Malek Z, Heba A K, Layali Y, Ahmad S, Farah T, Luai H, Hamzah H, Zaid A, Ahmad R A. The gene expression alterations in chronic hypoxic PANC-1 pancreatic cancer cell line. Pharmacy Practice. 2025 Oct-Dec;23(4):3238.
https://doi.org/10.18549/PharmPract.2025.4.3238

Original Research

The gene expression alterations in chronic hypoxic PANC-1 pancreatic cancer cell line

Malek Zihlif, Heba A Khader, Layali Younis, Ahmad Sharab, Farah Tahboub, Luai Hasoun, Hamzah Hajaj, Zaid Alawneh, Ahmad R. Alsayed

Received (first version): 18-March-2025, Accepted: 14- May-2025, Published online: 07-Nov-2025

Abstract

Aim: Cancer cells divide excessively, resulting in overpopulation and hypoxia. Tumor hypoxia drives tumor development and therapeutic resistance. Hypoxia dominates pancreatic tumor microenvironments. This study aimed to ascertain alterations in gene expression linked to chronic hypoxia in the pancreatic cancer cell (PANC-1) line. **Methods:** PANC-1 had eight-hour hypoxic events with oxygen levels below 1%. 40 episodes were exposed three times a week. Real-time PCR arrays were used to analyze gene expression changes. This investigation compared cells treated with 20 and 40 hypoxia episodes to normoxic cells. MTT cell proliferation assays assessed hypoxic cell doxorubicin resistance. Wound-healing assays measured cell migration. 20 and 40 hypoxia exposures altered gene expression patterns significantly. **Results:** No alterations were observed in either stage, as most genes exhibited a resurgence after 40 episodes. Following exposure to 20 episodes of hypoxia, the expression levels of the genes HIF1AN, HMOX-1, and PKM were significantly increased by factors of 6.9, 4.5, and 3.4, respectively. The IC50 value of Doxorubicin in PANC-1 cells exhibited a 2.7-fold increase and an approximately 3.6-fold increase after 20 and 40 episodes, respectively, compared to normoxic cells. This study demonstrates that subjecting cells to extended durations of hypoxia results in distinct alterations in gene expression compared to those induced by short-term hypoxia, specifically lasting less than 72 hours. Furthermore, it sanctioned the facilitation of chemotherapy resistance through prolonged exposure. **Conclusions:** The study suggests that HIF1AN, HMOX-1, and PKM may serve as promising biomarkers for hypoxia in pancreatic cancer and contributors to the cellular response to prolonged hypoxia.

Keywords: Cancer, Cell Line, Gene Expression, Hyoxia, PANC-1

INTRODUCTION

Pancreatic cancer is presently regarded as one of the most difficult forms of human malignancies. It continues to pose a significant unresolved public health challenge in the early years of the 21st century. Despite substantial reductions in cancer-related mortality in recent years, the prognosis for pancreatic cancer has remained persistently unfavorable[1]. Pancreatic ductal adenocarcinoma (PDAC) constitutes the predominant form of pancreatic cancer, comprising approximately 85-90% of all pancreatic tumor cases[2].

The PDAC is classified as the fourth most prevalent cause of mortality associated with cancer in the United States[2]. This malignancy is recognized as one of the most lethal among human diseases, exhibiting a mortality rate approaching nearly 100% within a short time[2]. It is one of the deadliest human malignancies, with a high mortality rate approaching 100%[3]. Pancreatic cancer is one of top ten leading cause of cancer death among Jordanian, accounting for 6.2 % of cancer deaths in 2012[4].

Currently, surgery is the only curative treatment for PDAC. Nevertheless, only a minority of PDAC patients are considered surgical candidates due to late-stage diagnosis common with this disease[5]. Chemotherapy is a standard treatment option in managing all stages of pancreatic cancer. In 1997, Burris et al. conducted a study to evaluate the efficacy of gemcitabine as the primary treatment option for patients with advanced, unresectable pancreatic cancer[6]. The use of gemcitabine (Gemzar®) or the combinations of gemcitabine with other cancer drugs, such as cisplatin, or erlotinib, has confirmed efficacy in treating pancreatic cancer[7]. The potential role of radiotherapy as a component of adjuvant therapy in the postoperative regimen remains debatable[8]. Pancreatic cancer is well recognized as a very challenging disease on many fronts. It is one of the major aggressive human malignancies, clinically marked by local invasion, early metastasis, and resistance to

Malek Zihlif*. Department of Pharmacology, School of Medicine, The University of Jordan, Amman 11942, Jordan.
Heba A Khader. Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmaceutical Sciences, The Hashemite University, Zarqa, Jordan.
Layali Younis. Department of Pharmacology, School of Medicine, The University of Jordan, Amman 11942, Jordan.
Ahmad Sharab. Department of Pharmacology, School of Medicine, The University of Jordan, Amman 11942, Jordan.
Farah Tahboub. Department of Pharmacology, School of Medicine, The University of Jordan, Amman 11942, Jordan.
Luai Hasoun. Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, Applied Science Private University (ASU) Amman, Jordan.
Hamzah Hajaj. Department of Pathology, Microbiology, and Forensic Medicine, School of Medicine, The University of Jordan.
Zaid Alawneh. Department of Pathology, Microbiology, and Forensic Medicine, School of Medicine, The University of Jordan.
Ahmad R. Alsayed. Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, Applied Science Private University (ASU) Amman, Jordan. a_alsayed@asu.edu.jo

www.pharmacypractice.org (eISSN: 1886-3655 ISSN: 1885-642X)

standard chemotherapy[1]. Hypoxia is a characteristic feature of solid tumors and define as a reduction in the normal level of tissue oxygen tension[9]. Cells go through genetic and adaptive alterations, which permit such cells to survive and proliferate in a hypoxic environment. Tumor hypoxia has been recognized as a potential therapeutic problem because it causes resistance to chemo- and radiotherapy and promotes a more aggressive phenotype, increasing mutation rates, invasion, and metastasis[10-14].

Genetic changes in the aforementioned observations have not yet been completely agreed [15]. Therefore, analyzing the modulated genes induced by hypoxia in cancer is greatly beneficial [16]. This study was designed to simulate actual hypoxic conditions in order to investigate the alterations in gene expression that occur in pancreatic cancer cells during prolonged exposure to hypoxia. The primary objective of this study is to elucidate novel biomarkers associated with tumor hypoxia. Our objective is to investigate the correlation between hypoxia and chemo-resistance and identify potential underlying mechanisms for this phenomenon.

MATERIALS AND METHODS

Cell culture

The Panc-1 human pancreatic cancer cell lines were purchased from the American Type Culture Collection (ATCC; USA), The Panc-1 cells were kept as an attached monolayer culture in the DMEM High Glucose (EuroClone,Via Figino, Italy), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Biowest, South America), 2 mM L-glutamine, and 100 U/mL and 100 µg/mL penicillin– streptomycin (EuroClone, Italy). The cells were grown on 75 cm2 attached types, filter-cap culture flasks (Membrane Solutions, USA). Exposure to hypoxia PANC-1 cells were exposed to an 8 hours of hypoxia (0.1% oxygen), three times a week for a total of 40 hypoxic episodes (continuous hypoxic PANC-1 cells). The experimental setup involved utilizing the AnaeroGen Compact system (Oxoid, UK), designed to generate an anaerobic atmosphere. This system was employed to create hypoxic conditions in the experimental environment[17]. The vented culture flasks were positioned within the plastic bags, while the paper sachets were opened and placed inside the bag. Subsequently, the bag was sealed using the plastic zip closure.

Throughout a five-month duration. In addition to administering hypoxic shots, the cells were subjected to incubation alongside their normoxic counterparts under normoxic conditions. The normoxia-exposed PANC-1 cells served as the control group.

Cell proliferation assay

7000 cells were seeded into coated 96-well plate (Greiner, Germany). Each of the hypoxic and normoxic cell line was seeded in triplicates in plain medium for at least 18 h. Then the media were aspirated from the wells. Then, gradually decreasing concentrations between 0.01 µM and 500 µM of Doxorubicin were added in fresh media. The rest of assay was done according to[14]. The MTT proliferation assay was done after the 20th, and 40th for hypoxic shots. As well, the MTT proliferation assay was done for the control PANC-1 cells which incubated under normoxic condition.

Wound Healing Experiment

A total of 50,000 cells were seeded in each well of a 6-well plate, originating from three different sources: control Panc-1 cells, Panc-1 cells after 20 hypoxic shots, and Panc-1 cells after 40 hypoxic shots. The cells were cultured until they reached confluence. A simulated wound was generated by a linear abrasion using a yellow pipette tip. To limit the width of the scratch, a deliberate angle of approximately 30 degrees was maintained while manipulating the pipette tip. Subsequently, the media underwent a process of alteration and cleansing utilizing a phosphate buffer solution in order to eliminate the deceased cellular entities. The experiment was conducted in a hypoxic environment with a 1% oxygen concentration. Photographs were captured at three distinct time intervals: 0 hours, 24 hours, and 48 hours.

RNA isolation and Real-time PCR

The RNA was extracted utilizing the RNeasy® Mini kit (Qiagen, Germany) in accordance with the instructions provided by the manufacturer.

The impact of hypoxia on the gene expression of the PANC-1 pancreatic cancer cell line was investigated utilizing a 96-well PCR array, specifically the RT2 Profiler PCR array (PAHS-032Z, Human Hypoxia Signaling Pathway PCR Array, Qiagen, USA). The array consists of 96-well plates containing a selection of primers for 84 genes identified as responsive to low oxygen levels, along with an additional set of 12 genes used for quality control purposes. The cDNA resulting from the First Strand Kit® reaction was mixed with RT2 SYBR® green master mix (Qiagen, USA) and nuclease free water (Biobasic, USA). Then, 20µl of the mix was placed in every well and the plate was subsequently centrifuged (Hettich, Germany) at 1000g for 1 minute to remove any air bubbles that could affect the results. CFX (Bio-Rad, USA) thermo-cycler performed the Real-time PCR; the program consisted of 95 °C for 10 min, followed by 40 cycles of 95 °C for 15.

RESULTS

Effect of hypoxia on PANC-1 cell line resistance to Doxorubicin

The investigation was conducted to verify the emergence of the hypoxic phenotype by assessing the resistance to Doxorubicin, a frequently employed chemotherapeutic agent. The IC50, which represents the concentration at which half of the maximal inhibitory effect is observed, was determined using the MTT cell proliferation assay, as described in the Materials and Methods section. The IC50 value exhibited a 2.7-fold increase following 20 instances of hypoxic episodes in comparison to the normoxic cells. Following a series of 40 hypoxic episodes, the cells exhibited an approximately 3.6-fold increase in their IC50 values when compared to the normoxic cells. Table 1 displays the IC50 values of the cells measured at different time points.

Table 1. Half maximal inhibitory concentrations (IC50) of Doxorubicin in hypoxic and normoxic cells measured at different stages of the experiment.

Cell Lines	Control Cells (H0) IC50 in nM(STD)	Hypoxic Cells(H--) IC50 in nM (STD)	Hypoxic Cells IC50/ Control Cells IC50 (fold)
H20/comparable age H0	344 (± 0.034)	934(± 0.014)	2.7 (INCREASE)
H40/comparable age H0	369 (±0.011)	1244(± 0.07)	3.6 (INCREASE)

Gene Expression Changes in H20-PANC-1 Cancer Cell Line

The changes in the gene expression of PANC-1 cells that were induced by hypoxia could be potentially used as biomarkers for hypoxia in pancreatic cells. RT-PCR was performed using a hypoxia-signaling pathway PCR array. An arbitrary cut-off point of 2 folds was chosen so that the changes identified would be stubstantial. When comparing cells exposed to 20 episodes of 8-hour hypoxia and similar passage-age normoxic cells, it was observed that three genes exhibited significant down-regulation, as indicated in Table 2. Conversely, eleven genes displayed significant up-regulation, as demonstrated in Table 3.

The alterations in gene expression in PANC-1 cells, which were subjected to 40 episodes of 8-hour hypoxia, were assessed using **PCR**. After conducting a comparison between the H40-PANC-1 pancreatic cancer cells and the H0-PANC-1 cells, it was observed that five genes exhibited down-regulation, while three genes showed up-regulation. This information is presented in Table 4 and Table 5, respectively.

Table 2. Genes Profoundly down-regulated in H20-PANC-1 compared to H0 -PANC-1 cells.

Gene Symbol	Gene Description	Fold Regulation	Gene Function (Qiagen,USA)
EGLN1	Egl-9 family hypoxia inducible factor 1	-6.0349	Angiogenesis, regulation of cell proliferation
VDAC1	Voltage dependent anion channel 1	-5.0159	Metabolism, regulation of apoptosis
NFKB 1	Nuclear factor kappa B subunit 1	-2.1628	Regulation of apoptosis, regulation of cell proliferation

Wound healing results

The utilization of the in vitro wound healing assay to monitor the migratory behavior of PANC-1 cells at the forefront of the scratch is exemplified in Figure 1. The photographs taken at the onset and conclusion of a 48-hour incubation period exhibited notable cellular migration towards the scratch. The photographs captured at the initial time point of 0 hours can be juxtaposed with the concluding photographs taken at 48 hours to ascertain the extent of closure for each individual scratch. The analysis of the images revealed that the control PANC-1 cells (located on the left side) and H40 PANC-1 cells (located on the right side) exhibited a slower migration rate in comparison to the H20 PANC-1 cells, where the scratch was nearly closed after 48 hours.

DISCUSSION

PANC-1 pancreatic cancer cells were subjected to recurring episodes of hypoxia lasting 8 hours, repeated three times per week, over a period of up to six months. Gene expression profiling was conducted at two-time points during the study: midway through the experiment and at its conclusion. The findings suggest that hypoxia is a significant factor in the emergence of a chemo-resistant phenotype in the PANC-1 pancreatic cancer cell line. It is evident that there are notable disparities in gene expression profiling between cells that were subjected to 20 episodes of hypoxia. We have achieved the enhancement of chemo-resistance to Doxorubicin through the induction of hypoxia in PANC-1 cells for a total of 20 episodes,

Table 3. Genes profoundly up regulated in H20-PANC1 compared to H0-PANC1 cells.

Gene Symbol	Gene Description	Fold Regulation	Gene Function (Qiagen,USA)
BTG1	B cell translocation gene 1 anti-proliferative	9.8383	Regulation of apoptosis, regulation of cell proliferation, angiogenesis.
HIF1AN	Hypoxia inducible factor 1 alpha subunit inhibitor	6.9431	HIF 1 & co-transcription factors
TP53	Tumor protein P53	6.6093	Regulation of apoptosis
HMOX1	Heme oxygenase (decycling) 1	4.529	Angiogenesis, apoptosis, metastasis
GPI	Glucose - 6- phosphate isomerase	3.9178	regulation of cell proliferation, regulation of apoptosis , metabolism
PKM	Pyruvate kinase, muscle	3.4092	Metabolism, apoptosis
SLC16A3	Solute carrier family 16 member 3	2.8492	Transporter, metabolism
ADORA2B	Adenosine A2b receptor	2.3495	Angiogenesis
TFRC	Transferrin receptor	2.1186	Regulation of cell proliferation
BHLHE40	Basic helix-loop-helix family member e40	2.0144	Cell differentiation, proliferation, apoptosis, and metabolism.
NCOA1	Nuclear receptor coactivator 1	2.0017	receptors, regulation of cell proliferation

Gene expression changes in H40-PANC-1 pancreatic cancer cell line

Table 4. Genes profoundly down-regulated in H40-PANC-1 cells.

Gene Symbol	Gene Description	Fold Regulation	Gene Function (Qiagen,USA)
ODC1	Ornithine decarboxylase	-2.5154	Regulation of cell proliferation
HIF3A	Hypoxia inducible factor 3 alpha subunit	-2.4014	HIF1 & Co-transcription factors
COPS5	COP9 signalosome subunit 5	-2.198	HIF1 & Co-transcription factors, regulation of cell proliferation
PDK1	Pyruvate dehydrogenase kinase 1	-2.1379	Regulation of cell proliferation
EGR1	Early growth response 1	-2.0106	Angiogenesis, regulation of cell proliferation

Table 5. Genes profoundly up-regulated in H40-PANC1 compared to H0-PANC1 cells.

Gene Symbol	Gene Description	Fold Regulation	Gene Function (Qiagen,USA)
MMP9	Matrix metallopeptidase 9	2.3345	Angiogenesis, wound healing, metastasis
EGLN1	EGL-9 family hypoxia inducible factor 1	2.0233	HIF1 & cell proliferation
ADM	Adrenomedullin	2.012	Regulation of apoptosis, regulation of cell proliferation

each lasting 8 hours. This observation was substantiated by a significant elevation in the IC50 values in comparison to the normoxic cells, exhibiting an approximate 2.7-fold increase. Our results are in agreement with[18] who reported that hypoxia induces chemo-resistance. Nevertheless, until this report is written, it is the first study to illustrate generation of chemo-resistance to Doxorubicin in PANC-1 pancreatic cancer cell line through chronic hypoxia.

One of the hypothesized mechanisms by which hypoxia prompts chemo- resistance is by decreasing in the formation of damaging ROS by Doxorubicin, which is decreased because of lacking oxygen[19]. As the MTT cell proliferation assay was done under normoxia, this theory is unlikely to contribute to our results. Another suggested theory relies on the rapid proliferation rate of cancer cells[20]. The theory in question is not applicable to our study, as the rate of cell proliferation in our sample has been found to be equivalent to that of normoxic cells of a similar age. This suggests that the genomic and proteomic characteristics of the cell are responsible for chemo-resistance development.

The primary finding of our study pertains to the up-regulation of the B cell translocation gene 1 anti-proliferative (BTG1) and Tumor protein P53 (TP53) in H20-PANC-1, with fold changes of 9.8383 and 6.6093, respectively, compared to H0-PANC-1. The induction of BTG1 by hypoxia and its observed promotion of apoptosis suggest that it is unlikely to play a role in chemo-resistance induction.

TP53 is one of the most prominent tumor suppressor genes[21], TP53 is inactivated by mutation in almost 50% of human cancer[22], including PDAC (50– 75%)[23]. TP53 is known to be induced by hypoxia[24]. The up-regulation of P53 in our results has been confirmed by[25] who found that P53 protein accumulates in cells exposed to hypoxia, this accumulation increases with the duration of the hypoxia incubation along with the decrease in the pO2 level[25].

Mutant p53 proteins interfere with regulation of cell survival, DNA damage repair and drug resistance[26]. Our results can also concurred with the work of [27] who have found that mutant p53 stimulates chemo-resistance of pancreatic adenocarcinoma cells to the standard drug gemcitabine. Heme oxygenase (decycling) 1 (HMOX1) was found to be up-regulated by 4.529 folds in H20 PANC-1 compared to H0 PANC-1, HMOX1 is involved in preventing chemo toxic-induced apoptosis, this concur with the findings of[28] who have indicated that HMOX-1 is involved in conferring the chemo-resistance to Doxorubicin in breast cancer cells by preventing apoptosis and autophagy. Another prominent gene in our study that is associated with apoptosis is Pyruvate kinase, muscle (PKM), which was up-regulated by 3.4092 folds in H20-PANC-1 compared to H0-PANC-1. Increased PKM levels have been associated with resistance to 5-fluorouracil in patients with colorectal cancer[29]. Also, genetic silencing of PKM potentiates the effects of docetaxel and cisplatin in in vitro and lung cancer xenograft models[30]. A recent study observed that a significant proportion (68%) of human pancreatic adenocarcinoma specimens exhibited a robust expression of PKM2. Additionally, nearly all of the analyzed pancreatic cancer cell lines also demonstrated a high level of PKM expression. Furthermore, the detection of PKM was found to have detrimental effects on cellular proliferation and enhanced apoptosis when examined in vitro[31]. Accordingly, we can conclude that HMOX-1 and PKM play an important role in defending PANC-1 cells from hypoxia- and chemotoxic-induced apoptosis.

In addition, there is another gene that is associated with apoptosis and notably down- regulated by -5.0159 folds in H20 compared with H0 is Voltage dependent anion channel 1(VDAC1), VDAC1 is anchor for pro- and anti-apoptotic proteins, respectively, of the hexokinase (HK)[32] and Bcl-2 families of proteins, which contribute to the balance between survival and cell death [33] found that hypoxia induces the appearance of a C-terminal truncated form of VDAC1,so our results is contrast with his finding as VDAC-1 was down –regulated. It has been confirmed that mitochondrial hexokinase I and II (HKI/II) function as anti-apoptotic proteins when bound to VDAC1, but their detachment enabled activation of apoptosis[34]. While VDAC1 was down-regulated by hypoxia, so most probably VDAC1 is not a player in chemo-resistance induction. Another prominent gene in our study is the Egl-9 family hypoxia inducible factor

1 (EGLN1), which is the most significantly down-regulated gene in H20-PANC-1 compared to H0-PANC-1 with -6.0349 folds, Prolyl hydroxylase 2 (PHD2) is an enzyme encoded by the EGLN1 gene and is considered the key oxygen sensor-regulating hypoxia-inducible factor (HIF)[35]. Another important gene that acts as cellular oxygen sensors, directly regulating the transcriptional complex HIF-1 activity that mediates adaptive responses to reduced oxygen level is hypoxia-inducible factor 1 alpha subunit inhibitor (HIF1AN)[36]. The HIF1AN mRNA has been one of the most obviously up-regulated genes in H20-PANC-1 compared to H0- PANC-1 with 6.9431 folds. FIH-1 which HIF1AN encodes. PHDs and FIH-1 are parts of the same superfamily of dioxygenases and both need the same set of cofactors, oxygen, 2-oxoglutarate and ferrous iron, but striking differences exist between these enzymes. FIH-1 posses a higher affinity to the molecular oxygen in comparison to the PHDs, which recommends that in mild hypoxia FIH-1 remains active while the PHDs are inactive[37]. Our results in agreement with[38] who reported that FIH-1 is capable to control the HIF-1 even under severe hypoxic conditions, when PHD enzymes fail to do so.

One of the surprising results of our study was the up-regulation of Matrix metallopeptidase 9 (MMP9), in H40 PANC-1 compared to H0-PANC-1 by 2.3345 folds while it was barely changed at the level of H20-PANC-1. These results against the expectation as the main function of MMP9 involved in metastasis and wound healing[39,40]. It was expected MMP9 to be up regulated in H20 as the wound closed at faster rate compared to H0 and to be down regulated in H40 when the wound migrated at slower rate compared to H0. These results agree with the huge body of evidence reporting that HMOX-1 is frequently up regulated in tumor tissues and promotes tumor growth and metastasis[41-44] have showed that HMOX1 as well promotes proliferation in pancreatic cancer cell lines. HMOX1 is induced by hypoxia[44,45]. GPI is an autocrine motility factor (AMF), and is also critical for migration, invasion and metastasis of tumor cells[46]. As mentioned in the literature review that the conversion of epithelial cells to more mesenchymal-like cells enabled cell migration, invasion, and metastasis. It has been demonstrated that GPI/AMF suppressed epithelial marker expression and improved mesenchymal marker expression[47]. GPI/AMF is up-regulated by hypoxia and is controlled by HIF-1[48]. As a result, we can suggest that the up regulation of HMOX1 and GPI in our results is consistent with the fact that hypoxia renders cancer cells more progressive and induces metastasis. Among our results Adenosine A2b receptor (ADORA2B) gene is up-regulated by 2.3495 folds in H20-PANC-1 compared to HO-PANC-1. Adenosine itself is a unanimously expressed purine nucleoside that is well-known to have the ability to bridge the gap between metabolic supply and demand[49]. It acts through four G coupled receptors: A1, A2A, A2B and A3 with A2B having the highest threshold for activation[50]. We have thoroughly discussed the role of ADORA2B in literature review under angiogenesis. ADORA2B is known to be induced in stressful conditions including hypoxia[51].

Stimulation of ADORA2B receptors in cancer cell lines up-regulates the production of angiogenic factors, indicating that tumor A2B receptors may promote revascularization[52-54]. It makes sense for ADORA2B to be up- regulated; not only to empower adenosine, but also to facilitate immune system evasion[55]. Activation of host ADORA2B receptors have been viewed as an inhibitor of solid tumors rejection and plays an essential role in promoting metastasis[56].

One of the key metabolic features of cancer cells is rapid utilization of glucose to fuel glycolysis. The metabolic reactions of glycolysis are catalyzed by numerous enzymes, one of the enzymes is PKM which is implicated in cancer[57]. In our study we have found that PKM is up regulated in H20 PANC-1 compared to H0 PANC-1 by 3.4 folds. The rationale behind the up-regulation of PKM is to meet the increased of glucose demand due to hypoxia-induced glycolysis. PKM dimer can translocate to the nucleus, where it will interact with HIF-1a and regulate expression of numerous proglycolytic enzymes[58]. PKM plays a crucial role in aerobic glycolysis[59]. Thus PKM drives the aerobic glycolysis in generating the energy needed for cellular processes

in tumors, a phenomenon termed “the Warburg effect”[60]. Our results agree with[61], who found that the mRNA levels of PKM was elevated with hypoxic treatment in numerous mouse and human hepatoma cell lines, although HIF-1 mRNA levels were not significantly elevated. In addition, our results found that Solute carrier family 16 member 3 (SLC16A3 also called MCT4), is up-regulated by 2.8 folds in H20-PANC-1. This up-regulation has been reported by[62] who found that SLC16A3 was up-regulated by hypoxia in bladder cancer. The up regulation of MCT proteins is noticed in tumor tissue and supports an apparent need for lactate shuttling to either fuel tumor growth or permit survival under stress conditions[63]. Our findings agree with[64] who found that SLC16A3 in general is elevated in Pancreatic ductal adenocarcinoma (PDAC), but the overexpression of Solute Carrier Family 16 Member 1 (SlC16A1) in PDAC was little versus other cancers[64]. Notable differences in genes were measured in cells exposed to 40 episodes of hypoxia such as Ornithine decarboxylase (ODC) gene. ODC gene is one of the most down-regulated gene in H40 cells compared to H0 cells by -2.5 folds. ODC1 is the first rate-limiting enzyme of polyamine biosynthesis, is found to be associated with cell growth, proliferation and transformation[65].

CONCLUSION

We also suggest that this reversal could be owed to the fact that other effectors take over when a stressful situation goes on beyond a specific point. Unfortunately, no other studies have exposed PANC-1cells for a similar period for comparison purposes. However, the precise determination of a mechanism behind chemo-resistance is beyond the scope of this report. Chemo-resistance was used as an end-point for transformation in light of absence of proliferation rate and morphological changes. Further research is necessary to investigate protein levels in order to uncover the currently elusive mechanism of hypoxia-induced chemotherapy resistance and identify a target for reversing this significant barrier in cancer treatment.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. Jul-Aug 2009;59(4):225-49. doi:10.3322/caac.20006
2. Siegel R, Naishadham D, Jemal A. Cancer statistics for Hispanics/Latinos, 2012. CA Cancer J Clin. Sep-Oct 2012;62(5):283-98. doi:10.3322/caac.21153
3. Lowenfels AB, Maisonneuve P. Risk factors for pancreatic cancer. J Cell Biochem. Jul 1 2005;95(4):649-56. doi:10.1002/jcb.20461
4. Registry JC. Cancer Incidence in Jordan Report. 2012;
5. Shaib YH, Davila JA, El-Serag HB. The epidemiology of pancreatic cancer in the United States: changes below the surface. Aliment Pharmacol Ther. Jul 1 2006;24(1):87-94. doi:10.1111/j.1365-2036.2006.02961.x
6. Burris HA, 3rd, Moore MJ, Andersen J, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol. Jun 1997;15(6):2403-13. doi:10.1200/jco.1997.15.6.2403
7. Yang ZY, Yuan JQ, Di MY, et al. Gemcitabine plus erlotinib for advanced pancreatic cancer: a systematic review with meta-analysis. PLoS One. 2013;8(3):e57528. doi:10.1371/journal.pone.0057528
8. Hazard L. The role of radiation therapy in pancreas cancer. Gastrointest Cancer Res. Jan 2009;3(1):20-8.
9. Vaupel P, Thews O, Hoeckel M. Treatment resistance of solid tumors: role of hypoxia and anemia. Med Oncol. 2001;18(4):243-59. doi:10.1385/mo:18:4:243
10. Vaupel P, Harrison L. Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. Oncologist. 2004;9 Suppl 5:4-9. doi:10.1634/theoncologist.9-90005-4
11. Yuan J, Glazer PM. Mutagenesis induced by the tumor microenvironment. Mutat Res. May 25 1998;400(1-2):439-46. doi:10.1016/s0027-5107(98)00042-6
12. Harris AL. Hypoxia--a key regulatory factor in tumour growth. Nat Rev Cancer. Jan 2002;2(1):38-47. doi:10.1038/nrc704
13. Rofstad EK. Microenvironment-induced cancer metastasis. Int J Radiat Biol. May 2000;76(5):589-605. doi:10.1080/095530000138259
14. Subarsky P, Hill RP. The hypoxic tumour microenvironment and metastatic progression. Clin Exp Metastasis. 2003;20(3):237-50. doi:10.1023/a:1022939318102
15. Alqawi O, Wang HP, Espiritu M, Singh G. Chronic hypoxia promotes an aggressive phenotype in rat prostate cancer cells. Free Radic Res. Jul 2007;41(7):788-97. doi:10.1080/10715760701361531
16. Bando H, Toi M, Kitada K, Koike M. Genes commonly upregulated by hypoxia in human breast cancer cells MCF-7 and MDA-MB-231. Biomed Pharmacother. Oct 2003;57(8):333-40. doi:10.1016/s0753-3322(03)00098-2
17. Hamdan FH, Zihlif MA. Gene expression alterations in chronic hypoxic MCF7 breast cancer cell line. Genomics. Dec 2014;104(6 Pt B):477-81. doi:10.1016/j.ygeno.2014.10.010
18. Greco O, Marples B, Joiner MC, Scott SD. How to overcome (and exploit) tumor hypoxia for targeted gene therapy. J Cell Physiol. Dec 2003;197(3):312-25. doi:10.1002/jcp.10374
19. Wardman P. Electron transfer and oxidative stress as key factors in the design of drugs selectively active in hypoxia. Curr Med Chem. Jun 2001;8(7):739-61. doi:10.2174/0929867013372959
20. Wouters A, Pauwels B, Lardon F, Vermorken JB. Review: implications of in vitro research on the effect of radiotherapy and chemotherapy under hypoxic conditions. Oncologist. Jun 2007;12(6):690-712. doi:10.1634/theoncologist.12-6-690
21. Lane DP. Cancer. p53, guardian of the genome. Nature. Jul 2 1992;358(6381):15-6. doi:10.1038/358015a0
22. Rivlin N, Brosh R, Oren M, Rotter V. Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis. Genes Cancer. Apr 2011;2(4):466-74. doi:10.1177/1947601911408889
23. Toledo F, Wahl GM. Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. Nat Rev Cancer. Dec 2006;6(12):909-23. doi:10.1038/nrc2012
24. Hammond EM, Giaccia AJ. The role of p53 in hypoxia-induced apoptosis. Biochem Biophys Res Commun. Jun 10 2005;331(3):718-25. doi:10.1016/j.bbrc.2005.03.154
25. Banasiak KJ, Haddad GG. Hypoxia-induced apoptosis: effect of hypoxic severity and role of p53 in neuronal cell death. Brain Res. Jun 29 1998;797(2):295-304. doi:10.1016/s0006-8993(98)00286-8
26. Acin S, Li Z, Mejia O, Roop DR, El-Naggar AK, Caulin C. Gain-of-function mutant p53 but not p53 deletion promotes head and neck cancer progression in response to oncogenic K-ras. J Pathol. Dec 2011;225(4):479-89. doi:10.1002/path.2971
27. Fiorini C, Cordani M, Padroni C, Blandino G, Di Agostino S, Donadelli M. Mutant p53 stimulates chemoresistance of pancreatic adenocarcinoma cells to gemcitabine. Biochim Biophys Acta. Jan 2015;1853(1):89-100. doi:10.1016/j.bbamcr.2014.10.003
28. Zhu XF, Li W, Ma JY, et al. Knockdown of heme oxygenase-1 promotes apoptosis and autophagy and enhances the cytotoxicity of doxorubicin in breast cancer cells. Oncol Lett. Nov 2015;10(5):2974-2980. doi:10.3892/ol.2015.3735
29. Shin YK, Yoo BC, Hong YS, et al. Upregulation of glycolytic enzymes in proteins secreted from human colon cancer cells with

5-fluorouracil resistance. Electrophoresis. Jun 2009;30(12):2182-92. doi:10.1002/elps.200800806
30. Guo W, Zhang Y, Chen T, et al. Efficacy of RNAi targeting of pyruvate kinase M2 combined with cisplatin in a lung cancer model. J Cancer Res Clin Oncol. Jan 2011;137(1):65-72. doi:10.1007/s00432-010-0860-5
31. Azoitei N, Becher A, Steinestel K, et al. PKM2 promotes tumor angiogenesis by regulating HIF-1α through NF-κB activation. Mol Cancer. Jan 6 2016;15:3. doi:10.1186/s12943-015-0490-2
32. Abu-Hamad S, Zaid H, Israelson A, Nahon E, Shoshan-Barmatz V. Hexokinase-I protection against apoptotic cell death is mediated via interaction with the voltage-dependent anion channel-1: mapping the site of binding. J Biol Chem. May 9 2008;283(19):13482-90. doi:10.1074/jbc.M708216200
33. Brahimi-Horn MC, Ben-Hail D, Ilie M, et al. Expression of a truncated active form of VDAC1 in lung cancer associates with hypoxic cell survival and correlates with progression to chemotherapy resistance. Cancer Res. Apr 15 2012;72(8):2140-50. doi:10.1158/0008-5472.Can-11-3940
34. Arzoine L, Zilberberg N, Ben-Romano R, Shoshan-Barmatz V. Voltage-dependent anion channel 1-based peptides interact with hexokinase to prevent its anti-apoptotic activity. J Biol Chem. Feb 6 2009;284(6):3946-55. doi:10.1074/jbc.M803614200
35. Berra E, Ginouvès A, Pouysségur J. The hypoxia-inducible-factor hydroxylases bring fresh air into hypoxia signalling. EMBO Rep. Jan 2006;7(1):41-5. doi:10.1038/sj.embor.7400598
36. Jokilehto T, Jaakkola PM. The role of HIF prolyl hydroxylases in tumour growth. J Cell Mol Med. Apr 2010;14(4):758-70. doi:10.1111/j.1582-4934.2010.01030.x
37. Koivunen P, Hirsilä M, Günzler V, Kivirikko KI, Myllyharju J. Catalytic properties of the asparaginyl hydroxylase (FIH) in the oxygen sensing pathway are distinct from those of its prolyl 4-hydroxylases. J Biol Chem. Mar 12 2004;279(11):9899-904. doi:10.1074/jbc.M312254200
38. Dayan F, Roux D, Brahimi-Horn MC, Pouyssegur J, Mazure NM. The oxygen sensor factor-inhibiting hypoxia-inducible factor-1 controls expression of distinct genes through the bifunctional transcriptional character of hypoxia-inducible factor-1alpha. Cancer Res. Apr 1 2006;66(7):3688-98. doi:10.1158/0008-5472.Can-05-4564
39. Roy R, Yang J, Moses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol. Nov 1 2009;27(31):5287-97. doi:10.1200/jco.2009.23.5556
40. Mohan R, Chintala SK, Jung JC, et al. Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. J Biol Chem. Jan 18 2002;277(3):2065-72. doi:10.1074/jbc.M107611200
41. Doi K, Akaike T, Fujii S, et al. Induction of haem oxygenase-1 nitric oxide and ischaemia in experimental solid tumours and implications for tumour growth. Br J Cancer. Aug 1999;80(12):1945-54. doi:10.1038/sj.bjc.6690624
42. Torisu-Itakura H, Furue M, Kuwano M, Ono M. Co-expression of thymidine phosphorylase and heme oxygenase-1 in macrophages in human malignant vertical growth melanomas. Jpn J Cancer Res. Sep 2000;91(9):906-10. doi:10.1111/j.1349-7006.2000.tb01033.x
43. Hara E, Takahashi K, Tominaga T, et al. Expression of heme oxygenase and inducible nitric oxide synthase mRNA in human brain tumors. Biochem Biophys Res Commun. Jul 5 1996;224(1):153-8. doi:10.1006/bbrc.1996.0999
44. Sunamura M, Duda DG, Ghattas MH, et al. Heme oxygenase-1 accelerates tumor angiogenesis of human pancreatic cancer. Angiogenesis. 2003;6(1):15-24. doi:10.1023/a:1025803600840
45. Panchenko MV, Farber HW, Korn JH. Induction of heme oxygenase-1 by hypoxia and free radicals in human dermal fibroblasts. Am J Physiol Cell Physiol. Jan 2000;278(1):C92-c101. doi:10.1152/ajpcell.2000.278.1.C92
46. Tsutsumi S, Yanagawa T, Shimura T, Kuwano H, Raz A. Autocrine motility factor signaling enhances pancreatic cancer metastasis. Clin Cancer Res. Nov 15 2004;10(22):7775-84. doi:10.1158/1078-0432.Ccr-04-1015
47. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. Jun 2002;2(6):442-54. doi:10.1038/nrc822
48. Funasaka T, Yanagawa T, Hogan V, Raz A. Regulation of phosphoglucose isomerase/autocrine motility factor expression by hypoxia. Faseb j. Sep 2005;19(11):1422-30. doi:10.1096/fj.05-3699com
49. Gessi S, Merighi S, Sacchetto V, Simioni C, Borea PA. Adenosine receptors and cancer. Biochim Biophys Acta. May 2011;1808(5):1400-12. doi:10.1016/j.bbamem.2010.09.020
50. Fredholm BB. Adenosine, an endogenous distress signal, modulates tissue damage and repair. Cell Death Differ. Jul 2007;14(7):1315-23. doi:10.1038/sj.cdd.4402132
51. Aherne CM, Kewley EM, Eltzschig HK. The resurgence of A2B adenosine receptor signaling. Biochim Biophys Acta. May 2011;1808(5):1329-39. doi:10.1016/j.bbamem.2010.05.016
52. Zeng D, Maa T, Wang U, Feoktistov I, Biaggioni I, Belardinelli L. Expression and function of A2B adenosine receptors in the U87MG tumor cells. Drug development research. 2003;58(4):405-411.
53. Feoktistov I, Ryzhov S, Goldstein AE, Biaggioni I. Mast cell-mediated stimulation of angiogenesis: cooperative interaction between A2B and A3 adenosine receptors. Circ Res. Mar 21 2003;92(5):485-92. doi:10.1161/01.Res.0000061572.10929.2d
54. Merighi S, Benini A, Mirandola P, et al. Caffeine inhibits adenosine-induced accumulation of hypoxia-inducible factor-1alpha, vascular endothelial growth factor, and interleukin-8 expression in hypoxic human colon cancer cells. Mol Pharmacol. Aug 2007;72(2):395-406. doi:10.1124/mol.106.032920
55. Lukashev D, Sitkovsky M, Ohta A. From “Hellstrom Paradox” to anti-adenosinergic cancer immunotherapy. Purinergic Signal.

Mar 2007;3(1-2):129-34. doi:10.1007/s11302-006-9044-9
56. Stagg J, Divisekera U, McLaughlin N, et al. Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. Proc Natl Acad Sci U S A. Jan 26 2010;107(4):1547-52. doi:10.1073/pnas.0908801107
57. Hirschhaeuser F, Sattler UG, Mueller-Klieser W. Lactate: a metabolic key player in cancer. Cancer Res. Nov 15 2011;71(22):6921-5. doi:10.1158/0008-5472.Can-11-1457
58. Luo W, Hu H, Chang R, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell. May 27 2011;145(5):732-44. doi:10.1016/j.cell.2011.03.054
59. Mazurek S. Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells. Int J Biochem Cell Biol. Jul 2011;43(7):969-80. doi:10.1016/j.biocel.2010.02.005
60. Yang W, Lu Z. Nuclear PKM2 regulates the Warburg effect. Cell Cycle. Oct 1 2013;12(19):3154-8. doi:10.4161/cc.26182
61. Kress S, Stein A, Maurer P, et al. Expression of hypoxia-inducible genes in tumor cells. J Cancer Res Clin Oncol. 1998;124(6):315-20. doi:10.1007/s004320050175
62. Ord JJ, Streeter EH, Roberts IS, Cranston D, Harris AL. Comparison of hypoxia transcriptome in vitro with in vivo gene expression in human bladder cancer. Br J Cancer. Aug 8 2005;93(3):346-54. doi:10.1038/sj.bjc.6602666
63. Parks SK, Chiche J, Pouysségur J. Disrupting proton dynamics and energy metabolism for cancer therapy. Nat Rev Cancer. Sep 2013;13(9):611-23. doi:10.1038/nrc3579
64. Baek G, Tse YF, Hu Z, et al. MCT4 defines a glycolytic subtype of pancreatic cancer with poor prognosis and unique metabolic dependencies. Cell Rep. Dec 24 2014;9(6):2233-49. doi:10.1016/j.celrep.2014.11.025
65. Hsu PC, Hour TC, Liao YF, et al. Increasing ornithine decarboxylase activity is another way of prolactin preventing methotrexate-induced apoptosis: crosstalk between ODC and BCL-2. Apoptosis. Mar 2006;11(3):389-99. doi:10.1007/s10495-006-4002-0