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Review Article

Molecular Genetics of Human PapillomaVirus– Significant for Cervical Cancer Diagnosis and Management

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ABSTRACT

Human Papilloma Virus (HPV) is a necessary causative agent for the cause and progression of cervical cancer. Diagnosis is difficult as HPV cannot be propagated in tissue culture, and therefore, in most cases its accurate identification relies on molecular biology techniques. With the advent of Nucleic Acid Amplification Techniques (NAATs) and other molecular diagnostics tools, have markedly increased essential parameters like sensitivity and specificity for the detection of HPV. The review article focuses on the different detection methods based on molecular tools for the detection & typing of HPV genomes and transcripts.

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1. INTRODUCTION

German virologist Harald zur Hausen, noble lauret proposed in 1976 that Human Papilloma Virus (HPV), not Herpes, was the cause of cervical cancer, a theory that other scientists originally rejected. In 1976, Harald zur Hausen, a German scientist and virologist published his famous hypothesis on HPV. In his hypothesis, he explored the association of HPV with cervical cancer. He later went on to prove the authenticity of his hypothesis. During 1983 and 1984, he and his team discovered two strains of HPV, HPV16 and HPV18. They also showed that these strains of HPV were the cause of cervical cancer. For his discovery, he won the Nobel Prize in Physiology or Medicine in 2008. After zur Hausen established a link between HPV and cervical cancer, researchers across the world began researching vaccines for the two strains of HPV most commonly linked to the cancer. Pharmaceutical companies developed two vaccines, Gardasil and Cervarix. The U.S. Federal Drug Administration approved Gardasil in 2006 and Cervarix in 2009. Human papillomavirus (HPV) is a virus from the papillomavirus family that is capable of infecting humans. Like all papillomaviruses, HPVs establish productive infections only in keratinocytes of the skin or mucous membranes. While the majority of the known types of HPV cause no symptoms in most people, some types can cause warts (verrucae), while others lead to cancers of the cervix, vulva, vagina, penis, oropharynx and anus [1, 2]. In addition, HPV 16 and 18 infections are strongly associated with an increased odds ratio of developing oropharyngeal (throat) cancer [3]. Over 120 HPV types have been identified and are referred to by number [4]. Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are carcinogenic "high-risk" sexually transmitted HPVs and may lead to the development of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), penile intraepithelial neoplasia (PIN), and/or anal intraepithelial neoplasia (AIN) [5-7]. More than 30 to 40 types of HPV are typically transmitted through sexual contact and infect the anogenital region. Some sexually transmitted HPV types may cause genital warts. Persistent infection with "high-risk" HPV types-different from the ones that cause skin warts-may progress to precancerous lesions and invasive cancer. HPV infection is a cause of nearly all cases of cervical cancer [8]. However, most infections with these types do not cause disease. Most of the cervical cancers are cause due to Human papillomavirus (HPV). HPVs are the members of family of viruses known as Papovaviruses. They are epitheliotropic viruses which promote cell proliferation. They are also known as shy virus because they can remain in dormant stage for many years in host body. It is very common infection, though most infected individuals eliminate evidence of the virus without ever developing clinically recognized manifestations. A well established factor that partially explains differential cervical cancer risk is HPV type. Over 40 HPV type infect the human anogenital tract based on pooled data from 11 case control studies of the association between cervical cancer and HPV infection from multiple countries, 15 HPV types have been classified as high risk for development of cervical cancer, 3 have been classified as probable high risk, 12 have been classified as low risk and 3 are considered to have undetermined risk (Table 1).

2. HPV PATHOGENESIS & GENETIC RELATIONSHIP

Two important types of the genes associated with HPV includes; Late Genes (L), mainly L1 & L2 and Early genes (E) which includes; E1, E2, E3, E4, E5, E6, E7, E8. Each HPV gene contributes to pathogenesis of this virus [9-11]. E_1 is essential for viral replication and is linked to genome maintenance. E_2 is involved with regulation of viral replication and transcription. This

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regulation is both positive and negative. E_4 interact with the cytoskeleton protein, more specifically, the keratin intermediate filament in epithelial cells. E_5 interacts with the cell membrane growth factors and is linked to the down regulation of MHC class I molecule.

Table 1: Disease/ Clinical Symptoms associated with different HPV type/s

Disease/Clinical Symptoms associated	HPVType/s responsible
Common warts	2, 7
Plantar warts	1, 2, 4, 63
Flat warts	3, 10, 8
Anogenital warts	6, 11, 42, 44
Anal lesions	6, 16, 18, 31, 53, 58
Genital cancers	Highest risk:16, 18,31, 45
Other high-risk	33, 35, 39, 51, 52, 56, 58, 59
Probably high-risk	26, 53, 66, 73, 82
Epidermodysplasia verruciformis	More than 15 types
Focal epithelial hyperplasia (oral)	13, 32
Oral papillomas	6, 7, 11, 16, 32
Oropharyngeal cancer	16
Verrucous cyst	-
Laryngeal papillomatosis	6, 11

E_6 and E_7 are oncogenes that are involved in host cell proliferation; they bind with *p53* and *pRB* genes respectively for the transforming procedure. L_1 encodes the major and L_2 encodes the minor portion of the capsid. L_2 is express before L_1 to allow for proper construction of the capsid. These genes facilitate the entry into the host cell and can differ from type to type. Some of the HPV "early" genes, such as E6 and E7, are known to act as oncogenes that promote tumor growth and malignant transformation [12].

2.1 E6/E7 proteins

The two primary oncoproteins of High Risk HPV types are E6 and E7. The "E" designation indicates that these two proteins are expressed early in the HPV life cycle, while the "L" designation indicates late expression.

The HPV genome is composed of six early (E1, E2, E4, E5, E6, and E7) ORFs, two late (L1 and L2) ORFs, and a non-coding long control region (LCR). After the host cell is infected viral early promoter is activated and a polycistronic primary RNA containing all six early ORFs is transcribed. This polycistronic RNA then undergoes active RNA splicing to generate multiple isoforms of mRNAs. One of the spliced isoform RNAs, E6*I, serves as an E7 mRNA to translate E7 protein [13, 14]. However, viral early transcription subjects to viral E2 regulation and high E2 levels repress the transcription. HPV genomes integrate into host genome by disruption of E2 ORF, preventing E2 repression on E6 and E7. Thus, viral genome integration into host DNA genome increases E6 and E7 expression to promote cellular proliferation and the chance of malignancy. The degree to which E6 and E7 are expressed is correlated with the type of cervical lesion that can ultimately develop [15].

2.2 Role in cancer

The E6/E7 proteins inactivate two tumor suppressor proteins, *p53* (inactivated by E6) and *pRb* (inactivated by E7). The viral oncogenes E6 and E7 are thought to modify the cell cycle so as to retain the differentiating host keratinocyte in a state that is favorable to the amplification of viral genome replication and consequent late gene expression. E6 in association with host E6-associated protein, which has ubiquitin ligase activity, acts to ubiquitinate p53, leading to its proteosomal degradation. E7 (in oncogenic HPVs) acts as the primary transforming protein [16, 17]. E7 competes for retinoblastoma protein (*pRb*) binding, freeing the transcription factor E2F to transactivate its targets, thus pushing the cell cycle forward. All HPV can induce transient proliferation, but only strains 16 and 18 can immortalize cell lines in vitro. It has also been shown that HPV 16 and 18 cannot immortalize primary rat cells alone; there needs to be activation of the ras

oncogene. In the upper layers of the host epithelium, the late genes L1 and L2 are transcribed/translated and serve as structural proteins that encapsidate the amplified viral genomes. Once the genome is encapsidated, the capsid appears to undergo a redox-dependent assembly/maturation event, which is tied to a natural redox gradient that spans both suprabasal and cornified epithelial tissue layers. This assembly/maturation event stabilizes virions, and increases their specific infectivity [18]. Virions can then be sloughed off in the dead squames of the host epithelium and the viral lifecycle continues. A 2010 study has found that E6 and E7 are involved in beta-catenin nuclear accumulation and activation of Wnt signaling in HPV-induced cancers [19, 20].

2.3 E2 Protein

In a recent study, 99.7% of one thousand cases of invasive cervical cancer were HPV positive to HPV16, being the most common followed by HPV-18 DNA. High Risk HPV E6 and E7 are more active than E2 in cellular transformation than low risk HPVs [21]. The oncogenes E7 and E6 have been found to change keratinocytes by altering their cell cycle. E6 binds to *p53* and degrades it preventing cell death apoptosis and promoting the replication of viral DNA. *p53* is a repair mechanism that destroys any abnormal cells or arrests the cell cycle. Genetic changes in the DNA, such as, the introduction of viral DNA, which transforms and destabilizes the cell. Additional research has been performed in the apoptotic effects of papillomavirus E2. The research findings that the E2 protein in HeLa cells induce *p53*, causing arrest of the cell cycle and apoptosis. But, the induce *p53* accumulation was not correlated to the cell growth arrest at G1 phase. This suggests that apoptosis and cell cycle arrest are independent of each other [22, 23]. Researchers used biochemical and genetic approaches to test the hypothesis that apoptosis by BPVI and HPV18 E2

proteins in HeLa cells is independent of *p53*. One experiment demonstrated that E2 induced apoptosis was set off by Bax, one of the best-known p53 promoting genes. Corroborating the independent pathway for cellular apoptosis and cell cycle arrest [24].

3. MOLECULAR DIAGNOSIS FOR HPV

HPV cannot be propagated in tissue culture, and therefore, in most cases its accurate identification relies on molecular biology techniques. With the advent of Nucleic Acid Amplification Techniques (NAATs) and other molecular diagnostics tools, have markedly increased essential parameters like sensitivity and specificity for the detection of HPV. Detection of HPV genomes and transcripts can be achieved with hybridization procedures including Southern and Northern blots, dot blots, in-situ hybridization, signal-amplification molecular technology (Hybrid Capture assay, version hc2; Digene, Gaithersburg, MD, USA), and DNA sequencing [25]. For proper treatment, follow-up and launching vaccination programmes, type specific HPV detection is very significant.

3.1 Hybrid Capture Assay II (HCA II)

Hybrid capture assay II (HCA II) is one of the method for diagnosing HPV. HCA II from Digene Diagnostics (Silver Spring, Md.) was used for the detection of HR-HPV. Following standardized procedure was used for HR-HPV detection (Digene Diagnostics, Silver Spring, Md.): An Invitro Nucelic Acid Hybridsiation Assay with signal amplification using microplate chemiluminiscence for the qualitative detection of HPV types 16,18,31,33, 35, 39, 45, 51, 52, 56, 58, 59, 68 was used. The Hybrid Capture 2 HR-HPV DNA Test using Hybrid Capture 2 Technology is a nucelic acid hybridization assay with signal amplification that utilizes micro plate chemiluminescent detection. Specimens containing the target DNA hybridize with a specific HPV RNA probe cocktail. The resultant RNA:DNA hybrids are captured on to the surface of a

micro plate well coated with antibodies specific for RNA:DNA hybrids. Immobilized hybrids are then reacted with alkaline phosphatase conjugated antibodies specific for the RNA:DNA hybrids, and detected with a chemiluminescent substrate. Several alkaline phosphatase molecules are conjugated to each antibody. Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted that is measured as relative light unit (RLUs) on a luminometer. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen. An RLU measurement equal to or greater than the Cutoff Value (CO) indicates the presence of high-risk HPV DNA sequences in the specimen. An RLU measurement less than the cutoff value indicate the absence of the specific high-risk HPV DNA sequence tested or HPV DNA level below the detection limit of the assay. All the, specimens were treated with the denaturing solution containing NaOH to denature the ds circular DNA of Human Papilloma Virus.

3.2 Dual Priming Oligonucleotide Technology (DPO)

This technique can simultaneously genotype (HPV-16 and 18) and screen out 16 high-risk HPV types (26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) and HPV type 6 and 11. DPO technology is a new addition to the Nucleic acid amplification techniques (NAAT) which provides freedom in primer designing, PCR optimization and maximizes PCR specificity and sensitivity by fundamentally blocking non-specific sequences. The assay includes internal control for the validation of the protocol which is introduced into every amplification reaction and is co-amplified with target DNA from the clinical specimen. Major drawback of PCR technique is false positive due to amplicons generation. Amplicon contaminations can be prevented by using 8-methoxypsoralen (8-MOP) which

will extinguish the template activity of contaminated DNA thus preventing false positive [26].

3.3 Nucleic acid-hybridization assays

Nucleic acid-hybridization assays such as Southern blotting, *in situ* hybridization, and dot-blot hybridization used radio-labeled nucleic acid hybridization assays to detect HPV infection in cervical samples. Although these techniques generated high-quality information, the disadvantages of these direct-probe approaches include low sensitivity, the need for relatively large amounts of purified DNA, and time-consuming procedures [27].

3.4 Polymerase Chain Reaction (PCR)

PCR use consensus primers such as PGMY09/PGMY1 and GP5+/GP6+, which allow amplification of a large number of HPV genotypes in a single reaction. The primers target conserved regions of the HPV genome, such as the L1 capsid gene [28]. After amplification, the HPV genotypes can be determined separately, using techniques such as restriction-fragment length polymorphism (RFLP), linear probe assays, direct sequencing, or genotype-specific primers [29]. Some researchers have used a type-specific PCR, with primers that amplify the long control region L1 and E6/E7 [30].

4. ACKNOWLEDGMENT

5. CONCLUSION

Recent days, more and extensive researches are going on against Human PapillomaVirus which established as one of the causative reason for cervical cancer. Meanwhile, our society and government should focus and encourage researchers for deep study involving the above mentioned different strains of virus.

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