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REVIEW ARTICLE

Epigenetic biomarkers in cancer diagnosis: advances and challenges in laboratory medicine

Biomarcadores epigenéticos en el diagnóstico del cáncer: avances y desafíos en la medicina de laboratorio

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ABSTRACT

Introduction: epigenetic biomarkers allow us to add a system of modifications to DNA within the information of genes, which will contribute to whether or not the same gene is expressed in different tissues or cells of the organism.
Objective: define the behavior of epigenetic biomarkers in cancer diagnosis in terms of advances and challenges in laboratory medicine.
Methods: a search was conducted in databases such as PubMed, ScienceDirect, and Scielo for articles published from January 1, 2023, to October 10, 2024. The PRISMA framework was used to systematize this selection. Finally, 19 articles were included.
Development: DNA methylation is the most important epigenetic mark due to its versatility in analyzing DNA regions. Regarding technology, epigenetic software has been developed that facilitates the location of the methylation site. Through the analysis of this study, it has been determined that the concentration of the β-HCG hormone shows a direct relationship with the aggressiveness of the cancer, which is why it is used as a tumor biomarker in various types of cancer. With these precedents, it is anticipated that epigenetic biomarkers will provide more information about the progression and metastasis of the cancer in which they are applied.
Conclusions: future studies on their role in other cancers will provide further insight into the role of epigenetic biomarkers in cancer progression and metastasis.

Keywords: Biomarkers; Epigenomics; Laboratory; Health; Methylation.

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RESUMEN

Introducción: los biomarcadores epigenéticos permiten añadir un sistema de modificaciones en el ADN dentro de la información de los genes, los mismo que van a contribuir a que un mismo gen se exprese o no en diferentes tejidos o células del organismo.
Objetivo: definir el comportamiento de los biomarcadores epigenéticos en el diagnóstico del cáncer en cuanto a avances y desafíos en la medicina de laboratorio.
Métodos: se hizo una búsqueda en bases de datos como Pubmed, ScienceDirect y Scielo de artículos publicados desde el 01 de enero de 2023 hasta el 10 de octubre de 2024. Se utilizó el esquema PRISMA para sistematizar esta elección. Finalmente se incluyeron 19 artículos.
Desarrollo: la metilación del ADN, es la marca epigenética más importante debido a su versatilidad de uso para analizar regiones de ADN. En cuanto a la tecnología, se ha implementado el desarrollo de un software epigenético que facilita la ubicación del sitio de metilación. Mediante el análisis de este estudio se ha determinado que la concentración de la hormona β-HCG, muestra una relación directa con la agresividad del cáncer, por lo cual se utiliza la misma como un biomarcador tumoral en varios tipos de cáncer. Con estos precedentes, se prevéquelos biomarcadores epigenéticos darán más información sobre la progresión y metástasis del cáncer en el que estos sean aplicados.
Conclusiones: los estudios futuros sobre su función en otros cánceres proporcionarán más información sobre el papel de los biomarcadores epigenéticos en la progresión y la metástasis del cáncer.

Palabras Clave: Biomarcadores; Epigenética; Laboratorio, Salud; Metilación.

INTRODUCTION

Epigenetic alterations, especially DNA methylation, are fundamental in the pathogenesis of cancer and can manifest in early stages, anticipating classic tumor-transforming events. Among the alterations recognized in tumors, silencing byhypermethylationof islandsCpGin promoters of suppressor genes. DNA methylation, which is widely studied, is analyzed using a variety of techniques, from restriction enzymes to high-throughput sequencing. These epigenetic changes offer promising therapeutic targets for cancer diagnosis and treatment, and also have potential as biomarkers.

Among the most recognized epigenetic alterations in tumors is silencing associated withhypermethylationof islandsCpGin the promoters of suppressor genes such as CDKN2A and RASSF1. In addition to this, the can also act as suppressors or oncogenes in different types of cancer. This is whythat epigenetic modifications are an important component in the etiology of cancer and due to their reversibility they constitute promising therapeutic targets for diagnosis or treatment and potential as possible biomarkers.[1]

With the development of the Human Genome Project, information on the structural organization of all the DNA sequences in our genome was completed. The genome consists of approximately 25,000 genes, many of which have a wide range of alleles. Alterations in many of these genes cause certain diseases, the compilation and description of which can be consulted in the OMIM (Online Mendelian Inheritance in Man) database, managed by Dr. Victor A. McKusick of the Johns Hopkins University School of Medicine in Baltimore.[2]

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(2025): e6789

Therefore, it has become necessary to develop new analytical tests to identify epigenetic changes through molecular biomarkers related to the active or silenced state of the regions where the candidate genes are located and thus facilitate the diagnosis of pathologies due to these modifications and the adoption of treatment strategies.[3]

In this direction, numerous investigations and trials are being carried out in order to detect changes in the methylation of cytosines, the acetylation of histones or the presence of micro-RNAs, related to certain pathologies, whose manifestation can be revealed prior to the emergence of the disease, including cancer in general.[4]

The importance of biomarkers in the biomedical field for the development of technologies that allow for the early diagnosis and treatment of diseases such as cancer. Three of the cancer biomarkers that have proven effective for treatment are highlighted: prostate-specific antigen (PSA), which is of utmost importance in determining prostate cancer recurrences and evaluating response to treatment; microRNA (miRNA), which is altered in cells;carcinogenic, and the hormone β-HCG.[5]

The latest advances in cancer pathogenesis and novel therapies are examined, revealing the importance of scientific research in the region and its contribution to oncological knowledge. The results highlight the identification of new therapeutic targets, a greater understanding of molecular mechanisms, and the identification of potential biomarkers.[6]

Given the importance of the topic, the objective of this research is to define the behavior of epigenetic biomarkers in cancer diagnosis in terms of advances and challenges in laboratory medicine.

METHODS

A search was made in databases such as Pubmed, ScienceDirect and Scielo with the following keywords and connectorsbiomarkers; epigenetics; laboratory, health; methylation. " of articles published from January 1, 2023 to October 10, 2024. 1044 articles related to the topic were collected, of which an evaluation was made and 400 were discarded for not meeting the objectives of the topic, 349 for not having open access, and 257 for not meeting the inclusion criteria. The PRISMA scheme was used to systematize this choice. Finally, 19 articles were included, from which information on the introduction, development, and conclusions was collected. The studies included are review articles, observational studies, meta-analyses, and clinical trials.

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(2025): e6789

DEVELOPMENT

DNA methylation

DNA methylation occurs predominantly in gene-rich regions of the genome, including satellite DNA and the two main types of dispersed repeated sequences, short ones, called Alu or SINES (Short Interspersed Transposable Elements), of 100-300 bp, and long ones, called L1 or LINES (Long Interspersed Trans-posable Elements), of 6,000 to 8,000 bp. Alu and L1 sequences correspond to a type of retrotransposons lacking the long terminal regions or LTRs, present at the ends of other mobile elements.[7]

Instead, they have one end that resembles the typical poly-adenine tails of gene transcripts after processing, due to the abundance of AT base pairs. In the human genome, it is estimated that there are about 50,000 copies of Ll sequences per haploid complement, which represents between 3,5 % and 13,5 % of the total genome mass (640 Mb in the total genome). In addition, there are more than 2,000,000 copies of Alu elements dispersed throughout all the chromosomes, which represents 20 % of the total genome mass (420 Mb in the total human genome). Alu elements tend to be found in regions rich in GC base pairs and LI elements are preferentially located in regions rich in AT.[7]

In the vicinity of the genes of vertebrate genomes, there are regions known as "CpG islands", which are located in cis position, upstream of the 5' region of the promoters and which constitute the areas of action of the activators (enhancers) and silencers of expression. These regions also extend through the UTR (non-translatable transcribed) regions and exon 1 and correspond to sequences of more than 500 bp, with a GC content = 55 %, and a proportion of CpG observed with respect toto the expected value of = 0,65 (15). CpG islands can be used for thebsearch and annotation of genes in the genomic studies of new species, as it constitutes an indicator of the presence of coding regions and given its distinction from pseudogenes, which are inactive genes due to deterioration of their sequences.[8]

- **DNA methylation process**

Epigenetic modification of the DNA molecule occurs by the enzymatic addition of a methyl group to carbon 5 of cytosine. Most 5-methylcytosines (5mC) in mammalian DNA are present at -CpG-35' dinucleotides.[8]

- **Establishment of methylation patterns**

A prerequisite for understanding the functions of DNA methylation is the recognition of heritable patterns of methylation status in the genome. Methylation patterns in somatic cells are generally stable and heritable; however, they are extensively reprogrammed in germ cells and during early embryonic development, with de novo methylation being particularly active at these stages.[9]

In general, the genome of female germ cells is less methylated than that of male cells. The methylation pattern of gametes is erased by widespread demethylation near the cellular stage. Thereafter, DNA methylation acquires specific patterns during embryonic development, and the methylation pattern of somatic cells is established.[9]

- **Cellular methylation machinery**

DNA methylation is a dynamic, efficiently regulated process; unmethylated sequences can be methylated and methyl groups can be lost, so the methylation patterns of somatic cells are the result of both methylation and demethylation activities.

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(2025): e6789

The DNA methylation reaction is catalyzed by DNA methyltransferases and involves the transfer of the methyl group from S-adenosyl-L-methionine to carbon 5 of cytosine. In mammalian cells, three different enzymes that carry out this reaction have been identified and classified into two groups: maintenance DNA methyltransferases (DNMT1) and de novo methylases (DNMT3A and DNMT3B).[10]

The action of maintenance DNA methyltransferases causes DNA to be methylated at the start of replication. Only the newly formed strand is methylated, and for this reason, the patterns are inherited in a semiconservative manner and can be perpetuated in the cell population. In mice, DNMT1 has a five- to 30-fold preference for hemimethylated substrates, so it has been assigned a role in maintaining methylation patterns. This enzyme is ubiquitously expressed in somatic tissues, and its main activity is observed during DNA replication, interacting with proliferating cell nuclear antigen (PCNA), the protein that anchors DNA polymerase at the replication fork.[11]

- **Functions of methylation**

DNA methylation is an epigenetic marker that identifies the template strand during DNA replication and the parental origin of imprinted regions. It regulates transposons, genomic imprinting, and gene expression. Methylation in non-coding regions, such as centromeric heterochromatin, appears to be crucial for maintaining chromosome conformation and integrity. Methylation also constitutes a defense mechanism of the genome against mobile genetic elements.[12]

- **Transcriptional repression**

There are two mechanisms by which methylation blocks transcription. 5mC inhibits the binding of certain transcription factors that contain CpG sequences in their recognition sites. For example, it modifies the binding activities of transcription factors such as E2F, CREB, AP2, cMyc/Myn, and NFkB. The other mechanism is more general and involves proteins or protein complexes that specifically bind to methylated CpGs and indirectly block the binding of transcription factors by limiting their access to regulatory elements. These proteins contain conserved methylated DNA-binding domains (MBDs).[13]

Generally, regions of genetic activity, CpG islands, are unmethylated, and genes are primed for expression by transcription activators. In contrast, when DNA methylation occurs in these regions, gene transcription is directly repressed, both by inhibiting the binding of transcription factors and indirectly by recruiting binding proteins to methylated CpG sites. This results in chromatin remodeling associated with repression of gene activity. Little is known about how DNA methylation is directed to specific regions, although the molecular mechanism is thought to involve interactions between DNA methyltransferases and one or more chromatin-associated proteins.[14]

Table 1 records the incidence of the application of epigenetic mechanisms in normal and altered functions.

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(2025): e6789

Table 1. Incidence of epigenetic mechanisms and their consequences.

Normal functions regulated by epigenetic mechanisms		Altered functions regulated by epigenetic mechanisms	
Chromatin organization	Control of active or silenced states of genes in somatic embryonic cells	DNA hypermethylation	Causes chromatin condensation and gene silencing
DNA-specific methylation and histone modifications	Control of gene-specific and tissue-specific epigenetic patterns	DNA hypomethylation	Activation of oncogenes; mobilization of transposable elements, resulting in chromosome instability
Silencing of repeated elements	Correct chromatin arrangement and maintenance of expression patterns	Mutations in menthylated cytosines	Alterations in gene expression
Genomic imprinting	Essential for development	Imprint defects	Loss of parental identity
Inactivation of an X chromosome in female mammals (lyonization)	Gene dosage compensation between males and females		

Note. Information obtained from the study "DNA hypomethylation can activate Xist expression and silence X-linked genes.
Fountain: Panning & Jaenisch,[14]

Certain microRNAs (miRNAs), primarily those that silence tumor suppressor genes and are present in higher copy numbers, have also been shown to promote oncogenesis. Multiple patterns of these epigenetic factors occur specifically in certain malignancies, allowing for their potential use as biomarkers. This review, presented in Table 2, presents examples of tests for each group of epigenetic factors that are currently available or under development for use in the early detection, prediction, prognosis, and treatment response of cancer. The availability of blood-based biomarkers is highlighted, as they allow for reduced invasiveness of sampling and simplify the sampling procedure. The article highlights the role of epigenetics as a crucial element of future cancer diagnosis and therapy as a promising option for detection.[15]

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(2025): e6789

Table 2. Methylation: prognostic biomarkers and predictions with diagnostic utility.

Methylation	Forecast	Prediction	Invasive/non-invasive diagnosis	Biological material	Cancer indicator
*MLH1*Hypomethylation	+	-	Invasive	FFPE	Colon
MGMT Hypermethylation	+	+	Invasive	FFPE	Glioblastoma
*1DH1*p.R132H and MGMT hypermethylation	+	-	Invasive	FFPE	Glioblastoma
*RB1*Hypermethylation	+	-	Invasive	FFPE	Retinoblastoma
*GSTP1, RASSF1, APC*in a state of methylation	+	-	Invasive	FFPE	Prostate
SEPT9	+	-	Non-invasive	Blood	Colon
MGMT-STP27	-	+	Invasive	FFPE	Oligodendrogliomas and oligoastrocytomas
ESR1	-	+	Non-invasive	Blood	Lung
ZNF331	+	-	Invasive	FFPE	Colon
SALL1	-	+	Invasive	FFPE	Head and neck

Note. Information obtained from the study "Prognostic and Predictive Epigenetic Biomarkers in Oncology**".**
FFPE formalin-fixed paraffin-embedded cancer tissue.
Fountain: Kamińska, K., et al.,[15]

The importance of studying the role of epigenetic biomarkers in cancer diagnosis, such as DNA methylation, is very relevant since recent research has allowed us to understand the relationship between DNA methylation and the modification of the histone machinery, also demonstrating the potential participation of small RNA molecules generated by the RNA interference .[16]

Using the results presented in Table 1, it was determined how epigenetic mechanisms affect two areas, such as normal and altered functions. This information is supported by what has been presented in studies of epigenetic modifications of the genome that regulate many cellular processes: they facilitate the specific expression of the necessary genes of each tissue; they provide a robust mechanism for long-term genetic silencing of undesirable transcriptional activity of non-specific genes of each tissue, already from embryonic development; they contribute to the correct expression of an X chromosome in women, by inhibiting the activity of the second through the Lyonization process, which represents a compensatory mechanism of the gene dosage in relation to males who only have one X chromosome.[17]

Within the study, discovery and analysis of new biomarkers, it has been identified that the development and progression of cancer are determined by the dynamic interaction between the tumor and its host. Tumors can trigger an adaptive antitumor immune response and, as is the case in colorectal cancer, the Immunoscore (which measures the densities of CD3+ T cells and CD8+ cytotoxic T cells in the tumor center and the invasive margin) has emerged as a promising predictor of prognosis. In a large international study of more than 2600 patients, the prognostic value of the Immunoscore surpassed that of the TNM classification.[18]

ISSN: 1561-3194 **RNPS:** 1877
Rev Ciencias Médicas. 2025; 29(2025): e6789

CONCLUSIONS

Future studies on their role in other cancers will provide further insight into the role of epigenetic biomarkers in cancer progression and metastasis.

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Conflict of Interest
The authors declare that they have no conflicts of interest.

Authorship Contribution
ÁPMV: conceptualization, data curation, formal analysis, research
VMOV: methodology, project management, software
DCChT: supervision, validation, visualization
JAMC: draft-original writing

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