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Modulated Hepatic Expression of NF-kB and CCN1 Genes in a Breast Cancer Mouse Model Induced by DMBA and High-Fat Diet

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Abstract

Background: The study investigates the combined effects of 7,12-dimethylbenz[a]anthracene (DMBA) and a high-fat diet (HFD) on hepatic gene expression in a breast cancer mouse model. Both DMBA and HFD are associated with oxidative stress, inflammation, and fibrosis, which may contribute to cancer progression. **Objective**: This study aims to investigate the combined effects of a high-fat diet and 7,12-dimethylbenz(a)anthracene (DMBA) exposure on hepatic gene expression, focusing on NF-κB and CCN1/CYR61, in a breast cancer mouse model. **Methods:** Female C57BL/6 mice were divided into three groups: standard diet (ND), ND + DMBA, and HFD + DMBA. Mice in the ND + DMBA and HFD + DMBA groups received DMBA injections weekly for six weeks. The HFD + DMBA group was also fed a high-fat diet for four weeks prior to DMBA administration. After a 12-week treatment period, liver tissues were collected and analyzed for NF-κB and CCN1/CYR61 gene expression using semi-quantitative RT-PCR. **Results:** Significant upregulation of NF-κB and CCN1/CYR61 was observed in the ND + DMBA and HFD + DMBA groups compared to the control group. The highest expression levels were found in the HFD + DMBA group, indicating a potential interaction between dietary factors and carcinogen exposure in modulating hepatic gene expression. **Conclusion:** The combination of HFD and DMBA exposure enhances hepatic NF-κB and CCN1/CYR61 expression, suggesting an influence on inflammatory and cellular stress pathways. These findings provide insights into the molecular response of the liver to dietary and carcinogenic factors, emphasizing the need for further exploration of their role in liver-related pathophysiology.

Keywords: breast cancer; CCN1/CYR61; DMBA; hepatic gene_ expression; high-fat diet; inflammation

Original Research Article

INTRODUCTION

Breast cancer is one of the most prevalent malignancies worldwide, known for its potential to metastasize and exert systemic effects beyond the primary tumor site (Siegel et al., 2023). The liver, a

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critical organ for metabolism and detoxification, is frequently affected by both primary cancers and their treatments. Systemic changes associated with breast cancer, including inflammation, metabolic alterations, and changes in liver function, can significantly influence disease progression and patient outcomes (Macedo et al., 2017). Therefore, understanding how breast cancer and its associated treatments affect distant organs, such as the liver, is crucial for developing comprehensive therapeutic strategies.

The use of a high-fat diet (HFD) and carcinogens such as 7,12-dimethylbenz[a]anthracene (DMBA) is well-established in creating cancer models to study the molecular mechanisms of carcinogenesis and metastasis. DMBA is a potent carcinogen that induces breast cancer in animal models and promotes oxidative stress, inflammation, and DNA damage (Kociba & Schwetz, 2018). Additionally, HFD is a significant risk factor for various cancers and metabolic diseases, including non-alcoholic fatty liver disease (NAFLD), and has been shown to exacerbate cancer progression by promoting a pro-inflammatory state and altering metabolic pathways (Lau et al., 2020; Sanyal et al., 2018).

Emerging evidence suggests that cancers such as breast cancer can influence distant organs like the liver by modulating gene expression and promoting a systemic pro-inflammatory and pro-fibrotic environment (Fisher et al., 2016). This is particularly relevant given the liver's role in metabolizing carcinogens and regulating systemic inflammation. The nuclear factor kappa B (NF-κB) pathway, a critical regulator of inflammation and immune responses, is activated in both cancer and liver diseases, suggesting a potential overlap in these conditions' molecular pathways (Sun & Karin, 2019). Similarly, CCN1/CYR61, a matricellular protein involved in tissue repair and fibrosis, has been implicated in cancer progression and liver fibrosis, indicating its dual role in oncogenesis and hepatic pathology (Jun & Lau, 2018; Zhou et al., 2020).

In this study, we investigated the expression of NF-κB and CCN1/CYR61 in the liver in a breast cancer mouse model induced by DMBA and exacerbated by an HFD. The rationale behind focusing on liver gene expression in a breast cancer model stems from the hypothesis that breast cancer, especially under conditions of carcinogen exposure and dietary stress, can create systemic effects that alter liver function and promote liver pathology. Understanding these interactions is crucial because the liver's response to cancer and its treatment can significantly influence overall disease outcomes, including metastasis and patient survival (Macedo et al., 2017; Friedman et al., 2018).

Our study aims to investigate the combined effects of a high-fat diet and 7,12-dimethylbenz(a)anthracene (DMBA) exposure on hepatic gene expression, focusing on NF-κB and CCN1/CYR61, in a breast cancer mouse model. This knowledge could provide new insights into the broader systemic effects of breast cancer and the role of diet and environmental factors in modulating these effects (Tsuchida & Friedman, 2017; Musso et al., 2018).

MATERIALS AND METHODS

Subjects

This study utilized a quantitative experimental design with animal models to evaluate the impact DMBA and HFD on the expression of NF-kB and CCN1/CYR61 genes in a breast cancer mouse model. Female C57BL/6 mice, aged 10-11 weeks, were sourced from iRAT Co. The sample size was calculated using Mead’s formula to ensure statistical power, with a total of 18 mice divided into three experimental groups (n=6 per group): Group 1 (Normal Diet, ND), Group 2 (Normal Diet + DMBA), and Group 3 (High-Fat Diet + DMBA).

Treatment and Induction

Mice were acclimated for 7 days before starting the treatment. Groups 1 and 2 were maintained on a standard diet, while Group 3 was given a high-fat diet (HFD) for 4 weeks. On day 29 of the experiment, mice in Groups 2 and 3 were treated with DMBA at a dose of 1 mg/kg body weight, dissolved in sesame oil, administered via subcutaneous injection once a week for 6 weeks. After completing the 12-week treatment period, liver tissues were collected for further analysis.

Tissue Collection and RNA Extraction

Liver tissues were extracted and stored in GENEzol™ reagent for RNA isolation. Tissue samples (50-100 mg) were homogenized in 1 mL of GENEzol™ and incubated at room temperature for 5 minutes. Following homogenization, 200 µL of chloroform was added, and the mixture was vortexed and centrifuged at 12,000-16,000 x g for 15 minutes at 4°C. The aqueous phase was carefully collected and RNA was precipitated by adding 1 mL of isopropanol. The RNA pellet was centrifuged, washed with 70% ethanol, and air-dried. The RNA was then resuspended in 20-50 µL of RNase-free water and stored at -80°C for further analysis.

Gene Expression Analysis

Gene expression levels of NF-kB and CCN1/CYR61 were measured using semiquantitative RT-PCR with the MyTaq™ One-Step RT-PCR Kit, following the manufacturer's instructions. The primers used were as follows: GAPDH (Forward: TTGATGGCAACAATCTCCAC; Reverse: CGTCCCGTAGACAAAATGGT; Annealing Temperature: 55°C), CCN1/CYR61 (Forward: GATGACCTCCTCGGACTCGAT; Reverse: CGTGCAGAGGGTTGAAAAGAA; Annealing Temperature: 55°C), and NF-kB (Forward: GGCCGGAAGACCTATCCTACT; Reverse: CTACAGACACAGCGCACACT; Annealing Temperature: 55°C).

Gel Electrophoresis and Quantification

RT-PCR products were analyzed by 2% agarose gel electrophoresis in Tris-Boric Acid EDTA buffer containing SYBR™ Safe DNA Gel Stain. Gels were run at 100 volts for 30 minutes and visualized under UV light. Band intensity was quantified and normalized to GAPDH to account for sample variability and PCR efficiency.

Ethical Considerations

The study was conducted following ethical guidelines for animal research, with approval obtained from the Maranatha Research Ethics Committee (No. 031/KEP/IV/2022). Adherence to the 3Rs (Replacement, Reduction, Refinement) principles was maintained. The number of animals was minimized to ensure statistical validity while prioritizing animal welfare.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism software. Differences between groups were assessed with ANOVA followed by post-hoc tests.

RESULTS

DMBA and High-Fat Diet Induce NF-κB Expression in Liver Tissues

Exposure to DMBA and a high-fat diet (HFD) resulted in a notable increase in NF-κB expression in liver tissues of the treated mice. Mice in the ND+DMBA group showed a 1.12-fold increase in NF-κB expression compared to those on a normal diet (ND), indicating a response to DMBA-induced carcinogenesis (Figure 1A). Interestingly, the combination of HFD and DMBA led to an even higher upregulation, with a 1.46-fold increase, suggesting a synergistic effect of dietary fat and DMBA on NF-κB activation. The differences between groups were statistically significant, particularly between the ND+DMBA and HFD+DMBA groups, highlighting the role of dietary fat in modulating NF-κB expression ($P < 0.05$ for ND+DMBA vs. ND; $^*P < 0.01$ for HFD+DMBA vs. ND) (Figure 1B).

CCN1/CYR61 Expression is Elevated in Response to DMBA and High-Fat Diet

Like NF-κB, CCN1/CYR61 gene expression was significantly increased in mice exposed to DMBA and a high-fat diet. In the ND+DMBA group, CCN1/CYR61 expression rose by 1.14-fold compared to the ND group, suggesting an initial response to DMBA exposure (Figure 2A). The HFD+DMBA group demonstrated an even greater increase of 1.55-fold, which was statistically significant, indicating a heightened response when both DMBA and a high-fat diet were present. These findings suggest that both NF-κB and CCN1/CYR61 pathways are activated under these conditions, potentially contributing to liver inflammation and fibrosis ($P < 0.05$ for ND+DMBA vs. ND; $^*P < 0.01$ for HFD+DMBA vs. ND) (Figure 2B).

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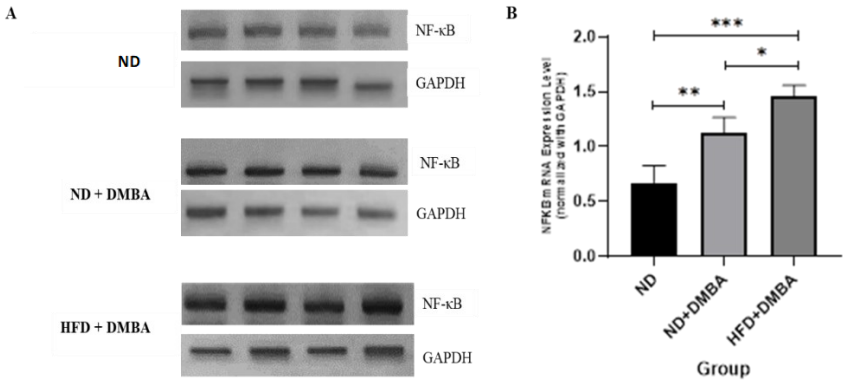

Figure 1: Gene expression of NF-κB in the standard diet group (ND, n=6), ND + DMBA group (n=6), and HFD + DMBA group (n=6). A) Electrophoresis results showing NF-κB and GAPDH expression levels. B) Histogram illustrating the differences according to the Post Hoc test results (* P<0.05; ** P<0.01; *** P<0.001). Data are presented as mean ± SD.

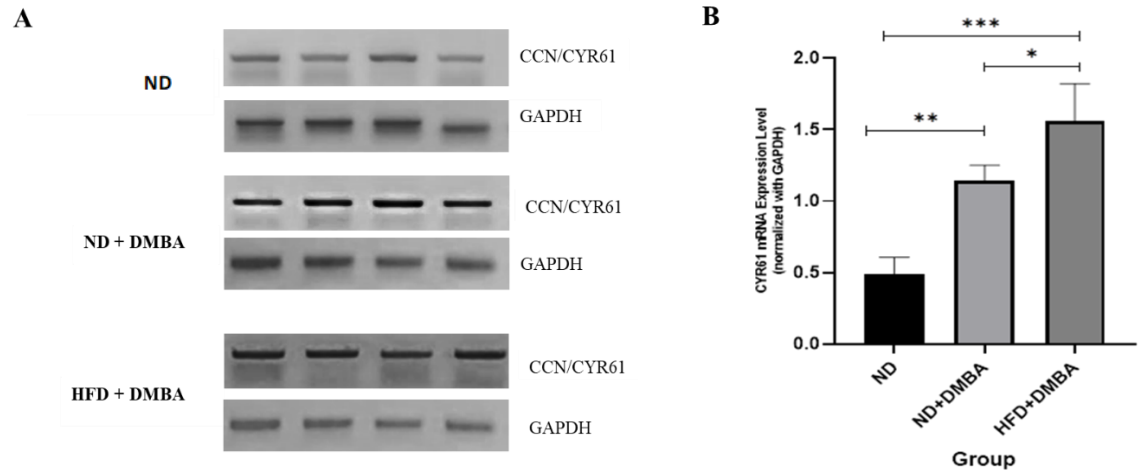

Figure 2: Gene expression of CCN1/CYR61 in the standard diet group (ND, n=6), ND + DMBA group (n=6), and HFD + DMBA group (n=6). A) Electrophoresis results showing CCN1/CYR61 and GAPDH expression levels. B) Histogram illustrating the differences according to the Post Hoc test results (* P<0.05; ** P<0.01; *** P<0.001). Data are presented as mean ± SD.

The results collectively suggest that the combination of DMBA and a high-fat diet synergistically enhances the expression of NF-κB and CCN1/CYR61 in liver tissues, potentially exacerbating inflammatory and fibrotic responses in the context of breast cancer progression.

DISCUSSION

Our study provides crucial insights into how exposure to 7,12-dimethylbenz[a]anthracene (DMBA) and a high-fat diet (HFD) influence hepatic gene expression, particularly in pathways related to inflammation and fibrosis in a breast cancer mouse model. Our results demonstrate a significant upregulation of NF-κB and CCN1/CYR61, suggesting a potential interaction between dietary fat and carcinogenic exposure that exacerbates liver pathology. These findings advance our understanding of how environmental and dietary factors contribute to disease progression, especially in relation to cancer and metabolic disorders.

NF-κB is a transcription factor that regulates immune and inflammatory responses. Its activation is crucial in the cellular response to stressors, such as oxidative stress and DNA damage from carcinogens like DMBA. This activation leads to the transcription of numerous genes involved in inflammation, including cytokines, chemokines, and adhesion molecules (Sun & Karin, 2019). Our findings support prior research demonstrating that DMBA exposure alone is sufficient to elevate NF-

κB expression in the liver, consistent with studies linking DMBA to hepatic inflammation and fibrosis (Kim et al., 2021).

The additional increase in NF-κB expression in the HFD + DMBA group indicates a synergistic interaction between dietary fat and DMBA in promoting hepatic inflammation. High-fat diets contribute to chronic, low-grade inflammation and metabolic stress, increasing the production of reactive oxygen species (ROS) and inflammatory mediators (Musso et al., 2018). These factors further activate NF-κB pathways. The combination of HFD and DMBA exposure likely amplifies lipid peroxidation and inflammatory processes, leading to a heightened inflammatory environment (Lau et al., 2020).

CCN1/CYR61 is a matricellular protein that plays a crucial role in cellular processes such as adhesion, migration, proliferation, and differentiation. Additionally, it is an essential regulator of tissue repair and fibrosis, particularly within the liver, where it mediates responses to injury and chronic inflammation (Leask & Abraham, 2016). Our study demonstrated a marked upregulation of CCN1/CYR61 expression in the DMBA and HFD groups, with the highest expression levels observed in the HFD + DMBA group. This pattern suggests that both dietary fat and carcinogen exposure may independently and synergistically contribute to hepatic fibrosis by stimulating hepatic stellate cells (HSCs) and enhancing extracellular matrix deposition, leading to tissue remodeling and fibrogenesis (Zhou et al., 2020).

In addition to its involvement in liver pathology, NF-κB is a critical factor in breast cancer progression and metastasis. This transcription factor is commonly activated in breast cancer cells and their microenvironment, playing a pivotal role in tumor development and immune evasion (Perkins, 2012). The sustained activation of NF-κB has been strongly associated with increased tumor cell proliferation, resistance to apoptosis, induction of angiogenesis, and epithelial-mesenchymal transition (EMT), which collectively facilitate tumor invasion and metastatic dissemination (Chae et al., 2020). Moreover, NF-κB signaling has been linked to resistance against chemotherapy and radiotherapy, making it an important target for potential therapeutic interventions (Hayden & Ghosh, 2014).

CCN1/CYR61 is also recognized as a significant modulator of breast cancer progression, particularly in the regulation of the tumor microenvironment. It has been shown to influence tumor cell adhesion, migration, and invasion, all of which are essential steps in metastasis (Lin et al., 2019). Elevated CCN1 expression has been correlated with increased tumor aggressiveness and metastatic potential, particularly in triple-negative breast cancer, a subtype known for its poor prognosis and limited treatment options (Jeong et al., 2021). The overexpression of CCN1/CYR61 may contribute to the enhancement of signaling pathways that support cancer cell survival, angiogenesis, and immune evasion, further promoting the spread of malignant cells.

The findings from our study indicate that the concurrent upregulation of NF-κB and CCN1/CYR61 in response to a high-fat diet and DMBA exposure may contribute to the creation of a hepatic microenvironment that favors breast cancer metastasis. These results align with previous research that has identified chronic hepatic inflammation as a key factor in increasing metastatic potential in various cancers, including breast cancer (Karin et al., 2017). By elucidating the molecular mechanisms underlying these interactions, our study underscores the importance of considering both dietary and environmental factors in cancer progression and highlights potential molecular targets for future therapeutic strategies.

CONCLUSION

In conclusion, our findings demonstrate that both DMBA and HFD independently and synergistically upregulate NF-κB and CCN1/CYR61 gene expression in the liver of a breast cancer mouse model, suggesting a compounded risk of liver inflammation and fibrosis. This interaction between diet, environmental toxins, and genetic expression highlights the importance of considering dietary interventions and environmental exposures in cancer management and prevention strategies.

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Further research is needed to explore the full spectrum of molecular changes induced by these factors and to develop targeted therapies that can mitigate their harmful effects.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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