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RAPID COMMUNICATION

Cardiac Rehabilitation With Dynamic Exercise Increases the Number of Muse Cells in the Peripheral Blood of Patients With Heart Disease

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Background: It is still unclear whether dynamic exercise increases the number of Muse cells, pluripotent stem cells, in the peripheral blood.

Methods and Results: The number of Muse cells, $SSEA3^+$ and $CD105^+$ double-positive cells, in the peripheral blood was measured using FACS before and after 40 min of cardiac rehabilitation with dynamic exercise in 6 patients with heart disease. The number of Muse cells significantly increased after cardiac rehabilitation in all patients. Muse cell mobilization may be related to the beneficial clinical outcome of cardiac rehabilitation.

Conclusions: Cardiac rehabilitation increases the number of Muse cells in the peripheral blood.

Key Words: Cardiac rehabilitation; Dynamic exercise; Muse cell

Multilineage-differentiating stress-enduring (Muse) cells can be isolated as cells double-positive for the pluripotent surface marker SSEA3 and mesenchymal stem cell surface marker CD105 from the bone marrow, peripheral blood, and various connective tissues.[1–4] We recently reported that endogenous Muse cells are mobilized into the peripheral blood after acute myocardial infarction (AMI); that AMI patients with a higher number of Muse cells in the acute phase had improvement in left ventricular (LV) function and remodeling in the chronic phase;[4] and that Muse cells given i.v. after AMI homed to the damaged myocardium and improved the LV function and remodeling through cardiomyocyte regeneration and paracrine effects in rabbits.[5] Muse cells could function as reparative stem cells. Given that cardiac rehabilitation has been reported to improve the prognosis of patients with coronary artery disease (CAD),[6] the beneficial effects of cardiac rehabilitation may be related to the mobilization of Muse cells. Therefore, in this study, we examined whether endogenous Muse cells are mobilized into the peripheral blood after cardiac rehabilitation with dynamic exercise.

Methods

Subjects and Study Protocol

Patients with heart disease who were receiving cardiac rehabilitation as outpatients at Gifu University Hospital were enrolled in the study. The patients consisted of 4 with old myocardial infarction and 2 with CAD treated with STENT or coronary artery bypass grafting. Mean patient age was 72.3±5.4 years. Blood samples were collected from the antecubital vein and collected into sterile tubes, immediately placed on ice, before and 40 min after a cardiac rehabilitation program. The program consisted of 10 min of warming up, 20 min of ergometer exercise, and 10 min of cooling down. The Ethics Committee of Gifu University Graduate School of Medicine approved this study (approval number: 28-26). All patients provided written informed consent before the study commenced. The investigation conformed to the principles outlined in the Declaration of Helsinki.[7] The public and trial registry number was R000032115.

Measurement of Muse Cells in the Peripheral Blood

The number of $SSEA3^+/CD105^+$ double-positive cells was measured on fluorescence-activated cell sorting (FACS Calibur, Beckton Dickinson, San Jose, CA, USA), as previously reported.[4] The number of Muse cells was expressed as absolute number of Muse cells (/100 μL)=white blood cells (/100 μL)×monocytes (%)×$SSEA3^+/CD105^+$ double-positive cells (%).

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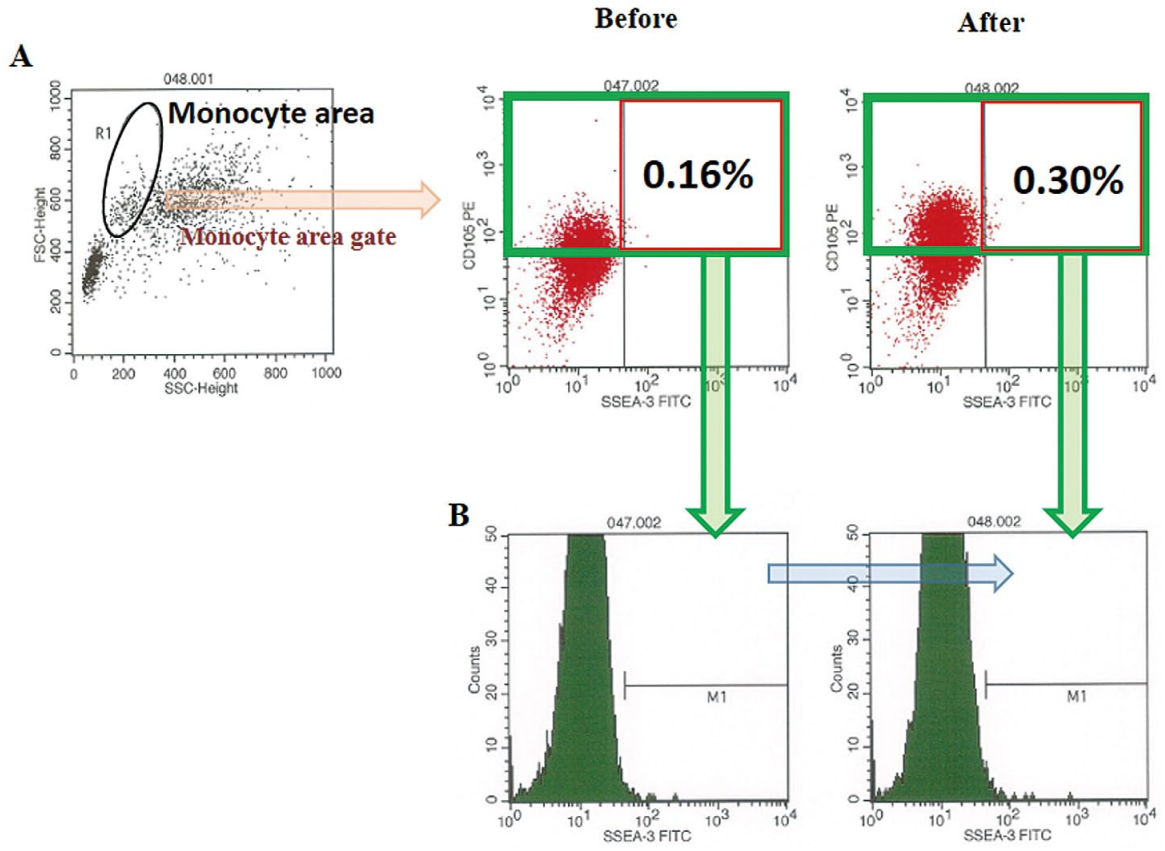

Figure 1. (**A**) A typical case involving a patient with old myocardial infarction. SSEA3+/CD105+ double-positive cells were measured in the monocyte area on fluorescence-activated cell sorting before and after cardiac rehabilitation with dynamic exercise. Bold green rectangle, CD105-positive cells in the monocyte area; right-upper red rectangle, SSEA3+/CD105+ double-positive cells. (**B**) Distribution of fluorescence intensity in SSEA3+ cells within the gating area (monocyte and CD105+ areas). M1, SSEA3+/CD105+ double-positive multilineage-differentiating stress-enduring (Muse) cells. FITC, fluorescein isothiocyanate; FSC, forware scatter; SSC, side scatter.

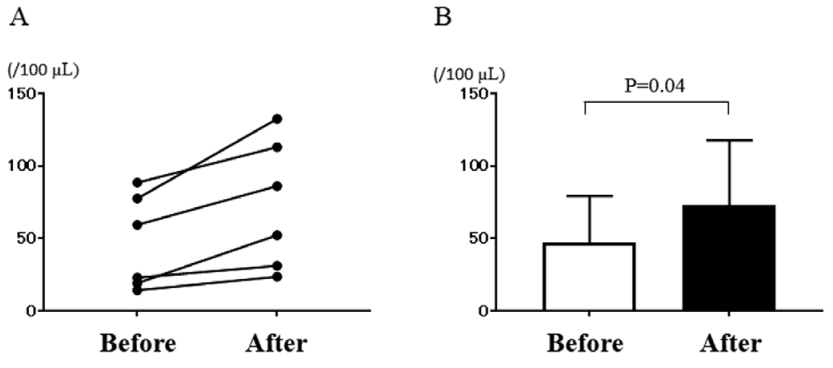

Figure 2. (**A**) Absolute and (**B**) mean±SD number of multilineage-differentiating stress-enduring (Muse) cells in peripheral blood before and after cardiac rehabilitation (n=6).

Statistical Analysis

The data were normally distributed based on Kolmogorov-Smirnov test and are given as mean±SD. The significance of the difference between 2 groups was determined using paired parametric Student's t-test. P<0.05 was considered significant. All statistical analyses were performed using GraphPad Prism7.

Results

Figure 1 shows typical measurements of SSEA3+/CD105+ double-positive Muse cells in the peripheral blood of a

patient with old myocardial infarction, in which most resided in the monocyte area[4] before and after cardiac rehabilitation. Cardiac rehabilitation with dynamic exercise increased the number of Muse cells in the peripheral blood in all patients (**Figure 2A**). The mean number of Muse cells after cardiac rehabilitation (73.2±44.5 cells/100 μL, P=0.04) was significantly higher than that before cardiac rehabilitation (47.0±32.3 cells/100 μL; **Figure 2B**).

Discussion

We have found for the first time that the number of Muse cells in the peripheral blood significantly increased after 40 min of cardiac rehabilitation with dynamic exercise (**Figure 2**). Similarly, it was previously reported that exercise induces the mobilization of endothelial progenitor cells and $CD34^+/133^+$ cells into the peripheral blood,[8,9] which might have served as a physiologic repair or compensation mechanism because these cell populations have the ability to promote angiogenesis and vascular regeneration.[8,9] In addition, Muse cells have the ability to regenerate cardiomyocytes, and to repair damaged cardiac tissue,[5] and AMI patients with a more marked mobilization of Muse cells have been shown to have improvement of LV function and remodeling.[4] Therefore, the better clinical outcome of patients with heart disease who underwent cardiac rehabilitation may be at least in part caused by the mobilization of Muse cells.

Conclusions

Cardiac rehabilitation with dynamic exercise accelerates the mobilization of Muse cells into the peripheral blood.

Acknowledgments

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Disclosures

The authors declare no conflicts of interest.

Author Contributions

Shinya M. designed the experiment; Shingo M., T.T., Y.Y., T.A., H.K., K.N., and M.K. obtained data; and Shingo M. and Shinya M. wrote the manuscript. All authors read and approved the final manuscript.

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