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FLCN: A new regulator of AMPK-dependent Warburg metabolic reprogramming

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Abbreviations: AMPK, AMP-activated protein kinase; BHD, Birt-Hogg-Dubé syndrome; FLCN, folliculin; FNIP, folliculin interacting protein; HIF, hypoxia-inducible factor; MEF, mouse embryonic fibroblast; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1 α; ROS, reactive oxygen species.

Tumor cells manage their energy to support aberrant proliferation by reprogramming their cellular metabolism, for example through the Warburg effect. Although AMPK is a major regulator of energy homeostasis, its role in cancer metabolic adaptation is unclear. We recently identified the tumor suppressor folliculin as a new regulator of AMPK-dependent metabolic transformation.

Sustaining rapid and persistent proliferation represents a bioenergetic and biosynthetic challenge for cancer cells, which must efficiently manage their energetic resources to survive and grow in unfavorable environments. This is achieved through metabolic reprogramming to stimulate aerobic glycolysis, a process known as the Warburg effect, which is now a well-appreciated hallmark of cancer.[1] However, the signaling pathways and key regulators of this metabolic transformation are still poorly defined. AMP-activated protein kinase (AMPK), a major physiological regulator of cellular energy homeostasis, is located at the center of a metabolic network.[2] Indeed, AMPK functions as a sensor of cellular energy fluctuation and a driver of pathways that minimize energy consumption and maximize energy production upon energetic stress. Despite its critical function in cell metabolism, the role of AMPK in cancer is controversial as both pro- and anti-tumorigenic effects have been described.[2] In fact, while AMPK activation might prevent tumor initiation through its ability to restrict cell proliferation, several reports have shown that AMPK gain of function acts as a driver of tumorigenesis by enhancing glycolytic energy production and cell survival under metabolic stress conditions.[2] Our recent work sheds light on how AMPK activation could drive metabolic transformation and tumorigenesis.[3]

Germline inactivating mutations in the folliculin (*FLCN*) tumor suppressor gene predispose to Birt-Hogg-Dubé (BHD) syndrome, an inherited cancer disorder associated with lung cysts, pneumothorax susceptibility, renal cell carcinoma, and skin tumors.[4] Although FLCN and its uncharacterized binding partners folliculin interacting protein 1 (FNIP1) and 2 (FNIP2) were identified as AMPK binding partners and phosphorylation tar binding partner and phosphorylation target, its physiological role and tumor suppressor mechanism and its impact on AMPK-dependent functions are still poorly characterized. We previously reported that loss of FLCN enhances transcriptional activity of hypoxia-inducible factor (HIF) and increases the glycolytic rate in human kidney cancer cells.[5] Moreover, a recent publication demonstrated that conditional deletion of *FLCN* in mouse kidney and muscle results in increased mitochondrial oxidative phosphorylation, suggesting that loss of FLCN enhances cellular metabolism.[6] To maintain cellular homeostasis under hypoxic conditions, HIF drives the transcription of genes that stimulate glycolysis, angiogenesis, and energy supply, which in turn promotes solid tumor growth, invasion, and metastasis.[7] However, how FLCN and AMPK regulate HIF activity and metabolic adaptation in normoxic conditions, and whether this effect is linked to tumorigenesis, is not defined.

Using untransformed *Flcn*$^{-/-}$ mouse embryonic fibroblasts (MEFs) and cancer cell lines naturally deficient for *FLCN* expression, we demonstrated that FLCN depletion constitutively activates AMPK independent of the cellular energy state. Using a non-phosphorylatable form of FLCN that is mutated at a previously identified AMPK phosphorylation and binding site we established that loss of FLCN binding to AMPK also results in constitutive AMPK activation.[8] Chronic AMPK activation leads to upregulation of

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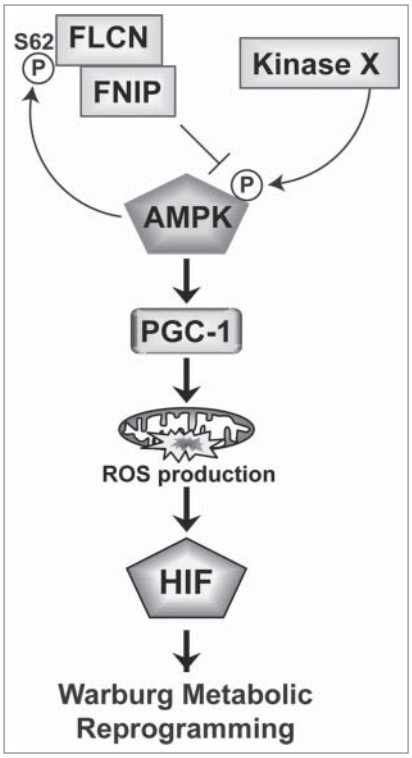

Figure 1. FLCN regulates AMPK activation and downstream Warburg metabolic reprogramming. Folliculin (FLCN) binds and inhibits phosphorylation (P) of AMP-activated protein kinase (AMPK) via serine 62 (S62), a previously described AMPK phospho-site. Upon loss of FLCN, AMPK is activated by phosphorylation by an unidentified kinase (Kinase X) and stimulates transcription and expression of peroxisome proliferator-activated receptor γ coactivator 1 (PGC-1), leading to enhanced mitochondrial biogenesis and reactive oxygen species (ROS) production. This drives hypoxia-inducible factor (HIF) transcriptional activation and enhances Warburg metabolic reprogramming.

the peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1α), a known target of AMPK, which in turn increases mitochondrial content and the oxidative phosphorylation rate, leading to enhanced production of reactive oxygen species (ROS). Surprisingly, elevated mitochondrial ROS production is not associated with increased oxidative protein and DNA damage, but rather acts as a signaling molecule to activate HIF transcriptional activity without affecting HIF-1α protein stability. Upon FLCN depletion, the active HIF complex mediates transcription of glycolytic effectors, thus enhancing glucose uptake and the glycolytic rate. Strikingly, knockout of AMPKα, knockdown of PGC-1α, or reduction of ROS levels by antioxidants abolish the HIF-dependent glycolytic increase (**Fig. 1**).

By concomitantly upregulating mitochondrial oxidative phosphorylation and glycolysis, the chronic AMPK activation induced by FLCN deficiency increases cellular ATP levels and biosynthetic precursors, mimicking the metabolic signature of highly proliferative cells. Interestingly, this metabolic transformation is not associated with a direct effect on cell proliferation or spontaneous transformation of primary cells per se. However, loss of FLCN significantly enhances the HIF-1α-dependent anchorage-independent and *in vivo* tumor growth of human cancer cell lines. Finally, HIF-1α nuclear translocation and upregulation of glycolytic effectors controlled by HIF were observed in a chromophobe tumor from a BHD patient, further suggesting that FLCN deficiency induces AMPK- and HIF-dependent metabolic reprogramming that confers a tumorigenic advantage *in vivo*.

Taken together, our recently reported data suggest a tumor suppressor mechanism for FLCN. We identify FLCN as a new physiological negative regulator of AMPK-dependent metabolic reprogramming, which might have wider implications for other cancers. Although AMPK was previously reported to increase the transcriptional activity of HIF without affecting its stability, the mechanism involved was not identified.[9] Strikingly, our findings confirm a link between AMPK and HIF transcriptional activation in normoxic conditions and identify PGC-1α as an intermediate effector. In addition, our data highlight a non-canonical role for ROS in HIF activation that differs from the classical ROS-dependent inhibition of prolyl-hydroxylases. Moreover, our work demonstrated that an increase in glycolysis is not necessarily associated with reduced mitochondrial oxidative phosphorylation and that both ATP generation processes could work simultaneously to provide metabolic flexibility, thus conferring bioenergetic and biosynthetic advantages on cancer cells. Our data are compatible with recent reports showing that AMPK and PGC-1α gain of function mutations are drivers of tumorigenesis by promoting the metabolic plasticity required to support malignant growth and survival under energetic stress conditions, such as hypoxia and nutrient limitation. This AMPK-dependent metabolic adaptation process might be particularly crucial during cancer progression and the development of metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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