INTRODUCTION

The real promise of a cell-based approach for muscle regeneration and repair lies in the promotion of cell survival at the site of graft to achieve both structural and functional benefits. We have showed recently that pig is an adequate large animal model for exploring myogenic precursor cell (MPC) transplantation strategies applicable in patients [1]. Nevertheless, a common observation in all the models tested so far is that MPC die very rapidly after transplantation. In our pig model, 90% of the transplanted cells died within the first five days after injection [1].

There is evidence that both inflammatory and immune processes contribute to the early cell death post injection [2]. In the study reported here, we genetically modified porcine MPC to express the anti-apoptotic/anti-inflammatory gene heme oxygenase-1 (HO-1) and investigated their survival after autologous transplantation in pigs.

MATERIAL AND METHODS

Gene transfer

We constructed a lentiviral vector encoding the human HO-1 gene containing a Flag sequence (HO-1/flag) under a PGK promoter. Cell transduction was performed by incubating freshly isolated porcine MPC [1] with HIV-HO-1/flag at a multiplicity of infection (MOI) of 3 (pilot experiments showed that this MOI allowed >60% cell transduction without loss of myogenicity) in the presence of polybrene (8 µg/mL).

Transduced cells were cultured for 5 days and used at passage 1 for all in vitro and in vivo experiments.

Western blot analysis

Total proteins were separated on an 8% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. Blots were incubated with the following primary antibodies: mouse anti human HSP70/72 (1:1000, Juro); rabbit anti human HO-1 (1:2000, Stressgen) and mouse anti tubulin (1:3000, Sigma). Blots were then incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit Ab (1:6000, BioRad). Blots were revealed using ECL reagents and Hyperfilm MP (Amersham Biosciences).

In vitro apoptosis assay

Control and HO-1/flag transduced cells were incubated with staurosporin (1 µM) for 24 h and the percentage of apoptotic cells was analyzed by flow cytometry using the Hoechst 33342/7AAD labeling technique. The percentage of FACS-positive apoptotic cells at MOI = 0 was set to 100%.

Cell transplantation

All in vivo experiments were done on 8 weeks old pigs. Care and use of animals were in compliance with national as well as international guidelines. Control, heat-shock (HS) pretreated (cell used 24 h after the thermal shock [1 h incubation at 42°C]), nls-LacZ transduced or HO-1/flag transduced porcine MPC were labeled with 3H-thymidine and transplanted (in triplicates, 2 x 10^5 cells per injection) in intact muscle. Biopsies were performed at day 0 and at day 5 post-injections. Statistical evaluation was performed using Student’s T test and results were considered significant if P-values were < 0.05.

RESULTS

In vitro

Western-blots analysis indicated a significant HO-1 over-expression 5 days after the lentiviral infection. HSP70/72 expression did not change in HO-1/flag porcine MPC as compared to uninfected cells (Fig 1). Transduced cells kept their ability to proliferate and fuse in vitro (data not shown). The expected protective cellular effects of HO-1/flag were assessed in vitro by quantifying apoptosis after 24 hours incubation with staurosporin (1µM). We demonstrated that HO-1/flag infected porcine MPC dose dependently inhibit staurosporin induced apoptosis in vitro (Fig 2, n=4). That apoptosis inhibition correlated ($r^2 = 0.97$) with an increased HO-1 expression in porcine cells.

In vivo

Survival of porcine MPC 5 days after autologous transplantation is shown in Fig 3. The 100% survival reference was defined as the quantity of donor MPC recovered from an injected intact muscle immediately after transplantation. Consistent with our previous report, a massive donor cell death occurred within 5 days after transplantation of untreated porcine MPC (10.8 ± 3%, n=6). Similar results were obtained after transplantation of nls-LacZ transduced cells (8.9 ± 2.6%, n=3). HO-1/flag transduced porcine MPC (n=7) showed a significant prolonged graft survival (47.5 ± 9.1%) as compared to untreated cells (P < 0.005) or to heat-shock pretreated cells (26.3 ± 5.5%; P = 0.03).

Figure 1: Porcine MPC were transduced with the lentiviral vector encoding the human HO-1/flag gene and analyzed by western blot 5 days after infection.

Figure 2: Expression of HO-1/flag in porcine cells correlates with protection from staurosporin-induced apoptosis. After infection with the HIV-HO-1/flag, porcine MPC expressed HO-1/flag (●) and dose dependently inhibited staurosporin mediated apoptosis (○) as assessed by FACS analysis (n=4).

Figure 3: Survival rate of 3H-thymidine labeled porcine MPC 5 days after autologous transplantation in intact porcine skeletal muscle.

DISCUSSION

In the present study, we demonstrated the feasibility of using anti-apoptotic human HO-1 gene transduction for creation of “resistant” porcine myogenic precursor cell grafts. HO-1 expression resulted in a markedly improved cell survival during the first five days after autologous transplantation. By increasing early cell survival, HO-1 over-expression may represent a potential strategy for improving muscle regeneration after injury.

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