OMTN, Volume *26*

Supplemental information

Suppression of endothelial miR-22 mediates non-small cell lung cancer cell-induced angiogenesis

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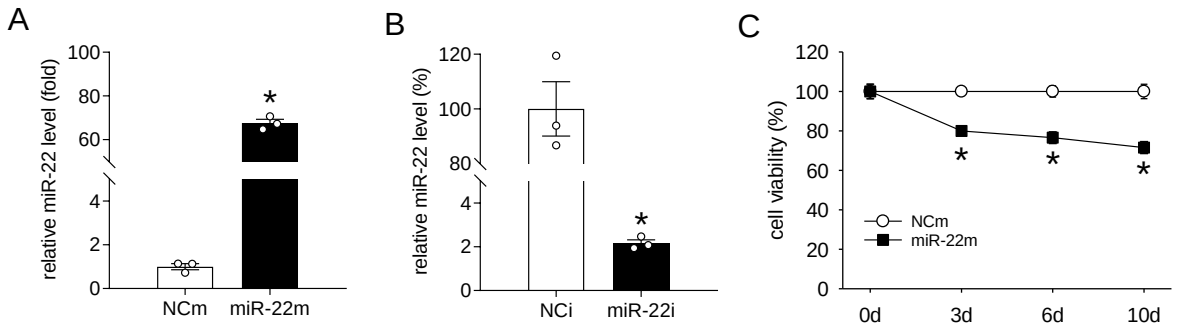


Figure S1. Expression of miR-22 and its effect on EC viability. A, **B**: Expression level of miR-22 (in fold of NCm or in % of NCi) in HDMECs transfected with miR-22m (**A**), miR-22i (**B**) or corresponding scrambled NCm (**A**) and NCi (**B**), as assessed by real-time PCR (n = 3). **C**: Viability (in % of NCm) of HDMECs transfected with miR-22m or NCm, as assessed by WST-1 assay (n = 4). After transfection, the cells were reseeded in 96-well plates and cultured for 0, 3, 6 or 10 days followed by WST-1 assay. Cell medium was replaced every 3 days. Means ± SEM. *P < 0.05 vs. NCm or NCi.

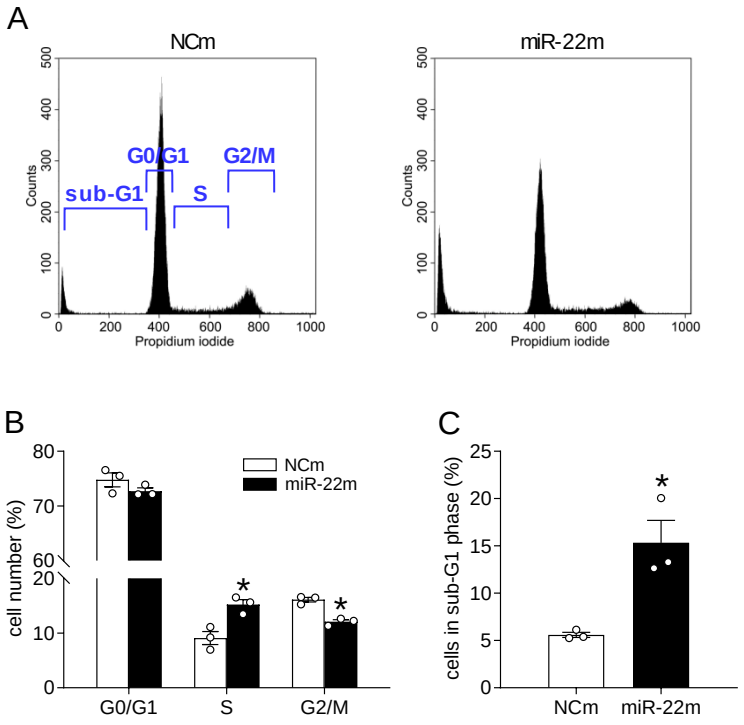


Figure S2. MiR-22 blocks HDMECs in the S phase. A: Representative cell cycle analysis of HDMECs transfected with NCm or miR-22m, as assessed by flow cytometry. **B**: Number of NCm- or miR-22m-transfected HDMECs in the G0/G1, S and G2/M phase (in % of total cell number), as assessed by flow cytometry (n = 3). **C**: Number of NCm- or miR-22m-transfected HDMECs in the sub-G1 phase (in % of total cell number), as assessed by flow cytometry (n = 3). Means ± SEM. *P < 0.05 vs. NCm.

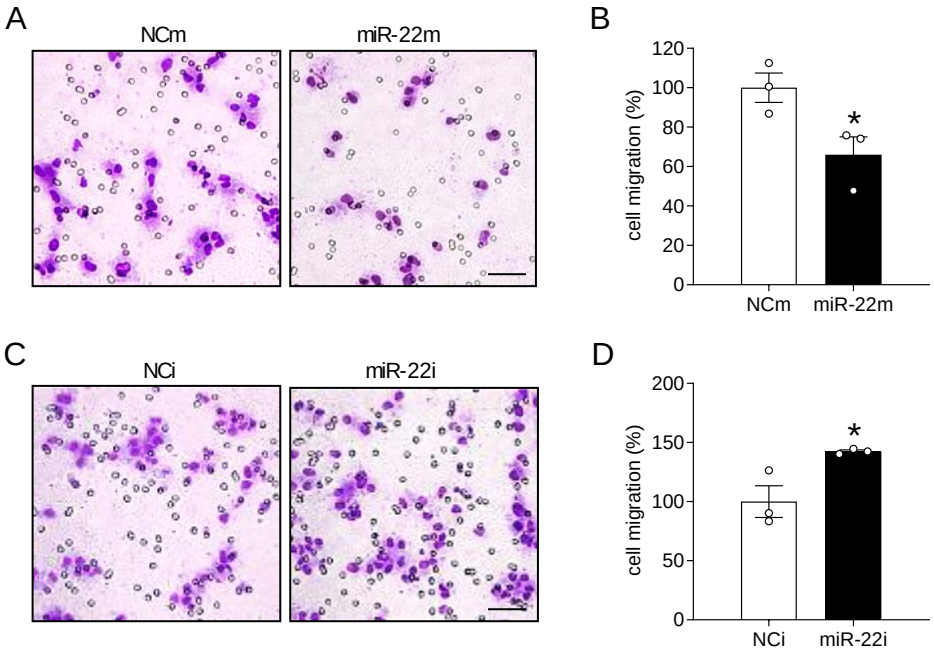


Figure S3. MiR-22 suppresses HDMEC migration. A, **C**: Light microscopic images of migrated HDMECs. The cells were transfected with miR-22m (**A**), miR-22i (**C**) or corresponding scrambled NCm (**A**) and NCi (**C**). Scale bars: 55 µm. **B**, **D**: Migration (in % of NCm or NCi) of HDMECs transfected with miR-22m (**B**), miR-22i (**D**) or corresponding scrambled NCm (**B**) and NCi (**D**), as assessed by transwell migration assay (n = 3). Means ± SEM. *P < 0.05 vs. NCm or NCi.

Table S1. Pathological characteristics of NSCLC patients.

Patient	Gender	Age	Tumor size	Tumor grade	Lymph nodes metastasis	Lymphatic invasion	Vascular invasion
1	female	52	pT2a	G2	pN0	no	yes
2	male	65	pT2a	G3	pN2	yes	yes
3	male	86	pT2a	G3	pN0	yes	no
4	female	66	PT3	G3	pN1	yes	yes
5	male	73	pT2b	G3	pN2	yes	yes
6	male	72	pT2a	G2	pNX	yes	yes
7	male	52	pT1a	G3	pN2	no	yes
8	male	70	pT2a	G2	pN0	yes	yes
9	male	70	pT3	G2	pN0	no	no
10	male	73	pT1b	G3	pN0	no	no
11	male	71	pT2a	G3	pNX	n.a.	n.a.
12	male	75	pT3	G2	pN2	no	no

n.a.:not available

Table S2. Correlation between endothelial miR-22 expression and clinical characteristics of NSCLC patients.

Characteristics of NSCLC patients		**Low miR-22 (<0.46)**	**High miR-22 (>0.46)**	***P*-Value (Fisher's exact)**
Tumor size	pT1	1/7 (14.3%)	1/5 (20.0%)	> 0.05
	pT2	5/7 (71.4%)	2/5 (40.0%)	> 0.05
	pT3	1/7 (14.3%)	2/5 (40.0%)	> 0.05
Tumor grade	G2	3/7 (42.8%)	2/5 (40.0%)	> 0.05
	G3	4/7 (57.1%)	3/5 (60.0%)	> 0.05
Lymph node metastasis	yes	4/6 (66.7%)	1/4 (25.0%)	> 0.05
Lymphatic invasion	yes	5/7 (71.4%)	1/4 (25.0%)	> 0.05
Vascular invasion	yes	7/7 (100.0%)	0/4 (0.0%)	0.0030

Table S3. Sequence details of human gene-specific primers for real-time PCR analyses.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
BDNF	TAACGGCGGCAGACAAAAAGA	GAAGTATTGCTTCAGTTGGCCT
CD45	ACCACAAGTTTACTAACGCAAGT	TTTGAGGGGGATTCCAGGTAAT
CD147	AAGTCGTCAGAACACATCAACG	AACTCACGAAGAACCTGCTCTC
CD151	ATGGGTGAGTTCAACGAGAAGA	GCAGGCTGATGTAGTCACTCT
CYR61	ACCGCTCTGAAGGGGATCT	ACTGATGTTTACAGTTGGGCTG
DDIT4	AGTGCCCTCCAAGACAGAGA	GTATGTGTCCCCAATGCACA
E-cadherin	CGAGAGCTACACGTTCACGG	GGGTGTCGAGGGAAAAATAGG
FGFR1	GGCTACAAGGTCCGTTATGCC	GATGCTGCCGTACTCATTCTC
GAPDH	ATGGGTGTGAACCATGAGAAGTA	GGCAGTGATGGCATGGAC
HMGB1	GCGGACAAGGCCCGTTA	AGAGGAAGAAGGCCGAAGGA
KDM3A	GTGCTCACGCTCGGAGAAA	AAACAGCTCGAATGGTCCCG
KRT20	GGACGACACCCAGCGTTTAT	CGCTCCCATAGTTCACCGTG
MTDH	CTCGGGCTGCTGCTGCTGTT	CAGCAAGGCCAGGTCGTCGG
NET1	TACAGTCATTTACCCTTCGTGG	TTCACCTCGGGACATTTCAT
NRAS	ATTTTTCCCGGCTGTGGTC	CCAACCACCACCAGTTTGTACT
PRKCB	AGCCCCACGTTTTGTGACC	GCTGGGAACATTCATCACGC
SIRT1	TAGCCTTGTCAGATAAGGAAGGA	ACAGCTTCACAGTCAACTTTGT
SP1	AGTTCCAGACCGTTGATGGG	GTTTGCACCTGGTATGATCTGT
SRPK1	ATGGAGCGGAAAGTGCTTG	GAGCCTCGGTGCTGAGTTT
TAK1	CCGGTGAGATGATCGAAGCC	GCCGAAGCTCTACAATAAACGC
Tie2	GGCTACAAGGTCCGTTATGCC	GATGCTGCCGTACTCATTCTC
TNFR2	CCAGTCTTGTGTCTGCGTCT	GAGGGGGAAGGCTGACTCTA
VEGFR2	GGCCCAATAATCAGAGTGGCA	CCAGTGTCATTTCCGATCACTTT
VEZF1	ACCCTTCAAATGCCAAACGTG	TGTATGCTGCACTCAGGAGC