TRANSPLANTATION OF PURIFIED HUMAN SKELETAL MUSCLE-DERIVED PERICYTES REDUCE FIBROSIS IN INJURED ISCHEMIC MUSCLE TISSUES

INTRODUCTION

Vascular pericytes are the mural cells that tightly encircle capillaries and microvessels throughout the body. In general, pericytes control blood vessel maturation, stability and contractility. Multi-lineage stem/progenitor cells have been identified within virtually all organs in both human and mouse and are named diversely [1]. However, due to the retrospective discovery of these multi-lineage stem/progenitor cells in culture, the true identity of these cells in situ remained obscure. It has been hypothesized that vascular pericytes are indeed, or at least contain, stem/progenitor cells that are able to differentiate into bone, cartilage, fat tissue and osteoblasts [2]. Recently, we and others laboratories have shown that vascular pericytes purified from multiple human organs not only express classic MSC markers but harbor stem cell properties such as myo-, osteo-, chondro-and adipogenic potentials [2]. Consequently, pericytes are assumed to be one of the developmental origins of MSC. Human skeletal muscle-derived pericytes were not only shown to regenerate skeletal muscle fibers in dystrophic as well as cardiotoxin-injured mouse muscle but also sustain impaired cardiac function after myocardial infarction in vivo [2]. Nevertheless, whether transplanted vascular pericytes contribute to the reduction of fibrosis at the site of injury remains to be elucidated. Matrix metalloproteinases (MMPs) are proteolytic enzymes responsible for extracellular matrix protein degradation with an important role in tissue remodeling processes. MMP-2 and MMP-9 activities are often implicated in fibrosis [3]. Using the animal model that has been established previously, we commenced to explore whether or not pericytes influences scar tissue formation within the damaged ischemic myocardium and attempt to elucidate the mechanism(s) of action [4].

METHODS

Cell Isolation and Cell Sorting: Fresh specimens of human fetal skeletal muscle were mechanically dissociated and digested with collagenases. After lysis of erythrocytes, single cell suspension was obtained by filtering with 70-μm cell strainer. For cell sorting, cells were incubated with the following directly-conjugated antibodies: anti-CD34-PE, anti-CD45-APC, anti-CD56-PE-Cy7, and anti-CD146-FITC. For dead cell exclusion, 7-amino-actinomycin D (7-AAD) was added to stained cells before running on a FACSAria flow cytometer. Flow cytometry with an identical gating strategy was used to verify the purity of long-term cultured muscle-derived pericytes.

Animal Model of Fibrosis: Permanent ligation of the left coronary artery was performed on NOD/SCID mice under open-chest surgery. Immediately after the ligation, skeletal muscle-derived pericytes from cultures were injected into the ischemic myocardium (3×10^5 cells/heart) and microvessels throughout the body. In general, pericytes control blood vessel maturation, stability and contractility. MMP-2 and MMP-9 activities are often implicated in fibrosis [3]. Using the animal model that has been established previously, we commenced to explore whether or not pericytes influences scar tissue formation within the damaged ischemic myocardium and attempt to elucidate the mechanism(s) of action [4].

Histology: Masson’s trichrome staining was employed to reveal myocardial fibrosis. The ratio of fibrotic area was estimated by total collagen deposition versus total sectional area using Image J software.

Hypoxic Culture: Pericytes were cultured in vitro under hypoxic conditions (2.5% oxygen) for 24 hours. Supernatants and cell pellets were subsequently collected for analysis.

RESULTS

Purification of Human Muscle-derived Pericytes: CD344556146^th muscle-derived pericytes were sorted and cultured as previously described [2]. To ensure long-term cultured muscle-derived pericytes remain homogeneous and retain their native properties, flow cytometry was employed to examine the expression of cell surface markers used to select these cells plus alkaline phosphatase (ALP), another pericyte marker. The result shows that after long-term culturing (>10 passages), all cells remain negative for CD34, CD45 and CD56, and virtually all pericytes are positive for CD146 and ALP (Figure 1a).

Transplantation of Muscle-derived Pericytes: Immediately after permanent ligation of the left coronary artery, muscle-derived pericytes from cultures were injected into the ischemic myocardium at a density of 3×10^5 cells/heart (Figure 1b) [4].

Scar Tissue Formation: Masson’s trichrome staining revealed myocardial fibrosis after infarction with collagen deposition stained in blue (Figure 2a). Quantification at 2 weeks post injection demonstrated a 38% reduction of the fibrotic area in pericyte-injected left ventricles, compared to saline-injection (Figure 2b).

Expression of MMP-2 by Muscle-derived Pericytes: Real-time quantitative PCR results revealed that cultured muscle pericytes have higher expression of MMP-2 gene than total muscle lysates, but not MMP-9. Under hypoxic culture conditions, muscle pericytes retain high expression of MMP-2 gene, while MMP-9 remained very low (Figure 3).

DISCUSSION

We demonstrated that transplantation of purified skeletal muscle-derived pericytes ameliorate fibrosis in injured ischemic cardiac muscle, possibly exerting a preventative effect. Reduced MMP-2 activity has been shown to contribute to cardiac fibrosis in pathological conditions [5]. We consequently hypothesized that the high expression level of MMP-2 by muscle-derived pericytes, even under hypoxic conditions, may play a key role in pericyte-mediated reduction of fibrosis. We are currently conducting experiments to demonstrate the efficacy of muscle pericyte transplantation in diminishing chronic fibrosis. We are also investigating the influence of increased angiogenesis, a beneficial effect displayed by muscle pericytes, on reduction of fibrosis. Overall, this study will shed light on the therapeutic value of muscle-derived pericytes for the treatment of fibrosis in the injured tissues including musculoskeletal tissues.

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REFERENCES