Molecular Pathways: BRAF Induces Bioenergetic Adaptation by Attenuating Oxidative Phosphorylation

Rizwan Haq[1,2], **David E. Fisher**[1,2], and **Hans R. Widlund**[3]

[1]Massachusetts General Hospital, Department of Dermatology, Cutaneous Biology Research Center, Harvard Medical School, Charlestown, MA

[2]Center for Melanoma, Massachusetts General Hospital Cancer Center, Boston, MA

[3]Brigham and Women's Hospital, Department of Dermatology, Harvard Skin Diseases Research Center, Harvard Medical School, Boston, MA

Abstract

Cancers acquire mutations in cooperating pathways that sustains their growth and survival. To support continued proliferation, tumor cells adapt their metabolism to balance energy production with their augmented biosynthetic needs. While most normal differentiated cells utilize mitochondrial oxidative phosphorylation (OXPHOS) as the bioenergetic source, cancer cells have been proposed to rely principally on cytoplasmic glycolysis. The molecular basis for this shift, termed the Warburg effect, is the subject of intense investigation, since mechanistic understanding may lead to novel approaches to target the altered metabolism of cancer cells. Recently, mutations BRAF(V600E) have emerged as a major regulator of metabolic homeostasis. Melanoma cells may utilize a metabolic shift to circumvent BRAF(V600E)-induced senescence though limiting their reliance on OXPHOS and promote proliferation. Furthermore, BRAF(V600E) acts to suppress expression of the melanocyte master regulator MITF and the mitochondrial biogenesis co-activator PGC1α. Accordingly, therapeutic inhibition of BRAF(V600E) reverses metabolic reprogramming in melanoma cells and elevates OXPHOS through increased MITF-PGC1α levels. BRAF-targeted drugs modulate the metabolic state of malignant melanoma cells, and counter-acting these adaptive responses using pharmacological agents may prove useful in combinatorial therapeutic strategies.

BACKGROUND

In 2002 the COSMIC team at the Sanger Center in the United Kingdom identified frequent gain-of-function BRAF(V600E) mutations in melanoma (1). Oncogenic mutations in the *BRAF* gene induce constitutive activation of its serine/threonine kinase activity and activation of the downstream mitogen-activated protein kinase (MAPK) signal transduction

pathway. BRAF mutations are also documented in other malignancies, for example, papillary thyroid carcinomas (2), hairy cell leukemias (3), non-small cell lung (4) and colorectal carcinomas (5), thus highlighting this pathway as a key oncogenic driver in multiple anatomical tissues. Overall, BRAF(V600E) is the most prevalent missense mutation in human cancer that in addition can be targeted therapeutically.

Identification of oncogenic driver mutations in human cancers such as the *KRAS* and *BRAF* genes has accelerated the development of small molecule inhibitors along the RAS-RAF-MEK-MAPK signaling pathway. Importantly, the first-in-class BRAF-specific inhibitor vemurafenib (PLX4032) (6) was approved in 2011 and extends melanoma survival by 4–6 months compared to standard chemotherapy (7,8). Despite dramatic initial tumor shrinkage in patients with melanoma, long-term efficacy is thwarted due to emergence of drug resistance (9). Alternative BRAF inhibitors, such as dabrafenib (GSK2118436 (10)), have shown similar response in melanoma patients followed by drug resistance and progression (11). However, the anti-tumor activity of vemurafenib in patients with BRAF(V600E)-mutant papillary thyroid or colorectal cancers remains dismal, possibly due to existence of compensatory pathways, such as EGFR signaling (12). Targeting the downstream target MEK1/2 has shown survival benefit in melanoma patients (13) and has been approved for treatment of patients with BRAF mutant melanoma. Collectively, targeted inhibition of the BRAF-MEK axis in malignant melanoma has extended patient survival, but responses are limited by emergence of drug resistance. Understanding how to prevent onset of resistance to oncogene targeted therapies and increase their initial therapeutic efficacy may provide key insights towards developing sustainable treatment against BRAF-mutant cancers in general and melanoma in particular. Because cancer cells have distinct metabolic demands from non-malignant cells, combinatorial inhibition of tumor-critical metabolic pathways is an attractive therapeutic approach to extend the usefulness of BRAF and MEK targeted therapies.

Metabolic shift in the transition from normal to cancer cell

In normal aerobic cells, glucose is the quintessential nutrient for energy production (Figure 1). Metabolic breakdown of glucose (glycolysis) drives catabolic ATP production through a pathway that couples the Krebs/tricarboxylic acid (TCA) cycle with oxidative phosphorylation (OXPHOS). In essence, glycolysis produces pyruvate, which is transported to the mitochondria for oxidation into acetyl-CoA to fuel the TCA cycle. Successive oxidation of acetate in the TCA cycle leads to transfer of electrons to NAD+ for the generation of NADH, which in turn is consumed by oxygen dependent reactions (respiration) producing carbon dioxide (CO_2), water (H_2O), and chemical energy. The released chemical energy, in the form of a proton gradient over the inner mitochondrial membrane, subsequently drives ATP re-generation from ADP. Overall, the TCA cycle and OXPHOS is a highly efficient mitochondrial chemical energy conversion pathway.

Proliferating cells must produce ATP like normal non-dividing cells, but also generate biomass and duplicate its genome to enable cell division. Hence, rapidly dividing cells, such as tumors and stem cells, must balance their catabolic ATP demand with anabolic building block production in order to maintain survival and sustain their proliferation, respectively.

Therefore, it is not surprising that cancer cells display a different metabolic phenotype and nutrient usage when compared to normal cells. Already observed by Dr. Otto Warburg in the early twentieth century, tumors metabolize glucose anaerobically despite plentiful oxygen present to generate lactate (14) - a phenomena now commonly referred to as the "Warburg effect". Specifically, lactate dehydrogenase consumes pyruvate to regenerate NAD+ from NADH produced by glucose breakdown, and hence enables a high rate of glycolysis to take place. Consequently, lactate is a byproduct in a reaction that sustains rapid production of building block intermediates and ATP sufficiency in proliferating cells. As lactate can easily be transported out and in between cells, and lactate dehydrogenase can generate NADH and pyruvate in reverse, this reaction is able to fuel mitochondrial oxidative phosphorylation among adjacent cells (15).

Role of mitochondrial oxidative phosphorylation in cancer

Despite the prevailing emphasis on glycolysis in cancer metabolism, leukemias, prostate and breast cancers, as well as melanomas, require oxidative phosphorylation for their growth (16,17,18,19). In fact, some melanomas have significantly higher oxygen consumption than melanocytes (20), indicating that OXPHOS itself may be an important metabolic target in some tumor contexts. Moreover, inhibition of OXPHOS using elesclomol provokes apoptosis initially, while prolonged in vitro selective growth drives enhanced glycolysis. Hence, this study indicates that melanomas are acutely dependent on OXPHOS, while they are able to adapt to alternate metabolism. Using a mouse malignant melanoma model it has also been demonstrated that selectively depleting mitochondrial DNA (mtDNA), which encodes key components of the electron transport chain and thus compromises OXPHOS, delays subcutaneous tumor growth and prevent metastasis (21). Similar results have been reported in a human breast cancer cell line (22). Together these observations suggest that in addition to a general glycolytic phenotype due to anabolic demands, there is significant heterogeneity in the metabolic requirements of cancer cells. Therefore, a molecular understanding of how OXPHOS is regulated by oncogenic pathways is critical for its rational therapeutic exploit in cancer treatment.

Activation of oncogenes and the shift to anabolic metabolism

Normal cells have a number of programs in place to reduce the likelihood for renegade growth (23). In particular oncogene-induced senescence (OIS) provides such a barrier that leads to premature terminal growth arrest in response to mutant RAS and RAF, which depends on intact pRB (*RB1*) and p53 (*TP53*) pathways (24,25,26). Interestingly, OIS by mutant RAS have been causally connected to induction of reactive oxygen species (ROS) (27). Because the major source for ROS is OXPHOS, aberrant metabolism may be important in triggering OIS. In order to escape OIS, cells may choose to reroute their metabolism towards glycolytic metabolism to reduce ROS. A key regulator that drives this metabolic shift is hypoxia-induced factor 1α (HIF1α), which in response to low oxygen gets stabilized and activates a program that reduces mitochondrial utilization through its function as a transcription factor. Specifically, HIF1α upregulates pyruvate dehydrogenase kinase 1 (PDK1) (28,29) that inactivates the pyruvate kinase complex, which is responsible for conversion of pyruvate to the main fuel for the TCA cycle: acetyl-CoA. Consistent with reducing OIS during hypoxic conditions (27), HIF1α acts as an oxygen sensor and drive

cellular metabolism towards anabolic pathways, which is in line with Warburg's original observation (14). Hence, adaptation to oncogenic mutations along the RAS-RAF-MEK pathway is causally linked to the metabolic shift observed in cancer cells.

Oncogenic BRAF(V600E) and senescence-like benign nevi

Seemingly a paradox at first, benign nevi was found to harbor BRAF(V600E) mutations in a frequency similar to that of malignant melanomas (30), offering some doubts as to its function as a cancer driver. However, subsequent functional studies using various models substantiated this oncogene as a melanoma formation driver and verified it as a key therapeutic target (31,32,33,34,35). Additionally, benign nevi have been demonstrated to exhibit hallmarks of OIS due to BRAF(V600E) activation (36), highlighting that fail-safe barriers to tumorigenesis could be visualized *in vivo*. Because oncogenic BRAF is not sufficient to induce tumorigenesis in the absence of additional genetic alterations, the vast majority of nevi represent an end-stage of local melanocyte proliferation. Hence, co-operating mutations such as PTEN loss-of-function mutations, which lead to constitutive activation of the phosphatidylinositol 3-kinase (PI3K) v-akt murine thymoma viral oncogene (AKT)-mammalian target of rapamycin (mTOR) pathway, is found in a substantial fraction of melanomas co-existing with mutant BRAF (37). Activation of the PI3K-mTOR pathway promote glycolysis by inducting the activity of HIF1α (38), and therefore also indicates a consistent "Warburg effect" to allow for continued proliferation through bypassing OIS (39).

Furthermore, recent data have revealed that BRAF(V600E)-induced senescence is accompanied by increased pyruvate entry into the TCA cycle via increased pyruvate dehydrogenase (PDH) complex activity (40). This effect was correlated to suppression of the PDH-inhibitory enzyme pyruvate dehydrogenase kinase 1 (PDK1) and induction of the PDH-activating enzyme pyruvate dehydrogenase phosphatase 2 (PDP2). Reversal of PDH activation enabled bypass of OIS and tumorigenic growth. Whether HIF1α activation would enable bypass of OIS in this context was not analyzed, but since PDK1 is a bona fide downstream target gene (28,29), it seems highly plausible and would favor Warburg's original observation. Nonetheless, these data highlight that BRAF(V600E) as an oncogene provoke OIS that requires coordination of metabolic re-programming to drive tumor initiation.

The oncogene MITF controls melanocyte development and melanoma metabolism

It is of critical importance to determine whether the effects of oncogene-targeted therapies intersects with the inherent cellular wiring of cancer cells to provide feedback control that limits or affords therapeutic effects. Given that melanoma cells are inherently sensitive to therapeutic inhibition of BRAF(V600E)-targeted agents, while papillary thyroid, non-small cell lung and colorectal carcinomas are not, a plausible explanation is that the metabolic state is dramatically different between these tumor cell-types.

Among mutations that co-segregate and co-operate with BRAF(V600E) in melanoma is amplification of the melanocyte master regulator *microphthalmia*-associated transcription factor (MITF) (35). MITF is required for melanocyte development (41,42) and regulates

differentiation-associated programs of pigment synthesis and transport, as well as survival cues via the BCL2-famly of anti-apoptotic proteins (43,44). As an oncogene in melanoma, MITF is target of frequent gene amplification (35) and point mutation within sporadic and germ-line predisposition (45,46), which consistently lead to its elevated transcriptional activity. Surprisingly, MITF expression is often reduced in melanomas as compared to normal melanocytes, suggesting that it may have both pro- and anti-cancer effects depending on the context. MITF is regulated by multiple signaling pathways, including the metabolic regulator HIF1α. Specifically, HIF1α suppress expression of *MITF* in melanoma cells through its transcriptional effects on the repressor *DEC1* (47,48).

Contrasting the effects of HIF1α to promote anabolic metabolism, MITF has recently been linked to promote catabolic metabolism by directly regulating the mitochondrial biogenesis co-activators PGC1α and PGC1β (49,50,51). Specifically, MITF's transcriptional regulation of PGC1α promotes mitochondrial respiration and resistance to oxidative stress in a defined subset of melanoma tumors with high MITF and PGC1α expression (49,50). Furthermore, BRAF and MEK inhibitors enhance *MITF* expression, leading to elevation of PGC1α levels and an increase in OXPHOS activity (50). Hence through simultaneous control of metabolism and cell survival cues, MITF poses to balance the effects of BRAF- and MEK-targeted therapies as a genuine melanoma oncogene and lineage master regulator (35,45,46).

Control of metabolism by cellular sensing mechanisms

The general metabolic sensing pathway in cells involves the tumor suppressor LKB1 acting on AMPK to respond to alterations in AMP/ATP levels in cells (52). Because activation of AMPK by LKB1 suppresses growth through inactivation of signaling onto mTOR when nutrients are scarce and ATP levels remains constant in cells, the activity is essentially affected by AMP levels alone. Interestingly, growth factors through their receptor tyrosine kinases and BRAF(V600E) have been shown to alter LKB1-AMPK signaling leading to reduced AMP sensing in melanoma cells (53,54). Specifically, downstream activation of MAPK and RSK have been shown to directly phosphorylate LKB1, leading to compromised ability to activate AMPK and thus decouples metabolic sensing (53), which have been shown to exhort important effects on melanoma growth. In addition, during conditions when the RAS-RAF-MEK pathway is not constitutively activated or mutated, metabolic sensing through AMPK may lead to specific suppression of BRAF activity (55). Hence, constitutive RAS-RAF-MEK signaling decouples energy sensing and may confer a sensitivity that offers mechanistic clues as to how cancer cells become sensitive to metabolic inhibition. Furthermore, mouse embryonic fibroblasts from LKB1 null mice are resistant to transformation by oncogenic RAS (56), suggesting that LKB1 tumor suppressor function may in fact be required to drive the metabolic shift, which further places emphasis on the intersection between energy sensing and cancer development.

CLINICAL-TRANSLATIONAL ADVANCES

The growing recognition of altered metabolism as a hallmark of cancer has kindled interest in approaches that target metabolic pathways in combination with current available anti-cancer treatment modalities (23). Chemical screens from over 20 years ago pointed to

approaches to modulate OXPHOS, and given the recognition of this pathway in cancer, it will be worthwhile to reevaluate these data in light of the current era of genomics and targeted therapies. In particular, recent work has revealed a number of metabolic intervention opportunities with regard to forestall resistance to targeted therapy against mutant BRAF.

Targeting the metabolic shift that prevents OIS

BRAF mutations are detected in more than half of all melanomas, and NRAS is mutant in another 15–25%, underscoring the NRAS-BRAF axis as causally linked to melanocytes neoplasia. As outlined above, the source of the metabolic shift seen in human cancer cells is believed to be caused by OIS. With particular interest on pRB's known effects on cellular metabolism (57) and governing OIS (24,25,26), reactivation of its function using a CDK4/6 inhibitor in melanoma cells have been shown to cause senescence (58). A current active area of exploration is to determine whether CDK4/6 inhibitors can synergize with BRAF and MEK targeted agents to increase therapeutic efficacy (59).

Deregulation of HIF1α contributes to the Warburg effect and consequently, therapeutic intervention of its activity and deregulated pathways that converge on its function is under intense scrutiny for cancer therapy (60). This pursuit have recently been accelerated as mutations in key metabolic enzymes in the TCA cycle is believed to deregulate important metabolites, such as succinate, which is expected to increase the stability of HIF1α due to interfering with its oxygen-dependent degradation.

Additionally, as OIS induced by BRAF(V600E) was recently shown to be dependent on increased pyruvate oxidation suggests that targeting PDK may be one possible approach (40). The preclinical data suggest that the generic inhibitor of PDK, dichroacetate (DCA) may in fact synergize with BRAF and MEK inhibitors to enhance the efficacy of inhibiting melanoma growth, even to resolve targeted drug resistance (40).

Metabolic intervention strategies with regard to modulation of OXPHOS

The recognition that melanoma cells may adapt to BRAF and MEK inhibitors by driving oxidative metabolism through MITF-PGC1α suggests that targeting this pathway may be an alternative approach (50). Specifically, mitochondrial uncoupling using poisons of the electron transport chain complexes, such as cyanide, do not have sufficient therapeutic index. The mitochondrial uncoupler, 2,4-dinitrophenol (2,4-DNP), which leads to dissemination of the proton gradient and generation of heat, has been used extensively used in diet pills but was discontinued due to dangerous side effects such as fatal hyperthermia. Although alternative mitochondrial uncouplers may be useful, they will likely lead to significant toxicities that possibly preclude their use for anti-cancer treatment regiments.

Well-tolerated inhibitors of OXPHOS may have greater therapeutic potential for cancer treatment, in particular the biguanides metformin and phenformin used in treatment of type II diabetes. These drugs are believed to act by inhibiting complex I of the electron transport chain (61). While biguanides have demonstrated anti-cancer activity both *in vitro* and *in vivo* (62), the efficacy of metformin may be limited by the expression of organic cation

transporters (OCT) required for uptake and OXPHOS inhibition (63). Notwithstanding, LKB1 mutant non-small cell lung cancer cells as well as an *in vivo* cancer model demonstrates robust tumor growth inhibition by phenformin (64). Given that constitutive RTK-RAS-BRAF imparts decreased LKB1 function in melanoma cells, phenformin was recently shown to enhance the effects of BRAF inhibitors both *in vitro* and in a mouse melanoma model (65). Hence, inhibition of OXPHOS using well tolerated biguanides, such as metformin and phenformin, may offer therapeutic effects in combination with BRAF- and MEK-inhibitors.

In addition, the repurposing of existing, FDA-approved drugs that target OXPHOS (66) could offer additional approaches. Of particular interest is the use of antibiotics such as anti-parasitic drugs such as atovaquone that inhibits cytochrome oxidase complex III of the electron transport chain (67), or the antimicrobials tigecycline that inhibits mitochondrial protein translation with shown anti-leukemic activity *in vitro* and *in vivo* cancer models (16).

Summary

Recent work have underscored that BRAF(V600E) attenuates oxidative phosphorylation (OXPHOS) consistent with Warburg's initial hypothesis of the metabolic shift of cancer cells. However, new mechanistic insights have revealed that the metabolic shift is caused by evasion of oncogene-induced senescence with important parallels to response to BRAF and MEK targeted therapy in melanoma cells. Additionally, modulating metabolic dependencies may have combinatorial efficacy. It is the current hope that targeting these metabolic pathways may prevent the onset of drug resistance, lead to durable melanoma treatment responses and extend the use of current BRAF-targeted agents to other human cancers.

Acknowledgments

References

1. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002; 417:949–54. [PubMed: 12068308]

2. Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA. High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Res. 2003; 63:1454–7. [PubMed: 12670889]

3. Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP, et al. BRAF mutations in hairy-cell leukemia. N Engl J Med. 2011; 364:2305–15. [PubMed: 21663470]

4. Brose MS, Volpe P, Feldman M, Kumar M, Rishi I, Gerrero R, et al. BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res. 2002; 62:6997–7000. [PubMed: 12460918]

5. Tol J, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer. N Engl J Med. 2009; 361:98–9. [PubMed: 19571295]

6. Sala E, Mologni L, Truffa S, Gaetano C, Bollag GE, Gambacorti-Passerini C. BRAF silencing by short hairpin RNA or chemical blockade by PLX4032 leads to different responses in melanoma and thyroid carcinoma cells. Mol Cancer Res. 2008; 6:751–9. [PubMed: 18458053]

7. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. BRIM-3 Study Group. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011; 364:2507–16. [PubMed: 21639808]

8. Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. N Engl J Med. 2012; 366:707–14. [PubMed: 22356324]

9. 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. [PubMed: 20818844]

10. Kefford R, Arkenau H, Brown MP, Millward M, Infante JR, Long GV, et al. Phase I/II study of GSK2118436, a selective inhibitor of oncogenic mutant BRAF kinase, in patients with metastatic melanoma and other solid tumors. J Clin Oncol. 2010; 28:15s. (suppl; abstr 8503).

11. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet. 2012; 380:358–65. [PubMed: 22735384]

12. 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. [PubMed: 22281684]

13. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med. 2012; 367:107–14. [PubMed: 22663011]

14. Warburg O. On the origin of cancer cells. Science. 1956; 123:309–14. [PubMed: 13298683]

15. Sonveaux P, Végran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, et al. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J Clin Invest. 2008; 118:3930–3942. [PubMed: 19033663]

16. Skrti M, Sriskanthadevan S, Jhas B, Gebbia M, Wang X, Wang Z, et al. Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. Cancer Cell. 2011; 20:674–88. [PubMed: 22094260]

17. Dakubo GD, Parr RL, Costello LC, Franklin RB, Thayer RE. Altered metabolism and mitochondrial genome in prostate cancer. J Clin Pathol. 2006; 59:10–6. [PubMed: 16394275]

18. Chang CY, Kazmin D, Jasper JS, Kunder R, Zuercher WJ, McDonnell DP. The metabolic regulator ERRα, a downstream target of HER2/IGF-1R, as a therapeutic target in breast cancer. Cancer Cell. 2011; 20:500–10. [PubMed: 22014575]

19. 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. [PubMed: 23043612]

20. 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. [PubMed: 22912665]

21. Berridge MV, Tan AS. Effects of mitochondrial gene deletion on tumorigenicity of metastatic melanoma: reassessing the Warburg effect. Rejuvenation Res. 2010; 13:139–41. [PubMed: 20370492]

22. Yu M, Shi Y, Wei X, Yang Y, Zhou Y, Hao X, et al. Depletion of mitochondrial DNA by ethidium bromide treatment inhibits the proliferation and tumorigenesis of T47D human breast cancer cells. Toxicol Lett. 2007; 170:83–93. [PubMed: 17391873]

23. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646–74. [PubMed: 21376230]

24. Kamijo T, Zindy F, Roussel MF, Quelle DE, Downing JR, Ashmun RA, et al. Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. Cell. 1997; 91:649–659. [PubMed: 9393858]

25. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell. 1997; 88:593–602. [PubMed: 9054499]

26. Zhu J, Woods D, McMahon M, Bishop JM. Senescence of human fibroblasts induced by oncogenic Raf. Genes Dev. 1998; 12:2997–3007. [PubMed: 9765202]

27. Lee AC, Fenster BE, Ito H, Takeda K, Bae NS, Hirai T, et al. Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. J Biol Chem. 1999; 274:7936–40. [PubMed: 10075689]

28. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. 2006; 3:177–85. [PubMed: 16517405]

29. Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab. 2006; 3:187–97. [PubMed: 16517406]

30. Pollock PM, Harper UL, Hansen KS, Yudt LM, Stark M, Robbins CM, et al. High frequency of BRAF mutations in nevi. Nat Genet. 2003; 33:19–20. [PubMed: 12447372]

31. Hingorani SR, Jacobetz MA, Robertson GP, Herlyn M, Tuveson DA. Suppression of BRAF(V599E) in human melanoma abrogates transformation. Cancer Res. 2003; 63:5198–202. [PubMed: 14500344]

32. Hoeflich KP, Gray DC, Eby MT, Tien JY, Wong L, Bower J, et al. Oncogenic BRAF is required for tumor growth and maintenance in melanoma models. Cancer Res. 2006; 66:999–1006. [PubMed: 16424035]

33. Karasarides M, Chiloeches A, Hayward R, Niculescu-Duvaz D, Scanlon I, Friedlos F, et al. B-RAF is a therapeutic target in melanoma. Oncogene. 2004; 23:6292–98. [PubMed: 15208680]

34. Patton EE, Widlund HR, Kutok JL, Kopani KR, Amatruda JF, Murphey RD, et al. BRAF mutations are sufficient to promote nevi formation, and cooperate with p53 in the genesis of melanoma. Curr Biol. 2005; 15:249–54. [PubMed: 15694309]

35. Garraway LA, Widlund HR, Rubin MA, Berger AJ, Ramaswamy S, Chen F, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. Nature. 2005; 436:117–22. [PubMed: 16001072]

36. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, et al. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature. 2005; 436:720–4. [PubMed: 16079850]

37. Tsao H, Goel V, Wu H, Yang G, Haluska FG. Genetic interaction between NRAS and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. J Invest Dermatol. 2004; 122:337–41. [PubMed: 15009714]

38. Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E, et al. Loss of PTEN facilitates HIF-1-mediated gene expression. Genes Dev. 2000; 14:391–6. [PubMed: 10691731]

39. Vredeveld LC, Possik PA, Smit MA, Meissl K, Michaloglou C, Horlings HM, et al. Abrogation of BRAFV600E-induced senescence by PI3K pathway activation contributes to melanomagenesis. Genes Dev. 2012; 26:1055–69. [PubMed: 22549727]

40. Kaplon J, Zheng L, Meissl K, Chaneton B, Selivanov VA, Mackay G, et al. A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. Nature. 2013; 498:109–12. [PubMed: 23685455]

41. Hodgkinson CA, Moore KJ, Nakayama A, Steingrímsson E, Copeland NG, Jenkins NA, et al. Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. Cell. 1993; 74:395–404. [PubMed: 8343963]

42. Haq R, Fisher DE. Biology and clinical relevance of the micropthalmia family of transcription factors in human cancer. J Clin Oncol. 2011; 29:3474–82. [PubMed: 21670463]

43. McGill GG, Horstmann M, Widlund HR, Du J, Motyckova G, Nishimura EK, et al. Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. Cell. 2002; 109:707–18. [PubMed: 12086670]

44. Haq R, Yokoyama S, Hawryluk EB, Jönsson GB, Frederick DT, McHenry K, et al. BCL2A1 is a lineage-specific antiapoptotic melanoma oncogene that confers resistance to BRAF inhibition. Proc Natl Acad Sci U S A. 2013; 110:4321–6. [PubMed: 23447565]

45. Yokoyama S, Woods SL, Boyle GM, Aoude LG, MacGregor S, Zismann V, et al. A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. Nature. 2011; 480:99–103. [PubMed: 22080950]

46. Bertolotto C, Lesueur F, Giuliano S, Strub T, de Lichy M, Bille K, et al. A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. Nature. 2011; 480:94–8. [PubMed: 22012259]

47. Feige E, Yokoyama S, Levy C, Khaled M, Igras V, Lin RJ, et al. Hypoxia-induced transcriptional repression of the melanoma-associated oncogene MITF. Proc Natl Acad Sci U S A. 2011; 108:E924–33. [PubMed: 21949374]

48. Cheli Y, Giuliano S, Fenouille N, Allegra M, Hofman V, Hofman P, et al. Hypoxia and MITF control metastatic behaviour in mouse and human melanoma cells. Oncogene. 2012; 31:2461–70. [PubMed: 21996743]

49. Vazquez F, Lim J-H, Chin H, Bhalla K, Girnun G, Pierce K, et al. PGC1α Expression Defines a Subset of Human Melanoma Tumors with Increased Mitochondrial Capacity and Resistance to Oxidative Stress. Cancer Cell. 2013; 23:287–301. [PubMed: 23416000]

50. Haq R, Shoag J, Andreu-Perez P, Yokoyama S, Edelman H, Rowe GC, et al. Oncogenic BRAF regulates oxidative metabolism via PGC1α and MITF. Cancer Cell. 2013; 23:302–15. [PubMed: 23477830]

51. Shoag J, Haq R, Zhang M, Liu L, Rowe GC, Jiang A, et al. PGC-1 coactivators regulate MITF and the tanning response. Mol Cell. 2013; 49:145–57. [PubMed: 23201126]

52. Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. 2005; 1:15–25. [PubMed: 16054041]

53. Zheng B, Jeong JH, Asara JM, Yuan YY, Granter SR, Chin L, Cantley LC. Oncogenic B-RAF negatively regulates the tumor suppressor LKB1 to promote melanoma cell proliferation. Mol Cell. 2009; 33:237–47. [PubMed: 19187764]

54. Esteve-Puig R, Canals F, Colomé N, Merlino G, Recio JA. Uncoupling of the LKB1-AMPKalpha energy sensor pathway by growth factors and oncogenic BRAF. PLoS One. 2009; 4:e4771. [PubMed: 19274086]

55. Shen CH, Yuan P, Perez-Lorenzo R, Zhang Y, Lee SX, Ou Y, et al. Phosphorylation of BRAF by AMPK Impairs BRAF-KSR1 Association and Cell Proliferation. Mol Cell. 2013; 52:161–72. [PubMed: 24095280]

56. Bardeesy N, Sinha M, Hezel AF, Signoretti S, Hathaway NA, Sharpless NE, et al. Loss of the Lkb1 tumour suppressor provokes intestinal polyposis but resistance to transformation. Nature. 2002; 419:162–7. [PubMed: 12226664]

57. Clem BF, Chesney J. Molecular pathways: regulation of metabolism by RB. Clin Cancer Res. 2012; 18:6096–100. [PubMed: 23154086]

58. Anders L, Ke N, Hydbring P, Choi YJ, Widlund HR, Chick JM, et al. A systematic screen for CDK4/6 substrates links FOXM1 phosphorylation to senescence suppression in cancer cells. Cancer Cell. 2011; 20:620–34. [PubMed: 22094256]

59. Sheppard KE, McArthur GA. The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma. Clin Cancer Res. 2013; 19:5320–8. [PubMed: 24089445]

60. Kaelin WG Jr. Cancer and altered metabolism: potential importance of hypoxia-inducible factor and 2-oxoglutarate-dependent dioxygenases. Cold Spring Harb Symp Quant Biol. 2011; 76:335–45. [PubMed: 22089927]

61. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J. 2000; 348:607–14. [PubMed: 10839993]

62. Pierotti MA, Berrino F, Gariboldi M, Melani C, Mogavero A, Negri T, et al. Targeting metabolism for cancer treatment and prevention: metformin, an old drug with multi-faceted effects. Oncogene. 2013; 32:1475–1487. [PubMed: 22665053]

63. Wang DS, Kusuhara H, Kato Y, Jonker JW, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in the lactic acidosis caused by metformin. Mol Pharmacol. 2003; 63:844–8. [PubMed: 12644585]

64. Shackelford DB, Abt E, Gerken L, Vasquez DS, Seki A, Leblanc M, et al. LKB1 inactivation dictates therapeutic response of non-small cell lung cancer to the metabolism drug phenformin. Cancer Cell. 2013; 23:143–58. [PubMed: 23352126]

65. Yuan P, Ito K, Perez-Lorenzo R, Del Guzzo C, Lee JH, Shen CH, et al. Phenformin enhances the therapeutic benefit of BRAFV600E inhibition in melanoma. Proc Natl Acad Sci U S A. 2013; 110:18226–31. [PubMed: 24145418]

66. Gohil VM, Sheth SA, Nilsson R, Wojtovich AP, Lee JH, Perocchi F, et al. Nutrient-sensitized screening for drugs that shift energy metabolism from mitochondrial respiration to glycolysis. Nat Biotechnol. 2010; 28:249–55. [PubMed: 20160716]

67. Painter HJ, Morrisey JM, Mather MW, Vaidya AB. Specific role of mitochondrial electron transport in blood-stage Plasmodium falciparum. Nature. 2007; 446:88–91. [PubMed: 17330044]
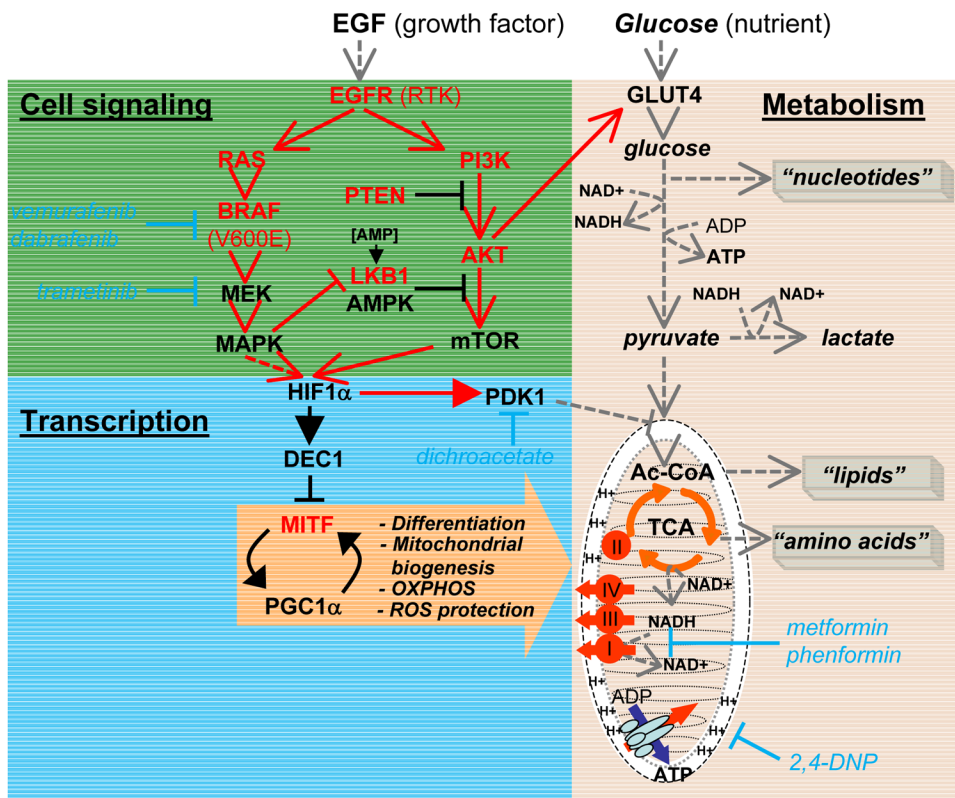
Figure 1. Intersection of signaling, transcription and metabolism regulate generation of ATP and building block availability to sustain survival and growth, respectively

Growth factor mediated activation (EGF) of a receptor tyrosine kinase (EGFR) leads to downstream pathway signaling along the RAS-RAF-MEK-MAPK and PI3K-AKT-mTOR pathways. Key convergence points are MAPK mediated suppression of LKB1/ AMPK energy sensing, and AKT activated translocation of the glucose transporter GLUT4 to the plasma membrane leading to augmented glucose import for anabolic (nucleotides, lipids, and amino acids) in addition to catabolism towards ATP regeneration in the mitochondria utilizing the proton gradient. Furthermore, MAPK stabilizes and mTOR increases translation of HIF1α, triggering the transcriptional function with effects on reducing pyruvate decarboxylation/oxidation to acetyl-CoA (Ac-CoA) destined for use in the TCA cycle. In melanoma cells, HIF1α activity reduces expression of MITF-PGC1α levels and thereby causes effects on reducing mitochondrial bioactivity (OXPHOS, ROS protection) in addition to differentiation associated cascades. Frequently mutant components (and pathways) in human cancers are indicted in red. Inhibitors of indicated pathway components are indicated in blue.