THE FATE OF TRANSPLANTED XENOGENEIC BONE MARROW-DERIVED STEM CELLS IN RAT INTERVERTEBRAL DISCS

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Introduction
Intervertebral disc (IVD) degeneration has been considered as the major cause of chronic low back pain. Recent advances in cell therapy using adult stem cells are promising approaches for the treatment of degenerative disc. Our previous study showed that rat bone marrow (BM) mononuclear cells (MNC) were able to survive and differentiate in IVDs for up to 21 days following disc injection (1). The type of BM cells surviving in IVD is unknown as MNC consist of both haemopoietic (CD34+) and non-haemopoietic (CD34-) stem cells. It is also unclear if human BM cells will survive in rodent IVD without immunosuppressive therapy. The present study was aimed to assess whether transplanted CD34+ and CD34- cells from human bone marrow would survive and differentiate in IVDs of a rodent model.

Materials and Methods

Human cells preparation: Human BM was collected from consented haematologically normal individuals. MNCs were isolated by Ficoll density centrifugation. CD34+ and CD34- cells were separated from MNCs using magnetic activated cell sorting into CD34+ (purity 89%) and CD34- cells, and then labelled with Cell Tracker Orange (CTO).

Animal experiments: The study was approved by the UNSW animal ethics committee. Twenty-eight male Sprague-Dawley rats weighting 350-400g were used. The intervertebral disc injection procedures were performed under fluoroscopic guidance with a 30 gauge needle. 5-10ul of either CD34+ or CD34- cells in PBS suspension (1x10^7 cells/ml) were injected into each coccygeal disc. For each rat, four discs were injected and one served as control. The rats were euthanased and the discs were harvested at day 1, 10, 21 and 42 post transplantation.

Assessment of survival cells: Discs were formalin fixed and both frozen and paraffin sections were prepared for histological and immunofluorescence assays. Survival of human cells was detected by visualization of bright red CTO-fluorescence cells and further confirmed by immunostaining with human specific marker to nuclear protein (HN, green). Expression of type II collagen was assessed in the transplanted cells using immunofluorescence method. Expression of CD68 and Fas-L were evaluated with Immunohistological staining.

Results
1. No major histological damage to the IVD as the result of the human cell injection (not show) was seen by standard light microscopy Hematoxylin Eosin (H&E) staining.
2. Fluorescence microscopy demonstrated significantly decreased number of CTO labelled human CD34+ cells by day 10 and no cells could be detected after day 21(Fig 1). However, the number of CTO+/CD34- cells dropped initially but survived up to Day 42 (Fig 2).
3. All CTO+ cells were confirmed to be of human origin by the expression of human specific nuclear antigen (HN). Expression of type II collagen was assessed in the transplanted cells using immunofluorescence method. Expression of CD68 and Fas-L were evaluated with Immunohistological staining.

Discussion and conclusion
To our knowledge, this was the first study to demonstrate that CD34- cells from human bone marrow but not CD34+ cells survived in rat IVDs for up 42 days. The surviving cells expressed collagen II after 21 days, providing preliminary evidence of differentiation of transplanted human cells in rodent IVDs without requiring immunosuppression. These findings suggest that the CD34- cells from human bone marrow have a broad differentiation potential and an ability to develop into disc-like cells which provides a potential source for cellular therapy for restoring degenerative disc. In addition, these data provide further in vivo evidence that the nucleus pulposus of intervertebral disc contains the properties of an immune privileged site. This relatively immune privileged nature of NP offers an adaptive environment for transplanted xenogenic or allogenic stem cells.

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Reference: (1) Brisyby H, Wei A, Chung S, Tao H, Ma D and Diwan A., 2004 ORSPN1075

Fig. 2. Detection of injected human CD34+ or CD34- cells (red dots, 10x) in IVD counterstained with DAPI (blue dots). (A)x(control), CTO+/CD34- cells were detected within the rat IVDs at Day 1 (B), 10 (C), 21 (D) and 42 (E100x). CTO+/CD34+ cells were not detected at day 21(F).

Fig. 3. Representative Immunofluorescence images of IVD section at day 21 post injection. A: Injected CTO+ cells (red). B: Injected cells were positive for human nuclear protein (HN, green). C: CTO+ cells were also HN+ (yellow). D: IVD tissue was counterstained with DAPI (blue, 40x). E: CTO/DAPI double stained cells (pink). F: CTO/DAPI triple-positive cells were detected in the middle of rat NP (yellow).

Fig. 4. Transplanted human CTO+/CD34- cells expressed collagen II in rat IVD at 21(A-C, 10X) and 42 days (D-F, 20X)

Fig. 5. Immunohistological analysis revealed CD68+ (brown) cells increased in the inner annulus fibrosus (AF) at day 1 (B,20x, C, 40x) compared with non injected (A). CD68+ were detected in outer AF at day 10 post injection (D: 10x), but was not detected in NP (E: 10x).

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