Low proteolytic processing of histone H3 in cervical tissue positive to hrHPV

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Abstract

During the different stages of cervical carcinogenesis there is an accumulation of epigenetic alterations leading to changes in gene expression. High-risk human papillomavirus (hrHPV) is a primary risk factor for cervical cancer (CC). Impaired proteolytic processing of histone H3 constitutes a potential epigenetic mechanism in CC. However, whether this event occurs in early stages of the HPV infection is unknown. Using human cervical samples with normal pathology but positive and negative to hrHPV, we identified that the H3 cleavage was low in the hrHPV positive cervix compared to the hrHPV negative cervix. These results suggest that low H3 processing previously observed in CC may be a primary effect of hrHPV infection.

Introduction

Worldwide, cervical cancer (CC) is the fourth most common neoplasm and could be accounted for approximately 7.5% of all cancer deaths in women. However, in low- and middle-income countries the CC impact is greater being the second most common cancer[1].

The most common viral infection of the reproductive tract is caused by human papillomaviruses (HPV). There are about 40 types of HPV that are associated with diseases of the genitals[2]. However, high-risk HPVs (hrHPV) are identified in CC and include HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66, HPV-68, HPV-73, HPV-82[3]. HPV-16 and HPV-18 are associated with 70% of invasive cervical neoplasms[2]. Various epigenetic processes are deregulated in early stages of carcinogenesis associated to hrHPV, these include DNA methylation[4], post-translational modifications (PTM) of histones[5] and noncoding RNA[6]. Commonly, the dysregulation of the epigenetic mechanisms associated with hrHPV generates changes in the expression of genes that may contribute to the progression to late stages of the disease[4].

A growing body of evidence suggests that the proteolytic cleavage of the amino terminal end of histone H3 (H3) is a conserved process in Eukaryotes[7–11]. H3 cleaved products may be involved in cell cycle promotion, DNA replication, cell proliferation, apoptosis and migration[8]. Notably, proteolytic cleavage of H3 is reported to occur during viral infections[12].

Proteolytic cleavage of the amino terminal end of H3 has been documented in cancer[13, 14]. Specifically, we reported that the proteolytic cut of H3 is low in CC[13]. However, it is unknown whether reduced H3 processing is product of advanced stages of the cancer or whether it is associated with the HPV infection before the onset of CC. Therefore, we sought to investigate the H3 processing status in the cervix infected or not infected with HPV but negative for CC.

Methods

Patients

Cervical samples from 8 patients diagnosed with abnormal uterine bleeding (AUB) or uterine myomatosis (UM) and adnexal tumor (AT) were obtained by hysterectomy procedures. The patients diagnosed with AUB were classified according to the International Federation of Gynecology and Obstetrics (FIGO). The information

concerning FIGO classification is included in Table 1. Additionally, all samples, were negative to pathological analysis to cervical cancer (NCC). Importantly 4 NCC samples (Lane P5 - P8) were positive for hrHPV by PCR, but negative for cervical intrapithelial neoplasia (CIN) by histopathological analysis (Table 1). In addition, the sample of each patient was divided into two parts; the first one for obtaining DNA and the remaining part was used to obtain total proteins extracts.

Table 1
Features of patients with NCC.

	P1	P2	P3	P4	P5	P6	P7	P8
Age (years)	49	36	40	38	51	50	39	47
IMC	16.44	27.38	26.17	37.8	31.24	26.14	30.84	37.67
Marital status	Single	Married	Married	Divorced	Married	Divorced	Married	Single
Place of residence	Mexico City	Mexico City	Michoacan	Mexico state	Mexico City	Mexico City	Michoacan	Mexico City
Employed	Not	Not	Not	Not	Not	Yes	Not	Yes
Diagnostic	AUB L	AUB L	AUB L	AUB L	AUB L	AUB L	AUB L	UM and AT
Surgery	TAH	TAH	TAH	TAH	TAH	TVH	TAH	TAH
CIN	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
HPV genotype	NEG	NEG	NEG	NEG	31	31, 66	18, 31	59

NCC, negative to pathological analysis to cervical cancer; IMC, body mass index; HPV, human papillomaviruses; UM, uterin myomatosis; AT, anexial tumoration; AUB L, abnormal uterine bleeding leiomyoma; TAH, total abdominal hysterectomy; TVH, total vaginal hysterectomy; CIN, cervical intraepithelial neoplasia; NEG, negative.

Immunoblot Analysis

Immunoblot analysis
Immunoblotting assays were performed using total proteins extracts from NCC tissue. All samples were obtained with RSB buffer (10 mM Tris-Cl, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 1% NP40) supplemented with complete protease inhibitor cocktail. Immunoblotting assays were performed as described elsewhere[13]. Briefly, samples were resuspended in 2X Laemmli-buffer with β-mercaptoethanol, heated to 95ºC for 8 min, and loaded onto a 15% SDS-polyacrylamide gel. Proteins were transferred to a PVDF membrane by damp blotting at 40 mA for 3 h. The PVDF membrane was blocked with 5% milk in TBS-T (10X TBS-T: 198 mM Tris, 1400 mM NaCl, 0.01% Tween 20, pH 7.6) for 1 h. Then the PVDF membrane was incubated overnight at 4ºC with the primary anti-H3 antibody (E.960.2, Thermo Fisher Scientific) or HRP conjugated-GAPDH antibody (14C10, Cell Signaling Technology). Anti-H3 antibody was used at a 1:500 dilution and GAPDH (used as loading control) at a 1:1000 dilution. The membrane was subsequently washed three times in TBS-T followed by incubation with

HRP-conjugated secondary antibody (Jackson ImmunoResearch) for 1 h at room temperature. Then, the membrane was washed three times in TBS-T. Signals were detected using by chemiluminescence C-Digit (LI-COR).

Genotyping Hpv

DNA extraction from the patient's tissue was performed following manufactures instructions (Invitrogen Thermo Fisher Scientific). After this, genotyping determination of HPV was carried out through Anyplex II HPV28 detection kit (Seegene) following the manufactures instructions. Briefly, in 20 µl PCR reaction 1x of HPV28 primer mix A (14 high-risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) or B (5 high risk HPV types: 26, 53, 69, 73, 82 and 9 low risk types: 6, 11, 40, 42, 43, 44, 54, 61, 70), 1x Anyplex master mix, and approximately 50 ng of DNA were added. The *thermocycling* conditions was as follow: an initial step at 50°C for 4 minutes and a denaturation step at 95°C for 15 minutes. The samples underwent 30 cycles at 95°C for 30 seconds, 60°C for 60 seconds and 72°C for 30 seconds. The PCR reaction were done in a CFX96 Real Time PCR System (Bio-Rad) as recommended by the manufacturer.

Results & Discussion

Histone Clipping has been observed in different cell lines[13, 14] including HeLa cells[13]. Previously, a report showed that H3 clipping is reduced in samples of CC[13]. However, if the impaired H3 processing occurred as a primary effect of the HPV infection or it is a consequence of cervical carcinogenesis as result of the high number of alterations is unknown. To address this, we analyzed the H3 clipping in samples of positive hrHPV cervical tissue with NCC. In addition, negative-HPV samples diagnosed with NCC were included as observed in Fig. 1. Characteristics of the analyzed samples are showed in the Table 1. Two forms of truncated H3 were detected principally in almost all the samples (Fig. 1). However, the presence of truncated H3 forms was reduced in positive hrHPV samples. These results indicate that H3 clipping is decreased in positive hrHPV NCC samples as compared with negative HPV NCC samples. Thus, this data suggest that aberrant H3 processing is inherently associated with hrHPV infection. Moreover, since low H3 clipping is also observed in samples with CC, it is possible that impaired H3 clipping is preserved during all the process of cervical carcinogenesis. Whether the low H3 processing is required for the development of CC remains to be demonstrated.

Alterations in histone clipping in host cells promoted by infectious microorganisms have been described previously[12, 15]. Proteins named adhesion and penetration protein (App) and meningococcal serine protease A (MspA) are secreted by *Neisseria meningitidis*, a bacterium that can cause meningitis and/or septicemia in children and young adults[16]. These proteins, involved in meningococcal pathogenesis, can bind histones of human cells and generate proteolytic processing of H3 due to their serine endopeptidase activity[15]. H3 clipping also can be generated by the 3C protease of the foot and mouth disease virus (FDMV). The truncated H3 losses 20 amino acids from the N-terminus[12].

Oncogenic viruses can promote aberrant epigenetic changes in the host[17]. HPV oncoprotein specifically can promote PTM[18]. It is known that E6 protein of HPV can bind cellular serine protease thought the PDZ domain[19] and we recently demonstrated that serine and aspartyl proteases are implicated in the H3 clipping[13].

Even though a limitation is the number of samples, the present work is the first to suggest that low H3 processing may not be a consequence of the gene expression alteration in CC but a primary step associate to the hrHPV infection. If this event is determinant for viral virulence in the progression to CC requires further investigation but if this is the case, it could offer a potential therapeutic preventive target for the development of the disease.

Abbreviations

hrHPV High-risk human papillomavirus

CC Cervical cancer

PTM Post-translational modification

H3 Histone H3

AUB Abnormal uterine bleeding

UM Uterine myomatosis

AT Adnexal tumor

FIGO International Federation of Gynecology and Obstetrics

NCC Non cervical cancer

CIN Intrapithelial neoplasia

Declarations

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Authors´ contributions

All authors listed in the present manuscript meet the ICMJE criteria for authorship. All authors certify that they have participated in the work to assume public responsibility for the content, including participation in the concept, design, formal analysis, project management, resources, supervision, writing or revision of the manuscript. The authors approved the submitted version.

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Availability of data and materials

The data analyzed in the study are in the archives of the Hospital Juárez de México and are included in this article.

Ethics approval and consent to participate

Ethical approval was obtained from the ethical committee of the Hospital Juarez de Mexico (HJM2231/13-B).

Consent for publication

Participants gave a written consent for participation and for their data to be published in a scientific journal.

Competing interests

We declared not conflict interests.

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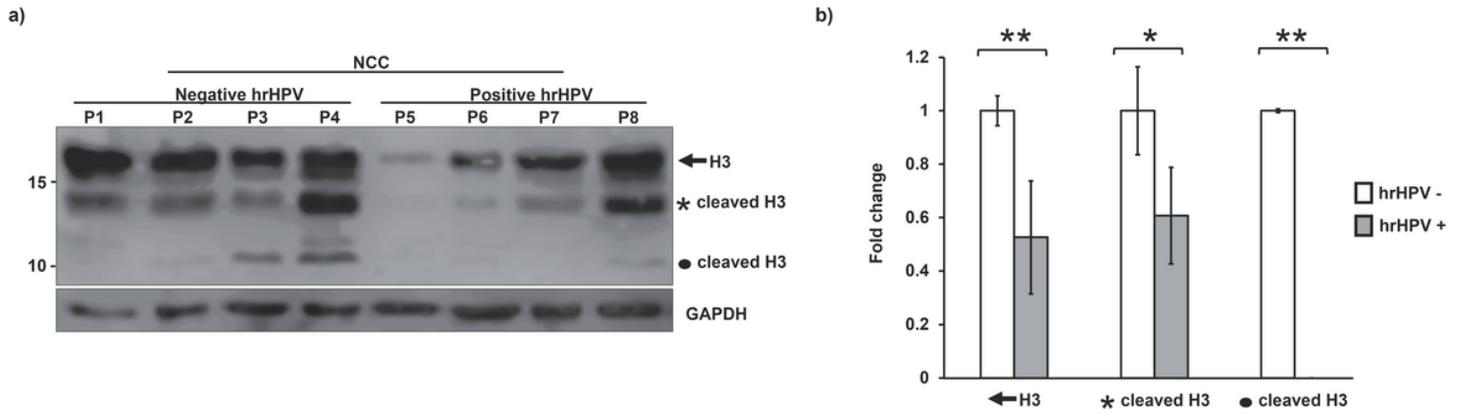
Figures

Figure 1

Low proteolytic processing of histone H3 in cervical tissue positive to hrHPV. (A) Immunodetection of histone H3 (H3) by western blot showed low levels of the two cleavage forms (asterisk and circle) in positive-hrHPV cervical tissue with negative to pathological analysis to cervical cancer (NCC; Lane P5 – P8) as compared with negative-hrHPV NCC samples (lane P1 – P4). Complete H3 (arrow) was identified in all samples (lane P1 – P8). Note the slight decrease of complete h3 in the positive-hrHPV NCC samples (lane P5 - P6). GAPDH expression was analyzed in parallel as an internal control. The running position of protein markers is shown on the left. The result is representative of at least three separate experiments. (B) Densitometry profile of complete and processed H3 (asterisk and circle) in cervical tissue samples NCC positive-hrHPV vs negative-hrHPV. The mean and variance of three separate experiments were plotted. The Wilconxon statistical test was applied to evaluate significant difference. **$P<0.01$ for complete H3 (arrow); *$P<0.05$ for H3 processed (asterisk) and **$P<0.01$ for H3 processed (circle).