Canine Placenta-derived Mesenchymal Stromal Cells for the Treatment of Neurological Disorders in Dogs

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Background

Neurological disorders such as **spinal cord injury (SCI)** can lead to permanent functional deficits in humans and their canine companions. More than 1,250,000 Americans are affected with SCI. Stem cell therapy for SCI has been investigated in preclinical and clinical trials with limited success, in part because of the lack of preclinical testing in realistic and spontaneous animal models. **Adapting new stem cell therapies** to treat companion animals with naturally occurring SCI could make **cutting-edge veterinary care** available to animals that would otherwise be euthanized, and **lay the groundwork for a therapy that can be used to treat human patients.**

Purpose

The Farmer/Wang lab has identified placental mesenchymal stromal cells **(PMSCs)** as a novel cell source for the treatment of SCI. Applying PMSCs in a fetal ovine model of SCI that mimics spina bifida (SB) dramatically and consistently cures the associated hind limb paralysis in the well-established fetal lamb model. **This study set out to isolate and characterize canine PMSCs (cPMSCs) and conduct an initial *in vitro* assessment of the potential therapeutic capacity of these stem cells for use in an eventual veterinary clinical trial.**

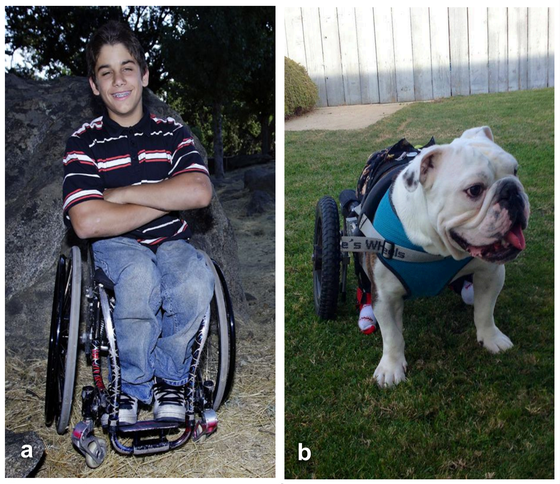

Figure 1: A comparison of human and canine spina bifida. Both children (a) and dogs (b) can be severely disabled with locomotor deficits and incontinence.

Methods

cPMSCs were isolated from term canine placentas (Figure 2). Cell phenotype was assessed by flow cytometry and multipotency assays. Growth kinetics were calculated during routine cell expansion. Cell secretion was detected using a quantitative cytokine array kit. Viability of cPMSCs in a collagen hydrogel was assessed using a fluorescent live/dead assay. cPMSC impact on neurogenesis of SH-SY5Y cells was analyzed by Wimasis imaging.

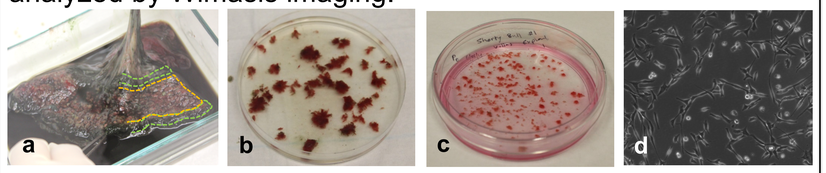

Figure 2: Isolation of cPMSCs. cPMSCs were isolated from term canine placenta obtained from seven donor puppies. Placental tissue (outlined in orange) was dissected from the marginal hematoma (outlined in green) (a) before further mincing and subsequent enzymatic digestions (b). The digested tissue and cells were then plated in tissue culture treated dishes (c) and harvested for subculture when dishes became confluent (d).

Results

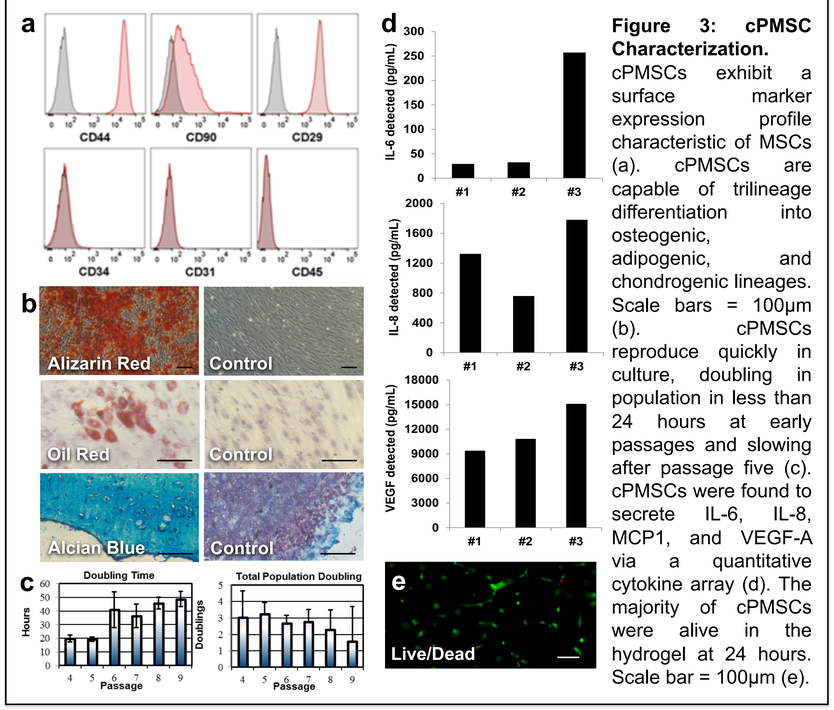

Figure 3: cPMSC Characterization. cPMSCs exhibit a surface marker expression profile characteristic of MSCs (a). cPMSCs are capable of trilineage differentiation into osteogenic, adipogenic, and chondrogenic lineages. Scale bars = 100µm (b). cPMSCs reproduce quickly in culture, doubling in population in less than 24 hours at early passages and slowing after passage five (c). cPMSCs were found to secrete IL-6, IL-8, MCP1, and VEGF-A via a quantitative cytokine array (d). The majority of cPMSCs were alive in the hydrogel at 24 hours. Scale bar = 100µm (e).

Results

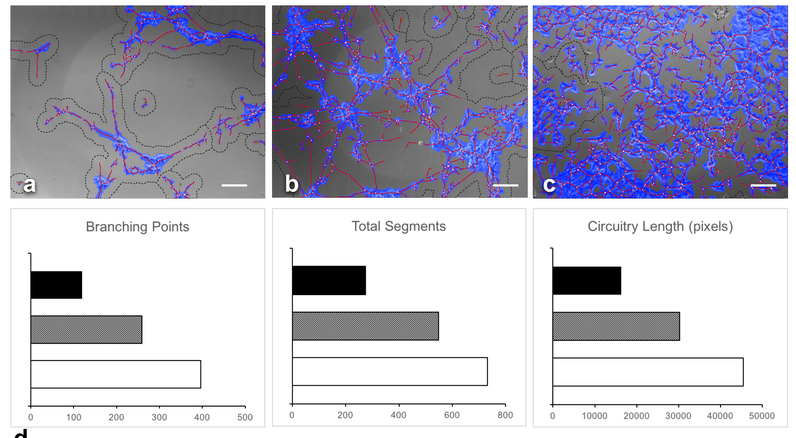

Figure 4: Co-culture with cPMSCs protects neuron-likeSH-SY5Y cells. SH-SY5Y cells damaged with staurosporine can be rescued if co-cultured with cPMSCs for 96 hours after staurosporine treatment. Damaged and untreated cultures exhibit the least proliferation and neurite outgrowth (a), in comparison to damaged cultures treated with cPMSCs (b) and undamaged controls (c). Scale bars = 100µm for a-c. Analysis with Wimasis software reveals that damaged cultures treated with cPMSCs more readily recover and express an increased number of neurite branching points and segments as well as an overall increase in total circuitry length when compared to damaged and untreated controls (d).

Conclusions

- cPMSCs meet the criteria to be considered MSCs and appear analogous to human PMSCs.
- cPMSCs show promise as a therapeutic due to viability in a collagen hydrogel delivery vehicle and secretion of immunomodulatory and neuroprotective cytokines and growth factors.
- Co-culture experiments with SH-SY5Y cells suggest cPMSCs can rescue damaged neurons.

Acknowledgements

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