Integration of organoids in peptide drug discovery: rise of the high-throughput screening

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Abstract

Organoids are three-dimensional cell aggregates with near-physiologic cell behaviors and can undergo long-term expansion in vitro. They are amenable to high-throughput drug screening processes, which renders them a viable preclinical model for drug development. The procedure of organoid-based high-throughput screening has been extensively employed to discover small molecule drugs, encompassing the steps of generating organoids, examining efficient drugs in organoid cultures, and data assessment. Compared to small molecules, peptides are more straightforward to synthesize, can be modified chemically, and demonstrate a high degree of target specificity and low cytotoxicity. Therefore, they have emerged as promising carriers to deliver drugs to disease-associated targets, and could be efficient therapeutic drugs for various diseases. To date, organoids have been used to evaluate the efficacy of certain peptide agents; however, no organoid-based high-throughput screening of peptide drugs has been reported. Given the advantages of peptide drugs, there is an urgent need to establish organoid-based peptide high-throughput screening platforms. In this review, we discuss the typical approach of screening small-molecular drugs with the use of organoid cultures, as well as provide an overview of the studies that have incorporated organoids in peptide research. Drawing on the knowledge gained from small molecular screens, we explore the difficulties and potential avenues for creating new platforms to identify peptide agents using organoid models.

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Running title: Organoid-based high-throughput peptide screening

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Abstract

Organoids are three-dimensional cell aggregates with near-physiologic cell behaviors and can undergo long-term expansion in vitro. They are amenable to high-throughput drug screening processes, which renders them a viable preclinical model for drug development. The procedure of organoid-based high-throughput screening has been extensively employed to discover small molecule drugs, encompassing the steps of generating organoids, examining efficient drugs in organoid cultures, and data assessment. Compared to small molecules, peptides are more straightforward to synthesize, can be modified chemically, and demonstrate a high degree of target specificity and low cytotoxicity. Therefore, they have emerged as promising carriers to deliver drugs to disease-associated targets, and could be efficient therapeutic drugs for various diseases. To date, organoids have been used to evaluate the efficacy of certain peptide agents; however, no organoid-based high-throughput screening of peptide drugs has been reported. Given the advantages of peptide drugs, there is an urgent need to establish organoid-based peptide high-throughput screening platforms. In this review, we discuss the typical approach of screening small-molecular drugs with the use of organoid cultures, as well as provide an overview of the studies that have incorporated organoids in peptide research. Drawing on the knowledge gained from small molecular screens, we explore the difficulties and potential avenues for creating new platforms to identify peptide agents using organoid models.

Key words

organoids, high-throughput screening, peptide-drug conjugates, therapeutic peptides, peptide-based radiopharmaceuticals

Introduction

Peptides consist of 5-50 amino acids and have a molecular weight between small molecules (500 Da) and antibodies (5000 Da)[1, 2]. Unlike small molecules, they are also not susceptible to degradation in the human body, and thus display longer tumor retention. Moreover, peptides can be rationally designed based on the sequence of protein targets or interaction binding sites, affording them higher specificity and selectivity than small molecules. Compared to antibodies, the smaller peptides have a shorter clearance time from healthy organs and can be easily synthesized or chemically modified [3-6]. Therefore, peptides are emerging as efficient treatment modalities for various diseases. They can be used as carriers for delivery of drugs to disease-associated targets[7]. For example, peptide-conjugated radionuclides are an efficient approach to delivering local radiation to cancer targets and killing tumor cells[1, 8, 9]. Cell-penetrating peptides are potential carriers to send drugs across the blood-brain barrier into the brain. Many peptides also show remarkable therapeutic efficacy, which could also serve as a treatment modality[10-12]. Multiple peptide-based drugs have been approved for clinical practice, highlighting the need for further studies to develop more efficient peptide drugs [6].

However, one of the largest gaps between basic research and clinical application for screening peptide drugs as potential treatments derives from the differences between existing preclinical models and human[13]. The animal models and two-dimensional (2D) cell lines cannot fully preserve characteristics of in vivo human cells, contributing to the high failure rate of tested drugs in phase I–III clinical trials, resulting in a significant waste of medical resources[14-16]. The emergence of organoids provides new insights into establishing a novel model system. Organoids are three-dimensional (3D) multicellular constructs primarily generated from pluripotent stem cells (PSCs) and adult stem cells (ASCs) through self-organization and self-renewal[13].

They can recapitulate the structural and functional features of their in vivo counterparts[17-19]. Compared to time-consuming animal models, organoids are easy to establish and manipulate[20-29]. Importantly, they can be used to study the mechanism of human diseases that are difficult to model in animals[25, 30, 31]. Although human cells can be maintained under two-dimensional conditions, they almost lose the features of native organs due to the lack of a similar microenvironment[24, 32, 33]. The three-dimensional cultured organoids exhibit near-physiologic cellular composition and behaviors, and maintain genome stability even after long-term expansion[18]. These advantages make organoids suitable preclinical models for high-throughput drug screening, which help to exclude a series of resistant drugs in the human body, which thus provides an effective approach to develop new drugs (Figure 1)[34-37].

A wide range of studies have focused on evaluating the feasibility and utility of organoids in small molecular drug screens, from investigating drug response to establishing organoid-based high-throughput screening platforms[13, 38, 39]. Although there are several applications of organoids in studying peptide-based drugs, a high-throughput organoid-based platform for screening peptides has not been reported. Given the advantages of peptides, we believe the organoid models will facilitate the development and clinical application of peptide-conjugated drugs and therapeutic peptides. This review discusses the advantages of organoids in peptide drug screens and how we learn from organoid-based small molecular drug screening platforms based on the feature of peptides. In the first part, the typical studies involving small molecular drug application in organoid models are introduced, and an overview of the methodology and theory of these studies are provided. The second part discusses the recent attempts to introduce peptide drugs in organoid models and presents the perspective and challenges of organoid-based high throughput peptide screening platforms.

Organoid-based small molecular drug screen platform

The successful establishment of multicellular organoids simulating the structure and function of native organs has highlighted their remarkable advantages in high-throughput drug screening. At first, studies were focused on investigating whether organoids are capable of responding to drugs, and whether the drug response was similar to traditional model systems and humans. Dekkers et al. used cystic fibrosis (CF) patient-derived rectal organoids expressing different cystic fibrosis transmembrane conductance regulator (CFTR) mutations to investigate their responses to two drugs, VX-770 and VX-809. The drug responses in the organoids matched with the clinical trial data, and the data from patient-derived rectal organoids provided evidence for selecting VX-770 as a treatment for patients carrying rare CFTR mutations[40]. Human primary liver cancer organoids were established and shown to preserve the histological architecture, gene expression pattern and native tumorigenesis of their parental tumors. Using the organoid cultures, ERK (extracellular regulated protein kinases) inhibitor was identified as a potential drug to treat primary liver cancer, suggesting the feasibility of primary liver cancer organoid models in drug screening and basic research[41]. In addition, Georgios et al. reported a living biobank of colorectal and gastroesophageal cancer patient-derived organoids, the drug response of these organoid models was matched to the tumor genotypes. They also compared the drug responses in organoids and organoid-derived tumor xenograft models with patients in clinical trials, demonstrating the viability of organoid models for modeling patient responses in clinical trials [42].

As progress in organoid culture technology has been made, establishing an organoid-based high-throughput drug screening platform has become the focus of organoid research[43-47]. Mills et al. described a high-throughput multicellular human cardiac organoid platform and used this system to identify pro-proliferative candidates (a 5000-compound library was screened) with minimized side effects on cardiac contractility and rhythm[48, 49]. Notably, the combination of this platform with contractile assays was the key point for the rapid assessment of the drug response in organoids. Human pluripotent stem cell-derived lung organoids and colonic organoids were used to establish infected models of COVID-19, confirming the applicability of both organoids in SARS-CoV-2 infection research.[50]. In addition, the organoids were used to screen a library of FDA-approved drug candidates. Several drugs were found to inhibit SARS-CoV-2 entry in organoids specifically. This study was reported in 2020, when the COVID-19 pandemic was just starting to spread worldwide, which thus further highlights the advantages of human organoid model systems for effective and rapid drug discovery, especially for severe acute infectious diseases.

Patient-derived tumor organoid-based high-throughput screening platforms are also widely used for discovering anti-cancer drugs[36, 51-54]. Yuan et al. established patient-derived gallbladder carcinoma (CBC) organoid lines recapitulating the original in vivo tissues[55]. Two effective anti-tumor compounds that suppress CBC organoids growth were identified by screening a panel of compounds targeting CBC-specific signaling pathways. The immunohistochemistry results from patients and healthy individuals suggested the therapeutic value of these anti-tumor drugs. This study proves that patient-derived organoids are amenable to investigating the sensitivity of a large quantities of compounds accurately. The major challenges in developing anti-cancer drugs include genetic heterogeneity, progressive growth, and metastasis of tumor cells. Patient-derived tumor organoids thus are valuable model systems for drug discovery and precision oncology [16]. A Food and Drug Administration (FDA)-approved drug with therapeutic potential was selected after high-throughput drug screening using treatment-resistant and metastatic breast tumor organoids from patients' tissues[56]. This study indicated the feasibility of patient-derived tumor organoid model systems to uncover treatment drugs for cancer patients with different tumor phenotypes, including rare ones. Similarly, Toshimitsu et al. reported a robust drug screening platform applicable to a wide range of patient-derived colorectal organoids. They used suspension culture with agitation, allowing for the efficient expansion of organoids, which substantially facilitates the implementation of fast, personalized, tumor-type-agnostic drug testing in a clinically relevant timeframe[57]. Tumor microenvironment plays an essential role in modulating tumor progressive growth and metastasis. Patient-derived tumor organoids can also be used to screen drugs through interaction with critical factors in microenvironments, such as immune cells. Tumor immunology has become a crucial aspect of targeted cancer therapy, which mainly relies on the activation and killing function of cytotoxic T cells. A high throughput drug screening platform based on the co-culture of patient-derived tumor organoids with tumor-specific $CD8^+$ cytotoxic T lymphocytes was developed to discover potential drugs for improving neoantigen presentation, and T-cell mediated cytotoxicity[58].

The prerequisite for establishing an organoid-based drug screen platform is optimal organoid culture conditions [13]. Organoids with a high degree of cell proliferation in vitro and without excessive cell death are suitable for living biobanking and high-throughput screens. Moreover, the organoids preserve the gene expression profile, genomic stability and histopathology features of their original parent tumors could serve as potential preclinical models for drug discovery. In addition, the fewest supplements in culture medium for maintenance of organoids were recommended to avoid alterations in tumor biology[53].

Secondly, the protocol of high-throughput drug screening suitable for organoids is the core [36, 59]. Despite there are differences between the reported protocols, the main procedures are generally similar. The procedures are briefly described below. The steady-state organoids (or dissociated single cells) are seeded in multiwell plates, such as 384-well plates and 96-well plates. Chemotherapeutic agents are easily dispensed to the multiwell plates using a drug dispenser. The component distribution layout including a series of drug candidates with different concentrations, positive control and negative control can be generated using the corresponding software. After incubation with drugs for several days (depending on the features of the original tissues and drugs), a CellTiter-Glo assay is used to detect cell viability as indicated by intracellular ATP levels. The luminescence signal from each well represents the cell viability readout. The IC_{50}, area under the curve (AUC) or growth rate inhibition (GR) metrics, which indicated the effect of drugs, could be measured using the readout.

Thirdly, despite cell viability, the corresponding function analysis of organoids after treatment with drug candidates is also critical to select effective therapies. Whether additional functional analysis is needed and how to perform the experiments depend on the biology of the disease. For cancers, the aim of therapeutic drugs is to kill the tumor cells, thus, cell viability is usually sufficient to assess the drug effects. However, additional assays are necessary for diseases for which the treatment aims to alter the cell behaviors, such as other kinds of readout reflecting the cell function or custom-designed luciferase reporter systems indicating the interaction of downstream pathways that are activated by drug application.

Applications of organoid technology in peptide drug screening

Peptide-based radiopharmaceutical therapy

Radiopharmaceutical therapy (RPT) is a novel treatment for cancer patients, involving the targeted delivery of radionuclides to tumors by conjugating to small molecules, peptides or antibodies[60-71]. RPT has advantages over existing therapeutic modalities, which makes it a safe, precise and effective option to treat many kinds of diseases, especially cancers[72, 73]. Unlike traditional radiotherapy, the radiation administered from radionuclides inside the tumor microenvironments minimizes the injury of normal tissues[60]. Importantly, by combining with molecular imaging, including positron emission tomography (PET) and single-photon emission computed tomography (SPECT), RPT allows diagnosis of the whole-body tumor status and effects of therapy in a non-invasive way, which is rapid and precise[74-76]. To our knowledge, organoid models used in RPT were mostly generated from three-dimensional cultures of tumor cell lines, which are mostly like tumor spheroids[77-94]. Due to the limited cell types, the cell line-derived tumor spheroids cannot present the characteristics of the corresponding tumors. Despite this, they also display some spatial features compared to monolayer cultured cell lines. Most of these studies focused on investigating the therapeutic efficacy on combination with radionuclide-conjugated drugs and potential radiosensitizers. For example, Rea et al. investigated whether disulfiram could promote the anti-cancer effect of ^{131}I-metaiodobenzylguanidine (^{131}I-MIBG) using tumor spheroids derived from human neuroblastoma and glioma cells. The results suggested that disulfiram facilitated the killing function of ^{131}I-MIBG to noradrenaline transporter-positive tumor spheroids, which could serve as a radiosensitizer[91].

Recently, ^{177}Lu-DOTATATE, a peptide receptor radionuclide therapy (PRRT), was approved by USA and European Union (EU) for the treatment of somatostatin-receptor (SSTR)-positive neuroendocrine tumors (NETs)[65, 95, 96]. Meanwhile,^{68}Ga-DOTATATE, ^{64}Cu-DOTATATE (US) and ^{68}Ga-DOTATOC (EU) were also approved as companion diagnostic agents for PET imaging of tumors in patients with SSTR-positive NETs, enabling the combination of diagnostic imaging with targeted therapy[60]. ^{177}Lu-DOTATATE is the only peptide-conjugated radionuclide applied in tumor spheroid study. Tesson et al. used tumor spheroids to evaluate the effectiveness of radiosensitizer drugs when combined with^{177}Lu-DOTATATE, and demonstrated a significantly increased cytotoxicity after combined treatment, indicated by the reduced tumor spheroid growth[97]. Similarly, Lundsten et al. cultured NET cell line-derived tumor spheroids to examine whether the heat shock protein 90 (HSP90) inhibitor onalespib could enhance the efficacy of ^{177}Lu-DOTATATE[93]. The results indicated that the combination of onalespib and^{177}Lu-DOTATATE significantly reduced spheroid growth compared to monotherapies. A p53-stabilizing peptide VIP116 was defined as a radiosensitizer to inhibit neuroblastoma growth and enhanced^{177}Lu-DOTATATE treatment. These results were also observed in tumor spheroid model systems[98]. Radiotracers can also be used to monitor the status of tumor spheroids. Seifert et al. incubated ^{68}Ga-DOTATATE with mCherry-transgenic mouse pheochromocytoma (MPC$^{\mathrm{mCherry}}$) derived tumor spheroids to study the morphologic and metabolic characteristics after HIF2α expression[84]. The reduced cellular uptake of ^{68}Ga-DOTATATE demonstrated the decreased expression level of SSTR2, suggesting that radiotracers can also be used to measure therapeutic effects in organoid-based drug screens.

Multiple potential peptide-conjugated radionuclides are undergoing preclinical tests, such as ^{177}Lu-FAP-2286. Thus, there is a huge gap in introducing patient-derived organoids to RPT, which may enable the discovery of more efficient modalities for the treatment of refractory diseases.

Tumor neoantigens

We have mentioned cancer immunotherapy in the section on small molecular drug screening. This part will overview the detailed mechanism of tumor neoantigens and cancer immunity, then discuss the application of organoids in identifying tumor antigenic peptides.

Tumor neoantigens (also known as tumor-specific antigens) are specifically present on tumor cells but not on normal cells and can be recognized by T lymphocytes [99]. Neoantigens are peptides with eight to ten amino acids produced by the degradation proteasome of intracellular proteins which are the translational products of mutant oncogenesis[100]. These antigenic peptides are then associated with major histocompatibility complex (MHC) class I molecules (human leukocytes antigen (HLA)) in the endoplasmic reticulum and migrate to the

cell membrane. Activated $CD8^+$ T lymphocytes recognize and bind to cancer cells and kill their target cells. This process is a cycle in which the tumor cell death results in the release of more antigens and improves the immune response to tumor cells. Notably, tumor neoantigens are captured by dendritic cells (DCs), then present to T lymphocytes for activating them. Therefore, the core of cancer immunotherapy is to activate the killing function of activated T lymphocytes or promote the presentation of neoantigens[101].

Currently, a wide range of studies focuses on identifying tumor neoantigens, which is critical for the development of new treatment modalities for cancers, such as peptide-based tumor vaccines and personalized drugs to kill tumor cells[102]. Mass spectrometry (MS)-based immunopeptidomics and computational predictions are the commonly used approaches to identify possible antigenic peptides[99]. The latter is based on computer-generated algorithms to identify peptides that could be produced by mutated genes in tumors and are likely to associate with MHC molecules[103]. The selected antigenic peptides are synthesized and used to activate T lymphocytes. The activated cytotoxic T lymphocyte (CTL, $CD8^+$ T lymphocytes) are then co-cultured with tumor cells to evaluate the CTL response for neoantigen screens[104, 105]. Owing to the limited source of patient-derived tumor tissues, studies have focused on using patient-derived organoids to find out the mutated oncogenes and investigate whether the activated CTLs can kill cancer cells. Newey et al. expanded patient-derived colorectal cancer (CRC) organoids and demonstrated the feasibility of MS-based immunopeptidomics of CRC organoids in investigating neoantigen presentation in vitro[106]. Wang et al. generated patient-derived hepatobiliary tumor organoids and found that they preserve most of the characteristics of their parental tissues, such as genetic features and neoantigen landscape. They used organoids as preclinical models to identify the predicted-peptide activated CTLs that exhibited anti-tumor activity[107]. This study provides evidence for the application of tumor organoids as preclinical models for rapid antigenic peptide validation through a prediction-based approach. Few cancer patients share the same neoantigens, and more than 99.95% of neoantigens are present in only one patient resulting from tumor heterogeneity[108, 109]. Patient-derived tumor organoids could avoid these issues and achieve personalized immunotherapy in the future. Since tumor heterogeneity is also characterized by different cell subgroups within the same tumor tissue, Demmers et al. cultured single cell-derived CRC organoid clones from the same patient and demonstrated that the HLA class I peptide presentation landscape was heterogeneous even within one individual[110]. They also indicated that highly conserved antigenic peptides in HLA presentation could be identified using the single-cell derived clonal organoids, which may be a suitable choice for designing anti-tumor vaccines.

The current studies that used organoids to perform tumor antigenic peptide screens are 1ow-throughput. Patient-derived organoids enable high-throughput screening of a large number of computationally-predicted peptides. Furthermore, organoids can undergo extensive expansion, allowing large quantities of material for MS-based immunopeptidomics analysis (Figure 2). Therefore, we propose that the utility of patient-derived organoids as preclinical models to high-throughput identify tumor neoantigens could be a powerful approach to the development of precision therapies for cancer patients.

Cell-penetrating peptides

Cell-penetrating peptides (CPPs) can cross tissues and cell surfaces without causing lethal injury to the membranes. The mechanism of the penetration process remains controversial. Most CPPs are endogenously produced proteins and peptides, including heparin-binding proteins, DNA-binding proteins, antimicrobial peptides and viral peptides[111]. Given the characteristics of CPPs to cross cell membranes, more and more researchers focus on whether they can pass the blood-brain barrier (BBB)[112]. The BBB is a complex microvasculature system mainly consisting of brain endothelial cells (ECs) that are tightly lined in the cerebral vascular lumens. The main function of BBB is to protect the brain. In addition to ECs, astrocytic glia and neurons together are organized into well-structured neurovascular units[113]. The brain ECs express high levels of tight junction proteins, efflux pumps, and specific transporters. The tight junctions between ECs prevent molecules in the blood from entering the central nervous system. The efflux pumps, including P-glycoprotein (PgP), exclude foreign substances from the brain. Specific transporters deliver essential nutrients to the brain, such as glucose and amino acids. Therefore, BBB is the main obstacle to delivering drugs to neural cells and developing effective treatments for central nervous system diseases. Although

CPPs are promising vectors to deliver drugs across the BBB, no CPP-based treatment is currently used clinical practice, partly due to the lack of suitable preclinical models that can accurately mimic the features of BBB to discover potential CPPs in a high throughput way[114]. The widely used in vitro models for BBB are co-culturing of brain ECs (top), astrocytes and pericytes (bottom) in a transwell system[115-117]. Microfluidics could introduce blood flow to stimulate BBB more dynamically[118]. However, these model systems require advanced equipment to establish the platform, increasing the experimental complexity of performing high-throughput drug screens.

Researchers recently reported three-dimensional multicellular structures through self-organization arrangement of brain ECs, pericytes and astrocytes[119]. The in vitro spheroids can recapitulate the complex interactions between each cell type, which is critical cell behavior to maintain the essential function of BBB. Lawler's group modified the method and established BBB organoids that could mimic the essential function of BBB[120]. They, for the first time, investigate whether the BBB organoids are suitable for screening BBB-penetrating drugs. BBB organoids were used to identify several CPPs that could cross the BBB, demonstrating their feasibility and utility as models for cost-effective and high-throughput drug screens. Many peptides are susceptible to being degraded by proteolytic, and show a relatively low ability to cross the BBB. The same group found that peptide macrocyclization could increase the cell uptake of CPPs and found that one macrocyclic analog of transpotan-10 displays improved capacity to deliver across the BBB organoids[121].

Together these studies demonstrated that three-dimensional multicellular BBB organoids can capitulate the complex interactions and arrangements of each cell type, and can reduce experimental complexity (Figure 2). These advantages make BBB organoids ideal preclinical models for high-throughput screening of CPPs that can cross BBB[114]. Furthermore, CPPs are also promising carriers to transfer therapeutic drugs into tumor cells. Therefore, it is necessary to establish tumor organoids that are amenable to the discovery of efficient CPPs with high stability, internalization ability and specificity.

Host defense peptides

The primary biological functions of the naturally-produced peptides, host defense peptides (HDPs, also known as antimicrobial peptides), are immunomodulatory, anti-inflammatory, and anti-bacterial[122]. HDP are peptides with 12–50 amino acids composed of cationic and hydrophobic amino acids that adopt an amphipathic conformation upon folding, usually after contact with membranes[123, 124]. Increasing antimicrobial resistance (AMR) organisms has become a severe issue for the treatment of inflammatory diseases due to the excessive use of antibiotics[125, 126]. The naturally-produced HDPs are promising candidates for developing treatments against the global threat caused by AMR organisms[126]. Similarly, there is also an urgent need to establish novel model systems to screen HDPs as potential drug candidates. Currently, an organoid system called air-liquid interface (ALI) construct are widely used in investigating HDPs[126]. ALI systems are comprised of a porous filter separating the apical and basolateral compartments. Cells cultured on the top chambers grow to multilayers and across the basal-apical threshold, where the medium remains on the bottom of the cell layers and the apical interface is surrounded by air. This system is ideal for studying the biology of tissues interacting with liquid and air in vivo[127]. Patients and healthy lung ALI models were used to examine the HDP expressions. The results demonstrated that they are suitable models to study the essential function of HDPs in respiratory diseases and enable the identification of drug candidates[128-130]. One study using primary ALI models reported that the frog skin-derived HDPs Esc (1-21) and its synthetic derivation both could protect the epithelial integrity when infected by P. aeruginosa[131]. Ritter et al. used ALI cultures with an aerosol delivery system to predict acute local lung toxicity through the assessment of various combinations of HDPs and nanocarriers[132]. This study also indicated the sensitivity of ALI models compared to submerged cultures.

Current studies introducing HDPs to organoid systems are all using ALI models, which involve complex manipulations and equipment, making them unsuitable for high-throughput screens. We believe that real three-dimensional organoid models (infected with microbes) are a potential approach to discovering effective HDPs and their derivations in a high-throughput way.

Other therapeutic peptides

A wide range of therapeutic peptides showed potential effects on various diseases [5, 133], and some of them were investigated using organoid systems. For example, the two Axin-derived staple peptides SAHPA1 and xStAx that target β-catenin were reported to promote the activity of Wnt/β-catenin signaling pathway[134]. The investigation of patient-derived tumor organoids showed that xStAx binds to the VHL ligand to promote intestinal tumor death, highlighting its potential as a novel anti-cancer drug.

Leucine-rich-repeat-containing G-protein-coupled receptor 5 (LGR5) positive intestinal adult stem cells are widely used to generate intestinal organoids. The culture technique is mature, and the derived organoids can undergo long-term expansion without changing the genetic features[135, 136]. The Frizzled 7 receptors (FZD7) are highly expressed in $LGR5^+$ intestinal stem cells and are critical in regulating self-renewal. The development of targeted drugs that bind to FZD7 is a potential approach to investigating FZD7 functions in cancer biology and developing novel regenerative therapy for intestinal epithelium. Nile et al. identified a potent peptide (dFz7-21) that specifically targets FZD7 and changes the conformation of the FZD domain[137]. The treatment of LGR5+ mice intestinal stem cells derived organoids with FZD7 suppressed stem cell function and disrupted the bud formation by impairing Wnt signaling, proving the utility and feasibility of organoids as preclinical models.

Perspective and challenges of organoid-based high-throughput peptide screening platforms

According to the methods of organoid-based small molecule drug screening platforms, we believe that organoids can also be applied in the high-throughput screening of peptides. This section presents the perspective and challenges of applying organoids in peptide screens based on their characteristics.

Owing to their remarkable binding affinity and specificity, peptides are suitable for transporting cytotoxic drugs to disease lesions[138-140]. However, there is still a wide gap between research and clinical application of peptide drugs. For example, the discovery of organ-specific targeted peptides may further decrease the side effect of this treatment modality. Given that peptides can be rapidly synthesized and easily modified, researchers can generate peptide libraries and perform high-throughput screening of the optimal candidates using organoid models, which is a promising direction for developing more effective peptide-based drugs[3]. Moreover, for peptides that deliver drugs in vivo, although the interactions are present on the cell surface, they may not alter the cell phenotype and cause a therapeutical effect. In RPT, the "cold" radionuclides without radioactivity are often used to label the peptide and prescreen the peptides with high binding affinity to reduce the experimental cost. The commonly used method, CellTiter-Glo assay, determines drug efficacy by measuring cell viability, which is not suitable for assessing the binding affinity of peptide candidates. Multiple approaches are used for traditional specific peptide discovery, such as using fluorescent labeled peptides. The future direction is to modify the existing methods and develop appropriate assays that match the high-throughput organoid culture technology, for direct assessment of peptide affinity. In addition, due to the difference in the emission range of radionuclides[141-143], the peptide-radionuclide conjugates not bound to the cell surface but located near the cells should be investigated to determine whether they can kill the targeted cells, which may cause false-positive results during screening.

Peptides hold high biological and chemical diversity; and most targeting peptides exhibit therapeutic effects on diseases[5]. For example, the expression of immune checkpoint proteins (PD-1 and PD-L1) can be suppressed by targeted peptides for increasing the immune response to kill tumor cells [144-148]. However, the high diversity of nature product peptides poses a challenge to the identification of the above-mentioned tumor neoantigens and bioactive peptides derived from venoms, which are produced by many kinds of organisms, such as plants, snakes, spiders and insects, and are found to interact with tumors and modulate the proliferation, migration, immune response of tumor cells, suggesting potential therapeutic agents for cancers[149-155]. Therapeutic peptides are promising candidates for treating various diseases, and more effective peptide drugs are required to address clinical demands. Thus, high-throughput screening of therapeutic peptides using organoid technology may significantly improve the efficiency for identifying and uncovering the optimal peptides with high binding affinity and ideal therapeutic effects. Furthermore, although peptides

are low-cytotoxic drugs compared to small molecules and antibodies, healthy organoid models can also be used to examine their side effects on normal cells, which is one of the advantages of organoid technology.

Conclusion

Owing to the straightforward synthesis and chemical modification, high target specificity, low toxicity, and broad range of targets, peptides have become a viable option for treating various diseases. This review provides an overview of the studies that employ organoids to assess the effects of peptide candidates, exploring the utility and challenges of high-throughput screening of peptide using organoids in light of the experiences from the organoid-based small molecule screens that have been extensively examined in recent years. Despite the limitations and difficulties in developing an organoid-based peptide high-throughput platform, various techniques can be employed to overcome these issues, including the advancement of organoid technology. Consequently, organoids will be a captivating and novel preclinical model for discovering peptide drugs in the future.

Conflict of Interest

All authors declare no conflict of interest.

Figure 1. The generation procedure and application of patient-derived organoids.

Figure 2. The application of patient-derived organoids in high-throughput screening of peptide conjugated drugs and therapeutic drugs. PDO, patient-derived organoids; CPP, Cell-penetrating peptides.

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Table 1. Overview of recent studies applying organoids in peptide research.

Applications	Organoid systems	Peptide drugs
RPT	NET cell lines derived spheroids	^{177}Lu-DOTATATE
	mouse derived tumor spheroids	^{68}Ga-DOTATATE
Tumor neoantigens	Patient-derived CRC organoids	–
	Patient-derived hepatobiliary tumor organoids	–
	Patient-derived single cell CRC organoids	–
Cell-penetrating peptides	Human primary cell derived multicellular BBB organoids	SynB1, L-2, PreS2-TLM Transpor
	Human primary cell derived multicellular BBB organoids	Perfluoroarene-based, macrocyclic
Host defense peptides	Patients and healthy lung ALI organoids	–
	Human primary cell derived lung ALI organoids	Esc (1-21)
	Human alveolar lung cell line derived ALI organoids	AA139, M33
Other therapeutic peptides	Patient-derived CRC organoids	staple peptides SAHPA1 and xStA
	Adult mice intestinal epithelium derived organoids	dFz7-21 (a peptide specifically bin

Abbreviations: RPT, Radiopharmaceutical therapy; CRC, Colorectal cancer; HLA, human leukocytes antigen; BBB, blood-brain barrier; CPP, Cell-penetrating peptides; ALI, air-liquid interface; HDP, Host defense peptides; FZD, Frizzled.