Circulating MicroRNAs in Delayed Cerebral Infarction After Aneurysmal Subarachnoid Hemorrhage

Gang Lu, MD, PhD; Man Sze Wong, MSc; Mark Zhi Qiang Xiong, PhD; Chi Kwan Leung, PhD; Xian Wei Su, PhD; Jing Ye Zhou, PhD; Wai Sang Poon, MD; Vera Zhi Yuan Zheng, MSc; Wai Yee Chan, PhD; George Kwok Chu Wong, MD

**Background**—Delayed cerebral infarction (DCI) is a major cause of morbidities after aneurysmal subarachnoid hemorrhage (SAH) and typically starts at day 4 to 7 after initial hemorrhage. MicroRNAs (miRNAs) play an important role in posttranscriptional gene expression control, and distinctive patterns of circulating miRNA changes have been identified for some diseases. We aimed to investigate miRNAs that characterize SAH patients with DCI compared with those without DCI.

**Methods and Results**—Circulating miRNAs were collected on day 7 after SAH in healthy, SAH-free controls (n=20), SAH patients with DCI (n=20), and SAH patients without DCI (n=20). We used the LASSO (least absolute shrinkage and selection operator) method of regression analysis to characterize miRNAs associated with SAH patients with DCI compared with those without DCI. In the 28 dysregulated miRNAs associated with DCI and SAH, we found that a combination of 4 miRNAs (miR-4532, miR-4463, miR-1290, and miR-4793) could differentiate SAH patients with DCI from those without DCI with an area under the curve of 100% (95% CI 1.000–1.000, P<0.001). This 4-miRNA combination could also distinguish SAH patients with or without DCI from healthy controls with areas under the curve of 99.3% (95% CI 0.977–1.000, P<0.001) and 82.0% (95% CI 0.685–0.955, P<0.001), respectively.

**Conclusions**—We found a 4-miRNA combination that characterized SAH patients with DCI. The findings could guide future mechanistic study to develop therapeutic targets. (*J Am Heart Assoc*. 2017;6:e005363. DOI: 10.1161/JAHA.116.005363.)

**Key Words:** biomarker • delayed cerebral infarction • miRNA • stroke • subarachnoid hemorrhage

Aneurysmal subarachnoid hemorrhage (SAH) accounts for about 3% to 5% of stroke and is an important cause of stroke in young populations, causing significant socioeconomnic burden worldwide. Up to two thirds of SAH patients experience cognitive impairment and impaired quality of life and may not be able to return to their previous work.2–8

Delayed cerebral infarction (DCI) occurs in up to 44% of SAH patients and typically starts at day 4 to 7 after the initial hemorrhage.9–11 DCI is a well-established and relevant clinical surrogate marker for neurological outcome after SAH.12 Numerous factors including age, initial neurological impairment, intraventricular hemorrhage, SAH load, and aneurysm size are reported to be associated with the development of DCI.13–16

MicroRNAs (miRNAs) are a family of small (19–23 base pairs), noncoding, and deeply conserved RNA molecules that regulate target gene expression at a posttranscriptional level by inhibiting mRNA translation17 or destabilizing mRNA molecules.18 There are 1881 miRNAs in human genomes, 296 of which are currently annotated as high confidence, according to miRBase 21.0.19 Circulating miRNAs have been demonstrated to be promising diagnostic or prognostic biomarkers for cerebrovascular conditions such as myocardial infarction,20 atherosclerotic diseases,21 stroke,22 cerebral infarction,23 hypertension,24 intracranial aneurysms,25–28 and SAH.29,30
In the current study, we aimed to investigate miRNAs that characterized SAH patients with DCI compared with those without DCI.

Methods
Patient Recruitment and Sample Collection
The study was approved by the Joint NTEC-CUHK (New Territories East Cluster-Chinese University of Hong Kong) Clinical Research Ethics Committee, and written informed consent was obtained from all participants or their next of kins. Circulating miRNAs were collected from healthy controls (n=20), SAH patients with DCI (n=20) at day 7 after SAH, and SAH patients without DCI (n=20) at day 7 after SAH. SAH patients were recruited from Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong Special Administrative Region, People’s Republic of China, between 2012 and 2013. Ruptured cerebral aneurysms were diagnosed by computer tomography angiography. For SAH patients, the healthy controls (n=20) were recruited from the family members of SAH patients with the no major medical problem (including no smoking history and hypertension). Participant characteristics are shown in Table 1.

Table 1. Participant Characteristics

| Demographics          | Control (n=20) | SAH With DCI (n=20) | SAH Without DCI (n=20) | P Value |
|-----------------------|---------------|---------------------|------------------------|---------|
| Age, y                | 50±17         | 59±12               | 59±11                  |         |
| Female, %             | 65 (13)       | 45 (9)              | 65 (13)                |         |
| WFNS grade on admission, % |               |                     |                        |         |
| 1–2                   |               | 60 (12)             | 75 (15)                | 0.311   |
| 3–5                   |               | 40 (8)              | 25 (5)                 |         |
| CT feature on admission |             |                     |                        |         |
| Fisher grade 3/4, %   |               | 100 (20)            | 100 (20)               | 1.000   |
| Risk factors, %       |               |                     |                        |         |
| Hypertension          |               | 55 (11)             | 30 (6)                 | 0.200   |
| Smokers               |               | 10 (2)              | 0 (0)                  | 0.487   |
| Outcome               |               |                     |                        |         |
| Infarction, %         |               | 65 (13)             | 0 (0)                  | <0.001* |
| mRS 3 mo, median      |               | 2                   | 1                      | 0.034*  |
| mRS 3 mo, >2          |               | 45 (9)              | 15 (3)                 | 0.082   |
| mRS 3 mo, ≤2          |               | 55 (11)             | 85 (17)                |         |

Data are % (N), mean±SD, or median. CT indicates computed tomography; DCI, delayed cerebral infarction; mRS 3 mo, modified Rankin Scale 3-month; SAH, subarachnoid hemorrhage; WFNS, World Federation of Neurosurgical Societies. *P<0.05.

Delayed Cerebral Infarction
DCI is defined as a new cerebral infarction identified on computed tomography after exclusion of procedure-related infarctions. Procedure-related infarction was defined as new hypodensity appearing on the posttreatment computed tomography at 12 to 24 hours after aneurysm treatment. All recruited patients had delayed computed tomography of the brain at 2 to 3 weeks after presentation available for assessment. The diagnoses of DCI were made by consensus of 2 neuroradiologists.

Quantitative Polymerase Chain Reaction
Peripheral blood samples were obtained using EDTA tubes with standard procedures. Samples were placed on ice immediately and centrifuged at 1000g for 15 minutes at 4°C. Plasma fraction was aliquoted and stored at −80°C. RNA isolation and quantitative real-time polymerase chain reaction were performed, as described previously. Briefly, serum was prepared by adding 20% wt/vol CaCl₂ (Sigma-Aldrich) into the plasma samples at a ratio of 1:100, followed by clotting overnight and centrifugation. Total RNA was isolated from pooled serum samples using the miRNeasy Serum/Plasma Kit following the manufacturer’s instructions (Qiagen). The quantity of the extracted RNA was determined using a Nanodrop 2000 UV-Vis spectrophotometer (Thermo Scientific). The results were analyzed with the Applied Biosystems SDS software for Ct values and melting curve analyses. Of the 99 possible deregulated miRNAs associated with SAH, we selected the top 20 differentially regulated miRNAs between SAH patients with and without DCI; the top 8 miRNAs between SAH patients with DCI and healthy controls (2 overlapped); and miR-132-3p and miR-324-3p, the 2 previously reported circulating miRNAs in SAH patients from a total of 28 unique miRNAs for expression analysis. Table S1 is the list of quantitative polymerase chain reaction primers for miRNA expression profiling used in this study.

Statistical Analyses
Heat maps with hierarchical clustering and principal component analysis (PCA) were computed and visualized using the functions pHeatmap and pcomp, respectively, from the stats package for R version 3.0.1 (R Foundation for Statistical Computing). The R package pROC was used to plot and visualize receiver operating characteristic (ROC) curves to compute the area under the curve (AUC) and confidence intervals to evaluate the performance of the miRNA-based
miRNA in SAH-Associated DCI  
Lu et al

Statistically significant, and P (Tables 1 and 2). A value of with and without DCI using ROC analysis.34 Based on the performance of individual miRNAs to characterize SAH patients

The possibility of characterizing the disease subtypes using a minimal overlap of the 2 SAH subtypes, highlighting the subtypes (Figure 1B). PCA generated 2 distinct clusters with recognition to evaluate the distance connectivity of the 2 SAH subtypes (Figure 1A) and used PCA for pattern array analyses to quantify the expression levels of the 28 miRNAs derived from the 3 cohorts for exploratory

Given the distinctive pattern of miRNA expression profile between the 2 SAH subtypes, we initially evaluated the performance of individual miRNAs to characterize SAH patients with and without DCI using ROC analysis.34 Based on the sensitivity (true-positive rate) and specificity (false-positive rate) at varied threshold levels, the ROC analysis allowed us to select possibly optimal models for DCI discrimination.

We plotted the ROC curves for individual miRNAs from a total of 28 miRNAs according to their quantitative polymerase

We postulated that a combination of miRNAs would show characteristics for SAH with versus without DCI. We examined each miRNA combination of the 28 SAH-associated miRNAs, based on their sensitivity, specificity, and accuracy, using the 4 different classifier algorithms: linear support vector machine, nonlinear support vector machine, linear discriminant analysis, and logistic regression. The results are shown

AUC indicates area under the receiver operating characteristic curve.

Table 2. Performance of Individual MicroRNAs in Subarachnoid Hemorrhage Subtypes Classification

| miRNA     | AUC (95% CI)        | P Value |
|-----------|---------------------|---------|
| miR-4532  | 0.9475 (0.873–1.000)| 1.80E-08|
| miR-4793  | 0.93 (0.854–1.000)  | 2.14E-07|
| miR-1290  | 0.9525 (0.897–1.000)| 3.54E-07|
| miR-421   | 0.9175 (0.836–0.999)| 3.24E-06|
| miR-4492  | 0.895 (0.798–0.992) | 7.26E-06|
| miR-574   | 0.8625 (0.753–0.972)| 2.70E-05|
| miR-4689  | 0.86 (0.748–0.972)  | 2.87E-05|
| miR-4449  | 0.855 (0.741–0.969) | 3.16E-05|
| miR-93-5p | 0.835 (0.713–0.957) | 9.31E-05|
| miR-1268  | 0.795 (0.648–0.942) | 2.69E-04|
| miR-4497  | 0.8175 (0.685–0.950)| 3.77E-04|
| miR-3178  | 0.7875 (0.640–0.935)| 6.71E-04|
| miR-4674  | 0.765 (0.602–0.928) | 7.94E-04|
| miR-297   | 0.81 (0.671–0.949)  | 9.15E-04|
| miR-4463  | 0.815 (0.677–0.953) | 1.03E-03|
| miR-4690  | 0.7325 (0.568–0.897)| 5.38E-03|
| miR-4459  | 0.73 (0.560–0.900)  | 9.75E-03|
| miR-3651  | 0.705 (0.534–0.876)| 1.99E-02|
| miR-132   | 0.7125 (0.547–0.878)| 2.37E-02|
| miR-4433  | 0.67 (0.497–0.843)  | 7.86E-02|
| miR-339-5p| 0.6625 (0.490–0.835)| 7.95E-02|
| miR-125a  | 0.66 (0.483–0.837)  | 1.58E-01|
| miR-324   | 0.59875 (0.419–0.779)| 2.29E-01|
| miR-4440  | 0.5675 (0.385–0.750)| 4.16E-01|
| miR-4454  | 0.5075 (0.321–0.694)| 7.66E-01|
| miR-27b   | 0.49 (0.304–0.676)  | 8.62E-01|
| miR-27a   | 0.485 (0.299–0.671) | 9.24E-01|
| miR-15a   | 0.525 (0.339–0.711) | 9.52E-01|

Results

Hierarchical Clustering of miRNAs Associated With SAH With and Without DCI

We performed unsupervised hierarchical clustering to graphically represent the averaged microarray expressions of the 28 miRNAs derived from the 3 cohorts for exploratory analysis: healthy controls, SAH with DCI, and SAH without DCI. The dendrogram represented with the heat map showing the entire data matrix of the 3 cohorts indicated that the expression profiles of the 28 miRNAs among the 3 cohorts are distinctive, with clear cluster dissimilarity (results not shown), and suggested that a subset of the 28 miRNAs could characterize SAH patients with DCI from those without DCI.

We next performed quantitative polymerase chain reaction array analyses to quantify the expression levels of the 28 SAH-associated miRNAs (Figure 1A) and used PCA for pattern recognition to evaluate the distance connectivity of the 2 SAH subtypes (Figure 1B). PCA generated 2 distinct clusters with minimal overlap of the 2 SAH subtypes, highlighting the possibility of characterizing the disease subtypes using a miRNA combination.

miRNAs That Characterized SAH With DCI

We plotted the ROC curves for individual miRNAs from a total of 28 miRNAs according to their quantitative polymerase chain reaction–measured expression levels in the SAH groups with DCI and without DCI. The results are shown in Table 2. The AUCs of 8 miRNAs can achieve >85% (P<0.0005), and 4 of them—miR-4532, miR-4793, miR-1290, and miR-421—are >90%. For miR-1290, the AUC, specificity, and sensitivity were 95.3% (95% CI 0.897–1.000), 0.900, and 0.850, respectively, at a cutoff level of 8.653.

We postulated that a combination of miRNAs would show characteristics for SAH with versus without DCI. We examined each miRNA combination of the 28 SAH-associated miRNAs, based on their sensitivity, specificity, and accuracy, using the 4 different classifier algorithms: linear support vector machine, nonlinear support vector machine, linear discriminant analysis, and logistic regression. The results are shown
in Figure 2. All 3 readouts improved while increasing the number of miRNAs contained in each classifier.

We next performed the LASSO regression analysis involving penalizing the absolute size of the regression coefficients to determine the accuracy using a combination of miRNAs to distinguish SAH with and without DCI (Figure 3A). The results are shown in Figure 3B. ROC analysis indicated that the AUC achieved 100% (95% CI 1–1, \( P < 0.0001 \)) using 4 miRNAs, namely, miR-4463, miR-4532, miR-4793, and miR-1290, according to the LASSO regression model and the ROC analysis using the following formula: 
\[
\ln(Y/1-Y) = -4.29759355 + \text{miR-4463} \times 0.16589606 + \text{miR-4532} \times 0.43352951 + \text{miR-4793} \times 0.09569081 + \text{miR-1290} \times 0.2602013.
\]
PCA analysis indicated that the 4-miRNA combination could produce 2 nonoverlapping clusters to unambiguously differentiate SAH with and without DCI.

Interestingly, the 4 miRNAs were strongly repressed in SAH blood samples with DCI but activated in the non-DCI
samples compared with healthy controls (Figure 4). The biphasic expressions of these 4 miRNAs in the SAH patients with or without DCI also provided discrimination with an AUC >80% by ROC analyses to differentiate the SAH patients with or without DCI from healthy controls with AUCs of 99.3% (95% CI 0.977–1, \( P < 0.0001 \)) and 82.0% (95% CI 0.685–0.955, \( P < 0.0005 \)), respectively. The results supported the disease-specific nature of the 4 miRNAs.

Discussion

In this study, both the heat map and PCA analysis suggested that a combination of miRNAs could characterize SAH patients with DCI from those without DCI (Figure 1). We demonstrated that a number of miRNAs could offer good to excellent discriminatory power between SAH with and without DCI (Table 2). LASSO regression analysis and PCA analysis further demonstrated that a 4-miRNA combination (miR-4463, miR-4532, miR-4793, and miR-1290) could produce 2 nonoverlapping clusters to unambiguously differentiate SAH with and without DCI, with an AUC of 100% (95% CI 1–1, \( P < 0.0001 \)) (Figure 3). In addition, the classifier can also distinguish the healthy controls from each SAH subtype with or without DCI (Figure 4), highlighting its disease specificity.

Recently, studies of miRNAs in serum and cerebrospinal fluid identified biomarkers associated with occurrence of SAH, including miR-92a, let-7b, miR-204-5p, miR-223-3p, miR-337-5p, miR-451a, miR-489, miR-508-3p, miR-514-3p, miR-516-5p,
miR-548, miR-599, miR-937, miR-1224-3p and miR-1301. Our previous genomewide serum miRNA expression profiling also suggested that miR-132-3p and miR-324-3p could be potential biomarkers for SAH. Styllis and others investigated cerebrospinal fluid miRNAs in 10 SAH patients with and 10 without cerebral vasospasm and identified miR-

Figure 3. A, LASSO (least absolute shrinkage and selection operator) regression analysis, (B) the box-and-whisker plot (upper panel), receiver operating characteristic (ROC) curve (bottom left panel), and principal component analysis (bottom right panel) of the 4 microRNAs (miR-4532, miR-4463, miR-1290, and miR-4793-3p) in the subarachnoid hemorrhage groups with and without delayed cerebral infarction (DCI) in the classification algorithm. AUC indicates area under the curve.
miRNAs associated with cerebral vasospasm, miR-516a-5p, miR-566, and miR-1197 as potential biomarkers for cerebral vasospasm but not DCI. The identification of miRNAs associated with DCI after SAH has been understudied, hindering understanding and development of therapeutic strategies for DCI after SAH.

Given that the downstream targets and functional specificities of the 4 miRNAs (miR-4463, miR-4532, miR-4793, and miR-1290) are uncharacterized, we performed gene-centric analysis to identify their common and unique potential targets using 3 algorithms: TargetScan, miRanda, and PITA. Considerable overlaps of their downstream targets were shared by these 4 miRNAs, inferring that these miRNAs may act on common regulatory pathways mediated by their shared targets for the development of DCI after SAH (Figure 5). KEGG (Kyoto Encyclopedia of Genes and Genomes) and gene ontology analyses also consistently revealed that the targets of these 4 miRNAs associated with multiple developmental pathways including Wnt signaling pathway, hedgehog and oxytocin signaling pathways. These pathways are broadly involved in neurogenesis and nervous system development, suggesting that these 4 miRNAs may function as causative factors in DCI pathology associated with SAH (Table S2).

The 4 novel miRNAs—miR-4463, miR-4532, miR-4793, and miR-1290—have not been associated with SAH pathology. miR-4532 has been associated with breast cancer cell chemotherapy resistance. miR-4793 expression was elevated in liver metastases of sporadic colorectal cancer patients. miR-1290 has been widely associated with different types of cancers including colorectal cancer, cervical cancer, non–small cell lung cancer, breast cancer, hepatocellular carcinoma, gastric cancer, laryngeal carcinoma, lymphoblastic leukemia, esophageal squamous cell carcinoma, lung adenocarcinoma, prostate cancer, pancreatic cancer, and bladder carcinoma, as well as oral submucosal fibrosis, nonalcoholic
fatty liver disease, and chronic rhinosinusitis.\(^ {39-41} \) miR-4463 has been shown to serve as a biomarker for a common female reproductive disease called polycystic ovary syndrome and arteriosclerosis obliterans.\(^ {42,43} \)

Our current study had several limitations. First, our SAH-associated miRNA pools were obtained from patients at day 7 after SAH, which is a commonly reported time point for etiological study of DCI. Time-course expression profiling of serum miRNAs in SAH patients at multiple time points could investigate stage/time-specific miRNAs for SAH with or without DCI in future. Second, the current study did not investigate the pathophysiological roles of these miRNAs in DCI after SAH and propose therapeutic targets. Third, the sample size was small, and subgroup analyses such as admission neurological grade and the mode of aneurysm treatment were not performed. In addition, the sample size did not allow meaningful matched group analyses. Fourth, 28 miRNAs were selected to find the important miRNAs involved to guide further mechanistic study to find therapeutic targets. Fifth, there could be a limitation in diagnosing DCI with computed tomography hypodensity, as magnetic resonance imaging was not routinely performed in our patients. Sixth, validation with another patient cohort would be warranted to confirm our findings. Seventh, cerebrospinal fluid samples were not collected for miRNA analysis and should be considered in future study.

Our work was important as it provides an understanding of the characteristics of circulating miRNAs associated with DCI after SAH. The 4 miRNAs might play distinct roles in DCI after SAH. Further investigations into their roles might bring new therapeutic targets for DCI after SAH. Future time-course studies of miRNAs might also identify SAH patients at risk of subsequent DCI development for stringent monitoring in neurocritical care units and timely treatment.

Conclusions

We found a 4-miRNA combination (miR-4532, miR-4463, miR-1290, and miR-4793) that characterized SAH patients with DCI. The findings could guide future mechanistic study to develop therapeutic targets.

Sources of Funding

This work was supported by the Chinese University of Hong Kong Direct Grant for Research (MD11782) and a collaborative funding from SDIVF R&D Centre.

Disclosures

None.

References

1. Lovelock CE, Rinkel GJ, Rothwell PM. Time trends in outcome of subarachnoid hemorrhage: population-based study and systematic review. Neurology. 2010;74:1494–1501.

2. Al-Khindi T, Macdonald RL, Schweizer TA. Cognitive and functional outcome after aneurysmal subarachnoid hemorrhage. Stroke. 2010;41:e519–e536.

3. Wong GK, Lam S, Ngai K, Wong A, Mok V, Poon WS; Cognitive Dysfunction after Aneurysmal Subarachnoid Haemorrhage I. Evaluation of cognitive impairment by the montreal cognitive assessment in patients with aneurysmal subarachnoid haemorrhage: prevalence, risk factors and correlations with 3 month outcomes. J Neurol Neurosurg Psychiatry. 2012;83:1112–1117.

4. Wong GK, Poon WS, Boet R, Chan MT, Gin T, Ng SC, Zee BC. Health-related quality of life after aneurysmal subarachnoid hemorrhage: profile and clinical factors. Neurosurgery. 2011;68:1556–1561; discussion 1561.

5. Scott RB, Eccles F, Molyneux AJ, Kerr RS, Rothwell PM, Carpenter K. Improved cognitive outcomes with endovascular coiling of ruptured intracranial aneurysms: neuropsychological outcomes from the International Subarachnoid Aneurysm Trial (ISAT). Stroke. 2010;41:1743–1747.

6. Wong GK, Wun TW, Yee YW, Zhu XL, Poon WS. Incidence and mortality of spontaneous subarachnoid hemorrhage in Hong Kong from 2002 to 2010: a Hong Kong hospital authority clinical management system database analysis. World Neurosurg. 2014;81:552–556.

7. de Rooij NK, Linn FH, van der Plas JA, Algra A, Rinkel GJ. Incidence of subarachnoid haemorrhage: a systematic review with emphasis on region, age, gender and time trends. J Neurol Neurosurg Psychiatry. 2007;78:1365–1372.
8. Passier PE, Visser-Meily JM, Rinkel GJ, Lindeman E, Post MW. Life satisfaction and return to work after aneurysmal subarachnoid hemorrhage. J Stroke Cerebrovasc Dis. 2011;20:324–329.

9. Schmidt JM, Rincon F, Fernandez A, Resor C, Kowalski RG, Claassen J, Connolly ES, Fitzsimmons BF, Mayer SA. Cerebral infarction associated with acute subarachnoid hemorrhage. Neurocrit Care. 2007;7:10–17.

10. Dorsch NW, King MT. A review of cerebral vasospasm in aneurysmal subarachnoid hemorrhage part I: incidence and effects. J Clin Neuroradiol. 1994;11:19–26.

11. Ionita CC, Baker J, Graffagnino C, Alexander MJ, Friedman AH, Zaidat OO. 10. Dorsch NW, King MT. A review of cerebral vasospasm in aneurysmal subarachnoid hemorrhage: the effect of treatment modality and clinical implications. J Stroke Cerebrovasc Dis. 2010;19:110–115.

12. Vergouwen MD, Vermeulen M, van Gijn J, Rinkel GJ, Wijdicks EF, Muizelaar JP, Ionita CC, Baker J, Graffagnino C, Alexander MJ, Friedman AH, Zaidat OO. 10. Dorsch NW, King MT. A review of cerebral vasospasm in aneurysmal subarachnoid hemorrhage: the effect of treatment modality and clinical implications. J Stroke Cerebrovasc Dis. 2010;19:110–115.

13. Sundt TM Jr, Piepgras DG, Fode NC, Meyer FB. Giant intracranial aneurysms. J Stroke Cerebrovasc Dis. 2014;10:971–977.

14. Kassell NF, Torner JC, Jane JA, Haley EC Jr, Adams HP. The international cooperative study on the timing of aneurysm surgery. Part 2: surgical results. J Neurosurg. 1990;73:37–47.

15. Rabenstein AA, Friedman JA, Weigand SD, McClelland RL, Fulgham JR, Manno EM, Atkinson JL, Wijdicks EF. Predictors of cerebral infarction in aneurysmal subarachnoid hemorrhage. Stroke. 2004;35:1862–1866.

16. Doria JS, Oldham JS. Subarachnoid hemorrhage: an update. Anesthesiol Clin. 2016;34:577–600.

17. Zhou J, Zhang J. Identification of miRNA-21 and miRNA-24 in plasma as novel biological markers for intracranial aneurysms. J Am Heart Assoc. 2014;3:e000972. DOI: 10.1161/JAHA.114.000972.

18. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell. 2009;136:642–655.

19. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 2014;42:D68–D73.

20. Li C, Chen X, Huang J, Sun Q, Wang L. Clinical impact of circulating miR-26a, miR-1290 and miR-1290 in human intracranial aneurysms. J Neurol Clin Neurosci. 2015;1:116–154.

21. Araldi E, Chamorro-Jorganes A, van Solingen C, Fernandez-Hernando C, Suarez Y. Therapeutic potential of modulating microRNAs in atherosclerotic vascular disease. Curr Vasc Pharmacol. 2015;13:291–304.

22. Leung LY, Chan CP, Leung YK, Jiang HL, A brigio JM, Wang de F, Chungs JS, Rainer TH, Graham CA. Comparison of miR-124-3p and miR-16 for early diagnosis of hemorrhagic and ischemic stroke. Clin Chim Acta. 2014;433:139–144.

23. Zhu J, Zhang J. Identification of miRNA-21 and miRNA-24 in plasma as potential early stage markers of acute cerebral infarction. Mol Med Rep. 2014;10:971–976.

24. Heggermont WA, Heymans S. MicroRNAs are involved in end-organ damage during hypertension. Hypertension. 2012;60:1088–1093.

25. Jin H, Li C, Ge H, Jiang Y, Li Y. Circulating microRNA: a novel potential biomarker for early diagnosis of intracranial aneurysm rupture a case control study. J Transl Med. 2013;11:296.

26. Li P, Zhang Q, Wu X, Yang X, Zhang Y, Li Y, Jiang F. Circulating microRNAs serve as novel biological markers for intracranial aneurysms. J Am Heart Assoc. 2014;3:e000972. DOI: 10.1161/JAHA.114.000972.

27. Liu D, Han L, Wu X, Yang X, Zhang Q, Jiang F. Genome-wide microRNA changes in human intracranial aneurysms. BMC Neuro. 2014;14:188.

28. Jiang Y, Zhang M, He H, Chen J, Zeng H, Li J, Duan R. microRNA/miRNA profiling and regulatory network of intracranial aneurysm. BMC Med Genomics. 2013;6:36.

29. Su WX, Chan AH, Lu G, Lin M, Sze J, Zhou YJ, Poon WS, Liu Q, Zheng VZ, Wong GK. Circulating microRNA 132-3p and 324-3p profiles in patients after acute aneurysmal subarachnoid hemorrhage. PLoS One. 2015;10:e0144724.

30. Muller AH, Povlsen GK, Bang-Berthelsen CH, Kruse LS, Nielsen J, Warnke F, Edvinsson L. Regulation of microRNAs miR-30a and miR-143 in cerebral vasculature after experimental subarachnoid hemorrhage in rats. BMC Genomics. 2015;16:119.

31. Robin X, Turkc N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, Muller M. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12:77.

32. Li XJ, Chong Y, Guo ZW, Xie C, Yang JX, Zhang Q, Li SP, Xiong Y, Yuan Y, Min J, Jiang WH, Jie Y, Chen MS, Chen MX, Fang JH, Zeng C, Zhang Y, Guo RP, Wu Y, Lin G, Zheng L, Zhang SM. A serum miRNA classifier for early detection of hepatocellular carcinoma: a multicentre, retrospective, longitudinal biomarker identification study with a nested case-control study. Lancet Oncol. 2015;16:804–815.

33. Friedeman J, Hastie T, Tibshirani R. Regularization paths for generalized linear models via coordinate descent. J Stat Softw. 2010;33:1–22.

34. Haji-Tilaki K. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. Caspian J Intern Med. 2013;4:627–635.

35. Powers Cj, Dickerson R, Zhang SW, Rink C, Roy S, Sen CK. Human cerebrospinal fluid microRNA: temporal changes following subarachnoid hemorrhage. Physiol Genomics. 2016;48:361–366.

36. Stylli SS, Adamides AA, Kolde RM, Luwor RB, Ritchie DS, Ziogas J, Kaye AE. Circulating microRNA expression profiling of cerebrospinal fluid in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg. 2017;126:1131–1139.

37. Du L, Hu WY, Ai NM, Yeap SK, Ky H, Chan KG, Yin WF, Sathasivang DA, Liew WC, Tan SW, Ong HK, Cheong SK. MiRNA transcriptome profiling of spheroid-enriched cells with cancer stem cell properties in human breast MCF-7 cell line. Int J Biol Sci. 2016;12:427–446.

38. Sayagues JM, Corchete LA, Gutierrez ML, Sarasquete ME, Del Mar Abad M, Bengoechea O, Ferriman E, Anduaga MF, Del Carmen S, Iglesias M, Esteban C, Angoso M, Alcazar JA, Garcia J, Orfao A, Munoz-Bellvis L. Genomic characterization of liver metastases from colorectal cancer patients. Oncotarget. 2016;7:72908–72922.

39. Huang X, Yuan T, Liang M, Du M, Xia S, Dittmar R, Wang D, See W, Costello B, Quevedo F, Tan W, Nandy D, Bevan GH, Longenbach S, Sun Z, Lu Y, Wang T, Thibodeau SN, Boardman L, Kohli M, Wang L. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. Eur Urol. 2015;67:33–41.

40. Mao Y, Liu J, Zhang D, Li B. Micro-R1290 promotes cancer progression by targeting nuclear factor-1/X(NF-1/X) in esophageal squamous cell carcinoma (ESCC). BioMed Pharmacother. 2015;76:82–93.

41. Zhang WC, Chin TM, Yang H, Nga ME, Lunnpy DN, Lim EK, Sun LL, Pang VH, Leow YN, Malasuy SR, Lim PX, Lee JZ, Tan BJ, Syh-Chang N, Lim EH, Lim WT, Tan DS, Tan EH, Tai BC, Soo RA, Tam WL, Lim B. Tumour-initiating cell-specific miR-1246 and miR-1290 expression converge to promote non-small cell lung cancer progression. Nat Commun. 2016;7:11702.

42. He XM, Zheng YQ, Liu SZ, Liu Y, He YZ, Zhou XY. Altered plasma microRNAs as novel biomarkers for arteriosclerosis obliterans. J Atheroscler Thromb. 2016;23:196–206.

43. Ding CF, Chen WQ, Zhu YT, Bo YL, Hu HM, Zheng RH. Circulating microRNAs in patients with polycystic ovary syndrome. Hum Fertil (Camb). 2015;18:22–29.
SUPPLEMENTAL MATERIAL
| miRNA      | Forward primer sequence (5' to 3') | Reverse primer sequence (5' to 3') |
|------------|-----------------------------------|-----------------------------------|
| hsa-miR-4433 | GGAGCCAGTTGGGACAG               | GGTCAGGTTTTTTTTTTTTTTTTATGTC    |
| hsa-miR-93-5p | GCAAAGTGGCTTGTGGCTG            | GTCCAGGTTTTTTTTTTTTTTTCTACCT    |
| hsa-miR-339-5p | CAGTTCCCTGCTCCTCAG           | GGTCAGGTTTTTTTTTTTTTTTCCGT     |
| hsa-miR-3651-5p | CATAGCCCAGGCTGCTCCTG     | GGTCAGGTTTTTTTTTTTTTTTCAATG    |
| hsa-miR-27a  | CAGTTCACAGTGGCTAAGTTC         | CAGTTTTTTTTTTTTTTTGCAGAA       |
| hsa-miR-27b  | CAGTTCACAGTGGCTAAGTTC         | TCCAGGTTTTTTTTTTTTTTTGCAGA    |
| hsa-miR-15a  | CAGTAGCAGCACTAATGCT          | GGTCAGGTTTTTTTTTTTTTTTCTAC     |
| hsa-miR-125a | CCCTGAGACCCCCTTAACCT         | GGTCAGGTTTTTTTTTTTTTTTCCAC     |
| hsa-miR-4454 | GCAGGGATCCGAGCTCAC            | GGTCAGGTTTTTTTTTTTTTTTCCAC     |
| hsa-miR-132-3p | GCAGTAAACAGTCTACAGCCA      | GGTCAGGTTTTTTTTTTTTTTTCCAC     |
| hsa-miR-4463 | GCAGGAGACTGGGGGTG           | GTTTTTTTTTTTTTTTGGCCCAC       |
| hsa-miR-324  | AGAACATTCTATGGTGGTCTG        | GGTCAGGTTTTTTTTTTTTTTTCCAC     |
| hsa-miR-421  | GCAGATCAACACAGACATTAATGGG   | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-mir-4492 | GGCTGGGGC                | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-miR-4793 | TGCACTGTGAGTTGGCT          | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-miR-4689 | CAGTTGAGGAGACATGGGT         | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-miR-4449 | GGCGTGACGCCGCA             | TCCAGGTTTTTTTTTTTGCAGAC       |
| hsa-miR-1290 | CGCACTGGATTTTTTGATCA       | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-miR-4497 | TCCGGGACGGCTG            | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-miR-4690-5p | GACACGGCGAGGCT           | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-miR-4459 | GGAGGCGAGGGAGGT           | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-miR-4674 | GCTGGGCTCAGGGAC           | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-miR-1268 | GGCGTGCTGGGAGT         | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-miR-3178 | GGGCGCGCCGGA             | GTTTTTTTTTTTTTTTTGCAGAC       |
| hsa-miR-297  | GCAGATGATGTGTGCACTG         | AGTGTGTTTTTTTTTTTTTGCAGAC     |
| hsa-miR-574  | GTGAGTGTGTGTGTGTGAG       | CAGTTTTTTTTTTTTTTTTTGCAGAC     |
| hsa-miR-4532 | CCCGGGCGGAGCC            | CAGTTTTTTTTTTTTTTTTTGCAGAC     |
| hsa-miR-4440 | TCGGAGGCTTGGCT            | CAGTTTTTTTTTTTTTTTTTGCAGAC     |
| cel-miR-39  | GTCACCGGAGTGAAATCG        | CAGTTTTTTTTTTTTTTTTTGCAGAC     |
Table S2. Gene ontology and KEGG enrichment analyses of the targets of miR-4532, miR-4463, miR-1290 and miR-4793-3p

| MapID  | MapTitle                          | Pvalue    | AdjustedPv | x    | y    | n   | N   | EnrichDirect | GeneIDs                                                                 |
|--------|----------------------------------|-----------|------------|------|------|-----|-----|--------------|------------------------------------------------------------------------|
| map04310 | Wnt signaling pathway            | 0.004005563 | 0.521424353 | 5    | 20   | 61  | 1069| Over         | ENST00000268459 ENST00000272164 ENST00000374806 ENST00000374894 |
| map05217 | Basal cell carcinoma             | 0.008067372 | 0.521424353 | 3    | 8    | 61  | 1069| Over         | ENST00000272164 ENST00000297261 ENST00000374694 |
| map05205 | Proteolytic enzymes in cancer    | 0.013652665 | 0.521424353 | 6    | 36   | 61  | 1069| Over         | ENST00000259438 ENST00000272164 ENST00000297261 ENST00000355822 ENST00000374694 |
| map04340 | Hedgehog signaling pathway       | 0.015921354 | 0.521424353 | 3    | 10   | 61  | 1069| Over         | ENST00000308595 ENST00000297261 ENST00000255641 |
| map04924 | Renin secretion                  | 0.026893481 | 0.625634513 | 3    | 12   | 61  | 1069| Over         | ENST00000374806 ENST00000245457 ENST00000368680 |
| map04925 | Aldosterone synthesis and secretion | 0.028655016 | 0.625634513 | 2    | 5    | 61  | 1069| Over         | ENST00000302909 ENST00000368680 |
| map04724 | Glutamatergic synapse            | 0.049227337 | 0.80639532  | 3    | 15   | 61  | 1069| Over         | ENST00000308595 ENST00000382159 ENST00000374806 |
| map04921 | Oxytocin signaling pathway       | 0.049245516 | 0.80639532  | 4    | 25   | 61  | 1069| Over         | ENST00000174806 ENST00000270458 ENST00000263026 ENST00000368680 |
| map00750 | Vitamin B6 metabolism            | 0.057026275 | 0.830578942 | 1    | 1    | 61  | 1069| Over         | ENST00000225573 |
| map04120 | Ubiquitin mediated proteolysis    | 0.068326741 | 0.898333442 | 4    | 30   | 61  | 1069| Over         | ENST00000399816 ENST00000492996 ENST00000264033 ENST00000291582 |
| map04550 | Signaling pathways regulating pluripotency of stem cells | 0.100843751 | 0.898333442 | 3    | 20   | 61  | 1069| Over         | ENST00000272164 ENST00000231121 ENST00000374694 |
| map04745 | Phototransduction - fly          | 0.110919583 | 0.898333442 | 1    | 2    | 61  | 1069| Over         | ENST00000308595 |
| map04122 | Sulfur relay system              | 0.110919583 | 0.898333442 | 1    | 2    | 61  | 1069| Over         | ENST00000452446 |
| map04390 | Hippo signaling pathway          | 0.113045039 | 0.898333442 | 3    | 21   | 61  | 1069| Over         | ENST00000272164 ENST00000374694 ENST00000590071 |
| map05412 | Arthrythmogenic right ventricular cardiomyopathy (ARVC) | 0.146390899 | 0.898333442 | 2    | 12   | 61  | 1069| Over         | ENST00000559488 ENST00000270458 |
| map04392 | Hippo signaling pathway - multiple species | 0.161747985 | 0.898333442 | 1    | 3    | 61  | 1069| Over         | ENST00000590071 |
| map03030 | DNA replication                  | 0.161747985 | 0.898333442 | 1    | 3    | 61  | 1069| Over         | ENST00000308418 |
| map04916 | Melanogenesis                    | 0.187886844 | 0.898333442 | 2    | 14   | 61  | 1069| Over         | ENST00000272164 ENST00000374694 |
| map05414 | Dilated cardiomyopathy           | 0.187886844 | 0.898333442 | 2    | 14   | 61  | 1069| Over         | ENST00000559488 ENST00000270458 |
| map01100 | Metabolic pathways               | 0.201576085 | 0.898333442 | 6    | 177  | 61  | 1069| Under        | ENST00000225573 ENST00000216484 ENST00000244043 ENST00000339600 ENST00000340941 ENST00000258873 |
| map05410 | Hypertrophic cardiomyopathy (HCM) | 0.209219076 | 0.898333442 | 2    | 15   | 61  | 1069| Over         | ENST00000559488 ENST00000270458 |
| map00910 | Nitrogen metabolism              | 0.209715502 | 0.898333442 | 1    | 4    | 61  | 1069| Over         | ENST00000454127 |
| map00061 | Fatty acid biosynthesis           | 0.209715502 | 0.898333442 | 1    | 4    | 61  | 1069| Over         | ENST00000258873 |
| map04976 | Bile secretion                   | 0.209715502 | 0.898333442 | 1    | 4    | 61  | 1069| Over         | ENST00000261196 |
| map00600 | Sphingolipid metabolism          | 0.209715502 | 0.898333442 | 1    | 4    | 61  | 1069| Over         | ENST00000216484 |
| map04146 | Peroxisome                       | 0.230795625 | 0.898333442 | 2    | 16   | 61  | 1069| Over         | ENST00000396385 ENST00000295930 |
| map05032 | Morphine addiction               | 0.230795625 | 0.898333442 | 2    | 16   | 61  | 1069| Over         | ENST00000308595 ENST00000382159 |
| map04145 | Phagosome                        | 0.242197418 | 0.898333442 | 3    | 30   | 61  | 1069| Over         | ENST00000559488 ENST00000355896 ENST000003555622 |
map03022  Basal transcription factors  0.254980623  0.898333442  1  5  61  1069  Over  ENST00000607778
map05340  Primary immunodeficiency  0.254980623  0.898333442  1  5  61  1069  Over  ENST00000291582
map00514  Other types of O-glycan biosynthesis  0.254980623  0.898333442  1  5  61  1069  Over  ENST0000396177
map03013  RNA transport  0.274309765  0.898333442  2  18  61  1069  Over  ENST00000542526 ENST00000281950
map04660  T cell receptor signaling pathway  0.274309765  0.898333442  2  18  61  1069  Over  ENST00000374806 ENST00000264033
map04919  Thyroid hormone signaling pathway  0.28685044  0.898333442  2  19  61  1069  Over  ENST00000594488 ENST00000396671
map04978  Mineral absorption  0.2976932  0.898333442  1  6  61  1069  Over  ENST00000233202
map03440  Homologous recombination  0.2976932  0.898333442  1  6  61  1069  Over  ENST00000359321
map05031  Amphetamine addiction  0.2976932  0.898333442  1  6  61  1069  Over  ENST00000374806
map05134  Legionellosis  0.2976932  0.898333442  1  6  61  1069  Over  ENST00000355622
map04918  Thyroid hormone synthesis  0.2976932  0.898333442  1  6  61  1069  Over  ENST00000392256
map04341  Hedgehog signaling pathway - fly  0.2976932  0.898333442  1  6  61  1069  Over  ENST00000255641
map04720  Long-term potentiation  0.2976932  0.898333442  1  6  61  1069  Over  ENST00000374806
map04130  SNARE interactions in vesicular transport  0.2976932  0.898333442  1  6  61  1069  Over  ENST00000367568
map04020  Calcium signaling pathway  0.317778521  0.898333442  2  20  61  1069  Over  ENST00000282018 ENST00000374806
map04144  Endocytosis  0.318859077  0.898333442  4  45  61  1069  Over  ENST0000085095 ENST00000264033 ENST00000356056 ENST00000304032
map04080  Neuroactive ligand-receptor interaction  0.33714738  0.898333442  4  47  61  1069  Over  ENST00000282018 ENST00000590320 ENST00000245457 ENST00000396671
map04142  Lysosome  0.339329413  0.898333442  2  21  61  1069  Over  ENST00000233202 ENST00000356056
map04071  Sphingolipid signaling pathway  0.360683922  0.898333442  2  22  61  1069  Over  ENST00000216444 ENST00000590320
map03420  Nucleotide excision repair  0.376019682  0.898333442  1  8  61  1069  Over  ENST00000607778
map03008  Ribosome biogenesis in eukaryotes  0.376019682  0.898333442  1  8  61  1069  Over  ENST00000341162
map04215  Apoptosis - multiple species  0.376019682  0.898333442  1  8  61  1069  Over  ENST00000318407
map01212  Fatty acid metabolism  0.376019682  0.898333442  1  8  61  1069  Over  ENST00000258873
map05014  Amyotrophic lateral sclerosis (ALS)  0.376019682  0.898333442  1  8  61  1069  Over  ENST00000374806
map05144  Malaria  0.41189414  0.898333442  1  9  61  1069  Over  ENST00000355622
map00590  Arachidonic acid metabolism  0.41189414  0.898333442  1  9  61  1069  Over  ENST00000244043
map04662  B cell receptor signaling pathway  0.41189414  0.898333442  1  9  61  1069  Over  ENST00000374806
map04391  Hippo signaling pathway - fly  0.41189414  0.898333442  1  9  61  1069  Over  ENST00000590971
map04360  Axon guidance  0.422132398  0.898333442  3  32  61  1069  Over  ENST00000297261 ENST00000374806 ENST00000245323
map05200  Pathways in cancer  0.442258613  0.898333442  6  78  61  1069  Over  ENST0000032159 ENST000003272164 ENST00000297261 ENST00000264033 ENST00000245457 ENST00000374694
map03320  PPAR signaling pathway  0.445737967  0.898333442  1  10  61  1069  Over  ENST00000258873
map00071  Fatty acid metabolism  0.445737967  0.898333442  1  10  61  1069  Over  ENST00000258873
map04370  VEGF signaling pathway  0.445737967  0.898333442  1  10  61  1069  Over  ENST00000374806
map04922  Glucagon signaling pathway  0.445737967  0.898333442  1  10  61  1069  Over  ENST00000374806

- Glucagon signaling pathway
- VEGF signaling pathway
- Fatty acid metabolism
- Axon guidance
- Arachidonic acid metabolism
- Hedgehog signaling pathway - fly
- Long-term potentiation
- SNARE interactions in vesicular transport
- Calcium signaling pathway
- Endocytosis
- Neuroactive ligand-receptor interaction
- Lysosome
- Sphingolipid signaling pathway
- Nucleotide excision repair
- Ribosome biogenesis in eukaryotes
- Apoptosis - multiple species
- Fatty acid metabolism
- Amyotrophic lateral sclerosis (ALS)
- Malaria
- Arachidonic acid metabolism
- B cell receptor signaling pathway
- Hippo signaling pathway - fly
- Axon guidance
- Pathways in cancer
- PPAR signaling pathway
- Fatty acid metabolism
- VEGF signaling pathway
- Glucagon signaling pathway
map00280  Valine, leucine and isoleucine degradation  0.445737967  0.898333442  1  10  61  1069  Over  ENST00000340941
map04750  Inflammatory mediator regulation of TRP channels  0.445737967  0.898333442  1  10  61  1069  Over  ENST00000245437
map04114  Oocyte meiosis  0.445737967  0.898333442  1  10  61  1069  Over  ENST00000374806
map05512  Tuberculosis  0.454617174  0.902346208  3  36  61  1069  Over  ENST00000374806 ENST00000355622 ENST00000256458
map04010  MAPK signaling pathway  0.463989936  0.906592628  3  37  61  1069  Over  ENST00000256080 ENST00000374806 ENST00000270458
map04214  Apoptosis - fly  0.477664298  0.906592628  1  11  61  1069  Over  ENST00000313407
map05166  HTLV-I infection  0.504242149  0.906592628  3  41  61  1069  Over  ENST00000272164 ENST00000374806 ENST00000374694
map04823  Regulation of lipolysis in adipocytes  0.507780062  0.906592628  1  12  61  1069  Over  ENST00000368680
map05140  Leishmaniasis  0.507780062  0.906592628  1  12  61  1069  Over  ENST00000355622
map04920  Adipokine signaling pathway  0.507780062  0.906592628  1  12  61  1069  Over  ENST00000258873
map03015  mRNA surveillance pathway  0.536186521  0.906592628  1  13  61  1069  Over  ENST00000238714
map04512  ECM-receptor interaction  0.536186521  0.906592628  1  13  61  1069  Over  ENST00000559488
map04713  Circadian entrainment  0.562978588  0.906592628  1  14  61  1069  Over  ENST00000382159
map05133  Pertussis  0.562978588  0.906592628  1  14  61  1069  Over  ENST00000355622
map04727  GABAergic synapse  0.562978588  0.906592628  1  14  61  1069  Over  ENST00000382159
map05130  Pathogenic Escherichia coli infection  0.562978588  0.906592628  1  14  61  1069  Over  ENST00000355622
map03460  Fanconi anemia pathway  0.562978588  0.906592628  1  14  61  1069  Over  ENST00000249408
map04260  Cardiac muscle contraction  0.562978588  0.906592628  1  14  61  1069  Over  ENST00000270458
map05321  Inflammatory bowel disease (IBD)  0.562978588  0.906592628  1  14  61  1069  Over  ENST00000355622
map04721  Synaptic vesicle cycle  0.588247125  0.906592628  1  15  61  1069  Over  ENST00000263354
map04723  Retrograde endocannabinoid signaling  0.588247125  0.906592628  1  15  61  1069  Over  ENST00000382159
map04620  Toll-like receptor signaling pathway  0.588247125  0.906592628  1  15  61  1069  Over  ENST00000355622
map04726  Serotonergic synapse  0.588247125  0.906592628  1  15  61  1069  Over  ENST00000382159
map05100  Bacterial invasion of epithelial cells  0.612077225  0.921633523  1  16  61  1069  Over  ENST00000264033
map05012  Parkinson's disease  0.612077225  0.921633523  1  16  61  1069  Over  ENST00000339600
map05010  Alzheimer's disease  0.642889256  0.944927224  2  24  61  1069  Over  ENST00000374806 ENST00000339600
map04810  Insulin signaling pathway  0.656299976  0.944927224  2  26  61  1069  Over  ENST00000452015 ENST00000264033
map04022  cGMP-PKG signaling pathway  0.656299976  0.944927224  2  26  61  1069  Over  ENST00000374806 ENST00000368680
map04150  mTOR signaling pathway  0.663613012  0.944927224  2  27  61  1069  Over  ENST00000272164 ENST00000374694
map04380  Osteoclast differentiation  0.663613012  0.944927224  2  27  61  1069  Over  ENST00000359448 ENST00000374806
map04024  cAMP signaling pathway  0.687211007  0.96785942  2  30  61  1069  Over  ENST00000245437 ENST00000368680
map04062  Chemo kinase signaling pathway  0.695449691  0.96785942  2  31  61  1069  Under  ENST00000382159
map05034  Alcoholism  0.716617786  0.96785942  1  35  61  1069  Under  ENST00000382159
map04015  Rap1 signaling pathway  0.716617786  0.96785942  1  35  61  1069  Under  ENST00000359448
| Pathway Name                                      | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Gene ID 1   | Gene ID 2   | Gene ID 3   | Gene ID 4   | Gene ID 5   |
|--------------------------------------------------|---------|---------|---------|---------|---------|-------------|-------------|-------------|-------------|-------------|
| Focal adhesion                                   | 0.716   | 0.968   | 1       | 3       | 34      | 61          | 1069        | Under       | ENST00000559488 |
| PI3K-Akt signaling pathway                       | 1       | 1       | 3       | 60      | 61      | 1069        | Under       | ENST00000559488 ENST00000382159 ENST00000355622 |
| HIF-1 signaling pathway                          | 1       | 1       | 17      | 61      | 1069    | Over        | ENST00000355622 |
| NF-kappa B signaling pathway                     | 1       | 1       | 17      | 61      | 1069    | Over        | ENST00000355622 |
| Salmonella infection                             | 1       | 1       | 17      | 61      | 1069    | Over        | ENST00000355622 |
| Purine metabolism                                | 1       | 1       | 20      | 61      | 1069    | Under       | ENST00000368680 |
| Cholinergic synapse                               | 1       | 1       | 18      | 61      | 1069    | Under       | ENST00000382108 |
| Platelet activation                              | 1       | 1       | 23      | 61      | 1069    | Under       | ENST00000554988 |
| Chagas disease (American trypanosomiasis)        | 1       | 1       | 20      | 61      | 1069    | Under       | ENST00000355622 |
| Vascular smooth muscle contraction               | 1       | 1       | 18      | 61      | 1069    | Under       | ENST00000368680 |
| Toxoplasmosis                                    | 1       | 1       | 31      | 61      | 1069    | Under       | ENST00000355622 |
| Ras signaling pathway                            | 1       | 1       | 2        | 42      | 61      | 1069        | ENST0000125080 ENST00000382108 |
| Insulin resistance                               | 1       | 1       | 18      | 61      | 1069    | Under       | ENST00000452015 |
| MicroRNAs in cancer                              | 1       | 1       | 23      | 61      | 1069    | Under       | ENST00000554988 |
| Non-alcoholic fatty liver disease (NAFLD)        | 1       | 1       | 26      | 61      | 1069    | Under       | ENST0000039600 |
| Huntington's disease                             | 1       | 1       | 29      | 61      | 1069    | Under       | ENST0000039600 |
| Neurotrophin signaling pathway                   | 1       | 1       | 20      | 61      | 1069    | Under       | ENST00000256458 |
| Hematopoietic cell lineage                       | 1       | 1       | 18      | 61      | 1069    | Under       | ENST00000559488 |
| Measles                                          | 1       | 1       | 18      | 61      | 1069    | Under       | ENST00000355622 |
| Rheumatoid arthritis                             | 1       | 1       | 25      | 61      | 1069    | Under       | ENST00000355622 |
| Tight junction                                    | 1       | 1       | 31      | 61      | 1069    | Under       | ENST00000216181 |
| Olfactory transduction                           | 1       | 1       | 55      | 61      | 1069    | Under       | ENST00000308595 ENST00000382159 ENST00000315453 |
| Natural killer cell mediated cytotoxicity        | 1       | 1       | 20      | 61      | 1069    | Under       | ENST00000374806 |
| ErbB signaling pathway                           | 1       | 1       | 20      | 61      | 1069    | Under       | ENST00000264033 |
| Influenza A                                      | 1       | 1       | 26      | 61      | 1069    | Under       | ENST00000355622 |
| Hepatitis B                                      | 1       | 1       | 21      | 61      | 1069    | Under       | ENST00000355622 |
| Amebiasis                                        | 1       | 1       | 18      | 61      | 1069    | Under       | ENST00000355622 |
| Transcriptional misregulation in cancer          | 1       | 1       | 23      | 61      | 1069    | Under       | ENST00000296921 |
| Regulation of actin cytoskeleton                 | 1       | 1       | 41      | 61      | 1069    | Under       | ENST00000559488 ENST00000382108 |
| Microbial metabolism in diverse environments     | 1       | 1       | 26      | 61      | 1069    | Under       | ENST000001225573 |
| AMPK signaling pathway                            | 1       | 1       | 20      | 61      | 1069    | Under       | ENST00000263026 |
| Adrenergic signaling in cardiomyocytes           | 1       | 1       | 23      | 61      | 1069    | Under       | ENST00000270458 |
| Oxidative phosphorylation                        | 1       | 1       | 22      | 61      | 1069    | Under       | ENST0000039600 |
| Chronic myeloid leukemia                         | 1       | 1       | 19      | 61      | 1069    | Under       | ENST00000264033 |
| Dopaminergic synapse                              | 1       | 1       | 19      | 61      | 1069    | Under       | ENST00000382108 |