Nuclear quiescence and histone hyper-acethylation jointly improve protamine-mediated nuclear remodeling in sheep fibroblasts

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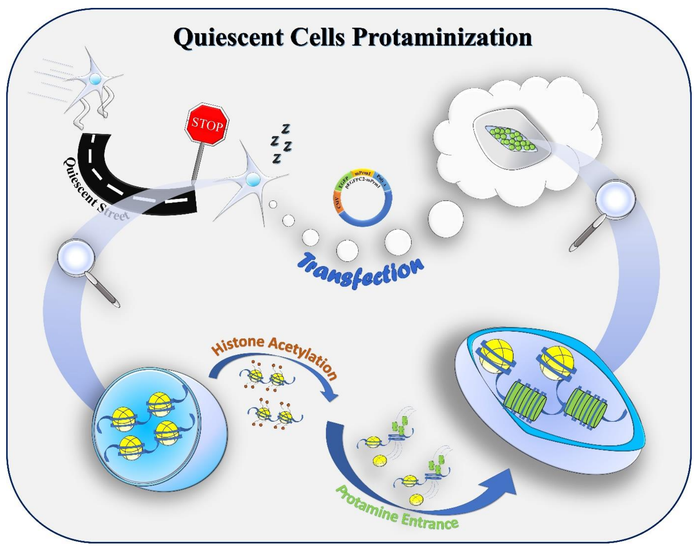
UNITE

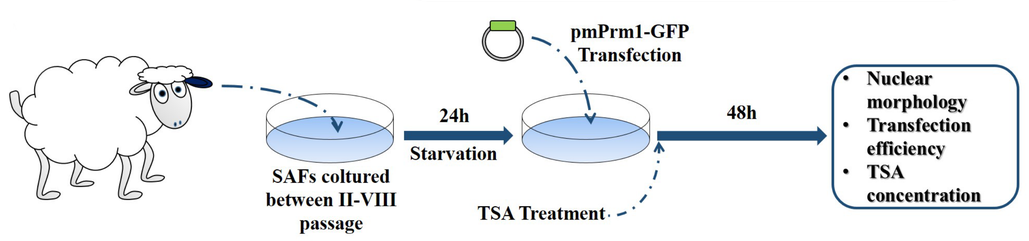
WCRB Okinawa, JAPAN 2017
September 27–29
2017
Okinawa Convention Center, Okinawa, Japan

Introduction: Recently we have demonstrated the possibility of the direct replacement of histone to protamine in somatic cells by the heterologous expression of the human protamine 1 (Prm1) gene in proliferating fibroblasts. Here we have further advanced our protocol, by mimicking the nuclear remodelling taking place in spermatogenesis. The spermiogenesis starts by cell quiescence and the open of nuclear chromatin (by histone-hyperacetylation) of post-meiotic round spermatids.



Aim 1: Post translation modifications of testis specific histone variants, namely genome-wide acethylation, facilitate the process. Our first aim was to optimize the protaminization of somatic nucleus by finding the best concentration and exposition time to Trichostatin A (TSA).

Aim 2: Since nuclear maturation in spermatids occurs in G0, our second aim was to test if protaminization of somatic nucleus increases when quiescent-stage fibroblasts are trasfected with mouse Prm1 (mPrm1) gene.



Result 1: We have found a greater number of spermatid-like cells with 50 nM of TSA concentration, comparing to 25 and 100 nM ($p<0.05$) (Fig.3). Moreover, Bromodeoxyuridine incorporation and gene expression analysis of quiescent cells mark (*Dicer1*, *Smarca 2*, *Ezh1*, *Ddx39*, *H2afz* and *Pink1*) demonstrated that the combination of low serum concentration in STarved group (ST-group, 0.5% FBS in MEM for 24h,) and 50 nM of TSA stop the cell proliferation respect the ConTRol (CTR-group, 10% FBS in MEM), driving the cells in a quiescent stage (Fig. 1).

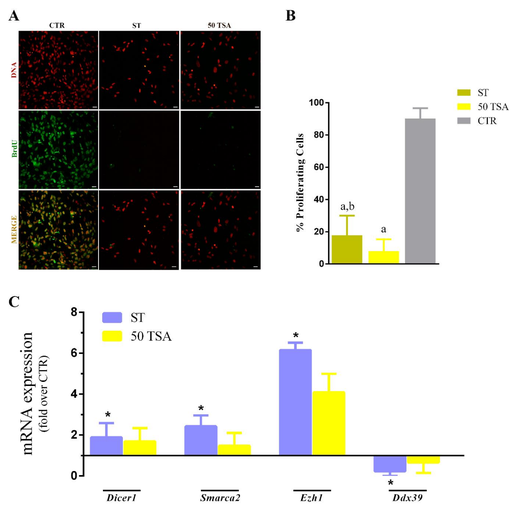


Fig. 1

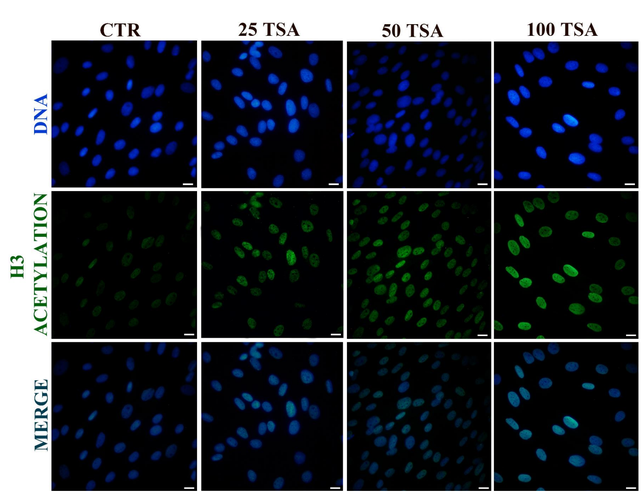


Fig. 2

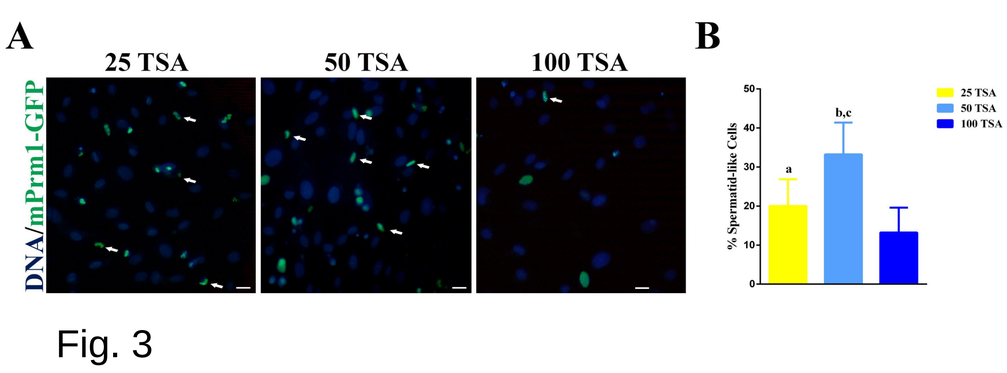


Fig. 3

Result 2: Ectopic mPrm1 traslocates into quiescent somatic nuclei, binds directly the somatic DNA and changes gradually the nuclear morphology into spermatid-like structure. The synergy of ST and 50 nM TSA increased the remodeling of somatic chromatin versus the 50 nM TSA ($p<0.05$) (Fig.5). Furthermore, the replacement of H3K9me3 with mPrm1, a critical epigenetic barrier of SCNT reprogramming, might be a further positive element to improve nuclear reprogramming (Fig. 4) .

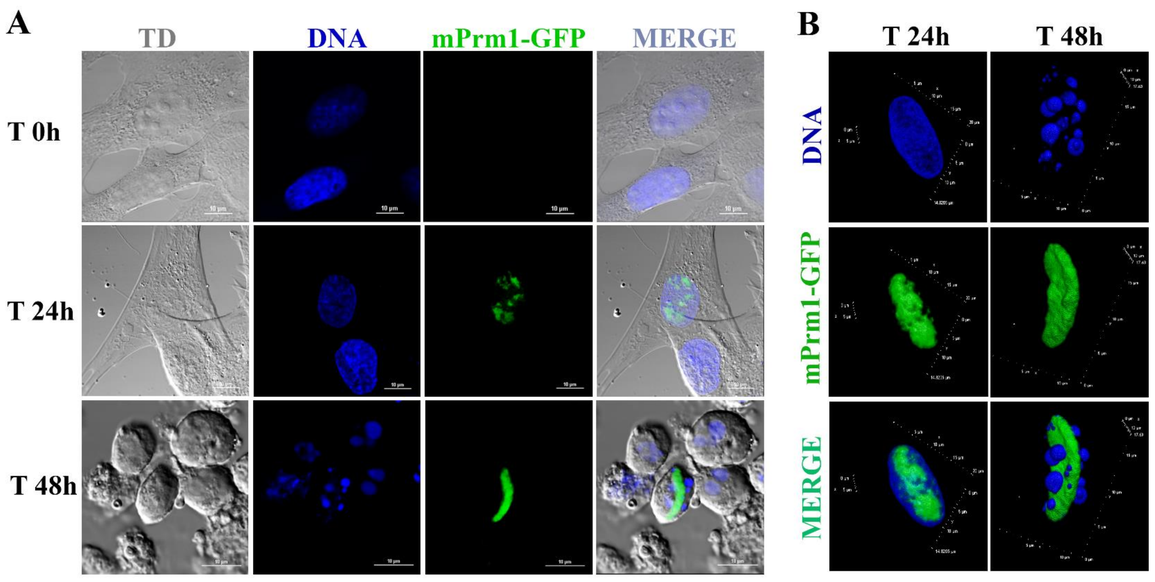


Fig. 4

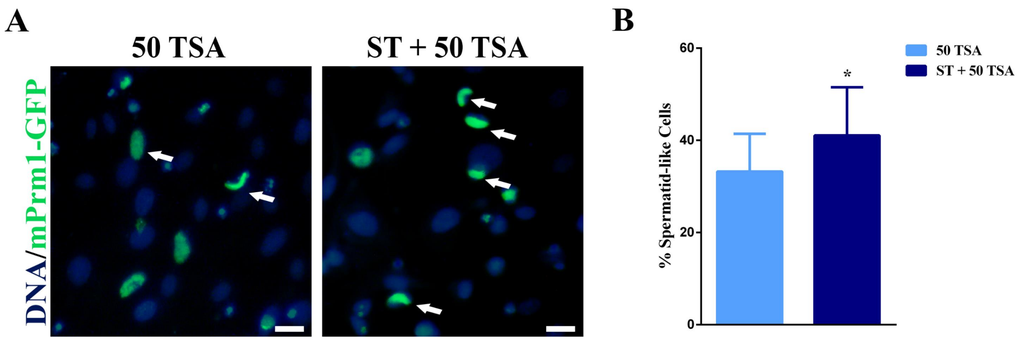


Fig. 5

Conclusion:

Here we have demonstrated that nuclear quiescence and TSA treatment resulted in a more efficient Prm1-mediated conversion of somatic nuclei into spermatid-like structures.
This approach mifght be valuable alternative for an improved nuclear reprogramming in SCNT.

Funding: The research presented is funded by MIUR/CNR and Program FIRB GA B81J12002520001 “GenHome”. This article also has received funding from the European Union’s Horizon 2020 research and innovation programme (H2020) Twinning action GA 698165 “EraofArt” and under grant agreement No 734434 “DryNet”. The authors are also participating in the COST Action CA16119.

Acknowledgement: The authors warmly acknowledge Prof. S. Khochbin, Institute Albert Bonniot, for providing the mPrm1-GFP plasmid and L. Valbonetti, University of Teramo, for help in the acquisitions the confocal images.