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Platelet-derived microparticles augment the adhesion and neovascularization capacities of circulating angiogenic cells obtained from atherosclerotic patients

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ABSTRACT

Objective: The neovascularization-related capacities of circulating angiogenic cells (CACs) are impaired in atherosclerotic patients, which may explain the unsatisfactory effects of therapeutic angiogenesis with atherosclerotic patient-derived CACs. Platelet-derived microparticles (PMPs) were reported to augment the re-endothelialization capacity of CACs. Accordingly, we investigated whether PMPs could augment the neovascularization-related capacities of atherosclerotic patient-derived CACs *in vitro* and *in vivo* and if so, the associated mechanisms.

Methods and results: We isolated mononuclear cells and PMPs from atherosclerotic patient-derived peripheral blood and generated PMP-pretreated CACs (PMP-CACs) by co-culture of the mononuclear cells and PMPs. Although the migration capacity of PMP-CACs was similar to that of CACs, the adhesion capacity of PMP-CACs was greater. PMPs released RANTES into the culture medium, and the receptors were similarly expressed on the surfaces of CACs and PMP-CACs. Intravenous injection of PMP-CACs to rats with hindlimb ischemia augmented neovascularization of the ischemic limbs more than the injection of CACs. The number of PMP-CACs incorporated into the capillaries of the ischemic limbs was greater than that of incorporated CACs. The augmented adhesion and neovascularization capacities by PMP-CACs were canceled out by a RANTES neutralizing antibody.

Conclusions: PMP-secreted RANTES may play a role in the augmenting adhesion and neovascularization capacities of CACs. Injection of PMP-CACs may be a new strategy to augment the effects of therapeutic angiogenesis for limb ischemia in atherosclerotic patients.

1. Introduction

Cell therapy for augmenting neovascularization in ischemic tissues is a promising therapeutic option to treat patients with ischemic cardiovascular disease [1]. Although various stem/progenitor cells were effectively used in experimental models, peripheral blood-derived mononuclear cells (MNCs), bone marrow-derived MNCs, and circulating angiogenic cells (CACs) [2] have been used in clinical studies [3–5]. MNCs and CACs have been reported to contribute to neovascularization through a multistep process composed of the following neovascularization-related capacities of the cells: chemotaxis and adhesion to mature endothelial cells, migration and invasion to the intracellular space in adjacent endothelial cells, and secretion of cytokines to stimulate sprouting new capillaries from pre-existing arteries [6]. Thus, the effects of therapeutic angiogenesis with MNCs or CACs may depend on the neovascularization-related capacities of the cells. We and others have previously reported the effects and safeties of therapeutic angiogenesis with MNCs or CACs in patients with myocardial ischemia or critical limb ischemia in large-scale clinical trials [3–5]; however, the effects have been unsatisfactory. This may be due to the injection of atherosclerotic patient-derived MNCs or CACs with impaired neovascularization-related capacities [7]. Indeed, Heeschen et al. reported that the impaired migration capacity of

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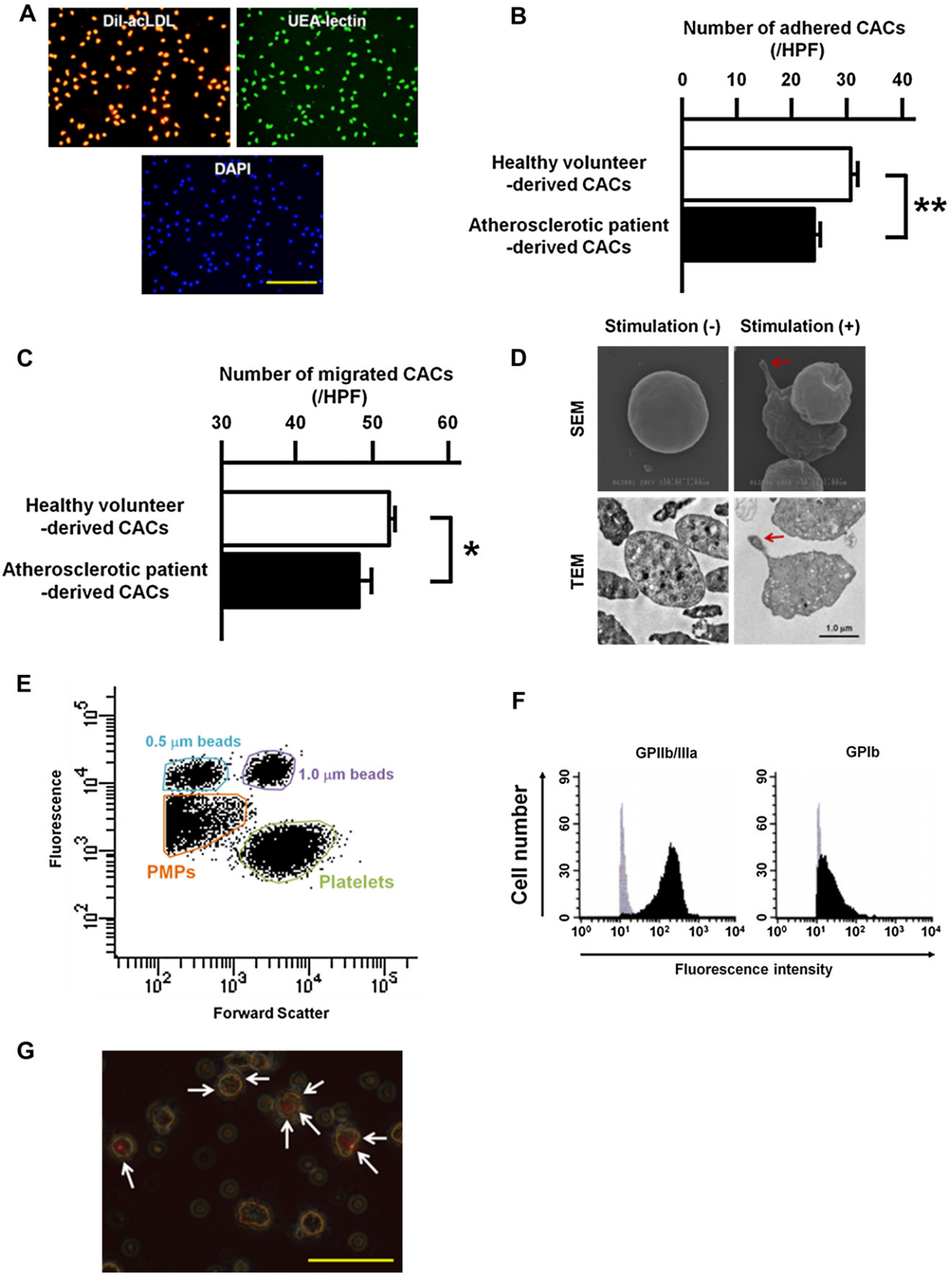

Fig. 1. (A) Representative microscopic images of CACs. CACs were stained by three fluorescent dyes Dil-acLDL (red), UEA-lectin (green), and DAPI (blue). The scale bar indicates 200 μm. (B) Bar graphs for pooled data of the number of CACs adhered to fibronectin. The number was significantly smaller for atherosclerotic patient-derived CACs than for healthy volunteer-derived CACs (**: $p < 0.005$, $n = 6$, each) (C) Bar graphs for pooled data of the number of migrated CACs for SDF-1α. The number was significantly smaller for