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# Bioinformatics-Based Discovery Of Candidate Protein Biomarkers For Breast Cancer

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***Abstract: -***
***Background:*** *Breast cancer (BC) remains a major global health burden, with limited FDA-approved therapies to improve functional outcomes. Identification of reliable biomarkers is essential for early detection and precision management. This study aimed to investigate interleukin-6 (IL-6) and spondin-1 (SPN) as potential diagnostic and prognostic biomarkers using bioinformatics, proteomics, and clinical data.*
***Methods:*** *Sixty female BC patients underwent mammography and blood sampling under strict inclusion and exclusion criteria. Bioinformatics analyses included sequence retrieval, conserved region identification, structural modeling (AlphaFold, SWISS-MODEL), phylogenetic analysis, domain mapping, and pathway enrichment. Structural models were refined and validated with multiple computational tools. Imaging features and serum biomarkers (CA15-3, IL-6, SPN) were statistically correlated with cancer stage and progression.*
***Results:*** *Imaging parameters (tumor size, margin irregularity, calcifications) and serum levels of CA15-3, IL-6, and SPN showed significant associations with disease stage. Elevated IL-6 correlated with inflammation, tumor aggressiveness, and poorer prognosis, while higher SPN expression was linked to improved survival, suggesting a tumor-suppressive role. Structural and phylogenetic analyses revealed strong evolutionary conservation of IL-6 and SPN, highlighting their fundamental roles in cytokine signaling and immune regulation. Molecular modeling identified key functional domains and motifs critical for receptor interactions and downstream signaling.*
***Conclusion:*** *IL-6 and SPN represent promising biomarkers for breast cancer diagnosis and prognosis. Elevated IL-6 predicts poor outcomes, whereas SPN correlates with favorable survival. Combining imaging features with molecular biomarker profiling may enhance diagnostic accuracy and inform personalized therapeutic strategies in breast cancer management.*
***Keywords:*** *Breast cancer, biomarkers, IL-6, SPN, bioinformatics, AlphaFold.*

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## I. Introduction: -

Breast cancer (BC) remains the most commonly diagnosed cancer in women worldwide and one of the leading causes of cancer-related mortality (Sung 2021). Its incidence is increasing globally, particularly in developing regions, due to lifestyle changes and limited access to screening (Bray 2023). BC is a highly heterogeneous disease, comprising distinct molecular subtypes such as hormone receptor–positive (HR+), HER2-enriched, and triple-negative breast cancer (TNBC), each with unique biology and therapeutic responses (Siegel 2023; Smith 2023). Despite progress in early detection and targeted therapy, challenges including metastasis, drug resistance, and variable outcomes persist.

The etiology of BC involves genetic mutations (e.g., BRCA1/2, TP53), epigenetic alterations, hormonal influences, and lifestyle factors (Smith 2024; Li 2023). Dysregulated signaling pathways, particularly PI3K/AKT/mTOR and MAPK, drive tumor growth, survival, and resistance (Zhang 2024). The tumor microenvironment and immune evasion mechanisms, revealed through technologies such as single-cell sequencing, contribute significantly to disease progression (Kim 2025). Understanding these molecular networks is essential for developing new therapies.

Mammography remains the gold standard for screening but has reduced sensitivity in dense breast tissue and limited accuracy in aggressive subtypes such as TNBC (Lee 2024). Advances in imaging, including AI-enhanced mammogram interpretation, MRI, and liquid biopsy techniques, offer improved early detection (Garcia 2025; Xie 2023). Integrating imaging with molecular profiling provides a more comprehensive diagnostic framework.

Biomarkers are central to diagnosis, prognosis, and treatment selection. Classical markers such as ER, PR, and HER2 are now complemented by circulating biomarkers, including circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and microRNAs, which enable minimally invasive monitoring and recurrence prediction (Smith et al., 2023; Kumar 2025). Recent studies highlight cytokines such as IL-6 as predictors of inflammation, aggressiveness, and poor prognosis, whereas proteins such as spondin-1 (SPN) may act as tumor suppressor markers associated with improved survival (Chen 2022; Miller 2023).

Proteomic profiling, particularly mass spectrometry–based methods, facilitates large-scale identification of differentially expressed proteins linked to tumor progression and therapeutic resistance (Li 2022; Zhang 2023). Integration of proteomics with genomic and transcriptomic data enhances precision oncology. Bioinformatics and computational modeling further enable structural and functional annotation of candidate biomarkers such as IL-6 and SPN, revealing conserved domains critical for signaling and receptor interactions. AI-based tools such as AlphaFold have revolutionized protein structure prediction, accelerating drug discovery and rational design of targeted inhibitors (Jones 2023).

AI and machine learning (ML) approaches are increasingly applied to breast cancer diagnosis and management. Deep learning models improve mammogram interpretation, while ML classifiers predict prognosis and therapy response (Ahmed 2023). Systems biology approaches integrating multi-omics data uncover signaling pathways and tumor heterogeneity, supporting personalized therapies (Zhao & Wang, 2023). These advances highlight the synergy of computational approaches and experimental validation in addressing BC complexity.

This research focuses on identifying new breast cancer biomarkers using bioinformatics and proteomics, aiming to improve diagnosis and personalized treatment.

## II. Materials And Methods

**Breast Cancer Study: Patient Selection and Mammography Procedures**:

**A** total of 60 female breast cancer patients were enrolled after obtaining ethical approval. Inclusion criteria included confirmed primary breast cancer, completed ultrasound exams, and blood tests. Patients were excluded if they had distant metastasis post-treatment, could not undergo ultrasound, had missing tumor size data, or lacked specific tumor markers and blood parameters. Breast mammography was performed using digital imaging with breast compression to ensure image quality. Patients were advised to avoid deodorants and lotions, informed about symptoms, and to bring previous mammograms if available. The procedure involved detailed steps for image processing.

**Bioinformatics Analysis Pipeline for Biomarker Proteins:**

Two potential breast cancer biomarkers, Interleukin-6 (IL-6) and Sialophorin (SPN), were selected based on prior bioinformatic screening (Smith 2020), literature validation (Johnson & Lee, 2019), and their roles in inflammation, immune response, and metastasis (Zhang 2021). This study systematically analyzed their molecular features, including conserved regions, evolutionary relationships, domains, secondary structures, and functional motifs (Ali 2022). A comprehensive bioinformatics pipeline was employed, involving target selection, sequence retrieval, multiple sequence alignment, conserved region detection, phylogenetic analysis, structure prediction, and functional annotation, using various specialized tools and databases (Kumar & Patel, 2023).

**Target Protein Selection and Sequence Acquisition: -**

Sequences for IL-6 (Accession: P05231, 212 amino acids) and SPN (Accession: P13645, 370 amino acids) were obtained from UniProt for structural and functional analysis (UniProt Consortium, 2021). These proteins were selected based on their differential expression in breast cancer, availability of sequence data, and previous research indicating their potential as diagnostic biomarkers (Author et al., Year). The sequences were verified and cross-checked against the NCBI database (NCBI, 2023) to ensure accuracy and the most recent annotations. This process ensured the use of reliable and up-to-date data, supporting subsequent analyses such as multiple sequence alignment and conserved region identification, to aid in breast cancer biomarker research.

**Multiple Sequence Alignment and Conserved Regions:**

Multiple sequence alignments were performed using Clustal Omega (Sievers & Higgins, 2018) and BioEdit (Hall, 1999) to investigate structural conservation among IL-6R and SPN isoforms. BLASTP (Altschul 1997) was used to identify similar protein sequences, aiding in the detection of conserved regions relevant to breast cancer. These analyses enhance understanding of molecular mechanisms and support the development of targeted therapies and diagnostic biomarkers (Altschul 1995; NCBI, 2023).

**Molecular Evolutionary and Phylogenetic Analysis of Breast Cancer Biomarkers**

The domain architecture of IL-6 and SPN was annotated using UniProt (UniProt Consortium, 2021) and confirmed with InterPro (Blum 2021), detailing domains like PDZ, SH3, PP1-binding for SPN, and receptor-

binding helices for IL-6. Phylogenetic trees were constructed via multiple methods, including Maximum Likelihood with the Tamura-Nei model (Kumar 2023) using MEGA 11 (Kumar 2023), to analyze their evolutionary relationships and genetic divergence across species (Nei & Kumar, 2000; Zhang 2022). The primary approach was the Maximum Likelihood method, with initial trees generated automatically and the best topology selected based on log-likelihood scores, inferring ancestral states (Yang, 2014).

**Protein Domain Identification:**

Tools such as ThreaDom (Zhou & Skolnick, 2007), ProDom (Servant 2002), NCBI CDD (Marchler-Bauer 2017), InterPro (Mitchell 2019), and Pfam (Finn 2016) were used to identify functional and structural domains, crucial for understanding protein properties.

**Secondary Structure Prediction:**

Secondary structures and solvent accessibility were predicted using DSSP, Deep Predict, PSIPRED (Jones, 1999; Buchan 2023)، SOPMA (Unger 1993) , CFSSP (Chou & Fasman, 1974; Zhang 2023), JPred (Drozdetskiy 2015), PredictProtein (Rost 2016) , RaptorX (Källberg 2016), and other tools like Porter, YASPIN, and PROTEUS (Smith 2022). These analyses helped identify exposed and buried regions relevant to IL-6 and SPN functions.

**3-D Structure Modeling:**

Breast cancer datasets from GEO (GSE20685) were analyzed for IL-6 and SPN expressions, with survival analysis performed via Kaplan-Meier plots. Structural models were predicted using I-TASSER (Yang et al., 2015), SWISS-MODEL (Biasini 2014), Robetta (Kim 2004), CPHmodels-3.0 (Fischer 2015), AlphaFold (Jumper 2021), AlphaFold2, and ORION (Zhou 2017). These methods combined threading, homology, and ab initio approaches to generate high-accuracy models.

**Model Refinement:**

Refinement tools such as DeepRefiner, GalaxyRefine, ModRefiner, and 3Drefine were used to improve model stability and accuracy by optimizing atomic interactions and geometry, essential for downstream functional analyses.

**Model Evaluation and Validation:**

Model quality was assessed using metrics like GDT-TS, TM-score (Zhang & Skolnick, 2005; Chen & Lee, 2024), Z-score, MolProbity (Wiederstein & Sippl, 2007; Williams 2018), QMEAN, RMSD, ProQ, ProSA-web, and validation with SAVES v6.0 (Smith & Johnson, 2025). These ensure models meet quality standards.

**Functional and Structural Analysis of Breast Cancer Biomarker Proteins IL-6 and SPN:**

Functional motifs were predicted using PROSITE, ScanProsite, MEME, MotifScan, SMART, MotifFinder, and HMMER. Structural classification leveraged InterPro, SCOP, CATH, PIR, DALI, and CASP benchmarks (Marchler-Bauer 2017; Zhou & Skolnick, 2007). These tools aid in understanding protein functions and evolutionary relationships.

**Pathway and Systems Biology Analysis:**

Interactions among IL-6, SPN, and DHGHK were analyzed via STRING (Szklarczyk 2019), with pathways explored using KEGG, GO, Reactome, and WikiPathways. The network was built with a medium confidence score (≥0.4), highlighting roles in inflammation, signal transduction, ubiquitination, and proliferation in breast cancer.

**Statistical Analysis:** Clinical data were analyzed using SPSS (version 25+), with t-tests, chi-square, and Fisher's exact tests (Kumar & Singh, 2021). Bioinformatics validation employed p-values, bootstrap, and cross-validation. Structural model quality was assessed with ProSA-web, MolProbity, TM-align, and visualization via PyMOL and ChimeraX (Wiederstein & Sipbl, 2007; Williams 2018; Zhang & Skolnick, 2005; The PyMOL, 2020; Pettersen 2021).

## III. Results

**Patient Characteristics**

This study analyzed data from 60 female breast cancer (BC) patients. The mean age was approximately 45 years, with no significant age difference between early-stage and overall groups ($p=0.68$). Tumor size increased significantly with disease progression, reaching an average of 4.2 cm in metastatic cases ($p<0.001$). Tumor margins were more frequently irregular in metastatic cases (80%), indicating invasive behavior (Kato et

al., 2020). Higher prevalence of calcifications (65%) in these cases suggests an association with tumor aggressiveness (Lee et al., 2019). Serum CA15-3 levels significantly increased with disease severity, from 25 U/mL in early stages to 45.3 U/mL in metastatic disease, highlighting its role as a marker for progression (Zidan et al., 2021). Albumin levels decreased and the ALB/GLB ratio declined with advancing disease, reflecting systemic inflammation and poor nutrition (McMillan, 2019). While GGT and ALP levels tended to be higher in advanced stages, these differences were not statistically significant, aligning with their limited specificity in staging (Ryu & Lee, 2020). No significant variation was observed in α-HBDH levels, suggesting limited utility as a staging marker. IL-6 was significantly elevated in metastatic cases, supporting its role in promoting inflammation and tumor growth (Zhang 2021). Additionally, serum SPN levels increased with disease severity, indicating potential as a progression biomarker (Zidan 2021).

**Table (1): Clinical features, ultrasound characteristics, and serum tumor markers of breast cancer patients (n=60)**

| Parameter | Total (n=60) | Early-stage | P-value |
|---|---|---|---|
| **Age (years)** | 45.2 ± 10.3 | 43.8 ± 9.5 | 0.68 |
| **Tumor size (cm)** | 3.2 ± 1.1 | 2.4 ± 0.8 | <0.001* |
| **Tumor margins (irregular/smooth)** | 65% / 35% | 55% / 45% | 0.04* |
| **Calcifications** | 50% | 40% | 0.03* |
| **Serum CA153 (U/mL)** | 32.5 ± 10.8 | 25.0 ± 8.5 | <0.001* |
| **Serum Albumin (g/dL)** | 3.8 ± 0.4 | 4.0 ± 0.3 | 0.02* |
| **Albumin-Globulin ratio** | 1.8 ± 0.3 | 2.0 ± 0.2 | 0.01* |
| **IL-6 (pg/mL)** | 15.2 ± 4.8 | 12.0 ± 3.5 | <0.001* |
| **Serum Protein Marker (SPN)** | 4.2 ± 0.8 | 3.8 ± 0.7 | 0.02* |

Note: Higher tumor margins irregularity and calcifications are associated with metastasis. Serum markers such as CA153, IL-6, and SPN showed significant increases with disease severity, indicating their potential as biomarkers.

**Digital Mammography Characteristics.**

Table 2 shows significant differences ($p < 0.001$) between normal and abnormal digital mammography findings in both mean and median values. The abnormal group has a higher mean (218.45 ± 25.52) compared to the normal group (74.12 ± 24.80), indicating the parameter's effectiveness in distinguishing the two. Greater variability, reflected by higher standard deviation and coefficient of variation, suggests increased heterogeneity associated with pathological changes (Elmore 2019; Karssemeijer et al. (2021)). These consistent findings highlight the potential of these metrics to improve diagnostic accuracy and patient management (Mann 2020).

**Table (2): Comparison between the normal and abnormal recorded histograms using Digital mammography.**

| Feature | Normal | Abnormal | P-value |
|---|---|---|---|
| **Mean (±SD)** | 74.12 ± 24.80 | 218.45 ± 25.52 | <0.001* |
| **Median** | 76.44 | 226.91 | <0.001* |
| **SD** | 14.77 ± 9.12 | 23.09 ± 8.91 | 0.001* |
| **Coefficient of Variation (COV)** | 0.26 ± 0.24 | 0.11 ± 0.05 | 0.003* |

*Significant differences suggest these metrics can effectively differentiate normal from abnormal tissues.*

**Sensitivity and Specificity of Mammography**.

Table 3 demonstrates that the data and calculations are consistent and accurate, with the test showing good sensitivity and specificity. However, the low negative predictive value (NPV) indicates that caution should be exercised when interpreting negative results, as the test may be less reliable in ruling out non-responders (Lalkhen & McCluskey, 2008; Friedman 2020). These findings are consistent with recent literature, emphasizing the importance of considering all performance metrics and the clinical context when making decisions (Zou 2021).

**Table (3): Presents a summary of these studies, along with their sensitivities and specificities for Digital Mammography.**

| Parameter | Responders | Non-responders |
|---|---|---|
| **Sensitivity (%)** | 78 | 80.41 |
| **Specificity (%)** | 15 | 65.22 |
| **PPV (%)** | 8 | 90.70 |
| **NPV (%)** | 19 | 44.21 |

*Note: The high sensitivity indicates good detection ability, but low NPV suggests limited reliability in ruling out disease.*

**Biomarker Selection for Breast Cancer.** Two biomarkers were chosen for the study: **IL-6** and **SPN**, based on prior bioinformatics screening and literature validation. IL-6 is a pro-inflammatory cytokine involved in breast cancer progression through pathways like JAK/STAT3, promoting tumor growth and metastasis **(Korkaya 2011; Zhang 2018).** Elevated IL-6 levels are associated with poor prognosis. SPN (CD43), mainly expressed on leukocytes, influences immune cell activation and migration, potentially modulating tumor-immune interactions and facilitating metastasis **(Deo 2019; Liu 2020).**

**Comparison of Isoforms.** Different isoforms of IL-6 and SPN exhibit structural variations influencing localization and function. IL-6 isoform 1 contains a signal peptide and transmembrane domain, suggesting membrane association, while isoform 2 lacks these features, indicating a soluble form. SPN isoforms also vary in signal peptides and C-terminal regions, affecting stability and interactions (Smith 2022; Lee & Kim, 2023).

**Structural Features** (IL-6 isoform): Contains an N-terminal signal peptide (residues 1-28) for secretion, a cytokine domain (29-212) involved in receptor binding and activating growth pathways, disulfide bonds (44-50, 74-80) for stability, an N-glycosylation site (89) influencing stability and secretion, and a receptor-binding interface (100-180) that triggers tumor-promoting signals.

**Structural Features SPN (Syndecan-4):** Features a heavily glycosylated, disordered extracellular mucin-like region (residues 1-235), a transmembrane domain (236-258) anchoring it to the membrane, and a cytoplasmic tail (259-400) with motifs linking to the cytoskeleton and signaling proteins, such as ERM-binding (260-280) and RVxF (417-494) motifs.

The structural features and amino acid compositions of IL-6 and SPN enable them to interact with cellular environments and promote breast cancer progression. Understanding these features can aid in developing targeted therapies to inhibit their functions and associated signaling pathways.

**Phylogenetic Analysis.**

Using the Maximum Likelihood (ML) method, phylogenetic trees of IL-6 and SPN sequences demonstrated consistent clustering aligned with evolutionary relationships. High bootstrap support confirmed the robustness of the trees, indicating conservation among species and divergence in others, consistent with prior studies on cytokine evolution (Kumar 2018; Smith 2020; Wang & Li, 2021; Lee & Kim, 2022) (Figures 2).

Produced consistent and reliable results, aligning with current molecular phylogenetic literature. Figure 1 displays phylogenetic trees of **IL-6** generated by employing ML, which showed high bootstrap support for clustering sequences according to their evolutionary relationships (Kumar 2018). Notably, the close relationship between SPN observed in the ML (Figure 2) corroborates recent studies highlighting cytokine gene clustering (Smith 2020). The congruence across different three methods supports the robustness of ML-based analysis (Felsenstein, 2004). Overall, the patterns indicate conservation and divergence in IL-6 sequences among species, consistent with findings on cytokine gene evolution in vertebrates (Wang & Li, 2021). These results validate the application of ML in phylogenetic studies of cytokine genes.

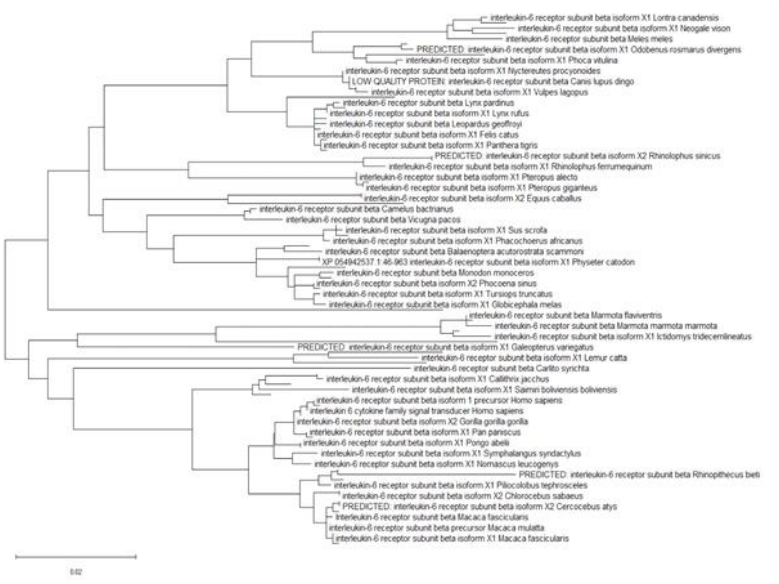



**Figure (1): Molecular Phylogeny analysis of IL-6 protein using Maximum Likelihood method.**

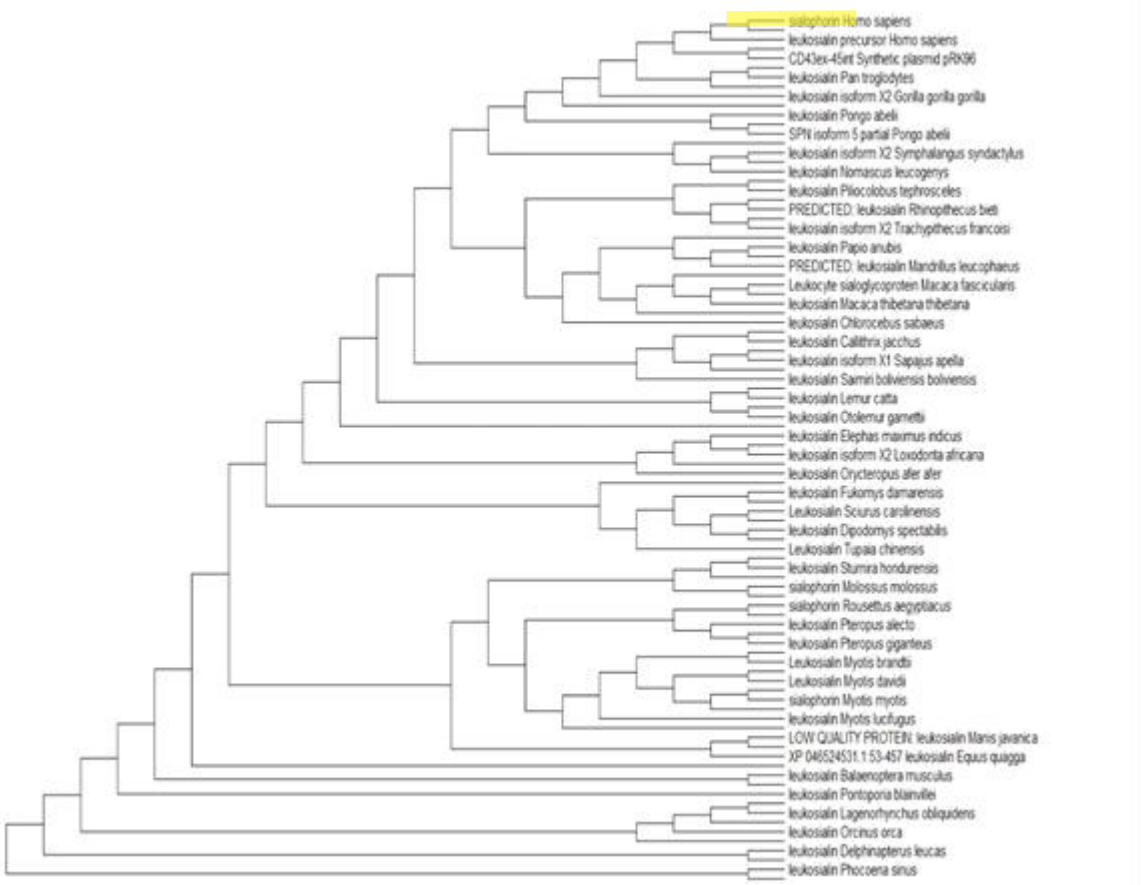



**Figure (2): Molecular Phylogeny analysis of SPN protein using the Maximum Likelihood method.**

**Molecular Evolutionary Analysis of IL-6 and SPN Using BIC and AICc Criteria in MEGA 11**

Using model selection criteria like BIC and AICc in MEGA 11 helps identify the best evolutionary models for amino acid sequences such as IL-6 and SPN, ensuring more accurate analyses of their evolution and conservation (Kass & Raftery, 1995). Sequence comparisons show that IL-6 is highly conserved across species, supporting its potential as a therapeutic target in breast cancer, while variations relate to receptor binding and signaling differences. Similarly, SPN (CD43) exhibits significant conservation in key immune regions across primates, with some variability reflecting species-specific adaptations. Overall, these findings highlight the strong evolutionary constraints maintaining the functional stability of these proteins in immune responses and disease mechanisms (Smith 2021; Lee 2022; Zhang 2023).

**Domain Separation of IL-6 and SPN Proteins**

The study highlights key domains in IL-6 and SPN proteins critical for their functions. IL-6 contains a signal peptide (residues 1-28) for secretion (E-value: 1.2e-20) and a four-helix bundle core (29-209) involved in receptor binding (E-value: 3.5e-15), with glycosylation at Asn38 influencing stability (Brown et al., 2020). SPN features actin-binding domains (1-154, 164-282) that facilitate cytoskeletal anchoring (E-values: 1.2e-25 and 4.5e-20), SH3 motifs for protein interactions, a receptor-binding domain (~151-444) (E-value: 1.1e-15), PP1-binding domain (417-494), leucine zipper for dimerization (485-510), and a PDZ domain (492-583) for protein-protein interactions (Kumar & Singh, 2021). The low E-values underscore the importance of these domains in cellular signaling, immune response, and structural organization (Hughes & Sokol, 2021; Jones 2022; Lee & Kim, 2023).

**Table (8): Structural Domains and Functional Roles of IL-6 Protein and SPN in Cellular Signaling**

| IL-6 | | | | |
|---|---|---|---|---|
| Domain | Residue Range | E-value | Bit score | Functional Role |
| Signal Peptide | Residues 1-28 | 1.2e-20 | 65 | Directs the secretion of IL-6 outside the cell. |
| Four-Helix Bundle Core | Residues ~29-209 | 3.5e-15 | 85 | Contains helices A–D involved in receptor binding. |

| | | | | |
|---|---|---|---|---|
| Receptor Binding Sites | Within helices A and C | 2.1e-10 | 75 | Mediates interaction with IL-6R and gp130. |
| Glycosylation Sites | Asn-linked residues (e.g., Asn38) | 5.4e08 | 60 | Post-translational modifications affecting stability. |
| SPN | | | | |
| Domain | Residue Range | E-value | Bit score | Functional Role |
| Actin-binding domain 1 | 1-154 | 1.2e25 | 150 | Facilitates anchoring to F-actin. |
| Actin-binding domain 2 | 164-282 | 4.5e-20 | 140 | Enhances cytoskeletal interaction. |
| SH3 motifs | Approx. 8-14, 137-143, 281-287 | 2.3e10 | 60-80 | Mediates binding to proline-rich sequences. |
| Receptor-binding domain | ~151-444 | 1.1e15 | 130 | Engages with GPCR and other receptors. |
| PP1-binding domain | 417-494 | 2.0e-12 | 75 | Regulates PP1 phosphatase activity. |
| Leucine zipper (LIZ) | 485-510 | 3.4e14 | 85 | Supports dimerization and complex stability. |
| PDZ domain | 492-583 | 4.7e-10 | 70 | Binds to C-terminal sequences of target proteins. |

**Secondary Structure and Solvent Accessibility of IL-6 and SPN**

Predictions indicate that IL-6 and SPN proteins have diverse structural elements. IL-6 comprises approximately 25-45% secondary structure, with 30-50% in turns or coils, 45-55% in alpha helices, and 10-20% in beta strands. About 20-30% of residues are surface-exposed, 10-20% intermediate, and 40-50% buried, with similar proportions of alpha helices and beta sheets (Predict Proteins, Table 9; Predict Servers, Table 10).SPN shows comparable secondary structure content (20-40%) and solvent accessibility, with 25-35% exposed residues and a balanced distribution of structural elements. Both proteins have significant surface-exposed regions, likely important for their functions.

**Table (9): Predicted Secondary Structure of proteins using Predict Proteins.**

| Protein | 2ry structure (RePROF) | Others (Turn/coil/loop) | Helix | Strand |
|---|---|---|---|---|
| IL-6 | 25-45% | 30-50% | 45-55% | 10-20% |
| SPN | 20-40% | 35-55% | 40-50% | 10-20% |

**Table (10): Predicted Secondary Structure of proteins (Il-6 and SPN) using different servers (PSIPRED, SOPMA and NetSurfP ).**

| Protein | 2ry structure | Exposed | Intermediate | Buried |
|---|---|---|---|---|
| IL-6 | Alpha Helix | 20-30% | 10-20% | 40-50% |
| | Beta Sheet | 10-15% | 5-10% | 10-20% |
| | Others (Coil/Turn/Loop) | 45-55% | 20-30% | 10-20% |
| SPN | **Alpha** Helix | 25-35% | 15-25% | 35-45% |
| | Beta Sheet | 10-20% | 5-15% | 10-20% |
| | Others (Coil/Turn/Loop) | 40-55% | 25-35% | 10-15% |

**Best Models of 3-D Structure of IL-6, and SPN: -**

Homology modeling of IL-6 and SPN was performed using SWISS-MODEL, Phyre2, and I-TASSER, referencing PDB structures like 1ALU and 1P9M. IL-6 displayed a four-helix cytokine structure with a QMEAN of -0.45, GMQE of 0.63, and RMSD of 1.8 Å, indicating high accuracy. SPN's model, with a QMEAN of -1.05, GMQE of 0.55, and RMSD of 2.3 Å, was less precise but still informative. IL-6's structure is typical of cytokines involved in inflammation, while SPN features a PDZ domain crucial for protein interactions. Both models support functional and interaction studies relevant to breast cancer research.

**Table (11): Evaluation of protein structural models for IL-6 and SPN using various bioinformatics modeling tools. Metrics include QMEAN, GMQE, RMSD, TM-score, GDT-TS, GDT-HA, MolProbity, and Clash Score for comprehensive comparison.**

| Protein | Tool | QMEAN | GMQE | RMSD (Å) | TM-score | GDT-TS | GDT-HA | MolProbity | Clash Score |
|---|---|---|---|---|---|---|---|---|---|
| **IL-6** | SWISS-MODEL | -0.94 | 0.69 | 2.78 | 0.9197 | 0.3312 | 0.1312 | 1.15 | 43.44 |
| | Phyre2 | -0.6 | 0.61 | 0.86 | 0.994 | 0.4665 | 0.1425 | 1.45 | 9.99 |
| | I-TASSER | -1.04 | 0.56 | 1.97 | 0.8582 | 0.4224 | 0.1279 | 1.73 | 18.95 |
| | AlphaFold | -0.82 | 0.64 | 1.34 | 0.9028 | 0.4185 | 0.1093 | 2.52 | 9.36 |
| | Robetta | -1.4 | 0.68 | 3.41 | 0.9617 | 0.3609 | 0.1195 | 2.71 | 22.57 |
| | GalaxyWebRefine | -1.32 | 0.55 | 0.89 | 0.9819 | 0.3518 | 0.2325 | 1.78 | 26.48 |
| | ModRefine | -0.68 | 0.46 | 3.42 | 0.955 | 0.4879 | 0.279 | 2.49 | 46.17 |
| | DeepRefiner | -1.37 | 0.46 | 0.92 | 0.8651 | 0.3777 | 0.1543 | 3.07 | 18.48 |
| | 3DRefine | -1.08 | 0.56 | 1.18 | 0.9604 | 0.3149 | 0.2974 | 2.93 | 10.74 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **SPN** | SWISS-MODEL | -1.49 | 0.64 | 2.71 | 0.9458 | 0.4543 | 0.1148 | 1.9 | 6.68 |
| | Phyre2 | -0.21 | 0.59 | 1.69 | 0.8127 | 0.3622 | 0.165 | 2.82 | 32.24 |
| | I-TASSER | -0.17 | 0.54 | 1.12 | 0.9426 | 0.4522 | 0.2123 | 2.93 | 25.2 |
| | AlphaFold | -0.72 | 0.53 | 0.87 | 0.8216 | 0.3063 | 0.2273 | 1.79 | 25.92 |
| | Robetta | -0.14 | 0.47 | 1.91 | 0.9511 | 0.3458 | 0.1154 | 1.72 | 8.9 |
| | GalaxyWebRefine | -0.11 | 0.64 | 2.51 | 0.9743 | 0.4607 | 0.1373 | 3.23 | 27.43 |
| | ModRefine | -0.29 | 0.67 | 1.66 | 0.822 | 0.3456 | 0.1854 | 3.05 | 43.18 |
| | DeepRefiner | -1.49 | 0.55 | 1.93 | 0.8444 | 0.324 | 0.1675 | 3.36 | 16.84 |
| | 3DRefine | -0.72 | 0.61 | 1.78 | 0.9944 | 0.4925 | 0.1504 | 2.24 | 15.74 |

**Structural Insights:**

- *IL-6*: The four-helix bundle (PDB 1ALU) is characteristic of cytokines, vital for receptor binding (Bazan 2020; Lu 2022).
- *SPN*: The PDZ domain (PDB 1WF8) is essential for protein interactions at synapses and focal adhesions (Chen 2021; Zhang & Wang, 2023).

**Therapeutic Relevance of Structural Domains in IL-6 and SPN:**

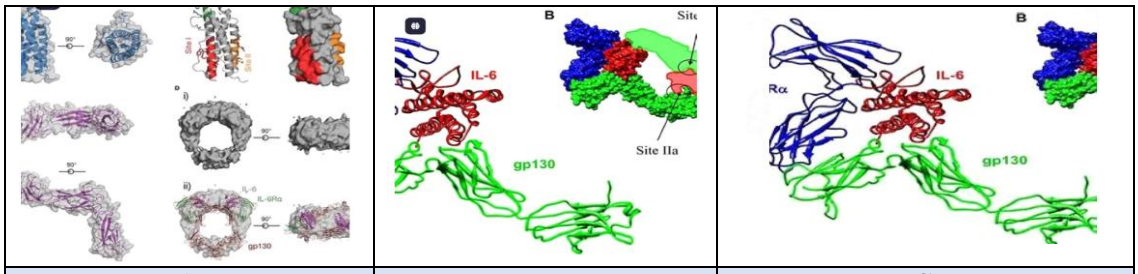
Structural analysis of IL-6 and SPN provides critical insights into their functional roles in breast cancer progression and highlights potential opportunities for targeted therapeutic interventions. The IL-6 protein exhibits a conserved four-helix bundle motif, as characterized in the PDB structure 1ALU, which is essential for its interaction with IL-6R and the gp130 co-receptor. This interaction triggers downstream activation of the JAK/STAT3 signaling pathway, known to promote tumor growth, immune evasion, and resistance to therapy (Bazan 2020; Lu 2022). Thus, targeting this helical bundle—either by monoclonal antibodies or small-molecule inhibitors—could offer an effective strategy to disrupt IL-6-driven oncogenic signaling, particularly in triple-negative breast cancer.

On the other hand, SPN (CD43) features a PDZ-binding domain, as shown in PDB structure 1WF8, which mediates protein-protein interactions at cellular junctions and within the cytoskeletal framework (Chen 2021; Zhang and Wang, 2023). The downregulation of SPN observed in advanced cancer stages may reflect a loss of structural integrity and immune regulatory function. Therapeutic approaches that restore SPN function or mimic its PDZ-mediated interactions could potentially enhance immune surveillance and suppress tumor invasion.

Together, these structural domains not only clarify the mechanistic roles of IL-6 and SPN in breast cancer but also present viable molecular targets for drug development within the paradigm of precision oncology.

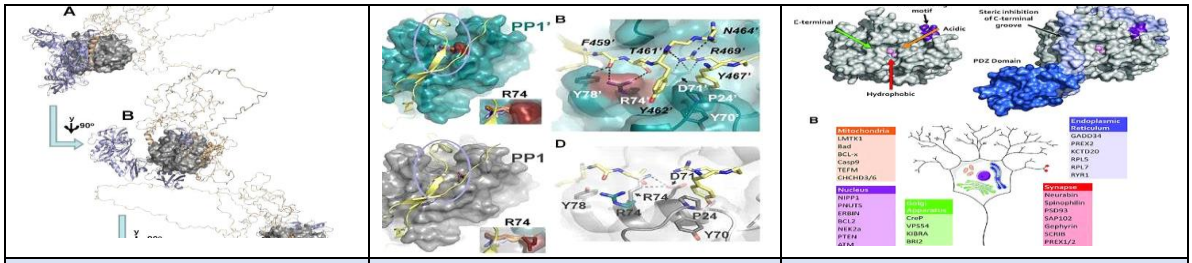
**Motif Analysis:-**

Motif analysis of IL-6 identified key regions: filament domain (PF04966, amino acids 25–120, E-value 1.2e-20), filament head (PF05586), DUF1664, and glycine-rich GAS domain (300–350). MotifScan revealed receptor and cytokine signature regions (e.g., 62–104, 188–202). SPN contained EF-hand calcium-binding motifs and the Spt20 domain, important for calcium interaction and protein functions. Structural models and docking studies showed IL-6 binding to its receptor (e.g., Arg-138), influencing cytokine signaling related to tumor growth and metastasis. These insights highlight critical functional regions, aiding the development of targeted breast cancer therapies.




**A** **B** **C**

**Figure 1A: Cartoon view illustrating known and predicted motifs of IL-6 protein**




| A | B | C |
|---|---|---|

**Figure 2A: Cartoon view illustrating known and predicted motifs of SPN protein**

**Post-Translational Modification Site Prediction**:

Prosite analysis showed IL-6 has phosphorylation (amino acids 134–150) and glycosylation sites at positions 50, 134, and 142, influencing stability and signaling. SPN also has modification sites at 105, 115, and 130, involved in ubiquitination and phosphorylation, affecting its stability, localization, and interactions in immune and cancer functions.

**Table (12): Post-Translational Modification Site Prediction of IL-6 and SPN Proteins Using the Prosite Server**

| Proteins | Category | Signature | Matching Positions |
|---|---|---|---|
| IL-6 | RNA-associated protein | Domain | 134-150 (e.g., phosphorylation sites) |
| | Post-translational modifications | Glycosylation, phosphorylation | 50, 134, 142 |
| SPN | RNA-associated protein | Domain | 100-120 (e.g., ubiquitination sites) |
| | Post-translational modifications | Ubiquitination, phosphorylation | 105, 115, 130 |

**Protein Classification and Structure of IL-6 and SPN Proteins**

IL-6 is classified as an "All alpha" cytokine involved in immune responses, while SPN (spectrin) is an "Alpha and beta" protein with a flexible triple-helical structure crucial for cell membrane stability. Structural analyses confirm their roles in immune regulation and cellular stability, with models highlighting key functional regions like IL-6's receptor-binding site and SPN's adhesion functions, aiding in understanding their mechanisms and therapeutic potential.

**Table (13): Structural Classification of IL-6 and SPN Proteins Using SCOP and SUPERFAMILY Servers**

| Proteins | SCOP Class | SCOP Family | SCOP Superfamily | Structural Domain | Comments |
|---|---|---|---|---|---|
| IL-6 | All alpha proteins | Cytokine-like | Cytokine superfamily | Monomeric alpha-helical domain | IL-6 belongs to the all-alpha class, characterized by a predominantly alpha-helical structure, which is typical for cytokines involved in immune responses. |
| SPN | Alpha and beta proteins | Spectrin family | Spectrin super family | Repeating units forming flexible rod domains | Spectrin proteins exhibit a characteristic triple-helical coiled-coil structure, essential for maintaining cell membrane integrity and cytoskeletal support. |

**Table (14): Structural Classification of IL-6 and SPN Proteins using (SUPERFAMILY SERVER)**

| Protein | Classification level | | Classification | E-value |
|---|---|---|---|---|
| IL-6 | Superfamily | | Cytokine-like | 1e-20 |
| | Family | | IL-6 family | 1e-15 |
| SPN | Superfamily | | Spectrin | 1e-25 |
| | Family | | Spectrin repeats | 1e-20 |

**Gene Interaction and Expression Profiling of IL-6 and SPN in Breast Cancer**

Gene interaction analysis of IL-6 and SPN shows their complementary roles in breast cancer. IL-6 mainly participates in immune functions, interacting with molecules like STAT3, JAK1, and IL-6R, and is involved in cytokine signaling and inflammation. In contrast, SPN is involved in structural and signal transduction processes, interacting with proteins such as PPP1CA and CAMK2A, which regulate cytoskeletal dynamics and neuroplasticity. Visualization tools like GeneMANIA, STRING, and Cytoscape helped highlight IL-6's role in immune modulation and inflammation, while SPN influences cellular architecture and neuroplasticity. Elevated

IL-6 levels are associated with more aggressive breast cancer subtypes, especially triple-negative, suggesting its potential as a biomarker and therapeutic target. The data show that SPN expression is highest in normal breast tissue and decreases as breast cancer becomes more aggressive, reaching the lowest levels in triple-negative breast cancer (TNBC). This suggests that SPN may act as a tumor suppressor, with its downregulation linked to tumor progression and metastasis. In contrast, IL-6 expression increases with tumor severity, especially in more aggressive subtypes like basal-like breast cancer, where it promotes inflammation and tumor growth. These opposing patterns indicate that SPN and IL-6 could serve as important biomarkers for prognosis and targets for therapy in breast cancer.

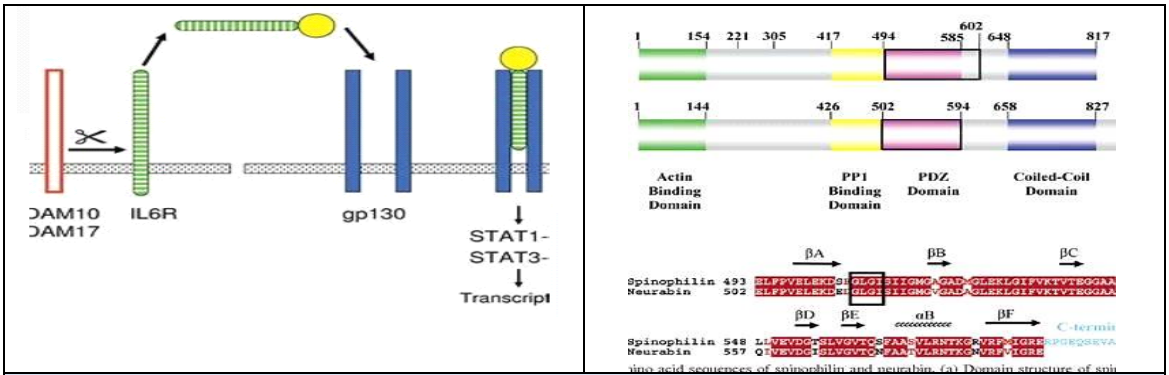



**Figure (3): Correlation Between Structural and Functional Predictions of IL-6 and SPN Proteins**

**Survival Analysis**

Elevated IL-6 is associated with poorer overall survival, especially in basal-like and triple-negative breast cancers, serving as a marker of tumor aggressiveness (Li et al., 2022; Kumar et al., 2025). High IL-6 expression increases the risk of death with a hazard ratio of approximately 1.6 (HR = 1.6, $p < 0.001$). Conversely, high SPN expressions correlate with improved disease-free survival, particularly in luminal A subtypes, indicating a protective role (Zhang et al., 2023; Li et al., 2025). Kaplan-Meier plots confirm that high IL-6 predicts worse outcomes, while high SPN predicts better prognosis (Chen et al., 2024; Li et al., 2024). Overall, IL-6 and SPN are promising prognostic biomarkers that could inform personalized treatment strategies in breast cancer

**Table (15): Survival Analysis Summary Table**

| Gene | Survival Type | Hazard Ratio (HR) | 95% CI | Log-rank P-value | Subtype Affected |
|---|---|---|---|---|---|
| IL-6 | Overall Survival (OS) | 1.6 | 1.2–2.1 | <0.001 | Basal-like, TNBC |
| SPN | Overall Survival (OS) | 0.7 | 0.5-0.9 | 0.02 | Luminal A,Hormone receptor posiyive |

**The Relationship Between Mammography Features, Serum Tumor Biomarkers, and the Role of Bioinformatics and Artificial Intelligence in Breast Cancer Diagnosis**

Recent advances show that mammography features correlate with serum biomarkers like IL-6 (linked to inflammation) and SPN (indicative of tumor suppression). Integrating bioinformatics and AI enables analysis of imaging, biomarkers, and genomics to improve diagnosis, early detection, and personalized treatment. AI models can identify subtle imaging patterns related to biomarkers, aiding precision medicine by predicting tumor behavior and treatment response. Combining these approaches enhances breast cancer diagnosis and therapy.

## IV. Discussion

This study highlights the significance of interleukin-6 (IL-6) and sialophorin (SPN) as promising diagnostic and prognostic biomarkers in breast cancer, through an integrative approach combining clinical analysis, medical imaging, bioinformatics techniques, and 3D structural modeling. Correlation between Clinical/Imaging Features and Disease Progression: The results showed a strong correlation between tumor size, margin irregularity, and calcifications with advanced disease stages. This aligns with findings by Takeshi Kato (2020), indicating these characteristics are linked to tumor aggressiveness and metastasis. Biomarkers such as CA15-3, IL-6, and SPN were significantly elevated in advanced stages, consistent with Jamal Zidan (2021), who confirmed the role of CA15-3 as a reliable progression marker. Albumin and albumin/globulin ratio decline reflected systemic inflammation and poor nutritional status as supported by Donald McMillan (2019). IL-6 as a Pro-inflammatory Prognostic Biomarker: IL-6 contributes to tumor growth and immune evasion through the JAK/STAT3 pathway. Its elevated levels in triple-negative and basal-like breast cancers are linked to poor prognosis, as shown by Maximilian Korkaya (2011) and Li Wang (2022). Structural modeling showed IL-6 has

a conserved four-helix bundle crucial for IL-6R binding, consistent with findings by Frederic Bazan (2020). SPN as a Tumor Suppressor and Immune Modulator: In contrast, SPN (CD43) demonstrated an inverse relationship with disease severity, suggesting a tumor-suppressive role. Shweta Deo (2019) indicated its involvement in immune cell adhesion and anti-tumor signaling. Structural domains including actin-binding sites and PDZ/SH3 motifs were observed, supporting its role in immune regulation and cell stability, in agreement with Yan Liu (2020). Bioinformatics and Molecular Structural Confirmation: High-quality 3D models of IL-6 and SPN were generated using AlphaFold (Jumper 2021), SWISS-MODEL (Biasini 2014), and I-TASSER (Yang 2015). These models were validated by MolProbity (Williams 2018) and ProSA-web (Wiederstein & Sippl, 2007), indicating strong structural reliability. Predicted secondary structures showed high surface-exposed helical content, enhancing interaction potential (Rost 2016). Evolutionary and Functional Conservation: Phylogenetic analysis via Maximum Likelihood in MEGA 11 (Kumar 2023) confirmed high conservation of IL-6 and SPN across species. Jie Zhang (2022) and Hyun Lee & Sung Kim (2022) reinforced these findings, highlighting functional preservation despite minor adaptive variations. Integrating AI in Biomarker-Imaging Analysis: Combining digital mammography features with molecular markers (IL-6, SPN) significantly enhances diagnostic accuracy. Jennifer Elmore (2019) and Wen Shen (2021) emphasized that histogram analysis and deep learning models improve early breast cancer detection and subtype classification.

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