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A Review of Liver Cancer Development Driven by the AP-1/c-Jun~Fra-2 Dimer through c-Myc

Song Liu[1*], Jinhuan Liu[1]

[1]The Fourth Affiliated Hospital of Soochow University, Suzhou, China
[2]The First Clinical Medical College, Wenzhou Medical University, Wenzhou, China
**Corresponding Author. Email: liusong19980307@163.com*

Abstract. Hepatocellular carcinoma (HCC) ranks among the leading causes of cancer-related mortality worldwide. Recent epidemiological data demonstrate a rising incidence of HCC, which is further compounded by its insidious onset, poor prognosis, and limited therapeutic options. Elucidating its molecular pathogenesis and identifying viable therapeutic targets remain critical unmet needs. While prior studies have implicated members of the AP-1 (Activator Protein-1) transcription factor family (e.g., c-Fos and c-Jun) in hepatocarcinogenesis, the roles of Fos-related antigens (Fra-1 and Fra-2) remain poorly characterized. This study investigates the mechanistic interplay between c-Jun/Fra-2 heterodimers and the oncogenic driver c-Myc—a well-established molecular nexus in HCC pathogenesis—unraveling their collective impact on HCC proliferation, inflammatory cascades, and tumorigenesis. Through a systematic literature review, this study delineates the pro-tumorigenic role of the c-Jun/Fra-2-Myc axis in murine HCC models. This paper elucidates how c-Jun-Fra-2 heterodimers transcriptionally regulate c-Myc to orchestrate core oncogenic programs. Integrative analysis of the HCC tumor microenvironment (TME) further reveals that c-Jun-Fra-2 overexpression induces TME remodeling, characterized by low-grade inflammation, mild fibrosis, and dyslipidemia—key permissive factors for HCC progression. The findings underscore the pivotal role of c-Jun-Fra-2 heterodimers in HCC pathogenesis, wherein their upregulation of c-Myc drives tumor initiation and progression. Notably, the c-Jun-Fra-2/c-Myc axis exhibits both reversibility and dependency, suggesting a therapeutic vulnerability. In HCC patients with elevated c-Jun-Fra-2 expression, pharmacological inhibition using the BET inhibitor JQ-1 significantly attenuates tumor growth, highlighting its potential as a precision therapy target. This work advances the molecular understanding of HCC and provides a rationale for targeted intervention in defined subsets.

Keywords: AP-1, c-Jun/Fra-2, HCC, c-Myc

1. Introduction

Hepatocellular carcinoma (HCC) ranks as the third leading cause of cancer-related mortality globally, with its incidence continuing to rise [1], particularly in low- and middle-income countries (LMICs) where risk factors such as chronic liver disease (e.g., viral hepatitis, metabolic dysfunction-

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associated steatotic liver disease [MASLD]) are highly prevalent. Despite its clinical burden, treatment options remain limited, underscoring the urgent need for mechanistic insights and targeted therapies [2,3]. Previous studies [4,5] have implicated transcription factor AP-1 (Activator Protein-1) family members in HCC pathogenesis—c-Jun regulating cell survival and c-Fos modulating metabolic reprogramming. However, the specific functions of AP-1 heterodimers (e.g., c-Jun/Fra-2) in HCC remain poorly understood, partly due to the limitations of conventional genetically engineered mouse models (GEMMs), which cannot dissect the individual contributions of specific dimer pairs, thereby hindering precision therapeutic development.

Previous studies have established that c-Jun promotes DEN-induced hepatocarcinogenesis through p53 suppression [4,6], while c-Fos induces precancerous lesions via cholesterol metabolic dysregulation [5]. Interestingly, Fra proteins (particularly Fra-2), though implicated in lipid metabolism, have unknown roles in liver cancer. c-Myc, a central oncogenic driver in HCC, promotes cell proliferation and tumor progression. While existing research indicates that c-Jun may indirectly regulate c-Myc through c-Fos inhibition [5], whether c-Jun/Fra-2 directly modulates c-Myc remains unclear. Notably, in colorectal cancer studies, Jun has been shown to cooperatively activate the MYC 3' enhancer (WRE) through β-catenin/TCF4 [7], but this mechanism awaits validation in HCC. This comprehensive review critically examines innovative approaches for studying "single-chain dimers" [8] to elucidate the role of c-Jun/Fra-2 dimers in HCC. It aims to clarify their mechanism in driving tumorigenesis through c-Myc regulation and explore novel stratified therapeutic strategies for clinical application.

2. Literature review

The pathogenesis of hepatocellular carcinoma (HCC) is highly complex, involving aberrant activation of multiple molecular pathways. Despite recent advances in diagnosis and treatment, the long-term survival rates of HCC patients remain suboptimal (5%-30% 5-year survival) due to tumor heterogeneity and therapy resistance. Elucidating the molecular mechanisms of HCC—particularly the regulatory networks of key transcription factors and their downstream effectors—is critical for developing novel targeted therapies.

2.1. Structure, function and advanced research of AP-1 transcription factor family

AP-1 (Activator Protein-1) is a dimeric transcription factor composed of Jun (c-Jun, JunB, JunD) and Fos (c-Fos, Fra-1, Fra-2) family proteins. It binds to specific DNA sequences (e.g., TGACTCA) to regulate target gene expression. Different AP-1 dimer combinations (e.g., c-Jun/c-Fos, c-Jun/Fra-2) exhibit distinct DNA-binding affinities and transcriptional regulatory properties, thereby diversifying their roles in cellular processes such as proliferation, inflammation, and tumorigenesis.

Efer et al. demonstrated that c-Jun plays pivotal roles in liver regeneration, oxidative stress response, and HCC development by suppressing p53 or activating anti-apoptotic signals [4]. Bakiri et al. later showed that c-Fos links metabolic dysregulation and DNA damage to hepatic cholesterol accumulation and preneoplastic lesions [6]. For Fra-1/Fra-2, Hasselblatt et al. identified their involvement in lipid metabolism (via PPARγ suppression) [9,10] and hepatoprotection [11], but their functions in HCC remain unclear.

Notably, AP-1 exhibits context-dependent duality in HCC: Smart et al. [12] reported that JunD could suppress hepatic fibrosis and steatosis under specific conditions. Min et al. [6] found that c-Jun promotes DEN (diethylnitrosamine)-induced hepatocarcinogenesis via p53 inhibition. These

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findings highlight how AP-1 may differentially regulate HCC progression through distinct downstream targets.

2.2. Comprehensive research in c-Jun-Fra-2

2.2.1. c-Jun-Fra-2 drives HCC development

This study reveals, for the first time, that hepatocyte-specific expression of c-Jun-Fra-2 dimers spontaneously induces HCC in mice, whereas Fra-2 alone or c-Jun-Fra-1 does not. This underscores the unique oncogenic role of c-Jun-Fra-2 among AP-1 dimers. Additionally, c-Jun-Fra-2 remodels the tumor microenvironment (TME), generating HCCs with mild inflammation and low-grade fibrosis—distinct from steatohepatitis-associated HCC.

2.2.2. c-Jun-Fra-2 activates c-Myc to fuel tumorigenesis

c-Myc, a central oncogene in HCC, interacts with IL-6/JAK/STAT3 and PI3K/AKT/GSK3β pathways to drive cell-cycle progression, metabolic reprogramming, and immune evasion [13,14]. This study demonstrates that c-Jun-Fra-2 directly upregulates c-Myc transcription by binding to its 3' enhancer (WRE), a mechanism validated in human HepG2 cells and TCGA datasets [15], suggesting broad relevance in human HCC.

Notably, most tumors regress upon c-Jun-Fra-2 silencing, indicating oncogene addiction. Residual escape tumors maintain c-Myc expression, potentially via compensatory AP-1 dimers (e.g., c-Fos), though this requires further validation.

2.2.3. Therapeutic targeting of the c-Jun-Fra-2/c-Myc axis

While c-Myc itself is "undruggable" due to its complex structure and pleiotropic functions, this study explores epigenetic modulation as an alternative strategy. The BET inhibitor JQ-1 effectively suppresses c-Jun-Fra-2-driven HCC [16] by downregulating c-Myc and its targets (e.g., Cyclin D1), and inhibiting proliferation and inducing apoptosis. Combining JQ-1 with sorafenib enhances antitumor efficacy, particularly in AP-1/Myc-high HCC subtypes.

2.2.4. Clinical implications

Detecting c-Jun/Fra-2 and c-Myc expression may predict BET inhibitor sensitivity, enabling patient stratification. The study’s findings also warrant exploration of combination therapies (e.g., JQ-1 + immune checkpoint inhibitors). By elucidating how c-Jun-Fra-2 directly activates c-Myc to drive HCC, this work provides both mechanistic insights and a novel target for precision therapy.

3. Discussion

The intricate pathogenesis of hepatocellular carcinoma (HCC) remains a major research focus. Increasing attention has been directed toward the role of the AP-1 transcription factor in HCC development. This study employs an innovative c-Jun-Fra-2 single-chain dimer (SCD) transgenic mouse model to elucidate the molecular mechanisms by which this dimer promotes HCC through c-Myc regulation and evaluates the therapeutic potential of targeting this pathway. This paper presents a detailed discussion of the experimental methodology, key findings, and their scientific significance.

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3.1. Experimental methodology

This study pioneered the use of single-chain dimer (SCD) technology to construct mouse models. Researchers developed a hepatocyte-specific c-Jun-Fra-2 SCD transgenic mouse using the Tet-On system, where c-Jun and Fra-2 were connected by a flexible peptide linker. This approach elegantly circumvented the limitations of conventional knockout models in dissecting specific dimer functions. Through doxycycline (Dox)-inducible expression, the study successfully mimicked the persistent AP-1 activation observed in chronic liver disease.

Experimental results revealed that 90% of c-Jun-Fra-2hep mice developed macroscopic liver tumors within 9 months after Dox withdrawal, whereas Fra-1hep or Fra-2hep mice showed no such phenotype. These tumors exhibited classic HCC pathological features, including cellular atypia and increased mitotic figures, along with elevated expression of HCC markers (AFP and GP73). This discovery not only provides the first evidence that c-Jun-Fra-2 dimers independently drive HCC, but also establishes an ideal platform for studying human HCC molecular characteristics.

3.2. Mechanistic studies

Chromatin immunoprecipitation (ChIP-qPCR) studies demonstrated that c-Jun-Fra-2 specifically binds to the distal 3' enhancer region (WRE) of c-Myc, with minimal promoter binding. Luciferase reporter assays showed that c-Jun-Fra-2 enhanced c-Myc-WRE activity by 5-fold, while Fra-2 alone had no effect. These results reveal the spatial specificity of AP-1-mediated c-Myc regulation - primarily through distal enhancers rather than promoters - and provide direct evidence for the AP-1-c-Myc axis in HCC. Notably, this regulatory mechanism differs from the JUN/β-catenin-MYC axis previously identified in colorectal cancer [7], suggesting tissue-specific regulation of c-Myc by AP-1.

3.3. Key finding-reversibility experiments

A particularly compelling finding emerged from reversibility experiments [17-18]. When c-Jun-Fra-2 expression was suppressed after 9 months of tumor formation, approximately two-thirds of mice showed complete tumor regression with normalized AFP levels, while one-third maintained tumor growth. Further analysis revealed that these "escape" tumors retained high c-Myc expression and exhibited c-Fos upregulation. These observations demonstrate the c-Jun-Fra-2-driven HCC displays oncogene addiction, consistent with previous findings on c-Myc-driven tumors; and functional redundancy among AP-1 family members, where c-Fos may compensate for c-Jun-Fra-2 loss. These findings have crucial clinical implications, suggesting that effective targeted therapies may need to simultaneously inhibit multiple AP-1 dimers to prevent resistance.

3.4. Exploration of treatment

The study evaluated the efficacy of BET inhibitor JQ-1 in both preventive and therapeutic settings. In the preventive group, JQ-1 treatment for 4 weeks (initiated 2 months after c-Jun-Fra-2 induction) significantly reduced tumor incidence. In established tumors, 8 weeks of JQ-1 monotherapy or combination with sorafenib resulted in 60% tumor volume reduction and decreased c-Myc protein levels. Mechanistically, JQ-1 selectively suppressed c-Myc downstream targets (e.g., Cyclin D1, FoxM1) without affecting c-Jun-Fra-2 expression. These results not only confirm that BET inhibitors can epigenetically disrupt the AP-1/c-Myc axis, but also suggest new clinical strategies for

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AP-1-high HCC, particularly the promising "BET inhibitor + targeted therapy" combination approach [19].

3.5. Other innovative meanings and challenges

This study makes several groundbreaking contributions. It transcends traditional single-gene approaches by revealing Fra-2 as c-Jun's essential oncogenic partner, significantly expanding our understanding of AP-1 functional diversity. It identifies the critical role of c-Myc distal enhancers, explaining why some c-Myc-high tumors are resistant to promoter-targeting drugs. It systematically elucidates the dynamic regulatory network of AP-1 dimers in tumor maintenance, providing key insights into tumor plasticity and drug resistance.

However, clinical translation faces challenges. Patient stratification requires new methods to accurately detect AP-1 dimer combinations (e.g., c-Jun/Fra-2 vs. c-Jun/c-Fos) for precision therapy. Overcoming resistance may necessitate pan-AP-1 inhibitors or complex combination strategies, given the compensatory c-Fos upregulation in escape tumors. Tumor microenvironment heterogeneity may affect treatment efficacy, demanding more comprehensive consideration of the tumor ecosystem in future studies.This work fundamentally advances our understanding of HCC pathogenesis while providing a framework for developing novel therapeutic strategies against AP-1-driven HCC.

4. Conclusion

This study elucidates the critical role of the specific c-Jun-Fra-2 heterodimer from the AP-1 transcription factor family in HCC pathogenesis. Through innovative construction of a c-Jun-Fra-2 single-chain dimer (SCD) transgenic mouse model, the author provides first evidence that this dimer directly binds to the 3' enhancer region of c-Myc, significantly upregulating its expression and subsequently activating multiple oncogenic signaling cascades that ultimately drive hepatocarcinogenesis. These findings not only reveal the central regulatory mechanism of the AP-1/c-Jun-Fra-2-c-Myc axis in HCC development, but more importantly establish a direct functional link between specific AP-1 dimers and oncogenes.

The c-Jun-Fra-2-driven HCC exhibits marked oncogene addiction features. Upon dimer suppression, most tumors regress, though a minority maintain growth through compensatory mechanisms involving other AP-1 members (e.g., c-Fos). Therapeutically, BET inhibitor JQ-1 demonstrates potent antitumor effects by selectively suppressing c-Myc and its downstream targets, showing synergistic efficacy when combined with sorafenib.

While representing a significant advance, several aspects require further investigation. Insufficient depth of mechanism: Studies are mainly based on literature integration, lack of in-depth experimental verification of the specific molecular mechanisms (such as coactivators and epigenetic modifications) of c-Jun-Fra-2 in regulating c-Myc, and the interaction between c-jun-Fra-2 and other key pathways (such as Wnt/β-catenin) has not been fully explored. Weak analysis of tumor microenvironment (TME): This article does not systematically analyze the effect of c-Jun-Fra-2 on key components of TME (such as immune cells, fibroblasts), which may affect HCC progression and treatment response, nor does it mention whether c-Jun-Fra-2 promotes HCC metastasis by regulating extracellular matrix (ECM) remodeling. Limitations of clinical transformation: although the potential efficacy of BET inhibitor JQ-1 was proposed, its clinical application challenges (such as drug resistance and patient stratification criteria) were not systematically analyzed, and the

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combination strategy with existing therapies (such as immunotherapy) was not discussed, which reduced the value of the conclusion for practice guidance.

Future research should focus on three key directions for deeper exploration. Mechanistic Elucidation and Multi-Omics Integration: Utilizing cutting-edge technologies such as ChIP-seq and CRISPR screening to systematically dissect the epigenetic mechanisms by which c-Jun-Fra-2 regulates c-Myc (e.g., enhancer-promoter interactions) and its crosstalk with pathways like Wnt/β-catenin. Additionally, single-cell sequencing should be employed to uncover the dynamic roles of AP-1 dimers in tumor heterogeneity. Tumor Microenvironment and Therapy Resistance: Leveraging humanized mouse models and spatial transcriptomics to elucidate how c-Jun-Fra-2 remodels the TME and investigate resistance mechanisms. Clinical Translation Optimization: Establishing an AP-1/c-Myc-based molecular stratification system for patient selection, designing combination therapies integrating JQ-1 with PD-1 inhibitors or targeted agents, and validating efficacy through organoid drug sensitivity platforms to advance precision medicine strategies. This structured approach will bridge mechanistic insights with clinical applicability, driving transformative progress in HCC therapeutics.

These advances will fundamentally deepen our understanding of HCC pathogenesis while providing actionable therapeutic targets. The current study lays crucial groundwork for AP-1 dimer-focused oncology research, opening new avenues for precision HCC treatment. Continued investigation along these lines promises to transform clinical management and improve patient outcomes.

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