Infection, Genetics and Evolution 61 (2018) 134–144

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Review

Understanding the HPV integration and its progression to cervical cancer

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ARTICLE INFO

Keywords:
HPV
Cervical cancer
Viral integration

ABSTRACT

Cervical cancer is one of the main causes of female cancer death worldwide, and human papilloma virus (HPV) its causal agent. To investigate viral oncogenesis several studies have focused on the effects of HPV oncoproteins E6 and E7 and the mechanisms by which these proteins stimulate the cellular transformation process. However, phenomena such as the physical state of the viral genome (episomal or integrated) and the effects of this integration on cell proliferation contribute new clues to understand how HPV infection causes carcinogenesis. New molecular technologies are currently facilitating these discoveries. This paper reviews the tumor development process initiated by HPV, recent findings on the process of viral integration into the host genome, new methods to detect HPV integration, and derived associated effects.

1. Introduction

Cervical cancer (CC) is the second most common type of cancer worldwide, with 500,000 cases per year (WHO | Human papillomavirus (HPV) and cervical cancer, 2015; Fact Sheets by Cancer, 2014). In Mexico, CC is the second most prevalent neoplasia in women, after breast cancer. In 2012 there were 5571 new cases of severe cervical dysplasia and CC *in situ*, mostly in women between 25 and 44 years of age (WHO | Human papillomavirus (HPV) and cervical cancer, 2015), being responsible for approximately 3880 deaths in women of reproductive age.

This neoplasia is a slow-evolving cellular alteration in the cervix that develops after the human papilloma virus (HPV) infection (Green, 1974; Cervical Cancer Home Page - National Cancer Institute, 2014). A persistent infection with HPV is considered a necessary but not sufficient event for the development of CC (Walboomers et al., 1999). Neoplasia progression has been extensively studied using molecular techniques, but recent research using next-generation sequencing (NGS) technologies have shed light on important aspects not previously described or analyzed. For example, it has been revealed where the host genome and the viral genome break, and it has been proposed how the HPV integration occurs. To understand how these new findings of HPV integration affect the progression of cervical cancer, it is necessary to identify novel and more precise biomarkers and to propose new treatment strategies.

In this review, we focus on describing and analyzing the evidence related to HPV integration, including the forms and integration sites in the host's genome, as well as the implications that such integration has for the host cell.

2. HPV as a causal agent

2.1. Characteristics of the Human Papilloma Virus

HPV is a circular DNA virus with an approximately eight thousand base pairs genome encoding eight genes, classified as early (E) or late (L) according to their expression patterns. There are six E genes (*E1*, *E2*, *E4*, *E5*, *E6*, and *E7*) and two L genes (*L1* and *L2*). HPV infects epithelial

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https://doi.org/10.1016/j.meegid.2018.03.003
Received 26 September 2017; Received in revised form 1 February 2018; Accepted 4 March 2018
Available online 06 March 2018
1567-1348/

Table 1
E6 and E7 important targets.

Oncogene	Target	Effect
E6	E6AP	Deregulates the translation signal in cell proliferation
	E6TP1	Inhibits Rap signaling
	E6BP	Inhibits the p53-independent antiapoptotic effect of terminal cellular differentiation
	hDIg	De-regulation of the cell cycle and loss of cellular differentiation
	hScrib	Involved in cellular adhesion and polarity; affects the MAGI-1/2/3-regulated p53-independent antiapoptotic effect
	Mcm7	Stopping point in early G1 phase
	XRCC1	Interferes with DNA repair efficiency
E7	p107, p130, p21, p48	Interruption of cell cycle regulation
	p27	Abrogates TGF-β-related growth and affects interferon-α signaling
	ATPase 4 subunit	Degradation of pRB by the proteasome
	TBP	Interferes with transcriptional initiation and Histone H1 kinase and affects the G2/M transition

cells via lesions in the epithelium, where the viral particles enter and spread to the basal layer. The infected cells start to produce viral particles, which initiate the development of low and high grade lesions or, cervical intra-epithelial neoplasia grade 1, 2 or 3, which can evolve into invasive cancer (Woodman et al., 2007; Munoz et al., 2003). During the infectious process, the virus can be present episomally, integrated into the host cell's genome or combined (episomal/integrated). In its integrated form, the virus can produce changes in cell functions that favor the replication of the viral particles and the cell malignant transformation. There are currently several hypotheses regarding the relationship between viral status and lesion stage. It has been suggested that the virus is completely integrated into the genome in advanced neoplastic lesions. Other studies failed to establish an unambiguous stage of full integration (Shirasawa et al., 1986; Choo et al., 1987a; Wei et al., 2015; Shukla et al., 2014; Ribeiro et al., 2014).

HPV is classified as HR-HPV (High risk Human Papilloma Virus) and LR-HPV (Low risk Human Papilloma Virus) according to its association to carcinogenic potential (Munoz et al., 2003). LR-HPV are found mainly in genital warts, meanwhile HR-HPV have been associated with invasive cervical cancer. These HPV are present in a singular or multiple infection (Del Rio-Ospina et al., 2017; Sohrabi et al., 2017).

A persistent infection with a HR-HPV is the main risk factor for cervical carcinogenesis (Walboomers et al., 1999). In addition, HR-HPV genome integration has been associated to the persistent infection (Manawapat et al., 2012), and this viral persistent infection could lead to a cancer progression. To better understand why HR-HPV are more frequently found to be integrated, it is necessary to understand their carcinogenic potential.

3. Viral oncogenes

E6 and *E7* genes products have shown to be primarily responsible for the cellular transformation process (Takebe et al., 1987; Cripe et al., 1987; Vousden et al., 1988; Hakura et al., 1989). It has been proposed that the integration of HPV into the host genome occurs following a break in the *E2* gene, which has been described as the main repressor of the expression of the *E6* and *E7* oncogenes (Choo et al., 1987b; zur Hausen, 2009). This break results in the loss of repression of these oncogenes, whose proteins interfere with the function of cellular proteins p53 and pRb, respectively. These oncogenes thus, directly and indirectly, influence cellular pathways such as apoptosis, proliferation, growth, and motility and their activation can lead to the onset of tumorigenesis (Rusan et al., 2015).

3.1. E1 and E2 genes

The HPV *E1* gene encodes a helicase protein, essential for the initiation of the viral DNA replication. This gene is expressed at early stages of the viral infection into the host cells. *E2* gene expression also occurs at an early stage of HPV infection, its overexpression reactivates the tumor suppressor pathway and inhibits the expression of the oncogenes *E6* and *E7* (Wu et al., 2000). The E2 protein alters the host gene splicing and it's been reported to affect cell movement and motility pathways in the host genome, this would lead to the loss of motility repression (Gauson et al., 2014). If the expression of E1/E2 is lost, there is no longer inhibition of the expression of E6 and E7 oncoproteins (Bechtold et al., 2003).

3.2. Oncogene E6

The product of the viral oncogene *E6* inhibits p53 and BAK, two key regulators of apoptosis (Boulet et al., 2007). As E6 also promotes cell proliferation by positively affecting telomerase and the SRC kinases family, its activities collectively favor cellular immortalization (zur Hausen, 2002). E6 also affects paxillin, which binds to multiple proteins involved in the regulation of actin cytoskeleton organization and is directly associated with tumor metastasis. The downstream effect is the rupture of the cytoskeleton that affects the cell's interactions with the extracellular matrix (Boulet et al., 2007; zur Hausen, 2002; Scheurer et al., 2005; Turner, 2000). E6 also affects other cellular factors such as IRF-3, thus decreasing the transcription of the *INF-β* gene, and targeting several PDZ proteins, increasing cell proliferation. Additionally, E6 modifies transcription by affecting the expression of *CBP/p300* (Scheurer et al., 2005). Other E6 targets include bax and c-myc, resulting in an anti-apoptotic effect.

3.3. Oncogene E7

Viral protein E7 mainly inhibits pRB, leading to an E2F transcription factor release and, in time, p16 upregulation, a cyclin-dependent kinase inhibitor (CKI) inactivation, that act as tumor suppressors (Boulet et al., 2007). E7 also stimulates cyclins A and E production, and inactivates the CKIs p21 and p27 and promote cell proliferation. This oncoprotein also induces the centrioles amplification, which can lead to aneuploidy (Boulet et al., 2007). All of these mechanisms act together to promote cell immortalization. Table 1 summarizes the most important targets of oncogenes *E6* and *E7* and their effects.

Overexpression of *E6* and *E7* oncogenes explains how the breakage of the *E2* regulator gene can initiate the cellular transformation process. However, this mechanism does not explain the cases of transformation in which no deregulation of viral oncogenes has occurred, i.e., where the viral genome breaks at sites other than *E2* or when the virus is found episomally rather than integrated.

To propose other cancer progression mechanisms, it is necessary to make a deeper study on how the HPV integration affects the normal functions of the host genome.

4. Alternative ways for tumorigenesis

Although several studies have reported increased expression of the viral oncogenes *E6* and *E7* in CC (zur Hausen, 1989), it is now thought that the cancer development can occur independently of these genes