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A Quick update from the Past to Current Status of Human Pluripotent Stem Cell-derived Hepatocyte culture systems

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ABSTRACT

Pluripotent stem cells (PSCs) may be offered as an unlimited cell source for the hepatocyte generation. The generation of hepatocytes from stem cells in vitro would provide an alternative cell source for applications in drug discovery and cell transplantation. In this review, we discuss different approaches to generate pluripotent stem cell-derived hepatocytes, advantages, limitations for each method and finally, how three-dimensional (3D) strategy can improve the maturation of PSCs -derived hepatocytes.

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Introduction

The liver exposes to different diseases, including inherited metabolic disorders and other disorders caused by variety of factors. Currently, orthotopic liver transplantation (OLT) is the only effective treatment for life-threatening liver problems. However, transplantation of allogeneic hepatocytes has now become an alternative approach, which is less invasive than OLT (1). Hepatocyte transplantation has been limited due to the shortage of organ donors, efficient isolation, and cryopreservation challenges. The liver is also a key organ to assess the pharmacokinetics and pharmacodynamics of drugs and toxicology studies of xenobiotics (2). All these issues have highlighted the need to explore other sources of cells, e.g., stem cells that can be expanded sufficiently and then differentiate them into functional hepatocytes. Human PSCs have opened novel pathways in the field of regenerative medicine in terms of cell therapy and tissue engineering. Moreover, hPSCs have created a unique model system for studying diverse fields, from basic scientific questions such as developmental processes to the practical application including drug screening (3).

Until now, various protocols have designed for hepatocyte differentiation and have tried to mimic the liver organogenesis during development by adding different substrates and growth factors, which are necessary for each developmental stage. However, the obtained hepatocytes showed an immature phenotype (4, 5). There are several ideas that can overcome the drawbacks in this regard to producing more mature hepatocytes (6).

Hepatocyte differentiation in two-dimensional culture

Currently, researchers have succeeded to develop the number of protocols for generating hepatocyte from hPSCs in 2D culture system. However, the hepatocyte-like cells (HLCs) generated in this protocols has indicated an immature phenotype with low hepatocyte functionality compared to primary human hepatocyte (7, 8). Monolayers of adherent 2D cells can grow on a coated flat and rigid surface such as polystyrene or glass (9). The 2D cell culture plays an essential role in the better understanding of the developmental biology and tissue morphogenesis. The monolayer cultures have faced many disadvantages regarding emulating in vivo conditions and providing physiological relevance. In addition, 2D cultures are unable to mimic the natural structures of tissues and the interactions will be missed in this type of culture that is responsible for cell differentiation, proliferation, viability, expression of genes and proteins, inducing stimuli, drug metabolism, and other cellular functions. Altogether, 3D approach has been used to improve maturation of HLCs and more emulation in vivo condition (10)

Hepatocyte differentiation in three-dimensional culture

Embryoid bodies (EBs) are 3D aggregates of hPSCs that can differentiate into three germ layers (endoderm, ectoderm, and mesoderm). EBs use as a model that can easily represent what is happening during in vivo development (11). A study has reported by Baharvand et al. that showed used EBs of hPSCs for hepatocyte differentiation (12). Using a dynamic 3D culture in a bioreactor for hepatocyte differentiation is the other method that can improve the hepatic maturation of hiPSC-derived (13). Vosough and his research team have succeeded to produce a scalable platform for hepatocyte differentiation from hPSCs using the mentioned system (14). Liver organoids provide a new class of biological model to serve as both tissue and organ. This approach could recapitulate many key parameters of in vivo conditions for instance, cell-cell and cell-ECM interactions and the complexities of the tissue (15). Recently Takebe et al. have indicated that mimicking the embryonic niche by culturing hepatic endoderm cells with stromal cells (endothelial and mesenchymal lineages), can be a new approach to hepatocyte differentiation and liver organoid generation from hPSCs (16).

Conclusion

In conclusion, numerous 3D cell culture systems have improved over the past two decades. The existing evidence strongly has proved the 3D cell cultures that develop cell-cell and cell-ECM interactions, can imitate the specificity of native tissue with further physiological relevance more than conventional 2D cultures.

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