INTEGRATIVE NETWORK-BASED CHARACTERIZATION OF SERUM BIOMARKER INTERACTIONS IN PROSTATE CANCER PROGRESSION
BIOMARKER NETWORKS IN PROSTATE CANCER

PROSTAT KANSERİ İLERLEMESİNDE SERUM BİYOBELİRTEÇLERİ ARASINDAKİ ETKİLEŞİMLERİN ENTEGRE AĞ TABANLI ANALİZİ
PROSTAT KANSERİNDE BİYOBELİRTEÇ AĞLARI

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ABSTRACT

Objective: This study investigates the interactions among key serum biomarkers—Hypoxia-inducible factor-1 alpha (HIF-1), Thrombospondin-1 (TSP1), Neuropilin-1 (NRP1), and Prostate-specific antigen (PSA)—and clinical parameters including age at diagnosis, diabetes mellitus (DM), hypertension (HT), and smoking status in patients with metastatic prostate cancer (mPCa). The objective was to identify structural and functional interdependencies among these variables using a network-based analytical approach.

Materials and Methods: Network analysis was conducted using JASP software (v0.19.3.0). Variables were modeled as nodes, and partial correlations between them as edges. Edge color represented the direction (positive or negative) of the correlation, while thickness indicated its strength. Network topology was evaluated using graph-theoretical metrics including degree, closeness, betweenness, and eigenvector centrality. Additional measures of density and sparsity were also calculated. Spatial visualization of the network was performed using the Fruchterman–Reingold algorithm.

Results: The network comprised eight variables and 27 connections, yielding a sparsity value of 0.036, indicating a highly dense structure. PSA and TSP1 exhibited the highest betweenness centrality, serving as critical bridging nodes. HT and DM had high degree and closeness centrality values, reflecting central positions within the network. NRP1 displayed the highest clustering coefficient, suggesting a localized regulatory role. A strong negative association was observed between TSP1 and HT.

Conclusion: This study highlights the utility of network analysis as a systems-level tool to explore complex biomarker interactions in mPCa. PSA, TSP1, and NRP1 emerged as key molecular regulators, while systemic conditions such as HT and DM significantly influenced network architecture. These findings warrant further validation through mechanistic and hypothesis-driven statistical studies.

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ÖZET

Amaç: Bu çalışma, metastatik prostat kanseri (mPK) hastalarında Hipoksiye duyarlı faktör-1 alfa (HIF-1), Trombospondin-1 (TSP1), Nöropilin-1 (NRP1) ve Prostat spesifik antijen (PSA) gibi temel serum biyobelirteçleri ile tanı yaşı, diyabetes mellitus, hipertansiyon ve sigara kullanımı gibi klinik parametreler arasındaki etkileşimleri ağ tabanlı analitik bir yaklaşımla değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntemler: Analiz, JASP yazılımı (v0.19.3.0) ile gerçekleştirilmiştir. Değişkenler düğüm (node), kısmi korelasyonlar ise kenar (edge) olarak modellenmiştir. Kenar renkleri ilişkinin yönünü (pozitif/negatif), kalınlıkları ise ilişkinin gücünü göstermektedir. Ağ yapısı; derece, yakınlık, aradalık ve özvektör merkezilik gibi grafik kuramı temelli ölçütlerle değerlendirilmiş; yoğunluk ve seyreklik değerleri hesaplanmıştır. Düğümlerin konumlandırılmasında Fruchterman–Reingold algoritması kullanılmıştır.

Bulgular: Toplam sekiz değişken ve 27 bağlantıdan oluşan ağın seyreklik değeri 0,036 olarak bulunmuştur; bu da yoğun bir yapı olduğunu göstermektedir. PSA ve TSP1 en yüksek aradalık merkezilik değerleriyle köprü rolü üstlenmiştir. Hipertansiyon ve diyabet, yüksek derece ve yakınlık merkezilik değerleriyle ağın merkezinde yer almıştır. NRP1 en yüksek kümeleme katsayısına sahip olup lokal bir düzenleyici rol üstlenmektedir. TSP1 ile hipertansiyon arasında güçlü negatif bir ilişki gözlenmiştir.

Sonuç: Bu çalışma, metastatik prostat kanserinde biyobelirteçler arası ilişkilerin sistem düzeyinde anlaşılmasında ağ analizinin etkili bir yöntem olduğunu ortaya koymaktadır. PSA, TSP1 ve NRP1 önemli moleküler düzenleyiciler olarak öne çıkarken; hipertansiyon ve diyabetes mellitus gibi sistemik hastalıklar ağ yapısının bütünlüğü üzerinde belirgin rol oynamaktadır. Bulguların mekanistik ve istatistiksel çalışmalarla doğrulanması gerekmektedir.

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Introduction

Prostate cancer (PCa) is one of the most frequently diagnosed malignancies and remains a leading cause of cancer-related mortality among men worldwide. The disease exhibits pronounced biological heterogeneity, with clinical manifestations ranging from indolent, organ-confined tumors to rapidly progressing and treatment-resistant metastatic lesions. This heterogeneity poses a significant challenge for accurate patient stratification and personalized therapeutic decision-making (1).

Currently, prostate-specific antigen (PSA) remains the cornerstone biomarker for PCa screening and disease monitoring. However, its limited specificity and prognostic utility are well-documented. PSA levels can fluctuate due to benign conditions such as benign prostatic hyperplasia (BPH), prostatitis, or even physical manipulation of the prostate. Moreover, PSA is often inadequate in distinguishing between indolent and aggressive tumor forms, potentially resulting in both overdiagnosis and overtreatment (2, 3). Consequently, the identification and integration of novel serum biomarkers with improved diagnostic and prognostic capabilities has become a key focus in PCa research (4).

In this context, accumulating evidence underscores the central roles of hypoxia, angiogenesis, and tumor microenvironmental remodeling in driving prostate cancer progression and therapeutic resistance. Among the biomarkers gaining increasing attention are Thrombospondin-1 (TSP1), Neuropilin-1 (NRP1), and Hypoxia-inducible factor-1 alpha (HIF-1α)—molecules involved in angiogenesis, hypoxia adaptation, and immune regulation.

TSP1 is a multifunctional matricellular glycoprotein known for its potent anti-angiogenic and tumor-suppressive properties, particularly through its interactions with CD36 and CD47, and modulation of TGF-β and VEGF signaling pathways (5, 6). In prostate cancer, TSP1 expression is frequently downregulated, correlating with increased angiogenesis, higher microvessel density, and enhanced tumor aggressiveness (7–9). NRP1 is a transmembrane glycoprotein that functions as a co-receptor for VEGF-A165 and semaphorins, facilitating angiogenesis, immune evasion, and tumor cell invasion (10, 11). Upregulation of NRP1 has been associated with metastatic potential, elevated Gleason scores, treatment resistance, and poor clinical prognosis, particularly in castration-resistant prostate cancer (12, 13). HIF-1α is a key transcription factor that orchestrates the cellular response to hypoxia. Its stabilization—either under low oxygen tension or due to oncogenic signaling—triggers metabolic reprogramming, angiogenesis, and resistance to apoptosis (14). Elevated HIF-1α expression has been linked to disease progression and resistance to androgen deprivation therapy and radiotherapy in PCa (15, 16).

Although traditional biostatistical methods and supervised machine learning models (e.g., random forest, decision trees) have offered valuable insights into biomarker performance, they often fall short in capturing the complex, non-linear, and multivariate interactions that define biological systems. In oncology, disease progression is seldom dictated by single biomarkers in isolation; rather, it reflects the dynamic interplay among numerous molecular and clinical factors within a highly interconnected network (17, 18). Therefore, an analytical framework capable of modeling these interdependencies is essential for developing a systems-level understanding of tumor behavior and biomarker function (19–21).

Network analysis has emerged as a powerful systems biology approach for visualizing, quantifying, and interpreting complex relationships among biological variables (17). In network theory, biomarkers or genes are represented as

nodes, while their pairwise associations—such as correlations, mutual information, or regulatory interactions—are represented as edges. Analysis of network topology, including metrics such as node degree, centrality, betweenness, and clustering coefficients, can reveal key regulatory hubs, identify modular subnetworks, and uncover latent biological signatures that may be overlooked by conventional methods (19, 22, 23).

In this study, we applied network analysis to systematically evaluate interactions among serum biomarkers (HIF-1α, TSP1, NRP1, and PSA) and clinical parameters (age at diagnosis, diabetes mellitus [DM], hypertension [HT], and smoking status) in patients with metastatic prostate cancer (mPCa). Our aim was to elucidate their central positions and potential regulatory influence within the disease network. By employing network-based algorithms, we sought to identify topological features that differentiate localized from metastatic PCa phenotypes.

We hypothesize that metastatic progression is associated with distinct network patterns—such as enhanced connectivity among pro-angiogenic factors and disruption of anti-angiogenic regulation—reflecting underlying biological mechanisms of disease advancement. This network-based framework complements traditional statistical and machine learning methods by enabling the identification of emergent properties within complex biomarker systems. Ultimately, by adopting a systems-level perspective, our goal is to improve the biological interpretability and clinical utility of biomarker data in prostate cancer stratification.

Materials and Methods

This study employed network analysis to uncover the structural relationships among clinical and demographic variables in patients with metastatic prostate cancer (mPCa). The aim was to characterize key network properties, identify strong and weak associations, quantify interaction intensity, and define the positional roles of each variable within the network. Network construction and visualization were conducted using JASP software (Version 0.19.3.0) [Computer software].

This retrospective observational study included 90 male patients diagnosed with mPCa and treated at İzmir Katip Celebi University between January 2019 and December 2023. Inclusion criteria were: age ≥50 years, histopathologically and radiologically confirmed mPCa according to international guidelines, availability of complete serum biomarker data (HIF-1α, TSP1, NRP1, PSA), and recorded clinical parameters including age at diagnosis, diabetes mellitus (DM), hypertension (HT), and smoking status. Exclusion criteria comprised: history of a second primary malignancy, prostate infection, rheumatologic, autoimmune, or metabolic disorders, uncontrolled comorbidities, ongoing immunosuppressive therapy, severe hepatic or renal dysfunction, and incomplete clinical or laboratory data. All patient data were anonymized prior to analysis to ensure confidentiality. The study was approved by the Institutional Ethics Committee of İzmir Katip Çelebi University Atatürk Training and Research Hospital (Approval No: 0165) and conducted in accordance with the principles of the Declaration of Helsinki.

In the constructed network, each variable was represented as a node, and partial correlations—controlling for all other variables—were represented as edges. Edge colors denoted the direction of the correlation: blue for positive and red for negative. The thickness and saturation of each edge reflected the strength of the partial correlation; thicker and more saturated edges indicated stronger associations. Edges with weights approaching zero were considered weak connections, potentially represent-

ing spurious or indirect associations rather than true interactions.

Node importance was assessed through centrality measures commonly used in network theory, including strength, closeness, influence (eigenvector centrality), and betweenness.

- **Node strength** reflects the total weight of connections associated with a node.
- **Closeness** quantifies how near a node is to all other nodes, indicating its accessibility within the network.
- **Betweenness** measures how often a node lies on the shortest path between other nodes, identifying potential regulatory "bridges."
- **Eigenvector centrality** captures both the quantity and quality of a node's connections, highlighting variables that are linked to other influential nodes.

This comprehensive analytical approach allowed for the identification of key variables and structural patterns within the biomarker-clinical parameter network of metastatic prostate cancer.

Results

In this study, the network positions of the variables HIF-1α, TSP1, NRP1, PSA, age at diagnosis, DM, HT, and smoking status were evaluated in patients with metastatic prostate cancer (mPCa). To assess their relative importance and connectivity within the network, core centrality measures—including degree, betweenness, closeness, and eigenvector centrality—were applied alongside network density metrics. Each of these centrality indices is based on different theoretical assumptions regarding node influence, providing complementary insights into which variables act as key players within the system. These metrics were used to identify the most active, central, and influential variables in the biomarker-clinical parameter interaction network.

The relationships among variables were visualized using a network diagram, presented in Figure 1. Each node represents one of the eight studied variables: Hypoxia-inducible factor-1 alpha (HIF-1α), Thrombospondin-1 (TSP1), Neuropilin-1 (NRP1), Prostate-specific antigen (PSA), age at diagnosis, diabetes mellitus (DM), hypertension (HT), and smoking status. Edges represent the partial correlations between variable pairs, controlled for all other variables in the network.

The layout of the network was generated using the Fruchterman–Reingold algorithm, a force-directed layout method that positions nodes based on the strength of their interconnections. Nodes that are more strongly connected are positioned closer together, while those with weaker or fewer connections are spaced farther apart. Edge thickness reflects the strength of partial correlations, with thicker lines indicating stronger associations. The direction of the correlation is indicated by color: blue edges represent positive correlations, and red edges represent negative correlations.

This network visualization provides a spatial representation of the interdependence among clinical and molecular variables, enabling the identification of both central hubs and peripheral actors within the mPCa interaction framework.

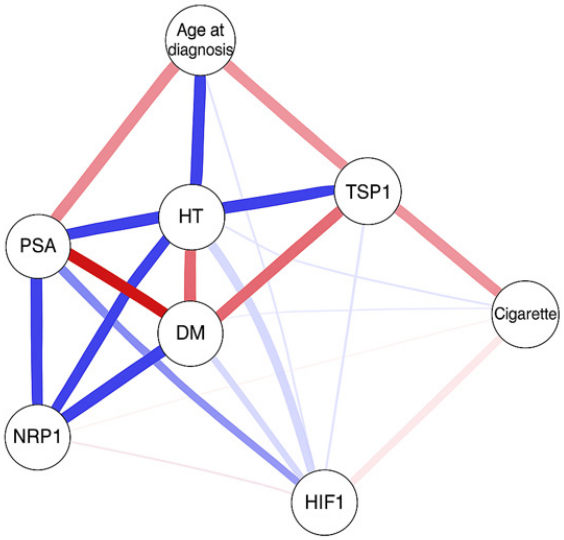

Figure 1. *Interaction network of serum biomarkers and clinical variables in metastatic prostate cancer.*

Interaction network of serum biomarkers and clinical variables in metastatic prostate cancer. Nodes represent individual variables: Hypoxia-inducible factor-1 alpha (HIF-1α), Thrombospondin-1 (TSP1), Neuropilin-1 (NRP1), Prostate-specific antigen (PSA), age at diagnosis, DM, HT, and smoking status. Edges represent partial correlations between variables, controlled for all other nodes. Edge color: Blue indicates positive correlation; red indicates negative correlation. Edge thickness: Represents the strength of the correlation (thicker = stronger).

In this study, the relationships among HIF-1α, TSP1, NRP1, PSA, age at diagnosis, diabetes mellitus (DM), hypertension (HT), and smoking status were evaluated within a network framework in patients with metastatic prostate cancer (mPCa). Graph-theoretic measures—including degree, closeness, and betweenness centrality—were applied to assess the influence and network position of each variable, alongside global metrics such as network density and sparsity.

Centrality measures are essential for interpreting a node's functional role in the network.

•**Degree centrality** reflects the number of direct connections a node has.

•**Closeness centrality** captures how efficiently a node can be reached from all other nodes.

•**Betweenness centrality** measures how often a node lies on the shortest paths between other node pairs, identifying potential "gatekeeper" variables (24).

In this analysis, **HT** and **DM** emerged as centrally positioned variables. Notably, HT displayed strong connections in both positive and negative directions, suggesting a high degree of interaction with other clinical and molecular markers. **DM**also exhibited meaningful associations, visually represented in Figure 1 through the thickness of connecting lines—where **thick blue edges** denote strong positive partial correlations, and **thick red edges** indicate strong negative associations.

The **Fruchterman–Reingold algorithm** was used for network visualization (25), optimizing node placement based on connection strength and achieving a balanced, interpretable layout. Spatial proximity between nodes in the diagram reflects the degree of interconnectivity.

In this context:

•**Blue edges** = positive partial correlations

•**Red edges** = negative partial correlations

•**Edge thickness** = strength of the association

For example, strong positive correlations were observed between **PSA–DM** and **PSA–HT** (thick blue edges), while a strong negative correlation was identified between **HT and TSP1** (thick red edge), suggesting a possible inverse clinical or biological relationship. These findings warrant further mechanistic and statistical validation.

A key global network property—**sparsity**—was also calculated. Sparsity is defined as one minus the ratio of observed edges to the maximum possible number of edges in the network (Newman, 2010). For a network of 8 variables, the total possible number of edges is 28. In

this study, **27 edges** were observed, yielding a **sparsity value of 0.036**, which indicates a **highly dense and interconnected structure**. A low sparsity value reflects a tightly structured system, potentially indicative of strong underlying clinical or biological associations.

Table 1 summarizes the key topological features of the network constructed for mPCa patients, including:

- Total number of nodes: 8
- Observed edges: 27
- Maximum possible edges: 28
- Calculated sparsity: 0.036

This high-density configuration supports the hypothesis that systemic conditions such as HT and DM, along with molecular biomarkers, interact within a cohesive and highly integrated clinical network in metastatic prostate cancer.

Table 1. *Descriptive Metrics of the Serum Biomarker–Clinical Variable Interaction Network in Metastatic Prostate Cancer*

	Number of nodes	Number of non-zero edges	Sparsity
Metastasis	8	27 / 28	0.036

Note: Number of nodes indicates the total number of variables included in the network (serum biomarkers and clinical parameters). Observed edges represent the number of non-zero partial correlations between variables, controlling for all other variables. Maximum possible edges are calculated using the formula n(n−1)/2 for an undirected network, where n is the number of nodes. Sparsity is defined as 1 − (observed edges / maximum possible edges); lower sparsity values indicate denser network structures. In this study, a sparsity value of 0.036 for a network of 8 variables suggests a highly connected and meaningful interaction structure.

Four types of centrality measures were used to determine the centrality levels of the variables. Structural features of the serum biomarker network in metastatic prostate cancer. Includes total number of nodes (variables), number of partial correlation edges, and calculated sparsity value. Sparsity is defined as 1 – (number of observed edges / number of possible edges); lower values indicate a denser network. These measures include degree centrality, closeness centrality, influence, and betweenness centrality. The centrality values for each variable are presented in Table 2. Centrality measures of serum biomarkers and clinical variables in the metastatic prostate cancer network. Metrics include: Betweenness centrality: Node's role as a bridge between other nodes. Closeness centrality: Average distance to all other nodes (higher = more central). Degree: Number of direct connections. Expected influence (eigenvector centrality): Influence of a node based on its connections to other influential nodes. Note: Some centrality values are normalized; negative values may reflect mean-centered or z-score transformed metrics and should be interpreted accordingly.

Table 2. *Graph-theoretical centrality metrics of the network nodes (standardized values using z-scores)*

	Metastasis			
Variable	Betweenness	Closeness	Degree	Expected influence
DM	0.114	0.769	0.833	-0.343
HIF-1α	-0.800	-1.414	-1.069	0.059
HT	0.114	1.018	0.967	1.980
NRP1	-0.800	-0.442	-0.403	0.844
PSA	1.486	1.210	1.200	-0.033
Smoking	-0.800	-1.163	-1.462	-0.825
TSP1	1.486	0.436	0.461	-1.117
Age at diagnosis	-0.800	-0.414	-0.527	-0.565

Note: Betweenness, closeness, degree, and expected influence (eigenvector centrality) metrics were used to evaluate the structural importance of each variable within the metastatic prostate cancer network. Betweenness centrality reflects how often a node acts as a bridge between other nodes; closeness centrality indicates the inverse of the average shortest distance from a node to all others; degree centrality represents the number of direct

connections a node has; and expected influence measures the relative influence of a node based on its connections to other influential nodes. Negative values result from z-score normalization and indicate variables with below-average centrality relative to the network mean.

In the current analysis, betweenness centrality values were derived using a standardized metric, specifically z-score normalization, in order to enhance interpretability and comparability across variables with differing scales. This approach may yield negative values, which reflect below-average centrality relative to the network's overall distribution. While raw betweenness values are inherently non-negative, normalization allows for more nuanced interpretation of relative node influence. Therefore, the presence of negative values in Table 2 should not be misinterpreted as computational error. This normalization procedure has been noted in the text and reflected in the updated table caption for clarity.

As shown in Table 2, four centrality measures—degree, closeness, betweenness, and influence (eigenvector centrality)—were used to evaluate the role of each variable within the metastatic network. Each measure reflects a different aspect of a variable's structural importance. Nodes with high betweenness centrality act as bridges between clusters of nodes that are otherwise not directly connected and are considered to have the potential to control the flow of information within the network. In the metastatic group, PSA and TSP1 exhibited the highest betweenness centrality values. Therefore, they can be regarded as relatively more influential. Accordingly, PSA and TSP1 appear to function as key active variables within the network, acting as bridges among otherwise unconnected variables (see Figure 1). As a result, it can be concluded that the overall network is composed of highly interrelated variables, with PSA and TSP1 representing the two most critical variables.

Closeness centrality indicates the proximity of one variable to all others. It is calculated as the reciprocal of the sum of the shortest distances to all other variables. A high closeness value reflects how quickly a node can interact with others, and also provides a measure of a node's independence or influence. According to the analysis, the variables with the highest closeness values are PSA and HT, while the lowest values are seen in HIF-1α and Smoking. This implies that PSA and HT are the most easily reachable nodes in the network, and reaching these nodes may also facilitate access to other nodes, suggesting their strategic position.

Degree centrality, which is calculated based on the number of direct connections, was highest for PSA with a value of 1.200, while the lowest value was observed for Smoking, with −1.462.

When eigenvector centrality (influence) values were examined, the variables with the highest influence were HT and NRP1, respectively. This indicates that HT and NRP1 exert a relatively higher influence over the rest of the network variables.

The clustering coefficient measures local group cohesion and is defined as the proportion of a node's neighbors that are also connected to each other. It reveals the strength of association among a node's immediate neighbors. This coefficient can also be interpreted as an indicator of network cohesiveness, measuring the density of triadic relationships. A low clustering coefficient suggests a higher level of interconnectivity among variables, whereas a higher value reflects more sparse or isolated connections. The clustering coefficients for each variable are presented in Table 3. Clustering coefficient values of individual variables across four algorithmic estimators: Barrat, Onnela, Watts–Strogatz (WS), and Zhang methods. The clustering coefficient quantifies the degree to which a node's neighbors are interconnected, reflecting local

network cohesion. Positive values indicate a tightly knit local structure, while negative values may result from normalization techniques.

Table 3. *Clustering Coefficients of Variables in the Metastatic Prostate Cancer Network*

Variable	Barrat	Onnela	WS	Zhang
DM	-0.906	0.626	-0.540	-0.062
HIF-1α	-0.305	-1.016	-0.540	-0.181
HT	-0.843	0.755	-0.540	-0.336
NRP1	1.360	0.069	0.620	1.813
PSA	-0.896	1.043	-0.540	-0.745
Smoking	0.765	-1.920	-0.540	-0.902
TSP1	-0.536	0.474	-0.540	-0.821
Age at diagnosis	1.360	-0.031	1.620	1.234

Note: Clustering coefficients were calculated using four algorithms—Barrat, Onnela, Watts–Strogatz (WS), and Zhang—to quantify the degree of interconnection among each node's neighbors, reflecting local network cohesion. Positive values indicate a tightly knit local structure, while negative values may result from normalization and indicate below-average local connectivity. Higher clustering coefficient values suggest a denser pattern of interactions among the variable's immediate neighbors.

Upon examining the clustering coefficients presented in Table 3, it was observed that NRP1 had the highest local density among its neighboring variables when all four coefficients were evaluated together. In contrast, smoking had the lowest clustering coefficient. These findings are consistent with the influence values reported in Table 2 and indicate that both variables exert substantial effects on other nodes in the network.

In this study, relationships among the variables HIF-1α, TSP1, NRP1, PSA, Age at Diagnosis, DM, HT, and Smoking in metastatic patients were evaluated within a network structure. The analysis was conducted using graph theory-based metrics such as centrality (degree, closeness, and betweenness), connection density, and sparsity.

Centrality measures are important for understanding the role of a variable within the network—specifically, its influence on information flow (24). Degree centrality refers to the total number of connections a node has; closeness centrality indicates how easily a node can be reached from other nodes; and betweenness centrality measures the extent to which a node lies on the shortest paths between other nodes. In this study, HT and DM emerged as variables occupying central positions in the network. In particular, HT showed strong connections in both positive and negative directions.

Discussion

In this study, the relationships between key serum biomarkers (HIF-1α, TSP1, NRP1, and PSA) and clinical variables (age at diagnosis, DM, HT, and smoking) in patients with mPCa were evaluated using a network-based approach. Through the application of graph theory-based metrics such as centrality measures, clustering coefficients, and sparsity, a systems-level analysis was conducted to explore how these variables interact within a biological context. The findings offer important insights into prostate cancer progression, biomarker evaluation, and clinical management.

One of the most notable findings is the central placement of PSA and TSP1 within the network, as evidenced by their high betweenness centrality scores. Nodes with elevated betweenness centrality function as critical bridges connecting otherwise unlinked clusters, thereby contributing significantly to the structural cohesion of the network. The prominent role of PSA aligns with its established status as a primary biomarker in the clinical screening of prostate cancer (1, 26). However, the comparable centrality of TSP1 highlights its potential significance in prostate cancer progression, particularly given its well-documented anti-angiogenic and immunomodulatory properties. This finding underscores the need for increased attention to

TSP1 in both research and clinical settings (1, 27, 28).

Previous studies have reported a downregulation of TSP1 expression in advanced prostate cancer, which correlates with increased vascularization and poor prognosis(29). The negative correlation identified in our network analysis supports the biological notion that as PSA levels increase, indicative of tumor progression, TSP1 expression tends to decline**,** reflecting a loss of anti-angiogenic control**.** This pattern suggests a reciprocal regulatory relationship**,** where rising PSA may reflect a microenvironment conducive to tumor growth, while falling TSP1 levels indicate diminished suppressive mechanisms (26). In support of our findings, TSP1 expression is significantly reduced in castration-resistant and neuroendocrine-transformed prostate cancer tissues, showing a negative correlation with markers of progression and aggressiveness This reduction often coincides with elevated PSA signaling, as androgen receptor (AR) activation indirectly promotes PSA expression while suppressing TSP1 transcription—consistent with observations that androgens repress TSP1, and that TSP1 levels rise following androgen deprivation(30, 31) .Functionally, the loss of TSP1 not only diminishes its anti-angiogenic suppression but may also facilitate VEGF-mediated neovascularization, supporting tumor growth and metastasis. High PSA alongside low TSP1 may therefore reflect a shift in the tumor microenvironment favoring angiogenesis and invasiveness (5, 6). The strong negative association observed between HT and TSP1 (indicated by a thick red edge) may suggest a biologically inverse regulatory mechanism. However, this relationship requires further biological validation. Notably, previous studies have also reported increased TSP1 levels in patients with treated HT, supporting the possibility of a mechanistic link (32).

Another key finding of the study is the central positioning of HT and DM within the network, as indicated by their high closeness and degree centrality values. This suggests that systemic diseases are not merely coexisting comorbidities, but also active contributors that shape tumor biology. HT and DM are known to influence the tumor microenvironment by promoting processes such as chronic inflammation, angiogenesis, and metabolic reprogramming(33, 34). TSP1, which typically inhibits angiogenesis and suppresses tumor development, is often downregulated in various tissues under conditions of elevated blood glucose levels (35, 36). In this study, the prominent positioning of these two variables within the network underscores the importance of considering them jointly in biomarker-based risk stratification and personalized clinical decision support systems.

NRP1 was identified in this study as the variable with the highest clustering coefficient. This finding indicates that NRP1 is highly interactive with its neighboring variables and functions as a local regulatory hub within the network structure. Studies have revealed that NRP1 plays a significant oncogenic role in PCa. It has been observed that NRP1 is highly expressed in PCa and positively associated with poor clinicopathological factors (12, 13, 37)

Furthermore, existing literature emphasizes the role of NRP1 as a pivotal regulatory molecule not only in angiogenesis but also in key oncogenic processes such as epithelial-mesenchymal transition (EMT), cellular migration and invasion, and the maintenance of stem cell-like phenotypes (38). From a methodological perspective, the calculated sparsity value of 0.036 reflects a highly interconnected network, indicating robust associations among variables. This result underscores the utility of network analysis as a complementary methodology to conventional statistical and machine learning approaches. In particular, for datasets charac-

terized by multicollinearity, variable synergy, or non-linear interactions, network-based methods provide a powerful framework for revealing biologically meaningful patterns that may be overlooked by traditional analyses.

Limitation

This study has several limitations that should be acknowledged. First, its single-center design may limit the generalizability of the findings to broader patient populations. Second, although network analysis provides valuable insights into the structural relationships between biomarkers and clinical parameters, it does not establish causality. The observed correlations may have been influenced by unmeasured confounding factors or secondary biological processes. The direction and biological significance of the observed positive and negative correlations require further mechanistic investigation. For instance, the negative association between HT and TSP1, or the dense connectivity pattern surrounding DM, may result not from direct effects but from secondary processes, compensatory responses, or confounding factors. It is therefore recommended that these relationships be validated using experimental models or longitudinal omics-based datasets. Third, statistical significance testing for network measures (e.g., permutation or bootstrap methods) was not performed, and such tests could have enhanced the robustness and reproducibility of the results. Finally, the analysis was restricted to serum biomarkers and selected clinical variables; the inclusion of additional molecular markers, genetic data, or longitudinal follow-up would allow for a more comprehensive understanding of biomarker interactions in metastatic prostate cancer.

Conclusion

This study demonstrates that network analysis is a powerful tool for understanding the interactions between clinical and molecular variables in the context of metastatic prostate cancer. The findings underscore the importance of integrating systemic conditions such as HT and DM into biomarker-based models and highlight molecules like TSP1 and NRP1 as promising candidates for further in-depth investigation. Future research is encouraged to validate the network structures identified here using experimental, longitudinal, and statistically robust data sources.

However, further discussion is warranted regarding the biological mechanisms underlying the direction and causality of the observed positive and negative associations. Additionally, it should be noted that the statistical significance of the network analysis findings—such as through permutation testing or bootstrap methods—was not reported, which represents a methodological limitation of the study. Future studies should incorporate permutation-based statistical testing or bootstrapping to validate the stability and robustness of network centrality metrics, thereby minimizing the risk of spurious associations.

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