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LIM-only protein FHL2 attenuates inflammation in vascular smooth muscle cells through inhibition of the NFκB pathway

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ABSTRACT

Despite the advent of new-generation drug-eluting stents, in-stent restenosis remains a significant problem in patients with coronary artery disease. In-stent restenosis is defined as the gradual re-narrowing of a stented coronary artery lesion due to arterial damage with subsequent local inflammation of the vessel wall and excessive growth of the vascular smooth muscle cells (vSMCs). Four-and-a-half LIM-domain protein 2 (FHL2) is a scaffold protein involved in regulating vSMC function and inflammation. Previously we have demonstrated that FHL2 prevents vSMC proliferation in a murine carotid artery ligation model. However, the effect of FHL2 on the inflammatory response of the vSMCs is not investigated. Therefore, we studied the inflammatory response in the vessel wall of FHL2-deficient (-KO) mice after carotid artery ligation. We found that circulating cytokines and local macrophage infiltration in the ligated carotid vessels were increased in FHL2-KO mice after carotid artery ligation. Moreover, FHL2-KO vSMCs showed increased secretion of cytokines such as SDF-1α and RANTES, and enhanced activation of the NFκB pathway. Finally, we found that blocking the NFκB signalling pathway abrogated this pro-inflammatory state in FHL2-KO vSMCs. Taken together, our results demonstrate that FHL2 decreases the inflammatory response of vSMCs through inhibition of the NFκB-signalling pathway.

1. Introduction

With age, atherosclerotic plaques build up inside the human cardiovascular system, preferentially at sites with disturbed flow. Especially when occurring inside coronary arteries, these plaques can be life-threatening when reaching a point when the vessel is too narrow to supply the heart with sufficient blood. Furthermore, rupture of instable plaques can cause acute vessel occlusion and can lead to myocardial infarction. In many cases, a bare-metal stent is placed to widen the lumen of the narrowed artery and restore blood flow. Unfortunately, a large number of the patients (\pm 6–43%) develop in-stent restenosis due to growth of the intima/media into the lumen of the stent, which can already occur within 1 month after stent placement [1,2].

Placing a stent causes de-endothelialization of the underlying surface, initiating a wound healing response [3]. Due to the arterial damage, endothelial cells become dysfunctional and secrete several growth factors, cytokines and chemokines which in turn promote recruitment of inflammatory cells such as leukocytes and neutrophils into the injury site [4]. Furthermore, the release of chemokines such as stromal cell derived factor-1α (SDF-1α/CXCL12) induces a smooth

muscle cell (SMC) phenotypic switch from contractile to synthetic phenotype allowing proliferation and migration of SMCs towards the damaged intima [5]. Moreover, macrophage infiltration and lipid deposition can create neoatherosclerosis within the restenotic tissue [3]. With time, these processes narrow the lumen of the artery requiring another intervention. Although it is well established that inflammation plays a pivotal role in the pathogenesis of in-stent restenosis [6,7], the factors that regulate the inflammatory process are still largely unknown.

Four-and-a-half LIM-domain 2 (FHL2) is a LIM-only protein that can act as a scaffold protein. By binding other proteins, it can affect signalling of pathways controlling many cellular processes such as inflammation, apoptosis and migration [8,9]. FHL2 has been shown to act as a cofactor for several transcription factors including Nur77, androgen receptor, LXR and NFκB [9–11]. FHL2-KO mice do not present with cardiovascular pathologies, but when triggered by for example a Western-Type diet, adrenergic stimulation or carotid artery ligation, the importance of FHL2 for homeostasis of the cardiovascular system becomes very apparent [12–14]. Carotid artery ligation in mice induces a vascular healing response similar to in-stent restenosis, involving

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intimal thickening, inflammation and extensive SMC proliferation [15]. We demonstrated previously that SMC proliferation and the formation of SMC-rich lesions are increased in FHL2-KO mice after carotid artery ligation, demonstrating that absence of FHL2 enhances disease progression [12].

As a scaffolding protein, FHL2 interacts with various proteins and modulates several pathways in a cell- and context-dependent fashion. For example, FHL2 has been shown to induce expression of IL-6 and IL-8 via activation of the NF κ B signalling pathway in liver and muscle cells [16]. In line with this, bone marrow derived macrophages from FHL2-KO mice exhibited decreased levels of IL-6 and TNF α in response to LPS [16]. In contrast, bone marrow derived macrophages from FHL2-KO also showed increased expression of TNF α , MCP-1 and SDF-1 α following LPS stimulation [17]. Interestingly, peritoneal macrophages from FHL2-KO mice displayed no change in pro-inflammatory cytokine expression following LPS stimulation [18]. However, under basal conditions, FHL2-KO mice exhibited higher serum protein levels of S100A8/A9, a well-established pro-inflammatory marker [18,19]. Taken together, these data suggests that FHL2 regulates the inflammatory response in a highly context- and tissue-dependent manner. Therefore, we investigated the role of FHL2 in the inflammatory response of vascular SMCs in vitro and in a murine carotid artery ligation model.

In this study, we found that both systemic and local vascular inflammation is increased in FHL2-KO mice after carotid artery ligation. In addition, FHL2 deficient SMCs secreted increased amounts of chemokines and cytokines, which was decreased to baseline when FHL2 levels were exogenously increased. Finally, we show that pharmacological inhibition of the NF κ B pathway significantly attenuated the increased inflammatory status of FHL2 deficient SMCs signifying that FHL2 interferes with this specific signalling pathway.

2. Results

2.1. FHL deficiency increases inflammation in carotid artery ligation

Previous studies showed that carotid artery ligation causes an increase in SMC-rich lesion area in FHL2-deficient mice compared to control mice [12]. Since inflammation is an important trigger for intimal hyperplasia, we determined the plasma levels of the pro-inflammatory cytokine SDF-1 α in FHL2-KO and WT mice 4 weeks after ligation. We found a significant increase of SDF-1 α in plasma of FHL2-KO mice compared to WT control (Fig. 1A). In addition, baseline plasma levels of SDF-1 α were elevated in FHL2-KO mice compared to WT animals (Fig. 1B). An increase in plasma SDF-1 α levels reflects a systemic increase in inflammation that may drive monocyte recruitment from the bone-marrow [4,20]. To determine if there was an influx of macrophages in the local microenvironment, we stained carotid lesions for MAC-3, a protein present on differentiating and activated macrophages, allowing the number of macrophages in the vessel to be quantified. In the first week after ligation the number of macrophages were the highest, and decreased over time in both WT and FHL2-KO mice (Fig. 1C–D). However, at all timepoints analyzed (1, 2 and 4 weeks after ligation), the number of macrophages was significantly increased in the FHL2-KO mice compared to WT animals. Next we determined mRNA levels of RANTES and SDF-1 α in ligated carotid artery tissue at these different timepoints. Both inflammatory mediators showed increasing expression over time in FHL2-KO and WT mice (Fig. 1E–F), but the expression levels of RANTES were significantly higher in FHL2-KO mice compared to WT animals under both basal and ligated conditions. Although SDF-1 α levels were unchanged at baseline in FHL2-KO mice, carotid artery ligation resulted in significantly higher the levels of SDF-1 α in FHL2-KO mice compared to WT counter parts.

2.2. FHL2-KO SMCs express increased inflammatory markers in vitro

Activated SMCs can produce an array of inflammatory cytokines that are instrumental in progression of vascular diseases, including restenosis [21,22]. Therefore, to further substantiate our in vivo observations, we isolated aortic SMCs from FHL-KO and WT mice. Upon serum-starvation, we measured the mRNA levels of SDF-1 α in cells and protein levels in the supernatants of WT and FHL2-KO SMCs. Consistent with the in vivo findings, both mRNA expression and protein levels of SDF-1 α were significantly increased in FHL2-KO SMCs compared to WT SMCs (Fig. 2A–B). Moreover, FHL2-KO SMCs showed a significant increase in expression of several other proinflammatory cytokines, namely protein expression of IL-6 and MCP-1, and mRNA expression of RANTES and CXCL-1 compared to WT SMCs (Fig. 2C–D). Altogether, FHL2-KO SMCs exhibit a pro-inflammatory phenotype in vitro.

2.3. FHL2 attenuates inflammation via inhibition of the NF κ B pathway

Previous reports showed that activation of NF κ B pathway plays a crucial role in neointima formation and cytokine production [6,23,24]. Since FHL2 can act as a cofactor for NF κ B in different cell types, we determined mRNA levels of the inhibitor of NF κ B signal transduction, I κ B α , and found that I κ B α levels were slightly decreased in serum-starved FHL2-KO SMCs compared to WT cells. Interestingly, stimulation with serum decreased I κ B α expression only in FHL2-KO SMCs, suggesting that the pro-inflammatory phenotype of the FHL2-KO SMCs is regulated via activation of the NF κ B pathway (Fig. 3A). To further study the effect of FHL2 on the NF κ B signalling pathway, we determined NF κ B transcription in FHL2-deficient SMCs by using two distinct NF κ B luciferase-reporter constructs. As expected, in normal culture conditions, NF κ B transcription was significantly higher in FHL2-KO SMCs versus WT SMCs (Fig. 3B). Moreover, NF κ B activity is markedly higher in FHL2-KO SMCs under TNF α stimulated conditions as demonstrated by both NF κ B reporter plasmids (Fig. 3C). To further elucidate the effect of FHL2 on NF κ B signalling in SMCs, we made use of a pharmacological inhibitor of NF κ B, BAY11-7085, which inhibits of I κ B α phosphorylation, thereby keeping the NF κ B dimer sequestered in the cytoplasm [25]. Inhibition of NF κ B with BAY11-7085 decreased mRNA expression of IL6, MCP-1, RANTES and SDF-1 α significantly in the FHL2-KO SMCs (Fig. 3D). Finally, when FHL2 levels were restored in FHL2-KO SMCs using FHL2 lentivirus, the expression levels of RANTES and SDF-1 α were significantly decreased (Fig. 3E). Taken together, these data support that FHL2-KO SMCs exhibit a high pro-inflammatory phenotype via sustained activation of the NF κ B pathway. In Fig. 4, we summarized our data in a schematic representations.

3. Discussion

Numerous studies have demonstrated that neointima formation is associated with increased cytokine and chemokine production by SMCs [26–28]. In this study we found that FHL2 deficiency increases both local and systemic inflammation after ligation of the carotid artery. Moreover, FHL2-deficiency induces the secretion of multiple inflammatory cytokines by SMCs, among which IL-6, MCP-1, CXCL1, RANTES and SDF-1 α . Furthermore, we show that blocking the NF κ B pathway, the inflammatory phenotype of FHL2-KO SMCs is reverted.

The NF κ B signalling pathway is a central regulator of inflammatory events associated with neointima formation. FHL2 has previously been shown to modulate NF κ B activity in osteoclasts, even though FHL2 does not directly interact with NF κ B [29]. We found that NF κ B transcriptional activity is also constitutively enhanced in FHL2 depleted SMCs and consistent with this, we observed diminished I κ B α expression in FHL2-KO SMCs. These data indicate that the function of endogenous FHL2 involves inhibition of the inflammatory response of SMCs. It has been shown that FHL2-KO mice show higher levels of inflammation in the serum under basal conditions [18]. In the current study, we also

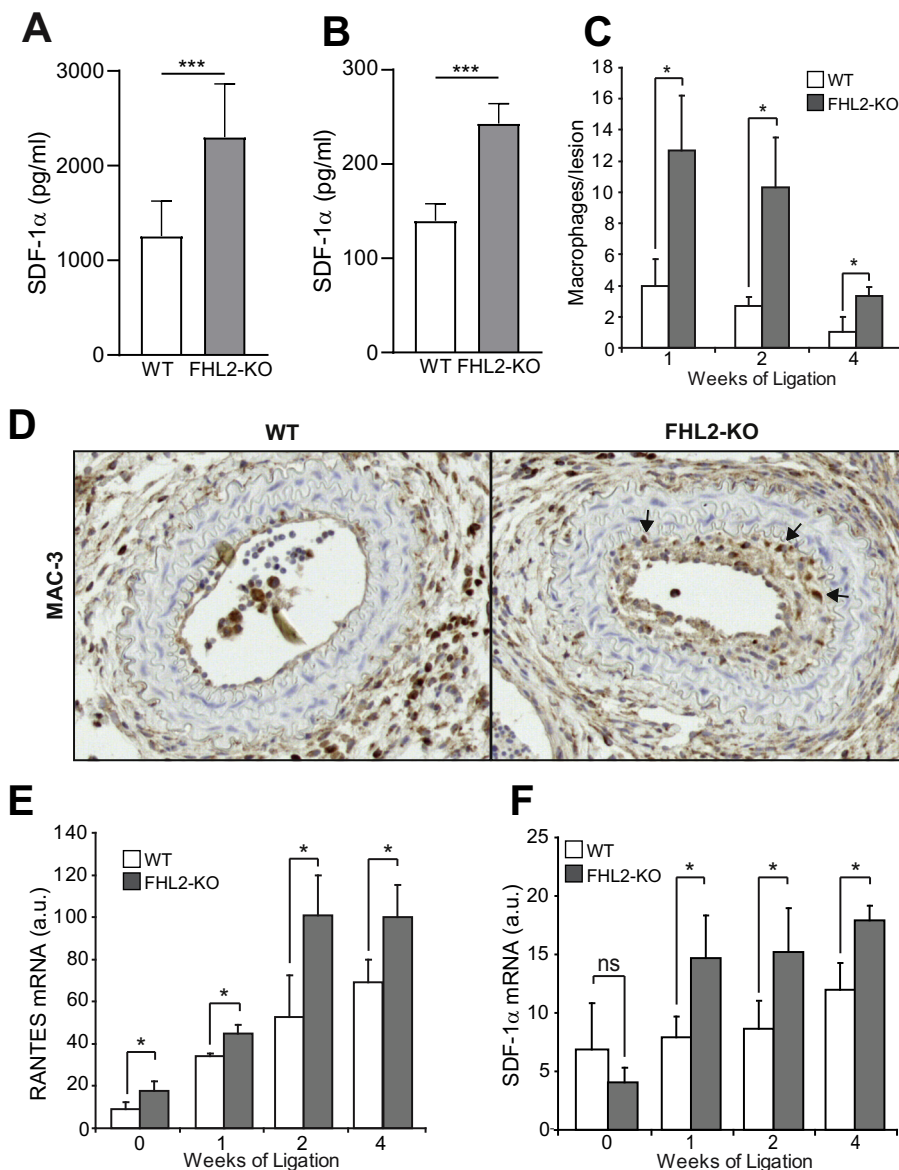


Fig. 1. FHL2 deficiency increases inflammatory protein expression. A. Plasma levels of SDF-1 α in the WT and FHL2-KO mice after 4 weeks of carotid artery ligation. B. Plasma levels of SDF-1 α in the WT and FHL2-KO mice at baseline. C. Quantification of MAC-3 immunostaining on WT and FHL2-KO carotid arteries ligated for 1, 2 or 4 weeks. D. Representative images of MAC-3 staining of WT and FHL2-KO carotid arteries ligated for 2 weeks. Arrows indicate positive cells. E & F. Graphs showing results of qRT-PCR for mRNA expression of RANTES (E) and SDF-1 α (F) in the ligated vessels from WT and FHL2-KO mice for the indicated time periods. Data represent means \pm SD, * p < .05, *** p < .005.

found that higher levels of SDF-1 α in the serum of FHL2-KO mice compared to WT mice under basal conditions. Furthermore, we demonstrated that FHL2 deficient SMCs exhibit a pro-inflammatory phenotype compared to WT SMCs through a sustained activation of NF κ B pathway. Previously it has been reported that FHL2 can induce or attenuate inflammation depending on the cell type involved in various disease models [16–19]. Therefore, FHL2 as a scaffold protein, can regulate inflammatory responses of multiple cell types in a cell- and context-dependent manner. Further research is warranted to delineate the molecular mechanism of FHL2 on inflammation in various cell types in different disease contexts.

We have reported previously that FHL-KO mice showed an increased SMC-rich lesion area in the ligated vessels *in vivo*, and increased proliferation of FHL2-KO SMCs was also observed *in vitro* [12]. Interestingly, our results concerning SDF-1 α complement the explanation for increased lesion area upon FHL2-deficiency. It has been shown that SDF-1 α is abundantly expressed after vascular injury and was identified in a genome-wide association study of myocardial infarction [30] and also associates with carotid artery disease [4,31]. After vascular injury, platelets account for short-term SDF-1 α release whereas SMCs mediate long-term SDF-1 α release to contribute to the process of vascular remodelling and repair [32–34]. SDF-1 α is also known to

recruit circulating SMC progenitor cells into the vessel wall, but this aspect has not been addressed in the current study [33]. We show SDF-1 α expression is markedly higher in the plasma of FHL2-KO mice than of WT mice. Furthermore, FHL2-KO SMCs displayed higher levels of SDF-1 α expression and overexpression of FHL2 restored SDF-1 α expression in FHL2-KO SMCs. Hence, we propose that enhanced SDF-1 α levels along with other inflammatory cytokines in FHL2-KO mice may contribute to neointima formation by promoting platelet activation, possibly by recruiting SM-progenitor cells, and by enhancing proliferation and migration of SMCs.

Previous research on ApoE $^{-/-}$ mice showed a decrease in atherosclerotic lesion size in the absence of FHL2 [13]. In addition, FHL2 $^{-/-}$ mice on a cholesterol-enriched diet show a reduction in lesion formation [35]. Atherosclerosis is, however, primarily initiated by lipid accumulation and activation of mononuclear cells and macrophages, whereas carotid artery ligation induces a SMC-based pathology. The latter model refers to SMC-specific pathologies such as in-stent restenosis, which is dominated by SMC proliferation. The seemingly distinct outcome in FHL2 $^{-/-}$ mice in these mouse models may therefore be explained by the fact that the mouse models represent intrinsically different vascular pathologies.

In conclusion, FHL2 deficiency causes an increased inflammatory

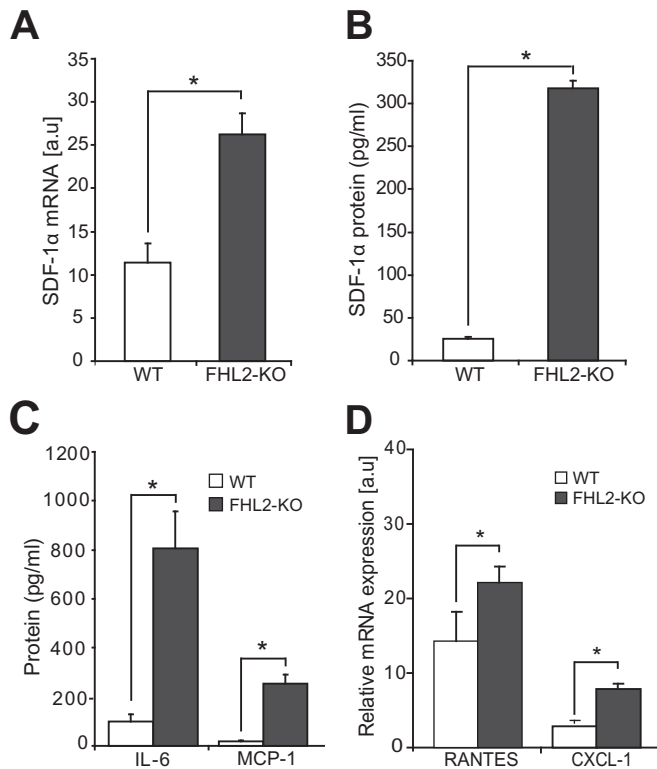


Fig. 2. FHL2-KO smooth muscle cells show increased inflammatory markers in FHL2-KO in vitro. A. Semiquantitative RT-PCR was performed to assess mRNA expression of SDF-1 α in serum-starved SMCs ($n = 2$). B. Protein levels in supernatants of serum-starved SMCs measured by ELISA ($n = 3$). C. Graph showing ELISA results of IL-6 and MCP-1 protein expression (C) or semi-quantitative RT-PCR for mRNA of RANTES and CXCL1 (D) of serum-starved SMCs stimulated with FCS for 8 h ($n = 2$). Data represent means \pm SD, $*p < .05$.

response after carotid artery ligation. In vitro, FHL2-KO SMCs secrete increased amounts of cytokines and show enhanced activation of NF κ B pathway. Exogenous FHL2 efficiently blocked the production of these cytokines. These results indicate that FHL2 inhibits the inflammatory response of vascular SMCs through attenuating NF κ B-signalling.

4. Methods

4.1. Animals and Left carotid artery ligation

All mice experiments were approved by the local animal ethic committee of the Amsterdam Medical Center, University of Amsterdam, The Netherlands (DBC102226) and were carried out according to the guidelines issued by the Dutch government. The procedure of murine left carotid artery ligation has been described previously [12].

4.2. Isolation and culturing of mouse aortic SMCs

Aortas from age and sex-matched WT and FHL2-KO mice were harvested and aortic SMCs were prepared as described previously [12].

4.3. Lentiviral transduction and Luciferase reporter assays in SMCs

Recombinant lentiviral particles encoding FHL2 were produced, concentrated, and titrated as described previously [11,12]. Transduction of SMCs was described previously [11,12]. Transient transfection and reporter assays were carried out in serum starved SMCs with the NF κ B luciferase reporter plasmids using Fugene6 transfection reagent (Roche) according to the manufacturer's protocol and described

previously [12].

4.4. Cytokines measurement by ELISA

Cytokine levels in mouse serum and cell supernatants were measured using the Cytometric Bead Array mouse inflammation kit (BD Biosciences, San Diego, CA). SDF-1 α secretion was measured by ELISA (RayBiotech).

4.5. qPCR

RNA was isolated from cells using the Total RNA mini kit (Bio-Rad) and from tissue using Trizol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was made using the iScript cDNA synthesis kit (Bio-Rad). Real-time reverse transcription PCR was performed using the MyiQ system (Bio-Rad) and the following mouse primers: SDF-1 α forw: 5'-CCTCGGTGTCCTCTGTGTGCC-3', SDF-1 α rev: 5'-GGCTCTGGCGATGTGGCTCTC-3', IL6 forw: 5'-GTTCTCTGGGA AATCGTGGA -3', IL6 rev: 5'-GGAAATTTGGGGTAGGAAGGA-3', MCP-1 forw: 5'-AGCAGGTGTCCCAAAGAAGC-3', MCP-1 rev: 5'-TGAAGACCT TAGGGCAGATGC-3'. RANTES forw: 5'-TCGTGCCACGTCAGGAGAT TTT-3', RANTES rev: 5'-TCTTCTCTGGGTGGCAGACACTT-3', CXCL-1 forw: 5'-GCTGGGATTACCTCAAGAA-3', CXCL-1 rev: 5'-AGGTGCCA TCAGAGCAGTCT-3'. As an internal control for cDNA content of the samples, acidic ribosomal phosphoprotein P0 was measured (P0 forw: 5'-GGACCCGAGAAGACCTCCTT-3', P0 rev: 5'-GCACATCACTCAGAAT TTCAATGG-3').

4.6. Immunostaining

Mouse carotid sections were fixed and stained as previously described [12]. Briefly, Paraffin sections were deparaffinized and rehydrated. Sections were boiled and blocked with 1% BSA in 0.1% Tween-PBS. Then sections were incubated overnight at 4 $^{\circ}$ C with primary antibodies directed against MAC3 (1:1000;M3/84, BD Pharmingen) followed by an HRP-conjugated secondary goat anti-rabbit antibody. DAB substrate was used for detection. After counterstaining with hematoxylin all the sections were embedded in pterex (HistoLab).

4.7. Statistical analysis

Statistical assays were performed using Graph Pad Prism (version 7). All tests were analyzed using Students *t*-test. Data are reported as mean \pm SD unless otherwise specified. *P* values $< .05$ were considered as statistically significant.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

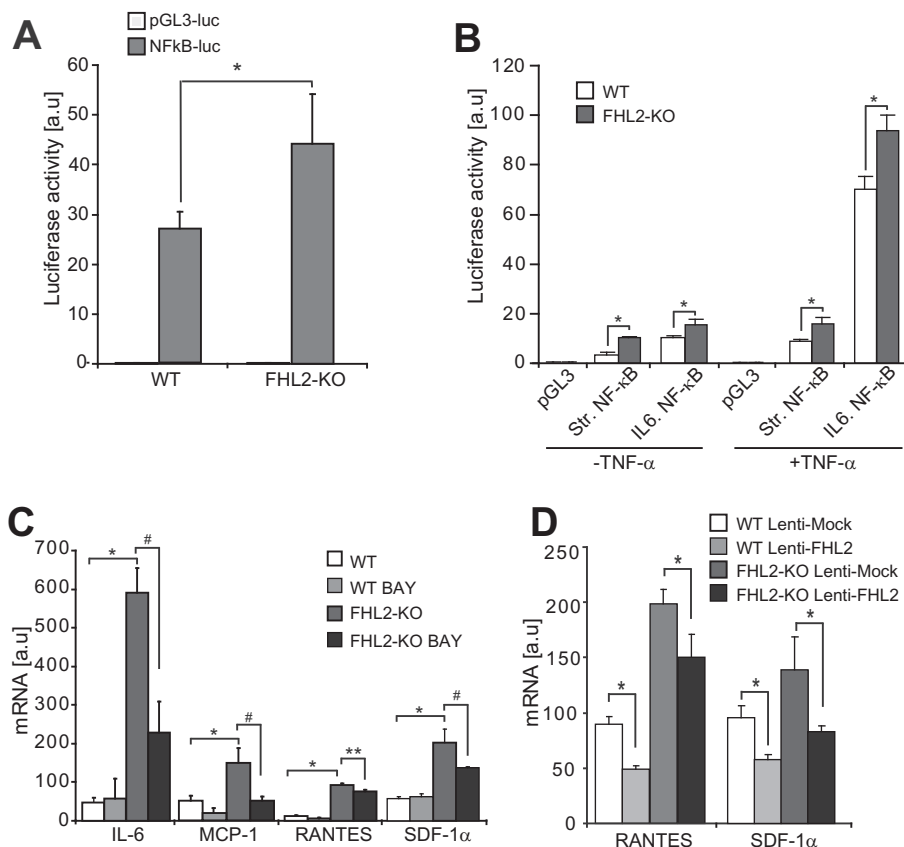


Fig. 3. FHL2 attenuates inflammation via the NFκB-pathway. A. Graph showing NFκB-response element reporter-plasmid luciferase activity in serum-starved SMCs stimulated with TNF-α (n = 3). B. Activity of NFκB was monitored in serum-starved SMCs transfected with a construct containing the NFκB response element of the Stratagene (Str.) or IL-6 promoter stimulated with or without TNF-α (n = 3). C. Graph showing qRT-PCR results for mRNA expression of IL-6, MCP-1, RANTES and SDF1-α in serum-starved SMCs treated with or without BAY11-7085 (n = 2). D. qRT-PCR results of mRNA expression of RANTES (n = 3) and SDF-1α (n = 2), in WT and FHL2-KO SMCs transfected with FHL2-lentivirus. Data represent means ± SD. *p < .05 for FHL2-KO versus WT. #p < .05 for BAY versus control.

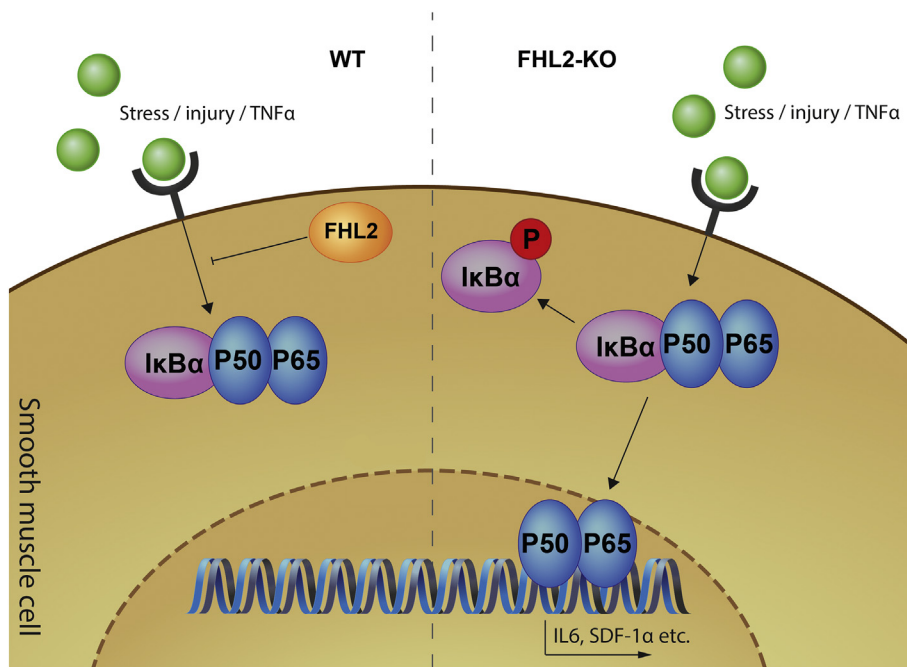


Fig. 4. Proposed mechanism of FHL2 in inhibition of inflammation in vascular smooth muscle cells. The left panel shows the normal situation where FHL2 inhibits NFκB-pathway in the cytosol, whereas the right panel represents the effect of FHL2 deficiency resulting in enhanced activity of NFκB-pathway.

References

[1] E. Solinas, E. Nikolsky, A.J. Lansky, A.J. Kirtane, M.C. Morice, J.J. Popma, J. Schofer, E. Schampaert, T. Pucelikova, J. Aoki, et al., Gender-specific outcomes after sirolimus-eluting stent implantation, *J. Am. Coll. Cardiol.* 50 (2007) 2111–2116, <https://doi.org/10.1016/j.jacc.2007.06.056>.
 [2] P.W. Serruys, H.E. Luijten, K.J. Beatt, R. Geuskens, P.J. de Feyter, M. van den Brand, J.H. Reiber, H.J. ten Katen, G.A. van Es, P.G. Hugenholtz, Incidence of restenosis after successful coronary angioplasty: a time-related phenomenon. A quantitative angiographic study in 342 consecutive patients at 1, 2, 3, and 4 months, *Circulation* 77 (1988) 361–371.
 [3] C. Chaabane, F. Otsuka, R. Virmani, M.L. Bochaton-Piallat, Biological responses in stented arteries, *Cardiovasc. Res.* 99 (2013) 353–363, <https://doi.org/10.1093/cvr/cvt115>.
 [4] J. Sjaarda, H. Gerstein, M. Chong, S. Yusuf, D. Meyre, S.S. Anand, S. Hess, G. Pare,

- Blood CSF1 and CXCL12 as causal mediators of coronary artery disease, *J. Am. Coll. Cardiol.* 72 (2018) 300–310, <https://doi.org/10.1016/j.jacc.2018.04.067>.
- [5] I. Mazin, G. Paul, E. Asher, Neoatherosclerosis - from basic concept to clinical implication, *Thromb. Res.* 178 (2019) 12–16, <https://doi.org/10.1016/j.thromres.2019.03.016>.
 - [6] F.G. Welt, C. Rogers, Inflammation and restenosis in the stent era, *Arterioscler. Thromb. Vasc. Biol.* 22 (2002) 1769–1776.
 - [7] M.S. Paiva, C.V. Serrano Jr., J.C. Nicolau, B.O. Jalbut, J.L. Fernandes, J.A. De Lemos, M.H. Blotta, R.T. De Oliveira, I.R. De Oliveira, A. De Oliveira Lda, et al., Differences in the inflammatory response between patients with and those without diabetes mellitus after coronary stenting, *J. Interv. Cardiol.* 21 (2008) 403–409, <https://doi.org/10.1111/j.1540-8183.2008.00385.x>.
 - [8] V. Wixler, The role of FHL2 in wound healing and inflammation, *FASEB J.* (2019), <https://doi.org/10.1096/fj.201802765RR> fj201802765RR.
 - [9] M.K. Tran, K. Kurakula, D.S. Koenis, C.J. de Vries, Protein-protein interactions of the LIM-only protein FHL2 and functional implication of the interactions relevant in cardiovascular disease, *Biochim. Biophys. Acta* 1863 (2016) 219–228, <https://doi.org/10.1016/j.bbamer.2015.11.002>.
 - [10] Kurakula, K.; van der Wal, E.; Geerts, D.; van Tiel, C.M.; de Vries, C.J. FHL2 protein is a novel co-repressor of nuclear receptor Nur77. *J. Biol. Chem.* 2011, 286, 44336–44343, doi:<https://doi.org/10.1074/jbc.M111.308999>
 - [11] K. Kurakula, D. Sommer, M. Sokolovic, P.D. Moerland, S. Scheij, P.B. van Loenen, D.S. Koenis, N. Zelcer, C.M. van Tiel, C.J. de Vries, LIM-only protein FHL2 is a positive regulator of liver X receptors in smooth muscle cells involved in lipid homeostasis, *Mol. Cell. Biol.* 35 (2015) 52–62, <https://doi.org/10.1128/mcb.00525-14>.
 - [12] K. Kurakula, M. Vos, I. Otermin Rubio, G. Marinkovic, R. Buettner, L.C. Heukamp, J. Stap, V. de Waard, C.M. van Tiel, C.J. de Vries, The LIM-only protein FHL2 reduces vascular lesion formation involving inhibition of proliferation and migration of smooth muscle cells, *PLoS One* 9 (2014) e94931, <https://doi.org/10.1371/journal.pone.0094931>.
 - [13] T. Ebrahimian, D. Simon, C.A. Lemarie, S. Simeone, M. Heidari, K.K. Mann, S. Wassmann, S. Lehoux, Absence of four-and-a-half LIM domain protein 2 decreases atherosclerosis in ApoE^{-/-} mice, *Arterioscler. Thromb. Vasc. Biol.* 35 (2015) 1190–1197, <https://doi.org/10.1161/atvbaha.114.305071>.
 - [14] Y. Kong, J.M. Shelton, B. Rothermel, X. Li, J.A. Richardson, R. Bassel-Duby, R.S. Williams, Cardiac-specific LIM protein FHL2 modifies the hypertrophic response to beta-adrenergic stimulation, *Circulation* 103 (2001) 2731–2738, <https://doi.org/10.1161/01.cir.103.22.2731>.
 - [15] A.W. Holt, D.A. Tulis, Experimental rat and mouse carotid artery surgery: injury & remodeling studies, *ISRN Minimally Invasive Surgery*, 2013 2013, <https://doi.org/10.1155/2013/167407>.
 - [16] J. Dahan, Y. Nouët, G. Jouvion, F. Levillayer, M. Adib-Conquy, A.M. Cassard-Doulcier, A. Tebbi, F. Blanc, L. Remy, J. Chen, et al., LIM-only protein FHL2 activates NF-κB signaling in the control of liver regeneration and hepatocarcinogenesis, *Mol. Cell. Biol.* 33 (2013) 3299–3308, <https://doi.org/10.1128/mcb.00105-13>.
 - [17] K. Kurakula, M. Vos, M. van Eijk, H.H. Smits, C.J. de Vries, LIM-only protein FHL2 regulates experimental pulmonary *Schistosoma mansoni* egg granuloma formation, *Eur. J. Immunol.* 45 (2015) 3098–3106, <https://doi.org/10.1002/eji.201545627>.
 - [18] A. Alnajjar, C. Nordhoff, T. Schied, R. Chiquet-Ehrismann, K. Loser, T. Vogl, S. Ludwig, V. Wixler, The LIM-only protein FHL2 attenuates lung inflammation during bleomycin-induced fibrosis, *PLoS One* 8 (2013) e81356, <https://doi.org/10.1371/journal.pone.0081356>.
 - [19] S. Wang, R. Song, Z. Wang, Z. Jing, S. Wang, J. Ma, S100A8/A9 in inflammation, *Front. Immunol.* 9 (2018) 1298, <https://doi.org/10.3389/fimmu.2018.01298>.
 - [20] W.D. Hill, D.C. Hess, A. Martin-Studdard, J.J. Carothers, J. Zheng, D. Hale, M. Maeda, S.C. Fagan, J.E. Carroll, S.J. Conway, SDF-1 (CXCL12) is upregulated in the ischemic penumbra following stroke: association with bone marrow cell homing to injury, *J. Neuropathol. Exp. Neurol.* 63 (2004) 84–96, <https://doi.org/10.1093/jnen/63.1.84>.
 - [21] P. Barath, M.C. Fishbein, J. Cao, J. Berenson, R.H. Helfant, J.S. Forrester, Tumor necrosis factor gene expression in human vascular intimal smooth muscle cells detected by in situ hybridization, *Am. J. Pathol.* 137 (1990) 503–509.
 - [22] S.K. Clinton, J.C. Fleet, H. Loppnow, R.N. Salomon, B.D. Clark, J.G. Cannon, A.R. Shaw, C.A. Dinarello, P. Libby, Interleukin-1 gene expression in rabbit vascular tissue in vivo, *Am. J. Pathol.* 138 (1991) 1005–1014.
 - [23] T. Yoshida, M. Yamashita, C. Horimai, M. Hayashi, Smooth muscle-selective inhibition of nuclear factor-kappaB attenuates smooth muscle phenotypic switching and neointima formation following vascular injury, *J. Am. Heart Assoc.* 2 (2013) e000230, <https://doi.org/10.1161/jaha.113.000230>.
 - [24] T.S. Blackwell, J.W. Christman, The role of nuclear factor-kappa B in cytokine gene regulation, *Am. J. Respir. Cell Mol. Biol.* 17 (1997) 3–9, <https://doi.org/10.1165/ajrcmb.17.1.fl32>.
 - [25] Tang, J.R.; Michaelis, K.A.; Nozik-Grayck, E.; Seedorf, G.J.; Hartman-Filson, M.; Abman, S.H.; Wright, C.J. The NF-κB inhibitory proteins IκBα and IκBβ mediate disparate responses to inflammation in fetal pulmonary endothelial cells. *Journal of immunology (Baltimore, Md. : 1950)* 2013, 190, 2913–2923, doi:<https://doi.org/10.4049/jimmunol.1202670>.
 - [26] N. Clausell, V.C. de Lima, S. Molossi, P. Liu, E. Turley, A.I. Gotlieb, A.G. Adelman, M. Rabinovitch, Expression of tumour necrosis factor alpha and accumulation of fibronectin in coronary artery restenotic lesions retrieved by atherectomy, *Br. Heart J.* 73 (1995) 534–539, <https://doi.org/10.1136/hrt.73.6.534>.
 - [27] R. Kornowski, M.K. Hong, F.O. Tio, O. Bramwell, H. Wu, M.B. Leon, In-stent restenosis: contributions of inflammatory responses and arterial injury to neointimal hyperplasia, *J. Am. Coll. Cardiol.* 31 (1998) 224–230, [https://doi.org/10.1016/s0735-1097\(97\)00450-6](https://doi.org/10.1016/s0735-1097(97)00450-6).
 - [28] K. Wasser, S. Schnaudigel, J. Wohlfahrt, M.N. Psychogios, M. Knauth, K. Groschel, Inflammation and in-stent restenosis: the role of serum markers and stent characteristics in carotid artery stenting, *PLoS One* 6 (2011) e22683, <https://doi.org/10.1371/journal.pone.0022683>.
 - [29] S. Bai, J. Zha, H. Zhao, F.P. Ross, S.L. Teitelbaum, Tumor necrosis factor receptor-associated factor 6 is an intranuclear transcriptional coactivator in osteoclasts, *J. Biol. Chem.* 283 (2008) 30861–30867, <https://doi.org/10.1074/jbc.M802525200>.
 - [30] S. Subramanian, C. Liu, A. Aviv, J.E. Ho, P. Courchesne, P. Muntendam, M.G. Larson, S. Cheng, T.J. Wang, N.N. Mehta, et al., Stromal cell-derived factor 1 as a biomarker of heart failure and mortality risk, *Arterioscler. Thromb. Vasc. Biol.* 34 (2014) 2100–2105, <https://doi.org/10.1161/ATVBAHA.114.303579>.
 - [31] A. Borghini, S. Sbrana, C. Vecoli, A. Mercuri, S. Turchi, C. Carpegiani, A. L'Abbate, M.G. Andreassi, Stromal cell-derived factor-1-3'A polymorphism is associated with decreased risk of myocardial infarction and early endothelial disturbance, *J. Cardiovasc. Med. (Hagerstown)* 15 (2014) 710–716, <https://doi.org/10.2459/jcm.000000000000068>.
 - [32] A. Schober, S. Knarren, M. Lietz, E.A. Lin, C. Weber, Crucial role of stromal cell-derived factor-1alpha in neointima formation after vascular injury in apolipoprotein E-deficient mice, *Circulation* 108 (2003) 2491–2497, <https://doi.org/10.1161/01.Cir.0000099508.76665.9a>.
 - [33] A. Zernecke, A. Schober, I. Bot, P. von Hundelshausen, E.A. Liehn, B. Mopps, M. Mericskay, P. Gierschik, E.A. Biessen, C. Weber, SDF-1alpha/CXCR4 axis is instrumental in neointimal hyperplasia and recruitment of smooth muscle progenitor cells, *Circ. Res.* 96 (2005) 784–791, <https://doi.org/10.1161/01.Res.0000162100.52009.38>.
 - [34] M. Chatterjee, M. Gawaz, Platelet-derived CXCL12 (SDF-1α): basic mechanisms and clinical implications, *J. Thromb. Haemost.* 11 (2013) 1954–1967, <https://doi.org/10.1111/jth.12404>.
 - [35] P.H. Chu, H.I. Yeh, H.H. Wu, R.C. Hong, T.F. Shiu, C.M. Yang, Deletion of the FHL2 gene attenuates the formation of atherosclerotic lesions after a cholesterol-enriched diet, *Life Sci.* 86 (2010) 365–371, <https://doi.org/10.1016/j.lfs.2010.01.007>.