The long arm of BRAF^{V600E} gets to mTORC1

Pablo Lopez-Bergami

e-mail: pablobergami@gmail.com

Despite the disappointing results of early clinical studies, targeting the BRAF/MEK/extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK) pathway is still a subject of investigation due to its biological relevance to melanoma. It is now evident that to antagonize redundant protein functions and compensatory signaling pathways BRAF inhibitors must be combined with inhibitors of other relevant pathways based on a strong rational basis. Thus, we must expand our understanding of MAPK effectors and how MAPK interacts with those other pathways. Two recent articles by Zheng et al. (2009) and Esteve-Puig et al. (2009) show that oncogenic BRAF^{V600E} negatively regulates the tumor suppressor LKB1 to promote both melanoma cell proliferation and attenuation of the apoptotic response to metabolic stress. Mechanistically, activation of the MAPK pathway by mutant BRAF leads to phosphorylation of LKB1 on Ser325 and Ser428 by ERK and p90 Ribosomal S6 Kinase (RSK), respectively (Zheng et al. 2009) (Figure 1). These phosphorylations affect the ability of LKB1 to bind and activate AMP-activated protein kinase (AMPK), a master kinase that regulates activity of many substrates functioning in macromolecule synthesis and cellular metabolism (Inoki et al., 2005). Among these substrates is Tuberous Sclerosis 2 (TSC2), which following phosphorylation activates the TSC1/TSC2 complex and inhibits the mammalian target-of-rapamycin complex 1 (mTORC1) by blocking the activity of the small GTPase Rheb (Figure 1). Substantial biochemical and genetic evidence indicates that inactivation of the LKB1/AMPK/TSC1/2 pathway drives cell proliferation and growth mainly by activation of mTORC1 signaling (Inoki et al., 2005). Conversely, pharmacological AMPK activation has recently been shown to be cytotoxic to many cancer cells both in vitro and in mouse xenograft models. mTORC1 kinase is activated in most human cancers and regulates numerous downstream targets, such as amino acid transporters, vascular endothelial growth factor (VEGF), p70 ribosomal protein S6 kinase 1 (S6K), and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) pathways, to increase protein translation and cell growth (Inoki et al., 2005).

The findings of Zheng et al. and Esteve-Puig et al. confirm a link between LKB1/AMPK/TSC1/2 and the MAPK pathway first delineated by Ma et al. (2005), who demonstrated in fibroblasts that active ERK (induced by either MEK1$^{S218D/S222D}$ or Ras^{G12V}) phosphorylates and inactivates TSC2 (Figure 1). Thus an emerging picture is that activation of MAPK, usually due to BRAF^{V600E} in melanoma, is responsible for mTORC1 activation, implicating the mTORC1 pathway as a new MAPK effector. Altogether, these observations reveal that two independent mechanisms enable MAPK signaling to activate mTORC1: inhibition of LKB1 and TSC2. Moreover, it is well known that Akt phosphorylates TSC2 at several sites, constituting a third mechanism to activate mTORC1 (Inoki et al., 2005). Remarkably, all three mechanisms are functional in a prototypical melanoma cell (Figure 1). Not surprisingly, mTORC1 has been found activated in melanoma cell lines (Karbowniczek et al., 2008). However, rapamycin, an mTORC1 inhibitor, shows only a partial inhibition of cell viability (25–35%, Werzowa et al., 2008) or cell growth (Lasithiotakis et al., 2008). These effects are less potent in melanoma cells than in other cancer cell types, possibly due to the fact that rapamycin increases Akt phosphorylation and enhances survival of some cancer cells via a negative feedback loop mediated by S6K or alternative mechanisms (Werzowa et al., 2008). In addition, a phase II clinical trial from the California Cancer Consortium concluded that the mTORC1 inhibitor CCI-779, an analog of rapamycin, is not active as a single agent in patients with metastatic melanoma. However, these observations should not preclude a possible beneficial effect of rapamycin when combined with other drugs such as phosphatidyl inositol 3-kinase (PI3K), VEGF or BRAF inhibitors. Interestingly, in view of findings described above, combining rapamycin analogs with BRAF inhibitors should not in principle confer an advantage over BRAF inhibitors alone, which are predicted to inhibit mTORC1. Nonetheless, while clinical trials are under way, preclinical studies have shown that combining the multi-kinase sorafenib (BAY 43-9006) with rapamycin inhibited cell growth modestly but significantly (13–27.8%) over sorafenib alone in six cell lines tested (Lasithiotakis et al., 2008). Similar results were obtained when rapamycin was combined with the MEK inhibitors U0126 or PD98059 (Lasithiotakis et al., 2008).

How can we interpret these results? The seemingly limited effect of rapamycin in melanoma is in line with the findings by Zheng et al. and Esteve-Puig et al. On the other hand, if we conclude that inhibiting mTORC1 and BRAF/MEK1 do have an additive effect, one explanation is that active ERK cannot completely inhibit the LKB1/AMPK/TSC/mTORC1 pathway. This interpretation seems to be in disagreement with data by Esteve-Puig et al. who showed

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Esteve-Puig R, Canals F, Colomé N, Merlino G and Recio JA. (2009). Uncoupling of the LKB1-AMPKa Energy Sensor Pathway by Growth Factors and Oncogenic BRAF^{V600E}. *PLoS ONE* **4**: e4771.

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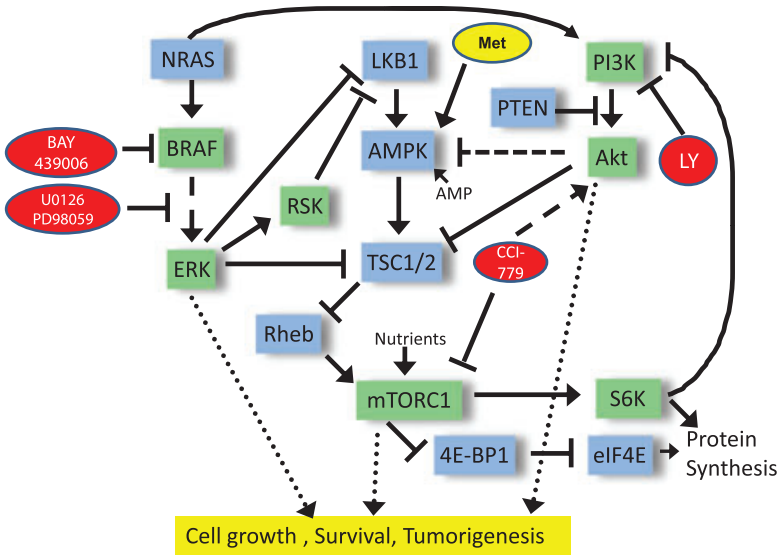

Figure 1. Reciprocal regulation of NRAS/BRAF/ERK, LKB1/AMPK/Tuberous sclerosis (TSC)/mammalian target-of-rapamycin complex 1 (mTORC1) and PI3K/Akt pathways in melanoma. Sharp and blocked arrows show activating and inhibitory effects, respectively. Protein kinases in green boxes are activated as a consequence of mechanisms operating in a prototypical melanoma cell ($BRAF^{V600E}$ and mutant phosphatase and tensin homolog deleted on chromosome Ten (PTEN). ERK and Akt activity activates mTORC1 through several mechanisms contributing to melanoma cell growth, survival and tumorigenesis. See text for details. Compounds usually used in melanoma to inhibit these pathways are show in red. Met, Methformin, LY, LY-294002.

complete inhibition of S6K phosphorylation by U0126, although the experiment was performed under metabolic stress conditions. Unfortunately, the study of Zheng et al. (2009), did not evaluate the effect of inhibiting MAPK downstream of TSC1/2. Also, none of the multiple studies analyzing MAPK inhibition in melanoma have exhaustively explored effects of such inhibition on mTORC1 activity. This gap in our knowledge not only makes difficult to assess how important the effects of MAPK on mTORC1 really are, but also opens the possibility that AMPK or TSC1/2 exerts its effects on cell growth via targets other than mTORC1. These open questions will lead undoubtedly to further explore the link between ERK and LKB1/TSC/mTORC1 pathways.

Another question brought about by the article from Zheng et al. is whether mutations inactivating LKB1 and TSC1/2 occasionally function in melanoma development. LKB1 and TSC1/2 are mutated in the germline of patients with the related neurocutaneous hereditary diseases Peutz–Jeghers syndrome (PJS) and Tuberous sclerosis (TSC), respectively (Inoki et al., 2005). Peutz–Jeghers syndrome and Tuberous sclerosis are benign tumor syndromes characterized by development of hamartomas, and they exhibit striking biochemical (both activate the mTORC1 pathway) and histopathological similarities. Whereas TSC lesions frequently express melanoma-associated antigens, including HMB-45, melan-A, CD63, and PNL2, PJS patients usually present with mucocutaneous hyper-pigmentation. The pigmented nature of PJS lesions has led investigators to evaluate LKB1 status in melanoma samples. Rowan et al. (1999) established that 4% of 50 melanoma samples analyzed showed somatic mutations (likely to be inactivating) in LKB1, suggesting that LKB1 contributes to tumorigenesis in a small fraction of malignant melanomas. To date, the occurrence of TSC1/2 mutations in melanoma has not been explored. Since the study of Rowan et al. was performed in 1999, BRAF status was not analyzed. Interestingly, a recent study of lung cancer showed that mutations in LKB1 are associated strictly with KRAS mutation but not with the $BRAF^{V600E}$ mutation (Mahoney et al., 2009). As is the case in melanoma with BRAF and NRAS mutations, BRAF and KRAS mutations in lung are mutually exclusive (Mahoney et al., 2009). The data presented by Zhang et al. suggest that mutations in LKB1 could provide a proliferative advantage to RAS (but not to BRAF) mutated cells. If the above holds true in melanoma, patients with LKB1 mutations may represent an important proportion of wild-type BRAF patients. Although it requires confirmation in a larger dataset, Karbowniczek et al. (2008) have established that the frequency of BRAF mutation in melanoma is markedly lower (41%) in patients with high phospho-S6K levels (an imperfect surrogate of LKB1 mutation) than in those with low phospho-S6K levels (80%). As LKB1/KRAS double mutant lung cancer cell lines are more sensitive to the MEK inhibitor CI-1040 than are KRAS single-mutated cells (Mahoney et al., 2009), identification of melanoma patients with this genetic profile may represent a favorable treatment opportunity.

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