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Identification of factors associated with increased *CRABP1* gene expression in patients with squamous intraepithelial lesions and cervical cancer

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Abstract

Cellular Retinoic Acid Binding Protein-1 (CRABP1) facilitates retinoid metabolism; however, an abnormal expression might influence cancers developed. Biopsy samples were evaluated from patients with cervical cancer (CC), high-grade (HSIL), and low-grade squamous intraepithelial lesions (LSIL) (n= 66) Clinic/pathological information was obtained from patients. *CRABP1* gene expression was examined by RT-PCR and CRABP1 protein by immunohistochemistry (IHC) techniques. The expression of the *CRABP1* gene is shown to be significantly lower in the HSIL group (p= 0.008). CRABP1 protein was expressed in 90.3% of tissues, and only in CC was presented absence of protein (9.7%). Overweight/obesity might increase the gene (p= 0.019) but not the protein expression (p= 0.053). Also, a gene expression increase was shown in high serum retinol concentration (p= 0.047) and the presence of HVP-16 (p= 0.011) (gene expression but not the protein, p > 0.05). Menopause patients were associated with an absent/weak CRABP1 immunostaining (p= 0.008). Finally, CRABP1 and associated factors might be used as biomarkers to track the progression and persistence of cervical cancer on tissue.

Keywords: cervical cancer, *CRABP1*, obesity, retinol, squamous intraepithelial lesion.

Identificación de factores asociados al aumento de la expresión génica de *CRABP1* en pacientes con lesiones intraepiteliales escamosas y cáncer de cuello uterino

Resumen

La unión celular de la proteína con el ácido retinoico-1 (CRABP1) facilita el metabolismo de los retinoides y de su expresión anormal podría resultar en diversos cánceres. Para el caso que nos ocupa se evaluaron muestras de las biopsias de pacientes con cáncer de cuello uterino (CC) y con lesiones intraepiteliales escamosas de alto (HSIL) y de bajo grado (LSIL) (n= 66), por lo que para realizar el estudio se obtuvo la información clínico-patológica de las mujeres. La expresión del gen *CRABP1* se examinó mediante RT-PCR y la proteína por inmunohistoquímica (IHC). La expresión de *CRABP1* resultó ser más baja en el grupo HSIL significativamente (p = 0.008). La expresión proteica de CRABP1 se observó en el 90.3% de los tejidos, y en el CC su ausencia fue de un 9.7%. El aumento de la expresión génica, así como la disminución de la cantidad de proteína en este tipo de cáncer varía en diferentes situaciones médicas de acuerdo a lo observado: en el sobrepeso/obesidad aumenta la expresión génica (p= 0.019), pero no en la proteína (p = 0.053). Además, se mostró un aumento de la expresión *CRABP1* con una concentración alta de retinol (p= 0.047) y la presencia de HVP-16 (p= 0.011) (expresión génica pero no la proteína, p > 0.05). Pacientes con menopausia se asociaron con la ausencia/débil inmunotinción de la proteína CRABP1 (p= 0.008). Finalmente, concluimos que *CRABP1* y los factores asociados pueden ser útiles como biomarcadores para rastrear la progresión y la persistencia del cáncer de cuello uterino en el tejido.

Palabras clave: cáncer de cuello uterino, *CRABP1*, obesidad, retinol, lesiones intraepiteliales escamosas.

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Introduction

Vitamin A and all retinoids take part in the reduction of tumor promotion and epithelial cancer prevention. Intracellular retinoids are transformed into retinoic acid (RA), which can act as a carcinogenesis inhibitor through three main mechanisms: induction of apoptosis, inhibition of cell proliferation, and induction of progenitor cells to differentiate correctly (Abu, Batuwangala, Herbert & Symonds, 2005).

Two Cellular Retinoic Acid Binding Protein (CRABP1 and CRABP2) bind with high affinity to all-trans retinoic acid, one of the primary retinol metabolites, and form part of the Retinol Binding Protein family (RBP). These proteins are essential in retinoid homeostasis and function (Napoli, 2017). CRABP1 has a high affinity for cytosolic RA, limiting its access to nuclear receptors (Nagpal & Wei, 2019). Additionally, this protein helps to distribute RA derivatives in other biological processes. These derivatives are more polar (e.g., 4-hydroxy retinoic acid and 4-oxo retinoic acid), and they may also bind with RA Receptors (RAR) that could generate other physiological roles (Idres, Marill, Flexor & Chabot, 2002). For example, it has been reported that CRABP1 can regulate the mitogen-activated protein kinase (MAPK) pathway that contributes to the regulation of stem cell proliferation, some cancers, adipocyte proliferation, and even neuroimmune regulation (Nhieu, Lin & Wei, 2022). Previous studies have shown that increases in CRABP1 expression enhance the antiproliferative effects of all trans-RA of tumor cells with or without endogenous expression transfected with this protein (Napoli, 2017).

In that sense, some studies have suggested that variation in CRABP1 expression could be attached to the severity of various human cancer types. However, the results are not conclusive; in the esophagus, ovary, thyroid, and kidney cancer, this protein was found down-expressed (Celestino *et al.*, 2018; Miyake *et al.*, 2011; Tanaka *et al.*, 2007; Pfoertner *et al.*, 2005) while in breast, stomach, and lung cancer it was found over-expressed (Sakata *et al.*, 2022; Favorskaya *et al.*, 2014; Liu *et al.*, 2015). The difference in expression in the different types of cancer may be due to the role that CRABP1 plays in each of the tissues, where in some, it can function as an expression regulator and in others as a retinoic acid transporter (Napoli, 2017).

In the case of cervix tissue, *The Human Protein Atlas* (www.proteinatlas.org) shows that the gene expression of the *CRABP1* in the cervix can remain low (The Human Protein Atlas, 2023a; Uhlén *et al.*, 2015), but when there is cervical cancer or some abnormality of the cervix, the expression can be modified, showing a decrease or overexpression in different patients (The Human Protein Atlas, 2023b; Uhlen *et al.*, 2017). This demonstrates that other factors can influence gene expression, not just the presence of the pathology. For example, a study with *Rattus norvegicus* in cervical epithelium showed that CRABP1 protein expression varies according to the estrous cycle, suggesting that the circulating estrogens could induce expression changes (Tannous-Khuri, Hillemanns, Rajan, Wright & Talmage, 1994). Indeed, alcohol intake, adiposity, and methylation in the promoter region are other factors associated with CRABP1 expression changes in human cervix tissue (Bi, Hu, Zhou & Wei, 2001; Tanaka *et al.*, 2007; Wei, 2012). On the other hand, it is known that CRABP1 protein expression has a differential distribution in basal, superficial, and differentiated layers of both standard and tumor cervical human epithelium (Hillemanns, Tannous-Khuri, Koulos, Talmage & Wright, 1992). However, there is a lack of evidence about CRABP1 protein and gene expressions change associated with the grade of epithelial lesions or cervical cancer or if its expression could be related to clinical features; moreover, if this molecule can be useful as a prognostic or treatment response biomarker. Therefore, this study aimed to determine the CRABP1 expression level in patients with cervical cancer and squamous intraepithelial lesions and their possible association with clinical features.

Materials and methods

Study population and samples collection

This descriptive research included 66 patients from the Dysplasia Clinic of Sanitary Jurisdiction II, Ciudad Juárez, Chihuahua, Mexico. The selection of patients was made by colposcopy and histopathological evaluation. There were classified as Low Squamous Intraepithelial Lesion (LSIL) (n= 22), High Squamous Intraepithelial Lesion (HSIL) (n= 28), and Cervical Cancer (CC) (n= 16). One normal tissue sample was used for control. The tissue sample was obtained by directed biopsy before administering any therapy or treatment to the patient. One section of the tissue sample was paraffin-embedded for histopathological examination, while the remaining section was used for nucleic acid extraction. Clinic-pathological information such as nutrition status was obtained from their medical records. A blood sample was obtained to analyze the retinol concentration by high-performance liquid chromatography (HPLC). This study was performed in line with the principles of the Declaration of Helsinki. Also, it was approved by the ethical Universidad Autónoma de Ciudad Juárez committee (CBE.ICB/004.01-14), and all selected patients signed informed consent.

CRABP1 gene expression analysis

The RNA was extracted from the cervical samples following the TRI Reagent® protocol (Molecular Research Center, USA). The precipitated RNA was dried at room temperature and resuspended in 30 µL of DEPC water (0.1% diethylpyrocarbonate). The RNA concentration was determined using the absorbance 260/280 nm ratio analyzed by the NanoDrop ™ 2000 spectrophotometer (Thermo Scientific, USA). The cDNA synthesis was performed by the reverse transcription method using ImProm-II ™ Reverse Transcription System kit reagent (Promega, USA).

The primers used were published before *CRABP1* Fw5'-GCACGCAAACTCTTCTTGAAG-3 'and CRABP1Rv 5'-CGGACATAAATTCTGGTGCAG-3' (132 bp) (Tanaka *et al.*, 2007). The StepONE Plus thermal cycler (Applied Biosystems, USA) was used. To determine relative expression of the *CRABP1* gene, the Ribosomal *18S* gene was used as the constitutive gene: 18sFw 5'- TTTGCGAGTACTCAACACCA-3' and 18sRv 5'- GTTGTCCAGACCATTGGCTA-3' (280bp). PCR reaction was used Power SYBR® Green Master Mix reagent (Applied Biosystems, USA). The alignment temperatures for amplifying the sequences in RT-PCR were 55 °C for *CRABP1* and 60 °C for Ribosomal 18s. The relative expression analysis was calculated by the Pfaffl method (Pfaffl, 2001).

Immunohistochemistry staining

The CRABP1 protein expression level was analyzed by the immunohistochemistry (IHC) technique. Twenty-eight paraffin-embedded tissues of patients were obtained from the Hospital General de Ciudad Juárez, Chihuahua (LSIL= 5, HSIL= 7, and CC= 16). Since it has been reported that the *CRABP1* gene expression does not vary in normal tissue of the cervix (The Human Protein Atlas, 2023a) a sample of normal cervical paraffin-embedded tissue was used for the analysis. The Paraffin blocks were sectioned (1 mm in diameter) to form a tissue microarray (TMA), using an automatic Tissue-Arrayer® ATA 100 equipment. The TMA was deparaffinized with xylene followed by ethanol, and rehydrated. Antigen was retrieved with Tris-EDTA (pH 9.0) for 7 min in a Pressure Cooker. Endogenous peroxidase was eliminated with methanol: hydrogen peroxide (0.3%) for 10 min and incubated (4 °C) overnight with an anti-CRABP1 monoclonal antibody, 1:100 dilution (GeneTex; Cat. GTX22816). The Vectastain Universal Quick Kit (Vector, USA) was used for biotinylated secondary antibody and streptavidin-peroxidase complex. Both were incubated for 30 min. DAB Peroxidase Substrate Kit (Vector, USA) was used to detect and counterstained with hematoxylin for 2 min. Derived from the fact that the staining was uniform through the tissue, it was not possible to determine the percentage of positive cells. Because of this, immunostains results were cataloged considering a semiquantitative ordinal method (Meyerholz & Beck, 2018): absent, weak, moderate, and strong. The immunostains were grouped into absent/weak and moderate/strong for data association. The microscopic analysis was evaluated by three different expert examiners who graded the results and blinded the patient's history. For the positive control, stomach tissue was used.

Retinol serum

The blood sample with EDTA was centrifugated at 3,000 rpm for 5 min to remove blood cells and obtain the serum. Retinol was extracted using 100 µL of serum with 10 µL of 0.1% ascorbic acid solution and 100 µl ethanol as precipitant. The samples were mixed for 1 min with 500 µL of hexane (Sigma-Aldrich, USA). The samples were centrifuged at 2,500 rpm for 10 min at 4 °C, and the organic phase was obtained. The hexane extract was evaporated to dryness under a stream of nitrogen. Chromato-graphic conditions were performed using an octadecylsilane column (Sigma-Aldrich, USA) and methanol: water solution (95:5) as mobile phase with a 1.2 mL/min flow rate in a device Waters 600E System Controller Millipore. Retinol was detected at an absorbance of 325 nm with the Waters 486 Tunable Absorbance Detector.

Human Papillomavirus analysis

Human Papillomavirus (HPV) analysis was determined by PCR using primer sequences reported by (Nishiwaki *et al.*, 2008): GP5+: 5'-TTTGTTACTGTGGTAGATACTAC-3' and GP6+: 5'-GAAAAATAAACTGTAAATCATATTC-3'. Because de HPV-16 is the most oncogenic and could change the gene expression (Aziz & Aziz, 2017), we determined this genotype using primer sequences by (Qu *et al.*, 1997): 16UFw: 5'-TCCTGCAGGTACCAATGGGGAAGAGG-3' and 16URv: 5'-TGCCATACCCGCTGTCTTCGCTTT-3'. For PCR amplification, 100 ng of DNA was added to a final volume of 25 µL PCR mix containing 12.5 µL GoTaq® Green Master Mix (Promega, USA), 1 µL of forward primer, and 1 µL of reverse primer (20 µM for each one). The alignment temperatures for the amplification were 44 °C for HPV and 65 °C for HPV-16. PCR products were loaded on 2.0% agarose gels, stained with ethidium bromide, and visualized under ultraviolet illumination.

Statistical analyses

Comparisons for statistical significance were analyzed using SPSS 23.0 software (SPSS Inc.). We used non-parametric tests when *CRABP1* gene expression levels were evaluated by the Kolmogorov-Smirnov (KS) test. As required, the significant differences were analyzed by Kruskal-Wallis or Mann-Whitney U tests. The association between immunostaining and clinic variables was analyzed using X^2 or Fisher's exact tests. For non-parametric analysis of the trend between *CRABP1* relative expression units or CRABP1 immunostainings levels, we used the Jonckheere-Terpstra test. All p values represented two-tailed tests and were considered significant at 0.05.

Results

***CRABP1* gene expression**

Gene expression was determined based on the results obtained by real-time PCR. The relative expression units of *CRABP1* did not show a normal distribution (KS= 0.309, $p < 0.001$). There was a significantly different relative expression among the groups, and HSIL showed a lower expression than the others ($p < 0.05$) (Figure 1). We observed a tendency towards of decrease in *CRABP1* expression from LSIL to HSIL, and then an increase for the CC group. The expression of CC shows a wide distribution compared to the other groups.

We analyzed their possible association to investigate further the relation between *CRABP1* expression levels and clinical

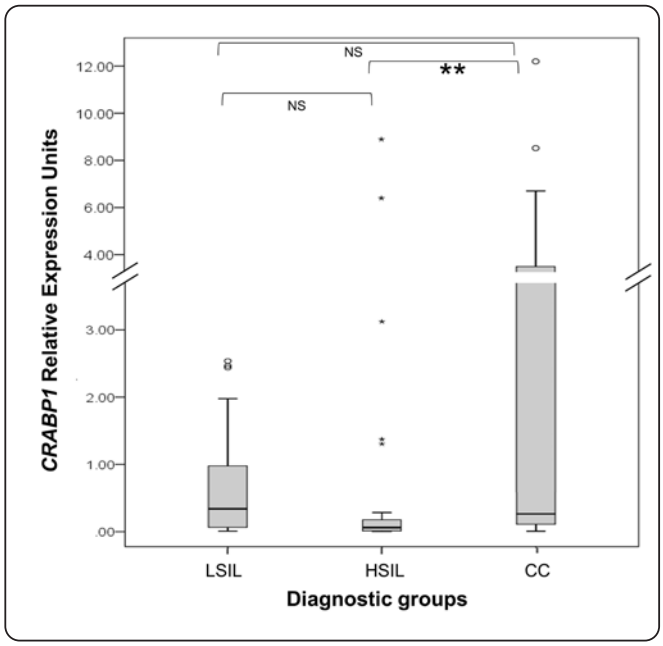

Figure 1. Relative expression units of *CRABP1* among diagnostic groups. A decrease in *CRABP1* expression from LSIL to HSIL and an increase in CC group were shown. RT-PCR analyzed gene expression, and the experiment was performed in triplicate. Circles and asterisks are extreme values and outliers, respectively. **p < 0.01 compare HSIL *vs.* CC. NS: not significant; LSIL: Low Squamous Intraepithelial lesion; HSIL: High Squamous Intraepithelial lesion; CC: Cervical cancer.

characteristics in diagnostic groups (Table I). According to the analysis, overweight/obesity, HPV-16, and serum retinol concentration were associated with *CRABP1* expression (Table II). Patients who presented overweight/obesity exhibit a high expression level of *CRABP1* compared with normal-weight patients (1.60 ± 2.7 *vs*. 0.44 ±1.3; p= 0.019). In addition, we separated the classes of obesity showing that the expression of *CRABP1* in overweight (p= 0.040) and obesity class I (p= 0.010) are statistically different from the expression in comparison with normal weight.

HPV-16 genotype positive shows a high expression level compared with HPV-16 negative (1.77 ± 3.1 *vs*. 0.80 ± 1.7; p= 0.011) and *CRABP1* high expression level was associated with a high serum retinol concentration compared with low serum retinol (2.40 ± 3.6 *vs*. 0.72 ± 1.7, p= 0.047). Also, a statistically significant trend shows that *CRABP1* expression increases with increasing serum retinol concentration (p= 0.049, Jonckheere-Terpstra test). We observed that the presence of overweight/obesity and HPV-16 increases the expression of *CRABP1*. For this reason, the analysis was stratified to identify if one variable influenced the result of the other. Analyzes show that the presence of HPV-16 increases *CRABP1* relative expression units (REUs) with or without overweight/obesity (overweight/obese group: HPV-16 negative, 1.07 ± 1.89 REU *vs*. HPV-16 positive, 2.60 ± 3.69 REU, p= 0.028; Non-overweight/obesity group: HPV-16 negative, 0.12 ± 0.24 REU *vs*. HPV-16 positive, 0.73 ± 0.52 REU, p= 0.051).

CRABP1 protein expression

IHC determined CRABP1 protein detection. Figure 2 shows the histological cervix's representative images in the four stages. The analysis of the normal squamous epithelium of the ectocervical shows a moderate immunohistochemical spectrum in the superficial and basal layer and decreases in the intermediate layer. The LSIL image shows abnormal cells in the basal and parabasal layers with a weak spectrum in the abnormal cells. The HSIL image shows abnormal cells throughout the squamous epithelium, nuclear enlargement, and lower cytoplasmic maturity. Finally, cervical cancer cells represented a superior nucleus with invasion into the stroma and absence of CRABP1 protein in the cytoplasm, as well as small undifferentiated infiltrating cells embedded in the stroma.

We obtained 28 of 66 samples for this assay and observed CRABP1 expression in 89.3% of tissues. A weak expression was observed in 32.1% of samples, while a moderate and strong expression was observed in 46.4% and 10.7%, respectively. Corresponding with the diagnostic group, CRABP1 immunostaining results were the following ones: LSIL (40.0% weak and 60.0% moderate), HSIL (28.6% weak, 57.1% moderate and 14.3% strong) and CC (18.8% absent, 31.13% weak, 37.5% moderate and 12.5% strong). According to an analysis of X^2, there were no associations between immunostaining levels with groups (p= 0.718). We compared *CRABP1* gene expression *vs*. CRABP1 protein expression levels, demonstrating no significant difference (Figure 3). However, we found a tendency to correlate increases in CRABP1 expression and immunostaining levels positively (p= 0.055, Jonckheere-Terpstra test).

To analyze the association between CRABP1 protein expression and the clinical characteristics, we grouped the expression level into absent/weak and moderate/strong (Table III). We observed that 100% of patients who presented menopause exhibited an absent/weak protein expression level; in contrast, most of the patients without menopause exhibited a moderate/strong expression level (69.6% *vs*. 30.4%, moderate/strong vs. absent/weak expression level; p= 0.008 95% CI). Moreover, CRABP1 moderate/strong expression level was more frequently found, but not statistically significant, in patients who presented overweight/obesity (75.0% *vs*. 25.0%; moderate/strong vs absent/weak expression level; p= 0.053 95% CI). No other clinical characteristics were found associated with CRABP1 protein expression.

Table I. General characteristics of patients.

Clinical variables	Total n (%)	LSIL n (%)	HSIL n (%)	CC n (%)
Age				
≥35 years	35 (53.0)	9 (40.9)	12 (42.9)	10 (62.5)
< 35 years	31 (47.0)	13(59.1)	16(57.1)	6(37.5)
Menopause				
Positive	6 (9.1)	0 (0)	4 (14.3)	2 (12.5)
Negative	60 (90.9)	22 (100)	24 (85.7)	14 (87.5)
Number of pregnancies (≥ 4)				
Positive	27 (40.9)	9 (40.9)	9 (32.1)	9 (56.3)
Negative	39 (59.1)	13 (59.1)	19 (67.9)	7 (43.8)
Family history of cancer				
Positive	22 (33.3)	5 (22.7)	11 (39.3)	6 (37.5)
Negative	44 (66.7)	17 (77.3)	17 (60.7)	10 (62.5)
Smoke				
Positive	16 (24.2)	6 (27.3)	5 (17.9)	5 (31.3)
Negative	50 (75.8)	16 (72.7)	23 (82.1)	11 (68.8)
Alcohol				
Positive	37 (56.1)	10 (45.5)	13 (46.4)	6 (37.5)
Negative	29 (43.9)	12 (54.5)	15 (53.6)	10 (62.5)
Hormonal contraceptive use				
Positive	18 (27.3)	5 (22.7)	8 (28.6)	5 (31.3)
Negative	48 (72.7)	17 (77.3)	20 (71.4)	11 (68.8)
HPV -16				
Positive	27 (40.9)	7 (31.8)	11 (39.3)	9 (56.3)
Negative	39 (59.1)	15 (68.2)	17 (60.7)	7 (43.8)
Overweight/obesity				
Positive	43 (65.2)	14 (63.6)	15 (53.6)	14 (87.5)
Negative	23 (34.8)	8 (36.4)	13 (46.4)	2 (12.5)
BMI classification (kg/m^2)				
Normal weight	23 (34.8)	8 (36.4)	13 (46.4)	2 (12.5)
Overweight	18 (27.3)	6 (27.3)	5 (17.9)	7 (43.8)
Obese class I	18 (27.3)	6 (27.3)	7 (25.0)	5 (31.3)
Obese class II	4 (6.1)	1 (4.5)	2 (7.1)	1 (6.3)
Obese class III	3 (4.5)	1 (4.5)	1 (3.6)	1 (6.3)
Serum retinol concentration (µg/dL)				
Quartile 1 (<28.5)	16 (24.2)	5 (22.7)	6 (21.4)	5 (31.3)
Quartile 2 (28.6-34.9)	20 (30.3)	10 (45.5)	9 (32.1)	1 (6.3)
Quartile 3 (35.0-47.9)	15 (22.7)	3 (13.6)	5 (17.9)	7 (43.8)
Quartile 4 (>48.0)	15 (22.7)	4 (18.2)	8 (28.6)	3 (18.8)
General characteristics were not significantly associated with diagnostic (p-value>0.05)				

Table II. Analysis of Relative Expression Units of *CRABP1* and personal characteristic variables.

	Relative Expression Units		
	M	**(± SD)**	***p value***
Age			
≥35 years (n= 31)	1.68	3.1	0.379
<35 years (n= 35)	0.78	1.5	
Number of pregnancies (≥ 4)			
≥ 4 (n= 27)	0.85	1.5	0.510
< 4 (n= 39)	1.43	2.8	
Menopause			
Positive (n= 6)	2.40	3.7	1.000
Negative (n= 60)	1.08	2.2	
Family history of cancer			
Positive (n= 22)	1.58	3.3	0.713
Negative (n= 44)	1.01	1.8	
Smoke			
Positive (n= 16)	1.30	2.6	0.255
Negative (n= 50)	1.16	2.3	
Alcohol			
Positive (n= 37)	1.20	2.7	0.108
Negative (n= 29)	1.19	2.1	
Hormonal contraceptive use			
Positive (n= 18)	1.46	2.7	0.730
Negative (n= 48)	1.10	2.3	
HPV-16			
Positive (n= 27)	1.77	3.1	**0.011**
Negative (n= 39)	0.80	1.7	
Overweight/obesity			
Positive (n= 43)	1.60	2.7	**0.019**
Negative (n= 23)	0.44	1.3	
BMI classification [§]			
Normal weight (n= 23)	0.44	0.3	-
Overweight (n= 18)	0.93	0.4	**0.040**
Obese class I (n= 18)	1.67	0.5	**0.010**
Obese class II (n= 4)	5.52	3.0	0.082
Obese class III (n= 3)	0.03	0.02	0.312
Serum retinol concentration (µg/dL) [§§]			
Quartile 1 (<28.5) (n= 16)	0.72	1.7	-
Quartile 2 (28.6-34.9)(n= 20)	0.59	0.9	0.217
Quartile 3 (35.0-47.9)(n= 15)	1.12	2.3	0.326
Quartile 4 (>48.0)(n= 15)	2.40	3.6	**0.047**

M, Media; **SD**, Standard Deviation. [§] Analysis was compared Normal weight (18.5-24.9 kg/m^2) *vs.* Overweight (25-29.9 kg/m^2), Obese **class I** (30-34.9 kg/m^2), **class II** (35-39.9 kg/m^2) and **class III** (≥ 40.0 kg/m^2). [§§] Analysis was compared Quartile 1 *vs.* Quartile 2, Quartile 3, and Quartile 4.

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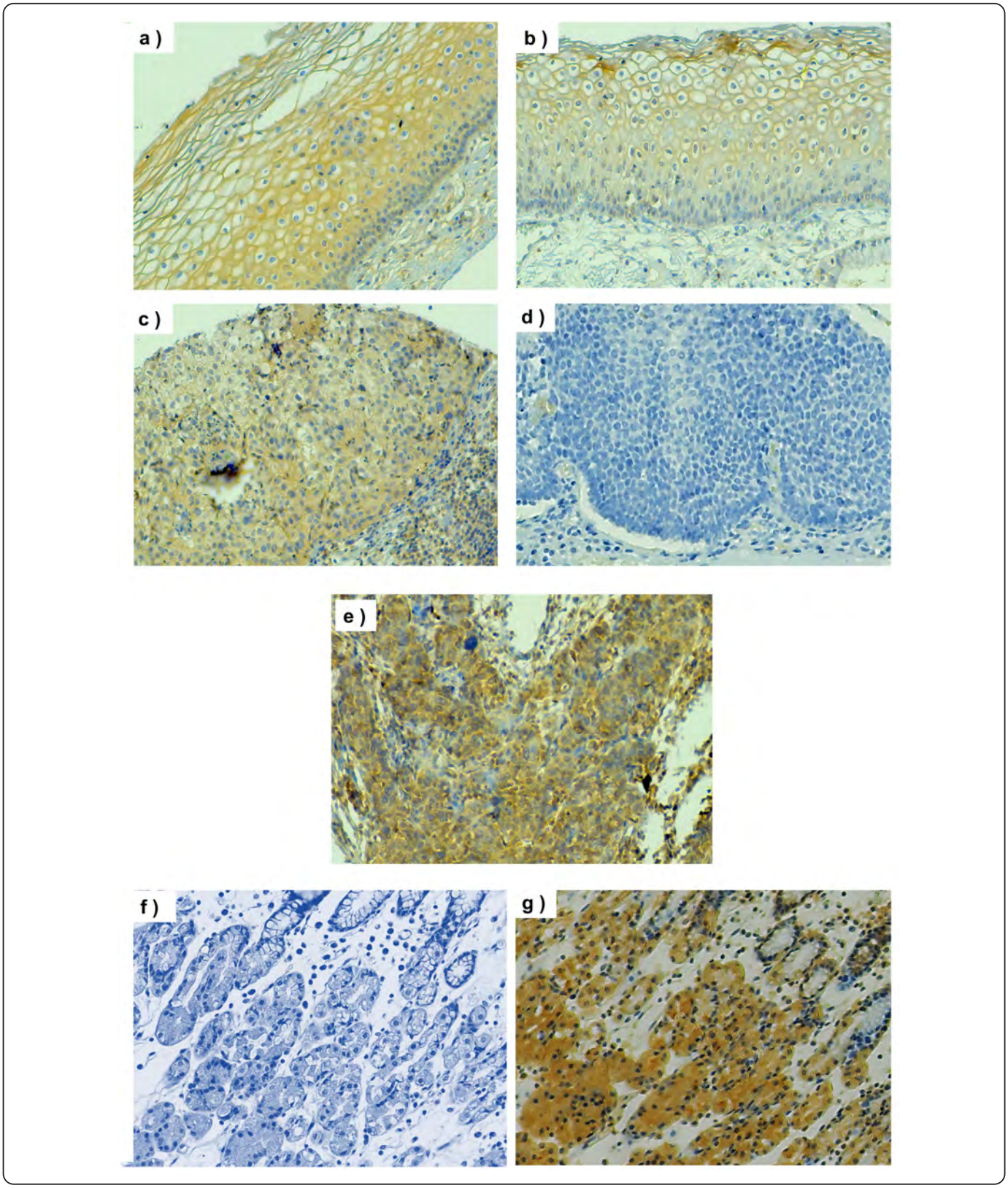

Figure 2. CRABP1 protein immunostaining in cervical tissues as examples. a) shows moderated staining in normal tissue. b) shows weak staining of one patient with LSIL. c) shows tissue with moderate staining in a HSIL. d) and e) show CC tissue with absent and strong staining, respectively. f) and g) show negative and positive control from stomach tissue. All tissues were viewed in 20X magnification.

Table III. Association between *CRABP1* immunostaining and personal characteristic variables.

	CRABP1 immunostaining		
Clinical variables	**Absent/weak (%)**	**Moderate/ strong (%)**	***p value***
Age			
≥35 years	7 (63.6)	4 (36.4)	0.121
< 35 years	5 (29.4)	12 (70.6)	
Menopause			
Positive	5 (100)	0 (0)	**0.008**
Negative	7 (30.4)	16 (69.6)	
Number of pregnancies (≥ 4)			
Positive	6 (33.3)	12 (66.7)	0.243
Negative	6 (60.0)	4 (40.0)	
Family history of cancer			
Positive	4 (33.3)	8 (66.7)	0.459
Negative	8 (50.0)	8 (50.0)	
Smoke			
Positive	4 (80.0)	1 (20.0)	0.133
Negative	8 (34.8)	15 (65.2)	
Alcohol			
Positive	5(45.5)	6(54.5)	1.000
Negative	7(41.2)	10(58.8)	
Hormonal contraceptive use			
Positive	1 (14.3)	6 (85.7)	0.184
Negative	11 (47.6)	10 (52.4)	
HPV -16			
Positive	6 (42.9)	8 (57.1)	1.000
Negative	6 (46.2)	7 (53.8)	
Overweight/obesity			
Positive	4 (25.0)	12 (75.0)	**0.053**
Negative	8 (66.7)	4 (33.3)	
BMI classification (kg/m^2) [§]			
Normal weight	8 (66.7)	4 (33.3)	-
Overweight	3 (30.0)	7 (70.0)	0.198
Obese class I	1 (25.0)	3 (75.0)	0.262
Obese class II	0 (0)	1 (100)	0.385
Obese class III	0 (0)	1 (100)	0.385
Serum retinol concentration (µg/dL)[§]			
Quartile 1 (<28.5)	3 (50.0)	3 (50.0)	-
Quartile 2 (28.6-34.9)	2 (40.0)	3 (60.0)	1.000
Quartile 3 (35.0-47.9)	3 (33.3)	6 (66.7)	0.622
Quartile 4 (>48.0)	4 (50.0)	4 (50.0)	1.000

[§]Analysis was compared Normal weight (18.5-24.9 kg/m2) *vs.* Overweight (25-29.9 kg/m2), Obese **class I** (30-34.9 kg/m2), **class II** (35-39.9 kg/m2) and **class III** (≥ 40.0 kg/m2).[§§] Analysis was compared Quartile 1 *vs.* Quartile 2, Quartile 3 and Quartile 4.[§]

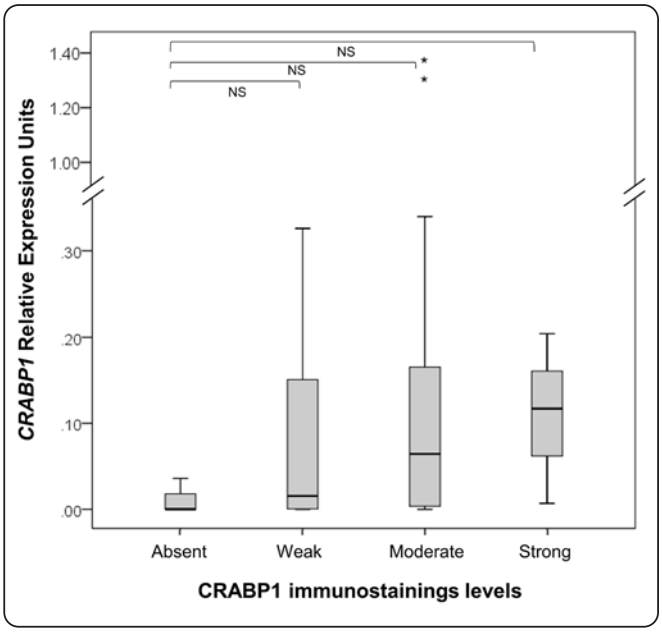

Figure 3. Relative expression units of *CRABP1* among CRABP1 immunostainings levels. There is no association between *CRABP1* gene expression *vs.* cytoplasmatic protein. RT-PCR analyzed gene expression, and the experiment was performed in triplicate. Immunostaining levels were determined by the immunohistochemistry (IHC) technique and classified considering a semiquantitative ordinal method (absent, weak, moderate, and strong immunostaining). Asterisks are outlier values. NS: not significant.

Discussion

CRABP1 protein involves the transport of retinoic acid in the cell for two purposes: normalizing the retinoic acid concentrations inside the cell and transporting it to the nucleus. Although CRABP1 expression changes have been observed in breast, lung, ovary, thyroid, stomach, and kidney cancer, low information about cervical cancer expression (CC) is available. Here, we described the CRABP1 gene and protein expression throw the cervical cancer process and the possible association between expression with some clinical characteristics of patients with premalignant or malignant lesions in the cervix epithelium.

Gene and protein expression level assays indicate that CRABP1 content in the epithelial cells on human tissue is independent of the degree of squamous intraepithelial lesions (SIL) and the presence of CC; moreover, CRABP1 protein expression was found throughout the epithelium, regardless of whether there were transformed cells in SIL or CC. In addition, we show that the relative units of mRNA and the level of protein expression are not associated, so there may be post-translational mechanisms that regulate protein synthesis, which can be explained by the clinical characteristics of the patients or distinct mechanisms of the cancer process.

First, in this study, a decrease and increase in the *CRABP1* gene expression were observed among the patients. When the clinical factors of each one were evaluated, the *CRABP1* relative expression units were associated with the presence of overweight/obesity (mainly those with overweight and obesity grade I), the presence of HPV16, and the serum retinol concentration.

Increased expression of *CRABP1* transcripts may increase the risk of mortality in patients with cervical cancer, as seen in the data represented previously (The Human Protein Atlas, 2023b). *CRABP1* overexpression has also been reported in other cancers, such as stomach cancer, where overexpression is associated with more aggressive cancer, which has been proposed for *CRABP1* as a marker of poor prognosis (Sakata *et al.*, 2022). In the present work, we show that the presence of overweight/obesity could increase the gene expression of *CRABP1* and, therefore, could be associated with a poor survival prognosis. Our results could support the hypothesis that mortality from the risk of cervical cancer could be associated with obesity or a high body mass index (BMI), as reported by other works (Clarke *et al.*, 2018; Poorolajal & Jenabi, 2016). As far as we know, this is the first study to show an association of changes in *CRABP1* gene expression with the presence of overweight/obesity in patients with CC or SIL, but not with the protein expression.

CRABP1 gene expression has been related to the cell proliferation of adipocytes as it is expressed in pre-adipocytes when they are in a differentiation process, and it is repressed when the differentiation ends (Wei, 2012). Also, it has been reported that CRABP1 may be a modulator of adipogenesis derived from the silencing of the *Crabp1* gene in mice (Lin, Park, Lin, Burton & Wei, 2020).

CRABP1 expression is regulated by thyroid hormone, a hormone that regulates adipogenesis, mainly triiodothyronine (T3) (Moreno-Navarrete & Fernández-Real, 2017). The *CRABP1* gene expression depends on the binding of T3 with the thyroid hormone receptor (TR) and retinoid X receptor (RXR) in the region of thyroid hormone response elements (TREs) (Wei, Lee, Filipcik & Chang, 1997). It has been reported that T3 and Thyroid stimulating hormone (TSH) can be elevated in obese patients by the adipogenesis process (Michalaki *et al.*, 2006; Muscogiuri *et al.*, 2013). So that overweight/obesity patients could induce the *CRABP1* gene expression in a generalized manner, including the epithelial cells of the cervix, which are in a constant process of differentiation and proliferation, not only in adipocyte cells.

CRABP1 gene expression has been reported to be regulated by the amount of holo-CRABP1 complex (bound with retinoic acid) in the adipocyte cell; concentrations of retinoic acid bound to the CRABP1 protein in the cervical epithelial cell could regulate the

CRABP1 gene. Patients with low serum retinol concentrations have less cellular retinoic acid availability and lower *CRABP1* concentrations. In addition, there is an association between the concentration of CRABP2 and CRABP1; it has been observed that when there is a low synthesis of the CRABP2 protein, the expression of CRABP1 is suppressed (Enikeev *et al.*, 2021). CRABP2 is an isoform expressed by retinoic acid concentrations in the cell (Balmer & Blomhoff, 2002); therefore, in patients with higher serum retinol concentrations, then transformed into retinoic acid in the cell, this could induce CRABP2-influenced.

Another factor associated with increased *CRABP1* gene expression was the presence of HPV-16. The *CRABP1* gene is found on the long arm of chromosome 15. It has been reported that there could be a chromosomal gain with a high squamous lesion, invasive cancer, or high-risk HPV infection are presented (Darroudi *et al*., 2010). The presence of the HPV-16 infection, when the genetic material is modified, generates a chromosomal instability that can repress the gene expression or increase the number of transcripts (Steenbergen, Snijders, Heideman & Meijer, 2014). Considering the previous information, in this study, we observed a significant increase in the expression of the *CRABP1* gene in patients who tested HPV-16 positive but not in the protein expression. This condition indicates that other regulatory pathways involved in translating the protein in the presence of HPV-16 are a future line of research. In addition, HPV-16 increases *CRABP1* expression regardless of overweight/obesity. As is well known, this oncological genotype can promote cervical cancer in patients with lesions, so HPV-16 alters the expression of many genes, including *CRABP1*.

This work did not show an association between CRABP1 gene expression and protein expression so post-translational modifications may prevent protein synthesis. However, these modifications could be generated by certain specific characteristics of the patients or the presence of cancer. The latter is explained by the fact that in the CC group, the protein is absent for some patients.

The only factor that was associated with protein expression was menopause (absent/weak CRABP1 immunostaining), which is an important factor related to the cervical cancer process (Aziz & Aziz, 2017). In addition, estrogens can increase the concentration of CRABP1 protein in the cervix (Tannous-Khuri, Hillemanns, Rajan, Wright & Talmage, 1994). Our analysis shows that protein expression is reduced in menopausal patients, even though some have overweight/obesity. Lack of CRABP1 expression affects retinoic acid metabolism and the antitumor effect of CRABP1. An interesting aspect is that the patients who presented menopause and with an absent/weak CRABP1 immunostaining also presented lower values of relative expression units. For future work, the association analysis of CRABP1 protein expression and menopause is recommended to see their association with survival.

The carcinogenic process, mainly in the cervix, could begin to block the expression of CRABP1 by avoiding its repressive action tumor formation. For example, it has been shown that the presence of this protein reveals an activity as a tumor suppressor since it induces apoptosis in cancer cells by regulating genes related to the apoptotic pathway, such as BcI-2 and Bax. In the presence of CRABP1, the expression of Bax is increased, which binds to and sequesters the Bcl-2 protein with an anti-apoptotic effect. Therefore, the absence of CRABP1 may promote cancer progression and reduce treatment sensitivity (Persaud *et al.*, 2016). In addition, this protein can be repressed by receptor-interacting protein 140 (RIP140) (Nautiyal, Christian & Parker, 2013). An increase in RIP140 has been reported in high squamous intraepithelial lesions (Vogelsang *et al.*, 2020) and has been observed in patients with a worse prognosis of cervical cancer (Vattai *et al.*, 2017). Therefore, the elevated presence of RIP140 could reduce CRABP1 gene and protein expression and induce cancer progression. However, we observed that overweight/obese patients present an increase in this protein despite having cancer or high-grade lesions, so the expression is not being repressed by RIP140. As mentioned, the presence of overweight/obesity may present higher thyroid hormone concentrations. RIP140 can be repressed by thyroid hormone (T3) and consequently would increase the CRABP1 (Park, Huang, Persaud & Wei, 2009).

This work presents two scenarios in which CRABP1 may influence the cervical cancer process. First, the lack of CRABP1 would not regulate the concentration of retinoic acid, and it can be degraded or used in another biochemical pathway (Idres *et al.*, 2002; Langton & Gudas, 2008). Therefore, the anticancer effect of retinoic acid would not work. This scenario could occur in menopausal patients and with some cancer patients. Second, the over-expression of the CRABP1 protein will not allow the retinoic acid transference to the nucleus nor the union with the nuclear receptors (RAR or RXR) (Nagpal & Wei, 2019); this could happen to overweight/obesity patients. Nevertheless, both the absence and over-expression of the protein could interfere with the correct transport of the retinoic acid, as some studies have demonstrated in non-small cell lung and breast cancer (Favorskaya *et al.*, 2014; Liu *et al.*, 2015). CRABP1 may indicate alterations in retinoic acid transport within the cell. If obesity causes an over-expression of CRABP1, it could sequester the retinoic acids in the cell or send it to its degradation, preventing the introduction of the retinoic acid to the nucleus (Blaese, Santo-Hoeltje & Rodemann, 2003). Consequently, the progression or persistence of the disease may be influenced by the inability of retinoic acid to have an antiapoptotic, differentiative, and cellular repression effect. Moreover, CRABP1 expression could affect treatment prognosis or survival in patients with lesions or cancer, as described in other cancers (Miyake *et al.*, 2011). We must consider that CRABP1 could be expressed differently in patients caused by single nucleotide polymorphisms (SNPs). For example, *CRABP1* polymorphisms have been seen in some

types of cancer, neurodegeneration, and immunological diseases (Nhieu *et al.*, 2022). Therefore, the polymorphism could affect the expression and be a factor that alters the post-translational mechanism. In addition, a polymorphism in patients could explain the possible associations described here, such as the association of overweight/obesity, menopause, and serum retinol concentrations with CRABP1 expression.

Conclusions

The results of gene and protein expression of CRABP1 in the process of cervical cancer obtained by RT-PCR and immunohistochemistry try to allow describing that overweight/obesity, HPV-16 genotype, menopause, and serum retinol content could be associated with changes in CRABP1 expression. Together, these factors could be important prevention markers to consider within the evolution of the cervical cancer process or prognosis and its interaction with the retinol pathway and the proliferative effect of the cells.

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Conflict of interest

The authors disclose no potential conflicts of interest.

References

Abu, J., Batuwangala, M., Herbert, K. & Symonds, P. (2005) Retinoic acid and retinoid receptors: potential chemopreventive and therapeutic role in cervical cancer. *Lancet Oncol.*, **6(9)**, 712–20.

Aziz, S. W. & Aziz, M. H. (2017). Cervical Cancer Metastasis. In A. Ahmad (Ed.), *Introduction to Cancer Metastasis*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-804003-4.00005-0

Balmer, J. E. & Blomhoff, R. (2002). Gene expression regulation by retinoic acid. *J. Lipid Res.*, **43(11)**, 1773–1808. https://doi.org/10.1194/jlr.r100015-jlr200

Bi, J., Hu, X., Zhou, F. C. & Wei, L. N. (2001). Upregulation of cellular retinoic acid-binding protein I expression by ethanol. *Dev. Growth Differ.*, **43(5)**, 553–561. https://doi.org/doi.org/10.1046/j.1440-169X.2001.00591.x

Blaese, M. A., Santo-Hoeltje, L. & Rodemann, H. P. (2003). CRABP I expression and the mediation of the sensitivity of human tumour cells to retinoic acid and irradiation. *Int. J. Radiat. Biol.*, **79(12)**, 981–991. https://doi.org/10.1080/09553000310001632949

Celestino, R., Nome, T., Pestana, A., Hoff, A. M., Gonçalves, A. P., Pereira, L., Cavadas, B., Eloy, C., Bjøro, T., Sobrinho-Simões, M., Skotheim, R. I. & Soares, P. (2018). CRABP1, C1QL1 and LCN2 are biomarkers of differentiated thyroid carcinoma, and predict extrathyroidal extension. *BMC Cancer*, **18(1)**, 68. https://doi.org/10.1186/s12885-017-3948-3

Clarke, M. A., Fetterman, B., Cheung, L. C., Wentzensen, N., Gage, J. C., Katki, H. A., Befano, B., Demarco, M., Schussler, J., Kinney, W. K., Raine-Bennett, T. R., Lorey, T. S., Poitras, N. E., Castle, P. E. & Schiffman, M. (2018). Epidemiologic Evidence That Excess Body Weight Increases Risk of Cervical Cancer by Decreased Detection of Precancer. *J. Clin. Oncol.*, **36(12)**, 1184–1191. https://doi.org/10.1200/JCO.2017.75.3442

Darroudi, F., Bergs, J. W. J., Bezrookove, V., Buist, M. R., Stalpers, L. J. & Franken, N. A. P. (2010). PCC and COBRA-FISH a new tool to characterize primary cervical carcinomas: To assess hall-marks and stage specificity. *Cancer Letters*, **287(1)**, 67–74. https://doi.org/https://doi.org/10.1016/j.canlet.2009.05.034

Enikeev, A. D., Komelkov, A. V., Axelrod, M. E., Galetsky, S. A., Kuzmichev, S. A. & Tchevkina, E. M. (2021). CRABP1 and CRABP2 Protein Levels Correlate with Each Other but Do Not Correlate with Sensitivity of Breast Cancer Cells to Retinoic Acid. *Biochemistry (Mosc.)*, **86(2)**, 217–229. https://doi.org/10.1134/S0006297921020103

Favorskaya, I., Kainov, Y., Chemeris, G., Komelkov, A., Zborovskaya, I. & Tchevkina, E. (2014). Expression and clinical significance of CRABP1 and CRABP2 in non-small cell lung cancer. *Tumour Biol.*, **35(10)**, 10295–10300. https://doi.org/10.1007/s13277-014-2348-4

Hillemanns, P., Tannous-Khuri, L., Koulos, J. P., Talmage, D. & Wright, T. C. (1992). Localization of cellular retinoid-binding proteins in human cervical intraepithelial neoplasia and invasive carcinoma. *Am. J. Pathol.*, **141(4)**, 973–980. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1886642/

Idres, N., Marill, J., Flexor, M. & Chabot, G. G. (2002). Activation of retinoic acid receptor-dependent transcription by all-trans-retinoic acid metabolites and isomers. *J. Biol. Chem.*, **277(35)**, 31491–31498. https://doi.org/doi: 10.1074/jbc.M205016200

Langton, S. & Gudas, L. J. (2008). CYP26A1 knockout embryonic stem cells exhibit reduced differentiation and growth arrest in response to retinoic acid. *Dev. Biol.*, **315(2)**, 331–354. https://doi.org/doi: 10.1016/j.ydbio.2007.12.021.

Lin, Y.-W., Park, S. W., Lin, Y.-L., Burton, F. H. & Wei, L.-N. (2020). Cellular retinoic acid binding protein 1 protects mice from high-fat diet-induced obesity by decreasing adipocyte hypertrophy. *Int. J. Obes. (Lond.)*, **44(2)**, 466–474. https://doi.org/10.1038/s41366-019-0379-z

Liu, R.-Z., Garcia, E., Glubrecht, D. D., Poon, H. Y., Mackey, J. R. & Godbout, R. (2015). CRABP1 is associated with a poor prognosis in breast cancer: adding to the complexity

of breast cancer cell response to retinoic acid. *Mol. Cancer*, **14(1)**, 129. https://doi.org/10.1186/s12943-015-0380-7

Meyerholz, D. K. & Beck, A. P. (2018). Principles and approaches for reproducible scoring of tissue stains in research. *Lab. Invest.*, **98**, 844–855. https://doi.org/10.1038/s41374-018-0057-0

Michalaki, M. A., Vagenakis, A. G., Leonardou, A. S., Argentou, M. N., Habeos, I. G., Makri, M. G., Psyrogiannis, A. I., Kalfarentzos, F. E. & Kyriazopoulou, V. E. (2006). Thyroid function in humans with morbid obesity. *Thyroid*, **16(1)**, 73–78. https://doi.org/10.1089/thy.2006.16.73

Miyake, T., Ueda, Y., Matsuzaki, S., Miyatake, T., Yoshino, K., Fujita, M., Nomura, T., Enomoto, T. & Kimura, T. (2011). CRABP1-reduced expression is associated with poorer prognosis in serous and clear cell ovarian adenocarcinoma. *J. Cancer Res. Clin. Oncol.*, **137(4)**, 715–722. https://doi.org/10.1007/s00432-010-0930-8

Moreno-Navarrete, J. M. & Fernández-Real, J. M. (2017). Adipocyte Differentiation. In M. E. Symonds (Ed.), *Adipose Tissue Biology* (pp. 69–90). Springer International Publishing. https://doi.org/10.1007/978-3-319-52031-5_3

Muscogiuri, G., Sorice, G. P., Mezza, T., Prioletta, A., Lassandro, A. P., Pirronti, T., Casa, S. della, Pontecorvi, A. & Giaccari, A. (2013). High-normal TSH values in obesity: Is it insulin resistance or adipose tissue's guilt?. *Obesity*, **21(1)**, 101–106. https://doi.org/10.1038/oby.2012.157

Nagpal, I. & Wei, L. N. (2019). All-trans retinoic acid as a versatile cytosolic signal modulator mediated by CRABP1. *Int. J. Mol. Sci.*, **20(15)**, 1–11. https://doi.org/10.3390/ijms20153610

Napoli, J. L. (2017). Cellular retinoid binding-proteins, CRBP, CRABP, FABP5: Effects on retinoid metabolism, function and related diseases. *Pharmacol. Ther.*, **173**, 19–33. https://doi.org/10.1016/j.pharmthera.2017.01.004

Nautiyal, J., Christian, M. & Parker, M. G. (2013). Distinct functions for RIP140 in development, inflammation, and metabolism. *Trends Endocrinol. Metab.*, **24(9)**, 451–459. https://doi.org/10.1016/j.tem.2013.05.001

Nhieu, J., Lin, Y.-L. & Wei, L.-N. (2022). CRABP1 in Non-Canonical Activities of Retinoic Acid in Health and Diseases. *Nutrients*, **14(7)**, 1528. https://doi.org/10.3390/nu14071528.

Nishiwaki, M., Yamamoto, T., Tone, S., Murai, T., Ohkawara, T., Matsunami, T., Koizumi, M., Takagi, Y., Yamaguchi, J., Kondo, N., Nishihira, J., Horikawa, T. & Yoshiki, T. (2008). Genotyping of human papillomaviruses by a novel one-step typing method with multiplex PCR and clinical applications. *J. Clin. Microbiol.*, **46(4)**, 1161–1168. https://doi.org/10.1128/JCM.00793-07.

Park, S. W., Huang, W. H., Persaud, S. D. & Wei, L. N. (2009). RIP140 in thyroid hormone-repression and chromatin remodeling of Crabp1 gene during adipocyte differentiation. *Nucleic Acids Res.*, **37(21)**, 7085–7094. https://doi.org/10.1093/nar/gkp780

Persaud, S. D., Park, S. W., Ishigami-Yuasa, M., Koyano-Nakagawa, N., Kagechika, H. & Wei, L.-N. (2016). All trans-retinoic acid analogs promote cancer cell apoptosis through non-genomic Crabp1 mediating ERK1/2 phosphorylation. *Sci. Rep.*, **6(1)**, 22396. https://doi.org/10.1038/srep22396

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.*, **29(9)**, 2002–2007. https://doi.org/10.1093/nar/29.9.e45

Pfoertner, S., Goelden, U., Hansen, W., Toepfer, T., Geffers, R., Ukena, S. N., von Knobloch, R., Hofmann, R., Buer, J. & Schrader, A. J. (2005). Cellular retinoic acid binding protein I: Expression and functional influence in renal cell carcinoma. *Tumour Biol.*, **26**, 313–323. https://doi.org/10.1159/000089262

Poorolajal, J. & Jenabi, E. (2016). The association between BMI and cervical cancer risk : a meta-analysis. *Eur. J. Cancer Prev.*, **25(3)**, 232–238. https://doi.org/https://doi.org/10.1097/CEJ.0000000000000164

Qu, W., Jiang, G., Cruz, Y., Chang, C. J., Ho, G. Y. F., Klein, R. S. & Burk, R. D. (1997). PCR detection of human papillomavirus: Comparison between MY09/MY11 and GP5+/GP6+ primer systems. *J. Clin. Microbiol.*, **35(6)**, 1304–1310. https://doi.org/10.1128/jcm.35.6.1304-1310.1997

Sakata, K., Kanda, M., Shimizu, D., Nakamura, S., Inokawa, Y., Hattori, N., Hayashi, M., Tanaka, C., Nakayama, G. & Kodera, Y. (2022). Expression of cellular retinoic acid binding protein 1 predicts peritoneal recurrence of gastric cancer. *Int. J. Oncol.*, **60(6)**, 63. https://doi.org/10.3892/ijo.2022.5353

Soprano, D. R. (1994). Serum and Cellular retinoid-binding proteins. In K. Dakshinamurti (Ed.), *Vitamin Receptors. Vitamins as Ligands in Cell Communication: Vol. XXXIII* (Issue 2, pp. 81–87). Cambridge University Press. https://doi.org/10.1017/CBO9780511525391.003

Steenbergen, R. D. M., Snijders, P. J. F., Heideman, D. A. M. & Meijer, C. J. L. M. (2014). Clinical implications of (epi) genetic changes in HPV-induced cervical precancerous lesions. *Nat. Rev. Cancer*, **14(6)**, 395–405. https://doi.org/10.1038/nrc3728

Tanaka, K., Imoto, I., Inoue, J., Kozaki, K., Tsuda, H., Shimada, Y., Aiko, S., Yoshizumi, Y., Iwai, T., Kawano, T. & Inazawa, J. (2007). Frequent methylation-associated silencing of a candidate tumor-suppressor, CRABP1, in esophageal squamous-cell carcinoma. *Oncogene*, **26(44)**, 6456–6468. https://doi.org/10.1038/sj.onc.1210459

Tannous-Khuri, L., Hillemanns, P., Rajan, N., Wright, T. C. & Talmage, D. A. (1994). Expression of Cellular Retinoic Acid-Binding Cervical Epithelium Is Stimuli During Normal Retinol- and Cellular Proteins in the Rat Regulated by Endocrine Squamous Metaplasia. *Am. J. Pathol.*, **144(1)**, 148–159. https://pubmed.ncbi.nlm.nih.gov/8291604/

The Human Protein Atlas (March, 2023a). Tissue expression

of CRABP1 Staining in cervix. The Human Protein Atlas. https://www.proteinatlas.org/ENSG00000166426-CRABP1/tissue/cervix.

The Human Protein Atlas (March, 2023b). Expression of CRABP1 in cervical cancer .The Human Protein Atlas. https://www.proteinatlas.org/ENSG00000166426-CRABP1/pathology/cervical+cancer#ihc

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å., Kampf, C., Sjöstedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C. A., Odeberg, J., Djureinovic, D., Takanen, J. O., Hober, S., Alm, T., Edqvist, P.H., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J.M., Hamsten, M., von Feilitzen, K., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., von Heijne, G., Nielsen, J. & Pontén, F. (2015). Proteomics. Tissue-based map of the human proteome. *Science*, **347(6220)**, 1260419. https://doi.org/10.1126/science.1260419.

Uhlen, M., Zhang, C., Lee, S., Sjöstedt, E., Fagerberg, L., Bidkhori, G., Benfeitas, R., Arif, M., Liu, Z., Edfors, F., Sanli, K., von Feilitzen, K., Oksvold, P., Lundberg, E., Hober, S., Nilsson, P., Mattsson, J., Schwenk, J. M., Brunnström, H., Glimelius, B., Sjöblom, T., Edqvist, P.H., Djureinovic, D., Micke, P., Lindskog, C., Mardinoglu, A. & Ponten, F. (2017). A pathology atlas of the human cancer transcriptome. *Science*, **357(6352)**, eaan2507. https://doi.org/10.1126/science.aan2507

Vattai, A., Cavailles, V., Sixou, S., Beyer, S., Kuhn, C., Peryanova, M., Heidegger, H., Hermelink, K., Mayr, D., Mahner, S., Dannecker, C., Jeschke, U. & Kost, B. (2017). Investigation of RIP140 and LCoR as independent markers for poor prognosis in cervical cancer. *Oncotarget*, **8(62)**, 105356–105371. https://doi.org/10.18632/oncotarget.22187

Vogelsang, T. L. R., Schmoeckel, E., Kuhn, C., Blankenstein, T., Temelkov, M., Heidegger, H., Kolben, T. M., Kolben, T., Mahner, S., Mayr, D., Jeschke, U. & Vattai, A. (2020). Regulation of LCoR and RIP140 expression in cervical intraepithelial neoplasia and correlation with CIN progression and dedifferentiation. *J. Cancer Res. Clin. Oncol.*, **146(7)**, 1847–1855. https://doi.org/10.1007/s00432-020-03178-x.

Wei, L. N., Lee, C. H., Filipcik, P. & Chang, L. (1997). Regulation of the mouse cellular retinoic acid-binding protein-I gene by thyroid hormone and retinoids in transgenic mouse embryos and P19 cells. *J. Endocrinol.*, **155(1)**, 35–46. http://www.ncbi.nlm.nih.gov/pubmed/9390004

Wei, L.-N. (2012). Chromatin remodeling and epigenetic regulation of the CrabpI gene in adipocyte differentiation. *Biochim. Biophys. Acta*, **1821(1)**, 206–212. https://doi.org/10.1016/j.bbalip.2011.03.003.