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Multi-functional adaptor SKAP1: regulator of integrin activation, the stop-signal, and the proliferation of T cells

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T-cell activation is a complex process involving a network of kinases and downstream molecular scaffolds or adaptors that integrate surface signals with effector functions. One key immune-specific adaptor is Src kinase-associated phosphoprotein 1 (SKAP1), which is also known as src kinase-associated protein of 55 kDa (SKAP55). This mini-review explains how SKAP1 plays multiple roles in regulating integrin activation, the “stop-signal”, and the optimization of the cell cycling of proliferating T cells through interactions with various mediators, including the Polo-like kinase 1 (PLK1). Ongoing research on SKAP1 and its binding partners will likely provide important insights into the regulation of immune function and have implications for the development of new treatments for disease states such as cancer and autoimmunity.

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T-cells, signalling, adaptor protein, integrin activation, SKAP1

Introduction

The activation of T cells involves the processing and presentation of peptide antigen bound to class I and II major histocompatibility complex (MHC) antigens on the surface of antigen-presenting cells (APCs) such as dendritic cells (DCs) (1, 2). The initial contact between a DC and a T cell involves random encounters, or responses to chemokines that can partially activate integrins. Integrins are transmembrane receptors that bind key surface ligands such as proteins needed for adhesion between cells or to extracellular matrix proteins. Of the 12 integrins that are expressed on lymphocytes, α L β 2 [leukocyte function-associated antigen-1 (LFA-1), also termed CD11a (α L chain of LFA-1)–CD18 (β 2 chain of LFA-1)] binds to the ligands intracellular adhesion molecules 1, 2, and 3 (ICAMs-1, 2 and 3) (3). Adhesion *via* integrins is needed for the migration of T and B cells to different tissues

and to sites of inflammation, for movement in lymph nodes and germinal centers and for the conjugation of T cells with APCs.

Importantly, the initial contact of a T cell with an APC ligates the T-cell receptor (TCR) complex that then induces “inside-out” signals for high-avidity adhesion (4). This is accompanied by more stable conjugation and the formation of an interface between the T cells and APCs, termed the “immunological synapse” (IS) (5). The process involves the formation of initial signaling micro-clusters that coalesce to form the supramolecular activation cluster (SMAC) (6, 7). Chemokines can enhance this process and increase the longevity of the adhesion between integrins and ligand (8). Atomic force microscopy has shown that conjugation forces develop over time and are highest when synapse formation is maximal (9).

When the TCR binds to peptide-loaded MHC molecules on APCs, it triggers the transcription of numerous genes related to T-cell development, differentiation, and effector functions. The specific genes transcribed may vary depending on the context such as the type of T cell, the strength and duration of the TCR signal, and the presence of co-stimulatory signals. Some of the most significant genes that are transcribed include IL-2, IFN- γ , TNF- α , CD69, and nuclear factor of activated T cells (NFAT). The earliest events induced by TCR ligation involve the induction of a tyrosine phosphorylation cascade in T cells (10, 11). Tyrosine phosphorylation is a relatively rare event accounting for less than 1% of total phosphorylation in cells (12). Nevertheless, it is crucial to virally induced phosphorylation events and activation by certain growth factor receptors (13). We first documented the binding of src kinase p56^{lck} to human CD4 and CD8, which initiates a phosphorylation cascade and leads to the phosphorylation of the antigen-receptor complex (10, 11, 14). We proposed that the CD4 and CD8 co-receptors would bring p56^{lck} into proximity of the TCR complex due to binding to non-polymorphic sequences in MHC antigens (15, 16). The presence of these activation complexes was also reported in murine T cells (17) while others have underscored a role for free p56^{lck} in the initiation process (18).

The CD4/CD8-p56^{lck} complexes and p56^{lck} alone can then phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) in the CD3 and T-cell receptor ζ chains for the recruitment of a second protein kinase ZAP-70 (ζ -chain associated protein kinase-70) kinase (19). Both p56^{lck} and ZAP-70 then phosphorylate the so-called “adaptor” proteins or molecular scaffolds that form complexes that integrate signals from the cell surface to the nucleus (10, 20). p56^{lck} phosphorylates a broader array of substrates than ZAP-70, many of which overlap with the substrates of other src-related kinases (11, 21). The major identified targets of ZAP-70 are immune-cell adaptors termed LAT (linker for activation of T cells) and SLP-76 (Src homology 2 domain-containing leukocyte protein of 76 kDa) (22, 23). ZAP-70 phosphorylates LAT, which recruits GRB2-related adaptor GADS, which in turn binds with high stoichiometry to SLP-76 (22). The LAT complex also recruits the kinase ITK (interleukin-2-inducible T-cell kinase), leading to the phosphorylation of the phospholipase C γ 1 and the mobilization of intracellular calcium (24). Recently, we showed that another integrin-linked kinase, FAK1 (Focal Adhesion Kinase 1), and PYK2

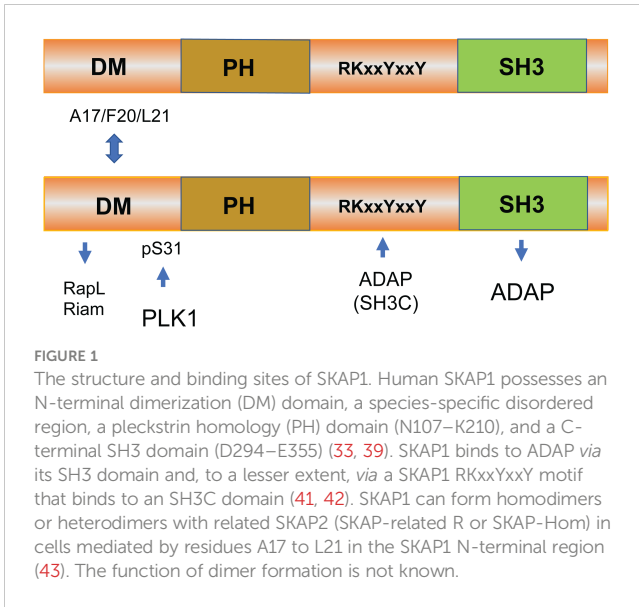
(proline-rich tyrosine kinase-2) could phosphorylate LAT on a specific Y-171 residue for GRB2 binding (25). This is a new model for LFA-1 in which the integrin can mediate both adhesion and de-adhesion events dependent on receptor cross-linking.

In addition to the activation of gene transcription, the TCR must activate integrins such as LFA-1 on the surface of T cells to generate stable conjugation with an APC (26). Integrins are inactive on resting cells but, in the “jackknife model”, unfold to form intermediate- and high-affinity binding (27). LFA-1 has been studied extensively where the α L subunit consists of a binding pocket containing an I-domain, a β -propeller, and an extracellular and cytoplasmic tail. The β 2 subunit is composed of an I-like domain that interacts with the β -propeller, and four integrin-epidermal growth factor-like (I-EGF) domains, which acts as a “leg” of the subunit (28). LFA-1 occupancy with surface ICAM-1 is needed for TCR conversion to an open headpiece high-affinity state (29). Interestingly, this appears not to be the case for chemokine-triggered LFA-1. During antigen presentation, LFA-1 is rearranged in a pSMAC ring that surrounds the TCR complex and other receptors in the central IS while binding to the ligand intercellular adhesion molecules (ICAMs) (30).

SKAP1 and T-cell adhesion

Although many upstream signals are needed to initiate the process (31), the downstream effector proteins in “inside-out” signaling for LFA-1 activation are only partly known. One advance in this area came with the discovery of immune-cell adaptors ADAP [previously known as Fyn T-binding protein (FYB)] and SKAP1. ADAP was cloned independently by the Koretzky and Rudd labs where it binds to the SH2 domain of the upstream adaptor SLP-76 (32–36). ADAP is also a preferred substrate of the src kinase p59^{fyn} (32, 37). SKAP1 was cloned independently by the Schraven and Rudd labs, the latter using ADAP as bait in a two-hybrid screen (33, 38). Human SKAP1 possesses an N-terminal dimerization (DM) domain, a species-specific disordered region, a pleckstrin homology (PH) domain (N107–K210), and a C-terminal SH3 domain (D294–E355) (33, 39). It is an intracellular immune adaptor protein expressed in thymocytes, T cells, and NK cells (40) (Figure 1). SKAP1 binds to ADAP *via* its SH3 domain and, to a lesser extent, *via* a SKAP1 RKxxYxxY motif binding to an ADAP SH3C domain (41, 42).

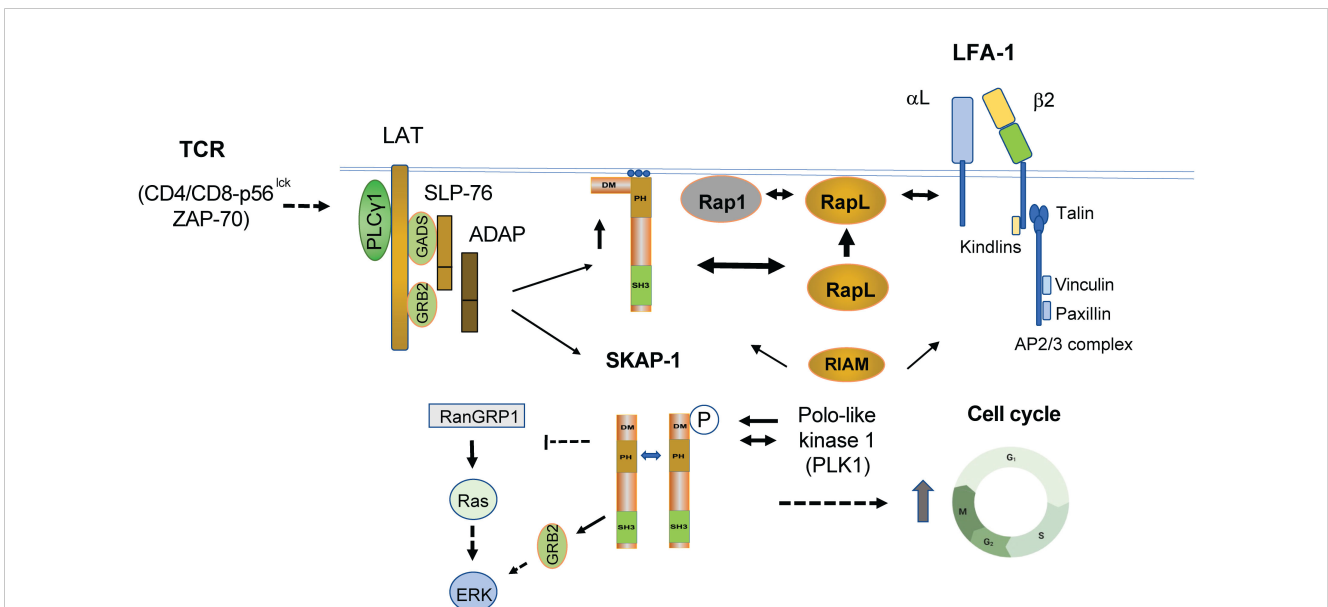
Early transfection and knock-down studies showed that SKAP1 promotes dwell times between T cells and dendritic cells (DCs) (26, 44–49). We showed that SKAP1, but not related SKAP2, regulated TCR-induced lymphocyte-associated antigen-1 (LFA-1) clustering and T cell–APC conjugation (48). No effect on TCR-CD3 clustering was seen (26, 48). SKAP1 enhances adhesion to both fibronectin and intercellular adhesion molecule-1 (ICAM-1), can colocalize with actin at the T cell–APC synapse, and can promote the clustering of LFA-1. The enhanced conjugation was comparable to that seen *via* adhesion and degranulation-promoting adaptor protein (ADAP), a binding partner of SKAP1, and is abrogated by the deletion of the SKAP1 SH3 domain. Conjugate formation is also



accompanied by the translocation of SKAP1 to membrane rafts (46, 47). The loss of SKAP1 in *skap1*^{-/-} mice had no obvious effect on thymic development, or the numbers of peripheral T, B, and natural

killer (NK) cells. Instead, the *skap1*^{-/-} T cells had impaired binding to ICAM1, more transient conjugation times, and a reduced localization of TCR-CD3 micro-clusters at the IS (49).

Mechanistically, we and others have shown that the adaptor can regulate LFA-1 activation via at least two non-exclusive pathways. One pathway involves SKAP1 binding to the SARAH domain of RapL (50–52), an immune cell isoform of the RASSF5 (Ras association domain family 5) family (53). The Kinashi lab has already done seminal work on the importance of Rap1–RapL binding in integrin activation (54, 55). Remarkably, we then found that RapL failed to form complexes with Rap1 in *skap1*^{-/-} T cells and that the SKAP1 PH domain plays a pivotal role in the pathway (44, 50). TCR and CD28 ligation can induce D-3 lipids, phospholipids with a phosphatidylinositol (PI) head group that is phosphorylated on the inositol ring at the 3-position. These lipids bind to the SKAP1 PH domain, thereby promoting its translocation to the inner face of the plasma membrane (56, 57). In this manner, the PH domain allowed SKAP1 to act as a kind of “shuttle” to facilitate the translocation of RapL to the inner face of the plasma membrane where it can interact with the GTP-binding protein Rap1, a potent stimulator of integrins including LFA-1 (50–52) (Figure 2). Further we found that the overexpression of RapL “slowed” T-cell motility in D011.10 transgenic T cells in lymph



nodes (LNs), an effect reversed by the L224A mutation needed for binding to SKAP1 (44). This implicated RapL and SKAP1 as co-regulators of the “stop-signal” in T cells. Furthermore, the addition of an N-terminal myr-tag to SKAP1 promoted the constitutive binding of RapL to the cell membrane and replaced the need for TCR ligation in the activation of LFA-1 in T cells. In keeping with this theme, Kliche and coworkers found that the disruption of the ADAP/SKAP1 binding led to a displacement of Rap1 from the plasma membrane and that membrane-targeted SKAP1 induced T-cell adhesion even in the absence of TCR-mediated ligation (58).

In terms of micro-cluster formation, the SKAP1 SH3 domain and associated ADAP have also been reported to stabilize the formation of SLP-76 micro-clusters (59, 60). We also found that LFA1 ligation and the activation of FAK1 to phosphorylate LAT led to the recruitment of SKAP1 to the signalosome (25).

In a minimal model, SKAP1 acts as a chaperone that transports RapL to the PM to interact with the GTP-binding protein Rap1. The RapL–Rap complex (including SKAP1) then binds to the α L chain of LFA-1, promoting its clustering and avidity for its ligand (46, 61). Complementary to this, the β 2 chain binds to Kindlins (1, 2, and 3) and TALIN that, in turn, binds directly to Rap1-interacting molecule (RIAM), Paxillin, and Vinculin. TALIN is a high-molecular-weight cytoskeletal protein that links integrins to the actin cytoskeleton (62). Although less well-understood, kindlin proteins are also integrin regulators where their loss or mutations result in defective integrin activation (63). The TALIN unfolded helical bundle R2R3 also binds to RIAM, a site distinct from the vinculin binding site (64). Furthermore, SKAP1 also binds to RIAM (58, 65–67). In this context, it cannot be excluded that SKAP1 acts as a shuttle or chaperone for RIAM. In keeping with these observations, we found that SKAP1 knockout T cells show reduced TALIN-1 and RIAM translocation to the IS (61). Furthermore, non-cleavable TALIN can rescue the defect in the T-cell conjugation of *skap1*^{-/-} T cells (61). Overall, the β 2 chain complex promotes the unfolding of LFA-1 to intermediate- and high- affinity forms, while the α L chain-associated SKAP1–RapL–Rap1 complex promotes LFA-1 clustering for high-avidity binding and participates in promoting greater affinity. Further studies will be needed to fully understand the mechanisms underlying these different interactions and their potential cooperative roles in regulating cell adhesion and migration.

SKAP1 dimerization

One intriguing feature of SKAP1 is its ability to form dimers as mediated by the N-terminal dimerization domain (DM) (43) (Figure 2). We showed that both SKAP1 and related SKAP2 [SKAP-R (related) or SKAP-Hom] can form homo- and heterodimers in cells (43, 59, 60). In our hands, homodimer formation of SKAP1 is mediated by residues A17 to L21 in the N-terminal region (43). Intriguingly, both RapL and RIAM have been reported to bind to a similar general region in SKAP1 (44, 65). This begs the question whether dimer formation controls the binding and engagement of the RapL or RIAM pathways in integrin activation. On one level, we found that SKAP1 dimer formation was not needed for RapL binding since dimerization

mutants still bind to RapL (43). Others have reported that SKAP1 dimers stabilize the formation and movement of micro-clusters of the key adaptor SLP-76 (59). In this model dimer, they found that dimer formation enabled adhesion *via* the TCR by mechanisms that were independent of RIAM, TALIN, and beta integrin activation. Different mechanisms may therefore be at play that determine the way in which SKAP1 and its dimerization regulate adhesion or other functions. In the context of integrin activation, SKAP1 primarily regulates cluster formation and, to a lesser extent, affinity, while RIAM, KINDLINS, TALIN, and other proteins primarily mediate LFA-1 conformation and affinity changes. It is still unknown whether dimer formation influences PLK1-mediated effects on cell cycling. Overall, these findings highlight the complexity of SKAP1 cellular signaling and the need for further research to fully understand the role of SKAP1 and its dimerization in various cellular functions.

SKAP1, polo-like kinase, and the cell cycle

In this context, we have found that SKAP1 can also regulate other pathways and cell functions. We found that SKAP1 is phosphorylated by and binds to polo-like kinase (PLK1) for the optimal cycling of T cells (Figure 2) (68). PLK1 is a serine/threonine kinase that regulates multiple steps of mitosis and the cell cycle progression of mammalian cells. Among multiple kinases including CDK1, CDK2, MAPK, Aurora B, CAMK, PLK3, PLK1, MST1, and ZAP-70, only PLK1 could phosphorylate SKAP1-GST *in vitro*. Furthermore, PLK1 bound to the N-terminal residue serine 31 (S31) of SKAP1 and the interaction is needed for optimal PLK1 kinase activity in T cells (68). Furthermore, siRNA knock-down of SKAP1 reduced the rate of T-cell division concurrent with a delay in the expression of PLK1, Cyclin A, and pH3. Reconstitution of KD cells with WT SKAP1, but not the SKAP1 S31 mutant, restored normal cell division. SKAP1–PLK1 binding is also seen to be dynamically regulated during the cell cycle of T cells. Our findings identified a novel role for SKAP1 in the regulation of PLK1 and optimal cell cycling needed for T-cell clonal expansion in response to antigenic activation (68).

SKAP1 and the ERK pathway

Lastly, SKAP1 also influences the activation of the p21^{ras}–extracellular signal-regulated kinase (ERK) pathway. p21^{ras} activity is regulated by several mediators that include the guanyl releasing protein 1 (RasGRP1). RasGRP1 exchanges GDP for active GTP on p21^{ras} in a cascade that activates ERKs (69, 70). The Mustelin and Rudd labs independently reported that the C-terminus of SKAP1 binds to RasGRP1 (43, 68). PLK1 and RasGRP1 therefore bind to opposite ends of SKAP1. Furthermore, *skap1*^{-/-} primary T cells had increased RasGRP1 in the trans-Golgi network (TGN) following CD3 ligation where p21^{ras} becomes activated (43). Consistent with this, SKAP1 overexpression impaired Ras-Erk activation with reduced AP-1 transcriptional activity (43). In T cells, the AP-1 transcription

factor regulates a wide range of genes related to differentiation and proliferation. However, others have reported that SKAP1 positively regulates the ERK pathway in the T-cell line Jurkat (71). In this pathway, tyrosine 271 played a central role for interaction with both Fyn kinase and adapter protein GRB-2 to mediate mitogen-activated protein kinase activation. In this context, the binding of SKAP1 to GRB2 has also been noted in our lab as mediated by LFA-1 cross-linking and activation of kinases FAK1 and Pyk2 (25). Each kinase phosphorylated a key site at Y-171 on LAT leading to the recruitment of the GRB2-SKAP1 complex. It is possible that the opposing effects of SKAP1 on ERK activation and adhesion are dependent on the concentration of SKAP1 within the cell, and on the presence or absence of other interacting proteins. Further research will be needed to fully understand the complex role of SKAP1 in these pathways.

SKAP1 and biology

The physiological impact of SKAP1 in regulating immunity is also being studied, although our current understanding is limited due to the early stage of research in this area. Using a mouse model of collagen-induced arthritis (CIA), we showed that *skap1*^{-/-} mice are resistant to the induction of arthritis CIA (69). This was observed in terms of both the incidence of disease and its severity. Furthermore, we noted a marked reduction of joint infiltrating T cells, in particular TH17-like cells. SKAP1 therefore represents a potential target in the therapeutic intervention in autoimmune and inflammatory diseases (69). Lakkis et al. found that *skap1* deficiency prolonged allograft survival; it did not seem to alter effector T-cell migration to pancreatic islet allografts (70). Lastly, in certain tumor models, *skap1*^{-/-} mice may also be more resistant to tumor growth (71). Genome-wide association studies (GWAS) have identified SKAP1 as one of eight risk loci for endometrial cancer (72, 73). It is presently unclear how one can reconcile these different findings. It is possible that differences in TCR ligation versus the involvement of chemokines differentially regulates the activity of SKAP1 and its function in different T-cell subsets and in different contexts.

Conclusion

Overall, while SKAP1 is an immune cell adaptor that regulates T-cell adhesion and optimal cell growth, much remains to be

discovered about the physiological impact of SKAP1 in regulating immunity. Further research is needed to fully understand the full range of mechanisms by which SKAP1 regulates T-cell signaling and immune function as well as the downstream effects of SKAP1 activation or inactivation. This may involve assessing the impact of SKAP1 on specific immune cell subsets or its place in various affected disease states such as autoimmunity, transplant rejection, and cancer. Overall, data so far demonstrate that it is an adaptor with multiple functions associated with different regions of the protein. Continued research on SKAP1 will provide important insights into the regulation of immune function and will have implications for the development of new therapies to treat disease states.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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