Vascular Pharmacology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Review

# Periadventitial local drug delivery to target restenosis

Belay Tesfamariam

*Division of Cardiovascular and Renal Products, Center for Drug Evaluation and Research, FDA, 10903 New Hampshire Ave, Bldg 22, Rm 4176, Silver Spring, MD, United States*

ARTICLE INFO

*Keywords:*
Neontimal hyperplasia
Restenosis
Periadventitial drug delivery systems
Microinjection catheters
Vascular injury

ABSTRACT

The adventitia functions as a dynamic compartment for cell trafficking into and out of the artery wall, and communicates with medial and intimal cells. The resident cells in the tunica adventitia play an integral role in the regulation of vessel wall structure, repair, tone, and remodeling. Following injury to the vascular wall, adventitial fibroblasts are activated, which proliferate and differentiate into migratory myofibroblasts, and initiate inflammation through the secretion of soluble factors such as chemokines, cytokines, and adhesion molecules. The secreted factors subsequently promote leukocyte recruitment and extravasation into the media and intima. The adventitia generates reactive oxygen species and growth factors that participate in cell proliferation, migration, and hypertrophy, resulting in intimal thickening. The adventitial vasa vasorum undergoes neovascularization and serves as a port of entry for the delivery of inflammatory cells and resident stem/progenitor cells into the intima, and thus facilitates vascular remodeling. This review highlights the contribution of multilineage cells in the adventitia along with de-differentiated smooth muscle-like cells to the formation of neointimal hyperplasia, and discusses the potential of periadventitial local drug delivery for the prevention of vascular restenosis.

## 1. Introduction

The adventitia is the complex outermost layer of the vessel wall and is composed of primarily fibroblasts, collagen-rich connective tissue, elastin and nerve fibers. In addition, the adventitia consists of a variety of cells, including dendritic cells, macrophages, lymphocytes, ganglionic cells, mast cells, stem cell-like vascular and hematopoietic lineage progenitors, and a surrounding layer of adipose tissue [1,2]. The heterogeneous resident adventitial cells play an integral role, along with medial and intimal layers in response to vascular wall injury. The adventitia contains a microvasculature that delivers nutrients and functions as a gateway for resident cells migration into the intima. The adventitia is also a major site of immune surveillance and inflammatory cell trafficking, and actively participates in the initiation of vascular inflammation and progression following vascular injury [3].

The adventitia is a metabolically active compartment and participates in the control of growth, injury repair, and remodeling [4]. The activated adventitia communicates with the inner layers of the vascular wall, and participates in the maintenance of vascular structure and tone. Mechanical forces, such as vessel overdistention or stretch, to the intimal and medial layers are transmitted to the adventitia by hydraulic conductance through convection and diffusion forces, and the adventitia becomes an early site for arterial response to injury. Vascular revascularization procedures are associated with a stimulus for maladaptive response. Grafts used for bypass and endarterectomy are subjected to trauma and distention that lead to a protracted course of healing. As the implication of adventitial involvement in injury response broadens, it becomes an important site in the regulation of vascular function and remodeling.

## 2. Adventitial fibroblasts

Adventitial fibroblasts play a pivotal role in vascular remodeling owing to their remarkable plasticity. Fibroblasts contribute to the maintenance of connective tissues for structural support by producing large amounts of collagen [5]. In response to mechanical stress and hypoxia/ischemia complications, the adventitial fibroblasts become activated and switch to a proinflammatory phenotype [5,6]. These phenotypic changes are accompanied by proliferation, differentiation, expression of contractile and extracellular matrix proteins, and release of factors that directly affect vascular function.

Activated fibroblasts are characterized by an increase in cellular proliferation, secretion of chemokines, cytokines, and growth and angiogenic factors that directly affect vessel wall growth and initiate inflammation (Fig. 1). Activated fibroblasts migrate to the intima, where they stain positive for cytoskeletal protein α-smooth muscle actin, indicating phenotypic switch and differentiation into myofibroblasts, induced by transforming growth factor-β1 (TGF-β1). Myofibroblasts are

*E-mail address:* belay.tesfamariam@fda.hhs.gov.

https://doi.org/10.1016/j.vph.2017.12.062
Received 28 August 2017; Received in revised form 18 October 2017; Accepted 7 December 2017
1537-1891/ Published by Elsevier Inc.

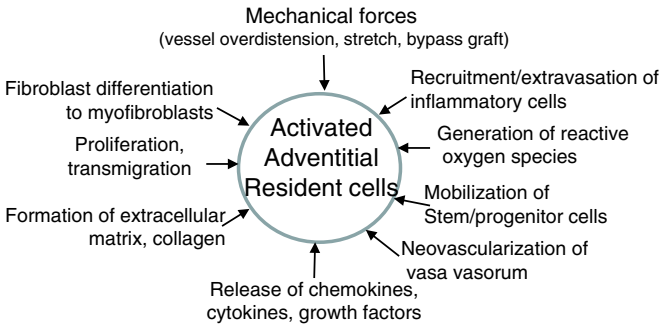


**Fig. 1.** The adventitia: The adventitia becomes activated in response to injury leading to a chain of events initiated by fibroblast activation, transformation to myofibroblast phenotype, proliferation and migration towards the intima. Adventitial fibroblasts upregulate cytokines, chemokines, and adhesion molecules, generate reactive oxygen species, and promote extravasation of inflammatory cells towards the intima. Adventitia produces collagen that participates in extracellular matrix formation and thickening of arterial wall. Resident stem/progenitor cells are mobilized and transported into the intima via neovascularized vasa vasorum and subsequently differentiate into endothelial or smooth muscle-like cells and participate in neointimal formation and arterial remodeling.

further distinguished from smooth muscle cells by the lack of smooth muscle markers, such as smooth muscle myosin heavy chains. Myofibroblasts are also derived from the transdifferentiation of endothelial cells through an endothelial-to-mesenchymal transition, a complex biological transition induced by TGF-β1. In such cases, endothelial cells lose specific markers, such as vascular endothelial cadherin and CD31, and acquire a myofibroblastic phenotype and express α-smooth muscle actin and type I collagen.

Myofibroblasts participate in crucial processes of adaptive fibrotic remodeling consisting of structural and functional reorganization, intimal thickening and healing (Table 1). In addition, myofibroblasts play an important role in the production and modification of the extracellular matrix, secretion of angiogenic and proinflammatory factors, and generation of tensile force. The contractile properties of myofibroblasts are indicated by the presence of stress fibers and cytoskeletal proteins, which are upregulated in the transformation from the non-proliferative fibroblastic phase [7]. Myofibroblast contraction is regulated by the activation of Rho-kinase, which inactivates myosin light chain phosphatase and phosphorylates myosin light chain, resulting in continued phosphorylation and persistent actin-myosin contraction. Unlike smooth muscle cells, which are characterized by a rapid and transient contraction, myofibroblasts exert a long-lasting isometric tension resulting in a slow and irreversible contraction. These observations indicate that the adventitia is a dynamic compartment and metabolically active layer of interacting cell types, capable of regulating vascular structure and tone.

### 2.1. Adventitia as stem/progenitor cell niche

The adventitia represents a niche for stem and progenitor cells with multilineage differentiation capacity that participates in vessel wall function [1,8]. The adventitial layer serves as a base for endothelial progenitor cells, mesenchymal progenitor cells, hematopoietic stem cells, smooth muscle cells, and pericyte progenitors [8,9]. Activated stem/progenitor cells undergo changes that include proliferation, differentiation, and migration. The progenitor cells are primed to respond to adventitial activation and to participate in regeneration and repair of vascular wall (Fig. 2). In addition, the adventitial progenitors possess paracrine capacity and spontaneously differentiate into pericytes, as well as into various vascular cells, including endothelial and smooth muscle cells [4].

The multipotent and lineage-committed progenitor cells have the ability to differentiate into various vascular cell lineages [10]. The adventitial stem/progenitor cells express stem cell markers such as stem cell antigen-1 (Sca-1), endothelial progenitor cell markers ($CD34^+/CD31^-$), smooth muscle progenitor cells ($Lin\text{-}Sca\text{-}1^+/CD45^-$), mesenchymal stem/stromal cells, and adventitial macrophage progenitor cells ($Lin\text{-}Sca\text{-}1^+/CD45^+$) [9]. Adventitial $Sca\text{-}1^+$ progenitors are embryonic hematopoietic cells and have the capacity to differentiate into endothelial and smooth muscle-like cells. Differentiated smooth muscle cells in the media migrate into the intima and express progenitor cell markers and contribute to a subpopulation of Sca-1 progenitor cells. Mesenchymal stem cell progenitors consisting of multipotent stromal cells differentiate into endothelial lineage ($CD34^+/CD31^-$) in the intima.

Adventitial progenitor cells respond to injury and participate in the repair of damaged tissues. Differentiated progenitors migrate into the intima, which becomes thickened [1]. Adventitial $Sca\text{-}1^+$ progenitor cells produce collagen, acquire a fibroblast-like phenotype, and promote vessel wall thickening. Sca-1 positive progenitors contribute to adventitial fibrosis involving transformation and recruitment of matrix-producing cells and play major roles in response to inflammation and injury-mediated adventitial remodeling and repair [11].

Multipotent adventitial stem/progenitor cells are involved in the repair of injured endothelium primarily through the migration of lineage-specific endothelial progenitor cells [12]. In addition, damaged endothelium is also repaired through the recruitment of circulating endothelial progenitor cells and proliferation of mature endothelial cells that re-enter the cell cycle via a dedifferentiation process. The adventitial resident stem/progenitor cells also contribute to neovascular growth during adventitial remodeling, accompanied by formation of microvascular networks. Thus, the resident adventitial stem/progenitor cells participate in endothelial repair as well as contribute to intimal thickening following vascular injury.

### 2.2. Periadventitial adipocytes

Periadventitial adipocytes participate in the regulation of vasomotor tone by releasing putative adipocyte-derived relaxing factors (ADRFs), which may include adiponectin, hydrogen peroxide, hydrogen sulfide, or prostacyclin [13]. ADRFs release nitric oxide (NO), upregulate endothelial NO synthase activity, activate $K^+$ channels, and inhibit myofibroblast migration [14,15]. The anti-inflammatory adiponectin produced by periadventitial adipose tissue plays an inhibitory role on neointimal growth, whereas pro-inflammatory adipokines such as leptin are associated with increased neointimal hyperplasia [16]. In

**Table 1**
Activated fibroblasts in vascular remodeling.

| Mediators | Role |
|---|---|
| • Differentiated myofibroblasts | • Functional reorganization, fibrotic remodeling, intimal thickening, neointimal formation |
| • Collagen deposition, Fibronectin, Tenascin | • Extracellular matrix formation, structural reorganization, intimal thickening |
| • Chemokine/cytokine secretion | • Inflammatory mediators, extravasation of leukocytes |
| • NADPH oxidase subunits | • Reactive oxygen species generation, oxidative stress, inactivation of NO |
| • Expression of growth factors, angiogenic factors | • Myofibroblastic phenotype, proliferation/migration, vasa vasorum neovascularization |
| • Upregulate adhesion molecules (ICAM-1, VCAM-1) | • Recruitment/extravasation macrophages, leukocytes |

ICAM-1, intercellular adhesion molecule-1; NDPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; VCAM-1, vascular cell adhesion molecule-1.