Real time visualization and kinetic measurement of somatic reprogramming

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ABSTRACT

Somatic reprogramming enables the generation of induced pluripotent stem cells (iPSCs) from non-germ cell lineages. This mirrors the somatic reprogramming of adult somatic cells to ES cell-like states. The three week long process is not yet fully understood. To gain a better understanding of the reprogramming process we used a combination of live cell imaging techniques and cell-free molecular analysis, in conjunction with real-time visualization methods. We also used kinetic methods for monitoring the reprogramming process in early pluripotency markers such as Alkaline Phosphatase Live Stain, SSEA4, TRA-1-60 and other reprogramming events which are measured by terminal AP staining of the colonies that have been generated using different reprogramming methods.

RESULTS

Figure 1. Traditional CytoTune™ reprogramming workflow

Figure 2. CD44 expression is very low in pluripotent cells

Figure 3. Surface markers distinguish reprogramming states

Figure 4. Use of combination of positive and negative PSC markers demonstrate full reprogramming

Figure 5. Live Cell Monitoring of reprogramming events

Figure 6. Using positive/negative pluripotency markers expression to track reprogramming kinetics

Figure 7. Dual marker expression can demonstrate differences across reprogramming methods

Figure 8. Utilizing surface marker expression and reprogramming kinetics to enrich for iPSC generation

CONCLUSIONS

- CD44 and pluripotent surface markers like AP Live Stain, SSEA4 and Tra-1-60 can be utilized to select fully reprogrammed and iPSC colonies from partially reprogrammed colonies based differential staining patterns confirming non-overlapping of the pluripotent marker expression and the somatic fibroblast marker expression. Reprogramming kinetics tracked through the increased expression of pluripotent markers like SSEA4 and the decreased expression of CD44 can be used to predict the speed and quality of reprogramming.
- Live cell monitoring can be used to look at the morphological changes associated with reprogramming and iPSC colony generation, in addition to surface marker expression.
- Looking at reprogramming kinetics, via cell surface marker expression can help elucidate the timing at which cells can be manipulated for downstream events such as cell enrichment/depletion and for ensuring proper selection of quality cells for cell engineering and other downstream applications.
- Further evaluation of early onset pluripotency (SSEA4) and late onset markers (Tra-1-60) against CD44 expression may lead to a better understanding on how different reprogramming systems, media systems, markers, small molecules and staging somatic populations can determine the quality of the reprogramming process.
- Live cell monitoring, reprogramming kinetic tracking and enrichment can potentially be applied to other somatic tissues like CD34+ blood cells or PBMCs, which also express CD44 at elevated levels.

REFERENCES

(2) RH Quintanilla, JST Asprer, C Vaz, V Tanavde, U Lakshmipathy (2014) CD44 is a negative cell surface marker for pluripotent stem cell identification during human fibroblast reprogramming. PLoS ONE 9(1); e85419

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