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Molecular Pathways: Fumarate Hydratase-Deficient Kidney Cancer: Targeting the Warburg Effect in Cancer

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Abstract

Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is a hereditary cancer syndrome in which affected individuals are at risk for development of cutaneous and uterine leiomyomas and an aggressive form of type II papillary kidney cancer. HLRCC is characterized by germline mutation of the tricarboxylic acid cycle (TCA) enzyme, *fumarate hydratase* (*FH*). *FH*-deficient kidney cancer is characterized by impaired oxidative phosphorylation and a metabolic shift to aerobic glycolysis, a form of metabolic reprogramming referred to as the Warburg effect. Increased glycolysis generates ATP needed for increased cell proliferation. In *FH*-deficient kidney cancer levels of AMPK, a cellular energy sensor, are decreased; resulting in diminished p53 levels, decreased expression of the iron importer, DMT1, leading to low cellular iron levels, and to enhanced fatty acid synthesis by diminishing phosphorylation of acetyl CoA carboxylase, a rate limiting step for fatty acid synthesis. Increased fumarate and decreased iron levels in *FH*-deficient kidney cancer cells inactivate prolyl hydroxylases, leading to stabilization of HIF1α, and increased expression of genes such as vascular endothelial growth factor (VEGF) and GLUT1 to provide fuel needed for rapid growth demands. Several therapeutic approaches for targeting the metabolic basis of *FH*-deficient kidney cancer are under development or are being evaluated in clinical trials, including the use of agents such as metformin, which would reverse the inactivation of AMPK, approaches to inhibit glucose transport, LDH-A, the anti-oxidant response pathway, the heme oxygenase pathway and approaches to target the tumor vasculature and glucose transport with agents such as bevacizumab and erlotinib. These same types of metabolic shifts, to aerobic glycolysis with decreased oxidative phosphorylation, have been found in a wide variety of other cancer types. Targeting the metabolic basis of a rare cancer such as fumarate hydratase-deficient kidney cancer will hopefully provide insights into the development of effective forms of therapies for other, more common forms of cancer.

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Background

Hereditary Leiomyomatosis and Renal Cell Carcinoma

Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is an autosomal dominant hereditary cancer syndrome in which affected individuals are at risk for the development of cutaneous and uterine leiomyomas and kidney cancer.(1, 2) Leiomyoma, which is a hallmark of HLRCC, is a benign smooth muscle neoplasm. We have detected HLRCC-associated cutaneous leiomyomas, in over 75% of our affected patients, The number of cutaneous leiomyoma per patient ranged from zero to more than 100 lesions. (3) Cutaneous leiomyomas most often occur in the *arrectores pilorum* muscles, which are attached to the hair follicles,(4) The *arrector pilori* can be considered an energy sensing organ; when it is cold, the *arrectores pilorum* contract to trap air and provide insulation. These raised lesions are can be painful and are often sensitive to touch or cold. There is currently no effective systemic or topical treatment for HLRCC-associated cutaneous leiomyomas. The treatment is most often symptomatic, sometimes including surgical resection. Females affected with HLRCC are at risk for development of multiple, early onset uterine fibroids. Uterine fibroids are detected in over 90% of affected HLRCC females, 70 to 80% of whom will undergo either a myomectomy (surgical removal of the uterine leiomyoma) or hysterectomy (removal of the uterus).(3, 5)

HLRCC-associated kidney cancer

Patients affected with HLRCC are at risk for the development of early onset, bilateral and/or multifocal renal cysts and papillary kidney tumors. Papillary kidney cancer has been reported to occur in 60% of HLRCC families.(3, 5) Papillary kidney cancer can be classified as type 1 or type 2. Type 1 papillary kidney cancer is characterized by an indolent growth pattern and is the histologic subtype found in patients with Hereditary Papillary Renal Carcinoma, which is associated with germline *MET* gene mutation.(6, 7) Type 2 papillary kidney cancer, however, is a lethal disease associated with rapidly aggressive growth and early metastasis. HLRCC tumors are classified as type 2 papillary kidney cancer and are characterized histologically by orangophilic nucleoli and prominent perinucleolar halo formation.(8) Because HLRCC renal tumors may occur in young individuals and have a propensity to spread when the tumors are very small, affected individuals have a life-long need to undergo annual abdominal imaging starting at age 10.(9) Patients affected with other familial kidney cancer syndromes, such as von Hippel-Lindau (germline *VHL* gene mutation), hereditary papillary renal cell carcinoma (germline *MET* gene mutation), or Birt-Hogg-Dubé syndrome (germline *FLCN* gene mutation) are managed with active surveillance until the largest renal tumor reaches the 3 cm threshold, at which time renal parenchyma-sparing surgical resection is recommended.(10, 11) However, due to the aggressive nature of HLRCC renal tumors, active surveillance is not recommended for the management of even small HLRCC-associated renal tumors; wide surgical excision is recommended when an HLRCC-associated renal tumor is detected.(9)

Fumarate hydratase: the HLRCC gene

Hereditary leiomyomatosis and renal cell carcinoma is characterized by germline mutation of the TCA (Krebs) cycle enzyme gene, *fumarate hydratase* (*FH*).(12) (Figure 1) Fumarate

hydratase, which exists in both a mitochondrial as well as a cytosolic form, is a homotetramer which catalyzes the hydration of fumarate to malate. Mutations of the *FH* gene, which may be missense, frameshift or complete or partial deletion, are detected in 90% of HLRCC families.(3, 5) *Fumarate hydratase*, localized to chromosome 1q42.2, is subject to a two-hit, loss of function in HLRCC tumors; loss of the somatic allele usually represents the second hit that is found in HLRCC-associated kidney tumors and uterine leiomyomas.(13-15)

Shift to aerobic glycolysis in *fumarate hydratase*-deficient kidney cancer: the Warburg effect

Fumarate hydratase-deficient kidney cancer undergoes a metabolic shift to aerobic glycolysis. The *FH*-deficient kidney cancer cell line UOK262, which is tumorigenic in a nude mouse model, is characterized by impaired oxidative phosphorylation and increased levels of glycolysis, as assessed by decreased oxygen consumption rate (OCR) and increased extracellular acidification (ECAR). Both the glycolytic and tumorigenic features of the *FH*-deficient cell line can be reversed by restoration of *FH* activity. In contrast to the cell line model derived from other forms of kidney cancer, such as *VHL*-deficient clear cell kidney cancer, *FH*-deficient kidney cancer cells are uniformly dependent on glucose and glycolysis for ATP production needed for rapid proliferation.(16-18) This is demonstrated in patients by the observation that, in contrast with other genetically defined types of kidney cancer, metastatic HLRCC-associated kidney cancer exhibits consistently high fluorodeoxyglucose uptake on PET scan imaging.(14) (Figure 1)

Inhibition of HIF prolyl hydroxylase results in stabilization of HIF1α

Hypoxia inducible factor 1α (HIF1α) and hypoxia inducible factor 2α (HIF2α) are transcription factors whose stability is regulated by HIF prolyl hydroxylase (PHD). In normoxic conditions, PHD (in the presence of α-ketoglutarate and iron) hydroxylate two HIF prolines, allowing the VHL complex to bind and facilitate ubiquitin-mediated degradation. In hypoxia (or with an inactivating mutation in *VHL*), HIF degradation is impaired and HIF1α and HIF2α accumulate.(19) HIF1α and HIF2α are transcription factors that increase the transcription of a number of genes critical to a cancer dependent on aerobic glycolysis, such as the glucose transporter, GLUT1, and vascular endothelial growth factor (VEGF), which would increase glucose transport and tumor vascularity. *FH*-deficient kidney cancer has an increased need for both vascularity and as well as glucose transport to provide increased nutrients for rapid growth and proliferation and for ATP production to compensate for a decrease in oxidative phosphorylation associated with a TCA cycle gene mutation. In *FH* deficient cells increased levels of fumarate inhibit the HIF prolyl hydroxylases and thus the activity of the VHL ubiquitination complex, leading to stabilization of HIF1α and increased transcription of its targets such as *VEGF* and *GLUT1*. (20) (Figure 1)

Diminished AMPK, a negative regulator of the Warburg effect in *FH*-deficient kidney cancer, facilitates increased fatty acid and protein biosynthesis needed for rapid tumor growth

The AMP-activated protein kinase (AMPK) is a cellular energy sensor which reflects cellular energy status by undergoing phophorylation and increasing activity when AMP levels increase and ATP levels decrease, indicative of energy deficiency.(21) Whereas HLRCC tumor cells might be expected to show AMPK activation because of their dependence on less efficient glycolysis and their rapid consumption of ATP during growth, unexpectedly, the reverse was observed, with markedly reduced phopho-AMPK levels, but also reduced levels of AMPK subunits AMPKα and AMPKβ1 at both transcriptional and protein levels. The reason for diminished AMPK levels is not known, but it was shown to result in reduced phosphorylation of acetyl CoA carboxylase (pACC), a state that normally renders the enzyme inactive, that would be expected to increase the synthesis of a key fatty acid biosynthetic intermediate, malonyl-CoA. In addition, the phospho-S6 ribosomal protein, an mTOR downstream effector, was also found to be activated. These results suggest that enhanced fatty acid and protein biosynthesis in *FH*-deficient kidney cancer results from decreased AMPK activity that promotes anabolic growth associated with cell growth and proliferation in tumors.(17)

Decreased AMPK leads to decreased iron and increased HIF1α levels

The glycolytic shift in *FH*-deficient kidney cancer is associated with low AMPK levels, which decreases p53 (14) and the iron transporter, DMT1, which appears to require p53 for expression (14). In the *FH*-deficient kidney cancer cell line, UOK262, the iron responsive proteins, IRP1 and IRP2, as well as the IRP target, transferrin receptor protein 1 (TFRC) are elevated, indicating that cytosolic iron concentrations decrease secondary to decreased DMT1 activity (14). Changes in cytosolic iron levels and corresponding IRP activities potentially have opposite effects on HIF1α and HIF2α. Because prolyl hydroxylases require iron for their catalytic activity, decreased cellular iron would be expected to stabilize both HIF1α and HIF2α.(22) However, while both *FH*-deficient kidney cancer cell line models UOK262 and UOK268 have increased HIF1α levels, both also have decreased HIF2α levels. Decreased HIF2α levels are consistent with increased IRP1 levels, as IRP1 is known to target the iron responsive element in the HIF2α 5′UTR and inhibit to HIF2α translation when cytosolic iron is low, in vitro (23) and in vivo.(24)

Activation of AMPK reduces the invasive potential of *FH*-deficient kidney cancer

As noted above, AMPK is decreased in UOK262 cells, resulting in activation of phospho-S6 ribosomal protein and a decrease in pACC (which would lead to an increase in fatty acid synthesis). In order to evaluate the potential role of activation of AMPK in *FH*-deficient kidney cancer, UOK262 cells were treated with either metformin or AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside). Both metformin and AICAR were found to significantly impair the invasiveness of *FH*-deficient kidney cancer cells (14).

Silencing of HIF1α reduces the tumorigenic potential of *FH*-deficient kidney cancer

Some studies have suggested that increased HIF2α levels are essential for tumorigenesis in VHL-deficient clear cell kidney cancer cells (25, 26) and a recent genetic and functional study has concluded that HIF1α actually functions as a tumor suppressor gene in clear cell kidney cancer.(27) However, in UOK262 *FH*-deficient kidney cancer cells HIF1α is increased, whereas HIF2α is decreased, so HLRCC cancer does not conform to this new model. It could be that the role of HIF1α in suppressing or enabling cancer development is disease-specific. HIF1α promotes glucose uptake, increases glycolysis and activates pyruvate dehydrogenase kinase 1 (PDK1), which inactivates the TCA cycle enzyme, pyruvate dehydrogenase (PDH), inhibiting the flow of glucose into the TCA cycle via the conversion of pyruvate to acetyl-CoA.(28) Blocking the conversion of pyruvate to acetyl-CoA would increase lactate production and could block utilization of glucose to provide carbon backbones in the anabolic synthesis of lipids.(29, 30) In a cancer that is dependent on oxidative metabolism of glucose for lipid production, such as a VHL-deficient kidney cancer that has an intact TCA cycle, HIF1α could potentially have a suppressive effect on cell growth by reducing energy production. However, in a cancer cell with a remodeled metabolic pathway that is 1) extremely dependent on efficient use of glucose for glycolysis for generation of ATP and is 2) dependent on carbons from glutamine, rather than from glucose, for lipid production and for carbon backbones of amino acid precursors such as oxaloacetate, increased HIF1α appears to be supportive of tumorigenesis. To evaluate the anti-tumor effectiveness of targeting the HIF1α pathway in *FH*-deficient kidney cancer, silencing of HIF1α by siRNA was performed in UOK262 cells. Silencing of HIF1α was found to significantly impair UOK262 invasiveness, suggesting that HIF1α is a critical oncoprotein in *fumarate hydratase* deficient kidney cancer.(17)

Fumarate hydratase-deficient kidney cancer is characterized by a metabolic shift to a reductive carboxylation, glutamine-dependent pathway

^{13}C metabolite analysis was utilized to further characterize the metabolic shift in fumarate hydratase-deficient kidney cancer. Even thought *FH*-deficient kidney cancer has a significant decrease in the flux of glucose-derived pyruvate to the mitochondria, it is still critically dependent on mitochondrial metabolic flux for critical biosynthetic reactions. Unlike other cancers that perform oxidative metabolism of both glucose and glutamine, *FH*-deficient kidney cancer is characterized by glutamine-dependent reductive carboxylation (see red arrows, figure 1), rather than the normal procession of the TCA cycle for citrate formation for the citrate/acetyl-CoA/lipid synthesis pathway. *FH*-deficient kidney cancer cells require glutamine to proliferate and glutamine was found to be the major carbon source for the increased fatty acid synthesis required for rapid cellular proliferation of this most aggressive form of kidney cancer.(31)

Clinical-Translational Advances

KEAP1 succination and Nrf2 signaling: antioxidant response in *FH*-deficient kidney cancer

In complementary studies, utilizing different approaches, Ooi et al. and Adam et al. delineated the role of the KEAP1 succination and Nrf2 signaling in *fumarate hydratase*-deficient kidney cancer and the role of fumarate as an oncometabolite.(32, 33) Nrf2 (nuclear

factor E2-related factor 2) is a transcription factor that that induces expression of a number of genes involved in antioxidant response, such as to counteract the effect of reactive oxygen species and that facilitate heme synthesis. KEAP1 (Kelch-like ECH-associated protein 1) is an electrophile that combines with CUL3 to facilitate degradation of Nrf2.(34) KEAP1 mutations have been found in a wide variety of cancers, including 34% of squamous lung cancers, and Nrf2 has been found to be elevated in a number of cancers, including head and neck, gallbladder, lung and pancreatic cancer.(35, 36) In a study of HLRCC (as well as sporadic) type 2 papillary kidney cancer, Ooi et al. found a gene expression pattern consistent with up-regulation of antioxidant response elements (ARE), including the aldo-keto reductase family member B10 gene (AKR1B10) as well as other ARE driven genes, NAD(P)H dehydrogenase (encoded by *quinone 1* also known as NQO1) and thioredoxin reductase 1 (TXNRD1).(33) Nrf2, which is known to bind the ARE enhancer and the electrophile sensor, KEAP1, forms a complex with the cullin 3 (CUL3) ubiquitin ligase to initiate ubiquitin-imediated degradation.(37) An exposed KEAP1 residue, Cys-151, reacts with fumarate, which has been shown to be elevated in *FH*-deficient HLRCC uterine fibroids (38) as well in *FH*-deficient kidney cancer cells,(33) resulting in a conformational change which inhibits KEAP1 from binding to NRF2, resulting in NRF2 accumulation and increases expression of antioxidant response genes.(33, 39, 40) The findings of Ooi et al. demonstrate that increased levels of fumarate induce succination of KEAP1 resulting in stabilization of NRF2 and increased expression of *AKR1B10*. Alderson, et al. showed that S-(2-Succinyl) cysteine (2SC) is formed by increased levels of fumarate, which inactivates the sulfhydryl enzyme, glycetaldehyde-3-phosphate dehydrogenase. These findings identify fumarate as an endogenous electrophile and indicate a role for fumarate as an oncoprotein in metabolic regulation.(41) Nagai et al. subsequently showed that increase in 2SC is the result of mitochondrial stress and that increased levels of fumarate modify cysteine residues in many proteins, a process called succination.(42) Bardella et al. showed that 2SC accumulates to high levels in *FH*-deficient cells and tumors and that 2SC staining could be a useful marker for detection of this disease.(43)

In a parallel study, Adam et al. showed that *fumarate hydratase*-deficient murine renal cyst formation is Hif and Phd independent and is associated with a pronounced up regulation of antioxidant pathways. In the *Fh1*-deficient mouse, activation of the NRF2 antioxidant pathway was found to arise as a direct consequence of *FH* inactivation. Critical residues of KEAP1 were found to be succinated in this model, indicating that NRF2 activation results from succination of KEAP1. Impairment of KEAP1 function by succination diminishes NRF2 proteosomal degradation and thereby increases NRF2 stability and activity.(32) (Figure 1)

Dysregulation of NRF2 degradation, a critical adaptive response that appears to occur early in hyperplastic cystic and tumor formation, could provide the foundation for the development of novel approaches for therapy of *FH*-deficient kidney cancer. Decreased KEAP1 function increases NRF2 activity, which would reduce oxidative stress and could provide a growth advantage to aggressive type 2 papillary kidney cancer cells. Thus, potential therapeutic approaches to target the oxidative response pathway could include inhibition of AKR1B10 or thioredoxin reductase 1.(33)

FH-deficiency induces HIF1α stabilization by generation of reactive oxygen species

Another potential therapeutic approach to target *FH*-deficient kidney cancer involves targeting the activated antioxidant pathway by increasing reactive oxygen. Sudarshan, et al. showed that inactivating mutations of *FH*-deficient kidney cancer cells results in glucose-mediated generation of cellular reactive oxygen species (ROS) and ROS-dependent HIF1α stabilization and that the metabolic shift to aerobic glycolysis is critical to HIF stabilization.(44) In order to evaluate a potential therapeutic approach for HLRCC-associated kidney cancer, Sourbier, et al. investigated the cytotoxic effects of increasing ROS levels using the proteosome inhibitor, bortezamib, which inhibits NFκB, in combination with cisplatin in *FH*-deficient cancer cells. Bortezamib was found to induce apoptosis *in vitro* and inhibited growth *in vivo*. Cellular ROS levels correlated with bortezomib-associated cytotoxicity. Combining other ROS inducers with bortezomib enchanced cytotoxicity, while combining a ROS scavenger with bortezomib inhibited its cytotoxic effect. When HLRCC murine xenografts were treated with cisplatin, a ROS inducer, and bortezomib dependent tumor regression resulted.(45)

Heme oxygenase is synthetically lethal in *FH*-deficient cells

Frezza, et al. described a glutamine to bilirubin pathway in *FH*-deficient cells which involves the biosynthesis and degradation of heme, which enables partial mitochondrial NADH production and ends with bilirubin excretion from the *FH*-deficient cells. They demonstrated that inhibition of heme oxygenase is synthetically lethal with *FH*-deficient cells, meaning that the *FH*- cells cannot survive without intact heme oxygenase. Thus, inhibition of heme oxygenase may cause specific death of tumor cells in HLRCC-associated kidney cancer, while sparing cells that are not *FH* deficient.(46)

Targeting glycolysis and LDH-A as a therapeutic strategy for *FH*-deficient kidney cancer

FH-deficient kidney cancer has undergone a metabolic shift to aerobic glycolysis and is dependent on high levels of glycolysis for ATP production needed for the rapid proliferation of this aggressive form of cancer.(17, 18) There are a number of different approaches for targeting glucose metabolism in a Warburg cancer, including targeting critical glycolytic enzymes such as HKII, PKM2 or LDH-A (reviewed in Hamanaka and Chandel).(47) In order to evaluate whether *FH*-deficient cells would be sensitive to LDH-A blockade, Xie et al. showed that LDH-A is over expressed in HLRCC kidney cancer, that LDH-A inhibition increased apoptosis in *FH*-deficient cells, that this effect is reactive oxygen species mediated and that LDH-A knockdown in *FH*-deficient cells resulted in a significant decrease in xenograft tumor growth.(48) Efforts are currently underway to identify an effective LDH-A inhibitor for evaluation in pre-clinical and clinical trials.

Englerin A: limiting the cell's access to glucose

Englerin A, a sesquiterpen isolated from the root bark and stem bark of *Phyllanthus engleri* Pax,(49) has been shown to have selective inhibition against renal cell carcinoma growth.(50, 51) Sourbier, et al. showed that englerin A binds to and activates protein kinase C-θ, inducing an insulin-resistant phenotype while limiting the cell's access to glucose. Simultaneously, englerin A induces PKCθ activation of the transcription factor, HSF1,

which induces glucose dependence. By starving the cells of glucose while simultaneously inducing glucose-dependence, englerin A is lethal to a number of types of kidney cancer, including *FH*-deficient RCC. *FH*-deficient RCC cells are very sensitive to treatment with englerin A, while cells with *FH* replaced are resistant.(52) Studies to further assess the effect of englerin A in *FH*-deficient as well as other forms of kidney cancer are in progress.

Bevacizumab and erlotinib: targeting *FH*-deficient kidney cancer tumor vasculature with anti-VEGF reagents in patients with metastatic HLRCC-associated kidney cancer

FH-deficient kidney cancer is characterized by impaired oxidative phosphorylation a shift to aerobic glycolysis and a dependence on glucose for ATP generation and oxidative stress. Increased oxidative stress and/or increased levels of fumarate inhibit HIF prolyl hydroxylase, resulting in HIF1α stabilization, leading to increased transcription of a number of hypoxia-induced pathway genes, such as vascular endothelial growth factor (VEGF) and GLUT1. HLRCC-associated kidney cancer, which is readily detected by [18] fluoro-deoxyglucose-based PET scanning, is a fast growing tumor that has a propensity to metastasize when the primary tumors are as small 1/2 centimeter in size.(9) In our experience managing HLRCC patients since 1989, we have not found this disease to be responsive to chemotherapy, immunotherapy or the conventional targeted therapeutic approaches in use for patients with other forms of advanced kidney cancer. Based in the knowledge that these cancer cells are critically dependent on tumor vasculature to ensure a high level of glucose transport needed for the increased glycolytic demand of this rapidly growing cancer, a clinical trial evaluating the effect of bevacizumab and erlotinib in patients with advanced HLRCC-kidney cancer is currently underway.(53)

Conclusions

Fumarate hydratase-deficient HLRCC-associated type II papillary kidney cancer, the most aggressive form of kidney cancer, may have an Achilles heel which can be successfully exploited for therapy. This early onset cancer, which has a high propensity for metastasis, has “precisely the properties expected of a Warburg tumor”.(54) In *FH*-deficient kidney cancer oxidative phosphorylation is impaired because the TCA cycle is blocked by *FH* deficiency; *FH*- cells undergo a metabolic shift to aerobic glycolysis to provide the ATP needed for maintenance of highly proliferating cells. Increased levels of fumarate and decreased levels of iron in *FH*-deficient RCC (14) inhibit HIF prolyl hydroxylase, resulting in stabilization of HIF1α levels (20, 55), which would drive vascularization and glucose transport. *FH*-deficient kidney cancer is characterized by a shift to a reductive glutamine-dependent pathway that provides citrate, which generates the acetyl CoA needed for lipid production in rapidly growing cells.(31) A number of potential therapeutic approaches to target this most aggressive form of kidney cancer, such as activation of AMPK, inhibition of LDH-A, repressing the anti-oxidant response and the heme oxygenase pathway have been developed. These approaches as well as the clinical trial underway targeting the vasculature and glucose transport in HLRCC-associated kidney cancer with bevacizumab and erlotinib will hopefully provide the foundation for the development of an effective form of therapy for patients affected with this most aggressive form of Krebs cycle mutation cancer and could provide insight into the management of patients with other forms of cancer

characterized by a metabolic shift to aerobic glycolysis and impaired oxidative phosphorylation.

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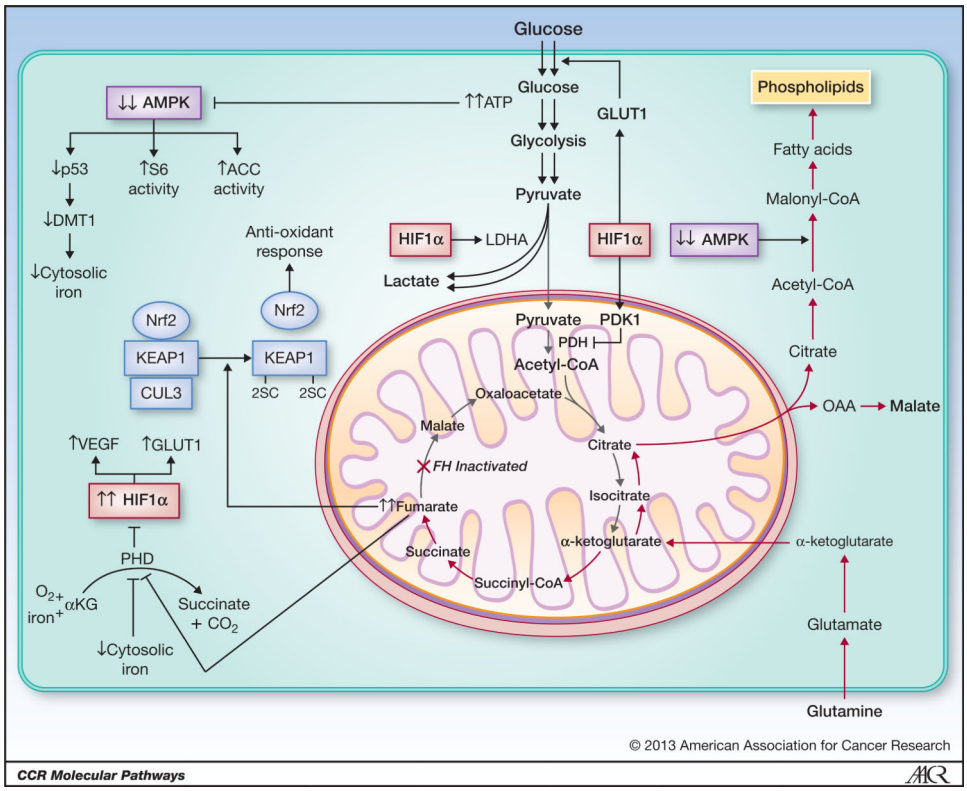

Figure 1.

AMPK is a negative regulator of the Warburg effect in *Fumarate hydratase*-deficient kidney cancer. *Fumarate hydratase* (*FH*)-deficient kidney cancer, characterized by impaired oxidative phosphorylation, and undergoes a metabolic shift to aerobic glycolysis to generate ATP required for the increased energetic demands of rapidly proliferating cells. The increased glycolysis suppresses expression and activation of AMPK which results in increased S6 and ACC activity, promoting anabolic growth and proliferation. Decreased AMPK results in decreased p53 and the iron transporter, DMT1. The iron responsive proteins, IRP1 and IRP2, as well as the IRP target, transferrin receptor protein 1 (TFRC) are elevated, indicating that cytosolic iron concentrations decrease secondary to decreased DMT1 activity. Prolyl hydroxylase, which is sensitive to iron levels, would be inhibited by decreased cytosolic iron levels, stabilizing HIF1α. Fumarate, which increases in *FH*-deficient cells, has been shown to inhibit prolyl hydroxylase, which would lead to further stabilization of HIF1α, increasing transcription of factors such as vascular endothelial growth factor (VEGF) and the glucose transporter, GLUT1. Increased fumarate has been shown to succinate KEAP1, thus altering it's conformation and disrupting its ability to induce degradation of Nrf2. Nrf2 transcription is increased activating anti-oxidant response and protecting against oxidative stress. Increased HIF1α would stimulate LDHA, increasing lactate production, and would stimulate PDK1, which inhibits PDH and would decrease entry of pyruvate into the TCA cycle. *FH*-deficient kidney cancer use a glutamine-dependent reductive carboxylation rather than rather than oxidative metabolism for citrate formation (red arrows). Glutamine is the major source for the increased fatty acid synthesis required for rapid proliferation in these cells with disabled normal oxidative

phosphorylation. Potential approaches for treatment of this aggressive form of kidney cancer include agents that stimulate AMPK, agents that target the tumor vasculature and glucose transport, agents that inhibit LDHA and agents that target the critical glutamine-dependent reductive fatty acid/lipid synthetic pathway.