Meta-analysis of trichostatin A treatment effects on mouse somatic cell nuclear transfer

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Received: 21 June 2018; Accepted: 26 October 2018

ABSTRACT

Improving somatic cell nuclear transfer (SCNT) efficiency is challenging, and trichostatin A (TSA) has been implemented to improve this technique, but it does not work for porcine and monkey SCNT. Thus, a meta-analysis was done to understand the relationship between TSA and mouse SCNT. Published articles were collected using PubMed and ScienceDirect from 2000 to 2018. Total 15 studies were included that suggest TSA can improve SCNT mouse blastocyst formation and live birth. Most TSA effects studied were on histone deacetylase (HDACs), hence the impacts of TSA on the cytoplasm, specifically cancer signaling pathways, endoplasmic reticulum, and HDACs localization were investigated. It is likely that TSA benefits mouse SCNT because the nucleus is easy to remove. Using fluorescent labeling to remove nuclei and TSA incorporation, SNCT may be improved for pig and monkey studies.

Key words: Blastocyst, Cloning, Nuclear transfer, Oocyte, Trichostatin A

RESULTS AND DISCUSSION

Our search yielded 15 studies from a total of 138 reports (Fig. 1). Table 2 lists descriptive details of every study (Kishigami et al. 2006, Kishigami et al. 2007, Li et al. 2008, Maalouf et al. 2009, Tsuji et al. 2009, Van Thuan et al. 2009, Bui et al. 2010, Costa-Borges et al. 2010, Dai et al. 2010, Ono et al. 2010, Hai et al. 2011, Kang and Roh 2011, Farifteh et al. 2014, Miyamoto et al. 2017, Qiu et al. 2017). TSA treatment can improve blastocyst rate remarkably [OR 2.01 (95% CI—1.79–2.26)] (Fig. 2). We established that control blastocysts formation was 32.84% (847/2,579), and variables reported within the dataset was settled by consultation with a third investigator.

Meta analysis: We assayed the effect of TSA on SCNT efficiency, specifically calculating blastocyst formation from embryonic cleavage and SCNT live births from embryonic transfer. We evaluated effect heterogeneity using Higgins statistic, ap-value, and an $I^2$ statistic (de la Cruz et al. 2017). Briefly, $I^2$ ranged from 0–100% and heterogeneity of 0–25% was low; 25–50% moderate; and > 50% indicated high heterogeneity (de la Cruz et al. 2017). A fixed effects model was used when $I^2$ was low or moderate. All of the data were calculated using Review Manager, Version 5.3 (Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen). To address publication bias, 3 methods, viz. visual inspection of funnel plots, Egger’s test, and Begg’s test were used, generated using Stata 12.0 (Stata Corp, College Station, TX) and $P<0.05$ was considered to be statistically significant.

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Table 1. Inclusion and exclusion criteria

| Inclusion                                                                 | Exclusion                                                                 |
|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Species evaluated must include, but are not limited to, mice             | Mice were not used                                                        |
| English literature                                                       | Non-English literature                                                    |
| TSA treatment of embryos but not limited to embryo treatment             | No TSA treatment of embryos                                               |
| Both donor cell and oocyte came from mice                                | Xenotransplantation                                                        |
| SCNT blastocyte formation data are available or SCNT birth data are available | Insufficient data                                                          |

Table 2. Characteristics of studies included in the review

| Study          | Year  | Mouse strain | Treat time (h)* | Medium |
|----------------|-------|--------------|-----------------|--------|
| Kishigami 2006b | B6D2F1 | A 6 + C 4    | KSOM            |
| Kishigami 2007a | B6D2F1 | A 6 + C 4    | KSOM            |
| Li 2008        | B6D2F1 | O 2 + A 6    | KSOM            |
| Tsuji 2009     | C57BL/6xDBA | O 2 + A 6 | KSOM            |
| Van Thuan 2009 | B6D2F1 | A 6 + C 4    | KSOM            |
| Maalouf 2009   | C57/CBA | A 6 + C 4   | M16             |
| Biu 2010       | B6D2F1 | A 6 + C 4    | KSOM            |
| Costa-Borges 2010 | Hybrid | O (2–3) + A 6 | KSOM            |
| Dai 2010       | B6D2F1 | O 2 + A 6 + C 2 | CZB            |
| Ono 2010       | B6D2F1 | A 6 + C 3    | KSOM            |
| Kang 2011      | B6D2F1 | A 6 + C 3    | KSOM            |
| Hai 2011       | B6D2F1 | A 6 + C 4    | MEM             |
| Farifteh 2014  | B6D2F1 | O 2 + A 6    | KSOM            |
| Miyamoto 2017  | B6D2F1 | A 6 + C 2    | KSOM            |
| Qiu 2017       | Kunming | A 6 + C 4    | KSOM            |

*Treatment time for TSA within different media. O, oocyte culture medium; A, activation medium; and C, embryo culture medium.

TSA improved this (50.64% or 1,312/2,591). Moreover, TSA increased the number of births (Fig. 3) in control and TSA groups by 0.56% (14/2,500) and 3.59% (61/1,697), respectively. We did not find heterogeneity. Funnel plot data did not indicate publication bias (Fig. 4). Egger’s test P = 0.261, Begg’s test Pr>|z| = 0.213. TSA can improve mouse SCNT blastocyst formation and increase live births.

When histone modification changes in SCNT embryos were observed, studies of histone modification of normally fertilized embryos were undertaken. Histone modification includes methylation, acetylation, phosphorylation, ubiquitination, deacetylation, or ADP ribosylation (Shanmugam et al. 2018). Two enzymes are involved in the regulation of histone acetylation namely histone acetyltransferase (HATs) and histone deacetylase (HDACs). If cellular acetylation and deacetylation are unbalanced, cell proliferation and differentiation are abnormal, and gene expression changes.

Use of TSA on oocytes (Li et al. 2008, Dai et al. 2010) is controversial as TSA can change HDACs in nuclei (Li et al. 2011). When oocyte nuclei are removed, there appears to be no overall effect (Rao and Rao 2013). Therefore, we assessed how TSA affects the cytoplasm, specifically cancer signaling pathways, the ER and HDAC localization. TSA may modify p21 protein expression and prevent the formation of cell cycle two polymer and cyclin-dependent kinase, which can block the cell cycle and induce cell differentiation, contributing to cancer. TSA is also involved in glioblastoma and human nasopharyngeal carcinoma cell p53 pathways. The PI3K/Akt signalling pathway is also linked to TSA. Thus, embryos may be affected in a manner similar to tumor cells.

Studies suggest that TSA affects ER function (Li et al. 2017). SER exits in 2 forms in oocytes, viz. vacuoles and small tubular aggregates, and SER is a calcium storage reservoir (Sfontouris et al. 2018). Smooth ER aggregate (SERAs) is very common in oocytes and calcium ion fluctuation can cause ER stress (ERS). SCNT technology must activate the genome after nuclear transplantation, and
this causes calcium fluctuations.

Why TSA is effective for mouse SNCT but not for porcine or monkey SCNT is of interest (Guo et al. 2018, Liu et al. 2018). Likely, the removal of mouse oocyte nuclei are easier than for other species; they are easily visualized using differential interference contrast (DIC) microscopy. For pig and monkey studies, the nuclear position is difficult to estimate. Thus, nuclear removal significantly reduces the cytoplasm. With the greater cytoplasmic loss, cloning efficiency is reduced. Thus, we assessed porcine hand clones and focused on nuclear/oocyte factors, the ER, and spindle wire. Other studies reported that proximity to the nucleus, the size of the spindle wire, and nuclear factor concentration (Ryu et al. 2017). SERa are more distributed near the nucleus (Itoi et al. 2016), and excessive cytoplasmic removal near the nucleus will reduce ER content.
TSA can increase mouse SCNT efficiency if fluorescent labeling is used to remove nuclei. This finding may be applied to porcine and monkey SCNT.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (Grant number 31671289). We would like to thank LetPub for providing linguistic assistance during the preparation of this manuscript.

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