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REVIEW

The emerging and diverse roles of the SLy/SASH1-protein family in health and disease—Overview of three multifunctional proteins

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Abstract

Intracellular adaptor proteins are indispensable for the transduction of receptor-derived signals, as they recruit and connect essential downstream effectors. The SLy/SASH1-adaptor family comprises three highly homologous proteins, all of them sharing conserved structural motifs. The initial characterization of the first member SLy1/SASH3 (SH3 protein expressed in lymphocytes 1) in 2001 was rapidly followed by identification of SLy2/HACS1 (hematopoietic adaptor containing SH3 and SAM domains 1) and SASH1/SLy3 (SAM and SH3 domain containing 1). Based on their pronounced sequence similarity, they were subsequently classified as one family of intracellular scaffold proteins. Despite their obvious homology, the three SLy/SASH1-members fundamentally differ with regard to their expression and function in intracellular signaling. On the contrary, growing evidence clearly demonstrates an important role of all three proteins in human health and disease. In this review, we systematically summarize what is known about the SLy/SASH1-adaptors in the field of molecular cell biology and immunology. To this end, we recapitulate current research about SLy1/SASH3, SLy2/HACS1, and SASH1/SLy3, with an emphasis on their similarities and differences.

KEYWORDS
adaptor proteins, apoptosis, cancer, cell biology, immune regulation, intracellular signaling, SLy/SASH1-protein family

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Abbreviations: BCR, B-cell receptor; CC, coiled-coil; CRKL, V-Crk Avian Sarcoma Virus CT10 Oncogene Homolog (Like) protein; DN, double-negative; DP, double-positive; EMT, epithelial-mesenchymal transition; Ig, immune globulin; NES, nuclear export signal; NLS, nuclear localization signal; SAM, sterile α motif; SH3, Src homology 3; SLy, Src homology domain 3 lymphocyte protein; SASH, SAM and SH3 domain containing; TCR, T-cell receptor.

1 | INTRODUCTION

Signaling from outside of the cell usually starts with a ligand-induced activation of cell surface receptors, leading to their oligomerization, and subsequent phosphorylation of cytoplasmic receptor regions. Many growth factors such as insulin, ephrin, or epidermal growth factor (EGF) signal through receptor tyrosine kinases (RTK) with inherent enzymatic activity, enabling receptor autophosphorylation upon ligand-binding.[1,2] By contrast, lymphocyte antigen-receptors apply intracellular non-receptor tyrosine kinases, which are recruited to the membrane after antigen-ligation.[3] Aside from these differences in early phosphorylation events, all receptors depend on the translation of the initial signal through downstream effectors, eventually resulting in reprograming of cell metabolism and gene transcription. These receptor-associated signaling molecules encompass enzymes such as tyrosine or serine/threonine kinases and phospholipase C_γ (PLC_γ), regulatory molecules such as the guanine nucleotide exchange factor Son of Sevenless (Sos) and adaptor proteins without any known catalytic activity, for example, growth factor receptor-binding protein 2 (Grb2).[2-4]

Adaptor proteins are a diverse group of signaling molecules, sharing conserved domains with well-defined biochemical properties. By the means of these domains, they serve as docking site for a range of effectors and mediate protein-protein interactions. Such proximal binding events are indispensable for the downstream transduction of receptor-derived signals. Some of the best-studied adaptor protein modules include phosphotyrosine-binding (PTB) and Src homology 2 (SH2) domains, both binding to phosphorylated tyrosine residues; the Src homology 3 (SH3) domain, binding to proline-rich sequences with the consensus PXXP; and the pleckstrin homology (PH) domain, interacting with phospholipids.[3,5] In addition, many cytoplasmic adaptor proteins contain a sterile α motif (SAM), an interaction domain with various functions including homo- and heterotypic association as well as RNA-binding.[6,7] For an in-depth discussion of the relevant adaptor protein modules, the reader is referred to previously published reviews.[6,8-12]

The SLy/SASH1-family of adaptor proteins consists of three highly homologous members, possessing a bipartite nuclear localization signal (NLS), a SH3, and a SAM domain, all of which enable their adaptor function. The first family member SLy1 (SH3 protein expressed in lymphocytes 1) was initially characterized by Beer et al in 2001, shortly followed by SLy2/HACS1 (hematopoietic adaptor containing SH3 and SAM domains 1) and SASH1 (SAM and SH3 domain containing 1).[13-15] Based on the pronounced structural homology and sequence similarity in their functional domains, SLy1, SLy2, and SASH1 were defined as one family of scaffolds.[14,16] Figure 1 provides a structural overview of the most important domains of all three adaptors in humans.

TABLE 1 Amino acid positions of the conserved motifs of the three SLy/SASH1-adaptor proteins in mice and men (italic) and overall homology between murine and human proteins (bold)

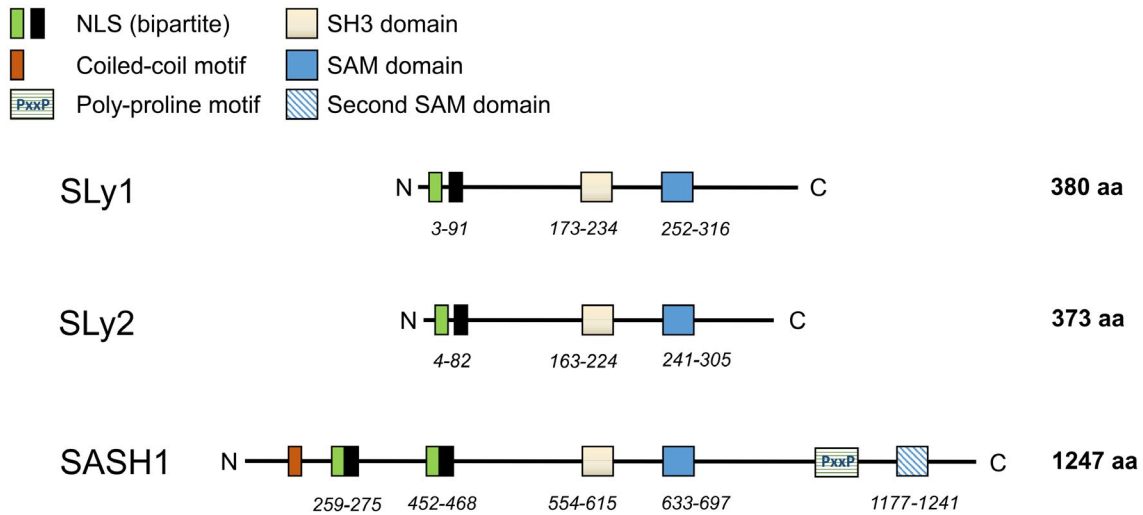| Member	Murine				Human				Protein homology murine/human
	NLS1	NLS2	SH3	SAM	NLS1	NLS2	SH3	SAM	
SLy1/SASH3	3-20	74-91	176-233	249-316	3-20	74-91	173-234	252-316	94%
SLy2/HACS1	4-21	65-82	166-223	239-305	4-21	66-82	163-224	241-305	87%
SASH1/SLy3	249-267	445-462	550-607	623-690/1157-1224	259-275	452-468	554-615	633-697/1177-1241	85%

FIGURE 1 Structural overview of the three SLy/SASH1-adaptor proteins in humans. All members of the SLy/SASH1-protein family contain a bipartite nuclear localization signal (NLS), a central Src homology 3 (SH3) domain and a sterile α motif (SAM) in their C-terminal region. In contrast to SLy1 and SLy2, SASH1 comprises additional features including an N-terminal coiled-coil (CC) domain and a second SAM domain. Human amino acid positions of the conserved adaptor domains are indicated. Murine amino acid positions are depicted in Table 1

Each of the three protein family members has its own expression pattern linked to diverse biological functions. What the SLy/SASH1-proteins have in common, is that they are frequently associated with proliferative disorders and cancer in humans, and SLy2 and SASH1 show a highly significant positive association with overall survival in the TCGA pan-cancer data set from 45 089 patients (Figure 2). In this review, we systematically point out the unique features of SLy1, SLy2, and SASH1 and discuss their emerging and diverse roles in health and disease.

2 | SLy1

SH3 protein expressed in lymphocytes 1, shortly termed SLy1 (alternatively SASH3) is the first member of the SLy-family of adaptor proteins and was described by Beer et al in 2001.[13] The human and the murine *SLy1* gene are both located on the X chromosome (Xq25-Xq26.3 and XA5 48,146,436-48,161,565, respectively). Interestingly, genes encoded in the same X chromosomal region are frequent subjects to mutations in lymphoid disorders, as for example CD40L and SH2D1A, associated with Hyper-IgM-Syndrome and X-linked lymphoproliferative disease in humans, respectively.[17,18] Murine SLy1 consists of 381 amino acids (aa) and has a size of 55 kDa. There is a 89% nucleotide sequence and a 94% aa sequence homology between the human and the murine cDNA and protein (Table 1), respectively.[13] SLy1 is comprised of a bipartite nuclear localization signal (NLS) at its N-terminus (aa 3-20 NLS1, aa 74-91 NLS2), a central SH3 domain, located within aa 176 and 233, and a C-terminal SAM domain (aa 249-316) (Figure 1).[19] At position 27, SLy1 contains a serine (Ser-27), targeted by activated serine kinases.[20] The SLy1 protein is exclusively expressed in lymphocytes and its mRNA can be detected in hematopoietic organs such as the bone marrow (BM), thymus, spleen, lymph nodes, and Peyer's patches. In the spleen, SLy1 is specifically restricted to the white pulp containing both, T and B cell areas, but is not expressed in the red pulp.[19] Within the cell, SLy1 is found in the cytoplasm as well as inside the nucleus, indicating intracellular shuttling of the protein.

The first evidences unveiling a crucial role of SLy1 in immune cell development and function were reported in 2005, when Beer and colleagues generated SLy1-mutant (SLy1^d/d) mice, harboring a deletion within *SLy1* from position 20 to 100. These mice express a truncated version of SLy1, lacking the Ser-27 phosphorylation site and the second part of the bipartite NLS. In contrast to wild-type (wt) SLy1, the mutant protein is exclusively located in the cytoplasm and no longer found inside the nucleus. Hence, cellular distribution of the SLy1-protein must depend on at least one or both, a functional NLS and the Ser-27 phosphorylation site. In lymphocytes, SLy1-phosphorylation at Ser-27 is likely to occur through a phosphatidyl- inositol 3-kinase (PI3K)/protein kinase C (PKC)-dependent signaling pathway.[19,20] As both, PI3K and PKC are important mediators during antigen receptor-induced signaling cascades this indicates a possible relevance of SLy1 during T- and B-cell receptor (TCR and BCR) signaling. Taking into account these findings, it stands to reason that SLy1 is phosphorylated in lymphocytes as a consequence of antigen-ligation and then translocates from the nucleus to the cytoplasm. In the latter, SLy1 is thought to be kept by binding to so called 14-3-3 proteins, which themselves serve as adaptor molecules and among others regulate conformation and localization of their targets.[21]
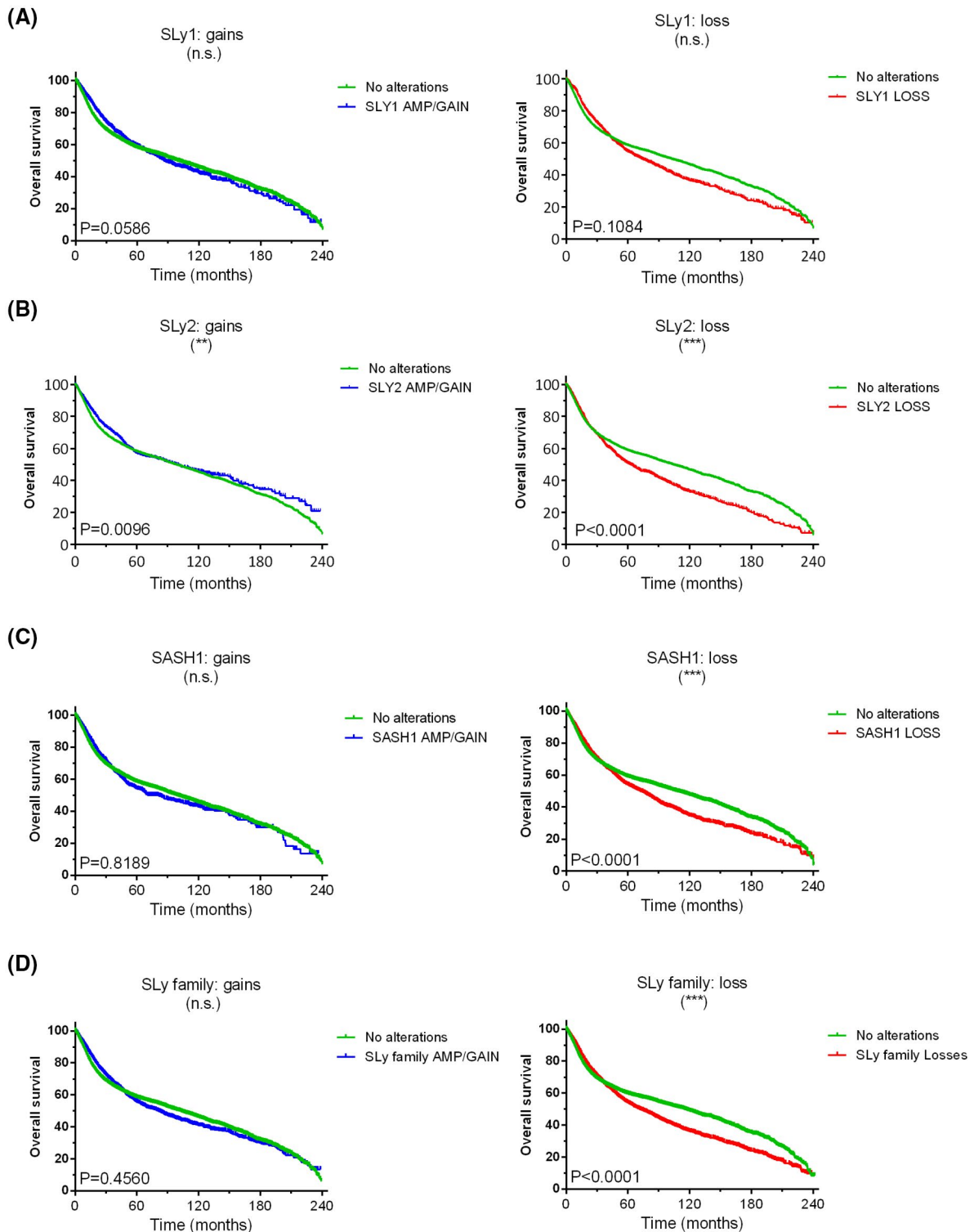
FIGURE 2 Correlation of genomic alterations within the coding regions of the SLy/SASH1-adaptor proteins and overall survival rates of human cancer patients. The overall survival (OS) trends of cancer patients in correlation with genomic gains, amplifications, and heterozygous or homozygous deletions that localize to the encoding regions of SLy1, SLy2, and SASH1 are shown. Data were assessed using the TCGA database (cBioPortal) and is based on 180 nonredundant pan-cancer studies, including 45 089 patients (47 497 samples). A, SLy1: Heterozygous or homozygous deletions that localize to the SLy1-coding region are significantly associated with worse survival prognosis. B, SLy2: Both, genomic gains as well as genomic losses corresponding to the SLy2-coding regions are significantly associated with survival trends: gains are prognostic for increased; losses for decreased OS of patients. C, SASH1: While amplifications and gains in the SASH1-encoding region do not show any correlation with OS, the heterozygous or homozygous loss of SASH1 significantly associates with worse OS of patients. D, SLy/SASH1-family: The combined loss of all three adaptor family members is highly significantly associated with reduced OS of cancer patients.

Strikingly, the size of various lymphoid organs such as thymus, spleen, peripheral lymph nodes, and Peyer's patches is dramatically reduced by up to 60% in SLy1$^{d/d}$ mice, associated with an overall reduction in lymphocyte counts. Concomitantly, the amount of macroscopically visible Peyer's patches is substantially lower in SLy1-mutant mice as compared to wt littermates. Reconstitution experiments using lethally irradiated congenic mice revealed that these effects most likely occur due to an impaired ability of hematopoietic cells to reconstitute lymphocyte populations in the absence of a functional SLy1-protein.[19] Thus, these initial studies on SLy1$^{d/d}$ mice prove that an aberrant subcellular localization of SLy1 causes severe alterations within the lymphocyte immune compartment.

Complementary, Reis et al generated a SLy1-knockout (Ko) mouse model in 2009, globally lacking the expression of *SLy1*. The reduced size of lymphatic organs initially observed in SLy1$^{d/d}$ mice could be confirmed in SLy1^{Ko} mice. Detailed investigation of SLy1^{Ko} mice revealed various defects in lymphocyte function and development.[22] As SLy1 fulfills specific roles within distinct lymphocyte subsets, these are separately described in the following parts.

2.1 | SLy1 in B cells

SLy1$^{d/d}$ mice hold normal amounts of B-cell precursors in the bone marrow, follicular (FO) B cells in the spleen and innate B-1 cells in the peritoneum. However, the SLy1-truncation induces a strong reduction of the splenic marginal zone (MZ) B cell pool. In addition, the proliferation of purified MZ B cells in response to stimulation with anti-IgM and anti-CD40 is impaired in SLy1$^{d/d}$ mice as compared to wt controls.[19]

MZ B cells represent a separate lineage of B lymphocytes, crucially involved in the rapid antibody response toward blood-borne pathogens such as encapsulated bacteria.[23,24] Following maturation in the bone marrow, newly formed naive B cells enter the spleen and develop from transitional 1 (T1) into T2 cells, which then further develop into either follicular (FO) or MZ B cells. The decision whether a T2 cell undergoes FO or MZ B cell differentiation is determined by multiple factors and among others depends on the Notch signaling pathway. Specifically, targeted deletions of the Notch signaling factors Notch2, recombination signal-binding protein for immune globulin kappa J region (RBP-J), or Msx2-interacting nuclear target protein (MINT) were reported to severely interfere with MZ B cell development.[25-28] In SLy1$^{d/d}$ mice, proportions of splenic T1, T2, FO, and pre-MZ B cells remain unaltered, indicating a partial block at the final step of cell differentiation from T2 cells to MZ B cells due to the SLy1-truncation. Consistently, the expression of RBP-J and respective target genes was found to be significantly lowered in MZ B cells derived from SLy1$^{d/d}$ mice.[29]

However, it is still to be investigated how the truncation of SLy1 may influence RBP-J expression levels. Since mutant SLy1 is solely placed in the cytoplasm but absent in the nucleus, it is tempting to speculate that the presence of SLy1 in the nucleus may be required for the full expression of RBP-J in MZ B cells.[29] Importantly, the significant reduction of the MZ B cell compartment could be confirmed in SLy1^{Ko} mice a few years later, further supporting the hypothesis of SLy1 as a regulator of MZ B cell immunity.[22] Other aspects that regulate MZ B development include the strength of B cell receptor (BCR) signaling and cell homing, mediated by adhesion molecules and chemokine receptors. However, neither BCR signaling nor chemokine receptor expression is affected in SLy1$^{d/d}$ mice.[19,25-27]

Considering the reduction of MZ B cells based on defective Notch signaling, it may not come as a surprise that mice harboring the SLy1 mutation display impaired natural and specific antibody production. Generally, the amounts of basal serum IgM and IgG$_1$ are significantly lowered in SLy1$^{d/d}$ mice, whereas the mutation does not have any effects regarding IgA, IgG$_{2a}$, and IgG$_3$.[19] As MZ B cells contribute to natural IgM titers under homeostatic conditions, the reduced MZ B cell pool of SLy1-mutant mice is likely to explain the lack in global serum IgM.[23] Despite unaltered numbers of innate B-1 cells in the peritoneum, SLy1-mutant mice also lack peritoneal IgM, implicating an additional effect through B-1 cell functional impairments.[19,30] Diminished IgG$_1$ titers might be caused by defects within the T helper (T$_h$) cell compartment, which will be discussed in detail in the next section.

Aside from homeostatic conditions, the expression of a truncated SLy1-protein also affects antigen-specific humoral immune responses upon vaccination in vivo. For instance, the amounts of IgM and IgG$_3$ antibodies produced in response to the thymus-independent (TI) type 2 antigen TNP-Ficoll are significantly reduced in these mutants.[19] Additionally, SLy1$^{d/d}$ mice cannot mount adequate levels of IgM toward pneumococcal polysaccharides if compared to wt littermates.[29] With regard to thymus-dependent (TD) antigens such as TNP-CGG, similar results could be observed. In SLy1$^{d/d}$ mice, the production of specific IgG$_1$ and IgG$_{2a}$ antibodies upon immunization with TNP-CGG is substantially impaired.

In summary, it can be stated that functional translocation of SLy1 into the nucleus is a prerequisite for both, MZ B cell development and proper antibody responses toward TI and TD antigens.

2.2 | SLy1 in thymocytes

In keeping with the severe reduction of thymus size observed in mice expressing the truncated SLy1-variant, a significantly lower absolute number of thymocytes was found in SLy1$^{d/d}$

mice as compared to wt controls.[19] Concomitantly, thymi of SLy1[Ko] mice show a 46% reduction in total cell number.

Assuming a specific role for SLy1 during thymocyte development, the different stages of developing T cells in the SLy1[Ko] model were further investigated using flow cytometry analysis. T cell development in the thymus is a complex multistep process. Initially, hematopoietic precursors enter the thymus via the bloodstream, being CD4 and CD8 double-negative (DN). DN cells are further subdivided into four different stages according to their surface expression of CD44 and Interleukin (IL)-2 receptor α-chain (CD25). Once passed through the stages DN1 (CD44^{+}CD25^{−}) and DN2 (CD44^{+}CD25^{+}), the cells downregulate CD44 and get irreversibly committed to the T-cell lineage (DN3 stage). DN3 cells pass through a complex selection process, characterized by TCR-gene rearrangement and eventually resulting in the expression of a pre-TCR (ß-selection). Cells that are subjected to programed cell death can be rescued by the expression of an intact pre-TCR, whereas cells failing to correctly rearrange their TCR-genes undergo apoptosis. During the last few years it became clear, that Notch-mediated signals are indispensable for the development of DN thymocytes. In conjunction with the pre-TCR, Notch ligand-engagement activates the mammalian target of rapamycin complex (mTORC), which in turn leads to the expression of nutrient receptors CD71 and CD98. Notch ligation additionally triggers activation of Phosphatidylinositol-Kinase 1 (PDK-1) and important AGC-serine kinases, thereby allowing for extensive proliferation of selected T cells.[31,32] After successfully passing the ß-selection checkpoint, the cells downregulate CD25 (DN4) and subsequently transit into the double-positive (DP) stage, hallmarked by simultaneous surface expression of CD4 and CD8. Finally, mature CD4 or CD8 single-positive (SP) T cells are able to exit the thymus.[33-35]

Both, counts of DP as well as SP T cells are significantly decreased in SLy1[d/d] and SLy1[Ko] mice.[19,22] On the contrary, the percentage of DN cells in SLy1[Ko] thymi was found to be doubled as compared to wt controls, indicating that SLy1 might be important for DN to DP cell transition. In line with this hypothesis, in vitro proliferation and upregulation of CD4 and CD8 in response to Notch ligand stimulation is significantly impaired in SLy1[d/d] and SLy1[Ko] DN thymocytes. These alterations in responsiveness are accompanied by reduced levels of serine phosphorylation and dampened upregulation of CD71 and CD98. Moreover, when stimulated with the Notch ligand delta-like 1 (DL-1) expressed on OP9 cells, specifically pre-TCR[+] DN3 thymocytes from SLy1[d/d] and SLy1[Ko] mice show a significant increase in overall induction of programed cell death, suggesting an anti-apoptotic role for SLy1 during DN3 development.[22]

Taken together, SLy1 is required for full activation of mTORC upon pre-TCR expression in DN T-cell precursors, thereby providing essential survival signals that eventually

allow DN to DP transition. In other words, a functional SLy1-protein is important to protect DN3 thymocytes from programed cell death.

Further, novel observations strongly suggest that SLy1 might be involved in signaling pathways essential for pre-TCR thymocyte development, namely Notch and IL-7 receptor (IL-7R) signaling. SLy1[Ko] thymocytes show an increase in Notch1 and Notch3 expression, while RAG1[Ko] x SLy1[Ko] mice relying solely on Notch signaling for survival do not show the decrease in thymocytes mentioned above, when compared to their wt counterparts (unpublished data, S. Beer-Hammer). Thus, SLy1-involvement in Notch signaling in DN1 to DN3 thymocytes is likely but may not be the main mechanism for reduced cell numbers in SLy1[Ko] thymi.

IL-7R signaling in DN thymocytes is important for cell proliferation and survival. By ways of Jak1/3 activation and phosphorylation of the transcription factor Stat5, Bcl-2 levels in the cell are increased.[36] In SLy1[Ko] thymocytes, IL-7R expression is increased, together with Janus kinase (Jak) 1-phosphorylated signal transducer and activator of transcription (STAT) 5 and Bcl-2 (unpublished data, S. Beer-Hammer). Overexpression of these pro-proliferative signaling pathways in the context of decreased cell numbers in SLy1[Ko] thymi guides us to further research questions currently being explored along the lines of SLy1-involvement in these signaling cascades.

2.3 | SLy1 in peripheral T cells

Beyond its role for thymocyte development, SLy1 has also been investigated in mature peripheral T cells. When stimulated with anti-CD3 in vitro, splenic T cells derived from SLy1[d/d] mice show an impaired proliferation rate, accompanied by reduced production of cytokines such as IL-2, IL-4, tumor necrosis factor (TNF), and interferon (IFN)-γ. These functional deficits can be compensated by the addition of exogenous IL-2, indicating that this effect is, at least in part, due to an impaired production of IL-2 caused by the SLy1-mutation. Nevertheless, TCR signaling itself is functional in SLy1[d/d] mice, as determined by the measurement of calcium-influx and relevant signaling molecules such as Erk, Jnk, and p38. In the same study, SLy1-mutant mice were shown to allow prolonged allograft survival of semi-allogenic heart transplants, indicating that the T-cell response is specifically impaired.[19]

A recent study focused on the formation of adaptive immune responses in SLy1[Ko] mice upon infection with *Listeria monocytogenes* and revealed increased susceptibility toward bacterial challenge in the absence of SLy1.[37] Both, the release of cytokines by innate immune cells as well as the T[H1]-driven differentiation of effector T cells upon infection are comparable in SLy1[Ko] mice and wt controls. In contrast, counts of *L monocytogenes*-specific splenic T cells are significantly lower

in SLy1^Ko mice at day 4 and 6 postinfection. In addition, the ex vivo proliferative capacity of CD4^+ and CD8^+ splenic effector T cells is significantly reduced in *SLy1*-targeted mice, accompanied by an increased burden of colony forming units (CFU) during infection with *L monocytogenes*. Interestingly, these differences are, at least in part, induced by enhanced expression of the cell-cycle inhibitors p130 and p27, which could be confirmed on mRNA and protein level.[37] The expression of p130 and p27 is initiated by the Forkhead box protein O (FOXO) transcription factor family. Upon TCR engagement, FOXO is phosphorylated by Akt and gets translocated into the cytoplasm, allowing the T cells to continue cell cycle progression (Figure 3). Akt phosphorylation itself and the export of FOXO1 seem to be fully functional in purified splenic T cells from SLy1-deficient mice. However, after 60 minutes

of anti-CD3/CD28 stimulation, the phosphorylation levels of FOXO1 are severely reduced in SLy1^Ko cells, accompanied by significantly accelerated nuclear reimport of the transcription factor as compared to wt cells. In line with these findings, Schäll and colleagues were able to demonstrate that the adaptor protein SLy1 shuttles from the nucleus into the cytoplasm upon TCR stimulation (Figure 3).[37]

Considering all these evidences, it is likely that SLy1 is part of a regulatory complex, mediating FOXO1-phosphorylation and inactivation by nuclear export as a response to TCR stimulation. Moreover, SLy1 is needed for FOXO1 to retain in the cytoplasm, thereby allowing for cell cycle progression. In its absence, premature reactivation of FOXO1, and thus, expression of cell cycle inhibitors prevents the effector T cells of proper expansion in response to bacterial infection (Figure 3).
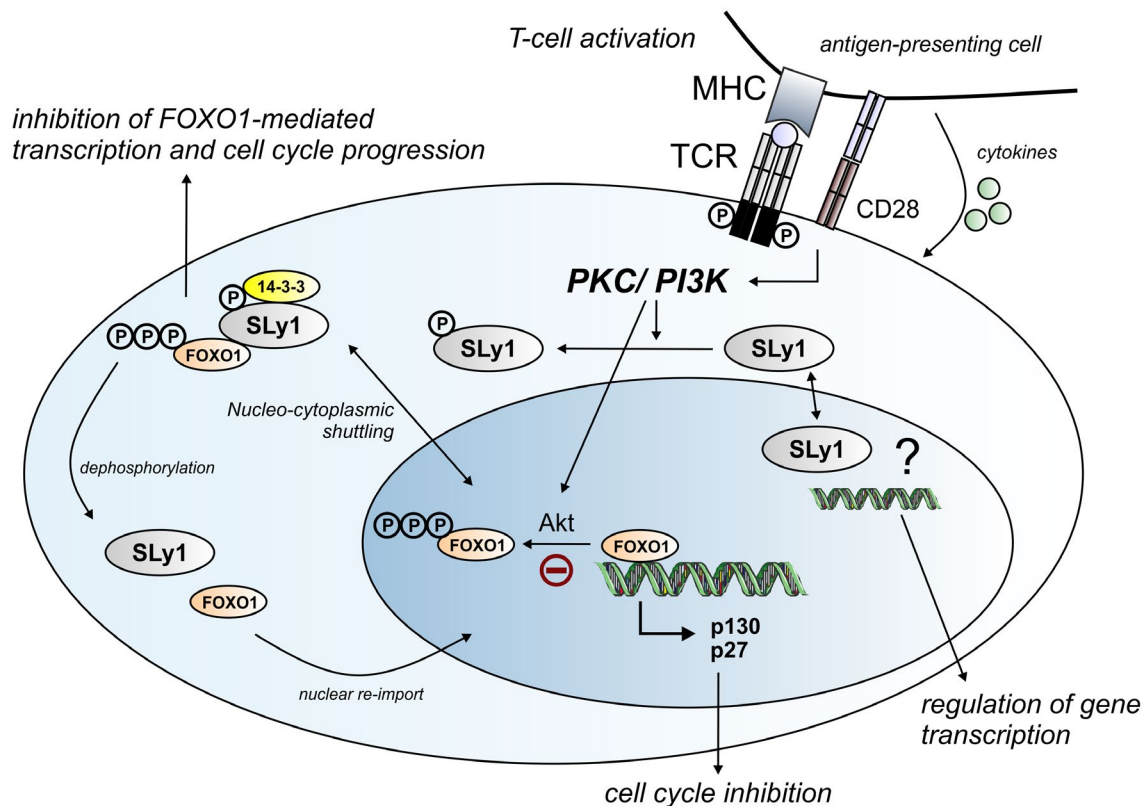

FIGURE 3 Molecular role of SLy1 in peripheral T cells. Under homeostatic conditions, unphosphorylated SLy1-protein is present in both, the cytoplasmic as well as the nuclear cell compartment of peripheral T lymphocytes. Evidence suggest a regulatory role of nuclear SLy1 in the context of gene transcription, however, its molecular interaction partners and target genes remain to be identified. At the same time, the transcription factor Forkhead box protein O (FOXO) 1 promotes the expression of the cell cycle inhibitors p130 and p27, thus, being an important negative regulator of cell expansion. Upon T-cell activation in response to T-cell receptor (TCR)-engagement and co-stimulation, SLy1 gets phosphorylated in the cytoplasm most likely in a phosphatidylinositol 3-kinase (PI3K)/protein kinase C (PKC)-dependent manner. As a consequence, phospho-SLy1 is no longer able to shuttle into the nucleus as it is kept within the cytoplasm by binding to 14-3-3 proteins. In addition, T-cell stimulation induces the activation of the Akt-kinase, which in turn phosphorylates and thereby inactivates FOXO1. Phospho-FOXO1 is consequently translocated into the cytoplasm, where it is bound and kept by the phospho-SLy1 and 14-3-3 protein complex. These events allow cell cycle progression and proliferation of the activated T cell. As soon as the T-cell response has been completed, both SLy1 and FOXO1 get dephosphorylated, the protein complex is resolved and the proteins are able to re-translocate into the nucleus. In SLy1-deficient T cells, the nuclear reimport of FOXO1 is accelerated and causes premature induction of cell cycle inhibitors that interferes with proper T-cell expansion in response to bacterial infection. Thus, SLy1 is crucially involved in the regulation of FOXO1-mediated cell cycle control.

2.4 | SLy1 in Natural Killer (NK) cells

In addition to B and T lymphocytes, the adaptor protein SLy1 is also expressed in mature peripheral Natural Killer (NK) cells.[38] NK cells are well-known for their important role in detection and elimination of virally infected and malignantly transformed cells. By means of various specific receptors, they efficiently interact with other cells and sense alterations in surface antigen expression.[39] In 2016, Arefanian et al reported a positive correlation between robust NK cell function and increased resistance toward lung cancer cells in mice.[38] Of interest, the NK cell-specific expression of SLy1 was found to be significantly lower in mouse strains being susceptible toward cancer cells as compared to those that are resistant. Moreover, SLy1[Ko] mice hold fewer numbers of peripheral NK cells. Proceeding from these observations, Arefanian and colleagues investigated NK cell function in SLy1[Ko] as compared to wt control mice upon intravenous injection of RMA-S lymphoma and Lewis lung carcinoma (LLC) cells. Growth of both RMA-S and LLC cells is accelerated in SLy1[Ko] mice, pointing to impaired NK cell function. Consistently, the in vitro clearance of malignant and nonmalignant NK cell targets is decreased in the absence of SLy1, accompanied by reduced degranulation activity and IFN-γ production of NK cells. Surprisingly, NK cell development in the BM and maturation of peripheral NK cells are unaffected by SLy1-deficiency. Nevertheless, isolated splenic NK cells from SLy1[Ko] mice display a severely altered activation status marked by reduced expression of activating surface receptors such as NK1.1, Nkp46, Ly49H, and NKG2D. In vitro stimulation of isolated splenic NK cells with IL-2 abrogates these differences. mRNA gene expression arrays of isolated NK cells revealed alterations in multiple pathways affecting NK metabolism, migration, and cytotoxicity in SLy1-targeted mice. Finally, SLy1-deficiency correlates with decreased percentages of viable NK cells in mice.[38]

Astonishingly, there is no fundamental difference within the signaling components of the activating NK cell receptor pathways in SLy1-deficient mice as compared to wt controls. However, when going into further detail, Arefanian and colleagues detected multiple ribosomal subunits and proteins that co-immunoprecipitate with endogenous SLy1. Moreover, SLy1 co-localized with the 80S ribosomal complex in activated NK cells and, in contrast to B and T lymphocytes, was solely placed in the cytoplasm (Figure 4). Mass spectroscopy further revealed an increase in multiple free ribosomal proteins specifically in SLy1[Ko] NK cells, including small ribosomal protein 3 (RPS3) and large ribosomal protein 5 (RPL5). Nevertheless, despite ribosomal instability, SLy1[Ko] NK cells seem to have an equivalent amount of mature assembled ribosomes as wt NK cells.[38]

Ribosomal stress associated with an accumulation of free ribosomal proteins such as RPL5 is known to interfere with proper function of Murine double minute 2 (Mdm2). Mdm2 itself is an E3 ubiquitin ligase that targets the pro-apoptotic factor p53 for sequestration under homeostatic conditions (Figure 4).[40,41] Indeed, in NK cells deficient for SLy1, higher overall levels of unbound p53 were detected, indicating that the overabundance of RPL5 in SLy1[Ko] cells inhibits Mdm2-mediated clearance of p53. p53 itself promotes apoptosis and downregulates several genes that are essential to NK cell function, including the master regulator Eomesodermin (EOMES), thus, leading to suppressed NK cell activating receptor expression.[42,43] Hence, SLy1 crucially contributes to the maintenance of ribosomal stability. Its deletion causes an X-linked ribosomopathy, resulting in the accumulation of the apoptosis inducer p53, which in turn drives NK cell dysfunction, diminished NK cell viability, and impaired tumor clearance (Figure 4).[38]

Notably, viral clearance was comparable between SLy1[Ko] and wt littermate mice in a MCMV viral infection model. Additionally, abovementioned functional and phenotypical defects of NK cells from SLy1[Ko] mice could be compensated by previous treatment of NK cells or mice with the pro-inflammatory cytokine IL-2, as shown by in vitro killing of LLCs and in vivo tumor growth, respectively. These results allow speculating that the ribosomal instability and p53-accumulation related to SLy1-deficiency might be negated under inflammatory conditions.[38] Interestingly, as already discussed above, the addition of IL-2 could also restore functional deficits of SLy1[d/d] T cells in vitro. This indicates a special role of the pro-inflammatory cytokine for SLy1-mediated signaling, hence, should be subject to future studies.

To expand our knowledge about the role of p53-accumulation in the context of SLy1-deficiency, we are currently investigating the effects of a NK cell-specific p53-deletion using a murine Cre/*loxP*-system. Novel findings indeed indicate a compensation of the SLy1[Ko]-induced deficits by p53-deletion (work in progress).

Taking all the information together, the expression of a functional SLy1-protein is essential for B, T, and NK cell immune responses. Thereby, functions of SLy1 are highly unique in each of the lymphocyte subsets and so far involve the regulation of Notch signaling in T and B cells, nucleocytoplasmic transport of the transcriptional regulator FOXO1 in T cells (Figure 3) and stabilization of ribosomal compartments in mature resting NK cells (Figure 4).

3 | SLy2

The second member of the SLy-protein family, SH3 protein expressed in lymphocytes 2 (SLy2), also termed HACS1 (hematopoietic adaptor containing SH3 and SAM domains 1) or SAMSN1 (SAM domain, SH3 domain, and NLS 1), was initially described in 2001 by Claudio
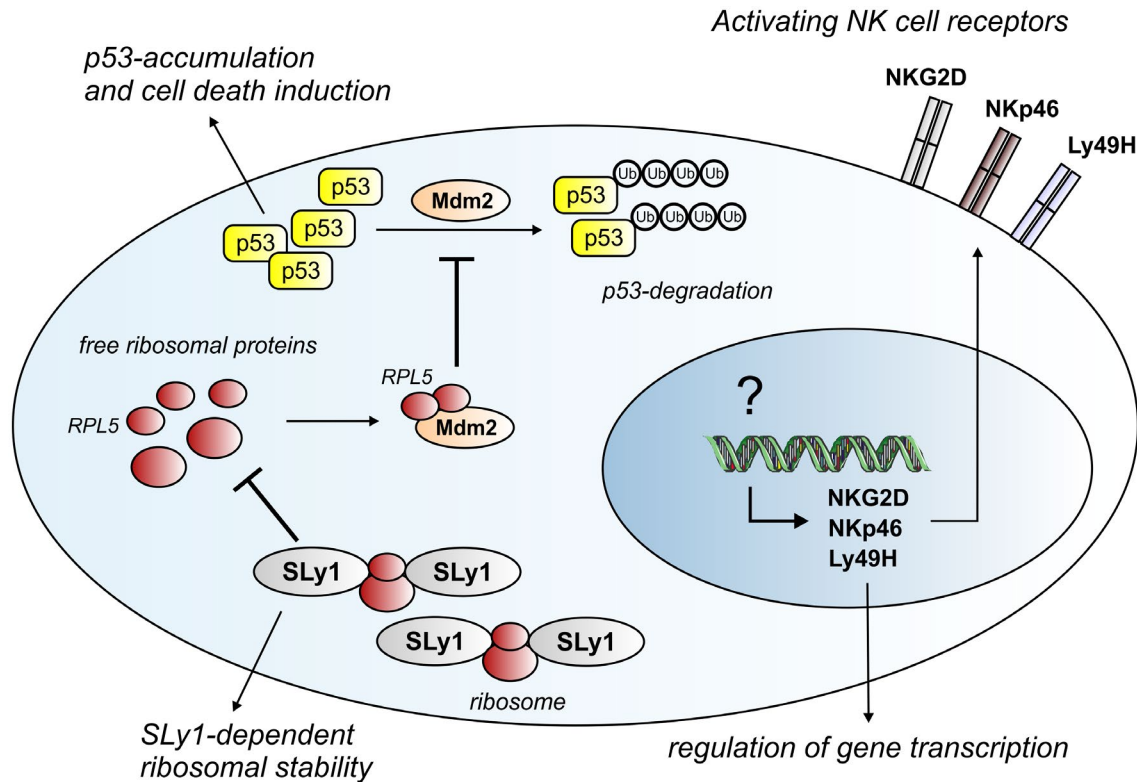
FIGURE 4 SLy1-dependent regulation of ribosomal stability in NK cells. In NK cells, SLy1 is solely placed in the cytoplasm where it co-localizes with and stabilizes the ribosomal compartment. Ribosomal stability is of high importance to the viability and functionality of NK cells, as free ribosomal proteins such as large ribosomal protein 5 (RPL5) cause cellular stress and promote p53-mediated cell death. More precisely, under homeostatic conditions, p53-protein levels are tightly controlled by the E3 ubiquitin ligase Murine double minute 2 (Mdm2). Through poly-ubiquitinylation, Mdm2 marks p53 for degradation. However, the presence of excessive RPL5 in the cytoplasm interferes with Mdm2-function and results in an accumulation of p53, eventually leading to the induction of apoptosis. As a consequence of ribosomal instability, both viability and tumor killing activity of SLy1-deficient NK cells are impaired, accompanied by a severely altered receptor expression profile. The mechanisms leading to the decreased expression levels of the activating surface receptors NKG2D, NKp46, and Ly49H on SLy1-deficient NK cells remain elusive.

et al in hematopoietic progenitors and patient-derived my-eloma cells.[14] Simultaneously, Uchida and colleagues re-ported the expression of a novel adaptor termed NASH1 (a NLS, SAM, and SH3 containing protein 1) in mast cells, most likely representing an alternative splicing form of SLy2.[44]

In humans, *SLy2* is encoded on chromosome 21q11.2, a region frequently disrupted by translocation events in hema-topoietic disorders.[45] In contrast, murine *SLy2* localizes to chromosome 16 and has an 87% overall similarity to human SLy2 on the protein level (Table 1). It is constituted of 372 aa with a molecular weight of 48 kDa. Structurally, SLy2 ex-hibits extensive homology to SLy1, with them sharing 50% aa identity.[13,14,16,44,46] Besides a central SH3 domain (aa 166-223), SLy2 also holds one SAM domain at its C-terminal part (239-305) and two NLS at its N-terminus (aa 4-21 NLS1, aa 65-82 NLS2) (Figure 1).[47] In addition, SLy2 contains a specific phosphorylation site at position serine 23 (Ser-23).[47] In contrast to SLy1, the SLy2-protein displays a broad ex-pression pattern. It is particularly highly expressed in homeo-static and malignantly transformed hematopoietic cells, but

can also be found at lower expression levels in endothelial cells, muscle tissue, heart, brain, lung, and pancreas.[14] Just like SLy1, SLy2 shuttles between the cytoplasmic and the nuclear cell compartment and interacts with 14-3-3 proteins in the cytoplasm.[47] In 2010, Brandt et al demonstrated the di-rect interaction of Ser-23-phosphorylated SLy2 with 14-3-3 and interestingly, co-transfection of 14-3-3ß into HEK293T cells stably expressing SLy2 drastically reduces the propor-tion of nuclear SLy2. Thus, it is reasonable to hypothesize that 14-3-3 proteins bind to phosphorylated SLy2, in order to regulate its subcellular distribution and prevent its nuclear translocation (Figure 5).

Once inside the nucleus, SLy2 was found to interact with Sin3A-associated polypeptide 30 (SAP30) and histone deacety-lase (HDAC) 1, both of which are conserved members of the Sin3A-HDAC gene repressor complex (Figure 5).[47,48] While SAP30 was shown to directly bind DNA, Sin3A serves as an adaptor for several transcription factors and enzymes, thereby en-abling the assembly of a large multi-protein.[48] Hyperacetylation of histone residues is an epigenetic modification that facilitates
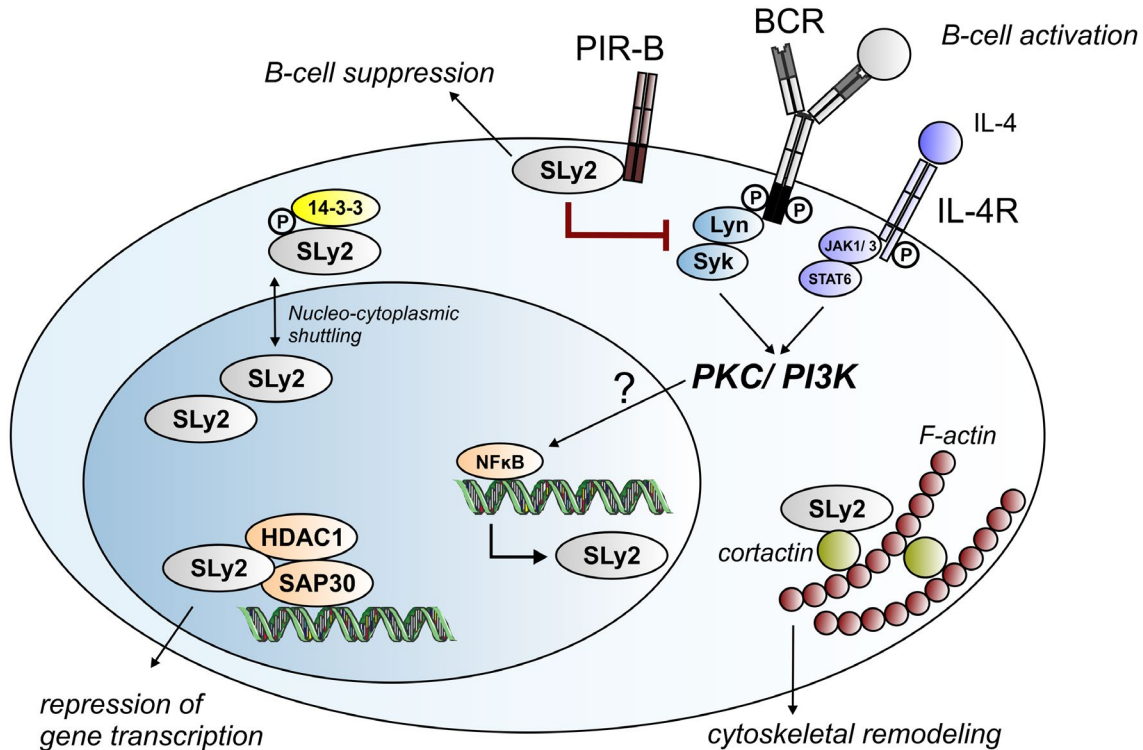
FIGURE 5 Molecular role of SLy2 in B cells. In resting B cells, the intrinsic expression of the SLy2-protein was found to be on a low level. However, upon B-cell activation through B-cell receptor (BCR)-engagement and/ or stimulation with Interleukin-4 (IL-4), SLy2-expression is significantly induced in a phosphatidylinositol 3-kinase (PI3K)/ protein kinase C (PKC)-dependent manner. IL-4-induced upregulation of SLy2 further involves the signaling molecules Signal transducer and activator of transcription (STAT) 6 and nuclear factor (NF) κB. The precise mechanisms of how SLy2-transcription is initiated remain to be solved. Inside the nucleus, SLy2 associates with Sin3A-associated polypeptide 30 (SAP30) and Histone deacetylase (HDAC) 1, both of which are part of a huge protein complex suppressing gene transcription. Although responsible kinases and phosphatases remain to be identified, it is known that the subcellular distribution of SLy2 is controlled in a phosphorylation-dependent manner. Specifically, phospho-SLy2 is restricted to the cytoplasm, where it is bound and kept by 14-3-3 proteins as already seen for its homolog SLy1. Within the cytoplasmic cell compartment, SLy2 binds to the immunoreceptor tyrosine-based inhibition motif (ITIM) of the inhibitory B-cell co-receptor paired immunoglobulin-like receptor B (PIR-B), and therefore, participates in the negative regulation of the B-cell response. Additionally, SLy2 co-localizes with filamentous (F-)actin and directly binds to the master regulator of actin dynamics, named cortactin. Thus, SLy2 is an adaptor protein that is implicated in B-cell signaling pathways and cytoskeletal reorganization.

the access of the transcriptional machinery; deacetylation through HDACs, on the contrary, usually represses gene transcription.[49,50] In keeping with the ternary complex formation between SLy2, SAP30, and HDAC1, the presence of SLy2 markedly increases HDAC1-activity in transfected HEK293T cells, identifying SLy2 as a novel putative member of the Sin3A-HDAC gene repressor complex (Figure 5).[47]

Investigation of SLy2 in a variety of research models including immortalized cell lines and genetically modified mouse strains demonstrated a pivotal role of the adaptor for several cellular processes. The following sections separately sum up, what we know until today.

3.1 | SLy2 in cytoskeletal reorganization

Experiments on transfected HeLa cells by our group in 2011 revealed that SLy2 is crucially involved in the regulation of

cell spreading and actin dynamics, strikingly similar to the third family member SASH1.[51,52] More precisely, ectopic expression of SLy2 triggers the emergence of actin-rich membrane ruffles in transfected cells and interestingly, SLy2 regionally co-localizes with filamentous (F)-actin in these structures. Further examination revealed that SLy2 interacts with cortactin, a key regulator of membrane dynamics and cytoskeletal remodeling, to promote Rac1-dependent membrane reorganization.[51] Of note, by making use of a SH3-domain-deficient SLy2-mutant (SLy2-ΔSH3), we were able to confirm that all these effects require a functional SH3 domain. Herewith, SLy2 was identified as an adaptor that acts in concert with cortactin, which in turn is an F-actin-binding protein that regulates nucleation and branching of actin networks (Figure 5).[51,53,54] Actin reorganization is necessary for cell spreading in response to internal and external stimuli. Consistent with this, SLy2-wt but not SLy2-ΔSH3 transfected HeLa cells display significantly reinforced

polarization and spreading associated with markedly reduced cell proliferation as compared to control transfected cells.[51] Thus, we can state that SLy2 is a regulator of actin dynamics and cell spreading behavior by direct interaction with cortactin. However, precise mechanisms remain to be solved.

3.2 | SLy2 in immune responses

The importance of SLy2 for B cells was first demonstrated by Zhu and colleagues in 2004.[16] While levels of SLy2 were found to be relatively low in naïve B cells, its expression is greatly upregulated in B220^{+} murine splenocytes and CD19^{+} peripheral human B cells upon in vitro treatment with stimulants such as IL-4 and CD40L. IL-4-induced upregulation of SLy2 was shown to involve PI3K-/PKC-dependent signaling, which is of high interest, since the same enzymes have been previously shown to mediate SLy1-phosphorylation (see sections above). Moreover, this upregulation of SLy2 depends on both, the STAT6 and nuclear factor (NF) κB transcription factors (Figure 5).[16]

Anti-IgM stimulation of B cells leads to endogenous association of SLy2 with several tyrosine-phosphorylated proteins, indicating a potential role of the adaptor downstream of the BCR. A putative-binding partner identified in this study was the paired immunoglobulin-like receptor B (PIR-B), an inhibitor of the BCR-associated kinases Syk and Btk, which negatively affects B-cell responses.[55] Interestingly, a recent publication by Kwan et al verified the molecular interaction between SLy2 and PIR-B by using a variety of pull-down and protein-binding assays. Specifically, SLy2 was shown to directly associate with the intracellular immunoreceptor tyrosine-based inhibition motif (ITIM) of PIR-B in a SH3 domain-dependent manner (Figure 5).[56] Accordingly, overexpression of SLy2 in murine splenic B cells strongly inhibits IL-4 and CD40L-dependent proliferation of B cells.[16] Also worth mentioning, SLy2-overexpressing B cells are severely defective in spreading on anti-IgM coated cover slips as compared to wt controls.[51] Inspired by these in vitro findings, subsequent studies focused on genetically modified mice, either being deficient for SLy2 (SLy2[Ko]) or specifically overexpressing the adaptor in T and B cells (SLy2-Tg).

SLy2[Ko] mice have normal numbers of B-cell progenitors in the BM as well as splenic mature and transitional B-cell populations. However, isolated splenic B cells from SLy2[Ko] mice display enhanced proliferation toward B cell stimulants such as IL-4, anti-IgM, and CD40L. Of importance, two studies independently reported alterations within the natural B-1 cell compartment of SLy2-deficient mice. These include a significant increase in peritoneal IgM^{+}CD5^{+} or B220^{+}CD5^{+} B-1a cells and increased rates of BM-resident CD19^{+}CD43^{+}CD5^{-} B-1b cells.[46,57] B-1 cells are a unique subset of innate-like B cells that constitutively produce protective antibody

and rapidly respond toward invading pathogens such as *Streptococcus pneumoniae*.[30,58,59] In line with that, we found natural IgM levels being significantly increased in the serum of SLy2[Ko] mice at steady state.[57] Complementary, the antibody responses toward immunization with TI and TD antigens are enhanced in these mice, as seen for TNP-Ficoll, TNP-KLH, and pneumococcal vaccine (Pneumovax23 and Prevenar13). Elevated levels of *Pneumococcus*-specific IgM and IgG[2] antibodies after immunization point to a role of SLy2 for immune responses toward *Streptococcus pneumoniae*.[57] Notably, upon ex vivo stimulation of SLy2[Ko] T cells, an overall increase in proliferation rate and cytokine production (IL-4 and IFN-γ) was observed. Thus, it cannot be excluded that the improved adaptive immunity seen in SLy2[Ko] mice partly relies on strengthened T cell responses.[46]

When it comes to bacterial infection, increased levels of *S. pneumoniae*-specific antibodies in SLy2[Ko] mice were not sufficient to provide survival advantages in the course of acute pneumococcal pneumonia.[57] However, humoral immunity is especially important in the context of septicemia, while nasopharyngeal clearance of *S. pneumoniae* has been shown to require CD4^{+} T helper cells.[60] As a consequence, the role of SLy2 should be additionally investigated in the context of pneumococcal sepsis, where the disease outcome mainly depends on antibody-mediated mechanisms.[60,61]

Investigation of SLy2-Tg mice in our group further highlighted the role of SLy2 for B-1 cells.[62] Not surprisingly, the overexpression of SLy2 in lymphocytes impairs proper B-1 cell function by lowering the percentage of peritoneal B-1 cells and levels of global serum IgM. Upon immunization with pure pneumococcal vaccine, SLy2-Tg mice show significant deficits regarding their specific antibody responses. Moreover, the IL-5-dependent IgM production in purified splenic B cells from SLy2-Tg mice is significantly decreased, accompanied by attenuated expression levels of IL5Rα on B-1a cells. IL-5/IL-5Rα-signaling is essential for survival, proliferation, and differentiation of B-1 cells and is promoted by the transcription factor OCT2.[63,64] Hence, one hypothesis is that SLy2 might negatively modulate IL-5-dependent differentiation of B-1 cells by suppressing OCT2-transcription or activity, resulting in decreased antibody formation. However, a physical interaction between SLy2 and OCT2 could not be shown, suggesting an indirect effect of the adaptor protein that remains to be solved.[62]

Intriguingly, *SLy2* belongs into a group of nine genes significantly amplified in Down syndrome (DS) patients.[65] These patients are highly susceptible toward infectious diseases and suffer from several immunodeficiencies. Alterations in the immune system of DS patients involve the lack of mature B cells, decreased natural serum IgM and IgG[2] as well as diminished responsiveness toward several vaccines.[62,65,66] Of interest, infectious pneumonia is one of the leading causes of death in DS patients.[66] Based on the severely impaired B-1

cell responses in SLy2-Tg mice, it is reasonable to hypothesize that the SLy2-overexpression in DS patients, at least in part, contributes to their strong susceptibility toward pneumococcal antigens.[62]

Fittingly, novel research findings have revealed drastically reduced counts of B cell progenitors within the BM of SLy2-overexpressing mice.[67] These observations apply to several developmental stages, including pro-B, pre-B, and immature B cells. This is of high interest, since various studies have reported defects in early development and expansion of B cells in DS children during their first year of life.[68-70]

Conclusively, SLy2 is involved in the regulation of both, innate and adaptive B cell responses toward B cell stimulatory signaling molecules and TI and TD antigens.

3.3 | SLy2 in cancer

In clinical terms, SLy2 has not only been associated with the immunodeficiency that is linked to DS, but is also frequently mentioned in connection with several types of cancers. Our own analysis shows that chromosomal gains of *SLy2* are significantly associated with better postoperative overall survival in the TCGA pan-cancer data set, whereas shallow or deep deletions (i.e., heterozygous or homozygous genomic losses) of the *SLy2* gene are highly significantly associated with decreased overall survival (Figure 2).

More specifically, *SLy2* expression was shown to be significantly lower in malignant tissue derived from hepatocellular carcinoma (HCC) patients as compared to noncancerous tissue probes. In these cases, decreased mRNA levels go ahead with hypermethylation of the *SLy2* promoter and proportionally decreased protein amounts, indicating defects in the regulation of *SLy2* gene transcription. The loss of *SLy2* further correlates with shorter overall survival and tumor recurrence in HCC patients.[71] Similar results were obtained in gastric cancer (GC), where diminished expression of *SLy2* in GC tissues was associated with larger tumor size and shorter disease free survival times of patients.[72] SLy2 was further identified as an independent prognostic factor for HCC and GC progression, indicating a putative role of the adaptor as tumor suppressor.[71,72] Additionally, several transcriptome studies revealed differential expression of SLy2 in the context of malignant transformations, including lung cancer and ulcerative colitis-associated colorectal cancer.[73,74]

Aside from solid tumors, SLy2 is also of relevance to the development of multiple myeloma (MM). MM is a hematopoietic disease that is characterized by a malignant transformation of plasma cells, which infiltrate and damage the BM of patients. It is frequently associated with genetic abnormalities such as chromosomal loss and translocation events.[75,76] In a Malaysian study in 2012, *SLy2* was identified

as a gene located within a chromosomal aberration region in 105 MM patients.[77] Subsequently, Amend and colleagues reported a large chromosome deletion in MM patients affecting the complete *SLy2*-coding gene section and probably leading to a total loss of SLy2.[78] Further, *SLy2* is homozygously deleted in KaLwRij mice, a murine strain spontaneously developing MM-like disease.[78] Complementary, the expression of *SLy2* is reduced in human myeloma cell lines as well as MM patient-derived CD138^{+} plasma cells as compared to healthy controls.[79] Intriguingly, novel findings of our group revealed significantly decreased rates and absolute numbers of BM-resident CD138^{+}TACI^{+} plasmablasts in SLy2-overexpressing mice.[67] In conjunction, these data allow speculating that SLy2 might serve as a negative regulator of plasma cell differentiation/ proliferation. Inspired by these results, SLy2 should be examined in the context of signaling pathways related to master regulators of plasma cell differentiation such as Blimp-1 and XBP-1.[80]

Strikingly, HDAC-inhibitors applied as anti-cancer drugs efficiently induce apoptosis in MM-derived plasma cells and show promising effects in MM patients.[81] These benefits have been shown to partly rely on inverse regulation of the MM plasma cell marker CD38, thereby increasing the sensitivity of malignant plasma cells toward therapeutic treatments.[82] Another crucial function of HDACs is the transcriptional regulation of cell cycle proteins such as p27 and p53, thereby modulating cell survival and apoptotic events.[83] Keeping in mind the physical interaction of SLy2 with HDAC1 discussed in the section above, it is likely that loss of *SLy2* could result in deregulation of HDAC-activity, thereby contributing to malignant cell transformation in MM. Surprisingly, upon stimulation with IL-6 and a proliferation-inducing ligand (APRIL), SLy2 is significantly upregulated in the human MM cell line RPMI 8226, accompanied by a regression in overall HDAC-activity and alterations in HDAC-target gene expression.[84] However, the precise mechanism of how SLy2 is associated with the development of MM is left to be investigated.

In summary, SLy2 is an inhibitory adaptor protein crucially involved in cytoskeletal actin remodeling, the formation of B cell responses and cancerous diseases. To date, known molecular interaction partners of SLy2 are the actin modulator cortactin, the Sin3A-HDAC gene repressor polypeptide, 14-3-3 proteins, and the PIR-B co-receptor. Nevertheless, the exact underlying signaling pathways remain to be identified.

4 | SASH1/SLy3

The third member of the SLy-protein family, SAM and SH3 Domain Containing 1 (SASH1), was first described in 2003 as a candidate tumor suppressor in breast cancer.[15] The human *SASH1* gene is located on chromosome 6q24.3,

has a size of 201 kb and consists of 20 exons, which undergo alternative splicing to generate two different *SASH1* transcripts, differing in their 3′-untranslated region.[15,85] As already seen for the first two SLy-proteins, a high degree of homology (85%) between the human and murine genes suggests an evolutionary conservation between species (Table 1). Human SASH1 comprises 1247 amino acids, thus, being the largest member of this protein family (Figure 1).[85] The central conserved protein region of SASH1 corresponds to SLy1 and SLy2 and includes the SH3 (aa 554-615) and the first SAM domain (aa 633-697).[52] In contrast to SLy1 and SLy2, SASH1 additionally features long N- and C-terminal stretches with a predicted coiled-coil sequence near the N-terminus, a proline-rich region and a second SAM domain in the C-terminal part (aa 1177-1241).[15,52,86] SASH1 also contains two bipartite NLS at its N-terminus (NLS1: aa 259-275; NLS2: aa 452-468), which have both been demonstrated experimentally to mediate nuclear localization of the adaptor protein.[87] A C-terminal deletion construct of SASH1 accumulates in the nucleus of several cell types, and genetic invalidation of either the first or the second NLS inhibits its nuclear uptake. Thus, it is likely that SASH1 also plays a functional role in the nucleus, by contribution to the regulation of gene transcription.[52,87] In contrast, deletion of the single predicted NES sequence did not result in any observable alterations of the intracellular localization of SASH1 (unpublished data). So far, no specific stimuli were identified that would control its nuclear import or export in different cell types.

SASH1 shows a broad tissue expression with the exception of lymphocytes, especially T cells, which appear to be devoid of SASH1 expression.[15] Several potential protein-protein interaction partners of SASH1 have been described. As already seen for SLy2, SASH1 has been shown to be associated with the F-actin cytoskeleton, most likely by a physical association with cortactin.[52] We have recently demonstrated by several orthogonal methods, including co-immunoprecipitation, yeast two-hybrid screen and dynamic mass redistribution-based quantitative measurements, that SASH1 interacts with the signal adaptor V-Crk Avian Sarcoma Virus CT10 Oncogene Homolog (Like) protein (CRKL) through a specific proline-rich motif in its C-terminal part, which is lacking in SLy1 and SLy2.[87,88] CRKL is the first interaction partner of SASH1 for which the binding affinity has been determined, binding to a proline-rich motif of SASH1 via its N-terminal SH3 domain. By binding and competitively inhibiting this domain of CRKL, SASH1 counteracts CRKL-mediated SRC/ FAK signaling, and thus, epithelial-mesenchymal transition (EMT) and metastasis formation in the context of colorectal cancer (Figure 6).[87,88] Further, SASH1 was recently described as scaffold protein in endothelial Toll-like receptor (TLR) 4 signaling, promoting LPS-induced

activation of the pro-inflammatory factors NFκB, JNK, and p38. SASH1 was reported to bind TNF receptor-associated factor (TRAF) 6 through a specific TRAF6-binding motif, which is in a region not conserved in the other two SLy-family members, as well as IκB kinase α and IκB kinase β (Figure 6).[89] The interaction with IκB kinase β was recently also verified independently by our own group in a yeast two-hybrid screen.[87] Concerning a potential physiological function, SASH1 was shown to bind β-arrestin 1, mediating Akt activation and nitric oxide formation downstream of TLR4 signaling in endothelial cells. Nitric oxide then may act on alveolar epithelial cells to promote maturation of lung parenchyma and induce the expression of surfactant-associated genes.[90] Further groups reported an association with MAP2K2, IQGAP1 and/or guanine nucleotide-binding protein subunit-alpha isoforms.[91-93] It is still unknown whether SASH1 associates with its various potential interaction partners simultaneously, especially since the specific binding sites are unknown for most interactions. While its exact physiological role is still not fully understood, there exists sound evidence demonstrating an involvement of SASH1 in cancer and several other pathologies, which will be discussed in detail below.

4.1 | SASH1 in cytoskeletal reorganization and integrin-mediated cell adhesion

In 2011, we have reported for the first time the subcellular localization of endogenous SASH1, and noted that SASH1 is strongly enriched at F-actin rich lamellipodia in human $CaCO_2$ colon cancer cells, as well as in murine CT26 rectal cancer cells.[52] This observation was confirmed by in vitro experiments on HEK293 and HeLa cells revealing that ectopically expressed SASH1, very similar to SLy2, is involved in regulation of the microfilament system and controls cell adhesion and migration.[51] As demonstrated by live-cell video microscopy and immunofluorescence microscopy, the expression of tagged versions of full-length human SASH1-induced membrane ruffling activity, lamellipodia protrusions and elongated, spindle-like cell shapes in HeLa cells, as well as enhanced actin polymerization.[52] Further, SASH1 co-localizes and co-precipitates with cortactin, a crucial regulator of membrane dynamics and cytoskeletal remodeling, highly similar to SLy2 and indicating an evolutionarily conserved role of the SLy protein family in the control of the actin cytoskeleton (Figure 6).[51,52] Of note, by making use of a series of deletion constructs, we could confirm that the central SH3-SAM1 domain of SASH1 is both necessary and sufficient to induce the actin-related effects on cell morphology, migration, adhesion, and induction of lamellipodia and
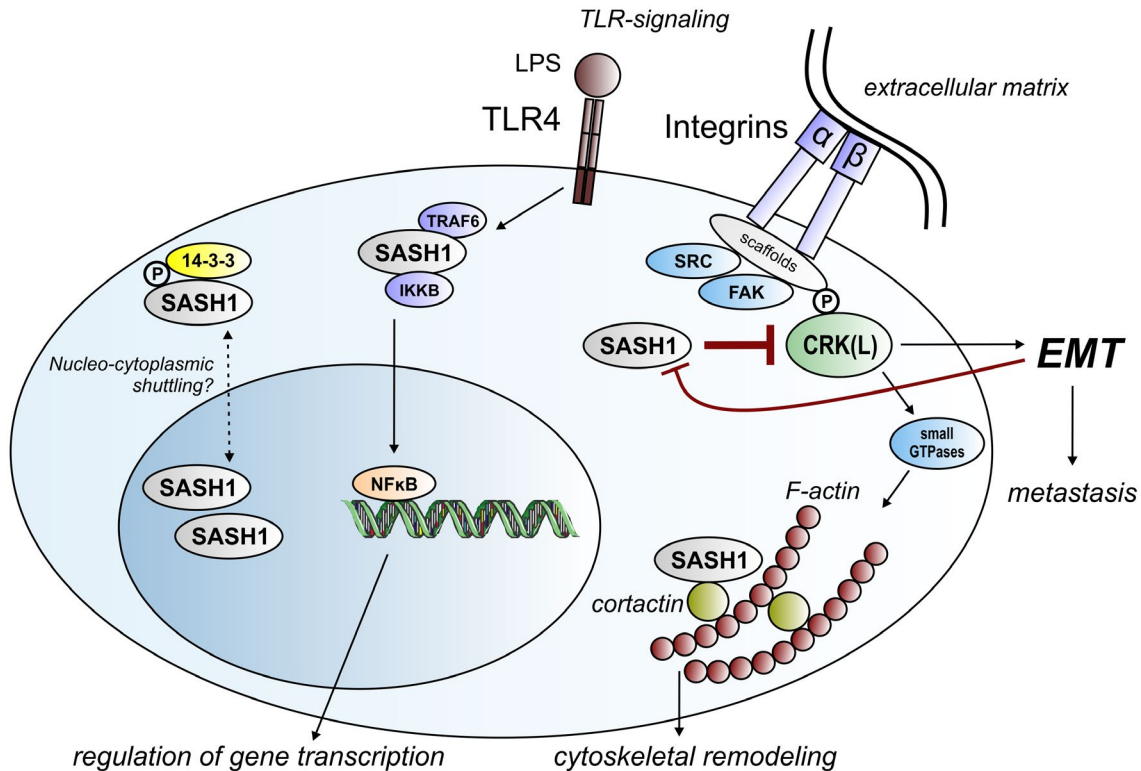
FIGURE 6 Molecular role of SASH1 in cell signaling. As already seen for the first two SLy-family members, the SASH1-protein is able to shuttle between the cytoplasmic and the nuclear cell compartment. Although molecular mechanisms underlying the subcellular transport of SASH1 remain to be identified, novel data strongly points to a direct interaction of SASH1 with 14-3-3 proteins. Based on what is already known in the context of SLy1 and SLy2, we hypothesize that phosphorylated SASH1 might be bound and kept by 14-3-3 proteins in the cytoplasm in order to prevent its nuclear translocation. In response to lipopolysaccharide (LPS)-sensing, SASH1 functions as an adaptor protein downstream of Toll-like receptor (TLR) 4 in endothelial cells, as it assembles with the pro-inflammatory factors TNF receptor-associated factor (TRAF) 6 and Inhibitor of nuclear factor (NF) κB kinase subunit ß (IKKB). Eventually, this signaling cascade leads to an activation of NFκB and corresponding alterations in gene expression. Besides, SASH1 binds the master regulator of actin dynamics cortactin, and is implicated in the control of cell morphology and actin remodeling. Another important interaction partner of SASH1 is the signal adaptor V-Crk Avian Sarcoma Virus CT10 Oncogene Homolog (Like) protein (CRKL). More precisely, SASH1 interferes with CRKL-mediated, SRC/ FAK-induced epithelial-mesenchymal transition (EMT) and epithelial cell metastasis. At the same time, EMT-induction was shown to downregulate *SASH1* transcription, indicating a reciprocal negative regulatory relationship between SASH1 and EMT. Accordingly, deletion of *SASH1* is associated with increased invasiveness and reduced patient survival in the context of various cancer entities. Thus, SASH1 is a tumor suppressor that is crucially involved in integrin-mediated cell adhesion and metastasis formation.

ruffles. Thus, the conserved sequence module in SASH1, which is also highly conserved among all three members of the protein-family, shows a high degree of functional overlap with SLy2. In contrast, the long sequence extensions unique to SASH1 may have divergent functions not seen in either SLy1 or SLy2. As stated earlier, our recent data indicate that SASH1 binds to the N-terminal SH3 domain of CRKL primarily through a PXXPXK motif (aa 984-989) with an affinity in the low micromolar range.[87] Through this interaction, SASH1 is crucially involved in the control of integrin-mediated signaling pathways that mediate cell motility and cell-matrix adhesion in cancer cells from different solid tumor entities.[87,88] Thus, the adaptor protein SASH1 is a regulator of actin cytoskeleton dynamics in epithelial cells and controls cellular adhesive and migratory behavior by direct interaction with cortactin and CRKL, and interference with SRC/FAK-signaling pathways.

4.2 | SASH1 in apoptosis

Similar to SLy1, SASH1 was reported to play a role in cell survival and apoptotic cell death. Several studies suggest that the expression level of SASH1 is associated with the promotion of apoptosis. Overexpression of SASH1 in human glioma cells, lung cancer cells, or osteosarcoma results in an increased rate of apoptotic cells and higher protein levels of activated caspase-3 or other related proteins like cyclin D and MMP-9/2.[94-96] Moreover, SASH1 was found to be cleaved directly by caspase-3, and a resulting

large C-terminal cleavage product of SASH1 was reported to translocate into the nucleus where it is proposed to activate NFκB and eventually apoptosis.[97] Conversely, SASH1 downregulation increases cell proliferation in human skin squamous cell carcinoma cells.[98] The upregulation of SASH1 was also seen in apoptosis induced through external stimuli, such as treatment with chemicals like chloropyramine and 4-hydroxytamoxifen, whereas UV irradiation of breast cancer cells induces cleavage and nuclear translocation of SASH1.[99,100] However, the contribution of SASH1-related cell apoptosis to tumor suppression is still controversial. A study using a stable melanoma cell line overexpressing SASH1 demonstrated that tumor suppressive effects of SASH1 occurred because of the G2/M arrest of cells rather than apoptosis.[101] SASH1 has not been linked to other types of cell death, including necroptosis, pyroptosis, and ferroptosis. Further, SASH1 was reported as direct target of microRNAs.[102,103] Inhibition of these microRNAs lead to lower cell proliferation and higher apoptotic rates as well as an increase in the protein expression of SASH1 in several cell lines, as shown recently.[104,105]

4.3 | SASH1 in cancer

SASH1 has been extensively studied in the context of human cancer. In 2003, SASH1 was found to be frequently lost or downregulated in breast cancer. Loss of heterozygosity of this region occurs in 30% of primary breast cancers and is associated with poor survival and increased tumor size. Correspondingly, SASH1 expression is reduced in the majority of breast tumors. Strikingly, sequencing of the entire SASH1-coding region from 66 human breast cancer samples revealed no inactivating mutations, indicating regulation events on the epigenetic level.[15] A multi-tissue tumor suppressor gene locus has been suspected at chromosome 6q24 for a long time, since the genomic region around 6q24.3 is frequently lost in various cancer entities, including B-cell non-Hodgkin's lymphoma, ovarian cancer, prostate cancer, endocrine pancreatic tumors, uterine cervical adenocarcinoma, and colorectal cancer.[106-111] Since its initial discovery in breast cancer, many studies demonstrated a clinical relevance for SASH1 in several types of tumors, including breast, colorectal and gastric cancer, glioma, and osteosarcoma as well as head and neck squamous cell carcinoma (HNSCC).[15,96,99,106,112-116] Our own analysis in the TCGA pan-cancer data set, based on more than 45,000 patients, shows that shallow or deep deletions (i.e., heterozygous or homozygous genomic losses) of the SASH1 gene locus are highly significantly associated with decreased overall survival (Figure 2). In primary colon cancer and liver metastases, SASH1 expression is strongly reduced on mRNA and protein level.[112] We have shown earlier that downregulation

of SASH1 is specifically associated with the formation of metachronous metastases and poor survival in colorectal cancer, highlighting SASH1 as an independent prognostic factor.[112,113] This was also observed independently in stage III colon cancer: in a cohort of 84 patients we could show that SASH1 expression is a valuable biomarker in stage III colon cancer as it significantly predicts distant metastasis occurrence and its expression is significantly associated with the expression of the canonical WNT-pathway surrogate marker osteopontin. In an earlier study, SASH1 expression in this stage III collective was significantly reduced as compared to a cohort of stage II colon cancer, however, a subgroup of patients in the stage III cohort showed increased distant metastasis occurrence risk although expressing relatively high levels of intratumoral SASH1.[117] While a similar correlation was also found in breast cancer, a recent study suggests subtype-specific differences, in which high SASH1 expression was associated with poor survival in a group of patients receiving multimodal therapy.[99] These subtype-specific alterations depending on the type of cancer entity can also be observed for SLy2 (see sections above).

In vitro, recombinant expression of SASH1 was demonstrated to counteract cell proliferation, viability, migration, and invasion in cell lines of several tumor entities, providing insights into how SASH1 might act as tumor suppressor.[94-96,99,101,118] Nevertheless, the underlying molecular mechanisms are somewhat controversially discussed. Several signaling pathways that may mediate the inhibitory function of SASH1 have been suggested, for example, PI3K/Akt, Notch1, or FAK signaling.[118-121] Several studies, including from our own group, further demonstrated a negative regulatory role of SASH1 in EMT, independent of the EMT-inducing stimulus.[87,118,120,122] EMT is a trans-differentiation process during which epithelial cells lose their epithelial characteristics and acquire a mesenchymal phenotype, increasing invasiveness, and promoting metastasis formation.[123,124] Indeed, loss of SASH1 induces a bona fide EMT with decreased epithelial and increased mesenchymal marker expression, as well as strongly pronounced migratory and invasive phenotypes, thus, produces highly metastatic cells in an orthotopic xenograft mouse model for colon cancer.[87] Therefore, based on these in vivo data, SASH1 should indeed be considered as a tumor suppressor, or more specifically, a suppressor of metastasis. Mechanistically, we further demonstrated within this study that SASH1 binds the signal adaptor CRKL, inhibiting CRKL-mediated SRC activation and EMT-induction to counteract metastasis formation, shown in vitro, as well as in vivo in mouse models (Figure 6). Accordingly, the highly aggressive, chemo-resistant, and pro-metastatic phenotype of SASH1-deficient cells is completely dependent on the presence of its interaction partner CRKL.[87] Data obtained on patient samples of colorectal cancer show a direct correlation of CRK family genes with the EMT master regulator ZEB1,

highlighting their role in EMT also in patients.[88] While loss of SASH1 is sufficient to induce EMT, *SASH1* transcription is downregulated upon EMT-induction through various stimuli such as TNF, suggesting a reciprocal negative regulatory relationship between SASH1 and EMT (Figure 6).[87,120,122]

Interestingly, Burgess et al recently demonstrated that increasing SASH1-protein levels by addition of chloropyramine is inhibiting the proliferation of lung cancer cells in a SASH1-dependent manner.[125] Moreover, this observation was accompanied by increased sensitivity of cancer cells toward cisplatin treatment. Therefore, the drug-mediated elevation of SASH1 concentrations should be considered as a potential future tool in anticancer therapy.[125]

In summary, SASH1 should be considered as a multi-tissue tumor suppressor with clinical relevance in a wide variety of cancerous diseases. *SASH1* downregulation is associated with reduced patient survival and increased metastasis formation, probably due to its pro-apoptotic properties and its ability to inhibit EMT through interfering with the adapter protein CRKL. Several pivotal cancer-related pathways have been shown to be involved in the suppressive action of SASH1. Nevertheless, it remains to be addressed which of these pathways are actually involved in cancer formation and if they are interconnected by SASH1, acting as an integrating scaffold protein.

4.4 | SASH1 in atherosclerosis

Besides its role as a tumor suppressor gene, SASH1 has been associated independently with atherosclerosis in humans. Atherosclerosis is a chronic pathological condition that evolves asymptomatically throughout aging, with atherosclerotic plaques accumulating in the arteries. These lipid-rich plaques can rupture in late stages of the pathology, leading to thrombosis, which can cause a variety of complications, including life-threatening events like stroke or myocardial infarction.[126] *SASH1* is ubiquitously expressed in human arterial walls, and its mRNA expression is increased in carotid plaques of smokers.[127] Furthermore, *SASH1* mRNA expression in circulating monocytes was found to be highly associated with smoking and plaque formation in carotid artery walls.[128] SASH1 negatively regulates human aortic endothelial cell migration, proliferation, and angiogenesis, indicating an inhibitory effect on these mechanisms also in atherosclerosis. Thus, upregulation of *SASH1* in smokers could be a cellular defense mechanism, specifically directed against the cigarette smoke-induced migration and proliferation of endothelial and myeloid cells.[127] Due to an observed reversibility of the effects in ex-smokers, it has been hypothesized that the variation in *SASH1* expression could be due to differential epigenetic modification of the *SASH1* promoter.[127,129] Indeed, a putative smoking-associated, hypermethylated

region was identified, causing increased expression of *SASH1* mRNA.[130] In accordance, data from the cancer field showed that the *SASH1* promoter is methylated in breast cancer and hepatocellular carcinoma. Using methylation microarrays, several methylation sites in the predicted *SASH1* promoter region were found to be increasingly methylated in tumors than in normal tissues.[131,132]

Taken together, *SASH1* expression is likely upregulated in circulating monocytes and in the arterial wall of smokers due to reversible hypermethylation. This may lead to an inhibition of migration, proliferation, and angiogenesis, suggesting a defense mechanism against the noxious agents of cigarette smoke.

4.5 | SASH1 in dermatological pathologies

The first report on an involvement of SASH1 in dermatological diseases was a study on Dyschromatosis Universalis Hereditaria (DUH) and Dyschromatosis Symmetrica Hereditaria (DSH), pathologies resulting in abnormal repartition of skin melanocytes, in which patients exhibit hyper- and hypopigmented macules (lentigines) covering face, trunk, and extremities. Here, the pigmentation phenotype of three non-consanguineous family members was associated with three heterozygous mutations encoding amino acid substitutions within *SASH1* in the affected individuals. Skin sections in one of these patients revealed a higher amount of melanocytes in basal and suprabasal layers of the epidermis compared to normal skin, indicating an increased migratory behavior of melanocytes into the epidermis.[91] In accordance, further germline point mutations in the *SASH1* gene locus were found to be associated with similar phenotypes such as genodermatoses and pigmentation disorders like DSH and DUH. Interestingly, eight out of nine mutations described in case studies are found in the conserved central region common to all SLy-family members.[91,133-136] Nevertheless, Courcet and colleagues described patients with a homozygous *SASH1* point mutation which is not localized in its central "SLy domain" but causes pigmentation disorders with additional alopecia, palmoplantar keratoderma, brittle teeth, and multiple recurrent spinocellular carcinoma of the skin (SCC).[137] This cancer entity, which is derived from squamous epithelia, is probably also under the influence of SASH1 and its known tumor suppressive function. One study refers to the influence of SASH1 on Akt signaling as an mechanistic approach to tumor suppression and shows a negative correlation of *SASH1* overexpression and Akt suppression.[98] The exact mechanisms behind these observations still remain unclear, although different approaches and findings are discussed, including a higher number and migration of melanocytes and increased epidermal cell proliferation. As a model for these complex processes in pigmentation and melanocyte biology,

melanoma cell lines such as A375 cells were used to investigate these mechanisms. The increased migration of A375 cells could be due to alterations in the Gαs-SASH1-IQGAP1-E-Cadherin pathway, resulting in the loss of E-Cadherin.[91,133] Furthermore, a p53-POMC-Gαs-SASH1 autoregulatory feedback loop has been described, in which point mutated *SASH1* leads to higher amounts of POMC and might, therefore, cause pigmentation disorders, as well as a SASH1/MAP2K2 crosstalk regulating the ERK1/2/CREB cascade through p53.[92,93] Another mechanism described propagates the inactivation of TGF-β1 via the downregulation of THSB1 due to mutated *SASH1* resulting in altered melanocyte migration and melanin synthesis in PIG1 cells.[138] In addition, an in vivo approach tried to investigate the function of SASH1 in melanogenesis by expressing a known human point mutation in the *SASH1* gene (Y551D) in BALB/cJ mice. This mutation is known to cause DUH in humans and was inserted into fertilized oocytes using a murine SASH1 vector under the control of its promoter. The authors conclude that SASH1 might induce accumulation of the transcription factor MITF in the nucleus, to regulate the signaling pathway of melanogenesis in vivo and in vitro.[139] Unpublished observations from our group indicate a central role of SASH1 in pigmentation in mouse models and human melanoma cells, although no altered migratory behavior of melanocytes could be detected. In addition, a recent investigation of six Japanese patients with lentigines identified five novel heterozygous mutations in the *SASH1* locus by using next-generation sequencing.[140] Thus, point mutations in *SASH1* cause autosomal-recessive or dominant genodermatoses, pigmentation disorders, and skin cancer, and therefore, underline the function of SASH1 in basic cellular and molecular processes.[137] Further studies are required to clarify whether gain-of-function or loss-of-function phenotypes are caused by the specific point mutations, and to identify the key pathways that mediate pigmentation defects upon SASH1 mutation.

In conclusion, SASH1 is involved in maintenance of cell and tissue homeostasis and counteracts tumor progression by regulating a variety of cellular signaling pathways.

(Epi-)genetic modifications or mutations of the *SASH1* gene are associated with cancer, atherosclerosis, and dermatological alterations in humans. Therefore, a precise identification of its cellular and tissue-specific functions should be the effort of future studies.

5 | CONCLUSIONS AND PERSPECTIVE

Although the members of the SLy/SASH1-protein family each fulfill a unique function by means of specific interactions and expression patterns, they all share conserved functional properties. Most importantly, we can state that the cytoplasmic-nuclear shuttling is a common feature of all three proteins. While the subcellular transport is likely to be controlled by 14-3-3 proteins in case of SLy1 and SLy2, the associated mechanisms for SASH1 remain to be identified. Impressively, recent findings gained from mass spectrometry analyses demonstrate a direct protein-protein interaction of SASH1 with all existing isoforms of 14-3-3 in colon cancer cells, suggesting a similar mechanism for the third SLy/SASH1-member and further emphasizing the conserved relationship within this protein family (unpublished data, F. C. Franke and K.-P. Janssen). Dependent on their localization, SLy/SASH1-proteins specifically participate in cell signaling. Both, SLy1 and SASH1 are functionally implicated in p53-mediated signaling, demonstrating that the adaptors control cell survival and programed cell death. Of interest, the importance of SLy1 for ribosomal stability in NK cells seems to be a function that is exclusive to the first member of the protein family. Beyond, both SLy2 and SASH1 have been shown to bind cortactin, a master regulator of actin dynamics and remodeling. Based on our current knowledge, we propose that SLy2 and SASH1 are essential to the homeostatic functionality of the cytoskeleton. As discussed above, this hypothesis is strongly supported by the fact that loss of SLy2 and SASH1 induces cytoskeletal abnormalities and promotes metastatic cell activity, respectively. Nevertheless, additional studies are needed to define a broader spectrum of interaction partners, such as CRKL, and to unveil the exact contribution of the SLy/SASH1-adaptor proteins to certain pathways.

Speaking of intranuclear mechanisms, no evidence of direct DNA-binding by SLy/SASH1-proteins exists to date. More probable, they indirectly contribute to gene regulation by stabilization of transcriptional machineries, as seen for SLy2 and the Sin3A-HDAC gene repressor complex. Also worth keeping in mind, SAM domains are able to bind RNA molecules, thereby regulating gene activity on a posttranscriptional level.[7] However, the role of the SLy/SASH1-specific SAM domain remains largely unknown and uncovering its function should be aim to future research.

In clinical terms, the most common ground of the three protein family members is their frequent association with the development and progression of various types of cancers. These include both, malignant transformations of the hematopoietic system and solid tumors. In this regard, the members of the SLy/SASH1-family are emerging as important tumor suppressors and valuable prognostic factors to estimate the risk and outcome of human disease. Expanding our understanding of the underlying mechanisms will pave the way to the development of novel therapeutic approaches.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Conceptualization: S. B.-H. and K.-P. J. Original manuscript draft: J.J. together with F. C. F. and A. S. Co-writing, revision, and finalization: All authors.

REFERENCES

1. Hubbard SR, Miller WT. Receptor tyrosine kinases: mechanisms of activation and signaling. *Curr Opin Cell Biol*. 2007;19(2):117-123.
2. Koch C, Anderson D, Moran M, et al. SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. *Science*. 1991;252(5006):668-674.
3. Norian LA, Koretzky GA. Intracellular adapter molecules. *Semin Immunol*. 2000;12(1):43-54.
4. Buday L, Wunderlich L, Tamas P. The Nck family of adapter proteins: regulators of actin cytoskeleton. *Cell Signal*. 2002;14(9):723-731.
5. Pawson T, Scott JD. Signaling through scaffold, anchoring, and adaptor proteins. *Science*. 1997;278(5346):2075-2080.
6. Qiao F, Bowie JU. The many faces of SAM. *Sci STKE*. 2005;2005(286):re7.
7. Aviv T, Lin Z, Lau S, et al. The RNA-binding SAM domain of Smaug defines a new family of post-transcriptional regulators. *Nat Struct Biol*. 2003;10(8):614-621.
8. Kay BK. SH3 domains come of age. *FEBS Lett*. 2012;586(17):2606-2608.
9. Kay BK, Williamson MP, Sudol M. The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains. *FASEB J*. 2000;14(2):231-241.
10. Feng S, Chen J, Yu H, et al. Two binding orientations for peptides to the Src SH3 domain: development of a general model for SH3-ligand interactions. *Science*. 1994;266(5188):1241-1247.
11. Zhou MM, Fesik SW. Structure and function of the phosphotyrosine binding (PTB) domain. *Prog Biophys Mol Biol*. 1995;64(2-3):221-235.
12. Scheffzek K, Welti S. Pleckstrin homology (PH) like domains - versatile modules in protein-protein interaction platforms. *FEBS Lett*. 2012;586(17):2662-2673.
13. Beer S, Simins AB, Schuster A, et al. Molecular cloning and characterization of a novel SH3 protein (SLY) preferentially expressed in lymphoid cells. *Biochim Biophys Acta*. 2001;1520(1):89-93.
14. Claudio JO, Zhu YX, Benn SJ, et al. HACS1 encodes a novel SH3-SAM adaptor protein differentially expressed in normal and malignant hematopoietic cells. *Oncogene*. 2001;20(38):5373-5377.
15. Zeller C, Hinzmann B, Seitz S, et al. SASH1: a candidate tumor suppressor gene on chromosome 6q24.3 is downregulated in breast cancer. *Oncogene*. 2003;22(19):2972-2983.
16. Zhu YX, Benn S, Li ZH, et al. The SH3-SAM adaptor HACS1 is up-regulated in B cell activation signaling cascades. *J Exp Med*. 2004;200(6):737-747.
17. Coffey AJ, Brooksbank RA, Brandau O, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet*. 1998;20(2):129-135.
18. Ramesh N, Morio T, Fuleihan R, et al. CD40-CD40 ligand (CD40L) interactions and X-linked hyperIgM syndrome (HIGMX-1). *Clin Immunol Immunopathol*. 1995;76(3 Pt 2):S208-S213.
19. Beer S, Scheikl T, Reis B, et al. Impaired immune responses and prolonged allograft survival in Sly1 mutant mice. *Mol Cell Biol*. 2005;25(21):9646-9660.
20. Astoul E, Laurence AD, Totty N, et al. Approaches to define antigen receptor-induced serine kinase signal transduction pathways. *J Biol Chem*. 2003;278(11):9267-9275.
21. Dougherty MK, Morrison DK. Unlocking the code of 14-3-3. *J Cell Sci*. 2004;117(Pt 10):1875-1884.
22. Reis B, Pfeffer K, Beer-Hammer S. The orphan adapter protein SLY1 as a novel anti-apoptotic protein required for thymocyte development. *BMC Immunol*. 2009;10:38.
23. Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. *Nat Rev Immunol*. 2013;13(2):118-132.
24. MacLennan ICM, Toellner K-M, Cunningham AF, et al. Extrafollicular antibody responses. *Immunol Rev*. 2003;194:8-18.
25. Pillai S, Cariappa A, Moran ST. Marginal zone B cells. *Annu Rev Immunol*. 2005;23:161-196.
26. Allman D, Srivastava B, Lindsley RC. Alternative routes to maturity: branch points and pathways for generating follicular and marginal zone B cells. *Immunol Rev*. 2004;197:147-160.
27. Lopes-Carvalho T, Kearney JF. Development and selection of marginal zone B cells. *Immunol Rev*. 2004;197:192-205.
28. Rolink AG, Andersson J, Melchers F. Molecular mechanisms guiding late stages of B-cell development. *Immunol Rev*. 2004;197:41-50.
29. Scheikl T, Reis B, Pfeffer K, et al. Reduced notch activity is associated with an impaired marginal zone B cell development and function in Sly1 mutant mice. *Mol Immunol*. 2009;46(5):969-977.
30. Baumgarth N. The double life of a B-1 cell: self-reactivity selects for protective effector functions. *Nat Rev Immunol*. 2011;11(1):34-46.
31. Ciofani M, Zuniga-Pflucker JC. Notch promotes survival of pre-T cells at the beta-selection checkpoint by regulating cellular metabolism. *Nat Immunol*. 2005;6(9):881-888.
32. Kelly AP, Finlay DK, Hinton HJ, et al. Notch-induced T cell development requires phosphoinositide-dependent kinase 1. *EMBO J*. 2007;26(14):3441-3450.
33. Hayday AC, Pennington DJ. Key factors in the organized chaos of early T cell development. *Nat Immunol*. 2007;8(2):137-144.
34. Ciofani M, Zuniga-Pflucker JC. The thymus as an inductive site for T lymphopoiesis. *Annu Rev Cell Dev Biol*. 2007;23:463-493.
35. von Boehmer H, Aifantis I, Feinberg J, et al. Pleiotropic changes controlled by the pre-T-cell receptor. *Curr Opin Immunol*. 1999;11(2):135-142.
36. Hong C, Luckey MA, Park JH. Intrathymic IL-7: the where, when, and why of IL-7 signaling during T cell development. *Semin Immunol*. 2012;24(3):151-158.
37. Schall D, et al. SLy1 regulates T-cell proliferation during Listeria monocytogenes infection in a Foxo1-dependent manner. *Eur J Immunol*. 2015;45(11):3087-3097.
38. Arefanian S, Schäll D, Chang S, et al. Deficiency of the adaptor protein SLy1 results in a natural killer cell ribosomopathy affecting tumor clearance. *Oncoimmunology*. 2016;5(12):e1238543.

39. Bjorkstrom NK, Ljunggren HG, Michaelsson J. Emerging insights into natural killer cells in human peripheral tissues. *Nat Rev Immunol*. 2016;16(5):310-320.

40. Dai M-S, Zeng SX, Jin Y, et al. Ribosomal protein L23 activates p53 by inhibiting MDM2 function in response to ribosomal perturbation but not to translation inhibition. *Mol Cell Biol*. 2004;24(17):7654-7668.

41. Sloan KE, Bohnsack MT, Watkins NJ. The 5S RNP couples p53 homeostasis to ribosome biogenesis and nucleolar stress. *Cell Rep*. 2013;5(1):237-247.

42. Gordon S, Chaix J, Rupp L, et al. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. *Immunity*. 2012;36(1):55-67.

43. Riley T, Sontag E, Chen P, et al. Transcriptional control of human p53-regulated genes. *Nat Rev Mol Cell Biol*. 2008;9(5):402-412.

44. Uchida T, et al. Identification of Nash1, a novel protein containing a nuclear localization signal, a sterile alpha motif, and an SH3 domain preferentially expressed in mast cells. *Biochem Biophys Res Commun*. 2001;288(1):137-141.

45. Mitelman F, Mertens F, Johansson B. A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. *Nat Genet*. 1997;15(S4):417-474.

46. Wang D, Stewart AK, Zhuang L, et al. Enhanced adaptive immunity in mice lacking the immunoinhibitory adaptor Hacs1. *FASEB J*. 2010;24(3):947-956.

47. Brandt S, Ellwanger K, Beuter-Gunia C, et al. SLy2 targets the nuclear SAP30/HDAC1 complex. *Int J Biochem Cell Biol*. 2010;42(9):1472-1481.

48. Viiri KM, Jänis J, Siggers T, et al. DNA-binding and -bending activities of SAP30L and SAP30 are mediated by a zinc-dependent module and monophosphoinositides. *Mol Cell Biol*. 2009;29(2):342-356.

49. Zhang YI, Sun Z-W, Iratni R, et al. SAP30, a novel protein conserved between human and yeast, is a component of a histone deacetylase complex. *Mol Cell*. 1998;1(7):1021-1031.

50. Gregoretti IV, Lee YM, Goodson HV. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *J Mol Biol*. 2004;338(1):17-31.

51. von Holleben M, Gohla A, Janssen K-P, et al. Immunoinhibitory adapter protein Src homology domain 3 lymphocyte protein 2 (SLy2) regulates actin dynamics and B cell spreading. *J Biol Chem*. 2011;286(15):13489-13501.

52. Martini M, Gnann A, Scheikl D, et al. The candidate tumor suppressor SASH1 interacts with the actin cytoskeleton and stimulates cell-matrix adhesion. *Int J Biochem Cell Biol*. 2011;43(11):1630-1640.

53. Daly RJ. Cortactin signalling and dynamic actin networks. *Biochem J*. 2004;382(Pt 1):13-25.

54. Cosen-Binker LI, Kapus A. Cortactin: the gray eminence of the cytoskeleton. *Physiology (Bethesda)*. 2006;21:352-361.

55. Maeda A, Scharenberg AM, Tsukada S, et al. Paired immunoglobulin-like receptor B (PIR-B) inhibits BCR-induced activation of Syk and Btk by SHP-1. *Oncogene*. 1999;18(14):2291-2297.

56. Kwan JJ, Slavkovic S, Piazza M, et al. HACS1 signaling adaptor protein recognizes a motif in the paired immunoglobulin receptor B cytoplasmic domain. *Commun Biol*. 2020;3(1):672.

57. Jaufmann J, Tümen L, Schmitt F, et al. SLy2-deficiency promotes B-1 cell immunity and triggers enhanced production of IgM and IgG2 antibodies against pneumococcal vaccine. *Immun Inflamm Dis*. 2020;8(4):736-752.

58. Haas KM, Poe JC, Steeber DA, et al. B-1a and B-1b cells exhibit distinct developmental requirements and have unique functional roles in innate and adaptive immunity to S. pneumoniae. *Immunity*. 2005;23(1):7-18.

59. Cotte C, Szczepanek SM. Peritoneal B-1b and B-2 B-cells confer long-term protection from pneumococcal serotype 3 infection after vaccination with Prevnar-13 and are defective in sickle cell disease mice. *Vaccine*. 2017;35(28):3520-3522.

60. Cohen JM, Khandavilli S, Camberlein E, et al. Protective contributions against invasive Streptococcus pneumoniae pneumonia of antibody and Th17-cell responses to nasopharyngeal colonisation. *PLoS One*. 2011;6(10):e25558.

61. Trzciński K, Thompson C, Malley R, et al. Antibodies to conserved pneumococcal antigens correlate with, but are not required for, protection against pneumococcal colonization induced by prior exposure in a mouse model. *Infect Immun*. 2005;73(10):7043-7046.

62. Schmitt F, Schäll D, Bucher K, et al. SLy2 controls the antibody response to pneumococcal vaccine through an IL-5Ralpha-dependent mechanism in B-1 cells. *Eur J Immunol*. 2015;45(1):60-70.

63. Moon B-G, Takaki S, Miyake K, et al. The role of IL-5 for mature B-1 cells in homeostatic proliferation, cell survival, and Ig production. *J Immunol*. 2004;172(10):6020-6029.

64. Emslie D, D'Costa K, Hasbold J, et al. Oct2 enhances antibody-secreting cell differentiation through regulation of IL-5 receptor alpha chain expression on activated B cells. *J Exp Med*. 2008;205(2):409-421.

65. Aït Yahya-Graison E, Aubert J, Dauphinot L, et al. Classification of human chromosome 21 gene-expression variations in Down syndrome: impact on disease phenotypes. *Am J Hum Genet*. 2007;81(3):475-491.

66. Englund A, Jonsson B, Zander CS, et al. Changes in mortality and causes of death in the Swedish Down syndrome population. *Am J Med Genet A*. 2013;161A(4):642-649.

67. Jaufmann J, Tümen L, Beer-Hammer S. SLy2-overexpression impairs B-cell development in the bone marrow and the IgG response towards pneumococcal conjugate-vaccine. *Immun Inflamm Dis*. 2021. https://doi.org/10.1002/iid3.413

68. Kusters MAA, Verstegen RHJ, Gemen EFA, et al. Intrinsic defect of the immune system in children with Down syndrome: a review. *Clin Exp Immunol*. 2009;156(2):189-193.

69. de Hingh YCM, van der Vossen PW, Gemen EFA, et al. Intrinsic abnormalities of lymphocyte counts in children with down syndrome. *J Pediatr*. 2005;147(6):744-747.

70. Ugazio AG, Maccario R, Notarangelo LD, et al. Immunology of Down syndrome: a review. *Am J Med Genet Suppl*. 1990;7:204-212.

71. Sueoka S, Kanda M, Sugimoto H, et al. Suppression of SAMSN1 expression is associated with the malignant phenotype of hepatocellular carcinoma. *Ann Surg Oncol*. 2015;22(Suppl 3):S1453-S1460.

72. Kanda M, Shimizu D, Sueoka S, et al. Prognostic relevance of SAMSN1 expression in gastric cancer. *Oncol Lett*. 2016;12(6):4708-4716.

73. Yamada H, Yanagisawa K, Tokumaru S, et al. Detailed characterization of a homozygously deleted region corresponding to a candidate tumor suppressor locus at 21q11-21 in human lung cancer. *Genes Chromosom Cancer*. 2008;47(9):810-818.

74. Watanabe T, Kobunai T, Yamamoto Y, et al. Predicting ulcerative colitis-associated colorectal cancer using reverse-transcription polymerase chain reaction analysis. *Clin Colorectal Cancer*. 2011;10(2):134-141.

75. Rajkumar SV. Multiple myeloma: 2016 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 2016;91(7):719-734.

76. Hideshima T, Mitsiades C, Tonon G, et al. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. *Nat Rev Cancer*. 2007;7(8):585-598.

77. Pau Ni IB, Ching Ching NG, Kian Meng C, et al. Translocation t(11;14) (q13;q32) and genomic imbalances in multi-ethnic multiple myeloma patients: a Malaysian study. *Hematol Rep*. 2012;4(3):e19.

78. Amend SR, Wilson WC, Chu L, et al. Whole genome sequence of multiple myeloma-prone C57BL/KaLwRij mouse strain suggests the origin of disease involves multiple cell types. *PLoS One*. 2015;10(5):e0127828.

79. Noll JE, Hewett DR, Williams SA, et al. SAMSN1 is a tumor suppressor gene in multiple myeloma. *Neoplasia*. 2014;16(7):572-585.

80. Shaffer AL, Lin K-I, Kuo TC, et al. Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. *Immunity*. 2002;17(1):51-62.

81. Eckschlager T, Plch J, Stiborova M, et al. Histone deacetylase inhibitors as anticancer drugs. *Int J Mol Sci*. 2017;18(7):1414.

82. Bat-Erdene A, Nakamura S, Oda A, et al. Class 1 HDAC and HDAC6 inhibition inversely regulates CD38 induction in myeloma cells via interferon-alpha and ATRA. *Br J Haematol*. 2019; 185 (5): 969–974.

83. Pang M, Ma LI, Liu NA, et al. Histone deacetylase 1/2 mediates proliferation of renal interstitial fibroblasts and expression of cell cycle proteins. *J Cell Biochem*. 2011;112(8):2138-2148.

84. Jaufmann J, Fleischmann M-T, Schmitt F, Beer-Hammer S. IL-6-mediated stimulation of RPMI 8226 cells induces upregulation of the inhibitory adaptor SLy2 and Is accompanied by alterations in HDAC-target gene expression. *J Hematol Mult Myeloma*. 2019;4(1):1019.

85. Janssen K. SASH1 (SAM and SH3 domain containing 1). *Atlas Genet Cytogenet Oncol and Heamatol*. 2014;18(4):236-238.

86. (SASH1_HUMAN), U.-O., https://www.uniprot.org/uniprot/O94885.

87. Franke FC, Müller J, Abal M, et al. The tumor suppressor SASH1 interacts with the signal adaptor CRKL to inhibit epithelial-mesenchymal transition and metastasis in colorectal cancer. *Cell Mol Gastroenterol Hepatol*. 2019;7(1):33-53.

88. Franke FC, Slusarenko BO, Engleitner T, et al. Novel role for CRK adaptor proteins as essential components of SRC/FAK signaling for epithelial-mesenchymal transition and colorectal cancer aggressiveness. *Int J Cancer*. 2020;147(6):1715-1731.

89. Dauphinee SM, Clayton A, Hussainkhel A, et al. SASH1 is a scaffold molecule in endothelial TLR4 signaling. *J Immunol*. 2013;191(2):892-901.

90. Coulombe P, Paliouras GN, Clayton A, et al. Endothelial Sash1 is required for lung maturation through nitric oxide signaling. *Cell Rep*. 2019;27(6):1769-1780 e4.

91. Zhou D, Wei Z, Deng S, et al. SASH1 regulates melanocyte transepithelial migration through a novel Galphas-SASH1-IQGAP1-E-Cadherin dependent pathway. *Cell Signal*. 2013;25(6):1526-1538.

92. Zhou D, Kuang Z, Zeng X, et al. p53 regulates ERK1/2/CREB cascade via a novel SASH1/MAP2K2 crosstalk to induce hyperpigmentation. *J Cell Mol Med*. 2017;21(10):2465-2480.

93. Zhou D, Wei Z, Kuang Z, et al. A novel P53/POMC/Galphas/SASH1 autoregulatory feedback loop activates mutated SASH1 to cause pathologic hyperpigmentation. *J Cell Mol Med*. 2017;21(4):802-815.

94. Yang L, Liu M, Gu Z, et al. Overexpression of SASH1 related to the decreased invasion ability of human glioma U251 cells. *Tumour Biol*. 2012;33(6):2255-2263.

95. Chen E-G, Chen Y, Dong L-L, et al. Effects of SASH1 on lung cancer cell proliferation, apoptosis, and invasion in vitro. *Tumour Biol*. 2012;33(5):1393-1401.

96. Meng Q, Zheng M, Liu H, et al. SASH1 regulates proliferation, apoptosis, and invasion of osteosarcoma cell. *Mol Cell Biochem*. 2013;373(1-2):201-210.

97. Burgess JT, Bolderson E, Adams MN, et al. Activation and cleavage of SASH1 by caspase-3 mediates an apoptotic response. *Cell Death Dis*. 2016;7(11):e2469.

98. Chen SZ, Zhang Y, Lei S-Y, Zhou F-Q. SASH1 suppresses the proliferation and invasion of human skin squamous cell carcinoma cells via inhibiting Akt cascade. *Onco Targets Ther*. 2020;13:4617-4625.

99. Burgess JT, Bolderson E, Saunus JM, et al. SASH1 mediates sensitivity of breast cancer cells to chloropyramine and is associated with prognosis in breast cancer. *Oncotarget*. 2016;7(45):72807-72818.

100. Fang QI, Yao S, Luo G, et al. Identification of differentially expressed genes in human breast cancer cells induced by 4-hydroxyltamoxifen and elucidation of their pathophysiological relevance and mechanisms. *Oncotarget*. 2018;9(2):2475-2501.

101. Lin S, Zhang J, Xu J, et al. Effects of SASH1 on melanoma cell proliferation and apoptosis in vitro. *Mol Med Rep*. 2012;6(6):1243-1248.

102. Zhu Y, Ma Y, Peng H, et al. MiR-130b promotes the progression of oesophageal squamous cell carcinoma by targeting SASH1. *J Cell Mol Med*. 2019;23(1):93-103.

103. Wu D, Zhang H, Ji F, et al. MicroRNA-17 promotes osteosarcoma cells proliferation and migration and inhibits apoptosis by regulating SASH1 expression. *Pathol Res Pract*. 2019;215(1):115-120.

104. Li X, Chen Y, Fu C, et al. Characterization of epigenetic and transcriptional landscape in infantile hemangiomas with ATAC-seq and RNA-seq. *Epigenomics*. 2020;12(11):893-905.

105. Tao W, Ma J, Zheng J, et al. Silencing SCAMP1-TV2 inhibited the malignant biological behaviors of breast cancer cells by interaction with PUM2 to facilitate INSM1 mRNA degradation. *Front Oncol*. 2020;10:613.

106. Alcock HE, Stephenson TJ, Royds JA, et al. Analysis of colorectal tumor progression by microdissection and comparative genomic hybridization. *Genes Chromosomes Cancer*. 2003;37(4):369-380.

107. Zhang Y, Weber-Matthiesen K, Siebert R, et al. Frequent deletions of 6q23-24 in B-cell non-Hodgkin's lymphomas detected by fluorescence in situ hybridization. *Genes Chromosomes Cancer*. 1997;18(4):310-313.

108. Shridhar V, Staub J, Huntley B, et al. A novel region of deletion on chromosome 6q23.3 spanning less than 500 Kb in high grade invasive epithelial ovarian cancer. *Oncogene*. 1999;18(26):3913-3918.

109. Srikantan V, Sesterhenn IA, Davis L, et al. Allelic loss on chromosome 6Q in primary prostate cancer. *Int J Cancer*. 1999;84(3):331-335.

110. Barghorn A, Speel EJM, Farspour B, et al. Putative tumor suppressor loci at 6q22 and 6q23-q24 are involved in the malignant progression of sporadic endocrine pancreatic tumors. *Am J Pathol*. 2001;158(6):1903-1911.

111. Acevedo CM, Henríquez M, Emmert-Buck MR, et al. Loss of heterozygosity on chromosome arms 3p and 6q in microdissected adenocarcinomas of the uterine cervix and adenocarcinoma in situ. *Cancer*. 2002;94(3):793-802.

112. Rimkus C, Martini M, Friederichs J, et al. Prognostic significance of downregulated expression of the candidate tumour suppressor gene SASH1 in colon cancer. *Br J Cancer*. 2006;95(10):1419-1423.

113. Nitsche U, Rosenberg R, Balmert A, et al. Integrative marker analysis allows risk assessment for metastasis in stage II colon cancer. *Ann Surg*. 2012;256(5):763-771.discussion 771.

114. Zhou N, Liu C, Wang X, et al. Downregulated SASH1 expression indicates poor clinical prognosis in gastric cancer. *Hum Pathol*. 2018;74:83-91.

115. Yang L, Zhang H, Yao Q, Yan Y, Wu R, Liu M. Clinical significance of SASH1 expression in glioma. *Dis Markers*. 2015;2015:383046.

116. Jin Y, Qin X. Paired like homeodomain 1 and SAM and SH3 domain-containing 1 in the progression and prognosis of head and neck squamous cell carcinoma. *Int J Biochem Cell Biol*. 2020;127:105846.

117. Sperlich A, Balmert A, Doll D, et al. Genetic and immunological biomarkers predict metastatic disease recurrence in stage III colon cancer. *BMC Cancer*. 2018;18(1):998.

118. Sun D, Zhou R, Liu H, et al. SASH1 inhibits proliferation and invasion of thyroid cancer cells through PI3K/Akt signaling pathway. *Int J Clin Exp Pathol*. 2015;8(10):12276-12283.

119. Chen H, Wang D, Liu Y. SASH1 inhibits cervical cancer cell proliferation and invasion by suppressing the FAK pathway. *Mol Med Rep*. 2016;13(4):3613-3618.

120. Zong W, Yu C, Wang P, Dong L. Overexpression of SASH1 inhibits TGF-beta1-induced EMT in gastric cancer cells. *Oncol Res*. 2016;24(1):17-23.

121. Stubblefield K, Chean J, Nguyen T, Chen C-j, Shively JE. The adaptor SASH1 acts through NOTCH1 and its inhibitor DLK1 in a 3D model of lumenogenesis involving CEACAM1. *Exp Cell Res*. 2017;359(2):384-393.

122. Pan JL, Liu Y. SASH1 inhibits hypoxia-induced epithelial-to-mesenchymal transition via suppression of the PI3K/Akt pathway in human pancreatic cancer cells. *Int J Clin Exp Pathol*. 2016;9:750-757.

123. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009;119(6):1420-1428.

124. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol*. 2014;15(3):178-196.

125. Burgess JT, Bolderson E, Adams MN, et al. SASH1 is a prognostic indicator and potential therapeutic target in non-small cell lung cancer. *Sci Rep*. 2020;10(1):18605.

126. Libby P, Buring JE, Badimon L, et al. Atherosclerosis. *Nat Rev Dis Primers*. 2019;5(1):56.

127. Weidmann H, Touat-Hamici Z, Durand H, et al. SASH1, a new potential link between smoking and atherosclerosis. *Atherosclerosis*. 2015;242(2):571-579.

128. Verdugo RA, Zeller T, Rotival M, et al. Graphical modeling of gene expression in monocytes suggests molecular mechanisms explaining increased atherosclerosis in smokers. *PLoS One*. 2013;8(1):e50888.

129. Tsaprouni LG, Yang T-P, Bell J, et al. Cigarette smoking reduces DNA methylation levels at multiple genomic loci but the effect is partially reversible upon cessation. *Epigenetics*. 2014;9(10):1382-1396.

130. Wan MA, Bennett BD, Pittman GS, et al. Identification of smoking-associated differentially methylated regions using reduced representation bisulfite sequencing and cell type-specific enhancer activation and gene expression. *Environ Health Perspect*. 2018;126(4):047015.

131. Sheyu L, Hui L, Junyu Z, et al. Promoter methylation assay of SASH1 gene in breast cancer. *J BUON*. 2013;18(4):891-898.

132. Peng L, Wei H, Liren L. Promoter methylation assay of SASH1 gene in hepatocellular carcinoma. *J BUON*. 2014;19(4):1041-1047.

133. Shellman YG, Lambert KA, Brauweiler A, et al. SASH1 is involved in an autosomal dominant lentiginous phenotype. *J Invest Dermatol*. 2015;135(12):3192-3194.

134. Zhong W-L, Wang H-J, Lin Z-M, et al. Novel mutations in SASH1 associated with dyschromatosis universalis hereditaria. *Indian J Dermatol Venereol Leprol*. 2019;85(4):440.

135. Wang J, Zhang J, Li X, et al. A novel de novo mutation of the SASH1 gene in a chinese family with multiple lentigines. *Acta Derm Venereol*. 2017;97(4):530-531.

136. Zhang J, Cheng R, Liang J, Ni C, Li M, Yao Z. Lentiginous phenotypes caused by diverse pathogenic genes (SASH1 and PTPN11): clinical and molecular discrimination. *Clin Genet*. 2016;90(4):372-377.

137. Courcet J-B, Elalaoui SC, Duplomb L, et al. Autosomal-recessive SASH1 variants associated with a new genodermatosis with pigmentation defects, palmoplantar keratoderma and skin carcinoma. *Eur J Hum Genet*. 2015;23(7):957-962.

138. Cui H, Guo S, He H, Guo H, Zhang Y, Wang B. SASH1 promotes melanin synthesis and migration via suppression of TGF-beta1 secretion in melanocytes resulting in pathologic hyperpigmentation. *Int J Biol Sci*. 2020;16(7):1264-1273.

139. Xu Z, Li Y, Wang D, et al. Mutated SASH1 promotes Mitf expression in a heterozygous mutated SASH1 knockin mouse model. *Int J Mol Med*. 2020;46(3):1118-1134.

140. Araki Y, Okamura K, Saito T, Matsumoto K, Natsuga K, Nishimoto J, Funasaka Y, Togawa Y, Suzuki T. Five novel mutations in SASH1 contribute to lentiginous phenotypes in Japanese families, *Pigment Cell Melanoma Res*. 2020. http://doi.org/10.1111/pcmr.12930