REVIEW ARTICLE

OCCURRENCE AND SIGNIFICANCE OF THE NUCLEAR TRANSCRIPTION FACTOR KRÜPPEL-LIKE FACTOR 4 (KLF4) IN THE VESSEL WALL

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Summary: Practically all mammalian cells including human can switch, according to micro- or macroenvironmental conditions, from states of cellular quiescence to inflammatory activation and *vice versa*. Along with recent knowledge, cellular quiescence is not a passive, but a highly active state with broad engagement of the cell synthetic and secretory machinery. Inflammatory activation is a beneficial process in cases of infection; however, if its control fails, it may degrade into autoimmune diseases or cancer growth. Control over cellular quiescence is exerted predominantly by a set of zinc-finger transcription proteins, referred to as Krüppel-like factors (KLFs). This review article offers recent information concerning activities of Krüppel-like factor 4 in the vascular wall.

Key words: *Endothelial phenotype; Inflammatory cytokines; Shear stress; Transcription factors; Arterial injury; Smooth muscle cells*

Pro-inflammatory activation of endothelial cells and its counter-regulation

In the setting of infection or wound healing, a tightly controlled pro-inflammatory and pro-thrombotic conversion of the endothelial phenotype is a beneficial biological response which helps to combat infection and renew organ integrity. However, prolonged endothelial activation, which has escaped control mechanisms of the host, may set off unfavorable sequelae, such as atherosclerosis and intravascular thrombosis (13).

Factors responsible for endothelial homeostasis involve both biochemical and biomechanical stimuli. The former include inflammatory cytokines, namely tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β), both of them known also as "proximal" cytokines which, in their turn, induce endothelial expression of adhesion molecules (e.g., E-selectin, vascular cell adhesion molecule-1 or VCAM-1, intercellular adhesion molecule-1 or ICAM-1) and pro-coagulant factors, most importantly tissue factor (TF). The net result is known as endothelial dysfunction (12, 18).

The central mediator which converges most inflammatory stimuli is the transcription factor nuclear factor κB (NF-κB). Its activation leads to the disruption of the non-inflammatory, non-thrombogenic vascular lining, which is transformed into a pro-inflammatory, pro-thrombotic endothelial surface (16). On the other hand, biomechanical factors, namely laminar shear stress (LSS), induce the expression of various protective factors, the most prominent ones being endothelial NOS (eNOS) and thrombomodulin (TM). Both of them are essential for regulation of vascular tone and maintenance of the quiescent state of endothelial cells (4, 25). In those areas of the vasculature where laminar shear stress does not fit in with its physiological range, expression of NF-κB is substantially enhanced (10). Consequently, these vascular segments are prone to the development of atherosclerosis. For example, branch points of the vascular tree are exposed to chaotic blood flow patterns, which are entirely distinct from regular laminar shear stress. Therefore, these branch points are highly susceptible to the formation of atherosclerotic lesions (19).

Krüppel-like factors: a family of transcription proteins with diverse functions

Krüppel-like factors (KLFs) are a subclass of the zinc-finger family of transcription factors. They are characterized by the DNA binding domain, which contains a conserved sequence CX2CX3FX5LX2HX3H. The zinc fingers are usually found at the C-terminus of the protein and bind to the consensus sequence 5'-CNCCC-3' of the target protein. The N-terminus is involved in transcriptional activation and repression (see Tab. 2) (3).

Many studies demonstrated that KLF proteins regulate critical aspects of cellular differentiation and tissue development. Original designation of individual KLFs reflected the tissue in which they were found most abundantly and in which they were supposed to exert their respective functions.

KLF1, also known under its older term EKLF or erythroid Krüppel-like factor, has been proved to be indispensable for red blood cell maturation (8).

By contrast, targeted disruption in mice of KLF2 revealed its essential role in programming quiescent phenotype of single-positive T cells and in normal development of the lungs. Hence its alternative denomination of LKLF or lung Krüppel-like factor (29). Nowadays, KLF2 stands out as a transcription factor which maintains quiescent phenotype of human vascular endothelium (6). Beyond doubt, KLF2 is one of the most important endogenous atheroprotective factors (7). Exogenously, KLF2 has been found to be up-regulated by statin treatment (26). Endogenously, beneficial activities of KLF2 are started off by elevated levels of laminar shear stress, such as are attained by physical exercise (27).

KLF4, also termed GKLF or gut-enriched Krüppel-like factor, was initially considered an epithelial-specific transcription factor which participated in the differentiation and growth of the epithelium, namely in the gut and in the skin. KLF4 is highly expressed in terminally differentiated, post-mitotic epithelial cells of the intestinal tract (24). Expression of KLF4 inhibits DNA synthesis and reduces cellular growth in colon cancer cells. Unfortunately, KLF4 expression is significantly decreased in multiple human cancers, including colon cancer and gastric cancer (14). By contrast, elevated KLF4 levels have been reported in mammary carcinoma (20). KLF4 displays a potential to switch from a growth-inhibiting tumor suppressor to a growth-promoting oncogene in response to changes in the cellular context (31). In this respect, its activities are reminiscent of those found in human vascular endothelium.

Recent studies have added an interesting piece of knowledge that KLF4 regulates pluripotent stem cell development (23). Most importantly, it has been confirmed that endothelial cells also express KLF4 and that endothelial KLF4 is also induced by laminar shear stress. Thus, in the vascular endothelium, KLF4 strongly resembles KLF2, since both KLFs are structurally interrelated and both hold under control critical steps responsible for endothelial cell inflammatory and thrombotic activation (11). Expression and functions of both KLF transcription factors are summarized in Tab. 1.

KLF4 activities in the vascular endothelium

Histologically, KLF4 is expressed by endothelial cells of small, medium, and large vessels, respectively, both arteries and veins, as well as by endocardial endothelium.

Just the same as KLF2, endothelial KLF4 is regulated by biomechanical forces and inflammatory cytokines. In human coronary arteries, KLF4 has been found to be induced by levels of laminar shear stress in the range of 12 to 20 dynes/cm^2, whereas in postcapillar venules by those of 2 dynes/cm^2.

KLF4 reduces secretion of various inflammatory mediators from endothelial cells. Experimental depletion of endothelial KLF4 produces an unopposed pro-inflammatory effect, which is manifest by decreased expression of eNOS, thrombomodulin, tissue plasminogen activator (tPA), and, consequently, by a prevailing impact of pro-inflammatory cytokines on the endothelium.

In such a condition, widespread elaboration of pro-inflammatory and pro-coagulant substances, namely TNFα, IL-Iβ, interferon-γ, thrombin, respectively, releases subsequent production of adhesion molecules, tissue factor (TF), and plasminogen activator inhibitor-1 (PAI-1), all of which corroborate the inflammatory and pro-coagulant phenotype of the endothelial cells.

In this stage of events, a counter-regulatory production of endothelial KLF4 sets in *in vivo*. KLF4 induced by an inflammatory micro-environment confers an anti-inflammatory expression pattern to endothelial cells. The ensuing endothelial phenotype results from the balance between inflammatory mediators and KLF4 expression, respectively, with the latter having the capacity to prevail over the former. This is in marked contrast to Krüppel-like factor 2, which is down-regulated by pro-inflammatory stimuli (2).

There is both a basal and a cytokine-induced expression of KLF4 in the endothelium. Under basal conditions, KLF4 induces eNOS and TM, and inhibits PAI-1.

Under the influence of pro-inflammatory cytokines, KLF4 inhibits the expression of a diverse set of pro-inflammatory factors, including monocyte chemoattractant protein-1, RANTES, C-reactive protein, PAI-1, IL-6, tissue

Tab. 1: Krüppel-like factors 2 and 4 regulation of gene expression in cells implicated in protective processes in atherosclerosis. For differences between the two see the text.

<table>
<tr><th>monocyte</th><th>T-cells</th><th>endothelial cell</th></tr>
<tr><td>chemokines
⇓ MIP-1
⇓ MCP-1
⇓ IL-8</td><td>reduced transition from naive to memory phenotype</td><td>inflammation
⇓ VCAM-1, E-selectin
⇑ eNOS</td></tr>
<tr><td>proinflammatory cytokines
⇓ TNFα
⇓ IL-1β</td><td rowspan="3"></td><td>coagulation
⇓ PAI-1, TF
⇑ eNOS, tPA</td></tr>
<tr><td>costimulatory interactions
⇓ CD40L</td><td rowspan="2">angiogenesis
⇓ E-selectin</td></tr>
<tr><td>proinflammatory mediators
⇓ COX-2</td></tr>
</table>

inhibitors of metalloproteinases 1 and 2, to name only some. Thus, KLF4 reduces in the vascular endothelium the deleterious effects of pro-inflammatory cytokines (21).

KLF4 and its target genes

Due to its capacity of a nuclear transcription factor, KLF4-inherent effects reside in the control of the promoter function of its target genes. KLF4 fine-tunes finite expression of its underlying genes, both activation and repression, via finally tailoring transcription of the genetic information. KLF4 transactivates the eNOS and TM promoters, the production of both proteins being significantly enhanced. By contrast, KLF4 down-modulates cytokine induction of the TF promoter. Furthermore, KLF4 inhibits p65-mediated induction of NF-κB. In particular, the synthesis of VCAM-1 and TF is dependent on NF-κB activity after the exposure of endothelial cells to diverse pro-inflammatory mediators. Taken together, KLF4 sets up an anti-inflammatory, anticoagulant milieu in endothelial cells (37). Adherence of leukocytes to TNFα-activated endothelium is profoundly inhibited. The ability of KLF4 to up-regulate thrombomodulin expression, even under inflammatory conditions, suggests that it supports blood fluidity which, in turn, acts to decrease leukocyte adhesion to endothelial surface. Additionally, KLF4 prolongs blood clotting time despite the presence of TNF. Thus, KLF4 decreases the formation of microthrombi within the vascular lumen (32).

KLF4 also increases the secretion of the tissue inhibitors of metalloproteinases 1 and 2. Metalloproteinase activity has been implicated in the formation of aortic aneurysms. It may be that endothelial KLF4 is endowed with beneficial effects that far exceed the vessel lumen (28).

Comparison of KLF4 and KLF2 in the vessel wall

As has been said repeatedly, both KLF4 and KLF2 are induced by laminar shear stress. By sharp contrast to KLF4, KLF2 expression is inhibited by inflammatory cytokines. Under basal conditions, endothelial KLF2 transcripts are present in about a 5–10-fold excess compared with KLF4. In the „plateau“ phase after treatment with TNF, KLF4 and KLF2 transcripts are present in approximately equal numbers. Laminar shear stress significantly induces both KLF4 and KLF2, with -fold induction of KLF4 being somewhat greater with both venous and arterial shear conditions. KLF4 and KLF2 have indisputably closely overlapping functions. It is therefore tempting to hypothetize that this overlap has been conserved during evolution in order to maintain sufficient levels of anti-inflammatory proteins both under basal and inflammatory conditions (9).

Smooth muscle cells in injured arterial wall

Smooth muscle cells (SMCs) of the arterial wall are another cell population that is implicated in the development of atherosclerosis according to the “response-to-injury” hypothesis. In normal mature blood vessels, SMCs are mostly differentiated cells which express smooth muscle (SM)-specific contractile proteins α-SM actin (α-SMA) and SM22α.

In mature arterial walls, SMCs exhibit also an extremely low rate of proliferation.

In response to vascular injury, such as blood flow perturbations, manual handling of the vessels during coronary artery bypass grafting (CABG) procedures, and development of restenosis after angioplasty, SMCs down-regulate their contractile proteins (α-SMA and SM22α) and revert to a dedifferentiated phenotype, in which the expression of an embryonic type MHC (SMemb/NMHC) gene, a dedifferentiated marker gene, is up-regulated. SMCs in injured vessels also increase their rates of proliferation, migration, and synthesis of extracellular matrix proteins, leading to neointima formation (15).

Impact of KLF4 on injured smooth muscle cells

Basal expression of KLF4 is low in vascular SMCs and does not seem to exert any significant function. Following injury, KLF4 expression is set off by platelet-derived growth factor β (PDGFβ) and oxidized phospholipids (5, 36). KLF4 binds to a DNA sequence that has either a CACCC homology or is rich in GC content. Biological effects of KLF4 on cellular proliferation and differentiation can be recognized as a SMCs growth repressor and a SMCs differentiation repressor (22).

Owing to KLF4 activity, expression of both SM α-actin and SM22α is markedly decreased in the medial layer of injured arteries. The promoter/enhancer regions of these differentiation markers contain common *cis* elements, including multiple CC(A/T-rich)6GG elements, and a transforming growth factor- (TGF-β) control element (17).

KLF4 binds to the TGF-β control element-containing promoter regions of the SM α-actin gene, and the SM22α gene.

In the medial layer of injured arteries, KLF4 positively regulates SM22α and α-SMA (the differentiation markers) and negatively regulates SMemb/NMHC (the dedifferentiation marker) (1). KLF4-induced growth suppression of SMCs, the hallmark of neointima formation, is caused by cell cycle arrest at the G1/S boundary (34). Inhibition of SMCs proliferation in the injured arteries is the result of KLF4-induced activation of the nuclear transcription protein p53 (35). Consequent to DNA damage, KLF4 is contributes substantially to mediating the p53-induced G1/S cell cycle arrest. Therefore, p53 stands out as an essential mediator of KLF4-activated differentiation, and KLF4-inhibited proliferation processes (30).

Moreover, it has been convincingly shown that in some cell lines, p53 is able to suppress the expression of matrix metalloproteinase-9 (MMP-9), collagenase-1 (MMP-1), and collagenase-3 (MMP-13). Since the promoter regions of

Tab. 2: Expression and regulatory roles of KLF transcription factors.

KLF No.	cell/tissue expression	function
KLF1	erythroid and mast cells	erythropoiesis, control of inflammation
KLF2	lung, blood vessels, lymphocytes	blood vessel, lung development, T-cell survival
	endothelial cells	essential for late stages of normal lung development
		low extression at aortic branch points
		induced by laminar flow, inhibited by proinflammatory cytokines
		overexpression induces eNOS
		inhibition of adhesion molecules expression
KLF3	erythroid tissue, brain	expressed in the myeloid lineage
KLF4	vascular smooth muscle cells, endothelial cells	induced in endothelial cells by laminar flow
KLF5	epithelial cells	cell growth
KLF6	ubiquitous	activate iNOS under stress
KLF7	ubiquitous	cell-cycle arrest
KLF8	ubiquitous	negative regulation of myeloid cells
KLF9	ubiquitous	not exactly known
KLF10	ubiquitous	apoptosis
KLF11	ubiquitous	antiproliferative
KLF12	brain, kidney, liver	negative regulators
KLF13	ubiquitous	antiproliferative
KLF15	ubiquitous	vascular smooth muscle cells, expressed in media
KLF16	ubiquitous	carcinogen metabolism

MMP-9 and MMP-2 include some putative KLF4-binding sites, these genes might also be target genes of KLF4 (33). Regulatory roles of KLFs are summarized in Tab. 2.

Concluding remarks

The presence of Krüppel-like factor 4 in the arterial wall, both intima (endothelial cells) and media (smooth muscle cells) layers, has been recognized only after the presence of KLF2 in human vasculature. Both transcription factors are active in maintaining the quiescent phenotype of the vessel wall, with KLF4 seemingly predominating over KLF2 due to the former's capacity to be expressed even in overt inflammatory conditions. However, both KLFs are closely interrelated both structurally and functionally. It may well be that other, as yet unknown mediators are operative in maintaining cellular quiescence. Further studies are needed to clarify this field of cellular biology in order to develop more effective treatment modalities to combat potentially malignant diseases, such as atherosclerosis, autoimmune disorders or cancer.

Acknowledgments

This work was supported by Ministry of Education, Czech Republic, project No. MSM0021620812.

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Received: 28/08/2009.
Accepted in revised form: 08/12/2009.

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