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Autumn 2020, Volume 5, Issue 19 (13-15)

Evaluation of CENPM Gene Expression in Cervical Cancer

Raziye Amuzgar[1*], Najme Shojaei[2]

[1] Kermanshah University of Medical Sciences, School of the Medicine, Kermanshah, Iran

[2] Personalized Medicine Research Center of AmitisGen, Tehran, Iran

**Corresponding author: Raziye Amuzgar. Kermanshah University of Medical Sciences, School of the Medicine, Kermanshah, Iran. Email: Raziye.Amuzgar@gmail.com*

DOI: 10.22034/pmj.2020.240046

Submitted: 2020/06/13
Accepted: 2020/08/09

Keywords:
Cervical cancer
Human papillomavirus
Chromosomal instability
CENPM Gene

Abstract

Cervical cancer is the most widely screened cancer globally, both in high- and middle-income countries. HPV has been implicated in 99.7% of cervical squamous cell cancer cases worldwide. Viral E6 and E7 gene expression leads to alterations in the cellular genome integrity, including structural and numerical chromosomal instability resulting in chromosomal mis-segregation and aneuploidy. Centromere protein M (CENPM) is an essential centromere component for chromosome separation. In this study, the gene expression was evaluated at the mRNA level using the qRT-PCR method on 20 cancer samples previously infected with feather papillomavirus. The results show a significant increase in gene expression in cancer samples.

INTRODUCTION

Cervical cancer is one of the most common gynecological cancers occurring in women, which can be detected and treated at precancerous stages completely [1]. Cervical cancer was the most important cancer among women in the past two decades. The findings revealed that female genital cancers are the fourth most common malignancy in women [2]. The incidence rate and prevalence of female genital cancers in various parts of the world are different. Among women in developing countries, this malignancy is one of the most important causes of female mortality, and after breast cancer, it is the most frequent malignancy [3]. Detection and timely treatment of these cancers can increase women's longevity. Cervical cancer is caused by sexually acquired infections with certain types of HPV [4]. Two HPV types (16 and 18) cause 70% of cervical cancers and precancerous cervical lesions. The association between genital HPV infections and cervical cancer was first demonstrated in the early 1980s by Harold Zur Hausen, a German virologist. Since then, the association between HPV and cervical squamous cell carcinoma has become well established [4]. The magnitude of the association between HPV and cervical squamous cell carcinoma is higher than the association between smoking and lung cancer. HPV has been implicated in 99.7% of cervical squamous cell cancer cases worldwide [5]. The HPV genome consists of a single molecule of double-stranded, circular DNA containing approximately 7,900 bp associated with histones [6]. All open reading frame (ORF) protein-coding sequences are restricted to one strand. The genome is functionally divided into three regions. First is a noncoding upstream regulatory region, second is an early region, consisting of ORFs E1, E2, E4, E5, E6, and E7, which are involved in viral replication and oncogenesis, and the third is a late region that encodes the L1 and L2 structural proteins for the viral capsid [7]. HR HPV can transform infected cells through the direct action of the products of two of its early genes: E6 and E7. The E6 protein of HR HPV binds with high affinity to the molecule known as p53, inducing its degradation [8]. The p53 protein is an important regulator of cell replication and is known as the main tumor suppressor in humans, which can detect damage to DNA and arrest cell replication. In high-risk papillomavirus infections, the cell cannot repair DNA errors and experience tumor development when the number of mutations increases [9].

On the other hand, the E7 protein binds specifically to the tumor suppressor gene product Rb. This is a cell-cycle regulatory factor and is directly associated with the E2F transcription factor that, in turn, induces the transcription of elements involved in cell replication [10]. Thus, E6 and E7 cooperate efficiently in cell transformation, stimulating chromosomal alterations in the uterine cervix. The profile of

chromosomal alterations is very heterogeneous in precursor lesions. Centromere protein M (CENPM) is an essential centromere component associated with several other centromere proteins, including CEN PA, CENPC, CENPI, and CENPH, and is required for chromosome separation [10]. The CENPM protein interacts with other proteins to form a vital complex that preserves kinetochore and spindle microtubule attachment during metaphase [11]. CENPM overexpression leads to unequal numbers of chromosomes in cells that subsequently exit mitosis and survive, leading to aneuploidy [12]. In addition, high CENPM expression has been reported to be associated with primary melanoma, bladder cancer, hepatocellular carcinoma, and head and neck squamous cell carcinoma [14]. In this study, CENPM gene expression was evaluated at mRNA level in cervical cancer samples.

METHOD AND MATERIALS

A total of 20 cervical cancer patients previously infected with a feather papillomavirus infection were selected as the case group, and 20 healthy vaginal specimens prepared during routine periodic visits were selected as a control group. None of the patients was treated with radiotherapy and chemotherapy before undergoing an operation. All patients were operated by a skilled clinician. A part of the tissue was used for pathological diagnosis, and the remaining part was frozen in liquid nitrogen for the extraction of mRNA. RNA was extracted by TRIzol™ Reagent (Thermo Fisher Scientific, USA). cDNA synthesis was done by random hexamer PrimeScript 1st strand cDNA synthesis kits (Takara Bio, Japan). Target gene mRNA expression was detected with the specific primers F: GCGGACTCGATGCTCAAAGA, R: TTCTGGAGACTGTATTTGCTGTG, and FastStart Universal SYBR Green Master. The data were analyzed using the 2^-ΔΔct equation.

RESULTS

CENPM gene expression was measured at mRNA level by qRT-PCR method on 20 cancer samples previously infected with feather papillomavirus infection and compared with healthy subjects The results showed a significant increase in this gene expression in cancer samples.

DISCUSSION

Cervical cancer is the most widely screened cancer globally, both in high- and middle-income countries. Population-based cervical cytology screening programs offering papanicolaou testing every 3 to 4 years have reduced cervical cancer incidence and mortality by up to 80% in developed countries [15]. The importance of human papillomavirus (HPV) infection and its role in cancer progress have been widely evaluated. Overexpression of the human papillomavirus (HPV) oncogenes E6 and E7 are necessary to develop distinct lower genital tract cancers [16]. Besides directly interfering with critical cell cycle pathways (p53, pRB) and thereby promoting cell proliferation, the E6 and E7 expression lead to alterations of the cellular genome integrity, including structural and numerical chromosomal instability resulting in chromosomal mis-segregation and aneuploidy [17]. Centromere protein M (CENPM) is an essential centromere component associated with several other centromere proteins, including CENPA, CENPC, CENPI, and CENPH, and is required for chromosome separation. The CENPM protein interacts with other proteins to form a vital complex that preserves kinetochore and spindle microtubule attachment during metaphase [17]. CENPM overexpression leads to unequal numbers of chromosomes in cells that subsequently exit mitosis and survive, leading to aneuploidy. CENPM gene expression has been reported to be associated with tumorigenesis in primary melanoma, bladder cancer, hepatocellular carcinoma, and head and neck squamous cell carcinoma [18]. In this study, the gene expression was evaluated at mRNA level by qRT-PCR method on 20 cancer samples previously infected with a feather papillomavirus infection. The results showed a significant increase in gene expression in cancer samples. Thus, it is suggested that further experiments should be conducted to explain which pathway contributes to the upregulation of CENPM in cervical cancer cells. Further studies are needed to elucidate the role of the HPV virus in altering this gene expression.

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