

Supplementary Materials

Table S1. Eligibility criteria for study selection.

General criteria
<ul style="list-style-type: none">• 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
<ul style="list-style-type: none">• 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
<ul style="list-style-type: none">• 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.

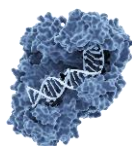


Table S3. Reverse transcription reaction setup.

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

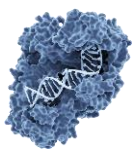
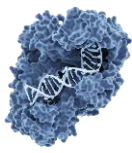


Table S6. The demographical and clinicopathological data of participants.

Variable	CRC patient n=24 (%)	Healthy individual n=8 (%)	p-value
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 \pm standard deviation	61.9 \pm 13.1	39.6 \pm 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)	-

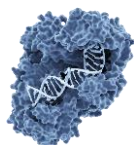


Table S7. List of disease association of selected miRNAs and the top 10 predicted target genes by each miRNA.

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: Neurogenin 1
		LHX8: LIM homeobox 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
		TGFB1: 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: Potassium channel modulatory factor 1
		ZNF462: Zinc finger protein 462
		KMT2D: Lysine methyltransferase 2D
		ISCU: Iron-sulfur cluster assembly enzyme

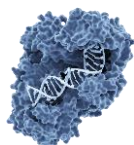
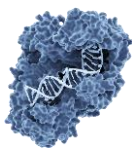


Table S8. GO and biological pathway of each of the miRNAs.

Type of miRNA	Gene ontology (<i>p</i> -value)			Biological pathway (<i>p</i> -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),	Human T-cell leukemia virus 1 infection (9.562×10 ⁻⁵),	Interleukin-4 and Interleukin-13 signaling (1.055×10 ⁻³),	Hepatitis B infection (3.312×10 ⁻⁴),
			Blood vessel development (2.932×10 ⁻²⁹),	Human cytomegalovirus infection (1.063×10 ⁻⁴),	Signaling by FGFR (3.1234×10 ⁻³),	Leptin signaling pathway (6.402×10 ⁻⁴),
			Vasculature development (3.692×10 ⁻²¹⁰),	Hepatitis	Signaling by FGFR4 (1.625×10 ⁻³),	IL-7 signaling pathway (9.397×10 ⁻⁴),
			Response to endogenous stimulus (1.071×10 ⁻³¹¹),	Pancreatic cancer (5.753×10 ⁻⁴),	FGFR3 mutant receptor activation (3.499×10 ⁻²),	EGFR tyrosine kinase inhibitor resistance (9.558×10 ⁻⁴),
			Response to organic substance (1.562×10 ⁻²¹²),	Transcriptional misregulation in cancer (1.124×10 ⁻³),	Signaling by FGFR1 (2.446×10 ⁻³),	Pancreatic adenocarcinoma pathway (1.151×10 ⁻³),
			Peptidyl-threonine phosphorylation (1.582×10 ⁻²¹³),	Pathways in cancer (1.273×10 ⁻³),	Insulin receptor signaling cascade (3.504×10 ⁻³),	TCA cycle
			Response to chemical (2.615×10 ⁻²¹⁴),	Proteoglycans in cancer (1.544×10 ⁻³),	Signaling by FGFR3 fusions in cancer (8.202×10 ⁻³),	nutrient use and invasiveness of ovarian cancer (2.960×10 ⁻³),
			Regulation of angiogenesis (3.673×10 ⁻²¹⁵),	AGE-RAGE signaling pathway in diabetic complications (1.713×10 ⁻³),	MAPK1 (ERK2) activation (8.202×10 ⁻³),	Spinal cord injury (3.443×10 ⁻³),
			Blood vessel morphogenesis (2.130×10 ⁻²¹⁶)	Bladder cancer (3.330×10 ⁻³), Gastric cancer (7.950×10 ⁻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)	VEGFA-VEGFR2 signaling pathway (3.867×10 ⁻³), Bladder cancer (3.979×10 ⁻³), IL6 signaling pathway (4.956×10 ⁻³),



					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}).		
		Regulation of metalloendopepti dase activity (1.007×10^{-5}), Regulation of metallopeptidase activity (1.435×10^{-4}), Negative regulation of metalloendopepti dase 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})		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}),			
Hsa-miR-21-5p activity (1.127×10^{-2})			NA				
Hsa-miR-210-3p	NA	NA	NA	NA	NA	NA	

*NA: not available.