In Silico Assessment of Human Beta-Glucuronidase (GUSB) Expression and Prognostic Outcomes in Breast Cancer: Evaluating Tumor-Intrinsic and Microbial Perspectives

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ABSTRACT

Background: Beta-glucuronidase (GUSB) is a lysosomal proteolytic enzyme that helps in the breakdown of glucuronide conjugates and the rejuvenation of estrogen that is of biological use. Although bacterial GUS released by the gut microbiome (the estrobolome) has been suggested to play a role in systemic estrogen recycling and breast cancer development, the prognostic value of human GUSB expression in breast tumor remains unknown. This research examined how human GUSB expression is related to overall survival in breast cancer, specifically treatment-specific subgroup and immune-related subgroup.

Methods: In silico survival analysis was done through Kaplan-Meier Plotter database (kmplot.com). Human GUSB mRNA levels were assessed in a group of breast cancer cases and overall survival (OS) was determined between a high and a low group. Further subgroup analyses were done according to the type of treatment and the immune cell infiltration such as chemotherapy treated patients with macrophage enriched tumors and endocrine treated patients with high CD8 + T-cell infiltration. We utilized the Kaplan-Meier Plotter platform to get log-rank p-values, 95% CIs, and hazard ratios (HRs).

Results: The overall survival rate was 0.87 (95% CI: 0.69-1.11, p = 0.27), which indicates that human GUSB expression was not significantly linked with breast cancer in the total group. On the other hand, trends toward better survival were shown by subgroup analyses. A greater GUSB expression was linked to an estimated 80% lower risk of death among chemotherapy-treated patients with tumors that were rich in macrophages (HR = 0.20, 95% CI: 0.02-1.80, p = 0.11). Similarly, endocrine-treated patients with high $CD8^+$ T-cell infiltration showed an estimated 59% reduction in mortality risk (HR = 0.41, 95% CI: 0.10–1.65, $p = 0.20$). There was no statistically significant relationship between these variables.

Conclusion: Given that human GUSB expression did not correlate with overall survival in the breast cancer community as a whole, it is very improbable that it serves as a standalone predictive biomarker. These findings are consistent with the concept that systemic estrogen reactivation is primarily influenced by microbial GUS activity in the gut rather than host **GUSB** expression. Although subgroup analyses indicated possible associations between elevated **GUSB** expression and improved survival in specific treatment and immune contexts, further experimental and clinical validation is required.

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INTRODUCTION

In addition to being the most often diagnosed cancer in women, breast cancer remains the leading cause of cancer-related deaths in this population globally.[1] The estrogen receptor (ER) positive subtype of breast cancer is the most prevalent, and estrogen signaling plays a major role in this subtype.. Thus, it is crucial to comprehend the processes that control estrogen metabolism to enhance disease management and determine new treatment targets. [2] Glucuronidation is one of the most important processes in estrogen

metabolism, and a phase II metabolic pathway that transforms active estrogen to an inactive glucuronide conjugate to be excreted. The enzyme that can reverse this process is known as beta-glucuronidase (GUS) that breaks down the glucuronides conjugates and restores the use of biologically active estrogen. [3][4]
Beta-glucuronidase activity has two different sources and biological functions. The initial one is microbial GUS which is the product of the enteral microbiome which is also referred to as the estrobolome. [5] These intestinal bacterial enzymes help in the deconjugation of estrogen in the intestine, which is reabsorbed into the circulation and might have effects on systemic estrogen rates. The second is human GUSB, a gene on chromosome 7, GUSB, which is an important lysosomal enzyme of intracellular degradation and normal cellular homeostasis. [8, 9]
Despite mounting evidence linking estrogen recycling by the gut microbiome to breast cancer, [10] the clinical relevance of the human GUSB expression in the breast tumor remains to be completely revealed. Specifically, it is not known yet whether GUSB expression has any impact on patient survival or whether the prognostic ability of GUSB expression depends with the type of treatment and tumor microenvironment immune composition. [11]
Thus, the current research had the purpose to assess human GUSB expression in correlation with the general survival in breast cancer based on the Kaplan-Meier Plotter database. Besides, treatment-specific and immune cell-based subgroup was also conducted to investigate how GUSB expression could be associated with clinical outcome in various tumor microenvironment settings. [13]

2. MATERIALS AND METHODS

2.1 Data Source and Survival Analysis

Human GUSB expression was examined for its prognostic relevance in breast cancer using the Kaplan-Meier Plotter database (kmplot.com), an online tool that integrates gene expression and clinical data from publicly available datasets like GEO and TCGA. As a clinical endpoint, overall survival (OS) was chosen. Based on the median expression threshold that was automatically selected by the Kaplan-Meier Plotter platform, the patients with high and low expression were separated into high-expression and low-expression groups, respectively. The standard analytical parameters produced log-rank p-values, 95% CIs, hazard ratios (HRs), and Kaplan-Meier survival curves. [12, 13]

2.2 Subgroup Analysis

Further subgroup analyses were conducted to examine whether or not the prognostic information of GUSB depended on treatment and the immune characteristics of tumors. [14]

The groups of patients tested included the following:

All breast cancer patients in the database without further stratification: This was the total breast cancer cohort.
Treatment with macrophage-enriched tumors: A subset of patients that underwent chemotherapy and their tumors had a high macrophage density. [15]
Patients who received endocrine therapy and whose tumors exhibited high $CD8^+$ T-cell infiltration. [16]
Hazard ratios with 95% CIs were calculated using Cox proportional hazards regression, and differences in group survival were assessed using the log-rank test.[17] A p-value less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Unselected Breast Cancer Population

Statistical analysis of the whole breast cancer cohort did not reveal a correlation between higher GUSB expression and overall survival (HR = 0.87, 95% CI: 0.69-1.11, p = 0.27; Figure 3.1). Based on these results, it appears that GUSB expression in host tumor tissue is not a reliable predictor of disease outcome in a breast cancer population that is not specifically targeted for research.

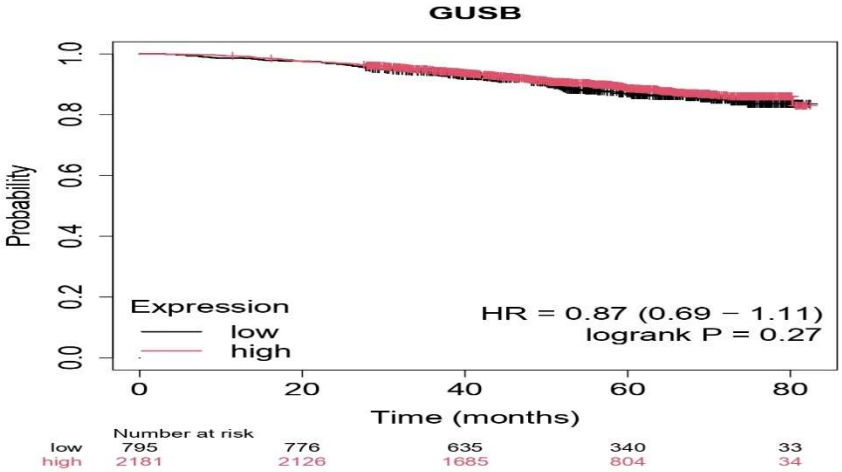

FIGURE: 3. 1

Patients with breast cancer may see their relapse-free survival rate, broken down by GUSB expression levels, on the Kaplan-Meier survival curve. The data comes from the KM Plotter database and includes 2976 individuals from all subtypes of the disease. Patients with high GUSB expression (n = 2181, pink) showed no significant difference in survival compared to low expression (n = 795, black) (HR = 0.87, 95% CI: 0.69–1.11, log-rank P = 0.27)

3.2 Microenvironment- and Treatment-Specific Subgroups

Subgroup analysis by treatment-type and immune cell-infiltration showed different patterns of survival.

1.Chemotherapy and macrophage infiltration: Improved overall survival was significantly seen in individuals with macrophage-enriched malignancies who had systemic chemotherapy and exhibited enhanced GUSB expression. Although the connection did not approach statistical significance, this led to an estimated 80% decrease in mortality risk (HR = 0.20, 95% CI: 0.02-1.80, p = 0.11; Figure: 3.2(1A)).

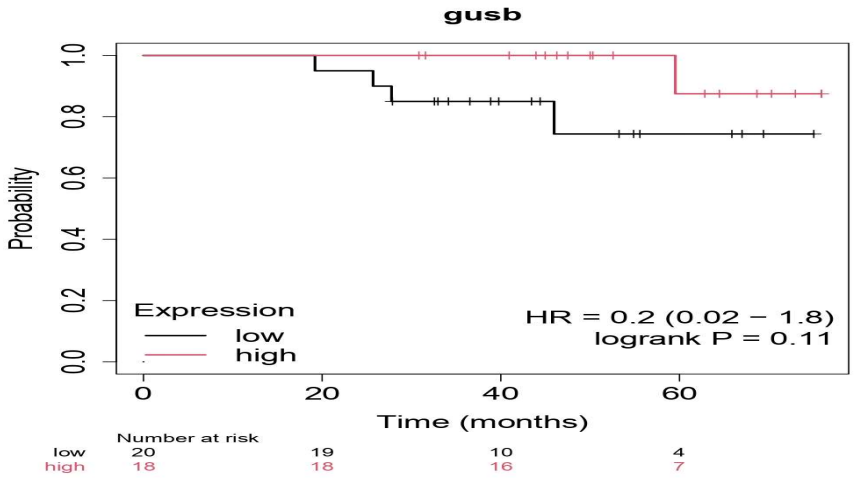

FIGURE: 3.2(1A)

Breast cancer patients with a tumor microenvironment rich in macrophages and who had systemic chemotherapy had a Kaplan-Meier survival curve that was stratified according on GUSB expression. The limited sample size within this subgroup likely contributed to the lack of statistical significance, however there was a tendency toward enhanced survival linked with high GUSB expression (HR = 0.20, 95% CI: 0.02-1.80, log-rank P = 0.11).

2.Endocrine therapy and CD8⁺ T-cell infiltration: Similarly, in patients treated with endocrine therapy whose tumors exhibited high CD8⁺ T-cell infiltration, improved overall survival was seen with higher GUSB expression. An estimated 59% lower risk of death was linked to this (HR = 0.41, 95% CI: 0.1-1.65, p = 0.2). Figure: 3.2(2B) but the result was not statistically significant.

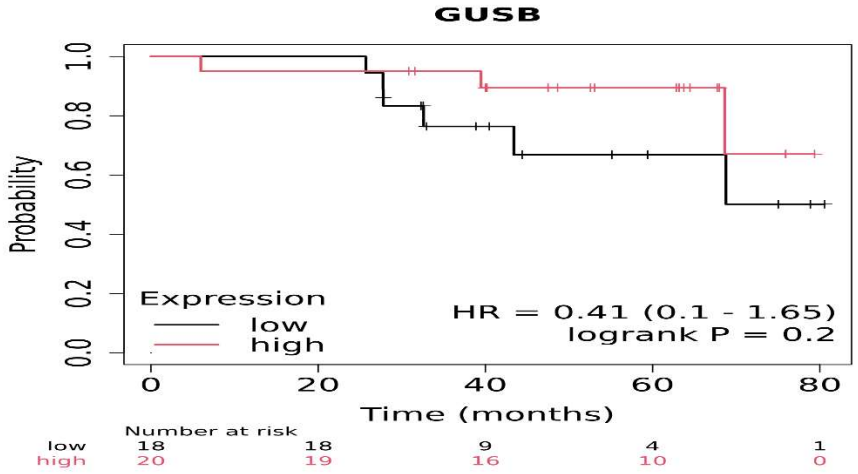

Figure 3.2(2B) Breast cancer patients stratified by GUSB expression and treated with endocrine treatment who have a high CD8⁺ T-cell infiltration had a better chance of overall survival, according to a Kaplan–Meier survival curve. Although the connection did not approach statistical significance, there was a tendency toward increased survival

associated with high GUSB expression. This corresponds to an estimated 59% decrease in mortality risk (HR = 0.41, 95% CI: 0.10-1.65, log-rank P = 0.2).

TABLE 1: Kaplan-Meier Survival Analysis of Human GUSB across Stratified Cohorts

Patient Cohort / Subgroup	Sample Filter	Hazard Ratio (HR)	95% Confidence Interval (CI)	Log-rank p-value	Clinical Significance / Trend
Unselected Breast Cancer	None (All)	0.87	0.69 – 1.11	0.27	Neutral standalone prognostic effect across broad cohorts
Chemotherapy Treated (HR,PgR)	Chemo + Macrophages Enriched	0.2	0.02 – 1.80	0.11	Strong protective trend (80% risk reduction) in chemo-treated macrophage TME
Endocrine Treated (HR,PgR)	Endocrine + CD8+ T-cells Enriched	**0.41**	0.1 – 1.65	**0.2**	Protective trend (59% risk reduction) in hormone-treated cytotoxic TME.

4. DISCUSSION

Although microbial GUS plays a role in deconjugation and recirculation of estrogens, human GUSB plays a primary role in normal lysosomal metabolism and cellular homeostasis. Surprisingly, subgroup analyses by the type of treatment and immune cell infiltration showed tendencies of better survival in groups of patients. The estimated risk of mortality was lower in patients undergoing chemotherapy of tumors enriched with macrophages and patients undergoing endocrine therapy with high levels of CD8+ T-cell infiltration when GUSB was increased. These associations were not found to be significant, but they indicate that GUSB might play context-specific roles in the tumor microenvironment.

A potential reason is that augmented lysosomal GUSB activity in immune cells, including tumor related macrophages and CD8 + T cells, can help cellular metabolism, lysosomal activity, or clearage of damaged cellular components in response to stress during treatment. These observations should be investigated to establish the role of GUSB in immune cell functions or response to treatment in breast cancer.

Limitations

This analysis relies on retrospective transcriptomic data and, thus, it cannot be causal. Moreover, the data on the expression of genes does not show the activity of the enzyme itself. These findings warrant future research with prospective clinical cohort, experimental validation, and functional assays between the activity of both human GUSB and microbial GUS to validate these results and help elucidate their biological relevance.

5. CONCLUSION

Conclusively, human GUSB expression did not significantly relate to overall survival in the general breast cancer population meaning that it is not likely to be a free-standing prognostic biomarker. These results also confirm that microbial gut b-glucuronidase (GUSB), as opposed to host GUSB, is a more pertinent target of a strategy that targets to minimize estrogen reactivation. Even though the subgroup analyses indicated possible relationships between the higher levels of GUSB expression and the better survival in immune-cell enriched tumors with chemotherapy or endocrine treatment, such results should be viewed with reservations since they were not found to be statistically significant. Further experiment studies and clinical research are needed to confirm the observations and determine the role of GUSB in the breast cancer tumor microenvironment better.

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