Charles University

Faculty of Pharmacy in Hradec Kralove

Department of Biological and Medical Sciences

The importance of membrane and soluble endoglin in atherosclerosis

(Diploma Thesis)

Diploma thesis mentor — Associate Professor Petr Nachtigal, PhD

Hradec Kralove 2017 — Constance Bontle Mphetang

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ABSTRAKT

Tato práce se zabývá významem membránového a rozpustného endoglinu (sEng) ve vzniku a progrese aterosklerózy. Ateroskleróza představuje hlavní rizikový faktor pro srdeční onemocnění a mrtvici, což činí vážný zdravotní problém. Úloha endoglinu při ateroskleróze je ještě třeba definovat a toto bude popsáno níže. Exprese endoglinu byla objevena v aterosklerotických cévách převážně v endoteliálních a hladkých svalových buňkách jak u pokusných zvířat, tak u lidí. Tento objev naznačuje, že endoglin hraje roli při aterogenezi a stabilizaci aterosklerotických plaků. Kromě aterosklerózy byly také zjištěny vysoké hladiny rozpustného endoglinu v případech hypercholesterolemie a infarktu myokardu a také se týkaly inhibice signalizace TGF-β ve stěnách postižených cév. Navíc, statiny rozumně snížily hladiny rozpustného endoglinu a usnadnily jeho expresi v aortě myší, což vedlo ke snížení aterosklerotických účinků a komplikací. Dále, pacienti s familiární hypercholesterolemií podstoupili extrakorporální eliminace, což pomohlo výrazně snížit hladiny rozpustného endoglinu. Ve závěru, hladiny rozpustného endoglinu může být kličem pro pochopení aterogeneze, její progrese a účinnosti zavedených léčebných strategií.

ABSTRACT

This thesis is about the importance of membrane and soluble endoglin (sEng) in atherosclerosis, both the development and the progression of the state. Atherosclerosis poses a major risk factor for heart diseases and stroke, which renders it a serious health issue. The role of endoglin in atherosclerosis is yet to be defined and this will be discussed below. Endoglin expression was discovered in atherosclerotic vessels predominantly in the endothelial and smooth muscle cells both in experimental animals and humans. This discovery suggests that endoglin plays a role in atherogenesis and stabilization of atherosclerotic plaques. In addition to atherosclerosis, high levels of soluble endoglin were also discovered in cases where hypercholesterolemia and myocardial infarction were observed and were also related to TGF-β signalling inhibition in the walls of the affected vessels. Additionally, statins reasonably lowered soluble endoglin levels and facilitated its expression in the aorta of mice, resulting in a decrease in the atherosclerotic effects and complications. Furthermore, patients with familial hypercholesterolemia underwent extracorporeal eliminations which helped significantly reduce the levels of soluble endoglin. In conclusion, knowing the levels of soluble endoglin and what that indicates in relation to atherosclerosis could be the tool to understanding atherogenesis, its progression and efficacy of treatment strategies implemented, which is what is yet to be determined in clinical studies.

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1 INTRODUCTION

This thesis is a review of the importance of membrane and soluble endoglin in atherosclerosis. Endoglin is a homodimeric trans-membrane glycoprotein which its role is to regulate TGF-β signalling. It exists in two forms, which are the membrane form, expressed in various tissues and the soluble endoglin form, which is found in the plasma of both the healthy people and those suffering from various illnesses including atherosclerosis.

The effects of soluble endoglin are not restricted to a possible role in atherosclerosis [1], as it also plays a role in disease conditions like cancer [2], hereditary haemorrhagic telangiectasia [3], and preeclampsia [4]. Endoglin effects on endothelial cells, and consequently its contribution to the development and progression of atherosclerosis are still being investigated and no concrete evidence has been established yet [1]. Endoglin in its soluble form which is found in blood plasma is a section of the full-length membrane endoglin which is found in elevated levels in cases where there are pathological conditions involving the vascular endothelium [5].

Atherosclerosis is a disease involving large arteries where these blood vessels harden due to the deposition of a fatty substance known as cholesterol. This deposition and hardening in turn hinders the normal physiological functioning of the affected vessels and presents a major risk factor for heart diseases and stroke. In western countries, this condition is responsible for 50% of the occurring deaths, rendering it a serious health issue. Atherosclerosis is a chronic condition which can result in an acute state on the event that an atherosclerotic plaque ruptures leading to embolism, or thrombosis [6].

Research to find a concrete answer on the contribution of endoglin in endothelial dysfunction and atherosclerosis is on-going. This focuses on both humans and experimental animals. The relationship between endoglin and atherosclerosis is what is yet to be elaborated

on in this write-up. The correlation between soluble endoglin levels in plasma and the development of atherosclerosis will be studied in detail. This information can also give an idea on the treatment strategies, a decreased atherosclerotic activity and evaluation of therapy in atherosclerosis management [7].

2 HISTOLOGICAL STRUCTURE OF BLOOD VESSELS

Blood vessels are tubular structures that contribute to homeostasis by providing means for blood to flow through to and from the heart. They also allow for the exchange of oxygen, nutrients and waste products between blood and tissues.

Five main types of blood vessels exist, named arteries, arterioles, capillaries, venules and veins. Arteries carry blood from the heart to the rest of the body parts. They are strong and elastic allowing them to carry out their functions effectively and to withstand the highest blood pressure as the heart pumps. Large arteries leave the heart and divide into medium-sized and eventually smaller vessels known as arterioles which upon entering tissues divide into even much smaller vessels known as capillaries. Capillaries are thin-walled, allowing for the exchange of nutrients, oxygen and waste products between blood and tissues. These capillaries subsequently join to form venules which also merge to form veins. Veins are responsible for transporting deoxygenated blood from peripheral tissues back to the heart [8].

2.1 ARTERIES

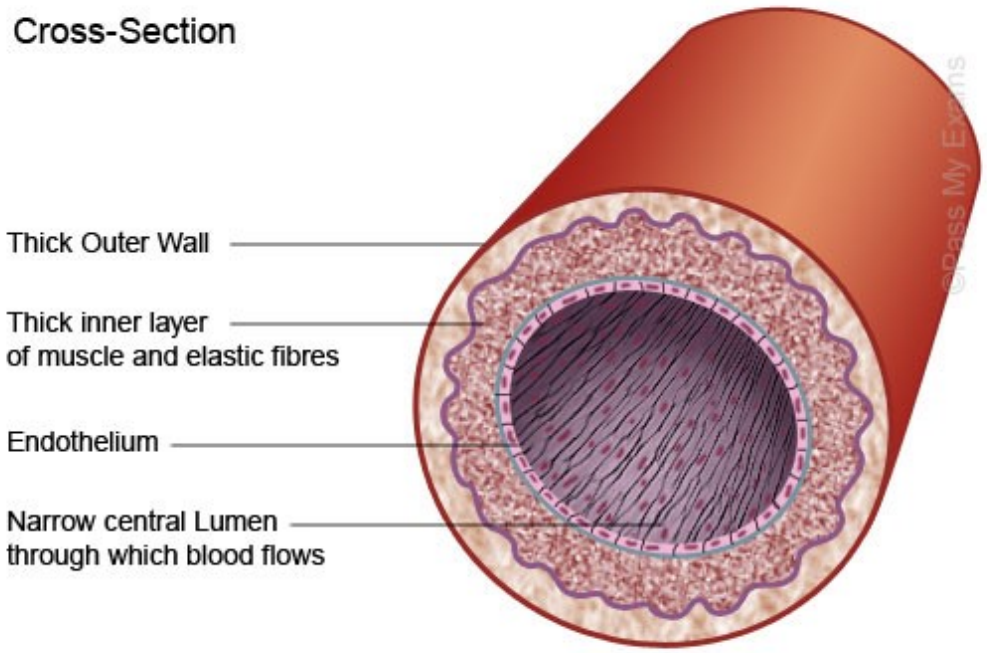

Figure 1: Shows the structure of an artery

http://www.passmyexams.co.uk/GCSE/biology/arteries.html

Retrieved 4 March 2017

Arteries transport oxygenated blood from the heart to the rest of the body parts. They are strong and have thick elastic walls (Figure 1), which allow them to expand and contract, hence allowing them to accommodate varying amounts of blood and also helps in maintaining blood pressure. The arterial elastic walls allow them to expand and contract, pushing blood along. This property also allows arteries to increase or decrease the vessel lumen diameter to increase or decrease tissue perfusion of a certain part of the body. Arteries eventually branch into finer structures known as arterioles which also possess the muscular and elastic properties, hence the ability to regulate tissue perfusion as well [8].

2.2 CAPILLARIES

Capillaries represent a bridge between arterioles and venules. They are made up of a single layer of endothelial cells (Figure 2) and are just wide enough to allow the passage of a single erythrocyte. Blood flow through capillaries is therefore very slow. These features allow for the effective exchange of oxygen, nutrients and waste materials between blood and body tissues [8].

2.3 VEINS

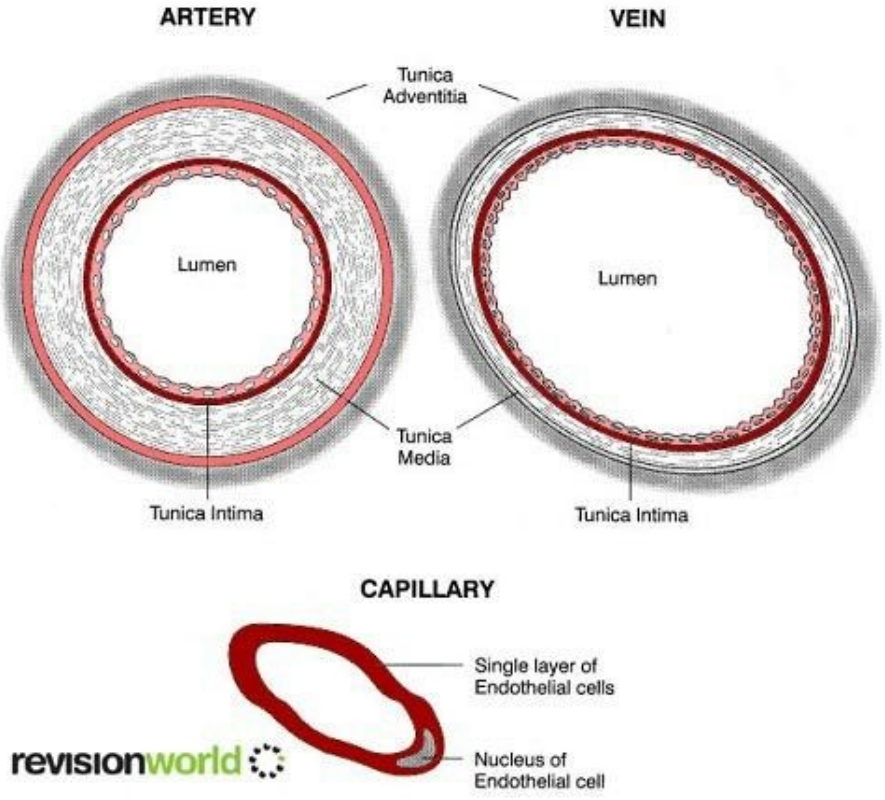

Figure 2: Shows the differences among the blood vessels arteries, veins and capillaries.

http://resizeandcrop.club/openphoto.php?img=http://media-cache-ak0.pinimg.com/736x/6d/f3/91/6df39126da7840fa1443272ca4f2db58.jpg

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On the contrary, veins carry deoxygenated blood from tissues back to the heart. Veins have thinner walls and larger lumens compared to arteries (Figure 2) because blood pressure in veins is relatively lower. Due to their large lumens, veins hold nearly two thirds of total blood in circulation. The structural differences will be discussed in more detail on the next paragraph. Veins can dilate to accommodate an increasing volume of blood. They have much thinner media compared to arteries and hence lack significant contraction and the elastic recoiling mechanism, which helps propel blood in arteries. Blood moves along the veins thanks to pressure gradients built during inspiration and expiration and also through some surrounding muscles as they contract. Some veins, like those on legs, have valves in them which help prevent backflow of blood. When these valves do not function well, there is a backflow of blood resulting in varicose veins [8].

2.3.1 BASIC HISTOLOGY OF BLOOD VESSELS

Blood vessel walls are made up of endothelial cells, smooth muscle cells and extra-cellular matrix, consisting of elastin and collagen. These form three coaxial layers named the intima, the media and the adventitia, from the inner most to the outer most layer [8].

2.3.1.1 THE INTIMA

It is the inner most layer of a blood vessel (artery or vein) consisting of only one layer of endothelial cells (Figure 3) and a sub-endothelial layer made up of connective tissue. It is relatively the thinnest layer. It being the inner most layer implies that it is in direct contact with blood. Between the intima and the adventitia lies a dense elastic membrane known as the internal elastic lamina (Figure 3). The intima and the inner part of the media get their nourishment and oxygen directly from blood via diffusion [8].

2.3.1.2 THE MEDIA

It is the layer between the intima and the adventitia. The media is the thickest layer (Figure 3) and is responsible for providing structural support, vasoreactivity and for the elastic properties of blood vessels. It is made up of varying amounts of elastic fibers, muscle fibers and connective tissue (depending on the location of the blood vessel). Veins have much thinner media compared to arteries. Elastic fibers are responsible for the elastic properties of this layer as they allow for the expansion of the vessel with systole and contraction with diastole, therefore pushing blood forward. Smooth muscles can undergo vasoconstriction and vasodilation which occur due to the influence of autonomic nervous system and some local metabolic factors. The external elastic lamina (Figure 3) , which is a dense elastic membrane, separates the media from the adventitia [8].

2.3.1.3 THE ADVENTITIA

This is the outermost layer (Figure 3) made up of connective tissue, nutrient vessels and autonomic nerves (nervi vasorum). This layer and the outer part of the media receive their nutrition from the nutrient vessels (vasa vasorum) [8].

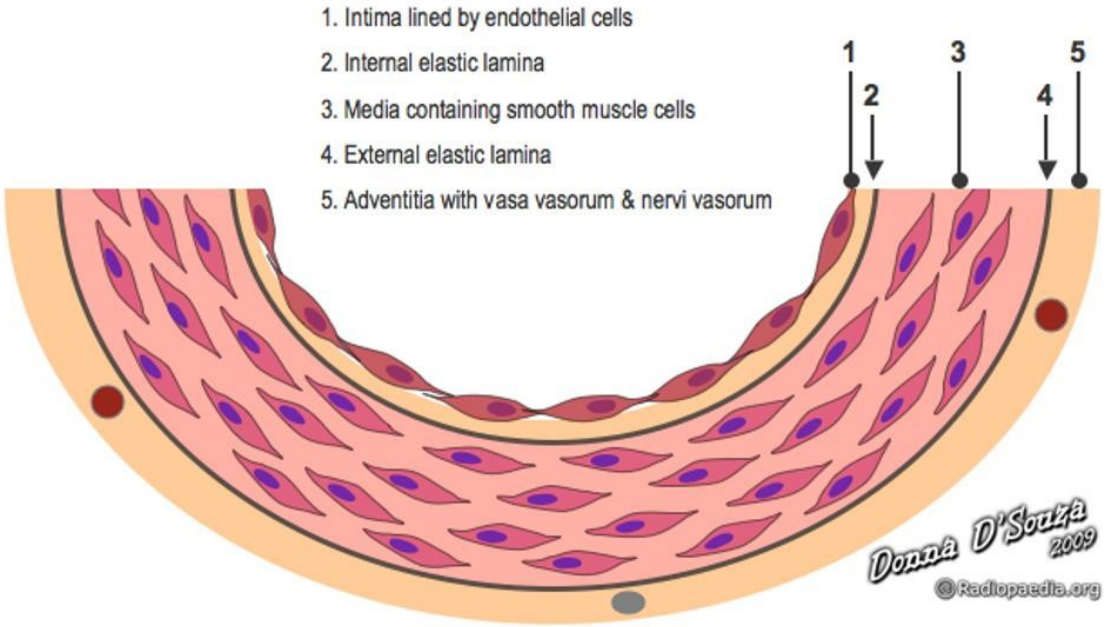

Figure 3: Diagram showing the structure of the layers of a blood vessel.

https://radiopaedia.org/cases/blood-vessel-wall-composition

Retrieved 4 March 2017

3 VASCULAR ENDOTHELIUM AND ITS FUNCTIONS

The vascular endothelium is a single layer of endothelial cells that line the whole circulatory system. This layer performs a wide range of functions and is absolutely necessary for the entire existence of an organism. The endothelial functions include regulation of vascular tone, exchange of materials between the blood and tissues and blood clotting, helps with blood flow and participates in angiogenesis. The endothelium forms a physical barrier between the blood and tissues and also secretes some regulatory substances like nitric oxide [9].

3.1 REGULATION OF BLOOD VESSEL TONE AND GROWTH

The endothelium secretes nitric oxide formed from L-arginine which is an amino acid and prostacyclin (Prostaglandin I_2 of the eicosanoid family) among other substances. These two substances are released in response to a stimulus, which can be physical, a hormone or some substances produced by platelets. This stimulation results in relaxation of vascular smooth muscles (vasodilation) and inhibition of platelet activation, hence clot formation [9]

Nitric oxide is continuously synthesized by nitric oxide synthase, a calcium-calmodulin dependent enzyme, from L-arginine. It is an important cardiovascular system master signaling molecule, which helps maintain homeostasis. Its functions include vascular tone regulation, regulation of local cell growth and protection of the vascular vessel from damage by circulating blood cells and platelets hence maintaining the integrity of the vessel and its physiological functions. Hypertension, hypercholesterolemia, diabetes and smoking, which are risk factors for atherosclerosis result in decreased nitric oxide levels either as a result of its decreased synthesis or increased oxidative degradation. Decreased nitric oxide levels greatly disrupt the endothelial equilibrium resulting in pathological states like atherosclerosis. It is for this reason that in clinical practice therapy aims to reverse endothelial dysfunction by stimulating nitric oxide release [10].Prostacyclin (PGI_2) is formed sequentially from arachidonic acid by phospholipase A_2, cyclooxygenase (COX) and specific prostaglandin synthases. Two forms of cyclooxygenase exist, which are COX-1 and COX-2. The latter is found in inflamed sites and in places where vascular injury occurred. Prostacyclin performs two important functions which are vasodilation and inhibition of platelet aggregation. Vasodilation results after prostacyclin binds to its prostacyclin receptors, which upon activation stimulate G-protein coupled rise in cAMP and protein kinase A, consequently leading to a decrease in calcium ions and eventually smooth muscle relaxation (vasodilation). Prostacyclin inhibits platelet aggregation by counteracting the effects of thromboxane A_2

(TXA_2). This effect of prostacyclin may make it eligible for use in the minimization of platelet-mediated thrombosis. It is important to maintain a balance between prostacyclin and thromboxane A_2, more especially during pregnancy and for newborns because a decrease in the PGI_2/TXA_2 ratio can lead to intrauterine growth restriction, preeclampsia and persistent pulmonary hypertension in the new-born (PPHN). An increase in the ratio can result in intra-ventricular hemorrhage and patent ductus arteriosus in preterm newborns [11, 12].

On the contrary, the endothelium also secretes some vasoconstrictive substances such as angiotensin II, thromboxane A_2 and endothelin. The above-mentioned substances resulting in vasodilation and those resulting in vasoconstriction participate in blood pressure regulation. Angiotensin II is an octapeptide hormone that maintains homeostasis of the circulatory system [13]. It works as an agonist on angiotensin 2 receptors, causing vasoconstriction. It also works hand in hand with renin through the renin-angiotensin system (RAS) which participates in immune and inflammatory responses and can also lead to pathophysiological states of the blood vessels. The effects of angiotensin 2 mediate pathological conditions like hypertension, diabetes, cardiac re-modelling, proliferative and inflammatory responses due to vascular injury and heart failure [14]. Pharmacological agents such as angiotensin converting enzyme inhibitors (ACE inhibitors) and angiotensin 2 receptor antagonists (satarns) are used in therapy to counteract angiotensin 2 effects and combat the resulting pathological conditions [13]. Angiotensin 2 contributes to the pathogenesis of atherosclerosis. In addition, cell growth/apoptosis of vascular cells, inflammation, migration of vascular smooth muscle cells and extra cellular matrix remodeling can also result as a consequence of angiotensin 2 influence [15-18].

In addition to the above groups of substances secreted, the endothelium also secretes some vascular growth promoters like platelet derived growth factors and thrombospondin and growth inhibitors like heparin derivatives such as heparin-derived oligosaccharides [19, 20].

3.2 ANGIOGENESIS AND CELL PROLIFERATIN

Angiogenesis is necessary for physiological and pathological processes. Vascular endothelial growth factor (VEGF) secreted by the endothelial cells is the main factor responsible for the formation of new, immature blood vessels from the pre-existing vasculature through angiogenesis or vasculogenesis. Further maturation of the blood vessels is the responsibility of angiopoietins (Ang), especially Ang1 and some members of the ephrin family, especially ephrinB2 whereupon the endothelium also integrates with pericytes and smooth muscles. The former is notably responsible for the maintenance of the mature vasculature stability [21]. These growth factor systems perform complementary functions.

Vascular endothelial growth factor (VEGF) also known as vascular permeability factor (VPF) has the potential to stimulate leaking and permeability on the endothelium. It also leads to endothelial proliferation [21]. It is a potent growth factor and it is found in elevated levels in tumor cells as it potentiates their angiogenesis. VEGF functions are not restricted to the vascular system as it also plays a role in some physiological processes like hematopoiesis [22], bone formation [23], development [24] and wound healing [25]. VEGF uses tyrosine kinase receptors, which are VEGFR1 (Flt-1), VEGFR2 (KDR, Flk-1) and VEGFR3. Flt-1 receptors are found in the vascular endothelium and monocytes and their abnormalities are lethal to the embryo, and so is any disruption of any allele on the VEGF. Embryo lethality results as a consequence of severe vascular abnormalities that develop due to disruptions of Flt-1 receptors and VGEF alleles. Flk-1/KDR receptors are found on endothelial cells and their abnormalities lead to disruptions in endothelial cell development and hematopoiesis [21, 26]. On the other hand, VEGFR3 receptors are found on the lymphatic endothelium [21].

Angiopoietins (Ang1) work with VEGF for a common goal, vascular formation. They also work through a different class of tyrosine receptors, known as Tie-1 and Tie-2 [27].

Ang1 predominantly works through the Tie-2 receptors and absence of either the Ang1 or the Tie-2 receptors does not result in any fatal consequences as is the case with VEGF and VGER-2, as a normal primary vasculature develops even though it fails to undergo maturity. Ang-1 works on the Tie-2 receptors to maintain the integrity of mature vasculature. Ang-2 exerts some inhibitory properties on Tie-2 receptors and is highly expressed in areas of vascular remodeling, especially in the female reproductive tract [21].

Ephrins work in yet another class of tyrosine kinase receptors whereupon they have to be membrane bound for the receptor to be activated. They show bidirectional signals which effect on both cells with the receptor and those expressing ephrin. Endothelial cells show the expression of ephrin A, ephrin B2 and ephB4. Studies suggest that ephrin B2 and its ephB4 receptors are expressed during vascular development [28]. Their disruption can result in pathological states like cancer. For this reason, further studies are conducted to find possible therapeutic interventions to combat the arising pathological states through understanding their complex biology [29].

3.3 COAGULATION AND ANTICOAGULATION PROPERTIES

As already stated, the endothelium maintains homeostasis which comprises inhibition of platelet aggregation, regulation of blood clotting and repairing damaged blood vessels. Maintaining homeostasis helps prevent thrombosis. The endothelium produces either prothrombotic or antithrombotic events depending on the local tissue needs and stress. In case of a damaged vessel, a cascade of events is initiated that aims to repair the damage and excessive clot formation is prevented by large amounts of anticoagulants that are secreted, hence preventing the occurrence of a thrombotic event. A healthy endothelium produces antiplatelet and anticoagulation factors whereas in case of endothelial dysfunction, fibrin

formation and platelet aggregation are supported. Finally, the endothelium produces pro-fibrinolytic agents which participate in clot dissolving or fibrinolysis [30].

3.4 VASCULAR INFLAMMATION AND CELL MIGRATION

The vascular endothelium is responsible for the maintenance of homeostasis in the vascular system. Its disturbances lead to a number of pathological states which usually start through inflammation and cell migration. Inflammation could be due to vascular infection or injury [31]. In the presence of an injury or infection, an inflammatory pathway is initiated, which helps with remodeling of the vessels leading to cell growth and proliferation of vascular smooth muscles. The inflammation process is accompanied by diapedesis of leukocytes, elevated oxidative stress, production of cytokines, an increase in the expression of adhesion molecules and ligands, activation of immune cells and pro-inflammatory cascades. The increased expression of the adhesion molecules Vascular Cell Adhesion Molecule-1 (VCAM-1) and Intercellular Adhesion Molecule-1 (ICAM-1) on the vascular membrane, accumulation of white blood cells including tissue infiltrating lymphocytes (B and T lymphocytes, monocytes/macrophages), natural killer cells and dendritic cells participate in the inflammation process following damage to the vascular tissue [32]. Patients presenting with cardiovascular diseases (CVD), including atherosclerosis, show increased levels of mediators and markers of inflammation [33-35]. Increased levels of inflammatory mediators such as ICAM-1, Interleukin-6 (IL-6) and C-reactive protein (CRP) may be hypertension risk factors [36, 37]. An ischemic cardiovascular event is probable in angina patients presenting some plasma levels of high-sensitivity CRP assay and is associated with vulnerable plaques. This may also indicate a possible cardiovascular event in an otherwise healthy individual [38-40].

4 ENDOTHELIAL DYSFUNCTION

This is a state when the endothelium shifts from performing its normal physiological functions as a response to cardiovascular risk factors which can also lead to atherosclerosis among other cardiovascular diseases. It is characterized by reduced vasodilation, pro-inflammatory state and the presence of a prothrombotic state. Endothelial dysfunction is related to cardiovascular diseases like atherosclerosis, hypertension, coronary and peripheral artery diseases, renal failure, coronary heart disease and diabetes. There is reduced vasodilation due to disturbances on the endothelium such as a decrease in nitric oxide production, excessive oxidation and a decrease in the production of hyperpolarizing factor. The inflammatory response, which has an input in the development of a prothrombotic state is a result of production of chemokines (macrophages chemo attractant peptide-1), adhesion molecules up regulation and release of plasminogen activator inhibitor-1. Hypercholesterolemia, hyperinsulinemia, alteration of insulin signaling, presence of an endogenous inhibitor of nitric oxide, asymmetric dimethylarginine and angiotensin 2 and endothelin-1, which are vasoactive all play a role in endothelial dysfunction, resulting in its detachment and subsequent apoptosis [41].

Endothelial dysfunction is a very important hallmark in the development and progression of atherosclerosis. In case of a dysfunctional endothelium, progenitor cells, which should be used for endothelial regeneration participate in endothelial pathophysiology instead. The severity of a dysfunctional endothelium is used in the prognosis of cardiovascular events and correction of the dysfunction leads to a decrease in the risks of the occurrence of a cardiovascular event [41]. Below are mechanisms involved in endothelial dysfunction.

4.1 PATHOPHYSIOLOGY OF ENDOTHELIAL DYSFUNCTION

4.1.1 NITRIC OXIDE

Nitric oxide (NO) is a vasodilator, prevents platelet aggregation and participates in the inhibition of inflammation. In case of endothelial dysfunction, there is a decreased amount of available nitric oxide, which could be because of decreased endothelial nitric oxide synthase activity or decreased levels of L-arginine as its substrate. Low nitric oxide levels could also be due to its reduced bioavailability [41]. A series of events result in endothelial nitric oxide synthase (eNOS) reduction property, which results in the formation of reactive oxygen species (ROS). More ROS is formed as nitric oxide synthase function shifts from producing nitric oxide through oxidation to producing ROS through reduction leading to oxidant excess [42] consequently leading to a vascular pro-inflammatory state. NO bioavailability drops due to inflammation as well. ROS lead to VCAM-1, ICAM-1 and chemotactic molecules up-regulation [43]. CRP as an inflammatory mediator decreases eNOS effects [44, 45].

4.1.2 ASSYMETRIC DIMETHYLARGININE

Asymmetric dimethylarginine (ADMA) results in a decrease of NO levels and hence decreased vasodilation and consequently endothelial dysfunction. It is an eNOS competitive inhibitor that causes endothelial dysfunction by indirectly reducing NO effects through inhibiting its synthesis. Patients presenting with hypercholesterolemia, which is a risk factor for atherosclerosis, showed ADMA levels, which were inversely related to vasodilation [46]. Administration of intravenous L-arginine helps with endothelial recovery. In a nutshell, high ADMA levels are associated with endothelial dysfunction and hypercholesterolemia [46]. They also predict acute coronary incidents [47].

4.1.3 OXIDATIVE EXCESS

Oxidative stress is characterized by the presence of excess free oxygen radicals. It was found that patients with primary hypertension presented with endothelial dysfunction as a result of reduced NO levels due to oxidative stress [48]. This function is also related to the extent of impairment of cardiovascular problems and of the endothelium-dependent vasodilation [49]. Endothelial dysfunction due to the excess of free oxygen radicals has also been observed in animal models with diabetes [50, 51]. Additionally, ROS also results in endothelial damage and detachment leading to endothelial apoptosis known as anoikis [52].

4.1.4 HYPERHOMOCYSTENEMIA

Patients with normal blood pressure with hyperhomocystenemia show endothelial dysfunction [53]. Hyperhomocystenemia can be reversed in children with chronic renal failure by the administration of folic acid which helps restore membrane integrity [54]. Studies suggest that homocysteine leads to a decrease in NO bioavailability through the formation of excess free oxygen radicals. It may also lead to ADMA [55] accumulation and hence endothelial dysfunction [56, 57].

4.1.5 ANGIOTENSIN 2

Angiotensin 2 (Ang 2) was introduced to lab rats and led to endothelial dysfunction [58-60], an increase in the levels of ROS [61] and also influences vascular inflammation. The use of an angiotensin converting enzyme inhibitor (ACE- inhibitor) leads to better endothelial recovery as compared to a beta blocker [62-64].

4.1.6 DIABETES MELLITUS TYPE II

Diabetes mellitus type II is one of the factors that can disturb the integrity of the endothelium. Hyperglycaemia produces advanced glycation end products (AGE), which lower NO levels and consequently leading to a dysfunctional endothelium [65]. AGE also

leads to the induction of ROS and promotion of vascular inflammation causing an increased expression of VCAM-1 and interleukin-6 [66]. Its clearance is delayed in renal failure promoting further renal and vascular injury [67] in patients with diabetic nephropathy. Decreased *in vivo* vasodilation in patients was observed as a consequence of hyperglycemia [68].

5 CELL ADHESSION MOLECULES, THEIR STRUCTURES AND FUNCTIONS

Cell adhesion molecules are proteins located on the surface of the cell that help bind the cell to other cells or to the extracellular matrix (ECM). They are necessary for both physiological and pathological processes. The adhesion is made possible by biochemical aspects of multiprotein complexes made up of three macromolecule classes named adhesion receptors, extracellular matrix components and adhesion plaque proteins [69]. Binding of the cell to the ECM or to other cells' counter receptors is typically achieved through cell adhesion receptors, which are transmembrane glycoproteins. The affinity between the cell-cell or cell-ECM adhesion is determined by these molecules. The ECM forms a complex network of proteins that is able to interact with multiple cell surface receptors simultaneously. Adhesion plaque proteins form a structural and functional link between adhesion receptors and the cell cytoskeleton. Intracellular biochemical events regulate the adhesion complexes through a two-way signaling process as the complexes are also exposed to the extracellular environment. Four families have been identified which are cadherins, integrins, selectins and the immunoglobulin superfamily which is also known as chemical adhesion molecules (CAMs) [70].

5.1 CADHERINS

Classical cadherins (Figure 4) is a class regulating adhesion of specific molecules. Many of them work through Ca^{2+}-dependent cell adhesion [71]. Extracellularly, they participate on cell-cell adhesion and intracellularly on the cadherin-cytoskeleton adhesion. The extracellular cell-cell adhesion is through swapping of β-strands between the adjacent cells' first extracellular domains (EC1). This swapping is typical for desmosomal and classical cadherins, but other swapping mechanisms are suggested for other cadherin types. Intracellularly, classical cadherins bind to β-catenin and p120 and the former binds to α-catenin, which is an F-actin binding protein [72]. This class of cell adhesion molecules is regulated by integrins analogous transmembrane signaling pathway. Cadherins participate in the sorting of cells, their rearrangements and their movements [71].

5.2 INTEGRINS

They are transmembrane, αβ heterodimeric receptors (Figure 4) that facilitate cell-ECM and cell-cell adhesion. They work through a two-way signal transduction mechanism that regulates many of activities in the cell after stimulation by either an extracellular or intracellular stimulus. These activities include signaling of growth factors, assembling the ECM, cell adhesion, cytoskeleton organization, apoptosis, and processes mediated by the cytoskeleton like contraction, phagocytosis and endocytosis. The regulation of these activities is via a complex system of adaptor proteins and signal kinases within the cell which works hand in hand with domains of the cytoplasmic and transmembrane subunits [73, 74].

5.3 SELECTINS

Selectins are transmembrane lectins (Figure 4) and are Ca^{2+} dependent. They mediate leukocyte extravasation. Three types of selectins exist namely the P-selectin which is found in activated platelets, L-selectin found in leukocytes and the E-selectin, which is expressed in

activated endothelial cells together with the P-selectin. Leukocyte extravasation involves promptly reversible interactions between selectins and glycosylated ligands on the cell surface which is controlled by force. It allows the interaction of leukocytes and immobile chemokines. Signal transduction during leukocyte extravasation is also through selectin ligands, which involves adaptor recruitment and the activation of kinases and leads to a reduction of leukocyte rolling velocity. *In vitro*, it was shown that selectin signaling results in myeloid cells responding to significantly low levels of signaling molecules including chemokines. This signaling results in effector cells reacting. The reactions include production of superoxide, synthesis of chemokines, degranulation and production of pro-inflammatory/procoagulation substances. Modulation of selectin-mediated cell adhesion could contribute to cardiovascular diseases including atherosclerosis and arterial and deep vein thrombosis in vivo [75].

5.4 IMMUNOGLOBULIN SUPERFAMILY (CAMs)

These are protein receptors with immunoglobulin (Ig)-like domains, which are found extracellularly, with one transmembrane domain and a tail in the cytoplasm (Figure 4) [76]. The domains are made up of two β-sheets consisting of β-strands, which are antiparallel to each other and kept in place by a disulphide bridge [77]. This class performs functions such as migration and adhesion of leukocytes and immune surveillance. The examples of this class include vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), ICAM-2 and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) [78]. These cell adhesion molecules are Ca^{2+} dependent and participate in both homophilic and heterophilic adhesion. Homophilic adhesion means binding to surfaces of opposite cells with similar structures whereas heterophilic adhesion is when they adhere to integrins and carbohydrates [79].

5.4.1 VCAM-1

VCAM-1, also known as cluster of differentiation 106 (CD106) is a membrane protein cell adhesion molecule, which is expressed upon endothelial stimulation by mediators like ROS and cytokines. When stimulated, VCAM-1 triggers signal transduction of the and participate on leukocyte transmigration. This process consequently results in an inflammatory state. The inhibition of signal transduction through VCAM-1 and VCAM-1-leukocyte inhibition both prevent the occurrence of an inflammatory state. Antioxidants inhibit VCAM-1 signal transduction and inflammation dependent on VCAM-1 hence their possible use in inflammatory diseases [80].

5.4.2 ICAM-1 AND ICAM-2

ICAM-1, also known as CD54, and ICAM-2 which is also known as CD102, are type one transmembrane glycoproteins. They bind to integrins. They participate in leukocyte migration across the endothelium. Only ICAM-1 participates in signal responses and inflammatory processes. ICAM-2 on the other hand does not appear to be important during the inflammation process [81].

5.4.3 MAdCAM-1

Mucosal addressin cell adhesion molecule-1 is an endothelial cell adhesion molecule that attracts leukocytes to mucosal as well as inflamed tissues. It is related to ICAM-1 and VCAM-1. MAdCAM-1 is expressed mostly by endothelial cells in venules in places of tissue infiltration by lymphocytes in mucosal murine lymphoid tissues and also in lamina propria. It binds lymphocytes for both mouse and humans that express the alpha 4 beta 7 integrin. In humans, the MAdCAM-1 RNA is restricted to the spleen, mucosal tissues and lymphoid tissues associated with the gut. Additionally, it selectively binds lymphocyte cell lineage for both the murine and humans, with alpha 4 beta 7 expression. Macaque and human

MAdCAM-1 possess two Ig-like domains similar to the two binding domains on the integrin amino-terminal of the murine MAdCAM-1 [82].

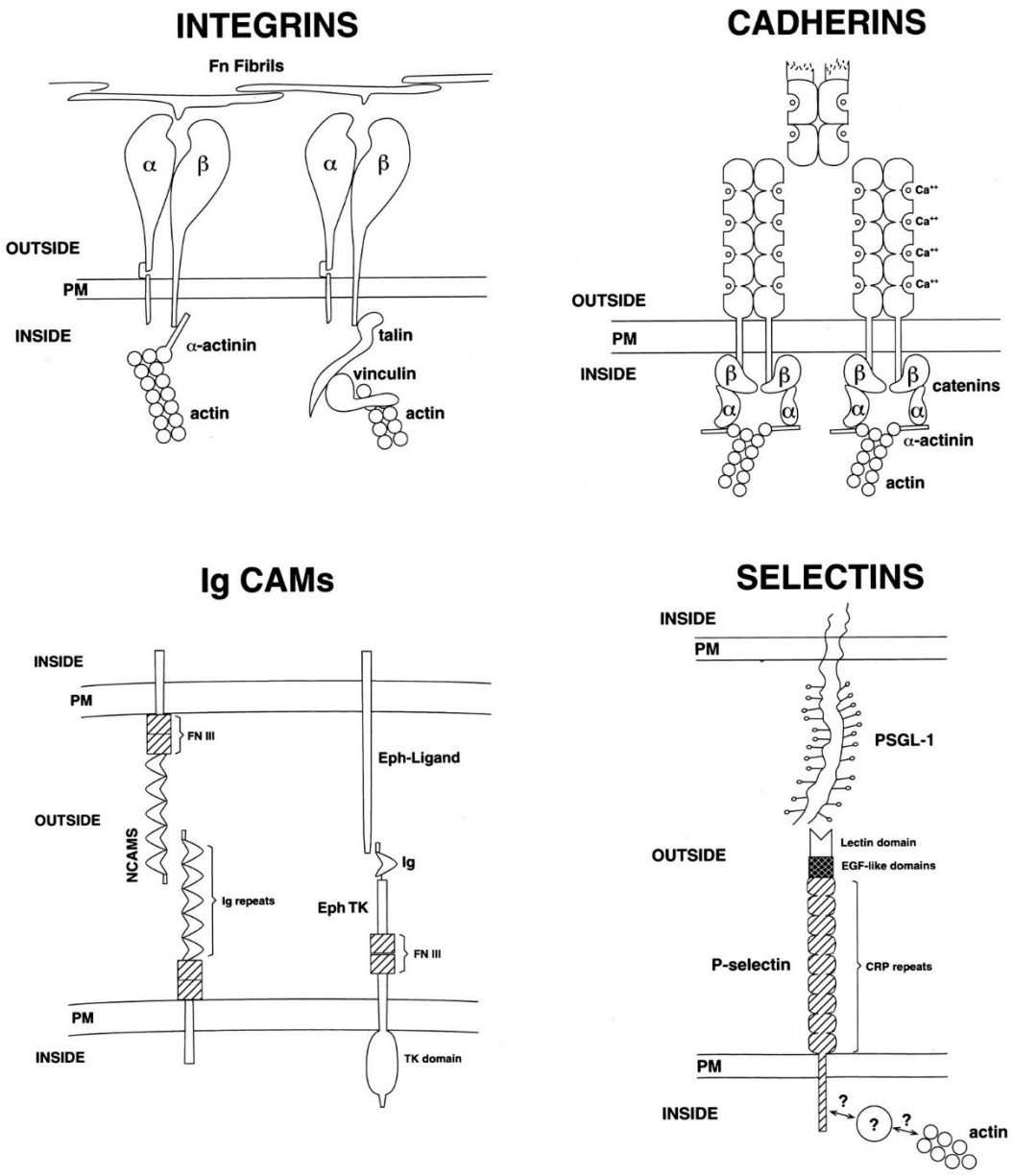

Figure 4: Shows the families of cell adhesion molecules, their structures and an indication of how they interact with their ligands.

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6 SUMMARY OF MOUSE MODELS OF ATHEROSCLEROSIS

Atherosclerosis is a chronic and inflammatory condition characterized by plaque build-up in arteries. The accumulated plaque hardens overtime, narrowing the lumen of the artery. This limits supply of oxygenated blood to the heart and other parts of the body. Atherosclerosis precedes very serious and potentially fatal complications like heart diseases and stroke, or even death. Vascular cells and white blood cells both participate in atherogenesis. Genetics, lipoproteins and the state of arterial blood flow influence atherogenesis. Hemodynamic environment of the arterial vessel and hypercholesterolemia are the two main factors contributing greatly to atherogenesis [83]. The chronic state can turn into an acute state upon plaque rupturing.

Almost 50% of deaths in the western societies are caused by atherosclerosis as a predecessor of fatal conditions like heart diseases and stroke. A number of genetic and environmental risk factors have been associated with atherosclerosis. In a quest to understand the ethiopathophysiology of the disease, animal models which have been genetically modified to present with the disease have been used and this gave a better understanding of the disease and possible therapeutic interventions. The elderly are at a higher risk of developing the disease [6].

Different animal models have been used in research but none of them is ideal because of interspecies differences. The results obtained from such studies are extrapolated to humans. This might not be accurate but it is very useful. Animals used include rats and mice and larger animals like rabbits and pigs. It is advantageous to use mice as it is possible to regulate their cells or proteins by either turning them on or deleting them in a temporal or tissue specific way [83]. This review will look at the use of mice models in atherosclerosis studies.

The mostly used in atherosclerosis studies is mice, despite differences in the factors that may lead to the development of atherosclerosis between mice and humans. These two species also show differences in the localization of lesions as the lesions are usually in peripheral vessels, carotids and coronary arteries in humans and in the innominate artery, aortic arch and aortic root in mice. However, some other features critical for the development of atherosclerosis are a common factor. Advantages of using mice include low maintenance as it is cheap to buy and maintain them, easy to breed and genetically manipulate, small consumption of materials as they are small in size and the ease of use in atherogenesis studies as they develop atherosclerosis over a short period of time. There is a large number of inbred strains of mice showing different susceptibility to atherogenesis and these can be used to identify genes showing different degrees of susceptibility to atherogenesis. This could be to find different genes' sensitivity or resistance to the development of atherosclerosis, mostly after the mice models have been genetically modified to have atherosclerosis [84-86].

Murine models of atherosclerosis are made by hypercholesterolemia that is not based on HDL (high density lipoprotein) by genetically ablating LDL (low density lipoprotein) receptor (LDLR) or apoE. The absence of LDLR in humans has been associated with familial hypercholesterolemia which is a risk factor for cardiovascular diseases [87]. Arterial lesions are present in humans with familial hypercholesterolemia and possess similar characteristics as lesions in mice. An unstable atherosclerotic plaque, which is often cause of acute clinical cardiovascular events is usually absent in murine models. Atherosclerotic lesions in humans have thick and fibrous caps and this feature is absent in murine models. Additionally, human lamellae in arterial media are big enough with the medial vasa vasora observed in large arteries whereas mice have small lamellae and no vasa vasora. As already stated, in humans, atherosclerosis develops mostly in arteries, whereas in mice it is more prominent in the aortic root which is likely a consequence of their notably rapid heart rate which leads to the

disturbance of blood flow. The wild type mice have HDL as their primary lipoprotein and this might result in them showing resistance to atherogenesis even in strains of the wild type mice manipulated to be susceptible. The small size of mice and their vessels is one of the limitations during their use in the study of atherosclerosis. Finally, cholesteryl ester transfer protein (CETP), a plasma protein with potential atheroprotective properties in humans, is absent in mice [88].

7 IMMUNOHISTOCHEMICAL METHODS

7.1 BASIC PRINCIPLES OF IMMUNOHISTOCHEMISTRY

Immunohistochemistry (IHC) is a technique used for endoglin detection in tissues. It is a procedure that uses antibodies to detect antigen distribution or localization in sections of tissues by way of immunological and chemical reactions. This procedure is very specific and sensitive. A large number of various antigens from various species can be detected through this process. It can be used to analyze both physiologic and pathologic tissue depending on the aim of the researcher [89, 90].

This technique is most important for pathological diagnostic procedures. Among other conditions, it is used in the diagnosis of cancer as specific tumor antigens tend to be more expressed or up-regulated. This technique gives a much greater insight, for instance, tissue examination in autopsy pathology is enough, but IHC use gives more insight. IHC can also be used in the estimation of time since death in the early post-mortem stages with different hormones showing positive results up to a certain time period. For example, glucagon cannot be stained 14 days and insulin 29 days after death. Proteins are degraded after death and this results in them showing reduced staining capacities [91]. IHC is very important in research and diagnostic laboratories.

Sample tissue is necessary to carry out this procedure which is then incubated with its corresponding antibody. Different types of microscopes are used to visualize the antigens where the antibodies are bound. Colloidal gold, fluorescent dye, radioactive substances and enzymes can be used. These can directly bind the primary antibody and in some cases, they bind secondary antibodies. Either a light microscope or an electron microscope can be used for visualizing.

The first IHC study was documented in 1941 where the localization of pneumococcal antigens in infected tissue was observed using a fluorescent dye. Enzyme labels like alkaline phosphate [92], peroxidase [93] and colloidal gold [94] were introduced. Colloidal gold is useful in both the electron and the light microscopes. Autoradiography visualization can also be used following the use of radioactive elements. The main goal of IHC is to use minimum amount of antibody and also carry out the procedure with minimal tissue damage [89].

7.2 EnVision™ SYSTEM

This is a staining procedure composed of two 2 steps. The first step is the primary antibody and the second step comprises of the sequential polymeric conjugate steps (Figure 5). On the latter step, great amounts of peroxidase and the secondary antibody are used. The enzyme and the secondary antibody bind to a dextran backbone which can hold up to 100 of the former and 20 of the latter. This technique can be used in the analysis of both the normal and pathological tissues. It is very sensitive, hence the use of very dilute primary antibodies. This makes it cheaper to use. Other advantages include prevention of background staining, preventing cross reactions that are unexpected and nonspecific bonding which is the case with polyclonal antibodies. Additionally, this technique requires less assay time. It is used in research and for daily analysis routines.

The procedure is slightly different depending on what tissue is being investigated. For example, for a bone marrow sample, it was put in 10% buffered formalin for 2 ½ hours, then washed in 70% alcohol for ½ an hour, ethylenediaminotetracetic acid (EDTA) bisodic salts were then used to decalcify for 2 ½ hours. The sample was once again washed with 70% alcohol and a VIP 2000 machine with paraffin embedding at 56^0C was used to process it. The final step is common for all tissue samples [95].

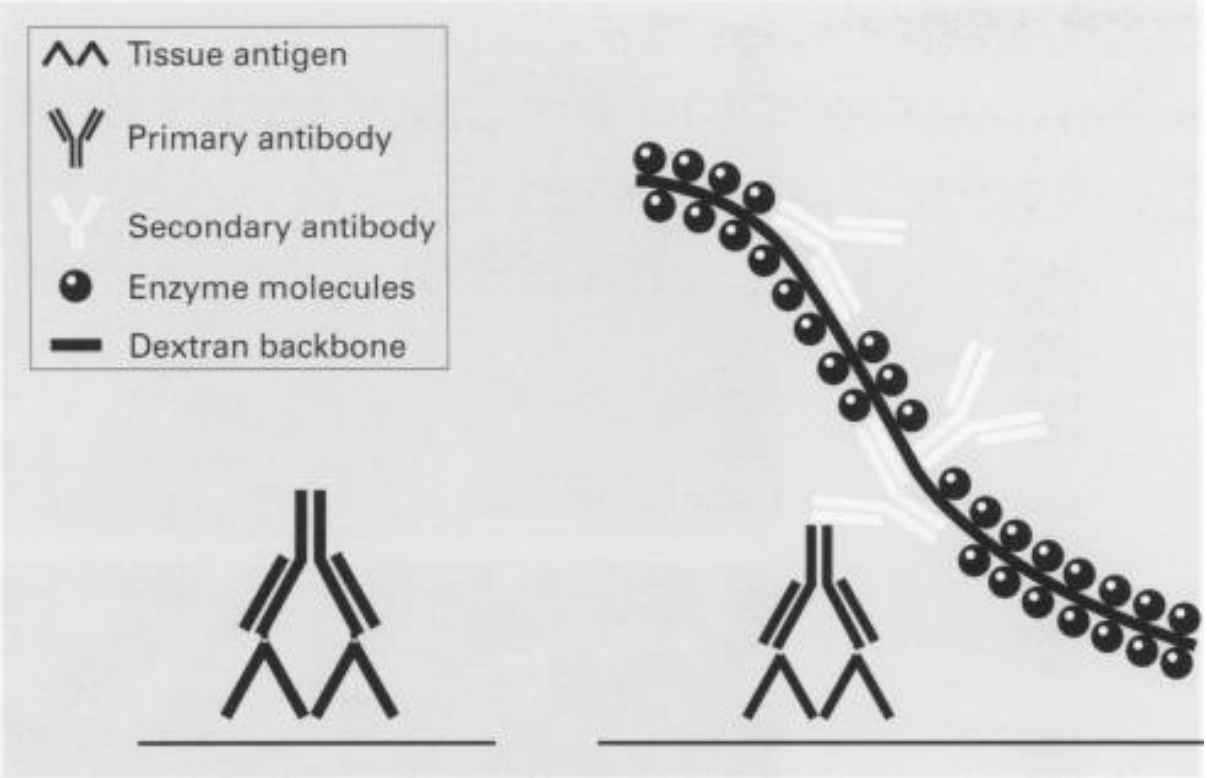

Figure 5: A schematic diagram showing how the EnVision™ system works.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC500802/figure/F1/

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7.3 ImmPRESS™ POLYMER DETECTION SYSTEM

This review is going to be based on this method. Unlike the EnVision™ method, this method proceeds in one step. It shows a higher quality of reaction intensity, it costs less and is generally better than the EnVision™ system. Both ImmPress™ and EnVision™ are non avidin-biotin techniques. In ImmPressTM, reporter molecules like peroxidase and secondary immunoglobulins attach to an inert backbone of a polymer [96]. This system uses tissues that have been formalin fixed and paraffin embedded and with the antigen to be investigated [97]. When working with tissues with a high activity of endogenous avidin-biotin, it is necessary to block the activity by the use of alkaline buffers, non-fat dry milk or preincubating the sections of tissues to be investigated with unlabelled biotin and avidin [96]. Commercial premade blocking preparations are also available which help save time. ImmPress™ is also preferred due to the fact that it showed relatively lower background staining, even though that might be dependent on the type of antibodies used, whether monoclonal or polyclonal. The latter antibodies have interestingly shown more background than the former [97].

7.4 AVIDIN-BIOTIN COMPLEX (ABC) METHODS

This is an indirect method that uses avidin, which is a large glycoprotein with four binding sites and high affinity for a low molecular weight vitamin, biotin, which has only one binding site for avidin. This method is relatively cheaper compared to the ImmPress™ and the EnVision™ methods. The other sites in biotin bind macromolecules like enzymes, antibodies or a label. Avidin and biotin form a strong bond between each other. Many variations of this IHC can be applied, but they all revolve around the same principle, which is to have a primary antibody attached to an epitope of an antigen in a cell (Figure 6). The complex of the cell and the primary antibody is then incubated with a secondary antibody which has an indicator necessary for visualization attached to it. The aim is for the antibody to recognize and bind its antigen on the tissue being examined. A positive result shows a gold

or brown staining of the product. This technique has non-specific background reactions which occur normally as a result of harsh antigen retrieving processes. This is a result of avidin binding to tissue lectins via electrostatic bonds as it has a pI of 10, or via its carbohydrate groups. The use of streptavidin can result in the reduction of the background as the only possible background that could result from using this method will be due to endogenous biotin. This is particularly likely to occur in tissues from the liver and kidney that are rich in biotin. The background reactions are the reason why non avidin-biotin polymer detection systems were developed. Avidin and labelled biotin are allowed to stand for thirty minutes before their application. This allows for the formation of a big complex. Another type of ABC method often used is the labelled avidin-biotin (LAB) or labelled streptavin-biotin (LSAB). The latter utilizes a secondary antibody that has been biotinylated and an alkaline phosphatase or a peroxidase (enzyme) labelled avidin as a third reagent. This method is much more sensitive than the standard ABC method [96, 98].

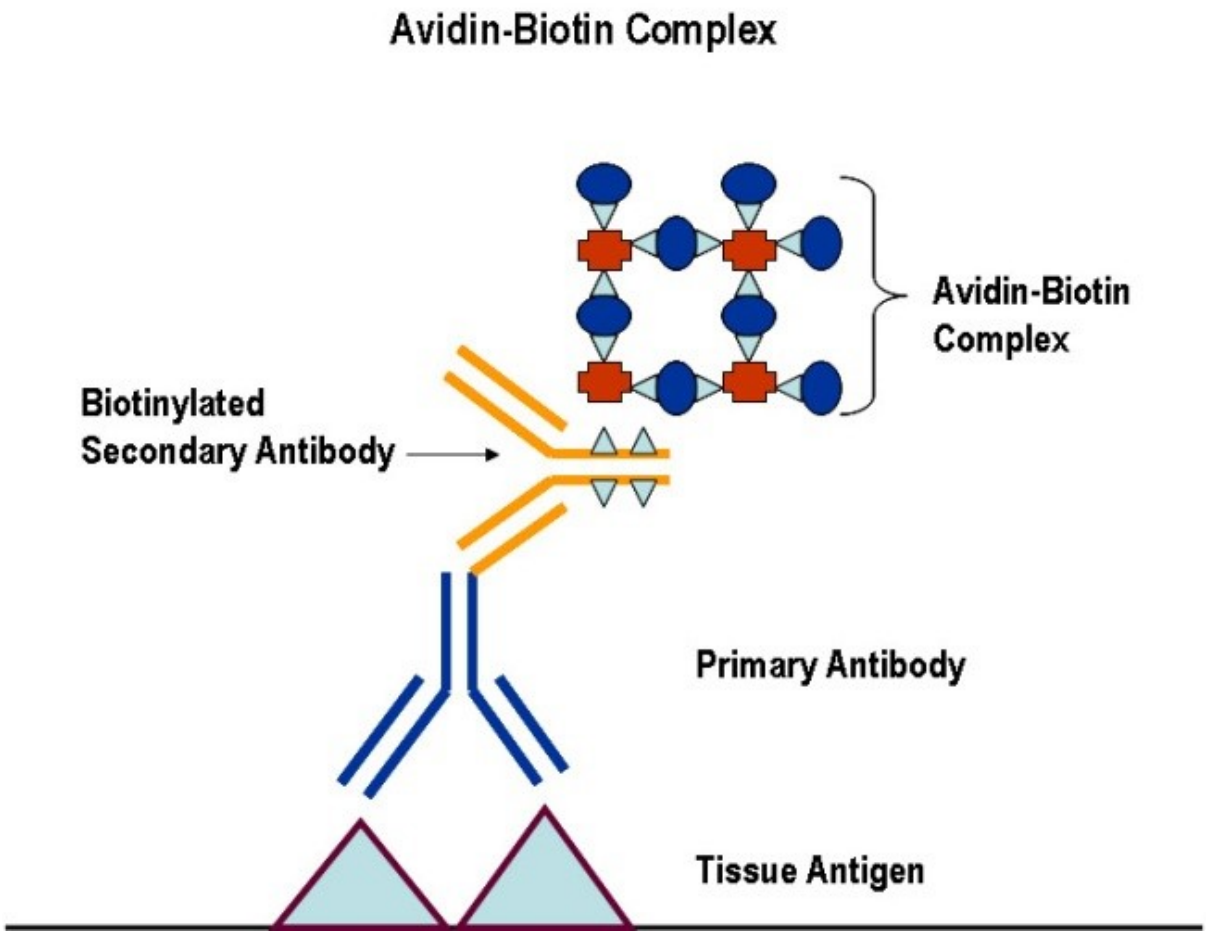

Figure 6: A schematic representation of how the Avidin-Biotin Complex (ABC) works.

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8 ENDOGLIN

Endoglin (TGF-β receptor III or CD105) exists in two forms named soluble form and the membrane form. It is a homodimeric transmembrane glycoprotein that functions as a regulator of TGF-β signalling. Membrane endoglin is expressed in various tissues whereas soluble endoglin, known as the full length extracellular domain of the membrane form is found in the plasma of both healthy people and those suffering from various cardiovascular events, particularly those involving atherosclerosis. High levels of endoglin have been found in pathological cases involving the vascular endothelium. Endoglin could possibly be a biomarker of the development and progression of cardiovascular events and possibly contribute to endothelial dysfunction as well [1, 5].

8.1 ENDOGLIN STRUCTURE

As already mentioned, endoglin is a transmembrane, homodimeric glycoprotein and has an extra-cellular domain made up of 561 amino acid residues. It also has a cytoplasmic tail rich in threonine or serine with 47 amino acid residues located near the transmebrane area. Based on its structure, endoglin is a member of the *zona pellucida* (ZP) family, which is a family of extracellular proteins with a common ZP domain made up of about 260 amino acids [99]. Endoglin amino acid arrangement shows some consensus motifs. The ZP extracellular domain has an RGD (arginine-glycine-aspartic acid) tripeptide, which is a prototypic family member in a family of motifs participating in integrin-based binding with other proteins [100]. There is also an orphan domain, which is found right next to the ZP domain, located at the amino protein terminus [101]. Both the orphan and the ZP domains are extracellular. The orphan domain binds the TGF-β super family. Endoglin is a supplementary TGF-β receptor [102]. Also present are four N-linked and some O-linked sites of glycosylation. The latter, found near the transmembrane area is the part rich in threonine and serine residues [103]. Since the endoglin extracellular domain is made up of a consensus ZP

domain, this thus suggests a critical role played by this domain in the overall functions of endoglin.

8.2 ENDOGLIN AND HEREDITARY HEMORRHAGIC TELANGIECTASIA TYPE 1

Hereditary hemorrhagic telangiectasia type-1 (HHT Type 1) is a hereditary disorder of blood vessels characterized by recurrent, severe mucocutaneous telangiectasia, nose bleeds and sometimes different serious arteriovenous malformations (AVM) which affect various organs like the liver, gastrointestinal tract, brain and lungs [104]. Telangiectasia of the gastrointestinal tract occurs more often and could lead to severe bleeding. HHT type 1 results as a consequence of mutations on chromosome 9, which is the chromosome that codes for endoglin. The mutated HHT genes encode the TGF-β superfamily proteins which are responsible for signaling in endothelial cells of blood vessels [105].

Endothelial cells show a predominant expression of endoglin and this implies that HHT pathogenesis starts with endothelial dysfunction. A study using mice was carried out in which targeted endoglin disruption resulted in embryo lethality [106]. Heterozygous mice ($Eng^{+/-}$) developed vascular lesions that were HHT-like of which severity, location and age of onset were unpredictable, which is the same as what is observed in HHT cases in humans. Histopathological features observed on the lesions included hemorrhage, dilated vessels with thin walls and fibrosis. The mice with $Eng^{+/-}$ had vascular walls abnormalities, dilated post-capillary venules and roughly 70% of their blood vessel walls had no smooth muscle cells [107].

Despite the knowledge on genes that cause HHT for over a decade now, the mechanism of HHT pathogenesis still remains unknown. Bourdeau et al. and Kang et al. hypothesized that other factors like environmental factors or modifier genes may influence the occurrence

of AVMs [108, 109]. A relationship between HHT and an imbalance in the proangiogenic factors (VEGF) and antiangiogenic factors has been proposed. This data suggests that therapies against angiogenesis may be of benefit in HHT as they target blood vessels with abnormalities, which contribute to HHT pathogenesis [110].

8.3 ENDOGLIN AND PREECLAMPSIA

Preeclampsia (PE) is a pathological condition affecting multiple systems and is characterized by hypertension development and proteinuria. It happens after the twentieth week of pregnancy in women who have otherwise been normotensive. This condition can also occur in women with pre-existing hypertension as well [111, 112].

The placenta appears to play a significant role in PE development. Insufficient placentation process leads to a change in the levels of angiogenic factors like the placental growth factor (PGF), sEng, the soluble type of vascular endothelial growth factor receptor type-1 (sFlt-1) and vascular endothelial growth factor (VEGF) [113]. PE has been divided into early and late PE, with regard to gestational age (GA). Early PE is at GA 34 weeks of pregnancy whereas late PE is GA≥34 weeks [114]. One other way to classify PE is with regard to the disease severity and this comprises the mild and the severe forms. The latter is characterized by blood pressure of >160/110 mmHg and proteinuria levels of ≥ 2mg/24h. It is suggested that each form of PE has its own distinct etiology.

PE development seems to be centralized on the placenta. This condition has been observed in cases where the placenta was present, but with no fetus, and in case of the presence of placental fragments still present in the uterus after delivery (improper removal of the placenta). Significant symptom improvement was observed upon the end of pregnancy and complete withdrawal of the placenta [112]. A vascular network is formed during pregnancy that maintains proper flow of blood between the mother and the fetus. Sequential

mechanisms of angiogenesis, vasculogenesis and pseudovasculogenesis are involved in this process [115].

A fault in the vascularization process leads to the production of antiangiogenic agents, which will be secreted into maternal circulation, consequently leading to endothelial dysfunction [116]. It is suggested that PE develops as a consequence of an imbalance between pro and anti angiogenic factors [117-119]. Vascular invasion is reduced in PE, reducing perfusion of the placenta and hence causing chronic hypoxia and restricted intrauterine growth [120]. Low oxygen levels result in the placenta producing antiangiogenic factors like sEng, sFlt, TGF-β1 and TGF-β3. These agents then cross to maternal circulation and result in endothelial dysfunction, proteinuria and hypertension.

Higher levels of sEng, which is found in abundance in the human placenta, were observed in women with preeclampsia as compared to normotensive pregnant women. Patients with severe PE also had higher sEng levels compared to those with mild PE. Additionally, sEng showed a correlation with markers of inflammation like TNF-α receptors even though it was found to be independently connected to the severity of the disease [121]. Levels of sEng vary depending on the stage of preeclampsia and this suggests that it could be used to predict PE. Finally, there are currently no successful therapeutic interventions for PE as the only available intervention is the interruption of pregnancy and complete removal of the placenta [113].

8.4 ENDOGLIN AND CANCER

As previously mentioned, endoglin participates in angiogenesis and its expression is up-regulated in proliferating endothelial cells and in case of hypoxia. Solid cancers need angiogenesis to grow and survive [122, 123]. They develop in two phases being the vascular phase and the avascular phase. The avascular phase, as the first phase of tumor development,

is characterized by tumor cells obtaining their nutrients and oxygen by simple diffusion. Solid cancer cells' progressive growth requires continuous angiogenesis, a process where new blood vessels are continuously formed. The angiogenesis process is necessary for tumor growth and metastasis. Therefore, the inhibition of angiogenesis in tumors is a potentially effective therapeutic intervention in the management of solid cancers. Generally, angiogenesis in adults is associated with pathological states like tumor growth, except in case of wound healing and ovulation [2].

Bevacizumab, a monoclonal antibody, is an antiangiogenic agent that has been used in cancer treatment. It works by inhibiting the proangiogenic factor VEGF in tumor cells. When used in conjunction with chemotherapy regimens, bevacizumab prolonged survival in patients with non-small-cell lung cancer and advanced colorectal cancer [124, 125]. Resistance often happens in the VEGR based cancer therapy pathway. Endoglin is the main angiogenic agent implicated in VEGF resistance. VEGF inhibitors can be used in combination with antiangiogenic agents targeting mainly endoglin to manage cancer [126].

Unsurprisingly, the use of anti-endoglin antibody to inhibit endoglin activation works synergistically with TGF-β in the inhibition of endothelial cell growth [126]. Just like any other new vasculature formation, tumor vasculature formation requires endoglin expression. Targeted endoglin inactivation leads to the development of a defective vasculature, leading to the death of mice at gestation day 10.5 due to the absence of angiogenesis [127]. Since endoglin is down-regulated in case of hypoxia, this leads to apoptosis of the hypoxic endothelium [128].

Endoglin expression is used in the prognosis of patients with solid tumors. Levels of sEng vary with each stage of the disease. Inhibition of VEGF led to an up-regulation of the expression of sEng in tumor cells, ensuring their continued growth [2].

8.5 ENDOGLIN AND ATHEROSCLEROSIS

8.5.1 ENDOGLIN EXPRESSION IN MICE AND HUMANS

Endoglin is expressed in normal tissue and in high levels in the presence of vascular endothelium related pathological states like inflammation. In atherosclerosis, endoglin was found predominantly in endothelial and smooth muscle cells in different types of blood vessels in both mice and humans. It is also found to be highly expressed in tissue undergoing angiogenesis or remodeling of blood vessels [1, 5]. High levels of endoglin have also been observed in cancer cells [129]. Islets mesenchyma stomal cells and endothelial cells express endoglin in islets of both mice and humans [130].

8.5.2 ENDOGLIN AND ITS RELATION TO eNOS FUNCTION AND FUNCTION OF THE ENDOTHELIUM

It has been reported that endoglin regulates the expression of endothelial nitric oxide synthase (eNOS). Endoglin deficient mice ($Eng^{+/-}$) show reduced levels of eNOS due to its (eNOS) reduced half-life. Endoglin therefore has a stabilizing effect on eNOS. Mice with endoglin deficiency tend to show a decreased NO synthesis and/or decreased eNOS levels. Low levels of eNOS result in decreased vasodilation. It was suggested that eNOS uncoupling results as a consequence of endoglin shortage in endothelial cells and is associated with an abnormal Enos/Heat shock protein 90 (Hsp90). Consequently, there is a decrease in NO production and an increase in O_2^- production [131], which is a risk factor for endothelial dysfunction.

9 SOLUBLE ENDOGLIN

This is the form of endoglin found in the plasma with the other form being the membrane endoglin form. Soluble endoglin is a transmembrane glycoprotein that works as a co-receptor for TGF-β1 and TGF-β3 responses modulation [132].

9.1 GENERATION OF SOLUBLE ENDOGLIN

It was proposed that sEng is a product of N-terminal endoglin cleavage cut off at position 586. The chipping is done mostly by membrane type metalloproteinase-14 (MMP-14). This test was done on human umbilical vein endothelial cells (HUVECs). MMP-14 is well expressed in endothelial cells and might play a vital role in the shedding of endoglin in patients with cancer or preeclampsia [133].

MMP-14 role in shedding endoglin was shown after the treatment of oxysterol which, resulted in the activation of LXR transcription factor and MMP-14 expression in placental explants and jar cells. The same study found sEng expressed in high levels in mice which also had a very high expression of MMP-14 [134]. Nonetheless, these results were not confirmed by Brownfoot et al. Even though HUVECs showed an up-regulation of sEng, none was shown by primary trophoblasts. Additionally, HUVECs did not show an upregulation of MMP-14 suggesting that there might be a primary cleavage protease other than MMP-14 [135]. Furthermore, there is currently no data supporting the role played my MMP-14 in cleaving endoglin in atherogenesis and/or hypercholesterolemia.

Cleavage of sEng at position 586 in HUVECs indicates that the whole extracellular domain was cleaved off [133]. Gregory et al. suggested that sEng cleavage in preeclampsia is not in position 586. The endoglin was also identified as having a lower molecular weight [136]. Currently no similar studies have been carried out regarding atherosclerosis, hence the unavailability of data on the structure of sEng cleared off arteries like aorta, which are prone

to atherosclerosis. Also, not fully understood yet is the tissue endoglin chipping position in different diseases.

9.2 SOLUBLE ENDOGLIN AND HYPERCHOLESTEROLEMIA

Hypercholesterolemia is a risk factor for endothelial dysfunction as well as atherosclerosis. Atherosclerotic patients presented with high levels of sEng. According to a study by Blann et al. there is a relationship between the levels of sEng and total levels of cholesterol, but no relationship was found between sEng and other endothelial dysfunction markers like E-selectin [137]. High sEg levels were observed in the early stages of atherosclerosis as a result of endothelial damage and dropped as the state progressed due to an increase in the formation of CD105/TGF-β1 complexes [138].

Patients with familial hypercholesterolemia showed elevated levels of sEng which increased with those of high total cholesterol [7]. Other risk factors for cardiovascular diseases like diabetes mellitus type 2 and arterial hypertension also affect the plasma levels of sEng [139]. Oxidative stress and inflammation play a very important role in the pathophysiology of both endothelial dysfunction and subsequent atherosclerosis. Tumor necrosis factor alpha (TNF-α), which is an inflammatory cytokine, was treated and oxidative stress was also induced by H_2O_2. These both resulted in high sEng levels [140]. A study was conducted using 288 patients with arterial hypertension, diabetes mellitus type 2 and healthy individuals. The results obtained indicated high endoglin levels in patients with arterial hypertension and diabetes mellitus type 2, and also in those with complications of diabetes like retinopathy, as compared to healthy individuals [139].

Heart vessels and coronary circulation were also studied where sEng was supposedly an indicator for oxidative stress, inflammation and endothelial senescence, where membrane endoglin cleavage reflected damage to the vessels which was directly proportional to the

sEng levels [140]. This corresponds to adverse events observed in coronary artery disease patients. Unstable angina pectoris, the morphology of the atherosclerotic plaque, remodeling of the heart after an infarction and acute myocardial infarction were accompanied by high levels of sEng as well [141].

sEng levels may reflect the membrane type endoglin being expressed in the heart. Low sEng levels in patients with the worst prognosis could possibly be due to decreased membrane or tissue endoglin expression, despite the expression of MMPs. This suggests a balanced ratio of some sort between the soluble endoglin form and its membrane form [142]. The same researchers also found out that patients with acute myocardial infarction had low levels of sEng as compared to their healthy counterparts. The low sEng levels could be a result of its participation in the formation of complexes in endothelial dysfunction. Additionally, they suggested that an early drop in sEng levels could be a prognostic tool and marker indicating the early stages of a cardiovascular death event [142]. Therapeutic interventions also resulted in lowered sEng levels. Blood cholesterol levels were lowered, and so was sEng and other endothelial dysfunction biomarkers like hs-CRP in familial hypercholesterolemia patients after LDL apheresis [7]. The levels of sEng dropped because of a decrease in the activity of the immune system and endothelial cells after the elimination of atherogenic elements, and not due to LDL apheresis [7].

Oxysterols lead to an increase in the sEng levels through the stimulation of its release form tissues whereas pravastatin had no effect on sEng levels [135]. No relationship between changes in the plasma endoglin levels and membrane endoglin expression in various specific organs like the aorta has been elucidated yet. This indicates that a direct relationship between the levels of Eng and therapeutic interventions is yet to be carefully studied.

Double knockout mice fed a diet rich in cholesterol showed increased cholesterol levels and sEng levels alike with bigger atherosclerotic plaques. Membrane endoglin expression in these mice aortas was reduced [143]. Atorvastatin was able to lower cholesterol levels, reduce the atherosclerotic plaque size and also decrease soluble endoglin levels while it simultaneously increased membrane endoglin expression levels in the aortas [144].

A study was conducted by [143-146] using atherosclerotic mouse models with the ability to develop spontaneous endothelial dysfunction, hypercholesterolemia and atherosclerosis [147]. These conditions can be stimulated by feeding the mice a diet rich in cholesterol [148]. These mice did not show any correlation between the sizes of the atherosclerotic plaque and cholesterol or sEng levels [5]. Rathouska et al. speculated that hypercholesterolemia in blood vessels could be associated with blood levels of sEng. sEng found in the blood may not be produced by just the aorta as Rathouska et al. also suggested that hypercholesterolemia might induce the cleavage of endoglin from other vessels. Atherogenesis and plaque composition and size in aorta were independent of sEng levels as per the above-mentioned statement [5].

Tissue endoglin is only expressed in endothelial cells of mice [149]. Rathouska et al. therefore suggested that hypercholesterolemia affects sEng plasma levels by endoglin chipping in various vascular endothelial blood vessels only [5].

9.3 SOLUBLE ENDOGLIN AND POSSIBLE INDUCTION OF ENDOTHELIAL DYSFUNCTION

Endothelial dysfunction is a pathological state characterized by a prothrombotic, vasoconstriction and inflammatory state [41]. As mentioned previously it is accompanied by an increase in adhesion molecules expression and a decrease in the amount of the substances promoting vasodilation. There is also an increase in vascular permeability, promoting the

diapedesis of immune cells which causes an inflammatory response to the gradually accumulating cholesterol molecules [150]. sEng is considered as an endothelial damage biomarker and whether or not it has an input in the development of endothelial dysfunction is yet to be established.

Venkatesha et al. suggested that sEng has antiangiogenic effects as it could hinder tube formation. They also administered recombinant sEng to rats, which interfered with the binding of TGF-β1 to TGF-β II receptors, as well as downstream signaling. This resulted in eNOS-dependent vasodilation inhibition in rat mesenteric and micro vessels isolated from the kidney [151]. This suggests that sEng might have hypertensive effects. This observation correlates with preeclampsia, which is also accompanied by endothelial dysfunction, where sEng is used as a biomarker to determine the severity of the disease [151, 152]. Walshe et al. carried out another study utilizing the expression of sEng by adenovirus on mesenteric venules, which resulted in the alteration of the endothelium and impaired vasodilation [153]. Rossi et al. on the other hand proposed that sEng inhibits the adhesion of leukocytes in venules, which is the exact opposite activity compared with membrane endoglin, but with no suggested mechanistic background [154]. Most of the data suggests that sEng could have the potential to induce signs typical for endothelial dysfunction. However, these studies have not shown the effects sEng has on arteries prone to atherosclerosis.

A recent study using a different approach to analyze the role of sEng was carried out on mice showing an overexpression of either human MMP-14 or sEng. Mice with an overexpression of human sEng showed increased systolic blood pressure as compared to their wild type counterparts. On a similar note, mice with overexpressed MMP-14 had a high expression of sEng and high systolic blood pressure relative to their wild type counterparts [134]. The exact mechanism of how high levels of sEng induce hypertension was not established in this review. Notwithstanding the fact that the exact mechanism for

hypertension induction by high sEng is not known nor revealed yet in the current studies, Rathouska et al. speculated that high sEng levels could be working through the interference with the TGF-β/TGF-β II receptor pathway together with the eNOS-dependent vasodilation inhibition that follows on the pathway [5].

Rathouska et al. also carried out another study using a strain of transgenic mice fed a standard diet in the laboratory. The results confirmed an elevated systolic blood pressure in mice with high sEng levels as compared to their littermates with lower sEng levels [5]. They also could not prove any contribution sEng high levels have on the aortic endothelial function alteration, whether at the functional or protein level, suggesting that as a single entity, sEng possibly has no input in the induction and progression of endothelial dysfunction [155].

It appears that sEng has different effects on different parts of the vascular bed including the veins or venules, aorta and muscular arteries. Additionally, Rathouska et al. indicated the necessity to evaluate any additive effects contributed towards membrane function/dysfunction by a combination of several various factors that can each lead to endothelial dysfunction like hypercholesterolemia, in conjugation with high sEng levels [5].

10 CONCLUSION

- Endoglin exists in two forms, being the soluble and membrane forms. Membrane endoglin is chipped to generate sEng. Endoglin is expressed in both normal and pathological tissues, with the latter showing an up-regulation of endoglin expression. In case of endothelial dysfunction and atherosclerosis, endoglin was expressed in all the affected blood vessels, predominantly in the endothelium and smooth muscle cells. Patients with diabetes mellitus type 2 and its complications, and arterial hypertension, which are endothelial dysfunction and consequently atherosclerosis

risk factors, showed high sEng levels in relation to normal people. sEng levels may reflect the expression of membrane endoglin.

- sEng is generated by the cleavage of membrane endoglin on the N-terminal at position 586 by MMP-14. Cleavage of membrane endoglin was shown to lead to blood vessel damage which was directly proportional to sEng levels. This suggests that high sEng levels cause endothelial damage. The above data also suggests an inverse relationship between sEng and membrane endoglin.
- sEng can be used as a biomarker for endothelial dysfunction and atherosclerosis as suggested by several data. High levels of sEng were observed in patients with atherosclerosis and its risk factors. The early stages of atherosclerosis showed high levels of sEng, which dropped as the state progressed, most likely due to sEng participation in forming the CD105/TGF-β1 complexes. It can also be a prognostic tool in predicting early stages of cardiac death.
- Endothelial dysfunction could result due to high levels of sEng which inhibits eNOS dependent vasodilation. This leads to hypertensive effects and consequently endothelial dysfunction.

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