INDUCED PLURIPOTENT STEM CELLS: PROGRESSION FROM "PRIMED" TO "NAÏVE" USING NOVEL LIF/6i MEDIA

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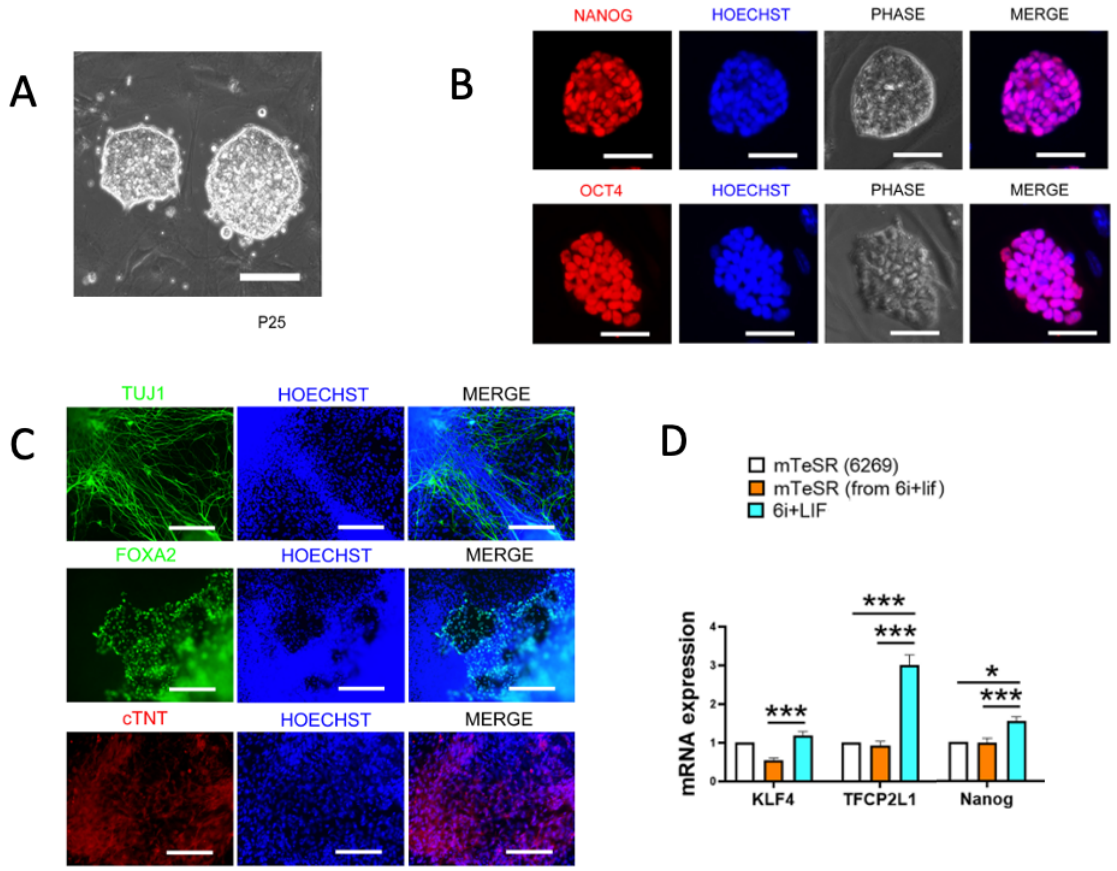
Background: Induced pluripotent stem cells (iPSCs) hold great promise for regenerative medicine, cell-based therapy, and the in vitro generation of functional gametes. Human PSCs can be derived from human blastocysts or reprogrammed from somatic cells [1]. Whereas *totipotent* stem cells have the ability to generate all embryonic and extraembryonic tissues, *pluripotent* stem cells can only differentiate into embryonic tissues. Key transcription factors (e.g., NANOG, KLF4, and OCT4) are associated with pluripotency, which is manifested in two distinct states: naïve and primed [2]. "Naïve" cells represent an earlier developmental state than "primed" cells and can differentiate into all three embryonic germ cell layers (ectoderm, endoderm, mesoderm) as well as primordial germ cells, while primed cells cannot produce germline lineages [3]. To date, culture conditions have been able to maintain human iPSCs in the primed state but have not successfully maintained authentic human naïve iPSCs.

Objective: The aim of this project is to derive and maintain authentic naïve human iPSCs using novel culture conditions.

Materials and Methods: Human cord blood cells were collected and transfected with the transcription factors OCT4, SOX2, KLF4, and C-MYC to reprogram them into naïve iPSCs, which were then cultured in our novel LIF/6i media. Quantitative PCR (qPCR) and immunofluorescence were utilized to detect pluripotency markers (NANOG, OCT4) in the naïve iPSCs. The naïve iPSCs were subsequently differentiated into cell types representing all three germ cell layers, which was confirmed through immunofluorescence. qPCR for both pluripotency and naïve state markers was performed in the widely used 6269 human iPSC line as well as the reprogrammed naïve iPSCs after culture in commercially available media (mTeSR) and our LIF/6i media. The results were compared using the Student's t-test, with $p<0.05$ considered statistically significant.

Results: The novel LIF/6i media maintained the naïve state of the reprogrammed iPSCs for up to 25 cell passages (Figure A). Immunofluorescence for the pluripotency markers NANOG and OCT4 in the iPSCs confirmed their pluripotent state (Figure B). The naïve iPSCs were successfully differentiated into the three germ cell layers, verified by the immunofluorescence markers TUJ1 (ectoderm), FOXA2 (endoderm), and cTNT (mesoderm) (Figure C). qPCR was used to detect both human pluripotency (KLF4, NANOG) and naïve (TFCP2L1) marker gene expression in the 6269 cell line and our reprogrammed cell line. Levels of both pluripotency and naïve markers were compared after culturing in mTeSR and LIF/6i media. Compared to mTeSR media, the expression levels of both naïve and pluripotency marker genes were significantly increased after culture in our media. Furthermore, when the reprogrammed iPSCs were transferred from LIF/6i media into mTeSR, the levels of naïve and pluripotency markers significantly decreased (Figure D). This suggests that our novel LIF/6i media can maintain the naïve state of iPSCs and is superior to the commercially available mTeSR media for deriving naïve iPSCs.

Conclusion: The development of an authentic naïve iPSC line represents an important step forward in regenerative medicine. Our novel LIF/6i cell culture media effectively sustains authentic naïve iPSCs and is superior to currently commercially available culture media.



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