

Factors related to the efficacy of skeletal muscle cell transplantation and future approaches with control-released cell growth factors and minimally invasive surgery

Keiichi Tambara MD^a, Yasuhiko Tabata PhD, DMedSci, DPharm^b, Masashi Komeda MD, PhD^{*,a}

^aDepartment of Cardiovascular Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

^bDepartment of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

1. Introduction

Cell transplantation to the diseased heart has emerged as a promising strategy for refractory heart failure that cannot successfully be treated by conventional therapies. Among various donor cells available, skeletal muscle cells form one of the cell sources about which some consensus of efficacy has been achieved: skeletal myoblast transplantation significantly attenuates left ventricular remodeling when myoblasts are implanted into the infarct area. Although clinically performed, skeletal myoblast transplantation has a number of aspects to be clarified such as indication, cell delivery method (timing, route, or cell condition), and working mechanism. In this paper, we outline the factors that affect the efficacy of skeletal muscle cell transplantation, and introduce very recent results in our research laboratory using control-released cell growth factors and minimally invasive cell delivery, which we believe is likely to contribute to solve some issues.

2. Factors related to the efficacy of skeletal muscle cell transplantation

There are a number of factors that affects the outcome of skeletal muscle cell transplantation. The followings are major ones:

1. donor cell number or graft volume [1,2]
2. graft cell death [3]
3. cell delivery method [4–6]

First, it is widely accepted that there is a positive correlation between the extent of improvement in cardiac function and the donor cell number or donor-derived muscle volume in skeletal myoblast transplantation [1,2]. Although it seems likely that a sizable muscular graft can be obtained if a large

number of cells are transplanted at one time, the procedure may run a risk of tissue overgrowth or massive cell death. Secondly, graft cell death is a critical problem especially in cardiomyocyte transplantation, in which dose escalation does not work. In contrast, skeletal muscle cells are much more tolerant to poor graft environments. However, because there is a report that less than 1% of delivered cells survived in human cases [3], the low survival rate of transplanted myoblasts remains to be solved. Finally, how myoblasts are transplanted is also a very important issue. This includes cells vs. tissues [4] vs. cell sheets [5], or surgical vs. transcatheter [6] vs. endoscopic approach.

These factors affect each other, and determine the efficacy of skeletal muscle cell transplantation.

3. Simultaneous administration of control-released cell growth factors

Cell growth factors are prerequisite peptides for cell maturation, proliferation, differentiation, kinetics, etc. Therefore, administration of these factors appears to be a reasonable approach to increase cell survival after transplantation. However, single-dose or bolus administration does not provide sufficient effect due to rapid reduction of tissue concentrations. In this context, we developed a drug-delivery system using gelatin hydrogel microspheres or sheets to enable various cell growth factors to be gradually released over 2 to 4 weeks [7,8]. In this system, growth factors are linked with gelatin molecules by electrostatic force, and released with biodegradation of gelatin.

We investigated in the following experiments of cell transplantation the efficacy of the control-release system using basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) in a rat myocardial infarction model. We used Lewis rats as both recipient and donor animals. All animals were treated 4 weeks after left coronary artery ligation.

* Corresponding author. Professor and Chairman, Graduate School of Medicine, Department of Cardiovascular Surgery, Kyoto University, 54 Kawaharacho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. Tel.: +81-75-751-3780; fax: +81-75-751-3098.

E-mail address: masakom@kuhp.kyoto-u.ac.jp

3.1. Basic fibroblast growth factor for increase in graft volume and vascular density

bFGF exerts functions to stimulate proliferation and repress differentiation in skeletal myoblasts. Therefore, in skeletal myoblast transplantation, its exogenous administration to the implantation site may facilitate the development of large muscular graft. In addition, bFGF may synergistically enhance the benefits of myoblast transplantation by inducing angiogenesis and arteriogenesis in and around the donor-derived tissue. Then, we investigated whether the efficacy of skeletal myoblast transplantation is enhanced by simultaneous administration of control-released bFGF [9].

Forty-five rats were randomized into 3 groups. In the Tx-bFGF group ($n=15$), neonatal skeletal myoblasts (5×10^6) were subepicardially transplanted into the infarct area, and a gelatin hydrogel sheet (20×20 mm) containing $100 \mu\text{g}$ bFGF, which enables controlled release of bFGF, was placed onto the left ventricular surface to cover the infarct and peri-infarct areas. In the Tx group ($n=15$), skeletal myoblasts were transplanted followed by application of a sheet impregnated with saline in the same manner. The control group ($n=15$) had culture medium injection with application of a sheet with saline. At 4 weeks, in the Tx-bFGF group left ventricular dimension (Tx-FGF, Tx, Control; 0.93 ± 0.01 vs. 0.96 ± 0.01 vs. 1.00 ± 0.01 cm, $P=0.01$) and infarct size were smallest ($P=0.05$), and left ventricular systolic and diastolic functions were most improved (Tx-FGF, Tx, Control; end-systolic elastance: 0.76 ± 0.04 vs. 0.51 ± 0.04 vs. 0.30 ± 0.03 mmHg/ μL ; τ : 15.8 ± 0.7 vs. 19.5 ± 0.8 vs. 20.5 ± 0.6 ms, both $P < 0.01$). Vascular density inside and around the graft was highest in the Tx-bFGF group. Graft volume in the Tx-bFGF group was 3 times larger than that in the Tx group. There was no difference in fibrotic area inside the graft between the Tx-bFGF and Tx groups. Therefore, we concluded that simultaneous administration of control-released bFGF may enhance the efficacy of skeletal myoblast transplantation by inducing angiogenesis inside and around the graft and greatly increasing graft volume.

In advance of this experiment, we reported that prevascularization with control-released bFGF enhances the benefits of cardiomyocyte transplantation [8]. Although skeletal myoblasts are much more ischemia-resistant than cardiomyocytes, it is still likely that administration of bFGF before transplantation benefits skeletal myoblast transplantation.

3.2. Hepatocyte growth factor for anti-apoptosis

Although HGF is known to be a potent angiogenic factor, its anti-fibrotic and anti-apoptotic functions may also exert favorable effects for cell survival. Therefore, we investigated whether simultaneous administration of control-released HGF enhances the efficacy of cell transplantation using cardiomyocytes.

Thirty-four rats were divided into 3 groups. In the Tx-HGF group ($n=12$), neonatal cardiomyocytes (5×10^6)

were transplanted to the infarct area with simultaneous application of a gelatin hydrogel sheet (20×20 mm) incorporating $40 \mu\text{g}$ HGF onto the left ventricular surface. In the Tx group ($n=12$), cardiomyocyte transplantation was performed in the same manner with a gelatin sheet containing saline only. In the Control group ($n=10$), culture medium was injected followed by application of a gelatin sheet with saline. Two rats each in the Tx-HGF and Tx groups were sacrificed 1 day after transplantation, and terminal deoxynucleotidyl transfer-mediated end labeling of fragmented nuclei (TUNEL assay) was performed on heart sections including donor cardiomyocytes. For the remaining 30 rats, hemodynamic and histological assessment was performed at 4 weeks. Although about one third of the donor cells were positive for TUNEL staining in the Tx group, only 10% were TUNEL-positive in the Tx-HGF group. Left ventricular end-diastolic dimension was smallest and fractional area change was largest in the Tx-HGF group (Tx-HGF, Tx, Control; 0.94 ± 0.04 vs. 0.98 ± 0.03 vs. 1.02 ± 0.03 cm, 40.9 ± 2.0 vs. 36.0 ± 1.8 vs. $30.5 \pm 1.5\%$, both $P < 0.05$). In addition, end-systolic elastance was highest and tau was lowest in the Tx-HGF group (both $P < 0.05$). Transplanted cardiomyocytes were found over the whole infarct area in the Tx-HGF group, whereas few donor cardiomyocytes were detected in the center of the scar in the Tx group. In conclusion, simultaneous application of control-released HGF may enhance the efficacy of cardiomyocyte transplantation by improving the survival rate of donor cells.

Again, although these findings are obtained in cardiomyocyte transplantation, we believe that similar effects can also be expected in skeletal myoblast transplantation. We are currently conducting an experiment.

4. Repetitive implantation with minimally invasive cell delivery

In the era of minimally-invasive robotic surgery, repetitive implantation of cells may be applicable. We assessed the hypothesis that repetitive implantation is an effective method for cell delivery in myoblast transplantation.

Thirty rats were randomized into 3 groups. In Group I ($n=10$), myoblasts (6×10^6) were subepicardially implanted in the infarct area at 0, 2, and 4 weeks. In Group II ($n=10$), myoblast transplantation was performed only at 0 weeks, and culture medium injection at 2 and 4 weeks. Group III (control, $n=10$) had culture medium injection at 0, 2 and 4 weeks. The transplanted cells at the second and third time were labeled with fluorescent dyes of PKH 26 (red) and PKH 2 (green), respectively. Four weeks after transplantation, only Group I showed reverse remodeling, in which left ventricular diastolic dimension decreased (0.96 ± 0.02 to 0.90 ± 0.02 cm, $P < 0.05$), fractional area change increased (35.0 ± 1.6 to $41.3 \pm 1.8\%$, $P < 0.05$), and the infarct size was decreased (29.3 ± 1.5 to $22.3 \pm 1.8\%$, $P < 0.05$). In Group II,

left ventricular remodeling was inhibited, but not reversed. Also, in Group I, end-systolic elastance was highest and tau was lowest of the 3 groups. Histological study showed that donor cells were extended over the whole infarct area in all the hearts from Group I, while not necessarily from Group II. Graft volume was 3 times larger in Group I than in Group II. The cells labeled with PKH 26 and PKH 2 showed a cluster formation. In conclusion, repetitive implantation may be a feasible and effective cell delivery method in skeletal myoblast transplantation.

We are now at the stage to start a simulation of minimally invasive surgery in a pig myocardial infarction model using a thoracoscopic approach. We performed minimally invasive myoblast transplantation in 2 pigs, and the preliminary results have been encouraging.

To note, it is another advantage in repetitive cell delivery that cells can be implanted in poorly engrafted parts, because there is a high variability in seeding efficiency in skeletal myoblast transplantation [10].

5. Future direction

Although cell transplantation with skeletal muscle cells is now being established as a standard tool for the treatment of chronically ischemic hearts, the efficacy is still limited and there is ample room for refinement. Multidisciplinary approaches, such as simultaneous administration of cell growth factors or combination with surgical procedures, may be prerequisite to achieve satisfactory outcome with which myoblast transplantation could replace heart transplantation.

References

- [1] Pouzet B, Vilquin JT, Hagege AA, et al. Factors affecting functional outcome after autologous skeletal myoblast transplantation. *Ann Thorac Surg* 2001;71:844–851.
- [2] Tambara K, Sakakibara Y, Sakaguchi G, et al. Transplanted skeletal myoblasts can fully replace the infarcted myocardium when they survive in the host in large numbers. *Circulation* 2003;108(Suppl II):259–263.
- [3] Pagani FD, DerSimonian H, Zawadzka A, et al. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. *J Am Coll Cardiol* 2003;41:879–888.
- [4] Pouzet B, Vilquin JT, Hagege AA, et al. Intramyocardial transplantation of autologous myoblasts: can tissue processing be optimized? *Circulation* 2002;102(Suppl III):210–215.
- [5] Miyagawa S, Sawa Y, Takano H, et al. Efficacy of a novel cellular cardiomyoplasty using cardiac sheet for myocardial regeneration: compared with needle injection. *Circulation* 2002;106(Suppl II):420.
- [6] Smits PC, van Geuns RJM, Poldermans D, et al. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure. *J Am Coll Cardiol* 2003;42:2063–2069.
- [7] Iwakura A, Tabata Y, Miyao M, et al. Novel method to enhance sternal healing after harvesting bilateral internal thoracic arteries with use of basic fibroblast growth factor. *Circulation* 2000;102(Suppl III):307–311.
- [8] Sakakibara Y, Nishimura K, Tambara K, et al. Prevascularization with gelatin microspheres containing basic fibroblast growth factor enhances the benefits of cardiomyocyte transplantation. *J Thorac Cardiovasc Surg* 2002;124:50–56.
- [9] Tambara K, Premaratne GU, Nakajima H, et al. Simultaneous administration of control-released basic fibroblast growth factor enhances the efficacy of skeletal myoblast transplantation by increasing vascular density and graft volume. *Circulation* 2003;108(Suppl IV):332.
- [10] Reinecke H, Murry CE. Transmural replacement of myocardium after skeletal myoblast grafting into the heart: too much of a good thing? *Cardiovasc Pathol* 2000;9:337–344.