Human Reproduction Update, Vol.27, No.2, pp. 421–422, 2021

human reproduction update

LETTER TO THE EDITOR

Will Single-Cell RNAseq decipher stem cells biology in normal and cancerous tissues?

Sir,

Single-cell RNAseq (scRNAseq) was selected as the Breakthrough of the Year 2018 by Science magazine and holds promise to transform scientific research and medical treatment as it allows dissection of the gene expression at the level of single-cell resolution. This technological advance can reveal cell-specific information which is not accessible when RNA is extracted from a heterogeneous population of cells. It is a tool to look at rare stem cells and disease associated cells e.g. circulating tumor cells in addition to tracking down cells during development and disease. Li *et al.* (2020) have published an excellent review on the advances in the field of reproductive biology using scRNAseq. The main focus was on primordial germ cells, their differentiation into gametes *in vivo*, gametogenesis along with the differentiation of human embryonic/induced pluripotent stem cells into gametes. Huge amounts of data have been compiled in a very comprehensive manner and the authors should be applauded for this but we have a few comments.

As mentioned in the review, scRNAseq failed to detect stem cells in adult ovary (Wagner *et al.*, 2020) and testes (Guo *et al.*, 2020). It is intriguing to point out that scRNAseq has failed to detect stem cells in other adult tissues as well including uterus (Mucenski *et al.*, 2019), prostate (Karthaus *et al.*, 2020) and cardiac tissue (Kretzschmar *et al.*, 2018). This needs to be understood and brain-stormed in order to obtain meaningful insight by undertaking scRNAseq studies. A careful review of the protocol used to prepare single cells suspension for scRNAseq experiments clarified why the stem cells have remained undetected by scRNAseq. The basic mistake scientific community commits is to put the stem and somatic cells in the same basket while processing. After preparing single cells suspension by enzymatic digestion, cells are usually collected by centrifuging at 200-600*g*. This speed of centrifugation ensures pelleting of the majority of somatic cells without affecting their viability. However, *stem cells being of smaller size remain buoyant at this speed and inadvertently get discarded*. As a result, they never get subjected to scRNAseq analysis resulting in negative data (Bhartiya and Sharma 2020).

Stem cells can be enriched by further centrifuging the supernatant at 1000*g* as reported recently for adult mouse testes (Kaushik and Bhartiya 2020), uterus (Singh and Bhartiya 2020), pancreas (Mohammad *et al.*, 2020), and multiple adult tissues (Bhartiya *et al.*, 2019). Wagner *et al.* (2020) centrifuged cells at 300*g* whereas Guo *et al.* (2020) centrifuged at 600*g* while preparing cells for scRNAseq from ovarian cortical slices or adult human testes and as a result discarded (unknowingly) the stem cells. Thus, more attention needs to be paid towards sample preparation prior to subjecting samples to scRNAseq in order to detect rare stem cells/cancer stem cells populations.

Conflict of interest

Authors declare no conflict of interest whatsoever.

References

Bhartiya D, Mohammad SA, Guha A, Singh P, Sharma D, Kaushik A. Evolving definition of adult stem/progenitor cells. *Stem Cell Rev Rep* 2019;**15:** 456–458.

Bhartiya D, Sharma D. Ovary does harbor stem cells - size of the cells matter. *J Ovarian Res*. 2020;**13**:39.

Guo J, Nie X, Giebler M, Mlcochova H, Wang Y *et al.* The dynamic transcriptional cell atlas of testis development during human puberty. *Cell Stem Cell* 2020;**26**:262–276.

Kaushik A, Bhartiya D. Additional evidence to establish existence of two stem cell populations including VSELS and SSCS in adult mouse testes. *Stem Cell Rev Rep* 2020;**16**:992–1004.

Karthaus WR, Hofree M, Choi D, Linton EL, Turkekul M *et al.* Regenerative potential of prostate luminal cells revealed by single-cell analysis. *Science* 2020;**368**:497–505.

Kretzschmar K, Post Y, Bannier-Hélaouët M, Mattiotti A, Drost J *et al.* Profiling proliferative cells and their progeny in damaged murine hearts. *Proc Natl Acad Sci U S A*. 2018;**115**:E12245–E12254.

Li L, Yang R, Yin C, Kee K. Studying human reproductive biology through single-cell analysis and in vitro differentiation of stem cells into germ cell-like cells. *Hum Reprod Upd* 2020:**26**:670–688.

Mohammad SA, Metkari S, Bhartiya D.Mouse pancreas stem/progenitor cells get augmented by streptozotocin and regenerate diabetic pancreas after partial pancreatectomy. *Stem Cell Rev and Rep* 2020;**16**:144–158.

Mucenski ML, Mahoney R, Adam M, Potter AS, Potter SS. Single cell RNA-seq study of wild type and Hox9,10,11 mutant developing uterus. *Sci Rep* 2019;**9**:4557.

Singh P, Bhartiya D. Pluripotent stem (VSELs) and progenitor (EnSCs) cells exist in adult mouse uterus and show cyclic changes across estrus cycle. *Reprod Sci* 2020;**24**. doi: 10.1007/s43032-020-00250-2.

Wagner M, Yoshihara M, Douagi I *et al.* Single-cell analysis of human ovarian cortex identifies distinct cell populations but no oogonial