**Table 1.** Correlation between H3K27 modification and clinicopathological features in patients with HCC who underwent hepatectomy.

| Clinical or pathological feature | Total N | H3K27 modification group | | | | P value |
|---|---|---|---|---|---|---|
| | | A | B | C | D | |
| All cases | 198 | 49 | 49 | 46 | 54 | |
| Age (years) | | | | | | 0.770 |
| ≤60 | 64 | 16 | 17 | 12 | 19 | |
| >60 | 134 | 33 | 32 | 34 | 35 | |
| Sex | | | | | | 0.831 |
| Male | 152 | 40 | 37 | 34 | 41 | |
| Female | 46 | 9 | 12 | 12 | 13 | |
| HBs Ag | | | | | | 0.913 |
| Positive | 45 | 11 | 11 | 9 | 14 | |
| Negative | 153 | 38 | 38 | 37 | 40 | |
| HCV Ab | | | | | | 0.435 |
| Positive | 132 | 31 | 37 | 31 | 33 | |
| Negative | 66 | 18 | 12 | 15 | 21 | |
| Cirrhosis | | | | | | 0.441 |
| Yes | 113 | 28 | 28 | 22 | 35 | |
| No | 85 | 21 | 21 | 24 | 19 | |
| Pathological stage | | | | | | 0.185 |
| Stage 1 | 18 | 5 | 6 | 5 | 2 | |
| Stage 2 | 86 | 24 | 25 | 16 | 21 | |
| Stage 3 | 74 | 14 | 14 | 18 | 28 | |
| Stage 4a | 20 | 6 | 4 | 7 | 3 | |
| Maximum tumor size (mm) | | | | | | 0.356 |
| ≤50 | 144 | 40 | 33 | 34 | 37 | |
| >50 | 54 | 9 | 16 | 12 | 17 | |
| Histological grade | | | | | | 0.003* |
| well | 52 | 19 | 14 | 13 | 6 | |
| mod | 115 | 27 | 30 | 27 | 31 | |
| por | 31 | 3 | 5 | 6 | 17 | |
| No. of lesions | | | | | | 0.603 |
| 1 | 130 | 30 | 35 | 32 | 33 | |
| ≥2 | 68 | 19 | 14 | 14 | 21 | |
| Vascular invasion | | | | | | 0.051 |
| Yes | 64 | 12 | 11 | 21 | 20 | |
| No | 134 | 37 | 38 | 25 | 34 | |
| Serum AFP level (ng/mL) | | | | | | 0.104 |
| ≤200 | 136 | 39 | 36 | 29 | 32 | |
| >200 | 62 | 10 | 13 | 17 | 22 | |
| Serum DCP level (mAU/mL) | | | | | | 0.082 |
| ≤100 | 111 | 35 | 25 | 22 | 29 | |
| >100 | 87 | 14 | 24 | 24 | 25 | |

AFP: alpha-fetoprotein DCP: des-gamma-carboxy prothrombin.
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ment was used [27]. Multivariate Cox proportional hazards regression models were used to control for confounding variables. The multivariate regression analysis was performed using the significant clinicopathological variables identified in the univariate analysis. The variable "pathological stage" was excluded in multivariate analysis because pathological stage was determined by other pathological variables, such tumor size and number and vascular invasion [19]. The time-dependent ROC analysis was performed to evaluate the discriminatory ability for the prediction of survival using R software (version 3.0.2, http://www.r-project.org) and its specific package as described previously [28,29]. The 5-year overall and recurrence-free models were used to perform

**Table 2.** Correlation between H3K27 modification and nuclear accumulation of p53 and β-catenin in patients with HCC who underwent hepatectomy.

| Molecular feature | Total N | H3K27 modification group | | | | P value |
|---|---|---|---|---|---|---|
| | | A | B | C | D | |
| All cases | 198 | 49 | 49 | 46 | 54 | |
| p53 | | | | | | 0.032* |
| Positive | 50 | 9 | 10 | 9 | 22 | |
| Negative | 148 | 40 | 39 | 37 | 32 | |
| β-catenin | | | | | | 0.312 |
| Positive | 43 | 11 | 7 | 9 | 16 | |
| Negative | 155 | 38 | 42 | 37 | 38 | |

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this analysis and the area under the curve (AUC) was calculated for measuring the prediction accuracy [30].

## Results

### Immunohistochemical analysis of H3K27ac and H3K27me3 in HCC

Immunohistochemical analyses of H3K27ac and H3K27me3 revealed positive staining of both in nuclei of HCC (**Figure 1a and 1b**). Approximately 40% of cases showed over 80% of cells containing vivid positive staining (IHC marker intensity 2–3, H-score >200), and 40% of cases showed various nuclear staining levels (IHC marker intensity 1–3, H-score 100–200). In the remaining 20% of cases, less than 50% cells exhibited weak or moderate positive staining (IHC marker intensity 1–2, H-score<100). As for the background liver, in over 80% of cases, few cells exhibited weak or moderate positive staining (IHC marker intensity 1–2, H-score<50). In total, the H-score of H3K27ac was 156.7±86.8 (mean±standard deviation [SD], range: 9–293) in HCC and 40.3±33.0 (range: 3–159) in background liver. The H-score of H3K27me3 was 151.8±78.1 in HCC (range: 9–296) and 64.7±45.6 (range: 8–211) in

background liver. Mann–Whitney test showed that H-scores of H3K27ac and H3K27me3 were higher in HCC than those in background livers (P<0.001) (**Figure 1c**). Western blotting analysis of frozen samples confirmed that H3K27ac and H3K27me3 levels were elevated in HCC (**Figure 1d**).

### Distributions of H3K27ac and H3K27me3 in HCC

Distributions of H3K27ac and H3K27me3 H-scores are shown in **Figure 2**. There was no significant correlation between these two modifications (r = 0.165). Cases were divided into two groups based on the H-scores, as in the previous study on H3K27 modification [24]: the low H-score group (H-score ≤150) and the high H-score group (H-score >150).

### H3K27 modification and correlation with clinicopathological characteristics

Based on the two-category classification (low/high H-score groups), the high H3K27me3 group correlated with poor differentiation, vascular invasion and serum alpha-fetoprotein (AFP) level, and the high H3K27ac group also had association with poor tumor differentiation (**Table S1**). To investigate the significance of concurrent activation of both histone modifications at the same residue of H3 in more detail, the cases were re-classified into four groups (Group A–D). Group A included cases with low H-scores for both H3K27ac and H3K27me3 (low-H3K27ac/low-H3K27me3 group), Group B included high-H3K27ac/low-H3K27me3 cases, Group C included low-H3K27ac/high-H3K27me3 cases, and Group D included high-H3K27ac/high-H3K27me3 cases. Representative photomicrographs of H3K27ac and H3K27me3 immunohistochemistry of Group A and Group D cases are shown in **Figure 3**.

The correlation of clinicopathological variables with the four groups (Group A–D) revealed that Group D (high-H3K27ac/high-H3K27me3 group) had a strong association with poor differentiation (**Table 1**). Group D also showed significant correlation with p53-positive staining (**Table 2, Figure S1**). The levels of β-catenin staining were not different among the four groups.

The median follow-up period was 45.0 months (range 2.3–196.2 months). The overall cumulative survival rate at five years was 40.4% and the recurrence free cumulative survival rate at five
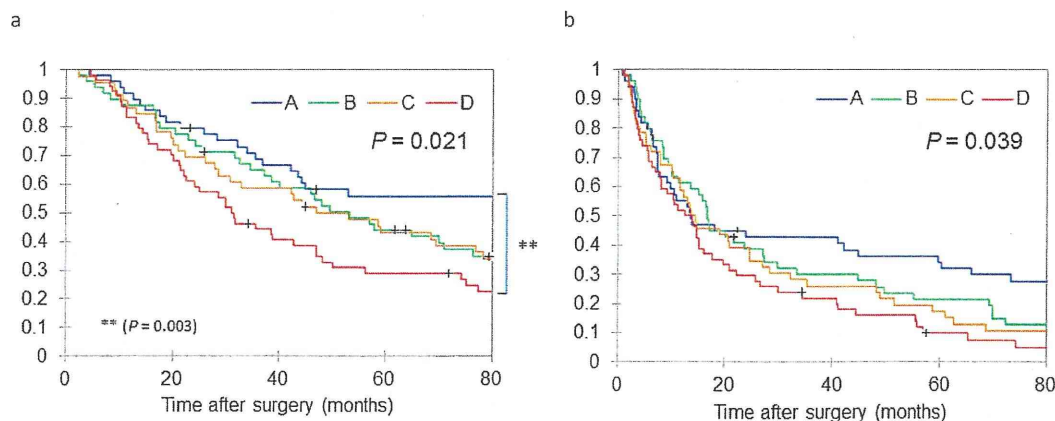
a



b



**Figure 4. Kaplan–Meier analysis of (a) overall survival and (b) recurrence-free survival after resection according to histone H3K27 modification in HCC.** Log-rank analysis showed significant differences in overall survival among the four groups (A–D) in both overall (P = 0.021) and recurrence free (P = 0.039) survival. Censored data were plotted as "+" in the Kaplan-Meier curve.
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**Table 3.** H3K27 modification in HCC and patient outcome (univariate analysis).

| | Overall survival | | | | Recurrence free survival | | | |
|---|---|---|---|---|---|---|---|---|
| | HR | 95% CI | | P-value | HR | 95% CI | | P-value |
| Age >60 years (vs ≤60) | 1.378 | 0.955 | − 1.988 | 0.087 | 1.247 | 0.902 | − 1.724 | 0.182 |
| Sex female (vs male) | 1.255 | 0.863 | − 1.827 | 0.235 | 1.034 | 0.729 | − 1.468 | 0.851 |
| HBs Ag positive (vs negative) | 0.951 | 0.632 | − 1.432 | 0.811 | 1.065 | 0.741 | − 1.533 | 0.733 |
| HCV Ab positive (vs negative) | 1.204 | 0.840 | − 1.727 | 0.312 | 1.233 | 0.893 | − 1.704 | 0.204 |
| Cirrhosis positive (vs negative) | 1.804 | 1.275 | − 2.554 | <0.001* | 1.529 | 1.127 | − 2.075 | 0.006* |
| Pathological stage | | | | <0.001* | | | | <0.001* |
| Stage 2 (vs Stage 1) | 1.948 | 0.885 | − 4.289 | 0.098 | 1.334 | 0.750 | − 2.374 | 0.327 |
| Stage 3 (vs Stage 1) | 4.683 | 2.131 | − 10.29 | <0.001* | 3.281 | 1.838 | − 5.857 | <0.001* |
| Stage 4 (vs Stage 1) | 6.286 | 2.644 | − 14.94 | <0.001* | 3.543 | 1.778 | − 7.061 | <0.001* |
| Maximum tumor size >50 mm (vs ≤50) | 1.517 | 1.060 | − 2.173 | 0.023* | 1.691 | 1.217 | − 2.349 | 0.002* |
| Histological grade | | | | 0.011* | | | | 0.011* |
| mod (vs well) | 1.149 | 0.769 | − 1.716 | 0.497 | 1.146 | 0.803 | − 1.635 | 0.453 |
| por (vs well) | 2.186 | 1.309 | − 3.649 | 0.004* | 2.009 | 1.261 | − 3.200 | 0.003* |
| No. of Tumor ≥2 (vs 1) | 2.219 | 1.580 | − 3.114 | <0.001* | 1.985 | 1.451 | − 2.717 | <0.001* |
| Vascular invasion positive (vs negative) | 2.592 | 1.843 | − 3.644 | <0.001* | 2.587 | 1.887 | − 3.548 | <0.001* |
| Serum AFP level >200 ng/mL (vs ≤200) | 1.697 | 1.198 | − 2.380 | 0.003* | 1.845 | 1.301 | − 2.591 | <0.001* |
| Serum DCP level >100 mAU/mL (vs ≤100) | 1.140 | 0.817 | − 1.586 | 0.438 | 1.206 | 0.863 | − 1.678 | 0.270 |
| p53 positive (vs negative) | 1.516 | 1.053 | − 2.183 | 0.025* | 1.502 | 1.072 | − 2.104 | 0.018* |
| β-catenin positive (vs negative) | 1.343 | 0.909 | − 1.986 | 0.139 | 1.345 | 0.942 | − 1.920 | 0.103 |
| H3K27 modification | | | | 0.023* | | | | 0.040* |
| Group B (vs Group A) | 1.347 | 0.820 | − 2.213 | 0.239 | 1.275 | 0.822 | − 1.980 | 0.278 |
| Group C (vs Group A) | 1.519 | 0.924 | − 2.499 | 0.099 | 1.537 | 0.986 | − 2.395 | 0.058 |
| Group D (vs Group A) | 2.054 | 1.285 | − 3.284 | 0.003* | 1.823 | 1.186 | − 2.802 | 0.006* |

CI, confidence interval; HR, hazard ratio.

years was 19.2%. Kaplan–Meier analysis revealed significant difference of overall and recurrence-free survival in the four H3K27 modification groups in HCC (log-rank $P = 0.021$ and $P = 0.039$, respectively) (**Figure 4**). Group D showed significantly poorer prognosis in overall survival than Group A ($P = 0.003$) even after the Bonferroni correction for multi-group comparison

**Table 4.** H3K27 modification in HCC and patient outcome (multivariate analysis).

| | Overall survival | | | | Recurrence free survival | | | |
|---|---|---|---|---|---|---|---|---|
| | HR | 95% CI | | P-value | HR | 95% CI | | P-value |
| Cirrhosis positive (vs negative) | 1.903 | 1.322 | − | 2.775 | <0.001* | 1.828 | 1.316 | − | 2.561 | <0.001* |
| Maximum tumor size >50 mm (vs 50≥) | 1.239 | 0.799 | − | 1.895 | 0.334 | 1.488 | 0.998 | − | 2.189 | 0.051 |
| Histological grade | | | | | 0.095 | | | | | 0.111 |
| mod (vs well) | 0.769 | 0.491 | − | 1.218 | 0.259 | 0.808 | 0.545 | − | 1.211 | 0.299 |
| por (vs well) | 1.261 | 0.674 | − | 2.337 | 0.466 | 1.279 | 0.735 | − | 2.204 | 0.381 |
| No. of Tumor ≥2 (vs 1) | 2.095 | 1.440 | − | 3.040 | <0.001* | 1.763 | 1.250 | − | 2.474 | 0.001* |
| Vascular invasion positive (vs negative) | 2.130 | 1.416 | − | 3.186 | < 0.001* | 2.212 | 1.502 | − | 3.235 | <0.001* |
| Serum AFP level >200 ng/mL (vs ≤200) | 1.184 | 0.776 | − | 1.788 | 0.429 | 1.038 | 0.697 | − | 1.527 | 0.851 |
| p53 positive (vs negative) | 1.072 | 0.703 | − | 1.606 | 0.743 | 1.206 | 0.822 | − | 1.742 | 0.333 |
| H3K27 modification | | | | | 0.171 | | | | | 0.686 |
| Group B (vs Group A) | 1.492 | 0.898 | − | 2.494 | 0.122 | 1.270 | 0.809 | − | 2.000 | 0.299 |
| Group C (vs Group A) | 1.546 | 0.907 | − | 2.652 | 0.101 | 1.253 | 0.783 | − | 2.010 | 0.347 |
| Group D (vs Group A) | 1.730 | 1.037 | − | 2.925 | 0.036* | 1.275 | 0.790 | − | 2.073 | 0.321 |

CI, confidence interval; HR, hazard ratio.
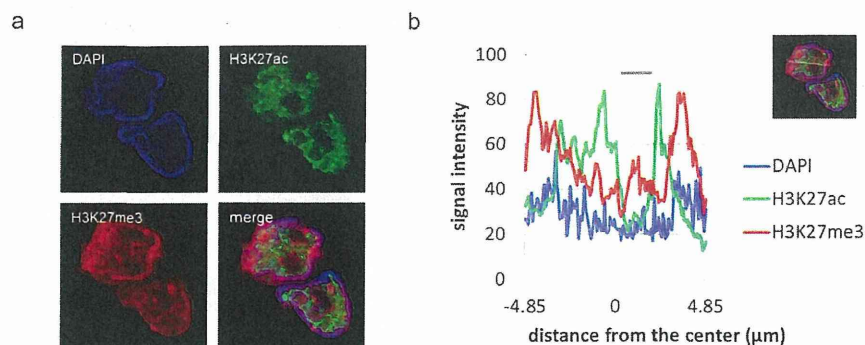doi:10.1371/journal.pone.0091330.t004

**Figure 5. Double immunofluorescent staining of H3K27ac and H3K27me3 in HCC.** (a) Images of double immunofluorescent staining of H3K27ac and H3K27me3 in frozen samples. (b) Distribution of immunofluorescent signal intensity in nucleus. Image analysis was performed of the dashed line shown in the merged image using LAS (Leica Microsystems, Mannheim, Germany). Nucleolus regions are shown by the gray bar.
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(**Table S2**). The univariate Cox regression analysis also showed that Group D had significantly poorer prognosis than Group A both in overall survival and in recurrence free survival (**Table 3**). In addition, the multivariate Cox regression analysis revealed that Group D had significant poorer prognosis than Group A in overall survival (**Table 4**).

The time-dependent ROC analysis showed that the H3K27 modification can predict 5-year recurrence-free survival (AUC = 0.662) especially in early-stage HCC (stage 1, 2) (AUC = 0.712) (**Figure S2**). The AUC of H3K27 modification is higher than other prognostic indicators or markers previously reported [31], such as AFP (AUC = 0.615 and AUC = 0.583, in all stage and in early-stage HCC, respectively). In the 5-year overall survival model, AUCs of H3K27 modification were nearly the same as those of other predictors.

## Immunofluorescent analysis of H3K27ac and H3K27me3 in HCC

The images of double immunofluorescence staining of H3K27ac and H3K27me3 in frozen sections are shown in **Figure 5a**. These two modifications were detected as mutually exclusive dot-like regions in the nucleus. H3K27ac tended to localize in the central euchromatin region of nuclei, whereas H3K27me3 was observed in the peripheral heterochromatin zone. Different immunofluorescent intensity peaks between H3K27ac and H3K27me3 were confirmed by quantitative intensity analysis using image analyzing software (**Figure 5b**).

## Discussion

This is the first study that evaluated the clinicopathological significance of concurrent increase of H3K27ac and H3K27me3 in HCC. Although both modifications appeared to occur independently, concurrent activation of acetylation and methylation at the same residue, H3K27, was observed in a subset of HCC with poorly-differentiated morphology and p53 abnormality, and the patients with this subset of HCC showed poor prognosis compared with others. These results could not be obtained without the precise quantification of immunohistochemical results in the present study. We confirmed that the system is feasible and reproducible [16]. The obtained data represented very well subtle differences among the cases, as illustrated in the scatter diagram (**Figure 2**).

Additionally, both modifications showed different nuclear localization patterns in individual cancer cells; H3K27ac was localized in the central euchromatin regions, while H3K27me3 was observed in the peripheral heterochromatin regions [32]. H3K27ac acts as an active enhancer, while H3K27me3 acts as a silencer [15]. Thus, the segregated localization might reflect compartmentalization of oncogenes related to proliferation or invasion and tumor suppressor genes related to apoptosis or differentiation within the nucleus [33,34].

H3K27ac modification is induced by p300/CBP and removed by HDACs, while H3K27me3 is induced by EZH2 and removed by JMJD3 [6]. Previous studies revealed the clinicopathological significance of expression of these modification enzymes. High expression of p300 in HCC correlated with poor differentiation and poor prognosis and high expression of EZH2 in HCC correlated with poor differentiation [35–38]. As for H3K27ac, high expressions of some HDACs in HCC also correlated with de-differentiation and worse survival [39,40]. These data are consistent with our results, but further studies are necessary to evaluate which targets are better for the patient stratification, either the modifying enzymes or the histone modifications.

The HCC subgroup with concurrently high H-scores of H3K27ac and H3K27me3 showed significant correlation with positive staining of p53. Positive p53 staining has been noted to correlate with p53 mutation in HCC [41]. The correlation of p53 abnormality with p300 and EZH2 has been recognized in several cancers. Nuclear accumulation of p53 was associated with p300 expression in breast cancer [42] and with EZH2 expression in squamous cell carcinoma of esophagus [43]. In an *in vitro* study, overexpression of mutant p53 upregulated EZH2 expression [44]. Di Agostino et al. demonstrated that mutant p53 is able to recruit p300 and cause histone acetylation in some promoter regions [45]. Therefore, some of the p53 abnormalities might cause concurrent increase of H3K27ac and H3K27me3 levels through mechanisms that have not been investigated yet.

In summary, concurrent activation of acetylation and methylation on H3K27 occurs in the subgroup of HCC, showing p53-positive poorly differentiated type. Further studies are necessary to evaluate the possible association between p53 and the concurrent increase of histone modification. Our findings demonstrate that image analyzer-assisted H-scores of H3K27ac and H3K27me3 identified an aggressive subgroup of HCC, and could serve as a prognostic marker for HCC. Additional ROC analysis supports

the possibility that the H3K27 modification might predict more accurately in HCC than other prognostic indicators or markers.

## Supporting Information

**Figure S1 Representative photomicrographs of p53 and β-catenin immunohistochemistry.**
(TIF)

**Figure S2 Time-dependent ROC analysis using (a) 5-year overall survival and (b) 5-year recurrence-free survival models in HCC.** Prognostic clinical and pathological valuables, tumor markers and H3K27 modification were entered; cirrhosis (positive or negative), tumor size (mm), tumor number (number), histological grade (well, moderate or poor), vascular invasion (positive or negative), AFP (ng/mL), DCP (mAU/mL), p53 (positive or negative), β-catenin (positive or negative) and H3K27 (group A, B, C or D). 95% confidence intervals were shown after AUC values.
(TIF)

**Table S1 Correlation between H3K27ac and H3K27me3 with clinicopathological features in patients with HCC who underwent hepatectomy.**
(DOC)

**Table S2 Multiple comparison in H3K27 modification groups using the log-rank test.**
(DOC)

## Author Contributions

Conceived and designed the experiments: AH NY SI MF. Performed the experiments: AH. Analyzed the data: AH JS TM AN. Contributed reagents/materials/analysis tools: HK JS SI GN HA NK YS. Wrote the paper: AH MF.

## References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. (2011) Global cancer statistics. CA Cancer J Clin 61: 69–90.
2. Research F/PoC (2011) Cancer Statistics in Japan 2011.
3. Takayasu K, Arii S, Ikai I, Omata M, Okita K, et al. (2006) Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. Gastroenterology 131: 461–469.
4. European Association For The Study Of The L, European Organisation For R, Treatment Of C (2012) EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 56: 908–943.
5. Garraway LA, Lander ES (2013) Lessons from the cancer genome. Cell 153: 17–37.
6. Shen H, Laird PW (2013) Interplay between the cancer genome and epigenome. Cell 153: 38–55.
7. Greer EL, Shi Y (2012) Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet 13: 343–357.
8. Selvi BR, Mohankrishna DV, Ostwal YB, Kundu TK (2010) Small molecule modulators of histone acetylation and methylation: a disease perspective. Biochim Biophys Acta 1799: 810–828.
9. Seligson DB, Horvath S, McBrian MA, Mah V, Yu H, et al. (2009) Global levels of histone modifications predict prognosis in different cancers. Am J Pathol 174: 1619–1628.
10. Chervona Y, Costa M (2012) Histone modifications and cancer: biomarkers of prognosis? Am J Cancer Res 2: 589–597.
11. Cai MY, Hou JH, Rao HL, Luo RZ, Li M, et al. (2011) High expression of H3K27me3 in human hepatocellular carcinomas correlates closely with vascular invasion and predicts worse prognosis in patients. Mol Med 17: 12–20.
12. He C, Xu J, Zhang J, Xie D, Ye H, et al. (2012) High expression of trimethylated histone H3 lysine 4 is associated with poor prognosis in hepatocellular carcinoma. Hum Pathol 43: 1425–1435.
13. Bai X, Wu L, Liang T, Liu Z, Li J, et al. (2008) Overexpression of myocyte enhancer factor 2 and histone hyperacetylation in hepatocellular carcinoma. J Cancer Res Clin Oncol 134: 83–91.
14. Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, et al. (2009) Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature 459: 108–112.
15. Ong CT, Corces VG (2011) Enhancer function: new insights into the regulation of tissue-specific gene expression. Nat Rev Genet 12: 283–293.
16. Braun M, Kirsten R, Rupp NJ, Moch H, Fend F, et al. (2013) Quantification of protein expression in cells and cellular subcompartments on immunohistochemical sections using a computer supported image analysis system. Histol Histopathol 28: 605–610.
17. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, et al. (2012) Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 44: 694–698.
18. Makuuchi M, Kosuge T, Takayama T, Yamazaki S, Kakazu T, et al. (1993) Surgery for small liver cancers. Semin Surg Oncol 9: 298–304.
19. Japan LCSGo (2003) General Rules for the Clinical and Pathological Study of Primary Liver Cancer, 2nd English edition. Tokyo: Kanehara.
20. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, et al. (1998) Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 4: 844–847.
21. Kimura H, Hayashi-Takanaka Y, Goto Y, Takizawa N, Nozaki N (2008) The organization of histone H3 modifications as revealed by a panel of specific monoclonal antibodies. Cell Struct Funct 33: 61–73.
22. Van Den Broeck A, Brambilla E, Moro-Sibilot D, Lantuejoul S, Brambilla C, et al. (2008) Loss of histone H4K20 trimethylation occurs in preneoplasia and influences prognosis of non-small cell lung cancer. Clin Cancer Res 14: 7237–7245.
23. Song L, Robson T, Doig T, Brenn T, Mathers M, et al. (2013) DNA repair and replication proteins as prognostic markers in melanoma. Histopathology 62: 343–350.
24. Tamagawa H, Oshima T, Numata M, Yamamoto N, Shiozawa M, et al. (2013) Global histone modification of H3K27 correlates with the outcomes in patients with metachronous liver metastasis of colorectal cancer. Eur J Surg Oncol 39: 655–661.
25. Torbenson M, Kannangai R, Abraham S, Sahin F, Choti M, et al. (2004) Concurrent evaluation of p53, beta-catenin, and alpha-fetoprotein expression in human hepatocellular carcinoma. Am J Clin Pathol 122: 377–382.
26. Salichs E, Ledda A, Mularoni L, Alba MM, de la Luna S (2009) Genome-wide analysis of histidine repeats reveals their role in the localization of human proteins to the nuclear speckles compartment. PLoS Genet 5: e1000397.
27. Bland JM, Altman DG (1995) Multiple significance tests: the Bonferroni method. BMJ 310: 170.
28. Blanche P, Dartigues JF, Jacqmin-Gadda H (2013) Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks. Stat Med 32: 5381–5397.
29. Heagerty PJ, Lumley T, Pepe MS (2000) Time-dependent ROC curves for censored survival data and a diagnostic marker. Biometrics 56: 337–344.
30. Ho WH, Lee KT, Chen HY, Ho TW, Chiu HC (2012) Disease-free survival after hepatic resection in hepatocellular carcinoma patients: a prediction approach using artificial neural network. PLoS One 7: e29179.
31. Tandon P, Garcia-Tsao G (2009) Prognostic indicators in hepatocellular carcinoma: a systematic review of 72 studies. Liver Int 29: 502–510.
32. Littau VC, Allfrey VG, Frenster JH, Mirsky AE (1964) Active and Inactive Regions of Nuclear Chromatin as Revealed by Electron Microscope Autoradiography. Proc Natl Acad Sci U S A 52: 93–100.
33. Chen X, Cheung ST, So S, Fan ST, Barry C, et al. (2002) Gene expression patterns in human liver cancers. Mol Biol Cell 13: 1929–1939.
34. Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, et al. (2009) Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Res 69: 7385–7392.
35. Yokomizo C, Yamaguchi K, Itoh Y, Nishimura T, Umemura A, et al. (2011) High expression of p300 in HCC predicts shortened overall survival in association with enhanced epithelial mesenchymal transition of HCC cells. Cancer Lett 310: 140–147.
36. Li M, Luo RZ, Chen JW, Cao Y, Lu JB, et al. (2011) High expression of transcriptional coactivator p300 correlates with aggressive features and poor prognosis of hepatocellular carcinoma. J Transl Med 9: 5.
37. Sudo T, Utsunomiya T, Mimori K, Nagahara H, Ogawa K, et al. (2005) Clinicopathological significance of EZH2 mRNA expression in patients with hepatocellular carcinoma. Br J Cancer 92: 1754–1758.
38. Sasaki M, Ikeda H, Itatsu K, Yamaguchi J, Sawada S, et al. (2008) The overexpression of polycomb group proteins Bmi1 and EZH2 is associated with the progression and aggressive biological behavior of hepatocellular carcinoma. Lab Invest 88: 873–882.
39. Quint K, Agaimy A, Di Fazio P, Montalbano R, Steindorf C, et al. (2011) Clinical significance of histone deacetylases 1, 2, 3, and 7: HDAC2 is an independent predictor of survival in HCC. Virchows Arch 459: 129–139.

40. Rikimaru T, Taketomi A, Yamashita Y, Shirabe K, Hamatsu T, et al. (2007) Clinical significance of histone deacetylase 1 expression in patients with hepatocellular carcinoma. Oncology 72: 69–74.

41. Hsu HC, Tseng HJ, Lai PL, Lee PH, Peng SY (1993) Expression of p53 gene in 184 unifocal hepatocellular carcinomas: association with tumor growth and invasiveness. Cancer Res 53: 4691–4694.

42. Vleugel MM, Shvarts D, van der Wall E, van Diest PJ (2006) p300 and p53 levels determine activation of HIF-1 downstream targets in invasive breast cancer. Hum Pathol 37: 1085–1092.

43. Yamada A, Fujii S, Daiko H, Nishimura M, Chiba T, et al. (2011) Aberrant expression of EZH2 is associated with a poor outcome and P53 alteration in squamous cell carcinoma of the esophagus. Int J Oncol 38: 345–353.

44. Tang X, Milyavsky M, Shats I, Erez N, Goldfinger N, et al. (2004) Activated p53 suppresses the histone methyltransferase EZH2 gene. Oncogene 23: 5759–5769.

45. Di Agostino S, Strano S, Emiliozzi V, Zerbini V, Mottolese M, et al. (2006) Gain of function of mutant p53: the mutant p53/NF-Y protein complex reveals an aberrant transcriptional mechanism of cell cycle regulation. Cancer Cell 10: 191–202.

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