# Endothelial-Mesenchymal Transition

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key event in vascular proliferative diseases by releasing cytokines and growth factors. This activation is mediated by Shh and PDGF-BB induced activation of Smo-dependent signalling and the selective inhibitor GDC-0449 may serve as a novel and promising therapeutic strategy to prevent neointima formation.

### P700 | BENCH
### The novel mineralocorticoid receptor antagonist Finerenone attenuates neointima formation after vascular injury

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**Background:** Ischemic cardiomyopathy as a result of coronary artery disease is the leading cause for heart failure. In consequence, the effect of novel heart failure therapeutics on vascular function and remodeling processes is of pivotal importance. Finerenone, a novel nonsteroidal mineralocorticoid receptor (MR) antagonist, holds the promise to be safe and efficient in the treatment of patients with heart failure and/or chronic kidney disease. However, the effects on vascular function remain elusive.
**Purpose:** The aim of this study was to determine the functional effect of selective MR antagonism by Finerenone in vascular cells in vitro and the effect on vascular remodeling following acute vascular injury in vivo.
**Methods and results:** Finerenone dose-dependently and significantly reduced aldosterone-induced human coronary artery smooth muscle cell (HCASMC) proliferation as quantified by BrdU incorporation. Furthermore, Finerenone dose-dependently and significantly prevented aldosterone-induced apoptosis in human umbilical vein endothelial cells (HUVEC) as measured with a flow cytometry based FLICA-assay.
In vivo, oral application of Finerenone dose-dependently and significantly inhibited intimal and medial cell proliferation following femoral artery wire-induced injury in C57BL/6J mice as quantified by staining for Ki-67 10 days following injury (vehicle vs. 1 mg/kg/d vs. 10mg/kg/d; each $p<0.01$). Concomitantly, Finerenone attenuated neointimal lesion formation following femoral artery wire-induced injury 21 days following injury (luminal stenosis, vehicle vs. Finerenone 1 mg/kg/d vs. Finerenone 10mg/kg/d: 90.2±1.1% vs. 60.1±17.3%; p=0.1063 to vehicle, vs. 35.3±10.0%; p=0.0061 to vehicle; n=8). Furthermore, there was a trend towards an accelerated re-endothelialization of the injured vessel segments in Finerenone-treated mice three days following electric injury of the murine carotid artery.
**Conclusion:** Finerenone treatment significantly attenuates HCASMC proliferation while simultaneously preventing apoptosis of endothelial cells in vitro. This is reflected by a significantly reduced neointima formation and reduction of luminal stenosis as well as a trend towards an accelerated endothelial healing of the injured vessels. Thus, apart from its beneficial effects in heart failure therapy, Finerenone might provide favorable vascular effects through restoring vascular integrity and preventing adverse vascular remodeling.
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### P701 | BENCH
### Endothelial protein tyrosine phosphatase-1B deletion enhances neointima formation in mice with diet-induced obesity

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**Background:** Obesity and metabolic dysfunction are associated with increased expression of protein tyrosine phosphate (PTP)-1B, a negative regulator of receptor tyrosine kinase signalling, and PTP1B overexpression may be causally involved in the development of insulin and leptin resistance in obesity. Previous studies have shown that systemic inhibition or genetic deletion of PTP1B protects against endothelial dysfunction in heart failure. However, the role of PTP1B expressed in endothelial cells for the cardiovascular risk in obesity has not been directly examined so far.
**Purpose:** To determine the effect of endothelial PTP1B deletion on the vascular response to injury in mice with diet-induced obesity.
**Methods:** Mice with loxP-flanked PTP1B alleles were mated with mice expressing a Cre recombinase-estrogen receptor fusion protein ER (T2) under control of the endothelial receptor tyrosine kinase (Tie2) promoter. Cre recombinase activity and endothelial-restricted PTP1B gene excision (End.PTP1B-KO) was induced by feeding mice tamoxifen-containing rodent chow for 6 weeks. Obesity was induced by feeding mice 45% high fat diet (HFD) for 4 weeks prior to vascular injury at the common carotid artery using 10% ferric chloride and continued until sacrifice 3 weeks later.
**Results:** HFD resulted in increased body weight, visceral obesity and total serum cholesterol, both in End.PTP1B-WT and End.PTP1B-KO mice and also was associated with elevated PTP1B protein levels in endothelial cells isolated from the lungs of obese mice (1.4-fold increase vs. lean; $P<0.05$). Morphometric analysis of serial sections through restenotic lesions revealed that deletion of PTP1B in endothelial cells was associated with a significant increase in the neointima area ($P<0.05$) and intima-to-media ratio ($P<0.05$). Because the total vessel area also was significantly enlarged in mice lacking endothelial PTP1B ($P<0.05$), the more pronounced lumen stenosis observed in End.PTP1B-KO mice (33±7.0 vs. 20±4.0% in End.PTP1B-WT) did not reach statistical significance. Analysis of re-endothelisation revealed significantly reduced numbers of luminal cell nuclei in End.PTP1B-KO animals ($P<0.05$), and (immuno-)histochemical analysis confirmed reduced luminal binding of endothelial lectin (10±4.4 vs. 25±5.1% in End.PTP1B-WT mice; P=0.065). Moreover, neointimal lesions of End.PTP1B-KO mice were characterised by an increased absolute number of cell nuclei ($P<0.05$ vs. End.PTP1B-WT mice) as well as elevated amounts of αSM-actin-positive SMCs (6315±2671 vs. 1729±496 $\mu m^2$; P=0.075).
**Conclusion:** Our findings suggest that endothelial PTP1B plays an important role for lesion re-endothelisation after vascular injury. The observed neointimal hyperplasia in mice lacking PTP1B in endothelial cells may be the consequence of failure to induce SMC quiescence resulting in uncontrolled neointimal SMC proliferation.

### P702 | BENCH
### Endothelial-Mesenchymal Transition: miR-101 as a new target to treat intimal hyperplasia

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**Introduction:** Endothelial-Mesenchymal Transition (EndMT) is a specific form of endothelial dysfunction wherein endothelial cells acquire a mesenchymal phenotype and lose their endothelial functions. We, and others, recently described that EndMT contributes to intimal hyperplasia and atherosclerosis.
Pro-fibrotic and inflammatory cytokines, such as IL-1β and TGFβ2 induce EndMT. We found that the mitogen activated protein kinase 7 (MAPK7, also known as Erk5) inhibits EndMT. MAPK7 activation decreases the expression of the histone methyltransferase Enhancer-of-Zeste homologue 2 (Ezh2) thereby maintaining endothelial quiescence.This decrease in Ezh2 expression may therefore be responsible for the protective effects of MAPK7 activation and may thus offer new therapeutic options for the treatment of endothelial dysfunction and intimal hyperplasia.
Ezh2 is the catalytic subunit of the Polycomb Repressive Complex 2 that methylates lysine 27 on histone 3 (H3K27me3). H3K27me3 is a repressive chromatin mark that inhibits gene expression. Currently, it is elusive how the crosstalk between MAPK7 and Ezh2 is regulated in the endothelium and if the balance between MAPK7 and EZH2 is disturbed during intimal hyperplasia.
**Methods and results:** We used in silico analysis to identify miRNAs that could evoke posttranscriptional silencing of Ezh2. In Luciferase reporter assays, miR-101 efficiently inhibited expression of the luciferase reporter by interacting with the 3'UTR of EZH2. Using a uniform laminar flow setup, we revealed that MAPK7 induced miR101 expression, which was blocked by the selective MAPK7 inhibitor BIX02189 ($p<0.05$). Furthermore, ectopic expression of miR-101 in endothelial cells reduced the expression of Ezh2.
In samples of human coronary artery stenosis Ezh2 levels are increased, whereas MAPK7 expression is reduced. Moreover, miR-101 expression is decreased, which associated with the increase of Ezh2 ($R^2=0.23$, p=0.051) and severity of the stenosis (Intima/Media Thickness, $R^2=0.45$, p=0.003).
**Conclusion(s):** Under uniform laminar flow MAPK7 inhibits Ezh2 expression via activation of miR-101. In coronary artery stenosis, endothelial cells are exposed by non-uniform shear stress which decreases MAPK7 activation, miR-101 expression and concurrently increases Ezh2 expression, which may cause EndMT and intimal hyperplasia. Therefore, the restoration of miR-101 expression or the silencing of Ezh2 in the endothelium might provide novel therapeutic approaches to treat intimal hyperplasia.
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### P703 | BEDSIDE
### TNF-antagonists improve arterial stiffness in patients with rheumatoid arthritis: a meta-analysis

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**Background:** Patients with rheumatoid arthritis (RA) have a higher arterial stiffness than their age-matched healthy counterparts and an increased inflammatory burden that might be associated with their increased cardiovascular risk. While tumor necrosis factor alpha (TNF)-antagonists have been found to reduce inflammatory markers in RA, it is debatable if they have favorable effects on surrogate markers of cardiovascular outcomes.
**Purpose:** We conducted a meta- analysis to assess the effect of TNF-antagonists on arterial stiffness, a predictor of cardiovascular events and mortality, in RA patients.
**Methods:** A search of PUBMED was conducted to identify studies into the ef-