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Review

The adaptor Lnk (SH2B3): An emerging regulator in vascular cells and a link between immune and inflammatory signaling

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ABSTRACT

A better knowledge of the process by which inflammatory extracellular signals are relayed from the plasma membrane to specific intracellular sites is a key step to understand how inflammation develops and how it is regulated. This review focuses on Lnk (SH2B3) a member, with SH2B1 and SH2B2, of the SH2B family of adaptor proteins that influences a variety of signaling pathways mediated by Janus kinase and receptor tyrosine kinases. SH2B adaptor proteins contain conserved dimerization, pleckstrin homology, and SH2 domains. Initially described as a regulator of hematopoiesis and lymphocyte differentiation, Lnk now emerges as a key regulator in hematopoeitic and non hematopoeitic cells such as endothelial cells (EC) moderating growth factor and cytokine receptor-mediated signaling. In EC, Lnk is a negative regulator of TNF signaling that reduce proinflammatory phenotype and prevent EC from apoptosis. Lnk is a modulator in integrin signaling and actin cytoskeleton organization in both platelets and EC with an impact on cell adhesion, migration and thrombosis. In this review, we discuss some recent insights proposing Lnk as a key regulator of bone marrow-endothelial progenitor cell kinetics, including the ability to cell growth, endothelial commitment, mobilization, and recruitment for vascular regeneration. Finally, novel findings also provided evidences that mutations in Lnk gene are strongly linked to myeloproliferative disorders but also autoimmune and inflammatory syndromes where both immune and vascular cells display a role. Overall, these studies emphasize the importance of the Lnk adaptor molecule not only as prognostic marker but also as potential therapeutic target.

Contents

Abbreviations: AGM, aorta–gonad–mesonephros; APS, adaptor protein with PH and SH2 domains; BM, bone marrow; CAM, cell adhesion molecules; CEBPα, CCAAT/enhancer-binding protein-α; CD, coeliac disease; c-Fms, macrophage colony stimulating factor receptor (M-CSFR); CH, calponin homology; DD, dimerization domain; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; EPO, erythropoietin; EPOR, erythropoietin receptor; ERK1/2, extracellular signal-regulated kinases1/2; ESC, embryonic stem cells; Gab2, Grb2-associated-binding protein 2; GH, growth hormone; Grb2, growth factor receptor bound-2; GSK3b, glycogen synthase kinase 3 beta; GWAS, genome-wide association study; HO-1, heme oxygenase-1; HPC, hematopoietic progenitor cells; HUVEC, human umbilical vein EC; HSC, hematopoietic stem cells; ICAM-1, intercellular adhesion molecule 1; IFNγ, interferon-γ; JAK2, Janus kinase 2; JNK, c-jun N-terminal kinase; KSL, c-Kit-positive, Sca-1-positive, lineage marker-negative cells; MAPK, mitogen-activated protein kinase; MHC, major histocompatibility; MPL, thrombopoietin receptor; MPN, myeloproliferative neoplasms; NFkB, nuclear factor kappa-B; PDGFR, platelet-derived growth factor receptor; PDZ, postsynaptic density 95: PSD-85, discs large: Dlg, zonula occludens-1: ZO-1; PH, pleckstrin homology; PI3 K, phosphatidylinositol 3 kinase; RA, rheumatoid arthritis; RNA, ribonucleic acid; SCF, stem cell factor; SH2,3, Src homology 2,3; SMC, smooth muscle cells; SNP, single nucleotide polymorphisms; STAT, signal transducers and activators of transcription; TCR, T-cell receptor; T1D, type 1 diabetes; TA, transplant arteriosclerosis; TNF, tumor necrosis factor alpha; TPO, thrombopoietin; Trk, neurotrophic tyrosine kinase receptor; VCAM-1, vascular cell adhesion molecule-1; VLA, very late antigen; WT, wild-type.

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1. Introduction

Inhibition of inflammation has become a primary goal of therapy irrespective of the underlying cause of the disease. For this reason, it is important to understand how inflammation develops and how it is regulated. The process by which extracellular signals are relayed from the plasma membrane to specific intracellular sites is a key step of cellular regulation. Cellular responses to external and intrinsic signals are organized and coordinated through specific protein–protein and protein–phospholipid interactions, commonly mediated by "adaptor proteins". In general, but not exclusively, adaptors are proteins without intrinsic enzymatic function that serve as molecular platforms for the coordination of signaling events [1–3]. Adaptors have multiple functions such as determining the localization of signaling proteins in the cell, coordinating the necessary but diverse signals involved in cell activation, and bringing together the required enzymes and substrates that drive the activation process. Adaptors include transmembrane proteins [4], cytoplasmic proteins under resting conditions that are recruited to the membrane upon activation, or proteins localized in specific intracellular compartments (endoplasmic reticulum, cytosol, etc.). The transmembrane adaptor proteins such as LAT, NTAL, PAG, LIME, TRIM, SIT and LAX organize complex membrane-proximal signaling assemblies and are therefore key mediators of immunoreceptor-mediated signaling [4]. Cytoplasmic adaptors are exemplified by the adaptors involved in TNF receptor signaling [5], innate immune signaling [6], integrin signaling [7], lymphocyte signaling [8] etc. A common feature of adaptor proteins is their modular structure that contains one or more dedicated domains allowing their interaction with several other proteins. Among the domains important for this function are Src homology 2 (SH2) [9] and phosphotyrosine binding (PTB) domains [10] which mediate interactions with phosphorylated tyrosine residues on target proteins, Src homology 3 (SH3) domains, which associate with proline-rich domains [11], and WW [12] and PDZ [13] domains which mediate other protein/protein interactions. Additionally, some adaptors mediate protein/lipid interactions through pleckstrin homology (PH) [3] domains. Expression of these adaptor proteins is either ubiquitous or restricted to selected cell types, where they play a specialized role by controlling differentiation and function. One of the first known and best characterized adaptors is the evolutionarily conserved Grb2 (growth factor receptor bound-2) protein that plays an essential role in the activation of the Ras pathway by growth factor receptors [1]. Grb2 consists of an SH2 domain flanked by two SH3 domains. Since the identification of Grb2, numerous other adaptors have been described [1]. The impact of the adaptors as positive and negative mediators of signaling has been progressively elucidated with the generation of genetically modified mouse [14–17]. This review focuses on Lnk (SH2B3) a member of the SH2B family of adaptor proteins which are implicated in integration and regulation of multiple signaling events (reviewed in [18]). Initially described as a regulator of hematopoiesis and lymphocyte differentiation, Lnk now emerges as a key signaling partner in hematopoieitic and non hematopoieitic cells in a broad array of signaling pathways. Recent findings also provided evidences that mutations in Lnk gene are strongly linked to myeloproliferative disorders but also autoimmune and inflammatory syndromes where both immune and vascular cells display a role. In this review, we discuss some recent insights into the functions of Lnk adaptor implicated in the control of signaling associated with vascular inflammation, remodeling and repair.

2. Lnk and the SH2-B family of adaptor proteins

Lnk, also termed SH2B3, is a member of the SH2-B (Src homology 2-B) protein family containing SH2B1 and SH2B2, originally named SH2-B and APS (adaptor protein with PH and SH2 domains), respectively. Lnk is structurally composed of a number of functional domains (Fig. 1 and Table 1), including a carboxyl-terminal Src homology 2 (SH2) domain, which is essential for specific binding to phosphotyrosine residue, a pleckstrin homology (PH) domain,

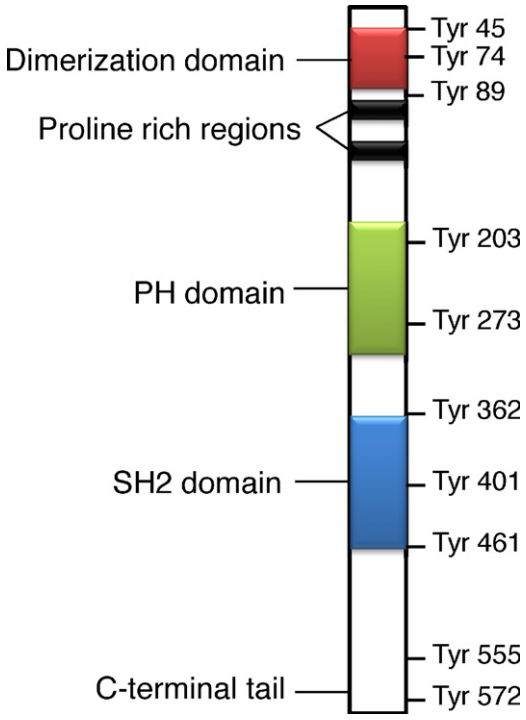

Fig. 1. Schematic representation of the structural organization of the Lnk (SH2B3) adaptor protein. PH: Pleckstrin homology domain, SH2: Src homology 2 domain, Tyr: tyrosine sites.

Table 1
Functional domains of the signaling adaptor Lnk.

Domain or region	Structure and function
Dimerization domain	The dimerization domain consists of about 60 amino acid residues and exhibits a phenylalanine zipper motif in its center. This particular structure consists of aromatic side chains from ten phenylalanine residues that are stacked within a hydrophobic core. This zipper mediates homo or heterodimerization of SH2-B protein members. Dimerization is a well known regulatory mechanism in signal transduction that mediates protein activation of a wide variety of transcription factors, cell surface proteins, and intracellular trafficking proteins.
Proline rich region	Proline rich region is a short motif that contains several prolin residues with the minimal consensus sequence Pro-X-X-Pro which is recognized by SH3 domain-containing protein.
PH domain	The Pleckstrin homology domains consist of about 120 amino acid residues that recognize phosphatidylinositol lipids within biological membranes and other proteins (protein kinase C, protein G). PH domain of Lnk protein is suggested to target the protein to cell membrane facilitating its function as regulator of signaling pathway.
SH2 domain	The Src homology 2 domain consists of about 100 amino acid residues originally identified in Src oncoprotein and represent the largest class of phosphotyrosine-selective recognition domains. SH2 domain binds phosphotyrosine-containing protein, generally serving to target kinases to their substrates.

which recognize phosphoinositides and control protein translocation to the cell membrane, proline-rich regions, dimerization domain (DD) and several putative tyrosine phosphorylation motifs. The DD domain possesses a motif refer as a 'phenylalanine zipper' [19,20] which mediates the formation of homo- and heterodimers between members of the SH2-B family [21].

Lnk has structural similarities to the other SH2-B adaptor proteins (SH2B1 and SH2B2), which all contain the multimerization, PH and SH2 domains. Alternative splicing of SH2B1 RNA at the 3′ end results in four isoforms, SH2B1α, β, γ, δ [22–25]. SH2B1 isoforms possess identical N-terminal region and domains but differ at their C-terminus regions after the SH2 domain. The specific function of the respective isoforms is not fully understood as they appear to interact with similar targets. However, affinity ligation seems to differ depending on variants, inducing distinct level of regulation by SH2B1 isoforms [23,25]. SH2B2 transcript gives rise to two proteins, SH2B2α and β [26], while no isoform was yet described for Lnk protein. The recently identified SH2B2β has a DD and PH domain but lacks a SH2 domain. SH2B2β bind to both SH2B1 and SH2B2α and appears to be an endogenous inhibitor of SH2B1 and/or SH2B2α, negatively regulating insulin signaling and Janus kinase-2 (JAK2)-mediated cellular responses [26].

Lnk was originally cloned from a rat lymph node cDNA library and shown to participate in T cell signaling [27]. It was initially reported that tyrosine-phosphorylated Lnk coimmunoprecipitates with the SH2 domain of Grb2, phospholipase Cγ-1, and phosphatidylinositol 3-kinase (PI3K) in activated T cells [27]. To further define the importance of Lnk in immune physiology, the structure of mouse Lnk was determined through isolation of the full-length cDNA encoding mouse Lnk and its nucleotide sequence [28]. Cloning of human cDNA for Lnk allowed the demonstration that Lnk functions as a negative mediator of the T-cell receptor signaling pathway [29].

Similar to Lnk, the other members of the SH2-B family were first identified as signaling molecules involved in immune cell activation [30,31]. However, subsequent studies have shown that SH2-B members participate to a broader variety of cellular processes notably by regulating activation and signaling of growth factors, hormones and cytokines. SH2B1 and SH2B2 have been reported to associate with different proteins including insulin receptor [26,32], neurotrophic tyrosine kinase receptor (Trk) family receptors [33,34], platelet-derived growth factor receptor (PDGFR) [25,30], nerve growth factor [35,36] and tyrosine Janus kinase (JAK) [37–39]. Lnk have been shown to negatively control receptors activation such as stem cell factor (SCF) receptor (c-kit) [40,41], thrombopoietin receptor (MPL) [42] erythropoietin receptor (EPOR) [43], platelet-derived growth factor receptor (PDGFR) [44] or more recently macrophage colony-stimulating factor receptor (c-Fms) [45].

Deletion of the SH2B1 gene results in severe obesity, leptin and insulin resistance as well as infertility [21,46] indicating that SH2B1 is required for maintaining normal energy metabolism and body weight in mice.

The inhibitory functions of Lnk in lymphopoiesis, erythropoiesis and megacaryocytopoiesis, initially revealed by the phenotype of Lnk-deficient mice, will be discussed in the following section. An overview of the major signaling pathways modulated by Lnk and effector targets reported yet is presented in Fig. 2.

3. Lnk is a negative regulator of hematopoiesis

Perlmutter laboratory was the first to generate Lnk-deficient mice (Lnk−/−) in order to elucidate the significance of Lnk protein [18]. The only noticeable phenotype of Lnk−/− mice was enlarged spleens containing about twice as many cells as those of wild-type (WT) mice. This observation was explained by a substantial accumulation of B-lineage cells (pre-B and B immature) in the spleens of Lnk−/− mice. In the bone marrow, B-lineage cells were also increased, reflecting enhanced production of pro-B cells that resulted in part from hypersensitivity of precursors to SCF, the ligand for c-kit. This study was the first to demonstrate the role of Lnk as a negative regulator of B lymphopoiesis at the early developmental stages. Surprisingly, while Lnk was originally described as expressed in T cells and implicated in T-cell receptor (TCR) signal transduction [27], lack of Lnk do not compromise T cell development or function [18].

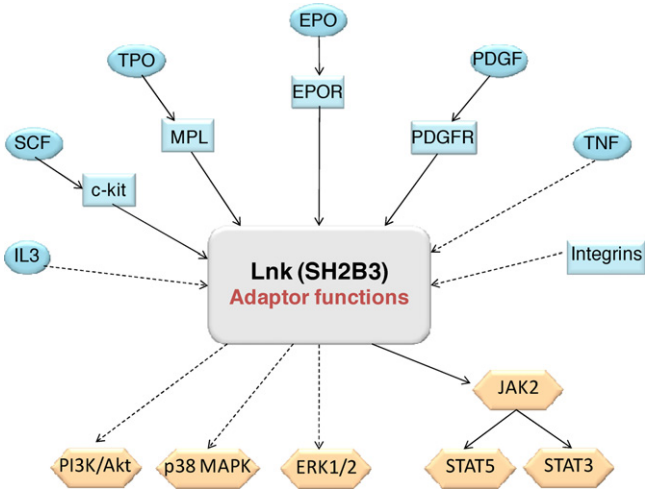

Fig. 2. A schematic overview of the major signaling pathways (in blue) under the regulatory control of the adaptor Lnk and effector targets (in orange). For details see text. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The generation of transgenic mice overexpressing Lnk has shown that lymphocyte B production was impaired in a dose-dependent manner upon overexpression of Lnk, confirming negative regulatory mechanism mediated by the Lnk adaptor protein in controlling B-lineage cell production [47]. Furthermore, abnormalities in B cell morphology and cell cycle status were observed, suggesting an additional role for Lnk in matured B cell function [47,48]. If the generation of Lnk−/− mice failed to underline the function of Lnk in T cell production, Lnk overexpression in lymphoid precursors impaired the expansion of pro-T cells in thymus. T cell reduction observed in transgenic mice implies that Lnk expression modulates both B and T lymphopoiesis. In order to elucidate disparity of results, authors proposed that Lnk, which is less expressed in T than in B lineage [28], regulates less finely T cell signaling, explaining the absence of abnormality of T cell expansion in deficient mice. Another possible explanation could be the compensation by other members of SH2-B family [47].

Lnk has also been found to be expressed in hematopoietic progenitors (HPC) and hematopoietic stem cells (HSC) [40,48]. Furthermore, the number and the proliferative capacity of hematopoietic progenitors and HSC are greatly increased in the absence of Lnk. Lnk−/− progenitors present hypersensitivity to several cytokines such as IL-3 and/or SCF, in part due to sustained mitogen-activated protein kinase (MAPK) activation reflected by high level of phospho-extracellular signal-regulated kinases1/2 (ERK1/2) [48]. This abnormal signaling modulation has also been observed by Takaki et al. where c-kit-mediated signaling contribute to the enhanced hematopoiesis by Lnk−/− cells. Takaki et al. have demonstrated that phosphorylated Lnk associates with c-kit and decreases phosphorylation of Grb2-associated-binding protein 2 (Gab2) and downstream MAPK cascade (phospho-ERK1/2) [40] (Fig. 3). Recent studies have further investigated the role of Lnk in SCF signaling and reported that Lnk associates with the c-kit receptor on tyrosine 567 through its SH2 domain, leading to the inhibition of the receptor downstream signaling [41,48–50]. Lnk has additionally been reported to control self-renewal of HSC by modifying thrombopoietin (TPO) signal. Hypersensibility to TPO of HSC Lnk−/− is related to increased activation of Akt and STAT5 and inactivation of p38 MAPK [42].

If Lnk deficiency increases the proliferative capacity of hematopoietic cells, the ability of HSC and hematopoietic progenitors cells to reconstitute the hematopoietic system of irradiated hosts is also significantly enhanced [40,51]. Takizawa et al. have taken advantage of this feature and have inhibited the functions of endogenous Lnk in HSC and HPC using a dominant-negative Lnk mutant. They demonstrated that even transient expression of dominant-negative Lnk significantly increases the repopulating ability of transduced cells, demonstrating that inhibition of Lnk potentiates HSC/HPC for engraftment [50]. Authors obtain a complete reconstitution of the lymphoid compartments of immunodeficient host animals under nonmyeloablative conditions. These results suggest that Lnk inhibitors could be a conceivable strategy to facilitate bone marrow transplantation.

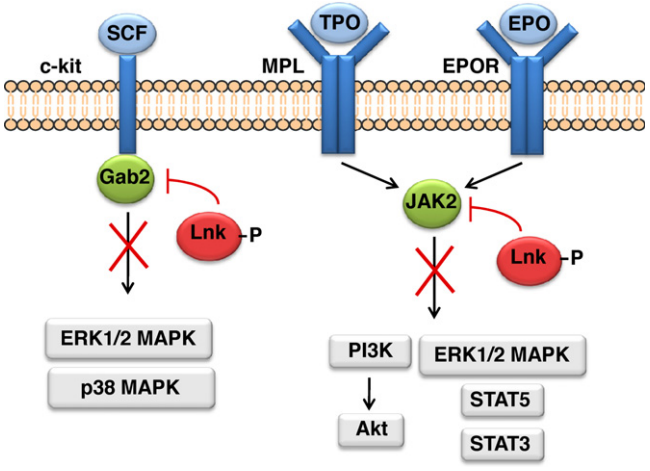

Fig. 3. Illustration of modulation of c-kit, MPL and EPOR surface receptors by Lnk through direct interaction with regulator proteins Gab2 and JAK2. For details see text.

The B lineage is the main but not the only population contributing to the increased cellularity in Lnk−/− mice spleens. Indeed, Lnk−/− mice exhibit hyperplasia of the megakaryocytic lineages, and increased numbers of erythroid cells with the latter contributes to splenomegaly [48]. Thrombocytosis in Lnk−/− animals is due at least in part to increased responsiveness of megakaryocytes to TPO [43,52]. The absence of Lnk causes enhanced and prolonged TPO induction of signal transducers and activators of transcription (STAT)3, STAT5, Akt, and MAPK signaling pathways whereas Lnk overexpression in bone marrow (BM)-derived megakaryocyte decreases TPO-dependent megakaryocyte growth. Those results demonstrate the role of Lnk as negative regulator of TPO-induced proliferation and signaling pathways in primary megacaryocytes. In splenic erythroid progenitors, lack of Lnk results in enhanced erythropoietin (EPO)-mediated signaling pathways. As in TPO-dependent signaling, Lnk blocks three major signaling pathways induced by EPO signal: Akt, STAT5 and MAPK [43]. Similarities in Lnk regulation of EPO and TPO signaling pathways results from the association of Lnk with a partner which is common to both pathways, the Janus protein JAK2. JAK2 is a member of the Janus family of cytoplasmic non-receptor tyrosine kinase. JAK proteins transduce cytokine-mediated signals through their association with cytokine receptor that lack intrinsic kinase activity such as MPL and EPOR. Ligand binding to cytokine receptor induces JAK phosphorylation causing recruitment and phosphorylation of STAT proteins and activation of downstream signaling pathways (review [53]). The impact of Lnk on TPO and EPO signaling is mediated through direct interaction with JAK2 [43,54]. In response to TPO and EPO stimulation, Lnk is phosphorylated and interacts with JAK2, leading to a negative-feedback regulation of JAK2 activation (Fig. 3). Therefore, Lnk negatively modulates TPO and EPO signaling by attenuating JAK2 activation, and regulates HSC self renewal, megakaryopoiesis and erythropoiesis [43,52,54].

All together, these data support that Lnk is an important negative regulator of lymphopoiesis, megakaryopoiesis, erythropoiesis as well as HSC expansion by moderating growth factor and cytokine receptor-mediated signaling.

4. Lnk is a negative regulator of signaling in endothelial cells (EC)

4.1. Endothelial cells as mediators of inflammation

Endothelial cells act as the major interface between blood and tissues. Forming the inner lining of blood vessels, they are uniquely positioned between circulating lymphocytes and the periphery and thereby regulate the trafficking of T lymphocytes from the bloodstream to sites of infection and inflammation [55–57]. EC at a site of inflammation are both active participants and regulators of inflammatory processes. The properties of EC change during the transition from acute to chronic inflammation. EC may respond, depending on the stimulus, by altering the expression of various genes whose products are central to endothelial cell matrix remodeling, coagulation, and fibrinolysis and angiogenesis as well

as interactions with polymorphonuclear leukocytes, platelets, and lymphocytes. Exposure of EC to cytokines, such as IL-1 or TNF, caused the EC to bind 20–40 times as many leukocytes as untreated EC [58]. The increase in EC adhesiveness results from the de novo induction of cell adhesion molecules (CAMs) that bind counter–receptor proteins expressed on leukocytes. Cytokine-treated EC are also a source of chemoattractant cytokines (chemokines) that contribute to adhesion by activating the affinity of leukocyte counter–receptors for endothelial CAMs thus amplifying the inflammatory loop [59]. These changes in EC phenotype and functions orchestrated by inflammatory cytokines and mediators result from activation of selective signaling pathways (nuclear factor kappa-B (NFkB), Pi3Kinase, MAPKinases) and transcription factors (mostly NFkB and AP-1) [58,60]. The implication of other signaling pathways including the Notch pathways has been recently reported [61–63]. Current efforts try to identify selective inhibitors that could prevent or reverse the signaling cascades leading to activation and ultimately dysfunction of EC [64–66].

4.2. Inflammation triggers endothelial dysfunction and transplant arteriosclerosis

EC are central to the rejection process that occurs when vascularized organs (heart, kidney, lung, pancreas, etc.) are transplanted into recipients [67]. Indeed, it has been reported that as a consequence of their activation, EC acquire a pro-inflammatory phenotype associated with the development of apoptotic features and a pro-coagulant status [58]. Transplant arteriosclerosis (TA) is the main limitation for long-term functioning of solid organ allografts [68]. In TA, allograft arteries characteristically develop severe, diffuse intimal hyperplasia that ultimately allow luminal stenosis and cause ischemic graft failure. Characteristics of the lesions include endothelial cell damage, mononuclear cell infiltration, smooth muscle cell proliferation, and matrix protein deposition in the intima of the vessel wall [69,70]. It is also noteworthy that neointimal formation, begins only after EC loss followed by smooth muscle cells (SMC) recruitment at the sites of injury suggesting that endothelial injury and/or denudation could be an important proximal step that initiate vasculopathy [71].

Recipient alloreactive T cells and antibodies, as well as infiltrating macrophages and NK cells, can all contribute to EC apoptosis and vascular injury [72]. Development of TA requires interferon-γ (IFNγ) and other proinflammatory cytokines such as TNFα [73,74]. Indeed, grafts with no expression of TNF receptors failed to develop TA, despite normal levels of IFNγ production. The effect was attributable to TNF signaling in donor vascular wall cells since preventing TNF signaling on host inflammatory cells or SMC precursors has no effect [73]. In addition to effects on infiltrating leukocytes, the local production of cytokines activates nearby EC to enlist their participation in the inflammatory response. Cytokine-induced up-regulation or de novo expression of immunomodulatory and proinflammatory endothelial molecules, such as major histocompatibility (MHC) antigens [75,76] and adhesion receptors, leads to the selective activation and recruitment of circulating leukocytes [77].

However, in some conditions, EC can also "adapt" to the transplant situation (referred to as "accommodation") particularly when such activation stimuli are progressively presented to these cells [78–81]. Furthermore, in vivo studies have previously demonstrated that approaches able to protect EC from rejection-mediated damage, for example by up-regulating anti-apoptotic genes such as heme oxygenase (HO-1) and the zinc finger protein A20, are capable of extending the survival of transplanted organs both in an allogeneic ABO incompatible setting and in a xenogeneic context [82,83]. Nevertheless, molecular mechanisms and signaling pathways conducting to endothelial protection and accommodation are still largely unknown.

4.3. Lnk is a negative regulator of signaling in endothelial cells

4.3.1. Endothelial expression of the adaptor Lnk and regulation upon inflammation

Identification of molecules that regulate or control inflammation and activation of the endothelium could provide novel and original targets to prevent endothelial cell dysfunction and damage, leading to enhanced protection of the transplanted organ from rejection [78,82,83].

In this light, we previously analyzed gene regulation in vascular EC stimulated with cytokines or antibodies directed against donor EC in a context of organ transplantation. These studies were conducted using transcriptome analysis and allowed us to identify a panel of molecules specifically regulated by EC in response to a pro-inflammatory stimulus [84–86]. We reported that the adaptor Lnk is expressed in EC and that the proinflammatory cytokine TNFα rapidly phosphorylates and subsequently upregulates Lnk at mRNA and protein level [87]. This study was the first to describe the expression and regulation of the adaptor Lnk in non hematopoietic human cells (i.e., EC) suggesting that Lnk may also contribute to regulatory mechanisms in other cells including vascular cells. Consistent with these findings, Nobuhisa et al. also reported that Lnk was predominantly expressed in the EC lining the dorsal aorta at embryonic day 11.5 (E11.5) in the midgestation mouse embryo [49] confirming that Lnk expression is not restricted to leucocytes. The ability of TNF to upregulate Lnk in the endothelium though a PI3Kinase-dependent manner was further observed in HUVEC [88]. Eicosapentaenoic acid, an n-3 polyunsaturated fatty acid, inhibited TNF-alpha-induced Lnk expression in HUVEC through the PI3K/Akt pathway [89]. This regulation may be a potential mechanism by which eicosapentaenoic acid acts in EC under inflammatory conditions.

4.3.2. Lnk is a negative regulator of TNF signaling in EC

Since Lnk is expressed and upregulated in vascular EC in response to TNFα [87], we hypothesized that the adaptor Lnk may be implicated in the TNFα signaling cascade. We showed that in EC, Lnk downregulates the expression, at both mRNA and protein levels, of the proinflammatory molecules VCAM-1 and E-selectin induced by TNFα. Mechanistically, our data indicated that, in response to TNFα, NFκB/p65 phosphorylation and translocation, as well as IκBα phosphorylation and degradation, were unchanged, suggesting that Lnk does not modulate NFκB activity. However, Lnk activates the PI3-kinase as reflected by Akt phosphorylation. Our results identify endothelial nitric oxide synthase (eNOS) as a downstream target of Lnk-mediated activation of the PI3-kinase/Akt pathway and HO-1 as a new substrate of Akt. We found that sustained Lnk-mediated activation of PI3-kinase in TNFα-activated EC correlated with the inhibition of ERK1/2 phosphorylation, while phosphorylation of p38 and JNK MAPKs was unchanged. ERK1/2 inhibition decreases VCAM-1 expression in TNFα-treated EC. Collectively, our results identify the adaptor Lnk as a negative regulator in the TNFα signaling pathway mediating ERK inhibition and suggest a role for Lnk in interplay between PI3-K and ERK triggered by TNFα in ECs [90].

4.3.3. Lnk a cytoprotective molecule for xenotransplantation?

The possibility to consider Lnk as a cytoprotective gene in the endothelium according to previous definition [64], i.e., a gene that is upregulated in response to inflammatory stimuli such as TNF and acts to protect EC from apoptosis and to limit the damage associated with activation, was recently examined. It was speculated that increased expression of Lnk in pig EC could

negatively regulate cell adhesion molecules and prevent EC activation in a pig-to-primate xenograft [66]. Significant overexpression of the intracellular adaptor protein Lnk was efficiently achieved in cultured pig EC isolated from wild type and genetically engineered Gal−/− pig [91]. Sustained Lnk expression in pig EC provides a negative regulation of EC activation by reducing the level of the adhesion molecule VCAM-1 implicated in the acquisition of a proinflammatory phenotype. Whether Lnk may regulate the expression of coagulation factors, which also impair endothelial cell functions in xenografts [92,93], still remains to be investigated. A role for Lnk in the protection of EC from apoptosis by a caspase3/7-dependent manner was also demonstrated [91]. However, the mechanism that account for this protection still remains to be elucidated. Moreover, Lnk was found to protect EC from both TNF-mediated apoptosis and anoïkis indicating that modulation of Lnk could prevent death of graft cells and restrict related inflammation. Finally, refinements to EC targeting in vivo could open the way to the design of effective gene transfer in vascularized organs at least in experimental animal models.

5. Lnk is a regulator of integrin signaling and cell migration

Members of the SH2-B family, SH2B1 and SH2B2, are shown to be involved in the regulation of the actin cytoskeleton. SH2B1β colocalizes with filamentous actin in growth hormone (GH)-induced membrane ruffles and interacts with Rac to potentiate GH-dependent actin rearrangement and cellular motility [94–97]. Adaptor SH2B2 may also impact on actin cytoskeleton rearrangement. Mice deficient in SH2B2 display reduced actin assembly in B-1 cell subset and mast cells [98]. In addition, SH2B2 binds the calponin homology (CH) domain of guanine nucleotide exchange factor Vav3, a member of the Vav family proteins that function as guanine nucleotide exchange factors for Rho/Rac. Rho and Rac GTPases are essential components of the integrin signaling pathway and play predominant role in actin cytoskeleton organization which affects adhesion and migration processes. First evidence of similar function for Lnk is its interaction with filamin A also termed actin binding protein 280 [99]. Whether Lnk may impact on integrin signaling is an important issue that recent studies of Takaki and Eto laboratories have highlighted.

As mentioned in above, inhibition of endogenous Lnk enhances engrafting potential of HSC and HPC. Transplantated HSC/HPC migrate from the blood circulation to the bone marrow and then localize and anchor in suitable microenvironments (niche) through interaction of adhesion molecules. Many reports have documented the importance of the integrins, particularly very late antigen (VLA)-4 (α4β1) and VLA-5 (α5β1), in regulating adhesive interactions between HSC/HPC and the cellular and extracellular matrix component that constitute the stem cell niche [100–104]. It Is has been proposed that Lnk inhibitors that block functions in HSC/HPC would be beneficial for reconstituting a blood system after irradiation or chemotherapy and for blood cell engraftment into immunodeficient hosts. Consistent with this hypothesis, Takizawa et al. demonstrated that Lnk deficiency decreases precursor migration on VCAM-1-coated membrane, suggesting that adhesion and migration of HSC/HPC on VCAM-1 is regulated by Lnk-mediated pathway [50]. VCAM-1 is known to interact directly with VLA-4 integrin and antibody against the α4 subunit of VLA-4 restore at least in part the defect migration observed in Lnk−/− cells. Therefore, these results support the idea that absence of Lnk increases interaction and trapping of HSC/HPC on cell adhesion molecules. Thus, beyond a role in progenitor cell growth and commitment, Lnk appears to be involved in cell motility and cellular interactions. Nevertheless, the mechanism by which Lnk control VLA-4/VCAM-1 interaction remains to identify, it will be important to address whether Lnk impact on integrin expression, engagement and/or signaling events.

Involvement of Lnk in integrin-mediated signaling pathways was also reported in megakaryocytes maturation process [105]. Mature megakaryocytes fragment and release thousands of platelets through mechanisms that are not well established. It was shown that platelets arise from long "proplatelet" processes that extend from the surface of mature megakaryocytes, through sinusoidal endothelial cells, and into blood vessels [106]. This process is regulated by various chemokines and cytokines as well as by adhesive interaction between megakaryocytes, integrin and endothelial cell adhesion molecules [107–109]. If TPO drives megakaryocyte proliferation and differentiation, it does not appear to directly promote platelet release from differentiated megakaryocytes. Takizawa et al. have investigated the possibility that Lnk might control thrombopoiesis by modulating integrin-mediated responses in addition to TPO growth signaling [105]. They have compared signaling events observed in megakaryocytes in contact with both TPO and VCAM-1 or with TPO alone. Addition of VCAM-1 modifies signaling events observed in TPO-stimulated megakaryocytes: STAT5 phosphorylation is decreased and MAPK activation is increased. In comparison, Lnk−/− megakaryocytes are not sensitive to VCAM-1 interaction and no change in STAT or MAPK signaling was detected in response to TPO stimulation. Furthermore, in the presence of TPO, WT megakaryocytes on VCAM-1 release greater number of platelets whereas number of platelets released by TPO-stimulated Lnk−/− cells is unchanged in contact or not with VCAM-1 molecules. This study suggests that spontaneous platelet release from megakaryocytes is regulated through integrin signaling and that Lnk modulates crosstalk between integrin- and cytokine-mediated signals, thus controlling thrombopoiesis [105].

Another unexpected function of Lnk is its role in stabilizing thrombus formation. Cellular events implicate in thrombi formation remain elusive owing to the difficulty of in vivo process analysis. In order to stop bleeding, activated platelets secrete soluble mediators to recruit additional circulating platelets and induce their aggregation and thrombus formation. Platelets activation is mediated through several signaling pathways, including the integrin αIIbβ3 pathway [110] that leads to platelets interaction with fibrinogen or Von Willebrand factor. Engagement of integrin αIIbβ3 induces recruitment and activation of various kinases or adaptor proteins such as Syk, Src, Fyn or Fyb [110–112]. The assembly of signaling complex triggers cytoskeleton rearrangement, allowing platelets spreading and thrombus stabilization. Using in vivo imaging, Takizawa et al. determined that Lnk is an essential contributor to the stabilization of developing thrombi within vessels. Spreading on fibrinogen is diminished in Lnk−/− platelets. Furthermore, absence of Lnk decreases tyrosine kinase Fyn binding to integrin αIIβ3 leading to reduced tyrosine phosphorylation of the β3 integrin. Those modifications impair stabilization of the developing thrombus, suggesting a new role for Lnk in facilitating integrin αIIbβ3 phosphorylation and signaling in order to promote platelet cytoskeleton rearrangement and spreading.

The cytoskeleton organization, highly regulated by the integrin signaling, is crucial for adhesion, migration, survival or proliferation of adherent cells such as endothelial cells. Recent experiments further defined the function of the Lnk adaptor in regard to endothelial cell signaling (Devallière J. et al., 2011, manuscript submitted). These findings indicate that Lnk expression induces cytoskeleton reorganization with EC morphological changes and increased number of focal adhesions, suggesting that Lnk may be implicated in signaling pathways involved in EC adhesion and motility. Consequently, a particular focus was given to identify the role that Lnk may play in EC integrin pathway. Firstly, we found

that integrin ligation and activation, using anti-β1 integrin (CD29), promotes Lnk upregulation and phosphorylation in human EC whereas β1 integrin ligation on Lnk−/− EC fails to activate integrin-mediated signal transduction via Akt and glycogen synthase kinase GSK3β phosphorylation. These data support a major regulatory function for Lnk in the integrin pathway. Using a gene transfer approach in primary EC cultures, we identified ILK as a new molecular partner for Lnk (Devallière J. et al., 2011, manuscript submitted). Functionally, sustained Lnk expression dramatically increases EC adhesion to extracellular matrix, inhibits EC migration in wound healing assays. The molecular mechanisms and interacting protein(s) implicated in the regulation of EC adhesion and migration trigerred by Lnk are under investigation. The regulating role of Lnk in cell motility is corroborated by Simon et al. study in which Lnk reduces mast cell migration in response to SCF through regulation of Rac/JNK and p38 MAPK pathways [41]. Collectively, these results identify the adaptor Lnk as a novel and effective key regulator of integrin-mediated signaling controlling EC adhesion/migration, both critical in vascular remodeling and regeneration.

All together, these studies present several evidences for a new role for the adaptor protein Lnk in integrin signaling and actin cytoskeleton organization. However, regulatory mechanisms by which Lnk modulates integrin pathway might differ according to cells (HSC/HPC, megakaryocytes, platelets, mast cells and endothelial cells). Identification of the protein targets and effectors of Lnk will be important to fully understand cellular functions of Lnk protein.

6. Lnk is a molecular target to enhance endothelial progenitor cells and vascular repair

Progenitor cells known as endothelial precursor cells (EPC), were initially described in 1997 [113]. EPC are immature cells that differentiate in situ into mature EC and support angiogenic and vasculogenic processes by secreting an array of growth factors, cytokines, and proteases. Consequently, circulating EPC have a high potential for cell therapy approaches aiming to vascular repair. Local delivery of EPC successfully promotes therapeutic neovascularization in both ischemic hind limbs as well as acute myocardial infarction models [114]. A number of studies have also indicated a strong correlation between EPC number and vascular function [115] and allograft outcome [116,117]. EPC number has been proposed as a surrogate measure of vascular injury since vascular pathologies [118] but also immunosuppressive regimens [117] have been shown to adversely affect EPC number and function. Thus EPC have been proposed as biomarkers to assess the risk of cardiovascular disease [119].

It was originally reported by Takaki et al. that Lnk-deficient mice display a significant increase in hematopoietic progenitor cells in the adult bone marrow [40]. In mice, long-term repopulating hematopoietic stem cells, which express several marker proteins, emerge in the aorta–gonad–mesonephros (AGM) region of the mouse at midgestation. Transcripts for Lnk were detected in the AGM region at E9.5, 11.5, and 14.5 and the fetal liver at E14.5, but not in the muscle of adult mice. Lnk was present in the EC lining the dorsal aorta indicating that Lnk is expressed in the AGM region at a stage of embryonic hematopoiesis. The expression pattern of Lnk overlaps with that of CD34 in the dorsal aorta, suggesting that Lnk might be involved in hematopoietic cell development from endothelial precursors [40]. Lnk was shown to function as a negative regulator of the development of hematopoiesis in the AGM region during mouse embryogenesis [40].

In human, the generation of hematopoietic cells from embryonic stem cells (ESC) has raised the possibility of using ESC as an alternative donor source for transplantation. Functional and molecular differences between CD34+ hematopoietic progenitor subsets derived from ESC and CD34+ subsets derived from umbilical cord blood that represents definitive hematopoiesis were compared recently. This study showed strong negative regulators of lymphopoiesis including the adaptor protein Lnk and CCAAT/enhancer-binding protein-α (CEBPα), were exclusively expressed in ESC–CD34+ subsets. Knockdown of Lnk lead to an increase in hematopoietic progenitors generated from ESC [120]. The particular molecular pattern observed in ESC–CD34+ cells could reflect the persistence of transcripts expressed in undifferentiated ESC and mesoderm progenitors, resulting in the blockage in definitive hematopoiesis from ESC.

As mentioned above, the inhibitory effect of Lnk was mediated by the binding of the Lnk SH2 domain to the phosphorylated c-Kit receptor. Both the number of HSC/HPC but also their self-renewal capacities were strongly increased in Lnk-deficient mice [51]. Consistent with both the reported ability of Lnk to directly inhibit TPO signaling [52], and the inability to rely increased HSC numbers in Lnk−/− mice to enhanced kit function [51], it was shown that Lnk is a physiological negative regulator of TPO-induced postnatal expansion of self-renewing HSC and myeloid progenitors [121] by modifying TPO-mediated signal transduction [42].

Postnatal HSC and EPC share common markers; however, the precise mechanisms regulating progenitor cell growth in adults, and endothelial commitment of stem cells and/or common precursors for hematopoietic cells and EC for postnatal vasculogenesis are still unclear. In mouse, the population of c-Kit-positive, Sca-1-positive, lineage marker-negative (KSL) cells in bone marrow represents a fraction of hematopoietic stem/progenitor cells [122]. The KSL cells are also believed to be a good source for EPC, since part of KSL cells differentiate into the endothelial lineage and thus contribute to vasculogenesis in vivo [122]. In vivo, the administration of Lnk−/− KSL cells to a mouse spinal cord injury model promoted significantly more effective angiogenesis, astrogliosis, axon growth, and functional recovery following injury that the administration of Lnk+/+ KSL cells [123]. Interestingly, this study also showed an overall reduction of the fibrous scar area possibly due to the restricted infiltration of inflammatory cells caused by a rapid migration of reactive astrocytes and vascular stabilization in the spinal cord treated with KSL cells. However, a possible direct impact of Lnk of fibrosis regulation still remains to be evaluated.

Moreover, Lnk deficiency in mice increases EPC kinetics in response to ischemia-related cytokines and factors (G-CSF, SDF-1γ, SCF, vascular endothelial growth factor (VEGF)) and improves neovascularization in vivo [124]. Lnk-deficient mice promote vasculogenesis/angiogenesis and osteogenesis by the mobilization and recruitment of HSC/EPC through activation of the SCF-c-kit signaling pathway in the perifracture zone [125]. Moreover, osteoblasts from Lnk-deficient mice also have a greater potential for terminal differentiation in response to SCF-c-kit signaling in vitro. These findings suggest that inhibition of Lnk may have therapeutic potential to enhanced fracture healing.

Together these findings provide strong evidence that Lnk is a key regulator of bone marrow-EPC kinetics, including the ability to cell growth, endothelial commitment, mobilization, and recruitment for vascular regeneration. Selective targeting of Lnk may be a safe and effective approach to augment therapeutic neovascularization by EPC transplantation.

7. Lnk gene mutations as genetic risk factors in cardiovascular and autoimmune diseases

Recently, genetic studies reported a role for Lnk gene polymorphism and mutations in various diseases including type 1 diabetes (T1D) [126–130], hypertension [131], myocardial

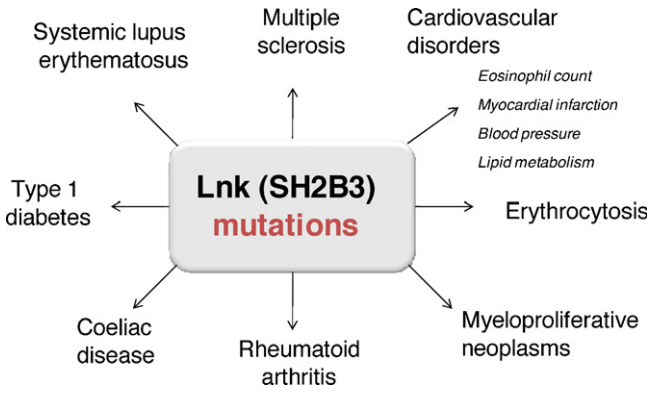

Fig. 4. Genetic associations between Lnk mutations and diseases.

infarction [132], coeliac disease [127,133], myleoproliferative diseases [134], erythrocyosis [135], systemic lupus erythematous [136], rheumatoid arthritis [137], multiple sclerosis [138]. An overview of previously reported disease associations for the identified Lnk mutations is presented in Fig. 4.

The gene SH2B3 maps on chromosome 12, at 12q24. Lnk's single nucleotide polymorphisms (SNPs) reported yet are located in exons 2, 3 and 5 and involve the Lnk PH and the SH2 domains. A missense mutation at position 262 (R262W) was initially found (SNP: rs3184504). The rs3184504 marker is a non-synonymous SNP in exon 3 of SH2B3 leading to a R262W amino acid change in the pleckstrin homology (PH) domain. The Lnk R262W mutation has been found associated with autoimmune and cardiovascular disorders (Fig. 4).

7.1. Type 1 diabetes and autoimmune diseases

A primary genome-wide association study (GWAS) on autoimmune type 1 diabetes (T1D), showed significant association ($P < 5 \times 10 - 7$) between T1D and six chromosome regions including 12q24 [126]. On chromosome 12q24, rs3184504 had the highest association score ($P = 1.73 \times 10 - 21$; odds ratio (OR) = 1.33, 95% confidence interval (c.i.) = 1.26–1.42) [126]. This single nsSNP was sufficient to model the association of the entire region [126]. Independent studies confirmed these findings in other populations [128–130]. Recent data suggested that carriage of SH2B3 784T > C correlates with T-cell proliferation in patients' peripheral mononuclear blood cells (PMBCs) stimulated with anti-CD28 and anti-CD3 antibodies [139]. Thus, the SH2B3 784T > C variant could contribute to the pathogenesis of T1D through impaired immune response that promotes activation and expansion of self-reactive lymphocytes in susceptible individuals.

In addition to type 1 diabetes, a strong association with coeliac disease was also reported in this region with rs3184504 (same allele and direction as T1D) [127,133]. This study further indicates that SH2B3 is strongly expressed in the small intestine, with a higher expression in inflamed coeliac biopsies that may reflect leukocyte recruitment and activation [133]. Association of rheumatoid arthritis with this coeliac disease risk variant in the SH2B3 was also observed, predominantly in the subgroup of rheumatoid factor-positive rheumatoid arthritis patients [137]. SH2B3 is also a genetic candidate for susceptibility to systemic lupus erythematous (SLE) [136], and possibly juvenile idiopathic arthritis [140]. SH2B variant rs3184504 was also associated with multiple sclerosis in GWAS [138]

7.2. Cardiovascular disorders

The SNP rs3184504 in SH2B3 is one of the blood pressure SNPs determining a risk allele for higher blood pressure [131]. A GWAS of systolic blood pressure, diastolic blood pressure, and hypertension provides evidence for a significant association signal between systolic and diastolic blood pressure and SH2B3 (rs3184504, $P = 1.7 \times 10 - 8$) [131]. The rs3184504T allele, which is associated with increased blood pressure, was recently shown to cause increased cytokine production [141,142]. Functional investigation of the effect of the SH2B3 genotype in response to lipopolysaccharide and muramyl dipeptide revealed that carriers of the SH2B3 rs3184504 risk allele showed stronger activation of the NOD2 recognition pathway [142]. This suggests that SH2B3 plays a role in protection against bacterial infection. Locus 12q24 was associated with coronary heart disease and hypertension. Lnk SNP rs3184504 was also associated ($P = 4.88 \times 10 - 11$) with retinal caliber changes reflecting microvascular disease and predictive of cardiovascular injury [143]. Interestingly, larger venular caliber has been associated with systemic markers of inflammation (C-reactive protein, IL-6) and dysfunction in glucose metabolism, obesity and dyslipidemia in epidemiologic studies [144]. Other studies demonstrated a link between the Lnk R262W variant and erythrocytosis [135,145], LDL cholesterol [146], myocardial infarction [132].

There are some evidences for a link between hypertension and inflammation [147,148], possibly involving complex interplay between systemic inflammation, vascular cells activation, and structural changes in the arteries. An attempt to link blood pressure with type 1 diabetes or coeliac disease was evaluated. No convincing association, between SNPs reported to be associated with T1D, coeliac disease or myocardial infarction, other than that for the SH2B3 missense SNP was observed [149].

A possible explanation is that the SH2B3 missense SNP impacts blood pressure through an action specific to cells outside of the immune system and that could support the hypothesis for a role of Lnk in vascular biology and homeostasis beyond its functions in the immune system. The association of this SNP with a panel of autoimmune diseases (T1D, coeliac disease, multiple sclerosis, rheumatoid arthritis, lupus, juvenile idiopathic arthritis etc.) may also suggest that immune response pathways may influence blood pressure by mechanisms not yet defined. Genetic variations can affect protein function by altering gene expression and protein levels or by altering the structure of the encoded protein. For Lnk both mechanisms can occur with the most described SNP, rs3184504, introducing and amino acid substitution in the PH domain whereas at least two other SNP (mutations E208X and C603G) correspond to a truncated protein with deletion of the PH and SH2 domains.

Current concepts on the pathogenesis of these autoimmune disorders attribute a crucial role to T and B cells inappropriately recognizing self antigens and initiating a cell-mediated or humoral reaction, or both, resulting in inflammatory tissue and vascular damage [150]. Autoimmune diseases can therefore be regarded as the final outcome of a series of events that likely include not only a genetic susceptibility, but also the failure of the checkpoints available to prevent autoimmunity following exposure to environmental challenges, such as infections. It is reasonable to postulate that the normalization of immune regulatory mechanisms could play a role in the suppression of autoimmunity. In this context, the impact of genetic variants for Lnk may indicate a role for Lnk in specific immune regulation associated with these diseases.

7.3. Novel Lnk mutations involved in myeloproliferative diseases

The myeloproliferative neoplasms (MPN) including polycythemia vera, essential thrombocytosis and primary myelofibrosis have been initially associated with a mutation in JAK2, the JAK2V617F mutation corresponding to a somatic substitution of a valine by a phenylalanine at codon 617 of JAK2 (reviewed in

[151,152]). These myeloproliferative neoplasms are human clonal disorders of hematopoietic progenitors, resulting in increased numbers of functional, mature and differentiated myeloid cells. JAK2V617F protein has constitutive kinase activity [53] and is constitutively phosphorylated in vitro [153] revealing that JAK2V617F is a gain of function mutation that confers cytokine hypersensitivity and cytokine-independent growth to hematopoietic cells. Interestingly, Lnk−/− mice display equivalent myeloproliferative abnormalities to those found in MNP patients, suggesting a potential implication of Lnk in the pathogenesis of these diseases. Recent study demonstrated that Lnk is upregulated in MNP patients and decreases JAK2V617F-dependent cell proliferation, confirming its role as negative regulator [154].

Additive mutations have been recently described in patients with myeloproliferative diseases [155], novel mutations in JAK2 (exon 12) but also in the thrombopoietin receptor gene MPL as well as in Lnk gene have been associated with MPN. Oh et al. provided the first evidences for Lnk mutations in JAK2V617F-negative and MPL-wild type myelofibrosis patients suggesting an alternative mechanism of JAK/STAT activation and clonal erythrocytosis [134]. Direct sequencing of the region of Lnk encompassing the PH and SH2 domains was performed on 33 JAK2 V617F – negative MPN samples, resulting in the identification of 2 novel mutations in exon 2 of Lnk. A 5-bp deletion and missense mutation (NM_005475.2:c.[603_607delGCGCT; C613G]), leading to a premature stop codon. This mutation results in the absence of both the PH and SH2 domains. In addition, a missense mutation (NM_005475.2:c.G622C) leading to a glutamic acid to glutamine substitution (E208Q) in the PH domain was also identified [134]. This study also functionally validated both Lnk mutations (E208Q and C613G) as loss of function mutations resulting in hyperactivation of the JAK-STAT signaling pathway [134]. Lnk point mutations affecting the PH domain impair Lnk translocation to the plasma membrane and partially reduce Lnk regulatory functions as demonstrated with Lnk mutants [156]. Lnk mutations affecting the SH2 domain prevent interaction and regulation of the JAK/STAT signaling and resulted in more severe phenotype [43,52,54,156]. Mutations of the Lnk SH2 domain also inhibit platelet-derived growth factor receptor signaling [44].

Subsequently, nine other heterozygous Lnk mutations were further identified in eight (13%) patients with MPN: six exon 2 missense mutations involving codons 215, 220, 223, 229 and 234, a synonymous mutation involving codon 208, and two deletion mutations involving exon 2 (685–691_delGGCCCCG) or exon 5 (955_delA); eight affected the pleckstrin homology (PH) domain [157]. Whereas the Lnk A215V mutation constitutes a conservative amino acid change, the Lnk E208X mutation leads to a truncation of both the PH and SH2 domains. The hypothesis that some of these mutations could behave like those of the Lnk−/− mouse model that has been characterized by several groups [40,48] was confirmed for some of these mutations [134,157]. Importantly, heterozygosity for the C613G mutation seems sufficient to initiate MPN pathogenesis [134]. Whether these mutations are present within the same individual or/and if the combination of these mutations affects the MPN phenotype or clinical outcome is still unknown [157].

Overall, the possible role of the SH2B3 variant associated in these studies to different autoimmune diseases is still undetermined, mainly because of the lack of knowledge on the functional role of the causal variant. However, the overexpression as well as the deficiency of this gene has consequences that could be of importance in the development of autoimmune diseases. Confirmation of common and/or specific roles for Lnk in the different immune-mediated diseases will be crucial to define the contribution of signaling pathways to the different disorders and to the development of novel therapies. Functional SNPs also could also provide useful diagnostic markers.

8. Future directions and conclusion

To conclude, identification and characterization of molecular targets able to provide a negative regulation of inflammatory signaling in EC without sensitizing cells to apoptosis is still a holy grail. The present review presents evidence for potent regulatory functions of the adaptor Lnk toward intracellular signaling associated with inflammation, cellular adhesion, migration and proliferation. Collectively, these findings suggest that Lnk could be an adaptor of interest for modulating inflammatory signaling pathways and to provide cytoprotection to vascular EC. Such approaches will most likely require specific cell targeting as well as temporal control of Lnk to avoid side effects. Moreover, negatively regulating the Lnk system may also have important implications for the development of new therapeutic strategies to enhance progenitor cell number and proliferation and subsequently to improve neovascularization/angiogenesis and vascular repair in regenerative medicine.

As suggested recently by genetic studies, the expression level of the Lnk adaptor seems crucial for its functions as demonstrated in myeloproliferative disorders, suggesting that an accurate screening of Lnk gene mutations in a broader array of diseases should be undertaken. The functional roles of the various Lnk mutations remain to be explored. Moreover, a detailed analysis of the mechanisms controlling Lnk gene expression, such as promoter structure for transcriptional control, miRNA-based regulation of gene expression and epigenetic modifications, is still needed for this gene. Further identification of exogenous factors, such as growth factors, cytokines and chemokines, that finely tune Lnk expression, is also needed. As an additional field of research, protein studies using proteomic-based analyses could clarify the crucial post-translational modifications (such as the presence of phosphorylated residues) that can contribute to the activation of specific signaling pathways by recruiting signaling partner molecules. The use of 'knockin' models will continue to provide important information regarding the physiological roles of particular Lnk domains, regulatory events and effector pathways. Finally, as genome and SNP-association studies gather pace, it could be expected that further links between SH2B adaptor proteins and human diseases will be discovered. Overall, these studies could thus emphasize the importance of the Lnk adaptor molecule not only as prognostic marker but also as potential therapeutic target.

Contributors

J.D. and B.C. contributed equally to the conception and redaction of the manuscript.

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