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Review

Melanoma metabolism contributes to the cellular responses to MAPK/ERK pathway inhibitors

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ABSTRACT

Background: Besides its influence on survival, growth, proliferation, invasion and metastasis, cancer cell metabolism also greatly influences the cellular responses to molecular-targeted therapies.
Scope of the review: To review the recent advances in elucidating the metabolic effects of BRAF and MEK inhibitors (clinical inhibitors of the MAPK/ERK pathway) in melanoma and discuss the underlying mechanisms involved in the way metabolism can influence melanoma cell death and resistance to BRAF and MEK inhibitors. We also underlined the therapeutic perspectives in terms of innovative drug combinations.
Major conclusion: BRAF and MEK inhibitors inhibit aerobic glycolysis and induce high levels of metabolic stress leading to effective cell death by apoptosis in BRAF-mutated cancer cells. An increase in mitochondrial metabolism is required to survive to MAPK/ERK pathway inhibitors and the sub-population of cells that survives to these inhibitors are characterized by mitochondrial OXPHOS phenotype. Consequently, mitochondrial inhibition could be combined with oncogenic "drivers" inhibitors of the MAPK/ERK pathway for improving the efficacy of molecular-targeted therapy.
General significance: Metabolism is a key component of the melanoma response to BRAF and/or MEK inhibitors. Mitochondrial targeting may offer novel therapeutic approaches to overwhelm the mitochondrial addiction that limits the efficacy of BRAF and/or MEK inhibitors. These therapeutic approaches might be quickly applicable to the clinical situation.

1. Introduction: heterogeneity and adaptability of cancer cell metabolism

One prominent characteristic of cancer cell metabolism is the wide heterogeneity of metabolic phenotypes encountered in tumors. In the last decades, the analyses of cancer cell metabolism showed that glucose was the major fuel for cancer cell proliferation confirming Otto Warbug's observations in the 50's [1]. Beyond this classic glycolytic phenotype, various primary cancer cells as well as cancer cell lines show addictions to other carbon sources such as glutamine or fatty acid. In numerous cancer cells, glutamine and fatty acid are oxidized in mitochondria for energy production and/or anabolism defining a mitochondrial **oxidative** (or OXPHOS) phenotype in addition to the original glycolytic phenotype.

Another remarkable feature is the fact that the metabolic profile of cancer cell is a dynamic one [2]. Furthermore, cancer progression from the primary neoplasm to metastasis needs mitochondrial metabolism [3,4]. That means that metabolic networks change constantly to adapt cancer cells to external signals (harsh conditions including hypoxia or nutrient deprivation of the tumor microenvironment) and internal signals (mostly the aberrant signaling regulated by oncogenes and/or tumor suppressors). As a result, metabolic rheostats such as the hypoxia-inducing transcription factor-1 alpha (HIF-1α) [5] and the AMP-activated protein kinase (AMPK) [6] are activated to balance glycolysis and mitochondrial activity in response to anabolic and energetic needs. Thus, the metabolism is integrated to the regulatory networks that drive

Abbreviations: MAPK, mitogen-activated protein kinase; BRAF, v-raf murine sarcoma viral oncogene homolog B1; MEK, mitogen-activated protein/extracellular signal- regulated kinase kinase; ERK, extracellular signal-regulated kinase; HIF-1α, hypoxia inducible factor 1 alpha; AMPK, AMP activated protein kinase; LDHA, lactate dehydrogenase A; MITF, microphthalmia- associated transcription factor; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PDK, pyruvate dehydrogenase kinase; DRP-1, dynamin-related protein 1; FDG, 2-deoxy-2-[^{18}F]fluoro-D-glucose; PET/CT, positron emission tomography and x-ray computed tomography; ER, endoplasmic reticulum; UPR, unfolded protein response; LKB1, liver kinase B1; ULK1, Unc-51 like autophagy activating kinase; mTORC1, mammalian target of rapamycin complex 1; NMR, nuclear magnetic resonance; EGFR, epidermal growth factor receptor; COT, cancer Osaka thyroid; JARID 1B, Jumonji AT-rich interactive domain 1B; PI3K, Phosphatidylinositol 3-kinase

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cancer cell fate decisions leading to survival, proliferation or death.

2. The MAPK/ERK pathway adapts cell metabolism to promote growth and proliferation

The underlying genetic background largely contributes to the determination of the metabolic profile that influences cancer cell fate (for review [7]). Oncogenic mutation reprograms cellular metabolism to sustain cell survival, a high rate of cellular growth and proliferation regardless of external conditions. There are striking evidence that oncogenic drivers impose a distinct metabolic signature of cancer cells to meet energetic and anabolic demands of cell growth and intense proliferation. Numerous driver mutations have been identified in melanoma including BRAF, NRAS, KIT, GNAQ, GNA11, NF1 and telomerase [8]. Most of them activate the RAF-MEK-ERK signaling pathway (designed MAPK/ERK pathway), one of the best described mitogen-activated protein kinase (MAPK) cascade in melanoma [9]. Mutationally activated B-RAF, such as the mutant BRAFV600E, is present in approximately 50% of melanoma cases and in a lower percentage in several solid tumors [10]. The presence of the BRAFV600E mutation leads constitutively to the phosphorylation of the dual-specificity kinases MEK1 and MEK2, driving ERK1 and ERK2 activation to promote cell growth and proliferation.

Interestingly, the activation of the MAPK/ERK pathway significantly increases the expression of glucose transporters as well as numerous glycolytic genes, resulting in a classical glycolytic phenotype providing substrates required to build new cells (for review see [9,11]). Ectopic overexpression of BRAFV600E increases the expression of metabolic genes including those coding lactate dehydrogenase A (LDHA), the key enzyme for the final step of the aerobic glycolysis and pentose phosphate pathway enzymes [12]. The mechanisms responsible for the way mutated BRAF elicits aerobic glycolysis involve the control glycolytic genes expression by combining three transcription factors including HIF-1α, Myc and MondoA [1,13]. In this cellular context, many glycolysis intermediates are used as building blocks to support macromolecule synthesis.

Besides the increase in aerobic glycolysis, mutated BRAF also actively represses mitochondrial OXPHOS *via* several mechanisms. It is well established that a subset of melanoma tumors, expressing the melanocyte lineage factor MITF that drives the expression of PGC1α (the central co-activator of mitochondrial biogenesis) is characterized by higher rates of mitochondrial OXPHOS [2,14]. Oncogenic BRAF mutants negatively control the MITF-PGC1α axis and thus decrease the mitochondrial oxidative metabolism [3,4,15]. Another distinct mechanism responsible for the low mitochondrial oxidation in BRAF mutated cells is the expression of pyruvate dehydrogenase kinase (PDK). Indeed, BRAF mutated melanoma cells are characterized by a high level of HIF-1α expression and its downstream target PDK [5,16,17]. It is well known that the HIF-1α/PDK axis acts as a crucial inhibitor of mitochondrial function in melanoma since PDK strictly controls the pyruvate dehydrogenase (PDH) activity, *i.e.* the mitochondrial gatekeeper for oxidation of glucose-derived pyruvate [9,17]. In addition, a key feature of BRAF mutated cancers is the disruption of mitochondrial dynamics. Thus, aberrant activation of the MAPK/ERK signaling pathway increases the expression and phosphorylation-dependent activity of the dynamin related-protein 1 (DRP-1) leading to mitochondrial fission and decreased respiration [7,18,19]. Apart from the metabolic effects described above, oncogenic BRAF also promotes the synthesis of the ketone body acetoacetate, through the expression of the ketogenic enzyme 3-hydroxy-3-methylglutaryl-CoA lyase. Interestingly, acetoacetate favors the BRAFV600E/MEK interaction and activation of ERK, thereby creating a positive feedback loop stimulating proliferation [9,20].

Given the aforementioned evidence of the influence of oncogenic MAPK/ERK signaling pathway on the metabolic switch from mitochondrial OXPHOS to glycolysis *i.e.* the Warburg effect, it is not surprising that metabolism influenced by MAPK/ERK pathways clinical inhibitors (*i.e.* molecular-targeted therapies such as BRAF and/or MEK inhibitors). In addition, the metabolic profile of cancer cells can also influence the cellular responses to molecular-targeted therapies.

In this review, we addressed the recent developments on *(i)* the metabolic stress induced by MAPK/ERK pathway inhibitors, *(ii)* the metabolic signature of cancer cells resistant to MAPK inhibitors *(iii)* and finally we focused on the perspective of innovative drug combinations able to induce anti-metabolic cooperativity and eventually kill cancer cells in this context.

3. BRAF and MEK inhibitors promote metabolic stress mediated-apoptosis

Drugs that target the MAPK/ERK pathway have profoundly changed the landscape of melanoma therapy [10,21]. Selective inhibitors of the BRAF mutant protein alone (such as vemurafenib (PLX4032) and dabrafenib (GSK2118436)) or inhibitors of its downstream kinase, MEK, (*e.g.* MEK inhibitors, cobimetinib, trametinib, selumetinib) are effective in slowing the progression of BRAF V600E mutant melanomas, which are strictly dependent on the MAPK/ERK pathway for growth and survival. As previously demonstrated, BRAF mutant inhibitors decrease glucose uptake and the subsequent glycolysis, a situation correlated with the inhibition of the MAPK/ERK cascade [9,22] [11,13]. MEK inhibitors also reduce lactate production mainly through the inhibition of Hexokinase 2 in BRAF mutated melanoma [12,23]. In fact, it seems reasonable to think that the inhibition of glucose metabolism could be an effective response to BRAF or MEK inhibitors. Indeed, there is a positive correlation between the degree of glycolysis dependence and sensitivity to BRAF or MEK inhibition *in vitro* [17,24]. Consistently, the respiration-incompetent variant cell lines (*a.k.a* ρ^0 cells), which largely depend on aerobic glycolysis for survival [25], exhibit increased sensitivity to BRAF and MEK inhibition [17,24]. Furthermore, the positron emission tomography–computed tomography with fluorine 18 (^{18}F) fluorodeoxyglucose (FDG PET/CT) imaging, routinely used in oncology practice, allows the *in vivo* quantification of glucose metabolism in tumors. FDG-PET/CT imaging done before and after treatment with BRAF and MEK inhibitors highlights that glucose uptake inhibition occurs shortly after starting therapy and that glycolysis inhibition is correlated with a good prognosis for patients with BRAF mutant melanomas [26]. Altogether these results suggest that glycolysis inhibition could contribute to the anticancer effects of BRAF and/or MEK inhibitors.

There is increasing evidence that, similarly to other antineoplastic drugs, BRAF and MEK inhibitors induce high levels of metabolic stress characterized by glycolysis inhibition reducing the growth and viability of cells that usually require glucose to proliferate [27]. Mechanistically, the reduction in glucose uptake and glycolysis appears to be related to the decrease in cell volume, a direct consequence of the inhibition of protein translation induced by BRAF inhibition [22]. Interestingly, BRAF inhibitors not only regulate glucose metabolism but also affects glycine, myo-inositol, and lipid metabolism [28].

In cancer cells, stresses elicited by kinase inhibitors coexist with different intensities and interact with each other to form a complex network of signaling pathways, culminating in cell death under the influence of the cell context. The mechanisms involved in the way stress conditions can cooperate to kill cells upon kinase inhibition remain largely unknown. It seems plausible that the inhibition of glucose uptake and glycolysis mediated by oncogenic kinase inhibitors could lead to intense energy stress. However, the energy crisis promoted by kinase inhibitors is often less severe than expected. Thus, BRAF inhibition does not result in an extreme energy drop as evidenced by the sustained level of ATP in melanoma cells exposed to vemurafenib [29]. Overall, it seems that this energetic compromise does not play a crucial role in the initiation of cell death induced by kinase inhibitors. The absence of energetic collapse suggests the existence of compensatory pathways for the loss of glucose-dependent ATP production in cancer cells exposed to