المركز العراقي لبحوث السرطان والوراثة الطبية / الجامعة المستنصرية

microRNAs: biogenesis and diagnosis

م. د. زينب سعد عبد الغني
رئيس قسم الاحياء الجزيئي

برعاية السيد رئيس الجامعة المستنصرية المحترم أ.د. حميد فاضل التميمي
وبإشراف السيد مدير عام المركز المحترم أ.م.د. عبير انور احمد

يتشرف قسم الاحياء الجزيئي وقسم العلاج التجريبي/ المركز العراقي لبحوث السرطان والوراثة الطبية/ الجامعة المستنصرية
بدعوتكم لحضور ورشة العمل الالكترونية العلمية الموسومة:

" MicroRNAs: Biogenesis and Diagnosis "

وذلك في يوم الجمعة الموافق 2020/5/22 في تمام الساعة الثالثة عصرا يلقيها
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والدعوة عامة للجميع

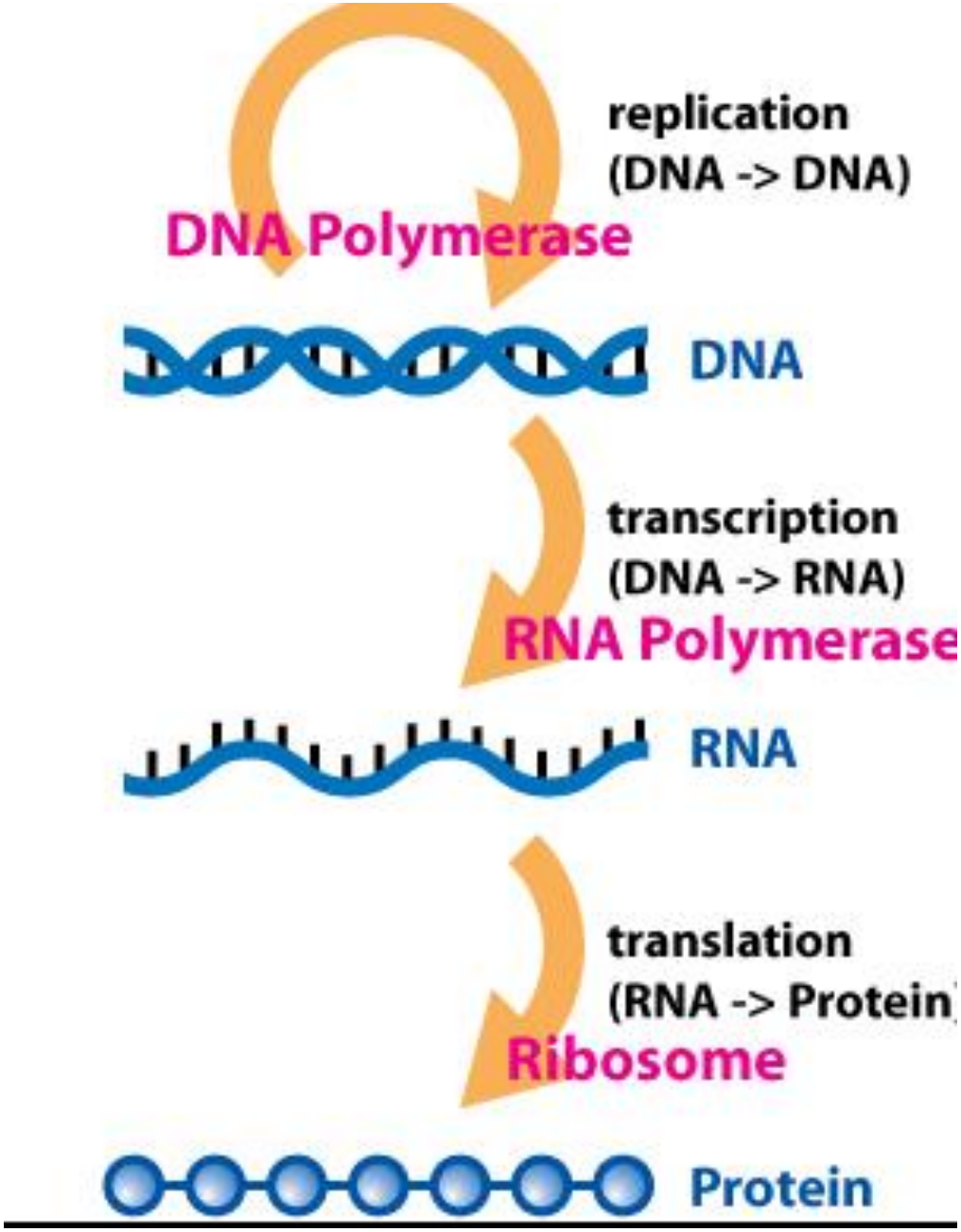
* Introduction : gene silencing
* Central dogma
* RNA interference
* Biomarker and screening
* Biogenesis
* Animation
* miRBase
* Treatment and trails
* ICCMGR observation

INTRODUCTION

- **Gene silencing** is a general term describing epigenetic processes of gene regulation.

- The term gene silencing is generally used to describe the "switching off" of a gene by a mechanism other than genetic modification.

- Gene silencing occurs when RNA is unable to make a protein during translation.


replication
(DNA -> DNA)
DNA Polymerase
DNA
transcription
(DNA -> RNA)
RNA Polymerase
RNA
translation
(RNA -> Protein
Ribosome
Protein

- Genes are regulated at either the transcriptional or post-transcriptional level.
- Transcriptional gene silencing is the result of histone modifications, creating an environment of heterochromatin around a gene that makes it inaccessible to transcriptional machinery (RNA polymerase, transcription factors etc.).

- Post-transcriptional gene silencing is the result of mRNA of a particular gene being destroyed or blocked.

- The destruction of the mRNA prevents translation to form an active gene product (in most cases, a protein).

- The blocking of the gene occurs through the activity of silencers, which bind to repressor regions. A common mechanism of post-transcriptional gene silencing is RNAi

Discovery of RNA interference (1998)

- silencing of gene expression with dsRNA

The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

Photo: L. Cicero

Andrew Z. Fire

Photo: J. Mottern

Craig C. Mello

RNA Interference

- RNA interference (RNAi), is a technique in which exogenous, double-stranded RNAs (dsRNAs) that are complimentary to known mRNAs, are introduced into a cell to specifically destroy that particular mRNA, thereby diminishing or abolishing gene expression .

- RNA interference was known by other names, including post transcriptional gene silencing and quelling .

Different classes of small RNA molecules

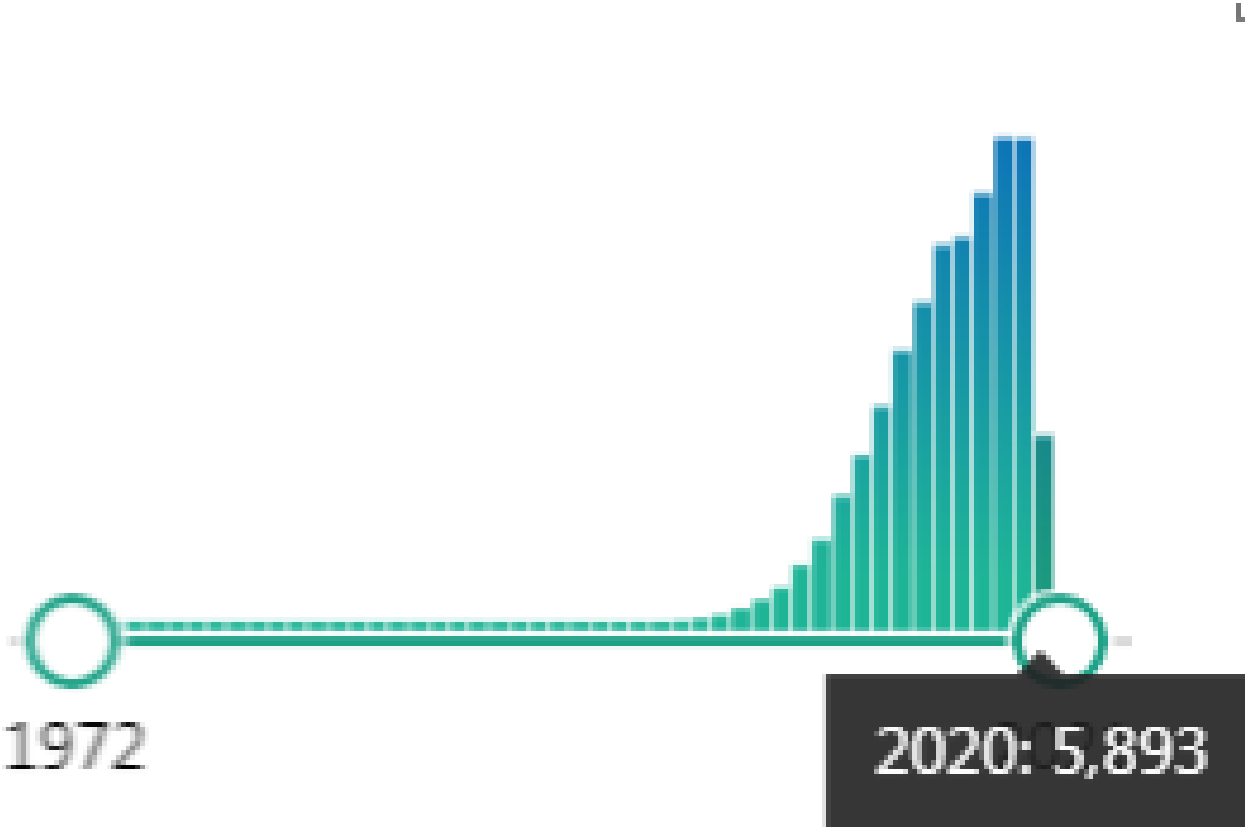
During dsRNA cleavage, different RNA classes are produced:

- siRNA
- miRNA

Small interfering RNA (siRNA):

- Short interfering RNA or silencing RNA
- 21–23 nucleotide-long double-stranded RNA
-
- Can be exogenously (artificially) introduced into cells by transfection

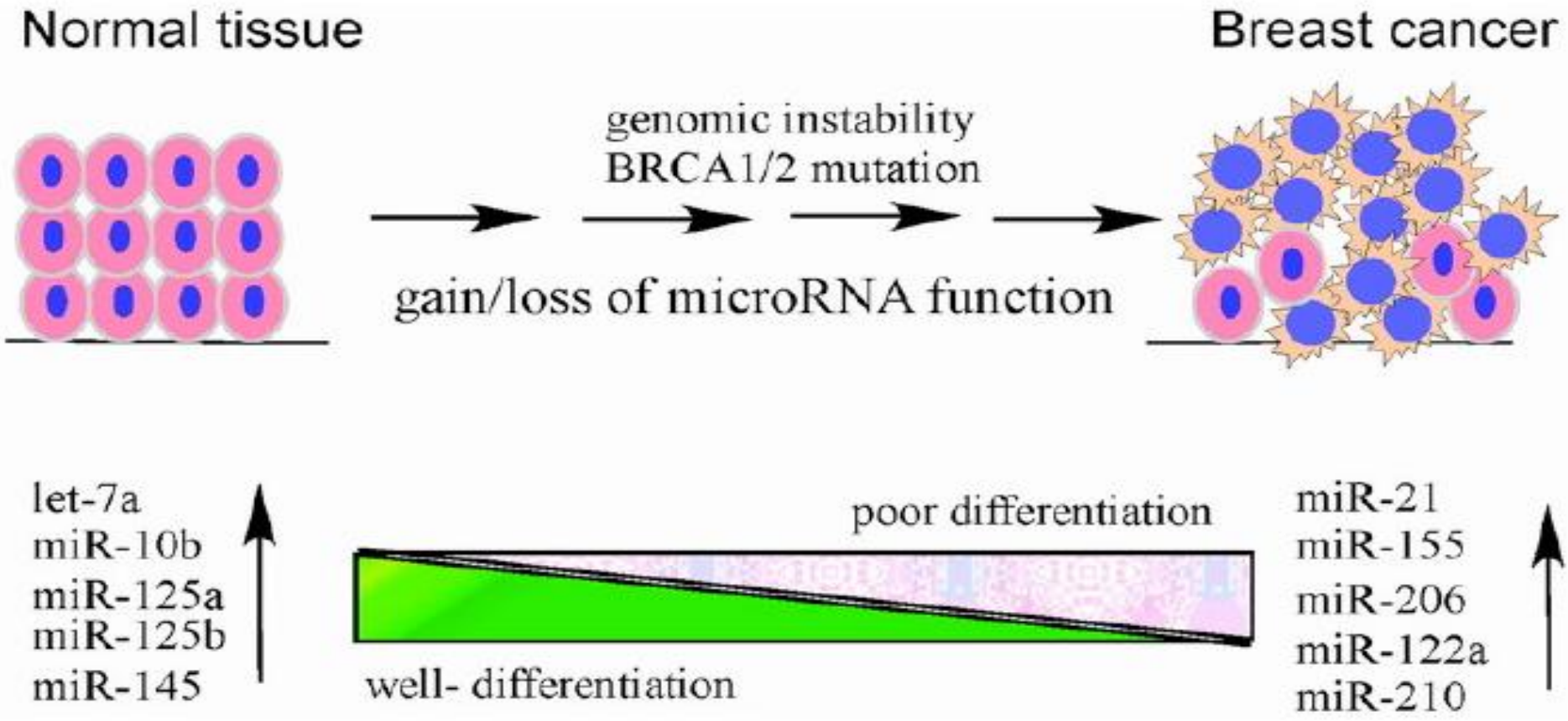
NCBI: microRNA



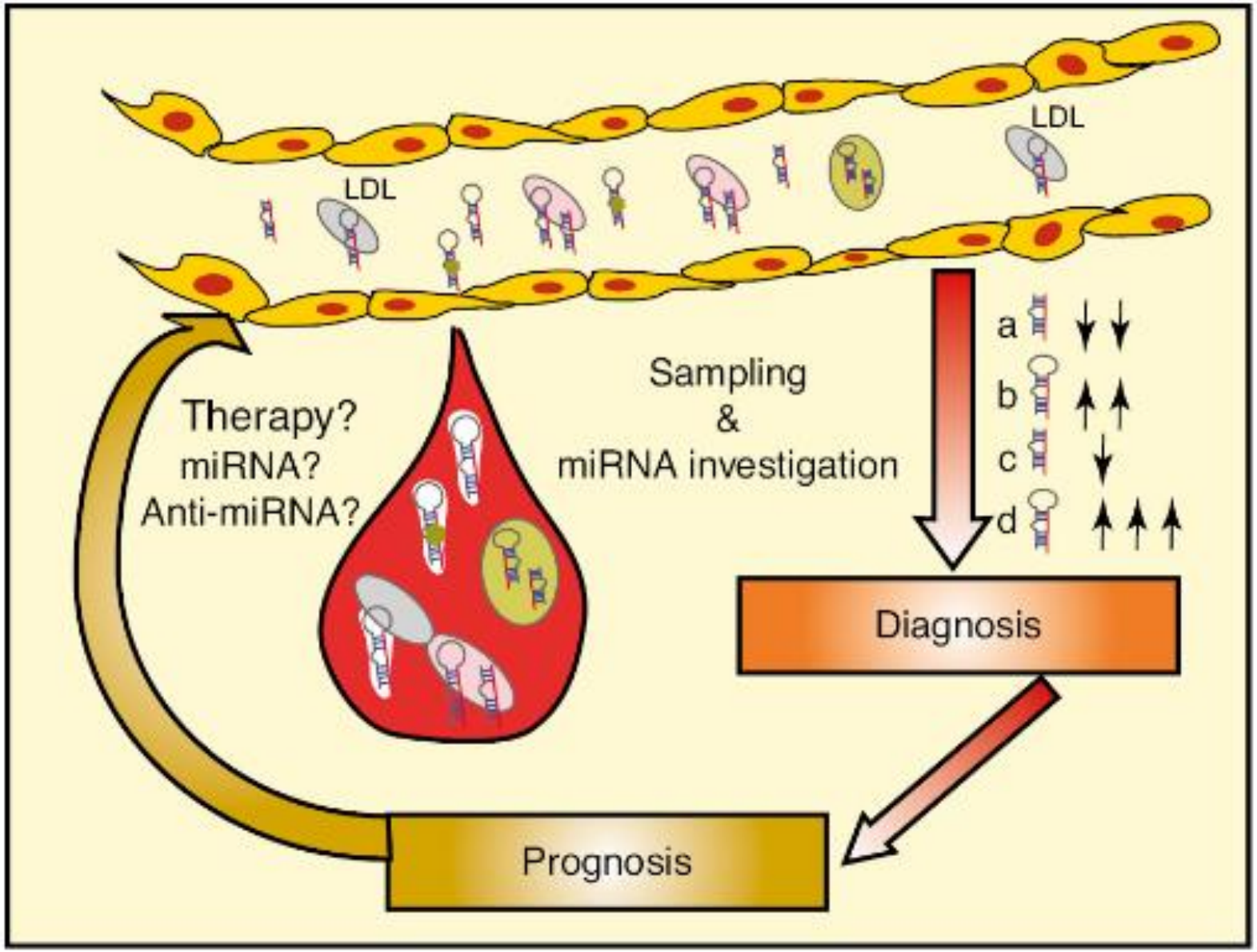
What is a miRNA?

- **Small single stranded RNAs (21-25 nucleotides) but derived from larger precursors (double stranded RNAs)**
- **Non-coding sequences**
- **Form imperfect stem-loop structures (hairpin)**
- **Hybridize by incomplete base-pairing to several sites in the 3'-untranslated regions of target mRNAs**
- **Negative regulators of gene expression (Postranscriptional and Translational regulation)**
- **Role in disease and cancer still in research**

The increasing number of studies that prove the presence of miRNAs in circulating (serum/plasma) increases the chance of using this miRNAs as a good biomarker for cancer and other diseases.



Samples used*	Method(s) used	Key miRNA changes	
		Up-regulated	Down-regulated
MCF-7, T47D breast cancer cell line	Northern blot		miR-143 miR-145 miR-16 let-7a-1
Breast cancer tumor tissues (6) *vs.* normal tissues (5) MCF-7 cell line	Bead-based flow cytometry Northern blot	miR-21	let-7
Breast cancer tumor tissues (76) *vs.* normal tissues (30) Breast cancer cell lines	miRNA microarray Northern blot	miR-21 miR-155 miR-206 miR-122a miR-210	let-7 miR-10b miR-125a miR-125b miR-145
Breast cancer tumor tissues (79) *vs.* normal tissues (6)	miRNA microarray	miR-21 miR-155 miR-206 miR-122a miR-210	let-7 miR-10b miR-125a miR-125b miR-145
Breast cancer tumor tissues (5) *vs.* normal tissues (5) MCF-7 cell line	TaqMan RT-PCR	miR-21	

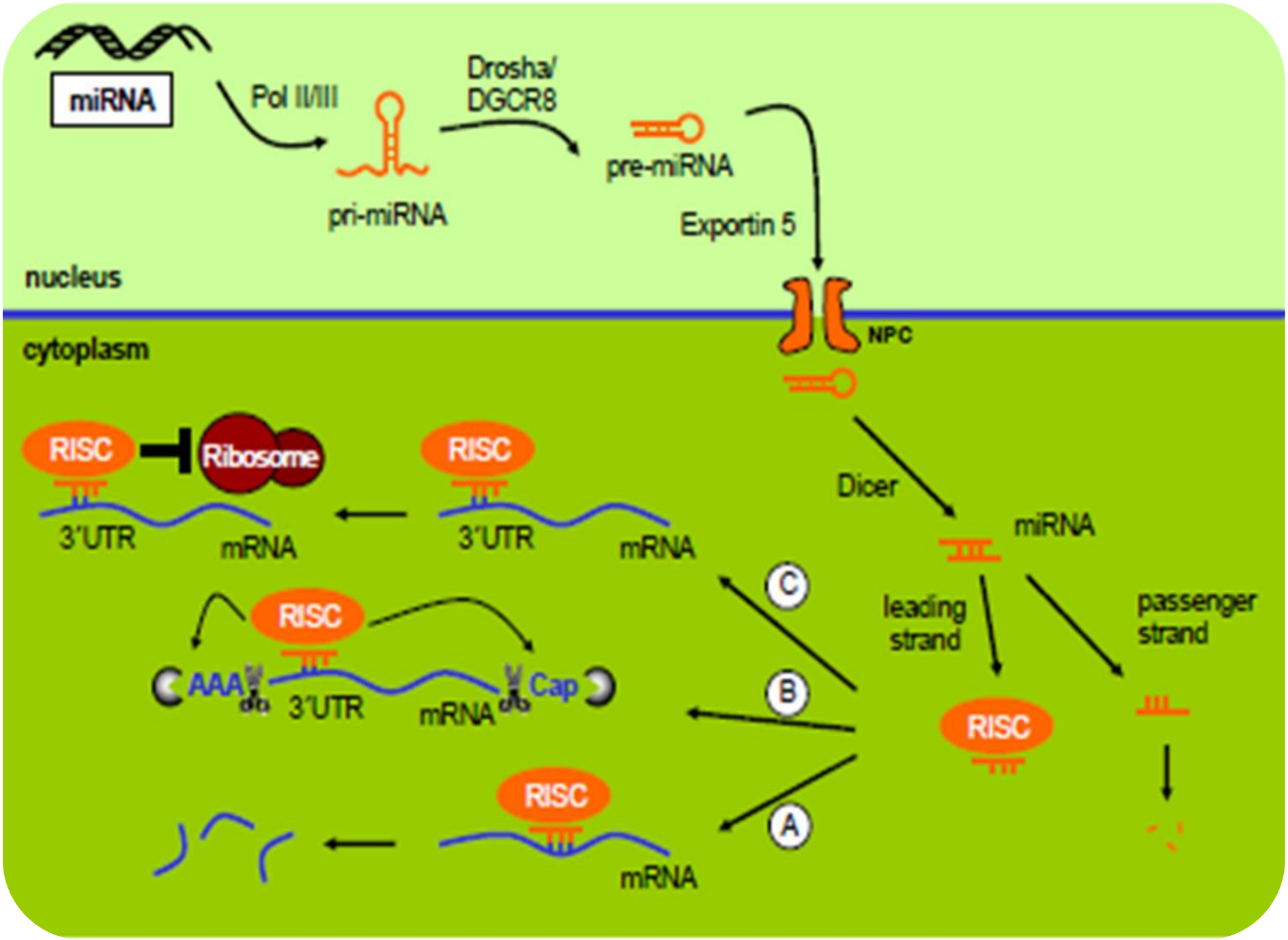

LDL
LDL
Therapy?
miRNA?
Anti-miRNA?
Sampling
&
miRNA investigation
a
b
c
d
Diagnosis
Prognosis

- A select number of miRNAs may serve as diagnostic markers for different tumor types and diseases.

- The ideal properties of biomarker should be:

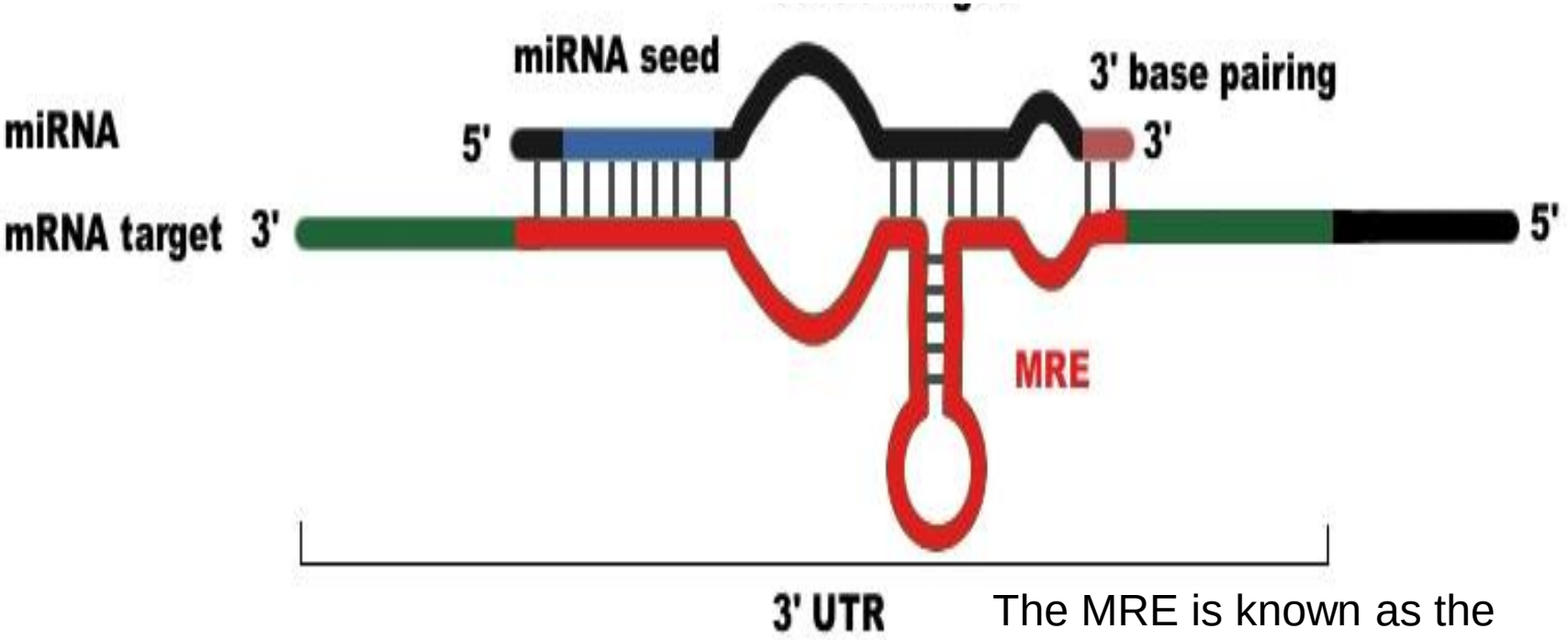
- disease specific
- able to differentiate between pathologies
- rapid and significant release during pathology development
- long life in sample, rapid, simple, accurate and inexpensive for detection
- un affected by environmental conditions
- present in accessible body fluid.

Cancer	Tumor marker	Sensitivity
Breast cancer	CEA	29%–53%[a]
	CA15-3	54%–90%[a]
Prostate cancer	PSA (>4 ng/ml)	20%–72%
Lung cancer	None	–
Colon cancer	CEA (>5 ng/ml)	26%
	CA19-9	18%
Uterine cancer	CA125	34.6%
Melanoma	S-100 protein	15%–65%*
Non-Hodgkin's lymphoma	None	–
Ovarian cancer	CA125	71%[a]
Bladder cancer	Urine cytology	71%
Renal cancer	None	–
Pancreas cancer	CA19-9	69%–93%


miRNA
Pol II/III
pri-miRNA
Drosha/
DGCR8
pre-miRNA
Exportin 5
nucleus
cytoplasm
NPC
RISC
Ribosome
3'UTR
mRNA
RISC
3'UTR
mRNA
Dicer
miRNA
leading
strand
passenger
strand
C
RISC
AAA
3'UTR
mRNA
Cap
B
RISC
RISC
A
mRNA

Symbol	Definition
Prefix: has and mmu	Indicates species
miR-XXX	All microRNA are identified by 3-4 numbers
Pri-miR	Primary miRNA: gene transcript with hairpin loop structure, capped with a specially modified nucleotide at the 5’ end, and polyadenylated, may contain 1-6 pre-miR
Pre- miR	Precursor miRNA: hairpin structure obtained from the pri-miRNA and is characterized by two nucleotide overhang at its 3’ end
miR-XXX	Mature miRNA that is the guide strand "-3p" anti sense
miR-XXX*	Passenger strand "5-p" sense
miR-XXXa and miR-XXXb	Indicates high homology
miR-XXX-1 and miR-XXX-2	Identical mature miRNAs originating from different regions of the genome
Seed sequence	Nucleotides 2-7 of miRNA complementary to target mRNA

miRNA Binding

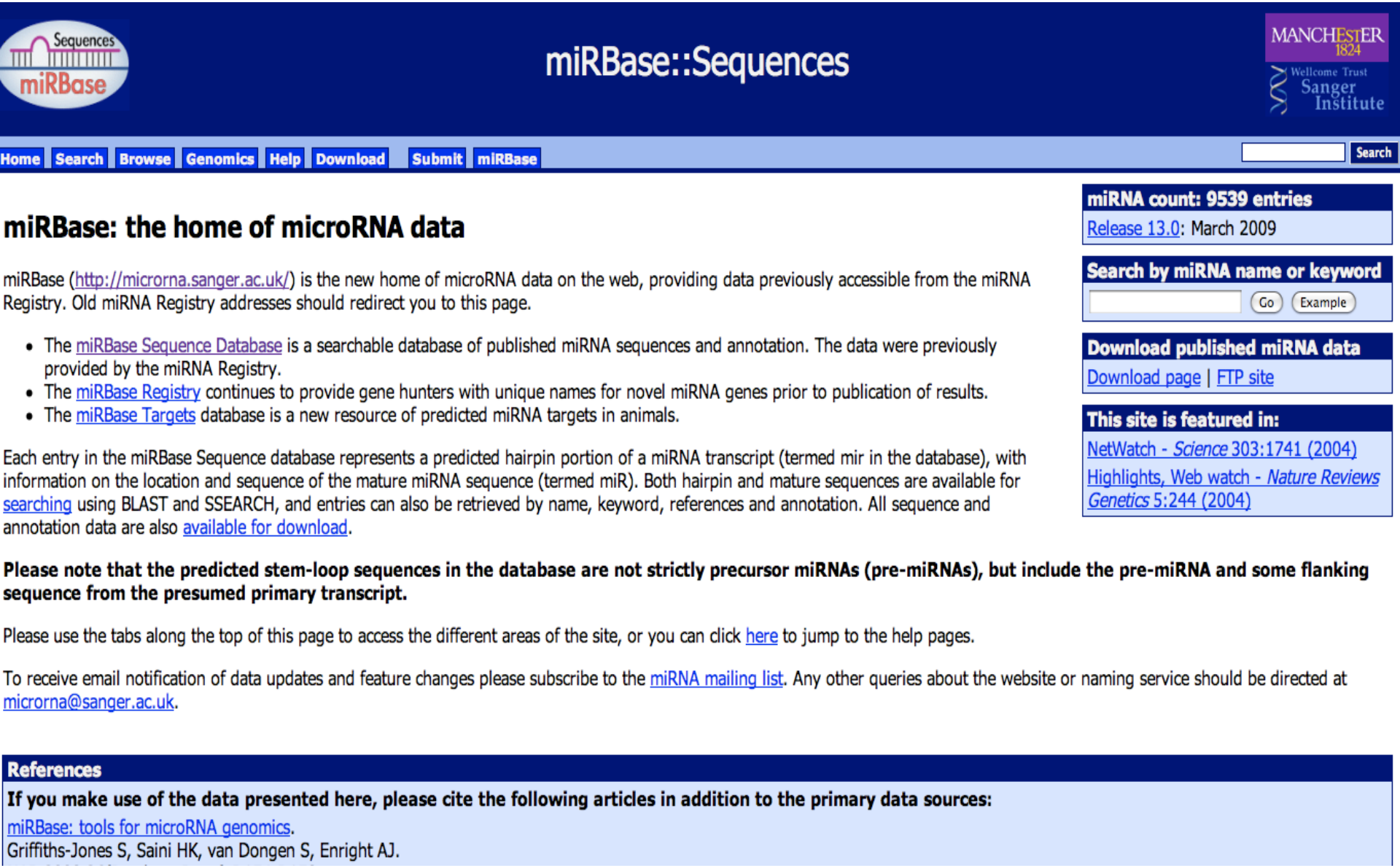


The MRE is known as the "miRNA recognition element." This is simply the sequence in the target that an miRNA binds to mRNA

The major players

- Dorsha and Pasha are part of the microprocessor protein complex
- Dorsha and Dicer are RNase III enzymes
- Pasha is a ds RNA binding protein
- Exportein 5 is a member of the nucleocytoplasmic transport factors
- Argonaute are RNase enzyme

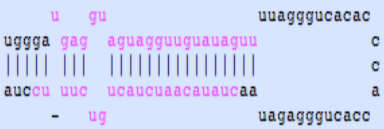
How to screen for your miRNA



Homo sapiens miRNAs (706 sequences)

ID	Accession	Chromosome	Start	End	Strand	Fetch
hsa-let-7a-1	MI0000060	9	95978060	95978139	+	
hsa-let-7a-2	MI0000061	11	121522440	121522511	-	
hsa-let-7a-3	MI0000062	22	44887293	44887366	+	
hsa-let-7b	MI0000063	22	44888230	44888312	+	
hsa-let-7c	MI0000064	21	16834019	16834102	+	
hsa-let-7d	MI0000065	9	95980937	95981023	+	
hsa-let-7e	MI0000066	19	56887851	56887929	+	
hsa-let-7f-1	MI0000067	9	95978450	95978536	+	
hsa-let-7f-2	MI0000068	X	53600878	53600960	-	
hsa-let-7g	MI0000433	3	52277334	52277417	-	
hsa-let-7i	MI0000434	12	61283733	61283816	+	
hsa-mir-1-1	MI0000651	20	60561958	60562028	+	
hsa-mir-1-2	MI0000437	18	17662963	17663047	-	
hsa-mir-7-1	MI0000263	9	85774483	85774592	-	
hsa-mir-7-2	MI0000264	15	86956060	86956169	+	
hsa-mir-7-3	MI0000265	19	4721682	4721791	+	
hsa-mir-9-1	MI0000466	1	154656757	154656845	-	
hsa-mir-9-2	MI0000467	5	87998427	87998513	-	
hsa-mir-9-3	MI0000468	15	87712252	87712341	+	
hsa-mir-10a	MI0000266	17	44012199	44012308	-	
hsa-mir-10b	MI0000267	2	176723277	176723386	+	
hsa-mir-15a	MI0000069	13	49521256	49521338	-	
hsa-mir-15b	MI0000438	3	161605070	161605167	+	
hsa-mir-16-1	MI0000070	13	49521110	49521198	-	

Stem-loop sequence MI0000060

Accession	MI0000060
ID	hsa-let-7a-1
Symbol	HGNC:MIRLET7A1
Description	Homo sapiens let-7a-1 stem-loop
Stem-loop	 Get sequence
Comments	let-7a* cloned in [6] has a 1 nt 3' extension (U), which is incompatible with the genome sequence.
Genome context	*Coordinates (NCBI36)*: 9: 95978060-95978139 [+] *Overlapping transcripts*: intergenic View flanking features
Clustered miRNAs	*< 10kb from hsa-let-7a-1* hsa-let-7a-1 — 9: 95978060-95978139 [+] hsa-let-7f-1 — 9: 95978450-95978536 [+] hsa-let-7d — 9: 95980937-95981023 [+]
Database links	EMBL: AJ421724 RFAM: RF00027; let-7 HGNC: 31476; MIRLET7A1 ENTREZGENE: 406881; MIRLET7A1
Gene family	MIPF0000002; let-7


This 3D animation is provided courtesy of Rosetta Genomics (www.rosettagenomics.com)

miRNAs in Treatment

- Since diseases result from the expression of undesired or mutated genes, or from overexpression of certain normal genes
- The discovery of siRNA and miRNA opens up a new therapeutic approach for the treatment of diseases by targeting genes that are involved in the pathological process

New example about miRNA / blood

GENERAL

NEW BLOOD TEST DEVELOPED TO DIAGNOSE ALZHEIMER'S DISEASE

Submitted by: MEMBS,

Apr 24, 2016 2:00 pm

University of Otago researchers have discovered a promising new marker that could help diagnose Alzheimer's disease—and all that might be required is a simple blood test.

Before this work began, blood plasma microRNA had been shown to reflect various disease processes, and specific microRNAs were linked to neurological diseases. This led Dr Joanna Williams of Otago's Department of Anatomy to suggest that blood microRNA levels may reflect changes in the brain.

The specific set of blood microRNAs that the Otago researchers have identified can detect Alzheimer's disease correctly 86 per cent of the time.

DIAMIR

DiamiR Receives Award from Alzheimer's Drug Discovery Foundation to Accelerate Development of microRNA Biomarkers for Alzheimer's Disease

Nov 20, 2019

MONMOUTH JUNCTION, N.J., Nov. 20, 2019 /PRNewswire/ — DiamiR, a developer of innovative blood-based diagnostic tests for neurodegenerative and other diseases, announced today a $492,000 award in support of the project entitled "Circulating brain-enriched microRNAs as peripheral biomarkers of neurodegeneration." The award will accelerate the development of DiamiR's targeted diagnostic technology for detection and prediction of Alzheimer's disease (AD) progression. The award is provided by the Alzheimer's Drug Discovery Foundation (ADDF) Diagnostics Accelerator, a fund set up in collaboration with Bill Gates and other philanthropic partners.

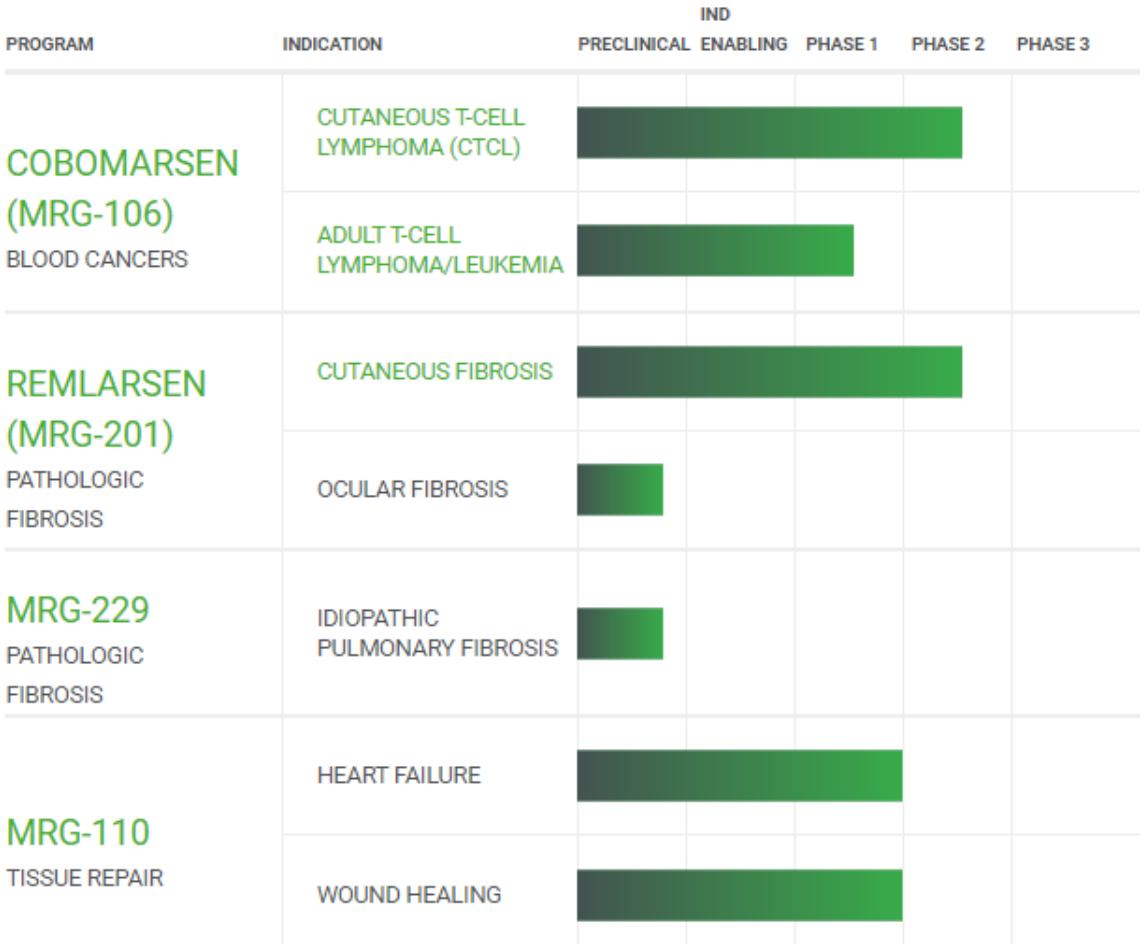
DiamiR Announces Additional $345,000 Funding from the NIH for Development of CogniMIR™

Sep 17, 2019

MONMOUTH JUNCTION, N.J., Sept. 16, 2019 /PRNewswire/ — DiamiR, a developer of innovative blood-based diagnostic tests for neurodegenerative and other diseases, announced today that the National Institute on Aging (NIA) of the National Institutes of Health (NIH) has awarded DiamiR $345,000 in supplemental funding under the ongoing Small Business

miRagen

ABOUT PROGRAMS SCIENCE CAREERS INVESTORS PATIENTS CLINIC



Citation: Molecular Therapy—Nucleic Acids (2015) 4, e252; doi:10.1038/mtna.2015.23
Official journal of the American Society of Gene & Cell Therapy

www.nature.com/mtna

REVIEW

siRNA Versus miRNA as Therapeutics for Gene Silencing

Jenny KW Lam[1,2], Michael YT Chow[1], Yu Zhang[1] and Susan WS Leung[1,2]

Table 5 A summary of siRNA therapeutics in clinical trials (registered with *clinicaltrials.gov*, last accessed 13 June 2015)

Name	Indications	siRNA target	Phase	Delivery system	Route of administration	Trial ID (reference)
Cancer						
ALN-VSP02	Advanced solid tumors with liver involvement	KSP and VEGF	1, completed	Lipid nanoparticles	Intravenous	NCT01158079; NCT00882180[2]
Atu027	Advanced solid tumor	PKN3	1, ongoing	Liposomal particles (AtuPLEX®)	Intravenous	NCT00938574[3]
	Pancreatic ductal carcinoma		1/2, ongoing			NCT01808638
CALAA-01	Solid tumor	RRM2	1, terminated	Polymer-based targeted nanoparticles	Intravenous	NCT00689065[185]
DCR-MYC (Dicer-substrate siRNA)	Solid tumor, multiple myeloma, non-Hodgkin's lymphomas	MYC oncogene	1, ongoing	Lipid nanoparticles (EnCore)	Intravenous	NCT02110563
	Hepatocellular carcinoma		1/2, ongoing			NCT02314052
siG12D LODER	Advanced pancreatic cancer	mutated KRAS oncogene	1, completed; 2, ongoing	Biodegradable polymer-based scaffold	Local implantation	NCT01188785; NCT01676259
siRNA-EphA2-DOPC	Advanced cancers	EphA2	1, ongoing	Neutral liposomes	Intravenous	NCT01591356
TKM-080301 (TKM-PLK1)	Primary or secondary liver cancer	PLK1	1, completed	Lipid nanoparticles	Intravenous	NCT01437007
	Neuroendocrine tumors and adrenocortical carcinoma		1/2 ongoing			NCT01262235

Table 6 A summary of miRNA therapeutics in clinical trials (registered with *clinicaltrials.gov*, last accessed 13 June 2015)

Name	Indications	miRNA	Phase	Delivery system	Route of administration	Trial ID
MRX34	Primary liver cancer or liver metastasis from other cancers	miRNA-34a	1, ongoing	Liposomes (SMARTICLES)	Intravenous	NCT01829971
TargomiRs	Malignant pleural mesothelioma; non–small-cell lung cancer	miRNA-16	1, ongoing	Nanoparticles (nonliving bacterial minicells)	Intravenous	NCT02369198

frontiers in Genetics

Front Genet. 2019; 10: 478.
Published online 2019 May 16. doi: 10.3389/fgene.2019.00478

PMCID: PMC6532434
PMID: 31156715

The Potential for microRNA Therapeutics and Clinical Research

Johora Hanna,[1] Gazi S. Hossain,[1] and Jannet Kocerha[2,*]

Abstract

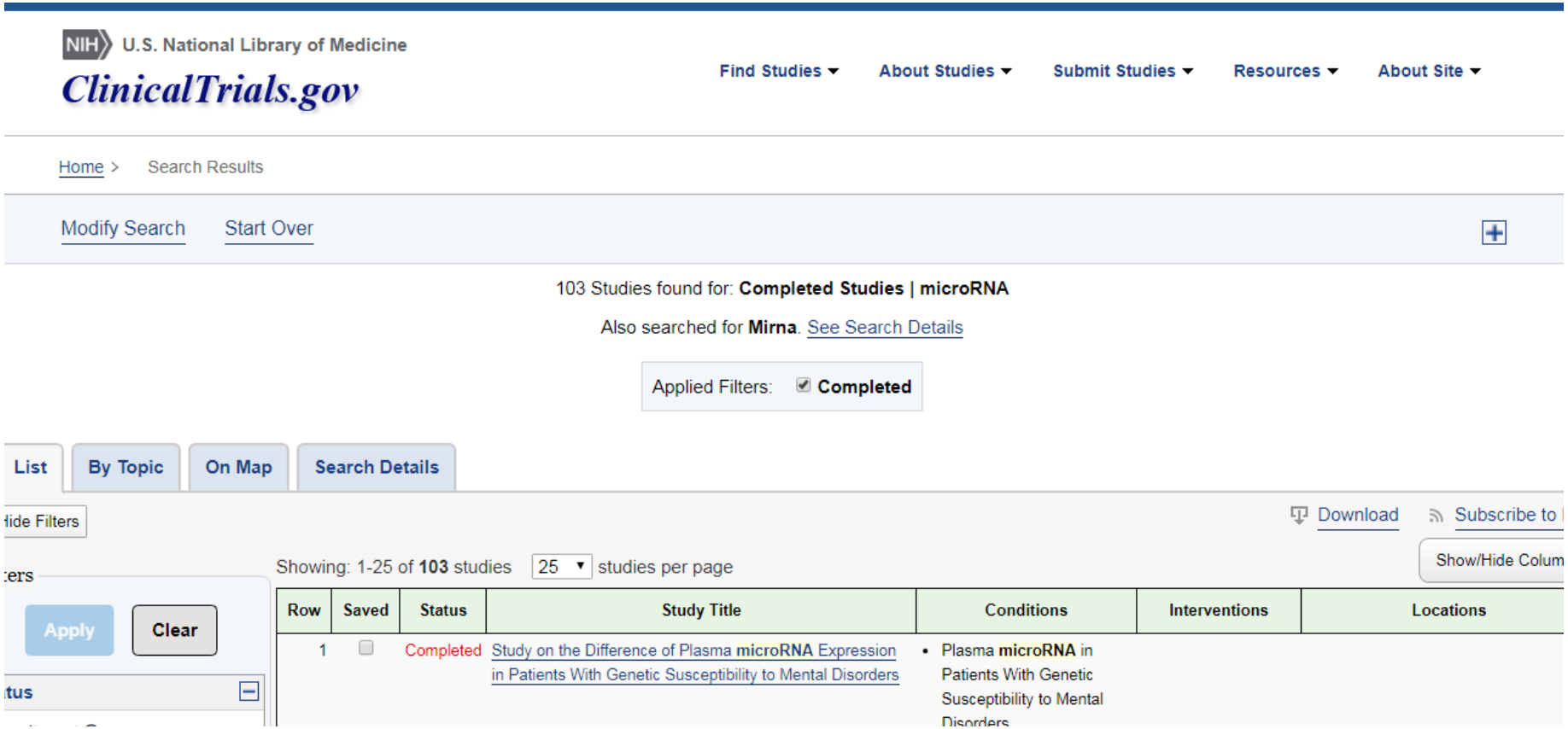
Go to:

As FDA-approved small RNA drugs start to enter clinical medicine, ongoing studies for the microRNA (miRNA) class of small RNAs expand its preclinical and clinical research applications. A growing number of reports suggest a significant utility of miRNAs as biomarkers for pathogenic conditions, modulators of drug resistance, and/or as drugs for medical intervention in almost all human health conditions. The pleiotropic nature of this class of nonprotein-coding RNAs makes them particularly attractive drug targets for diseases with a multifactorial origin and no current effective treatments. As candidate miRNAs begin to proceed toward initiation and completion of potential phase 3 and 4 trials in the future, the landscape of both diagnostic and interventional medicine will arguably continue to evolve. In this mini-review, we discuss miRNA drug discovery development and their current status in clinical trials.

Table 1

Interventional clinical trials for miRNAs.

miRNA gene; drug name	Clinical trial number; phase status	Disease/disorder investigated
miR-34; MRX34	NCT01829971; phase 1 (terminated)	Liver cancer, lymphoma
	NCT02862145; phase 1 (withdrawn)	melanoma
miR-92; MRG 110	NCT03603431; phase 1 (recruiting)	wound healing, heart failure
miR-16; MesomiR-1	NCT02369198; phase 1 (completed)	Mesothelioma, lung cancer
miR-122; Miravirsen	NCT02508090; phase 2 (completed)	Hepatitis C virus
	NCT02452814; phase 2 (completed)	
	NCT01200420; phase 2 (completed)	
	NCT01872936; phase 2 (unknown status)	
	NCT01727934; phase 2 (unknown status)	
	NCT01646489; phase 1 (completed)	
miR-29; MRG-201	NCT03601052; phase 1 (recruiting)	Keloid, fibrous scar tissue formation
miR-21; RG-012	NCT02855268; phase 2 (suspended, sponsor decision)	Alport syndrome
	NCT03373786; phase 1 (active, not recruiting)	
miR-155; Cobomarsen (MRG-106)	NCT03713320/phase 2 (recruiting)	T-cell lymphoma/mycosis fungoides
	NCT03837457/phase 2 (new/not yet recruiting)	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Also searched for Mirna. See Search Details
Applied Filters: Completed
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25
studies per page
Show/Hide Colum
Apply
Clear
Row
Saved
Status
Study Title
Conditions
Interventions
Locations
1
Completed
Study on the Difference of Plasma microRNA Expression in Patients With Genetic Susceptibility to Mental Disorders
Plasma microRNA in Patients With Genetic Susceptibility to Mental Disorders

Status	Study Title	Conditions	Interventions	Locations
		Diseases		
Completed	microRNA Profile in Early-stage Cervical Cancer	• Cervical Cancer		
Completed	Blood microRNA Depression Change After DHCA	• Aortic Aneurysm	• Other: collect blood	• Xuanwu Hospital Beijing, Beijing, China
Completed	The Potential Role Of MicroRNA-155 And Telomerase Reverse Transcriptase In Diagnosis Of Non-Muscle Invasive Bladder Cancer And Their Pathological Correlation	• Bladder Cancer • Bladder Disease • Bladder Neoplasm • Micro-RNA	• Diagnostic Test: MicroRNAs-155 • Diagnostic Test: Human telomerase reverse transcriptase	• Urology and Nephrology Center Mansourah, Aldakahlia, Egypt
Completed	Establishment of a Signature of Circulating microRNA as a Tool to Aid Diagnosis of Primary Brain Tumors in Adults	• Brain Tumors		• Groupe Hospitalier Pitié-Salpêtrière Paris, France
Completed	Expression Profiling of microRNA Following Administration of Dexmedetomidine	• Coronary Disease	• Drug: Dexmedetomidine Injection	• Jinqiao Qian Kunming, Yunnan, China
Completed	Microarray Analysis of microRNA Expression in Basal Cell Carcinoma	• Basal Cell Carcinoma		• Department of Dermatology, Venereology and Allergology, Ruhr-University Bochum Bochum, NRW, Germany
Completed	Tocilizumab Effect on microRNA Expression and Adipokine Levels in Rheumatoid Arthritis Patients	• Rheumatoid Arthritis	• Other: laboratory blood tests	
Completed	MicroRNA Diagnostics in Subarachnoid Hemorrhage 2	• Subarachnoid Hemorrhage • Delayed Cerebral Ischemia • Acute Lung Injury		• Rigshospitalet Copenhagen, Denmark

Future observation of ICCMGR

- Develop
- Understand
- Target

Novel expression of microRNAs in serum samples of Iraqi breast cancer women

Saad, Zaynab, Arif, Muhammad, Yassen, Nahi, Jasim, Hameed, Jelawe, Majed and Brown, James (2014). Novel expression of microRNAs in serum samples of Iraqi breast cancer women. *American Journal of Biomedicine*, 2 (5), pp. 567-574.

Abstract

Although a lot of hard work against cancer to reduces its spread but it still continues to kill with abandon. The need for a biomarker for cancer early detection becomes the most mind concentrated scientists. MicroRNAs the tiny non coding RNA molecules opened new path for the scientists to determine the cancer in its early stages. Expression of microRNAs profiles has been investigated to be involved in cancer development. Here we determined the expression of microRNAs in serum of Iraqi healthy volunteers and other women diagnosed with breast cancer. MicroRNAs expression has been determined by using real time qPCR and delta method has been

EJSO EUROPEAN JOURNAL OF SURGICAL ONCOLOGY THE JOURNAL OF CANCER SURGERY

ABSTRACT ONLY | VOLUME 42, ISSUE 5, PS20, MAY 01, 2016

A differential expression of miRNA in plasma and breast tissue: A potential biomarker

M.Arif Nasir • Zaynab Abdul-Ghany • Zartasht Carmichael • James E.P. Brown • Amtul R. Carmichael

DOI: https://doi.org/10.1016/j.ejso.2016.02.083

Article Info

Related Articles

Introduction: Breast cancer is a complex disease and is the leading cause of cancer mortality in women after lung cancer. A non-coding class of RNA called micro RNA (miRNA) is implicated in many diseases including breast cancer where it has been suggested as a potential biomarker.

Thank you for your very kind attention!

Hope it will be easy for you

You can fell free to contact me

zaynab.saad@iccmgr.org

Thank you for attending this workshop