Sumoylation and Acetylation Play Opposite Roles in the Transactivation of PLAG1 and PLAGL2

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PLAG1 (pleomorphic adenoma gene 1) and PLAGL2 (PLAG-like 2) are oncogenes involved in various malignancies. Thus the study of their regulatory mechanisms may lead to identification of novel therapeutic targets. In this study, we provide supporting evidence that sumoylation and acetylation regulate functions of PLAG1 and PLAGL2. A conserved transrepression repression domain exists in both PLAG1 and PLAGL2, whose activity depends on the presence of three sumoylation motifs and an intact sumoylation pathway. In vivo sumoylation assays confirmed that lysines 244, 263, and 353 of PLAG1 and lysines 250, 269, and 356 of PLAGL2 are indeed sumoylation sites. Further study showed that sumoylation inhibits PLAG1-induced IGF-II expression in reporter assays. The repression mediated by sumoylation may be partially explained by its effect on the cellular localization of PLAG1 and PLAGL2, because sumoylation-deficient but not wild-type PLAG1 and PLAGL2 concentrate in the nucleolus. PLAG1 and PLAGL2 are also regulated by acetylation. They are acetylated and activated by p300 and deacetylated and repressed by HDAC7. Interestingly, the sumoylation-deficient mutant of PLAG2 is acetylated at a lower level than its wild-type counterpart, suggesting that some of the lysine residues may be targets for both modifications. Finally, mutation of three lysine residues in sumoylation motifs significantly impairs the transcriptional activity of PLAG1 and PLAGL2, suggesting the essential roles of these sites in the oncogenic potential of PLAG1 and PLAGL2. Taken together, the activities of PLAG1 and PLAGL2 are tightly modulated by both sumoylation and acetylation, which have opposite effects on their transactivation. To our knowledge, this is the first demonstration that oncoproteins can be regulated by both sumoylation and acetylation.

The pleomorphic adenoma gene 1 (PLAG1) is a developmentally regulated (1) transcription factor that plays an important role in tumorigenesis. Dysregulated PLAG1 expression, which results from chromosomal translocation, is crucial in the formation of pleomorphic adenomas of the salivary glands (2) and lipoblastomas (2–4). PLAG1 overexpression is also detected in tumors without chromosomal translocation, such as uterine leiomyomas, leiomyosarcomas, and smooth muscle tumors (5). It was shown recently that both PLAG1 and its related molecule, PLAGL2, play important roles in the pathogenesis of acute myeloid leukemia in cooperation with CbB-MYH11 (6, 7).

Both PLAG1 and PLAGL2 consist of an N-terminal zinc finger DNA binding domain and a C-terminal transactivation domain. The consensus DNA binding site comprises a core sequence (GRGGCG) and a G-cluster (RGGK) (8). Although several potential target genes of PLAG1 have been identified (9), the regulatory mechanisms of transcriptional activation mediated by PLAG1 and PLAG2 remain unknown. One of the mechanisms of regulation of the activity of transcription factors is post-translational modification, such as phosphorylation (10), acetylation (11), methylation (12), ubiquitination (11), isoglycerolysis (13), neddylation (14), and sumoylation (11). Sumoylation is a three-step enzymatic pathway analogous to that of ubiquitin conjugation, which results in the transfer of SUMO from Ubc9 to target proteins (11). The functional consequences of sumoylation are distinct from ubiquitination. Instead of being marked for degradation by ubiquitination, sumoylation has diverse substrate-specific functions. Several transcription factors, including androgen receptor (15), Sp3 (16), c-Myb (17), and Elk-1 (18), are sumoylation targets, and sumoylation represses their transcriptional activities. The exact mechanism of how sumoylation represses transactivation remains unclear, although SUMO-dependent recruitment of histone deacetylases (HDACs) has been implicated in the transcriptional repression of p300 (19) and Elk-1 (20).

In addition to ubiquitination and sumoylation, lysine residues of transcription factors can be covalently modified by acetylation, a process known to enhance DNA binding (21), change protein–protein interaction (22), and regulate transactivation (23, 24). Numerous nuclear histone acetyltransferases (HATs) have been identified. Among them, p300 and the closely related CREB-binding protein (CBP) are the most potent and versatile of all the acetyltransferases. Consistent with its role as a global co-activator, p300 acetylates and regulates various non-histone transcription factors, such as GATA-1 (25), MyoD (26), E2F-1 (27), and p53 (23). On the other hand, HDACs reverse the acetylation process to maintain the balance between the acetylated and deacetylated states of chromatin and other non-histone proteins. HDACs are divided into four classes: class I HDACs (HDACs 1, 2, 3, and 8) localize to the nucleus; class II HDACs (HDACs 4, 5, 6, 7, 9, and 10) are found in both the nucleus and cytoplasm; class III HDACs are NAD+-dependent enzymes that are similar to the yeast SIR2 proteins; and class IV (HDAC11) is the smallest of the HDAC members (28).

In this study, we have identified regulatory mechanisms for PLAG1 and PLAGL2. Sumoylation occurs in a conserved repression domain and inhibits transactivation of both PLAG1 and PLAGL2, which may be explained by changes of subnuclear localization. Moreover, PLAG1 and PLAGL2 are also regulated by acetylation. They are acetylated and activated by p300, while deacetylated and repressed by HDAC7, suggesting opposite roles of acetylation and sumoylation in the transactivation of PLAG1 and PLAGL2. Interestingly, sumoylation-deficient PLAGL2...
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FIGURE 1. Characterization of the repression domain in PLAG proteins. A, structures of PLAG1, PLAGL2, and their GAL4-fusion derivatives. B, PLAGL2 has a repression domain. HEK293 cells in 12-well plates were transfected with 0.1 μg of pGS5-luc reporter, 2 ng of pRV-SV40 (internal control), and 0.1 μg of PM-PLAGL2-(387–496) or PM-PLAGL2-(387–496) as indicated. Luciferase activities were measured 48 h after transfection. Luciferase assays are representative of at least three independent experiments.

shows decreased acetylation, suggesting that some lysine residues could be targets for both modifications. Importantly, mutation of the sumoylation sites greatly reduced transformation abilities of both PLAG1 and PLAGL2, suggesting the importance of these modifications for PLAG1 and PLAGL2 as oncoproteins.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—Anti-Myc (9E10) and Anti-Ubc9 (Sc-10759) were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Anti-FLAG (M2) was purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Anti-HA antibody was from BAbCo (Richmond, CA). Anti-acetylated-lysine was from Cell Signaling (Beverly, MA). L8G5-luc and LexA-VP16 were generous gifts from Dr. Andrew D. Sharrocks (18). PCMV6-SSP3 and pcDNA3-DNUbc9 were obtained from Dr. Ronald T. Hay (19). pCG-PLAG1 and PCG-PLAG2 were gifts from Dr. Shigeru Taketani (29). Human IGF-II-luciferase reporter construct Hup3-luc was provided by Dr. P. Elly Holthuizen.

Transient Transfection, Immunoprecipitation, and Western Blot Analysis—HEK-293 cells were transfected by the calcium phosphate method. 24–48 h later, cells were lysed in a denaturing buffer (2% SDS, 10 mM Tris-HCl pH 8.0, 150 mM NaCl), and analyzed by SDS-PAGE and Western blotting. Luciferase assays were performed according to the manufacturer’s instructions (Promega). Renilla luciferase internal control plasmid was cotransfected with the plasmids as indicated. The relative luciferase units were corrected based on Renilla luciferase activity. For GAL4 fusion–driven luciferase reporter gene assays, 0.1 μg/well of reporter (pG5-luc) was cotransfected with 0.1 μg of GAL4 fusion expression plasmid in 12-well plates. 0.2 μg/well of pcDNA3-DNUbc9 or pCMV6-SSP3 construct was used if indicated. For IGF-II-luciferase reporter assays, 0.05 μg/well of reporter plasmid was cotransfected with effectors of plasmids of indicated amounts in 12-well plates.

Plasmid Construction—The PLAG Constructs used in mammalian cells were generated by polymerase chain reactions with primers containing restriction sites for cloning. Every construct was sequenced fully to verify the fidelity of the polymerase chain reaction. PLAG cDNAs were fused in-frame to the DNA binding domain of GAL4 using the pHis tag. PcDNA3-PLAG1-Myc and subfragments of PLAG1 were constructed using Xhol and EcoRI sites. PcDNA3-PLAGL2-Myc and subfragments of PLAG2 were constructed using EcoRI and BglII sites. PEGFP-PLAG1 or its mutants were constructed by inserting full-length or mutant PLAG1 into Xhol and EcoRI sites of pEGFP-C1 (Clontech). PEGFP-PLAG2 or its mutants were constructed by inserting wild-type or mutant PLAG2 into the EcoRI and BamHI sites of pEGFP-C1.

Immunostaining—HEK293 cells were seeded in chamber slides (0.5 × 10^5 cells/ml) and transfected 24 h later with 2 μg of respective plasmids by the calcium phosphate method. 24–48 h later, cells were washed in cold PBS, fixed with 3.7% formaldehyde for 30 min and permeabilized with 0.5% Triton X-100 for 5 min. Cells were then blocked.
for 30 min in blocking buffer (PBS, 5% bovine serum albumin, 0.3% Triton X-100), incubated with primary antibody for 1 h and washed three times with PBS. After incubation with secondary antibody and washing three times with PBS, cells were examined under confocal immunofluorescence microscope.

Focus Formation Assay—NIH-3T3 cells (in 6-well plates) were transfected with 2 μg of empty vector, expression plasmid for PLAG1, PLAG1 mutant, PLAGL2, PLAGL2 mutants, or Ha-RAS. The day after transfection, cells were split at 3 × 10^4/plate in 10-cm plates and selected in 300 μg/ml G418 and 10% serum in Dulbecco’s modified Eagle’s medium for 3 days. Then cells were fed once every 4 days with DMEM plus 1% serum and 300 μg/ml G418. After 3 weeks, cells were fixed and stained with methylene blue, and the number of foci was determined.

RESULTS

Identification of Repression Domains in PLAG1 and PLAGL2—In this study, PLAG1 and PLAGL2 were found to share similar regulatory mechanisms. To avoid redundancy, the data for PLAGL2 is under “Results” wherever indicated; the rest of the data is under supplemental materials. The structures of PLAG1, PLAGL2, and their GAL4 fusion derivatives are shown in Fig. 1A. Both PLAG proteins have a zinc finger domain in the N terminus, and a transactivation domain in the C terminus. A previous study by Kas et al. (30) identified a region in the middle of PLAG1 and PLAGL2 with a transcriptional repression function. As shown in Fig. 1B, when PLAGL2-(387–496), which includes only the transactivation domain, and PLAGL2-(238–496), which includes both the transactivation domain and the middle region, were fused with the GAL4 DNA binding domain, there was a 10-fold difference in the transactivation capacity between these two constructs. Similarly, PLAG1-(361–500) exhibited about 10 times more transactivation potential compared with PLAG1-(232–500) (supplemental Fig. S1). These results suggest that PLAG1-(232–361) and PLAGL2-(238–387) have repressive activity. The repression domains of some transcription factors such as Elk-1 have been shown to act in trans (18); that is, when bound to a promoter, the repression domain itself is able to repress the activity of another adjacent transcription factor. To test whether the repression domains of PLAG proteins also repress transcription in trans, we performed transrepression assays in which GAL4 fusion proteins were used to repress the activity of a Lex-VP16 activated reporter (18). We found that both PLAG1-(232–361) (data not shown) and PLAGL2-(238–387) (supplemental Fig. S2) have no significant repression activity in the assay, suggesting that the repression domains of PLAG1 and PLAGL2 function differently from that of Elk-1.

Potential SUMO Modification Sites Play a Repressive Role in PLAG1 and PLAGL2—Because repression activities are conserved in the middle regions of PLAG1 and PLAGL2, we aligned their sequences to find the possible conserved sites that may be crucial for the repressive activ-

![Figure 2](image-url)
PIAS1 promotes sumoylation of PLAGL2.

A. PLAGL2 is sumoylated. HEK293 cells in 6-well plates were transfected with 0.5 μg of PLAGL2-Myc in the presence or absence of 0.5 μg of FLAG-SUMO-1 as indicated. 48 h after transfection, cells were lysed with denaturing buffer and analyzed with indicated antibodies. All data are representative of at least three independent experiments.

B. PIAS1 promotes sumoylation of PLAGL2. HEK293 cells in 6-well plates were transfected with 0.5 μg of HA-PLAGL2, 0.5 μg of FLAG-SUMO-1, and 0.5 μg of FLAG-PIAS1 or its mutant either alone or in combination as indicated. 48 h after transfection, cells were lysed with denaturing buffer and analyzed with indicated antibodies.
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Next we investigated whether the sumoylation pathway is involved in the activity of the repression domains of PLAG1 and PLAGL2. We used two approaches to block the sumoylation pathway: a catalytically inactive form of the SUMO-conjugating enzyme, Ubc9C93S (DNUbc9), which acts as a dominant-negative mutant; and SSP3, which is a SUMO-specific protease. As shown in Fig. 2C, transfection of pcDNA3-DNUbc9 completely rescued the difference between GAL4-PLAGL2-(238–496) and GAL4-PLAGL2-(387–496), suggesting that the repressive activity of PLAGL2-(238–387) was abolished. A similar observation was made using SSP3 (Fig. 2D). When we converted the luciferase activity to fold induction (the ratio between transfected and untransfected cells), each PLAGL2 single mutant still retained the ability to be sumoylated, suggesting that sumoylation targets, wild-type and mutant PLAGL2 were cotransfected with FLAG-SUMO-1 and FLAG-PIAS1. PLAGL2K250R, partially, and the triple mutant PLAGL2K250R/K269R/K356R completely, lost their abilities to be sumoylated (Fig. 3D), which correlates with the loss of repression activity (Fig. 2A). Similar results were obtained for PLAG1 (supplemental Fig. S6). We also tested single mutants of PLAGL2. As shown in Fig. 3E, each PLAGL2 single mutant still retained the ability to be sumoylated, suggesting that all three lysine residues are sumoylation targets. PLAG1L is another member in the PLAG family. Unlike PLAG1 and PLAGL2, PLAGL1 is a tumor suppressor gene rather than an oncogene. As shown in Fig. 3F, no sumoylation was detected for PLAG1L even when FLAG-SUMO-1 was overexpressed. These results suggest that both PLAG1 and PLAGL2, but not PLAGL1, can be sumoylated, and lysine residues inside the sumoylation motifs are indeed sumoylation targets.

Sumoylation Pathway Represses PLAG1/PLAGL2-mediated Transcription—Because both the intact sumoylation motifs and the sumoylation pathway are required for the repression domains of PLAG1 and PLAGL2, PLAG1 and PLAGL2, but Not PLAGL1, Are Modified by Sumoylation—To verify that sumoylation of PLAGL2 occurs in cells, we cotransfected HEK293 cells with expression plasmids for PLAGL2-Myc and FLAG-SUMO-1 and analyzed sumoylation of PLAGL2 by immunoblotting with anti-Myc antibody. A slower migrating band reacting with anti-Myc antibody was clearly detected in cells ectopically expressing FLAG-SUMO-1 (Fig. 3A). Because PIAS1 is a well established E3 ligase in sumoylation, we tested whether PIAS1 promotes sumoylation of PLAGL2. HEK293 cells were transfected with PLAGL2-Myc, FLAG-SUMO-1, and FLAG-PIAS1 or FLAG-PIAS1C335A, a construct encoding an inactive form of PIAS1. As shown in Fig. 3B, cotransfection of FLAG-PIAS1, but not FLAG-PIAS1C335A, strongly enhanced the sumoylation of PLAGL2. Moreover, DNUbc9 abolished the appearance of the slower migrating bands (Fig. 3C), suggesting that the higher molecular weight bands correspond to sumoylated PLAGL2. To test whether lysine residues in the consensus sumoylation motifs are indeed sumoylation targets, wild-type and mutant PLAGL2 were cotransfected with FLAG-SUMO-1 and FLAG-PIAS1. PLAGL2K250R, partially, and the triple mutant PLAGL2K250R/K269R/K356R completely, lost their abilities to be sumoylated (Fig. 3D), which correlates with the loss of repression activity (Fig. 2A). Similar results were obtained for PLAG1 (supplemental Fig. S6). We also tested single mutants of PLAGL2. As shown in Fig. 3E, each PLAGL2 single mutant still retained the ability to be sumoylated, suggesting that all three lysine residues are sumoylation targets. PLAG1L is another member in the PLAG family. Unlike PLAG1 and PLAGL2, PLAGL1 is a tumor suppressor gene rather than an oncogene. As shown in Fig. 3F, no sumoylation was detected for PLAG1L even when FLAG-SUMO-1 was overexpressed. These results suggest that both PLAG1 and PLAGL2, but not PLAGL1, can be sumoylated, and lysine residues inside the sumoylation motifs are indeed sumoylation targets.

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their ability to activate IGF-II expression in reporter assays, we found that PLAG1K244,263,353R has lower transactivation activity than wild-type PLAG1 (Fig. 6A). We speculated that besides being the sumoylation sites, these lysine residues may play other critical roles in transactivation. Because lysine residues can also be acetylated, and acetylation has been shown to regulate transactivation of transcription factors, we considered the possibility that one or more of the three lysine residues of PLAG1 and PLAGL2 are also targets for acetylation. To determine whether PLAG1 and PLAGL2 can be acetylated in cells, GFP-PLAG1 or FLAG-PLAGL2 were transiently transfected into HEK293 cells with or without HA-p300, a well studied lysine acetyltransferase. Cell lysates were immunoprecipitated with anti-FLAG or anti-GFP antibody, and acetylated forms of PLAGL2 and PLAG1 were detected by Western blotting with anti-acetyl lysine antibody (Fig. 6B and supplemental Fig. S9). P300 significantly enhanced acetylation of both PLAG1 and PLAGL2. TSA, which is a histone deacetylase inhibitor, further increased the acetylation level of PLAG1 (Fig. 6C), suggesting that HDACs may regulate the acetylation of PLAG1 and PLAGL2. Indeed, HDAC7, a histone deacetylase, significantly decreased acetylation of PLAGL2 (lane 4 compared with lane 3, Fig. 6D). Interestingly, the triple mutant PLAGL2K250,269,356R was less acetylated than the wild-type (lane 3 compared with lane 6, Fig. 6D), suggesting that these lysine residues may also be the targets for acetylation, which could explain why the sumoylation-deficient mutant PLAG1 has weaker activity than the wild-type PLAG1 (Fig. 6A). To investigate the functional consequences of acetylation, we tested whether p300 affects the transactivation of PLAG1 and PLAGL2. When GAL4-fused full-length PLAG1 and PLAGL2 were transfected alone or with p300, p300 significantly enhanced the transactivation potential of both PLAG proteins (Fig. 6E and data not shown). Moreover, transfection of p300 also enhanced PLAG1-induced IGF-II expression in luciferase reporter assays (supplemental Fig. S10). Because acetylation is a reversible process and HDAC7 decreased p300-induced PLAGL2 acetylation (Fig. 6D), we also tested the effect of HDAC7 on PLAG1-mediated IGF-II expression in reporter assays. As shown in Fig. 6F, ectopic expression of HDAC7 repressed the PLAG1-induced IGF-II expression in a dose-dependent manner. Taken together, these results suggest that acetylation activates, whereas deacetylation represses PLAG1 and PLAGL2.

**Lysine Residues Responsible for Sumoylation/Acetylation Are Important for the Transforming Activity of PLAG1 and PLAGL2**—Both PLAG1 and PLAGL2 are oncogenes involved in the pathogenesis of different malignancies. Overexpression of PLAG1 or PLAGL2 transforms NIH-3T3 cells to low serum growth (31). To evaluate the significance of sumoylation/acetylation in their transforming activity, expression plasmids for PLAG1, PLAGL2, or their lysine mutants were transfected into NIH-3T3 cells. One day after transfection, cells were split at the density of 3 × 10^6 cells/10-cm plate, and grown as a monolayer in the medium containing 10% serum and G418 (300 μg/ml). After 3 days the medium was changed to 1% serum plus 300 μg/ml G418. The ability of transfected cells to form foci was analyzed 3 weeks following selection. As shown in Fig. 7, both activated Ha-RAS oncogene, PLAG1 and PLAGL2 promoted focus formation in NIH-3T3 cells in low serum condition. It was noticed that although RAS group and PLAG1/PLAGL2 groups had similar numbers of focus formation, RAS transformed foci were bigger in size than the ones transformed by PLAGs (data not shown), which suggests that PLAG1 and PLAGL2 are weaker oncogenes than RAS. Importantly, whereas all the single mutants of PLAGL2 still retained partial transforming ability, the abilities of PLAG1/PLAGL2 sumoylation-deficient mutants to transform NIH-3T3 cells were greatly reduced (Fig. 7). These results suggest that lysine...
residues that are sumoylation/acetylation targets are important for the transforming ability of PLAG1 and PLAGL2.

**DISCUSSION**

In our study, we found that PLAG1 and PLAGL2 are modulated by both sumoylation and acetylation. Sumoylation occurs in a conserved repression domain and is required for the repression activity. In contrast, acetylation by p300 activates the transcriptional activity of PLAG1 and PLAGL2. Mutation of the sumoylated lysine residues severely impairs the transforming ability of PLAG1 and PLAGL2, suggesting the importance of these modifications on the transforming potential of these proteins.

It was shown that sumoylation pathway is activated under stress situations, such as hypoxia or genotoxic condition (32, 33, 34), and involved in DNA repair and maintenance of genome stability (35). Several important enzymes involved in DNA repair, such as thymine DNA glycosylase (36), DNA topoisomerase I (37) and II (38), are regulated by sumoylation. At the same time, sumoylation prevents further cell...
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There are several possible mechanisms that could explain the repression effect exerted by sumoylation. The first one is that sumoylation regulates subcellular localization of the substrates. Early studies have suggested that sumoylation is involved in regulating nucleocytoplasmic transport. For example, RanGAP1 is targeted to the nuclear pore complex after being sumoylated (40). Recent studies indicated that sumoylation may play a more important role in regulating subnuclear distribution of proteins. Interestingly, sumoylation is essential for PML (promyelocytic leukemia) to accumulate in the nuclear bodies (41), and for Sp3 to localize in the nuclear periphery and nuclear dots (16). Our data suggest that sumoylation affects the subnuclear localization of PLAG1 and PLAGL2 (Fig. 5), which may contribute to its transcriptional repression effect. Another possible mechanism for sumoylation-mediated transcriptional repression is that sumoylation may abolish some of the crucial protein-protein interactions thus rendering the substrates inactive. One of such examples is that sumoylation of ZNF76 affects its interaction with TATA-binding protein (TBP) (42), which is a critical component for transcription initiation. Finally, it is also possible that sumoylation may cause a gain of function such that sumoylated PLAG1/PLAGL2 may recruit novel repressors to repress their transcriptional activity. It was shown that SUMO alone in a promoter is sufficient to inhibit promoter activity (18). Because SUMO itself does not have repression activity, it suggests that other factors are recruited by SUMO to repress transcription. HDACs and HP1 have been shown to be recruited to p300 (19), Elk-1 (18), and histones (43) after sumoylation to affect gene expression. Importantly, these mechanisms are not mutually exclusive. Although sumoylation affects the subnuclear localization of PLAG1/PLAGL2, whether changes in protein-protein interactions or recruitment of novel repressors by sumoylation affect PLAG-mediated transactivation requires further investigation.

Acetylation has been shown to modulate activities of a broad range of transcription factors including DNA binding, protein-protein interactions, protein stability, and transcriptional potency. In our study, we found that PLAG1 and PLAGL2 can be activated by acetylation and repressed by deacetylation. Interestingly, sumoylation-deficient PLAG2 is partially acetylation-defective. We propose the functions of acetylation are 2-fold: first, to compete lysine residues for sumoylation, second, to activate transcription factors possibly through enhancing DNA binding or changing protein-protein interactions such as recruitment of co-activators. Thus, a model emerges whereby PLAG1 and PLAGL2 can respond to activating signals by desumoylation and subsequent acetylation at the same lysine residues, which not only eradicates the function of the repression domain, but also enhances transcriptional activation.

During the preparation of the current report, Van Dyck et al. (44) published their study on the biochemical characterization of PLAG1 sumoylation. Our study not only agrees with the published data but also provides additional novel findings: first, our study shows that both PLAG1 and PLAGL2 (7, 45), but not PLAG1, which is a tumor suppressor rather than an oncogene, are regulated by sumoylation. Thus regulation by sumoylation may be one of the mechanisms to differentiate the functions of these three PLAG family members. Second, using the GAL4 reporter system, our data clearly demonstrate that sumoylation is required for the function of the repression domains of PLAG1 and PLAGL2 described previously (30). Third, our cellular localization studies suggest that sumoylation may play a role in the nuclear localization of PLAG1 and PLAGL2 (Fig. 5). Fourth, we identify acetylation as another post-translational modification for PLAG1 and PLAGL2, which has an opposite function from sumoylation. Finally, in the transcription assay, our data clearly show that acetylation/sumoylation sites of PLAG1 and PLAGL2 are important for their transforming ability.

Notably, both sumoylation and acetylation are reversible processes in which SUMO-specific proteases and deacetylases can desumoylate and deacetylate modified proteins, respectively. Acetylation and sumoylation of PLAG1/PLAGL2 may differentially modulate their affinity for different interacting protein partners, which contributes to different functional consequences of the two modifications: sumoylation causes a switch to a repressed state, whereas acetylation of PLAG1/PLAGL2 causes a switch to an activated state. Thus, the transactivation potential of PLAG1 and PLAGL2 can be regulated by a dynamic interplay of acetylation, deacetylation, sumoylation, and desumoylation in response to various biological signals. In our study, sumoylation and acetylation not only affect the transactivation of PLAG1 and PLAGL2, but also affect their transforming abilities (Fig. 7), suggesting a profound biological effect of these modifications. Given that both PLAG1 and PLAGL2 are oncoproteins involved in the pathogenesis of certain cancers, the enzymes involved in regulating their sumoylation and acetylation could be considered as potential therapeutic targets in associated malignancies.

Acknowledgments—We thank Dr. Ke Shiuai for pCMV5–PIAS1; Dr. Andrew D. Sharrocks for L8G5–luc and Lexa–VP16 constructs; Dr. Ronald T. Hay for pCMV6–SSP3 and pcDNA3–DNIibc9; Dr. Shigeru Taketani for pCG–PLAGL1 and pCG–PLAGL2; Dr. P. Elly Holthuizen for the human IGF-II–luciferase reporter construct Hup3–luc; Dr. Alan Wolfman for Ha–RAS construct; and Shweta Mandrekar, Xiaoxue Zhang, and Jinying Ning for reading the report.

FIGURE 7. Lysine residues responsible for sumoylation/acetylation are important for the transcriptional activity of PLAG1 and PLAGL2. NIH-3T3 cells in 6-well plates were transfected with 2 μg of pcDNAs, pcDNA3–PLAG1 or its mutants, pcDNA3–PLAG1 or its mutant, or expression plasmid for Ha–RAS. After selection in medium with 1% serum and 300 μg/ml G418 for 3 weeks, cells were fixed and stained with methylene blue, and the number of transformed foci was determined (S.D. is shown, n = 2). The data shown here are representative of three independent experiments.
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