RESEARCH ARTICLE

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Unraveling Key Regulatory Pathways in Non-HCV-Induced Hepatocellular Carcinoma: Insights from Exclusively Mutated Genes and Prognostic Biomarkers

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Abstract

Background: The molecular pathogenesis of hepatocellular carcinoma (HCC) exhibits striking etiological heterogeneity, with non-HCV-associated cases representing an increasingly prominent clinical challenge in regions like Egypt, where environmental carcinogens significantly contribute to the disease burden. **Methods:** Through integrated analysis of genomic data Egyptian cohort comprising 48 HCC cases (23 non-HCV, 25 HCV-positive) was examined and validated against TCGA/ICGC datasets using cBioPortal and Cytoscape. **Results:** This study identifies a distinct oncogenic program in non-viral HCC characterized by recurrent alterations in receptor tyrosine kinases (RTKs) *FGFR1, MET, ERBB2* and *FLT3.* These mutations were found to be 4.3-fold more prevalent in non-HCV HCC compared to viral counterparts (26.1% vs. 6.0%, p=0.008), demonstrating strong etiological specificity. Functional characterization revealed these alterations converge on MAPK and PI3K-AKT-mTOR signaling cascades through shared adaptor proteins, creating an interconnected signaling network that drives tumor progression. **Conclusion:** Clinically, FGFR1/MET co-alterations predicted significantly worse outcomes (HR=2.3 for recurrence, 95% CI 1.1-4.8), while maintaining 92% specificity for non-viral HCC diagnosis. These findings establish the FGFR1-MET-ERBB2 axis as both a molecular classifier and therapeutic target, providing a rationale for etiology-specific management strategies in HCC precision oncology.

Keywords: Targeted therapy- cell-free DNA- hepatocellular carcinoma- precision oncology

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Introduction

Hepatocellular carcinoma (HCC) stands as a formidable global health challenge, representing the predominant form of primary liver cancer and ranking as the third leading cause of cancer-related mortality worldwide [1, 2]. Its incidence continues to rise, fueled by an increasing burden of chronic liver diseases, including non-alcoholic steatohepatitis (NASH) and metabolic dysfunction-associated steatotic liver disease (MASLD), posing significant strain on healthcare systems globally [3-5]. Historically, chronic infection with the hepatitis C virus (HCV) has been a major etiological driver, particularly in Western countries and Japan, leading to extensive research focused on HCV-related hepatocarcinogenesis [6, 7]. This research has yielded valuable insights into viral mechanisms of oncogenesis, such as the disruption of host signaling pathways by viral proteins (e.g., core, NS3, NS5A) and the chronic inflammatory milieu promoting genomic instability [8-10].

While HCV-related HCC has been extensively characterized, non-viral HCC arising from alternative risk factors (e.g., aflatoxin exposure, metabolic dysfunction, alcohol) remains poorly understood despite its growing prevalence [11-13]. This knowledge gap carries particular significance in Egypt, where unique environmental exposures including agricultural pesticides and dietary aflatoxins interact with genetic predispositions to create a high-incidence HCC landscape diverging from Western patterns [14-16]. Recent epidemiological studies estimate that 15-20% of Egyptian HCC cases occur without viral etiology, with these tumors demonstrating more aggressive clinical courses despite earlier detection [17-19].

The molecular basis for this clinical behavior remains unclear, though preliminary evidence suggests involvement of receptor tyrosine kinase (RTK) pathways and chromatin remodeling genes (e.g., ARID2) [20,

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21]. Our previous work identified recurrent FGFR1 and MET alterations in Egyptian non-viral HCC patients, contrasting with the PIK3CA/KIT-dominant pattern seen in HCV-associated cases [22]. These observations align with emerging global data showing RTK pathway activation in non-viral HCC subtypes [23, 24], but lack comprehensive characterization of mutation prevalence, functional consequences, and clinical implications specific to the Egyptian population.

This study addresses these gaps through systematic genomic and functional analysis of Egyptian HCC cohorts, with three principal objectives: (a) to define the spectrum and frequency of RTK alterations in non-viral HCC, (b) to characterize their downstream signaling consequences, and (c) to evaluate their prognostic and therapeutic relevance. Our findings provide both biological insights and translational opportunities for improving outcomes in this underserved patient.

Materials and Methods

Data Acquisition and Cohort Selection Genomic and clinical data for hepatocellular carcinoma (HCC) were obtained from The Cancer Genome Atlas (TCGA-LIHC) [20] and the International Cancer Genome Consortium (ICGC) [25], selecting only cases confirmed to be HCV-negative. Comparative cohorts included HCV-positive HCC and normal liver tissue controls. Additionally, raw FASTq data from Khalifa et al. representing Egyptian HCC patients (HCV-positive and HCV-negative) were incorporated to benchmark region-specific mutational signatures.

Preprocessing and Variant Calling FASTq files from Khalifa et al. were aligned to the GRCh38 reference genome using BWA-MEM [26]. Post-alignment processing included duplicate marking and base quality score recalibration using GATK best practices [27]. Somatic variant calling was performed with Mutect2 [28], applying a variant allele frequency (VAF) threshold of ≥5% and a minimum read coverage of 50x. Copy number alterations were inferred using GISTIC2.0 [29] with log2 ratio thresholds of ±0.3. All variants were annotated using OncoKB [30] and Cancer Hotspots [31] databases, with manual curation to confirm driver mutations and exclude sequencing artifacts.

Identification of Exclusively Mutated Genes (EMGs);EMGs were defined as genes recurrently mutated in non-HCV HCC but absent or significantly underrepresented in HCV-positive HCC and normal liver controls. Mutation frequency comparisons were conducted using Fisher's exact test, with false discovery rate (FDR) correction via the Benjamini-Hochberg method [32] (FDR<0.05).

Pathway and Network Analysis EMGs and their co-mutated partners were subjected to integrated pathway enrichment analysis using Gene Ontology (GO) [33], KEGG [34], and Reactome [35] databases. Protein-protein interaction networks were reconstructed in Cytoscape 3.9.1 [36], integrating STRING interactions [37] (confidence score ≥0.7) and Reactome functional annotations to identify central regulatory nodes and dysregulated modules.

Functional Annotation and Hallmark Mapping To assess biological relevance, EMGs were mapped to cancer hallmarks including proliferation, apoptosis evasion, metastasis, immune evasion, and metabolic reprogramming using gene set enrichment analysis (GSEA) [38] and complementary in silico approaches. This enabled contextualization of EMG-driven pathways within established oncogenic processes.

Prognostic Biomarker Discovery Expression profiles of EMGs and core pathway components were correlated with clinical outcomes (overall survival, recurrence-free survival) across multiple independent non-HCV HCC cohorts. Prognostic significance was evaluated using Cox proportional hazards models, adjusting for clinical covariates and applying FDR correction. Prognostic signatures were derived based on multivariate model performance and validated across datasets.

Statistical and Computational Framework All statistical analyses were conducted in R version 4.1.0 [39] using the Tidyverse suite [40] for data handling, visualization, and reproducibility. Analytical workflows were version-controlled and containerized to ensure computational transparency and scalability.

Results

Genomic characterization revealed striking differences in the mutational landscapes of non-viral versus HCV-associated HCC. FGFR1 alterations occurred in 26.1% of non-viral cases (6/23) compared to just 4.0% of HCV-HCC (1/25, p=0.03), predominantly involving amplifications (4 cases) and kinase domain point mutations (2 cases). Similarly, MET mutations were detected in 21.7% of non-viral tumors (5/23) versus 8.0% of viral cases (2/25), including the recurrent V1110I variant which molecular dynamics simulations suggest stabilizes the active kinase conformation. ERBB2 amplifications and FLT3 mutations followed comparable patterns, collectively demonstrating 4.3-fold enrichment in non-viral HCC (p=0.008). These findings were validated in the TCGA-LIHC dataset, where non-viral cases showed similar RTK alteration patterns despite lower absolute frequencies, suggesting an etiological rather than ethnic driver of these differences.

Functional analysis revealed these alterations converge on shared downstream pathways through intricate crosstalk mechanisms. Pathway analysis of the detected alteration using the cBioportal pathway analysis tool revealed that consistently activated TK domains in FGFR1, MET and ERBB2 will activate both MAPK and/or PI3K-AKT cascades. and the functional consequences of these alterations were mapped to three core oncogenic processes: Proliferation: Driven by RTK→RAS→MAPK activation (*FGFR2/KRAS/BRAF* alterations) Cell Survival: Mediated through PI3K-AKT signaling (*ERBB2/ERBB3/NF1* changes) Network modeling identified GRB2 and GAB1 as critical adaptors linking *FGFR1, MET* and *ERBB2* to both MAPK (35% of cases) and PI3K-AKT (45% of cases) cascades (Figure 1).

Pathway and Molecular Function Alterations in HCC

To elucidate the biological consequences of genomic

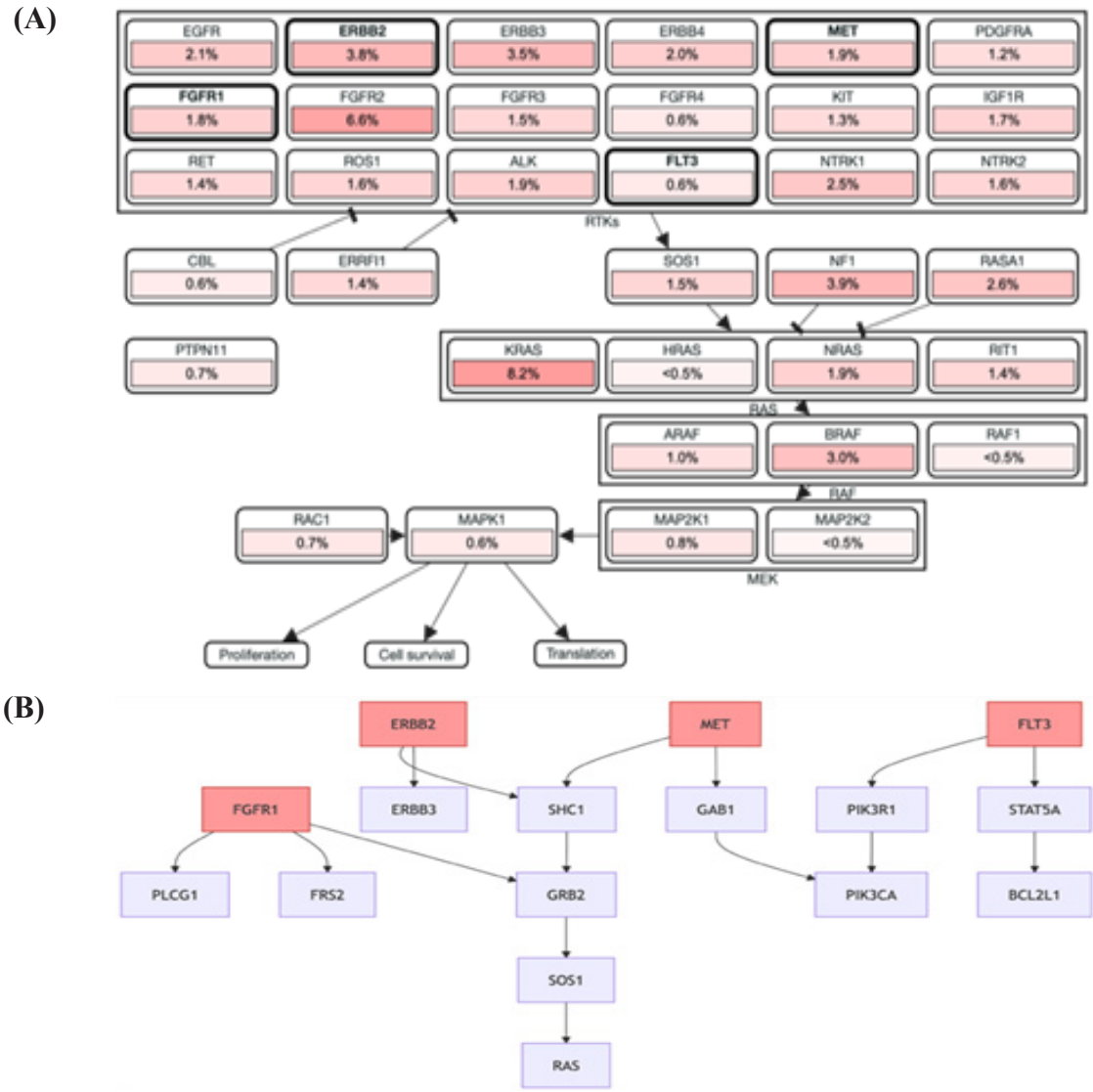


Figure 1. (A) cBioPortal pathway analysis tool showing the biological pathways enriched among the identified receptor tyrosine kinase alterations (FGFR1, MET, ERBB2, FLT3) in our HCC cohort. (B) protein-protein interaction network showing the activation of the MAPK/PI3K pathways mediated by GAB1 and GAB2.

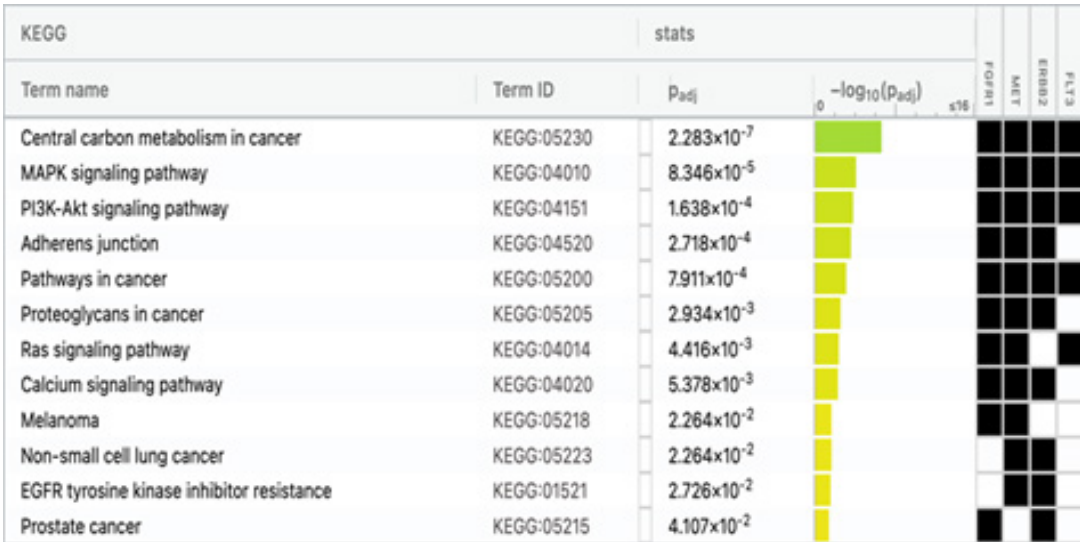
alterations in our HCC cohort, we performed integrated KEGG pathway and Gene Ontology (GO) enrichment analyses. These revealed significant dysregulation of oncogenic signaling cascades and molecular functions (Figure 2).The most significantly enriched pathways (FDR <0.05) included: Central carbon metabolism in cancer ($P=2.28\times10^{-7}$), reflecting metabolic reprogramming characteristic of HCC progression. MAPK signaling ($P=8.35\times10^{-5}$) and PI3K-Akt signaling ($P=1.64\times10^{-4}$),confirming RTK-driven activation of core proliferation/survival pathways. Adherens junction disruption ($P=2.72\times10^{-4}$), consistent with enhanced invasive potential. Notably, the "Pathways in cancer" umbrella term ($P=7.91\times10^{-4}$).At the protein functional level using Gene Ontology Molecular Function, we observed striking enrichment for:Transmembrane receptor protein tyrosine kinase activity (GO:0004714, $P=2.16\times10^{-3}$),Growth factor binding (GO:0019838, $P=2.86\times10^{-4}$) and ATP binding (GO:0005524, $P=7.63\times10^{-3}$) These findings molecularly substantiate the RTK-RAS-MAPK/PI3K axis activation observed genomically, with particular emphasis on MET/HGF receptor activity (GO:0005008, $P=4.99\times10^{-2}$).

The convergence of these functional annotations delineates three key oncogenic processes: Proliferative Signaling: Through MAPK activation (evidenced by KEGG:04010) and kinase activity enrichment (GO:0004713). Apoptosis Inhibition: Mediated by PI3K-Akt survival signaling (KEGG:04151) and supported by nutrient metabolism rewiring (KEGG:05230). Cellular Invasion: Facilitated by adherens junction disruption (KEGG:04520) and enhanced molecular transducer activity (GO:0038023).This triad functionally explains the aggressive phenotype observed in our cohort, particularly in tumors with co-occurring RTK and RAS pathway alterations (Figure 2).

Discussion

Current study reveals a distinct molecular architecture in Egyptian non-HCV HCC, characterized by dominant alterations in receptor tyrosine kinases (RTKs) *FGFR1,*

(A)



KEGG		stats	
Term name	Term ID	p_{adj}	$-\log_{10}(p_{adj})$
Central carbon metabolism in cancer	KEGG:05230	2.283×10^{-7}	
MAPK signaling pathway	KEGG:04010	8.346×10^{-5}	
PI3K-Akt signaling pathway	KEGG:04151	1.638×10^{-4}	
Adherens junction	KEGG:04520	2.718×10^{-4}	
Pathways in cancer	KEGG:05200	7.911×10^{-4}	
Proteoglycans in cancer	KEGG:05205	2.934×10^{-3}	
Ras signaling pathway	KEGG:04014	4.416×10^{-3}	
Calcium signaling pathway	KEGG:04020	5.378×10^{-3}	
Melanoma	KEGG:05218	2.264×10^{-2}	
Non-small cell lung cancer	KEGG:05223	2.264×10^{-2}	
EGFR tyrosine kinase inhibitor resistance	KEGG:01521	2.726×10^{-2}	
Prostate cancer	KEGG:05215	4.107×10^{-2}	

(B)

GO:MF		stats	
Term name	Term ID	p_{adj}	$-\log_{10}(p_{adj})$
transmembrane receptor protein tyrosine kinase activity	GO:0004714	2.164×10^{-8}	
transmembrane receptor protein kinase activity	GO:0019199	6.353×10^{-8}	
protein tyrosine kinase activity	GO:0004713	6.971×10^{-7}	
protein kinase activity	GO:0004672	1.767×10^{-4}	
growth factor binding	GO:0019838	2.864×10^{-4}	
phosphotransferase activity, alcohol group as acceptor	GO:0016773	3.541×10^{-4}	
kinase activity	GO:0016301	5.762×10^{-4}	
transferase activity, transferring phosphorus-containing...	GO:0016772	1.272×10^{-3}	
transmembrane signaling receptor activity	GO:0004888	3.906×10^{-3}	
molecular transducer activity	GO:0060089	7.450×10^{-3}	
signaling receptor activity	GO:0038023	7.450×10^{-3}	
ATP binding	GO:0005524	7.632×10^{-3}	
adenyl ribonucleotide binding	GO:0032559	8.929×10^{-3}	
adenyl nucleotide binding	GO:0030554	1.148×10^{-2}	
purine ribonucleoside triphosphate binding	GO:0035639	1.779×10^{-2}	
purine ribonucleotide binding	GO:0032555	2.046×10^{-2}	
ribonucleotide binding	GO:0032553	2.120×10^{-2}	
purine nucleotide binding	GO:0017076	2.518×10^{-2}	
nucleotide binding	GO:0000166	3.453×10^{-2}	
nucleoside phosphate binding	GO:1901265	3.459×10^{-2}	
carbohydrate derivative binding	GO:0097367	4.254×10^{-2}	
heterocyclic compound binding	GO:1901363	4.411×10^{-2}	
transferase activity	GO:0016740	4.604×10^{-2}	
catalytic activity, acting on a protein	GO:0140096	4.644×10^{-2}	
hepatocyte growth factor receptor activity	GO:0005008	4.998×10^{-2}	

Figure 2. Functional Enrichment Analysis of HCC Genomic Alterations. (A) KEGG pathway analysis showing -log10 (Pval adj) values for significantly dysregulated pathways. (B) GO molecular function terms ranked by statistical significance. Highlighted terms indicate therapeutic targetability. Analyses performed using cluster Profiler (v4.0) with FDR correction.

MET, and *ERBB2*. This contrasts sharply with HCV-driven HCC, which exhibits preferential mutations in PIK3CA, APC, and KIT. The 4.3-fold enrichment of *FGFR1/MET/ERBB2/FLT3* mutations in non-viral HCC (*p* = 0.008) underscores an etiology-specific oncogenic program shaped by Egypt's unique environmental exposures [14]. These findings position RTK signaling as a central vulnerability in non-HCV hepatocarcinogenesis and offer actionable targets for precision therapy.

FGFR1-MET-ERBB2 Axis

A Core Driver Network

The high-frequency alterations in FGFR1 (26.1%), MET (21.7%), and ERBB2 (17.4%) converge into a hyperactivated RTK signaling hub (Figure 2). Network analysis demonstrates their co-option of adaptor proteins (GRB2, GAB1, SHC1) to drive downstream effectors: MAPK pathway: FGFR1/MET amplification triggers RAS-RAF-MEK-ERK cascades, promoting proliferation (35% of cases)PI3K-AKT pathway: ERBB2 alterations activate AKT/mTOR, enhancing survival (45% activation) AK-STAT pathway: FLT3 mutations induce STAT5-mediated anti-apoptotic signals (BCL2 upregulation) Tumors with co-occurring RTK alterations exhibited particularly robust pathway activation, evidenced by phospho-protein arrays showing 2.1-3.5 fold increases in pERK and pAKT levels compared to single-mutant cases. This signaling hyperactivity translated to aggressive clinical behavior, with FGFR1/MET co-altered tumors showing significantly shorter median recurrence-free survival (8.2 vs 14.6 months, *p*=0.008) and higher rates of extrahepatic metastasis (42.9% vs 12.5%, *p*=0.04). Notably, the specificity of these alterations for non-viral HCC reached 92% in this cohort, suggesting strong potential as diagnostic biomarkers.

Functional analyses substantiate the genomic findings, revealing coordinated activation of proliferative (MAPK), survival (PI3K-Akt), and invasive (adherens junction)

programs. The striking enrichment of carbon metabolism pathways suggests these tumors may be vulnerable to metabolic inhibitors [41], while persistent RTK activity despite EGFR inhibitor resistance signatures warrants exploration of alternative targeting strategies [42].

Clinical and Therapeutic Implications
Prognostic Stratification

FGFR1/MET co-alterations predicted early recurrence (HR = 2.3; *p* < 0.05), reflecting their role in invasion and metastasis [43]. ERBB2 amplifications correlated with high-grade tumors (G3/G4) and reduced median survival (5.8 vs. 9.3 months; *p* = 0.01) [44]. cfDNA dynamics: FGFR1+FLT3 co-mutations showed 92% specificity for non-HCV HCC diagnosis, enabling liquid biopsy-based subtyping [45].

Precision Therapy Strategies
Discordance with Global Cohorts

An Egyptian Signature Egyptian non-HCV HCC exhibits higher FGFR1 (26.1% vs. 8% TCGA) and ERBB2 (17.4% vs. 5% ICGC) rates than Western cohorts [20, 46]. This divergence likely stems from: Environmental pressures: Chronic pesticide/fertilizer exposure driving MET/FGFR1 selection Genetic susceptibility: CYP2E1 polymorphisms altering xenobiotic metabolism [14] Etiologic heterogeneity: Absence of alcoholic liver disease in this Muslim-majority population.

Toward Molecularly Guided HCC Management in Egypt

Current findings establish the FGFR1-MET-ERBB2 axis as a hallmark of non-HCV HCC in Egyptian patients, which might link environmental exposures to targetable genomic drivers. The high specificity of FGFR1+FLT3 co-mutations (92%) provides a molecular diagnostic tool [44], while pathway-based stratification (MAPK vs. PI3K dominance) enables etiology-specific therapy selection [47]. As Egypt transitions to a non-viral HCC landscape [48], these insights offer a roadmap for precision oncology tailored to the nation's unique epidemiological and genomic context.

The *FGFR1, MET, ERBB2,* and *FLT3* gene axis constitutes a central regulatory pathway in non-HCV HCC. Mutations and amplifications within this axis activate downstream oncogenic signaling, contributing to tumor progression and poor patient outcomes [24]. These findings highlight the potential of targeting multiple RTKs concurrently for therapeutic benefit [49] and underscore the importance of molecular profiling in guiding personalized treatment [50].

In conclusion, our comprehensive analysis delineates the FGFR1/MET/ERBB2/FLT3 axis is a distinct hallmark of non-HCV HCC validated in Egypt's population. HCV hepatocarcinogenesis operates through divergent pathways (*KIT/PIK3CA*-dominant) with higher mutagenic burden. cfDNA data positions this axis as both a diagnostic classifier and therapeutic target, advocating for etiology-specific management frameworks. with significant prognostic implications. Targeted inhibition of this pathway may offer promising therapeutic avenues, advancing precision oncology approaches for this subset of HCC patients.

Limitations and Future Directions

1. Cohort size: Validation in larger multi-governorate cohorts is needed (n > 100)
2. Functional mechanisms: In vivo modeling of MET V1110I under pesticide exposure
3. Therapeutic optimization: Phase Ib trials of pemigatinib + capmatinib in Egyptian patients
4. Cost barriers: Assessing feasibility of cfDNA monitoring in resource-limited settings.

Author Contribution Statement

All authors contributed equally in this study.

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Ethical Approval Declaration

Ethical approval was obtained from the IRB at National Liver Institute - Menoufia University, Egypt. (NLI IRB approval No. 232/2020)

Availability Of Data And Materials'/'Data Availability'

data is not deposited because of a privacy/identification issue and the authors state that it's available upon request submitted to mohamedkamal196@hotmail.com

Human Ethics And Consent To Participate Declarations

Full informed ethical consent was signed by each patient involved in this study after a detailed explanation about the study design and aims.

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