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Correlation between the presence of high-risk human papillomaviruses and Id gene expression in Syrian women with cervical cancer

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Abstract

Infection by high-risk human papillomaviruses (HPVs) is considered to be the central cause of invasive cervical cancer. Previously reported studies have shown that Id genes regulate cell invasion and metastasis in several human carcinomas including cervical cancer. In order to investigate the correlation between high-risk HPVs and Id genes in human cervical cancer, the presence of high-risk HPVs and their association with Id gene expression was examined using PCR methods and tissue microarray analyses in a cohort of 44 cervical cancer patients from Syria. This study showed that high-risk HPVs were present in 42 samples (95%) that represent invasive cervical cancers and that the most frequent high-risk HPV types in Syrian women were 33, 16, 18, 45, 52, 58, 35, 51 and 31. Furthermore, the expression of E6 oncoprotein of high-risk HPVs was found to correlate with overexpression of Id-1, but not of Id-2, Id-3 or Id-4 in the majority of invasive cervical cancer tissue samples. These data suggest that high-risk HPVs can enhance the progression of human cervical cancer through Id-1 regulation.

Keywords: Cervical cancer, high-risk HPV, Id genes, Syrian women
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Introduction

High-risk HPVs are important risk factors for human cervical cancer; approximately 96% of cervical cancers score positive for high-risk HPVs [1,2]. In addition, the presence of high-risk HPVs serves as a prognostic factor in early-stage cervical cancers, and are associated with vascular invasion, lymph node metastases and tumour size [2–5]. The E6 and E7 oncoproteins of high-risk HPVs, which are expressed in cervical cancers, inactivate p53 and pRb tumour suppressors, respectively [6]. E6 facilitates the degradation of p53 through its association with accessory protein E6-AP, a component of the ubiquitin proteolytic pathway [7]. E7 proteins of high-risk HPVs bind to Rb [8], as well as to other pocket proteins, such as p107 and p130 [9], leading to cell cycle deregulation. This results in genomic instability and has been implicated in the transformation of normal cells and the progression of cancerous cervical cells.

Id (inhibitors of differentiation and DNA binding) genes are members of the helix-loop-helix transcription factor family and have multiple functions including inhibition of differentiation, induction of proliferation and delaying replicative senescence [10,11]. Moreover, Id-1 and Id-3 have been indicated as potential oncogenes because they are overexpressed in several human cancers including cervical, colorectal, breast, pancreatic and prostate cancer [12–16]. The up-regulation of Id-1 is clearly associated with more aggressive behaviour of tumour cells, particularly in cervical cancers [12]. These studies suggest that Id genes play an important role in the progression of human cervical cancers.

This investigation aims to identify the specific types of high-risk HPV infections present in cervical cancers of Syrian women and their association with Id gene expression and tumour aggressiveness.

Materials and Methods

HPV detection and type specification

Formalin-fixed, paraffin-embedded blocks of cervical tumour samples were obtained from 44 Syrian patients with an average age of 57 (range 38–82) years. Formalin-fixed (buffered, neutral aqueous 10% solution), paraffin-embedded cervical tumour samples were supplied by the Department of Pathology, Faculty of Medicine at the University of Aleppo, Syria. The specimens and data used in this research were approved by the Ethics Committee of the Faculty of Medicine of Aleppo University. Five micrograms of purified genomic DNA (Qiagen GmbH, Hilden, Germany) were taken from each sample and analysed for HPVs by multiplex PCR targeted to the conserved L1 region of the viral genome using PGMY09/11 L1 primer pools [3,13]. In parallel, specific primers for the E7 gene were used to detect HPV types 16, 18, 31, 33, 35, 45, 51, 52 and 58, while specific primers for the gene encoding for glyceraldehyde-3-phosphate dehydrogenase were used as an internal control [17] (Table 1). PCR products were denatured in 0.13 N NaOH and hybridized to an immobilized HPV probe array using an extended reverse line-blot assay for HPV genotyping (Roche Molecular Systems, Inc., Alameda, CA, USA) of nine high-risk HPVs (types 16, 18, 31, 33, 35, 45, 51, 52 and 58), as classified by Begum *et al.* [3].

Tissue microarray

The tissue microarray (TMA) construction was performed as described by Kuefer *et al.* [18]. Cervical tumour samples were embedded in a virgin paraffin TMA block using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD, USA). Each block was assembled without prior knowledge of associated clinical or pathological staging information. An average of two TMA cores (range two to six) 1.0 mm in diameter were sampled from a cohort of 44 Syrian patients diagnosed with cervical carcinoma. After construction, 4-μm sections were cut and stained with haematoxylin and eosin to verify the histological diagnosis. Slides of the finished blocks were used for immunohistochemistry analysis.

TABLE 1. Gene-specific primer sets for E7 genes of high-risk HPVs used for PCR amplification

HPV types	Region	Primers
16	E7	5′-ATGCATGGAGATACACCTACATTGCAT-3′ 5′-GTTTCTGAGAACAGATGGGGCACAC-3′
18	E6	5′-GCTTTGAGGATCCAACACGG-3′ 5′-TGCAGCACGAATGGCACTGG-3′
31	E7	5′-GGGCTCATTTGGAATCGTGTG-3′ 5′-AACCATTGCATCCCGTCCCC-3′
33	E7	5′-TGAGGATGAAGGCTTGGACC-3′ 5′-TGACACATAAACGAACTGTG-3′
35	E7	5′-CTATTGACGGTCCAGCT-3′ 5′-TACACACAGACGTAGTGTCG-3′
45	E7	5′-CCC ACG AGC CGA ACC ACA G-3′ 5′-TCT AAG GTC CTC TGC CGA GC-3′
51	E7	5′-TAC GTG TTA CAG AAT TGA AG-3′ 5′-AAC CAG GCT TAG TTC GCC CAT T-3′
52	E7	5′-GCA GAA CAA GCC ACA AGC AA-3′ 5′-TAG AGT ACG AAG GTC CGT CG-3′
58	E7	5′-CGA GGA TGA AAT AGG CTT GG-3′ 5′-ACA CAA ACG AAC CGT GGT GC-3′

Primers specific for the glyceraldehyde-3-phosphate dehydrogenase gene, 5′-GAAGGC-CATGCCAGTGAGCT-3′ and 5′-CCGGGAAACTGTGGCGTGAT-3′, were used as an internal control.

Immunohistochemistry

Immunohistochemical procedures examining the expression of Id-1, Id-2, Id-3, Id-4 and E6 were carried out using the following standard procedures. The protein expression levels and immunolocalization of Id-1, Id-2, Id-3, Id-4 and E6 in the TMA of Syrian cervical tumour specimens were analysed by mounting 4-μm sections of the TMA on saline-coated slides (Sigma, St Louis, MO, USA), which were subsequently dried overnight at 37°C. The TMA sections were then deparaffinized in graded alcohol, rehydrated, and boiled in 10 mM citrate buffer (pH 6.0) for antigen retrieval. The TMA slides were subsequently incubated for 32 min at 37°C with primary polyclonal rabbit antihuman Id-1, Id-2, Id-3 and Id-4 antibodies (sc-488, sc-489, sc-490 and sc-491, respectively; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:25, or E6 (mouse monoclonal; clone C1P5; Calbiochem, ON, Canada) at a dilution of 1:20, using the NexES automated immunostainer (Ventana Medical System, Tucson, AZ, USA). The automated Ventana Medical System uses an indirect biotin–avidin system with a universal biotinylated immunoglobulin secondary antibody. Diaminobenzidine was used as a chromogen, and slides were counterstained with haematoxylin prior to mounting. All staining procedures were performed according to the manufacturers' recommendations. Negative controls were obtained using specific blocking peptides from Santa Cruz Biotechnology with anti-Id antibodies at a ratio of 10:1 and by omitting the specific primary antibody for E6.

Following immunohistochemistry, all TMA slides were manually scored for Id-1, Id-2, Id-3, Id-4 and E6 expression and categorized using a three-tiered system (0 = negative, 1 = weak, 2 = strong) by two independent observers.

Results

In order to categorize the presence of high-risk HPV in human cervical cancer in Syrian women, we investigated the presence of high-risk HPV types 16, 18, 31, 33 35, 45, 51, 52