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Research Article

Tau protein expression and phosphorylation in a glucose-repressed yeast model: Insights into the cancer-alzheimer's disease link

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Abstract

Objectives: The microtubule-associated protein tau, responsible for stabilizing microtubules, plays a role in the pathology of neurodegenerative diseases called tauopathies, including Alzheimer's disease. In Alzheimer's disease, neurofibrillary tangle formation is observed as a result of tau hyperphosphorylation. Although it is known that tau protein plays a role in many cellular processes, all of its functions have not yet been elucidated. The inverse relationship between Alzheimer's disease and cancer has been a topic of research that has attracted attention in recent years. In addition, the role of tau protein in cancer has also gained importance with the determination of its direct relationship with DNA. In particular, the negative correlation between Alzheimer's disease and cancer points to two extremes of a common mechanism. Discovering a common molecule or pathway will allow understanding the cause of both diseases and developing treatments.

Methods: In this study, we obtained a cell model that mimics cancer metabolism by creating aerobic glycolysis-like conditions with glucose repression in *S. pombe* cells heterologously expressing human tau protein. We examined tau protein expression and phosphorylation (S262, S396 and S404) and various cellular processes (glucose metabolism, stress response, ER stress, autophagy, 20S proteosome activity, intracellular oxidation) at the molecular level in model cells.

Results: Under aerobic glycolysis-like conditions, we observed an approximately 2-fold increase in tau protein expression. In addition to this increase, we determined that the amount of phosphorylation at S396 residue of tau protein was decreased, while phosphorylation at S262 and S404 residues was increased.

Conclusion: These findings suggest a potential divergence in tau regulation under altered metabolic conditions, warranting further investigation.

Keywords: Cancer, glucose repression, heterologous expression, tauopathy, tau protein

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Microtubules play a role in morphogenesis, cell division, intracellular transport of macromolecules and organelles, and motility [1]. These processes occur through dynamic restructuring of microtubules mediated by microtubule-associated proteins such as tau. Tau protein is responsible for the stabilization of microtubules [2]. There is great interest in tau protein because it plays a major role in neurodegenerative diseases referred to as tauopathies, including Alzheimer's disease (AD) [3]. There is increasing evidence that tau protein, in addition to its microtubule stabilizing function, is implicated in many significant signaling pathways, including proliferation, morphogenesis, and cell differentiation [4]. A lesser-known property of tau is its ability to bind to cancer-associated protein kinases. This suggests that tau has a potential role in regulating microtubule-independent cellular pathways [3]. It has also been determined that tau protein can bind to nu-

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cleic acids [5]. Tau has been suggested to be a DNA protector, specifically by preventing stress-induced DNA breaks [6, 7].

Over 55 million individuals globally suffer from dementia, with AD constituting 60–70% of these instances. The global prevalence of Alzheimer's disease is projected to surpass 138 million by 2050 [8]. Another leading disease with a high mortality rate is cancer. Approximately 10 million deaths attributed to cancer were reported globally in 2020, along with 19.3 million newly diagnosed cases of cancer. [9, 10]. In AD, alterations in behavior and cognitive impairments linked to neurodegeneration typically manifest around a decade following the beginning of proteinopathy. Its two characteristic features are Aβ plaques, and neurofibrillary tangles caused by tau hyperphosphorylation [11]. Cancer is a disease in which some cells uncontrolled divide by genetic mutations and they spread to other parts of the body. Brown and colleagues define cancer as a disease characterized by the uncontrolled multiplication of altered cells that undergo evolution through natural selection [12]. Multiple epidemiological studies indicate a negative correlation between cancer and AD [13]. This intriguing correlation is limited to certain types of cancer and neurodegenerative diseases. However, the underlying mechanisms of the two diseases are very different. While cancers evade cell death, neurodegeneration leads to cell death. As a result, it is possible that people with a neurodegenerative disease might have a lower likelihood of developing specific kinds of cancer, and the opposite might also be true [14]. Even though the underlying physiological processes of cancer and AD have been extensively researched, they are not yet distinctly understood [10].

Dysregulation of cellular energy metabolism is one of the hallmarks of cancer [15]. The Warburg effect refers to the transition from aerobic respiration to aerobic glycolysis, resulting in the production of lactate from glucose as the end-product. Warburg reported that even when oxygen is sufficient in an environment, cancer cells generally use glucose via glycolysis rather than oxidative phosphorylation [16]. Cancer cells typically exhibit increased glycolysis and diminished oxidative phosphorylation. While this long-term metabolic reprogramming is known as the Warburg effect, the short-term, reversible alteration of this metabolic process is known as the Crabtree effect [17]. The Warburg effect provides an advantage for cancer cells to survive and progress. In order to achieve high aerobic glycolysis rates, some types of cancer cells are induced to possess specific isoforms of glycolytic enzymes [18]. This metabolic adaptation helps cancer cells to proliferate and become more invasive. The relationship between high glucose and cancer development is still being investigated [19].

Glucose is the preferred carbon source for most living cells, and glucose metabolism provides the energy necessary for cell survival [20]. In yeast, glucose can induce the expression of various genes encoding some glucose transporters, ribosomal proteins, and glycolytic enzymes, by a process known as glucose induction. At the same time, the expression of numerous genes involved in respiration, gluconeogenesis, and the utilization of alternative carbon sources can be suppressed, in the process named glucose repression. Yeasts are easy to use as models in studies examining glucose perception and signal transduction because of their similarity to complex multicellular eukaryotes in terms of glucose metabolism. Although yeast cells can use a wide variety of carbon sources, the presence of abundant glucose suppresses the utilization of alternative carbon sources, cellular respiration, and gluconeogenesis [21]. *Schizosaccharomyces pombe*, the fission yeast, is a single-celled model organism that can be used in studies on glucose sensing, intracellular signaling, and signal response processes. It shows high similarity with multicellular eukaryotic organisms in terms of molecular processes [22, 23].

Genes involved in various processes in human have orthologs in *S. pombe*, and *S. pombe* has 1514 orthologous transcripts (proteins and ncRNAs) associated with human diseases [24, 25]. Phosphorylation of tau protein, which plays a role in various diseases, is carried out by glycogen synthase kinase 3 beta (GSK-3β), cyclin-dependent kinase 5 (CDK-5), and cAMP-dependent protein kinase A (PKA) and the orthologous of the human GSK3β gene, which is the major kinase in the phosphorylation of tau protein, exists in the *S. pombe* genome [26–28]. Moreover, in our previous studies, we showed that human tau protein is phosphorylated in the *S. pombe* model, and we detailed several cellular processes [29, 30].

In this study, based on the inverse correlation seen in cancer and neurodegenerative diseases, we investigated the relationship between tau protein and the aerobic fermentation process observed in cancer, which we mimicked by glucose repression metabolism in yeast. We hypothesize that one of the factors affecting the dysregulation of glucose metabolism in neurodegenerative diseases and cancer may be the tau protein, which stands out with its role in microtubule stabilization and has a potential effect on glucose metabolism. In the present study, the conditions of glucose repression in *S. pombe* were referred to as "aerobic glycolysis-like state". For this purpose, we cultured *S. pombe* cells heterologously producing human tau protein under high glucose conditions (5%) to create glucose-repression and mimicked aerobic fermentation in cancer cells. We studied various processes at the molecular level in model cells with aerobic glycolysis-like metabolism in the presence of tau protein. In our model cells, a decrease in stress response was seen, as in cancer cells. We determined that under aerobic glycolysis-like conditions, the expression of tau protein in cells increased approximately 2-fold. Additionally, we observed a decrease in the phosphorylation level at the S396 site of tau protein and an increase in the phosphorylation levels at the S404 and S262 regions. Contrary to expectations under aerobic glycolysis-like conditions, the increase in tau level and the decrease in hexokinase, which plays a role in glycolysis, suggested that tau protein has a greater role in glucose metabolism than is known. The findings suggest that the inverse relationship between neurodegenerative diseases and cancer may be due to differences in the phosphorylation level of tau protein, which plays a role in

Table 1. Primer sequences used in real-time PCR

Gene	Forward Primer	Reverse Primer	Tm (°C)
gpdh	5' ggtgacaaccactcctccat 3'	5' tcaacaacacggtgggagta 3'	55
mapt	5' caagtgtggctcaaaggataat 3'	5' ggtttatgatggatgttgcctaa 3'	55
hxk2	5' caacaaggactttgcccaat 3'	5' aaggtgtcgctctcctttga 3'	55
fbp1	5' gtatggtgcttcggctcatt 3'	5' ttcatgtttcgatgggtcaa 3'	55
sod1	5' attggccgtaccattgtcat 3'	5' gacaccacaagcgttacgtg 3'	55
ctt1	5' atcctcaatccgaccacttg 3'	5' aacgtcggtaatttcgtcca 3'	55
ire1	5' attctcgacattcttcgggt 3'	5' aacttgtgaatccgtctggt 3'	55
atg14	5' tcaccctagtttactctcaaca 3'	5' cggcaaatgtccataaaaactc 3'	55
gsk3	5' gatgcttctcctcgtcatt 3'	5' catcaagtttcacgggtaaag 3'	55
pp2a	5' tattgttatcgctgtggtaatc 3'	5' ggtgtccttcgagctatt 3'	55

the molecular mechanisms of both diseases, or to an as-yet-unknown function of tau protein in glucose metabolism.

Materials and Methods

Yeast strain and culture conditions

In the present study, we used *S. pombe* cells (pMS-mapt) that produce human tau protein, which we obtained in our previous study [29]. pMS-mapt cells are auxotrophic for guanine and carry the human *MAPT* gene in the pSGP572 plasmid. pSGP572 contains the *ura4* marker gene, and the *GFP* gene at the 3'-terminal in the cloning site. So, the tau protein produced is a fusion protein with GFP. The *MAPT* and *GFP* genes are under the control of the *nmt* promoter, which is an inducible promoter and is repressed by thiamine [31].

In this study, we investigated the relationship between aerobic fermentation conditions and tau protein. Firstly, tau protein production was induced in pMS-mapt cells [24], then these cells were grown under different glucose conditions. For this purpose, cells were grown in standard EMM (1% glucose) medium containing thiamine (15 µM) and guanine (50 mg/L) at 30°C for 24 hours. Then, the cells were washed with PBS and cultured in standard thiamine-free EMM medium with guanine for 20 hours. After incubation, cells were washed with PBS and cultured in EMM containing different concentrations of glucose (3% and 5%) at 30°C for 4 hours.

Cell densities of pMS-mapt cells were measured spectrophotometrically at 600nm wavelength for 32 hours, and their growth under different conditions were compared. Cells grown under different glucose concentration conditions were examined under the microscope.

Gene expression analysis

After cells collected, total RNA isolation was performed by using "Thermo Scientific GeneJET RNA Purification Kit", according to the manufacturer's instructions with a minor modification. Cells in PBS were mechanically homogenized by using glass beads. After RNA isolation, cDNA was synthesized from 2 µg of total RNA by using a "Roche Transcriptor High Fidelity cDNA Synthesis Kit", according to the manufacturer's instructions.

We examined the expression level of genes related to various cellular mechanisms, including glucose metabolism (*hxk2, fbp1*), stress response (*sod1, ctt1*), ER stress (*ire1*), autophagy (*atg14*), and regulators of tau phosphorylation (*gsk3, pp2a*). Additionally, *mapt* gene expression was also examined.

Real-Time PCR was performed using "ThermoScientific Applied Biosystems PowerUp SYBR™ Green PCR Master Mix" kit and the primers, which we used in our previous study, listed in Table 1 [24]. Pairs of primers were designed using the "IDT PrimerQuest Tool". *S. pombe gapdh* gene was used as the reference gene. We applied the Pfaffl equation to analyse the relative expression levels of genes and we used *gapdh* gene expression levels for normalization [32]. All experiments were performed in three biological and technical replicates.

Immunoblotting analysis

Protein extraction from cells grown under different glucose concentration conditions was performed according to the method of Forsburg and Rhind (2006) with minor modifications [31]. The cells were mechanically homogenized by vigorous shaking using glass beads and lysis buffer (150 mM NaCl, 1mM PMSF, 0.5% (w/v) Nonidet-P40, 5 mM EDTA 50 mM Tris).

SMART™ bicinchoninic acid (BCA) protein assay kit (iNtRON Biotechnology) was used to quantify total protein. Equal amounts of protein (30 µg/well) was loaded on 10% SDS-PAGE, and transferred to PVDF membrane (Thermo Fisher Scientific). After blocking, the membrane blots were incubated overnight with the following primary antibodies: Anti-tau rabbit IgG (1/1000 dilution, Tau Rabbit mAb, Abclonal) and anti-phospho-tau (Ser396) rabbit IgG (E178) (1/1000 dilution, ab32057, Abcam), phospho-tau S404 Rabbit mAb (1/1000 dilution, AP1378 Abclonal) and phospho-tau S262 Rabbit pAb (1/1000 dilution, AP0397 Abclonal). Anti-GAPDH mouse IgG (1/2500 dilution, MA5-15738 Invitrogen) was used as internal control. The washed membranes were incubated with HRP-linked secondary antibody (1/5000 dilution anti-rabbit HRP Goat Anti-Rabbit IgG, Abclonal). Following washing, the blots were visualized using SuperSignal West Pico PLUS ECL reagent (Thermo Fisher Scientific). Immunoblots (heterologous MAPT protein levels) were imaged

using the ChemiDoc XRS system (BioRad). We performed quantification by using ImageLab 6.0.1 software (Bio-Rad).

Measurement of 20S proteasome activity

We determined 20S proteasome activity as suggested by Reinheckel et al. [33]. After protein extraction from grown cells under stated conditions, we diluted protein concentration to 50 µg/mL with assay buffer (50 mM Tris, 20 mM KCl, 0.5M DTT, 5 mM CH3(COO)2). To measure peptidase activity, we added 99 µL assay buffer and 1 µL Suc-Leu-Leu-Val-Tyr-AMC (Sigma) (stock 40 mM) to 100 µL of diluted protein sample. After 1 h incubation at 37°C, the reaction was stopped by adding an equal volume of cold ethanol and 1.6 mL of 125 mM sodium borate buffer (Na2B4O7.10H2O, pH 9.0). Then, we measured 20S proteasome activity by a spectrofluorometer plate reader (ex: 390nm, em: 470 nm).

Determination of intracellular oxidation level

We determined the intracellular oxidation level as recommended by Inoue et al. [34]. In this method, intracellular oxidation level is measured by conversion of DCFH-DA (2',7' dichlorofluorescein diacetate) to the fluorescent compound 2',7'-dichlorofluorescein. pMS-mapt cells grown in EMM media containing guanine and different glucose concentrations (3%, 5%) were centrifuged for 5 minutes at 3000 g, and cells were dissolved in 1 mL EMM media with guanine. 40 µM DHCF-DA (Sigma) solution dissolved in ethanol was added and incubated at 30°C for 1 h, and the samples were washed with PBS. We kinetically measured intracellular oxidation level with a spectrofluorometer plate reader (Bio-tek FLx800) with excitation and emission wavelengths of 490 and 524 nm.

Imaging by microscopy

S. pombe cells grown under the stated conditions were fixed using 10% formaldehyde, and the cells were washed with PBS. Then, cells were examined under the microscopy (Olympus BX53).

Statistical data

We expressed all of our data as mean±SD. We used GraphPad Prism 9 software for statistical comparisons based on Student's t-test. The value of $p<0.05$ was considered statistically significant.

Results

Microscopic examination of the cells

We examined pMS-mapt cells grown under different glucose concentrations under the microscope, and observed morphological differences in cells grown under aerobic glycolysis-like conditions for 4 hours (Fig. 1a). Additionally, the absorbance graph of cell cultures measured every two hours at 600 nm wavelength for 32 hours is given in Figure 1b.

Aerobic glycolysis-like conditions

When pMS-mapt cells grown under standard glucose concentration conditions were compared with cells under aerobic glycolysis-like conditions, a 3.58-fold decrease in the expression of the *hxk2* gene, which encodes the hexokinase 2 enzyme that plays a role in glycolysis in glucose metabolism, was observed under aerobic glycolysis-like conditions. A 4.52-fold decrease was observed in the expression of the *fbp1* gene, which encodes the fructose-1,6-bisphosphatase 1 enzyme that plays a role in gluconeogenesis under aerobic glycolysis-like conditions (Fig. 2).

Tau protein and its phosphorylation

When the expression of tau protein in cells grown under standard and aerobic glycolysis-like conditions was examined by western blot analysis, an increase in total tau was observed under repression conditions, consistent with gene expression analysis. However, it was determined that the phosphorylation level in the S396 region of the tau protein was lower in cells grown under aerobic glycolysis-like conditions, while the phosphorylation level in the S262 and S404 regions of the tau protein was higher in cells grown under aerobic glycolysis-like conditions (Fig. 3a).

Under aerobic glycolysis-like conditions, *MAPT* gene expression increased 2.68-fold. However, the expression of the *gsk3* gene, which is responsible for the phosphorylation of tau protein and encodes the glycogen synthase kinase 3 enzyme, decreased 2.67-fold. In addition, the expression of the *pp2a* gene, which encodes the protein phosphatase 2a enzyme that removes phosphate groups from the tau protein, decreased 2.78-fold (Fig. 3b).

Cellular stress response

When the intracellular oxidation level was examined, a significant 1.86-fold increase was observed under aerobic glycolysis-like conditions (Fig. 4a). The expression of *sod1* and *ctt1* genes, which encode superoxide dismutase and catalase enzymes that play a role in the stress response in the cell, decreased 1.68 and 3.39 times, respectively (Fig. 4b).

Under aerobic glycolysis-like conditions, 20S proteasome activity, one of the pathways responsible for the degradation of proteins in the cell, was found to be 10% higher than standard conditions (Fig. 5a). When the expression of the *atg14* gene, which is related to autophagy, another degradation pathway, was examined, no significant change was observed under aerobic glycolysis-like conditions. The expression of the *ire1* gene, a marker of endoplasmic reticulum stress, decreased 2.14-fold under glucose suppression conditions. (Fig. 5b).

Discussion

In recent years, interest in the relationship between Alzheimer's disease and cancer has increased. There are studies showing that the risk of developing cancer decreases in Alzheimer's patients and, conversely, the risk of developing AD decreases in cancer patients [10, 13, 35, 36]. In their study, Musicco and colleagues reported that the risk of cancer was

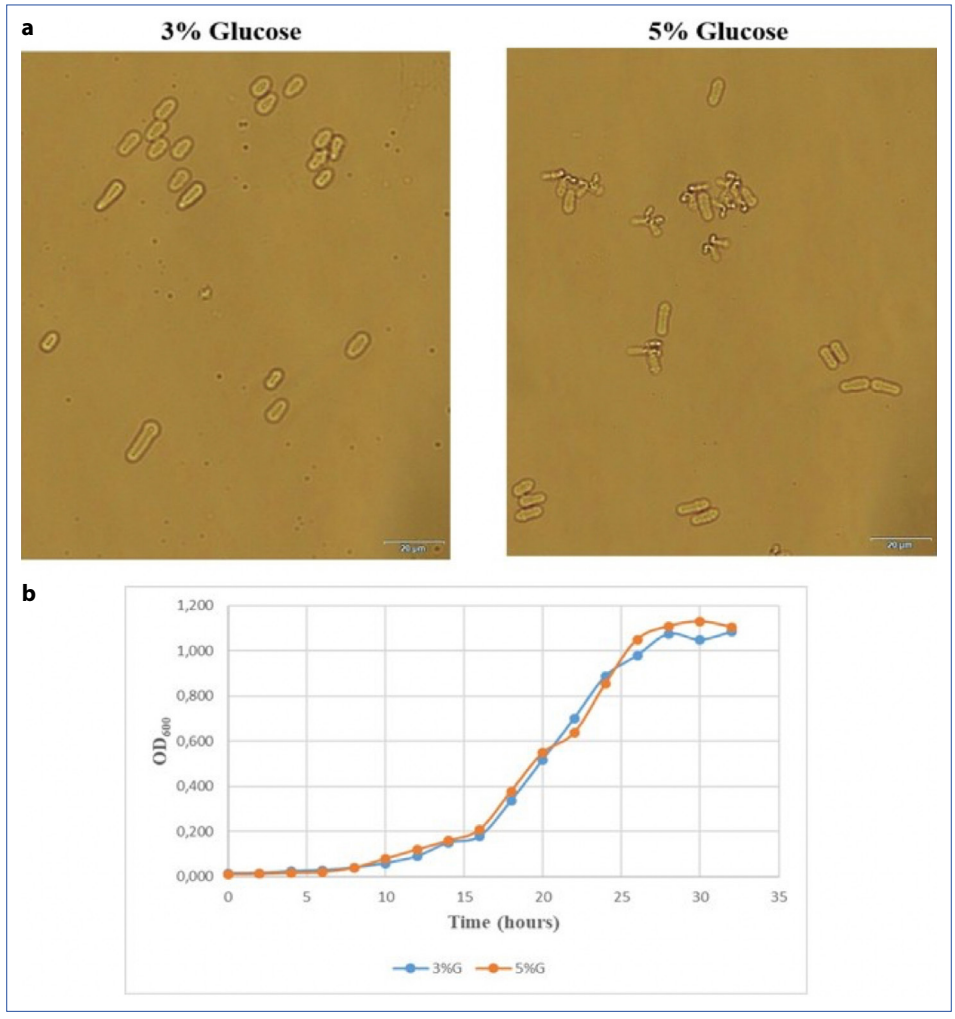

Figure 1. Growth and imaging of pMS-mapt cells under different conditions. (a) Microscope images of pMS-mapt cells grown under different glucose concentrations (40X magnification and bar represents 20 µm). (b) Growth curves of pMS-mapt cells grown under different glucose concentrations.

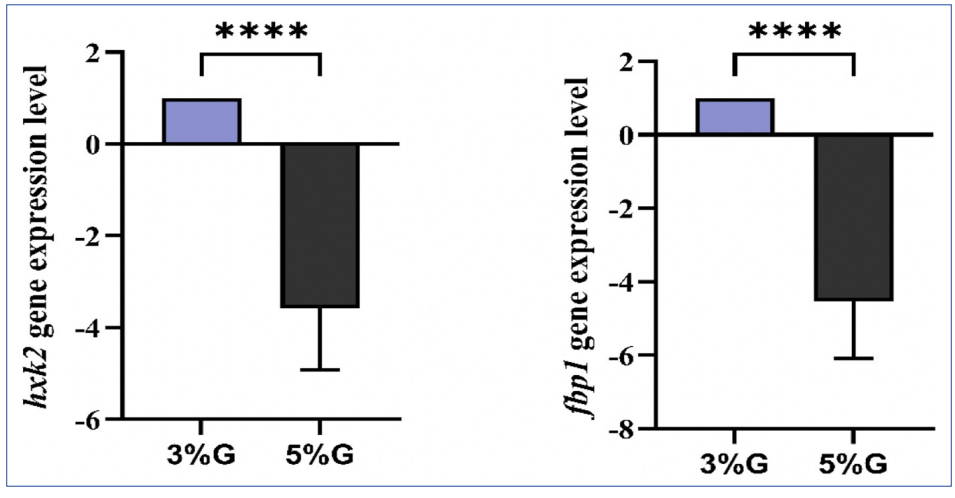

Figure 2. Expression levels of *hxk2* and *fbp1* genes related to glucose metabolism in pMS-mapt cells grown at different glucose (G) concentrations (3%G, 5%G).

Data (mean±SD) was derived from three independent experiments and analyzed using unpaired Student's t-test (****p<0.0001).

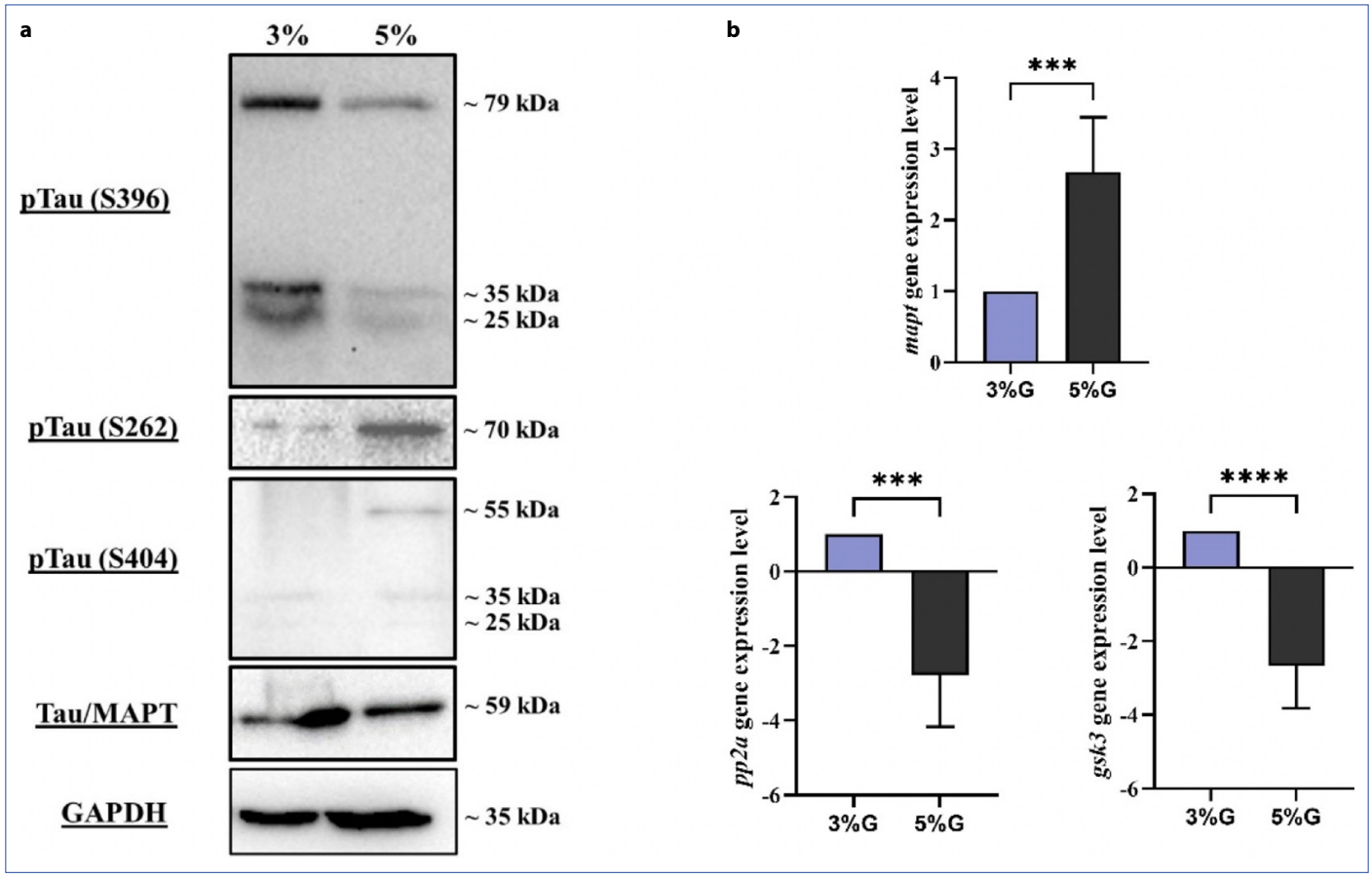

Figure 3. Indication of tau protein expression and phosphorylation in pMS-mapt cells grown under different conditions. (a) Membrane images as a result of western blot of total tau and phosphorylated tau protein (S262, S396 and S404) in pMS-mapt cells. (b) Expression levels of the *MAPT* gene encoding tau protein and the *gsk3* and *pp2a* genes encoding glycogen synthase kinase 3 and protein phosphatase 2a, which are responsible for the phosphorylation and dephosphorylation of tau protein.

Data (mean±SD) was derived from three independent experiments and analyzed using unpaired Student's t-test (***p<0.001; ****p<0.0001). SD: Standard deviation.

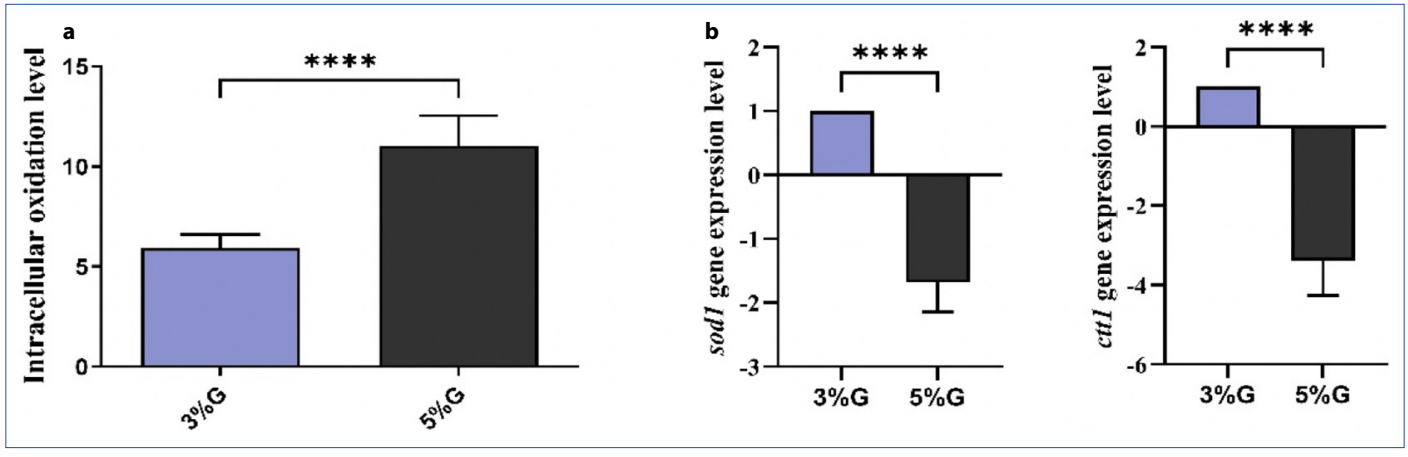

Figure 4. Intracellular oxidative stress response in pMS-mapt cells grown under different conditions. (a) Intracellular oxidation level in cells (b) Expression levels of stress response genes *sod1* and *ctt1*.

Data (mean±SD) was derived from three independent experiments and analyzed using unpaired Student's t-test (****p<0.0001).

halved in AD patients and the risk of AD in cancer patients was reduced by 35% [37]. Although the pathophysiological mechanisms of both cancer and AD have been widely studied, they have not been elucidated. However, an inverse relationship between them is noticed. It has been determined that patients with AD have a 61% lower risk of cancer [10].

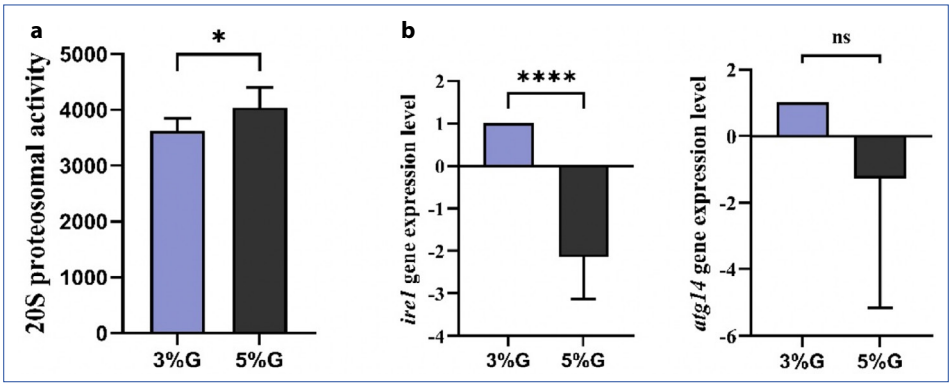

Figure 5.20S proteasome activity in pMS-mapt cells grown under different conditions. (a) 20S proteasome activity in cells (b) Expression levels of ire1 genes, which play a role in ER stress response, and atg14 genes, which play a role in autophagy.

Data (mean±SD) was derived from three independent experiments and analyzed using unpaired Student's t-test (*p<0.05; ****p<0.0001). ns: Non-significant.

Tau protein is recognized for its involvement in neurodegenerative disorders. Research indicates that Tau may contribute to the advancement of several tumors and the resistance to cancer therapies [3]. Numerous studies have documented abnormal levels of tau in cancer cells of the brain, breast, stomach, and prostate [38]. Moreover, the level of tau expression has been associated with resistance to anti-microtubule drugs in cancer [39, 40]. Gargini and colleagues showed that higher levels of MAPT were inversely correlated with glioma aggressiveness [41].

Aerobic glycolysis (Warburg effect) is observed in cancer cells under high glucose conditions. In some types of cancer, glycolytic enzymes are stimulated to achieve high rates of aerobic glycolysis [18]. Glycolysis is promoted, and this metabolic adjustment enables cancer cells to reproduce and invade more rapidly. This strengthens the competition between cancer cells with normal cells [19].

Yeasts also prefer fermentation despite the presence of oxygen, similar to cancer cells, when there is plenty of glucose in the environment. Additionally, the expression of numerous genes that play a role in gluconeogenesis and respiration, the use of alternative carbon sources, and stress response pathways are suppressed in cells [21, 41].

There are both similarities and differences between aerobic glycolysis, where cancer cells turn to glycolysis even in the presence of oxygen (the Warburg effect), and processes where yeast cells prefer fermentation over cellular respiration in the presence of high glucose and oxygen (the Crabtree effect). In both cancer cells and yeast, this fermentative metabolism is associated with rapid growth and proliferation [17]. In both processes, fermentation occurs in an oxygenated environment; however, the end products are lactate (resulting from aerobic glycolysis) or ethanol (resulting from fermentation). Additionally, aerobic glycolysis and fermentation, glycolysis is rapid, and mitochondrial respiration is repressed. Besides their similarities, one of the most important differences is that the Warburg effect is permanent in cancer cells, but in yeast, when glucose is depleted in the environment, aerobic respiration begins [42]. Santos and Hartman (2019) mimicked the Warburg effect by repressing respiration in the presence of glucose in *S. cerevisiae*. They examined the effects of doxorubicin used in chemotherapy in a yeast model and reported that glucose-repressed yeasts could be a suitable model for cancer research [43]. Although yeast, a single-celled organism, cannot fully provide the Warburg effect, it has the potential to elucidate cancer mechanisms due to the similarities between the processes.

In *S. pombe*, glucose is sensed by G protein-coupled receptor (GPCR), and signal transduction occurs via cAMP-dependent protein kinase A (PKA) [22, 44]. Glucose repression signaling represses *fbp1* gene expression via activation of the cAMP-dependent PKA pathway [45]. In our study, under aerobic glycolysis-like conditions, the expression of the *fbp1* gene encoding the fructose-1,6-bisphosphatase-1 enzyme that plays a role in gluconeogenesis was repressed.

Hexokinase-2 enzyme is encoded by the *hxk2* gene and is a rate-limiting enzyme that functions in the first step of the glycolytic cascade [19]. Hxk2 is an important enzyme in glucose repression. Under high glucose conditions, PKA causes hyperphosphorylation of Rgt1, followed by expression of the *hxk2* gene [46, 47]. In our study, transcription of the *hxk2* gene was unexpectedly decreased under aerobic glycolysis-like conditions in pMS-mapt cells. This may be due to the increase in the expression of the *MAPT* gene.

When total tau and the phosphorylation status of tau in the S262, S396, and S404 regions were examined by immunoblot analysis, aerobic glycolysis-like caused an increase in the expression of tau protein, while a decrease in phosphorylation was observed at S396 residue. Tau phosphorylation at S396 and S404 residue is one of the earliest events in AD [48, 49]. We observed that under aerobic glycolysis-like conditions, the increase in *MAPT* gene expression in cells is consistent with the increase in tau protein. However, under aerobic glycolysis-like conditions, higher phosphorylation occurred at residues S262 and S404 of tau protein. Tau phosphorylation at sites such as Ser262 in the proline-rich region and Ser396/404 at the edges of the microtubule binding

site induces conformational change of tau protein and weakens the binding of tau protein to microtubules [50]. Full-length tau has 85 potential phosphorylation sites [51]. Since only three phosphorylation sites (S262, S396, and S404) were analyzed in the present study, our interpretation remains limited. Future studies should include additional AD-relevant phosphorylation sites to better assess tau modification patterns.

Glycogen synthase kinase-3 (GSK3) is a protein kinase composed of GSK3α and GSK3β subunits that phosphorylates a large number of substrates. Increased GSK3 expression has been seen in the brains of AD patients and models. GSK3 directly promotes tau phosphorylation, modulates amyloid precursor protein (APP) breakage, results in Aβ production, and either directly or indirectly incites neuroinflammation and oxidative injury [52]. In our study, under aerobic glycolysis-like conditions, the expression of the *gsk3* gene, which encodes GSK3, which phosphorylates tau protein, was reduced in cells, and the decrease in the phosphorylation level of tau protein was associated with this decrease in the expression of the *gsk3* gene. PP2A, a serine/threonine protein phosphatase, is a tumor suppressor [53] and regulates the cell cycle by interacting with more than 300 cell cycle-related substrates [54]. Expression of protein phosphatase 2A (PP2A) is reduced in both cancer and neurodegenerative diseases. A decrease in PP2A-Aα subunit expression to approximately 50% of the normal level induces tumor formation, while a decrease in PP2A-Aα expression by more than 63% results in apoptosis [14]. Similar to cancer conditions, we observed that decreased expression of the *pp2a* gene in pMS-mapt cells under aerobic glycolysis-like conditions.

It was seen that the cells were both smaller and had different cellular shapes compared to cells in normal conditions. However, these differently shaped cells are not dead cells, because the cells were observed to reproduce for 32 hours.

Under glucose repression conditions, the stress response in the cell is repressed [22, 55, 56]. We also observed a decrease in the gene expression of stress response genes *sod1* and *ctt1*, as expected. Suppression of stress response genes in pMS-mapt cells resulted in an increase in the level of intracellular oxidation. ER stress and unfolded protein response (UPR) are molecular events in the development of AD. Failure of these mechanisms results in the formation of pathological structures such as neurofibrillary tangles formed by hyperphosphorylated tau. ER stress is an imbalance between protein synthesis and ER protein folding capacity. This results in the accumulation of misfolded proteins in the ER. As a result of ER stress, unfolded proteins are proteasomally degraded [57–59]. In the present study, we observed that the expression of the ire1 gene, which is involved in the ER stress response, was reduced under aerobic glycolysis-like conditions. A decrease in autophagy is an expected result due to decreased ER stress in pMS-mapt cells, and this may explain the decrease in *atg14* gene expression. No significant change in 20S proteasome activity was observed under aerobic glycolysis-like conditions in pMS-mapt cells. Decreases in ER stress response and autophagy are the expected results under repression conditions.

Several studies on diabetes and tau protein suggest a role of tau protein, particularly in regulating glucose homeostasis. In some studies, Alzheimer's disease has been referred to as type III diabetes. However, the relationship between impaired glucose signaling and tau has not yet been elucidated [60]. One of the limitations of this study is that the effect of insulin, one of the most important components of glucose metabolism, could not be demonstrated in model cells. Another limitation is that the DNA protection function of tau protein has not been examined in our model. Emerging evidence for tau's functions in P53 regulation and DNA repair suggests that it is associated with cancer. Studies have shown that the MAPT gene is a potential factor in many types of cancer. However, the role of tau in cancer is still unclear. Whether tau is positively or negatively associated with cancer type may be because it plays a role in different cellular processes [7].

In the present study, aerobic fermentation metabolism in cancer was mimicked by creating glucose repression conditions in the tau protein-producing *S. pombe* model organism. Under aerobic glycolysis-like conditions, we observed an increase in tau protein expression and a decrease in its phosphorylation. In contrast, it is the increase in tau phosphorylation that causes pathology in tauopathies. Thus, under cancer-mimicking conditions, a situation in tau phosphorylation that was opposite to that in neurodegeneration was observed. The fact that a different response than expected occurred also in glucose metabolism in these tau-producing cells suggests that tau protein may be a common component that plays a greater role in the mechanisms underlying the diseases than thought. In our study, we examined only one phosphorylation site, and although this is not sufficient for a definitive conclusion, it provides data on the change of phosphorylation status. In future studies, target phosphorylation sites in Alzheimer's disease should be examined in more detail in cancer. Based on previous studies and our findings, tau protein may play a more important and multifunctional role in cellular mechanisms than thought. Therefore, the cellular mechanisms in which tau protein is involved and the molecular components it interacts with should be investigated in more detail.

Conclusion

More detailed studies are needed to understand the molecular and cellular mechanisms related to the different or common features between cancer and neurodegeneration. Both diseases are common and have high mortality rates. Most studies investigating the relationship between cancer and neurodegenerative diseases demonstrate the potential for one disease to protect against the other. At this point, it is of great importance to determine the mechanism that brings together two opposing processes, such as uncontrolled cell proliferation and degeneration, and the intermediary molecules involved in this mechanism. Understanding the underlying mechanisms linking AD and cancer will enable the development of prevention strategies and treatment for both diseases.

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