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The Influence of HPV on the Expression Levels of miRNAs in Cervical Cancer

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Abstract

Cervical cancer is the third most common type of cancer in women and the fourth cause of death in the world, being Human Papillomavirus infection the main cause of the disease. Beyond to cancer developmental pathways, the virus has been described as influencing the expression levels of microRNAs, which also play an important role in oncogenesis.

Keywords: ***Cervical cancer; microRNA; HPV***

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Introduction

Cervical cancer is the third most common type of cancer in women and the fourth leading cause of death in the world, with 90% of deaths in developing countries [1].

Human papillomavirus (HPV) infection is the main cause of cervical cancer and an important agent for other types of cancer, such as vaginal, vulvar, anogenital, oropharynx and penis [2,3]. Other risk factors are the number of sexual partners, the early onset of sexual life, smoking habit and the presence of Human Immunodeficiency Virus (HIV) [3,4]. Although the HPV DNA was identified in approximately 99% of cases of cervical cancer, the HPV infection is a necessary but not sufficient cause for the development of cancer and has been reported to influence the expression levels of microRNAs (miRNA) [5-7].

HPV

HPV is a small virus of double-stranded circular DNA composed of 8000 base pairs, a member of the *Papilomaviridae* family [8,9] and infects the undifferentiated basal epithelial cells of the stratified epithelium [10]. The HPV genome is divided into three regions: the early (E) region, where genes E1, E2, E4, E5, E6, E7; the late (L) region, composed of L1 and L2, and the upper regulatory region (URR) which does not encode proteins but contains elements for gene regulation, genome replication and packaging in viral particles [5,8].

The E6 and E7 genes play an important role in the development of cancer, they act in intracellular dysregulation of the pathways involved with cell proliferation, apoptosis and genetic and epigenetic instability [11,12]. The expression of these proteins is

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considered responsible for initiating and maintaining the phenotypic transformation of cancer. The E6 onco-protein is responsible for inducing ubiquitination and degradation of the tumor suppressor protein p53, in addition to stimulating the activity of the enzyme telomerase [2,11-15]. The p53 protein binds to DNA and stimulates the production of p21 that interacts with cdk2, a cell division stimulating protein - the p21-cdk2 complex prevents the cell from advancing in its division. When mutated, they do not send the stop signal and the cell begins to divide continuously and uncontrolled and form the tumors. Telomerase is an enzyme that synthesizes the telomeric tandem repeats (TTAGGG) at the end of the chromosomes, which, in addition to other functions, control the cells' replication capacity and senescence [16]. E7 protein acts on the degradation of the tumor suppressor protein retinoblastoma (pRB). The pRB controls the entry of the cell into the cell cycle synthesis phase (S). The control of cell cycle is mediated by the binding and inhibition of pRB with E2F-family gene regulatory proteins required in the transcription of genes that will encode proteins for S phase activation. If inactivated, it favors inappropriate cell entry into the division cellular, leading to the formation of cancer [2,12,14,17].

In addition to the features described above for HPV, some studies suggest that virus infection may modify the expression of certain microRNAs (miRNAs), as well as variations in biologic behavior [13,18-20].

miRNA

The miRNAs are a class of endogenous, non-coding RNAs with approximately 22 nucleotides acting on the regulation of gene expression in a post-transcriptional way. They were described in a 1993 study in an experiment with Caenorhabditis elegans, in which was observed that a small RNA, called lin-4, was found to negatively regulate the level of lin-14 protein. A few years later, the second miRNA, let-7, was discovered [21]. After this, several studies appeared in the search of the understanding of the performance and the function of miRNAs. It is known that they can act both as tumor suppressor genes and as oncogenes, depending on the target gene and tumor type that is involved [21,22]. A classic example is miR-15a and miR-16a, which in chronic lymphocytic leukemia act as tumor suppressors and in pancreatic cancer act as oncogenic [23,24].

miRNAs play an important role in several biological processes, such as cell cycle, cell growth, apoptosis, viral infection and cancer development [25,26]. These influences suggest that circulating miRNAs are potential candidates as biomarkers, including has been described as a marker for the diagnosis and treatment of cancer [20]. Changes in its expression have been associated with several types of pathologies such as cardiovascular disease, diabetes, sepsis and cancer; among them cervical cancer [13,27]. Approximately 50% of miRNAs are located in fragile sites, on chromosomes that are often deleted, amplified or rearranged in some cancers and play a crucial role in disease progression [28,29].

The biogenesis occurs in two stages: in the nucleus and in the cytoplasm. In the nucleus, the miRNA genes undergo RNA polymerase II/III action, forming the pri-miRNA (in the form of a hairpin, with hundreds of nucleotides); then an enzyme called DROSHA acts by transforming the pri-miRNA into pre-miRNA (contains approximately 70 nucleotides). The Exportin 5 (XPO5) acts on the migration of pre-miRNA from the nucleus to the cytoplasm. In the cytoplasm, the pre-miRNA, called miRNA duplex undergoes action of another enzyme, the DICER, which is responsible for forming two chains with approximately 22 nucleotides - a complementary (miRNA*), which can be degraded or used for another target, and a main miRNA. This binds to the RNA silencing complex (RISC) that will act on target mRNA by binding in its 3 'untranslated region (UTR). Depending on the level of complementarity the inhibition of protein translation can be accomplished by blocking the mRNA or by its degradation [2,22,28,30,31].

When there is a complete/perfect complementarity the target mRNA is degraded and when complementarity is imperfect/incomplete a repression of mRNA translation is observed. In most cases there is imperfect complementarity [28,32]. To bind to the target mRNA, a sequence of 2-8 nucleotides, known as the seed region, located in the 5 ' end of the mature miRNA, is required which binds preferentially to the 3'UTR end of the mRNA [32,33].

Levels of miRNAs and HPV infection

miRNAs have been in the focus of cancer studies because of their important roles in several cellular processes and in this context its expression can be modified by genetic and/or epigenetics mutations leading to the development of neoplasias [34, 35]. Specifically in cervical cancer, the presence of HPV appears to influence the expression levels of certain miRNAs [35]. The virus genotyping has been used to try to understand the role of HPV on miRNAs, but the mechanism by which the virus interferes with its expression is not fully elucidated. Studies suggest that the miRNA levels are altered in the presence of certain types of high-risk HPV and its oncoproteins [10,20].

Liu et al. [12] determined the expression of E6 and E7 transcripts from 13 high-risk HPV types in 101 cervical carcinomas and correlated with the expression profile of miRNAs. They identified nine miRNAs with different levels of expression, with miR-9 being the most active by E6 action, p53 independent. miR-9 is significantly elevated in HPV 16 tumors when compared to HPV 18 tumors and HPV negative tumors and was considered a favorable biomarker for CC in another study by the same author. The activation was verified in a functional study related to targets involved in cell migration, favoring the progression of the disease, but the mechanisms of activation by HPV have not been described by the author [12].

A similar study also observed high levels of miR-9 in HPV positive cervical cancer samples when compared with HPV negative. A functional study was performed and the authors observed that HPV E6 oncoprotein was related to the increase levels of miR-9. The author cites other studies that found no significant change and related the divergence of results with the possible types of HPV, considering that the work did not genotype the HPV. It reinforces that miR-9 was elevated in the presence of HPV in both tumor tissues and normal tissues, suggesting that activation is a result of HPV infection and not cancer development [1].

The increase in miR-9 expression, in addition to miR-21 and miR-155 was verified in 52 formalin-fixed paraffin-embedded primary cervical cancer tissue samples and 50 normal cervical tissue samples was verified by Park et al. [11]. They also evaluated the expression of the 3 miRNAs described with the mRNA expression of positive, negative HPV E6/E7 samples and normal samples. They found a significant increase between the HPV E6/E7 positive samples and the normal samples for the three miRNAs. When comparing HPV E6/E7 negative with normal samples, miR-21 and miR-155 were significantly increased and miR-9 was not significant. The data suggest the possibility of using miRNAs as biomarkers because they are involved in the development of cancer [11]. Similar result, increased miR-9 expression associated with HPV infection has been reported in oropharyngeal cancer, demonstrating that miRNA alteration is related to HPV and not just to cervical cancer [36].

Martinez et al. [13] also suggested that there is a relation of some HPV types with the expression levels of miRNAs. A study performed in cervical cell line HPV-16 (Caski, SiHa) with integrated DNA compared to normal cervix (C-33A) showed an increased expression of 3 miRNAs and decrease of 24. Among the miRNAs evaluated, miR-218 was significantly reduced in the presence of HPV-16 E6 which increases the expression of LAMB3 mRNA, but the same does not happen with low-risk HPVs, such as HPV-6. LAMB3 is related with cell migration and tumorigenesis in mice, if associated with its ligand α6β4-

integrin can be promote tumorigenesis in human. Among other miRNAs analyzed, they suggested that miR-218 would be a specific cellular target for HPV and LAMB3 is a possible target of miR-218 [13].

Other study did HPV genotyping was performed in samples of epithelial tissue from vaginal cervix, and also reported an association between miR-218, high-risk HPV infection and the degree of CIN. The more advanced the disease, the lower the expression of miR-218, suggesting its association with carcinogenesis and describes LAMB3 mRNA as a miR-218 target [19]. Wang [37] studied the expression of miR-34 and miR-218 in the diagnosis of cervical cancer and found too that the low expression of miR-218 is related to the degree of tumor differentiation, invasion, metastasis and clinical staging and the low expression of miR-34 is related to the same processes, except clinical staging. While miR-34a (suppressor tumor) has its expression inhibited by p53 destabilization, caused by the E6 oncoprotein of HPV 16 and HPV 18, influencing cell proliferation [38]. Both miRNAs are closely related to clinicopathological parameters and both can be used as markers for diagnosis and for the evaluation of cervical cancer prognosis [37].

A comparative study among normal and cancer cervical tissues evaluated the expression of 29 miRNAs and they found 13 miRNAs up-regulated and 16 down-regulated, highlighting the miR-20b and miR-106-b like up-regulated. The patients evaluated were HPV positive, however, they did not correlate the type of HPV and miRNAs, being unable to determine if the expression was related to HPV infection or the type of oncoprotein involved [26].

A study by Liu et al. [18], comparing normal tissue without HPV, normal tissue HPV-16 and cervical carcinoma with HPV-16 positive, identified increased expression of miR-193a and reduction of others 18 miRNAs. Among them, 3 were significantly reduced: miR-196a, miR-133a and miR-133b, when comparing normal tissue with normal tissue HPV positive. The same result was verified in cancer samples. The miR-196a was the one that had the most significant reduction, so to validate this data, a cell line study was performed (cell line HPV 16 positive: CaSki and SiHa, HPV 18 positive: Hela and HPV negative: C33a). The results in vitro were consistent with those observed in the patients' tissues, where the presence of HPV-16 E5 protein reduced the expression of miR-196a by bind to HOXB8 mRNA. The increase of HOXB8 expression is associated with others cancers and influence on cell proliferation, cell growth and apoptosis [18].

Although the studies have shown association of the presence of HPV with changes in miRNAs levels, some mechanisms are not clear, and the regulation may be an indirect effect of the progression of the disease. Knowledge about the targets may help elucidate the HPV action in relation to the miRNAs and the processes in which they are involved.

However, in some cases, it has been suggested that there is no relationship with the pathway of the disease, since even in cases of normal tissues infected with HPV, there was a change in miRNA expression [1,11,18].

The alteration of miRNA expression from tissue lesions also interferes with their expression in the bloodstream. Therefore, the level of circulating miRNAs is considered good biomarkers and help in the diagnosis in a non-invasive, and they can also be used in the monitoring of the disease [37].

Considerations

Increasing or decreasing the expression of a miRNA may interfere with the behaviour of the disease. More functional studies should be performed to determine which types of HPV are able to regulate the expression of miRNAs and identify the mechanisms of action or if there are other pathways related to the disease that may interfere with the expression of the miRNAs.

Understanding the molecular mechanisms and new biomarkers is essential for the identification of possible therapeutic targets for cervical cancer.

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