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# Identification of Novel microRNA Prognostic Markers Using Cascaded Wx, a Neural Network-Based Framework, in Lung Adenocarcinoma Patients

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**Supplementary Materials:**

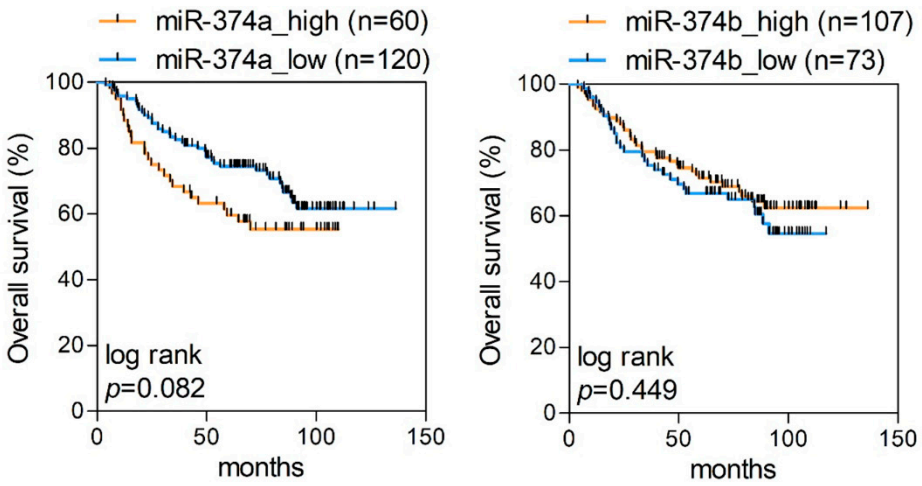


**Figure S1.** miR-374 and miR-374b are poor prognostic markers in LUAD patients. Overall survival of LUAD patients based on miR-374a and miR-374b expression. LUAD patients were divided into two groups (high and low) based on expression levels of miR-374a (left) or miR-374b (right) obtained from NanoString.

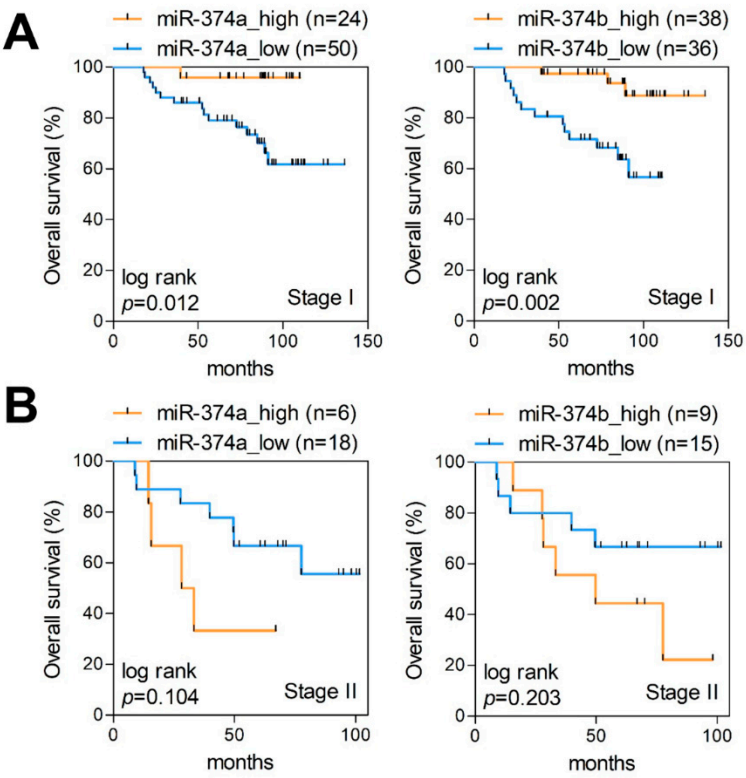


**Figure S2.** The effects of miR-374a and miR-374b on patient overall survival are cancer stage-specific. (**A**) Overall survival of stage I-LUAD patients based on miR-374a and miR-374b expression. Patients (n = 74) were divided into two groups (high and low) based on the expression levels of miR-374a (left) or miR-374b (right) obtained from NanoString. (**B**) Overall survival of stage II-LUAD patients based on miR-374a and miR-374b expression. Patients (n = 24) were divided into two groups (high and low) based on the expression levels of miR-374a (left) or miR-374b (right) obtained from NanoString.

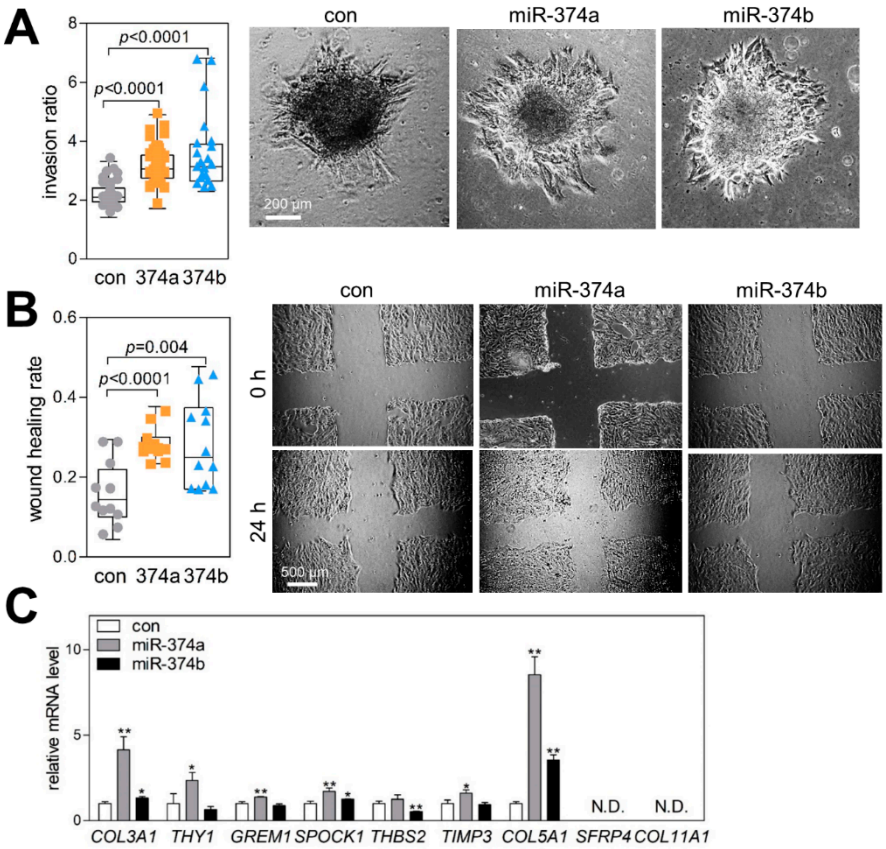


**Figure S3.** miR-374a and miR-374b promote invasion of H1792 lung cancer cells. (**A**) Spheroid invasion assay in miR-374 mimic-transfected cells. Spheroids made from hanging-drop cultures of miR-374 mimic-transfected H1792 cells were seeded on collagen gels and then cultured for 24 h. Spheroid invasion ratios (ratio of whole cell area to central spheroid area) were measured using ImageJ. Box-and-whisker plots denote median and upper/lower quartiles+1.5×IQR (interquartile range). con, n = 45; 374a, n = 42; 374b, n = 20. *p*, two-tailed Student's t-test. (**B**) Wound-healing assay in miR-374 mimic-transfected H1792 cells. Cells were subjected to scratch wounds and then incubated for 24 h with mitomycin C (1 μg/mL) to block proliferation-related effect. Wound healing rates (1−[wound area ratio of 24 h to 0 h]) were measured using ImageJ (n = 12). *p*, two-tailed Student's *t*-test. (**C**) qRT-PCR of EMT and invasiveness signature genes in miR-374 mimic-transfected cells. H1792 cells were transiently transfected with miR-374a or miR-374b mimic, and expression levels of the signature genes were measured by qRT-PCR. Expression levels were normalized to *RPL32* level. Relative values to those of H1792 cells transfected with negative control (set at 1.0) are presented. Data are mean+SD (n = 3). **p* < 0.05, ** *p* <0.01; two-tailed Student's t-test. N.D., not detected.

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**Table S1.** The CWx miRNA list.

| rank | miRNAs | CWx score | rank | miRNAs | CWx score | rank | miRNAs | CWx score |
|---|---|---|---|---|---|---|---|---|
| 1 | hsa-mir-374a | 466 | 67 | hsa-mir-183 | 155 | 133 | hsa-mir-1301 | 49 |
| 2 | hsa-mir-374b | 448 | 68 | hsa-mir-505 | 152 | 134 | hsa-mir-452 | 49 |
| 3 | hsa-let-7f-1 | 436 | 69 | hsa-mir-155 | 150 | 135 | hsa-mir-3130-1 | 48 |
| 4 | hsa-mir-101-1 | 421 | 70 | hsa-mir-34a | 149 | 136 | hsa-mir-19b-1 | 46 |
| 5 | hsa-mir-21 | 415 | 71 | hsa-mir-421 | 148 | 137 | hsa-mir-146b | 46 |
| 6 | hsa-mir-22 | 398 | 72 | hsa-mir-3607 | 147 | 138 | hsa-mir-1254 | 43 |
| 7 | hsa-mir-15a | 386 | 73 | hsa-mir-106a | 146 | 139 | hsa-mir-132 | 43 |
| 8 | hsa-mir-141 | 386 | 74 | hsa-mir-125b-2 | 145 | 140 | hsa-mir-134 | 43 |
| 9 | hsa-mir-98 | 376 | 75 | hsa-mir-582 | 145 | 141 | hsa-mir-199a-1 | 42 |
| 10 | hsa-mir-182 | 367 | 76 | hsa-mir-375 | 141 | 142 | hsa-mir-550a-2 | 41 |
| 11 | hsa-mir-142 | 364 | 77 | hsa-mir-628 | 140 | 143 | hsa-mir-625 | 39 |
| 12 | hsa-mir-146a | 349 | 78 | hsa-mir-152 | 136 | 144 | hsa-mir-196b | 38 |
| 13 | hsa-mir-218-2 | 346 | 79 | hsa-mir-3677 | 135 | 145 | hsa-mir-140 | 37 |
| 14 | hsa-mir-200c | 343 | 80 | hsa-mir-429 | 134 | 146 | hsa-mir-20b | 37 |
| 15 | hsa-let-7f-2 | 340 | 81 | hsa-mir-9-2 | 131 | 147 | hsa-mir-2277 | 35 |
| 16 | hsa-mir-708 | 332 | 82 | hsa-mir-19a | 126 | 148 | hsa-mir-378c | 35 |
| 17 | hsa-mir-29b-1 | 331 | 83 | hsa-mir-503 | 126 | 149 | hsa-mir-3117 | 34 |
| 18 | hsa-mir-32 | 327 | 84 | hsa-mir-511-2 | 125 | 150 | hsa-mir-181d | 34 |
| 19 | hsa-mir-590 | 310 | 85 | hsa-mir-301a | 119 | 151 | hsa-mir-33a | 34 |
| 20 | hsa-mir-19b-2 | 309 | 86 | hsa-mir-92b | 118 | 152 | hsa-mir-1266 | 33 |
| 21 | hsa-mir-210 | 303 | 87 | hsa-mir-27b | 116 | 153 | hsa-mir-548b | 32 |
| 22 | hsa-mir-20a | 300 | 88 | hsa-mir-143 | 113 | 154 | hsa-mir-9-1 | 31 |
| 23 | hsa-mir-128-1 | 276 | 89 | hsa-mir-1307 | 112 | 155 | hsa-mir-581 | 29 |
| 24 | hsa-mir-17 | 257 | 90 | hsa-mir-379 | 110 | 156 | hsa-mir-424 | 29 |
| 25 | hsa-let-7g | 257 | 91 | hsa-mir-203 | 109 | 157 | hsa-mir-145 | 28 |
| 26 | hsa-mir-101-2 | 256 | 92 | hsa-mir-148b | 107 | 158 | hsa-mir-3614 | 27 |
| 27 | hsa-mir-335 | 252 | 93 | hsa-mir-103-2 | 103 | 159 | hsa-let-7b | 27 |
| 28 | hsa-mir-148a | 250 | 94 | hsa-mir-338 | 103 | 160 | hsa-mir-942 | 27 |
| 29 | hsa-mir-200b | 250 | 95 | hsa-mir-339 | 103 | 161 | hsa-mir-223 | 26 |
| 30 | hsa-mir-542 | 249 | 96 | hsa-mir-769 | 101 | 162 | hsa-mir-337 | 25 |
| 31 | hsa-mir-100 | 249 | 97 | hsa-mir-511-1 | 98 | 163 | hsa-mir-382 | 24 |
| 32 | hsa-mir-454 | 248 | 98 | hsa-mir-191 | 98 | 164 | hsa-mir-501 | 24 |
| 33 | hsa-mir-128-2 | 242 | 99 | hsa-mir-24-2 | 97 | 165 | hsa-mir-1255a | 24 |
| 34 | hsa-mir-4326 | 236 | 100 | hsa-mir-195 | 94 | 166 | hsa-mir-361 | 23 |
| 35 | hsa-mir-2355 | 234 | 101 | hsa-mir-212 | 91 | 167 | hsa-mir-362 | 22 |
| 36 | hsa-mir-29a | 234 | 102 | hsa-mir-99a | 90 | 168 | hsa-mir-450a-1 | 22 |
| 37 | hsa-mir-340 | 228 | 103 | hsa-mir-330 | 90 | 169 | hsa-mir-221 | 21 |
| 38 | hsa-mir-107 | 224 | 104 | hsa-mir-30a | 89 | 170 | hsa-mir-30c-1 | 20 |
| 39 | hsa-mir-3913-1 | 223 | 105 | hsa-mir-127 | 88 | 171 | hsa-mir-181a-1 | 18 |
| 40 | hsa-let-7a-2 | 218 | 106 | hsa-mir-3136 | 88 | 172 | hsa-mir-1293 | 18 |
| 41 | hsa-let-7a-3 | 217 | 107 | hsa-mir-1-2 | 86 | 173 | hsa-mir-3065 | 17 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 42 | hsa-mir-103-1 | 214 | 108 | hsa-mir-126 | 85 | 174 | hsa-let-7i | 17 |
| 43 | hsa-let-7a-1 | 208 | 109 | hsa-let-7c | 84 | 175 | hsa-mir-760 | 17 |
| 44 | hsa-mir-135b | 207 | 110 | hsa-mir-598 | 82 | 176 | hsa-mir-381 | 16 |
| 45 | hsa-mir-29c | 207 | 111 | hsa-mir-29b-2 | 82 | 177 | hsa-mir-939 | 16 |
| 46 | hsa-mir-23b | 206 | 112 | hsa-mir-3613 | 79 | 178 | hsa-mir-3682 | 16 |
| 47 | hsa-mir-151 | 197 | 113 | hsa-mir-451 | 77 | 179 | hsa-mir-3934 | 14 |
| 48 | hsa-mir-7-1 | 191 | 114 | hsa-mir-550a-1 | 75 | 180 | hsa-mir-1287 | 12 |
| 49 | hsa-mir-26a-2 | 189 | 115 | hsa-mir-589 | 73 | 181 | hsa-mir-194-1 | 11 |
| 50 | hsa-let-7e | 188 | 116 | hsa-mir-144 | 72 | 182 | hsa-mir-654 | 11 |
| 51 | hsa-mir-497 | 184 | 117 | hsa-mir-199a-2 | 71 | 183 | hsa-mir-450a-2 | 10 |
| 52 | hsa-mir-96 | 177 | 118 | hsa-mir-425 | 70 | 184 | hsa-mir-181b-2 | 9 |
| 53 | hsa-mir-181a-2 | 176 | 119 | hsa-mir-219-1 | 70 | 185 | hsa-mir-324 | 9 |
| 54 | hsa-mir-629 | 174 | 120 | hsa-mir-1274b | 67 | 186 | hsa-mir-214 | 9 |
| 55 | hsa-mir-130b | 174 | 121 | hsa-mir-23a | 67 | 187 | hsa-mir-33b | 8 |
| 56 | hsa-mir-150 | 174 | 122 | hsa-mir-455 | 65 | 188 | hsa-mir-491 | 6 |
| 57 | hsa-mir-200a | 174 | 123 | hsa-mir-181c | 65 | 189 | hsa-mir-130a | 6 |
| 58 | hsa-mir-27a | 165 | 124 | hsa-mir-624 | 64 | 190 | hsa-mir-192 | 6 |
| 59 | hsa-mir-31 | 165 | 125 | hsa-mir-660 | 64 | 191 | hsa-mir-500b | 5 |
| 60 | hsa-mir-16-1 | 163 | 126 | hsa-mir-10a | 64 | 192 | hsa-mir-153-2 | 4 |
| 61 | hsa-mir-181b-1 | 162 | 127 | hsa-mir-3158-2 | 63 | 193 | hsa-mir-10b | 4 |
| 62 | hsa-mir-93 | 161 | 128 | hsa-mir-30d | 62 | 194 | hsa-mir-185 | 4 |
| 63 | hsa-mir-18a | 159 | 129 | hsa-mir-125b-1 | 60 | 195 | hsa-mir-320b-2 | 3 |
| 64 | hsa-mir-450b | 159 | 130 | hsa-mir-34b | 54 | 196 | hsa-mir-186 | 2 |
| 65 | hsa-mir-199b | 158 | 131 | hsa-mir-342 | 53 | 197 | hsa-mir-576 | 1 |
| 66 | hsa-mir-30e | 156 | 132 | hsa-mir-26b | 51 | | | |

**Table S2.** Univariate and multivariate analysis using the Cox proportional hazards model for recurrence-free survival.

| Variables | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *p*-value | HR | 95% CI | *p*-value |
| Gender (**female** vs. male) | 1.165 | 0.722–1.881 | 0.532 | | | |
| Age **(>65** vs. ≤65) | 1.215 | 0.752 – 1.963 | 0.426 | | | |
| Differentiation(**poor/mod** vs. good) | 2.376 | 1.243–4.539 | 0.009 | 1.583 | 0.794–3.155 | 0.192 |
| T stage (**T3/4** vs. T1/2) | 2.380 | 1.297–4.368 | 0.005 | 1.481 | 0.791–2.773 | 0.220 |
| N stage (**N1-3** vs. N0) | 3.974 | 2.444–6.461 | < 0.001 | 1.757 | 0.925–3.340 | 0.085 |
| Lymphatic invasion (**yes** vs. no) | 1.139 | 1.007–1.288 | 0.039 | 2.424 | 1.276–4.605 | 0.007 |
| Vascular invasion (**yes** vs. no) | 1.067 | 0.913–1.248 | 0.412 | | | |
| Peri-neural invasion (**yes** vs. no) | 1.042 | 0.876–1.239 | 0.642 | | | |
| High miR-374a (**high** vs. low) | 1.593 | 0.977–2.596 | 0.062 | 1.642 | 0.995–2.709 | 0.053 |
| High miR-374b (**high** vs. low) | 1.568 | 0.936–2.628 | 0.088 | 1.218 | 0.713–2.080 | 0.471 |

**Table S3.** Univariate and multivariate analysis using the Cox proportional hazards model for overall survival.

| Variables | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *p*-value | HR | 95% CI | *p*-value |
| Gender (female vs. male) | 0.828 | 0.508–1.351 | 0.450 | | | |
| Age (>65 vs. ≤65) | 1.332 | 0.818–2.170 | 0.249 | | | |
| Differentiation(poor/mod vs. good) | 1.114 | 0.787 – 1.576 | 0.175 | | | |
| T stage (T3/4 vs. T1/2) | 1.957 | 1.020–3.758 | 0.044 | 1.253 | 0.631–2.487 | 0.520 |
| N stage (N1-3 vs. N0) | 2.774 | 1.676–4.590 | < 0.001 | 1.482 | 0.750–2.927 | 0.258 |
| Lymphatic invasion (yes vs. no) | 1.235 | 1.108–1.378 | < 0.001 | 2.317 | 1.388–3.868 | 0.001 |
| Vascular invasion (yes vs. no) | 1.199 | 1.066–1.349 | 0.002 | 1.072 | 0.465–2.470 | 0.871 |
| Peri-neural invasion (yes vs. no) | 1.189 | 1.053–1.341 | 0.005 | 1.886 | 0.571–6.229 | 0.298 |
| High miR-374a (high vs. low) | 1.548 | 0.942–2.544 | 0.085 | 1.804 | 1.084–3.003 | 0.023 |
| High miR-374b (high vs. low) | 0.828 | 0.508–1.350 | 0.449 | | | |

**Table S4.** Sequences of qRT-PCR primers used in this study.

| Genes | Forward (5′→3′) | Reverse(5′→3′) |
|---|---|---|
| *RPL32* | ACAAAGCACATGCTGCCCAGTG | TTCCACGATGGCTTTGCGGTTC |
| *SNAI1* | CTGAGGCCAAGGATCTCCAG | ATCTGCGGCAAGGCGTTTTCCA |
| *SNIA2* | ATCTGCGGCAAGGCGTTTTCCA | GAGCCCTCAGATTTGACCTGTC |
| *CDH1* | GCCTCCTGAAAAGAGAGTGGAAG | TGGCAGTGTCTCTCCAAATCCG |
| *INADL* | ACAAGGCAGATTTGACGACCTGG | CTTTGAGCCACAACAGGAAGGTC |
| *CRB3* | CTTCTGCAAATGAGAATAGCACTG | GACCACGATGATAGCAGTGATGG |
| *SPOCK1* | GTTCTACTGGCAAAAGCCTCGC | AGGTTCCGCAACTCCTTGTCTG |
| *COL3A1* | TGGTCTGCAAGGAATGCCTGGA | TCTTTCCCTGGGACACCATCAG |
| *TIMP3* | TACCGAGGCTTCACCAAGATGC | CATCTTGCCATCATAGACGCGAC |
| *COL5A1* | GGAGATGATGGTCCCAAAGGCA | CCATCATCTCCTTTGTCACCAGG |
| *SFRP4* | CTATGACCGTGGCGTGTGCATT | GCTTAGGCGTTTACAGTCAACATC |
| *THBS2* | CAGTCTGAGCAAGTGTGACACC | TTGCAGAGACGGATGCGTGTGA |
| *COL11A1* | ATGGACCAGCAGGATTACGTGG | TGTACCTGCTGACCCACGTTCT |
| *GREM1* | TCATCAACCGCTTCTGTTACGGC | CAGAAGGAGCAGGACTGAAAGG |