doi:10.2478/enr-2025-0020

Hypoxic regulation of insulin receptor substrate 2 gene expression is differently regulated by endoplasmic reticulum stress and hydrocortisone in normal human astrocytes and glioblastoma cells

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Objective. The insulin receptor substrate 2 (IRS2) is phosphorylated by the tyrosine kinase activity of the insulin receptor and the insulin-like growth factor I (IGF-1) receptor upon receptor stimulation. It mediates insulin signaling controlling metabolism as well as cell proliferation and invasion in tumors. Hypoxia and endoplasmic reticulum (ER) stress are significant factors in regulating the growth of malignant tumors including glioblastoma. The present study aims to investigate the regulation of the IRS2 gene expression in normal human astrocytes and U87MG glioblastoma cells by hypoxia and ER stress in the context of the native stress hormone hydrocortisone, which is widely used for the co-treatment of glioblastoma.

Methods. The normal human astrocytes (line NHA/TS) and U87MG glioblastoma cells were used. Hypoxia was introduced by the HIF1A prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG), which mimics the effects of hypoxia under normoxic conditions. Tunicamycin and thapsigargin were used for the induction of ER stress. Hydrocortisone-water soluble BioReagent, suitable for cell culture (cyclodextrin-encapsulated hydrocortisone) was used. Cells were treated with DMOG, tunicamycin, thapsigargin, and hydrocortisone for 4 h. RNA was extracted with TRIzol reagent. IRS2 gene expression was examined by quantitative real-time RT-PCR and normalized to beta-actin mRNA.

Results. It was found that hypoxia decreased the IRS2 gene expression in normal human astrocytes, but upregulated it in glioblastoma cells. At the same time, hydrocortisone did not significantly change the expression of this gene in both normal astrocytes and glioblastoma cells. However, hypoxia in combination with hydrocortisone strongly increased IRS2 gene expression in both cell types. Tunicamycin decreased the expression of the IRS2 gene in normal astrocytes, but increased it in glioblastoma cells and this effect of tunicamycin was not significantly altered by hypoxia in both cell types. At the same time, thapsigargin did not significantly alter the expression of the IRS2 gene in normal astrocytes, but it strongly upregulated it in glioblastoma cells. Hypoxia modified the effect of thapsigargin on this gene expression in both cell types, but by different ways: decreased in normal astrocytes and increased in glioblastoma cells. In addition, the impact of tunicamycin and thapsigargin on IRS2 gene expression was significantly upregulated by hydrocortisone in normal astrocytes and especially in glioblastoma cells. At the same time, the combined effect of hypoxia and hydrocortisone enhanced the expression of the IRS2 gene in tunicamycin-treated normal astrocytes, especially in the glioblastoma cells. Hydrocortisone also increased the effect of hypoxia on this gene expression in thapsigargin-treated normal astrocytes and decreased it in glioblastoma cells.

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Conclusion. Our findings provide evidence that hypoxic regulation of IRS2 gene expression is modified by inducers of ER stress and hydrocortisone, but differently in normal astrocytes and glioblastoma cells and that the combined effect of hypoxia with ER stress and hydrocortisone greatly enhanced this gene expression in both cell types, especially in the glioblastoma cells.

Keywords: hypoxia, hydrocortisone, tunicamycin, thapsigargin, IRS2, normal human astrocytes, U87MG glioblastoma cells

The insulin receptor substrate 2 (IRS2) is a signaling adapter protein that participates in the signal transduction from the insulin receptor (INSR) and insulin-like growth factor I (IGF-1) receptor. It mediates the control of various cellular processes by insulin and IGF-1 including cell metabolism, proliferation, and invasion in tumors (Mercado-Matos et al. 2018; Tan et al. 2020; Chen and Chen 2021; Ahmed et al. 2023; Greenberg et al. 2025). IRS2 is phosphorylated by the tyrosine kinase activity of these receptors upon their stimulation. Phosphorylated IRS2 plays an important role not only in the glucose homeostasis, but also in the development, growth, and tumorigenesis (Copps and White 2012; Porter et al. 2013; Manohar et al. 2020; Kubota et al. 2025).

Glioblastoma is the most common and aggressive malignant primary brain tumor with a limited response to therapy (McFaline-Figueroa and Lee 2018; Lah et al. 2020). The endoplasmic reticulum (ER) stress and hypoxia are essential factors in glioblastoma progression as well as other malignant tumors (Denko 2008; Chevet et al. 2015; Hetz et al. 2020; Ma et al. 2023). Cancer cells maintain their malignancy through metabolic reprogramming and adaptations to hypoxia primarily via ER stress signaling pathways enabling them to survive in hypoxic conditions (Almanza et al. 2019; Lebeaupin et al. 2020; Ediriweera and Jayasena 2023; Lin et al. 2023). ER stress intensifies the survival of glioblastoma cells and their polyresistance including chemoresistance (Chevet et al. 2015; Logue et al. 2018; Papaioannou and Chevet 2018). Hypoxia is known to reprogram glucose metabolism through specific changes in the expression of numerous genes that contribute to malignant tumor growth and its resistance to therapy (Minchenko et al. 2002; Batie and Rocha 2020; Bao and Wong 2021; Sebestyen et al. 2021; Taneja et al. 2024). Hypoxia increases the alpha subunit of the transcription factor HIF, which regulates the expression of genes with hypoxia-responsive element in promoter region (Minchenko and Caro 2000; Minchenko et al. 2004; Infantino et al. 2021). However, more than 150 proteins have been identified that can interact with HIF1A altering its stability and transcriptional activity through different mechanisms including phosphorylation (Semenza 2017). We have previously demonstrated that HIF-1 mediates the hypoxic induction of endothelin-1 gene expression in microvascular endothelial cells and that the protein kinase inhibitor genistein abrogates the effect of hypoxia on this gene's expression (Minchenko and Caro 2000).

Glucocorticoids are widely used in cancer patients as part of their antitumor treatment; however, these compounds can also contribute to the development of pathological processes that are closely linked to malignant tumor progression and metastasis possibly through diverse mechanisms (Azher et al. 2016; Lin and Wang 2016; Obradovic et al. 2019; Hirko and Eliassen 2021; Butz and Patocs 2022). Glucocorticoids are primary stress hormones that control various aspects of metabolism as well as stress, development, and inflammatory responses through multiple mechanisms of transcriptional regulation in a physiological context (Kadmiel and Cidlowski 2013; Frank et al. 2021). Numerous effects of glucocorticoid hormones are mediated through a specific receptor, which represents a transcription factor NR3C1. The glucocorticoid receptor binds to the glucocorticoid hormone for activation and regulates transcription of target genes through the glucocorticoid response elements in their promoter regions (Frank et al. 2021). Moreover, the glucocorticoid receptor can modulate the activity of other transcription factors (Ratman et al. 2013; Beaupere et al. 2021). However, much remains to be understood about the function of this receptor in cancer including implications for cancer progression and drug resistance as well as about the duality of glucocorticoid action in malignant tumors (Kadmiel and Cidlowski 2013; Mayayo-Peralta et al. 2021; Khadka et al. 2023).

The effect of glucocorticoid hormones on gene expression depends on the level and activity of the glucocorticoid receptor, which can be modulated by several coregulators (Leonardi et al. 2019). It has also been demonstrated that inhibition of the ERN1 signaling protein leads to upregulation of glucocorticoid receptor NR3C1 expression in glioblastoma cells (Minchenko et al. 2016). Hypoxia also increases the expression of the *NR3C1* gene in glioblastoma cells; however, suppression of ERN1 activity decreased the effect of hypoxia on its expression (Minchenko et al. 2016). Thus, ER stress and hypoxia, as important factors in tumor growth, affect the expression of the

glucocorticoid receptor as well as several proteins associated with its function (Minchenko et al. 2016). These results have shown that the glucocorticoid receptor-mediated effect of glucocorticoid hormones on gene expression in glioblastoma cells is also dependent on ER stress and hypoxia.

However, the dependence of the effect of hydrocortisone on gene expression on ER stress and hypoxia has not yet been sufficiently studied not only in normal cells, but also in malignant cells. Recently, it has been demonstrated that hydrocortisone interacts with hypoxia and ER stress in the regulation of *INSR* and its target genes *IRS2* and *INSIG2* expressions as well as ER stress-dependent genes *XBP1* and *DNAJB9* in kidney embryonic cells line HEK293 (Minchenko et al. 2024b). It has also been demonstrated that hydrocortisone controls the regulation of *PSAT1* gene expression by hypoxia and ER stress and that the combined effect of hydrocortisone and hypoxia greatly enhanced this gene expression in tunicamycin-treated normal astrocytes and glioblastoma cells (Minchenko et al. 2025).

ER stress and hypoxia are important factors of malignant tumor progression, metabolic reprogramming, and therapeutic resistance, but very little is known about their interaction with glucocorticoids used for co-treatment of glioblastoma. Furthermore, there are no available data regarding the interaction of these factors in controlling *IRS2* gene expression in normal astrocytes and glioblastoma cells. In this study, we aimed to investigate the regulation of the *IRS2* gene expression in normal human astrocytes and U87MG glioblastoma cells by ER stress induced by two different compounds and hypoxia induced by dimethyloxalylglycine (DMOG) dependent on the action of native stress hormone hydrocortisone, which is widely used for the co-treatment of glioblastoma and other malignant tumors.

Materials and Methods

Cell lines and culture conditions. In this investigation, normal human astrocytes, line NHA/TS, and U87MG glioblastoma cells were used. Cells were grown as described (Auf et al. 2013). Cells were treated for 4 h with hydrocortisone (10 μM), tunicamycin (0.5 μg/ml) and thapsigargin (2 μM) for induction of ER stress, and DMOG (0.5 mM), a HIF1A prolyl hydroxylase inhibitor, which mimics the effects of hypoxia under normoxic conditions as described previously (Minchenko et al. 2002, 2024a). Hydrocortisone was received from Sigma-Aldrich (St. Louis, MO, USA) as a Hydrocortisone-Water Soluble BioReagent, suitable for cell culture (cyclodextrin-encapsulated hydrocortisone, H0396). Tunicamycin and thapsigargin were also received from Sigma-Aldrich, and DMOG from Selleck Chemicals, Huston, TX, USA.

In this investigation, we used twelve groups of both normal human astrocytes and glioblastoma cells: 1 – control, 2 – separate impact of hydrocortisone, 3 – separate impact of hypoxia, 4 – separate impact of tunicamycin, 5 – separate impact of thapsigargin, 6 – combined impact of hydrocortisone with hypoxia, 7 – combined impact of tunicamycin with hydrocortisone, 8 – combined impact of tunicamycin with hypoxia, 9 – combined impact of tunicamycin with hydrocortisone and hypoxia, 10 – combined impact of thapsigargin with hydrocortisone, 11 – combined impact of thapsigargin with hypoxia, and 12 – combined impact of thapsigargin with hydrocortisone and hypoxia, which covered all separate and combined effects of four used conditions (hydrocortisone, hypoxia, tunicamycin, and thapsigargin).

RNA isolation. Total RNA was isolated from normal human astrocytes and glioblastoma cells using the TRIzol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). The RNA pellets were washed twice with 75% ethanol and dissolved in nuclease-free water. Concentration of RNA and its spectral characteristics were measured using NanoDrop Spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative PCR analysis. The Thermo Scientific Verso cDNA Synthesis Kit (Germany) was used for reverse transcription as described (Minchenko et al. 2024c). The expression levels of *IRS2* and *ACTB* mRNAs were measured in normal human astrocytes and U87MG glioblastoma cells by quantitative polymerase chain reaction using the Luna Universal qPCR Master Mix (New England Biolabs, Ipswich, MA, USA) and "QuantStudio 5 Real-Time PCR System" (Applied Biosystems, USA). Polymerase chain reaction was performed in triplicate. As a control of the analyzed mRNA quantity the expression of *ACTB* mRNA was used. Primers for *IRS2* and *ACTB* were described previously (Minchenko et al. 2024b).

The results of quantitative PCR were analyzed using the "Differential Expression Calculator". Statistical analysis of the obtained results was performed using GraphPad Prism 8.0.1. The values of studied gene expression were normalized to the expression of ACTB mRNA and expressed as a percentage of controls (100%). All values were expressed as mean±SEM from triplicate measurements performed

in 4 independent experiments. A value of $p<0.05$ was considered significant in all cases. All experimental qPCR data were analyzed for the normality of distribution using a graphical tool (normal probability plot) and a histogram as described previously (Rudnytska et al. 2021). A normal distribution was observed for all analyzed datasets.

Results

We studied the impact of hypoxia (induced by DMOG) and hydrocortisone first separately and then in combination on *IRS2* gene expression in normal human astrocytes to evaluate the interaction of these factors in *IRS2* gene expression regulation. As shown in Figure 1, the expression level of this gene was down-regulated by hypoxia (by 16%; $p<0.05$) in normal human astrocytes in comparison with control cells. At the same time, no significant changes were detected in the expression of this gene in normal astrocytes exposed to hydrocortisone (Figure 1). Although hydrocortisone did not significantly change the level of *IRS2* gene expression, it significantly modified the effect of hypoxia on the expression of this gene in normal human astrocytes. From the data presented in Figure 1, it can be seen that under conditions of hypoxia, this steroid hormone increased the expression level of the *IRS2* gene by 51% ($p<0.01$) compared to the control and by 80% ($p<0.001$) compared to the action of hypoxia alone.

Completely different results were obtained when studying the separate and combined effects of hypoxia and hydrocortisone on the expression of the *IRS2* gene in U87MG glioblastoma cells (Figure 2). Thus, the expression of this gene in glioblastoma cells was significantly increased by hypoxia (by 47%; $p<0.01$). At the same time, the *IRS2* gene expression in these cells was resistant to hydrocortisone action as compared to control glioblastoma cells (Figure 2). However, in hypoxic conditions, the expression of this gene was strongly induced by hydrocortisone. Thus, under the combined action of hydrocortisone with hypoxia, the level of the *IRS2* gene expression increased by 259% ($p<0.001$) compared to the control and by 144% ($p<0.001$) compared to the action of hypoxia alone (Figure 2).

We also studied the impact of tunicamycin alone and in combination with hypoxia and hydrocortisone on the expression of the *IRS2* gene in normal human astrocytes. As shown in Figure 3, the expression level of this gene is decreased (by 22%; $p<0.05$) in normal astrocytes treated with tunicamycin compared to the control cells. At the same time, hypoxia did not significantly change the effect of tunicamycin on *IRS2* gene expression in normal human astrocytes as compared to the action of tunicamycin alone (Figure 3). However,

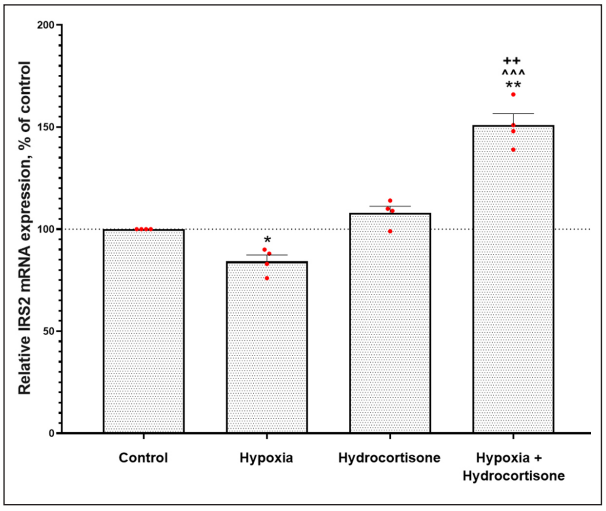

Figure 1. The impact of dimethyloxalylglycine (hypoxia), hydrocortisone and their combination on the expression level of insulin receptor substrate 2 (*IRS2*) in normal human astrocytes (line NHA/TS) measured by qPCR. The values of this mRNA expression were normalized to ACTB mRNA and presented as a percentage of the control. Data are presented as mean±SEM; *$p<0.05$ and **$p<0.01$ vs. control; ^^^$p<0.001$ vs. hypoxia; ++$p<0.01$ vs. hydrocortisone.

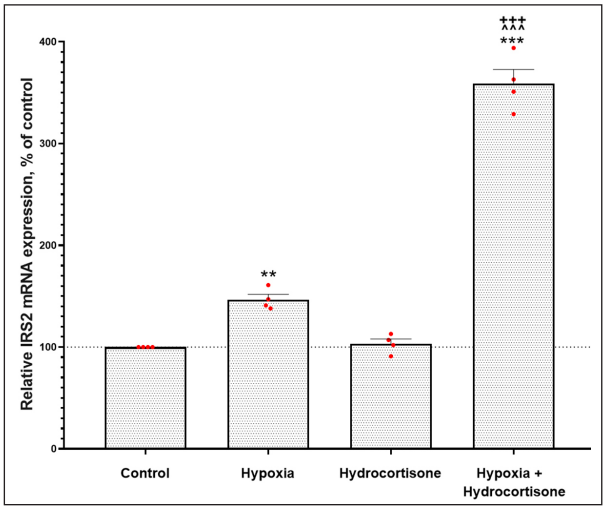

Figure 2. The impact of hypoxia (dimethyloxalylglycine), hydrocortisone and their combination on the expression level of insulin receptor substrate 2 (*IRS2*) in glioblastoma cells measured by qPCR. The values of *IRS2* mRNA expression were normalized to ACTB mRNA and presented as a percentage of the control. Data are presented as mean±SEM; **$p<0.01$ and ***$p<0.001$ vs. control; ^^^$p<0.001$ vs. hypoxia; +++$p<0.001$ vs. hydrocortisone.

hydrocortisone significantly increased the expression level of the *IRS2* gene in tunicamycin-treated normal astrocytes by 21% ($p<0.05$) as compared to control cells and by 55% ($p<0.01$) as compared to the action of tunicamycin alone (Figure 3), although in the absence of tunicamycin, hydrocortisone did not significantly change the expression of this gene in normal astrocytes (Figure 1).

The opposite changes in the expression of the *IRS2* gene were observed in glioblastoma cells under induction of ER stress by tunicamycin compared to normal astrocytes. We demonstrated that tunicamycin increased the expression of the *IRS2* gene in glioblastoma cells by 17% ($p<0.05$) compared to control cells indicating the involvement of ER stress signaling pathways in positive control of this gene expression in glioblastoma cells (Figure 4). At the same time, the expression of the *IRS2* gene in glioblastoma cells was resistant to hypoxia in the presence of tunicamycin; however, it was increased by hydrocortisone by 57% ($p<0.001$) as compared to control cells and 34% ($p<0.01$) as compared to the action of tunicamycin alone (Figure 4). At the same time, in the absence of tunicamycin, hydrocortisone did not significantly change the expression of the *IRS2* gene in these cells (Figure 2). Strong up-regulation of the *IRS2* gene expression was observed in glioblastoma cells simultaneously treated with tunicamycin, hydrocortisone, and hypoxia by 336% ($p<0.001$) as compared to control cells, 257% ($p<0.001$) as compared to cells treated with tunicamycin and hypoxia, and 178% ($p<0.001$) as compared to the action of tunicamycin with hydrocortisone (Figure 4).

As shown in Figure 5, thapsigargin did not significantly change the expression of the *IRS2* gene in normal astrocytes. However, hypoxia down-regulated the level of *IRS2* gene expression by 15% ($p<0.05$) in thapsigargin-treated normal human astrocytes compared to the control. At the same time, hydrocortisone significantly increased the expression level of this gene (by 82%; $p<0.001$) in thapsigargin-treated normal astrocytes as compared to control cells (Figure 5). Even a greater increase in *IRS2* gene expression was observed in normal astrocytes simultaneously treated with thapsigargin, hydrocortisone, and hypoxia by 122% ($p<0.001$) as compared to control, 161% ($p<0.001$) as compared to the combined effect of thapsigargin and hypoxia, and 22% ($p<0.05$) as compared to cells simultaneously treated with thapsigargin and hydrocortisone (Figure 5).

We also studied the impact of thapsigargin on *IRS2* gene expression in glioblastoma cells. As shown in Figure 6, the expression level of this gene is strongly increased by thapsigargin (by 202% $p<0.001$) as

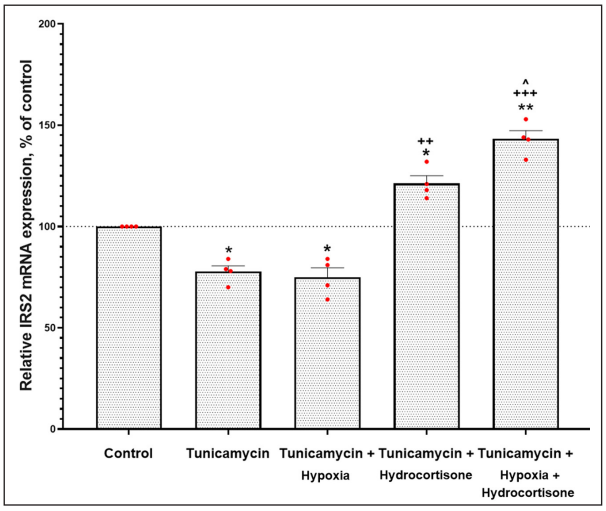

Figure 3. The impact of tunicamycin and its combination with hypoxia and hydrocortisone on the expression of insulin receptor substrate 2 (*IRS2*) mRNA in normal human astrocytes measured by qPCR. The values of this mRNA expression were normalized to beta-actin mRNA and presented as a percentage of the control. Data are presented as mean±SEM; *$p<0.05$ and **$p<0.01$ vs. control; $^{++}p<0.01$ and $^{+++}p<0.001$ vs. tunicamycin and tunicamycin+hypoxia; ^$p<0.05$ vs. tunicamycin+hydrocortisone.

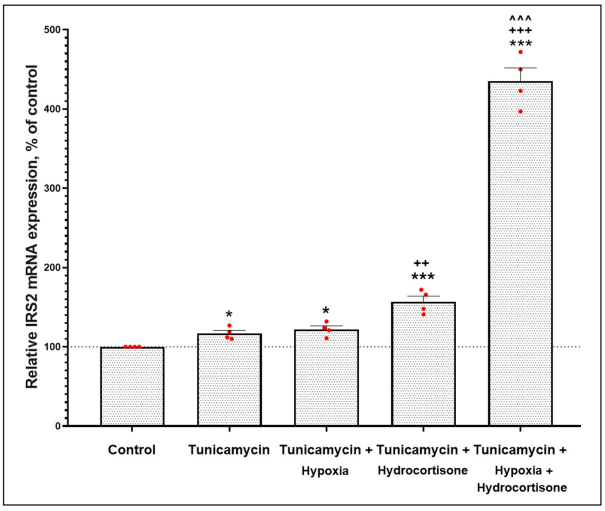

Figure 4. The impact of tunicamycin and its combination with hypoxia and hydrocortisone on the expression of insulin receptor substrate 2 (*IRS2*) mRNA in glioblastoma cells measured by qPCR. The values of *IRS2* mRNA expression were normalized to beta-actin mRNA and presented as a percentage of the control (Vector; 100%). Data are presented as mean±SEM; *$p<0.05$ and ***$p<0.001$ vs. control; $^{++}p<0.01$ and $^{+++}p<0.001$ vs. tunicamycin and tunicamycin+hypoxia; ^^^$p<0.001$ vs. tunicamycin+hydrocortisone.

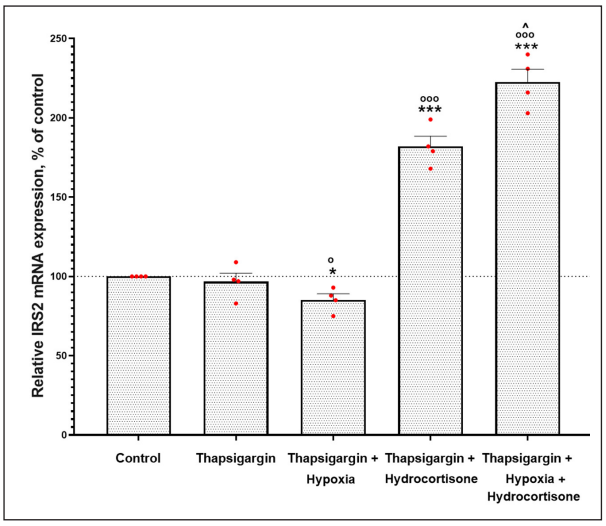

Figure 5. The impact of thapsigargin and its combination with hypoxia and hydrocortisone on the expression of insulin receptor substrate 2 (*IRS2*) mRNA in normal human astrocytes measured by qPCR. The values of this mRNA expression were normalized to beta-actin mRNA and presented as a percentage of the control. Data are presented as mean±SEM; *p<0.05 and ***p<0.001 vs. control; °p<0.05 and °°°p<0.001 vs. thapsigargin and thapsigargin+hypoxia; ^p<0.05 vs. thapsigargin+hydrocortisone.

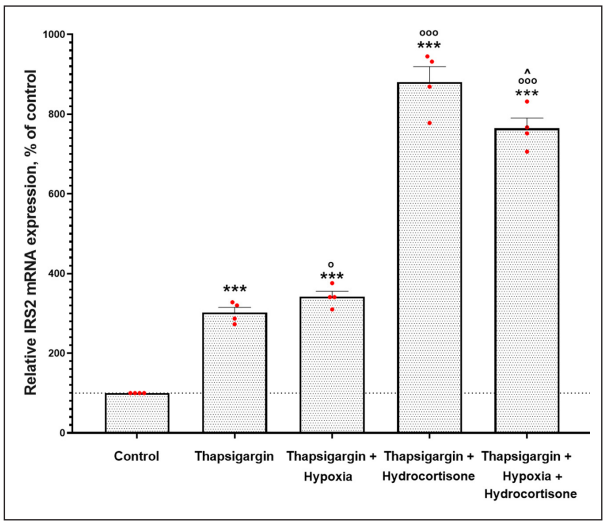

Figure 6. The impact of thapsigargin and its combination with hypoxia and hydrocortisone on the expression of insulin receptor substrate 2 (*IRS2*) mRNA in glioblastoma cells measured by qPCR. The values of *IRS2* mRNA expression were normalized to beta-actin mRNA and presented as a percentage of the control. Data are presented as mean±SEM; ***p<0.001 vs. control; °p<0.05 and °°°p<0.001 vs. thapsigargin and thapsigargin+hypoxia; ^p<0.05 vs. thapsigargin+hydrocortisone.

compared to control. Hypoxia also increased the expression of the *IRS2* gene in thapsigargin-treated glioblastoma cells by 13% (p<0.05) as compared to the effect of thapsigargin alone (Figure 6). However, a more significant increase in the level of the *IRS2* gene expression was observed in glioblastoma cells under the simultaneous action of thapsigargin and hydrocortisone by 781% (p<0.001) as compared to control glioblastoma cells. However, the synchronized action of thapsigargin, hydrocortisone, and hypoxia decreased the *IRS2* gene expression by 13% (p<0.05) as compared to glioblastoma cells simultaneously treated with thapsigargin and hydrocortisone (Figure 6). At the same time, the level of *IRS2* gene expression in glioblastoma cells under the simultaneous action of thapsigargin, hydrocortisone, and hypoxia is high compared to the action of thapsigargin alone by 153% (p<0.001), and compared to the combined action of thapsigargin and hypoxia 123% (p<0.001, Figure 6).

Thus, the effect of hydrocortisone on *IRS2* gene expression is differentially dependent on ER stress and hypoxia in normal astrocytes and glioblastoma cells. Induction of ER stress by tunicamycin and thapsigargin differentially alters the efficacy of hydrocortisone on *IRS2* gene expression in normal astrocytes and glioblastoma cells. Moreover, during induction of ER stress by tunicamycin, hypoxia dramatically enhances the effect of hydrocortisone on *IRS2* gene expression in glioblastoma cells; however, in combination with thapsigargin this effect is reduced.

Discussion

The major finding reported here is that the expression of the gene encoding the IRS2 is resistant to hydrocortisone in both normal human astrocytes and glioblastoma cells and that hypoxia and ER stress significantly modify its effect on *IRS2* expression in cell-dependent manner. The results of this investigation are summarized in Figure 7. These results indicate that *IRS2* gene expression is sensitive to hypoxia and ER stress differently in normal astrocytes and glioblastoma cells and that the two different inducers of ER stress, tunicamycin, and thapsigargin, differentially alter the efficacy of hydrocortisone and hypoxia on *IRS2* gene expression in normal astrocytes and glioblastoma cells.

We demonstrated that hypoxia increases the expression of the *IRS2* gene in glioblastoma cells, but suppresses in normal astrocytes. At the same time, hydrocortisone significantly modifies the effect of

hypoxia on this gene expression. We observed strong induction of the *IRS2* gene expression in both glioblastoma cells and normal astrocytes (Figure 7). These data are in good agreement with previous findings that hypoxia (3% oxygen, 16 h) enhanced *IRS2* gene expression in glioblastoma cells (Minchenko et al. 2013). Previously, it was also demonstrated that hydrocortisone and hypoxia affect the expression of different genes differently and that hypoxia modifies the impact of hydrocortisone on the *INSR* (insulin receptor) and *INSIG2* gene expressions, but not the *IRS2* in HEK293 cells (Minchenko et al. 2024b). Hypoxic regulation of gene expression is realized preferentially through transcription factor HIF, but the changes in gene expression under hypoxia are gene-specific (Minchenko et al. 2004; Denko 2008; Wicks and Semenza 2022; Taneja et al. 2024).

Moreover, there are HIF-independent mechanisms of regulating metabolism in hypoxic tumor cells (Minchenko et al. 1994; Minchenko and Caro 2000; Lee et al. 2021). It is known that many proteins interacting with HIF1A can alter hypoxic regulation of gene expression in diverse ways (Semenza 2017). The interaction of these proteins, which have different mechanisms of action with the HIF1A protein, can either reduce or enhance its degradation and alter its transcriptional activity differently (Semenza 2017). Among the proteins interacting with HIF1A, co-activators and co-repressors have been identified, which can enhance and suppress the transcriptional activity of this transcription factor. It is possible that the glucocorticoid receptor and associated proteins also exhibit co-activator and co-repressor activities, since their expression under hypoxia changes differently in magnitude and direction in glioblastoma cells (Minchenko et al. 2016).

Moreover, the effect of hypoxia on the expression of numerous genes depends on ER stress, particularly its ERN1 signaling pathway, which is responsible for alternative splicing of the transcription factor XBP1 (Minchenko et al. 2016, 2019, 2020, 2021; Sliusar et al. 2023). The transcription factor XBP1s regulates ERN1-dependent expression of numerous genes and is a co-activator of HIF1A (Semenza 2017). Moreover, tunicamycin, an inducer of ER stress, alters the expression of the *IRS2* gene in normal astrocytes and glioblastoma cells differently; however, in combination with hydrocortisone and hypoxia, it markedly enhances the expression of this gene in both cell types with stronger effect in glioblastoma cells (Figure 7).

These data indicate that ER stress and hypoxia, two key pro-oncogenic factors, alter the level of *IRS2* gene expression in normal astrocytes to the tumor cell type. This may be the basis for the development of pathophysiological processes that are strictly

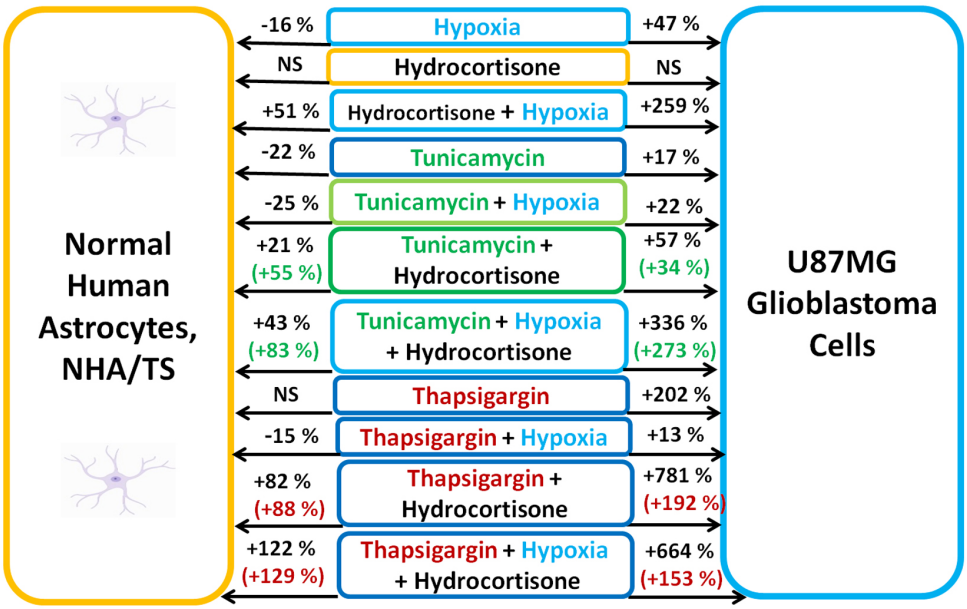

Figure 7. Schematic demonstration of the *IRS2* mRNA expression sensitivity in normal human astrocytes and glioblastoma cells to different treatments: hypoxia, hydrocortisone, and inductor of endoplasmic reticulum stress (tunicamycin or thapsigargin) as well as to their different combinations vs. non-treated control cells or vs. cells treated with tunicamycin or thapsigargin alone (in green and red brackets, respectively).

related to malignancy, tumor progression, and metastasis when using glucocorticoids for co-therapy of malignant tumors (Azher et al. 2016; Obradovic et al. 2019; Manohar et al. 2020; Hirko and Eliassen 2021; Mayayo-Peralta et al. 2021; Afshari et al. 2022; Butz and Patocs 2022). These results agree well with previously reported data concerning the involvement of ERN1 signaling protein in the control of hypoxic regulation of gene expression (Minchenko et al. 2016, 2019, 2020; Sliusar et al. 2023).

In conclusion, the data of the present study showed that the sensitivity of *IRS2* gene expression to ER stress and hypoxia is lower in normal human astrocytes than in U87MG glioblastoma cells and that ER stress in combination with hypoxia strongly enhances the sensitivity of this gene expression to hydrocortisone action less pronounced in normal astrocytes than glioblastoma cells. Furthermore, glucocorticoids are important regulators of hypoxia-associated ER stress especially in normal astrocytes. This may shed light on the development of complications during co-therapy of tumors with these hormones. However, the detailed molecular mechanisms of the interaction of glucocorticoids with ER stress, its signaling pathways, and hypoxia in the control of gene expression and tumor growth are not are not yet fully understood and require further study.

Acknowledgement

This work was funded by the State Budget Program "Support for the Development of Priority Areas of Scientific Research" (Code: 6541030).

Conflict of interest: *The authors declare no conflicts of interest.*

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