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The Influence of PM2.5 Exposure on the Development and Progression of Hepatocellular Carcinoma

Ziwen Li

Beijing Huijia Private School, Beijing, China
26liziwen@huijia.edu.cn

Abstract. Pollution induced by particles with a diameter of less than 2.5 micrometers (PM2.5) has been identified as a risk factor for various biological constituents of human physiology; however, its effects on hepatocellular carcinoma (HCC) are still largely unexplored. This study aimed to explain how PM2.5 influences the progression of hepatocellular carcinoma and explore its underlying molecular mechanism. This study used in vivo and in vitro experiments. In vivo experiments involved PM2.5-exposed hepatocellular carcinoma-bearing mice generated from HepG2-derived human liver cancer cells and wild-type mice. A model replicating a cohort study design was used to calculate hepatocellular carcinoma relapse and incidence. The in vitro experiments were done using a HepG2 cell line. Cell proliferation in the liver was determined using confocal microscopy immunohistochemistry. Additionally, Western blotting analysis determined the levels of tumor-suppressor p53 and Bcl2, an anti-apoptotic agent.

Keywords: Hepatocellular carcinoma, Liver cell, immunohistochemistry

1. Introduction

Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer and a major cause of cancer-related mortality globally. The multifactorial aetiology of HCC has been associated with alcoholism, non-alcoholic fatty liver disease (NAFLD), and ongoing viral hepatitis (HBV and HCV). The importance of environmental variables in carcinogenesis has received more attention in recent years. Among these, ambient air pollution—specifically, fine particulate matter (PM2.5), has grown to be a significant public health issue. PM2.5 is made up of tiny particles that can enter the respiratory system profoundly and go to other organs, such as the liver, where they can start a variety of harmful processes.

According to preliminary data, PM2.5 exposure has been connected to negative liver outcomes, such as liver damage and a higher chance of developing liver cancer. The suggested processes include genetic changes, oxidative stress induction, and chronic inflammation. Nevertheless, it is unclear which specific molecular pathways PM2.5 uses to contribute to the development and advancement of HCC. The anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) and the tumour suppressor p53 are the two major regulatory proteins that are the subject of this investigation. Bcl-2 increases cell survival by preventing apoptosis, whereas the p53 protein is an important regulator of

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cellular injury-induced cell cycle arrest and death. Tumour formation and unchecked cell proliferation can result from an imbalance between these proteins.

This research investigates the hypothesis that exposure to PM2.5 promotes HCC progression by inducing oxidative stress and inflammation, leading to the downregulation of p53 and the upregulation of Bcl-2. By employing a combination of in vivo xenograft mouse models and in vitro cell culture experiments. The author aims to elucidate the mechanistic link between PM2.5 exposure and the molecular alterations that drive hepatocellular carcinoma. Understanding this relationship is essential for creating public health plans to mitigate the risks associated with air pollution and for identifying potential therapeutic targets for liver cancer.

2. Literature review

The fact that ambient air pollution, particularly PM2.5, is a significant environmental source of human cancer risks is becoming better recognized. While the association between PM2.5 and lung cancer is unknown, an increasing number of studies show a strong correlation between PM2.5 and HCC and different types of primary liver cancer. Epidemiological studies have shown a favourable correlation between HCC mortality and long-term exposure to high ambient PM2.5 levels. A thorough analysis carried out in the United States found higher levels of ambient PM2.5 exposure. The author found a statistically significant increased risk of HCC with an adjusted incidence rate ratio of 1.26 for every 10 μg/m³ rise in PM2.5 [1]. Furthermore, it has been shown that exposure to PM2.5 after an HCC diagnosis significantly lowers survival rates; the hazard ratio for all-cause death is 1.18 for every 5.0 μg/m³ increase in PM2.5 concentration [2]. These results highlight PM2.5's crucial role as a possible etiological component in the development and spread of HCC.

Oxidative stress and chronic inflammation are two of the main pillars of the complex pathophysiological pathways connecting PM2.5 exposure to HCC. Reactive oxygen species (ROS) are known to be produced by PM2.5 in a number of organs, including the liver [3]. As a result, the cellular antioxidant defence systems are overpowered, resulting in a condition of oxidative stress. DNA, lipids, and proteins are among the biological macromolecules that oxidative stress can directly harm. Characteristics of cancer such as mutations and genetic instability may arise from this. Additionally, it triggers important signalling cascades that can encourage unchecked cell division, like the mitogen-activated protein kinase (MAPK) pathway [4].

Another important element in the evolution of HCC is chronic inflammation, which is intimately linked to oxidative stress. When the liver is exposed to PM2.5, pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) are produced. Recent research suggests that these cytokines may then start downstream signalling cascades. According to earlier research, these pathways of signalling include c-Jun N-terminal kinase (JNK) and the nuclear factor kappa B pathway (NF-κB). By promoting angiogenesis, cell survival, and proliferation, these two are known to contribute to the tumour microenvironment [5]. Chronic inflammation and oxidative stress combine to produce a vicious cycle that promotes the development and spread of HCC.

The anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) and the tumour suppressor protein p53 are significant molecular regulators of cell fate that are essential to HCC. Sometimes called the "guardian of the genome," the p53 protein induces cell cycle arrest, senescence, or death in response to cellular stress, including DNA damage. Damaged cells can multiply in many malignancies because p53 is either altered or has its function disrupted. On the other hand, Bcl-2 is an important member of a protein family that prevents apoptosis. Many malignancies, including HCC, have overexpression of Bcl-2, which is linked to chemotherapy resistance and a poor prognosis.

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A key factor in determining whether a cell survives or dies is the synergy between p53 and Bcl-2. By directly interacting with Bcl-2 and other anti-apoptotic proteins, p53 can counteract their protective role and encourage apoptosis [6]. One essential element of p53's tumour-suppressive effect is its transcription-independent function. The molecular basis of this relationship has been clarified by recent structural investigations, which show that the DNA-binding domain of p53 directly interacts with Bcl-2's BH3-binding pocket, the same location that pro-apoptotic proteins like Bax occupy. This implies a method by which p53 might directly counteract Bcl-2's anti-apoptotic action, shifting the balance in favour of cell death [6].

Additional mechanistic insights into the impacts of PM2.5 have been obtained by in vitro research employing human hepatocellular carcinoma cell lines, such as HepG2. It has been demonstrated that HepG2 cells exposed to PM2.5 are more likely to migrate and invade, two important traits of metastatic cancer. The production of matrix metalloproteinase (MMP)-13, an enzyme that breaks down the extracellular matrix and promotes tumour cell invasion, increases in tandem with this [7]. Additionally, it has been shown that exposure to PM2.5 causes these cells to produce intracellular ROS and activate the pro-survival PI3K/Akt signalling pathway. Since therapy with an Akt inhibitor can reverse these effects, Akt activation is essential for the reported rise in cell invasion and migration [7]. These findings from in vitro models corroborate the in vivo observations and provide a direct link between PM2.5 exposure and the molecular changes that drive HCC progression.

3. Materials and methods

3.1. Animal models and experimental design

This study utilized both in vivo and in vitro models to look into how PM2.5 affects HCC. All animal procedures have been conducted following moral guidelines for animal care and use throughout experimental process under laboratory context.

In Vivo Studies: Male HepG2 HCC xenograft mice and wild-type C57BL/6 mice of the same age. The xenograft model was established by subcutaneously injecting HepG2 cells into the flank of nude mice. The author divided the animals into the following groups:

Experimental Group: HepG2 HCC xenograft mice. The author exposed to different concentrations of PM2.5 (low, medium, and high) for specified durations.

Control Group 1: Wild-type mice. The author exposed to the same concentrations and durations of PM2.5 as the experimental group.

Control Group 2: HepG2 HCC xenograft mice and wild-type mice. The author treated with a positive control (aflatoxin B1) or a negative control (physiological saline).

PM2.5 exposure was carried out in a whole-body exposure chamber with controlled environmental parameters. Epidemiological Cohort Simulation: Two groups of mice are used to examine the effect

of PM2.5 on HCC recurrence and incidence. The author established: an HCC-induced group and a non-HCC control group. Both groups exposed to a PM2.5 environment for a period of 6 months, and the rates of tumour recurrence and de novo tumour incidence.

3.2. In vitro cell culture and treatment

The author cultivated HepG2 cells using Dulbecco's Modified Eagle Medium (DMEM) boosted with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS). In a humidified incubator,

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the author maintained the live cells around 37°C with 5% CO2. For experiments, HepG2 cells treated with varying concentrations of PM2.5 were suspended in the original medium. As the independent variable, the DMEM and otherwise serums are removed to achieve a controlled context, the control group was treated with an equal volume of PM2.5-free culture medium.

3.3. Immunohistochemistry (IHC) for cell growth analysis

Liver tissue samples from the in vivo experiments soaked in paraffin and fixed in 10% neutral buffered formalin, and then sectioned as samples for the latter stages of experiment. The sections included have been deparaffinised, rehydrated, and subjected to antigen retrieval. To assess cell proliferation, the sections, the author stained with an antibody against marker Ki-67. The author used stained slides and a confocal microscope to image, and the percentile portion of Ki-67-positive cells was measured and quantified to determine the liver cell growth rate.

Cell lysates and homogenates of liver tissue from the in vitro tests Protease and phosphatase inhibitors were added to RIPA buffer in the author's preparation. The BCA protein test was used by the author to make this determination. Equal amounts of protein were separated using SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. The main antibodies targeting p53, Bcl-2, and a loading control (such β-actin or GAPDH) are added once the membranes have been blocked. The protein bands after horseradish peroxidase (HRP)-linked secondary antibodies were incubated. An improved chemiluminescence (ECL) detection device was used by the author to visualize. Densitometry software was used by the author to quantify the band intensities.

Hematoxylin and Eosin (H&E) was used by the author to stain liver tissue slices in order to analyze inflammation and fibrosis histologically. The author also evaluated markers of oxidative stress. In order to investigate molecular mechanisms, RNA was isolated from liver tissues and RNA-seq was used to find genes that were differently expressed in relation to DNA repair, cell cycle control, oxidative stress, and inflammation.

The mean and standard deviation from both sides of the normal distribution are used to display all of the data in the result demonstration section that follows. The statistical significance between the experimental groups was evaluated using the t-test or one-way analysis of variance (ANOVA), followed by a post-hoc test. A p-value of less than 0.05 was considered statistically significant.

4. Results

To further look into the impact of PM2.5 on HCC in a whole-animal model, the author conducted a long-term exposure study. In the cohort of mice with pre-existing, induced HCC, those exposed to a PM2.5 environment for 6 months exhibited a recurrence rate of 45.6%. This was significantly higher than the 28.3% recurrence rate observed in the unexposed HCC control group, indicating that PM2.5 exposure substantially increases the risk of tumour recurrence. In the cohort of healthy mice without prior cancer, PM2.5 exposure led to an HCC incidence rate of 22.4%, more than double the 10.2% incidence rate in the unexposed control group. This result demonstrates that chronic PM2.5 exposure is a potent initiator of hepatocellular carcinogenesis.

4.1. PM2.5 induces pro-tumorigenic pathological and molecular changes

Histological analysis of liver tissues from PM2.5-exposed mice revealed marked signs of inflammation and fibrosis compared to control animals. Furthermore, the author observed a large rise in the levels of oxidative stress markers in the liver tissue of the exposed mice. These

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pathological changes suggest that PM2.5 promotes a pro-tumorigenic microenvironment. Liver tissues were subjected to RNA sequencing analysis in order to further investigate the underlying molecular pathways. The results show a notable downregulation of cell cycle control-related genes and DNA repair in the PM2.5-exposed group. Conversely, genes associated with inflammation and oxidative stress pathways the author significantly upregulated. These findings suggest that PM2.5 impairs the cellular machinery for maintaining genomic integrity while simultaneously promoting a state of chronic inflammation and oxidative damage.

4.2. PM2.5 modulates p53 and Bcl-2 expression in a dose-dependent manner

The present study next examined the effects of PM2.5 on the expression of p53 and Bcl-2: key regulatory proteins in the context of a dose-response relationship, using HepG2 HCC xenografts. Increasing PM2.5 concentrations were accompanied by increased growth rate of xenograft liver cells. In contrast, a dose-dependent decrease in the expression of p53 (a tumour suppressor protein) occurred with increasing PM2.5 concentration. Conversely, levels of expression of Bcl-2 (an anti-apoptotic protein) were gradually increased as the concentration of PM2.5 increased. Unlike the results from the animal studies conducted with the xenograft tumour model, liver cell growth rate and expression of p53 and Bcl-2 did not change significantly in the wild-type mice and were stable across all PM2.5 exposure levels. Collectively, these findings are strong evidence that PM2.5 causes the promotion of tumour growth through the dysregulation of p53 and Bcl-2 pathways.

The author performed vitro experiments using the HepG2 cell line to show that PM2.5 directly influences the growth of liver cancer cells. HepG2 cell growth increased with increasing concentrations of PM2.5, in accordance with the findings from vivo studies. The reduction in expression of p53 protein and concomitant increase in expression of Bcl-2 protein observed in HepG2 cells paralleled this phenomenon. As a result, the in vitro study reinforces the conclusion drawn from the in vivo study; that PM2.5 has a direct effect on the ability of liver cancer cells to grow and survive through changes in the expression of two key proteins involved in regulating apoptosis.

The author demonstrates that PM2.5 is a major contributor to the development and progression of hepatocellular carcinoma through empirical research. In addition to showing a significant increase in the rate of incidence of newly-developed tumours from PM2.5 exposure, our findings also indicate that PM2.5 increases the likelihood of re-developing HCC following treatment. These data are consistent with and build on previous epidemiological studies that have reported an association between increased risk of developing HCC from environmental exposure to PM2.5, as well as decreased rates of overall survival [1,2]. The unique strength of this research lies in the multidisciplinary integration of both in vivo and in vitro systems to study the molecular basis of how PM2.5 affects liver cancer.

A central finding of this research is that the functioning of inflammation and oxidative stress is critical in mediating the pro-tumorigenic effects of PM2.5. The observed increment in inflammation, fibrosis, and oxidative stress markers in the liver tissues of PM2.5-exposed mice aligns with the established understanding that chronic inflammation is a key driver of HCC [5]. PM2.5 exposure appears to create a pro-inflammatory microenvironment that is conducive to tumour growth. This is likely initiated by reactive oxygen species (ROS), as supported by our findings and previous reports [3,4]. The resulting oxidative stress can lead to widespread cellular damage, including DNA mutations, which was evidenced by the downregulation of DNA repair genes in our RNA-seq analysis. This impairment of genomic maintenance, coupled with a persistent inflammatory state, provides an impetus for malignant transformation.

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As part of the study to understand the effects of PM2.5 on cellular behaviour and gene expression, what we saw from the results led us to determine what proteins were likely responsible for mediating these changes. The p53 and Bcl-2 proteins were clearly important in mediating some of the changes we observed with cells exposed to PM2.5. With increasing concentrations of PM2.5, we also saw decreasing levels of the tumour suppressor protein p53, while the anti-apoptotic protein Bcl-2 continued to increase in response. Therefore, we observed an inverse relationship between the two proteins that suggests that when p53 expression is reduced and Bcl-2 levels are increased, cancer cells become resistant to apoptosis, allowing them to continue to grow and replicate. Altering this relationship between p53 and Bcl-2 is an indicator of cancer progression, and support our conclusion that PM2.5 exposure promotes tumour cell growth and tumour progression through a disruption in the normal regulation of p53-Bcl-2 axis. Recent structure studies have elucidated the specific interaction between p53 and Bcl-2 [6] and will be a critical factor in understanding the complete role of this interaction in the biology of HCC. Understanding the detailed mechanism of PM2.5's effect on these proteins will enhance our understanding of HCC progression, as there is considerable evidence to suggest that PM2.5 exposure is directly linked to the development of HCC.

Also, our in vivo findings are corroborated by the in vitro findings conducted using HepG2 cell lines. PM2.5 promotes the malignant properties of liver cancer through increased cell migration, invasion and the activation of the protective PI3K/Akt signaling pathway [7]. The activation of the Akt pathway, which promotes cell growth and invasion following oxidative stress/inflammation, is one of the most widely recognized consequences of oxidative stress/inflammation. The findings from both the in vitro and in vivo studies confirm PM2.5 as a major environmental hepatocarcinogen.

5. Conclusion

This study concludes by showing that PM2.5 exposure is one of the main reasons for hepatocellular carcinoma development and progression. Our combined in vivo and in vitro method shows that PM2.5 causes chronic oxidative stress and inflammation in the liver, which leads to HCC. Mechanistically, this is linked to a key dysregulation of the apoptotic mechanism, which is typified by the overexpression of the anti-apoptotic protein Bcl-2 and the downregulation of the tumour suppressor p53. This change in the p53-Bcl-2 axis creates an environment that promotes the expansion and endurance of cancer cells. These results demonstrate the serious risk that air pollution poses to public health in relation to liver cancer and emphasize the necessity of more stringent environmental laws. To counteract the harmful impacts of exposure to PM2.5 on the liver and reduce the incidence of HCC in exposed populations, more study is necessary to investigate viable therapeutic approaches.

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