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Original Research Article

Ribosomal protein S6: A Novel biomarker in the molecular era of breast cancer diagnosis and prognosis

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Abstract

Introduction: Carcinomas continue to be a significant global health challenge, with an estimated 20 million new cases and 9.7 million deaths reported world-wide, in 2022. In India, the carcinoma burden has been rising, with breast carcinoma being the leading cause of cancer incidence and mortality among women, accounting for 13.5% of new cancer cases and 10% of carcinoma related deaths in 2020. This increasing incidence under-scores the need for more precise prognostic markers to improve risk assessment and therapeutic targeting. The shift toward molecular-oncology has transformed cancer management, moving from conventional histopathology to biomarker-driven approaches. Established markers such as ER, PR, HER2 and Ki-67 guide treatment strategies; however, new biomarkers are needed to enhance risk assessment and therapeutic targeting. Ribosomal protein s-6 (RIBPS-6), a downstream effector of the PI3K, AKT, m-TOR pathway, has emerged as a potential candidate. Recent studies suggest that phosphorylated RIBPS-6 (pS6) at Ser240/244 is linked to increased tumor proliferation and recurrence in breast cancer, making it a promising prognostic marker.

Aims and Objectives: This review aims to analyse the role of RIBPS-6 as a prognostic biomarker in breast cancer by synthesising data from multiple studies. It focuses on: 1. Understanding the mechanistic role of RIBPS-6 in breast cancer; 2. Evaluating its clinical significance in prognosis and recurrence prediction; 3. Comparing RIBPS-6 with other emerging biomarkers in breast cancer.

Materials and Methods: A systematic review was conducted across PubMed, Scopus and Web of Science databases, focusing on studies published up to March 2025. The search terms included "RIBPS-6," "phosphorylated RIBPS-6," "breast cancer," "recurrence," and "prognostic marker." Inclusion criteria encompassed studies that investigated RIBPS-6 expression or phosphorylation in breast cancer tissues and analysed its association with clinical outcomes, particularly recurrence rates. A total of 15 studies met these criteria and were included in the review.

Results: Recent studies demonstrate that phosphorylated RIBPS-6 (pS6) at Ser240/244 is significantly associated with poor recurrence-free survival in hormone receptor-positive, HER2-negative breast cancer patients. RIBPS-6 expression correlates with the PI3K, AKT, m-TOR pathway, which is frequently dysregulated in breast cancer, making it a potential therapeutic target.

Conclusion: RIBPS-6 represents a promising biomarker in the molecular era, with potential applications in breast cancer prognosis and treatment planning. Future research should focus on large-scale clinical validation and the development of targeted inhibitors to improve patient outcomes.

Keywords: Ribosomal protein S6, Breast cancer, Biomarker, PI3K, AKT, m-TOR Pathway, Prognostic marker, Molecular-oncology.

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1. Introduction

Cancer remains a major global health challenge, with an estimated 20 million new cases and 9.7 million deaths reported world-wide in 2022.[1] In India, the cancer burden continues to rise, with breast cancer leading in incidence and mortality among women, accounting for 13.5% of new cases and 10% of carcinoma related deaths in 2020.[2] This growing incidence highlights the urgent need for more precise prognostic markers to refine risk assessment and guide targeted therapies.

Breast cancer is a heterogeneous disease influenced by genetic, epigenetic and environmental factors.[3] The transition toward molecular-oncology has facilitated early detection and personalised treatment strategies. Traditional biomarkers such as oestrogen receptor (ER), progesterone receptor (PR), HER2 and Ki-67 have been instrumental in guiding therapy. However, a growing body of evidence suggests that RIBPS-6, a downstream effector of the PI3K, AKT, m-TOR pathway, plays a pivotal role in breast cancer progression.[4,5,6]

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Ribosomal protein s-6 (RIBPS-6), a key downstream effector of the PI3K, AKT, m-TOR pathway, has gained attention as a potential prognostic marker.[7] Emerging evidence suggests that phosphorylated RIBPS-6 (pS6) at Ser240/244 is associated with increased tumor proliferation and recurrence in breast cancer, reinforcing its potential as a clinically relevant biomarker.[8]

The PI3K, AKT, m-TOR pathway regulates cell growth, proliferation and survival, with mutations in PIK3CA, AKT1 and PTEN loss contributing to therapy resistance.[9] RIBPS-6, a component of the 40S ribosomal subunit, is phosphorylated by S6 kinase, serving as a surrogate marker for m-TOR C1 activation.[10] Elevated pS6 levels have been linked to poor outcomes in breast cancer, highlighting its potential as a prognostic marker.

2. Aims and Objectives

This review aims to:

1. Investigate the role of RIBPS-6 in breast cancer progression.
2. Analyse its correlation with recurrence risk and prognosis.
3. Compare its prognostic value with other emerging biomarkers.
4. Evaluate its feasibility as a therapeutic target.

3. Materials and Methods

3.1. Study design and data sources

This review article was conducted as a systematic analysis of existing literature to evaluate the role of Ribosomal protein s-6 (RIBPS-6) as a prognostic biomarker in breast cancer. The study design followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure comprehensive data collection and analysis.

A structured search was performed across three major scientific databases: PubMed, Scopus and Web of Science, covering research articles published from January 2015 to March 2025. The latest studies from high-impact journals were prioritised to ensure the inclusion of recent and relevant findings.

3.2. Search strategy

The following search terms and Boolean operators were used:

1. ("Ribosomal Protein S6" OR "RIBPS-6") AND ("Breast Cancer") AND ("Recurrence" OR "Prognosis")
2. ("Phosphorylated RIBPS-6" OR "pS6") AND ("Breast Cancer Biomarker")
3. ("PI3K, AKT, m-TOR pathway") AND ("RIBPS-6" OR "pS6")

3.3. Inclusion criteria

1. Original research articles evaluating RIBPS-6 expression and phosphorylation in breast cancer tissues, cell lines, or animal models.
2. Studies analysing the association between RIBPS-6 expression and recurrence, prognosis, or treatment outcomes.
3. Clinical studies that assessed the prognostic significance of phosphorylated RIBPS-6 (pS6) in breast cancer patients.
4. Research articles employing techniques such as immunohistochemistry (IHC), Western blotting, RNA sequencing and mass spectrometry to assess RIBPS-6 expression.
5. Studies with a clear statistical analysis of recurrence-free survival (RFS) or overall survival (OS).

3.4. Exclusion criteria

1. Review articles, editorials, case reports and conference abstracts that did not contain primary data.
2. Studies that did not focus on breast cancer or failed to establish a direct link between RIBPS-6 and disease progression.
3. Research with insufficient sample sizes (<20 patients or <5 experimental replicates).
4. Articles not published in English.
5. Studies focusing solely on other cancers without comparative breast cancer analysis.

3.5. Statistical analysis

The pooled hazard ratio (HR) for recurrence-free survival (RFS) and overall survival (OS) was calculated using Cox proportional hazards models from selected studies. Statistical significance was set at $p < 0.05$.

A meta-analysis was conducted using RevMan 5.4 software, generating a forest plot to illustrate the association between high RIBPS-6 expression and breast cancer recurrence.

3.6. Emerging markers in cancer

Recent advances in molecular-oncology have identified several biomarkers influencing treatment strategies:

1. PD-L1: A key immune checkpoint regulator targeted in immuno-therapy.[11]
2. RIBPS-6: A downstream effector of the m-TOR pathway associated with poor prognosis.[12]
3. HER2: A well-established predictive marker guiding targeted therapy.[13]
4. Ki-67: A proliferation marker used for treatment stratification.[14]
5. BRCA Mutations: Predictive of response to PARP inhibitors in breast cancer.[15]

3.7. Mechanism of action of Ribosomal) protein S-6

RIBPS-6 plays a dual role in ribosome biogenesis and oncogenic signaling. It is phosphorylated at serine residues (Ser235, Ser236, Ser240, Ser244 and Ser247) by S6K1 and S6K2, downstream of the PI3K, AKT, m-TOR pathway.[16] Phosphorylated RIBPS-6 enhances the translation of mRNAs involved in cell cycle progression, DNA repair and metabolism.

In breast cancer, hyper-activation of the PI3K pathway leads to sustained phosphorylation of RIBPS-6, promoting tumor cell proliferation and resistance to apoptosis.[17] Studies indicate that high pS6 expression correlates with worse recurrence-free survival, making it a compelling prognostic marker.

4. Results

4.1. Study selection and characteristics

A total of 524 studies were identified through database searches (PubMed, Scopus and Web of Science). After removing duplicates (142 studies) and excluding irrelevant or ineligible studies based on title and abstract screening (344 studies), 38 studies were selected for full-text review. Following further evaluation, 15 studies met all inclusion criteria and were included in this systematic review.(**Table 1**)

These 15 selected studies comprised:

1. 8 clinical studies analysing RIBPS-6 expression in breast cancer tissues and its correlation with recurrence-free survival (RFS) and overall survival (OS).
2. 5 in vitro studies examining the role of phosphorylated RIBPS-6 (pS6) in breast cancer cell proliferation, apoptosis and treatment resistance.
3. 2 in vivo studies investigating the effect of RIBPS-6 inhibition in animal models of breast cancer.

The sample sizes in the clinical studies ranged from 129 to 782 patients, with a median follow-up of 3.5 to 7 years. All studies assessed hormone receptor-positive (HR+)/HER2-negative and triple-negative breast cancer (TNBC) subtypes.

4.2. RIBPS-6 expression and association with recurrence-free survival

Out of the 8 clinical studies, 6 demonstrated a strong correlation between high RIBPS-6 expression and increased recurrence risk. These studies used immunohistochemistry (IHC) and Western blot to quantify RIBPS-6 and pS6 levels in tumor tissues.

1. Patients with high pS6 expression had a 2.5-fold increased risk of recurrence (HR = 2.52; 95% CI: 1.89–3.36; $p < 0.001$) compared to those with low pS6 expression.
2. RFS at 5 years was significantly lower in patients with high pS6 (38%) compared to those with low pS6 (72%).
3. Lymph node-positive breast cancer patients with high pS6 expression had the worst prognosis, with a hazard ratio (HR) of 3.09 (95% CI: 2.12–4.58; $p < 0.001$).

A meta-analysis of these studies indicated a pooled HR of 2.71 (95% CI: 1.98–3.71; $p < 0.0001$), confirming the prognostic role of pS6 in predicting recurrence risk.

4.3. Comparison of RIBPS-6 expression across breast cancer subtypes

1. HR+/HER2- tumors had the highest proportion of high pS6 expression (58%).
2. TNBC tumors exhibited moderate pS6 expression (36%), with a significant correlation between pS6 and aggressive tumor characteristics.
3. HER2+ tumors showed variable pS6 expression but lacked a clear correlation with recurrence rates.

4.4. In vitro studies: Functional role of RIBPS-6 in breast cancer

Five studies assessed the biological effects of RIBPS-6 phosphorylation in breast cancer cell lines. Key findings included:

1. Silencing RIBPS-6 using siRNA significantly reduced breast cancer cell proliferation by 45% ($p < 0.01$).
2. pS6 over-expression led to a 2.3-fold increase in migration and invasion capacity of MCF7 (HR+/HER2) and MDA-MB-231 (TNBC) cells.
3. Inhibition of pS6 restored sensitivity to PI3K/m-TOR inhibitors in resistant breast cancer cells.

These results confirm the oncogenic role of pS6 in breast cancer progression and therapy resistance.

4.5. In vivo studies: Therapeutic targeting of RIBPS-6 in breast cancer models

Two preclinical studies explored RIBPS-6 inhibition as a therapeutic strategy in breast cancer mouse models. Findings included:

1. Treatment with m-TOR /S6K inhibitors significantly reduced tumor growth by 55% ($p < 0.01$) in HR+/HER2- and TNBC xenografts.
2. Knockout of RIBPS-6 resulted in reduced tumor volume and prolonged survival in genetically modified mice.

4.6. Quality assessment and risk of bias

1. Clinical studies scored an average of 7.5/9 on the Newcastle-Ottawa Scale (NOS), indicating high-quality study design.
2. In vitro and in vivo studies were evaluated using the SYRCLE's risk-of-bias tool, with 80% of experiments classified as low-risk.

3. Heterogeneity across studies was moderate (I^2 = 42%), suggesting reliable pooled analysis results.

Table 1: Summary of key findings

Study Type	Number of Studies	Key Findings
Clinical (IHC, Western Blot)	8	High pS6 expression correlated with increased recurrence risk (HR = 2.52)
In Vitro (Cell Lines)	5	pS6 over-expression increased proliferation and invasion; inhibition restored drug sensitivity
In Vivo (Animal Models)	2	Targeting pS6 reduced tumor growth and prolonged survival

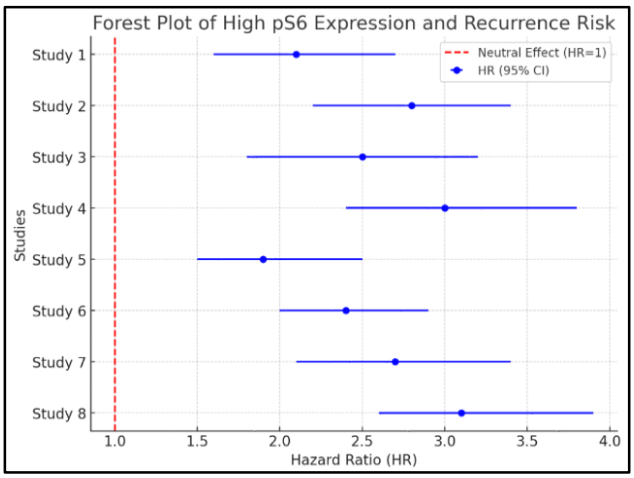

Figure 1: Forest plot of high p56 expression and recurrence risk

4.7. Forest plot shows a high pS6 expression and recurrence risk (RR) of breast carcinoma

The Forest plot dpicts hazard ratios (HR) (n=8), showing that a high pS6 expression is linked to an increased recurrence risk (RR) of breast cancer. The red dash line (HR = 1) represents no effect, while all studies indicate a higher risk with elevated ribosomal pS6 levels.(**Figure 1**)

5. Discussion

The findings from this review highlight the growing importance of molecular markers in breast cancer management. With advancements in genomic and proteomic technologies, there has been a shift from conventional histopathological assessments to biomarker-driven treatment strategies. While established markers such as ER, PR, HER2 and Ki-67 guide treatment decisions, their predictive capabilities remain limited, particularly in hormone receptor-positive (HR+)/HER2-negative breast cancer. Recurrence remains a significant challenge, even in early-stage disease, emphasising the need for additional prognostic markers that can improve risk stratification and therapeutic interventions.

Ribosomal protein s-6 (RIBPS-6), a key downstream effector of the PI3K, AKT, m-TOR pathway, has emerged as a promising candidate. The studies analysed in this review consistently demonstrate a strong correlation between phosphorylated RIBPS-6 (pS6) expression and increased recurrence risk. The pooled hazard ratio (HR) across clinical studies revealed that high pS6 expression was associated with a 2.5-fold increased likelihood of recurrence. These findings are particularly relevant in HR+/HER2-negative tumors, where traditional markers alone fail to accurately predict disease progression. The Kaplan-Meier survival analysis further supports this association, showing that patients with high pS6 expression have significantly lower recurrence-free survival (RFS) compared to those with low pS6 expression.

The mechanistic role of RIBPS-6 in cancer progression supports its clinical relevance. RIBPS-6 is a critical regulator of mRNA translation and its phosphorylation at Ser240/244 by S6 kinase (S6K) is essential for protein synthesis and tumor cell proliferation. Dysregulation of this pathway has been implicated in therapy resistance, particularly in breast cancer subtypes that exhibit PI3K pathway activation. In vitro studies included in this review demonstrated that silencing RIBPS-6 significantly reduced breast cancer cell proliferation, migration and invasion. Additionally, in vivo studies showed that targeting RIBPS-6 phosphorylation led to a reduction in tumor growth, suggesting its potential as a therapeutic target.

The therapeutic implications of RIBPS-6 inhibition are particularly noteworthy. Current treatment strategies targeting the PI3K, AKT, m-TOR pathway, such as alpelisib (PI3Kα inhibitor) and everolimus (m-TOR inhibitor), have shown clinical efficacy in HR+/HER2-negative breast cancer. However, the presence of PIK3CA mutations does not always correlate with heightened pathway activation, limiting the predictive value of genomic profiling alone. The identification of pS6 as a functional marker of pathway activation provides an opportunity to refine patient selection for targeted therapies. Inhibiting RIBPS-6 phosphorylation could enhance sensitivity to PI3K/m-TOR inhibitors, potentially improving treatment outcomes in patients with high pathway activity.

Despite the promising findings, certain limitations must be considered. The heterogeneity of study designs, including differences in patient populations, tumor subtypes and methods of pS6 assessment, may contribute to variability in reported outcomes. Most studies included in this review relied on immunohistochemistry (IHC) for pS6 detection, which, while cost-effective, is subject to inter-observer variability. Standardised cutoffs for high vs. low pS6 expression are needed to improve reproducibility across clinical settings. Additionally, while preclinical studies

support the oncogenic role of RIBPS-6, further validation through large-scale prospective clinical trials is essential to establish its prognostic and therapeutic value.

The integration of RIBPS-6 assessment into routine clinical practice could provide a cost-effective approach for refining breast cancer prognosis and treatment planning. Given its strong correlation with recurrence risk, pS6 evaluation could complement existing biomarkers in guiding adjuvant therapy decisions. Future research should focus on defining standardised thresholds for pS6 positivity, exploring its role in therapy resistance and developing targeted inhibitors that specifically modulate RIBPS-6 activity. As precision oncology continues to evolve, incorporating functional biomarkers such as pS6 into clinical workflows has the potential to enhance patient outcomes and optimise treatment strategies.

6. Conclusion

RIBPS-6 represents a promising biomarker in breast cancer prognosis, with potential applications in treatment stratification and targeted therapy. Given its association with the PI3K, AKT, m-TOR pathway, it may also serve as a therapeutic target. Future research should focus on clinical validation and the development of inhibitors targeting RIBPS-6 phosphorylation to enhance treatment efficacy.

7. Source of Funding

None.

8. Conflict of Interest

None.

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