Endothelial to Mesenchymal Transition Represents a Key Link in the Interaction between Inflammation and Endothelial Dysfunction

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Endothelial cells that line the inner walls of blood vessels are in direct contact with blood and display remarkable heterogeneity in their response to exogenous stimuli. These ECs have unique location-dependent properties determined by the corresponding vascular beds and play an important role in regulating the homeostasis of the vascular system. Evidence suggests that vascular endothelial cells exposed to various environments undergo dynamic phenotypic switching, a key biological program in the context of endothelial heterogeneity, but that might result in EC dysfunction and, in turn, cause a variety of human diseases. Emerging studies show the importance of endothelial to mesenchymal transition (EndMT) in endothelial dysfunction during inflammation. EndMT is a complex biological process in which ECs lose their endothelial characteristics, acquire mesenchymal phenotypes, and express mesenchymal cell markers, such as alpha smooth muscle actin and fibroblast-specific protein 1. EndMT is induced by inflammatory responses, leading to pathological states, including tissue fibrosis, pulmonary arterial hypertension, and atherosclerosis, *via* dysfunction of the vascular system. Although the mechanisms associated with inflammation-induced EndMT have been identified, unraveling the specific role of this phenotypic switching in vascular dysfunction remains a challenge. Here, we review the current understanding on the interactions between inflammatory processes, EndMT, and endothelial dysfunction, with a focus on the mechanisms that regulate essential signaling pathways. Identification of such mechanisms will guide future research and could provide novel therapeutic targets for the treatment of vascular diseases.

Keywords: endothelial dysfunction, inflammatory process, endothelial to mesenchymal transition, endothelial heterogeneity, vascular disease

INTRODUCTION

Endothelial cells (ECs) play a key role in maintaining vascular homeostasis in response to various stimuli. They can regulate vascular tone, permeability, coagulation, and inflammation through the regulation of numerous mediators, such as endothelium-derived relaxing and contracting factor, cell-adhesion molecules, cytokines, and chemokines (1, 2). However, vascular injuries resulting from procedures and conditions, such as angioplasty, stenting, diabetes, hypertension, and immune-mediated damage, can lead to endothelial dysfunction, resulting in disturbance or loss of normal endothelial functions (1, 3–5). Many studies have revealed an association between

endothelial dysfunction and inflammatory stress in vascular biology. Under conditions of chronic inflammation, sustained activation of ECs by inflammatory stimuli, such as interleukin (IL)-6, tumor necrosis factor-α (TNF-α), IL-1β, and pathogens, cause alterations in normal endothelial function, resulting in impaired endothelial-dependent immune response, which is the hallmark of endothelial dysfunction (6–9). Indeed, endothelial dysfunction due to inflammatory stress contributes to the pathogenesis of many diseases, including fibrosis, atherosclerosis, pulmonary arterial hypertension (PAH), and pathological angiogenesis (10–17). In addition, emerging evidence shows that the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome not only has a role as a critical sensor in immune response, but also has a critical role in endothelial dysfunction and the pathogenesis of vascular diseases, such as atherosclerosis and metabolic syndrome (18–20). Assembly and activation of the NLRP3 inflammasome results in the conversion of the inactive procaspase-1 into active caspase-1, with subsequent secretion of mature IL-1β and IL-18 in such diseases (21–25).

Accumulating evidence suggests that endothelial to mesenchymal transition (EndMT) represents a key link in the complex interactions between inflammatory stress and endothelial dysfunction. EndMT is a phenotypic switching process by which ECs lose their characteristics and acquire mesenchymal traits (26, 27). EndMT exhibits features similar to those of epithelial to mesenchymal transition (EMT) and is often considered a specific form of EMT (26, 28). Although both processes use the same signaling pathways and result in cells with a mesenchymal phenotype, studies of the differences between EMT and EndMT are needed due to differences in the origin, fundamental function, and microenvironment of ECs and epithelial cells (29). EndMT was first discovered and has been studied in heart development and emerging studies show that EndMT can occur in postnatal pathologies associated with several diseases, such as fibrosis, cancer, neointima formation, cerebral cavernous malformations, atherosclerosis, and PAH (26, 28–30). Indeed, it has been reported that EndMT contributes to endothelial dysfunction during inflammatory conditions, and that some inflammatory mediators, such as IL-1β, TNF-α, nuclear factor kappa B (NF-κB) transcription factor, and endotoxins, can activate ECs and convert them to mesenchymal-like cells through the EndMT process (6, 7, 31). However, how EndMT contributes to disease progression remains unclear (32), and the specific role of EndMT in inflammatory stimulus-induced endothelial dysfunction has not been fully elucidated due to the dynamic nature of the EndMT process, which consists of multiple steps.

A single layer of ECs lining blood vessels displays heterogeneity in function, morphology, gene expression, and antigen composition depending on location (2) and behaves differently based on its exposure to different microenvironments (33). Therefore, it is also important to understand the molecular basis of inflammation-induced EndMT in the context of endothelial heterogeneity, because this can be critical for developing personalized vascular therapies for patients with vascular bed specific diseases (33).
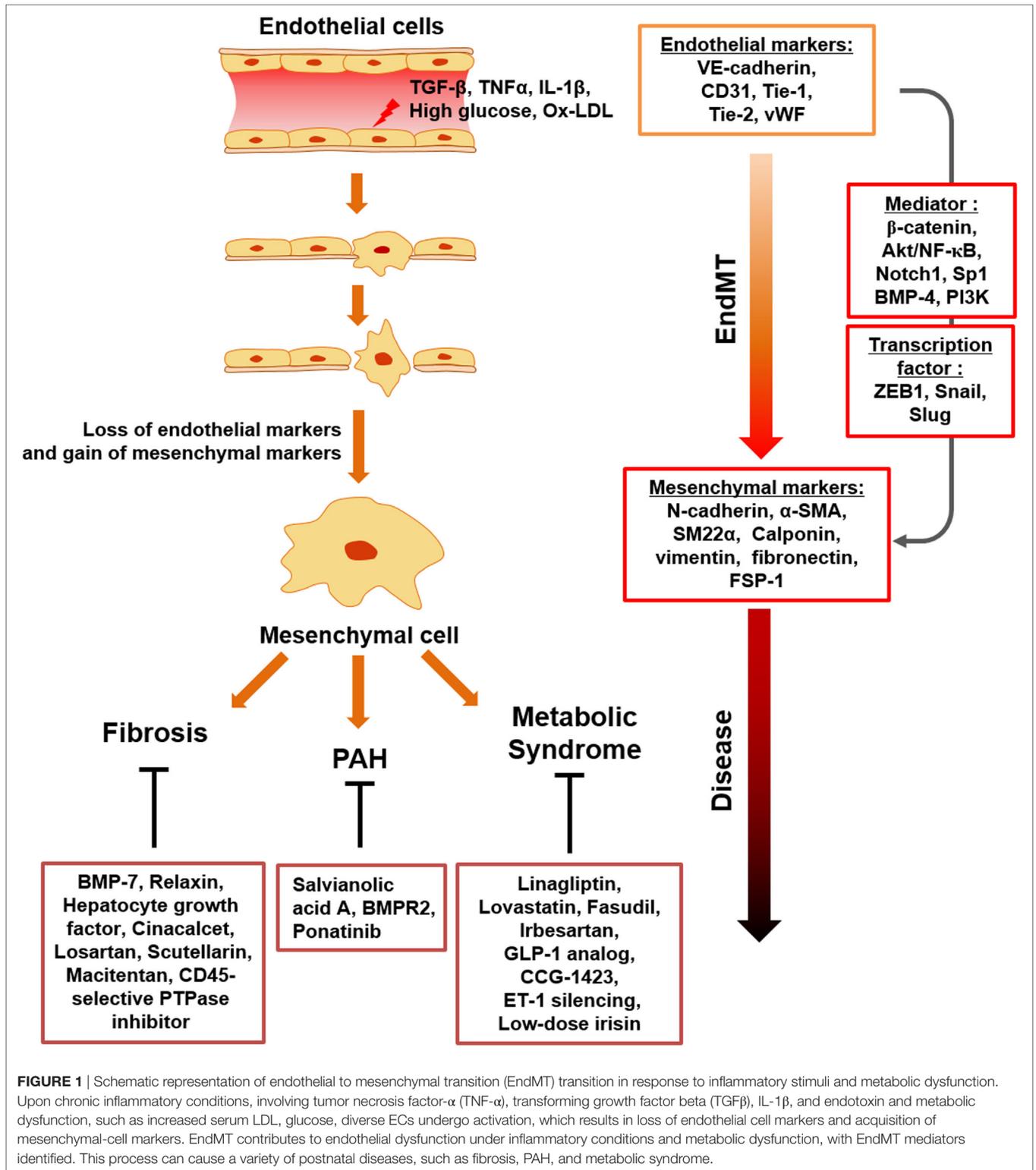
In this review, we summarize the knowledge currently available regarding the role of EndMT in inflammatory processes and discuss endothelial heterogeneity in the context of inflammation.

ENDMT MEDIATORS AND SIGNALING PATHWAYS DURING INFLAMMATION

Endothelial cells play an important role in the maintenance of homeostasis across the entire vascular system (10, 34). ECs actively participate in the regulation of immune responses to various stimuli. To this end, the inflammation-mediated signaling pathway has been extensively studied (6, 17). However, cell signaling associated with inflammation-induced EndMT remains poorly understood. Nevertheless, the molecular mechanisms underlying inflammation-induced EndMT have been gradually identified based on observations of EMT processes that are relatively well studied on inflammatory responses (6). Current evidence suggests that inflammation-induced EndMT, similar to that of EMT, is largely governed by two signaling pathways: the transforming growth factor beta (TGFβ) pathway and the non-TGFβ pathway (35). TGFβ is the most well-known EndMT inducer and upregulates the expression of transcription factors, such as snail, slug, and zinc finger E-box-binding homeobox 1 (ZEB1). These transcription factors then upregulate the expression of mesenchymal markers, such as alpha smooth muscle actin (α-SMA), smooth muscle protein 22 alpha (SM22α), calponin, vimentin, type I collagen, fibronectin, fibroblast-specific protein 1 (FSP-1), N-cadherin, matrix metalloprotein (MMP)-2, and MMP-9 (6, 36, 37).

It has been identified that EndMT related to direct immune responses is triggered in response to pro-inflammatory cytokines, such as TNF-α, IL-1β, and their combinations. Similarly, inflammation-induced EndMT is characterized by the loss of endothelial phenotypes and gain of mesenchymal-like characteristics, and endothelial/mesenchymal markers are tightly controlled by EndMT mediators, such as ZEB1, β-catenin, Akt/NF-κB, snail, slug, Notch1, bone morphogenetic protein (BMP)-4, Sp1, phosphoinositide 3-kinase (PI3K), and enhancer of zeste homolog 2 (EZH2) (**Figure 1**).

Tumor necrosis factor-α, a pro-inflammatory cytokine, plays an important role in the regulation of various cellular activities (38). In ECs, TNF-α responses are initiated by the binding of one of two receptors, TNF receptor type 1 and TNF receptor type 2, allowing these receptors to activate transcription factors, such as NF-κB, which leads to the induction of transcription of multiple genes such as vascular cell adhesion molecule 1 (*VCAM-1*) and intercellular adhesion molecule 1 (31, 39–42). TNF-α also induces EndMT through activation of multiple signaling pathways in various ECs types (39, 40, 43, 44). However, future studies will be needed to clarify what type of TNF-α receptor is involved in EndMT. In lymphatic endothelium, TNF-α-induced EndMT occurs through inhibition of vascular endothelial (VE)-cadherin expression while increasing the expression of β-catenin, N-cadherin, and ZEB1, key molecules involved in the EndMT processes (44). A previous study (40) showed that TNF-α drives

FIGURE 1 | Schematic representation of endothelial to mesenchymal transition (EndMT) transition in response to inflammatory stimuli and metabolic dysfunction. Upon chronic inflammatory conditions, involving tumor necrosis factor-α (TNF-α), transforming growth factor beta (TGFβ), IL-1β, and endotoxin and metabolic dysfunction, such as increased serum LDL, glucose, diverse ECs undergo activation, which results in loss of endothelial cell markers and acquisition of mesenchymal-cell markers. EndMT contributes to endothelial dysfunction under inflammatory conditions and metabolic dysfunction, with EndMT mediators identified. This process can cause a variety of postnatal diseases, such as fibrosis, PAH, and metabolic syndrome.

EndMT through Akt/NF-κB activity in both embryonic and adult-valve endothelium, finding that EndMT-related protein expression involving α-SMA and snail was significantly upregulated, whereas VE-cadherin was significantly downregulated in response to TNF-α in porcine aortic valve ECs (PAVECs), but not porcine aortic ECs (40); suggesting the importance of determining the molecular mechanism of EndMT in the context of endothelial heterogeneity during inflammation. The same group also demonstrated heterogeneous susceptibility to EndMT in PAVECs in response to TNF-α. Under TNF-α stimulation, non-transforming

cells that maintain endothelial-cell marker expression and transforming cells that acquire mesenchymal-marker expression were isolated using membrane-based three-dimensional culture systems. Transforming cells decreased endothelial marker expression, such as VE-cadherin and endothelial nitric oxide synthase and acquired mesenchymal markers, such as α-SMA, Notch1, MMP-9, BMP-4, and TGFβ in PAVECs (**Figure 2**) (39).

Interleukin-1β is a proinflammatory cytokine (45) involved in endothelial dysfunction (46) and a key inducer of EndMT. IL-1β-induced phenotypic changes in ECs were first demonstrated in IL-1β-treated human dermal microvascular ECs undergoing morphological changes and cytoskeletal reorganization, in addition to decreased expression of typical endothelial markers, such as von Willebrand Factor (vWF) and CD31 (47). In addition, long-term exposure of human dermal microvascular ECs to IL-1β induces the expression of mesenchymal markers such as α-SMA, type I collagen, and calponin and inhibits the expression of vWF (48). Maleszewska et al. (49) reported that the molecular mechanism underlying IL-1β-induced EndMT involves increased expression of SM22α, which is encoded by

TAGLN. Their results demonstrated the epigenetic regulation of *TAGLN via* EZH2, which acts as a key negative regulator in IL-1β-induced EndMT (49). Moreover, in corneal ECs, IL-1β induced an EndMT phenotype by increasing fibroblast growth factor (FGF) expression through the PI3K-signaling pathway (50, 51) in accordance with changes in the actin cytoskeleton and cellular morphology (**Figure 2**). The most recent study has shown that the NLRP3 inflammasome, closely associated with mature IL-1β secretion, is involved in mechanical stretch-induced EndMT in lung fibrosis and NLRP3 inactivation could inhibit EndMT, suggesting novel therapeutic options against mechanical ventilation-induced pulmonary fibrosis (52).

Several studies reported that a combination of cytokines, including TNF-α, IL-1β, and TGFβ, is more powerful than a single cytokine at inducing EndMT. The combination of TGFβ1, IL-1β, and TNF-α induces EndMT *via* the Sp1 transcription factor, which is a key transcriptional regulator of EndMT-related genes in human intestinal microvascular endothelial cells (31). TGFβ2 and IL-1β synergistically induce EndMT through increased expression of mesenchymal markers while decreasing the
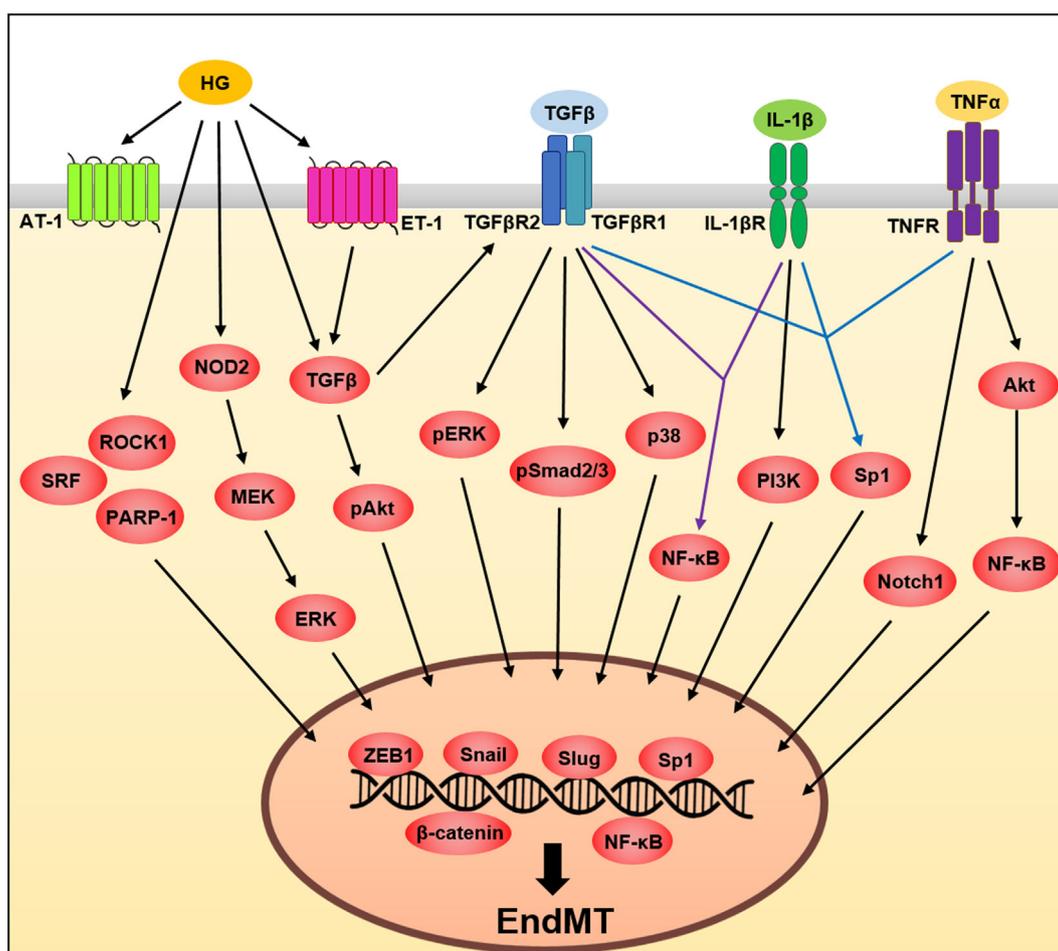
FIGURE 2 | A schematic illustration of the signaling pathways governing endothelial to mesenchymal transition (EndMT). Tumor necrosis factor-α (TNF-α), transforming growth factor beta (TGFβ), interleukin (IL)-1β, and high glucose influences EndMT by regulating signaling pathways. These pathways converge and induce the expression of transcription factors involving Slug, Snail and zinc finger E-box-binding homeobox 1 (ZEB1) (see text for details).

expression of endothelial markers in human esophageal microvascular endothelial cells and human umbilical vein ECs (HUVECs) (53, 54). The combination of TNF-α, IL-1β, and TGFβ1 also induces EndMT in pulmonary artery ECs, with EndMT cells exhibiting morphological changes, as well as changes in endothelial and mesenchymal markers (7).

Emerging evidence has shown that endothelial dysfunction induced by metabolic disorders such as obesity, hyperglycemia, and dyslipidemia is critically associated with induction of EndMT. Several studies have demonstrated that high glucose induces EndMT, which leads to increased expression of mesenchymal markers, such as α-SMA, FSP-1, type I collagen, fibronectin, vimentin, and MMP-2 along with decreased expression of endothelial markers CD31 and VE-cadherin in various EC types (55–57). It was shown that high-glucose-induced EndMT occurs through positive regulators, such as Smad2/3, Snail, Rho-associated kinase 1 (ROCK1), serum response factor (SRF), nucleotide-binding oligomerization domain-containing protein 2 (NOD2), and ERK in glomerular ECs (57–60). In human aortic ECs (HAECs) and HUVECs, high glucose also induces EndMT through positive regulators, such as angiotensin II, poly (ADP-ribose) polymerase 1 (PARP-1), endothelin 1 (ET-1), Smad, Akt, p38, and ERK, contributing to diabetic cardiomyopathy (55, 56, 61–63). It has also been shown that oxidized low-density lipoprotein (ox-LDL) accelerates radiation-induced EndMT in HAECs

and contributes to radiation-induced atherosclerosis (64), whereas high-density lipoprotein (HDL) inhibits TGFβ1-induced EndMT in HAECs suggesting anti-fibrotic effects of HDL (**Figure 2**) (65).

Although much attention has recently been directed to EndMT because of its importance in many diseases, most studies have been limited to the identification of endothelial and mesenchymal markers in response to inducers of EndMT. Therefore, elucidation of the potential molecular mechanisms regulating pathological EndMT induced by inflammatory stimuli will be important in the future. **Table 1** shows a summary of the main studies exploring EndMT under specific inflammatory stimuli and metabolic dysfunction, including EndMT mediators, endothelial and mesenchymal markers, and EC types.

EC HETEROGENEITY DURING INFLAMMATION

Endothelial cells line the inner wall of blood vessels and exhibit diverse subtypes (2, 8, 33). Different ECs have different structural and functional characteristics based on their exposure to distinct microenvironments (2, 8, 9, 33, 66). They are activated in response to inflammatory stimuli, with this activation resulting in the expression of adhesion molecules necessary for leukocyte binding (33).

TABLE 1 | Summary of the key studies exploring EndMT under specific inflammatory stimuli and metabolic dysfunction.

Stimuli	Endothelial markers	Mesenchymal markers	Endothelial cell types	Positive regulator of EndMT	Reference
TNF-α	VE-cadherin	N-cadherin	LEC	ZEB1 and β-catenin	(44)
TNF-α	VE-cadherin, CD31, eNOS	α-SMA and MMP-9	PAVEC and eQEE	Akt/NF-κB, Snail, Slug, TGFβ, Notch1, and BMP-4	(39, 40)
IL-1β	vWF	α-SMA, collagen I, and calponin	HDMEC	Non determined	(47, 48)
IL-1β	Non determined	SM22α	HUVEC	pSmad2 and TGFβ2	(49)
IL-1β	Non determined	Cell shape change and actin cytoskeleton	CECs	PI3K	(50, 51)
TNF-α, IL-1β and TGFβ1	VE-cadherin, CD31, and vWF	α-SMA, FSP-1, vimentin, N-cadherin, and fibronectin	HIMEC	Sp1	(31)
TGFβ2 and IL-1β	CD31, vWF, and VE-cadherin	SM22α, FSP-1, collagen 1 A2, vimentin, and α-SMA	HEMEC	Snail	(53)
TGFβ2 and IL-1β	eNOS and vWF	SM22α, calponin	HUVEC	NF-κB	(54)
TNF-α, IL-1β and TGFβ1	vWF, CD31, VE-cadherin, and Occludin	Calponin, α-SMA, and collagen I	PAECs	Non determined	(7)
High glucose	CD31 and VE-cadherin	α-SMA, α-SMA, FSP-1, and fibronectin	GEnC	TGFβ, pSmad2/3, Snail, ROCK1, NOD1, MEK/ERK, SRF, and Snail	(57–60)
High glucose	CD31 and VE-cadherin	α-SMA, FSP-1, collagen I, collagen III, and MMP-2/9	HAEC	Angiotensin II, Snail, and PARP-1	(55, 61)
High glucose	VE-cadherin and CD31	α-SMA, collagen I, FSP-1, vimentin, and MMP-2/9	HUVEC	TGFβ1, ERK, pSmad2/3, and MAPK (p38 and ERK)	(56, 63)
High glucose	VE-cadherin	FSP-1 and collagen I	HUVEC and HAEC	ET-1, TGFβ1, pSmad3, pAKT, and Snail	(62)
ox-LDL + Radiation	VE-cadherin and CD31	α-SMA, FSP-1, and vimentin	HAEC	Non determined	(64)

TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; TGFβ, transforming growth factor-β; VE-cadherin, vascular endothelial cadherin; eNOS, endothelial nitric oxide synthase; vWF, von Willebrand Factor; α-SMA, α-smooth muscle actin; SM22α, smooth muscle protein 22-α; FSP-1, fibroblast-specific protein 1; LEC, lymphatic endothelial cell; PAVEC, porcine aortic valve endothelial cell; eQEE, embryonic quail endocardial explant; HDMEC, human epithelioid dermal microvascular endothelial cell; HUVEC, human umbilical vein endothelial cell; CEC, corneal endothelial cell; HIMEC, human intestinal microvascular endothelial cell; HEMEC, human esophageal microvascular endothelial cell; PAEC, pulmonary artery endothelial cell; ZEB1, zinc finger E-box-binding homeobox 1; NF-κB, nuclear factor kappa B; BMP-4, bone morphogenetic protein 4; EZH2, enhancer of zeste homolog 2; FGF-2, fibroblast growth factor 2; PI3K, phosphatidylinositol 3-kinase. GEnC, glomerular endothelial cell; ROCK1, Rho-associated kinase 1; HAEC, human aortic endothelial cell; GLP-1, glucagon-like peptide-1; PARP-1, Poly (ADP-ribose) polymerase 1; SRF, Serum response factor; ET-1, endothelin-1; ox-LDL, oxidized low-density lipoprotein; NOD2, Nucleotide-binding oligomerization domain-containing protein 2.

Many studies showed that each EC subtype responds differently to different inflammatory stimuli *in vitro* (33). Viemann et al. (67) suggested that genes differentially regulated upon TNF-α stimulation between human microvascular ECs and HUVECs exhibit functional differences, and that genes whose expression was altered only in the human microvascular ECs group were associated with signaling and transcription factors, apoptosis, cell proliferation, immune response, and cell structure. However, genes showing altered expression only in HUVECs were associated with chemokines, cytokines, cell-surface molecules, and signaling and transcription factors (67). VCAM-1 expression was only increased in response to TNF-α in HUVECs and glomerular ECs, but not in dermal microvascular ECs (68). In addition, Scott et al. (69) reported changes in heterogeneous gene expression in response to TNF-α, lipopolysaccharide (LPS), and IL-1β in HUVECs, human pulmonary microvascular ECs, HAECs, carotid artery ECs, coronary artery ECs, subclavian artery ECs, and brachiocephalic artery ECs. TNF-α, and IL-1β-stimulated organ-specific endothelial heterogeneity has also been reported (70).

These findings suggest that each EC subtype might respond differently to different inflammatory stimuli in the context of EndMT. Indeed, Pinto et al. (71) compared responses to TGFβ1 or TGFβ2 between human coronary artery ECs and microvascular pulmonary artery ECs, finding that in human coronary artery ECs, both TGFβ1 and TGFβ2 upregulated the expression of the mesenchymal markers α-SMA and SM22α, but only TGFβ1 had an effect on α-SMA expression in human pulmonary microvascular ECs. In addition, TNF-α increased the expression of the mesenchymal markers in PAVECs, although this response was not observed in porcine aortic ECs (40).

Importantly, other inflammation-associated endothelial activators, such as shear stress or protein kinase C (PKC), have also been studied in this context (33). Methe et al. (72) showed that venous and coronary artery specific flows differentially regulate the expression of endothelial adhesion molecules as well as KLF2/KLF4 transcription factors in human saphenous vein ECs and human coronary artery ECs. Two ECs have also been reported to show heterogeneity in adhesion molecule expression in response to PKC. Here, PKC activation induces E-selectin and VCAM-1 expression in HUVECs, but not in human dermal microvascular ECs (73).

Differences in the behavior of various EC subtypes in response to inflammatory stimuli have also been reported *in vivo* (33). Tamaru et al. (74) showed that induction of adhesion molecule expression in response to IL-1β stimulation is both tissue- and cell-type specific. However, no changes were observed in VCAM-1 expression in brain and liver microvascular ECs in response to LPS stimulation (75, 76). Furthermore, van Meurs et al. (77) showed that E-selectin and VCAM expression in human glomerular ECs differs from that in other ECs. In CD31-deficient mice, apoptosis of peritubular-capillary ECs occurs upon LPS administration, although other microvessel ECs were unaffected (78). Given the extent of endothelial heterogeneity found both *in vitro* and *in vivo*, studying the molecular mechanisms and functions associated with the EndMT process in inflammation in the context of endothelial heterogeneity will

eventually enable us to better understand vascular diseases and develop more sophisticated and effective therapeutic drugs.

TARGETING ENDMT FOR THERAPEUTIC AND CLINICAL APPLICATIONS IN VASCULAR DISEASES

Endothelial to mesenchymal transition is recognized to not only occur during development but also it is now clear that EndMT underlies pathological processes associated with multiple diseases (6, 26, 79, 80). EndMT is also controlled by a variety of stimuli, including inflammation, growth factors, and hypoxia (81–83). Particularly, inflammation-induced EndMT aggravates inflammation and destroys vascular homeostasis, leading to pathogenesis of several diseases, such as cardiac fibrosis, PAH, and atherosclerosis (31, 84, 85). Given the involvement of EndMT in multiple inflammatory diseases, preventing EndMT may represent a useful approach to treat inflammatory diseases.

Several factors have been identified as the negative regulators of EndMT signaling pathways (86–88). Vascular endothelial growth factor-A reverses TGFβ2-induced EndMT (89), and HDL and the extracellular-matrix protein fibulin 1 and kallistatin also exert inhibitory effects on TGFβ-induced EndMT (65, 90, 91). Furthermore, the most common aldosterone receptor antagonist, spironolactone has a protective role against TGFβ-induced EndMT in HUVECs (92) and, rapamycin suppresses mechanistic target of mTOR signaling, leading to the inhibition of EndMT (93). Although emerging studies report multiple EndMT mediators that play critical roles in EndMT induction, the targeting of EndMT mediators requires careful evaluation due to the modulation of EndMT exhibiting differential effects in different ECs based on endothelial heterogeneity. For example, IL-1β upregulates FGF2 expression through PI3K activation, which leads to EndMT of corneal ECs (51). Lee et al. (50) suggested that blocking the IL-1β and FGF2 pathways would prevent inflammation-induced EndMT in corneal ECs; however, FGF2 exerts an inhibitory effect on TGFβ-mediated EndMT *via* miR-20a in HUVECs (81, 94). Moreover, another study showed that FGF receptor-1 is a key inhibitor of TGFβ-driven EndMT in HUVECs (95), and the endogenous antifibrotic peptide N-acetyl-seryl-aspartyl-lysyl-proline restores FGF receptor levels and upregulates levels of the let-7, resulting in the inhibition of EndMT in human dermal microvascular ECs (96). Therefore, further studies are needed to completely elucidate the mechanisms associated with FGF2, as well as many mediators involved in EndMT (81).

In the context of diseases, studies on the inhibitory effect of EndMT have mainly focused on fibrosis. The common feature of many fibro-proliferative diseases is inflammation (97). Fibrosis results from chronic inflammation, possibly owing to infection, autoimmune reactions, or allergic reactions, which results in the release of inflammatory mediators, abnormal cell proliferation, and deposition of extracellular-matrix components (81, 82, 84, 97). BMP7 has been shown to exhibit anti-EndMT effects and reduce cardiac fibrosis; however, most other BMPs are positive regulators of EndMT (81, 85, 98, 99). Hepatocyte growth factor

reduces cardiac fibrosis by suppressing TGFβ1-mediated EndMT (100). Cinacalcet, a calcimimetic agent, reduces serum levels of parathyroid hormone and suppresses EndMT, leading to attenuation of cardiac fibrosis (101). Similarly, losartan and irbesartan, two angiotensin II-receptor type 1 blockers, ameliorate cardiac fibrosis by inhibiting EndMT (61, 102). Scutellarin and relaxin are also EndMT inhibitors and prevent cardiac fibrosis by regulating Notch1 and Jagged-1 (86, 103). Furthermore, inhibition of CD45 protein tyrosine phosphatase leads to reduced EndMT in TGFβ1-treated mitral valve ECs (104). The anti-fibrotic effects of linagliptin, which blocks EndMT, have also been reported *in vitro* and in diabetic kidneys (105). Similarly, macitentan inhibits endothelin-1 or TGFβ1-induced EndMT in systemic sclerosis (106).

Cytokine-induced inflammation is widely considered a major cause of PAH development (107). Moreover, remodeling of the pulmonary artery under inflammatory conditions is a major feature of PAH (12–15). Recent evidence suggests that inflammation-induced EndMT is a key contributor to pathological pulmonary vascular remodeling associated with transition of ECs to α-SMA-expressing mesenchymal-like cells in obstructive vascular lesions of PAH (7, 29). Clinical data also indicate that the serum levels of IL-1, -6, -8, -10, and TNF-α are elevated in PAH patients (107). In this context, salvianolic acid A, a polyphenol compound, inhibits EndMT in PAH, thereby attenuating inflammation associated with monocrotaline-induced PAH (108). Another study showed

that the delivery of BMP receptor-2 resulted in less right-ventricle hypertrophy, pulmonary vascular resistance, and improved cardiac function through attenuation of EndMT (109). Kang et al. (87) suggested that ponatinib, a multi-target tyrosine-kinase inhibitor, delays TGFβ1-mediated EndMT and has therapeutic potential for use in PAH therapy, where it could act by regulating Wnt signaling.

Atherosclerosis is a vascular disease mediated by a typical inflammatory response. Inflammatory stimuli continuously lead to calcified plaque formation (110). Atherosclerosis lesions mostly comprise EndMT-derived fibroblast-like cells, which are regulated by various EndMT mediators, such as snail, slug, and β-catenin (84, 111, 112). Other pathways might also lead to atherosclerosis by inducing EndMT *via* TGFβ, oxidative stress, hypoxia, Wnt/β-catenin signaling, and BMP signaling (81, 84, 113). These data suggest that EndMT is a major source of neointimal hyperplasia and plays a role in the progression of arteriosclerosis through inflammation. In particular, excessive BMP activity promotes the calcification of atherosclerotic lesions through EndMT and serine-protease inhibitors also reduce EndMT and vascular calcification (88, 114). Consistent with this report, vascular calcification was found to be reduced in response to a BMP inhibitor in matrix-gla-protein-deficient mice (115).

It has been shown that metabolic syndrome, which is associated with metabolic dysfunction such as obesity, hyperglycemia, insulin resistance, and dyslipidemia has a central role in the

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TABLE 2 | Summary of the key studies exploring endothelial to mesenchymal transition (EndMT) as a therapeutic target in various diseases.

Model of Study	Negative regulator of EndMT	Clinical relevance	Reference
Isoproterenol-induced myocardial fibrosis rat model	Relaxin	Cardiac fibrosis	(86)
Bleomycin-induced PAH model	Ponatinib (multi-targeted tyrosine-kinase inhibitor)	Pulmonary arterial hypertension (PAH)	(87)
TGFβ1-induced EndMT	HDL	Non-determined	(65)
TGFβ-induced EndMT	Spironolactone (aldosterone receptor antagonist)	Non-determined	(92)
Mouse models of pressure overload and chronic allograft rejection	BMP-7	Cardiac fibrosis	(85)
Heterotopic heart transplantation model	BMP-7	Endocardial fibroelastosis	(99)
Pressure-overload mouse model	HGF	Cardiac fibrosis	(100)
Rat model of uremia and secondary hyperparathyroidism	Cinacalcet (calcimimetic agent)	Cardiac fibrosis	(101)
TGFβ1-induced EndMT	Losartan (angiotensin II receptor type 1 blocker)	Non determined	(102)
Isoproterenol -induced myocardial fibrosis rat model	Scutellarin	Cardiac fibrosis	(103)
Ovine inferior myocardial infarction model	CD45-selective PTPase inhibitor	Myocardial infarction	(104)
STZ-induced diabetic mice	Linagliptin (DPP-4 inhibitor)	Diabetic kidney fibrosis	(105)
TGFβ and ET-1-induced EndMT	Macitentan (ET-1 receptor antagonist)	Systemic sclerosis	(106)
MCT-induced PAH model	Salvianolic acid A	Pulmonary arterial hypertension	(108)
Hypoxia, MCT-induced PAH model	Delivery of *BMPR2*	Pulmonary arterial hypertension	(109)
STZ-induced diabetic rats	Lovastatin	Diabetic nephropathy	(58)
db/db diabetic mice	Fasudil (ROCK1 inhibitor)	Diabetic nephropathy	(59)
STZ-induced SHR diabetic rats	Irbesartan (angiotensin II receptor type 1 blocker)	Diabetic cardiomyopathy	(61)
STZ-induced diabetic mice	GLP-1 analog	Diabetic cardiomyopathy	(55)
STZ-induced diabetic rats	CCG-1423 (SRF inhibitor)	Diabetic nephropathy	(57)
STZ-induced diabetic ET-1$^{f/f}$; Tie2-Cre(+) mice	ET-1 silencing	Diabetic cardiomyopathy	(62)
STZ-induced diabetic mice	Low-dose irisin	Diabetic cardiomyopathy	(63)
TGFβ1-induced EndMT	HDL	Non determined	(65)

TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; TGFβ, transforming growth factor-β; VE-cadherin, vascular endothelial cadherin; eNOS, endothelial nitric oxide synthase; vWF, von Willebrand factor; α-SMA, α-smooth muscle actin; SM22α, smooth muscle protein 22-α; FSP-1, fibroblast-specific protein 1; LEC, lymphatic endothelial cell; PAVEC, porcine aortic valve endothelial cell; eQEE, embryonic quail endocardial explant; HDMEC, human epithelioid dermal microvascular endothelial cell; HUVEC, human umbilical vein endothelial cell; CEC, corneal endothelial cell; HIMEC, human intestinal microvascular endothelial cell; HEMEC, human esophageal microvascular endothelial cell; PAEC, pulmonary artery endothelial cell; ZEB1, zinc finger E-box-binding homeobox 1; NF-κB, nuclear factor kappa B; BMP-4, bone morphogenetic protein 4; EZH2, enhancer of zeste homolog 2; FGF-2, fibroblast growth factor 2; PI3K, phosphatidylinositol 3-kinase. STZ, streptozotocin; SHR, spontaneously hypertensive rats; HDL, High-Density Lipoproteins; HGF, hepatocyte growth factor; MCT, monocrotaline.

pathogenesis of cardiovascular disease, diabetes mellitus type 2, and tissue remodeling (116). Abnormalities associated with chronic inflammation are key risk factors of metabolic dysfunction, leading to the endothelial dysfunction that is critically involved in the development of such diseases (64, 116–119). In the context of metabolic syndrome, studies on EndMT induced by metabolic dysfunction have mainly focused on diabetic nephropathy and cardiomyopathy. Several studies have shown that EndMT contributes to diabetic nephropathy, while inhibition of EndMT by lovastatin, fasudil, and CCG-1423 could ameliorate diabetic nephropathy in streptozotocin (STZ)-induced diabetic animal models (57–59). In addition, inhibition of EndMT by Irbesartan, glucagon-like peptide-1 analog, ET-1 inhibition and low-dose irisin could prevent diabetic cardiomyopathy in diabetic animal models (55, 61–63).

Taken together, the currently available data indicate that EndMT plays a key role in various fibrosis-related and cardiovascular diseases (**Figure 1**; **Table 2**). Considering the large number of studies that suggest targeting EndMT as a novel therapeutic approach for many diseases, clarifying the underlying signaling mechanisms associated with EndMT and establishing strategies to regulate EndMT are urgently needed.

CONCLUSION

Endothelial to mesenchymal transition plays an important role not only during the development process but also in adults under physiological and pathological conditions. A central role for EndMT emerges from the complex network of interactions that underlie inflammation-induced endothelial dysfunction. There is accumulating evidence indicating that EndMT is a key feature in inflammation-related endothelial dysfunction. It is through this phenotypic switch that EndMT causes diverse vascular diseases, such as atherosclerosis, PAH, and fibrosis. Therefore,

the modulation of EndMT might yield new therapeutic strategies for the treatment of diverse diseases. Although our current understanding of the molecular mechanisms underlying EndMT in the context of inflammation is advancing, further studies are needed in the future to completely understand the molecular mechanism associated with EndMT in inflammation-related diseases. Given that heterogeneity is apparent in ECs of different organs in response to different inflammatory stimuli, it will also be important to determine the molecular mechanisms associated with EndMT in the context of endothelial heterogeneity during inflammation in future studies. In conclusion, the study of EndMT will provide valuable insights into the molecular mechanisms leading to various human diseases and will help develop more sophisticated and effective therapeutic drugs for patients suffering from these diseases.

AUTHOR CONTRIBUTIONS

JK, JC, and AL wrote the manuscript. JK, WC, and MS-L were critically involved in the design of the work and the discussion of the content. All the authors approved the final manuscript.

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