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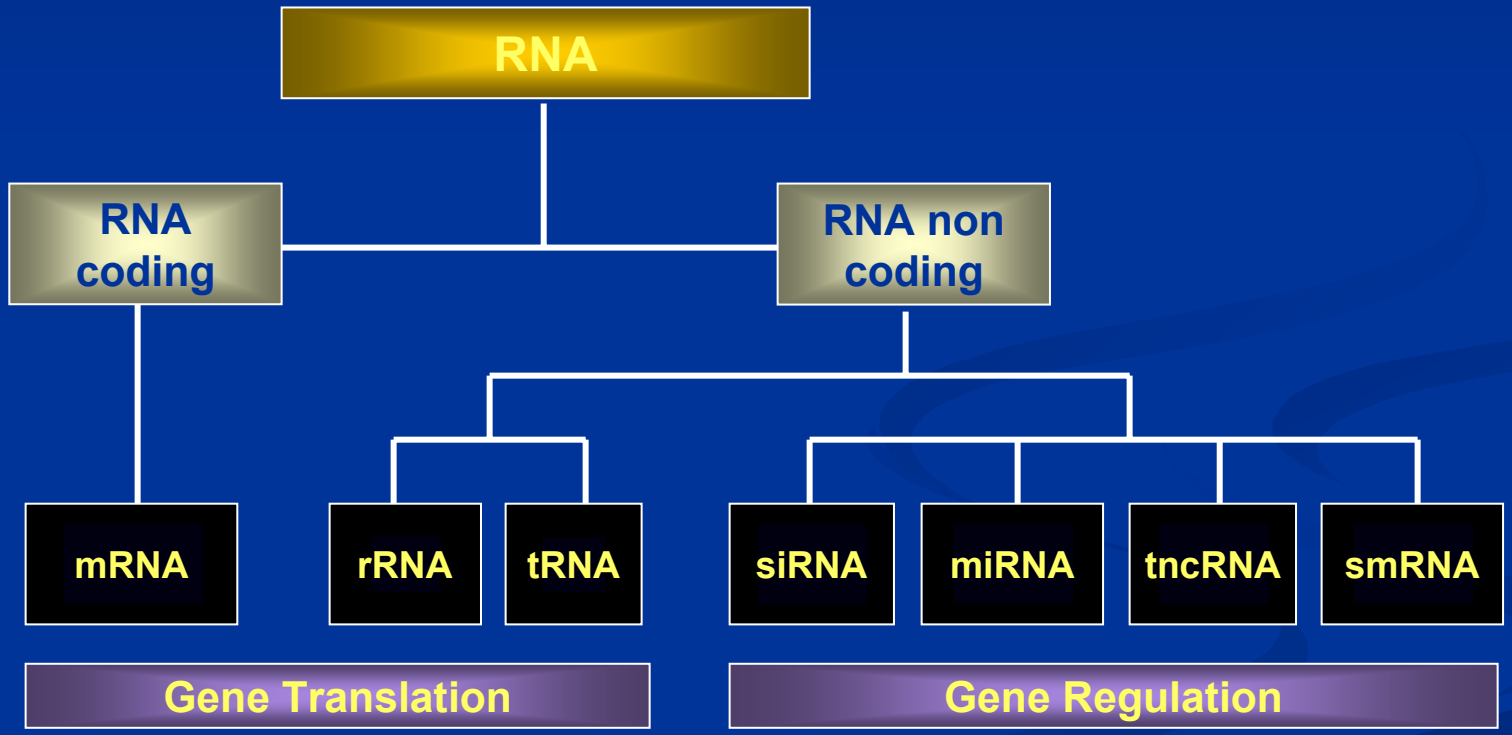
MicroRNA e tumorigenesi: diagnosi, prognosi e terapia

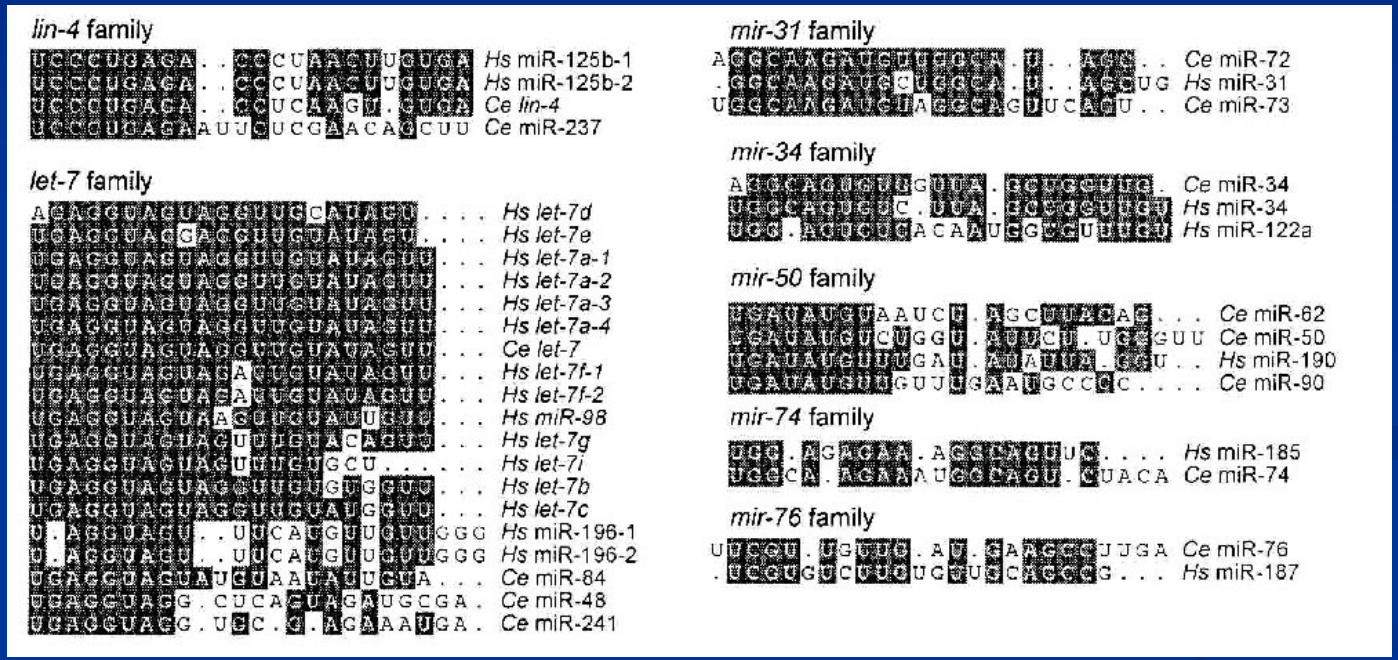
Massimo Negrini

Dipartimento di Medicina Sperimentale e Diagnostica
e Centro Interdipartimentale per la Ricerca sul Cancro
Università di Ferrara

miRNA is a new class of genes encoding short RNAs able to regulate gene expression



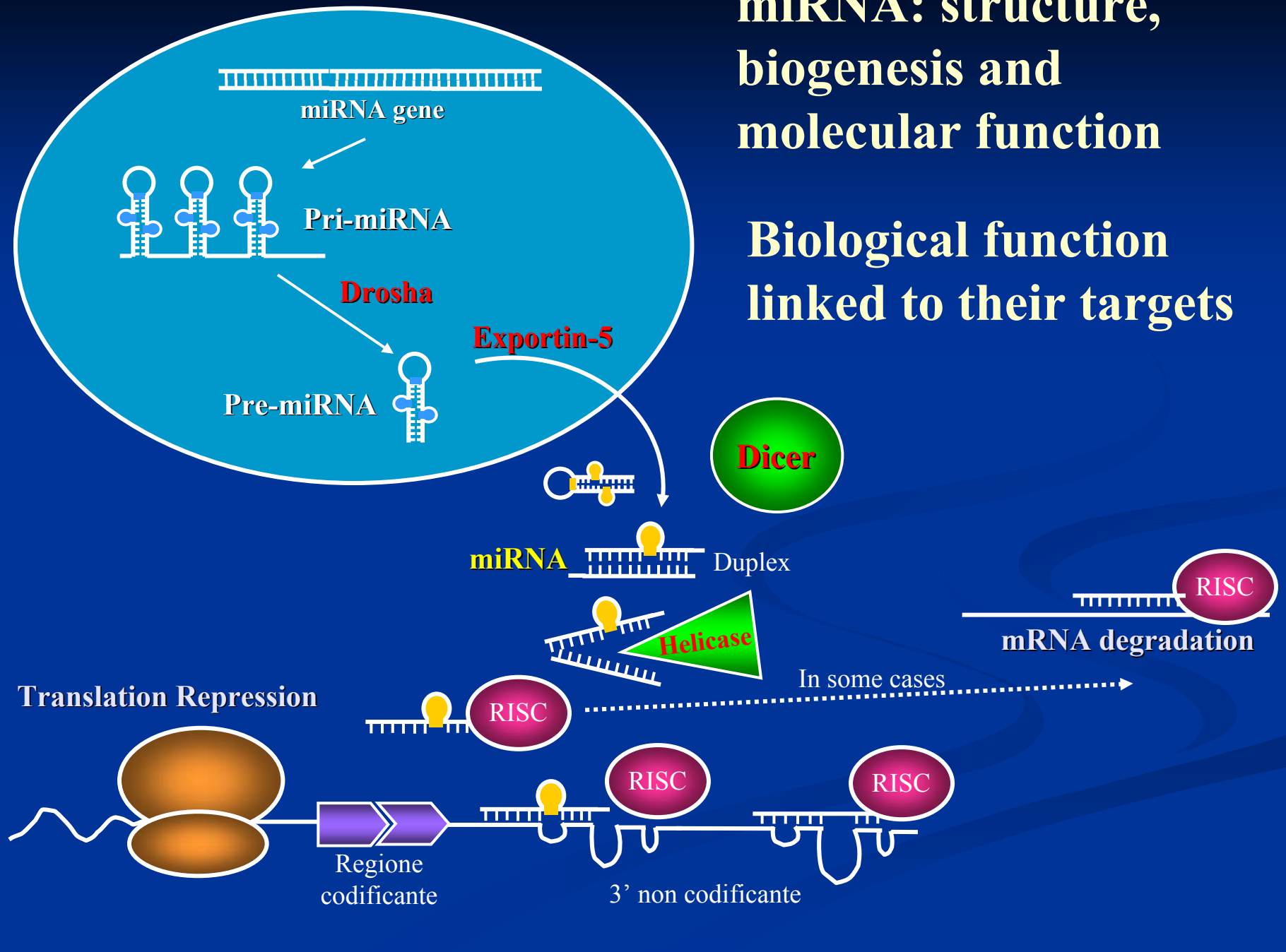
Evolutionary Conservation of miRNA sequences



Lim et al. compared the identified miRNA sequences from *C. elegans* to the human genome, and found that over 1/3 of these genes had homologs in humans.

miRNA: structure, biogenesis and molecular function

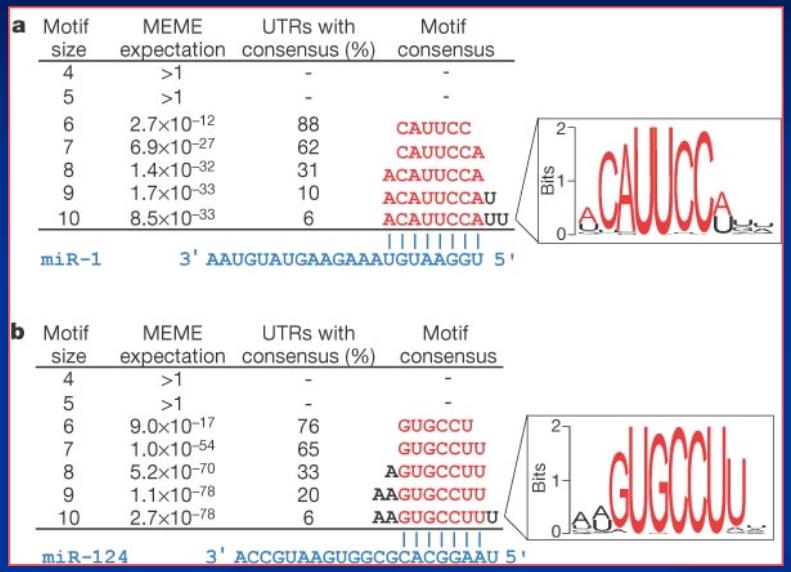
Biological function linked to their targets



Target genes by computational analyses

Many programs claim to discover miRNA targets in mammals:

- miRanda
- DIANA-MicroT
- TargetScan
- PicTar



They all use:

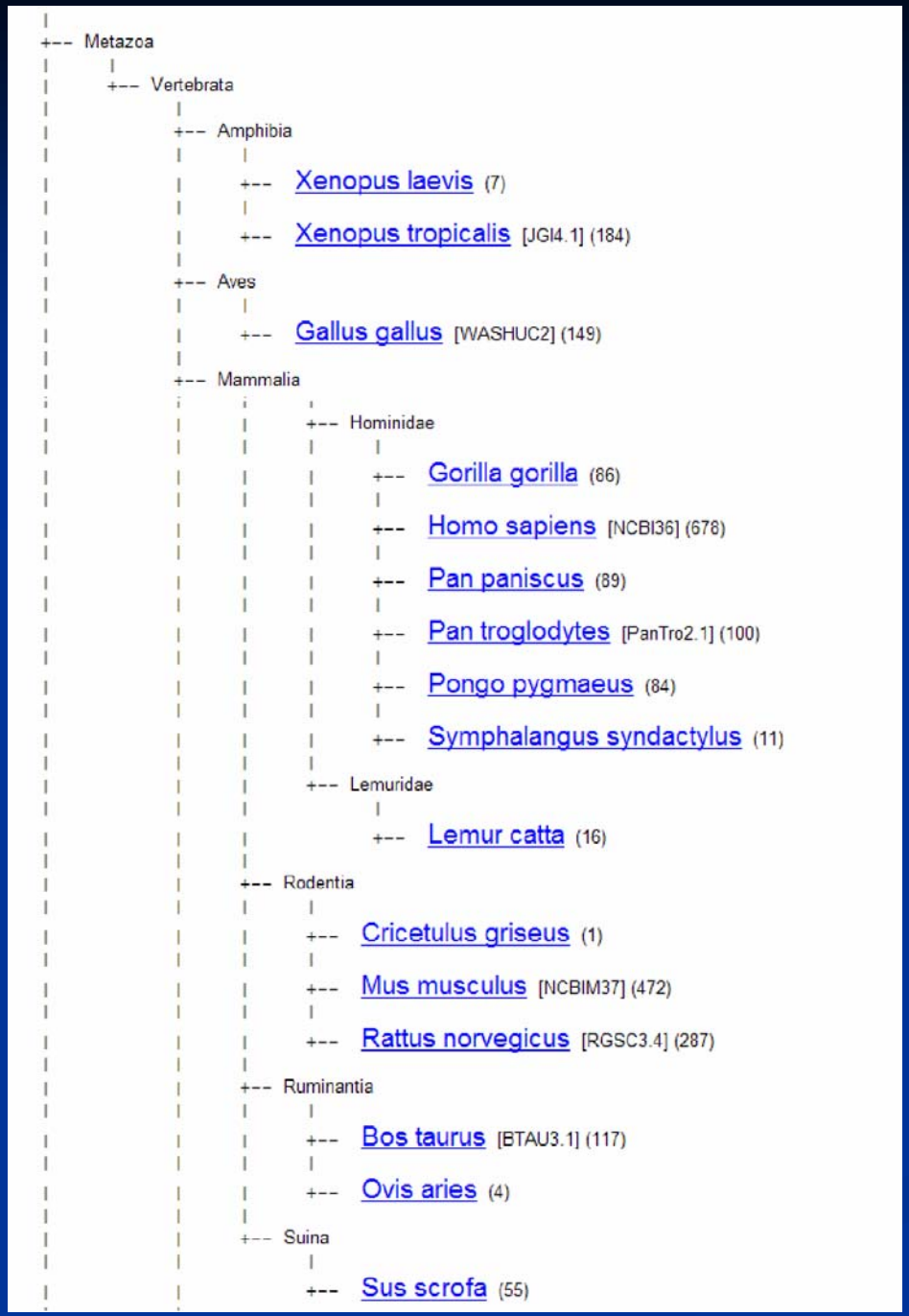
(i) the presence of the miRNA homology region in the 3' UTR of target gene;

(ii) the precise annealing of seed sequence;

(iii) filogenetic conservation of the target regions in the 3'UTR.

- Some, but not all methods consider the role of homology with 3' region of miR and the role of mRNA secondary structure that might hide target sequence

More than 100 targets per miRNA are predicted.
How reliable is the prediction ? Experimental validation required



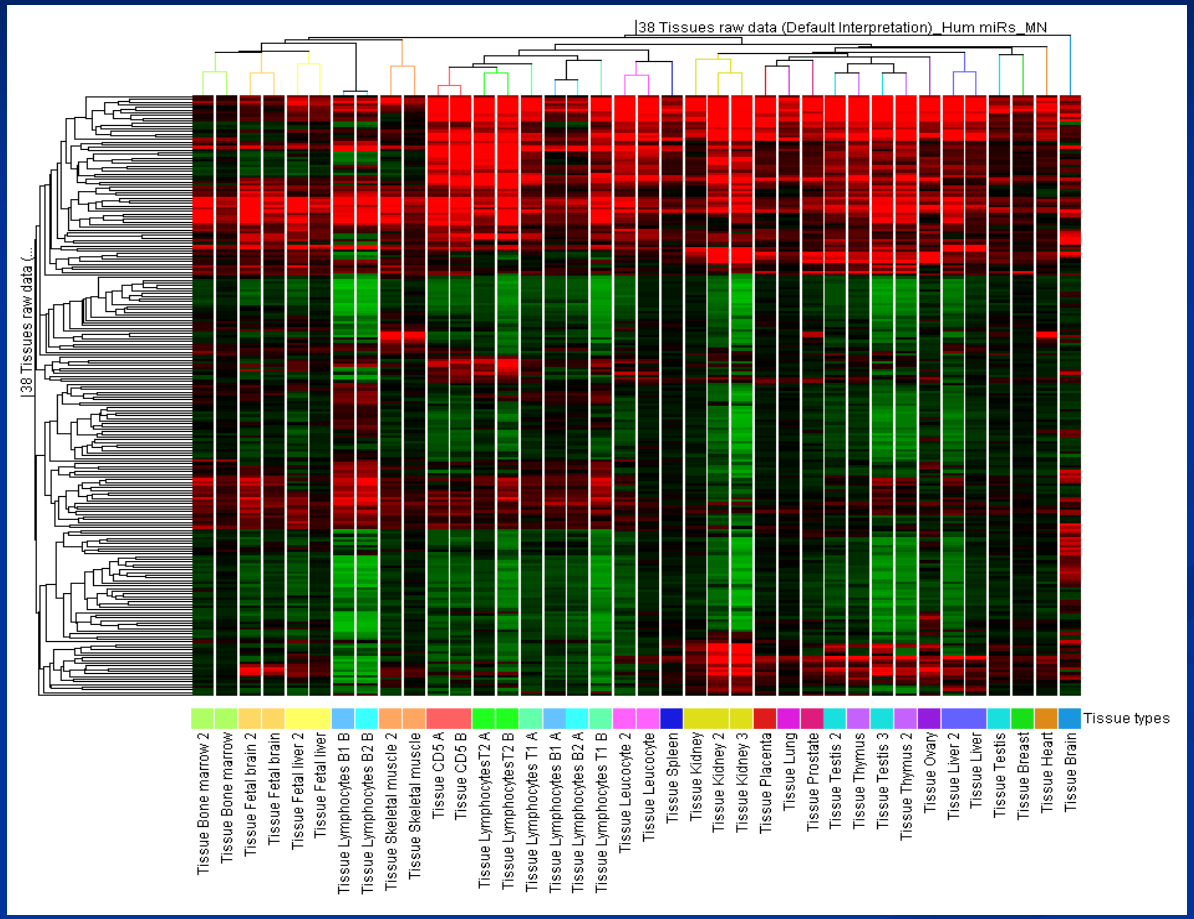
How many microRNAs are presently known ?

miRNA registry

If we assume about 100 mRNA targets recognized by each miRNA, about 70.000 targets are expected

Under a simple Poisson distribution, we should expect about 90% of the known 25.000 human genes regulated by the 678 miRNAs

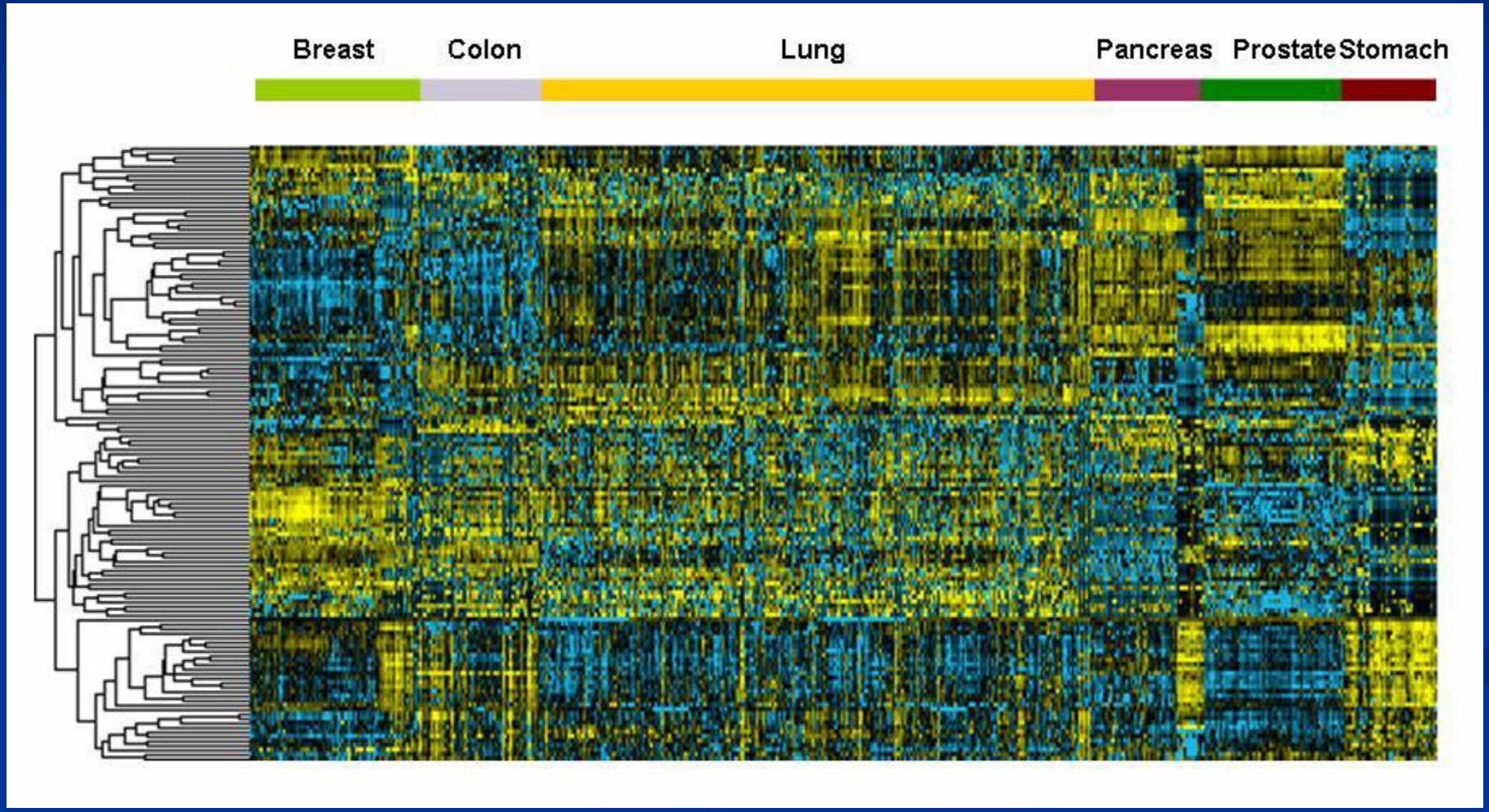
Consistency of Tissue specific expression of human miRNAs



Expressed miRNA:mRNA of each tissue may combine in ways that may generate different biological outcomes

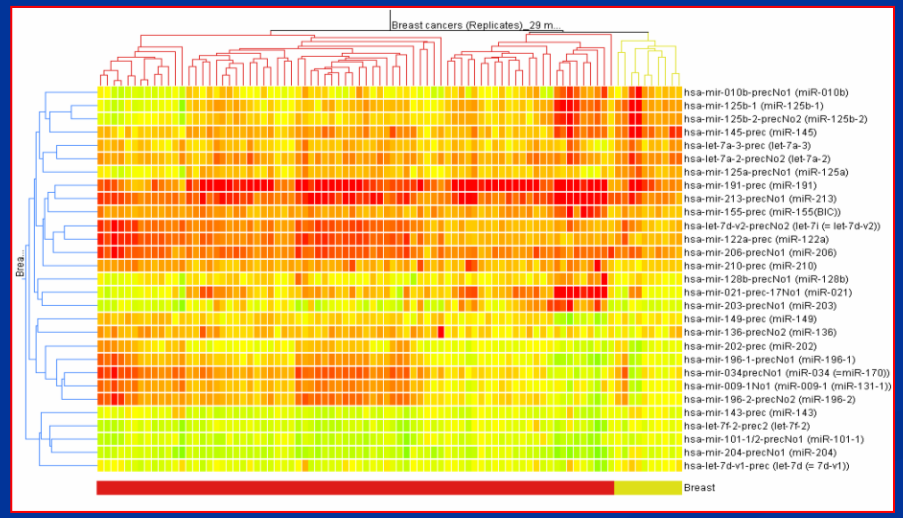
microRNAs are widely involved in human cancer

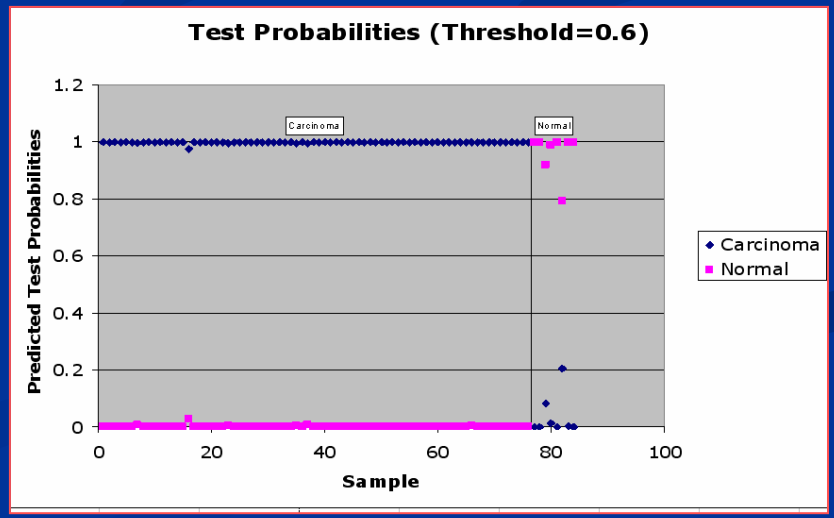
miRNA signatures in human cancer



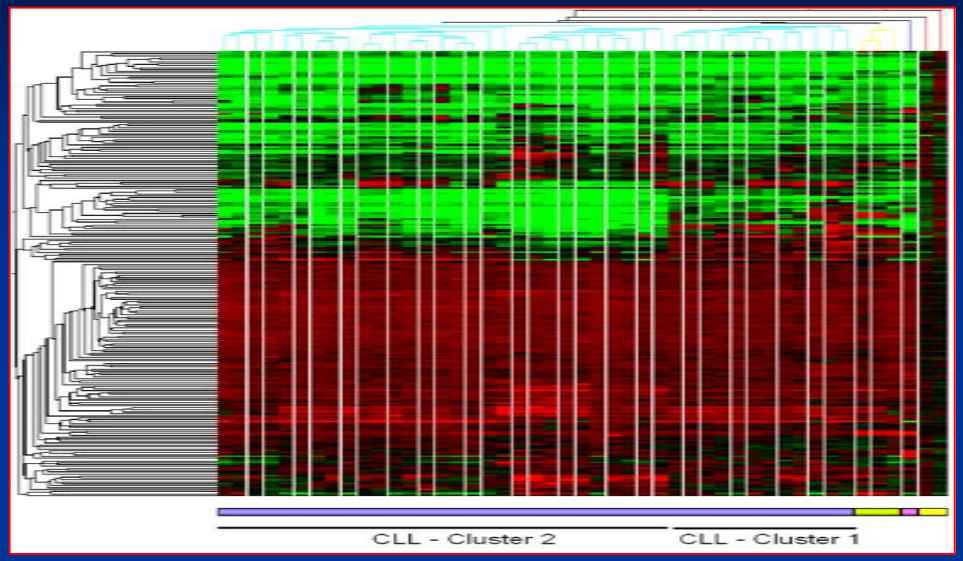
A miRNA expression signature of human breast cancer

miRNA name	Median expression		ANOVA[a] Probability	SVM prediction strength[b]	PAM score[c]		Chromos map
	Cancer	Normal			Cancer	Normal	
miR-009-1	1.36	1.01	0,0091	8.05	0.011	-0.102	1q22
miR-010b	1.11	1.7	0,0449	8.7	-0.032	0.299	2q31
miR-021	1.67	1.08	0,0047	10.2	0.025	-0.235	17q23.2
miR-034	1.67	1.09	0,0106	8.05	0.011	-0.106	1p36.22
miR-102 (miR-29b)	1.36	1.14	> 0.10	8.92	0.000	-0.004	1q32.2-32.3
miR-123 (miR-126)	0.92	1.13	0,0940	9.13	-0.015	0.138	9q34
miR-125a	1.2	1.73	0,0033	8.99	-0.04	0.381	19q13.4
miR-125b-1	1.3	2.87	0,0265	14.78	-0.096	0.915	11q24.1
miR-125b-2	1.26	2.63	0,0233	17.62	-0.106	1.006	21q11.2
miR-140-as	0.93	1.1	0,0695	11.01	-0.005	0.05	16q22.1
miR-145	1.52	3.61	0,0040	12.93	-0.158	1.502	5q32-33
miR-155(BIC)	1.75	1.37	0,0012	10.92	0.003	-0.03	21q21
miR-194	0.96	1.09	> 0.10	11.12	-0.025	0.234	1q41
miR-204	0.78	0.89	0,0022	8.1	-0.015	0.144	9q21.1
miR-213	3.72	2.47	0,0108	9.44	0.023	-0.22	1q31.3-q32.1

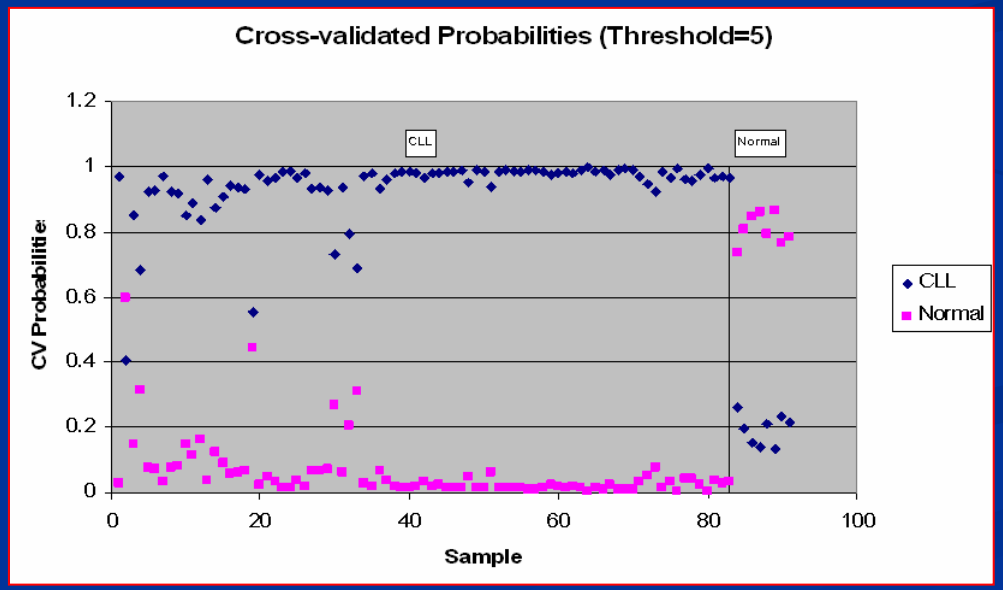




A predictive miRNA signature for CLL

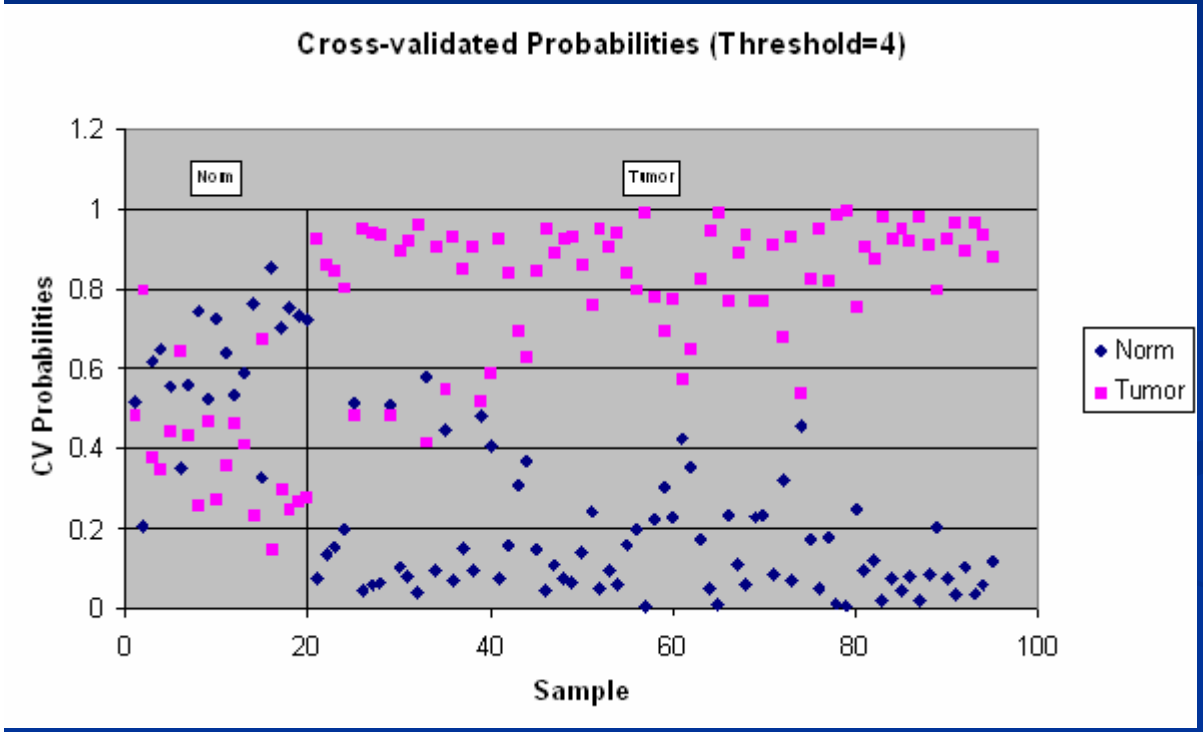


CLUSTER analysis



PREDICTION analysis

Eight microRNAs compose the signature that predicts lung cancer status.



MicroRNA	Lung Cancer Expression
hsa-mir-021	Up
hsa-mir-210	Up
hsa-mir-205	Up
hsa-mir-030d	Down
hsa-mir-123	Down
hsa-mir-191	Up
hsa-mir-030a	Down
hsa-mir-155	Up

Ten fold cross-validation was performed to assess misclassification error. Most divergent miRNAs in the two classes are listed on the top.

miRNA commonly deregulated in human neoplasms

Up-regulated miRs may act as oncogenes

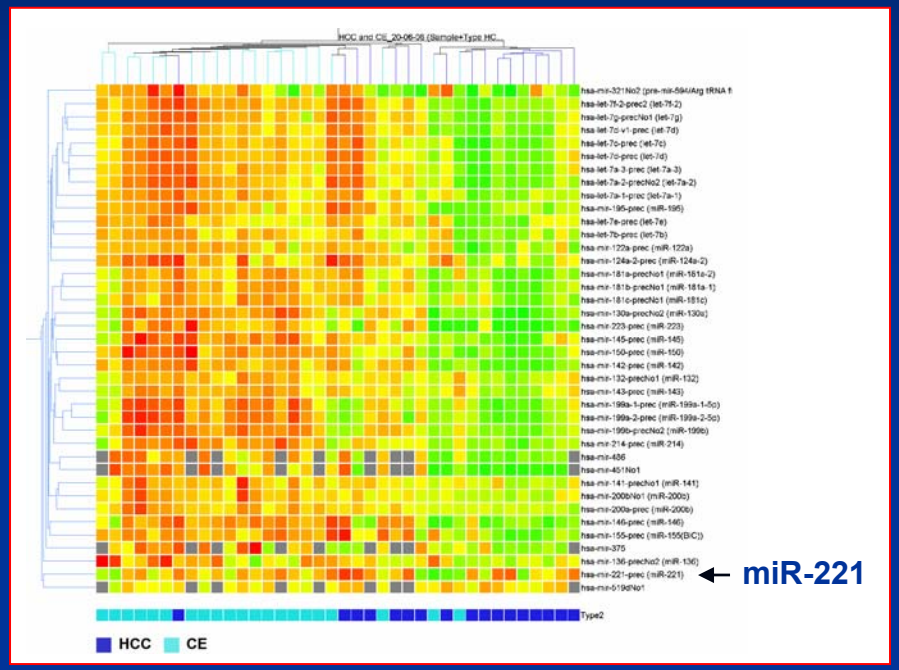
Down-regulated miRs may act as tumor suppressors

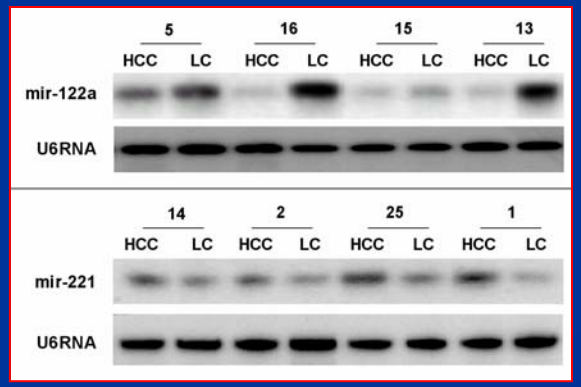
HOW ?

miRNA	Tipo di neoplasia	Espressione rispetto al tessuto normale
let-7a	Mammella	Sotto espresso
let-7a	Polmone	Sotto espresso
let-7e	Colon	Sotto espresso
mir-009-3	Colon	Sotto espresso
mir-010b	Mammella	Sotto espresso
mir-017	Colon	Sovra espresso
mir-020	Colon	Sovra espresso
mir-021	Mammella	Sovra espresso
mir-021	Colon	Sovra espresso
mir-021	Polmone	Sovra espresso
mir-023a	Colon	Sovra espresso
mir-023b	Colon	Sovra espresso
mir-024-1	Colon	Sovra espresso
mir-026a	B-CLL	Sovra espresso
mir-030a	Polmone	Sotto espresso
mir-030b	Polmone	Sotto espresso
mir-030d	Polmone	Sotto espresso
mir-103	Colon	Sovra espresso
mir-106	Colon	Sovra espresso
mir-107	Colon	Sovra espresso
mir-123	Polmone	Sotto espresso
mir-125a	Mammella	Sotto espresso
mir-125b-1	Mammella	Sotto espresso
mir-125b-2	Mammella	Sotto espresso
mir-128a	Colon	Sotto espresso
mir-138-2	Colon	Sotto espresso
mir-145	Mammella	Sotto espresso
mir-145	Colon	Sotto espresso
mir-145	Polmone	Sotto espresso
mir-150	B-CLL	Sovra espresso
mir-155	Linfomi	Sovra espresso
mir-155	Mammella	Sovra espresso
mir-155	Polmone	Sovra espresso
mir-185	B-CLL	Sotto espresso
mir-191	Colon	Sovra espresso
mir-210	B-CLL	Sotto espresso
mir-210	Polmone	Sovra espresso
mir-212	Colon	Sotto espresso
mir-213	B-CLL	Sotto espresso
mir-213	Mammella	Sovra espresso
mir-221	Colon	Sovra espresso
mir-223	Colon	Sovra espresso

Some microRNAs can act as oncogenes

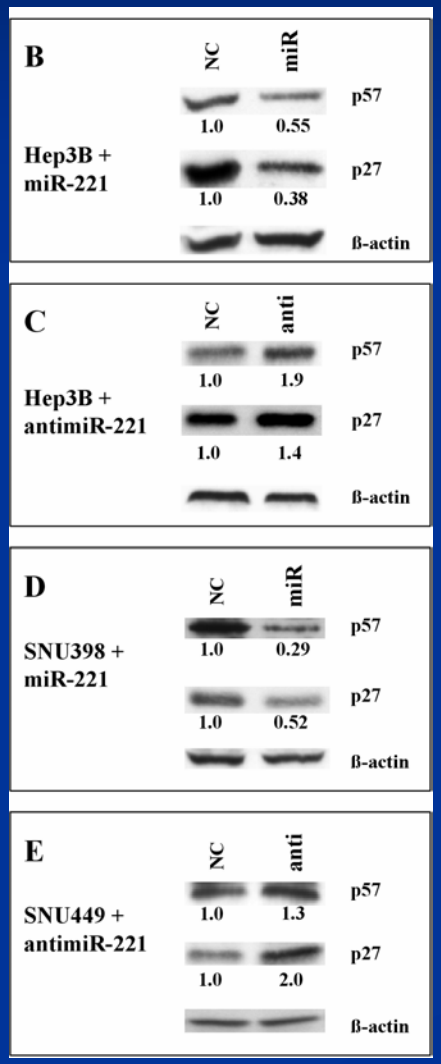
miR-221 is up-regulated in human hepatocellular carcinoma and in several other human cancers



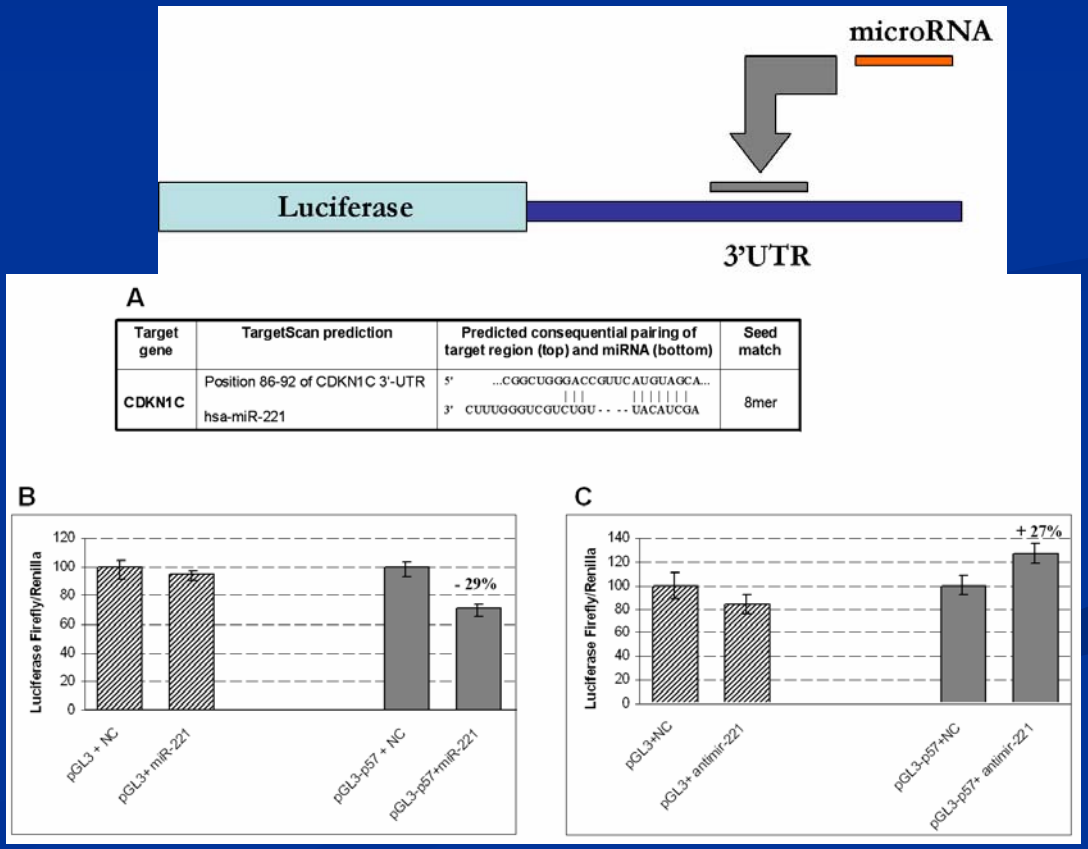


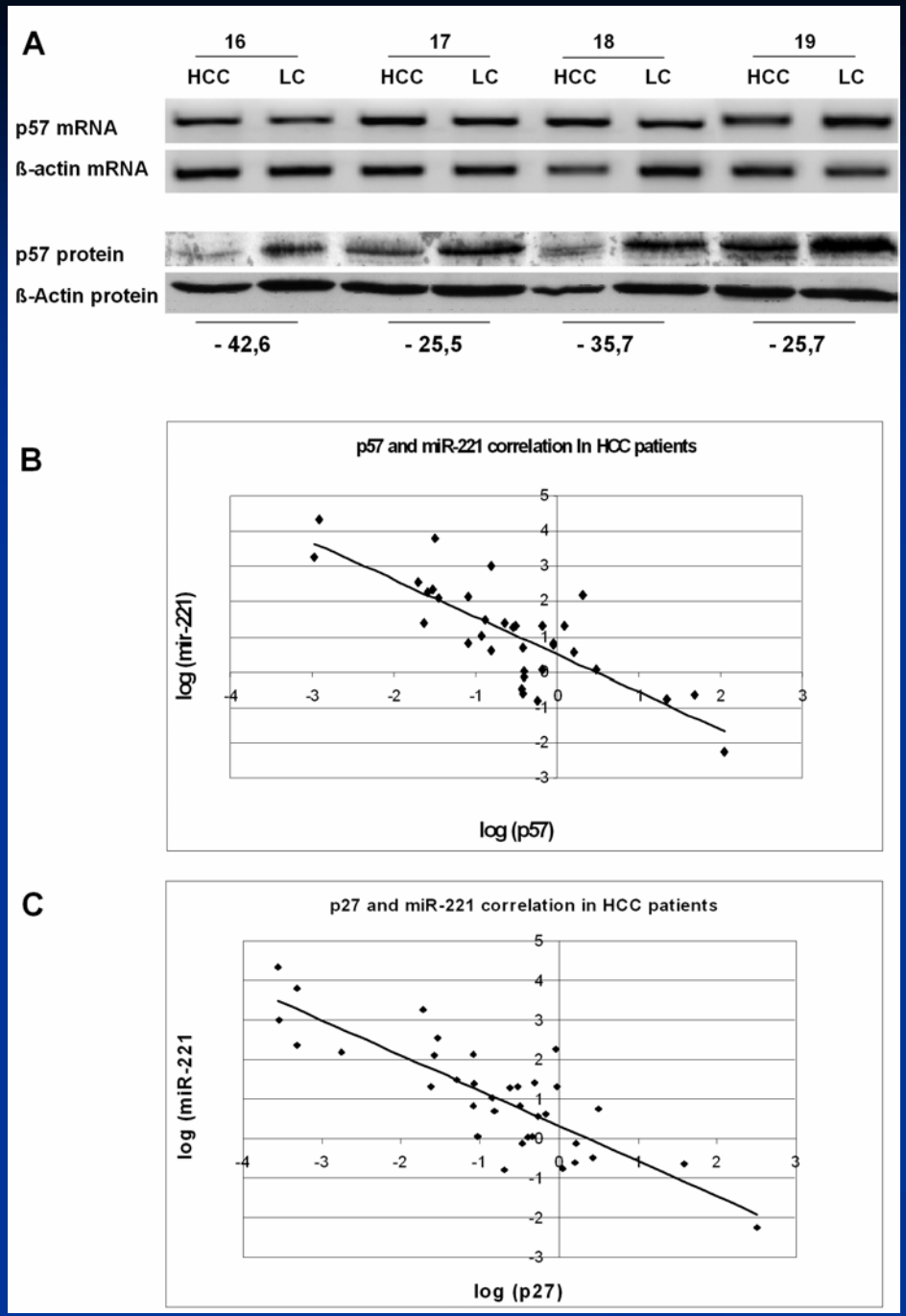
In addition to hepatocellular carcinomas, miR-221 is also up-regulated in breast, colon, pancreas, stomach, thyroid, bladder carcinomas and in glioblastomas

The CDK inhibitors p27 and p57 are both controlled by miR-221



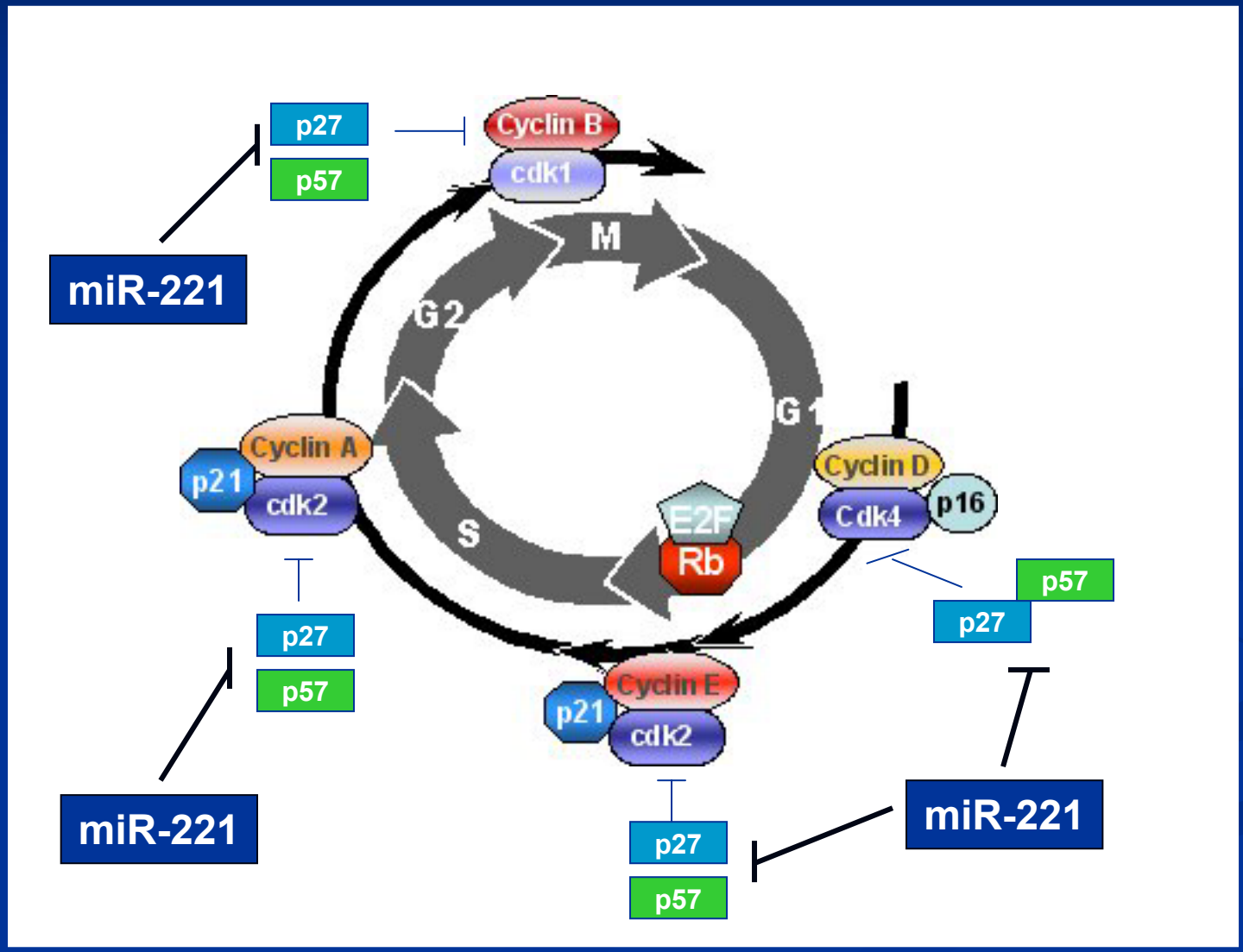
Luciferase assay using miR-221 or anti-miR-221





The CDK inhibitors p27 and p57 are controlled by miR-221 in primary HCCs

Cell cycle is controlled by CDKs and their inhibitors

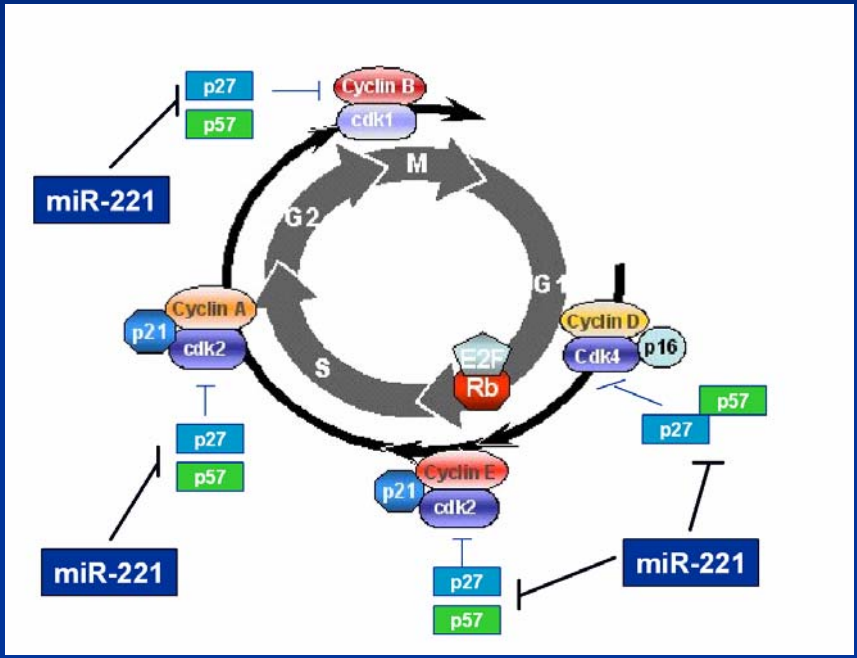


The up-regulation of miR-221

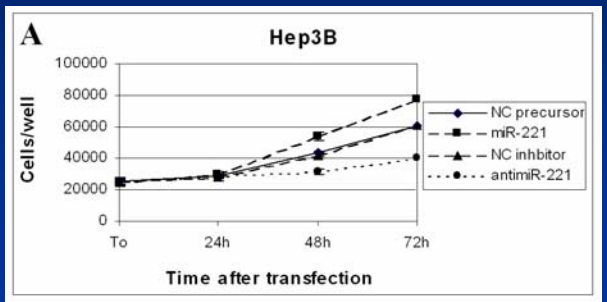
may block two important cell cycle inhibitors

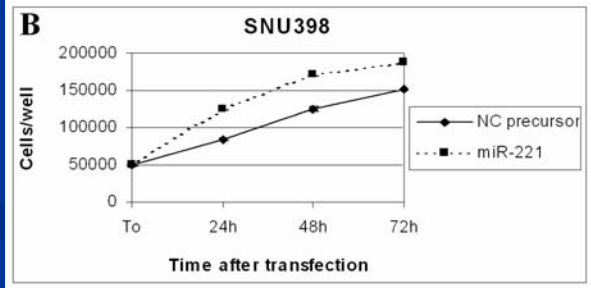
and promote cell cycle progression

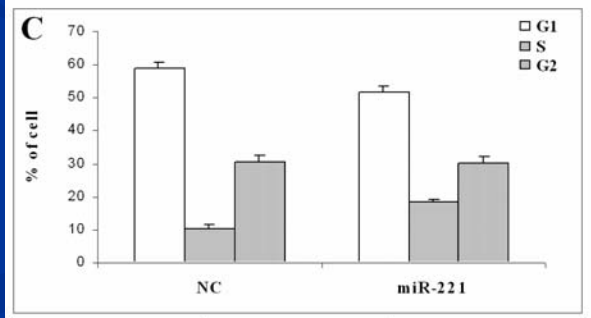
Oncogenic action of miR-221



The up-regulation of miR-221 blocks two important cell cycle inhibitors and promote cell cycle progression







	NC (%)	miR-221 (%)
G1	59,0	51,5
S	10,4	18,1
G2/M	30,6	30,4

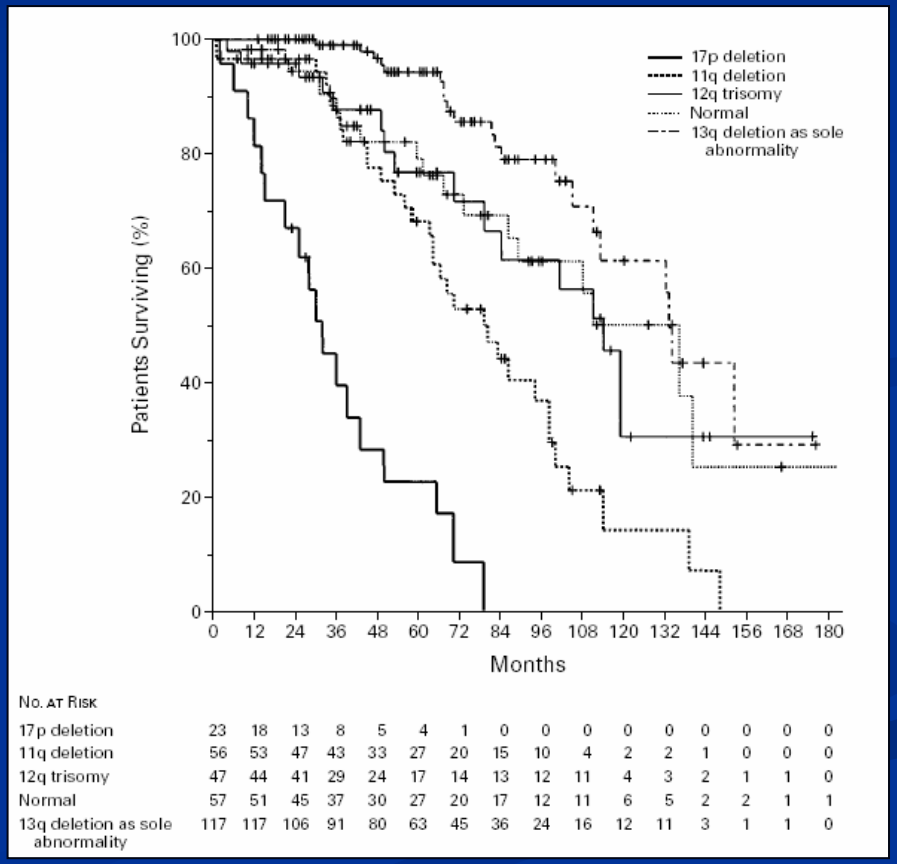
Some microRNAs can act as tumor suppressors

Deletion 13q14 is the most common chromosomal aberration associated with human CLL

TABLE 1. INCIDENCE OF CHROMOSOMAL ABNORMALITIES IN 325 PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.

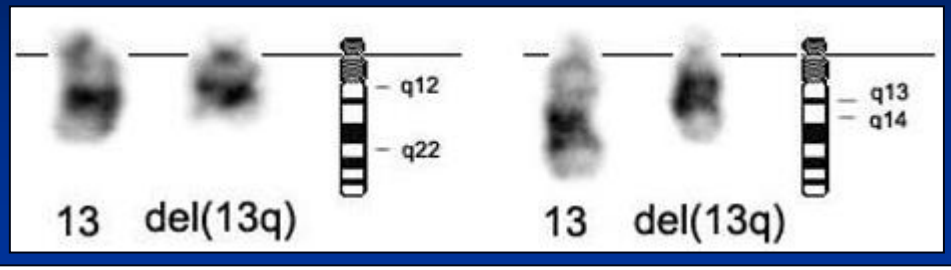
ABERRATION	NO. OF PATIENTS (%)*
13q deletion	178 (55)
11q deletion	58 (18)
12q trisomy	53 (16)
17p deletion	23 (7)
6q deletion	21 (6)
8q trisomy	16 (5)
t(14q32)	12 (4)
3q trisomy	9 (3)
Clonal abnormalities	268 (82)
Normal karyotype	57 (18)

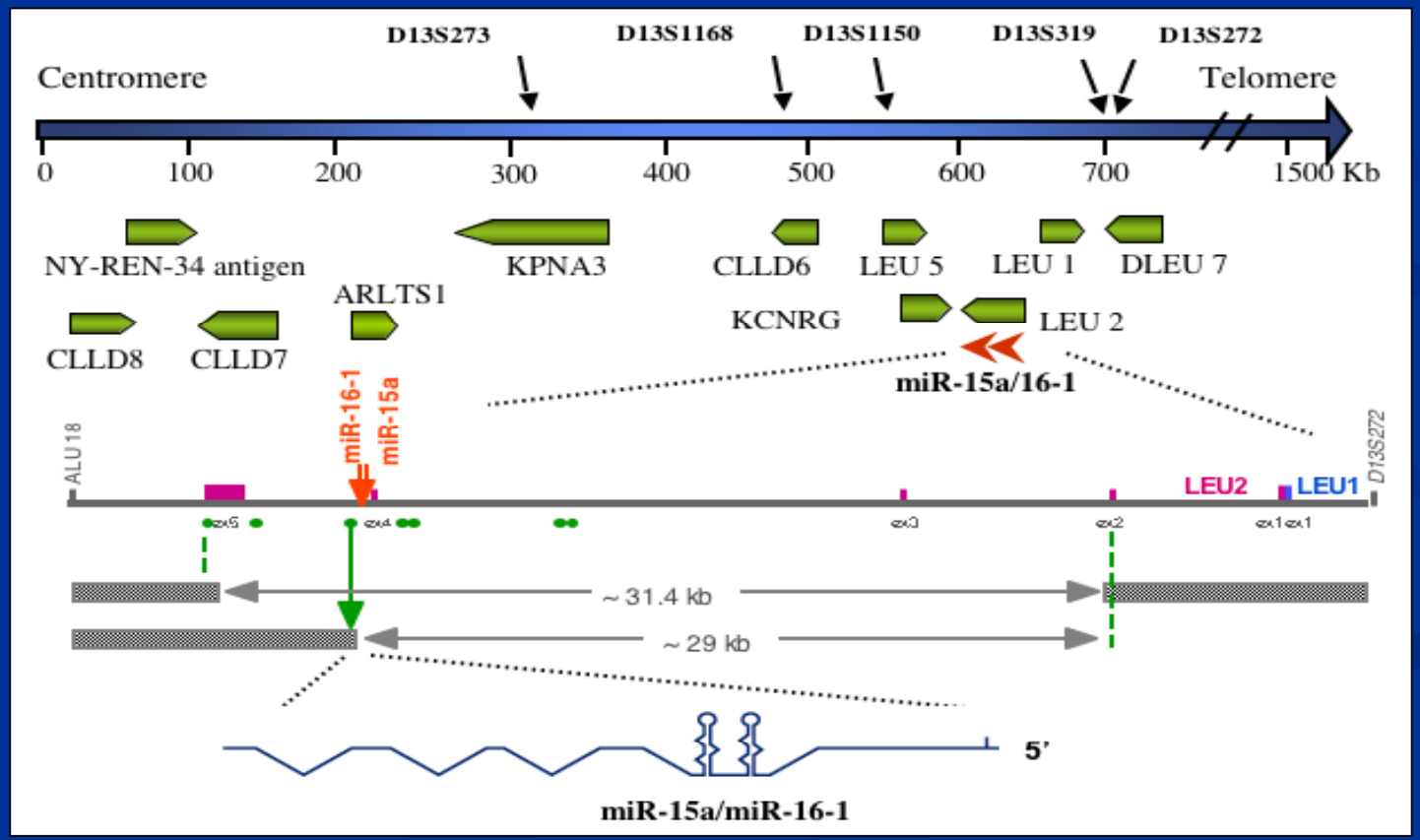
*One hundred seventy-five patients had one aberration, 67 had two aberrations, and 26 had more than two aberrations.



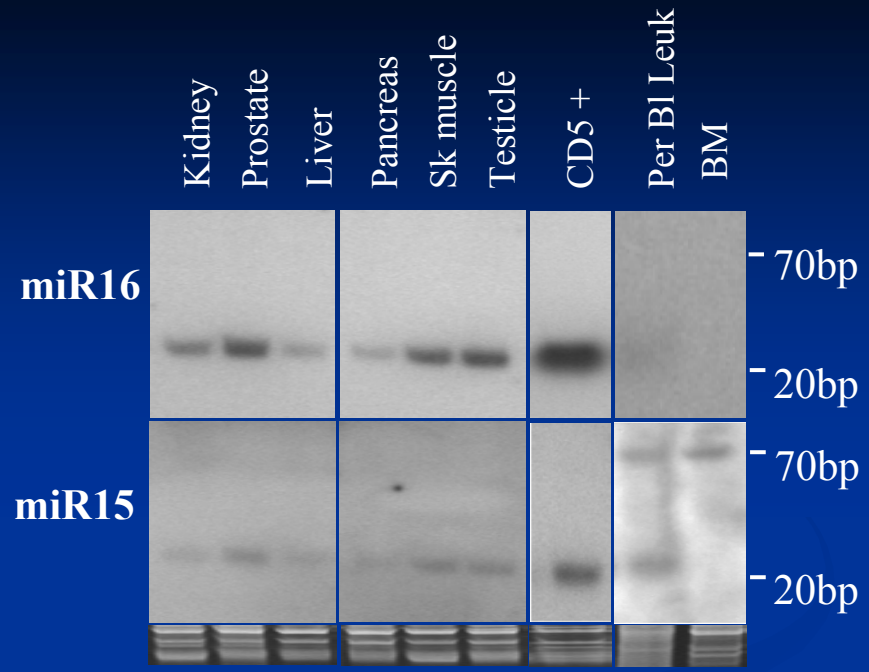
Dohener et al. N Engl J Med, 2000

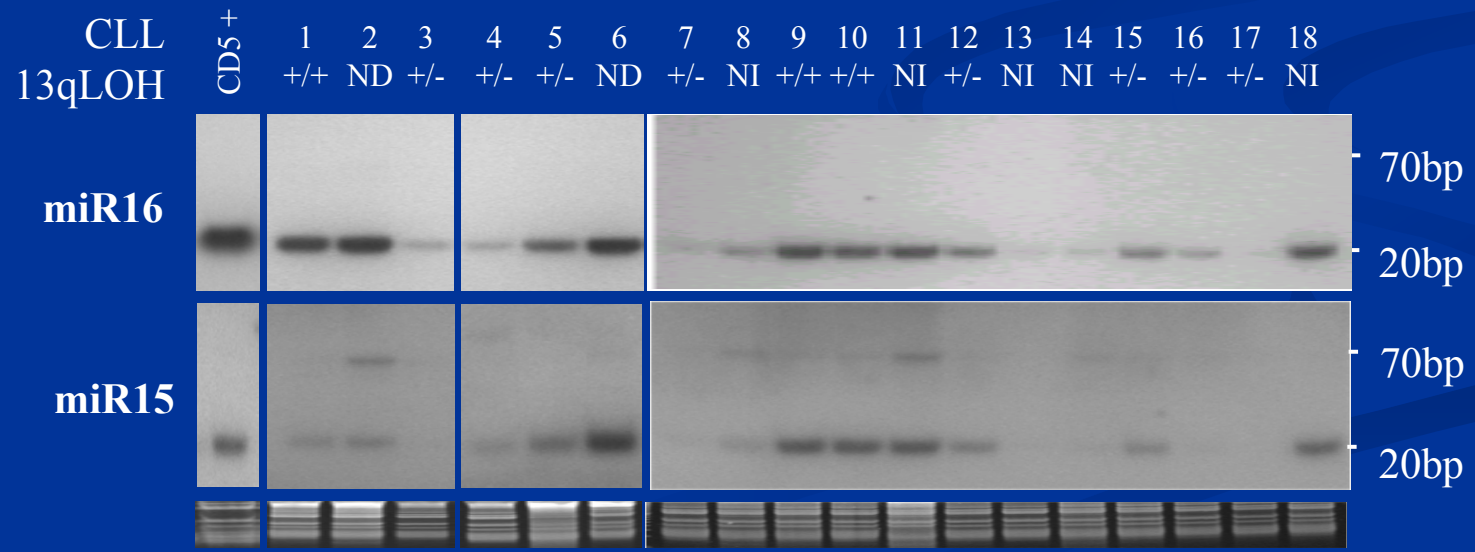
mir-15 and mir-16 are within the minimal region of 13q deletion in human CLL





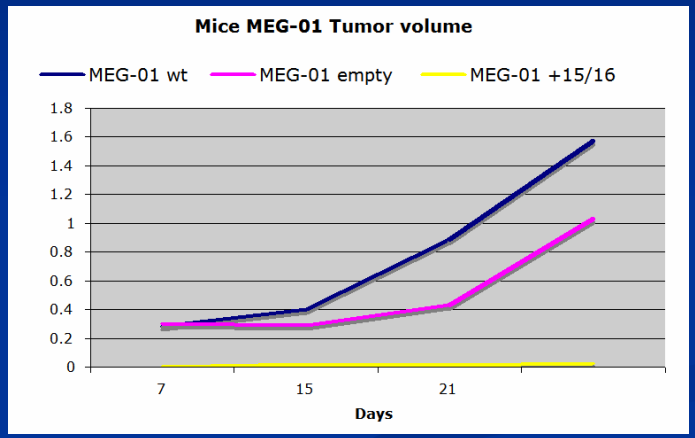
Expression of mir-15 and mir-16 in normal tissues and human CLLs

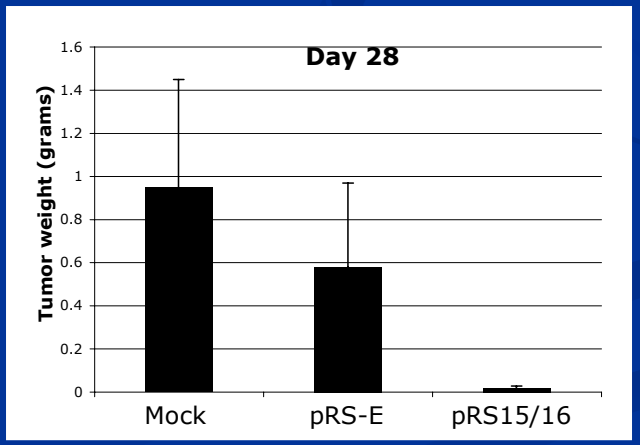




miR-15a/16-1 suppress tumorigenicity

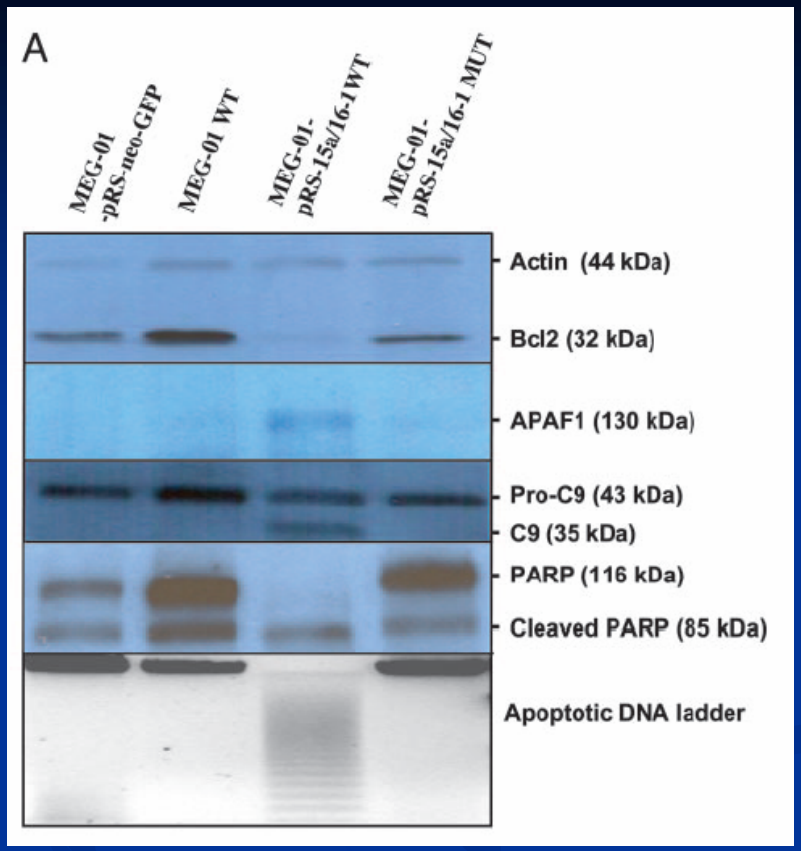
MEG-01 is a megakaryocytic leukemic cell line that lacks miR15/16 because of homozygous deletions

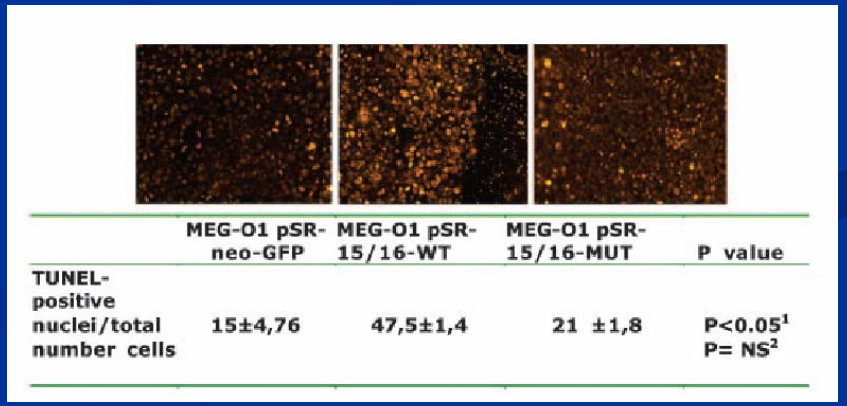




Effect of miR-15a / miR-16-1 restoration in MEG-01 human leukemic cells

Expression of miR-15/16 promotes apoptosis in the megakaryocytic cell line MEG-01

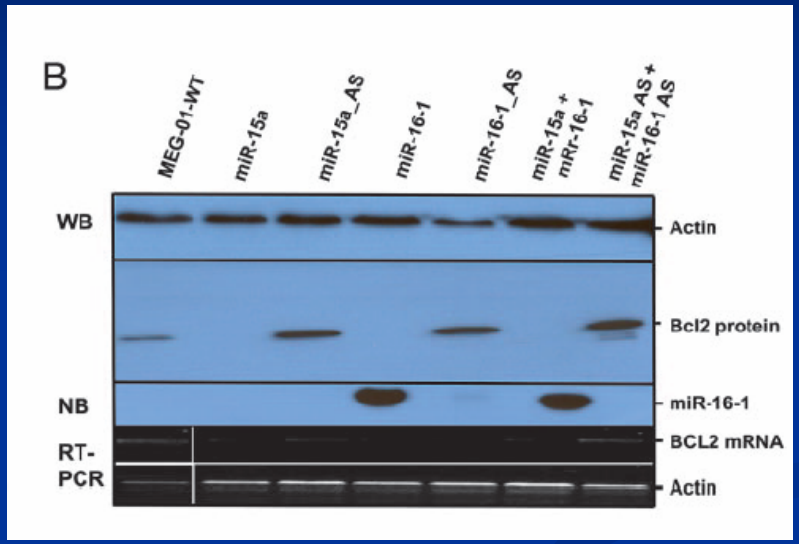




	MEG-O1 pSR-neo-GFP	MEG-O1 pSR-15/16-WT	MEG-O1 pSR-15/16-MUT	P value
TUNEL-positive nuclei/total number cells	15±4,76	47,5±1,4	21 ±1,8	P<0.05[1] P= NS[2]

Cimmino et al., PNAS 2005

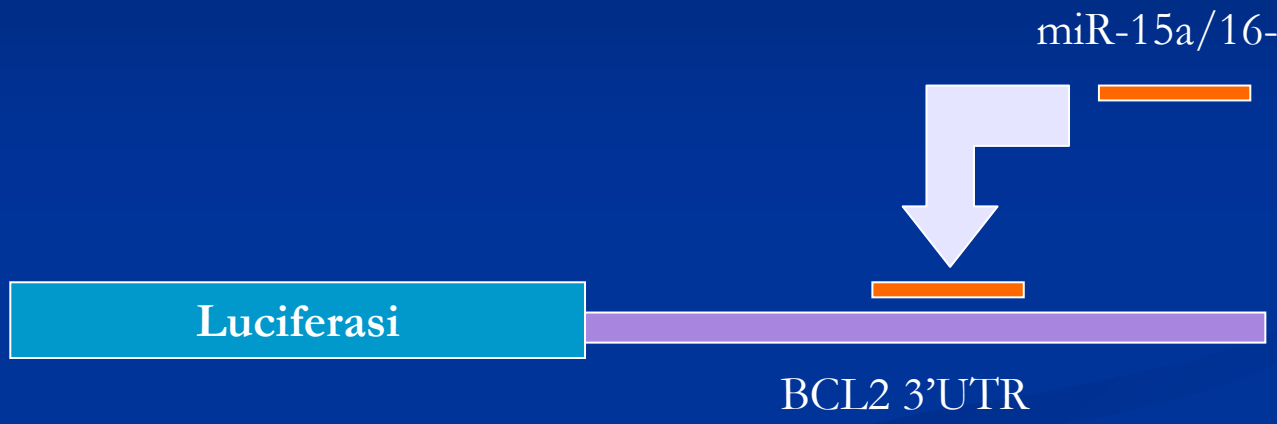
BCL2 is target of mir-15/16

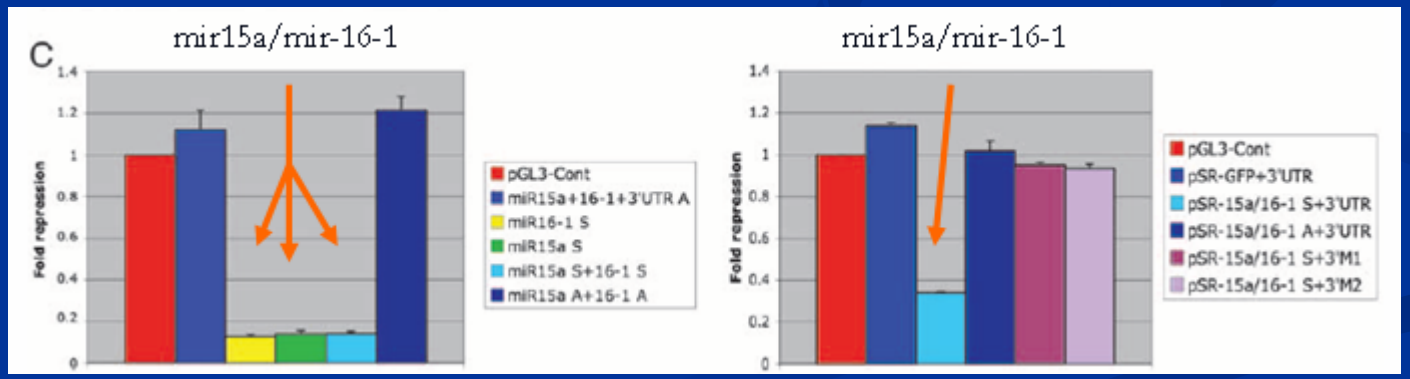


These results suggest that loss of miR-15a/miR-16-1 in human CLL may lead to the activation of the anti-apoptotic oncogene BCL2 in human CLL

Cimmino et al., 2005

Luciferase assay confirms that BCL2 3'UTR is targeted by miR-15a and miR-16-1

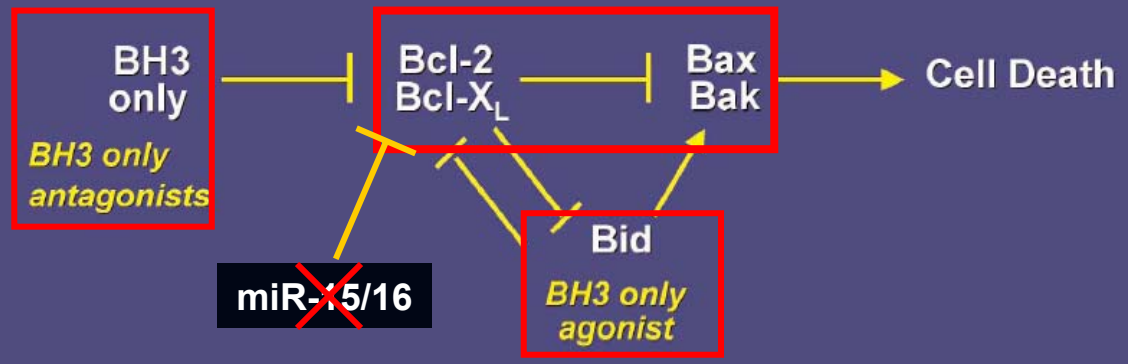


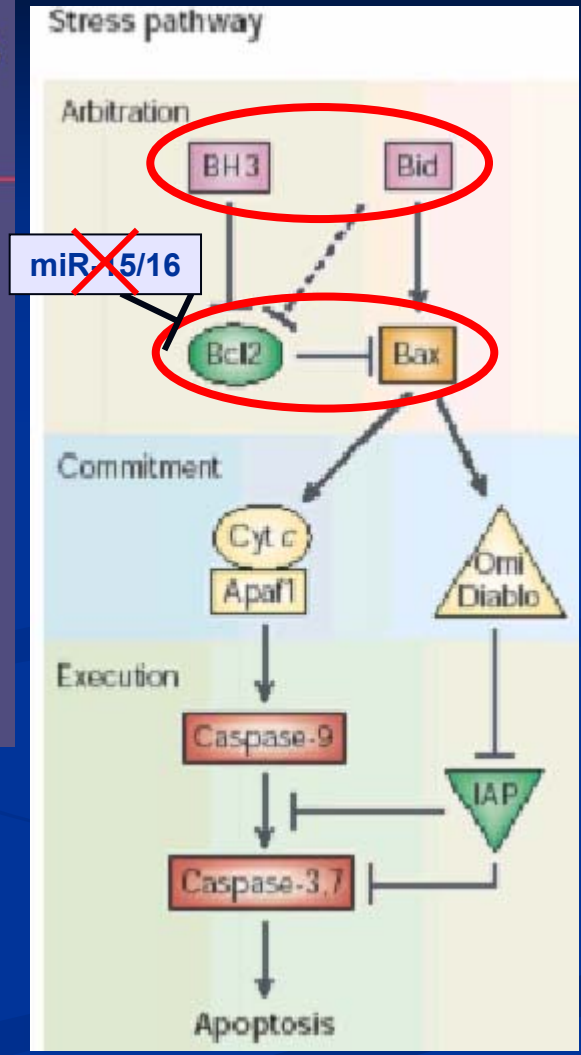


Sense and anti-sense miR-15/16

WT and mutant (5nt del) BCL2 3'UTR

Functional and Physical Interactions of Bcl-2 Family Proteins





The loss of miR-15a/miR-16-1 in human CLL may lead to the activation of the anti-apoptotic oncogene BCL2,

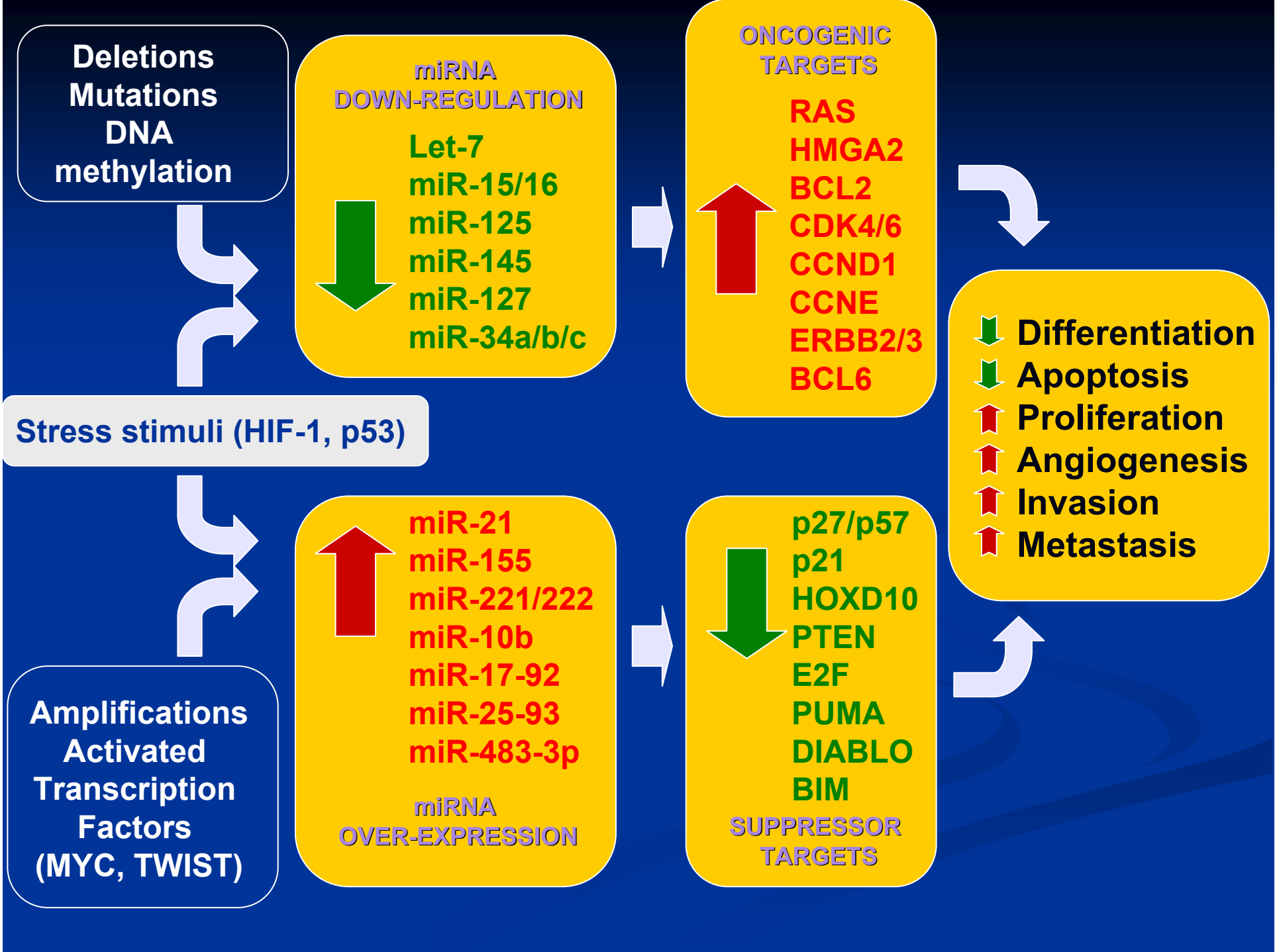
This mechanism may be responsible for the accumulation of a clonal cell population more resistant to cell death stimuli

Nature Medicine, published online 19 October 2008

The *miR-15a–miR-16-1* cluster controls prostate cancer by targeting multiple oncogenic activities

Désirée Bonci[1], Valeria Coppola[1], Maria Musumeci[1], Antonio Addario[1], Raffaella Giuffrida[2], Lorenzo Memeo[2], Leonardo D'Urso[3], Alfredo Pagliuca[1], Mauro Biffoni[1], Catherine Labbaye[1], Monica Bartucci[1], Giovanni Muto[3], Cesare Peschle[1,4] & Ruggero De Maria[1,2]

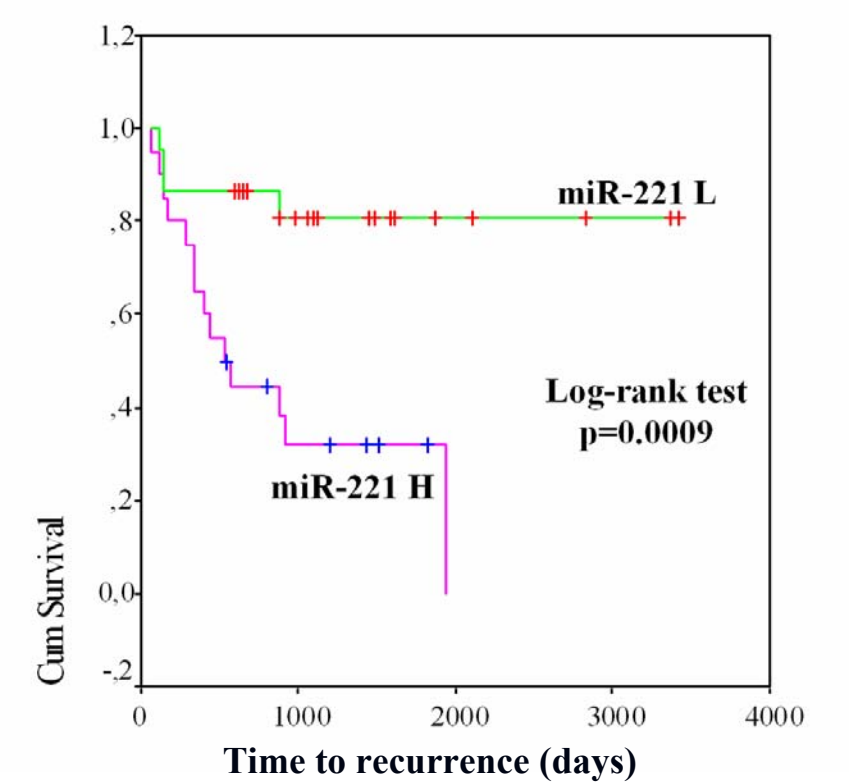
- Delivery of antagomirs specific for miR-15a and miR-16 to normal mouse prostate results in marked hyperplasia, and knockdown of miR-15a and miR-16 promotes survival, proliferation and invasiveness of untransformed prostate cells, which become tumorigenic in immunodeficient NOD-SCID mice.
- restoration of miR-15a and miR-16 expression in prostate cancer cells resulted in dramatic tumor regression.
- cyclin D1 and Wnt3a were shown to be targets of miR-15a and miR-16-1.
- **A single miRNA cluster miR-15 / miR-16 can modulate at least two biological functions relevant in cancer: apoptosis and cell cycle**

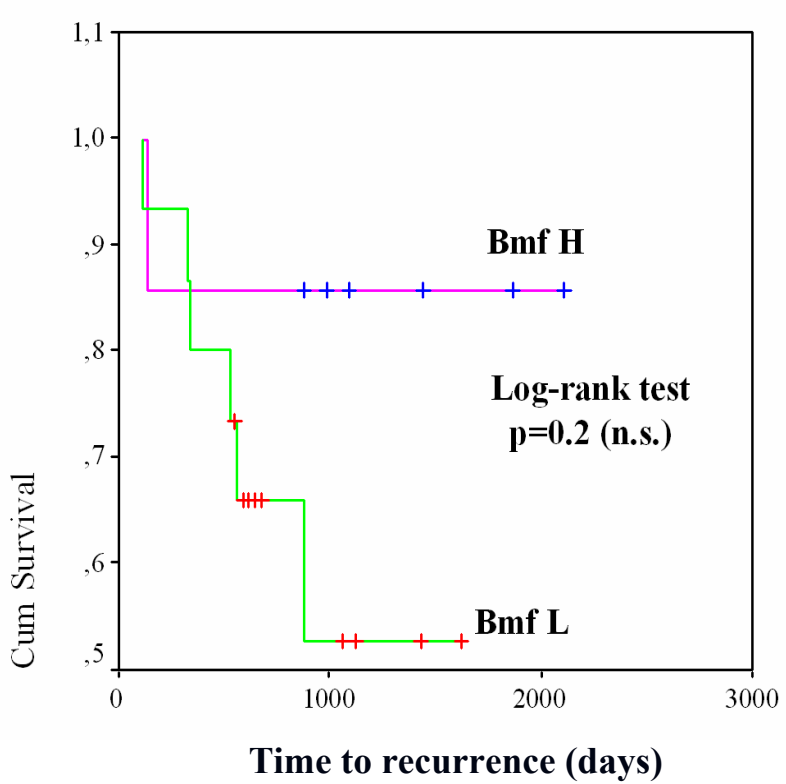

Deletions
Mutations
DNA
methylation
miRNA
DOWN-REGULATION
Let-7
miR-15/16
miR-125
miR-145
miR-127
miR-34a/b/c
ONCOGENIC
TARGETS
RAS
HMGA2
BCL2
CDK4/6
CCND1
CCNE
ERBB2/3
BCL6
Differentiation
Apoptosis
Proliferation
Angiogenesis
Invasion
Metastasis
Stress stimuli (HIF-1, p53)
Amplifications
Activated
Transcription
Factors
(MYC, TWIST)
miR-21
miR-155
miR-221/222
miR-10b
miR-17-92
miR-25-93
miR-483-3p
miRNA
OVER-EXPRESSION
p27/p57
p21
HOXD10
PTEN
E2F
PUMA
DIABLO
BIM
SUPPRESSOR
TARGETS

Early cellular transforming events	**Evading Apoptosis**	miR-15a/16-1 → BCL2 miR-21 → PDCD4 miR-17/92 → E2F, BCL2L11/BIM
	Promoting Cell cycle	miR-15a/16-1 → CCND1 miR-17/92 → CDKN1A/p21 miR-221/222 → CDKN1B/p27 miR-221/222 → CDKN1C/p57
	Independence from external control through the activation of RTKs, RAS, PI3K/AKT signaling	miR-125a/b → ERBB2, ERBB3 miR-199a* → MET miR-1 → MET Let-7 → KRAS, NRAS, HRAS miR-21 → PTEN
Advanced cancer features modulated by interaction with surrounding environment	**Promoting Angiogenesis**	miR-17/92 → CTGF miR-17/92 → TSP1
	Promoting Invasion EMT Metastasis	miR-21 → PDCD4 miR-199a* → MET miR-10b → HOXD10 miR-373 → CD44 miR-520c → CD44 miR-335 miR-126 miR-206 miR-200 → ZEB1/2 miR-210

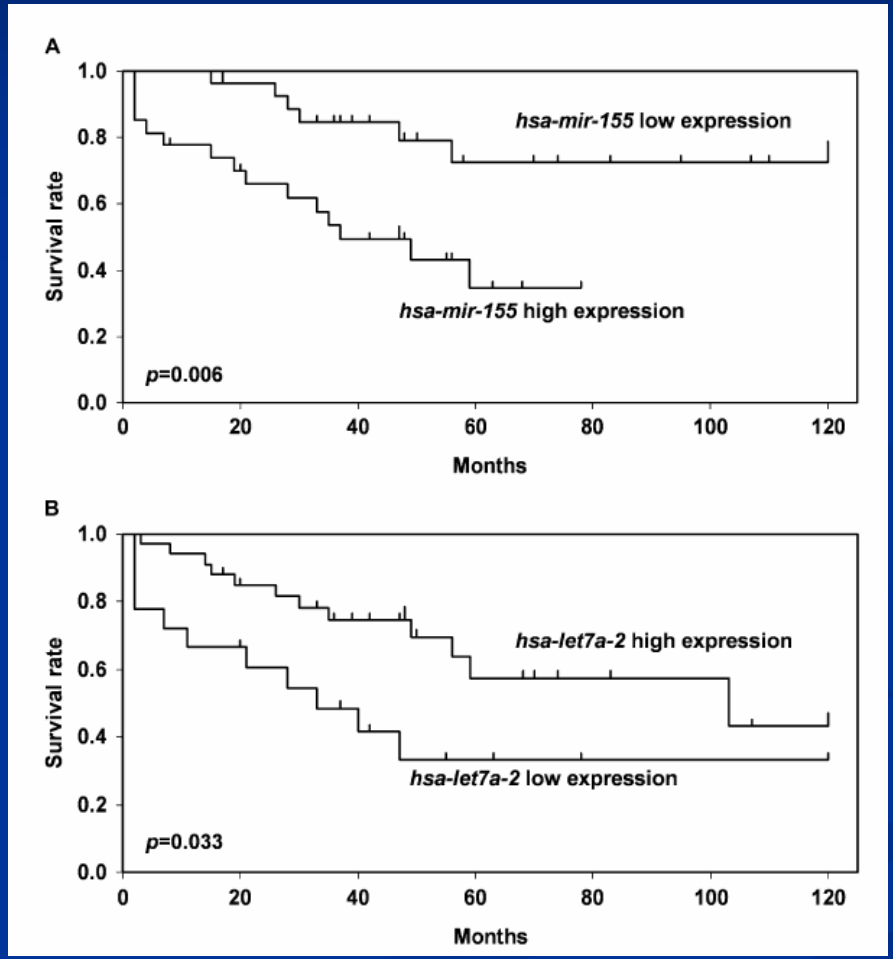
microRNA expression as prognostic marker

High expression of miR-221 is associated with a shorter time to recurrence in HCC



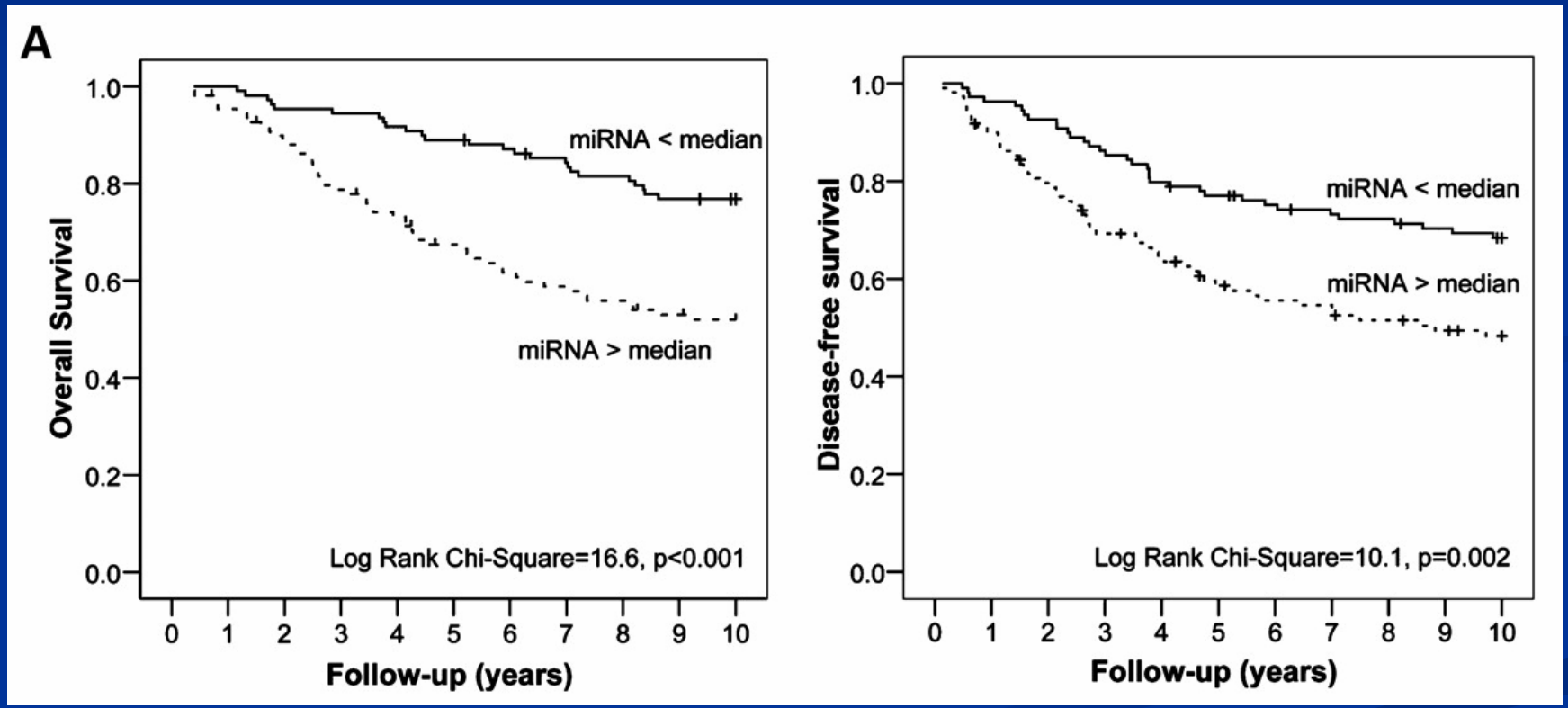


miR-155 and Let-7a expression associated with lung adenocarcinoma patients' survival



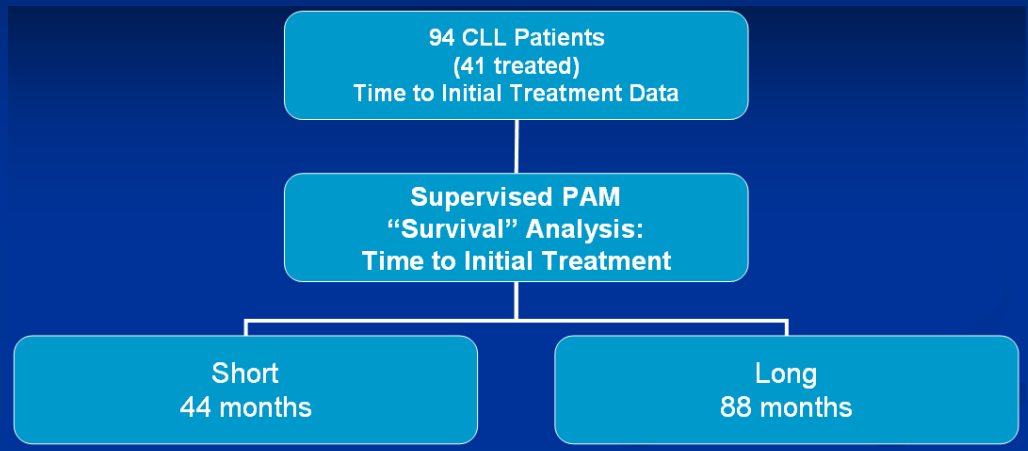
Yanaihara et al., Cancer Cell, 2006

miR-210 expression inversely correlated with overall and disease-free survival in breast cancer patients



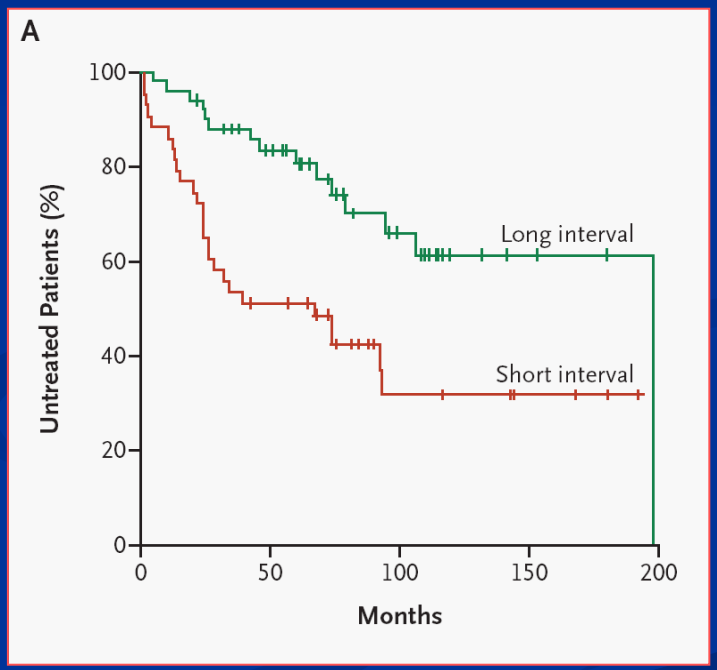
Camps et al., Clin Cancer Res, 2008

microRNA prognostic signature in CLL: Time to initial treatment signature



B

MicroRNA	Level of Expression of MicroRNA	
	Short interval	Long interval
miR-181a	High	Low
miR-155	High	Low
miR-146	High	Low
miR-24-2	High	Low
miR-23b	High	Low
miR-23a	High	Low
miR-222	High	Low
miR-221	High	Low
miR-29c	Low	High



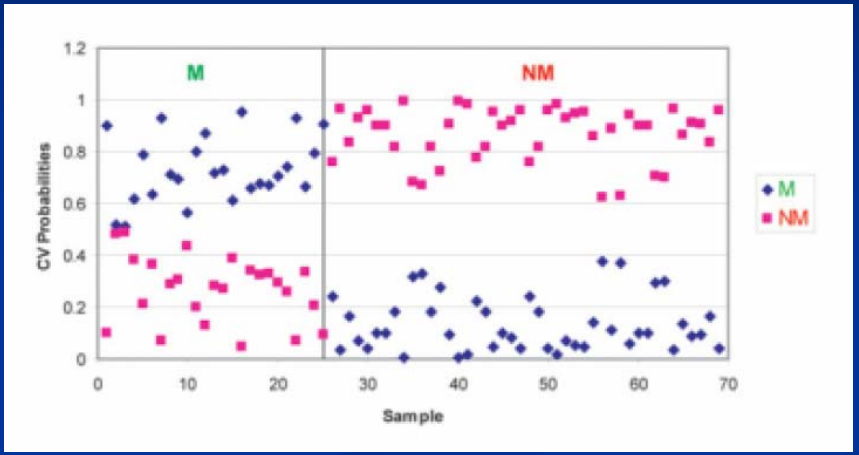
N Eng J Med 353:1793, 2005

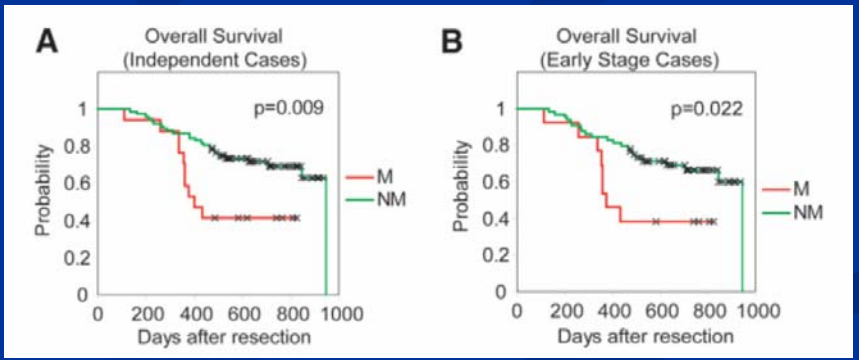
A 20-miRNA signature associated with metastasis and survival in HCC

No	Metastasis-miRNA	Genomic Location	Parametric *P*-Value	Mean Intensities in M	Mean Intensities in NM	Ratio (M/NM)	Expressed in Metastatic HCC	Host targets*	Survival-miRNA
1	mir-338	17q25.3	0.0001	356	250	1.42	up	IRF2	mir-338
2	mir-219-1	6p21.32	0.0002	578	391	1.48	up	ADD2	mir-219-1
3	mir-207	9p21.1	0.0002	3676	2432	1.51	up	n.a.	
4	mir-185	22q11.21	0.0009	461	346	1.33	up	KCNN3	
5	mir-30c-1	1p34.2	0.0001	813	1618	0.50	down	**KIAA0063**	mir-30c-1
6	mir-1-2	18q11.2	0.0002	294	571	0.51	down	**G3BP2**; **GCLC**; HAND2; TMSB4X; HDAC4; GJA1; KCNJ2	mir-1-2
7	mir-34a	1p36.2	0.0004	261	539	0.48	down	**SPTBN2**; E2F3, DLL1, NOTCH1	
8	mir-19a	13q31.3	0.0004	535	947	0.56	down	PTEN	mir-19a
9	mir-148a	7p15.2	0.0004	539	1084	0.50	down	**GTF2H1**; **PSCD3**	mir-148a
10	mir-124a-2	8q12.3	0.0004	236	448	0.53	down	**VAMP3**; MTPN; MAPK14	mir-124a-2
11	mir-9-2	5q14.3	0.0005	197	347	0.57	down	**RAB8A**; **SLC20A2**; **VAMP3**	mir-9-2
12	mir-148b	12q13.13	0.0005	578	1063	0.54	down	**GTF2H1**; **PSCD3**	mir-148b
13	mir-122a	18q21.31	0.0005	466	781	0.60	down	**GYS1**; CAT-1	mir-122a
14	mir-125b-2	21q21.1	0.0007	1346	2337	0.58	down	**ITGA9**; **YES1**; LIN28	mir-125b-2
15	mir-194	1q41	0.0008	406	689	0.59	down	**HBEGF**	mir-194
16	mir-30a	6q13	0.0008	2915	4572	0.64	down	KIAA0063; VERATIN; TMEM2; THBS1, SLC7A6; PRO2730; TUBA3; CYR61; CDK6	mir-30a
17	mir-126	9q34.3	0.0009	226	395	0.57	down	n.a.	mir-126
18	let-7g	3p21.2	0.0009	582	838	0.69	down	**PSCD3**; KRAS; NRAS	
19	mir-15a	13q14.2	0.0010	294	461	0.64	down	**ASPH**; **SLC20A2**; **SPTBN2**; DMTF1; BCL2	mir-15a
20	mir-30e	1p34.2	0.0010	960	1512	0.63	down	KIAA0063	mir-30e

*The experimentally proved host target genes are based on Tarbase. Potential host target genes in bold are based on TargetScan (release 3.1, November 2006) and are part of the 153-gene metastasis signature described in Ye et al., Nat Med 2003;9:416-423.

Abbreviation: n.a., not available.





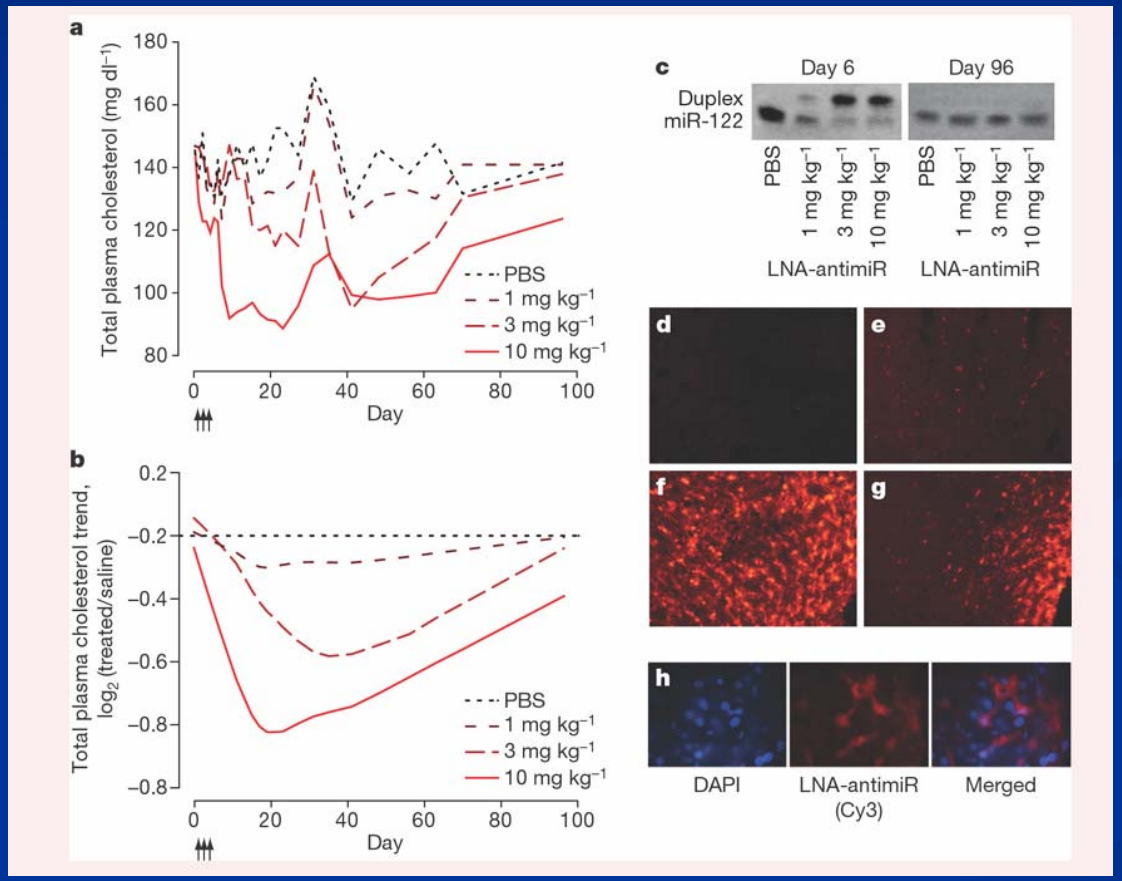
Budhu et al., Hepatology, 2008

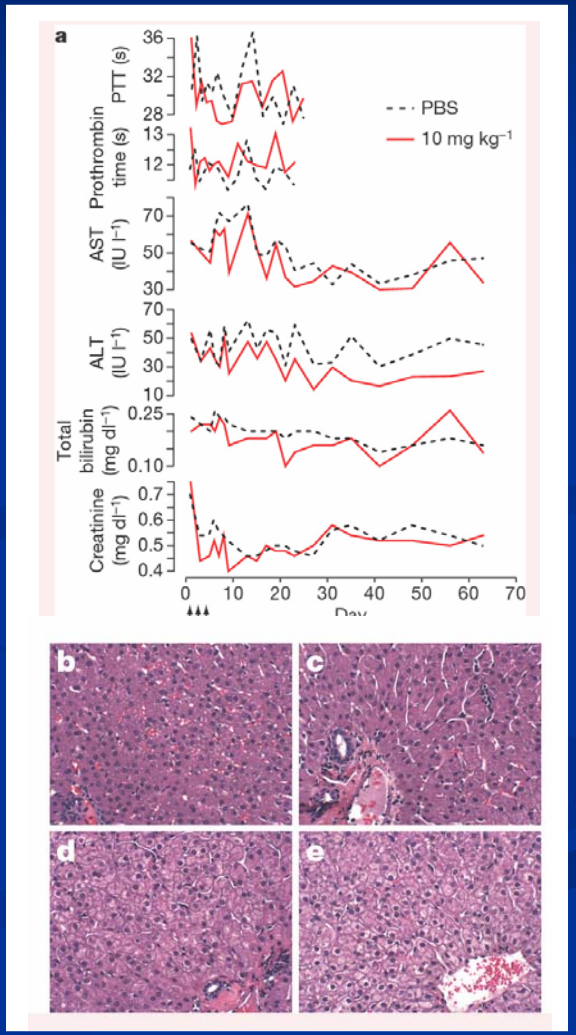
miRNAs as cancer diagnostic tools

- Clearly these results established the potential usefulness of microRNA expression in cancer stratification and risk assessment
- Prospectic studies are still required to validate their use in clinics
- However, it remains surprising that such a small number of genes (little more than 500) could be used to gain a level of information that is only achievable by the use of genome-wide microarray investigations

microRNAs as targets of molecular therapy ?

Silencing of miR-122 in non-human primates by LNA-antimiR





from ***Elmén*** et al. ***Nature* 452**, 896-899(17 April 2008)

Anti-cancer anti-miR therapy ?

- these results established the basis (safety and efficacy) for the use of anti-miR in clinical trials

Antagomir-*17-5p* Abolishes the Growth of Therapy-Resistant Neuroblastoma through p21 and BIM

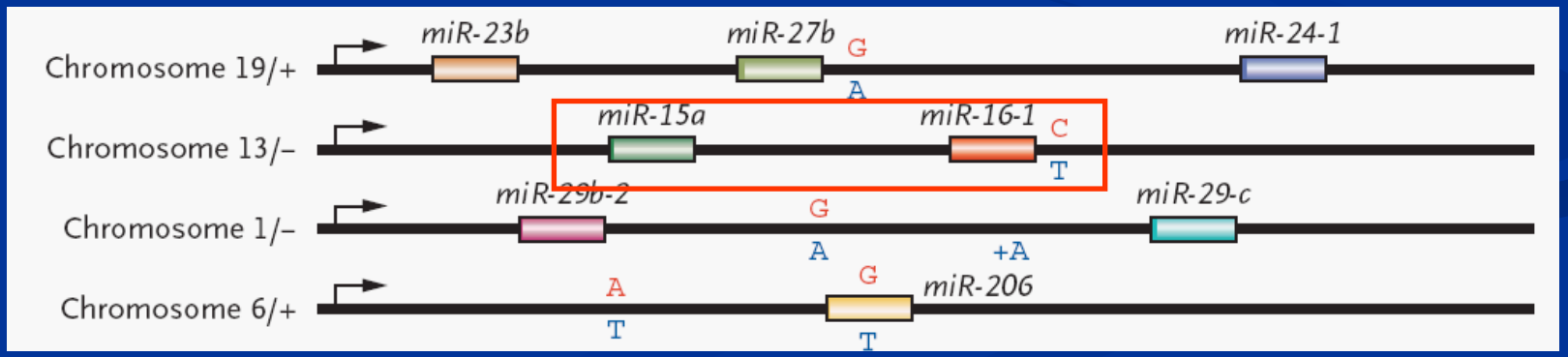
Laura Fontana[1*], Micol E. Fiori[1☯], Sonia Albini[2☯], Loredana Cifaldi[2], Serena Giovinazzi[1], Matteo Forloni[2], Renata Boldrini[3], Alberto Donfrancesco[4], Valentina Federici[5], Patrizio Giacomini[6], Cesare Peschle[1,7¶], Doriana Fruci[2¶]

- Additional anti-miRs may potentially be used in anti-cancer therapy: the several "oncogenic" miRs (miR-21, miR-221, miR-10b, miR-373 and others) could become targets of anti-miRs therapy

Germ-line mutations in miRNA genes

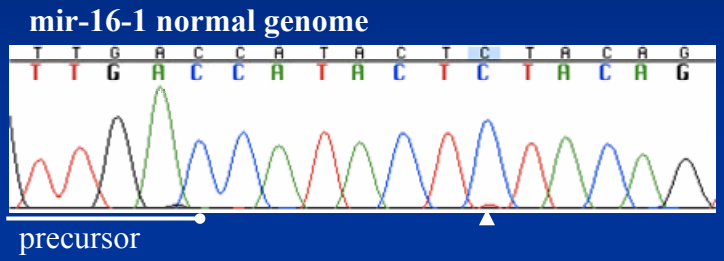
Table 3. Genetic Variations in the Genomic Sequences of MicroRNAs in Patients with CLL.*

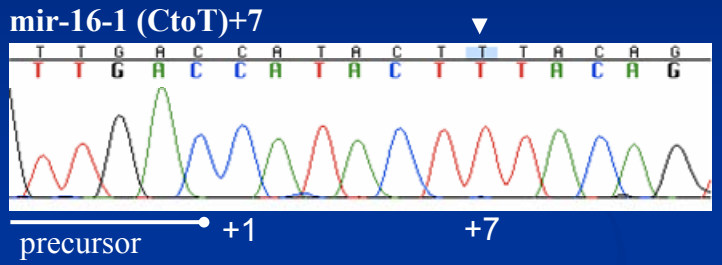
MicroRNA	Location†	Patients with CLL	Control Subjects	MicroRNA-Microchip Expression	Comment
		no./total no.			
miR-16-1	Germ-line pri-microRNA (C→T)+7-bp in the 3' direction	2/75	0/160	15 percent and 40 percent of normal levels, respectively	Normal allele deleted in CLL cells in both patients (by FISH and LOH); history of breast cancer in 1 patient; mother died with CLL; sister died with breast cancer
miR-27b	Germ-line pri-microRNA (G→A)+50-bp in the 3' direction	1/75	0/160	Normal	Throat and lung cancer diagnosed in mother at 58 yr; lung cancer diagnosed in father at 57 yr
miR-29b-2	Pri-microRNA (G→A)+212 in the 3' direction	1/75	0/160	75 percent of normal	Breast cancer diagnosed in sister at 88 yr (still living); "some type of blood cancer" diagnosed in brother at 70 yr
miR-29b-2	Pri-microRNAs insertion (+A)+107 in the 3' direction	3/75	0/160	80 percent of normal	Family history of unspecified cancer in 2 patients
miR-187	Pri-microRNA (T→C)+73 in the 3' direction	1/75	0/160	Not available	Unknown

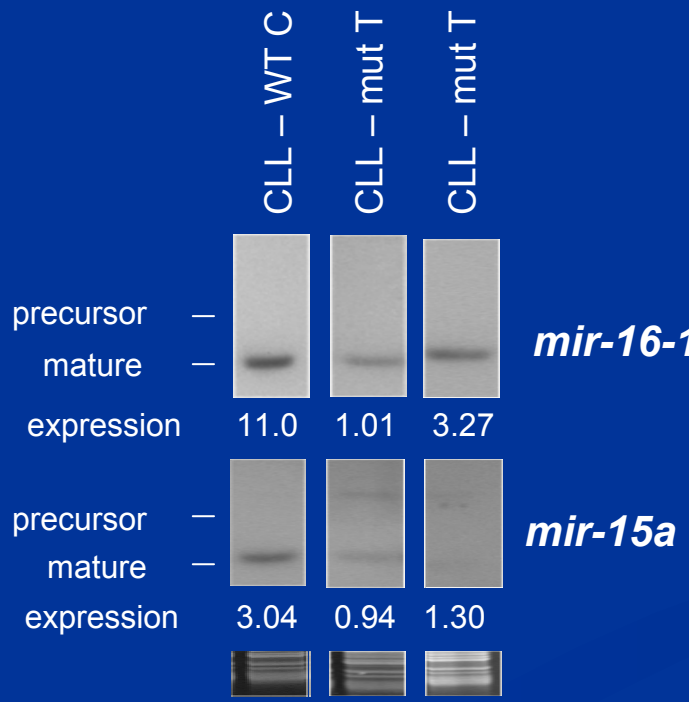


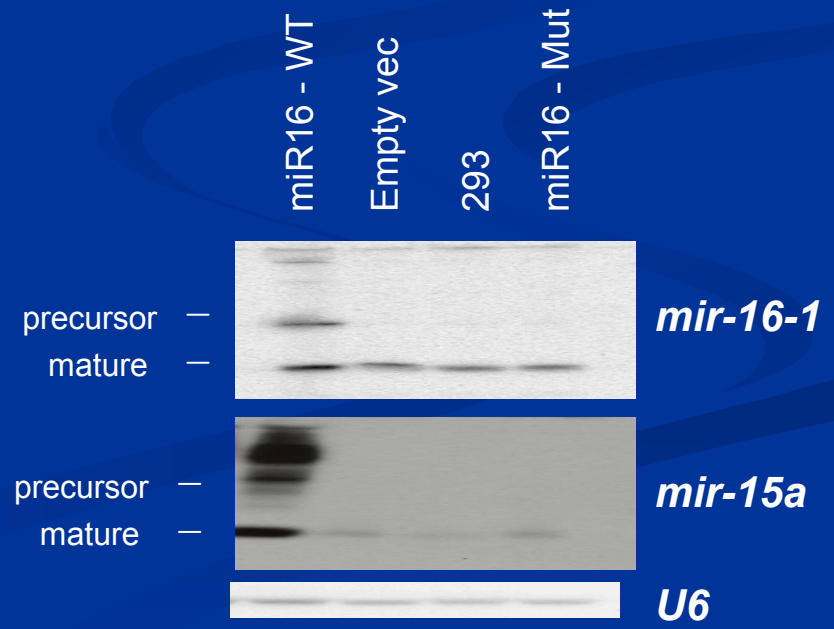
NEJM 353:1793, 2005

Genetic changes can affect miRNA stability or maturation









About humans and mice: same genes - same disease

Blood First Edition Paper, prepublished online March 9, 2007; DOI 10.1182/blood-2007-02-071225

Abnormal microRNA-16 Locus with Synteny to Human 13q14 Linked to CLL in NZB Mice*†

Elizabeth S. Raveche[1], Erica Salerno[1], Brian J. Scaglione[1], Vijaya Manohar[2], Fatima Abbasi[3], Yi-Chu Lin[1], Torgny Fredrickson[4], Pablo Landgraf [7], Sumant Ramachandra[1,8], Konrad Huppi[5], Jorge R Toro[6], Vincent E. Zenger[3], Robert A. Metcalf[3] and Gerald E. Marti[3]

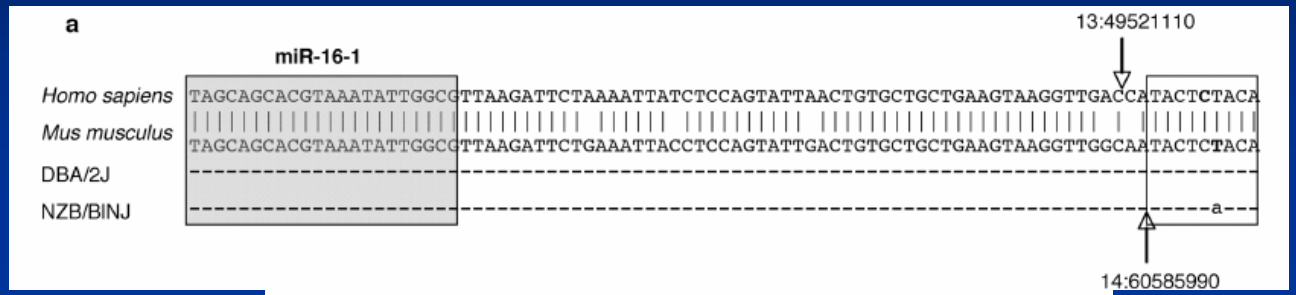
[1]UMDNJ/NJMS, Pathology and Lab Med, Newark, NJ [2]Georgetown Univ Med Ctr, Dept of Physiology and Biophysics, Washington, DC [3]CBER/FDA,Bethesda, MD, [4]Lab of Immunopathology, NIAID/NIH, Bethesda, MD, [5]Gene Silencing Sec,ATS/NCI/NIH Gaithersburg, MD, [6]Genetic Epidemiology Branch, DCEG/NCIPS Rockville, MD, [7]Lab of RNA Mol Biol, Howard Hughes Med Inst, The Rockefeller Univ, New York, NY, [8]Present Address: Sumant Ramachandra, MD, PhD,VP, Global Development/Schering-Plough Res Inst, Kenilworth, NJ

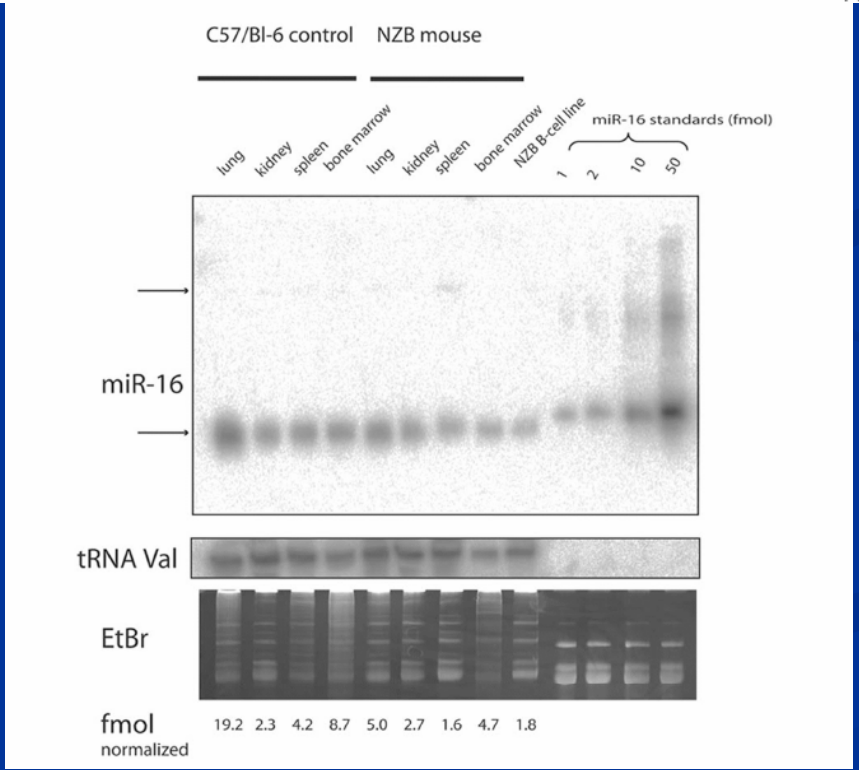
**** NZB strain naturally develop CLL-like disease during aging;***

NZB mouse strain presents a point mutation near the 3' end of miR-15/16 pre-miRNA at 1 nucleotide difference from the mutation found in human CLL

Raveche et al., Blood (2007)

NZB mouse strain presents a point mutation near the 3' end of miR-15/16 pre-miRNA and a reduced expression of miR-16-1





Raveche et al., Blood (2007)

Mir-15a/16-1 mutation is responsible for CLL in the NZB mouse strain

- The mutation found in the NZB strain is not found in any other mouse strain, including the nearest neighbor NZW
- Exogenous miR-16 delivered to NZB malignant B-1 cells resulted in cell cycle alterations and increased apoptosis
- The authors conclude that the altered expression of miR-15a/16-1 cluster caused by the point mutation is responsible for CLL in the NZB mouse strain

Raveche et al., Blood (2007)

Could germline mutations in miRNAs be involved in cancer predisposition ?

microRNA	Variation	Cancer association	Functional consequences	Reference
miR-15a/16-1 cluster	Germ-line (C-to-T) + 7 nt	Sporadic and familial CLL	Decreased expression of mature miRNA and failure to decrease BCL2 protein levels	Calin GA, et al.: A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 2005, 353:1793-1801.
miR-15a/16-1 cluster	NZB strain specific (A-to-T) + 6 nt	B-lymphoproliferative disease in mice	Decreased levels of miR-16 expression in lymhoproliferative tissues	Raveche ES, et al.: Abnormal microRNA-16 locus with synteny to human 13q14 linked to CLL in NZB mice. Blood 2007, 109:5079-5086.
miR-196a	C to T	Non-small cell lung cancer	Decreased mature miRNA	Hu Z, et al.: Genetic variants of miRNA sequences and non-small cell lung cancer survival. J Clin Invest 2008.
miR-146	G to C	Thyroid cancer	Decreased mature miRNA	Jazdzewski K, et al: Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci U S A 2008, 105:7269-7274.
Let-7e	G to A	Various cancers	Decreased mature miRNA	Wu M, et al: Genetic variations of microRNAs in human cancer and their effects on the expression of miRNAs. Carcinogenesis 2008, 29:1710-1716.
miR-17	C to T	Familial breast cancer	Increased mature miRNA	Shen, J., et al. (2009) Novel genetic variants in microRNA genes and familial breast cancer. Int J Cancer, 124:1178-1182.

Moreover, could germline "polymorphisms" in 3'UTRs affect miRNA interactions and, therefore, gene expression ?

Gene	Variation	miRNA target	Type of cancer	Reference
ITGB4	G/A	miR-34a	Breast cancer	Brendle, A., et al (2008) Polymorphisms in predicted microRNA-binding sites in integrin genes and breast cancer: ITGB4 as prognostic marker. Carcinogenesis, 29:1394-1399.
CD86	G/C	miR-337, -582, -200a*, -184, -212	Colorectal cancer	Landi, D., et al (2008) Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer. Carcinogenesis, 29:579-84
KRAS	T/G	Let-7	Non-small cell lung cancer	Chin, L.J., et al (2008) A SNP in a let-7 microRNA complementary site in the KRAS 3' untranslated region increases non-small cell lung cancer risk. Cancer Res, 68:8535-8540.
KIT	G/A	miR-221/222	Thyroid cancer	He, H., Jet al (2005) The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci U S A, 102:19075-19080.

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Il mio laboratorio

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Dip. Medicina Interna e Gastroenterologia
Università di Bologna

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Ospedale Bambin Gesù, Roma