Commagene Journal of Biology

Aydoğan Türkoğlu et al., (2025) *Comm. J. Biol.* 9(1), 33-41.
DOI: 10.31594/commagene.1590025

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

Experimental and *In silico* Analysis of the Hypoxic Response of Human HTR1B Expression in Human Cell Lines and Its Ortholog Ser-4 Expression in *Caenorhabditis elegans*

Sümeyye AYDOĞAN TÜRKOĞLU[1]*, Canberk TOPRAK[2], Aysu BOZKURT[2], Fatma POYRAZLI[2]

[1]*Balikesir University, Faculty of Science and Literature, Department of Molecular Biology and Genetics, 10145 Balikesir, TÜRKIYE*
[2]*Balikesir University, Institute of Science, Department of Molecular Biology and Genetics, 10145 Balikesir, TÜRKIYE*
ORCID ID: Sümeyye AYDOĞAN TÜRKOĞLU: https://orcid.org/0000-0003-1754-0700; Canberk TOPRAK: https://orcid.org/0009-0000-0575-4360; Aysu BOZKURT: https://orcid.org/0000-0003-0165-528X; Fatma POYRAZLI: https://orcid.org/0000-0001-8069-6447

Received: 23.11.2024 **Accepted:** 24.02.2025 **Published online:** 10.03.2025 **Issue published:** 30.06.2025

Abstract: The relationship between serotonin receptors and cancer has been particularly investigated in recent years. Some studies suggest that serotonin receptors may promote the growth, spread, and metastasis of cancer cells. Serotonin also plays an important role in *C. elegans*. The simple nervous system of *C. elegans* provides an ideal system to study to understand the functions of neurons and neuromodulators. The Serotonin system is highly conserved evolutionarily in humans and *C. elegans*.

A decrease in oxygen levels in cells is called hypoxia and hypoxia promotes tumor growth and is associated with treatment resistance. The usability of *C. elegans* as a new model in the investigation of cancer-related genes in hypoxic studies is important. For this purpose, hypoxic conditions were created in two different models (human cell lines (HUVEC and PC-3) and *C. elegans*) and the expression changes of serotonin receptors HTR1B and its ortholog Ser-4 were examined. Bioinformatic analyses showed that these two genes were 87% similar and affected similar cellular signaling pathways. The expression of HTR1B was increased in the HUVEC cell line at 48 and 72 hours under hypoxic conditions. A hypoxic response was observed in the PC-3 cell line at 48 hours. The expression of Ser-4, the HTR1B *C. elegans* ortholog gene, was also increased in hypoxia at 1 hour.

The effects of the HTR1B gene on various cell lines play a critical role in understanding the complex dynamics of the serotonergic system. In conclusion, the effects of the HTR1B gene on various cell lines constitute an important step in understanding the functionality of this gene in cancer and its potential therapeutic uses.

Keywords: Serotonin, HTR1B, Ser-4, HUVEC, PC-3, *C. elegans*, cancer, hypoxia.

İnsan HTR1B İfadesinin İnsan Hücre Hatlarında ve Ortoloğu Ser-4'ün *Caenorhabditis elegans*'da Hipoksik Koşullarda Cevabının Deneysel ve *In silico* İncelenmesi

Öz: Serotonin ve serotonin reseptörleri ile kanser arasındaki ilişki son yıllarda özellikle araştırılmıştır. Bazı çalışmalar, serotonin reseptörlerinin kanser hücrelerinin büyümesini, yayılmasını ve metastazını destekleyebileceğini ileri sürmektedir. Serotonin ayrıca *C. elegans*'ta önemli bir rol oynar. Bu organizmada serotonin, sinir sistemi ve diğer dokulardaki çeşitli fizyolojik süreçlerin düzenlenmesinde kullanılan bir nörotransmitter olarak işlev görür. *C. elegans*, serotonin sisteminin evrimsel olarak oldukça korunduğunu ve insanlarda benzer biyolojik işlevlere sahip olduğunu göstermektedir.

Hücrelerdeki oksijen seviyelerinde azalmaya hipoksi denir ve bazı klinik çalışmalar hipoksinin tümör büyümesini desteklediğini ve tedavi direnciyle ilişkili olduğunu göstermiştir. HIF, oksijen seviyelerindeki değişikliklere yanıt olarak gen ekspresyonunu düzenler ve hipoksi koşulları altında hücrelerin yanıt verdiği DNA'nın belirli bölgelerine bağlanarak gen ekspresyonunu etkiler. Hipoksik çalışmalarda kanserle ilişkili genlerin araştırılmasında yeni bir model olarak *C. elegans*'ın kullanılabilirliği önemlidir. Bu amaçla iki farklı modelde (insan hücre hatları (HUVEC ve PC-3) ve *C. elegans*) hipoksik koşullar oluşturuldu ve son yıllarda kanserle ilişkili olduğu belirlenen serotonin reseptörleri HTR1B ve onun ortologu Ser-4'ün ekspresyon değişiklikleri incelendi. Yapılan biyoinformatik analizlerde bu iki genin %87 benzer olduğu ve benzer hücresel sinyal yollarını etkilediği görülmüştür. HTR1B'nin hipoksik koşullarda 48 ve 72. saatlerde HUVEC hücre hattında ekspresyonunun normal koşullara kıyasla arttığını bulduk. PC-3 hücre hattında ise 48. saatte hipoksik yanıt gözlendi. HTR1B *C. elegans* ortolog geni olan Ser-4'ün ekspresyonu da 1. saatte hipokside artmıştır.

HTR1B geninin çeşitli hücre hatları üzerindeki etkileri, serotoninerjik sistemin karmaşık dinamiklerini anlamada kritik bir rol oynamaktadır. Sonuç olarak, HTR1B geninin çeşitli hücre hatları üzerindeki etkileri, bu genin kanserdeki işlevselliğini ve potansiyel terapötik kullanımlarını anlamada önemli bir adım oluşturmaktadır.

Anahtar kelimeler: Serotonin, HTR1B, Ser-4, HUVEC, PC-3, *C. elegans*, kanser, hipoksi.

1. Introduction

Serotonin ($C_{10}H_{12}N_2O$ and 5-hydroxytryptamine) is a neurotransmitter that plays an important role in the central nervous system and other body tissues. It is synthesized by the raphe nuclei in nerve cells and by the enterochromaffin cells in tissues such as the intestine. It is particularly important in regulating many processes such as mood, sleep, appetite, sexual activity, learning, memory, and social behavior (Kitson, 2007; Veenstra-

Corresponding author: sumeyye_ayd@hotmail.com

WanderWelee et al., 2000). Therefore, a deficiency or imbalance of serotonin can contribute to depression, anxiety, sleep disorders, and other mental health problems.

Serotonin synthesis begins with tryptophan, an amino acid taken in through food. In the liver, it is metabolized by the enzyme tryptophan hydroxylase (TPH1) to an intermediate product called 5-hydroxytryptophan (5-HTP). 5-hydroxytryptophan (5-HTP), which is formed as a result of the metabolism of tryptophan by the enzyme tryptophan hydroxylase, is then converted to 5-HTP by another enzyme, amino acid decarboxylase (AADC). This reaction occurs in the areas where tryptophan hydroxylase functions (skin, intestine, and pineal gland). 5-HTP is then converted to the molecule 5-hydroxytryptamine, called serotonin, by another enzyme, amino acid decarboxylase (Veenstra-WanderWelee et al., 2000).

Serotonin metabolism is a key process in how this chemical is produced, broken down, and used in the body. Imbalances in serotonin can contribute to a range of mental health problems, and therefore research on serotonin is important in understanding the physiology and pathology of such disorders and is critical to their treatment. Serotonin receptors are found in various tissues throughout the body and receive serotonin signals, causing cellular responses (Walther et al., 2003).

Serotonin is broken down by an enzyme called monoamine oxidase (MAO). MAO breaks down serotonin molecules and removes them from the body. This metabolic process is important in keeping serotonin levels balanced (Smith et al., 2020). Additionally, medications that increase serotonin reuptake, such as selective serotonin reuptake inhibitors (SSRIs), can also affect serotonin metabolism, which is used to treat conditions such as depression (David & Gardier, 2016).

The relationship between serotonin and serotonin receptors has been particularly investigated in recent years. Serotonin can have an effect on the cell through its receptors. It is important to understand the functions of serotonin and its cellular effects because recent studies have shown that serotonin is effective not only in physiological conditions but also in pathophysiological processes such as cancer. Studies show that serotonin receptors can be found in cancer cells and may play a role in cancer development. For example, some studies suggest that serotonin receptors may promote the growth, spread, and metastasis of cancer cells. However, the relationship between serotonin and cancer is quite complex and not fully understood. Serotonin has been shown to have a potential effect on cancer cell proliferation, invasion, spreading, and tumor angiogenesis (Balakrishna et al., 2021). Different receptor subtypes mediate the effect of serotonin in prostate cancer at different tumor stages (Dizeyi et al., 2004). An in vitro study using an androgen-independent cell line demonstrated that serotonin has a dose-dependent stimulatory effect on cell proliferation (Siddiqui et al., 2006). Serotonin shows its effects through different types of receptors found on pre-synaptic and post-synaptic cell membranes. These receptors are 5-Hydroxytryptamine receptors (5-HTR1-5-HTR7). Serotonin function may vary depending on the region and receptor. Serotonin signaling promotes tumor progression by stimulating the proliferation of cancer cells and inhibiting apoptosis via the MAPK and PI3K/Akt signaling axis (Karmakar et al., 2021).

Caenorhabditis elegans (*C. elegans*) is a microscopic nematode (roundworm) species. *C. elegans* is considered a very important model organism in the scientific world because these worms are easily genetically modified, reproducing rapidly, transparent, and have contributed to the understanding of many basic biological processes with their simple structure and completely sequenced genome (Savaş et al., 2018; Riddle et al., 1997).

Serotonin also plays an important role in *C. elegans*. In this organism, serotonin functions as a neurotransmitter used in the regulation of various physiological processes in the nervous system and other tissues. In *C. elegans*, serotonin is secreted from nerve cells and plays a role in synaptic transmission. *C. elegans* is a model organism used in studies of the functioning of the nervous system and the regulation of behavioral responses. Serotonin plays an important role in various processes in this organism, such as behavioral regulation, movement control, development, and aging. The simple nervous system of *C. elegans* provides an ideal system to study to understand the functions of neurons and neuromodulators (White et al., 1986; Dag et al., 2023). However, studies on *C. elegans* show that the serotonin system is highly conserved evolutionarily and has similar biological functions in humans (Fig. 1.) In the *C. elegans* serotonin pathway, serotonin is synthesized by tryptophan hydroxylase (TPH1) and degraded by an enzyme homologous to monoamine oxidase (MAOA). Therefore, studies on *C. elegans* can also provide important clues in research aimed at understanding and treating human health and diseases. The Ser-4 gene, the human HTR1B *C. elegans* ortholog, is a part of the serotonin receptors and these receptors play an important role in locomotor activity. Serotonin and serotonin receptors, such as Ser-4, may have a role in the functions of cells that regulate development and food sensing. This may affect postembryonic development and food-seeking behavior in *C. elegans* (Gürel et al., 2012).

Oxygen plays a critical role in the energy metabolism of cells. Anabolic processes, signaling pathways, and enzymatic reactions are dependent on ATP produced by mitochondrial oxidative phosphorylation and glycolysis. However, decreased oxygen levels in cells are called hypoxia and are especially common in solid tumors. In hypoxic conditions, tumor cells adapt to genetic changes and some clinical studies have shown that hypoxia promotes tumor growth and contributes to treatment resistance (Türkoğlu et al., 2021; Türkoğlu & Kockar, 2016).

The molecular mechanism of hypoxia is governed by a key protein called Hypoxia-Inducible Factor (HIF). HIF regulates gene expression in response to changes in oxygen levels. In particular, HIF-1α affects gene expression by binding to specific regions of DNA to which cells respond under hypoxia conditions. This mechanism can affect the survival strategies of tumor cells, causing them to behave aggressively in a hypoxic environment (Türkoğlu et al., 2021).

Caenorhabditis elegans can exhibit various physiological and genetic responses when exposed to hypoxic environments. These responses provide

researchers with clues to understand how the worms' metabolism and gene expression change under hypoxic stress (Nystul et al., 2003; Miller & Roth, 2009). In addition, *C. elegans* lifespan is extended by hypoxia (Rascón & Harrison, 2010; Leiser et al., 2013). However, understanding the defense mechanisms of *C. elegans* against hypoxia may contribute to the development of potential therapeutic strategies for the treatment or prevention of hypoxia-related diseases in humans.

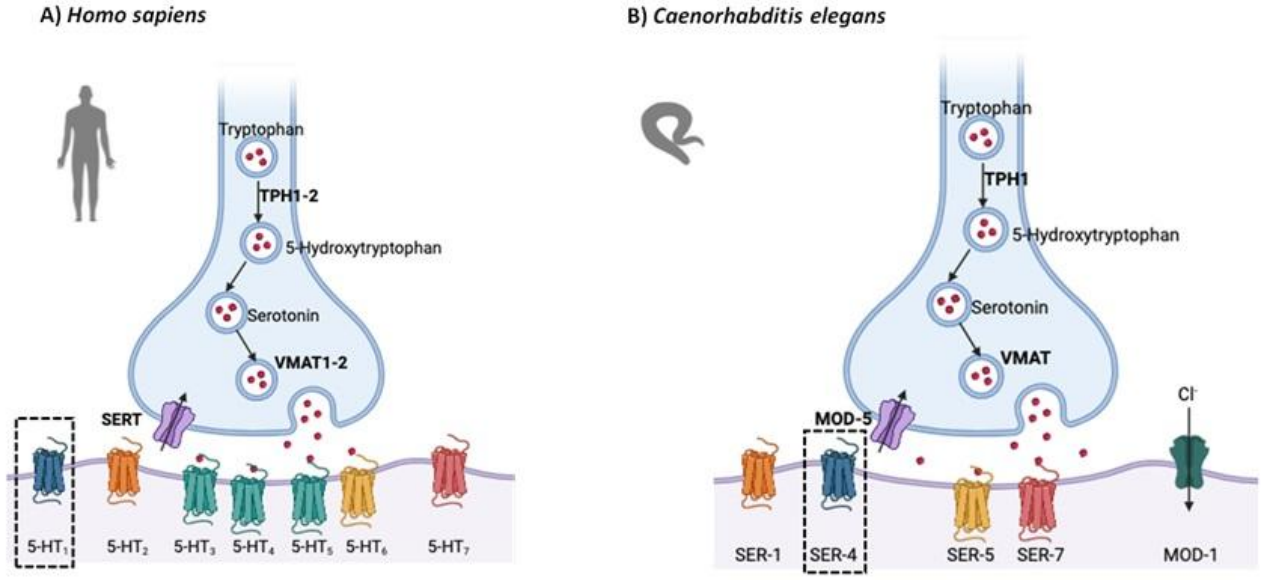

Figure 1. Human (A) and *C. elegans* (B) serotonin pathway (Curran & Chalasani, 2012).

Caenorhabditis elegans is a valuable model organism used in hypoxia research and studying the physiological and genetic responses of this worm under hypoxic conditions plays an important role in basic science and medical research. Especially considering that the cancer microenvironment is hypoxic, the usability of *C. elegans* as a new model in the investigation of cancer-related genes is important. Serotonin, which acts on cells through its receptors, is effective not only in physiological conditions but also in pathophysiological processes such as cancer. Studies on its effect on cancer are associated with changes in the expression of serotonin receptors. In this context, in our study, hypoxic conditions were created in two different models and the expression changes of serotonin receptors HTR1B and its ortholog Ser-4, which have been identified to be associated with cancer in recent years, were examined.

2. Material and Method

2.1. Cell Culture

Human vein endothelial cell line (HUVEC) and Prostate Cancer Cell Line (PC-3) were cultured using a routine passaging method in DMEM (Sigma, Dulbecco's Modified Eagle's Medium) containing 10% FCS (Fetal Calf Serum). Cells were incubated at 37°C in an environment containing 5% CO_2.

2.2. Cultivation of *C. elegans*

First, for the preparation of NGM (Nematode Growth Medium), 2.5 g peptone, 3 g NaCl, and 20 g agar were weighed and mixed with one liter of pure water and the mixture was autoclaved at 121°C for 15 minutes and then cooled to 55°C. 1 mL cholesterol, 1 mL 1M $MgSO_4$, 25 mL 1.5M KH_2PO_4, and 1 mL 1M $CaCl_2$ were added to the cooled mixture and mixed until the medium became homogeneous and poured into petri dishes and allowed to solidify. For the cultivation of E. coli OP50 strain, LST (Lauryl Sulfate Broth) 9.125 g Lauryl Sulfate Broth was weighed on a precision balance and mixed with 250 mL of pure water. The mixture was autoclaved at 121°C for 15 minutes and cooled to 37°C. A colony of E. coli was seeded into the cooled mixture and kept in a shaking incubator at 37°C for 24 hours. 400µL of *E. coli* OP50 strain was spread onto the solidified *C. elegans* medium and allowed to dry.

2.3. Creation of Hypoxic Model

The HUVEC and PC-3 cell lines were seeded in 25 cm^2 flasks with 2,000,000 cells. Control groups that were not treated with any substance were labeled as normoxia and experimental groups were treated with 150 µM $CoCl_2$ and were labeled as hypoxic experimental groups for 24, 48, and 72 hours (Türkoğlu & Köçkar 2016). To examine the hypoxic effect, cell pellets were taken at 24, 48, and 72 hours and stored at -80^{0}C before RNA isolation.

For *C. elegan's* hypoxic experiment, embryos were removed from adult *C. elegans* with hypochlorite and L1 stage organisms were obtained 16 hours later. The worms were then kept on NGM plates for 3 days. To create the *C. elegans* hypoxic model, sodium sulfite solution was prepared in M9 buffer at a concentration of 1 g/L. Worms were washed with the prepared M9 buffer after 3 days and the plates were transferred to 15 mL falcons and incubation was provided at different time intervals. At the end of incubation, the organisms were transferred to new NGM plates and waited for 24 hours for recovery.

2.4. Total RNA Isolation and cDNA Synthesis

For cell lines, RNA isolation was performed according to the previous studies. 1µg of RNA was used as a template to obtain cDNA according to the protocol. cDNAs were checked using Hb2 microglobulin primers for HUVEC and PC-3 cells (Poyrazlı et al., 2024; Türkoğlu et al., 2020). CDC primers were used for *C. elegans* control PCR analysis (Control PCR, Fig. 2A).

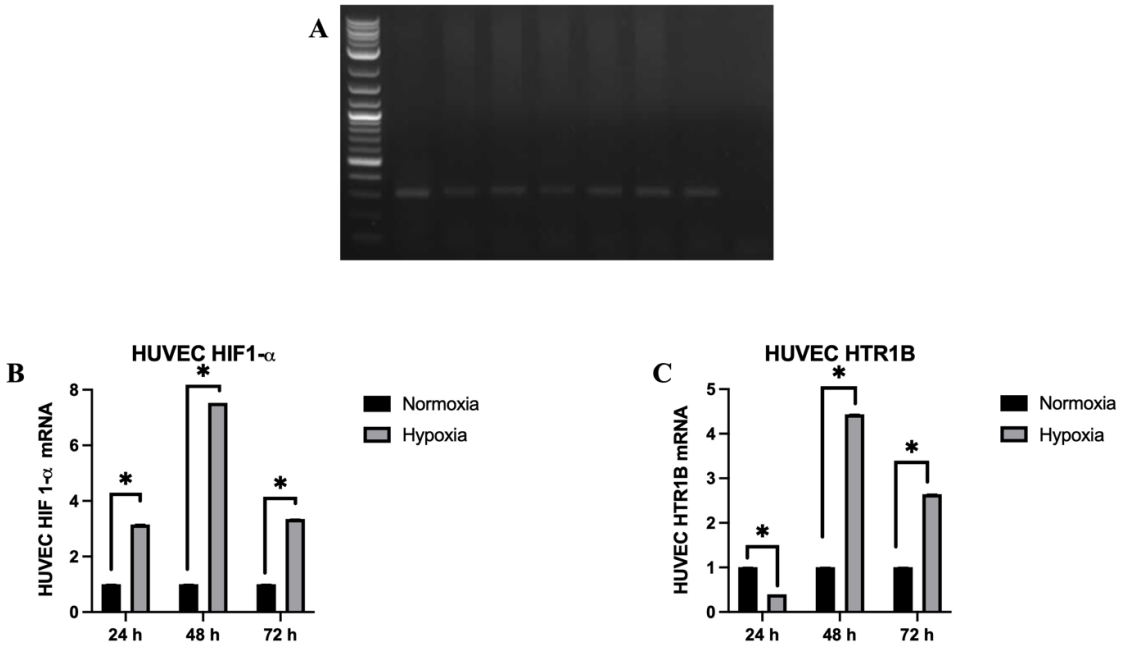

Figure 2. Expression of HIF1-α and HTR1B in HUVEC cell line (24, 48 and 72 hours); A: HB2 HUVEC control PCR image (M: 1 kb marker, 1: 24 h Normoxia, 2: 24 h Hypoxia, 3: 48 h Normoxia, 4: 48 h Hypoxia, 5: 72 h Normoxia, 6: 72 h Hypoxia, 7: Positive Control, 8: Negative Control), B: HIF1-α Expression, C: HTR1B Expression.

Caenorhabditis elegans were washed with M9 buffer and collected from the media. Then, the living organisms were placed in a falcon tube and the M9 on them was aspirated. Fresh M9 was added and the falcon tube was kept on ice. The living organisms were placed in the tubes and centrifuged to form a pellet. After the supernatant was removed, Trizol was added and the tubes were quickly frozen by throwing them into liquid nitrogen and RNA isolation was performed according to the protocol (Riccio, 2019). cDNAs were checked using CDC primers for *C. elegans* (Control PCR, Fig. 3A).

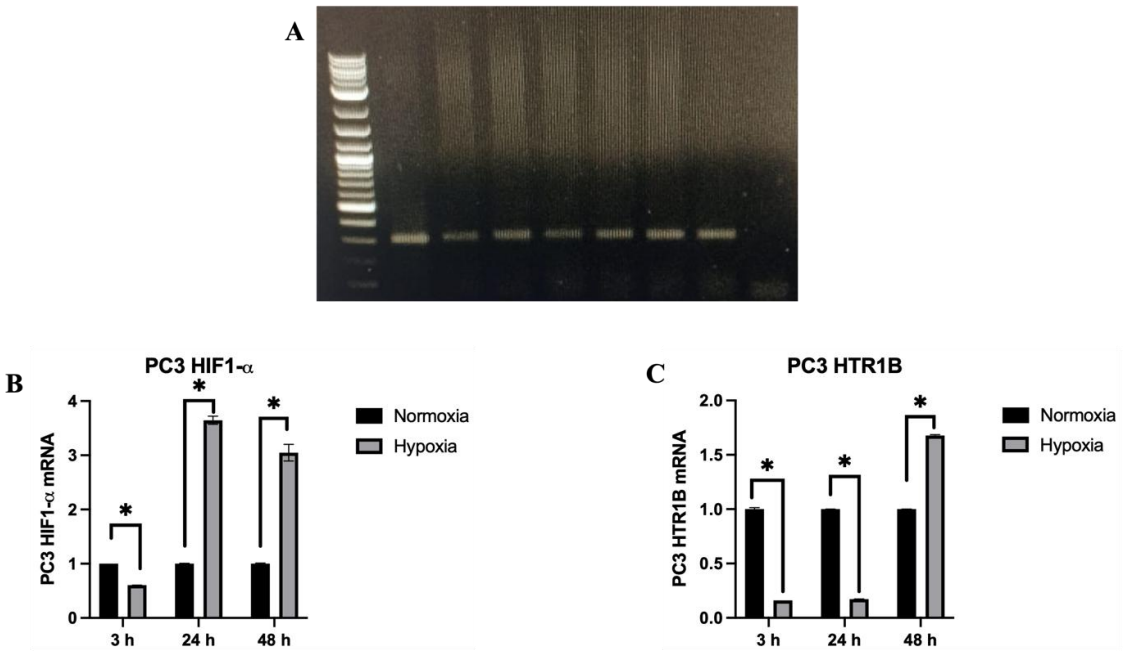

Figure 3. Expression of HIF1-α and HTR1B in PC3 cell line (24, 48 and 72 hours); A: HB2 PC3 control PCR image (M: 1 kb marker, 1: 3 h Normoxia, 2: 3 h Hypoxia, 3: 24 h Normoxia, 4: 24 h Hypoxia, 5: 48 h Normoxia, 6: 48 h Hypoxia, 7: Positive Control, 8: Negative Control), B: HIF1-α Expression, C: HTR1B Expression.

2.5. Real-Time PCR

To determine the HTR1B and Ser-4 mRNA expression, Real-Time PCR (Light Cycler 485 (Roche Diagnostic)) was performed by using 0.5 µl (100 ng/µl) for each of the forward and reverse primers, 6.25 µl RealQ Plus 2x Master Mix Green (Ampliqon) and 4.25 µl dH2O in a volume of 1µl from the obtained cDNAs. The experiments established with these cDNAs were performed in 3 repetitions and the Ct values were evaluated according to the LIVAK method.

2.6. Bioinformatics Analyses

The sequence of the human HTR1B gene (NM_000863.3)

and the *C. elegans*, Ser-4 (NM_065051.8) sequences were accessed from the website http://www.ncbi.nlm.nih.gov/. Primers were designed using Blast and OligodT Analyzer programs. In this context, the designed primers for the HTR1B gene are forward 5′-AAGAAGAAACTCATGGCCGCTAGGG-3′ reverse 5′-GGGGTTGATGAGGGAGTTGAGATAG-3′ and for the Ser-4 gene, forward 5′-CAGGTTTTCTCCACAGCGAC-3′ reverse 3′-CTTGATTCATTGTGGCGTGGA-5′ and the annealing temperatures are 55ºC. HTR1B and Ser-4 aa sequences were compared with NCBI and Bioedit Programme (Fig. 4).

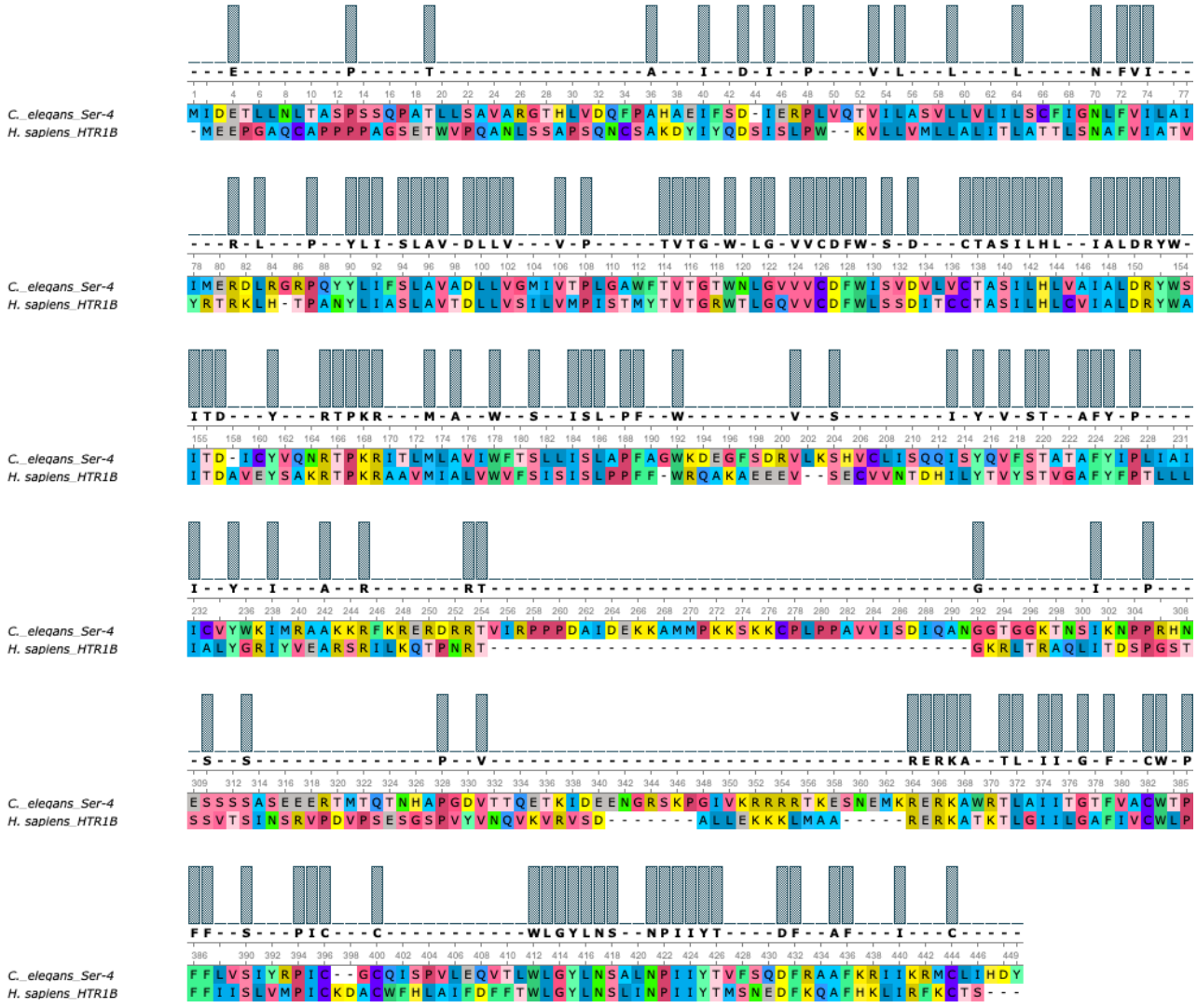

Figure 4. Bioinformatic comparison of *H. sapiens* HTR1B and *C. elegans* ser-4 amino acid sequence.

STRING database was used to draw the associated protein network diagram of HTR1B and Ser-4 genes and then analysis was performed with the KEGG database to display the basic biological processes they are involved in (Figs. 5-6).

Statistical analysis

Determination of HIF-1α, HTR1B, and Ser-4 mRNA levels were performed in cell lines and *C. elegans* in independent experiments at different time periods. HIF-1α and HTR1B levels were normalized by comparing with hβ2 and averaged from three replicate groups. Ser-4 levels were normalized with CDC42 and averaged from three replicate groups. Ct values obtained from Real Time PCR were analyzed according to LIVAC method. The results were graphed using the GraphPad Prism 8 program and evaluated statistically with One Way Anova in the program ($p<0.05$* was considered significant). The experiment was carried out in 3 repetitions.

3. Results

3.1. Similarities of HTR1B and ser-4 Genes

Human and *C. elegans* serotonin synthesis and signaling pathways are quite similar. As can be seen in Figure 1, serotonin is synthesized from the amino acid tryptophan via TPH enzymes in both organisms. Storage, release, and reuptake mechanisms are also quite similar. There are also similarities in terms of the receptors that initiate the effect of serotonin, which is very important in these signaling pathways, in cells. In particular, human HTR1b and its *C. elegans* ortholog Ser-4, which are among the active receptors, were compared in terms of gene and amino acid similarity.

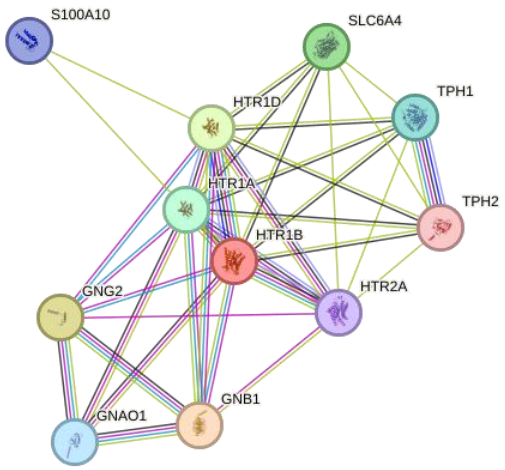

Figure 5. Pathway analysis of the HTR1B gene (HTR1B: 5-hydroxytryptamine receptor 1B, GNB1: Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1, GNG2: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2, HTR1D: 5-hydroxytryptamine receptor 1D, SLC6A4: Sodium-dependent serotonin transporter, HTR1A: 5-hydroxytryptamine receptor 1A, TPH1: Tryptophan hydroxylase 1, GNAO1: Guanine nucleotide-binding protein G(o) subunit alpha, S100A10: Protein S100-A10, HTR2A: 5-hydroxytryptamine receptor 2A, TPH2: Tryptophan hydroxylase 2.)

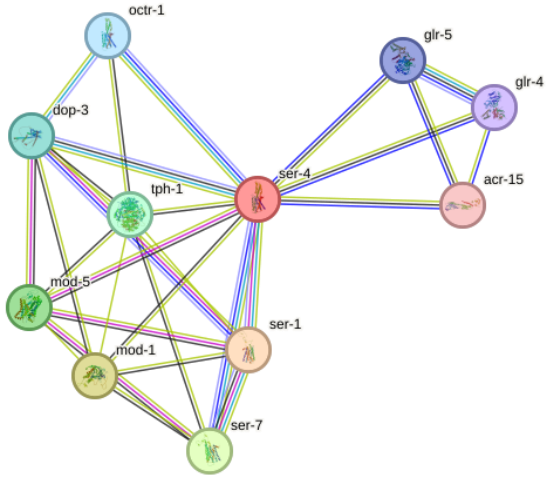

Figure 6. Pathway analysis of the ser-4 gene (ser-4: G_PROTEIN_RECEP_F1_2 domain-containing protein, ser-1: G_PROTEIN_RECEP_F1_2 domain-containing protein, mod-1: Serotonin-gated chloride channel, ser-7: G_PROTEIN_RECEP_F1_2 domain-containing protein, mod-5: Transporter, tph-1: BH4_AAA_HYDROXYL_2 domain-containing protein, dop-3: Dopamine receptor 3, octr-1: G_PROTEIN_RECEP_F1_2 domain-containing protein, glr-5: GLutamate Receptor family, glr-4: GLutamate Receptor family, acr-15: Acetylcholine Receptor.)

The similarity of *Homo sapiens* HTR1B and *C. elegans* Ser-4 genes were analyzed using both the UGENE program and NCBI blast programs. The similarity rate of HTR1B and Ser-4 aa sequence were compared and 87% aa identity was found (Fig. 4). It is seen from the Figure that the similarity between the 81st and 192nd amino acids is highly conserved.

3.2. HTR1B and HIF1-α Expression in HUVEC and PC-3 Cell Lines

Firstly, to confirm the hypoxic condition in the selected cell lines, the expression of HIF1-α, the main regulatory transcription factor of the hypoxic condition, was analyzed with Real-Time PCR. As seen in Figure 7, the expression of HIF1-α increased in hypoxic conditions at 24, 48, and 72 hours and it was confirmed that hypoxia occurred in HUVEC cell line. The expression of the serotonin receptor gene HTR1B under hypoxic conditions was also analyzed. HTR1B is regulated in hypoxic conditions at 48 and 72 hours and its expression increases compared to normal conditions in HUVEC cell line (Fig. 2). In the PC-3 cell line, no hypoxic response occurred at 3 hours. It can be observed in Figure 3 that hypoxia occurred at 24 and 48 hours. When the HTR1B mRNA level was examined at these time periods, an increase was detected at 72 hours compared to normal conditions.

3.3. Ser-4 and CeHIF Expression in *C. elegans*

To confirm whether our *C. elegans* hypoxic model was formed, this time *C. elegans* CeHIF expression was analyzed with Real-Time PCR. As shown in Figure 7, CeHIF expression could not be detected in all time periods. CeHIF expression increased only at 1 hour and the chemical hypoxia model was confirmed at 1 hour in *C. elegans*. When the expression of Ser-4, the HTR1B *C. elegans* ortholog gene, was examined, it was shown that its expression increased at 1 hour depending on the hypoxic condition.

3.4. Pathways in which the HTR1B Gene is Involved and Genes It Interacts with

HTR1B is an important receptor in serotonin metabolism. As a result of pathway analysis studies conducted with the String program, it was seen that the HTR1B gene was associated with Serotonergic synapse, folate biosynthesis, tryptophan metabolism, taste transmission, GABAergic synapse, morphine addiction, circadian entrainment, cAMP signaling pathway, and neuroactive Ligand-Receptor interaction pathways. It also plays a role in the cancer pathway together with the GNG2 gene it interacts with.

3.5. Pathways in Which Ser-4 Gene Takes Part and Genes it Interacts with

As a result of the analyses, it was observed that the Ser-4 gene takes part in the Neuroactive Ligand-Receptor interaction, calcium signaling pathway, and axon regeneration pathways. The genes selected in both models were found to be related to the G protein-coupled receptor signaling pathway, cellular response to dopamine, chemical synaptic transmission, cellular response to chemical stimulation, regulation of multicellular organism processes, serotonin binding, G protein-coupled serotonin receptor activity, neurotransmitter receptor activity, and GPCR ligand binding signaling pathways.

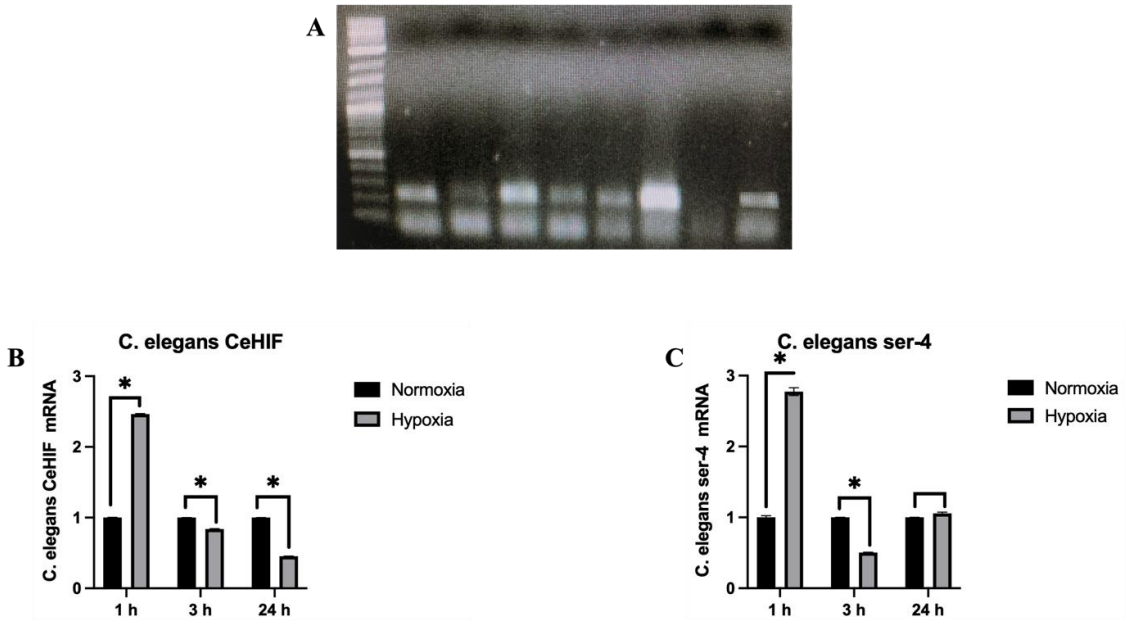

Figure 7. *C. elegans* CeHIF and ser-4 expression (1, 3 and 24 hours); A: CDC-42 *C. elegans* control PCR image (M: 1 kb marker, 1: 1-hour Normoxia, 2: 1-hour Hypoxia, 3: 3 hours Normoxia, 4: 3 hours Hypoxia, 5: 24 hours Normoxia, 6: 24 hours Hypoxia, 7: Negative Control, 8: Positive Control), B: cEHIF Expression, C: Ser-4 Expression.

4. Discussion and Conclusion

In recent years, studies on the potential roles of serotonin and serotonin receptors in cancer treatment have provided important findings. In studies conducted with prostate cancer cell lines, it was observed that hormone-independent DU145 and PC3 cell lines were more sensitive to 5-HT1A, 5-HT2B, and 5HT4 antagonists compared to androgen-dependent LNCaP cell lines. In particular, 5-HT1B receptor antagonists NAN-190 and SB224289 induce apoptosis in PC3 cell lines, indicating that they may have potential in the treatment of prostate cancer (Sarrouilhe et al., 2015).

Studies on the effects of serotonin and its receptors, especially 5-HT1A and 5-HT1B, on bladder cancer cell growth emphasize that these receptors have potential for treatment. It is thought that serotonin administration promotes cell growth in the HT1376 cell line with dose changes and may play a role in bladder cancer progression. In addition, 5-HT1B receptor antagonists, which significantly inhibit cell growth in the HT1376 cell line, suggest that this receptor may be a potential target in bladder cancer (Sarrouilhe et al., 2015).

In studies conducted with Small Cell Lung Carcinoma (SCLC), the effect of serotonin and its receptors on cell proliferation was examined and although the 5-HT1 antagonist methysergide was shown to be a potential treatment for SCLC, the 5-HT1D agonist sumatriptan was shown to increase SCLC proliferation. More studies should be conducted to elucidate the effect of serotonin and its receptors on SCLC (Sarrouilhe et al., 2015).

In the antagonist studies on colorectal cancer, Y25130 for 5-HT3 is seen as a potential target for treatment because it has a strong apoptotic effect. The effect of sulforaphane, which reduces the expression of serotonin receptors 5-HT1A, 5-HT2C 5-HT3, and SERT, on colorectal cancer is shown. Immunohistochemical analyses in colorectal cancer show that the 5-HT1B receptor needs to be investigated further (Sarrouilhe et al., 2015).

Cholangiocarcinoma is a type of cancer that occurs in the bile duct. Studies in this cancer show that serotonin receptors play an important role. Experiments have found that cholangiocarcinoma cell lines express all serotonin receptors and down-regulate 5-HT1B, 1F, 2B, 3C, and 7 and up-regulate all other receptors compared to the H69 cell line (Sarrouilhe et al., 2015).

Studies on serotonin and serotonin receptors in breast cancer have shown that serotonin signal imbalance plays a role in the onset and progression of breast cancer through disruption of the epithelial homeostatic system. In the MCF-7 cell line, 5-HT2A has been shown to have a proliferation-promoting property when serotonin levels increase. However, the increase in the expression of TPH1, which plays a role in serotonin biosynthesis, is associated with changes in tumor progression. However, immunohistochemical analyses do not show that 5-HT1A, 5-HT1B, and 5-HT2B receptors are associated with tumor grade (Sarrouilhe et al., 2015).

The most common form of liver cancer is hepatocellular carcinoma (HCC) and the role of the serotonin system, especially the receptors, in the development and prognosis of liver cancer is being studied significantly. In studies where different concentrations of serotonin were applied to Huh7 and HepG2 cell lines, cell survival and proliferation were increased. In the same cell lines, 5-HT1B and 5-HT2B antagonists (SB216641 and LY272015) were shown to have strong cytotoxic effects. Thus, serotonin receptors are thought to be of great importance in the treatment of hepatocellular cancer (Sarrouilhe et al., 2015).

In the study conducted with carcinoid tumors, typical bronchopulmonary NET cell line (NCI-H727), atypical bronchopulmonary NET cell line (NCI-H720), small intestine NET cell line (KRJ-I), and functional human pancreatic carcinoid cell line (BON) were used. As a result of the studies, exogenously added serotonin increased cell proliferation in four human carcinoid cell lines in a concentration-dependent manner. It was shown that the

proliferative effect of serotonin on carcinoid cells was mediated by 5-HT1A, 1B, and 5-HT2. This suggests that serotonin promotes the growth of carcinoid cells in an autocrine manner. In addition, the tumor growth-regulating effects of serotonin-release inhibitors such as Sandostatin LAR® demonstrate the potential of this mechanism in terms of treatment. In conclusion, these findings support that serotonin-related pathways may be therapeutic targets for carcinoid tumors (Sarrouilhe et al., 2015).

The effects of the HTR1B gene on various cell lines play a critical role in understanding the complex dynamics of the serotonergic system. In conclusion, the effects of the HTR1B gene on various cell lines constitute an important step in understanding the functionality and potential therapeutic uses of this gene. More comprehensive studies of HTR1B in various disease models may contribute to the development of the targeted treatment strategies.

The Ser-4 gene, the human HTR1B *C. elegans* ortholog, is part of the serotonin receptors and these receptors play an important role in locomotor activity. Serotonin activates these receptors, slowing down movement (Gürel et al., 2012). Serotonin may also affect the immune system of *C. elegans*. The function of serotonin receptors, such as Ser-4, is to regulate G-protein communication in serotonin epithelial cells and may affect the immune system response to infections (Anderson et al. 2013). Serotonin and serotonin receptors, such as Ser-4, may have a role in the functions of cells that regulate development and food sensing. This may affect postembryonic development and food-seeking behavior (Gürel et al., 2012).

In our study, the expression of serotonin receptor HTR1B was compared in both healthy human endothelial cells and prostate cancer cell line PC-3 cells, which are observed to be more sensitive to 5-HT1A, 5-HT2B, and 5HT4 antagonists due to hypoxia, an important mechanism for cancer microenvironment. The response of the Ser-4 gene, which was revealed to be %87 similar in bioinformatic analyses performed by us in *C. elegans*, which is planned to be a model in cancer studies related to serotonin, to hypoxia was also investigated. It was observed that HTR1B and Ser-4 were studied in both human cell line models and *C. elegans*, responded to hypoxia. HIF-1 binding site was detected in TF binding regions of the promoter regions of the genes. In the literature, no studies have been found on the chemical hypoxia model of this gene in HUVEC and PC-3 cell lines under hypoxic conditions and the *C. elegans* ortholog of this gene, Ser-4. Investigating the responses of these genes to hypoxic conditions and revealing their similarities in humans and *C. elegans* provides new information about serotonin and its metabolism in the evolutionary process. It also showed that hypoxia, a cancer-related condition, should be addressed in the creation of therapeutic protocols in different cancer cell types where these genes are effective. All these results indicate that further regulation studies are needed to reveal the status of serotonin metabolism in cancer. The effects of the HTR1B gene on various cell lines play a critical role in understanding the complex dynamics of the serotonergic system. In conclusion, the effects of the HTR1B gene on various cell lines constitute an important step in understanding the functionality of this gene and its potential therapeutic uses. In our study, the selected genes were examined at the mRNA level. Examining the response of these genes at the protein level and also at the promoter level can provide information about whether there are changes in different gene regulation steps of the hypoxic response. A more comprehensive examination of HTR1B in various disease models may contribute to the development of the targeted treatment strategies.

Ackowledgement: This work was supported by Health Institutes of Türkiye (TUSEB) project (TUSEB 2023-A4-04 38374).

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

Author Contributions: Conception – S.A.T.; Supervision – S.A.T.; Fund – S.A.T.; Materials – S.A.T.; Data Collection and Processing – S.A.T., C.T., A.B., F.P.; Analysis Interpretation – S.A.T., C.T., A.B., F.P.; Literature Review – S.A.T., C.T., A.B., F.P.; Writing – S.A.T., C.T.; Critical Review – S.A.T., C.T.

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