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Trophoblast stem cells, trophoblast invasion, and organoids – advancements in gynecology

Trofoblastové kmeňové bunky, invázia trofoblastu a organoidy – pokroky v gynekológii

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Summary: The human placenta serves as a vital barrier between the mother and the developing fetus during pregnancy. A defect in the early development of the placenta is associated with severe pregnancy disorders. Despite its complex development, various molecular processes control placental development, and the specialization of trophoblast cells is still not fully understood. One primary obstacle is the lack of suitable cell model systems. Traditional two-dimensional (2D) cell cultures fail to mimic *in vivo* conditions and do not capture the intricate intercellular interactions vital for studying placental development. However, three-dimensional (3D) organoid models derived from stem cells that replicate natural cell organization and architecture have greatly improved our understanding of trophoblast behavior and its medicinal applications. Organoids with relevant phenotypes provide a valuable platform to model both placental physiology and pathology, including the modeling of placental disorders. They hold great promise for personalized medicine, improved diagnostics, and the evaluation of pharmaceutical drug efficacy and safety. This article provides a concise overview of trophoblast stem cells, trophoblast invasion, and the evolving role of organoids in gynecology.

Key words: trophoblast stem cells – trophoblast invasion – organoids – pregnancy complications

Súhrn: Ľudská placenta predstavuje životne dôležitú bariéru medzi matkou a vyvíjajúcim sa plodom počas tehotenstva. Porucha včasného vývoja placenty je spojená so závažnými poruchami tehotenstva. Napriek jej komplexnému vývoju stále nie sú úplne objasnené rôzne molekulárne procesy riadiace vývoj placenty a špecializáciu buniek trofoblastu. Jednou z hlavných prekážok je nedostatok vhodných bunkových modelových systémov. Tradičné dvojrozmerné (2D) bunkové kultúry nedokážu imitovať podmienky *in vivo* a nezachytávajú zložité medzibunkové interakcie nevyhnutné na štúdium vývoja placenty. Avšak trojrozmerné (3D) modely organoidov, odvodené z kmeňových buniek, ktoré replikujú prirodzenú organizáciu a architektúru buniek výrazne zlepšili naše chápanie správania sa trofoblastov a ich medicínskych aplikácií. Organoidy s relevantnými fenotypmi poskytujú cennú platformu na modelovanie fyziológie a patológie placenty, vrátane modelovania porúch placenty. Sú veľkým prísľubom pre personalizovanú medicínu, zlepšenie diagnostiky a hodnotenia účinnosti a bezpečnosti farmaceutických liečiv. Tento článok poskytuje stručný prehľad trofoblastových kmeňových buniek, invázie trofoblastu a rozvíjajúcej sa úlohy organoidov v gynekológii.

Kľúčové slová: trofoblastové kmeňové bunky – invázia trofoblastu – organoidy – komplikácie tehotenstva

Introduction

In the field of gynecology, significant advances have been made in recent years, particularly in understanding trophoblast stem cells, trophoblast invasion, and the application of organoids. These areas of research hold great promise for better understanding reproductive health, pregnancy complications, and the development of novel therapeutic approaches.

The complex process of placental implantation and development is shaped by trophoblast progenitors and uterine cells, and is regulated by transcription factors, cytokines, adhesion receptors, and their associated ligands [1]. However, the molecular mechanisms involved in placental formation and trophoblast cell specification and differentiation are not yet fully understood. One of the main challenges in exploring these processes is the lack of suitable cell models [2]. Conventional two-dimensional

(2D) cell cultures fail to capture the complex intercellular interactions required to study placental development. The emergence of three-dimensional (3D) organoid models has opened new avenues for understanding trophoblast behavior and its medical applications [3].

The purpose of this article is to provide an overview of trophoblast stem cells, trophoblast invasion, and the growing role of organoids in gynecology.

Trophoblast stem cells

Stem cells are undifferentiated cells present in the embryonic, fetal, and adult stages of life. They give rise to differentiated cells that are essential components of tissues and organs [4]. Stem cells are essential for maintaining organ size, structure, and function through processes such as cell renewal, migration, differentiation, and apoptosis. They reside in specific environments known as stem cell niches, which provide the necessary structural support, nutrients, and mechano-chemical signals that regulate stem cell fate [5].

The most important characteristics of stem cells are self-renewal (the ability to reproduce extensively), clonality (usually arising from a single cell), and potency (the ability to differentiate into different cell types). These characteristics can vary among different types of stem cells [4].

Trophoblast stem cells are pluripotent cells whose differentiated derivatives are restricted to trophoblast lineages [6]. They represent a unique population of cells derived from the trophectoderm that play a crucial role in early embryonic development and placenta formation [7]. They have self-renewal capacity and can differentiate into various trophoblast lineages [8]. Recent studies have shed light on the characterization and regulation of trophoblast stem cells, including key transcription factors and signaling pathways involved in their maintenance and differentiation [7]. Induced trophoblast stem cells closely resemble those derived from human blastocysts or first-trimester placentas, both in terms of molecular characteristics and functional properties [9].

Human placenta, trophoblast, and trophoblast invasion

The human placenta serves as a vital barrier between the mother and the developing fetus, performing a range of essential functions during pregnancy [2,10]. These functions include the physiological adaptation of the mother, immunological acceptance, nutrition, and support for embryo growth. Placental villi play a crucial role as transport units of the placenta. Immersed in the mother's blood, they efficiently deliver vital nutrients and oxygen to the developing fetus while removing metabolic waste products. These villi undergo dynamic morphological changes during pregnancy. At the early stages, they begin as mesenchymal structures and gradually transform into highly vascularized formations capable of extracting essential substances from the maternal circulation. The extensive branching morphogenesis of the villi results in an epithelial surface estimated to be approximately 12–14 square meters. This extensive surface ensures a consistent supply of nutrients, crucial during a period of rapid fetal growth. In addition, the placenta releases specific hormones into the fetal bloodstream that influence fetal development, growth, and timing of delivery [2].

The normal development and functioning of the placenta are essential for a successful pregnancy [11]. Defects in early placental development are the primary cause of common pregnancy disorders, including recurrent miscarriage, fetal growth restriction, pre-eclampsia, and stillbirth. Furthermore, adverse gestational conditions can have lasting effects on lifelong fetal health through developmental programming [12].

Currently, placental delivery is the only known treatment for many placenta-related diseases and complications, emphasizing the need for further extensive research into the underlying processes contributing to the pathophysiology of these conditions [7]. Understanding the physiological mechanisms involved in the development of the placenta is crucial in clarifying the pathogenesis of various pregnancy complications [13,14].

The trophoblast, representing the major cell type in the placenta, plays a crucial role in facilitating interactions between the fetus and the mother at the feto-maternal interface. These specialized cells are derived from the trophectoderm, which forms the outer layer of the human blastocyst and serves as a source of trophoblast progenitor cells [1,11,15].

Uterine trophoblast invasion is an essential process during implantation and placental development, beginning early in pregnancy and continuing until the 20th week of gestation [16,17]. Trophoblast invasion marks the initial stage of human blastocyst implantation, essential for remodeling the uterine spiral arteries in the decidua to ensure adequate nutrition and oxygenation. Inadequate or excessive trophoblast invasion has been associated with various pregnancy complications, such as preeclampsia, fetal growth restriction, and placenta accrete [1].

Ethical and legal concerns limit our ability to explore intrauterine development of the human placenta [10]. In addition, knowledge about the human placenta is also limited due to the lack of representative functional models [18]. Our current knowledge of the cellular and molecular mechanisms of placental epithelial cells and trophoblasts has been primarily obtained using various methods. These methods include primary cultures, placental villous explants, choriocarcinoma cell lines, as well as immortalized cell lines derived from the placenta or mouse trophoblast stem cells [14,19]. However, human and other animal placentas show significant struc-

tural and functional differences, making data from animal models irrelevant to understanding human physiology [18].

Researchers have been investigating the molecular mechanisms underlying trophoblast invasion, including the role of specific genes, microRNAs, and extracellular matrix remodeling. Understanding the complex processes of trophoblast invasion may lead to better diagnostic and therapeutic strategies for pregnancy-related disorders.

Organoids in gynecological medicine

Organoids are three-dimensional, *in vitro* models ranging in size from micrometers to millimeters. They faithfully replicate the structural and functional characteristics of specific *in vivo* tissues or organs [20,21]. Composed of cells that form small clusters capable of self-organizing and differentiating into functional cell types, they are often referred to as "mini-organs". These organoids can be derived from embryonic stem cells, induced pluripotent stem cells, or neonatal and adult stem cells through a process similar to how an organ achieves its characteristic organization [21]. Stem cells are capable of producing organoids from all three germ layers, including extraembryonic membranes [22].

The origin of organoids dates back to 1907 when H. V. Wilson dissected sponge cells and observed their remarkable ability to self-organize and regenerate into new organisms [23]. The term "organoid" was first used in 1987 to describe *in vitro* cultures from neuroblastomas and lungs [24]. Nowadays, stem cells can be used to create organoids that closely resemble the structure and function of the original organs [23].

Organoid self-assembly occurs due to spatially restricted lineage commitment and cell sorting, which requires activation of various signaling pathways mediated by internal cellular components or external environments such as the extracellular matrix and media [21].

Unlike monolayer cells, organoids better represent the *in vivo* structure of organs, significantly affecting cell signaling networks. In 2D cultures, cells grow attached to a substrate, which is popular, but many *in vitro* studies have shown that it does not faithfully represent the *in vivo* situation because it differs substantially from the organization of tissues and cell connections in a living organism [3].

Organoids have additional benefits: they support the growth of both stem cells and progenitor cell cultures, promoting significant cell-cell interactions. This is a feature that traditional 2D cultures lack [25].

Due to the ability of stem cells to self-organize into 3D tissue structures, organoids have shown tremendous potential for modeling human physiology [26]. The current surge in organoid research also results from the ability to grow organoids from cells or tissues derived from individuals, promising advancements in human biology and medical research [27].

From a scientific and ethical perspective, organoids offer a more accurate and morally acceptable alternative to traditional research using animal and embryo models. Substituting organoids for animal models aligns with the ethical principle of 'reduction' of animal experiments, minimizing the number of laboratory animals required. Similarly, organoid technology can serve as a morally acceptable substitute for the controversial use of embryos for certain research purposes. However, it's essential not to view organoids as ethically neutral alternatives; they are cultured from cells and tissues from human individuals, raising ethical concerns at various levels, including the organoids themselves, individual patients or donors, and society in general [28].

In gynecology, organoids have gained attention as valuable tools for studying reproductive organs, pregnancy, and related diseases [29]. Recently, the creation of trophoblast organoids has provided a platform to study trophoblast differentiation, invasion, and interactions with the maternal environment. Trophoblast organoids can contribute to the understanding of placental development, disease modeling, and drug discovery [19,30].

Applications in gynecology

Trophoblast stem cells, trophoblast invasion research, and organoid integration hold promise for clinical applications in gynecology leading to:

Improved diagnostics: The study of trophoblast invasion using trophoblast stem cells and organoids enables detailed molecular investigations. These provide a new platform for research on the molecular mechanisms involved in placental development and early embryogenesis, relevant to human pregnancy diseases occurring during early development [6]. It may potentially offer new biomarkers for early detection and risk assessment of pregnancy complications.

Personalized Medicine: Organoids offer a unique opportunity to practice personalized medicine [31]. They can serve as a base for testing individualized therapeutic interventions and optimizing treatment strategies for conditions related to trophoblast function.

Drug Development and Screening: Trophoblast organoids provide a physiologically relevant model for testing the efficacy and safety of pharmaceutical agents targeting placental disorders [18]. They can also be used to study maternal-fetal interactions and the transfer of nutrients, drugs, and pathogens [23].

Disease Modeling and Research: Disease modeling at the cellular level is a powerful method for discovering the underlying causes of various health conditions and creating innovative therapies [32]. Trophoblast stem cells and organoids offer valuable tools to study the mechanisms underlying placental dysfunction and pregnancy-related disorders.

Discussion

Organoids have emerged as invaluable tools for investigating trophoblast stem cells, trophoblast invasion, and intricate interactions between trophoblasts and uterine cells [10]. These 3D cell models offer closer-to-the-physiological environment, enabling the observation of intercellular interactions and enhancing the study of placental development [33]. By incorporating various cell types, including trophoblast progenitors and uterine cells, organoids can better replicate the dynamic cellular processes during placental implantation and invasion.

Utilizing organoids offers several advantages over traditional 2D cell culture systems. First, they provide a more accurate representation of the *in vivo* environment, facilitating the study of trophoblast behavior under conditions closer to physiological norms. This phenotypic accuracy makes organoids a valuable tool for investigating placental physiology and modeling placental diseases [5]. Second, organoids can be tailored to individual patients, allowing for the development of personalized therapies. By using patient-derived cells, organoids can mimic the unique characteristics of an individual's placenta, enabling personalized drug screening and treatment optimization [20,31]. Finally, organoids provide an improved platform for toxicity studies, as their 3D architecture and cellular complexity allow for more accurate assessment of drug toxicity, potentially reducing the need for animal models and expediting drug development processes [34].

The study and creation of organoids have revolutionized our understanding of trophoblast stem cells, trophoblast invasion, and interactions with uterine cells. These advancements have the potential to greatly expand our understanding of placental physiology and offer valuable insights into placental diseases. Organoids serve as powerful tools for exploring the pathogenesis of placental disorders, identifying new therapeutic targets, and developing personalized treatment strategies [33,35]. Continuous research and refinement of organoid models contribute to the advancement of gynecology and enhancement of patient care.

In recent years, *in vitro* trophoblast models, including trophoblast stem cells and trophoblast organoids, still lack key components such as blood vessels, stromal cells, and immune cells, which are present in *in vivo* chorionic villi. In the future, innovative bioengineering systems are expected to be used to develop models that more closely resemble chorionic villi or the blood-placenta barrier. Additionally, elucidating the communication between the endometrium and trophoblasts could be achieved using endometrial organoids [14].

Conclusion

The integration of trophoblast stem cells, trophoblast invasion, and utilization of organoids has led to significant progress in the field of medicine. The 3D cell models provided by organoids offer a more physiologically relevant and versatile platform for studying placental development, modeling diseases, and developing personalized therapies. The ongoing exploration of trophoblast behavior and development of organoid models hold great promise for enhancing our understanding of placental physiology and improving health outcomes in the field of gynecology.

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