Original Paper

Characterization of Hepatocellular Carcinoma Patients with *FGF19* Amplification Assessed by Fluorescence in situ Hybridization: A Large Cohort Study

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Keywords

FGF19 · Fluorescence in situ hybridization · Hepatocellular carcinoma · High copy number amplification · Prognosis

Abstract

Background: *FGF19* amplification is a relatively novel type of genetic aberration that has been proposed to be a driver of hepatocarcinogenesis. Selective inhibitors of *FGFR4*, a receptor of *FGF19*, have been developed as targeted therapies for hepatocellular carcinoma (HCC). Despite the role of *FGF19* in mediating HCC progression, the clinicopathological characterization of patients exhibiting *FGF19* amplification remains unclear. Immunohistochemical staining is the simplest and most widely used method of identifying aberrations in the *FGF19* gene, although its specificity is very low. **Methods:** This study investigated the prognostic significance of *FGF19* amplification in a large cohort of 989 HCC patients using fluorescence in situ hybridization (FISH), which has a high degree of specificity. In addition, FISH data from formalin-fixed, paraffin-embedded sections were compared with copy number variation (CNV) data

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obtained from fresh frozen sections to validate the use of FISH as a diagnostic tool. **Results:** *FGF19* amplifications were detected by FISH in 51 (5.15%) of the 989 patients, and were independently associated with poor survival and a higher risk of tumor recurrence, as well as with poor prognostic factors such as a high α -fetoprotein level, hepatitis B or C virus infection, a large tumor size, microvascular invasion, and necrosis. In addition, *FGF19* amplification was associated with *TP53* mutation, and was mutually exclusive with *CTNNB1* mutation. The results of the FISH and CNV analyses exhibited a significant concordance rate of 96% ($\kappa = 0.618$, $p < 0.001$). **Conclusions:** These data indicate that *FGF19* amplification represents a unique molecular subtype associated with poor prognostic characteristics, which supports the hypothesis that the *FGF19*-*FGFR4* signaling pathway plays an important role in hepatocarcinogenesis. We have also demonstrated that FISH is a viable alternative to CNV analysis, offering a number of advantages in the clinical setting.

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Introduction

Hepatocellular carcinoma (HCC) is the third most frequent cause of cancer-related mortality worldwide and its incidence is associated with hepatitis B and/or C infection, alcoholism, and metabolic syndrome [1]. Recent genomic studies have demonstrated that hepatocarcinogenesis involves genetic and epigenetic changes in multiple molecular pathways [2–6]. For example, somatic mutations in the *TP53* and *TERT* promoter regions have been identified as major mechanisms by which HCC progression can be facilitated [7, 8]. Similarly, aberrant methylation of *SEPT9* and/or *EFNB2* has been reported to drive hepatocarcinogenesis at the epigenetic level [9], and copy number amplification (e.g., *FGF19*) and deletion (e.g., *Rb* deletion) of certain genes are also associated with hepatocarcinogenesis [2, 10, 11].

FGF19 amplification was recently identified as a genetic aberration that may promote hepatocarcinogenesis [12, 13]. Normally, *FGF19* binds to *FGFR4* on the surface of hepatocytes and regulates bile acid metabolism [14]; the activation of this pathway also leads to hepatocyte proliferation [15]. Accordingly, *FGF19* amplification results in the abnormal proliferation of hepatocytes, thereby driving hepatocarcinogenesis and leading to the development of HCC [16, 17]. As a result, selective *FGFR4* inhibitors have been investigated as potential targeted therapies for HCC associated with *FGF19* amplification or overexpression [3, 18, 19]. However, studies that have demonstrated an association between *FGF19* amplification and poor HCC prognosis have had notable limitations, such as small study populations or low sensitivity and specificity associated with the immunohistochemistry used to detect *FGF19* expression [13, 16, 20].

In a previous study of 231 cases of HCC, we reported the potential clinical relevance of *FGF19* amplification for patient stratification [10]. In our current study, we have examined the presence of *FGF19* amplification further, using fluorescence in situ hybridization (FISH) analysis in a large cohort of 989 HCC patients. Our main goal was to investigate the prognostic significance of *FGF19* amplification in HCC using FISH. In addition, FISH data obtained from formalin-fixed, paraffin-embedded sections were compared with copy number variation (CNV) data obtained from fresh frozen sections to validate the use of FISH as a diagnostic tool.

Subjects and Methods

Patients and Samples

This retrospective study included a large cohort of 989 patients diagnosed with HCC by surgical resection at the Asan Medical Center, Seoul, Republic of Korea, between 2005 and 2013. All clinical information was

Fig. 1. Fluorescence in situ hybridization micrographs showing *FGF19* (red signal) and *CEP11* (green signal) fluorescence in tumor cells and non-tumor cells. **a** *FGF19* positive in tumor cells: an increase in the number of red signals in tumor cells, indicating amplification of *FGF19* (left upper inset). Normal *FGF19* or *CEP11* signals served as the internal positive control in lymphocytes (left lower inset). Magnification, $\times 1,000$. **b, c** *FGF19* negative in tumor cells (**b**) and *FGF19* negative in non-tumor cells (**c**): no increase in red signals in tumor and non-tumor cells, indicating no amplification of *FGF19*, respectively (insets). Magnification, $\times 1,000$.

collected from the database of the Asan Liver Center. The 989-patient cohort included 227 patients from our previous study, and the results of the *FGF19* CNV analysis obtained from these patients were used in this study [10]. The median follow-up time was 56 months (range 0–148). The study was approved by the Institutional Review Board of the Asan Medical Center (IRB No. 2017-0875).

Tissue Microarrays

All slides and histological data were reviewed by two pathologists (E.Y. and H.J.K.). A total of 56 tissue microarrays were prepared from formalin-fixed, paraffin-embedded tissue samples. In brief, two cores from tumorous tissue and one core from nonneoplastic liver tissue (both with a diameter of 2.0 mm) were sampled for each HCC case and placed into recipient blocks with holes made by an arraying machine (TMArrayer; Pathology Devices, Westminster, MD, USA).

FGF19 Fluorescence in situ Hybridization

Consecutive 5- μ m sections were cut from the microarray blocks. FISH was performed with two probes targeting *FGF19* and *CEP11* (Abbott Molecular, Des Plaines, IL, USA) on tissue microarrays, according to the manufacturer's recommendations. The slides were examined with an Olympus BX51 fluorescence microscope (Olympus, Tokyo, Japan) using a 100 $\times$ objective. Each image was obtained through fluorescence isothiocyanate and Texas Red band-pass filters on an Olympus DP70 camera and analyzed using DP Controller software (version 3.3.1.292).

Scoring Criteria

The two experienced pathologists (E.Y. and H.J.K.) independently interpreted all FISH slides. Discordant cases were resolved by reevaluation of the *FGF19* FISH status on whole sections. Normal *FGF19* or *CEP11* signals served as the internal positive control in lymphocytes (Fig. 1a). The FISH signals were visualized as single copies, multiple copies, and clusters. A discrete dot was counted as a single copy of *FGF19* or *CEP11*. The size of these single dots was used as a reference to determine the relative number of amplified copies in the cancer cell nuclei. In some nuclei, clusters of dots representing many copies of the *FGF19* gene were apparent. In such cases, a small cluster of multiple signals was counted as 6 signals and a large cluster was counted as 12 signals. The results were determined according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) 2013 guidelines and interpreted as positive, equivocal, or negative [21].

FGF19 and *CEP11* signals were enumerated in this manner in 50 cell nuclei within a given HCC area, and the *FGF19*/*CEP11* ratio was calculated. All cases were visually assessed, and classified as *FGF19* positive if they met the following criteria: (1) dual-probe *FGF19*/*CEP11* ratio ≥ 2.0 with an average *FGF19* copy number

≥4.0 signals/cell; (2) dual-probe *FGF19/CEP11* ratio ≥2.0 with an average *FGF19* copy number <4.0 signals/cell; or (3) dual-probe *FGF19/CEP11* ratio <2.0 with an average *FGF19* copy number ≥6.0 signals/cell. They were interpreted as *FGF19* negative if the dual-probe *FGF19/CEP11* ratio was <2.0 with an average *FGF19* copy number <4.0 signals/cell. Results such as a single-probe FISH average *FGF19* copy number ≥4.0 and <6.0 signals/cell or a dual-probe *FGF19/CEP11* ratio <2.0 with an average FISH *FGF19* copy number ≥4.0 and <6.0 signals/cell were interpreted as equivocal. For these results, an additional examination was performed in 50 nuclei at a different site from those initially observed. The *FGF19/CEP11* ratio was then calculated in a total of 100 cell nuclei from the first and the second site.

Somatic Mutation and CNV Analysis

The results of the *FGF19* FISH were compared with the data on somatic mutations and CNV data obtained in our previous study [10]. The platform used in the previous study was the Affymetrix CytoScan HD Array. As previously noted, CNV analyses were performed using the Nexus Copy Number software (version 6.1; BioDiscovery, El Segundo, CA, USA) with default parameters. In addition, whole exome sequencing data from 28 significantly mutated genes (*TP53*, *CTNNB1*, *HMCN1*, *PCLO*, *ADGRV1*, *LRP1B*, *APOB*, *RYR3*, *VCAN*, *SYNE1*, *DNAH6*, *RYR2*, *FBN2*, *XIRP2*, *PKHD1L1*, *C17ORF97*, *FLG*, *OBSCN*, *AXIN1*, *KIAA1109*, *AHNAK2*, *MGAM*, *CENPF*, *COL7A1*, *COL6A6*, *PXDN*, *KMT2D*, and *RB1*), which had shown a frequency of more than 5% in our previous study, were included in this evaluation.

Statistical Analysis

Statistical analyses were performed using the SPSS statistical software program (version 18.0; SPSS Inc., Chicago, IL, USA). Concordance rates between the different methods were determined and κ statistics were calculated using Cohen's kappa coefficient, according to which a κ value of 1 denotes complete agreement, a value of 0.75 denotes excellent agreement, values between 0.4 and 0.75 denote fairly good agreement, and values <0.4 denote poor agreement. A χ^2 test was performed to compare the frequencies of categorical variables between the groups.

The overall survival (OS) was designated to extend from the day of surgical tumor resection to the date of the patient's death from any cause. The follow-up period for the surviving patients was assessed from the last follow-up date. Disease-free survival (DFS) was assessed as the length of time from the day of surgical tumor resection to disease recurrence or the last follow-up date. Both the OS and DFS times were calculated by the Kaplan-Meier method, and their statistical significance was evaluated by the log-rank test and the Cox proportional-hazards regression model. All tests were two-sided, and *p* values <0.05 were considered to indicate statistical significance.

Results

Clinicopathological Characteristics

Table 1 summarizes the patient characteristics and pathologic features of the evaluated tumors. The mean patient age at diagnosis was 56 years (range 25–83) and 790 patients (79.9%) were male. Recurrence occurred in 529 patients (53.5%) and early recurrence within 2 years in 464 (46.9%). The estimated 5-year OS and DFS rates were 405 (40.9%) and 317 (32.0%), respectively.

*Correlation between *FGF19* Amplification by FISH and Clinicopathological Variables*

The results of *FGF19* FISH in tumor cells were positive in 51 cases (5.16%; Fig. 1a) and negative in 938 cases (94.84%; Fig. 1b). No cases showed *FGF19* amplification in nontumorous cells (Fig. 1c). *FGF19* amplification was significantly correlated with high serum α -fetoprotein levels (*p* = 0.001), hepatitis B or C virus infection (*p* = 0.030), a tumor size >5 cm (*p* = 0.003), microvascular invasion (*p* = 0.012), necrosis (*p* = 0.030), and early disease recurrence (*p* = 0.010; Table 2). In contrast with our previous observations, *FGF19* amplification showed no association with cirrhosis (*p* = 0.386). An analysis comparing the FISH results with our previously published whole exome sequencing data revealed that *FGF19*

Table 1. Demographic summary of the study patients and characteristics of the hepatocellular carcinoma samples ($n = 989$)

Age, years	56 (25–83)
≤50 years	292 (29.5)
>50 years	697 (70.5)
Sex	
Male	790 (79.9)
Female	199 (20.1)
Serum AFP, ng/mL	12,970.81 (0.41–1,284,740.0)
≤100 ng/mL	590 (59.7)
>100 ng/mL	399 (40.3)
Hepatitis virus	
Hepatitis B	824 (83.3)
Hepatitis C	54 (5.5)
NBNC	111 (11.2)
Liver cirrhosis	
Absent	433 (43.8)
Present	556 (56.3)
Tumor diameter, cm	5.33 (0.7–22.0)
≤5 cm	625 (63.2)
>5 cm	364 (36.8)
Multiplicity	
Solitary tumor	863 (87.3)
Multiple tumors	126 (12.7)
Edmondson-Steiner grade (worst)	
I or II	261 (26.4)
III or IV	728 (73.6)
Microvascular invasion	
Absent	613 (62.0)
Present	376 (38.0)
Glisson capsule invasion	
Absent	842 (85.1)
Present	147 (14.9)
Necrosis	
Absent	523 (52.9)
Present	466 (47.1)
Child-Pugh class	
A	935 (94.5)
B	46 (4.7)
C	8 (0.8)
BCLC stage	
0	49 (5.0)
A	692 (70.0)
B	103 (10.4)
C	137 (13.9)
D	8 (0.8)

Values are presented as n (%) or mean (range). AFP, α -fetoprotein; NBNC, neither hepatitis B virus nor hepatitis C virus.

amplification was significantly associated with *TP53* mutations ($p = 0.040$) and mutually exclusive with *CTNNB1* mutations ($p < 0.001$; Fig. 2).

Prognostic Significance of *FGF19* Amplification

FGF19 amplification was significantly associated with lower 5-year OS (43.70% in *FGF19*-positive vs. 71.01% in *FGF19*-negative patients, $p < 0.001$; Fig. 3a) and lower 5-year DFS (26.26% in *FGF19*-positive vs. 44.97% in *FGF19*-negative patients, $p < 0.001$; Fig. 3b). Univariate analysis showed that *FGF19* amplification was inversely associated with OS

Table 2. Correlation between *FGF19* amplification and clinicopathological characteristics

	<i>FGF19</i> negative (<i>n</i> = 938)	<i>FGF19</i> positive (<i>n</i> = 51)	<i>p</i>
Age			0.082
≤50 years old	271 (28.9)	21 (41.2)	
>50 years old	667 (71.1)	30 (58.8)	
Sex			0.857
Male	750 (80.0)	40 (78.4)	
Female	188 (20.0)	11 (21.6)	
Serum AFP			0.001*
≤100 ng/mL	571 (60.9)	19 (37.3)	
>100 ng/mL	367 (39.1)	32 (62.7)	
Hepatitis virus			0.030*
Hepatitis B virus	777 (82.8)	47 (92.2)	
Hepatitis C virus	50 (5.3)	4 (7.8)	
NBNC	111 (11.8)	0 (0.0)	
Liver cirrhosis			0.386
Absent	414 (44.1)	19 (37.3)	
Present	524 (55.9)	32 (62.7)	
Tumor diameter			0.003*
≤5 cm	603 (64.3)	22 (43.1)	
>5 cm	335 (35.7)	29 (56.9)	
Multiplicity			0.517
Solitary tumor	820 (87.4)	43 (84.3)	
Multiple tumors	118 (12.6)	8 (15.7)	
E-S grade (worst)			0.191
I or II	252 (26.9)	9 (17.6)	
III or IV	686 (73.1)	42 (82.4)	
Microvascular invasion			0.012*
Absent	590 (62.9)	23 (45.1)	
Present	348 (37.1)	28 (54.9)	
Glisson capsule invasion			0.418
Absent	801 (85.4)	41 (80.4)	
Present	137 (14.6)	10 (19.6)	
Necrosis			0.030*
Absent	504 (53.7)	19 (37.3)	
Present	434 (46.3)	32 (62.7)	
Early recurrence ^a			0.010*
Absent	507 (54.1)	18 (35.3)	
Present	431 (45.9)	33 (64.7)	

Values are presented as *n* (%). AFP, α-fetoprotein; NBNC, neither hepatitis B virus nor hepatitis C virus; E-S grade, Edmondson-Steiner grade. * Significant *p* value. ^a Early recurrence is defined as recurrence within 2 years after surgical tumor resection.

(hazard ratio [HR] = 2.722, 95% confidence interval [CI] 1.889–3.922, *p* < 0.001; Table 3) and DFS (HR = 1.901, 95% CI 1.349–2.680, *p* < 0.001; Table 4). In the multivariate analysis, *FGF19* amplification was predicted as an independent prognostic factor for OS (HR = 2.257, 95% CI 1.558–3.269, *p* < 0.001; Table 3), along with BCLC stage (*p* < 0.001), a large tumor size (*p* = 0.016), multiple tumors (*p* < 0.001), microvascular invasion (*p* < 0.001), Glisson capsule invasion (*p* = 0.001), and necrosis (*p* < 0.001). In addition, *FGF19* amplification was predicted

Fig. 2. Recurrent gene mutations in patients with hepatocellular carcinoma exhibiting/lacking *FGF19* amplification (as determined by fluorescence in situ hybridization analysis).

Fig. 3. Comparison of survival rates according to *FGF19* amplification status. The positive group, with *FGF19* amplification, was associated with poorer overall survival (**a**) and disease-free survival (**b**) rates.

Table 3. Univariate and multivariate analyses of overall survival according to the clinicopathological variables

	HR	95% CI	p
<i>Univariate analysis</i>			
Age ≤50 years	1.320	1.052–1.658	0.017*
Male gender	0.920	0.708–1.197	0.536
Serum AFP >100 ng/mL	1.694	1.365–2.101	<0.001*
Hepatitis B	1.112	0.845–1.463	0.449
Liver cirrhosis	1.081	0.870–1.344	0.483
BCLC stage C or D	3.047	2.391–3.884	<0.001*
Tumor diameter >5 cm	2.665	2.145–3.310	<0.001*
Multiple tumors	1.832	1.387–2.420	<0.001*
E-S grade III or IV (worst)	1.399	1.215–1.610	<0.001*
Microvascular invasion	3.201	2.570–3.987	<0.001*
Glisson capsule invasion	2.396	1.873–3.066	<0.001*
Necrosis	2.676	2.132–3.360	<0.001*
<i>FGF19</i> amplification	2.722	1.889–3.922	<0.001*
<i>Multivariate analysis</i>			
Age ≤50 years	0.962	0.762–1.215	0.746
Serum AFP >100 ng/mL	1.093	0.867–1.379	0.450
BCLC stage C or D	1.845	1.424–2.391	<0.001*
Tumor diameter >5 cm	1.367	1.060–1.764	0.016*
Multiple tumors	1.747	1.319–2.315	<0.001*
E-S grade III or IV	1.197	0.888–1.614	0.239
Microvascular invasion	2.012	1.570–2.577	<0.001*
Glisson capsule invasion	1.539	1.190–1.990	0.001*
Necrosis	1.600	1.239–2.066	<0.001*
<i>FGF19</i> amplification	2.257	1.558–3.269	<0.001*

AFP, α-fetoprotein; HR, hazard ratio; CI, confidence interval; E-S grade, Edmondson-Steiner grade. * Significant *p* value.

as an independent prognostic factor for DFS (HR = 1.644, 95% CI 1.163–2.324, *p* = 0.005; Table 4), along with BCLC stage (*p* = 0.017), a large tumor size (*p* < 0.001), multiple tumors (*p* < 0.001), microvascular invasion (*p* < 0.001), and necrosis (*p* = 0.008).

Comparison of the FISH Method with the CNV Results

We evaluated the reproducibility of the *FGF19* amplification previously detected by CNV analysis using CytoScan HD data analysis on 227 HCC cases [10]. Cohen's kappa statistics showed agreement between the two methods for 219 of the 227 cases (concordance percentage: 96%, κ = 0.618, *p* < 0.001). Of the 8 discrepant cases, 3 were positive for *FGF19* amplification by FISH and negative in the CNV analysis, whereas 5 were negative by FISH and positive in the CNV analysis (Table 5).

Discussion

This study evaluated *FGF19* amplification using FISH in a large cohort of patients with HCC. Our main finding was that *FGF19* amplification was independently associated with poor survival and with a higher risk of tumor recurrence. In accordance with this observation, *FGF19* amplification was found to be significantly associated with poor prognostic factors, such as a high α-fetoprotein level, hepatitis B or C infection, a large tumor size, microvascular

Table 4. Univariate and multivariate analyses of disease-free survival according to the clinicopathological variables

	HR	95% CI	p
<i>Univariate analysis</i>			
Age ≤50 years	1.268	1.058–1.520	0.010*
Male gender	1.002	0.809–1.241	0.985
Serum AFP >100 ng/mL	1.474	1.242–1.751	<0.001*
Hepatitis B	0.972	0.773–1.223	0.810
Liver cirrhosis	1.090	0.917–1.295	0.330
BCLC stage C or D	2.080	1.673–2.585	<0.001*
Tumor diameter >5 cm	2.685	2.261–3.189	<0.001*
Multiple tumors	1.539	1.215–1.951	<0.001*
E-S grade III or IV (worst)	1.487	1.213–1.823	<0.001*
Microvascular invasion	2.187	1.842–2.597	<0.001*
Glisson capsule invasion	1.778	1.425–2.218	<0.001*
Necrosis	2.026	1.704–2.408	<0.001*
<i>FGF19</i> amplification	1.901	1.349–2.680	<0.001*
<i>Multivariate analysis</i>			
Age >50 years	0.923	0.766–1.112	0.399
Serum AFP >100 ng/mL	1.090	0.908–1.309	0.355
BCLC stage C or D	1.324	1.052–1.668	0.017*
Tumor diameter >5 cm	1.957	1.605–2.385	<0.001*
Multiple tumors	1.646	1.295–2.092	<0.001*
E-S grade III or IV (worst)	1.110	0.894–1.378	0.343
Microvascular invasion	1.535	1.267–1.861	<0.001*
Glisson capsule invasion	1.158	0.918–1.459	0.215
Necrosis	1.305	1.073–1.587	0.008*
<i>FGF19</i> amplification	1.644	1.163–2.324	0.005*

AFP, α-fetoprotein; HR, hazard ratio; CI, confidence interval; E-S grade, Edmondson-Steiner grade. * Significant *p* value.

Table 5. Concordance between the FISH and CNV results

CNV analysis	FISH				<i>p</i> value <0.001
	positive (amplification)	negative (no amplification)	equivocal	total	
No amplification	3	212	0	215	
Amplification	7	5	0	12	
Total	10	217	0	227	

FISH, fluorescence in situ hybridization; CNV, copy number variation.

invasion, and necrosis. This finding supports the hypothesis that the *FGF19-FGFR4* signaling pathway plays an import role in hepatocarcinogenesis [11, 18].

We also investigated the tentative relationship between *FGF19* amplification and cirrhosis, especially in hepatitis B virus-related HCC, as observed in our previous study [10]. In the present study, which included a larger cohort, we found no relationship between *FGF19* amplification and cirrhosis. However, *FGF19* amplification was significantly associated with chronic HBV infection. Interestingly, a recent study reported an association between increased

Fig. 4. Comparison of the detailed results between fluorescence in situ hybridization (FISH) and copy number variation (CNV) analyses. Among 227 cases, 219 (96%) were matched by the CNV analysis and FISH results. Of the 8 (3%) discrepant cases, 3 (1%) were positive by FISH with one copy gain in the CNV analysis, whereas 5 (2%) were negative by FISH with high copy amplification in the CNV analysis.

FGF19 level and noncirrhotic patients [22]. Additional studies will be required to provide further clarification of the significance of the *FGF19*-cirrhosis relationship.

Aberrant *FGF19-FGFR4* signaling in HCC has been the focus of a number of clinical studies. BLU9931, a selective small molecule *FGFR4* inhibitor, was recently shown to inhibit aberrant *FGF19-FGFR4* signaling [3, 23, 24]. In another study, treatment with an anti-*FGF19* antibody in liver and colon cancer xenograft models has suggested that inactivation of *FGF19*-mediated signaling may be beneficial for the treatment of liver cancer and other malignancies involving the interaction of *FGF19* and *FGFR4* [25]. We believe our current study outlines an optimal approach to identifying the most appropriate candidates for treatment, but further studies are required to investigate the clinical application of anti-*FGF19* agents.

Previous investigators have reported the rate of *FGF19* amplification by CNV analysis to be 5–9% [4, 10], but the correlation between *FGF19* gene status assessed by CNV and that assessed by FISH has not previously been reported. Our study showed a concordance rate between CNV results and FISH assays of approximately 96%, suggesting that CNV data are largely reproducible using FISH, thus providing confirmation of our main study findings. However, a slight discrepancy (8 cases, 3%) was observed (Fig. 4). From our retrospective review of the detailed results of both FISH and CNV analyses for all cases, 3 of the 19 cases with one copy gain in the CNV analysis showed *FGF19* amplification according to FISH, which may be due to the low sensitivity of CNV arrays at lower tumor purity levels [26, 27]. We selected only high copy amplifications in CNV analysis to minimize false positives and increase the specificity. In addition, 5 cases with high copy amplification in CNV analysis were seen to be FISH negative. This discrepancy may be due to the limited resolution with FISH or issues associated with the formalin fixing process, such as a delay between tissue sampling and processing, or incomplete fixation. Reducing the time between tissue collection and fixation, as well as ensuring complete fixation, will improve the quality of FISH assays.

In summary, this study is the first to demonstrate a relationship between *FGF19* amplification and the clinicopathological characteristics of HCC in a large cohort using FISH. Our findings support the hypothesis that the *FGF19-FGFR4* signaling pathway plays an important role in hepatocarcinogenesis and that HCC with *FGF19* amplification represents a unique molecular subtype of patients with poor prognostic characteristics who may benefit from anti-*FGF19* therapeutic strategies. In addition, the observed correlation between FISH and CNV results suggests that the FISH methodology can be used with confidence to detect *FGF19* amplification in patients with HCC.

Statement of Ethics

This study was approved by the Institutional Review Board of Asan Medical Center with a waiver of informed consent (IRB No. 2017-0875).

Disclosure Statement

No potential conflicts of interest are disclosed.

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