DNA Methylation of Cellular Retinoic Acid-Binding Proteins in Cervical Cancer

Ana L. Arellano-Ortiz[1], Mauricio Salcedo-Vargas[2], Claudia L. Vargas-Requena[1], José A. López-Díaz[1], Antonio De la Mora-Covarrubias[1], Juan C. Silva-Espinoza[1] and Florinda Jiménez-Vega[1]

[1]Laboratorio de Biotecnología, Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez, Ciudad Juárez, Chihuahua, México. [2]Unidad de Investigación Médica de Enfermedades Oncológicas, Hospital de Oncología, Centro Médico Nacional-Siglo XXI, IMSS, Ciudad de México, México.

ABSTRACT: This study determined the methylation status of cellular retinoic acid-binding protein (*CRABP*) gene promoters and associated them with demographic characteristics, habits, and the presence of human papilloma virus (HPV) in patients with cervical cancer (CC), low and high squamous intraepithelial lesions, and no intraepithelial lesion. Women (n = 158) were selected from the Colposcopy Clinic of Sanitary Jurisdiction II in Ciudad Juarez, Chihuahua, Mexico. Demographic characteristics and habit information were collected. Cervical biopsy and endocervical scraping were used to determine methylation in promoter regions by methylation-specific polymerase chain reaction technique. We found hemi-methylation patterns in the promoter regions of *CRABP1* and *CRABP2*; there was 28.5% hemi-methylation in *CRABP1* and 7.0% in that of *CRABP2*. Methylation in *CRABP1* was associated with age (≥35 years, $P = 0.002$), family history of cancer ($P = 0.032$), the presence of HPV-16 ($P = 0.013$), and no alcohol intake ($P = 0.035$). These epigenetic changes could be involved in the CC process, and *CRABP1* has the potential to be a predictive molecular marker of retinoid therapy response.

KEYWORDS: cervical cancer, squamous intraepithelial lesions, methylation, *CRABP*

CITATION: Arellano-Ortiz et al. DNA Methylation of Cellular Retinoic Acid-Binding Proteins in Cervical Cancer. *Genetics & Epigenetics* 2016:8 53–57 doi:10.4137/GEG.S40847.

TYPE: Original Research

RECEIVED: August 29, 2016. **RESUBMITTED:** October 9, 2016. **ACCEPTED FOR PUBLICATION:** October 15, 2016.

ACADEMIC EDITOR: Christian Bonner, Editor in Chief

PEER REVIEW: Four peer reviewers contributed to the peer review report. Reviewers' reports totaled 678 words, excluding any confidential comments to the academic editor.

FUNDING: This work was supported by CONACyT [Fondo Sectorial de Investigación y Seguridad Social SSA/IMSS/ISSSTE-CONACyT, SALUD-2014-01-233271]. During this work, ALAO and JCSE were recipients of a CONACyT fellowship. The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: fjimenez@uacj.mx

Paper subject to independent expert single-blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

Published by Libertas Academica. Learn more about this journal.

Introduction

Cervical cancer (CC) is the second leading cause of death from malignancy in women in Mexico and the fourth in the world.[1] Over the years, CC has been studied to define specific characteristics involved in the cancer process to determine the best time for interventions. One of the processes that is currently being studied is the process of DNA methylation and its involvement in cancer treatment. During the cancer process, promoter methylation profiles of tumor suppressor genes are commonly methylated, creating a mechanism for the promotion and development of cancer.[2–4] In CC, it is known that the presence of human papilloma virus (HPV) oncoproteins, such as E6 and E7, increases DNA methyltransferase activity and causes global methylation.[4] Nevertheless, other factors may impinge on this process.

Epidemiological and lifestyle factors are implicated in methylation, such as age,[5,6] obesity,[7] smoking and alcohol intake,[8,9] physical activity,[10] epigenomic inheritance,[11] and circulating estrogens.[12,13] However, there are no studies that have evaluated the relationship between lifestyle factors and the methylation processes in CC.

DNA methylation has been useful in identifying the presence of a tumor, as well as determining its status, subtype, and responsiveness to specific therapies such as retinol.[14] In CC, it has been shown that there are epigenetic alterations in genes related to retinol metabolism, such as the retinoic acid receptor (*RAR*) and the cellular retinol-binding protein (*CRBP1*).[15,16] Nevertheless, it has not been reported whether methylation status can occur in cellular retinoic acid-binding protein-1 (*CRABP1*) and 2 (*CRABP2*) and silence their gene expression.

Retinoic acid (RA) or vitamin A is a metabolite that has an effect on embryonic development, cell growth, differentiation, and apoptosis.[17] These effects are regulated by CRABPs that are related to RA transport within the cell. There are two isoforms, CRABP1 that is expressed in almost all tissues and CRABP2 that is expressed in the skin, nervous system, breast, uterus, and ovary.[18] Both proteins protect amphipathic molecules of RA from oxidative degradations and they also control the availability of retinoids in several metabolic processes. The presence of RA is essential for cell cycle regulation blocking the carcinogenesis process.

Therefore, if methylation in the promoter region of these genes exists, the retinol metabolism could change and affect retinol treatment in CC patients, a commonly used therapy for this cancer. Moreover, knowing other personal factors that promote methylation may allow future interventions for risk populations. The aim of this study is to determine the methylation status of *CRABP* genes and its association with the evolution of the type of squamous intraepithelial lesion (SIL) and CC, as well as the relation with risk factors such as demographic characteristics, habits, and the presence of HPV.

Materials and Methods

Tissue collection. A total of 158 women were selected from the Colposcopy Clinic of Sanitary Jurisdiction II in Ciudad Juarez, Chihuahua, Mexico. Patients were selected by colposcopic and histopathological evaluation, and each patient then signed a consent form for the study. The cervix sample of each patient was obtained by biopsy and by endocervical scraping for the control group. Samples were distributed as low squamous intraepithelial lesion (LSIL; n = 42), high squamous intraepithelial lesion (HSIL; n = 69), and CC (n = 25). Women who showed no intraepithelial lesion (NIL) (n = 22) were selected as control group. All tissue samples were stored in 50 µL of RNAlater® at –20°C (Invitrogen). The ethics committee of Universidad Autónoma de Ciudad Juárez approved this study (CBE.ICB/004.01-14). This research complied with the principles of the Declaration of Helsinki. Patients diagnosed with CC by histopathological evaluation were not on any treatment at that moment. Some demographic characteristics and habit information have been reported to influence the methylation process.[6] Therefore, we collected information about age, family history of cancer, hormonal contraceptive use, smoking, and alcohol intake from patient interviews.

DNA extraction and HPV genotyping method. DNA of tissue samples was extracted by the phenol–chloroform–isoamyl alcohol-adapted technique. Before DNA extraction, tissue was treated with 500 µL of lysis buffer (0.2 M Tris–HCL pH 8; 10 mM ethylenediaminetetraacetic acid pH 8; 0.5 M NaCl; 1% sodium dodecyl sulfate) and 2.5 µL of proteinase K (20 mg/mL) and incubated for 30 minutes at 56°C. Then, the phenol–chloroform–isoamyl alcohol technique was used.[19] HPV genotyping was determined by polymerase chain reaction (PCR). PCR conditions and primer sequences used have been reported elsewhere.[20,21]

Bisulfite treatment and methylation-specific PCR. Extracted DNA was treated with bisulfite using the DNA Methylation-Gold Kit (Zymo Research Corp.) and following the manufacturer protocol. After bisulfite treatment, modified DNA was used as a template for the methylation-specific PCR (MSP) technique. For PCR amplification, 50 ng of bisulfite-modified DNA was added to a final volume of 25 µL PCR mix containing 12.5 µL GoTaq® Green Master Mix (Promega), 1 µL of forward primer, and 1 µL of reverse primer (4 µM for *CRABP1* and 20 µM for *CRABP2*). Primer sequences are shown in Table 1. The unmethylated and methylated regions of *CRABP1* (–193 to +19 bp) and *CRABP2* (–265 to –179 bp) were determined in typical PCR conditions. Annealing temperature for methylated and unmethylated *CRABP1* primers was 60°C. For *CRABP2*, the annealing temperature was 60°C for methylated and 65°C for unmethylated primers. 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 15.0 software (SPSS Inc.). A two-proportion *z*-test was used to analyze the proportions of methylation status among groups. Association between methylation status and personal characteristics of groups was analyzed using χ^2 or Fisher's exact test, as appropriate. Correlations between the process of cancer (NIL to CC) and the presence of methylation were analyzed with Spearman's correlation coefficient (Rho). All *P*-values represent two-tailed tests and were considered significant at 0.05.

Results

Methylation and hemi-methylation of *CRABPs*. The epigenetic modifications were evaluated in Mexican population distributed in three different groups, according to the grade of

Table 1. Primer sequences for *CRABP1* and *CRABP2*.

			5′ → 3′	AMPLICON SIZE (bp)
*CRABP1**	Methylated	Fw	GGAGGTTTTTTAGTTGGAGAGC	212
		Rv	CTCGCAAAACGAAAACTAACG	
	Unmethylated	Fw	GAGGTTTTTTAGTTGGAGAGTGG	211
		Rv	AACTCACAAAACAAAAACTAACACT	
*CRABP2***	Methylated	Fw	CGTTTTCGCGGAGAGCGCG	87
		Rv	AACCGAAATAACCTTCTCCTACGC	
	Unmethylated	Fw	TTTGTTTTTGTGGAGAGTGTGA	86
		Rv	TCCAAAATAACCTTCTCCTACACT	

Notes: *Primer sequences were designed by Wu et al.[23] **Primer sequences design based on Calmon et al.[24]
Abbreviations: Fw, primer forward; Rv, primer reverse.

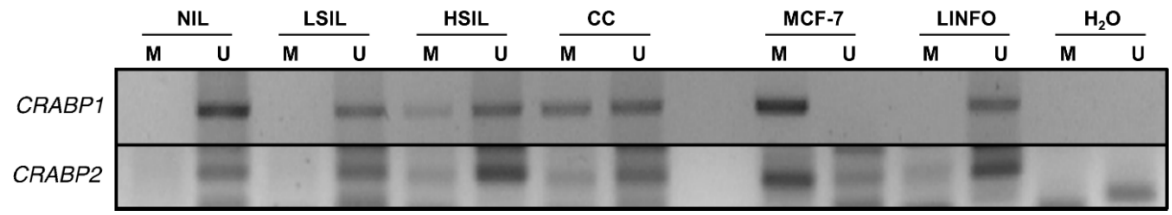

Figure 1. MSP amplification products of representative samples from each group. Patients with CC (16) and HSIL (18) showed hemi-methylation (methylation and unmethylation) patterns in *CRABP1*, and *CRABP2* hemi-methylation was observed in CC (5) and HSIL (3). MCF-7 cell lines were used as methylated (M) positive controls and LINFO (lymphocytes) as unmethylated (U) positive controls.

SIL or CC. The DNA was modified by bisulfite treatment, and PCR test was performed to determine the methylation status of *CRABP1* and *CRABP2*. The amplification products are shown in Figure 1. Amplification results show hemi-methylation (methylation and unmethylation) patterns in the promoter regions of *CRABP1* and *CRABP2*, compared with the control cell line MCF-7. According to the results, 28.5% (45/158) of the samples showed methylation in the promoter region of *CRABP1*, 7.0% (11/158) in that of *CRABP2*, and only 2.5% (4/158) methylation in both genes at once (as shown in Table 2). Analysis indicated that *CRABP1* methylation is significantly associated with CC ($\chi^2 = 19.7$, $P < 0.001$) and with increases in the degree of injury (rho = 0.290, $P < 0.001$). Analysis of proportions showed significant differences between CC and the other groups (NIL, $P = 0.004$; LSIL, $P < 0.001$; HSIL, $P = 0.001$). In contrast, *CRABP2* methylation was observed to be higher in women with CC but significantly different in women with HSIL ($P = 0.047$). The linear correlation of *CRABP2* methylation and degree of injury showed no statistical significance (rho = 0.145, $P = 0.069$).

Risk factor associated with methylation profiles. Important factors involved in CC and methylation were considered in this study (age, family history of cancer, hormonal contraceptive use, HPV infection, and habits as alcohol and smoke). The statistical multivariate analysis shows in Table 3 an association analysis that determines whether the presence of methylation is related to certain personal characteristics. Results of methylation in the promoter of *CRABP1*, adjusted by diagnosis, were found to be 3.6-fold increased when age was ≥35 years (95% confidence interval [95% CI] = 1.58–8.16), 2.2-fold increased with family history of cancer (95% CI = 1.05–4.64), and 2.9-fold increased with the presence of HPV-16 (95% CI = 1.24–6.73). In contrast, methylation of *CRABP1* was found to be 0.4-fold decreased in the presence of alcohol consumption (95% CI = 0.18–0.95). The presence of methylation of *CRABP2* was not statistically significantly associated with any personal characteristics.

Table 2. Percentage of patients with presence of hemi-methylation in *CRABP1* and *CRABP2* gene promoters.

	CRABP1 HEMI-METHYLATION		*CRABP2* HEMI-METHYLATION	
	% (n)	rho (*P*)§	% (n)	rho (*P*)§
NIL	18.2 (4/22)*	0.290 (<0.001)	4.6 (1/22)	0.145 (0.069)
LSIL	16.7 (7/42)*		4.8 (2/42)	
HSIL	26.1 (18/69)*		4.6 (3/69)*	
CC	64.0 (16/25)		20.0 (5/25)	

Notes: §Statistically significant Spearman's correlation coefficient (rho) ($P < 0.05$). *Analysis of proportions showing significant differences between CC and the other groups ($P < 0.05$).

Discussion

Retinoids are commonly used as a chemopreventative and a chemotherapeutic agent for cancer.[17] Nevertheless, the effects of retinoids may be altered by epigenetic changes in CC. Mendoza et al[16] showed that *CRBP1* is methylated in this cancer and that the gene expression is reduced. Additionally, the presence of *RARβ2* methylation increases from low grade to invasive in CC patients.[15] Therefore, metabolism of all retinols has the potential to be changed and generate a resistance to retinoid therapy.[22]

The aim of this study was to determine whether the methylation status was present in *CRABP1* and *CRABP2* gene promoters in CC. First, the proportion of patients with the presence of methylation in *CRABP1* was statistically greater in CC patients than in SIL and NIL patients. This result is similar to that reported in another study.[23] *CRABP2* methylation studies have also described different types and samples of cancer line cells and cancer tissue.[24] In this study, the CC group showed methylation in the promoter region of *CRABP2*, but there was no significant difference when compared with LSIL and NIL. In addition, the proportion of patients with methylation of *CRABP2* was less than that of *CRABP1*. This shows that the process of methylation may be different for the two *CRABPs*. This study analyzed the association of personal characteristic variables that could contribute to methylation in *CRABPs*. The results showed a statistical association between methylation and age, family history of cancer, and HPV-16 genotype infection. Researchers have reported that the presence of global methylation can increase in older people, and this is known as age-related methylation.[5] A family history of cancer may have a genetic influence on methylation,[25] which may contribute to methylation in other

Table 3. Association between promoter hemi-methylation of *CRABPs* and personal characteristic variables.

	CRABP1, n (%)				*CRABP2*, n (%)			
	HM	U	OR* (95% CI)	*P*	HM	U	OR* (95% CI)	*P*
Age								
≥35 years	28 (42.4)	38 (57.6)	3.6	**0.002**	7 (10.6)	59 (89.4)	2.3	0.238
<35 years	17 (18.5)	75 (81.5)	(1.58–8.16)		4 (4.3)	88 (95.7)	(0.58–9.52)	
Family history of cancer								
Positive	23 (39.7)	35 (60.3)	2.2	**0.032**	3 (5.2)	55 (94.8)	0.5	0.392
Negative	22 (22.0)	78 (78.0)	(1.05–4.64)		8 (8.0)	92 (92.0)	(0.12–2.23)	
Hormonal contraceptive use								
Positive	13 (35.1)	24 (64.9)	1.4	0.406	2 (5.4)	35 (94.6)	0.6	0.578
Negative	32 (26.5)	89 (73.5)	(0.61–3.28)		9 (7.4)	112 (92.6)	(0.12–3.18)	
HPV§								
Positive	37 (90.2)	88 (83.8)	1.8	0.434	10 (90.9)	115 (85.2)	1.7	1.000
Negative	4 (9.8)	17 (16.2)	(0.53–7.77)		1 (9.1)	20 (14.8)	(0.22–79.2)	
HPV 16§§								
Positive	22 (44.0)	28 (56.0)	2.9	**0.013**	7 (14.0)	43 (86.0)	3.4	0.081
Negative	15 (20.0)	60 (80.0)	(1.24–6.73)		3 (4.0)	72 (96.0)	(0.79–14.9)	
Smoke								
Positive	8 (22.9)	27 (77.1)	0.6	0.273	2 (5.7)	33 (94.3)	0.65	0.618
Negative	37 (30.1)	86 (69.9)	(0.21–1.53)		9 (7.3)	114 (92.7)	(0.13–3.37)	
Alcohol								
Positive	11 (25.0)	50 (81.9)	0.4	**0.035**	2 (3.3)	59 (96.7)	0.3	0.181
Negative	33 (34.4)	63 (65.6)	(0.18–0.95)		9 (9.4)	87 (90.6)	(0.07–1.71)	

Notes: *Analysis of odds ratio (OR) is adjusted with diagnostics. §Analysis of 146 patients. §§Analysis of positive HPV (n = 125). Bold values show statistical significance ($P \leq 0.05$).
Abbreviations: HM, hemi-methylated; U, unmethylated.

chromosome regions, such as the *CRABP1* gene promoter. Nevertheless, this result must be studied in more depth to find a correlation between both variables. On the other hand, it is well known that high-risk HPV oncoproteins, such as E6 and E7, increase DNA methyltransferase activity and cause global methylation. In addition, host cells increase the methylation process by regulating regions of E6 and E7 oncogenes as a defense mechanism, which may also be affecting other regions.[26] Therefore, this study suggests that the methylation pattern of *CRABP1* is changed by personal characteristics and HPV-16 infection. Alcohol consumption has a positive effect on the absence of methylation of *CRABP1*, and studies have reported that it has an influence on methylation development, especially hypomethylation. Alcohol alters DNA transmethylation and homocysteine metabolism by enzymatic inhibition.[27] However, this study suggests that more analysis is needed on the effects of alcohol on the methylation process.

DNA methylation can be used as a marker to diagnose cancer, evaluate prognosis, or predict a therapy response.[28] Considering this, *CRABP1* might be an epigenetic marker. The results of this study determined that the *CRABP1* gene has epigenetic changes that are in response to personal characteristics of the patient. Consequently, this methylation in the *CRABP1* gene promoter may repress gene expression and disturb retinol metabolism. For example, CRABP1 is the protein that regulates cytoplasmic RA concentration and allows the interaction of RA with other proteins.[29] The absence of *CRABP1* gene expression may alter the correct use of RA and be counterproductive in the use of retinoid treatment.

Conclusion

CRABP1 may be a predictive marker of retinoid therapy response. Nevertheless, we propose to extend this study to determine whether methylation in *CRABP1* and the presence of older age, family history of cancer, HPV-16 infection, and alcohol intake could affect the retinoid treatment in CC. Finally, abnormal methylation processes are of recent interest for many researchers who want to generate epigenetic markers for early detection of cancer or therapeutic prognosis. This study showed that *CRABP1* may be a marker and an important regulator of the retinol pathway in CC.

Acknowledgments

M.D. CDH and patients who contributed to this study of the Colposcopy Clinic of Sanitary Jurisdiction II in Ciudad Juarez, Chihuahua, Mexico, are acknowledged.

Author Contributions

Conceived and designed the experiments: FJV and MSV. Analyzed the data: ALAO, ADMC. Wrote the first draft of the manuscript: ALAO, FJV and JCSE. Contributed to the writing of the manuscript: ADMC, JALD and CLVR. Agree with manuscript results and conclusions: FJV and MSV. Jointly developed the structure and arguments for the paper: FJV and ALAO. Made critical revisions and approved final version: FJV. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Bruni L, Barrionuevo-Rosas L, Albero G, et al. *Human Papillomavirus and Related Diseases in the World*. ICO Information Centre on HPV and Cancer (HPV Information Centre); Barcelona, Spain; 2016.
2. Darroudi F, Bergs JWJ, Bezrookove V, Buist MR, Stalpers LJ, Franken NA. PCC and COBRA-FISH a new tool to characterize primary cervical carcinomas: to assess hall-marks and stage specificity. *Cancer Lett*. 2010;287(1):67–74.
3. Vedham V, Verma M. Cancer-associated infectious agents and epigenetic regulation. In: Verma M, ed. *Cancer Epigenetics. Risk Assessment, Diagnosis, Treatment, and Prognosis*. Vol 1238. Hatfield, Hertfordshire, UK: Humana Press; 2015:335.
4. Jiménez-Wences H, Peralta-Zaragoza O, Fernández-Tilapa G. Human papilloma virus, DNA methylation and microRNA expression in cervical cancer. *Oncol Rep*. 2014;31:2467–2476.
5. Issa J. CpG—island methylation in aging and cancer. *Curr Top Microbiol Immunol*. 2000;249:101–118.
6. Lim U, Song M. Dietary and lifestyle factors of DNA methylation. In: Dumitrescu R, Verma M, eds. *Cancer Epigenetics: Methods and Protocols*. Vol 863. Springer Science+Business Media. Switzerland; 2012:359–376.
7. Dick KJ, Nelson CP, Tsaprouni L, et al. DNA methylation and body-mass index: a genome-wide analysis. *Lancet*. 2014;383:1990–1998.
8. Lim U, Flood A, Choi S, et al. Genomic methylation of leukocyte DNA in relation to colorectal adenoma among asymptomatic women. *Gastroenterology*. 2008;134(1):47–55.
9. Smith IM, Mydlarz WK, Mithani SK, Califano JA. DNA global hypomethylation in squamous cell head and neck cancer associated with smoking, alcohol consumption and stage. *Int J Cancer*. 2007;121(8):1724–1728.
10. Nakajima K, Takeoka M, Mori M, et al. Exercise effects on methylation of ASC gene. *Int J Sports Med*. 2010;31(9):671–675.
11. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev*. 2002;16(1):6–21.
12. Campesi I, Sanna M, Zinellu A, et al. Oral contraceptives modify DNA methylation and monocyte-derived macrophage function. *Biol Sex Differ*. 2012;3(4):1–11.
13. Kovalchuk O, Tryndyak VP, Montgomery B, et al. Estrogen-induced rat breast carcinogenesis is characterized by alterations in DNA methylation, histone modifications, and aberrant microRNA expression. *Cell Cycle*. 2007;6(16):2010–2018.
14. Mulero-Navarro S, Esteller M. Epigenetic biomarkers for human cancer: the time is now. *Crit Rev Oncol Hematol*. 2008;68(1):1–11.
15. Ivanova T, Petrenko A, Gritsko T, et al. Methylation and silencing of the retinoic acid receptor-beta 2 gene in cervical cancer. *BMC Cancer*. 2002;2:4.
16. Mendoza-Rodriguez M, Arreola H, Valdivia A, et al. Cellular retinol binding protein 1 could be a tumor suppressor gene in cervical cancer. *Int J Clin Exp Pathol*. 2013;6(9):1817–1825.
17. Bushue N, Wan Y-JY. Retinoid pathway and cancer therapeutics. *Adv Drug Deliv Rev*. 2010;62(13):1285–1298.
18. Noy N. Retinoid-binding proteins: mediators of retinoid action. *Biochem J*. 2000; 348:481–495.
19. Ausubel F, Brent R, Kingston RE, et al. *Short Protocols in Molecular Biology*. 5th ed. New York: John Wiley & Sons; 2002.
20. Nishiwaki M, Yamamoto T, Tone S, et al. Genotyping of human papillomaviruses by a novel one-step typing method with multiplex PCR and clinical applications. *J Clin Microbiol*. 2008;46(4):1161–1168.
21. Qu W, Jiang G, Cruz Y, et al. PCR detection of human papillomavirus: comparison between MY09/MY11 and GP5+/GP6+ primer systems. *J Clin Microbiol*. 1997;35(6):1304–1310.
22. Freemantle SJ, Spinella MJ, Dmitrovsky E. Retinoids in cancer therapy and chemoprevention: promise meets resistance. *Oncogene*. 2003;22(47):7305–7315.
23. Wu Q, Lothe RA, Ahlquist T, et al. DNA methylation profiling of ovarian carcinomas and their in vitro models identifies HOXA9, HOXB5, SCGB3A1, and CRABP1 as novel targets. *Mol Cancer*. 2007;6:45.
24. Calmon MF, Rodrigues RV, Kaneto CM, et al. Epigenetic silencing of CRABP2 and MX1 in head and neck tumors. *Neoplasia*. 2009;11(12):1329–1339.
25. Gertz J, Varley KE, Reddy TE, et al. Analysis of DNA methylation in a three-generation family reveals widespread genetic influence on epigenetic regulation. *PLoS Genet*. 2011;7(8):1–10.
26. Burgers WA, Blanchon L, Pradhan S, de Launoit Y, Kouzarides T, Fuks F. Viral oncoproteins target the DNA methyltransferases. *Oncogene*. 2012;26(11): 1650–1655.
27. Varela-Rey M, Woodhoo A, Martinez-Chantar M-L, Mato J, Lu S. Alcohol, DNA methylation, and cancer. *Alcohol Res Rev*. 2011;35(1):25–36.
28. Strathdee G. Epigenetic markers and response to chemotherapy in cancer. *Dis Markers*. 2007;23(1–2):43–49.
29. Pfoertner S, Goelden U, Hansen W, et al. Cellular retinoic acid binding protein I: expression and functional influence in renal cell carcinoma. *Tumour Biol*. 2005;26:313–323.