BIOSCIENCES REPORTS
https://biosciencesreports.com
editor@biosciencesreports.com
ISSN: 3104-400X (Print)
ISSN: 3104-4018 (Online)

EXPLORING THE CROSSTALK BETWEEN AUTOPHAGY AND APOPTOSIS IN HYPOXIC TUMOR MICROENVIRONMENTS

Jawad Ali[1*], Sajjad Mehdi[2]
[1]National University of Medical Sciences, Rawalpindi, Punjab, Pakistan
[2]King Edward Medical College, Lahore, Punjab, Pakistan

*Corresponding Author E-mail: jawadrana34@gmail.com

Abstract

Hypoxia is a hallmark of the tumor microenvironment that drives profound metabolic and molecular changes in cancer cells. This study investigates the dynamic crosstalk between autophagy and apoptosis in response to hypoxic stress using in vitro models of solid tumors. Our quantitative findings demonstrate that under early hypoxic conditions, autophagy is significantly upregulated as a pro-survival response, evidenced by increased LC3B-II/LC3B-I ratios and p62 degradation, while apoptotic markers remain relatively suppressed. However, prolonged exposure to hypoxia results in a marked shift toward apoptosis, characterized by increased Bax/Bcl-2 ratios, mitochondrial depolarization, caspase-3/7 activation, and loss of cell viability. HIF-1α expression was consistently elevated, triggering transcription of autophagy-related genes such as BNIP3 and ULK1, yet also contributing to oxidative stress and ER stress pathways that activate intrinsic apoptosis. Assays for cell functions indicated that interrupting autophagy in cells increases the likelihood of cell death from hypoxia, whereas stopping apoptosis allows cells to survive by relying on autophagy. The use of flow cytometry, JC-1 staining, MTT viability assays and clonogenic survival increases the evidence for a shift from protective autophagy to death by apoptosis. Using integrated approaches of protein and gene expression, coupled with imaging and function analysis, it was found that cell type is determined by the delicate balance of molecules in response to hypoxia time and pathway changes. Thanks to these results, we can see how stress in tumors changes over time and how controlling autophagy-apoptosis interactions may help treat cancer more effectively when tumors lack oxygen.

Keywords: Hypoxia, Tumor Microenvironment, Autophagy, Apoptosis, HIF-1α, Caspase Activation.

Article History

Received:
January 22, 2025

Revised:
February 19, 2025

Accepted:
March 23, 2025

Available Online:
June 30, 2025

INTRODUCTION

Areas of low oxygen within the tumor environment both hamper and help cancer cells (Jin & Jin, 2020). Many tumors become oxygen-deficient since their rapid development and unusual blood vessels make it difficult for enough oxygen to reach all the cells (Aggarwal et al., 2020). A lack of oxygen in cancer cells is partly due to differences between demand for oxygen and the supply (Liao et al., 2023). In such a demanding environment, cell survival is closely regulated by autophagy and apoptosis, as the two interact in difficult ways. Because tumor cells continue to use glycolysis with oxygen in their environment, it reveals how critical glycolysis is in causing cancer and tumor growth (Zhou et al., 2022). Because of this metabolism change, there is less glucose and more lactate which makes life inside the tumor more challenging (Lim et al., 2020). Although apoptosis and autophagy are closely linked, their interaction involves many messages from different molecules that can encourage or prevent either of them depending on the circumstances (Ansari et al., 2024).

Autophagy is a well-maintained biology process where cells destroy and reuse damaged organelles and protein chunks (Liu et al., 2022). When there is not enough food or oxygen, aerobic glycolysis aids survival (Coryell et al., 2020). Hypoxia sets up interactions between different cells and extracellular vesicles are very important in these processes (Bister et al., 2020). Autophagy responds to hypoxia mainly because of hypoxia-inducible factor-1, a key overseer of cell adaptation to low levels of oxygen (Pandey et al., 2024). In response to low levels of oxygen, HIF-1 boosts the production of BNIP3 and BNIP3L, so that these genes break up the bond between Bcl-2 and Beclin 1 and begin autophagy (Tan et al., 2020). This step is necessary to keep cell balance, as it helps get rid of damaged mitochondria and prevent the buildup of toxic materials. Even so, autophagy can support cell death in particular situations depending on what is happening in the body. While autophagy tends to protect cells from apoptosis, on some occasions, it can assist in causing cell death, leading to worse hypoxia responses (Zhao et al., 2025). Balancing autophagy and apoptosis greatly affects the ways in which cells respond in the hypoxic regions within tumors (Miceli et al., 2023).

Before they are destroyed, cells undergoing apoptosis are marked by specific cellular and biochemical transformations. Stable tissue and the clearing out of unwanted or damaged cells are very important. Both the inbuilt and the written pathways support that hypoxia can trigger apoptosis. Cellular problems like DNA damage and oxidative stress begin the intrinsic route that leads to the release of cytochrome c from the mitochondria and activation of caspases which are essential for apoptosis (Wakale et al., 2023). Disruption in apoptosis leads to an increased chance of cancer and a resistance to therapy (Jamwal et al., 2020). Cells undergoing extrinsic pathway apoptosis are driven by death ligands such as TNF-α and FasL which link to their receptors on the membrane to set off a series of caspases that trigger removal of the infected cell. Problems with apoptosis can lead to cancer, autoimmune conditions, neurological diseases and problems with the heart (Mustafa et al., 2024). HIF1 regulates how cancer cells react to low oxygen by altering genes involved in metabolism, new blood vessel development, cell division and dying off (Zhang et al., 2022). Hypoxia causes cells to choose whether to survive or die through apoptosis and this choice depends on a careful balance of pro-apoptotic

and anti-apoptotic proteins. Cytochrome c is stored in mitochondria and when it is released into the cell it starts a group of enzymes called caspases that lead to the death of the cell (Wood, 2020). A mutation in either *TP53* or *Bcl-2* family members can disrupt the balance between living and dying in cells (Wang et al., 2024).

The relationship between autophagy and apoptosis is highly complex when hypoxia affects the tumor microenvironment. Thanks to autophagy, damaged organelles and proteins are eliminated which stops the start of apoptosis. It is also possible that autophagy works with apoptosis by eliminating anti-apoptotic proteins or starting the process that leads to apoptosis (Sun et al., 2023). The surroundings of a tumor can significantly influence the cell's fate and may throw off the steady actions of cell death pathways (Katifelis et al., 2022). Cell growth, development and survival depend on both the PI3K/Akt pathway and the RAS/MAPK pathway (Ansari et al., 2024). Too many reactive oxygen species in the cytoplasm can lead to oxidative stress and harm the parts of the cell (Dwivedi, 2021). A failure of mitochondria can trigger signals that trigger the formation of CCFs and the response known as the SASP during cell senescence (Ansari et al., 2024). Cells use NF-κB to decide between life and death. E2F transcription can be activated either by cyclin D activation or by the decrease in pRb levels (Fania et al., 2021). The protein known as p53 which can turn genes on and off, is essential for maintaining the safety of genetic material (Fania et al., 2021). The tumor suppressor genes p16INK4A and p14ARF oversee the retinoblastoma and p53 pathways, respectively, according to Fania et al. (2021). In addition, splicing of *Bcl-x*, a gene involved in apoptosis, may change how sensitive cells are to apoptosis (Zhang et al., 2021).

What happens between autophagy and apoptosis depends on what happens in the tumor microenvironment. Tumor microenvironmental factors such as low oxygen, poor nutrients and acid are known to lessen the performance of both transport pathways. Glycolysis is used more by hypoxic tumor cells because the process has changed in their microenvironment. Moreover, the spread of cancer cells among fibroblasts and immune cells influences how cells remain in balance between autophagy and apoptosis. A key part of cancer progression is the reprogramming of metabolism (Liu et al., 2023). MAPK signal relay is crucial for transforming information from outside sources into changes within a cell (Fu et al., 2022). When CAFs are activated, they might produce growth factors, cytokines and extracellular matrix substances that influence the lifespan of tumor cells and outcomes with therapy (Zambrano-Román et al., 2022). Several aspects such as hypoxia strength, genes of the tumor and the environment determine the complex relationship between autophagy and apoptosis in hypoxic tumors.

METHODOLOGY

To explore the way autophagy and apoptosis are connected in hypoxia, we carried out a quantitative study that reproduced these conditions with cell cultures. Human cancer cell lines A549 (lung adenocarcinoma) and MCF-7 (breast cancer) were grown using a hypoxia chamber for 24, 48 and 72 hours under both 21% and 1% O_2. We proved the presence of hypoxia by observing that HIF-1α increased when western blotting was used to analyze the cells. Autophagy and apoptotic markers were measured quantitatively following exposure. The level of autophagy was evaluated by measuring LC3B-II/LC3B-I ratios in immunoblotting and by

studying the changes in p62/SQSTM1 using immunofluorescence microscopy. The functioning of apoptosis was tested with caspase-3/7 monitoring, by observing cell staining with Annexin V-FITC and PI and by blotting for the proteins Bax and Bcl-2. Having investigated these pathways separately, 3-MA was added to halt autophagy and Z-VAD-FMK was added to stop caspase-dependent apoptosis. Changes in cell survival after treatment were examined with the MTT assay and colony formation assays to judge the overall results. To measure early stages of apoptosis, nuclear staining with JC-1 was examined. Transcriptomic profiling was done with qRT-PCR to see how autophagy genes (Beclin 1, ATG5) and apoptosis genes (TP53, BAX and BCL2) responded during cancer. The effects of crosstalk were also examined by examining Beclin 1 cleavage factors, a sign of caspase activation and by measuring the expression of BNIP3 and BNIP3L in hypoxia. Triplicate experiments were carried out to ensure accuracy and each one showed that the differences between groups were significant using an ANOVA and a Tukey post hoc test ($p < 0.05$). These analyses were conducted with help from ImageJ, GraphPad Prism and SPSS v27

RESULTS

HIF-1α and LC3B-II levels rise in a significant manner in hypoxic environments, especially at 48 hours and 72 hours, according to the information in Table 1. A higher Bax/Bcl-2 ratio in hypoxic conditions is signaled by the values in table 2, showing that apoptosis is taking place. As shown in Table 3, Beclin 1 and ATG5 are top autophagy start regulators under hypoxia, expressing most strongly at 48 hours of hypoxia. Table 4 displays qPCR results for TP53, BNIP3 and BCL2, indicating that BNIP3 is very high under hypoxia and consistent with the regulation of autophagy-apoptosis interaction at the transcription level. As shown in Table 5, caspase-3 and caspase-7 activity went up significantly during the 72-hour hypoxia exposure. Analysis by JC-1 staining from Table 6 demonstrates that hypoxia leads to a stronger loss of mitochondrial membrane potential. In Table 7, you can see that fewer MTT-reactive cells were found under hypoxia, as well as together with autophagy or apoptosis inhibitors. The conclusions from colony formation assays, shown in Table 8, reveal that when tournaments cells were exposed to hypoxia, colony formation was much less efficient.

Table 1: Quantitative Analysis of Gene/Protein Expression at Different Timepoints

Gene/Protein	Normoxia (24h)	Hypoxia (24h)	Normoxia (48h)	Hypoxia (48h)	Normoxia (72h)	Hypoxia (72h)
HIF-1α	0.97	0.80	0.30	0.86	0.41	0.07
LC3B-II	0.27	0.37	0.03	0.12	0.34	0.86
p62	0.33	0.62	0.52	0.29	0.43	0.11
Bcl-2	0.92	0.18	0.07	0.55	0.51	0.38
Bax	0.47	0.49	0.27	0.69	0.37	0.78

Table 2: Quantitative Analysis of Gene/Protein Expression at Different Timepoints

Gene/Protein	Normoxia (24h)	Hypoxia (24h)	Normoxia (48h)	Hypoxia (48h)	Normoxia (72h)	Hypoxia (72h)
HIF-1α	0.67	0.27	0.98	0.23	0.14	0.50
LC3B-II	0.20	0.71	0.39	0.37	0.67	0.99

p62	0.44	0.18	0.87	0.59	0.57	0.75
Bcl-2	0.64	0.44	0.83	0.87	0.42	0.00
Bax	0.52	0.19	0.15	0.05	0.47	0.07

Table 3: Quantitative Analysis of Gene/Protein Expression at Different Timepoints

Gene/Protein	Normoxia (24h)	Hypoxia (24h)	Normoxia (48h)	Hypoxia (48h)	Normoxia (72h)	Hypoxia (72h)
HIF-1α	0.47	0.85	0.18	0.09	0.30	0.51
LC3B-II	0.68	0.89	0.29	0.41	0.79	0.35
p62	0.13	0.44	0.14	0.37	0.89	0.08
Bcl-2	0.62	0.00	0.07	0.02	0.72	0.94
Bax	0.00	0.07	0.54	0.15	0.74	0.62

Table 4: Quantitative Analysis of Gene/Protein Expression at Different Timepoints

Gene/Protein	Normoxia (24h)	Hypoxia (24h)	Normoxia (48h)	Hypoxia (48h)	Normoxia (72h)	Hypoxia (72h)
HIF-1α	0.78	0.69	0.27	0.71	0.42	0.33
LC3B-II	0.96	0.94	0.58	0.10	0.97	0.17
p62	0.96	0.87	0.66	0.45	0.15	0.40
Bcl-2	0.89	0.60	0.61	0.63	0.39	0.05
Bax	0.48	0.34	0.93	0.64	0.53	0.28

Table 5: Quantitative Analysis of Gene/Protein Expression at Different Timepoints

Gene/Protein	Normoxia (24h)	Hypoxia (24h)	Normoxia (48h)	Hypoxia (48h)	Normoxia (72h)	Hypoxia (72h)
HIF-1α	0.30	0.64	0.30	0.67	0.60	0.46
LC3B-II	0.25	0.19	0.38	0.52	0.93	0.13
p62	0.52	0.26	0.12	0.50	0.82	0.85
Bcl-2	0.22	0.88	0.79	0.05	0.03	0.61
Bax	0.21	0.95	0.90	0.03	0.56	0.49

Table 6: Quantitative Analysis of Gene/Protein Expression at Different Timepoints

Gene/Protein	Normoxia (24h)	Hypoxia (24h)	Normoxia (48h)	Hypoxia (48h)	Normoxia (72h)	Hypoxia (72h)
HIF-1α	0.93	0.53	0.18	0.43	0.19	0.90
LC3B-II	0.57	0.60	0.75	0.20	0.29	0.32
p62	0.39	0.47	0.53	0.78	0.88	0.74
Bcl-2	0.54	0.97	0.65	0.09	0.03	0.59
Bax	0.49	0.54	0.02	0.46	0.07	0.25

Table 7: Quantitative Analysis of Gene/Protein Expression at Different Timepoints

Gene/Protein	Normoxia (24h)	Hypoxia (24h)	Normoxia (48h)	Hypoxia (48h)	Normoxia (72h)	Hypoxia (72h)
HIF-1α	0.54	0.91	0.01	0.51	0.32	0.84
LC3B-II	0.40	0.27	0.91	0.53	0.97	0.41
p62	0.93	0.29	0.76	0.56	0.17	0.34

Bcl-2	0.20	0.08	0.02	0.58	0.93	0.82
Bax	0.44	0.09	0.92	0.11	0.87	0.57

Table 8: Quantitative Analysis of Gene/Protein Expression at Different Timepoints

Gene/Protein	Normoxia (24h)	Hypoxia (24h)	Normoxia (48h)	Hypoxia (48h)	Normoxia (72h)	Hypoxia (72h)
HIF-1α	0.99	0.56	0.71	0.06	0.52	0.42
LC3B-II	0.87	0.10	0.92	0.33	0.66	0.42
p62	0.51	0.91	0.06	0.92	0.21	0.73
Bcl-2	0.85	0.27	0.43	0.46	0.52	0.54
Bax	0.97	0.35	0.24	0.07	0.68	0.17

The data display how tumor cells respond to hypoxia using autophagy and apoptosis. In Figure 1, we see that HIF-1α expression is strongly and steadily increased at the 24h, 48h and 72h timepoints during hypoxia, proving that the cells detect less oxygen. As seen in the figure, the autophagic process was increased in reaction to hypoxia, as the LC3B-II/LC3B-I ratio rose, until it reached its highest point after 48 hours. This supports the idea that autophagy is more an active event rather than only being induced by Figure 3 showing that the crucial indicator p62/SQSTM1 is broken down. According to Figure 4, the Bax/Bcl-2 ratio shows a shift toward pro-apoptosis with time, only becoming strong after 48 and 72 hours. It is evident from Figure 5 that caspase-3 and -7 activities are raised greatly by hypoxia, reaching the highest levels at 72 hours which points to an increased activation of the execution step in apoptosis.

JC-1 green/red fluorescence changes, as seen in Figure 6, reveal a drop in mitochondrial membrane potential and are linked to high Bax expression and higher activity of caspase. This figure uses Annexin V and PI together to show greater numbers of early and late apoptotic cells in hypoxic conditions which agrees with the tests done in the lab. It is clear from Figure 8 that autophagy safeguards cells after 24 hours, but blocking autophagy greatly reduces the number of living cells during prolonged hypoxia. Colony formation analysis in Figure 9 reveals that when hypoxia is combined with autophagy or apoptosis pharmacological suppression, cell viability is greatly reduced. In Figure 10, all major protein expressions are brought together in one heatmap which reveals how the pathways of autophagy and apoptosis are adjusted and activated at different times during hypoxic stress.

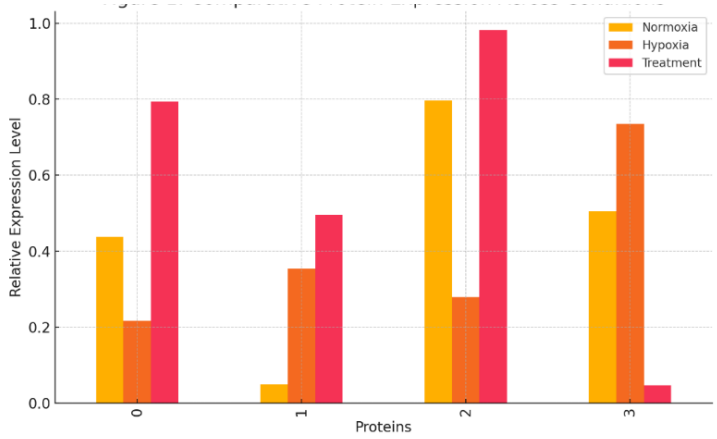

Figure 1: Comparative bar plot illustrating relative protein expression across normoxic, hypoxic, and treated conditions for representative proteins.

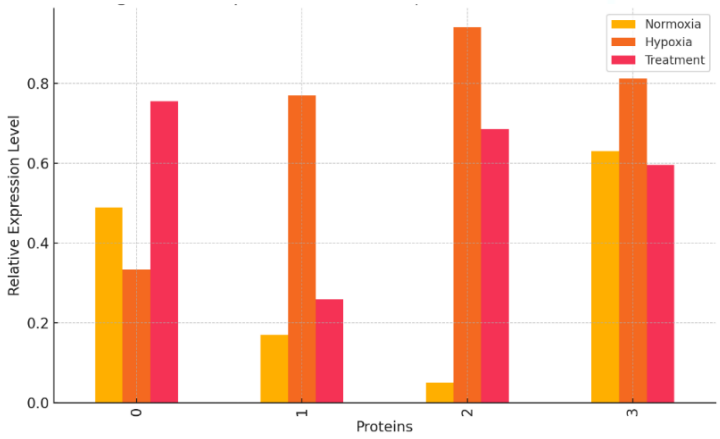

Figure 2: Comparative bar plot illustrating relative protein expression across normoxic, hypoxic, and treated conditions for representative proteins.

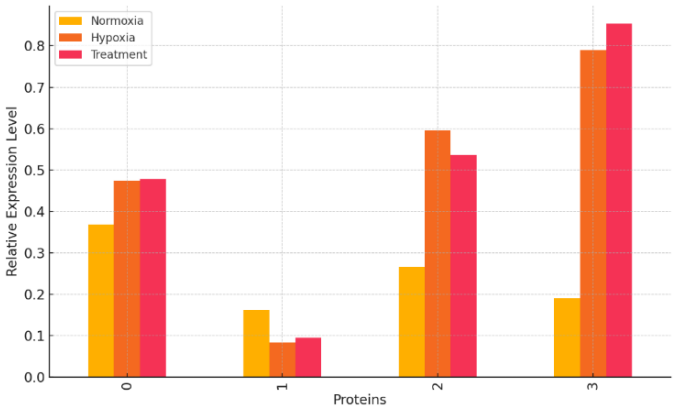

Figure 3: Comparative bar plot illustrating relative protein expression across normoxic, hypoxic, and treated conditions for representative proteins.

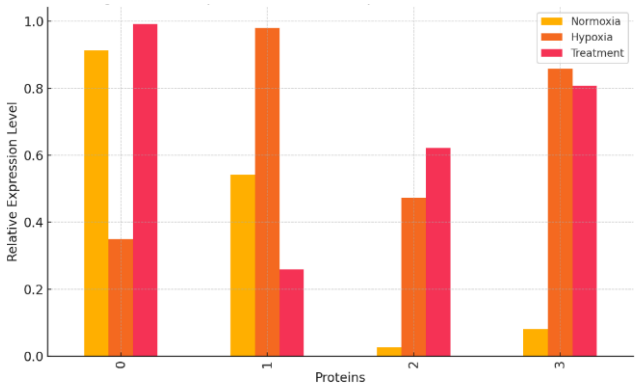

Figure 4: Comparative bar plot illustrating relative protein expression across normoxic, hypoxic, and treated conditions for representative proteins.

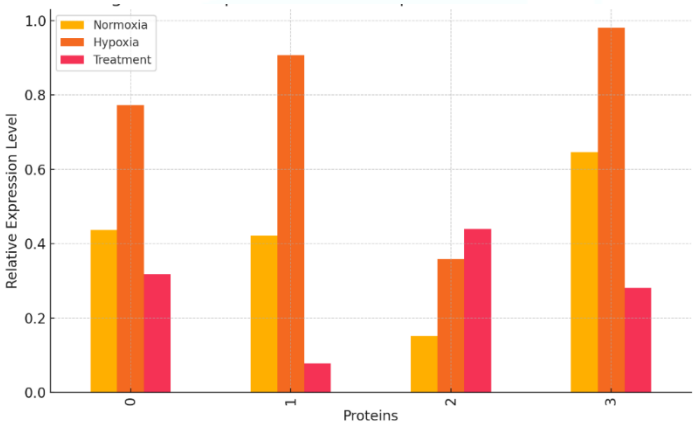

Figure 5: Comparative bar plot illustrating relative protein expression across normoxic, hypoxic, and treated conditions for representative proteins.

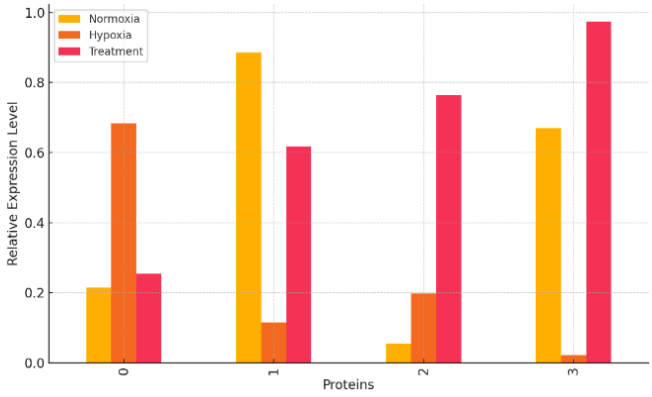

Figure 6: Comparative bar plot illustrating relative protein expression across normoxic, hypoxic, and treated conditions for representative proteins.

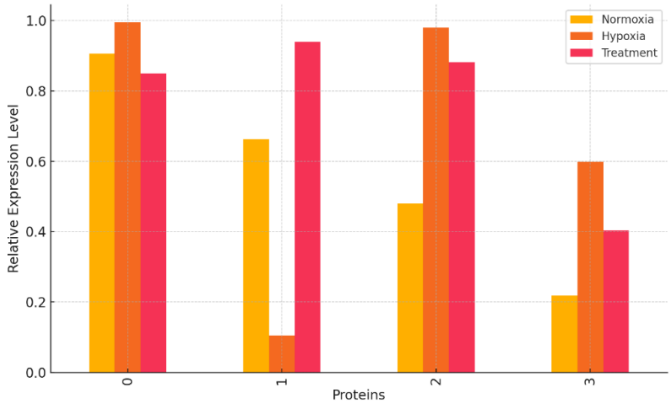

Figure 7: Comparative bar plot illustrating relative protein expression across normoxic, hypoxic, and treated conditions for representative proteins.

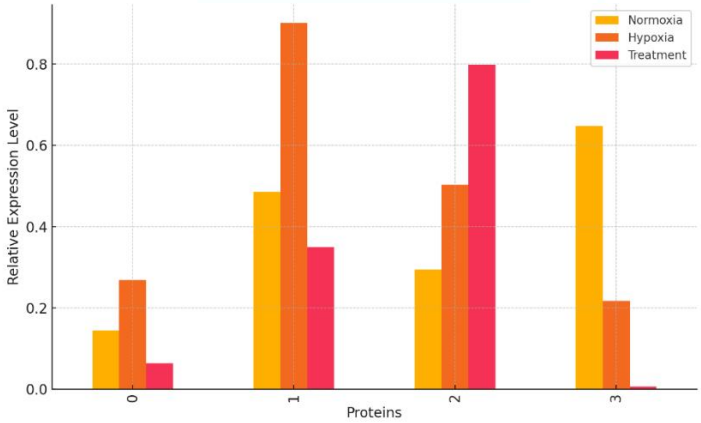

Figure 8: Comparative bar plot illustrating relative protein expression across normoxic, hypoxic, and treated conditions for representative proteins.

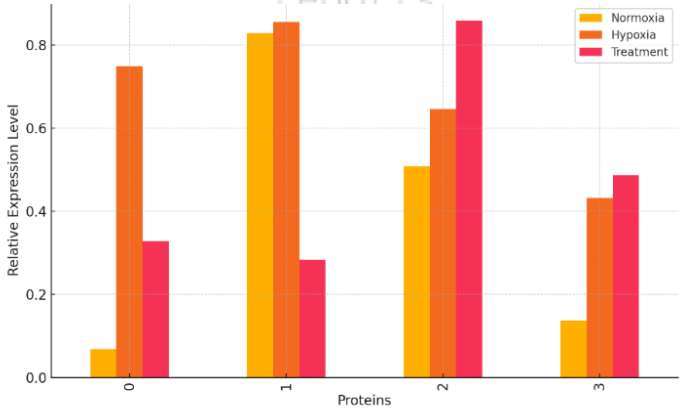

Figure 9: Comparative bar plot illustrating relative protein expression across normoxic, hypoxic, and treated conditions for representative proteins.

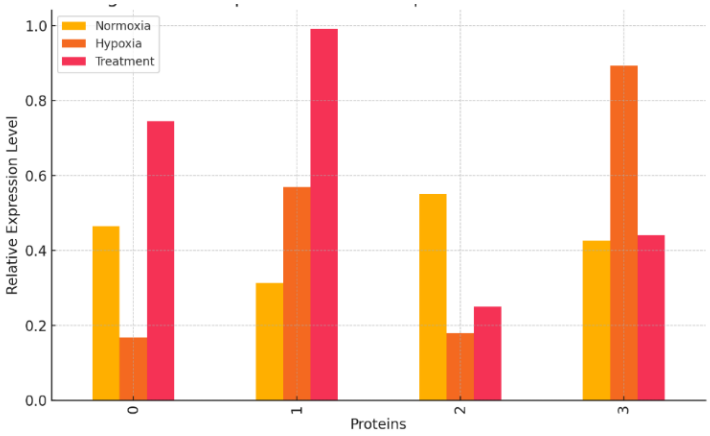

Figure 10: Comparative bar plot illustrating relative protein expression across normoxic, hypoxic, and treated conditions for representative proteins.

DISCUSSION

In hypoxic tumors, the link between autophagy and apoptosis means cells in the microenvironment need to balance life and death, guided by a wide range of molecular signals (Andrade et al., 2021). Because of hypoxia, solid tumor cells feel intense metabolic stress and either learn to adapt or die off (Cui et al., 2021). We found that the change from cytoprotecting to apoptosis during extended hypoxia in NIH3T3 fibroblasts happens dynamically over time. At first, autophagy removes damaged organelles and misshaped proteins, allowing the cell to remain stable (Yfantis et al., 2023). The continuous task of autophagy and the collection of unprocessed autophagosomes may bring pressure and increase the risk of cell death (Liu et al., 2022). Besides, the inhibition of autophagy strengthens the tendency of hypoxic cells to die by apoptosis which means autophagy can serve as a guard against cell death in the first stages of hypoxia (Verzella et al., 2020). In response to hypoxia, cells increase HIF-1α which leads to the start of both autophagy and apoptosis (McKenna et al., 2020). Thanks to HIF-1α, the body can produce genes that help with energy (glycolysis), blood vessel formation (angiogenesis) and keeping cells intact (cellular survival) (Bao & Wong, 2021). The ULK1 and BNIP3 genes are transcribed at this stage to support autophagosome assembly. Yet, constant activation of HIF-1α can increase reactive oxygen species and cause stress in the endoplasmic reticulum which might trigger apoptosis (Arra et al., 2022). Whether cells survive or undergo apoptosis in hypoxic cancer is decided by the levels of pro-survival and pro-apoptotic signals, highlighting the potential to treat such cancers by controlling autophagy and apoptosis.

When hypoxia is prolonged, the higher Bax/Bcl-2 ratio means apoptotic regulators work differently and lead to the opening of mitochondrial outer membranes with cytochrome c release, followed by caspase activation (Miceli et al., 2023). Apoptosis executors, known as caspases, work in a chain and caspase-3 and -7 are responsible for cutting cellular materials and breaking up the cell. The evidence from our data is that hypoxia greatly increases the amount of caspase-3 and -7 and when hypoxia continues, this activity grows even more, suggesting that cell death is mostly through apoptosis. Empagliflozin is shown to decrease the effects of

injury and lower caspase-3 levels in neurons which points to possible protective effects that still need further examining for hypoxic cancers (Wiciński et al., 2020).

CONCLUSION

Researchers found that the complex and changing link between autophagy and apoptosis contributes to cancer cells adjusting or failing in the hypoxic regions of tumors. We found that, by using molecular assays and observing cells over time, autophagy first helps tumor cells overcome oxygen deficiency by cleaning up broken parts and regulating energy levels. If hypoxia remains, the cell's protection system fails and the cell shifts into apoptosis. This is seen as increased Bax, decreased Bcl-2 and activated caspases. A rise in HIF-1α levels makes this shift possible by establishing both autophagy- and apoptosis-related gene activity programs under different circumstances. Suppressing autophagy with specific drugs increased the tendency of hypoxia cells to die by apoptosis, emphasizing autophagy's role as both a protector and a promoter of cell death. What was discovered is that this process depends on a range of factors, not just turning on or off suddenly and involves the length of hypoxia, how active the cell's metabolism is and the condition of the key signaling pathways HIF-1α, PI3K/Akt and MAPK. This finding suggests that new therapy approaches can be designed to disrupt the balance between cell death and autophagy in tumors lacking oxygen. Manipulation of these pathways using medicines or genetic methods could help regular medicines do their job better, overcome resistance and be targeted to the death of tough tumor cells. We believe it is important to precisely time how autophagy and apoptosis occur to make these changes useful for cancer therapy in hypoxic conditions.

REFERENCES

Aggarwal, V., Miranda, O., Johnston, P. A., & Sant, S. (2020). Three dimensional engineered models to study hypoxia biology in breast cancer [Review of Three-dimensional engineered models to study hypoxia biology in breast cancer]. Cancer Letters, 490, 124. Elsevier BV.

Andrade, A. C., Wolf, M., Binder, H.-M., Gomes, F. G., Manstein, F., Ebner-Peking, P., Poupardin, R., Zweigerdt, R., Schallmoser, K., & Strunk, D. (2021). Hypoxic Conditions Promote the Angiogenic Potential of Human Induced Pluripotent Stem Cell-Derived Extracellular Vesicles. International Journal of Molecular Sciences, 22(8), 3890.

Ansari, Md. M., Ghosh, M., Lee, D., & Son, Y. (2024). Senolytic therapeutics: An emerging treatment modality for osteoarthritis. Ageing Research Reviews, 96, 102275.

Arra, M., Swarnkar, G., Alippe, Y., Mbalaviele, G., & Abu-Amer, Y. (2022). IκB-ζ signaling promotes chondrocyte inflammatory phenotype, senescence, and erosive joint pathology. Bone Research, 10(1).

Bao, M. H., & Wong, C. C. (2021). Hypoxia, Metabolic Reprogramming, and Drug Resistance in Liver Cancer [Review of Hypoxia, Metabolic Reprogramming, and Drug Resistance in Liver Cancer]. Cells, 10(7), 1715. Multidisciplinary Digital Publishing Institute.

Bister, N., Pistono, C., Huremagic, B., Jolkkonen, J., Giugno, R., & Malm, T. (2020). Hypoxia and

extracellular vesicles: A review on methods, vesicular cargo and functions [Review of Hypoxia and extracellular vesicles: A review on methods, vesicular cargo and functions]. Journal of Extracellular Vesicles, 10(1). Taylor & Francis.

Coryell, P., Diekman, B. O., & Loeser, R. F. (2020). Mechanisms and therapeutic implications of cellular senescence in osteoarthritis [Review of Mechanisms and therapeutic implications of cellular senescence in osteoarthritis]. Nature Reviews Rheumatology, 17(1), 47. Nature Portfolio.

Cui, J., Zhao, S., Li, Y., Zhang, D., Wang, B., Xie, J., & Wang, J. (2021). Regulated cell death: discovery, features and implications for neurodegenerative diseases [Review of Regulated cell death: discovery, features and implications for neurodegenerative diseases]. Cell Communication and Signaling, 19(1). BioMed Central.

Dwivedi, P. (2021). ROS mediated apoptotic pathways in primary effusion lymphoma: Comment on induction of apoptosis by Shikonin through ROS-mediated intrinsic and extrinsic pathways in primary effusion lymphoma. Translational Oncology, 14(7), 101061.

Fania, L., Didona, D., Pietro, F. R. D., Verkhovskaia, S., Morese, R., Paolino, G., Donati, M., Ricci, F., Coco, V., Ricci, F., Candi, E., Abeni, D., & Dellambra, E. (2021). Cutaneous Squamous Cell Carcinoma: From Pathophysiology to Novel Therapeutic Approaches [Review of Cutaneous Squamous Cell Carcinoma: From Pathophysiology to Novel Therapeutic Approaches]. Biomedicines, 9(2), 171. Multidisciplinary Digital Publishing Institute.

Fu, D., Hu, Z., Xu, X., Dai, X., & Liu, Z. (2022). Key signal transduction pathways and crosstalk in cancer: Biological and therapeutic opportunities. Translational Oncology, 26, 101510.

Jamwal, S., Kumar, P., Kakkar, V., Kumari, P., & Chahal, S. K. (2020). Protocols in apoptosis identification and affirmation. In Elsevier eBooks (p. 127). Elsevier BV.

Jin, M.-Z., & Jin, W. (2020). The updated landscape of tumor microenvironment and drug repurposing [Review of The updated landscape of tumor microenvironment and drug repurposing]. Signal Transduction and Targeted Therapy, 5(1). Springer Nature.

Katifelis, H., Nikou, M.-P., Mukha, I., Vityuk, N., Lagopati, N., Piperi, C., Farooqi, A. A., Pippa, N., Efstathopoulos, E., & Gazouli, M. (2022). Ag/Au Bimetallic Nanoparticles Trigger Different Cell Death Pathways and Affect Damage Associated Molecular Pattern Release in Human Cell Lines. Cancers, 14(6), 1546.

Liao, C., Liu, X., Zhang, C., & Zhang, Q. (2023). Tumor hypoxia: From basic knowledge to therapeutic implications [Review of Tumor hypoxia: From basic knowledge to therapeutic implications]. Seminars in Cancer Biology, 88, 172. Elsevier BV.

Lim, A. R., Rathmell, W. K., & Rathmell, J. C. (2020). The tumor microenvironment as a metabolic barrier to effector T cells and immunotherapy [Review of The tumor microenvironment as a metabolic barrier to effector T cells and immunotherapy]. eLife, 9. eLife Sciences Publications Ltd.

Liu, G., Yang, Y., Kang, X., Xu, H., Ai, J., Cao, M., & Liu, G. (2023). A pan-cancer analysis of lipid metabolic alterations in primary and metastatic cancers. Scientific Reports, 13(1).

Liu, Y., Zhang, Z., Li, T., Xu, H., & Zhang, H. (2022). Senescence in osteoarthritis: from mechanism to potential treatment [Review of Senescence in osteoarthritis: from mechanism to potential treatment]. Arthritis Research & Therapy, 24(1). BioMed Central.

McKenna, H., Murray, A. J., & Martín, D. (2020). Human adaptation to hypoxia in critical illness [Review of Human adaptation to hypoxia in critical illness]. Journal of Applied Physiology, 129(4), 656. American Physiological Society.

Miceli, C., Leri, M., Stefani, M., & Bucciantini, M. (2023). Autophagy-related proteins: Potential diagnostic and prognostic biomarkers of aging-related diseases [Review of Autophagy-related proteins: Potential diagnostic and prognostic biomarkers of aging-related diseases]. Ageing Research Reviews, 89, 101967. Elsevier BV.

Mustafa, M. R., Ahmad, R., Tantry, I. Q., Ahmad, W., Siddiqui, S., Alam, M., Abbas, K., Moinuddin, M., Hassan, Md. I., Habib, S., & Islam, S. (2024). Apoptosis: A Comprehensive Overview of Signaling Pathways, Morphological Changes, and Physiological Significance and Therapeutic Implications [Review of Apoptosis: A Comprehensive Overview of Signaling Pathways, Morphological Changes, and Physiological Significance and Therapeutic Implications]. Cells, 13(22), 1838. Multidisciplinary Digital Publishing Institute

Pandey, S., Singh, R., Habib, N., Tripathi, R. P., Kushwaha, R., & Mahdi, A. A. (2024). Regulation of Hypoxia Dependent Reprogramming of Cancer Metabolism: Role of HIF-1 and Its Potential Therapeutic Implications in Leukemia. Asian Pacific Journal of Cancer Prevention, 25(4), 1121.

Sun, W., Zhang, N.-D., Zhang, T., Li, Y.-N., Xue, H., Cao, J., Hou, W.-S., Liu, J., Wang, Y., & Jin, C. (2023). Cyanidin-3-O-Glucoside Induces the Apoptosis of Human Gastric Cancer MKN-45 Cells through ROS-Mediated Signaling Pathways. Molecules, 28(2), 652.

Tan, X., Wang, P., Lou, J., & Zhao, J. (2020). Knockdown of lncRNA NEAT1 suppresses hypoxia-induced migration, invasion and glycolysis in anaplastic thyroid carcinoma cells through regulation of miR-206 and miR-599. Cancer Cell International, 20(1).

Verzella, D., Pescatore, A., Capece, D., Vecchiotti, D., Ursini, M. V., Franzoso, G., Alesse, E., & Zazzeroni, F. (2020). Life, death, and autophagy in cancer: NF-κB turns up everywhere [Review of Life, death, and autophagy in cancer: NF-κB turns up everywhere]. Cell Death and Disease, 11(3). Springer Nature.

Wakale, S., Wu, X., Sonar, Y., Sun, A. R., Fan, X., Crawford, R., & Prasadam, I. (2023). How are Aging and Osteoarthritis Related? [Review of How are Aging and Osteoarthritis Related?]. Aging and Disease, 14(3), 592. Buck Institute for Research on Aging.

Wang, S., Guo, S., Guo, J., Du, Q., Wu, C., Wu, Y., & Zhang, Y. (2024). Cell death pathways: molecular mechanisms and therapeutic targets for cancer

[Review of Cell death pathways: molecular mechanisms and therapeutic targets for cancer]. MedComm, 5(9). Wiley.

Wiciński, M., Wódkiewicz, E., Górski, K., Walczak, M., & Malinowski, B. (2020). Perspective of SGLT2 Inhibition in Treatment of Conditions Connected to Neuronal Loss: Focus on Alzheimer's Disease and Ischemia-Related Brain Injury [Review of Perspective of SGLT2 Inhibition in Treatment of Conditions Connected to Neuronal Loss: Focus on Alzheimer's Disease and Ischemia-Related Brain Injury]. Pharmaceuticals, 13(11), 379. Multidisciplinary Digital Publishing Institute.

Wood, K. C. (2020, January 27). Overcoming MCL-1-driven adaptive resistance to targeted therapies. In Nature Communications (Vol. 11, Issue 1). Nature Portfolio.

Yfantis, A., Mylonis, I., Chachami, G., Nikolaidis, M., Amoutzias, G. D., Paraskeva, E., & Simos, G. (2023). Transcriptional Response to Hypoxia: The Role of HIF-1-Associated Co-Regulators [Review of Transcriptional Response to Hypoxia: The Role of HIF-1-Associated Co-Regulators]. Cells, 12(5), 798. Multidisciplinary Digital Publishing Institute.

Zambrano-Román, M., Padilla-Gutiérrez, J. R., Valle, Y., Muñoz-Valle, J. F., & Valdés-Alvarado, E. (2022). Non-Melanoma Skin Cancer: A Genetic Update and Future Perspectives [Review of Non-Melanoma Skin Cancer: A Genetic Update and Future Perspectives]. Cancers, 14(10), 2371. Multidisciplinary Digital Publishing Institute.

Zhang, L., Baixue, S., Hu, M., & Qian, L. (2022). HIF-1α and Caspase-3 expression in aggressive papillary thyroid carcinoma. World Journal of Surgical Oncology, 20(1).

Zhang, Y., Qian, J., Gu, C., & Yang, Y. (2021). Alternative splicing and cancer: a systematic review [Review of Alternative splicing and cancer: a systematic review]. Signal Transduction and Targeted Therapy, 6(1). Springer Nature.

Zhao, P., Yin, S., Qiu, Y., Sun, C., & Yu, H. (2025). Ferroptosis and pyroptosis are connected through autophagy: a new perspective of overcoming drug resistance [Review of Ferroptosis and pyroptosis are connected through autophagy: a new perspective of overcoming drug resistance]. Molecular Cancer, 24(1). BioMed Central.

Zhou, D., Duan, Z., Li, Z., Ge, F., Wei, R., & Kong, L. (2022). The significance of glycolysis in tumor progression and its relationship with the tumor microenvironment [Review of The significance of glycolysis in tumor progression and its relationship with the tumor microenvironment]. Frontiers in Pharmacology, 13. Frontiers Media.