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REVIEW

NF-κB signaling pathway as target for antiplatelet activity

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ABSTRACT

In different nucleated cells, NF-κB has long been considered a prototypical proinflammatory signaling pathway with the expression of proinflammatory genes.
Although platelets lack a nucleus, a number of functional transcription factors are involved in activated platelets, such as NF-κB. In platelet activation NF-κB regulation events include IKKβ phosphorylation, IκBα degradation, and p65 phosphorylation. Multiple pathways contribute to platelet activation and NF-κB is a common pathway in this activation. Therefore, in platelet activation the modulation of NF-κB pathway could be a potential new target in the treatment of inflammation-related vascular disease therapy (antiplatelet and antithrombotic activities).

1. Introduction

Platelets represent an important link between inflammation and thrombosis [1–5]. Activated platelets stimulate thrombus formation in response to a rupture of the atherosclerotic plaque, promoting cardiovascular diseases (CVD) [6]. Multiple pathways contribute to platelet activation, including those triggered by thrombin, arachidonic acid, adenosine diphosphate (ADP) and collagen, among others [7,8]. As a result, platelets release different inflammatory mediators such as soluble P-selectin (sP-selectin, CD62P), soluble CD40 ligand (sCD40L), interleukin (IL)-1β, transforming growth factor-β1 (TGF-β1), chemokine (C-C motif) ligand 5 (CCL5), matrix metalloproteinases, tumor necrosis factor alpha (TNF-α) and IL-6 [9–13]. These molecules participate in the development of atherosclerosis, from early lesion development to vulnerable plaque formation [14,15].

Atherosclerosis is a chronic inflammatory disease [16,17]. Many inflammatory pathways that contribute to the initiation and progression of atherosclerosis are regulated by the nuclear factor (NF)-κB, regulator of innate and adaptive immune responses [18–20]. Activated NF-κB has been identified in human atherosclerotic plaques and has been enhanced in unstable coronary plaques [21,22]. In different cells, NF-κB has long been considered a prototypical proinflammatory signaling pathway with the expression of proinflammatory genes, such as cytokines, chemokines and adhesion molecules [23,24]. Therefore, the inhibition of NF-κB may have a great impact for the treatment of various inflammatory diseases [25].

This may have a great impact when these types of drugs are considered for the treatment of cancer and various inflammatory diseases.

Different inflammatory mediators regulate the expression of cellular genes through NF-κB [26–28]. The pleiotropic NF-κB normally exists as an inactive cytoplasmic complex; its predominant form is a heterodimer composed of p50 and p65 subunits. These subunits are tightly bound to inhibitory proteins of the IκB family. The activation of NF-κB is when IκBα is phosphorylated by the IKK complex. It starts dissociating of IκBα from NF-κB subunits, and then IκBα is ubiquitinated and rapidly degraded by the proteasome [29–32].

Although platelets lack a nucleus, a number of functional transcription factors including signal transducer and activator of transcription 3 (STAT3) and NF-κB are involved in activated platelets [33,34]. NF-κB activation is another signaling pathway involved part of classical agonist-mediated platelet activation [34–38]. Yet, the mechanisms by which the NF-κB pathway may contribute to platelet activation are yet to be fully elucidated. In this article we explore the potential impact of inhibiting NF-κB function in platelet activation.

2. NF-κB and platelet activation

NF-κB is a redox-sensitive transcription factor that regulates inflammation and plays a critical role in the vascular response to injury [39]. Activated NF-κB is detected in human atherosclerotic and restenotic lesions of smooth muscle cells, monocytes, endothelial cells and platelets, among others [21]. NF-κB may have a function independent of gene regulation in platelets. Three IKK family members (α, β, and γ) are

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expressed in platelets, with the β form being the most strongly expressed [34].

There is recent evidence about alternative pathways of NF-κB-dependent regulation of platelet function. Thus NF-κB has an important dual regulatory role on platelet function. Whereas NF-κB activation induces platelet activation, it also seems to be essential for shedding surface glycoprotein (GP) Ibα by activated platelets and protein kinase Ac (PKAc) activation (platelet inhibitory pathway) [36,40].

Platelet–monocyte interactions via extracellular matrix metalloproteinase inducer (EMMPRIN) stimulate NF-κB-driven inflammatory pathways in monocytes, such as matrix metalloproteinases and cytokine induction, thus representing another potential drug target to inhibit NF-κB platelet–monocyte interactions [41]. Platelets are activated on increase of cytosolic Ca^{2+} activity, accomplished by store-operated Ca^{2+} entry (SOCE) involving the pore-forming ion channel subunit Orai1. In this context, recent observations unravel serum- and glucocorticoid-inducible kinase 1 (SGK1) as novel regulator of platelet function, effective at least in part by NF-κB-dependent transcriptional up-regulation of Orai1 in megakaryocytes and increasing platelet SOCE [42].

NF-κB activation during the late stage of inflammation is associated with the resolution of inflammation and anti-inflammatory gene expression [40]. Thus NF-κB activation limits platelet–leukocyte interaction by promoting a disintegrin and metalloprotease domain 17 (ADAM17)-mediated GPIbα shedding [40].

In platelet activation NF-κB signaling events included IKKβ phosphorylation, IκBα degradation and p65 phosphorylation [43]. IKKβ phosphorylation has been proposed as a major upstream regulator for IκBα phosphorylation leading to NF-κB activation during platelet activation [34,37].

Platelets contain all three members of the SNAP-23/25/29 gene family and its phosphorylation provides a critical link between activation and secretory processes. SNAP-23 is the most highly enriched of these proteins in platelets and is required for exocytosis from platelet alpha, dense, and lysosomal granules [44,45]. IKKβ, in response to platelet activation, phosphorylates SNAP-23 resulting in enhanced SNARE complex formation, enhanced membrane fusion and granule release [38]. Meanwhile inhibition of IKKβ blocked SNAP-23 phosphorylation and platelet secretion.

Several platelets agonists/receptors modulate NF-κB pathway, such as thrombin/PAR4, sCD40L/CD40L, receptor for advanced glycation end products (RAGE) axis, toll-like receptors (TLRs) and peroxisome proliferator-activated receptors (PPARs) (Fig. 1).

2.1. Thrombin/PAR4

Activation via thrombin/PAR4 is involved in Ca^{2+}-dependent release of platelet granules, activation of GPIIb/IIIa, adhesion, aggregation and thrombus formation [46]. Also thrombin induces the release of platelet inflammatory mediators such as sP-selectin, sCD40L, IL-1β, TGF-β1 and CCL5 [9–11].

In platelet activation, the binding of thrombin to PAR4 triggers the activation of sphingomyelinase (nSMase) with increased of C24:0-ceramide level and NF-κB activation [47]. Thrombin not only causes platelet activation but also appears to fine-tune this response by initiating downstream NF-κB-dependent PKA activation, as a novel feedback inhibitory signaling mechanism for preventing undesired platelet activation [36]. The PKA activation phosphorylates multiple target proteins in numerous platelet inhibitory pathways that have a very important role in maintaining circulating platelets in a resting state [48].

2.2. sCD40L/CD40L

The ligand CD40L is similarly expressed in the plasma membranes of endothelial cells, T-lymphocytes and platelets [49,50]. Platelets constitute the major source of sCD40L, and trigger endothelial cell activation and atherosclerosis progression [51,52].

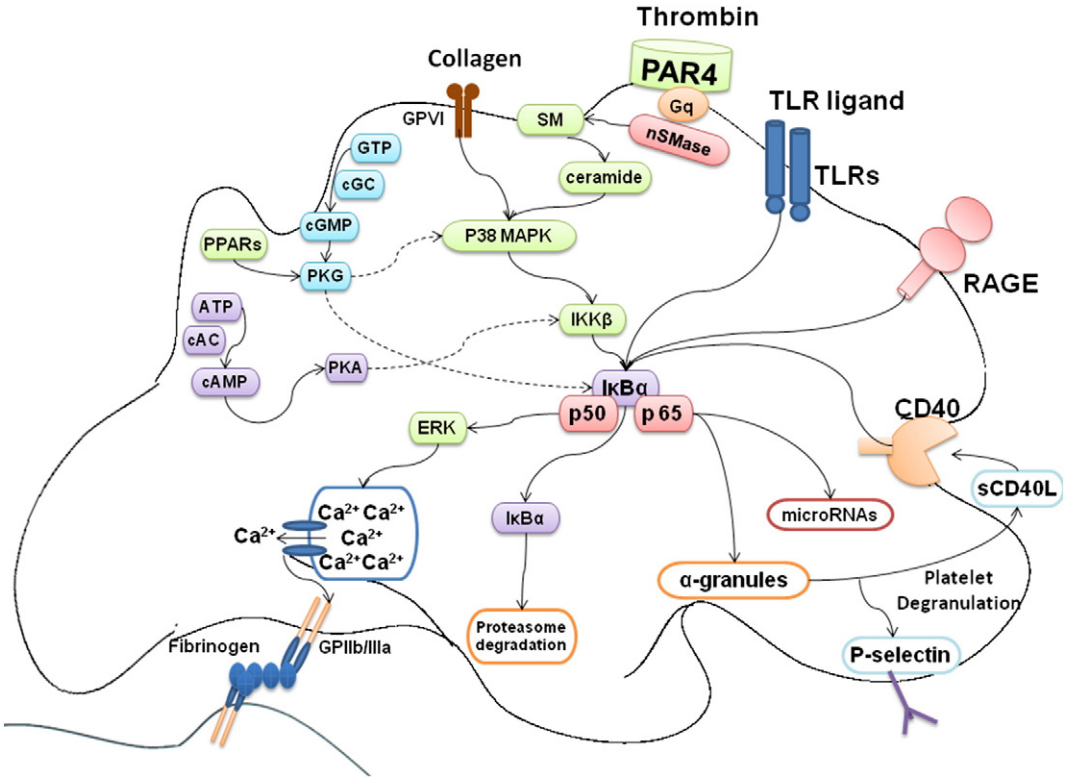

Fig. 1. Main regulatory mechanisms of NF-κB (p50 and p65) signaling pathway in platelets. ATP, adenosine triphosphate; cAC, adenylate cyclase; cGC, guanylate cyclase; GP, glycoprotein; Gq, G protein-coupled receptors; GTP, guanosine triphosphate; nSMase, sphingomyelinase; PKA, protein kinase A; PKG, protein kinase G; PPARs, Peroxisome proliferator-activated receptors; RAGE, receptor for AGEs; sCD40L, soluble CD40 ligand; SM, sphingomyelin; TLRs, toll-like receptors. NF-κB is p50 and p65. Continuous lines: activation and dotted line: inhibition.