ABSTRACTS

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Canine fibroblasts expressing human SOX2: Preliminary results on the production of canine induced pluripotent stem cells

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OBJECTIVES AND METHODS: The main objective of this study was to produce canine cell lineages expressing four known pluripotency-related transcription factors (OCT4, SOX2, c-MYC and KLF4, or OKSM) (3) associated to fluorescent reporter genes (2) individually or in combination in order to access the optimal stoichiometry needed for the induction of pluripotency in canine species. Fetal fibroblasts were obtained from one fetus at approximately thirty days of gestation derived from an elective hysterectomy. After the removal of head and organs, fetal tissue was digested with collagenase IV (Sigma). The resulting cells were washed and in vitro cultured (DMEM, Invitrogen, supplemented with fetal bovine serum and antibiotics). Lentiviral production consisted on the lipofection of 293FT cells (Invitrogen) with Lipofectamine reagent (Invitrogen) following manufacturer’s suggestions. Supernatant was recovered at 48h and 72h after transfection, filtered and concentrated by ultracentrifugation. Stable transgenic cell lineages were produced by transducing 2 x 10^5 cells canine fibroblasts previously plated the day before with 100µl or 250µl of supernatant containing lentivirus produced with each of the transcription factors supplemented with 6µg/ml polibrene (Sigma). Percentage of cells expressing the fluorescent reporters was analyzed by flow cytometry (FACSAria, BD Biosciences) and positive cells were sorted and in vitro cultured or cryopreserved for further studies.

RESULTS: Stable cell lineages transgenic for the pluripotency-related factors were produced and the results concerning SOX2-mCitrine gene were analyzed and detailed here. The percentage of positive cells was calculated using non-transduced cells as controls. After incubation with two different supernatant concentrations, canine cells were successfully responsive to the transduction. No difference was observed between cell cultures, however, cells transduced with a higher amount of virus presented a diminished percentage of positive cells when compared to those that received 250µl of supernatant containing lentivirus produced with each of the transcription factors supplemented with 6µg/ml polibrene (Sigma). Percentage of cells expressing the fluorescent reporters was analyzed by flow cytometry (FACSARia, BD Biosciences) and positive cells were sorted and in vitro cultured or cryopreserved for further studies.

CONCLUSION: The amount of cells in which the reprogramming factors stably integrated into the canine genome was determined by the fluorescent reporter gene expression detected by flow cytometry. Canine cells showed to be responsive to the integration and expression of human SOX2, probably in a higher magnification when compared to other species (1). Such positive results are essential to the establishment of pluripotency induction through the incorporation of known transcription factors into the genome of somatic cells. As already known, species other than humans and mice are refractory to the establishment of true embryonic stem cells. Therefore, optimization and characterization of direct reprogramming in domestic animals such as dogs are extremely welcome and are needed in order to dissect mechanisms involved in cellular reprogramming pathways.

Table 1: Flow cytometry analysis of stable SOX2-mCitrine transgenic canine fibroblasts

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<thead>
<tr>
<th>Supernatant volume</th>
<th>SOX2-mCitrine positive cells</th>
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<tr>
<td>100 µl</td>
<td>96,3%</td>
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<tr>
<td>250µl</td>
<td>90,2%</td>
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