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Original Article

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High Ki67 Gene Expression Is Associated With Aggressive Phenotype in Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) with high Ki67 protein expression, the most commonly used cell proliferation marker, is associated with an aggressive biologic phenotype; however, conventional immunostaining is hampered by variability in institutional protocol, specific antibody probe, and by assessor subjectivity. To this end, we hypothesized that Ki67 gene (*MKi67*) expression would identify highly proliferative HCC, and clarify its association with oncologic outcome, tumor progression, and immune cell population in the tumor microenvironment (TME). Furthermore, we sought to identify the cell-cycle gene expression profile that confers this aggressive phenotype.

Methods: A total of 473 HCC patients with clinicopathological data associated with transcriptome were selected for this study: 358 patients from The Cancer Genome Atlas (TCGA) as the testing cohort, and 115 from GSE76427 as the validation cohort. Each cohort was divided into a highly proliferative group (MKi67-high) and the low MKi67 group (MKi67-low) by the median of Ki67 gene (*MKi67*) expression levels.

Results: MKi67-high HCC patients had worse disease-free survival (DFS), disease-specific survival (DSS), and overall survival (OS) independent of histological grade in the TCGA cohort. MKi67 expression correlated with histological grade and tumor size. MKi67 expression increased throughout the HCC carcinomatous sequence from normal liver, cirrhotic liver, early HCC, and advanced HCC. MKi67-high HCC was associated with higher intratumor heterogeneity, homologous recombination deficiency, and altered fraction as well as intratumoral infiltration of T helper type 1 (Th1) and Th2 cells, but lower interferon-gamma response and M2 macrophage infiltration. Cell proliferation-related gene sets in the Hallmark collection (E2F targets, G2M checkpoint, Myc target v1 and mitotic spindle), MTORC1 signaling, DNA repair, PI3K MTOR signaling, and unfolded protein response were all enriched in the MKi67-high HCC (false discovery rate (FDR) < 0.25).

Conclusions: High *MKi67* gene expression identified highly proliferative HCC with aggressive biology involving classical pathways in cell cycle regulation and DNA repair, as well as poor overall oncologic outcomes. This suggests potential for personalized treatment strategies, but validation and refinement of these observations require further research to elucidate the underlying mechanisms and validate therapeutic targeting of these pathways in MKi67-high HCC tumors.

Keywords: *MKi67*; Gene expression; HCC; Outcomes; Signaling pathways

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Introduction

Hepatocellular carcinoma (HCC) ranks as the fourth leading cause of cancer-related deaths worldwide and is currently the fastest growing cause of cancer-specific mortality in the United States [1, 2]. HCC frequently arises in individuals with chronic liver disease, commonly due to persistent hepatoviral infection or nonalcoholic fatty liver disease (NAFLD) [3, 4]. The prognosis of patients with HCC is generally poor with an overall 5-year survival of approximately 18% [5]. This is because most patients (60%) are diagnosed with an advanced disease, which makes them ineligible for curative intent treatment [6]. Consequently, the identification of precise prognostic biomarkers holds the potential to enhance patient selection and identify those who are more likely to benefit from aggressive HCC treatment.

Ki67 is a protein predominantly found in the nucleolar cortex and exhibits high expression in the majority of proliferating malignant cells, while being seldom expressed in normal cells. Ki67 is recruited into chromosomes during cell division, and its concentration rises during the transition from grade 1 (G1) to mitosis with a rapid decrease in later phases [7-9]. As a result, it is one of the most employed clinical markers for cell proliferation in many malignancies [10-14]. Prior studies have shown that Ki67 expression is correlated with worse tumor

biology and poorer overall outcomes in patients with central nervous system malignancies, renal cell carcinoma, adrenocortical carcinoma, and uterine cancer [15, 16]. In patients with HCC, a recent meta-analysis showed that high Ki67 protein expression was associated with larger tumor size, higher number of lymph node metastases, cirrhosis, vascular invasion, and presence of distant metastasis [17].

The most widely adopted method by which Ki67 is assessed and reported is through immunostaining. However, conventional immunostaining is subject to user variability and subjective interpretation, and results are histology-specific [18-20]. In this context, the expression of the Ki67 gene (*MKi67*), which is more objective and accurately quantifiable than immunohistochemistry, can be employed to identify highly proliferative HCC [21]. We therefore hypothesized that HCC with high MKi67 expression correlates with overall oncologic outcomes, and we sought to investigate its association with aggressive tumor biology.

Materials and Methods

Clinical and transcriptomic data acquisition

We utilized The Cancer Genome Atlas (TCGA) that collected the untreated bulk tumors and their associated transcriptomes with the clinicopathological data of each patient as we previously described [22-36]. TCGA is a comprehensive, collaborative initiative that systematically characterizes gene expression profiles across various cancer types. It provides a vast repository of genomic and clinical data, facilitating in-depth analyses to better understand the molecular basis of cancer. There are 358 HCC patients included in TCGA cohort that we used in our previous studies [37-43]. It is important to note, however, that the TCGA dataset lacks certain demographic and clinical characteristics such as patients' medical history or treatment modalities. Similarly, factors such as smoking, socioeconomic status or diet were inaccessible to the authors.

This dataset was further validated using an additional 115 patients from the Gene Expression Omnibus (GEO) GSE76427 cohort [44]. Tumor characteristics: grade, size, node and staged were obtained from the Genomic Data Commons (GDC) Data Portal and reported according to the American Joint Committee on Cancer (AJCC) classification. We compared the GSE6764 (n = 75) cohort to the GSE89377 (n = 107) cohort [45, 46] to investigate the association between MKi67 expression, clinicopathological characteristics and outcomes from GEO repository. HCC pathological classification in GSE6764 followed the guidelines of the International Working Party and was defined as: 1) Very early HCC (n = 8), well-differentiated tumors < 2 cm in diameter with no vascular invasion/satellites (size range: 8 - 20 mm); 2) Early HCC (n = 10), tumors measuring < 2 cm with microscopic vascular invasion/satellites; well to moderately differentiated tumors measuring 2 - 5 cm without vascular invasion/satellites; or 2 - 3 well-differentiated nodules measuring < 3 cm (size range: 3 - 45 mm); 3) Advanced HCC (n = 7), poorly differentiated tumors measuring > 2 cm with microvascular invasion/satellites or tumors measuring > 5 cm; and 4) Very advanced HCC (n = 10), tumors with macrovascular invasion or diffuse liver involvement. For the GSE89377 cohort (n = 107), they were defined as: normal (n = 13), dysplasia (n = 22), cirrhosis (n = 12), low-grade chronic hepatitis (n = 8), high-grade chronic hepatitis (n = 12), early HCC (n = 5), HCC grade 1 (n = 9), HCC grade 2 (n = 12) and HCC grade 3 (n = 14) [47].

Patients were divided into two groups based on their *MKi67* gene expression levels. Those whose *MKi67* gene expression levels exceeded the median were designated as the highly proliferative group (MKi67-high), whereas the remaining patients in each cohort were grouped as the MKi67-low group.

All genomic analyses used were log2 transformed normalized transcriptomic data. The TCGA and all GEO cohorts used in this study are deidentified and available within the public domain, therefore the Institutional Review Board was waived. The declaration of ethical compliance with human study was not applicable.

Tumor microenvironment

The xCell algorithm obtained through the xCell website [48], was used to calculate the immune cell infiltration in the tumor microenvironment through transcriptomic data as we described previously [43, 49-51]. The scoring of homologous recombination, intratumor heterogeneity, fraction altered, silent mutation, non-silent mutation, single-nucleotide variant (SNV) neoantigens, indel neoantigens, leukocyte fraction, lymphocyte infiltration, and interferon (IFN)- response score was performed as published by Thorsson et al [52].

Gene set enrichment analysis (GSEA)

The publicly-available software (GSEA version 4.0.3) and the GSEA algorithm were used in this study [53]. Statistical significance was determined to have a false discovery rate (FDR) of 0.25.

Statistical analysis

Statistical significance for comparison analysis between groups was set at P less than 0.05 by the Kruskal-Wallis test, the Mann-Whitney U test and two-tail Fisher's exact tests. Survival analysis was performed with the Kaplan-Meier method and log rank test. Statistical analyses and data plotting were performed in R software version 4.2.3 (R Project for Statistical Computing).

Results

MKi67-high HCC patients had worse disease-free, disease-specific, and overall survival (OS) in the TCGA cohort

First, relationships between MKi67 expression and survival outcomes were assessed. MKi67-high expression was sig-

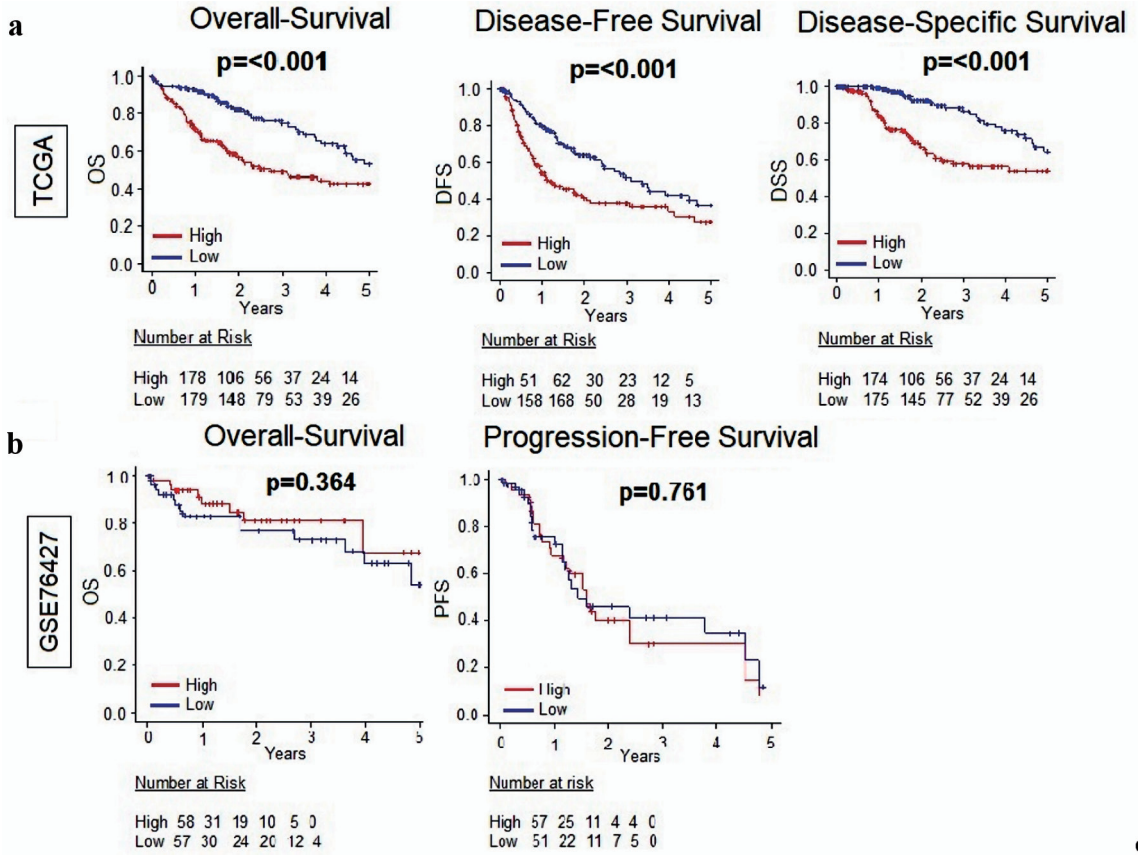

Figure 1. Relationship between MKi67 and survival outcomes in patients with HCC. (a) Kaplan-Meier survival curves comparing high- vs. low-MKi67 expression in HCC to determine disease-free survival (DFS), disease-specific survival (DSS), and overall survival (OS) in the TCGA (n = 358) cohort. (b) Kaplan-Meier survival curves comparing high- vs. low-MKi67 expression in HCC to determine overall survival and progression-free survival in the GSE76427 (n = 115) cohort. The P value was calculated using a log rank test. Significant P value < 0.05. HCC: hepatocellular carcinoma; TCGA: The Cancer Genome Atlas.

nificantly associated with worse disease-free survival (DFS), disease-specific survival (DSS) and OS, when compared to MKi67-low expression in TCGA ($P < 0.001$ for all comparisons), although these results were not validated in in GEO GSE766427 cohort (OS $P = 0.364$ and PFS $P = 0.751$) (Fig. 1).

Given that higher histological grade is a pathological determination of cancer cell proliferation, we did a subgroup analysis of MKi67-high vs. low stratified by histological grade (Fig. 2). Our results demonstrated that MKi67-high had worse oncologic outcomes across all histological grades, but it is more pronounced in histological grade 3 (G3) tumors. Nevertheless, DFS in G1 and OS in grade 2 (G2) tumors did not reach statistical significance ($P = 0.978$ and $P = 0.072$, respectively).

MKi67 expression was positively correlated with HCC progression

Given that MKi67 was associated with worse oncologic outcomes, we hypothesized that MKi67 expression increases in a stepwise progression from normal liver to early HCC to advanced HCC. MKi67 expression was therefore measured at each phase of histological progression in the GSE6764 and GSE89377 cohorts (Fig. 3). We found that MKi67 expression increased in a stepwise fashion from early to advanced HCC ($P \leq 0.001$) with no significant difference between the normal, dysplastic, and cirrhotic liver in the GSE6764 cohort (normal liver vs. early HCC $P = 0.015$; normal vs. advanced HCC $P < 0.001$; normal vs. very advanced HCC, $P < 0.001$). We validated these results with the GSE89377 cohort, showing MKi67 expression was also significantly enhanced in higher grades of HCC compared with normal, dysplastic, and cirrhotic liver and low- and high-grade chronic hepatitis (normal vs. G1, $P = 0.025$; normal vs. G2, $P = 0.025$; normal vs. G3, $P < 0.001$).

MKi67 expression correlated with histological grade, tumor size, lymph node metastasis and AJCC stage in HCC

Next, we investigated whether MKi67 expression was associated with aggressive tumor characteristics in HCC. To this end, we evaluated the differences in *MKi67* gene expression stratified by histological grade, T-category, N-category, and staging by AJCC as a clinical marker of tumor aggressiveness. Child-Pugh classification was also assessed as a marker of severity of liver cirrhosis. As expected, MKi67 expression strongly correlated with

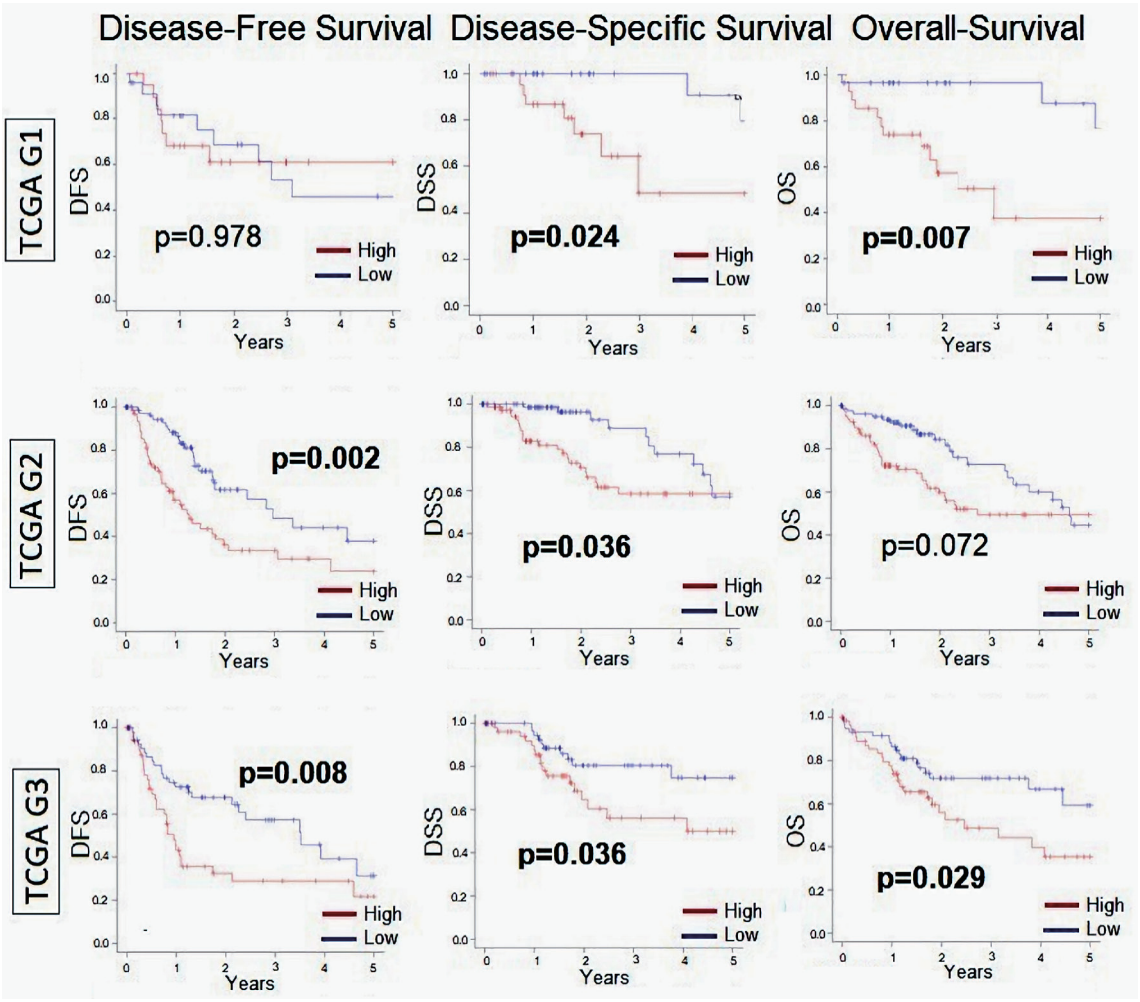

Figure 2. Relationship between MKi67 and survival outcomes in patients with HCC broken down by histological grade. Kaplan-Meier survival curves comparing high- vs. low-MKi67 expression in HCC to determine disease-free survival (DFS), disease-specific survival (DSS), and overall survival (OS) in grade 1 (n = 53), grade 2 (n = 168), and grade 3 (n = 121) from TCGA cohort. The P value was calculated using a log rank test. Significant P value < 0.05. HCC: hepatocellular carcinoma; TCGA: The Cancer Genome Atlas.

histological grade (Fig. 4) ($P \leq 0.001$). We found that MKi67 expression correlated with a higher T-stage ($P < 0.001$), although it was less clear in T4 tumors noting the small sample size. Node-positive tumors showed a non-significant trend toward higher expression of MKi67 ($P = 0.053$). MKi67 expression significantly correlated with higher stage consistently in both TCGA and GSE76427cohorts ($P \leq 0.001$), with the exception of metastatic (stage IV) tumors. In terms of cirrhosis, normal liver showed the highest MKi67 expression. Notably, there was no statistically significant difference in MKi67 expression among any Child-Pugh class in the GSE76427 cohort ($P = 0.208$).

MKi67-high was significantly associated with intratumor heterogeneity, homologous recombination defects, and altered fraction

To understand the genomic profile in HCC with high versus low expression of MKi67, we evaluated the underlying mutation rate of HCC using scores pre-calculated by Thorsson et al [52]. We found that homologous recombination defects, intratumor heterogeneity and altered fraction were significantly higher in the MKi67-high HCC ($P \leq 0.001$), but not silent mutation rate ($P = 0.601$), non-silent mutation rate ($P = 0.830$), SNV neoantigens ($P = 0.438$), or indel neoantigens ($P = 0.745$) in the TCGA cohort (Fig. 5).

MKi67-high HCC was not consistently associated with immune cell infiltrations, except for T helper type 1 (Th1) and Th2 cells

In view of the higher homologous recombination defects but not mutation rates in MKi67-high HCC tumors, we sought to evaluate the relationship between MKi67 expression and immune cell infiltration in the tumor microenvironment. Over-

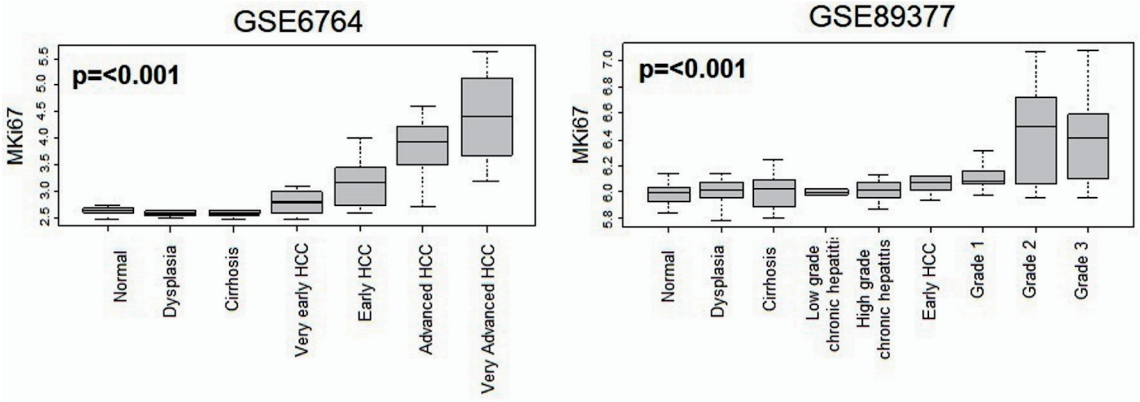

Figure 3. Relationship between MKi67 expression and histological progression of HCC. Boxplots of the MKi67 expression by multistep hepatocarcinogenesis, including normal liver tissue (n = 10), dysplasia (n = 17), cirrhosis (n = 13), very early HCC (n = 8), early HCC (n = 10), advanced HCC (n = 7), and very advanced HCC (n = 10) in the GSE6764 (n = 75), The P value was calculated using a Kruskal-Wallis test. HCC: hepatocellular carcinoma.

all, there was no association between MKi67 expression and lymphocyte infiltration, leukocyte fraction, or transforming growth factor (TGF)-beta response and (Fig. 6a). Interestingly, IFN-gamma response was attenuated in MKi67-high tumors (P = 0.002). The only immune cells that were highly represented in MKi67-high tumor microenvironment were Th1 and Th2 cells. In contrast, M2 macrophages were significantly overrepresented in the MKi67-low tumor microenvironment. Moreover, while alpha-fetoprotein (AFP) exhibited a significant elevation in MKi67-high tumors within the TCGA cohort, this association did not reach statistical significance in the GSE76427 cohort (Fig. 6b).

MKi67-high HCC is associated with cell proliferation-related and cell-cycle gene sets

Considering MKi67-high HCC was associated with aggressive clinical characteristics, we conducted a GSEA in the TCGA and GSE76427 cohorts to investigate enriched pathways associated with MKi67-high tumors. As expected, we found significant enrichment of cell proliferation-related gene sets

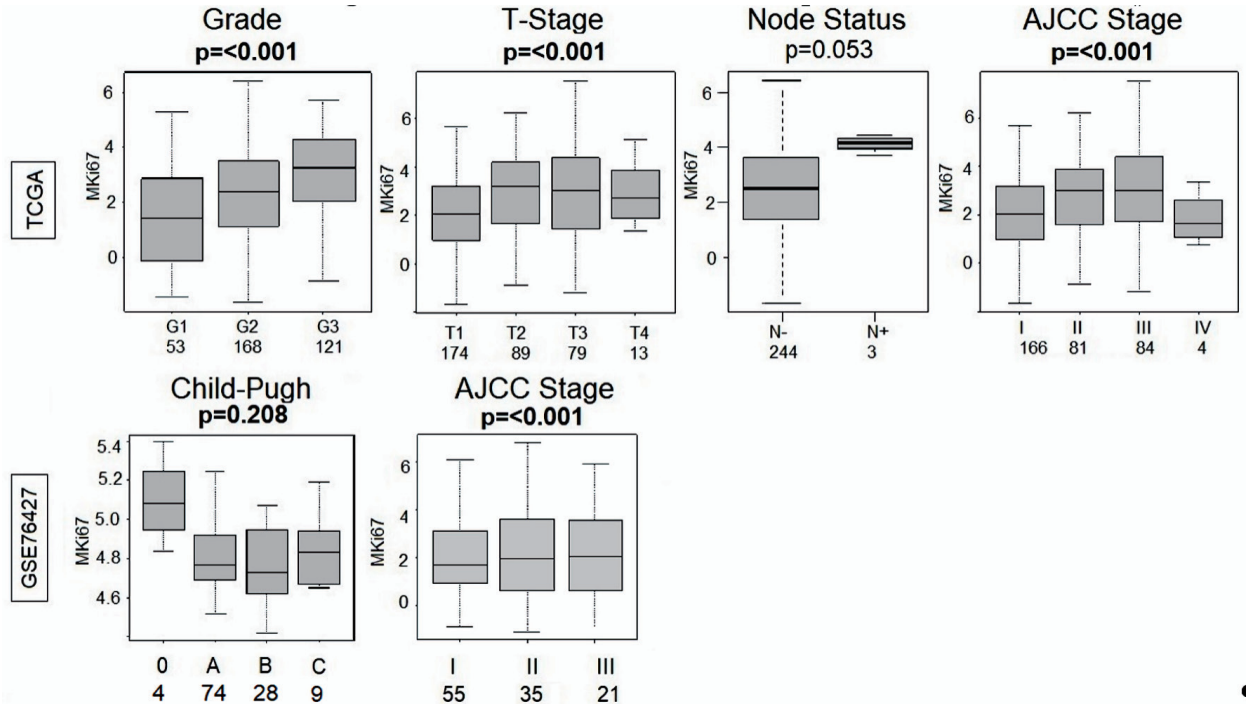

Figure 4. Comparison of the MKi67 expression and grade, T-stage, node status and AJCC stage in the TCGA (n = 358) cohort and MKi67 expression and Child-Pugh classification and AJCC stage in the GSE76427 (n = 115) cohort. P value < 0.05 was considered statistically significant. TCGA: The Cancer Genome Atlas; AJCC: American Joint Committee on Cancer.

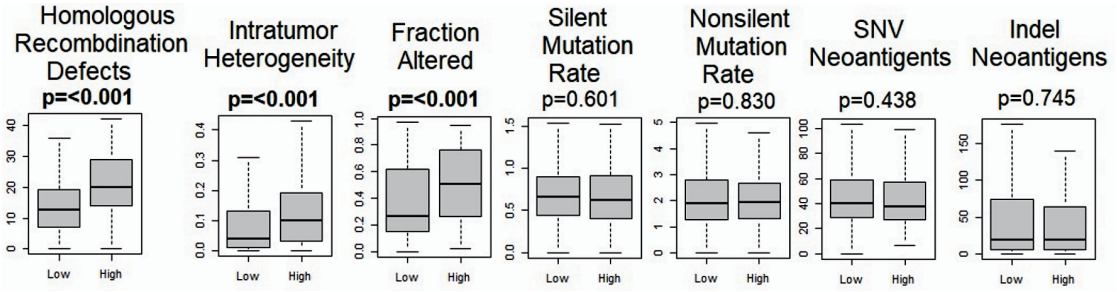

Figure 5. Relationship between MKi67 and mutation related scores. Boxplots of the comparison of the high- vs. low-MKi67 expression and homologous recombination deficiency, intratumor heterogeneity, fraction altered, Silent and non-silent mutation rates, single-nucleotide variant (SNV) neoantigens, and indel neoantigens. The P value was calculated using the Mann-Whitney U test. Significant P value < 0.05.

in the Hallmark collection: E2F targets, mitotic spindle, G2M checkpoints, Myc targets V1 (Fig. 7). Furthermore, we found that gene sets that reflect aggressive tumor biology: unfolded protein response (UPR), PI3K MTOR signaling, MTORC1 signaling and DNA repair, were all enriched to MKi67-high HCC in both TCGA and GSE76427 cohorts.

Discussion

In this study, we found that high expression of MKi67 was associated with biologically aggressive HCC. Not only was higher expression of MKi67 positively correlated with aggressive clinical tumor characteristics (histological grade, tumor size, and AJCC staging), but also intratumor heterogeneity, homologous recombination defects, and altered fraction. Further, in our analysis of the GSE6764 and GSE89377 cohorts, MKi67 expression appeared to correlate with progression of HCC from early to advanced and histological grade from lower (G1) to higher (G2 and G3) in a stepwise fashion. Interestingly, despite higher cell proliferation in MKi67-high HCC, there was no association with the degree of immune response (except for increased infiltrating Th1 and Th2 cells). More importantly, high expression of MKi67 in HCC was associated with enriched expression of multiple genes involved in the cell cycle and DNA repair pathways.

Tumor markers play a crucial role in the diagnosis and prognosis of various cancers. In the context of HCC, AFP is the most extensively utilized biomarker for both diagnosis and prognostication [54]. Our study demonstrated a positive correlation of AFP levels and MKi67 expression in the TCGA cohort but not in the GSE76427. While not directly connected to Ki67, AFP exhibits a dual regulatory role in cell proliferation. The impact of AFP on growth regulation, whether enhancing or inhibitory, is contingent upon the concentration of AFP and the levels of cytokines, hormones, and growth factors in the culture system [55]. However, it is worth noting that approximately 30-40% of HCC cases are AFP-negative. While a lack

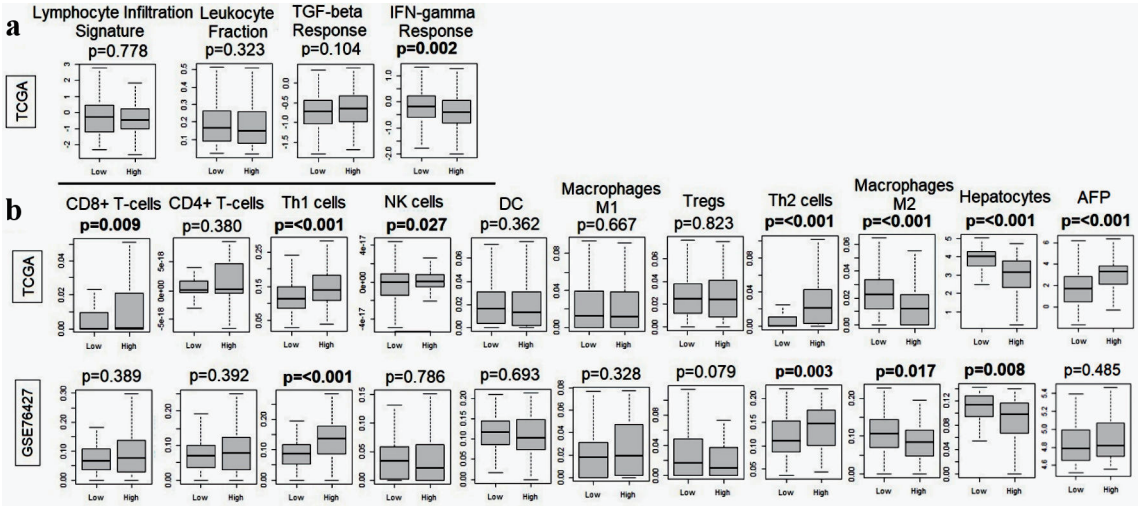

Figure 6. Relationship between MKi67 and immune response-related genes in the TCGA and GSE76427. (a) Boxplots of lymphocyte infiltration signature, leukocyte fraction, TGF-beta response, and IFN-gamma response. (b) Boxplots of anticancer immune cells: $CD8^+$ T cell, $CD4^+$ T cell, T helper type 1 (Th1) cells, M1 macrophages and dendritic cells and pro-cancer immune cells including regulatory T cells (Treg), T helper type 2 (Th2) cells, M2 macrophages, hepatocytes and alpha-fetoprotein (AFP). The P value was calculated using the Mann-Whitney U test. Significant P value < 0.05. TGF: transforming growth factor; IFN: interferon; TCGA: The Cancer Genome Atlas; NK: natural killer; DC: dendritic cell.

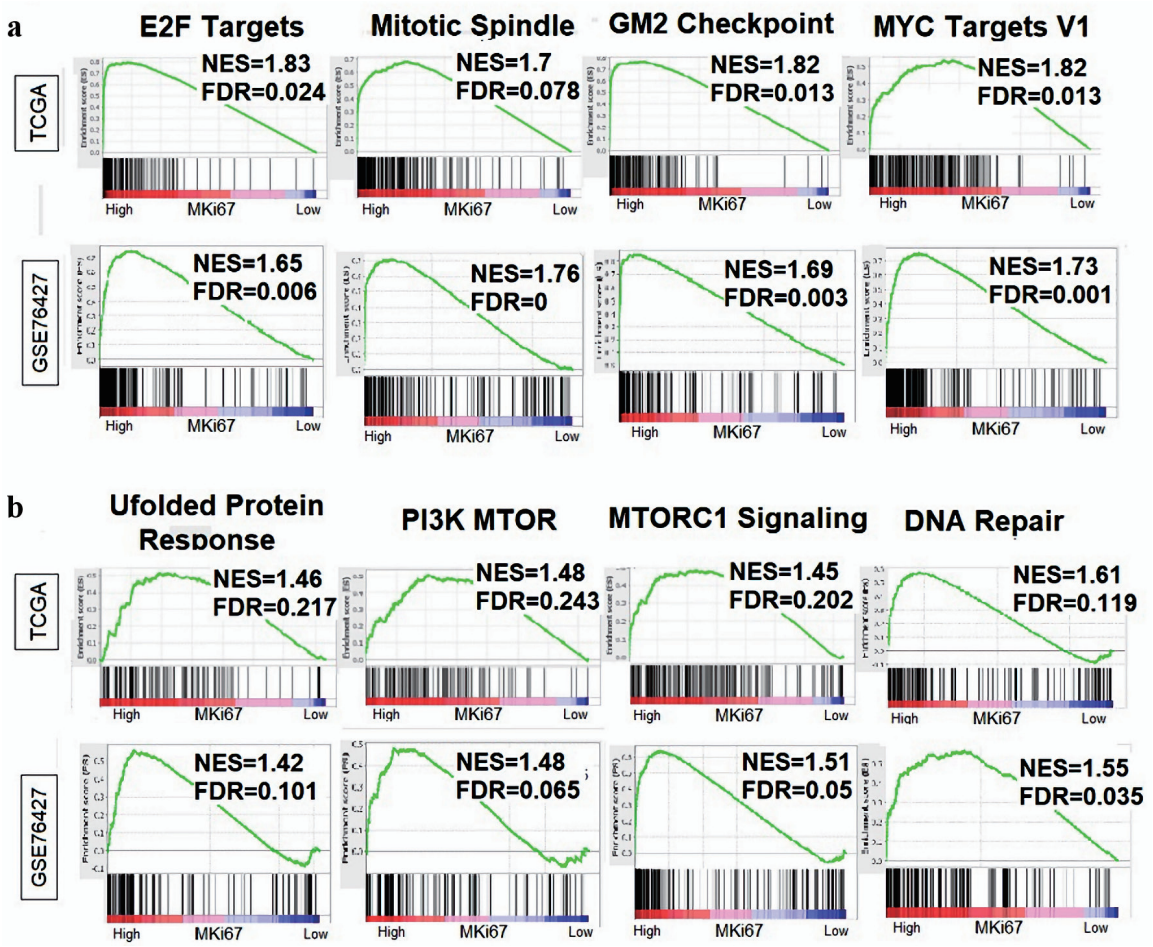

Figure 7. Relationship between MKi67 expression and cell proliferation-related gene sets. Gene Set Enrichment Analysis (GSEA) was performed on the Hallmark gene sets, comparing MKi67-high vs. low scores in HCC across the TCGA and GSE76427 cohorts Enrichment plots was generated displaying the normalized enrichment score (NES) and false discovery rate (FDR) for proliferation-related gene sets. An FDR of 0.25 was determined statistically significant as recommended by the GSEA software. (a) Cell proliferation-related gene sets in the Hallmark collection. (b) Genes related to aggressive tumor biology. HCC: hepatocellular carcinoma; TCGA: The Cancer Genome Atlas.

of detectable AFP in the serum may be indicative of positive outcomes in liver cancer cases, it can also lead to an inaccurate diagnosis of liver cancer as a whole [56].

Ki67 expression, on the other hand, serves as a comprehensive surrogate measure of cell proliferation, and heightened expression is indicative of aggressive tumor behavior. Numerous studies support the pivotal role of Ki67 in cancer prognostics, as its expression is strongly correlated with the aggressiveness of various tumors, including those affecting the breast, pancreas, lungs, central nervous system, prostate, and salivary glands [57-63]. In resonance with this study, Luo et al found that high Ki67 protein expression was associated with more advanced HCC stages, poorer differentiation, larger tumors, as well as poorer DFS, RFS and OS [17]. However, in our study, Ki67 expression was evaluated by directly examining *MKi67* gene expression. This approach has been proven to be more accurate in gauging tumor proliferation compared to assessing protein expression through immunohistochemistry (IHC). This superiority is attributed to its higher sensitivity, reproducibility, and reduced variability among users [64].

We found that intratumor heterogeneity, homologous recombination defects and altered fraction were significantly higher in the MKi67-high HCC. Intratumor heterogeneity has been found to influence tumor growth, metastasis, recurrence, and resistance to cytotoxic chemotherapy [65-68]. Similarly, homologous recombination defects have been associated with genomic scarring and a poor prognosis in HCC [69]. Additionally, as reported by Liu et al, HCC tumors with higher altered fraction are associated with rapid proliferation and immune evasion and could result in an attenuated response to immunotherapy [70, 71].

This study revealed no association between lymphocyte infiltration, leukocyte fraction or TGF-beta response and MKi67 expression. Additionally, there was no significant increase in pro-cancer immune cell proliferation except for Th1 and Th2 cells in the MKi67-high tumors. A plausible explanation for this weakened anticancer immune response might involve T cell exhaustion, a phenomenon previously explored

by Wu et al [72]. Despite the association of MKi67 upregulation with increased infiltration of B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, dendritic cells, and other functional T cells, MKi67 may concurrently contribute to T cell exhaustion. This dual effect could result in a diminished anticancer immune response, characterized by the upregulation of critical genes such as *TIM-3* and *TIGIT*. These genes, comparable to the therapeutic targets programmed death ligand-1 (PD-1) and cytotoxic T lymphocyte antigen (CTLA), play a role in regulating T-cell responses and could act as therapeutic targets for immunotherapy drugs theoretically with fewer toxicities due to their more targeted activity [72].

To validate the association between pathological and biological characteristics with MKi67 expression, we conducted a gene-set enrichment analysis. We found that in both TCGA and the GSE76427 cohorts, MKi67-high HCC had significant enrichment of E2F targets, mitotic spindle, G2M checkpoints, Myc targets V1, UPR, PI3K MTOR signaling, MTORC1 signaling, and DNA repair pathways. These cell proliferation-related and cell-cycle gene pathways genes have an important role in the pathogenesis of HCC. Our previous study showed that upregulation of individual E2F targets was associated with worse OS and DFS in HCC, especially the alterations in E2F3, E2F5 and E2F6 [73]. Furthermore, a metanalysis of c-Myc overexpression in HCC was associated with worse oncologic outcomes [74]. Even though the PI3K/Akt/mTOR pathway is upregulated in approximately 50% of HCC patients and plays an important role in the pathogenesis of this malignancy, clinical studies have failed to demonstrate any capacity of mTOR inhibitors to decrease tumor growth or recurrence [75, 76]. Moreover, our group's prior study on the role of the UPR in HCC demonstrated that the upregulation of UPR was associated with multiple parameters of cell proliferation, including MKi67 expression, enrichment of cell proliferation-related gene sets and mutational load that ultimately translated to worse survival of patients with HCC [39]. Similarly, Oshi et al showed that the DNA repair pathway was enhanced in higher histological grade HCCs, which also burdened with elevated tumor heterogeneity and higher mutational load ultimately yielding worse survival outcomes compared to HCCs without enriched DNA repair pathway [77].

This study provides evidence supporting the potential use of MKi67 expression as a prognostic marker in HCC and sheds light on its underlying biological mechanisms. These findings suggest that HCC tumors with high MKi67 expression may exhibit unique molecular characteristics. These characteristics, particularly those related to DNA repair pathways and immune responses, could potentially guide treatment strategies. For instance, we speculate that HCC with high MKi67 expression might be more responsive to therapies targeting cell cycle regulation, DNA repair pathways, or immunomodulation. We highlight the possibility that these findings could inform personalized treatment strategies, with further research needed to validate and refine these speculations.

There are some limitations to our study. As we used publicly available cohorts, our results may not reflect the heterogeneity among the patients in the population. Unfortunately, our group did not have access to information regarding the patients' medical history or treatment modalities such as surgery, local ablative, loco-regional or systemic therapy to the cohorts we analyzed. Additionally, the absence of detailed clinical follow-up data in some cases limits the ability to draw robust conclusions about responses to treatments. Similarly, factors such as smoking, socioeconomic status or diet were inaccessible to the authors.

In conclusion, our study provides compelling evidence that high expression of MKi67 in HCC is closely linked to a more aggressive biological phenotype driven by the upregulation of multiple interconnected pathways involved in cell cycle regulation, DNA repair and oncogenic signaling. Further clinical studies are warranted to elucidate the underlying mechanisms and validate the therapeutic potential of targeting these pathways in *MKi67*-high HCC tumors. Such efforts hold the promise of improving patient outcomes and advancing precision medicine approaches for the management of HCC.

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Financial Disclosure

None to declare.

Conflict of Interest

The authors have no potential conflicts of interest to disclose.

Informed Consent

Not applicable.

Author Contributions

Conceptualization: V. Ramos-Santillan, M. Oshi, and K. Takabe. Data analyses: M. Oshi, I. Endo, and K. Takabe. Writing - original draft preparation: V. Ramos-Santillan. Writing - review and editing: E. Nelson, M. Oshi, I. Endo, and K. Takabe. Supervision: I. Endo, and K. Takabe. Funding acquisition: K. Takabe. All authors have read and agreed to the published version of the manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author, KT, upon reasonable request.

Abbreviations

HCC: hepatocellular carcinoma; TME: tumor microenvironment; TCGA: The Cancer Genome Atlas; GEO: Gene Expression Omnibus; DFS: disease-free survival; DSS: disease-specific survival; OS: overall survival; NAFLD: nonalcoholic fatty liver disease; GDC: Genomic Data Commons; AJCC: American Joint Committee on Cancer; SNV: single-nucleotide variant; IFN: interferon; GSEA: Gene enrichment analysis; G1: grade1; G2: grade 2; G3: grade 3

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