Can histone proteins promote the growth of melanoma?

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The melanoma burden

Melanoma incidence has been rising steadily over the past 30 years, and it is expected to rise faster than any other cancer in the coming decades. Despite recent therapeutic advances, melanoma remains a major clinical challenge. While surgery can be curative in early stage disease, curative treatments for patients with metastatic disease are lacking, as classical chemotherapeutic agents and radiotherapy are minimally effective. The development of novel targeted agents have raised hope for a ‘melanoma cure’, however they can only be used in particular subgroups of patients (i.e., combination of BRAF and MEK inhibitors for *BRAF*^{V600E}-mutant melanoma) [1]. Moreover, efficacy tends to be transient with resistance and relapse rapidly ensuing for the majority of patients [2]. In contrast, immunomodulatory therapies can produce durable responses, but this also occurs in

only a fraction of patients (i.e., 20% of anti-CTLA4-treated patients; ~40% combined with anti-PD1) [3]. Available treatments have been unable to substantially reduce mortality in metastatic melanoma patients, thus there is a clear need for new therapeutic targets and strategies.

Epigenetic regulation of melanoma

Recent targeted therapeutic approaches are based on the identification of genetic alterations in key signaling pathways that are common drivers of melanoma tumorigenesis [4]. Much of the research effort to date has focused on dissecting aberrant signaling cascades (receptors/ligands, kinases); however, the malignant transformation of melanocytes is not only driven by genetic changes, but also by abnormal chromatin configurations and alterations in DNA methylation patterns. The latter underscores the centrality of epigenetics

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in cancer biology, and therefore we favor the hypothesis that treatment should be guided by both genetic and epigenetic alterations.

A growing body of evidence suggests that epigenetic changes lead to the deregulation of transcriptional programs that are directly associated to melanoma pathogenesis. Recent studies have highlighted a role for ‘chromatin writers’ (enzymes that add chemical modifications to histones) such as the histone methyltransferases SETDB1 and EZH2, which catalyze methylation of lysine 9 and 27 in histone H3, respectively. SETDB1 is amplified in human melanoma and its expression accelerates melanoma development in a zebrafish *BRAF*^{V600E} model system [5]. EZH2 expression is elevated and associated with poor survival in melanoma, and its conditional ablation inhibits tumor growth and metastases in a melanoma mouse model [6]. In addition, genome sequencing efforts have identified mutations in *ARID2*, a chromatin remodeler, and *TRRAP*, a component of histone acetyltransferase (HAT) and histone chaperone [7,8]. Further, we and others have elucidated a critical role for a number of ‘chromatin readers’ in melanoma. These are proteins capable of detecting a specific chromatin modification and subsequently initiating regulatory downstream processes. For example, the BET (bromodomain and extraterminal domain) proteins (BRD2, BRD3, BRD4 and BRDT) bind to acetylated lysine residues in histones and function as scaffolds to recruit chromatin regulators, thereby coupling histone acetylation to transcription [9]. Among them, BRD2 and BRD4 are overexpressed in melanoma tissues [10,11] and required for tumor maintenance by supporting cellular proliferation and controlling the expression of key cell-cycle and survival genes [10,11]. Finally, the loss of 5-hydroxymethylcytosine of DNA, mediated by IDH and TET enzymes in melanoma, has also recently been reported [12].

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Histone variants in melanoma

In addition to chromatin writers and readers, components of the nucleosome mediate melanoma progression. Histone variants are sequence and structural variations of the canonical histones, which replace their conventional counterparts within the nucleosome [13]. Histone variants confer unique functions within the chromatin template, and in turn increase chromatin complexity. They can have temporal and tissue-specific expression, and their incorporation into

the nucleosome requires the assistance of additional factors known as histone chaperones. It has become evident in recent years that histone variants contain a unique ability to regulate key cellular and developmental processes, and their deregulation may contribute to cancer initiation and progression [13]. Remarkably, the first histone variant mutations have recently been uncovered in cancer [14,15].

Work from our laboratory has shed light on the role of histone variants in melanoma. We first identified the histone variant macroH2A as a tumor suppressor and showed that its expression is epigenetically silenced during the radial growth phase (RGP) to vertical growth phase (VGP) transition of melanoma progression [16]. This important finding was further supported by the absence of macroH2A in metastatic melanoma. Functional assays demonstrated that macroH2A acts as a barrier to tumor growth and metastasis. Gene expression profiling analysis showed that macroH2A directly regulates the expression of genes that are critical for melanoma maintenance including *CDK8*, a reported oncogene in colorectal cancer. Rescue experiments showed that the tumor suppressive function of macroH2A is mediated, at least in part, by the upregulation of *CDK8*. Finally, we showed that the chromatin remodeler ATRX (Alpha Thalassemia/Mental Retardation Syndrome X-Linked), a regulator of macroH2A [17], can be used as a prognostic marker of melanoma [18].

More recently, we have reported a role of a different variant of the H2A family, namely H2A.Z.2, in promoting proliferation of melanoma cells [11]. This is the first evidence for H2A.Z.2 function in any cancer type. H2A.Z.2 is an isoform of H2A.Z that is distinct from the well-studied isoform H2A.Z.1. Despite their highly similar sequence, H2A.Z.1 mouse knock-out studies suggest that the two genes are nonredundant [19]. We have demonstrated that both H2A.Z isoforms are highly expressed in melanoma specimens and that high levels correlate with shorter survival of patients. While we do not exclude a role for H2A.Z.1 in melanoma pathogenesis, we identified a unique role for H2A.Z.2 as a mediator of melanoma cell proliferation. Through loss-of-function approaches, we showed that H2A.Z.2 exerts a key role in promoting cell cycle progression by controlling the transcriptional output of E2F target genes. Our integrated genomic and transcriptomic analyses further revealed that these genes are

highly expressed and display a unique signature of H2A.Z occupancy. To further dissect H2A.Z function in melanoma, we performed quantitative mass spectrometry. We identified the BET protein BRD2, which is also overexpressed in melanoma, as an H2A.Z interacting protein. We then showed that depletion of BRD2 causes a G1/S arrest, leading us to hypothesize that H2A.Z.2 and BRD2 work together to promote expression of E2F target genes. Indeed, chromatin immunoprecipitation experiments revealed that H2A.Z, BRD2 and E2F1 co-occupy the promoters of H2A.Z.2-regulated genes, and that BRD2 and E2F1 are recruited in a H2A.Z.2-dependent manner. We also found evidence of hyperactivation of the H2A.Z.2–BRD2–E2F1 axis in a subset of melanoma patients, which was not present in benign nevi. Intriguingly, while the BET inhibitor JQ1 [20] exerts a cytostatic effect in melanoma cells, combined depletion of H2A.Z.2 and JQ1 treatment induces apoptosis. H2A.Z.2 deficiency also sensitizes melanoma cells to chemotherapy and to MEK inhibitors.

Conclusion & perspective

In closing, an aberrant expression and/or chromatin incorporation of histone variants can disrupt the cellular epigenetic balance and lead

to altered gene expression programs, ultimately contributing to melanoma initiation and/or progression. Thus, epigenetic regulators may provide new therapeutic targets to be used as alternatives or in a complementary fashion to the classical inhibition of signaling pathways. Our findings implicate histone variants as mediators of tumor growth and metastasis, as well as drug sensitivity, in malignant melanoma. Owing to the fact that histone deposition and chromatin modification are reversible processes, these collective studies hold invaluable therapeutic potential for this challenging cutaneous neoplasm.

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