

Review Article

Aberrant DNA Methylation in Breast Cancer Cells

Emanuel Martín Campoy Sergio Laurito Guillermo Urrutia
María Teresita Branham María Roqué

Laboratory of Cellular and Molecular Biology, IHEM-CCT-CONICET, Mendoza, Argentina

Key Words

Aberrant DNA methylation · Breast cancer · Cancer epigenetics · Tumoral heterogeneity · Tumor environment

Abstract

The epigenome is regulated by a large number of macromolecular machines that are dynamically involved in various processes, including DNA methylation, histone modification and non-coding RNA signals, all of them working together to regulate the proper expression of the genome. Thus, in contrast with the genome, whose sequence is carefully conserved during cell life, the epigenome is highly dynamic. The epigenomic modifications are acquired during normal cell differentiation, replicated during mitosis and passed to daughter cells. A fundamental epigenetic attribute is that this plasticity occurs in response to environmental signals. It is therefore now accepted that the environment influences modifications in the cellular transcriptome through the epigenome. In developmental and evolutionary terms, the regulation of gene expression through epigenomic modifications is an advantageous shortcut and a highly conserved mechanism. However, it implies an increased risk for misregulation, as, for example, aberrant epigenomic modifications associate with the development of different human diseases, i.e. lupus, asthma, neurological diseases and cancer. Although epigenetic alterations in breast cancer have been deeply studied and discussed in the last decades, apparently contradictory results are yet often observed. Consequently, in this review, we will briefly discuss the latest findings of aberrant DNA methylation in breast tumorigenesis. Emphasis will be given to the discussion of the idea that different environments could explain paradoxical biological and pathobiological behaviors in individual patients and thus should be taken into consideration for the design and implementation of diagnosis, prognosis and predictive biomarkers.

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María Roqué
Laboratory of Cellular and Molecular Biology
IHEM-CCT-CONICET
Mendoza 5500 (Argentina)
E-Mail mroque@mendoza-conicet.gob.ar

Introduction

Aberrant DNA Methylation in Breast Cancer

Breast cancer is still the most common malignancy among women in western countries [1]. During the development of breast cancer, a variety of genetic alterations occur, including point mutations, copy number variations, chromosomal rearrangements and aneuploidies. An explosion of data has revealed that these genetic alterations are accompanied by epigenetic changes, which cooperate specially in either the silencing or activation of cell cycle regulatory genes. Global hypomethylation of DNA as well as gene-specific hypermethylation leads gradually to the acquirement of the cancer cell hallmarks well defined by Hanahan and Weinberg [2]. Although aberrant methylation is a common feature across many types of cancers and the last mentioned hallmarks are shared by almost all solid tumors, there seem to exist epigenetic marks most often found in distinct types of tumors, e.g. the hypermethylation of RASSF1A in breast cancer [3], Rb in retinoblastoma [4], p16 in melanoma [5], VHL in renal carcinoma [6] and APC in colon cancer [7]. These observations have led investigators to propose subsets of aberrant methylated genomic regions as epigenetic tumor markers. Prognostic markers for breast cancer are of high interest because of the wide variety in outcome of the disease. Aberrant methylation of specific genes has been associated with prognosis [8], response to treatment [9], survival [10], lymph node affection [11] and general outcome [12] in breast cancer. Based on this observation, many efforts have been made over the last few years to establish prognostic, predictive and monitoring markers for breast cancer, including the detection of epigenetically marked, circulating tumor DNA [13, 14]. The aberrant methylation of RASSF1 could help to define patients' outcomes [8] and to detect the early stages of the disease in patients' serum [15, 16]. Thus, the prediction that methylation changes would become a powerful diagnostic tool is becoming a reality [17].

Why Is There Controversial Information about DNA Methylation in Cancer?

DNA hypermethylation is a frequent alteration in cancer cells, which is known to induce silencing of cell cycle regulator genes [18]. An enormous number of studies in the last decade have reported how aberrantly methylated genes are associated with different tumorigenic processes [15, 19, 20]. However, some controversial observations have appeared regarding the observed methylation frequencies in distinct genes and their transcriptional impact. When considering breast cancer, for example, some authors report methylation [11, 21] with reduced expression of WT1 [21], while others communicate overexpression of this gene in the same tumor types [22]. Interestingly, some publications indicate methylation frequencies in this gene, which are highly different from those communicated by authors in other geographical regions [23]. Likely, various reasons may explain these apparent controversies, some of which we would like to subsequently consider. First, not all CpG islands have an impact on gene expression. Although most transcriptional regulatory regions are located around the transcription start sites, many times, the crucial regions within a CpG island are localized down or upstream of the transcription start sites [17]. Therefore, it is fundamental to consider the *topology* of the genomic region on which experimental measurements have been performed. Second, tumors are highly heterogeneous. The tumorigenic process starts with a single cell, but during progression, diverse cell populations arise, carrying genetic and epigenetic differences on which selection acts in a similar manner as in evolutionary processes [24]. So, when a tumor sample is epigenetically studied, the information could be biased by the portion of tissue under study, a phenomenon commonly known as tumor sampling bias. Finally, not many authors take the impact of their patients' environment on their epigenetic

tumor profiles into consideration. Moelans et al. [25] found in breast cancer invasive ductal carcinomas of Dutch patients that MSH6 was the most frequently methylated gene. By using the same methodology and studying the same CpG islands on invasive ductal carcinomas of Argentinian patients, our group detected WT1 as the most frequently methylated gene [11]. Thus, in epigenetic studies, the environments (i.e. diet, ethnicity and lifestyle) of populations should be recognized as likely differential contributors to the epigenetic differences that give rise to variant molecular, cellular and functional phenotypic features.

Role of Environment in DNA Methylation

The incidence of breast cancers is rising among premenopausal women, presenting more aggressive tumor types and worse response [26]. The World Health Organization (WHO) has identified factors that could explain the differences in breast cancer incidence in different countries, some of them interestingly related to nutrition (e.g. obesity, alcohol consumption, birth weight and height) [26]. The biological fundament of these environmental influences relays on two concepts: (1) transcriptional responses to particular nutrients are known to differ from one individual to another, depending on the cellular epigenomic profile, and (2) diverse nutrients can induce the writing, reading and erasing of different epigenetic marks, which consequently generate alternative transcriptomes [27, 28]. Besides nutrition, an increasing number of studies have been revealing the influence of stress [28], exercise [29] and air pollution [30] on an individual's epigenome and transcriptome. Research on epigenetics of monozygotic twins shows how time increases the differences between their methylation profiles [31], supporting the concept that epigenetics act as an interface between environment and the individual phenotype. The proposal which challenges the classical Darwinian paradigms of inheritance and evolution is that these changes could be inherited in a transgenerational manner [32]. On the other hand, the evidence supports Jean-Baptiste Lamarck's paradigm, which proposes that changes acquired during the life of an organism are transmitted to the offspring. This theory was proposed contemporaneously to Darwin's postulates on evolution but did not reach strong widespread popularity until the birth of modern epigenetics. Therefore, environmental events that impact on the transcriptome of an individual could be transferred to the offspring, who are no longer exposed to the external factor.

Role of DNA Methylation in Breast Cancer Heterogeneity

Human breast cancer is considered a heterogeneous disease, with different outcomes and individual responses to treatments likely due to variations in gene expression profiles [33]. There are evident differences among the incidence rates of these cancers in distinct continents, with an apparent increase in incidence in high-income countries [34, 35]. In addition, within patient subgroups that share the same tumor type, one finds differences in morbidity and mortality. It has been proposed that each tumor presents a unique epigenetic signature [11]. Thus, the differences are not only restricted to occur between populations of different geographic regions, but also among individuals of the same population, which supports the validity of the new paradigm of individualized medicine. These observations urge both researchers and practitioners to consider whether candidate methylation markers for breast cancer diagnosis, prognosis and/or treatment response prediction, found in individual studies or distinct patient series, could be extrapolated as a general feature to all breast cancer patients and how they can be validated in specific populations.

Concluding Remarks

Breast cancer is a heterogeneous disease, which has a better outcome upon early diagnosis and when specific therapeutic targets are identified. An increasing number of studies are contributing candidate epigenetic markers for diagnosis, prognosis and prediction of disease outcome. However, emerging evidence also proposes an important role of the external environment in influencing the epigenomic landscape of individual breast tumors. This could explain the broad variation observed in incidence rates, survival outcome, aggressiveness and response to treatments. Thus, association of methylation marks with clinicopathological features should be evaluated with caution. In particular, it is critical to define which CpG islands are under study and if these marks have a real impact on the tumor transcriptome. Otherwise, wrong associations can contribute to the misinterpretation of epigenetic differences. Furthermore, when epigenetic markers are proposed, it becomes important to also define the patient population on which the studies were performed, since, in the highly diverse world we live in in the 21st century, most large-scale research datasets mainly originate from studies performed in high-income countries and may not be extrapolated to other nations with different ethnic and economic backgrounds. Additional misinterpretations could also be generated if those observations are extrapolated to the general breast cancer population rather than considering their application in a setting of individualized diagnostics and therapeutics. Thus, as the field of epigenetics continues fueling studies in breast and other cancers, emphasis on these variations will be key to its success as a useful tool toward understanding and treating this disease.

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References

- 1 Widschwendter M, Jones PA: DNA methylation and breast carcinogenesis. *Oncogene* 2002;21:5462–5482.
- 2 Hanahan D, Weinberg RA: Hallmarks of cancer: the next generation. *Cell* 2011;144:646–674.
- 3 Dammann R, Yang G, Pfeifer GP: Hypermethylation of the CpG island of Ras association domain family 1A (RASSF1A), a putative tumor suppressor gene from the 3p21.3 locus, occurs in a large percentage of human breast cancers. *Cancer Res* 2001;61:3105–3109.
- 4 Sakai T, et al: Allele-specific hypermethylation of the retinoblastoma tumor-suppressor gene. *Am J Hum Genet* 1991;48:880–888.
- 5 Gonzalez-Zulueta M, et al: Methylation of the 5' CpG island of the p16/CDKN2 tumor suppressor gene in normal and transformed human tissues correlates with gene silencing. *Cancer Res* 1995;55:4531–4535.
- 6 Herman JG, et al: Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc Natl Acad Sci USA* 1994;91:9700–9704.
- 7 Esteller M, et al: Analysis of adenomatous polyposis coli promoter hypermethylation in human cancer. *Cancer Res* 2000;60:4366–4371.
- 8 Jiang Y, Cui L, Chen WD, Shen SH, Ding LD: The prognostic role of RASSF1A promoter methylation in breast cancer: a meta-analysis of published data. *PLoS One* 2012;7:e36780.
- 9 Gil EY, et al: Promoter methylation of RASSF1A modulates the effect of the microtubule-targeting agent docetaxel in breast cancer. *Int J Oncol* 2012;41:611–620.
- 10 Ulirsch J, et al: Vimentin DNA methylation predicts survival in breast cancer. *Breast Cancer Res Treat* 2013;137:383–396.
- 11 Marzese DM, et al: DNA methylation index and methylation profile of invasive ductal breast tumors. *J Mol Diagn* 2012;14:613–622.

- 12 van Hoesel AQ, et al: Primary tumor classification according to methylation pattern is prognostic in patients with early stage ER-negative breast cancer. *Breast Cancer Res Treat* 2012;131:859–869.
- 13 Fiegl H, et al: Circulating tumor-specific DNA: a marker for monitoring efficacy of adjuvant therapy in cancer patients. *Cancer Res* 2005;65:1141–1145.
- 14 Muller HM, et al: DNA methylation in serum of breast cancer patients: an independent prognostic marker. *Cancer Res* 2003;63:7641–7645.
- 15 Jones PA, Baylin SB: The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002;3:415–428.
- 16 Dulaimi E, Hillinck J, Ibanez de Caceres I, Al-Saleem T, Cairns P: Tumor suppressor gene promoter hypermethylation in serum of breast cancer patients. *Clin Cancer Res* 2004;10:6189–6193.
- 17 van Vlodrop IJ, et al: Analysis of promoter CpG island hypermethylation in cancer: location, location, location! *Clin Cancer Res* 2011;17:4225–4231.
- 18 Balleara E, Esteller M: The impact of chromatin in human cancer: linking DNA methylation to gene silencing. *Carcinogenesis* 2002;23:1103–1109.
- 19 Laird PW: Cancer epigenetics. *Hum Mol Genet* 2005;14 Spec No 1:R65–R76.
- 20 Laird PW, Jaenisch R: The role of DNA methylation in cancer genetic and epigenetics. *Annu Rev Genet* 1996;30:441–464.
- 21 Huang TH, et al: Identification of DNA methylation markers for human breast carcinomas using the methylation-sensitive restriction fingerprinting technique. *Cancer Res* 1997;57:1030–1034.
- 22 Loeb DM, et al: Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. *Cancer Res* 2001;61:921–925.
- 23 Marzese DM, Gago FE, Vargas-Roig LM, Roque M: Simultaneous analysis of the methylation profile of 26 cancer related regions in invasive breast carcinomas by MS-MLPA and drMS-MLPA. *Mol Cell Probes* 2010;24:271–280.
- 24 Feinberg AP, Ohlsson R, Henikoff S: The epigenetic progenitor origin of human cancer. *Nat Rev Genet* 2006;7:21–33.
- 25 Moelans CB, Verschuur-Maes AH, van Diest PJ: Frequent promoter hypermethylation of BRCA2, CDH13, MSH6, PAX5, PAX6 and WT1 in ductal carcinoma in situ and invasive breast cancer. *J Pathol* 2011;225:222–231.
- 26 Teegarden D, Romieu I, Lelievre SA: Redefining the impact of nutrition on breast cancer incidence: is epigenetics involved? *Nutr Res Rev* 2012;25:68–95.
- 27 Waterland RA: Early environmental effects on epigenetic regulation in humans. *Epigenetics* 2009;4:523–525.
- 28 Petherick A: Environment and genetics: making sense of the noise. *Nature* 2012;485:S64–S65.
- 29 Corliss J: Exercise: powering up. *Nature* 2012;485:S62–S63.
- 30 Jardim MJ: microRNAs: implications for air pollution research. *Mutat Res* 2011;717:38–45.
- 31 Fraga MF, et al: Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci USA* 2005;102:10604–10609.
- 32 Bergman Y, Cedar H: DNA methylation dynamics in health and disease. *Nat Struct Mol Biol* 2013;20:274–281.
- 33 Perou CM, et al: Molecular portraits of human breast tumours. *Nature* 2000;406:747–752.
- 34 Maxmen A: The hard facts. *Nature* 2012;485:S50–S51.
- 35 Dunn BK, Gurs-Collins T, Browne D, Lubet R, Johnson KA: Health disparities in breast cancer: biology meets socioeconomic status. *Breast Cancer Res Treat* 2010;121:281–292.