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Chapter 1 : Morphogenesis of Endothelium : Werner Risau :

*Vascular Endothelium in Human Physiology and Pathophysiology (Endothelial Cell Research) [Patrick J Vallance, David J. Webb] on blog.quintoapp.com *FREE* shipping on qualifying offers. Endothelial dysfunction is now regarded as an early marker of vascular disease and therefore an important target for therapeutic intervention and discovery of novel treatments.*

This is an open access article distributed under the Creative Commons Attribution License , which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Abstract Activation of NF-E2-related factor 2 Nrf2 is a potential therapeutic intervention against endothelial cell oxidative stress and associated vascular disease. We hypothesized that treatment with the phytochemicals in the patented dietary supplement Protandim would induce Nrf2 nuclear localization and phase II antioxidant enzyme protein in human coronary artery endothelial cells HCAECs , protecting against an oxidant challenge in an Nrf2- dependent manner. Nrf2 silencing significantly decreased the Protandim-induced increase in HO-1 protein. These results show that Protandim induces Nrf2 nuclear localization and antioxidant enzyme expression, and protection of HCAEC from an oxidative challenge is Nrf2 dependent. Increased production of reactive oxygen species ROS and oxidative damage in the vascular endothelium contribute to CAD initiation and progression. Specifically, increased vascular superoxide causes oxidation of lipids, decreased nitric oxide availability, increased expression of adhesion molecules and inflammatory mediators, and recruitment of monocytes to the endothelium [5 â€” 8]. Endothelium-bound superoxide dismutase is also decreased in CAD patients compared to healthy controls, impairing the cellular response to excessive ROS production [9]. Atherosclerotic coronary arteries isolated from humans display increased superoxide production compared to nonatherosclerotic human coronary arteries, and in a mouse model of atherosclerosis, attenuation of superoxide production by decreased expression of NADPH oxidase NOX results in a decrease in atherosclerotic lesion size [10 , 11]. Initial studies examining the effects of decreasing oxidative stress in several diseases, including cardiovascular disease, have used exogenous antioxidant supplements such as vitamins C and E. However, the protective effect of exogenous antioxidants has been disappointing and in some cases supplementation increased mortality [12 â€” 14]. A novel approach to decreasing disease-associated oxidative stress involves augmenting endogenous antioxidant defense systems rather than relying on exogenous antioxidant supplementation. Protandim is a commercially available dietary supplement consisting of phytochemicals derived from five widely studied medicinal plants including silymarin from milk thistle, curcumin from turmeric, bacopa extract, ashwagandha, and green tea extract. The five phytochemical components of Protandim have a synergistic effect to induce phase II antioxidant enzymes and protect cells from oxidative stress through activation of the transcription factor NF-E2-related factor 2 Nrf2 [15 , 16]. Nrf2 is constitutively expressed but is marked for ubiquitination by association with Kelch-like ECH-associated protein 1 Keap1 in the cytosol. Activation of Nrf2 occurs when it is released from Keap1 and translocates to the nucleus. In the nucleus, Nrf2 heterodimerizes with small Maf or Jun proteins and binds to the antioxidant response element ARE in the promoter region of several hundred genes including many phase II antioxidant enzymes subsequently initiating transcription [17 , 18]. Protandim likely activates Nrf2 through activation of various kinases with subsequent Nrf2 phosphorylation [16 , 19]. Although acute activation of Nrf2 occurs in vivo in response to oxidized phospholipid signaling, increased ROS production, hyperglycemia, and shear stress [20 â€” 22], in chronic disease states the antioxidant response is often insufficient to maintain redox balance and prevent disease progression [22 â€” 24]. For example, Landmesser et al. In contrast, decreased SOD activity was observed in coronary arteries from CAD patients compared to age-matched controls [9]. Data show that upregulation of phase II antioxidant enzymes can protect against oxidative stress in vitro and in humans [16 , 25 , 26]. It was also recently reported that Protandim protected a human saphenous vein ex vivo culture from oxidative stress-induced hyperplasia and vessel wall thickening [

27]. Thus, phytochemical-induced Nrf2 activation is a potential therapeutic intervention against endothelial cell oxidative stress and associated vascular disease initiation and progression. Limited research 8 publications exists examining whether Protandim treatment can minimize the pathologies associated with chronic diseases. The effects of Protandim on Nrf2 and oxidative stress in human coronary vascular cells have not been investigated. The purpose of this study was to determine 1 if treatment with Protandim-induces Nrf2 nuclear translocation and phase II antioxidant enzyme protein expression in human coronary artery endothelial cells HCAEC , 2 if treatment with Protandim protects HCAEC from apoptosis induced by an oxidant challenge, and 3 if Nrf2 mediates Protandim induced protection from an oxidative challenge. We hypothesized that Protandim treatment would induce Nrf2 nuclear localization and phase II antioxidant enzyme protein expression, and Protandim treatment prior to an oxidant challenge would afford cells protection in a Nrf2 dependent manner. Materials and Methods 2. Protandim treatments ranged from 1 hr to 12 hrs as indicated, and H2O2 treatments were 4 hrs. Protein concentrations were determined using a BCA assay, and samples were diluted with Laemmli sample buffer. Cells were initially treated with Protandim for 1 hr, 2 hrs, 4 hrs, 8 hrs, and 12 hrs to determine the optimal duration of treatment for visualizing Nrf2 nuclear localization. Following time course experiments, all Protandim treatments were for 1 hr. The coverslips were mounted on slides using DAPI containing mounting medium for identification of cell nuclei and visualized by fluorescence microscopy Nikon TE using Metamorph data acquisition software Molecular Devices, Sunnyvale, CA. Cells were grown to confluence on fibronectin-coated coverslips prior to Protandim and H2O2 treatment. The coverslips were then mounted on slides using DAPI containing mounting medium to identify cell nuclei. Signals were visualized using fluorescence microscopy Nikon TE Nrf2 siRNA or control RNA was added to the transfection solution for a final concentration of 50 nM and incubated at room temperature for 15 min. The volume of transfection reagent used caused minimal distress to the cells as assessed by minimal changes to cell morphology. After 24 hrs, the transfection solution was removed and the cells were rinsed with PBS, treated with Protandim, and assayed as indicated. Statistical Analysis Unpaired t-tests were used to compare control versus Protandim treatments. Statistical significance was set at. HO-1 protein was visible after 1 hr of Protandim treatment and became significant and sustained from 4 hrs through the longest treatment period of 12 hrs data not shown. The HO-1 signal was verified using a purified HO-1 protein extract positive control. Nrf2 content and nuclear localization were elevated as soon as 1 hr after Protandim treatment initiation. Induction remained with treatments of 2, 4, 8, and 12 hrs 12 hrs was the longest treatment duration examined Figure 2. Protandim induced Nrf2 expression and nuclear localization. HCAECs were treated with Protandim for 1 hr, following which immunofluorescence was used to visualize changes in Nrf2 expression and localization. Following Protandim treatment Nrf2 signal was greater and became visible in the nucleus. Protandim was removed prior to H2O2, exposure. Figures 4 a and 4 b. Silencing of Nrf2 abrogated Protandim-induced increases in HO-1 expression. Nrf2 was then silenced prior to Protandim treatment and an oxidative challenge. The number of apoptotic cells in the no RNA condition compared to the control RNA condition was not significantly different with or without Protandim no Protandim, with Protandim. Protandim prior to Nrf2 siRNA also significantly protected cells from apoptosis ; however the amount of protection afforded by the Protandim in this condition was significantly less than in no RNA and control RNA conditions and , resp. Protandim treatment following Nrf2 silencing protected HCAEC against an oxidative challenge; however, protection following Nrf2 silencing was significantly diminished compared to controls. Discussion The novel findings of this study were that Protandim treatment of HCAEC induced Nrf2 nuclear localization and phase II antioxidant enzyme expression, Protandim treatment prior to an oxidative challenge was protective against apoptosis, and this protection was dependent on Nrf2. Our data show that the phytochemical components of Protandim increase Nrf2 in the cytosol and nucleus of HCAEC within an hour of treatment. In cardiac myocytes we have observed increases in Nrf2 within 15 minutes of Protandim treatment unpublished data. Nrf2 remains elevated through treatment durations up to 12 hours, which was the longest duration examined. Protandim Induction of Antioxidant Enzymes The components in Protandim work synergistically

to activate Nrf2 and induce antioxidant enzyme expression. Until recently, the antioxidant properties of HO-1 were thought to be through production of the ROS scavenger bilirubin, but data now suggest HO-1 may have other antioxidant qualities and be important in multiple cell types in the vasculature. It has also been demonstrated in macrophages that HO-1 functions as an antioxidant by decreasing heme availability, which decreases expression of the NADPH oxidase heme containing subunit and subsequently decreases superoxide production [28]. Decreasing NOX superoxide production has important implications in both macrophages and endothelial cells. Substantial evidence exists demonstrating a role for NOX and increased superoxide production in obesity and atherosclerosis. Compared to nonobese controls, overweight, and obese subjects demonstrate increased NOX subunit expression and augmented oxidative stress in the vascular endothelium [29]. NOX subunit expression is elevated in lesions of coronary arteries in bypass graft patients, particularly in the vicinity of macrophages, and NOX expression levels correlate with severity of atherosclerosis [10]. Whether HO-1 also functions as a protective antioxidant in endothelial cells through decreasing heme availability, inhibiting NOX, and decreasing superoxide production, or via an alternative mechanism also is yet to be determined. Greater baseline HO-1 expression in aortic endothelial cells also results in decreased effects of oxidized phospholipids on inflammatory genes [31], suggesting that presence of HO-1 may delay progression of disease-related phenotypic changes when cells are faced with chronic lipid and oxidative stresses. While it was beyond the scope of this study, determining whether HO-1 is essential to the Nrf2-dependent Protandim induced protection against an oxidative challenge is warranted. These are the first data indicating increases in GR and NQO1 in response to Protandim; increases in SOD1 expression and activity have been previously reported [15 , 27]. In an ex vivo preparation of human saphenous veins, Protandim treatment increased SOD activity 3-fold, HO-1 activity 7-fold, and catalase activity fold [27]. Catalase has been shown to be increased by Protandim and to mediate protective effects in erythrocytes and saphenous vein preparations, as well as protect against skin cancer development in an animal model [15 , 27 , 32]. Collectively, these data suggest that the antioxidant enzymes increase following Protandim treatment in a cell type specific manner. There are multiple potential explanations for this observation. First, diminished but incomplete protection may be due to the rapid turnover rate in Nrf2. Nrf2 is constitutively expressed and rapidly degraded. In unstimulated cells the half-life of Nrf2 is less than 30 minutes [33]. Second, the amount of siRNA that can be delivered is limited by the ability of the cells to tolerate the transfection procedure. The capacity of the delivered Nrf2 siRNA to silence Nrf2 may be exceeded by the previously noted continuous turnover and subsequent increases in response to Protandim. Finally, antioxidant enzymes can also be activated independently of Nrf2 by other proteins including p53 and sirtuins [34 , 35]. However, our data show that significantly more cells undergo apoptosis in response to an oxidative challenge if Nrf2 is silenced regardless of Protandim treatment indicating the importance of Nrf2 in cytoprotection. We used apoptosis in endothelial cells as an outcome because it has direct translation to vascular disease development and outcomes. Endothelial cell apoptosis contributes to plaque progression and rupture and may be an independent risk factor for thrombosis [36]. Endothelial cell apoptosis can be induced by oxidized lipids as well as by ROS, and the role of endothelial cell apoptosis in atherosclerosis has been extensively reviewed [37 â€” 39]. Translation and Future Studies Our data suggest positive effects of Protandim in healthy coronary artery endothelial cells supporting future examination of how Protandim may affect cells that have been chronically exposed to oxidative and lipid challenges. Therefore, it is of interest to determine if Protandim can slow or reverse disease related endothelial cell phenotypic changes using in vivo models of chronic oxidative stress. Supplementation with Protandim in humans is safe, with no reported adverse side effects [15 , 16]. It has been shown that with oral Protandim supplementation in humans, circulating TBARs, a measure of lipid peroxidation, decrease in 5â€”12 days, an effect that persists with continued supplementation as measured at 30 and days [15]. In addition, erythrocytes isolated from subjects who ingested Protandim for days had greater SOD and catalase activity compared to controls [15]. Thus the effects observed in our cell culture model directly mirror those seen in vivo indicating that exposure of cells to the components of Protandim in

human subjects is likely similar to what we used in vitro. Studies in vivo will need to be performed to determine if these results are translatable to intact coronary arteries. Conclusion Our current investigation shows for the first time that Protandim treatment in HCAEC induces Nrf2 nuclear localization, phase II antioxidant enzyme expression, and Nrf2-dependent protection from an oxidant stress. Oxidative stress has a well-established role in CAD initiation and progression, and our data support further research on phytochemical activation of Nrf2 and the endogenous antioxidant response as a potential therapeutic approach.

Chapter 2 : Endothelium | Revolvy

Indeed, abnormal activity of a number of endothelial mediators may be a feature of many forms of cardiovascular disease. The present understanding of the role of the endothelium in health and disease has resulted from both animal experiments and cl.

Advanced Search Abstract The endothelium plays a central role in cardiovascular regulation. Endothelial cells produce a variety of vasculoregulatory and vasculotropic molecules that act locally or at distant sites. Alteration of the vascular endothelium is a primary event in the pathogenesis of vascular diseases, such as atherosclerosis, as well as systemic and pulmonary hypertension. For these reasons, the study of human endothelium has become central in cardiovascular research. Unfortunately, technologies handling endothelial cells in vitro are often criticized due to the uncertain transferability of results to intact organs and, importantly, to humans. Although methods to assess endothelial function non-invasively have been available for decades, cell-based approaches for the direct ex vivo evaluation of endothelial cell biology in humans have been devised only recently. The discovery of endothelial progenitor cells opened the way for studies on vascular regeneration, while it has been recognized that mature circulating endothelial cells mainly represent a consequence of the ongoing vascular damage. Coupled with a minimally invasive way to obtain fresh human endothelial cells through an endovascular biopsy, these new methods provide a novel outlook on human endothelial cells as close as possible to their natural environment. In this review, we will summarize the current knowledge and the methodological perspective of these cell-based methods. Endothelial cell biology and pathology Once viewed as a passive inner cover of the vessel wall, the endothelium is now recognized as an active organ with many important functions, such as regulation of vascular tone and permeability, coagulation, inflammation, and angiogenesis. For this reason, the assessment of the endothelium has progressively become a cornerstone of modern cardiovascular research. They produce a number of vasoactive substances that can mediate either vasoconstriction e. Endothelial cells are exposed to a variety of nutritive and toxic substances carried by the bloodstream, thus being easily subject to pathological alterations. This gas is derived from l-arginine through a reaction catalysed by a family of enzymes, called the endothelial NO synthases eNOS. Indeed, endothelial dysfunction can be associated with apoptosis of endothelial cells, which tend to detach from the vessel wall, leaving a thrombogenic and pro-inflammatory subendothelial surface. This kind of research needs reliable methods to assess the anatomical and functional integrity of the endothelium. Although this can be easily explored in animals, it is much more difficult to have direct information on endothelial biology in humans. Endothelial function is usually assessed non-invasively as the NO-dependent vasodilatory capacity of a given arterial segment to an endothelial stimulus, such as shear stress [e. As a surrogate of the human endothelium, most researchers use cultured human endothelial cell lines, which allow extensive and detailed biochemical studies and manipulations. However, uncertainties always remain regarding the transferability of results obtained in vitro to the in vivo clinical picture. In this review, we will discuss three relatively new cell-based methods to study endothelial cells [circulating endothelial cells CECs , circulating endothelial progenitor cells EPCs , and endothelial biopsy with the aim of providing some indications for a comprehensive evaluation of endothelial biology in humans. Circulating endothelial cells Mature endothelial cells circulate in the bloodstream at a very low concentration. The exact origin of these CECs is presently not entirely defined. It is believed that they are derived from the vessel wall itself, by sloughing of resident endothelial cells into the circulation, as part of their normal turnover process, or as an effect of damaging factors, either mechanical e. In more recent times, the clinical and pathophysiological meaning of these cells has gained renewed attention. Resident endothelial cells might detach from the vessel wall as viable integral cells or as cell fragments. As a result, CECs often display features of apoptotic cells, such as externalization of phosphatidylserine and binding of the early apoptotic marker Annexin-V, or frank evidence of cell death, such as loss of plasma membrane integrity and propidium iodine staining. Moreover,

enhanced release of CECs might also take place during normal body growth or at sites of tissue regeneration, either physiological e. In normal steady-state conditions, the amount of CECs in the bloodstream is very low, due to the fact that endothelial turnover is a very slow process in the absence of pathological stimuli and that non-viable CECs are likely rapidly cleared by the reticulo-endothelial system. The level of CECs is expected to increase as a consequence of any type of damage to the vessel wall. Obviously, CEC measure cannot distinguish where the cells come from and therefore the site of endothelial damage, e. Anyway, a high CEC level, for instance, in the setting of acute coronary syndrome, has been shown to identify subjects at higher risk for subsequent cardiovascular events. This method is based on the incubation of blood cells with dynabeads conjugated to an anti-endothelial antibody, such as anti-CD In addition, the enumeration of CECs fulfilling the above-described criteria requires time-consuming microscopic inspection and is not automated. More recently, immunomagnetic bead isolation has been almost completely replaced by a flow cytometry approach that can be fully standardized and automated. After excluding cell debris with a morphological gate, cells are stained with the pan-leucocyte antigen CD45 to exclude haematopoietic cells and with a nuclear dye e. The remaining cells are assayed for the expression of the endothelial antigen CD in this case, contamination by T-lymphocytes is ruled out by CD45 negativity. In addition, they have shown by a molecular biology approach that these cells express vascular endothelial VE -cadherin mRNA. Despite the many advantages of flow cytometry over immunomagnetic bead selection, it should be recognized that no single marker can unambiguously identify CECs. Notwithstanding these limitations, it appears that the methods to analyse CECs have been substantially improved over the last years and that a reliable approach is available, based on flow cytometry. It should be, however, noted that a considerable amount of work is usually needed to achieve a good standard in each laboratory and that the learning curve can be long and demanding.

Chapter 3 : Endothelium: Journal of Endothelial Cell Research

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The expression of 6 of these structures -1,6-GlcNAc branching, high-mannose N-glycans, N-acetyllactosamine, -2,6-sialic acid, -1,4-galactose increased significantly after TCM treatment. In particular, the -1,6-GlcNAc branching glycan expression level was greatly elevated after the stimulation [7]. This -1,6-GlcNAc branching glycan was demonstrated to initiate endothelial cell contraction and gap formation, and these events lead to subsequent biological events such as tumorigenesis. Increasing surface N-linked mannose by inhibiting N-glycan processing potentiated monocyte adhesion under flow during TNF-stimulation. Conversely, enzymatic removal of high-mannose N-glycans or masking mannose residues with lectins significantly decreased monocyte adhesion under flow. These results, therefore, indicate that surface N-linked mannose on ECs is a novel ligand for monocyte adhesion during atherogenesis. Glycosphingolipids A glycosphingolipid GSL is composed of a glycan structure attached to a lipid tail containing the sphingolipid ceramide. GSLs are widely expressed on cell membranes in lower and higher eukaryotic organisms. GSLs have frequently been used as important developmental marker molecules and have been suggested to have important biological functions [3 , 9]. By contrast, IL-1 increases the cell content of neutral and acidic GSLs but does not alter their surface expression. ECs are believed to play an important role in the pathogenesis of hemolytic uremic syndrome HUS. EC damage by Escherichia coli verocytotoxin in vitro is potentiated by additional exposure to inflammatory mediators such as TNF-. ECs demonstrated that the level of EC sensitivity to verotoxin depends on the expression of Gb4Cer synthase [14]. This suggests that LacCer is important in VEGF-implicated angiogenesis associated with coronary heart disease, vascular complications in diabetes, inflammatory vascular diseases, and tumor metastases. The functional involvement of other GSLs in angiogenesis is also implied. Thus, this report concludes that GM3 has antiangiogenic action in ECs and may possess therapeutic potential for reducing tumor angiogenesis. This paper indicates the functional importance of SGPG expression for brain-associated ECs in neuroinflammatory diseases. Recently, the SGPG cell signaling pathways were investigated. Proteoglycans Proteoglycans PGs are macromolecules composed of a specific core protein substituted with covalently linked glycosaminoglycan GAG chains, namely, chondroitin sulfate CS , dermatan sulfate DS , and heparan sulfate HS. GAGs are linear, negatively charged polysaccharides comprised of repeating disaccharides of acetylated hexosamines N-acetyl-galactosamine or N-acetyl-glucosamine and mainly uronic acids D -glucuronic acid or L -iduronic acid sulfated at various positions. In HA, there are no chemical modifications such as sulfation and epimerization. PGs can be classified into two main groups according to their localization: The secreted group consists of PGs involving large aggregating PGs, namely, hyalectans e. Cell-surface-associated PGs are divided into two main subfamilies: PGs perform numerous biological functions, act as structural components in tissue organization, and affect several cellular parameters, such as cell proliferation, adhesion, migration, and differentiation. PGs interact with growth factors and cytokines, as well as with growth factor receptors, and they are implicated in cell signaling. Heparan Sulfate HS To clarify the roles of HS in endothelium under pathological conditions, HS expression on human ECs was studied following stimulation by inflammatory or hyperglycemic conditions. Inflammation is pivotal in atherosclerosis, and a key early step is endothelial dysfunction. Also, C-reactive protein, the prototypic marker of inflammation and cardiovascular risk marker, has been shown to promote atherogenesis, and increased levels of C-reactive protein are associated with endothelial dysfunction. C-reactive protein treatment caused the expression of HS to significantly reduce [24]. In diabetes, the endothelium is exposed chronically or transiently to hyperglycemic conditions. In addition, endothelial dysfunction in diabetes is related to changes in the inflammatory response and turnover of the extracellular

matrix. In hyperglycemic conditions, short-term inflammatory stimuli affected both the size and the sulfation pattern of HS on ECs, with the outcome depending on the type of stimulus [25]. Thus, modification of HS under pathological conditions is considered a major cause of endothelial dysfunction, resulting in the disturbance of vascular integrity and barrier properties, due to decreased negative charge and increased permeability, and the consequent release of bioactive substances such as cytokines, enzymes, and growth factors. To date, several studies have investigated the binding capabilities of HS on human ECs. In another study, P. Binding via HS followed by sequestration may be related to the severity of the diseases. Functional studies of HS on human ECs have been performed. HSulf-1 downregulation also enhanced downstream signaling through the extracellular signal-regulated kinase pathway, when compared with untreated cells [30]. Chondroitin Sulfate CS CD97, which is highly expressed on various inflammatory cells and some carcinomas, contributes to inflammation-mediated angiogenesis and possibly tumor progression. CD97 acts as a potent chemoattractant for the migration and invasion of ECs, and this function is integrin dependent. Hyaluronan HA Chronic inflammation has a critical role in the onset of several diseases, including atherosclerosis, and endothelial dysfunction is a key early step in these diseases. A study [33] has shown that the expression of HA on HUVECs is induced by IL, which has been suggested to play a role in the setting of the chronic autoimmune disease, particularly in the recruitment and activation of synovial T-cells. The results of the study suggest that IL can regulate EC function and thereby enable a CDinitiated adhesion pathway that facilitates the entry of activated T lymphocytes into inflammatory sites. In HAECs, C-reactive protein, the expression of which is related to inflammation as well as endothelial dysfunction, dose dependently increased HA release. This is thought to result in EC dysfunction by, for example, increasing monocyte-EC adhesion [24]. Moreover, it has been verified that U monocyte adhesion to stimulated ECs depends strongly on HA [34]. Thus, in chronic inflammation, elevation of HA expression via inflammatory stimulation promotes adhesion of leucocytes to ECs, resulting in vascular-related diseases, such as atherosclerosis. Recent in vivo studies revealed that the inner blood vessel surface is lined with an endothelial surface layer at least 0. HA seems to be an essential component that is related to the atheroprotective properties of this surface structure. It has also been shown that HA is increased in a shear-stress-dependent manner via the phosphatidylinositol 3-kinase-Akt pathway [35 , 36]. In particular, pulsatile, arterial-like shear stress conditions effectively induced HA [36]. Thus, fluid shear stress stimulates the incorporation of HA into the glycocalyx, which may contribute to its vasculoprotective effects against proinflammatory and pro-atherosclerotic stimuli. Extracellularly Secreted PGs 4. The functions of HS in endothelial perlecan were further investigated [38 , 39]. The studies show that the binding abilities of FGF-1 and FGF-2 to endothelial perlecan differ depending on the HS structures in the different cell types. Hyperglycemia is an independent risk factor for diabetes-associated cardiovascular disease. One potential mechanism involves hyperglycemia-induced changes in arterial wall extracellular matrix components leading to increased atherosclerosis susceptibility. In addition, endothelial dysfunction in diabetes is related to changes in the inflammatory response and the turnover of extracellular matrix. In hyperglycemic conditions, secretion of perlecan from ECs was increased after IL-1 stimulation [25]. Perlecan, but not other HSPGs, is dramatically downregulated in ECs treated with antiangiogenic cleaved and latent forms of antithrombin [41 , 42]. The previously established key role of perlecan in mediating FGF-2 stimulation of EC proliferation and angiogenesis suggests that a primary mechanism by which antiangiogenic antithrombins exert their effects is through the downregulation of perlecan expression. The role of perlecan in the antiangiogenesis function of NK4, an angiogenesis inhibitor, was also studied [43]. In this study, knockdown of perlecan expression in ECs by RNAi significantly reduced the inhibitory effect of NK4 on fibronectin assembly and cell spreading. Thus, this study indicates that the association of NK4 with perlecan plays a key role in angiogenesis inhibition by NK4. The results suggest that endocan is preferentially expressed in tumor endothelium in vivo and that its expression is regulated by tumor-derived factors. Now, it is highlighted that endocan is a marker of EC activation during growth of the new vessels required for tumor progression [46]. Decorin Decorin, a member of the small leucine-rich repeat proteoglycan SLRP family, is

expressed by sprouting ECs during inflammation-induced angiogenesis in vivo and by human ECs cocultured with fibroblasts in a collagen lattice. As function of decorin in human ECs, it has been reported that decorin core protein can bind to and activate insulin-like growth factor-I receptor IGF-IR [48] and that decorin promotes 2 1 integrin-dependent EC adhesion and migration on fibrillar collagen type I [49]. It is now understood that modulation of cell-matrix interactions by decorin plays a key role during angiogenesis [50]. Induced versican synthesis and de novo V3 expression were also observed in ECs induced to migrate in a wound-healing model in vitro and in angiogenic ECs forming tubule-like structures in Matrigel or fibrin clots. Thus, in activated conditions, versican expression in human ECs is altered [51]. This study indicates that versican produced from ECs plays a key role in the pathological conditions such as inflammation, angiogenesis, and wound healing. TNF- , responsible for changing the morphology of the cells from a polygonal to a spindle shape and for stimulating the detachment of the cells from the culture dish, markedly decreased the synthesis of biglycan. Although the functional roles of biglycan and PG are not yet clearly understood, their different responses to the stimuli may be critical for the progression of vascular diseases. Another study has shown that antiangiogenic antithrombin treatment significantly decreases biglycan in HUVECs [42], suggesting that a mechanism of antiangiogenic antithrombins is through the downregulation of proangiogenic biglycan. Cell Surface PGs 4. Glypicans Glypican-1 is the only glypican expressed in the vascular system. Recently, the contribution of glypican-1 to the cell cycle and proliferation has been demonstrated in ECs [54]. Syndecans Proteoglycans PGs are important constituents of the plasma membrane and of the basement membrane supporting the EC layer. Changes in the amounts or the structures of PGs in the endothelium may affect important functions, such as turnover of lipoproteins, filtration properties, and regulation of chemokines during inflammation, which are all relevant to diabetes. In HUVECs, exposure to high glucose hyperglycemic condition leads to decreased secretion of syndecan-1 [55]. Thrombospondin-1 TSP-1 , an extracellular matrix protein, modulates focal adhesion in mammalian cells and exhibits dual roles in angiogenesis. There are indications that binding of TSP-1 to syndecan-4 proteoglycan mediates tubulogenesis and their protection from apoptosis [56]. Syndecan-2, the major syndecan expressed by human microvascular ECs HMECs , is regulated by growth factors and extracellular matrix proteins, in both bidimensional and tridimensional culture conditions [57]. Downregulation of syndecan-2 reduced the spreading and adhesion of HMECs, and it not only enhanced their migration but also impaired the formation of capillary-like structures. Therefore, syndecan-2 has an important function in some of the necessary steps in the angiogenic process. Syndecan-1 is a critical regulator of v 3 and v 5 integrins during angiogenesis and tumorigenesis, and it is inhibited by the novel peptide called synstatin [58]. Galectins Galectins are a family of lectins that bind to -galactosides via a carbohydrate recognition domain containing many conserved sequence elements [59]. There are currently 15 known mammalian galectins [60], which are involved in a variety of biological processes [61]. Expression of galectin-1 in cultured human ECs was first shown in by Baum et al. Treatment of cells with dsRNA in vitro mimics viral infection and regulates the expression of various genes. Thus, it has been proposed that upregulation of galectin-9 expression by poly IC in the vascular endothelium may be part of the mechanism for leukocyte trafficking through the vascular wall after viral infection. Subsequently, galectin-1, -3, -8, and -9 expression levels in quiescent ECs were measured, and the expression and distribution of these galectins changed after activation with tumor-derived culture medium [64]. Recently, it was reported that galectin-9 protein expression is positively regulated by histone deacetylase 3 in ECs [65]. Some functional analyses of galectins in human ECs have been reported. Cancer-associated carbohydrate T antigen plays a leading role in docking breast and prostate cancer cells onto endothelium by specifically interacting with EC-expressed galectin-3 [66]. Thus, D-LDO may be a new galectin inhibitor for blocking angiogenesis [67]. A direct interaction was detected on the plasma membrane between galectin-3 and VEGF-R2, and this interaction was dependent on the expression of Mgat5.

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While much has been written within recent years about the role of endothelial cells in cardiovascular control, it is unusual to find an entire volume focused on the physiology and pathophysiology of the endothelium in humans.

Chapter 5 : Glycoconjugates and Related Molecules in Human Vascular Endothelial Cells

Hypercholesterolemia, hypertension, and insulin resistance contribute to endothelial dysfunction and inflammation in the vascular wall, as well as to increased lipoprotein oxidation, smooth muscle cell proliferation, extracellular matrix deposition, cell adhesion, and thrombus formation [].