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Vascular smooth muscle cell differentiation from human stem/progenitor cells

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ABSTRACT

Transplantation of vascular smooth muscle cells (VSMCs) is a promising cellular therapy to promote angiogenesis and wound healing. However, VSMCs are derived from diverse embryonic sources which may influence their role in the development of vascular disease and in its therapeutic modulation. Despite progress in understanding the mechanisms of VSMC differentiation, there remains a shortage of robust methods for generating lineage-specific VSMCs from pluripotent and adult stem/progenitor cells in serum-free conditions. Here we describe a method for differentiating pluripotent stem cells, such as embryonic and induced pluripotent stem cells, as well as skin-derived precursors, into lateral plate-derived VSMCs including 'coronary-like' VSMCs and neural crest-derived VSMC, respectively. We believe this approach will have broad applications in modeling origin-specific disease vulnerability and in developing personalized cell-based vascular grafts for regenerative medicine.

1. Introduction

1.1. Overview

Vascular smooth muscle cells (VSMCs) are a type of smooth muscle that controls the diameter of medium and large blood vessels. VSMCs contract or relax to control blood pressure and act to distribute blood to areas where tissue oxygenation and nutrients are needed. From a regenerative medicine perspective, VSMCs are a therapeutically relevant cell type shown to enhance angiogenesis and wound healing, and improve heart function after myocardial infarction [1–5].

A normal vasculature is essential to maintaining tissue homeostasis and to providing the necessary oxygenation and nutrients to cells of the human body. When tissue homeostasis is disrupted, such as occurs with impaired vascular function, various complications arise. Cardiac and peripheral vascular diseases, often caused by atherosclerosis, cause a great deal of morbidity and mortality in the Western world. Current treatment strategies include stents or tissue grafts to restore blood flow to affected tissues. However,

Abbreviations: VSMCs, vascular smooth muscle cells; VEGF, vascular endothelial growth factor; PAH, pulmonary arterial hypertension; TGF-β, transforming growth factor beta; IL-1β, interleukin 1 beta; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitor of metalloproteinases; hPSCs, human pluripotent stem cells; SKP, skin-derived precursor; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; BMP, bone morphogenetic protein; T, Brachyury; KDR, kinase insert domain receptor; Flk1, fetal liver kinase 1; VEGFR2, vascular endothelial growth factor receptor 2; PDGFRα, platelet derived growth factor receptor alpha; DKK1, Dickkopf homolog 1; EBs, embryoid bodies; IMDM, Iscove's Modified Dulbecco's Medium; MTG, monothioglycerol; bFGF, basic fibroblast growth factor; ASMA, alpha smooth muscle actin; MRTFA/B, myocardin related transcription factor A/B; SRF, serum response factor; qRT-PCR, quantitative real-time polymerase chain reaction; hASMC, human aortic smooth muscle cell; hBSMC, human bladder smooth muscle cell; FITC, fluorescein isothiocyanate.

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the use of grafts can be insufficient for the recovery of blood flow and to restore functional integrity of the affected tissue. This may be due to a multitude of factors, including cell death, the development of graft disease and incomplete functional integration. We posit that it may be possible to mitigate these issues by finding appropriate progenitor cell types that might serve as the ideal starting material for regenerative applications.

The ideal progenitor cell type for regenerative therapies must meet certain criteria. It must be able to expand to limitless numbers, be disease free, resistant to developing disease, be immune tolerant/compatible and must integrate functionally into their milieu. To date, the "holy grail" of progenitor cells has not been found, however studies are currently underway to deal with these challenges by engineering or modifying progenitor cell sources. In designing new cellular therapies to treat the multitude of diseases for which they may represent a cure, novel strategies need to be employed. For example, in designing progenitor cells to expand to high numbers, one might accelerate or deregulate the cell cycle with the unintended risk of cancer. Because of this, progenitors need to be designed such that they can be geared to enter the cell cycle *in vitro*, but kept in check *in vivo*. Similarly, in designing vascular grafts that promote angiogenesis, one might inadvertently promote the blood supply of a malignant tumor. For example, protein therapies such as VEGF have been shown to promote the survival of vascular grafts, but have also been shown to promote angiogenesis in cancer [6]. Genetic modification of various progenitor cell populations has also been attempted. In the treatment of pulmonary arterial hypertension (PAH), endothelial progenitor cells have been introduced with angiopoietin-1, VEGF, adrenomedullin, calcitonin gene-related peptide and endothelial nitric oxide synthase [7]. All of these manipulations have improved the pathophysiology of PAH, however, genetic manipulation can be costly, introduces the toxicity risk of viral and/or non-viral transfection vectors and can be difficult to scale [7]. Autologous adult stem cells present the least risk, however they also present the least potential for trans-differentiation and regeneration. By contrast, pluripotent stem cells have the greatest potential for differentiation and regeneration, but pose the greatest risk in terms of neoplasia. In the case of VSMCs, their relevant progenitors are derived from many different lineages as will be described below. These differences in lineage have been shown to have differential effects on function. It is currently unknown if matching the lineage of VSMC to the graft site would improve integration and therefore functional recovery.

1.2. Embryonic origins of VSMCs

Using fate mapping approaches and chimeras, 8 independent embryonic sources of VSMCs have been identified [8]. These include the secondary heart field, the neural crest, the pro-epicardium, the somitic mesoderm, splanchnic mesoderm, mesothelium, mesoangioblasts and various adult stem/progenitor cells [8]. In particular, VSMCs from the aortic root are derived from the secondary heart field [9,10], those of the aortic arch are derived from the neural crest [11], while the pro-epicardium gives rise to VSMCs of the coronary arteries [12,13]. Somitic mesoderm gives rise to VSMCs in the descending thoracic aorta [14], and VSMCs of the abdominal aorta are derived from the splanchnic mesoderm [14]. Various adult stem/progenitor cells are found heterogeneously throughout the vasculature and their origins may be diverse [15–20].

VMSCs of different embryonic origins have been observed to respond differentially to different growth conditions such as serum and TGF-β. For example, neuro-ectoderm-sourced VSMCs proliferate in serum-free conditions, whereas mesoderm-sourced VSMCs require serum for proliferation [21]. Moreover, on the addition of TGF-β1, cell proliferation was increased in neural crest-derived VSMCs, in contrast to mesoderm-derived VSMCs which were either unresponsive or growth inhibited [22]. Also, c-Myb expression was induced on the addition of TGF-β1 in neural crest-derived VSMCs, but not in mesoderm-derived VSMCs [22]. VSMCs isolated from different parts of the aorta, and thus of different embryonic origin, display differential responses to IL-1β, an atherogenic cytokine [23]. Gene expression levels, and corresponding protein levels of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) vary in response to IL-1β treatment depending on the VSMC lineage being treated [24,25]. This may have significant implications, as MMPs play a critical role in vascular remodeling, aneurysm formation and atherogenesis [24]. This is based on the ability of MMPs to degrade vessel wall connective tissue, which precedes the development of many vascular diseases.

These differential effects in VSMC function appear relevant to atherosclerosis. Aortic homograft transplantation studies of animals fed a high fat diet, where the atherosclerosis prone abdominal aorta (of splanchnic origin) has been transplanted to the atherosclerotic resistant thoracic aorta (of somitic origin) have demonstrated that atherosclerosis susceptibility is dependent on intrinsic differences within the vessel wall [26,27]. This supports the premise of lineage-dependent VSMC diversity. Similar studies transplanting the abdominal aorta into the pulmonary circulation (of secondary heart field origin) have yielded similar results in response to an atherogenic diet [28]. This phenomenon can be translated to humans whereby it has been shown that different vascular segments display their own unique atherogenic response [29]. Moreover, aortic dissections occur preferentially at the interfaces where VSMCs of distinct embryological origins meet [8]. Together, these studies support the notion that intrinsic VSMC differences may be attributed to their lineage heterogeneity.

1.3. Pluripotent stem cells

If we accept that the key to defining optimal functional integration may be lineage specification, we must ask how we might best achieve origin-dependent lineage restriction. Should this be through pluripotent stem cells (PSCs) or through adult progenitors such as the skin-derived precursor (SKP, see below) [30]? Since PSCs represent the cellular source with greatest regenerative and differentiation potential, we have focused this review on embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs).

Developing different differentiation protocols using PSCs provides investigators with the prospect of developing cell-based therapies for many genetic and non-genetic human diseases, to perform drug screens, to study the earliest stages of human development and to model disease *in vitro*. The cardiovascular lineage in particular has been an area of concentrated interest as cardiovascular disease is a leading cause of death, congenital heart abnormalities are relatively common, and unanticipated cardiac toxicity prevents many new drugs from reaching clinical application. Using *in vivo* model systems, scientists have been able to identify the key regulatory pathways that control the establishment of several embryonic lineages in the embryo. At the present time, the most successful differentiation strategies recapitulate key regulatory pathways *in vitro*. The pluripotent nature of ESCs and iPSCs makes them attractive candidate sources for developing different VSMCs of distinct sources/origins and therefore differential function and disease susceptibility. Protocols to differentiate pluripotent stem cells into somite, lateral plate and neural crest-derived VSMCs have been devised and have produced VSMC with discrete functional/biological differences [25]. In the case of iPSCs, the power of this technology lies in its potential to generate a limitless number of genetically modifiable and personalized PSC-derived VSMC obtained from autologous patient tissue. This provides a strong platform for disease modeling and for generating

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