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Adenosine Monophosphate Activated Protein Kinase (AMPK) Enhances Chemotherapy Response in Acute Myeloid Leukemia (AML)

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Abstract

Adenosine monophosphate activated protein kinase (AMPK) is a master regulator of cell metabolism and is involved in cancer as both a tumor suppressor and a source of resistance to metabolic stress. The role of AMPK in response to chemotherapy has been examined in solid tumor models but remains unclear in acute myeloid leukemia (AML). To determine the role of AMPK in chemotherapy response, AML cell lines were generated lacking AMPK activity. AMPK knock out cells demonstrated significant resistance to cytarabine and doxorubicin both *in vitro* and *in vivo*. Mitochondrial mass and function were unchanged in AMPK knockout cells. Mechanistically, AMPK knock out cells demonstrated a diminished DNA damage response with significantly lower γH2AX foci, p53 and p21 induction as well as decreased apoptosis following chemotherapy exposure. Most importantly, TCGA datasets revealed that patients expressing low levels of the *PRKAA1* subunit of AMPK had significantly shorter survival. Finally, AML cells were sensitized to chemotherapy with the addition of the AMPK activator AICAR. These data demonstrate that AMPK sensitizes AML cells to chemotherapy and suggests a contribution of the cellular metabolic state to cell fate decisions ultimately affecting therapy response.

1. Introduction:

Acute myeloid leukemia (AML) is characterized by poor outcomes and resistance to therapy. AML is an aggressive malignancy leading to the accumulation of immature myeloid

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Declaration of interests

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Declaration of Competing Interest

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precursors, progressive marrow failure and death[1]. It affects more than 20,000 people per year in the United States; despite decades of research, the overall 5-year survival remains less than 30%[2]. Older age, typically defined as 60 years of age or older, is a poor prognostic factor for AML. Clinical trials show an estimated 5-year survival of less than 10% in these patients[3]. Ten-year survival is even worse with one study estimating only 2.4% of patients over 60 years of age alive when treated with chemotherapy[4]. Likewise, frailty and poor performance status is linked to high treatment related mortality and poor outcomes[5,6]. Outcomes outside of clinical trials are similar; a SEER database study reported patients 65 years of age or older had a 5-year survival rate of under 5%[7]. This poor outcome of older patients is the result of disease that is resistant to therapy[8]. Resistance to therapy is a central clinical issue in AML, especially in the elderly and those with TP53 disruptions[9], however; the molecular mechanisms remain largely unclear. There is a desperate need to increase our understanding of the molecular mechanisms of resistance.

AMPK is a master regulator of cell metabolism[10]. When the energy charge of the cell is low resulting in a high AMP/ATP ratio, AMPK is activated by phosphorylation of threonine 172. This results in activated AMPK phosphorylating a number of downstream substrates that decrease anabolic processes and increase energy generating ones (review by Jeon[11]). AMPK is a heterotrimeric complex that contains a catalytic α subunit and two regulatory β and ϒ subunits (reviewed by Herzig and Shaw[10]). There are two isoforms of the α subunit encoded by the *PRKAA1* and *PRKAA2* genes. Previously, AMPK was identified as a source of resistance to AML cell metabolic stress[12]. How the energy status of an AML cell is altered by exposure to chemotherapy and how AMPK contributes to cellular response is not well understood. Previous studies have shown that AMPK is destabilized by chemotherapy in some AML cell lines but not others[13]. Studies in solid tumors have shown that AMPK is activated by chemotherapy and influences the DNA damage response (reviewed by Sanli[14]) however; the role of AMPK in AML response to chemotherapy remains unclear. The present study utilizes genetically defined *Prkaa1* knockout murine AML cells to assess the role of AMPK in chemotherapy response. AML cells without functional AMPK were more resistant to therapy, had a diminished DNA damage response and normal AML cells could be made more sensitive to chemotherapy with the AMPK activating small molecule AICAR.

2. Materials and Methods:

2.1 Cas9/CRISPR Gene Deletion:

MFL2 cells were infected with the Cas9 expressing vector, MSCV_Cas9_puro (gift from Christopher Vakoc) (Addgene plasmid # 65655) and infected cells selected with puromycin. Resistant cells were then transfected with sgRNA expressing vector LRG (Lenti_sgRNA_EFS_GFP) also a gift from Christopher Vakoc (Addgene plasmid # 65656) tagged with GFP targeting the indicated genes, *Prkaa1* and the safe harbor locus, *Rosa26*. Gene deletion was confirmed by western blot. Clonal populations of deleted cells were obtained by serial dilution.

2.2 Cell Culture:

All MFL2 cell lines were grown in stem cell media, 45% DMEM, 45% IMDM, 10% FBS, supplemented with 1% penicillin, 1% streptomycin, 0.11% IL-6, 0.02% IL-3, and 0.11% SCF, at 37° C with 5% CO2.

2.3 Competition Assay:

Mixed GFP+/− cell population were plated at a density of ~50,000 cells/mL in 24-well plates. Mixed populations were exposed to the indicated therapy for 72 hours and GFP-positive percentage was quantified in the live cell population by using a BD Accuri C6 Analyzer flow cytometer (BD Bioscience, San Jose, CA).

2.4 Cell Viability:

MFL2 cell lines were plated at a density of ~50,000 cells/mL in 24-well plates. Cell lines were exposed to the indicated therapy for 72 hours. Viability assays were done using the EZQuant assay (ALSTEM, Richmond, CA) according to the manufacturer's protocol.

2.5 TMRM/Mitotracker Deep Red Assay:

MFL2 cell lines were pelleted at a density of 1×10^6 cells. Pellets were resuspended in 1mL of PBS. Mitrotracker deep red dye was added to the cell suspension (ThermoFisher Scientific, #M22426, 5μL of 5mM stock) and incubated at 37° C with 5% CO2 for fifteen minutes. TMRM dye was added to cell suspension (ThermoFisher Scientific, #T668, 5μL of 40mM stock) and incubated at 37° C with 5% CO2 for thirty minutes. Cell suspension was centrifuged, supernatant was aspirated, and pellet was washed with 1mL of PBS. After centrifugation, pellet was resuspended in 500μL of PBS. Samples were analyzed by flow cytometry.

2.6 Annexin V/ Propidium Iodide Assay:

MFL2 cell lines were plated at a density of ~50,000 cells/mL in 24-well plates. Cell lines were exposed to the indicated therapy for 72 hours. Annexin V assay was done using APC Annexin V, 5μL per sample, (BD Pharmingen, #550474) according to the manufacturer's protocol. A counterstain, PI, 1 μL per sample, (BD Pharmingen, #556463), was used. Samples were analyzed by flow cytometry.

2.7 Western Blots:

Cultured and treated cells were pelleted and washed in cold PBS. Pellets were lysed in Laemmli buffer, separated by SDS-PAGE, and transferred to an Immobilon PVDF membrane (Millipore). Antibodies against AMPK α1 subunit (Abcam, #Y365, 1: 1,000), AMPK (Cell Signaling, #2532, 1:1000), phosphorylated AMPK (Cell Signaling, #2531, 1:1000), p53 (Cell Signaling, #9282, 1:1,000), p21(Abcam, #EPR3993, 1:1,000), ACC (Cell signaling, #3662, 1:1,000), phosphorylated ACC (Cell signaling, #3661, 1:1,000), and actin (Abcam #AC-15, 1:5000 or Abcam #Ab8227, 1:5,000) were used. Relative protein expressions were acquired by ImageJ software and the protein expression of each sample was normalized based on the corresponding α-actin levels.

2.8 ɣ-H2AX Assay:

MFL2 cell lines were plated at a density of ~3×10^6 cells/mL in 6-well plates. Cell lines were exposed to the indicated therapy for four, six, or eight hours. ɣ-H2AX assay was done using phosphorylated ɣ-H2AX (Cell signaling, #9720, 1:50) according to manufacturer's protocol. Rabbit IgG was used as an isotype control (Cell signaling, #3452, 1:50). Samples were analyzed by flow cytometry.

2.9 Mitochondrial Respiration Assays:

All oxygen consumption rate assays were performed using the XF24 Extracellular Flux Analyzer (Seahorse Bioscience) as per the manufacturer's instructions. Cells were plated at a density of 400,000 viable cells per well for oxygen consumption assays. Data was normalized to viable cell number and viability was routinely between 96% and 99% as determined by trypan blue exclusion assay (Countess automated cell counter, ThermoFisher). The media used for oxygen consumption assays was the mitochondrial stress test media, specifically: DMEM with 5.5mM glucose, 2.0mM Sodium Pyruvate, and 2mM L-glutamine – warmed to 37oC and pH 7.35. Cells were placed in assay media and incubated for 2 hours prior to being placed in the assay. For the oxygen consumption experiments oligomycin at a final concentration of 1 μM, FCCP at 0.5 μM, and Antimycin A and Rotenone each at 1 μM were used. Assay was done in triplicate (3 wells per condition each measured in triplicate) and repeated in three independent experiments.

2.9 Mouse Studies:

The Wake Forest University Institutional Animal Care and Use Committee approved all mouse experiments. 1×10^6 luciferase-tagged leukemia cells were transplanted into 10 to 11 week old sub-lethally irradiated (4Gy) female recipient mice, C57BL/6 (Taconic Laboratories), by tail-vein injection. Mice were monitored by bioluminescent imaging performed using an IVIS100 imaging system (Caliper LifeSciences, Hopkinton, MA). Mice were injected with 150 mg/kg D-Luciferin (Gold Biotechnology, St. Louis, MO), anesthetized with isoflurane, and imaged for 2 min. Chemotherapy was initiated upon detection of clear signals. Mice were treated with 100 mg/kg ARA-C for 5 days and 3 mg/kg DOX for the first three days (both from Bedford Laboratories, Bedford, OH) by IP injection. Control animals were injected with PBS. Toxicity was measured by weighing the mice daily during treatment, if mouse had lost more than ten percent of body weight treatment was withheld. Following completion of therapy mice were followed for survival.

2.10 Statistical Analysis:

All means were compared by two-tailed student's T test or Two-way ANOVA. Survival curves were estimated by the Kaplan-Meier method and p values were determined by the log rank test. P values below 0.05 were considered significant. Analysis was performed using Graph Pad Prism version 7 (Graph Pad Software Inc). Significance is indicated by asterisks, p-values, * = $p < 0.05$, ** = $p < 0.01$, *** = $p< 0.001$, **** = $p < 0.0001$.

3. Results:

3.1 Chemotherapy activates AMPK leading to increased sensitivity.

Murine AML cell line (MFL2), driven by the MLL-ENL and FLT3-ITD oncogenes, was exposed to cytarabine for 16 hours and AMPK phosphorylation at Thr172 was assessed by Western blot. There was a dose dependent increase in the phosphorylation of AMPK at Thr172 with a 6-fold increase at 200nM (Figure 1A+B). To determine the role of AMPK in chemotherapy response, Cas9 expressing MFL2 cells were partially infected with a GFP tagged vector expressing an sgRNA targeting *Prkaa1*. Importantly, MFL2 cells do not express *Prkaa2* determined by microarray (data not shown). The partially infected pool of cells was exposed to cytarabine or doxorubicin for 72 hours, and the percentage of GFP+ cells in the viable fraction was assessed by flow cytometry. The GFP+, sgRNA-containing population increased in the presence of either cytarabine or doxorubicin. These results are consistent with *Prkaa1* deleted cells being more resistant to chemotherapy (Figure 1C+D). These data suggest that AMPK is activated following chemotherapy treatment and is a source of sensitivity.

3.2 *Prkaa1* deleted MFL2 cells have no AMPK activity.

To better understand the role of AMPK activity in response to chemotherapy, clonal isolates of MFL2 cells with sgRNA targeting either *Prkaa1* (AMPK KO) or the safe harbor locus *Rosa26* (Rosa26) were obtained. An α1-subunit specific antibody was used to confirm *Prkaa1* deletion (Figure 2A). To determine if there was any detectable *Prkaa2* protein expression, a Western blot using a pan-specific antibody that recognizes both α1-and-α2-subunits was performed. There was no detectable expression of either subunit in the isolated *Prkaa1* knock out clone (Figure 2B). To confirm loss of AMPK activity, Rosa26 and AMPK KO cells were stressed with nutrient poor conditions by exposure to Hank's balanced salt solution for 4 hours and the downstream target of AMPK, acetyl-CoA carboxylase (ACC1), was assessed for phosphorylation. To further confirm that ACC1 phosphorylation was due to AMPK activity in ROSA26 cells the AMPK inhibitor compound C was used to treat cells. Rosa26 but not AMPK KO cells had phospho-ACC1 that was attenuated by the presence of compound C consistent with a lack of AMPK activity in KO cells (Figure 2C). As AMPK induces p53 and cell cycle arrest at low glucose conditions[15] growth in the presence of the glycolysis inhibitor 2-deoxy-glucose was determined. Consistent with a lack of AMPK activity, AMPK KO cells were significantly more proliferative in the presence of 2-DG compared to Rosa26 cells (Figure 2D). These data demonstrate that *Prkaa1* deleted MFL2 cells do not express either *Prkaa1* or *2* and have no detectable AMPK activity.

3.3 AMPK KO cells are resistant to chemotherapy.

AMPK KO and Rosa26 cells were exposed to either cytarabine or doxorubicin for 72 hours and viability was assessed. These agents were chosen as cytarabine and an anthracycline are the main chemotherapeutics used clinically for the treatment of AML. Consistent with the competition assay results, AMPK KO cells were significantly more resistant to both cytarabine and doxorubin (Figure 3A). To confirm these results and rule out a clonal artifact, a pooled population of eight independently generated *Prkaa1* deleted clones were treated with cytarabine and doxorubicin. Pooled isolates also demonstrated a significant increase

in resistance to chemotherapy (Figure 3B). To determine if the differences in viability were a result of decreased apoptotic response, AMPK KO and Rosa26 cells were assessed by Annexin V/Propidium iodide staining following chemotherapy exposure. AMPK KO cells had a higher basal apoptotic level but a clearly blunted apoptotic response following exposure to chemotherapy (Figure 3C and Supplemental Figure 1). These data demonstrate a cell autonomous effect of AMPK on chemotherapy response but AML cells are affected by their microenvironment and *in vitro* experiments are unable to account for these micro-environmental influences. To assess the role of AMPK in chemotherapy response *in vivo*, C57Bl/6 mice were sub-lethally irradiated and injected with 1×10^6 AMPK KO or Rosa26 cells. These cells express firefly luciferase to allow for *in vivo* imaging. Upon engraftment, 8 days for Rosa26 and 11 days for AMPK KO cells, as determined by bioluminescence imaging, treatment was initiated. The regimen consisted of cytarabine 100 mg/kg I.P. for 5 days and doxorubicin 3mg/kg I.P. for the first 3 days; this combination mimics the treatment received by AML patients in clinic[16]. Pre-treatment luciferase signals were quantified to ensure that the disease burden was comparable between cohorts. No significant difference was noted between cohorts pre-treatment (Figure 3D). Mice were treated with vehicle or chemotherapy and followed for survival. Vehicle treated mice injected with AMPK KO cells survived significantly longer than Rosa26 injected mice consistent with previous reports that AMPK is required for efficient AML engraftment[12] (median survival of 8 versus 5.5 days, Figure 3E). However, in chemotherapy treated animals AMPK KO injected mice had a shorter survival when compared to those injected with Rosa26 (median survival of 11 versus 12 days, Figure 3E). This was further illustrated by the smaller survival benefit from chemotherapy seen in the AMPK KO injected animals where median survival increased by only three days compared to over seven days improvement in survival for ROSA injected animals (Figure 3E). This data is consistent with the chemotherapy resistance phenotype observed *in vitro*.

3.4 AMPK KO cells have normal mitochondria.

As AMPK is known to have a role in mitochondrial biogenesis and turnover[17] the mitochondrial effects of AMPK deletion were evaluated. To assess mitochondrial content, AMPK KO and Rosa26 cells were stained with the mitochondrial dye, mitotracker red, and analyzed by flow cytometry. Mitochondrial content did not differ in AMPK KO and Rosa26 cells (Figure 4A). Mitochondrial membrane potential (MMP) is the driver of ATP synthesis and a key indicator of mitochondrial health. To determine if MMP was affected by AMPK, cells were stained with the MMP dependent dye TMRM. There was no significant difference in TMRM uptake between AMPK KO and Rosa26 cells (Figure 4B). A known source of AML cell resistance is increased mitochondrial oxygen consumption in response to chemotherapy[18]. To determine if AMPK plays a role in this response, AMPK KO and Rosa26 cells were treated with cytarabine and mitochondrial oxygen consumption rates (OCR) determined. There was no significant difference in induced mitochondrial OCR following chemotherapy exposure (Figure 4C). These data suggest no significant changes in mitochondria in AMPK KO cells.

3.5 AMPK KO cells have a blunted DNA damage response.

Previous work has implicated AMPK in the DNA damage response, with AMPK KO mouse embryonic fibroblasts having diminished ATM activity, including decreased γ-H2AX formation following ionizing radiation exposure[19]. To assess if AMPK played a similar role in chemotherapy induced DNA damage in AML, AMPK KO or Rosa26 cells were exposed to 50 nM doxorubicin for increasing amounts of time, stained for H2AX formation and analyzed by flow cytometry. AMPK KO cells demonstrated significantly lower levels of γ-H2AX staining following chemotherapy exposure (Figure 5A and Supplemental Figure 2). We next measured p53 induction in response to DNA damage caused by doxorubicin. As with γ-H2AX, AMPK KO cells displayed a reduced induction of p53 in response to doxorubicin when compared to Rosa26 control cells (Figure 5B+C). To confirm and extend this result the canonical p53 target, p21, was also assessed following doxorubicin exposure. Consistent with a reduction in p53 activity, AMPK KO cells demonstrated diminished induction of p21 following exposure to doxorubicin (Figure 5D+E). These findings suggest that AML patients with higher AMPK activity, would have better responses to chemotherapy. Expression levels of *PRKAA1* were assessed in the TCGA database of AML patients[20] and survival compared. Patients in the lowest expressing quintile had a significantly worse median overall survival of 243 days compared to 647 days in the high expressing patients (p=0.0369, Figure 5F). Finally, to directly test if higher AMPK activity increased the response to chemotherapy, the small molecule 5-Aminoimidazole-4-carboxamide riboside (AICAR) that mimics AMP and activates AMPK was tested in the parental AMPK WT cell line MFL2. Consistent with the hypothesis AICAR sensitizes MFL2 cells to doxorubicin (Figure 5G). To determine the effect of AICAR on p53 and p21 induction, MFL2 cells were exposed to AICAR with and without doxorubicin for 4 hours, harvested and lysates blotted for p53 and p21. Consistent with AMPK increasing induction of p53 there was an increase in p53 and p21 induction in the presence of both doxorubicin and AICAR over either agent alone (Supplemental Figure 3). These data suggest that AMPK activity is required for efficient DNA damage response and induction of apoptosis.

4. Discussion:

Chemotherapy resistance is a critical factor in the treatment of various cancers including AML. Emerging evidence has indicated that chemotherapy resistance is a complex process influenced by stress response proteins[21]. Multiple studies have shown the direct role of metabolism in AML resistance to chemotherapy. Our group has previously published that mitochondrial metabolism is a source of resistance to therapy[22]. This finding was independently confirmed in an elegant *in vivo* study showing that AML cells resistant to cytarabine are enriched in mitochondrial mass and oxidative capacity[23]. The role of mitochondria in resistance to therapy is not limited to chemotherapy as sensitivity to the BCL-2 inhibitor, venetoclax, was increased with inhibition of mitochondrial function[24,25]. AMPK is the main cellular energy sensor known to coordinate metabolic shifts under stressful conditions[26]. As such, it is a prime candidate to influence the cellular response to chemotherapy. Treatment of AML cells with metabolic inhibitors causes the cells to become metabolically stressed leading to the activation of AMPK[12] and once activated AMPK promotes a shift towards catabolic processes[27] along with the inhibition of anabolic

processes[28] for restoration of cellular energy homeostasis. AMPK activation suppress cell growth and proliferation suggesting that AMPK may function as part of a tumor suppressor pathway[29,30]. However, the role of AMPK in response to chemotherapy, particularly in AML, is not known. Our data show that AMPK deleted AML cells are more resistant to chemotherapy in comparison to control cells; observed by competition (Figure 1) and viability assays (Figure 3). It is also important to note that the same chemotherapeutic resistance phenotype was observed both *in vivo* (Figure 3) and in the clinical setting with low *PRKAA1* expressers (Figure 5).

Recent studies have demonstrated that AMPK activation in cancer cells following exposure to chemotherapy leads to the activation of p53 and p21[14]. p53 is important for the regulation of the cell cycle, DNA damage response and is an important tumor suppressor[31]. It has three main functions including growth arrest, DNA repair and apoptosis. These three primary functions work synergistically to prevent the proliferation of cells with critically damaged DNA[32]. AMPK directly phosphorylates p53 on serine15[19] which in turn leads to the transcriptional regulation of primary target genes such as p21, which mediate G1/S or G2/M phase cell cycle arrest. Additionally, γH2AX becomes phosphorylated on serine139 as a reaction to double strand breaks where it is primarily responsible for the recruitment of repair complexes at the specific site of the DNA damage[33]. Induction of p53, p21, and γH2AX were attenuated in AMPK deleted cells following exposure to DNA damaging agents (Figure 5). AMPK activation has also been associated with apoptosis regulation following exposure to metabolic stressors[34]. The loss of AMPK in our system leads to decreased induction of apoptosis (Figure 3). Taken together this data suggests that AMPK establishes an additional metabolic input into cell fate decisions. When AMPK is functional, cells that are without the appropriate metabolic resources to repair their DNA damage end up becoming more apoptotic. In this model, AMPK adds an additional input to the cell's DNA damage response; allowing cell fate decisions to take into account not only the degree of damage but also the metabolic reserves of the cell (Figure 6). This allows a more nuanced cell fate decision allowing earlier commitment to apoptosis for AML cells without the resources to complete the repair process. This provides additional insights into the mechanism of chemotherapy response and suggests a strategy whereby an AMPK activating agent added to a chemotherapy regimen could improve responses for patients suffering from this devastating disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- AMPK activity can protect AML cells from metabolic stress
- DNA damage response was impaired in AML cells without AMPK activity leading to reduced induction of p53 and apoptosis following chemotherapy
- Lower expression of AMPK led to decreased survival in AML patients
- AMPK activating small molecule AICAR increased AML cell response to chemotherapy
- AMPK provides additional metabolic input into AML cell fate decisions following chemotherapy treatment

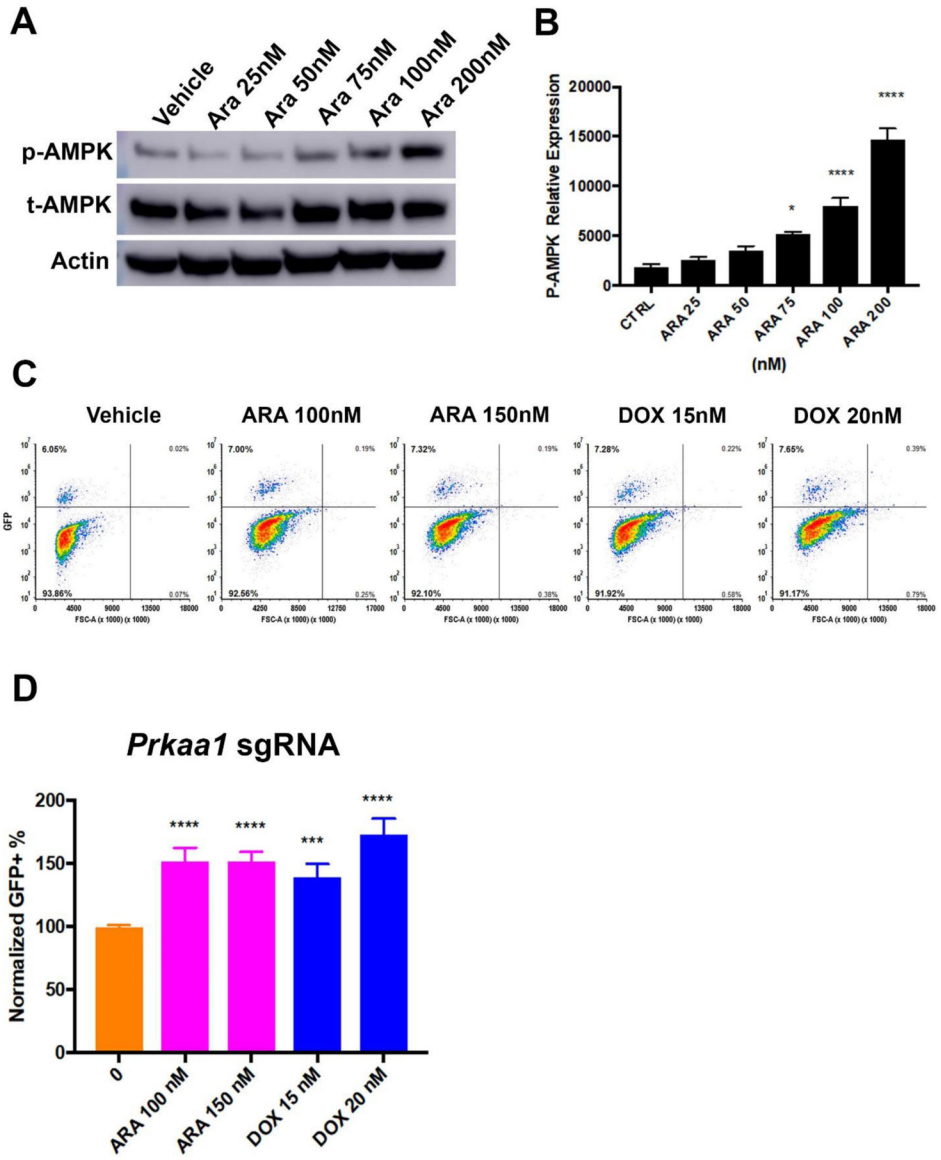

Figure 1. AMPK is activated following chemotherapy and is a source of sensitivity.

A) Western blot for phosphorylated (p-AMPK) or total (t-AMPK) AMPK alpha subunit. Murine AML cells (MFL2) were treated with the indicated amount of cytarabine (Ara) for 16 hours and lysates blotted as indicated. Actin served as a loading control. B) Densitometry of triplicate Western blots as done in (A) normalized to actin. C) Competition assay. Cas9 expressing Murine AML cells were partially infected with an sgRNA targeting *Prkaa1* with a GFP reporter. Cells were treated as indicated for 72 hours and viable fraction analyzed for GFP expression by flow cytometry. Representative contour plots are shown for each treatment tested. D) Competition assay. Normalized GFP+ percentages for each conditioned tested. Shown are the mean values of three independent experiments each done in triplicate. *=p value <0.05, ***=p value <0.005, ****=p value <0.001.

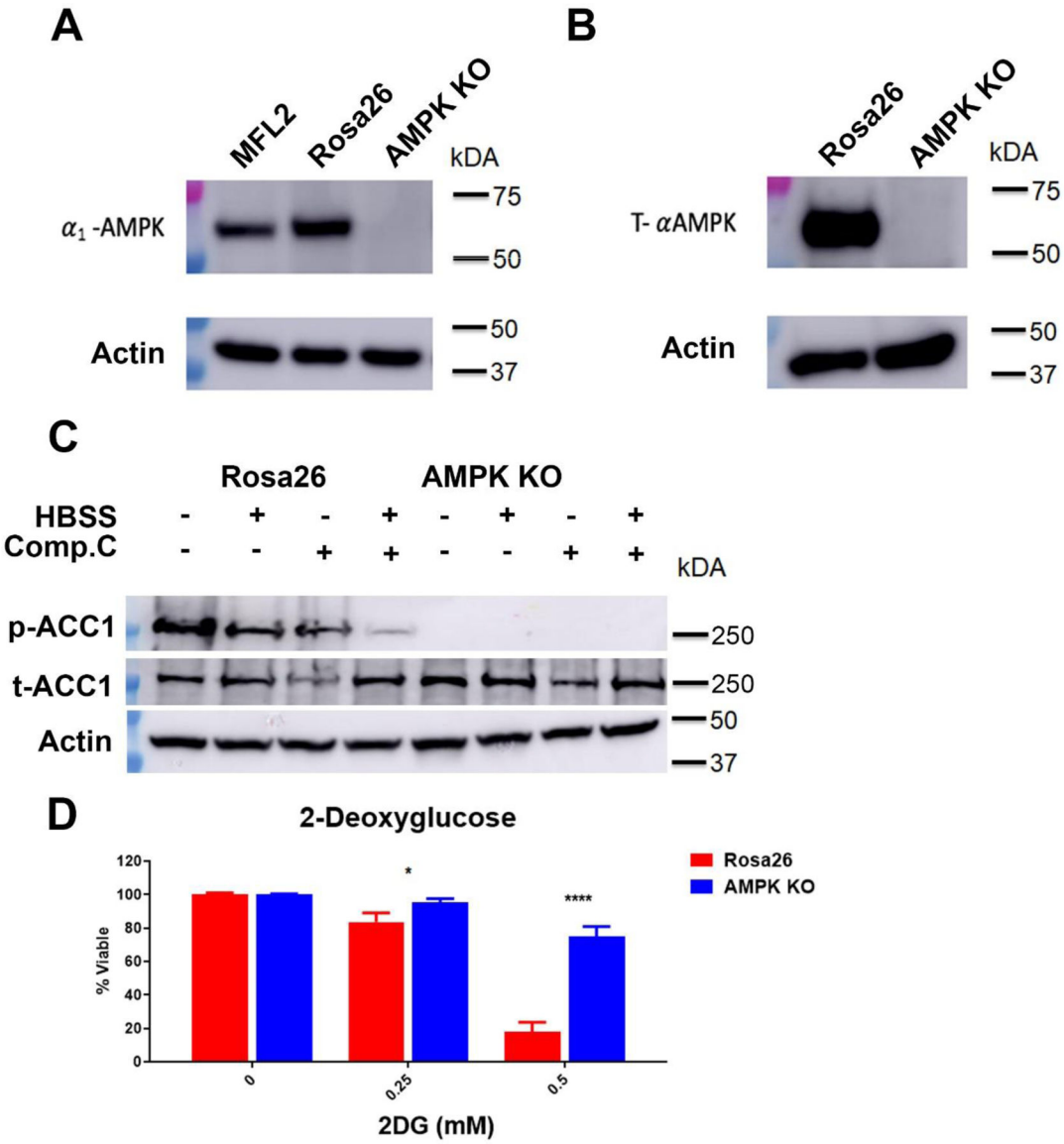

Figure 2. *Prkaa1* deleted cells have no AMPK activity.

A) Western blot for *Prkaa1* (α_1-AMPK) from the parental line (MFL2), control *Rosa26* targeted control cells or *Prkaa1* deleted cells (AMPK KO). Actin served as a loading control. B) Western blot for *Prkaa1* and Prkaa2 (T-αAMPK). Actin served as a loading control. C) Western blot for phosphorylated ACC1 (p-ACC1) and total ACC1 (t-ACC1) in Hank's balanced salt solution (HBSS) with and without compound C (Comp.C). Actin served as a loading control. D) Viability assays. Rosa26 control or AMPK KO cells were treated with the indicated amount of the glycolysis inhibitor 2-deoxyglucose (2DG) for 72 hours and viability assessed. *=p value <0.05, ****=p value <0.001.

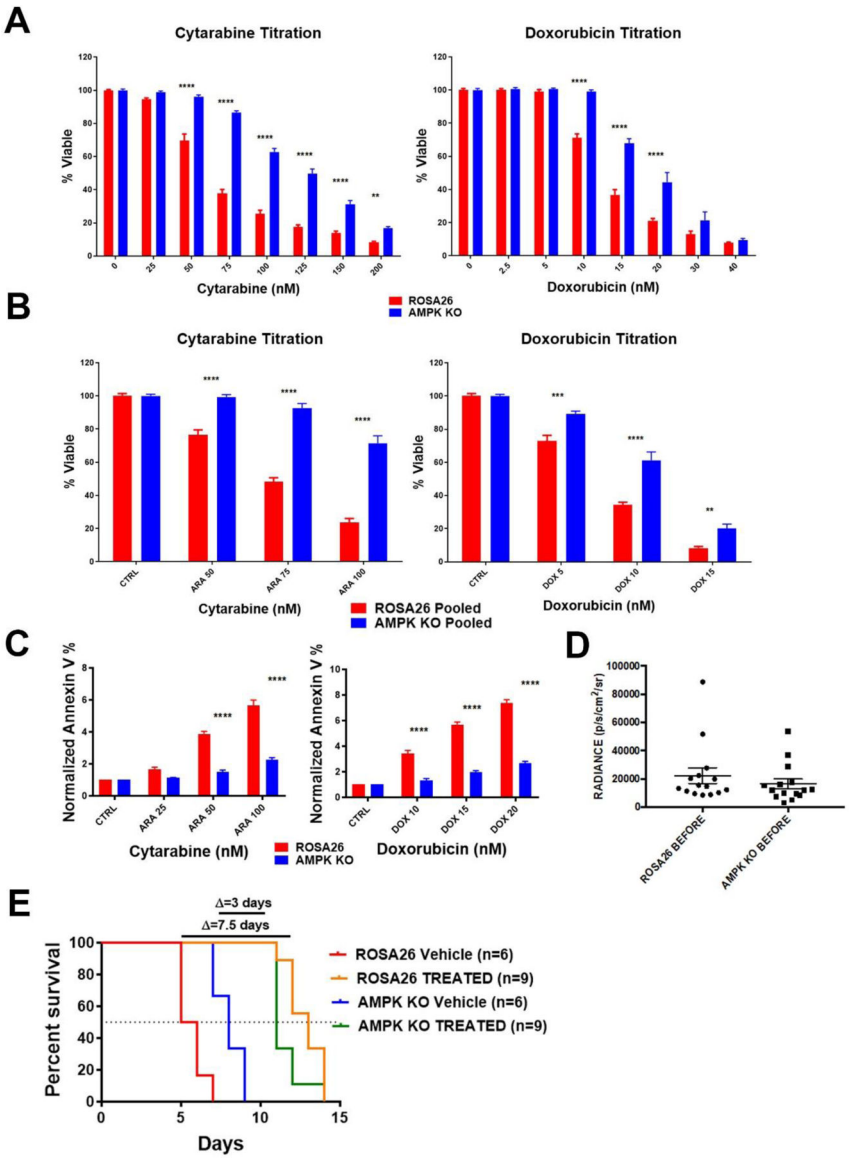

Figure 3. AMPK is a source of sensitivity to chemotherapy in AML.

A) Viability assays. AMPK KO and Rosa26 clonal isolates were exposed to the indicated chemotherapy for 72 hours and viability assessed. B) Pooled clones of AMPK KO or control Rosa26 cells were exposed to the indicated chemotherapy for 72 hours and viability assessed. C) AnnexinV assays. AMPK KO and Rosa26 cells were exposed to the indicated chemotherapy for 72 hours, stained with annexin V and analyzed by flow cytometry. Shown are the means of three independent experiments with the data normalized to each vehicle. D) Quantified bioluminescence. Mice were injected with either AMPK KO or control Rosa26 cells and subjected to bioluminescence imaging. Engraftment at time of treatment initiation is shown. E) Kaplan-Meier curves for vehicle or chemotherapy treated mice. Change in median survival in days is shown above the graph. *=p value <0.05, **=p value <0.01, ***=p value <0.005, ****=p value <0.001.

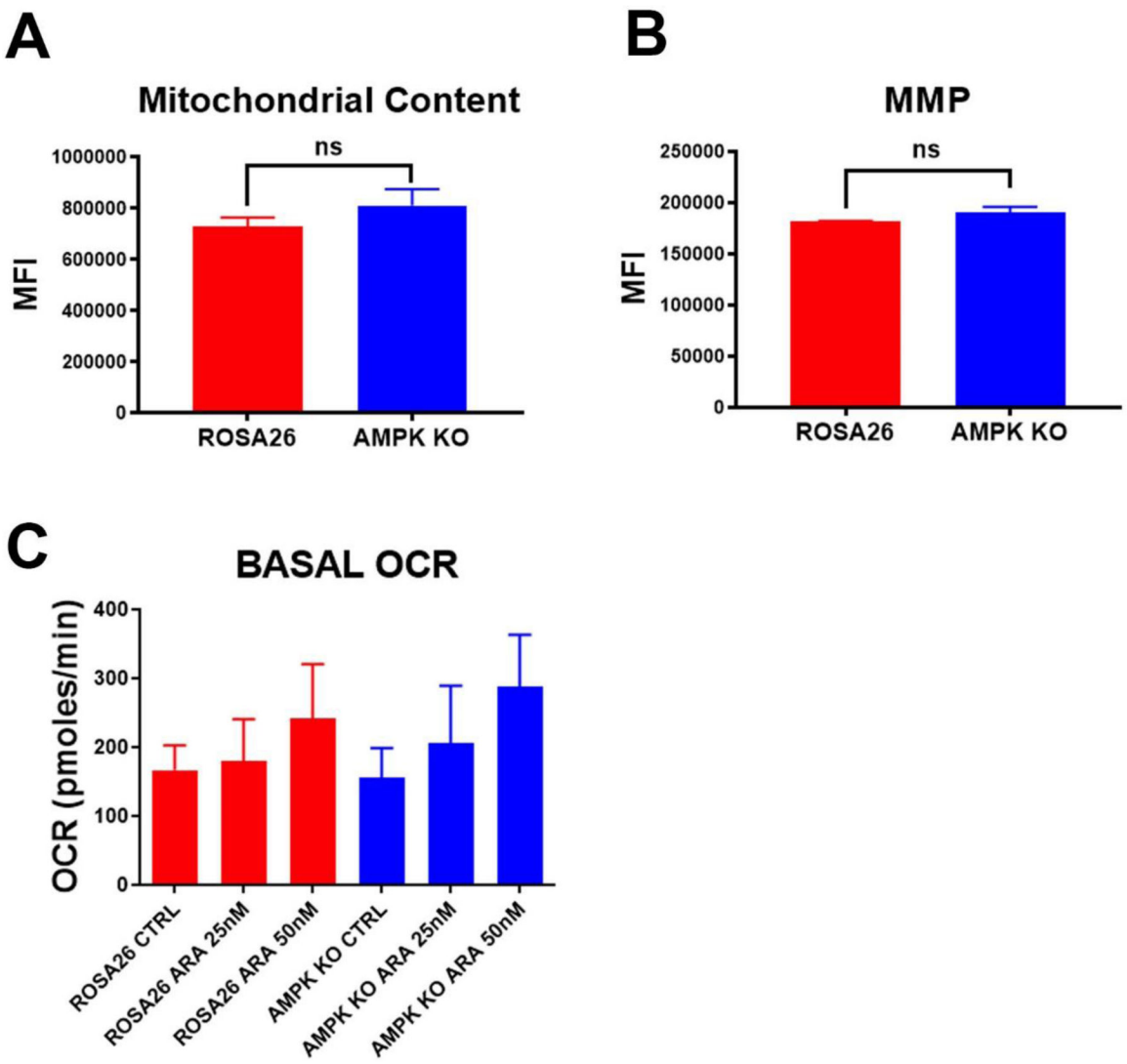

Figure 4. AMPK KO AML cells have intact mitochondria.

A) Mitotracker staining. AMPK KO and Rosa26 control cells were stained with the mitochondrial dye, mitotracker red. Shown are the mean values of the median florescence intensity (MFI) of three independent experiments. B) TMRM staining. AMPK KO and Rosa26 control cells were stained with the mitochondrial membrane dependent dye, TMRM. Shown are the mean values of the median florescence intensity (MFI) of three independent experiments. C) Basal oxygen consumption rates. AMPK KO and Rosa26 control cells were treated with the indicated amounts of cytarabine (ARA) for 16 hours and basal oxygen consumption rates (OCR) determined. Shown are means of three independent experiments each done in triplicate. There was no significant difference of OCR for any chemotherapy concentration.

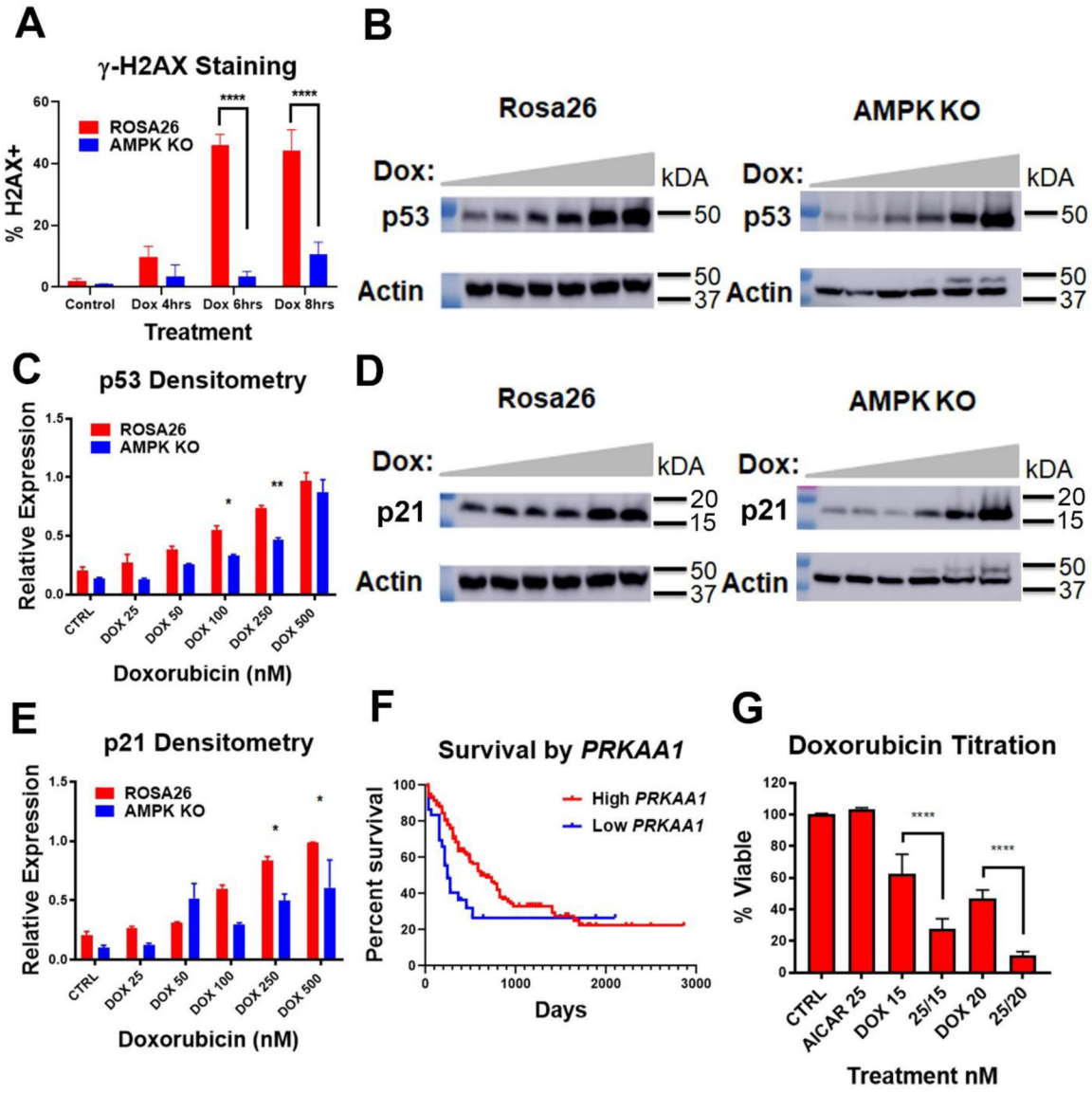

Figure 5. AMPK KO cells have an impaired DNA damage response.

A) γH2AX staining. AMPK KO and Rosa26 control cells were treated with 50 nM doxorubicin for the indicated amount of time and stained for γH2AX. Following staining cells were analyzed by flow cytometry. B) Western blots. AMPK KO and Rosa26 control cells were treated with increasing amounts of doxorubicin for 4 hours and lysates blotted for p53. Actin was used as a loading control. C) Densitometry of triplicate Western blots as done in (B) normalized to actin. D) Western blots. AMPK KO and Rosa26 control cells were treated with increasing amounts of doxorubicin for 4 hours and lysates blotted for p21. Actin was used as a loading control. E) Densitometry of triplicate Western blots as done in (D) normalized to actin. F) Overall survival of AML patients by *PRKAA1* expression. AML patients from the TCGA database were analyzed for survival by expression level of *PRKAA1*. Low *PRKAA1* (bottom tertile, blue line), High *PRKAA1* (Top 4 tertiles, red line). G) Viability assays. Parental MFL2 cells were treated with the nM amounts of AICAR

or doxorubicin (DOX) or both as indicated for 72 hours and viability assessed. *=p value <0.05, **=p value <0.01, ***=p value <0.005, ****=p value <0.001.

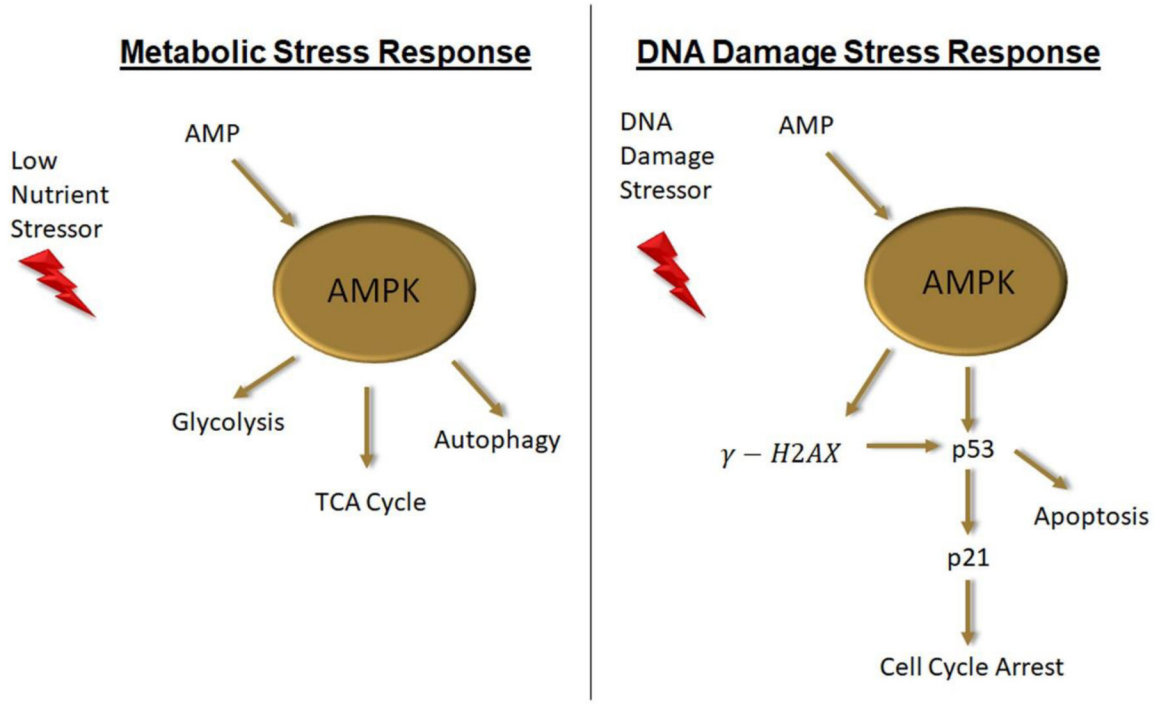

Figure 6. Model of AMPK role in metabolic stress and DNA damage response.

When nutritional stores are low AMP levels raise activating AMPK which in turn activates the energy producing pathways of glycolysis, TCA cycle and autophagy. When DNA damage occurs AMPK integrates nutritional reserve data by stimulating H2AX foci formation and p53 induction leading to increased apoptosis when nutritional reserve is insufficient to repair the damage.