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*Review*

# Pathophysiological Aspects of Vascular Remodeling in Cardiopulmonary Lesions: Influence of Inhibitor of DNA Binding/Differentiation-3 (ID3) & Estrogenic Endocrine Disruptors

**Vincent Avecilla[1*], Mayur Doke[1]**

[1]Department of Environmental & Occupational Health, Florida International University, Miami, FL, USA
*Correspondence should be addressed to Vincent Avecilla; vavec001@fiu.edu

**Abstract** : Cardiopulmonary lesions, which manifest from various types of diseases such as pulmonary arterial hypertension, atherosclerosis, pulmonary arteriovenous malformations, lymphangioleiomyomatosis, and peripheral arterial disease, pose a public health problem. Vascular remodeling, which refers to alternations to the structure of the vessel is an important pathophysiological feature of these diseases. The Inhibitor of DNA-binding/Differentiation-3 (ID3), which is part of the ID family of transcriptional regulators, has been demonstrated to play an essential role in the vasculature and therefore may influence the alterations of these lesions. This review will highlight the existing understanding of how ID3 may contribute to cardiopulmonary lesion perturbations via involvement in vascular remodeling. Furthermore, based on the accumulative number of reports that suggest oxidative stress plays a critical role in the pathophysiology of vascular remodeling, we will also consider the impact of exposure to estrogenic endocrine disruptors (EEDs) such as polychlorinated biphenyls (PCBs) and bisphenol A (BPA) on ID3 & cardiopulmonary disease. Improved understanding of how ID3 pathways contributes to these molecular mechanisms in the lesion will likely provide useful knowledge in the mediation of vascular remodeling associated with ID3 & EED exposure, which may play an essential role in cardiopulmonary disease prevalence.

Keywords: cardiopulmonary lesions; endocrine disruptors; ID3; vascular dysfunction; vascular remodeling

## 1. Introduction

Inhibitor of DNA Binding/Differentiation-3 (ID3) is a transcriptional regulator known to prevent stem cell differentiation and promote cell cycle progression. A member of the ID family of helix-loop-helix proteins programmed by an immediate-early gene responsive to oxidative stress and mitogenic signals, ID3 via accumulative evidence suggests that it may be involved in vascular remodeling, a process in which alterations in the structure of the vessel and vessel wall occur including four processes: cell death, cell growth, synthesis or degradation of the extracellular matrix, and cell migration [1-4]. Vascular remodeling is supported upon dynamic interactions between hemodynamic stimuli, vasoactive substances, and growth factors, which may contribute to the pathophysiology of cardiopulmonary disease and cardiac disorders [5]. For instance, ID3 has been studied in combination with lipoxygenase (12/15-LO), which is demonstrated to produce pro-inflammatory changes in blood vessels that lead to the development of atherosclerosis [6]. Furthermore, 12/15-LO has been seen as an essential intermediary of VSMC growth and its growth-

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promoting effect has been established to be mediated by ID3 transcription [7]. With regard to these vasculature interactions, it is also significant that ID3 is important to embryonic vasculogeneis as well as endothelial cell activation [1-3].

Thus, we intend to discuss the current understanding of how ID3 may influence these various components in vascular remodeling that lead to alterations in the vessel causing obstruction & further damage. Since many of these vascular remodeling mechanisms are oxidative stress dependent [4], exposure to estrogenic endocrine disruptors (EEDs) may also contribute to perturbations that take place during vascular remodeling. Estrogenic endocrine disruptors are mainly synthetic chemicals ubiquitously discovered in our environment that act by modifying hormonal events. EEDs such as estrogenic polychlorinated biphenyls (PCBs), Bisphenol A (BPA), phthalates, and diethylstilbestrol (DES) have been implicated to interfere with metabolic health during vital periods of development and adulthood. Epidemiological studies have reported associations between estrogenic endocrine disruptors and various cardiovascular & vascular diseases [8-10]. Based on recent findings that demonstrated ID3 dependent endothelial cell activation via estrogenic PCB153 exposure, we will discuss how exposure to estrogenic endocrine disruptors (EEDs) may contribute to complex vascular lesions, in which cardiopulmonary disease manifest from via ID3 [11]. Previously, we elucidated association between ID3 & EEDs in metabolic syndrome (MetS) perturbations via adipose tissue that bioaccumulate, which can modify various chronic diseases such as vascular, neurological, cancer, and autoimmune [12-13]. This may help clarify additional factors contributing to vascular alternations in the blood vessel. Further study in these areas may reveal novel avenues of therapeutic modalities as well as provide prevention, control, & treatment strategies of vascular remodeling with exposure to estrogenic endocrine disruptors & dysregulation of ID3.

## 2. Inhibitor of DNA-Binding/Differentiation-3 (ID3)

ID3 (Inhibitor of DNA-Binding/Differnation-3) is part of a family of small proteins that comprise of four genes (ID1, ID2, ID3, & ID4). This family share a widespread amino acid sequence homology within their HLH (helix-loop-helix) domain (69-78%), however the remaining constitutes are non-related. Originally identified as a serum-inducible immediate-early gene, ID3 peaks transcriptionally at 1 hour. Subsequently, expression of ID3 has been identified to be biphasic with maximal stimulation at 1 hour succeeding a secondary burst at 24 hour in context of tissue regeneration after injury [14-15]. Previous experimental studies have revealed the importance of ID3 in cell differentiation and embryonic development. Dual ID1-ID3 knockout mice have demonstrated cardiac deficiencies, neuronal differentiation, and deviant vascularization of the brain that were embryonically fatal [16-17]. ID3 is highly expressed in embryonic tissue however decreases as cells differentiate. Expression of ID3 has been reported to be induced by various stimuli in numerous cell types [18]

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Previously, Felty and Das demonstrated that vascular endothelial cells exposed to E2 (17β-estradiol) or estrogenic polychlorinated biphenyl 153 (PCB153) resulted in protein phosphorylation, endothelial neovascularization, and increased ID3 expression. Furthermore, PCB153 increased oxidative stress or ROS (reactive oxygen species) that facilitate ID3 expression. Estrogenic chemical exposure has been demonstrated to increase ROS, altering surrounding DNA essential for transcriptional stimulation of cell growth genes. ID3 protein-protein interactions occur via the HLH motif. During this, ID proteins dimerize and block the DNA binding activity of basic HLH transcription factors such as E proteins, which include: E12, E47, E2-2, and HEB, encoded by the TCF3, TCF4, and TCF12 gene [19]. ID3 protein-protein interactions can regulate transcription by E-proteins preventing subsequent binding and activation of target gene promoters. Furthermore, E-proteins suppress the expression of embryonic genes SOX2, OCT4, and NANOG leading to cell differentiation as demonstrated in Figure 1 [19]. Additionally, ID3 promotes cells to pass via cell cycle checkpoints by inhibiting the expression of cell cycle inhibitor gene p21Cip21. Research has demonstrated that ectopic overexpression of ID3 increased SOX2 and OCT4 expression and resulted in cell population positive for molecular stem cell markers $CD133^{+}$ $VEGFR3^{+}$ CD34. Based on these findings, ID3 maintains cells in a noncommittal or undifferentiated state by preventing the repression of pluripotency factors by TCF3 [20-21]. Since ID3 is a transcriptional regulator of genes involved in stemness and cell proliferation, it is plausible for EEDs to contribute to vascular remodeling mechanisms such as uncontrolled proliferation through ID3, thus contributing to the prevalence of cardiopulmonary lesions, in which vascular diseases manifest from.

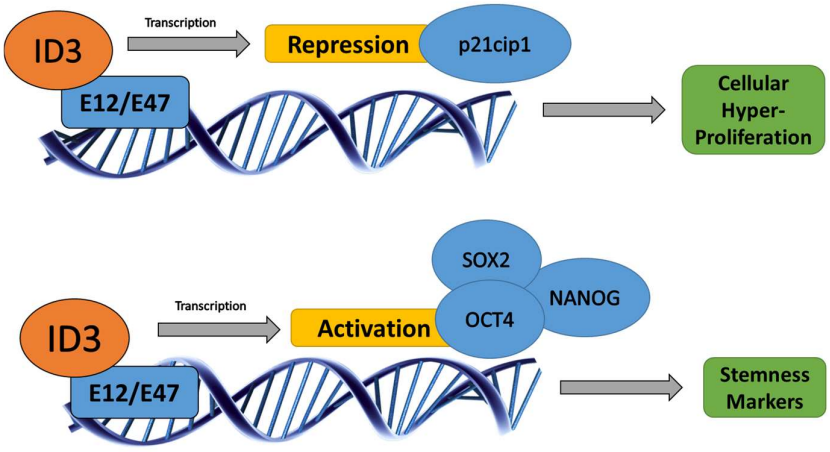


**Figure 1.** ID3 Transcriptional Regulation. Figure illustrates various roles in cellular functions such as apoptosis, angiogenesis, differentiation, & cellular growth. ID3 regulates transcription genes such as p21cip1, OCT4, SOX2, & NANOG.

### 3. Vascular Remodeling

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### 3.1 Vessel Patterning & Perturbations

Cardiopulmonary lesions manifest from both proliferative and obliterative vascular diseases, which are categorized by the disruption of the normal patterning of vessels and their cellular components. Various pathways that lead to these end-stages are infrequently noticeable. Due to the assembly of normal vessels that may trace development, developmental biology, which combines the embryologic component of cell-to-cell interactions, cellular origins, & lineage with genetic studies, holds a possibility of demonstrating the role of various individual genes in pathogenesis [22]. The endothelium of the blood vessel plays a crucial role in considerations of cardiopulmonary diseases. A vital foundation of current vascular biology is that the endothelial lining is a changeable interface [22]. Overall, the endothelium appears at best to function in this specific capability given its distinctive position between blood & tissue as well as its ability to generate biological effectors. Certain aspects such as imbalances in the interaction and production of these different mediators appear to contribute and influence various vital functions of the endothelium. One particular aspect, the role as a non-thrombogenic vessel for not only blood but anti-inflammatory, antithrombotic, & growth inhibitory behaviors help to maintain the reliability of the vascular wall, in the face of numerous disease risk factors and stimuli that may lead to injury [22]. The vessel is comprised of three structural layers. The innermost layer, the tunica intima is lined with endothelial cells that attach and produce to the basal lamina with collagen type IV and laminin supported by an elastic lamina. These are anchored and connected by a collagen fiber network, elastic fibers, and fibrillin [23-24]. The middle layer, the tunica media is mainly composed of smooth muscle cells and elastin, which is arranged in a 3-D continuous network between collagen fibers and thin layers of proteogylycan-rich extracellular matrix (ECM) [25]. The outermost layer, the tunica adventitia is a collagen-rich area comprised of myofibroblast cells. The high content of collagen fibrils (chiefly collagen types I and III) helps prevent vascular rupture at high pressures. The overall amount of collagen determines the tensile strength of the artery.

### 3.2 Inflammatory & Vascular Dysfunction

An essential inflammatory process categorizes numerous cardiopulmonary diseases. The pathogenesis of these cardiopulmonary lesions consists of a variable increase in numbers of both lymphocytes & macrophages and intimal smooth muscle cell proliferation. This response can be attributed to perturbations in the signal transduction events that regulate inflammation. Outcomes of this response consist of generation of reactive oxygen & nitrogen species, which can lead to cellular proliferation, antithrombotic properties that can be altered, and abnormal vascular plasticity. While procedures (invasive) are essential in some cases, lifestyle adjustments (physical activity & diet) & medical treatment result in benefits that may contribute to decrease in inflammatory process production [26]. Endothelial cells (EC) prevent adhesion of leukocytes. Nonetheless the perturbations of atherosclerosis can initiate various expressions of adhesion molecules on ECs causing leukocyte adhesion to the arterial wall. VCAM-1 (Vascular Cell Adhesion Protein-1), a protein that acts as a

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mediator and functions in leukocyte-endothelial cell sign transduction is a key part of this interaction. Oxidized lipids has the ability to prompt gene expression by the pathway initiated by the NF-$\kappa$B (nuclear transcription factor $\kappa$B) such as TNF-$\alpha$ and IL-1$\beta$ [27]. Increased levels of cell adhesion molecules (CAM) are predicative of cardiac events and are an independent risk factor in men with coronary disease [4].

Chemokines are cytokines responsible for intermediating the maturation, differentiation, & migration of cells involved in inflammatory response. Chemokines can also promote ROS (reactive oxygen species) production and various cytokines via leukocyte penetration of the vessel wall. One particular chemokine MCP-1 (monocyte chemotactic protein-1) has the ability to regulate movement & infiltration of monocytes & macrophages into the inflammation site. In the presence of cardiovascular risk factors, it is overexpressed specifically in atherosclerotic lesions. Activation induces NF-$\kappa$B and AP-1, which leads to the release of IL-6 and proliferation of VSMC [28]. Proteins that demonstrate a multifaceted of signaling networks critical for distinctive regulation and adaptive inflammatory responses are known as cytokines. Through their influence on the development, growth, and activation of leukocytes, cytokines have the ability to modulate inflammatory responses. A vital moderator in systemic inflammation is TNF-$\alpha$, which activities include cell migration, activation of metalloproteinases (MMP), and production of interleukin CAM expression. TNF-$\alpha$ is detected in smooth muscle cells at all stages of the formation of athermanous plaques as well as endothelial cells [29]. The alteration from a vascular homeostasis inflammatory state is influenced by a difference between pro-inflammatory and anti-inflammatory events of interleukins. Some interleukins include: IL-1 which includes the stimulation of CAM, growth factors, tissue factors, chemokines, and various other cytokines. IL-6 is a multifunction cytokine with a central role in inflammation. Higher levels of IL-6 increase the risk of both mortality in patients with coronary heart disease and risk of myocardial infarction [30].

### 3.3 Vascular Remodeling, Extracellular Matrix (ECM), & Cell Adhesion

Arterial stiffness due to perturbations in the extracellular matrix is one of the mechanisms responsible for increased peripheral resistance in cardiopulmonary lesions. Evidence suggests arterial stiffness is an independent predictor of CVD events. The extracellular matrix (ECM), a collection of extracellular molecules secreted by cells, which provides structure and biochemical support to the surrounding cells deliver mechanical integrity to the vessel wall. Furthermore the ECM contains ligands that induce cell signaling to control migrations, proliferation, differentiation, and survival [31]. Cells respond to the ECM by remodeling their microenvironment that develops dysregulation in various diseases such as atherosclerosis, pulmonary arterial hypertension, & peripheral arterial disease. Metalloproteinases (MMP) are used for preserving homeostasis of extracellular structures, which comprise of cell-cell and cell-matrix interactions. MMPs with signaling molecules can modify cell-cell interactions through activation of signal transmission and release certain cytokines and chemokines. Due to these effects, these signaling molecules can transmit various inflammatory responses. Another essential component in the vascular remodeling process is the RAAS (renin-angiotensin-aldosterone system) [32-33]. Both inflammation and oxidative stress contribute to an increased CVD morbidity and mortality associated with the activation of RAAS.

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Aldosterone and angiotensin II (Ang-II) have previously been highlighted as an important regulator of cardiovascular homeostasis and pathogenesis of various CVD diseases. In order to evaluate its role, previous investigation of the expression of AT1R/AT2R at the vascular level was demonstrated [4]. Results showed the decreased expression of AT2R and increased expression of ATR1, which promotes vascular hypertrophy endothelial dysfunction, and growth.

**3.4 Additional Factors Contributing to Vascular Remodeling**

Other factors can contribute to vascular remodeling such as metabolic perturbations characterized by obesity, insulin resistance, hyperglycemia, and hypertension [34-35]. Grassi et al demonstrated that normotensive subjects suffering from severe obesity would also present vascular remodeling and endothelial dysfunction of small resistance arteries. Lean (age: 49.6 ± 2.9 years, BMI: 22.9 ± 0.3 kg/m2, mean ± s.e.m.) and age-matched severely obese (BMI: 41.1 ± 2.3 kg/m2) normotensive subjects were examined. The media thickness, media cross-sectional area (CSA), media-to-lumen ratio values of resistance arteries, and superimposable blood pressure were significantly greater in obese compared to lean subjects (media thickness 26.3 ± 0.6 vs. 16.2 ± 0.6 μm, CSA 22,272 ± 1,339 vs. 15,183 ± 1,186 μm2, and media-to-lumen ratio 0.113 ± 0.006 vs. 0.059 ± 0.001, respectively, $P < 0.01$). Stiffness of small arteries as evaluated by the stress/strain connection was comparable in severely obese and lean subjects. Based on these lines of evidence, results show that severe obesity is associated with significant alterations in functional and structural features of small arteries, which may be responsible for the manifestation of elevated risk factors of CVD and increased incidence of coronary, renal, and cerebrovascular events conveyed in obesity. [36].

Increased methylglyoxal (MG) production in vascular tissues is one of the causative factors for vascular remodeling in various subtypes of MetS that includes insulin resistance and hypertension. Up-regulation of fructose-induced aldolase-B (Aldo-B) contributes to increased production of vascular MG, however the fundamental mechanisms are uncertain. MG serum and fructose levels were determined in diabetic patients with hypertension. Significant levels of MG demonstrated positive correlations with blood pressure and fructose level correspondingly. C57BL/6 mice were fed with control or fructose-enriched diet for 3 months and ultrasonographic and histologic analyses were performed to evaluate arterial structural changes. Fructose-fed mice demonstrated hypertension and high levels of serum MG with normal glucose level. Fructose intake increased blood vessel wall thickness and vascular smooth muscle cell (VSMC) proliferation. Furthermore Cao et al demonstrated that Aldo-B level was suggestively higher in both frutocse-treated VSMCs and the aorta of fructose-fed mice, while aldolase-A (Aldo-A) expression was unchanged. Fructose induced translocation of ChREBP (carbohydrate-repsonsive element-binding protein) from the cytosol to nucleus demonstrated activated Aldo-B gene expression, which was inhibited by the knockdown of ChREBP. Furthermore, fructose caused FoxO1/3$\alpha$ transporting from the nucleus to cytosol and inhibited its binding to Aldo-B promoter region. Evidently this data suggests that fructose activates ChREBP and inactivates FoxO1/3$\alpha$ pathways to up-regulate both MG production and Aldo-B expression, leading to vascular remodeling [37].

Small artery pathophysiology is frequently invoked as a cause of obesity-related diastolic heart failure. However, evidence to support this hypothesis is insufficient, particularly in humans. To address this, Khvandi et al studied human small artery structure and function in obesity and

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looked for correlations between vascular parameters and diastolic function. 17 obese patients with MetS and 5 control participants underwent echocardiography and subcutaneous gluteal fat biopsy. Small arteries were isolated from the biopsy and pressure myography was used to study endothelial function and wall structure. In comparison with the control group, small arteries from obese participants exhibited significant endothelial dysfunction, assessed as the vasodilatory response to acetylcholine and also pathological growth of the wall. For the obese participants, multiple regression analysis revealed an association between left atrial volume and both the small artery wall thickness (β=0.718, *P*=0.02) and wall-to-lumen ratio (β=0.605, *P*=0.02). Furthermore, the E:E' ratio was associated with wall-to-lumen ratio (β=0.596, *P*=0.02) and inversely associated with interleukin-6 (β=–0.868, *P*=0.03). By contrast, endothelial function did not correlate with any of the echocardiographic parameters studied. Although the small arteries studied were not cardiac in origin, our results support a role for small artery remodeling in the development of diastolic dysfunction in humans. Further direct examination of the structure and function of the myocardial resistance vasculature is now warranted, to elucidate the temporal association between metabolic risk factors, small artery injury, and diastolic impairment [38].

Additionally, oxidative stress is a moderator of endothelial dysfunction and vascular remodeling. Marchesi et al investigated vascular dysfunction in MetS and the various oxidant mechanisms involved. New Zealand Obese (NZO) mice with MetS and New Zealand (NZ) black control mice were studied. NZO mice demonstrated increased visceral fat & blood pressure alongside insulin resistance when compared with NZ black mice. Mesenteric resistance arteries from NZO mice demonstrated increased media-lumen ratio and media cross-sectional area, representing hypertrophic vascular remodeling. Furthermore, vascular superoxide and peroxynitrite production was increased, as well as adhesion molecule expression. Perivascular adipose tissue of NZO mice showed increased superoxide production and NADPH oxidase activity, as well as adipocyte hypertrophy, associated with inflammatory Mac-3–positive cell infiltration. Marchesi et al suggest that this rodent model of MetS is associated with perivascular adipose inflammation & oxidative stress, endothelial dysfunction, and hypertrophic resistance artery remodeling, concluding a result of decreased nitric oxide (NO) and improved superoxide generated by uncoupled endothelial NO synthase [39].

Docosahexaenoic acid (DHA), a peroxisome proliferator–activated receptor-$\alpha$ (PPAR$\alpha$) activator, decreases blood pressure in some hypertensive models. Deip et al established the hypothesis that DHA would prevent blood pressure elevation and improve vascular dysfunction in angiotensin (Ang)-II–infused rats by modifying of inflammation and NADPH oxidase activity in the vascular wall. Systolic blood pressure (mm Hg), elevated in Ang-II–infused rats (172±3) vs. controls (108±2, P<0.01), was decreased by DHA (112±4). In mesenteric small arteries evaluated in a pressurized myograph, acetylcholine-induced relaxation impaired in Ang-II–infused rats (P<0.05) and media/lumen; both were standardized by DHA. In blood vessels of Ang-II–infused rats, NADPH oxidase activity measured by chemiluminescence and expression of adhesion molecules intercellular adhesion molecule and vascular cell adhesion molecule-1 were increased significantly. PPAR$\alpha$ activator DHA reduced the development of hypertension, altered structural abnormalities, and

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enhanced endothelial dysfunction induced by Ang-II. Overall these effects are connected with decreased oxidative stress and inflammation in the vascular wall [40].

Vascular endothelial growth factor receptor antagonist, Sugen 5416 & chronic hypoxia combined is known to cause pronounced pulmonary hypertension with lesions in rat and mice models [41]. Vitali et al determined whether weekly SU5416 injections during 3 weeks of hypoxia leads to long-term development of angioobliterative lesions and sustained or progressive PH in mice. SU5416 (SuHx) or vehicle (VehHx) was injected into male C57BL/6J mice weekly during 3 weeks of exposure to 10% oxygen. Following 3 weeks of hypoxia and 10 weeks of follow-up in normoxia, tricuspid annular plane systolic excursion was decreased showing decreased systolic RV (right ventricular) function. Few angioobliterative lesions were discovered at the 10-week follow-up time point in SuHx mouse lungs. Based on these lines of evidence, SU5416 combined with 3 weeks of hypoxia causes a more overwhelming PH phenotype in mice than hypoxia alone. Over 10 weeks of normoxic follow-up in SuHx mice showed PH persists, however significant angioobliterative lesions do not occur, and neither PH nor RV dysfunction worsens. The SuHx mouse model is a useful adjunct to other PH models, but the search will continue for a mouse model that better recapitulates the human phenotype [41]. Furthermore Al-Husseini et al demonstrated the aspect of the model, specifically, that treatment of rats with the anti-angiogenic vascular endothelial growth factor (VEGF) receptor 1 and 2 kinase inhibitor, Sugen 5416, when combined with chronic hypoxia, causing angioproliferative pulmonary vascular disease. Data demonstrated in the failing right ventricle of SuHx rats there was a decrease in the expression of VEGF-B and VEGF-D as well as reduction in VEGF-A expression. MAZ51, an inhibitor of VEGFR3 phosphorylation and VEGFR3 signaling, mainly prohibited the development of angio-obliteration in the SuHx model; however, obliterated vessels did not reopen when animals with PAH were treated with the VEGFR3 inhibitor. A portion of the mechanism of vasoobliteration in the SuHx model transpires via VEGFR3. VEGFR1/VEGFR2 inhibition can be originally anti-angiogenic via lung vessel endothelial cell apoptosis; nonetheless, it can be consequently angiogenic via VEGF-C and VEGF-D signaling through VEGFR3 [42].

**3. ID3 and cardiopulmonary disease**

ID3 involvement in cardiopulmonary diseases has been studied through various types of models. Previously, Forrest et al demonstrated during vascular lesion formation in rats, an alternate isoform of ID3 produced by intron retention is abundantly expressed. ID3 is expressed early in lesion formation when the proliferation index of the neo-intima is highest and stimulates smooth muscle cell (SMC) proliferation alongside S-phase entry inhibiting transcription of the cell-cycle inhibitor p21Cip1. Forrest et al furthermore showed that ID3a protein is induced during vascular lesion formation and that the expression peaks late when the proliferative index is low or diminishing and extensive apoptosis is observed, thus defining a innovated feedback loop in which an ID3 isoform is created that acts to limit SMC growth. Overall, this delivers the first indication that regulated intron preservation can modify a pathologic process *in vivo* [43]. ID3 has also been studied together with the lipoxygenase (12/15-LO), which is known to produce pro-inflammatory alterations in blood vessels that lead to the development of atherosclerosis [44]. 12/15LO expression in the vessel wall is demonstrated to increase in animal models of MetS & diabetes. Increased expression of 12/15LO increases cultured vascular smooth muscle cell (VSMC) proliferation, an effect that is intermediated

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by ID3. Overexpressed 12/15LO transgenic mice had larger post-injury carotid ID3 and KI-67 expression, cell number, & deposition compared with C57BL/6 mice. Deliri et al results demonstrated p21cip1 as a potential target of the 12/15LO-ID3 pathways and suggest a variation of this pathway may have beneficial placement for targeting the increased risk of restenosis in diabetic patients [45]. By the use of ubiquitous E-proteins as stimulus, Matsumura et al determined ID3 and a novel isoform of ID3 (ID3a) were cloned. Balloon injury revealed ID3a was abundantly expressed throughout the neo-intimal layer. ID3 overexpression of adenovirus-mediated isoforms in cultured rat aortic SMCs showed that infection of SMCs with an adenovirus overexpressing ID3a (in contrast comparison to ID3) stemmed in a significant decrease in cell number versus AdLacZ-infected cells. These outcomes deliver indication that alternate splicing of the ID3 gene may represent an important mechanism by which neo-intimal SMC growth is weakened during vascular lesion formation [46]. Primary aortic VSMCs from leukocyte-type 12/15-LO transgenic, leukocyte-type 12/15-LO knockout (KO), and control mice were plated in equal densities and assayed for growth, ID3 transcription, & ID3 protein expression. Results established that 12/15-LO transgenic VSMCs cultivated quicker while 12/15-LO KO VSMCs cultivated slower comparative to control VSMCs [7]. Western blots showed ID3 protein increase in 12/15-LO transgenic VSMCs, whereas luciferase promoter reporter assays showed ID3 transcription increase. The growth-promoting effects of 12/15-LO are moderately mediated through stimulation of ID3 transcription [7].

Reactive oxygen species (ROS), such as superoxide are involved in the abnormal growth of numerous cell types. Angiotensin-II (Ang-II) is one of the most effective inducers of oxidative stress in the vasculature. Ang-II as well as xanthine/xanthine oxidase (X/XO) led to enhanced DNA synthesis and proliferation of VSMCs. VSMCs were incubated with X/XO, and change of gene expression was monitored by differential display, leading to the identification of ID3, which was up regulated within 30 minutes by X/XO and Ang-II. It was demonstrated that ID3 overexpression of antisense through transfection in VSMCs entirely obliterated Ang-II and X/XO-induced cell proliferation. Overexpression of sense ID3, Ang-II, and X/XO also demonstrated down-regulated protein expression of p21WAF1/Cip1, p53, & p27Kip1. Ang-II and overexpression of sense ID3 caused hyper-phosphorylation of the retinoblastoma protein. Ang II–induced phosphorylation of the retinoblastoma protein was diminished by antisense ID3 overexpression. Ang-II furthermore induced proliferation of VSMCs via production of superoxide, which increases the expression of ID3. ID3 regulates the downstream mitogenic processing through depression of p21WAF1/Cip1, p53, & p27Kip1. These overall findings reveal a novel redox-sensitive pathway involved in growth control [47]. ID3 also plays a role in high fat diet stimulated visceral adipose VEGFA expression, micro-vascular blood volume, & depot expansion [48]. ID3 is essential to obesity due to its demonstration to stimulate angiogenesis that is considered an important factor of HFD (high-fat diet)-induced visceral adiposity [48]. ID3 knockout (KO) mice demonstrated a significant protective effect from HFD-induced visceral fat depot expansion when compared to control. Furthermore adipose tissue neighboring major arteries (perivascular adipose tissue or PVAT) demonstrates evidence to specify vessel insulation and support. Developing evidence elucidates that PVAT regulates artery pathology and physiology, such as atherosclerosis development via production of inflammatory cytokines. It was previously demonstrated by Harmon et al that C57BL6 mice with B cell specific insufficient ID3 had larger IgM production and visceral adipose tissue B-1b cells and less inflammation of adipose

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tissue when to WT littermate controls [49]. Prasad et al recently demonstrated that ID3 also regulates the number of B-1b cells in the PVAT (peri-vascular adipose tissue) [50]. Furthermore ID proteins (ID1-4) are major downstream transcriptional targets of BMP signaling. The impact of BMPR-II mutation on the expression of the range of ID proteins and the contribution of individual ID proteins to abnormal PASMC function remain uncertain. The BMP-stimulated induction of both ID1 and ID3 was markedly reduced in BMPR-II mutant pulmonary arterial smooth muscle cells (PASMCs) and in control PASMCs following siRNA silencing of BMPR-II. Pulmonary arteries in BMPR-II mutant mice and patients with heritable PAH demonstrated reduced levels of ID3 compared with control subjects. Particularly ID1 and ID3 are critical downstream effectors of BMP signaling in PASMCs and both regulate the proliferation of PASMCs via cell cycle inhibition, an effect that may be exacerbated by inflammatory stimuli [51]. Registries report a greater incidence of PAH in women; mutations in the bone morphogenic protein type II receptor (BMPR-II) occur in approximately 80% of patients with heritable PAH (hPAH). Maier et al examined the BMPR-II signaling pathway in hPASMCs derived from men and women with no underlying cardiovascular disease (non-PAH hPASMCs). The development of pulmonary hypertension in male and female mice deficient in SMAD1 was also determined. Female non-PAH hPASMCs exhibited reduced messenger RNA and protein expression of BMPR-II, the signaling intermediary SMAD1, and the downstream genes, inhibitors of DNA binding proteins, ID1 and ID3. Induction of phospho-Smad1/5/8 and ID protein by BMP4 was also reduced in female hPASMCs. BMP4 induced proliferation in female, but not male, hPASMCs. Male hPASMCs demonstrated estrogen decreased messenger RNA and protein expression of ID genes. The estrogen metabolite 4-hydroxyestradiol decreased phospho-Smad1/5/8 and ID expression in female hPASMCs while increasing these in males proportionate with a decreased proliferative effect in male hPASMCs. Female Smad1 (+/-) mice developed pulmonary hypertension (reversed by ovariectomy). Results demonstrated that estrogen-driven suppression of BMPR-II signaling in non-PAH hPASMCs derived from women contributes to a pro-proliferative phenotype in hPASMCs that may predispose women to PAH [52].

Coronary artery disease, which is damage or disease to the heart's major blood vessels, causes approximately 1 of every 7 deaths in the United States. Population-based studies have found SNP (single nucleotide polymorphisms) rs11574 in the coding region of the human ID3 gene associated with subclinical atherosclerosis in the Diabetes Heart Study [53]. ID3 SNP rs11574 has also demonstrated a significant association of coronary artery disease for Caucasians and to an abridged extend African Americans and Hispanics [54]. Previously, genes have been demonstrated to be involved in CAD including: LFNG, ID3, PLA2G7, FOLR3, PADI4, ARG1, IL1R2, NFIL3 and MGAM, were differentially expressed according to the analysis [55]. These genes showed connection to CAD via statistical and biological pathway analysis. Shi et al concluded that pathways related to immune responses, especially neutrophil degranulation, were associated with coronary heart disease [55]. Angiogenic gene expression of outgrowth endothelial cells (OECs), an endothelial progenitor cell (EPCs) subtype capable to shape vessel structures have been introduced as a novel therapeutic model for cell-based treatments for stroke. OECs (at colony or mature stages) showed higher expression of CCL2, ID3, IGF-1, MMP9, TGFBR1, TNFAIP2, TNF and TGFB1. ID3 ($p=0.008$) and TGFBR1 ($p=0.03$) genes remained significantly overexpressed in colony-OECs compared to mature-OECs or hCMEC/D3. MMP9 levels were significantly increased in colony-OECs ($p=0.025$) compared to

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mature-OECs. Results demonstrated that OECs from stroke patients present higher levels of pro-angiogenic factors at early stages, decreasing in mature OECs when they become more similar to mature microvascular endothelial cells [56]. Furthermore ID3 may be a predictive factor for stroke, which is sudden death to the brain cells due to inadequate blood flow. Expression levels of 10 candidate genes (ANTXR2, STK3, PDK4, CD163, MAL, GRAP, ID3, CTSZ, KIF1B, and PLXDC2) were data mined, compared between groups, and evaluated for their predicative ability at each time point in 23 ischemic stroke patients. Results demonstrated direct expression levels of the candidate genes were able to distinguish between stroke patients and controls with levels of sensitivity and specificity upwards of 90% across all three time points. Based on these lines of evidence, these findings confirm the diagnostic strength of the pattern of differential expression in an independent patient population, and further suggest that it is temporally stable over the first 24h of stroke [57]. ID3 expression also demonstrated a molecular stemness signature comprising of $CD133^{+}$ $VEGFR3^{+}CD34^{+}$ cells. SU5416 exposure to the cells showed positive protein expression of ID3, CD34, VEGFR3, and increased expression of pluripotent transcription factors SOX2 and OCT4. Overexpressing ID3 cells reinforce the formation of a 3-D microvascular lesion co-cultured with smooth muscle cells. Further investigations into how stem-like cells apply ID3 may lead to novel possibilities for an improved understanding of the molecular mechanisms which are essential to the pathological development of microvascular diseases [20].

**4. Estrogenic Endocrine Disruptors (EEDs) in cardiopulmonary disease**

There is a growing concern that estrogenic endocrine disruptors may also contribute to the pathology of cardiopulmonary disease such as polychlorinated biphenyls (PCBs) and bisphenol A (BPA). Epidemiological studies have shown a linkage between EED exposure and increased cardiopulmonary disease risk. Studies have revealed association between PCBs and hypertension participants in the 1999-2004 National Health and Nutrition Examination Survey [58]. PCB serum concentrations by demographic characteristics were different but demonstrated on average greater concentrations among those with hypertension. Further analyses identified categories of PCBs such as 66, 101, 118, 128 and 187 significantly associated with increased risk of hypertension [58]. Additionally circulating levels of environmental pollutants were related to hypertension in a population-based sample of men and women. The population, which included 1,016 subjects aged 70 years old were investigated in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. When these environmental pollutants were treated as continuous variables and adjusted for gender only, two PCBs (PCB 105 & 118) were related to prevalent hypertension. These results further strengthened the experimental findings that these pollutants might influence blood pressure [59]. Additionally the Seguimiento Universidad de Navarra project is a Spanish cohort of university graduates. The cohort included 14,521 participants, initially free of hypertension, who were followed-up for a median of 8.3 years. The published concentration levels of polychlorinated biphenyls measured in samples of food consumed in Spain were used to estimate dietary intake. During follow-up, 1497 incident cases of medically diagnosed hypertension were identified. After adjustment for total energy intake and potential confounders, participants in the fifth quintile of total PCBs intake were at higher risk of developing hypertension (adjusted hazard ratio, 1.43 [95% confidence interval, 1.09-1.88; P for trend 0.017]) compared with those in the first quintile.

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Results demonstrated that dietary intake of polychlorinated biphenyls, estimated using a food frequency questionnaire showed associated with a higher risk of developing hypertension during follow-up [60].

The effects of PCB126 on vascular inflammation has been linked to hepatic dysfunction utilizing a liver injury mouse model. Male C57Bl/6 mice were fed either an amino acid control diet (CD) or a methionine-choline deficient diet (MCD) in this 14-week study. Mice were exposed to PCB126 (0.5 mg/kg) and analyzed for inflammatory, calorimetric and metabolic parameters. MCD diet-fed mice demonstrated steatosis, indicative of a compromised liver. Mice fed the MCD-diet and subsequently exposed to PCB126 manifested lower body fat mass, increased liver to body weight ratio and alterations in hepatic gene expression related to lipid and carbohydrate metabolism, implicating metabolic disturbances. PCB126 induced steatosis irrespective of the diet type, but only the MCD+PCB126 group exhibited steatohepatitis and fibrosis. Furthermore, PCB126 exposure in MCD-fed mice led to increased plasma inflammatory markers such as ICAM-1, PAI-1 and pro-atherogenic trimethylamine-N-oxide (TMAO), suggesting inflammation of the peripheral vasculature that is characteristic of atherosclerosis. Taken together, data provide new evidence of a link between a compromised liver, PCB-mediated hepatic inflammation and vascular inflammatory markers, suggesting that environmental pollutants can promote crosstalk between different organ systems, leading to inflammatory disease pathologies [61]. Alterations of epigenetic marks can be induced by exposure to environmental pollutants and may contribute to vascular disease risks. Human vascular endothelial cells were exposed to physiologically relevant concentrations of several PCBs congeners (77, 118, 126 and 153) followed by quantification of inflammatory gene expression and changes of histone methylation. Only exposure to coplanar PCBs 77 and 126 induced the expression of histone H3K9 trimethyl demethylase jumonji domain-containing protein 2B (JMJD2B) and nuclear factor-kappa B (NF-κB) subunit p65, activated NF-κB signaling as evidenced by nuclear translocation of p65, and up-regulated p65 target inflammatory genes, such as interleukin (IL)-6, C-reactive protein (CRP), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and IL-1$\alpha$/$\beta$. Based on these lines of evidence, it is suggestive that coplanar PCBs may exert endothelial cell toxicity through changes in histone modifications [62].

PCB153 has been shown to bind to the estrogen receptor alpha, prompt vessel formation, and increase the development of reactive oxygen species (ROS) in endothelial cells. Since PCB153-induced phenotypic changes are comparable to estradiol, it is postulated that PCB153 stimulates redox-signaling pathways common to 17$\beta$-estradiol [63]. We previously determined a gene network demonstrating estrogenic chemicals modulation in human vascular endothelial cells. Planar and coplanar polychlorinated biphenyls (PCBs) induce the expression of various genes compared to estradiol. Results demonstrated that exposure of vascular endothelial cells to environmentally relevant concentrations of estrogenic PCBs induce gene networks implicated in the process of inflammation and adhesion. The data suggests that PCBs can promote vascular lesion formation by activating gene networks involved in endothelial cell adhesion, cell growth, and pro-inflammatory molecules which were different from natural estrogen. While inflammation and adhesion are a hallmark in the pathology of endothelial cell dysfunction, reconstructing gene networks provide insight into the potential mechanisms that may contribute to the vascular risks associated with estrogenic environmental chemicals [64].

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Previous studies have also shown a linkage between exposure to Bisphenol A (BPA), another EED and many disorders that affect the vascular system. BPA is a chemical produced at high volumes and used widely in food and drink packaging. Shankar et al examined the association between urinary BPA levels and PAD in a nationally representative sample of U.S. adults. The analysis of 745 participants in the National Health and Nutritional Examination Survey 2003–2004 was used. A positive association was demonstrated between growing levels of urinary BPA and PAD before and after adjusting for confounders. Multivariable-adjusted odds ratio for PAD associated with the highest versus lowest tertile of urinary BPA was 2.69 (95% confidence interval: 1.02, 7.09; *p*-trend = 0.01). These results demonstrate that urinary BPA levels were significantly associated with PAD, independent of traditional CVD risk factors [65]. Recruitment 886 subjects (12-30 years of age) from a population-based sample of adolescents and young adults based on a mass urine screening to determine the relationship between serum levels of BPA and carotid intima-media thickness (CIMT). After confounding factors were controlled, linear regression analyzes showed a 1-unit increase in natural log BPA was significantly associated with an increase in mean CIMT (mm) ($\beta = 0.005$, 95% C.I. = 0.003-0.007, $p < 0.001$) and other measurement of CIMT (including right and left side of common carotid artery, carotid bulb and internal carotid artery). Higher serum concentrations of BPA were associated with increased CIMT in this cross-sectional study of adolescents and young adults [66].

Various animal models have demonstrated an association between BPA and the vasculature. BPA-treated rabbits exhibited insulin resistance, hepatic steatosis, and prominent adipose accumulation. BPA exposure also triggered myocardial injury and augmented the development of atherosclerosis in the aortic arch with increased advanced lesion areas (69%) and macrophage number (86%). Increase in expression of inflammatory genes found in the liver of BPA-treated rabbits alongside the up-regulation of ER stress, lipid & glucose homeostasis, and inflammatory genes in cultured HepG2 cells and HUVECs suggest that BPA may induce metabolic disorders and enhance atherosclerosis through regulating above molecular pathways in both liver and the endothelium. [67]. Furthermore BPA demonstrated stimulated proliferation and migration of cultured cardiac fibroblasts and collagen production in a concentration-dependent manner, as revealed by wound healing assay, MTT, and collagen assay. Estrogen receptor inhibitor ICI182780 or ERK inhibitor PD98059 prohibited the enhanced phosphorylation of ERK1/2, and inhibited up-regulation of transforming growth factor-β1 (TGF-β1) expression induced by BPA. Taken together, results demonstrated that BPA act as a promoting factor in proliferative process and collagen production of cardiac fibroblasts via activating ERK1/2 [68]. Progeny were maintained on a defined diet containing BPA (0.03, 0.3, 3, 30, or 300 ppm) that resulted in BPA exposures from 4-5 to approximately 5000 μg/kg·d or a diet containing 17$\alpha$-ethinyl estradiol (EE; ~0.02, 0.2, and 0.15 μg/kg · d) as an oral bioavailable estrogen control (Belcher et al. 2015). Exposure-related changes in the rates of ventricular contraction, suggest toward increased parasympathetic activity, were detected in males. Decreased systolic blood pressure was observed in males exposed to BPA above 5 μg/kg·d and in females from the highest BPA exposure group. Based on these lines of evidence, results show significant sex-specific changes in gene expression in response to BPA that were consistent with the observed exposure-related phenotypic changes in the collagenous/non-collagenous extracellular matrix, cardiac remodeling, altered autonomic responses, and lipid metabolism [69].

BPA may also play a role in aortic and coronary atherosclerotic vascular remodeling. Over

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12 weeks Watanabe heritable hyper-lipidemic (WHHL) rabbits were exposed to 400-μg/kg BPA per day being administered orally by gavage, compared to the vehicle group using histological and morphometric methods. The atherosclerotic lesion area in the aortic arch was enlarged by 57% compared to the vehicle group. Histological and immune-histochemical analyses revealed marked increases in advanced lesions (37%) accompanied by smooth muscle cells (60%). Coronary atherosclerosis and coronary stenosis incidents increased by 11% and smooth muscle cells increased by 73% compared to the vehicle group. These results demonstrated for the first time that BPA exposure may increase susceptibility to atherosclerosis in WHHL rabbits (Fang et al. 2014). Higher urinary BPA concentrations can also be associated with arterial hypertension. Eight (8) week-old CD11 mice were administered with BPA (4 nM to 400 μM in drinking water). Mice established high blood pressure (dosage-dependent) (systolic 130 ± 12 vs. 170 ± 12 mmHg; EC50 0.4 μM), a 1.7-fold increase in arterial angiotensin II (Ang-II); significant eNOS-dependent superoxide and peroxynitrite accumulation impairment of acetylcholine (AcH)-induced carotid relaxation (0.66 ± 0.08 vs. 0.44 ± 0.1 mm), an 8.7-fold increase in eNOS mRNA and protein; and Ang-II inhibition with 0.5 mg/ml losartan reduced oxidative stress and normalized blood pressure and endothelium-dependent relaxation. These lines of evidence suggest that Ang-II uncouples eNOS and contributes to the BPA-induced endothelial dysfunction by promoting oxidative and nitrosative stress [70]. Additionally Lind & Lind examined circulating levels of phthalate metabolites and BPA in a cross-sectional study related to atherosclerosis. In the population-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (1016 subjects all aged 70), the prevalence of overt plaques and echogenectity (grey scale median, GSM) of carotid artery plaques were recorded by ultrasound in both of the carotid arteries. Bisphenol A (BPA) and 10 phthalate metabolites were analyzed in serum by a API 4000 liquid chromatograph/tandem mass spectrometer. Mono-methyl phthalate (MMP) was related to carotid plaques in an inverted U-shaped manner. This pattern was significant after adjustment for gender, body mass index, blood glucose, blood pressure, HDL and LDL-cholesterol, serum triglycerides, smoking, antihypertensive treatment and statin use ($p=0.004$). High levels of BPA, mono-isobutyl phthalate (MiBP) and MMP were associated with an echogenic IM-GSM and plaque GSM, while high levels of mono-2-ethylhexyl phthalate (MEHP) were associated with an echolucent IM-GSM and plaque GSM ($p<0.0001$ after adjustment). The phthalate metabolite MMP was related to atherosclerotic plaques in an inverted U-shaped manner independently of CV risk factors. Some phthalates and BPA were also related to the echogenicity of the plaques, suggesting a role for plaque-associated chemicals in atherosclerosis [71].

**5. ID3 & Estrogenic Endocrine Disruptors in Vascular Remodeling**

Previously a mechanism by which estrogen-induced oxidant regulated differentiation in endothelial cells into tube-like structures via ID3 was examined. Overexpression of the superoxide scavenger MnSOD and the hydrogen peroxide scavenger catalase inhibited formation of the tube in estrogen treated endothelial cells. Since tube formation on matrigel is not endothelial cells specific, Felty and Porther established results in a co-culture model that better represents tube formation in vivo. Tube formation of Estrogen-induced was inhibited by the actin cytoskeleton disruptor cytochalasin D and the microtubule destabilizer colchicine. Estrogen increased ID3 phosphorylation, which was reduced by catalase and *N*-acetylcysteine treatments. The functional role of ID3 in tube

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formation was determined by RNA interference and showed ID3 siRNA to inhibit tube formation in estrogen exposed cells. Overall, estrogen-induced tube formation requires the presence of ID3 factors and estrogen increases ID3 phosphorylation via a redox-dependent process. [2].

Furthermore, mechanisms responsible for initiating micro-vascular damage continue to be inadequately defined, although several inciting factors have been proposed, including environmental toxicants-induced oxidative stress. Enhanced neovascularization has been implicated in either the development or progression of proliferative vascular lesions. Overall, results demonstrated that PCB-induced ROS mediated a highly tube branched neo-vascular phenotype that also depended on ID3 and Pyk2. Furthermore, PCB153 treatment caused the size of endothelial spheroids to increase under circumstances normally used for clonal selection of stem cell spheroids. High ID3 protein expression correlated with an increased level of malignancy and oxidative DNA damage marker 8-OHdG in blood vessels from human subjects. PCB153 treatment also increased both serine and tyrosine phosphorylation of endothelial ID3. Stable ID3 overexpression increased cell survival of human micro-vascular endothelial cell line hCMEC/D3. [11].

Additionally, since micro-vascular diseases are characterized by unwarranted vessel growth, it is rational that estrogen-produced neovascularization contributes to the development of micro-vascular lesions. Suggestion for how ID3 overexpression in endothelial cells contributes to the progression of an estrogen-induced neo-vascular phenotype with emphasis on Pyk2 kinase was demonstrated. Data showed that overexpression of ID3 increased spheroid growth, neovascularization, & cell migration of human cerebral micro-vascular endothelial cells, hCMEC/D3. Overexpressing ID3 cells presented significant estrogen-induced G2/M phase transition. Estrogen treatment increased phosphorylation of ID3 and total protein that was inhibited by tamoxifen; and Pyk2 mediated estrogen-induced ID3 mRNA expression. These results suggest that Pyk2 signals ID3 expression and ID3 is crucial for estrogen-induced neovascularization in hCMEC/D3 cells [72]. Since cardiopulmonary diseases also affects the brain such as arteriovenous malformations, intracranial atherosclerosis, and vascular dementia, further research is warranted between ID3, EED exposure and disease outcomes. As demonstrated in Figure 1, exposure of EED may trigger ID3 to modulate mechanisms in vascular remodeling. Furthermore, these alterations may affect various cellular and non-cellular components such as cell growth, cell adhesion, cell migration, ECM alterations, and cell death as demonstrated in Figure 2.

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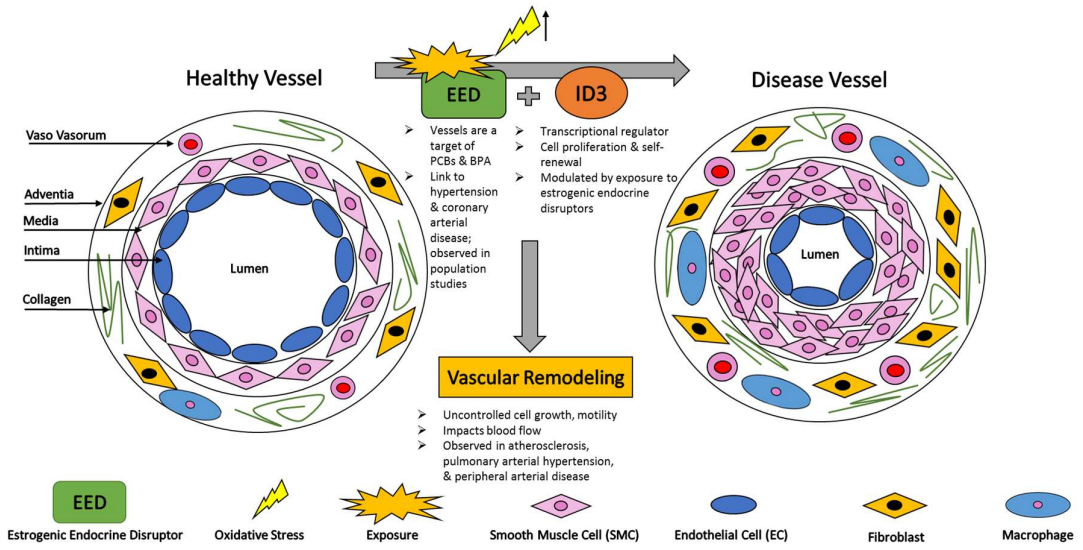


**Figure 2.** EEDs & ID3 influence on vessel. Left panel shows healthy vessel & right panel shows potential effect on diseased vessel with exposure to EEDs & modulation of transcriptional regulator ID3. Exposure of EEDs increases oxidative stress causing modulation of ID3, which mediates vascular remodeling: uncontrolled cell growth & narrowed lumen which impacts blood flow.

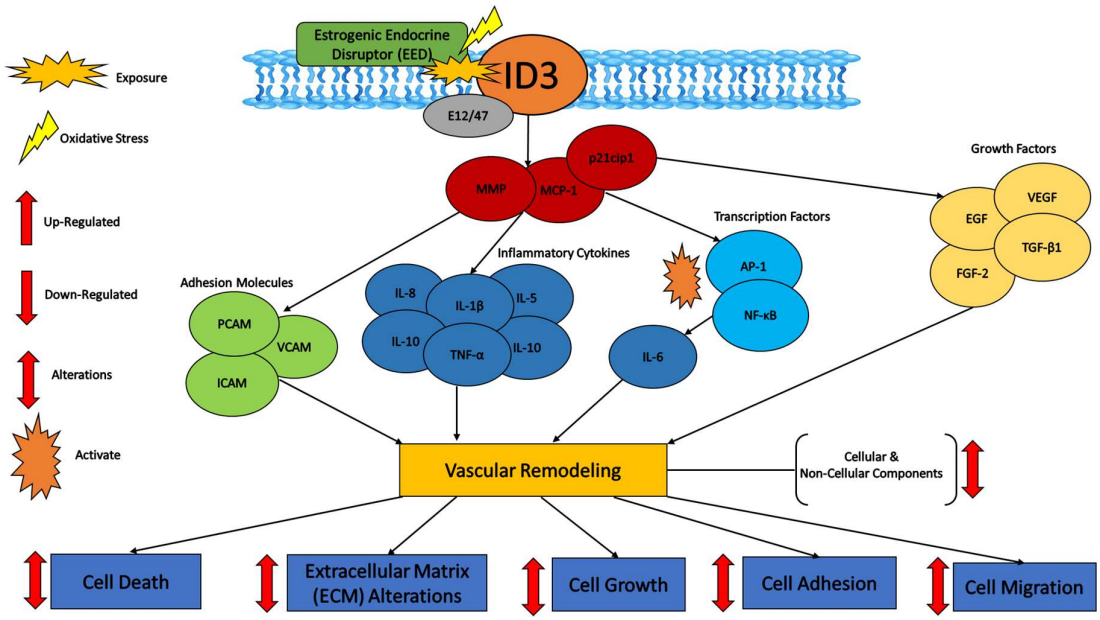


**Figure 3.** Molecular mechanisms of EEDs & ID3 in vascular remodeling. Exposure to EEDs increases oxidative stress causing the modulation/activation of transcriptional regulator, ID3. Mediation of various vascular remodeling factors via ID3 may elucidate to alterations in cell death, extracellular matrix, cell growth, cell adhesion, & cell migration.

## Conclusion

We have comprehensively reviewed the existing evidence to illustrate the association between ID3 & vascular remodeling. Furthermore, we extended this understanding of how ID3 & vascular remodeling by environmental pollutants such as estrogenic endocrine disruptors (EEDs) may modify vascular lesions. ID3 has been previously seen to be involved in cardiopulmonary diseases such as atherosclerosis, coronary arterial disease, & arteriovenous malformation. Research is warranted to better explain the influence of estrogenic endocrine disruptors to ID3-induced vascular remodeling. This may lead to novel pathways for how the interaction of ID3, EEDs, and vascular remodeling mediates cardiopulmonary diseases and can further help to treat and prevent these detrimental diseases.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest

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