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Full Length Research Paper

HPV pathogenesis and biomarkers of viral progression and cervical cancer: A literature review

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The critical sensitivity and specificity of the screening system for detection of HPV and cervical cancer indicates a necessity to study new biomarkers of early viral infection. This study aimed to review the literature evidence of novel biomarkers of viral progression and cervical cancer. To conduct this review, the electronic databases Medline, SciELO, Pubmed, in Portuguese and English, referring to biomarkers of viral progression and HPV pathogenesis were consulted. The viral infectious and carcinogenic action of HPV among the biomarkers of tumor progression described in the literature was observed, with highlights to microRNAs (miRNA) Transglutaminase Type 2 (TG2), Ki-67, p16^{INK4a} and Dynamin2 and HPV L1 capsid Protein. Based on scientific evidence, it was concluded that it is possible to improve the process by feasible techniques of high predictive value, thus highlighting the need for further studies to prove the biomarker effectiveness for subsequent use in the national screening system.

Key words: Uterine cervical neoplasms, HPV, biological markers.

INTRODUCTION

The infection for HPV is becoming more incident and prevalent in population. It is known that HPV infects the organism in two different ways, inflammatory and carcinogen, however we are seeing new details and markers from both processes, besides, the critical sensitivity and specificity of the screening system for detection of HPV and cervical cancer indicates the need to study new early biomarkers of viral infection (Conway et al., 2009; Zur Hausen, 2002; Garland et al., 2007; Faridi et al., 2011).

In this study, we aimed to review, in a narrative way, current data about HPV infection and the disclosure of new early biomarkers for HPV.

To initiate the study, the following question was formulated: what has the scientific production been presenting about the thematic of HPV pathogenesis? For this review, the data bases of Medline, SciELO, and Pubmed were consulted, in Portuguese and English, referring to biomarkers of viral progression and HPV pathogenesis, using the describers: HPV, biomarkers, cancer.

The virion of human papillomavirus (HPV) is a DNA virus, from the *Papillomaviridae* family, not enveloped, with 72 capsomers. It has capsid with 55 nm of diameter and is presented in an icosahedral form. Its genome is circular, with a double-stranded DNA measuring 7,900 Kb and mass of 5,000 KDa. It contains nine open reading frames (ORF), in which are placed not only on the early reading genes E1, E2, E4, E5, E6 and E7, but also those of late reading, L1 and L2. There is also a non coding region of large control region (LCR) that controls the other genes. Each one of those viral genes works transcribing proteins of different functions as shown in Table 1(Zur Hausen, 2002; Garland et al., 2007; Faridi et al., 2011; Louvanto et al., 2013).

Currently, different viral kinds of HPV have already been identified, and are split into two groups, being

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Table 1. Description of the specific functions of each HPV gene (Zur Hausen, 2002; Garland et al., 2007; Faridi et al., 2011).

HPV gene	Function
E1	Operates in the episomal replication of the virus
E2	Negatively regulates the functions of proteins E6 and E7
E4	Produces the protein secondary viral capsid
E5	Induces proliferation of the virus infected cell
E6	Directly affects the p53 of the host cell ubiquitin and keeps the telomerase length above its critical point, protecting cells against apoptosis.
E7	Inactivates the pRB protein of the host cell, preventing cycle lock Cell
L1	Synthesizes the main protein of the viral capsid.
L2	Expresses the viral capsid secondary protein

considered of low carcinogen risk, which are frequently associated with intraepithelial lesions of low-grade and to accumulated condylomata that are the viral subtypes of HPV: 6, 11, 40, 42, 43, 54, 61, 70, 72, 81: and the group of high-level oncogenic risk, for being frequently associated with high-level of intraepithelial neoplasm and the invasive neoplasms are the viral subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 (Crawford et al., 2011).

HPV infection is related to proliferative lesions, malignant or benign. The benign lesions include common warts, plantar or genital (condyloma acuminata) and oral lesions and oropharynx in the conjunctiva. The malignant lesions associated with HPV include squamous-cell cancers of the respiratory and oral tract (Crawford etal., 2011).

The HPV virus is connected to the cells through proto cutaneous receptors highly prevalent in cell membranes (Roden et al., 1994; Moody et al., 2010; Schiller and John, 2014). The HPV acts mostly on the squamous tissue cells, the L1 gene contains a major determinant for the entry of the virus in the intracellular. Some studies support the hypothesis that heparan sulfate proteoglycan is essential for early interactions between the HPV virus and epithelial cells, whereas administration of heparinase inhibited viral inclusion (Joyce et al., 1999; Giroglou et al., 2001).

A viral inclusion is not substantially detected within 24 h after capsid binding, the viral internalization occurs primarily in a slow phase which lasts from 2 to 4 h after binding to the cell membrane. It is known that the L2 gene is determinant for this stage, being it structurally modified by the Furin protein; in the presence of these protein inhibitors, the virus loses its capacity of cellular invasion (Culp et al., 2004; Richards et al., 2006).

The pathways of endocytosis and intracellular transport are extensively studied, it has become clear that these pathways directly depend on the viral genotype, and the role of a protein called Claritin in the endocytic process was clarified as an important step in viruses like HPV - 16. It is known that Claritin inhibitors block the viral infection. In contrast, several studies have shown that HPV 31 does not have the same endocytosis pathway, this viral genotype has its process dependent of caveolae, but research failed to block infection by inhibiting viral caveolae (Laniosz et al., 2008; Bousarghin et al., 2003).

Traffic to the late endosome is dependent on L1, intranuclear transport currently is not well elucidated and it is known that this process is founded on L2 gene by interacting with the cellular microtubules. Complexes based on the nuclear domains promote transcription of the viral genome, the reorganization of these domains is observed in cervical wounds, these are produced by the L2 gene (Schelhaas et al., 2008; Maul, 1998; Florin et al., 2002; Darshan et al., 2004).

Several promoters are shown to be related with the generation of Messenger RNA (mRNA) from HPV that infects the genital tract. There are significant differences in the expression of E6 and E7 proteins from HPV classified as being of low and high oncogenic risk. In cases of high-risk HPVs, there is only one promoter which stimulates the synthesis of E6 and E7. In the low-risk group, there is a genetic influence of two different promoters for transcription of E6 and E7 (Korzeniewski et al., 2011).

The noncoding region of HPV has been identified as having important elements for the initial expression of viral genes; however this same region appears to be important in the viral latency process (Korzeniewski et al., 2011).

Viral replication occurs in three possible models. The first model happens during the initial infection of basal keratinocytes, with the amplification of 50 to 100 viral copies, the next stage is defined as genome maintenance in these infected cells, viral DNA is conserved in multiple copies of stable plasmid. Viral replication is synchronized with cell cycle, which ensures a latent infection. The last type of viral replication occurs in a vegetative way in the already differentiated epithelial cells. At this point it is possible to detect HPV late gene expression and release of new virions. The regulation genome at this stage is not

well understood and can be influenced or not by the cell controlling factors (Conway et al., 2009; Korzeniewski et al., 2011).

HPV is a very prevalent virus; warts are very frequent viral diseases with an estimated incidence of 7 to 10% in the European population and 1% in the U.S. population (Hengee, 2004). In immunocompromised and patients recipients of renal transplant, this number increases 50 to 100 times, reaching 90% after 15 years of transplantation (Lindelof et al., 2000). Warts can occur at any age and the incidence increases during school age, peaking in youth and young adults.

A recent study conducted in Tanzania with 3603 women from urban and rural population of the country, revealed the prevalence of HPV in 20.1% of the population, its detection being higher in HIV-positive women (46.7%) (Dartell et al., 2012). In the presence of cervical cancer, HPV detection was observed in 100% of the population in New Guinea (Tabone et al., 2012).

Recently, HPV prevalence has been studied in a lot of country, such as Indonesia, which have a high prevalence of HPV infection (Panigoro et al., 2013). Boublenza et al. (2013) also demonstrated in a retrospective analysis of data about cervical cancer from 2006 through 2010 in the province (wilaya) of Tlemcen (Algeria) that cervical cancer is the second leading cancer among women with an incidence of 13.3 per 100 000 women.

A study conducted in the cities of São Paulo, Porto Alegre and Campinas showed prevalence of genital HPV infection of high risk in 17.8% of the studied population (2,300 women 15-65 years). The rate of infection was observed as 27.1% (below 25 years), 21.3% (25-34), 12.1% (35-44), 12.0% (45-54 years) and 13.9% (55-65 years). Subjects with the highest number of sexual partners during lifetime had higher frequency of infection (Rama et al., 2008).

The HPV virus prevalence evaluated at the Federal University of Rio Grande was 66.3%, in which 60% of those were related to HIV virus coinfection (Entiauspe et al., 2010).

A systematic review of the literature about epidemiology of cervical cancer and human papilloma virus infection among Iranian women demonstrated that the mean cervical cancer age-standardized incidence was 2.5 per 100,000 in pathology-based cancer registries. The same study showed HPV types isolated in Iranian women, the results indicate that HPV 16 (54%), 18(14%), and 31 (6%) were the most commonly detected in Iranian cervical cancer patients (Khorasanizadeh et al., 2013).

Presently, during diagnosis, HPV infection is detected directly by Polimerase chain reaction (PCR). In Addition to this, studies indicate that histopathological analysis of biopsy demonstrates 100% accuracy (Souza et al., 2001; Patel et al., 2012).

Epidemiological studies carried out on samples collected using the DNA-CITOLIQ system (DIGENE - Brazil) showed cases with negative results in the cytological screening, which were positive in HPV DNA tests, and thus evidenced the high sensitivity of biomolecular techniques (Utagawa et al., 2004).

The Pap smear test is held annually as a screening test for possible injuries caused by HPV virus, but, with a critical sensitivity, indicates the need for new biomarkers of early viral infection (Souza et al., 2010).

The use of biomarkers in cervical cytology and histology has been demonstrating high predictive values, several biomarkers for the detection of cervical diseases have been indentified, many of them are involved in the cell cycle regulation, transduction cascades, DNA replication and cell proliferation (Malinowski, 2005a; 2007b).

Recently, Patel et al (2012) demonstrated that the high methylation of candidate genes particularly of HPV 16 is associated with chances of abnormal cervical cytology results.

METHODOLOGY

This study is in a narrative review, in which the methodology was based on the pursuit of experimental scientific articles, review articles and clinical between the years 1994 and 2013 in the data bases of Medline, SciELO and Pubmed.

RESULTS AND DISCUSSION

The role of biomarkers in screening tests improvement

The screening by cervical cytology has been reducing morbidity and mortality from cervical cancer, but its limitations in terms of sensitivity and specificity indicate the need for improvement in the screening method (Bulet et al., 2008). Recently, Andrade (2012) points in his review, limitations in the process of cervical cancer diagnosis in Brazil, among these stands the lack of standardization in cytological reading and low specificity of colposcopy, corroborating the need for new tools in the diagnosis of cervical cancer.

This contemporary need for early diagnosis was confirmed by Whilock et al. (2011), they demonstrated the ineffectiveness of liquid cytology as a screening test for cervix cancer, thus concluding the need for new scientific evidence for progression biomarkers.

The Pap smear screening has been proved as a limiting exam on the screening process for cervical cancer, and its sensitivity has been decreased due to the determination of atypical squamous cells of undetermined significance, elucidating again the need for accessory diagnostic parameters to the traditional screening method in order to contribute to the sensitivity and specificity levels of the procedure (Briet et al., 2010).

The use of biomarkers of dysplastic progression is reported as a facilitator for abnormal cell detection together with Pap smear cytopathological screening. Recent publications demonstrate the predictive power of several biomarkers in screening tests, showing high levels of protein expression in high-grade precursor wounds (Brown et al., 2012).

Furthermore, Alonso et al. (2012) demonstrated through semi-quantification by immunohistochemical $p16^{INK4a}$ a significant relationship between expressional levels of the protein and development of malignancies in the genital tract caused by HPV, evidencing important diagnostic tools in the progression of viral infection.

Zagels et al (2010) investigated, using proteomics technique, the cervical fluid in order to identify biomarkers for pathologies of the genital system, and elucidated in his study the correlation of the technique with the disclosure of new biomarkers for various diseases.

Therefore, a clear need for new biomarkers with sensitivity and specificity complementary to this screening system is identified in order to corroborate the already performed procedure in the female population and improve the early detection of cervical cancer. Based on this need, the potential biomarkers demonstrated in recent studies of the literature are thus explained.

Biomarkers and screening

Ki-67

Ki-67 is a protein located in the nucleus and nucleolus expressed during G1, S, G2 and M phases. Its cellular function is not evidenced in literature; however it has its protein expression related to cell proliferation (Endl et al., 2000). Because HPV induces high rates of cell proliferation, there is a described correlation between the expressional levels of Ki-67 and the progression of infectious HPV in dysplasia and neoplasia (Keating et al., 2001).

Recently, associations were identified of expressional levels of $p16^{INK4a}$ and Ki-67 in patients diagnosed with HPV, reaching high positive and negative predictive values, thus it can be used as an adjunct to the gynecological routine (Ziemke et al., 2012).

$p16^{INK4a}$

The $p16^{INK4a}$ protein has a regulatory role in the cell cycle, and its expression is highly controlled by normal cells. $p16^{INK4a}$ acts as a tumor suppressor by inhibiting cycle-dependent kinase 4 and 6, which phosphorylate the retinoblastoma (Rb) that is usually bonded to E2F which regulates the entry of the cell in stage S of the cell cycle. On HPV infection, the E7 protein breaks the binding of Rb with E2F, resulting in an expressional increase of $p16^{INK4a}$ (Khleif et al., 1996; Sano et al., 1998).

Godoy et al. (2008) recently demonstrated a correlation between the expressional levels of $p16^{INK4a}$ and cervical wounds caused by HPV, indicating $p16^{INK4a}$ as a biomarker for neoplastic lesions caused by HPV.

p16/Ki-67

Recently, a study in Germany with women of over thirty years old demonstrated a new screening model for HPV using the marking for the p16 and ki-67 proteins simultaneously, the efficiency of this type of screening was verified as an important factor in the progression of viral infection and cancer pathogenesis (Petry et al., 2011).

Donà et al. (2012) analyzed cervical smears from 140 women, the samples went through ki-67 and p16 quantification process simultaneously via CINtec® PLUS, the results evidenced in this study corroborate previous data, showing that the immunohistochemistry for these markers may have an important diagnostic value, and the positivity for these biomarkers is highly related to the presence of high-grade premalignant lesions.

Other immunohistochemistry kits with simultaneous action for p16 and Ki-67 have been proven effective in the early diagnosis of cervical cancer, the use of ProEx™ immunohistochemistry demonstrated results with sufficiently effective accuracy, and also highlighting the need for repetition in only one third of the samples (Walts et al., 2009).

miR-100

MicroRNAs (miRNA) are RNA molecules that act by inhibiting messenger RNA (mRNA) in several biological processes, including proliferation, differentiation, development, metabolism and cell death. Studies show that miRNA is often super expressed in malignancies and its role was evidenced in the emergence and development of tumors. As a potential tumor suppressor, the reduction of miR-100 expression has been evidenced in some types of tumors, such as low level bladder cancers, oral, ovarian and liver cancer. However, the expression and function of miR-100 in cervical cancer is still poorly studied (Catto et al., 2009; Henson et al., 2009; Yang et al., 2008; Thorgeirsson, 2011).

Li et al. (2011) investigated different miRNAs through microarray and as a result, the miRNAs are separated into groups according to their expression level, miR-100 showed to be the miRNA with lower expression based on the associations described above. The same study investigated their relationship in cervical cancer by analyzing 125 cases of cervical cancer, cervical intraepithelial neoplasm, normal tissues and also evaluating their role in cell culture. This study showed a negative proportional relationship between miR-100 levels and the development of cervical cancer, and its lower expression was identified in high-grade lesions. In cell culture, the inhibitor of miR-100 increased apoptosis

and decreased cell proliferation as well as accelerated G2 / M phase in HaCaT cells, whereas the increase of miR-100 had the reverse effect, miRNA increased apoptosis and decreased malignant cell proliferation.

The miR-100 targets are not known, some associations are identified in other types of tumor, for example, in ovarian tumor it was observed that overexpression of miR-100 suppressed the mTOR (mammalian target of rafamicina), which acts in cell cycle progression, death and size (Nagaraja et al., 2010).

Another molecule strongly associated with miR-100 inhibitory action is PKL1, a protein, which regulate cell mitosis, being the checkpoint of the M/G2 stage; its alteration can cause chromosomal instability. Its expression levels are shown to be high in several types of tumors (Schmit et al., 2009; Nappi et al., 2009; Feng et al., 2009), including cervical cancer (Gao et al., 2006). Studies have shown that depletion caused by interference RNAs results in inhibition of cell proliferation and increased apoptosis.

Recently, an inverse relationship was demonstrated between the expression of miR-100 and PKL1 contributing to nasopharyngeal cancer progression (Shi et al., 2010). This correlation was observed only in high-grade lesions and cervical tumors. However, more studies are needed to evaluate the expressional levels of these proteins.

Transglutaminase type 2

The transglutaminase enzyme type 2 (TG2) has been identified as an influencing factor in HPV infection (Jeon et al., 2006). TG2 is involved in a wide range of functions, such as cell death, cytoskeleton rearrangement and extracellular stabilization. Its altered activation has been associated with several pathologies (Fesus et al., 2002; Facchiano et al., 2006; Falasca et al., 2005a 2008b).

TG2 is also associated with viral pathogenesis; several proteins involved in the infection process demonstrate to be modified by the TG2 protein (Amendola et al., 2001; Lu et al., 2001; Jeon et al., 2006). A recent study indicated that TG2, with its catalytic activity, can incorporate a polyamine in the E7 protein of HPV18, thus inhibiting its binding to pRb protein in cytosol and nucleus. TG2 also has the ability to translocate from the cytosol to the nucleus incorporating polyamines of retinoblastoma protein (Rb) thereby protecting the degradation by caspases (Boehm et al., 2002; Milakovic et al., 2004).

Del Nonno et al. (2011) demonstrated that TG2 can incorporate E7 oncoprotein from HPV18 virus, thereby participating in the cellular response to the virus. Low-grade lesions have high levels of the enzyme, while in high-grade lesions those levels are diminished. This enzyme is now referred to as an early phenomenon that is not restricted to high-risk genotypes.

Dynamin2

Dynamin2 represents one of the proteins from the family of GTP-binding proteins that is associated with microtubules and is involved in endocytosis processes of cell motility and membrane changes. Dynamin binds to proteins that bind to actin and other cytoskeletal proteins (Durieux et al., 2011).

Lee et al. (2012) recently investigated the correlation between the expressional levels of dynamin2 and the emergence of cervical tumors in HPV-infected patients; there were results of association between marker and cancer progression, thus demonstrating a new possible early marker for infectious and cancerous lesion by HPV.

HPV L1 capsid protein

Recently, new findings indicate that HPV L1 capsid protein is also associated with the productive phase of HPV infection; nevertheless, expression pattern in distinct HPV-associated cervical lesion and its association to p16 expression is not still well implicit. Izadi-Mood et al. (2013) demonstrated that L1 capsid protein was positive in 63.6% of low-grade CINs and 9.1% of high-grade CINs on 89 paraffin-embedded tissue samples.

Another study on Pap smears from 65 women support that detection of HPV-L1 protein could represent prognostic information about the development of early dysplastic cervical lesions (Sarmadi et. al., 2012).

Conclusion

Continuous improvement of the screening process is a global need, the institution of new biomarkers of HPV infection progression and its consequent cervical neoplasm indicate, with proven effectiveness, the improvement of sensitivity and specificity of the screening process.

Based on the scientific evidence cited in this study, it is possible to improve the program through feasible and high predictive techniques, highlighting the importance and need for new studies to prove the full effectiveness of biomarkers for later use in the national screening system.

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