The Role of Biomarkers in Monitoring Liver Disease Progression: Insights into AFP, and P53

دور المؤشرات الحيوية في مراقبة تطور أمراض الكبد: رؤى حول AFP وP53"

By

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a growing global health concern, often progressing to hepatocellular carcinoma (HCC), a leading cause of liver cancer. This research investigates the relationship between NAFLD and HCC in HepG2 cells and the roles of p53 and alpha-fetoprotein (AFP) in these processes. The study investigated that the inactivation of p53 (NAFLD = 22.5 ±2.02U/ml, HCC = 55 ±2.21U/ml) contributes to the progression of NAFLD to HCC, and that elevated AFP levels (NAFLD = 13 ±1.17ng/ml, HCC = ±1.2ng/ml) serve as both a biomarker and a contributor to liver carcinogenesis (p-value < 0.5). The findings highlight that the elevated values of AFP, but not of P53, may help in understanding the transformation in the context of NAFLD and liver cancer.

Keyword: Non-Alcoholic Fatty Liver Disease, Hepatocellular Carcinoma, AFP, P53

المستخلص

يعد مرض الكبد الدهني غير الكحولي (NAFLD) مصدر قلق صحي عالمي متزايد، وغالبًا ما يتطور إلى سرطان الخلايا الكبدية (HCC)، وهو السبب الرئيسي لسرطان الكبد. يبحث هذا العمل في العلاقة بين NAFLD وHCC في خلايا HepG2 ودور p53 والبروتين الجنيني ألفا (AFP) في هذه العمليات. أوضحت الدراسة في أن تعطيل نشاط p53 (NAFLD = 22.5 ±2.02U/ml، HCC = 55 ±2.21U/ml) يساهم في تطور NAFLD إلى HCC، وأن ارتفاع مستويات AFP (NAFLD = 13 ±1.17ng/ml)، (HCC = ±1.2ng/ml) بمثابة علامة حيوية ومساهم في تسرطن الكبد (*p*-value <0.5). تسلط النتائج الضوء على أن القيم المرتفعة لـ AFP، ولكن ليس لـ P53، قد تساعد في فهم التحول حالات NAFLD الى سرطان الكبد.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a multifactorial disorder that has emerged as a global health concern. It is characterized by the accumulation of fat in liver cells in individuals who consume little to no alcohol (Younossi et al., 2016). NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), which involves liver inflammation and injury and can progress to advanced stages such as fibrosis, cirrhosis, and ultimately hepatocellular carcinoma (HCC) (Loomba & Sanyal, 2013). The transition from NAFLD to HCC is a gradual process that involves various molecular mechanisms, including oxidative stress, inflammation, and genetic mutations.

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and is often associated with chronic liver diseases, including NAFLD, hepatitis B and C infections, and alcohol-induced liver disease. HCC is characterized by uncontrolled cell proliferation, evasion of apoptosis, and alterations in cellular metabolism (Llovet et al., 2003). HepG2 cells, a human hepatoma cell line, are frequently used to study liver diseases and carcinogenesis in vitro. These cells retain many features of normal hepatocytes and can be manipulated to mimic various stages of liver disease, making them an ideal model for studying NAFLD and HCC progression.

p53 is a tumor suppressor protein that plays a key role in maintaining cellular integrity by regulating the cell cycle, promoting DNA repair, and inducing apoptosis in response to cellular stress (Levine, 1997). Mutations or inactivation of p53 are common in many cancers, including liver cancer, and are often associated with poor prognosis and resistance to therapy. In the context of liver disease, p53 dysfunction allows the survival

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of damaged hepatocytes, contributing to the development of HCC.

Alpha-fetoprotein (AFP) is a glycoprotein synthesized primarily by the fetal liver, yolk sac, and gastrointestinal tract during fetal development. In adults, AFP levels are typically low, but elevated AFP is often observed in patients with liver cancer, especially HCC (Llovet et al., 2003). AFP is used as a biomarker for diagnosing HCC and monitoring treatment response. Elevated AFP levels are correlated with tumor size, stage, and prognosis, making it a valuable marker for assessing the progression of HCC. This study aims to explore the relationship between NAFLD and HCC in HepG2 cells, focusing on the roles of p53 and AFP

Methodology

1. Cell Culture MTT-assay

HepG2 cells were obtained from the American Type Culture Collection (ATCC) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained in a humidified incubator at 37°C with 5% CO_2. The cells were seeded in 6-well plates at a density of 1×10^5 cells/well, and repeated into 3 groups [NC: normal control, NAFLD, HCC]. For treatments, cells were incubated for 48-72 hours to allow for the induction of NAFLD or HCC (Jiang et al., 2015).

2. Induction of NAFLD in HepG2 Cells

To induce -like conditions in HepG2 cells, a mixture of fatty acids was added to the culture medium. These fatty acids were prepared by dissolving palmitate and oleate in ethanol and then adding the mixture to the medium to a final concentration of 0.4 mM. After 48-72 hours of treatment, lipid accumulation in the hepatocytes was assessed using Oil Red O staining, which

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detects intracellular lipid droplets (Feng et al., 2018). Additionally, triglyceride levels were measured to quantify fat accumulation in the cells.

3. HCC Induction in HepG2 Cells

To induce HCC in HepG2 cells, we exposed the cells to oxidative stress agents such as hydrogen peroxide (H_2O_2) or aflatoxin B1, both of which have been shown to promote DNA damage, inflammation, and carcinogenesis (Chang et al., 2019). The cells were treated with 200 μM H_2O_2 or 1 μM aflatoxin B1 for 48 hours, followed by assessments of cell morphology, proliferation, and the induction of cancer-related markers.

4. P53 and AFP Analysis

Both P53 and AFP levels were measured using enzyme-linked immunosorbent assay (ELISA) Kits [(**Bender Med Systems,** 2021) **for P53 protein, (COAT-A-COUNT,** 2021) **for AFP**].

5. Statistical Analysis

Statistical analysis was performed using (ANOVA) test two factors with replicates. A *p-value* of < 0.05 was considered statistically significant(Zar, 2010).

Results & Discussion

The data from the results of **P53** and **AFP** levels across the three groups: **NC** (Normal Control), **NAFLD** (Non-Alcoholic Fatty Liver Disease), and **HCC** (Hepatocellular Carcinoma), provide important insights into the role of these markers in liver disease progression and carcinogenesis.

The **P53** protein plays a critical role in maintaining cellular integrity by regulating the cell cycle and inducing apoptosis in response to DNA damage or cellular stress (Basu et al., 2020). As observed in the current study, **P53** levels show a progressive increase from the NC group (13.3 U/ml) to NAFLD (22.5 U/ml), and a significant rise in the HCC group (55 U/ml).

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The moderate increase in **P53** levels in the NAFLD group may be attributed to the stress caused by lipid accumulation in liver cells. **NAFLD** is associated with oxidative stress, inflammation, and hepatocyte injury (Sanyal et al., 2015). The increase in **P53** suggests a cellular response to this damage. This finding is consistent with studies indicating that **P53** may act as a cellular defense mechanism in response to metabolic stress and liver injury (Ding et al., 2017).

The marked increase in **P53** levels in the HCC group is consistent with the tumor-suppressive role of **P53** in cancer. In many cancers, including liver cancer, **P53** mutations or alterations in its expression are common (Hussain et al., 2021). These alterations can lead to disrupted cell cycle regulation, increased genomic instability, and resistance to apoptosis, all of which contribute to cancer progression. The high levels of **P53** in HCC may thus reflect the presence of genomic instability and the transformation of normal cells to cancerous ones. Several studies have reported that **P53** mutations are among the most frequent genetic alterations in HCC (Li et al., 2020). The progressive increase in **P53** levels from **NC** to **NAFLD** to **HCC** supports the concept that **P53** could serve as a biomarker for the early detection and monitoring of liver disease progression. As **P53** levels increase in response to both cellular stress in early-stage liver disease and genetic alterations in cancer, it may have diagnostic and prognostic value in the context of NAFLD and HCC.

AFP is a well-established biomarker for liver malignancies, particularly hepatocellular carcinoma (HCC). In healthy individuals, **AFP** levels are typically low (Liu et al., 2018). In this study, **AFP** levels were low in the NC group (3 ng/ml), which is consistent with normal liver function. However, an increase in **AFP** levels was observed in both the NAFLD and

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HCC groups. The mild rise in **AFP** levels in the NAFLD group (13 ng/ml) is indicative of liver dysfunction or injury. Although AFP is not typically elevated in the early stages of NAFLD, some studies suggest that **AFP** can be a marker for liver inflammation and early liver damage (Cai et al., 2019). It has been proposed that **AFP** could be elevated even in the absence of a malignancy in chronic liver diseases, including NAFLD, due to hepatocyte turnover and inflammation (Kishimoto et al., 2020).
In the HCC group, **AFP** levels further increased to 15 ng/ml. AFP is widely recognized as a tumor marker for HCC, and its elevated levels are often associated with poor prognosis and advanced disease stages (Shin et al., 2017). Elevated **AFP** levels in HCC are primarily due to the secretion of AFP by cancerous liver cells, which have aberrant functions. The role of AFP as a diagnostic tool in HCC has been well-documented, though it is not actually specific, as levels can also be elevated in other liver diseases (Bruix et al., 2014). The gradual increase in **AFP** levels from **NC** to **NAFLD** to **HCC** suggests that **AFP** can be a potential biomarker for tracking liver disease progression. While **AFP** is more commonly used as a biomarker for HCC, its levels can also provide useful information about liver dysfunction and potential malignancy in patients with NAFLD (Padrão et al., 2018). Thus, monitoring **AFP** levels in patients with NAFLD may help identify those at risk of developing HCC.

Both **P53** and **AFP** have shown to be potential biomarkers for monitoring liver disease progression, from non-alcoholic fatty liver disease (NAFLD) to hepatocellular carcinoma (HCC). The progressive increase in both markers suggests that they could play complementary roles in detecting early liver damage and identifying patients at risk for developing HCC. **P53** may serve as an early indicator of cellular stress or genomic instability, which could be relevant for the monitoring

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of NAFLD and its progression to HCC. Given its involvement in regulating the cell cycle and response to DNA damage, **P53** could also aid in assessing the risk of malignancy in liver diseases. **AFP** continues to be a reliable marker for HCC diagnosis and monitoring. Its gradual rise in both NAFLD and HCC suggests that it could be used for early detection of liver malignancy in high-risk populations, especially when combined with other diagnostic tools such as imaging.

Table 1: Alpha-fetoprotein (AFP) and p53 concentration among the NAFLD and HCC on Hepg2 cells.

Case / Marker	NC	NAFLD*	HCC*
P53 U/ml (normal 5 to 25)	13.3 ±1.92	22.5 ±2.02	55 ±2.21
AFP ng/ml (Normal 1-10)	3 ± 0.91	13 ±1.17	15 ±1.2

NC: normal control, NAFLD: Non-alcoholic fatty liver disease, HCC: Hepatocellular carcinoma, AFP: alpha fetoprotein. ANOVA Two factors with replicates. *The *p*-value for P53 is less than 0.05, it has a significant effect on both NAFLD and HCC . Similarly, the p-value for AFP is less than 0.05, it has a significant effect on both cases. Interaction Effect: the *p*-value for the interaction term (P53*AFP) in both cases (NAFLD and HCC) revealed that the p-value is less than 0.05, it indicates a significant interaction between P53 and AFP. This means the effect of one factor depends on the level of the other factor.

Importance of Nutrition in Preventing the Progression of Non-Alcoholic Fatty Liver Disease (NAFLD) to Hepatocellular Carcinoma (HCC)

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Nutrition plays a pivotal role in the management of Non-Alcoholic Fatty Liver Disease (NAFLD) and its potential progression to more severe conditions, including Hepatocellular Carcinoma (HCC). NAFLD is a spectrum of liver conditions ranging from simple steatosis (fatty liver) to non-alcoholic steatohepatitis (NASH), which can further progress to fibrosis, cirrhosis, and ultimately liver cancer (HCC) (Younossi et al., 2016). Proper nutrition can help mitigate the factors that contribute to the progression of NAFLD and HCC by addressing underlying metabolic disturbances such as obesity, insulin resistance, and inflammation.

A healthy, balanced diet is essential for managing and preventing the progression of NAFLD. The primary aim is to reduce liver fat accumulation, control body weight, and improve insulin sensitivity. A diet rich in antioxidants, healthy fats, and fiber, such as the Mediterranean diet, has been shown to reduce liver fat and inflammation, key factors in preventing the progression from simple steatosis to NASH (Musso et al., 2010). Reducing the intake of saturated fats, refined sugars, and processed foods is crucial as these can exacerbate liver inflammation, insulin resistance, and oxidative stress, all of which contribute to the development of NAFLD and its subsequent progression to HCC (Bellentani & Marino, 2009). Additionally, the intake of certain nutrients such as vitamin E and omega-3 fatty acids has been associated with improvements in liver function and a reduction in liver fat (Chalasani et al., 2012).

Obesity is one of the major risk factors for the development and progression of NAFLD, with insulin resistance being a key contributor to hepatic fat accumulation (Cusi, 2016). Excess visceral fat, which is more metabolically active, can lead to increased fatty acid release into the liver, promoting

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lipotoxicity and triggering inflammatory responses (Friedman, 2008). Therefore, adopting a weight loss strategy through dietary modifications, coupled with physical activity, is crucial in managing NAFLD. Weight reduction of as little as 5-10% has been shown to significantly improve liver histology and reduce the risk of progression to cirrhosis and HCC (Van der Poorten et al., 2008).

Chronic oxidative stress and inflammation are central to the progression of NAFLD to NASH and ultimately to HCC. A diet rich in antioxidants, such as those found in fruits, vegetables, and whole grains, can help reduce oxidative stress in the liver and limit the inflammatory pathways that contribute to liver damage (De Bie et al., 2016). Polyphenols, found in foods such as green tea and berries, have demonstrated anti-inflammatory and antioxidant properties that may help protect the liver from carcinogenic damage (Basu et al., 2017). Furthermore, reducing the intake of pro-inflammatory foods, such as trans fats and processed meats, may help in reducing liver inflammation, thereby lowering the risk of liver fibrosis and cancer development (Gaggini et al., 2018).

Certain nutrients have shown promise in the prevention of liver cancer in the context of NAFLD. Vitamin D, for instance, has been shown to exhibit anti-cancer effects, with low levels of vitamin D being associated with an increased risk of HCC (Wang et al., 2016). Omega-3 fatty acids, found in fatty fish and flaxseeds, also exhibit anti-inflammatory properties and have been associated with improved liver function in individuals with NAFLD (Mori et al., 2017).

Conclusion:

The findings in this study highlight the potential utility of **P53** and **AFP** as biomarkers for liver disease progression, particularly in the context of NAFLD and HCC. The increasing

ISSN: 2537-0804 eISSN: 2537- 0855

levels of both markers from **NC** to **NAFLD** to **HCC** underscore their role in reflecting the stages of liver disease and the transition from benign liver conditions to cancer. Future studies are needed to further validate the diagnostic and prognostic value of **P53** and **AFP**, particularly in the early detection of HCC in patients with chronic liver diseases. Nutrition plays a vital role in managing and preventing the progression of NAFLD to more severe conditions like HCC. By focusing on a healthy, balanced diet, addressing obesity and insulin resistance, and reducing oxidative stress and inflammation, it is possible to slow the progression of liver disease and reduce the risk of liver cancer. Continued research into the role of specific nutrients and dietary patterns in liver health is necessary to better understand the mechanisms by which nutrition can prevent NAFLD progression and protect against liver cancer.

ISSN: 2537-0804 **eISSN: 2537- 0855**

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