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# Targeting the IL-1R/MyD88 Signaling Axis: Unraveling Inflammatory Mediators Driving Breast Cancer Progression and Therapy Resistance

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**ABSTRACT**

Breast cancer is the second leading cause of cancer-related death among women, with treatment efficacy hindered by the heterogeneity of the disease. Understanding the molecular signaling pathways that govern tumor progression and therapy resistance is essential for improving clinical outcomes. One critical pathway is the IL-1 receptor (IL-1R)/MyD88 axis, which regulates immune responses and inflammation. In this study, we investigated the role of key inflammatory mediators, including IRAK1, TRAF6, NF-κB, p38MAPK, and JNK, in the progression of breast cancer. We compared the expression of these molecules in MCF7 breast cancer cells and normal human primary dermal fibroblasts (HPDF) at 24- and 72-hour time points. Our results demonstrate significantly higher expression levels of IRAK1, TRAF6, NF-κB, p38MAPK, and JNK in MCF7 cells compared to HPDF cells. These findings highlight the contribution of MyD88-mediated inflammatory signaling to breast cancer cell proliferation, survival, and resistance to apoptosis. Additionally, elevated expression of these signaling components was associated with increased activation of pro-inflammatory and pro-survival pathways, suggesting that they play a key role in tumorigenesis and therapy resistance. This study provides valuable insights into the molecular mechanisms underlying breast cancer progression and emphasizes the potential of targeting the IL-1R/MyD88 signaling pathway for therapeutic intervention, particularly in breast cancer subtypes where these pathways are dysregulated.

***Keywords:*** *Breast cancer, IL-1R/MyD88 axis, IRAK1, TRAF6, NF-κB, MAPK, Inflammation, Therapeutic resistance, Tumor progression, MCF7 cells.*

## 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). While conventional treatments such as surgery, radiotherapy, chemotherapy, and

endocrine therapy (Xu et al., 2020) have improved outcomes, therapeutic efficacy remains inconsistent due to the heterogeneity of breast cancer subtypes. Thus, there is a critical need to understand the molecular signaling mechanisms that drive tumor progression and influence treatment response.

One pathway of particular interest involves immune-mediated signaling via the IL-1 receptor (IL-1R)/MyD88 axis, which regulates inflammatory responses and has been implicated in various cancers. Upon activation, MyD88 recruits IL-1 receptor-associated kinases (IRAKs), notably IRAK1, leading to the activation of TNF receptor-associated factor 6 (TRAF6) and downstream signaling cascades including NF-κB and MAPK pathways (Zheng et al., 2022; Alcoceba et al., 2022). These molecular interactions contribute to cell proliferation, survival, and resistance to apoptosis, thereby supporting tumor growth and metastasis (Bennett et al., 2023; Rhyasen et al., 2014; Walsh et al., 2015).

TRAF6 and IRAK1 are known to mediate key immune and inflammatory pathways through the activation of transcription factors such as NF-κB and kinases such as p38 and JNK, which upregulate genes that sustain tumor-promoting inflammation (Li et al., 2020). Moreover, aberrant activation of these pathways can lead to enhanced production of vascular endothelial growth factor (VEGF), supporting angiogenesis and immune evasion (Noort et al., 2014). Elevated IL-1R/MyD88 signaling has also been linked to resistance against conventional therapies like tamoxifen, through persistent activation of pro-survival and anti-apoptotic mechanisms (Zheng et al., 2024).

To explore the involvement of these inflammatory mediators in breast cancer, the current study compares the expression levels of IRAK1, TRAF6, NF-κB, p38, p38MAPK, and JNK in MCF7 breast cancer cells and normal human primary dermal fibroblasts (HPDF). Additionally, cell viability at 24 and 72 hours was assessed to determine the functional impact of these molecular changes. This investigation aims to provide further insight into the role of IL-1R/MyD88 downstream signaling components in breast cancer progression and their potential as therapeutic targets.

## 2. METHODS AND MATERIALS Chemicals

All reagents used were the same as those described in the first article, including Dulbecco's phosphate-buffered saline (PBS), MTT (Sigma, St. Louis, MO, USA), DMEM, FBS, and antibiotics (Gibco, Grand Island, NY, USA). DMSO was obtained from Merck (Germany), and other reagents such as PI, sodium citrate, and Triton X-100 were from Sigma (USA).

### Cell Culture and Treatments

MCF7 cells (luminal A subtype) and HPDF cells (PCS-201-012, ATCC, USA) were cultured under identical conditions to those previously described: DMEM with 10% FBS, 100 IU/mL penicillin-streptomycin, and 200 mM L-glutamine. Cultures were maintained at 37°C in a 5% $CO_2$ incubator. Proliferation was monitored at 0 h, 24 h, and 48 h.

### Cell Viability

MTT assay was performed following the standard protocol as outlined in the previous study (Patria et al., 2019). Cells were seeded at $1 \times 10^4$ cells/well in 96-well plates. After 24 and 48 hours, MTT was added, and formazan crystals were solubilized in DMSO. Absorbance was read at 540 nm. DMSO controls (1% and 4%) were included to evaluate cytotoxic effects.

### Western Blot Analysis

To assess the expression levels of NFkβ, and p38 MAPK in MCF7 and HPDF cells following 72 h, 1×106 cells were collected and undergone homogenization process. This process was performed in 800 µl of lysis 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 phenylmethylsulphonyl fluorite (PMSF), and 0.1 mg aprotinin/mL The homogenate was centrifuged at 14,000 g for 40 min at 4ºC. The total protein contents of the ice-chilled supernatant were analyzed by the Bradford method (Bio-Rad Laboratories, Inc.) in a plate spectrophotometer. Next, the samples were submitted to 10% SDS–polyacrylamide gel electrophoresis and transferred into nitrocellulose membrane. A 1% BSA was used to block the membranes (with 2 h incubation at room temperature). The primary antibodies for MyD88 (Elabsciences, USA, Cat N: E-AB-93306, NFkβ (Elabsciences, USA, Cat N: E-AB-67677), p38 MAPK (Elabsciences, USA, Cat N: E-AB-66279), and β-Actin (Elabsciences, USA, Cat N: E-AB-40517) were used to detect the target protein at 1:1000 dilution, overnight at room temperature. Subsequently, the membranes were incubated with appropriate secondary antibodies: horseradish peroxidaseconjugated anti-rat IgG for 1 h. In order to quantify the densities of the bands obtained from the chemiluminescent membrane, the Arash Pishroo Teb Image Software for Windows was used as arbitrary unit. Target protein normalization was performed with the housekeeping beta-actin.

### mRNA Extraction, cDNA Synthesis, and qRT-PCR

To highlight the expression levels of MyD88, IRAK1, TRAF6, IL-1β, IL-1βR, TLR1, p38, and JUNK the quantitative PCR after retro-transcription (qRT-PCR) was used. Briefly, 1 × 106 cells were used and total RNA was extracted using TRIZOLchloroform as previously described (Ibrahim and Salah-Eldin, 2019). The quantity and quality of extracted RNA

were assessed using a nanodrop spectrophotometer (Thermo ScientificTM, USA, 260 nm and A260/280 ratio = 1.8-2.0). Next, cDNAs were synthesized by reverse transcription according to the protocol of the commercial kit used (Fermentas, GmbH, Germany). Next, 0.5 µl (i.e. approx. 5-10 ng) of cDNA was amplified in 10 µl 1X SYBR GREEN master mix (High ROX,

Noavaran Teb-Beinolmelal, Iran), and 0.5 µl (600 nM) of each reverse and forward primers of the targeted genes (see Table 1). PCR conditions were run as follows: 1 cycle of general denaturation at 95°C for 5 min, followed by 45 cycles (95°C for 20sec, annealing temperature: 60°C for 15 sec, elongation: 72°C for 1 min). The mean threshold cycle (TC) values of the target PCR fragment from triplicate reactions were normalized by subtracting the mean TC value of the housekeeping GAPDH transcript. The relative expression level of the mRNA of interest was calculated by the following equation: $2^{(-\Delta\Delta Ct)}$.

**Table 1: primer sequences for corresponding genes**

| Gene | Forward | Reverse |
|---|---|---|
| IRAK (Interleukin-1 receptorassociated kinase) | 5'-GACCGACTTCTACAGCCACA-3' | 5'-CCTGAGGTGAAGTCGACGAA-3' |
| TRAF6 | 5'-AGCTGAGGACTGGGTGTTCA-3' | 5'-CGGGTGATGGCTGGTACTT-3' |
| NF-κB (Nuclear factor kappa B) | 5'-AGCACAGATACCACCAAGAC-3' | 5'-CAGCTGAAGGGCCTTCTGTT-3' |
| p38 (Mitogen-activated protein kinase) | 5'-TGGTGACATGGAACAGTGAGG-3' | 5'-GCTGTGTTGTTGAGTGGGTC-3' |
| JNK (c-Jun N-terminal kinase) | 5'-TGGATTCGAGACTGGACCGT-3' | 5'-TGTGTGGGACTTTTGGAGGG-3' |
| ERK (Extracellular signalregulated kinase) | 5'-GTCCTACATCGTCCTGGATTG-3' | 5'-CAGCTTCATCTTCTCCATGTCC-3' |
| B-Actin | 5'-CCCGCGAGTACAACCTTCT-3′ | 5′-CGTCATCCATGGCGAACT-3′ |

**Statistical Analyses**

All statistical procedures were identical to the first article. Kolmogorov-Smirnov and Levene tests checked for normality and homogeneity. One-way ANOVA and Tukey's post-hoc test analyzed group differences ($p < 0.05$ considered significant). Results are shown as mean ± SD and visualized using GraphPad Prism.

## 3. RESULTS

*The cellular viability in different time intervals*

Cell viability was assessed using the MTT assay. No significant difference in the mean percentage of viable cells was observed between HPDF and MCF7 cells at 24 and 72 hours (Fig. 1A, 1B).

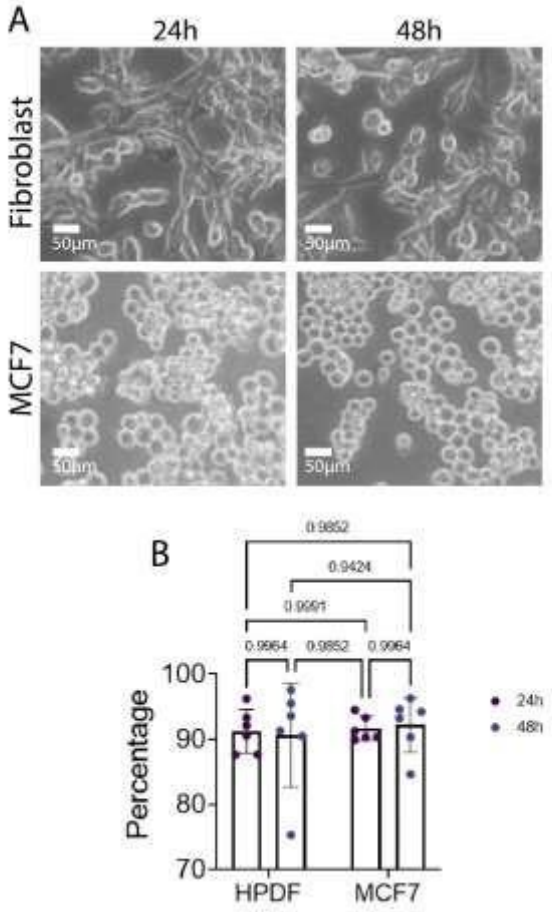


**Fig. 1: The Human Primary Dermal Fibroblasts (HPDF) and MCF7 cell lines following 24 and 72 hours: (A) photomicrograph of the cells under invert microscope following 24 and 72 hours, and (B) the results of the MTT test following 24- and 72-hour time points. All data are presented in Mean±SD (n=6 samples/each group).**

***The expression levels of TRAF6 and IRAK1***

The expression levels of IRAK1 and TRAF6 in HPDF and MCF7 cells were evaluated using qRT-PCR. The results demonstrated a significant increase ($p < 0.05$) in IRAK1 mRNA levels in MCF7 cells compared to HPDF cells at both the 24- and 72-hour time points. No significant differences ($p > 0.05$) were observed between the 24- and 72-hour time points within the HPDF and MCF7 cells (Fig. 2A). Additionally, MCF7 cells exhibited significantly higher ($p < 0.05$) mRNA levels of TRAF6 compared to HPDF cells at both time points, with no significant differences between the 24- and 72-hour time points within either cell line (Fig. 2B).

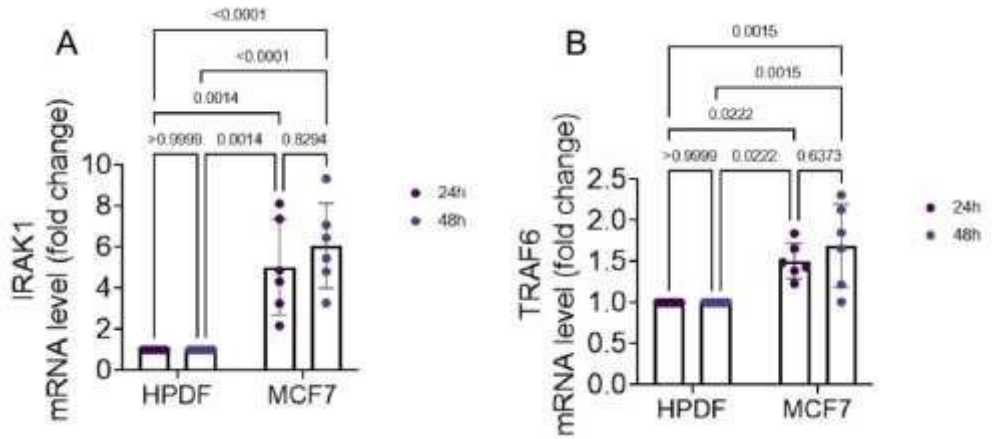

**Fig. 2: Expression levels (A) IL-1 receptor-associated kinases-1 (IRAK1), and (B) TNF receptor-associated factor 6 (TRAF6) in HPDF and MCF7 cells following 24- and 72-hour time points. All data are presented in Mean±SD (n=6 samples/each group).**

***Expression level of Nf-kB***

To investigate the expression levels of NF-kB in MCF7 cells compared to HPDF cells, both protein (after 72 hours) and mRNA levels (after 24 and 72 hours) were assessed. The results showed that MCF7 cells exhibited significantly higher ($p < 0.05$) NF-kB mRNA and protein levels compared to HPDF cells (Fig. 3A, 3B, 3C).

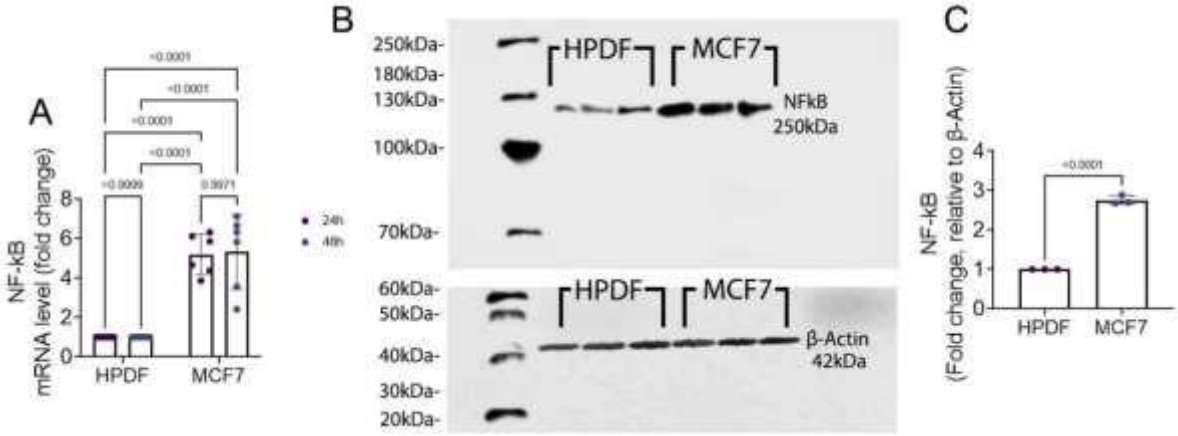


**Fig. 3: Expression level of NF-kB: (A) the mRNA levels of NF-kB in HPDF and MCF7 cells, (B) photomicrograph of the western blot bands for NF-kB protein in the HPDF and MCF7 cells following 72-hour time point, and (C) the quantitative fold change of the NF-kB protein relative to β-Actin and fold change to the HPDF cells. All data are presented in Mean±SD (n=3 samples/each group).**

***Expression level of p38MAPK***

To investigate the expression levels of *p38MAPK* in MCF7 cells compared to HPDF cells, the protein levels were assessed following 72-hour time point. The results showed that MCF7 cells exhibited significantly higher ($p < 0.05$) *p38MAPK* protein levels compared to HPDF cells (Fig. 4A, 5B).

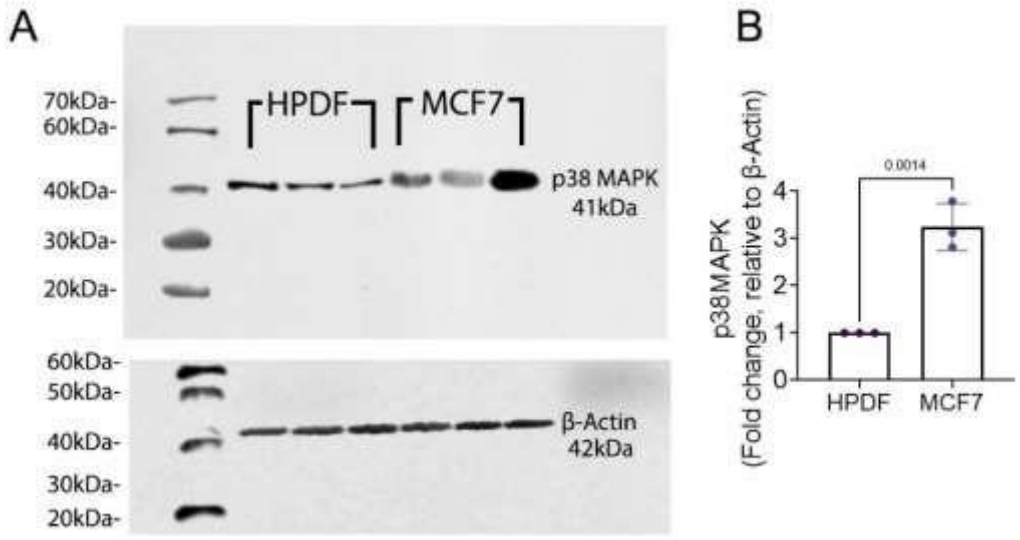


**Fig. 4: Expression level of p38MAPK: (A) photomicrograph of the western blot bands for p38MAPK protein in the HPDF and MCF7 cells following 72-hour time point, and (B) the quantitative fold change of the p38MAPK protein relative to β-Actin and fold change to the HPDF cells. All data are presented in Mean±SD (n=3 samples/each group).**

***The expression levels of p38 and JUNK***

The expression levels of p38 and JUNK in HPDF and MCF7 cells were evaluated using qRT-PCR. The results demonstrated a significant increase ($p < 0.05$) in p38 mRNA levels in MCF7 cells compared to HPDF cells at both the 24- and 72-hour time points. No significant differences ($p > 0.05$) were observed between the 24- and 72-hour time points within the HPDF and MCF7 cells (Fig. 4A). Additionally, MCF7 cells exhibited significantly higher ($p < 0.05$) mRNA levels of JUNK compared to HPDF cells at both time points, with no significant differences between the 24- and 72-hour time points within either cell line (Fig. 6A, 6B).

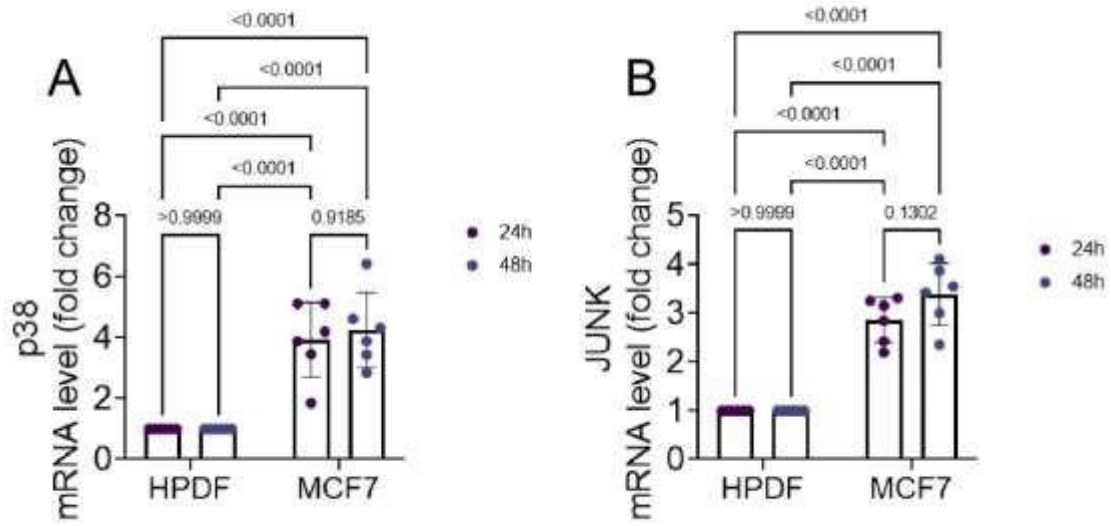


**Fig. 5: Expression levels (A) p38, and (B) JUNK in HPDF and MCF7 cells following 24- and 72-hour time points. All data are presented in Mean±SD (n=6 samples/each group).**

## 4. DISCUSSION

This study examined the MyD88/IL-1β signaling pathway in MCF7 breast cancer cells, with a focus on key downstream mediators such as IRAK1, TRAF6, NF-κB, and MAPK. By comparing MCF7 cells with normal HPDF cells, we sought to investigate the role of MyD88-driven inflammatory signaling in breast cancer cell proliferation and survival. The results suggest that MCF7 cells exhibit a significantly higher expression of MyD88 and related signaling molecules, underscoring the involvement of the IL-1β/IL-1R/MyD88 axis in promoting tumor growth and resistance to apoptosis in this breast cancer subtype.

Previous studies have highlighted MyD88 as a crucial adaptor protein in immune signaling, particularly in activating TLRs and IL-1Rs, which trigger downstream signaling to produce pro-inflammatory cytokines and modulate tumor progression (Atre et al., 2023; Alcoceba et al., 2022). In this context, our findings that MCF7 cells exhibit significantly higher levels of IRAK1 and TRAF6 compared to HPDF cells support the notion that the MyD88 pathway is a key driver of inflammatory responses in breast cancer. TRAF6, known for its role in activating NF-κB and MAPK pathways, was found to be overexpressed in MCF7 cells, suggesting that these cells are primed for sustained immune and inflammatory signaling, which promotes tumor growth and resistance to chemotherapy and radiotherapy (Walsh et al., 2015; Li et al., 2020).

The activation of NF-κB, a central regulator of inflammation and survival, is another critical step in the inflammatory signaling cascade initiated by MyD88. Our data show that MCF7 cells exhibit significantly higher levels of both NF-κB mRNA and protein compared to HPDF cells, indicating a robust inflammatory response that supports tumorigenesis. This is consistent with findings from other cancers, where NF-κB activation leads to increased expression of pro-inflammatory cytokines, angiogenic factors like VEGF, and resistance to apoptosis, thus contributing to tumor progression and metastasis (Noort et al., 2014). Elevated NF-κB expression also plays a pivotal role in mediating immune evasion, allowing cancer cells to resist immune surveillance and escape therapeutic interventions (Zheng et al., 2024).

The MAPK signaling pathway, particularly through the p38 and JNK kinases, also emerged as a critical mediator in the inflammatory response of MCF7 cells. The significant increase in p38MAPK protein levels and p38 and JNK mRNA expression in MCF7 cells compared to HPDF cells highlights the activation of these pathways in response to MyD88mediated signaling. MAPK pathways regulate key cellular processes such as growth, differentiation, and survival, and their

dysregulation is a hallmark of many cancers (Cargnello et al., 2011; Bahar et al., 2023). Our findings that MCF7 cells display higher MAPK pathway activation compared to HPDF cells suggest that these signaling cascades contribute to the increased proliferative capacity and resistance to stress seen in breast cancer cells. This is consistent with studies in other cancers, where MAPK pathway dysregulation facilitates tumor progression and therapeutic resistance (Ferreira et al., 2022).

Interestingly, while the MTT assay revealed no significant difference in cell viability between MCF7 and HPDF cells at the 24- and 72-hour time points, this may suggest that the cells are in a stable state regarding basic viability, but the underlying inflammatory signaling mechanisms play a more prominent role in driving long-term survival, proliferation, and resistance to therapy. This points to the importance of targeting the inflammatory signaling pathways, particularly MyD88, IRAK1, TRAF6, NF-κB, and MAPK, in developing therapeutic strategies for breast cancer, especially for the ER+, PgR+/−, HER2− subtype, where these pathways may be particularly active.

In conclusion, the results from this study provide evidence that MyD88/IL-1β signaling pathways, through the activation of TRAF6, IRAK1, NF-κB, and MAPK, contribute significantly to the proliferation and survival of MCF7 breast cancer cells. These pathways support tumorigenesis by promoting inflammation, cell survival, and resistance to apoptosis. Targeting these inflammatory signaling cascades may represent a promising therapeutic approach to treating breast cancer, particularly in subtypes where these pathways are dysregulated. Given the involvement of MyD88 and MAPK in multiple cancers, including melanoma, colorectal, and lung cancers, their inhibition could offer broad potential in cancer therapy (Castellani et al., 2023; Ferreira et al., 2022).

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