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Supplemental information

Silencing of lncRNA XIST impairs angiogenesis and exacerbates cerebral vascular injury after ischemic stroke

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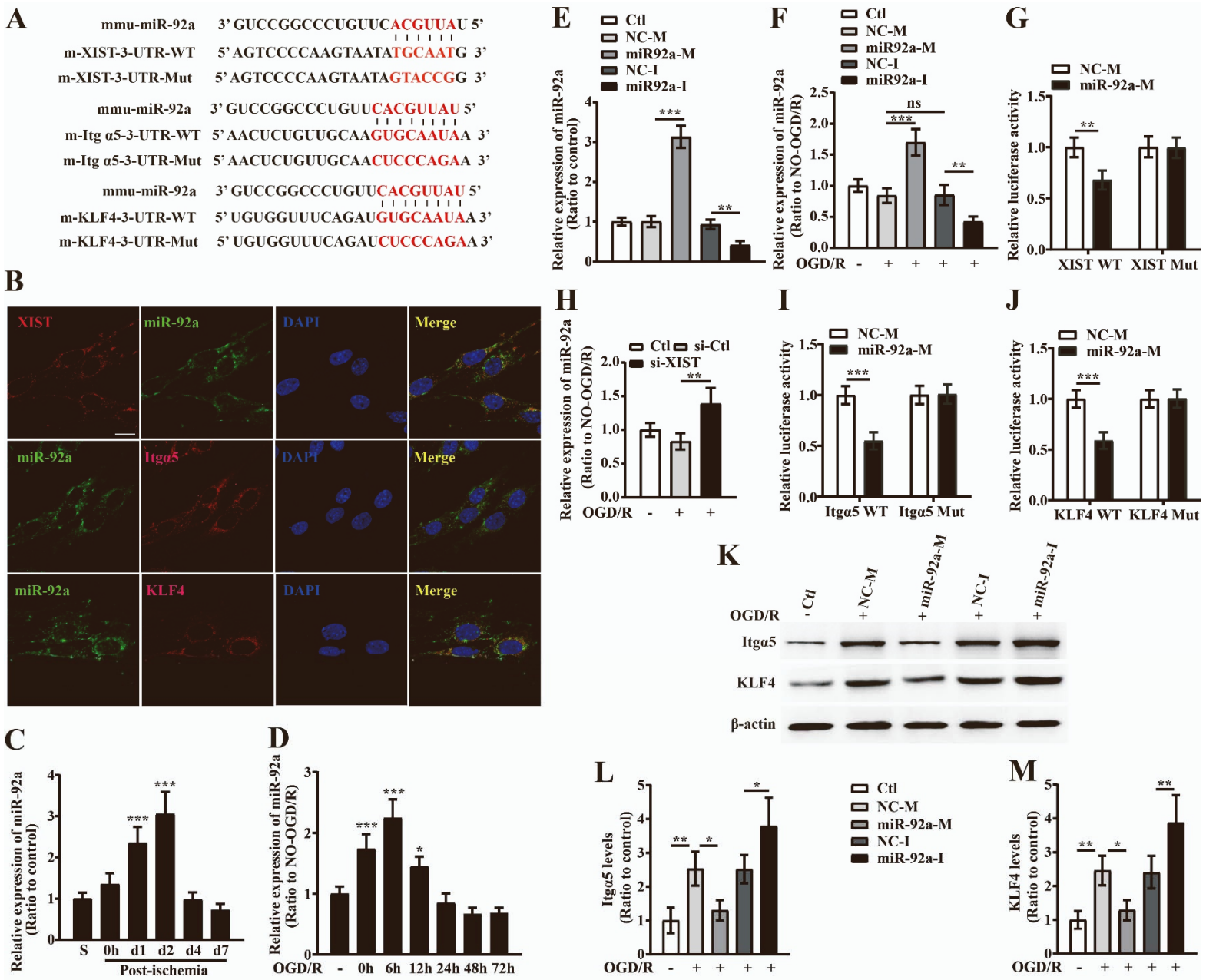
A
mmu-miR-92a 3' GUCCGGCCCUGUUCACGUUAU 5'
m-XIST-3-UTR-WT 5' AGTCCCCAAGTAATATGCAATG 3'
m-XIST-3-UTR-Mut 5' AGTCCCCAAGTAATAGTACCGG 3'
mmu-miR-92a 3' GUCCGGCCCUGUUCACGUUAU 5'
m-Itg α5-3-UTR-WT 5' AACUCUGUUGCAAGUGCAAUAA 3'
m-Itg α5-3-UTR-Mut 5' AACUCUGUUGCAACUCCCAGAA 3'
mmu-miR-92a 3' GUCCGGCCCUGUUCACGUUAU 5'
m-KLF4-3-UTR-WT 5' UGUGGUUUCAGAUGUGCAAUAA 3'
m-KLF4-3-UTR-Mut 5' UGUGGUUUCAGAUCUCCCAGAA 3'
B
XIST
miR-92a
DAPI
Merge
miR-92a
Itgα5
DAPI
Merge
miR-92a
KLF4
DAPI
Merge
C
Relative expression of miR-92a (Ratio to control)
S 0h d1 d2 d4 d7
Post-ischemia
D
Relative expression of miR-92a (Ratio to NO-OGD/R)
OGD/R - 0h 6h 12h 24h 48h 72h
E
Ctl
NC-M
miR92a-M
NC-I
miR92a-I
Relative expression of miR-92a (Ratio to control)
F
Ctl
NC-M
miR92a-M
NC-I
miR92a-I
Relative expression of miR-92a (Ratio to NO-OGD/R)
ns
OGD/R - + + + +
G
NC-M
miR-92a-M
Relative luciferase activity
XIST WT XIST Mut
H
Ctl si-Ctl
si-XIST
Relative expression of miR-92a (Ratio to NO-OGD/R)
OGD/R - + +
I
NC-M
miR-92a-M
Relative luciferase activity
Itgα5 WT Itgα5 Mut
J
NC-M
miR-92a-M
Relative luciferase activity
KLF4 WT KLF4 Mut
K
OGD/R - Ctl + NC-M + miR-92a-M + NC-I + miR-92a-I
Itgα5
KLF4
β-actin
L
Itgα5 levels (Ratio to control)
OGD/R - + + + +
Ctl
NC-M
miR-92a-M
NC-I
miR-92a-I
M
KLF4 levels (Ratio to control)
OGD/R - + + + +

Figure S1. The cellular localization and interactive relationship between lncRNA XIST, miR-92a and Itgα5 or KLF4

A, Predicted binding sites of lncRNA XIST with miR-92a, and miR-92a with Itgα5 or KLF4. **B**, RNA FISH assay showed that lncRNA XIST colocalized with miR-92a and miR-92a had a colocalization relationship with Itgα5 or KLF4 in bEnd3 cells. **C**, The expression of miR-92a in the ipsilateral ischemic cerebral cortex from sham-operated mice (S, control) or mice at days 0, 1, 2, 4, and 7 post-ischemia was examined by qPCR. Results are expressed as the mean ± standard deviation (n =6 per experimental group). Note that cerebral ischemia induced a strong increase in the expression of miR-92a in the ischemic hemisphere, reaching a peak at day 2 and then declining at day 4. ***$P < 0.001$ compared with sham control. **D**, qRT-PCR analysis for miR-92a levels after BECs was subject to OGD/R. Results are expressed as the mean ± standard deviation (n =4 per experimental group). Note that OGD/R induced a significant increase in the expression of miR-92a in bEnd3 cells, with this effect maximal 6 h post-restoration. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control. **E&F**, Upregulation or downregulation of miR-92a was achieved by transfection with miR-92a mimics (miR-92a-M) or inhibitors (miR-92a-I) in bEnd3 cells in normoxia (E) or at 24 h restoration from OGD (F) and the efficiency were validated by RT-qPCR. **$P < 0.01$, ***$P < 0.001$; ns, not significant. **G**, Dual-luciferase report assay was conducted to detect luciferase activity after co-transfection of HEK293T cells with XIST-wild type (WT) or XIST-mutant (Mut) and normal control mimics (NC-M), miR-92a-M. **$P<0.01$, n=4. **H**, miR-92a expression was measured using qRT-PCR after transfection with si-Ctl or si-XIST at 24 h restoration from OGD. Results are expressed as the mean ± standard deviation (n =4 per experimental group). Note that miR-92a levels were significantly elevated in si-XIST-treated cells as compared to that of the si-Ctl-treated cells after 24 h restoration. **$P<0.01$.

I&J, Dual-luciferase report assay revealed that miR-92a mimic (miR-92a-M) reduced the luciferase activity of Itgα5-WT (I) or KLF4-WT (J), but not of Itgα5-Mut (I) or KLF4 Mut (J). ***P<0.001, n=4. **K**, The expression of Itgα5 and KLF4 was measured using western blot in bEnd3 cells transfected with miR-92a-M or miR-92a-I at 24 h restoration from OGD. **L-M**, Bar graphs show the quantitative analyses of western blots as ratios of Itgα5/β-actin (**L**) and KLF4/β-actin (**M**) (n=4 per experimental group). Note that overexpression of miR-92a (miR-92a-M) decreased, but inhibition of miR-92a (miR-92a-I) increased the expression of Itgα5 and KLF4 in the bEnd3 cells at 24 h restoration from OGD**.** *P<0.05, **P<0.01

Supplementary Table 1. Baseline clinical characteristics of acute stroke patients and healthy controls

Characteristics	Controls (n=60)	CIS (n=77)	*P* value
Age mean ±SD (years)	67.52±11.43	68.19±12.25	0.744
Sex Male n (%)	31 (51.67 %)	41 (53.25%)	0.865
Hypercholesterolemia n (%)	16 (26.67 %)	39 (50.65 %)	**0.005**
Hypertension n (%)	28 (46.67 %)	53 (68.83 %)	**0.014**
Diabetes n (%)	5 (8.33 %)	21 (27.27 %)	**0.008**
Active Smoker n (%)	12 (20 %)	23 (29.87 %)	0.237
Alcohol n (%)	10 (16.67 %)	16 (20.78%)	0.662
Atrial fibrillation n (%)	5 (8.33 %)	23 (29.87 %)	**0.002**
Coronary disease n (%)	-	13 (16.88 %)	-
Previous antiplatelet treatment n (%)	14 (23.33 %)	24 (31.17 %)	0.341
Previous treatment with statin n (%)	15 (25 %)	28 (36.36 %)	0.195

Values are shown as number (%), mean ± standard deviation (SD).