The Expression of Oct3/4A mRNA and Not Its Isoforms is Upregulated by the HPV16 E7 Oncoprotein

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Abstract

Oct3/4 a transcription factor is involved in maintaining the characteristics of cancer stem cells. Oct3/4 can be expressed differentially with respect to the progression of CC. In addition, Oct3/4 can give rise to three isoforms by alternative splicing of the mRNA Oct3/4A, Oct3/4B and Oct3/4B1. The aim of this study was to evaluate the mRNA expression from Oct3/4A, Oct3/4B and Oct3/4B1 in low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), cervical cancer (CC) samples, and measure the effect of the HPV16 E7 oncoprotein on the mRNA expression from Oct3/4 isoforms in the C-33 A cell line. The expression levels of Oct3/4A, Oct3/4B and Oct3/4B1 mRNA were analyzed by reverse transcription quantitative polymerase chain reaction (RT-qPCR) in patients with LSILs, HSILs and CC. Additionally, C-33 A cells that expressed the HPV16 E7 oncoprotein were established to evaluate the effect of E7 on the expression of Oct3/4 mRNA isoforms. Oct3/4A (p=0.02), Oct3/4B (p=0. 001) and Oct3/4B1 (p<0. 0001) expression is significantly higher in patients with LSIL, HSIL and CC than in woman with non-IL. In the C-33 A cell line, the expression of Oct3/4A mRNA in the presence of the E7 oncoprotein increased compared to that in nontransfected C-33 A cells. Oct3/4B and Oct3/4B1 mRNA were expressed at similar levels among the different groups. These data indicate that only the mRNA of Oct3/4A is upregulated by the HPV16 E7 oncoprotein.

Introduction

Cervical cancer (CC) is a worldwide public health problem, ranking fourth in incidence and mortality among cancers [1]. CC is a multifactorial disease [2, 3]. However, persistent high-risk human papillomavirus (HR-HPV) infection, mainly HPV types 16 and 18, is the most important risk factor and is mainly related to oncogenic potential of the E6 and E7 oncoproteins [4, 5]. The E7 oncoprotein of HPV promotes cell proliferation and inactivates the G1-S checkpoint of the cell cycle [6]. In addition to HPV oncoproteins, genes associated with stem cells, including Oct3/4, Sox2 and Nanog, have also been shown to be related to CC and are overexpressed in CC cells [7–9].

Octamer-binding transcription factor 4 (Oct3/4), also known as POU5F1, is a nuclear transcription factor of the POU family that binds to the 5'-ATTTGCAT-3' octamer motif, forms a trimeric complex with Sox2 in the DNA and controls the expression of several genes involved in embryonic development [10]. The human Oct3/4 gene consists of five exons that can generate three isoforms, Oct3/4A, Oct3/4B and Oct3/4B1 [11, 12], by alternative mRNA splicing, and these isoforms generate four proteins: Oct3/4A, Oct3/4B-190, Oct3/4B-265 and Oct3/4B-164 [11]. Oct3/4 is overexpressed in CC [8, 9]. Oct3/4 has also been reported to be overexpressed in HR-HPV-positive CC cell lines (HeLa and Caski) compared to HPV-negative cell lines (C-33A). Therefore, Oct3/4 overexpression is closely related to HPV-AR infection (HPV16 and HPV18)[13].

Previously, it was observed that the E7 oncoprotein upregulates the expression of factors such as Oct3/4, Sox2 and Nanog both *in vivo* and *in vitro* [14]. However, it is unknown which isoform is deregulated by the HPV16 E7 oncoprotein. While the expression of Oct3/4 has been detected in several cancer cell lines and tumors, little information exists about the differences in the expression of Oct3/4 splice variants in CC. Indeed, almost nothing is known about the expression of Oct3/4 isoform variant in cervical tissues. Thus, we investigated the expression of Oct3/4 isoforms in a series of cervical samples, and we analyzed the effect of the E7 oncoprotein of HPV16 on the level of mRNA expression of the Oct3/4 isoforms, contributing to understanding the importance of Oct3/4 expression in the development of CC and improving its application in clinical diagnosis.

Materials And Methods

Public expression data download and analysis

Gene expression data related to CC were obtained from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO: www.ncbi.nlm.nih.gov/geo). TCGA analysis was performed using cBioPortal [15, 16] and Gene Expression Profiling Interactive Analysis (GEPIA) [17] software. GEO analysis was performed with the GSE7803 dataset [18] (platform: GPL96 [HG-U133A] Affymetrix Human Genome U133A Array) in GEO2R software [19]. Data for nonmalignant cervical tissues and various International Federation of Gynecology and Obstetrics (FIGO) stages of carcinomas from the TCGA dataset were used to assess the differences in Oct3/4 mRNA expression levels.

Patients and clinical samples

A total of 67 females aged 19-88 years were enrolled in this study from September 2017 to March 2019. All participants were from the Servicio de Diagnóstico Integral en la Detección Oportuna de Cáncer Cérvico Uterino y VPH of the Facultad de Ciencias Químico Biológicas at the Universidad Autónoma de Guerrero and the Servicio de Ginecología Oncológica at the Instituto Estatal de Cancerología (Guerrero, Mexico).

The patient samples were further divided into two groups: (1) those sent to the histopathology service for diagnosis according to the classification system of the FIGO [non-intraepithelial lesion (non-IL; N= 18), low-grade squamous intraepithelial lesion (LSIL; N= 13), high-grade squamous intraepithelial lesion (HSIL; N= 15) and CC (N= 21)] and (2) those sent for DNA and RNA extraction for HPV16 typing and analysis of expression of the Oct3/4 isoforms. The exclusion criteria for the study were as follows: patients with a history of chemotherapy or radiotherapy; patients with previous physical treatment of the cervix; and patients with autoimmune diseases. This study was reviewed and approved by the ethical committee of each participating institution. All procedures were in accordance with the Helsinki Declaration. Informed consent was obtained from all participants. Data confidentiality was maintained throughout the study.

Sample collection and DNA extraction

Sample collection was performed as described previously [20]. Briefly, for HPV detection, cytobrushes with cervical scrapes (non-IL and LSIL) were placed in lysis buffer (10 mM Tris pH 8.0, 20 mM EDTA pH 8.0, and 0.5% sodium dodecyl sulfate) and removed after the cervical material was separated; the samples were stored at −20°C until analysis. For HPV detection in women with HSIL or CC, biopsy specimens were eluted in phosphate-buffered saline (PBS) and stored at −70°C until analysis. DNA purification from cervical samples was performed with the standard SDS-proteinase K-phenol-chloroform method [21] .

HPV16 detection

HPV16 DNA was detected and identified by the INNO-LiPA HPV Genotyping Extra CE assay. Briefly, PCR was performed in a final reaction volume of 50 µl containing 40 µl of PCR master mix and 10 µl of the extracted DNA. The amplification program was as follows: 9 min at 94°C, 40 cycles of 30 sec at 94°C, 45 sec at 52°C and 45 sec at 72°C, with a final extension step at 72°C for 10 min. The PCR amplicons were subsequently analyzed by reverse hybridization on a nitrocellulose strip following the manufacturer's instructions. The line probe assays were evaluated by two independent observers and were adjudicated by a third observer when different results were reported, which did not occur in this study.

Cell culture and transfection

The C33-A cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) containing 10% FBS (Gibco, Life Technologies, Grand Island, NY, USA) and an antibiotic-antimycotic mixture (100 U/ml penicillin and 100 µg/ml streptomycin; Invitrogen, Carlsbad, CA, USA). The cells were cultured in plates and maintained at 37°C with 5% CO2. The cells were transfected with PEGFP-N1-E7 (Organista, 2017) or PEGFP-N1 using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol. Nontransfected C-33 A cells were used as a control.

Total RNA extraction and quantitative real-time PCR (RT-qPCR)

Total RNA (large- and small-sized RNAs) from clinical specimens and from cultured cells was extracted using TRIzol Reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's recommended protocol. The A260/A280 ratio was used to assess the purity of the RNA (>1.8 indicated high purity), and the RNA concentration was determined using a spectrophotometer (NanoDrop 2000 Thermo Scientific).

Total RNA (1 mg) was reverse transcribed into cDNA with oligo (dT) primers and the Superscript II First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) for RT-PCR according to the manufacturer's instructions. Quantitative real-time PCR was performed with a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Calif., USA) using TaqMan Universal PCR Master Mix II (Applied Biosystems, Foster City, CA, USA) and the protocol provided by the manufacturer.

PCR was carried out in a final reaction volume of 15 µl including 7.5 µl 2X TaqMan Universal PCR Master Mix II (containing Taq DNA polymerase, reaction buffer, dNTP mix, 1 mM MgCl2), 0.5 µM of each primer, 5 µl of the template (300 ng of template per reaction) and a variable volume of ultrapure water. All primer sequences and product sizes are described in Table 1. The expression levels of the mRNAs were determined from the threshold cycle (Ct), and the relative expression levels were calculated using the 2ΔΔCt method [22]. For mRNA quantification, the Ct values were normalized to the mRNA expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). mRNA quantification was performed in triplicate, and both negative and positive controls (data not shown) were included in each reaction.

Table 1
Oligonucleotide sequences and probes used in this work.

Isoformas	Oligonucleótide sense (5' a 3')	Oligonucleótido antisense (5' a 3')	Probe	Size (bp)	Ref.
RT-qPCR					
OCT4A	CGCAAGCCCTCATTTCAC	CATCACCTCCACCACCTG	CTTCGGATTTCGCCTTCTCGCCC	111	(Asadi et al., 2011).
OCT4B	CAGGGAATGGGTGAATGAC	AGGCAGAAGACTTGTAAGAAC	AGTTAGGTGGGCAGCTTGGAAGGCA	177	
OCT4B1	GGGTTCTATTTGGTGGGTTCC	TCCCTCTCCCTACTCCTCTTCA	ATTCTGACCGCATCTCCCCTCTAAG	128	
GAPDH	GTGAACCATGAGAAGTATGACAAC	CATGAGTCCTTCCACGATACC	CCTCAAGATCATCAGCAATGCCTCCTG	123	
RT-PCR					
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCCTGTTGCTGTA		450	Xu et al., 2015
E7	CCCAGCTGTAATCATGCATG	TGCCCATTAACAGGTCTTCC		269	Aguilar-Lemarroy et al.,2002

Statistical analysis

The data are presented as the mean ± standard deviation (SD) and were analyzed using GraphPad Prism software (v5.0; GraphPad Software, Inc., CA, USA). The differences between experimental groups were analyzed using a Mann-Whitney test. P values less than 0.05 were considered to indicate a statistically significant difference.

Results

Oct3/4 is expressed in patients with cervical tumors.

Analysis of the mRNA from tumor and nontumor samples from the TCGA in GEPIA revealed that the expression of Oct3/4 in tumor samples was upregulated compared with that in nontumor samples (Figure 1A). Oct3/4 expression was compared between different types of cervical carcinoma samples in cBioPortal and was found to be upregulated in cervical squamous cell carcinoma and endocervical adenocarcinoma (Figure 1B).

Oct3/4A and Oct3/4B1 are highly expressed in CC.

To analyze the Oct3/4 isoforms in CC and compare their expression between them, we used forward, and reverse primers and probes previously reported by Asadi et al., 2011. The primers used prevent the nonspecific amplification of Oct3/4 pseudogenes [23]. To identify whether Oct3/4 isoforms are differentially expressed between LSIL, HSIL and CC patients, samples of LSIL (n=13), HSIL (n=15), CC (n= 21) patients and women with non-ILs positive for Oct3/4A, Oct3/4B and Oct3/4B1 expression (n= 18) were examined. The analysis clearly demonstrated that Oct3/4 isoforms expression were significantly increased in LSIL, HSIL and CC patients (Figure 3A).

Our data revealed Oct3/4A expression was detected in 27.00% (5/18) of women with non-ILs. Oct3/4A mRNA was observed in 38.00% (5/13) of LSIL patients and in 40.00% (6/15) of HSIL patients. Oct3/4A mRNA expression was observed in 71.00% (15/25) of CC patients (Figure 2). The average expression of Oct3/4A mRNA was 7.88±1.15-fold (mean ± standard error [SE]) higher in LSIL patients than in patients with non-ILs (p=0.004). In patients with HSILs, the Oct3/4A mRNA expression level was also significantly increased, with an average of 8.83±6.55-fold increase compared with that detected in women with non-ILs (P=0.006). Compared with that in women with non-ILs, the expression level of Oct3/4A mRNA in CC patients was also significantly increased by an average of 82.11±55.51-fold (P=0.0004; Figure 3A).

Oct3/4B was detected in LSIL, HSIL and CC patients and in women with non-ILs. Oct3/4B mRNA expression was observed in 22.00% (4/18), 38.00% (5/13), 27.00% (4/15) and 38.00% (8/21) of women with non-ILs, LSILs, HSILs and CC, respectively (Figure 2). However, the expression level was much higher in the LSIL, HSIL and CC samples than in the non-IL samples. The average expression of Oct3/4B mRNA was 2.14±0.56-fold higher in patients with LSILs than in women with non-ILs (p=0.016; Figure 3B). In patients with HSILs, the Oct3/4B mRNA expression level was also increased by an average of 7.09 5.27-fold compared with that in the women with non-ILs (p=0.029; Figure 3B). Compared with that in the women with non-ILs, the expression level of Oct3/4B mRNA in the CC patients was significantly increased by an average of 13.75±8.49-fold (P=0.004; Figure 3B).

On the other hand, Oct3/4B1 mRNA was observed in 22.00% (4/18) of women with non-ILs, in 31.00% (4/13) of LSIL patients, in 33.00% (5/15) of HSIL patients and in 67.00% (14/21) of CC patients. The average expression of Oct3/4B1 mRNA was 8.25±3.40-fold higher in LSIL patients than in women with non-ILs women (p=0.033; Figure 3C). In patients with HSIL, the Oct3/4B1 mRNA expression level was also increased by an average of 11.95±4.79-fold compared with that in the women with non-ILs (p=0.009; Figure 3C). Compared with that in the women with non-ILs, the expression level of Oct3/4B1 mRNA in the CC patients was significantly increased by an average of 15.01±7.31-fold (p<0.0001; Figure 3C). Our data revealed that Oct3/4 isoforms (A, B and B1) are expressed in a higher percentage of CC patients than in women with non-ILs.

To obtain a broader view of these results, the level of expression of the Oct3/4A and Oct3/4B isoforms was evaluated from data available in the TCGA and data obtained from a GSE7803 dataset. From this analysis, we observed an increase in Oct3/4A and Oct3/4B mRNA levels in CC samples, although this increase was not significant (Figure 3D to 3G). It was previously shown that Oct3/4A functions as the active isoform, while Oct3/4B is more closely related to the regulation of the expression of the active isoform [8], which could explain why the level of expression of Oct3/4B remains constant, while Oct3/4A increases, favouring the maintenance of the CC.

Increased levels of Oct3/4A mRNA are observed in cells expressing the HPV16 E7 oncoprotein.

Previously, it was observed that the oncoprotein HPV16 E7 increases the level of Oct3/4 expression both *in vivo* and *in vitro* [14]. However, it is unknown which isoform is deregulated by the HPV16 E7 oncoprotein. Here, we observed an effect on the expression of Oct3/4A and Oct3/4B1 mRNA with the increase's degree of lesions in HPV16-positive samples. To investigate the effects of the HPV16 E7 oncoprotein on the expression of Oct3/4 mRNA isoforms, we established C-33 A cells that transiently express the E7 oncoprotein, according to a previously reported method [14]. As a positive control for the Oct3/4 mRNA PCR assay, we used cDNAs derived from gastric cancer samples (where it has been reported that Oct3/4 mRNA isoforms are highly expressed [23], data not shown). We used the forward and reverse primers previously reported by Aguilar-Lemarroy et al.,2002 and Xu et al., 2015 [24, 25] to analyse the expression of HPV16 E7 mRNA, which was only observed in C-33 A cells that transiently expressed the E7 oncoprotein (PEGFP-N1-E7 cells; Figure 4D).

As shown in Figure 4A, the cells transfected with the HPV16 E7 oncoprotein expressed significantly higher levels of Oct3/4A ($P<0.05$); the average Oct3/4A mRNA level was 4.07±1.24-fold (mean ± SE) higher in the C-33 A-PEGFP-N1-E7 cells than in the C-33 A cells ($p=0.017$) and PEGFP-N1 cells ($p=0.032$). On the other hand, the E7 oncoprotein had no effect on Oct3/4B and Oct3/4B1 mRNA expression. Regarding Oct4B, there was no change in the level of expression in C-33 A-PEGFP-N1-E7 cells [1.42±0.39-fold; $p>0.05$] (Figure 4B). Likewise, analysis of the data concerning the expression of Oct3/4B1 mRNA did not show any significant change in C-33 A cells transfected with PEGFP-N1-E7 (1.60 ± 0.56; $p=0.34$) (Figure 4C). Therefore, only Oct3/4A mRNA was positively affected by the presence of the HPV16 E7 oncoprotein. These data show that Oct3/4A could actively participate in the development of CC.

Discussion

In the present study, Oct3/4A, Oct3/4B and Oct3/4B1 mRNA expression was investigated in non-ILs, LSILs, HSILs and CCs. In addition, we used a cell line that expresses the HPV16 E7 oncoprotein [C-33 A cells] to evaluate the effect of the HPV16 E7 oncoprotein on the mRNA expression of Oct3/4A, Oct3/4B and Oct3/4B1. Our results suggest that the HPV16 E7 oncoprotein is involved in the overexpression of Oct3/4A mRNA in CC.

The E7 oncoprotein is an important factor in cervical carcinogenesis, and it is also involved in the alteration of a large number of cellular genes [26] and increases the expression of Oct3/4 and stemness-related genes [14]. The human Oct3/4 gene can generate three isoforms by alternative mRNA splicing, Oct3/4A, Oct3/4B and Oct3/4B1 [11, 12]. Oct3/4 plays an important role in maintaining the totipotentiality and pluripotentiality of human embryonic cells [12]. Previous studies have shown that Oct3/4 is overexpressed in CC [8, 9, 27]. Moreover, Oct3/4 can promote carcinogenesis and the development of tumors, while the loss of its expression leads to the loss of self-renewal and proliferation capabilities [28, 29] .

On the other hand, it has been reported that the HPV E7 oncoprotein binds to Oct3/4 protein, forming a complex both *in vitro* and *in vivo* and that Oct3/4 is active and expressed in HR-HPV-transformed cells [30, 31], However, it is unknown which isoform is deregulated by the HPV16 E7 oncoprotein. It has also been observed that the HPV16 E7 oncoprotein positively regulates the expression of Oct3/4 at the mRNA and protein levels both *in vivo* and *in vitro*, so the HPV16 E7 oncoprotein is proposed as a critical factor for cell self-renewal that facilitates the overexpression of factors related to maintenance of stem features [14, 30]. In 2015, Li et al. observed the expression of Oct3/4 (A and B) isoforms in CC and reported that Oct3/4A is responsible for cell self-renewal, which has also been attributed to the onset of CC, while Oct3/4B increases proliferation and tumor formation due to antiapoptotic activity [8]. Therefore, it is suggested that the expression level of each Oct3/4 isoform could be different in CC due to the presence of oncoproteins, such as the HPV16 E7 oncoprotein. To meet the stated objective, the PEGFP-N1 vector and PEGFP-N1-E7 construct were transiently transfected into HPV-negative C-33 A epithelial cells derived from CC.

In this study, the detection of Oct3/4A, Oct3/4B and Oct3/4B1 mRNA expression was performed to identify which of the Oct3/4 isoforms are expressed in samples of patients with LSILs, HSILs and CC positive for HPV16 infection. Oct3/4A and Oct3/4B1 mRNA were mostly expressed in samples from patients with HSILs and CC. Our results are similar to those of Li et al., 2015 and Assadi et al., 2011, who reported overexpression of Oct3/4A mRNA in samples from CC patients [8] and Oct3/4B1 mRNA in samples from gastric cancer patients [23], which could be due to the presence of the E7 oncoprotein of HPV16. Oct3/4 mRNA overexpression is closely related to HPV-AR infection, and the HPV16 E7 oncoprotein plays an important role in Oct3/4 overexpression [8, 9, 14, 30].

On the other hand, our results showed higher expression of Oct4A mRNA in C-33 A cells in the presence of the HPV16 E7 oncoprotein than in C-33 A cells that were not transfected or in cells transfected with the vector PEGFP-N1. A similar behavior was observed for the expression of Oct3/4B and Oct4B1 mRNA since their expression was very similar between the study groups, so the HPV16 E7 oncoprotein seemed to have no effect on the expression levels of Oct4B and Oct4B1 mRNA. Our data are consistent with those reported by Wang et al., who showed basal expression of Oct3/4 in C-33 A cells, which agrees with our expression data of the different isoforms in C-33 A cells without transfection [9]. There is also similarity in our results and those reported by Liu et al., who showed that the expression of Oct3/4 was higher in HR-HPV-positive cells, such as CasKi (HPV16-positive) and HeLa (HPV18-positive) cells, compared to C-33 A cells (HPV-negative) [13]. Taken together, these data indicate that the HPV16 E7 oncoprotein is directly or indirectly involved in the upregulation of Oct3/4A, which supports the idea that the transformation of cells mediated by the HPV16 E7 oncoprotein is closely related to the overexpression of Oct3/4A.

On the other hand, it has been reported that Oct3/4 can act as an oncogene and is associated with the triggering of cancerous stem cells [8, 32]. Likewise, during HPV-AR infection, viral DNA can be integrated into host DNA and initiate the synthesis of the E6 and E7 oncoproteins, promoting proliferation by inactivating the p53 and pRB tumor suppressors, respectively [33]. Moreover, it has been shown that the increase in cell proliferation leads to high expression of Oct4 in a pRB-dependent manner [34], while the degradation of p53 leads to the upregulation of Nanog [35]. Nanog is a positive regulator of Oct3/4 expression [36]. In addition, it has been observed that the HPV16 E7 oncoprotein also binds to the Oct3/4 protein [30] and that expression of the HPV16 E7 oncoprotein stimulates the Oct3/4 promoter [31]. In addition, it was found that the Oct3/4 gene is autoregulated by the Oct3/4 protein [37]. These data could help to understand why the high levels of Oct3/4 expression in patient samples could be due to the degradation of p53 by the HPV-AR E6 oncoprotein, leading to overexpression of Nanog, which could lead to an increase in the expression of Oct3/4 in the presence of HPV16 infections. Likewise, pRB degradation by the HPV-AR E7 oncoprotein could also lead to overexpression of Oct3/4. In addition, the interaction between the Oct3/4 protein and HPV16 E7 oncoprotein could modify the transcription of the Oct3/4 gene by directly binding to its promoter region, suggesting that the HPV16 E7 oncoprotein promotes self-renewal through the positive regulation of the Oct3/4 gene, thus maintaining the population of CC stem cells (Figure 5).

The data shown here suggest that Oct3/4A mRNA expression is positively regulated by the HPV16 E7 oncoprotein. However, future studies needed to clarify the effect of the HPV16 E7 oncoprotein on the Oct3/4 isoforms and to reveal new perspectives on the potential roles of the HPV16 E7 oncoprotein in Oct3/4 protein regulation during CC development. In conclusion, we showed that the presence of the E7 oncoprotein of HPV16 increases the expression of Oct4A mRNA.

Declarations

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Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Bioethical Committee of the Universidad Autónoma de Guerrero and Instituto Estatal de Cancerología “Dr. Arturo Beltrán Ortega” (approval no. FO-INV-AUT2018).

Consent to participate

Written informed consent was obtained from all participants prior your registration in this study. Data confidentiality was maintained throughout the study.

Consent for publication

All authors consent to the publication of this manuscript.

Author Contributions

Conceptualization, J. O.-N. and B. I.-A.; methodology, Y. G.-G., J. O.-N., S.I. C.-P., A. L.-M., D. V.-M., D.Y. A.-R., L.D.L. A.-R., J. O.-O., M.A. L.-V. and. M.A. J.-L.; validation, Y. G.-G. and J. O.-N.; formal analysis, Y. G.-G., E.G. S.-B. and J. O.-N. writing—original draft preparation, Y. G.-G., and S.I. C.-P.; writing—review and editing, J. O.-N. and B. I.-A.; supervision, J. O.-N. and B. I.-A.; project administration, B. I.-A.; funding acquisition, B. I.-A. All authors have read and agreed to the published version of the manuscript.

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Figures

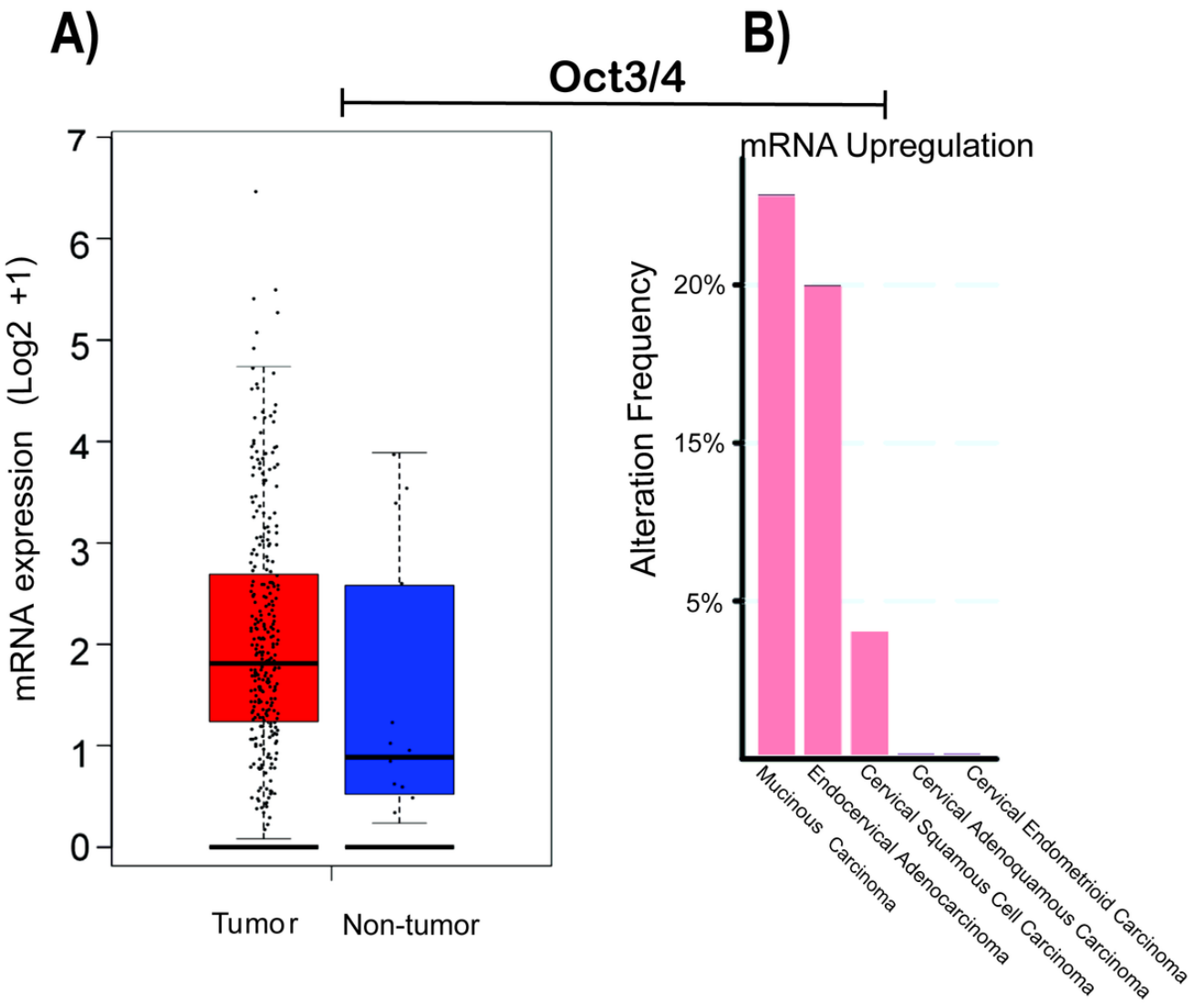

Figure 1

Oct3/4 expression was compared between different types of cervical carcinoma samples in cBioPortal and was found to be upregulated in cervical squamous cell carcinoma and endocervical adenocarcinoma

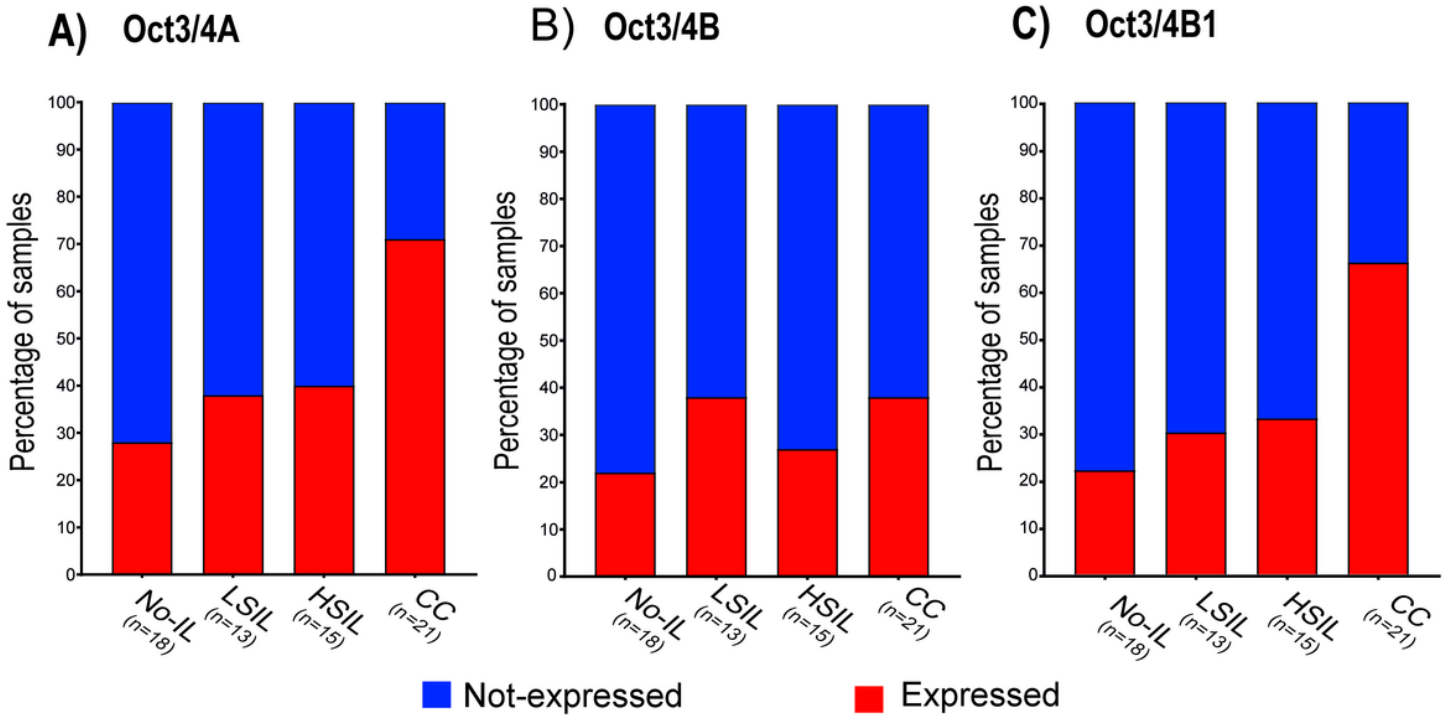

Figure 2

Our data revealed Oct3/4A expression was detected in 27.00% (5/18) of women with non-ILs. Oct3/4A mRNA was observed in 38.00% (5/13) of LSIL patients and in 40.00% (6/15) of HSIL patients. Oct3/4A mRNA expression was observed in 71.00% (15/25) of CC patients

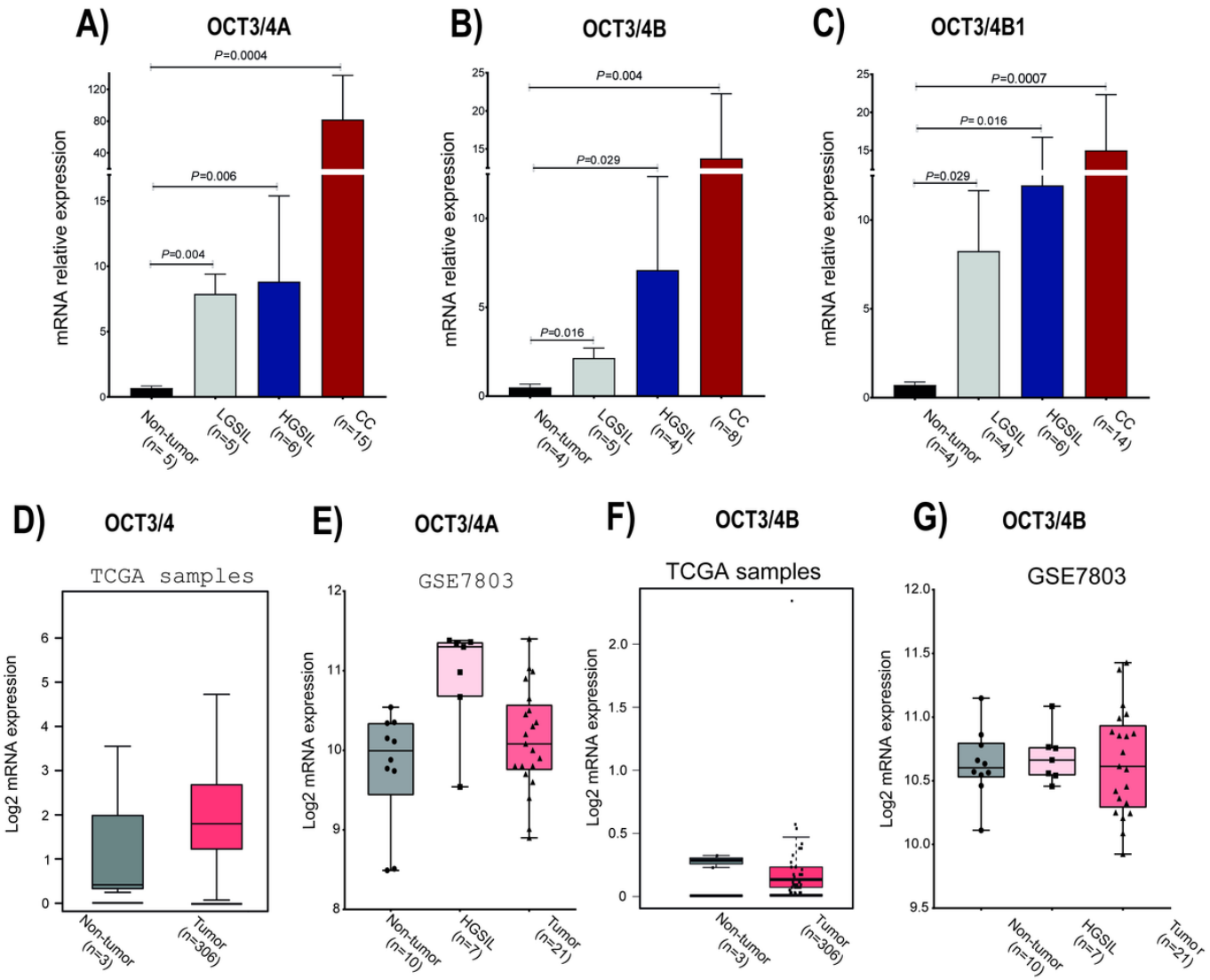

Figure 3

The average expression of Oct3/4A mRNA was 7.88±1.15-fold (mean ± standard error [SE]) higher in LSIL patients than in patients with non-ILs (p=0.004). In patients with HSILs, the Oct3/4A mRNA expression level was also significantly increased, with an average of 8.83±6.55-fold increase compared with that detected in women with non-ILs (P=0.006). Compared with that in women with non-ILs, the expression level of Oct3/4A mRNA in CC patients was also significantly increased by an average of 82.11±55.51-fold

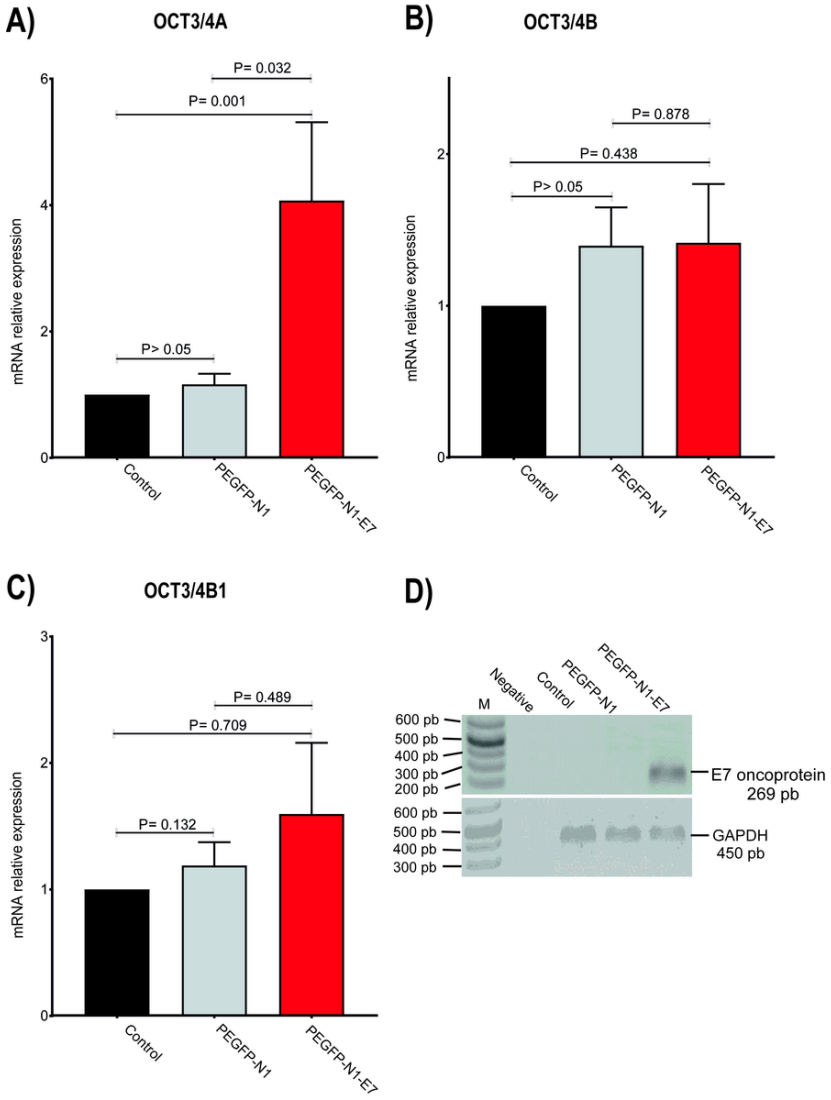

Figure 4

the cells transfected with the HPV16 E7 oncoprotein expressed significantly higher levels of Oct3/4A ($P<0.05$); the average Oct3/4A mRNA level was 4.07±1.24-fold (mean ± SE) higher in the C-33 A-PEGFP-N1-E7 cells than in the C-33 A cells ($p=0.017$) and PEGFP-N1 cells ($p=0.032$). On the other hand, the E7 oncoprotein had no effect on Oct3/4B and Oct3/4B1 mRNA expression. Regarding Oct4B, there was no change in the level of expression in C-33 A-PEGFP-N1-E7 cells [1.42±0.39-fold; $p>0.05$]

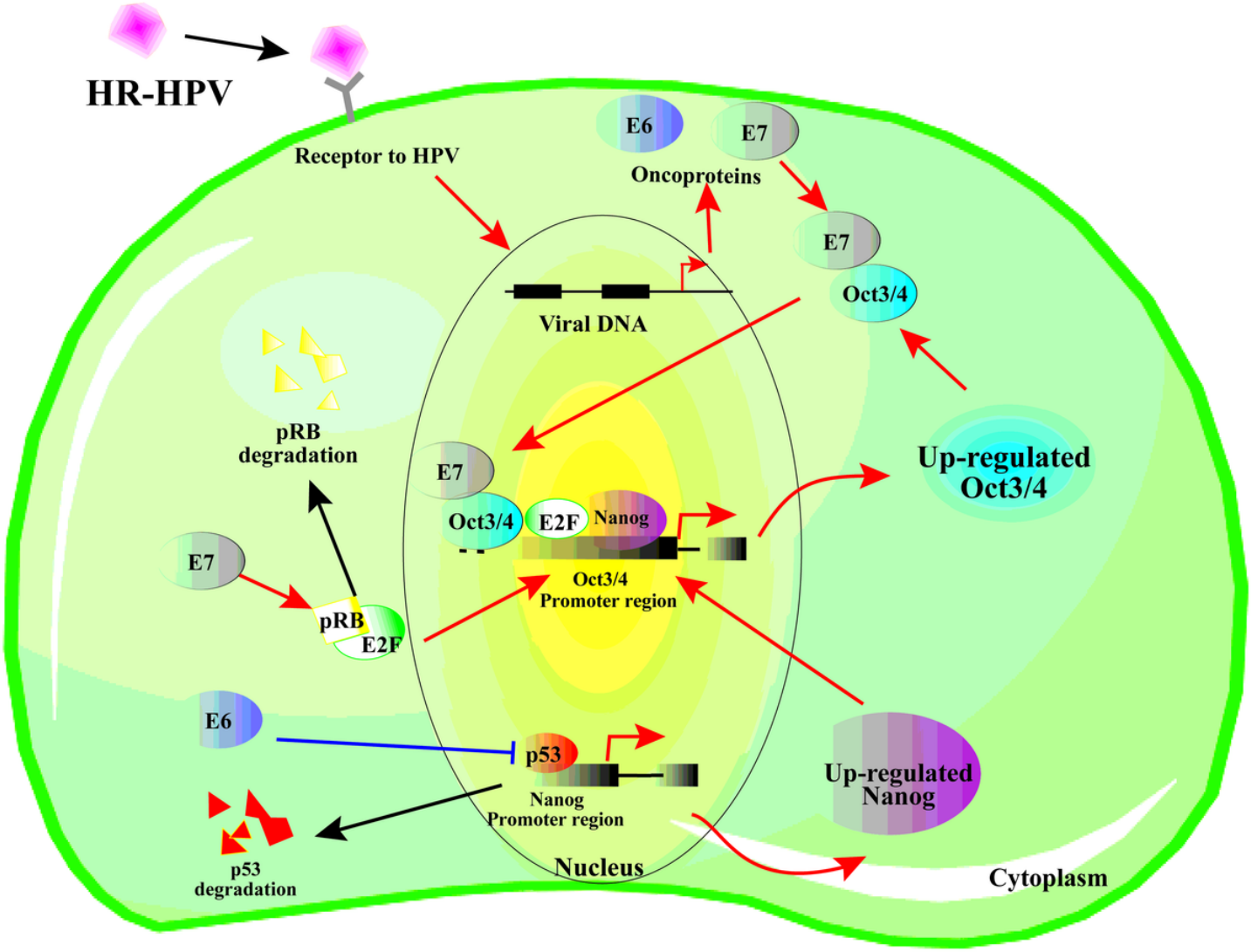

Figure 5

In addition, the interaction between the Oct3/4 protein and HPV16 E7 oncoprotein could modify the transcription of the Oct3/4 gene by directly binding to its promoter region, suggesting that the HPV16 E7 oncoprotein promotes self-renewal through the positive regulation of the Oct3/4 gene, thus maintaining the population of CC stem cells