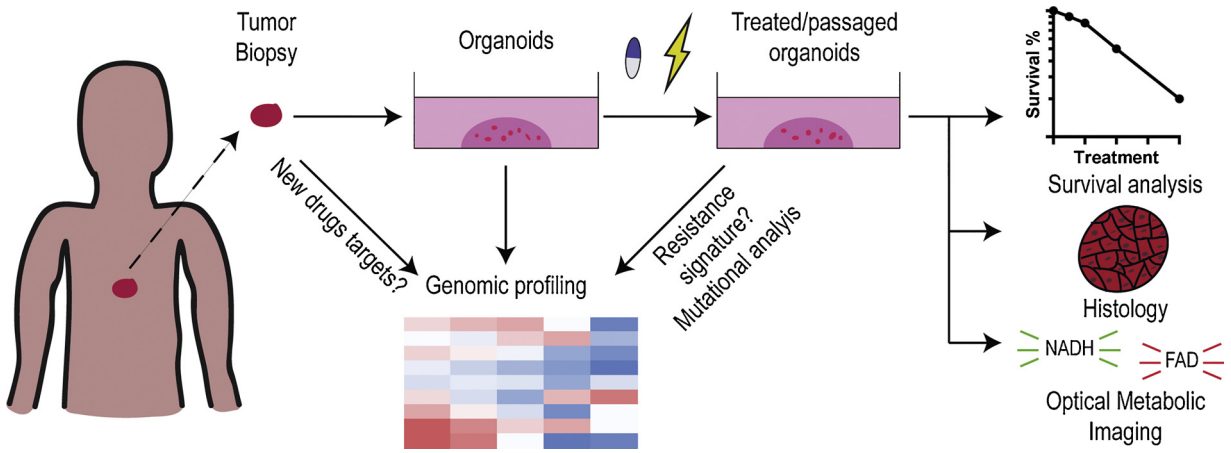

Fig. 2. The possibilities with (tumor) organoids. Following biopsy collection, cells can be isolated and cultured as organoids. Many read-outs can be obtained using organoid technologies. Genomic profiling can be used to identify new drug targets [96] from the biopsy/early passage organoids, mutations following treatment [93] or to potentially identify treatment resistance signatures of cancer stem cells in treated organoids. Techniques such as survival assays [73], histological [93] or immunofluorescent staining [61] and optical metabolic imaging (OMI) [79,80] can also be performed on cultured/treated organoids.

allow for prediction of a 'personalized therapeutic ratio'.

Pancreatic cancer is also known to be a notoriously difficult cancer to predict and treat [88]. However, although some very illuminating patient-derived xenograft models [89] and animals [90] have been established, there is still a need for more complementary preclinical tools for assessing response, as these models are expensive and can't be used to quickly determine/predict patient responses. Recent developments in culturing of pancreatic ductal adenocarcinoma organoids which physiologically resemble the development of pancreatic cancer [91] may address some of the drawbacks of the current models.

An exciting new development in the field of organoids is the creation of "Living biobanks". These depositories have been proposed/created for tumors of various origin, including breast, colorectal and bladder [59,60,92,93], to enable access to organoid systems on a larger scale. Biobanks of patient-derived cancer organoids have the potential to be accessible worldwide to advance research and treatment. Since organoid cultures mimic the heterogeneity of cancer subtypes better than cell lines, biobanks could potentially offer a platform for treatment screening with a more personalized response. Organoid depositories can better encompass the genetic diversity between tumors which can be used to identify specific drug-genetics interactions.

Although less work has been performed on organoids derived from cancers than normal tissue, the work is highly revealing and indicative of a strong future for organoids in cancer biology research. The advantages of more accurate response prediction than traditional two-dimensional cell culture models, the capacity to reveal heterogeneity even within individual patients, combined with the more facile nature of high throughput screening than xenografts, should enable organoids to take their place amongst the most important tools for prediction of cancer treatment responses in the future. The majority of cancer organoid models are limited to adenocarcinomas, and thus far less opportunities are currently available for squamous cell carcinomas for instance. However, as our knowledge of cancer development expands, and more cancer stem cell markers are identified, organoids can only become a stronger research tool covering all cancer types.

3.3. Limitations of organoid models

While organoid models potentially offer new insights into the development and treatment of cancer, they are not the final piece in the puzzle and many limitations to organoid models still remain. Cancer development and treatment responses are highly complex processes that involve many other factors. Tumors are, in general, highly vascularized. These blood vessels further influence the response of cancer to treatment [40]. Furthermore, treatment response is also influenced by the tumor microenvironment, such as localized regions of hypoxia [94], and by the surrounding immune system [95]. While tumor organoids can be cultured under hypoxic conditions, this can't recapitulate the differing gradients of hypoxia found within a tumor. Growth factors and cytokines can be added to media to elicit an immune response. However, this remains a highly artificial response. In the future, it may be interesting to perform co-cultures with blood vessels and immune cells to further mimic a truer environment of the tumor, and thus capture a more realistic tumor treatment response. Despite these limitations the results from organoid cultures faithfully recapitulate the *in vivo* response of patient-derived xenograft models, as shown in recent studies by Pauli et al. [92] and Lee et al. [93] using bladder cancer-derived organoids.

4. Concluding remarks

Organoids, derived from both normal tissue stem cells and cancer stem cells, have advanced our understanding of both disease development and drug discovery. They have added to our wealth of knowledge already obtained from decades of priceless work using the more traditional two-dimensional cell lines. The added complexity of organoids, with their different cell-cell interactions, cell-matrix interactions and the potential for cellular differentiation within organoid cultures [29,30], have complemented and strengthened this data, while also enabling us to overcome some of the limitations of cell lines, such as an overestimation of drug and irradiation responses [52,53,73]. The heterogeneity of cell subtypes found in organoids, and their treatment responses being more similar to *in vivo* than those seen in cell lines, means that organoids can also be used to potentially test new upcoming cancer treatments, such as immunotherapy [6–8], with higher accuracy, while organoids also offer a more reliable platform for assessing drug responses of 'new' drugs which can be identified by genetic profiling on a personalized basis [96]. As mentioned above, extending the therapeutic window of treatment is the primary aim of drug and/or irradiation treatment studies. Comparing the response of tumor-derived organoids from a patient with the response of normal tissue derived organoids from the same patient, or possibly even co-cultures of the two, could in the future offer a predictive therapeutic window on an individual personalized basis. The initiation of accessible organoid biobanks for research purposes similar to cell line depositories can only further advance our understanding of the role of cancer stem cells in therapeutic responses [59,60,92,93].

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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