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Research Communication

Deregulated LAP2α Expression in Cervical Cancer Associates with Aberrant E2F and p53 Activities

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Summary

Lamina-associated polypeptide 2 alpha (LAP2α) plays a role in maintaining nuclear structure, in nuclear assembly/disassembly, and in transcriptional regulation. Elevated LAP2α mRNA expression has been previously reported to associate with certain cancer types. The aim of this study was to investigate LAP2α expression in cervical cancer and transformed cells and to identify factors that associate with its differential expression. LAP2α expression was found to be elevated in cervical cancer tissue by microarray, qRT-PCR, and immunofluorescence analyses. LAP2α also showed elevated expression in cervical cancer cell lines and in transformed fibroblasts compared with normal cells. To determine factors associated with elevated LAP2α in cervical cancer, the effect of inhibiting HPV E7 and E6 oncoproteins was investigated. E7 inhibition resulted in a decrease in phosphorylated Rb and an associated decrease in LAP2α, suggesting a role for E2F in regulating LAP2α expression. This finding was confirmed by inhibiting DP1, a co-activator of E2F, which resulted in decreased LAP2α levels. Inhibition of E6 resulted in elevated p53 and an associated decrease in LAP2α, suggesting that p53 associates with the negative regulation of LAP2α expression. This hypothesis was tested by inhibiting p53 in normal cells, and a resultant increase in LAP2α expression was observed. In conclusion, this study provides evidence for elevated LAP2α expression in cervical cancer and suggests that E2F and p53 activities associate with the positive and negative regulation of LAP2α expression, respectively.
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Keywords cervical cancer; lamina-associated polypeptide 2 alpha; E2F; p53.

INTRODUCTION

Cervical cancer is the second most common form of cancer in women worldwide (*1*). Between 90% and 100% of cervical cancer cases are caused by infection with the human papillomavirus (HPV) (*2*), whereby high levels of viral-encoded oncoproteins, E6 and E7, trigger cancer development by binding to and inactivating the growth regulatory proteins, p53, and retinoblastoma (Rb), respectively (reviewed in Ref. *2*). This leads to global changes in gene expression and ultimately causes cellular transformation, which can result in the development of cervical cancer. In order for alterations in gene expression in the cancer state to be studied, we performed expression profiling of normal and cervical cancer tissue. cDNA microarray analysis revealed a significant upregulation of the nuclear structural protein, Lamina-associated polypeptide 2 alpha (LAP2α), in cervical cancer tissue compared with normal.

LAP2α is a member of the LAP family of nuclear proteins and encoded by the LAP2 gene. There are six different LAP2 isoforms (LAP2-α , β, γ, δ, ε, ζ) encoded by LAP2, which are alternatively spliced to produce proteins of differing molecular weights (*3, 4*). The different LAP2 isoforms share a common N-terminal region, and the alpha isoform contains a unique C-terminal tail that lacks transmembrane regions (*3*). This makes LAP2α nonmembrane bound and essentially nucleoplasmic, localizing to the interior of the nucleus unlike its β, γ, δ, and ε counterparts that contain a transmembrane domain and are thus anchored in the inner nuclear membrane (*5, 6*). The function of LAP2α is still being elucidated, but evidence so far has shown that it plays a role in maintaining nuclear architecture through interacting with A-type lamins (*7*); it facilitates postmitotic nuclear assembly through interacting with chromosomes (*8*), and it is involved in regulating transcriptional activity through anchoring hypophosphorylated Rb in the nucleus (*9*). Additionally, it has recently been shown that LAP2α associates with the heat shock protein, Hsp70, suggesting that it could play a role in resistance against stress (*10*).

LAP2α expression has been previously found to associate with cell growth, as in a study by Markiewicz et al. (2002), negligible LAP2α expression was observed in quiescent fibroblasts; however, its expression increased substantially once cells

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Table 1
Genes up-regulated in cervical cancer tissue compared with normal cervix ($P < 0.0005$)

Gene	Description	Fold change	*P*-value
KPNA2	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	4.4	7.80E-09
CCT5	Chaperonin containing TCP1, subunit 5 (epsilon)	3.5	2.20E-06
MTHFD2	Methylenetetrahydrofolate dehydrogenase 2	3.4	2.80E-04
RAN	RAN, member RAS oncogene family	3	2.10E-06
SNRPB	Small nuclear ribonucleoprotein polypeptides B and B1	2.9	5.80E-06
HDGF	Hepatoma-derived growth factor (high-mobility group protein 1-like)	2.8	5.50E-05
	heat shock 60kD protein 1 (chaperonin)	2.7	8.70E-05
SLC25A5	Solute carrier family 25 (mitochondrial carrier)	2.7	1.40E-04
PCNA	Proliferating cell nuclear antigen	2.6	8.40E-07
STMN1	Stathmin 1/oncoprotein 18	2.6	7.50E-07
TMPO	***Thymopoietin/LAP2α***	***2.6***	***2.90E-04***
TPM3	Tropomyosin 3	2.5	2.40E-09
THBS2	Thrombospondin 2	2.5	4.70E-05
ITGB1	Integrin, beta 1	2.4	4.00E-04
HSP90B1	Heat shock protein 90kDa beta (Grp94), member 1	2.4	6.50E-05
	protocadherin alpha 9	2.4	3.70E-07
PSMD11	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	2.3	2.40E-05
LDHA	Lactate dehydrogenase A	2.3	4.40E-04
H2AFZ	H2A histone family, member Z	2.3	2.10E-05
PKM2	Pyruvate kinase, muscle	2.3	2.70E-05

were induced to re-enter the cell cycle (*9*). Furthermore, LAP2α expression was also found to decrease progressively in confluent and postconfluent cultures, co-inciding with entry into G_0 (*11*). These findings imply that LAP2α expression is regulated in a cell cycle-dependent manner, pointing to its potential deregulation in cancer. In line with this, LAP2α expression has been previously shown to be elevated in medulloblastoma (*12*) and larynx, lung, stomach, breast, and colon primary tumors, although only at the mRNA level (*13*). However, LAP2α expression has not been previously determined in cervical cancer material nor has its protein expression been evaluated in any tumor tissue to date. The aim of this study was, therefore, to confirm our microarray study showing increased LAP2α mRNA expression in cervical cancer, to determine its expression at the protein level, and to investigate the molecular mechanisms associated with increased LAP2α expression in cervical cancer.

EXPERIMENTAL PROCEDURES

Patient Material

Patient material was collected from Groote Schuur Hospital, Cape Town, South Africa. Cancer tissue was collected from patients with cervical carcinoma, and "normal" tissue from patients admitted for hysterectomies for reasons other than cervical abnormalities. A pathologist confirmed normal or diseased status. Samples were obtained with patient consent, and the study was approved by the Research Ethics Committee of the University of Cape Town (REC REF:153/2004) (*14*).

RNA Isolation and Microarray Analysis

Total RNA from patient tissue was isolated using Trizol reagent (Invitrogen) and amplified using the Eberwine RNA amplification procedure (*15*), as described previously (*16*). Human reference RNA (Stratagene) served as a control. Microarray analysis was performed using 11,000-element cDNA microarray slides produced by the Microarray Core Facility, National Cancer Institute, USA, using Incyte Genomics UniGEM clones, as described (*14*). Normal and cancer group comparisons were performed in MaDB (*17*) using a multivariate permutation test.

Immunohistochemistry of Patient Tissue Sections

Normal and cancer tissue sections were obtained from archived paraffin-embedded sections and heat-fixed prior to rehydration and de-waxing. Antigen retrieval was performed by pressure-cooking in EDTA, pH 8.0, and slides blocked in 0.2% gelatin. LAP2α specific antibody (5162; Abcam) was added at 1:100 for 1 H in a humidified chamber at RT. Slides were washed and incubated in 0.3% Sudan Black before a Cy3-conjugated goat antirabbit secondary antibody (Jackson ImmunoResearch) was added at 1:300 and incubated for 45 Min. Slides were incubated with DAPI (Sigma), mounted in Mowiol 4-88 (Calbiochem), and visualized on a Zeiss fluorescent microscope.

Table 2
Genes down-regulated in cervical cancer tissue compared with normal cervix ($P < 0.0005$)

Gene	Description	Fold change	*P*-value
	Vimentin	0.4	3.90E-04
AHSG	Alpha-2-HS-glycoprotein	0.6	2.50E-05
CXCL3	Chemokine (C-X-C motif) ligand 3	0.7	6.10E-06
ARID4A	AT rich interactive domain 4A (RBP1-like)	0.7	6.20E-05
C16orf52	Chromosome 16 open reading frame 52	0.7	5.60E-05
B3GNTL1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase-like 1	0.7	3.50E-04
RND2	Rho family GTPase 2	0.7	4.20E-04
CCL7	Chemokine (C-C motif) ligand 7	0.7	1.90E-04
TNFSF13	Tumor necrosis factor (ligand) superfamily, member 13	0.7	3.50E-04
PER2	Period homolog 2 (Drosophila)	0.7	2.60E-04
SLITRK2	SLIT and NTRK-like family, member 2	0.7	3.80E-05
S100A4	S100 calcium binding protein A4	0.7	4.20E-04
	bromodomain adjacent to zinc finger domain, 1B	0.7	4.40E-04
GRIP2	Glutamate receptor interacting protein 2	0.7	1.40E-04
NSMAF	Neutral sphingomyelinase (N-SMase) activation associated factor	0.7	2.80E-04
TSPAN8	Tetraspanin 8	0.7	2.90E-05
SLC1A1	Solute carrier family 1	0.8	7.20E-05
SURF5	Surfeit 5	0.8	4.60E-05
TUBGCP2	Tubulin, gamma complex associated protein 2	0.8	4.20E-04
PDCD11	Programmed cell death 11	0.8	3.20E-04

Images were viewed using Axiovision 4.6 software, and fluorescence intensity quantitated using six fields of view per slide.

Cell Lines and Cell Culture

Cell lines used included: normal primary cervical epithelial cell culture, HCX, its transformed counterpart, HCX-E6/E7 (Dr. C. Baker, NIH, USA (*18*)), cervical cancer cell lines, CaSki, HeLa, ME-180, MS751, SiHa and C33A (American Type Culture Collection (ATCC)), normal lung fibroblasts, WI38, transformed SVWI38 and CT-1 (*19*), normal breast skin fibroblasts, CCD1068SK, and normal skin fibroblasts, FG_0 (UCT). SVWI38 fibroblasts are WI38 cells that were transformed using the SV40 virus (*20*), while CT-1 fibroblasts are WI38 cells that were transformed by gamma irradiation (*19*). All cell lines were maintained in DMEM supplemented with 10% foetal calf serum (Gibco) and 100 units/mL penicillin and 100 μg/mL streptomycin, with the exception of HCX and HCX-E6/E7, which were maintained in keratinocyte serum-free medium (Invitrogen), containing 50 μg/mL bovine pituitary extract, 26 ng/mL epidermal growth factor, and penicillin and streptomycin. HCX-E6/E7 cells were grown in the presence of 50 μg/mL G418. All cells were kept at 37 °C in a 5% CO_2 incubator.

Real-Time RT-PCR

qRT-PCR was performed using primers specific for LAP2α: F 5′-GCAGGCAGACATTAGTCAAGC-3′, R 5′-CGACCTACAGTGGCATTTCC-3′; p53: F 5′-CAACAAGATGTTTTGCCAACTG-3′, R 5′-ATGTGCTGTGACTGCTTGTAGATG-3′; CyclophilinD: F 5′-TGAGACAGCAGATAGAGCCAAGC-3′, R 5′-TCCCTGCCAATTTGACATCTTC-3′; and β-glucuronidase: F 5′-CTCATTTGGAATTTTGCCGATT-3′, R 5′-CCGAGTGAAGATCCCCTTTTTA-3′. qRT-PCR was performed using KAPA SYBR qPCR Master Mix (KAPA Biosystems) and the StepOne Real-Time PCR machine (Applied Biosystems). The $\Delta\Delta C_T$ method was used to calculate gene expression relative to that of the average of cyclophilin D and β-glucuronidase (*21*).

Immunofluorecence of Cultured Cells

Cells were plated on coverslips, grown to 80% confluency, and fixed in 4% paraformaldehyde. Fixed cells were incubated in 0.1% Triton X-100 in PBS followed by 50 mM NH_4Cl in PBS and blocked in 0.2% gelatin. Cells were subsequently incubated with 1:100 LAP2α primary antibody (5162; Abcam) for 45 Min in a humidified chamber. After washing in PBS, 1:300 Cy3-conjugated goat antirabbit secondary antibody (Jackson ImmunoResearch) was applied for a further 45 min. Cell nuclei were stained with 100 ng/mL DAPI and coverslips mounted in Mowiol.

RNA Interference

Cells were transfected with 20 nM LAP2 siRNA (sc-43386), DP1 siRNA (sc-37813), p53 siRNA (sc-29435), or Control

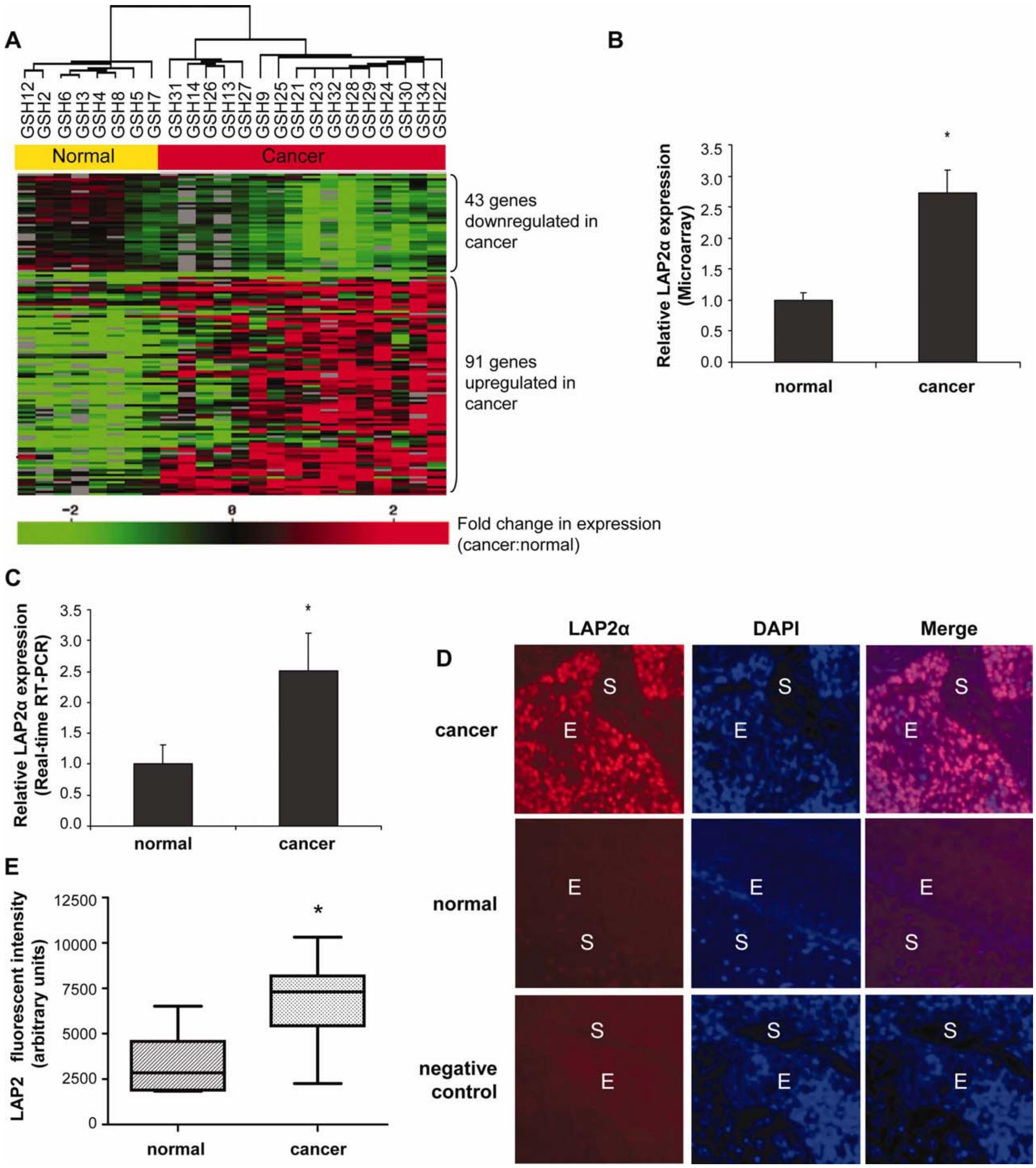

Figure 1. Relative LAP2α mRNA and protein levels in normal and cervical cancer tissues. (A) A heat map view of the expression patterns of down- or up-regulated genes in the cancer specimens compared with the normal specimens (green: low expression, red; high expression). (B, C.) LAP2α mRNA expression determined by (A) microarray analysis (normal: $n = 8$, cancer: $n = 16$, $*P < 0.0001$) and (B) real-time RT-PCR analysis (normal: $n = 9$, cancer: $n = 16$, $*P < 0.05$). β-glucuronidase and cyclophilin D were used as normalizers of the real-time PCR data. Results shown for both microarray and real-time RT-PCR analyses represent the mean ± standard error of the mean (SEM). (D) Immunofluorescence showing LAP2α protein expression in representative normal and cervical cancer tissue sections (400× magnification). (E: epithelium; S: stroma) (E) Quantification of LAP2α protein expression in normal and cancer tissue sections (normal: $n = 9$, cancer: $n = 16$, $*P < 0.05$). Box and whisker plots were generated in Graphpad Prism and represent the mean ± minimum and maximum values. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

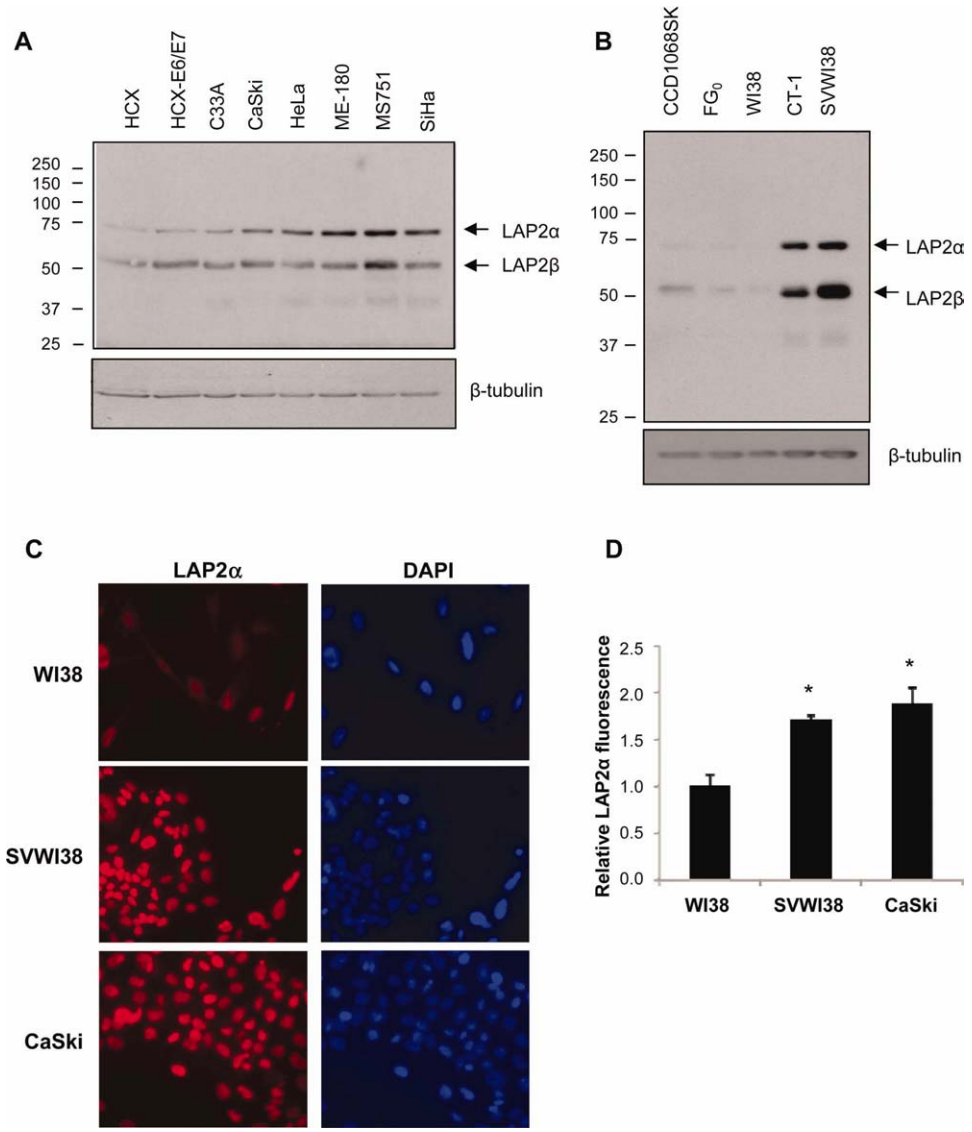

Figure 2. LAP2α protein expression in cultured cell lines. Western blot analysis showing LAP2α and LAP2β expression in (A) cervical cancer cells (C33A, CaSki, HeLa ME-180, MS751, SiHa) compared with normal cervical epithelial cells, HCX, and HCX-E6/E7-transformed cells; and (B) transformed lung fibroblasts (CT-1 and SVWI38) compared with normal fibroblasts (CCD1068SK (breast), FG_0 (skin), and WI38 (lung)). β-tubulin was used to control for protein loading. (C) Immunofluorescence showing LAP2α expression in WI38, SVWI38 and CaSki cells, using a LAP2α specific antibody. (D) Quantification of LAP2α fluorescence obtained from at least 100 cells per cell line, $*P < 0.05$. Experiments were performed at least two independent times. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

siRNA-A (sc-37007) (Santa Cruz Biotechnology), using TransFectin lipid reagent (Bio-Rad). HPV16 E6 and E7 siRNAs were designed as described in Ref. (22). RNA oligos were synthesized by Ella Biotech GmBH (Germany) and annealed in a reaction containing 30 μL each RNA oligo (50 μM) and 15 μL annealing buffer (100 mM potassium acetate, 30 mM Hepes, pH 7.4, 2 mM magnesium acetate) at 90 °C for 1 Min, followed by 37 °C for 1 H, to give a 20 μM siRNA concentration. Cells were transfected with 20 nM E6 siRNA or E7 siRNA.

Western Blot Analysis

Protein was harvested in RIPA buffer (10 mM Tris–Cl, pH 7.4, 150 mM NaCl, 1% sodium deoxycholate, 0.1% SDS, 1% Triton X-100, 1X complete protease inhibitor cocktail (Roche) and 1 mM Na_3VO_4 phosphatase inhibitor). Western blots were performed using antibodies against LAP2 (sc-28541, Santa Cruz Biotechnology), HPV16 E7 (sc-6981, Santa Cruz Biotechnology), phospho-RB (ser 807/811) (#9308S, Cell Signaling), p53 (M7001, DakoCytomation), p21 (sc-756, Santa Cruz

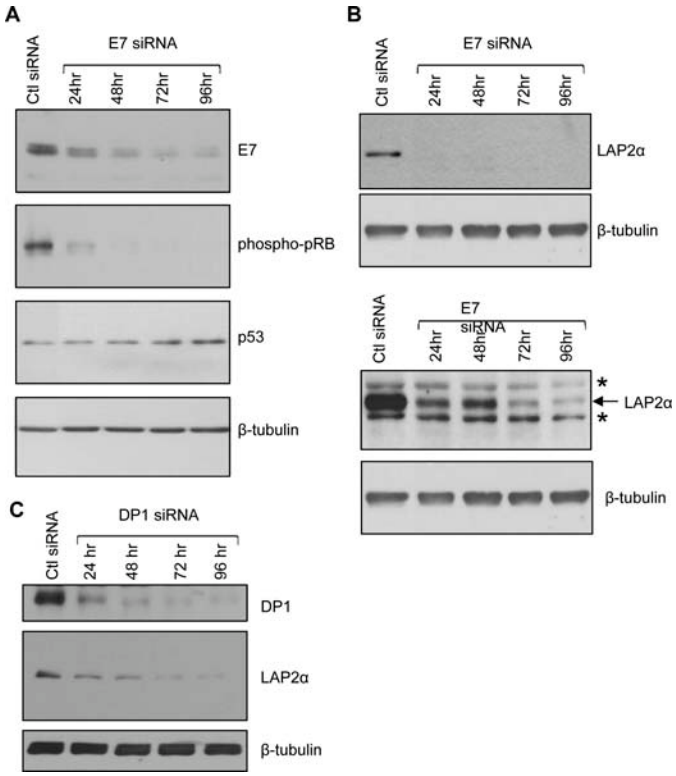

Figure 3. LAP2α expression associates with E2F. (A) Western blot analysis showing E7, phospho-RB, and p53 levels in response to E7 inhibition using 20 nM E7 siRNA. To control for the nonspecific effects of siRNA transfection, 20 nM control (ctl) siRNA was used. (B) Western blots showing decreased LAP2α expression within 24 H of E7 inhibition. Lower panel: A longer exposure of the Western blot, showing LAP2α expression in response to E7 siRNA treatment, reveals a time-dependent decrease in LAP2α levels. (C) Western blots showing decreased LAP2α expression after DP1 inhibition. β-tubulin was used as a control for protein loading.

Biotechnology), DP1 (sc-610, Santa Cruz Biotechnology), and β-tubulin (sc-9104, Santa Cruz Biotechnology).

RESULTS

Differential LAP2α mRNA and Protein Expression in Normal and Cervical Cancer Patient Specimens

To identify genes with differential expression patterns in cervical cancer and normal tissue cDNA, microarrays were performed using RNA derived from eight normal and 16 cervical cancer tissue specimens. Many genes were identified to have either up- or down-regulated expression in cervical cancer tissue compared with normal (Tables 1 and 2, Fig. 1A), and among the genes identified to have significantly enhanced levels of expression in the cancer samples was LAP2α, also known as Thymopoietin (TMPO) (Table 1 and Fig. 1B). To confirm increased LAP2α expression in the cancer specimens, real-time RT-PCR was performed using RNA from 16 cervical cancer and nine normal biopsy specimens. Results showed that LAP2α expression was significantly increased in the cervical cancer patient material compared with normal, validating the microarray data (Fig. 1C).

Having established that cervical cancer tissue displays elevated LAP2α mRNA, immunofluorescence was next used to assay LAP2α protein expression in archival patient tissue sections. High LAP2α expression was detected in the cervical cancer cells, and its expression was evident compared with that in the surrounding stromal tissue, which showed little staining (Fig. 1D). In contrast, very low LAP2α expression was detected within the epithelium of normal cervical tissue. Quantification of LAP2α fluorescence intensity in 16 cancer and nine normal tissue sections revealed a significant increase in LAP2α expression in the cancer specimens (Fig. 1E). These findings support the microarray and real-time RT-PCR data, and together suggest that enhanced LAP2α mRNA and protein expression associate with cervical cancer.

LAP2α Protein Expression in Normal, Cervical Cancer, and Transformed Cell Lines

To determine whether LAP2α expression is elevated in cervical cancer cells grown in culture, its expression was determined in a panel of cervical cancer cell lines (C33A, CaSki, HeLa, ME-180, MS751, and SiHa) and compared with that in a normal primary cervical epithelial cell culture, HCX, and its transformed counterpart HCX-E6/E7. Western blot analysis revealed that LAP2α expression was elevated in all of the cancer cell lines compared with normal (Fig. 2A). As the antibody used for Western blot analysis recognizes the common N-terminal region of the human LAP2 isoforms, LAP2β expression was also detected in the cancer cells and found to be elevated in the cervical cancer cell lines. Expression of LAP2α and LAP2β was also increased in the HPV E6/E7-transformed cell line compared with the normal HCX cells, suggesting that expression of LAP2 may associate with cellular transformation.

To investigate the possibility that LAP2α expression associates with cellular transformation, its expression was determined in normal (CCD1068SK, FG_0, and WI38) and transformed (SVWI38 and CT-1) fibroblasts. Western blots showed barely detectable LAP2α in the normal fibroblasts, while the transformed fibroblasts showed substantially higher levels of expression (Fig. 2B), again suggesting that elevated LAP2α expression associates with cellular transformation. Similar results were seen for LAP2β. The increase in LAP2α expression was independently confirmed by immunocytochemistry, using a LAP2α-specific antibody, with transformed SVWI38 cells showing elevated LAP2α expression compared with the untransformed WI38 cells and comparable with that of the cervical cancer cell

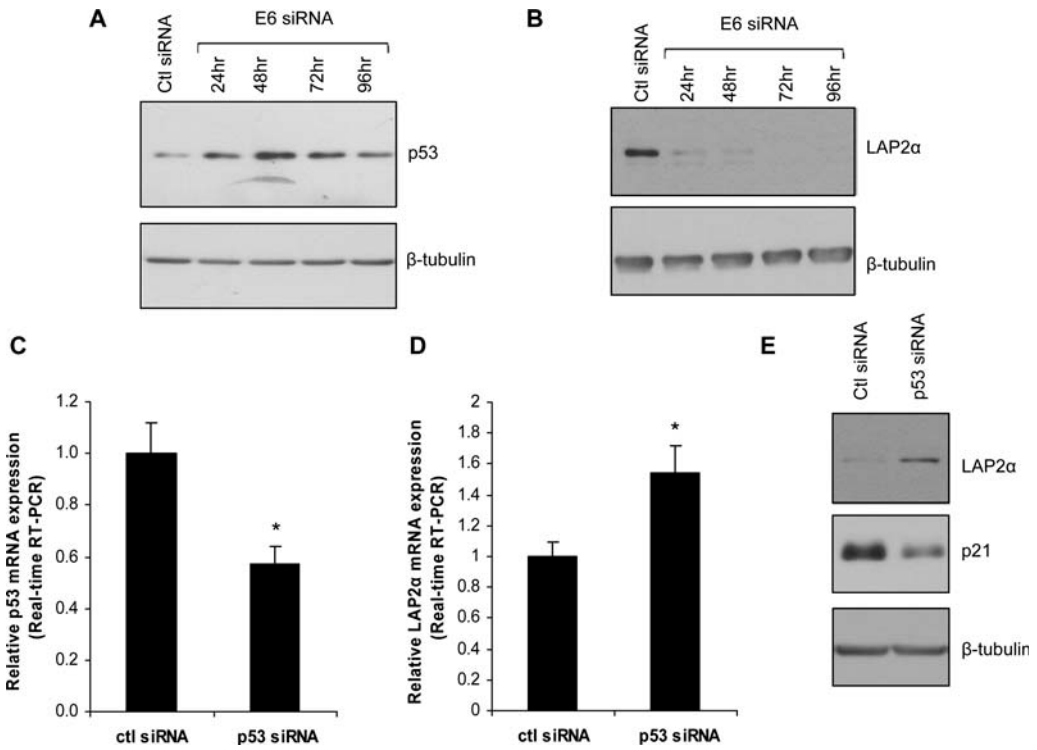

Figure 4. LAP2α expression associates with p53 expression. (A) Western blot analysis showing an increase in p53 levels in CaSki cell lysates after E6 inhibition. (B) Western blots showing decreased LAP2α expression after E6 siRNA transfection. (C,D) Real-time RT-PCR data showing (C) p53 and (D) LAP2α mRNA expression in WI38 cells transfected with p53 siRNA (*$P < 0.05$). (E) Western blot analysis showing LAP2α protein expression in p53 inhibited cells. p21 levels decreased confirming the inhibition of p53 activity. β-tubulin was used as a control for protein loading.

line, CaSki (Fig. 2C). Quantification of the fluorescence showed significantly higher LAP2α levels in the cancer and transformed cells compared with the normal cells (Fig. 2D).

Elevated LAP2α Expression in Cervical Cancer Cells Associates with Aberrant E2F/RB and p53 Activities

As we had observed high LAP2α expression in cervical cancer, we next investigated the factors that could account for its elevated expression. Previous studies in osteosarcoma cells reported that LAP2α expression is regulated by members of the E2F family (*13*). In cervical cancer, the HPV oncoproteins, E6 and E7, bind to and block the functions of the tumor suppressor proteins, p53 and RB, respectively. E6 binds p53, targeting p53 for degradation (*23*). E7 binding to RB interferes with the function of RB (*24*), where under normal circumstances, hypophosphorylated RB forms a complex with the transcription factor E2F and inhibits E2F activity. The binding of E7 to RB sequesters RB and causes its phosphorylation and degradation, resulting in the untimely release of active E2F (*25*). Hence, we postulated that inhibition of E7 in cervical cancer cells would result in hypophosphorylated RB, which in turn would inhibit E2F activity and affect LAP2α expression. E7 siRNA was thus used to inhibit E7 expression in CaSki cervical cancer cells, and a substantial reduction in phosphorylated RB was observed (Fig. 3A). E7 inhibition and hypophosphorylated RB associated with a significant reduction in LAP2α expression (Fig. 3B), likely as a result of the ability of hypophosphorylated RB to bind and inhibit E2F activity. A longer exposure of the Western blot showing LAP2α expression in response to E7 siRNA treatment reveals a time-dependent decrease in LAP2α levels (Fig. 3B, lower panel). To investigate the requirement of E2F for high LAP2α expression in cervical cancer, the activity of the E2F proteins was inhibited, by silencing the expression of DP1, a co-activator essential for E2F function. Inhibition of DP1 resulted in a reduction in LAP2α expression within 24 H of DP1 siRNA transfection (Fig. 3C). These results suggest that high E2F activity is required for the elevated levels of LAP2α observed in cervical cancer cells. We also observed an increase in the levels of p53 after inhibition of E7 in cervical cancer cells (Fig. 3A), suggesting an involvement of wildtype p53 in the regulation of LAP2α, as increasing p53 levels associated with a decrease in expression of LAP2α.

To investigate a possible role for p53 in regulating LAP2α expression, the expression of HPV E6, the negative regulator

of p53 in cervical cancer, was inhibited using siRNA. E6 inhibition resulted in elevated p53 levels (Fig. 4A) and was shown to associate with a significant decrease in expression of LAP2α (Fig. 4B), suggesting that p53 might play a role suppressing LAP2α expression under normal conditions. To test this hypothesis, p53 expression was inhibited in normal WI38 cells, containing wildtype p53 and low LAP2α expression. p53 inhibition using siRNA (Fig. 4C) resulted in an increase in both LAP2α mRNA and protein expression (Figs. 4D and 4E). To confirm interference with p53 activity, the expression of p21, a downstream target of p53, was determined, and a decrease in p21 was observed in p53 siRNA transfected cells (Fig. 4E).

DISCUSSION

This study provides evidence for elevated LAP2α mRNA and protein expression in cervical cancer and describes an association of E2F and p53 with its expression in cervical cancer cells. We also show that expression of LAP2α is increased in transformed cells in culture compared with their untransformed counterparts, suggesting that LAP2α overexpression is likely a feature of the transformed phenotype.

LAP2α has been previously reported to be regulated by E2F in osteosarcoma cells through the binding of E2F to the LAP2 promoter (*13*), and our results support of a role for E2F in regulating LAP2α expression in cervical cancer cells. Interestingly, LAP2α plays a role in the negative regulation of E2F activity, as it has been found to anchor hypophosphorylated Rb in the nucleus, and this in turn enhances Rb stability and Rb silencing activity, resulting in the decreased activation of E2F target genes (*9, 26*). Hence, it appears that LAP2α and E2F relate in the form of a negative feedback loop. We propose that in cervical cancer, however, where Rb is inactivated by the HPV E7 protein, it is likely that the feedback loop is broken, hence contributing to deregulated LAP2α expression.

In line with this, we show that LAP2α levels decrease when HPV E7 is inhibited. Published reports show that after inhibition of E7 in CaSki cervical cancer cells, the cells undergo a G1 arrest and accumulate hypophosphorylated RB (*27*). Based on RB function, hypophosphorylated RB results in a subsequent inhibition of E2F and we postulate that this causes the decrease in LAP2α expression. However, it is difficult to distinguish whether the changes in LAP2α expression upon E7 inhibition are due to the fact that E2F function is inhibited or, alternatively, due to cell cycle arrest (via an E2F-independent manner).

Our results are a first to suggest a potential role for p53 in the negative regulation of LAP2α expression. As cancer cells commonly contain mutations in the p53 gene (*28*), it is likely that this contributes to the deregulation of LAP2α expression observed in several cancer types (*12, 13*). We show that inhibition of p53 affects LAP2α at the mRNA and protein levels. Whether p53 directly or indirectly regulates LAP2α at the promoter level is unclear at present, and future work is required to address this.

Together, the findings presented in this study suggest that the growth regulatory proteins, E2F and p53, associate with the positive and negative regulation of LAP2α expression, respectively. We propose that activation of E2F and inactivation of p53 by HPV E7 and E6 proteins, respectively, are key contributors to LAP2α overexpression in cervical cancer. The functional significance of LAP2α overexpression in cancer, however, remains to be elucidated, and the contradictory reports in literature regarding its potential role in cell proliferation highlight the need for further work (*11, 26, 29*).

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