DNA METHYLATION: A REVIEW OF NEW PERSPECTIVES FOR EARLY DETECTION OF CERVICAL CANCER

METILAÇÃO DE DNA: UMA REVISÃO SOBRE NOVAS PERSPECTIVAS PARA DETECÇÃO PRECOCE DO CÂNCER DO COLO DO ÚTERO

Maria Cristina de Melo Pessanha Carvalho[1], Maria Paula Leite da Rocha[2]

ABSTRACT

Introduction: Cervical cancer is a serious public health problem and the fourth most common type of cancer in the female population. Persistent human papillomavirus infection is a risk factor for this tumor. However, epigenetic abnormalities occur in the carcinogenic process, in which DNA methylation is an important mechanism for gene silencing. That tends to lead to premalignant changes, a fact which can be investigated through the use of biomarkers for early detection and prevention of cervical cancer. **Objective:** To identify studies that analyzes epigenetic science, DNA methylation, and research on molecular biomarkers as new perspectives for early detection of cervical cancer. **Methods:** This is a literature review including articles published in the last 5 years in English and Portuguese, using the LILACS, Medline, SciELO, Cochrane Library, and PubMed databases. **Results:** The study determined a process of convergence in the analyzed subject. After reading various studies on DNA methylation and cervical cancer, the team highlighted the inactivation of tumor suppressor genes and the activation of oncogenes, which have an important role in cervical carcinogenesis, caused by epigenetic changes found in women with cervical intraepithelial neoplasia and cervical cancer. **Conclusion:** This review showed several screening strategies associated with cytological and molecular tests; however, there are knowledge gaps and the need for further investigation. Potential biomarkers are suggested to allow the monitoring of molecular events that complement the program of cervical cancer control, reducing mortality in the female population.

Keywords: DNA methylation; papillomaviridae; cervical intraepithelial neoplasia; biological markers.

RESUMO

Introdução: O Câncer do colo do útero é um sério problema de saúde pública e o quarto tipo mais comum na população feminina. A infecção persistente por HPV é um dos fatores de risco para o desenvolvimento deste tipo de tumor. No entanto, anormalidades epigenéticas ocorrem no processo carcinogenico, em que a metilação do DNA é importante mecanismo para silenciamento gênico que tende a levar a alterações pré malignas, fato que pode ser explorado com a utilização de biomarcadores para detecção precoce do cancer cervical. **Objetivo:** Identificar estudos que exploram a ciência epigenetica, metilação de DNA e investigações acerca de biomarcadores moleculares como novas perspectivas para detecção precoce do câncer cervical. **Métodos:** Trata-se de uma revisão bibliográfica em que foram incluidos artigos publicados nos últimos 5 anos em ingles e portugues, utilizando as bases de dados LILACS, Medline, SciELO, Biblioteca Cochrane e Pubmed. **Resultados:** Encontrou-se convergência sobre a temática estudada. Após a leitura de vários estudos sobre a metilação do DNA e câncer cervical, destacou-se a inativação de genes supressores tumorais e ativação de oncogenes, que desempenham um importante papel na carcinogênese cervical causada por alterações epigenéticas encontrados em mulheres com neoplasia intraepitelial cervical e câncer cervical. **Conclusão:** Esta revisão mostrou várias estratégias de triagens associadas com testes citológicos e moleculares, no entanto ainda existem lacunas de conhecimento e necessidade de maiores investigações. Propostas de biomarcadores foram ilustradas permitindo monitorar eventos moleculares que complementam programa de controle do câncer cervical, reduzindo a mortalidade na população feminina.

Palavras-chave: metilação de DNA; papillomaviridae; neoplasia intraepitelial cervical; marcadores biológicos.

INTRODUCTION

This study is based on the increasing research on epigenetic science, human papillomavirus (HPV), cervical cancer, and molecular markers, indicating new perspectives for early detection of cervical cancer. This topic is relevant in view of the carcinogenic effects that may be associated with DNA methylation and molecular changes in the development of this type of tumor[1].

The carcinogenic process comprises a series of modifications that are accumulated in the cell and eventually allow unregulated growth. The presence of mutations in key genes is involved in the cell-cycle regulation and cell growth and they are among the genetic exchanges that take place[2].

Persistent high-risk HPV infection is essential, but not a prerequisite, for cervical carcinogenesis. There are genetic and epigenetic changes that also operate in the development of precursor lesions and invasive cancer[2].

Thus, cervical cancer is a serious public health problem in women worldwide. It is the second most common type of cancer among the women and has higher incidence in less developed countries. This difference is also seen in relation to survival, as, in poor countries, diagnosis is most often made in advanced stages[3].

According to the statistical data, 528,000 new cases and 266,000 deaths were estimated in 2012. In Brazil, 15,590 new cases of cervical cancer were estimated in 2014, with an estimated risk of 15.33 cases per 100,000 women. Other factors related to immunity, genetics, and sexual behavior also influence the mechanisms that determine the regression or persistence of the infection[3].

Therefore, understanding epigenetic events of cervical cancer, such as the methylation of several genes, becomes relevant, which promises to aid the diagnosis and prognosis of cervical tumors. DNA

[1]PhD in Nursing (Women's Health) from the Anna Nery School of Nursing of the Universidade Federal do Rio de Janeiro (EEAN/UFRJ); Professor at the Centro Universitário Barra Mansa; Researcher and Nurse at the Ministry of Health and Municipal Secretariat of Health of Rio de Janeiro – Rio de Janeiro (RJ), Brazil.

[2]Postgraduate student of the Family Health Program of the Universidade Estácio de Sá; Nurse at the Municipal Secretariat of Health of Rio de Janeiro – Rio de Janeiro (RJ), Brazil.

methylation is an important epigenetic mechanism for gene silencing, which tends to cause cervical cancer and premalignant changes[2].

In addition, epigenetic abnormalities occur in the carcinogenic process, a fact that can be investigated through the use of molecular markers for diagnosis. Tumor biomarkers are indicators of the physiological state and the changes that occur during the neoplastic process[4]. Accordingly, the identification of a set of hypermethylated genes in cytological smears may offer new means for the detection of cervical cancer[4].

DNA methylation and cervical cancer

Research advances in genetics have brought new discoveries and approaches in the context of molecular and cellular biology. Epigenomic investigations are leading to a better understanding of factors associated with complex disease processes and have elucidated revelations and definitions for diseases such as cancer. Malignant neoplasms contain genetic and molecular bases which, because of genomic instability, may cause certain complications and may lead to the development of malignant tumors[5].

Therefore, epigenetics is the study of DNA and histone modifications that are inheritable and do not alter the sequence of DNA bases. In other words, epigenetic changes are chemical alterations in the genome that do not change the DNA sequence; they may lead, however, to a specific phenotype, disease state, or other observable characteristics[5].

In this regard, methylation is based on a methyl grouping (CH3), which is transferred from S-adenosylmethionine to a cytosine at the carbon 5 position (5-MeC), usually preceded by a guanine (CpG dinucleotides), by the action of a family of enzymes named DNA methyltransferase (DNMT)[6].

Methylation is an epigenetic process that prevails during development and may be modulated along the postnatal life. In addition, methylation is an important event because it leads to gene silencing[1].

With regard to cervical cancer, genetic and epigenetic changes favor the progression of precancerous changes to invasive cancer. A number of changes occur during all stages of cervical carcinogenesis, affecting the expression of the HPV as well as the host genes that represent stages of the process in carcinogenesis. These include global DNA hypomethylation, hypermethylation of key tumor suppressor genes, and histone modifications[7]. The increase in DNA methylation occurs in the promoter region of genes during the progression of the lesion[8]. In cervical cancer, methylation of various genes has been described. DNA methylation has been established as deregulatory in the development of cervical cancer[8].

According to Lodi et al.[9], although high-risk HPV infection is necessary for the development of cervical cancer, epigenetic changes must also be considered when occurring in the viral genome. They can also influence the carcinogenic process directed by the virus and epigenetic changes in the host genome. HPV can silence genes activation, reducing the host defense and enabling the persistent infection[9].

In view of that fact, epigenetic science emerges as an essential factor to foster interest in research and discoveries in the field of molecular genetics, leading to diagnostics and prognostics as well as creating new ways to care for women's health. The control of persistent HPV infection should be a priority, benefiting women with prevention by HPV vaccination, early detection, and treatment of precursor lesions and cervical cancer.

Thus, detection and the use of tools that can complement both diagnosis and screening of precursor lesions of cervical cancer are suggested by several studies, which make them highly relevant. The choice for this study is justified by the importance of focusing on stimulating the research on new means of early detection for this type of tumor.

OBJECTIVE

Considering the situation, the objective is to identify studies that explore epigenetic science, DNA methylation, and research on molecular biomarkers as new perspectives for early detection of cervical cancer.

METHODS

This is a literature review of articles published between 2010 and 2015 to search for the information on the topic and to gather information for the survey of relevant publications. We used the LILACS, MEDLINE, SciELO, Cochrane Library, and PubMed databases. We used the following keywords as the search strategy: *methylation, cervical cancer, cervical intraepithelial neoplasia, DNA, human papillomavirus, genetics*. To combine the terms, we used the words *and, or, and not* as identifiers. As limits, we used articles published in English and Portuguese. We found 74 bibliographical productions, of which 69 were in English; from this total, 25 met the criteria established to achieve the objectives of the study. Some of the selected articles are outlined in **Chart 1**. The highlighted inclusion criteria are the publication period, the suggestion approach to biomarkers as means of cervical cancer detection, as well as the study of the DNA methylation of the human genome and the HPV genome. We excluded articles that did not investigate and/or suggest molecular biomarkers for the detection of precursor lesions and cervical cancer from the study. The team read scientific publications, classified them by author and year of publication, method used, relevant findings and suggestions of genes as biomarkers.

RESULTS

The team conducted an analytical and a selective reading as the criteria to provide data on the subject to meet the proposed objective. This study indicates some suggested biomarkers in **Chart 1** as new perspectives for early detection of cervical cancer.

DISCUSSION

HPV methylation with different approaches and specific methodologies to identify biomarkers that may contribute to the early detection of cervical cancer has been widely investigated in the studies.

We found a significant convergence of several authors consulted on the subject studied. The analysis of several studies on DNA methylation and cervical cancer provides a comprehensive overview revealing that the inactivation of tumor suppressor genes and the activation of oncogenes have an important role in cervical carcinogenesis caused

Chart 1 – Highlight of some studies found in this review on DNA methylation and cervical cancer.

Authorship (year)	Method	Relevant findings	Suggested biomarkers
Yang[2] (2014)	Review that summarized DNA methylation in the HPV genome and identified its clinical implications.	Methylation of the HPV long control region (LCR) and the L1 gene is common during cervical carcinogenesis and increases with the severity of the neoplasia.	Hypermethylation of the L1 gene of HPV16 and HPV18
Nye et al.[12] (2013)	Study with 213 women with CIN I, II, or III or cervical cancer. Data collected from the questionnaire, biopsies peripheral blood, cervical smears, HPV, and HIV infections. Assessed methylation status of PEG3 by bisulfite sequencing and treatment.	The data confirm that the methylation of the PEG3 gene is a potential molecular target for inclusion in the screening of cervical intraepithelial neoplasia, to assess disease progression.	Methylation of the PEG3 gene
Carestiato et al.[14] (2013)	The study assessed 141 cervical samples from patients. HPV detection and genotyping were performed using the PCR technique and methylation of the p16(INK4A) gene, through nested methylation-specific PCR (MSP).	HPV infections and epigenetic alterations showed strong statistical association with cervical carcinoma.	Methylation of the p16(INK4A) gene
Xiong et al.[16] 2014	Studies eligible at PubMed, Web of Science, EMBASE, and CNKI were systematically assessed using meta-regression, subgroup analysis, and sensitivity analysis. Sources of heterogeneity were analyzed. The odds ratio (OR) and the 95% confidence interval (95% CI) were calculated by meta-analysis in R.	Association between methylation of the DAPK1 promoter gene.	Methylation of the DAPK1 gene
Murakami et al.[17] (2013)	Cervical cells were collected from 54 HPV52-positive and 41 HPV58-positive women. The HPV genome was examined using bisulfite modification as well as amplification and sequencing of the polymerase chain reaction.	The increase in methylation of the CpG regions of the L1 gene of HPV52/58 L1 was correlated with the severity of the cervical neoplasia, similar to HPV16 and HPV18.	Methylation of the HPV 52L1 and 58L1 genes
Louvanto et al.[19] (2015)	The methylation status was examined of selected loci in HPV16 and human genes in DNA extracted from exfoliated cervical cell samples from 244 women with cancer who were HPV16-positive or had cervical intraepithelial neoplasia (CIN) or who tested negative for intraepithelial lesion and malignancy (NILM). The methylation of the CpG regions of the L1 gene of HPV16 (CpG 6367 and 6389) as well as of the EPB41L3 (CPG 438, 427, and 425) and LMX1 (CPG 260, 262, 266, and 274) human genes was quantified after bisulfite treatment and sequencing.	The methylation of the LMX1 and EPB41L3 genes of the host and the viral loci of the HPV16L1 viral gene has the potential to distinguish between precancerous lesions and invasive diseases.	LMX1 and EPB41L3 host genes HPV16L1 viral gene
Brebi et al.[20] (2014)	Cervical smear samples were assessed using methylation-specific PCR (MSP) and quantitative MSP (qMSP).	Brebi et al. tested the methylation of regions of the ZAR1 and SFRP4 promoter genes as potential biomarkers for the diagnosis of cervical preneoplastic and neoplastic lesions.	Methylation of the ZAR1 and SFRP4 genes
Vasiljević et al.[21] (2014)	Methylation of 26 genes: APC, CADM1, CCND2, CDH13, CDKN2A, CTNNB1, DAPK1, DPYS, EDNRB, EPB41L3, ESR1, GSTP1, HIN1, JAM3, LMX1, MAL, MDR1, PAX1, PTGS2, RARB, RASSF1, SLIT2, SOX1, SPARC, TERT, and TWIST1 was measured by sequencing in cytology samples from a group of women with normal histological results or CIN III results.	High methylation of the EPB41L3 gene in CIN II and III neoplasias. ($p < 0.0001$)	Methylation of the EPB41L3 gene
De Strooper et al.[22] (2014)	We analyzed the methylation of the FAM19A4 gene through quantitative methylation-specific PCR (qMSP). Cervical hrHPV-positive smears from 43 women with cervical intraepithelial neoplasia III (CIN III) and 135 with cervical intraepithelial neoplasia I (CIN I) were used.	Highly efficient methylation of the FAM19A4 gene in the detection of cervical carcinomas, CIN II and CIN III.	Methylation of the FAM19A4 gene
Van der Meide et al.[25] (2011)	Methylation of the promoter gene of nine Wnt-antagonists (APC, AXIN2, DKK3, SFRP2, SFRP4, SFRP5, SOX17, WIF1, and WNT5A) was assessed through methylation-specific PCR (MSP) in a small number of cervical samples, including cervical adenocarcinoma and adenocarcinoma in situ. To assess the diagnostic potential of more frequently methylated genes, they were analyzed through quantitative MSP (qMSP).	The frequency of methylation of the DKK3 and SFRP2 promoter genes establishes promising screening markers for HPV-positive women at risk cervical adenocarcinoma and adenocarcinoma in situ.	Methylation of the DKK3 and SFRP2 genes

Continue...

Chart 1 – Continuation.

Authorship (year)	Method	Relevant findings	Suggested biomarkers
Zuo et al.[26] (2014)	Methylation-specific PCR (MSP) and HPV-specific (HPV16 and HPV18) PCR were performed in 110 cervical samples: 40 normal cervical tissues, 10 CIN I tissues, 10 CIN II tissues, 10 CIN III tissues, and 40 cervical cancer tissues. The expression of both genes was determined by reverse transcription PCR (RT-PCR) for CIN III and 40 cervical cancer tissues.	The CCNA1 and HS3ST2 genes may have an important role in cervical cancer induced by HPV. Patients with specific hypermethylated genes may be at increased risk of progression to invasive cervical cancer.	Methylation of the CCNA1 and HS3ST2 genes
Chujan et al.[27] (2014)	A histopathological method was used as the gold-standard method for samples separated into the following groups: negative (n=31), low-grade squamous intraepithelial lesion (n=34), and high-grade intraepithelial lesions (n=32). High-risk HPV was detected by Hybrid Capture 2 (HC2), and the methylation of the CCNA 1 promoter gene was analyzed through CCNA 1 methylation-specific duplex-PCR.	The assessment of the prevalence of the methylation of the cyclin-A1 promoter gene (CCNA 1) in residual cervical cells isolated from cytology in liquid medium enable to distinguish negative histology, low-grade lesions, and high-grade lesions.	Methylation of the CCNA 1 gene
Jung et al.[28] (2011)	Methylation levels for the vimentin promoter (VIM) and the expression of the VIM gene were analyzed in seven cervical cancer cell lines and 50 samples of human tissues with a different grade of malignant formation.	The hypermethylated promoter gene in cervical cancer cells and epigenetic alterations of the VIM gene are associated with the development of cancer cells as well as gene silencing.	Methylation of the vimentin promoter gene (VIM)

CIN: cervical intraepithelial neoplasia; PCR: polymerase chain reaction; CpG: phosphate-cytosine-guanine; hrHPV: high-risk human papillomavirus.

by genetic and epigenetic changes[7]. According to the survey, we observe that some methylated genes have an important role in the development of cervical cancer, suggesting the benefits of using relevant biomarkers in the carcinogenic process. According to a study by Clarke et al.[10], the detection of methylated genes in cervical samples is technically feasible and it is a source to identify biomarkers for cervical carcinogenesis.

Therefore, there was an increase in information sources, with concatenated approaches. We expanded concepts and redefined them and thus, contributing to new proposals for the detection of cervical cancer.

The Papanicolaou test, also called colpocytology or Pap smear test, is considered a more appropriate, practical, and cost-effective instrument to screen cervical cancer. However, there is still a need to search for new alternatives for early detection of this type of tumor among women[11].

Considering the various epigenetic change in profiles, many researchers seek to propose new, more sensitive, and more specific alternatives for cervical cancer screening[7]. As a result, we understand that even with the evidence of the success of the program of cervical cancer control and with a substantial reduction in the incidence of cervical cancer, there are still few limitations. A frequent example of that is the inadequacy and poor quality of smears for the cytological analysis, which lead to increased costs and higher sensitivity when the collection is associated with the HPV test.

For Nye et al.[12], cytology screening for invasive cervical cancer (ICC) requires sensitivity and specificity to distinguish a process of persistent cervical intraepithelial neoplasia (CIN) from a regression process[12].

Cytology through the Papanicolaou method has good sensitivity and high specificity when used as a screening method. However, method sensitivity and specificity are reduced if analyzed in patients with cervical alterations[13]. In terms of prevention, HPV prophylactic vaccine currently offers the possibility of action at the primary level, because, thus far, prevention only occurred at secondary level.

In the field of epigenetics, for example, the study on other screening methods for cervical cancer is being conducted in order to detect the tumor in an early stage. In view of that context, it is clear that the issue is being addressed as a priority in recent years. We emphasize the finding of several studies aimed at reducing morbidity and mortality caused by cervical cancer.

The review by Termini and Villa[4], for example, stresses some of the main lines of research on biomarker identification and their possible use in the screening of cervical cancer and its precursor lesions. For that reason, there are many studies that seek to identify biomarkers associated with cervical neoplasia, which may be used to perform the screening of women who are carriers of HPV. This article only discusses the most outstanding biomarkers in scientific production.

Among the studies included in this review, we highlighted the use of possible biomarkers; some mention the hypermethylation of the p16(INK4A) gene. Carestiato et al.[14] indicated that the hypermethylation of the p16(INK4A) gene is an important cofactor of cervical carcinogenesis, eliminating the tumor suppressor function of the p16 protein in malignant lesions. Methylation was identified only in 10.7% of the normal epithelium samples, 22.9% of the low-grade lesions, 57.1% of the high-grade lesions, and 93.1% of the carcinomas ($p<0.0001$)[14].

There was an association between the completion of the p16(INK4A) gene and the infection by different types of HPV. The most prevalent type was HPV16 (37%), followed by HPV18 (16.3%), and HPV33/45 (15.2%). We found a correlation between methylation and HPV infection ($p<0.0001$), high-risk genotypes ($p=0.01$), high-grade lesions ($p<0.0007$), and cancer ($p<0.0001$)[14]. Considering such findings, we established a strong statistical association with cervical carcinoma.

Mirabello et al.[15] added that, from their age-stratified analysis, women who are above the average age of 28 years have an increased risk of developing precancerous lesions associated with high methylation. Therefore, high levels of HPV16 DNA methylation can be useful for the early diagnosis of precursor lesions of cervical cancer[15].

By demonstrating the detection of the overexpression of p16(INK4a) as an indirect test of the expression of E6/E7, which is used to confirm cervical neoplasia, Grce et al.[13] reported that the detection of viral oncogenes transcribed from E6/E7 as a marker of productive infection is a promising tool for the follow-up of women with HPV.

In addition to such findings, Nye et al.[12], studying 213 women diagnosed with CIN I, II, and III, outlined as the first evidence an important cofactor of the risk of developing invasive cancer; they stressed a 5% increase in the 5% DNA methylation of the PEG3 gene, which is associated with an increased 1.6-fold risk of cervical cancer and is useful as a biomarker.

With regard to the DAPK1 promoter gene, Xiong et al.[16], from a systematic review comprising 818 samples of tumor tissues and 671 samples of normal tissue, established the methylation frequency of the DAPK1 promoter gene, which ranged from 30.0 to 78.6% (median of 59.3%) in cervical cancer tissue and from 0.0 to 46.7% (median of 7.8%) in normal cervical tissue, indicating that DAPK1 methylation may be a biomarker during carcinogenesis and may serve as an early indication of cervical cancer[16].

The review by Barbaresco et al.[6] supplemented the data for the DAPK1 gene by the report that the hypermethylation of the DAPK1 gene was observed in 33.3% of CIN I cases, 50% of CIN III cases, and 71.4% of cervical samples. It was observed that methylated genes may have a significant role in the onset of cancer and that the methylation of some genes is associated with a more advanced stage of the disease. The authors consider that this information may be useful to predict cervical neoplasia, to prevent disease progression, and to be used as a tool in the disease treatment[6].

Yang[2] showed that the L1 gene of HPV16 and HPV18 is consistently hypermethylated in ICC and may be used as a marker in the clinical progression of this type of cancer.

Corroborating that study, Murakami et al.[17] introduced a research with 54 women with HPV type 52 and 41 women with HPV type 58. There was a hypomethylation in the long control region of HPV52/58; the methylation of the L1 gene of HPV52 had a correlation with the prognosis of CIN I and II, with a percentage mean of 15% and 35% for regression and persistence, respectively ($p < 0.05$). Moreover, the methylation state of the L1 gene of HPV58 had a correlation with the severity of cervical neoplasia, with a percentage mean of 12%, 38%, and 61% for CIN I, II, and III, respectively[17].

Johannsen and Lambert[18] summarized that in the case of HPV16, it is documented that the methylation status of the viral genome changes not only in the context of the viral life cycle but also in the context of the progressive neoplasia that culminates in cancer. They also specify the recent implementation of the methodologies of ChIP-seq and RNA-seq analysis to study cervical cancer, which offer a new opportunity to identify epigenetic markers for tumors with viral genomes and correlate such signs with the expression of viral genes in the context of neoplastic diseases caused by these viruses[18].

Louvanto et al.[19] examined the methylation status of selected loci in HPV16 and human genes in the DNA extracted from the cervical cell samples of the 244 women with cancer with HPV16 or CIN or who tested negative for intraepithelial lesion and malignancy. The methylation of the CpG regions of the L1 gene of HPV16 as well as of the EPB41L3 and LMX1 human genes was quantified. Methylation in all loci significantly increased according to the severity of the lesion ($p<0.0001$)[19].

Brebi et al.[20] also reported from their study that the methylation frequency of the ZAR1 and SFRP4 genes increased as the grade of the lesions increased, and the differences between normal and cervical cancer are statistically significant ($p<0.0001$). Therefore, the more severe the lesion, the greater the possibility of identifying DNA methylation.

There is, in addition, the research by Vasiljević et al.[21], which measured the methylation of 26 genes originating from the material obtained from two previous studies. That study highlighted the methylation of the EPB41L3 gene, which was significantly high in CIN, reporting it as a potential and significant diagnostic biomarker for CIN in high-grade HPV[21].

De Strooper et al.[22], assessing the marker FAM19A4 in the screening of women with high-risk HPV based on women with CIN I and III, found that all carcinomas and advanced intraepithelial neoplasias had methylation of the FAM19A4 gene in comparison with 42.1% of CIN II and III of early lesions. Furthermore, Steenbergen et al.[23] assessed the increase in methylation levels for six genes, that is, FAM19A4, LHX1, NKX2-8, NPTX-1, PHACTR3, and PRDM14, with disease progression in cervical tissue samples. All six methylated genes frequently occurred in cervical carcinomas; however, they assessed higher frequencies of up to 100% for FAM19A4, PHACTR3, and PRDM14[23].

Regarding the LDOC1 tumor suppressor gene, Buchholtz et al.[24] concluded that after the study of four of the six cervical cancer cell lines, the LDOC1 expression was silenced. Gene methylation analysis revealed a significant association between the methylation of the tumor promoter gene, indicating that LDOC1 silencing is a frequent event in cervical cancer and may be of interest as a molecular marker for it[24].

Van der Meide et al.[25] presented new biomarkers from the Wnt/β-catenin protein, which are activated during the carcinogenesis of cervical cancer. After the analysis of samples of cervical tissue to assess the diagnostic potential of more methylated genes, the study established the frequency of DKK3 and SFRP2 methylation, which was significantly higher in adenocarcinomas in comparison with squamous cell carcinomas, that is, 82% against 18% ($p < 0.01$) and 84% against 39% ($p < 0.01$), respectively. That fact reveals promising screening biomarkers for women who are carriers of HPV[25]. Zuo et al.[26], after assessing the suitability of HS3ST2 and CCNA1 genes as biomarkers for early detection of cervical cancer, found that hypermethylated genes were correlated with HPV16 and HPV18 infection in high-grade lesions and cervical cancer (both at <0.05). They also reported that the expression of the HS3ST2 and CCNA1 genes was lower in cervical tissues with positive methylation than in cervical tissues with negative methylation[26].

It is important to outline that CCNA1 methylation is a marker that can distinguish negative, low-grade, and high-grade results through cervical cytology samples. Results of a study by Chujan et al.[27] showed that the methylation frequencies of the promoter gene were 0.00, 5.88, and 83.33%, whereas the high-risk HPV percentages were

66.67, 82.35 and 100.00% in the negative, low-grade, and high-grade groups, respectively[27].

Some studies have addressed the methylation of the VIM promoter gene (vimentin). Jung et al.[28] investigated the correlation between the methylation levels of the VIM promoter gene and the effect of methylation in the expression of the VIM gene (vimentin) during the development of cervical cancer.

Vimentin is a protein-coding gene, known for being associated with several biological processes, including cell maintenance as well as cytoskeletal interactions and stabilization. Methylation of the VIM promoter gene appears in CIN I and II, during relatively early stages of carcinogenesis. Therefore, methylation of the VIM promoter gene is suggested as an effective biomarker for cervical diagnosis.

Some studies also indicate new treatment perspectives for cervical cancer through molecular events. That is exemplified by Nogueira-Rodrigues and Melo[29], who presented an opinion piece while studying the search for molecular alterations to find new therapeutic strategies for the group of tumors with HPV-dependent molecular signature. These changes, which are influenced by HPV infection, have been studied as cellular targets for the development of new treatment technologies. These examples demonstrate that there is the commitment not only to find innovative ways to achieve better results in early detection of cervical cancer, but also to research on new therapeutic perspectives for cervical cancer.

CONCLUSION

This literature review is a foundation for conducting further studies on new perspectives for the early detection of cervical cancer precursor lesions and cervical cancer. Considering the strategies for the control of cervical cancer, such as HPV vaccination for prevention and oncologic colpocytology for early detection of precursor lesions, technological advances may be used, reducing the number of cases of the disease in women.

In that scenario, this study establishes that most of the research reports that methylation begins in the early stages of the carcinogenic process. We observed an increase in the methylation pattern as the lesion progresses. A variety of different markers were analyzed from several studies and the most important criterion for the potentiality of a biomarker is its reliability.

Therefore, from this broader view, the relevance of the knowledge and discoveries from studies being conducted worldwide is proved, as they seek new alternatives to benefit the female population with additional care toward their health. Generally, having a Pap smear test proves insufficient. The proposal of complementary strategies for the detection of premalignant changes and cervical cancer is necessary. Despite the advances and discoveries in the field of epigenetics, there are still gaps in the knowledge about the relationship of DNA methylation in HPV and in the host, which need to be clarified.

Factors associated with the complex process of cervical carcinogenesis and new proposals for the detection of cervical cancer through biomarkers will enable the monitoring of such molecular events, redefining and complementing the program of cervical cancer control, thus reducing the morbidity and mortality caused by the disease in the female population.

Therefore, we stress the importance on studies that analyze the topic, to aid the reduction of the incidence of cervical cancer, and to introduce the concepts and definitions that lead to the implementation of preventive actions for this type of tumor in women.

Conflict of interests

The authors report no conflict of interests.

REFERENCES

1. Wright ML, Ralph JL, Ohm JE, Anderson CM. DNA methylation in complex disease: applications in nursing research, practice, and policy. Nurs Outlook. 2013 Jul-Aug;61(4):235-241.e4.
2. Yang HJ. Aberrant DNA methylation in cervical carcinogenesis. Chin J Cancer. 2013;32(1):42-8.
3. Instituto Nacional do Cnacer José Alencar Gomes Silva. Coordenação de prevenção e Vigilancia. Estimativa Incidencia de Câncer no Brasil - 2014. Rio de Janeiro; 2014;124. Disponível em: http://www.inca.gov.br/estimativa/2014/estimativa-24042014.pdf Acesso em: 18/03/2015.
4. Termini L, Villa LL. Biomarcadores na triagem do câncer do colo uterino. DST – J bras Doenças Sex Transm. 2008;20(2):125-31.
5. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. Genes Dev. 2009;23(7):781-3.
6. Barbaresco AA, Freitas-junior R, Michelin MA, Murta EFC. Metilação aberrante de DNA, câncer cervical e HPV. Femina. 2012;40(5):247-52
7. Saavedra KP, Brebi PM, Roa JCS. Epigenetic alterations in preneoplastic and neoplastic lesions of the cervix. Clin Epigenetics. 2012;4(1):13.
8. Wentzensen N, Bergeron C, Cas F, Eschenbach D, Vinokurova S, von Knebel Doeberitz M. Evaluation of a nuclear score for p16INK4a-stained cervical squamous cells in liquid-based cytology samples. Cancer. 2005;105(6):461-7.
9. Lodi CT da C, Michelin MA, Murta EFC, Lima MIM, Melo VH. Metilação genética, neoplasia intraepitelial cervical e câncer do colo uterino. Femina. 2012;40(5):287-93.
10. Clarke MA, Wentzensen N, Mirabello L, Ghosh A, Wacholder S, Harari A, et al. Human Papillomavirus DNA Methylation as a Potential Biomarker for Cervical Cancer. Cancer Epidemiol Biomarkers Prev. 2012;21(12):2125-37.
11. Kitchener HC, Castle PE, Cox JT. Chapter 7: Achievements and limitations of cervical cytology screening. Vaccine. 2006;24 Suppl 3:S3/63-70.
12. Nye MD, Hoyo C, Huang Z, Vidal AC, Wang F, Overcash F, et al. Associations between Methylation of Paternally Expressed Gene 3 (PEG3), Cervical Intraepithelial Neoplasia and Invasive Cervical Cancer. PLoS One. 2013;8(2):e56325.
13. Grce M, Matovina M, Milutin-Gasperov N, Sabol I. Advances in cervical cancer control and future perspectives. Coll Antropol. 2010;34(2):731-6.
14. Carestiato FN, Afonso LA, Moysés N, Almeida Filho GL, Velarde LGC, Cavalcanti SMB. An upward trend in DNA p16ink4a methylation pattern and high risk HPV infection according to the severity of the cervical lesion. Rev Inst Med Trop Sao Paulo. 2013;55(5):329-34.
15. Mirabello L, Schiffman M, Ghosh A, Rodriguez AC, Vasiljevic N, Wentzensen N, et al. Elevated methylation of HPV16 DNA is associated with the development of high grade cervical intraepithelial neoplasia. 2014;132(6):1412-22.
16. Xiong J, Li Y, Huang K, Lu M, Shi H, Ma L, et al. Association between DAPK1 promoter methylation and cervical cancer: a meta-analysis. PLoS One. 2014;9(9):e107272.
17. Murakami I, Fujii T, Dan K, Saito M, Ohno A, Iwata T, et al. Methylation of human papillomavirus-52 and -58 is a candidate biomarker in cervical neoplasia. J Clin Virol. 2013;58(1):149-54.
18. Johannsen E, Lambert PF. Epigenetics of Human Papillomaviruses. Virology. 2014;445(1-2):205-12.
19. Louvanto K, Franco EL, Ramanakumar AV, Vasiljević N, Scibior-Bentkowska D, Koushik A, et al. Methylation of viral and host genes and severity of cervical lesions associated with human papillomavirus type 16. Int J Cancer. 2015;136(6):E638-45.

20. Brebi P, Hoffstetter R, Andana A, Ili CG, Saavedra K, Viscarra T, et al. Evaluation of ZAR1 and SFRP4 methylation status as potentials biomarkers for diagnosis in cervical cancer: exploratory study phase I. Biomarkers. 2014;19(3):181-8.
21. Vasiljević N, Scibior-Bentkowska D, Brentnall AR, Cuzick J, Lorincz AT. Credentialing of DNA methylation assays for human genes as diagnostic biomarkers of cervical intraepithelial neoplasia in high-risk HPV positive women. Gynecol Oncol. 2014;132(3):709-14.
22. De Strooper LM, Meijer CJLM, Berkhof J, Hesselink AT, Snijders PJF, Steenbergen RDM, et al. Methylation analysis of the FAM19A4 gene in cervical scrapes is highly efficient in detecting cervical carcinomas and advanced CIN2/3 lesions. Cancer Prev Res (Phila). 2014;7(12):1251-7.
23. Steenbergen RDM, Ongenaert M, Snellenberg S, Trooskens G, van der Meide WF, Pandey D, et al. Methylation-specific digital karyotyping of HPV16E6E7-expressing human keratinocytes identifies novel methylation events in cervical carcinogenesis. J Pathol. 2013;231(1):53-62.
24. Buchholtz M-L, Jückstock J, Weber E, Mylonas I, Dian D, Brüning A. Loss of LDOC1 expression by promoter methylation in cervical cancer cells. Cancer Invest. 2013;31(9):571-7.
25. Van der Meide WF, Snellenberg S, Meijer CJLM, Baalbergen A, Helmerhorst TJM, van der Sluis WB, et al. Promoter methylation analysis of WNT/β-catenin signaling pathway regulators to detect adenocarcinoma or its precursor lesion of the cervix. Gynecol Oncol. 2011;123(1):116-22.
26. Zuo Q, Zheng W, Zhang J, Pan Z, Liu Y, Long H, et al. Methylation in the promoters of HS3ST2 and CCNA1 genes is associated with cervical cancer in Uygur women in Xinjiang. Int J Biol Markers. 2014;29(4):e354-62.
27. Chujan S, Kitkumthorn N, Siriangkul S, Mutirangura A. CCNA1 Promoter Methylation: a Potential Marker for Grading Papanicolaou Smear Cervical Squamous Intraepithelial Lesions. Asian Pacific J Cancer Prev. 2014;15(18):7971-5.
28. Jung S, Yi L, Kim J, Jeong D, Oh T, Kim C-H, et al. The role of vimentin as a methylation biomarker for early diagnosis of cervical cancer. Mol Cells. 2011;31(5):405-11.
29. Rodrigues AN, Melo AC. Perspectivas no Tratamento do Câncer do colo do Útero: Explorando o Bloqueio da Sinalização Celular. Rev bras cancerol. 2012;58(3):529-32.

Correspondence address:
MARIA CRISTINA DE MELO PESSANHA CARVALHO
Avenida Nossa senhora de Copacabana, 827, apto. 402 – Copacabana
Rio de Janeiro (RJ), Brazil
Zip Code: 22050-002
Tel.: +55 (21) 99404-1317
E-mail: mcrismelo4@hotmail.com

Received on: 6.24.2015
Accepted on: 12.15.2015