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ENDOTHELIAL AND VASCULAR CELLS

Autologous cell sources in therapeutic vasculogenesis: *In vitro* and *in vivo* comparison of endothelial colony–forming cells from peripheral blood and endothelial cells isolated from adipose tissue

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Abstract

Background aims. Autologous endothelial cells are promising alternative angiogenic cell sources in trials of therapeutic vasculogenesis, in the treatment of vascular diseases and in the field of tissue engineering. A population of endothelial cells (ECs) with long-term proliferative capability, endothelial colony–forming cells (ECFCs), can be isolated from human peripheral blood. ECFCs are considered an endothelial precursor population. They can be expanded in cell factories in sufficient numbers for clinical applications, but because the number of isolated primary ECs is low, the culture period required may be long. Another EC population that is easily available in the autologous setting and may be expanded *in vitro* through several population doublings are ECs from adipose tissue (AT-ECs). *Methods.* Through extensive comparisons using whole-genome microarray analysis, morphology, phenotype and functional assays, we wanted to evaluate the potential of these EC populations for use in clinical neovascularization. *Results.* Global gene expression profiling of ECFCs, AT-ECs and the classical EC population, human umbilical vein ECs, showed that the EC populations clustered as unique populations, but very close to each other. By cell surface phenotype and vasculogenic potential *in vitro* and *in vivo*, we also found the ECFCs to be extremely similar to AT-ECs. *Conclusions.* These properties, together with easy access in the autologous setting, suggest that both AT-ECs and ECFCs may be useful in trials of therapeutic neovascularization. However, AT-ECs may be a more practical alternative for obtaining large quantities of autologous ECs.

Key Words: *adipose tissue-derived endothelial cells, cell therapy, endothelial colony–forming cells, microarray analysis*

Introduction

Therapeutic angiogenesis, the treatment of tissue ischemia by promoting the proliferation of new blood vessels, has recently emerged as a promising treatment option. However, such pro-angiogenic therapy is not limited to vascular disease; it is also highly relevant in the field of tissue engineering, where clinical use of engineered tissues and tissue substitutes is often hampered by a lack of vascular perfusion. One approach promoting angiogenesis in ischemic diseases and vascularization of tissue-engineered constructs is the use of cells with angiogenic potential. Human umbilical vein derived endothelial cells (HUVEC) have been one of the most important sources of vascular endothelial cells since the first successful culture by Jaffe et al. in 1973 [1]. However, HUVECs are not suited for therapeutic use because of their allogenic nature. A source of autologous endothelial cells (ECs) with potential for use in therapeutic applications are

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the endothelial progenitor cells (EPCs), which were first isolated from human peripheral blood (PB) by Asahara et al. [2]. EPCs exhibit a spindle-like morphology and are able to differentiate into ECs and incorporate into sites of active angiogenesis in animal models [2]. Later, based on new clonogenic assays, two distinct EPC populations with different growth characteristics were observed, referred to as early- and late-outgrowth EPCs [3–5]. Early-outgrowth EPCs appeared in culture after 4 to 7 days, had low proliferation potential and have been found to be hematopoietic in origin [6]. Late-outgrowth EPCs are now also known as endothelial colony–forming cells (ECFCs) or outgrowth endothelial cells (OEC) [3,6]. Colonies of these cells appear after 14 to 21 days in culture, have a cobblestone appearance in monolayer culture and have high proliferative potential [7].

EPCs/ECFCs have been considered extremely rare in human PB, with reported frequencies as low as 0.05 EPCs/mL of blood [6,8,9]. However, culturing unfractionated PB, Reinisch et al. recently quantified ECFCs at four colonies per milliliter of blood and showed how these cells could be expanded to clinically relevant numbers in cell factories [10]. This strategy would enable the use of autologous ECFCs in trials of therapeutic angiogenesis. Another EC population that is easily accessible in the autologous setting and may be expanded *in vitro* through relatively few population doublings (PDs) to yield $\geq 10^8$ cells are the ECs from adipose tissue (AT) [11–13]. In the context of clinical applications, other EC populations may not be expanded to sufficient numbers because of they can supply only small amounts of starting material (dermal microvascular cells), are not accessible (ECs from the arterial side) or are allogeneic (HUVECs and foreskin microvascular cells) and therefore likely to be rejected by alloimmune mechanisms. Thus, at this time, the ECs most likely to have a place in trials of therapeutic angiogenesis are ECFCs and AT-ECs.

In the present study, we performed an extensive comparison of these two EC populations to evaluate their potential for use in clinical applications. For comparison of expression profiles between the two EC populations, we include HUVECs as an endothelial reference cell population. We show that ECFCs and AT-ECs are extremely similar in morphology, phenotype and their expression of EC-relevant genes. Furthermore, both EC populations showed similar potential for *in vivo* angiogenesis, as demonstrated using a mouse model.

Methods

All reagents were purchased from Sigma Aldrich unless otherwise stated.

Ethics statement

Biological samples were collected after written informed consent in accordance with the guidelines of the Declaration of Helsinki. The study was approved by the Regional Committee for Medical Research Ethics, Southern Norway, Section A, and by the Institutional Review Board of the Medical University of Graz (protocol numbers 19–252 ex 07/08 and 18–243 ex 06/07). Animal experiments were approved by the Animal Care and Use Committee at the Veterinary University of Vienna on behalf of the Austrian Ministry of Science and Research according to the Guide for the Care and Use of Laboratory Animals (BMWF-66.010/0082-II/10b/2009).

Cells

AT-ECs from liposuction material, ECFCs from PB and HUVECs from HUVs were isolated as described previously [10,12,14], with details also described later in the article. All comparisons were performed using cells from three donors for each EC population.

Isolation and in vitro humanized expansion of AT-ECs

AT was obtained from liposuction material from abdominal regions of three healthy female donors (aged 22–35 years; BMI: 23–30) undergoing cosmetic surgery. The stromal vascular fraction (SVF) was separated from AT as described previously [15]. Briefly, lipoaspirate was washed and digested using 0.1% collagenase A type 1. After centrifugation, the cell pellet was filtered through 100-μm and then 40-μm cell sieves (Becton Dickinson). SVF cells were obtained from the interface after Lymphoprep gradient separation (Axis Shield).

The acquisition of AT-ECs from the SVF has been described previously [12]. The strategy chosen included removal of $CD44^+$ cells from SVF because this gave superior purity and yield compared with other isolation procedures. $CD44^+$ cells were removed using Dynabeads (Dynabeads Pan Mouse IgG, Invitrogen Dynal AS) according to the manufacturer's description. Dynabeads were washed then pre-coated with monoclonal anti-CD44 antibody (Southern Biotech) at the concentration of 0.6 μg antibody per 25 μL Dynabeads and incubated for 2 h with gentle tilting and rotation. Dynabeads were then washed again, and approximately 400×10^6 magnetic CD44 beads were added to 100×10^6 SVF cells.

AT-ECs were plated at 2×10^6 cells per 75-cm^2 tissue culture flask (Nunc). Cells were maintained at 37°C in an atmosphere of 5% CO_2 in humid air using EC growth medium (MCDB 131, Gibco) supplemented with 1% L-glutamine, 1 ng/mL basic fibroblast growth factor, 1 μg/mL hydrocortisone, 10 ng/mL epidermal growth factor, 50 μg/mL gentamicin,