Michael K. Danquah · Ram I. Mahato
Editors

Emerging Trends in Cell and Gene Therapy

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ISBN 978-1-62703-416-6 ISBN 978-1-62703-417-3 (eBook)
DOI 10.1007/978-1-62703-417-3
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013940099

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

To my parents George and Elizabeth: my lifelong teachers, who have devoted their lives to making the world a better place by caring for others and whose constant encouragement and sublime standards serve to inspire me. To the loving memory of Gabriel: my brother and hero. For a brief moment in time you lit up our world. To Jennifer: for layers of love and extraordinary patience.

Michael K. Danquah

I dedicate this book to my wife Subhashini; my children Kalika and Vivek for their love and support; my late mother Sarswati for believing in me; and to my students and mentors who have always helped me in my quest for learning and in achieving higher goals.

Ram I. Mahato

Preface

Emerging Trends in Cell and Gene Therapy is meant for those who seek the golden thread that runs through the fields of cell therapy, gene therapy, and tissue engineering, yet have found other books too specialized to do so. This book aims to arm basic scientists and clinicians with this golden thread so they are better positioned to address the debilitating diseases presently plaguing mankind.

Cell and gene therapies are promising approaches for treating genetic and acquired diseases. To date, numerous biological barriers and ethical issues have limited their clinical translation. Nonetheless, active research in cell and gene therapy in both academia and industry is continually providing fresh insight that promises to bring these potentially potent therapies to our doorstep. While there are several books already available covering cell and gene therapy, most of these deal with both subject areas separately. Furthermore, many of these books only address various aspects such as fundamental principles and delivery or application of cell or gene therapy. This current situation has the tendency of leaving the interested readers with a fragmented understanding regarding these two areas and the flexible and powerful therapeutic platforms which can be developed when various aspects of cell and gene therapy are combined. Hence, there is a great demand from the scientific community for a book providing a holistic perspective on novel and important areas at the interface of cell and gene therapy, as well as potential synergistic therapeutic benefit obtained when both therapeutic approaches are combined with delivery strategies. Here is what this book offers you.

First, it is broadly organized to provide critical and in-depth review in the following three key areas: (1) basic biological aspects of stem cell sources, differentiation, and engineering, (2) application of stem cells and gene therapy to specific human disease, and (3) utilization of biomaterials and stem cells in regenerative medicine. This arrangement allows the readers to observe the common theme involved in the integration of cell, gene therapy, and tissue engineering and how it can be used to guide future research.

Second, this book covers a range of topics including recent advances in embryonic stem cell engineering towards tailored lineage differentiation, the human amniotic membrane as a potential tissue and cell source for cell therapy and regenerative

medicine, emerging strategies for the selection of vectors, delivery techniques and therapeutic targets for gene transfer to the heart, application of microfluidics to study stem cell dynamics, biomimetic multiscale topography for cell alignment, and spinal cord repair by means of tissue engineered scaffolds. The contents of *Emerging Trends in Cell and Gene Therapy* are contributed by leading international research and clinical experts and therefore represent current understanding, practice, and state of the fields of cell therapy, gene therapy, and tissue engineering. Hence, this book offers, in a single volume, the required comprehensive understanding regarding the connecting thread running through cell therapy, gene therapy, and tissue engineering for veterans and newcomers to the field.

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Michael K. Danquah graduated with BS in Chemical Engineering from Kwame Nkrumah University of Science and Technology, Ghana. He received his MS in Chemical Engineering from The University of Kentucky, Lexington, KY, and PhD in Pharmaceutics and Drug Delivery from The University of Tennessee Health Science Center, Memphis, TN, USA. He is currently an Assistant Professor in the Department of Pharmaceutical Sciences, Chicago State University, Chicago. His research interests include small molecule and gene-based combination therapy for treating cancer, design and synthesis of novel biomaterials, and application of nanobiotechnology for enhanced drug delivery.

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Chapter 1
The Mechanism of Stem Cell Differentiation into Smooth Muscle Cells

Russell Simpson and Qingbo Xu

Abstract Stem cells represent one of the most promising areas in biological and medical research for the treatment of vascular disease; by taking advantage of their unique ability to undergo unlimited self-renewal and to differentiate into specific cell lineages, they potentially provide an unlimited cell source for vascular tissue repair and for the construction of engineered vessels. Emerging evidence indicates that the mobilisation and recruitment of circulating or tissue-resident stem/progenitor cells give rise to smooth muscle cells (SMCs) which participate in numerous cardiovascular diseases such as atherosclerosis. Understanding the regulatory mechanisms that control smooth muscle differentiation and their recruitment from vascular progenitors is essential for stem cell therapy for vascular diseases and regenerative medicine. In this chapter, we examine the differentiation process of SMCs from pluripotent stem cells, highlighting the environmental cues and signalling pathways that control phenotypic modulation within the vasculature. We highlight the potential targets for promoting/inhibiting SMC differentiation and discuss their application for vessel-tissue engineering and treatment of cardiovascular pathologies.

Keywords Stem cell • Stem cell differentiation • Atherosclerosis • Epigenetic modification • MicroRNA

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M.K. Danquah, R.I. Mahato (eds.), *Emerging Trends in Cell and Gene Therapy*,
DOI 10.1007/978-1-62703-417-3_1,

1.1 Introduction

Blood vessels are composed mainly of two types of cells, endothelial cells that line the lumen and vascular smooth muscle cells (SMCs) that form the structure of the media [1]. Vascular SMCs refers to the particular type of smooth muscle found within and composing the majority of the wall of blood vessels. In addition to providing structural integrity within the vasculature, the main role of SMC is to regulate blood flow and pressure in blood vessels, a mechanism that is responsible for the redistribution of the blood within the body to areas where it is needed. Vascular smooth muscle can contract or relax through highly regulated contractile machinery which in the differentiated cell is composed of specific contractile proteins. A host of human diseases including cancer, atherosclerosis, hypertension and restenosis [2, 3] can be directly attributed in part to dysfunctionality of SMCs. Deciphering the cellular and molecular mechanisms which control the differentiation and phenotypic plasticity of SMCs is vital to develop new strategies to prevent and ameliorate these diseases particularly those effecting vasculogenesis. The limited lifespan of adult vascular SMCs and the difficulty in obtaining adult and mature arteries from patients present limitations for constructing autologous human vessels in vitro to regenerate a diseased adult cardiovascular system. Finding alternative cell sources to obtain large amounts of functional SMCs for development of vascular tissue engineering has generated much interest and research in the clinical use of stem cells.

Stem cells are characterised by the unique capacity for unlimited growth and self-renewal whilst maintaining the potential to differentiate into specialised cells. Generally stem cells can be divided into embryonic stem cells and tissue-resident or adult stem cells [4, 5]. Aside from their origin, the major distinction between different forms of stem cells is their "pluripotency", that is to say their ability to develop into any cell type from the three germ layers endoderm (interior stomach lining, gastrointestinal tract, lungs), mesoderm (muscle, bone, blood, urogenital) or ectoderm (epidermal tissues and nervous system) [6–9]. Embryonic stem cells (ESCs) are the pluripotent derivatives of the inner cell mass of blastocytes, hollow sphere-shaped embryos of 200–250 cells [5, 10]. They are the most promising pluripotent stem cell sources and give rise to all types of mature tissue cells in the human body [8, 9]. The isolation of the first ESCs from mouse embryos [11] led to the revolutionary knockout mouse technology which is still widely used today [12]. Alternatively, adult stem cells are derived from blood, bone marrow, vessel wall and other tissues, but unlike ESCs, they display variable capacities for differentiation and are not pluripotent in the true sense of the word [13]. Other stem cells of non-human sources are embryonic germ cells derived from the gonad ridge of primordial germ cells and recently discovered post-implantation epiblast-derived stem cells in mouse [14, 15]. Mesoangioblasts have also been characterised recently as stem cells that can differentiate into SMC [16, 17].

Elucidating the underlying mechanisms for stem cell differentiation has been a considerable challenge for researchers. Yamamoto et al. demonstrated that mechanical force produced by fluid flow can induce ESC differentiation into endothelial

cells [18], whilst Wang et al. [19] revealed that shear stress induced and suppressed angiogenic growth factors and SMC-associated growth factors, respectively. In addition to shear stress, growth factors and cytokines have been shown to directly regulate ESC differentiation [13], and the expression levels of cytokines and growth factors are likewise altered during differentiation of mesenchymal stem cells, for example [20]. Coculture of mouse neural stem cells with human endothelial-like cells gives rise to neural stem cells that have the potential to form capillary networks [21], highlighting the role of cytokines in stem cell differentiation.

In the last several years, a major achievement has been the ability to differentiate ESCs into vascular endothelial cells, SMCs and cardiomyocytes in vitro, providing not only an understanding of the development process but also a potential source for cardiovascular tissue repair [22]. The limited lifespan of adult vascular smooth muscle cells and difficulty in sourcing them present challenges for constructing human vessels in vitro to replace diseased or injured vasculature. The progress of SMC differentiation from stem cells has led to increased interest in their clinical potential to create tissue-engineered vascular grafts to treat terminal cardiovascular diseases. Furthermore, accumulating evidence indicates that the mobilisation and recruitment of circulating or tissue-resident progenitor cells that give rise to SMCs can participate in many vascular diseases including atherosclerosis, angioplasty restenosis and neointima hyperplasia after arterial injury and transplant arteriosclerosis [5, 23, 24]. Hence, in recent years, much effort has been made to understand the regulatory mechanisms which promote stem cell and progenitor cell differentiation towards SMC lineage for improving current therapeutic avenues for cardiovascular disease and vascular tissue engineering.

1.2 Smooth Muscle Cell Phenotypic Switching in Atherosclerosis

Arterial SMCs normally reside in the arterial wall in a differentiated contractile state where they provide structural support to the vasculature and control blood pressure and blood flow through highly regulated contractile mechanisms. Differentiated SMCs in adult blood vessels proliferate at an extremely low rate, exhibit low synthetic activity and express a unique repertoire of ion channels, signalling molecules and contractile proteins required for the cell's contractile function [25, 26]. Differentiated SMCs express a variety of SMC-specific contractile and contractile-associated proteins that contribute to these functions including SM-myosin heavy chain [27, 28], SM22α [29], calponin [29, 30] and SM α-actin [3, 31, 32]. Although this repertoire is specifically expressed in the fully differentiated SMC, most of these markers are expressed at least transiently in other cells during repair or pathological conditions [33], making identification of mature SMCs problematic.

Differentiation of SMCs is necessary for maturation and remodelling of the vasculature [34–36], and in addition, they secrete important components of the

extracellular matrix (ECM) such as elastin and collagen, which assist in regulating mechanical properties of blood vessels [37, 38]. Unlike the cardiac and skeletal muscle cells, adult SMCs demonstrate remarkable plasticity, and in response to vascular injury, during remodelling to changes in blood flow or in different disease states, SMCs in the arterial wall can undergo profound and reversible phenotypic alterations, a process called "phenotypic switching" [39] (reviewed by Owens [25]). These dedifferentiated or "synthetic" SMCs are characterised by decreased SMC differentiation marker gene expression and increased SMC proliferation, migration, ECM synthesis [40, 41], contractile SMCs and can synthesise up to 25–46 times more collagen [42, 43] probably as a result of increased responsiveness to growth factors. Differentiation and phenotypic modulation of SMCs are controlled by a dynamic array of extrinsic cues. The fact that vascular SMCs are not terminally differentiated and retain the ability to modulate their phenotype to changing environmental cues likely evolved in higher organisms as it conferred a survival mechanism for vascular repair. Paradoxically, an unfortunate consequence of this plasticity is that it allows rapid adaptation to fluctuating environmental cues during development and progression of vascular diseases; asthma, hypertension, cancer and development of irreversible atherosclerotic lesions have all been shown to be attributed in part to phenotypic switching [39, 40, 44]. Hence, because it is believed that transition to the "synthetic" state facilitates many of the pathogenic roles of SMCs, an understanding of the factors regulating SMC differentiation is paramount for treatment strategies [45]. Whilst much is known regarding factors and mechanisms that control SMC differentiation in cultured cells, we still have an incomplete knowledge of the transcription regulatory mechanisms that ultimately regulate SMC phenotypic switching in vivo, and this is by no means made easier by the plasticity of this cell type or the fact that SMCs derive from multiple precursors throughout the embryo [46]. Unlike cardiac and skeletal muscle cells, during embryonic development, SMCs are derived from numerous distinct populations of precursor cells. Coronary artery SMCs in the vasculature, for example, are derived from proepicardial cells, whereas the aortic arch and thoracic aorta contain SMCs which have originated from the neural crest [46]. It is this origin-associated diversity which may account for the distinct structural and functional properties analogous with SMCs [46] such as the variant expression of contractile proteins with SMCs from various tissues [47, 48].

A major challenge has been to elucidate not only the environmental cues that regulate phenotypic switching in SMCs but how these processes become disrupted in disease states. A further complexity is that the precise nature of phenotypic switching is highly variable in these different diseases, with changes in atherosclerosis involving profound changes in SMC morphology, function and gene expression patterns, compared with the much more subtle changes in contractility associated with asthma and hypertension [40], for example. Moreover, the precise role of the SMC varies greatly depending on the stage of these diseases, and this is best illustrated in atherosclerosis which is probably the best-known example of a disease in which SMC phenotype switching plays a critical role.

Arteriosclerosis is an overlying term covering all pathologies in which arteries become harder and less elastic. Arteriosclerosis is characterised by SMC hyperplasia or hypertrophy and matrix protein accumulation in the intima or media or both, with or without lipid deposition, resulting in thickening and stiffness of the arterial wall [49]. Arteriosclerosis includes spontaneous atherosclerosis, accelerated (transplant) arteriosclerosis, vein graft atherosclerosis and restenosis after percutaneous transluminal coronary angioplasty [50]. Atherosclerosis, the most common form of arteriosclerosis, is a disease responsible for over 55 % of all deaths in Western civilisation [51]. In atherosclerosis lesions, the three major cell components are the SMCs, which are the most abundant cell type around the necrotic core, and the lymphocytes (intracellular and extracellular lipid) [52]. It has been estimated that up to 70 % of lesion development mass is made up of SMCs or SMC products such as ECM [25, 53]. Atherosclerosis is a progressive disease characterised by the formation of atheromatous plaques within the walls of large- and medium-sized arteries. Early lesions, otherwise known as fatty streaks, may occur in the intima as early as childhood and develop into plaques with a lipid-rich core within the central portion of the thickened intima in adults. The characteristic feature of the advanced atherosclerotic plaque is irregular thickening of the arterial intima by inflammatory cells, extracellular lipid (atheroma) and fibrous tissue (sclerosis) [54]. A large part of the lesions comprise seemingly inert and acellular fibrous tissue, but there is often a distinct and highly cellular fibrous cap which arises from the migration and proliferation of vascular smooth muscle cells and from matrix deposition [53]. The fibrous cap undoubtedly contributes something to luminal encroachment, but its importance has recently been emphasised as a strong determinant of the likelihood of plaque rupture at later stages. Rupture leads to the release of lipids which results in a signal cascade that leads to thrombus formation [53, 55–57], thereby contributing to arterial occlusions, coronary disease, myocardial infarction and stroke. It is now known that within the fibrous cap of advanced atherosclerotic plaques, SMCs may play either a beneficial role or detrimental role in determining plaque stability, depending on the cells' phenotypic state [58, 59]. In their synthetic state, SMCs are the primary cells responsible for stabilising fibrous caps by virtue of their proliferation and production of extracellular proteins. However, in response to environmental signals that are poorly characterised, these cells can become apoptotic and activate expression of matrix metalloproteinases and inflammatory mediators that can act together in promoting end-stage disease events such as plaque rupture and thrombosis [58, 59]. It had been argued that the accumulation of smooth muscle cells in the tunica intima was a negative feature of plaque progression [51, 60]. Recently, however, pathologists and cardiologists have come to see the formation and survival of a fibrous cap consisting of smooth muscle cells and connective tissue as a good thing, as part of an attempt by the vessel wall to encapsulate the toxic products accumulating in the necrotic core [61]. It is known that medial SMCs and those within arteriosclerotic lesions differ dramatically and there has been extensive work made in an attempt to study this phenotypic switching between normal and diseased states [62, 63]. During formation of arteriosclerosis, it is believed that

before SMCs can migrate from the media into intima, a transition in their phenotype is required [64]. Medial non-proliferating SMCs have a contractile phenotype which they need to maintain vascular tone. When SMCs proliferate, they take on a synthetic phenotype which is associated with modulated gene expression and generation of proteins. The prevailing theory for the pathogenesis of arteriosclerosis suggests that during atherosclerotic plaque or neointima formation or both, SMCs from the media migrate to the intima and assume the synthetic phenotype, proliferate, produce extracellular matrix and participate in fibrous cap formation [51, 53]. According to this view, intimal SMCs in transplant arteriosclerotic lesions should originate from the donor vessels; however, there is now growing evidence to support the recipient origin of SMCs in neointimal lesions in animal models [65–68], whilst it has been argued that SMCs in human transplant arteriosclerosis are derived from both donors and recipients [5].

There is now growing evidence that stem cells and smooth muscle progenitor cells also contribute to arteriosclerosis by differentiating into SMCs in the intima [65, 67–72]. Derivation from these different sources may be the main reason as to why SMCs in arteriosclerotic lesions display a diversity of phenotypes, characteristics and behaviours. Since this is an important issue for understanding the pathogenesis of arteriosclerosis, the sections that follow concentrate on smooth muscle origins and the mechanism of SMC differentiation from stem cells.

1.3 Smooth Muscle Progenitors

It is now appreciated that adult stem cells are present in a host of tissues and organs (Fig. 1.1) [73, 74]. SMC accumulation in the intima is a key event in the development of arteriosclerosis [75], and as described above, the most accepted theory had been that the majority of intimal SMC are derived from the media of the vessel [76]. This long-standing dogma is being revisited following the discovery that different sources of cells may be responsible for smooth muscle accumulation in atherosclerosis. Emerging evidence has demonstrated the existence of a population of vascular stem/progenitor cells in a variety of tissues including circulating bone marrow-derived stem cells [67, 77] and/or resident $Sca1^+$ adventitial cells [74, 78]. There is also evidence demonstrating that SMC or SMC-like cells may be derived from a variety of sources, including transdifferentiation of endothelial cells [79] and adventitial fibroblasts [80–82] as well as medial SMC [83]. Specifically, bone marrow- and vessel wall-derived progenitors have been shown to have the ability to differentiate into SMCs which can participate in angiogenesis and vascular remodelling [84–88]. Furthermore, these cells may be directly or indirectly involved in cardiovascular disease development [89, 90] and participate in atherosclerotic plaque development and neointima formation [74, 91–95]. The lack of definitive SMC lineage-tracing studies in the context of atherosclerosis and problems in pinpointing phenotypically modulated SMC within lesions that have attenuated SMC marker genes and/or induced expression of markers of alternative cell types, that is,

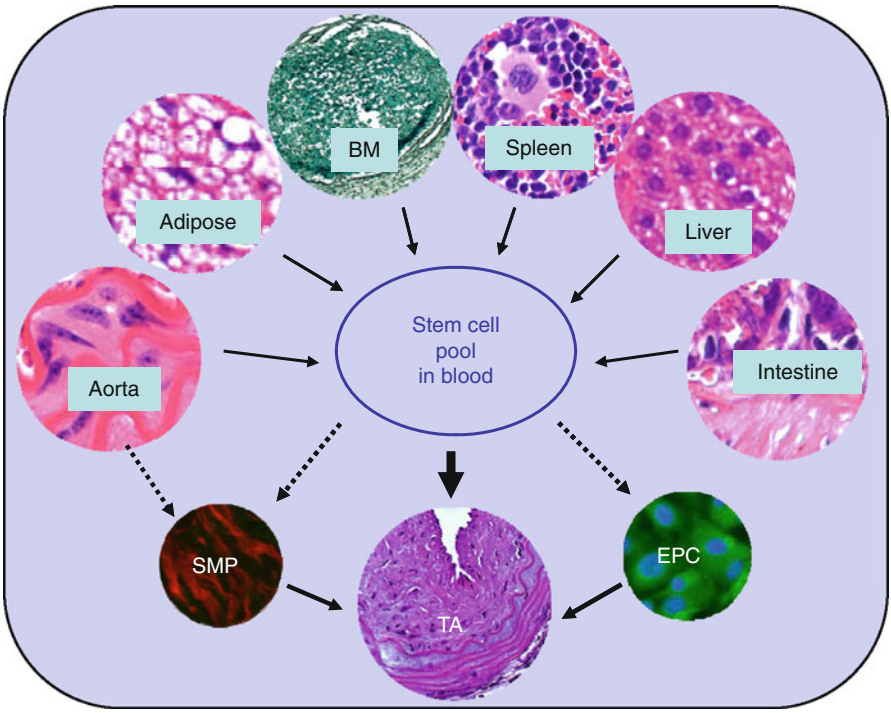

Fig. 1.1 Stem/progenitor cell origins. Stem/progenitor cells could be released from arterial wall, adipose tissue, bone marrow (*BM*), spleen, liver and intestine into blood, where they form circulating stem cell pool in blood. Smooth muscle progenitors (*SMPs*) and endothelial progenitor cells (*EPC*) accumulate within the intima, where they differentiate into SMCs contributing to the lesion formation of arteriosclerosis

macrophages, raise major questions regarding the contributions of SMC at all stages of atherogenesis. The precise frequency and roles of progenitor cell-derived SMCs in arteriosclerosis remain uncertain, but it is however widely agreed that progenitors can contribute to SMC accumulation in lesions, depending on the differential degrees of vessel damage [1]. Yet, there is still uncertainty about the origin and residency sites of smooth muscle progenitors in vivo, and given the innate heterogeneity of SMCs, it is not surprising that there is conflicting data. It was demonstrated that hematopoietic stem cells could give rise to arterial SMCs after injection into the border zone of experimental myocardial infarcts in mice [69]. In native atherosclerosis, Sata et al. demonstrated that SMCs in atherosclerotic plaques were shown to originate from bone marrow progenitors, implying that SMCs were derived from hematopoietic stem cells [67]. One group showed the majority of neointimal SMCs within plaques of experimental atherosclerosis in sex-matched chimeric scenarios and transgenic bone marrow transplant settings are derived from the bone marrow [66]. Other investigators failed to identify bone marrow-derived SMCs in atherosclerosis [68, 83, 96]. Early on, Benditt and Benditt [97]