**Table S1. Association between SMYD3, ANKHD1 expression and patient's clinicopathologic features in HCCs**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Clinicopathological Variables | Case Number(n=243) | SMYD3 expression | *P* Value | ANKHD1 expression | *P* Value |
| negative（n=55） | positive（n=188） | negative（n=93） | positive（n=150） |
| Age |  |  | 51.27±11.5 | 50.8±12.3 | 0.80 | 52.05±11.9 | 50.19±12.2 | 0.244 |
| Sex | Female | 21 | 7 | 14 | 0.273 | 8 | 13 | 0.986 |
| male | 222 | 48 | 174 | 85 | 137 |
| Serum AFP | ≤400 ng/mL | 148 | 37 | 111 | 0.346 | 64 | 84 | 0.058 |
| >400 ng/mL | 95 | 18 | 77 | 29 | 66 |
| HBsAg | Negative | 24 | 17 | 7 | <0.001\* | 10 | 14 | 0.825 |
| Positive | 219 | 38 | 181 | 83 | 136 |
| Cirrhosis | Absent | 70 | 18 | 52 | 0.500 | 23 | 47 | 0.309 |
| Present | 173 | 37 | 136 | 70 | 103 |
| Tumor size | ≤5 cm | 104 | 29 | 75 | 0.121 | 65 | 39 | <0.001\* |
| >5 cm | 139 | 26 | 113 | 28 | 111 |
| Microvascular invasion | Absent | 92 | 29 | 63 | 0.012\* | 65 | 27 | <0.001\* |
| Present | 151 | 26 | 125 | 28 | 123 |
| Number of tumors | Single | 180 | 41 | 139 | 0.928 | 77 | 103 | 0.016\* |
| Multiple | 63 | 14 | 49 | 16 | 47 |
| Tumor differentiation | I-II | 142 | 44 | 98 | <0.001\* | 70 | 72 | <0.001\* |
| III-IV | 101 | 11 | 90 | 23 | 78 |
| TNM stage | I-II | 141 | 39 | 102 | 0.030\* | 73 | 68 | <0.001\* |
| III-IV | 102 | 16 | 86 | 20 | 82 |

Abbreviations: AFP, α-fetoprotein; HBsAg, hepatitis B surface antigen; TNM, tumor-lymph node-metastasis. \* *P*<0.05

**Table S2. Univerate and multivariate analysis of factors associated with survival and recurrence of 243 HCCs**

|  |  |  |
| --- | --- | --- |
| **Variables** | **Survival** | **Recurrence** |
| **Univariate Analysis** | **Multivariate Analysis** | **Univariate Analysis** | **Multivariate Analysis** |
|  | HR | 95% CI | *P* value | HR | 95% CI | P value | HR | 95% CI | P value | HR | 95% CI | *P* value |
| **Age** (≤50.0 vs. >50.0) | 0.933 | 0.653-1.334 | 0.704 |  |  |  | 0.784 | 0.575-1.069 | 0.124 |  |  |  |
| **Sex**(female vs. male) | 1.048 | 0.548-2.002 | 0.888 |  |  |  | 1.121 | 0.658-1.908 | 0.675 |  |  |  |
| **Serum AFP**(≤400 vs. >400ng/mL) | 1.653 | 1.154-2.366 | 0.006\* |  |  |  | 1.531 | 1.117-2.098 | 0.008\* |  |  |  |
| **HBsAg**(negative vs. positive) | 0.933 | 0.514-1.694 | 0.819 |  |  |  | 0.991 | 0.582-1.686 | 0.972 |  |  |  |
| **Cirrhosis**(absent vs. present) | 1.141 | 0.767-1.696 | 0.516 |  |  |  | 1.322 | 0.929-1.882 | 0.121 |  |  |  |
| **Tumor size**(≤5 vs. >5cm) | 2.585 | 1.757-3.804 | <0.001\* |  |  |  | 2.030 | 1.469-2.807 | <0.001\* |  |  |  |
| **Microvascular invasion**(absent vs. present) | 2.207 | 1.491-3.267 | <0.001\* |  |  |  | 1.727 | 1.244-2.396 | 0.001\* |  |  |  |
| **Number of tumors**(single vs. multiple) | 1.568 | 1.061-2.318 | 0.024\* |  |  |  | 1.511 | 1.077-2.120 | 0.017\* |  |  |  |
| **Tumor differentiation**(I-II vs. III-IV) | 1.940 | 1.355-2.776 | <0.001\* |  |  |  | 1.550 | 1.134-2.118 | 0.006\* |  |  |  |
| **TNM stage**(I-II vs. III-IV) | 3.581 | 2.484-5.162 | <0.001\* | 2.481 | 1.584-3.887 | <0.001\* | 3.216 | 2.339-4.422 | <0.001\* | 2.597 | 1.748-3.858 | <0.001\* |
| **SMYD3 expression****(** negative vs. positive) | 2.343 | 1.384-3.966 | 0.002\* | 1.987 | 1.159-3.405 | 0.012\* | 1.745 | 1.153-2.643 | 0.009\* | 1.617 | 1.054-2.480 | 0.028\* |

Abbreviations: AFP, α-fetoprotein; HBsAg, hepatitis B surface antigen; TNM, tumor-lymph node-metastasis. \* *P*<0.05

**Table S3. Mass spectrometry analysis of the proteins interacted with H3K4me3 when SMYD3 was overexpressed**

|  |  |  |
| --- | --- | --- |
| Number | Protein name | Full name |
| 1 | ANKHD1 | Ankyrin repeat and KH domain containing 1 |
| 2 | TRIP13 | Thyroid hormone receptor interactor 13 |
| 3 | RNF168 | Ring finger protein 168 |
| 4 | NLRC4 | NLR family CARD domain containing 4 |
| 5 | CDKL4 | Cyclin dependent kinase like 4  |
| 6 | NCOA5 | Nuclear receptor coactivator 5 |
| 9 | INO80 | INO80 complex subunit |
| 10 | Vimentin | Vimentin |
| 11 | OTX2 | Orthodenticle homeobox 2 |
| 12 | FCF1 | rRNA-processing protein |
| 14 | POLB | DNA polymerase beta |
| 15 | RRM1 | Ribonucleotide reductase catalytic subunit M1 |
| 16 | YTHDC1 | YTH domain containing 1 |
| 17 | MRE11A | MRE11 homolog, double strand break repair nuclease |

|  |
| --- |
|  |

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | SMYD3 | *P* value |
| Negative | Positive |
| Slug | HCC  | Negative | 7 | 6 | 0.030 |
| Positive | 1 | 11 |
| MVI and mPVTT | Negative | 6 | 4 | 0.007 |
| Positive | 1 | 14 |
| E-cadherin | HCC | NegativePositive | 26 | 143 | 0.010 |
| MVI and mPVTT | NegativePositive | 34 | 171 | 0.012 |

**Table S4. Association between SMYD3 and Slug expression in HCC, MVI and mPVTT**

|  |  |  |
| --- | --- | --- |
|  | SMYD3 | *P* value |
| Negative | Positive |
| ANKHD1 | Negative | 2629 | 67121 | 0.155 |
| Positive |

**Table S5. Association between SMYD3 and ANKHD1 expression in 243 HCCs**

**Table S6. Association between the expression of ANKHD1 and Slug in SMYD3 positive/ negative HCC.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **SMYD3 positive** | ANKHD1 | *P* value |
| Negative (n=67) | Positive (n=121) |
| Slug | Negative (n=88) | 42 | 46 | 0.001\* |
| Positive (n=100) | 25 | 75 |
|  | **SMYD3 negative** | ANKHD1 | *P* value |
| Negative (n=26) | Positive (n=29) |
| Slug | Negative (n=43) | 21 | 22 | 0.659 |
| Positive (n=12) | 5 | 7 |

\* *P*<0.05

**Table S7. siRNA/shRNA sequences used in the study.**

|  |  |
| --- | --- |
| Name | sequences |
| **siRNA:** |  |
| siSMYD3-1 | sense: | 5'-UCACAGCUGUGACCCCAACTT-3' |
|  | santisense: | 5'-GUUGGGGUCACAGCUGUGATT-3' |
| siSMYD3-2 | sense: | 5'-AGCCUGAUUGAAGAUUUGATT-3' |
|  | antisense: | 5'-UCAAAUCUUCAAUCAGGCUTT-3' |
| siANKDH1-1 | sense: | 5’-GGAGAAAGCCUGCUGUGUUTT-3’ |
|  | antisense: | 5’-AACACAGCAGGCUUUCUCCTT-3’ |
| siANKDH1-2 | sense: | 5’-GCAGGCAUCAACACUCAUUTT-3’ |
|  | antisense: | 5’-AAUGAGUGUUGAUGCCUGCTT-3’ |
| **shRNA:** |  |  |
| shSMYD3 | sense: | 5’-CCGGAGCCTGATTGAAGATTTGATTCTCGAGAATCAAATCTTCAATCAGGCTTTTTTG-3’ |
|  | antisense: | 5’-AATTCAAAAAAGCCTGATTGAAGATTTGATTCTCGAGAATCAAATCTTCAATCAGGCT-3’ |

**Table S8. Primer sequences used in the study.**

|  |  |
| --- | --- |
| Primer name | Primer sequences |
| **Primers for real-time PCR:** |  |
|  | SMYD3 sense: | 5'-GTCTTCAAACTTATGGATGGAGC-3' |
|  | SMYD3 antisense: | 5'-GGCATCCTGTATTTCTTCTCTCA-3' |
|  | ANKHD1 sense | 5’-CACTGTCCTCACGAGTTGCT-3’ |
|  | ANKHD1 antisense | 5’-AGAAACTCGCTGGGAAGGTG-3’ |
|  | Slug sense: | 5'-TGCTGCCAAATCATTTCAACTG-3' |
|  | Slug antisense: | 5'-CAACCAGACAACCGACATGT-3' |
|  | Snail sense: | 5'-GTAATGGCTGTCACTTGTCG-3' |
|  | Snail antisense: | 5'-TGTAAACATCTTCCTCCCAGG-3' |
|  | ZEB1 sense: | 5'-GGCATACACCTACTCAACTACGG-3' |
|  | ZEB1 antisense: | 5'- TGGGCGGTGTAGAATCAGAGTC-3' |
|  | ZEB2 sense: | 5'- GCTGGACACGATTGCACATT-3' |
|  | ZEB2 antisense: | 5'- AGAACTAAGCGTGTGGGAAG-3' |
|  | Twist sense: | 5'- GCCGGAGACCTAGATGTCATTG-3' |
|  | Twist antisense: | 5'- CACGCCCTGTTTCTTTGAATTT-3' |
|  | hTERT sense: | 5'- TGTCAAGGTGGATGTGACGG-3' |
|  | hTERT antisense: | 5'- CATGTACGGCTGGAGGTCTG-3' |
|  | C-met sense: | 5'- CTGGTGCCACGACAAATGTG-3' |
|  | C-met antisense: | 5'- GTCAGCCTTGTCCCTCCTTC-3' |
|  | C-myc sense: | 5'- CATCAGCACAACTACGCAGC-3' |
|  | C-myc antisense: | 5'- CGTTGTGTGTTCGCCTCTTG-3' |
|  | Cyclin A2 sense: | 5'- TGAGCATGTCACCGTTCCTC-3' |
|  | Cyclin A2 antisense: | 5'- CAGCTGGCTTCTTCTGAGCT-3' |
|  | ANKHD1 sense | 5’-CACTGTCCTCACGAGTTGCT-3’ |
|  | ANKHD1 antisense | 5’-AGAAACTCGCTGGGAAGGTG-3’ |
|  | GAPDH sense: | 5’-GAAGGTGAAGGTCGGAGTCAACG-3’ |
|  | GAPDH antisense: | 5’-TGCCATGGGTGGAATCATATTGG-3’ |
| **Primers for Slug promoter mutation construction:**  |  |
|  | binding site 1 mutation sense: | 5’-GCTCCTGCGCCaagaCTAGCTCCCAG-3’ |
|  | binding site 1 mutation antisense: | 5’-CGGGGTCTCTGCCCTGCCCGCC-3’ |
|  | binding site 2 mutation sense: | 5’-GAAAAAAAAACaagaCCAGCCAAAAC-3’ |
|  | binding site 2 mutation antisense: | 5’-CTCTCTTTTGCAAGAAAGATCCAATC-3’ |
| **Primers used for ChIP in the Slug promoter:** |  |
|  | SMYD3 binding site 1 sense: | 5’-CCTCTCCAGATGCCACTTCC-3’ |
|  | SMYD3 binding site 1 antisense: | 5’-GATCCACGCTCTCTGGGA-3’ |
|  | SMYD3 binding site 2 sense: | 5’-TCAGCTGTGATTGGATCTTTCT-3’ |
|  | SMYD3 binding site 2 antisense: | 5’-GTCCCTACAGCATCGCGG-3’ |