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Phenotypic modulation of human cardiospheres between stemness and paracrine activity, and implications for combined transplantation in cardiovascular regeneration

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ABSTRACT

As the search for new cell types for cardiovascular regeneration continues, it has become increasingly important to optimize *ex vivo* cell processing. We aimed to develop an optimal processing strategy for human cardiac progenitor cells. We hypothesized that enhancing the stemness potential and promoting the secretory activity for paracrine effects are mutually exclusive routes. Therefore, we investigated the two divergent cell processing methods to enhance cellular potency and humoral activity, respectively. We obtained human right ventricular tissues and sequentially generated primary cardiosphere (CS), primary CS-derived cells (PCDC) and secondary CSs. During secondary CS formation, inhibiting the ERK pathway, using selective RTK1 and TGF-β inhibitors, *Oct4* increased 20 fold and *VEGF* was decreased. When the ERK pathway was stimulated by addition of EGF and TGF-β, *VEGF* expression was upregulated and *Oct4* was downregulated, indicating that the ERK pathway serves a directional role for cellular potency *versus* paracrine capacity. Transplantation of PCDCs or secondary CSs into the infarcted heart of immunocompromised mouse showed significant angiogenic effects compared with PBS injection. Interestingly, combined transplantation of the two differently-processed, dual-purpose secondary CSs resulted in an additional increase in neovascularization. Human VEGF was primarily produced from secondary CSs under ERK stimulating conditions. Cardiomyocyte-like cells were produced from secondary CSs under ERK inhibitory conditions. These findings indicate that combined transplantation of specifically-processed human secondary CSs enhances infarct repair through the complementary enhancement of cardiopoietic regenerative and paracrine protective effect. Furthermore, these results underscore the fact that optimal cell processing methods have the potential to maximize the therapeutic benefits.

1. Introduction

Heart failure following myocardial infarction (MI) is a major cause of morbidity and mortality [1]. To meet the significant clinical unmet need for new therapeutic modalities, cell therapy has been considered as a promising strategy to repair the infarcted failing heart. In terms of translational research, a number of cardiogenic or cardioprotective cell types have been identified, enabling bench-to-bedside clinical application [2,3]. So far, in clinical trials by the administration of bone marrow-derived cells, mainly intracoronary infusion of minimally-processed (simple fractionation) mononuclear cells into MI patients, the feasibility and safety of cell therapy have been excellent, whereas modest or inconsistent improvement in heart function has been reported [4]. To overcome the current therapeutic limitations of cell therapy, resident cardiac stem/progenitor cells have been investigated. Cardiac stem/progenitor cells presumably hold enormous potential for

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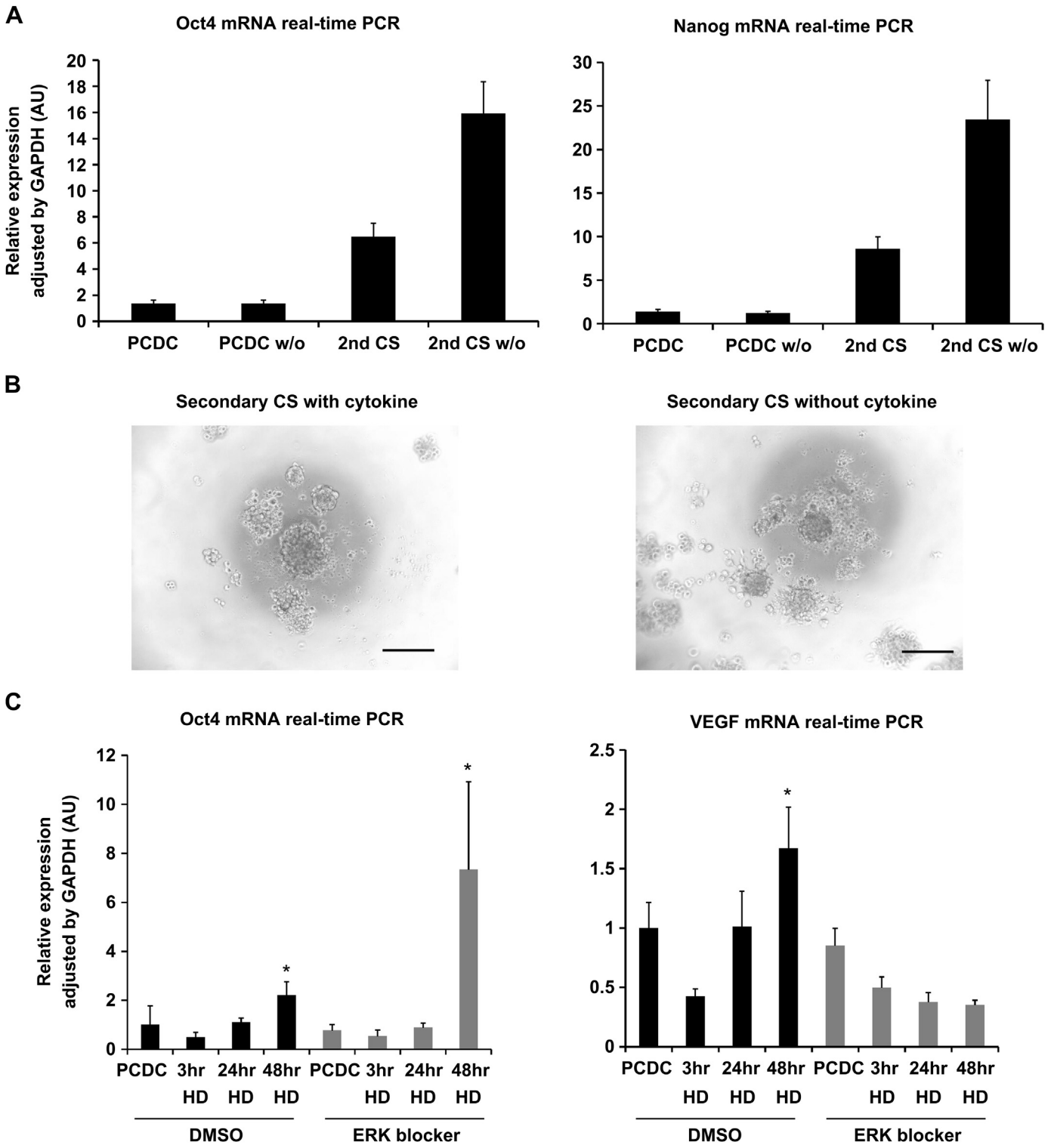

Fig. 1. The upregulation of stemness gene without cytokines during secondary cardiosphere generation. (A) Enhanced stemness of secondary cardiospheres under cytokine deprivation. (B) Different size of secondary cardiosphere with or without cytokines (scale bar, 200 μm). (C) Enhanced stemness and decreased secretory activity of secondary CSs under ERK inhibition. *Oct4* and *VEGF* expression, as determined by real-time PCR (*$P < 0.05$ *versus* PCDC).

cardiovascular regeneration, more so than extra cardiac cell and thus have been targeted to enhance/improve therapeutic efficacy [5–7]. The promising results of recent small-scale, phase I clinical studies using resident cardiac progenitor cells have prompted large-scale trials [8,9], however, with this approach *ex vivo* cell expansion techniques are required prior to transplantation.

As the search for optimal cell types continues, it has become increasingly clear that efforts to optimize the processing of cells for transplantation have lagged. Because the very limited number of resident adult cardiac progenitor cells are acquired from biopsy tissues, *in vitro* and *ex vivo* expansion of these cells is necessary for further application. To address this limitation, we have undertaken

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