Canine adult stem cells as a model for human cardiac stem cell research

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Problem and Background

Rodent modelling systems allow for cost effective, high throughput and rapid analysis, however the results obtained rarely directly translate into human disease systems. Larger animal models are required to study cardiac stem cell biology, and the dog is an ideal subject. The dog is a species which develops naturally occurring cardiac disease, including some clinical syndromes seen in humans. The dog is a relatively inbred species which therefore limits genetic variation when looking for genetic causes of disease. Moreover the pet dog is a close human companion, and therefore shares common environmental factors of disease.

ASCs are of key interest in terms of understanding tissue biology and for the development of autologous tissue repair systems (Smith *et al,* 2007). Unlike ESCs they do not form teratomas, and are therefore considered safer for clinical applications. Development of canine specific stem cell culture may act as an animal sparing procedure for pharmaceutical testing.

Therefore we have aimed to isolate and characterise adult stem cells from the dog, and make direct comparisons with cardiac stem cells described from humans.

Materials and Methods

Atrial cardiac explants were taken from dogs *post-mortem* and cultured using established techniques (Messina *et al* 2004). Putative stem cell harvests were collected from these tissues and characterised; following this cardiac lineage directed differentiation was performed using the de-methylating agent 5' Azacytidine (Oh *et al* 2003). Gene expression profiles of stem cell markers, mid-differentiation markers and cardiac lineage markers were investigated at both the RNA and protein level to determine the outcome of the differentiation procedure.

Results and Discussion

Large phase bright cells grew directly from cardiac explant tissue in culture. These cells were isolated and transferred to serum free stem cell culture conditions and were capable of successive passages (Fig. 1A-D). The stem cells demonstrated clonal expansion (Fig. 1E-I)), a key characteristic of stem cells. The cells formed large spherical clusters termed 'cardiospheres' previously described for human cardiac stem cells, which could be harvested and were labelled for c-kit using immunofluorescence (IFA) (Messina *et al* 2004) (Fig. 2A-D and 2E). Marker expression analysis demonstrated a maintenance of expression patterns over extended stem cell culture suggesting a standard profile; c-kit, GATA 4, flk-1 positive, cardiomyocyte lineage negative (Fig. 2G). IFA of individual stem cells showed expression for c-kit, and flk-1 (Fig. 2F).

Following cardiac directed differentiation (Fig. 3A) morphological changes were seen in the cultured cells, with the suggestion of alignment in the differentiated cells. Control wells of cells maintained in stem cell media continued to form cardiospheres over the three week experimental period (Fig. 3B-G). RT-PCR analysis demonstrated an altered expression profile toward a cardiac lineage (Fig. 3H). A decrease in the stem cell marker c-kit, and the endothelial marker flk-1, with an upregulation of the cardiac markers NKx2.5 and cardiac troponin T (CTT) was suggestive of cardiac lineage directed differentiation. Maintenance of GATA 4 was seen, with a decrease in the endothelial markers VEGF and von Willibrands Factor (vWF). Full cardiomyocyte differentiation was not achieved however, as key functional markers of cardiomyocytes such as the ryanodine receptor (RyR), cardiac troponin I (CTI) and the • 1- adrenergic receptor (B1 Ad) were not upregulated. Analysis of key markers was performed using quantitative RT-PCR (Fig. 4), which confirmed results suggested using standard RT-PCR.

Conclusions

It is possible to isolate adult stem cells from the canine heart which behave similarly to human cardiac stem cells. Furthermore we have demonstrated a change in marker expression profile consistent with cardiac directed differentiation. The combination of clonal expansion and gene expression profiles supports and justifies further characterisation of this cell population. We conclude that canine adult stem cells are directly comparable to human stem cells and are therefore an appropriate large animal modelling system for cardiac stem cell research.

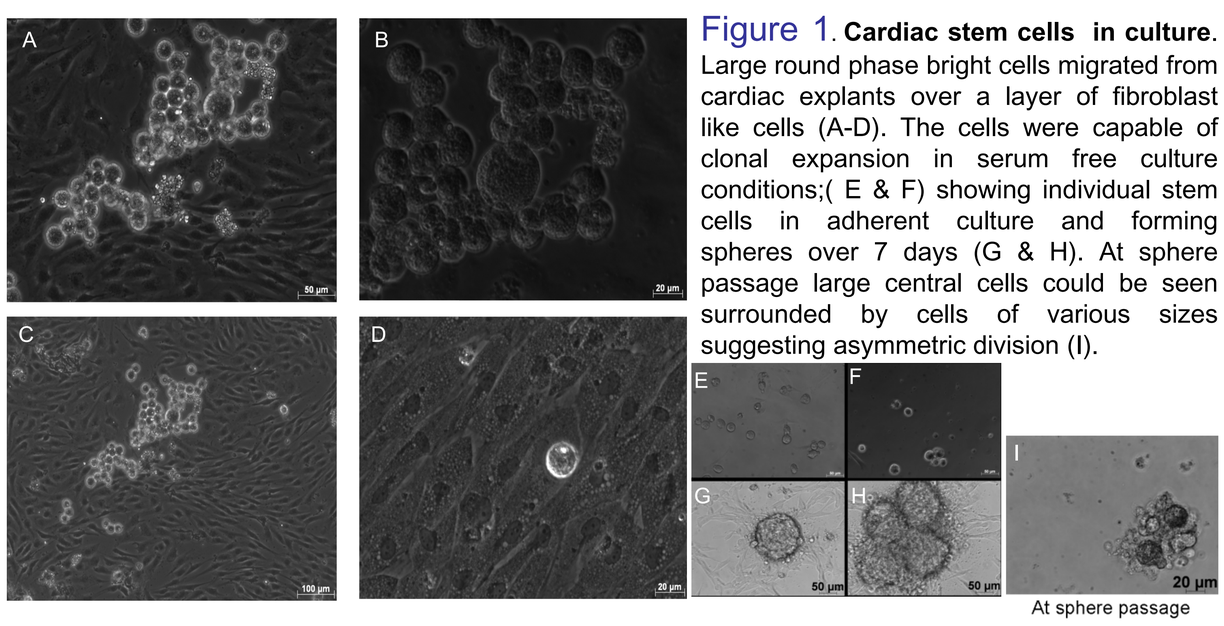

Figure 1. **Cardiac stem cells in culture.** Large round phase bright cells migrated from cardiac explants over a layer of fibroblast like cells (A-D). The cells were capable of clonal expansion in serum free culture conditions;(E & F) showing individual stem cells in adherent culture and forming spheres over 7 days (G & H). At sphere passage large central cells could be seen surrounded by cells of various sizes suggesting asymmetric division (I).

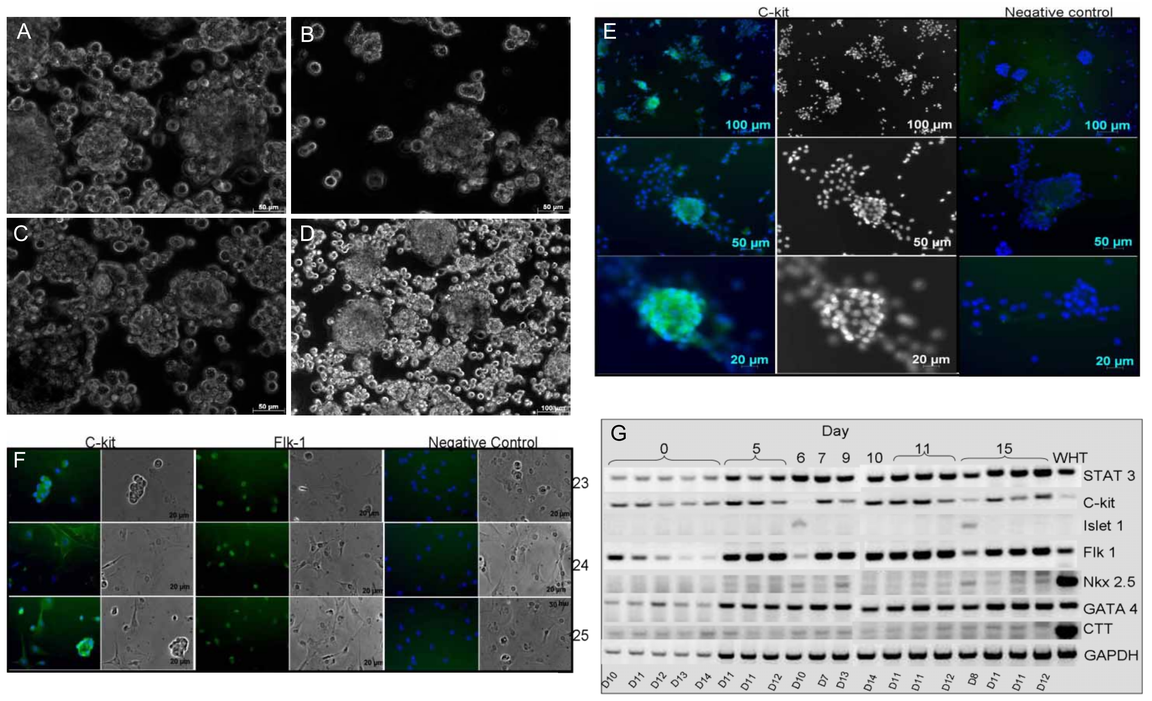

Figure 2. **Characterisation of cardiac stem cells.** Cardiac stem cells formed large clusters, 'cardiospheres' in serum free culture conditions (A-D). These spheres were strongly positive for C-kit using both RT-PCR and IFA (E). Early culture stem cells showed positive for c-kit and flk-1 using IFA (F). Semi-quantitative RT-PCR of extended culture cardiac stem cells in serum free media suggested a standard expression profile at RNA level (G) (Day – days in serum free media; multiple dogs used, labelled along the bottom).

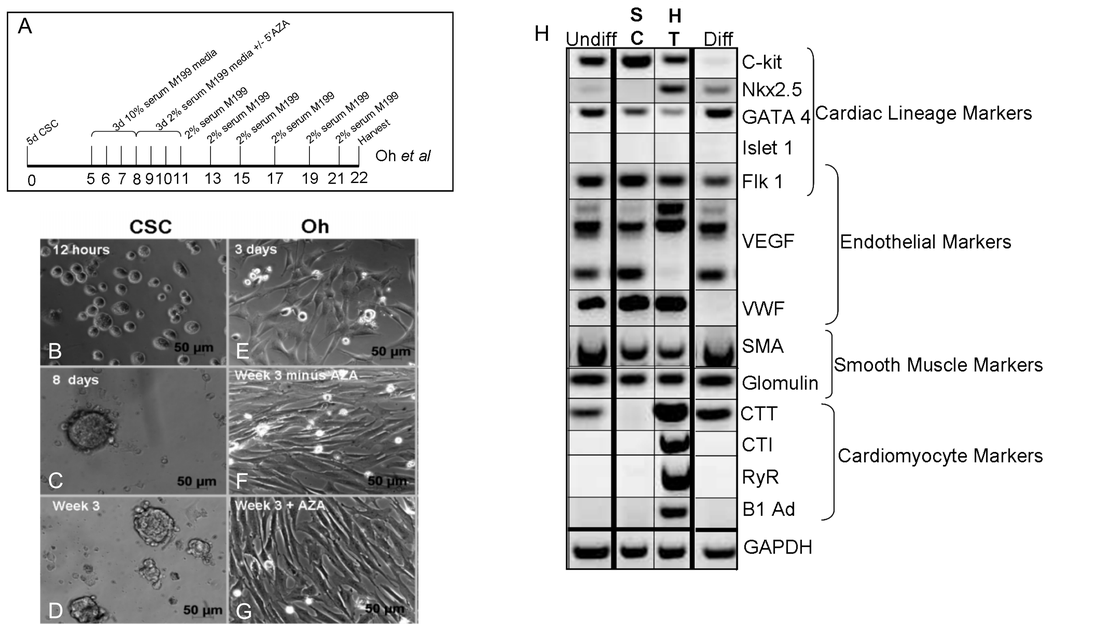

Figure 3. **Cardiac directed differentiation of cardiac stem cells.** Following a protocol using 5' AZA and serum changes adopted from Oh *et al* (2003) cardiac stem cells were driven toward a cardiac lineage. A modified protocol was run concurrently, not including 5'AZA (A). Cells were cultured for 3 weeks and subsequently analysed. A control culture of cardiac stem cells in serum free media produced cardiospheres, as seen previously (B-D) . Initially all cells exposed to serum media formed adherent expanding cultures (E). Over three weeks, morphologically the differentiated cells appeared to grow in a more organised pattern (G) compared to the modified protocol (F). Semi-quantitative RT-PCR analysis suggested a difference in expression pattern between the differentiated and modified protocols when compared to controls (H); cardiac stem cells (CSC) and whole heart tissue (WHT).

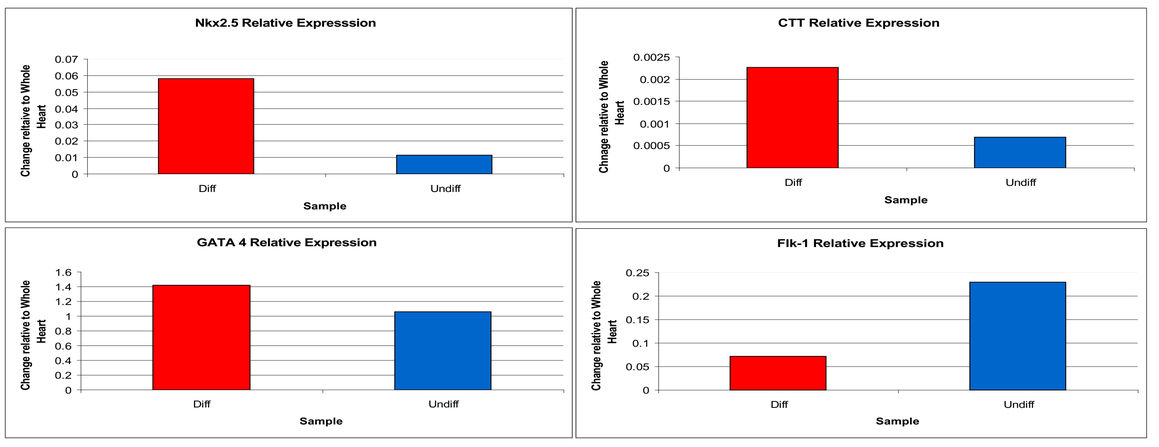

Figure 4. **Quantitative analysis of cardiac directed differentiation.** A 5 fold and 3.5 fold increase in expression of Nkx2.5 and CTT (respectively) was seen in the differentiated samples, compared to controls. No significant alteration in GATA 4 expression was seen between samples, however a 3 fold reduction in flk-1 expression was seen in differentiated cells compared to controls.

Selected References

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Disclosure; This work has been submitted to The Veterinary Journal for publication