**Supplementary Table 1. The expression levels of nine ARs in HCC tissues and paired normal liver tissues from inhouse (n=11) and TCGA (n=50) data sets**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Inhouse data set** | | | |  | **TCGA data set** | | | |
| **Mean Expression level (Tumor tissues)** | **Mean Expression level (Normal tissues)** | **Difference** | **P value** |  | **Mean Expression level**  **(Tumor tissues)** | **Mean Expression level**  **(Normal tissues)** | **Difference** | **P value** |
| ADRA1A | 13.340 | 1.013 | 12.327 | <0.0001 |  | 9.382 | 4.975 | 4.408 | <0.0001 |
| ADRA1B | 4.351 | 1.416 | 2.935 | 0.0005 |  | 8.381 | 6.251 | 2.130 | <0.0001 |
| ADRA1D | 0.040 | 0.544 | -0.504 | 0.0549 |  | 1.564 | 2.797 | -1.234 | 0.0044 |
| ADRA2A | 1.188 | 0.971 | 0.217 | 0.2681 |  | 5.424 | 5.350 | 0.074 | 0.8389 |
| ADRA2B | 2.294 | 0.388 | 1.906 | 0.0007 |  | 7.607 | 5.424 | 2.183 | <0.0001 |
| ADRA2C | 0.548 | 5.702 | -5.154 | 0.1132 |  | 3.886 | 5.415 | -1.529 | 0.0002 |
| ADRB1 | 0.470 | 0.172 | 0.298 | 0.0886 |  | 4.630 | 3.049 | 1.582 | <0.0001 |
| ADRB2 | 4.949 | 1.970 | 2.979 | 0.0005 |  | 8.364 | 6.950 | 1.414 | <0.0001 |
| ADRB3 | 0.010 | 0.012 | -0.002 | 0.3957 |  | 0.120 | 0.298 | -0.178 | 0.2137 |

**Supplementary Table 2. The correlation between the expression levels and methylation levels of ADRA1A from inhouse data set (n=22) and TCGA data set (n=231).**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Inhouse data set** | | |  | **TCGA data set** | | |
| **Gene** | **Probe ID** | **Pearson r value** | **P value** |  | **Probe ID** | **Pearson r value** | **P value** |
| ADRA1A | cg09557462 | -0.60 | 0.0033 |  | cg09557462 | -0.53 | < 0.0001 |
| ADRA1A | cg02409177 | -0.57 | 0.0058 |  | cg02409177 | -0.49 | < 0.0001 |
| ADRA1A | cg21230493 | -0.52 | 0.0141 |  | cg21230493 | -0.45 | < 0.0001 |
| ADRA1A | cg07645844 | -0.56 | 0.0062 |  | cg07645844 | -0.52 | < 0.0001 |
| ADRA1A | cg17963840 | -0.58 | 0.0051 |  | cg17963840 | -0.45 | < 0.0001 |
| ADRA1A | cg23008606 | -0.40 | 0.0624 |  | cg23008606 | -0.32 | < 0.0001 |
| ADRA1A | cg20303399 | -0.46 | 0.0330 |  | cg20303399 | -0.44 | < 0.0001 |

**Supplementary Table 3. The correlation between the methylation levels of ADRA1A gene and clinical characteristics of HCC patients**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **CpG Sites** | **Items** | **Mean (%)** | ***P* value** | **Items** | **Mean (%)** | ***P* value** | **Items** | **Mean (%)** | ***P* value** |
|  | **Alcohol intake (≥50mg/day)** | | | **AFP** | | | **Liver cirrhosis degree** | | |
| CpG\_1.2 | Yes | 29.25 | 0.0043 | Positive | 34.81 | 0.2200 | 0 | 31.15 |  |
| 1 | 33.72 | 0.9495 |
| No | 36.13 | Negative | 31.75 | 2 | 31.44 | 0.9495 |
| 3 | 41.27 | 0.1357 |
| CpG\_3.4 | Yes | 16.55 | 0.0044 | Positive | 23.71 | 0.0858 | 0 | 19.15 |  |
| 1 | 19.97 | 0.8950 |
| No | 24.45 | Negative | 18.26 | 2 | 20.44 | 0.8282 |
| 3 | 33.82 | 0.0828 |
| CpG\_5 | Yes | 32.56 | 0.0429 | Positive | 37.37 | 0.2555 | 0 | 42.85 |  |
| 1 | 32.28 | 0.0665 |
| No | 38.34 | Negative | 34.04 | 2 | 34.44 | 0.1133 |
| 3 | 41.09 | 0.8186 |
| CpG\_7 | Yes | 22.20 | 0.0034 | Positive | 27.97 | 0.0207 | 0 | 18.23 |  |
| 1 | 27.31 | 0.0629 |
| No | 28.31 | Negative | 22.75 | 2 | 25.50 | 0.0775 |
| 3 | 34.36 | 0.0053 |
| CpG\_8 | Yes | 29.25 | 0.0043 | Positive | 34.81 | 0.2200 | 0 | 31.15 |  |
| 1 | 33.72 | 0.6124 |
| No | 36.13 | Negative | 31.75 | 2 | 31.44 | 0.9495 |
| 3 | 41.27 | 0.1357 |
| CpG\_13 | Yes | 13.31 | 0.1235 | Positive | 18.29 | 0.0032 | 0 | 14.23 |  |
| 1 | 15.33 | 0.8372 |
| No | 17.24 | Negative | 11.55 | 2 | 13.24 | 0.8149 |
| 3 | 27.64 | 0.0849 |
| CpG\_14 | Yes | 10.04 | 0.0810 | Positive | 14.32 | 0.0105 | 0 | 13.85 |  |
| 1 | 10.61 | 0.5166 |
| No | 13.91 | Negative | 9.43 | 2 | 11.46 | 0.5625 |
| 3 | 22.00 | 0.3067 |
| CpG\_15.16 | Yes | 18.45 | 0.0107 | Positive | 24.95 | 0.0569 | 0 | 18.62 |  |
| 1 | 21.56 | 0.6119 |
| No | 25.36 | Negative | 19.62 | 2 | 20.94 | 0.6632 |
| 3 | 37.27 | 0.0345 |
| CpG\_17 | Yes | 22.20 | 0.0034 | Positive | 27.97 | 0.0207 | 0 | 18.23 |  |
| 1 | 27.31 | 0.0629 |
| No | 28.31 | Negative | 22.75 | 2 | 25.50 | 0.0775 |
| 3 | 34.36 | 0.0053 |
| CpG\_18 | Yes | 18.62 | 0.0515 | Positive | 24.55 | 0.0110 | 0 | 16.38 |  |
| 1 | 19.81 | 0.4962 |
| No | 23.97 | Negative | 17.77 | 2 | 20.68 | 0.4072 |
| 3 | 32.36 | 0.0173 |
| CpG\_19 | Yes | 14.80 | 0.0583 | Positive | 20.69 | 0.0328 | 0 | 13.69 |  |
| 1 | 16.17 | 0.6830 |
| No | 20.29 | Negative | 14.38 | 2 | 16.86 | 0.5860 |
| 3 | 32.36 | 0.0403 |
| Mean | Yes | 21.37 | 0.0061 | Positive | 27.02 | 0.0280 | 0 | 22.39 |  |
| 1 | 24.29 | 0.6819 |
| No | 27.38 | Negative | 22.15 | 2 | 23.62 | 0.7744 |
| 3 | 34.92 | 0.0585 |

**Supplementary Table 4. The AUC area values in the CpG sites of *ADRA1A* promoter for different groups.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **CpG sites** | **All patients** | |  | **AFP (-)** | |  | **AFP (+)** | |  | **Alcohol intake (-)** | |  | **Alcohol intake (+)** | |
| AUC | P |  | AUC | P |  | AUC | P |  | AUC | P |  | AUC | P |
| CpG\_1.2 | 0.75 | <0.0001 |  | 0.72 | 0.0001 |  | 0.76 | <0.0001 |  | 0.82 | <0.0001 |  | 0.62 | 0.0357 |
| CpG\_3.4 | 0.61 | 0.0005 |  | 0.54 | 0.4225 |  | 0.65 | 0.0002 |  | 0.66 | 0.0001 |  | 0.54 | 0.4764 |
| CpG\_5 | 0.70 | <0.0001 |  | 0.65 | 0.0077 |  | 0.73 | <0.0001 |  | 0.76 | <0.0001 |  | 0.58 | 0.1466 |
| CpG\_7 | 0.69 | <0.0001 |  | 0.64 | 0.0134 |  | 0.72 | <0.0001 |  | 0.74 | <0.0001 |  | 0.60 | 0.0615 |
| CpG\_8 | 0.75 | <0.0001 |  | 0.72 | 0.0001 |  | 0.76 | <0.0001 |  | 0.82 | <0.0001 |  | 0.62 | 0.0357 |
| CpG\_13 | 0.54 | 0.2173 |  | 0.45 | 0.3334 |  | 0.59 | 0.0213 |  | 0.57 | 0.0857 |  | 0.49 | 0.8557 |
| CpG\_14 | 0.48 | 0.5875 |  | 0.40 | 0.0824 |  | 0.52 | 0.5696 |  | 0.50 | 0.9169 |  | 0.45 | 0.3585 |
| CpG\_15.16 | 0.67 | <0.0001 |  | 0.65 | 0.0053 |  | 0.69 | <0.0001 |  | 0.72 | <0.0001 |  | 0.58 | 0.1698 |
| CpG\_17 | 0.69 | <0.0001 |  | 0.64 | 0.0134 |  | 0.72 | <0.0001 |  | 0.74 | <0.0001 |  | 0.60 | 0.0615 |
| CpG\_18 | 0.62 | 0.0004 |  | 0.51 | 0.8851 |  | 0.68 | <0.0001 |  | 0.65 | 0.0003 |  | 0.56 | 0.3144 |
| CpG\_19 | 0.59 | 0.0057 |  | 0.51 | 0.8135 |  | 0.63 | 0.0011 |  | 0.62 | 0.0032 |  | 0.54 | 0.4877 |
| Mean | 0.70 | <0.0001 |  | 0.65 | 0.0084 |  | 0.73 | <0.0001 |  | 0.76 | <0.0001 |  | 0.59 | 0.1103 |

** Supplementary Figure 1.** The methylation levels of *ADRA1B* and *ADRB2* from inhouse and TCGA data sets.(a) The methylation levels of *ADRA1B* from our inhouse data set. (b) The methylation levels of *ADRA1B* from TCGA data set. (c) The methylation levels of *ADRB2* from our inhouse data set. (d) The methylation levels of *ADRB2* from TCGA data set. \*P<0.05, NS: not significant.



**Supplementary Figure 2.** The methylation levels of *ADRA2B* in 12 HCC patients. \*P<0.05, NS: not significant.



**Supplementary Figure 3.** The methylation level ofADRA1A in other tumors from TCGA data set. (a) melanoma; (b) colon cancer.



**Supplementary Figure 4.** The analyses of *ADRA1A* promoter methylation level in different subgroups and ADRA1A expression in different isoforms. (a) ADRA1A methylation of different CpG sites between alcohol intake and non-alcohol intake in normal tissues for HCC patients. (b) Expression of different ADRA1A isoforms in HCC patients with alcohol intake. (c) The mean methylation level of ADRA1A in HCC patients with different TNM stages. \*P<0.05, \*\*\*P<0.0001, NS: not significant.



**Supplementary Figure 5.** ROC curves of methylation levels in 14 CpG sites of *ADRA1A* promoter between HCC tissues and ANT in different subgroups: (a) AFP negative patients; (b) AFP positive patients; (c) Alcohol intake patients; (d) non-alcohol intake patients.



**Supplementary Figure 6.** Heat map of differently expressed genes in cytokine-cytokine receptor interaction pathway. (a) TNF genes; (b) IL genes; (c) ILR genes.



**Supplementary Figure 7.** KEGG and Gene Ontology (GO) analyses of the correlated genes from cBioPortal (<http://www.cbioportal.org/>). (a) KEGG signaling pathway; (b) GO analysis. MF: molecular function; CC: cellular component; BP: biological process.