The Role of 5' Adenosine Monophosphate-Activated Protein Kinase in the Chemo-Sensitivity and Metabolic Behaviour of Breast Cancer Cells Exposed to Hypoxia and Hyperglycaemia

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Abstract: *Background*: 5' adenosine monophosphate-activated protein kinase (AMPK) is a key enzyme for maintaining energy homeostasis in the cell and is associated with many downstream targets of metabolic processes such as mTORC1, p53 and fatty acid synthase (FASN) and insulin-like growth factor binding protein-2 (IGFBP-2).

Aim: To investigate the interactions between AMPK, FASN and IGFBP-2 and how the activity of AMPK affects the metabolism and response of breast cancer cells to chemotherapy with changes in oxygenation and under different glucose concentrations.

Methods: MCF-7 breast cancer cells were exposed to different glucose levels (5mM and 25mM) in the presence or absence of doxorubicin under normoxic and hypoxic conditions with and without AMPK silenced using siRNA. Changes in protein abundance were monitored using Western Immunoblotting. Cell death was measured by the Muse® Cell Analyser using a count and viability assay. Hypoxia was chemically induced using cobalt chloride or with low levels of oxygen (2%). Lactate and citrate levels were measured using commercially available kits.

Results: In normoxic conditions**,** AMPK activity was higher in normal levels of glucose (5mM) compared with high levels of glucose (25mM). Under hypoxic conditions, AMPK phosphorylation remained high in 5mM glucose with levels in 25 mM glucose being equivalent. Upregulation of AMPK in normoxic and hypoxic conditions was associated with a reduction in FASN and IGFBP-2, which resulted in a better response to chemotherapy. Moreover, the cells increased the production of lactate and reduced production of citrate under normoxic conditions in 25mM glucose compared to 5mM glucose. Silencing AMPK under normoxic conditions or inducing hypoxia promoted a more glycogenic phenotype. However, silencing AMPK under hypoxic conditions reduced levels of lactate comparable to normoxic levels. The citrate profile was unaffected by silencing AMPK or altering levels of oxygen.

Conclusions: AMPK plays an important role in regulating metabolic signalling and this alters the sensitivity of breast cancer cells to chemotherapy.

Keywords: AMPK, hyperglycaemia, hypoxia, insulin-like growth factor binding protein 2, chemo-resistance, fatty acid synthase.

INTRODUCTION

Cancer is characterised by unrestrained proliferation where the cells are no longer responsive to normal growth controlling mechanisms [1]. As the tumour develops, it can frequently outgrow the blood supply resulting in cancer cells being exposed to hypoxia [2]. In order to support rapid proliferation, cancer cells adopt altered metabolic behaviour and develop mechanisms to sustain their growth and proliferation.

5' adenosine monophosphate-activated protein kinase (AMPK) is an important enzyme that maintains energy homeostasis in the cell. AMPK can be activated by metabolic stress such as hypoxia, lack of glucose and exercise [3]. Activation of AMPK preserves energy for essential energy demand by stimulation of the fatty acid oxidation pathway to generate ATP and inhibition of ATP-dependent anabolic processes [3]. AMPK can also increase insulin sensitivity, inhibit hepatic gluconeogenesis, enhance glucose uptake in myocytes, and inhibit fatty acid synthesis [4]. Thus, activation of AMPK has long been considered a way of treating metabolic syndrome and type 2 diabetes [3, 5].

AMPK is associated with many downstream targets of metabolic processes such as mTORC1, p53 and fatty acid synthase (FASN). AMPK is thus able to control intracellular energy and sustain normal growth levels [6, 7].

High glucose inhibits the expression of AMPK, which then abrogates the inhibitory effect of AMPK on acetyl CoA carboxylase (ACC). Activated ACC converts acetyl-CoA to malonyl-CoA, which is a precursor for FASN. Malonyl-CoA is an inhibitor of carnitine palmitoyl transferase 1 (CPT1), which is an enzyme that converts acetyl-CoA in the mitochondria to

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citrate. This shifts the cells to aerobic glycolysis and away from the β-oxidation cycle [8].

We have shown previously that high levels of glucose reduce the sensitivity of cancer cells to chemotherapy and this was attributed to increased abundance and activation of FASN and ERα and a consequent ERα-induced increase in IGFBP-2 in breast cancer cells [9, 10] and prostate cancer cells [11].

Glucose serves as a substrate for FASN, which is highly expressed in malignant cells including breast cancer cells [12]. We and others have shown that FASN knockdown using siRNA leads to improved sensitivity of breast cancer cells to chemotherapy [9, 13] and suppression of the oncogenic activity of HER2 [14] acting through the PI3K pathway [9]. FASN plays an important role in metabolism since it catalyzes the synthesis of the fatty acid palmitate from its substrate glucose [15]. Palmitate itself is a fundamental precursor for ceramide synthesis [16].

Controlling the levels of glucose and insulin in the blood could be beneficial for cancer patients who suffer from type 2 diabetes and might increase their responsiveness to chemotherapy. There is evidence suggesting that control of hyperglycaemia during chemotherapy could improve toxicity and outcome of treatments in patients with haematological and solid tumours [17]. Accumulating epidemiological and laboratory studies have reported that the use of antidiabetic therapy may improve responsiveness of cancer cells, cancer recurrence prevention and enhanced effectiveness to chemotherapy. Agents such as metformin have increasingly been of interest in cancer treatment though the mechanisms of how these agents act and their anticancer effects are still being investigated and defined [18].

In this study we aimed to investigate the interactions between AMPK, FASN and IGFBP-2 and how the activity of AMPK could affect the response of breast cancer cells to chemotherapy with changes in oxy-genation and under different glucose concentrations.

MATERIALS AND METHODS

Compounds were purchased from Sigma-Aldrich, Dorset, UK unless otherwise stated.

Cell Maintenance and Treatment

Breast cancer cells (MCF-7) were maintained in 4.5g glucose (or 25mM glucose) DMEM growth medium supplemented with 50ml 10% FBS and 5ml 2mM L-glutamine. The cells were seeded at $0.1x10^6$ cells in 1g glucose (or 5mM glucose) DMEM growth medium then serum starved for 24h in 5mM or 25mM glucose DMEM serum-free media prior to treatment with 1μM Doxorubicin. Hypoxia was induced either chemically with 500μM cobalt chloride ($CoCl_2$) or with exposure to 2% oxygen levels using a hypoxic chamber.

Transfection of Cells with siRNA

The expression of AMPKα1 and AMPKα2 were silenced by transfecting the cells with siRNA (Qiagen, #SI02622228 and #SI02758595, accordingly). Transfection was conducted following the manufacturer's instructions- Synvolus Therapeutics (Leiden, The Netherlands).

Western Immunoblotting

A protein assay was performed to estimate the concentrations present in a sample. Western immunoblotting was performed to assess changes in protein abundance using anti-IGFBP-2 (goat, 1:1000) Santa Cruz, Heidelberg, Germany, anti-AMPK and anti-p-AMPK (rabbit, 1:1000; Cell Signaling Hertfordshire, UK), anti-FASN (mouse, 1:2000), and anti-HIF-Iα (mouse, 1:500) purchased from BD Biosciences, Oxford, UK. Anti-α-tubulin (mouse, 1:5000) was purchased from Merck Millipore Hertfordshire, UK and was used as a loading control.

Cell Counting: A Count and Viability Assay

Cell death was measured using the Muse® cell analyser using a cell count and viability assay as outlined by the manufacturer (EMD-Millipore, Burlington USA). The cells were diluted 1:10 prior to assessment.

Lactate and Citrate Assays

Lactate production was measured using L-Lactate Colorimetric Assay Kit (BioVision #K627-100, USA). Citric acid production was measured using Citrate Colorimetric Assay Kit (BioVision #K655-100, USA). The assays were conducted as per the manufacturer's instructions.

Statistical Analysis

Data in this study represent the mean ± SEM of three independent experiments each repeated in triplicate, unless otherwise stated. For multiple

comparisons, one-way ANOVA post hoc LSD test was used, and differences between samples were considered significant at *P<0.05, **P<0.01, and ***P<0.001.

RESULTS

The Role of AMPK in the Response of Breast Cancer Cells to Chemotherapy

The expression of AMPK was knocked down in MCF-7 cells using AMPK siRNA and the cells were treated with 1μM doxorubicin in 5mM and 25mM glucose for 24h under normal levels of oxygen. Consistent with our previously published data, MCF-7 cells were more chemo-resistant in 25mM glucose where the level of cell death was 37.5%, compared to 52.7% in 5mM glucose, in response to doxorubicin. Although the protective effect of high glucose was not affected when AMPK was silenced, the level of cell death in 5mM glucose was significantly reduced where cell death decreased from 52.7% to 43.8% (Figure **1A** and **B**).

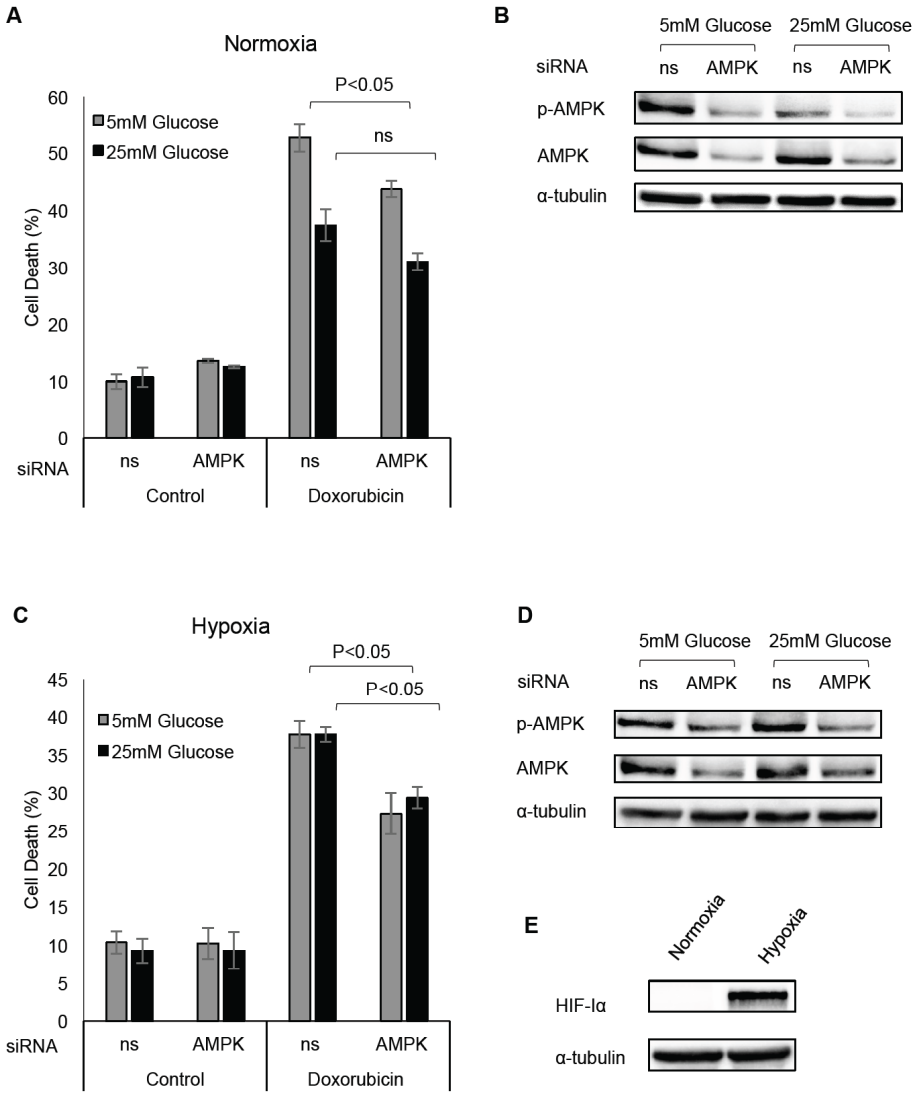


Figure 1: The role of AMPK in the response of breast cancer cells to chemotherapy.

(**A**) MCF-7 cells were transfected with AMPK siRNA to silence its expression then were treated with doxorubicin for 24h in 5mM and 25mM glucose. The cells were incubated at normal oxygen levels. The levels of cell death were measured by count and viability assay. (**B**) Western immunoblotting showing efficiency of AMPK knock-down and basal level of AMPK activity in different glucose levels under normal oxygen level. The graph represents the mean ± SEM of three independent repeats, each performed in triplicate. (**C**) MCF-7 cells were transfected with AMPK siRNA then treated with doxorubicin under hypoxic conditions. Hypoxia was induced chemically by 500μM $CoCl_2$. The levels of cell death were measured by count and viability assays. (**D**) Western immunoblotting showing efficiency of AMPK knock-down and basal level of AMPK activity in different glucose levels under hypoxia. (**E**) Western immunoblotting was used to detect the abundance of HIF-Iα; reflecting the cells were hypoxic. The graph represents the mean ± SEM of three independent repeats, each performed in triplicate.

We confirmed our previous work that the differential effect of glucose on the response of MCF-7 cells to chemotherapy was negated under hypoxia where levels of cell death were not statistically significant between 5mM and 25mM glucose [10]. With hypoxia, when AMPK expression was silenced, the levels of cell death dropped significantly in both levels of glucose and there was still no differential effect of the glucose (Figure **1C** and **D**). HIF-Iα was a positive indicator of successful hypoxia induction (Figure **1E**).

Effect of Glucose and Hypoxia on AMPK Activation

We studied the activity of AMPK in 5mM and 25mM glucose and confirmed that the phosphorylation of AMPK was significantly higher in 5mM compared with 25mM glucose (Figure **2A** and **C**), consistent with AMPK being activated when energy supply was limited and being less active when energy supply was plentiful. When the cells were exposed to hypoxia, AMPK activity not only remained active in 5mM glucose but was also increased in 25mM glucose relative to levels

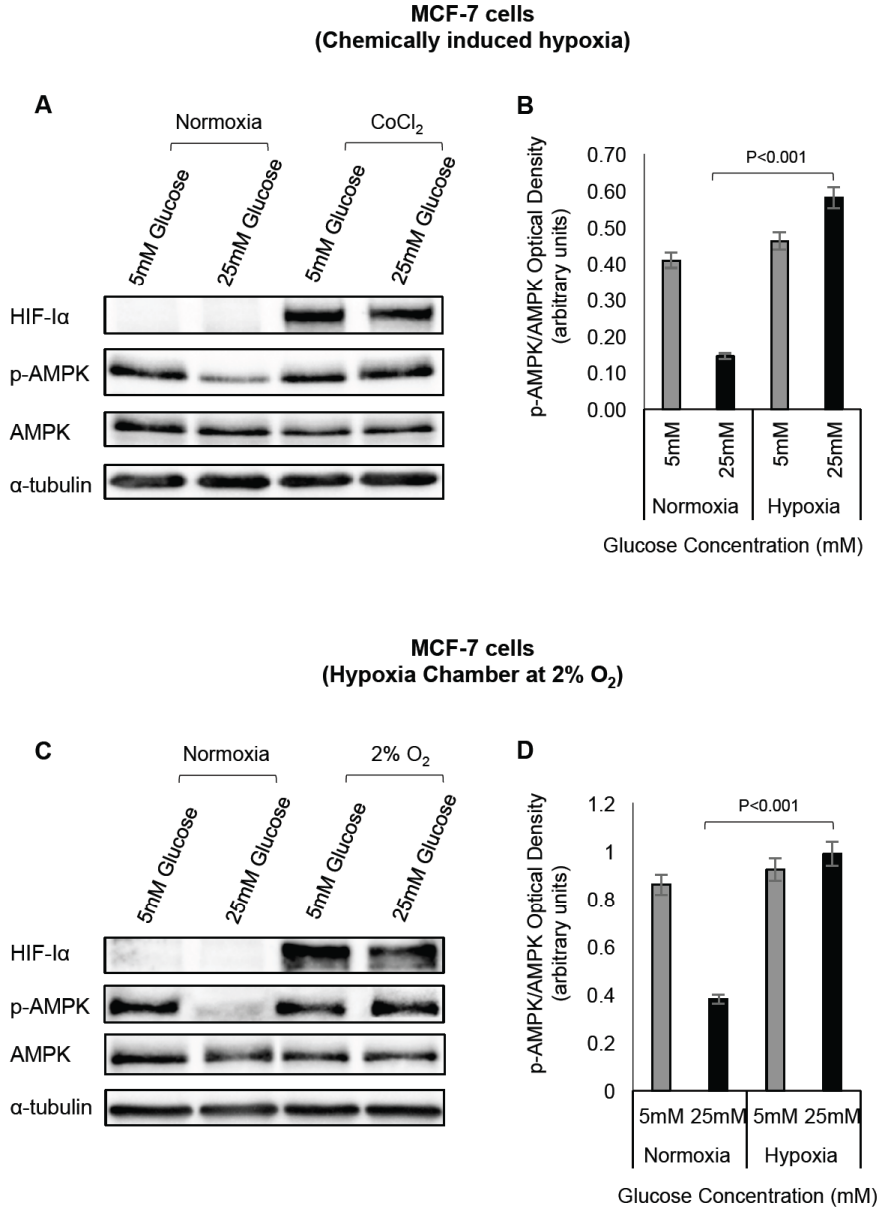


Figure 2: The effect of glucose and hypoxia on AMPK activity.

(**A**) MCF-7 cells were incubated in 5mM and 25mM glucose for 24h in normal oxygen levels following treatment with $CoCl_2$ to induce hypoxia. Changes in HIF-Iα and AMPK activity were monitored by Western immunoblotting. HIF-Iα was a positive indicator of successful hypoxia induction. (**B**) The densitometry of changes in AMPK activity in normoxia and chemically-induced hypoxia by $CoCl_2$. (**C**) MCF-7 cells were incubated in 5mM and 25mM glucose for 24h in normal oxygen levels and low oxygen levels at 2% to induce hypoxia. Changes in HIF-Iα and AMPK activity were monitored by Western immunoblotting. (**D**) The densitometry of changes in AMPK activity in normoxia and hypoxia induced by 2% O_2.

in normoxia and at a similar level to that observed with 5mM glucose in normoxia. Hypoxia induction using either a hypoxia chamber or chemically using cobalt chloride were consistent and resulted in an increase in activity of AMPK in 25mM glucose (Figure **2A**, **B**, **C**, and **D**).

The Involvement of AMPK in Hyperglycaemia-Induced Up-Regulation of FASN and IGFBP-2 Abundance under Normoxic and Hypoxic Conditions

Previously, we have shown that FASN and IGFBP-2 mediate hyperglycaemia-induced chemoresistance [9]. We also previously reported that under hyperglycaemic conditions, IGFBP-2 abundance is down-regulated resulting in an increased sensitivity of cancer cells to chemotherapy, comparable to that observed in normal levels of glucose [10]. Given that FASN is an important component of the fatty acid synthesis pathway and that the activity of FASN is highly regulated by the activity of AMPK; we investigated the effect of AMPK on FASN. Consistent with our previously published data [9], in normoxia, the abundance of FASN increased in 25mM glucose compared with 5mM glucose. FASN abundance also increased in both levels of glucose when AMPK expression was silenced (Figure **3A** and **B**) but the increase was only significant at 5mM glucose (Figure **3A** and **B**). Hypoxia negated the high glucose-induced increase in FASN levels. Interestingly, similar to under normoxic conditions, silencing the expression of AMPK under hypoxic conditions significantly increased the abundance of FASN up to 9.8% in 5mM glucose and 9.9% in 25mM glucose (Figure **3C** and **D**).

In normoxia, as anticipated, AMPK activity increased in 5mM glucose compared to 25mM glucose and the abundance of IGFBP-2 increased in 25mM glucose compared to 5mM glucose. Silencing the expression of AMPK resulted in a further increase in IGFBP-2 abundance at both 5mM and 25mM and the differential effect of glucose was retained (Figure **3E** and **F**).

We also investigated the effects of AMPK activity on IGFBP-2 under hypoxic conditions where the glucose mediated increase in IGFBP-2 is negated. Silencing the expression of AMPK increased the abundance of IGFBP-2 up to 11.7% in 5mM and 12.4% in 25mM glucose (Figure **3G** and **H**) and there was still no differential effect of the glucose.

The Effect of Glucose and Hypoxia on Glucose Metabolism and the Role of AMPK

Lactate and citrate concentrations were measured in 5mM and 25mM glucose under normoxic and hypoxic conditions in the presence or absence of AMPK.

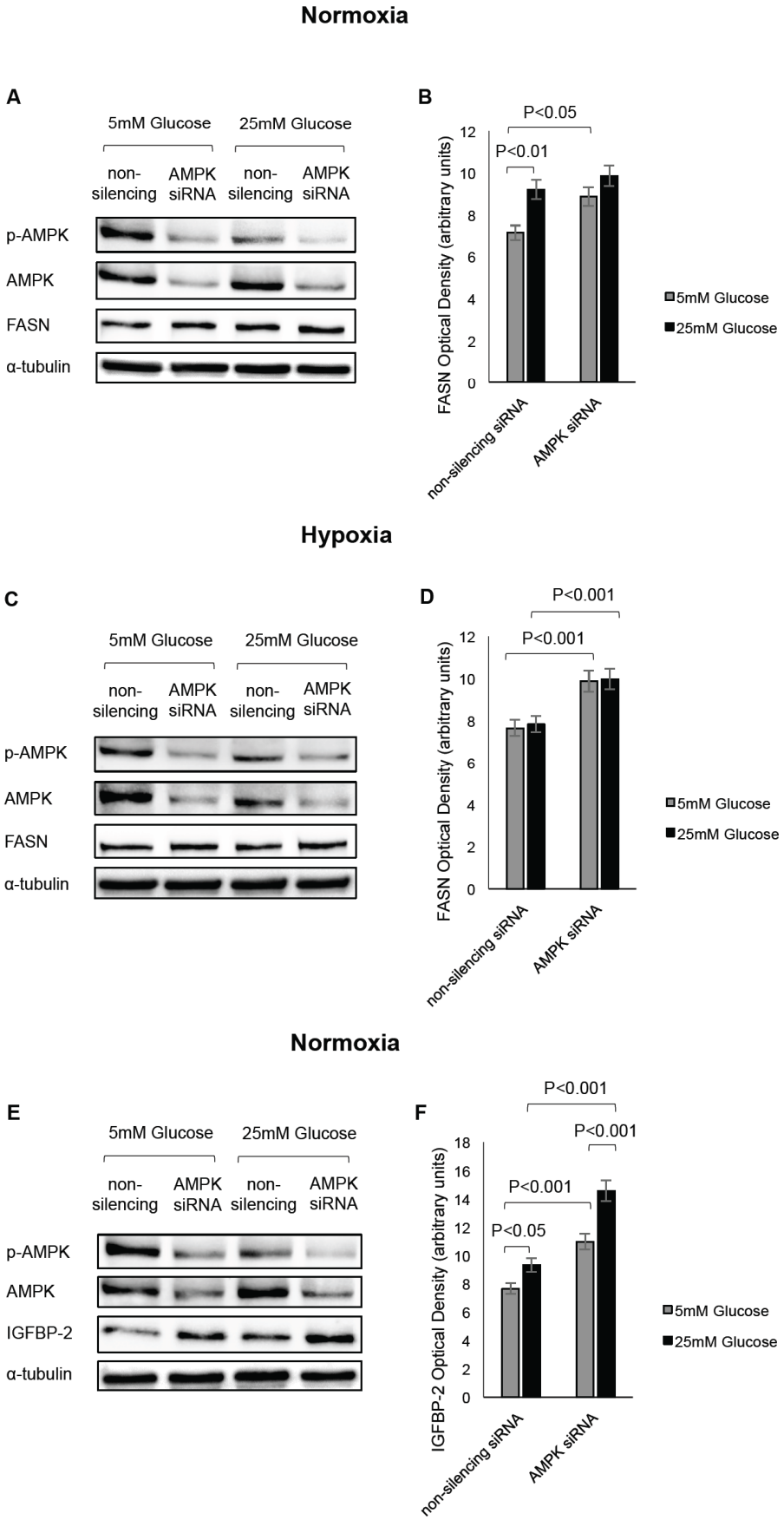
Lactate concentrations increased significantly in 25mM glucose compared with 5mM glucose in normoxic conditions (Figure **4A**). With hypoxia lactate production increased in both 5mM and 25mM glucose when compared with normoxic conditions and there was no difference in concentration of lactate between normal and high glucose (Figure **4B**).

In normoxia, silencing the expression of AMPK increased levels of lactate in 5mM glucose in normoxia and hence negated the differential effect of secreted lactate between 5mM and 25mM glucose; the cells reverted to the same metabolic pattern of lactate secretion observed under hypoxic conditions (Figure **4A**). Silencing the expression of AMPK in hypoxic conditions lowered lactate levels in 5mM and 25mM glucose and restored the differential effect of secreted lactate between 5mM and 25mM glucose; the cells reverted to the metabolic phenotype of lactate secretion observed under normoxic conditions (Figure **4B**).

Under normal oxygen levels, citrate concentrations were higher in 5mM glucose compared to 25mM glucose. This occurred with the increase in lactate production in 25mM glucose (Figure **4C**). With hypoxia, the differential effect of glucose on citrate production was not affected (Figure **4D**). Under normoxia, silencing the expression of AMPK lowered citrate concentrations in 5mM glucose but not in 25mM glucose (Figure **4C**) and the differential effect of glucose on citrate production was not affected by silencing the expression of AMPK (Figure **4C**). Under hypoxic conditions, the metabolic phenotype of citrate secretion was unaffected when silencing the expression of AMPK (Figure **4D**). The efficiency of AMPK knock-down and induction of hypoxia (represented by increased HIF-Iα abundance) are illustrated in Figure **4E** and **F**, respectively.

DISCUSSION

Obesity is considered an important risk factor for breast cancer. Hyperglycaemia, which is a consequence of obesity, induces resistance to chemotherapy. This was shown in our previous study


Normoxia
A
5mM Glucose
25mM Glucose
non-silencing
AMPK siRNA
non-silencing
AMPK siRNA
p-AMPK
AMPK
FASN
α-tubulin
B
P<0.05
P<0.01
FASN Optical Density (arbitrary units)
0
2
4
6
8
10
12
non-silencing siRNA
AMPK siRNA
5mM Glucose
25mM Glucose
Hypoxia
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5mM Glucose
25mM Glucose
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5mM Glucose
25mM Glucose
Normoxia
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25mM Glucose
non-silencing
AMPK siRNA
non-silencing
AMPK siRNA
p-AMPK
AMPK
IGFBP-2
α-tubulin
F
P<0.001
P<0.001
P<0.001
P<0.05
IGFBP-2 Optical Density (arbitrary units)
0
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18
non-silencing siRNA
AMPK siRNA
5mM Glucose
25mM Glucose

(Figure 3). Continued.

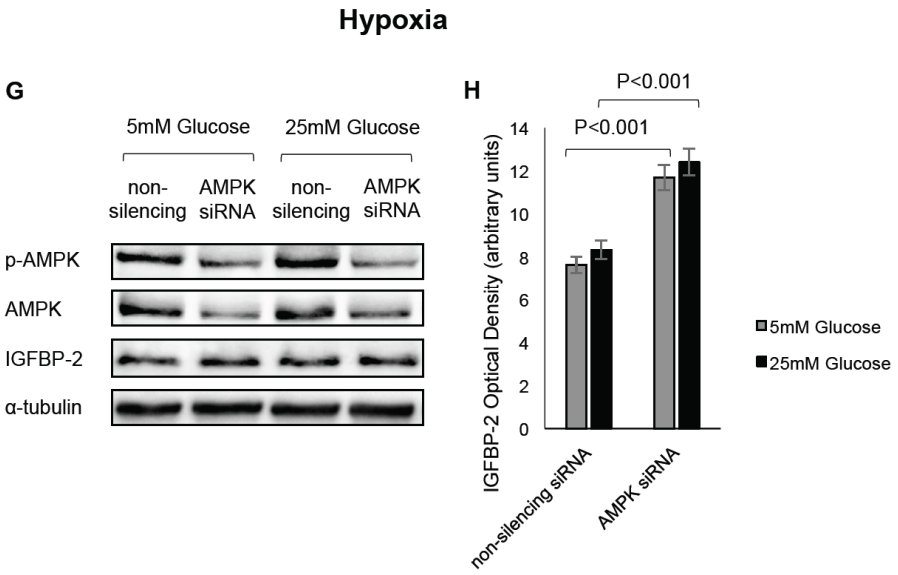

Figure 3: The effect of AMPK on FASN and IGFBP-2.

MCF-7 cells were transfected with AMPK siRNA to silence its expression then were treated with doxorubicin for 24h in 5mM and 25mM glucose. The cells were incubated at normal oxygen levels. Changes in AMPK and FASN (**A**) and IGFBP-2 (**E**) levels were examined by Western immunoblotting. Optical densitometry of FASN (**B**) and IGFBP-2 (**F**) in normoxic conditions. The graphs represent the mean ± SEM of three independent repeats, each performed in triplicate. MCF-7 cells were transfected with AMPK siRNA to silence its expression and then treated with doxorubicin for 24h in 5mM and 25mM glucose under hypoxic conditions. Hypoxia was induced chemically by 500μM $CoCl_2$. Changes in FASN (**C**) and IGFBP-2 (**G**) levels were examined by Western immunoblotting. Optical densitometry of FASN (**D**) and IGFBP-2 (**H**) in the presence and absence of AMPK under hypoxic conditions. The graphs represent the mean ± SEM of three independent repeats, each performed in triplicate.

where increases in FASN [9] and IGFBP-2 [11, 10] mediated hyperglycaemia-induced chemo-resistance. As the tumour develops, it often outgrows the blood supply exposing the cells to hypoxia. Previously, we have shown that hypoxia negated the hyperglycaemia-induced chemo-resistance through down-regulation of FASN and IGFBP-2 [10].

AMPK is a well-characterised energy sensor in living cells [12]. AMPK activity increases in response to low energy in order to maintain an appropriate balance between energy consumption and production [12].

We have studied the activity of AMPK in 5mM and 25mM glucose in normoxic and hypoxic conditions. With normoxia we confirmed that the phosphorylation of AMPK is significantly higher in 5mM compared with 25mM glucose consistent with the fact that the cells activate AMPK when energy supply is limited and deactivate AMPK when energy is plentiful. This also correlated with a higher level of chemosensitivity in lower levels of glucose compared with high. When the cells were exposed to hypoxia, AMPK activity not only remained active in 5mM glucose but also increased in 25mM glucose to a similar level observed with 5mM glucose. This suggests that the cells were stressed and required more energy since glucose oxidation was not possible in the absence of oxygen. As we showed previously [9, 10] hypoxia negated the high glucose-induced chemoresistance, with levels of induced cell death being the same in normal and high levels of glucose.

To further support a role for AMPK in chemosensitivity, we silenced AMPK under normoxic conditions and found that whilst the differential effect of glucose remained, with high glucose conferring chemo-resistance, the overall levels of cell death were reduced compared with cells expressing AMPK. Under hypoxic conditions with AMPK silenced, levels of cell death were reduced to the same degree in both normal and high glucose. These data indicate that under normoxic and hypoxic conditions activation of AMPK is associated with a better response to chemotherapy.

This is consistent with other reports indicating that activation of AMPK sensitizes cancer cells to chemotherapy including those of the breast [13] and non-small cell lung cancer [14].

We showed previously that FASN and IGFBP-2 were involved in mediating the effects of hyperglycaemia on chemoresistance. We observed that silencing AMPK in altered levels of glucose and

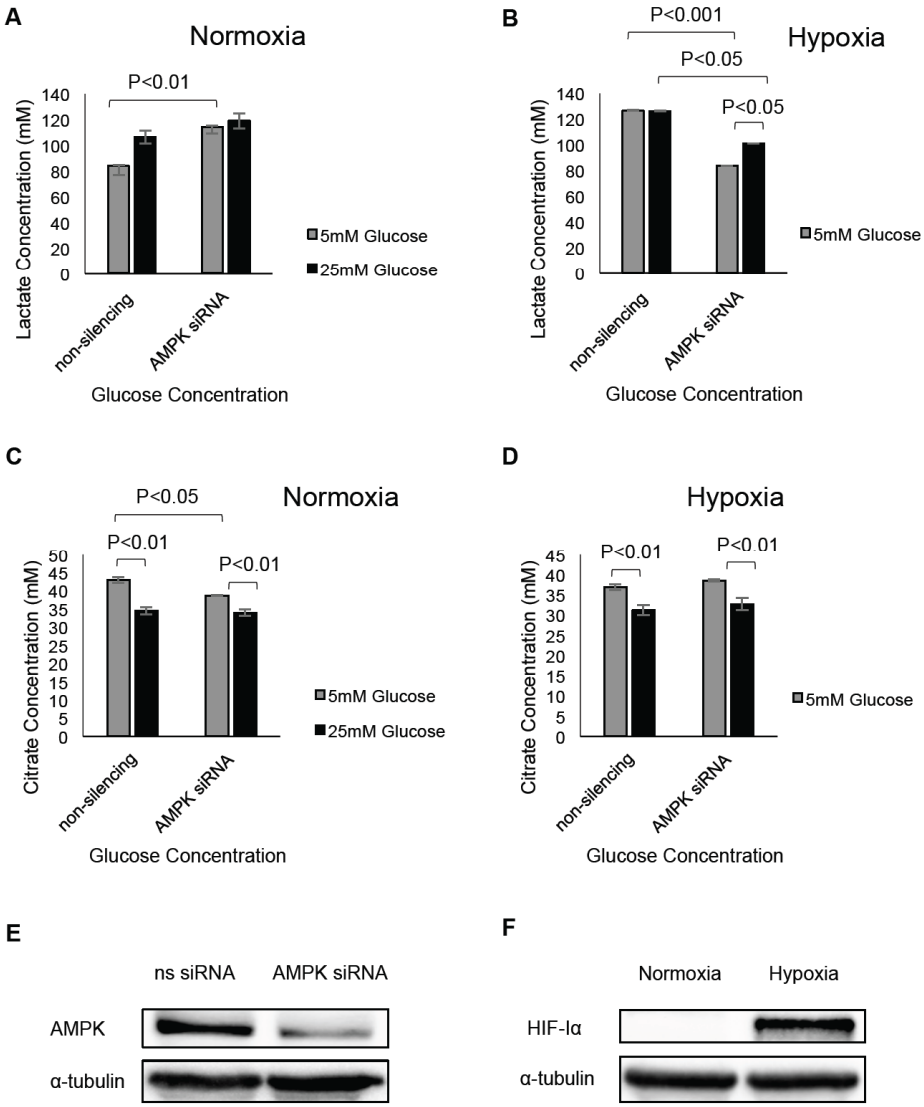

Figure 4: The role of AMPK on glucose metabolism in normoxia and hypoxia.

(**A+B**) MCF-7 cells were seeded at $0.1x10^6$ cells and were transfected with AMPK siRNA to silence AMPK expression. The cells were then incubated in 5mM and 25mM glucose under normal oxygen levels (**A**) and hypoxic conditions (**B**) for 48h. Lactate concentrations were measured using a lactate assay. Hypoxia was chemically induced by 500μM $CoCl_2$. (**C+D**) MCF-7 cells were seeded at $0.1x10^6$ cells and were transfected with AMPK siRNA to silence AMPK expression. The cells were then incubated in 5mM and 25mM glucose under normal oxygen levels (**C**) and hypoxic conditions (**D**) for 48h. Citrate concentrations were measured using a citrate assay. (**E**) Western immunoblotting illustrating efficient knock-down of AMPK using siRNA in MCF-7 cells. (**F**) Western immunoblotting illustrating successful induction of hypoxia represented by increased expression of HIF-Iα. The graphs represent the mean ± SEM of three independent repeats, each conducted in triplicate.

oxygenation further inhibited the ability of the cells to respond to chemotherapy and that this chemoresistance was associated with a further increase in FASN and IGFBP-2 abundance.

These data are consistent with the results from studies in prostate cancer where a high-fat diet group of mice exhibited higher expression of FASN, enhanced phosphorylation of MAPK in addition to inactivation of AMPK signalling. This promoted tumour growth and survival. Inhibition of FASN reduced cancer proliferation through down-regulation of PI3K/MAPK signalling and activation of the AMPK pathway. Enhanced activation of AMPK by the pharmacological AMPK activator, 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) down-regulated the expression of FASN and phosphorylation of PI3K/MAPK signalling. Moreover, overexpression of FASN was clinically associated with high Gleason scores and late pathological T stage [15].

Regardless of oxygen availability, cancer cells mostly rely on glycolysis that takes place in the cytosol to generate energy over oxidative phosphorylation in

the mitochondria in a phenomenon known as the Warburg effect [16]. Although glycolysis yields less ATP molecules than oxidative phosphorylation, increased glycolysis provides cancer cells with many advantages for growth and survival [17]. For example, high levels of glycolysis are not only required for ATP production but also is essential for providing intermediate substrates for anabolic processes such as glycogen, amino acids, nucleic acids, and lipids biosynthesis [17, 18]. In addition, increased glycolysis levels provide the cells with a robust mitochondrial membrane owing to upregulation of hexokinase I and II that bind to the outer mitochondrial membrane. This is particularly critical in protecting cancer cells from cytochrome C that could otherwise leak into the cytosol leading to subsequent activation of caspases. This gives cancer cells robustness and resistance to apoptosis induced by intrinsic apoptotic pathways. Moreover, elevated lactic acid secretion forms an acidic milieu, which provides cancer cells with invasive capability [8]. In normoxic conditions we found that in high, compared with lower glucose conditions the cells increased their production of lactate and reduced that of citrate. Accumulating evidence suggests that cancer cells are able to utilize both glycolysis and oxidative phosphorylation, depending on the cellular context and that AMPK and HIF-I are the master regulators of these processes [19]. In our models of hypoxia, HIF-I, was stabilized and this correlated with the increased glycolytic activity of the cells characterised by increased lactate production, that is known to further stabilize HIF-I, thus forming self-enforcing feedback loops of HIF-I activity [20]. A recent study used computational modelling to demonstrate the metabolic plasticity that exists in cancer cells and showed that cancer cells are able to use three stable steady states, (HIF-I^{high}/pAMPK^{low}), (HIF-I^{low}/pAMPK^{high}), and (HIF-I^{high}/pAMPK^{high}) corresponding to a glycolysis phenotype, an oxidative phosphorylation phenotype, and a hybrid metabolic phenotype, in which cancer cells use both processes [21]. Our data highlighted the plasticity of the metabolic processes under different conditions. Under normoxic conditions, we found that silencing AMPK promoted a more glycogenic phenotype, increasing levels of lactate, consistent with AMPK being a negative regulator of aerobic glycolysis [22]. This increase in lactate correlated with enhanced chemoresistance consistent with reports that lactate induces resistance to chemotherapy and promotes metastasis in breast cancer [23].

Hypoxia alone induced a glycogenic phenotype by stabilising HIF-I. However, on silencing AMPK, levels of lactate were reduced comparable to the normoxic profile of lactate levels, despite the additional resistance to chemotherapy that was observed. Perhaps indicating another metabolic fuel other than lactate was mediating the chemoresistance.

Presumably silencing AMPK shifts the balance between HIF-I and AMPK and whilst lactate and citrate levels were both altered in response to glucose, as anticipated only lactate levels were significantly affected by silencing AMPK.

Controlling levels of glucose in cancer patients who suffer from type 2 diabetes has proven to be beneficial. There is evidence that effective control of hyperglycaemia during chemotherapy can improve tumor-toxicity and outcome of treatments in patients with haematological and solid tumours [24]. Accumulating epidemiological and laboratory studies have reported that the use of antidiabetic therapies, such as metformin, may improve responsiveness of cancer cells, cancer recurrence prevention and enhanced effectiveness to chemotherapy [25, 26, 27, 28, 29, 30, 31].

Metformin can act on cancers in many different ways: the two major pathways are the IGF pathway where metformin reduces the levels of circulating IGF-1 in the blood therefore deactivating PI3K/AKT/mTOR signaling pathway, which in turn inhibits tumour proliferation [32]. The second pathway is the AMPK pathway in which metformin directly acts on tumour cells causing an increase in AMPK levels and inhibition of downstream mTOR [32].

CONCLUSION

AMPK plays an important role in regulating metabolic signalling and this alters the sensitivity of breast cancer cells to chemotherapy. Our data highlights that chemosensitivity is affected by the plasticity that exists within energy metabolism in cancer cells in relation to altered availability of glucose and oxygenation. The data in this paper suggest that perhaps a targeting strategy that impacts on both glycolysis and oxidative phosphorylation may be the best way forward.

AUTHOR CONTRIBUTIONS

The research experiments were performed by AMS. Al-Qahtani. The manuscript was written by AMS. Al-Qahtani and was revised by CM. Perks and JMP. Holly. CM. Perks and JMP. Holly supervised the study.

CONFLICTS OF INTEREST

The authors declare there are no conflicts of interest related to this work.

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ABBREVIATIONS

AMPK = 5' Adenosine monophosphate-activated protein kinase

CPT1 = Carnitine palmitoyltransferase 1

IGF = Insulin-like growth factor

$CoCl_2$ = cobalt chloride

FASN = fatty acid synthase

HIF-I = hypoxia-inducible factor-I

IGFBP-2 = insulin-like growth factor binding protein-2

HIGHLIGHTS

- Increased expression of AMPK negates the protective effect of hyperglycaemia-induced chemo-resistance in breast cancer cells.
- AMPK is involved in FASN and IGFBP-2 upregulation in breast cancer cells.
- AMPK plays a role in glucose metabolism in breast cancer cells.

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