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Expresión de HIF-1α y genes blanco *in vitro* e *in silico* en cáncer cervicouterino

T E S I S

Que para obtener el grado de
Maestría en Ciencias Biomédicas
P R E S E N T A:

Q.B.P. Víctor Daniel Priego Hernández

Directora de tesis: Dra. Luz del Carmen Alarcón Romero

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FACULTAD DE CIENCIAS QUÍMICO BIOLÓGICAS
FACULTAD DE MEDICINA
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MAESTRÍA EN CIENCIAS BIOMÉDICAS

ACTA DE APROBACIÓN DE TESIS

En la ciudad de Chilpancingo, Guerrero, siendo los 04 días del mes de julio del año dos mil veintidós, se reunieron los miembros del Comité Tutorial designado por la Academia de Posgrado de la Maestría en Ciencias Biomédicas, para examinar la tesis titulada **Expresión de HIF-1α y genes blanco *in vitro* e *in silico* en cáncer cervicouterino**, presentada por el alumno **Víctor Daniel Priego Hernández**, para obtener el Grado de Maestría en Ciencias Biomédicas. Después del análisis correspondiente, los miembros del comité manifiestan su aprobación de la tesis, autorizan la impresión final de la misma y aceptan que, cuando se satisfagan los requisitos señalados en el Reglamento General de Estudios de Posgrado e Investigación Vigente, se proceda a la presentación del examen de grado.

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Este trabajo se realizó en el laboratorio de Biomedicina Molecular y el laboratorio de Investigación en Biomoléculas, de la Facultad de Ciencias Químico Biológicas de la Universidad Autónoma de Guerrero en Chilpancingo de los Bravo, Gro.

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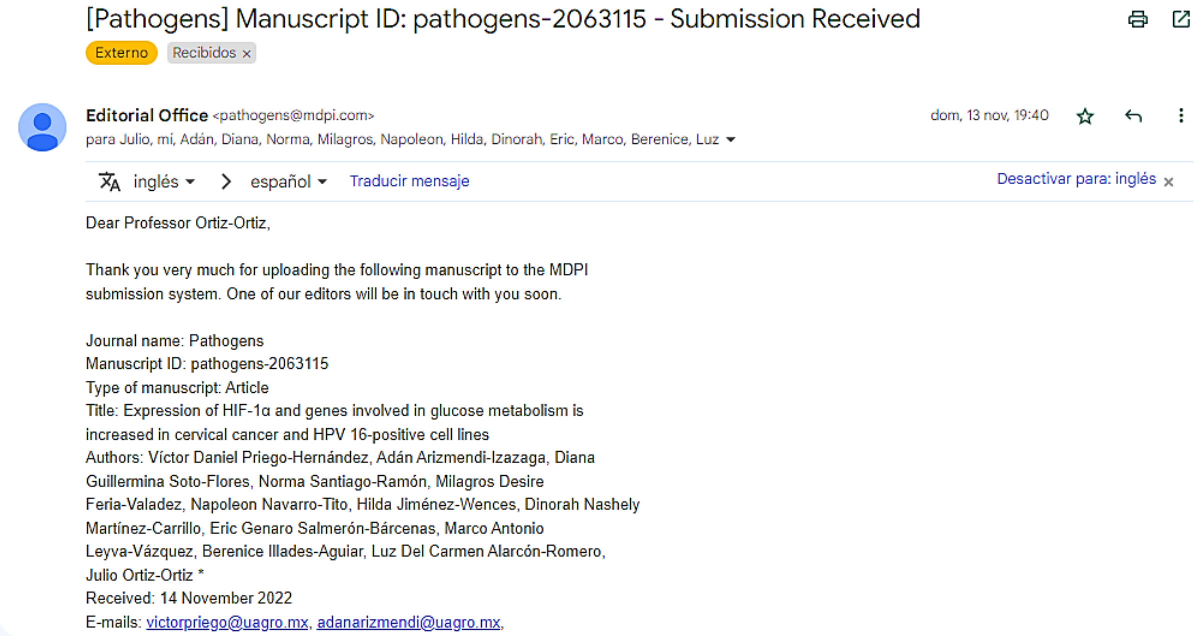
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Title page

Expression of HIF-1α and genes involved in glucose metabolism is increased in cervical cancer and HPV 16-positive cell lines

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Abstract

Cervical cancer (CC) is the most common cancer in women in the lower genital tract. The main risk factor for developing CC is persistent infection with HPV 16. The E6 and E7 oncoproteins of HPV 16 have been related to metabolic reprogramming in cancer through the regulation of the expression and stability of HIF-1α and consequently of the expression of its target genes such as HIF1A (HIF-1α), SLC2A1 (GLUT1), LDHA, CA9 (CAIX), SLC16A3 (MCT4) and BSG (Basigin or CD147), which are involved in glucose metabolism. This work aimed to evaluate the expression of HIF-1α, GLUT1, LDHA, CAIX, MCT4, and Basigin in patient samples and CC cell lines. To evaluate the expression level of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG genes in tissue from pa-tients with CC and normal tissue, the TCGA dataset was used. To evaluate the expression level of these genes by RT-qPCR in CC cell lines, HPV negative (C-33A) and HPV 16 positive (SiHa and Ca Ski) cell lines were used. Increased expression of HIF1A, SLC2A1, LDHA, SLC16A3, and BSG was found in Ca Ski and CA9 in SiHa compared to C-33A. Similar results were observed in CC tissues compared to normal tissue obtained by bioinformatics analysis. In conclusion, the expression of HIF-1α, GLUT1, LDHA, CAIX, MCT4, and BSG genes is increased in CC and HPV 16-positive cell lines.

Keywords: HPV 16; HIF-1α; glucose metabolism; cervical cancer

I. Introduction

Cervical cancer (CC) is the fourth leading cause of death in women worldwide, with approximately 604,127 new cases and 341,831 deaths annually [1]. Human papilloma-virus 16 (HPV 16) is present in more than 50% of cases of CC [2]. The oncogenicity of HPV results mainly from the action of E6 and E7 oncoproteins. E6 promotes carcinogenesis by inducing the degradation of the tumor suppressor protein p53 and activating the PI3K/AKT/mTOR [3]. In contrast, E7 contributes to carcinogenesis by inducing the reti-noblastoma tumor suppressor protein (pRb) degradation by releasing the cell cycle tran-scription factor E2F1 [4]. Several studies have revealed the association between HIF-1α overexpression and worse prognosis in patients with this type of cancer [5]. In different HPV 16-positive cancers, other active HIF1-regulated genes are overexpressed, which en-code proteins that play an essential role in immortalization [6,7], cell proliferation, metas-tasis, and metabolic reprogramming [8].

Metabolic reprogramming is an essential feature of cancer. Tumor cells reprogram their metabolism through a process known as the Warburg effect; this process is defined as the ability of cells to have high rates of glycolysis for the generation of ATP and precur-sors of different biomolecules independent of O2 [5,9]. The active HIF1 transcription factor is a master transcription factor of genes in response to hypoxia [10]. Active HIF1 is a het-erodimer consisting of 2 subunits: HIF-α, which is induced by hypoxia and has 3 isoforms: HIF-1α, HIF-2α and HIF-3α; and the HIF-1β subunit, which is constitutively ex-pressed [10]. The transcription factors, such as the c-Myc-MAX heterodimer, the ISGF3 complex, composed of STAT1/STAT2/IRF9, STAT3, NF-κB, and even active HIF1 itself, bind to the promoter region of the HIF-1α gene to activate the initiation of its transcription [11]. Active HIF1 binds to the promoters of genes containing the 5'-RCGTG-3' sequence, known as hypoxia response elements (HRE) [12]. Together with the coactivators CBP and p300 [13] they can regulate the expression of more than 70 genes, including some involved in glucose metabolism such as HIF-1α, GLUT1, LDHA, CAIX, MCT4, and BSG (Basigin) [12,14]. HIF-1 upregulates the expression of GLUT1 and GLUT3 genes, both necessary for glucose uptake, and enhances

lactate production by the enzyme lactate dehydrogenase A (LDHA), thereby decreasing intracellular pH. This mechanism regulates intracellular ac-idosis and is essential for maintaining homeostasis is the carbonic anhydrase IX (CAIX)-dependent mechanism. Together with MCT4 and BSG, they facilitate the release of lactate and H+ to the extracellular milieu to neutralize intracellular acidosis in a HIF-1-dependent manner [15], which is associated with a hyperglycolytic and acid-fast phenotype in cancer [14,16,17] and the release of lactate into the extracellular milieu [18]. It has been shown in cancer in vitro that MCT4 is regulated by HIF1, unlike MCT1 and MCT2, because it has 2 HRE in its promoter [19].

It has been reported that under hypoxia conditions in CC, E7 is associated with in-creased expression of HIF-1α via ROS, ERK1/2, and NF-□B [2,11]. In turn, E6 positively regulates HIF-1α, preventing its ubiquitination by VHL and subsequent degradation via proteasome [20]. Thus, E7 and E6 could indirectly promote increased glycolysis and re-sistance to intracellular pH changes through increased expression of HIF-1α and its target genes such as GLUT1, LDHA, CAIX, MCT4, and BSG [3,9,21]. However, the role of HPV 16 oncoproteins E6 and E7 in metabolic reprogramming is not entirely clear.

Therefore, in this work, we evaluated the expression of active HIF1 target genes; HIF-1α, GLUT1, LDHA, CAIX, MCT4, and BSG in HPV 16-positive CC cell lines and biop-sies of CC patients using data from The Cancer Genome Atlas (TCGA). Likewise, an over-all survival analysis was performed using the Kaplan-Meier Plotter database (https://kmplot.com/analysis/). Increased expression of HIF1A, SLC2A1, LDHA, SLC16A3, and BSG was observed in cell lines and biopsies of patients with CC and an association with poor survival prognosis. This information will contribute to a better understanding of the mechanisms that favor metabolic reprogramming in HPV 16-positive CC.

II. Material and Methods

Gene expression analysis in CC samples using the TCGA dataset

For gene expression analysis in CC patient samples, data were obtained from The Cancer Genome Atlas (TCGA) dataset and the GEPIA database [22]. The total

was n= 306 biopsies from patients with CC and n= 13 from normal tissue. Graphs showed the expres-sion levels of HIF-1α, GLUT1, LDHA, CAIX, MCT4, and BSG. Expression was log2 trans-formed (TPM+1), differences were calculated using a one-way ANOVA test, and a p-value <0.05 was considered statistically significant.

Correlation analysis

Correlation analysis between HIF1A expression and SLC2A1, LDHA, SLC16A3, and BSG expression was performed on CC samples from the TCGA dataset using the GEPIA database [22]. The correlation was calculated using Spearman and R^2 coefficients; a value of $p < 0.05$ was considered statistically significant.

Cell culture

C-33A, SiHa, and Ca Ski cell lines were cultured in DMEM (Dulbecco's Modified Ea-gle's Medium) medium supplemented with 10% fetal bovine serum (Gibco, Life Technol-ogies, USA) and 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Corp.) were added. The cells were maintained at a temperature of 37ºC with an atmosphere of 5% CO_2.

RNA extraction

Total RNA extraction was performed using TriZol® Reagent (Ambion® by Life Technologies, USA), following the manufacturer's instructions. RNA integrity was verified by 1.5% agarose gel electrophoresis. The concentration and purity of the RNA obtained were determined by spectrophotometry using the Nanodrop 2000c (Thermo Fisher Scien-tific).

Determination of gene expression in C-33A, SiHa, and Ca Ski cells by RT-qPCR

Determination of gene expression in C-33A, SiHa, and Ca Ski cells was performed

by RT-qPCR using the TaqMan® RNA-to-Ct™ 1-Step Kit (4392938). Each 10 μL reaction con-tained 1 μL of total RNA (50 ng), 5.0 μL of TaqMan® RT-PCR Mix reaction mix (2✕), which contains AmpliTaq Gold® DNA Polymerase, UP (Ultra Pure), dNTPs (dATP, dCTP, dGTP, dTTP, and dUTP), ROX™ passive reference and Optimized buffer compo-nents; 0. 25 μL of TaqMan® RT Enzyme Mix (40✕) containing: ArrayScript™ UP Reverse Transcriptase and RNase inhibitor; 0. 5 μL of HIF1-α (ID: Hs0015153153_M1), GLUT1 (ID:), LDHA (ID: Hs01378790_g1), MCT4 (ID: Hs00358829_m1), CAIX (ID: Hs00154208_m1) and BSG (ID: Hs00936295_m1) probes respectively. The probe used as endogenous was GAPDH (ID: Hs99999905_05); and 3. 25 μL of nuclease-free H2O. Thermal cycling condi-tions were as follows: 48°C for 15 min for retrotranscription, 95°C for 10 min to activate DNA polymerase, 40 cycles of 95°C for 15 s for cDNA denaturation; 60°C for 1 min for alignment and extension on the 7500 Fast System real-time PCR system (Applied Biosys-tems, South San Francisco, CA 94080 U.S.A). Gene expression was expressed as averages ± SD and determined using the 2-ΔΔCT method (Kenneth and Thomas 2001).

Overall and relapse-free survival analyses

Survival (OS) analyses were obtained from the Kaplan-Meier Plotter database (https://kmplot.com/analysis/) [23]. Survival curves were estimated using the Kaplan-Meier estimator. Survival curves were compared with the log-rank test. Data were analyzed for CC using the pan-cancer expression option. 304 patients were analyzed from the database repository.

Statistical analysis

The analysis of data obtained from the cell lines was performed by multivariate analysis using ANOVA. Post-hoc Tuckey tests considered a value $p < 0.05$ as statistically significant. Survival analyses with a $p < 0.05$ were considered statistically significant Log-Rank test. A $p < 0.01$ was considered statistically significant for expression analyses in patient samples.

III. Results

HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG expression is increased in samples from patients with CC

Metabolic reprogramming is a key feature in cancer progression. For this reason, the expression of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG genes was analyzed in samples from patients with CC and normal tissue was performed using the TCGA and GEPIA datasets [22]. The expression level of the genes of interest was obtained in 13 sam-ples from normal tissue and 306 from patients with CC. Increased expression levels of SLC2A1, LDHA, CA9, and SLC16A3 were observed in CC. The differences were statistically significant compared to normal tissue samples (Figure 1).

We also found that HIF-1α and BSG expression levels increased in CC compared to normal tissue.

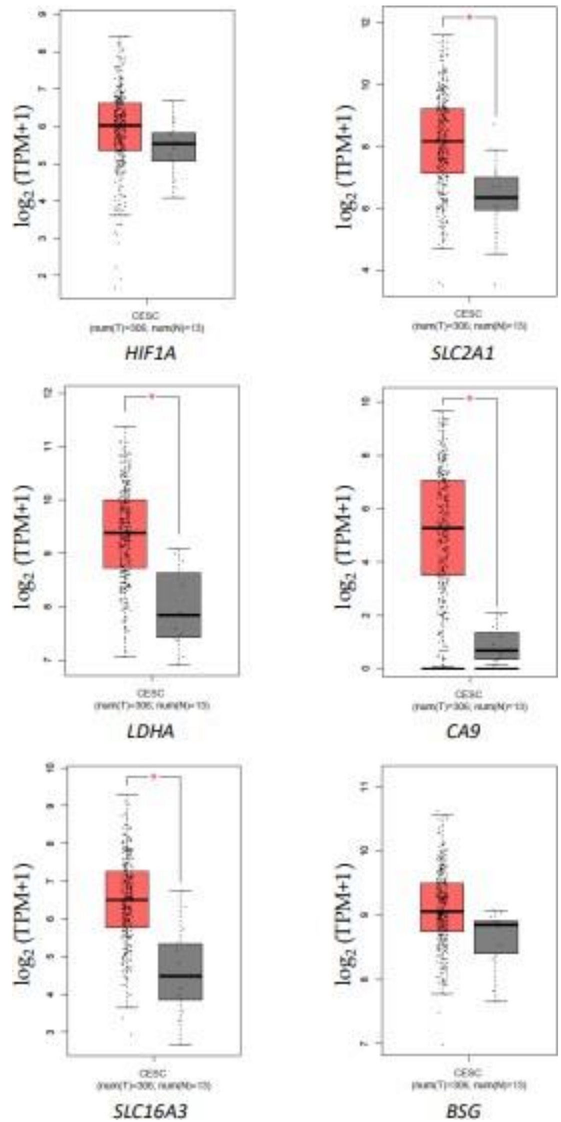

Figure 1. Expression of HIF1A and its target genes SLC2A1, LDHA, CA9, SLC16A3, and BSG in biopsies of patients with CC (T) and normal tissue (N), obtained from the TCGA dataset, is shown. *p< 0.01. Expression was log2 transformed (TPM+1).

High expression of HIF1A correlates with increased expression of SLC2A1, LDHA, CA9, SLC16A3, and BSG in CC

SLC2A1, LDHA, CA9, SLC16A3, and BSG are transcriptional targets of the active tran-scription factor HIF1. To evaluate whether increased expression levels of HIF-1α correlate with increased expression levels of the target genes of active HIF1, a correlation analysis was performed between expression levels of HIF-1α

and SLC2A1, LDHA, CA9, SLC16A3, and BSG in the GEPIA database (Table 1). It was observed that SLC2A1, LDHA, CA9, and SLC16A3, expression show a positive correlation with HIF1A expression, i.e., when HIF1A expression levels increase, SLC2A1, LDHA, CA9, and SLC16A3 expression levels also in-crease, in all cases the data were statistically significant. However, in the case of BSG, when HIF1A levels are low, BSG expression levels increase, although the data were not statistically significant. These data suggest that in CC, the high expression of SLC2A1, LDHA, CA9, SLC16A3, and BSG is related to the high expression of the HIF-1α subunit of the active HIF1 complex.

Table 1. Correlation analysis between HIF-1α expression levels with HIF1A, LC2A1, LDHA, CA9, SLC16A3, and BSG expression levels.

HIF-1α target genes	**CESC (Cervical squamous cell carcinoma and endocervical adenocarcinoma)**	
	Tumor	
	R	***P***
SLC2A1	0.27	***
LDHA	0.41	***
CA9	0.14	**
SLC16A3	0.31	***
BSG	-0.26	0.66

Tumor, tissue correlation analysis TCGA. (*) p< 0.05, (**) p< 0.01, (***) p< 0.001. R (correlation) and P (p-value).

mRNA expression of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG is increased in CC cell lines

To determine whether, as in patient samples, the expression levels of HIF-1α and its target genes SLC2A1, LDHA, CA9, SLC16A3, and BSG are increased in CC cell lines, their expression was evaluated in the SiHa and Ca Ski (HPV 16-positive)

and C-33A (HPV-negative) cell lines. The results obtained show that the mRNA expression level of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG increases in the Ca Ski cell line compared to C-33-A. However, a higher increase of CAIX was observed in SiHa compared to Ca Ski cells. Furthermore, in SiHa cells, an upregulation in the expression of HIF-1α and SLC16A3 was observed compared to C-33A. On the other hand, a slight decrease in LDHA and BSG expression was observed in SiHa compared to C-33A; however, this de-crease was not statistically significant (Figure 2). These results suggest that HPV 16 is involved in the overexpression of HIF1A, which in turn induces the overexpression of SLC2A1, LDHA, CA9, SLC16A3, and BSG involved in metabolic reprogramming in CC.

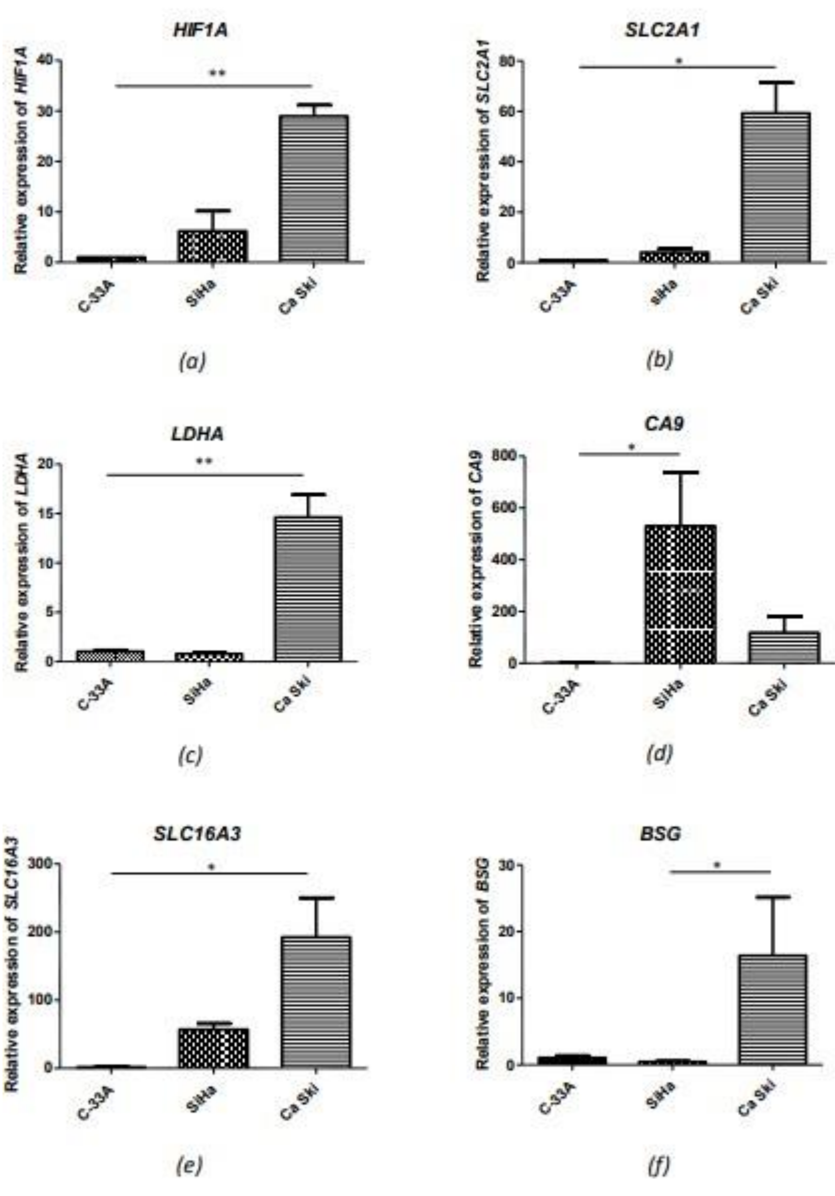

Figure 2. Relative expression of genes involved in metabolic reprogramming. The relative ex-pression level of HIFA1 (a), SLC2A1 (b), LDHA (c), CA9 (d), SLC16A3 (e), and BSG (f) in the SiHa and Ca SKi cell compared to C33-A. A value of $p < 0.05$ was considered statistically significant through a one-way ANOVA test and using mean and standard error. Data were measured in three inde-pendent experiments in triplicate in RT-qPCR and calculated by the $2^{-\Delta\Delta CT}$ method. Expression of the six transcripts was normalized to endogenous GAPDH. Relative expression levels were analyzed in GraphPad Prism software. * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$.

High expression of HIF1A, SLC2A1, LDHA, CA9, and SLC16A3 correlates with lower survival in CC

Here, we found that the expression of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG is increased in CC samples (Figure 1) and in HPV 16-positive CC cell lines (Figure 2). Furthermore, high expression of HIF-1α was observed to correlate with increased expres-sion of SLC2A1, LDHA, CA9, and SLC16A3 in CC patient samples (Table 1). To determine whether high expression of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG is involved in survival in patients with CC, overall survival analyses were performed using the Kaplan-Meier Plotter database (https://kmplot.com/analysis/). High expression of HIF1A, SLC2A1, LDHA, CA9, and SLC16A3 genes was associated with shorter survival in patients diagnosed with CC, and these differences were statistically significant (Figure 3).

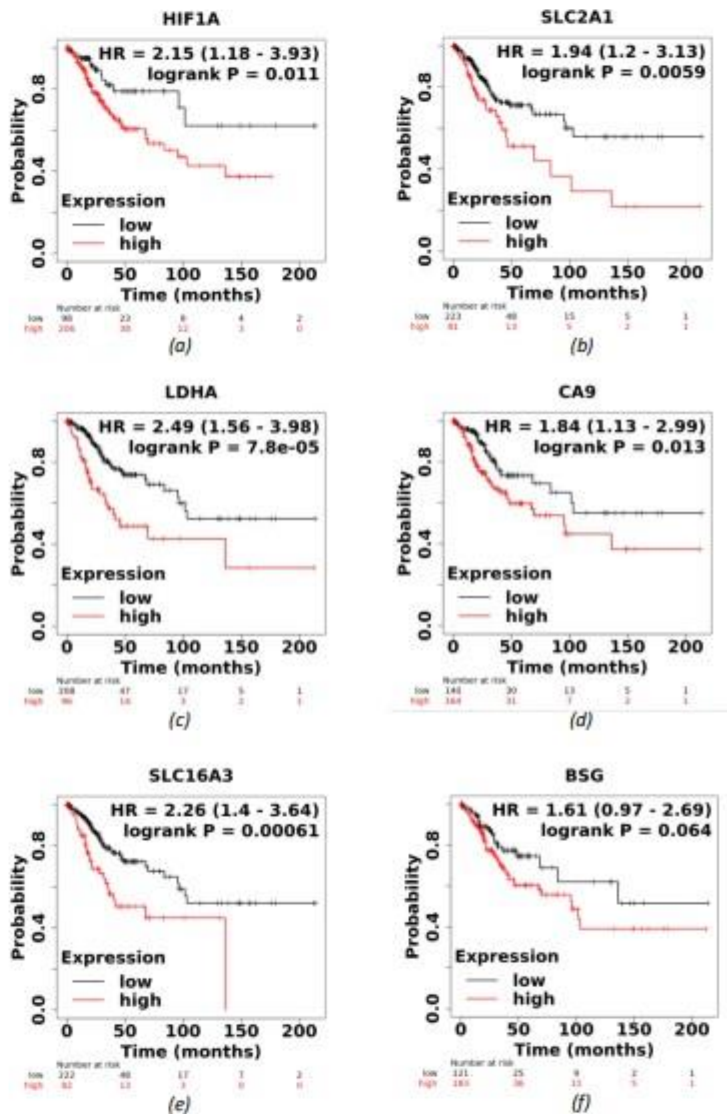

Figure 3. Overall survival analysis. Kaplan-Meier curve of overall survival by expression of HIF1A (a), SLC2A1 (b), LDHA (c), CA9 (d) SLC16A3 (e) and BSG (f) in patients with CC. The data show the probability of survival for 200 months, the time during which the levels of the transcripts were studied. The lines in red show high levels, and the gray color shows low levels of gene expression. Numbers below the plots indicate the number of patients during baseline, 50, 100, 100, 150, and 200 months of expression analysis. $p < 0.05$ were considered statistically significant.

IV.Discussion

Under hypoxia conditions in CC, it has been observed that HPV 16 oncoproteins E6 and E7 positively regulate HIF-1α. On the one hand, E7 promotes its gene expression, and on the other hand, E6 prevents its ubiquitination by VHL and its subsequent degradation via proteasome [20]. Several studies show that active HIF1 regulates gene expression in different hallmarks of cancer. The deregulated genes are grouped into tumor suppressor genes and oncogenes. In CC, deregulation of the expression of several genes is a mecha-nism that promotes tumor development and progression. These genes are known to code for proteins involved in processes such as metabolic reprogramming [9,21], angiogenesis [24], cell migration, invasion, and metastasis [25,26]. High expression of GLUT1, LDHA, and MCT4 proteins has been observed in biopsies from patients with invasive cervical cancer [27]. Additionally, LDHA inhibition has resulted in cell cycle inhibition and apop-tosis in nasopharyngeal carcinoma [28]. It also suppresses cell migration, increases chemo- and radiosensitivity in cancer cells [29], induces cell cycle arrest in the G2/M phase, and activates the mitochondrial apoptosis pathway in CC cells [30]. On the other hand, there is evidence that HIF1A mRNA is overexpressed in CC [31] and laryngeal squamous cell carcinoma [32], while GLUT1 is overexpressed in CC [33] and colorectal cancer [34]. However, overexpression of LDHA, CAIX, MCT4, and BSG has only been re-ported in other types of cancer but not in CC. LDHA overexpression has been reported in lung adenocarcinoma [35]; CAIX in breast cancer [36] and oral squamous cell carcinoma [37]; MCT4 in bladder [38] and breast cancer [39]; and BSG in acute myeloid leukemia [40]. In this work, the expression of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG was evalu-ated in tissue from patients with CC and normal tissue using the TCGA dataset and in HPV-negative (C-33A) and HPV 16-positive (SiHa and Ca Ski) cell lines.

The expression of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG was found to be increased in 306 samples from patients with CC compared with 13 samples of normal tissue. The increased expression of SLC2A1, LDHA, CA9, and SLC16A3 is

statistically sig-nificant. However, the increase in HIF1A and BSG expression was not statistically signifi-cant. Increased expression of SLC2A1 [41], LDHA [42], CA9, SLC16A3, BSG [43] and HIF-1α [31,44]. Active HIF1 plays an important role in metabolic reprogramming in cancer by ac-tivating the transcription of genes encoding proteins involved in glucose metabolism, which promotes glucose uptake, conversion of pyruvate to lactate; pyruvate detour from mitochondria, and selective mitochondrial autophagy [45]. Active HIF1 regulates the ex-pression of GLUT1, LDHA, CAIX [14] MCT4, and CAIX [12]. It can also bind to the pro-moter region of HIF-1α and promote its expression [11]. HIF-1α is the regulatory subunitin the formation of active HIF1, it is expressed under hypoxic conditions, and its expres-sion is related to the Warburg effect in cancer [46]. These data suggest that the increased expression of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG could be related to HIF1 ac-tivation triggered by increased HIF1A expression. These data were confirmed in the corre-lation analysis, where a positive correlation between the expression of HIF-1α and SLC2A1, LDHA, CA9, and SLC16A3 was observed. Interestingly, when HIFA1 expression is increased, SLC2A1, LDHA, CA9, and SLC16A3 expression is also increased. A negative correlation was observed between HIF1A expression and BSG expression, although the data were not statistically significant.

On the other hand, in The Human Protein Atlas (https://www.proteinatlas.org/), gene expression data for HIF1A, SLC2A1, LDHA, SLC16A3, and BSG were found in 69 human cell lines. Interestingly, no data related to CA9 expression were found in SiHa, HeLa, and HaCaT cell lines (Figure S1). The data found show an increase in the expression of HIF1A, SLC2A1, and SLC16A3 transcripts, a slight increase in BSG expression, and a decrease in LDHA expression in the SiHa cell line, while in the HeLa cell line (HPV 18 positive) only increased expression of SLC16A3 was observed, compared to the immortalized human keratinocyte cell line (HaCaT). These data suggest that high-risk HPV could regulate the expression of HIF1A, SLC2A1, SLC16A3, and BSG genes in these cell lines, which are re-quired to carry out metabolic reprogramming. Importantly, no reports on the expression levels of these transcripts in the C33-A and Ca Ski cell lines were found on this platform.

This work evaluated the expression of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG transcripts in cervical cancer cell lines with and without HPV 16. The HPV-negative C-33A tumor cell line, SiHa, with 1 to 2 integrated copies per cell of the HPV 16 genome, and Ca Ski with 600 integrated copies per cell of the HPV 16 genome. Increased expression of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG transcripts was observed in the Ca Ski cell line compared to C-33A, with statistically significant differences in the expression of all genes evaluated, except CA9. In the SiHa cell line, there were only statistically signifi-cant differences in the expression of CA9 compared to C-33A. There is also an increase in the expression of HIF1A and SLC16A3 compared to C-33A; however, a slight decrease in LDHA and BSG was observed. When comparing the data obtained in this study with the data from The Human Protein Atlas, it is observed that there is a similar behavior in the expression of LDHA. On the other hand, the expression observed in the Ca Ski and C-33A cell lines was similar to that observed in the 306 CC samples and the 13 normal tissue samples reported in the TCGA dataset, in which the expression of the six genes evaluated was found to be increased with statistically significant differences in SLC2A1, LDHA, CA9 and SLC16A3 (Figure 1), as in primary advanced uterine cervical carcinoma [31], human papilloma virus type 16-positive and -negative cervical cancer [33] and cervical cancer [38].

These results support the theory that HPV 16 could be favoring the gene expression of HIFA1 through E7 and the formation of active HIF1 through E6 and that, in turn, active HIF1 may be inducing the expression of its target genes, such as HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG. This effect could be affected by viral load, E6, and E7 variants, or at the stage of tumor progression. Importantly, SiHa and Ca-Ski cells, in addition to being HPV 16 positive, show two different stages of cancer progression, as they were derived from primary cervical carcinoma and metastatic tumor cells, respectively [47]. Ca Ski is a cervical cancer cell line established from cells from metastasis in the mesentery of the small intestine and containing the integrated HPV 16 genome of about 600 copies per cell and the E-G131/G350 variants of E6 and E7-Prototype [48]. On the other hand, the SiHa cell line was established from primary uterine squamous cell

carcinoma tissue. It con-tained the integrated HPV 16 genome of 1 to 2 copies per cell and the E-G350/C442 vari-ants of E6 and E7-C645 [49]. These particularities of the cell lines could explain why, alt-hough the two cell lines contain HPV 16, in Ca Ski, there is a high expression of HIF1A, SLC2A1, LDHA, SLC16A3, and BSG, whereas, in SiHa, there is a higher expression of CA9.

Changes in mRNA expression of various genes are often used to establish associa-tions between gene transcription and disease stage. Previous studies have shown that high expression of LDHA is involved in cell proliferation and survival, migration, inva-sion, angiogenesis, and immune evasion in cancer, indicating that LDHA may be a poten-tial prognostic marker and therapeutic target in cancer [7,50]. High SLC2A1 expression and HPV 16 have been reported to be independent prognostic factors in patients with CC [33]. On the other hand, increased expression of MCT1 and MCT4 is generally associated with poor prognosis. MCT4 is overexpressed in different types of cancer, such as breast, bladder, colorectal, and CC cancers. Moreover, high expression of MCT4 is closely associ-ated with increased expression of CAIX and BSG [15,21].

Regarding CAIX, its expression has been reported to regulate epitheli-al-mesenchymal transition and cell migration in CC [51]. In contrast, high expression of BSG has been correlated with radioresistance in the CC cell line SiHa [52]. Also, it has been reported that shorter survival of patients in all types of breast cancer, especially in those with the triple-negative phenotype, is associated with high expression of HIF-1α [53], CA9 [54] and BSG [55]; LDHA in lung adenocarcinoma [35]; SLC16A3 in bladder can-cer [38]; and SLC2A1 in colorectal cancer [34]. Likewise, in this study, the overall survival analysis with Kaplan Meier curves shows that high expression of HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG is associated with worse survival in patients with CC (Figure 3), in which HPV 16 is the leading etiological agent. All these data support the theory that a higher expression of the transcripts evaluated here is associated with HPV 16 and a worse prognosis in CC.

V. Conclusions

In conclusion, these results suggest that HPV 16 increases the expression of active HIF1 target genes: HIF1A, SLC2A1, LDHA, CA9, SLC16A3, and BSG in the Ca Ski cell line and in patients with CC. On the other hand, the high expression of these genes is related to lower survival in patients with CC, denoting the importance of studying these genes and their possible use as prognostic biomarkers. This poor survival could be related to viral load, HPV 16 E6 and E7 variants, or stage of tumor progression; however, further studies are needed in this regard.

VI. References

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