**Supplementary Tables**

Supplementary Table S1. Clinicopathologic information of 12 multifocal HCC patients.

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**Supplementary Table S1. Clinicopathologic information of 12 multifocal HCC patients.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient ID** | **Gender** | **Age** | **Viral**  **infection** | **HBV (IU/ml)** | **Tumor number** | **The longest diameter (cm)** | | **AFP (ug/L)** | **OS**  **(days)** | **Antiviral therapy**  **(Before surgery)** |
| **Large tumor** | **Small tumors** |
| HCC372 | Male | 43 | HBV | 4410 | 2 | 18 | 1 | 37571 | 195 | No |
| HCC374 | Female | 46 | HBV | 884000 | 2 | 12 | 1 | 471206 | 424 | No |
| HCC393 | Male | 32 | HBV | 83800 | 2 | 13 | 1 | 1336 | 566 | No |
| HCC401 | Male | 73 | HBV | 15400 | 2 | 5 | 1 | 139 | 325 | No |
| HCC406 | Male | 32 | HBV | 10000 | 2 | 16 | 7 | 259 | 432 | No |
| HCC416 | Male | 37 | HBV | <100 | 2 | 13 | 1.5 | 17069 | 133 | Yes |
| HCC591 | Male | 44 | HBV | 520000 | 5 | 8.5 | 1.8; 1.5; 1; 1 | 2383 | 251 | No |
| HCC612 | Male | 44 | HBV | <100 | 7 | 13.1 | 4.5; 3; 3; 2; 2; 1 | 9 | 293 | No |
| HCC624 | Male | 65 | HBV | 101 | 5 | 10.4 | 5.4; 4; 2; 1 | 431 | 90 | No |
| HCC642 | Male | 46 | HCV | <100 | 6 | 6 | 1; 1; 1; 1; 1 | 1040 | 127 | No |
| HCC817 | Male | 65 | HBV | 966 | 7 | 10 | 2.5; 2; 2; 1; 1; 1 | 731 | 180 | No |
| HCC1030 | Female | 61 | HBV | <100 | 3 | 5 | 1; 1 | 343582 | 56 | No |

NOTE: All the 12 patients were classified as Child-Pugh class A liver function.

**Supplementary Table S2. PCR primers used for validation of HBV integration sites.**

|  |  |
| --- | --- |
| **HBV integration sites** | **primers** |
| HCC372-site 1-F | TCTTTGTACTGGGAGGCTGTAG |
| HCC372-site 1-R | AGAACCTGTGGGCTCTGATT |
| Length | 161bp |
|  |  |
| HCC372-site 2-F | TTGTGGGAAAGCAGGGAAGT |
| HCC372-site 2-R | TATGCCTCAAGGTCGGTCGT |
| Length | 217bp |
|  |  |
| HCC374-site 1-F | AGGAGTTGGGGGAGGAGATT |
| HCC374-site 1-R | TGGAAGGTGAAGGGGCAG |
| Length | 166bp |
|  |  |
| HCC374-site 2-F | AAGATGAGGCATAGCAGCAGGA |
| HCC374-site 2-R | TGGGTGATTAACAGATTTGGGG |
| Length | 125bp |
|  |  |
| HCC406-site 1-F | TCGGCTGTGCTGCCAACT |
| HCC406-site 1-R | GAAGCAGAAGATTATGCTGGAGT |
| Length | 176bp |
|  |  |
| HCC406-site 2-F | AGTATTATGGGTGCAGTTAGTGTG |
| HCC406-site 2-R | GTCAGAAGGCAAAAAAGAGAGTAC |
| Length | 142bp |
|  |  |
| HCC416-F | GATTAGATTAAAGGTCTTTGTACTG |
| HCC416-R | CTTATTTTAGATTAAGTGGTTAATTAC |
| Length | 215bp |

**Supplementary Table S4. HBV integration analysis in WGS samples.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Integration\_ID** | **Sample** | **Left\_chr** | **Left\_pos** | **Left\_strand** | **Right\_chr** | **Right\_pos** | **Right\_strand** |
| HCC372-site 1 | HCC372 | HBV | 179 | + | chr19 | 36213899 | + |
| HCC372-site 2 | chr19 | 36213896 | + | HBV | 1564 | + |
| HCC374-site 1 | HCC374 | HBV | 1819 | + | chr5 | 1295396 | - |
| HCC374-site 2 | HBV | 359 | - | chr5 | 1295854 | + |
| HCC406-site 1 | HCC406 | HBV | 1501 | + | chr5 | 1296741 | - |
| HCC406-site 2 | chr5 | 1296816 | + | HBV | 1862 | + |
| HCC416 | HCC416 | HBV | 1829 | + | chr5 | 1301895 | - |

**Supplementary Table S8. *P*-value of ssGSEA analysis.**

|  |  |  |
| --- | --- | --- |
| **Pathways** | ***P*-value** | **FDR** |
| ANGIOGENESIS | 1.16009E-05 | 9.39498E-05 |
| VEGF\_SIGNALING\_PATHWAY | 1.39469E-05 | 9.39498E-05 |
| HEDGEHOG\_SIGNALING\_PATHWAY | 1.78952E-05 | 9.39498E-05 |
| MYC\_TARGETS\_V1 | 1.51736E-05 | 9.39498E-05 |
| PI3K\_AKT\_MTOR\_SIGNALING | 0.000500642 | 0.001501926 |
| TNFA\_SIGNALING\_VIA\_NFKB | 0.001331536 | 0.002542023 |
| INTERFERON\_ALPHA\_RESPONSE | 0.000329788 | 0.00138511 |
| ALLOGRAFT\_REJECTION | 0.001072248 | 0.002542023 |
| INFLAMMATORY\_RESPONSE | 0.001189869 | 0.002542023 |
| T\_CELL\_RECEPTOR\_SIGNALING\_PATHWAY | 0.000431063 | 0.001501926 |
| B\_CELL\_RECEPTOR\_SIGNALING\_PATHWAY | 0.001304862 | 0.002542023 |
| T\_CELL\_SIGNAL\_TRANSDUCTION | 0.003474354 | 0.005612418 |
| CD8\_TCR\_PATHWAY | 0.033794325 | 0.041103272 |
| POSITIVE\_REGULATION\_OF\_IL\_8\_SECRETION | 0.035231376 | 0.041103272 |
| REGULATION\_OF\_IL\_1\_BETA\_PRODUCTION | 0.017000852 | 0.023801193 |
| CHRONIC\_INFLAMMATORY\_RESPONSE | 0.014484034 | 0.021726051 |
| REGULATION\_OF\_IL\_1\_SECRETION | 0.032244883 | 0.041103272 |
| Immune Stimulator Genes | 0.00160812 | 0.00281421 |
| Immune Inhibitor Genes | 0.133833765 | 0.140525453 |
| 28-Gene\_IFN\_Signature | 0.048364116 | 0.053455076 |

**Supplementary Table S10. Correlation between the number of rearrangements and immune activity.**

|  |  |  |
| --- | --- | --- |
| **Immune Activity** | **Coefficient** | ***P*-value** |
| TNFA\_SIGNALING\_VIA\_NFKB | -0.2534278 | 0.4267 |
| INTERFERON\_ALPHA\_RESPONSE | 0.3379722 | 0.2826 |
| ALLOGRAFT\_REJECTION | 0.1811048 | 0.5732 |
| INFLAMMATORY\_RESPONSE | -2.44E-05 | 0.9999 |
| T\_CELL\_RECEPTOR\_SIGNALING\_PATHWAY | 0.2708072 | 0.3946 |
| B\_CELL\_RECEPTOR\_SIGNALING\_PATHWAY | -0.3012526 | 0.3413 |
| T\_CELL\_SIGNAL\_TRANSDUCTION | -0.4395263 | 0.1528 |
| CD8\_TCR\_PATHWAY | -0.2662332 | 0.4029 |
| POSITIVE\_REGULATION\_OF\_IL\_8\_SECRETION | 0.05891741 | 0.8557 |
| REGULATION\_OF\_IL\_1\_BETA\_PRODUCTION | -0.2947776 | 0.3523 |
| CHRONIC\_INFLAMMATORY\_RESPONSE | -0.1530027 | 0.635 |
| REGULATION\_OF\_IL\_1\_SECRETION | 0.09396505 | 0.7715 |
| B cells naive | 0.2743534 | 0.3882 |
| T cells CD8 | -0.3390697 | 0.281 |
| T cells regulatory (Tregs) | -0.1979378 | 0.5375 |
| Monocytes | -0.2217346 | 0.4886 |
| Macrophages M1 | -0.3406833 | 0.2785 |
| Macrophages M2 | -0.4131434 | 0.1819 |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Case** |  | **Treatment** |  | **The longest diameter of large tumor** | | |  | **The longest diameter of small tumor** | | |
|  |  | **Pre-treatment**  **(cm)** | **Post-treatment**  **(cm)** | **Treatment**  **response** |  | **Pre-treatment**  **(cm)** | **Post-treatment**  **(cm)** | **Treatment**  **response** |
| Case 1 |  | Anti-PD-1 monotherapy |  | 2.24 | 2.89 | SD |  | 1.43 | 0.59 | PR |
| Case 2 |  | Anti-PD-1 monotherapy |  | 7.0 | 10.31 | PD |  | 1.12 | 0.4 | PR |
| Case 3 |  | Anti-PD-1 monotherapy |  | 7.07 | 8.12 | SD |  | 1.34 | 0.72 | PR |
| Case 4 |  | Anti-PD-1 monotherapy |  | 2.35 | 2.36 | SD |  | 1.51 | 0.75 | PR |
| Case 5 |  | Anti-PD-1 monotherapy |  | 6.76 | 9.24 | PD |  | 1.75 | 1.76 | SD |
| Case 6 |  | Anti-PD-1 monotherapy |  | 5.06 | 5.22 | SD |  | 3.12 | 3.06 | SD |
| Case 7 |  | Anti-PD-1 monotherapy |  | 2.59 | 2.41 | SD |  | 1.43 | 1.33 | SD |
| Case 8 |  | Combination therapy of  anti-PD-1 and lenvatinib |  | 7.05 | 2.89 | PR |  | 3.05 | 0 | CR |

**Supplementary Table S11.** **Detailed clinical data of patients with anti-PD-1 therapy.**

**Notes:** The first seven cases (Case 1 to Case 7) were patients with anti-PD-1 monotherapy. Among them, five cases (Case1 to Case 5) showed various treatment responses in different foci, with a better response in the small tumor than the large tumor, while the small and large tumors were both evaluated as SD in other two cases (Case 6 and Case 7).

Abbreviations: PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.