A tissue-engineered artificial human thymus from human iPSCs to study T cell immunity

A tissue engineering method using a 3D scaffolding enables the generation of an artificial human thymus from inducible pluripotent stem cells (iPSCs). The artificial thymus can be used to study human T cell development in hematopoietic humanized mice.

The problem

Hematopoietic humanized (hu) mice (also known as immunocompromised mice engrafted with human hematopoietic stem cells) are a powerful preclinical model for studying human immune responses and diseases, such as cancer and COVID-19 (refs. 1,2). However, modeling human T cell immunity in hu mice has been challenging, mainly because hu mice lack a human thymus—the primary lymphoid organ responsible for the generation and selection of T cells. Although the endogenous mouse thymus can provide limited support for human T cell development, human T cells generated in mouse thymus cannot properly engage with other human immune cells and, therefore, mount effective immune responses. The only existing solution is to transplant pieces of human fetal thymus into hu mice, but this method is impeded by ethical concerns and tissue scarcity. Generating an artificial human thymus using a renewable cell source, such as inducible pluripotent stem cells (iPSCs), would enable the construction of a functional human T cell compartment in hu mice and broaden the use of this model for biomedical research and drug discovery.

The solution

The thymus contains two major cellular compartments: the transient populations of developing T cells originating from hematopoietic progenitor cells (HPCs) and residential stromal cells, which include thymic epithelial cells (TECs). TECs are essential in regulating T cell lineage differentiation and education. We had developed a tissue engineering method that enabled us to construct a functional mouse artificial thymus by repopulating a decellularized thymus scaffold with isolated TECs. We adapted this method to generate artificial human thymus, and to overcome the inaccessibility of human TECs, we differentiated iPSCs into thymic epithelial progenitors cells (iPSC-TEPCs) using a multistep induction protocol. A 3D alginate encapsulation technology was used to promote more effective TEC differentiation and maturation. Artificial human thymus was constructed with iPSC-TEPCs and HPCs and tested for its ability to support human T cell development in hu mice (Fig. 1a).

Expression of key TEC markers, such as FOXP1 and KRT8, was substantially higher in 3D iPSC-TEPCs than in those derived from 2D differentiation, demonstrating the advantages of using the 3D technology (Fig. 1b). These findings are in line with results from single-cell transcriptome analysis of iPSC-TEPCs. Flow cytometry analysis of key markers of T cell development demonstrated the capability of the artificial human thymus to support the progression of T cell lineage programming in vitro. Most importantly, analysis of hu mice engrafted with the human artificial thymus showed the development of a diverse population of human T cells expressing a complex repertoire of T cell receptors (TCRs). T cell-mediated cellular and/or humoral immune responses, such as proinflammatory responses upon TCR engagement, inhibition of allogeneic tumor graft growth and immunoglobulin class switching, were restored in these mice, further indicating the development of a functional human T cell compartment.

The implications

Translating findings from animal studies to the clinic is often hampered by the species-specific differences between models and humans. Generating a functional human T cell population in hu mice engrafted with artificial human thymus will greatly increase the accessibility of this model for studying human adaptive immune responses and accelerate the translation of preclinical research. With the rapid expansion of iPSC repositories, T cell immunity of an individual patient with a specific HLA composition could be modeled in hu mice to facilitate personalized medicine. Theoretically, improvement of the thymus engineering technology would enable one to generate an artificial thymus from a patient’s own iPSCs, for treatment of primary and acquired immune deficiency disorders.

TECs can be generally categorized into two functional distinct subsets on the basis of their localization within the thymus: cortical TECs and medullary TECs. Our next approach will focus on reproducing the geometric organization of the artificial thymus to improve its function. iPSC-TEPCs will be induced to differentiate into specific TEC subsets. New tissue-engineering tools will also be developed to repopulate TEC subsets to their specific regions within the thymus scaffolds. Another area of focus will be to improve the interactions between T cells and B cells to gain more robust adaptive immune responses in hu mice.

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**EXPERT OPINION**

The studies provide new insights that move the field closer to making functional TECs from iPSCs, an achievement that could eventually lead to the generation of T cells in vivo that could have clinical benefits. The main addition to the differentiation protocol is the use of scaffolds to create a 3D structure, and the 2D comparison cultures suggest that the 3D approach makes a difference.” Gay Crooks, University of California Los Angeles, Los Angeles, CA, USA.

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**BEHIND THE PAPER**

I have a long-held interest in thymus tissue-engineering, with the goal of redefining an individual patient’s immunological self to correct their existing immunodysregulation. After our success with mouse thymus engineering, the logical next step was to adapt the technology to construct an artificial human thymus, using iPSC-derived TEPCs as surrogates for human TECs. With us having no previous experience in hu mice, the project quickly showed signs of being an engineering nightmare. It takes at least 4 months to generate the thymus-engrafted hu mice, from iPSC encapsulation and differentiation, thymus scaffold preparation and repopulation, and CD34+ HPCs isolation and cryopreservation to mouse preconditioning and engraftment, with possible missteps at each step. Nevertheless, thanks to the diligent efforts of both the Banerjee and the Fan laboratories and the continued institutional support, we were able to overcome all the technical challenges and bring the project to fruition. Y.F.

**FROM THE EDITOR**

This paper uses human thymic organoids derived from iPSCs to reconstruct the T cell environment in immunodeficient humanized mice. Recapitulating aspects of the adaptive immune system still remains a challenge, and I think that this paper will spur further development in this space.” Madhura Mukhopadhyay, Associate Editor, Nature Methods