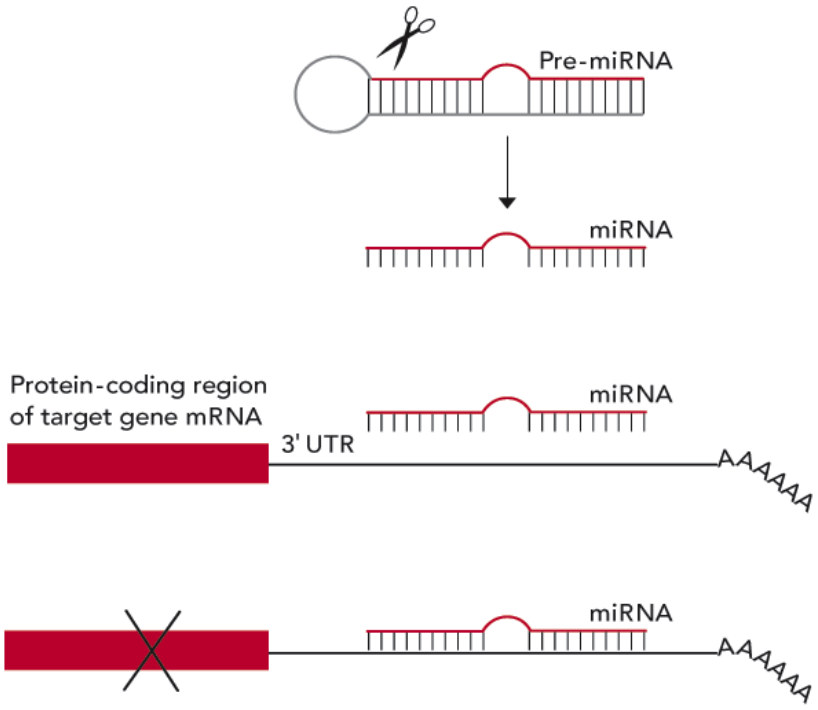
Asian Society of Gynecologic Oncology

3rd ASGO workshop, Seoul, Korea



miRNA strategy in ovarian cancer

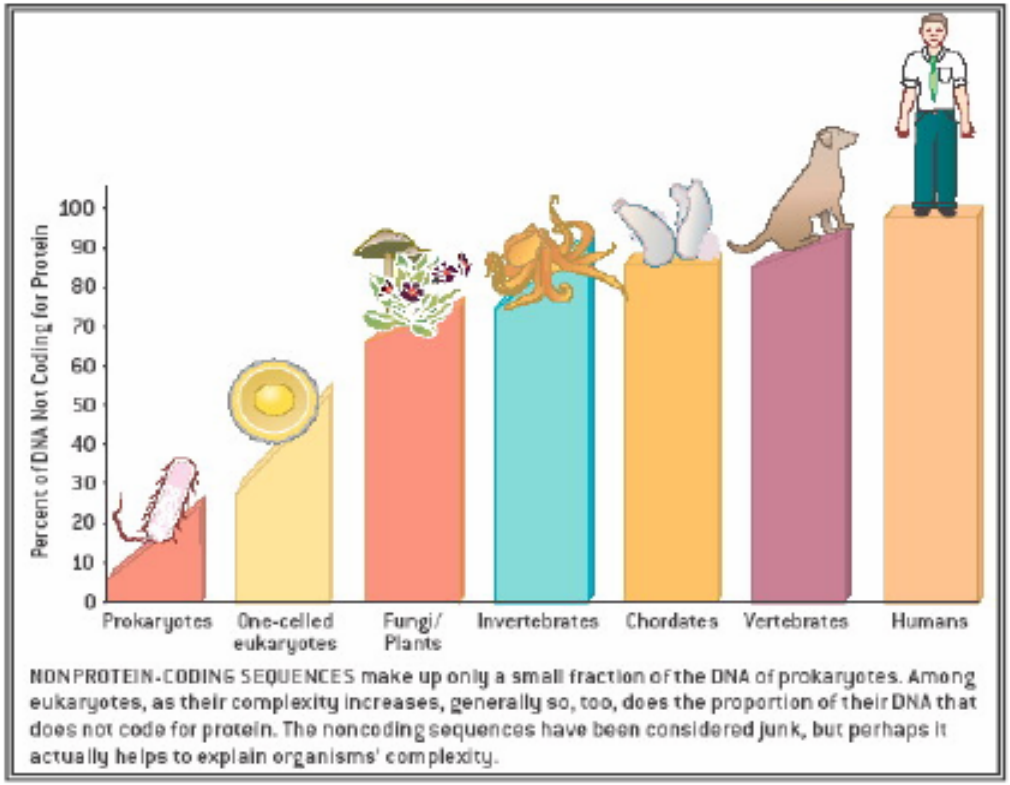
Seoul St. Mary's hospital

Keun Ho Lee, M.D.

Agenda

- **miRNA introduction**
- **miRNA expression in cancer**
  - Examples of miR functions in tumorigenesis
  - miRNAs as prognostic factors for cancer patients
  - miRNAs in body fluids and their potential as prognostic and diagnostic biomarkers
- **miRNAs as targets**

Non-Coding RNA: A Key to Eukaryotic Complexity



NONPROTEIN-CODING SEQUENCES make up only a small fraction of the DNA of prokaryotes. Among eukaryotes, as their complexity increases, generally so, too, does the proportion of their DNA that does not code for protein. The noncoding sequences have been considered junk, but perhaps it actually helps to explain organisms' complexity.

Organism	Percent of Transcriptional Output	
	Protein Coding RNA	Non Coding RNA
E.*coli*	84	16
S. *cerevisiae*	71	29
C.*elegans*	27	73
D. *melanogoster*	13	87
H. *sapiens*	2	98

Data suggesting role in diverse mechanisms:

- RNAi
- Gene co-suppression
- Imprinting/DNA Methylation

Possible roles in:

- Cancer
- Neurological Disorders
- Host-pathogen interactions

What is microRNA (miRNA)?

- **Small non-coding RNAs of 20–22 nucleotides**

- First discovered in **Caenorhabditis elegans**
- Present and **highly conserved** among a wide range of species (plants, animals, and some viruses)
- Functions in **RNA silencing and post-transcriptional regulation of gene expression**

- Since 2000, miRNA research has revealed multiple roles for miRNAs in plant and animal development and in many other biological processes.

History

- **lin-4,** first miRNA to be described in C. elegans; important in **development of the worm** from larva to adult.

Victor Ambros 1993

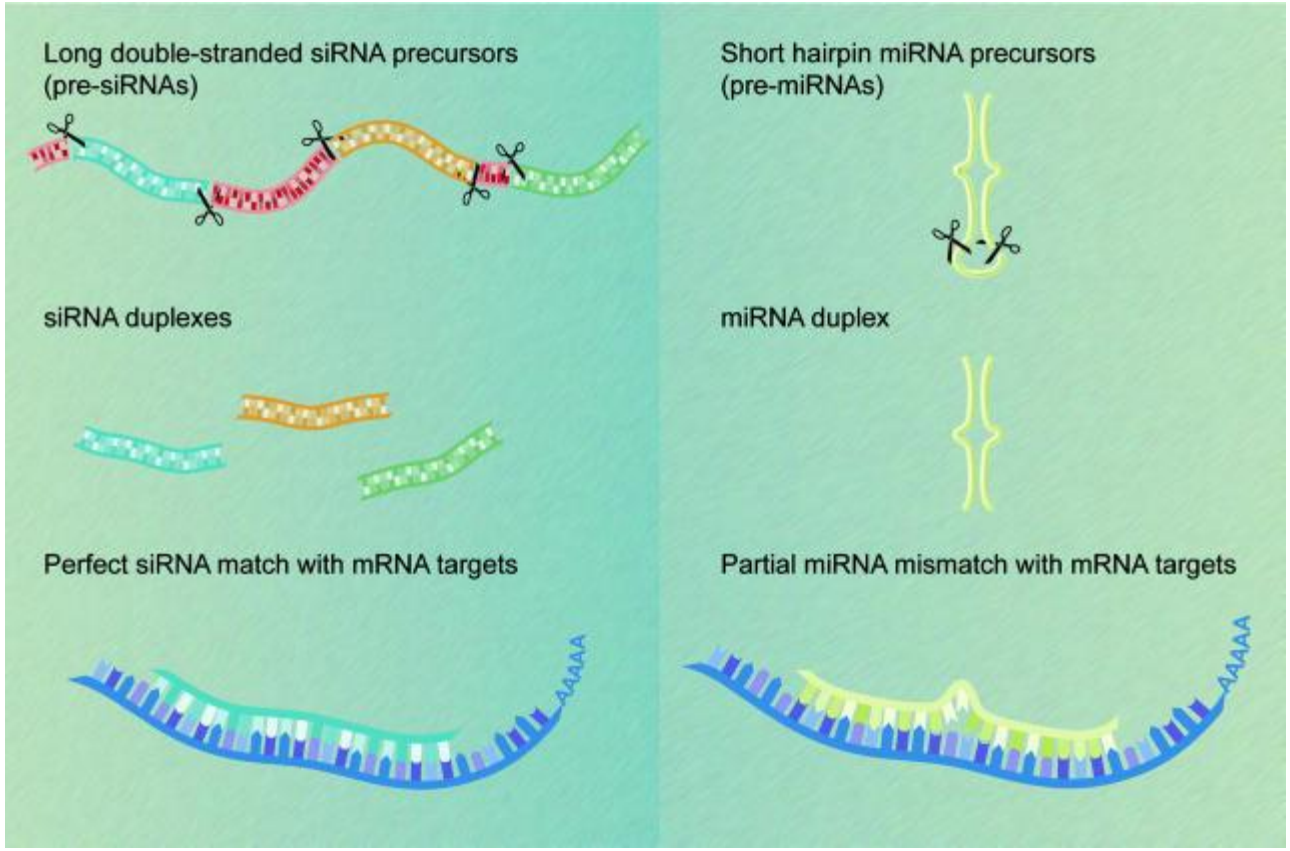
- **let-7**, was also described in C. elegans as critical to stop the stem-cell-like divisions of seam cells and induce their fully differentiated state. Reduced let-7 expression is associated with human cancers and cancer stem cells, thus suggesting that let-7 in humans also promotes terminal differentiation and is a tumor suppressor.

Reinhard BJ, 2000

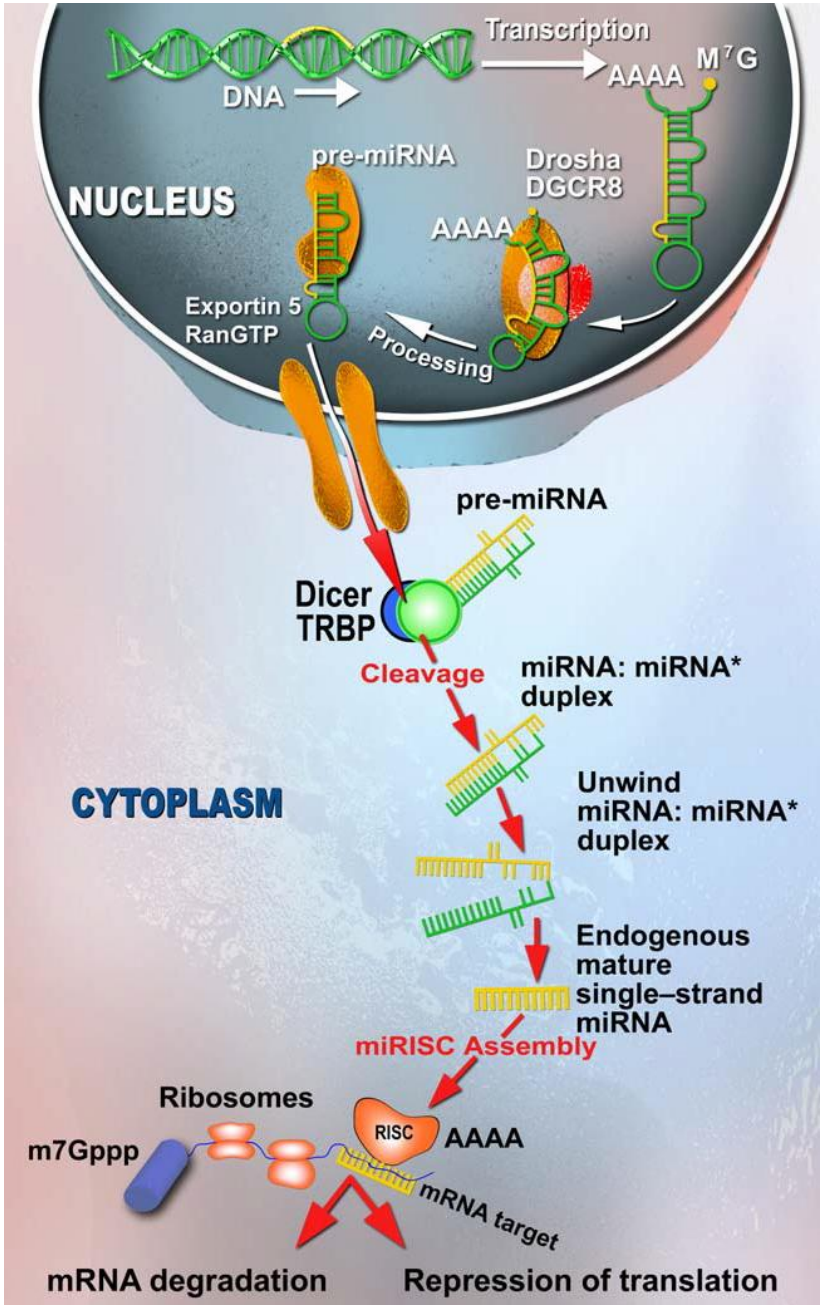
- **1998-Fire and Mello**, experiments in C. elegans, first to show that dsRNA is much more potent at inhibiting gene expression than antisense RNA. Set the stage for understanding the role of miRNAs in development and gene regulation.

Nobel Prize in Physiology and Medicine, 2006

siRNA **miRNA**



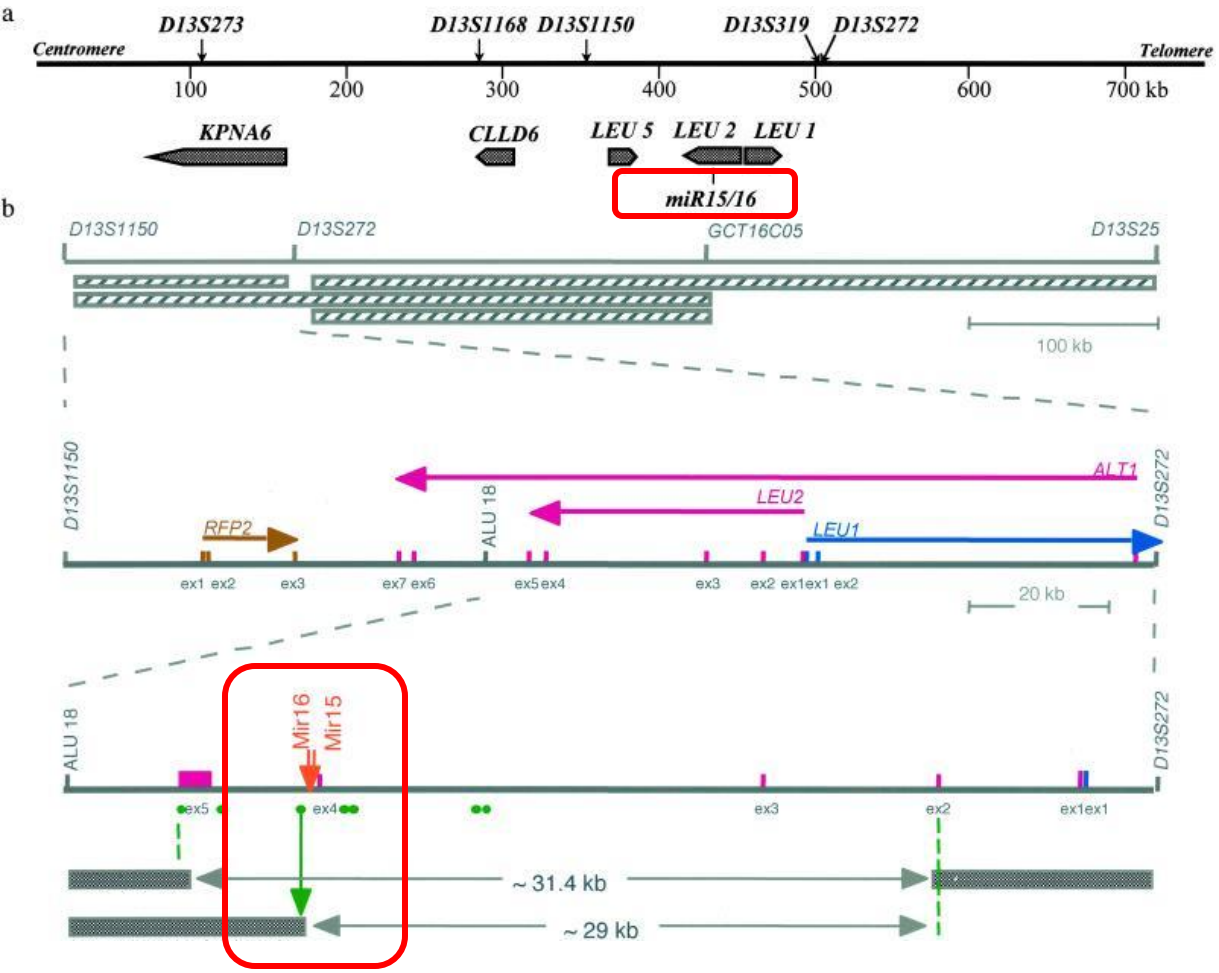
	Length	Where Found?	Target Recog	Mechanism
miRNA	19-25 nt	Endogenous	Imperfect Match	Translational Repression
siRNA	19-21 nt	Exogenous	Exact Match	mRNA Cleavage



miRNA processing

- RNA polymerase II
- Primary miRNA (pri-miRNA)
- Drosha, Pasha
- Pre-miRNA
- Exportin 5
- Dicer
- RNA induced silencing complex (RISC)

miRs and cancer

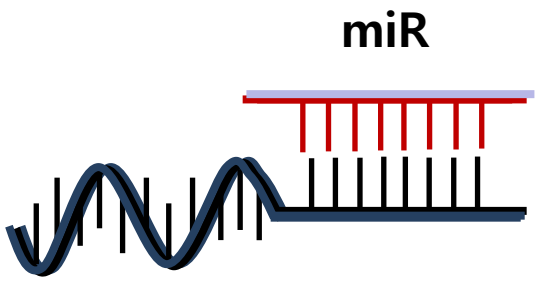


- First reported in **chronic lymphocytic leukemia**, where **miR-15 and miR-16** were found to be deleted or down-regulated in the vast majority of tumors

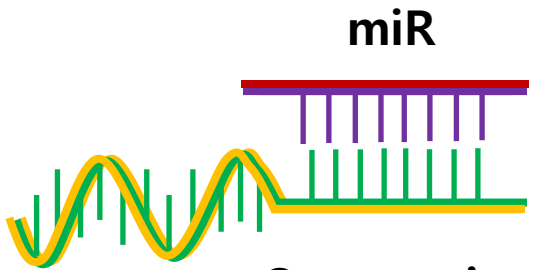
t(2;13)(q32;q14) translocation

Calin GA, PNAS 2002

MicroRNA ACTIVITY IN CANCER
: TUMOR SUPPRESSIVE OR ONCOGENIC



Tumor suppressive

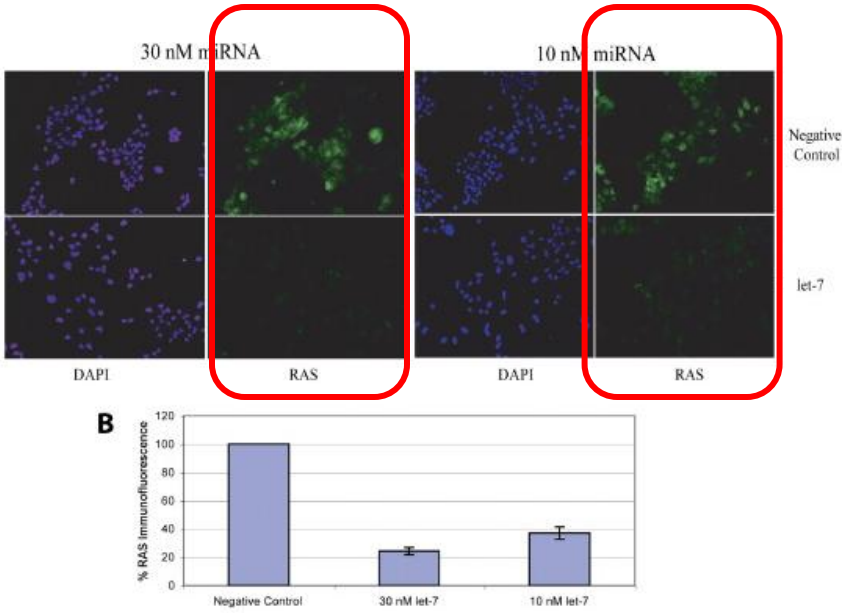


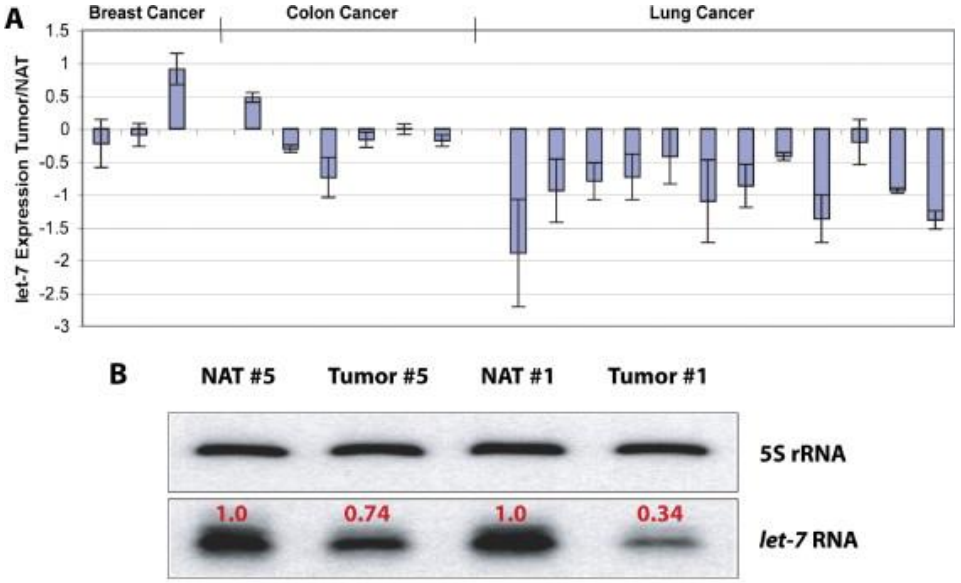
Oncogenic

- Suppress expression of oncogenes, growth promoting, survival and angiogenic genes (low in tumors)
- Suppress expression of tumor suppressor, growth inhibitory, proapoptotic genes (high in tumors)

miRs are involved in RAS oncogenic pathways

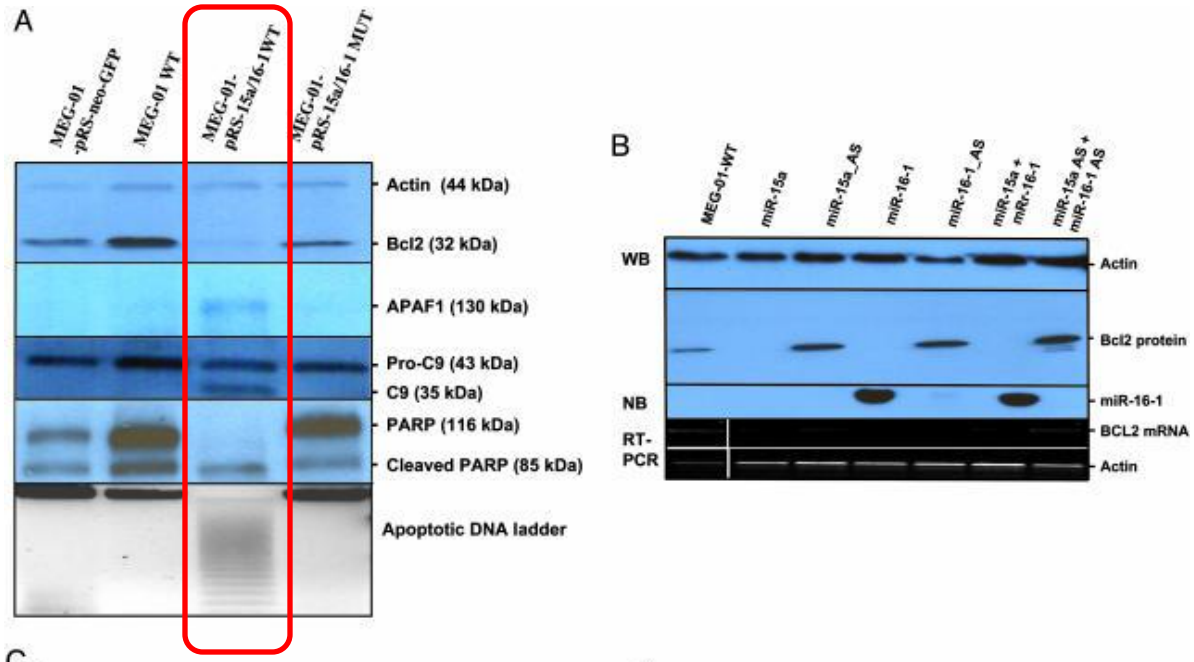
- the three human RAS oncogenes (H-, K-, and N-RAS) all contain **let-7** sites in their 3′ UTR
- the let-7 family of miRs, which is typically down-regulated in various tumors, has been shown to negatively regulate the RAS oncogenes in lung tumors, therefore acting as tumor suppressor genes





Johnson SM, Cell 2005

miR-15 and miR-16 induce apoptosis by targeting BCL2



- miR-15 and miR-16 have been shown to target the BCL2 oncogene, leading to its down regulation and apoptosis in leukemic cells

Cimno A, PNAS 2005

Example of miRs acting as oncogenes

- **miR-221 and miR-222** can target and inhibit the expression of the **p27Kip tumor suppressor**
  - le Sage et al. 2007

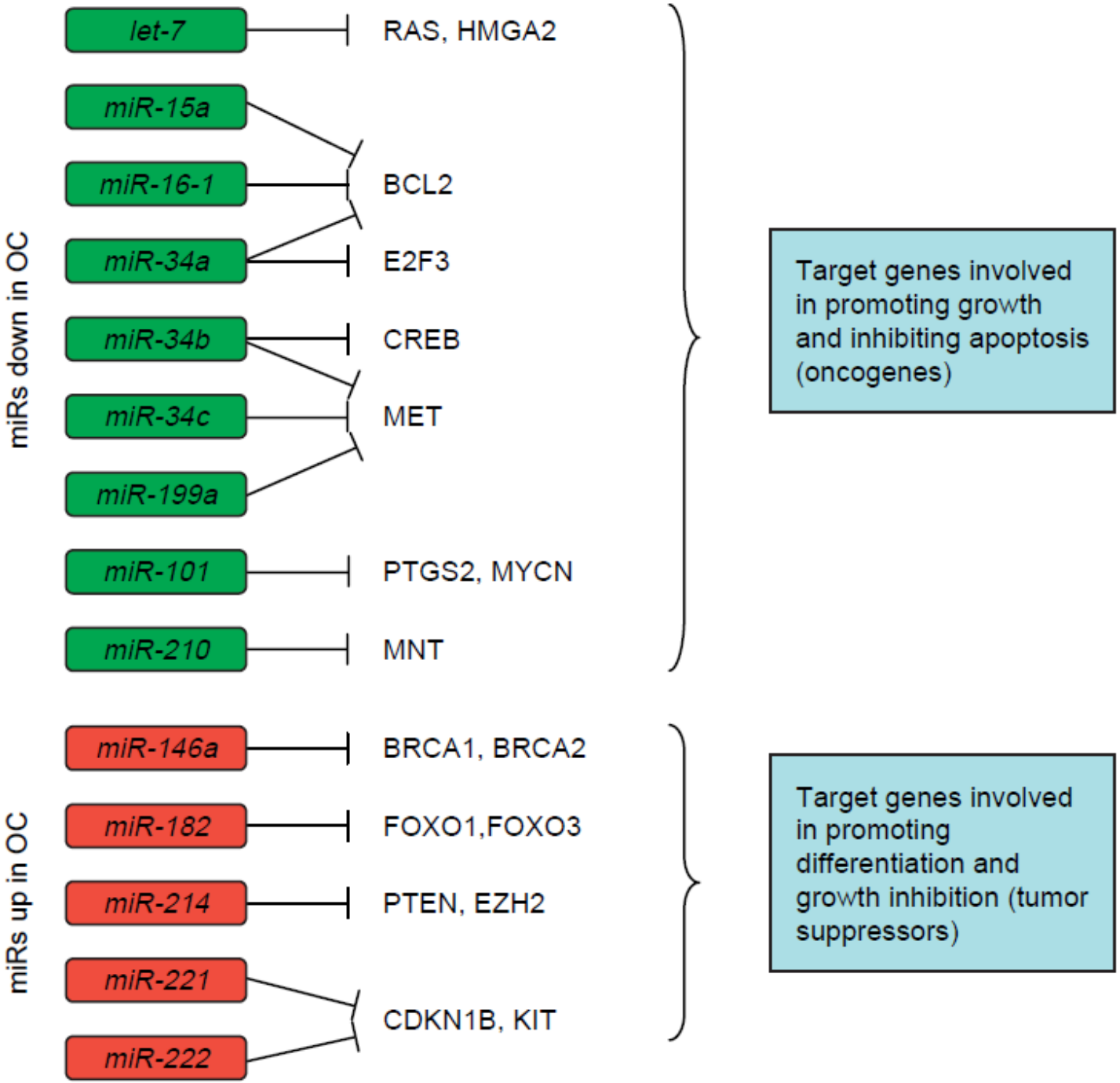
- **p53 tumor suppressor** has been shown to transcriptionally induce **miR-34** following genotoxic stress and this induction is important in mediating p53 function
  - Chang et al. 2007, He et al. 2007, Raver-Shapira et al. 2007, Tarasov et al. 2007

Established Profiling Methods to Quantify miRNAs

	ADVANTAGES	LIMITATIONS
qRT-PCR	• Semi-high throughput • Good quantification • Amplification enables superior sensitivity (fM)	• Difficult to distinguish single nucleotide differences • Not for discovery of ncRNAs
Microarray	• Very high throughput • Good ratio between cost and generated information • Easy to perform data analyses • Results are often validated with qRT-PCR	• In some cases fair specificity • Medium sensitivity (pM) • Sensitivity and specificity can be improved by LNA modification of the probes • Limited for quantification • Not useful for discovery of ncRNAs
In situ hybridization	• Locate miRNA in tissue and cell compartments • miRNA and target identification on the same slide	• Low throughput • Invasive sample collection • Limited sensitivity • Very limited quantification
RNA sequencing	• High throughput due to barcoding • High sensitivity(<fM) • High specificity • Can be used for discovery of novel ncRNAs	• Large amount of complex data that need to be analyzed • High cost

Up-regulated	Down-regulated	Comparison	Reference
miR-221, miR-146b, miR-508	*let-7f, miR-106b, miR-134, miR-155, miR-21, miR-346, miR-422a, miR-424, miR-519a, miR-648, miR-662*	Ovarian carcinoma cell lines and tissues versus normal	Dahiya *et al.* (2008)
miR-26b, miR-182, miR-103, miR-26a	*miR-127, miR-134, miR-154*, miR-410, miR-377, miR-100, miR-432, miR-368, miR-154, miR-495, miR-376a, miR-323, miR-376b, miR-370, miR-299, let7d, miR-155, miR-140, miR-222, miR-337, miR-124a, miR-99a, miR-331, miR-104, miR-150, miR-184, miR-152, miR-145, miR-424, miR-224, miR-302c*	EOC cell lines versus normal	Zhang *et al.* (2008)
None	*miR-509, miR-514, miR-513, miR-196, miR-376a, miR-184, miR-519d, miR-495, miR-424, miR-1, miR-368 miR-362 miR-22, miR-376b, miR-337, miR-133a, miR-508, miR-492, miR-137, miR-95, miR-448, miR-518, miR-491, miR-455, miR-365, miR-147, miR-488, miR-34a, miR–372, miR-202, miR-503, miR-520e, miR-410, miR-519e, miR-375, miR-346, miR-15a, miR-507, miR-450, miR-377, miR-34b, miR-518a, miR-432, miR-516*	Early-stage cancer versus late-stage cancer	Zhang *et al.* (2008)
None	*miR-514, miR-509, miR-508, miR-34c, miR-513, miR-368, miR-379, miR-154, miR-337, miR-507, miR-503, miR-376*	Low-grade cancer versus high-grade cancer	Zhang *et al.* (2008)
miR-200b, miR-21, miR-200c, miR-141, miR-20a, miR-27a, miR-16, miR-93	*miR-145, miR-125b, miR-100, miR-99a, miR-26a, miR-10b, miR-143, miR-214, let-7b, miR-29a, miR-125a*	Serous ovarian carcinoma versus normal ovarian tissues	Nam *et al.* (2008*a*)
miR-182, miR-200c, miR-142-3p, miR-200b, miR-135b, miR-200a, miR-195, miR-126, miR-26b, miR-10b, miR-126, miR-199b-5p, miR-107, miR-30b, miR-192, miR-335, miR-32, miR-20a, miR-30c, miR-143, miR-92a, miR-199b-3p, miR-99a, miR-26a, miR-18a, miR-16, miR-15a, miR-30e, miR-194, miR-29c, miR-30d, miR-106b*	*miR-127-3p, miR-377*, miR-382, miR-493, miR-409-3p, miR-193a-5p, miR-210, miR-935, miR-100, miR-31, miR-22, miR-152, miR-379, miR-185, miR-221, miR-744, miR-21*, let-7a*, miR-574-5p, miR-31*, miR-130b, miR-149, miR-423-5p, miR-1308, miR-629, miR-320a*	Stage III/IV epithelial ovarian carcinoma versus normal (only miRs differentially expressed between all OC subtypes versus normal are included in this table)	Wyman *et al.* (2009)
miR-223, miR-206, let-7i, miR-30a3p, miR-368, miR-10b, miR-338, miR-195, miR-93, miR-23a, miR-185, miR-22, miR-339, miR-321, miR-29b, miR-186, miR-128a, miR-374, miR-193, miR-106b, miR-194, miR-370, miR-128b, miR-198, miR-224, miR-222, miR-29c, miR-21, miR-34c, miR-139, miR-197, miR-15a, miR-218, miR-106a, miR-340, miR-219, miR-155, miR-92, let-7g, miR-328, miR-149, miR-23b, miR-221, miR-150, miR-190, miR-107, miR-331, miR-181c, miR-133b	*miR-326, miR-30d, miR-125b, miR-31, miR-99a, miR-100, miR-137, miR-9*	Primary versus recurrent serous papillary ovarian carcinomas	Laios *et al.* (2008)
miR-200a, miR-200b, miR-200c, miR-141	*miR-140, miR-199a, miR-199b, miR-145, miR-143, miR-125a, miR-125b, miR-101, miR-212, miR-222*	Normal versus cancer	Iorio *et al.* (2007)
miR-199a, miR-424, miR-302d, miR-320, miR-214, miR-200a, miR-29a	*miR-493, miR-494, miR-125b, miR-100, let-7a, let-7b, let-7c*	Normal versus primary tumors	Yang *et al.* (2008*a*)

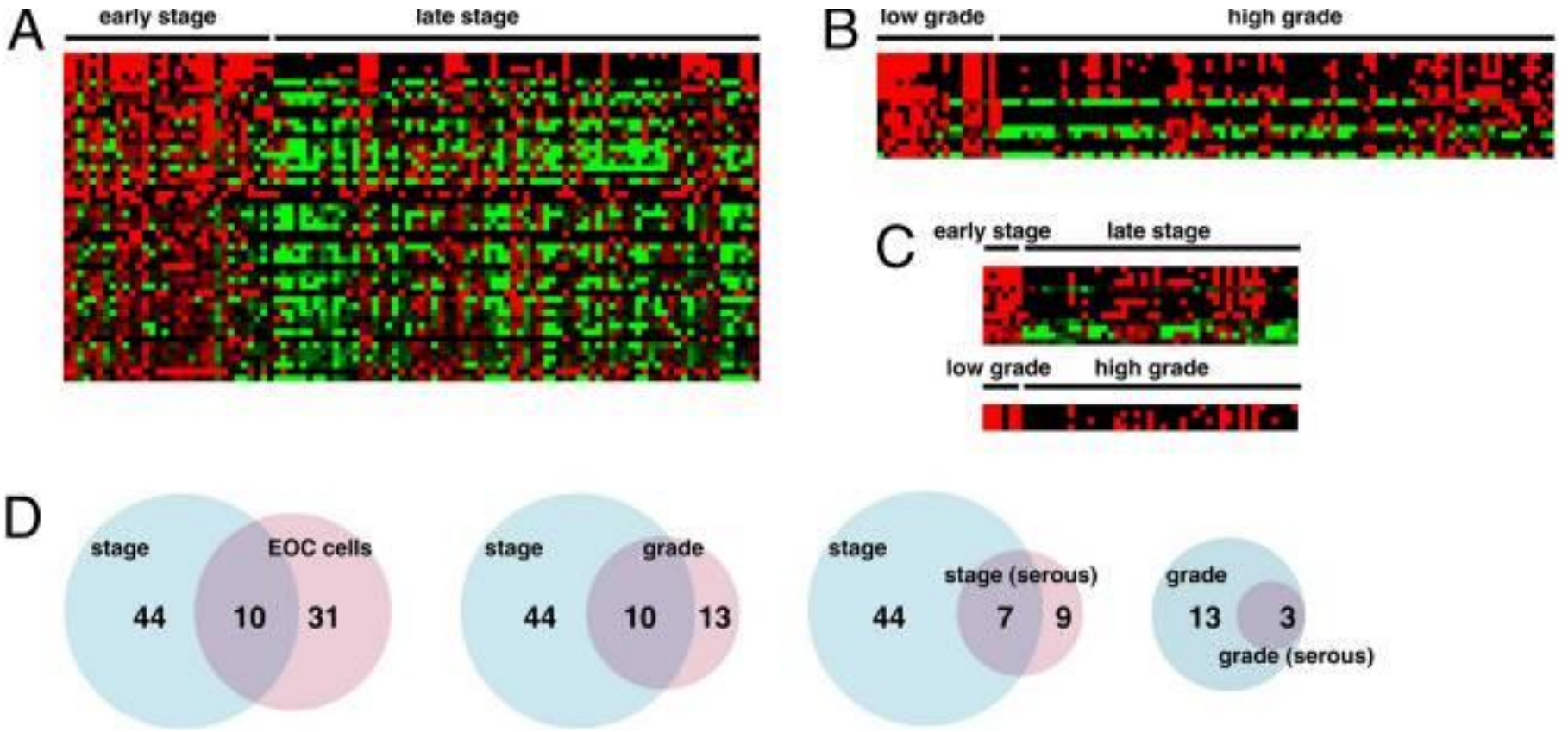
- **A number of studies have used various gene expression profiling approaches to study miR expression in ovarian carcinoma.**


miRs down in OC
let-7
RAS, HMGA2
miR-15a
miR-16-1
BCL2
miR-34a
E2F3
miR-34b
CREB
miR-34c
MET
miR-199a
miR-101
PTGS2, MYCN
miR-210
MNT
Target genes involved in promoting growth and inhibiting apoptosis (oncogenes)
miRs up in OC
miR-146a
BRCA1, BRCA2
miR-182
FOXO1,FOXO3
miR-214
PTEN, EZH2
miR-221
miR-222
CDKN1B, KIT
Target genes involved in promoting differentiation and growth inhibition (tumor suppressors)

Mechanisms leading to abnormal expression of miRs in cancer

- **Chromosomal rearrangements**
  - (Calin et al. 2005, Tagawa & Seto 2005, Calin & Croce 2007)
- **Genomic copy number change**
  - (Calin et al. 2004, Zhang et al. 2006, Giannakakis et al. 2008)
- **Epigenetic modifications**
  - (Saito et al. 2006, Iorio et al. 2007)
- **Defects in miR biogenesis pathway**
  - (Kumar et al. 2007)
- **Regulation by transcriptional factors**
  - (Ho et al. 2007)

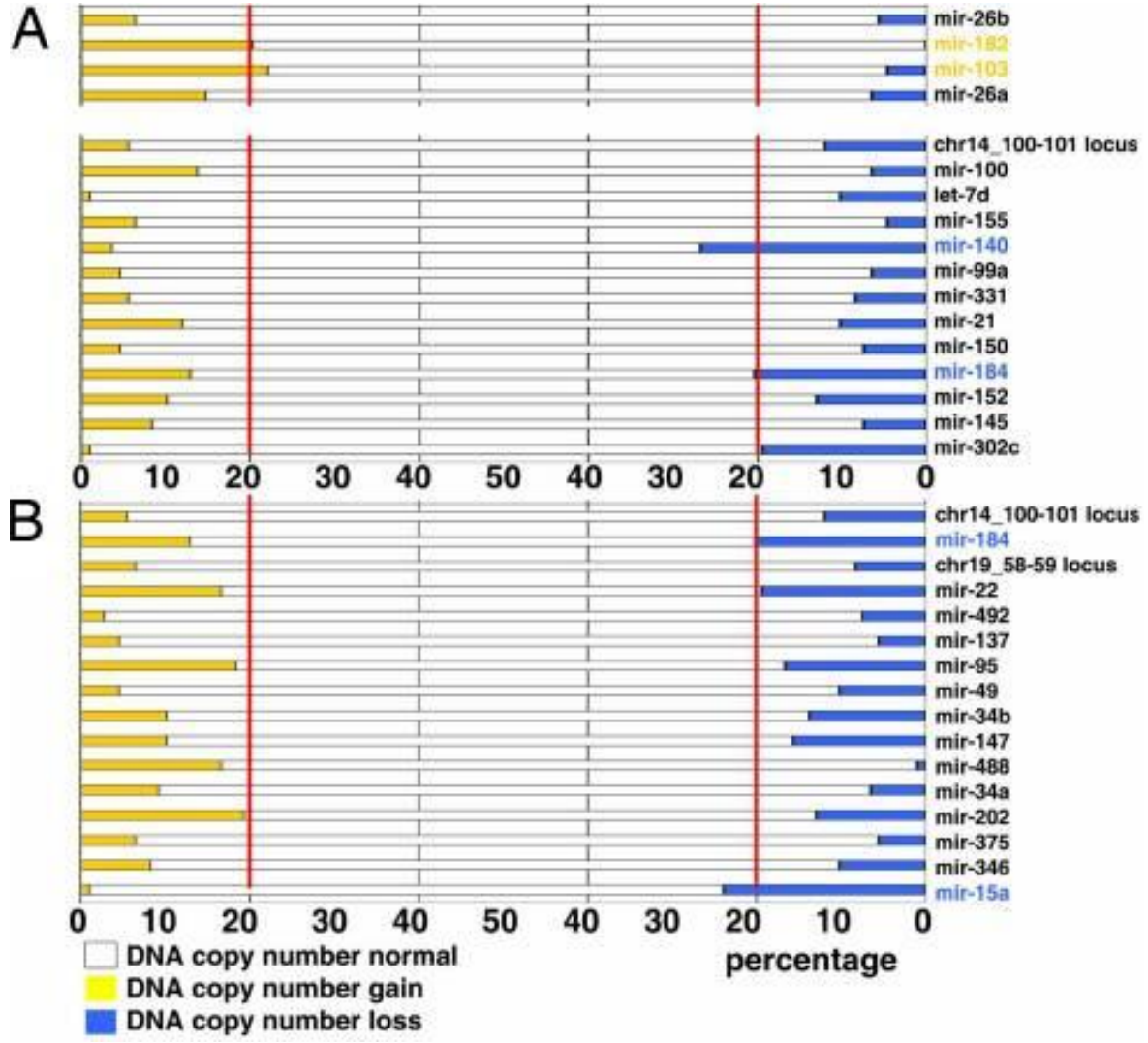
Both genomic losses and epigenetic alterations may be responsible for miR down-regulation.



- Numerous miRNAs are down-regulated in late-stage or high-grade ovarian cancer

Zhang L, PNAS 2008

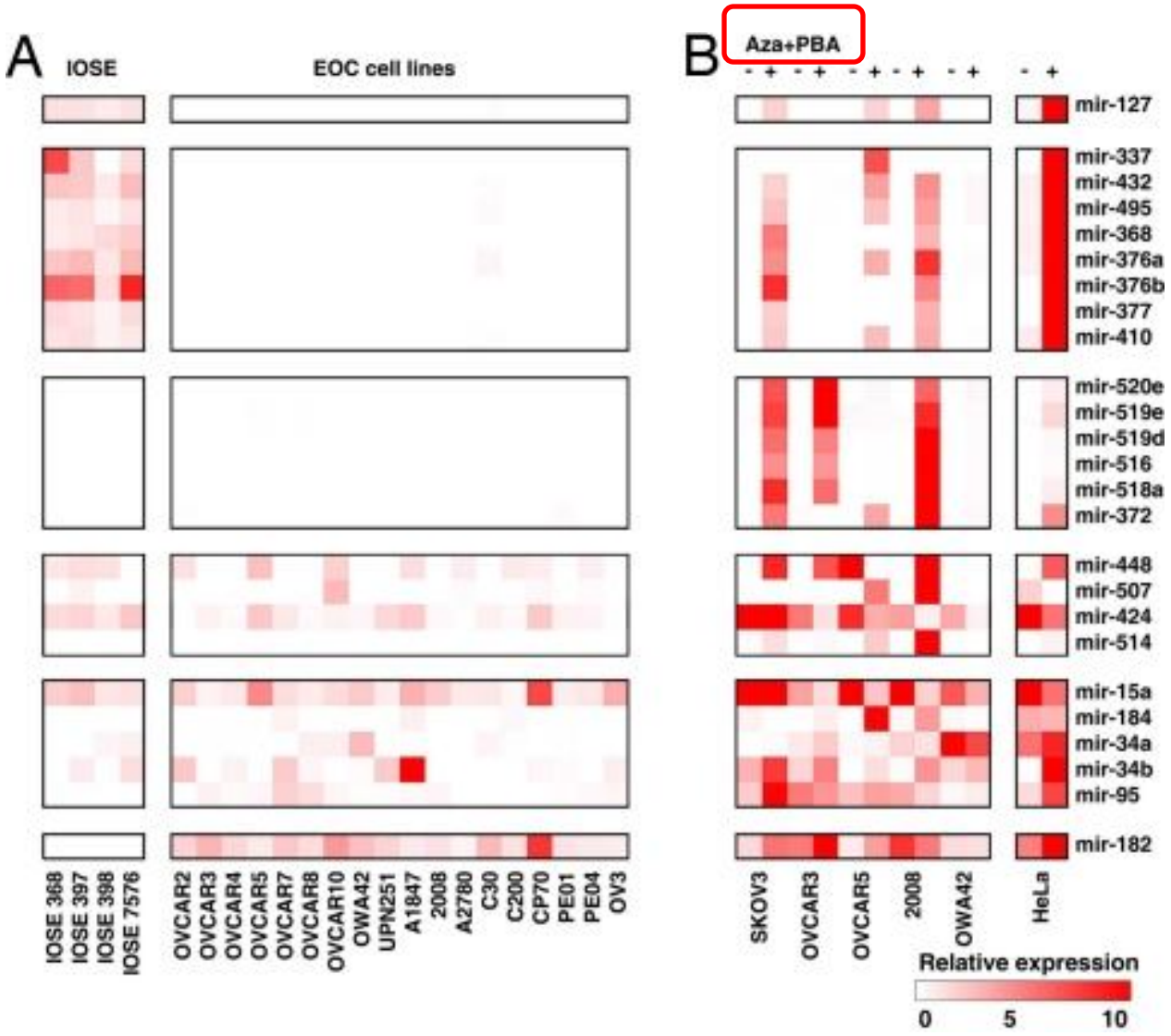
DNA copy number deletions contribute to down-regulation of miRNAs



- DNA copy number amplification and deletion were correlated with **miR-182 and miR-15a expressions** respectively, in both primary tumors and cell lines

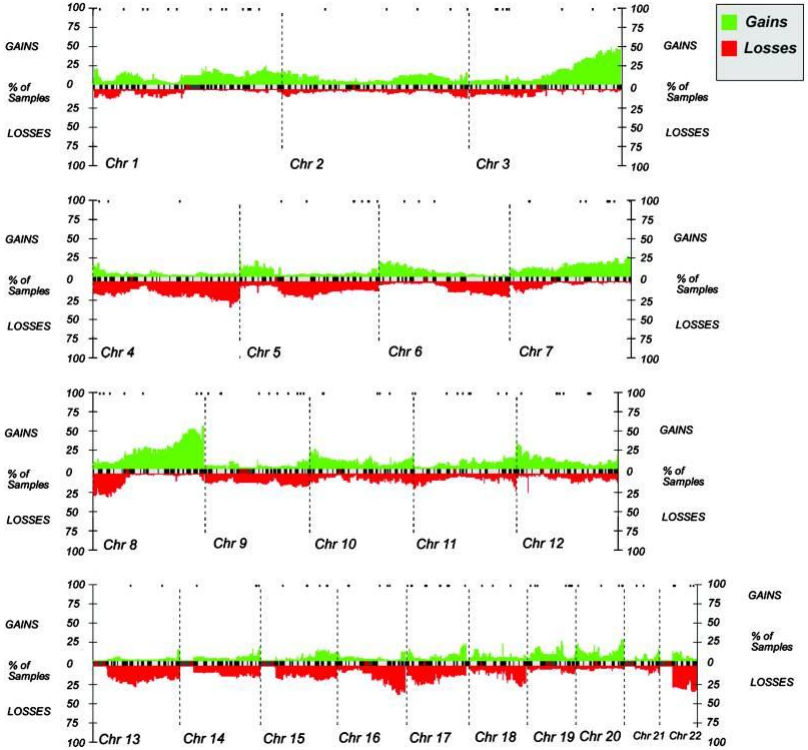
Zhang L, PNAS 2008

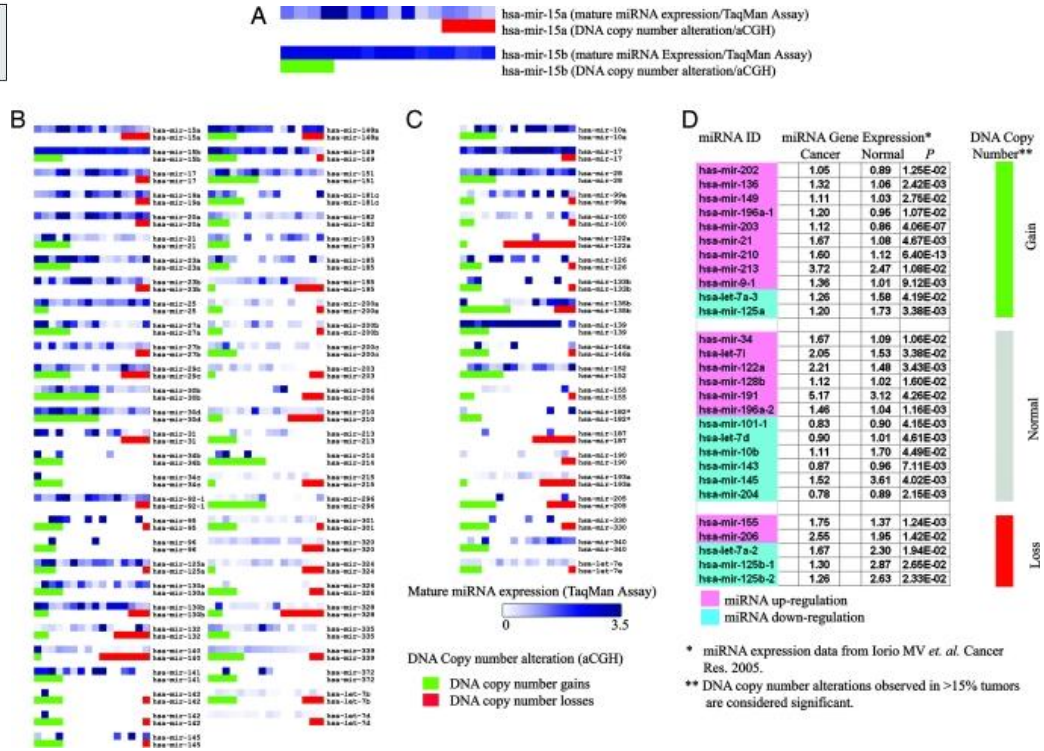
Epigenetic alterations silence miRNA expression in ovarian cancer



- EOC cell lines that were treated with DNA **demethylating and histone deacetylase (HDAC) inhibitors** exhibited up-regulation of 16 miRs, which suggests **epigenetic modification** as another crucial factor determining the expression of miRs in EOC.

microRNAs exhibit high frequency genomic alterations in human cancer

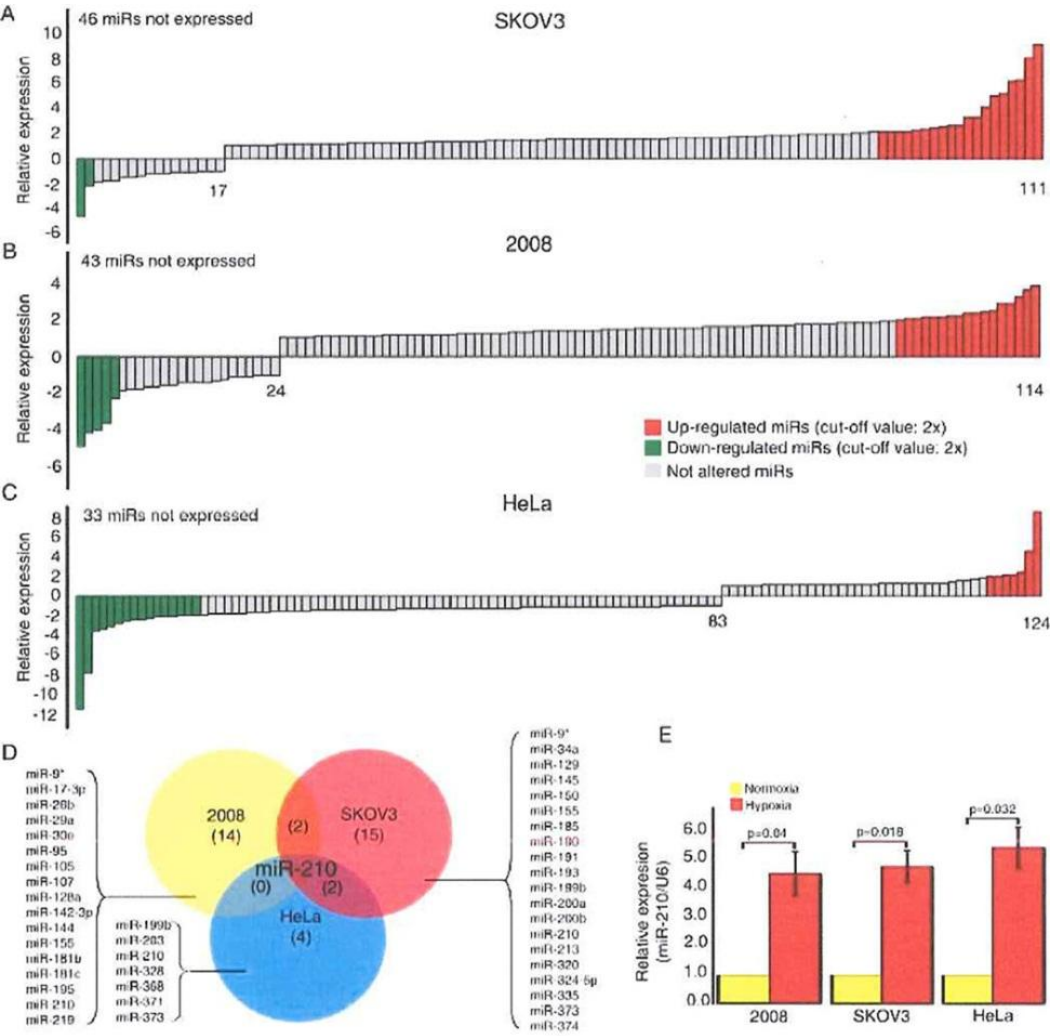




- Genomic regions containing miR genes frequently exhibited copy number abnormalities
- Copy number losses of the region containing miR-218-1 and SLIT2 were observed in 15.5% of ovarian carcinomas
- Positive correlation between miR copy number changes and the miR expression levels of 73.1% of the miR genes

Zhang L, PNAS 2006

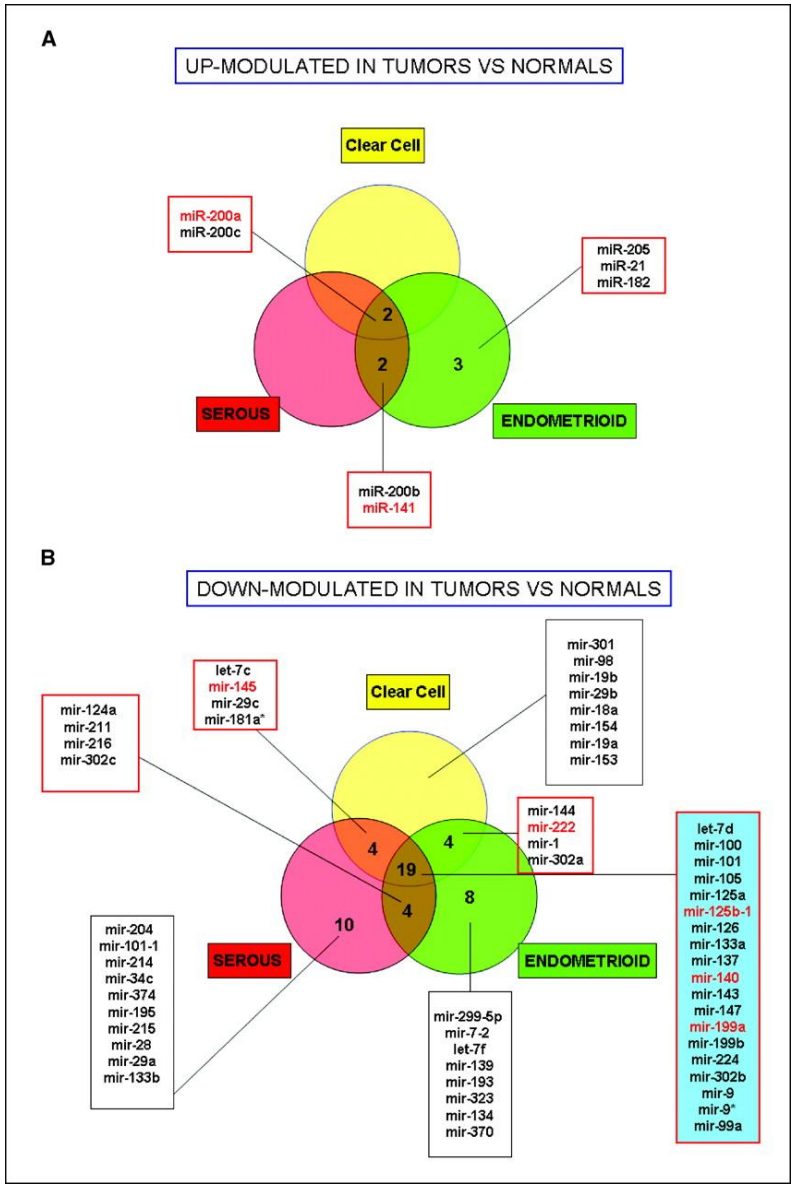
Hypoxia-responsive miRs in ovarian carcinoma



- **miR-210** links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer.
- e2f transcription factor 3 (e2f3), a key protein in cell cycle, is regulated by miR-210. E2F3 was further confirmed to be downregulated at the protein level upon induction of miR-210.

Giannakakis A, Cancer biology & therapy 2008

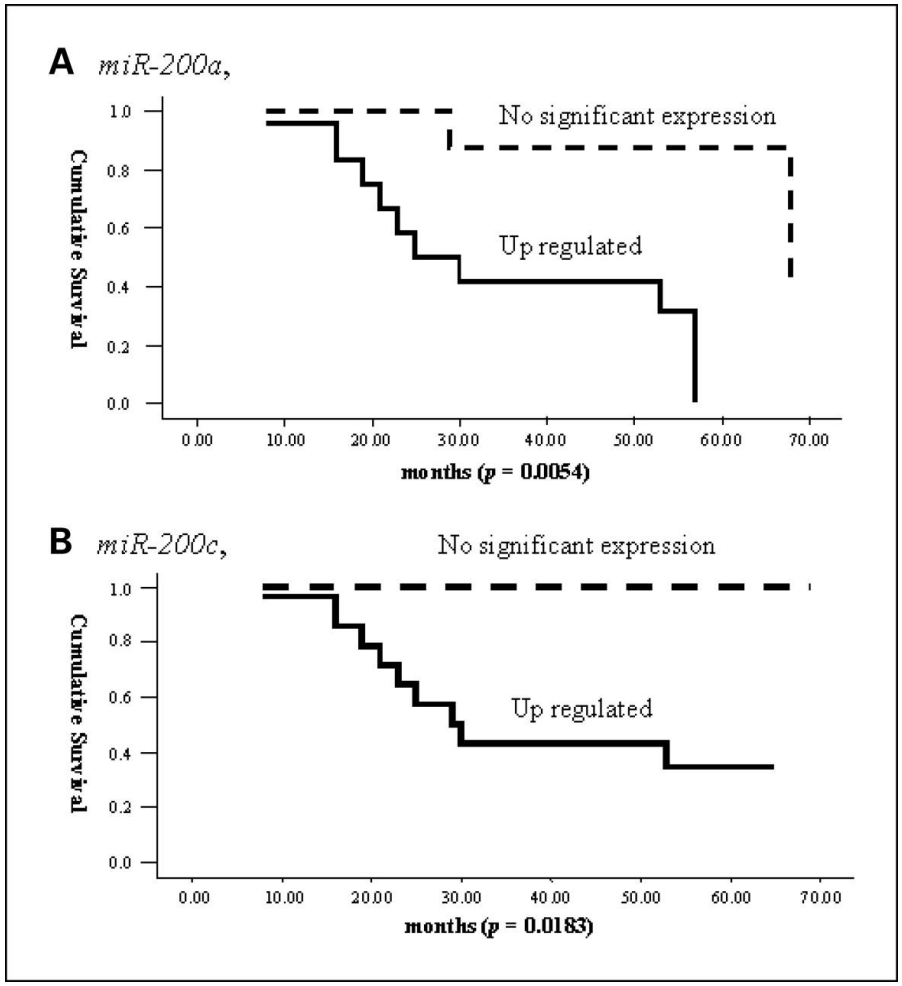
miR expression signature in ovarian carcinoma



- A total of 29 and 39 miRs were found to be aberrantly expressed by significance analysis of microarrays (SAM) and prediction analysis of microarrays (PAM) analysis respectively. **MiR-200a and miR-141 were highly up-regulated**, whereas **miR-199a, miR-140, miR-145, and miR-125b1** were most significantly down-regulated.
- miRNA signatures characterizing different ovarian carcinoma **histotypes** (serous, endometrioid, and clear cell)

Iorio MV, Cancer research 2007

miR expression profiles in serous ovarian carcinoma



- Higher expression of **miR-200, miR-141, miR-18a, miR-93, and miR-429**, and lower expression of **let-7b, and miR-199a** were significantly correlated with a poor prognosis ($P < 0.05$).

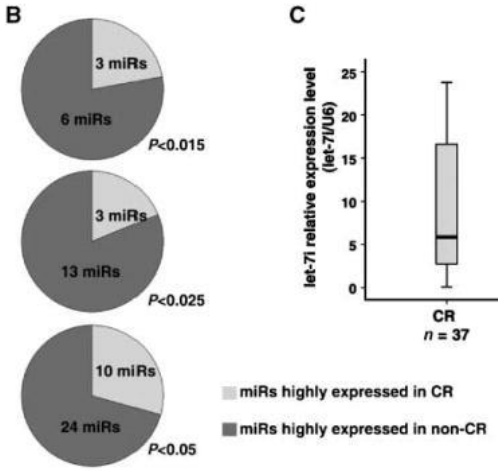
Nam EJ, Clinical Cancer research 2008

Roles of miRs in ovarian carcinoma chemotherapy

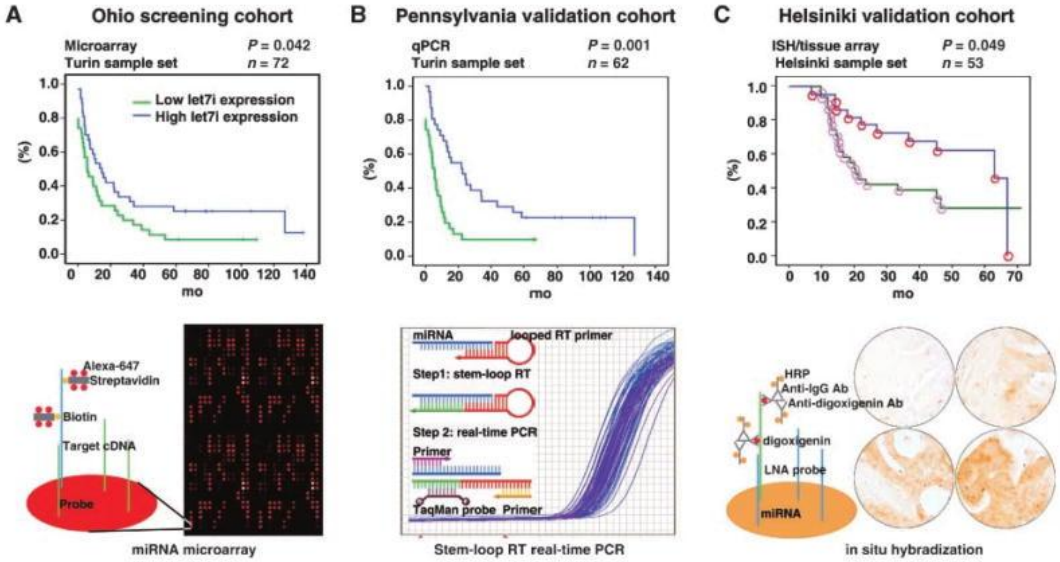
A

miRNA ID	p-value	Ratio (CR/non-CR)
let-7i	0.003	9.284
mir-321 #	0.005	1.383
mir-223	0.006	0.502
mif-216	0.008	0.315
mir-198	0.008	0.606
mir-22	0.008	0.249
mir-126	0.012	0.683
mir-509	0.012	4.883
mir-370	0.014	0.291
mir-214	0.016	0.639
mir-520e	0.017	0.3
mir-519e	0.017	0.315
mir-1b-1	0.021	0.302
mir-152	0.021	0.623
mir-196a	0.022	0.603
mir-521	0.023	0.287

mir-321 is a fragment of a Arg-tRNA



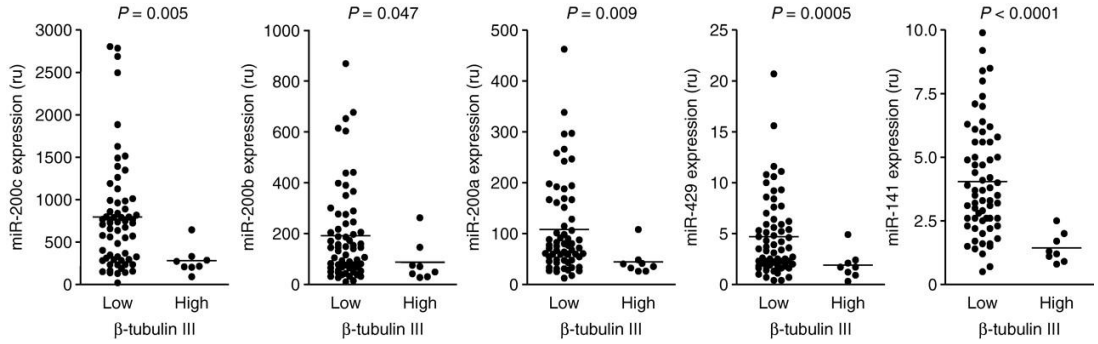
- **Let-7i** was the most down-regulated miR in the chemotherapy resistant patients.

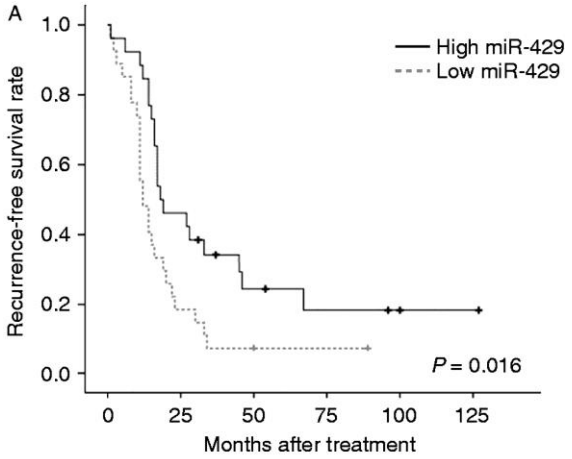


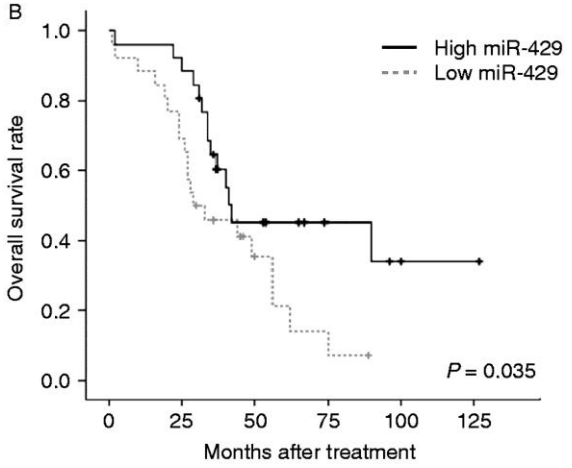
Yang H, Cancer research 2008

The miR-200 family controls β-tubulin III expression and is associated with paclitaxel-based treatment response and progression-free survival in ovarian cancer patients

- Women with stages III and IV serous ovarian carcinoma (n=557) **without a complete response** to a regimen of paclitaxel and carboplatin have tumors with significantly **lower miR-200c** levels when compared with those who achieved a complete response (HR, 1.43; 95% CI, 1.02- 1.99 [P5.037]).

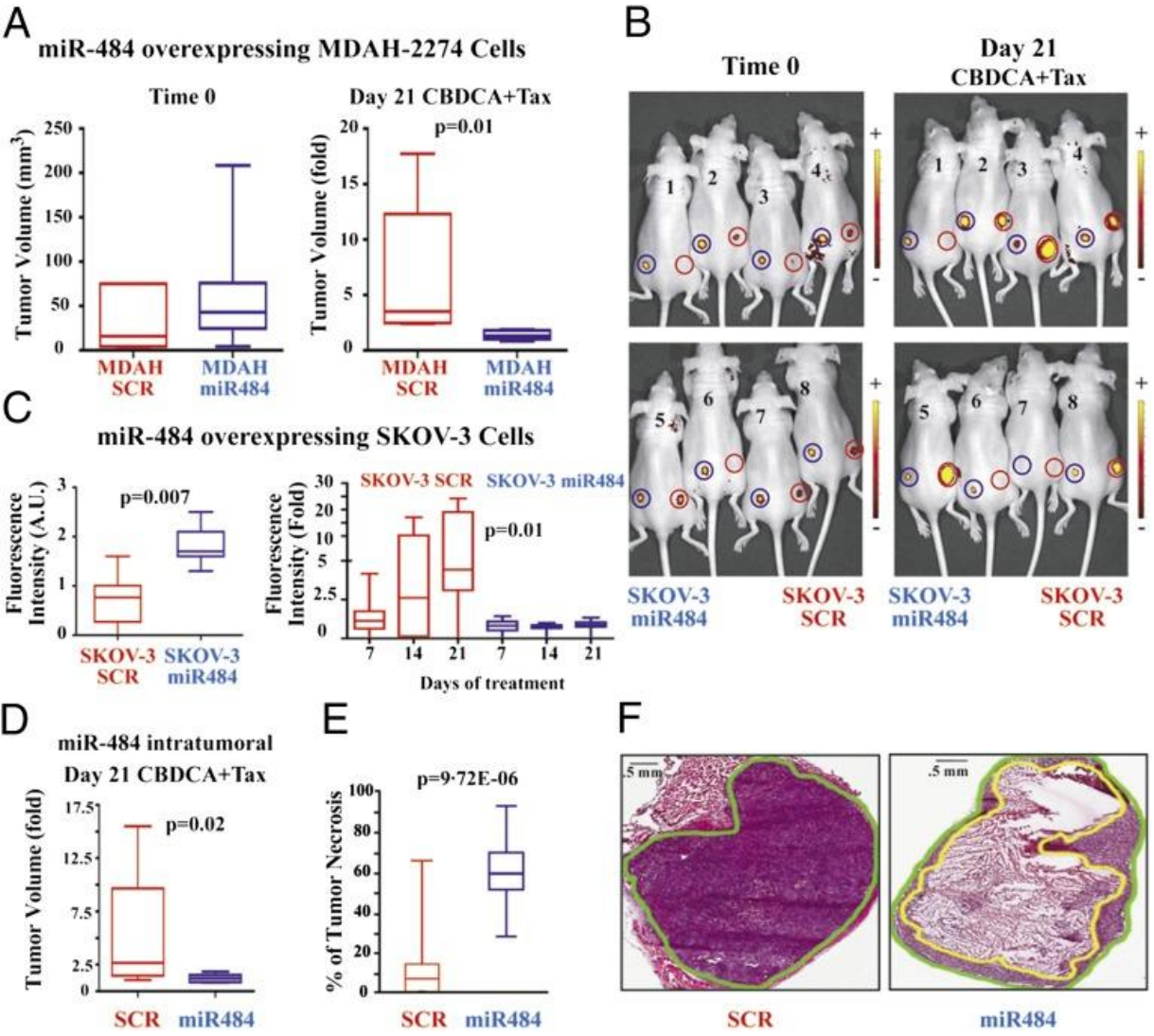






Leskela S, Endocr Relat Cancer 2010

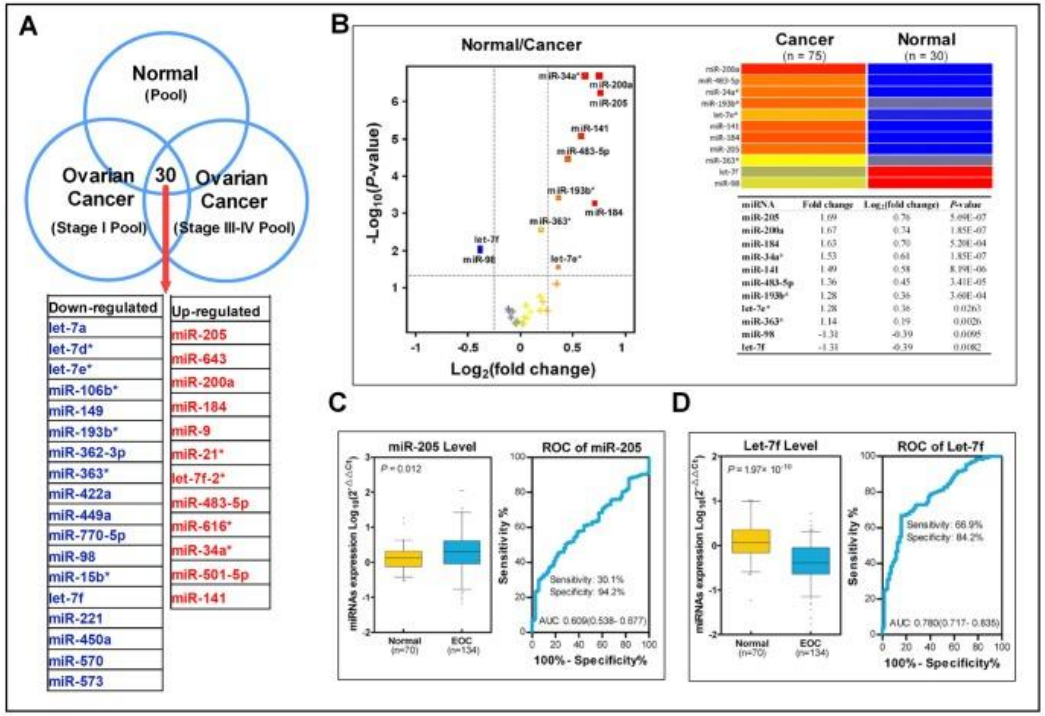
A microRNA signature defines chemoresistance in ovarian cancer through modulation of angiogenesis



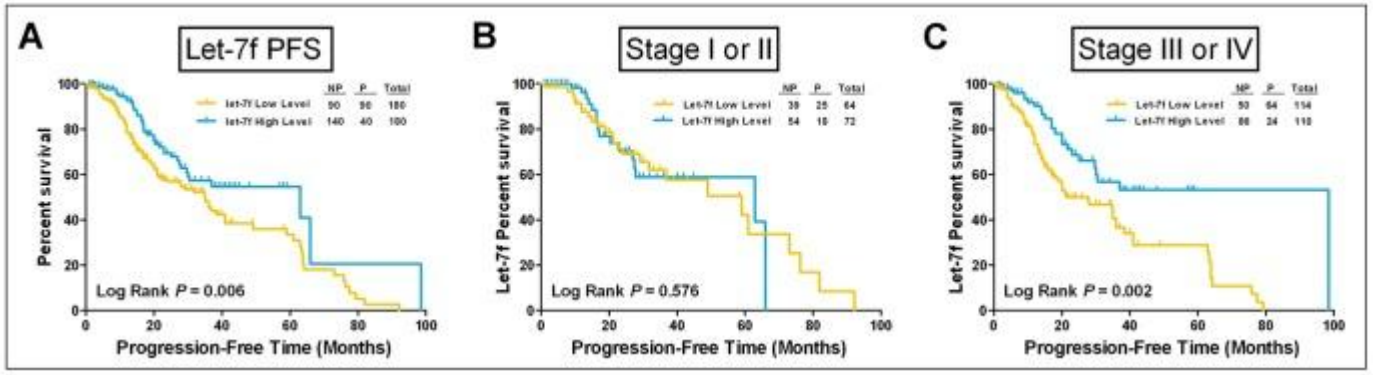
- three miRs **(miR-484, -642, and -217**) were able to predict chemoresistance
- miR-484 modulates in vivo response to chemotherapy
- miR-484 directly targets VEGFB and VEGFR2

Vechione A, PNAS 2013

Plasma miRNAs as diagnostic and prognostic biomarkers for ovarian cancer

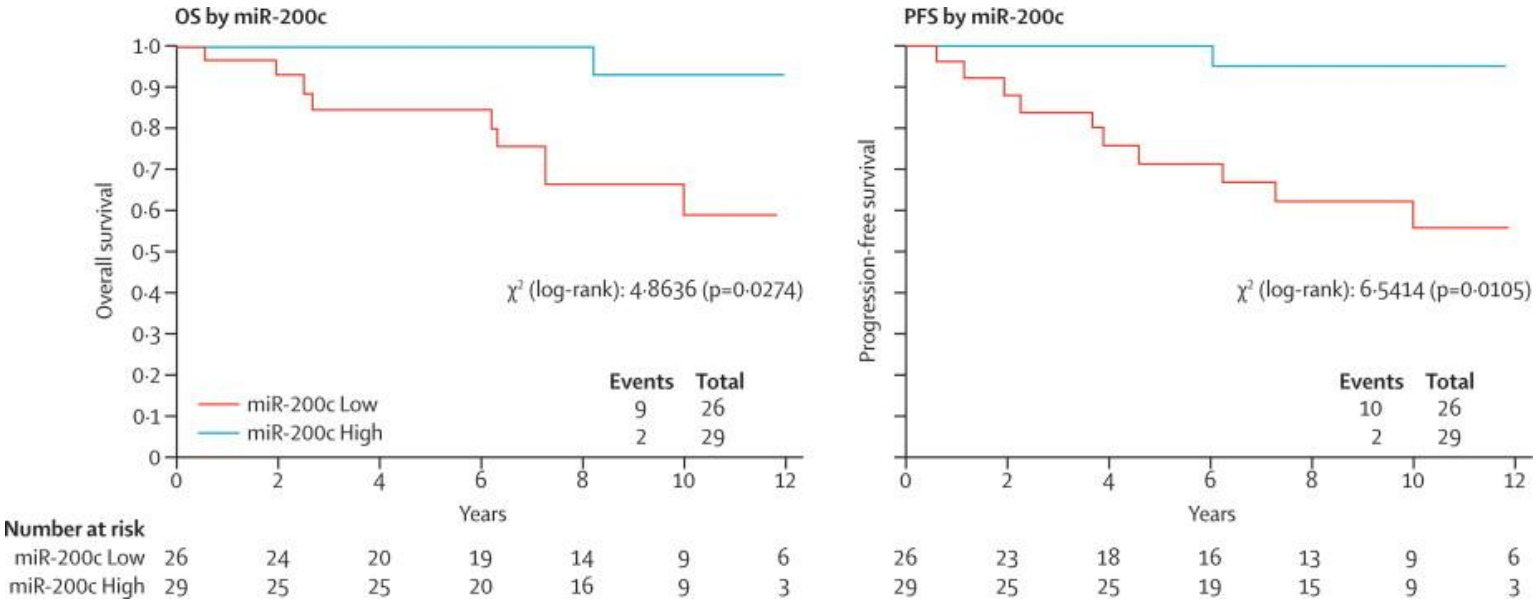


- **plasma miR-205 and let-7f** are biomarkers for ovarian cancer detection that complement CA-125; let-7f may be predictive of ovarian cancer prognosis.



Zheng H, 2013

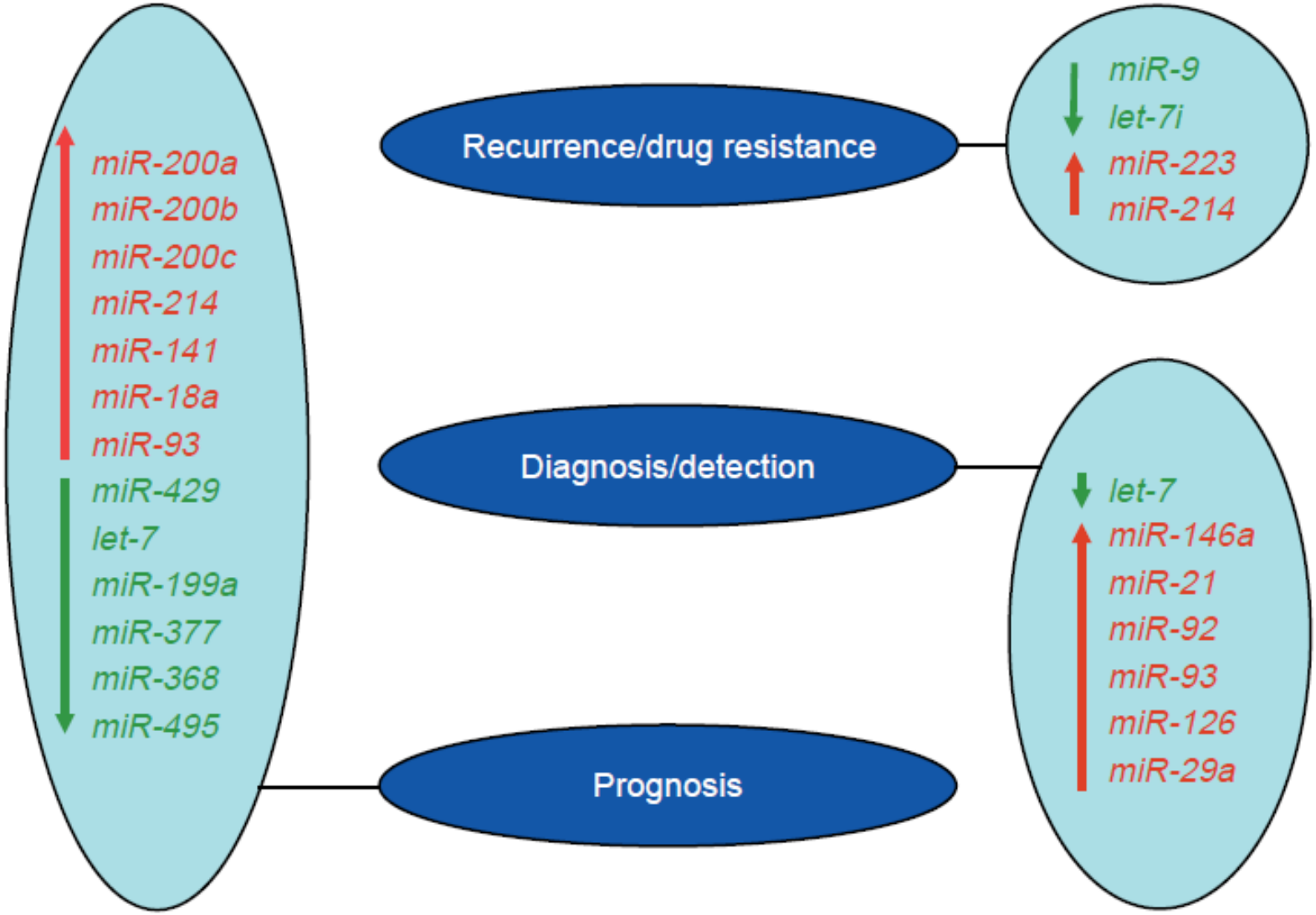
Association between miR-200c and the survival of patients with stage I epithelial ovarian cancer



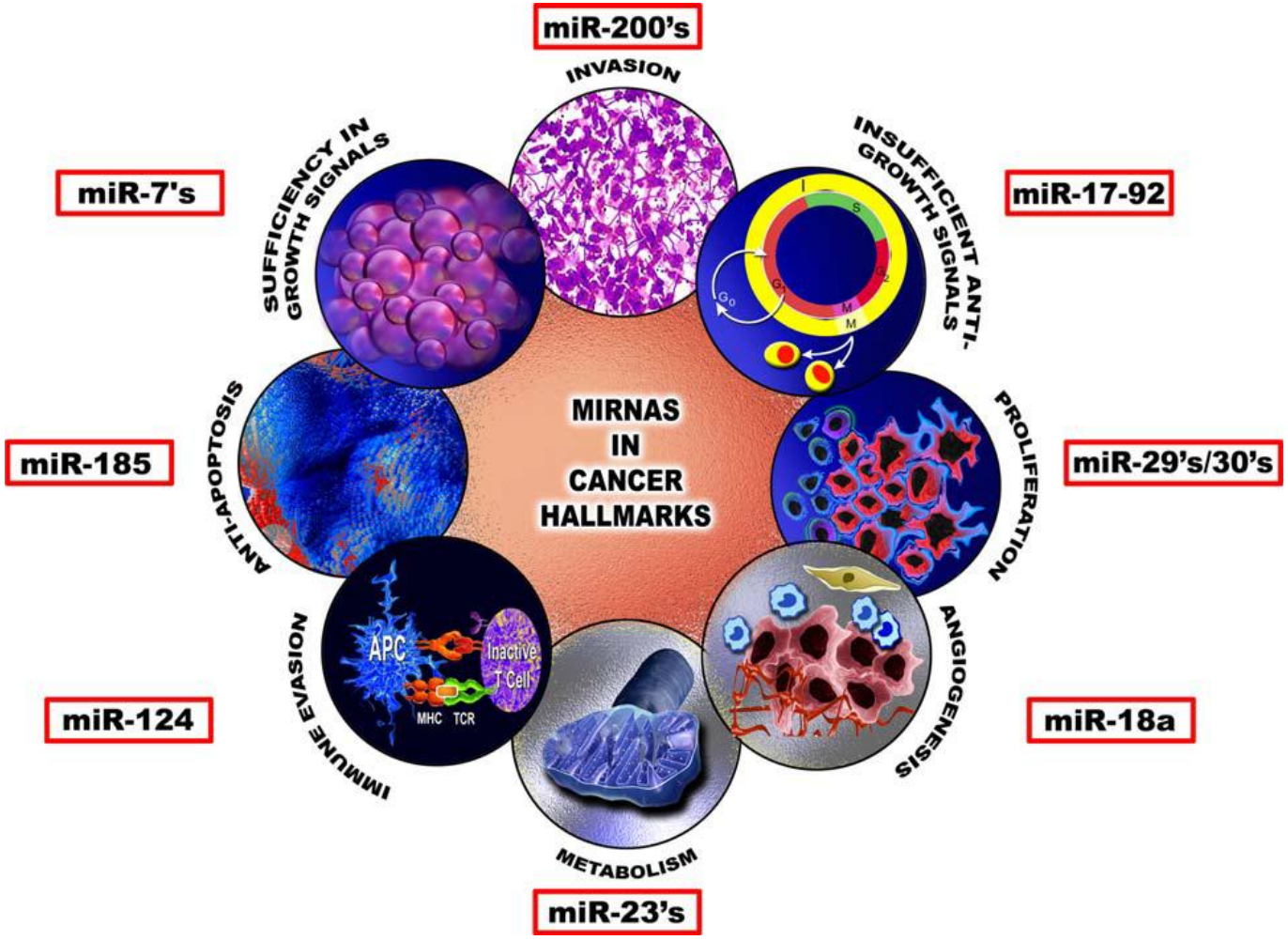
- Multivariate analysis confirmed that **down regulation of miR-200c** was associated with overall survival (HR 0·094, 95% CI 0·012-0·766, p=0·0272) and PFS (0·035, 0·004-0·311; p=0·0026), independent of clinical covariates.

Marchini S, Lancet Oncol 2011

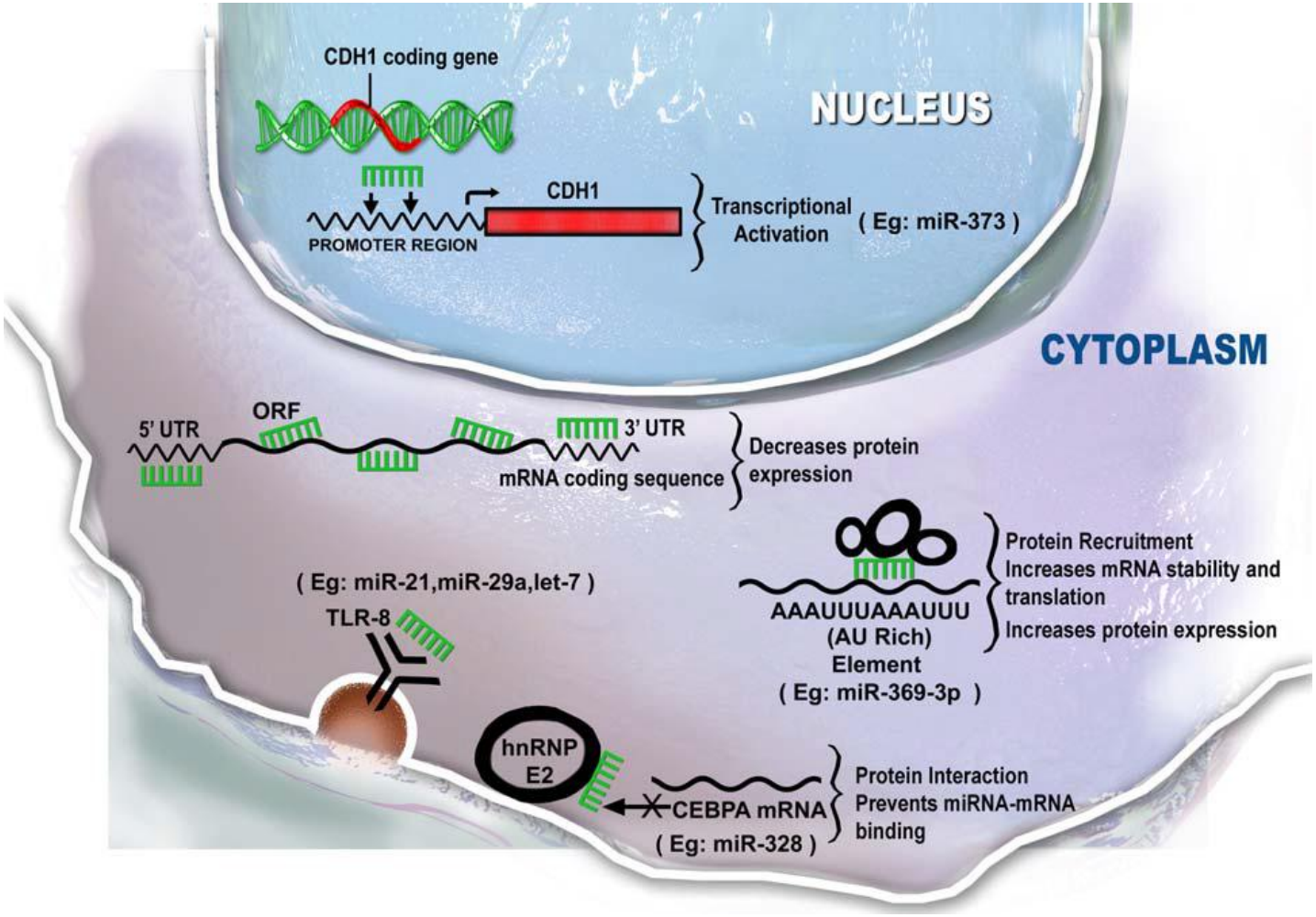
miRs with potential clinical use in ovarian carcinoma



MicroRNAs Involved in the Cancer Hallmarks

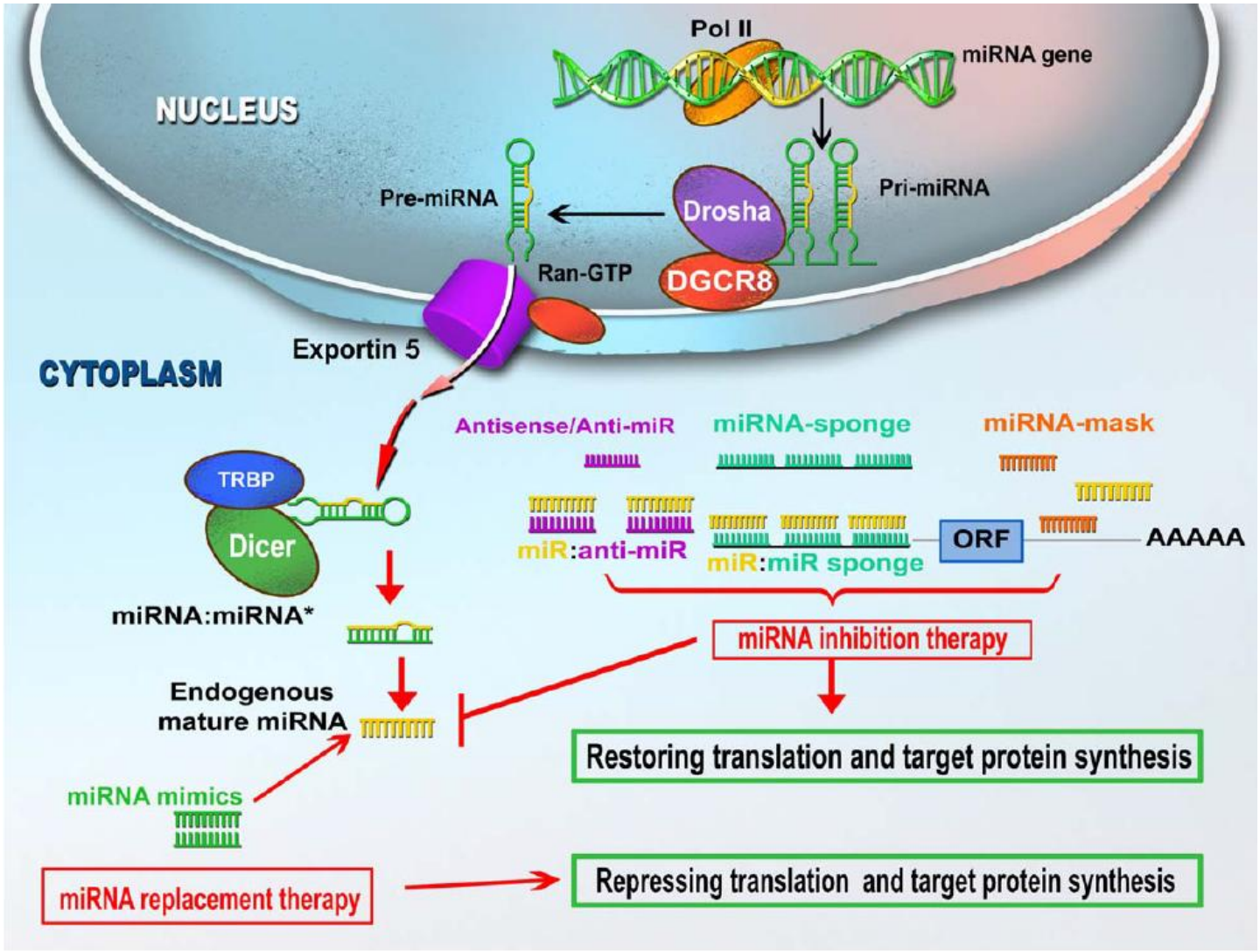


Mechanisms of Action of MicroRNAs



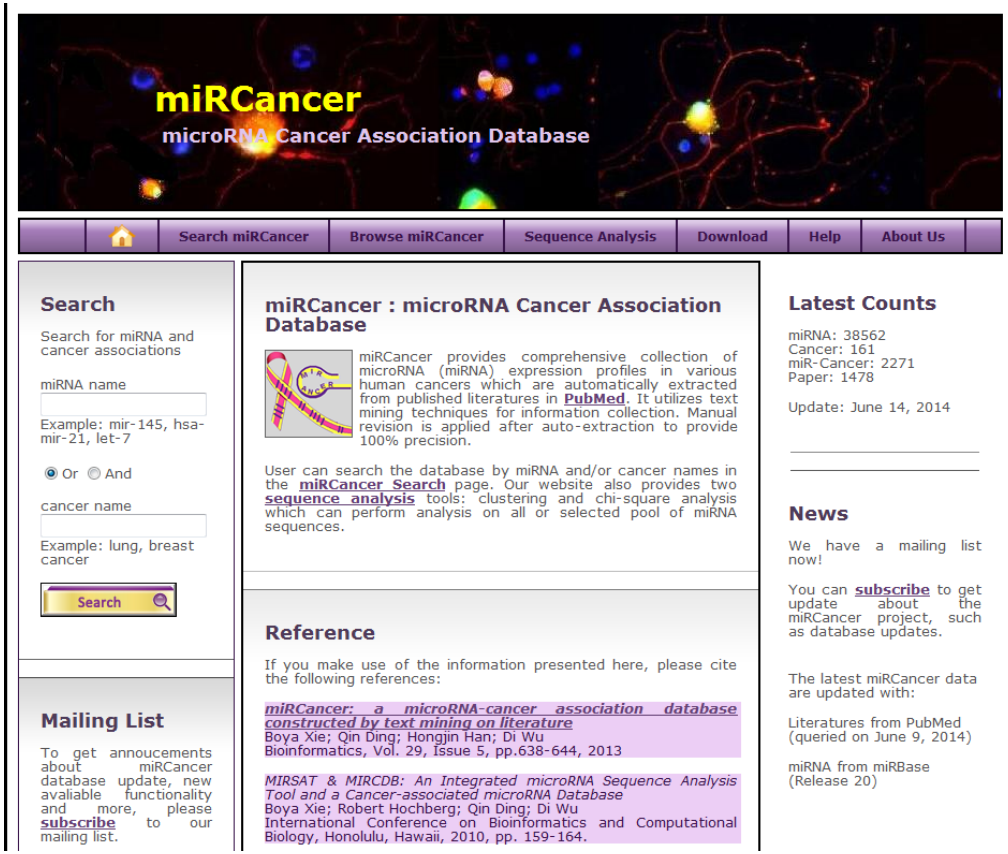
(A) direct binding (B) positive regulation of translation(C) direct interaction with promoter sequences (miR-373); (D) direct agonism of receptors (E) direct interaction with protein

MicroRNAs as Targets for Anticancer Drug Therapy

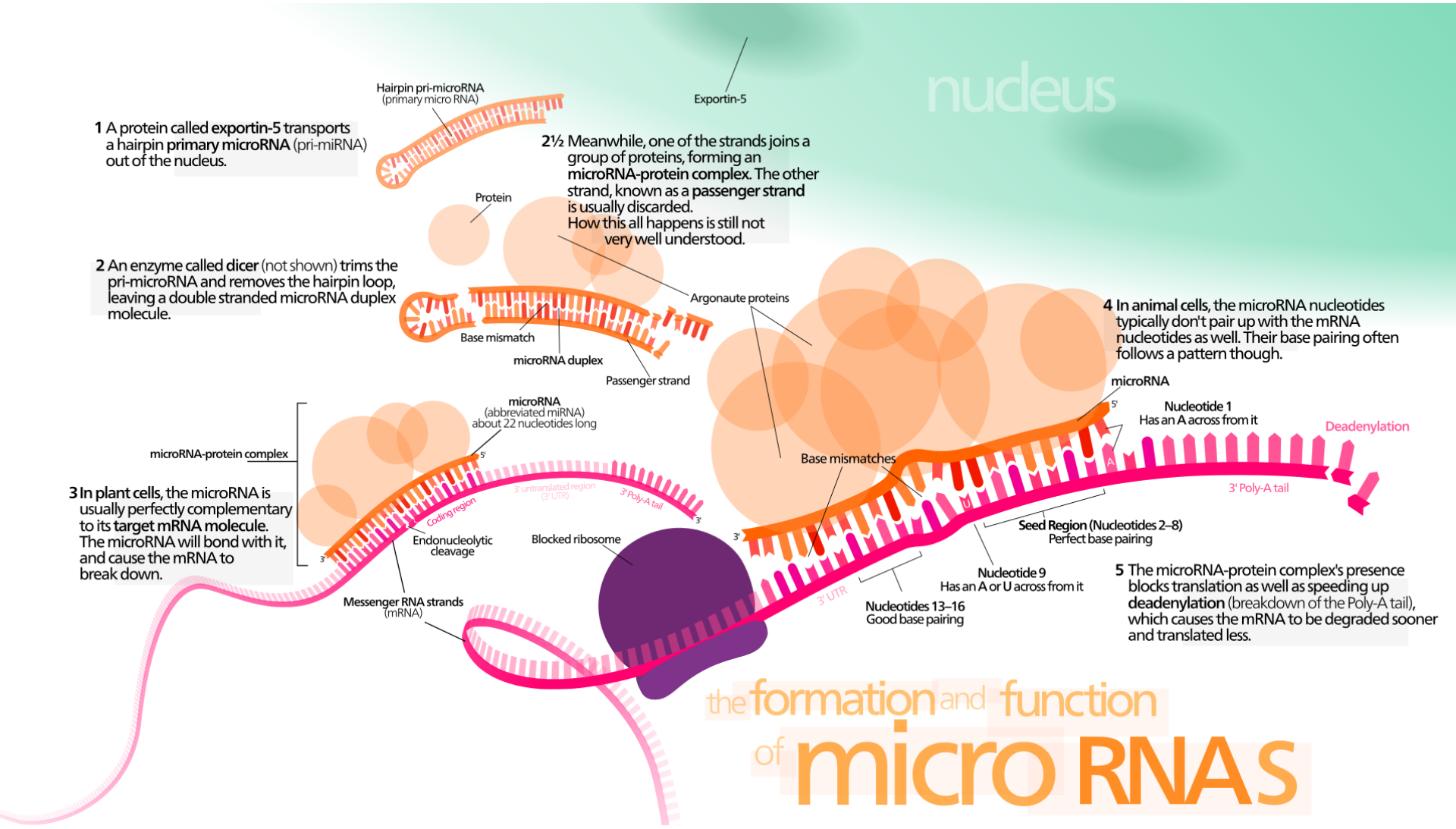


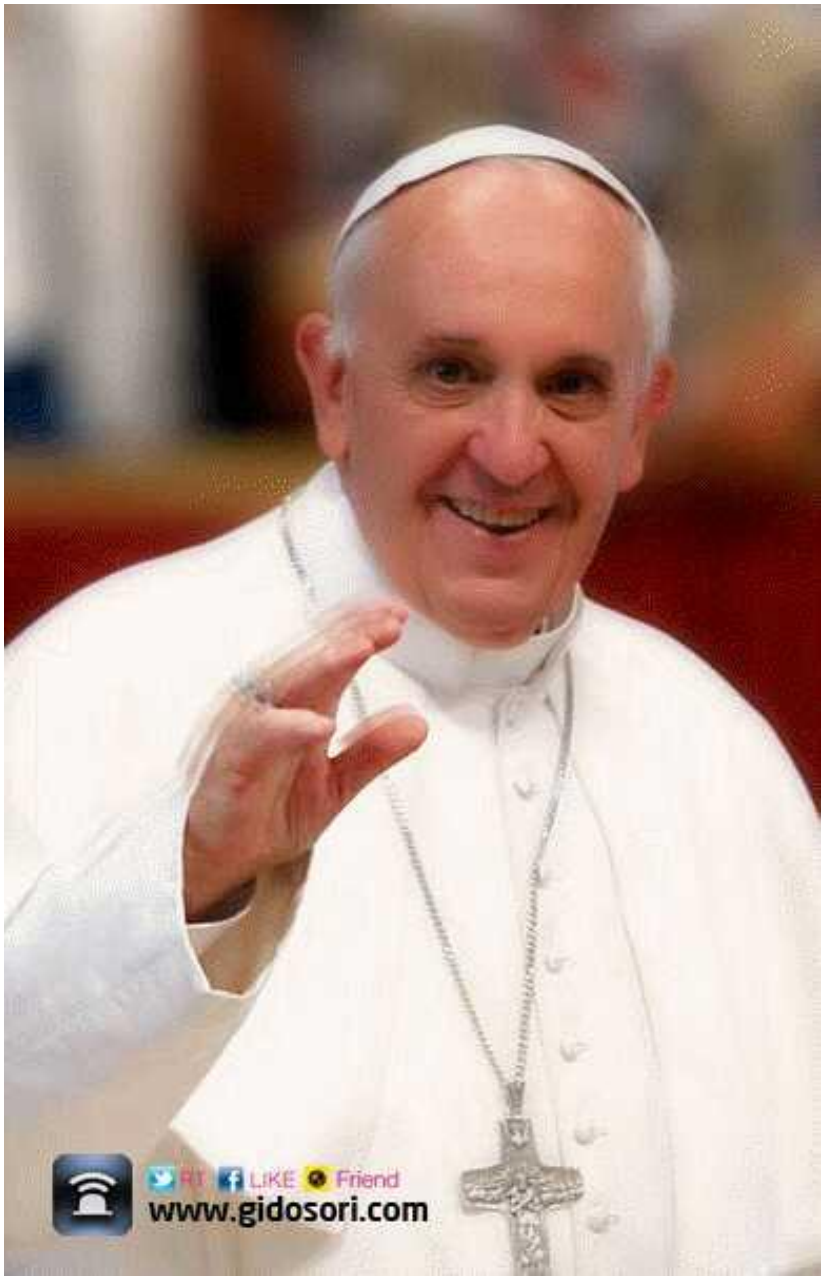
Information and target prediction tools

- Targetscan
- miRBase
- Argonaute



http://mircancer.ecu.edu/


nucleus
Hairpin pri-microRNA
(primary micro RNA)
Exportin-5
1 A protein called exportin-5 transports a hairpin primary microRNA (pri-miRNA) out of the nucleus.
2½ Meanwhile, one of the strands joins a group of proteins, forming an microRNA-protein complex. The other strand, known as a passenger strand is usually discarded.
How this all happens is still not very well understood.
Protein
2 An enzyme called dicer (not shown) trims the pri-microRNA and removes the hairpin loop, leaving a double stranded microRNA duplex molecule.
Argonaute proteins
Base mismatch
microRNA duplex
Passenger strand
4 In animal cells, the microRNA nucleotides typically don't pair up with the mRNA nucleotides as well. Their base pairing often follows a pattern though.
microRNA
(abbreviated miRNA)
about 22 nucleotides long
microRNA-protein complex
3 In plant cells, the microRNA is usually perfectly complementary to its target mRNA molecule. The microRNA will bond with it, and cause the mRNA to break down.
5'
3'
Coding region
3' untranslated region
(3' UTR)
3' Poly-A tail
Endonucleolytic
cleavage
Blocked ribosome
Messenger RNA strands
(mRNA)
microRNA
5'
Nucleotide 1
Has an A across from it
Deadenylation
Base mismatches
A
3' Poly-A tail
Seed Region (Nucleotides 2–8)
Perfect base pairing
3'
3' UTR
Nucleotide 9
Has an A or U across from it
Nucleotides 13–16
Good base pairing
5 The microRNA-protein complex's presence blocks translation as well as speeding up deadenylation (breakdown of the Poly-A tail), which causes the mRNA to be degraded sooner and translated less.
the formation and function
of microRNAs



Viva Papa!
Gracias a la vida