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Association between the stages of cervical cancer and chromosome 1 aneusomy

Elva I. Cortés-Gutiérrez[a,b,*], Martha I. Dávila-Rodríguez[a], Marycarmen Muraira-Rodríguez[a], Salvador Said-Fernández[c], Ricardo M. Cerda-Flores[a,d]

[a]*Genetics Division, Centro de Investigación Biomédica del Noreste (CIBIN), Instituto Mexicano del Seguro Social (IMSS), Administración de Correos No. 4, Apartado postal 20, C.P. 64720 Monterrey, Nuevo León, Mexico*
[b]*Facultad de Ciencias Biológicas (FCB), Universidad Autónoma de Nuevo León (UANL), Monterrey, Nuevo León, Mexico*
[c]*Cellular Biology and Molecular Division, CIBIN, IMSS, Monterrey, Nuevo León, Mexico*
[d]*Facultad de Enfermería, UANL, Monterrey, Nuevo León, Mexico*

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Abstract The high-risk human papillomavirus is known to play a pivotal role in cervical carcinogenesis. Numerical and structural aberrations are known to be related to different behaviors of malignant cervical lesions. The aims of this study were (1) to assess the number of cervical cells with chromosome 1 aneusomy (monosomy, trisomy, and tetrasomy) in 20 women with cervical intraepithelial neoplasia (CIN 1, CIN 2, CIN 3, and invasive cancer) and three women without CIN by fluorescence in situ hybridization (FISH), (2) to determine the heterogeneity of aneusomy among women within each of the five groups studied, (3) to determine the association between the four progressive stages of cervical cancer and the number of cells with and without aneusomy, (4) to determine the association between number of cells with and without aneusomy and human papilloma virus (HPV) infection, and (5) to determine its usefulness as a biomarker of cancer risk. A hospital-based unmatched case–control study in a sample of 23 women grouped by disease stage and selected by histology from the Obstetrics and Gynecology Hospital of the Instituto Mexicano del Seguro Social (IMSS) in Mexico was conducted in 2002. Numerical aberrations of chromosome 1 in cervical smears were detected with FISH. HPV was detected with polymerase chain reaction (PCR) and typing was performed with restriction fragment length polymorphism (RFLPs). Analysis of chromosome 1 aneusomy revealed (1) homogeneity among women within each one of the five groups, (2) a positive linear trend between the aneusomy frequency and grade of lesion, and (3) an association between aneusomy and high-risk HPV infection. These findings suggest the usefulness of the number of cervical cells with chromosome 1 aneusomy as a biomarker. In order to validate this biomarker we suggest a larger prospective study of cytological samples of patients with a longer follow-up.

1. Introduction

Cervical cancer represents the second most common malignant neoplasia in women worldwide. In Mexico, cervical cancer is the most common female malignancy [1].

Cervical intraepithelial neoplasia (CIN) is morphologically classified as mild (CIN 1), moderate (CIN 2), and severe (CIN 3) or as in situ carcinoma. CIN 3, if left untreated, has a high probability of progressing into invasive carcinoma [2].

Risk factors have been correlated to development of CIN, suggesting that the human papillomavirus (HPV) types 16, 18, 31, and 33 (high risk) play a pivotal role; however, additional chromosomal alterations seem to be necessary for development and progression [3].

Numerical changes in specific chromosomes (aneusomy) can involve a gain (e.g., trisomy) or a loss (e.g., monosomy), with respect to a normal condition (disomy). A misdivision gives rise to an amplification of the whole genome (polyploidy) [4].

Chromosomal anomalies in cervical tumors are either numerical or structural or a mixture of both. By conventional cytogenetic techniques (e.g., G-banding), structural rearrangements of chromosome 1 (e.g., deletions, translocations, and

* Corresponding author. Tel.: +52-81-81904035.
E-mail address: elvacortes@cibinmty.net (E.I. Cortés-Gutiérrez).

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isochromosomes) have been described as the most frequent karyotypic changes; for example, >95% of patients show rearrangements in this chromosome [5]. On the other hand, aneusomy and polyploidy of chromosome 1 have been detected as the most frequently numerical changes in cervical cancer [5].

From fluorescence in situ hybridization (FISH) and interphase cytogenetics findings, aneusomy in chromosomes 1, 7, 8, 11, 17, and X-chromosome [6–13] has been proposed as an early event in squamous cell carcinoma.

Until the development of the FISH technique, accurate identification of chromosome gain or loss in solid tumor was difficult, because direct chromosome analysis is frequently hampered by the absence or a small number of metaphases [14]. In patients with cancer of the uterine cervix, a significant increase in the number of cells with chromosome 1 aneusomy has been reported [6–7]; however, the association between chromosome 1 aneusomy and cancer and its use as a biomarker have not been established.

Our objectives were to (1) assess the number of cervical cells with chromosome 1 aneusomy in women with and without CIN, using FISH, (2) determine the heterogeneity of aneusomy among women within each one of the five studied groups, (3) determine the association between the four progressive stages of cervical cancer and the number of cells with and without aneusomy, (4) determine the association between the number of cells with and without aneusomy and high-risk HPV infection, and (5) determine its usefulness as a biomarker of uterine cervical cancer.

2. Materials and methods

2.1. Study population

A hospital-based unmatched case–control study in a sample of 23 women grouped by disease stage and selected by histology (control, CIN 1, CIN 2, CIN 3, and invasive) from the Obstetrics and Gynecology Hospital of the Instituto Mexicano del Seguro Social (IMSS) in Mexico was conducted in 2002. This study was approved by the Ethical Committee of the Centro de Investigación Biomédica del Noreste, IMSS.

The International Federation of Gynecology and Obstetrics (FIGO) criterion was used for the histological diagnoses [15].

The patients had no previous chemotherapy or radiotherapy. None had a clinical history of chronic infection, drug use (including contraceptives), cigarette smoking, or radiation exposure. The four grandparents of the patients were born in Northeastern Mexico. The average age was 43.04 (range 31–55) and 45.51 years (range 34–56) for the patients and control, respectively.

For detecting the presence of HPV, the polymerase chain reaction (PCR) was performed using commercial probes MY09/MY11 (MWG Biotech; NC). HPV typing was done by restriction fragment length polymorphisms (RFLPs) analyses with a combination of *Hae*III, *Pst*I, and *Rsa*I restriction endonucleases.

2.2. FISH detection

The cervical smears were hybridized with a DNA probe containing complementary centromeric α-satellite sequences of chromosome 1 (CEP 1 [D1Z5] SpectrumOrange) (Vysis, Downers Grove, IL) and contrasted using 4′,6-diamidino-2-phenylindole (DAPI) [16].

Hybridization signals were viewed with a triple-band-pass filter set (fluorescein isothiocyanate [FITC]/Texas Red/DAPI) on a standard epifluorescence-equipped microscope (Zeiss Axiophot). Photomicrographs were taken with Kodak Ektachrome ASA 1600 color slide film.

2.3. Scoring methodology

Only slides with morphologically well-demarcated and well-preserved nonoverlapping nuclei were evaluated. The criteria for evaluating FISH signals were that nuclei should not overlap and that signals should have the same homogeneous fluorescence intensity [17].

Detection of any variation in probe signal number (i.e., differing from 2; D1Z5×2) was considered positive. This includes monosomy (one signal; D1Z5×1), trisomy (three signals; D1Z5×3) and tetrasomy (four signals; D1Z5×4). For each woman, 300 cells were examined [18].

In addition, smears were also read by a second, blinded cytogeneticist to assess the intralaboratory diagnostic agreement rate ($\kappa = 0.92$).

Table 1
Distribution of the frequency of cervical cells with and without aneusomy of chromosome 1, by study group

			Cervical cells[a]				
Group	*n*	Cells, no.	Disomy, %	Monosomy, %	Trisomy, %	Tetrasomy, %	*p*
Control	3	900	96.08	1.57	2.35	0.00	0.954
CIN 1	2	600	92.56	2.93	4.39	0.12	0.918
CIN 2	9	2,700	90.59	2.78	6.28	0.35	0.997
CIN 3	7	2,100	80.16	10.78	6.79	2.35	0.506
Invasive	2	600	74.35	13.91	10.43	1.30	1.000

Abbreviations: CIN, cervical intraepithelial neoplasia; *P*, probability of heterogeneity per group using R × C with 10,000 simulations.
[a] Disomy: nuc ish 1 cen (D1Z5×2). Monosomy, nuc ish 1 cen (D1Z5×1). Trisomy, nuc ish 1 cen (D1Z5×3). Tetrasomy, nuc ish 1 cen (D1Z5×4).