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Potential implications of artificial miRNAs in cancer therapy

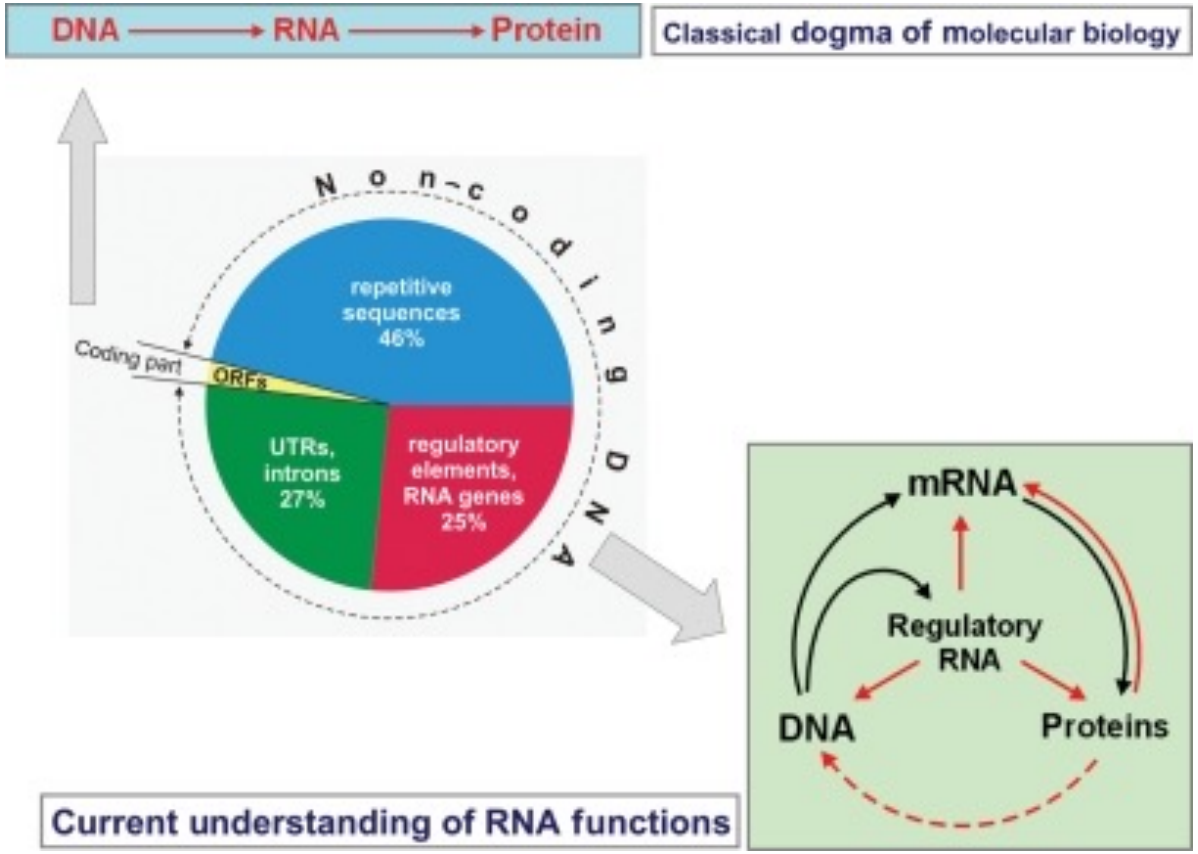
Sergiu Chira, PhD

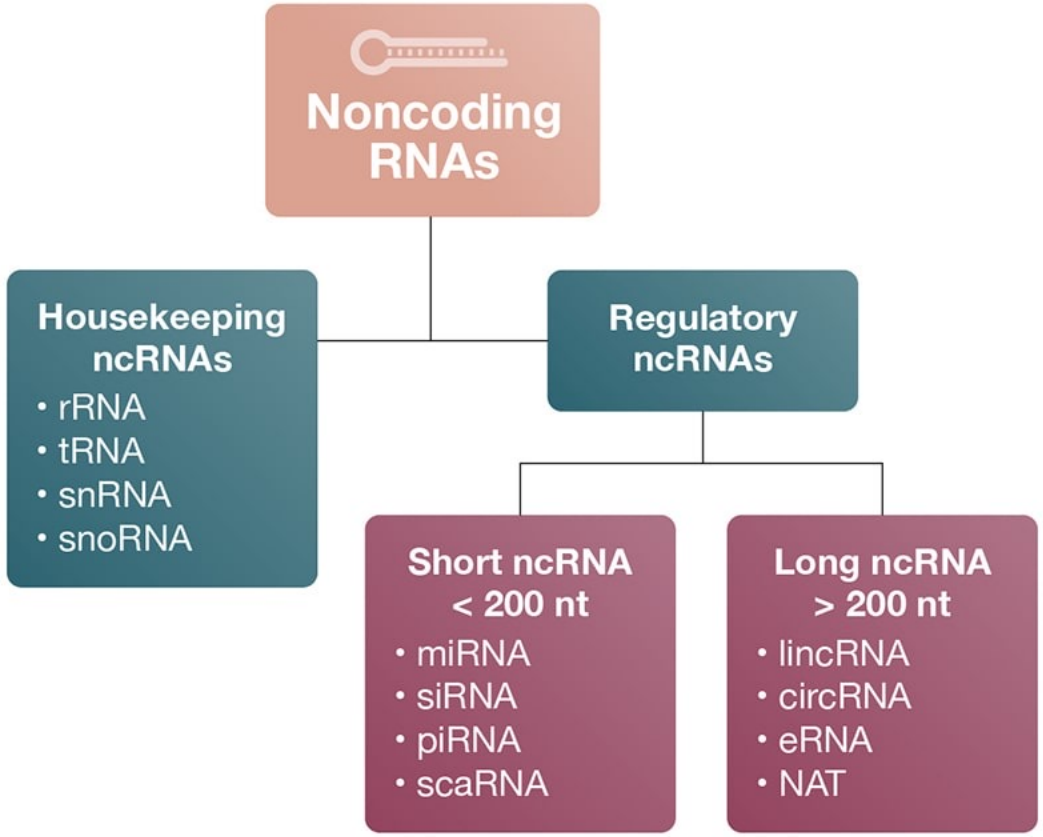
Oslo, September 19

UMF
UNIVERSITATEA DE MEDICINĂ ŞI FARMACIE IULIU HAŢIEGANU CLUJ-NAPOCA

GENOMICA

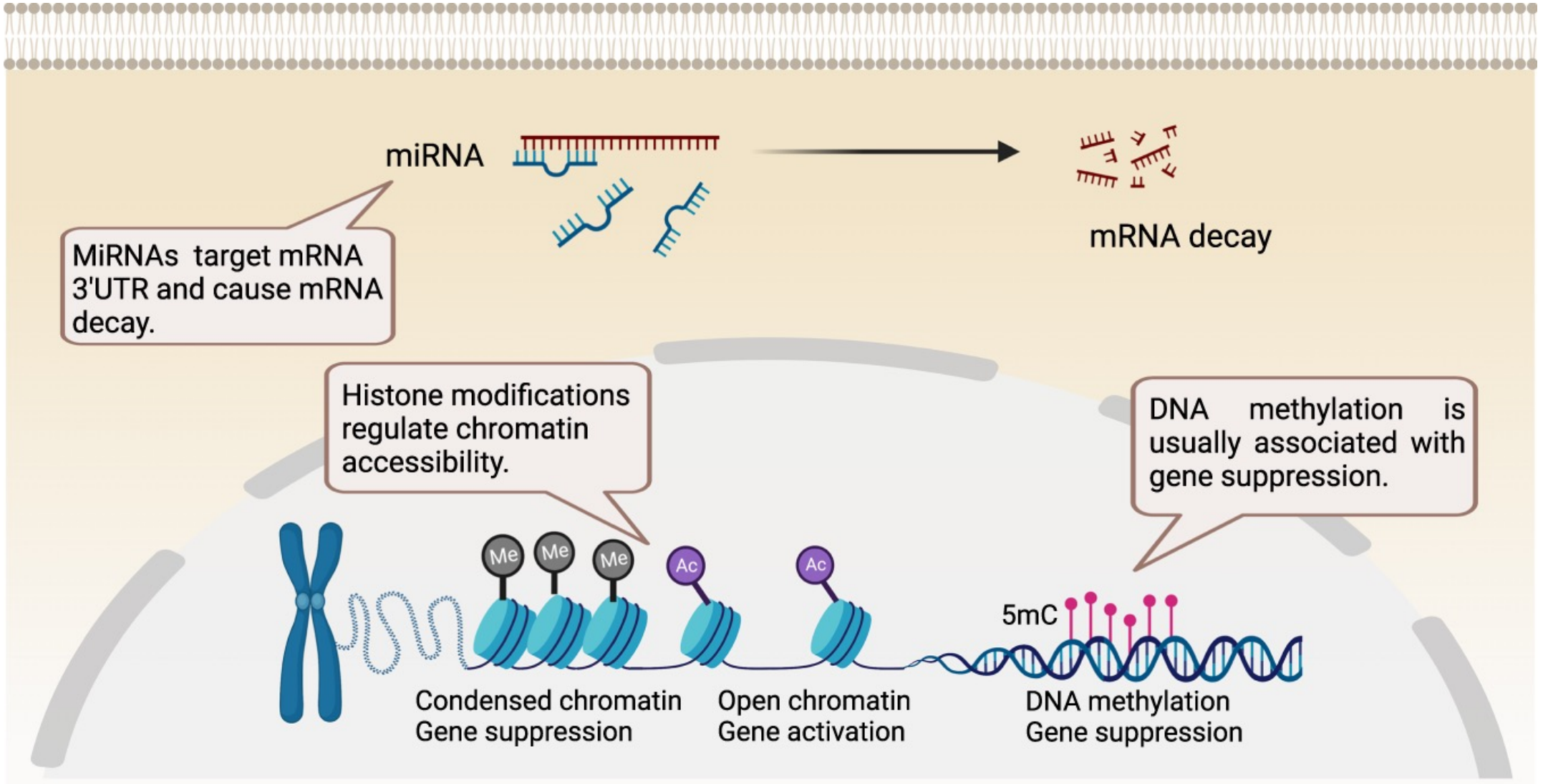
Non-coding DNA covers most of the human genome





Poller W et al, Cardiovascular Research 2019
https://www.bio-rad.com/

microRNAs (miRNAs) represent one of the epigenetic factors regulating gene expression

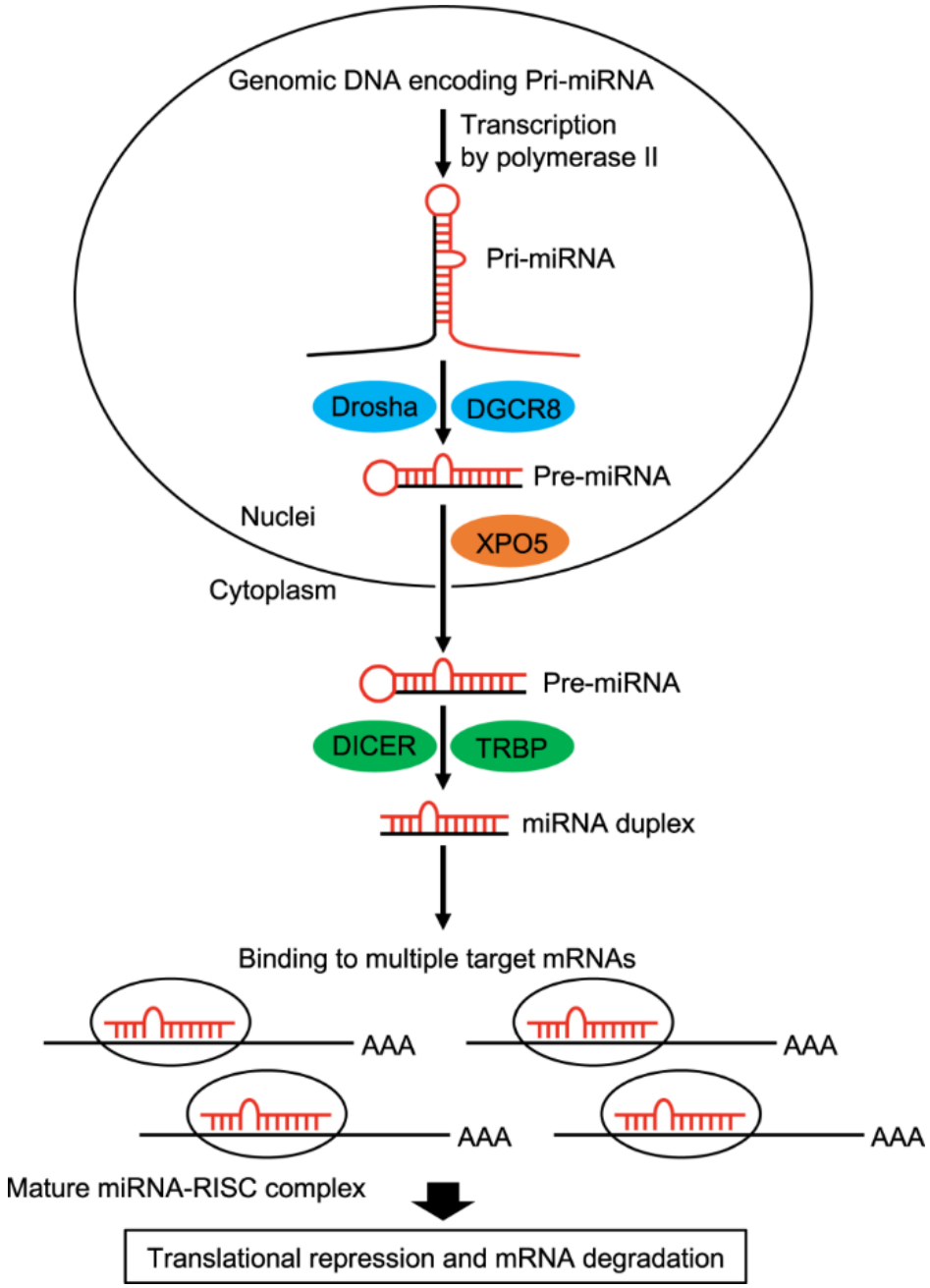


Xiao F et al, Cell. & Mol. Imm. 2022

miRNA biogenesis and mechanism of gene silencing

- One miRNA can target multiple genes
- One gene can be targeted by multiple mRNAs

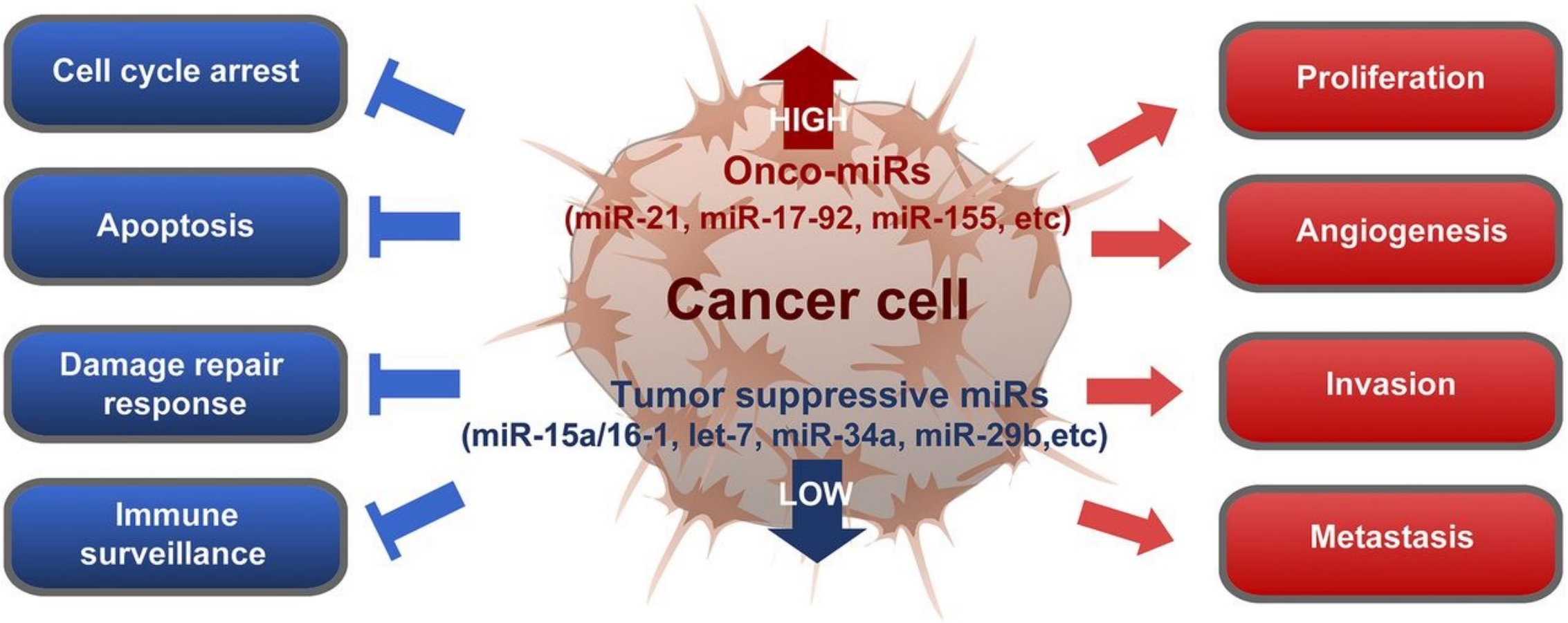
Cell fate specification	Fat metabolism
DNA repair	Insulin secretion
DNA methylation	Stem cell maintenance
Cell proliferation'	Resistance to viral infection
Cell differentiation	Inflammation
Developmental timing	Immunomodulation
Cell cycle control	Apoptosis
Angiogenesis control	Proinflammatory stimuli
Pattern formation	Antiinflammatory stimuli
Morphogenesis regulation	Neuronal differentiation
Circardian rhythm	Neurogenesis
Synapse function	Neuroprotection



Inoue J & Inazawa J, J. Hum. Gen. 2021

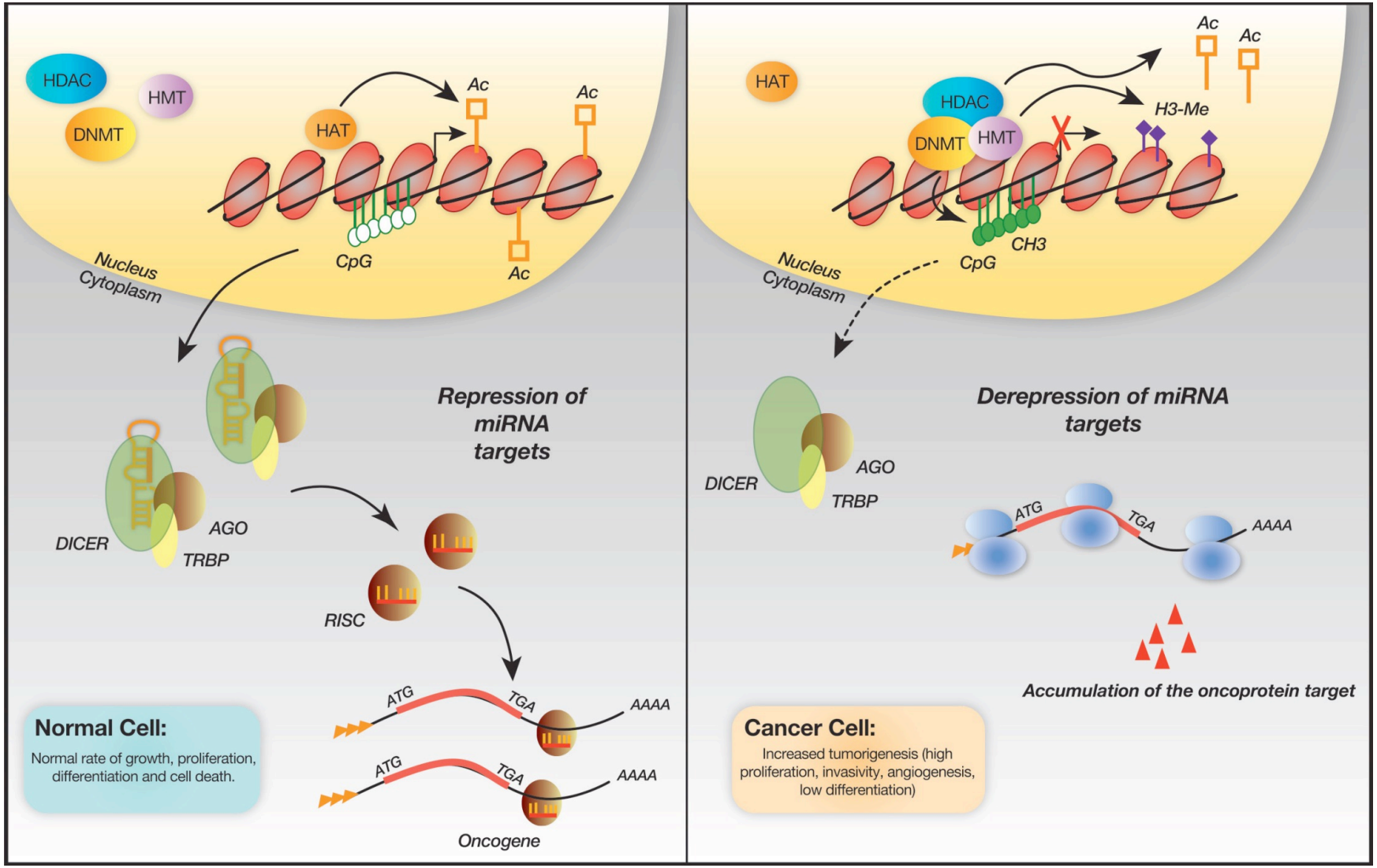
Ha TY, Immune Network 2011

Deregulation of miRNA expression patterns is associated with hallmarks of cancers



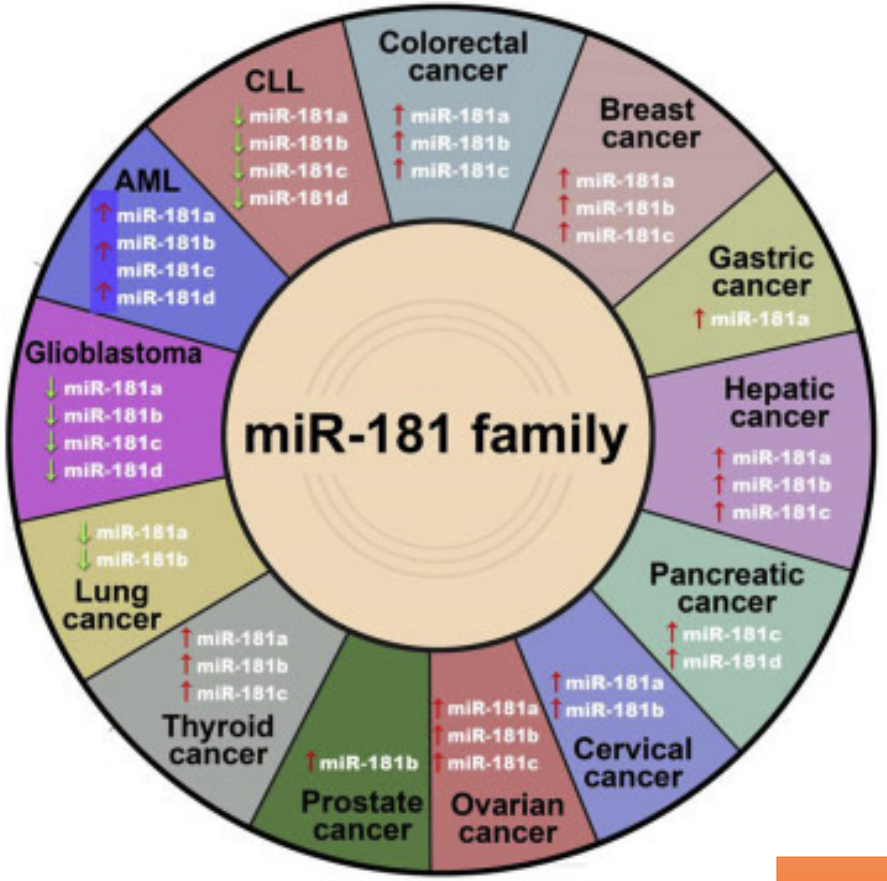
Lee JT et al, Pharma. Rev. 2020

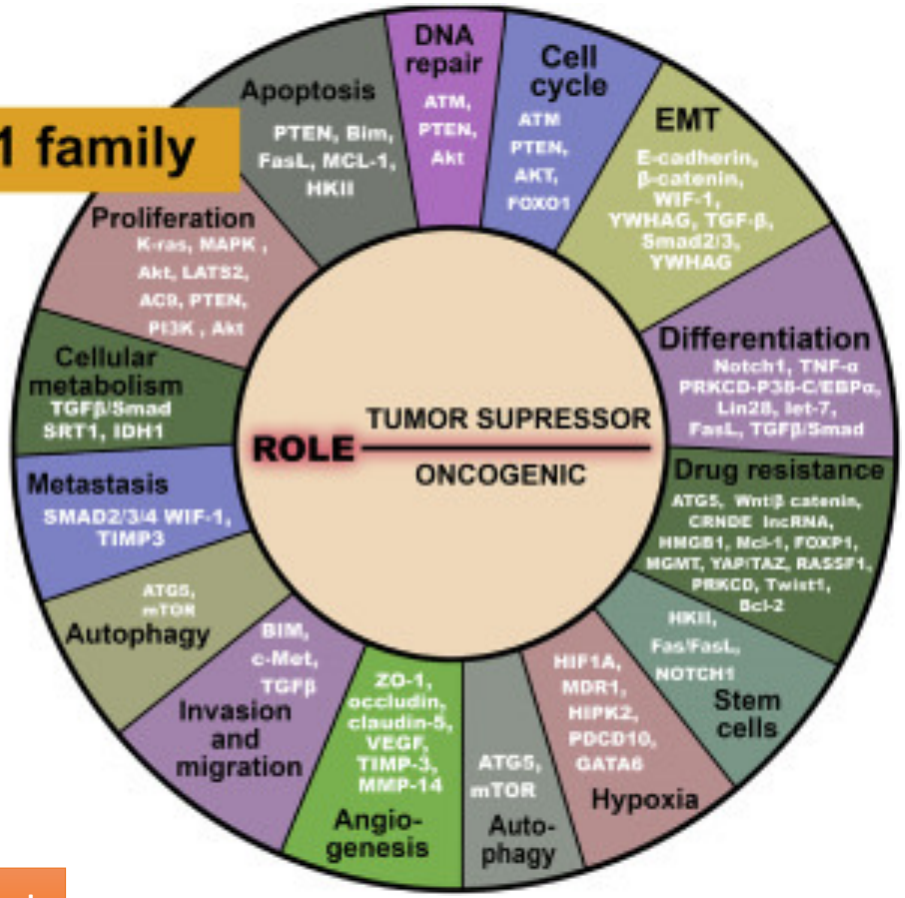
Cancer cells exhibit epigenetic changes of miRNA genes



Zavesky l et al, Neoplasma 2015

Dysregulation of miR-181 expression is widely distributed in a large array of human cancers

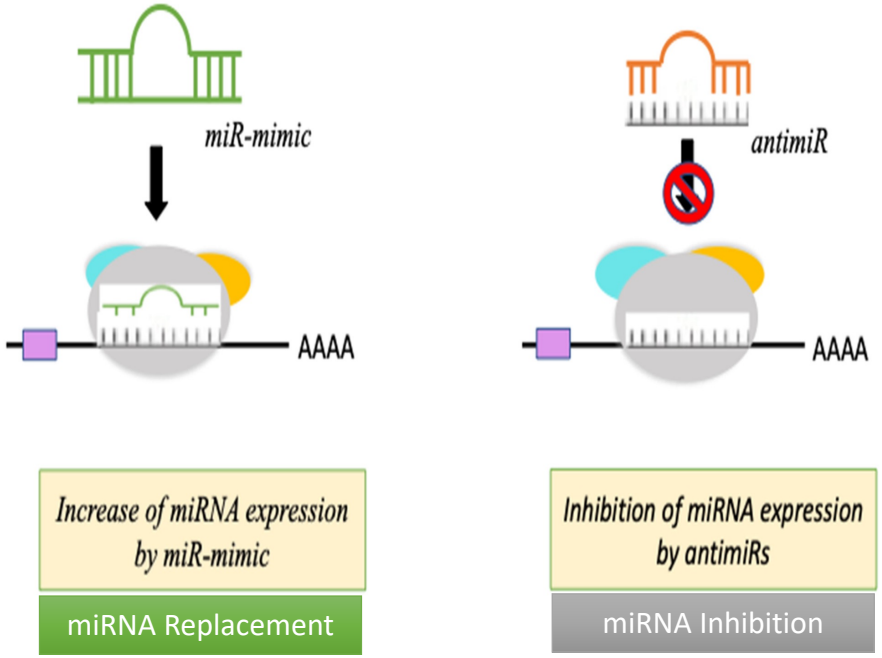


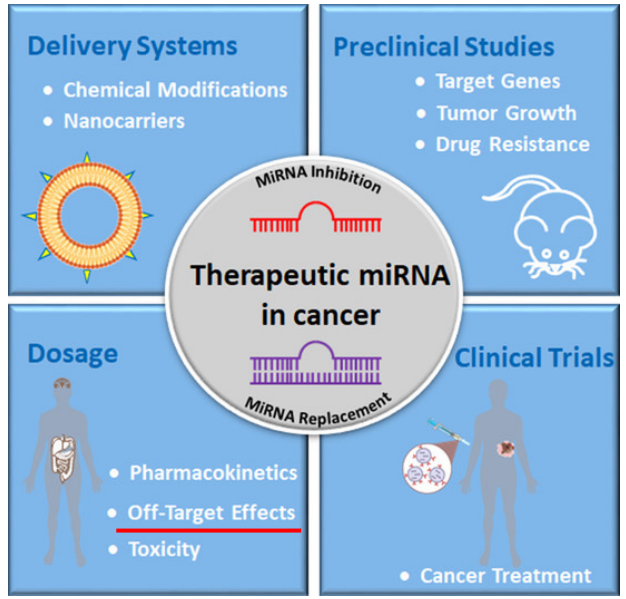


Is miR-181 a potential target for cancer therapy?

Braicu C et al, Mol. Asp. Med. 2019

miRNA-based approaches and challenges for cancer therapy





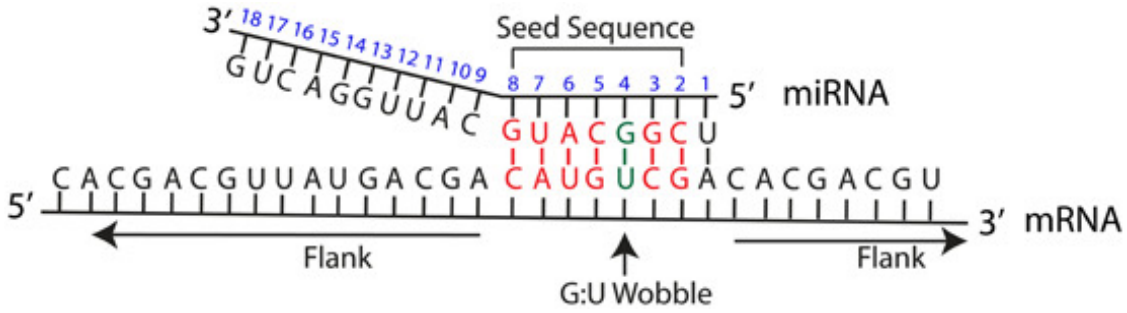
miRNA therapeutics in cancer clinical trials.

Drug name	Therapeutic agent	Disease	Delivery system	Administration route	Company	Current status	Clinical trials identifier
MRX34	miR-34a mimic	Multiple solid tumors	LNPs-Liposomes	IV	Mirna Therapeutics, Inc.	Phase I (Terminated-2016) Phase I/II (Withdrawn-2016)	NCT01829971 NCT02862145
TargomiRs	miR-16 mimic	Malignant pleural mesothelioma and non-small cell lung cancer	EGFR-antibody targeted minicells	IV	EnGeneIC Limited	Phase I (Completed-2017)	NCT02369198
Cobomarsen	Anti-miR-155	T-cell leukemia/lymphoma	LNA-mediated	Subcutaneous injection or IV	miRagen Therapeutics, Inc.	Phase I (Completed-2020) Phase II (Terminated-2020) Phase II (Terminated-2020)	NCT02580552 NCT03713320 NCT03837457

Kargutkar et al, Clin. Gen. 2020

Kara G et al, Adv. Drug Del. Rev. 2022

Understanding the basis of miRNA-mRNA interaction might help design artificial miRNAs with reduced off-target effects



It is though that miRNA's "seed" region is key element for target mRNA selectivity

Can this model explain the marked differences in numbers of miRNA's targets?

Peterson SM et al, Front. Genet. 2014

Twenty-five miRNAs analyzed in the RNA-seq experiments

hsa-let-7c-5p	UGAGGUAGUAGGUUGUAUGGUU	31
hsa-miR-107	AGCAGCAUUGUACAGGGCUAUCA	35
hsa-miR-10a-5p	UACCCUGUAGAUCCGAAUUUGUG	32
hsa-miR-124-3p	UAAGGCACGCGGUGAAUGCC	151
hsa-miR-126-3p	UCGUACCGUGAGUAAUAAUGCG	11
hsa-miR-126-5p	CAUUAUUACUUUUGGUACGCG	48
hsa-miR-133b	UUUGGUCCCCUUCAACCAGCUA	108
hsa-miR-142-3p	UGUAGUGUUUCCUACUUUAUGGA	108
hsa-miR-145-5p	GUCCAGUUUUCCCAGGAAUCCCU	82
hsa-miR-146a-5p	UGAGAACUGAAUUCCAUGGGUU	42
hsa-miR-155-5p	UUAAUGCUAAUCGUGAUAGGGGU	154
hsa-miR-15a-5p	UAGCAGCACAUAAUGGUUUGUG	108
hsa-miR-16-5p	UAGCAGCACGUAAAUAUUGGCG	122
hsa-miR-17-5p	CAAAGUGCUUACAGUGCAGGUAG	74
hsa-miR-193b-3p	AACUGGCCCUCAAAGUCCCGCU	102
hsa-miR-200a-3p	UAACACUGUCUGGUAACGAUGU	35
hsa-miR-200b-3p	UAAUACUGCCUGGUAAUGAUGA	126
hsa-miR-200c-3p	UAAUACUGCCGGGUAAUGAUGGA	93
hsa-miR-206	UGGAAUGUAAGGAAGUGUGUGG	206
hsa-miR-210-3p	CUGUGCGUGUGACAGCGGCUGA	43
hsa-miR-21-5p	UAGCUUAUCAGACUGAUGUUGA	11
hsa-miR-31-5p	AGGCAAGAUGCUGGCAUAGCU	85
hsa-miR-34a-5p	UGGCAGUGUCUUAGCUGGUUGU	155
hsa-miR-9-3p	AUAAAGCUAGAUAACCGAAAGU	182
hsa-miR-9-5p	UCUUUGGUUAUCUAGCUGUAUGA	106

Liu W & Wang X, Gen.. Biol. 2019

Higher order structures of miRNAs might impose an additional constrain for target genes selection

SCIENTIFIC REPORTS
nature research

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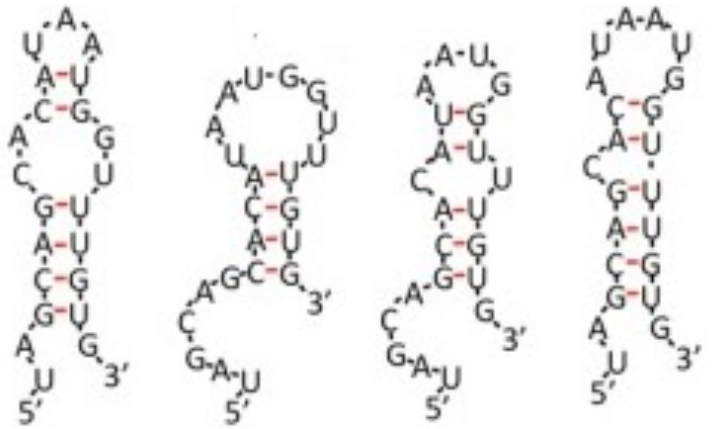
Published online 2020 Jan 16. doi: 10.1038/s41598-019-57289-8
PMCID: PMC6965629
PMID: 31949213

Endogenous and artificial miRNAs explore a rich variety of conformations: a potential relationship between secondary structure and biological functionality

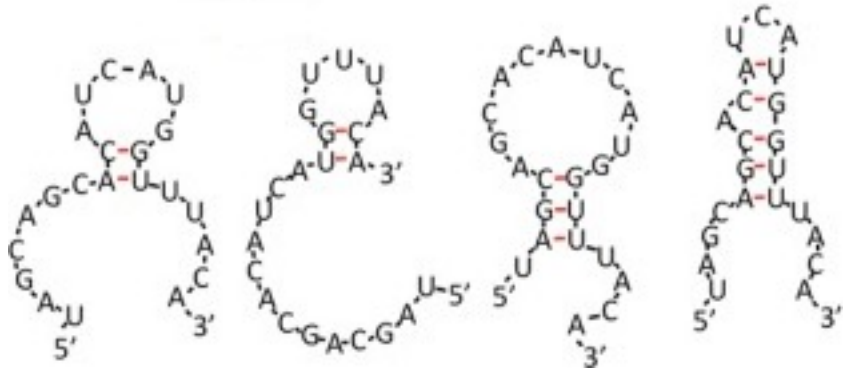
C. M. A. Gangemi,[1] S. Alaimo,[2] A. Pulvirenti,[2] Sara García-Viñuales,[3] D. Milardi,[3] A. P. Falanga,[4] M. E. Fragalà,[1] G. Oliviero,[4] G. Piccialli,[5] N. Borbone,[5] A. Ferro,[2] A. D'Urso,[1] C. M. Croce,[6] and R. Purrello[1]



miR-15a

miR-15a: 5′-*UAG CAG CAC* AU**A** AUG GUU U**GU G**-3′

miR-15b: 5′-*UAG CAG CAC* AU**C** AUG GUU U**AC A**-3′



miR-15b

Same seed region, distinct biological activity

Is there any relation between miR-181 family members primary sequence, secondary structure and biological function?

FEBS Letters

FEBS PRESS science publishing by scientists

RESEARCH LETTER

Artificial miRNAs derived from miR-181 family members have potential in cancer therapy due to an altered spectrum of target mRNAs

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2 Translational Molecular Pathology, MD Anderson Cancer Center, Texas State University, Houston, TX, USA
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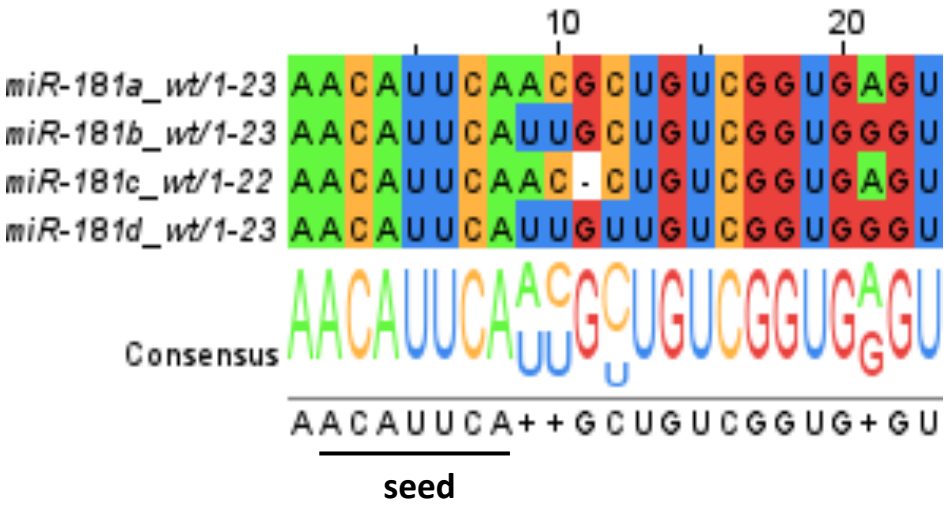
Edited by Qinghua Cui

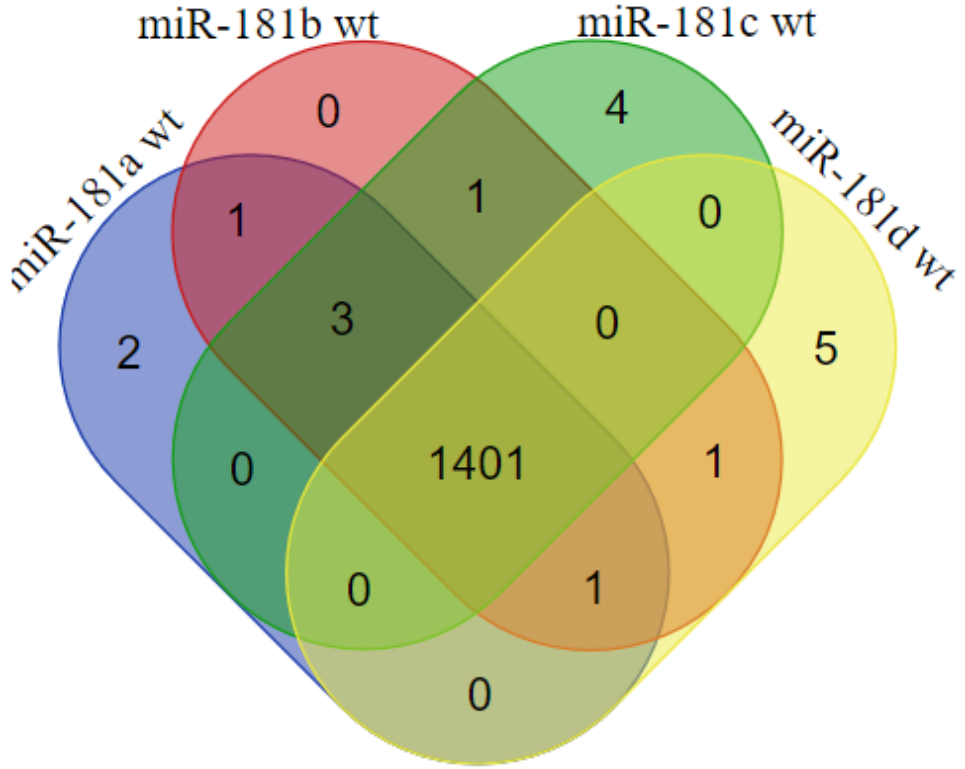
miRNAs are a class of noncoding RNAs with gene regulation properties, and they function as key factors in cell homeostasis. The interaction of miRNAs with their target mRNAs is largely considered to rely on sequence complementarity; however, some evidence indicates that mature miRNAs can adopt diverse conformations with implications for their function. Using the oncogenic miR-181 family as a study model, we suggest that a potential relationship between the primary sequence and secondary structure of miRNAs may have an impact on the number and spectrum of targeted cellular transcripts. We further emphasize that specific alterations in miR-181 primary sequences might impose certain constraints on target gene selection compared with the wild-type sequences, leading to the targeting of new transcripts with upregulated function in cancer.

Keywords: cancer therapy; miR-181; miRNAs; secondary structure; sequence alterations; target genes

miR-181 family members' primary sequence and predicted target genes



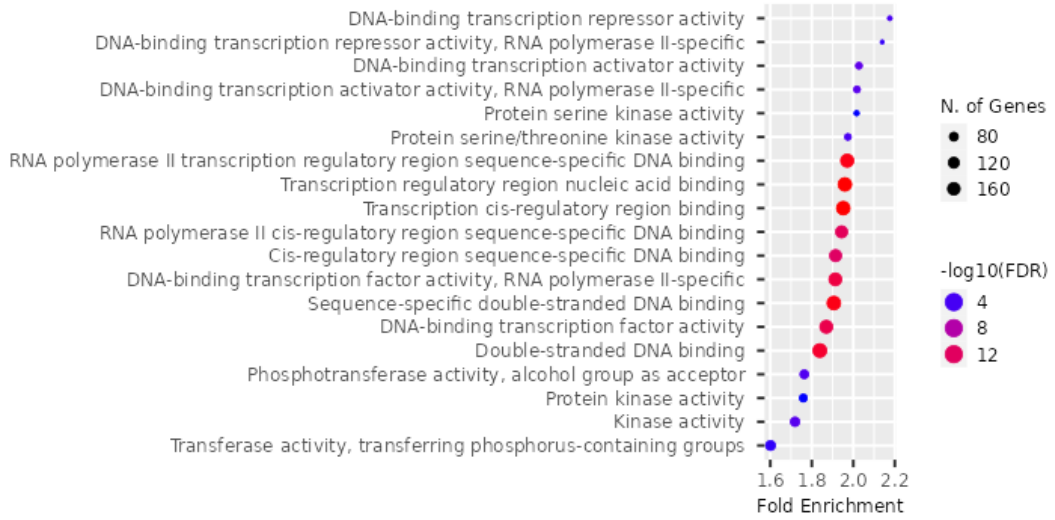
miR-181 family members are highly conserved in the seed region



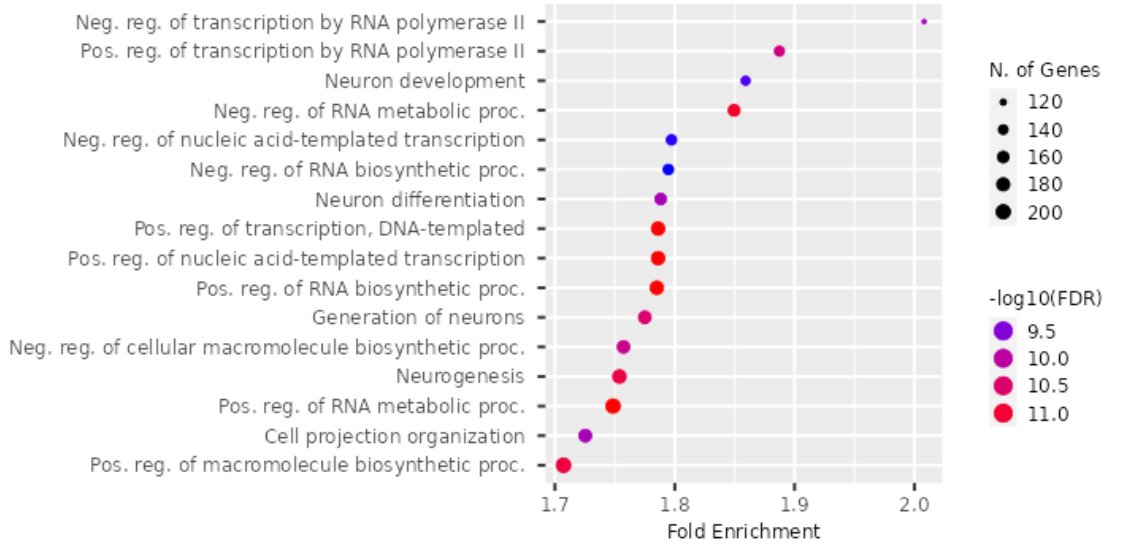
miR-181 family members share a high number of predicted target genes

Functional enrichment analysis of shared target genes between miR-181 members

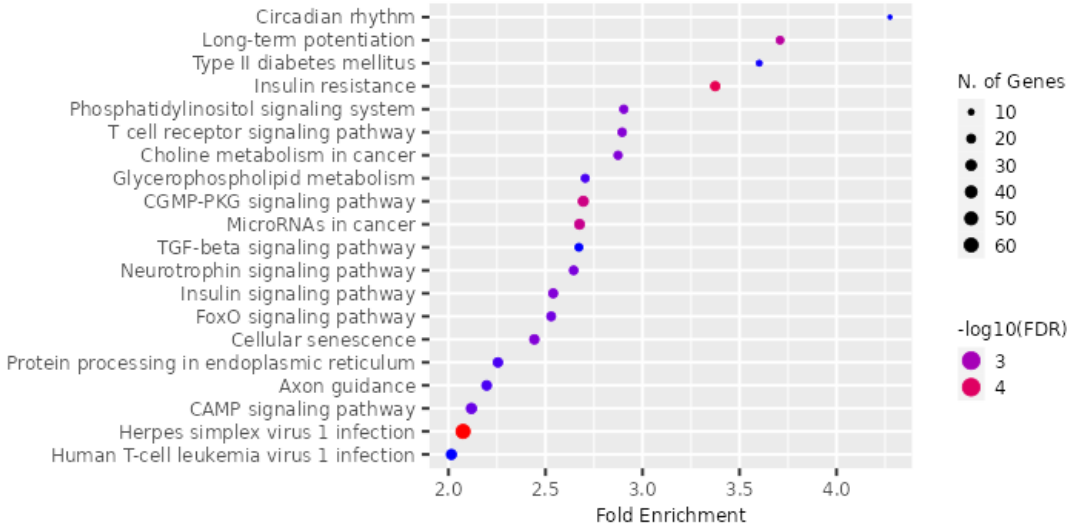
GO Molecular Function



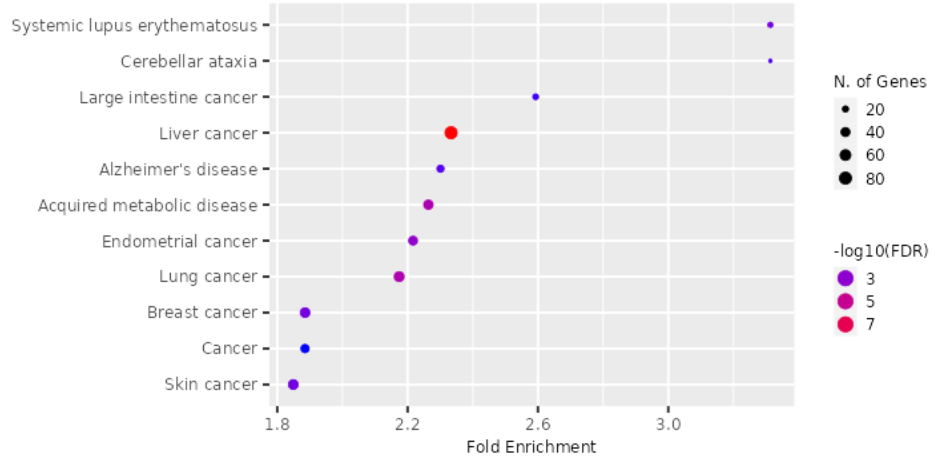
GO Biological Process



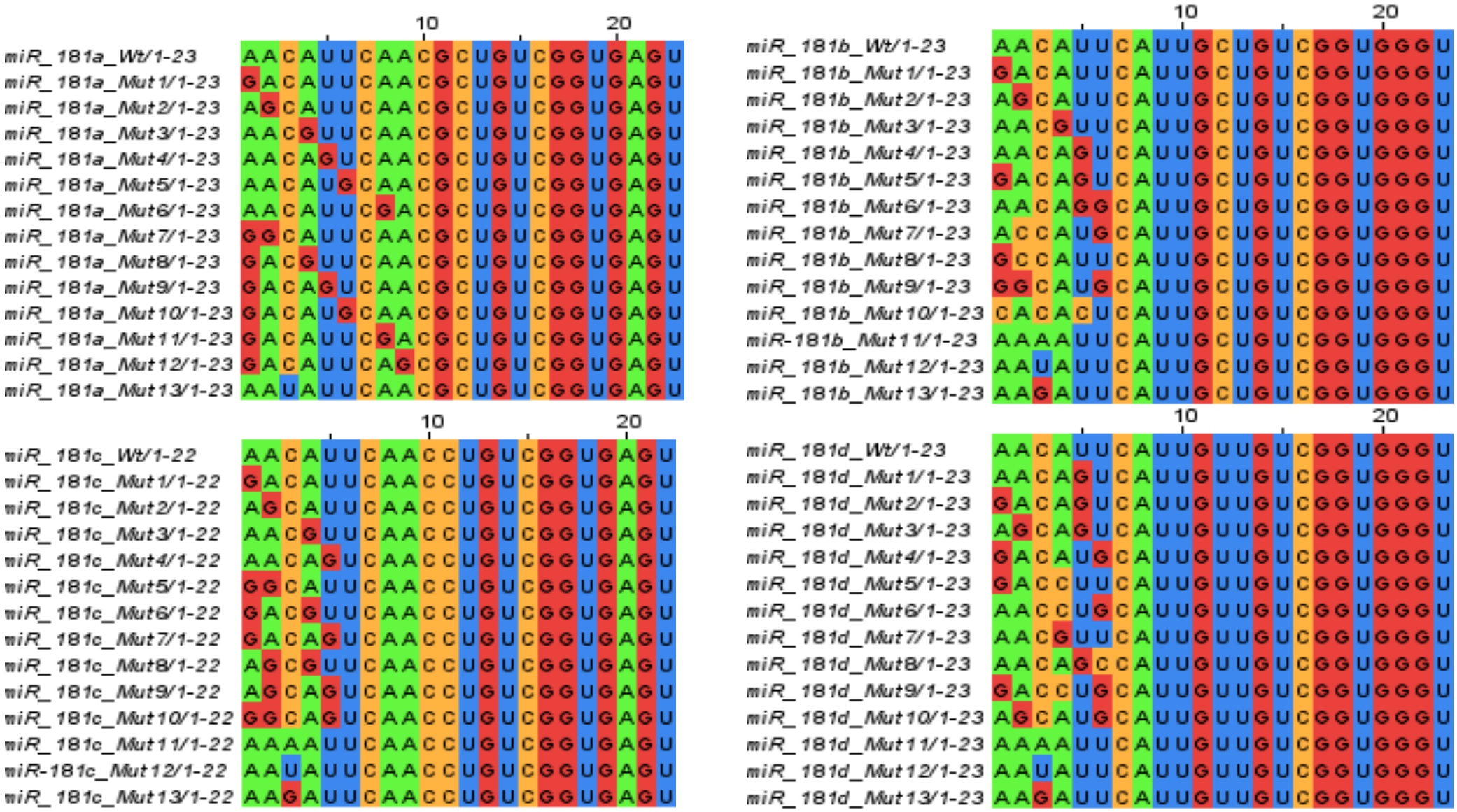
KEGG



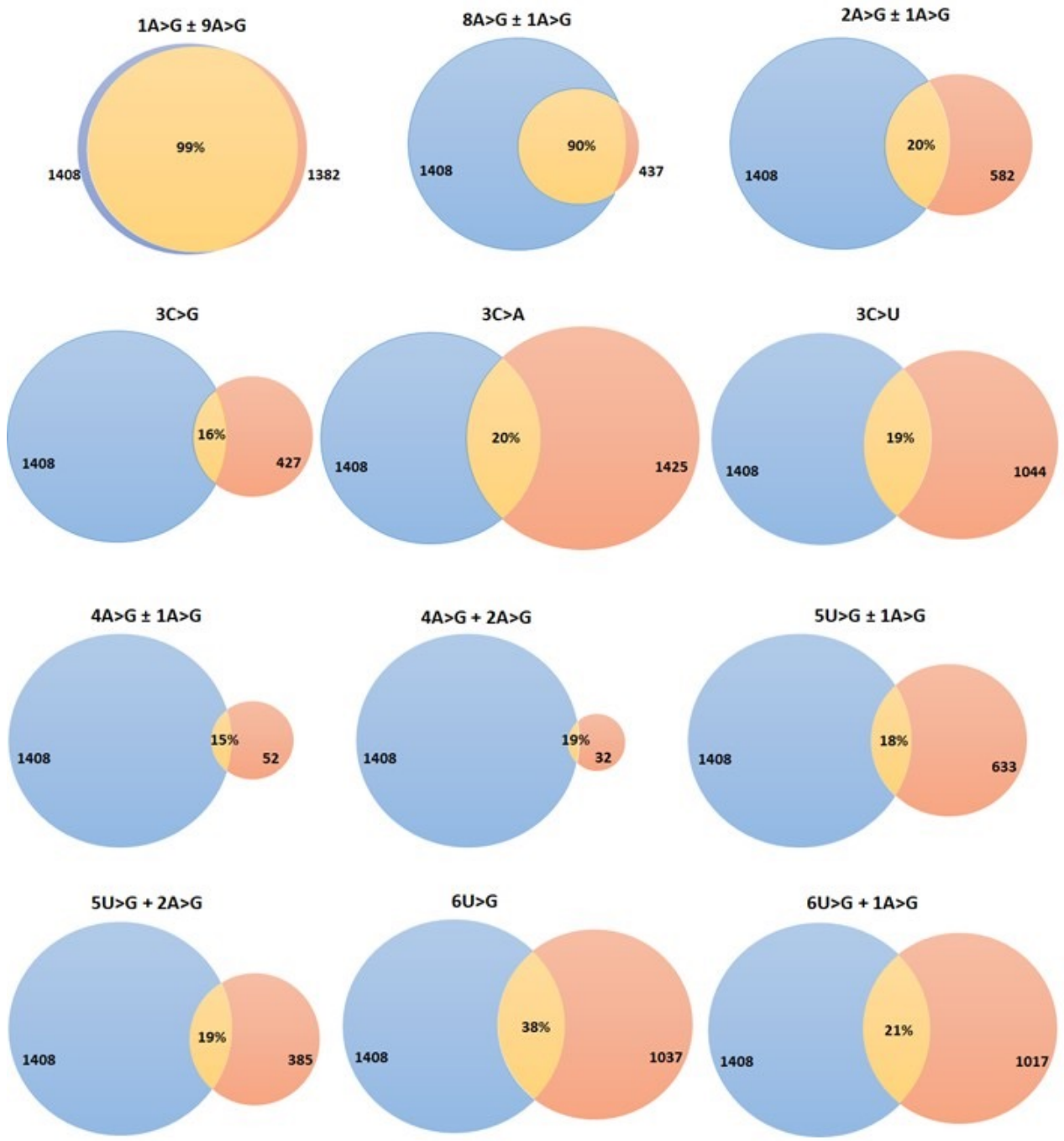
Jensen Diseases database



Thirteen mutant variants of the seed region for each miR1-181 family members

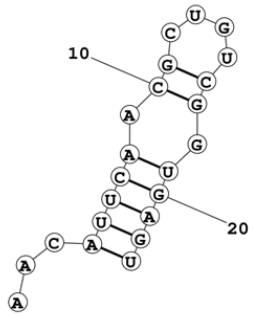


Position and type of the substitution can have a dramatic effect on the number and selectivity of predicted target genes



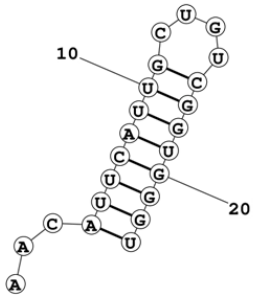
Mutant variants of miR-181 members adopt diverse secondary structures with distinct number and pattern of predicted targets genes

wt



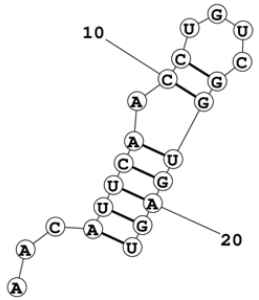
miR-181a Energy = -2.4

1408



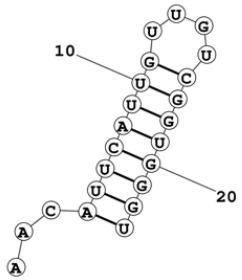
miR-181a Energy = -3.5

1408



miR-181a Energy = -4.2

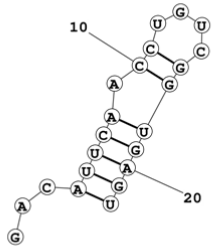
1409



miR-181a Energy = -4.6

1408

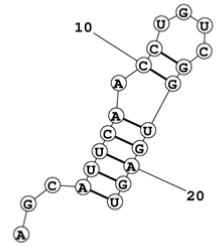
1A>G



miR-181c mut1 Energy -4.2

1386 – 1381 (99.1%)

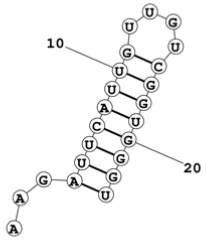
2A>G



miR-181c mut2 Energy -4.2

586 – 128 (21.8%)

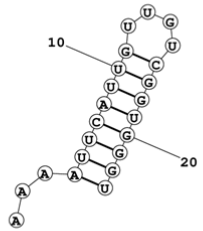
3C>G



miR-181d mut13 Energy -4.7

428 – 69 (16.1%)

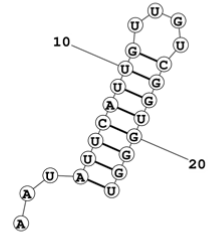
3C>A



miR-181d mut11 Energy -4.6

1431 – 294 (20.5%)

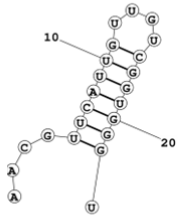
3C>U



miR-181d mut12 Energy -4.5

1047 – 205 (20.5%)

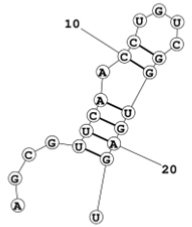
4A>G



-181d mut7 Energy -3.5

52 – 8 (15.4%)

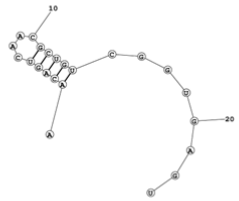
2A>G; 4A>G



miR-181c mut8 Energy -3.1

32 – 6 (18.7%)

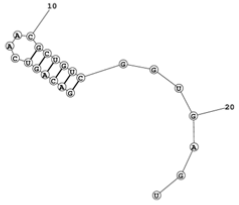
5U>G



miR-181a mut4 Energy -3.6

639 – 115 (15.4%)

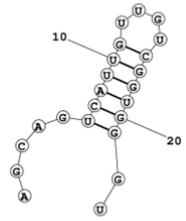
1A>G; 5U>G



miR-181a mut9 Energy -7.5

621 – 112 (18%)

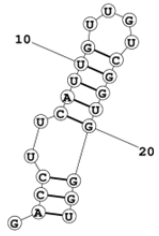
2A>G; 5U>G



miR-181d mut3 Energy -3.2

385 – 75 (19.6%)

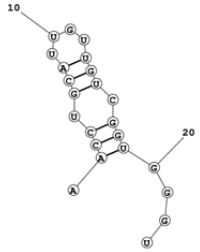
1A>G; 4A>C



miR-181d mut5 Energy = -4.0

373 –63 (16.9%)

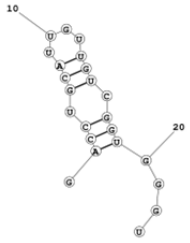
4A>C; 6U>G



miR-181d mut6 Energy = -5.1

284 –45 (15.8%)

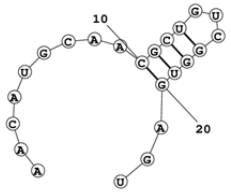
1A>G; 4A>C; 6U>G



miR-181d mut9 Energy = -5.2

272 –44 (15.8%)

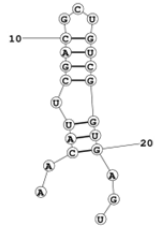
6U>G



miR-181a mut5 Energy -5.8

1037 – 212 (38%)

8A>G



miR-181a mut6 Energy -4.3

438 – 395 (90%)

Predicted targets for specific miR-181 mutant variants include genes with differentially expression patterns in human cancers – TCGA analysis

		LUSC		LUAD		Lower grade glioma		Glioblastoma		Breast cancer	
miR-181 sequence	Gene name	EXP	FC	EXP	FC	EXP	FC	EXP	FC	EXP	FC
181a mut3&mut8, 181b mut3, 181c mut3&mut6	*RGS5*	Down	−5.44	Down	−2.38						
181a mut3&mut8, 181b mut3, 181c mut3&mut6, 181d mut7	*SLC4A1*	Down	−6.95	Down	−5.53						
181a mut3&mut8, 181b mut3, 181c mut3&mut6, 181d mut7	*C5orf34*	Up	6.31	Up	4.33						
	CTSE	Down	−12.53								
	ENPP5	Down	−3.06								
	LILRB2	Down	−3.34	Down	−2.01						
	LRP1	Down	−2.43	Down	−2.08						
	PLXDC2	Down	−2.27	Down	−2.08						
	SPSB4			Down	−2.49						
181a mut3&mut8, 181c mut3&mut6, 181d mut7	*CBR1*	Up	2.35								
	PNPLA2	Down	−2.68								
181a mut3, 181c mut3, 181d mut7	*TRIM5*							Up	8.32		
181a WT&mut3&mut8, 181b WT&mut3, 181c WT&mut3&mut6, 181d WT&mut7	*B3GNT5*	Up	2.24							Down	−2.87
	MTMR10	Down	−2.13	Down	−2.22						
	TNIK	Down	−3.28	Down	−2.35						
	ZNF559									Down	−2.07
	METAP2							Up	2.03		
181c mut8	*CDK2AP1*							Up	3.53		
	DLK1					Down	−2.20				
	DMD	Down	−3.19	Down	−2.67					Down	−13.97
	HPS5			Down	−2.30						
	MARVELD3	Up	3.04	Up	2.36	Up	2.55			Up	2.33
	MATN2			Down	−2.17					Down	−10.89
	SCNN1A	Down	−2.57								
	SFTPB	Down	−64.75	Down	−5.30						
	SLC6A1	Down	−2.47								
	TBX3	Down	−2.52	Down	−5.08						
	ULBP3	Up	2.46								

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; EXP, expression; FC, fold change of expression (malignant vs adjacent normal tissue)

Upregulated genes with oncogenic potential highlighted by TCGA analysis

Gene name	Gene symbol	Cancer type	Oncogenic function	Clinical significance
Chromosome 5 open reading frame 34	*C5orf34*	LUAD	Activation of MAPK signaling pathway Proliferation and migration	Poor prognosis in LUAD patients
MARVEL domain containing 3	*MARVELD3*	Colorectal cancer Pancreatic cancer Hepatocellular carcinoma (HCC)	Cell migration and invasion	Poor prognosis in OSCC patients
Cyclin-dependent kinase 2 associated protein 1	*CDK2AP1*	Glioma	Cell proliferation and tumor growth in xenograft models	Associated with aggressive subtypes and poor prognosis in HCC and prostate cancer
UL16 binding protein 3	*ULBP3*	Diverse cancer types	Modulation of NK cytotoxic activity	Upregulated levels of cell surface and soluble ULBP3 in cancer patients
Methionyl aminopeptidase 2	*METAP2*	Fibrosarcoma Melanoma Breast cancer Glioma	Increase of vascular mimicry	
Beta-1,3-N-acetylglucosaminyltransferasee	*B3GNT5*	Breast cancer Glioma	Cancer cell stemness	

Highlights of the study

- Target genes selectivity by miRNAs might be a function of both sequence composition of the seed region and higher order secondary structure
- Sequence alterations that result in stem structures with a 5’ and 3’ free arm might increase the selectivity for target genes. Sequence composition and length of the free arms could further provide a tuning role.
- Artificial miRNAs with a reduced number of targets enriched in oncogenes might provide novel miRNA-based therapeutics for cancer treatment with less off-target effects

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