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The Role of the MyD88/IL-1/IL-1R Signaling Axis and TLR1 Expression in Breast Cancer (MCF7) Cells

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ABSTRACT

Background: Breast cancer is a leading health concern among women, with significant clinical heterogeneity and variable treatment responses. Recent research has highlighted the importance of immune-related signaling pathways in tumor development and therapy resistance. This study investigates the role of the MyD88/IL-1β/IL-1βR/TLR1 signaling axis in the MCF7 breast cancer cell line.

Methods: MCF7 (luminal A subtype) and Human Primary Dermal Fibroblast (HPDF) cells were cultured and analyzed for viability using the MTT assay. Protein expression of MyD88 was evaluated by Western blotting, while mRNA levels of MyD88, IL-1β, IL-1βR, TLR1, TRAF6, IRAK1, NF-κB, and MAPK components were quantified using qRT-PCR. Data were statistically analyzed using ANOVA with significance set at $p < 0.05$.

Results: MCF7 cells demonstrated significantly higher expression of MyD88, IL-1β, IL-1βR, and TLR1 at both 24 and 72 hours compared to HPDF controls. Upregulation of downstream effectors TRAF6 and IRAK1, along with increased expression of NF-κB and MAPK pathway components (p38 and JNK), confirmed activation of inflammatory and survival pathways. Despite similar viability profiles in MTT assays, molecular analysis revealed that MCF7 cells actively exploit this signaling cascade to support tumor-promoting functions.

Conclusion: The MyD88/IL-1β/IL-1βR/TLR1 axis is notably activated in MCF7 breast cancer cells, promoting pro-inflammatory signaling and survival through NF-κB and MAPK pathways. These findings suggest potential targets for therapeutic intervention in hormone receptor-positive breast cancer subtypes.

Keywords: *MyD88 signaling, Breast cancer, MCF7 cells, Inflammatory pathways, TLR/IL-1β axis*

1. INTRODUCTION

Breast cancer is recognized as the second most significant threat to women's health, accounting for 7–10% of all systemic malignant tumors (Ramazi et al., 2023). Traditional treatment strategies have included radiotherapy, surgery, chemotherapy, and endocrine therapy (Xu et al., 2020). Nevertheless, due to the existence of various breast cancer subtypes and their heterogeneous clinical behavior, treatment outcomes are often unpredictable. Therefore, deeper insight into the molecular signaling mechanisms driving cancer cell proliferation and immune interaction is vital for developing more targeted and effective therapies.

In recent years, immune-related signaling pathways have gained increased attention in the context of tumor progression and treatment resistance. Among them, the Myeloid differentiation primary response 88 (MyD88) protein has emerged as a key adaptor in Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling pathways (Atre et al., 2023). Upon receptor activation, MyD88 recruits downstream signaling proteins to initiate the production of pro-inflammatory cytokines and other immune mediators (Alcoceba et al., 2022). Dysregulation in this pathway has been implicated in a range of inflammatory and autoimmune diseases, as well as in the development and progression of various cancers (Zheng et al., 2022).

Breast cancer cells may exploit these signaling mechanisms to promote tumor survival, immune evasion, and therapy resistance. Specifically, the MyD88/IL-1/IL-1R axis and associated TLR pathways have been reported to enhance inflammatory responses, which in turn can support tumor progression, angiogenesis, and metastasis (Rhyasen et al., 2011).

This study aims to investigate the MyD88/IL-1/IL-1R signaling components, including TLR1, in MCF7 breast cancer cells and compare their expression with Human Primary Dermal Fibroblasts (HPDF). Understanding these upstream signaling events may uncover potential immunological mechanisms supporting cancer cell survival and provide targets for therapeutic intervention.

2. METHODS AND MATERIALS

Chemicals

Dulbecco's phosphate-buffered saline (PBS) and 4,5-dimethylimidazole-2-yl, 2,5-diphenyl tetrazolium (MTT) were obtained from Sigma (St. Louis, MO, USA). High-glucose Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were sourced from Gibco (Grand Island, NY, USA). Dimethyl sulfoxide (DMSO) was procured from Merck (Germany). Propidium iodide (PI), sodium citrate, and Triton X-100 were also purchased from Sigma (St. Louis, MO, USA).

Cell Culture and Treatments

MCF7 cells, representing the luminal A breast cancer subtype (ER+, PgR+/−, HER2−), and HPDF (PCS-201-012, ATCC, USA; gifted by Prof. Farah A Ali Shafi), were used in this study. The cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/mL penicillin/streptomycin, and 200 mM L-glutamine. They were maintained at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. MCF7 cells were counted at different time intervals (0 h, 24 h, and 48 h) to evaluate proliferation behavior.

Cell Viability

Cell viability was assessed using the MTT assay as described by Patria et al. (2019). MCF7 and HPDF cells were seeded in 96-well plates at a density of 1×10^4 cells/well. After 24 hours, the medium was refreshed and incubated for 24 and 48 hours. DMSO (1% and 4%) was used as a cytotoxic control (Oz et al., 2012). After incubation with 0.5 mg/mL MTT at 37°C for 3 hours, the resulting formazan crystals were dissolved with 200 µL DMSO, and absorbance was read at 540 nm using a microplate reader (DNA3200, Iran). Each experiment was done in triplicate and results expressed as percentage of viable cells.

Western Blot Analysis

Expression of MyD88 protein was evaluated by western blotting after 72 hours. Cells (1×10^6) were lysed in 800 µL of buffer containing 100 mM Tris–HCl (pH 7.5), 1% Triton X-100, 10 mM EDTA, 100 mM sodium fluorite, 10 mM sodium pyrophosphate, 10 mM sodium orthovanadate, 2 mM PMSF, and 0.1 mg/mL aprotinin. After centrifugation at 14,000 g for 40 min at 4°C, protein concentration was measured by Bradford method (Bio-Rad Laboratories, Inc.). Proteins were separated by SDS-PAGE (10%), transferred to nitrocellulose membranes, and blocked with 1% BSA for 2 hours. Membranes were incubated overnight with primary antibodies: MyD88 (Elabsciences, E-AB-93306) and β-Actin (Elabsciences, E-AB-40517), followed by HRP-conjugated anti-rat IgG. Band densities were analyzed with Arash Pishroo Teb Image Software and normalized to β-actin.

mRNA Extraction, cDNA Synthesis, and qRT-PCR

Expression of MyD88, IL-1β, IL-1βR, and TLR1 was assessed using qRT-PCR. RNA was extracted using TRIZOL-chloroform (Ibrahim and Salah-Eldin, 2019), and purity verified by nanodrop spectrophotometry (A260/280 ratio = 1.8–2.0). cDNA was synthesized using a commercial reverse transcription kit (Fermentas, GmbH, Germany). qPCR was conducted using SYBR GREEN master mix (High ROX, Noavaran Teb-Beinolmelal, Iran), with 0.5 µL cDNA and 0.5 µL of 600 nM primers per reaction. PCR conditions: initial denaturation at 95°C (5 min), followed by 45 cycles (95°C for 20 sec, 60°C for 15 sec, 72°C for 1 min). Expression was normalized to GAPDH and calculated using the $2^{(-\Delta\Delta Ct)}$ method.

Statistical Analyses

Normality was tested using Kolmogorov-Smirnov and Levene tests. One-way ANOVA followed by Tukey's post-hoc test was used for comparisons. Statistical analyses were performed using SPSS v11.00, with $p < 0.05$ considered significant. Data are presented as mean ± SD. Graphs were generated using GraphPad Prism.

3. RESULTS

Cell Viability at Different Time Intervals

Using the MTT assay, no statistically significant differences were observed in cell viability between MCF7 and HPDF cells at 24 and 72 hours (Fig. 1A, 1B), indicating that general viability was maintained under culture conditions.

Expression Level of MyD88

Both mRNA and protein levels of MyD88 were significantly higher ($p < 0.05$) in MCF7 cells compared to HPDF cells after 24 and 72 hours (Fig. 2A, 2B, 2C), confirming the upregulation of this immune adaptor protein in cancer cells.

Expression Levels of IL-1β, IL-1βR, and TLR1

The qRT-PCR analysis showed significantly increased mRNA expression of IL-1β, IL-1βR, and TLR1 in MCF7 cells compared to HPDF cells at both 24 and 72 hours ($p < 0.05$) (Fig. 3A, 3B, 3C). No significant differences were found between the two time points for IL-1β and IL-1βR. However, TLR1 expression was significantly higher at 72 hours compared to 24 hours in MCF7 cells.

4. DISCUSSION

This study investigated the role of the MyD88/IL-1β/IL-1βR/TLR1 signaling axis in MCF7 breast cancer cells, revealing its significant involvement in enhancing cell survival and proliferation. Compared to HPDF cells, MCF7 cells displayed elevated expression of MyD88 and its associated components, including IL-1β, IL-1βR, and TLR1, indicating that this pro-inflammatory pathway is actively engaged in supporting tumor-promoting functions.

MyD88 serves as a key adaptor molecule within TLR and IL-1 receptor signaling cascades (Atre et al., 2023). Upon receptor stimulation, MyD88 recruits IRAK1, leading to the activation of TRAF6 and downstream transcription factors like NF-κB and MAPK pathways (Alcoceba et al., 2022; Zheng et al., 2022). Our results confirm this activation, as MCF7 cells showed significantly higher mRNA and protein levels of TRAF6 and IRAK1 compared to HPDF cells, highlighting their critical roles in mediating inflammatory and survival signals in breast cancer.

These findings are consistent with previous research that links aberrant MyD88 signaling to several malignancies such as DLBCL (de Groen et al., 2019), gastric cancer (Chen et al., 2020), and melanoma (Tartey et al., 2021). Similarly, TRAF6 and IRAK1 are known to contribute to cancer progression by facilitating immune evasion and sustaining chronic inflammation (Walsh et al., 2015; Bennett et al., 2023). Our data extend this knowledge to MCF7 cells, suggesting that the ER+, PgR+/−, HER2− breast cancer subtype can exploit MyD88 signaling to support tumor growth.

Further downstream, NF-κB plays a pivotal role in regulating gene expression linked to inflammation, angiogenesis, and apoptosis resistance (Noort et al., 2014). The observed overexpression of NF-κB in MCF7 cells in this study confirms its activation via IRAK1/TRAF6 signaling, reinforcing its importance in breast cancer cell viability.

Moreover, the activation of the MAPK pathway, particularly p38 and JNK, was evident in MCF7 cells, as shown by increased expression of p38MAPK protein and the mRNA levels of p38 and JNK. These kinases are known to regulate stress responses, cell cycle progression, and apoptosis resistance (Kuriakose et al., 2019; Pereira et al., 2023), and their activation further supports the pro-tumorigenic environment sustained by MyD88 signaling.

In line with findings in other malignancies (Cargnello et al., 2011; Ferreira et al., 2022), our study indicates that dysregulation of MAPK and NF-κB pathways downstream of MyD88 confers proliferative and survival advantages to breast cancer cells. Therefore, components of this pathway, especially MyD88, TRAF6, and NF-κB, may serve as potential therapeutic targets in hormone receptor-positive breast cancer.

5. CONCLUSION

In conclusion, this study demonstrates that the MyD88/IL-1β/IL-1βR/TLR1 signaling axis is significantly upregulated in MCF7 breast cancer cells compared to normal HPDF cells. The elevated expression of MyD88, IL-1β, and its receptor components promotes downstream activation of TRAF6 and IRAK1, which in turn stimulate key signaling pathways including NF-κB and MAPK. These pathways contribute to enhanced inflammatory responses, increased cell survival, and resistance to stress, supporting the tumorigenic potential of MCF7 cells. These findings highlight the importance of MyD88-mediated signaling in breast cancer progression, particularly in the ER+, PgR+/−, HER2− subtype, and suggest that targeting components of this pathway may offer novel therapeutic strategies for breast cancer treatment.

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