Cancer Letters 342 (2014) 170–177

Contents lists available at SciVerse ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Epigenetic biomarkers in skin cancer

Edward S. Greenberg, Kelly K. Chong, Kelly T. Huynh, Ryo Tanaka, Dave S.B. Hoon *

Department of Molecular Oncology, John Wayne Cancer Institute at Saint John's Health Center, Santa Monica, CA, USA

ARTICLE INFO

Keywords:
Epigenetics
Biomarker
Methylation
MicroRNA
Melanoma
Prognosis

ABSTRACT

Epigenetic aberrations have been associated with cutaneous melanoma tumorigenesis and progression including dysregulated DNA gene promoter region methylation, histone modification, and microRNA. Several of these major epigenetic aberrations have been developed into biomarkers. Epigenetic biomarkers can be detected in tissue and in blood as circulating DNA in melanoma patients. There is strong evidence that biomarkers in cutaneous melanoma will have an important role as companions to therapeutics and overall patient management. Important progress has been made in epigenetic melanoma biomarker development and verification of clinical utility, and this review discusses some of the key current developments and existing challenges.

1. Introduction

Worldwide, major skin cancers predominantly include basal cell carcinoma (BCC), cutaneous melanoma and cutaneous T-cell lymphoma (CTCL). This review will focus specifically on cutaneous melanoma, the most malignant form of skin cancer with the most extensive epigenetic analysis on biomarkers (BMs) conducted to date. Cutaneous malignant melanoma is a highly aggressive disease, comprising less than 5% of skin cancers but accounting for a majority of the deaths from skin malignancies [1]. Overall, the incidence rates of melanoma have been rising in the United States over the past 10 years, with patient survival dependent on early detection and diagnosis. Patients with metastatic melanoma have a poor prognosis, with 5-year overall survival (OS) for patients with regional or distant metastasis less than 70% and 20% respectively, as compared to over 95% for those with localized disease [2]. In patients with advanced melanoma, although some promising new therapies have recently emerged, a better understanding of the molecular alterations involved in melanoma progression, particularly from localized tumors to metastasis, such as genomic and epigenomic aberrations, will aid in early detection and development of BMs and future targeted treatment strategies.

Melanoma, like other solid tumors, is thought to arise from a series of genetic and epigenetic events. Genetic aberrations have been identified in the past decade and have potential utility as BMs [3–9]. Recently, multiple studies have revealed that epigenetic events, such as genomic promoter region methylation of CpG islands, histone modification, and microRNA (miRNA) expression, have been shown to be important regulators of melanoma progression, and that these epigenetic changes can potentially serve as molecular BMs in tumor tissues and in blood as circulating DNA, for diagnosing disease and predicting disease outcome and progression (Fig. 1) [10–13].

2. DNA methylation

Epigenetics refers to heritable changes in gene expression that are not caused by changes in the genomic DNA sequence. DNA methylation is one of the hallmark epigenetic events most studied in cancers. DNA methylation involves the addition of a methyl group to the 5′ carbon of a cytosine ring located 5′ to a guanosine base in a CpG dinucleotide and is catalyzed by DNA methyltransferases (DNMTs) [1]. These CpGs are often clustered in short CpG-rich DNA stretches; deemed CpG islands, and the majority are found in the promoter region of genes [14]. Methylation events of promoter regions have been strongly implicated in cutaneous melanoma progression [10,12]. Hypermethylation of CpG islands in the promoter region leads to gene silencing through the inhibition of transcription or via recruitment of chromatin remodeling

Abbreviations: BC, biochemotherapy; BCC, basal cell carcinoma; BM, biomarker; SBM, sodium bisulfite modification; CAE, capillary array electrophoresis; cf-CNA, cell-free circulating nucleic acid; ChIP, chromatin immunoprecipitation; CIMP, CpG island methylator phenotype; CoBRA, combined bisulfite restriction analysis; CTC, circulating tumor cell; CTCL, cutaneous T-cell lymphoma; DNMT, DNA methyltransferase; IHC, immunohistochemistry; LINE-1, long interspersed nuclear element-1; MCC, merkel cell carcinoma; MINT, methylated-in-tumor; miRNA, micro-RNAs; MSP, methylation-specific realtime PCR; OS, overall survival; PCR, polymerase chain reaction; ROC, receiver operating characteristics; SCC, squamous cell carcinoma; TMZ, temozolomide; TRG, tumor-related genes; UTR, untranslated region.

* Corresponding author. Address: Department of Molecular Oncology, John Wayne Cancer Institute at Saint John's Health Center, 2200 Santa Monica Blvd., Santa Monica, CA 90404, USA. Fax: +1 310 449 5282.

E-mail address: hoon@jwci.org (D.S.B. Hoon).

0304-3835/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2012.01.020

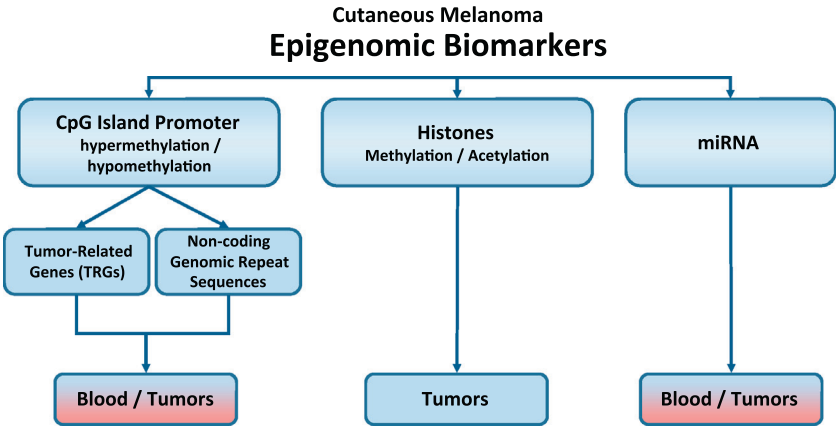

Fig. 1. Epigenomic BMs and studied applications.

co-repressor complexes [15]. Silencing of tumor suppressor genes or tumor-related genes (TRGs) can occur during melanoma development or later in advanced stage melanoma [12,16]. Epigenetic inactivation of multiple TRGs has been implicated in the establishment of malignancy and throughout stages of melanoma progression and metastasis [1,14]. Many of these genes are involved in cell cycle control, cell signaling, migration and invasion, apoptosis, angiogenesis, and metastasis [14,17]. At the same time there are TRGs that are activated in melanoma that are silenced in melanocytes. This transcription activation can be attributed to hypomethylation of the CpG islands in the promoter region and histone modifications.

2.1. DNA methylation detection techniques

One of the challenges in evaluating the DNA methylation status of genes is the fact that several techniques exist for evaluation of CpG island methylation. A recent review by Laird provides an excellent overview of the main principles of DNA methylation analysis, dividing these techniques into various types of pretreatment (enzyme digestion, affinity enrichment, sodium bisulfite) followed by different analytical steps (locus-specific analysis, gel-based analysis, array-based analysis, and next-generation sequencing-based analysis) [18]. Technique selection depends on the quality and quantity of input DNA needed, purity and type of tissue or fluid DNA is being extracted from, extent of genome coverage, and overall assay reproducibility, sensitivity, specificity, accuracy, and quantification. Moreover, despite advances in this field and development of multiple platforms for studying genomic methylation, uniformity and standardization remain significant issues in evaluating and comparing results.

Sodium bisulfite modification (SBM) of genomic DNA is one of the most well-utilized techniques for assessing CpG methylation status, based on the modification of genomic unmethylated cytosines to uracil [19,20]. Bisulfite conversion requires DNA denaturation before treatment and subsequent purification to remove the sodium bisulfite, thus causing substantial DNA degradation and often times requiring a large amount of high-purity input DNA. Other limitations of SBM include incomplete bisulfite conversion and differential PCR efficiency for methylated versus unmethylated sequences [18]. Bisulfite-based DNA methylation analysis is currently regarded as the "gold standard" and offers the advantage of quantitative assessment, detection sensitivity and the ability to analyze a wide variety of samples, though it is limited by the amount of DNA isolated from SBM as described above. The most commonly used SBM assays for assessing epigenomic BMs include: bisulfite sequencing, bisulfite pyrosequencing, combined bisulfite restriction analysis (CoBRA), methylation-specific realtime PCR (MSP), and gel electrophoresis [15,21]. The advantages of MSP include that it can be performed on very small quantities of DNA, such as that from paraffin-embedded tissues or free-circulating DNA in blood, and its products can be assessed as BMs by various platforms including gel electrophoresis, capillary array electrophoresis (CAE), MassARRAY, or real-time quantitative MSP [22]. CAE, MassARRAY and real-time MSP offers the ability to quantitatively evaluate a promoter region; moreover, with the MassARRAY method, one can determine the specific CpG island that best correlates with gene expression [22].

A newer approach of BM assessment is methylation-sensitive restriction enzyme digestion, based on the sensitivity of sequence-specific restriction enzymes that can recognize methylated cytosine within their cleavage recognition site. Although it is a cost effective and sensitive approach when coupled with PCR following enzyme digestion, this technique is limited to the analysis of CpG sites located within the enzyme recognition site(s) and is prone to false-positive results secondary to incomplete enzyme digestion. Affinity enrichment assays use antibodies specific for methylated CpGs or methyl-binding proteins with affinity for methylated genomic DNA. These methods allow for genome-wide assessment of DNA methylation but are limited by lack of specificity in areas of low CpG density and cannot be used to obtain information on individual CpG sites [16,18].

Another major challenge is identifying the key regulator CpG islands in the promoter region. There can be more than one gene or a specific repetitive sequence region that controls mRNA transcription. It is also possible that no CpG regulatory site exists in a gene promoter region. The majority of promoter region regulatory CpG sites are near the open-reading frame. It takes methodical analysis of CpG region sequencing in conjunction with mRNA expression to determine key regulatory sites. Studies may often report hyper- or hypo-methylation in the gene promoter region that plays little role in affecting respective gene transcription.

2.2. DNA methylation BMs for diagnosis and prognosis

Aberrantly methylated melanoma TRGs can serve as BMs for early diagnosis of cancer, evaluation of cancer progression, and as prognostic indicators in melanoma patients. Our group identified and verified the inactivation of RAS association domain family protein 1A (RASSF1A), a tumor suppressor gene, in melanoma [23]. RASSF1A is involved in the regulation of apoptosis, migration, and metastasis [1]. An increase in RASSF1A methylation positively correlates with advancing tumor stage, suggesting that RASSF1A may be a useful BM of melanoma progression [12]. Separately, RASSF1A