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Thymosin β 4 mobilizes mesothelial cells for blood vessel repair

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Abstract

Mesothelium is the simple squamous epithelium covering all abdominal organs and the coeloms in which those organs reside. While the structural characteristics of this cell type were documented a century ago, its potential in development, disease, and wound healing is only now becoming apparent. In the embryo, mesothelia provide vasculogenic cells for the developing heart, lungs, and gut. Furthermore, adult mesothelial cells can be reactivated using Thymosin β 4 and mobilized to aid in tissue repair. Despite their positive role in development and repair, mesothelia are also susceptible to adhesion and tumor formation. With knowledge that the mesothelium is an important mediator of tissue repair as well as disease, it will be important to identify other factors like Thymosin β 4 that have the ability to potentiate these cells. Future use of chemical and genetic agents in conjunction with mesothelial cells will lead to enhanced therapeutic potential and mitigation of deleterious outcomes.

Keywords

mesothelium; Thymosin β 4; smooth muscle regeneration; blood vessel injury

Introduction

In the 1880, Minot first described the epithelial component of the lateral plate mesoderm that comprises the body cavities of vertebrate organisms [1]. After more than a century of characterization, we have arrived at the term mesothelium to describe the most superficial mesodermally-derived layer composed of a simple squamous epithelium that covers all organs contained in the peritoneal, pleural, and pericardial cavities of the adult organisms as well as the cavities themselves. Despite its early identification, it is only recently that a better appreciation of mesothelial development and diversity and its roles in tissue repair as well as disease has come to light. Since this initial description, much progress has been made in our understanding of the origin and function of cardiac mesothelium (the epicardium). Yet, only recently have in depth studies been devoted to examination of the pleural and peritoneal mesothelia. While similarities exist between these tissues, they are not as Mutsaers suggests “essentially similar regardless of species or anatomical site” [1]. Rather, these distinct tissues have different points of origin as well as different biological potentials. In addition, it is becoming apparent that this normally quiescent lining has the ability to be therapeutic as well as deleterious under certain conditions. Here, we give an overview of mesothelial biology and how Thymosin β 4 can be used to potentiate mesothelial-mediated repair.

Mesothelia During Development

The development of the mesothelium of the pericardial cavity, the epicardium, has been extensively studied. It is known that these mesothelial cells originate outside of the heart in the proepicardium [2, 3]. These cells then migrate over the developing looped heart tube where some of them undergo an epithelial to mesenchymal transformation and dive into the myocardium to contribute smooth muscle [4] and endothelial cells [5] to the coronary vasculature [6] [7, 8]. Other cells remain on the surface of the heart to form the epicardium proper, a tissue that remains relatively quiescent in adult organisms.

Recently, it has come to light that this may not be the convention for all types of mesothelium. Gut mesothelium does not originate from a proepicardium-like organ. Rather, a resident population of cells embedded in the splanchnic mesoderm of the developing alimentary canal differentiates into the mesothelium that encompasses the gut (Winters et al. in revision). Some of these cells subsequently undergo an epithelial to mesenchymal transformation and provide the majority of smooth muscle cells for the gut vasculature [9]. The origin of pleural mesothelium is unknown as of yet. However, it has been shown that pleural mesothelial cells are a source of vascular smooth muscle as well as mesenchyme for the developing lungs [10]. These data suggest that while mesothelia may differ on their points of origin, a conserved role for mesothelium in development of coelomic organ vasculature exists.

Functions of Adult Mesothelia

Historically, mesothelium was thought to be the coelom's protective barrier against physical damage and pathogens [11]. Indeed, mesothelial cells secrete glycosaminoglycans, phosphatidylcholine, and surfactant, all of which act as lubricants to reduce friction between coelomic organs and the body wall. In addition, mesothelium is involved in transportation of fluid across coelomic cavities. The luminal aspect of pleural and peritoneal mesothelia is carpeted by microvilli, which increases surface area and promotes fluid absorption [12, 13]. Furthermore, stomatal pores are located at mesothelial cell junctions and provide a portal for fluid and cells to be shuttled into the underlying lymphatic system and away from the coelomic space [14]. While these functions were the first to be described, it is evident that mesothelium is actively involved in other aspects of homeostasis and tissue repair.

Mesothelium and Disease

Serosal adhesions can form following post-operative injury to the mesothelium. Under normal circumstances, if the mesothelium is damaged mesothelial cells secrete fibrin in order to initiate tissue repair. Within days, the mesothelial lining is regenerated and the fibrin is reabsorbed. However, if normal regeneration is perturbed, patches of fibrous, vascularized, and innervated tissue form between adjacent organs or between organs and the body wall, which can lead to pain, intestinal obstructions, and infertility [15].

Cancer of the mesothelium is referred to as mesothelioma. Predominantly found in pleural mesothelium, mesothelioma can also occur at lower frequencies in the peritoneum and pericardial cavity as well [16]. It has been reported that the most common etiological cause of mesothelioma is asbestos exposure. However tumors can occur without asbestos exposure suggesting other causative agents. An aggressive form of cancer, mesothelioma is generally unresponsive to conventional chemotherapeutic and radiation therapies.

Mesothelia and Wound Healing

The healing power of mesothelium has been recognized in clinical circles for well over a century [17]. The omentum, a visceral mesothelial tissue, has been transplanted onto a variety of injured tissues including ischemic hearts [18], gastric ulcers [19], and the brains of Alzheimer patients [20]. These procedures demonstrated the omentum's remarkable ability to stimulate new blood vessel formation. In addition, mesothelial cells play a role in modulating coelomic inflammation [21]. Mesothelial secretion of chemokines promotes leukocyte migration into the coelom in response to pathogens or tissue damage [22]. Additionally, in response to injury, mesothelial cells can release growth factors including transforming growth factor beta (TGF- β) family members, fibroblast growth factors (FGFs), epidermal growth factors (EGFs), and platelet-derived growth factor (PDGF) [23]. These growth factors can then regulate aspects of cell proliferation, cell migration, and extracellular matrix deposition in order to promote wound healing [1]. Taken together, these studies illustrate a paracrine role for mesothelium in tissue repair.

Thymosin β 4 and Wound Healing

Thymosin β 4 (T β 4) was originally identified as a G-actin sequestering protein [24] and is now known to have a wide range of biological activities including influencing cell migration. As a regulator of actin cytoskeletal dynamics T β 4 can greatly impact a cell's ability to move [25]. In addition, T β 4 can activate AKT and PKC signaling cascades, potent inducers of cell migration [26–28] (Figure 1). Finally, T β 4 can regulate components of the basement membrane like laminin-5, which is known to affect cell adhesion and migration [29].

T β 4 was also identified as a mediator of cell differentiation. Labeled an angiogenic molecule, T β 4 was shown to induce endothelial and smooth muscle cell differentiation as well as tube formation [27] (Figure 1). Furthermore, various mouse models demonstrated that T β 4 promoted keratinocyte and hair follicle differentiation leading to dermal repair and increased hair growth respectively [30, 31].

Because of its ability to modulate cell migration and differentiation, T β 4 was thought to be clinically relevant. Indeed, it has shown promise in promoting corneal [32, 33] and dermal [34, 35] wound healing. Furthermore, T β 4 was found to be cardio-protective and can promote cardiac regeneration following infarct [36–38]. Specifically, addition of T β 4 to a mouse infarct model mobilized adult epicardial cells to invade the myocardium and establish new coronary vessels (Figure 1) [39].

Potentiating Omental Grafts with Thymosin β 4

Until recently, the mesothelium was only thought to play a paracrine role in wound healing [11]. And while several studies demonstrated that adult omental mesothelium retained the ability to differentiate into vasculogenic cells [28, 40], we were the first group to demonstrate a cellular contribution of omentum to blood vessel repair [28]. In studies performed in mice, when omentum was grafted onto injured arteries, omental mesothelium differentiated into smooth muscle and remained adjacent to the vessel wall as it healed (Figure 2 A,B). While no graft integration into the injured vessel was observed, the mesothelial cells did provide paracrine signaling that accelerated wound healing [28]. Knowing that T β 4 can promote cell migration, vessel development, and tissue repair, agarose beads soaked in T β 4 were added to omental grafts that were transplanted onto injured blood vessels (Figure 2C). Interestingly, T β 4 was able to potentiate mesothelial grafts in several ways (Figure 1). First, it enhanced the ability of omental cells to differentiate into vascular smooth muscle and endothelial cells in vitro. In addition, it

promoted omental cell migration. Finally, T β 4 mobilized mesothelial graft cells to integrate into the wounded vessel and repopulate the smooth muscle layers of the vessel thereby significantly decreasing healing time of grafted vessels in vivo. Furthermore, Myographic studies revealed that vessels that incorporated mesothelial derived smooth muscle cells were functionally indistinguishable from uninjured vessels [28]. This was the first indication that omentum was capable of cellular contributions to healing tissues. In addition, it was the first report that factors like T β 4 could be used to potentiate omental grafts and improve therapeutic outcomes.

Conclusions and Future Directions

Coelomic epithelia was identified well over 100 years ago, however, we have only recently begun to appreciate the impact mesothelia can have on coelomic organ development, repair, and disease. As new data emerges, it is becoming clear that all mesothelium is not created equal. While most studies have concentrated on characterizing the origin, developmental contribution, and wound healing potential of the epicardium, the nuances of pleural and peritoneal mesothelia are less well defined. A recent report suggests that at least two types of mesothelial tissues are derived from different source tissue (Winters et al. in revision), and yet, despite variations in their developmental origins, all mesothelium studied to date is able to provide vasculogenic cells to the organs it surrounds [4, 9, 10]. Similarly, the ability to reactivate adult epicardial and omental mesothelium has been established [28, 39]. And in both instances, T β 4 is able to enhance the ability of each tissue to facilitate wound healing by promoting cell mobilization and differentiation (Figure 1). Understanding how to potentiate mesothelial cells in order to maximize their therapeutic potential will be important for future studies. To this end, advances in the field of small organic molecule development will be instrumental in harnessing mesothelial potential. Likewise, as not all migration and differentiation associated with mesothelial cells is beneficial, understanding the mechanisms of mesothelial disease will also be of broad interest to the clinical and basic science communities.

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Figure 1. Thymosin $\beta 4$ reactivates adult mesothelial cells to aid in tissue repair

Thymosin $\beta 4$ (T $\beta 4$) can activate PKC and AKT signaling in adult epicardial and omental mesothelial cells. In epicardial cells, T $\beta 4$ promotes proliferation, migration, differentiation into vasculogenic lineages, and endothelial tube formation all of which aid in repair of the myocardium following infarct. Similarly, in omental mesothelial cells, T $\beta 4$ promotes proliferation, migration, and differentiation into vasculogenic lineages in order to accelerate the repair of injured blood vessels.

Figure 2. Thymosin $\beta 4$ mobilizes omental cells for blood vessel repair

Murine carotid arteries were subjected to cryo-injury rendering them devoid of vascular smooth muscle and endothelial cells (A). When omental mesothelial grafts were transplanted onto injured arteries, the grafts differentiated into smooth muscle and accelerated vessel healing via a paracrine mechanism. However, no integration of mesothelial cells into healing vessels was observed (B). Agarose beads soaked in Thymosin $\beta 4$ were transplanted onto injured arteries in conjunction with omental grafts. Thymosin $\beta 4$ mobilized omental-derived smooth muscle cells and promoted their incorporation into the medial layers of vessels as they healed thereby significantly accelerating the healing process (C).