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Original Research

Investigating the Correlation Between THBS2 Expression and Metastasis in Breast Cancer: An Immunohistochemical Analysis

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Abstract

Thrombospondin-2 (THBS2) plays a pivotal role in regulating tumor progression and metastasis. This study investigates the expression of THBS2 in breast cancer tissue and its correlation with tumor grade and localization in core and marginal regions of the tumor. An observational, retrospective study was conducted on 100 archived breast cancer tissue specimens, comprising 48 grade 1, 37 grade 2, and 15 grade 3 tumors. Immunohistochemical analysis was performed to evaluate THBS2 expression, and the extent and intensity of staining were scored by two independent observers. Statistical comparisons between core and marginal regions, as well as between tumor grades, were analyzed.

Results demonstrated a significant increase in THBS2 expression with tumor grade, with grade 3 tumors showing the highest levels of positivity. Additionally, marginal tissue regions exhibited consistently higher THBS2 staining compared to core regions, irrespective of tumor grade. These findings suggest that THBS2 is involved in tumor invasion and metastasis, with a pronounced role at the invasive tumor margins. The differential expression of THBS2 across tumor grades and tissue regions highlights its potential as a biomarker for breast cancer aggressiveness and metastatic potential.

This study underscores the need for further investigation into the mechanistic role of THBS2 in breast cancer progression and its potential as a therapeutic target.

Keywords: Thrombospondin-2, Breast Cancer, Tumor Grade, Tumor Microenvironment, Cancer Biomarkers, Immunohistochemistry

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Introduction

Breast cancer remains one of the most common and clinically challenging malignancies globally, accounting for a significant proportion of cancer-related morbidity and mortality in women.[1] Despite advances in early detection and treatment, metastatic breast cancer continues to represent a major cause of poor prognosis and treatment resistance. Metastasis, the spread of cancer cells from the primary tumor to distant organs, is a multifactorial process driven by complex molecular and cellular alterations that facilitate tumor cell invasion, survival, and colonization at secondary sites. [2] Understanding the molecular mechanisms underlying metastasis is crucial for identifying potential biomarkers that can aid in prognosis, early detection, and targeted therapies.

Thrombospondin-2 (THBS2) is a glycoprotein belonging to the thrombospondin family, which plays a critical role in regulating extracellular matrix (ECM) remodeling, cell adhesion, migration, and angiogenesis. THBS2 has been implicated in various physiological processes, including tissue repair, immune response, and tumor progression. [3] Its role in cancer biology has garnered attention due to its dual function as both a tumor suppressor and promoter, depending on the context and cancer type. In particular, THBS2 is thought to interact with key molecules involved in the metastatic cascade, including matrix metalloproteinases (MMPs), integrins, and growth factors, which are involved in the degradation of the ECM and the promotion of tumor cell motility.[4,5]

In breast cancer, the expression levels of THBS2 are altered in both primary tumors and metastatic lesions, suggesting its potential involvement in metastasis. [6] However, the precise mechanisms through which THBS2 contributes to the metastatic process remain incompletely understood. Several studies have shown that increased expression of THBS2 may correlate with aggressive tumor behavior, including enhanced invasion and migration, while other research suggests that THBS2 may exert anti-tumor effects by inhibiting angiogenesis and promoting ECM stability. [7–9] This dual role complicates its classification as either a pro- or anti-metastatic factor in breast cancer, necessitating a more nuanced investigation into its functional significance during different stages of tumor progression.

Recent advances in high-throughput technologies, such as RNA sequencing and proteomics, have allowed for a more comprehensive analysis of gene and protein expression profiles in metastatic breast cancer. These technologies provide a valuable tool for identifying novel biomarkers and therapeutic targets associated with metastasis. THBS2, in particular, has been identified as a potential candidate biomarker for metastatic disease in various cancer types, including breast cancer. Despite its association with tumor progression, there remains a gap in understanding how changes in THBS2 expression at different stages of breast cancer progression relate to metastatic potential and patient outcomes.

This study aims to investigate the correlation between THBS2 expression and metastasis in breast cancer using IHC analysis. By elucidating the relationship between THBS2 and metastatic potential, we hope to contribute to the growing body of knowledge on the molecular determinants of breast cancer progression and identify potential biomarkers for therapeutic intervention and prognosis.

Materials and Methods

Study Design

This observational, retrospective study was conducted using archival tissue blocks from breast cancer patients. The aim was to investigate the extent of THBS2 expression in core and marginal tissue regions of breast cancer specimens.

Sample Collection

A total of 100 tissue specimens were obtained from archival blocks stored in the pathology department. These samples included both the core tumor tissue and the marginal tissue surrounding the tumor. The samples were anonymized to maintain patient confidentiality.

Immunohistochemical Analysis

Immunohistochemistry (IHC) was performed to assess the expression of THBS2 in the collected specimens. The standard IHC protocol was followed, which involved

deparaffinization, rehydration, antigen retrieval, blocking, incubation with the primary antibody specific for THBS2, and subsequent application of secondary antibody and chromogenic substrate. Slides were counterstained with hematoxylin and mounted for analysis.

Slide Scoring

Two independent observers scored the IHC-stained slides. The extent of THBS2 positivity was quantified based on staining intensity and percentage of positive cells in the core and marginal tissue regions. Discrepancies between the observers were resolved through discussion and consensus.

Data Analysis

The difference in positive staining between the core and marginal tissues was noted and statistically analyzed. Descriptive and comparative statistics were used to evaluate the staining patterns and identify significant differences in THBS2 expression.

Ethical Considerations

Ethical clearance was deemed unnecessary due to the use of anonymized archival specimens, as outlined in our institutional policy. The study protocol complied with the Declaration of Helsinki and the ethical guidelines for retrospective studies.

Results

Demographic Data

The study analyzed a total of 100 breast cancer tissue specimens. Among these, 48 cases (48%) were classified as grade 1, 37 cases (37%) as grade 2, and the remaining 15 cases (15%) as grade 3. This distribution provided a comprehensive representation of varying tumor grades, enabling the assessment of THBS2 expression across a spectrum of disease severities.

THBS2 Expression Across Tumor Grades

The analysis revealed a significant variation in the levels of thrombospondin-2 (THBS2) expression across different histological grades of breast cancer. Grade 3 tumors exhibited the highest levels of THBS2 expression, followed by grade 2, while grade 1 tumors demonstrated comparatively lower expression levels. This gradient of expression suggests a correlation between increased THBS2 levels and higher tumor grade, potentially reflecting its role in aggressive tumor behavior and progression.

Comparison of THBS2 Expression in Core and Marginal Tissue Regions

When comparing the core tumor tissue to the marginal tissue surrounding the tumor, the marginal regions consistently displayed higher intensity of THBS2 staining. This observation was evident across all grades of the tumor and was quantified through scoring by two independent observers. The enhanced staining in the marginal tissue could indicate a localized upregulation of THBS2 in the tumor microenvironment, which might contribute to processes such as invasion and metastasis.

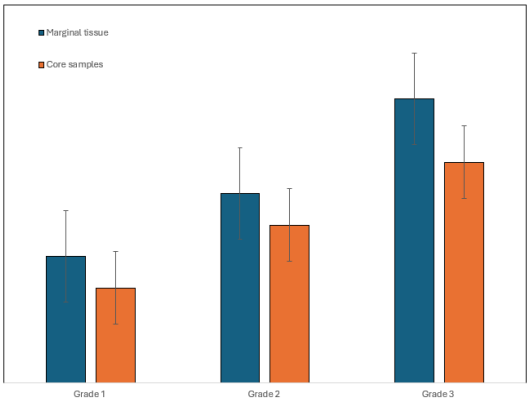

Figure 1: Comparison of THBS2 expression intensity in core and marginal tissue regions across different breast cancer tumor grades. The bar chart shows higher THBS2 staining in marginal tissues compared to core tissues, with grade 3 tumors exhibiting the strongest expression in both regions.

Statistical Significance

The difference in THBS2 expression levels between tumor grades and between core and marginal tissue regions was statistically analyzed. The findings confirmed that the observed variations were significant ($p < 0.05$), highlighting the potential of THBS2 as a biomarker for both tumor aggressiveness and metastatic propensity.

Observational Trends

Qualitatively, the staining intensity patterns revealed that THBS2 expression was predominantly cytoplasmic, with occasional membranous localization. The core tissues exhibited patchy and less intense staining compared to the more uniform and pronounced staining in marginal tissues.
These results underscore the potential role of THBS2 in tumor progression and its differential expression across grades and tissue regions, providing valuable insights for further exploration as a prognostic and therapeutic target.

Discussion

This study investigated the expression of thrombospondin-2 (THBS2) in breast cancer tissue samples, with a focus on its correlation with tumor grade and localization in core and marginal tissue regions. The results indicate a clear association between increased THBS2 expression and higher tumor grade, with grade 3 tumors showing the highest levels of expression. This finding supports the hypothesis that THBS2 may play a role in the progression and aggressiveness of breast cancer. Previous research has suggested that thrombospondins, including THBS2, contribute to tumor growth, invasion, and metastasis through mechanisms such as extracellular matrix remodeling, angiogenesis, and cell migration. The increase in THBS2 expression from grade 1 to grade 3 tumors observed in our study is consistent with the notion that THBS2 may be involved in more advanced stages of cancer, where the tumor is more likely to invade surrounding tissues and metastasize.[8,10]

Furthermore, the comparison between core and marginal tissue regions revealed significantly higher THBS2 expression in the marginal tissues, regardless of the tumor grade. This finding may suggest that THBS2 plays a crucial role in the invasive front of tumors, where the interaction between tumor cells and the surrounding microenvironment is critical for metastasis.[4,11] The enhanced staining in marginal regions could reflect a localized upregulation of THBS2 in response to the tumor's need to invade neighboring tissues, which is a key step in the metastatic cascade. The results provide further support for the idea that THBS2 may contribute to the establishment of a metastatic niche, potentially facilitating the migration of tumor cells to distant sites.

Statistical analysis confirmed that the differences in THBS2 expression between tumor grades and between core and marginal tissues were significant, highlighting the robustness of the observed trends. These findings are in line with studies that have linked THBS2 expression to poor prognosis in various cancers, including breast cancer.[6,12,13] The ability to assess THBS2 expression using immunohistochemistry provides a valuable tool for identifying aggressive tumors and predicting their potential to metastasize.

This study has certain limitations inherent to its retrospective design. Firstly, the data was derived from archival tissue specimens, which might lack comprehensive clinical information that could influence THBS2 expression. Additionally, as a single-center study, the findings may not fully represent the broader patient population, necessitating validation in larger, multi-center cohorts. The results of this study add to the growing body of evidence suggesting that THBS2 may serve as a useful biomarker for both prognostic and therapeutic purposes in breast cancer. The differential expression of THBS2 in different tumor grades and tissue regions underscores its potential role in tumor progression and metastasis. Further studies, particularly those involving larger and more diverse cohorts, are needed to better understand the precise mechanisms through which THBS2 contributes to breast cancer biology and to explore its potential as a therapeutic target.

Conclusion

In conclusion, this study demonstrates a significant correlation between thrombospondin-2 (THBS2) expression and tumor grade in breast cancer, with higher levels of THBS2 observed in grade 3 tumors compared to grade 1 and grade 2 tumors. Additionally, THBS2 expression was notably

higher in the marginal tissue regions compared to the core tumor regions across all grades, suggesting its involvement in the invasive front of tumors. These findings support the potential role of THBS2 as a biomarker for tumor progression and metastasis, providing valuable insights into its contribution to breast cancer aggressiveness. Given its differential expression in tumor tissues, THBS2 may serve as a prognostic marker to identify high-risk patients, and its role in metastasis warrants further exploration for potential therapeutic targeting. Further studies with larger cohorts and functional assays are necessary to fully elucidate the mechanisms by which THBS2 influences breast cancer progression and metastasis.

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References

1. Lemij AA, Bastiaannet E, De Glas NA, van den Bos F, Portielje JE, Liefers GJ, Derks MG. Breast cancer in the older population: a global challenge—an epidemiological perspective. Annals of Breast Surgery. 2023 Jun 30;7. http://dx.doi.org/10.21037/abs-21-89

2. Arvelo F, Sojo F, Cotte C. Cancer and the metastatic substrate. ecancermedicalscience. 2016;10. https://doi.org/10.3332/ecancer.2016.701

3. Liao X, Wang W, Yu B, Tan S. Thrombospondin-2 acts as a bridge between tumor extracellular matrix and immune infiltration in pancreatic and stomach adenocarcinomas: An integrative pan-cancer analysis. Cancer cell international. 2022 Jun 14;22(1):213. http://dx.doi.org/10.1186/s12935-022-02622-x

4. Huang J, Wang C, Hou Y, Tian Y, Li Y, Zhang H, Zhang L, Li W. Molecular mechanisms of Thrombospondin-2 modulates tumor vasculogenic mimicry by PI3K/AKT/mTOR signaling pathway. Biomedicine & Pharmacotherapy. 2023 Nov 1;167:115455. http://dx.doi.org/10.1016/j.biopha.2023.115455

5. Lepucki A, Orlińska K, Mielczarek-Palacz A, Kabut J, Olczyk P, Komosińska-Vassev K. The role of extracellular matrix proteins in breast cancer. Journal of Clinical Medicine. 2022 Feb 25;11(5):1250. http://dx.doi.org/10.3390/jcm11051250

6. Wang J, Zhan X, Luo Q, Kuang Y, Liang X, Wang J, Tao X, Sun E, Chen B. Comprehensive Analysis of the Expression and Prognostic Significance of THBSs in Breast Cancer.http://dx.doi.org/10.21203/rs.3.rs-620437/v1

7. Yang H, Sun B, Fan L, Ma W, Xu K, Hall SR, Wang Z, Schmid RA, Peng RW, Marti TM, Gao W. Multi-scale integrative analyses identify THBS2+ cancer-associated fibroblasts as a key orchestrator promoting aggressiveness in early-stage lung adenocarcinoma. Theranostics. 2022;12(7):3104. http://dx.doi.org/10.7150/thno.69590

8. Qu HL, Hasen GW, Hou YY, Zhang CX. THBS2 promotes cell migration and invasion in colorectal cancer via modulating Wnt/β-catenin signaling pathway. The Kaohsiung Journal of Medical Sciences. 2022 May;38(5):469-78. http://dx.doi.org/10.1002/kjm2.12528

9. Drev D, Bileck A, Erdem ZN, Mohr T, Timelthaler G, Beer A, Gerner C, Marian B. Proteomic profiling identifies markers for inflammation-related tumor–fibroblast interaction. Clinical Proteomics. 2017 Dec;14:1-6. http://dx.doi.org/10.1186/s12014-017-9168-7

10. Qian Z, Zhang G, Song G, Shi J, Gong L, Mou Y, Han Y. Integrated analysis of genes associated with poor prognosis of patients with colorectal cancer liver metastasis. Oncotarget. 2017 Apr 4;8(15):25500. http://dx.doi.org/10.18632/oncotarget.16064

11. Liu Y, Lv H, Liu X, Xu L, Li T, Zhou H, Zhu H, Hao C, Lin C, Zhang Y. The RP11-417E7. 1/THBS2 signaling pathway promotes colorectal cancer metastasis by activating the Wnt/β-catenin pathway and facilitating exosome-mediated M2 macrophage polarization. Journal of Experimental & Clinical Cancer Research. 2024 Jul 17;43(1):195. https://doi.org/10.1186/s13046-024-03107-7

12. Weng TY, Wang CY, Hung YH, Chen WC, Chen YL, Lai MD. Differential expression pattern of THBS1 and THBS2 in lung cancer: clinical outcome and a systematic-analysis of microarray databases. PloS one. 2016 Aug 11;11(8):e0161007. http://dx.doi.org/10.1371/journal.pone.0161007

13. Deng B, Liu XP, Wang X. Prognostic and immunological role of THBS2 in colorectal cancer. BioMed Research International. 2021;2021(1):1124985. http://dx.doi.org/10.1155/2021/1124985