|  |  |
| --- | --- |
| Classification | BTC patients（n=75） |
| Tumor category |
|  | GBC | 37（49.33 %） |
|  | BDC | 34（45.33 %） |
|  | AVC | 4 （ 5.33 %） |
| Pathology grade |
|  | Low differentiation | 12（16.00 %） |
|  | Low-middle differentiation | 34（45.33 %） |
|  | Middle differentiation | 18（24.00 %） |
|  | Middle-high differentiation | 5 （ 6.67 %） |
|  | High differentiation | 6 （ 8.00 %） |
| Histological type |
|  | Adenocarcinoma | 69（92.00 %） |
|  | Squamous carcinoma | 1 （ 1.33 %） |
|  | Neuroendocrine carcinoma | 2 （ 2.67 %） |
|  | Others | 3 （ 4.00 %） |
| cTNM |
|  | Ⅰ | 6 （ 8.00 %） |
|  | Ⅱ | 2 （ 2.67 %） |
|  | Ⅲ | 29（38.67 %） |
|  | Ⅳ | 38（50.67 %） |

Table S1. Distribution of patients with BTC

|  |
| --- |
| **Total RNA (100ng) + Labeling Spike-In (optional)** |
| ↓phosphatase treatment, incubate 30 minutes, 37℃ |
| **Dephosphorylated RNA** |
| ↓add DMSO |
| ↓heat, ice |
| ↓assemble labeling reaction, incubate 2 hours, 16℃  |
| **Labeled RNA** |
| ↓desalt with Spin Column (optional) |
| **Desalted Labeled RNA** |
| ↓dry sample with vacuum concentrator, 2 to 3 hours (1 hour with columns), 45℃ to 55℃  |
| ↓assemble hybridization mixture+ Hyb Spike-In (optional) |
| ↓heat, ice |
| ↓hybridize 20 hours, 55℃, 20RPM |
| ↓wash, scan |
| **miRNA Profile** |

Table S2. The specific experimental process of low-density chip sequencing

|  |  |  |
| --- | --- | --- |
| Constituent  | Volume (μL) per Reaction | Volume (μL) per 9 Reaction |
| 10× calf intestinal alkaline phosphatase buffer |  0.4 |  3.6 |
| Labeling of spike-in solution (3 times dilution) |  1.1 |  9.9 |
| Calf intestinal alkaline phosphatase |  0.5 |  4.5 |
| Total volume |  2.0 |  18.0 |

Table S3. Preparation of dephosphorylated mixture

|  |  |  |
| --- | --- | --- |
| Constituent  | Volume (μL) per Reaction | Volume (μL) per 9 Reaction |
| 10× T4 RNA ligase buffer | 0.4 | 3.6 |
| Cyanine 3-pCp | 1.1 | 9.9 |
| T4 RNA ligase | 0.5 | 4.5 |
| Total volume | 2.0 | 18.0 |
| The reaction mixture should be prepared before use, placed on ice and used within 15 minutes |

Table S4. Combination reaction mixture preparation method

|  |  |
| --- | --- |
| Test Variable | The malignant group (n=75) *vs* The benign group（n=20） P value |
| Gender  | 0.686 |
| Age (≥60 years) | 0.203 |
| Gallstones  | 0.926 |
| PTCD | 0.210 |
| Hypertension  | 0.163 |
| Diabetes  | 0.283 |
| Laboratory detection |
| Hb  | 0.007 |
| RBC | 0.025 |
| WBC | 0.633 |
| Neutrophils | 0.359 |
| TBIL | 0.308 |
| DBIL | 0.031 |
| LDH | 0.321 |
| Urobilinogen  | 0.995 |
| Urobilin  | 0.382 |
| Hepatitis B | 1.000 |
| Ferr  | 0.280 |
| CA19-9 | 0.004 |
| CEA | 0.990 |
| AFP | 1.000 |
| CA242 | 0.417 |

Table S5. Statistical description of BTC and BBD

|  |  |  |  |
| --- | --- | --- | --- |
| Test Variable | the malignant group *vs* the benign group | the malignant group *vs* the control group | the benign group *vs* the control group |
| hsa-miR-10a | 0.923 | 0.012 | 0.087 |
| hsa-miR-21 | 0.667 | 0.006 | 0.014 |
| hsa-miR-23a | 0.116 | 0.357 | 0.091 |
| hsa-miR-106a | 0.551 | 0.902 | 0.474 |
| hsa-miR-130a | 0.072 | 0.676 | 0.052 |
| hsa-miR-135b | 0.435 | 0.006 | 0.015 |
| hsa-miR-143 | 0.740 | 0.272 | 0.338 |
| hsa-miR-145 | 0.119 | 0.074 | 0.166 |
| hsa-miR-200c | 0.421 | 0.134 | 0.136 |
| hsa-miR-221 | 0.041 | 0.022 | 0.068 |
| hsa-miR-214 | 0.926 | 0.031 | 0.047 |
| hsa-miR-483 | 0.001 | 0.588 | 0.003 |
| hsa-miR-627 | 0.037 | 0.097 | 0.075 |

Table S6. Pairwise comparison of miRNAs between groups

|  |  |  |  |
| --- | --- | --- | --- |
| Test Variable | the malignant group *vs* the benign group | the malignant group *vs* the control group | the benign group *vs* the control group  |
| hsa-miR-10a | 0.517 | <0.001 | 0.016 |
| hsa-miR-21 | 0.542 | <0.001 | 0.004 |
| hsa-miR-135b | 0.121 | <0.001 | 0.001 |
| hsa-miR-214 | 0.386 | 0.002 | 0.004 |
| hsa-miR-221 | 0.005 | <0.001 | 0.401 |
| hsa-miR-483 | 0.003 | 0.981 | 0.003 |

Table S7. Pairwise comparisons of miRNAs in the malignant, the benign, and the control groups in verification test

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Test Variable | area under curve (AUC) | standard error (SE) | P value | 95% confidence interval |
| lower limit | upper limit |
|  hsa-miR-10a | 0.677 | 0.063 | 0.012 | 0.554 | 0.800 |
|  hsa-miR-21 | 0.700 | 0.062 | 0.005 | 0.578 | 0.821 |
|  hsa-miR-135b | 0.655 | 0.064 | 0.028 | 0.530 | 0.781 |
|  hsa-miR-214 | 0.635 | 0.066 | 0.048 | 0.506 | 0.765 |
|  hsa-miR-221 | 0.724 | 0.064 | 0.002 | 0.598 | 0.850 |

Table S8. Distribution information of the ROC of five miRNAs subject characteristics