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To use canine induced pluripotent stem cells (ciPSCs) for further application, it is important to generate many ciPSC lines from not only experimental dogs, but also companion animal dogs, and from healthy as well as patient dogs. Previously reported ciPSCs were generated from fibroblasts or mesenchymal stem cells, and involved invasive techniques (de Figueiredo Pessôa et al., 2019), owing to which owners of patient dogs often refuse the procedure. Hence, it is important to be able to reprogram other cell types that can be prepared by non-invasive techniques. We recently reported the generation of footprint-free ciPSCs from canine embryonic fibroblasts (CEFs) using auto-erasable Sendai virus vector, SeVdp(KOSM)302L (Tsukamoto et al., 2018). In this study, using SeVdp(KOSM)302L, we reprogrammed canine peripheral blood mononuclear

cells (PBMCs), which can be prepared easily.

Canine PBMCs were isolated from whole fresh blood of a 7-year-old laboratory bred beagle bitch using lymphocyte separation solution and infected by SeVdp(KOSM)302L at multiplicity of infection of 2. The PBMCs were then reseeded onto inactivated mouse embryonic fibroblasts and incubated in 20% KSR supplemented with 10 ng/ml LIF and bFGF as described in our previous report (Tsukamoto et al., 2018). Fourteen days later, primary colonies were obtained. After several passages, we verified that SeVdp(KOSM)302L was not present in our ciPSCs by RT-PCR (Fig 1A). The morphologies of the ciPSCs were similar to human iPSCs and were maintained after multiple passages (Fig. 1B). ciPSCs from PBMCs had alkaline phosphatase activity (Fig. 1C), and expressed pluripotent markers such as SSEA1, NANOG, and OCT3/4 (Fig. 1D). This expression pattern coincided with previously reported ciPSCs from CEFs (Tsukamoto et al., 2018). Immunocytochemistry of embryoid bodies (EBs) formed by ciPSCs at passage 16 revealed that our ciPSCs differentiated into all three germ layers *in vitro* (Fig. 1E). Karyotype analysis revealed that at passage 22, ciPSCs had normal 78 XX karyotype, with 38 matched pairs of autosomes (data not shown). These results proved that PBMCs were one of the good cell sources to generate ciPSC lines from companion and patient dogs.

Unfortunately, we could not confirm the differentiation of all three germ layers *in vivo* and the injection of ciPSCs at passage 20 into the testicular capsule of NOD/SCID mouse resulted in the development of incomplete teratomas, which is consisted of nothing but well differentiated neuronal tissue (Fig. 1F). The phenotype of the transplanted cells may affect the results of the teratoma assay, and transplantation of a relatively homogeneous pluripotent stem cell population is expected to promote higher teratoma formation efficiencies (Gropp et al., 2012). From this point of view, although we did not assess the heterogeneity and tendency to differentiate, the ciPSCs in this study might have been heterogeneous, and the tendency to differentiate might have led to the development of incomplete teratomas. Furthermore, we generated only one ciPSC line, and the reprogramming efficiency was 0.00037%. This might indicate that reprogramming and culture conditions should be improved using small molecule compounds to promote reprogramming or other media to maintain the pluripotency (Dakhore et al., 2018). In addition, selection of CD34-positive cells from canine PBMCs might result in a high reprogramming efficiency similar to that observed in humans (Okumura et al., 2019).

In conclusion, this is the first study to report the generation of ciPSC line from canine PBMCs. Although further study will be needed, we believe that this is a significant step toward the application of ciPSCs for regenerative medicine, elucidation of etiology, and drug discovery in veterinary medicine.

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Conflict of Interests

The authors declare that there are no conflict of interests.

References

Okumura, T., Horie, Y., Lai, C. Y., Lin, H. T., Shoda, H., Natsumoto, B., Fujio, K., Kumaki, E., Okano, T., Ono, S., Tanita, K., Morio, T., Kanegane, H., Hasegawa, H., Mizoguchi, F., Kawahata, K., Kohsaka, H., Moritake, H., Nunoi, H., Waki, H., Tamaru, S., Sasako, T., Yamauchi, T., Kadowaki, T., Tanaka, H., Kitanaka, S., Nishimura, K., Ohtaka, M., Nakanishi, M., & Otsu, M. (2019). Robust and highly efficient hiPSC generation from patient non-mobilized peripheral blood-derived CD34+ cells using the auto-erasable Sendai virus vector. *Stem Cell Research & Therapy*, *10*, 185.
doi: 10.1186/s13287-019-1273-2

Dakhore, S., Nayer, B., & Hasegawa, K. (2018). Human pluripotent stem cell culture: current status, challenges, and advancement. *Stem Cells International*, *2018*, 7396905.
doi: 10.1155/2018/7396905

de Figueiredo Pessôa, L. V., Bressan, F. F., & Freude, K. K. (2019). Induced pluripotent stem cells throughout the animal kingdom: Availability and applications. *World Journal of Stem Cells*, *11*, 491–505.
doi: 10.4252/wjsc.v11.i8.491

Gropp, M., Shilo, V., Vainer, G., Gov, M., Gil, Y., Khaner, H., Matzrafi, L., Idelson, M., Kopolovic, J., Zak, N. B., & Reubinoff, B. E. (2012). Standardization of the teratoma assay for analysis of pluripotency of human ES cells and biosafety of their differentiated progeny. *PLoS One*, *7*, e45532.
doi: 10.1371/journal.pone.0045532

Tsukamoto, M., Nishimura, T., Yodoe, K., Kanegi, R., Tsujimoto, Y., Alam, M. E., Kuramochi, M., Kuwamura, M., Ohtaka, M., Nishimura, K., Nakanishi, M., Inaba, T., Sugiura, K., & Hatoya, S. (2018). Generation of footprint-free canine induced pluripotent stem cells using auto-erasable

sendai virus vector. *Stem Cells and Development*, *27*, 1577–1586.
doi: 10.1089/scd.2018.0084

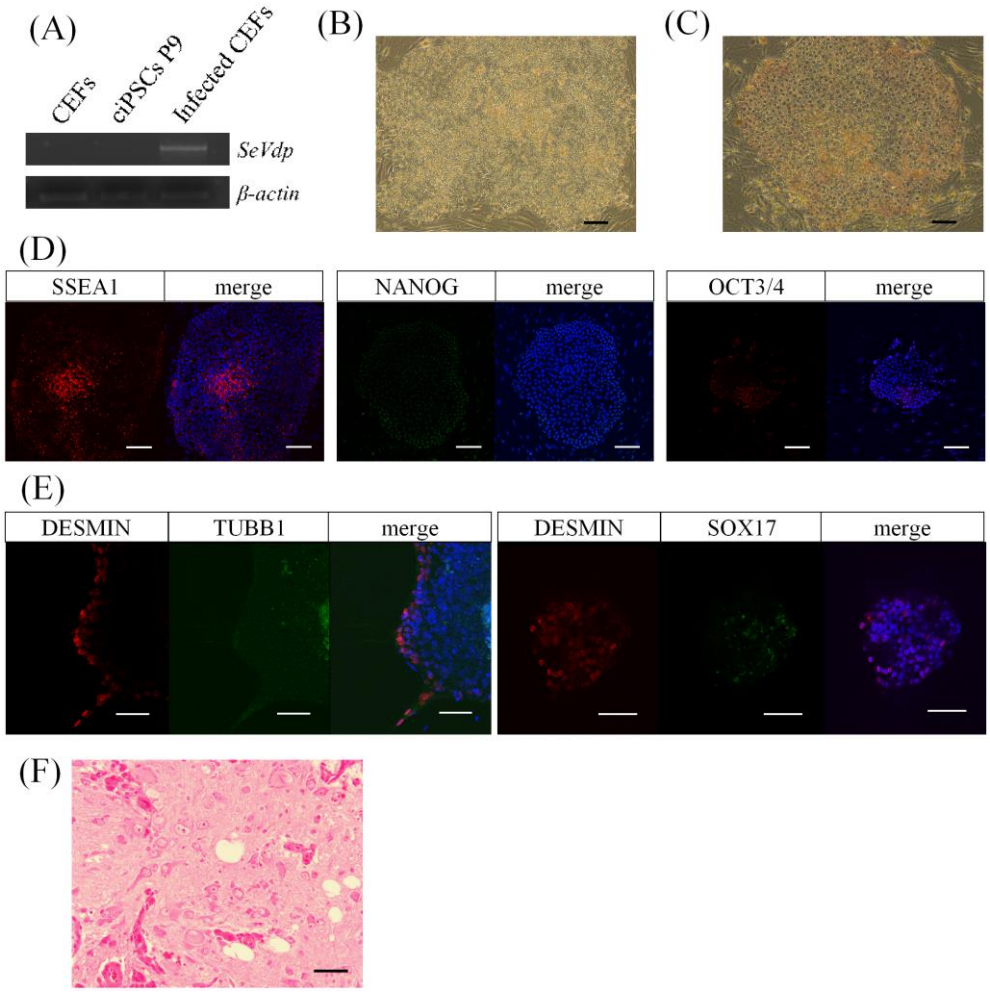

Figure legend

Fig. 1 (A) RT-PCR for checking presence of SeVdp(KOSM)302L. CEFs; negative control, infected CEFs; positive control. (B) The morphology of ciPSC line at passage 21 (C) Alkaline phosphatase activity of ciPSCs at passage 29. (D) Immunocytochemistry for each pluripotent marker at passage 19. Right panel of each marker shows the merge with DAPI. In (B)-(D), scale bar = 100 μm. (E) Immunocytochemistry for each differentiation marker. DESMIN; mesoderm, TUBB1; ectoderm, SOX17; endoderm. Right panel of each marker shows the merge with DAPI. Scale bar = 50 μm. (F) Hematoxylin and Eosin staining of incomplete teratomas. Scale bar = 50 μm.