

REVIEW

Potential Role of E4 Protein in Human Papillomavirus Screening: a Review

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Abstract

In 2006, cervical cancer was reported as the second most common cancer in women of Malaysia. This type of cancer has been shown to correlate with persistent high risk human papillomavirus (HPV) infection. Although HPV is well known to induce cervical cancer, knowledge of pathways that link the latent stage of the viral replication cycle to precancerous and cancerous stages remains incomplete. However, it is interesting to note that the virus can be isolated from tissues ranging from normal to low-grade squamous intraepithelial lesions as well as high-grade intraepithelial lesions (HSILs), thus prompting scientists to develop HPV detection methods for screening. Detection of HPV using viral proteins such as L1 and E1 is proposed to be very useful in assisting the management of high risk infection and cervical cancer. These tests however can lead to false positive results, largely due to the existence of asymptomatic or transient HPV infections within any given individual. Some observation indicate that use of HPV proteins such as E6 and E7 might lead to false positive results. However, one particular HPV protein, E4 shows potential as an accurate marker of the tissue state following HPV infection. E4 expression has been shown to correlate with the levels of HPV DNA incorporation by the host. Thus, it is possible that E4 could serve as a useful marker to define stages of viral carcinogenesis.

Keywords: HPV- E4- cervical cancer- screening

Asian Pac J Cancer Prev, **18 (2)**, 315-319

Introduction

Cervical cancer has caused 275,000 deaths in 2008, with an estimated 529,000 new cases in the same year (WHO, 2008) with persistent infection by HPV shown to be the main cause of cervical carcinogenesis. Interestingly, HPV infection is common as its DNA can be detected in cervical tissues ranging from normal, low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) to carcinoma (De Sanjosé et al., 2007; Smith et al., 2007; Prétet et al., 2008).

The virus replication is in correlation with the host cell differentiation stages. This association is an important determinant not only for effective viral replication, but also potentially crucial in viral-host immune interaction (Doorbar 2005). During natural infection, HPV produces viral protein to assist viral replication and production. Such proteins include E1, E2, E4, E5, E6, E7, L1 and L2. It was proposed that the virus gains entry into host cells at the basal lamina through the acquisition of micro wounds across the epithelial barrier (Schiller et al., 2010). The virus then gains entry into host cells by the action of the icosahedral capsid protein, L1 and L2 (Johnson et al., 2009). Then, the viral genome is released into the host cell, followed by an initial amplification phase controlled

by HPV protein E1 and E2 (Parish et al., 2006; McBride 2008; Pyeon et al., 2009). The amplification is followed by the expression of E6 and E7, which lead to an increase in cell proliferation. E6 enhances cell proliferation due to its ability to down-regulate p53 (Fu et al., 2010) and to activate telomerase activity (Gewin et al., 2001). HPV E7 also contributes to viral replication by increasing the host cell proliferation by down-regulating members of the Retinoblastoma (Rb) protein family (Roman 2006; Barrow-Laing et al., 2010). The expression of both E6 and E7 protein thus causes cells to overcome the cell cycle block and re-enter the S-phase, which is conducive for viral replication.

Both E4 and E5 also contribute to viral replication. E5 was shown to induce the formation of pores and abrogate apoptosis (Kabsch et al., 2004), as well as proposed function as to enhance the activity of EGF signals and MAP kinase in order to facilitate genome amplification (Pim et al., 1992; Crusius et al., 2000; Genter et al., 2003). During the later stages of viral replication, viral genome is packaged and released into the environment. These processes are facilitated by the minor coat protein, L2 and major coat protein, L1. An increase in the availability of E2 causes the production of the both L1 and L2 protein to initiate, thus leading to viral packaging,

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producing infective virion (Johansson et al., 2012). In some instances, the viral productive cycle can progress into neoplasia due to de-regulation of the viral protein. This was proposed to be related to the change in E6 and E7 expression. It was shown that as E6 and E7 expressions are elevated, the associated severity of neoplasia also increases (Fontecha et al., 2016).

HPV E4

HPV E4 protein is synthesised as a E1^{E4} fusion protein as a result of mRNA splicing, where the first initial sequence of amino acid including the initiation codon contains sequences from E1 open reading frame (ORF) (Wang et al., 2011). The protein was found to be highly expressed in HPV infected biopsies as well as in HPV associated productive warts (Breitburd et al., 1987). Initially thought to be an early protein of HPV (Chen et al., 1982), no evidence was shown to support the hypothesis that E4 protein involves in the early stages of HPV viral replication cycle. This, and factors such as the first appearance of E4 protein in relation to the start of viral propagation, thus suggest a role of E4 in the late stages of HPV replication cycle (Breitburd et al. 1987; Doorbar et al., 1996; Peh et al., 2002).

During the viral replication cycle, E4 was shown to have predominant expression within cells that resides in the middle and upper parts of the epithelial layer (Peh et al., 2004; Maglennon et al., 2011) and appears in granules within the cytoplasmic of infected host cells (Croissant et al., 1985; Breitburd et al. 1987; Peh et al., 2002). These E4 containing granules were proposed to be one of the main contributors of the “cytopathic effect” defined by pathologists. These so called E4 granules associated “cytopathic effect” differs in terms of their morphology depending on the types of HPV that infected the host (Rogel-Gaillard et al., 1993; Doorbar et al. 1996; Roberts et al., 2003).

It was assumed that E4 proteins from different HPV types have similar functionality and mechanisms of action. E4 from HPV1, HPV16, HPV18 and HPV31 exist as E1^{E4} form. During the early viral replication cycle, E1^{E4} protein is undetectable within the host cell. As host cell initiates late viral promoter activation, E1^{E4} expression occurs (Doorbar 2013). The knowledge regarding the functions of E1^{E4} during the viral life cycle remains incomplete. However, extensive examinations of the functionality of E4 protein carried out in high risk HPV types 16, 18 and 31 has suggested multiple roles during the late stages of the viral replication cycle. E4 protein, in the form of E1^{E4} was shown to contain a ‘leucine cluster’ motif in close proximity to the N-terminus of the protein, which is integral in the protein association with keratin (Roberts et al., 1997). Keratin binding by E4 was suggested to be integral in viral release, however, this claim has yet to be successfully explored (Brown et al., 2006). The leucine structure within E1^{E4} protein from various HPV types was also shown to be important in E4 protein self-association through its interaction with the C-terminal domain of other E4 protein (McIntosh et al., 2008). E4 self-association allows E4 to form structures resembles amyloid fibres, allowing for the manipulation

of host cell organisation, including the cyokeratin network (McIntosh et al., 2010). In its constrained form, however, E4 has reduced capacity to bind keratin or to undergo multimerisation, and was proposed to be the E4 prevalent type during the early stages of viral infection (Doorbar 2013). As the virus replication cycle transitions into the genome application phase, the E4 protein experiences post-translational modification by means of phosphorylation. Studies by McIntosh et al. (2010) and Wang et al. (2009) suggested that E4 is phosphorylated at the amino acid Threonine 57 by S-phase associated p42 ERK and other MAP kinase (Wang et al., 2009; McIntosh et al. 2010). The study conducted by Wang (2009) also further showed the importance of phosphorylation as means to stabilise E4 protein and increase in its capacity to bind keratin. As E4 protein accumulates during viral genome amplification stage, the protein is cleaved by calpain at the amino acids 17 and 18, releasing E4 from its constrained form (McIntosh et al. 2008), which then leads to E4-associated disorganisation of the keratin network (Khan et al., 2011).

The functionality of E4 as to cause disruption in keratin organisation suggests the role of E4 in viral release. Although the exact function of E4 in viral replicative cycle has yet to be determined, scientists suggested that E4 might have an important function in arresting the cell cycle. Epithelial cells with the expression of E4 from HPV types 16 and 18 as well as HPV1 were shown to experience cell growth arrest at G2 due to the inhibition of nuclear accumulation of Cyclin/Cdk1 protein in the nucleus (Davy et al., 2002; Davy et al., 2005; Knight et al., 2006). In high risk HPV type 16, E4-associated induction of cell cycle arrest might be associated to the inhibition of E6 and E7 function as cells transit from basal to the mid layer of the epithelia (Doorbar 2013).

Current HPV testing and E4

Cervical cancer is considered as one of the major health issue concerning women worldwide, with more than 500,000 registered cases (Jemal et al., 2011). In the year 2006, the National Cancer Registry of Malaysia has reported that cervical cancer is the second most common cancer in Malaysia women (National Cancer Registry, Ministry of Health 2006). HPV infection has been determined to be the main contributor to the development of this type of cancer due to the presence of HPV DNA within 95% of cervical cancer cases (Walboomers et al., 1999). This apparent aspect of cervical cancer oncogenesis has led scientists to develop methods for HPV detection in order to assist in patient diagnosis and management. To date, detection of HPV by using viral proteins such as L1 and E1 is becoming very useful in assisting the detection and management of high risk HPV infection and cervical cancer (Clifford et al., 2005; Smith et al. 2007). Currently, HPV DNA testing involving detecting L1 or E1 protein or genes has become useful tool for the triage of women with abnormal cervical pap smears and as follow up on women undergoing cancer treatment (Petry et al., 2003; Chansaenroj et al., 2010).

Mainstream HPV DNA testing, for example The cobas® HPV Test was shown to be able to identify and

differentiate 13 high risk HPV types on the basis of the genetic sequence of the major capsid protein, L1 (Heideman et al., 2011). The cobas® test was also shown to be consistent in the detection of high risk HPV types (Mateos et al., 2010) as well as clinically approved to be used for ASC-US triage (Martínez et al., 2012). Other forms of HPV tests include the utilisation of microarray analysis to assist in HPV detection. The PapilloCheck® HPV test developed by Greiner Bio-One utilises DNA microarray chips to amplify HPV E1 gene followed by the gene analysis by CheckScanner™ and Check-Report™ system (Bryant et al., 2011). The test was shown to be valuable in the identification of high risk and low risk HPVs (Didelot et al., 2011), despite a hefty cost to run (Dalstein et al., 2009; Schopp et al., 2010). Considered to be the future of HPV and cervical screening, these tests however are expensive to run. The equipment required to run either of these tests are very expensive in addition to the necessary expertise required to run and analyse the tests.

Tests that utilise DNA and mRNA analysis of both E6 and E7 from HPV were made possible through the availability of polymerase chain reaction (PCR) and real-time PCR (qPCR). However, tests involving the detection of E6 and E7 protein can lead to false positive results, largely due to the existence of asymptomatic or momentary HPV infections within any given individuals (Cattani et al., 2009). Studies published by Molden (2005 and 2006) for example, showed that E6 and E7 mRNA can be detected in women with normal cytology (Molden et al., 2005; Molden et al., 2006). This observation thus indicates that the use of HPV protein such as E6 and E7 might lead to false positive results.

However, one particular HPV protein, E4 shows potential as a marker to suggest tissue state following HPV infection. The potential role of E4 as a tool to diagnose cervical cancer has been reported in multiple publications. For instance, the presence of sequence variety between E4 from different types of HPV can be used to distinguish different HPV types, thus allowing us to establish the causative HPV type associated with a particular disease manifestation (Griffin et al., 2012). E4 expression was also shown to correlate with the levels of HPV DNA incorporation by the host (Chow et al., 2009), suggesting that E4 is a promising candidate to be used as an indicator for defective HPV life cycle that progressed towards cervical neoplasia, particularly in combination with another biomarker, p16INK4a (Griffin et al., 2015). In another study by Supchokpul, E4 mRNA was detected in biopsy samples associated with low grade squamous intraepithelial lesion (LSIL) and high grade intraepithelial lesion (HSIL) samples, but not negative for intraepithelial lesion (NIL) samples (Supchokpul et al., 2011). In association to these observations, it is possible that E4 could be used as a marker to define stages of viral carcinogenesis.

Although the knowledge on the roles of E1^E4 in cancer progression remain incomplete, the fact that E4 protein can be readily detected in HPV infected cervical samples suggests a potential utility in HPV screening. The

facts that E4 is available at the end of the viral replication cycle as well as its ability in negatively affecting the keratinocytes suggest that E4 could be used as an indicator of the severity of HPV infection. This will potentially pave way to replace the current Pap smear screening, which is less specific, time consuming and in some cases require re-testing and colposcopy. Thus, development of a diagnostic tool that allows rapid detection without requiring the need for re-testing and sample biopsy will play an important role in improving patient management. This will ultimately assist in reducing the cost involved in HPV and cervical screening at large.

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