Review

Molecular Cancer Therapeutics

The Slow Cycling Phenotype: A Growing Problem for Treatment Resistance in Melanoma

Antonio Ahn[1], Aniruddha Chatterjee[1,2], and Michael R. Eccles[1,2]

Abstract

Treatment resistance in metastatic melanoma is a longstanding issue. Current targeted therapy regimes in melanoma largely target the proliferating cancer population, leaving slow-cycling cancer cells undamaged. Consequently, slow-cycling cells are enriched upon drug therapy and can remain in the body for years until acquiring proliferative potential that triggers cancer relapse. Here we overview the molecular mechanisms of slow-cycling cells that underlie treatment resistance in melanoma. Three main areas of molecular reprogramming are discussed that mediate slow cycling and treatment resistance. First, a low microphthalmia-associated transcription factor (MITF) dedifferentiated state activates various signaling pathways. This includes WNT5A, EGFR, as well as other signaling activators, such as AXL and NF-κB. Second, the chromatin-remodeling factor Jumonji/ARID domain-containing protein 1B (JARID1B, *KDM5B*) orchestrates and maintains slow cycling and treatment resistance in a small subpopulation of melanoma cells. Finally, a shift in metabolic state toward oxidative phosphorylation has been demonstrated to regulate treatment resistance in slow-cycling cells. Elucidation of the underlying processes of slow cycling and its utilization by melanoma cells may reveal new vulnerable characteristics as therapeutic targets. Moreover, combining current therapies with targeting slow-cycling subpopulations of melanoma cells may allow for more durable and greater treatment responses. *Mol Cancer Ther; 16(6); 1002–9. ©2017 AACR.*

Downloaded from http://aacrjournals.org/mct/article-pdf/16/6/1002/1854108/1002.pdf by guest on 11 October 2024

Introduction

Targeted therapy of metastatic melanoma carrying *BRAF* oncogenic mutations is heralded as a breakthrough into an era of personalized medicine (1, 2). Inhibition of the hyperactive MAPK pathway in *BRAF*-mutant melanomas induces rapid tumor regression that significantly improves patient survival. However, clinical management of melanoma using targeted therapy remains beset by short-lived treatment responses (2, 3). This is largely due to subpopulations of tumor cells that survive drug cytotoxicity, and quickly acquire new mechanisms of resistance to enable tumor regrowth. Even upon tumor remission, where no trace of melanoma is detectable by clinical examinations or radiographic imaging, relapse can subsequently occur, which suggests that a small residual number of melanoma cells remains undamaged throughout the treatment (4).

Tumor heterogeneity is regarded as one of the main reasons for mixed responses to treatment. A frequently observed pattern in melanoma patients is tumor regression followed by treatment resistance. Furthermore, different tumors from the same patient may display a wide variety of treatment responses, despite sharing the same oncogenic mutations, illustrating the complexity of melanoma heterogeneity that extends beyond genomics (5).

Accumulating evidence suggests that distinct tumor cell phenotypes underlie heterogeneous treatment responses in patients, independent of oncogenic mutations. Slow-cycling or growth-arrested melanoma cells, which appear senescent, are increasingly described as a major determinant for treatment resistance (6–8). The majority of treatment strategies, including chemotherapy and targeted therapies, target the bulk of the tumor with a proliferative phenotype, whereas slow-cycling cancer cells may be left undamaged (8, 9). Consequently, proliferating melanoma cells undergo rapid apoptosis, while those that either retain or switch to a slow-cycling phenotype escape therapeutic stress. Indeed, studies demonstrate that *BRAF*-mutant melanoma cells exhibit increased features of senescence upon exposure to targeted therapies such as vemurafenib (which targets the *BRAFV600E* mutation; ref. 10) and chemotherapy (11).

Although it has long been thought that inhibition of tumor cell growth is a favorable outcome, and is analogous to tumor cell killing, more recent studies indicate that tumor cells with a slow-cycling phenotype may be metabolically active and highly aggressive, with increased potential to form new tumors and metastasize (8, 12–14). This suggests an urgent need to therapeutically target the slow-cycling phenotype in tumor cells, along with the bulk of the tumor that is proliferative, to increase tumor cell death and reduce the risk of developing treatment resistance. Recently, there have been several studies that may offer insight into the molecular mechanisms underlying slow-cycling cells. This review briefly overviews molecular changes associated with the slow-cycling phenotype in melanoma, and potential therapeutic implications. We discuss several key features of molecular reprogramming in melanoma, accompanied by the rewiring of signaling cascades mediating

[1]Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand. [2]Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, New Zealand.

Corresponding Author: Michael R. Eccles, Department of Pathology, Dunedin School of Medicine, University of Otago, 56 Hanover Street, Dunedin, Otago 9054, New Zealand. Phone: 643-479-7878; Fax: 643-479-7136; E-mail: michael.eccles@otago.ac.nz

doi: 10.1158/1535-7163.MCT-16-0535

©2017 American Association for Cancer Research.

dedifferentiation, chromatin remodeling, and a shift in the metabolic state of oxidative phosphorylation (12).

Altered regulation of cell cycling in melanoma cells results from low MITF-mediated dedifferentiation

Microphthalmia-associated transcription factor (MITF) is a master regulator of development and differentiation in the melanocytic lineage (15). In melanoma, MITF also plays a critical role in cell phenotype and tightly controls the transcriptional activity and repression of a large array of genes. Indeed, low and high MITF levels, respectively, have been associated with distinct global gene expression patterns (16, 17). Low levels of MITF have been shown to drive dedifferentiation in melanoma cells as demonstrated by the effect of depletion of MITF leading to increased expression of stem cell markers, while decreasing expression of melanocytic differentiation markers (6, 18). In parallel to a dedifferentiated phenotype, depleting MITF has been shown to reduce cell growth and induce senescence and/or apoptosis (6, 11, 18–21). MITF silencing in human melanoma cell lines leads to increased expression of cell-cycle inhibitors including p21 (CDKN1a) and p27 (CDKN1b; refs. 6, 11, 21). Moreover, silencing MITF triggers a DNA damage response (DDR) pathway that upregulates p53, which then mediates cell-cycle arrest (11). The characteristics of dedifferentiation and a slow-cycling phenotype are key features of malignant melanoma stem or initiating cells (MMIC), which have crucial roles in tumor initiation, progression, metastasis, and treatment resistance. While MITF is an important regulator of the differentiation of melanocytes from a neural crest precursor cell lineage, a full discussion of MMICs in the context of melanoma is beyond the scope of this article and has been reviewed by others (22–24).

The "low MITF" slow-cycling state has important therapeutic implications in melanoma. Low MITF activity confers intrinsic and acquired resistance to MAPK pathway inhibitors in *BRAF*-mutant melanoma cell lines and is associated with vemurafenib-resistance in patient biopsies (25–28).

The therapeutic resistance associated with the low MITF dedifferentiated state suggests that mechanisms promoting survival in response to MAPK pathway inhibitors have been activated. Current clinical strategies involving targeted treatment generally target melanoma cell populations that are "addicted" to the hyperactive MAPK pathway for survival and rapid cell division. However, increasing evidence suggests that melanomas in a low MITF–mediated dedifferentiated state have "rewired" signaling cascades that redistribute oncogenic signaling toward multiple pathways, including non-MAPK pathways, in addition to the MAPK pathway (25, 26, 28). In other words, instead of total dependence on a single growth-regulatory pathway, dedifferentiation allows usage of multiple pathways to confer drug resistance (12).

Upon MAPK pathway inhibition, a low MITF state in small subpopulations of slow-cycling cells within tumors allows them to utilize alternative mechanisms for survival. This correlates with various signaling activators and confers treatment resistance, and therefore permits time for slow-cycling cells to acquire additional mutations and/or epigenetic aberrations that subsequently can allow tumor regrowth and tumor relapse (29). In the following, we discuss several of the key signaling activators, including WNT5A and EGFR, which are associated with low MITF expression and with the slow-cycling phenotype.

Therapeutic stress promotes the survival and metastasis of WNT5A-expressing cells

WNT5A signaling occurs via a noncanonical WNT pathway and elicits dedifferentiation by suppressing MITF levels (30), promoting a senescence-like cellular phenotype (14). Melanoma cells with high WNT5A expression were shown to respond to therapeutic stress induced by vemurafenib treatment by further increasing WNT5A signaling, which then led to cell-cycle arrest via upregulation of the cell-cycle inhibitor, p21. Increased levels of conventional senescence markers including senescence-associated β-galactosidase (SA-β-gal), senescence-associated heterochromatic foci (SAHF), H3K9Me chromatin markers, and PML bodies accompanied the growth arrest (14). However, although cell division was suppressed, cells expressing high levels of WNT5A were able to reenter the cell cycle, indicating they were not truly senescent (thus termed senescence-like).

High WNT5A expression levels in melanoma cell lines and tumors from patients prior to treatment, were associated with significantly poorer response to vemurafenib compared with tumors and cell lines with low WNT5A expression levels, which suggests that WNT5A signaling may be functionally linked to innate resistance (31, 32). In addition, following BRAF inhibitor treatment, melanoma cell lines and tumors generally had increased expression of WNT5A (31, 32). Potential BRAF inhibitor resistance mechanisms associated with WNT5A signaling include reactivation of the MAPK pathway; indeed, not only does WNT5A signaling lead to resistance to BRAF inhibitors, but elevated WNT5A expression also increases ERK activity in melanoma cell lines (32). Mechanisms that result in activation of the MAPK pathway lead to reduced dependency on the activity of mutant *BRAF*, including switching to an alternative RAF isoform such as CRAF (33, 34), and activation of the FGF receptor 3/RAS–signaling axis (35). WNT5A has also been shown to activate the PI3K–AKT pathway via the WNT receptors FZD7 and RYK (31).

WNT5A-expressing growth-arrested cells were found to possess enhanced invasiveness upon increasing therapeutic dosage (14). Given that WNT5A suppresses MITF, the increased metastatic potential of WNT5A-expressing cells is consistent with reports that low MITF also confers increased invasiveness (16, 21, 32, 36). This indicates that targeted therapy may rapidly kill proliferative cells, but that it may simultaneously promote invasiveness and metastasis in a subpopulation of slow-cycling WNT5A-high cells that are able to adaptively resist therapeutic stress. Indeed, WNT5A-high cells were capable of reentering the cell cycle and forming new tumor colonies at distant body sites in *in vivo* experiments (14). There are a number of reports supporting the notion that BRAF inhibitors promote metastasis. For example, proliferative melanoma cell lines adopt invasive characteristics upon MAPK pathway inhibition in *in vitro* experiments (27), and resistance to vemurafenib selects for a highly malignant phenotype with increase metastatic potential to the lungs in *in vivo* mouse experiments (37). Consistent with a dedifferentiated state, the metastatic cells in the lungs of these mice were shown to express higher levels of cancer stem cell markers such as CD271, JARD1B, and fibronectin. Also in the clinical setting, targeted therapy has been reported to promote the development of new metastases during treatment relapse (38).

In contrast to regulation of a slow-cycling phenotype by WNT5A, there are also reports that WNT5A is associated with

Downloaded from http://aacrjournals.org/mct/article-pdf/16/6/1002/1854108/1002.pdf by guest on 11 October 2024

proliferation (31). In two melanoma cell lines, WNT5A overexpression increased proliferation, whereas knockdown of WNT5A reduced cell division (31), highlighting the complexity of the regulation of cell division by WNT5A.

WNT5A is a potential therapeutic target for targeting slow-cycling cells as a first-line combination therapy. Knockdown of WNT5A inhibits invasive potential and ablates the formation of *in vivo* metastases of primary human melanoma cell lines injected into the tail vein of athymic nude mice (14, 30).

EGFR signaling confers treatment resistance in slow-cycling melanoma cells

EGFR signaling and enhanced expression of EGFR induce a slow-cycling phenotype, and elevated levels of cell-cycle inhibitors including $p21^{Cip1}$ (CDKN1A), $p27^{Kip1}$ (CDKN1B), as well as activated hypophosphorylated retinoblastoma protein (7). Activation of various receptor tyrosine kinase (RTK) signaling pathways has been demonstrated to confer resistance to MAPK pathway inhibitors (7, 26, 28). However, EGFR signaling is reported as being crucial for the development of resistance to MAPK pathway inhibitors in melanoma (7, 28, 39).

When EGFR signaling was assessed in post-treatment patient biopsies compared with pre-treatment tumors, 6 of 16 patients had increased EGFR activation as detected by IHC (7). Treatment resistance attributed to EGFR signaling was also shown in melanoma cell lines (28). EGFR signal transduction pathways have been shown to be part of the same transcriptional program as WNT5A, promoting resistance to vemurafenib in whole-transcriptome microarray analysis (31). In colorectal cancer (CRC), resistance to vemurafenib treatment of *BRAF*-mutant CRC is mediated by EGFR expression, which is a consequence of the epithelial lineage of CRC. Intrinsically activated EGFR signaling pathways are found to promote innate resistance to BRAF inhibitors in CRC (40, 41). It has been demonstrated that upon BRAF inhibition, EGFR signaling enables feedback reactivation of the MAPK pathway (42) and activation of the PI3K–AKT pathway (41). Increased EGFR transcription results following the demethylation of DNA enhancer elements in the EGFR promoter (39). While the EGFR signaling pathway is normally inactive in the melanocyte neural crest lineage (43), dedifferentiation in melanoma as a result of low MITF levels leads to increased activity of the EGFR signaling pathway (28). Indeed, EGFR-mediated resistance in melanoma is associated with profound alterations in gene expression patterns, which correlate with dedifferentiation, as defined by a reduction in MITF levels together with reduction in downstream targets of MITF and other important melanocytic regulators, such as SOX10, PAX3, and LEF1. Knockdown of MITF leads to the activation of an autocrine drug resistance loop that consists of increased secretion of EGF ligand and increases EGFR expression (28). Forced expression of MITF in melanoma inhibits EGFR signaling and increases sensitivity to BRAF and MEK inhibitors (28). Interestingly, increased expression of MITF in CRC cells was also found to increase sensitivity to BRAF inhibitors (28).

In one study, slow-cycling populations of melanoma cells with high EGFR expression were associated with low levels of the transcription factor SOX10, which is known to both upregulate, and interact with MITF to regulate melanocyte lineage development (7). Like low MITF, low expression of SOX10 promotes TGFβ signaling, which in turn activates numerous receptor tyrosine kinases, including EGFR, PDGFR-B, ERBB3, and AXL (7, 26). Cells expressing high EGFR and low SOX10 were resistant to BRAF and MEK inhibitors and exhibited slow cycling. However, upon treatment with MAPK inhibitors, cells with high EGFR and low SOX10 were selectively enriched, while cell division was increased. Interestingly, upon discontinuation of treatment with MAPK inhibitors, there was a depletion of cells with high EGFR and low SOX10. These data suggest that the high EGFR and SOX10 axis is only beneficial in the presence of MAPK inhibitors and without MAPK inhibitors, the cells return to a drug-sensitive state (7).

Combination treatment of melanoma cells with gefitinib, an EGFR inhibitor, and vemurafenib was unable to inhibit proliferation of the drug-resistant cells, indicating that other routes of drug resistance were also present (7). As RTKs, such as PDGFR-B and ERBB3, are expressed in melanoma cells with low SOX10, and given that many RTKs stimulate both MAPK and PI3K–AKT downstream pathways, the combined inhibition of MAPK and PI3K–AKT pathways using BRAF and PI3K inhibitors was found to restore inhibition of proliferation in the drug-resistant melanoma cells (7).

Other signaling activators

A range of other signal activators was found to be associated with a low MITF dedifferentiated state and treatment resistance in melanoma. The expression of AXL tyrosine receptor kinase expression was significantly increased by low MITF levels, and conferred both intrinsic and acquired resistance in melanoma cell lines (25, 26). Expression and activation of AXL, as determined by phosphorylation, as well as other RTKs was further increased upon exposure to vemurafenib. AXL was able to maintain MAPK pathway activity and activate the PI3K–AKT pathway when exposed to BRAF or MEK inhibition (25). The high AXL/low MITF expression profile was also observed in TCGA data, which includes transcriptional profiles for 356 patients, as well as in tumor samples obtained from patient-derived xenografts (26). Other RTKs that increased with high AXL included EGFR and PDGFR. It is thought that MITF suppresses these RTKs either indirectly or directly, and thus reduced MITF levels lead to the activation of these various RTKs (26). Inhibiting AXL did not result in significant tumor cell death in drug-resistant cell lines suggesting that, although AXL contributes to the drug-resistant phenotype, other RTK activity may also need to be cotargeted (25).

The activation of NFκB was shown to be an important player in promoting treatment resistance in MITF-low cells (25). In treatment-naïve melanoma cell lines, cells with high NFκB and low MITF expression were intrinsically resistant to both single-agent inhibition at multiple points in the MAPK pathway, and to combination BRAF/MEK inhibition (25). Furthermore, NFκB signaling was sufficient to promote a switch to a low MITF state that enabled resistance to MAPK pathway inhibition (25).

The dual phenotypic role of MITF and its therapeutic implications

As previously discussed, the depletion of MITF mediates senescence via dedifferentiation in melanoma (21). However, MITF activity has been shown to be a central regulator of distinct cellular phenotypes, highlighting its complex role. For instance, MITF plays an essential role in cell division by suppressing the cell-cycle inhibitor $p27^{Kip1}$ (*CDKN1B*; refs. 20, 21, 44, 45). However, other studies have reported that high MITF levels suppress cell division by activating $p16^{Ink4a}$

Downloaded from http://aacrjournals.org/mct/article-pdf/16/6/1002/1854108/1002.pdf by guest on 11 October 2024

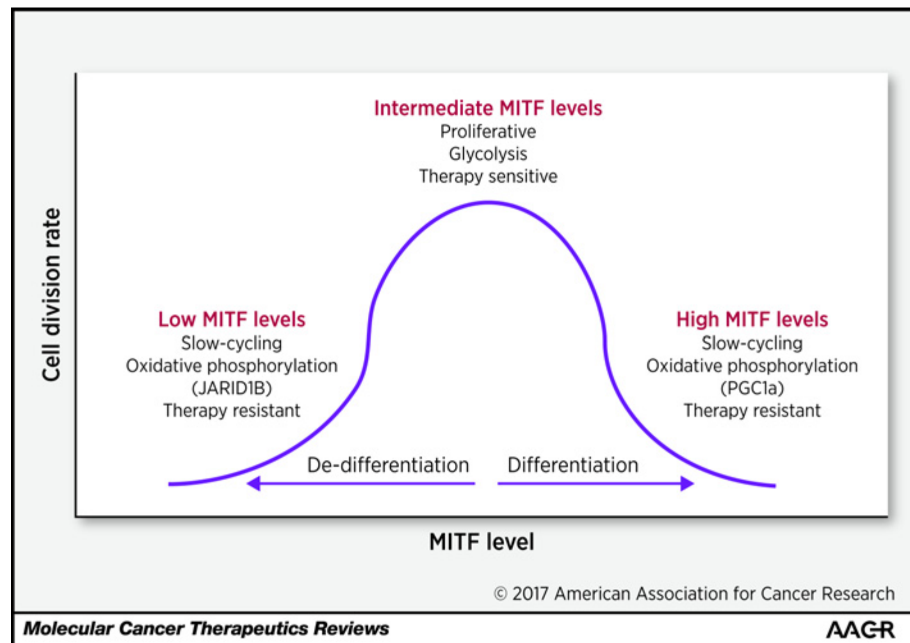

Figure 1.
Two states, corresponding to either low or high levels of MITF, contribute to cells with a slow-cycling phenotype in melanoma. The melanoma rheostat model (21) proposes that different levels of MITF regulate distinct phenotypic states: low MITF is associated with dedifferentiation and controls a senescent-like phenotype, while intermediate MITF controls proliferation, and high MITF is associated with differentiation-mediated senescence. The proliferative phenotype of an intermediate MITF level is dependent on glycolysis to fuel cell division. Cells with intermediate MITF are addicted to the MAPK pathway, and consequently rapid apoptosis is elicited upon inhibition of MAPK signaling. In contrast, the slow-cycling phenotypes, mediated by either dedifferentiation or differentiation, are therapy resistant, and utilize oxidative phosphorylation to maintain survival. In the dedifferentiated state, the oncogenic burden is distributed to various signaling pathways, which reduces the melanoma cell's dependency on the MAPK pathway, and consequently leads to resistance to MAPK pathway inhibition.

(*CDKN2A*; ref. 46) and p21^{Cip1} (*CDKN1A*; refs. 46, 47). To explain the paradoxical function of MITF in regulating both proliferation and cell-cycle arrest, the rheostat model was proposed (21). This model proposes that different MITF levels drive distinct cellular phenotypes. Low levels of MITF induce dedifferentiation resulting in a senescence-like phenotype, intermediate MITF levels favor cell proliferation, and higher levels of MITF result in differentiation-mediated senescence (ref. 21; Fig. 1).

Increasing numbers of studies have expanded the rheostat model with therapeutic implications. High MITF expression and increased expression of its melanocyte-specific target genes were reported to be associated with treatment resistance to various single-agent MAPK pathway inhibitors, as well as to combined inhibition of BRAF and MEK (26, 48–52). Ectopic MITF overexpression was reported to promote resistance to BRAF, MEK, or ERK inhibitors (25, 48). Moreover, genomic amplification of MITF is found in 15.2% of metastatic melanoma patients and is associated with poorer prognosis and resistance to chemotherapeutic agents (44).

In contrast, MITF expression has also been shown to be necessary in *BRAF*-mutant melanomas to facilitate the response to MAPK pathway inhibition (25–27). *BRAF*-mutant melanoma cell lines were responsive to MAPK pathway inhibition only in those cell lines that exhibited MITF activity (25, 26). The association of MITF with both treatment resistance and susceptibility is reminiscent of the dual role of MITF in proliferation and differentiation-mediated senescence. According to the rheostat model, intermediate MITF levels regulate the proliferative phenotype and confer susceptibility to MAPK pathway inhibition, whereas higher MITF expression levels mediate differentiation-mediated senescence and confer drug resistance (Fig. 1).

Chromatin remodeling orchestrates slow cycling and intrinsic treatment resistance

Chromatin remodeling provides a major mechanism to control gene transcription. The chemical modification of chromatin opens up DNA regions, which are otherwise condensed, for access to transcription factors and RNA polymerase (53). Cancer cells are capable of epigenetically reprogramming their transcriptional processes through chromatin-remodeling factors to maintain an oncogenic phenotype (54). In melanoma, aberrant chromatin remodeling through histone demethylation has been shown to play an important role in switching to a slow-cycling state.

In melanoma cell lines and in tumors of patients with advanced melanoma, the chromatin-remodeling factor, JARID1B (KDM5B), was highly expressed in small subpopulations of cells (8, 55, 56). JARID1B is a member of the highly conserved family of jumonji/ARID1 and it functions to remove methyl groups from histone 3 lysine 4 (H3K4) residues, which in turn alters the chromatin structure and transcriptional expression patterns. JARID1B-high cells exhibit slow-cycling properties, with a cell division doubling rate of more than 4 weeks (13). Moreover, JARID1B-high cells lack protein expression of proliferation marker Ki-67 (13) and have increased activity of

Downloaded from http://aacrjournals.org/mct/article-pdf/16/6/1002/1854108/1002.pdf by guest on 11 October 2024

retinoblastoma protein, which downregulates E2F target genes and induces cellular senescence (57).

Slow-cycling JARID1B-positive cells display intrinsic resistance to various chemotherapeutic drugs (cisplatin, bortezomib, temozolomide), and to vemurafenib (8). This resistance mechanism results in enrichment of JARID1B-positive cells upon vemurafenib exposure in melanoma cell lines and in *in vivo* human melanoma xenografts. Moreover, melanoma biopsies obtained before and after relapse from the same patients, who were treated with vemurafenib, displayed increased JARID1B expression post-treatment (8). Further investigations revealed that the primary mechanism of enrichment of JARID1B-high cells following drug exposure was through the selection of preexisting JARID1B-positive slow-cycling cells. In addition, induction via an adaptive mechanism also occurred, although to a lesser degree (8). Pathway analysis revealed increased PI3K–AKT pathway signaling in JARID1B-high cells, which supports the notion that slow-cycling cells have altered their dependency on growth-stimulatory pathways to allow them to escape programmed cell death or senescence when the MAPK pathway is inhibited (8). A quiescent cellular state with drug resistance has also been associated with histone demethylase JARID1A (*KDM5A*) in numerous cancer types, including melanoma (58). This is associated with increased IGFR1 signaling, which is again indicative of activation of an alternative growth-stimulatory pathway, and escape from therapeutic stress resulting from MAPK pathway inhibition.

As alluded to above, adaptive mechanisms may occur during induction of drug resistance in contrast to the enrichment of preexisting drug-resistant cells in an initial cell population (8). For example, in one study where vemurafenib-induced drug-tolerant cells (IDTC) were generated, global chromatin modifiers were also induced, including upregulation of JARID1A, JARID1B, and the activity of various other histone demethylases (12). In the IDTCs, the transcription of genes involved in cell proliferation and DNA replication was reduced, as well as cell growth rates, which is characteristic of a quiescent phenotypic state. Dedifferentiation was demonstrated by the increased expression of putative melanoma stem cell markers, such as CD271, and the loss of melanocyte-specific antigens including tyrosinase and melan-A (12). Moreover, multiple signaling pathways were upregulated including the MAPK pathway, the PI3K–AKT pathway, and the mTOR pathway.

Cells with a shift in metabolic state due to oxidative phosphorylation are resistant to therapy

Cancer cell proliferation is associated with a shift in cell metabolism toward glycolysis, a phenomenon commonly referred to as the Warburg effect (59, 60). This process is also termed aerobic glycolysis given that glycolysis is maintained, despite sufficient oxygen supply and an intact mitochondrial respiration system (61, 62). Glycolysis permits fast production of ATP and the molecular building blocks required to support rapid cell division and biomass accumulation (61). Indeed, *BRAF*-mutant malignant melanomas are dependent on glycolysis to permit continuous cell division, as demonstrated by reduced oxygen consumption rate, and increased levels of lactate and glycolytic enzymes (63, 64).

Recently, there has been a growing body of evidence supporting the notion that cancers use multiple strategies of metabolism (59). For instance, oxidative phosphorylation underlies a subpopulation of slow-cycling cells in melanoma (8). Subpopulations of slow-cycling cells with high levels of JARID1B chromatin-remodeling factor were shown to feature a high level of oxidative phosphorylation. Proteomic profiling revealed the JARID1B-overexpressing cells had elevated expression of mitochondrial enzymes of electron transport chain complexes, including ATP-synthase and NADPH dehydrogenase (complex I). Accordingly, the JARID1B-high cells also had higher mitochondrial energy production, consumed more oxygen, and generated more peroxide compared with control cells (8).

Increasing evidence suggests that oxidative phosphorylation mediates treatment resistance in various cancers including melanoma (65). For example, treatment of *BRAF*-mutant melanoma cell lines with vemurafenib enriched for resistant cells that had increased oxidative phosphorylation (8, 66, 67). Oxidative phosphorylation was demonstrated with upregulation of numerous mitochondrial proteins, increased oxygen consumption rates (8, 67), increased mitochondria density (66), and reduced levels of lactate (66, 67). Consistently, *BRAF*-mutant melanoma samples excised from patients who have developed treatment resistance were found to exhibit increased expression of oxidative phosphorylation gene expression (66). Overall, this suggests that metabolic heterogeneity in melanoma may underlie different cellular phenotypes as well as distinct responses to therapeutic drugs.

While proliferative melanoma cells utilizing glycolytic pathways are susceptible to therapeutic drugs, oxidative phosphorylation in a subpopulation of slow-cycling melanomas allows the energy usage to be diverted from cell division toward more efficient energy production, favoring cell survival (8, 65, 66). Consequently, slow-cycling cells with an enhanced potential for oxidative phosphorylation, and suppression of proliferative potential, are selected upon therapeutic stress. Slow-cycling cancer cells that utilize oxidative phosphorylation for drug resistance have also been observed in pancreatic tumors (68, 69), glioblastoma (70), and acute myelogenous leukemia (71). In pancreatic tumors, subpopulations of slow-cycling tumor cells with cancer stem cell–like features were identified that depended on mitochondrial respiration to survive the toxic effects of therapeutic drugs, such as doxycycline (68). These drug-resistant cells characteristically expressed cancer stem cell markers, and displayed increased mitochondrial respiration (68). Drug-resistant tumor cells can remain dormant for months in a slow-cycling state until tumor relapse.

Like JARID1B, PGC1α has a role in oxidative phosphorylation and resistance to therapy. PGC1α is one of the key drivers mediating oxidative phosphorylation and treatment resistance in melanoma (66, 72). PGC1α is a transcriptional coactivator known for its role in the regulation of mitochondrial biogenesis. It has a central role in upregulating oxidative phosphorylation in various tissue types (73). *BRAF*-mutant melanoma cell lines treated with vemurafenib exhibit significantly increased levels of PGC1α-mediated oxidative phosphorylation. In addition, an upregulated gene expression program of mitochondrial oxidative phosphorylation, an increased oxygen consumption rate, and a reduction of lactate secretion, glycolysis, and glucose uptake are observed in vemurafenib-treated melanoma cells (66, 72). PGC1α also has a role in the detoxification of reactive oxygen species (72).

Even though JARD1B and PGC1α both govern oxidative phosphorylation, these two factors do not cooperate in the same

Downloaded from http://aacrjournals.org/mct/article-pdf/16/6/1002/1854108/1002.pdf by guest on 11 October 2024

pathway, and current data suggest they represent two distinct mechanistic pathways. More than one mechanism controls oxidative metabolism during treatment resistance in melanoma. Melanoma cell lines treated with vemurafenib upregulate oxidative metabolism regardless of PGC1α expression (67). In addition, JARID1B and PGC1α appear to represent two distinct states of differentiation (Fig. 1). JARID1B is reported to be a putative stem cell marker with roles in stem cell biology and in inducing pluropotency (74, 75). High JARID1B–expressing cells were shown to have increased tumorigenic potential with increased ability to sustain continuous tumor growth (13). Throughout serial xenograft transplantation where human melanoma is grown through serial passage in mice, JARID1B-negative cells eventually reach a replicative ceiling and become exhausted, whereas JARID1B-positive cells can be continuously passaged indefinitely. In contrast, in a subpopulation of melanoma cells, a high MITF melanocyte-specific differentiation program drives PGC1α-mediated oxidative phosphorylation, as well as the downstream targets of MITF, such as *TYR*, *MLANA*, and *DCT* (66). MITF was found to directly stimuate PGC1α expression by binding to its promoter (66, 72).

The rheostat model and regulation of oxidative phosphorylation

Collectively, the JARID1B and MITF–PGC1α axes could regulate oxidative phosphorylation and drug resistance mechanisms at different ends of the rheostat model. Slow cycling occurs with either low MITF (dedifferentiation) or with high MITF (differentiation). JARID1B mediates oxidative phosphorylation in slow-cycling cells that are dedifferentiated. In contrast, differentiated slow-cycling cells utilize the MITF–PGC1α axis to mediate oxidative phosphorylation and treatment resistance (Fig. 1). Interestingly, the MITF–PGC1α axis correlates with a proliferation expression signature. However, the forced expression of PGC1α did not lead to enhanced cell division, indicating that it does not regulate proliferation (66).

Oxidative phosphorylation serves as a valuable therapeutic target, as slow-cycling cells depend on it for metabolic energy. Mitochondrial inhibitors including oligomycin A, an ATP synthase inhibitor, and other mitochondrial inhibitors were able to suppress the enrichment of a JARID1B-positive subpopulation (8). Tumor cell death was also enhanced in melanoma cells that utilized a MITF–PGC1α axis–mediated treatment resistance pathway, when treated with mitochondrial inhibitors in combination with chemotherapeutic agents, or with vemurafenib (66). However, drugs that inhibit the mitochondrial complexes typically have a narrow therapeutic index, which means that frequently they are too toxic to be used in the clinic. The pro-oxidative drug, elesclomol, has a wider therapeutic index, and demonstrates antitumor activity in vemurafenib-resistant melanoma cells *in vitro* and *in vivo* (67). Elesclomol induces apoptosis by increasing oxidative stress (76–78); apoptosis is induced by elesclomol in slow-cycling cells with high JARID1B, preventing the enrichment of treatment-resistant JARID1B-positive cells (79). Cancerous cells with oxidative phosphorylation already have increased levels of endogenous reactive oxygen species (8). Therefore, this generates increased dependence on anti-oxidation for survival, making slow-cycling melanoma cells more sensitive to pro-oxidative drugs compared with normal cells. Elevated levels of reactive oxygen species selectively elicit cell death in tumors with high levels of oxidative phosphorylation. These data demonstrate that multidrug resistance in slow-cycling cells can be overcome by combining elesclomol with therapeutic drugs such as the BRAF inhibitor, vemurafenib (79). Elesclomol has shown clinical efficacy in a phase III trial in a subgroup of metastatic melanoma patients with normal baseline lactate dehydrogenase (LDH; ref. 80). Normal LDH levels reflect slow-cycling cancerous cells that depend on oxidative phosphorylation, and which are susceptible to elesclomol. In contrast, high LDH levels are associated with rapidly proliferating tumor cells, which are largely dependent on glycolysis, have low levels of reactive oxygen species, and are more resistant to elesclomol (79, 81). Therefore, combining targeted therapy with drugs that target oxidative phosphorylation may be a promising therapeutic strategy.

Conclusions

Melanoma cells that are slow cycling are highly resistant to conventional chemotherapy and to current targeted therapy regimes. While keeping cancerous cells in a senescent nonproliferative state has been suggested as an efficient therapeutic option, increasing evidence suggests that these cells could be wrongly interpreted as being senescent, but rather are slow cycling, as they can re-gain proliferative characteristics. Slow-cycling cells can rapidly acquire enhanced levels of proliferation leading to cancer relapse. The characterization of the slow-cycling phenotypes may therefore lead to the identification of vulnerable characteristics of tumor cells that may serve as therapeutic targets to overcome treatment resistance and ultimately improve melanoma therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

The authors acknowledge financial support from the University of Otago (to A. Ahn), and the New Zealand Institute for Cancer Research Trust (to A. Chatterjee, M.R. Eccles), and the Maurice Wilkins Centre for Molecular Biodiscovery (to A. Chatterjee, M.R. Eccles).

Received August 10, 2016; revised September 27, 2016; accepted January 20, 2017; published online June 2, 2017.

Downloaded from http://aacrjournals.org/mct/article-pdf/16/6/1002/1854108/1002.pdf by guest on 11 October 2024

References

1. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 2010;363:809–19.
2. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011;364:2507–16.
3. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med 2012;367:1694–703.
4. Tolk H, Satzger I, Mohr P, Zimmer L, Weide B, Schad S, et al. Complete remission of metastatic melanoma upon BRAF inhibitor treatment - what happens after discontinuation? Melanoma Res 2015;25:362–6.
5. Shi H, Hugo W, Kong X, Hong A, Koya RC, Moriceau G, et al. Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. Cancer Discov 2014;4:80–93.
6. Cheli Y, Giuliano S, Botton T, Rocchi S, Hofman V, Hofman P, et al. Mitf is the key molecular switch between mouse or human melanoma initiating cells and their differentiated progeny. Oncogene 2011;30:2307–18.

7. Sun C, Wang L, Huang S, Heynen GJ, Prahallad A, Robert C, et al. Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. Nature 2014;508:118–22.
8. Roesch A, Vultur A, Bogeski I, Wang H, Zimmermann KM, Speicher D, et al. Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B(high) cells. Cancer Cell 2013;23:811–25.
9. Moore N, Houghton J, Lyle S. Slow-cycling therapy-resistant cancer cells. Stem Cells Dev 2012;21:1822–30.
10. Haferkamp S, Borst A, Adam C, Becker TM, Motschenbacher S, Windhovel S, et al. Vemurafenib induces senescence features in melanoma cells. J Invest Dermatol 2013;133:1601–9.
11. Giuliano S, Cheli Y, Ohanna M, Bonet C, Beuret L, Bille K, et al. Microphthalmia-associated transcription factor controls the DNA damage response and a lineage-specific senescence program in melanomas. Cancer Res 2010;70:3813–22.
12. Ravindran Menon D, Das S, Krepler C, Vultur A, Rinner B, Schauer S, et al. A stress-induced early innate response causes multidrug tolerance in melanoma. Oncogene 2015;34:4448–59.
13. Roesch A, Fukunaga-Kalabis M, Schmidt EC, Zabierowski SE, Brafford PA, Vultur A, et al. A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. Cell 2010;141: 583–94.
14. Webster MR, Xu M, Kinzler KA, Kaur A, Appleton J, O'Connell MP, et al. Wnt5A promotes an adaptive, senescent-like stress response, while continuing to drive invasion in melanoma cells. Pigment Cell Melanoma Res 2015;28:184–95.
15. Hartman ML, Czyz M. MITF in melanoma: mechanisms behind its expression and activity. Cell Mol Life Sci 2015;72:1249–60.
16. Jeffs AR, Glover AC, Slobbe LJ, Wang L, He S, Hazlett JA, et al. A gene expression signature of invasive potential in metastatic melanoma cells. PLoS One 2009;4:e8461.
17. Hoek KS, Schlegel NC, Brafford P, Sucker A, Ugurel S, Kumar R, et al. Metastatic potential of melanomas defined by specific gene expression profiles with no BRAF signature. Pigment Cell Melanoma Res 2006;19: 290–302.
18. He S, Li CG, Slobbe L, Glover A, Marshall E, Baguley BC, et al. PAX3 knockdown in metastatic melanoma cell lines does not reduce MITF expression. Melanoma Res 2011;21:24–34.
19. Kido K, Sumimoto H, Asada S, Okada SM, Yaguchi T, Kawamura N, et al. Simultaneous suppression of MITF and BRAF V600E enhanced inhibition of melanoma cell proliferation. Cancer Sci 2009;100:1863–9.
20. Du J, Widlund HR, Horstmann MA, Ramaswamy S, Ross K, Huber WE, et al. Critical role of CDK2 for melanoma growth linked to its melanocyte-specific transcriptional regulation by MITF. Cancer Cell 2004;6: 565–76.
21. Carreira S, Goodall J, Denat L, Rodriguez M, Nuciforo P, Hoek KS, et al. Mitf regulation of Dia 1 controls melanoma proliferation and invasiveness. Genes Dev 2006;20:3426–39.
22. Nguyen N, Couts KL, Luo Y, Fujita M. Understanding melanoma stem cells. Melanoma Manag 2015;2:179–88.
23. Murphy GF, Wilson BJ, Girouard SD, Frank NY, Frank MH. Stem cells and targeted approaches to melanoma cure. Mol Aspects Med 2014;39:33–49.
24. Schatton T, Frank MH. Cancer stem cells and human malignant melanoma. Pigment Cell Melanoma Res 2008;21:39–55.
25. Konieczkowski DJ, Johannessen CM, Abudayyeh O, Kim JW, Cooper ZA, Piris A, et al. A melanoma cell state distinction influences sensitivity to MAPK pathway inhibitors. Cancer Discov 2014;4:816–27.
26. Muller J, Krijgsman O, Tsoi J, Robert L, Hugo W, Song C, et al. Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma. Nat Commun 2014;5:5712.
27. Zipser MC, Eichhoff OM, Widmer DS, Schlegel NC, Schoenewolf NL, Stuart D, et al. A proliferative melanoma cell phenotype is responsive to RAF/MEK inhibition independent of BRAF mutation status. Pigment Cell Melanoma Res 2011;24:326–33.
28. Ji Z, Erin Chen Y, Kumar R, Taylor M, Jenny Njauw CN, Miao B, et al. MITF Modulates Therapeutic Resistance through EGFR Signaling. J Invest Dermatol 2015;135:1863–72.
29. Chatterjee A, Stockwell PA, Ahn A, Rodger EJ, Leichter AL, Eccles MR. Genome-wide methylation sequencing of paired primary and metastatic cell lines identifies common DNA methylation changes and a role for EBF3 as a candidate epigenetic driver of melanoma metastasis. Oncotarget 2016;8:6085–101.
30. Dissanayake SK, Olkhanud PB, O'Connell MP, Carter A, French AD, Camilli TC, et al. Wnt5A regulates expression of tumor-associated antigens in melanoma via changes in signal transducers and activators of transcription 3 phosphorylation. Cancer Res 2008;68:10205–14.
31. Anastas JN, Kulikauskas RM, Tamir T, Rizos H, Long GV, von Euw EM, et al. WNT5A enhances resistance of melanoma cells to targeted BRAF inhibitors. J Clin Invest 2014;124:2877–90.
32. O'Connell MP, Marchbank K, Webster MR, Valiga AA, Kaur A, Vultur A, et al. Hypoxia induces phenotypic plasticity and therapy resistance in melanoma via the tyrosine kinase receptors ROR1 and ROR2. Cancer Discov 2013;3:1378–93.
33. Montagut C, Sharma SV, Shioda T, McDermott U, Ulman M, Ulkus LE, et al. Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. Cancer Res 2008;68:4853–61.
34. Villanueva J, Vultur A, Lee JT, Somasundaram R, Fukunaga-Kalabis M, Cipolla AK, et al. Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. Cancer Cell 2010;18:683–95.
35. Yadav V, Zhang X, Liu J, Estrem S, Li S, Gong XQ, et al. Reactivation of mitogen-activated protein kinase (MAPK) pathway by FGF receptor 3 (FGFR3)/Ras mediates resistance to vemurafenib in human B-RAF V600E mutant melanoma. J Biol Chem 2012;287:28087–98.
36. Eccles MR, He S, Ahn A, Slobbe LJ, Jeffs AR, Yoon HS, et al. MITF and PAX3 play distinct roles in melanoma cell migration; outline of a "genetic switch" theory involving MITF and PAX3 in proliferative and invasive phenotypes of melanoma. Front Oncol 2013;3:229.
37. Zubrilov I, Sagi-Assif O, Izraely S, Meshel T, Ben-Menahem S, Ginat R, et al. Vemurafenib resistance selects for highly malignant brain and lung-metastasizing melanoma cells. Cancer Lett 2015;361:86–96.
38. Menzies AM, Haydu LE, Carlino MS, Azer MW, Carr PJ, Kefford RF, et al. Inter- and intra-patient heterogeneity of response and progression to targeted therapy in metastatic melanoma. PLoS ONE 2014;9:e85004.
39. Wang J, Huang SK, Marzese DM, Hsu SC, Kawas NP, Chong KK, et al. Epigenetic changes of EGFR have an important role in BRAF inhibitor-resistant cutaneous melanomas. J Invest Dermatol 2015;135:532–41.
40. Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995;19:183–232.
41. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 2012;483:100–3.
42. Corcoran RB, Ebi H, Turke AB, Coffee EM, Nishino M, Cogdill AP, et al. EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. Cancer Discov 2012;2:227–35.
43. Real FX, Rettig WJ, Chesa PG, Melamed MR, Old LJ, Mendelsohn J. Expression of epidermal growth factor receptor in human cultured cells and tissues: relationship to cell lineage and stage of differentiation. Cancer Res 1986;46:4726–31.
44. Garraway LA, Widlund HR, Rubin MA, Getz G, Berger AJ, Ramaswamy S, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. Nature 2005;436:117–22.
45. Widlund HR, Horstmann MA, Price ER, Cui J, Lessnick SL, Wu M, et al. Beta-catenin-induced melanoma growth requires the downstream target Microphthalmia-associated transcription factor. J Cell Biol 2002;158:1079–87.
46. Loercher AE, Tank EM, Delston RB, Harbour JW. MITF links differentiation with cell cycle arrest in melanocytes by transcriptional activation of INK4A. J Cell Biol 2005;168:35–40.
47. Carreira S, Goodall J, Aksan I, La Rocca SA, Galibert MD, Denat L, et al. Mitf cooperates with Rb1 and activates p21Cip1 expression to regulate cell cycle progression. Nature 2005;433:764–9.
48. Van Allen EM, Wagle N, Sucker A, Treacy DJ, Johannessen CM, Goetz EM, et al. The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. Cancer Discov 2014;4:94–109.
49. Smith MP, Ferguson J, Arozarena I, Hayward R, Marais R, Chapman A, et al. Effect of SMURF2 targeting on susceptibility to MEK inhibitors in melanoma. J Natl Cancer Inst 2013;105:33–46.
50. Haq R, Yokoyama S, Hawryluk EB, Jonsson GB, Frederick DT, McHenry K, et al. BCL2A1 is a lineage-specific antiapoptotic melanoma oncogene that

Downloaded from http://aacrjournals.org/mct/article-pdf/16/6/1002/1854108/1002.pdf by guest on 11 October 2024

confers resistance to BRAF inhibition. Proc Natl Acad Sci U S A 2013;110: 4321–6.
51. Johannessen CM, Johnson LA, Piccioni F, Townes A, Frederick DT, Donahue MK, et al. A melanocyte lineage program confers resistance to MAP kinase pathway inhibition. Nature 2013;504:138–42.
52. Smith MP, Brunton H, Rowling EJ, Ferguson J, Arozarena I, Miskolczi Z, et al. Inhibiting drivers of non-mutational drug tolerance is a salvage strategy for targeted melanoma therapy. Cancer Cell 2016;29:270–84.
53. Clapier CR, Cairns BR. The biology of chromatin remodeling complexes. Annu Rev Biochem 2009;78:273–304.
54. Nair SS, Kumar R. Chromatin remodeling in Cancer: A Gateway to regulate gene Transcription. Mol Oncol 2012;6:611–9.
55. Roesch A, Becker B, Meyer S, Wild P, Hafner C, Landthaler M, et al. Retinoblastoma-binding protein 2-homolog 1: a retinoblastoma-binding protein downregulated in malignant melanomas. Mod Pathol 2005;18: 1249–57.
56. Roesch A, Becker B, Schneider-Brachert W, Hagen I, Landthaler M, Vogt T. Re-expression of the retinoblastoma-binding protein 2-homolog 1 reveals tumor-suppressive functions in highly metastatic melanoma cells. J Invest Dermatol 2006;126:1850–9.
57. Chicas A, Kapoor A, Wang X, Aksoy O, Evertts AG, Zhang MQ, et al. H3K4 demethylation by Jarid1a and Jarid1b contributes to retinoblastoma-mediated gene silencing during cellular senescence. Proc Natl Acad Sci U S A 2012;109:8971–6.
58. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010;141:69–80.
59. Jose C, Bellance N, Rossignol R. Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? Biochim Biophys Acta 2011; 1807:552–61.
60. Berridge MV, Herst PM, Tan AS. Metabolic flexibility and cell hierarchy in metastatic cancer. Mitochondrion 2010;10:584–8.
61. Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol 2011;27: 441–64.
62. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009;324:1029–33.
63. Hall A, Meyle KD, Lange MK, Klima M, Sanderhoff M, Dahl C, et al. Dysfunctional oxidative phosphorylation makes malignant melanoma cells addicted to glycolysis driven by the (V600E)BRAF oncogene. Oncotarget 2013;4:584–99.
64. Parmenter TJ, Kleinschmidt M, Kinross KM, Bond ST, Li J, Kaadige MR, et al. Response of BRAF-mutant melanoma to BRAF inhibition is mediated by a network of transcriptional regulators of glycolysis. Cancer Discov 2014;4: 423–33.
65. Viale A, Corti D, Draetta GF. Tumors and mitochondrial respiration: a neglected connection. Cancer Res 2015;75:3685–6.
66. Haq R, Shoag J, Andreu-Perez P, Yokoyama S, Edelman H, Rowe GC, et al. Oncogenic BRAF regulates oxidative metabolism via PGC1alpha and MITF. Cancer Cell 2013;23:302–15.
67. Corazao-Rozas P, Guerreschi P, Jendoubi M, Andre F, Jonneaux A, Scalbert C, et al. Mitochondrial oxidative stress is the Achille's heel of melanoma cells resistant to Braf-mutant inhibitor. Oncotarget 2013;4:1986–98.
68. Viale A, Pettazzoni P, Lyssiotis CA, Ying H, Sanchez N, Marchesini M, et al. Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. Nature 2014;514:628–32.
69. Sancho P, Burgos-Ramos E, Tavera A, Bou Kheir T, Jagust P, Schoenhals M, et al. MYC/PGC-1alpha balance determines the metabolic phenotype and plasticity of pancreatic cancer stem cells. Cell Metab 2015;22:590–605.
70. Janiszewska M, Suva ML, Riggi N, Houtkooper RH, Auwerx J, Clement-Schatlo V, et al. Imp2 controls oxidative phosphorylation and is crucial for preserving glioblastoma cancer stem cells. Genes Dev 2012;26: 1926–44.
71. Lagadinou ED, Sach A, Callahan K, Rossi RM, Neering SJ, Minhajuddin M, et al. BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. Cell Stem Cell 2013;12: 329–41.
72. Vazquez F, Lim JH, Chim H, Bhalla K, Girnun G, Pierce K, et al. PGC1alpha expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. Cancer Cell 2013;23:287–301.
73. Kelly DP, Scarpulla RC. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes Dev 2004;18:357–68.
74. Dey BK, Stalker L, Schnerch A, Bhatia M, Taylor-Papidimitriou J, Wynder C. The histone demethylase KDM5b/JARID1b plays a role in cell fate decisions by blocking terminal differentiation. Mol Cell Biol 2008;28: 5312–27.
75. Stewart MH, Albert M, Sroczynska P, Cruickshank VA, Guo Y, Rossi DJ, et al. The histone demethylase Jarid1b is required for hematopoietic stem cell self-renewal in mice. Blood 2015;125:2075–8.
76. Barbi de Moura M, Vincent G, Fayewicz SL, Bateman NW, Hood BL, Sun M, et al. Mitochondrial respiration–an important therapeutic target in melanoma. PLoS ONE 2012;7:e40690.
77. Blackman RK, Cheung-Ong K, Gebbia M, Proia DA, He S, Kepros J, et al. Mitochondrial electron transport is the cellular target of the oncology drug elesclomol. PLoS ONE 2012;7:e29798.
78. Hasinoff BB, Yadav AA, Patel D, Wu X. The cytotoxicity of the anticancer drug elesclomol is due to oxidative stress indirectly mediated through its complex with Cu(II). J Inorg Biochem 2014;137:22–30.
79. Cierlitza M, Chauvistre H, Bogeski I, Zhang X, Hauschild A, Herlyn M, et al. Mitochondrial oxidative stress as a novel therapeutic target to overcome intrinsic drug resistance in melanoma cell subpopulations. Exp Dermatol 2015;24:155–7.
80. O'Day SJ, Eggermont AM, Chiarion-Sileni V, Kefford R, Grob JJ, Mortier L, et al. Final results of phase III SYMMETRY study: randomized, double-blind trial of elesclomol plus paclitaxel versus paclitaxel alone as treatment for chemotherapy-naive patients with advanced melanoma. J Clin Oncol 2013;31:1211–8.
81. Ho J, de Moura MB, Lin Y, Vincent G, Thorne S, Duncan LM, et al. Importance of glycolysis and oxidative phosphorylation in advanced melanoma. Mol Cancer 2012;11:76.

Downloaded from http://aacrjournals.org/mct/article-pdf/16/6/1002/1854108/1002.pdf by guest on 11 October 2024