Molecular Mechanisms of Cervical Cancer Induced by the Human Papillomavirus: Recent Proposals for Early Detection and Treatment

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Authors' contributions

This work was carried out in collaboration between both authors. Author MCMPC designed the study, performed the study analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Author LABC managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

Review Article

ABSTRACT

Background: Cervical cancer is considered an important public health problem, and the main risk factor for this type of tumor is human papillomavirus infection. However, the search for new proposals for disease control and prevention has brought new findings and approaches in the context of molecular biology indicating innovations and perspectives in the early detection of the disease. Thus, this study aimed to identify studies that explore events and molecular mechanisms in cervical tissues and cells and to address the main methods used, bringing new perspectives and proposals for the early detection of cervical cancer.
Methods: This is a bibliographical review of articles published between 2014 and 2017. Medline, Scielo, Web Science and PubMed databases were used. Publication period. The cell cycle theme, gene expression, protein action, precursor lesions, cervical cancer and human papillomavirus, and the suggestion of molecular markers for treatment and / or diagnosis were highlighted as inclusion criteria.

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Results: There were 63 publications found, of which 58 were in the English language. Of this total, 38 corresponded to the inclusion criteria and the proposed objectives, being selected the 16 main articles. It was concluded that most of the studies have brought about molecular mechanisms, highlighting the process of carcinogenesis between the initial phase, with the precursor lesions until invasive cervical cancer.

Conclusions: In this review, the highlight was the study on chromosome aberration found in cervical squamous cell carcinoma and important markers for prognosis and early detection of cervical cancer, which contributes to the reduction of morbidity and mortality from this type of cancer in the female population.

1. INTRODUCTION

The growing number of research on cervical cancer, human papillomavirus (HPV) and the carcinogenic process have brought new findings and approaches in the context of molecular biology, indicating innovations and perspectives in early detection and control of disease [1]. The main risk factor of cervical cancer is HPV infection, and persistent infection by this virus can lead to premalignant lesions called cervical intraepithelial neoplasia (CIN) that when not treated can develop into cancer of the uterine cervix [2].

On average, there is a period of 12 to 15 years between the time of HPV infection and tumor development, highlighting the various stages of carcinogenesis. This process is defined as an event which comprises a series of molecular modifications which are accumulated in the cell and end up allowing an unregulated growth of this neoplasm [3].

In this way, the cervical cancer is considered an important public health problem. Knowing the events involved in the development of cervical cancer such as gene silencing and the expression of genes and the proteins, is relevant for better understanding the factors associated with the complex processes of this disease.

The genetic changes that occur in the neoplastic process can be explored with the investigation of molecular markers and may be targets of precursor lesions and cervical cancer control [4]. It is worth mentioning that a study on the gene expression profile contributes to know the markers of the process of carcinogenesis and identify molecular signatures, as well as helping in the treatment and prognosis of the disease [5].

However, screening of cervical cancer and the HPV vaccine brought the possibility of primary level prevention, although new technologies are still required, especially in the area of molecular biology [6]. Thus, this study was carried out due to its relevance, because although Pap test is used as a method of sorting and presents a good sensitivity and high specificity, it is not sufficient when there are persistent cervical changes [7].

1.1 Genetic Changes and Cervical Carcinogenesis

Gene expression and the simultaneous analysis of various messenger RNA expression patterns and their relationship with biological functions is a powerful tool to identify the biological action of cancer and important genes involved in this biomolecular event development [8,9]. It is understood that HPV can influence the process of carcinogenesis by changing the host's genome, as gene silencing and activation, reducing the defense of the host and favoring the persistent infection of the virus. It is relevant to understand the mechanism of genes with greater or lesser expressivity in the process of cervical cancer development. Thus, in this study we will discuss some investigations that stood out and brought important results about genes and proteins involved in the development of cervical cancer.

Given this scenario, the present work objective is to identify studies that explore events and molecular mechanisms in cervical tissues and cells and address the main methods that bring new perspectives and proposals for cervical cancer early detection.

2. MATERIALS AND METHODS

This is a literature review of articles published from 2014 to 2017 that explore the topic about molecular mechanisms related to human papillomavirus (HPV) and carcinogenesis. We used Medline, Scielo, Web Science and Pubmed databases. To search the journals, we used the

following key words: cell cycle, expression genes, proteins, cervical cancer, human papillomavirus, and methods. Most of the articles were published in the English language. As inclusion criteria we determined the publication period, the theme expression cell cycle genes/proteins, precursor lesions, cervical cancer and human papillomavirus and suggestion of molecular markers for treatment and/or diagnosis.

3. RESULTS

63 publications were found, 58 of them in English. 38 of them met the criteria chosen to achieve the proposed objectives, and 16 articles were selected, as shown in Table 1. The 16 articles chosen were the articles that presented the best description of the methods used to identify molecular mechanisms, better presentation of the objectives and results for proposals for the early detection of cervical cancer. From the studies found, an analytic and selective reading is done as a criterion to obtain data related to the topic. In this study we point out some relevant findings, such as researches about biomolecular mechanisms in the cervical carcinogenesis process, the most used methods and suggestions of biomolecular markers in therapy and diagnosis in the early detection of cervical cancer. Many important articles have not been added in the review, remaining only the articles were limited to specific keywords.

The research of different approaches and methodologies about molecular mechanisms shown in Fig. 1, like gene and protein expression, clinical significance and the biomarkers identification were part of the major topics that have been widely explored about cervical cancer and HPV development.

It was observed a significant convergence of various authors on the subject investigated in this study. The varied research analysis, illustrated the relevance of the subject on the search of methodological innovations in the early detection and better understanding the process of carcinogenesis of uterine cervical cancer (UCC). Understanding the molecular mechanisms of cervical cancer development and identifying new biomarkers is crucial to detect and treat this type of tumor [10].

The studies have brought a range of discussions and highlight the effect of genes that are more or less expressed in cellular proliferation and development of cervical cancer, as well as correlations between normal and cancerous cells. It was possible to emphasize the role of proteins expression that are also fundamental in the process of the cell cycle related to this malignant neoplasm. Previously unpublished themes were also addressed, highlighting not only the apoptosis effects, but the identification of chromosomal aberrations in women with cervical cancer.
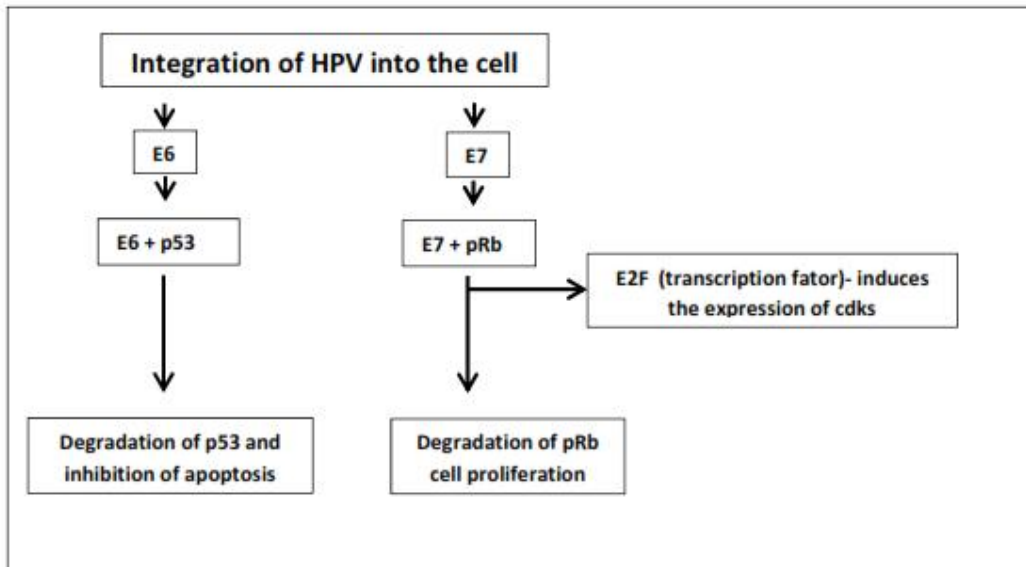

Fig. 1. Molecular mechanisms related to human papillomavirus and carcinogenesis

Table 1. Studies found in this review on biomolecular mechanisms about cervical cancer

Author/ year	Objectives	Description method	Method	Genes expression	Relevant results	Suggestions, therapies proposed and biomarkers
Zhao et al., (2015) [13]	To study the expression and significance of tumor suppressor in gene methylation (TSLC1), on protein expression in TSLC1 of cervical cancer and precancerous lesions, as well as its relationship with the DNA of high-risk HPV infection.	The technique Immunohistochemistry (IHC) was used to detect the protein expressions TSLC1 in cervical lesions and the hybrid capture method of second generation (HC2) was used to detect high-risk HPV in cervical lesions.	MSP-PCR IHC HC2	TSLC1	In the CIN progression for invasive cervical cancer, there was no significant correlation between the TSLC1 gene methylation and DNA infection of HR-HPV (P = 0.919), the TSLC1 protein expression and DNA HR-HPV infection (P = 0.664). The analysis showed a negative correlation between gene methylation TSLC1 and TSLC1 and protein expression (r =-0.674, P < 0.001).	The methylation of gene promoter TSLC1 can be an early event in cervical carcinogenesis, and a marker in early detection, prevention and prognostic forecast for cervical cancer.
Cheah et al.,(2016) [14]	To assess the correlation between HR-HPV detection using a commercial in situ hybridization essay (ISH) and p16 immunoexpression in cervical squamous intraepithelial lesions and squamous carcinoma.	27 cervical squamous intraepithelial lesions of low grade, fixed in formalin paraffin embedded, LSIL, 21 high grade squamous intraepithelial lesions (HSIL) and 51 squamous carcinomas (SCC) were searched. In-situ hybridization (ISH) for HPV detection was used. The immunohistochemical staining for p16 was performed using the CINtec Histology Kit.	In situ hybridization Immunohistoch emical (IHC)	HRrHPV p16INK4a	HR-HPV (high-risk HPV) significantly more frequent in HSIL (76,2%) and SCC (88,2%) when compared with LSIL (37,0%).it was significantly more frequently detected in HSIL and SCC when compared with LSIL(p<0.05). The expression of p16 was similarly more frequent in HSIL (95,2%) and SCC (90,2%) when compared with LSIL (3,7%).	This study enables to use a pathological laboratory diagnostic system in a surgical routine
Liu et al., (2016) [15]	To determine the genes associated with the development of cervical cancer	The microarray data (GSE55940 and GSE46306) were downloaded from the Gene Expression Omnibus. Overlap between the differently expressed genes (DEGs) in GSE55940 (identified by package Limma) and differentially methylated genes were tracked. Enrichment analysis was subsequently performed for these genes using the	Microarray	DEGs, DPP4, EDN3, FGF14, TAC1 e WNT16	The study identified 91 DEGs under expressed and 151 over expressed in the data set GSE55940. In GSE46306, 561 overlap genes differentially methylated based on analysis of differential methylation in CpG	The human papillomavirus methylated carcinogenic DNA can be used as a predictive and diagnostic biomarker for risk of cervical cancer

Author/ year	Objectives	Description method	Method	Genes expression	Relevant results	Suggestions, therapies proposed and biomarkers
		ToppGene database.			site, CpG island level and gene level DPP4, EDN3, FGF14, TAC1 and WNT16 may be involved in the pathogenesis of cervical cancer. fang	
Hou et al., (2016) [10]	To investigate the significance of the expression COL6A1 in cervical cancer	COL6A1 expression in cervical cancer and normal tissue was determined by RT-PCR. Total RNA was extracted from tumor and normal tissue using Trizol reagent. The expression of the protein COL6A1was examined in 162 samples of cervical cancer by Immunohistochemistry and the correlation of the expression of COL6A1 with clinicopathologic factors was also examined.	RT-PCR Immune-hystochemical	COL6A1	The high expression of COL6A1 was significantly correlated with the tumor stage (P < 0.001), the size of the tumor (P = 0.025) metastasis (P = 0.028).	COL6A1 can be used as a marker in the progression of cervical cancer. COL6A1 can serve as oncogene in the beginning and progression of cervical cancer
Wang et al., (2015) [16]	To investigate the expression of cyclin D1 in cervical intraepithelial neoplasia (CIN) and squamous cell carcinoma and its relationship with the gene expression E7of human papillomavirus 16 (HPV16).	The cell lineages SiHa and Hcc94 were obtained from cervical epithelial cells of squamous cell carcinoma. The E6/E7 gene was silenced in Hcc94. SiRNA was built to direct the promoter of HPV16 E7 and then transfected into SiHa cells to establish the line cm-16 with stable silencing of E7. The line of control cells B3 was obtained by transfection of blank plasmid in SiHa cells. The RT-PCR and Western blot were used to detect the mRNA of cyclin D1 and the protein expression in cells and SiHa, B3 and cm-16, respectively.	QT-PCR Immune-hystochemical RT-PCR Western blot	Ciclina D1	Both ARNmas cyclin D1 protein in cells E7 (+) SiHa were lower than cm-16 and Hcc94 cells. The squamous cell carcinoma with high expression of HPV E7 shows low level of cyclin D1, suggesting that the gene HPV16 E7 inhibits the expression of cyclin D1.	The relationship between cyclin D1 and E7 gene launched bases for revealing molecular mechanisms in cancer formation process
Fang et al., (2016) [17]	To investigate the expression of miR-155 in cervical cancer and explore the biological functions of miR-155 in cervical cancer.	Tissues from 129 cancer patients and cancer-free tissue were collected. The cell lines Hela of cervical cancer were obtained. We used quantitative real-time PCR (qRT-PCR) to determine the expression of miR-155. We used a Trizol reagent (Invitrogen, EUA) to extract total RNA from tumor tissues. (si-miR-155) and a coded negative Control (si-NC) were provided. We used small intereferencia RNA	qRT-PCR	microRNA-155	Cell growth was significantly reduced in si-miR-155 transfected cells compared to the si-NC (P < 0.05). Univariate analysis revealed that the relative level of miR-155 (P = 0.000), FIGO stage (P = 0.003), metastasize to lymph nodes (P	It was noted that the miR-155 is a new molecule involved in the progression of cervical cancer, which provides a potential prognostic biomarker and therapeutic target

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		(siRNA) to suppress the expression of miR-155 in cervical cancer cells. In vitro assays were performed to explore the biological functions of miR-155 in cervical cancer			= 0.019), and vascular invasion (P = 0.022) were significantly associated with overall survival of cervical cancer	
Jimenez-wences et al., (2016) [18]	To analyze the methylation of the promoter of miRNAs and the expression of miRNAs	With infection by HPV16 were 7 without injury (non-SIL), 16low grade (LSIL) and 16 with cervical cancer. It was determined the state of methylation using miARN EpiTect Methyl II Signature PCR Array ® and the expression of miR-124, miR-218 and miR-193b was determined by qRT-PCR using individual TaqMan trials.	PCR qRT-PCR	miRNAs-miR-124 miR-128 miR-193-b	The expression of miR-124 and miR-218 was significantly lower in the cervical cancer than in samples of low-grade lesions (LSIL). The expression of miR-193b was significantly higher in the cancer of the cervix than in LSIL and samples without injuries.	Our results suggest that methylation and abnormal expression of miR-124, miR-218 and miR-193b promoter are common events during cervical carcinogenesis.
Huang et al., (2016) [19]	To confirm expression of miR-224 in cervical cancer tissue is greater than in normal tissue samples and demonstrate that miR-224 plays a role promoting the proliferation, migration and invasion of cervical cancer cells	A total of 190 samples of cervical tissue, including 126 of squamous cell carcinoma and 64 normal tissues were collected to determine the relative amount of miRNA and mRNA expression. We used the method Quantitative RT-PCR (qRT-PCR). The SiHa and CaSki cell lines, were compares for uncovering the influence of miR-224 on cervical cell lines proliferation, 96 wells plates were applied to culture cells SiHa and CaSki. Cell viability was assessed by MTT trial. Total protein was extracted from cells by western blot. The antibodies used to analyze the transfer of Western blot and IHC were anti-RASSF8 antibodies	qRT-PCR Western blot Immune-hystochemical	miR-224	The expression of miR-224 promoted cell proliferation, migration and invasion in cell lines SiHa and CaSki of cervical cancer. High expression was significantly associated with metastasis, tumor size, vascular involvement and stromal invasion (all P < 0.05), suggesting that the higher level of expression of miR-224 may be involved in the progression and metastasis of cervical cancer. RASSF8 served as a target of miR-224 in cervix cancer cells. Western blot analysis showed that the amount of expressive level of SiHa cells RASSF8 protein and CaSki transfected with copycat of miR-224 decreased	The miR-224 acts as a tumor promoter in cancer of the cervix and miR-224 and its target protein RASSF8 can be potentially used as predictors of prognosis or therapeutic targets in cancer patients.

Author/ year	Objectives	Description method	Method	Genes expression	Relevant results	Suggestions, therapies proposed and biomarkers
					significantly	
Manawapat -Klopfer et al., (2016) [4]	To identify biomarkers in the progression of the normal uterine cervix to the CIN III in women with persistent infection by HPV16.	From a cohort study accompanied by up to 19 years cervical cytology samples were obtained from women tested for HPV DNA by Hybrid Capture 2 (HC2) and positive samples were genotyped by INNO-LiPA. Genotyping (Innogenetics, Ghent, Belgium). To identify markers in progression to CIN III, it was analyzed microarrays in RNA extracted from cervical swabs of 30 women with persistent infection by HPV16 and 11 women negative to infection. Six genes were selected and validated by PCR. Three genes were subsequently validated within a different group of women from the same Cohort. Regression analyses of Kaplan-Meier and Cox were used to investigate whether expression levels of these 3 genes predict progression to CIN III	PCR Microarray	TMEM45A SERPINB5 P16NK4A	The reasons of risk of NIC3 basal expression level was (TMEM45A: HR = 1.6, 95% CI: 1.1-2.3; SERPINB5: HR = 1.8, 95% CI: 1.2-2.7; p16INK4a: HR = 1.6, 95% CI: 1.1-2.5). High levels of expression of mRNA of SERPINB5 p16INK4a TMEM45A, were associated with increased risk of NIC III in women infected with HPV16. TMEM45A levels, SERPINB5 and p16INK4a. MRNA in women persistently infected with HPV16 with normal cervical cytology increases the risk to progress to CIN III	Potential markers, besides risk stratification, as well as to understand the biological processes that cause progression to CIN III. High expression. Levels of TMEM45A, SERPINB5 and p16INK4a mRNA in women persistently infected with HPV16 and with normal cervical cytology increases the risk of developing future CIN III.
Roychowdh ury et al., (2017) [20]	To identify frequent chromosome aberrations in patients with advanced SCCC (Squamous Cell Cervical Carcinoma) in Indian women	Tumor tissues were highlighted for RNA isolation. SiHA (CSCC cells line positive for HPV-16) were acquired. The DNA was isolated by standard phenol/chloroform. Total samples RNA was isolated by TRIzol ®. Detection of HPV in cervical lesions was detected by PCR. The microarray blade was scanned using Agilent Scanner. Initially, the analysis of high resolution CGH-SNP microarrays (comparative genomic hybridization-single nucleotide polymorphism) pointed frequent CNVs (variations in number of prints) followed by genes significantly altered. After comparison with the TCGA data set (Cancer Genome Atlas), the expressions of genes were checked to identify key genes followed by Ingenuity ®. Then, property analysis of Node effect was done. It was applied in	PCR Microarray	PARP1 ATR	Integrative analysis found 78 key genes, including several new ones that have been associated with cancerwang and that can regulate DNA repair and metabolic pathways. PARP1 and ATR were among the main proteins interfering. Frequent amplification and over-expression of ATR and PARP1 were confirmed in cervical lesions, indicating its association with poor prognosis for patients with advanced CSCC.	3q gain can be used as a potential Biomarker for CC's progression. The biological relevance of these changes was evidenced. Between these 78 genes altered, the amplification and overexpression of ATR (3q23) and PARP1 (1q 42.12) was validated in independent samples of CSCC, paving the way for studying all these newly identified genes in detail to understand its synergistic role in pathogenesis of cervical

Author/ year	Objectives	Description method	Method	Genes expression	Relevant results	Suggestions, therapies proposed and biomarkers
		PPI network (protein-protein interaction) built to sort key proteins. Ass validations in independent samples were performed.				cancer to better therapeutic intervention and prognosis.
Ditttman J et al., (2017) [21]	To analyze the expression of ITIH5 inter-á-trypsin-inhibitor heavy chain 5) during cervical carcinogenesis in biopsies and cell culture.	Functional analyses were performed for the ectopic expression of ITIH5 in different cell lines. Tthe validity of the microarray data differential expression was confirmed by qPCR. ITIH5 protein loss, was assessed by Immunohistochemistry	Microarray qPCR Immune-hystochemical	(ITIH5) inter-á-trypsin-inhibitor heavy chain 5)	The loss of protein ITIH5, evaluated by immunohistochemistry, was evident in 81% of the (CCs), while the ITIH5 presented weak to moderate cytoplasmic staining in 91% of cases NIC2/3. ITIH5 was strongly reduced or absent in seven cervical cancer cell lines and in three cell lines of immortalized keratinocytes.	The ITIH5 gene silencing is a frequent event during the progression of the disease, thus providing evidence of a tumor suppressor role in cervical carcinogenesis.
Suman et al., (2017) [22]	To identify genes that are differentially expressed in cervical intraepithelial neoplasia I compared to normal cells.	A Bioinformatics approach with gene expression data, analysis, biostatistics, computational tools and databases were used to extract the key genes involved in CIN I and identify differentially expressed genes (DEGs). Key genes involved in the CIN I were extracted. It was built a gene regulatory network incorporating DEGs, (miRNAs) and transcription factors (TFs). The analysis of enrichment of DEGs via were followed by construction of a protein-protein interaction (PPI).	Microarray	Differently expressed genes (DEGs)	4 genes were found, known as PABPC1, RPS27, RPL13A and RPL21, that overlap between us with greater centrality (hubs) and the genes of the network modules PPI and also part of significant motives (motifs) –of the regulatory network. Genetic regulation of differentially expressed genes (DEGs) also revealed important transcription factors (TFs) e miRNAs as ELF1, SRF, has-mir-125b-5p and has-mir-644a.	PABPC1, RPS27, RPL13A and RPL21 can serve as potential biomarkers for CIN I and as prospective targets for therapeutic approaches
Iancu et al., (2015) [11]	To evaluate the role of the gene E5 in cervical oncogenesis in patients with abnormal cytology and cytology HPV 16	HPV DNA was detected and genotyped using the Linear Array HPV genotyping tests (Roche Molecular Biochemicals, Mannheim, Germany) and E2, E6, E5 HPV16, p21 and EGFR were investigated by qRT-PCR.	Microarray qRT-PCR	E5	The expression E5 was significantly high in cytology of low-grade lesion. The expression ofE5 correlated with moderate levels of E6 and low levels of p21. All specimens of	These findings indicate a potential role for the expression pattern of E5 on discrimination between lesions that can progress to cancer.

Author/ year	Objectives	Description method	Method	Genes expression	Relevant results	Suggestions, therapies proposed and biomarkers
	in the context of viral status correlated with potential targets (p21, EGFR).				high grade lesion presented integrated and mixed viral forms. Mixed forms presented moderate expression of E5, high levels of p21 correlate with high expression of oncogenes E6.	
Yang et al., (2014) [23]	To iinvestigate the Sox2 expression in normal cervix and cervical squamous cell carcinoma (SCC), and evaluate the prognostic significance of the expression of Sox2 in the International Federation of Gynecology and Obstetrics (FIGO) in stage I-II of SCC.	Immunohistochemistry was performed to define the expression of Sox2 in 20 normal cervical tissue samples and 55 samples of cervical SCC. The prognostic impact of Sox2 expression in terms of disease-free survival (Disease-free survival-DFS) was determined by the Kaplan-Meier method. The correlation with clinicopathologic characteristics was determined by chi-square test.	Immune-hystochemical	Sox2	The expression of Sox2 di not correlate with clinical factors (p > 0.05) DFS rates with negative and positive expression of Sox2 were 35.7 and 29.3%, respectively (p = 0.360).	The results show that Sox2 was overexpressed in stage I-II of SCC, indicating that overexpression of Sox2 may play an important role in carcinogenesis of squamous cell carcinoma.
Lee et al., (2017) [24]	To analyze the expression of CK7, CK19 p16and, of HPV in CIN III and SCC investigate the relation in cervical carcinogenesis.	25 cases CIN III and 30 cases of SCC were collected by cone biopsy, Loop Electrosurgical Excision Procedure (LEEP) or hysterectomy. It was analyzed the pattern of expression of CK19, CK7 and p16 using Immunohistochemistry and HPV infection by in situ hybridization	Immune-hystochemical in situ hybridization	CK7, CK19, p16, e HPV em CIN3		CK7/CK19 staining can be used as a marker to predict the physical state of HPV and the level of oncoprotein E7 in cervical tumor
Wang et al., (2014) [25]	To identify a subset of microRNAs hosts specifically regulated by HPV16 or HPV18 infection in vitro model systems.	Primary keratinocyte cultures and their derived tissues with or without infection by HPV16 or HPV18 was prepared. The purified total RNA was used for microarray miARN and miARN-Seq. miRNA profiles resulting from each tissue were validated by Northern blotting and TaqMan miRNA RT-qPCR. Expression of miR-16, miR-25, miR-92a, miR-378,	Microarray Northern blot RT-qPCR	miRNA-HPV	Infection with HPV16 led to a decrease of the expression De 20 miRNAs hosts including miR-34a (18), and increase the expression 22 miRNAs hosts (P < 0.05), including miR-16 Northern blot analysis of miR-	The increase of miR-25 and miR-92a correlated with the progression of cervical lesions, making them potential biomarkers CINs and CC.

Author/ year	Objectives	Description method	Method	Genes expression	Relevant results	Suggestions, therapies proposed and biomarkers
		miR-22, miR-27a, miR-29a and miR-100 in 38 normal cervical tissue and in 13 CIN I, 39 CIN III and 68 CC tissues were examined by RT-qPCR			181a, MiR-455-3 p, miR-203, miR-375 and miR-16 confirmed the expression change. It was observed the increased expression of miR-16, miR-25, miR-92a e miR-378 in CIN and CC with HPV infection. The high expression of miR-16, miR-25, miR-92a e miR-378 and decreased expression of miR-22, miR-miR-27a, 29a and miR-100 have been assigned to viral oncoprotein E6 or E7.	

Legend: CIN- Cervical Intraepithelial Neoplasia; CC - Cervical Cancer; PCR- Polymerase chain reaction; RT-PCR- Real time polymerase chain reaction HR-HPV- High-risk Human papillomavirus; RT-qPCR- Real time quantitative Polymerase chain reaction; SCC - Squamous Cell Carcinoma; LSIL - Low grade squamous intraepithelial lesion; HSIL - High grade squamous intraepithelial lesion; SIL - Squamous intraepithelial lesion

It was found that literature brings an important approach, especially about the main roles of genes related to human papillomavirus in the development of cancer, since the beginning of the pre-malignant lesions up to invasive cancer. It is noticed that inactivation or activation of genes responsible for cell protection, changes in molecular bases due to endocrine effects of HPV-related proteins, play a significant role in cervical carcinogenesis, and often lead to inactivation of tumor suppressor genes. The study of Iancu et al. [11] is an example which investigates the role of E5 gene in cervical oncogene.

In this way, a collection of information with concatenated and scientific visibility is built up leading to information and understanding expansion, reworking concepts and searching for new investigations. That fact contributes to innovative proposals in the early detection of the UCC according to molecular underpinnings of human papillomavirus (HPV)-related carcinogenesis [12].

4. DISCUSSION

In the face of discoveries about the genetic alterations and the development of cervical cancer, numerous studies are being conducted in the search for more specific alternatives for disease prevention [26]. However, although the screening program for cervical cancer control deserves recognition, there are still obstacles such as the lack of infrastructure, inadequacy and lack of quality of the cytology collected, showing the need for new technologies to promote the diagnosis precocious [27].

Some studies have documented that in screening strategies of cervical cancer, HPV tests are more sensitive than cytological screening, however, even in the face of these two methods, there is a need to improve cervical screening [7,28].

Regarding the control of cervical cancer in Brazil, unfortunately, most regions of Brazil still demonstrate high mortality rates, ranking CC as the fourth most common cancer among Brazilian women. Cervical cancer mortality presents reducing trends, however these are unequally distributed throughout in the country, where the North and Northeast regions present the highest mortality rates [28]. In Brazil, there are still no organized programs for the implementation of molecular screening for the early detection of cervical cancer and HPV tests that are already in use in many developed countries. This implementation is hampered by the confirmation of diagnoses and strategies [29].

In recent years has developed several studies on methods for HPV DNA test, however the implementation of the new technique as cervical cancer screening strategy, still presents obstacles to be overcome, such as the cost-effectiveness analysis. Mitteldorf et al. [30], refer that there are still important issues to be discussed and improved in defining future strategies, including application in different socioeconomic contexts, definition of the best test to be applied in the context of Brazilian women [30].

Thus, in this review are highlighted studies that focus on identifying expressed genes relating to cervical changes due to HPV and various methods used in molecular biology. For an overview on the search of patterns of gene expression, microarray analysis or DNA chips techniques was largely evident. The method still allows the identification of molecular markers for diagnosis, prognosis and treatment of this neoplasm [8].

However, this technique can also be accompanied by other methods such as Polymerase chain reaction (PCR) in situ hybridization and Northern blotting. The technique of PCR allows the amplification of segments of DNA and study the gene expression. To analyze the expression, it is highlighted the real time PCR (RT-PCR) that uses an enzyme called reverse transcriptase to convert a sample of RNA into cDNA before the amplification step. The quantitative RT-PCR is used to quantify the mRNA in relative and absolute terms [31].

It is worth noting that to measure changes in expression of genes in samples from patients, the reverse transcription quantitative polymerase chain reaction (RT-qPCR) is a valuable method due to its high sensitivity, flexibility, low cost and easy to use [32].

Regarding the in-situ hybridization, this technique is based on pairing of nucleic acid sequences complementary to each other. The in-situ hybridization method have been used in numerous areas, including gene expression analysis [33].

When it comes to Northern blotting technique, it is a method that allows the detection of RNA sequences (total or messenger). It can be used to study the temporal pattern of gene expression during the development of the tumor. Few studies refer to this technique. On the study of Wang et al. [25], the northern blot was used to confirm the change of expression of miRNAs. It was evidenced that the oncogenic human papillomavirus (HPVs) induce aberrant expression of many cell miRNAs and the infection by HPV18 does not produce detectable viral miRNA.

The MicroRNAs (miRNAs or miRs) are a small non-coding RNAs class with 20 to 24 nucleotides in length that regulate the expression of genes and proteins in the post-transcriptional level through the 3, non-translated region of target messenger RNAs (mRNAs) to suppress the translation or induce the degradation of mRNAs [34]. MiRNAs are involved in a variety of biological processes, including development, proliferation, differentiation and apoptosis. In addition, certain miRNAs are demonstrated to function as oncogenes or tumor suppressors [35]. The altered expression of miRNAs in many types of cancer has been explored as a marker for possible diagnosis and therapy [26]. In cervical cancer, many miRNAs are specifically detected. For Huang et al. [19], the level of expression of miR-224 was significantly increased (1.82 times, $P = 0.0025$) in cervical cancer compared to normal tissues. Huang et al. [19] showed similar results to the study by Wang et al. [25], in which a large increase in the expression of MiR-25 and MiR-92 was observed, correlating with the progression of precursor lesions. Both authors used RT-PCR method to determine miRNA expression.

We can add the research of Fang et al. [17] which pointed out that the levels of expression of miR-155 were significantly higher in tissues of cervical cancer compared with non-cancerous tissues ($P < 0.05$). The result indicated that a MiR-155 promoted cell proliferation of cervical cancer and suggest that miR-155 can work as an oncogenic miRNA in the progress of cervical carcinogenesis.

As for the expression and identification of proteins, the study brought investigations that involved techniques such as Western blotting and Immunohistochemistry. The analysis by Western blotting allows to detect, identify and quantify proteins separated by electrophoresis. It

also says that, with this technique, it is possible to check whether a specific protein is present in the sample under analysis [36].

Immunohistochemistry or IHC refers to the process of localizing antigens/proteins in tissues, exploiting the principle of specific binding of antibodies to antigens in biological tissues [37].

In the face of all these techniques illustrated, it is possible to mention, in this review, the main studies relating to gene and protein expression induced by HPV, as well as other molecular events and the methods used to identify carcinogenesis processes in the development of cervical cancer.

It is noticed during the analysis of this review that a large number of studies focuses on the development of cervix cancer, considering the intraepithelial neoplasia of grade I, II and III, invasive cancer, as references of tumor staging. The study of Dittmann et al. [21], investigated the expression based on microarrays showing that levels of mRNA of chain 5 (ITIH5) of trypsin-trypsin inhibitor in cervical cancers were significantly lower than in CIN III.

The research of Wang et al. [16], also brings the expression of cyclin D1 that was expressed in the basal cells of normal cervical squamous epithelium gradually decreasing in the progression of CIN to CIN III. The squamous cell carcinoma presented negative or fuzzy expression of cyclin D1 ($P < 0.05$). The authors stressed that the term E7 on cell proliferation in cervical cancer depends on the via E7-pRb which inhibits the expression of cyclin D1 [16].

In contrast, Lee et al. [24], analyzed the protein expression, noting that the expression of CK19, p16 and HPV was positive in all cases of CIN III and squamous cell carcinoma. The expression of CK7 was positive in all CIN III cases and in 66% of carcinomas.Comparative studies related to normal and altered cervical cells were highlighted. Suman et al. [22], brought the differential expression analysis to identify Genes that are differentially expressed in CIN I compared to normal cells. The authors claim that microarrays analysis revealed 71 differentially expressed genes, including 39 overexpressed and 32 under expressed.

It is noteworthy that Liu et al. [15], used microarray analysis to identify methylated genes by determining the associated genes in the development of cervical cancer. However,

Suman et al. [22], used the microarray technique to identify genes differentially espressos in NIC I highlighting genes that can act in the early detection of cervical cancer. But in front of the results, the authors emphasize the necessity of other experimental studies to prove reliable value in the finding.

The study of Hou et al. [10] noted the expression of COL6A1 in normal and tumor cervical tissue demonstrating an overexpression of COL6A1 in tissues of cervical cancer compared with non-tumor tissue. To the authors, the COL6A1 gene has an important role in cellular proliferation and progression. The investigation brings the relationship with the clinical prognosis about the tumor, unlike the investigation by Yang et al. [23] in which the expression of Sox2 did not correlate with any clinical factor of the disease. However, the study was limited by the small number of participants in the research.

The authors verified that the Sox2 expression was significantly higher in SCC than in normal cervix. They pointed out that the rate of positive expression in the SCC was 74.5% (41/55), while in normal cervix was 20.0% (4/20; p = 0.000).

Another highlight in comparative studies about the development of cervical cancer, is Manawapat-Klopfer et al. [4] study, that identified markers on the progression of normal uterine cervix to CIN III, emphasizing the HPV 16 infection. It was found that in women with normal cytology and persistent infection by HPV16 the expression mRNA of TMEM45A, SERPINB5 and p16INK4a increased, and was associated with an increased risk of progression to CIN III.

The identification of p16INK4a as a marker for neoplastic transformation of cervical squamous epithelial cells by HPVs allows the identification of HPV-transformed cells in histopathology or cytopathology specimens. Compared to HPV DNA tests, immunochemical detection of p16INK4a-stained cells demonstrates a significantly improved specificity with remarkably good sensitivity, enabling p16INK4a in the triage of HPV-positive women. In this way, it is understood how a molecular mechanism that can be used in the early detection and diagnosis of cervical cancer [38]. The Fig. 2 shows the p16INK4a is overexpressed in HPV-transformed cells we review the accumulated clinical evidence suggesting that p16INK4a can serve as a useful biomarker in the routine diagnostic.

There are other examples of markers related to HPV-induced molecular mechanisms that may be useful in the early detection of cervical cancer and disease control, however in this review the p16INK4 protein has been highlighted because it is the most studied marker for the progression of cervical intraepithelial neoplasia.
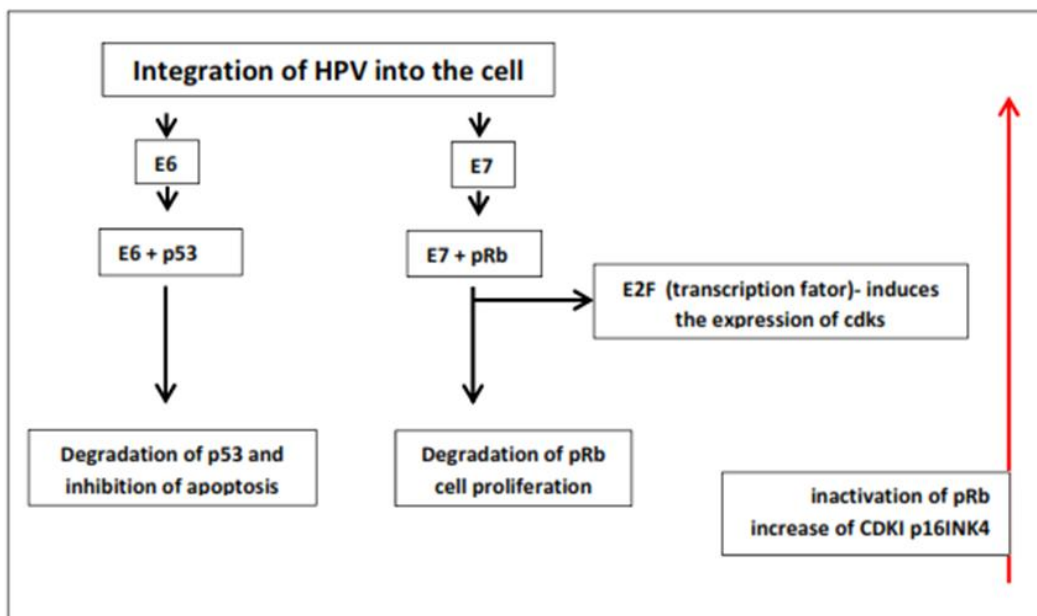

Fig. 2. Effects of HPV E7 on the levels of expression of p16INK4a

Some studies addressed the methylation as a molecular mechanism associated with cervical cancer. The hypomethylation of DNA and hypermethylation of various cancer-related genes are extensively studied. DNA methylation, a process of epigenetics, may increase the risk of cervical cancer by regulating gene expression and chromatin structure. Numerous studies have reported that the methylation of certain genes is associated with the pathogenesis of cervical cancer [39,40,15].

In the article by Zhao et al. [13], it was investigated TSLC1 gene methylation in cervical lesions through the methylation specific polymerase chain reaction (MSP-PCR). The methylation-specific PCR technique is sensitive and specific being widely used to detect methylation of CpGs DNA sites [1].

Jimenez-Wences et al. [18], examined methylation of promoter of 22 miRNAs and the expression of 3miRNAs in 10 no squamous intraepithelial lesions without infection with HPV16. The levels of methylation of promoters miR-124-2, miR-218-1, miR-218-2 and miR-34b/c were significantly higher in cervical cancer than in low-grade lesions samples.

In this review, we also highlight the study of Roychowdhury et al. [20], where the authors identified in patients with cervical squamous cell carcinoma (CSCC) frequent chromosomal amplifications followed by deletions. Chromosomal regions changed ranged from 6 kb (19p13.3) up to 105 Mb (1q21.1-1q44). The research shown that the gain in chromosome 3q (3q25.2-3q26.1; 3q26.1-3q26.31; 3q26.32-3q29) was the most important chromosomal aberration (100%) found in the CSCC.

A long latency period (about 15-20 years) is seen between HPV infection and the development of invasive lesion. This indicates that in addition to the persistent HPV infection, chromosomal changes are needed to cause additional carcinogenicity. These observations suggest that genes located within these chromosomal loci, frequently changed, can play a crucial role in the development of aggressive tumor [20].

With this set of information about detection methods early diagnosis of cervical cancer, it have more knowledge of the mechanisms molecules that are involved on cervical oncogenesis due to use of advanced techniques molecular biology. Some techniques allow the identification and HPV viral genotyping, such as such as PCR, qRT-PCR and microarrays, which are relevant for the detection of HPV genotyping [41].

However, in Brazil these techniques are used in laboratories equipped, but they are very expensive high financial cost [42]. The integration of these techniques with existing methods, such as papanicolaou, can improve the early detection of cervical neoplasias, as well as contribute to the creation of new therapies for diagnosis and treatment. Thus, the implementation of strategies and campaigns to clarify the efficacy of molecular methods are important points for the reduction of morbidity and mortality due to cervical cancer in developing countries, especially Brazil, but this evolution still depends on well established economic and political factors. Therefore, understanding the mechanisms of cervical carcinogenesis enables find new alternatives for prevention and treatment of this type of tumor among women.

5. CONCLUSIONS

The data obtained in this review suggest the importance of research that identifies molecular markers for the early detection of cervical cancer. This review brought the approach of several studies about new perspectives for early detection of cervical cancer. The search for more specific alternatives to prevent the development of cervical cancer allowed to glimpse various techniques in the field of molecular biology, with methods that allow to understand the process of cervical carcinogenesis with an emphasis on molecular mechanisms of association with cervical changes due to HPV infection.

Given this panorama, it was possible to realize that much of the research referred to molecular events in the initial phase of the process of carcinogenesis, highlighting the progression of precursor lesions to invasive cervical cancer. We observed mechanisms such as protein expression more or less expressive in varying degrees of cervical intraepithelial neoplasia, emphasizing the approaches of some studies on methylation as one of the mechanisms associated with cervical cancer. This study also highlighted the proposal of different biomarkers that can be used as diagnostic and therapeutic targets about cervical cancer.

Therefore, starting from this premise, we can emphasize the importance of encouraging

research and discoveries that culminate in new proposals for early detection of cervical cancer. Despite the existence of a program to control cervical cancer, with oncotic colpocytology and HPV vaccine as main highlights, other complementary strategies that guarantee the reduction of morbidity and mortality for this type of cancer in the feminine population are still required.

This context emphasizes the relevance of studies that explore the science of molecular biology with technology improvements, adding new concepts and methods of prevention and early detection, aimed at implementing new measures to prevent and treat the disease as a benefit to this segment of the population.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Oliveira CM, Levi JE. The biological impact of genomic diversity in Cervical Cancer Development. Acta Cytologica. 2016; 60(6):513-517.
2. De Brot L, Pellegrini B, Moretti ST, Carraro DM, Soares FA, Rocha RM. et al. Infections with multiple high-risk HPV types are associated with high-grade and persistent low-grade intraepithelial lesions of the cervix. Cancer. 2017;125(2):138-143.
3. Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ, et al. HPV-mediated cervical carcinogenesis: Concepts and clinical implications. J Pathol. 2006; 208(2):152-64.
4. Manawapat-klopfer A, Thomsen LT, Martus P, Munk C, Russ R, Gmuender H et al. TMEM45A, SERPINB5 and p16INK4A transcript levels are predictive for development of high-grade cervical lesions. American Journal of Cancer Research. 2016;6(7):1524-1536.
5. Thomas A, Mahantshetty U, Kannan S, Deodhar K, Shrivastava SK, et al. Expressions profiling of cervical cancers in Indian women at diferente stages toidentify gene signatures during progression of the disease. Cancer Medicine. 2013;2(6): 836-848.
6. Lew JB, Simms KT, Smith MA, Hall M, Yoon-Jung Kang, Xu XM, et al. Primary HPV testing versus cytology-based cervical screening in women in Australia vaccinated for HPV and unvaccinated: effectiveness and economic assessment for the National Cervical Screening Program. The Lancet Public Health. 2017; 2(2):e96–e107.
7. Austin RM, Zhao C. Is 58% sensitivity for detection of cervical intraepithelial neoplasia 3 and invasive cervical cancer optimal for cervical screening? Cytojournal. 2014;11:14.
8. Wadlow R, Ramaswamy S. DNA microarrays in clinical cancer research. Curr Mol Med. 2005;5(1):111-120.
9. Zhu MY, Chen F, Niyazi M, Sui S, Gao D, et al. Variation in apoptotic gene expression in cervical cancer through oligonucleotide microarray profiling. J Low Genit Tract Dis. 2015;19(1):46-54.
10. Hou T, Tong C, Kazobinka G, Zhang W, Huang X, Huang Y, et al. Expression of COL6A1 predicts prognosis in cervical cancer patients. Am J Transl Res. 2016; 8(6):2838-2844.
11. Iancu IV, Pleşa A, Botezatu A ,Huică I, Stănescu AD, Socolov D, et al. E5HPV16 mRNA expression. Pattern analysis in patients with cervical lesions in viral status contexto. Roum Arch Microbiol Immunol. 2015;74(1):33-9.
12. Faraji F, Zaidi M, Fakhry C, Gaykalova DA. Mechanisms of human papillomavirus-related carcinogenesis in head and neck cancer. Microbes and Infection. 2017; 19(10-9):464-475.
13. Zhao X, Li Y, Cui Y, Wei L, et al. Significance of TSLC1 gene methylation and TSLC1 protein expression in the progression of cervical lesions. Zhonghua Zhong Liu Za Zhi. 2015;37(5):356-60.
14. Cheah PL, Koh CC, Nazarina AR, Teoh KH, Looi LM. Correlation of p16INK4a immunoexpression and human papillomavirus (HPV) detected by in-situ hybridization in cervical squamous neoplasia. Malaysian J Pathol. 2016;38(1): 33–38.
15. Liu MY, et al. Identification of key genes associated with cervical cancer by comprehensive analysis of transcriptome microarray and methylation microarray. Oncology Letters. 2016;12(1):473–478.
16. Wang P. Expression of cyclin D1 in cervical intraepithelial neoplasia and squamous cell carcinoma and its

relationship with HPV16 E7 gene. Zhonghua Bing Li Xue Za Zhi. 2015; 44(12):884-8.

17. Fang H, Shuang D, Yi Z, Sheng H, Liu Y, et al. Up-regulated microRNA-155 expression is associated with poor prognosis in cervical cancer patients. Biomedicine & Pharmacotherapy. 2016; 83:64–69.

18. Jimenez-Wences H, DN martínez-Carrilo, Zaragoza OP, Campos-Viguri GE, Hernadez-Sotelo D, Jimenez-Lopez MA, et al. Methylation and expression of miRNAs in precancerous lesions and cervical cancer with HPV16 infection. Oncology Reports. 2016;35(4):2297–2305.

19. Huang Y, Li Y, Wang FF, Lv W, Xie X, Cheng X, et al. Over-Expressed miR-224 promotes the progression of cervical cancer via targeting RASSF8. PLoS ONE. 2016;11(9):1-17.

20. Roychowdhury A, Samadder S, Das P, Mandloi S, Addya S, Chakraborty C, et al. Integrative genomic and network analysis identified novel genes associated with the development of advanced cervical squamous cell carcinoma. Biochimica et Biophysica Acta. 2017;1861(1):2899–2911.

21. Dittman J. Gene expression analysis combined with functional genomics approach identifies ITIH5 as tumor suppressor gene in cervical carcinogenesis. Molecular Carcinogenesis. 2017;56(6):1578-1589.

22. Suman S, Mishra A, Kulshrestha AA. A systems approach for the elucidation of crucial genes and network constituents of cervical intraepithelial neoplasia 1 (CIN1). Molecular Bio Systems. 2017;13:549–555.

23. Yang Z. Expression of Sox2 in cervical squamous cell carcinoma. J Buon. 2014; 19(1):203-6.

24. Lee H, Lee H, Cho YK. Cytokeratin7 and cytokeratin19 expression in high grade cervical intraepithelial neoplasm and squamous cell carcinoma and their possible association in cervical carcinogenesis. Diagnostic Pathology. 2017;12(18):1-9.

25. Wang X, Wang HK, Li Y, Hafner M, Banerjee NS, Tang S, et al. microRNAs are biomarkers of oncogenic human papillomavirus infections. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(11): 4262–7.

26. Saavedra KP, Brebi PM, Roa JCS. Epigenetic alterations in preneoplastic and neoplastic lesions of the cervix. Clinical Epigenetics. 2012;4(1):2-7.

27. Catarino R, Petignat P, Dongui G, Vassilakos P. Cancer screening in developing countries at a crossroad: Emerging technologies and policy choices. World Journal of Clinical Oncolog. 2015; 6(6):281-290.

28. Arbyn M, Ronco G, Anttila A, Meijer CJ, Poljak M, Ogilvie G, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine. 2012;30(5):88-99.

29. Barbosa IR, Souza DLB, Berna MM, Costa ICC. Desigualdades regionais na mortalidade por câncer de colo de útero no Brasil: tendências e projeções até o ano 2030. Ciência & Saúde Coletiva. 2016; 21(1):253-262.

30. Mitteldorf CATF. Cervical cancer screening: from Pap smear to future strategies. J Bras Patol Med Lab. 2016; 52(4):238-245.

31. Lebuln M, Derenkó J, Rademacher A, Helbig S, Munk B, Pechtl A, et al. DNA and RNA Extraction and Quantitative Real-Time PCR-Based Assays for Biogas Biocenoses in an Interlaboratory Comparison. Bioengineering. 2016;3(7): 1-24.

32. Fjeldbo CS, Aarnes EK, Malinen E, Kristensen GB, Lyng H, et al. Identification and validation of reference genes for RT-qPCR studies of hypoxia in squamous cervical cancer patients. PLoS ONE. 2016; 11(5):1-15.

33. Silva –Venezuela MG, et al. Hibridização in situ com sonda não-radioativa para mRNA:princípios e aplicações em patologia. J Bras Patol Med Lab. 2006; 42(3):207-213.

34. Inui M, Martello G, Piccolo G. MicroRNA control of signal transduction. Nat Rev Mol Cell Biol. 2010;11(4):252-63.

35. Li J, Wang X, Zhang Y, Zhang Y, et al. E3 ubiquitin ligase isolated by differential display regulates cervical cancer growth in vitro and in vivo via microRNA-143. Experimental and Therapeutic Medicine. 2016;12(2):676-682.

36. Bass JJ, Wilkinson DJ, Rankin D, Phillips BE, Szewczyk NJ, Smith K, et al. An overview of technical considerations for Western blotting applications to physiological research. Scandinavian

Journal of Medicine and Science in Sports. 2016;27(1):4–25.

37. Junior JE, Giraldo PC, Gonçalves AK. Marcadores imunoistoquímicos de lesões precursoras do câncer de colo uterino associadas ao HPV: o papel da proteína de supressão tumoral p16ink4a. DST – J bras Doenças Sex Transm. 2006;18(1): 62–65.

38. Bergeron C, Ronco G, Reuschenbach M, Wentzensen N, Arbyn M, Stoler M, von Knebel Doeberitz, et al. The clinical impact of using p16(INK4a) immunochemistry in cervical histopathology and cytology: An update of recent developments. Int J Cancer. 2015;136(12):2741-51

39. Chen CC, Lee KD, Pai MW, Chu PY, Hsu CC, Chiu CC, et al. Changes in DNA methylation are associated with the development of drug resistance in cervical cancer cells. Cancer Cell Int. 2015;15(98): 1-9.

40. Lando M. Interplay between promoter methylation and chromosomal loss in gene silencing at 3p11-p14 in cervical câncer. Epigenetics. 2015;10(10):970-980.

41. Assunção JGF, Correia AKA. Análise comparativa das técnicas de biologia molecular para genotipagem do papilovírus humano- HPV. Revista Cientifica da Escola de Saúde. 2014;3(2): 1-9.

42. Rivoire WA, Eye Corleta HVE, Brum IS, Capp E. Molecular biology of cervical cancer. Rev. Bras. Saúde Matern. Infant. 2006;6(4):447-451.