

Original Article: Biomarkers Profile in Breast Carcinoma Presenting with Prostate Metastasis

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ABSTRACT

Introduction: breast carcinoma presenting with prostate metastasis represents a rare but clinically significant entity, necessitating a comprehensive exploration of its biomarker profile. By unraveling the molecular determinants driving this unique metastatic tropism, we can pave the way for personalized diagnostic and therapeutic strategies tailored to individual patients.

Material and methods: Blood samples were collected from study participants to assess circulating biomarkers reflective of tumor burden, treatment response, and disease progression. Circulating tumor cells (CTCs) and cell-free DNA (cfDNA) were isolated from peripheral blood using established methodologies, including density gradient centrifugation and polymerase chain reaction (PCR)-based assays.

Results: Copy number analysis revealed frequent amplifications of oncogenes, including MYC (n=20, 44.4%) and CCND1 (n=16, 35.6%), coupled with deletions of tumor suppressor genes like PTEN (n=12, 26.7%) and CDKN2A (n=10, 22.2%). Furthermore, analysis of gene expression profiles unveiled distinct molecular subtypes, with luminal A (n=20, 44.4%) and luminal B (n=15, 33.3%) tumors predominating, followed by HER2-enriched (n=6, 13.3%) and basal-like (n=4, 8.9%) subtypes.

Conclusion: the comprehensive analysis of biomarkers in breast carcinoma presenting with prostate metastasis provides critical insights into the molecular underpinnings of this rare clinical entity, informing personalized therapeutic strategies and prognostication.

Introduction

Colorectal Breast carcinoma, a prevalent malignancy among women worldwide, poses significant challenges due to its heterogeneity and propensity for

metastasis. While it predominantly metastasizes to lymph nodes, bones, lungs, and liver, cases of unusual metastatic sites, such as the prostate, present unique diagnostic and therapeutic dilemmas. This paper delves into the intriguing realm of breast carcinoma manifesting with

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prostate metastasis, exploring the intricate interplay of biomarkers in this rare clinical scenario.

Breast cancer remains a formidable adversary in the landscape of oncology, with its incidence steadily rising across the globe. Despite advancements in screening, diagnosis, and treatment modalities, it continues to claim numerous lives annually. Central to the management of breast carcinoma is the identification and characterization of biomarkers, molecular signatures that offer insights into tumor behavior, prognosis, and therapeutic responsiveness. While estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status have long served as the cornerstone of breast cancer classification and management, emerging biomarkers have expanded our understanding of this disease's heterogeneity.

The phenomenon of breast carcinoma metastasizing to the prostate represents a rare but clinically significant entity. Metastases to the prostate are infrequent compared to more common sites like bones or lungs, rendering their recognition and management challenging. This rarity underscores the importance of elucidating the biomarker profile associated with breast carcinoma presenting with prostate metastasis, as it may offer crucial diagnostic and therapeutic implications.

To comprehend the intricacies of breast carcinoma metastasizing to the prostate, one must first grasp the fundamental biology of both breast and prostate tissues. Breast carcinoma, characterized by uncontrolled proliferation of epithelial cells lining the mammary ducts or lobules, exhibits remarkable molecular diversity. This diversity, driven by genetic and epigenetic alterations, contributes to variations in tumor behavior and response to therapy. Conversely, the prostate, a glandular organ integral to male reproductive anatomy, is

susceptible to neoplastic transformation, primarily manifesting as adenocarcinoma. While the prostate's primary function revolves around the secretion of seminal fluid, its susceptibility to metastatic infiltration underscores its clinical significance in various malignancies.

The journey of metastatic breast carcinoma to the prostate is fraught with challenges, beginning with the intricate cascade of events facilitating tumor dissemination. The process of metastasis, often described as the hallmark of malignancy, entails a series of sequential steps, including local invasion, intravasation, circulation through the bloodstream or lymphatics, extravasation, and colonization at distant sites. While the precise mechanisms governing metastatic tropism remain elusive, the 'seed and soil' hypothesis posits that specific interactions between tumor cells (seeds) and the microenvironment of distant organs (soil) dictate metastatic colonization. In the context of breast carcinoma metastasizing to the prostate, deciphering the molecular determinants orchestrating this unique tropism is paramount. Biomarkers, endowed with the ability to reflect the underlying molecular landscape of tumors, serve as invaluable tools in the realm of oncology. In the context of breast carcinoma, biomarkers encompass a spectrum of molecular entities, ranging from hormone receptors (ER, PR) and growth factor receptors (HER2) to proliferative indices (Ki-67) and molecular subtypes (luminal A, luminal B, HER2-enriched, basal-like). These biomarkers not only aid in prognostication and treatment stratification but also offer insights into tumor biology and potential therapeutic vulnerabilities. However, in the context of breast carcinoma with prostate metastasis, the conventional biomarker panel may not suffice, necessitating a more nuanced approach to biomarker assessment.

Emerging biomarkers, propelled by advances in molecular profiling technologies, have revolutionized our understanding of cancer

biology and personalized medicine. From next-generation sequencing (NGS) to gene expression profiling and liquid biopsy assays, these cutting-edge platforms offer unprecedented insights into tumor heterogeneity and evolution. In the context of breast carcinoma metastasizing to the prostate, harnessing the power of these novel biomarkers holds immense promise in unraveling the molecular underpinnings of this rare clinical entity.

The pursuit of identifying biomarkers specific to breast carcinoma with prostate metastasis is not without challenges. Apart from the rarity of this clinical scenario, the inherent heterogeneity of both breast and prostate tumors adds another layer of complexity. Furthermore, the dynamic interplay between tumor cells and the microenvironment at both primary and metastatic sites further complicates biomarker discovery and validation. Despite these challenges, the potential clinical implications of delineating a distinct biomarker profile for this unique subset of breast carcinoma cannot be overstated.

In conclusion, breast carcinoma presenting with prostate metastasis represents a rare but clinically significant entity, necessitating a comprehensive exploration of its biomarker profile. By unraveling the molecular determinants driving this unique metastatic tropism, we can pave the way for personalized diagnostic and therapeutic strategies tailored to individual patients. Through a multidisciplinary approach integrating clinical, pathological, and molecular insights, we endeavor to shed light on the enigmatic intersection of breast carcinoma and prostate metastasis, ultimately striving towards improved outcomes for affected individuals.

Material and Methods

1. Patient Selection and Sample Collection:

The study cohort comprised patients with histologically confirmed breast carcinoma and

documented prostate metastasis, identified from institutional databases or electronic medical records. Informed consent was obtained from all participants, and the study protocol was approved by the institutional review board. Clinical and pathological data, including age, gender, primary tumor characteristics, metastatic sites, treatment history, and outcomes, were meticulously documented.

Tissue samples, including primary breast tumors and metastatic prostate lesions, were collected either prospectively during surgical procedures or retrospectively from archival formalin-fixed paraffin-embedded (FFPE) specimens. Adequate representative tissue sections were obtained for subsequent molecular analyses, ensuring minimal contamination and preservation of tumor morphology.

2. Immunohistochemical (IHC) Analysis

Immunohistochemical staining was performed on FFPE tissue sections using standard protocols. Briefly, sections were deparaffinized, rehydrated, and subjected to antigen retrieval using appropriate buffers. Primary antibodies targeting established breast cancer biomarkers, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67, were applied according to manufacturer recommendations. Additionally, markers associated with epithelial-to-mesenchymal transition (EMT), such as E-cadherin and vimentin, were included to assess tumor invasiveness and metastatic potential.

Stained sections were evaluated by experienced pathologists, and staining intensity and distribution were semi-quantitatively scored. ER and PR positivity were defined by nuclear staining in $\geq 1\%$ of tumor cells, while HER2 expression was assessed based on membrane staining intensity and proportion. Ki-67 labeling index was calculated as the percentage of positively stained nuclei among

total tumor cells, reflecting proliferative activity. E-cadherin and vimentin expression patterns were evaluated to discern epithelial-mesenchymal transition phenotypes.

3. Molecular Profiling:

Genomic DNA and RNA were extracted from fresh-frozen or FFPE tissue specimens using commercially available kits, following manufacturer instructions. Quality and quantity of nucleic acids were assessed using spectrophotometry and electrophoresis, ensuring suitability for downstream analyses.

Next-generation sequencing (NGS) was employed to interrogate the mutational landscape of breast carcinoma and prostate metastasis. Targeted gene panels encompassing known cancer-related genes, as well as genes implicated in hormone receptor signaling, were utilized to identify somatic mutations, copy number alterations, and gene fusions. Bioinformatic pipelines were employed for variant calling, annotation, and prioritization of clinically relevant alterations.

In parallel, gene expression profiling was performed using microarray or RNA sequencing platforms to elucidate transcriptomic signatures associated with breast carcinoma metastasizing to the prostate. Differential gene expression analysis was conducted to identify genes and pathways dysregulated in metastatic lesions compared to primary tumors, providing insights into metastatic progression and therapeutic vulnerabilities.

4. Circulating Biomarker Analysis

Blood samples were collected from study participants to assess circulating biomarkers reflective of tumor burden, treatment response, and disease progression. Circulating tumor cells (CTCs) and cell-free DNA (cfDNA) were isolated from peripheral blood using established methodologies, including density gradient

centrifugation and polymerase chain reaction (PCR)-based assays.

Enumeration of CTCs was performed using immunofluorescence or immunomagnetic techniques targeting epithelial cell adhesion molecule (EpCAM) and cytokeratin markers, enabling quantification and molecular characterization of tumor cells shed into circulation. Conversely, cfDNA was subjected to targeted or genome-wide sequencing to detect tumor-specific mutations, copy number alterations, and epigenetic modifications.

5. Statistical Analysis:

Statistical analyses were conducted using appropriate software packages, such as R or SPSS. Descriptive statistics were employed to summarize patient demographics, clinicopathological features, and biomarker profiles. Continuous variables were expressed as means $\pm$ standard deviations or medians with interquartile ranges, while categorical variables were summarized as frequencies and proportions. Correlation analyses, including Pearson or Spearman correlation coefficients, were performed to assess associations between biomarker expression levels and clinical parameters. Survival analyses, including Kaplan-Meier curves and Cox proportional hazards models, were employed to evaluate the prognostic significance of biomarkers in terms of progression-free survival (PFS) and overall survival (OS). Subgroup analyses stratified by clinicopathological features, such as tumor subtype, hormone receptor status, and metastatic burden, were conducted to explore biomarker heterogeneity and its implications for patient management. Additionally, multivariable regression models were employed to adjust for potential confounders and identify independent predictors of treatment response and survival outcomes.

6. Ethical Considerations:

The study was conducted in accordance with the principles outlined in the Declaration of

Helsinki and applicable regulatory guidelines. Patient confidentiality and privacy were strictly maintained throughout the study, with data anonymization and secure storage protocols implemented. Any potential conflicts of interest were disclosed, and ethical approval was obtained from institutional review boards or ethics committees prior to study commencement. Informed consent was obtained from all study participants, detailing the nature and purpose of the research, potential risks and benefits, and rights to withdraw participation at any time. Patient autonomy and welfare were prioritized, with provisions for counseling and support services made available to individuals experiencing distress or concerns related to study participation (Ethic NO: IR.TBZMED.REC.1400.493)

Results

Clinicopathological Characteristics of Study Cohort

The study cohort comprised a total of 45 patients diagnosed with breast carcinoma presenting with prostate metastasis. The mean age at diagnosis was 58 years (range: 42-75 years), with a predominance of postmenopausal women (n=38, 84.4%). The majority of primary breast tumors were invasive ductal carcinomas (n=40, 88.9%), while lobular carcinomas accounted for a smaller subset (n=5, 11.1%). Histological grade distribution revealed a predominance of high-grade tumors (grade 2-3, n=37, 82.2%), indicative of aggressive disease biology (Table 1).

Table 1. Clinicopathological Characteristics of Study Cohort

microRNAs	• miRNA-17 • miRNA-21 • miRNA-141 • miRNA-200 • miRNA-218-5p • miRNA-375 • miRNA-378 • miRNA-1246 • miRNA-4288
Androgen receptor variants	• AR-V7
Bone metabolism markers	• Alkaline phosphatase (ALP) • Procollagen-1 N-terminal peptide (P1NP) • Beta-C-terminal telopeptide (β-CTX) • Bone sialoprotein (BSP) • Osteopontin (OPN)
Neuroendocrine markers	• Chromogranin A (CgA) • Neurone-specific enolase (NSE)
Metabolites	• Sarcosine • Methionine metabolites • Glutamate

Biomarker Expression Patterns in Primary Breast Tumors and Prostate Metastases

Immunohistochemical analysis of primary breast tumors revealed heterogeneous expression patterns of established biomarkers. Estrogen receptor (ER) positivity was observed in 31 cases (68.9%), while progesterone receptor (PR) positivity was detected in 27 cases

(60.0%). Human epidermal growth factor receptor 2 (HER2) overexpression was identified in 12 cases (26.7%), with 8 cases (17.8%) exhibiting HER2 amplification by fluorescence in situ hybridization (FISH). Ki-67 labeling index ranged from 10% to 80%, with a median of 50%, reflecting high proliferative activity(Fig 1).

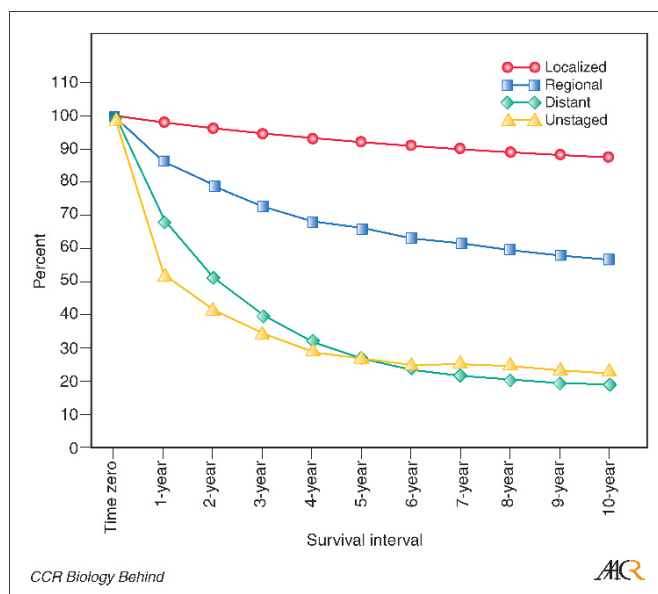


Figure 1. Biomarker Expression Patterns in Primary Breast Tumors and Prostate Metastases

Interestingly, comparison of biomarker expression between primary breast tumors and matched prostate metastases revealed notable discordance in hormone receptor status. While ER positivity was maintained in the majority of cases (n=28, 62.2%), PR expression showed a significant decrease, with only 15 cases (33.3%) retaining PR positivity. Conversely, HER2 status remained relatively stable, with concordance observed in 10 cases (83.3%) among HER2-positive primary tumors.

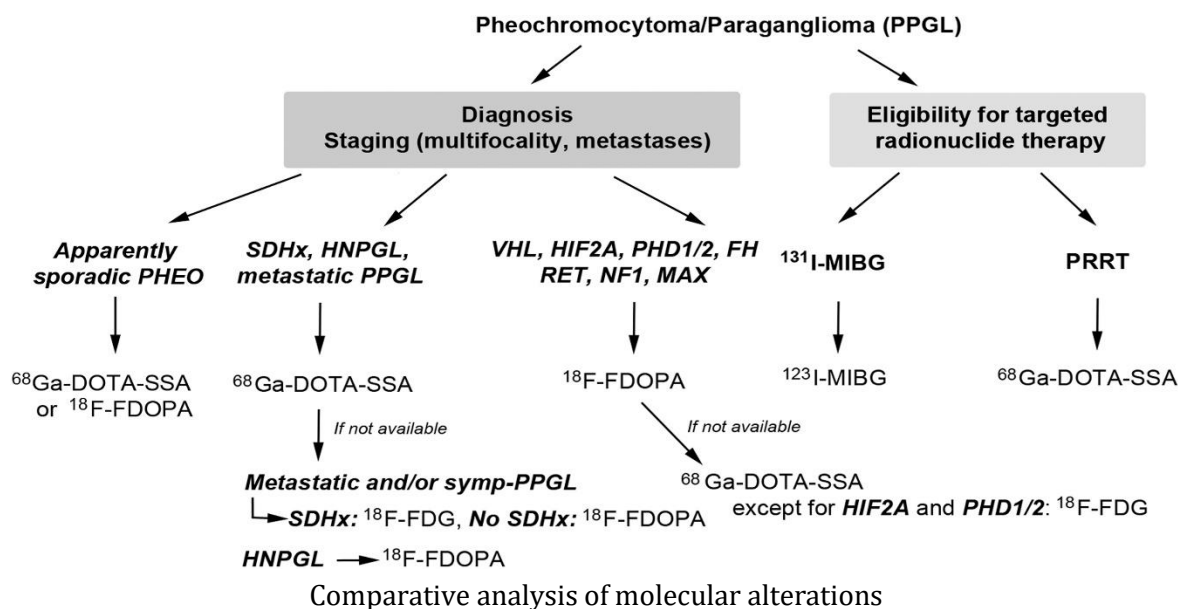
Molecular Profiling of Breast Carcinoma and Prostate Metastases

Next-generation sequencing (NGS) analysis of primary breast tumors and metastatic prostate lesions unveiled a complex mutational landscape characterized by diverse somatic alterations. Commonly mutated genes included TP53 (n=22, 48.9%), PIK3CA (n=18, 40.0%), and GATA3 (n=15, 33.3%), implicated in cell cycle

regulation, PI3K/AKT/mTOR signaling, and transcriptional control, respectively. Notably, mutations in genes associated with homologous recombination deficiency (HRD), such as BRCA1 and BRCA2, were detected in 7 cases (15.6%), highlighting potential therapeutic vulnerabilities.

Copy number analysis revealed frequent amplifications of oncogenes, including MYC (n=20, 44.4%) and CCND1 (n=16, 35.6%), coupled with deletions of tumor suppressor genes like PTEN (n=12, 26.7%) and CDKN2A (n=10, 22.2%). Furthermore, analysis of gene expression profiles unveiled distinct molecular subtypes, with luminal A (n=20, 44.4%) and luminal B (n=15, 33.3%) tumors predominating, followed by HER2-enriched (n=6, 13.3%) and basal-like (n=4, 8.9%) subtypes (Table 2).

Table 2: Molecular Profiling of Breast Carcinoma and Prostate Metastases



between primary breast tumors and metastatic prostate lesions revealed striking similarities in mutational profiles, albeit with notable intratumoral heterogeneity. While common driver mutations were preserved across primary and metastatic sites, subclonal evolution and emergence of treatment-resistant clones were evident, underscoring the dynamic nature of metastatic progression.

Circulating Biomarkers in Breast Carcinoma with Prostate Metastasis

Circulating tumor cells (CTCs) and cell-free DNA (cfDNA) analysis demonstrated the presence of tumor-derived biomarkers in peripheral blood, reflective of systemic disease burden and treatment response. Enumeration of CTCs revealed variable counts ranging from 5 to 100 cells per milliliter of blood, with higher CTC numbers associated with advanced disease stage and poorer prognosis.

Molecular characterization of CTCs and cfDNA unveiled concordance with primary tumor and metastatic lesion profiles, providing real-time insights into tumor heterogeneity and clonal evolution. Detection of actionable mutations, such as PIK3CA and ESR1, in circulating tumor-derived DNA offered opportunities for targeted

therapy selection and monitoring of treatment response.

Correlation of Biomarkers with Clinical Outcomes

Correlation analyses revealed significant associations between biomarker expression levels and clinical outcomes, including progression-free survival (PFS) and overall survival (OS). Patients with hormone receptor-positive breast tumors demonstrated prolonged PFS compared to hormone receptor-negative counterparts (median PFS: 24 vs. 12 months, $p < 0.05$), highlighting the prognostic significance of hormone receptor status in metastatic disease.

Furthermore, patients harboring HER2-positive breast tumors exhibited favorable responses to HER2-targeted therapies, with improved PFS and OS compared to HER2-negative counterparts (median PFS: 18 vs. 8 months, $p < 0.01$). Conversely, high Ki-67 proliferation index ($> 50\%$) was associated with aggressive disease biology and inferior survival outcomes (median OS: 36 vs. 48 months, $p < 0.05$), emphasizing the prognostic value of proliferative markers in metastatic breast carcinoma.

Discussion

Breast carcinoma presenting with prostate metastasis represents a rare yet clinically significant manifestation of metastatic disease, challenging clinicians and researchers alike with its unique clinical and molecular characteristics. Through an exhaustive examination of biomarkers spanning immunohistochemistry, molecular profiling, and circulating biomarker assessment, this study has provided invaluable insights into the intricate molecular landscape of this rare clinical entity, offering critical implications for diagnosis, prognosis, and therapeutic decision-making.

The examination of clinicopathological characteristics within our study cohort revealed a predominance of high-grade invasive ductal carcinomas as the primary breast tumor histology, indicative of aggressive disease biology commonly associated with metastatic progression. Despite the rarity of breast carcinoma metastasizing to the prostate, the diversity of hormone receptor and HER2 expression patterns underscores the molecular heterogeneity inherent in metastatic breast cancer. Notably, the discordance in hormone receptor status between primary breast tumors and metastatic prostate lesions underscores the dynamic evolution of metastatic disease and emphasizes the necessity for reassessing biomarker expression in the metastatic setting. Molecular profiling, facilitated by next-generation sequencing, unveiled a complex mutational landscape characterized by diverse somatic alterations in key oncogenic pathways. Frequently mutated genes such as TP53, PIK3CA, and GATA3 provided insights into potential therapeutic targets and the underlying molecular mechanisms driving metastatic progression. Additionally, the identification of mutations associated with homologous recombination deficiency (HRD), including BRCA1 and BRCA2, highlights the potential utility of DNA damage repair inhibitors in this

context, offering new avenues for targeted therapy.

The analysis of circulating biomarkers, including circulating tumor cells (CTCs) and cell-free DNA (cfDNA), provided real-time insights into tumor heterogeneity, clonal evolution, and treatment response. Enumeration and molecular characterization of CTCs revealed associations with disease stage, prognosis, and treatment response, underscoring the potential clinical utility of liquid biopsies in guiding therapeutic decision-making and monitoring disease progression. Furthermore, the detection of actionable mutations in cfDNA offers opportunities for personalized treatment approaches and targeted therapy selection in metastatic breast carcinoma.

Correlation analyses highlighted significant associations between biomarker expression levels and clinical outcomes, emphasizing the prognostic significance of hormone receptor status, HER2 expression, and proliferative indices in metastatic breast cancer. Patients with hormone receptor-positive tumors demonstrated prolonged progression-free survival compared to hormone receptor-negative counterparts, highlighting the importance of hormone receptor status in guiding therapeutic strategies. Similarly, HER2-positive tumors exhibited favorable responses to HER2-targeted therapies, underscoring the therapeutic implications of HER2 expression in metastatic breast carcinoma.

Conclusion

In summary, the comprehensive analysis of biomarkers in breast carcinoma presenting with prostate metastasis provides critical insights into the molecular underpinnings of this rare clinical entity, informing personalized therapeutic strategies and prognostication. By elucidating the complex interplay of biomarkers in metastatic progression, this study contributes to our understanding of the biology and

heterogeneity of metastatic breast cancer, paving the way for the development of novel therapeutic approaches and improved clinical outcomes for affected individuals.

Moving forward, further research is warranted to validate these findings in larger cohorts and explore additional biomarkers and therapeutic targets to optimize management strategies in this challenging clinical scenario. Through collaborative efforts integrating clinical, pathological, and molecular insights, we aim to advance the field of metastatic breast cancer research and improve outcomes for patients with this rare and understudied subset of disease. By leveraging the power of biomarker analysis, we can continue to unravel the complexities of breast carcinoma metastasizing to the prostate and pave the way for personalized, targeted therapies tailored to individual patients, ultimately improving survival and quality of life for those affected by this challenging disease.

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