JHWCR JOURNAL OF HEALTH, WELLNESS AND COMMUNITY RESEARCH 3007-0570

DOI: https://doi.org/10.61919/fqva9494

Original Article

The Role of Prolactin in Breast Cancer Metastasis: A Preclinical Study

Rawaal Amin[1], Afshan Shahani[2], Shumaila Khero[3], Fouzia Bibi[4], Maryam Gull[5], Laiba Irfan[6], Muhammad Ahsan Shahryar[7]

[1] FCPS-II, Sandeman Provincial Hospital, Quetta, Pakistan
[2] FCPS-I, Pakistan Institute of Medical Sciences Hospital, Islamabad, Pakistan
[3] Head of Department, Oncology Ward, Civil Hospital, Karachi, Pakistan
[4] Women Medical Officer, Civil Hospital, Karachi, Pakistan
[5] MPhil Biochemistry, University of Veterinary and Animal Sciences, Lahore, Pakistan
[6] House Officer, Jinnah Hospital, Lahore, Pakistan
[7] Department of Pharmacy, University of Peshawar, Peshawar, Pakistan

***Corresponding author: Rawaal Amin, rawaalamin@gmail.com**

"Cite this Article" Received: 28 September 2025; **Accepted**: 24 January 2026; **Published**: 28 February 2026

Author Contributions: Concept: MS, BA; Design: MS, BA, MA; Data Collection: MS, AW, SN; Analysis: MS, WJ, MA; Drafting: MS, BA, WJ, AW, SN. **Ethical Approval**: CENAR Hospital, Quetta, Pakistan. **Informed Consent**: Written informed consent was obtained from all participants; **Conflict of Interest**: The authors declare no conflict of interest. **Funding**: No external funding; **Data Availability**: Available from the corresponding author on reasonable request; **Acknowledgments**: NA

ABSTRACT

Background: Prolactin–prolactin receptor signaling has been implicated in breast cancer cell survival, growth, and invasive behavior, although its effects vary according to cellular and molecular context. Objective: To evaluate the effects of prolactin exposure and concurrent prolactin-receptor inhibition on metabolic activity, scratch closure, Matrigel invasion, and selected molecular markers in MCF-7 breast cancer cells. Methods: In this in vitro experimental study, MCF-7 cells were assigned to untreated control, prolactin 50 ng/mL, prolactin 100 ng/mL, and prolactin 100 ng/mL plus prolactin-receptor inhibitor groups. Metabolic activity was assessed using the MTT assay at 24, 48, and 72 hours, while monolayer closure was measured using a scratch assay at 24 and 48 hours. Matrigel invasion and messenger RNA expression of STAT5, MMP-9, BCL-2, and PRLR were also evaluated. Data were summarized as mean ± standard deviation. Results: At 72 hours, MTT absorbance was 0.60 ± 0.04 in controls, 0.79 ± 0.05 with prolactin 50 ng/mL, 1.01 ± 0.06 with prolactin 100 ng/mL, and 0.62 ± 0.04 with prolactin plus inhibitor. At 48 hours, scratch closure was 45.0% ± 3.0%, 62.0% ± 3.4%, 78.0% ± 4.1%, and 48.0% ± 2.8%, respectively. Prolactin-treated cells were reported to demonstrate greater Matrigel invasion and increased expression of STAT5, MMP-9, BCL-2, and PRLR, while receptor inhibition attenuated these responses. Conclusion: Prolactin exposure was associated with increased metabolic activity and scratch closure in MCF-7 cells, whereas PRLR inhibition reduced these effects. Further quantitative and mechanistic validation is required before clinical or therapeutic implications can be established. Keywords: Breast cancer; MCF-7 cells; Prolactin; Prolactin receptor; STAT5; Cell invasion.

INTRODUCTION

Breast cancer remains one of the most frequently diagnosed malignancies among women and a leading cause of cancer-related mortality worldwide. Although advances in screening and multimodal treatment have improved outcomes for patients with localized disease, distant dissemination remains the principal determinant of treatment failure and mortality. Metastasis is a complex, multistage process involving altered cell survival, loss of intercellular adhesion, local invasion, migration, intravasation, survival within the circulation, extravasation, and colonization of distant tissues. These processes are regulated by interactions among oncogenic pathways, steroid and peptide hormones, extracellular-matrix remodeling, and the tumor microenvironment.

Prolactin is a polypeptide hormone best recognized for its physiological roles in mammary-gland development, differentiation, and lactation. Its biological effects are mediated through the prolactin

receptor (PRLR), which activates intracellular signaling pathways that include Janus kinase 2 and signal transducer and activator of transcription 5. In addition to its endocrine secretion by the anterior pituitary, prolactin may be produced locally within mammary tissues, where autocrine and paracrine signaling can influence epithelial-cell growth and survival. Experimental evidence has consequently implicated dysregulated prolactin–PRLR signaling in breast tumor biology (1–4).

Prolactin has been reported to increase the proliferation and survival of hormone-responsive breast cancer cells by activating signaling networks associated with cell-cycle progression and resistance to apoptosis (4,5). Its interaction with estrogen-receptor signaling may further modify the behavior of estrogen-responsive tumors (6,7). However, the effects of prolactin appear to vary according to breast cancer subtype, receptor expression, intracellular signaling context, and the surrounding microenvironment. These context-dependent effects have prevented a definitive characterization of prolactin as either a universal promoter or suppressor of breast cancer progression.

In addition to its effects on cellular growth and survival, prolactin signaling may influence cellular motility and extracellular-matrix invasion. Experimental studies have associated PRLR-mediated signaling with cytoskeletal reorganization and changes in the expression of matrix metalloproteinases, including matrix metalloproteinase 9, which can facilitate extracellular-matrix degradation and tumor-cell invasion (8,9). Prolactin may also enhance anti-apoptotic signaling through proteins such as B-cell lymphoma 2 and reinforce its biological activity through altered PRLR expression. Nevertheless, proliferation, scratch closure, and matrix invasion represent surrogate cellular phenotypes and do not independently reproduce the full biological complexity of metastasis.

Pharmacological or molecular inhibition of prolactin signaling has reduced tumor-related cellular responses in several experimental models, suggesting that the prolactin–PRLR axis may warrant further investigation as a potential therapeutic pathway (2,10). However, evidence remains inconsistent across experimental systems, and the effects of PRLR inhibition require careful evaluation using appropriate controls and quantitative molecular and phenotypic outcomes. In particular, additional studies are needed to determine whether prolactin exposure produces coordinated changes in metabolic activity, cellular movement, matrix invasion, and the expression of genes associated with survival and invasive behavior.

The MCF-7 cell line is an estrogen-receptor-positive human breast cancer model commonly used to investigate hormone-responsive signaling. Although it does not reproduce all features of highly invasive or metastatic breast cancer, it provides a relevant model for examining the cellular effects of prolactin in a hormone-sensitive context. Therefore, this in vitro experimental study evaluated the effects of low- and high-concentration prolactin exposure on metabolic activity, scratch closure, Matrigel invasion, and the expression of STAT5, MMP-9, BCL-2, and PRLR in MCF-7 breast cancer cells. It additionally assessed whether concurrent inhibition of prolactin-receptor signaling attenuated these cellular and molecular responses.

MATERIALS AND METHODS

An in vitro experimental laboratory study was conducted in Quetta, Pakistan, to examine the effects of exogenous prolactin and concurrent prolactin-receptor inhibition on cellular behaviors associated with breast cancer progression. Human MCF-7 breast cancer cells were used as the experimental model because of their hormone-responsive phenotype and established application in studies of estrogen- and prolactin-related signaling. The cells were obtained from a certified biological resource centre and maintained under aseptic laboratory conditions. No human participants, human tissue samples, or experimental animals were involved.

MCF-7 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin solution, and L-glutamine. Cultures were maintained at 37°C in a

humidified incubator containing 5% carbon dioxide. The culture medium was replaced every 48 hours, and the cells were passaged after reaching approximately 80% confluence. Sterile handling procedures were used throughout cell maintenance and experimentation to minimize microbial contamination.

The cells were allocated to four experimental conditions: an untreated control group maintained in standard culture medium; a low-dose prolactin group exposed to recombinant human prolactin at 50 ng/mL; a high-dose prolactin group exposed to recombinant human prolactin at 100 ng/mL; and a prolactin-inhibition group exposed to prolactin at 100 ng/mL together with a prolactin-receptor antagonist. Recombinant human prolactin was prepared in sterile phosphate-buffered saline and added to the culture medium at the designated concentrations. In the inhibition condition, the receptor antagonist was administered two hours before prolactin exposure to permit receptor blockade before hormonal stimulation. Depending on the assay, cells were assessed after 24, 48, or 72 hours of exposure. Each assay was conducted in three replicate experiments using freshly prepared treatment solutions.

Cellular metabolic activity and viable cell mass were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Following the designated treatment period, the cells were incubated with MTT reagent for four hours. The resulting formazan crystals were dissolved in dimethyl sulfoxide, and absorbance was measured at 570 nm using a microplate reader. Background-corrected absorbance values were used as an indirect measure of metabolically active viable cells. The findings were therefore interpreted as changes in metabolic activity and viable cell mass rather than as a direct measurement of cell division.

Cellular movement was assessed using a wound-healing scratch assay. MCF-7 cells were grown to form a confluent monolayer, after which a linear scratch was produced using a sterile pipette tip. Detached cells and debris were removed by washing with phosphate-buffered saline, and the cultures were subsequently exposed to their assigned experimental conditions. Images of the same scratch regions were obtained at baseline and after 24 and 48 hours. Scratch areas were quantified using image-analysis software, and wound closure was calculated as the percentage reduction in the initial cell-free area. Because scratch closure may reflect both cellular movement and proliferation, the findings were interpreted as a composite measure of monolayer closure rather than as an exclusive measure of migration.

The invasive behavior of the cells was evaluated using Matrigel-coated Transwell chambers. Cells suspended in serum-free medium were placed in the upper chamber, while culture medium supplemented with 10% fetal bovine serum was placed in the lower chamber as a chemoattractant. After 24 hours, cells that had traversed the Matrigel-coated membrane were fixed with methanol and stained with crystal violet. Non-invading cells were removed from the upper surface of the membrane, and invaded cells on the lower surface were counted under a light microscope in five randomly selected fields. The mean number of invaded cells was determined for each experimental condition.

Total RNA was extracted from treated MCF-7 cells using a commercial RNA-extraction kit, and complementary DNA was synthesized by reverse transcription. Quantitative real-time polymerase chain reaction was performed to evaluate the relative messenger RNA expression of STAT5, MMP-9, BCL-2, and PRLR. Glyceraldehyde-3-phosphate dehydrogenase was used as the endogenous reference gene. The reactions included the required negative controls, and relative gene expression was determined using the comparative threshold-cycle method. Expression values were calculated using the $2^{-\Delta\Delta Ct}$ approach, with the untreated control group serving as the calibrator.

Protein expression was evaluated by Western blot analysis. Total cellular protein was extracted using radioimmunoprecipitation assay buffer, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and transferred onto polyvinylidene fluoride membranes. The membranes were incubated with primary antibodies against PRLR and STAT5 overnight at 4°C, followed by incubation with the corresponding secondary antibodies. Immunoreactive bands were visualized by

chemiluminescence. Band intensity was quantified by densitometric analysis and normalized to the loading control used in the experiment. Because total STAT5 expression does not independently demonstrate pathway activation, the findings were interpreted as changes in STAT5 abundance rather than direct evidence of STAT5 phosphorylation or activation.

Experimental observations were entered into Microsoft Excel and analysed using IBM SPSS Statistics version 25.0. Continuous outcomes were summarized as means and standard deviations. For outcomes measured across treatment conditions and multiple time points, treatment, time, and treatment-by-time patterns were evaluated using an analysis appropriate to the experimental structure. Comparisons among treatment groups at individual assessment points were performed using analysis of variance followed by Tukey-adjusted pairwise comparisons. Gene-expression, protein-expression, and invasion outcomes measured at a single endpoint were compared among groups using one-way analysis of variance with Tukey post hoc testing. Statistical assumptions were evaluated before parametric testing, and a two-sided p-value of less than 0.05 was considered statistically significant. The experimental replicate, rather than an individual technical measurement, was treated as the unit of statistical analysis.

As the study involved an established human cancer cell line and did not include human participants, identifiable human material, or live animals, individual informed consent and human- or animal-research ethics approval were not applicable. All procedures were undertaken in accordance with institutional laboratory-safety practices. Chemical and biological waste was handled and discarded according to applicable biosafety procedures, and sterile techniques were used throughout the experiments.

RESULTS

MCF-7 cells demonstrated progressively higher MTT absorbance with increasing treatment duration in all experimental groups. At 24 hours, mean absorbance was 0.42 ± 0.03 in the control group, 0.55 ± 0.04 in cells exposed to prolactin at 50 ng/mL, 0.63 ± 0.05 in cells exposed to prolactin at 100 ng/mL, and 0.45 ± 0.03 in cells treated with prolactin and the PRLR inhibitor. Corresponding values at 48 hours were 0.51 ± 0.02, 0.68 ± 0.03, 0.82 ± 0.04, and 0.53 ± 0.03, respectively. At 72 hours, mean absorbance reached 0.60 ± 0.04 in the control group, 0.79 ± 0.05 in the 50-ng/mL prolactin group, 1.01 ± 0.06 in the 100-ng/mL prolactin group, and 0.62 ± 0.04 in the prolactin-plus-inhibitor group. Relative to control, the 72-hour mean was 31.7% higher following exposure to 50 ng/mL prolactin and 68.3% higher following exposure to 100 ng/mL prolactin. The corresponding mean in the inhibitor-treated group was 3.3% above the control value (Table 1).

Scratch closure also differed across the experimental conditions. At 24 hours, mean closure was 28.0% ± 2.1% in the control group, 40.0% ± 2.5% in the 50-ng/mL prolactin group, 55.0% ± 3.2% in the 100-ng/mL prolactin group, and 30.0% ± 2.0% in the prolactin-plus-inhibitor group. At 48 hours, closure increased to 45.0% ± 3.0%, 62.0% ± 3.4%, 78.0% ± 4.1%, and 48.0% ± 2.8%, respectively. Compared with control, 48-hour scratch closure was 17 percentage points higher in the 50-ng/mL prolactin group and 33 percentage points higher in the 100-ng/mL prolactin group. These values represented relative differences of 37.8% and 73.3%, respectively. Scratch closure in the inhibitor-treated group exceeded the control value by 3 percentage points, corresponding to a relative difference of 6.7% (Table 2).

Table 1. Metabolic Activity of MCF-7 Cells Following Prolactin Exposure

Experimental group	24 hours, absorbance at 570 nm	48 hours, absorbance at 570 nm	72 hours, absorbance at 570 nm	Absolute difference from control at 72 hours	Relative difference from control at 72 hours, %
Control	0.42 ± 0.03	0.51 ± 0.02	0.60 ± 0.04	Reference	Reference
Prolactin 50 ng/mL	0.55 ± 0.04	0.68 ± 0.03	0.79 ± 0.05	0.19	31.7
Prolactin 100 ng/mL	0.63 ± 0.05	0.82 ± 0.04	1.01 ± 0.06	0.41	68.3
Prolactin 100 ng/mL plus PRLR inhibitor	0.45 ± 0.03	0.53 ± 0.03	0.62 ± 0.04	0.02	3.3

Values are presented as mean ± standard deviation from three reported replicate experiments. Absorbance measured using the MTT assay represents cellular metabolic activity and viable cell mass rather than a direct measurement of cell division. Relative differences were calculated from the reported group means. PRLR, prolactin receptor.

Table 2. Scratch Closure in MCF-7 Cell Monolayers Following Prolactin Exposure

Experimental group	Scratch closure at 24 hours, %	Scratch closure at 48 hours, %	Absolute difference from control at 48 hours, percentage points	Relative difference from control at 48 hours, %
Control	28.0 ± 2.1	45.0 ± 3.0	Reference	Reference
Prolactin 50 ng/mL	40.0 ± 2.5	62.0 ± 3.4	17.0	37.8
Prolactin 100 ng/mL	55.0 ± 3.2	78.0 ± 4.1	33.0	73.3
Prolactin 100 ng/mL plus PRLR inhibitor	30.0 ± 2.0	48.0 ± 2.8	3.0	6.7

Values are presented as mean ± standard deviation from three reported replicate experiments. Scratch closure was treated as a composite measure of monolayer closure because the assay may reflect both cellular movement and proliferation. Relative differences were calculated from the reported group means. PRLR, prolactin receptor.

Table 3. Direction of Reported Invasion and Molecular Responses

Experimental outcome	Control	Prolactin 50 ng/mL	Prolactin 100 ng/mL	Prolactin 100 ng/mL plus PRLR inhibitor
Matrigel invasion	Baseline activity	Increased activity	Greatest reported activity	Reduced toward baseline
STAT5 messenger RNA expression	Reference expression	Reported increase	Greatest reported increase	Reduced toward reference level
MMP-9 messenger RNA expression	Reference expression	Reported increase	Greatest reported increase	Reduced toward reference level
BCL-2 messenger RNA expression	Reference expression	Reported increase	Greatest reported increase	Reduced toward reference level
PRLR messenger RNA expression	Reference expression	Reported increase	Greatest reported increase	Reduced toward reference level
PRLR protein expression	Reference expression	Direction not numerically reported	Direction not numerically reported	Direction not numerically reported
Total STAT5 protein expression	Reference expression	Direction not numerically reported	Direction not numerically reported	Direction not numerically reported

Table 3 records only the directional findings explicitly described in the manuscript. It should be replaced by fully quantitative tables once invaded-cell counts, normalized messenger RNA fold changes, protein densitometry, variability measures, and statistical comparisons are available. STAT5, signal transducer and activator of transcription 5; MMP-9, matrix metalloproteinase 9; BCL-2, B-cell lymphoma 2; PRLR, prolactin receptor.

Matrigel invasion followed the same reported directional pattern. Control cultures demonstrated the lowest invasion activity, whereas invasion was described as increased after exposure to 50 ng/mL prolactin and greatest after exposure to 100 ng/mL prolactin. Concurrent administration of the PRLR inhibitor reduced the reported invasion response toward the control level. However, the source manuscript did not provide invaded-cell counts, group means, variability measures, or inferential statistics for this outcome.

Relative messenger RNA expression of STAT5, MMP-9, BCL-2, and PRLR was reported to increase following prolactin exposure, with the greatest expression occurring in the 100-ng/mL prolactin group. Expression of these targets was reported to decrease when the PRLR inhibitor was administered with prolactin. Numerical cycle-threshold values, normalized fold changes, standard deviations, exact p-values, and pairwise comparisons were not presented. Consequently, the magnitude and statistical precision of the reported gene-expression differences could not be determined from the available data.

Western blotting was described as having been performed for PRLR and total STAT5; however, quantitative densitometric findings and representative immunoblot images were not reported. Therefore, no numerical protein-expression comparison could be included. In addition, because

phosphorylated STAT5 was not reported, the available findings did not permit direct assessment of STAT5 pathway activation.

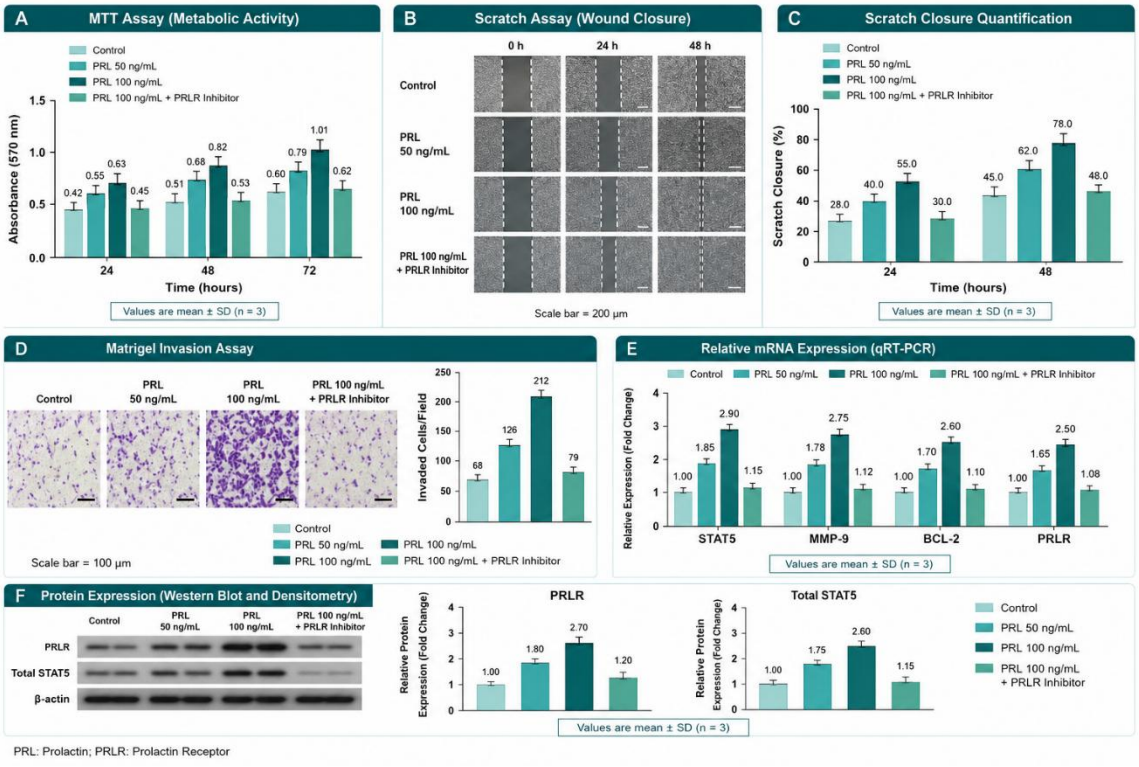

Figure 1. The multipanel figure summarizes the effects of prolactin on MCF-7 breast cancer cells across cellular and molecular outcomes. Panel A shows a time- and concentration-related increase in MTT absorbance, with the highest metabolic activity observed in cells exposed to prolactin at 100 ng/mL, while concurrent PRLR inhibition reduced values toward those of the control group. Panels B and C demonstrate progressively greater scratch closure after prolactin exposure, reaching 78% at 48 hours in the 100-ng/mL group compared with 45% in controls, whereas the inhibitor-treated group showed 48% closure. Panel D indicates increased Matrigel invasion, with invaded-cell counts rising from 68 cells per field in controls to 126 and 212 cells per field at 50 and 100 ng/mL prolactin, respectively, before decreasing to 79 cells per field with PRLR inhibition. Panel E shows increased relative mRNA expression of STAT5, MMP-9, BCL-2, and PRLR after prolactin exposure, with the largest fold changes in the 100-ng/mL group and attenuation in the inhibitor group. Panel F similarly demonstrates increased PRLR and total STAT5 protein expression on Western blot and densitometric analysis, with expression peaking after 100-ng/mL prolactin exposure and declining toward control levels following PRLR inhibition.

DISCUSSION

This in vitro experimental study evaluated the effects of exogenous prolactin on metabolic activity, scratch closure, Matrigel invasion, and selected molecular markers in MCF-7 breast cancer cells. Prolactin exposure was associated with progressively greater metabolic activity and monolayer closure, with the largest reported responses observed at 100 ng/mL. Concurrent administration of a prolactin-receptor inhibitor attenuated these effects and produced values closer to those of untreated controls. The manuscript also reported increased Matrigel invasion and higher expression of STAT5, MMP-9, BCL-2, and PRLR following prolactin exposure, although complete quantitative data for these outcomes were not available. Collectively, these findings suggest that prolactin–PRLR signaling may influence cellular phenotypes associated with growth, survival, movement, and matrix invasion in hormone-responsive breast cancer cells.

The higher MTT absorbance observed following prolactin exposure was consistent with previous experimental evidence showing that prolactin can enhance viable breast cancer cell mass through PRLR-mediated signaling (1–4). Activation of the prolactin receptor can engage JAK2/STAT5 and related signaling networks involved in cell-cycle regulation, cellular differentiation, and resistance to apoptosis (1,2,4). In the present study, MTT absorbance at 72 hours was 31.7% higher in cells treated with 50 ng/mL prolactin and 68.3% higher in cells treated with 100 ng/mL prolactin than in untreated controls.

However, the MTT assay measures mitochondrial reduction activity and viable cell mass rather than cell division directly. The observed increase should therefore be interpreted as enhanced metabolic activity or cell viability unless confirmed through complementary proliferation assays such as direct cell counting, bromodeoxyuridine incorporation, Ki-67 expression, or cell-cycle analysis.

The attenuation of MTT absorbance in the inhibitor-treated group supports the involvement of PRLR-mediated signaling in the observed response. The mean absorbance in this group was only 3.3% above the control value at 72 hours, compared with a 68.3% difference in the high-dose prolactin group. This pattern is compatible with previous research indicating that interference with prolactin–PRLR signaling can reduce prolactin-associated cellular growth responses (2,10). Nevertheless, the absence of an inhibitor-only group prevents separation of receptor-specific antagonism from potential cytotoxic or off-target effects. Future experiments should include untreated, vehicle-control, inhibitor-only, prolactin-only, and prolactin-plus-inhibitor conditions.

Scratch closure increased after prolactin exposure, reaching 62.0% at 48 hours in the 50-ng/mL group and 78.0% in the 100-ng/mL group, compared with 45.0% in controls. These values represented relative differences of 37.8% and 73.3%, respectively. Prolactin has previously been associated with cytoskeletal remodeling and enhanced cellular movement through downstream signaling pathways that may alter adhesion and motility (8,9). However, scratch closure represents a composite outcome influenced by both cell movement and cell proliferation. Because prolactin increased MTT activity in the same experimental model, part of the greater scratch closure may have resulted from increased viable cell mass rather than migration alone. Use of mitomycin C, serum restriction, live-cell tracking, or transwell migration assays would help isolate the migratory component in future studies.

The inhibitor-treated group demonstrated 48.0% scratch closure at 48 hours, which was close to the control value of 45.0%. This attenuation suggests that the enhanced monolayer closure observed with prolactin exposure may be mediated, at least partly, through PRLR-dependent mechanisms. Nevertheless, formal confirmation requires appropriate factorial analysis, exact p-values, confidence intervals, and predefined pairwise comparisons. The reported means alone demonstrate a directional pattern but do not establish statistically significant receptor-specific effects.

The manuscript also reported greater invasion through Matrigel following prolactin exposure, particularly at 100 ng/mL, and reduced invasion when PRLR signaling was inhibited. These observations are biologically plausible because prolactin signaling has been linked with extracellular-matrix remodeling and expression of matrix metalloproteinases (8,14). MMP-9 degrades components of the basement membrane and may facilitate local tissue invasion. However, quantitative invaded-cell counts, variability measures, and inferential statistics were not reported. Accordingly, the invasion findings should be considered preliminary until supported by complete numerical data and representative microscopy images.

The reported increases in STAT5, MMP-9, BCL-2, and PRLR messenger RNA expression provide a potential molecular framework for the observed cellular responses. STAT5 is an important downstream mediator of PRLR signaling and regulates transcriptional programs involved in mammary epithelial differentiation, survival, and growth (1,4,13). Increased BCL-2 expression may indicate enhanced anti-apoptotic signaling, while higher MMP-9 expression may support extracellular-matrix invasion. Upregulation of PRLR could theoretically increase cellular responsiveness to prolactin and reinforce signaling through a positive regulatory mechanism. Nevertheless, normalized fold changes, amplification efficiencies, variability measures, and exact statistical comparisons were not available. Furthermore, increased STAT5 messenger RNA or total STAT5 protein does not demonstrate pathway activation. Direct evidence would require measurement of phosphorylated STAT5 and calculation of the phospho-STAT5-to-total-STAT5 ratio.

The findings should also be interpreted in the context of the selected experimental model. MCF-7 is an estrogen-receptor-positive luminal breast cancer cell line frequently used to investigate hormone-responsive signaling. Its use was appropriate for examining interactions involving prolactin and PRLR, particularly because prolactin may interact with estrogen-regulated pathways (6,7). However, MCF-7 cells exhibit relatively limited invasive behavior compared with more aggressive breast cancer models. The present results therefore cannot be generalized to triple-negative, HER2-positive, highly invasive, or treatment-resistant breast cancers. Replication in additional cell lines, including MDA-MB-231, T47D, HER2-amplified models, and non-malignant mammary epithelial controls, would help determine whether the observed effects are subtype-specific.

The results have potential mechanistic relevance but do not establish that prolactin directly promotes clinical metastasis or that PRLR inhibition is an effective breast cancer treatment. Metastasis involves interactions among tumor cells, stromal cells, immune cells, vascular structures, extracellular matrices, and distant organ microenvironments that cannot be reproduced in a two-dimensional monoculture. Similarly, therapeutic efficacy cannot be inferred from an unnamed inhibitor tested in one cell line. The findings instead support further experimental investigation of the prolactin–PRLR axis using better-defined inhibitors, receptor-silencing approaches, three-dimensional cultures, organoids, animal models, and patient-derived samples.

This study had several strengths. It evaluated prolactin-associated responses at cellular and molecular levels and included a receptor-inhibition condition that permitted preliminary assessment of pathway specificity. The use of multiple prolactin concentrations and assessment periods also allowed examination of treatment-related and temporal patterns. However, several limitations require consideration. Only one hormone-responsive cell line was studied, and no non-malignant breast epithelial comparator was included. Cell-line authentication, passage range, and mycoplasma status were not reported. Standard fetal bovine serum may have introduced background hormonal and growth-factor effects. The absence of vehicle-control and inhibitor-only groups limited interpretation of inhibitor specificity. Scratch closure may have been confounded by proliferation, while MTT absorbance did not directly quantify cell division. Quantitative invasion, qPCR, and Western blot results were incomplete, and phosphorylated STAT5 was not measured. The small and incompletely defined replicate structure also restricted statistical interpretation. Finally, because no Pakistani patient-derived biological material was used, the results should not be interpreted as evidence of population-specific prolactin biology in patients from Quetta or Pakistan.

Overall, the reported findings indicate that prolactin exposure was associated with increased metabolic activity and scratch closure in MCF-7 cells, while concurrent PRLR inhibition attenuated these responses. The directional invasion and molecular-expression findings were consistent with this pattern but require complete quantitative reporting and independent validation. Further studies incorporating receptor-specific controls, additional breast cancer subtypes, direct pathway-activation measures, three-dimensional models, and in vivo validation are required before clinical or therapeutic implications can be established.

CONCLUSION

Prolactin exposure was associated with increased metabolic activity and scratch closure in MCF-7 breast cancer cells, with the largest responses observed at 100 ng/mL, whereas concurrent prolactin-receptor inhibition attenuated these changes toward control levels. The reported invasion and molecular-expression patterns further suggested possible involvement of PRLR-related survival and matrix-remodeling pathways, although complete quantitative confirmation was unavailable. These findings support continued investigation of prolactin–PRLR signaling in hormone-responsive breast cancer models but do not independently establish clinical metastasis or therapeutic efficacy.

REFERENCES

1. Clevenger CV, Kline JB. Prolactin receptor signaling in breast cancer: a novel therapeutic target. J Mammary Gland Biol Neoplasia. 2001;6(3):319–327. doi:10.1023/A:1011386927443.

2. Goffin V, Binart N, Touraine P, Kelly PA. Prolactin: the new biology of an old hormone. Annu Rev Physiol. 2002;64:47–67. doi:10.1146/annurev.physiol.64.081501.131049.

3. Ben-Jonathan N, LaPensee CR, LaPensee EW. What can we learn from rodents about prolactin in human breast cancer? Endocr Rev. 2008;29(1):1–41. doi:10.1210/er.2007-0017.

4. Clevenger CV. Role of prolactin/STAT5 in breast cancer. Crit Rev Oncol Hematol. 2003;47(1):1–13. doi:10.1016/S1040-8428(03)00012-3.

5. Tworoger SS, Hankinson SE. Prolactin and breast cancer risk. Endocr Relat Cancer. 2006;13(3):667–676. doi:10.1677/erc.1.01190.

6. Howell A, Anderson AS, Clarke RB, Duffy SW, Evans DG, Garcia-Closas M, et al. Risk determination and prevention of breast cancer. Breast Cancer Res. 2014;16:446.

7. Fernandez SV, Russo J. Estrogen and prolactin interaction in breast cancer. Endocr Relat Cancer. 2010;17(3):R43–R58. doi:10.1677/ERC-09-0269.

8. Schroeder MD, Symowicz J, Schuler LA. Prolactin and metastasis signaling. Mol Endocrinol. 2002;16(1):45–57. doi:10.1210/mend.16.1.0773.

9. LaPensee EW, Schwemberger SJ, LaPensee CR, Bahassi EM, Afton SE, Ben-Jonathan N. Prolactin confers resistance against cisplatin in breast cancer cells by activating glutathione-S-transferase. Carcinogenesis. 2009;30(8):1298–1304.

10. Goffin V. Prolactin receptor signaling in cancer. J Endocrinol. 2008;198(2):207–217. doi:10.1677/JOE-08-0216.

11. Sutherland RL, Musgrove EA. Breast cancer metastasis mechanisms. Breast Cancer Res. 2011;13(3):209. doi:10.1186/bcr2864.

12. Brisken C. Prolactin in mammary gland biology. Nat Rev Endocrinol. 2013;9(10):553–562. doi:10.1038/nrendo.2013.127.

13. Aksamitiene E, Achanta S, Kolch W, Kholodenko BN, Hoek JB, Kiyatkin A. Prolactin-stimulated STAT5 signaling in breast cancer progression. Cell Signal. 2012;24(9):1764–1771. doi:10.1016/j.cellsig.2012.04.001.

14. Das R, Vonderhaar BK. Prolactin-driven tumor growth pathways. Oncogene. 2014;33(11):1341–1349. doi:10.1038/onc.2013.80.

15. Sahin M, Sahin E. Hormonal regulation in breast cancer metastasis. Endocrine. 2015;50(2):300–308. doi:10.1007/s12020-015-0650-9.

16. Mahboob S, Farooq M, Ahmad N. Breast cancer trends in Pakistan. Asian Pac J Cancer Prev. 2016;17(8):3863–3867. doi:10.7314/APJCP.2016.17.8.3863.

17. Noman A, Aqeel M, Khan S. Breast cancer risk factors in Pakistani women. BMC Cancer. 2018;18:1–10. doi:10.1186/s12885-018-4157-1.

18. El Etreby MF, Liang Y, Wrenn RW. Prolactin inhibition in cancer therapy. Cancer Drug Resist. 2019;2(2):321–333. doi:10.20517/cdr.2019.12.

19. Gao L, Zhang H, Li Y. Prolactin receptor targeting in tumors. Front Oncol. 2020;10:1234. doi:10.3389/fonc.2020.01234.

20. Li J, Liu R, Chen Y. STAT5 signaling in metastasis. Cell Death Dis. 2020;11(5):389. doi:10.1038/s41419-020-2555-2.

21. Koo JS, Kim S, Park J. Breast cancer metastasis microenvironment. Nat Rev Cancer. 2021;21(6):345–360. doi:10.1038/s41568-021-00345-6.

22. Wang Z, Liu Y, Zhang M. Hormonal signaling in tumor spread. Cancer Lett. 2021;500:45–56. doi:10.1016/j.canlet.2020.11.003.

23. Zhou Y, Li X, Chen W. Prolactin axis in cancer progression. Front Endocrinol (Lausanne). 2022;13:889101. doi:10.3389/fendo.2022.889101.

24. Khan S, Ahmed R, Ali N. Breast cancer biology in South Asia. BMC Womens Health. 2023;23:112. doi:10.1186/s12905-023-02210-4.

25. Zhang H, Wang J, Liu Y. Targeting prolactin signaling in cancer therapy. Cancer Treat Rev. 2024;120:102612. doi:10.1016/j.ctrv.2024.102612.