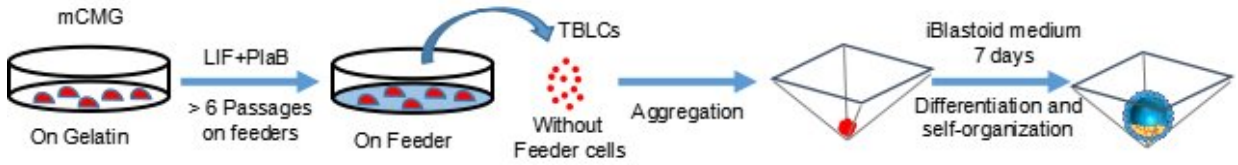
Generation of blastocyst-like structures from spliceosomes represses mouse totipotent blastomere-like cells

February 24 2023



To assess whether TBLCs possess the ability to form blastoids, 5, 10, 25, and 50 cells of mCMG-TBLCs were aggregated per microwell of the AggrewellTM 400 in the reported medium for generating EPS-blastoid (iBlastoid medium). After 5 days of culture, some of the microwells initiating with 5 and 10 cells turned out to be empty because of cell death. Nevertheless, most of the remaining cell clumps with different initiating cell numbers started to form a cavity. The cavities gradually grew bigger to form a blastocyst-like structure in another 2 days. Credit: Science China Press

A study led by Dr. Man Zhang (Guangzhou Laboratory, Guangzhou Medical University) has been published in the journal *Science China Life Sciences*.

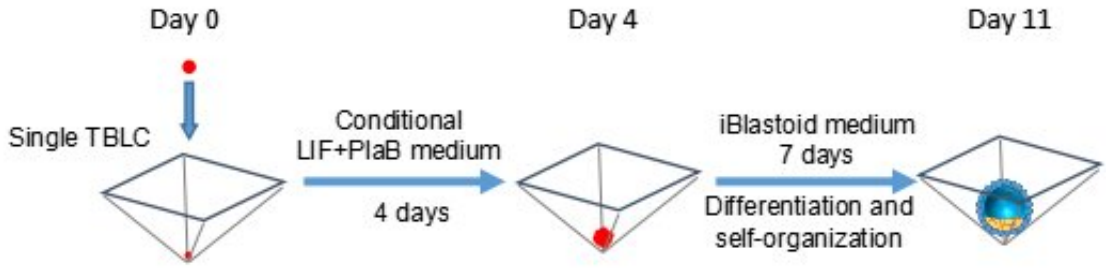
Previous studies have shown that the efficiency of the blastocyst-like structures (blastoids) formation from extended pluripotent cells (EPS) was pretty low (15%). Additionally, EPS-blastoids contained few TE

cells and a considerable amount of mesoderm-like cells. In order to increase the efficiency of blastoid generation, spliceosomal repression-induced totipotent blastomere-like cells (TBLCs) were used to generate blastoids.

It was reported that TBLCs have similar transcriptomes to 2C-4C embryos. The team found that blastoids were observed in nearly 80% of micro-wells when aggregated in the iBlastoid medium, which is much higher than the efficiency of EPS-blastoid formation. "This is an unexpected promotion," Man Zhang said. immunofluorescence staining revealed that around 71% of TBLC-blastoids expressed the markers of early cell lineages with a similar location to natural embryos.

Meanwhile, Dr. Pengfei Zhang together with the lab director Man Zhang sought to determine whether a single TBLC could give rise to blastocyst-like structures. As it's critical to generate blastoid from a single cell for high-throughput screening of the effects of gene manipulation in early embryogenesis. Accordingly, 9.3% of microwells (multiplied 18.6% by 50%) from a single TBLC can form blastocyst-like structures that expressed all 3 lineage markers in a similar pattern to that of natural blastocysts.

In addition, scRNA-seq revealed that TBLC-blastoids shared a similar lineage composition and cell transcriptome to blastocysts. Compared to the blastoids generated from other reported cell types (EPS, ETS and TPS), TBLC-blastoids contained more TE lineage cells but fewer PrE-like cells. Additionally, TSC-like and PrESC-like cell lines were established from TBLC-blastoids.



For high-throughput screening of the effects of gene manipulation in early embryogenesis, generating blastoids from a single TBLC is crucial. They cultured the single TBLC in a PlaB media supplemented with 50% irradiated mouse embryonic fibroblasts (iMEFs) conditional media in microwells for 4 days when the cell numbers were 16–22 and then changed the medium to iBlastoid medium. After another 7 days of culture, although around 34.8% of the microwells were blank, almost 30% of the surviving cell clumps formed blastocyst-like structures. Credit: Science China Press

The researchers also evaluated whether TBLC-blastoid can develop beyond implantation and recapitulate some aspects of post-implantation development both in vitro and in vivo. TBLC-blastoids cultured in vitro in the IVC medium can transit to the egg-cylinder stage. When transplanted into the uterus of pseudopregnant mice, TBLC blastoids can induce deciduae in vivo. They obtained an E7.5 embryo-like structure from the deciduae.

However, they were unable to develop normally in vivo. The authors indicate that the abnormal gene expression profile of extra-embryonic lineages (TE and PrE) of blastoids might be the major cause of their abnormal in vivo development. Further studies to optimize the medium to obtain an adequate proportion and faithful PrE and TE cells in TBLC-blastoids are necessary to generate fully functional synthetic embryos.

More information: Pengfei Zhang et al, Highly efficient generation of

blastocyst-like structures from spliceosomes-repressed mouse totipotent blastomere-like cells, *Science China Life Sciences* (2023). DOI: 10.1007/s11427-022-2209-3

Provided by Science China Press