Progress in Microbes and Molecular Biology

HH PUBLISHER

Supplementary Materials

Table S1. Eligibility criteria for study selection.

General criteria

- Participants who attend Hospital Universiti Sains Malaysia for treatment purpose
- Age 18 years and above
- Voluntarily giving consent (either self-volunteer or represented by relatives)
- Possibly matched the age and gender between study groups

Colorectal cancer (CRC) group

- Diagnosed with CRC (regardless of stages)
- Did not receive any blood donation within 3 months prior recruited
- Does not undergo chemotherapy prior recruited

Healthy control group

- Does not have any history of malignant
- Does not receive any blood donation within 3 months prior recruited

Table S2. The components for 1x reaction of reverse transcription.

Component	Volume per reaction (µl)
5x iScript reaction mix	4l
iScript reverse transcriptase	1
Nuclease-free water*	x
RNA template (100 fg to 1µg total RNA)*	x
Total	20

Note: * The volume is adjustable following the concentration and volume needed.

Table S3. Reverse transcription reaction setup.

Step	Time (min)	Temperature (℃)
Priming	5	25
Reverse transcription	20	46
RT inactivation	1	95
Hold (Optional step)	~	4

Table S4. The components of the qPCR master mix.

Component	Volume per reaction (µl)
Buffer	5
Primer	1 (10µM)
cDNA	2 (10ng)
Nuclease free water	3
Total	11

Table S5. The 2-step cycling of the qPCR program.

Cycles	Temperature (℃)	Time	Notes
1	95	2 min	Polymerase activation
2	95	5 second	Denaturation
	60-65	15-30 second	Annealing/Extension

Table S6. The demographical and clinicopathological data of participants.

	CRC patient n=24 (%)	**Healthy individual n=8 (%)**	***p*-value**
Variable			
Gender			>0.05
Male	16.0 (66.7)	3.0 (37.5)	
Female	8.0 (33.3)	5.0 (62.5)	
Age			<0.05
Median age	65.5	37.0	
Mean ± standard deviation	61.9 ±13.1	39.6 ±9.7	
TNM Stage			
I	3.0 (12.5)	-	
II	8.0 (33.3)	-	
III	6.0 (25.0)	-	
IV	7.0 (29.2)	-	
Metastasis			
Liver/Lung	10.0 (41.7)	-	
Others	-	-	
None	14.0 (58.3)	-	
Tumor lesion			
Rectosigmoid	17.0 (70.8)	-	
Rectal	6.0 (25.0)	-	
Caecum	1.0 (4.2)	-	
Histology			

Adenocarcinoma	23.0 (95.8)	-
Mucous	1.0 (4.2)	-

Table S7. List of disease association of selected miRNAs and the top 10 predicted target genes by each miRNA.

Type of miRNA	Disease association	Targeted gene
Hsa-miR-20a-5p	Colorectal cancer	FOXQ1: Forkhead box Q1 ATP1A2: ATPase NA+/K+ transporting subunit alpha 2 SLC4A4: Solute carrier family 4 member NEUROG1: Neirogenin 1 LHX8: LIM homeobax 8 CADM2: Cell adhesion molecule 2 KIF26B: Kinesin family member 26B MAPK4: Mitogen-activated protein kinase 4 GPR6: G protein-coupled receptor 6 SCN2B: Sodium voltage-gated channel beta subunit 2.
Hsa-miR-21-5p	Colorectal cancer, clear renal cell carcinoma, esophageal carcinoma, hepatocellular carcinoma, laryngeal squamous cell carcinoma, lung adenocarcinoma, rectal cancer	SLC30A10: Solute carrier family 30 member 10 BMP3: Bone morphogenetic protein 3 BEST3: Bestrophin 3 TGFBI: Transforming growth factor beta-induced ALX1: ALX homeobox 1 IL6R: Interleukin 6 receptor THRB: Thyroid hormone receptor beta OSR1: Odd-skipped related transcription factor 1 FGF18: Fibroblast growth factor 18 PDCD4: Programmed cell death
Hsa-miR-210-3p	Glioblastoma, non-small cell lung cancer, prostate cancer	FGFRL1: Fibroblast growth factor receptor-like 1 ELFN2: Extracellular leucine-rich repeat and fibronectin type III domain containing 2 SCARA3: Scavenger receptor class A member 3 GPD1L: Glycerol-3-phosphate dehydrogenase 1 like DENND6A: DENN domain-containing 6A KCMF1: Pottasium channel modulatory factor 1 ZNF462: Zinc finger protein 462 KMT2D: Lysine methyltransferase 2D ISCU: Iron-sulfur cluster assembly enzyme

Table S8. GO and biological pathway of each of the miRNAs.

Type of miRNA	**Gene ontology (*p*-value)**			**Biological pathway (*p*-value)**		
	Molecular function	**Cellular component**	**Biological process**	**KEGG**	**REACTOME**	**WikiPathways**
Hsa-miR-20a-5p	Cytokine receptor binding (3.740×10^{-3}), Glycosaminoglycan binding (1.998×10^{-2}), Interleukin-8 receptor binding (4.154×10^{-2}), Transferase activity, transferring phosphorus-containing groups (3.219×10^{-2}), Kinase activity (1.329×10^{-2}), Phosphotransferase activity, alcohol group as acceptor (9.305×10^{-3})	NA	Angiogenesis (1.215×10^{-28}), Blood vessel development ($2.932\times10^{-2}9$), Vasculature development (3.692×10^{-210}), Response to endogenous stimulus (1.071×10^{-311}), Response to organic substance (1.562×10^{-212}), Peptidyl-threonine phosphorylation (1.582×10^{-213}), Response to chemical (2.615×10^{-214}), Regulation of angiogenesis (3.673×10^{-215}), Blood vessel morphogenesis (2.130×10^{-216})	Human T-cell leukemia virus 1 infection (9.562×10^{-5}), Human cytomegalovirus infection (1.063×10^{-4}), Hepatitis (4.913×10^{-4}), Pancreatic cancer (5.753×10^{-4}), Transcriptional misregulation in cancer (1.124×10^{-3}), Pathways in cancer (1.273×10^{-3}), Proteoglycans in cancer (1.544×10^{-3}), AGE-RAGE signaling pathway in diabetic complications (1.713×10^{-3}) Bladder cancer (3.330×10^{-3}), Gastric cancer (7.950×10^{-3})	Interleukin-4 and Interleukin-13 signaling (1.055×10^{-3}), Signaling by FGFR (3 1.234×10^{-3}), Signaling by FGFR4 (1.625×10^{-3}), FGFR3 mutant receptor activation ($3.499X10^{-2}$), Signaling by FGFR1 (2.446×10^{-3}), Insulin receptor signaling cascade (3.504×10^{-3}), Signaling by FGFR3 fusions in cancer (8.202×10^{-3}), MAPK1 (ERK2) activation (8.202×10^{-3}), Signaling by FGFR2 (1.018×10^{-2}), Signaling by FGFR4 in disease (1.054×10^{-2}), Signaling by Insulin receptor (1.276×10^{-2})	Hepatitis B infection (3.312×10^{-4}), Leptin signaling pathway (6.402×10^{-4}), IL-7 signaling pathway (9.397×10^{-4}), EGFR tyrosine kinase inhibitor resistance (9.558×10^{-4}), Pancreatic adenocarcinoma pathway (1.151×10^{-3}), TCA cycle nutrient use and invasiveness of ovarian cancer (2.960×10^{-3}), Spinal cord injury (3.443×10^{-3}), VEGFA-VEGFR2 signaling pathway (3.867×10^{-3}), Bladder cancer (3.979×10^{-3}), IL6 signaling pathway (4.956×10^{-3}),

Hsa-miR-21-5p	Metalloendopeptidase inhibitor activity (1.127×10^{-2})	Regulation of metalloendopeptidase activity (1.007×10^{-5}), Regulation of metallopeptidase activity (1.435×10^{-4}), Negative regulation of metalloendopeptidase activity (4.452×10^{-3}), Positive regulation of vascular-associated smooth muscle cell apoptotic process (7.416×10^{-3}), Endothelial cell apoptotic process (8.540×10^{-3}), Circulatory system development (1.328×10^{-2})	NA	Proteoglycans in cancer (5.345×10^{-7}), MicroRNAs in cancer (9.334×10^{-6}), Neurotrophin signaling pathway (1.664×10^{-3}), FoxO signaling pathway (2.429×10^{-3}), Hepatitis C (4.933×10^{-3}), Pathways in cancer (5.110×10^{-3}), Hepatitis B (5.573×10^{-3}), Non-small cell lung cancer (1.047×10^{-2}), EGFR tyrosine kinase inhibitor resistance (1.380×10^{-2}), Human cytomegalovirus infection (1.913×10^{-2}),	Signaling by FGFR1 in disease (1.722×10^{-3}), Signaling by FGFR in disease (9.477×10^{-3}), Signaling by FGFR3 fusions in cancer (1.032×10^{-2}), Signaling by FGFR4 in disease (1.326×10^{-2}), PI-3K cascade: FGFR3 (2.866×10^{-2}), Interleukin-15 signaling (3.342×10^{-2}), PI-3K cascade: FGFR1 (3.853×10^{-2}), FGFR3 mutant receptor activation (4.401×10^{-2}), Signaling by FGFR3 in disease (4.985×10^{-2}), PI-3K cascade: FGFR4 (4.985×10^{-2}).	MET in type 1 papillary renal cell carcinoma (6.819×10^{-3}), Ras signaling (9.367×10^{-3}), Non-small cell lung cancer (1.304×10^{-2}), EGFR tyrosine kinase inhibitor resistance (2.064×10^{-2}).
Hsa-miR-210-3p	NA	NA	NA	NA	NA	NA

*NA: not available.