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（要　旨）
(Summary)

Classic cell culture media do not accurately represent the availability of the nutrients in plasma. They usually contain a supraphysiological concentration of nutrients such as glucose, amino acids, etc. These high nutrients can alter the metabolism of cultured cells and induce metabolic phenotypes that don't reflect in vivo conditions.
Refinement of media formulations has a potential application in maturity modulation of stem cell-derived β cells (SC-β) generation in vitro. This has the potential importance in regenerative medicine for curative diabetes treatment. Previous reports from mice studies have shown that amino acid levels progressively decreased between embryonic day 19 and postnatal day 9. Moreover, elevated amino acid levels have been linked to the activated mammalian target of rapamycin complex 1 (mTORC1) pathway, an increase in islet cell proliferation, accompanied by a low level of maturation. It has been demonstrated that late-stage culture in low levels of amino acids promoted the maturation of SC-β and improved their glucose-stimulated insulin secretion (GSIS) function. To the best of my knowledge, the effect of the physiological level of amino acids on the whole differentiation process has not been investigated. In this study, I asked whether SC-β cells could be derived in media that better recapitulate the composition of human plasma amino acids. And whether it would promote differentiation.
To address these questions, I established for the first time a defined culture system to derive SC-β cells using a blood-like amino acids medium (BALM) using RPChiPS771-3G cell line. RT-PCR, immunohistochemistry, western blot analysis, and GSIS, were performed to evaluate differentiation efficiency and maturation level. This study shows that by refining the nutrient composition of BALM-based medium, I successfully promoted hiPSCs differentiation into the DE. The physiological-like culture medium formulation significantly reduced the undifferentiated cell population. Moreover, pluripotency markers (*POU5F1*, *NANOG*) expression was considerably lower in cells cultured in BALM than in those cultivated in DMEM F12-based medium. When comparing cells grown in BALM to those cultured in DMEM F12, the DE marker genes (*SOX17*, *FOXA2*, *GATA4*) exhibited a

significant increase in cells cultured in a BALM-based medium. These findings indicate that supraphysiological concentrations of nutrients suppressed DE differentiation. The use of physiological levels of nutrients can lead to modulating cell function.

I demonstrated that supraphysiological nutrients level interferes with efficient endodermal differentiation through upregulation of the mTOR pathway. Moreover**,** human pluripotent stem cells (hPSCs) can be efficiently differentiated into definitive endoderm, pancreatic progenitors, endocrine progenitors, and SC-β in BALM. The differentiated cells secreted C-peptide *in vitro* in response to high glucose levels and expressed several pancreatic β-cell markers. Therefore, I conclude that the physiological level of amino acids is sufficient for the derivation of functional SC-β cells.

These results are consistent with the previous report that showed that the activated mTORC1 pathway sustains pluripotency and negatively affects differentiation.

these findings reveal that supplementing the medium with amino acids different from those present in serum resulted in corresponding changes in their uptake/excretion and metabolism. The results show that the supraphysiological concentrations of nutrients typically found in conventional media skew the metabolism of hiPSCs. The results agreed with previously reported results in cancer cells. My finding may have potential translational application in developing hPSCs models for studying metabolic regulation of pancreatic β-cell differentiation. This study also examined the application of albumin-free culture for pancreatic differentiation. Albumin-free cell culture refers to a type of cell culture where the growth media used to culture the cells does not contain any albumin. Albumin is a protein that is often used in cell culture media as a supplement because it can bind to various nutrients and growth factors, thereby increasing their stability and availability to the cells. However, there are some situations where the use of albumin in cell culture media may be undesirable.

For example, some cell types may be sensitive to the presence of albumin in the media, and the protein may interfere with experimental outcomes. Additionally, albumin can introduce variability into cell culture experiments because the concentration and quality of albumin can vary from batch to batch.

Albumin-free cell culture media is typically formulated using alternative protein sources, such as transferrin or bovine serum albumin (BSA)-free formulations. These media are designed to support the growth and survival of cells in the absence of albumin.

Researchers may choose to use albumin-free cell culture media in a variety of applications, including drug discovery, toxicology, and biomanufacturing. By eliminating albumin from the media, they can reduce experimental variability and ensure the reproducibility of their results. chemically defined bovine serum albumin-free specified culture system was developed to generate SC-β cells using a blood amino acids-like medium (BALM). BALM-based medium successfully converts hiPSCs into the definitive endoderm, pancreatic progenitors, endocrine progenitors, and functional SC-β.

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