Applications and perspectives of tumor organoids in radiobiology (Review)

JIN YU[1*], KAILUN WANG[2*], YONGJIANG TANG[3] and DALIN ZHENG[1]

[1]Department of Hematology, Panzhihua Central Hospital, Panzhihua, Sichuan 617067, P.R. China;
[2]Emergency Department, Panshihua Central Hospital, Panzhihua, Sichuan 617067, P.R. China;
[3]Department of Vascular Surgery, Panzhihua Central Hospital, Panzhihua, Sichuan 617067, P.R. China

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Abstract. Radiotherapy exhibits significant versatility and efficacy in cancer treatment, thereby playing a crucial role in the field of oncology. However, there remains an urgent need for extensive research on various aspects of radiotherapy, including target selection, damage repair and its combination with immunotherapy. Particularly, the development of *in vitro* models to replicate *in vivo* tumor lesion responses is vital. The present study provides a thorough review of the establishment and application of tumor organoids in radiotherapy, aiming to explore their potential impact on cancer treatment.

Contents

1. Introduction

Nearly 2.0 million new cancer cases are predicted to occur in 2024 in the USA (1). With increasing patient numbers, medical and caregiving costs over the next 30 years are projected to reach a staggering 25 trillion US dollars globally (2). To improve the long-term prognosis of cancer patients using existing treatment modalities optimally, it is essential to consider factors such as age, physical condition, tumor location, staging and treatment outcomes comprehensively (3). Despite the efficacy of conventional treatments such as chemotherapy in cancer therapy, challenges remain, including precision issues, high costs and severe side effects. For specific tumor types, such as brain tumors or those near the genitourinary tract, surgical complications often make radiotherapy the preferred option (4,5).

Radiotherapy directly impacts the DNA of tumor cells or generates highly reactive oxygen radicals, leading to cell mutations and cell death for therapeutic purposes (6). Although this approach has a long history in clinical practice and relatively mature technology, it still encounters challenges, including tissue damage and treatment failures. The shift from general to precision medicine and the application of radiation research findings in clinical practice have been bottlenecks in the field (7). Emerging *in vitro* models hold the potential to address these challenges.

Research models associated with radiotherapy predominantly comprise cell and animal models. Cell models are advantageous for cell culture, genetic research and high-throughput screening, but are limited in their ability to replicate cell-cell interactions and *in vivo* microenvironments over extended periods. This limitation results in an incomplete representation of primary tumors, thus raising concerns about the accuracy of experimental data (8). By contrast, animal models, including genetically engineered mice and patient-derived xenograft mice, offer clinical relevance, genetic stability, tumor heterogeneity and complete internal environmental systems, encompassing neural control and immune responses (9). Gene-editing techniques can be used to investigate the association between specific tumor mutation genes, such as tumor protein p53 (10) or ATM serine/threonine kinase (11), and radiotherapy. However, some oncogenes, such as lncGRS-1, are highly conserved in primates but absent in rodents, making traditional mouse models unsuitable for subsequent experimental research (12). Similarly, genes such as APC regulator of WNT signaling pathway in colorectal cancer or KRAS proto-oncogene GTPase in pancreatic cancer cannot be replicated in animal models due to the insidious onset, high degree of malignancy and rapid progression. Once found,

Correspondence to: Dr Jin Yu, Department of Hematology, Panzhihua Central Hospital, 34 Yikang Street, Panzhihua, Sichuan 617067, P.R. China
E-mail: yj20200330@163.com

*Contributed equally

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most cases are at an advanced stage (13,14). Additionally, the differences in genetics, anatomy and physiology reduce the reproducibility of animal models and fail to accurately represent the development of human organs and systems. This is particularly evident in the study of rare diseases such as fragile X syndrome (15), where animal experiments have proven ineffective. For castration-resistant prostate cancer that does not rely on the androgen receptor signaling mechanism, animal models have not established yet (16), and ethical concerns, such as reducing the number of animals used, cannot be ignored (17). Three-dimensional organoid models overcome these limitations, offering advantages in accuracy and efficiency, and promoting the transition of basic research into clinical applications (18,19).

Organoid technology, recognized as the Life Science Method of the Year by 'Nature Methods', constructs multicellular, self-organizing, three-dimensional structures using stem cells, such as embryonic stem cells, induced pluripotent stem cells (iPSCs) or tissue-resident stem cells/progenitor cells (18-20). Tumor organoids are created by *in vitro* three-dimensional culturing of tumor stem cells, allowing them to self-assemble into 'minimal systems' that closely mimic the morphology and function of corresponding *in vivo* tumor tissues. Categorization of organoids based on distinct germ layers includes: i) the ectoderm, which mainly encompasses the nervous system, with glioblastoma organoids (21) predominantly in radiotherapy, ii) the mesoderm, comprising organoids from the urogenital system, notably breast cancer (22) and renal organoids (23), with the former widely used in high-throughput drugs screenings, mechanistic explorations and pharmacological assessments, and the latter primarily focusing on post-transplantation functionality; and iii) the endoderm, primarily encompassing gastrointestinal tract organoids (24), known for their early development stages, standardized cultivation techniques and extensive applications in radiotherapy. Additionally, the rapidly advancing field of organoid technology has introduced new forms such as cardioids (25), trophoblasts (26) and bone marrow organoids (27). In addressing prior challenges associated with the lack of immune components, Bouffi *et al* (28) made a significant breakthrough in 2023 by developing an intestinal organoid that incorporates an immune system (Fig. 1).

In contrast to models focused on single tumor types, biobanks provide a comprehensive view of various pathological stages of tumors. For instance, the gastrointestinal tumor organoid (GITO) biobank (24) spans stages ranging from inflammation and erosion to ulcers, atrophy, intestinal metaplasia, hyperplasia, carcinogenesis and even metastasis. These biobanks are particularly effective in integrating genomic and functional data from clinical sources, predominantly derived from patient-derived tumor subtypes, to identify potential therapeutic targets, thus playing a pivotal role in bridging basic research with clinical applications. Currently, an array of organoid biobanks is being established, catering to cancers such as prostate (16), bladder (5), liver (29) and pancreatic (14) cancer, among others.These biobanks differ in terms of tissue sources, cellular composition, cultivation methods, the duration of generation and maintenance, and their number and applications. More crucially, the role of biobanks as a repository is fundamental in precision medicine (Table I) (30-38).

2. Methodologies of tumor organoids

Materials for tumor organoids are typically derived from sources such as fine-needle aspirations, biopsies, resection specimens, circulating tumor cells and PSCs (24,39). A key factor for successful cultivation is obtaining an adequate quantity of tumor cells. It is important to note that these diverse techniques for obtaining organoids balance simplicity and ease of use with precision.

Submerged culture. This method involves culturing the material within gels of extracellular matrix (ECM), submerged beneath tissue culture media (40). Submerged culture is predominantly suited for tumor tissues such as those from the digestive tract, glands and urogenital reproduction system.

Induced stem cells. This category includes both iPSCs and embryonic stem cells from human or murine sources. This approach involves using customized tissue-specific differentiation protocols to generate the corresponding organ type. However, it is important to note that this process generally requires a significant duration, ranging from weeks to months (41).

Air-liquid interface culture. In this technique, the top of the Transwell is directly exposed to air, which enhances oxygen diffusion and facilitates the growth of larger organoids (41). This method is extensively used in respiratory system studies and is crucial when combined with gene-editing technology for investigating tumor mechanisms or signaling pathways (42).

Co-culture. This approach enriches the culture system by incorporating a mix of cells, tissues and organs, fostering a more complex and interactive environment (43).

Bioreactors. These devices are especially efficient in quickly providing cells with the essential nutrients and growth factors; they are primarily used for rapid organoid generation in the nervous system over a brief period of 2-4 weeks (44).

Organoid-on-a-chip. Originating from the field of microfluidics, this innovative device simulates human organ functional units *ex vivo*. Organoid-on-a-chip enables precise control over the physical and biochemical microenvironment, managing aspects such as cytokine concentration gradients and nutrient supply, and modeling interactions between tissues and multiple organs (45).

3. Common applications of tumor organoids

Patient-derived tumor organoids (PDTOs) reflect the *in vitro* mutational modeling of all stages of malignancy, and their construction is the same as that of the other tumor cells such as iPSCs and embryonic stem cells. PDTOs are useful in precision medicine as they are able to guide patient-specific therapies. The organoids are celebrated for their ability to include various cell types, accurately reflect the corresponding native tissues and replicate definitive functionalities. A pivotal feature of PDTOs is their capacity to display the genomic and phenotypic heterogeneity within and between

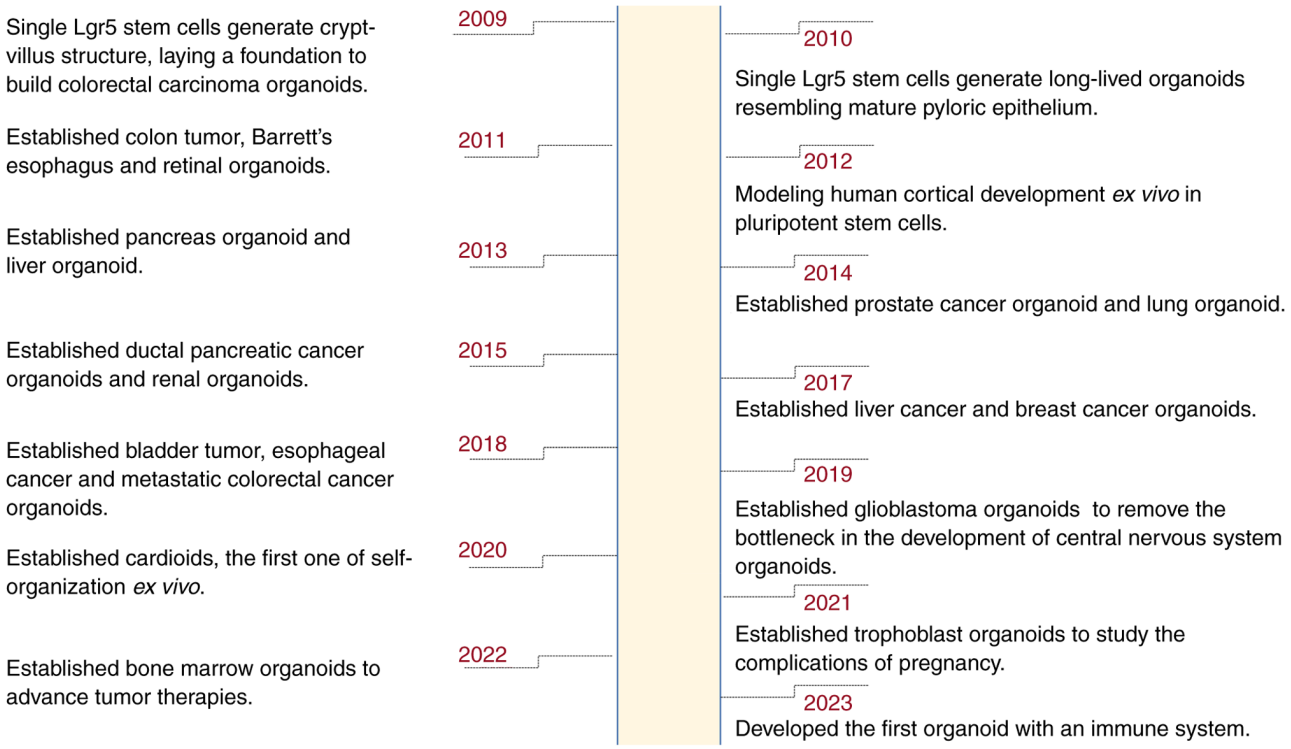

Figure 1. Evolutionary timeline of organoid development (2009-2023). A comprehensive timeline of organoid development from 2009 to 2023 highlighting the key milestones, including the introduction of colorectal carcinoma organoids in 2009, glioblastoma organoids in 2019, cardioids in 2020, trophoblast organoids in 2021, bone marrow organoids in 2022 and organoids with an immune system in 2023. Lgr5, leucine-rich repeat-containing G protein-coupled receptor 5.

tumors. Employing advanced sequencing analyses, such as whole-genome sequencing and RNA sequencing, PDTOs maintain the unique somatic mutations of the patient (18,19). The preservation of genetic profiles, combined with rapid chemosensitivity testing post-diagnosis, is crucial in precision medicine (46). Besides, PDTOs can mimic the tumor under controlled laboratory conditions, provide results closer to those of the clinic than cell lines and animals, and last but not least, reduce the requirement for animal testing. Currently, there is a lack of established guidelines for PDTOs between different labotories, meaning that reproducibility cannot be ensured. Meanwhile, the ethical considerations (17) of obtaining patient-derived tumor materials also cannot be ignored.

In pancreatic cancer specifically, PDTOs demonstrate a matching rate of up to 89%, showing therapeutic responses similar to those of the patient they originate from. This similarity offers a promising indicator for predicting patient responses to treatments in clinical settings (14). PDTOs can accurately replicate complex inter- and intra-tumoral heterogeneity. Notably, some organoid lines exhibit biomarker overexpression, such as that of human epidermal growth factor receptor 2 (HER2) in breast cancer PDTOs, which consequently results in insensitivity to HER2-targeted therapies (22). Likewise, lung cancer PDTOs with epidermal growth factor receptor mutations may exhibit resistance to erlotinib, while activin receptor-like kinase 1 mutations may show no response to crizotinib. Conversely, Erb-B2 receptor tyrosine kinase 2-mutated cancer organoids have shown sensitivity to both erlotinib and gefitinib (36). These variations suggest that predictive mutation biomarkers may sometimes be off-target or influenced by sequencing biases.

Notably, insights from patient-derived glioblastoma organoids have highlighted the cytotoxic effects of targeted therapies such as everolimus (a mammalian target of rapamycin inhibitor) and cobimetinib (a mitogen-activated protein kinase inhibitor) (21). These findings underscore the importance of clinicians being acutely aware of potential adverse effects when administering these drugs to patients. In summary, PDTOs offer a transformative approach to oncology, blending tumor biology intricacies with personalized medicine precision. The workflow of generation, characterization and applications of tumor organoids is illustrated in Fig. 2.

4. GITOs

Animal models for predicting human gastrointestinal responses to radiotherapy have previously been limited due to disparities in gut microbiota, the microenvironment, dietary structure, and anatomical variances between mice models carrying carcinogenic genes and tumor lesions in patients, and advanced stage tumors have also resulted in a shortened mouse lifespan (47,48). Emerging GITOs have effectively addressed the previous contradictions by achieving high fidelity and precise matching of corresponding tumor structure in patients. Yet, it is important to note that the cultural systems vary significantly across different laboratories.

Comparison of the cultural systems of GITOs. The [epidermal growth factor (EGF), Noggin and R-Spondin 1] (ENR) culture system developed by Sato *et al* (49) is recognized as the simplest method for cultivating intestinal organoids, and is primarily used in primary tissue cultures. This system

Table I. Overview of various tumor organoid biobanks (2011-2022).

Tumor tissue	Cell composition	Culture methods	Generated/ maintaining time	Organoid numbers	Applications	Publication year	(Refs.)
Colon	Colonic cancer epithelial	Submerged culture	10 days/within 3 months	22	Developing a technology to culture the gastrointestinal tract	2011	(30)
Prostate	Prostate cancer biopsy specimen/ circulating tumor cells	Submerged culture	3 days-3 weeks/>6 months	6	Investigating relevant genetic and pharmacological studies	2014	(16)
Posterior tongue	Adult taste stem cells/progenitor cells	Induced pluripotent stem cells	2-3 days/ 1 month	43	Generating the functional taste bud model from stem cells	2014	(31)
Pancreatic duct	Human PDA endoscopic needle biopsies	Submerged culture	3 days-2 weeks/up to 1 months	19	Investigating PDA pathogenesis and identifying molecular pathways with disease progression	2015	(14)
Fallopian tube	Gynecological tissue specimens	Submerged culture	>16 months	7	Investigating the signaling routes	2015	(32)
Liver	Patient-derived liver tumor tissue of surgical resection samples	Submerged culture	Around 1 year	8	Identifying biomarker and screening drug	2017	(29)
Endometrium	Human adult stem cell	Submerged culture	>6 months	25	Study diseases (such as endometriosis and endometrial cancer) and the physiology of early gestation	2017	(33)
Gastrointestinal tract	Patient-derived biopsies of gastrointestinal cancers	Submerged culture	>3 months	63	Implementing in personalized medicine	2018	(24)
Breast	Patient tissue underwent lumpectomy	Submerged culture	>4 months	95	Discovering drug and cancer mechanism	2018	(22)
Bladder	Patient-derived biopsies of bladder cancers	Submerged culture	>7 weeks	12	Studying tumor evolution and treatment response	2018	(5)
Esophageal	Esophageal adenocarcinoma tissue samples of esophagectomy	Submerged culture	>6 months	10	Screening drugs and studying tumor clonality	2018	(34)
Epidermis	Murine keratinocytes	Induced adult epidermal stem cells	>7 months	23	Studying the biology of skin diseases	2019	(35)
Lung	Lung cancer tissues of surgically resected/a small biopsy tissue	Submerged culture	Around 4 weeks/>2 months	39	Predicting patient-specific drug responses	2019	(36)

Table I. Continued.

Tumor tissue	Cell composition	Culture methods	Generated/ maintaining time	Organoid numbers	Applications	Publication year	(Refs.)
Renal	Surgical specimens	Submerged culture	>120 days	10	Improving therapeutic treatments	2019	(23)
Biliary tract	Biliary tract carcinoma of patients	Submerged culture	>1 year	6	Screening drugs as potential therapeutic agents	2019	(37)
Glioblastoma	Patient-derived glioblastoma resection samples	Submerged culture and co-culture	>2 months (>1 year of continuous culture without passaging)	70	Describing a novel organoid culture system and studying the heterogenoous cell-cell relationships	2019	(21)
Nasopharyngeal	Surgical specimens	Submerged culture	>6 months	16 primary NPC, 23 recurrent NPC, 13 normal mucosa samples	Exploring the pathogenesis and developing precision medicine	2022	(38)

PDA, pancreatic ductal adenocarcinoma; NPC, nasopharyngeal cancer.

triggers the expression of the leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) gene, which acts as a receptor for the Wnt activator R-spondin 1; it also utilizes specific molecules such as Noggin, EGF and Wnt to promote the growth of intestinal crypts (50). Notably, R-spondin 1, serving as a Wnt modulator, can substitute for the role of Wnt3a, meaning that organoid formation does not always require Wnt induction, and their combined use does not lead to synergistic effects (51). Moreover, the composition of culture systems varies among research teams. Some studies suggest that adding IL-22 to a culture system lacking EGF can prevent radiation-induced damage to ISCs by inducing Stat3 phosphorylation in $Lgr5^+$ ISCs. This method not only protects ISCs from radiation damage, but also promotes the proliferation of remaining stem cells, aiding in tissue repair (52).

In contrast to the traditional ENR culture system, the 8C culture system [LDN193189, glycogen synthase kinase 3 inhibitor XV, pexmetinib, valproic acid, EPZ6438, EGF, R-Spondin 1 conditioned medium and basic fibroblast growth factor (bFGF)], proposed by Qu *et al* (53), exhibited a nearly 130-fold increase in expression of the stem cell antigen-1 gene associated with organ regeneration after radiation. Additionally, the repair genes clusterin, annexin A1 and regenerating islet-derived β showed nearly 300-, 160- and 22-fold increases, respectively. Consequently, this system significantly accelerates the restoration of the crypt structure in the intestinal epithelium following radiation injury (53). Furthermore, the Yap pathway plays a crucial role in crypt regeneration post-ionizing radiation by suppressing the Wnt signaling pathway while inducing the Egfr signaling pathway, ultimately promoting the proliferation of $Lgr5^+$ ISCs (54).

Besides $Lgr5^+$ stem cells, Farin *et al* (55) discovered that a culture medium containing Wnt11 could increase organoid proliferation following radiation damage by activating the Wnt signaling pathway. Therefore, Wnt11 holds promise as a potential target for radiation injury repair. Consequently, growth factors are not only crucial for the development of organoid culture systems, but also influence the outcomes of radiotherapy. For instance, R-spondin 1 can protect $Lgr5^+$ stem cells in the intestine from radiation-induced damage (56). Insulin-like growth factor 1 (IGF-1) and FGF-1 both inhibit p53-dependent apoptosis and promote the survival of ISCs after exposure to radiation (57).

GITOs for radiotherapy. Current research utilizing organoids in fields such as biobanking, phenotype validation and drug screening is thriving. However, research on radiotherapy using organoids is relatively limited (34). The development of tumor organoids provides a comprehensive understanding of clinical heterogeneity in patients, offering a method for predicting patient responsiveness to radiotherapy. The organoids developed by Ganesh *et al* (58) not only align with rectal cancer in terms of tissue pathology, but also maintain consistent expression of intestinal epithelial cell differentiation markers, aiding in the assessment of patient reaction to radiotherapy. Yao *et al* (59) effectively used gastrointestinal organoids for screening highly effective clinical radiochemotherapy regimens, achieving an efficacy rate of up to 85% for tumor

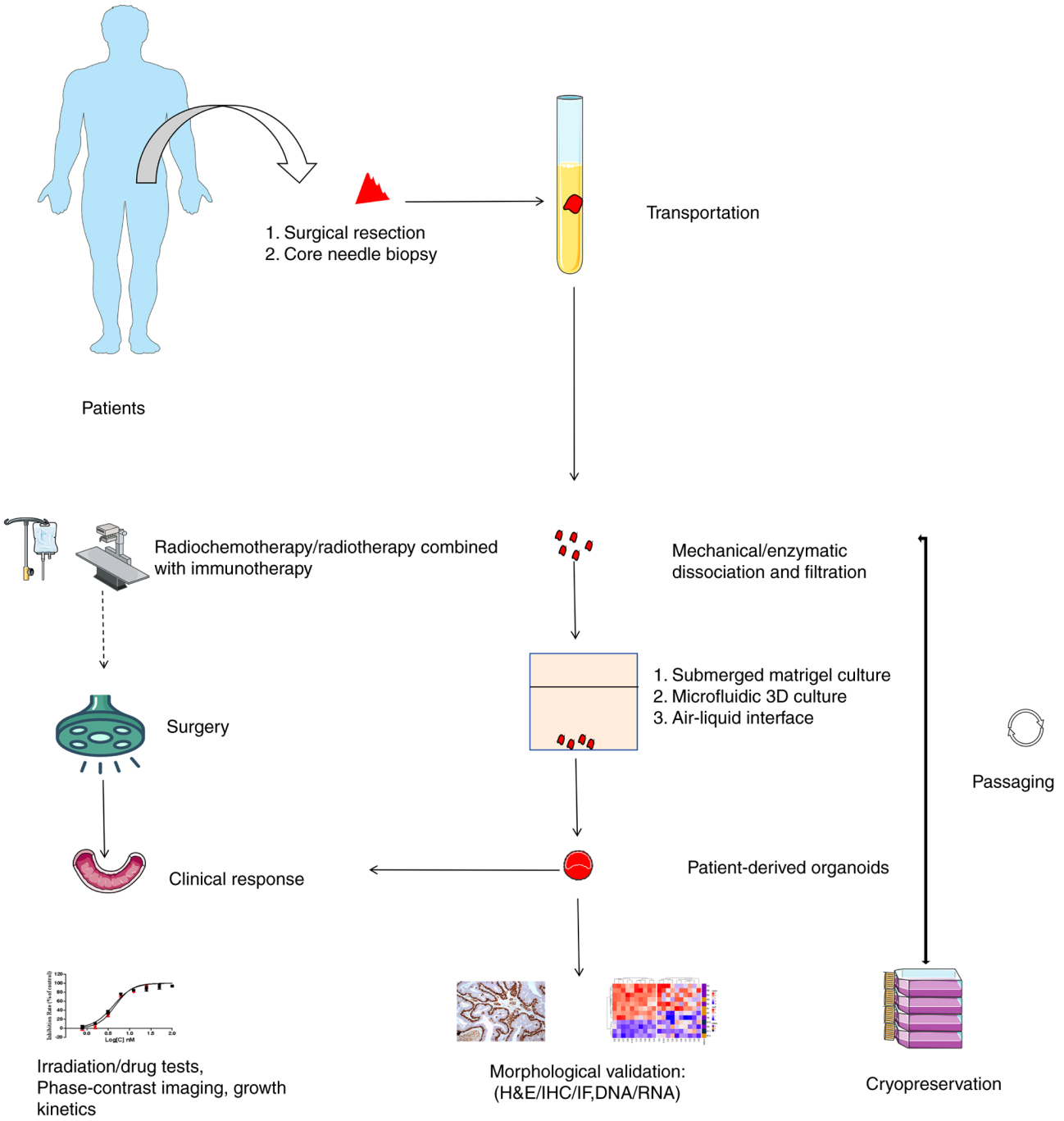

Figure 2. Workflow of PDO generation, characterization and applications. PDO generation begins with the acquisition of tumor fragments via surgical resection or core needle biopsy. These are then dissociated and filtered into a single-cell suspension upon arrival at the laboratory. Cells are seeded in conditional medium using methods such as submerged Matrigel culture, microfluidic 3D culture or air-liquid interface. Subsequently, PDOs are processed for cryopreservation to establish a patient biobank and subjected to morphological assays, including H&E/IHC/IF, DNA/RNA analysis, coupled with irradiation, drug tests, phase-contrast imaging and growth kinetics. A special focus is placed on neoadjuvant therapies such as radiochemotherapy and radiotherapy combined with immunotherapy, aimed at reducing tumor size, alleviating symptoms or improving surgical outcomes. Finally, experimental results are applied to patients for precision medicine. PDO, patient-derived organoid; H&E, hematoxylin and eosin; IHC, immunohistochemistry; IF, immunofluorescence.

patients who had experienced treatment failures or disease progression. Given the significant benefits of drug screening in organoids, this model is often combined with chemotherapy to improve treatment effectiveness.

Additionally, research by Al Bitar *et al* (60) demonstrated that using a radiation sensitizer alone has minimal impact on tumor lesions. However, when combined with low-dose radiation (2 Gy), just 10 μM of the sensitizer belinostat is sufficient to induce damage in colon cancer organoids. This model can also be used to investigate pathways associated with radiation resistance, including the PI3K/Akt/mTOR, MEK/ERK and Notch activation pathways, as well as pathways linked to damage repair, such as the DNA-dependent protein kinase, RAD51 homolog recombination and breast cancer type 2 susceptibility protein pathways (61). Considering the increased susceptibility of rectal cancer to the cumulative effects of

Table II. Different tumor organoids contained in reviews, especially applications in radiotherapy.

Tumor organoids	RT dose, Gy	Applications	(Refs.)
RC	0-8	RC tumoroids display varying sensitivity to ionizing radiation, which corresponds to clinical radiotherapy responses	(58)
Locally advanced RC	8	PDOs predict LARC patient responses in the clinic and may represent a companion diagnostic tool in RC treatment	(59)
Colorectal cancer	Combining a low dose of TQ (3 μM) with IR (2 Gy)	TQ sensitizes cancer cells and stem/progenitor cells to radiation mainly through the inhibition of cell survival, DNA repair and stemness in addition to regulating major pathways implicated in this process	(60)
Duodenum, ileum, jejunum and colon	0.003 (Gy/h)-30(Gy/h)	Cell competition, through apical junctions and extracellular ligands, might contribute to the dose-rate effect on $Lgr5^+$ cell replenishment	(61)
NPC	0.2-30	Study the radioresistance of the hypoxic sub-volumes in recurrent radioresistant NPC	(64)
Head and neck squamous cell carcinoma	0-10	Recapitulating genetic, histological and functional features for future therapy screening	(65)
Esophageal cancer	5	Reflecting clinical response following neoadjuvant radiotherapy	(66)
Breast cancer	20	Co-culture macrophages with irradiated mammary glands, as a model for studying tumor-stromal interactions, infiltration of immune cells and macrophage polarization within an irradiated microenvironment	(67)
Glioblastoma	3	Stem and non-stem glioblastoma cell populations can be simultaneously cultured to explore new facets of microenvironmental influences and cancer stem cell biology, depending on receiving critical maintenance cues from their microenvironment	(68)

RT, radiotherapy; RC, rectal cancer; PDO, patient-derived organoids; LARC, locally advanced rectal cancer; TQ, thymoquinone; IR, irradiation; NPC, nasopharyngeal cancer; Lgr5, leucine-rich repeat-containing G protein-coupled receptor 5.

radiation (23), improvements in intestinal protectors and radiation techniques, such as intensity-modulated radiotherapy and volumetric-modulated arc therapy, can partially reduce damage to the surrounding pelvic tissues. However, radiation proctitis remains a challenging issue, and a definitive cure is still elusive.

In 2017, Schwartz *et al* (62) applied intestinal organoids to decellularized matrices, forming a monolayer epithelial structure reminiscent of the intestinal surface, which could be used to mend sites affected by radiation-induced injury. Subsequently, Jee *et al* (63) successfully transplanted colon organoids onto the irradiated mucosa of mouse rectums with damaged tissue, leading to the restoration of the epithelial structure. This model can even indirectly highlight the importance of the internal environment during radiotherapy. When exposed to 4 Gy of radiation, *in vitro* intestinal organoids experience a significant decrease in the number of $Lgr5^+$ stem cells, making lesion repair challenging. However, at the same radiation dose *in vivo*, reserve stem cells are activated, resulting in a rapid increase in $Lgr5^+$ cells and expediting the repair of damaged tissue (57,61). Table II provides a detailed summary of tumor organoids that are presently being utilized in studies, focused on radiotherapy dose (58-61,64-68).

Radiotherapy with immunotherapy of GITOs. Radiotherapy elicits a range of immune-related responses, both locally and systemically. A key objective of our research has been to induce antitumor immune effects that improve the ability of the immune system to recognize and combat malignant cells, ultimately aiming to control and eradicate tumors. The immune system primarily achieves this by promoting the infiltration of effector T cells, mesenchymal stem cells (MSCs) and macrophages into the tumor microenvironment (TME). This process involves upregulating transforming growth factor-β (TGF-β) to activate the signal transducer and activator of transcription-3 (STAT3) pathway, thereby influencing the post-radiotherapy immune-related biological behaviors (62-63,69).

Radiotherapy generates free radicals and oxidative stress through high-energy generation, causing cellular damage. It has been observed that high doses of radiation (>10 Gy), can lead to adverse effects, including vascular endothelial damage, reduced blood supply and reduced oxygen-carrying capacity (70). This, in turn, reduces the recruitment of effector

T cells, reducing the immunogenicity of the tumor and its abscopal effect (63). Consequently, single high-dose radiotherapy does not seem conducive to triggering a sustained systemic immune response.

MSCs, integral to the tissue microenvironment, exhibit multifunctionality and immunoregulatory capabilities. The cells can stimulate vascular regeneration, which is highly relevant for repairing radiation-induced lesions. Cultivating MSCs alongside digestive tract tumor organoids results in a more biologically pertinent model (71). Radiotherapy directly eradicates tumor cells, and immunotherapy aims to strengthen the patient's antitumor abilities; therefore, obviously, the combination of radiotherapy and MSCs can synergistically leverage their respective strengths and enhance the therapeutic outcome (72).

Gong *et al* (73) observed that MSC transplantation promotes the Wnt/Notch signaling pathways, encouraging the proliferation of ISCs and promoting the regeneration of the epithelium in mice with irradiation-induced intestinal injuries (74). Similarly, Moussa *et al* (75) found that this model overexpresses genes such as gremlin 1 DAN family BMP antagonist and twisted gastrulation BMP signaling modulator 1, which by inhibiting the BMP signal, co-operatively promote the proliferation of $Lgr5^+$ stem cells, contributing to lesion repair after irradiation. This is particularly significant in mice subjected to extracorporeal irradiation after allogeneic bone marrow transplantation.

Moreover, the IL-22 dimer/Fc fusion protein F-652 has been shown to significantly protect intestinal $Lgr5^+$ cells from radiation-induced damage *in vivo*. This treatment also helps mitigate intestinal pathological damage and reduces mortality associated with graft-vs.-host disease (76).

5. Other systems of tumor organoids in radiotherapy

GITOs are widely employed in radiotherapy, for both clinical treatment and fundamental research. Additionally, the development of tumor organoids for other areas, such as the nervous system, head and neck, and reproductive system, has been increasing. However, developing cerebral cortex organoids involves addressing complex challenges related to nutrient supply and neural regulatory functions. Furthermore, the graded regulation and feedback mechanisms of the reproductive system still require further refinement. Research on head and neck tumor organoids is currently quite comprehensive. Tumors originating in the head and neck region are characterized by high invasiveness, a strong potential for metastasis and a high recurrence rate (1). Historically, they were often treated as a single type of solid tumor, which led to treatment failures. Extensive two-dimensional cell experiments have deepened our understanding of the heterogeneity of head and neck cancers (62,63). Presently, the primary focus of organoid models for head and neck cancers is on oropharyngeal and nasopharyngeal cancers. The former exhibits notable differences in terms of the outcomes of radiotherapy, while the culture systems of the latter show variations in composition, concentration and product batch numbers (64).

Ionizing radiation is widely employed to slow the growth of glioma; however, its clinical efficacy is limited due to brain edema and radiation-induced brain conditions, including loss of consciousness (77). Liu *et al* (12) identified long non-coding RNAs (lncRNAs) as potential therapeutic targets through glioma organoid screening. The knockout of these lncRNAs increased tumor cell sensitivity to radiotherapy. Additionally, research indicates that treatment regiments combining vemurafenib (Zelboraf) and cobimetinib (Mekinist) can significantly improve the low remission rates of postoperative radiotherapy and temozolomide chemotherapy for patients, expediting clinical decision-making (78-81). Driehuis *et al* (65) found that pharyngeal cancer organoids exhibit particular sensitivity to radiotherapy in clinical practice. There are reports of patients remaining recurrence-free for up to 5 months after receiving 48 Gy of radiation treatment, aligning with the results of complete local remission in organoids following radiotherapy (65). Patients with oropharyngeal cancer often experience reduced saliva production and xerostomia, post-radiotherapy. Peng *et al* (82) discovered that reducing the secretion of senescence-associated secretory phenotype factors in salivary gland organoids can delay the aging of salivary gland stem/progenitor cells, providing a targeted solution for radiotherapy-induced salivary secretion dysfunction. Seol *et al* (83) analyzed the effects of radiation doses (0-12 Gy) on cervical cancer organoids and found that the organoids could serve as a suitable *in vitro* platform for predicting radiation sensitivity. While most of the aforementioned solid tumor lesions are often localized, lymph nodes are distributed throughout the body and are frequently affected during tumor metastasis. After breast cancer surgery, adjuvant radiotherapy and lymph node dissection are often necessary, but these procedures can cause substantial damage to lymphatic vessels, resulting in complications such as blocked lymphatic drainage and edema in the affected areas. Lenti *et al* (84) transplanted lymphatic organoids into areas of the mouse where lymph nodes had been removed. These transplants fully integrated into the endogenous lymphatic system, restoring lymphatic drainage and alleviating edema problems. Therefore, injecting lymphatic organoids into the lesion sites may help restore lymphatic drainage.

In clinical practice, radiotherapy is the primary treatment modality for nasopharyngeal cancer. However, complications during treatment can further reduce the 5-year survival rate of patients. Therefore, it is crucial to comprehensively address the issue of radiotherapy dosage, considering the patient's disease stage and physical condition. Insufficient dosage may lead to suboptimal treatment effects, while excessive dosage could exacerbate damage to the surrounding tissues (85). Nasopharyngeal cancer organoids exhibit an impressive 88% positive predictive value and a perfect 100% negative predictive value, providing crucial insights for accurately modeling the association between nasopharyngeal cancer tissue and radiotherapy dosage. This promotes the translation of basic research into clinical practice (86).

Nasopharyngeal cancer is categorized as a lymphoepithelial tumor, with its growth and proliferation intricately involving immune cells and the TME (9). Microfluidic technology enables the simulation of this growth environment, gradually diffusing oxygen, growth factors and nutrients, while modeling cell heterogeneity resulting from changes in microenvironment concentration. Compared with conventional 2D culture methods, microfluidic technology more

faithfully replicates the actual proliferation rate of *in vivo* tumor cells (45). Current research predominantly centers on oxygen supply. Hypoxia results in a decrease in reactive oxygen species in irradiated cells, leading to the induction of hypoxia-related transcription factors and subsequent radiotherapy resistance (87). In clinical practice, a fractionated irradiation approach can progressively oxygenate hypoxic tumor cells, resulting in a gradual therapeutic effect. Moreover, clinical cases of local recurrences in pleomorphic glioblastomas and breast cancer, in addition to nasopharyngeal cancer, are often linked to unresected hypoxic cancer cells. Rycaj and Tang (88) showed that increasing the radiation dose to hypoxic organoids by 1.4 times could effectively eradicate recurrent tumor lesions. Consequently, utilizing nasopharyngeal cancer organoid models can be crucial for optimizing radiation dosage and adjusting radiotherapy plans. Hill *et al* (89) indicated that the platelet-derived growth factor receptor-β/vascular endothelial growth factor-2/STAT2 signaling pathway induces radiotherapy resistance in ovarian cancer organoids by influencing the oxygen-dependent glycolysis pathway. Additionally, in hypoxic environments and culture systems, various cytokines, such as TGF-β, the FGF family, EGF and hepatocyte growth factor, can induce or increase the receptiveness of nasopharyngeal cancer organoids to epithelial-mesenchymal transition induction signals. This aids in studying the impact of radiotherapy on tumor metastasis (90).

6. Discussion

Tumor organoids have played a crucial role in various aspects of radiotherapy-related research, including organ development, injury repair, regeneration studies and microenvironment homeostasis. The ability of organoids to maintain stable genetic traits throughout long-term passages offers significant advantages in constructing patient resource banks, gaining substantial attention in cancer research and clinical translation. However, the considerable size differences between tumor organoids and *in vivo* tissue organs present challenges. For example, the extensive digestive tract, which can reach up to 16 to 23 feet in length, dwarfs the considerably smaller tumor organoids (49). Additionally, complex, multi-tiered organs, such as the lungs and kidneys, pose challenges for effectively integrating organoids into *in vivo* tumor tissues due to their limited maturity and integrity. Additionally, the absence of neuroregulatory factors further complicates the modeling of diseases with high neural regulation, such as ulcerative colitis. These challenges highlight several issues that need to be addressed (91).

Several factors, including specimen source, culture components, immune cells, stromal cells and the microenvironment, are crucial in the construction of tumor organoids. Compared with the use of iPSCs and surgically resected tissues, core biopsies contain a lower percentage of tumor cells, resulting in a cultivation success rate of <20% (9). Furthermore, uncleared normal tissue can compete with tumor growth, interfering with the intended formation of organoids. Certain tumor tissues, such as esophageal cancer tissues, are particularly susceptible to bacterial and/or fungal contamination due to local narrowing and food obstruction resulting from pathological conditions when attempting to culture organoids from primary tissues (92). For rare malignancies such as chordomas, although organoid biobanks have been successfully established, research related to radiotherapy remains unexplored (93).

Patient tissue specimens serve as sources of tumor stem cells, with the ECM in the culture medium containing varying types and concentrations of factors that influence organoid quality and area parameters (94). While Matrigel derived from Engelbreth-Holm-Swarm mouse sarcoma cells is commonly used in constructing organoid culture systems, its tumorigenic potential renders it unsuitable for clinical-grade cultures. Consequently, studies such as that by Jee *et al* (63) have explored the use of fibronectin from connective tissues, showing their promise for clinical regenerative therapy and the treatment of complex conditions such as fistulas and refractory ulcers (95). Furthermore, cellular experiments indicate that the ECM significantly affects tumor growth, invasion and metastasis. Tumor cells that survive radiation exhibit increased invasiveness, migrating to distant sites independently of enzymatic conditions. Thus, tumor organoids provide a valuable platform for studying tumor invasion and culture matrices, effectively addressing a significant proportion (up to 90%) of clinical concerns (96). In addition to the ECM, various additives are essential for constructing organoids, for freshly obtained specimens, the ϱ-kinase inhibitor, Y-27632, is necessary to reduce anoikis-related apoptosis (22). Within tumor organoid cultures, selective removal of Wnt3a is crucial to prevent overgrowth. Therefore, the adjustment of cytokines within the culture system is essential (30). Although stem cells, the ECM and additive components form the foundation of the organoid culture system, a significant gap exists between this system and the growth environment of solid tumors. Introducing immune cells through co-culturing is a preliminary step in enriching the system. For instance, co-culturing radiation-exposed breast cancer organoids with macrophages can induce chemokine ligand 2 chemotaxis, increasing invasive phenotypes and promoting cancer cell migration (67).

Upregulating cytokines in the culture system can also trigger TGF-β signaling, leading to increased secretion of IL-10 by macrophages and inhibiting immune responses. Co-culturing tumor cells post-radiation with monocytes can also downregulate cytochrome P450 family 1 subfamily A polypeptide 1, impacting tumor cell transcription (97). These findings underscore the significance of immune cell-organoid interactions in more accurately replicating actual tumor responses.

When utilizing tissue-cultured organoids, the persistence of uncleared fibroblasts can lead to their activation and dominance. These fibroblasts may then consume the cytokines needed by the organoids and encase the entire structure, thereby inhibiting organoid growth (98). Additionally, immune cells act as essential intermediaries connecting stromal cells and the microenvironment. Stromal cells influence the migration, adhesion and activation of immune cells in the context of radiotherapy. Microfluidic technology can integrate all three components within a single system where fluid flow velocity in the microenvironment can impact the immune functionality of T cells. This offers a platform for deeper exploration of radiotherapy and immunotherapy in tumor organoid models (99).

Hypoxic damage to the vascular endothelium and poor blood circulation also contribute to increase the resistance to radiotherapy. Reduced oxygen supplementation can alter the TME, thus affecting organoid responses to radiotherapy and immunotherapy. For instance, head and neck tumors, which have a rich blood supply, demonstrate significantly higher sensitivity to radiotherapy compared with tumors in the extremities. Furthermore, with prolonged culture periods, aging cells may appear within the interior of the organoid and its proliferative boundaries, indicating that oxygen gradients in the microenvironment and cell proliferation can contribute to cellular aging. Despite the millimeter-level precision achieved by stereotactic body radiotherapy, which minimizes radiation doses to surrounding structures, including bone marrow, cancer-related fatigue (CRF) is inevitable. CRF is a subjective sense of fatigue commonly experienced by radiotherapy patients, which cannot be replicated using organoid models (100).

7. Challenges and future prospects

Radiotherapy is a cornerstone in the arsenal of treatments against malignant tumors and is crucial for ~70% of patients with cancer at various stages of their therapeutic journey (101). Radiotherapy is particularly vital in advanced stages or in cases of recurrent malignancies, where it serves as a key strategy not only in mitigating clinical symptoms, but in significantly prolonging patient survival and enhancing quality of life. The field of radiotherapy has seen considerable evolution in recent years, marked by rapid technological advancements and continuous refinement of its underlying physical principles. This evolution has propelled tumor radiotherapy into an era of heightened precision and sophisticated intelligence. The innovative progression of radiotherapy techniques, along with interdisciplinary synergy, heralds a transformative era in cancer treatment.

The landscape of radiation oncology has undergone significant transformation, evolving from conventional two-dimensional radiotherapy, which focused on precise target delineation and meticulous radiation delivery, to cutting-edge integration of genomics- and radiomics-guided precision radiotherapy. Today, we are witnessing a groundbreaking shift towards integrating artificial intelligence and systems biology, marking a new era of intelligent radiotherapy. This rapid advancement is further underscored by the introduction of novel equipment and technologies in the field. A prime example is the incorporation of innovative flash technology (102) in medical linear accelerators. This advanced technology enables the administration of ultra-high radiation doses with unprecedented precision in beam flux control, showing profoundly beneficial biological effects in clinical settings. Such advancements not only redefine the potential of radiotherapy but also emphasize its evolution towards a more patient-centric approach.

Moreover, the integration of radiotherapy with surgical procedures, chemotherapy, immunotherapy and targeted antibody-drug conjugates (103) represents a synergistic approach that significantly enhances treatment effectiveness. The emergence of tumor organoids marks a pivotal advancement in clinical applications. There have been previous reviews of organoids. For example, Lv *et al* (104) systematically reviewed the construction of the TME, cell components and methods for creating tumor organoids. Radiotherapy, especially in CRC, rectal cancer and cervical cancer, just has an applicational role. The review by Nagle and Coppes (9) aimed to explore radiotherapy with organoids, and the shortcomings of existing organoid models in radiotherapy and immunity in the field of radiation biology research. The present review placed an emphasis on transformation and the clinical applications in radiotherapy of constructed diverse tumor organoids (not only for CRC, but all included diseases), especially in immunotherapy after irradiation.

By facilitating the acquisition of preclinical data on radiotherapy (and/or other therapeutic modalities) in an *ex vivo* setting, these organoids provide a forward-looking framework for precise treatment strategy selection. This innovative approach fosters a customized treatment landscape for diverse cancer patients, optimizing therapeutic outcomes; it maximizes patient benefits, strategically reduces healthcare costs, and mitigates the incidence and severity of side effects, thereby enhancing the overall treatment experience.

However, research exploring the intersection of tumor organoids and radiotherapy remains relatively limited. Given the inherent genomic instability of cancer cells, mutations may occur during their treatment with radiotherapy. Therefore, future research endeavors could focus on investigating the genomic evolution within cancer organoid models before and after radiotherapy.

In the coming years, investigations associated with tumor organoids may primarily focus on elucidating the mechanisms underlying cancer resistance in radiotherapy, refining screening and staging methodologies, identifying prognostic and treatment-associated biomarkers, optimizing therapeutic interventions for distinct tumor subtypes and spearheading novel therapeutic modalities.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JY, KW and YT conceptualized the study. KW was responsible for investigation of the literature. YT provided resources. DZ supervised the study and assisted in revising the manuscript. JY wrote the original draft. JY and KW reviewed and edited the manuscript. All authors have read and approved the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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