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REVIEW

PAG - a multipurpose transmembrane adaptor protein

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Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), also known as Csk-binding protein (Cbp), is a ubiquitously expressed transmembrane adaptor protein present in lipid rafts and involved in a number of signaling pathways. It helps recruit cytoplasmic C-terminal Src kinase (Csk) to lipid raft-associated Src kinases, mediates a link to actin cytoskeleton and interacts with several other important cytoplasmic and plasma membrane-associated proteins. In recent years, PAG has been implicated in various aspects of cancer cell biology. Our review covers all so far published data on this interesting protein.

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INTRODUCTION

Many biologically important receptors, for example, lymphocyte antigen-specific receptors (T cell receptor (TCR); B cell receptor (BCR)), Fc-receptors or some cytokine receptors, are more or less complex assemblies of two types of transmembrane subunits: those responsible for specific ligand recognition and those mediating signal transduction (for example, ζ-chain of the TCR complex,[1] γ-chain of several Fc receptors,[2] and DAP12 or DAP10 chains of several NK-/myeloid cell activating receptors[3]). The signaling subunits usually contain very short extracellular parts, single transmembrane segments and intracellular domains with multiple tyrosine-based phosphorylation motifs. Phosphorylation of the tyrosine residues by associated protein tyrosine kinases enables other cytoplasmic proteins involved in receptor signaling to bind via their SH2 domains and initiate signaling cascades in response to receptor–ligand interaction.

In addition to receptor-associated signaling subunits, a number of similar proteins (called transmembrane adaptor proteins, TRAPs) exist, which are not directly associated with any receptor but still may be directly or indirectly involved in the regulation of receptor signaling. Some of these TRAPs are palmitoylated and thereby targeted to lipid rafts, which are membrane microdomains characterized by specific lipid and protein composition. Membrane microdomains are functionally implicated in receptor signaling and many other physiologically important phenomena.[4–6] Several recent reviews exist, dealing with TRAPs in general or specifically with some of them.[2,3,7–18]

In this review, we address one of the raft-associated TRAPs, named phosphoprotein associated with glycosphingolipid microdomains (PAG), also known as Csk-binding protein (Cbp). The official human gene symbol is *PAG1* (human gene ID: 55824). In the past decade, PAG has attracted much attention as a widely expressed multifunctional regulator of Src family kinases (SFKs) and has been linked to many cellular processes and malignant conditions. Within 5 years after the PAG literature was reviewed,[19] about 30 new research articles have been published, contributing significantly to our understanding of PAG cellular functions. Therefore, we provide a comprehensive overview of the published literature with the main focus on the new findings.

INITIAL CHARACTERIZATION AND BIOCHEMICAL PROPERTIES OF PAG

Discovery and cloning of PAG

Since the early 1990s, a heavily tyrosine phosphorylated protein (~80 kDa) was repeatedly observed as a major constituent of *in vitro*-labeled immunoprecipitates of detergent-resistant membrane microdomains (DRMs).[20–23] Concurrently, the same protein entity was found in a protein complex containing SFK Fyn.[24,25] Later, the identity of this phosphoprotein was simultaneously revealed by two groups. In our laboratory and in collaboration with Burkhart Schraven, we identified the phosphoprotein as a ubiquitously expressed TRAP and cloned the cDNA from a human B-cell line.[26] Owing to its particular membrane localization, we named it PAG. Kawabuchi *et al.* studied how Csk (C-terminal Src kinase) is recruited to the plasma membrane. They found the phosphoprotein to be strongly associated with Csk in detergent-solubilized membranes of the brain.[27] Thus, they termed it Csk-binding protein (Cbp).

Gene/protein structure/protein interaction motifs

PAG is very well conserved in evolution and found virtually in all vertebrate species with known genome sequence. The human PAG protein (432 aa) is composed of a 16 aa extracellular peptide, 20 aa transmembrane segment followed by a consensus palmitoylation motif CxxC and 393 aa cytoplasmic domain containing 10 tyrosine residues, which when phosphorylated might create binding sites for SH2 domains. In addition, the cytoplasmic domain contains also two proline-rich motifs for potential interaction with SH3 domains (a class I motif: [R/K/Y]xxPxxP, aa 131–137; and a class II motif: PxxPx[K/R], aa 263–258), and a class I PDZ domain-binding motif (VTRL) at the C terminus. The mouse ortholog, frequently mentioned in this review, is only 3 aa shorter and contains basically the same sequence elements (Figure 1).

PAG expression

PAG is a ubiquitous protein, but its expression is variable in different cell types. *PAG* mRNA (messenger RNA) was detected in

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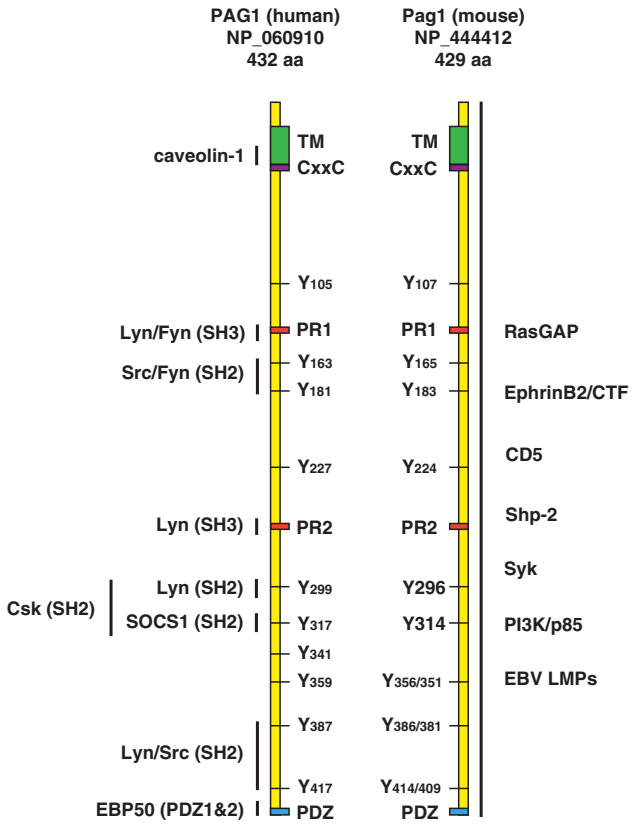

Figure 1. Schematic representation of human and mouse PAG proteins with protein interaction motifs. The known binding sites for interaction partners of PAG are indicated by vertical lines on the left side of the figure. The long vertical line on the right side represents interactions, which have not been mapped. TM (green), transmembrane part; PR1, PR2 (red), proline-rich motifs; CxxC (violet), palmitoylation motif; PDZ (blue), PDZ domain-binding motif; Y (with numbers), position of tyrosine residues in PAG amino acid sequence. The tyrosine residues in the rat PAG sequence have the same positions as in mouse PAG protein except for the last three tyrosine residues (indicated by the number with slash; mouse/rat).

almost all tissues with relatively highest expression in the immune system, lungs, heart and placenta.[26] PAG is a major phosphoprotein in resting T cells.[26] In T cells from old mice, *PAG* mRNA expression is reduced by 70%, correlating with decreased proliferative capacity of older T cells.[28] PAG is well expressed in primary B cells and B-cell lines.[29,30] Interestingly, in B-cell development, *PAG* mRNA and protein levels increase with acquisition of the maturation marker CD19 and then slightly decrease in mature peripheral B cells.[31] A remarkably high expression of PAG has been reported in human germinal centers.[30,32] In osteoclasts, PAG expression is downregulated by the RANKL/TRAF6/NFAT2 signaling pathway, and this blockade requires active Erk, JNK, p38 and NF-kB signaling.[33] In oligodendrocytes (a type of glia cells), the expression of PAG (and Csk) might be regulated by laminin.[34] Differential expression of PAG was observed in various types of cancer cells, as discussed in detail in the section 'PAG IN MALIGNANT CELLS'.

Biochemical properties of PAG

The theoretical molecular weight of PAG calculated from the amino acid composition (~48 kDa) obviously does not correspond to the observed sodium dodecyl sulfate–polyacrylamide gel electrophoresis mobility. In lymphoid cell detergent lysates, PAG migrates as a broad fuzzy band of ~80–85 kDa. This is probably because of the combined effects of heavy phosphorylation (*in vitro* dephosphorylated PAG ~68 kDa), lipid modifications (palmitoylation) and unusually high content of acidic amino acid residues (PAG pI = 4.2), which can cause anomalously low sodium dodecyl sulfate binding.[35]

PAG is palmitoylated presumably on its cytoplasmic membrane–proximal cysteine residue(s),[26] and this lipid modification is vital for PAG targeting into lipid rafts (or more precisely into raft-derived detergent-resistant membrane complexes, DRMs).[36] Interestingly, the association of PAG with DRMs is remarkably strong and preserved even in the presence of lipid raft-disrupting drugs MβCD or filipin.[29] In addition, PAG-containing membrane complexes can be isolated by affinity-based methods using a cholesterol-binding protein probe[37] or anti-ganglioside GD3 (R24).[38] In contrast to another raft-associated TRAP NTAL (non-T-cell activation linker) (also known as LAB or LAT2), PAG is not co-internalized with the B-cell receptor (BCR) but remains at the cell surface following BCR cross-linking. This, as well as biochemical characteristics of the PAG- or NTAL-containing detergent-resistant complexes, suggests that PAG molecules reside in raft microdomains, which are physically distinct from those containing BCR and NTAL.[39]

Phosphorylation of PAG

The cytoplasmic domain of PAG contains 10 tyrosine residues, nine of which fall into the phosphorylation motif preferred by SFKs. The tyrosine phosphorylation of PAG is dynamically regulated in response to engagement of specific plasma membrane receptors (for example, immunoreceptors, growth factor receptors and integrins). The peak phosphorylation in various cell types (with the exception of T cells where PAG is dephosphorylated upon TCR stimulation) is generally achieved within 5–15 min of cell stimulation, and then it slowly returns to basal levels.[39–42] In T cells, PAG is phosphorylated mainly by SFK Fyn.[43] A recent quantitative phosphoproteomic study revealed a negative TCR feedback loop originating from SLP-76 that regulates PAG phosphorylation at Tyr-181, 227, 341, 359 and 417.[44] In the SLP-76-deficient Jurkat cell line, basal phosphorylation of PAG is reduced. This might be perhaps because of an aberrant phosphorylation of PAG by Fyn or because of increased activity of PAG phosphatases.[44] In B cells, mast cells and erythroid cells, PAG seems to be phosphorylated mainly by SFK Lyn.[42,45,46] In other cell types, PAG is phosphorylated by one or more SFK members, as discussed in the following sections. Phosphorylation of PAG by SFKs and Csk recruitment to membrane ruffles can be monitored by live-cell FRET microscopy. It seems that PAG senses the activation status of SFKs specifically in lipid rafts, and thus could serve as a useful indicator of SFK activation in living cells.[47] Surprisingly, under specific *in vitro* conditions, PAG can serve as a substrate even for Csk.[48] In addition, the cytoplasmic domain of PAG also contains several putative serine and threonine phosphorylation motifs and therefore might serve as a substrate for protein kinases other than SFKs.

In contrast to PAG tyrosine phosphorylation, which is mediated only by one family of enzymes (SFKs), PAG dephosphorylation seems to be controlled by multiple phosphatases. Receptor-like protein tyrosine phosphatase (PTP) CD45 (PTPRC) has been identified as the main phosphatase of PAG in primary mouse T cells.[49] However, PAG can be dephosphorylated in CD45-deficient Jurkat T cells,[26] indicating that in this T-cell line other phosphatases substitute for CD45. The non-receptor PTP Shp-2 (PTPN11) can bind and dephosphorylate PAG in PAG-transfected HEK293 cells, and PAG is constitutively hyper-phosphorylated in Shp-2-deficient cells.[50] In addition, PTP-α (RTPRA) has been shown to regulate PAG phosphorylation indirectly via raft-resident Fyn. In PTP-α-deficient mouse thymocytes, Fyn is more active and

hyper-phosphorylates PAG, which in turn recruits more Csk (a negative regulator of SFKs) to the plasma membrane. As a result, the long-term proliferative response of PTP-α-deficient thymocytes (but not the lymph node T cells) is reduced.[51]

Interaction partners of PAG

Several potential protein interactions can be deduced from the amino acid sequence of PAG. Indeed, PAG has been shown to interact with a number of proteins in various experimental systems and different cellular contexts. All reported interaction partners of PAG are summarized in Table 1 and Figure 1, and the most important of them are discussed here.

In vitro, PAG binds recombinant SH2 domains of signaling proteins Lck, Fyn, Lyn, Csk, Shc, Vav, GAP, PI3K (phosphatidylinositol 3-kinase), ZAP-70 and Syk, and weakly also Grb2, SLP-76, SHP-1, SHP-2 and rasGAP.[26,52] *In vivo*, association with some of these molecules could be demonstrated by immunoprecipitation.

The association with Csk, a negative regulator of SFKs, requires human PAG phosphorylation at Tyr-317 (which corresponds to Tyr-314 in mouse PAG) in the consensus Csk SH2 domain-binding motif YxSV.[26,27] In addition, the region upstream of Tyr-317/Tyr-314 (encompassing Tyr-299/Tyr-296) is also recognized by Csk and presumably stabilizes the interaction of these two proteins *in vivo*.[53,54] Interestingly, binding to PAG not only localizes Csk to the plasma membrane but also potentiates Csk enzymatic activity most likely by an induced conformational change in the catalytic domain.[48] The complex of PAG and Csk is very stable and preserved even in 1.5 M NaCl *in vitro*.[35] Although the Csk affinity for PAG is high, the SOCS1 ubiquitin ligase can replace Csk during erythroid cell signaling to negatively regulate Lyn by ubiquitylation. For this interaction, both the SH2 domain of SOCS1 recognizing Tyr-314 of mouse PAG and the proline-rich region of SOCS1 seem to be important.[42]

SFKs were reported to bind PAG via cooperative interactions of SH3 and SH2 domains. The SH3 domain of Fyn recognizes the first proline-rich motif of PAG (RxLPxxP, aa 131–137; the class I SH3 ligand). Thereafter, the SH2 domain of Fyn binds PAG phosphorylated at tyrosine residues Tyr-163 or Tyr-181. This dual-domain docking renders Fyn less sensitive to Csk-mediated negative regulation.[55,56] In TCR-stimulated T cells, the PAG–Fyn–Csk membrane complex rapidly disassembles, with Fyn dissociating first.[57] Binding preferences of Lyn SH3 and SH2 domains for PAG seem to be cell-type specific. In erythroid cells, the SH3 domain of Lyn prefers the second PAG proline-rich motif (aa 255–260 in rat PAG), and its SH2 domain binds phosphorylated Tyr-381 or Tyr-409 (rat PAG).[35,49] On the other hand, in transformed B cells, Lyn SH3 domain shows strong preference for the first proline-rich motif of PAG (which is also indispensable for PAG phosphorylation by Lyn *in vitro*) and Lyn SH2 domain for PAG phosphorylated at Tyr-299.[58,59] Finally, the SH2 domain of Src has been shown to bind rat PAG overexpressed in mouse fibroblasts, which was phosphorylated on Tyr-165/183 or Tyr-381/409.[60] It seems likely that PAG also binds all other SFKs in a similar way.[52,53] In the plasma membrane, PAG is localized almost exclusively to the raft membrane microdomains owing to its palmitoylation, as mentioned above. Furthermore, PAG also associates with a subtype of membrane rafts, caveolae, via a constitutive interaction (mediated by motif FVITFLIF in the PAG transmembrane segment) with caveolin-1.[41]

CELLULAR FUNCTIONS OF PAG

PAG as an adaptor for Csk

The two initial studies describing PAG at the molecular level[26,27] defined PAG as an adaptor protein recruiting the cytoplasmic tyrosine kinase Csk, the major negative regulator of SFKs, to plasma membrane lipid rafts (Figure 2). Moreover, it has been proposed that the negative regulatory role of the PAG–Csk complex may be further enhanced by phosphatases PEP or PTP-PEST.[61] The hematopoietic-specific phosphatase PEP (or its human ortholog LYP; PTPN22) and ubiquitous PTP-PEST (PTPN12) were reported to associate constitutively with the SH3 domain of Csk.[62–64] Therefore, they might negatively regulate SFK activity coordinately with Csk. Although Csk phosphorylates the negative regulatory tyrosine of SFKs, PEP/PTP-PEST would at the same time dephosphorylate their positive regulatory tyrosine. As a result, SFKs would acquire fully closed conformation and remain enzymatically inactive. However, there is a surprising lack of experimental evidence for the formation of such functional inhibitory PAG–Csk–PEP/PTP-PEST module *in vivo*. Moreover, the recent report indicates that, under resting conditions, the PAG-bound Csk and LYP are rather uncoupled in human T cells.[65]

The beauty of the model is further underlined by the inducible manner of PAG–Csk interaction, which creates a genuine negative feedback loop in SFK signaling.[27] Following a signaling event, SFKs are activated and they phosphorylate their target proteins including PAG. Phosphorylated PAG then recruits Csk to plasma membrane lipid rafts to the vicinity of active SFKs. Once the SFKs

Table 1. Reported PAG protein interactions

Protein	*Interaction motifs*	*Cell types*	*Methods*
Csk[34,44]	pY317 and pY296 of PAG (rat); SH2 domain of Csk	A431 (epidermoid cell line)	Co-IP
Fyn[56]	1st proline-rich motif + pY163/181 of PAG; SH3 and SH2 domains of Fyn	T cells	Co-IP
Src[60]	pY165/Y183 or pY381/Y409 of PAG (rat); SH2 domain of Src	Fibroblasts	Co-IP
Caveolin-1[41]	FVITFLIF motif in TM domain of PAG; TM domain of caveolin-1	A431 cells	Co-IP
Lyn[42,58]	Both pro-rich motifs + pY381/409 of PAG; SH3 and SH2 domain of Lyn	Erythroblasts, B cells	Y2H
Co-IP			
SOCS1[42]	pY314 of PAG; SH2 domain + Pro-rich motif of SOCS1	J2E (erythroid cell line)	Co-IP
RasGAP[74]		T cells	Co-IP
SFKs[101]		Fibroblasts	Co-IP
EphrinB2/CTF2[90]		Neurons	Co-IP
CD5[72]		T cells	Co-IP
Shp-2[50]	Phospho-PAG	HEK293	Co-IP
Syk[109]	Phospho-PAG	B-NHL lymphoma	Co-IP
P85 PI3-K[109]	Phospho-PAG	B-NHL lymphoma	Co-IP
EBV LMPs[109]	Phospho-PAG	Raji (B cell line)	Co-IP
EBP50[92,93]	PDZ-binding motif of PAG, both PDZ domains of EBP50	T cells, B cells	Y2H Co-IP

Abbreviations: B-NHL, non-Hodgkin B lymphoma; Co-IP, complex immunoprecipitation; EBV, Epstein–Barr virus; LMPs, latent membrane proteins; PAG, Phosphoprotein associated with glycosphingolipid-enriched microdomains; PI3, phosphatidylinositol 3-kinase; SFKs, Src family kinases; TM, transmembrane.

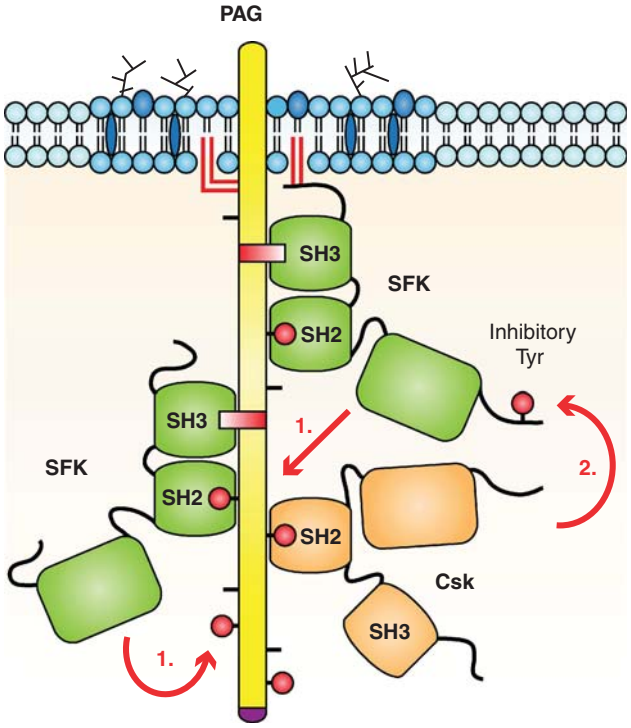

Figure 2. Model of negative regulation loop in SFK signaling mediated by PAG–Csk. PAG present in lipid rafts can associate with and become phosphorylated by SFKs (1). Phosphorylation of PAG creates docking sites for SH2 domains of SFKs and their negative regulator Csk. Csk bound to PAG then phosphorylates the inhibitory tyrosine residue at the C termini of SFKs (2) and inactivates them.

are inactivated by the action of Csk (and possibly PEP/PTP-PEST), phosphorylation of PAG returns to the basal level and Csk dissociates from the membrane. The real model must be, of course, much more complex because of the existence of multiple PTPs, which dephosphorylate both PAG and SFKs (in the latter case the PTP effect might be either activating or inhibitory). The details of these SFK regulatory circuits are beyond the scope of this review and are discussed elsewhere.[66,67]

PAG–Csk module in immunoreceptor signaling

TCR signaling. The basic model described above is applicable in various cell types, except in T lymphocytes. In resting T cells, phosphorylated PAG together with associated Csk is present in lipid rafts and might help to set an activation threshold for the initiation of TCR signaling. The basal phosphorylation of PAG in T cells is likely maintained by the lipid raft-resident SFK Fyn.[43] TCR engagement in primary human T cells rapidly decreases PAG phosphorylation and its association with Csk. This suggests that transient release from PAG–Csk-mediated inhibition by displacement of Csk from PAG-containing rafts may be involved in the initiation of T-cell activation.[26,68] Csk dissociated from PAG during T-cell stimulation is sequestered in cytosol away from the immunological synapse by the adaptor protein G3BP. Functionally, G3BP overexpression augments and G3BP knockdown inhibits IL-2 production by T cells.[69]

A number of overexpression studies in primary T cells and T-cell lines repeatedly validated that the net effect of PAG overexpression on TCR signaling is inhibitory. For example, overexpression of PAG in Jurkat T cells clearly decreases the activity of the NF-AT promoter after TCR stimulation.[26] Overexpressed PAG (but not PAG with mutated PDZ-binding motif) in Jurkat cells also prevents the formation of a functional immunological synapse and inhibits IL-2 production.[70] In agreement with this, T cells from transgenic mice expressing increased amounts of PAG show inhibited responses to TCR stimulation, and the mutant of PAG unable to bind Csk has a strong dominant-negative effect. As demonstrated by the experiments in which PAG was co-expressed with constitutively active Fyn, PAG inhibitory effects in T cells are solely because of the downregulation of SFK activity in lipid rafts.[49] However, PAG inhibitory effects are not limited to TCR signaling but appear to be relevant also to other signaling processes in which SFKs are involved. For instance, antibody-mediated cross-linking of the CD4 co-receptor results in the inhibition of TCR-induced tyrosine phosphorylation of downstream substrates most likely owing to a markedly increased activity of Csk associated with PAG.[71] Similarly, inhibition of TCR signaling upon ligation of the inhibitory receptor CD5 depends in part on relocation of CD5 dimers into lipid rafts, which may interfere with dissociation of Csk from PAG and activation of Fyn.[72]

Strong TCR activation in the absence of proper co-stimulation results in T-cell unresponsiveness or T-cell anergy.[73] In anergic T cells, the interaction of PAG with Fyn is augmented and ectopic expression of the PAG tyrosine mutant, which binds Fyn but is unable to bind Csk (Tyr-314 mutated to Phe), leads to strengthening of calcium response and anergy induction.[57] Moreover, the anergy-promoting protein complex composed of hyper-phosphorylated PAG, Fyn, a multifunctional adaptor Sam68 (KHDRBS1) and RasGAP (RASA1) is formed in activated T cells. This model is further supported by the observation that overexpression of PAG and Fyn in Jurkat T cells suppresses Ras activation. Knockdown of PAG, in contrast, augments Ras activation. Thus, PAG might negatively regulate not only SFKs but also Ras.[74]

The association of PAG with Csk in T cells is regulated also via cAMP. Prostaglandin E2 treatment of T cells elevates cAMP levels and inhibits T-cell activation. Under such conditions, PAG phosphorylation and Csk recruitment to lipid rafts are increased and the PAG–Csk complex is stabilized.[75] Moreover, the activity of Csk already potentiated by binding to raft-associated PAG is further increased (2–4 fold) upon phosphorylation of Ser-364 by kinase protein kinase A type I, which is activated by cAMP. These interactions are apparently facilitated by a raft-associated supramolecular signaling complex consisting of protein kinase A type I, EBP50, PAG and Csk, which contributes to the TCR signaling inhibition mediated by cAMP.[76–78]

In apparent contrast to PAG overexpression studies in T cells stand unaffected phenotypes of PAG-deficient mice. The first indication that PAG might be indeed dispensable for Csk membrane targeting in T cells came from the analysis of the immune system of mice after a transfer of bone-marrow cells overexpressing dominant-negative PAG mutant.[49] Later, at least three research groups independently generated and studied PAG-deficient mice.[79–81] Neither mice with constitutively abolished PAG[79] nor mice with PAG deleted conditionally in CD4-positive T cells[80] showed any developmental defects. Moreover, except for slightly increased numbers of thymocytes in the conventional knockout, the T- and B-cell development, T-cell responses (TCR-proximal signaling, proliferation, cytokine production), humoral immune responses and B- or T-cell tolerance after a superantigen (staphylococcal enterotoxin B) challenge were normal in PAG-deficient mice. Interestingly, although the first study demonstrated that Csk is excluded from lipid raft fractions in resting thymocytes in PAG-deficient mice,[79] the second study did not find any difference in Csk compartmentalization to lipid rafts in thymocytes.[80]

BCR signaling. In contrast to T cells, the tyrosine phosphorylation of PAG is markedly increased (not decreased) after BCR stimulation.[29] This finding may indicate a different role of PAG in BCR signaling as compared with TCR signaling. In B cells, SFKs (especially Lyn) might phosphorylate mainly the negative signaling regulators (for example, Siglecs); thus, increased PAG

phosphorylation leading to increased Csk activity may decrease these inhibitory influences and support BCR signaling (as may be the case also in aged T cells discussed above). Two main SFKs are involved in BCR signaling: Fyn and Lyn.[82] Lyn deficiency results in the hyper-responsive phenotype of B cells, which might be partially explained by decreased PAG phosphorylation, less PAG–Csk membrane complexes and increased PI3K activity. In such mutant B cells, Fyn activity is increased.[45] However, why increased levels of Fyn are not able to phosphorylate PAG under such circumstances is currently unknown; one may speculate that Fyn-containing lipid rafts may not interact well with the PAG-rich ones. An additional recent study points to the rather negative regulatory role for PAG in BCR-triggered activation of B lymphocytes and excludes the possibility that PAG maintains tonic inhibition in BCR-proximal signaling.[58]

FcεRI signaling. In mast cells, PAG regulates immunoreceptor signaling by a mechanism more similar to the situation in B cells. Instead of setting an activation threshold like in T cells, PAG in mast cells probably acts as a genuine feedback-loop inhibitor. Shortly after FcεRI triggering, SFKs (mainly Lyn) hyper-phosphorylate PAG, which results in recruitment of Csk to lipid rafts and inactivation of SFKs.[46] PAG overexpression in rat basophil cells effectively impairs cell activation and degranulation induced by FcεRI.[83] Mast cells derived from ASK mutant mice, which are epilepsy-resistant variants of EL mice, a widely used model of autism and epilepsy, show a phenotype similar to those from Lyn-deficient mice: enhanced proliferation and production of TNF-α and IL-2, presumably owing to the lack of the Lyn-dependent negative regulatory loop. In these cells, PAG is hypo-phosphorylated and recruits less Csk to lipid rafts, resulting in hyperactive Fyn and Src.[84] In contrast to ASK-derived mast cells, mast cells from Hck-deficient mice upregulate Lyn activity, which results in increased PAG phosphorylation and inhibition of signaling events evoked by FcεRI.[85]

PAG–Csk module in growth factor signaling. Given the previous findings that PAG and Csk form an SFK inhibitory unit, PAG has been extensively studied also in fibroblasts; the cells are often used as a cellular model of growth factor signaling, migration and transformation. In fibroblasts, PAG remains hypo-phosphorylated until the cells adhere to the extracellular matrix (for example, fibronectin),[40] or until they are treated with growth factors EGF or PDGF.[41,47] Expression of PAG mutant that is unable to bind Csk (a dominant-negative mutant) or PAG knockdown leads to impaired recruitment of Csk to lipid rafts. Overexpression of PAG in fibroblast cell lines can suppress EGF-induced Erk and Akt phosphorylation, cell transformation and colony formation in soft agar.[41] Dephosphorylation of PAG by PTP Shp-2 is required for normal growth factor-evoked SFK activation. In growth factor signaling, Shp-2 opposes the action of SFKs by binding and dephosphorylation of PAG. In Shp-2-deficient fibroblasts, PAG is hyper-phosphorylated and the activity of SFKs is upregulated because of defective recruitment of Csk to the plasma membrane.[50] Phosphorylation of PAG might be influenced also by RPTP-α, but in contrast to Shp-2, only indirectly via enhanced Fyn activity. The receptor phosphatase RPTP-α positively regulates SFKs in fibroblasts by dephosphorylation of their C-terminal regulatory tyrosine (opposing the action of Csk). A fraction of RPTP-α is present in lipid rafts where it efficiently regulates SFK activity.[86]

PAG–Csk module in integrin signaling. Integrin activation at focal adhesions controls fibroblast adhesion to the extracellular matrix and prevents cell migration. Recently, a direct involvement of PAG in inside-out β1 integrin signaling via dioxin receptor/aryl hydrocarbon receptor (AhR) has been described.[87] In its promoter region, PAG contains xenobiotic responsive element for binding of active transcriptional repressor AhR. Fibroblasts derived from AhR-null mice show increased β1 integrin activity and impaired migration presumably because of upregulated PAG expression. Increased levels of PAG target more Csk to the plasma membrane and negatively regulate the activity of Src and Src targets focal adhesion kinase and caveolin. This finding might have implications in transformed fibroblasts as an additional mechanism of SFK deregulation.

PAG in erythropoietin (Epo) signaling in differentiation of erythroid cells. Differentiation of erythroid cells is induced upon treatment with Epo. PAG influences this process by a two-step regulation of SFK Lyn. In mouse erythroblasts, Epo stimulation induces PAG phosphorylation via the pre-associated Lyn. PAG phosphorylation results in the recruitment of Ctk (MATK), the hematopoietic homolog of Csk, and feedback inhibition of Lyn kinase activity. Moreover, another negative regulator of Lyn expressed after Epo stimulation, SOCS1, later competes with Ctk for PAG binding. SOCS1 is involved in ubiquitylation and proteasomal degradation of many signaling molecules including Lyn. The J2E erythroid cells overexpressing PAG have significantly reduced colony-forming ability, Epo-induced hemoglobin synthesis (cell differentiation) and phosphorylation of several signaling proteins.[35,82]

PAG–Csk in angiotensin II signaling in podocytes. In podocytes, specialized kidney epithelial cells involved in the first step of renal blood filtration, PAG might be one of the key proteins in angiotensin II-induced cell damage. Nephrin, the major structural protein of the slit diaphragm between podocytes, has also signaling and anti-apoptotic functions regulated by Fyn phosphorylation. Angiotensin II-treated podocytes tend to overproduce Csk, which readily forms a plasma membrane complex with hyper-phosphorylated PAG and inhibits Fyn functions. Indeed, following angiotensin II treatment, phosphorylation of nephrin is decreased and thus its anti-apoptotic functions are substantially abolished.[88]

PAG–Csk in bone resorption. Effective osteoclastic bone resorption requires high activity of Src, and thus mice deficient in Src develop severe osteopetrosis.[89] As PAG recruits Csk to lipid rafts and interferes with Src activity, osteoclasts specifically downregulate PAG expression by the RANKL signaling pathway. As expected, ectopic expression of PAG in osteoclasts inhibits Src activation and prevents bone resorption.[33]

PAG and regulation of SFK activities in the brain. Although PAG phosphorylated by SFK Fyn was discovered as a Csk-binding protein expressed in the brain,[27,40] the functional role of PAG in the nervous system remained unasked for almost a decade. Currently, several lines of evidence suggest a putative role for PAG in Csk-dependent negative regulation of SFKs in various types of brain cells and their developmental processes. For instance, the PAG–Lyn complex is specifically enriched in the growth cone fraction of developing neurons and might be of functional importance.[38] The analysis of a neonatal nervous system in PAG-deficient mice revealed decreased Csk lipid-raft recruitment and increased Fyn and Src activities, but the brain developed normally.[81] Remarkably, no such differences were found in adult brains, indicating that other mechanisms of SFK regulation may compensate for PAG deficiency during brain development. This conclusion evidently holds good more generally to explain the lack of a clear phenotype in any cell type in the PAG-deficient mice.

In rat neurons, PAG is also involved in ephrinB2 signaling as a negative regulator of Src. Upon EphB2 receptor binding, ephrinB2 ligand is cleaved by the γ-secretase proteolytic complex. After the cleavage, the released fragment (ephrinB2/CTF2) associates with PAG and promotes PAG dephosphorylation, Csk release and Src activation. Active Src then phosphorylates ephrinB2 ligand.

Moreover, PAG downmodulation by siRNA results in impairment of EphB2-induced ephrinB2 ligand phosphorylation.[90] This PAG function could be potentially relevant to human neurodegenerative disorders.

Oligodendrocytes from the laminin-deficient mice express double amount of PAG and Csk, the negative regulators of Fyn. Laminin is an important structural component of the basal lamina. In natural laminin α2 mutant mice (dy/dy mice), the development of oligodendrocytes is significantly delayed presumably owing to suppressed Fyn activity. This suggests that laminin might regulate PAG and Csk expression and thus Fyn kinase activity.[34]

Negative regulation of SFKs by PAG independent of Csk binding

The inhibitory effect of PAG on SFK signaling might not be mediated solely by the PAG-associated Csk. Clearly, PAG unique localization to plasma membrane microdomains alone and interaction with raft molecules might also come into play, at least after PAG overexpression. In mouse embryonic fibroblasts, ectopic expression of PAG strongly inhibits PDGFR-Src mitogenic signaling independently of Csk. The N-terminal part of PAG (aa 1–97) somehow (probably via the interactions with sialidase Neu3 and ganglioside GM1) excludes PDGFR from caveolae, which are indispensable for PDGFR signal propagation. These effects are dependent on the intact caveolin-1-binding site within the transmembrane domain of PAG.[91]

PAG links lipid rafts to the cytoskeleton

In addition to SH2- and SH3-binding motifs, PAG contains a C-terminal PDZ-binding motif, which can be recognized by both PDZ domains of the scaffolding protein EBP50/NHE-RF (SLC9A3R1).[82,92] Via this interaction, PAG becomes a component of the EBP50–ezrin complex, and thus may anchor lipid rafts to the cortical cytoskeleton (Figure 3). *In vivo*, however, only a minor fraction of EBP50 is bound to PAG.[93] This PAG link to the cytoskeleton is reversible. Upon T-cell stimulation, the association of PAG and EBP50 is lost and the presence of EBP50 in the DRM fraction is decreased.[70] Also in B cells, ezrin seems to be recruited to lipid rafts via phosphorylated PAG, and this interaction is transiently lost after BCR stimulation.[94]

A consequence of such interactions might be a PAG-mediated regulation of lipid raft dynamics, which could be important for the initiation of immunoreceptor signaling. Indeed, overexpressed PAG markedly decreases the surface mobility of the lipid-raft glycolipid marker GM1 and prevents the formation of a functional immunological synapse between Jurkat T cells and Raji B cells in an EBP50-dependent manner.[70] In line with this observation, the palmitoylation-defective (raft-displaced) PAG mutant (CxxC motif mutated to AxxA) is unable to inhibit proximal TCR signaling, in spite of intact plasma membrane localization, phosphorylation and formation of the PAG–Fyn–Csk–EBP50 complex.[36] Consistently with all PAG overexpression studies in T cells, it seems plausible that PAG exerts its negative regulatory roles on TCR signaling by a combined action of Csk targeting and modulation of raft dynamics (aggregation).

Another reason for the PAG–EBP50 interaction might be targeting of protein kinase A type I to the vicinity of the PAG–Csk complex. The ezrin-bound protein kinase A type I is activated by cAMP and negatively regulates TCR signaling, probably via phosphorylation of Csk at Ser-364, which stimulates Csk activity.[95]

Among important components of lipid rafts are the GPI-anchored proteins.[96] Although devoid of any cytoplasmic domains, these proteins are able to transmit signals inside the cell upon surface antibody cross-linking.[96,97] Evidently, this is because of their indirect associations with some raft-associated transmembrane proteins, possibly including TRAPs. When analyzed by single-molecule tracking techniques, membrane trajectories of the GPI-bound proteins seem to stop for milliseconds in a process termed 'transient anchorage', which indicates short-lived interactions with the cytoskeleton. Interestingly, PAG has been shown to interact with Thy1 (an abundant GPI-anchored protein of rodent T cells) at the plasma membrane[52] and is important for transient anchorage of cross-linked Thy1 clusters.[98]

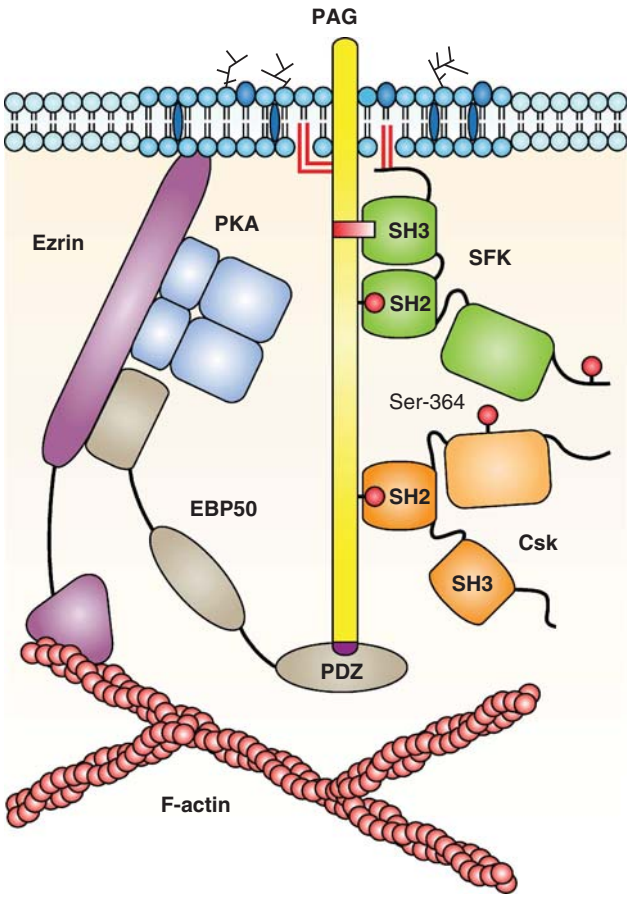

Figure 3. PAG links lipid rafts to the actin cytoskeleton. Lipid raft-resident PAG can associate via its C-terminal PDZ-binding motif with the PDZ domains of scaffold protein EBP50. EBP50 is further linked to actin-binding protein ezrin. Interestingly, PKA type I is also present in the complex and thus can phosphorylate Csk (Ser-364) to potentiate its enzymatic activity toward SFKs.

PAG IN MALIGNANT CELLS

The role of PAG in regulation of SFKs implicates its possible involvement in cell transformation and pathogenesis of human malignant diseases. In various cellular contexts, PAG expression is either downregulated or upregulated, which might reflect two different strategies used by deregulated tumor cells. The first strategy (PAG downregulation) seems to be more intuitive, as PAG has been repeatedly validated as a negative regulator of SFKs. Thus, removal of this negative regulation might potentially lead to hyper-activation of SFKs and cell transformation (Figure 4). The other strategy (PAG upregulation), instead, could be related to the fact that in some cell types SFKs may be more important in activation of negative regulators of cell proliferation. Alternatively, PAG, uncoupled from Csk, may gain novel positive signaling functions (Figure 5). Although PAG downregulation can be explained by epigenetic histone modifications[99] and transcriptional repression by dioxin receptor AhR,[87] the mechanism of PAG upregulation remains elusive.

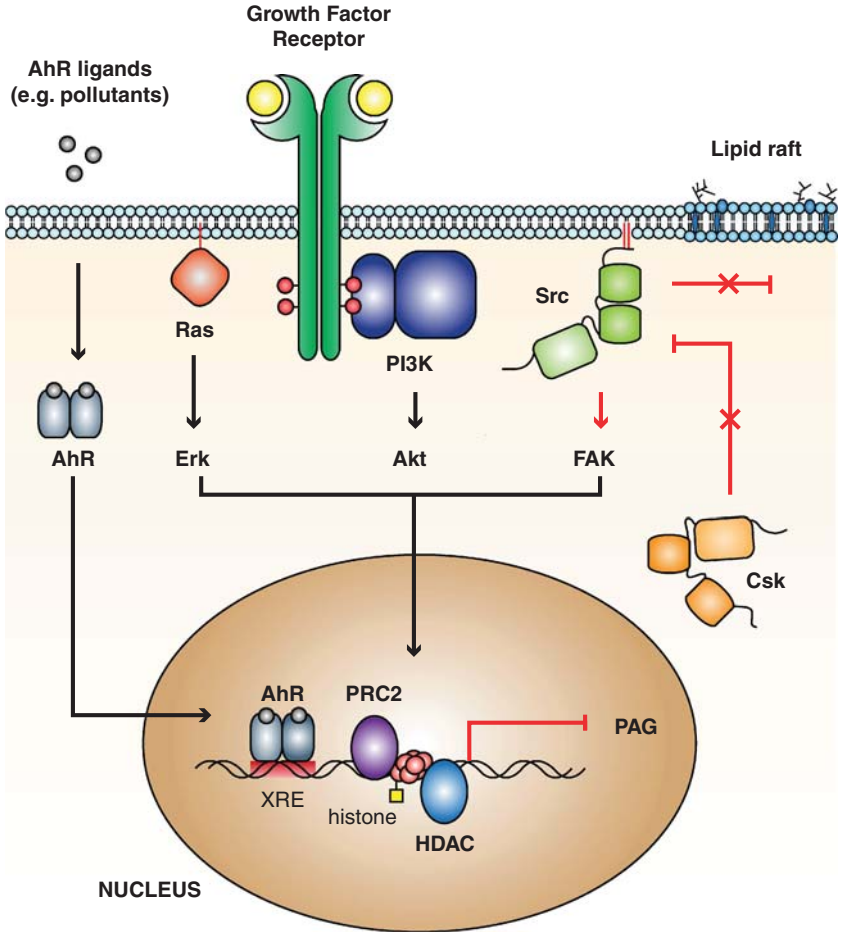

Figure 4. Mechanisms and consequences of PAG downmodulation in cancer cells. In some types of cancer, PAG expression is downmodulated by growth factor oncogenic signaling, which results in histone modifications (by PRC2 and HDAC enzymes) in *PAG* promoter and suppression of *PAG* transcription. In addition, various AhR ligands from the environment can activate AhR, which binds to xenobiotic responsive element in *PAG* promoter and functions as *PAG* transcription repressor. The lack of PAG leads to upregulated SFKs oncogenic activities, because they cannot be suppressed by PAG-associated Csk or sequestered to lipid rafts by PAG itself.

Cancer cells with downregulated PAG

The oncogenic EGFR, Src and Ras signaling exceedingly stimulates PI3K/AKT and Erk/p38 MAP kinase pathways. This eventually leads to specific deacetylation and trimethylation of histones in the PAG promoter by HDAC1 and PRC2 complexes. Such modifications suppress PAG mRNA synthesis, creating a positive-feedback loop in oncogenic signaling (Figure 4).[99] PAG expression might be repressed also directly by transcription factor AhR.[87] Binding of ligands to AhR (various environmental pollutants) leads to AhR dimerization, nuclear translocation and binding to promoter regions of target genes (xenobiotic responsive element in the case of PAG). It is tempting to speculate that this link of environmental factors to PAG expression might contribute to cell transformation and cancerogenesis (Figure 4).

Uncontrolled Src activity in fibroblasts can result in transformation and tumorigenesis, that is, changed cell morphology and anchorage-independent growth. These effects are even more pronounced in Csk-deficient fibroblasts. In such cells, PAG is constitutively hyper-phosphorylated, and reexpression of Csk leads to PAG dephosphorylation. In addition, spreading and migration is reduced in Csk-deficient cells, and siRNA-mediated knockdown of PAG enhanced cell spreading. Therefore, PAG might function as a sensor of SFK activity during fibroblast adhesion and mediate SFK feedback inhibition by recruiting Csk to lipid rafts.[40] In Csk-deficient fibroblasts, endogenous Src alone is unable to induce transformation owing to existence of other control mechanisms. Nevertheless, exogenously increased levels of Src easily overcome this block. In such Src-transformed fibroblasts, PAG expression is strongly downmodulated presumably by the epigenetic mechanisms. Surprisingly, PAG reexpression, even in the absence of Csk, can prevent Src-induced transformation.[60] This points to an important aspect of PAG-mediated control of SFK signaling independent of Csk. Src, itself being a non-raft SFK, can only induce transformation if it phosphorylates other non-raft proteins (for example, integrin-associated signalosomes in focal contacts). Thus, if sequestered into lipid rafts away from its substrates by phosphorylated PAG, Src oncogenic potential is disabled.[60,100]

The oncogenic potential of all SFKs apparently depends on the membrane microenvironment. Fyn and Yes, the raft-associated SFKs (recovered in DRMs), cannot transform Csk-deficient fibroblasts, whereas other SFKs, distributed (Src and Blk exclusively) also in non-raft fractions, can. The raft-localized PAG has the potential to associate with all active SFKs, and thus it generally serves as a suppressor of SFK-mediated cell transformation. Interestingly, LIME, another raft-localized TRAP, which can bind both SFK and Csk, cannot prevent SFK-induced cell transformation, suggesting a unique role of PAG in SFK membrane homeostasis and suppression of their transforming potential.[101]

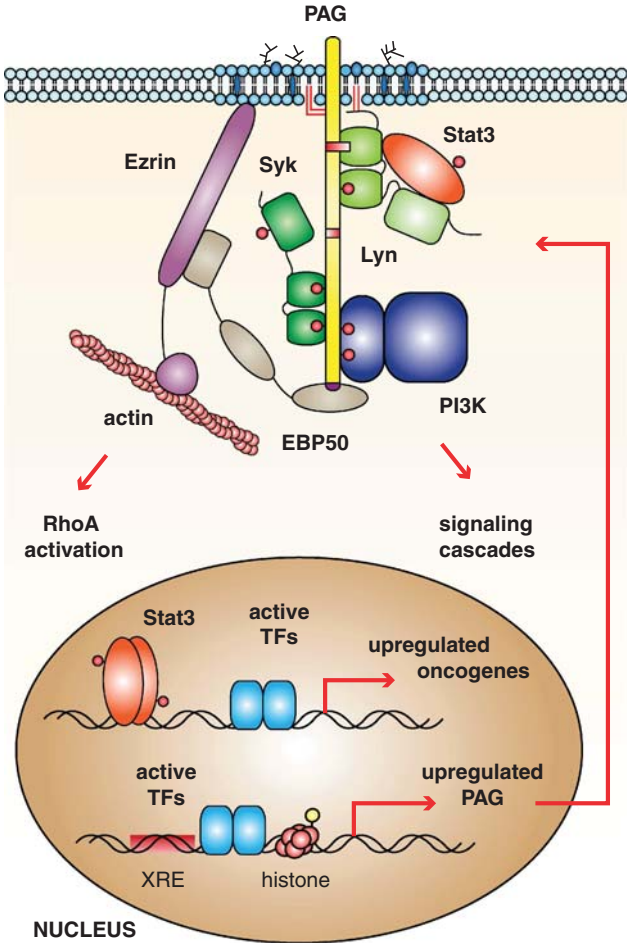

Figure 5. Mechanisms and consequences of PAG upregulation in cancer cells. In some cancer cells, PAG is upregulated by unknown mechanisms. At the plasma membrane, PAG organizes a lipid raft-resident oncogenic signalosome variably composed of Lyn, Stat3, Syk and PI3K. The signalosome activates multiple signaling cascades and stimulates transcription of other oncogenes. The Lyn-associated transcription factor Stat3 is activated by phosphorylation and contributes to cell transformation. The link to actin cytoskeleton via associated EBP50 might contribute to RhoA activation and enhanced cellular invasiveness.

In other transformed cell types, however, Csk-dependent regulation of SFK activity by PAG seems to prevail. In colorectal cancer cell lines, Csk mislocalization has been proposed as a novel mechanism of Src-driven oncogenesis. Targeting Csk to lipid rafts by lipid modifications specifically inhibits cellular invasiveness, but the cell growth may remain unaffected. In various colorectal cell lines and primary tumor biopsies,[60,102] the expression of PAG is reduced and reexpression of PAG increases Csk membrane localization with concomitant reduction of cellular invasiveness, functionally mimicking raft-targeted Csk. Importantly, these effects are lessened by the Csk inhibitor ASN2324598 or Csk knockdown, arguing for the crucial role of the PAG–Csk complex in limiting SFK oncogenicity.[102]

One can speculate that the combination of both PAG inhibitory mechanisms (Csk-dependent and independent) might apply in human non-small cell lung cancer cells, where PAG expression is downregulated and Src activity is upregulated. Ectopic expression of PAG in non-small cell lung cancer cells recruits both Csk and Src into lipid rafts and suppresses Src kinase activity and the ability of these cells to invade *in vitro* and metastasize *in vivo*.[103] Downmodulation of *PAG* mRNA and protein levels have been reported also in esophageal carcinoma samples.[104]

Insights into the molecular aspects of bovine B lymphocyte transformation by *Theileria parva* infection might provide a useful model of leukemia-like phenotype induction accompanied by PAG downregulation. The B cells transformed by this protozoan parasite markedly downregulate PAG expression, causing Csk displacement from SFK-rich lipid rafts, and thereby increased activity of SFKs (namely Hck) excessively stimulating the AP1 signaling pathway.[105] A similar mechanism based on decreased PAG expression might contribute to pathogenesis in human mantle cell lymphoma.[106]

Cancer cells with upregulated PAG

In contrast to transformed mouse fibroblasts and human colorectal and lung cancers, various human lymphomas rather overexpress than downmodulate PAG (Figure 5). Several extensive immunohistochemical studies of malignant human lymphomas revealed that the expression of PAG is strongly increased in most germinal center-like diffuse large B-cell lymphomas but is almost absent in mantle cell lymphomas and chronic lymphocytic leukemias. Thus, PAG might serve as a positive marker of germinal centers of secondary lymphatic follicles and follicular malignant lymphomas and negative marker of mantle cell lymphomas.[79,80,104] In the TEL/AML1 subgroup ($CD19^+$, $CD10^+$) of childhood B-cell precursors of acute lymphoblastic leukemias, PAG expression on both mRNA and protein levels is significantly higher than in other subgroups of acute lymphoblastic leukemias or non-malignant controls.[31] In addition, PAG is overexpressed in most human renal cell carcinomas (more than 70% of cases) and human renal cell carcinomas cell lines.[107] In laryngeal carcinoma cells, PAG is differentially upregulated in a subtype resistant to radiotherapy, and overexpression of PAG in originally radiosensitive cells renders the cells resistant to radiotherapy.[108]

The increased expression of the negative regulator PAG by some transformed cells might be explained by the formation of raft-resident PAG-based oncogenic signalosome (Figure 5).[59] In such a configuration, Csk is apparently somehow uncoupled from PAG, and permanently phosphorylated PAG acquires novel positive signaling functions. Interestingly, different lymphoma cell lines contain distinct PAG-based lipid raft signaling complexes containing invariably phosphorylated PAG, Lyn and Lyn-bound STAT3, and variably Syk and PI3K.[109] In follicular lymphoma cell line (DoHH2), maximally activated PI3K and Syk generate strong anti-apoptotic signals. The complex in mantle-cell lymphoma cell line (Jeko-1) contains only weakly phosphorylated PI3K. Finally, in Burkitt-derived lymphoma cell line (Raji), only Syk is bound to PAG. In these cells, the latent membrane proteins encoded by the transforming Epstein–Barr virus may associate with PAG and prevent PI3K from binding. Under such conditions, Syk can be activated directly by the Lyn present in the same complex, independently of the signals emanating from BCR. Functionally, disruption of the PAG–Lyn–STAT3 oncogenic complex in non-Hodgkin B lymphoma cells by inhibition of Lyn activity or PAG knockdown results in decreased cell survival.[59] Similarly, downmodulation of PAG in renal cell carcinomas cell lines can effectively suppress proliferation, cell motility and invasiveness *in vitro* and in model nude mice.[107] In addition, in agreement with the previous findings, knockdown of PAG in radio-resistant laryngeal carcinoma cells is able to decrease their resistance.[108]

Lipid raft composition seems to be important in the oncogenic process. The PAG–Lyn complex is not formed in anaplastic lymphoma kinase-positive lymphomas, and Lyn is less active than in non-Hodgkin B lymphoma cells. It seems that the membrane sphingolipids abundantly present in the lipid rafts from anaplastic lymphoma kinase-positive lymphomas interfere with PAG–Lyn complex formation and oncogenic signaling.[110] This finding might

be relevant to therapeutic effects of Rituximab, an antibody generated against the tetraspanin protein CD20. Aggregation of CD20 by the antibody leads to stabilization of sphingolipid-enriched membrane microdomains and inhibition of oncogenic Lyn kinase activity. Strikingly, only the lymphoma cell lines that express high levels of PAG are sensitive to Rituximab effects.[111] This suggests that the formation of oncogenic PAG–Lyn complex is compatible only with moderate amounts of membrane sphingolipids and with specific membrane environment.

Finally, PAG oncogenic effect might be partially dependent on its link to the cortical actin cytoskeleton via PDZ–EBP50–ezrin interaction. Overexpression of PAG with intact PDZ-binding motif in renal carcinoma cells leads to a significant increase of RhoA activity and enhanced cell migration. PAG depletion (or overexpression of a PAG mutant defective in its PDZ-binding motif), on the other hand, can inhibit RhoA activation and drastically alter cell morphology.[107]

Possible clinical implications

The findings on differential PAG expression in various types of lymphoma may be of diagnostic value.[30,32] A remarkable study demonstrating that the PAG–SOCS1 fusion construct can degrade active Lyn may point to possible usefulness of such constructs in gene therapy of SFK-dependent malignancies.[112] The PAG–Lyn oncogenic signalosome may be viewed more generally as a potential therapeutic target for therapy of lymphomas.

CONCLUSIONS AND OUTSTANDING QUESTIONS

The results obtained during the past years of studies on the transmembrane adaptor protein PAG (alias PAG1, Csk-binding protein) clearly indicate that its roles are not limited to regulation of Csk activity. Although the importance of PAG in a number of Csk-dependent and -independent regulatory pathways has been clearly demonstrated in many *in vitro* and *in vivo* experimental systems, the crucial test of its *in vivo* essentiality based on examination of PAG-deficient mice surprisingly demonstrated that PAG is actually dispensable.

The ample evidence for an important role of PAG in negative regulation of SFKs obtained in isolated cells or cell lines *in vitro* is in a striking contrast with the whole-animal studies. On the basis of the strong functional association of PAG with Csk, it was assumed that PAG knockout mice would have a strong (or lethal) phenotype reminiscent of the Csk knockout.[113,114] However, none of the defects observed in Csk-null mice (embryonic lethality, deregulated SFKs, impaired T-cell development and function and so on) were recapitulated in PAG-deficient mice. It is of course possible that subtle or even substantial phenotypic effects in the PAG-deficient mice might be revealed under some so far untested conditions (for example, sensitivity to some pathogens or tumors, specific brain functions). For example, based on the model discussed in section 'PAG–Csk module in immunoreceptor signaling', it may be expected that the PAG-deficient mice may differ from the wild-type mice in the effects of cAMP on T-cell activation.

This is not an exception—many apparently essential gene products turned out to be dispensable in similar gene knockout experiments. It can be argued that many vitally important gene products may be backed up by alternative ones that may almost instantly take over the function(s) of the missing protein, especially when the gene in question is absent from the very beginning of the organism development. It can well be the case for the PAG function as the adaptor mediating Csk localization to plasma membrane raft microdomains involved in SFK regulation. Actually, a number of possible other Csk adaptors were described that may possibly replace the missing PAG, such as LIME,[115,116] caveolin-1,[117,118] SIT,[119] VE-cadherin,[120] ZO-1/2[92] or SCIMP.[121] Paxillin and focal adhesion kinase recruit Csk specifically to focal adhesions,[122,123] and Dok-1 interaction with Csk is important for mitogenic signaling.[124,125] Thus, it would be interesting to know whether some of these Csk-binding proteins are upregulated and thus compensate for PAG deficiency also in PAG knockout mice. In addition, the generation of PAG-inducible knockout might be very informative to determine whether acute deletion of PAG will influence Csk localization and perhaps how fast the compensatory mechanisms may occur.

One compensatory mechanism involving another Csk-binding protein caveolin-1 has been already described in detail, albeit in a fibroblast cell line. In caveolin-deficient fibroblasts, PAG is upregulated and compensates for the partial loss of Csk membrane recruitment. Accordingly, PAG depletion leads to increased caveolin phosphorylation and Csk membrane localization. Moreover, depletion of PAG in caveolin-deficient cells markedly upregulated Src kinase activity.[126] This finding provides the evidence that cells use multiple mechanisms to control excessive Src activation via Csk. LIME, another raft-localized TRAP, which can bind both SFKs and Csk, cannot prevent SFK-induced cell transformation; this indicates that the regulatory role of PAG in SFK signaling might be quite specific.[101]

The *in vivo* studies on PAG-null mice demonstrated that PAG is probably not the main adaptor for Csk. However, it seems possible that PAG might function as a dispensable Csk 'buffer' under normal conditions, which might be called into action at the outset of cell transformation, for example, when SFKs get upregulated. The literature is very consistent regarding the role of PAG in tumor cells. Apart from its Csk-binding function, PAG might inhibit SFKs in fibroblasts by sequestering them to lipid rafts[60] or, on the other hand, activate them in lymphoma cells by the formation of oncogenic signalosomes.[59] How exactly is Csk uncoupled from the PAG complex under such circumstances is not known. A remarkable, so far isolated observation is that the N-terminal part of PAG, in collaboration with specific glycolipids, may negatively regulate a receptor kinase by excluding it from raft microdomains.[91] Unfortunately, little *in vivo* evidence supports the various mechanisms proposed for PAG on the basis of *in vitro* experiments. It would be certainly interesting to address PAG involvement in tumorigenesis *in vivo* using PAG-deficient mice and various mice tumor models.

In addition, the regulation of membrane microdomain dynamics by modulation of GM1 content in the raft compartment by PAG would deserve more attention. This might have profound consequences on our understanding of regulation of membrane microdomains and their content of important signaling molecules.

ABBREVIATIONS

ALL, acute lymphoblastic leukemia ; BCR, B-cell receptor; Cbp, Csk-binding protein; DRM, detergent-resistant membrane microdomain; Epo, erythropoietin; NSCLC, non-small cell lung cancer; PAG, phosphoprotein associated with glycosphingolipid-enriched microdomains; PTP, protein tyrosine phosphatase; RCC, renal cell carcinomas; SH2, SH3, Src homology domain; SFK, Src family kinase; TCR, T-cell receptor; TRAP, transmembrane adaptor protein

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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