Hypoxic environment mitigates glucose deprivation-induced cell death by regulating reactive oxygen species

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Hypoxic environment mitigates glucose deprivation-induced cell death by regulating reactive oxygen species

Directed by Professor Kyung-Sup Kim

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June, 2016

Yu Shin Lee

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

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Overcoming metabolic stress is a critical step for solid tumor growth. However, the underlying mechanisms of cell death and survival under metabolic stress are not well understood. In this study, we observed that hypoxia-exposed cancer cells showed increased survival under glucose deprivation accompanied by reduced

SAPK/JNK signaling pathway, high NADPH/NADP ratio compared with glucose-deprived cells under normoxic condition. Since antioxidant NAC (sodium acetyl cysteine) rescued glucose deprivation-induced cell death, we measured ROS level of hypoxia-exposed cells under glucose deprivation. Glucose-deprived cells showed drastic increase of ROS production in normoxia whereas hypoxia-exposed cells showed moderate increase of ROS after glucose deprivation although basal ROS production is higher in hypoxic cells under normal glucose condition. These data indicate that hypoxic cells are resistant to glucose deprivation-induced cell death probably through regulating redox balance.

Key words : hypoxia, glucose deprivation, ROS

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Ⅰ. INTRODUCTION

Solid tumors encounter metabolic stress such as glucose depletion or hypoxia, requiring alternative pathway for survival and proliferation.[1] Under glucose-deprivation condition, transformed human cells appear to be more susceptible to glucose deprivation-induced cytotoxicity and oxidative stress than untransformed

human cells.[2] Several studies also demonstrated that glucose deprivation-induced cell death have been observed in skeletal muscle cells [3] and a variety of cancer cells including glioblastoma cell lines, ovarian cancer cells, breast cancer, prostate and colon cancer cells through modulating cellular signaling pathway such as AMPK-Akt signaling pathway, p53-induced apoptotic pathway, and tyrosine kinase signaling concomitant with reactive oxygen species (ROS) generation.[4-9]

Particularly, excess production of ROS, oxidative stress, caused by various cellular stresses including nutritional defects, ER stress, and inflammation has been known to induce cell death.[10,11] Whereas excessive ROS production damages cellular components such as DNA, proteins and lipids and induces cell death, ROS could also act as signaling molecules at low to moderate levels to maintain cell survival, proliferation and differentiation. Therefore, balancing of intracellular ROS level is critical for homeostasis.

In addition, it has been known that solid tumors are often poorly vascularized resulting in cancer cells exposed to low oxygen condition, hypoxia, undergo a variety of adaptive biological responses through activating transcription factor hypoxia-inducible factor (HIF) in order to survive and proliferate under hypoxic tumor microenvironments.[12] Activation of HIF-1 mediates highly glycolytic metabolic switching through inducing sets of genes involved in glycolytic pathway such as glucose transporters (GLUT), hexokinase 2 (HK2), lactate dehydrogenase A (LDHA) could lead to glucose depleted condition around the solid tumor. Therefore,

it is important to understand how glycolytic cancer cells in hypoxia promote metabolic adaptation during energy stress in respect to therapeutic intervention.

In this study, we examined the effects of hypoxia on the cell death caused by glucose deprivation to determine whether there are overcoming mechanism to adapt both oxygen and nutrient insufficiency. We observed that hypoxia-exposed cancer cells are tolerant to glucose deprivation-induced cell death accompanied by marked decrease in JNK/p38 activation, p-Bcl-xL level, high NADPH/NADP ratio and moderate increase of ROS after glucose deprivation compare to normoxic cells. These data indicate that hypoxic cells are resistant to glucose deprivation-induced cell death probably through attenuating excess ROS production.

Ⅱ. MATERIALS AND METHODS

1. Cell culture

U2OS, 786-0, Hep3B were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with high glucose (25 mM) or DMEM without glucose or DMEM without glutamine (Gibco, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (Gibco), 100 μg/ml streptomycin (Gibco), and 100 U/ml penicillin (Gibco). Six-well, 60-mm, and 100-mm culture dishes (TPP, St. Louis, MO, USA) were used for cell culture. The cells were incubated at 37°C with 5% CO_2. Hypoxic conditions (1% O_2) were established in a hypoxia incubator (Forma Scientific, Inc., Marietta, OH, USA) where N_2 was used to compensate for the reduced O_2 level.

2. Cell proliferation analysis

Cells were plated in 12-well plates. On the next day, the cells were washed with PBS and cultured in glucose-free or glutamine-free DMEM. Cell numbers were counted at 72 hr after glucose or glutamine deprivation using ADAM-MC (NANOENTEK, South Korea).

3. Western blot analysis

Cultured cells were washed with ice-cold PBS and harvested in whole-cell lysis

buffer [1% SDS-60 mM Tris (pH 6.8)]. Protein concentrations were measured by the bicinchoninic acid assay. Equal amounts of protein extracts were subjected to SDS-PAGE and transferred on to nitrocellulose transfer membranes. The membranes were blocked in Tris-buffered saline (pH 7.4) containing 0.1% Tween 20 (Sigma–Aldrich, St.Louis, MO, USA) and 5% non-fat skimmed milk, followed by incubation with the primary antibodies. Primary antibodies were : anti-AMPK, anti-p-AMPK, anti-p-JNK, anti-JNK, anti-p-c-Jun, anti-p38, anti-p-p38 (Cell Signaling Technology, Danvers, MA, USA), anti-p-Bcl-xL (Invitrogen, Carlsbad, CA, USA), anti-Bcl-xL, anti-HIF1α (Santa Cruz Biotechnology, Dallas, TX, USA), anti-IDH_2 (Abcam, Cambridge, UK), anti-α-tubulin antibodies (Millipore, Tumecula, CA, USA). For secondary antibodies, IRDye 800CW goat anti-rabbit and IRDye 680 goat anti-mouse antibodies (LI-COR Biosciences, Lincoln, Nebraska, USA) were used. The proteins were visualized in conjunction with the Odyssey CLx Imaging System (LI-COR Biosciences) for signal detection.

4. Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from cultured cells using TRIzol® (Invitrogen) according to the manufacturer's instructions. For qRT-PCR, cDNAs were synthesized from 4 μg of total RNA using random hexamer primers and SuperScript reverse transcriptase Ⅱ (Invitrogen) following the manufacturer's instructions. An aliquot (1/50) of the reaction was used for quantitative PCR using the SYBR Green PCR

Master Mix (Applied Biosystems, Foster City, CA, USA) and gene-specific primers, and this was then subjected to RT-PCR quantification using the ABI PRISM7300 RT-PCR System (Applied Biosystems). All reactions were performed in triplicates. The relative amounts of the mRNAs were calculated using the comparative cycle-time method [Applied Biosystems User Bulletin number 2 (2001);Applied Biosystems]. 9S ribosomal RNA (abbreviated 9S rRNA) level was also measured as an invariant control.

5. RNA interference

Silencing RNA (siRNA) oligonucleotides for IDH2 and control siRNA were purchased from Genolution Pharmaceuticals Inc. (Seoul, Korea). Transfection was performed using Lipofectamine RNA iMax (Invitrogen) according to the manufacturer's protocol. Briefly, 20 nmol of siRNA and 3 μl of Lipofectamine RNA iMax were mixed in 200 μl of serum-free media. After 15 min. of incubation, the siRNA-Lipofectamine RNAiMax mixture was added to the cultured cells. The cells were incubated for 48 h before protein preparation.

6. Quantification of reactive oxygen species

The intracellular levels of H_2O_2 and $O_2^{\bullet-}$ were measured with H_2-DCFDA. Quantification of mitochondrial superoxide was detected by utilizing MitoSox Red. Cells were treated with 5 mM of H_2-DCFDA for 30 min. The cells washed with

PBS two times and harvested for fluorescence-activated cell sorting (FACS) analysis. FACSverse was used for FACS analysis. The mean fluorescence intensity (MFI) of 10,000 cells was analyzed in each sample and corrected for autofluorescence from unlabeled cells.

7. Quantification of NADPH/NADP

The intracellular levels of NADPH and NADP were measured with previously described enzymatic cycling methods with slight modifications. Cells were lysed in 400 µl of extraction buffer (20 mM nicotinamide, 20 mM $NaHCO_3$, 100 mM Na_2CO_3), sonicated and centrifuged. For NADPH extraction, 150 µl of the supernatant was incubated at 60°C for 30 min. Next, 160 µl of NADP-cycling buffer (100 mM Tris-HCl pH8.0, 0.5 mM thiazolyl blue, 2 mM phenazine ethosulfate, 5 mM EDTA) containing 1.3 U of G6PD was added to a 96-well plate containing 20 ml of the cell extract. After a 1-min incubation in the dark at 30℃, 20 ml of 10 mM glucose 6-phosphate (G6P) was added to the mixture, and the change in absorbance at 570 nm was measured every 30 sec for 4 min at 30℃ using Infinite® F200 PRO (Tecan, Switzerland). The concentration of NADP was calculated by subtracting [NADPH] from [total NADP].

8. Treatment of N-acetylcysteine

786-0 cells were seeded for 24 hr, washed with PBS and media was changed to

glucose-free DMEM with or without 2 mM N-acetylcysteine (NAC) (Sigma–Aldrich) for 72 hr.

9. Treatment of JNK inhibitor in cell culture

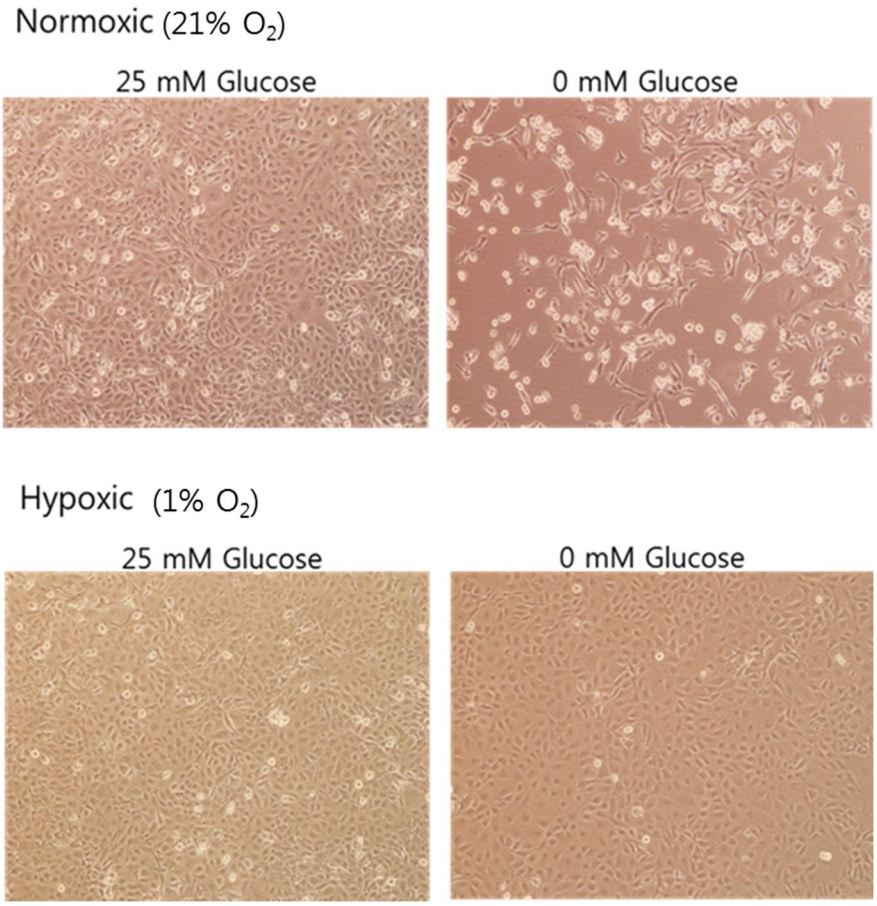
SP600125 (Sigma–Aldrich) is used for inhibiting Jun N-terminal kinase (JNK). As a general guide, for every 10 μM SP600125, it is recommended to include 0.1% dimethyl sulfoxide (DMSO) in the culture media. DMSO was used as a control instead of SP600125.

Ⅲ. RESULTS

1. Glucose deprivation-induced cell death is protected under hypoxia in U2OS cells

The effect of hypoxia on glucose deprivation-induced cytotoxicity was determined in human osteosarcoma U2OS cells. We examined the cell growth and cell viability after exposure to hypoxia and glucose deprivation. U2OS cell survival was decreased dramatically after 72 hr of glucose deprivation in normoxia, consistent with previous reports.[4,6] In hypoxia, however, U2OS cells were protected from glucose deprivation-induced cell death (Figure 1A-B). In addition, we tested whether glutamine affects the cell survival under normoxic or hypoxic condition. Glutamine, which is highly transported into proliferating cells, is a major source of energy and nitrogen for biosynthesis, and a carbon substrate for anabolic processes in cancer cells.[13,14] Glutamine deprivation caused no significant change in total cell number under hypoxia compared with normoxic condition (Figure 1B). Interestingly, during glutamine deprivation, U2OS cells maintained high cell viability, whereas total number of cells was significantly less than was seen in glucose deprivation (Figure 1C). These results indicate that cells in hypoxia are resistant to nutrient deprivation-induced death, such a phenomenon is dependent on glucose, not glutamine.

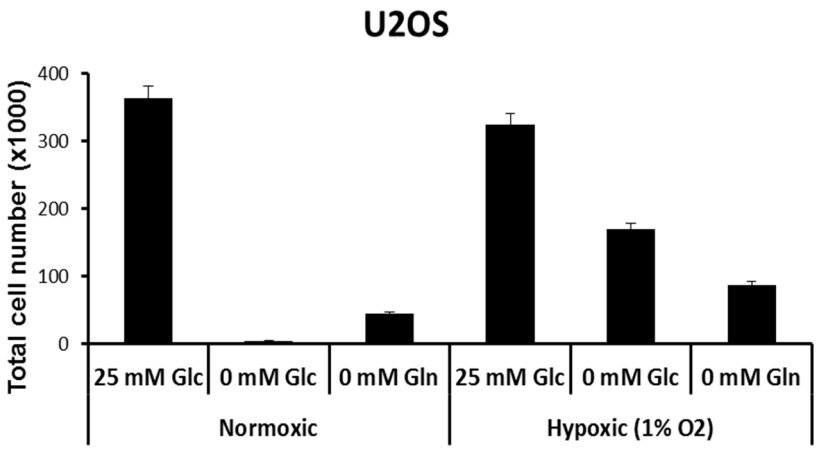
A



B

Cell culture medium	D(+)Glucose (Glc)	L-Glutamine (Gln)	Sodium pyruvate
Normal	25 mM	2 mM	0
No Glucose	0 mM	2 mM	0
No Glutamine	25 mM	0 mM	0

+ amino acids, vitamins, etc
+ 10 % FBS



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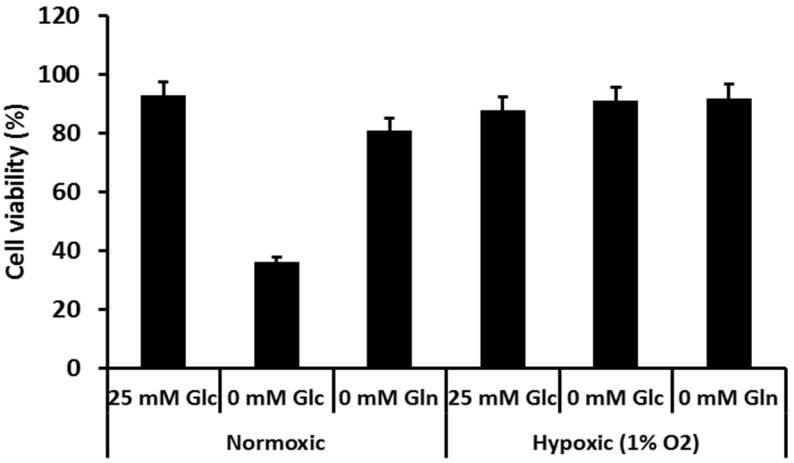


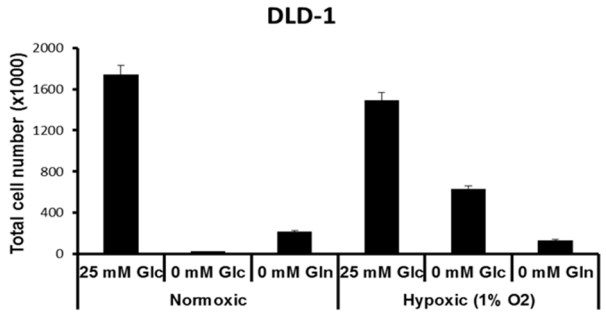
Figure 1. Glucose deprivation-induced cell death is protected under hypoxia in U2OS cells. U2OS cells were plated and on the next day, cells were cultured in DMEM with high glucose, DMEM without glucose containing 2 mM glutamine, DMEM without glutamine containing 25 mM glucose, respectively, and then exposed to normoxic or hypoxic (1% O_2) condition during 72 hr. (A) The microscopic appearance of U2OS cells. (B) Total cell numbers were counted at 72 hr after glucose or glutamine deprivation using ADAM-MC. (C) Cell viability was measured by ADAM-MC.

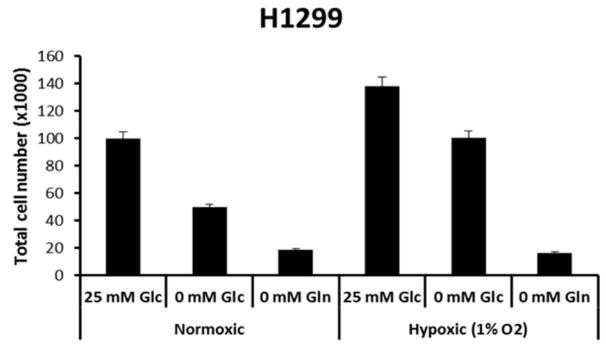
2. Hypoxic cancer cells under glucose deprivation showed increased cell survival

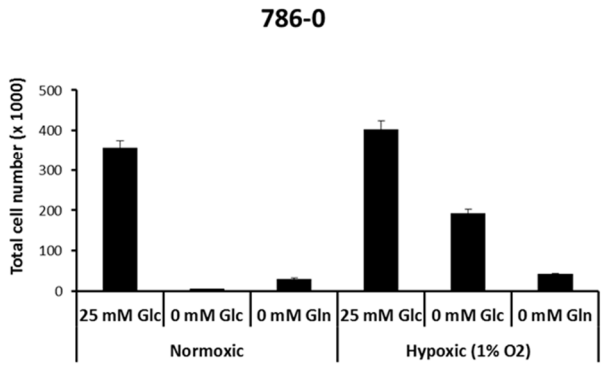
We examined the glucose deprivation-induced cell death in a variety of human cancer cell lines (DLD-1 colorectal adenocarcinoma cells, 786-O renal cell carcinoma cells, RKO colon cancer cells, H1299 non-small lung carcinoma cells).

Similarly to U2OS cells, DLD-1, 786-O, RKO, H1299 cells showed that glucose deprivation-induced cell death is inhibited in hypoxic condition. In contrast, glutamine deprivation did not affect cell survival.

These results confirm that hypoxia protect cancer cells from glucose deprivation-induced cell death.







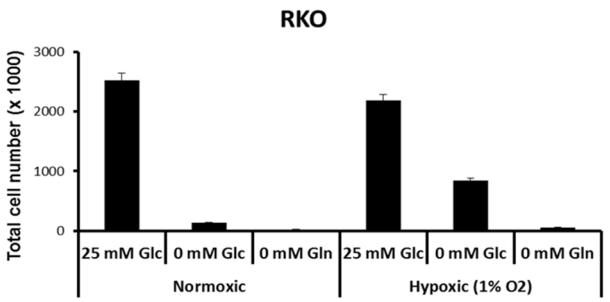
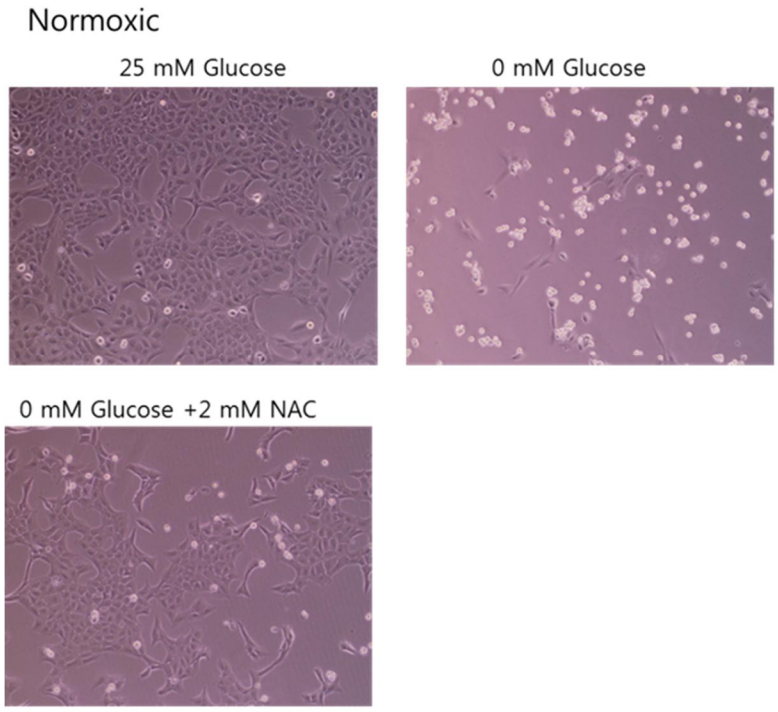


Figure 2. Hypoxic cancer cells under glucose deprivation showed increased cell survival. DLD-1 colorectal adenocarcinoma cells, 786-0 renal cell carcinoma cells, RKO colon cancer cells, H1299 non-small lung carcinoma cells were plated and on the next day, cells were cultured in DMEM with high glucose, DMEM without glucose containing 2 mM glutamine, DMEM without glutamine containing 25 mM glucose, respectively, and then exposed to normoxic or hypoxic (1% O_2) condition during 72 hr. Total cell numbers were counted at 72 hr after glucose or glutamine deprivation using ADAM-MC.

3. N-acetylcysteine rescued glucose deprivation-induced cell death

ROS generation during glucose deprivation can result in cell death.[15,16] We therefore investigated whether ROS mediate glucose deprivation-induced cell death. 786-0 cells were grown for 24 hr and media was changed to glucose-free DMEM with or without an antioxidant, N-acetylcysteine. Cells were microscopically analyzed at 72 hr after glucose deprivation. In normoxic condition, NAC rescued glucose deprivation-induced cell death (Figure 3A). Similar results were obtained in cell counting assay (Figure 3B). These results suggest that glucose deprivation-induced cell death is associated with ROS.

A



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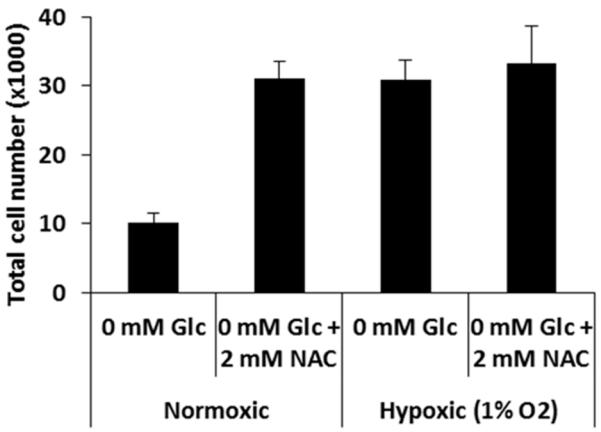


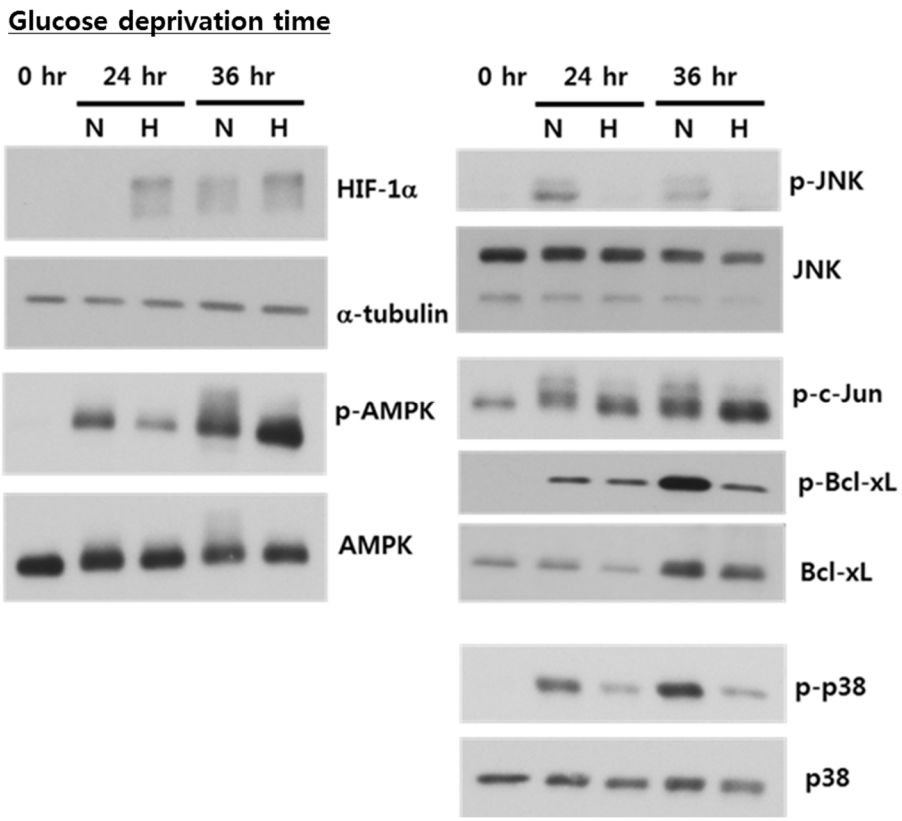
Figure 3. N-acetylcysteine rescued glucose deprivation-induced cell death. 786-0 cells were cultured for 1 day in basal medium. On the next day, the culture medium was replaced with DMEM with high glucose or DMEM without glucose and incubated in normoxic or hypoxic condition. 2 mM of NAC was treated during glucose deprivation. (A) Microscopic appearance of 786-0 cells in normoxic condition. (B) Total cell numbers were counted at 72 hr after glucose deprivation using ADAM-MC.

4. Glucose deprivation-induced cell death is related to JNK pathway

ROS-induced apoptosis requires the participation of other cell death signaling pathways, including Jun N-terminal kinase (JNK).[17] JNK is a stress-activated protein kinase that can be induced by inflammatory cytokines, bacterial endotoxin, osmotic shock, UV radiation, and hypoxia.[18] Mitochondrial translocation of JNK occurs in stressed cells,[19] and thus mitochondrially localized JNK provides the proximity to mitochondria-generated ROS and many apoptosis regulatory proteins such as Bcl-2 family proteins.[20,21]

Therefore, we examined whether JNK pathway activation is associated with glucose deprivation-induced cell death. Hypoxia-inducible factor-1α (HIF-1α) was detected as a maker of hypoxia and phosphorylation of AMP-activated protein kinase (AMPK), a cellular stress marker, was also detected during glucose deprivation. Activation of JNK and its downstream targets, including c-Jun, Bcl-xL, p38 were decreased by their phosphorylation state under hypoxia, whereas higher activation was induced in normoxia (Figure 4A). Furthermore, a treatment of JNK inhibitor SP600125 significantly inhibited glucose deprivation-induced cell death (Figure 4B). These findings indicate that JNK signaling pathway mediates glucose deprivation-induced cell death.

A



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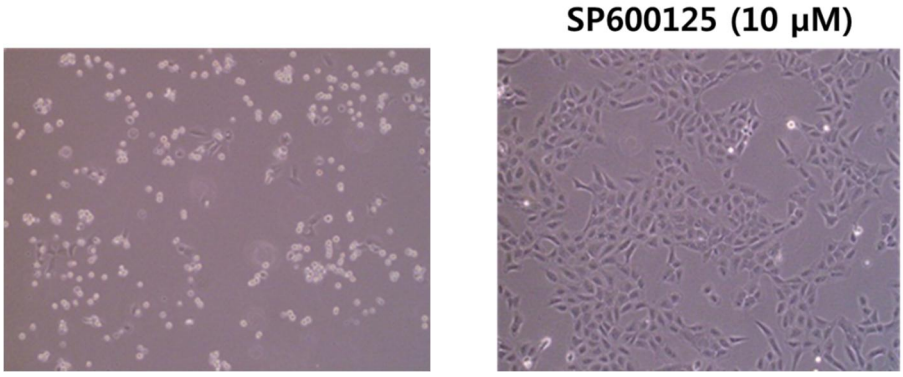

Figure 4. Glucose deprivation-induced cell death is related to JNK pathway. U2OS cells were grown for 1 day in basal medium. On the next day, the culture medium was replaced with DMEM with high glucose or DMEM without glucose and incubated in normoxic or hypoxic conditions for 24 hr or 48 hr. (A) Western blot analysis was performed for proteins that are associated with JNK pathway (p-JNK, JNK, p-c-Jun, p-Bcl-xL, Bcl-xL, p-p38, p38), hypoxia marker (HIF-1α), cellular stress marker (AMPK). The levels of α-tubulin were used as a loading control. (B) Microscopic appearance of glucose-deprived U2OS cells treated with or without 2 mM of SP600125.

5. Glucose deprivation-induced ROS is decreased in hypoxia

In normoxic condition, glucose deprivation induced a nearly two-fold increase in the mean fluorescent intensity (MFI) of the oxidation-dependent fluorogen 2', 7'-dichlorodihydrofluorescein diacetate (DCF-DA). In contrast, in hypoxia, glucose deprivation exhibited negligible increase in DCF-DA signal although the basal level of ROS is slightly higher than the level of normoxia (Figure 5A). We next tested whether levels of mitochondrial ROS can be changed following glucose deprivation. Similarly to DCF-DA assay, we found that glucose deprivation induced significant increase in mitochondrial ROS level under normoxia, and the level was decreased in hypoxia (Figure 5B).

Supporting this observation, treatment with NAC reduced mitochondrial ROS following glucose deprivation in normoxia. Thus, decreased level of ROS contributes to the cell survival after glucose deprivation in hypoxia.

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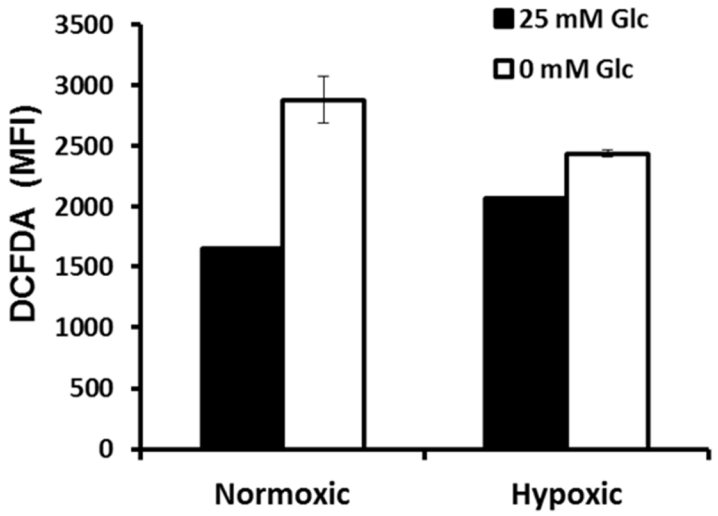

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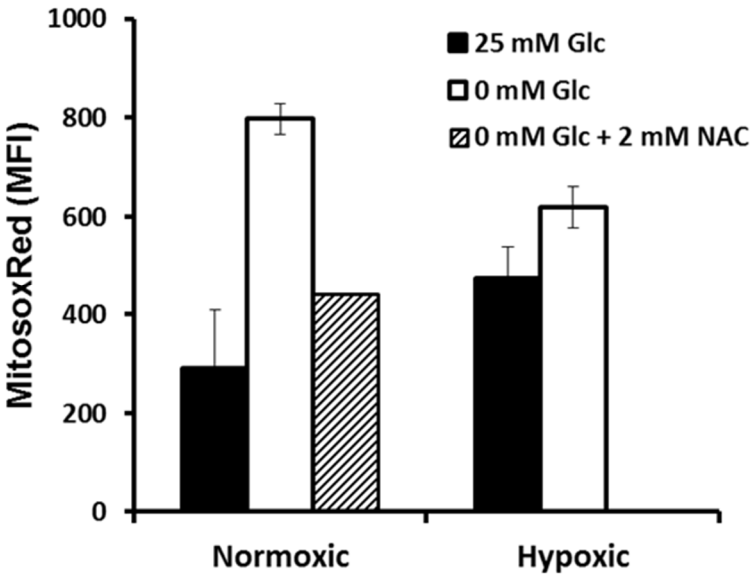

Figure 5. Glucose deprivation-induced ROS is decreased in hypoxia. Intracellular total ROS and mitochondrial superoxide level were assessed. (A) The intracellular levels of ROS were measured with H_2-DCFDA. Cells were treated with 5 mM of H_2-DCFDA for 30 min. (B) The level of mitochondrial superoxide production via FACS analysis of MitoSox Red intensity. 2 mM of NAC was treated during glucose deprivation under normoxia. The mean fluorescence intensity (MFI) of 10,000 cells was analyzed in each sample.

6. Glucose-deprived cells in hypoxia showed higher NADPH/NADP ratio compared with cells in normoxia

We speculated that decreased level of ROS under hypoxia may be associated with the ability of ROS detoxification. There are several antioxidant pathways for the elimination of ROS, and these involve reduced glutathione (GSH), thioredoxin (TXN). TXN, a protein that reduces ROS levels, can be regenerated by thioredoxin reductase (TXNRD) using the metabolite NADPH. GSH, which can also be regenerated by NADPH via glutathione reductase (GSR), is derived from the metabolites glutamate and cysteine. GSH acts directly on eliminating ROS through the action of glutathione peroxidase (GPX) and glutathione S-transferase (GST).[22-24]

To investigate the role of NADPH in hypoxia, we examined the NADPH /NADP ratio after glucose deprivation under normoxic or hypoxic condition in U2OS cells. As a result, Glucose deprivation rapidly depleted NADPH/NADP in normoxia, whereas cells in hypoxia maintained high NADPH/NADP.

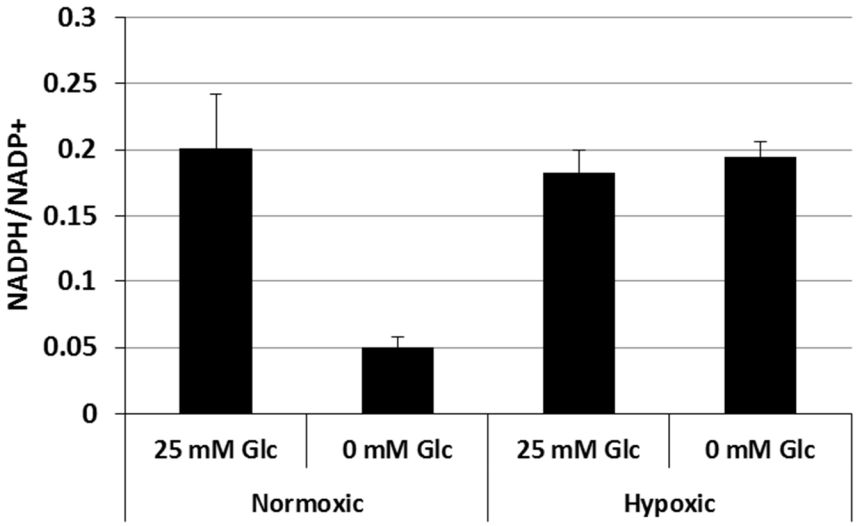

Figure 6. Glucose-deprived cells in hypoxia showed higher NADPH/NADP ratio compared with cells in normoxia. NADPH/NADP ratio after glucose deprivation in normoxic or hypoxic condition for 48 hr in U2OS cells. Infinite® F200 PRO was used.

7. The effect of NADPH-producing enzymes on hypoxia-induced cell survival

We then asked whether decreased glucose deprivation-induced cell death in hypoxia might due to upregulation of NADPH-producing enzymes, such as Glucose-6-phosphate Dehydrogenase (G6PD), 6-Phosphogluconate dehydrogenase (6PGD), malic enzyme 1/2 (ME1 and ME2), isocitrate dehydrogenase 1/2 (IDH1 and IDH2), nicotinamide nucleotide transhydrogenase (NNT). We determined NADPH-producing enzymes expression in U2OS exposed to glucose deprivation. The results have shown that hypoxia induced IDH2 expression (Figure 7A). Similar results are obtained by immunoblotting analysis (Figure 7B). However, IDH2 knockdown in U2OS cells had no effects on hypoxia-induced cell survival (Figure 7C), explaining NAPDH producing enzymes have no effects on hypoxia-induced cell survival upon glucose deprivation.

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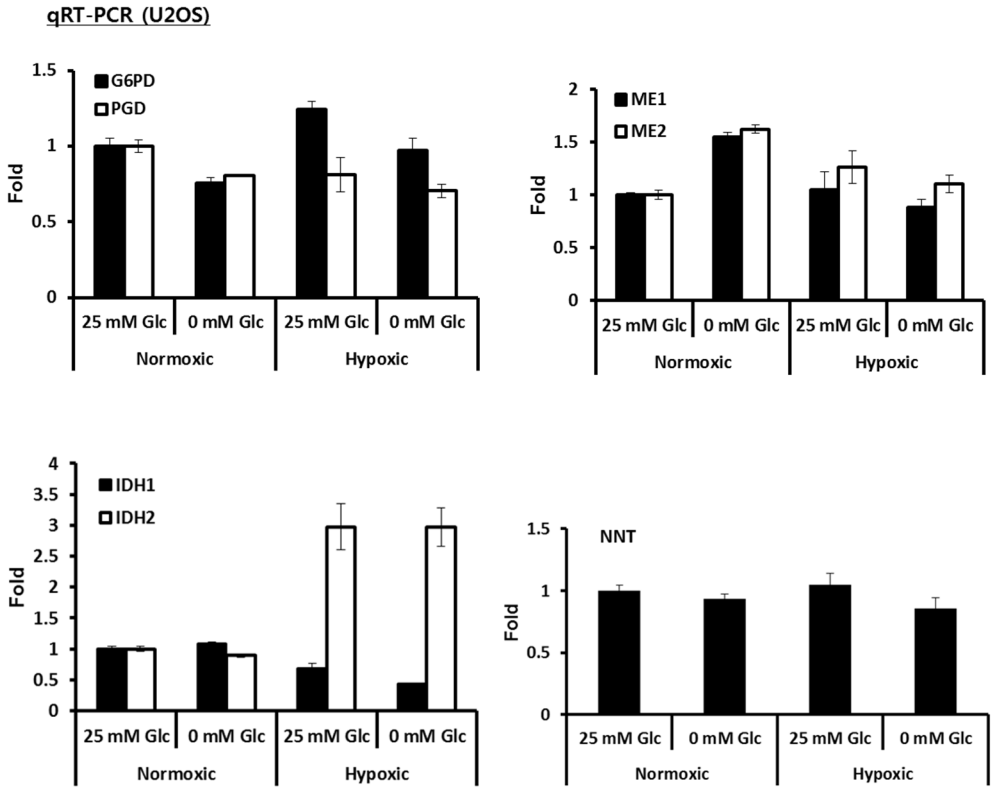

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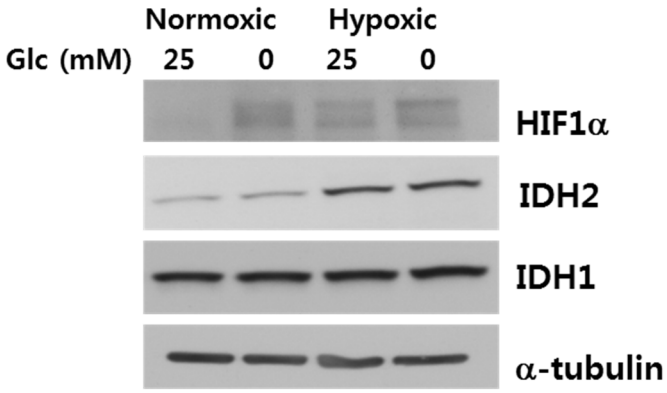

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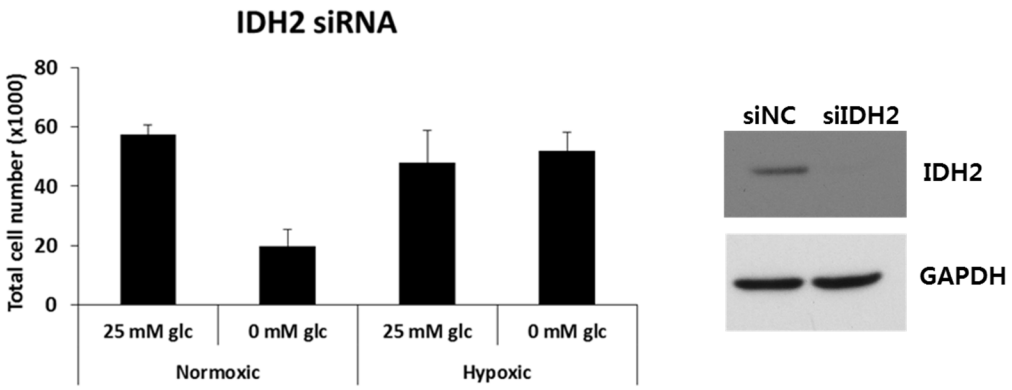

Figure 7. The effect of NADPH-producing enzymes on hypoxia-induced cell survival. (A) qRT-PCR of genes involved in NADPH production (G6PD, PGD, ME1, ME2, IDH1, IDH2, NNT) in U2OS cells. The relative amount of mRNA in each sample was normalized to 9S ribosomal RNA levels. (B) Western blot analysis for IDH1 and IDH2 in U2OS cells after glucose deprivation under normoxic or hypoxic condition for 48 hr. The levels of α-tubulin were used as a loading control. (C) Using ADAM-MC, cell counting for IDH2 siRNA-treated U2OS cells after glucose deprivation under normoxic or hypoxic condition for 72 hr.

Ⅳ. DISCUSSION

Solid tumors encounter metabolic stress such as glucose depletion or hypoxia, requiring alternative pathway for survival and proliferation. However, the underlying mechanisms of cell death and survival under insufficient both oxygen and nutrients are largely unknown. In this study, we demonstrate that hypoxia-exposed cancer cells are resistant to glucose deprivation-induced cell death through attenuating excessive ROS production.

We show that various cancer cells exhibit cell death in glucose free medium but not in glutamine deficient medium although cell proliferation rates were decreased in both medium. Notably, cells exposed to hypoxic condition showed complete cell viability upon glucose deprivation. Glucose deprivation-induced cell death has been observed in various cancer cells and skeletal muscle cells through several mechanisms.[3-9] CREB binds with p53 to repress transcription of MDM2 resulting in p53-induced cell death in wild type p53-expressing U2OS cells,[8] ovarian cancer cells activates AMPK concomitant with Akt inhibition and cell death,[5] excess ROS generation due to decreasing NADPH generation and stimulating tyrosine kinase signaling.[4,9]

The imbalance between ROS generation and elimination through antioxidant defense mechanism and consequent cellular oxidative stress mediates cell death. Oxidative stress could occur in response to various cellular stresses such as UV irradiation, ER stress, proinflammatory cytokines including tumor necrosis factor-α

(TNF-α) and interleukin-1 and glucose deprivation.[2,4,6,7] Under the sustained excess ROS generation, c-Jun N-terminal kinase (JNK)/p38 signaling pathway is activated by apoptosis signal activating kinase 1 (ASK1) and ASK1-independent pathway contributes to cell death.[17,25]

Since we observed that treatment of antioxidant and JNK inhibitor rescued glucose deprivation-induced cell death in normoxic condition, we speculated that hypoxia relieves glucose deprivation-induced cell death through ameliorating oxidative stress. As might be expected, hypoxic cells in glucose free medium did not exhibit decrease of NADPH/NADP ratio which indicates high detoxifying capacity is maintained during sustained glucose depletion in hypoxia. Furthermore, increase of ROS in response to glucose withdrawal in hypoxia is significantly smaller than that in normoxic condition, although basal level of ROS in glucose containing medium in hypoxia is increased as reported in other studies.[26,27] Whether cells produce increased amount of ROS during hypoxia remains controversial.[28] Mitochondrial ROS production during hypoxia is observed in skeletal muscle [3] and is necessary for hypoxia-induced transcriptional activation in hepatocellular carcinoma Hep3B cells [26] and HIF stabilization,[27] whereas there is a report in which no evidence for increased ROS production under moderate hypoxia (0.4-1.6%) [29] and even decrease of ROS due to reduction in mitochondrial function.[30]

Studies in respect to adaptation mechanism of hypoxic cells during sustained glucose deprivation are scarce except that there is a report demonstrates nitric oxide

treatment confer tolerance to glucose deprivation in a 5'-AMP-activated protein kinase manner.[31] In this study, we propose that the mechanism by which survival of hypoxic cancer cells in prolonged glucose depletion is related to decreased level of cellular ROS. Suppression of the exacerbating ROS production might be explained by several possibilities. In hypoxic conditions, the HIF-1 regulates switching of cytochrome c oxidase 4-1 (COX4-1) subunit to COX4-2 to maximize the efficient use of available oxygen thereby protects cells from oxidative damage.[32] Other possibility is that reduced mitochondrial function conserves oxygen for non-mitochondrial oxygen consumption.[33,34]

Our findings suggest that hypoxia suppress sustained activation of JNK through suppressing excess increase of ROS during prolonged glucose deficiency as a overcoming mechanism during both glucose and oxygen deficiency.

Ⅴ. CONCLUSION

Our findings suggest that reduction of intracellular ROS is key modulator of cancer cell survival from glucose deprivation under hypoxic condition, which accompanied by down-regulation of JNK pathway and the high level of NADPH relative to normoxic condition.

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ABSTRACT (IN KOREAN)

저산소 환경에서 활성산소종 조절을 통한 포도당 결핍에 의해 유도되는 암세포 사멸 억제

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이 유 신

고형암의 성장에 있어 대사 스트레스를 극복하는 것은 매우 중요하다. 하지만, 대사 스트레스 상태로부터 세포가 사멸하거나 살아남는 기전에 대해서 정확히 알려진 바 없다. 본 연구에서는 저산소 환경에 노출된 암세포가 포도당 결핍에 의한 세포죽음으로부터 살아남을 수 있으며 이러한 기전에는 활성산소가 관여한다는 것을 밝혔다. 또한 저산소 환경에서

의 이러한 현상은 정상 산소환경에서 암세포에 포도당 결핍을 준 경우와 비교했을 때 SAPK/JNK 신호경로의 감소, 높은 NADPH/NADP ratio를 수반한다는 것을 확인할 수 있었다. 항산화제인 NAC를 처리하였을 때 포도당 결핍으로 인한 세포사멸이 억제되는 것을 확인하였고, 저산소 환경에 노출된 암세포에 포도당 결핍을 준 후에 세포 내 활성산소 양을 측정하였다. 정상산소 환경에서는 포도당 결핍에 의해 암세포 내 활성산소 생성이 현저하게 증가하였다. 반면, 저산소 환경에 노출된 암세포의 경우 포도당 결핍에 의한 활성산소의 생성양은 미미한 증가를 보였다. 하지만 저산소 환경에 노출된 세포 내 기본적인 활성산소양은 정상 산소 환경일 때에 비해 높게 나타나는 것을 관찰할 수 있었다. 위의 실험 결과들을 통해 저산소 환경에 노출된 암세포는 산화환원 반응을 조절함으로써 포도당 결핍에 의해 유도되는 세포사멸로부터 살아남는 것임을 예측할 수 있다.

핵심되는 말 : 저산소 환경, 포도당 결핍, 활성산소종