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Application note

The effects of X gene for the enhanced stem cell survival

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Introduction

Stem cell-based cell therapy has shown promising results to cure and apply various disease such as myocardiac infarction, hindlimb ischemia, and stroke for biomedical treatment. Although stem cells show a remarkable therapeutic effect, it has some problematic factors, like cell expansion before the treatment and low survival of cells treated at disease site. To overcome the limitation of these disadvantages, X gene, which is a family of secreted glycoprotein hormones that function as the inhibition of anti-apoptotic and oxidative damage, and the induction of pro-proliferation. Here, we delivered X gene into human adipose-derived stem cell (ADSC) to prolong stem cell survival.

Materials and methods

Live and dead cells

To examine the viability of X gene expressing stem cells after hydrogen peroxide (H_2O_2) treatment, both attached and floating stem cells treated with NC, H_2O_2, pcDNA+H_2O_2, and pcDNA/X+H_2O_2 were harvested and fixed in 70% ethanol at 4°C over 20 hrs. Following fixation, the cells were incubated at 4°C with a mixture of propidium iodide (PI; Sigma-Aldrich) solution (50 μg/mL) and RNase (0.5 mg/mL) for 1 hr, and then, after PBS washing 2 times, cells were analyzed by Arthur™ Image-Based Cytometer (NanoEnTek) according to the manufacturer`s instruction. Each experiment was repeated at least three times.

Results

Live and dead cell populations

After X gene delivery, we evaluated cell viability after cellular damage. Cell viability was evaluated by the counting of the cells stained by PI capable of discriminating live and dead cells. After staining, cells were analyzed by Arthur imaged-based cytometer. The quantification of live and dead cells treated with NC, H_2O_2, pcDNA+H_2O_2, and pcDNA/X+H_2O_2 as the cellular concentration was showed as percentage of cell population.

Figure shows the percent of the total population for each cells stained by PI, side by side with the percent of the total population that is both viable and expressing PI. In results, live and dead populations in parental cells were measured 81 and 19%, respectively. Dead cell populations were observed higher in cells treated with H_2O_2 (25% for live and 75% for dead cells). On the other hand, increased live cell and decreased dead cell populations were showed in cells treated with pcDNA/X+H_2O_2 (67 and 33%) compared with that of cells treated wih pcDNA+H_2O_2 (45 and 55%). These data demonstrate that X gene expressed in ADSC elicits enhanced cell survival compared with empty vector transfected cells, against oxidative stress.

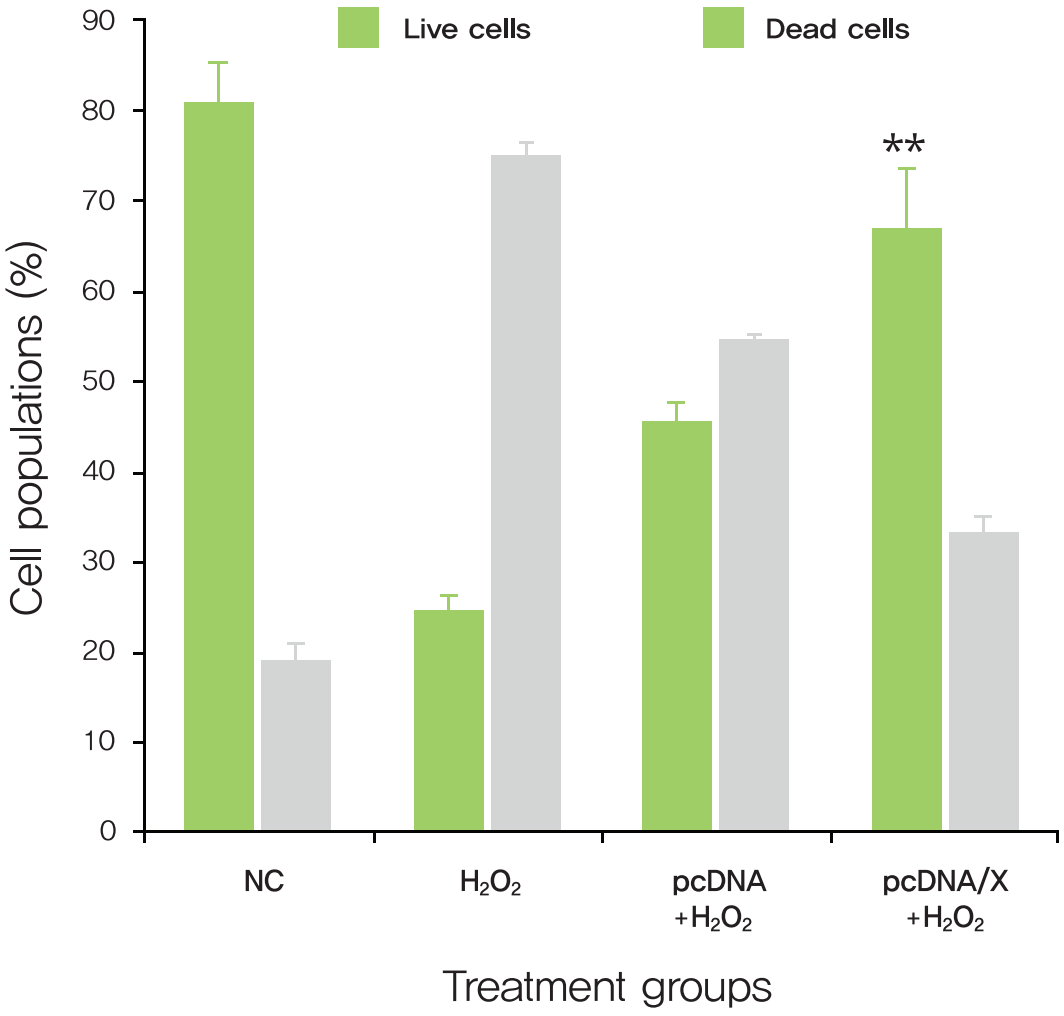

Figure. Enhancement of live/dead cell populations by X gene. Cell viability of ADSCs was observed by Arthur image-based cytometer in cells stained with PI solution after the treatment of X gene and H_2O_2. Dead cells were stained by PI as red color. **P < 0.02 versus H_2O_2 groups.

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