Epigenomics

Predictive significance of circulating histones in hepatocellular carcinoma patients treated with sorafenib

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Background: Predictive biomarkers for advanced hepatocellular carcinoma are lacking. EZH2 drives sorafenib resistance through H3K27me3 and is counteracted by SETD2, which catalyzes H3K36me3. The authors tested the predictive power of circulating H3K27me3 and H3K36me3 in advanced hepatocellular carcinoma patients treated with sorafenib. **Methods:** A total of 80 plasma samples were tested for histone variants by ELISA. Changes from baseline to best response or progressive disease were correlated with patient survival. **Results:** A higher *EZH2/SETD2* ratio predicted worse prognosis in this setting. H3K27me3 and H3K36me3 decreased from baseline to best response. The H3K27me3/H3K36me3 ratio increased from baseline to progressive disease. Higher ratios at best response were associated with shorter progression-free survival. **Conclusion:** The authors suggest that circulating H3K27me3/H3K36me3 ratio level acts as a predictive biomarker for sorafenib treatment outcomes in patients with advanced hepatocellular carcinoma.

Plain language summary: Hepatocellular carcinoma (HCC) is responsible for approximately 10% of all cancer-related deaths worldwide. It is caused mainly by dysmetabolic syndrome, which is the presence of multiple risk factors: abdominal obesity, high blood pressure, hypercholesterolemia and diabetes. The authors aimed to identify new and predictive factors for sorafenib treatment outcomes in advanced HCC patients. The authors enrolled 85 patients who received sorafenib at two Italian oncological institutions, testing their blood for the following epigenetic biomarkers: H3, H3.1 variant, H3K27me3 and H3K36me3. The authors found that H3K27me3 and H3K36me3 decreased from baseline to maximum tumor shrinkage, H3K27me3/H3K36me3 ratio increased from baseline to progressive disease and higher ratios were associated with shorter progression-free survival. The authors suggest that circulating H3K27me3/H3K36me3 ratio level acts as a predictive biomarker for sorafenib treatment outcomes in patients with advanced HCC, and its role warrants further investigation in different HCC therapeutic strategies.

Future Medicine

Hepatocellular carcinoma (HCC) represents the fourth leading cause of cancer-related deaths worldwide, with an annual incidence of 4.7% new cases and an expected 5- and 10-year survival rate of 20 and 19%, respectively [1–3]. Sorafenib, a tyrosine kinase inhibitor targeting the Raf/Mek/Erk pathway and interacting with multiple cell surface kinases involved in angiogenesis, has long been the standard of care for advanced HCC [4]. Despite increasing first-line options, including lenvatinib [5] and atezolizumab/bevacizumab combination [6], sorafenib represents a first-line alternative in case of contraindication to more recent combination therapies. Of note, no predictive biomarkers of treatments' outcome have yet been identified by the scientific comunity. Similarly, most robust prognostic factors, including high alfa-fetoprotein (AFP) levels, poor liver function and presence of macrovascular invasion or extrahepatic spread, rely on clinical features that are not treatment-specific [7]. Sorafenib-related adverse events have been reported as on-treatment prognostic factors by mainly retrospective studies [8,9], whereas no treatment-specific prognostic factors have thus far been identified at baseline. Because of the relative scarcity of hot-spot mutations, circulating tumor DNA has shown limited applicability in HCC compared with other malignancies. By contrast, epigenetic modifications on cell-free circulating nucleosomes have shown great diagnostic and prognostic potential in several malignancies, including HCC [10].

Available evidence indicates that HCC pathogenesis is driven by the interaction between genetic and epigenetic alterations [11]. Histone post-translational modifications (HPTMs) are epigenetic alterations occurring predominantly at the N-terminal tail of histones [12]. HPTMs alter the local chromatin structure, thereby regulating the transcriptional activity of specific genes. Some modifications, such as histone acetylation, reduce chromatin compaction, increasing gene expression. Other HPTMs (e.g., histone methylation) produce different effects depending on the target residue. For example, the trimethylation of histone H3 at lysine 27 (H3K27me3) induces a more compact chromatin state (gene silencing). By contrast, trimethylation at lysine 36 of the same protein (H3K36me3) causes gene activation. It has recently been shown that H3K27me3 and H3K36me3 play antagonistic roles in human malignancies [13], but the function of this interplay in HCC has not been fully elucidated. H3K27me3 is catalyzed by the polycomb gene *EZH2*, which is a known oncogene and driver of sorafenib resistance [14] and, when inhibited, enhances sorafenib's antitumor effects [15]. SETD2, the main enzyme that catalyzes H3K36me3 [14], has been described as a potential oncosuppressor in HCC [16]. Its inhibition has been shown to trigger spontaneous development of HCC in mouse models [17], but its role in mediating response to sorafenib has never been investigated.

Recently, a range of immunoassays have been used to profile various histone modifications and variants associated with cell-free circulating nucleosomes in an approach referred to as Nucleosomics™ (Belgian Volition SRL, Gembloux, Belgium). These assays, which are based on 96-well plates coated with epitope-specific antibodies and a biotinylated detection antibody specific for intact nucleosomes, have been explored for early detection of malignancies in serum [18–20]. H3K36me3 has been assessed alongside H3.1 variant and H3 citrullinated nucleosomes as a potential panel for early detection of kidney transplant rejection [21]. In this study, the authors applied this emerging technology to investigate sorafenib resistance in HCC. Specifically, the authors employed prototype immunoassays specific for H3K27me3 and H3K36me3 alongside commercially available Nu.Q™ assays (Belgian Volition SRL) for H3.1- and total H3-containing nucleosomes. These assays have never been used to monitor response to specific cancer treatments. This approach allowed the authors to test the hypothesis that circulating levels of H3K27me3, H3K36me3 and total histone H3 levels predict response to sorafenib in advanced HCC patients.

Methods

Bioinformatics analyses

Gene expression & survival correlations according to the University of Alabama at Birmingham Cancer database

The University of Alabama at Birmingham Cancer interactive web server was engaged to determine the mRNA expression of SETD2 and EZH2 in HCC cells [22,23]. The University of Alabama at Birmingham Cancer database provides gene expression data analysis of cancer and normal tissues from The Cancer Genome Atlas, MET500 and Clinical Proteomic Tumor Analysis Consortium data portal [24–26]. Using the logrank test, the Kaplan–Meier plotter [27] was used to understand the prognostic value of EZH2 and SETD2 in the median progression-free survival (mPFS) and median overall survival (mOS) of 29 sorafenib-treated patients in a cohort of 364 HCC patients from The Cancer Genome Atlas [28].

Identification of co-expressed EZH2 & SETD2 genes & pathway analyses

The Cancer Genome Atlas Firehose Legacy HCC dataset in cBioPortal [29] was used to understand co-expressed *EZH2* and *SETD2* genes in 429 samples that had a higher R^2 value (>50). To provide the pathway activity map of the co-expressed genes, the genes were analyzed with the help of the ConsensusPathDB-human database. ConsensusPathDB-human data for interactions identified in the literature originate from 32 public resources [30,31].

Clinical data

The authors enrolled 85 advanced HCC patients treated with first-line sorafenib at Pisa University Hospital, Pisa, Italy, and Istituto di Ricovero e Cura a Carattere Scientifico, Modena, Italy. The drug was administered orally daily, as per clinical practice, on a 28-day cycle. Starting dose was 800 mg/day, with dose reductions allowed according to the pivotal trial [4]. Patients had signed informed consent forms for translational research projects within the INNOVATE trial [32] relative to baseline (i.e., within 28 days from first sorafenib administration), best response (BR) and progressive disease (PD) according to Response Evaluation Criteria in Solid Tumors 1.1 [33].

Efficacy and activity data were described in terms of mPFS, mOS, best radiological response and AFP serum levels. Patients were defined as 'responders' if they achieved partial response or stable disease or 'nonresponders' if PD occurred at first assessment. PFS and OS were estimated using the Kaplan–Meier method and calculated from the day of treatment initiation until the day of disease progression or death or until the day of death, respectively. 80 patients who were evaluable in terms of HPTMs concentrations and radiological responses during sorafenib treatment were included in the longitudinal biomarker analysis; five patients were excluded because of lack of most clinical, laboratory or survival data.

Sample processing & biomarkers

One 6-ml vial with EDTA and one 6-ml vial with coagulation activator were collected at each time point to obtain serum and plasma for storage in 2-ml Eppendorf Tubes (Eppendorf, Hamburg, Germany) and preserved at -80°C. The 85 samples were measured by specific ELISA assays for nucleosomes containing H3 and the canonical H3.1 variant (Active Motif, CA, USA) as well as H3K27me3 and H3K36me3 (prototype Nu.Q™ assays) at The Open University, London, UK. Assays were performed according to the manufacturers' instructions and displayed selectivity for the specific histone variant or post-translational modification using a range of recombinant nucleosomes. The kits enable detection and quantification of nucleosomes in serum or plasma [34].

Statistical analysis

Analyses of biomarker levels as well as activity and efficacy were performed for patients who received ≥1 cycle of sorafenib. Clinical nominal variables were described as frequencies and compared across different biomarker level groups through Chi-square test. Continuous AFP values were dichotomized according to the literature cut-off (≥400 vs <400 ng/ml) [4]. H3, H3.1, H3K27me3, H3K36me3 and H3K27me3/H3K36me3 ratios were described using their mean, median and range values and were treated as continuous variables using rank-sum test and as dichotomous with respect to their median values since they were not normally distributed. The predictive value of epigenetic markers was tested with chi-square and rank-sum tests, and their prognostic role in terms of PFS and OS was estimated using the Kaplan–Meier method and compared with the logrank test. A multivariate Cox proportional hazards model was built to identify predictors of outcome; only factors with a two-sided p < 0.05 were included. With regard to on-treatment comparison of biomarker concentrations, two time point confrontation (i.e., baseline vs BR; baseline vs PD; BR vs PD) was performed with Wilcoxon signed-rank test or paired *t*-test for not normally and normally distributed differences, respectively. Statistical software used was MedCalc version 14.8.1 (MedCalc Software Ltd, Oostende, Belgium) and SciPy 1.0.

Results

Bioinformatics analysis: *EZH2* & *SETD2* expression profiles & prognostic role

To confirm the relevance of the genes of interest, the authors analyzed a public dataset to measure expression of *EZH2* and *SETD2* in 371 HCC samples versus 50 adjacent normal tissues [22]. Both transcripts were significantly upregulated in HCC (p < 0.0001) (Figure 1A & B). To develop a systematic understanding of the similarities between *EZH2* and *SETD2*, the authors used correlation analysis of co-expressed genes in *EZH2* and *SETD2*. *EZH2* had 36 co-expressed genes with a higher regression value (R^2 >50), and *SETD2* had nine co-expressed genes with a higher regression value (R^2 >50). No common genes were co-expressed, as plotted with a Venn
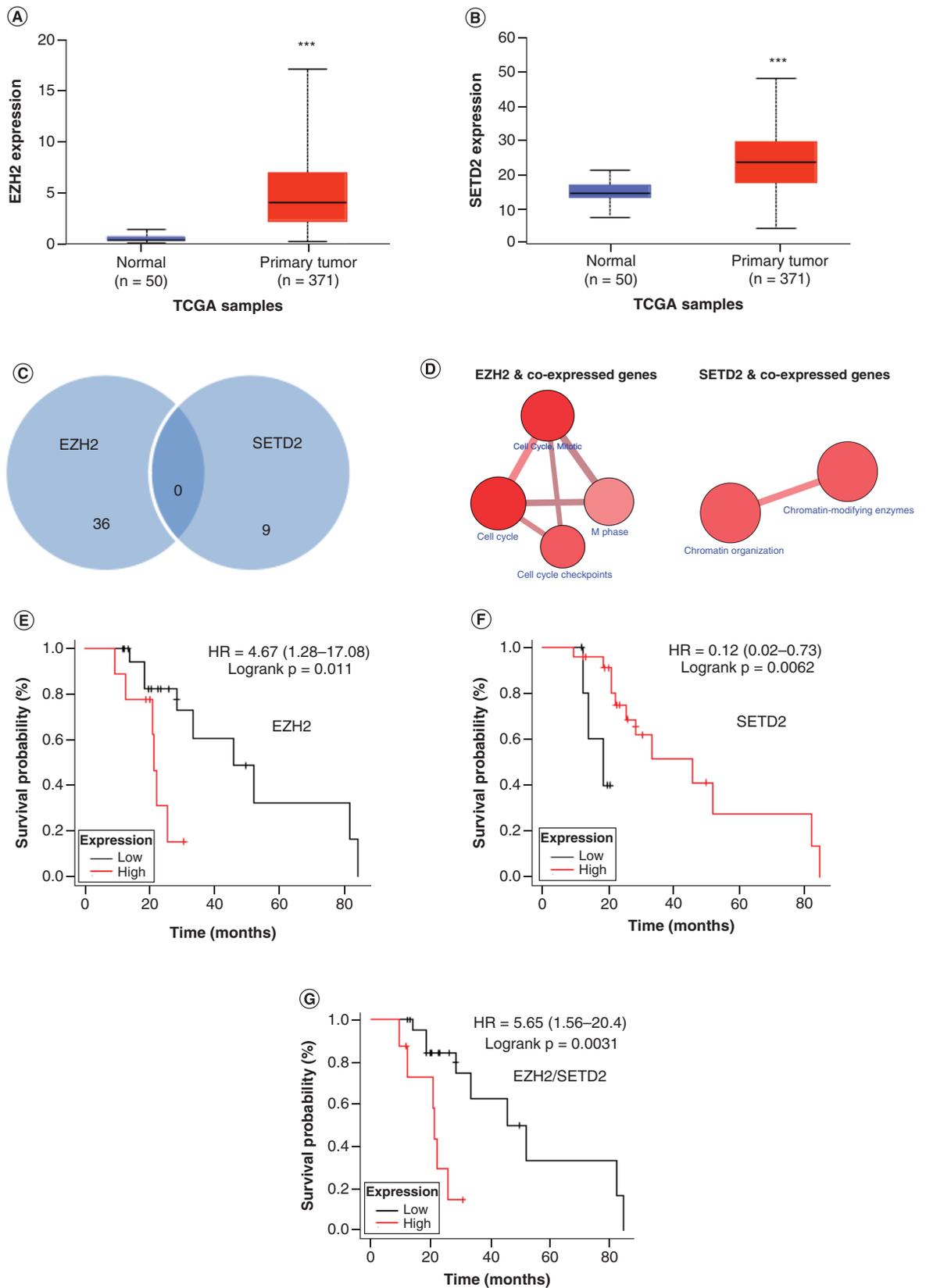
Figure 1.   Bioinformatics data on *EZH2* and *SETD2*. (A) *EZH2* and **(B)** *SETD2* expression in HCC and normal tissues. **(C)** *EZH2* and *SETD2* co-expressed genes. **(D)** Functional pathway map of co-expressed *EZH2* and *SETD2* genes. Correlation of **(E)** *EZH2*, **(F)** *SETD2* and **(G)** *EZH2/SETD2* with OS.
***p < 0.05.
HCC: Hepatocellular carcinoma; HR: Hazard ratio; OS: Overall survival; TCGA: The Cancer Genome Atlas.

Table 1. H3, H3.1, H3K27me3, H3K36me3 and H3K27me3/H3K36me3 ratio descriptive statistics at studied time points.			
	Baseline	BR	PD
H3			
N evaluable	80	21	24
Median (95% CI)	0.45 (0.32–0.79)	0.32 (0.17–0.50)	0.36 (0.21–0.71)
Mean (95% CI)	0.82 (0.64–1.00)	0.35 (0.25–0.45)	0.65 (0.34–0.97)
Range	0.02–3.16	0.037–0.75	0.00–2.75
H3.1			
N evaluable	80	21	24
Median (95% CI)	0.50 (0.39–0.63)	0.24 (0.14–0.42)	0.41 (0.27–0.56)
Mean (95% CI)	0.79 (0.61–0.96)	0.36 (0.15–0.58)	0.52 (0.31–0.74)
Range	0.07–3.08	0.00–2.13	0.00–2.37
H3K27me3			
N evaluable	80	21	24
Median (95% CI)	0.28 (0.20–0.33)	0.09 (0.06–0.12)	0.13 (0.08–0.32)
Mean (95% CI)	0.37 (0.28–0.47)	0.11 (0.06–0.16)	0.25 (0.14–0.33)
Range	0.00–2.39	0.00–0.48	0.00–0.75
H3K36me3			
N evaluable	80	21	24
Median (95% CI)	122.90 (104.70–149.57)	22.90 (6.70–43.23)	19.73 (6.68–55.60)
Mean (95% CI)	137.38 (117.68–147.09)	22.67 (15.48–39.85)	55.52 (26.64–85.40)
Range	0.00–273.34	0.00–100.66	2.34–222.51
(H3K27me3/H3K36me3)*10³			
N evaluable	78	20	24
Median (95% CI)	0.0023 (0.0019–0.0026)	0.0051 (0.003–0.012)	0.0032 (0.002–0.008)
Mean (95% CI)	0.0034 (0.0026–0.0042)	0.0065 (0.0028–0.010)	0.0067 (0.003–0.001)
Range	0.00–0.153	0.00–0.034	0.00–0.340

H3, H3.1 and H3K27me3 values are expressed in μg/ml. H3k36me3 values are expressed in ng/ml. H3K27me3/H3K36me3 ratio is dimensionless.
BR: Best response; PD: Progressive disease.

diagram (R^2 >50) (Figure 1C). Next, a pathway analysis was performed to identify *EZH2* and *SETD2* co-expressed genes. *EZH2* co-expressed genes were involved in pathway activities such as cell cycle, M phase and cell cycle checkpoints (Figure 1D), whereas *SETD2* co-expressed genes were involved in chromatin modification and chromatin-modifying enzyme activities. Subsequently, the authors investigated the prognostic relevance of these two transcripts in patients exposed to sorafenib. The authors' results showed that higher expression of *EZH2* was associated with worse prognosis, whereas higher expression of *SETD2* was associated with better prognosis (Figure 1E & F). Notably, the *EZH2/SETD2* ratio was more precise than either gene alone in determining prognosis with sorafenib (p = 0.0031) (Figure 1G).

Taken together, these results indicate that both *EZH2* and *SETD2* are selectively upregulated in HCC. However, intratumoral *EZH2* expression is associated with worse response to sorafenib, whereas intratumoral *SETD2* expression is associated with sensitivity to sorafenib.

Clinical cohort

After a median follow-up of 20.82 months (95% CI: 18.06–20.82), a median of 3.71 cycles of sorafenib was administered (95% CI: 2.98–5.42). Treatment interruption occurred at disease progression, with the exception of two patients who interrupted sorafenib 1 month earlier as the result of a therapeutic pause. At the time of observation, 93.7% of patients had progressed and 81.0% had died, with mPFS and mOS of 3.42 (95% CI: 2.76–4.93) and 11.41 (95% CI: 8.19–15.62) months, respectively. AFP median baseline values were 104.10 ng/ml (95% CI: 16.2–447.4). As expected based on the literature, AFP <400 ng/ml and Child–Pugh score A were significantly associated with OS (univariate analysis), with the former confirming its independent prognostic role in the Cox multivariate regression model (p = 0.04) [35,36].

Circulating histones: descriptive analysis

Descriptive statistics of circulating levels of H3K27me3 and H3K36me3 and total levels of histone H3 and H3.1 at different time points are shown in Table 1. The latter two epigenetic biomarkers were employed to corroborate the hypothesis that a specific histone modification, rather than total histone level, is associated with treatment response.

Table 2. Univariate analysis of overall and progression-free survival and H3, H3.1, H3K27me3, H3K36me3 and H3K27me3/H3K36me3 ratio baseline values.

	mOS, months	p-value	mPFS, months	p-value
H3 Median ≥ vs <	14.37 vs 8.82	0.50	4.51 vs 2.93	0.52
H3.1 Median ≥ vs <	8.79 vs 12.47	0.10	2.76 vs 4.77	0.11
H3K27me3 Median ≥ vs <	9.23 vs 12.27	0.77	3.09 vs 3.62	0.99
H3K36me3 Median ≥ vs <	14.60 vs 9.23	0.80	3.85 vs 3.0	0.43
(H3K27me3/H3K36me3)*10³ Median ≥ vs <	8.59 vs 13.0	0.26	2.77 vs 4.90	0.29

H3, H3.1 and H3K27me³ values are expressed in µg/ml. H3K36me³ values are expressed in ng/ml. H3K27me³/H3K36me³ ratio is dimensionless.
mOS: Median overall survival; mPFS: Median progression-free survival.
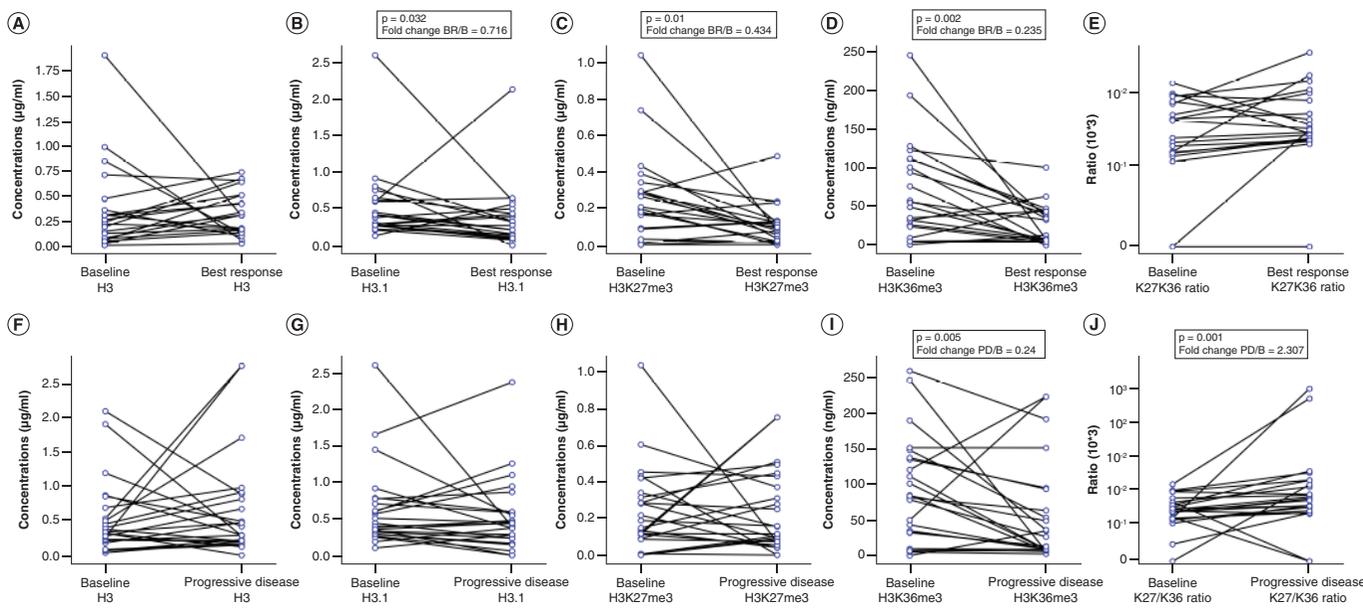
Figure 2.   Epigenetic biomarker variations from baseline to best response or progressive disease. Variations in **(A)** H3, **(B)** H3.1, **(C)** H3K27me3, **(D)** H3K36me3 and **(E)** H3K27me3/H3K36me3 ratio (K27/K36 ratio) from B to BR in responders. Variations in **(F)** H3, **(G)** H3.1, **(H)** H3K27me3, **(I)** H3K36me3 and **(J)** H3K27me3/H3K36me3 ratio (K27/K36 ratio) from B to PD in all evaluable patients. **(E & J)** Because of the high dynamic range of values for the H3K27me3/H3K36me3 ratio plot, a mixed linear/log scale was used for the y-axis. Fold change obtained as median biomarker value at BR or PD/median biomarker value at B. Data compared using Wilcoxon signed-rank or paired *t*-test.
B: Baseline; BR: Best response; PD: Progressive disease.

Predictive & prognostic value of baseline circulating histones

Levels of epigenetic biomarkers at baseline did not show any predictive or prognostic value. Indeed, no association with PFS was observed for any of the biomarkers dichotomized according to median values. Similar results were obtained for OS. Results are summarized in Table 2. No correlations were found between BR or PD and biomarker baseline values (data not shown).

Analysis of circulating histones in sorafenib responders versus nonresponders

H3.1, H3K27me3 and H3K36me3 levels significantly decreased from baseline to BR in matched samples for 21 responders (Figure 2A–E).

Among 17 matched nonresponder samples, the authors observed that H3K36me3 and H3K27me3/H3K36me3 ratio varied significantly, with the former decreasing (median: 83.6 vs 35.9 ng/ml; p = 0.08) and the latter increasing (median: 0.002 vs 0.005 ng/ml; Wilcoxon signed-rank test: p = 0.0001).

Circulating histones at sorafenib failure

24 patients were evaluable at baseline and PD irrespective of best response. In this case, the authors' longitudinal analysis (baseline vs failure) revealed results that were similar to those observed in the nonresponder cohort. H3K36me3 levels were significantly reduced (mean: 89.4 vs 55.2 ng/ml; p = 0.005), and the H3K27me3/H3K36me3 ratio was significantly increased from baseline to PD (median: 0.002 vs 0.005 ng/ml; p = 0.001), reinforcing the hypothesis of a biologically antagonistic role between these two histone marks (Figure 2F–J).

H3K27me3/H3K36me3 ratio as a prognostic biomarker

In light of these results, the authors sought to explore the prognostic role of H3K27me3/H3K36me3 ratio at BR and PD. H3K27me3/H3K36me3 ratio values were dichotomized according to their median and mean at each time point and tested against PFS and OS with Kaplan–Meier plots. At BR, no statistical significance was found for H3K27me3/H3K36me3 ratio based on its median value cut-off of 0.003 (mPFS < vs ≥ median: 5.6 vs 4.7 months; p = 0.14; mOS < vs ≥ median: 12.5 vs 10.3 months; p = 0.25). However, significantly longer PFS and OS were found for H3K27me3/H3K36me3 ratio values lower than the mean value of 0.0065 (mPFS < vs ≥ mean: 5.6 vs 3.1 months; p < 0.05; mOS < vs ≥ mean: 12.5 vs 5.8 months; p = 0.05), confirming the negative prognostic role of this marker. The same analyses were performed at PD, where the prognostic role of H3K27me3/H3K36me3 ratio was confirmed for OS with respect to its median value of 0.005 (mOS < vs ≥ median: 12.3 vs 4.7 months; p = 0.05). Because of the numerosity difference of the two groups (22 vs 2), no survival analyses were performed at PD with respect to H3K27me3/H3K36me3 ratio mean value. Receiver operating characteristic curves were conducted to confirm the prognostic ability of different H3K27me3/H3K36me3 ratio cut-offs, and stratification of mean H3K27me3/H3K36me3 ratio at BR was confirmed (area under the curve = 0.76; p = 0.04). Kaplan–Meier curves for statistically significant survival results are shown in Figure 3.

Discussion

The authors have identified the histone H3K27me3/H3K36me3 ratio as a potentially noninvasive predictive biomarker for HCC patients treated with sorafenib. In the nucleus of a cell, histones constitute the core of the nucleosome, which is the structural unit of eukaryotic chromatin [37]. Each histone has a long N-terminal tail, which can be modified by acetylation, methylation and other HPTMs. These modifications are catalyzed by epigenetic enzymes, which control gene expression. Many epigenetic enzymes are deregulated in cancer [38]. The authors' bioinformatics analyses show that the epigenetic enzymes catalyzing both H3K27me3 (EZH2) and H3K36me3 (SETD2) are significantly upregulated in HCC versus non-neoplastic tissues. Previous studies have indicated that *EZH2* is a driver of sorafenib resistance in HCC [14], whereas *SETD2* seems to antagonize the biological effects of *EZH2* [13]. In line with this model, the authors have found that *EZH2* and *SETD2* intratumoral expression levels have opposite prognostic significance in HCC patients exposed to sorafenib. Interestingly, the *EZH2/SETD2* ratio predicts survival outcomes more accurately than either gene alone. Consistent with the authors' observations, such an antagonistic relationship between these two epigenetic regulators also held true for a prostate cancer mouse model, in which *SETD2* downregulation resulted in increased levels of *EZH2* and tumor metastatic potential via a polycomb-repressed chromatin state [39].

Nucleosomes are released in the circulation as a consequence of necrosis and apoptosis [40]. Cancerous tissues release nucleosomes at higher rates as a result of increased cell turnover [41]. It has therefore been proposed that circulating nucleosomes and histone modifications could be used as diagnostic, predictive and prognostic biomarkers. Therefore, the authors decided to examine the possibility that H3K27me3 and H3K36me3 plasma ratio levels are readout of *EZH2* and *SETD2* activity in HCC. In line with this hypothesis, the authors observed that circulating levels of both H3K27me3 and H3K36me3 were significantly decreased in patients with reduced tumor mass (best response). More interestingly, the H3K27me3/H3K36me3 ratio at best response predicted patient survival and was significantly increased at tumor progression. These observations are in line with the authors' original biological hypothesis; namely, that *EZH2* drives sorafenib resistance, whereas *SETD2* plays an opposite role.
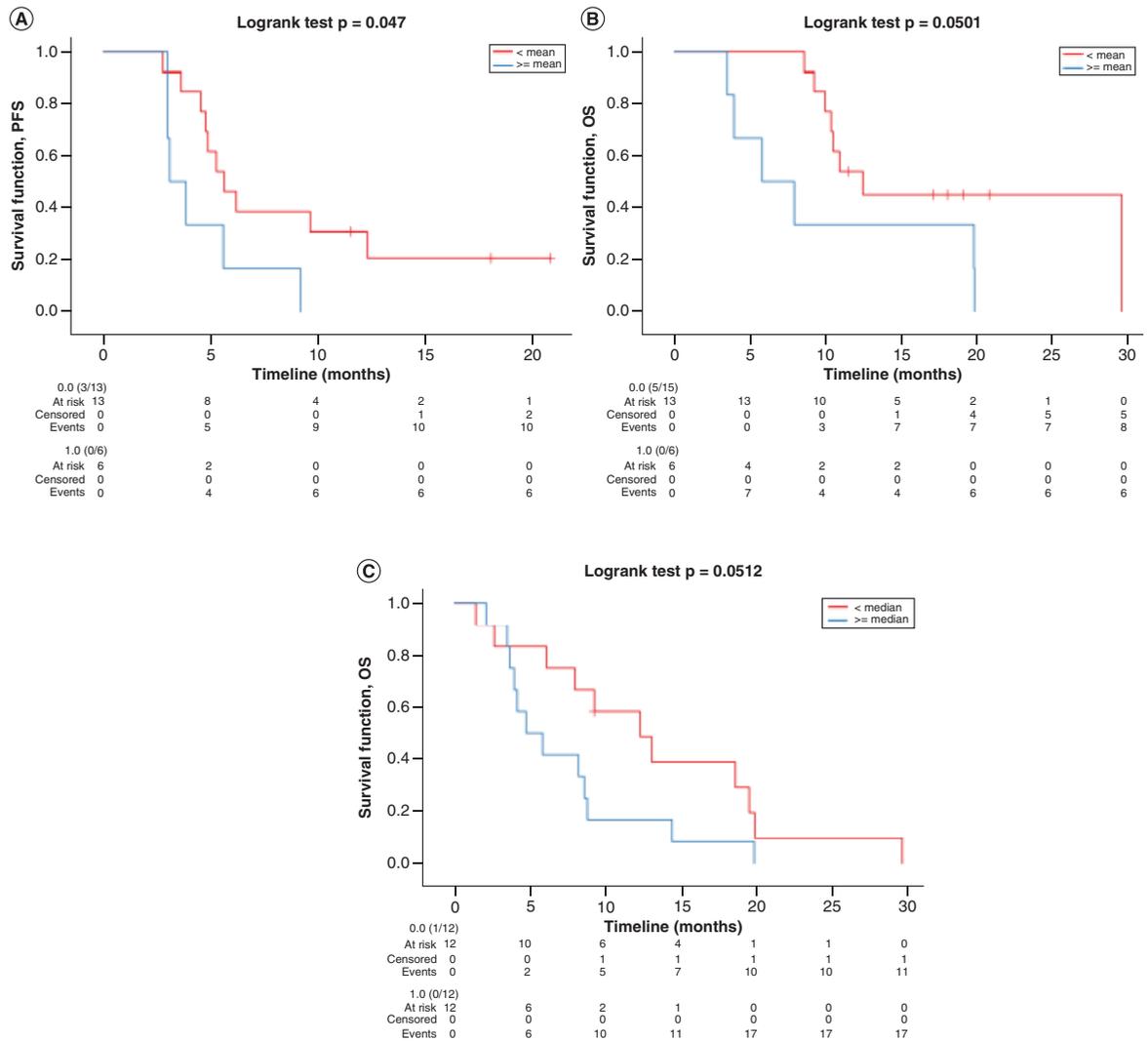
Figure 3. Kaplan–Meier curves for progression-free and overall survival according to H3K27me3/H3K36me3 ratio values at best response and progressive disease. Kaplan–Meier curves for **(A)** PFS according to H3K27me3/H3K36me3 ratio mean values at BR, **(B)** OS according to H3K27me3/H3K36me3 ratio mean values at BR and **(C)** OS according to H3K27me3/H3K36me3 ratio median values at PD. Data for all curves compared using log rank test.
BR: Best response; OS: Overall survival; PD: Progressive disease; PFS: Progression-free survival.

This study has some limitations. First, confirmation of the authors' results should be obtained in larger cohorts, particularly with regard to longitudinal analyses of serial samples collected from the same patient during treatment. Second, subsequent studies should clarify whether the H3K27me3/H3K36me3 ratio also predicts benefit from salvage tyrosine kinase inhibitors (e.g., regorafenib or cabozantinib). Finally, it would be of interest to explore circulating H3K27me3/H3K36me3 in patients receiving alternative first-line therapies (e.g., lenvatinib and, primarily, atezolizumab plus bevacizumab). The authors have previously predicted that *EZH2* activity could be associated with response to immune checkpoint inhibitors [42].

The authors' study also has some strengths. The OS observed reflects that reported in clinical trials. This means that second-line drugs had limited impact, so the correlation between putative biomarkers and outcome is not confounded by the effect of additional therapies. Moreover, serial sampling from single patients allows for monitoring of the biomarker during treatment, thus strengthening the association with drug efficacy. Finally, measuring circulating HPTMs in peripheral blood offers the advantage of a noninvasive technique that is able to anticipate the course of the disease even when tumor tissue is lacking, as often occurs in HCC.

Conclusion

To our knowledge, we have reported the first clinical observation of the predictive role of circulating levels of H3K27me3, H3K36me3 and H3K27me3/H3K36me3 ratio in advanced HCC patients treated with sorafenib, shedding light on the value of these biomarkers in monitoring systemic treatment and pointing out the opportunity to prospectively explore such soluble epigenetic biomarkers in future therapeutic strategies.

Future perspective

The study of epigenetics for oncological applications is becoming more and more promising in oncology. Within this framework, our results suggest that the epigenetic regulators *EZH2* and *SETD2* play a role in HCC prognosis and that their corresponding readouts, H3K27me3, H3K36me3 and H3K27me3/H3K36me3 ratio, act as predictive factors for first-line sorafenib treatment outcomes. Since the identification of predictive factors for treatment outcomes represents the most promising strategy for treatment personalization, we plan to test the predictive value of H3K27me3/H3K36me3 ratio with regard to different therapeutic modalities for HCC, such as other first- and further-line tyrosine kinase inhibitors (e.g., lenvatinib and regorafenib) and the new standard of care – combination atezolizumab/bevacizumab.

Summary points

- Treatments for advanced hepatocellular carcinoma lack predictive biomarkers.
- Epigenetics modifications, underpinning cancer plasticity, may provide biomarkers for hepatocellular carcinoma treatments.
- Both *EZH2* and *SETD2* are significantly upregulated in hepatocellular carcinoma versus normal tissue.
- *EZH2*/*SETD2* is a negative prognostic marker for sorafenib in bioinformatics data.
- *EZH2*/*SETD2* confirmed its role in the authors' experimental setting.

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Papers of special note have been highlighted as: ● of interest; ●● of considerable interest

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