

Regulation of cellular fate and establishment of novel bioresource for avian species

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Abstract

Somatic cells have great potential as a bioresource for avian species. In this study, we tried to establish iPSCs (induced pluripotent stem cells) and immortalized cells for this purpose. In addition to the establishment of these cells, we investigated the optimal basal medium for avian cell culture.

1. Establishment of iPSCs from chicken fibroblasts.

iPSC technology converts terminally differentiated cells into pluripotent stem cells through the expression of defined reprogramming factors. Although, iPSCs have been established for mammalian species like mouse, human, and monkey, studies on iPSCs derived from avian species are still very limited. To establish chick iPSCs, six factors were used within a poly-cistronic reprogramming vector (named PB-R6F) — M3O (MyoD-derived transactivation domain fused with Oct3/4), Sox2, Klf4, c-Myc, Lin28, and Nanog. The PB-R6F-derived iPSCs were positive for alkaline-phosphatase and SSEA-1, which are markers of pluripotency. Histological analysis of teratomas revealed that the established chick iPSCs had the ability to differentiate into three-germ-layer derived tissues. In this study, we thus established iPSCs from chicken fibroblasts using the poly-cistronic reprogramming vector¹.

2. Establishment of immortalized cells from chicken and Okinawa rail fibroblasts.

Although immortalized cultures are useful for various functional assays or transcriptomic analysis, highly efficient and reproducible immortalization methods have not been developed for avian-derived cells. We attempted to use an immortalization method involving the co-expression of mutant cyclin-dependent kinase 4 (CDK4), cyclin D, and TERT (K4DT method) in these avian cells. We succeeded in obtaining immortalized cells exhibiting K4DT expression and concluded that it can be used for the immortalization of avian-derived cells².

3. Investigation of the optimal basal medium for avian cell culture.

Avian cell culture requires an optimized basal medium, and there are currently relatively few options for this. This means that there is still an opportunity to develop an optimal basal medium for this application. We compared KAv-1 medium, DMEM, and Medium 199 for the culture of chick fibroblasts and determined that KAv-1 is the optimal medium for these assays³.

References

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