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Thyroid organoids: advances and applications

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Abstract

Organoids are derived from stem cells under three-dimensional culture conditions through self-assembly, and they can recapitulate the structural and functional characteristics of organs *in vivo* during culture. Organoids can be generated from both normal and malignant tissues. Those derived from normal tissues are widely used in the field of regenerative medicine. Meanwhile, tumour-derived organoids retain the phenotypic heterogeneity and atypia of the primary tumour, thereby providing a reliable *in vitro* model for the study of tumour pathogenesis and treatment. The thyroid gland is one of the most important endocrine organs regulating the body's energy metabolism and growth; however, it is also associated with a high incidence of malignancy. Organoid is an effective tool for thyroid research. Thyroid tumour-derived organoids can inherit the histopathological properties of primary tumours, and thyroid tissue-derived organoids can form follicular structures and secrete thyroid hormones. The above characteristics of organoids provide a reliable way to study the mechanism of thyroid genesis and tumour development *in vitro*. In this review, we focus on current knowledge and strategies for the establishment of thyroid organoids in thyroid regeneration and tumour research aiming to increase our understanding of the pathogenesis of thyroid tumours and the regenerative treatment of patients with hypothyroidism. **(Endokrynol Pol 2023; 74 (2): 121–127)**

Key words: *organoids; thyroid; regenerative medicine; tumour research*

Introduction

Organoids are three-dimensional (3D) *in vitro* cultures derived from tissue stem cells. They form through stem cell-driven self-organization and can simulate the structure and function of natural organs [1]. Organoid culture originated from the isolation and culture of Lgr5+ intestinal stem cells by Clevers et al. in 2009, who constructed intestinal crypt villous organoids that could produce a continuously expanding and self-organizing epithelial structure similar to the human intestine. This novel *in vitro* model paved the way for regenerative medicine and gene therapy [2]. However, the study of thyroid morphogenesis and development lacks a stem cell-derived thyroid organoid model system that can recapitulate the differentiation and assembly of thyroid follicular cells into functional thyroid follicles. In 2012, Antonica et al. induced mouse embryonic stem cells (ESCs) to produce thyroid organoids displaying the morphological and functional properties of thyroid follicles for the first time, and demonstrated their ability to metabolize iodide, thereby establishing the applicability of stem cell regenerative medicine in the treatment of hypothyroidism [3]. In addition to its putative application in thyroid regeneration, organoid technology also has potential for use in research on the occurrence and development of thyroid tumours. The thyroid is an organ with a high incidence of malignant tumours, among which papillary thyroid carcinoma (PTC) is the most common. PTC patients generally have a good prognosis, with a 5-year survival rate of 95–97%; however, approximately 20% exhibit tumour recurrence, metastasis, and radioactive iodine-refractory disease (RAIRD) within 10 years [4]. In addition, some patients with total bilateral thyroid resection experience severe side effects during hormone replacement therapy [5]. Anaplastic thyroid cancer (ATC) is a very rare and aggressive form of thyroid carcinoma, with a median survival of only 6 months due to its highly invasive nature [6]. Organoids can be utilized to clarify the mechanisms underlying tumour occurrence and development as well as for the identification of effective intervention

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targets that can be applied to individualized diagnosis and treatment of patients with refractory and highly invasive thyroid tumours. In addition, thyroid regeneration using organoids may help patients with congenital hypothyroidism or postoperative patients with thyroid cancer, who experience serious side effects after hormone replacement therapy. In this review, we focus on the difficulties associated with thyroid organoid technology, as well as on the current applications of thyroid organoids in regenerative medicine and tumour research. We also discuss the limitations and prospects of this technology.

Organoid technology

Since Clevers et al. [2] first generated intestinal organoids from Lgr5+ intestinal adult stem cells (ASCs), organoid technology has been applied to a variety of human organs. Organoids have unique advantages over traditional two-dimensional (2D) culture and animal models (Fig. 1). First, compared with 2D culture, organoids are complex, 3D, multicellular, self-organizing structures with specific tissue organization and function, which allows for the close connection and frequent interaction of multiple cell types. For instance, interactions among a variety of cell types result in intestinal crypt villous organoids forming a central lumen lined with villous epithelium surrounded by crypt-like domains [2]. Secondly, organoids can be widely amplified during culture while maintaining genomic stability [7]. For instance, the number of nephron progenitor cells (NPCs) is limited, and these cells are difficult to obtain with poor *in vitro* expansion capacity, such as that seen in 2D culture, which hampers research into kidney development and diseases. Under organoid culture conditions, long-term *in vitro* expansion of NPCs can be achieved, while maintaining genome stability, molecular homogeneity, and renogenic potential [8]. Thirdly, compared with animal models, organoids better replicate human physiology and have a higher degree of operability and experimental flexibility. For example, the microinjection of *Helicobacter pylori* into human gastric organoids can successfully recapitulate the typical symptoms of infection by this bacterium, whereas *H. pylori* infection in mice does not progress to ulceration and cancer as it does in humans [9]. Traditional 2D culture does not have accurate cell–cell and cell–extracellular matrix (ECM) interactions [10], uniform distribution of cells in the culture dish, or a nutritional gradient, which weakens the physiological correlation between cells [11]. Furthermore, differences in cell structure and physiology mean that animal models cannot accurately reflect the regulatory mechanisms and cell interactions occurring in human tissues [12, 13]. Combined, these observations indicate that organoids are more suitable for the construction

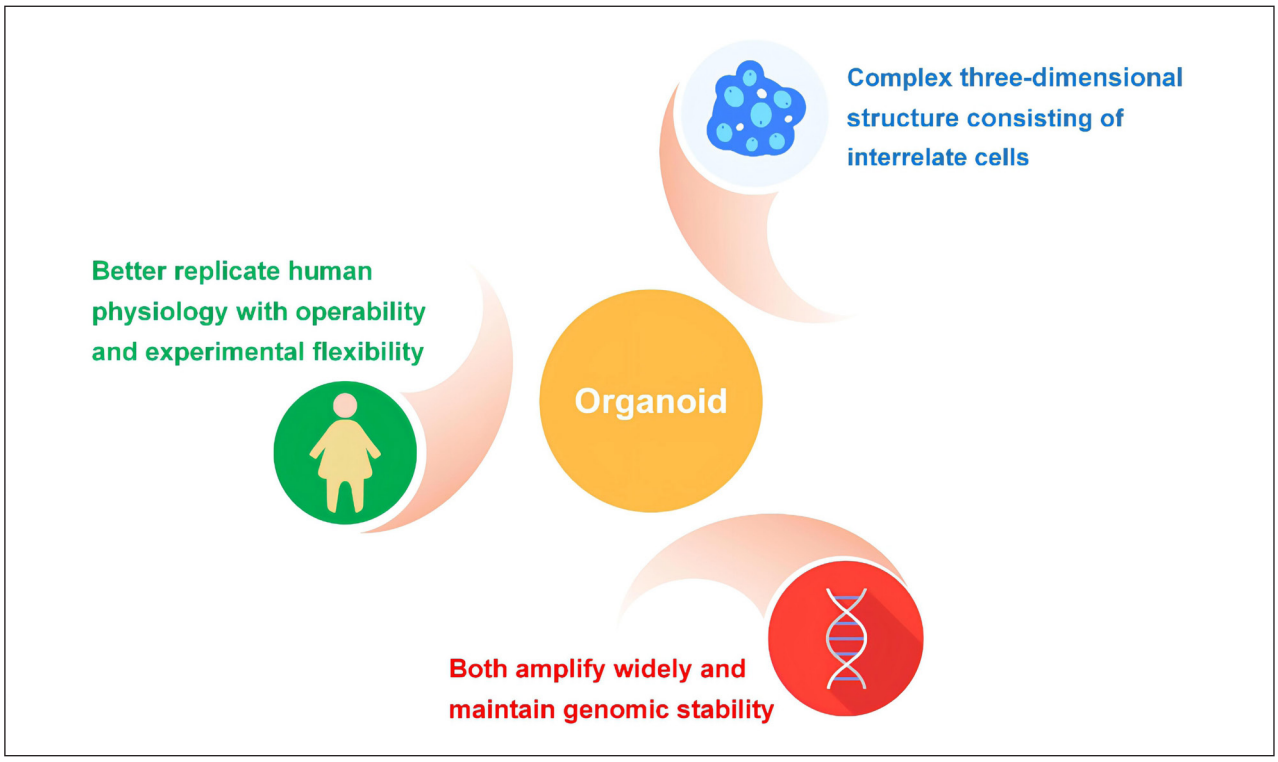

Figure 1. *Advantages of organoids over traditional 2D culture and animal models*

of biobanks and high-throughput screening, and have gradually become a representative tool for studying living organs *in vitro*.

Organoids can be derived from 2 types of stem cells: pluripotent stem cells (PSCs), which include ESCs and induced PSCs (iPSCs); and ASCs, which are resident stem cells specific to differentiated tissues [14]. PSCs can form all tissues of the body and will spontaneously differentiate *in vivo* into a disorganized mass of differentiated tissue, called a teratoma, through the manipulation of factors that control embryonic organogenesis [15]. Methods have been developed to guide the stepwise differentiation of PSCs into embryonic germ layer-restricted organoids, organ-specific organoids, and even specific cell types such as hepatocytes, neurons, and cardiomyocytes [15]. The induction of PSCs for organoid culture requires their differentiation into the 3 germ layers (endoderm, mesoderm, and ectoderm) under specific differentiation signals, followed by multiple induction steps toward a fully differentiated structure, which requires a specific growth factor mixture at each step [16]. Gastric organogenesis requires the addition of activin to generate endothelial progenitor cells from PSCs, followed by the addition of Wnt3a and fibroblast growth factor 4 (FGF4) activators and bone morphogenetic protein (BMP) inhibitors to differentiate these cells into foregut cells, and then retinoic acid to yield posterior foregut cells [17]. Finally, high concentrations of epidermal growth factor (EGF) are applied to generate gastric organoids [17]. The whole process of organogenesis in the human embryonic period is simulated in PSC-derived organoids, rendering them representative of living organs for *in vitro* studies. ASCs are found in differentiated tissues and have the capacity for division and self-renewal as well as for tissue regeneration [18]. Generating organoids from ASCs does not require transcription factor transduction; organoids can be directly induced, making them physiologically compatible with the normal tissues of the host [19, 20]. Stem cells must grow in a specialized microenvironment; namely, a stem cell niche, composed of fibroblasts, immune cells, endothelial cells, perivascular cells or their precursors, ECM, cytokines, and growth factors [21]. Additionally, the growth of stem cells must be regulated by providing cell-to-cell contact and secretory factors [22]. Thyroid organoid culture involves an artificially created microenvironment that allows for the formation of stem cell niches by providing the corresponding cytokines and ECM. The ECM can support cell proliferation, enable cell adherence, and allow nutrient and growth factor diffusion [23]. Stem cells must be in strict contact with ECM components, such as collagen, laminin, and fibronectin, which are important regulators of stem cell behaviour, migration, and differentiation [24]. The cytokines required for thyroid organoid generation include Wnt-3a, R-spondin-1, Noggin (a BMP antagonist), EGF, FGF, thyroid-stimulating hormone (TSH), and Y-27632, a specific ROCK protein inhibitor [25]. Various classes of cytokines play different roles during organogenesis. Wnt and R-spondin-1 play a key role in the self-renewal of multiple types of adult stem or progenitor cells [26]. When Wnt proteins bind to members of the Frizzled family of receptors, the canonical Wnt pathway is activated [27], and this signalling pathway can be further enhanced through the activity of the R-spondin protein [28]; jointly, these factors drive the differentiation of stem cells in culture. Noggin interferes with the binding of BMP to its receptor, thereby antagonizing the function of cytokines that limit stem cell proliferation [29]. EGF is a growth factor for epithelial tissues, and its binding to EGF receptors can induce hyperplasic changes [30]. Saito et al. exposed thyroid cells to different concentrations of TSH for thyroid organoid culture and found that TSH could significantly promote organoid formation in a concentration-dependent manner [25]. Y-27632 has been reported to inhibit premature apoptosis in organoids, enhance the ability of cells to form spheroids, and improve the ability of stem cells to survive and proliferate *in vitro* [31]. To date, organoid technology has been applied to the *in vitro* culture of a variety of human organs, including the establishment of an organoid culture system for thyroid-related research.

Applications of thyroid organoids

Regenerative medicine

Since its establishment, organoid culture technology has experienced rapid development and shown strong application value in the field of regenerative medicine, including by providing a new graft source. Many types of organ models have been applied to regenerative medicine to date, including liver, intestine, pancreas, kidney, skin, and, importantly, thyroid models [32–37]. Longmire et al. reported the directed differentiation of thyroid cells from mouse ESCs in 2012. To achieve this, the authors inhibited bone morphogenetic protein (BMP) and transforming growth factor-beta (TGF-β) in ESC-derived endodermal cells, yielding NK2 homeobox 1 (Nkx2.1)+ endothelial progenitor cells. The subsequent combinatorial induction of BMP and FGF signalling in these Nkx2.1+ cells promoted thyroid lineage specification [37]. This report laid the foundation for ensuing studies on the construction of thyroid organoids. Later, Antonica et al. reported that mouse ESCs could be directed to differentiate into thyroid cells through the transient overexpression of

the transcription factors Nkx2.1 and paired-box gene 8 (PAX8) under doxycycline induction, and that these thyroid cells formed thyroid organoids with 3D follicular structures under thyrotropin treatment [38]. This study confirmed for the first time that mouse ESC-derived thyroid follicular cells could form thyroid organoids *in vitro* [38]. Moreover, based on the results obtained by Longmire et al. with differentiated thyroid cells [37], Kurmann et al. differentiated mouse PSC-derived endoderm cells into thyroid follicular organoids [39]. In the 2 studies [38, 39], the constructed thyroid organoids were transplanted into mice with hypothyroidism induced by radioiodine ablation of their thyroid tissue, leading to the generation of follicular-like tissue that secreted thyroid hormone and the restoration of the level of thyroid hormone to normal; moreover, the regenerated thyroid tissue could be regulated by TSH. These studies suggest that PSC-derived thyroid organoids have a strong regenerative capacity *in vivo* and can compensate for the hypothyroidism caused by the lack of thyroid tissue *in situ*.

In addition to PSCs, organoids can also be generated from ASCs derived from differentiated tissues (Fig. 2A). Lan et al. isolated a side population of cells that can express adenosine triphosphate binding box transporter G2 (ABCG2) from differentiated thyroid cells [40]. These cells had a high nucleus/cytoplasm ratio; they had strong expression of the stem cell markers ABCG2 and octamer binding transcription factor 4 (Oct4); and they could proliferate, form spheres, and differentiate into thyroid cells under the action of TSH [40]. Oct4 is the major transcription factor determining the fate of ESCs as well as that of some ASCs [41]. Although no specific marker of thyroid ASCs has been identified, the above-described research suggests that ASCs may exist in the thyroid. Thyroid organs can be directly cultured from thyroid tissue without undergoing cell reprogramming. Saito et al. cultured surgically excised mouse thyroid tissue fragments after mechanical and enzymatic digestion and established mouse thyroid organoids with normal thyroid function under the action of TSH [25]. These functions included thyroglobulin synthesis, iodine uptake, and the production and release of thyroid hormone [25]. The authors transplanted cultured thyroid organoids into hypothyroic mice, resulting in the generation of thyroid follicle-like tissue capable of iodide uptake [25]. In addition to their normal thyroic function, the thyroid organoids stably expressed thyroid immune markers during both *in vitro* and *in vivo* culture [25]. Ogundipe et al. used mouse and human thyroid tissue for organoid culture and found that the thyroid markers NKX2.1, PAX8, sodium iodide symporter (NIS), thyroglobulin (TG), and thyroxine (T4) were expressed in constructed organoids and remained stable in passage [42]. The transplantation of organoid-derived cells under the renal capsule of hypothyroidic mice led to the formation of thyroid follicular structures that expressed NKX2.1, TG, and T4 [42]. The above results show that thyroid organoids derived from mature thyroid tissue also have a strong regenerative ability. Furthermore, regenerated thyroid tissue can function

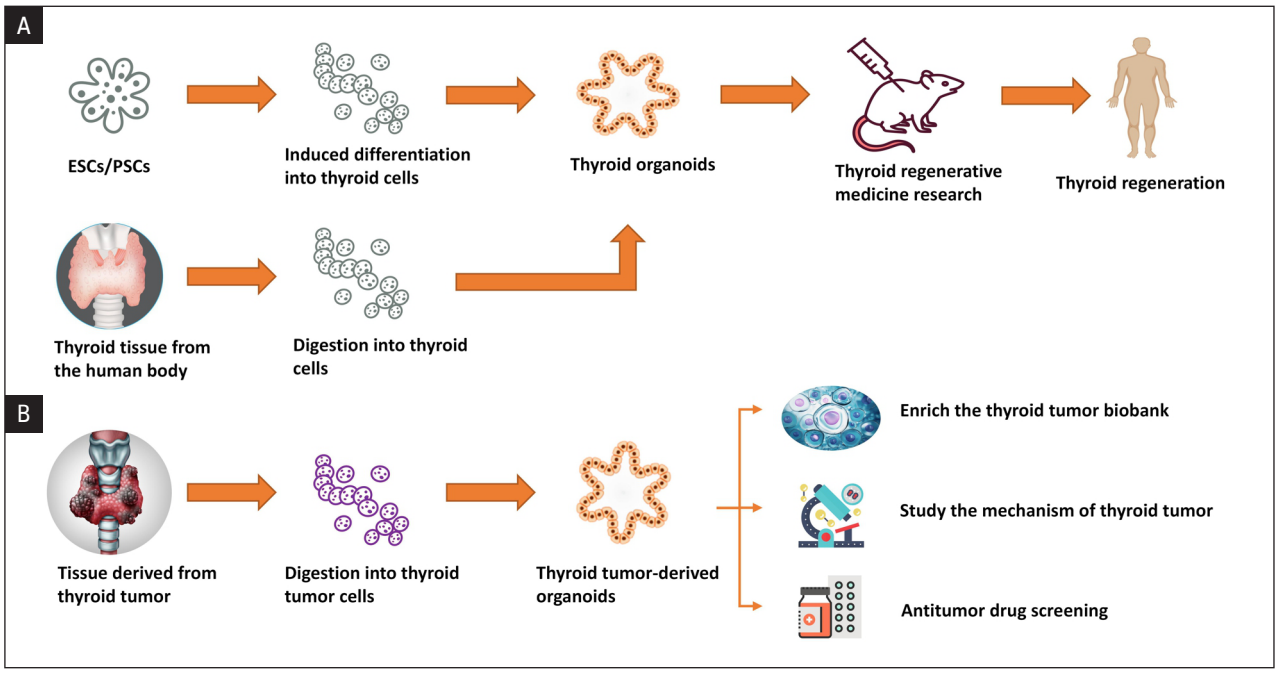

Figure 2. *Culture and application of organoids in thyroid.* **A.** *Normal thyroid organoids;* **B.** *Thyroid tumour organoids*

normally *in vivo* and restore thyroid hormone levels in hypothyroic mice.

Congenital hypothyroidism is the most common congenital endocrine disease in humans, caused by a dysfunctional thyroid gland (15%) or dysplasia (85%) [43]. The incidence rate of neonatal hypothyroidism is approximately 1/2000 [43]. Patients who have undergone bilateral thyroidectomy for thyroid cancer treatment require lifelong hormone replacement therapy owing to the absence of thyroid hormone postoperatively. However, some patients experience serious side effects from hormone replacement therapy after total bilateral thyroidectomy [5]. Organoids consisting of PSC-derived thyroid cells or primary thyroid gland-derived cells can be transplanted into the body and form follicular-like tissue with normal thyroid function, and they may represent an effective means of treating hypothyroidism. Yang et al. [44] developed a method for microencapsulating porcine thyroid cells in alginate-poly-L-ornithine-alginate microcapsules as a thyroid hormone replacement approach. The semipermeable microcapsule membrane allowed the diffusion of thyroid hormones, TSH, nutrients, oxygen, electrolytes, and wastes while blocking that of immunoglobulins, antibodies, and host cells that mediate the immune response [44]. Under TSH stimulation, the encapsulated porcine thyroid cells formed 3D follicular spheres in the inner core of the liquefied alginate microcapsules that released thyroid hormone [44]. In the future, microencapsulation technology may be combined with organoids for *in vivo* transplantation, thereby allowing the transplant recipient to retain normal thyroid function without immunosuppression. This provides a basis for the application of thyroid organoids in the field of regenerative medicine.

Organoids and thyroid tumours

Tumour cells have unique mutational and epigenetic features, exhibiting heterogeneity in gene expression, metabolism, proliferation, and metastatic potential [45]. Organoid cultures can recapitulate this heterogeneity *in vitro* while maintaining genomic stability during passaging. Patient-derived organoids (PDOs) can be used to enlarge small tumour samples and enable the analysis of cancer at any stage in culture, greatly expanding the types of tumour samples that can be propagated and studied in the laboratory [46]. Studies on thyroid cancer organoids are scarce and have mainly focused on surgically removed thyroid cancer tissues. Sondorp et al. generated patient-derived organoids from 13 patients with PTC and 3 with RAIRD, and found that the marker expression of PTC and RAIRD organoids was more like that of the tumour tissue of origin [47]. Furthermore, NIS was not expressed in RAIRD organoids, which may explain why postoperative iodine 131 treatment in patients with PTC is ineffective, leading to patient relapse [47]. DNA sequencing of thyroid cancer organoids combined with drug screening can be applied to individualized treatment in thyroid cancer patients. Chen et al. [48] created organoid cultures of tumour tissues from PTC patients and carried out whole-exome sequencing (WES) in PTC organoids and parental tumour tissues to identify significant mutant genes. The results showed that PTC-derived organoids harboured the same mutated genes as the parental tumours and remained unchanged in long-term *in vitro* culture [48]. The authors then applied antitumour drugs to evaluate the drug sensitivity of PTC organoids, and found that there was a correlation between the sensitivity of PTC organoids and their mutation spectrum [48]. Although most of the PTC-derived organoids with BRAF gene mutation were sensitive to the $BRAF^{V600E}$ inhibitors vemurafenib and dabrafenib, a few were resistant to these drugs, despite harbouring BRAF gene mutation [48]. This observation highlights the value of the combination of WES and organoid drug screening for identifying tumours that are insensitive to targeted drugs [48]. Gene editing can also be applied to thyroid cancer organoids. For instance, Saito et al. introduced the cDNA of $NRAS^{Q61R}$, the main oncogenic driver of thyroid cancer, into thyroid organoids established from p53 knockout (KO) mice, and then transplanted the resulting $NRAS^{Q61R}/p53^{KO}$ thyroid organoids into mice to generate poorly differentiated thyroid cancer (PDTC) [25]. This study not only enriches the thyroid cancer biobank but also reduces the ethical risk and provides a reliable model for thyroid cancer research.

The above-mentioned studies indicate that thyroid carcinoid organoids cultured *in vitro* retain the mutational profile and histopathological characteristics of the primary tumour [47,48]. Accordingly, they can represent living tumours, thus allowing the establishment of cancer models *in vitro* for relevant studies (Fig. 2B). Additionally, thyroid organoids can produce PDTCs in mice through gene editing and transplantation [25], indicating that tumour organoids can be xenografted and that tumour samples can be enriched for laboratory-based analysis. Finally, thyroid cancer organoids, combined with DNA sequencing, can be used to predict the sensitivity of drugs in the human body [48], suggesting their potential as an effective tool for the individualized treatment of patients with thyroid tumours. Several studies have been undertaken on organoid culture and drug screening for gastrointestinal, liver, lung, and breast tumours [49–52]. For example, Vlachogiannis et al. tested the effects of anticancer agents on ex vivo PDOs, and compared the responses of patients in clinical with PDO-based mouse xenograft mod-

els to anticancer agents, they concluded that PDOs can recapitulate drug response of patients and have the potential to be executed in personalized medicine programs [49]. Research on thyroid cancer organoids is currently mainly focused on PTC, while studies on poorly differentiated and undifferentiated thyroid cancer organoids are lacking. More research on this kind of thyroid cancer and its organoids is needed to better understand thyroid cancer pathogenesis and identify effective intervention targets. Organoids will likely become a new tool for physiological and pathological research of thyroid cancer.

Discussion

Unlike traditional 2D culture and animal models, organoid culture technology can lead to the establishment of an ideal model that highly recapitulates the structure, function, and immunophenotype of the thyroid gland and thyroid cancer *in vitro*. Accordingly, organoids can play an important role in tumour research and regenerative medicine. However, the technique is not fully mature, and many limitations in thyroid organoid culture remain. First, there is no uniform criterion regarding the substances required for the culture of thyroid organoids, and the factors used for culture vary slightly in a study-dependent manner. The organoid formation rate is also relatively low. Sondorp et al. reported that the formation efficiency of PTC-derived organoids is stable at about 7%, while that of RAIRD organoids is approximately 5% [47]. Secondly, organoid formation should ideally mimic the development of the whole organ; however, differences in the timing of the action of the various growth factors involved and the concentrations of these growth factors make it difficult to produce organoids that perfectly match the relevant organs *in vivo* [53]. There are also minor differences in gene expression patterns between established organoids and the corresponding tissues *in vivo* [54]. Finally, generated organoids lack vascularization [55]. Increasing organoid volume is accompanied by increasing levels of hypoxia and metabolic waste, which kills the cells; consequently, the organoids currently cultured *in vitro* cannot fully represent the real organs. In conclusion, the culture and application of thyroid organoids require further development. Current research is mainly concentrated on thyroid- and PTC-derived organoids, and studies on organoids relating to highly invasive, high-mortality, poorly differentiated, and undifferentiated cancers are lacking. Future research on thyroid organoids must focus on improving culture systems and developing more comprehensive *in vitro* models, thereby promoting the development of precision medicine.

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References

1. Li Mo, Izpisua Belmonte JC. Organoids - Preclinical Models of Human Disease. N Engl J Med. 2019; 380(6): 569–579, doi: 10.1056/NEJMra1806175, indexed in Pubmed: 30726695.
2. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009; 459(7244): 262–265, doi: 10.1038/nature07935, indexed in Pubmed: 19329995.
3. Antonica F, Kasprzyk DF, Opitz R, et al. Generation of functional thyroid from embryonic stem cells. Nature. 2012; 491(7422): 66–71, doi: 10.1038/nature11525, indexed in Pubmed: 23051751.
4. Mazzaferri E, Jhiang S. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. Am J Med. 1994; 97(5): 418–428, doi: 10.1016/0002-9343(94)90321-2, indexed in Pubmed: 7977430.
5. Garber JR, Cobin RH, Gharib H, et al. American Association of Clinical Endocrinologists and American Thyroid Association Taskforce on Hypothyroidism in Adults. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Endocr Pract. 2012; 18(6): 988–1028, doi: 10.4158/EP12280.GL, indexed in Pubmed: 23246686.
6. Saini S, Tulla K, Maker AV, et al. Therapeutic advances in anaplastic thyroid cancer: a current perspective. Mol Cancer. 2018; 17(1): 154, doi: 10.1186/s12943-018-0903-0, indexed in Pubmed: 30352606.
7. Behjati S, Huch M, van Boxtel R, et al. Genome sequencing of normal cells reveals developmental lineages and mutational processes. Nature. 2014; 513(7518): 422–425, doi: 10.1038/nature13448, indexed in Pubmed: 25043003.
8. Li Z, Araoka T, Wu J, et al. 3D Culture Supports Long-Term Expansion of Mouse and Human Nephrogenic Progenitors. Cell Stem Cell. 2016; 19(4): 516–529, doi: 10.1016/j.stem.2016.07.016, indexed in Pubmed: 27570066.
9. Bartfeld S, Bayram T, van de Wetering M, et al. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. Gastroenterology. 2015; 148(1): 126–136.e6, doi: 10.1053/j.gastro.2014.09.042, indexed in Pubmed: 25307862.
10. Samimi H, Atlasi R, Parichehreh-Dizaji S, et al. A systematic review on thyroid organoid models: time-trend and its achievements. Am J Physiol Endocrinol Metab. 2021; 320(3): E581–E590, doi: 10.1152/ajpendo.00479.2020, indexed in Pubmed: 33427048.
11. Bonnier F, Keating ME, Wróbel TP, et al. Cell viability assessment using the Alamar blue assay: a comparison of 2D and 3D cell culture models. Toxicol In Vitro. 2015; 29(1): 124–131, doi: 10.1016/j.tiv.2014.09.014, indexed in Pubmed: 25300790.
12. Hartung T. Thoughts on limitations of animal models. Parkinsonism Relat Disord. 2008; 14 Suppl 2: S81–S83, doi: 10.1016/j.parkreldis.2008.04.003, indexed in Pubmed: 18585949.
13. Shanks N, Greek R, Greek J. Are animal models predictive for humans? Philos Ethics Humanit Med. 2009; 4(1), doi: 10.1186/1747-5341-4-2, indexed in Pubmed: 19146696.
14. Dutta D, Heo I, Clevers H. Disease Modeling in Stem Cell-Derived 3D Organoid Systems. Trends Mol Med. 2017; 23(5): 393–410, doi: 10.1016/j.molmed.2017.02.007, indexed in Pubmed: 28341301.
15. Takebe T, Wells JM. Organoids by design. Science. 2019; 364(6444): 956–959, doi: 10.1126/science.aaw7567, indexed in Pubmed: 31171692.
16. Li Y, Tang P, Cai S, et al. Organoid based personalized medicine: from bench to bedside. Cell Regen. 2020; 9(1): 21, doi: 10.1186/s13619-020-00059-z, indexed in Pubmed: 33135109.
17. McCracken KW, Catá EM, Crawford CM, et al. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. Nature. 2014; 516(7531): 400–404, doi: 10.1038/nature13863, indexed in Pubmed: 25363776.
18. Dutta D, Heo I, Clevers H. Disease Modeling in Stem Cell-Derived 3D Organoid Systems. Trends Mol Med. 2017; 23(5): 393–410, doi: 10.1016/j.molmed.2017.02.007, indexed in Pubmed: 28341301.
19. Hussein SM, Batada NN, Vuoristo S, et al. Copy number variation and selection during reprogramming to pluripotency. Nature. 2011; 471(7336): 58–62, doi: 10.1038/nature09871, indexed in Pubmed: 21368824.
20. Laurent LC, Ulitsky I, Slavin I, et al. Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. Cell Stem Cell. 2011; 8(1): 106–118, doi: 10.1016/j.stem.2010.12.003, indexed in Pubmed: 21211785.

21. Korkaya H, Liu S, Wicha MS. Breast cancer stem cells, cytokine networks, and the tumor microenvironment. J Clin Invest. 2011; 121(10): 3804–3809, doi: 10.1172/JCI57099, indexed in Pubmed: 21965337.
22. Plaks V, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? Cell Stem Cell. 2015; 16(3): 225–238, doi: 10.1016/j.stem.2015.02.015, indexed in Pubmed: 25748930.
23. Brown JW, Mills JC. Implantable synthetic organoid matrices for intestinal regeneration. Nat Cell Biol. 2017; 19(11): 1307–1308, doi: 10.1038/ncb3635, indexed in Pubmed: 29087385.
24. Vazin T, Schaffer DV. Engineering strategies to emulate the stem cell niche. Trends Biotechnol. 2010; 28(3): 117–124, doi: 10.1016/j.tibtech.2009.11.008, indexed in Pubmed: 20042248.
25. Saito Y, Onishi N, Takami H, et al. Development of a functional thyroid model based on an organoid culture system. Biochem Biophys Res Commun. 2018; 497(2): 783–789, doi: 10.1016/j.bbrc.2018.02.154, indexed in Pubmed: 29470983.
26. de Lau WBM, Snel B, Clevers HC. The R-spondin protein family. Genome Biol. 2012; 13(3): 242, doi: 10.1186/gb-2012-13-3-242, indexed in Pubmed: 22439850.
27. MacDonald BT, He Xi. Frizzled and LRP5/6 receptors for Wnt/β-catenin signaling. Cold Spring Harb Perspect Biol. 2012; 4(12), doi: 10.1101/cshperspect.a007880, indexed in Pubmed: 23209147.
28. Mah AT, Yan KS, Kuo CJ. Wnt pathway regulation of intestinal stem cells. J Physiol. 2016; 594(17): 4837–4847, doi: 10.1113/JP271754, indexed in Pubmed: 27581568.
29. Gehart H, Clevers H. Tales from the crypt: new insights into intestinal stem cells. Nat Rev Gastroenterol Hepatol. 2019; 16(1): 19–34, doi: 10.1038/s41575-018-0081-y, indexed in Pubmed: 30429586.
30. Xu H, Lyu X, Yi M, et al. Organoid technology and applications in cancer research. J Hematol Oncol. 2018; 11(1): 116, doi: 10.1186/s13045-018-0662-9, indexed in Pubmed: 30219074.
31. Tilson SG, Haley EM, Triantafillu UL, et al. ROCK Inhibition Facilitates In Vitro Expansion of Glioblastoma Stem-Like Cells. PLoS One. 2015; 10(7): e0132823, doi: 10.1371/journal.pone.0132823, indexed in Pubmed: 26167936.
32. Takebe T, Sekine K, Enomura M, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature. 2013; 499(7459): 481–484, doi: 10.1038/nature12271, indexed in Pubmed: 23823721.
33. Spence JR, Mayhew CN, Rankin SA, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature. 2011; 470(7332): 105–109, doi: 10.1038/nature09691, indexed in Pubmed: 21151107.
34. Hohwieler M, Illing A, Hermann PC, et al. Human pluripotent stem cell-derived acinar/ductal organoids generate human pancreas upon orthotopic transplantation and allow disease modelling. Gut. 2017; 66(3): 473–486, doi: 10.1136/gutjnl-2016-312423, indexed in Pubmed: 27633923.
35. Taguchi A, Nishinakamura R. Higher-Order Kidney Organogenesis from Pluripotent Stem Cells. Cell Stem Cell. 2017; 21(6): 730–746.e6, doi: 10.1016/j.stem.2017.10.011, indexed in Pubmed: 29129523.
36. Coraux C, Hilmi C, Rouleau M, et al. Reconstituted Skin from Murine Embryonic Stem Cells. Curr Biol. 2003; 13(10): 849–853, doi: 10.1016/s0960-9822(03)00296-3, indexed in Pubmed: 12747834.
37. Longmire TA, Ikonomou L, Hawkins F, et al. Efficient derivation of purified lung and thyroid progenitors from embryonic stem cells. Cell Stem Cell. 2012; 10(4): 398–411, doi: 10.1016/j.stem.2012.01.019, indexed in Pubmed: 22482505.
38. Antonica F, Kasprzyk DF, Opitz R, et al. Generation of functional thyroid from embryonic stem cells. Nature. 2012; 491(7422): 66–71, doi: 10.1038/nature11525, indexed in Pubmed: 23051751.
39. Kurmann AA, Serra M, Hawkins F, et al. Regeneration of Thyroid Function by Transplantation of Differentiated Pluripotent Stem Cells. Cell Stem Cell. 2015; 17(5): 527–542, doi: 10.1016/j.stem.2015.09.004, indexed in Pubmed: 26593959.
40. Lan L, Cui D, Nowka K, et al. Stem cells derived from goiters in adults form spheres in response to intense growth stimulation and require thyrotropin for differentiation into thyrocytes. J Clin Endocrinol Metab. 2007; 92(9): 3681–3688, doi: 10.1210/jc.2007-0281, indexed in Pubmed: 17609303.
41. Tai MH, Chang CC, Kiupel M, et al. Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. Carcinogenesis. 2005; 26(2): 495–502, doi: 10.1093/carcin/bgh321, indexed in Pubmed: 15513931.
42. Ogundipe VML, Groen AH, Hosper N, et al. Generation and Differentiation of Adult Tissue-Derived Human Thyroid Organoids. Stem Cell Reports. 2021; 16(4): 913–925, doi: 10.1016/j.stemcr.2021.02.011, indexed in Pubmed: 33711265.
43. Grüters A, Krude H. Detection and treatment of congenital hypothyroidism. Nat Rev Endocrinol. 2011; 8(2): 104–113, doi: 10.1038/nrendo.2011.160, indexed in Pubmed: 22009163.
44. Yang Y, Opara EC, Liu Y, et al. Microencapsulation of porcine thyroid cell organoids within a polymer microcapsule construct. Exp Biol Med (Maywood). 2017; 242(3): 286–296, doi: 10.1177/1535370216673746, indexed in Pubmed: 27708182.
45. McGranahan N, Swanton C. Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. Cancer Cell. 2015; 27(1): 15–26, doi: 10.1016/j.ccell.2014.12.001, indexed in Pubmed: 25584892.
46. Tuveson D, Clevers H. Cancer modeling meets human organoid technology. Science. 2019; 364(6444): 952–955, doi: 10.1126/science.aaw6985, indexed in Pubmed: 31171691.
47. Sondorp LHJ, Ogundipe VML, Groen AH, et al. Patient-Derived Papillary Thyroid Cancer Organoids for Radioactive Iodine Refractory Screening. Cancers (Basel). 2020; 12(11), doi: 10.3390/cancers12113212, indexed in Pubmed: 33142750.
48. Chen D, Tan Y, Li Z, et al. Organoid Cultures Derived From Patients With Papillary Thyroid Cancer. J Clin Endocrinol Metab. 2021; 106(5): 1410–1426, doi: 10.1210/clinem/dgab020, indexed in Pubmed: 33524147.
49. Vlachogiannis G, Hedayat S, Vatsiou A, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science. 2018; 359(6378): 920–926, doi: 10.1126/science.aao2774, indexed in Pubmed: 29472484.
50. Broutier L, Mastrogiovanni G, Verstegen MMa, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nat Med. 2017; 23(12): 1424–1435, doi: 10.1038/nm.4438, indexed in Pubmed: 29131160.
51. Kim M, Mun H, Sung CO, et al. Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening. Nat Commun. 2019; 10(1): 3991, doi: 10.1038/s41467-019-11867-6, indexed in Pubmed: 31488816.
52. Walsh AJ, Cook RS, Sanders ME, et al. Quantitative optical imaging of primary tumor organoid metabolism predicts drug response in breast cancer. Cancer Res. 2014; 74(18): 5184–5194, doi: 10.1158/0008-5472.CAN-14-0663, indexed in Pubmed: 25100563.
53. Kim J, Koo BK, Knoblich JA. Human organoids: model systems for human biology and medicine. Nat Rev Mol Cell Biol. 2020; 21(10): 571–584, doi: 10.1038/s41580-020-0259-3, indexed in Pubmed: 32636524.
54. Pollen AA, Bhaduri A, Andrews MG, et al. Establishing Cerebral Organoids as Models of Human-Specific Brain Evolution. Cell. 2019; 176(4): 743–756.e17, doi: 10.1016/j.cell.2019.01.017, indexed in Pubmed: 30735633.
55. Nagle PW, Plukker JT, Muijs CT, et al. Patient-derived tumor organoids for prediction of cancer treatment response. Semin Cancer Biol. 2018; 53: 258–264, doi: 10.1016/j.semcancer.2018.06.005, indexed in Pubmed: 29966678.