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Previews

Taking the Stress out of Melanoma

Matthew J. Martin,[1] David Carling,[2,*] and Richard Marais[1,*]
[1]Signal Transduction Team, The Institute of Cancer Research, London SW3 6JB, UK
[2]Cellular Stress Group, MRC Clinical Sciences Centre, Imperial College London, London W12 ONN, UK
*Correspondence: david.carling@csc.mrc.ac.uk (D.C.), richard.marais@icr.ac.uk (R.M.)
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A recent study published in *Molecular Cell* describes a mechanism whereby oncogenic BRAF inhibits AMPK in melanoma cells. This may explain why cancer cells expressing oncogenic BRAF grow under conditions of metabolic stress and may provide new therapeutic opportunities to treat this life-threatening disease.

During cancer progression, tumor cells acquire the so-called "hallmarks of cancer," including self-sufficiency in growth signals and insensitivity to anti-growth signals (Hanahan and Weinberg, 2000). Most human melanomas achieve self-sufficiency in growth through acquisition of activating mutations in the protein kinase BRAF (Wellbrock et al., 2004), which delivers proliferation signals through the MEK/ERK pathway. However, a key question that remains is how melanoma cells adapt their glucose metabolism to sustain growth in the stressful conditions of the tumor microenvironment. A recent study in *Molecular Cell* by Zheng et al. (2009) describes an intriguing link between BRAF and AMP-activated protein kinase (AMPK), the key sensor of metabolic stress in eukaryotic cells. This study may explain how oncogenic BRAF (^{V600E}BRAF) overwhelms the metabolic stress signals that normally inhibit cell growth.

AMPK is activated in conditions of "low energy," when the AMP:ATP ratio increases. To conserve energy, AMPK promotes catabolism (e.g., increased glucose uptake and glycolysis) and blocks anabolism (e.g., protein and lipid synthesis). It also inhibits cell growth by activating TSC2, an exchange factor/activator for the small G protein Rheb, which in turn inhibits the protein kinase mammalian target of rapamycin (mTOR), thereby reducing protein translation. AMPK is phosphorylated and activated by a protein kinase called LKB1 (Hong et al., 2003), a tumor suppressor that is mutated in Peutz-Jeghers syndrome, a rare genetic condition characterized by the presence of benign harmatomas and an increased lifetime risk of cancer (Hemminki et al., 1998). It is thought that LKB1 is constitutively active and that its ability to activate AMPK is mediated by AMP binding to AMPK, which prevents its dephosphorylation and allows the active enzyme to accumulate.

Zheng and colleagues start by showing that the cell-permeable AMPK activator AICAR (5-aminoimidazole-4-carboxamide ribonucleoside) does not activate AMPK in ^{V600E}BRAF melanoma cells but does activate AMPK in wild-type BRAF cells. This suggests that ^{V600E}BRAF suppresses AMPK activity in melanoma cells. Accordingly, BRAF depletion by RNA interference or MEK inhibition with small molecules promotes AMPK activation in ^{V600E}BRAF cells. Critically, AMPK activation still requires LKB1, and the authors show that ERK and RSK, two kinases constitutively activated downstream of ^{V600E}BRAF, phosphorylate LKB1 on S325 and S428, respectively. Mutation of these residues allows AICAR to activate AMPK in the presence of ^{V600E}BRAF, and strikingly, this LKB1 double mutant blocks ^{V600E}BRAF-driven proliferation and soft agar colony formation.

Notably, even though ERK is activated in the majority of melanoma cells, it only phosphorylates LKB1 when ^{V600E}BRAF is present, because ^{V600E}BRAF stimulates formation of a ternary complex between itself, LKB1, and ERK, whereas wild-type BRAF cannot form this complex. ERK signaling occurs within highly ordered complexes coordinated by specialized scaffold proteins (Kolch, 2005), and these data suggest that ^{V600E}BRAF alters the architecture of this pathway to allow ERK to phosphorylate LKB1.

Although AMPK blocks cell growth, it also promotes some events that could be advantageous to tumor cells. Under nutrient (particularly glucose) deprivation or in hypoxia, AMPK stimulates glucose uptake and increases expression of key glycolytic enzymes (Ashrafian, 2006). Thus, while inhibition of AMPK is necessary to permit cell growth, when tumors get large and nutrients and oxygen become scarce, AMPK reactivation may actually promote anabolic pathways and cell growth. In their study, Zheng et al. do not model the sustained elevation of AMP that occurs in hypoxic or glucose-deprived tumors, but they do show an inverse correlation between AMPK and ERK phosphorylation in human tumor samples. While these data were not correlated to BRAF mutation status, they do suggest that the BRAF-driven mechanism is physiologically relevant. Nevertheless, it will be intriguing to determine whether AMPK is still inhibited within the hypoxic regions of ^{V600E}BRAF melanomas.

Taken together, the data above suggest that ^{V600E}BRAF regulates metabolic signaling directly, describing a ^{V600E}BRAF-AMPK "axis" that allows proliferation under metabolic stress (Figure 1A). They suggest that this axis undermines normal growth control to promote tumorigenesis and imply that metabolic signals are therapeutic targets in melanoma. They also describe a mechanism of LKB1 regulation by phosphorylation, although the functions of S325 and S428 in LKB1 regulation and cell growth are controversial because previous studies have failed to show clear roles for these sites in LKB1 regulation and effects (Sapkota et al., 2002; Fogarty and Hardie, 2009; Denison et al., 2009). Perhaps they have context-specific roles that are only revealed, for example, in the presence of ^{V600E}BRAF. It should also be noted that the axis may be absent in some ^{V600E}BRAF cells. ^{V600E}BRAF cannot inhibit AMPK when LKB1 is reintroduced into LKB1-deficient G361 melanoma cells (Fogarty and Hardie, 2009), suggesting that these cells can still grow when AMPK is active or can inhibit AMPK through

alternative mechanisms. Finally, the RAF-AMPK axis may be absent in RAS mutant melanoma cells because RAS signals exclusively through CRAF (Dumaz et al., 2006), which, like wild-type BRAF, may be unable to support the axis.

One aspect of the Zheng et al. study yet to be explored is the consequence of impaired AMPK activation in BRAF mutant melanoma cells. The effects of AICAR on cell growth are not described by Zheng et al., and although nonphosphorylatable LKB1 is shown to block cell growth, the authors do not show whether this is mediated by AMPK or one of the twelve other protein kinases that LKB1 activates. Expression of an active version of AMPK could answer this question. Similarly, intriguing questions relating to the broader signaling network should be answered. For example, AMPK is thought to mediate growth suppression through inhibition of mTOR, and the authors argue that inhibition of AMPK is necessary to allow mTOR activation. However, ^{V600E}BRAF also activates RSK, which is a direct activator of mTOR (Figure 1A), so why is LKB/AMPK inhibition necessary when RSK is activated? Presumably AMPK dominates RSK in mTOR regulation, so RSK performs the dual role of inhibiting the mTOR inhibitor while simultaneously activating mTOR. Clear answers to these questions will be important to assist therapeutic strategy design based on these studies.

And what of the potential for therapeutic intervention? The authors propose that BRAF/MEK inhibitors together with AMPK activators should offer an effective therapeutic approach. This is interesting and timely because the antidiabetic drug metformin, which activates AMPK indirectly, has been found to reduce lifetime risk of cancer (Evans et al., 2005), and potent and selective BRAF and MEK drugs are in clinical development. However, no data are provided to support this notion, and it is unclear whether such a combination would be better than monotherapies that target BRAF/MEK. As Zheng et al. show, MEK inhibitors activate AMPK, so why would AMPK activators provide any additional benefit (Figure 1B)? This question goes to the heart of combination therapy design. Will better responses be achieved by inhibiting several pathways simultaneously, or will "belt and braces" approaches that target single, critical pathways at several nodes provide the best response? Happily, in this case, we have the tools and wherewithal to perform the preclinical studies necessary to answer the question.

Finally, what of other cancers harboring mutations in BRAF, such as colorectal (15% of cases), thyroid (30% of cases), and ovarian (30% of cases) cancers? Does ^{V600E}BRAF also rewire ERK to phosphorylate LKB1 in those cancers, and if so, can they also be treated using therapies designed for melanoma? Like all good studies, the work presented by Zheng et al. raises as many questions as it answers, but importantly, it points to strategies that may provide therapeutic benefit by combining drugs that target growth and metabolic signaling pathways. The potential of these approaches should now be tested.

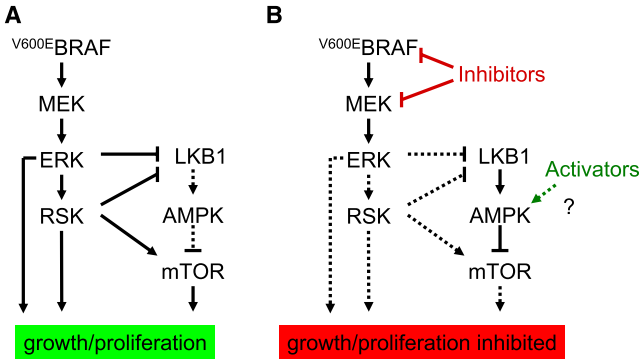

Figure 1. The ^{V600E}BRAF-AMPK Axis in Melanoma Cells

(A) ^{V600E}BRAF rewires the signaling pathway to allow ERK and RSK to phosphorylate and inhibit LKB1, thereby preventing AMPK activation. Together with a positive signal from RSK, this stimulates mTOR activation, leading to cell growth and proliferation.

(B) Inhibition of BRAF/MEK signaling causes a collapse in the downstream network, allowing LKB1 to activate AMPK, which then inhibits mTOR. The loss of these signaling pathways leads to inhibition of cell growth/proliferation. Since these enzymes are part of the same pathway, it is unclear whether AMPK activators will enhance the consequences of MEK/BRAF inhibition.

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