miRNAs在醫藥上的創新與應用

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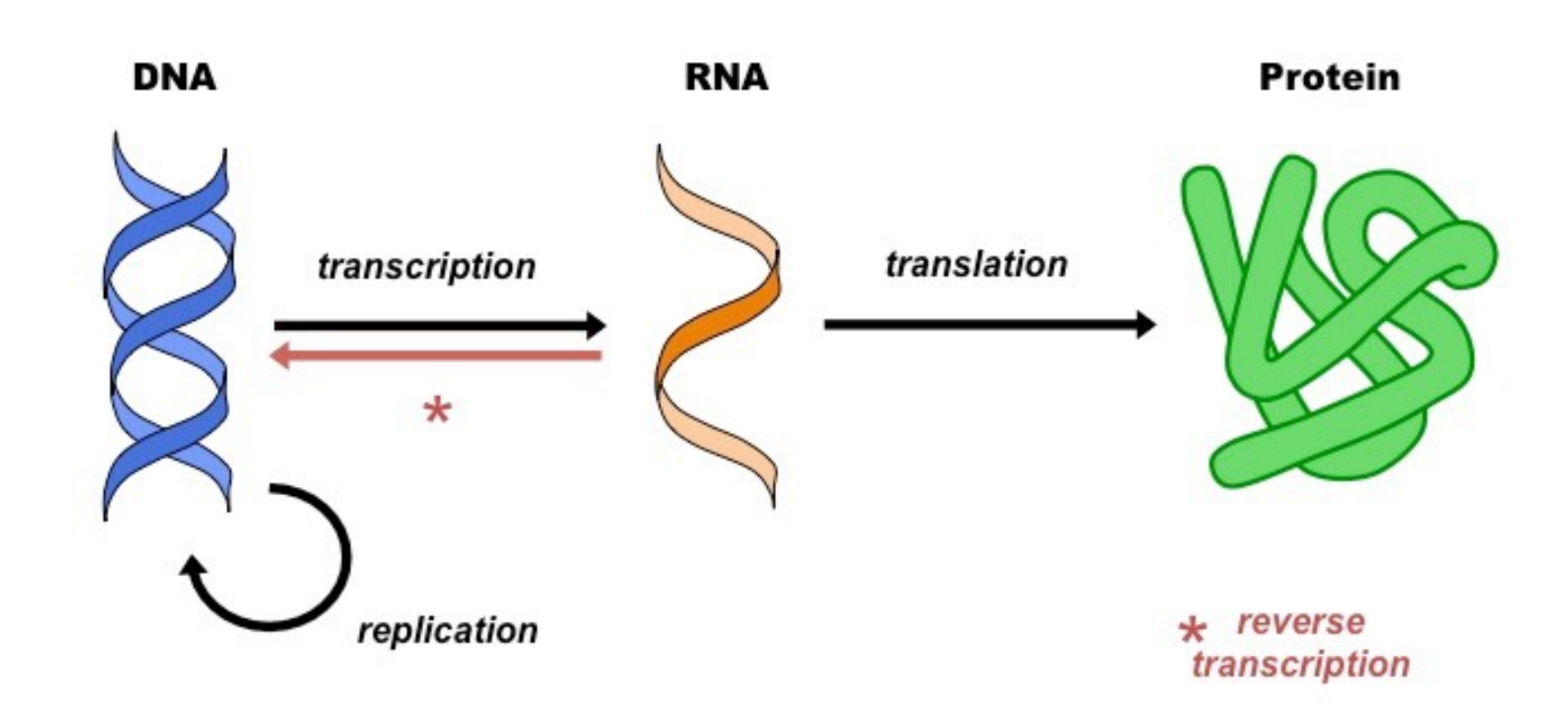
系統生物與生物資訊碩博班
03/17/2020

Central Dogma

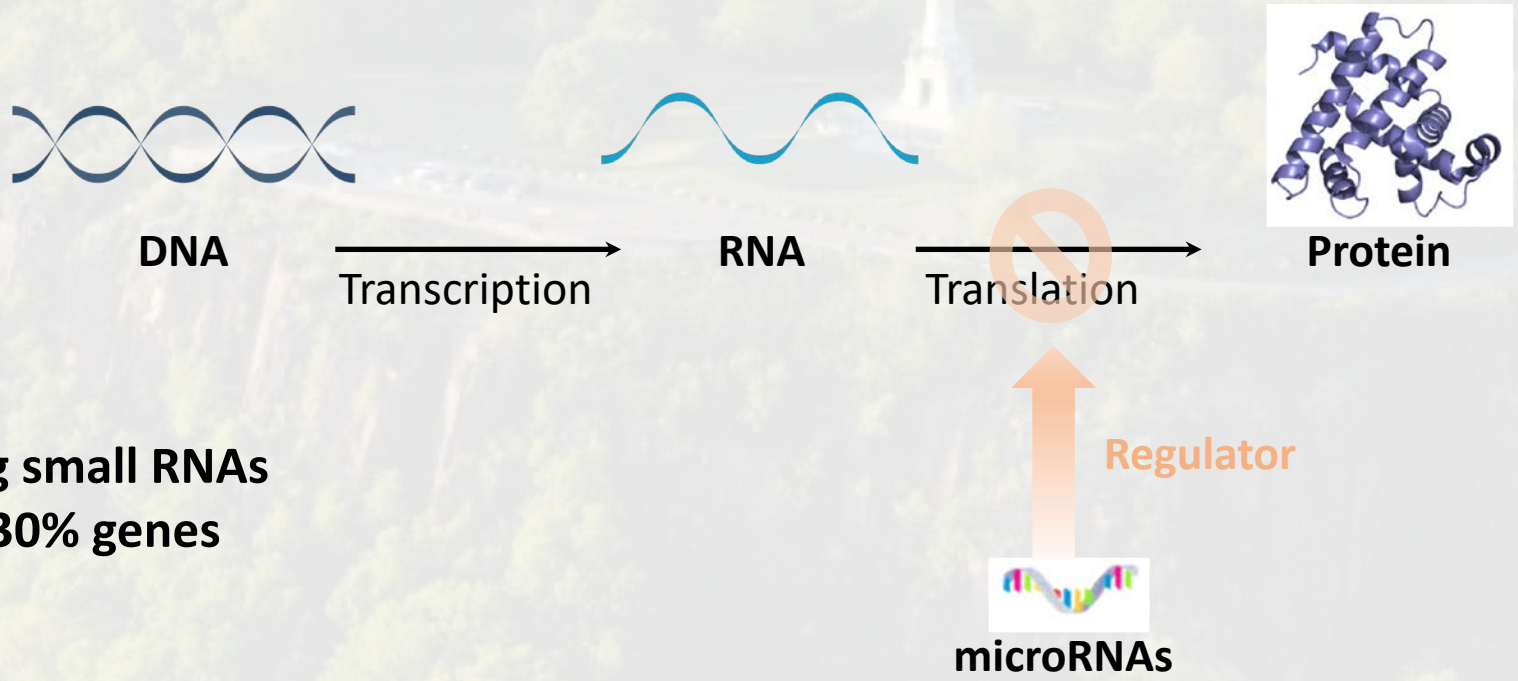


https://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/27-dna-replication-transcri/central-dogma.html

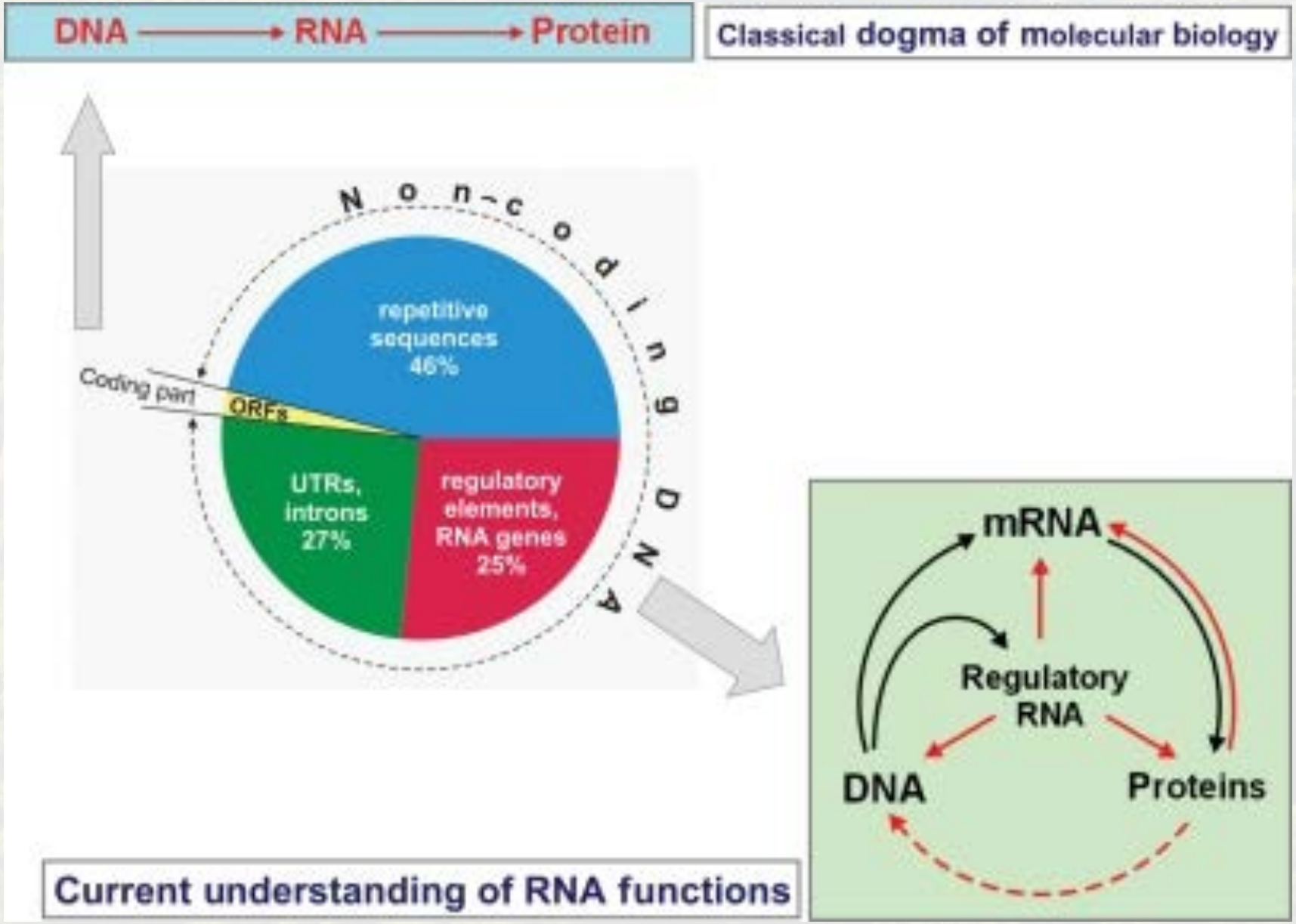
MicroRNAs (non-coding RNAs) are important regulators of gene expression

A new paradigm of gene regulation

The functions of microRNAs

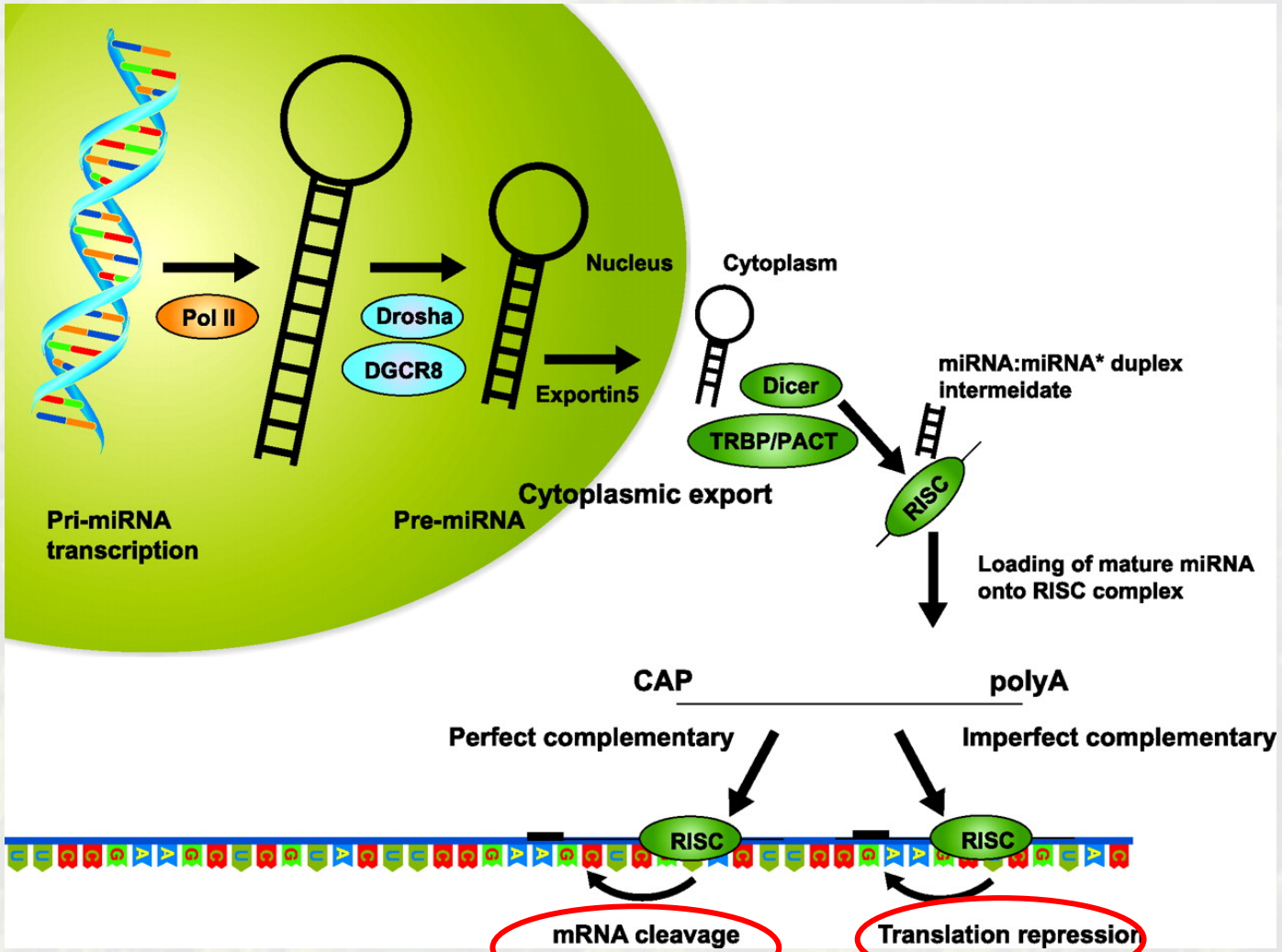


- **Non-coding small RNAs**
- **Regulate ~30% genes**



February 2010 Cardiovascular Research 86(3):353-64

miRNAs biogenesis process



pri-miRNA = primary microRNA transcript

pre-miRNA = precursor microRNA
~ 70 nt

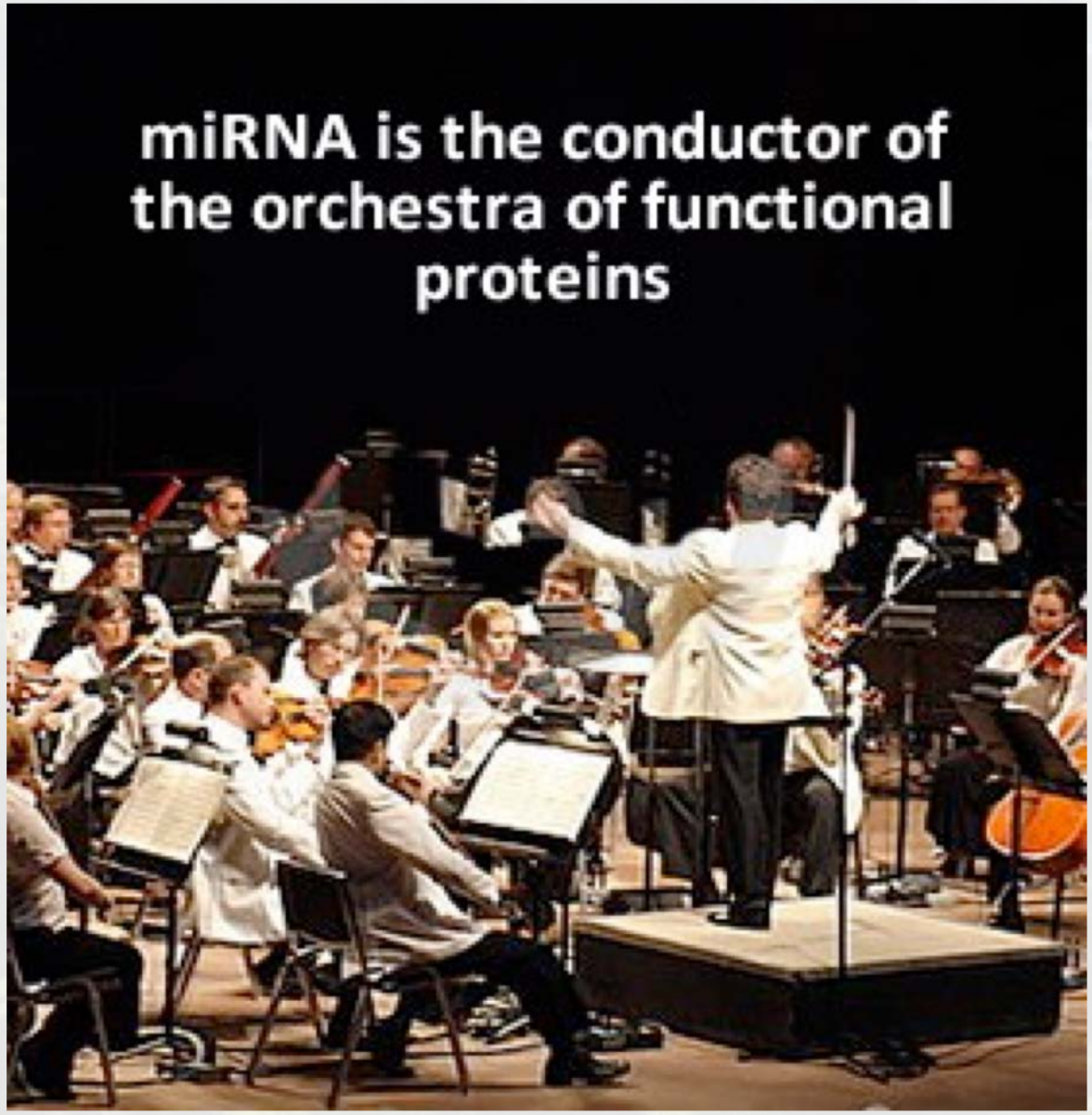
miRNA* = antisense microRNA
~22 nt (now -3p or -5p)

Amount of Protein

Kristen M. Nelson, and Glen J. Weiss Mol Cancer Ther 2008;7:3655-3660

miRNAs –What do we know ?

- primarily bind to the 3' UTR of mRNA
- mRNA degradation and translation repression
- > 55% genes –one or more target sites
- A miRNA can regulate more than one gene targets
- Affect > 30% gene expressions
- Involved in many aspects in numerous biological process

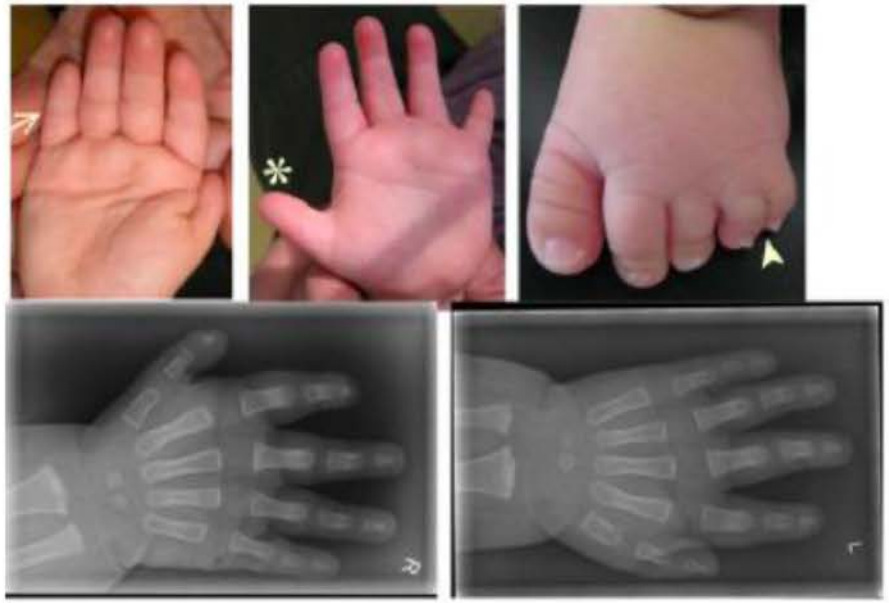


Physiol Genomics 2011 43(10):517

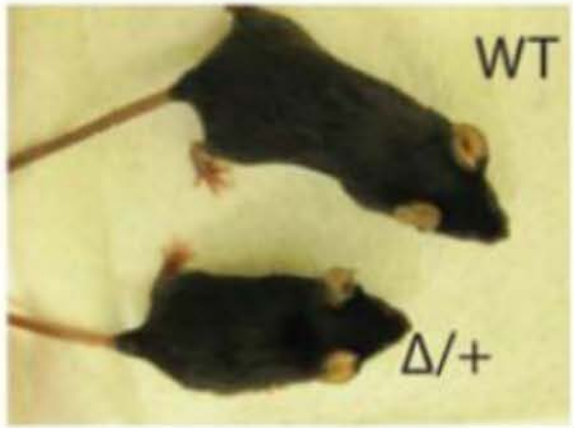
miRNA and inherted disease

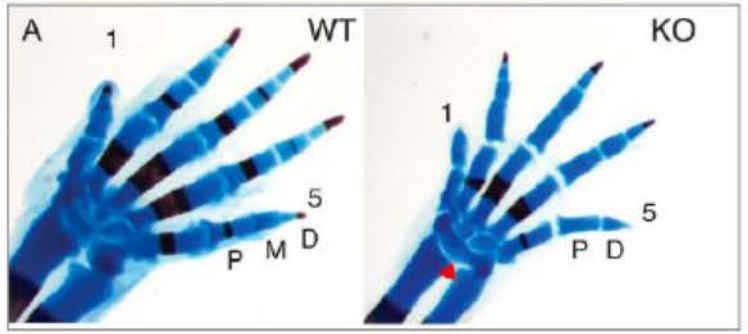
miR-17-92 polycistron

Example of miRNA mutation responsible for a developmental defect in humans
(de Pontual Nat Genet. 2011)



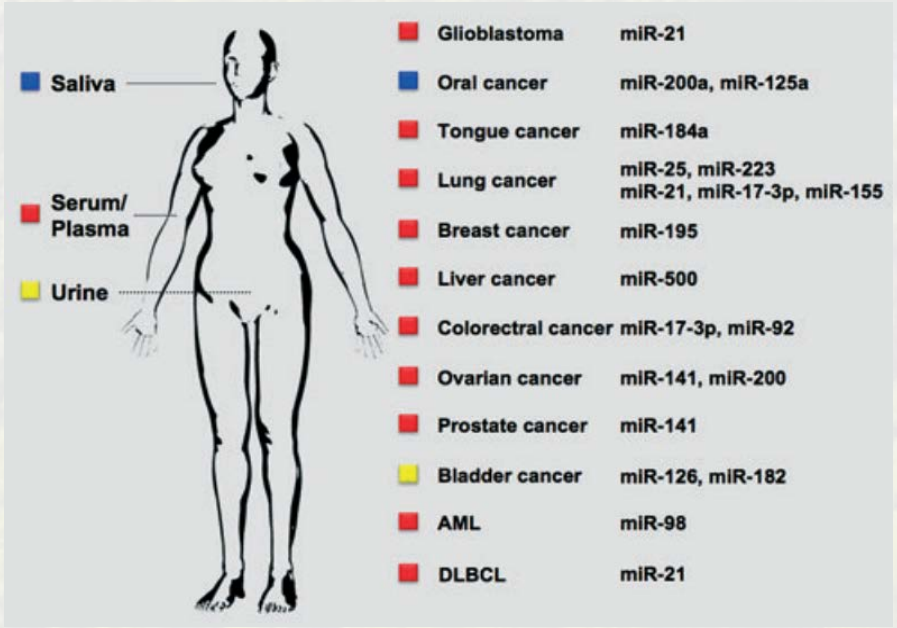
Hemizygous deletion –
Short stature and digital abnormalities





MicroRNAs applications

microRNA-based diagnosis



Cancer Science, Volume 101, Issue 10, 2010

microRNA-based therapeutic

santaris pharma a/s

RNA Medicines for the 21st Century

Miravirsen: miR-122 inhibitor
(Clinical trail: Phase II)

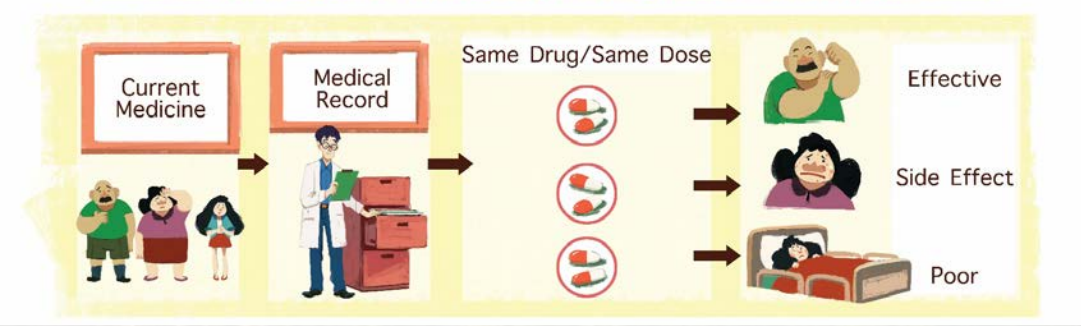
Precision Medicine: https://www.youtube.com/watch?v=HQKFgfMO5Sw

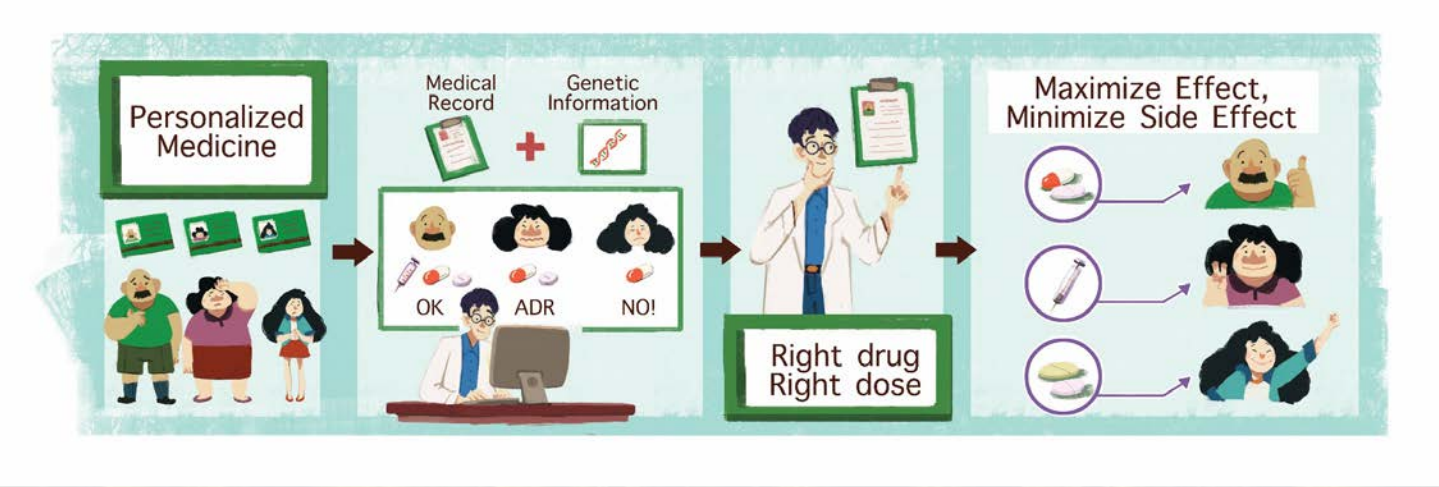
Biomakers: https://www.youtube.com/watch?v=7Ud1vsLcXag

Personalized and Precision Medicine

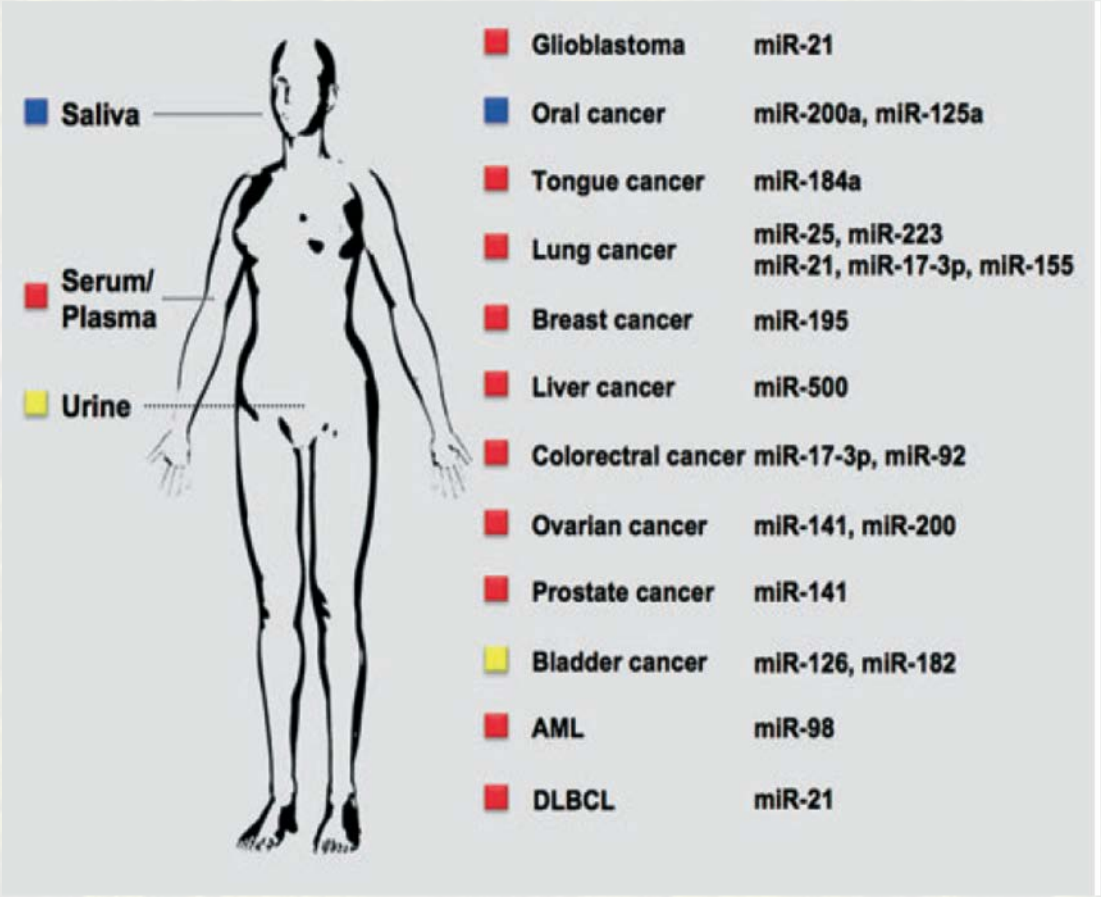
the simplest but important example.

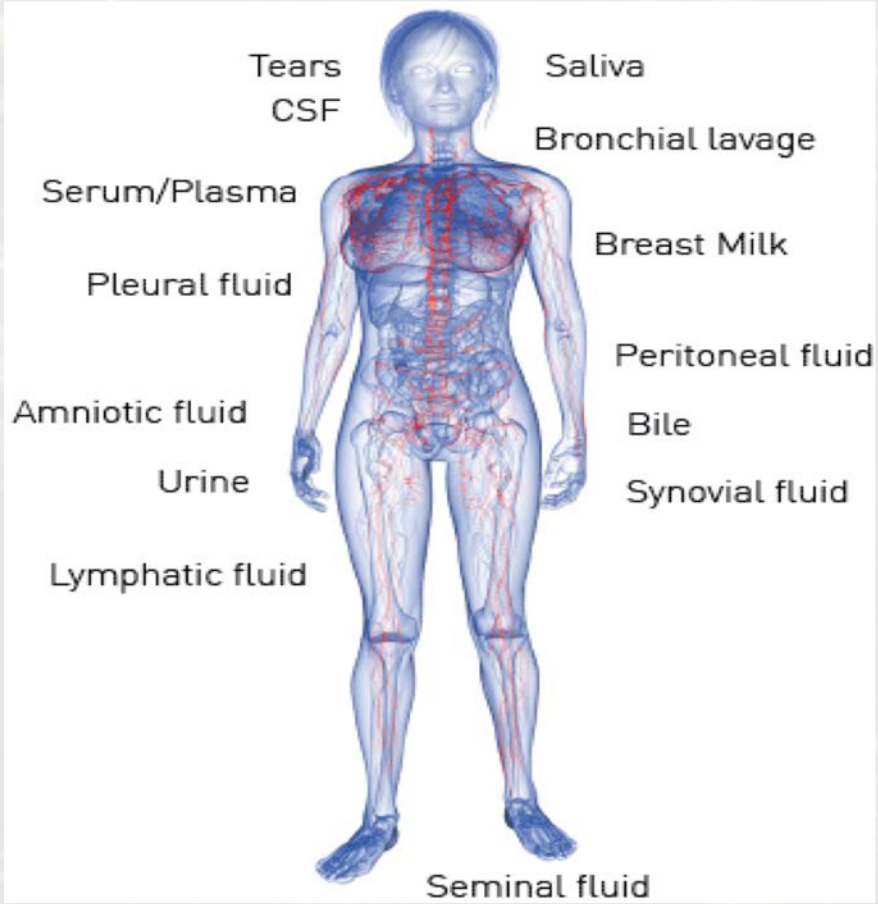
- *Preventing Adverse Drug Reaction by biomarkers*





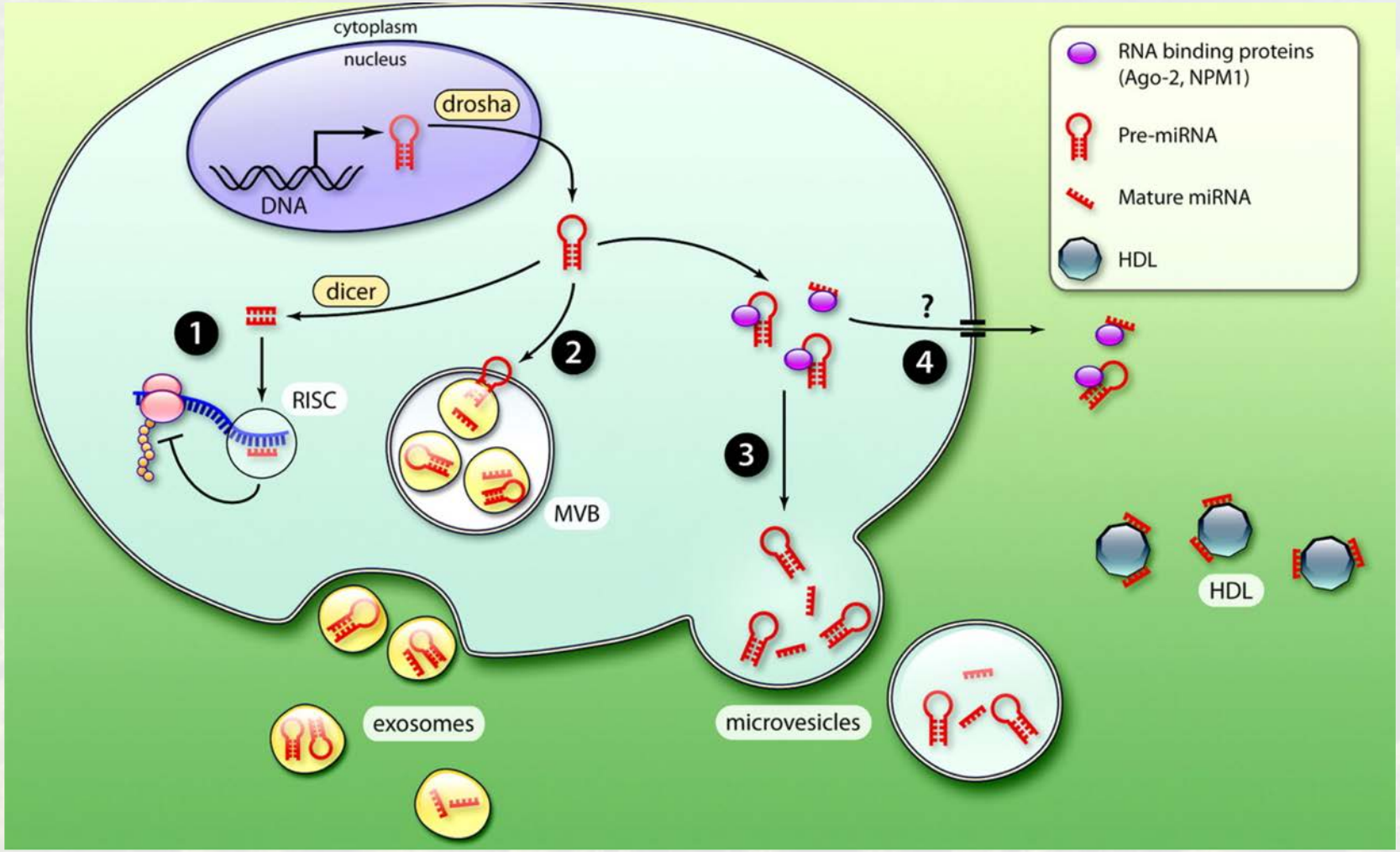
➢Circulating microRNAs in the body fluid: a new potential biomarker for cancer diagnosis and prognosis





Cancer Science 2010, vol.101, p2087-2092

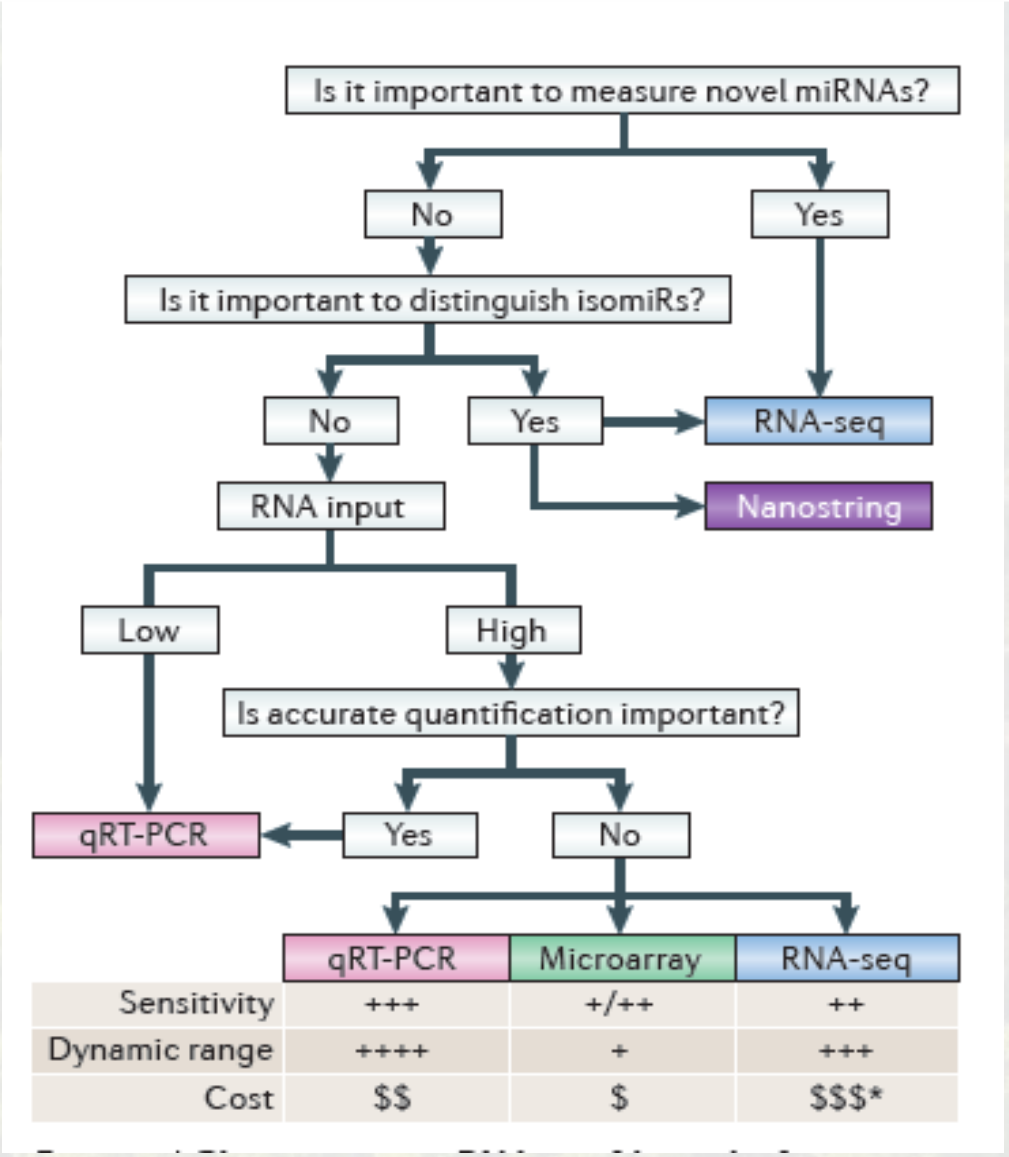
Cellular Release and Stability of Extracellular miRNAs



Circulation Research. 2012;110:483-495

NON-CODING RNA

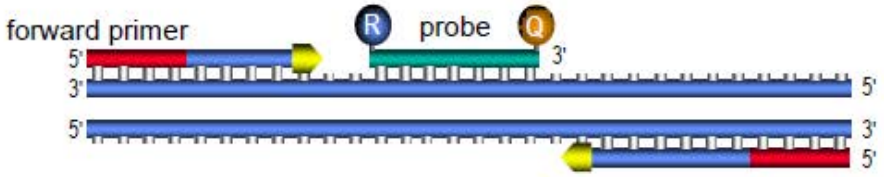
MicroRNA profiling: approaches and considerations



	qRT-PCR	Microarray	RNA-seq
Sensitivity	+++	+/++	++
Dynamic range	++++	+	+++
Cost	$$	$	$$$*

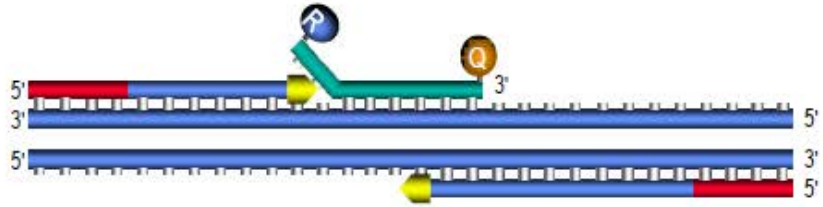
Nature Reviews Genetics 2012, 13,358-369

➢ TaqMan® Assay: Fluorogenic 5‘-nuclease Assay

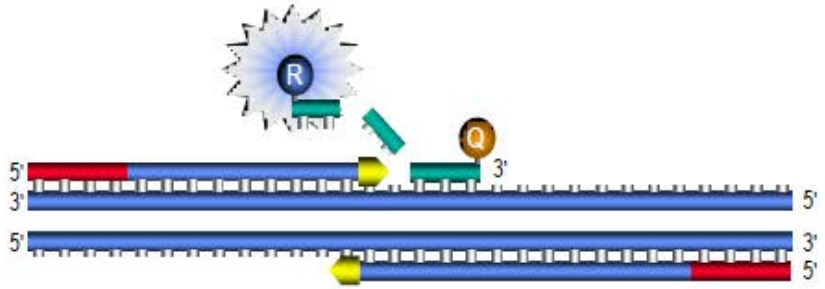


1. Polymerization

reverse primer



2. Strand displacement



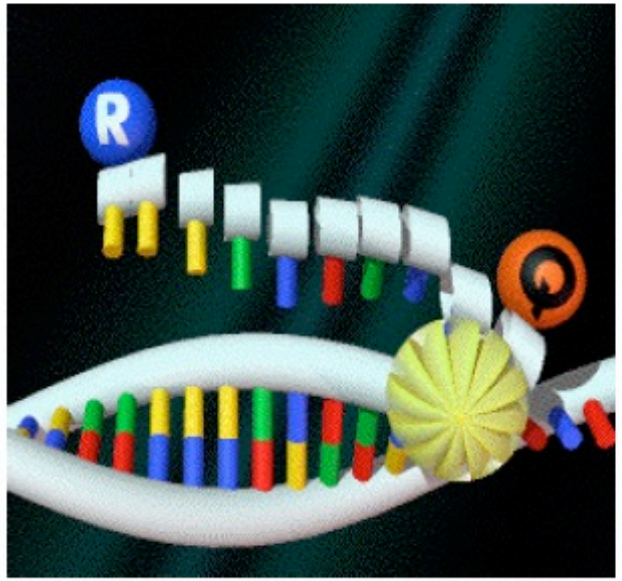
3. Cleavage

4. Polymerization completed

R = Reporter (FAM, VIC, etc.)
Q = Quencher (NFQ/MGB, etc.)

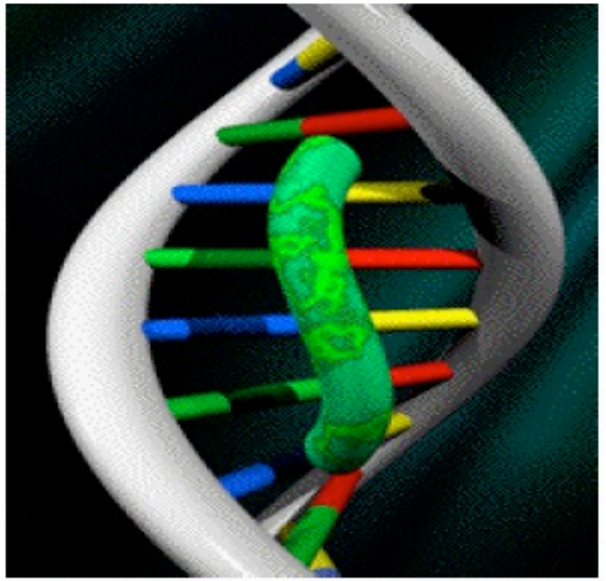
➢ Real-time PCR Chemistries

TaqMan® and TaqMan® MGB



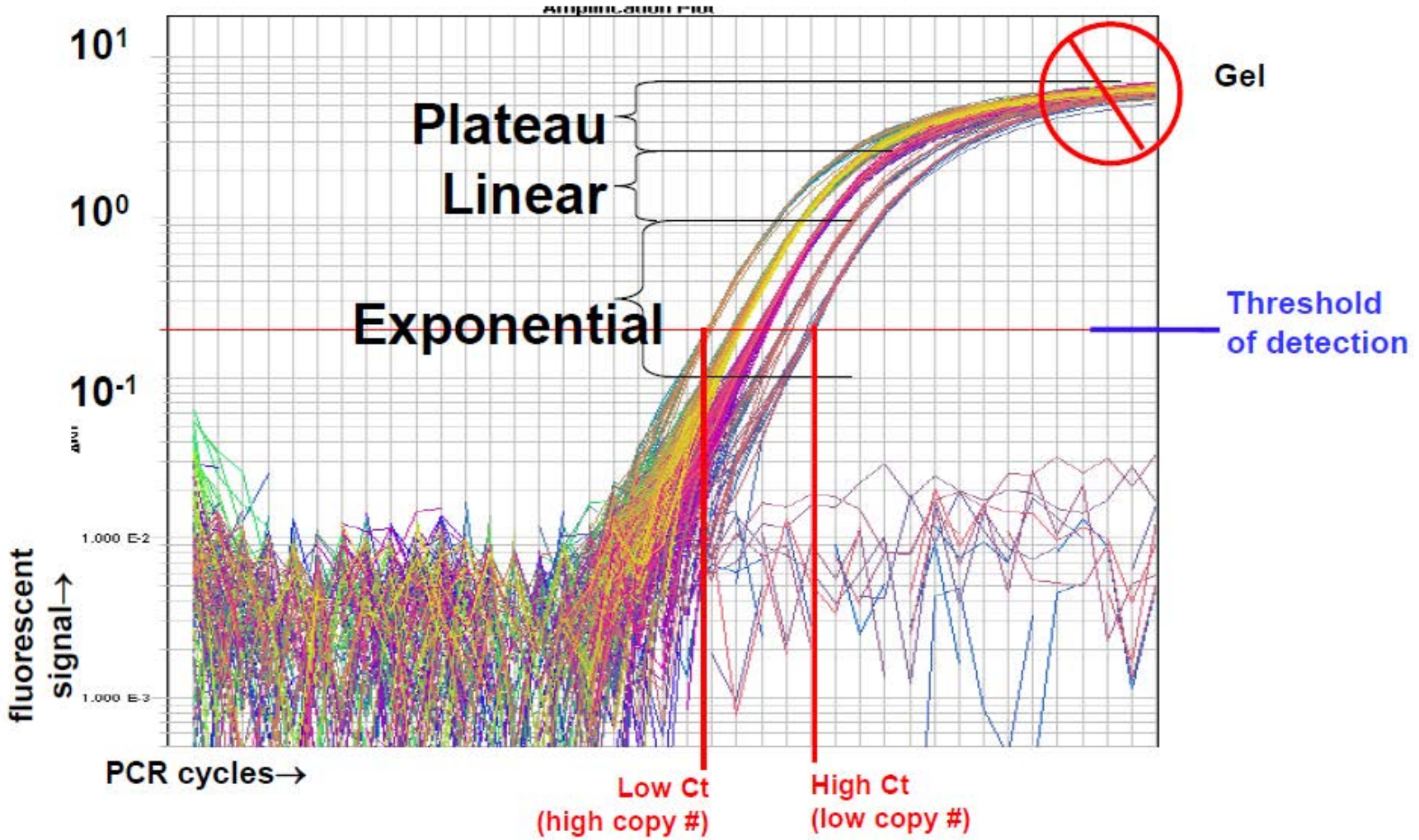
Fluorogenic 5’ Nuclease Assay

SYBR® Green I dye



Binds Double-stranded DNA

➢ Real-time PCR Signal Detection: Exponential Phase

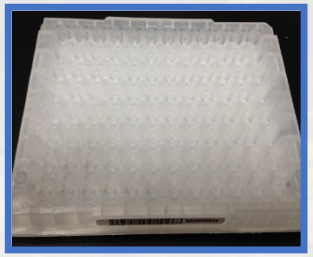


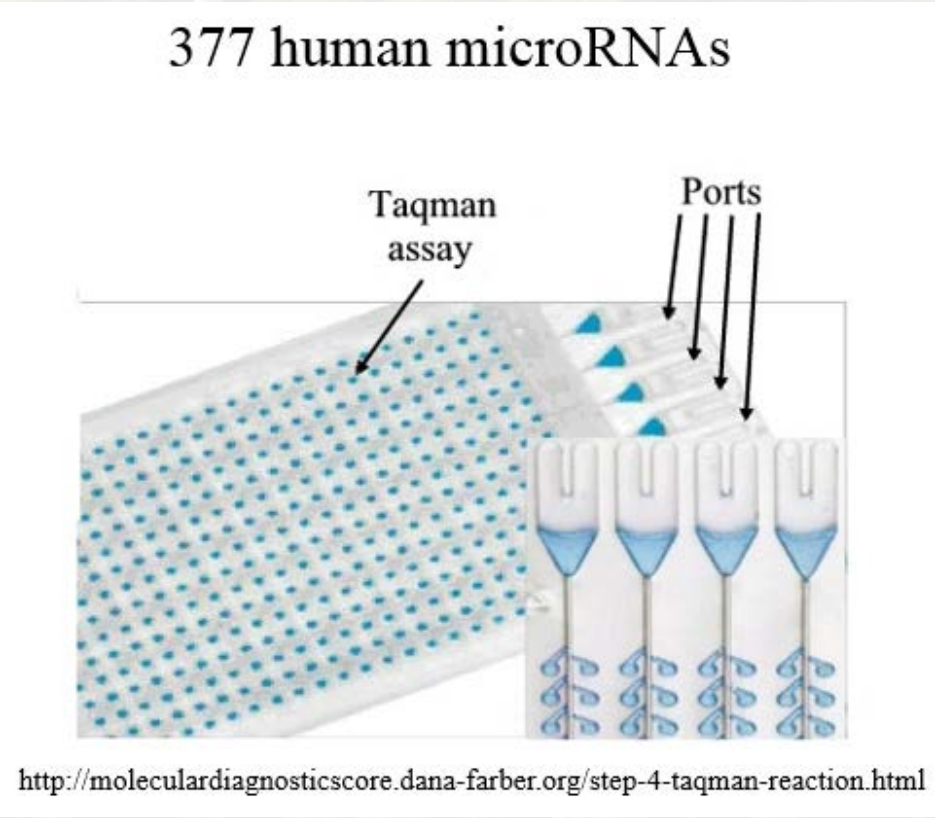
$Y = N_0 2^n$，C_T 與起始濃度之對數值成反比

如何檢測微量的檢體?

高通量PCR聚合酶連鎖反應

ViiA™ 7 Real Time PCR System





The TaqMan® Array Human MicroRNA Card (Containing a total of 754 human microRNAs

Cancer Treatment

50%↑

Surgery

Chemotherapy

Radiation therapy

What is the clinical phenomenon of cancer radiotherapy ?

Response

n= 47

Mr. Lin

Age: 57
Cancer: H&N ca.
Staging: IV
Total Dosage: 7020

Poor response

n= 15

Mr. Dong

Age: 58
Cancer: H&N ca.
Staging: IV
Total Dosage : 7000

Develop the plasma microRNAs panel as the prognostic marker for cancer radiotherapy.

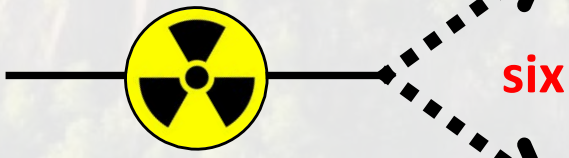
➢Radiotherapy Biomarker

Response assessed by Response Evaluation Criteria in Solid Tumors (RECIST), v 1.1 (CT, MR, PET imaging)

miRNA as biomarker

Cancer patients

Head & Neck cancer
Rectal cancer



six months

Poor Response patient

Response patient

Study : miRNA expression and correlation to Radiation Response

Method

Before R/T

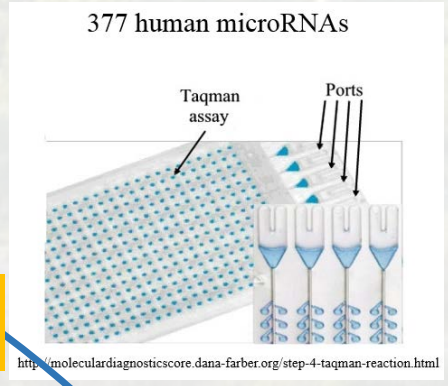
After R/T

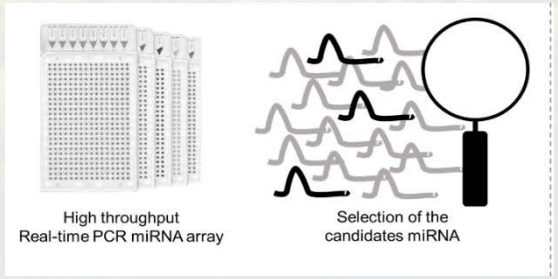
6 mon

Poor RT response

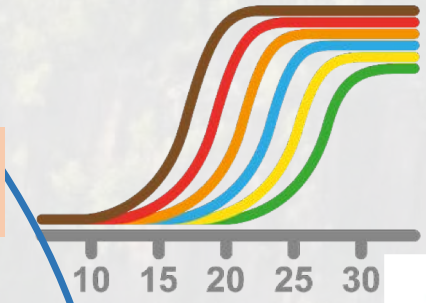
Good RT response

Screening set



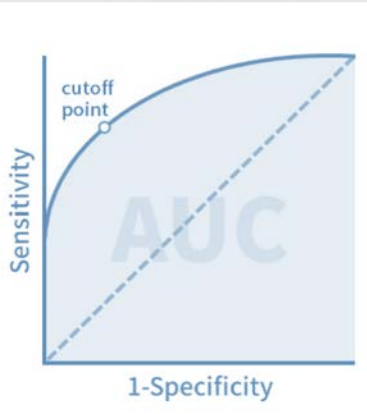


Training set



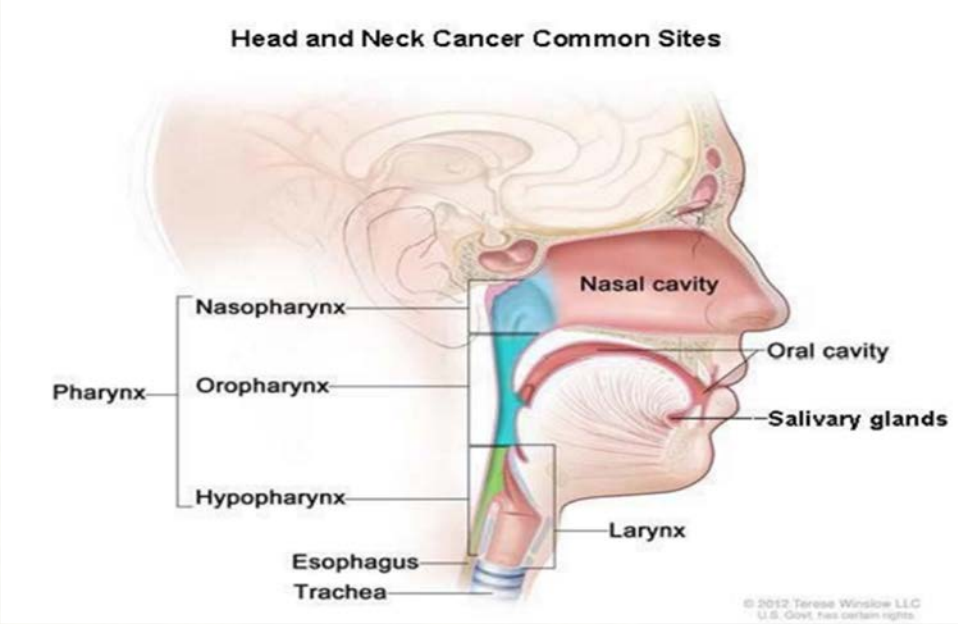
miRNA PCR assay

Testing set

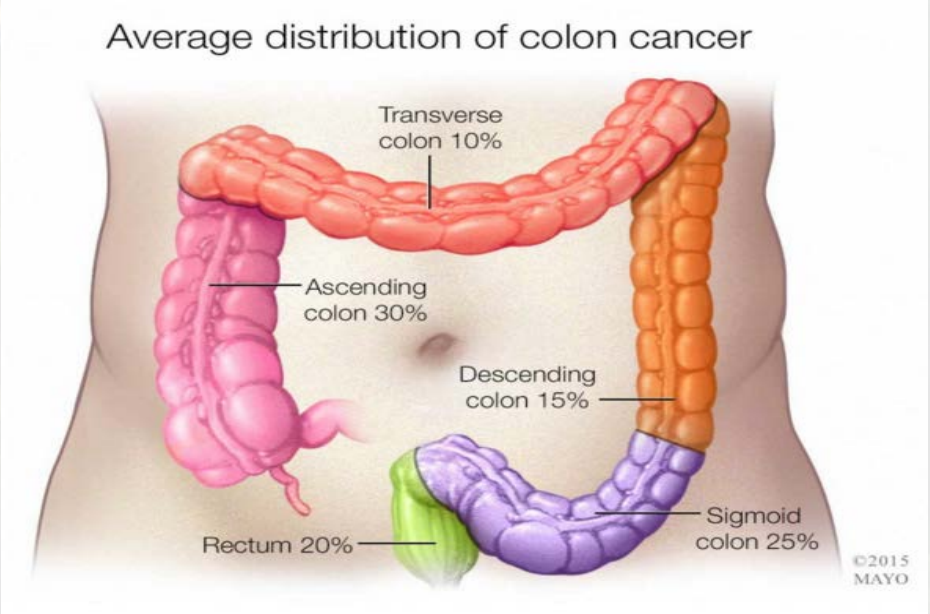


➢ The case of cancers:

➢Head and Neck cancer (HNC)



➢Rectal cancer.

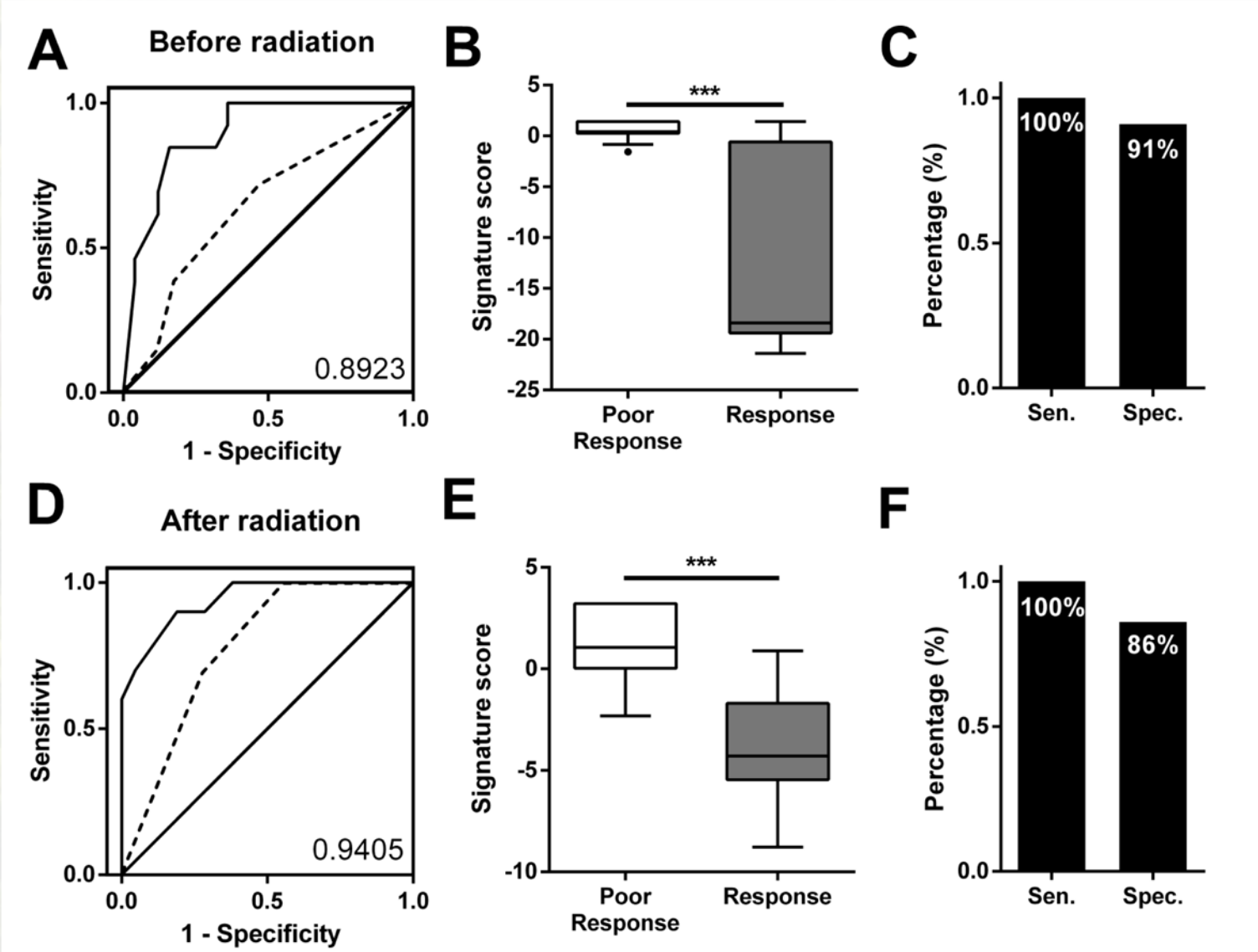


➢Radiotherapy Biomarker

Training n=38 **Testing** n=24

Four miRNAs

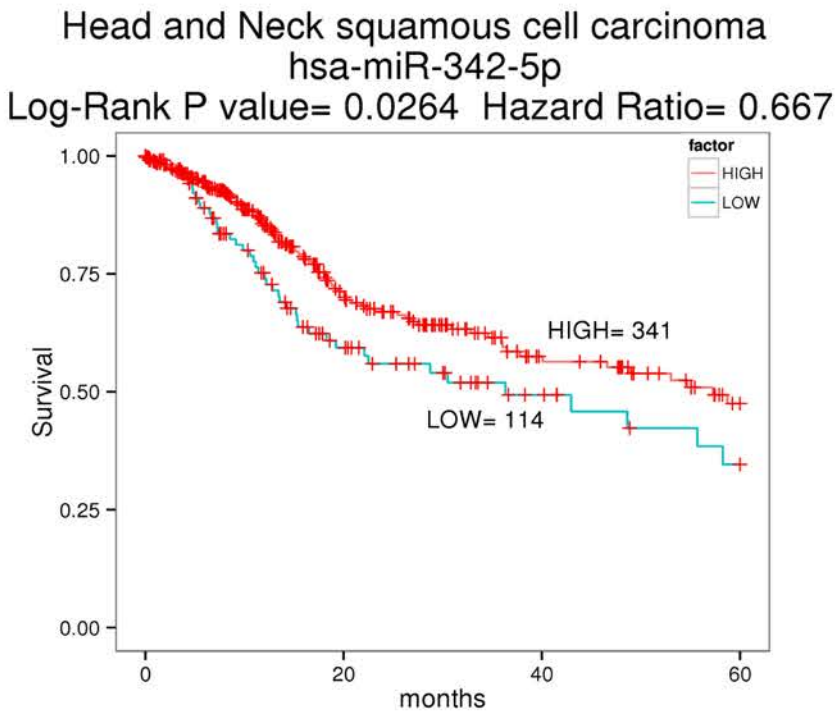
Three miRNAs

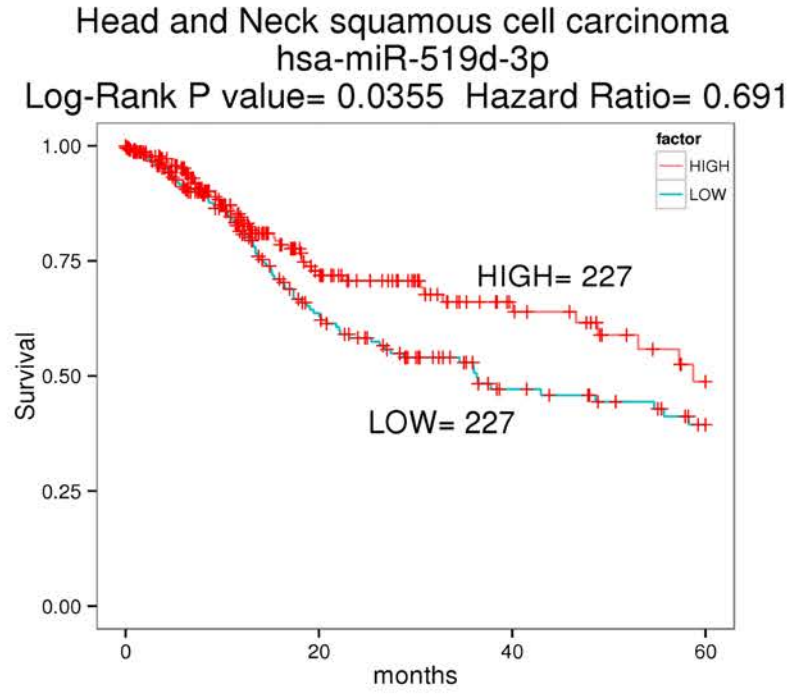


Li AL et al. , Journal of Translational medicine. 2018,16 (1): 341

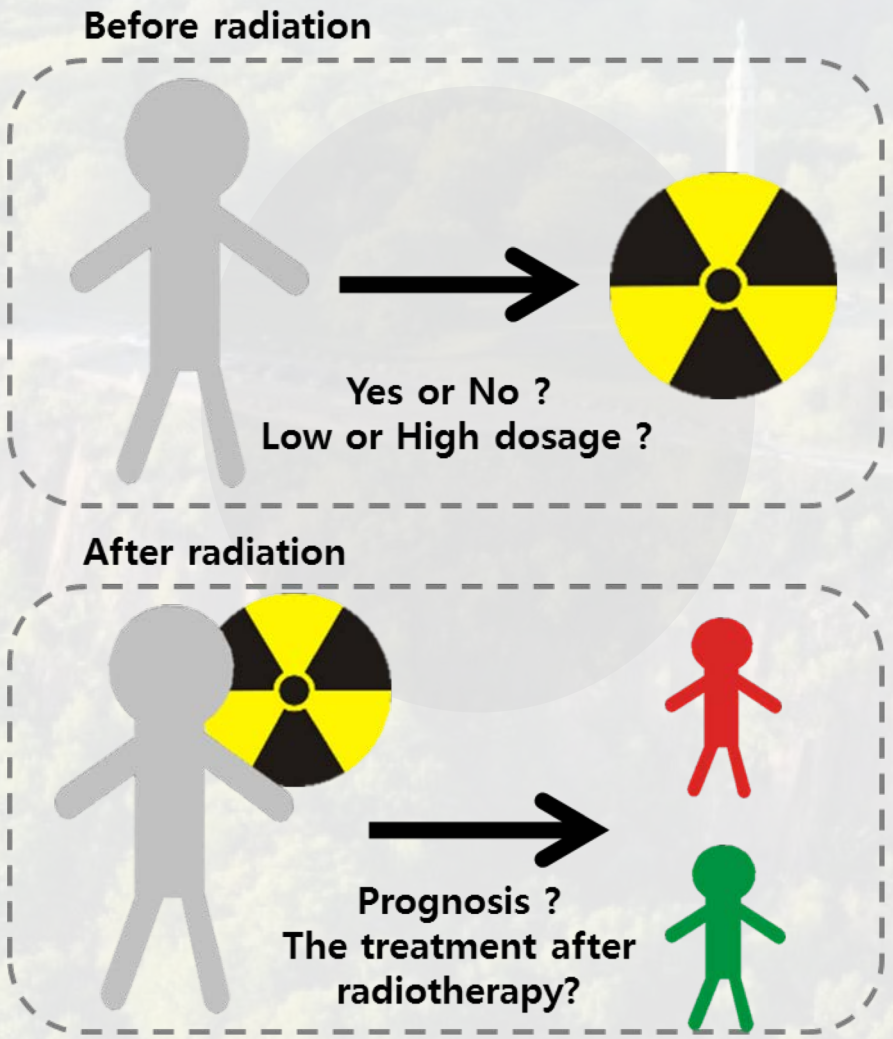
➢Radiotherapy Biomarker

- **5-year Survival curve**



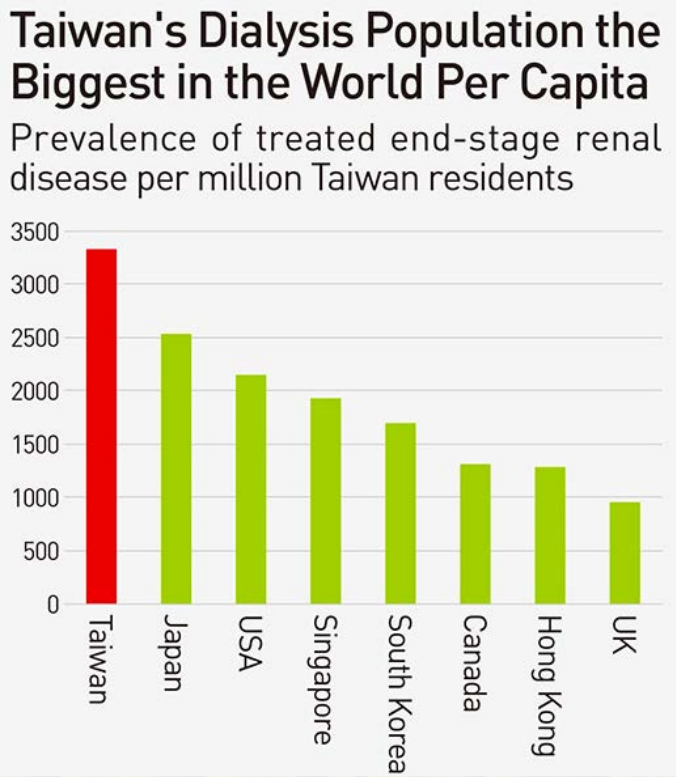


➢Radiotherapy Biomarker



- *Patent :Taiwan Approved (#I614629)*
  US and China applying
- *Technology Transfer*

702: 偵測慢性腎臟病得到泌尿道上皮癌趨勢
703: 泌尿上皮癌的檢測套組及檢測方法



國人10大病排名

資料來源：中央健保署

排名	疾病	健保給付總額	總人數
1	慢性腎衰竭	469.6億元	25.8萬人
2	牙齒相關疾病	395.4億元	1070.9萬人
3	糖尿病	256.4億元	142.3萬人
4	高血壓	227.1億元	248.4萬人
5	急性上呼吸道感染	219.2億元	1318.4萬人
6	成人呼吸衰竭	141.5億元	4.7萬人
7	椎間盤突出或下背痛	138.5億元	292.2萬人
8	肺炎	125.5億元	63.2萬人
9	腦出血	120.9億元	28.3萬人
10	思覺失調症及其他精神疾患	119.7億元	13.7萬人

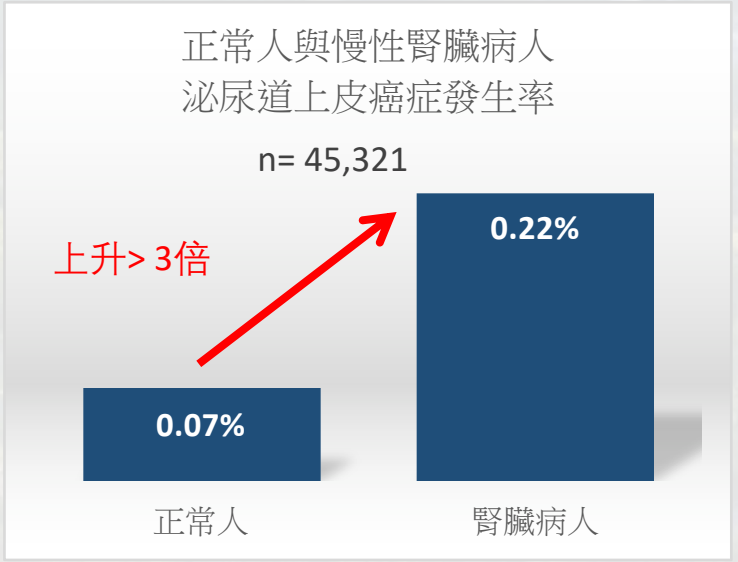
註 上列為去年健保給付金額最多的前10項疾病，與前年相較僅9、10名排名互換

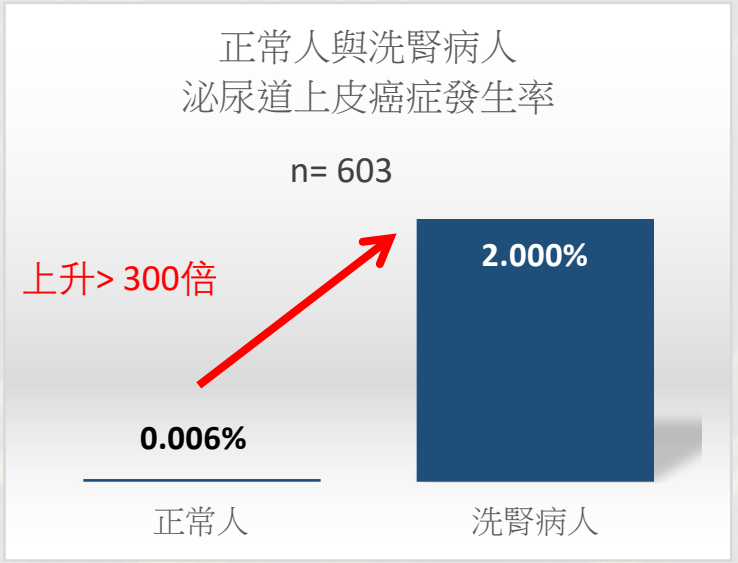
台灣慢性腎臟病發生率不僅為全世界第一名，且每年佔健保資料庫排名均為給付總額第一名，平均每年花費將近500億元，顯示慢性腎臟病及其相關的共同疾病 (癌症)臨床評估都具有極大市場發展

2018 天下雜誌 https://english.cw.com.tw/article/article.action?id=1839

➢ Urothelial Cancer (including cancer in urinary system)

- 泌尿道上皮癌通常經由腰痛、血尿等臨床表徵察覺，確診時經常為癌症晚期
- 泌尿道上皮癌的檢測需要透過尿液細胞學或膀胱鏡影像檢查，這些項目因特異性低或侵入性高，降低了臨床檢查的常規性

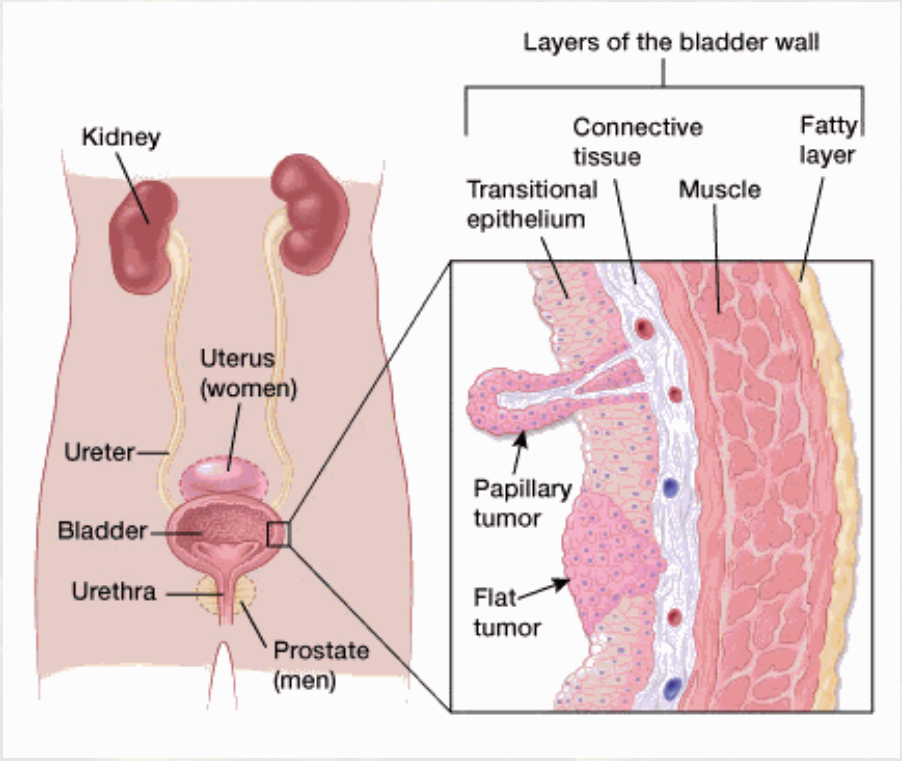




Jeng-Sheng Chen, et. al.; Medicine, 2016
蒲永孝醫師；2010 腎衰竭與泌尿上皮癌的關係

CKD: chronic kidney disease

- Current diagnosis depends on gross painless hematuria / cystoscopy



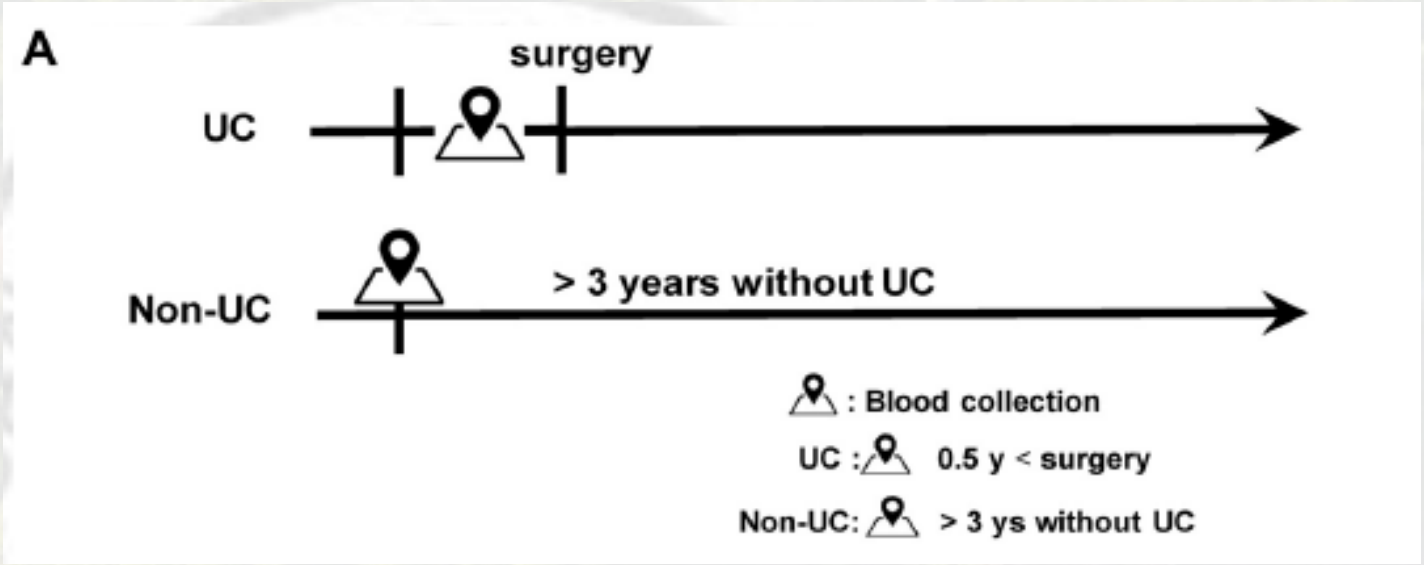
(www.cancer.org/cancer/bladdercancer)

UCC Taiwan UC Consortium

臺灣 Taiwan Biobank

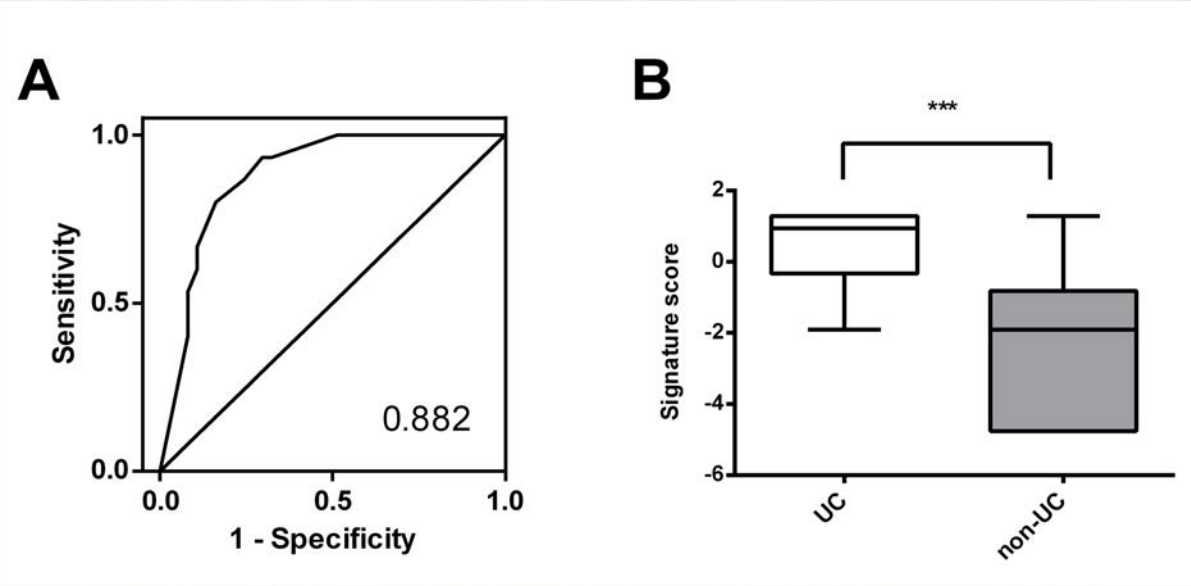
➢ UC Biomarker for Hemodialysis

To predict the possibility of UC within 3 years



➢ UC Biomarker for Hemodialysis

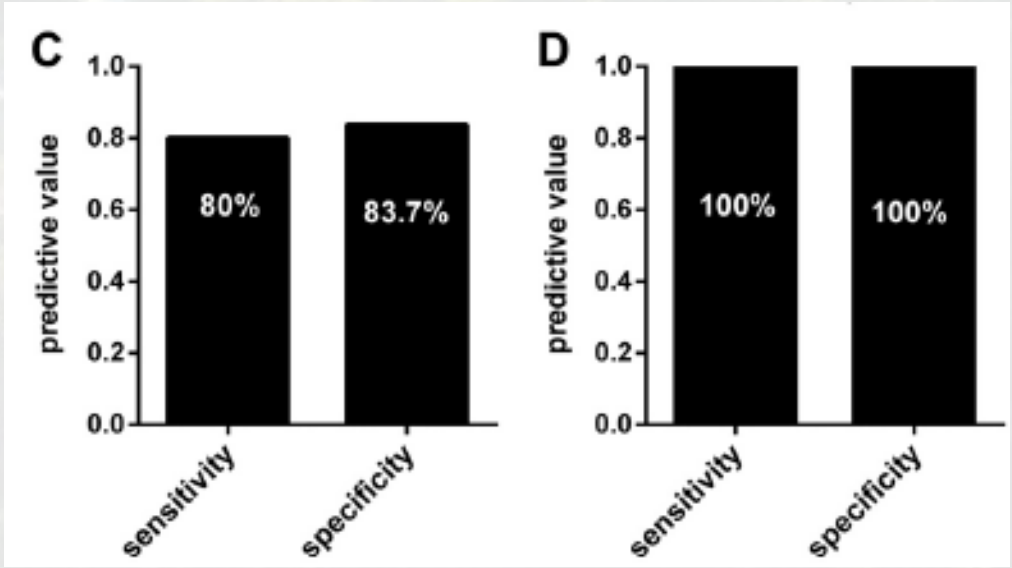
5 miRNAs



N=52

Training

Testing

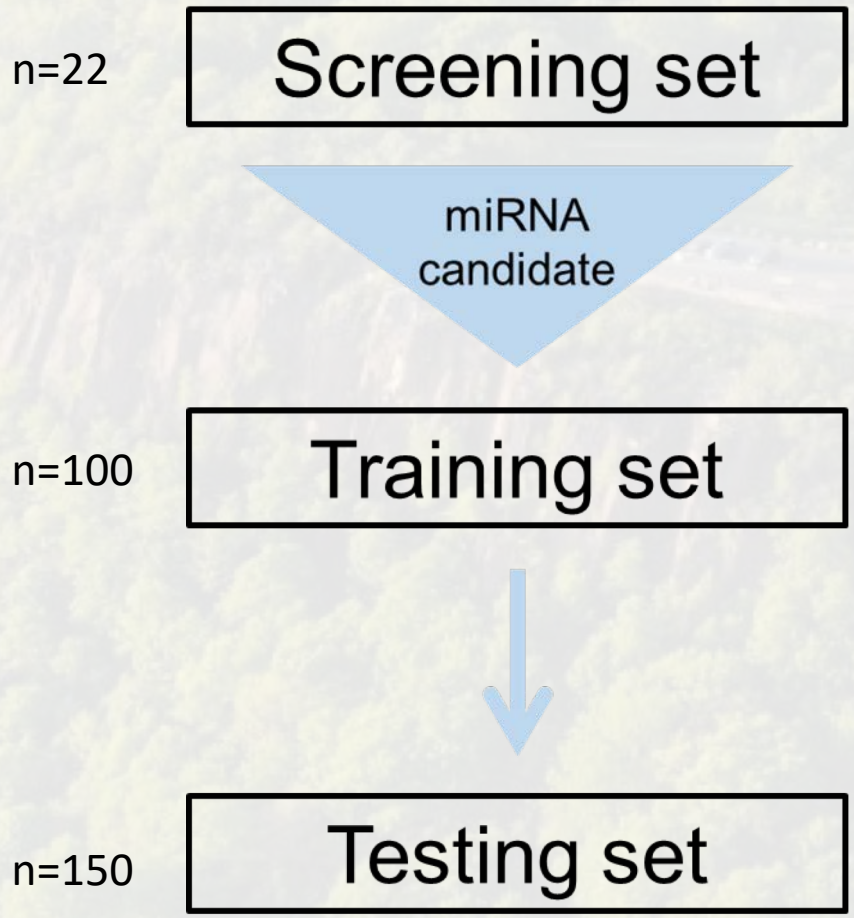


N=52

N=9

Chen CL et al. Am J physiol Renal physiol, 2019
Patent :Taiwan Approved (#107140464)
US and China applying

➢ Urotheiial Carcinoma (UC) Biomarker for CKD

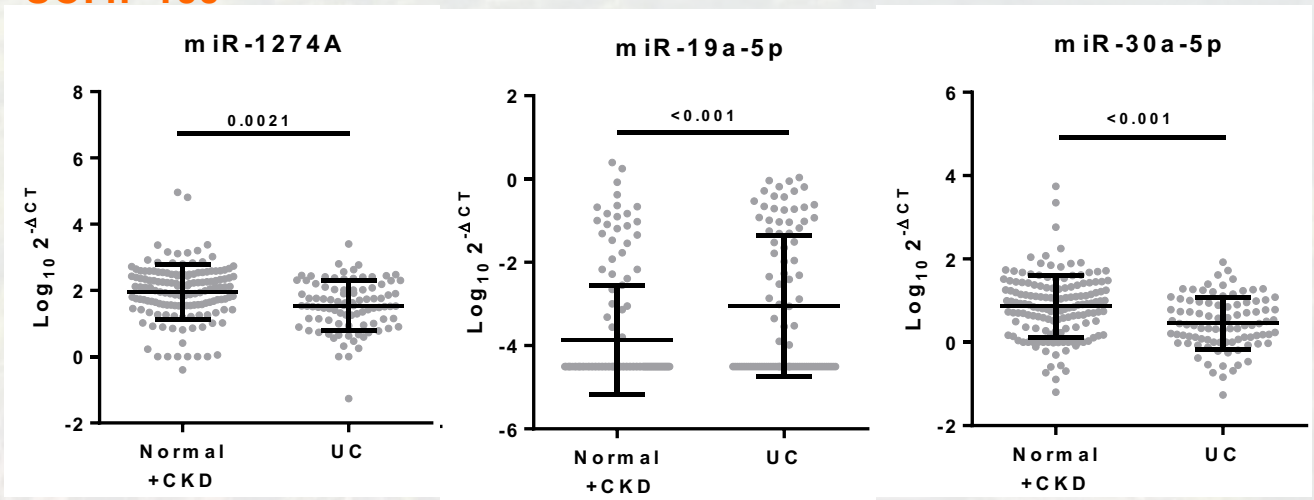


n	Urine samples			Plasma samples	
	Normal	CKD	UC	CKD	UC
Screening set		11	11	8	8
Training set		50	50	47	41
Testing set	50	50	50	34	48

➢ miRNA Expression in Urine

Normal+CKD: n=50+100

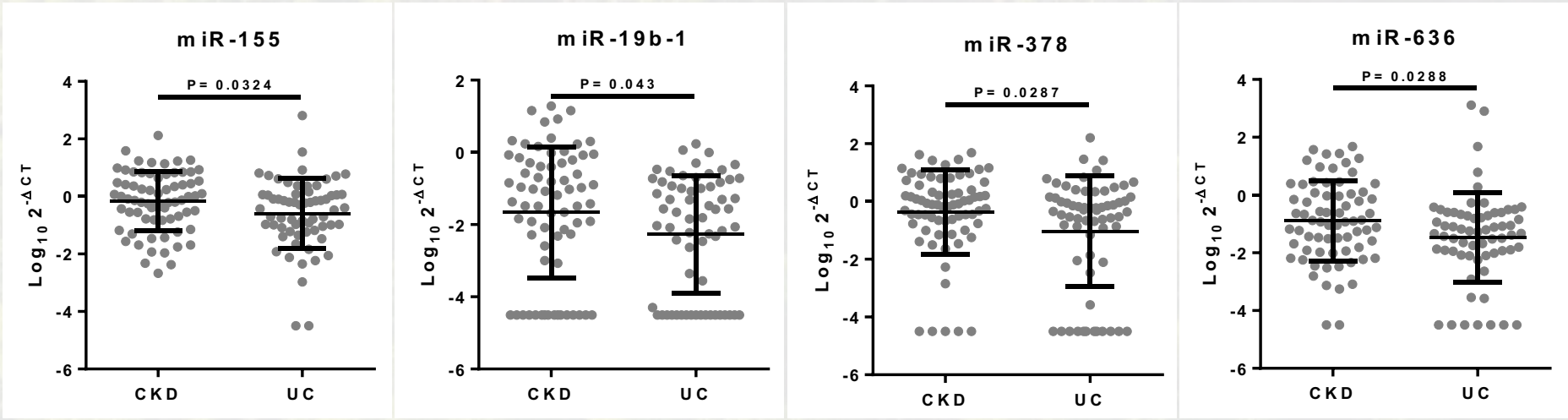
UC: n=100



➢ miRNA Expression in Plasma

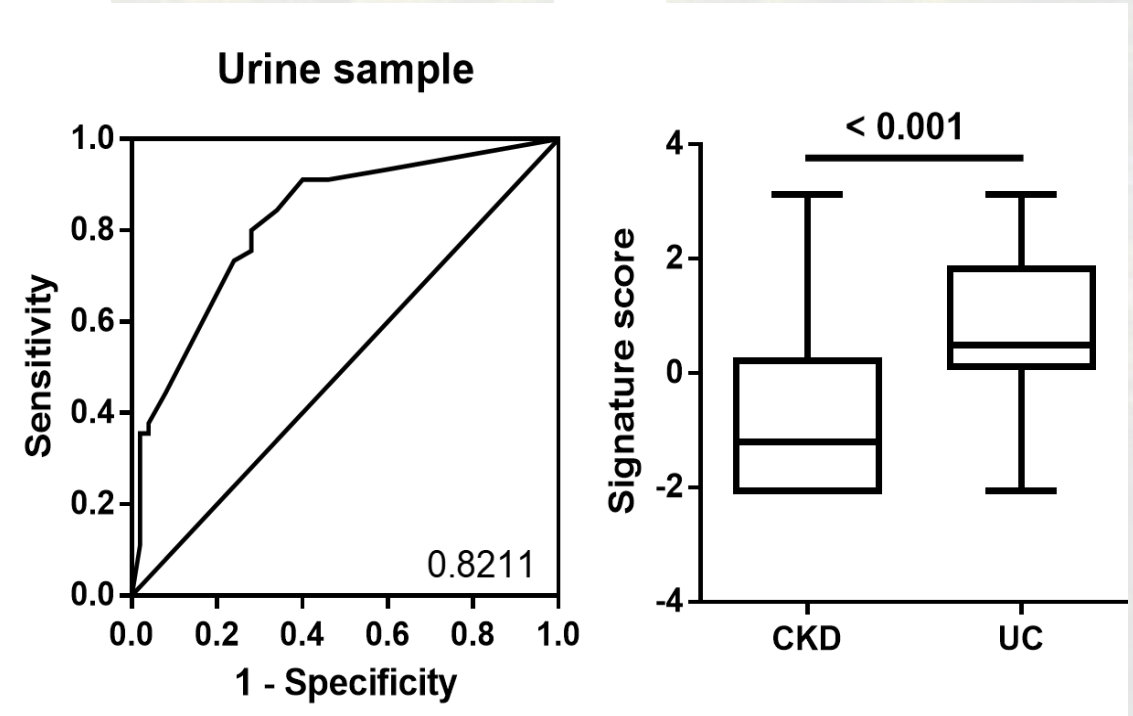
CKD: n=70

UC=64

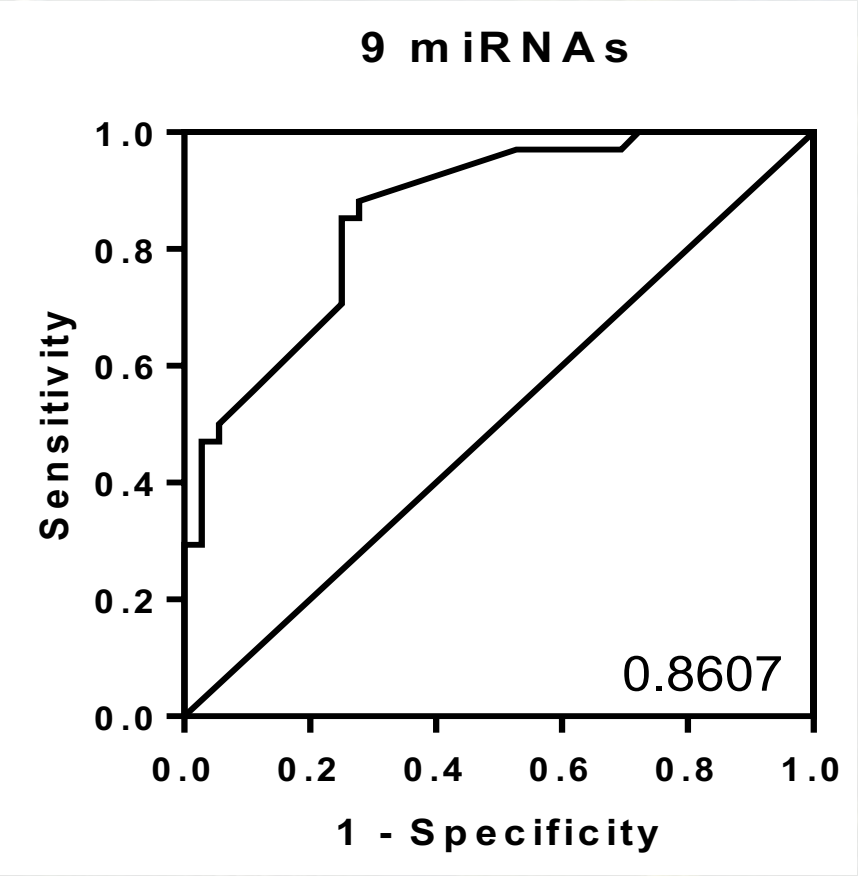


➢ Urotheiial Carcinoma (UC) Biomarker for CKD

Urine

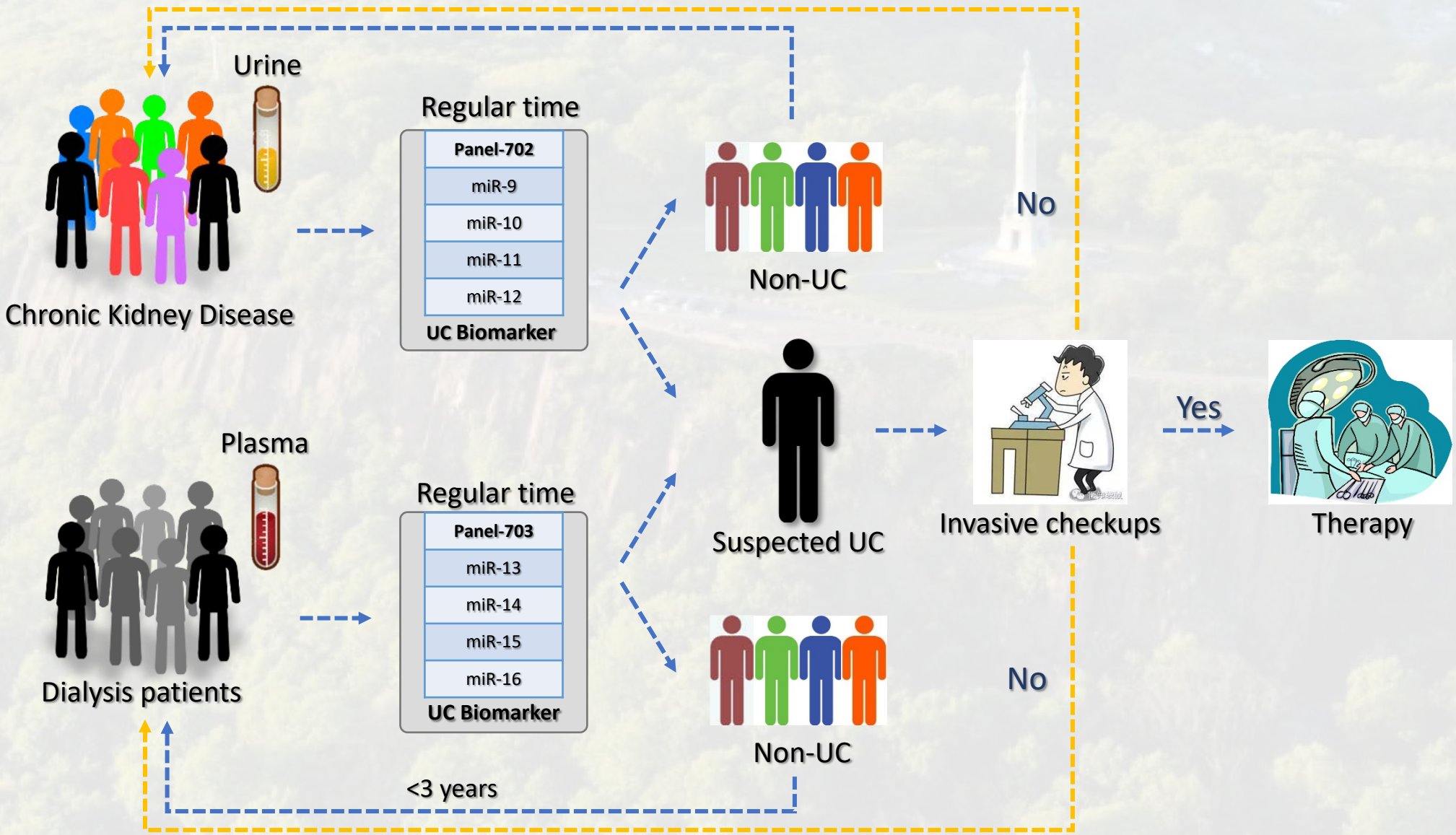


Urine +Plasma



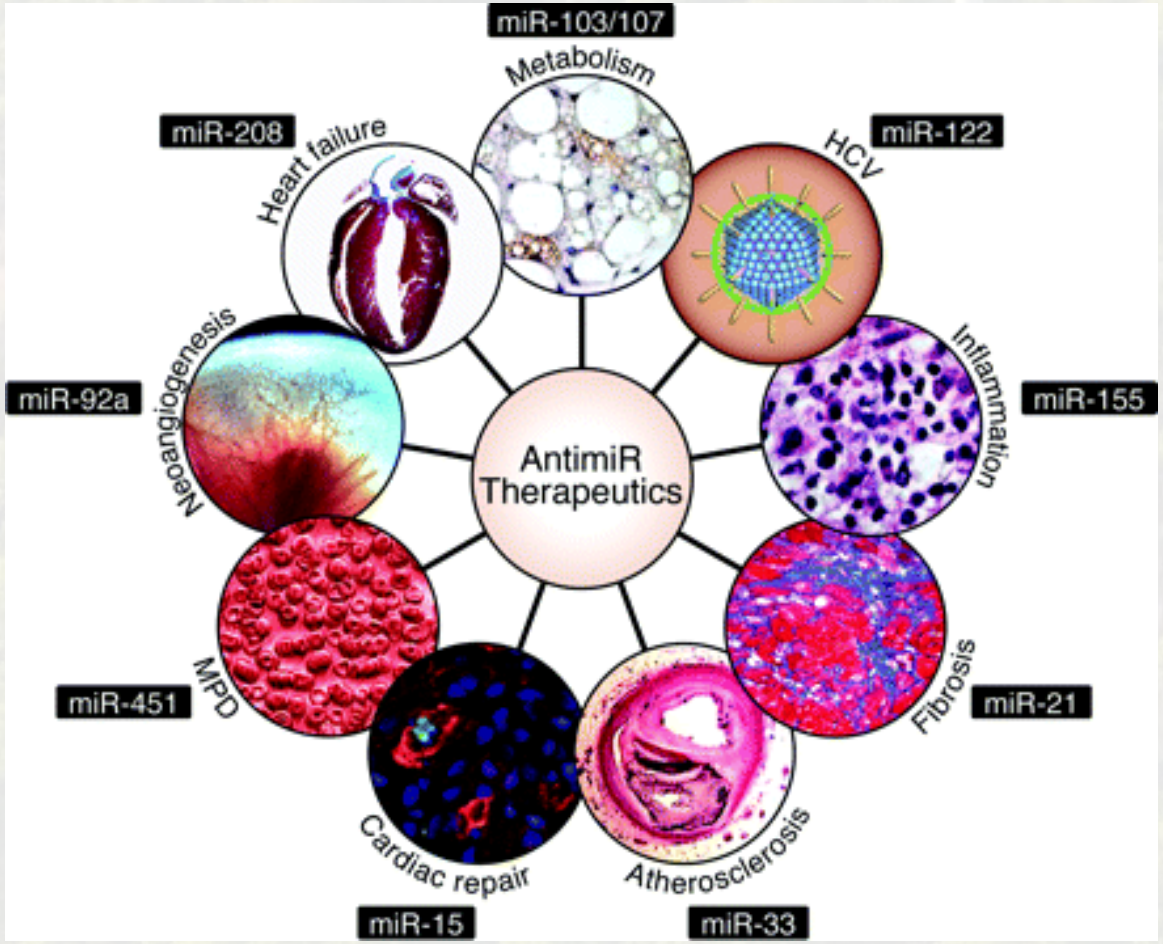
Patents are applying….

UC biomarker



microRNA vs. disease

- **Specific miRNAs that are currently being pursued as clinical candidates.**



Circulation Research. (2012) vol. 110 no. 3496-507

RAS-MAPK pathway epigenetic activation in cancer: miRNAs in action.

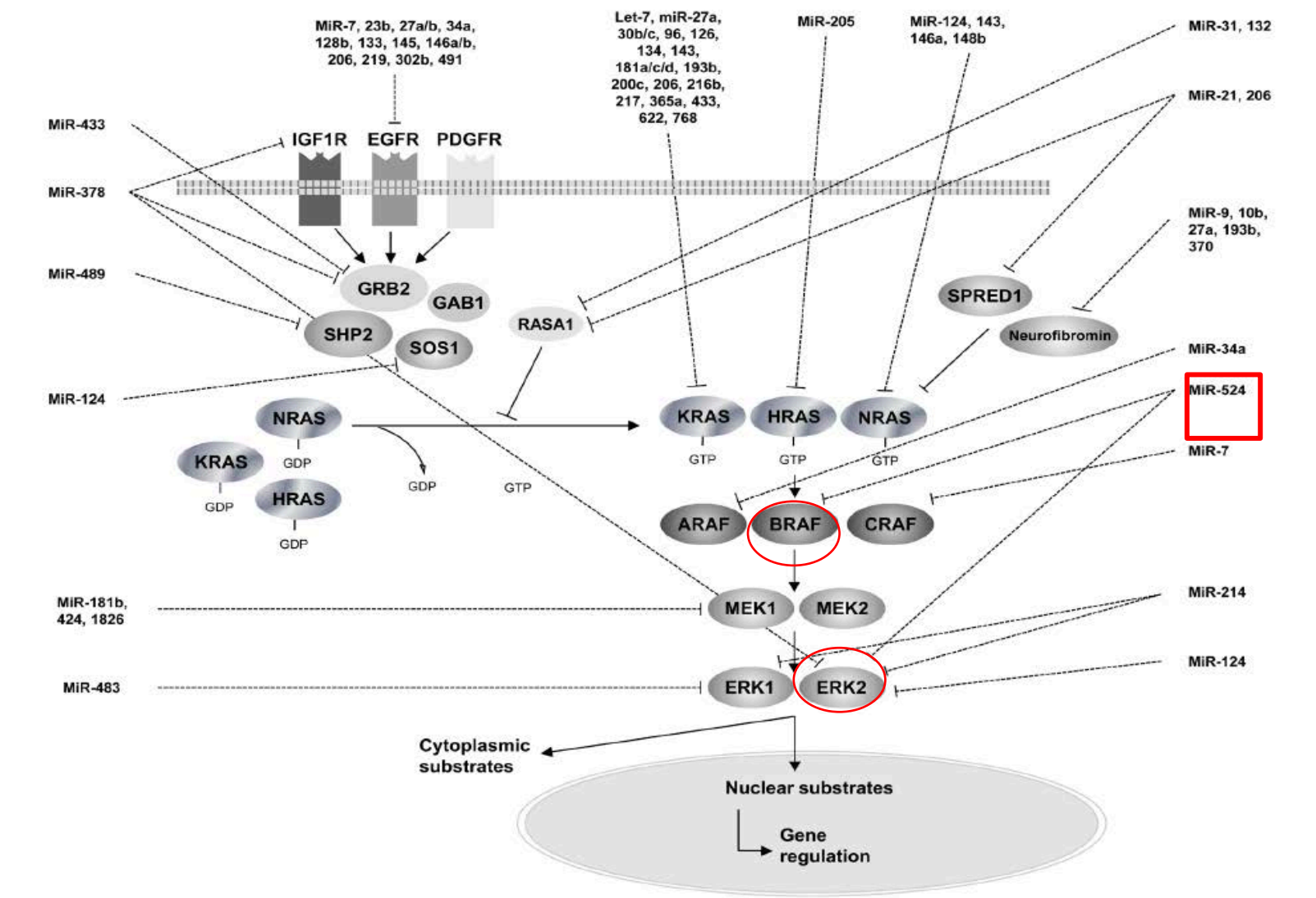
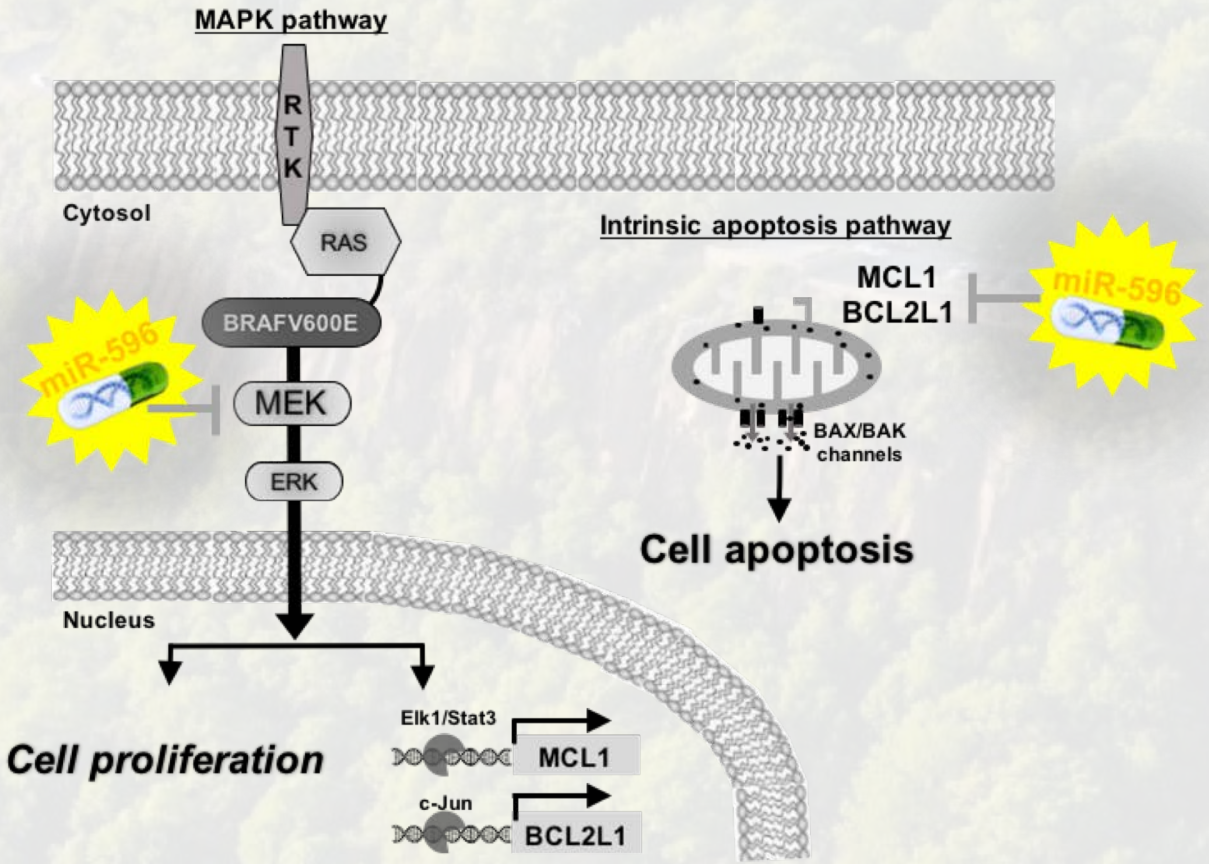


Figure 2: An overview of RAS-MAPK pathway regulation by microRNAs.

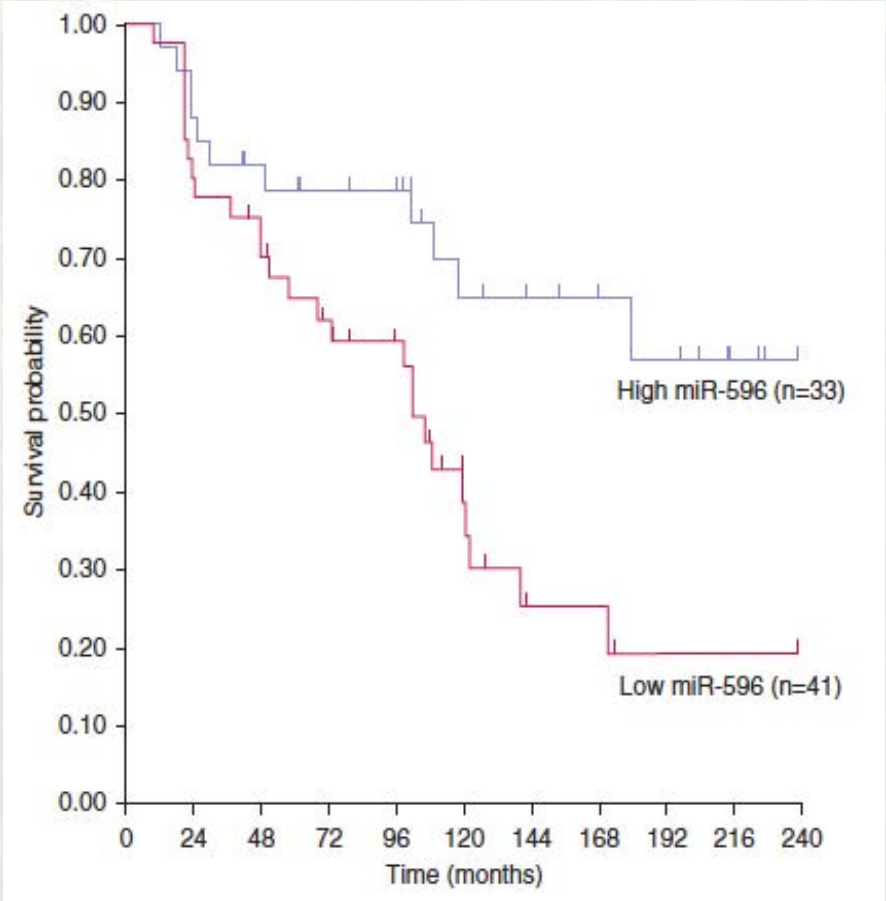
Oncotarget 2016 Jun 21;7(25):38892-38907. doi: 10.18632/oncotarget.6476.

The model of miR-596 function



Kaplan-Meier curve

20-years Survival

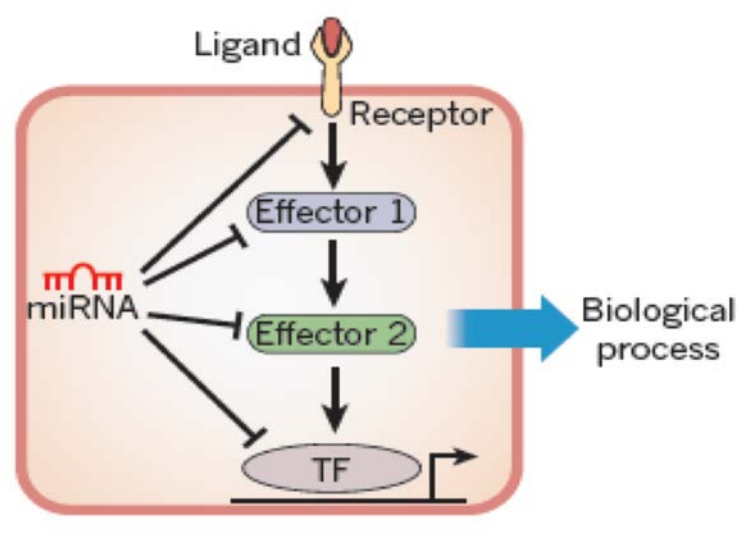


P=0.008

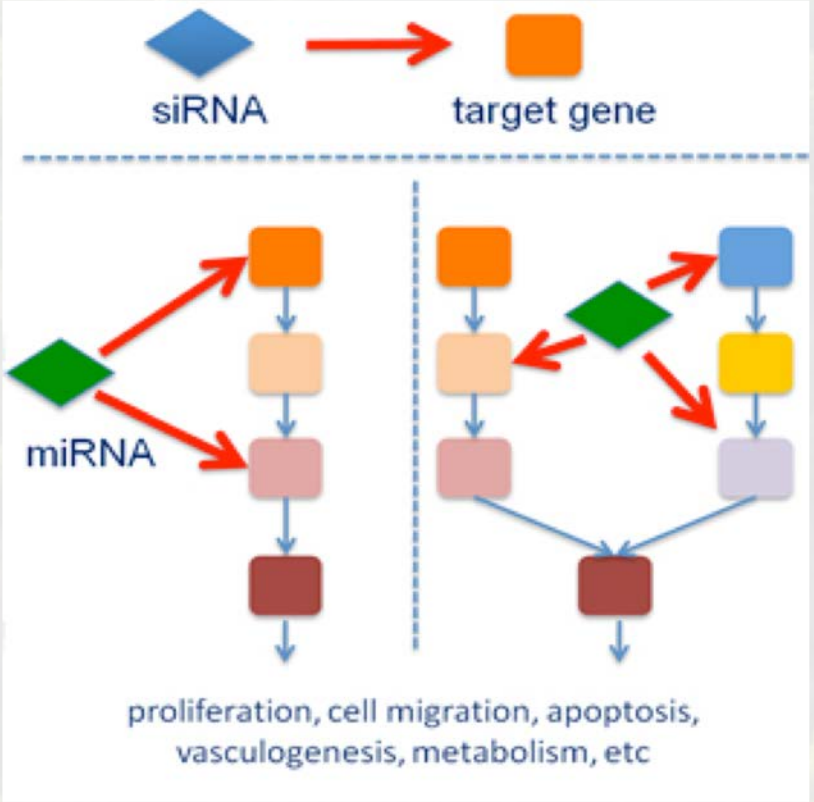
GSE59334

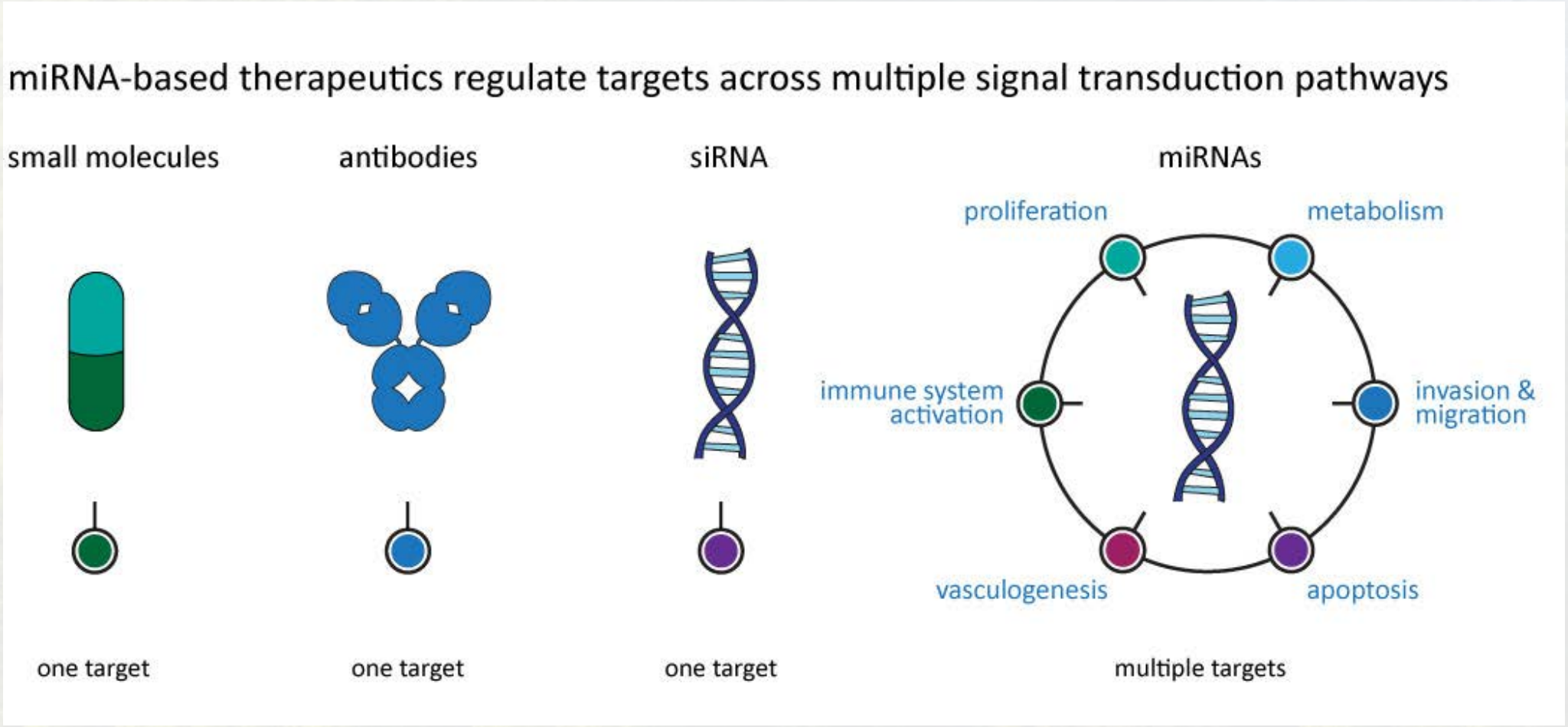
Liu SM, et al. ,Journal of Investigative Dermatology, 2018, 138(4), 911-921

MicroRNAs regulate signaling transduction pathways



Modified from Nature, 2011, vol.469, p336-342

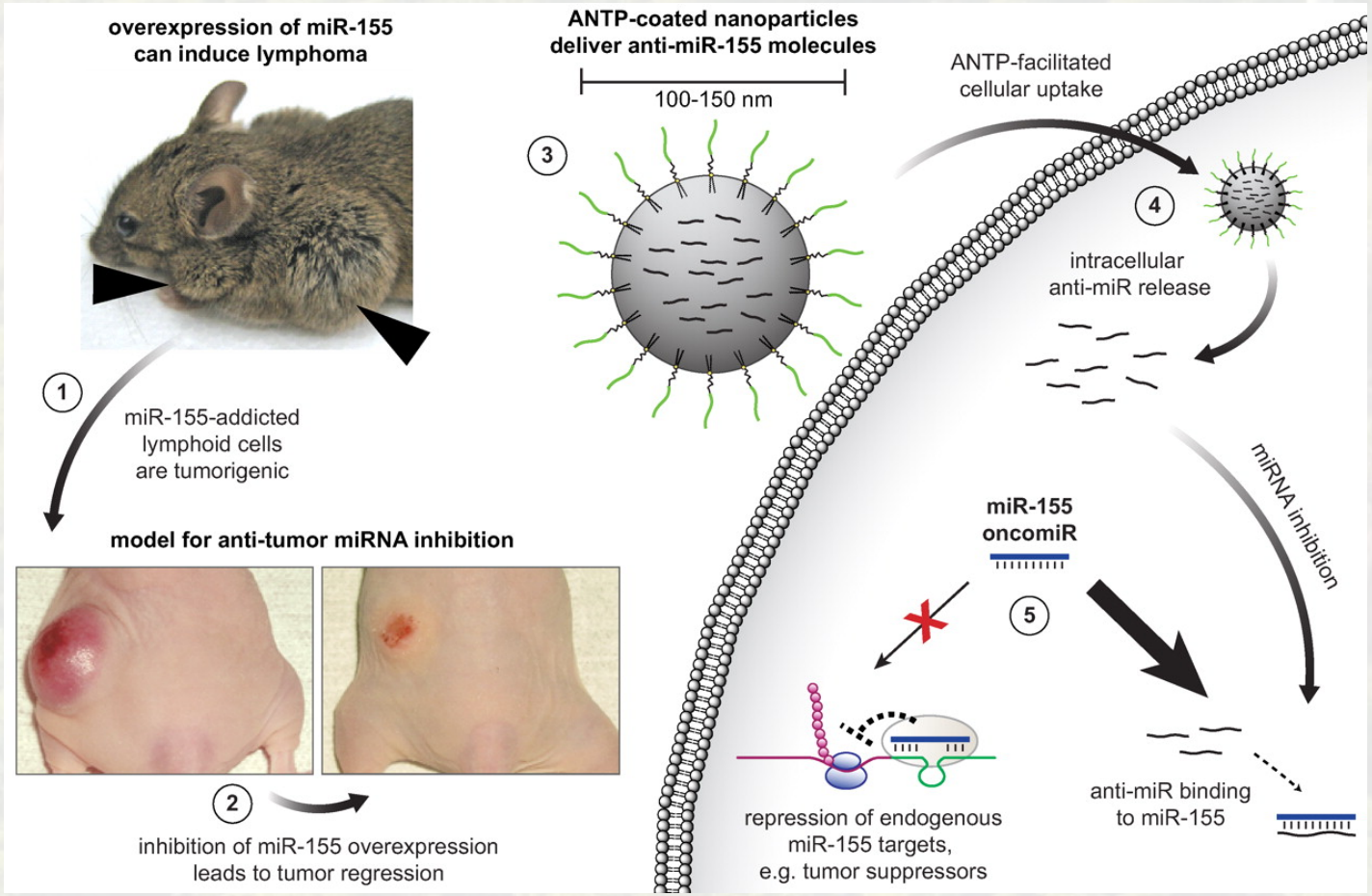




https://interna-technologies.com/mirnas-and-cancer/

Nanoparticle-based therapy in an in vivo microRNA-155 (miR-155)-dependent mouse model of lymphoma

Imran A. Babar[a,1], Christopher J. Cheng[b,c,1], Carmen J. Booth[d], Xianping Liang[a], Joanne B. Weidhaas[e], W. Mark Saltzman[c], and Frank J. Slack[a,2]



Synthetic materials for miRNA and anti-miRNA oligonucleotide delivery

- **Poly(lactide-co-glycolide) (PLGA) particles**

PNAS (2012)

ANY Questions?

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