Epigenetic markers in melanoma

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Practice points

- Many aberrant DNA methylated genes either hypermethylated or hypomethylated have been identified in melanoma and detection of methylation status of certain gene panels have been tested.
- Ten–eleven translocation-mediated DNA hydroxymethylation marker 5-hydroxymethylcytosine is a putative epigenetic biomarker of melanoma that correlates with degree of dysplasia, predicts biological behavior, and demonstrates significant clinical utility in the histopathologic evaluation of sentinel lymph node biopsies.
- Increasing evidence implicates dysregulated histone modification and chromatin remodeling play in melanoma pathogenesis.
- Extensive studies indicate that an array of miRNAs regulate melanoma progression through a multitude of either oncogenic or tumor suppressive pathways.
- Recent studies implicate the potential pathogenic role of long noncoding RNAs in melanoma.

Melanoma, one of the most virulent forms of human malignancy, is the primary cause of mortality from cancers arising from the skin. The prognosis of metastatic melanoma remains dismal despite targeted therapeutic regimens that exploit our growing understanding of cancer immunology and genetic mutations that drive oncogenic cell signaling pathways in cancer. Epigenetic mechanisms, including DNA methylation/demethylation, histone modifications and noncoding RNAs recently have been shown to play critical roles in melanoma pathogenesis. Current evidence indicates that imbalance of DNA methylation and demethylation, dysregulation of histone modification and chromatin remodeling, and altered translational control by noncoding RNAs contribute to melanoma tumorigenesis. Here, we summarize the most recent insights relating to epigenetic markers, focusing on diagnostic potential as well as novel therapeutic approaches for more effective treatment of advanced melanoma.

Melanoma, a genetically heterogeneous malignant neoplasm of melanocytes, is one of the few human cancers whose incidence is steadily increasing, with more than 232,000 new cases diagnosed in 2012 [1]. Despite the deployment of therapies directed at specific genetic mutations in melanoma, mortality rates from this deadly disease also continue to rise [2]. Accordingly, novel biomarkers for earlier and more accurate detection of melanoma, and for novel therapeutic strategies for the more effective treatment of advanced disease are urgently needed.

Over the past decade, enormous advancements have enhanced our understanding of the immunologic and genetic underpinnings of melanoma pathobiology, leading to the development of several groundbreaking therapies (e.g., pathway targeted therapy, check-point immunotherapy) [3–5]. In addition, evidence is rapidly accruing that implicates epigenetic events in cancer biology [6–11].

KEYWORDS

- DNA demethylation
- DNA methylation
- epigenetic biomarkers
- histone modification
- 5-hydroxymethylcytosine
- long RNAs • melanoma
- miRNAs • noncoding RNA
- ten–eleven translocation

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An epigenetic trait was defined as a ‘stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence’ at the meeting hosted by the Banbury Conference Center and Cold Spring Harbor Laboratory [12]. Epigenetic mechanisms regulate gene expression by altering the conformation of chromatin through DNA methylation [13] and histone modification [14]. Noncoding RNAs regulate gene expression by influencing a number of complex cellular process [15]. Recent studies have accelerated our understanding of epigenetic dysregulation in melanoma, with specific focus on DNA methylation/demethylation, histone modification and noncoding RNAs (Figure 1) [16–18]. The high frequency, early occurrence and reversibility of epigenetic modifications have made this biological machinery an important area of investigation with respect to cancer biology, which has unveiled numerous attractive candidates for potential biomarkers and therapeutic targets [19,20]. In this review, we summarize the most recently discovered epigenetic markers to enhance approaches to diagnosis and therapeutic targeting in melanoma.

Histone modification & chromatin remodeling

At the core of chromatin structure are highly conserved histone proteins (H3, H4, H2A, H2B and H1) that function as building blocks to package eukaryotic DNA into nucleosomes [21]. Post-translational modifications (e.g., acetylation, phosphorylation, methylation and ubiquitination) occur on the histone protein tails by a variety of catalytic enzymes [14]. Histone modifications cause structural changes to chromatin and regulate the expression of the genes in the affected anatomical segments [14]. Aberrant histone modifications such as histone acetylation have been well-documented in melanoma tumorigenesis. Histone hypoacetylation has been shown to downregulate PI3K/Akt pathway tumor suppressor genes (phosphatidylinositol 4,5-bisphosphate 5-phosphatase A) in melanoma [22]. In addition, aberrant histone acetylation has been demonstrated to affect tumor cells and tumor-infiltrating lymphocytes by regulating the apoptotic pathway TRAIL/Apo2L [23] or Bcl-2 family proapoptotic proteins (Bim, Bax and Bak) in melanoma [24]. These studies provide a putative

Figure 1. Schematic representation of aberrant DNA methylation and histone modification. Key components of the DNA methylation and histone modifying machinery are indicated.

DNMT: DNA methyltransferase; HAT: Histone acetyltransferase; HDAC: Histone deacetylase; PcG: Polycomb group; Pol III: DNA polymerase III. Reprinted with permission from [16] © Oxford University Press (2014).

mechanism for the potential therapeutic efficacy of HDAC inhibitors in melanoma. Other post-translational histone modifications, such as histone methylation and demethylation, also play important roles in melanoma. The followings are a few examples of epigenetic markers of histone modification in melanoma that have been reported.

- **Histone demethylase JHDM1D (also known as KDM7A)**

JHDM1D participates in epigenetic gene regulation by modifying the level of methylation in histone. Increased expression of histone demethylase JHDM1D in nutrient-deprived conditions was found to suppress tumor growth by downregulating angiogenesis [25]. In addition, it was reported that trimethylation of histone H3 lysine 79 and phosphorylation of H3 threonine 80 (H3K79me3T80ph) could be an indicator of a subset of primary cutaneous melanomas with more aggressive clinical behavior [26].

- **SETDB1**

SETDB1, an enzyme that methylates histone H3 on lysine 9 (H3K9), was demonstrated to significantly accelerate melanoma formation in the zebrafish model, supporting its oncogenic role in melanoma [27]. Moreover, one study identified SETDB1 overexpression in over half of their melanoma patient samples. A significant relationship between overexpression of SETDB1, as documented through immunohistochemical means and aggressive tumor behavior such as lymph node metastasis and/or distant metastasis, was observed in a series of 67 cases of melanoma [28]. Interestingly, cytoplasmic SETDB1 expression also has been shown to significantly correlate with a higher frequency of methylation and suppressed expression of the tumor suppressor p16 [29].

- **EZH2**

EZH2 is the H3K27 methyltransferase catalytic subunit of PRC2. EZH2 expression was associated with high proliferation rate through catalyzing the transcriptionally repressive methylation of H3K27 in melanoma [30]. Moreover, EZH2 depletion had a pro-apoptotic effect in melanoma cells by normalizing acetylation of the CDKN1A locus and removal of histone deacetylases [31]. Significant increase of EZH2 expression by immunohistochemistry has been reported in melanoma compared with nevi [32].

More recent investigations have demonstrated enhanced EZH2 expression at the invasive front of human primary cutaneous melanomas in histopathologic samples, providing a unique contextual insight into epigenetic mechanisms in melanoma pathobiology [31]. Further interrogation into the potential pathogenic role of EZH2 in melanoma as well as its therapeutic targeting are active areas of ongoing investigation [33,34].

DNA methylation

The methylation of DNA at the 5-carbon position of cytosine is a critical epigenetic mechanism that regulates gene expression. In total, 5-methylcytosine (5-mC) constitutes 2–8% of all cytosines in human genomic DNA and impacts a broad range of both biological functions and pathological processes [35]. Three DNA methyltransferases (DNMT), namely DNMT1, DNMT3a and DNMT3b, are responsible for transcriptional silencing through the addition of methyl groups to cytosine residues preceding guanine (CpG), which, during normal development and in certain pathological conditions, occur on areas concentrated with CpGs, called ‘CpG islands’ just upstream of gene promoters in mammalian cells [36]. Aberrant DNA hypermethylation in the normally unmethylated promoter regions of tumor suppressive genes has been proposed as one mechanism of their suppression in cancer cells [37–39]. Tumor cells of various cancer types have also been found to exhibit global hypomethylation, which may correspond to a worse prognosis [40]. Some lines of evidence suggest that hypomethylation of specific proto-oncogenes can lead to their activation (Table 1) [41,42]. Others indicate that DNA hypomethylation of pericentromeric satellite regions may induce chromosomal instability [43–45]. DNA hypomethylation has been suggested to have potential utility for cancer classification and prediction of clinical course [46]. Selective hypermethylation at the promoters of a number of tumor suppressor genes have been well-documented in melanoma (Table 1) [47–66].

To date, many aberrantly methylated genes have been identified in melanoma (Table 1) [69]. Promoter hypermethylation of a number of well-known tumor suppressor genes has been documented in melanoma, including *p16(INK4A)*, the causative gene in familial atypical multiple mole melanoma (FAMMM) syndrome, as well as *PTEN* [62,70–71]. Moreover, methylation profiles also have been shown to correlate

Table 1. Hyper- and hypo-methylated genes in malignant melanoma.		
Gene	Translational relevance and potential	Biological evidence and significance
Hypermethylated genes		
<i>APC</i>	Diagnostic/prognostic marker; therapeutic target	APC gene promoter hypermethylation in melanoma cell lines and patient melanoma tissue samples; leads to APC's transcriptional silencing which in turn increases cell proliferation without compromising invasiveness; associated with poor prognosis [51]
<i>ASC/PYCARD</i>	Therapeutic target	Plays stage-dependent role in tumorigenesis by differential regulation of NF- κ B and IL-1 β [52]
<i>p16(INK4A)/Caspase-8</i>		Methylation and silencing in cutaneous melanoma is induced by Cadmium and results in resistance to apoptosis [53]
<i>CDH1</i>	Prognostic marker	Inversely correlated with melanoma stage and Breslow thickness, higher expression is associated with favorable prognosis [57]
<i>CDKN2A</i>	Diagnostic marker; therapeutic target	Major high-risk melanoma susceptibility gene; tumor-suppressor capable of blocking cell cycle at the G1-S checkpoint and activating RB [54]
<i>FRZB</i>		Inhibits melanoma migration and invasion via Wnt5a signaling; part of multilocus DNA-methylation signature that discriminates melanomas from nevi [55]
<i>LXN</i>		Densely methylated in melanoma cell lines and tumors; increased expression inhibits proliferation and alters stem cell-like properties [58,59]
<i>PTEN</i>	Prognostic marker, therapeutic target	Tumor suppressor, the silencing of which results in activation of the AKT pathway and thereby promotes melanoma growth, proliferation, and development; hypermethylated <i>PTEN</i> is found in blood of patients bearing metastatic melanoma and is associated with poor survival; <i>PTEN</i> loss also correlates with <i>BRAF</i> activation [60–62]
<i>RAR-b2</i>	Diagnostic marker, therapeutic target	Methylated in human melanoma cell lines and clinical specimens; RAR-b2 can be reactivated by 5-aza 2'-deoxycytidine to inhibit cell growth through the regulation of ATRA [63]
<i>RASSF1A</i>	Staging and prognostic marker; therapeutic target	Degree of methylation varies with tumor stage; silencing leads to suppression of mitochondrion-dependent apoptosis [64]
<i>SOC51</i>	Staging marker; therapeutic target	Hypermethylation increases with advancing clinical tumor stage; overexpression inhibits proliferation by causing G1/S blockage [65,66]
<i>TFAP2A</i>	Therapeutic target	Down-regulated in melanomas compared with nevi; epigenetically silenced in advanced melanoma [67]
Hypomethylated genes		
<i>CD2</i>	Prognostic marker; therapeutic target	Higher levels associated with lower recurrence rate and improved overall survival [42,55]
<i>IL2</i>	Therapeutic target	Patients treated with IL2 showed better survival [55]
<i>LINE-1</i>	Prognosis marker	Hypomethylated LINE-1 is associated with increased overall survival [68]

tightly with prognostic pathologic features. For instance, primary melanomas with intermediate and high methylation phenotypes have been found to be the most deeply invasive and most mitotically active [71–73]. In addition, the greatest extent of DNA methylation-mediated silencing of *TFAP2A* was shown to occur in late stage melanomas [67]. In addition, investigations into the promoter methylation status of *LINE-1* in melanoma revealed that hypomethylation at a specific CpG site was a predictor for improved overall survival, whereas hypermethylation of this location was associated with worse prognosis [68]. These findings emphasize the prognostic potential of such epigenetic features while reinforcing the notion that melanoma is a genetically and epigenetically heterogeneous malignancy.

Of interest, recent findings indicate a more intimate relationship between epigenetic mechanisms and known oncogenic cell signaling pathways than was previously appreciated. A recent report, for instance, demonstrated that the oncogenic *BRAF* V600E mutation could influence the methylation profile and expression of key epigenetic genes in melanoma cells, including *DNMT1* and *EZH2* [74]. In addition, Guo *et al.* recently demonstrated that melanomas harboring *BRAF* V600E mutations harbored greater global DNA hypomethylation compared with *BRAF* wild-type melanomas. Dahl *et al.* recently demonstrated that increasing methylation of the receptor tyrosine kinase *KIT* promoter occurred alongside stimulation and growth of melanoma cells with stem cell factor (SCF) [75]. Further investigations delineating the relationship between epigenetic mechanisms and cell signaling pathways will be critical to exploit the full therapeutic potential of epigenetic dysregulation in melanoma.

Imbalance of DNA methylation and demethylation, whether it be excessive or deficient, is involved in the early stage of carcinogenesis [76]. However, the process of demethylation was largely unknown until breakthrough studies showed that 5-mC could be converted to 5-hydroxymethylcytosine (5-hmC) by the ten–eleven translocation (TET) family genes in 2009 (Figure 2) [77–80]. While aberrant methylation status of gene promoter regions and global hypomethylation have been tightly linked with malignancies such as melanoma, quantitation of 5-mC is insufficient for their distinction from their benign counterparts. In contrast, using genome-wide mapping, we have found that the

loss of 5-hmC is an epigenetic event that distinguishes melanoma from the benign nevus and that also is associated with melanoma virulence. Moreover, we have demonstrated using animal models that restoration of the 5-hmC landscape through *TET2* overexpression inhibits tumor growth, suggesting a tumor suppressive role for this 5-mC hydroxylase and the DNA demethylation pathway (Figure 2) [81,82]. The correlation of loss of 5-hmC with tumor virulence is not unique to melanocytes, as similar relationships have also been made established between the malignant and benign counterparts of other tissue types [83]. Indeed, loss of 5-hmC has now been documented as a hallmark of many solid tumors including breast, prostate, colon cancer and hematologic myeloid malignancies and invariably predicts poor prognosis [35,81,84–87].

Regarding the use of epigenetic alterations as biomarkers, we have employed immunofluorescence and immunohistochemical (IHC) staining to establish that benign melanocytic nevi retain strong 5-hmC staining whereas virtually all tumor cells in primary and metastatic melanomas exhibit a dramatic loss of 5-hmC [81]. Moreover, we find that increasingly atypical dysplastic melanocytic nevi, which clinically, histologically and biologically may be intermediate between benign nevi and melanoma, show increasing loss of 5-hmC and also corresponds to increasing nuclear diameter, a correlate for increasing dysplasia [82]. 5-hmC levels can be easily detected by IHC and hopefully will provide an adjunct to clinical practice during evaluation for premalignant potential as well as in confirming primary malignancy and the presence of ambiguous metastases [35,81,88]. Our observations have been confirmed by several other groups, who found that 5-hmC levels were significantly decreased in advanced melanomas compared with nevi and thin melanomas as well as that 5-hmC could differentiate between malignant melanomas of distinct biological origins and other benign nevic subtypes [89,90].

Based on these observations and the role of *TET* family enzymes in enabling DNA demethylation, this critical epigenetic regulator has generated significant scientific interest. *TET* family enzymes are α -ketoglutarate (α -KG) and iron-dependent dioxygenase enzymes, and thus depend on metabolic pathways to sustain their function [18]. Of interest, isocitrate dehydrogenase (IDH) mutations have been demonstrated to lead to the accumulation of oncometabolites

Figure 2. Active DNA demethylation pathway. The pathway involved in the TET-dependent generation of 5-hmC, an epigenetic mark that is lost in melanoma (pictorially rendered to lower right). 5-hmC: 5-hydroxymethylcytosine; BER: Base excision repair; DNMT: DNA methyltransferase; IDH: Isocitrate dehydrogenase; SDH: Succinate dehydrogenase; TDG: Thymine DNA glycosylase; TET: Ten–eleven translocation.

that can directly compete with α -KG and inhibit TET function [91]. Further indication of the pathogenic role of dysfunctional TET2 in melanoma has also come from genome-wide association studies, which have found that a single nucleotide polymorphism (SNP) located within the intron of the *TET2* gene on chromosome 4q24 was among the most tightly associated with increased melanoma risk in 2298 cases [92]. However, direct mutation to the genes encoding TET or IDH family enzymes appears insufficient to explain the near universal loss of 5-hmC, the most stable epigenetic mark of the DNA demethylation pathway. In fact, the frequency of somatic mutation to the *TET* and *IDH* family genes within melanoma tissue samples is much lower than the observed loss of 5-hmC in most, if not all malignant melanomas [92]. Collectively, these observations indicate that mechanisms beyond direct mutation, either through metabolic and/or higher regulatory control, are responsible for 5-hmC-related dysfunction in the malignant context.

In this regard, recent studies demonstrate that *TET* family genes are direct targets of several miRNAs in various cancers, including melanoma. A recent study identified a novel cancer germline transcript harboring oncogenic miR-767, which represses TET1/3 miRNA and protein expression in melanoma and lung cancer [93]. Additional studies also have shown that *TET* family genes were direct targets of oncogenic miR-22, promoting metastasis in breast cancer [94,95]. While these particular miRNAs exhibit oncogenic potential, other studies demonstrate their potential tumor suppressive function, including the miR-29 family members, which have the potential to inhibit metastasis by upregulating TET1 in hepatocellular cancer cell lines and tumor samples [95,96]. The role of miRNAs in oncogenesis and tumor suppression in the context of melanoma will be discussed in greater detail below.

Noncoding RNA

ncRNAs are classified into two categories based on length: small ncRNA (<200 bp) and long

ncRNA (lncRNA, >200 bp). Noncoding RNAs are not translated into proteins but, nonetheless, are potent regulators of gene expression [91]. Given the importance of ncRNA effects on multiple molecular pathways and hundreds of downstream target genes involving cell proliferation, invasion and migration, among others, the role of ncRNAs in cancer and melanoma pathobiology has been an active area of investigation [97].

• miRNAs

miRNAs are the most well documented small ncRNAs, with a length of approximately 22 nucleotides. miRNAs regulate gene expression by binding to miRNA response elements within the 3'-untranslated region (3'-UTR), 5'-UTR or coding region of protein-encoding mRNA [15]. Thus, miRNA-mediated gene regulation occurs through inducing mRNA degradation and/or repressing mRNA translation, and thus has enormous potential to regulate multiple biological and pathophysiological processes [98].

miRNAs may function in either an oncogenic or tumor suppressive manner and play important roles in melanoma biology by regulating critical pathways/genes, including those involved in cell proliferation, apoptosis, migration and invasion, drug resistance and angiogenesis (Figure 3) [99]. Extensive insight into the pathobiology of miRNAs in melanoma suggests that these important epigenetic components bear significant translational potential. For example, recent reports have indicated that serum levels of certain miRNAs (miR-150, miR-15b, miR-199a-5p, miR-33a and miR-424) may be used as circulating biomarkers for identifying primary melanoma patients with high recurrence risk and as well as for monitoring melanoma tumor burden over time [100]. In this review, we summarize the existing literature of deregulated miRNAs in melanoma tissue samples and/or cell lines, organized based on their proposed biological effect(s) (Table 2) [101–136]. These important epigenetic regulators have been shown to

Figure 3. Pathogenic miRNAs in melanoma. Schematic summary displays miRNAs shown to be pathogenic in melanoma and the mechanisms involved and enabled, including proliferation, drug resistance, invasion/metastasis, angiogenesis and anti-apoptosis.

modulate a number of complex pathways, as detailed below, and continued investigation in these areas will have significant biomarker, translational and therapeutic potential.

Cell proliferation & growth

Multiple miRNAs have been shown to play an important role in regulating melanoma tumor cell proliferation and growth, including miR-15b, miR-99a, miR-137, miR-148, miR-149, miR-193b, miR-211, miR-221 and miR-506-514 (Table 2). As an example, ectopic transfection of miR-137 inhibited uveal melanoma cell growth and proliferation by downregulating multiple target genes, including MITF, a transcription factor known to control melanocyte development and melanogenesis; c-MET, the oncogenic tyrosine kinase protein receptor; and CDK6, a cell cycle-related protein [137]. In addition, miR-211, which is regarded to have tumor suppressive function [138], was shown to inhibit melanoma invasion and migration through the regulation of NUA1, MITF and BRN2 [121,122].

Apoptosis

Several miRNAs with effects on melanoma apoptosis resistance have been identified, including miR-18b, miR-26a, miR-34a, miR-34b/c, miR-137, miR-203 and miR-205, (Table 2). For instance, miR-26a was found to be strongly downregulated in melanoma cell lines compared with primary melanocytes, and miR-26a transfection of melanoma cell lines led to apoptosis in a silencer of death domain (SODD)-dependent manner [107]. Conversely, ectopic expression of miR-34b in melanoma cells has also been shown to promote apoptosis and reduce cell invasiveness and motility rates [139]. miR-137, mentioned above, has also been shown to induce tumor cell apoptosis by decreasing BCL2 levels [104,119].

Invasion & metastasis

Several miRNAs have been shown to play important roles in melanoma metastasis by promoting tumor cell invasion, including miR-214, miR-30b/30d, miR-182, let-7a, miR-126, miR-145, miR-137, miR-18b, miR-34a/c, miR-211, miR-9 and miR-31, among others (Table 2). Increased expression of miR-200c, miR-205 and miR-211, for instance, was shown to inhibit melanoma cell migration *in vitro* [138]. In addition, overexpression of miR-205 impeded melanoma cell migration and invasion in both *in vitro* and *in vivo* models. miR-205 expression has

also been shown to progressively decrease from the benign to dysplastic nevi, and even further in malignant melanomas in both clinical specimens and melanoma cell lines [140]. Decreased miR-205 expression in melanoma cells also has been shown to associate with significantly shorter melanoma-specific survival in patients, independent of melanoma stage, age, gender and Breslow depth [141]. miR-18b is another example of a potential tumor suppressor in melanoma. miR-18b expression is significantly decreased in melanoma tissue and cell lines, and its overexpression reduced *in vivo* tumor growth, inhibited cell viability, induced apoptosis and suppressed melanoma migration and invasiveness [106].

Angiogenesis

Angiogenesis plays a critical role in enhancing the viability of human malignancies. Of interest, several miRNAs have been shown to promote this function, which is essential to the malignant phenotype. miR-1908, miR-199a-5p and miR-199a-3p, for example, have been shown to be endogenous promoters of invasion, angiogenesis and colonization in melanoma through ApoE signaling inhibition and by suppressing heat shock factor DNAJA4 [128]. These miRNAs may be of therapeutic and prognostic interest in melanoma.

Drug resistance

Recent studies indicate that miR-146a may promote the initiation and progression of melanoma by activating Notch signaling, a pathway known to enhance tumor cell capacity for drug resistance [131]. In addition, miR-200c has been shown to have tumor suppressive function in melanoma not only by inhibiting cell proliferation and migratory capacity [138], but also by reducing the expression of key transporter genes involved in melanoma drug resistance, including BMI-1, ABCG2, ABCG5 and MDR1 [132].

• lncRNAs

Recent studies demonstrate the potential role of lncRNAs in gene regulation, adding another layer of complexity to higher, epigenetic mechanisms of gene-expression regulation. lncRNAs can be classified into five categories based on their loci positions: sense lncRNAs are transcribed from a locus that overlaps with the protein-coding gene; antisense lncRNAs loci overlap with the antisense strand of protein coding gene; bidirectional lncRNAs are located on the antisense strand of a protein-coding gene whose start codon is <1000 base

Table 2. Dysregulated miRNAs in melanoma.

Function	Level	Regulator	miRNA	Target gene	Notes	Ref.
Sustaining proliferative signaling	↑	PLZF [‡]	miR-221	c-Kit [‡]	p53 mediates a prosurvival pathway by upregulation of miRNA-149 [‡] (miR-149 [‡]), which, in turn, targets GSK3 α , leading to stabilization of Mcl-1 in melanoma cells under endoplasmic reticulum stress	[101–104]
			miR-15b	BCL2 [‡]		
	↓	p53	miR-149 [§]	GSK3a [‡]		
			miR-506–514	HOXB7, PBX		
			miR-137	MITF [‡]		
Resisting cell death	↓		miR-193b	CCND1 [‡]	miR-18b showed tumor suppressive activity through MDM2-p53 pathway, it suppressed melanoma cell viability, induced apoptosis and reduced tumor growth <i>in vivo</i> miR-26a induces cell death in multiple cell lines, through targeting anti-apoptotic protein SODD	[105,136]
			miR-148	MITF [‡]		
			miR-18b	MDM2 [‡]		
Enabling replicative immortality	↓	p53	miR-26a	SMAD1 [‡]	miR-34a suppresses invasiveness, miR-34c reduces proliferation	[106,107]
			miR-34a [§]	CDK6 [‡]		
			miR-34b/c [§]	CDK4, CDK6 [‡]		
Activating invasion and metastasis	↑	Twist-1	miR-203	E2F3 [‡]	GALNT7 suppression reduces immune function	[111–115]
			miR-205	E2F1, E2F5 [‡]		
			miR-214 [§]	ITGA3, TFAP2C [‡]		
			miR-30b/d	MET, ALCAM [§]		
			miR-182	GALNT7 [‡]		
	↓	p53 p53 MITF	miR-20b [‡]	MITF, FOXO3, c-Met [‡]	miR-9 inhibits melanoma proliferation and metastasis through downregulation of the NF- κ B–Snail1 pathway	[116–122]
			let-7a	PAR-1 [‡]		
			miR-126	ITGB3 [‡]		
			miR-145 [§]	ADAM9, MMP7 [‡]		
			miR-137	FSCN1 [‡]		
			miR-18b	EZH2 [‡]		
			miR-34a/c [§]	MDM2 [‡]		
			miR-211 [§]	c-Met [‡]		
			iR-9	NUAK1, BRN2 [‡]		
			miR-125b	NK-kB, Snail [‡]		
Cell migration and invasion	↓		miR-31	c-Jun [‡]	miR-573 regulates MCAM, a cell-surface glycoprotein that mediates heterotypic and homeotypic cell–cell adhesion	[123–126]
			miR-124a	EZH2 [‡]		
			miR-375	CDK6, cyclin D2, EZH2 [‡]		
			miR-573	MCAM [‡]		
	↑		miR-195	WEE1 [‡]	Overexpression of miR-195 in SK-Mel-28 caused WEE1 reduction and reduced stress-induced G2-M cell cycle arrest	[127,128]
			miR-199a	ApoE, DNAAJ4 [‡]		

[‡]Expression level uncertain.[‡]Inhibition.[§]Stimulation.[‡]: Upregulated; ↓: Downregulated.

Table 2. Dysregulated miRNAs in melanoma (cont.).					
Function	Level	Regulator	miRNA	Target gene	Notes
Melanocyte differentiation, cell cycle progression, proliferation and invasion	↓		miR-101 miR-196a miR-146a	MITF, EZH2, HOX-C8 [†] NUMB [‡]	NUMB is a repressor of Notch signaling
Cell proliferation and migratory capacity as well as drug resistance	↑		miR-200c	BMI-1, ABCG2, ABCG5 and MDR1 [‡]	Overexpression of miR-200c in melanoma cells resulted in significantly decreased cell proliferation and migratory capacity as well as drug resistance
Cell proliferation	↓		miR-99a	mTOR	The PI3K-AKT-mTOR pathway is constitutively activated in many melanomas, leading to increased cell growth, proliferation and survival
Cell cycle exit and epidermis differentiation	↑		miR-449a	HDAC-1	
Suppress tumorigenesis by changing the methylation status of DNA	↓	NF-κB [‡]	miR-29	DNMT3A/B [‡]	Hypermethylation status of TRGs and non-coding MINT loci in different stages of melanoma showed an inverse association with miR-29c expression

[†]Expression level uncertain.
[‡]Inhibition.
[§]Stimulation.
↑: Upregulated; ↓: Downregulated.

pairs away; intronic lncRNAs are transcribed from introns of protein-coding genes; and long intergenic (or intervening) noncoding RNAs loci are not within the proximity of a protein-coding gene [33,34]. Most lncRNAs have been demonstrated to function within the nucleus, where they modify transcription by recruiting or sequestering proteins [34]. However, several lncRNAs have been demonstrated to exert their function in the cytoplasm and regulate gene expression by either stabilizing mRNAs as well as their localizing proteins [34]. Although the functions and roles of lncRNAs remain largely unknown in melanoma, certain lncRNAs have been suggested to play important roles in its pathobiology [83]. Several studies of melanoma-associated lncRNAs have been summarized below.

HOX transcript antisense RNA

Studies have shown that the lncRNA *HOX* transcript antisense RNA (HOTAIR) plays an important role in solid tumors, such as breast cancer [142,143] and one recent report suggested that HOTAIR may play a prometastatic role in melanoma. This study demonstrated overexpression of HOTAIR in three pairs of primary melanomas and matched lymph node metastases. In addition, HOTAIR knockdown inhibited the motility and invasiveness of melanoma cells, with reduced capacity to degrade the extracellular matrix [144]. However, no statistically significant differences in expression levels of HOTAIR were found in another study that compared melanoma and adjacent normal tissue [145]. Nonetheless, expression of lncRNAs urothelial carcinoma-associated 1 (UCA1) and lung adenocarcinoma transcript 1 (Malat-1) has been documented to be increased in metastatic melanomas [145]. lncRNA HOTAIR has also been shown to suppress expression of HOXD, which is known to produce miR-7 that inhibits the expression of the histone methyltransferase SETDB1 [146].

BRAF-activated noncoding RNA

BRAF-activated noncoding RNA (BANCR) is located on chromosome 9 and can be induced by *BRAF* V600E mutation in melanocytes. The overexpression of BANCR in melanomas has been reported, and BANCR depletion could inhibit melanoma cell migration, suggesting a potential role in melanoma while providing a link between this novel epigenetic mechanism and well-established cell signaling pathways [147]. A recent study also indicated

that BANC1 could promote melanoma proliferation by activating the ERK1/2 and JNK MAPK pathways that are known to correlate with tumor stage and that represent potential therapeutic targets [148].

SPRY4-IT1

SPRY4 has been suggested to have tumor suppressive function by inhibiting the MAPK signaling pathway [149]. lncRNA SPRY4-IT1 was identified by comparing lncRNAs in melanoma cell lines, melanocytes and keratinocytes [149]. Overexpression of SPRY4-IT1 was reported in the melanoma cell lines, and its knockdown was shown to promote apoptosis and inhibit cell growth and differentiation in melanoma cell lines [149].

Antisense noncoding RNA in INK4 locus

The antisense lncRNA antisense noncoding RNA in INK4 locus (ANRIL) was identified relative to the p15/CDKN2B-p16/CDKN2A-p14/ARF gene cluster on the chromosome 9p21 locus [150]. Various short and long isoforms and a recently discovered circular isoform of ANRIL have been found to be expressed in melanoma cell lines [151]. Polymorphism of a single nucleotide variant (rs1011970) of ANRIL has been identified to be associated with increased melanoma risk in a recent GWAS study [152]. These data suggest the potential importance of ANRIL in melanoma pathogenesis, and further studies are indicated.

Conclusion

Significant progress in our understanding of epigenetic mechanisms and their role in melanoma has been made over the past decade. Epigenetic mechanisms, as discussed above, can exert oncogenic or tumor suppressive effects in melanoma by altering chromatin structure through covalent modification of DNA bases or histone proteins, as well as by regulating translation through the site-specific actions of noncoding miRNAs and lncRNAs. Here, we have provided an up-to-date summary of recent discoveries implicating epigenetic mechanisms in melanoma, and highlight their potential translational relevance as biomarkers and therapeutic targets. In doing so, we highlight the key regulators of DNA methylation, DNA hydroxymethylation, histone modification and noncoding RNAs, including several miRNAs and lncRNAs.

Future perspective

While this rapidly accumulating body of evidence has enhanced our understanding of the critical pathogenic roles of epigenetic mechanisms in melanoma, our knowledge remains in its early stages. Furthermore, our increasingly detailed understanding continues to reveal the complexity of these mechanisms as well as their relationships with well-established cell signaling pathways. Two critically important aspects will need to be thoroughly investigated. First, clarification of how the multiple and interwoven layers of epigenetic mechanisms interact with one another should be explored. Second, how this machinery regulates or is regulated by, known cell signaling pathways will require further understanding. Identifying the dysregulated epigenetic mechanisms unique to malignant tissues may potentially lead to the development of novel targeted therapies that may be deployed in combination with existing targeted and immunologic therapeutic strategies. Enhancing our understanding of the dysregulated epigenetic and molecular pathways unique to an individual's disease, or, perhaps, common to definable subpopulations of patients, should fuel innovative discoveries of epigenetic biomarkers with value to personalized approaches for more precise application of a growing armamentarium of therapeutic options.

Although our understanding remains in its infancy, significant progress in our understanding of epigenetic mechanisms in melanoma will be accelerated by the availability of novel technologies, genome-wide approaches and computational models for big data analysis. We are therefore at the cusp of a new era in oncogenomics, one that should result in an integrated understanding of how the epigenome, genome and proteome interact to distinguish benign from malignant. This understanding will have immense translational implications for benefiting patients afflicted with advanced melanoma and other forms of cancer.

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