

Evaluation of the use of hyperbaric oxygen in suppression of hyper-proliferation in hypoxic NSCLC cells

Jiun-Rung Chen^{1,2}, Haseeb Khan^{3*}, Yigiter Umarzai³, Abbaas Rajput³, Tokalp Mamund³¹Division of Pulmonary & Respiratory Medicine, Department of Internal Medicine, E-Da Cancer Hospital, I-Shou University, Kaohsiung, Taiwan²School of Chinese Medicine for Post Baccalaureate, College of Medicine, I-Shou University, Kaohsiung, Taiwan³Internal Medicine, Ziauddin Medical University, Karachi, Pakistan

ARTICLE INFO

Original paper

Article history:

Received: June 15, 2023

Accepted: August 23, 2023

Published: December 20, 2023

Keywords:

Hypoxic NSCLC cells, Hyper-proliferation, "Hyperbaric oxygen therapy (HBO)"

ABSTRACT

"Hyperbaric oxygen therapy (HBO)" is being researched as a potential adjuvant therapy for solid malignancies, such as NSCLC. It can reduce tumour hypoxia and has been found to slow tumour growth, stop dedifferentiation, and reduce apoptosis resistance in hypoxic NSCLC cells. Though HBO has shown promise in treating various cancers, more study is required to determine its precise mechanism of action in NSCLC. Examine the result of "hyperbaric oxygen therapy" on the proliferation of hypoxic "non-small cell lung cancer" cells. In addition, the "NSCLC cell lines A549 and H1299" were employed for the in vitro analysis of aerobic glycolysis. Warburg effect testing included glucose absorption, lactate, "adenosine triphosphate (ATP)", and pyruvate measurements. Using a quantitative glycolytic flow model, we also analyzed the effect of HIF-1-induced genes on the flux of glucose metabolism. "Lewis lung carcinoma (LLC)" animal models in C57BL/6J mice were used to examine the development of lung tumours. The effects of pcDNA and HIF1A on glucose uptake, lactate production, pyruvate, and ATP levels were studied in "A549 and H1299 NSCLC cells". The glucose absorption of A549 cells exhibited a gradual increase throughout the course of the experiment, but H1299 cells had a significant decrease in glucose absorption following HBO therapy. The levels of pyruvate were shown to be notably higher in the H1299 cell line, particularly under hypoxic conditions, and were observed to decrease in reaction to "hyperbaric oxygen therapy (HBO)". In A549, the lactate content was more effective. After HBO treatment, glucose absorption was reduced while intracellular ATP levels were maintained. Overexpression of HIF-1 α was able to counteract the effect of HBO on glycolytic gene expression. PFKFB3 is a possible therapeutic target because HBO reduces the "Warburg effect in NSCLC cells" by downregulating "HIF-1".

Doi: <http://dx.doi.org/10.14715/cmb/2023.69.14.21>Copyright: © 2023 by the C.M.B. Association. All rights reserved. 

Introduction

The consumption of oxygen beneath promoted atmospheric pressure or at a pressure higher than that observed on the world cover at sea level, defined as 1 atm (1), is known as "hyperbaric oxygen therapy (HBO)". Hypoxic and ischemic diseases are the most common pathological situations for which "hyperbaric oxygenation" is employed as an additional therapy (2). According to the usual protocol for "hyperbaric oxygen therapy (HBOT)", patients are required to inhale sterile oxygen at a pressure range of around 1.5 to 2.5 "atmospheres absolute (ATA)". This pressure encompasses both the total atmospheric pressure outside the hyperbaric chamber and the gauge pressure within it (3).

There are two distinct, well-known processes by which oxygen is delivered from the blood to tissues: Hemoglobin forms a compound with carbon dioxide in "red blood cells (RBCs)" and is also dissolved in the "blood plasma". Around 97% of the haemoglobin available is oxygen-saturated in normal atmospheric circumstances (4). Conversely, plasma typically only has "0.32% dissolved oxygen" (2,5). Therefore, the authority of "hyperbaric oxygen

therapy (HBO)" may enhance haemoglobin-independent transport; nevertheless, it does not significantly affect oxygen delivery through red blood cells. When tissue hypoxia is of cardiovascular origin, "hyperbaric oxygen therapy (HBO)" has further beneficial benefits, including enhanced flexibility of "red blood cells (RBCs)" and reduced aggregation of platelets (1,6). Henry's law states that an increase in oxygen partial pressure (pO_2) will increase tissue oxygen content (3). The distance over which oxygen diffuses increases when pO_2 is higher; HBOT improves tissue oxygenation by raising the blood's dissolved oxygen concentration (6).

Lung cancer is a prominent global cause of mortality (7). Approximately 85% of fatalities associated with lung cancer can be attributed to "non-small-cell lung cancer (NSCLC)", a condition characterized by an inferior prognosis for patients (7). The attribution of unfavourable prognoses is often ascribed to delayed diagnoses, tumour metastases, and the limited efficacy of conventional therapy modalities (8). Hence, improved knowledge of the mechanism(s) underlying NSCLC tumour formation and metastasis is essential for developing more efficient diagnostic and treatment strategies.

* Corresponding author. Email: haseebkhan731.ksu@gmail.com

The local tumor microenvironment is indicated by the fact of low oxygen partial pressures, also known as hypoxia. This condition arises as a result of the fast proliferation of malignant cells and the unusual formation of blood vessels (angiogenesis) within the tumor (9). Tumor cells initiate hypoxia-induced signalling pathways in response to a limited supply of oxygen in the local environment. This adaptive response serves to support “cellular proliferation”, “invasion”, and “metastasis”, as well as to promote the formation of new blood vessels through angiogenesis and vasculogenesis (9). The role of “Hypoxia-Inducible Factor 1 Alpha (HIF-1 α)” as a vital mediator of these pathways has been well established (10). The promotion of the Warburg effect and the “epithelial-to-mesenchymal transition (EMT)” is facilitated by the binding of activated HIF-1 α to “Hypoxia Response Elements (HREs)” located in the booster region of certain genes, as part of the cellular response to hypoxia (11). Several cellular phenotypes are produced due to these alterations, which increase the aggressiveness and proliferation of malignant cells (11).

As a result, treatment approaches such as the potential effectiveness of “Hyperbaric Oxygen Therapy (HBO)” and “normobaric oxygen therapy (NBO)” in the therapy of cancer patients, by reducing tumour hypoxia, have been identified. HBO involves the management of 100% oxygen at forces ranging from 1.5 to 3.0 bars, while NBO entails the delivery of 100% oxygen at ambient pressure (12). According to Moen et al. based on a thorough examination of the available literature, it can be concluded that HBO therapy is deemed to be safe, exhibiting a minimal likelihood of complications and lacking any stimulatory impact on the growth of malignant tumours (13). Furthermore, “hyperbaric oxygen (HBO)” therapy has been demonstrated to exhibit the potential to decelerate the progression of tumour growth in certain types of malignancies, including breast carcinoma. However, the available data is limited in supporting the efficacy of this treatment in addressing other types of malignancies, such as cervical and bladder carcinomas (13). The current focus of research involves a comprehensive investigation of HBO as an adjuvant treatment, seeking to improve the efficacy of traditional “radio- and photodynamic” treatments in the treatment of certain solid tumours (14). HBO has been demonstrated to prevent dedifferentiation and apoptosis resistance in CoCl₂-induced hypoxic NSCLC cells *in vitro* (15), despite the lack of clinical data supporting its use in NSCLC patients. HBO affects hypoxic NSCLC cells, although the exact mechanism(s) through which it works is still unknown. The benefit of HBOT in the therapy of various lung tumours and the mechanisms underlying HBO's actions in hypoxic NSCLC cells are highlighted in this article's summary of the available data on the medical service of HBO in cancer cure.

Materials and Methods

Study design

The study was performed in the pathology laboratory from March 2022 to January 2023. The specimens were procured from the “E-Da Cancer Hospital pathology laboratory”, “I-Shou University, Kaohsiung, Taiwan”. During our *in-vitro* research, we used the “NSCLC cell lines A549 and H1299”. Aerobic glycolysis was evaluated using glucose uptake, lactate, “adenosine triphosphate (ATP)”, and

pyruvate tests (Warburg effect). HIF-1 α -induced genes' contributions to glucose metabolism were examined using a “quantitative glycolytic flux model”. To gauge the development of lung tumours in C57BL/6J mice, we used a “Lewis lung carcinoma (LLC)” mouse model.

Firstly, aerobic glycolysis was evaluated using several tests, including glucose uptake, lactate production, ATP levels, and pyruvate tests (Warburg effect), separately for A549 and H1299 cell lines. These measurements assessed metabolic alterations associated with aerobic glycolysis in NSCLC cells. Following that, the impact of genes activated by HIF-1 α on glucose metabolism was investigated using a quantitative model of glycolytic flux. The present study offers valuable insights into the influence of genes activated by HIF-1 α on the rate of glucose flow within the glycolysis pathway. In addition to the *in vitro* experiments, the development of lung tumours was assessed using an LLC mouse model. C57BL/6J mice were used for this purpose. The LLC mouse model is well-established and widely used to study lung tumour development. The model involves the implantation of LLC cells in the mice, which subsequently form tumours resembling lung carcinomas. By utilizing this model, the researchers could gauge the development and progression of lung tumours *in vivo*.

Statistical analysis

The statistical analysis in this study was conducted using SPSS 25, a software widely recognized for its effectiveness in statistical analysis. The continuous data has been reported using the mean value together with its corresponding standard deviation. On the other hand, the discrete data has been provided in terms of frequency and the corresponding percentage. The study utilized “analysis of variance (ANOVA)” as the statistical method for conducting its analysis. The chosen level of significance was determined to be $p < 0.05$.

Ethical approval

Ethical approval is received from the ethical committee for all protocols of animals. All institution and government regulations were followed, and the NIH Guidebook executed animal protocols for the “Use and Care of Laboratory Animals”.

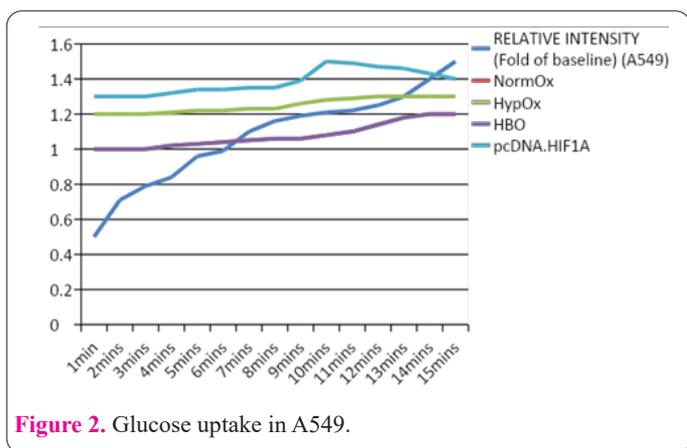
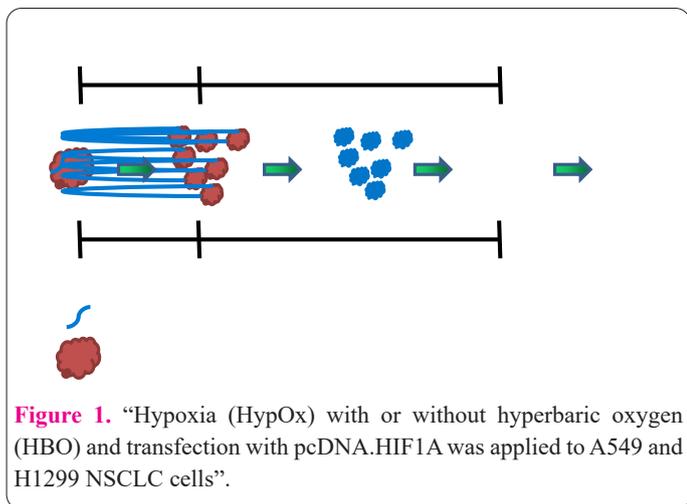
Results

Glucose absorption, lactate release into the supernatant, intracellular pyruvate levels, and intracellular ATP levels were quantified in “A549 and H1299 non-small cell lung cancer (NSCLC)” cells following exposure to pcDNA. The two constructs under consideration are HIF1A and pcDNA. Control transfection of plasmids, including pcDNA. Controlled plasmid DNA transfection (Ctrl) and plasmid DNA (pcDNA) transfection.Ctrl (Figure 1).

Figure 2 shows the uptake of glucose in both A549 and H1299. The relative intensity of uptake of glucose in A549 has increased gradually over time from a baseline in hyperbaric oxygen (HBO), hypoxia (HypOx), Normal oxygen (NormOx), and pcDNA.HIF1.

Figure 3 shows the uptake of glucose in H1299. HBO has a significant reduction in the uptake of glucose. The relative intensity of glucose uptake is gradually increasing after 3 minutes.

Table 1 shows the levels of pyruvate intracellularly in



phorylation (OXPHOS)” in response to changes in their environment. In this study, the researchers employed Oligomycin A, a well-known inhibitor that specifically targets mitochondrial ATP synthase, to pretreat “non-small cell lung cancer (NSCLC)” cells. The objective was to eliminate their activation capacity for “oxidative phosphorylation (OXPHOS)”. Even while internal ATP levels in hypoxic cells remained high, we found that oligomycin A significantly reduced these levels after the addition of HBO. All seven glucose metabolism was also measured in “A549 and H1299” by “quantitative polymerase chain reaction (qPCR)”. Gene upregulated expression of all seven glycolytic markers was significantly suppressed by HBO, but the effects of HIF-1a overexpression were able to reverse this.

Discussion

“NSCLC cell lines A549 and H1299” were investigated and used by Zhang *et al.* (16) in 2021 for *in vitro* investigations. “Glucose uptake”, “pyruvate”, “lactate”, and “adenosine triphosphate (ATP)” tests (the Warburg effect) were used to measure “aerobic glycolysis”. Using a “quantitative glycolytic flux model”, we analyzed the contributions of the HIF-1-induced genes involved in glucose metabolism to the flux. We utilized a “Lewis lung carcinoma (LLC)” mouse model to evaluate tumour growth in “C57BL/6J mice”. HBO inhibited “hypoxia-induced HIF-1” expression and “HIF-1 signalling in NSCLC cells”. Phosphofructokinase, “platelet (PFKP)”, a “HIF-1-induced

both A549 and H1299. The pyruvate levels are high in H1299 compared to A549. The highest is seen in hypoxia-induced uptake of pyruvate, and it is reduced significantly in HBO.

Figure 4 shows the lactate concentration in A549 and H1299. The levels of lactate are high in A549 compared to H1299. High levels of pyruvate are seen in pcDNA. HIF1A and there is a significant reduction in HBO. Hypoxic cells showed increased amounts of intracellular ATP. Interestingly, intracellular ATP levels persisted after HBO treatment despite a decrease in “glucose uptake”. Even, Metabolic flexibility is a familiar feature of cancer cells, allowing them to switch between glycolysis and “oxidative phos-

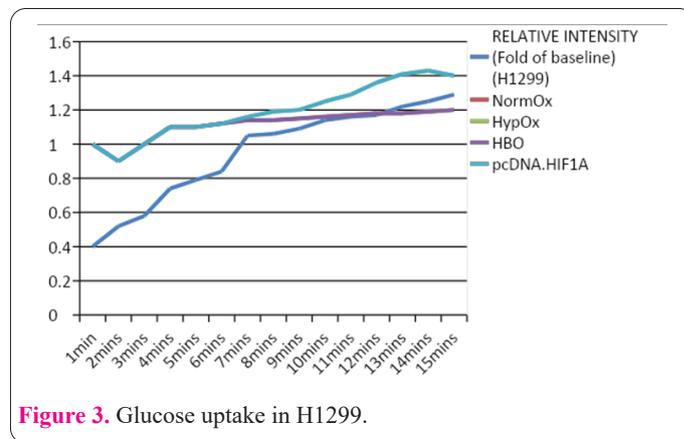


Table 1. “Intracellular levels of pyruvate in H1299 and A549 cells”.

Levels of	NormOx	HypOx	HBO	pcDNA.HIF1A
Pyruvate				
A549	0.9	1.2	0.8	1.3
H1299	0.92	1.6	1	1.5
Lactate (mM)				
A549	0.24	0.3	0.2	0.35
H1299	0.15	0.3	0.15	0.25
Adjusted ATP concentration (NormOx's factor)				
A549	1	1.8	0.9	2
H1299	1	1.7	1	1.8
OIIA (the OIIA fold of NormOx) normalized the ATP concentration.				
A549	1	1.5	0.9	1.7
H1299	1	1.8	1	1.5

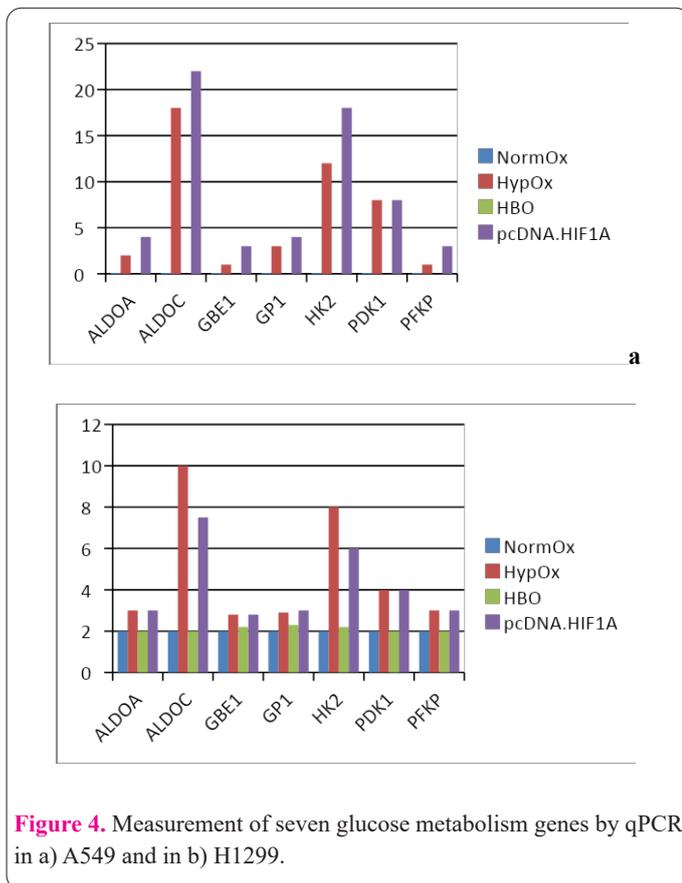


Figure 4. Measurement of seven glucose metabolism genes by qPCR in a) A549 and in b) H1299.

glucose metabolism gene”, considerably improved glycolytic flow under low and high glucose conditions (17,18). In response to hypoxia, HBO inhibited HIF-1's ability to regulate PFKP transactivation and gene expression. The “Warburg effect”, hyper-proliferation, and “epithelial-to-mesenchymal transition (EMT)” in hypoxic NSCLC cell lines are all suppressed by HBO, and this impact is mediated by the “HIF-1/PFKP axis”. In a PFKP-dependent way, HBO therapy inhibited the growth of LLC lung tumours in mice in vivo. The suppression of the Warburg effect, hyper-proliferation, and EMT in hypoxic NSCLC cells relies on HBO's capacity to inhibit HIF-1. In “hypoxic NSCLC cells”, the “HIF-1 target gene PFKP” mediates the effects of HBO, suggesting that this gene may be a metabolic vulnerability in NSCLC tumours (14,16).

Paclitaxel (50 mg/m), carboplatin, and 10% glucose were administered intravenously to 22 patients with NSCLC who had numerous lung metastases every week for three out of four weeks in a study by Ohguri *et al.* (15) Weekly intravenous carboplatin infusions were also combined with “hyperthermia (HT)” of the whole thoracic area in all patients. In addition, 16 (72%) of the 22 patients underwent “hyperbaric oxygen therapy (HBO)” right after each weekly chemotherapy session. Six patients without HBO received 27 cycles, while 16 patients with HBO received 107 cycles. A retrospective analysis was done on the toxicity and effectiveness of these individuals (15,16). One patient experienced leukopenia/neutropenia of grade 3 and another experienced pneumonitis of the same severity. Minimal toxicity was observed in both the hematologic and non-hematologic systems. Of the 22 patients, 14 (or 64%) had an objective response. In 16 individuals using HBO, the median time to disease progression was nine months rather than eight months for all patients. Four of the nine patients (44%) had received previous treatment

that included paclitaxel and carboplatin (16,17). Because of this, the innovative combination treatment of “paclitaxel, carboplatin, HT, and HBO” may be a viable and effective treatment option for NSCLC with numerous pulmonary metastases. The findings call for further research to determine the therapeutic advantages of this regimen (15,16).

Wang *et al.* (17) researched and looked at solid tumours; hypoxia promotes rapid growth and a more robust malignant phenotype. Hypoxia can be significantly reduced with hyperoxic treatment utilizing “hyperbaric oxygen (HBO)”, as has been demonstrated in earlier studies. Researchers set out to look at how HBO affected the malignancy that hypoxia caused in lung cancer cells. The “lung cancer cell line A549” was subjected to “chemical hypoxia using cobalt chloride (COCl₂)”. The evaluation encompassed the examination of the expression of “inducible factor-1 (HIF-1 α)” and “lactate dehydrogenase (LDH)” activity, as well as the assessment of motility and invasion capacity, expression profiles of “epithelial-mesenchymal transition (EMT)” indicators, and apoptotic markers in “A549 cells treated with COCl₂”, both with and without HBO treatment (18-21). The induction of chemical hypoxia through COCl₂ exposure resulted in several notable effects, including elevated expression of GRP78, augmented motility and invasion capabilities, reduced “ratio of E-cadherin to N-cadherin”, intensified “epithelial-mesenchymal transition (EMT)” phenotype, heightened LDH activity, and diminished “E-cadherin/N-cadherin ratio” (17,18). The application of HBO therapy exhibits the capacity to significantly mitigate the impact of “hypoxia on LDH activity”, “migration”, and “invasion”, as well as on the “epithelial-mesenchymal transition (EMT)” phenotype, the “ratio of E-cadherin to N-cadherin”, the “ratio of Bcl-2 to Bax”, and the expression of GRP78. The potential efficacy of HBO as an adjuvant cure for solid tumours that specifically target the hypoxic microenvironment is worth considering (20).

Overall, it was discovered that HIF-1 α down-regulation is necessary for HBO to suppress the “Warburg effect”, “hyper-proliferation”, and “EMT in hypoxic NSCLC cells”. Subsequent research revealed that HBO's impacts on “hypoxic NSCLC cells” are mediated mainly by the HIF-1 α target “gene PFKP” (21,22). This study identified PFKP as a significant contributor to the proliferation, invasion, and metastasis of “NSCLC tumour cells” caused by “hypoxia/HIF1 α ”, which may indicate a metabolic vulnerability in “NSCLC malignancies”. Apart from PFKP, HIF-1 α gene targets are known to regulate “glycolysis”, “proliferation”, and “EMT” by Chen *et al.* (8). Additionally, HBO's impacts on “hypoxic NSCLC cells” might be mediated by HIF-1 α -independent mechanisms. Future research should therefore focus on finding additional important mediators of EMT and hyper-proliferation in “hypoxic NSCLC cells”.

Conclusion

The research findings indicate that HBO treatment effectively suppresses the “Warburg effect”, “hyperproliferation”, and “epithelial-mesenchymal transition (EMT)” in hypoxic “non-small cell lung cancer (NSCLC)” cells through the downregulation of “hypoxia-inducible factor 1-alpha (HIF-1 α)”. Additionally, it was found that the target gene PFKP of HIF-1 α serves as a pivotal mediator

of the effects of “hyperbaric oxygen therapy (HBO)” in hypoxic “non-small cell lung cancer (NSCLC)” cells. The outcomes of this study reveal that PFKP plays an important role in the advancement of “NSCLC tumour cell proliferation”, invasion, and metastasis under conditions of hypoxia and HIF1 α activation. The identification of PFKP as a potential metabolic vulnerability in “non-small cell lung cancer (NSCLC)” tumours suggests that targeting this pathway could be a promising strategy for therapeutic intervention. Nevertheless, it is significant to recognise the limitations of our analysis. First, our study was conducted *in vitro*, and it is not clear whether the findings would be replicated *in vivo*. Second, our study only examined the effects of HBO on a single HIF-1 α target gene. Other HIF-1 α target genes may also affect HBO's effects on hypoxic NSCLC cells. The main limitation of this study is that it was conducted *in vitro*, and it is not clear whether the findings would be replicated *in vivo*.

Additionally, the study only examined the effects of HBO on a single HIF-1 α target gene. Other HIF-1 α target genes may also affect HBO's effects on hypoxic NSCLC cells. Despite these limitations, the results of this study present that PFKP may be a profitable target for therapeutic intervention in NSCLC. Future studies should investigate HBO's effects on PFKP expression and activity *in vivo*. Additionally, future studies should investigate the role of other HIF-1 α target genes in HBO's effects on hypoxic NSCLC cells.

References

- Jain KK. Physical, physiological, and biochemical aspects of hyperbaric oxygenation. In: Jain KK, editor. Textbook of hyperbaric medicine. Germany: Hogrefe Huber 2009; 4:9-20.
- Daruwalla J, Christophi C. Hyperbaric oxygen therapy for malignancy: a review. World J Surg 2006; 30(12):2112-31.
- Gill AL, Bell CN. Hyperbaric oxygen: its uses, mechanisms of action and outcomes. QJM 2004; 97(7):385-95.
- Tibbles PM, Edelsberg JS. Hyperbaric-oxygen therapy. N Engl J Med 1996; 334(25):1642-48.
- Chaves JC, Fagundes DJ, SimoesMde J, Bertolotto PR, Oshima CT, Taha MO. Hyperbaric oxygen therapy protects the liver from apoptosis caused by ischemia-reperfusion injury in rats. Microsurg 2009; 29(7):578-83.
- Ilhan H, Eroglu M, Inal V, Eyi YE, Arziman I, Yildirim AO. Hyperbaric oxygen therapy alleviates oxidative stress and tissue injury in rats' renal ischemia/reperfusion injury. Ren Fail 2012; 34(10):1305-8.
- Nawaz K, Webster RM. The Non-Small-Cell Lung Cancer Drug Market. Nat Rev Drug Discov 2016; 15(4):229-30.
- Chen YL, Zhang Y, Wang J, Chen N, Fang W, Zhong J. A 17 Gene Panel for Non-Small-Cell Lung Cancer Prognosis Identified Through Integrative Epigenomic Transcriptomic Analyses of Hypoxia-Induced Epithelial-Mesenchymal Transition. Mol Oncol 2019; 13:1490-502.
- Michiels C, Tellier C, Feron O. Cycling Hypoxia: A Key Feature of the Tumor Microenvironment. Biochim Biophys Acta (BBA)-Reviews Cancer 2016; 1866:76-86.
- Soni S, Padwad YS. HIF-1 in Cancer Therapy: Two Decade Long Story of a Transcription Factor. Acta Oncol 2017; 56:503-15.
- Icard P, Shulman S, Farhat D, Steyaert J-M, Alifano M, Lancet H. How the Warburg Effect Supports Aggressiveness and Drug Resistance of Cancer Cells? Drug Resistance Updates 2018; 38:1-11.
- Kim SW, Kim IK, Lee SH. Role of Hyperoxic Treatment in Cancer. Exp Biol Med 2020; 245:851-60.
- Moen I, Stuhr LE. Hyperbaric Oxygen Therapy and Cancer—a Review. Targeted Oncol 2012; 7:233-42.
- Wu X, Zhu Y, Huang W, Li J, Zhang B, Li Z. Hyperbaric Oxygen Potentiates Doxil Antitumor Efficacy by Promoting Tumor Penetration and Sensitizing Cancer Cells. Advanced Sci 2018; 5:1700859.
- Ohguri T, Imada H, Narisada H, Yahara K, Morioka T, Nakano K. Systemic Chemotherapy Using Paclitaxel and Carboplatin Plus Regional Hyperthermia and Hyperbaric Oxygen Treatment for non-Small Cell Lung Cancer With Multiple Pulmonary Metastases: Preliminary Results. Int J Hyperthermia 2009; 25:160-7.
- Zhang L, Ke J, Min S, Wu N, Liu F, Qu Z, Li W, Wang H, Qian Z and Wang X. Hyperbaric Oxygen Therapy Represses the Warburg Effect and Epithelial-Mesenchymal Transition in Hypoxic NSCLC Cells via the HIF-1 α /PFKP Axis. Front Oncol 2021; 11:691762.
- Wang Y, Qi Y, Wei X, Chen S, Jia N, Zhou Q. Hyperbaric Oxygen Rescues Lung Cancer Cells From Chemical Hypoxia-Induced Low Differentiation and Apoptosis Resistance. Exp Lung Res 2018;44:417-23.
- Wu J, Song J, Yin X, Tang J, Zhang J, Wang X, Ji Y, Zhao Y, Chen D, Sheng J, Bai X, Liang T. Recent Advancements of Nanotechnology-Based Strategies for Overcoming Tumor Microenvironment Hypoxia. Front Biosci (Landmark Ed). 2022 May 6;27(5):145.
- Nurwidya F, Takahashi F, Kobayashi I, Murakami A, Kato M, Minakata K, Nara T, Hashimoto M, Yagishita S, Baskoro H, Hidayat M, Shimada N, Takahashi K. Treatment with insulin-like growth factor 1 receptor inhibitor reverses hypoxia-induced epithelial-mesenchymal transition in non-small cell lung cancer. Biochem Biophys Res Commun. 2014 Dec 12;455(3-4):332-8.
- Wang X, Liang X, Liang H, Wang B. SENP1/HIF-1 α feedback loop modulates hypoxia-induced cell proliferation, invasion, and EMT in human osteosarcoma cells. J Cell Biochem. 2018 Feb;119(2):1819-1826. doi: 10.1002/jcb.26342.
- Wei L, Sun JJ, Cui YC, Jiang SL, Wang XW, Lv LY, Xie L, Song XR. Twist may be associated with invasion and metastasis of hypoxic NSCLC cells. Tumour Biol. 2016 Jul;37(7):9979-87. doi: 10.1007/s13277-016-4896-2.
- Wang H, Zhang H, Tang L, Chen H, Wu C, Zhao M, Yang Y, Chen X, Liu G. Resveratrol inhibits TGF- β 1-induced epithelial-to-mesenchymal transition and suppresses lung cancer invasion and metastasis. Toxicology. 2013 Jan 7;303:139-46. doi: 10.1016/j.tox.2012.09.017.