Regulation of Hypoxia-inducible Factor 1α (HIF-1α) by Lysophosphatidic Acid Is Dependent on Interplay between p53 and Krüppel-like Factor 5*

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Background: LPA induces HIF-1α expression, but the underlying mechanism remains unknown.

Results: LPA induced HIF-1α by decreasing p53 expression, and additionally, KLF5 transactivated HIF-1α expression.

Conclusion: KLF5 and p53 reciprocally regulate HIF-1α expression.

Significance: This study provides a new dimension to understanding how LPA promotes colon cancer.

Hypoxia-inducible factor 1α (HIF-1α) and p53 are pivotal regulators of tumor growth. Lysophosphatidic acid (LPA) is a lipid mediator that functions as a mitogen by acting through LPA receptors. We have shown previously that LPA stimulates lipid mediator that functions as a mitogen by acting through regulators of tumor growth. Lysophosphatidic acid (LPA) is a tumor cell response by undergoing multiple modifications to adaptation to hypoxia. The importance of HIF-1α in tumorigenesis is underscored by the finding that HIF-1α is overexpressed in a variety of tumors and its expression is often associated with poor prognosis (1–3). HIF-1 activity is involved in multiple aspects of tumorigenesis including tumor cell proliferation, angiogenesis, metastasis, and chemotherapy resistance. HIF-1 is composed of a constitutively expressed β-subunit and a hypoxia-inducible α-subunit (3). HIF-1α is subjected to oxygen-dependent hydroxylation and proteasomal degradation (4). However, hypoxia is not the only condition for HIF-1α induction; in cancer cells, various growth factors, activated oncogenes, or loss-of-function mutations of tumor suppressor genes can induce HIF-1α expression under nonhypoxic conditions (5, 6). Evidence supports the idea that HIF-1α promotes nonhypoxic growth of colon cancer cells and prevents prematurity senescence and aging of cells, particularly following γ-irradiation (7, 8).

LPA is a bioactive lysosphopholipid that evokes multiple growth factor-like responses that modulate cell proliferation, migration, survival, and secretion of cytokines (9, 10). LPA signals through a distinct family of G protein-coupled receptors located on the cell surface. To date, six LPA receptors, LPA₁–LPA₆, have been identified and validated. These receptors activate multiple downstream effectors, including mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), phospholipase C, Rho, and Rac (9, 11, 12). LPA₂ is overexpressed in several types of cancer, and the tumor promoting activity of LPA₂ is of tremendous clinical interest (13–15). LPA₂ deficiency protects mice from colitis-induced colon cancer and decreases tumor burden in ApcMin/⁺ mice (16, 17). In addition,
LPA2-deficient intestinal tumors show reduced expression levels of cyclooxygenase 2, β-catenin, and Krüppel-like factor 5 (KLF5) (16, 17). It has been shown that LPA stimulates vascular endothelial growth factor (VEGF) expression, whereas transgenic expression of LPA2 in mouse ovaries produces VEGF and urokinase-type plasminogen activator (18, 19). It is now known that LPA enhances the secretion of VEGF through the induction of HIF-1α (20). Moreover, hypoxia enhances the effects of LPA on VEGF expression and the metastasis of ovarian cancer cells (20, 21). We observed previously that loss of LPA2 function results in decreased HIF-1α expression in intestinal tumors (16). Similarly, mouse embryos lacking LPA-generating autotaxin lack HIF-1α expression, and the absence of HIF-1α is independent of oxygen tension (22). These results indicate that LPA is a potent regulator of HIF-1α expression, but the underlying mechanism of how LPA induces HIF-1α expression is not well understood. In this study, we report that LPA-mediated HIF-1α expression is dependent on the down-regulation of p53. In addition, we identified KLF5 as a positive transactivator of HIF-1α.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Plasmids**—HCT116, LoVo, HT-29, and SW480 cells were grown as described previously (23). HIF-1α−/− HCT116 cells were described previously (7). p53−/− HCT116 cells were kindly provided by Dr. Vogelstein. The pLenti6 plasmids harboring V5-p53 and V5-p53_R175H (24) were obtained from Addgene (Cambridge, MA). pcDNA/HA-KLF5 was a kind gift from Dr. Vincent Yang (Stony Brook University). Transient transfection was performed using Lipofectamine 2000 (Invitrogen). LPA2 with N-terminal fusion of vesicular stomatitis virus glycoprotein (VSVG-LPA2) was described previously (14). Stable expression of LPA2 was achieved by transduction with lentiviral pCDH/VSVG-LPA2 (11). Lentiviral pCDH was used as a control for transfection of LPA2. pLKO.1 plasmids harboring shRNA targeting LPA2, p53, or HIF-1α were from Sigma. pLKO.1-puro with non-target shRNA was used to generate the control lentivirus. siRNA against Mdm2 or KLF5 and control siRNA were from Sigma.

**Chemicals and Antibodies**—LPA (18:1 Lyso PC; 1-oleoyl-2-hydroxy-sn-glycero-3-phosphate) was purchased from Avanti Polar Lipids (Abalaster, AL). For the in vitro study, LPA was used at a final concentration of 1 μM in PBS containing 0.1% fatty acid-free bovine serum albumin (BSA) unless specified otherwise. An equal volume of PBS containing 0.1% BSA was added as a control. When needed, actinomycin D (ActD, 10 nm), LY294002 (10 μM), or U0126 (10 μM) was used, and an equal volume of dimethyl sulfoxide was added as a vehicle control. Mouse anti-VSVG P5D4 antibody was described previously (14). The following antibodies were purchased: mouse anti-Mdm2 and mouse anti-HIF-1α antibodies (BD Biosciences); mouse anti-actin antibodies (Sigma-Aldrich); rabbit anti-V5 and mouse anti-HA (Covance, Princeton, NJ); and rabbit anti-p53 and rabbit anti-pMdm2 (Ser-166) (Cell Signaling Technology, Danvers, MA).

**Cell Proliferation**—Cells seeded at a density of 2 × 10⁵ cells/well were synchronized by serum starvation for 36 h. Cells were treated with 1 μM LPA once a day for up to 3 days, and the number of cells was counted daily using a hemocytometer.

**Western Immunoblot and Immunoprecipitation**—Immunoprecipitation and Western blotting were performed as described previously (11). Isolation of nuclear proteins for the detection of p53 was done using a NE-PER reagents kit (Thermo Fisher Scientific). Co-immunoprecipitation of Mdm2, p53, and HIF-1α was performed using the Catch and Release® system (EMD Millipore, Billerica, MA) according to the manufacturer’s instructions.

**HIF-1α and p53 Transcription Activity**—The transcription activities of HIF-1α and p53 were performed using a DuoSet IC activity assay kit (R&D Systems, Minneapolis, MN). Briefly, biotinylated double-stranded oligonucleotides containing a consensus HIF-1α or p53 binding site were incubated with 2 mg/ml nuclear extracts from HCT116 cells. Oligonucleotide-bound HIF-1α or p53 complex was captured by an immobilized antibody specific for HIF-1α or p53. After unbound material was washed away, bound oligonucleotides were isolated using streptavidin-horseradish peroxidase (HRP).

**Quantitative RT-PCR (qRT-PCR)**—Total RNA was isolated from cells using TRIzol (Invitrogen), and cDNA was synthesized using the first strand synthesis kit (Invitrogen). qRT-PCR was performed as described (16). The following primer pairs were used for HIF-1α: 5′-CACCTACCTGCGCACCAGT-3′ and 5′-CCCCTTCTCCTGCTTTG-3′.

**Chromatin Immunoprecipitation (ChIP)—ChIP was performed using the EZ-ChIP kit (EMD Millipore) according to the manufacturer’s protocol. Briefly, cells were treated with 1% formaldehyde for 15 min to cross-link proteins to DNA, lysed, and then sonicated. The lysate was incubated with primary antibodies overnight at 4°C. The immunocomplex was purified by incubation with 60 μl of protein G-agarose beads for 1 h and eluted for DNA purification. qRT-PCR was performed with primers for the HIF-1α promoter flanking the putative p53 and KLF5 binding sites (Table 1). Anti-RNA polymerase II and normal mouse IgG were used as the positive and negative control for immunoprecipitation, respectively. The human HIF-1α promoter sequence was found using the Eukaryotic Promoter Database. The putative binding sites were predicted using Algen Promo software, version 3.0.2 (25, 26).

**Ras Activation Assay**—The activation of Ras by LPA was determined using a G-LISA Ras activation assay kit (Cytoskeleton, Denver, CO). Cells were treated with 1 μM LPA for 15 min, and lysates (1 mg/ml) were added to 96-well plates coated with Ras GTP-binding protein (Raf-RBD). After incubation with light shaking at 4 °C for 30 min, the plate was washed three times with washing buffer before the addition of antigen-presenting buffer. The captured GTP-bound Ras was incubated with the anti-Ras antibody followed by HRP-conjugated secondary antibody. Ras activity was quantified by measuring absorbance at 490 nm.

**Confocal Immunofluorescence Microscopy**—Confocal immunofluorescent labeling of HCT116 cells was performed as described (27). Briefly, cells were washed twice with cold PBS, fixed in 4% paraformaldehyde in PBS for 10 min at room temperature, permeabilized in 0.2% Triton X-100 in PBS for 5 min, and blocked in PBS containing 5% normal goat serum for 30
The Mechanism of HIF-1α Induction by LPA

RESULTS

LPA Induces HIF-1α Expression—We have shown previously that exposing HCT116 cells to LPA overnight results in increased HIF-1α expression under normoxic conditions (16). Fig. 1A shows that LPA acutely induced HIF-1α expression and maintained the elevated level for at least 36 h. The induction of HIF-1α by LPA was blocked by ActD (Fig. 1B), indicating that the regulation is at the transcription level. Our previous study showed that loss of LPA2 function attenuates HIF-1α expression in intestinal tumors, suggesting the pivotal importance of LPA2 in this regulation (16). To confirm the role of LPA2, we stably expressed shRNA targeting LPA2 (shLPA2) in HCT116 cells, which resulted in at least a 60% decrease in LPA2 expression (11). In cells transfected with shLPA2, the effect of LPA on HIF-1α mRNA was significantly attenuated compared with control shRNA-transfected cells (Fig. 1C). Increased binding of oligonucleotides containing a HIF-1α response element indicated that HIF-1α induced by LPA was transcriptionally active (Fig. 1D). HIF-1α transcription activity was enhanced by over-expression of VSVG-LPA2 compared with pCDH-transfected control cells, whereas shLPA2 resulted in almost complete loss of the activity. To ascertain that the effect was not HCT116 cell-specific, we determined the effect of LPA on other colon cancer cell lines. LPA markedly increased HIF-1α expression in LoVo cells, but the effect in HT-29 and SW480 cells was so marginal that we could observe only a slight increase in the level of HIF-1α. Similarly, LPA did not induce HIF-1α expression in Caco-2 cells (data not shown). These results suggest that the effect of LPA is cell line-dependent (Fig. 1E).

LPA Negatively Regulates p53 via Mdm2 Induction—The tumor suppressor p53 is frequently mutated in cancer cells. Unlike HCT116 and LoVo cells, which express wild type p53 (wtP53), HT-29 and SW480 cells harbor mutant p53 (mutP53) (28). It was shown previously that LPA decreases p53-dependent transcription (29), and hence we determined p53 expression in cells treated with LPA. Although p53 levels at the resting state were low, LPA clearly decreased p53 protein levels in HCT116 and LoVo cells. In contrast, the expression levels of mutP53 in SW480 and HT-29 cells were considerably higher, and importantly these were not significantly altered by LPA (Fig. 2A). To ascertain the effect of LPA on p53, we determined p53 transcriptional activity in HCT116 cells. LPA led to a significant decrease in p53 transcriptional activity (Fig. 2A). Con-
expression only in wtp53-expressing HCT116 and LoVo cells but not in HT-29 and SW480 cells (Fig. 4D). Interestingly, we could not observe the induction of Mdm2 in p53<sup>−/−</sup> HCT116 cells (Fig. 4E). Taken together, our results show that LPA regulates p53 via a negative feedback loop involving Mdm2.

Mutations in the KRAS gene occur in ~40% of colorectal cancers, and both HCT116 and LoVo cells harbor oncogenic K-Ras (32). LPA stimulates the MAPK and PI3K pathways (10, 14), and so we examined whether LPA regulates p53 and Mdm2 via the MAPK or PI3K effector pathway. Fig. 5A shows that the MEK inhibitor U0126 blocked Mdm2 induction by LPA in HCT116 cells, but the PI3K inhibitor LY290042 did not. Consistently, U0126 blocked LPA-induced changes on p53 and HIF-1α expression (Fig. 5B). Because HCT116 and LoVo cells express oncogenic K-Ras, we questioned whether Ras activity in these cells was altered by LPA. Fig. 5C illustrates that the basal Ras activity was high, but a significant increase in total Ras activity was observed in the presence of LPA. These results suggest that LPA suppresses p53 by activating the Ras pathway, despite the presence of an oncogenic Ras.

**LPA Stimulates Nuclear Translocation of p300**—The activity of HIF-1α under hypoxic conditions is enhanced by recruitment of the transcriptional coactivator p300 (33). To determine whether HIF-1α regulation by LPA involves p300, we assessed the interaction between HIF-1α and p300 by co-immunoprecipitation. As shown in Fig. 6A, LPA significantly increased the interaction between HIF-1α and p300 without changing p300 expression. Increased interaction between HIF-1α and p300 was abolished by the silencing of LPA<sub>2</sub>, further underscoring the importance of LPA<sub>2</sub>. The interaction between HIF-1α and p300 was confirmed by immunofluorescence analysis, which showed an overlapping of HIF-1α and p300 labels in the nucleus (supplemental Fig. 2). In contrast to the p300-HIF-1α interaction, the interaction between p53 and p300 was decreased in response to LPA (Fig. 6B). These results demonstrate that p300 interacts reciprocally with HIF-1α and p53.

**LPA-induced HIF-1α Promotes Colon Cancer Cell Proliferation**—To determine whether HIF-1α induction by LPA is functionally relevant, we assessed proliferation of HCT116 cells. As we showed previously, LPA stimulated proliferation of HCT116 cells. As we showed previously, LPA stimulated proliferation of HCT116 cells via LPA<sub>2</sub> (Fig. 7, A and B) (23, 34). Silencing of HIF-1α significantly attenuated the proliferation of HCT116 cells in the presence of LPA (Fig. 7C). Similarly, anchorage-independent colony formation of colon cancer cells by LPA was attenuated in Hif1α<sup>−/−</sup> cells or in HCT116 cells transfected with shHIF-1α (Fig. 7D) (7). Together, these data demonstrate that HIF-1α plays a critical role in colon cancer cell growth induced by LPA.

**Induction of HIF-1α by LPA Is KLF5-dependent**—Our results thus far showed that down-regulation of p53 is obligatory for the induction of HIF-1α transcription by LPA. However, p53 is a negative regulator of HIF-1α transcription and not an inducer. We have shown previously that the transcription factor KLF5 plays an important role in the proliferation of colon cancer cells in response to LPA (23). Because silencing of either KLF5 or HIF-1α shows equally striking effects on HCT116 cell proliferation, we asked whether KLF5 has any role in HIF-1 regulation or vice versa. As shown previously (23), LPA induced KLF5...
expression in colon cancer cells, but unlike the effect of HIF-1α, KLF5 induction was independent of the p53 status (Fig. 8A). To determine whether KLF5 plays a role in HIF-1α induction by LPA, HIF-1α induction was examined in cells treated with siRNA against KLF5 (23). Fig. 8, B and C, shows that silencing of KLF5 significantly attenuated the induction of HIF-1α mRNA and protein expression in HCT116 and LoVo cells. However, KLF5 knockdown had no effect on HIF-1α in HT-29 cells. These results were corroborated by immunofluorescence analysis, where LPA-induced nuclear expression of HIF-1α was
abrogated by siKL5 (supplemental Fig. 3). The importance of KL5 in HIF-1α expression was confirmed by expressing HA-KL5 in HCT116 cells. HA-KL5 increased the basal expression level of HIF-1α, which was further enhanced by LPA compared with the control-transfected cells (Fig. 8D). On the contrary, the induction of KL5 by LPA was not different between HCT116 and HCT116/HIF1α−/− cells (Fig. 8E), indicating that KL5 expression is not influenced by HIF-1α. These results demonstrate that KL5 is an upstream regulator of HIF-1α in colon cancer cells harboring wtP53.

The results in Fig. 5 show that Mdm2 regulation by LPA is MAPK-dependent. Similarly, we found previously that LPA-mediated KL5 induction is in part regulated via the MAPK pathway (23). Hence, we examined the possibility that KL5 is necessary for Mdm2 regulation. However, knockdown of KL5 did not alter Mdm2 induction by LPA (Fig. 8F), and similarly siMdm2 failed to modulate KL5 induction, suggesting that LPA regulates Mdm2 and KL5 independently downstream of MAPK.

KL5 and p53 Compete for the Hif1α Promoter—To determine whether KL5 directly regulates HIF-1α mRNA expression, we performed Hif1α promoter region analysis using Algen Promo software (25, 26). The Hif1α promoter contains several putative KL5 and p53 binding sites, including three overlapping sites (Fig. 9A). Because of the negative role of p53 in LPA-mediated HIF-1α induction, we postulated that KL5 and p53 compete for the same binding site(s). To determine whether KL5 or p53 binds the Hif1α promoter, we performed a ChIP assay followed by qRT-PCR in HCT116 cells. The direct interaction of KL5 or p53 with the Hif1α promoter was assayed in cells transfected with HA-KL5 or V5-p53. We used four sets of primers that included three KL5/p53-overlapping sites and the region proximal to −893 of the start site of the Hif1α promoter (Table 1). Of these primer sets tested, only the primer pair ranging between −714 and −515 resulted in an amplicon with the predicted size of 200 bp from the anti-HA immunoprecipitates (Fig. 9B). Similarly, a ChIP assay on HCT116 expressing HA-wtp53 or HA-mutp53 using anti-V5 antibody resulted in a PCR amplicon corresponding to the same region. These results suggest that KL5 and p53 bind to the same region of the Hif1α promoter. We next determined whether LPA regulates the occupancy of the Hif1α promoter by KL5 or p53. Fig. 9C shows that LPA stimulated binding of the Hif1α promoter to KL5, whereas the occupancy by p53 was decreased. To test whether p53 competes with KL5 for the Hif1α promoter, we determined whether overexpression of HA-p53 or HA-mutp53 affects the binding of KL5 to the Hif1α promoter. Fig. 9D shows that HA-p53 or HA-mutp53 attenuated LPA-induced binding of KL5 to the Hif1α promoter. Conversely, expression of HA-KL5 further decreased binding of p53 to the Hif1α promoter (Fig. 9E). Together, these results demonstrate that LPA reciprocally regulates KL5 and p53 occupancy of the Hif1α promoter by differentially regulating the expression of KL5 and p53.

However, it remains unanswered as to how HT-29 or SW480 cells escape the induction of HIF-1α by LPA. We postulated that the basis of this difference is the inability of LPA to decrease mutp53 expression. To test this idea, we compared p53 expression in HCT116 and SW480 cells. As shown earlier, LPA had a marginal effect on mutp53 expression in SW480 cells (Fig. 9F). Even with shp53, a relatively small decrease in mutp53 expression was observed, so that mutp53 expression in SW480 was considerably greater than in HCT116 cells. Consequently, shp53 resulted in a small increase in HIF-1α mRNA, which did not reach statistical significance (Fig. 9G). We next overexpressed HA-KL5 together with shp53 to mimic the circumstances of LPA treatment. This resulted in a statistically significant increase in HIF-1α mRNA compared with shCont-transfected cells. However, the increase was smaller compared with the increase in HCT116 cells (Fig. 9G). Hence, we concluded that in colon cancer cells such as HT-29, the high expression of mutp53 that is resistant to LPA-induced down-regulation dilutes the positive effect of KL5, preventing an increase in HIF-1α expression.

DISCUSSION

HIF-1 is an essential mediator of cellular responses to hypoxia, but hormone and growth factors can induce HIF-1 under nonhypoxic conditions (5, 6). A body of evidence has shown that LPA
is such a regulator of HIF-1. In this report, we have demonstrated that LPA-mediated regulation of HIF-1 is p53-dependent. Our data show that LPA down-regulated p53 expression, which was a prerequisite for HIF-1 induction. Down-regulation of p53 was in turn dependent on Mdm2. Mdm2 was initially identified on double-minute chromosomes of spontaneously transformed 3T3 fibroblast and was later found to interact physically with p53 (35, 36). Compelling evidence supports the role of Mdm2 in ubiquitin-dependent proteasomal degradation of p53 (37). In the current study, we did not examine ubiquitination of p53 in response to LPA, but the temporal changes in Mdm2 and p53 expression in response to LPA correlated with the necessity for Mdm2 in the regulation of p53. It has been shown that Mdm2 ubiquitinates p53 in both the nucleus and the cytoplasm (38). LPA treatment primarily increased nuclear Mdm2 expression in HCT116 cells, suggest-
ing that Mdm2 interacts functionally with p53 in the nucleus. It is noteworthy that Mdm2 itself is a transcription target of p53 (39, 40). However, as LPA decreased p53 expression, the effect on Mdm2 did not appear to be a direct result of p53-mediated transcription. On the other hand, the p53 dependence on Mdm2 induction by LPA suggests the presence of a negative regulatory feedback loop through which p53 regulates its own expression level.

Our finding that LPA down-regulated p53 in colon cancer cells is consistent with a previous report that LPA decreased nuclear and cellular p53 expression in A549 lung carcinoma and HepG2 hepatocellular carcinoma cells (29). Consistently, LPA induces HIF-1α and VEGF expression in SK-Hep1 cells with wtp53 but not in p53-null Hep-3b cells (41, 42). On the other hand, it is reported that LPA stimulated HIF-1α expression in ovarian OVCAR-3 and CAVOV-3 cells, despite the presence of mutp53 (41, 43). The basis for the differential cellular responses by the different cell types is not known. Additional factors such as PI3KCA or PTEN status might contribute to HIF-1α regulation. In keeping with this idea, LPA-induced HIF-1α in ovarian cancer OVACAR-3 and pancreatic cancer PC-3 cells was PI3K-dependent, whereas we found that LY290042 does not attenuate HIF-1α induction in colon cancer cells (41). Alternatively, HIF-1α induction by LPA could be controlled by the relative level of mutp53 (see below).

It is known that HIF-1 and p53 bind to the transcription coactivator p300, which is necessary for the full activation of these transactivators (33, 44). Blagosklonny et al. (45) show that the binding of p53 to p300 is needed for the inhibition of HIF-1 activity by p53, so that p53 that is defective in binding with p300 (p53 with mutations at residues 22 and 23) does not inhibit HIF-1α activity (45). Our observation that LPA induced nuclear translocation of p300 and enhanced in its binding to HIF-1α is in agreement with the report by Blagosklonny et al. (45). However, it is unlikely that p300 influenced p53 function in our study in view of decreasing p53 expression. On the other hand, p300 is known to enhance p53 ubiquitination by Mdm2 (30) and p300 could bind to Mdm2 to form part of its degradation complex. Further experiments are needed to validate the involvement of p300 in LPA-mediated p53 regulation.

KLF5 is a transcription factor expressed predominantly in the crypt compartment of the intestinal tract (46, 47). KLF5 has been implicated in Ras-mediated transformation, and intestine-specific deletion of KLF5 in adult mice resulted in distorted villus and crypt structures and defective barrier functions (48, 49). Our previous study identifies KLF5 as a mediator of LPA2-mediated proliferation of colon cancer cells, and studies using LPA2-deficient mice demonstrate the correlation between LPA2 and KLF5 expression in vivo (16, 17, 23). The salient finding of this study is that KLF5 is a transcription factor that regulates HIF-1α expression in cells treated with LPA. The Hif1α promoter contains three overlapping docking sites for KLF5 and p53, but we found that only the region between 252 and 1452 bp upstream of the transcription start was occupied by both KLF5 and p53. LPA treatment enhanced KLF5 binding while decreasing p53 interaction with the Hif1α promoter in HCT116 cells. These data indicate that the primary determinant of which transactivator occupies the Hif1α promoter is the relative expression level of KLF5 and p53. A previous report has shown that survivin expression is under the control of KLF5 and p53 (50). KLF5 binds to the survivin promoter to increase survivin expression while suppressing p53-dependent repression of survivin. However, the Zhu et al. (50) show that p53 does not directly repress the survivin promoter but rather does so through interaction with KLF5. Recently, Yang et al. (51) have reported that KLF5 and p53 regulate Notch1 in the context of esophageal squamous cell transformation. We propose the following model (see Fig. 9H). The transactivation of Hif1α is low when p53 occupies the Hif1α promoter (Fig. 8E). When cells are exposed to LPA, p53 expression is reduced, whereas the KLF5 level increases such that KLF5 out-competes p53, thereby transactivating Hif1α and promoting cancer cell growth. Similarly, mutp53 binds the Hif1α promoter to block Hif1α transcription. However, unlike wtp53, mutp53 is not degraded by LPA, and KLF5 is unable to displace mutp53. Hence, LPA-mediated HIF-1α induction is determined by the balance between KLF5 and p53 expression.

In the series of genetic alterations leading to colorectal cancer, mutation in the p53 gene or loss of p53 function is closely related to the adenoma–carcinoma transition, but these alterations are a late event occurring after mutations in the adenomatous polyposis coli and KRAS genes (32). However, it has been shown that mutational activation of Ras enables cells to survive p53-dependent apoptosis and overexpression of H-Ras(G12V) attenuates p53 expression in NIH3T3 cells (52). Moreover, despite the presence of the KRAS mutation, no accumulation of p53 occurs in aberrant crypt foci (53). The Ras-MEK-Erk cascade is a major signaling pathway activated by LPA. A previous study shows that LPA2 protects colon cancer cells from etoposide-induced apoptosis by activation of Erk and protects mouse intestine from γ-irradiation-induced injury (54, 55). Hence, together with the current observation that LPA2 down-regulates p53 expression, we postulate that LPA2 potentiates the progression of colorectal cancer by activating the Ras-MEK pathway and repressing p53 function.

In summary, we have identified KLF5 as a transcription factor that competitively binds the Hif1α promoter. The current study shows that the pro-oncogenic effects of LPA are compounded by its ability to repress p53 and induce HIF-1α, which enhances tumor growth and neovascular processes.

**TABLE 1**

| Primers used in ChIP assay | Primer sequence |
|---------------------------|-----------------|
| 5′-CTTCTCTCCAGGCTTTCCTCC-3′ | 1452 to −893 |
| 5′-CTGACCCGAGAAATGGACTT-3′ | −913 to −654 |
| 5′-GCTCTGCAAGTTGGACCA-3′ | −714 to −515 |
| 5′-CTGCTGGCTCTCTTTC-3′ | −893 Forward |
| 5′-TGTGACTGAAGCTGAGG-3′ | −320 to −1 |

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