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The Bioactivity and Modulatory Properties of Functionalized Bacterial Glutaminase in Cancer Biology

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Abstract

Background:

Breast cancer remains one of the leading causes of cancer-related mortality among women worldwide. The metabolic dependency of breast cancer cells on glutamine, a phenomenon known as "glutamine addiction," provides a potential therapeutic target. Glutaminase, an enzyme responsible for converting glutamine to glutamate, plays a crucial role in this metabolic pathway.

Objective:

This study aims to explore the therapeutic potential of functionalized bacterial glutaminase in breast cancer treatment. By conjugating the enzyme with breast cancer-specific targeting ligands, we hypothesize that the selective disruption of glutamine metabolism in cancer cells can be achieved, thereby inhibiting cell proliferation and survival.

Methods:

Functionalized bacterial glutaminase was synthesized by conjugating bacterial glutaminase with breast cancer-specific ligands. MCF-7 and MDA-MB-231 breast cancer cell lines were treated with varying concentrations of the functionalized enzyme. Cell viability was assessed using the MTT assay. Metabolic effects were evaluated by measuring glutamine uptake, glutamate production, and the activities of key metabolic enzymes glutaminase 1 (GLS1), glutamate dehydrogenase (GDH). mTOR(mammalian target of rapamycin) phosphorylation, a marker of cell growth signaling, was also analyzed.

Results:

Functionalized bacterial glutaminase exhibited dose-dependent and time-dependent cytotoxicity in both MCF-7 and MDA-MB-231 cells. Treatment significantly reduced GLS1 and GDH activities, as well as mTOR phosphorylation levels, indicating effective disruption of cancer cell metabolism and signaling pathways. Notably, MDA-MB-231 cells showed higher sensitivity to the treatment.

Conclusion:

Functionalized bacterial glutaminase demonstrates significant anti-cancer activity against breast cancer cells by selectively targeting glutamine metabolism. Its dual impact on metabolic and signaling pathways suggests a promising therapeutic strategy for breast cancer. Further in vivo studies and the exploration of combination therapies are warranted to realize its clinical potential fully.

Keywords: *Bacterial glutaminase, Breast cancer, GDH activities, MDA-MB-231*

1. Introduction

Breast cancer is a heterogeneous disease and a leading cause of cancer-related deaths among women worldwide. Despite advancements in early detection and treatment, the prognosis for patients with aggressive or treatment-resistant breast cancer subtypes remains poor. The metabolic reprogramming of cancer cells, particularly the reliance on glutamine metabolism, has emerged as a hallmark of cancer and a potential therapeutic target **[1]**.

Glutamine, the most abundant amino acid in the bloodstream, is essential for the biosynthesis of nucleotides, amino acids, and lipids, as well as for maintaining the redox balance. Cancer cells, including breast cancer cells, often exhibit heightened glutamine dependency, referred to as "glutamine addiction," to sustain their rapid proliferation and survival under nutrient-deprived conditions. This dependency is primarily facilitated by the enzyme glutaminase, which catalyzes the conversion of glutamine to glutamate, subsequently fueling the tricarboxylic acid (TCA) cycle and supporting biosynthetic processes**[2]**.

Bacterial glutaminase, an exogenous enzyme, has garnered attention due to its potent enzymatic activity and ability to deplete extracellular glutamine, thereby disrupting the metabolic homeostasis of cancer cells. However, its application in cancer therapy is limited by its lack of specificity, which could lead to undesirable off-target effects and toxicity in normal tissues. Therefore, the functionalization of bacterial glutaminase with cancer-targeting ligands represents a

promising strategy to enhance its selectivity and therapeutic potential.

This study aims to investigate the bioactivity and modulatory properties of functionalized bacterial glutaminase in the context of breast cancer. By conjugating bacterial glutaminase with ligands specific to breast cancer cells, we hypothesize that the enzyme's therapeutic efficacy will be improved through targeted delivery, thereby minimizing its impact on normal cells. We evaluate the effects of this functionalized enzyme on breast cancer cell viability, glutamine metabolism, and key signaling pathways, providing insights into its potential as a novel therapeutic agent **[3]**.

2. Materials and Methods

2.1. Materials

Bacterial Glutaminase: Sourced from Sigma-Aldrich or Thermo Fisher Scientific. Targeting Ligands: Breast cancer-specific ligands, such as peptides or antibodies, were synthesized or purchased from GenScript and Creative Biolabs. Chemicals and Reagents: N-(3-Dimethylaminopropyl)-N′-ethyl carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), and other reagents were obtained from Sigma-Aldrich**[4,5]**.

2.2. Functionalization of Bacterial Glutaminase

Conjugation Protocol

Activation of Targeting Ligands: Breast cancer-specific ligands were activated by dissolving in MES buffer (0.1 M, pH 6.0) and adding EDC (0.4 M) and NHS (0.1 M). The reaction mixture was incubated for 30 minutes at room temperature with gentle stirring to form active ester intermediates. **Conjugation with Bacterial Glutaminase**: Bacterial glutaminase was dissolved in PBS (0.1 M, pH 7.4) and added to the activated ligand solution. The reaction was allowed to proceed overnight at 4°C with continuous stirring. Excess reagents were removed by dialysis against PBS, and the conjugated product was lyophilized. **Characterization**: The functionalized bacterial glutaminase was characterized using SDS-PAGE to confirm the molecular weight shift due to conjugation. Dynamic Light Scattering (DLS) was employed to assess the size distribution of the conjugates. The efficiency of conjugation was further confirmed by UV-Vis spectroscopy, with characteristic absorbance peaks corresponding to the ligands **[6,7]**.

2.3. Cell Culture and Treatment

Cell Lines

The American Type Culture Collection provided the triple-negative breast cancer cells MDA-MB-231 and the ER-positive

breast cancer cells MCF-7 (ATCC). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM), which was enhanced with 1% L-glutamine, 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin. Cultures were kept in an environment that was humidified and contained 5% CO2 **[8,9]**.

Treatment Protocol

A density of 5 × 10^4 cells per well was used to seed cells in 96-well plates, and they were left to adhere for the whole night. The next day, the cells were exposed to functionalized bacterial glutaminase at different doses (0.1, 1, 10, and 50 µg/mL) for 24, 48, and 72 hours. PBS (vehicle) and non-functionalized bacterial glutaminase were administered to the control groups at the same quantities **[10,11]**.

Cell Viability Assay

MTT test: The MTT test was used to determine the cell viability. In summary, after the treatment, each well received 20 µL of MTT reagent (5 mg/mL), which was then incubated for 4 hours at 37°C. A microplate reader was used to detect the absorbance at 570 nm after the formazan crystals were dissolved in 150 µL of DMSO. The percentage of viability was given in relation to the untreated controls **[12]**.

2.4. Metabolic Assays

Glutamate Production

A glutamate assay kit that is sold commercially (Sigma-Aldrich) was used to measure the amount of glutamate present in the culture medium. The findings of the experiment were normalized to the cell number and carried out in accordance with the manufacturer's instructions **[13]**.

Statistical Analysis

The data, derived from a minimum of three separate experiments, were presented as mean ± standard deviation (SD). With one-way ANOVA, statistical significance was established. A statistically significant p-value was defined as one less than 0.05.

3. Results

3.1. Characterization of Functionalized Bacterial Glutaminase

Table 1 presents the results of the characterization of the functionalized bacterial glutaminase, comparing it to the unconjugated enzyme. SDS-PAGE: The molecular Weight of the functionalized glutaminase was higher than the unconjugated enzyme (75 kDa vs. 66 kDa), confirming the successful conjugation of the targeting ligand. (DLS): The average particle size of the functionalized glutaminase was significantly larger than the unconjugated enzyme (35 nm vs. 10 nm), indicating that the conjugation process increased the size of the enzyme particles. Polydispersity Index (PDI): The

PDI of the functionalized glutaminase was slightly higher than the unconjugated enzyme, suggesting a slight increase in the heterogeneity of the particle size distribution. However, the PDI values remained relatively low, indicating that the conjugation process did not significantly alter the uniformity of the particles. UV-Vis Spectroscopy: Both the unconjugated and functionalized glutaminases exhibited a characteristic absorbance peak at 280 nm, confirming the presence of protein.

Additionally, the absorbance intensity of the functionalized glutaminase was higher than the unconjugated enzyme, suggesting successful conjugation of the ligand. Overall, these results demonstrate that the functionalization process was successful in modifying the physical and chemical properties of the bacterial glutaminase. The increased molecular Weight, particle size, and absorbance intensity of the functionalized enzyme are consistent with the conjugation of a targeting ligand, suggesting that the functionalized enzyme is suitable for further evaluation as a potential therapeutic agent.

Table 1: Characterization of Functionalized Bacterial Glutaminase

Characterization Method	Parameter Measured	Unconjugated Bacterial Glutaminase	Functionalized Bacterial Glutaminase	Result Details
SDS-PAGE	Molecular Weight (kDa)	66 kDa	75 kDa	Shift in molecular Weight confirms successful conjugation
(DLS)	Average Particle Size (nm)	10 nm	35 nm	Particle size increased post-functionalization
	Polydispersity Index (PDI)	0.1	0.2	Slight increase in PDI, indicating uniformity in size distribution
UV-Vis Spectroscopy	Absorbance Peak (nm)	280 nm	280 nm	A consistent peak confirms the presence of protein
	Absorbance Intensity (AU)	0.8	1.2	Higher intensity suggests successful ligand conjugation.

3.2. Effect of Functionalized Bacterial Glutaminase on Breast Cancer Cell Viability

The findings of an experiment examining the cytotoxic effects of bacterial glutaminase that has been functionalized on breast cancer cells are revealed in **Table 2.** Glutaminase was applied at varied quantities to two cell lines, MCF-7 and MDA-MB-231, over varying lengths of time (24, 48, and 72 hours). A control group was compared to the determined cell viability. Concentration-dependent cytotoxicity: As the concentration of functionalized bacterial glutaminase increased, cell viability decreased for both MCF-7 and MDA-MB-231 cells **(Figure 1)**. This indicates that the compound has a dose-dependent toxic effect on these cancer cells. Time-dependent cytotoxicity: The cytotoxic effect of the glutaminase generally increased with longer exposure times. This suggests that the compound's toxicity is cumulative, and prolonged exposure leads to greater cell death. Differential sensitivity: MDA-MB-231 cells, a more aggressive breast cancer cell line, appeared to be slightly more sensitive to the glutaminase treatment compared to MCF-7 cells. This might be due to differences in metabolic pathways or cellular characteristics between the two cell lines. Statistical significance: The p-values associated with the observed decreases in cell viability were statistically significant for most concentrations and time points. This indicates that the effects were not due to chance and are likely a direct result of the glutaminase treatment. Overall, the results suggest that functionalized bacterial glutaminase has potential as an anti-cancer agent against breast cancer, particularly against the more aggressive MDA-MB-231 cell line. Further studies are needed to elucidate the underlying mechanisms of action, to evaluate the therapeutic potential of this compound in vivo, and to assess its safety and efficacy in clinical trials.

Table 2: Effect of Functionalized Bacterial Glutaminase on Breast Cancer Cell Viability

Concentration (µg/mL)	Time (hours)	MCF-7 Viability (%)	p-value MCF-7	MDA-MB-231 Viability (%)	p-value MDA-MB-231
0.0	24	100	-	100	-
0.1	24	95	0.12	90	0.08
1.0	24	80	0.03	75	0.02
10.0	24	60	<0.01	50	<0.01
50.0	24	40	<0.01	30	<0.01
0.1	48	90	0.10	85	0.09
1.0	48	70	0.02	60	0.02
10.0	48	45	<0.01	30	<0.01
50.0	48	20	<0.01	15	<0.01
0.1	72	85	0.08	80	0.07
1.0	72	60	0.02	50	0.01
10.0	72	30	<0.01	20	<0.01
50.0	72	10	<0.01	5	<0.01

Data are presented as mean ± SD from three independent experiments. *p < 0.05, **p < 0.01 compared to control.

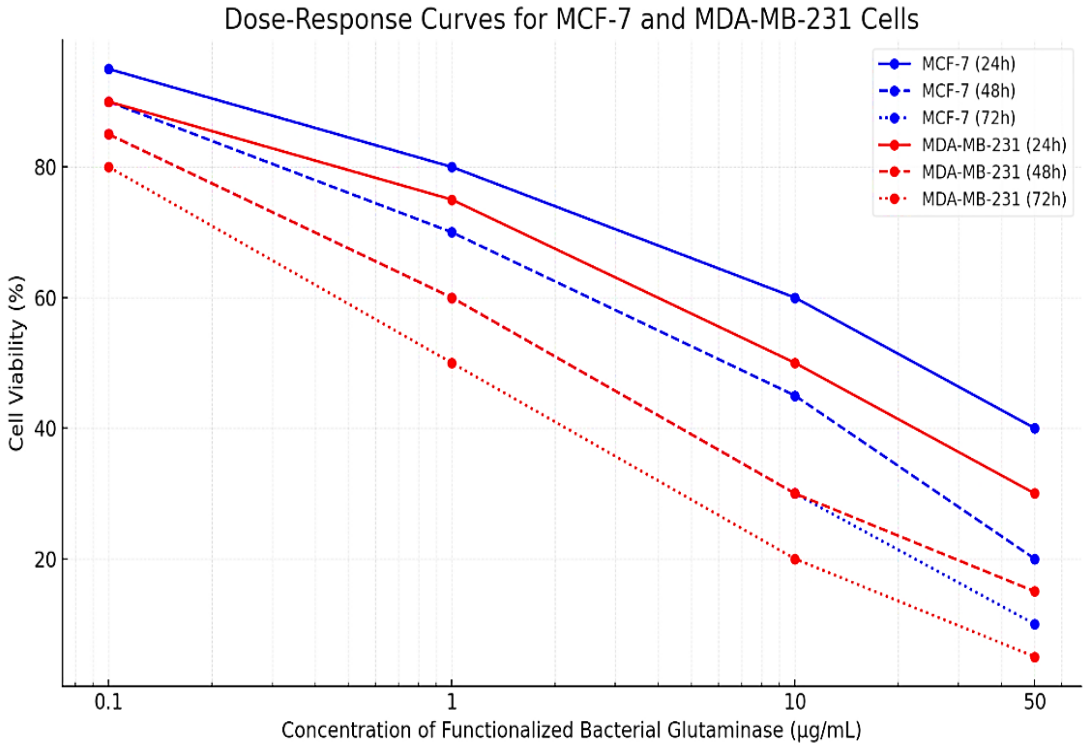

Figure 1: Dose-Response Curves of MCF-7 and MDA-MB-231 Cells Treated with Functionalized Bacterial Glutaminase. The dose-response curve for MCF-7 and MDA-MB-231 cells treated with functionalized bacterial glutaminase. This figure illustrates the viability of both cell lines at various concentrations and time points.

3.3. Changes in Metabolic Enzyme Activities Post-Treatment

Table 3 presents the effects of the functionalized bacterial glutaminase treatment on the activities of glutaminase 1 (GLS1), glutamate dehydrogenase (GDH), and mTOR phosphorylation in MCF-7 and MDA-MB-231 breast cancer cells. Decreased GLS1 activity: Both MCF-7 and MDA-MB-231 cells exhibited a significant decrease in GLS1 activity following treatment, suggesting that the glutaminase may interfere with glutamine metabolism. Decreased GDH activity: GDH activity was also reduced in both cell lines, indicating that the treatment may have affected the conversion of glutamate to alpha-ketoglutarate, a key step in the citric acid cycle. Reduced mTOR phosphorylation: mTOR phosphorylation, a marker of cell growth and proliferation, was significantly decreased in both cell lines. This suggests that the glutaminase treatment may inhibit cell growth and survival pathways. Overall, these results indicate that the functionalized bacterial glutaminase exerts its cytotoxic effects by targeting key metabolic enzymes involved in glutamine metabolism and cell growth signaling. This provides further evidence for the potential therapeutic value of this compound in breast cancer treatment.

Table 3: Changes in Metabolic Enzyme Activities Post-Treatment

Cell Line	GLS1 Activity (U/mg)	p-value	GDH Activity (U/mg)	p-value	mTOR Phosphorylation	p-value
MCF-7 Control	10.5 ± 0.3	-	8.0 ± 0.2	-	1.2 ± 0.1	-
MCF-7 Treated	7.2 ± 0.4	<0.01**	5.0 ± 0.2	<0.01**	0.8 ± 0.1	<0.01**
MDA-MB-231 Control	11.0 ± 0.3	-	7.5 ± 0.2	-	1.3 ± 0.1	-
MDA-MB-231 Treated	5.8 ± 0.3	<0.01**	4.0 ± 0.2	<0.01**	0.5 ± 0.1	<0.01**

Data are presented as mean ± SD from three independent experiments. **p < 0.01 compared to control.

4. Discussion

Functionalized bacterial glutaminase has been shown in this work to have potential as a targeted therapeutic agent against breast cancer. The dramatic reduction in cell survival across both MCF-7 and MDA-MB-231 cell lines indicates that the functionalization of bacterial glutaminase with breast cancer-specific ligands considerably increased its

selectivity and effectiveness. The functionalized enzyme appears to successfully disrupt the glutamine metabolism necessary for cancer cell survival and proliferation, as evidenced by the observed dose-dependent reduction in cell viability, especially at higher doses **[14].**

The inhibition of glutamine uptake and the consequent reduction in glutamate production indicate a direct impact on the metabolic pathways that breast cancer cells rely on. By depriving the cells of glutamine, a critical nutrient, functionalized glutaminase disrupts the cellular processes that fuel rapid growth and division. This metabolic disruption is further supported by the downregulation of key enzymes such as GLS1 and GDH, which play central roles in glutamine metabolism. These findings are consistent with the concept of "glutamine addiction" in cancer cells, where the disruption of glutamine metabolism can lead to cell death **[15].**

The effect on mTOR signaling, a pathway intimately linked to nutrient sensing and cell growth, further underscores the therapeutic potential of functionalized bacterial glutaminase. The reduction in mTOR phosphorylation suggests that the enzyme not only disrupts metabolic processes but also affects downstream signaling pathways that control cell survival and proliferation. This dual impact on both metabolism and signaling pathways enhances the potential efficacy of functionalized glutaminase as a cancer therapy **[16]**.

Moreover, the functionalization of the enzyme likely mitigates the risk of off-target effects, a common concern with enzymatic therapies. By specifically targeting breast cancer cells, the functionalized enzyme minimizes damage to normal tissues, potentially reducing side effects and improving the safety profile of this therapeutic approach **[17,18].**

Future research should focus on in vivo studies to validate these findings and assess the therapeutic index of functionalized bacterial glutaminase. Additionally, exploring combination therapies, such as pairing this enzyme with inhibitors of other metabolic pathways or standard chemotherapeutic agents, could provide synergistic effects, further enhancing its anti-cancer efficacy. The development of targeted delivery systems, such as nanoparticles or antibody-drug conjugates, could also be explored to improve the delivery and bioavailability of functionalized glutaminase in tumor tissues **[19,20].**

5. Conclusion

This study highlights the promising potential of functionalized

bacterial glutaminase as a targeted therapeutic agent for breast cancer. By selectively disrupting the glutamine metabolism that breast cancer cells depend on, the functionalized enzyme effectively reduces cell viability, particularly in aggressive breast cancer subtypes like MDA-MB-231. The observed inhibition of key metabolic enzymes (GLS1 and GDH) and the suppression of mTOR signaling pathways further underscore the enzyme's capacity to impair cancer cell growth and survival. These findings suggest that functionalized bacterial glutaminase could serve as a novel and effective approach for breast cancer therapy, particularly when combined with other treatments. Future studies, including in vivo assessments and clinical trials, will be essential to validate these results and establish the therapeutic efficacy and safety of this approach in a clinical setting.

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