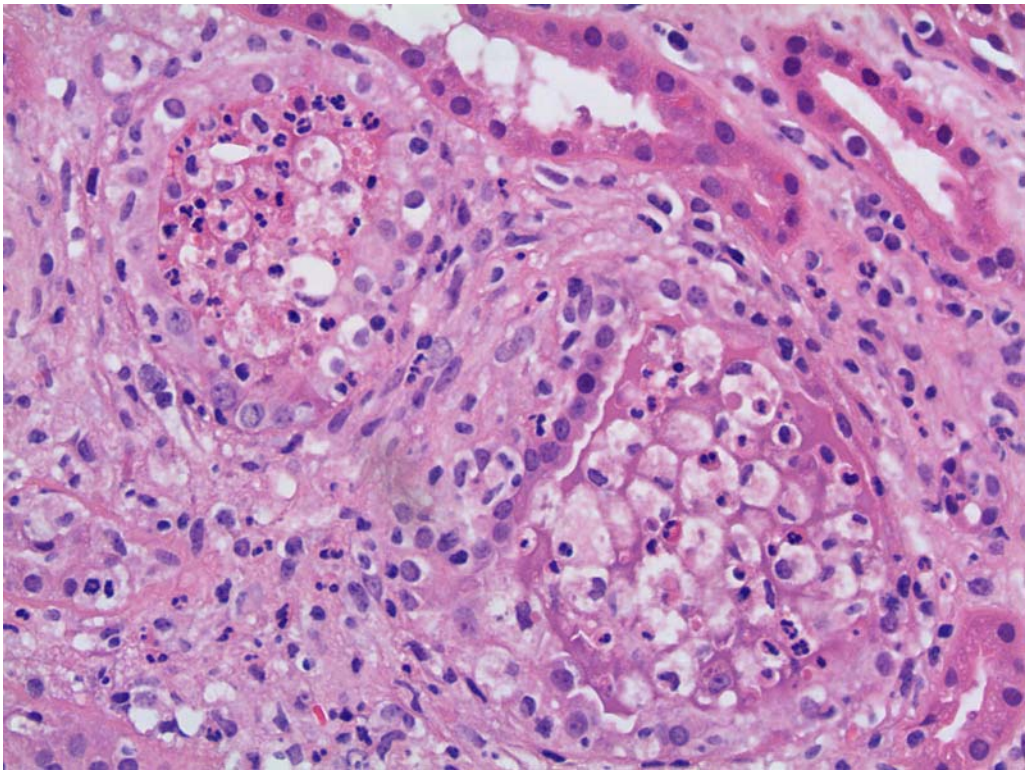
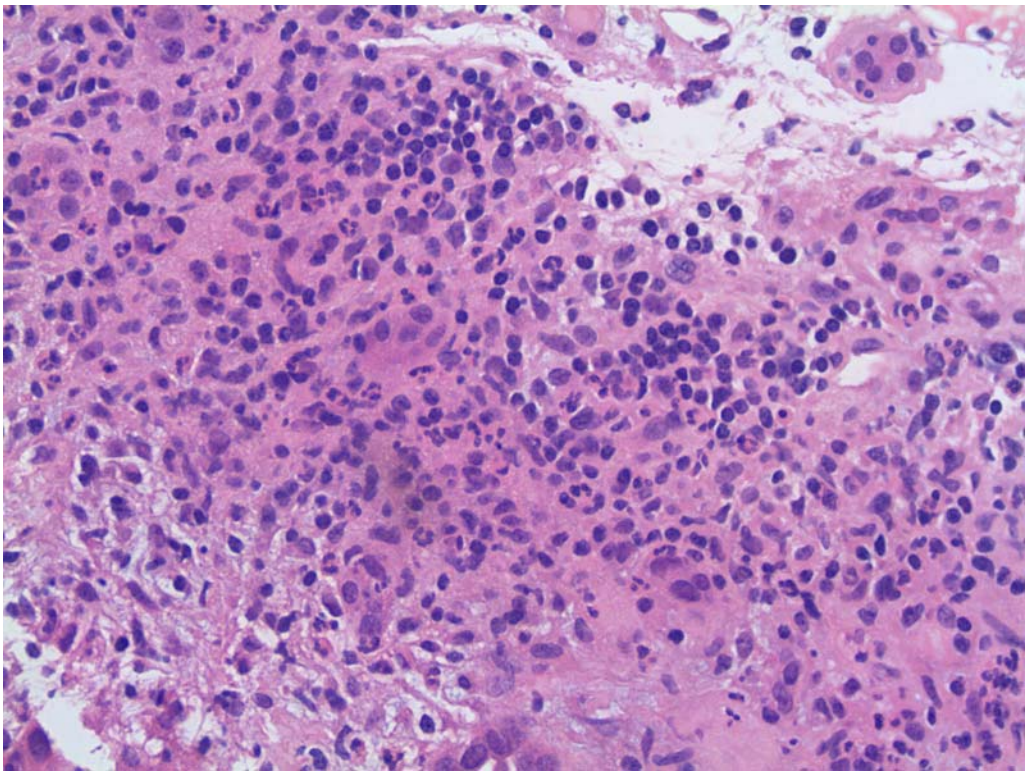
SDC Figure S1A. H&E stained biopsy I5 from Table 4 (400x). Polymorphonuclear cells in tubules and in the interstitium.



SDC Figure S1B. H&E stained biopsy I8 from Table 4 (400x). Prominence of polymorphonuclear leukocytes in the interstitium, infiltrating and destroying tubules.



SDC Table 1. The 10 of 49 patients with graft loss within one year of the diagnostic biopsy.



| | Age | Race | Sex | Transplant type | Duration transplant to biopsy | Biopsy diagnosis | Duration between biopsy and urine culture | Urine culture result | CFU/ml | Baseline S. cr. before biopsy | S. cr. 1 month post-biopsy | S. cr. 1 year post-biopsy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 21 | AA | M | Cad | 3 weeks | Mixed inflammation with PMNs, microabscesses, possible concomitant rejection | 13 days before biopsy | C. lusitanae | 1000 to 5000 | DGF | 3.5 | lost |
| 2 | 27 | AA | F | Cad | 1 month | Pyelonephritis, mild C4d positive, possible early rejection | 1 minth, 13 days before biopsy | E. fecalis | > 100,000 | DGF | lost | n/a |
| 3 | 44 | AA | M | Cad | 2 weeks | Pyelonephritis, Banff Grade 2A rejection, C4d negative | day of biopsy | E. fecalis | 100,000 | DGF | lost | n/a |
| 4 | 68 | AA | M | Cad | 4 months, 3 weeks | Pyelonephritis | 3 months, 10 days before biopsy | Enterobacter | 100,000 | 2 | lost | n/a |
| 5 | 44 | C | M | Cad | 1 year, 2 months | Mixed inflammation with PMNs, cannot exclude pyelonephritis, mild C4d positive, transplant glomerulopathy | 7 days before biopsy | E. fecalis, P. rettgeri | 100,000 | DGF | lost | n/a |
| 6 | 26 | C | F | Cad | 1 week | Mixed inflammation with PMNs, suggestive of pyelonephritis. | 11 days after biopsy | E. fecalis | 100,000 | DGF | lost | n/a |
| 7 | 30 | AA | F | Cad | 2 weeks | Mixed inflammation with PMNs, suggestive of pyelonephritis | 1 month after biopsy | K. oxytoca, C. koseri | < 50,000 | 5 | lost | n/a |
| 8 | 15 | C | F | LRD | 1 year | Pyelonephrits, recurrent aggressive IgA nephropathy | 7 days before biopsy | E.coli | 50,000 to 100,000 | 6.2 | 4.2 | lost |
| 9 | 71 | C | F | LRD | 1 year 1 month | Mixed inflammation with PMNs, neutrophilic tubulitis, mild interstitial fibrosis, mild focal C4d staining, history of desensitization protocol | 6 months before biopsy | E.coli | > 100,000 | 1.5 | 1.7 | lost |
| 10 | 76 | AA | M | Cad | 3 months | Acute pyelonephritis, moderate interstitial fibrosis | 1 month 15 days before biopsy | E.coli | > 100,000 | 1.3 | lost | n/a |

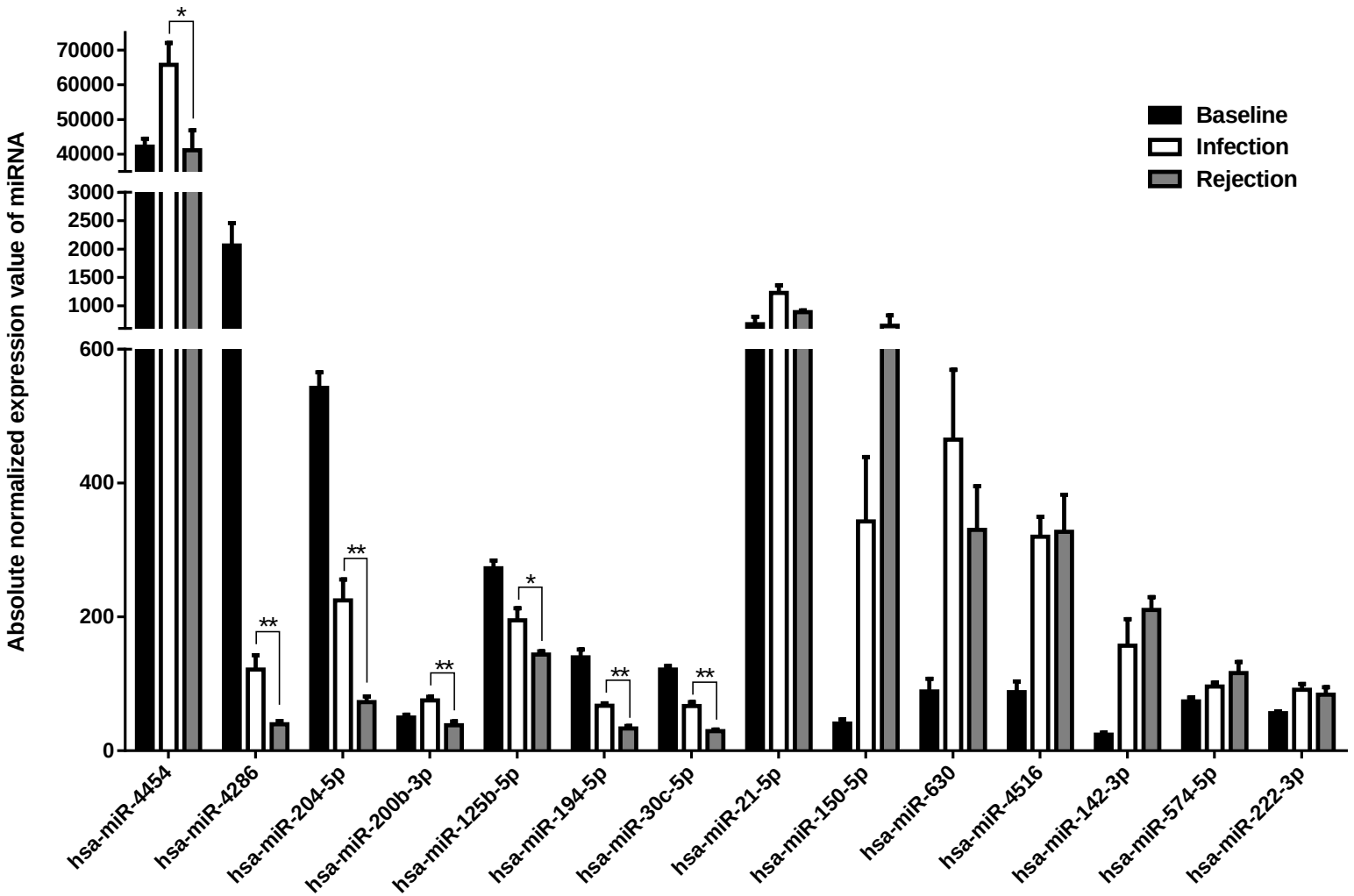
DGF= delayed graft function, PMNs=polymorphonuclear leukocytes, C. koseri=Citrobacter koseri, C. lusitaniae=Candida lusitaniae, P. rettgeri=Providencia rettgeri, K. oxytoca=Klebsiella oxytoca, S. cr=serum creatinine in mg/dl.

SDC Table 2. Baseline biopsies, and unequivocal rejection biopsies analyzed by Nanaostring nCounter. Biopsy features and follow up serum creatinine levels at 1 year are described.

| | Biopsy | Age | Race | Sex | Type of transplant | Duration between transplant to biopsy | Biopsy diagnosis | Duration between biopsy and urine culture result | Urine culture result | Colony count (CFU) | Baseline S. Cr. before biopsy | S. cr. 1 month post biopsy | S. cr. 1 year post biopsy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | B1 | 46 | C | F | LUD | 0 days | Baseline biopsy, unremarkable renal cortex | N/A | Negative | N/A | N/A | N/A | N/A |
| 2 | B2 | 48 | C | F | LUD | 0 days | Baseline biopsy, unremarkable renal cortex | day of biopsy | Negative | N/A | N/A | N/A | N/A |
| 3 | B3 | 61 | C | M | LRD | 0 days | Baseline biopsy, unremarkable renal cortex | N/A | Negative | N/A | N/A | N/A | N/A |
| 4 | B4 | 58 | C | M | LUD | 0 days | Baseline biopsy, unremarkable renal cortex | 5 days after biopsy | Negative | N/A | N/A | N/A | N/A |
| 5 | R1 | 44 | C | F | Cad | 0 days | Acute cellular rejection Banff grade 2B | on day of biopsy | Negative | N/A | DGF | 1.3 | 1.1 |
| 6 | R2 | 29 | C | F | Cad | 6 months | Acute cellular rejection Banff grade IIA, mild diffuse C4d | 4 days before biopsy | Enterococcus fecalis (UTI) | 10,000 to 50,000 | 0.7 | 1.5 | dialysis |
| 7 | R3 | 23 | C | F | LUD | 4 months | Acute cellular and humoral rejection Banff grade 1B | 2 days before biopsy | Negative | N/A | 0.9 | 1.2 | 0.9 |
| 8 | R4 | 61 | AA | F | Cad | 19 days | Acute cellular rejection Banff grade IIB | 6 days before biopsy | Negative | N/A | DGF | 1.3 | 1.5 |
| 9 | R5 | 51 | C | F | Cad | 1 month, 9 days | Acute cellular rejection Banff grade1B | 7 days before biopsy | MRSE; E. coli (UTI) | > 100,000 | 0.9 | 1.8 | dialysis |

B=baseline; R=rejection, Ph=Phillipino; LUD=living unrelated donor; LRD=living related donor; Cad=cadaveric donor; S. cr.=serum creatinine in mg/dl. MRSE = methicillin resistant Staphylococcus epidermidis; UTI=urinary tract infection.

SDC Figure S2. 14 miRs showing statistically significant differential expression between normal kidney (baseline biopsies) and the four biopsies with unequivocal pyelonephritis (I1, I2, I3, I6).




Graph shows means (+/- SEM) of absolute normalized values of miRNA expression for baseline, infection and rejection biopsies. Graphs were plotted using GRAPHPAD PRISM software and p values were calculated by unpaired t test. (* ***p < 0.05, ** p < 0.01)***

SDC Table 3. Thirty differentially expressed miRNAs between acute rejection and normal baseline biopsies. 14 of these miRNAs are differentially expressed between rejection and pyelonephritis. Two-tailed, two-sample unequal variance T-test, using cut-off p-value of 0.05).

| | Baseline biopsies (normal kidney) | | | | Acute Rejection | | | | | Acute pyelonephritis | | | | Rejection vs baseline | | Infection vs rejection | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | B1 | B2 | B3 | B4 | R12 | R2 | R3 | R4 | R5 | I1 | I2 | I3 | I6 | t-test | fold change | t-test | fold change |
| hsa-miR-1260a | 1114.88 | 2447.8 | 3501.8 | 3850.72 | 1891.32 | 2045.91 | 904.3 | 1754.93 | 1481.85 | 2305.54 | 2465.99 | 2843.62 | 2105.34 | **0.0399035** | 0.4945743 | **0.0116104** | 1.5711104 |
| hsa-miR-125b-5p | 631.86 | 1178.05 | 1625.38 | 1408.18 | 698.19 | 922.49 | 892.13 | 899.48 | 789.05 | 1000.77 | 2662.19 | 2188.41 | 1254.12 | **0.0376787** | 0.5985369 | 0.152749 | 2.3212316 |
| hsa-miR-150-5p | 15.15 | 30.57 | 39.69 | 52.02 | 261.42 | 650.12 | 209.34 | 1138.62 | 968.52 | 66.36 | 513.28 | 407.1 | 383.71 | **0.030812** | 15.839156 | 0.2159742 | 0.5094661 |
| hsa-miR-630 | 69.51 | 90.53 | 119.92 | 55.65 | 482.19 | 345.6 | 119.28 | 441.41 | 263.69 | 569.44 | 680.66 | 200.06 | 409.96 | **0.0182907** | 3.7252988 | 0.4113685 | 1.4628842 |
| hsa-miR-4286 | 532.04 | 2307.9 | 2592.49 | 1289.62 | 51.81 | 41.08 | 25.56 | 32.12 | 48.13 | 70.84 | 123.67 | 116.19 | 174.69 | **0.0361201** | 0.0192601 | **0.0507684** | 2.6061064 |
| hsa-miR-4516 | 48.12 | 101.11 | 106.12 | 55.65 | 247.87 | 367.03 | 323.75 | 510.42 | 187.49 | 403.54 | 320.8 | 284.8 | 270.61 | **0.0102828** | 3.7353013 | 0.8943912 | 1.0277045 |
| hsa-miR-1972 | 127.44 | 47.03 | 50.9 | 131.87 | 291.71 | 271.48 | 267.76 | 536.59 | 436.13 | 420.58 | 181.32 | 76.88 | 155.5 | **0.0039929** | 4.7093211 | 0.3233549 | 0.6272212 |
| hsa-miR-125a-5p | 144.37 | 255.13 | 269.17 | 293.98 | 143.46 | 161.64 | 131.45 | 140.39 | 141.37 | 168.59 | 160.87 | 214.04 | 236.28 | **0.0026147** | 0.5266975 | 0.1427883 | 1.2610618 |
| hsa-miR-204-5p | 230.82 | 545.52 | 501.24 | 580.69 | 87.67 | 71.44 | 43.82 | 70.2 | 91.24 | 185.63 | 175.74 | 312.75 | 224.17 | **0.0008012** | 0.1343341 | **0.0701865** | 3.0834957 |
| hsa-miR-126-3p | 60.6 | 191.64 | 215.68 | 156.06 | 103.61 | 100.02 | 58.42 | 129.69 | 105.27 | 118.37 | 377.52 | 254.22 | 167.62 | **0.0147669** | 0.5293159 | 0.1791373 | 2.5154088 |
| hsa-miR-342-3p | 71.3 | 104.64 | 89.72 | 65.33 | 156.22 | 303.63 | 141.18 | 177.28 | 145.38 | 79.81 | 353.35 | 234.13 | 195.89 | **0.0291523** | 2.1341369 | 0.6910166 | 1.2040295 |
| hsa-miR-30a-5p | 142.59 | 348.01 | 339.05 | 216.55 | 76.51 | 107.16 | 76.68 | 76.15 | 84.22 | 201.77 | 245.48 | 217.53 | 253.45 | **0.0339377** | 0.2793595 | **0.002666** | 2.6335013 |
| hsa-miR-142-3p | 9.8 | 22.34 | 19.84 | 30.24 | 203.24 | 201.82 | 148.49 | 258.18 | 241.63 | 76.22 | 265.01 | 134.54 | 152.47 | **0.0004898** | 8.727092 | 0.4543819 | 0.7527816 |
| hsa-miR-99b-5p | 106.05 | 174 | 199.29 | 156.06 | 143.46 | 114.31 | 70.59 | 108.27 | 76.2 | 201.77 | 200.85 | 187.83 | 168.63 | **0.0080673** | 0.5812751 | **0.0012802** | 1.918927 |
| hsa-miR-30d-5p | 129.22 | 273.94 | 270.03 | 180.26 | 117.96 | 133.06 | 62.07 | 95.18 | 81.21 | 231.36 | 254.78 | 183.46 | 209.02 | **0.02792** | 0.4055176 | **0.0099113** | 2.2799706 |
| hsa-miR-223-3p | 12.48 | 28.22 | 12.94 | 25.41 | 102.02 | 95.55 | 51.12 | 184.42 | 121.32 | 77.12 | 221.31 | 83.87 | 78.76 | **0.0135894** | 4.9971158 | 0.7709154 | 1.1492283 |
| hsa-miR-4443 | 75.75 | 47.03 | 63.84 | 79.85 | 115.57 | 118.77 | 114.41 | 89.23 | 101.26 | 50.22 | 69.74 | 87.36 | 76.74 | **0.0215786** | 1.6964346 | **0.0468403** | 0.6407784 |
| hsa-miR-130a-3p | 59.71 | 102.29 | 92.31 | 100.41 | 41.44 | 74.12 | 38.95 | 59.49 | 51.13 | 82.5 | 185.04 | 104.83 | 138.34 | **0.0010064** | 0.5392292 | **0.1446966** | 2.3408014 |
| hsa-miR-888-5p | 61.49 | 65.84 | 81.1 | 42.34 | 145.85 | 175.92 | 155.79 | 96.37 | 69.18 | 156.03 | 108.79 | 104.83 | 67.65 | **0.029356** | 2.0385989 | 0.840814 | 0.957975 |
| hsa-miR-1276 | 40.1 | 25.87 | 25.02 | 38.71 | 93.25 | 115.2 | 93.72 | 105.89 | 120.31 | 160.52 | 61.37 | 48.92 | 45.44 | **4.271E-05** | 3.538192 | 0.706688 | 0.8542309 |
| hsa-let-7i-5p | 32.08 | 32.92 | 38.82 | 19.36 | 68.54 | 78.59 | 64.51 | 65.44 | 66.17 | 98.64 | 172.95 | 67.27 | 70.68 | **0.0106967** | 2.2607025 | 0.292634 | 1.6453508 |
| hsa-miR-23b-3p | 44.56 | 89.35 | 112.15 | 127.03 | 27.9 | 27.68 | 35.3 | 33.31 | 40.1 | 71.74 | 115.3 | 95.22 | 95.93 | **0.0163503** | 0.3000457 | **0.0358985** | 2.8634325 |
| hsa-miR-99a-5p | 41.89 | 77.6 | 87.14 | 105.25 | 35.87 | 42.86 | 36.51 | 35.69 | 29.08 | 47.53 | 125.53 | 104.83 | 110.06 | **0.0163781** | 0.400037 | **0.1347428** | 2.5729126 |
| hsa-miR-30c-5p | 48.12 | 131.68 | 119.92 | 112.51 | 22.32 | 36.61 | 25.56 | 32.12 | 29.08 | 50.22 | 71.6 | 70.76 | 75.73 | **0.0008794** | 0.2400758 | **0.0263116** | 2.2030796 |
| hsa-miR-423-5p | 23.17 | 48.2 | 55.21 | 54.44 | 47.82 | 41.97 | 40.16 | 51.16 | 40.1 | 47.53 | 71.6 | 63.77 | 56.55 | **0.0419063** | 0.8408362 | **0.1316638** | 1.3780269 |
| hsa-miR-194-5p | 56.15 | 148.14 | 154.43 | 116.14 | 32.68 | 46.44 | 25.56 | 28.55 | 34.09 | 63.67 | 76.25 | 60.28 | 68.66 | **0.0077571** | 0.239765 | **0.0047437** | 1.9941828 |
| hsa-miR-100-5p | 37.43 | 50.56 | 58.67 | 71.38 | 31.88 | 34.83 | 26.78 | 42.83 | 41.11 | 43.04 | 98.57 | 71.64 | 47.46 | **0.0354604** | 0.5894358 | 0.1524619 | 2.0031374 |
| hsa-miR-1290 | 57.04 | 45.85 | 46.59 | 25.41 | 82.09 | 102.7 | 87.63 | 54.73 | 39.1 | 103.13 | 63.23 | 54.16 | 56.55 | **0.0458973** | 1.8646585 | 0.9898017 | 1.003504 |
| hsa-miR-495 | 32.97 | 38.8 | 31.06 | 44.76 | 74.12 | 70.55 | 76.68 | 44.02 | 61.16 | 72.64 | 48.35 | 47.18 | 43.42 | **0.0090591** | 1.7092828 | 0.4154472 | 0.8583693 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| hsa-miR-27b-3p | 49.02 | 88.18 | 101.8 | 65.33 | 29.49 | 34.83 | 25.56 | 22.61 | 21.05 | 58.29 | 84.62 | 75.13 | 60.59 | **0.0263964** | 0.3138302 | **0.0187283** | 2.721282 |