CHARLES UNIVERSITY

FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

DEPARTMENT OF BIOLOGICAL AND MEDICAL SCIENCES

DOCTORAL THESIS

POSSIBLE CHANGES OF ENDOGLIN EXPRESSION IN ENDOTHELIAL CELLS

Mgr. Katarína Tripská

HRADEC KRÁLOVÉ, 2022

STATEMENT OF AUTHORSHIP

I hereby declare that I am the sole author of this thesis. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made in the thesis itself. All the literature and other resources from which I drew information are listed in the list of references. This work has not been used to get another or the same title.

In Hradec Králové

Katarína Tripská

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Although this thesis bears my name on the cover, it would not have been possible without the assistance and support of other people. Therefore, I want to express my gratitude to everyone who helped me to get to this point.

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ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Biological and Medical Sciences

Doctoral Degree Program: Pharmacology and Toxicology

Candidate: Mgr. Katarína Tripská

Supervisor: prof. PharmDr. Petr Nachtigal, Ph.D.

Title of Doctoral Thesis: Possible changes of endoglin expression in endothelial cells

Endoglin (Eng) is a co-receptor for ligands of the Transforming Growth Factor β superfamily. Multiple studies have demonstrated that Eng is associated with metabolic syndrome-related pathological conditions, such as hypercholesterolemia, hyperglycemia, hypertension, or obesity. However, the exact role of Eng in these pathologies is still unknown. Therefore, the main purpose of this thesis was to elucidate and summarize the role of membrane and soluble Eng in the selected pathologies associated with metabolic syndrome.

We demonstrated that soluble Eng (sEng) levels, together with plasma lipids and markers of inflammation, were reduced after every lipoprotein apheresis in patients with familial hypercholesterolemia. This result, in combination with knowledge acquired by the review article, indicates that increased sEng levels are associated with cardiometabolic disorders. Regarding the role of membrane Eng, we showed that blockage of Eng expression by TRC105 results in a decrease in hypercholesterolemia- and hyperglycemia-induced Eng expression, followed by inhibition of Eng-mediated signaling and reduced adhesion and transendothelial migration of monocytes. This suggests that the interaction between membrane Eng and monocytes, responsible for adhesion and transendothelial migration of monocytes, might be the potential pharmacological target in diseases associated with elevated levels of glucose and/or cholesterol.

In conclusion, this doctoral thesis helped to extend the knowledge regarding the role of Eng in metabolic syndrome-related disorders. We suggest that sEng can be considered a biomarker of cardiometabolic diseases and that lowering its levels should be considered in patients who are prone to develop these diseases. However, the membrane Eng role is still not fully elucidated, and more studies are necessary to understand all the mechanisms involved in its regulation and function.

ABSTRAKT

Univerzita Karlova, Farmaceutická fakulta v Hradci Králové

Katedra biologických a lékařských věd

Doktorský študijný program: Farmakológia a toxikológia

Kandidát: Mgr. Katarína Tripská

Školiteľ: prof. PharmDr. Petr Nachtigal, Ph.D.

Názov dizertačnej práce: Možné zmeny expresie endoglínu v endotelových bunkách

Endoglín (Eng) je ko-receptor pre ligandy zo superrodiny transformujúceho rastového faktora β. Viaceré štúdie preukázali, že Eng je spojený s patologickými stavmi súvisiacimi s metabolickým syndrómom, ako sú hypercholesterolémia, hyperglykémia, hypertenzia alebo obezita. Avšak presná úloha Eng pri týchto patologických stavoch stále nie je známa. Preto bolo hlavným cieľom tejto práce objasniť a zhrnúť úlohu membránového a solubilného Eng vo vybraných patologických stavoch spojených s metabolickým syndrómom.

Preukázali sme, že hladiny solubilného Eng (sEng), spolu s plazmatickými lipidmi a markermi zápalu, sa po každej aferéze lipoproteínov znížili u pacientov s familiárnou hypercholesterolémiou. Tento výsledok v kombinácii s poznatkami získanými v prehľadovom článku naznačuje, že zvýšené hladiny sEng súvisia s kardiometabolickými ochoreniami. Pokiaľ ide o úlohu membránového Eng, ukázali sme, že blokovanie expresie Eng pomocou TRC105, má za následok zníženú hypercholesterolémiou- a hyperglykémiou-indukovanú proteínovú expresiu Eng, inhibíciu Eng-sprostredkovanej signalizácie a zníženú adhéziu a transendotelovú migráciu monocytov. To naznačuje, že interakcia medzi membránovým Eng a monocytmi, zodpovedná za adhéziu a transendotelovú migráciu monocytov, môže byť potencionálnym farmakologickým cieľom u ochorení spojovaných so zvýšenými hladinami glukózy a/alebo cholesterolu.

Záverom možno konštatovať, že táto dizertačná práca prispela k rozšíreniu poznatkov týkajúcich sa úlohy Eng pri poruchách súvisiacich s metabolickým syndrómom. Navrhujeme, že sEng možno považovať za marker kardiometabolických ochorení a že zníženie jeho hladiny by sa malo zvážiť u pacientov, ktorí sú náchylní na vznik týchto ochorení. Úloha membránového Eng však stále nie je úplne objasnená a na pochopenie mechanizmov jeho regulácie a funkcie sú potrebné ďalšie štúdie.

LIST OF ABBREVIATIONS

ALK	Activin receptor-like kinase
APC	activation of protein C
apoB	apolipoprotein B
$ApoE^{-/-}/LDLR^{-/-}$	Apolipoprotein E and LDL receptor-deficient
ARBs	angiotensin 2 receptor blockers
AT2	angiotensin 2
AVMs	arteriovenous malformations
BMPs	bone morphogenic proteins
CD40L	CD40 ligand
CRP	C reactive protein
CVD	cardiovascular disease
DM	diabetes mellitus
ED	endothelial dysfunction
EMPs	endothelial microparticles
Eng	endoglin
eNOS	endothelial nitric oxide synthase
FH	familial hypercholesterolemia
GC	guanine-cytosine
HDL	high-density lipoprotein
HHT	Hereditary Hemorrhagic Telangiectasia
HMGCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase
HMVECs	human microvascular endothelial cells
hsCRP	high sensitivity C reactive protein
HUVECs	human umbilical vein endothelial cells
ICAM-1	intercellular cell adhesion molecule 1
IL	interleukin
KLF6	Kruppel-like factor 6
LDL	low-density lipoprotein
L-endoglin	long endoglin isoform
LOX	lectin-like oxidized low-density lipoprotein
LXR	liver X receptor
MCP-1	monocyte chemoattractant protein 1
MetS	metabolic syndrome

MMPs	matrix metalloproteinases
NF-κB	nuclear factor kappa B
NO	nitric oxide
NOXs	nicotinamide adenine dinucleotide phosphate oxidases
OD	orphan domain
PAI-1	plasminogen activator inhibitor 1
PCOS	polycystic ovary syndrome
PECAM-1	platelet and endothelial cell adhesion molecule 1
RAAS	renin-angiotensin-aldosterone system
RGD	arginyl-glycyl-aspartic tripeptide
ROCK	RhoA/Rho-associated protein kinase
ROS	reactive oxygen species
S-endoglin	short endoglin isoform
sEng	soluble endoglin
Smad	Suppressor of Mothers against Decapentaplegic
SP	signaling peptide
Sp1	specific protein 1
sP-selectin	soluble P-selectin
TC	total cholesterol
TF	tissue factor
TGF-β	Transforming Growth Factor beta
TNF-α	tumor necrosis factor alpha
VCAM-1	vascular cell adhesion molecule 1
VEGF	vascular endothelial growth factor
VSMCs	vascular smooth muscle cells
ZP	zona pellucida domain
ZP-C	zona pellucida domain closer to the C-terminal region
ZP-N	zona pellucida domain closer to the N-terminal region

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1 Theoretical background

1.1 Endoglin

1.1.1 Expression and structure of membrane endoglin

Endoglin (CD105) is a 180 kDa transmembrane glycoprotein made of two identical subunits bound together by disulfide bonds. Endoglin is a co-receptor for ligands of the Transforming Growth Factor beta (TGF-β) superfamily, and it is expressed predominantly on endothelial cells (1), but has also been detected in vascular smooth muscle cells (2), activated monocytes and macrophages (3), fibroblasts (4), and hepatic stellate cells (5).

Endoglin's extracellular region consists of 561 amino acids, and the transmembrane part is made of 26 amino acids (6). The extracellular region can be divided into an orphan domain (OD) and the zona pellucida domain (ZP) (Fig. 1). OD sequence does not display significant homology to any other protein family, and it is responsible for the binding of TGF-β ligands, such as TGF-β1, TGF-β3, bone morphogenic proteins (BMPs) and activin-A. ZP domain can be further divided into ZP-N (closer to the N-terminal region of endoglin) and ZP-C (closer to the C-terminal region). In the ZP-N domain, at position 399-401 aa, we can find the arginyl-glycyl-aspartic tripeptide (RGD), which can bind to integrins and other RGD-recognizing receptors (6, 7).

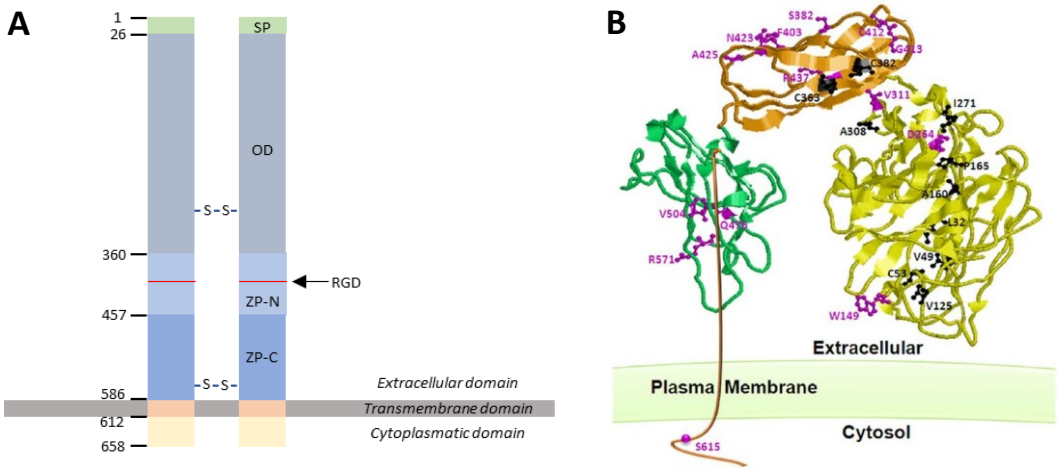

Figure 1. *Endoglin structure. A) Schematic representation of endoglin structure. Endoglin is composed of the extracellular, transmembrane, and cytoplasmatic domains. The extracellular domain can be divided into zona pellucida domain C (ZP-C), zona pellucida domain N (ZP-N), with an Arg-Gly-Asp tripeptide (RGD), orphan domain (OD) and signaling peptide (SP) (picture created by the author). B) 3D structure of endoglin (8).*

Depending on the phosphorylation status and the number of amino acids in the cytoplasmatic domain, we distinguish two different isoforms of endoglin: a long form (L-endoglin) with 47 amino acids and a short form (S-endoglin) with 14 amino acids (9, 10). L-endoglin has been shown to stimulate migration, proliferation, and angiogenesis. On the other hand, S-endoglin has been proposed to inhibit the migration and proliferation of endothelial cells, resulting in endothelial cell senescence. However, the exact role of S-endoglin has not been fully elucidated yet (11, 12). Since L-endoglin is a predominant form and there is insufficient knowledge regarding the role of S-endoglin, in the subsequent chapters of this thesis, we will discuss L-endoglin exclusively and refer to it as endoglin (Eng), as it is done in most of the literature.

1.1.2 Regulation of endoglin expression

In 1998, a genomic DNA clone containing the Eng sequence was isolated in order to understand the mechanisms involved in the regulation of Eng expression. This led to the finding that Eng gene lacks TATA and CAAT boxes but instead contains two guanine-cytosine (GC) rich regions and consensus motifs for Specific protein 1 (Sp1), nuclear factor kappa B (NF-κB), TGF-β- or estrogen-responsive elements, and others (13).

A few years later, another Sp1 site was identified as a critical element for the basal transcription of Eng. Mutation in this sequence or addition of Sp1 inhibitor resulted in the elimination of basal transcriptional activity and the responsiveness of Eng promoter to TGF-β. Moreover, the synergistic cooperation between Sp1, TGF-β, or Suppressor of Mothers against Decapentaplegic 3 (Smad3) on promoter activity was demonstrated (14).

In the meantime, new data about high Eng expression in the neovasculature under hypoxic conditions (such as ischemic tissues or tumors) were discovered, but the mechanisms underlying this upregulation were unknown. Therefore, Sánchez-Elsner et al. investigated the role of hypoxia in Eng regulation. They demonstrated that the Eng promotor is activated under hypoxic conditions, which results in the upregulation of Eng mRNA transcript and Eng protein expression on the cell surface. This led to the discovery of a hypoxia-responsive element in Eng gene that can regulate Eng

expression at the transcriptional level. Since hypoxia and TGF-β signaling pathways are mediated by hypoxia-inducible factor 1 (HIF-1) and Smad proteins, respectively, the involvement of these factors was investigated in combination with Sp1. As a result, multiprotein Sp1/Smad3/HIF-1 complex on the Eng promoter was identified (15).

The fact that Eng and Kruppel-like factor 6 (KLF6) (a nuclear protein that contains three zinc fingers, which are responsible for binding to GC motif in promotors of different genes (16, 17)) are both induced during vascular injury, together with the knowledge that Eng transactivation is dependent on GC boxes, led Botella et al. to examine if there is any connection between them. They were able to colocalize KLF6 and subsequent Eng induction in rats after carotid balloon injury. Furthermore, endothelial denudation of endothelial cells resulted in immediate KLF6 induction, followed by Eng upregulation. Moreover, endothelial cells transfected with KLF6 vector expressed 3.2-fold more Eng mRNA than cells transfected with empty vector. Finally, they demonstrated that KLF6 stimulates Eng promoter activity, which relies on the region that overlaps with the Sp1 site (18). This KLF6-Sp1 cooperation was shown to be enhanced by the TGF-β/Smad pathway, likely through the formation of the KLF6/Sp1/Smad3 complex (19).

It has been demonstrated that KLF6 can also act as a coactivator of nuclear factor kappa B (NF-κB); more specifically, it can interact with p65 in the nuclei and bind to the promoters of p65 target genes (20). However, KLF6 is not necessary for the activation of NF-κB, as other mechanisms can also activate it. NF-κB represents a family of five structurally related proteins, namely: p65 (also called RelA), RelB, c-Rel, p50, and p52 (21). They can be found in the cytoplasm of most cell types, where they are bound to inhibitory proteins named I kappa B. Upon receiving a signal (mediated mainly by the generation of reactive oxygen species (ROS)), I kappa B undergoes degradation, resulting in the entry of NF-κB to the nucleus, where it can bind to DNA and modulate the expression of different genes (22), among others also HIF-1 (23) and Eng (24).

When Henry-Berger et al. were working on identifying novel targets of liver X receptor (LXR) in the placenta to improve understanding of trophoblast invasion, they also discovered that LXR is a transcription factor of Eng. They confirmed it when the treatment of cells by T0901317, an LXR agonist, resulted in a significant increase in

Eng mRNA and protein levels. They also demonstrated that LXR, as a heterodimer with retinoid X receptor, was able to bind to Eng promoter on LXR response element and thus mediate activation of Eng expression (25). Other groups also confirmed the involvement of the LXR pathway in the release of sEng (26, 27).

All this evidence suggests that the regulation of Eng expression is interconnected and involves many different mechanisms and pathways and does not exclude that more mechanisms of Eng regulation are yet to be found.

1.1.3 Endoglin signaling and function

As previously mentioned, Eng is a member of the TGF-β superfamily. TGF-β receptors can be divided into three distinct subtypes: type I receptors, also called activin receptor-like kinases (ALK1-7), type II receptors, and type III receptors - Eng and β-glycan**.** (1, 28).

Even though type I and type II receptors form a functional complex, the type III co-receptor can modulate the transmitted signal (29). In general, the type II receptor transphosphorylates and activates the type I receptor (30-34). Type I receptor can then phosphorylate Smad transcription factors, which transmit signal to the nucleus (35, 36). Eng (an accessory receptor that lacks intrinsic kinase activity and requires the co-expression of type I and type II receptors in order to bind its ligands) can bind to either ALK1 or ALK5 receptor and thus modulate which Smad signaling pathway will be activated (1, 37). The binding of Eng to ALK1 leads to the activation of the ALK1/Smad1/5 signaling pathway, which stimulates the proliferation and migration of endothelial cells (38). Eng binding to ALK5 results in the activation of the ALK5/Smad2/3 signaling pathway that inhibits the proliferation and migration of endothelial cells (39). By this mechanism, Eng maintains the balance between ALK1/Smad1/5 and ALK5/Smad2/3 signaling, therefore, regulating whether endothelial cells are activated or quiescent (Fig. 2) (39).

Eng also plays an essential role in cardiovascular development and vascular remodeling. Specifically, Eng expression is increased during heart development in mesenchymal cells of the atrioventricular channel during heart septation and valve formation in early human development (40, 41). This is supported by the fact that

embryos of Eng-deficient mice die during the 10—11th day of intrauterine development as a result of vascular and heart abnormalities (42, 43).

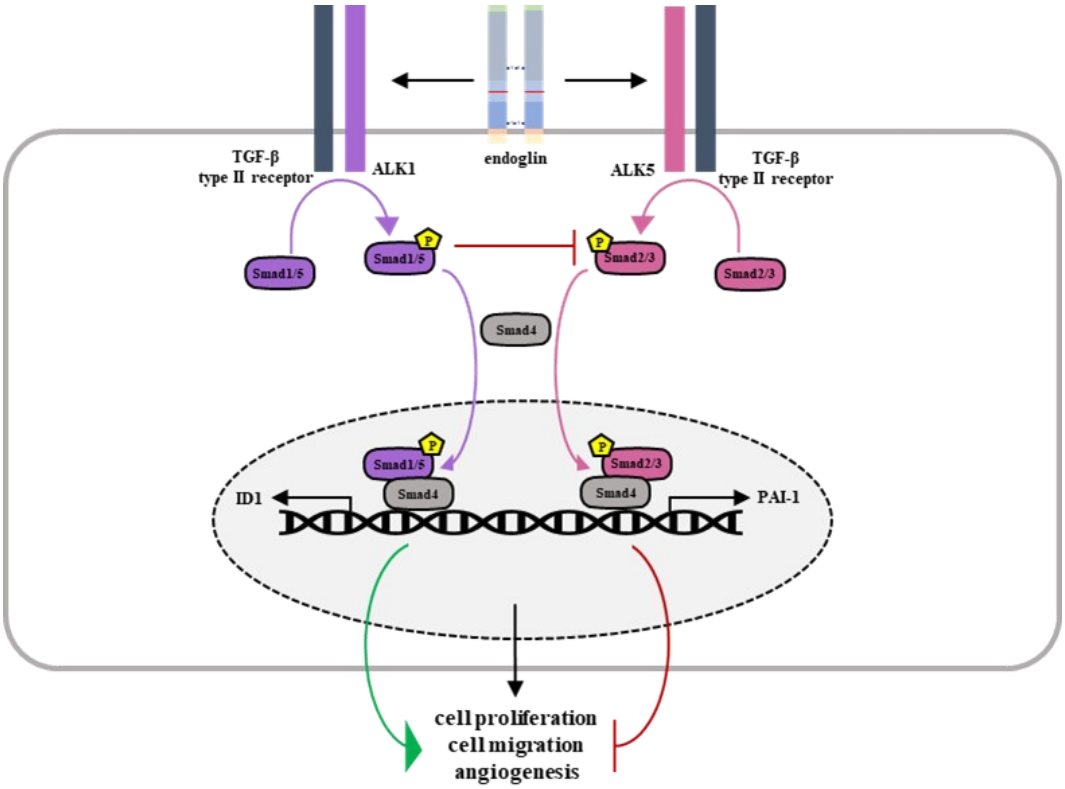

Figure 2. *Role of endoglin in TGF-β signaling. Eng mediates the balance between the activation of ALK1/Smad1/5 and ALK5/Smad2/3 signaling pathways. After Eng binds to the type I TGF-β receptor (either ALK1 or ALK5), respective Smad transcription factors are phosphorylated. Then they create a complex with Smad4 and translocate to the nucleus, where they activate the transcription of different genes. Activation of the ALK1/Smad1/5 signaling pathway results in increased cell proliferation, cell migration, and angiogenesis; meanwhile, activation of ALK5/smad2/3 inhibits these processes (picture created by the author).*

The mutation in the Eng gene is a cause of the specific type of autosomal dominant disease called Hereditary Hemorrhagic Telangiectasia 1 (HHT1) (44). Mutation in Eng prevents downstream signaling and disrupts angiogenesis, promoting dysfunctional remodeling of vascular endothelium. This results in a loss of elasticity in the vessels, which are then permanently dilated (45). Continuous pressure combined with the loss of vascular integrity causes the formation of telangiectasias (dilated microvessels) and arteriovenous malformations (AVMs) (dilated macrovessels) (46, 47). Because of the decreased elasticity and permanent dilatation of the vascular lumen, telangiectasias are fragile and susceptible to hemorrhage (46). Telangiectasias can be most commonly found in the nasal mucosa and oral cavity (47); meanwhile, AVMs can be formed in

the brain, lungs, gastrointestinal tract, or liver (46). Rupture of AVMs may result in severe complications, such as internal bleeding, stroke, seizure, migraine, and brain abscess (48). Untreated AVMs in the lungs and liver can lead to arteriovenous shunting and pulmonary hypertension (49).

1.1.4 Soluble endoglin

Soluble Eng (sEng) is a product of enzymatic cleavage of membrane Eng by matrix metalloproteinases (MMPs). So far, two MMPs have been demonstrated to cleave Eng – MMP14 and MMP12. The cleavage site is located at the aa 586-587, which means that a nearly full-length Eng extracellular domain is released into the circulation (Fig. 3) (50).

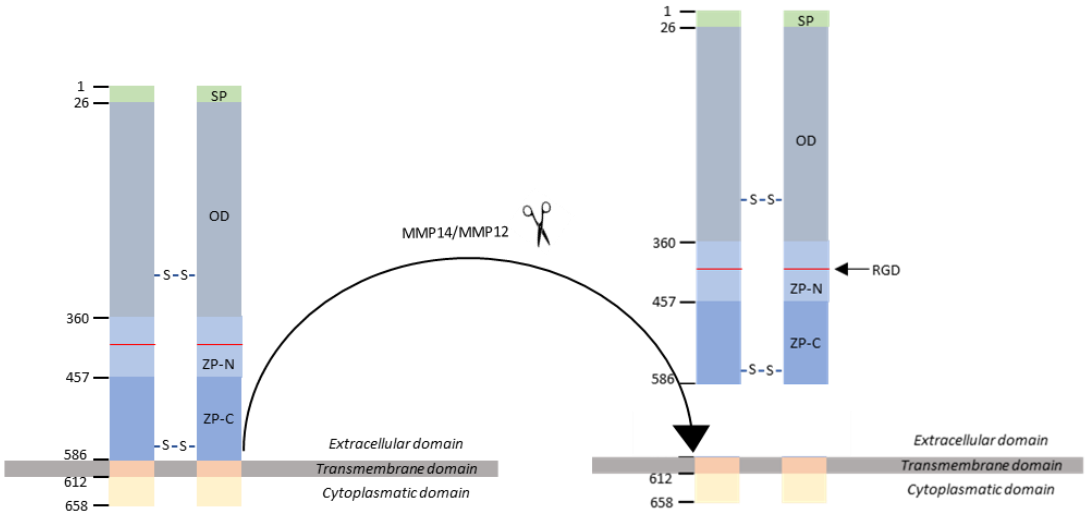

Figure 3. *Generation of soluble Eng. Cleavage of membrane Eng by MMP14 or MMP12 at the position 586-587 aa results in the release of the nearly full-length extracellular domain (sEng) into the circulation (picture created by the author).*

Increased levels of sEng in circulation have been associated with cardiometabolic pathologies such as hypercholesterolemia (51, 52), diabetes mellitus (DM) (53, 54), hypertension (55), or obesity (56). The role of sEng in these processes will be discussed in more detail in the following chapters.

1.2 Endothelium

1.2.1 The physiological function of the endothelium

The endothelium is composed of a single layer of endothelial cells that line the entire inner surface of the cardiovascular system (Fig.4) (57). Endothelial cell structure and functional integrity are crucial in order to maintain proper endothelial function (58).

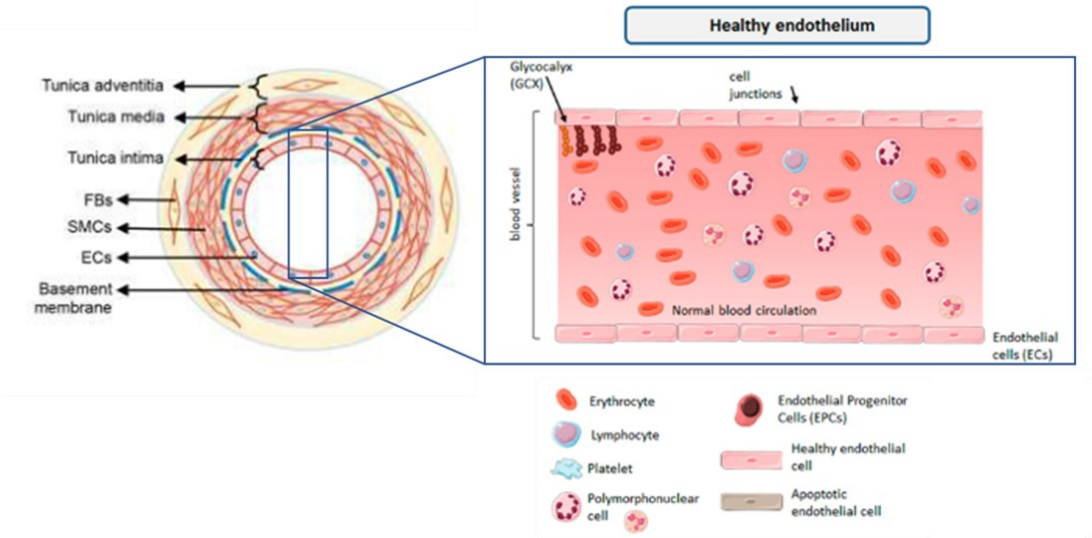

***Figure 4**. Healthy endothelium. The anatomical structure of blood vessels from inside out – tunica intima is composed of a layer of endothelial cells (ECs) on top of the basement membrane; tunica media is made of mostly smooth muscle cells (SMCs), and tunica adventitia consists mostly of fibroblasts (FBs) (59). Healthy endothelium is characterized by endothelial cells with a glycocalyx (GCX), tight cell junctions, and normal blood flow (60).*

One of the endothelium's main functions is to regulate the transendothelial migration of leukocytes via junctions between endothelial cells during inflammation. This happens due to leukocytes interactions with cell adhesion molecules located on the surface of endothelial cells, such as E-selectin, intercellular cell adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) or platelet and endothelial cell adhesion molecule 1 (PECAM-1) (61).

Another crucial function of the endothelium is the regulation of vascular tone. Endothelial cells are able to mediate vascular tone by producing vasodilation and vasoconstriction substances. The release of nitric oxide (NO) plays a key role during the induction of vasodilation (62). Molecules participating in vasodilation include

prostacyclin (63) and endothelium-derived hyperpolarizing factor (64, 65). Endothelial cells also produce vasoconstrictive mediators such as endothelin-1 (ET-1) (66, 67).

In addition, the endothelium is also able to produce a variety of vasoactive substances, making it an endocrine, paracrine, and autocrine organ with numerous functions (68).

Another essential function of the endothelium is to maintain proper hemostasis and, thus, optimal blood flow by regulating the delicate balance between pro-coagulation vs. anticoagulation and prothrombogenic vs. anti-thrombogenic environment (69).

Last but not least important function of endothelial cells is angiogenesis, the process of formation of new vessels from previous endothelium. Angiogenesis is mediated mostly by vascular endothelial growth factor (VEGF), an angiogenic factor produced by many cell types, including endothelial cells (58).

The healthy endothelium functions in quiescent mode when it maintains a relaxed vascular tone and inhibits vascular smooth muscle cells (VSMCs) growth, leukocyte and platelet adhesion and aggregation, as well as thrombosis (66).

1.2.2 Endothelial dysfunction

Endothelial dysfunction (ED) is a condition characterized by the disturbed function of the endothelium, resulting in the transition of endothelium from a quiescent mode to a proinflammatory mode. Proinflammatory mode is defined by increased endothelial permeability, aggregation of platelets, adhesion of leukocytes to endothelium, and production of proinflammatory cytokines (70). The most important hallmark of ED is decreased production and activity of NO, which leads to impaired vasodilation (Fig. 5) (71).

ED is considered an early step in the development of atherosclerosis (72), DM type II, arterial hypertension (73), and other pathologies underlying metabolic syndrome (74), as well as other cardiovascular-related diseases, such as peripheral vascular disease, stroke, venous thrombosis and others (69).

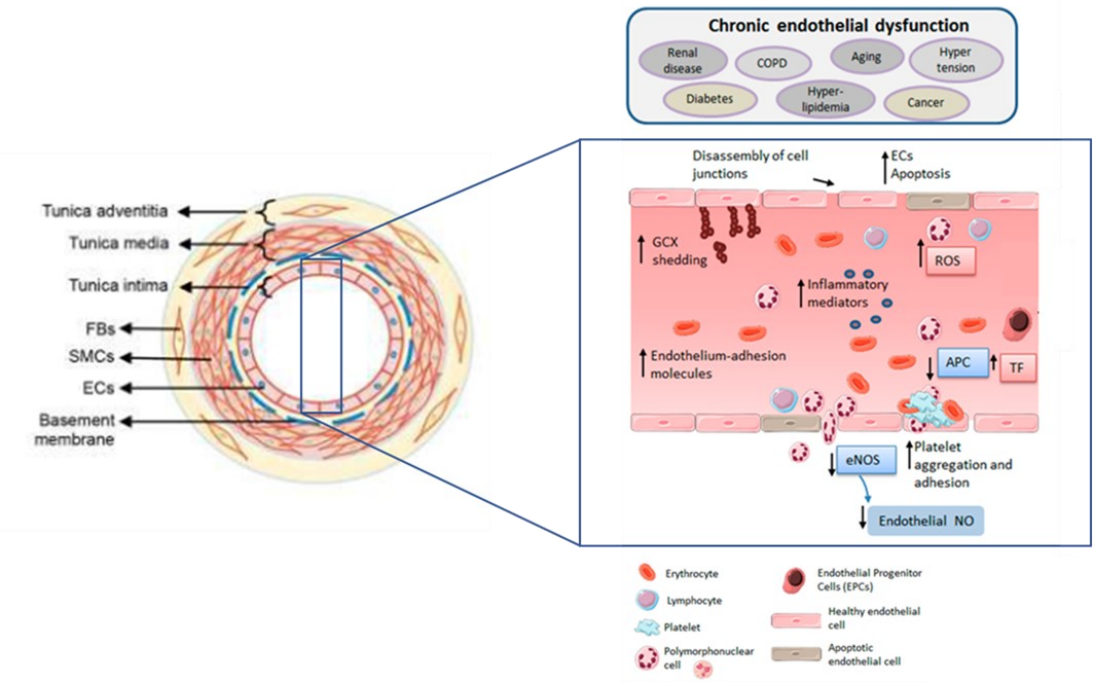

Figure 5. *Endothelial dysfunction. Endothelial dysfunction can be induced by increased oxidative stress/production of reactive oxygen species (ROS) and/or inflammation, which results in the disassembly of endothelial cell junctions, apoptosis of endothelial cells (ECs), increased expression of adhesion molecules, which promotes leukocyte adhesion to endothelial cells, induction of a pro-coagulant and anti-fibrinolytic state with diminished activation of protein C (APC) and increased production of tissue factor (TF), as well as a decrease in the production of nitric oxide (NO) by the endothelial nitric oxide synthase (eNOS) (59, 60).*

1.2.2.1 Biomarkers of endothelial dysfunction

Biomarkers of ED might provide more information about the risk of cardiovascular disease development (75, 76) and search for new biomarkers is very important since ED precedes and possibly stimulates the development of atherosclerotic plaques (71). ED is most often initiated by different cardiovascular risk factors such as hypertension, hyperglycemia, or hyperlipidemia. These lead to increased production of tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), and C reactive protein (CRP). These proinflammatory cytokines bind to their respective receptors, resulting in the activation of the NF-κB, followed by the stimulation of transcription of E-selectin, ICAM-1, and VCAM-1. CRP also down-regulates transcription of endothelial nitric oxide synthase (eNOS) and destabilizes eNOS mRNA, followed by the decrease of NO. Furthermore, the reorganization of actin filaments (through other signaling pathways) leads to the opening of intercellular

junctions, which allows leukocytes to transmigrate into the subendothelial space (Fig. 6) (77, 78).

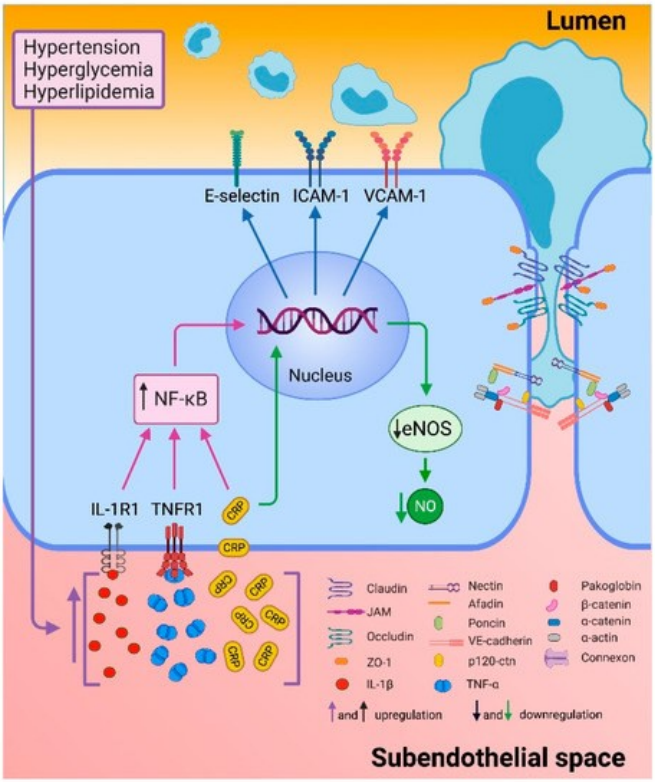

Figure 6. *Biomarkers of endothelial dysfunction. Endothelial dysfunction can be initiated by hypertension, hyperglycemia, and hyperlipidemia, which leads to increased production of interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), and C reactive protein (CRP). These proinflammatory cytokines activate nuclear factor kappa B (NF-κB) that stimulates the transcription of biomarkers of endothelial dysfunction, such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), or vascular cell adhesion molecule-1 (VCAM-1) (79).*

Selectins are responsible for the initial tethering and rolling of leukocytes on the surface of vascular endothelial cells. E-selectin is usually absent in resting endothelium, but it is transcriptionally induced by inflammatory mediators. On the other hand, P-selectin is constitutively synthesized and deposited in secretory granules in platelets, from which it can quickly translocate to the surface upon stimulation with inflammatory mediators (80). The expression of P-selectin was also demonstrated in damaged endothelium of atherosclerotic plaque but not in healthy endothelium (81), which suggests that increased expression of P-selectin can be considered an early indicator of ED.

Another typical hallmark of activated endothelium is increased protein expression of VCAM-1. VCAM-1 is expressed predominantly by endothelial cells activated by proinflammatory cytokines, ROS, or oxidized low-density lipoprotein (LDL) cholesterol (82, 83). Its increased expression was also found in vasculature at the sites, where atherosclerosis is typically present (84). VCAM-1 is the key regulator of leukocyte adhesion and transendothelial migration through interaction with very late antigen-4, also called α4β1 (85).

Despite ICAM-1 being constitutively expressed at low levels on endothelial cells (and other cell types), activation of endothelium by inflammatory cytokines (TNF-α, IL-1, interferon gamma) and ROS can lead to a significant increase in ICAM-1 expression (86). ICAM-1 can also be found in atherosclerotic plaques, where it participates in their progression (87). The soluble form of ICAM-1 was detected in the plasma of patients with cardiovascular disease (CVD), and it also correlated with the severity of the disease (88, 89). Therefore, these findings suggest that both ICAM-1 and its soluble form might be valuable biomarkers reflecting the development of ED and atherosclerosis.

1.2.2.2 Risk factors of endothelial dysfunction

Cardiovascular risk factors, including smoking, hyperglycemia, hypercholesterolemia, hypertension, and aging, have all been implicated in the alteration of endothelial function (Fig. 7) (90-92), and specifically, hyperglycemia, hypercholesterolemia and hypertension have also been shown to affect the expression of Eng and/or sEng.

Hyperglycemia has been shown to activate nicotinamide adenine dinucleotide phosphate oxidases (NOXs) (93, 94), which in turn leads to excess production of ROS (95-97). ROS can catalyze oxidation, which results in decreased levels of tetrahydrobiopterin (a cofactor crucial for proper eNOS function), thus decreasing its availability for eNOS activation (98). This leads to eNOS uncoupling and reduced production of NO (99). Furthermore, NF-κB has been demonstrated to be a direct activator of NOX4 and a stimulus for NOX1 activation (100). Additionally, protein kinase C was shown to be activated by oxidative stress and also to be able to phosphorylate NOX1 and NOX2, thus promoting increased production of superoxide anion (101).

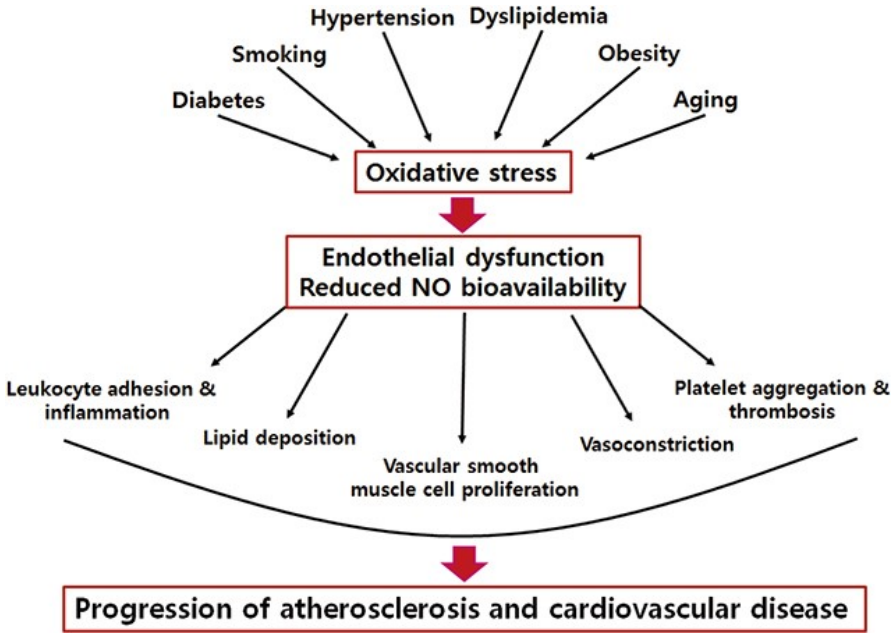

***Figure** 7. Cardiovascular risk factors. Progression from risk factors to atherosclerosis and other cardiovascular diseases is mediated by oxidative stress, followed by endothelial dysfunction characterized by a reduction in nitric oxide (NO) availability (102).*

Hypercholesterolemia, similarly to hyperglycemia, leads to an increase in the production of superoxide anion, therefore resulting in decreased availability of NO, as well as promoting the oxidation of LDL (103, 104). Moreover, asymmetric dimethylarginine, which is an endogenous inhibitor of eNOS, has been found highly increased in plasma of hypercholesterolemic patients (105-107).

There are several mechanisms implicated in the development of ED during hypertension. Primarily, endothelial activation in patients with essential hypertension seems to be activated by the cyclooxygenase pathway, resulting in decreased NO availability via oxidative stress production (108). Another mechanism involved is the initiation of the RhoA/Rho-associated protein kinase (ROCK) pathway. As a result, NO bioavailability is impaired through decreased stability of eNOS mRNA and reduced eNOS phosphorylation via the inhibition of protein kinase B (also known as Akt) (109, 110). Several studies have also shown an interaction between ROS and RhoA/ROCK signaling pathway (111, 112).

1.2.2.3 Role of membrane endoglin in endothelial dysfunction

There is contradictory evidence regarding Eng role in ED. On one hand, multiple studies have demonstrated that Eng participates in integrin-mediated cell adhesion via the RGD peptide located in its extracellular domain. RGD peptide is a recognition motif for integrins that are present on the cell surface of extracellular matrix proteins such as fibronectin, thrombospondin, von Willebrand factor, as well as prothrombin and fibrinogen (113-115). Therefore it is no surprise that Eng has been demonstrated to interact via its RGD motif with integrin α5β1 on leukocytes (24, 116) and mural cells (117), as well as with integrin αIIbβ3 on platelets (118). Numerous lines of evidence support the Eng role in leukocyte trafficking since a) Eng expression is upregulated during inflammation in endothelial cells (119), b) mice lacking Eng show an impaired immune response (120, 121), and c) despite the fact that Eng is present in entire vascular endothelium, its expression is stronger in capillaries (compared to veins or arteries), where most leukocyte infiltration takes place (122).

On the other hand, several studies demonstrated that reduced Eng expression is associated with eNOS-dependent ED (24, 123). For instance, levels of eNOS were significantly reduced in kidneys, femoral arteries, and aortic endothelial cells from $Eng^{+/-}$ mice compared to $Eng^{+/+}$ mice. $Eng^{+/-}$ mice also displayed reduced hypotensive and vasodilatory response induced by acetylcholine and bradykinin, and also urinary and plasma concentrations of nitrites (NO metabolites) were lower in $Eng^{+/-}$ mice (123). Reduced Eng expression accompanied by a decrease in eNOS expression was also demonstrated in HUVECs after stimulation with proinflammatory cytokine TNF-α (124). Furthermore, cells from $Eng^{+/-}$ mice showed a decreased levels of protein stabilizing factors, such as p21 activated kinase 1, VEGF receptor 2, VE-cadherin or Rac family small GTPase 2, and upregulation of destabilizing factors like CD148 and Thrombospondin 1. This leads to increased endothelial permeability and impairment of endothelial barrier function, which was confirmed by increased transmigration of neutrophils through the monolayer of endothelial cells (125).

It has been suggested that these contradictory results might be caused by different Eng roles in microvasculature compared to macrovasculature (126).

1.2.2.4 Role of soluble endoglin in endothelial dysfunction

The role of sEng during ED is no less ambiguous than the role of its membrane counterpart. The above-mentioned publications studying Eng interactions via RGD motif with leukocytes, mural cells, and platelets by Rossi et al., demonstrated that increased adhesion/transmigration could be blocked by the addition of sEng. Since sEng consists of a nearly full-length extracellular domain of membrane Eng, it also contains an RGD motif in its structure, and thus sEng is able to interact with integrins on leukocytes, mural cells, and platelets. Because integrins are occupied by sEng, they will no longer be available for interaction with membrane Eng, and thus will not participate in adhesion and transmigration to endothelial cells (Fig. 8) (116-118).

However, sEng treatment of HUVECs resulted in the activation of interleukin 6 (IL-6) and NF-κB expression, signifying stimulation of proinflammatory phenotype (127). Another study on primary placental and endothelial cells showed that oxysterols are able to induce sEng levels (26). Additionally, mice that were pretreated with adenovirus expressing sEng exhibited increased capillary permeability in the lungs, liver, and kidney. Furthermore, the administration of recombinant sEng to rats resulted in an inhibition of eNOS-dependent vasodilatation (128). Another study with sEng adenovirus showed that increased sEng levels lead to induced expression of P-selectin and leukocyte rolling to the endothelium, elevated levels of soluble E-selectin and soluble VCAM-1, as well as impaired vasodilatation (129). Moreover, many studies demonstrated increased levels of sEng during ED in various cardiovascular-related pathologies, such as atherosclerosis (52, 130), familial hypercholesterolemia (51), hypertension, and DM (55).

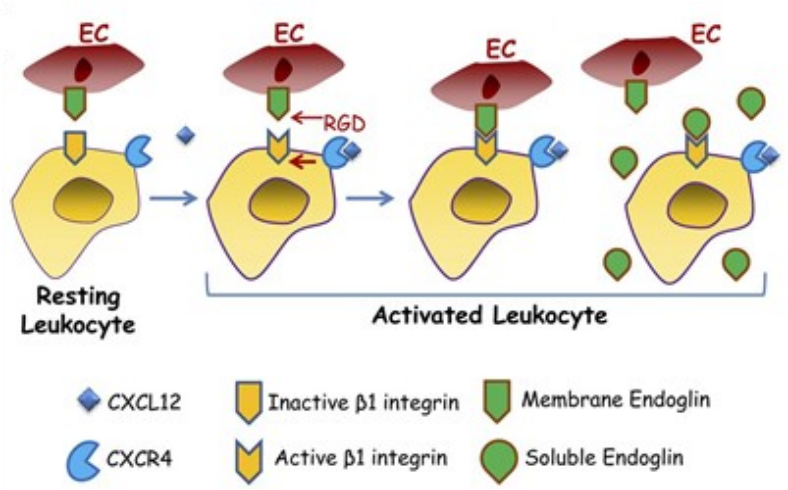

***Figure 8**. Role of Eng and sEng in transendothelial migration of leukocytes. The transmigration process is initiated by the binding of C-X-C motif chemokine 12 (CXCL12) to its receptor C-X-C chemokine receptor type 4 (CXCR4), which activates β1 integrins. Upon activation, they bind to the RGD motif in membrane Eng on the surface of the endothelial cells, thus allowing the transmigration of leukocytes through the endothelium. However, when sEng is present, it competes for the binding to integrins on leukocytes and therefore interferes with leukocytes' adhesion to endothelial surface and subsequent transendothelial migration (116).*

1.2.3 Endothelial cell types used in cardiovascular research

The appropriate and reliable *in vitro* models are important in order to better understand molecular mechanisms underlying different cardiovascular pathological conditions and also to utilize simple and reproducible techniques to discover new therapies. To achieve these goals, multiple cell types have been used, most often cardiovascular cells, but also non-cardiovascular cells (human embryonic kidney 293 cells or buccal mucosa cells) or embryonic stem cells (131).

The cardiovascular system, with its hierarchical and complex architecture, is comprised of multiple cell types, which together participate in the proper function of the entire system. For example, the effective cooperation of the cardiomyocytes and non-myocyte cells is crucial for the correct cardiac physiology. In the vessels, endothelial cells and smooth muscle cells have different roles, but both are essential for the right vessel function. Dysfunction of a single cell type might result in the development of various pathologies.

One of the methods used to characterize a pathology is to use a primary human culture, which is composed of cells isolated from the organ of interest (131).

However, one of the disadvantages is that primary endothelial cells have a finite life span, and in the past, their accessibility was quite limited. Nevertheless, throughout the years, the process of cell isolation from different tissues, such as the umbilical vein, aorta, or coronary arteries, became more accessible (131).

The human umbilical vein endothelial cells (HUVECs) (Fig. 9A) are one of the most used cell types in cardiovascular research (132). They are characterized "by the presence of Weibel-Palade bodies, pinocytic vesicles, small amounts of 100 Å fibrils located near the nucleus, mitochondria with tubular shapes which rarely showed ramifications, an ellipsoid nucleus with a fine granular pattern of condensed chromatin and one to three nucleoli present in each nucleus" as cited from Haudenschild et al. (133).

However, venous and arterial endothelial cells have different origins and show different molecular and functional properties (134). For example, when Deng et al. studied the different predisposition of arteries and veins to atherosclerosis, they found that the basal gene expression profile of endothelial cells from veins displays higher protection against ED compared to endothelial cells from arteries, suggesting that arterial cells are a better model of atherosclerosis (135).

Therefore, using human aortic endothelial cells (HAECs) (Fig. 9B) might be more accurate for studying diseases such as atherosclerosis, which does not naturally occur in veins. HAECs produce thrombotic and antithrombotic factors such as plasminogen activator inhibitor 1 (PAI-1) and tissue plasminogen activator, respond to stimulation with TNF-α by expressing ICAM-1 and also producing NO and ET-1 (136). This was the reason why we decided to use these cells for the *in vitro* studies in this thesis.

Another endothelial cells frequently used in cardiovascular research are human microvascular endothelial cells (HMVECs) (Fig. 9C). They are usually isolated from the skin, lungs, or heart. They express all the classical markers of endothelial cells and also exhibit typical cobblestone morphology. Nevertheless, they are most often used for studying angiogenesis, wound healing, inflammation, and intercellular communication, not as much for atherosclerosis (137-141).

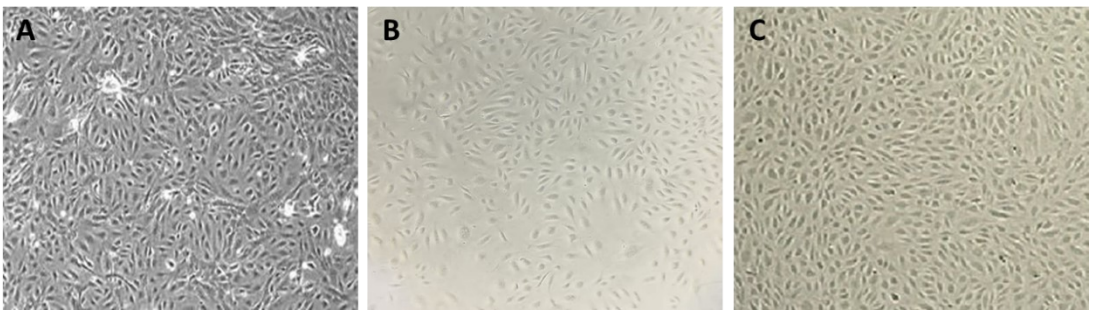

***Figure 9**. Endothelial cell types used in cardiovascular research. A) Human umbilical vein endothelial cells (HUVECs) (142). B) Human aortic endothelial cells (HAECs) (own picture). C) Human microvascular endothelial cells (HMVECs) isolated from lungs (143).*

1.3 Metabolic syndrome

In Czech population, metabolic syndrome (MetS) is defined as the presence of three or more of the following criteria:

i. Large waist circumference — a waistline that measures > 80 cm for women and > 94 cm for men
ii. Triglyceride level ≥ 1.7 mmol/l or hypolipidemic therapy
iii. Reduced high-density lipoprotein (HDL) cholesterol — < 1.3 mmol/l in women or < 1.00 mmol/l in men or hypolipidemic therapy
iv. Increased blood pressure — ≥ 130/ ≥ 85 mm Hg or antihypertensive therapy
v. Elevated fasting blood sugar ≥ 5.6 mmol/l or impaired glucose tolerance or diabetes mellitus type II or antidiabetic therapy (144)

When MetS is present, the risk of the development of cardiometabolic disorders, such as coronary heart disease or stroke, is highly increased (145, 146). The prevalence of MetS is varied around the world, and it seems to be increasing in parallel with increasing prevalence of obesity (147). Age, race/ethnicity, and gender are responsible for the high variation in the MetS prevalence. MetS affects approximately a third of the adult population of the USA (148), one-tenth of US adolescents (149), and around a quarter of the European population (147). Currently, there is a lower prevalence of MetS in Southeast Asia, but the trend is rapidly increasing towards rates in the western world (150). Race- and gender-dependent variations in MetS prevalence are also very common. The prevalence in African-American women is around 57% higher

compared to African-American men and approximately 26% higher in women of Hispanic origin compared to Hispanic men (147).

Race/ethnicity differences are also observed among the individual components of MetS. For instance, insulin resistance is more common in Hispanics, dyslipidemia in Whites, and hypertension in African-Americans (147).

The pathogenesis of MetS is highly complex and remains to be fully elucidated. If the individual conditions of MetS represent separate pathologies or they are a result of a single common mechanism is still not clarified. The differences in the geographical distribution of MetS and the increasing trend in developing countries (151), suggest that lifestyle and environmental factors (excess calorie intake and physical inactivity) are major contributors. It has been demonstrated that visceral adiposity is a primary trigger involved in most of the MetS pathways, thus again pointing to a high intake of calories as the main causative factor (152). From all the suggested mechanisms, neurohumoral activation, insulin resistance, and chronic inflammation seem to be the key processes involved in the initiation, progression, and transition from MetS to CVD (Fig. 10) (153).

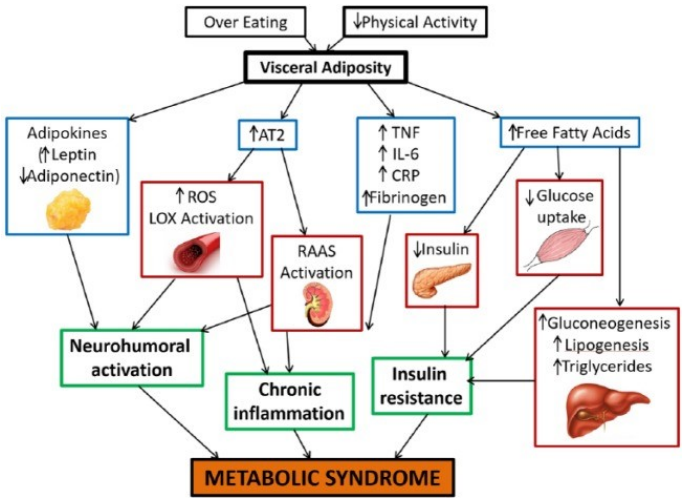

Figure 10. *Pathological mechanisms involved in MetS. Overeating and reduced physical activity led to increased visceral adiposity, which in turn triggered the release of selected adipokines, angiotensin 2 (AT2), pro-inflammatory molecules, and free fatty acids. Together they induce neurohumoral activation, chronic inflammation, and insulin resistance, followed by the development of MetS. CRP, C-reactive protein; IL-6, interleukin 6; LOX, lectin-like oxidized low-density lipoprotein; RAAS, renin-angiotensin-aldosterone system (153).*

1.3.1 Risk factors of metabolic syndrome

The following risk factors precede the development of MetS and its individual components (insulin resistance, hyperlipidemia, hyperglycemia, hypertension):

i. Modifiable risk factors

- Lifestyle habits – smoking, being inactive, eating unhealthy and large portion sizes, not getting enough good quality sleep, excessive drinking of alcohol (154)
- Occupation – shift workers have a higher risk of MetS caused by a disturbance in their circadian rhythm, which leads to perturbations in the metabolism of nutrients (155)

ii. Non-modifiable risk factors

- Age – the risk of MetS increases with higher age (156)
- Gender - In older adults, women were shown to have a higher risk of MetS than men because of the changes in their hormone levels after menopause (157)
- Environment – low socioeconomic status might lead to an unhealthy diet, inactive lifestyle, and not enough sleep (158)
- Family history – the presence of MetS in close relatives increases the risk of the development of MetS (159)
- Genetics – genetic background can affect how the organism responds to insulin (160)
- Other medical conditions – obesity, polycystic ovary syndrome (PCOS) (161), psoriasis (162), sleeping issues, and medications used to treat allergies, bipolar disorder, depression, human immunodeficiency viruses, and schizophrenia can also increase the risk of MetS (163, 164)

1.3.2 Role of membrane endoglin in metabolic syndrome

Eng has been demonstrated to play a role in all of the individual components of MetS, such as hypertension, hyperglycemia, obesity, and hypercholesterolemia.

As mentioned above, multiple studies described interactions between Eng and eNOS (123, 124, 165, 166), the enzyme responsible for the production of NO. Therefore, changes in Eng expression are reflected in the availability of NO, and thus might contribute to ED and subsequently to hypertension (167).

There is also evidence for the Eng role in hyperglycemia, although it is less clear. Eng has been increased in DM patients with a lower risk of nephropathy compared to patients with a higher risk of nephropathy, suggesting a protective role of Eng towards the development of diabetic nephropathy (168). Another study has demonstrated that HUVECs treated with constant or oscillating high glucose (25 mmol/L) led to increased Eng mRNA expression (169).

When exploring the role of Eng in adipose tissue, Jilkova et al. found that induction of low-grade inflammation and obesity in mice resulted in increased membrane Eng expression (170).

Finally, there is also an association between Eng and hypercholesterolemia. Apolipoprotein E and LDL receptor-deficient ($ApoE^{-/-}/LDLR^{-/-}$) mice, model of spontaneous hypercholesterolemia, showed decreased Eng/eNOS expression in the aorta, as well as decreased NO production. Surprisingly, treatment of HAECs with 7-ketocholesterol resulted in increased Eng/eNOS expression, supporting the different roles of Eng *in vitro* vs. *in vivo* (24).

1.3.3 Role of soluble endoglin in metabolic syndrome

The role of sEng in the MetS is more described than the role of membrane Eng. Blazquez-Medela et al. demonstrated that sEng levels correlate with systolic blood pressure, pulse pressure, left ventricular hypertrophy, and others, suggesting that sEng might be an indicator of hypertension (55). sEng has also been shown to induce hypertension in the context of preeclampsia (27, 128) and other studies proposed sEng role in different types of pulmonary arterial hypertension (PAH) (171-173). Gallardo-Vara et al. partially elucidated the underlying mechanism when they discovered that sEng acts through its downstream mediator BMP4 (174), which increases the production of ROS via activation of NOX and cyclooxygenase 2 (175, 176).

There are also a few studies regarding the role of sEng during hyperglycemia/DM. sEng was found to be increased in patients with advanced DM and its concentration correlated with the severity of diabetic nephropathy (53, 54), diabetic retinopathy (55), and diabetic neuropathy (177). Another study showed that both hyperglycemia and hypoglycemia in patients with DM were able to increase sEng levels, and the authors suggested that this increase was related to oxidative stress, which is developed in both conditions (178). Cawyer et al. demonstrated that high glucose levels also significantly increased levels of sEng (179, 180).

A study related to the role of sEng in adipose tissue by Lappas et al. showed that levels of sEng are increased in the omental adipose tissue of women with pre-existing maternal obesity and gestational DM (56). On the other hand, the study by Vieira et al. found that sEng levels were decreased in the plasma of obese women compared to non-obese pregnant women (181). These rather contradictory results might be explained by the different number of women in the respective groups (834 vs. 3106) in the study by Vieira et al., as well as the fact they evaluated the levels of sEng from plasma samples at the 14 - 16 week of pregnancy, meanwhile in the study by Lappas et al. samples were taken from the omental adipose tissue after the delivery.

Last but not least, levels of sEng were also increased in patients with hypercholesterolemia (52), as well as familial hypercholesterolemia (51). Additionally, sEng levels were also increased in the mouse model of hypercholesterolemia with atherosclerosis (24, 182, 183).

1.4 The pharmacological approach to affect endoglin expression

1.4.1 The pharmacological approach to affect endoglin expression in metabolic syndrome

1.4.1.1 Antihyperlipidemic therapy

From the category of antihyperlipidemic agents, the group that is most widely used with respect to Eng is 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) inhibitors, also called “statins.” Inhibition of HMGCR results in the reduction of cholesterol synthesis in the liver and, thus, increased expression of low-density lipoprotein (LDL) receptors, which are responsible for the uptake of LDL from plasma (184).

There are multiple studies regarding the statins' effects on membrane Eng expression. It has been demonstrated that atorvastatin can increase membrane Eng in HUVECs (124, 185), in endothelial cells covering the atherosclerotic plaque (186), and in mice aorta (182, 187). However, other studies have shown that atorvastatin can also lead to decreased levels of Eng in the myocardium (188), in mice endothelium (189), and decreased Eng promoter activity in cardiac fibroblasts (190). These ambiguous results might be partially explained by another study, which demonstrated that atorvastatin treatment results in decreased Eng expression in mice endothelium after four weeks of treatments but increased Eng expression after eight weeks, suggesting that Eng expression might be time-dependent (191). Fluvastatin treatment has been shown to decrease the release of Eng^{+} endothelial microparticles (EMPs) (192), and simvastatin was demonstrated to reduce Eng mRNA expression in a model of liver fibrosis (193).

Several studies have also been performed to evaluate statins' effects on sEng. Treatment with pravastatin, rosuvastatin, and simvastatin resulted in increased sEng levels in HUVECs, with simvastatin having the most potent effect (194, 195). On the other hand, pravastatin was able to decrease sEng levels in endothelial colony-forming cells (196), trophoblast cell line (197), and mice model of preeclampsia (198). Another study showed that treatment of $ApoE^{-/-}/LDLR^{-/-}$ mice with atorvastatin also resulted in the decrease of sEng levels simultaneously with reduced atherosclerosis (182).

Taken together, studies regarding the effects of statins on membrane Eng are quite contradictory, and thus no conclusion should be made right now. Nevertheless, most of the studies with respect to sEng levels seem to be in agreement that satins are able to decrease levels of sEng.

1.4.1.2 Antidiabetic therapy

The most used antidiabetic medication affecting Eng expression is metformin. Metformin belongs to the biguanide class, and the mechanism of action is not fully elucidated yet. However, metformin has been shown to decrease hepatic production of glucose, thus decreasing levels of glucose in the blood and also increasing insulin sensitivity by the increase of peripheral glucose uptake and its utilization (199). One study has shown that metformin in combination with pemetrexed decreased membrane Eng expression in mice lung tissue (200). Other studies were focused on the metformin

effects on sEng levels. Most of those demonstrated that metformin treatment resulted in decreased sEng levels in primary endothelial cells, villous cytotrophoblast cells (201), in HUVECs supernatants (202, 203), and in mice serum (in combination with pemetrexed) (200). On the other hand, few studies also showed that metformin did not induce a significant change in sEng levels in supernatant from a human trophoblast cell line (204), in preeclamptic patients' plasma (205) or in EMPs from patients with PCOS before and after treatment with metformin (206). There are contradicting results regarding the metformin effect on sEng levels in placental explant (201, 203, 207).

Other medications used to affect Eng expression are thiazolidinediones, which act as selective agonists of peroxisome proliferator-activated gamma receptors, as well as phosphoinositide 3-kinase activators, leading to increased sensitivity to insulin in peripheral tissues (208). Rosiglitazone has been shown to reduce membrane Eng expression in white adipose tissue extracts (170) and also to decrease sEng levels in trophoblast cell line supernatants (179). Only study evaluating the effects of pioglitazone demonstrated that pioglitazone was able to increase sEng levels in endothelial progenitor cells (209).

Therapy with insulin or its analogs is unavoidable in patients with type I DM and often recommended in patients with type II DM. The study by Cudmore et al. showed that insulin was able to reduce sEng levels in HUVECs supernatant (210).

One study with glucagon-like peptide 1, an incretin able to stimulate the formation and release of insulin upon food ingestion (208), demonstrated that glucagon-like peptide 1 was able to decrease sEng in patients with both hypo- and hyperglycemia (178).

Finally, a study with empagliflozin, a sodium-glucose co-transporter-2 inhibitor that blocks the reabsorption of glucose mediated by sodium-glucose co-transporter-2 in the proximal tubule in kidneys (208), showed that sEng levels were not significantly changed in the EMPs from PCOS patients before treatment compared to after treatment with empagliflozin (206).

In conclusion, not much is known about the effects of antidiabetic treatment on membrane Eng, but two published studies show that antidiabetics can decrease

membrane Eng expression. Similarly to statins, most antidiabetic drugs have been demonstrated to decrease sEng levels.

1.4.1.3 Antihypertensive therapy

From the available antihypertensive drugs, angiotensin II receptor blockers (ARBs) are most commonly used to affect Eng expression. ARBs act as antagonists on the angiotensin 2 type 1 receptor (211). Valsartan, candesartan and losartan have been demonstrated to reduce membrane Eng in rat cardiac fibroblasts (212). Additionally, valsartan has been shown to decrease Eng expression in the myocardium (188), and treatment with losartan resulted in decreased Eng levels in human coronary artery endothelial cells (213). One study showed no changes in Eng expression in the trophoblast cell line treated with losartan (214).

There are only two studies regarding the ARBs effect on sEng levels. The first study with losartan demonstrated decreased sEng levels in trophoblast cells (215), and the second one with candesartan similarly showed a decrease in sEng levels in vascular endothelial cells isolated from mice (216).

Two studies evaluated the effects of angiotensin-converting enzyme inhibitors on the membrane and soluble Eng levels. Prieto et al. demonstrated that trandolapril reduced the expression of membrane Eng in a rat model of renal fibrosis (217) and Buda et al. showed that perindopril treatment leads to decreased levels of sEng in patients' plasma (218).

Three studies in total were found while searching for β-blockers effects on Eng expression. One, studying the effects of propranolol on membrane Eng levels, found that treatment with propranolol led to reduced Eng expression in endothelial cells (219). The remaining two studies evaluated β-blockers effects on sEng levels. Binder et al. showed that: a) metoprolol decreased sEng levels in HUVECs, but not in placental explants; b) bisoprolol reduced levels of sEng in explants but not in HUVECs; and c) carvedilol did not significantly change sEng levels neither in HUVECs nor explants (220). Gangooly et al. demonstrated that labetalol did not change levels of sEng in placental explants (221).

Only one study was found using diuretics, specifically non-selective mineralocorticoid receptor blocker spironolactone and selective mineralocorticoid

receptor blocker eplerenone. Both of them were shown to decrease levels of membrane Eng in cardiac tissue, as well as sEng levels in plasma (222).

No relevant studies were found when searching for the effects of calcium channel blockers on Eng expression.

In summary, most of the available antihypertensive pharmaceuticals have been shown to decrease both membrane and soluble Eng levels.

1.4.2 The pharmacological approach to directly affect endoglin expression

1.4.2.1 TRC105

The combination of the fact that Eng is highly expressed in proliferating endothelial cells (223) and solid tumors, as well as being essential for angiogenesis (224), makes Eng an attractive target for angiogenesis-related cancer therapy. One of the agents developed for this purpose is TRC105 (carotuximab), an anti-endoglin chimeric monoclonal antibody that binds to Eng with high avidity and modulates Smad signaling (225). TRC105 has been shown to act through a few different mechanisms. First, binding of TRC105 to Eng leads to the induction of antibody-dependent cell-mediated cytotoxicity, which is mediated by neutrophils, natural killer cells, or monocytes and results in the killing of the target cells. Moreover, it has been demonstrated that TRC105 blocks the binding of BMP9 to Eng, thus inhibiting the downstream signaling of Eng. Finally, TRC105 has also been shown to induce cleavage of Eng by MMP14, thus increasing the levels of sEng (Fig. 11) (226).

A lot of studies have been published with regard to TRC105 effects on Eng and sEng expression, and they are nicely summarized in the recent reviews by Liu et al. (226), Schoonderwoerd et al. (11), and Jeng et al. (227). Most of these studies are related to TRC105 targeting Eng in the therapy of cancer; however, there are no studies available about the use of TRC105 to modulate Eng expression in pathologies associated with MetS.

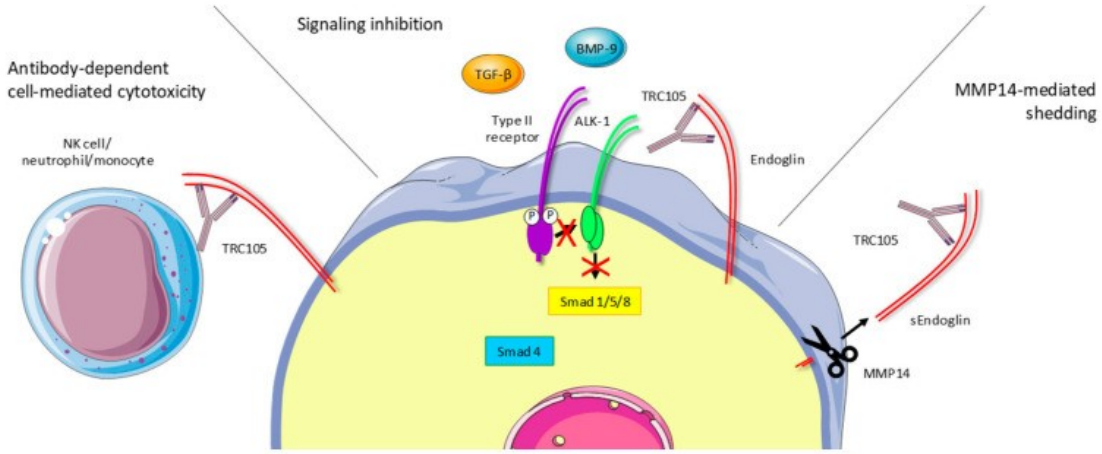

***Figure 11**. Mechanism of action of TRC105. The binding of TRC105 to Eng results in the induction of antibody-dependent cell-mediated cytotoxicity, which is mediated by neutrophils, natural killers (NK) cells, or monocyte and leads to the killing of the target cells. TRC105 also blocks the binding of BMP9 to Eng and therefore inhibits downstream signaling otherwise mediated by Eng. Finally, TRC105 can induce cleavage of Eng by MMP14, thus increasing the levels of sEng (226).*

1.4.2.2 Specific estrogen receptor modulators

Other therapeutics able to modulate Eng expression are specific estrogen receptor modulators, raloxifene and bazedoxifene. This was discovered thanks to the observation that pre-menopausal women have less frequent epistaxis than women after menopause. This theory was then tested by treating 40 HHT patients suffering from gastrointestinal bleeding with estradiol and to avoid the feminizing effect of estradiol, men were treated with ethinylestradiol/norethindrone and danazol. Results showed that the condition of most of these patients was improved as they needed fewer blood transfusions (228). The mechanism of this phenomenon was later explored, and it was shown that raloxifene is able to increase Eng mRNA and protein expression, as well as stimulate Eng promotor activity in endothelial cells (229). Similarly, bazedoxifen was able to increase Eng mRNA levels in cultured endothelial cells and in cells derived from the patients' blood (230).

1.4.2.3 Resveratrol

Studies evaluating the effects of resveratrol, a plant-derived polyphenol with antioxidant and anti-inflammatory effects (231, 232), showed that resveratrol is able to decrease Eng mRNA and protein levels of membrane Eng in lung adenocarcinoma

cell line and in xenograft mice (233), as well as decrease Eng mRNA in kidney tissue from rats (234). When studying sEng levels, Hannan et al. demonstrated that resveratrol could decrease sEng in trophoblast cells and HUVECs (235); however, this was not confirmed in placental explants exposed to 1% O_2 when sEng levels remained unchanged (236).

1.4.2.4 Magnesium sulfate

How magnesium sulfate affects Eng expression was also studied by a few research groups. Lee et al. showed that magnesium sulfate reduces membrane Eng expression in microvascular endothelial cells isolated from adipose tissue of control patients or patients with preeclampsia (237). Korish et al. demonstrated that magnesium sulfate is able to decrease sEng levels in preeclamptic rats (238), yet no effect on sEng levels was found in preeclamptic patients before and after treatment with magnesium sulfate (239).

2 Aims of the dissertation thesis

Considering that levels of membrane and soluble Eng have been demonstrated to change during pathological conditions related to metabolic syndrome and the results of the individual studies are rather ambiguous, we formulated the following aims to help us better understand the role of Eng in metabolic syndrome:

1. Analyze the data collected during the last fifteen years from patients with familial hypercholesterolemia treated by lipoprotein apheresis in Faculty Hospital in Hradec Králové to determine the value of this procedure regarding levels of soluble Eng, plasma lipoproteins and biomarkers of inflammation and endothelial dysfunction.

2. Summarize current knowledge regarding the membrane and soluble Eng role in metabolic and cardiovascular disorders that are related to the metabolic syndrome.

3. Evaluate Eng expression, signaling, and function during endothelial dysfunction induced by hypercholesterolemia and hyperglycemia and study how treatment with pharmacological agent TRC105 will affect these processes *in vitro*.

3 Commentary on published articles

3.1 Monitoring of up to 15 years effects of lipoprotein apheresis on lipids, biomarkers of inflammation, and soluble endoglin in familial hypercholesterolemia patients

Visek J, Blaha M, Blaha V, Lasticova M, Lanska M, Andrys C, Duintjer Tebbens J, Igreja Sa IC, **Tripska K**, Vicen M, Najmanova I, Nachtigal P. Orphanet J Rare Dis. 2021 Feb 27;16(1):110. doi: 10.1186/s13023-021-01749-w. PMID: 33640001. IF=4.307 (Q2); AIS=1.349 (Q2)

In this paper, we analyzed the association of sEng, plasma lipoproteins, and biomarkers of inflammation and endothelial dysfunction with the data collected during the past 15 years from patients diagnosed with familial hypercholesterolemia (FH) who underwent lipoprotein apheresis (LA).

Ten heterozygous and four homozygous patients with FH underwent long-term treatment with LA. Lipids (total cholesterol (TC), LDL cholesterol (LDL), HDL cholesterol (HDL), apolipoprotein B (apoB), triglycerides), biomarkers of inflammation and endothelial dysfunction (high sensitivity C-reactive proteins (hsCRP), soluble P-selectin (sP-selectin), monocyte chemoattractant protein 1 (MCP-1) and CD40 ligand (CD40L)) and sEng levels were examined.

Results showed that LA is associated with significantly decreased levels of sEng, as well as TC, LDL, HDL, and apoB, along with selected biomarkers of inflammation and endothelial dysfunction after every single LA in most of the patients. Long-term LA (up to 15 years) was also associated with decreased levels of sEng, together with TC, LDL, HDL, and certain biomarkers of inflammation and endothelial dysfunction in individual patients. Additionally, we showed that sEng levels correlate with selected biomarkers of inflammation in certain FH patients.

Taken together, these results suggest that LA might contribute to the improvement of cardiovascular prognosis after every procedure in most FH patients, as well as in the long-term period in some of the patients. Furthermore, the correlation of sEng with selected biomarkers of inflammation suggests that sEng might also reflect an endothelial inflammatory state in some of the FH patients treated with LA.

3.2 Membrane and soluble endoglin role in cardiovascular and metabolic disorders related to metabolic syndrome

Vicen M, Igreja Sa IC, **Tripska K**, Vitverova B, Najmanova I, Eissazadeh S, Micuda S, Nachtigal P. Cell Mol Life Sci. 2021 Mar;78(6):2405-2418. doi: 10.1007/s00018-020-03701- w. PMID: 33185696. IF=9.207 (Q1); AIS=2.069 (Q1)

In this review article, we summarized the current knowledge about the role of membrane and soluble Eng in MetS-related pathologies, such as endothelial dysfunction, hypercholesterolemia, hyperglycemia/DM/insulin resistance, obesity, and arterial hypertension.

The current literature regarding the role of membrane Eng in endothelial dysfunction, inflammation, and hypercholesterolemia, seems to be in favor of the proinflammatory potential of Eng in the microvasculature, and the opposite is true in macrovasculature, where Eng might have a protective role. Therefore, we proposed that membrane Eng plays a different role in endothelial dysfunction, inflammation, and hypercholesterolemia, depending on its localization in the vascular bed. Regarding the role of membrane Eng in hyperglycemia/DM and arterial hypertension, not much is known, and the few studies published presented rather contradictory data; therefore, no conclusions have been made so far.

The increased levels of sEng have been linked to hypercholesterolemia, and they also reflect hyperglycemia, as well as hypertension, and thus sEng could be considered as a possible biomarker of these pathologies. However, the precise mechanism of how sEng induces these changes still remains to be elucidated. Furthermore, there is no conclusive evidence regarding the sEng levels in the development of atherosclerotic lesions or its association with obesity.

In conclusion, the role of membrane Eng in the pathologies related to MetS seems to be dependent on its localization in the vasculature. However, sEng seems to be increased in most of the mentioned pathologies, and therefore, sEng levels should be taken into account in patients who already have hypercholesterolemia, DM, and hypertension or are at a higher risk of developing them.

3.3 Monoclonal anti-endoglin antibody TRC105 (carotuximab) prevents hypercholesterolemia and hyperglycemia-induced endothelial dysfunction in human aortic endothelial cells

Tripska K, Igreja Sa IC, Vasinova M, Vicen M, Havelek R, Eissazadeh S, Svobodova Z, Vitverova B, Theuer C, Bernabeu C, Nachtigal P. Front Med. 2022 Sep 7; 9:845918. doi: 10.3389/fmed.2022.845918. PMID: 36160139. IF=5.058 (Q2); AIS=1.233 (Q2)

In this article, we used human aortic endothelial cells (HAECs), an appropriate *in vitro* atherosclerosis model, which were treated with 7-ketocholesterol to simulate hypercholesterolemia or D-glucose to simulate hyperglycemia. 300 μg/mL of TRC105, a pharmacological agent that is able to modulate Eng expression, has been used for 12 and 13 hours in hypercholesterolemia and hyperglycemia, respectively. Time and concentrations have been chosen based on the previously published articles and/or our preliminary data. Adhesion and transmigration, which simulate adhesion and diapedesis of leukocytes during atherosclerosis, were used to assess the functional status of the endothelium.

Induction of hypercholesterolemia and hyperglycemia resulted in increased Eng protein levels, activation of Smad signaling pathways, and induction of endothelial dysfunction (characterized by increased expression of cell adhesion molecules, as well as increased adhesion and transmigration of monocytes through HAECs monolayer).

Treatment with TRC105 did not affect mRNA levels of Eng or its transcription factors; however, it decreased Eng protein levels (suggesting non-transcriptional regulation) and prevented activation of Smad-mediated Eng signaling induced by hypercholesterolemia and hyperglycemia. Despite increased protein levels of cell adhesion molecules, TRC105 treatment also prevented hypercholesterolemia- and hyperglycemia-induced adhesion and transmigration of monocytes through endothelial monolayer.

These results suggest that blockage of Eng by TRC105 was able to prevent hypercholesterolemia- and hyperglycemia-induced endothelial dysfunction, which makes Eng and its interactions interesting targets in pathologies related to elevated levels of cholesterol and glucose.

4 Discussion

Multiple studies have demonstrated that Eng is associated with MetS-related pathological conditions, such as hypercholesterolemia (51, 52), DM (53, 54), hypertension (55), or obesity (56). However, the exact role of Eng in these pathologies is still unknown. Therefore, the main purpose of this thesis was to elucidate and summarize the role of Eng and sEng in the selected pathologies associated with MetS.

In line with our first aim, we focused on the analysis of data collected during the past 15 years from patients with FH who were treated by LA to clarify what is the benefit of this procedure regarding the levels of plasma lipids, sEng, and biomarkers of inflammation and endothelial dysfunction. We demonstrated for the first time that both homozygous and heterozygous patients with FH, who are treated with LA for up to 15 years, have decreased levels of total cholesterol not only after each procedure, which is anticipated and in agreement with other studies (240-243), but also gradually every year they undergo LA. Moreover, we showed that biomarkers of inflammation and endothelial dysfunction (hsCRP, MCP-1) are also reduced after LA in most of the patients, which is in line with other papers (244, 245). These findings suggest that treatment with LA might reduce the risk of cardiovascular complications and thus improve cardiovascular prognosis in most patients with FH. We also observed that sEng levels were reduced after every LA in most of the patients, and interestingly, they were reduced in a higher percentage of patients than other traditional markers of inflammation and endothelial dysfunction. Additional analysis showed that sEng levels correlate with biomarkers of inflammation, implying that sEng levels reflect an inflammatory state in some FH patients treated with LA.

Given the results generated by our first study, we have decided to analyze the current literature, with a focus on the role of the membrane and soluble Eng in different cardiometabolic disorders. In this review, we concluded that most of the studies exploring the role of sEng in cardiometabolic disorders showed that sEng levels are increased during these pathologies, and therefore sEng could be considered a possible biomarker of these disorders. Additionally, sEng has also been shown to aggravate MetS-related pathologies, such as endothelial dysfunction (246) or nonalcoholic steatohepatitis (247). In clinical practice, this would mean that sEng levels should be taken into account in patients who already have cardiometabolic-related pathologies

(especially hypercholesterolemia, DM, or hypertension) or are at a higher risk of developing them. Furthermore, we suggested that discrepancies in the role of membrane Eng might be due to the fact that Eng function is dependent on its localization in the vasculature. Especially during endothelial dysfunction, inflammation, and hypercholesterolemia, membrane Eng seems to play a pro-inflammatory role in microvasculature by mediating adhesion and transendothelial migration of leukocytes; however, the opposite is true in macrovasculature, where it seems to have a protective role by regulating eNOS expression. Another important point was that acute exposure to hypercholesterolemia *in vitro* generated the opposite results as chronic exposure to hypercholesterolemia *in vivo*, at least with respect to levels of membrane Eng (24). This suggests that Eng expression might be time-dependent, as was shown previously when eight weeks old $ApoE^{-/-}$ mice displayed higher levels of membrane Eng compared to sixteen weeks old $ApoE^{-/-}$ mice (191).

Keeping in mind these contradictory results regarding the role of membrane Eng in cardiometabolic pathologies, we decided to further deepen the knowledge in this specific field. We demonstrated that stimulation of HAECs with hypercholesterolemia and hyperglycemia resulted in increased Eng protein levels, activation of Eng-dependent Smad signaling, as well as induction of endothelial dysfunction (defined as increased expression of proinflammatory cell adhesion molecules, along with increased adhesion and transendothelial migration of monocytes through HAECs monolayer). This is in agreement with other previously published studies (24, 169), implying that membrane Eng is indeed expressed together with proinflammatory markers during acute exposure to hypercholesterolemia and hyperglycemia *in vitro.* Additionally, we used for the first time the pharmacologic agent TRC105 to block membrane Eng expression in cardiometabolic disorders, which then allowed us to explore specifically the role of Eng in these processes. We showed that blockage of Eng led to a decrease in Eng protein levels but not in mRNA expression of Eng or its transcription factors, which confirmed that TRC105 does not regulate Eng expression on a transcriptional level, as previously suggested by Liu et al. (225). Furthermore, we demonstrated that TRC105 is able to prevent the activation of both pSmad1/5 and pSmad2/3 signaling axis, which confirmed the results described by Liu et al. (248), despite that Kumar et al. observed that treatment with TRC105 resulted in the inhibition of pSmad1/5, but enhanced pSmad2/3 signaling (249). Finally, we showed

that even though TRC105 treatment resulted in increased levels of cell adhesion molecules, the blockage of Eng was essential for the prevention of adhesion and transmigration of monocytes through the endothelial layer. This can seem quite controversial; however, it is supported by another study of our research group, where we decreased Eng expression by small interfering RNA and observed the same result (24). Similarly, when Rossi et al. injected the $Eng^{+/-}$ and $Eng^{+/+}$ mice with carrageenan to stimulate the transmigration of leukocytes into the peritoneal cavity, they observed that infiltration of leukocytes was significantly lower in $Eng^{+/-}$ compared to $Eng^{+/+}$ mice (116). In the context of atherosclerosis, this could mean that fewer monocytes will transmigrate through the endothelium, and therefore fewer monocytes will be present in subendothelial space, where they can transform into foam cells and further induce inflammation and atherosclerosis progression. Taken together, these results suggest that Eng plays a crucial role in the development of endothelial dysfunction under hyperglycemic and hypercholesterolemic conditions, which makes Eng and its interactions attractive pharmacological targets in pathologies associated with elevated glucose and cholesterol levels.

Given the information acquired by all the papers that are part of this doctoral thesis, we suggest that decreasing sEng levels in patients with cardiometabolic-related disorders is desirable and should be considered, at least in patients who are prone to develop them. Regarding the membrane Eng, the reduction of its expression is associated with decreased transendothelial migration, which is a good thing from the perspective of atherogenesis. However, in the wider context, this would also mean decreasing Eng expression in large arteries, which could result in reduced eNOS expression and, thus, induction of vascular dysfunction. Therefore, one of the possible approaches could be to reduce Eng-mediated transendothelial migration by targeting the specific integrins on leukocytes that are responsible for the interaction with Eng on the endothelium. This could then result in a decreased number of monocytes/macrophages that can transform into foam cells in subendothelial space, but at the same time, the basal levels of membrane Eng, and thus, eNOS in large arteries would remain unchanged.

5 Conclusions

In conclusion, the collection of articles that make up this doctoral thesis helped to extend the knowledge regarding the role of membrane and soluble Eng in disorders related to metabolic syndrome.

In spite of the contradictory results regarding the general role of Eng in cardiometabolic disorders, we can conclude that increased sEng levels were associated with cardiometabolic disorders in the vast majority of the studies. Therefore, we suggest that sEng can be considered a biomarker of cardiometabolic diseases and a potential inducer of their development; thus, lowering its levels should at least be considered in patients who are prone to develop these diseases.

Regarding the role of membrane Eng, we suggest that decreasing the undesirable interaction between Eng and leukocytes while keeping the basal expression of Eng in arteries intact, could be a possible approach how to alleviate the negative effects of increased Eng expression during cardiometabolic pathologies. However, since the role of membrane-bound Eng is still not fully understood, this must be taken with caution, and also future studies in animal models are necessary to either confirm or disprove this idea.

6 Contribution of the candidate to the published work included in the thesis

I. Visek J, Blaha M, Blaha V, Lasticova M, Lanska M, Andrys C, Duintjer Tebbens J, Igreja Sa IC, **Tripska K**, Vicen M, Najmanova I, Nachtigal P. **Monitoring of up to 15 years effects of lipoprotein apheresis on lipids, biomarkers of inflammation, and soluble endoglin in familial hypercholesterolemia patients.** Orphanet J Rare Dis. 2021 Feb 27;16(1):110. IF=4.307 (Q2); AIS=1.349 (Q2)

- Contribution to the interpretation of the data
- Contribution to the writing, review, and editing of the manuscript

II. Vicen M, Igreja Sa IC, **Tripska K**, Vitverova B, Najmanova I, Eissazadeh S, Micuda S, Nachtigal P. **Membrane and soluble endoglin role in cardiovascular and metabolic disorders related to metabolic syndrome.** Cell Mol Life Sci. 2021 Mar;78(6):2405-2418. IF=9.207 (Q1); AIS=2.069 (Q1)

- Contribution to the writing, review, and editing of the manuscript

III. **Tripska K**, Igreja Sa IC, Vasinova M, Vicen M, Havelek R, Eissazadeh S, Svobodova Z, Vitverova B, Theuer C, Bernabeu C, Nachtigal P. **Monoclonal anti-endoglin antibody TRC105 (carotuximab) prevents hypercholesterolemia and hyperglycemia-induced endothelial dysfunction in human aortic endothelial cells.** Front Med. 2022 Sep 7; 9:845918. IF=5.058 (Q2); AIS=1.233 (Q2)

- Conceptualization of the project
- Data curation
- Formal analysis of the acquired data
- Manuscript preparation – writing of original draft and visualization

7 List of other outputs of the candidate

7.1 Overview of publication activity of the candidate

Vitverova B, Najmanova I, Vicen M, **Tripska K**, Igreja Sa IC, Hyspler R, Pericacho M, Nachtigal P. **Long-term effects of soluble endoglin and mild hypercholesterolemia in mice hearts.** PLoS One. 2020 May 29;15(5):e0233725. doi: 10.1371/journal.pone.0233725. PMID: 32470058. IF=3.240 (Q2); AIS=1.011 (Q2)

Igreja Sa IC, **Tripska K**, Hroch M, Hyspler R, Ticha A, Lastuvkova H, Schreiberova J, Dolezelova E, Eissazadeh S, Vitverova B, Najmanova I, Vasinova M, Pericacho M, Micuda S, Nachtigal P. **Soluble Endoglin as a Potential Biomarker of Nonalcoholic Steatohepatitis (NASH) Development, Participating in Aggravation of NASH-Related Changes in Mouse Liver.** Int J Mol Sci. 2020 Nov 27;21(23):9021. doi: 10.3390/ijms21239021. PMID: 33261044. IF=5.924 (Q2); AIS=1.123 (Q2)

Visek J, Blaha M, Blaha V, Lasticova M, Lanska M, Andrys C, Duintjer Tebbens J, Igreja Sa IC, **Tripska K**, Vicen M, Najmanova I, Nachtigal P**. Monitoring of up to 15 years effects of lipoprotein apheresis on lipids, biomarkers of inflammation, and soluble endoglin in familial hypercholesterolemia patients**. Orphanet J Rare Dis. 2021 Feb 27;16(1):110. doi: 10.1186/s13023-021-01749-w. IF=4.307 (Q2); AIS=1.349 (Q2)

Vicen M, Igreja Sa IC, **Tripska K**, Vitverova B, Najmanova I, Eissazadeh S, Micuda S, Nachtigal P. **Membrane and soluble endoglin role in cardiovascular and metabolic disorders related to metabolic syndrome**. Cell Mol Life Sci. 2021 Mar;78(6):2405-2418. doi: 10.1007/s00018-020-03701-w. PMID: 33185696. IF=9.207 (Q1); AIS=2.069 (Q1)

Nejmanova I, Vitverova B, Eissazadeh S, **Tripska K**, Igreja Sa IC, Hyspler R, Nemeckova I, Pericacho M, Nachtigal P. **High Soluble Endoglin Levels Affect Aortic Vascular Function during Mice Aging**. J Cardiovasc Dev Dis. 2021 Dec 4;8(12):173. doi: 10.3390/jcdd8120173. PMID: 34940528. IF=4.415 (Q2); AIS=09.914 (Q2)

Tripska K, Draessler J, Pokladnikova J. **Heart rate variability, perceived stress and willingness to seek counselling in undergraduate students**. J Psychosom Res. 2022 Sep; 160:110972. doi: 10.1016/j.jpsychores.2022.110972. PMID: 35728339. IF=4.620 (Q2); AIS=1.149 (Q2)

Tripska K, Igreja Sa IC, Vasinova M, Vicen M, Havelek R, Eissazadeh S, Svobodova Z, Vitverova B, Theuer C, Bernabeu C, Nachtigal P. **Monoclonal anti-endoglin antibody TRC105 (carotuximab) prevents hypercholesterolemia and hyperglycemia-induced endothelial dysfunction in human aortic endothelial cells.** Front Med. 2022 Sep 7;9:845918. doi: 10.3389/fmed.2022.845918. PMID: 36160139. IF=5.058 (Q2); AIS=1.233 (Q2)

7.2 Awards and scholarships attained during the studies

2020 **3rd place for the Best Poster** in 24th Congress on Atherosclerosis (CSAT) – Pilsen, Czech Republic.

2021 **Young Investigator Fellowship** for the 89th EAS Virtual Congress, Finland, from the European Atherosclerosis Society (EAS)

1st Place for the Best Poster in 25th Congress on Atherosclerosis (CSAT) – Prague, Czech Republic.

2022 **Travel grant** for the 90th EAS Congress in Milan from the Czech Society for Atherosclerosis (CSAT)

7.3 Grant projects

Principal investigator

2020 – 2022 Grant Agency of Charles University
Carotuximab effects on endoglin expression, signaling and function in inflammation and oxysterol-induced endothelial dysfunction in endothelial cells

Team member

2017 – 2020 Czech health research council
The relationship of soluble endoglin and hypercholesterolemia in patients with type 2 diabetes mellitus and the impact of the therapeutical intervention.

2022 - 2024 Grant Agency of Czech Republic
Impact of direct endoglin modulation on the development of major complications of metabolic syndrome: atherogenesis and non-alcoholic steatohepatitis.

7.4 Oral/poster presentations related to the topic of the thesis

2019 **Presented poster –** "Endoglin role in endothelial dysfunction after the induction of inflammation."
23rd Congress on Atherosclerosis (CSAT) – Pilsen, Czech Republic.

2020 **Lecture –** "Endoglin expression, signalization and function in inflammation induces endothelial dysfunction."
10th Postgraduate and Postdoc Conference – Hradec Králové, Czech Republic.
Poster – "Endoglin is important for monocytes transmigration through endothelial cells during inflammation."
88th EAS Congress – Geneva, Switzerland.
Presented poster – "Carotuximab affects 7-ketocholesterol induced endothelial dysfunction in HAEC via endoglin? – a pilot study."
24th Congress on Atherosclerosis (CSAT) – Brno, Czech Republic.

2021 **Lecture** – "Carotuximab affects endoglin expression and adhesion and transmigration of monocytes via endothelial cells in 7-ketocholesterol induced endothelial dysfunction."
11th Postgraduate and Postdoc Conference – Hradec Králové, Czech Republic
Poster – "Carotuximab effects on endoglin expression and signaling in 7-ketocholesterol induced endothelial dysfunction in HAECs."
89th EAS Virtual Congress – Helsinki, Finland
Presented poster – "Endoglin blockage is essential in hypercholesterolemia and hyperglycemia-induced endothelial dysfunction in HAECs."
25th Congress on Atherosclerosis (CSAT) – Prague, Czech Republic.

2022 **Lecture** – "Effects of anti-endoglin antibody (TRC105) on endoglin expression, signaling and function in 7-ketocholesterol and high glucose-induced endothelial dysfunction in HAECs."
12th Postgraduate and Postdoc Conference – Hradec Králové, Czech Republic.
Poster – "Critical impact of endoglin blockage in endothelial dysfunction induced by hypercholesterolemia and hyperglycemia in human aortic endothelial cells."
90th EAS Congress – Milan, Italy
Poster – "Blockage of endoglin is crucial in the prevention of endothelial dysfunction induced by hypercholesterolemia and hyperglycemia in aortic endothelial cells."
14th HHT International scientific conference – Cascais, Portugal

8 List of figures

9 List of references

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10 List of annexes

I. Visek J, Blaha M, Blaha V, Lasticova M, Lanska M, Andrys C, Duintjer Tebbens J, Igreja Sa IC, **Tripska K**, Vicen M, Najmanova I, Nachtigal P**. Monitoring of up to 15 years effects of lipoprotein apheresis on lipids, biomarkers of inflammation, and soluble endoglin in familial hypercholesterolemia patients**. Orphanet J Rare Dis. 2021 Feb 27;16(1):110. doi: 10.1186/s13023-021-01749-w. IF=4.307 (Q2); AIS=1.349 (Q2)

II. Vicen M, Igreja Sa IC, **Tripska K**, Vitverova B, Najmanova I, Eissazadeh S, Micuda S, Nachtigal P. **Membrane and soluble endoglin role in cardiovascular and metabolic disorders related to metabolic syndrome**. Cell Mol Life Sci. 2021 Mar;78(6):2405-2418. doi: 10.1007/s00018-020-03701-w. PMID: 33185696. IF=9.207 (Q1); AIS=2.069 (Q1)

III. **Tripska K**, Igreja Sa IC, Vasinova M, Vicen M, Havelek R, Eissazadeh S, Svobodova Z, Vitverova B, Theuer C, Bernabeu C, Nachtigal P. **Monoclonal anti-endoglin antibody TRC105 (carotuximab) prevents hypercholesterolemia and hyperglycemia-induced endothelial dysfunction in human aortic endothelial cells.** Front Med. 2022 Sep 7;9:845918. doi: 10.3389/fmed.2022.845918. PMID: 36160139. IF=5.058 (Q2); AIS=1.233 (Q2)