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Application of Gadph in Human Tumor Research

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Abstract. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has long considered as a crucial enzyme that involved in glycolysis and production of ATP and pryuvate. Also, since it has relatively stable expression in cells, therefore it is been widely applied as a reference gene. Recent research shows that GADPH take part in more function in regular cells and tumor cell researches. This review compiles knowledge of GADPH, by discussing GADPH function and performance in tumor cell research, and it can be found that GADPH is a crucial enzyme of glycolysis, and involved in cellular stress response, also, GADPH affects gene regulation indirectly. In tumor cell researchs, GADPH functions as an widely used housekeeping gene, cause of Warburg effect, an factor affects cellular drug tolerance, and a potential biomarker. Not only can this research improve the shortcomings of current research, also future research area can be expanded.

Keywords: GADPH, metabolism, oxidative stress response, housekeeping gene

1. Introduction

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a crucial enzyme involved in various cellular processes and is widely distributed across different tissues. Its significance has grown in recent years due to the increasing interest in tumor cell research. As an essential enzyme in tumor cell metabolism, GAPDH may hold the key to understanding the metabolic mechanisms of cancer [1]. Additionally, in cancer-related studies, GAPDH is frequently used as a reference gene or protein in experimental techniques such as RT-PCR, qPCR, and Western blotting.

Beyond these well-established roles, an increasing number of studies have revealed additional mechanisms and functions of GAPDH and its corresponding gene [1]. Therefore, this review aims to summarize and synthesize previous research on GAPDH. First, an overview of GAPDH's functions will be provided, including its roles in cellular metabolism, stress response, and gene regulation. Next, the application of GAPDH in tumor-related research will be discussed, focusing on its use as an internal reference gene, its involvement in the Warburg effect, its influence on drug tolerance, and its potential as a biomarker.

2. GADPH related mechanism

By examining these mechanisms and effects, this review seeks to consolidate existing knowledge of GAPDH's functions and applications. Finally, the paper will identify current research gaps and propose directions for future studies. The findings of this review will contribute to the current body

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of knowledge by offering new insights into the functions of GAPDH. Moreover, it opens avenues for further investigation, particularly in exploring GAPDH's uncharacterized cellular and molecular mechanisms in tumor cells.Application of GADPH

2.1. Metabolism

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) plays a crucial role in metabolism, which is evident in its involvement in glycolysis and various cellular processes. In glycosis, Glyceraldehyde-3-phosphate dehydrogenase converts Glyceraldehyde 3-phosphate to 1,3-Bisphosphoglycerate and produce NADH and proton in sixth step of glycolysis, represents the beginning of ATP production statement [2]. Specific progress is described in Figure 1 below:

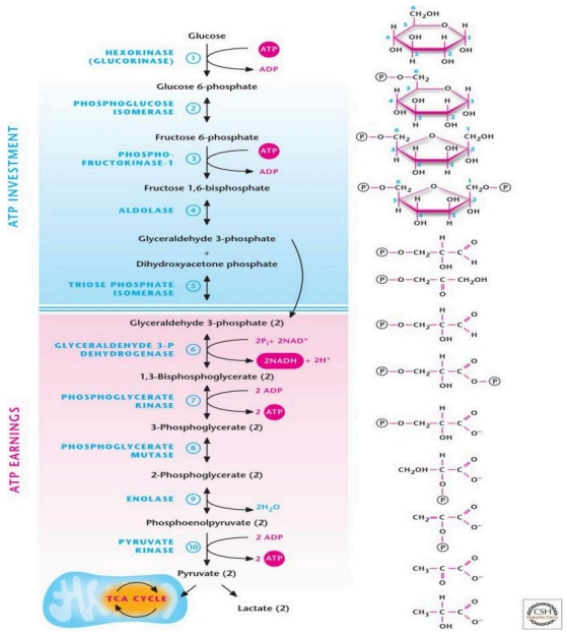


Figure 1. 10 steps of Glycolysis [1]

Moreover, previous research has shown that GADPH is also participated in non-glycolysis metabolic pathway. For instance, Yang et al's research demonstrated that GADPH activity can be inhibited by submicromolar concentrations of palmitoyl-CoA, therefore producing palmitoylated GADPH, which was demonstrated to facilitate the translocation of GAPDH to either lipid vesicles or naturally occurring biologic membranes. Since hallmark of lipotoxicity is the acculmation of fatty acids and their acyl-CoA metabolin beyond a cell's capacity to conduct a proper metabolism for them [3]. Furthermore, GAPDH can be indirectly involved in some metabolic pathways by adjusting the ratio of NAD+/NADH to influence metabolic status. For example, a prior research conduct by Zhang et al shows that the expression level of GAPDH is changed, it may lead to a change in the total GAPDH protein level inside the cell, which in turn affects the ratio of ATP/ADP and NAD+/NADH, and metabolism of specific process will be affected as well [4].

2.2. Stress response

Previous research has also shown that GADPH has been involved in various non-metabolic processes. Prior researches have indicated that under oxidative stress conditions, the modification and aggregation of GAPDH are related to cell death. For instance, Nakajima, Hidemitsu et al's

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research on GAPDH demonstrated that GADPH is able to mediate cell death under oxidative stress. Through specific and detailed mechanism is still elusive, they still found that active-site cysteine (Cys-152) of GAPDH has a strong relationship with cell death associated and oxidative stress-induced GADPH accumulation. Moreover, they found that a type of C152A-GAPDH mutant has ability to interfering the aggregation of wild type GADPH to save cell from nitric oxide (NO)-induced cell death [5].

In addition, more research done GADPH has found that when level of GADPH has been regulated, the resistance of cells to oxidative stress changes, and the number of GAPDH aggregates also changes accordingly. For instance, from Lazarev et al's research, they applied the model of oxidative stress based on SK-N-SH human neuroblastoma cells treated with hydrogen peroxide, and found that lentivirus-mediated up or down regulation of GADPH amount caused enhancement or inhibition of protein aggregation level, which will lead to increase or reduce of cellular death rate. Their findings indicated that lentivirus-mediated upregulation or downregulation of GAPDH expression led to a corresponding increase or decrease in protein aggregation, which in turn affected the rate of cell death.

Importantly, treatment with RX409 or RX429 reduced both GAPDH aggregation and the associated cell death rate, suggesting that GAPDH not only serves as a metabolic enzyme but also plays a key role in protecting cells from oxidative damage, as Figure 2 shows [6].

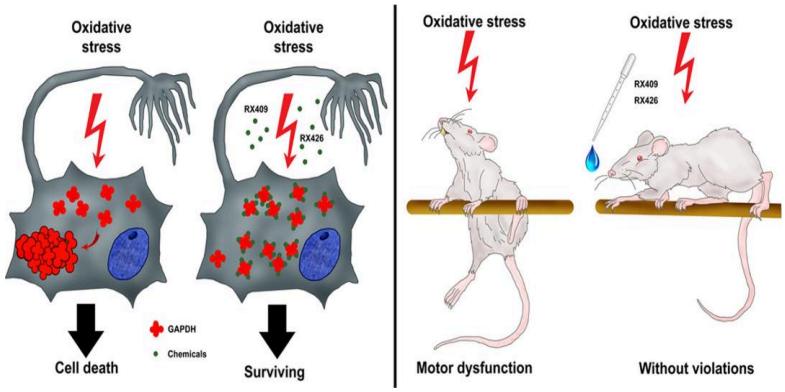


Figure 2. GADPH aggregation under oxidative stress and treatment [6]

In brief, under cellular stress, GADPH also participate in regulation by mediating level of protein aggregation to control cell response against oxidative stress.

2.3. Gene regulation

GADPH has ability to assist gene regulation in cell. Previous research have demonstrated that GADPH is able to bind with mRNA to have an impact on expression of specific gene. For instance, according to Millet et al, GADPH can regulate transcription of TNF mRNA. TNF is a inflammatory cytokine which its expression is tightly controlled, during endotoxin tolerance, transcription of TNF mRNA is repressed, although not entirely eliminated, however, production of TNF cytokine is further restricted due to mechanism of posttranscriptional repression of TNF mRNA by GAPDH binding to the TNF 3′ untranslated region. Increase of the binding is related to the low glycolysis state in cell, which can be reversed by increasing glycolysis or knocking down GAPDH expression. During further research, they concluded that based on cellular metabolic state, GAPDH–TNF mRNA binding can regulate expression level of cytokine TNF [7] .

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Besides binding with mRNA, GADPH also participates in DNA repair. In accordance with Azam et al, knocking down GAPDH expression led to a higher number of spontaneously generated apurinic/apyrimidinic (AP) sites in cells, highlighting its essential role in maintaining genomic stability [2]. Moreover, on the basis of Kosova et al, in the nucleus, GADPH takes part in genes which involved in antiapoptotic pathways and cell multiplication. Moreover, GAPDH can interact with proteins such as APE1, PARP1, HMGB1 and HMGB2, whose are participating in DNA repair [8]. In conclusion, through GADPH cannot directly participate in gene regulation, it still has its own mechanism to indirectly regulate gene expression.

3. Gadph in tumor cells related researches

GADPH has played multiple roles in tumor cell related researches, such as internal reference gene, probable cause of Warburg effect, influence factor of drug tolerance and potential biomarker.

3.1. Internal reference gene (housekeeping gene)

Internal reference genes, also known as housekeeping genes, refer to genes that are consistently expressed and have relatively stable expression levels in almost all cell types. They are crucial for maintaining basic cellular functions and are commonly used for data standardization in gene expression analysis [9,10]. GADPH is one such gene that has stable expression level in common cells and widely used in research. For instance, in previous research, by applying descriptive data analysis and the software programs Normfinder and geNorm to assess express stability of GAPDH, B2M and ACTB, therefore they found that GADPH is a stable reference gene [11]. Moreover, previous research conducted by Lemma et al. showed that GADPH is one of the most stable reference genes at different pH by verifying stability of 15 commonly used housekeeping genes through five algorithms [12]. However, expression level of GADPH is not always stable. For instance, research by Lin et al. found that neither GAPDH mRNA nor β-actin mRNA is consistently expressed at high levels in all tissues, with some organs showing notably low expression levels [13]. Therefore, instead of relying sorely on GADPH as housekeeping gene, multiple housekeeping gene combinations are often chosen by researchers to improve the accuracy of the results. To illustrate, Jiang et al's research found that in the fetal rat hippocampus, the combination of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and hypoxanthine phosphoribosyltransferase (HPRT) shows the best result as compound reference genes [14]. Overall, while GAPDH is one of the most commonly used housekeeping genes in tumor research, its limitations necessitate careful validation or the use of complementary reference genes in certain experimental contexts.

3.2. Warburg effect

Warburg effect refers to the tendency of tumor cells to convert glucose to lactate through glycolysis, even under conditions of sufficient oxygen, which is a significant hallmark of cancer cell metabolism [15]. GADPH, as a key enzyme in glycolysis, it catalyzes the conversion of D-glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate and regulate metabolic flux during this process [16]. During Warburg effect, this step related to GADPH is a rate-limiting step, according to Liberti et al, by performing MCA on lactate production flux and evaluating thermodynamics for each step in glycolysis, the result indicate that only the FCC(Flux control coefficients) value of GAPDH increases during the Warburg effect [17]. Besides, inhibition of GAPDH can suppress the occurrence of Warburg effect, since mitochondrial malfunction in cancer cells cause hyperglycolysis

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resulting in NADH.H accumulation, which is produced during GAPDH step, forces LDH to become an irreversible reaction in the direction of lactate formation, causing Warburg effect [18]. Therefore, it can be seen from the research above that GAPDH is crucial for maintaining high levels of glycolysis in cancer cells, making it a potential target for treating cancer and autoimmune diseases [16].

3.3. Drug tolerance factor

GAPDH has been found to be associated with drug resistance in various tumor cells. More specifically, regulation of GADPH expression level may enhance or weaken cellular drug tolerance. On one side, knockdown of GAPDH can enhance cell sensitivity to drugs through various mechanisms, thereby overcoming drug resistance. According to Guan et al, tumor cells' chemotherapeutic drug resistance under hypoxic conditions is caused by inhibition of autophagy apoptosis by and drug efflux through activation of ATP-dependent transporter, however, by knockdown GADPH, GADPH expression level will be disrupted, autophagy and ATP levels in tumor cells can be reduced, thus drug resistance can be overcome [19]. On the other hand, overexpression of GADPH may promote the development of cellular drug resistance. In accordance of Guo et al, though specific mechanism is still not fully explained, by constructing a stable cell line overexpressing GAPDH and study its impact on drug tolerance, therefore, they discovered that overexpression of GAPDH leads to increased drug tolerance and survival ability of breast cancer cells [20]. In summary, change in expression of GADPH effects drug tolerance in a tumor cell.

3.4. Potential biomarker

Biomarker is a objective and quantitative feature that used in assess normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention [21]. GADPH is not an ideal biomarker itself, since it's relatively stable expression under different cell types and conditions, it's commonly used as housekeeping genes. However, in certain specific situations, GAPDH can serve as a potential biomarker. For instance, according to Zhang et al, researchers synthesized a new photoaffinity labeled cADPR agonist PAL cIDPRE and used it to purify binding proteins. GAPDH was identified as the essential cADPR binding protein that mobilizes cADPR-mediated Ca2+ from ER via RyRs. This finding indicates that GADPH might be the biomarker in Ca2+ involved biological process [22]. Moreover, in Crow et el's research, GAPDH is used to evaluate the changes in serum extracellular vesicle miRNA in Ewing sarcoma family tumor patients, which can help the diagnosis and monitoring of ESFT [23]. However, still, due to the widespread presence of GAPDH in most cell types and its relatively constant expression level, it is not suitable as an independent biomarker for disease diagnosis or prognosis prediction. Therefore, many studies combine GAPDH with other more specific molecules to improve detection accuracy. For example, in Zhu et al's research on Lung adenocarcinoma, GADPH is applied with uroplakin 2(UPK 2) mRNA to investigate postoperative recurrence rate of lung adenocarcinoma [24]. In summary, GAPDH can serve as an auxiliary biomarker in certain specific scenarios, however, it is not an ideal biomarker independently.

4. Conclusion

In this review, a brief overview was provided on GAPDH's functions in cellular metabolism, stress response, and gene regulation. Additionally, the review examined the expanding role of GAPDH in

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tumor cell research, specifically focusing on the following aspects: (1) discussing GADPH's role being a stable reference gene (housekeeping gene) and its instability in some case; (2) its involvement in the Warburg effect and its regulatory relationship with tumor metabolism; (3) its association with drug tolerance in tumor cells, including strategies to modulate drug resistance through GAPDH expression levels; (4) its potential as a biomarker in certain tumor-related diseases.

Indeed, GADPH plays an unique role in tumor research. Recent studies suggest that GAPDH is closely linked to tumor metabolism, indicating its potential as a target in the development of anti-cancer therapies. However, it has to be acknowledged that, current the understanding of the specific mechanism of GAPDH in tumors is not comprehensive enough, moreover, and more research is needed in the future to clarify this point. Therefore, future research can be improved in the following directions. Firstly, further exploration the specific mechanisms of GADPH under different pathological conditions can be made. Additionally, the interactions between GADPH and other proteins can be a potential research area. Moreover, elucidation of the specific molecular mechanisms of GADPH in non metabolic functions can be further discussed. Furthermore, it's important to find more stable alternative reference genes to improve the reliability of qPCR and other techniques. Lastly, specific inhibitors or activators for GAPDH is also a considerable area to investigate.

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