PROSPECTS
Journal of Cellular Biochemistry 113:404–409 (2012)

Journal of Cellular Biochemistry

Targeting AMPK in the Treatment of Malignancies

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ABSTRACT

The AMPK pathway is a metabolic stress-related and energy censor pathway which plays important regulatory roles in normal and malignant cells. This cellular cascade controls generation of signals for initiation of mRNA translation via the mTOR pathway and exhibits regulatory roles on the initiation of autophagy. AMPK activators have been shown to suppress mTOR activity and to negatively control malignant transformation and cell proliferation of diverse malignant cell types. Such properties of AMPK inducers have generated substantial interest for the use of AMPK targeting compounds as antineoplastic agents and have provoked extensive research efforts to better define and classify the mechanisms controlling AMPK activity and its functional consequences in malignant cells. J. Cell. Biochem. 113: 404–409, 2012.

KEY WORDS: AMPK; mTOR; CANCER

The AMP-activated protein kinase (AMPK) is composed of a catalytic subunit (α subunit) and two regulatory subunits (β and γ subunits) [Davies et al., 1994; Mitchelhill et al., 1994; Carling et al., 2011; Xiao et al., 2011]. In order for the kinase activity of AMPK to be induced, phosphorylation of residue Thr172 located in the α subunit of the complex is required [Hawley et al., 1996]. Such phosphorylation is mediated by the upstream LKB1 kinase in response to changes in the AMP/ATP ratio [Hawley et al., 1996]. Increased levels of AMP result in binding of AMP to the γ subunit of AMPK, promoting a conformational change of the AMPK protein to expose the Thr172 position as a substrate for LKB1 [Hawley et al., 1996; Carling et al., 2011]. Phosphorylation of AMPK on that site also occurs in response to Ca^{2+} changes, via engagement of the calmodulin-dependent protein kinase kinase-beta (CAMKKβ) [Hawley et al., 2005]. In such Ca^{2+}-mediated activation of AMPK, the Thr172 phosphorylation can occur in the absence of any apparent changes in the AMP levels [Carling et al., 2008]. The interaction between CAMKKβ and AMPK occurs via the kinase domains of these kinases and it is specific for CAMKKβ, as previous work failed to show interactions of CAMKKα with AMPK [Green et al., 2011].

Engagement of AMPK results in downstream signals that ultimately control processes important for regulation of metabolism, including fatty acid oxidation and mRNA translation/protein synthesis [Carling et al., 2008; Carling et al., 2011; Hardie 2011]. Previous studies have demonstrated that AMPK suppresses activation of the mTOR pathway via indirect inhibitory effects on the mTORC1 complex, involving phosphorylation and activation of the tuberous sclerosis complex 2 (TSC2), which in turn inhibits Rheb [Inoki et al., 2002]. There is also evidence for direct modulation of the mTOR complex, via AMPK-induced phosphorylation of Raptor, a key component of the mTORC1 complex. AMPK regulates the mTORC1 complex via phosphorylation of Raptor on Ser792 and Ser722, followed by 14-3-3 binding to Raptor and mTORC1 inhibition [Gwinn et al., 2008].

AMPK physiologically inhibits mTOR to optimize homeostasis in the context of decreased available energy sources to the cell [Carling et al., 2011]. However, as mTOR complexes play key roles in proliferation and survival of malignant cells [Bjornsti and Houghton, 2004; Sabatini, 2006] there has been substantial interest in AMPK activators as potential antineoplastic agents. The best known AMPK activators are AICAR and metformin and both of these agents have been used extensively in studies to define the roles of AMPK signaling in various cellular processes. Upon transport in the cell, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) is converted to ZMP, ultimately resulting in AMPK activation and fatty acid oxidation [Corton et al., 1995; Merrill et al., 1997]. The mechanism of metformin-mediated AMPK activation is still uncertain; however, one possible mechanism is the inhibition of the mitochondrial complex I, thereby decreasing ATP production leading to an indirect increase of the AMP/ATP ratio [Owen et al., 2000]. A recent study suggested a novel mechanism by which metformin activates AMPK involving inhibition of AMP deaminase (AMPD), followed by inhibition of AMP breakdown and

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Received 9 September 2011; Accepted 12 September 2011 • DOI 10.1002/jcb.23369 • © 2011 Wiley Periodicals, Inc.
Published online 16 September 2011 in Wiley Online Library (wileyonlinelibrary.com).

accumulation of AMP [Ouyang et al., 2011]. This study also demonstrated that AMPD knockdown results in abrogation of metformin stimulation of glucose transport, underscoring the relevance of this deaminase in the generation of metformin responses [Ouyang et al., 2011].

AMPK AS A THERAPEUTIC TARGET IN MALIGNANCIES

AMPK has been implicated in the regulation of glucose homeostasis [Mor and Unnikrishnan, 2011] and skeletal muscle metabolism [Jorgensen et al., 2006]. However beyond such functions and because of the critical regulatory roles of AMPK in cell metabolism, this kinase is an important target for the development of therapeutic approaches in type II diabetes and the metabolic syndrome [Rutter and Leclerc, 2009; Viollet et al., 2009; Zhang et al., 2009] and, in fact, metformin is one of the major drugs used in the treatment of type II diabetes. However, as there has been emerging evidence that many of the pathways deregulated in cancer affect cell metabolism [Cairns et al., 2011], an increasing number of studies have focused on the link between AMPK regulation and tumorigenesis. Below, we discuss basic studies focusing on AMPK in different malignancies and clinical-translational implications and efforts resulting from such work [Fig. 1].

RENAL CELL CARCINOMA

There is substantial evidence for aberrant activation of the AKT/mTOR pathway in renal cell carcinoma (RCC), while mTORC1 inhibitors have shown significant clinical activity in the treatment of this malignancy [Hudes et al., 2007; Dancey, 2010]. Moreover, increased phosphorylation of AKT and reduced PTEN expression appears to correspond with lower survival rates in RCC [Hager et al., 2009]. Such evidence has raised the possibility that modulation of AMPK may provide an additional approach to target the mTOR pathway in RCC cells. Consistent with this, a recent study demonstrated that activation of AMPK by metformin or AICAR in RCC lines correlates with suppression of mTOR effectors and generation of antineoplastic responses [Woodard et al., 2010]. Interestingly, concomitant treatment with statins, which in previous studies were shown to block mTOR activation [Woodard et al., 2008], enhanced the anti-RCC effects of metformin or AICAR suggesting that such combinations may provide an approach to enhance targeting of RCC cells [Woodard et al., 2010]. Another recent study demonstrated that metformin induces G_0/G_1 cell cycle arrest and suppresses renal carcinoma growth in a xenograft model in nude mice [Liu et al., 2011]. Altogether these recent studies have suggested that AMPK targeting may provide an effective approach for the treatment of RCC, a concept further reinforced by the emerging evidence that renal cancer is a metabolic disease, as shown among other things by the fact that all known kidney cancer genes are involved in pathways engaged in response to metabolic stress or to nutrient stimulation [Linehan et al., 2010].

BREAST CANCER

There is evidence for dysregulation of the PI3K/AKT/mTOR pathway in breast cancer, resulting in substantial interest on the therapeutic potential of agents that target mTOR [O'Regan and Hawk, 2011]. As AMPK activation results in mTOR suppression, this has raised the potential of AMPK targeting for the treatment of breast cancer, while the importance of exploring the anticancer effects of metformin has been articulated [Hadad et al., 2008]. Notably, other studies have shown that AMPK is dysfunctional in primary breast cancer and that

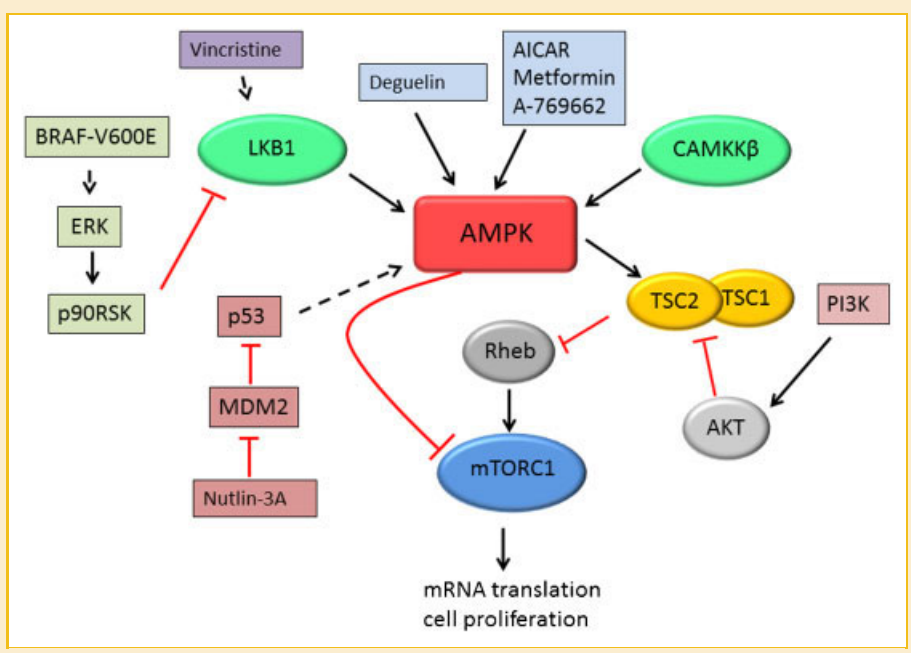

Fig. 1. Overview of signaling pathways and compounds regulating AMPK in malignancies.

decreased AMPK signaling has an inverse relationship with histological grade and axillary lymph node involvement, further supporting the potential value of AMPK activators in therapeutic approaches for breast cancer [Hadad et al., 2009]. In vitro studies have shown that metformin activates AMPK in breast cancer cell lines and suppresses activation of elements of the mTOR cascade and inhibits malignant cell proliferation [Zakikhani et al., 2006]. In addition, there is recent evidence that siRNA-mediated knockdown of AMPK in breast cancer cells reverses the effects of metformin [Zakikhani et al., 2006]. Other studies have demonstrated that this agent induces cell cycle arrest of breast cancer cells independently of p53 or HER2 mutations [Zhuang and Miskimins, 2008]. Such effects appear to be mediated by reduction in cyclin D1 levels, in a $p27^{Kip1}$ and $p21^{Cip1}$-dependent manner [Zhuang and Miskimins, 2008]. Importantly, AMPK targeting appears to have suppressive effects on breast cancer stem cells. Metformin has been shown to selectively inhibit breast cancer stem cells [$CD44^{high}/CD24^{low}$], while it acts synergistically with doxorubicin, which inhibits cancer cells but not stem cells [$CD44^{low}/CD24^{high}$], resulting in decrease in the numbers of both cell populations [Hirsch et al., 2009]. Other work has shown inhibitory effects of metformin on cell proliferation of triple negative (ER, PR, HER2/neu negative) breast cancer cells, associated with diverse molecular responses [Liu et al., 2009].

Beyond laboratory studies, there has been evidence from epidemiological studies suggesting that AMPK activation may be effective in the treatment of breast cancer. For instance, diabetic patients on metformin have a lower incidence of breast cancer [Evans et al., 2005]. In addition, among diabetic patients with breast cancer on neoadjuvant therapy, those receiving metformin were found to have higher chance to achieve complete remissions (CR) than those not receiving metformin [Jiralerspong et al., 2009]. A recent clinical trial [Hadad et al., 2011] examined the effects of metformin on Ki67 and gene expression in non-diabetic women with primary operable invasive breast cancer. This study defined biological effects of metformin in vivo, demonstrating that metformin decreased tumor proliferation assessed by Ki67 staining and suppressed expression of phosphodiesterase 3B, while induction of several genes such as p53 and BRCA1 was also affected [Hadad et al., 2011]. Thus, it appears that AMPK activation using metformin may be of value in future efforts to devise new approaches for the treatment of breast cancer. It will be also of interest to examine in more detail the effects of other agents that have been reported to activate AMPK in breast cancer cells in vitro, such as the ginseng saponin metabolite compound K [Kim et al., 2010], quercetin [Lee and Park, 2010] and resveratrol [Lin et al., 2010].

MALIGNANT MELANOMA

There is evidence that malignant melanoma cells harboring the B-RAF V600E mutation on the BRAF kinase which leads to over-activation of the RAF/MEK/ERK signaling cascade have significantly reduced AMPK activity [Zheng et al., 2009]. The mechanism by which such suppression occurs appears to reflect enhanced activation of the downstream effectors of B-RAF, ERK and p90RSK, resulting in phosphorylation and inactivation of LKB1 [Zheng et al., 2009]. Previous studies have demonstrated that inhibiting the AKT/mTOR pathway enhances the pro-apoptotic effects of certain chemotherapeutic agents in melanoma cells [Sinnberg et al., 2009]. Recent work has also shown that AICAR and metformin exhibit antiproliferative effects on malignant melanoma cell lines and suppress anchorage-independent growth [Woodard and Platanias, 2010]. In addition, the pro-apoptotic effects of these agents were enhanced by concomitant statin-treatment [Woodard and Platanias, 2010]. Interestingly, another recent study demonstrated that vincristine, exerts its cytotoxic effects on melanoma cells via reactive oxygen species [ROS]-mediated activation of AMPK by LKB1. The pro-apoptotic effects of vincristine were shown to depend on AMPK activation and were further enhanced by combination with AICAR, thereby inhibiting the mTOR pathway and activating the p53 pathway, ultimately resulting in apoptotic cell death [Chen et al., 2011]. Beyond in vitro effects, metformin has also been shown to suppress melanoma growth in vivo in a B16 mouse melanoma model [Janjetovic et al., 2011b].

LUNG CANCER

Inactivation of LKB1, one of the two main AMPK kinases has been shown to cooperate with Kras mutations to promote lung tumorigenesis [Ji et al., 2007]. Remarkably, homozygous inactivation of LKB1 was found to result in stronger cooperation with Kras for transformation than loss of p53 or Ink4a/Arf [Ji et al., 2007]. Other studies demonstrated that AICAR reduces cell death of LKB1-wild-type but not LKB1-mutant lung carcinoma cells under reduced glucose conditions [Carretero et al., 2007]. Increasing AMPK activity may provide an approach to prevent development and/or target lung tumors as suggested by a study demonstrating that, by activating AMPK and suppressing AKT, the chemo-preventative agent deguelin inhibits translation of survivin and suppresses lung tumorigenesis [Jin et al., 2007]. Treatment with metformin has also been shown to prevent lung carcinogenesis in mice exposed to a tobacco carcinogens [Memmott et al., 2010]. Interestingly, although AMPK activation in that case was found in the liver but not lungs of such mice, mTOR activity was suppressed in lung tissue, raising the possibility that inhibition of lung tumorigenesis in this case might reflect direct effects on mTOR, rather than AMPK induction [Memmott et al., 2010]. AMPK is also activated by ionizing radiation in an LKB1-independent manner, resulting in $p21^{(waf/cip)}$ and cell cycle arrest [Sanli et al., 2010], while treatment of lung cancer cells with lovastatin also results in AMPK induction, and may be a mechanism by which lovastatin sensitizes cells to ionizing radiation [Sanli et al., 2011].

OVARIAN CANCER

The potential use of metformin as an anti-neoplastic agent in ovarian cancer is supported by studies demonstrating in vitro inhibitory effects [Gotlieb et al., 2008], as well as dose-dependent antitumor effects in a mouse xenograft model for ovarian cancer [Rattan et al., 2011a]. In that study, tumors from mice injected with A2780 ovarian cells were treated with metformin, resulting in 50–60% reduced size as compared to tumors from untreated mice. Such in vivo effects of metformin correlated with AMPK activation and inhibition of protein biosynthesis as a result of suppression of the mTOR pathway [Rattan et al., 2011a]. In addition, metformin

resulted in a significant reduction of angiogenesis and metastasis, while combination of metformin and cisplatin results in synergistic cytototoxicity [Rattan et al., 2011a]. There is also evidence that ovarian carcinoma cells are sensitive to glucose deprivation, as a result of induction of AMPK activity, further suggesting that AMPK activators may ultimately prove to be of value in the treatment of ovarian cancer [Priebe et al., 2011]. Other studies have provided evidence that metformin may generate antiproliferative effects on ovarian cells via both AMPK-dependent and AMPK-independent mechanisms [Rattan et al., 2011b]. Altogether, these studies suggest that engagement of AMPK may provide a novel clinical-translational approach in the treatment of ovarian cancer. However, there should be also some caution, as one study demonstrated that AMPK activation by lipophospatidic acid (LPA) promotes tumor metastasis in ovarian cancer [Kim et al., 2011].

OTHER SOLID TUMORS

Engagement of AMPK and/or known activators such as AICAR or metformin have been shown to generate inhibitory responses in several different types of solid tumor cells. These include models using pancreatic cancer cells [Kisfalvi et al., 2009], thyroid cancer cells harboring the BRAF-V600E mutation [Choi et al., 2011], and glioblastoma cells [Guo et al., 2009]. There is also evidence that the AMPK activator phenformin suppresses the growth of colon cancer cells [Lea et al., 2011], while induction of apoptosis of colon cancer cells by 20(S)-ginsenoside Rg3 [20(S)-Rg3)] is reversible by the AMPK inhibitor compound C or by siRNA targeting AMPK, indicating a key role for AMPK in the process [Yuan et al., 2010].

HEMATOLOGICAL MALIGNANCIES

Studies in which various childhood acute lymphoblastic leukemia (ALL) cell lines were treated with the AMPK activator AICAR have shown that this agent induces cell cycle arrest and apoptotic cell death in ALL cells [Sengupta et al., 2007]. In addition, combination of AICAR with the mTOR inhibitor rapamycin further enhanced suppression of cell proliferation [Sengupta et al., 2007]. AMPK also appears to be an attractive target in mantle cell lymphoma [MCL] cells. Studies using an MDM2 inhibitor, nutlin-3A, demonstrated that the resulting p53 activation down regulates the AKT/mTOR cascade via an AMPK-dependent mechanism, leading to G1-S cell cycle arrest and apoptosis of MCL cells [Drakos et al., 2009]. More recently, metformin was shown to have potent inhibitory effects on various acute myeloid leukemia [AML] cell lines, primary AML cells, as well as AML xenografts in nude mice, associated with decreased mTOR signaling [Green et al., 2010]. These studies have raised the potential of targeting the LKB1/AMPK pathway for the treatment of AML [Green et al., 2010]. There is also evidence that AMPK activation suppresses the growth of multiple myeloma cells [Baumann et al., 2007], suggesting that multiple myeloma may be another malignancy worth exploring potential therapeutic approaches to target AMPK.

FUTURE PROSPECTS FOR AMPK TARGETING IN MALIGNANCIES

The AMPK pathway has gained increasing interest in the cancer research field over the last decade. AMPK is an appealing target as a therapeutic for a variety of tumors, mainly because of its inhibitory effect on one of the most important metabolic pathways, the PI3K/AKT/mTOR signaling pathway. The effects of AMPK activators in the reduction of tumorigenesis have been established in many systems. In particular, the AMPK activator, metformin, has been the subject of many recent studies in various tumor models, starting from efforts to establish its inhibitory effects on cell lines and animal models, ultimately leading to human clinical trials. A potential drawback in studies using metformin and AICAR is that beyond effects on AMPK, some of their antitumor properties may reflect modulation of other pathways, in a cell-type specific manner [Robert et al., 2009; Kalender et al., 2010; Santidrian et al., 2010; Janjetovic et al., 2011a]. Although in most cases this does not appear to be the case and AMPK induction results in potent antitumor effects, this emphasizes the need for designing and developing more potent and selective AMPK activators. A new compound, A-769662, identified from a large chemical screen, might also be an attractive, more direct method of targeting AMPK, as it is has a significantly lower EC_{50} than either AICAR or metformin and has been illustrated to induce AMPK activation at concentrations lower than AMP [Cool et al., 2006]. Studies using A-769662 and other similar compounds that may emerge are warranted and may provide powerful new tools in the future treatment of malignancies.

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