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FOXP1 – a gatekeeper of endothelial cell inflammation

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Keywords

FOXP1; endothelial cells; inflammation; atherosclerosis

Endothelial cells, as the innermost layer in all vessels, play an essential role in regulating tissue homeostasis by controlling the extravasation of circulating cells into tissues. By altering their production of cytokines, chemokines and adhesion molecules endothelial cells control the traffic of immune cells into sites of injury[1]. Injury or dysfunction of the endothelial layer is known to contribute to many pathologies, including atherosclerosis. Atherosclerosis is a chronic inflammatory disease in which recruitment and trapping of immune cells, especially monocytes, is a hallmark[2, 3]. The recent CANTOS trial (Canakinumab Antiinflammatory Thrombosis Outcome Study) has highlighted the importance of inflammation; specifically, inflammasome-derived inflammation in cardiovascular disease[4]. Macrophages are thought to be the main producers of inflammasome-derived mediators and less is known about inflammasome activation in endothelial cells and how that contributes to disease.

In this issue of *Circulation Research*, Zhuang and co-workers uncover that forkhead box protein P1 (FOXP1) is a key regulator of endothelial cell inflammation and atherosclerosis (Figure)[5]. FOXP1 is a large transcriptional repressor that binds highly conserved regions of the DNA[6] and previous work has indicated that endothelial FOXP1 is critical for an organism to develop[7]. Consistent with the idea that FOXP1 is important in endothelial cells; the same group recently reported that endothelial FOXP1 is a key regulator of pathological myocardial fibrosis[8], but the role of FOXP1 in atherosclerosis is unknown.

The first clue that FOXP1 might be involved in endothelial cell dysfunction and atherosclerosis came when the authors demonstrated that FOXP1 is downregulated in areas prone to atherosclerosis and areas that already exhibit atherosclerosis, in both mice and humans[5]. Targeted deletion of FOXP1 in adult mice, selectively in endothelial cells, resulted in increased atherosclerosis at two different time points in mice that were also deficient in APOE[5]. The increase in atherosclerosis was associated with greater macrophage accumulation within the lesion. FOXP1 deletion augmented monocyte adhesion and conditioned media from endothelial cells without FOXP1 stimulated monocyte migration,

Conflicts of interest

None

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clearly indicating that FOXP1 is critical for regulating endothelial-monocyte interactions and highlights the importance of endothelial cells in regulating monocyte trafficking in atherogenesis. Importantly, Zhuang et al. could show that overexpression of FOXP1 had the opposite effect to that of FOXP1 deletion.

To mechanistically understand how FOXP1 in endothelial cells could control monocyte recruitment RNA-sequencing was carried out. Endothelial cell FOXP1 deletion resulted in upregulation of genes associated with inflammasome activation such as *Nlrp3*, *Casp1* and *Il1b*. The NLRP3-inflammasome is a multimeric complex that processes pro-IL-1β into mature IL-1β[9]. Full activation of the NLRP3-inflammasome normally requires 2 signals; a priming and an activating signal. In atherosclerotic lesions, cholesterol crystals have been proposed to be the main activator[9, 10]. To test if FOXP1 deficiency turns on the NLRP3 inflammasome in vivo, the authors blocked NLRP3 activation using 3 different approaches: endothelial cell-specific NLRP3 deficient mice (crossed into their FOXP1-deficient mice), the NLRP3 inhibitor MCC950 and the Caspase-1/IL-1β inhibitor Diacerein. Little is known about activation of the NLRP3-inflammasome in endothelial cells in vivo and whole-body inhibition has generated some contradictory results[11–13]. Many investigators would have predicted that the majority of the inflammasome activation would be derived from the hematopoietic compartment[9] and thus one would not have expected such a profound effect of the endothelial-selective deficiency in NLRP3. Therefore it was somewhat surprising that all three approaches including the targeted deletion of NLRP3 in endothelial cells reduced atherosclerosis and monocyte recruitment in APOE-deficient mice[5], arguing that endothelial NLRP3-derived inflammation is more important than previously appreciated. Furthermore, the decrease in atherosclerotic burden was greater in mice where FOXP1 had been deleted. From this, the authors concluded that FOXP1 downregulation accelerates atherogenesis via enhanced inflammasome activation. As stated above, the inflammasome typically requires 2 signals. The deletion of FOXP1 serves as the priming signaling as evident by increased expression of the genes involved, but what is the activating signal in endothelial cells? Hyperlipidemia can induce cholesterol crystal formation in endothelial cells[14] and disturbed flow induces sterol regulatory element binding protein 2 in endothelial cells resulting in increased cholesterol accumulation[15], potentially suggesting that altered flow might serve as both primer and activator of the inflammasome which is then potentiated by hyperlipidemia (Figure).

To complicate things, in addition to inflammasome activation, the RNA-sequencing data indicted the FOXP1 deletion was associated with an abundance of inflammatory changes, such as elevated chemokine expression. For example, one of the most highly upregulated genes in FOXP1-deficient endothelial cells was the chemokine *Ccl8*, which was also demonstrated to be a direct target of FOXP1. Thus, part of the effect of FOXP1 deletion could potentially be independent of NLRP3-inflammasome activation and might be driven by increased chemokine expression. But either way, the data demonstrate that FOXP1 is a critical player in regulating endothelial cell behavior, although the target(s) appears to be context depend[8].

Why and how is FOXP1 downregulated in atherosclerotic lesions? Zhuang and colleagues went on to show that FOXP1 is a target of the laminar flow sensor Krüppel-like factor 2

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(KLF2), explaining why the expression of FOXP1 is lower in areas of disturbed flow. Furthermore, the authors demonstrate that at least some of the pro-inflammatory effects of reduced KLF2 are mediated via FOXP1[5]. In summary, Zhuang and colleagues have highlighted the importance of endothelial cell inflammatory status in atherosclerosis and demonstrated that FOXP1 is a key regulator of endothelial inflammation. In future research, it would be of interest to expand these studies to conditions associated with exaggerated endothelial cell dysfunction such as diabetes-associated complications.

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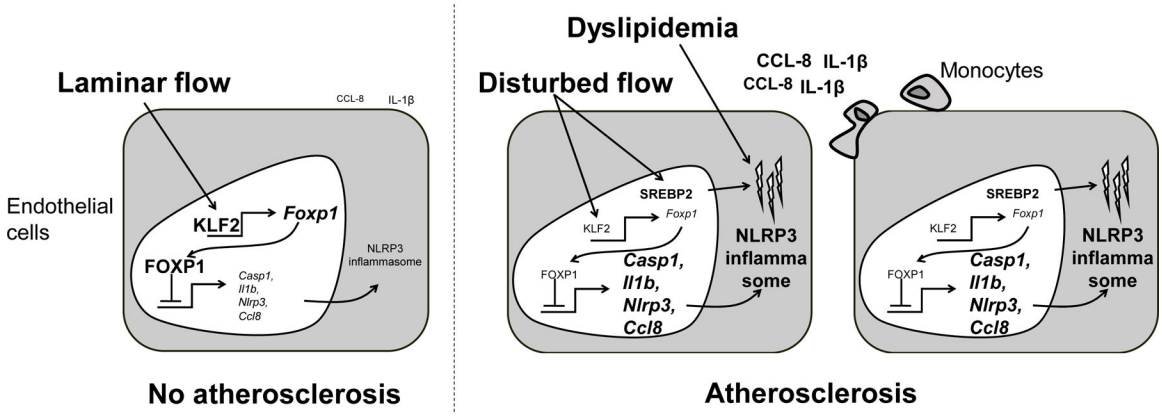

Figure.

FOXP1 acts as a gatekeeper of endothelial inflammation. In areas where laminar flow is present the Krüppel-like factor 2 (KLF2) is expressed which in turn stimulates expression of FOXP1. FOXP1 suppresses the expression of components of the NLRP3-inflammasome and *Ccl8*. In areas of disturbed flow, which also develops atherosclerosis, FOXP1 levels are reduced allowing for increased expression of inflammasome components and chemokines. Disturbed flow triggers expression of SREBP2 (sterol regulatory element binding protein 2) which increases cholesterol accumulation which might act as the second signal to activate the NLRP3 inflammasome. Also, dyslipidemia induces cholesterol crystal formation which might further activate the inflammasome in endothelial cells. All of which results in increased monocyte recruitment and acceleration of atherosclerosis.