Mobilization of Pluripotent Multilineage-Differentiating Stress-Enduring Cells in Ischemic Stroke

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Goal: This prospective study was aimed to prove the hypothesis that multilineage-differentiating stress-enduring (Muse) cells are mobilized from bone marrow into peripheral blood in patients with ischemic stroke. *Materials and Methods:* This study included 29 patients with ischemic stroke. To quantify the circulating Muse cells, peripheral blood was obtained from all patients on admission and at days 7 and 30. Using fluorescence-activated cell sorting, Muse cells were identified as stage-specific embryonic antigen-3-positive cells. The control values were obtained from 5 healthy volunteers. Separately, immunohistochemistry was performed to evaluate the distribution of Muse cells in the bone marrow of 8 autopsy cases. *Findings:* The number of Muse cells robustly increased within 24 hours after the onset, compared with the controls, but their baseline number and temporal profile widely varied among patients. No clinical data predicted the baseline number of Muse cells at the onset. Multivariate analysis revealed that smoking and alcohol intake significantly affect the increase in circulating Muse cells. The odds ratio was .0027 ($P = .0336$) and 1688 ($P = .0220$) for smoking and alcohol intake, respectively. The percentage of Muse cells in the bone marrow was .20% ± .17%. *Conclusion:* This study shows that pluripotent Muse cells are mobilized from the bone marrow into peripheral blood in the acute stage of ischemic stroke. Smoking and alcohol intake significantly affect their temporal profile. Therapeutic interventions that increase endogenous Muse cells or exogenous administration of Muse cells may improve functional outcome after ischemic stroke. **Key Words:** Ischemic stroke—pluripotent stem cell—Muse cell—bone marrow—mobilization.

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Introduction

Stroke is one of leading causes of human death. Among all stroke patients, more than 80% suffer from ischemic stroke.[1] Despite a number of research, stroke treatment is still limited to thrombolytic therapy using tissue plasminogen activator within 4.5 hours after the onset. Only supportive care and rehabilitation are established for patients with ischemic stroke in the chronic phase. Therefore, alternative therapeutic approaches are warranted to improve their functional outcome.

Recent studies have demonstrated that a variety of stem/progenitor cells are mobilized from the bone marrow into the peripheral blood in a variety of disorders, including ischemic stroke. Nowadays, stem/progenitor cells are believed to originate mainly from the nonhematopoietic bone marrow stromal cells (BMSCs). Once mobilized into the peripheral blood, they migrate toward the ischemic tissue. They include endothelial progenitor cells (EPCs) and contribute to angiogenesis in the brain. The other cells release neurotrophic factors and enhance functional recovery after ischemic stroke.[2] More importantly, they also contain a small fraction of multi- or pluripotent cells that can differentiate into neural cells.[3] Recently, Dezawa and coworkers[4] have discovered a unique type of stem cells in adult human mesenchymal cells such as dermal fibroblast and BMSCs. They correspond to several percentages of total BMSCs and are stress tolerant, named as multilineage-differentiating stress-enduring (Muse) cells.[4] They can efficiently be isolated as the cells positive for a well-known human embryonic stem cell marker, stage-specific embryonic antigen-3 (SSEA-3). Using SSEA-3 antibody, fluorescence-activated cell sorting (FACS) can efficiently separate Muse cells from the human bone marrow and fibroblasts.[4] Muse cells can self-renew, express the genes associated with pluripotency such as Nanog, Oct3/4, and Sox2, and differentiate into endodermal-, ectodermal-, and mesodermal-lineage cells from a single cell. Under cytokine induction, Muse cells differentiate into neuronal maker positive cells with a very high ratio of ~90%.[5] In animal experiments, they act as tissue repair cells when transplanted in vivo; they migrate toward damaged tissues and spontaneously differentiate into cells compatible with the homed-into tissue in the animal models of several disorders.[4] In fact, Muse cells are integrated into the host brain, express the neuronal markers, and significantly enhance functional recovery when directly injected into the murine infarct brain.[6] Unlike well-known pluripotent stem cells such as embryonic stem cells and induced pluripotent stem cells, the telomerase activity of Muse cells is low and do not form teratoma in immunodeficient mice testes.[5,7]

Based on these observations, the present study was aimed to prove the hypothesis that Muse cells are mobilized from bone marrow into peripheral blood in patients with ischemic stroke.

Materials and Methods

Patients

This prospective study included 29 adult patients who were admitted to our hospitals due to ischemic stroke in the supratentorial region within 24 hours after the onset. The patients with lacunar infarction were excluded. There were 16 men and 13 women. Their mean age was 71.4 ± 13.3 years, ranging from 41 to 93 years. The present study was approved by the Ethical Review Board of Toyama University Hospital and Saiseikai Toyama Hospital, and written informed consent was obtained from each individual participant.

We collected clinical data from each patient, including age, gender, National Institutes of Health Stroke Scale (NIHSS) score on admission, past history, smoking status, alcohol intake, subtypes of ischemic stroke, the location and size of the cerebral infarct, and modified Rankin Scale (mRS) score at 1 month after the onset. Past history included ischemic and hemorrhagic strokes, hypertension, diabetes mellitus, and hyperlipidemia. Hypertension was defined as blood pressure higher than 140/90 mmHg or current use of antihypertensive agents. Diabetes mellitus was defined as a hemoglobin A1C value higher than 6.5% or current use of antiglycemic medications. Patients with serum low-density lipoprotein cholesterol levels higher than 140 mg/dL or current use of lipid-lowering agent were considered as having hyperlipidemia. Current smoking was defined as any tobacco smoking on a daily basis within 3 months before admission. Current alcohol intake was defined as alcohol consumption of more than 150 g/week within 3 months.

Physiological and Laboratory Data

On admission, blood pressure, electrocardiogram, and laboratory data were recorded in all patients. These examinations were repeated at 7 and 30 days after the onset.

Radiological Examinations

On admission, diffusion-weighted, T2-weighted, and fluid-attenuated inversion recovery images and magnetic resonance angiography were obtained from all patients using a 1.5-Tesla magnetic resonance apparatus. The size of the cerebral infarct was divided into 3 groups: small, moderate, and large. The size of the cerebral infarct was graded as large when the lesion was located in the area of more than 2 cortical branches, moderate when the lesion was located in the area of one cortical branch, and small when the lesion was smaller (Fig 1).

Quantification of Circulating SSEA-3$^+$ Cells

To quantify the circulating SSEA-3$^+$ cells, a total of 3 mL of peripheral blood was obtained from all patients on