LÉKAŘSKÁ FAKULTA MASARYKOVY UNIVERSITY
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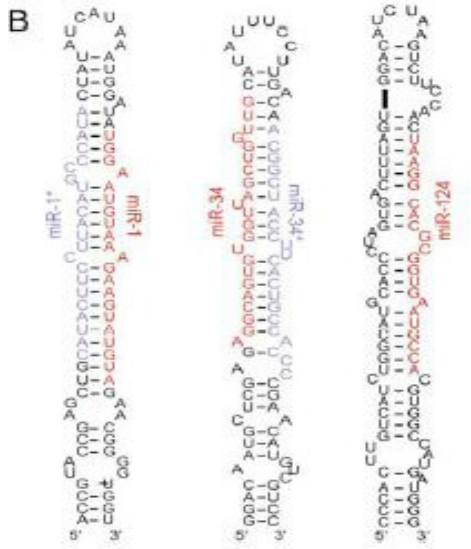
Centrum molekulární biologie a genové terapie

Význam microRNA (v onkologii)

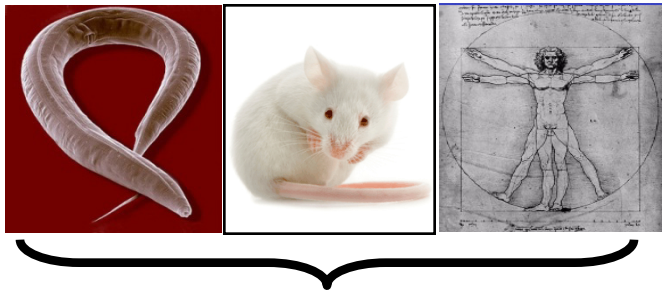
MUDr. Mgr.Marek Mráz



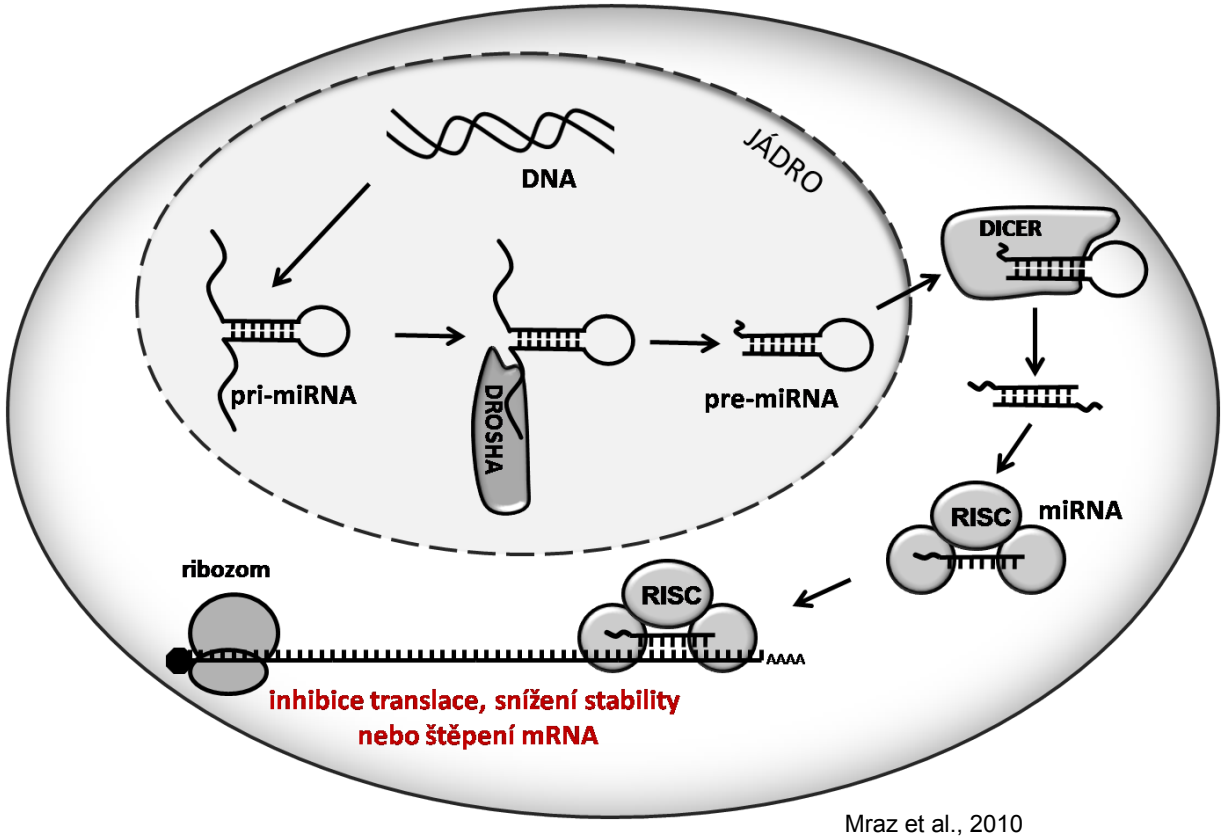
- Lidské miRNA geny: cca 1000

microRNA (miRNA)

- krátké RNA molekuly ~22 nukleotidů
- komplementární vazba k cílové mRNA
- inhibují translaci a snižují stabilitu mRNA

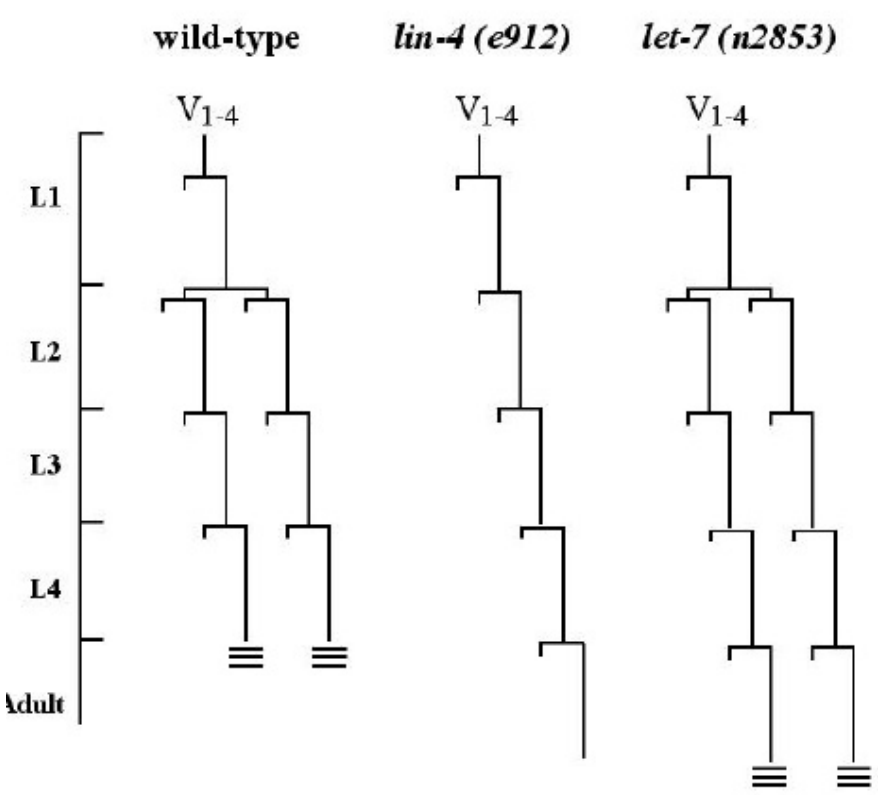


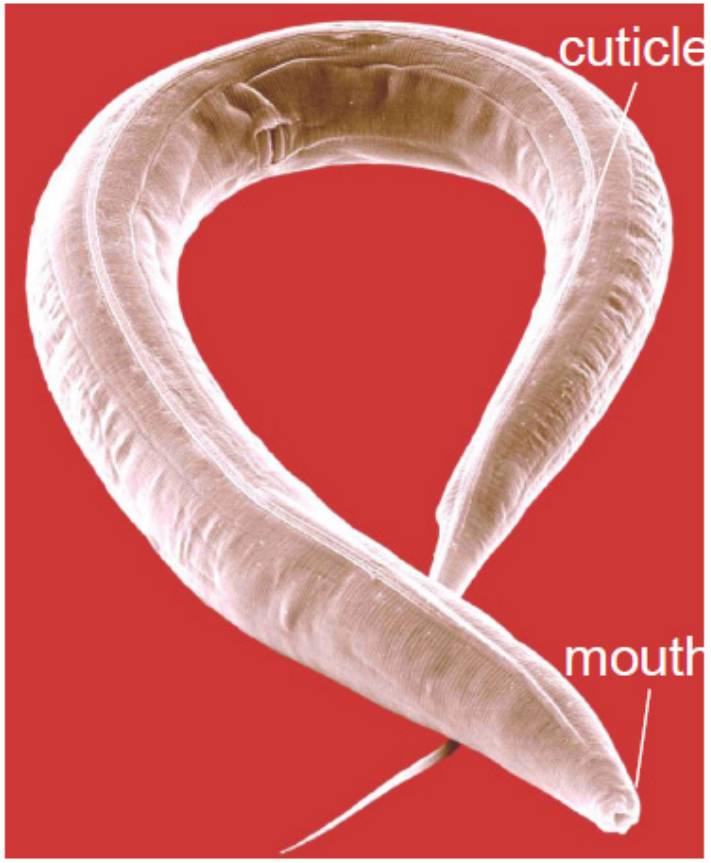
Stovky evolučně konzervovaných microRNA



Mraz et al., 2010

MicroRNAs were discovered by V. Ambros and G. Ruvkun in *C. elegans*





Lee et al. 1993 *Cell*

Reinhart et al. 2000 *Nature*

courtesy of F. Slack

microRNA genes map to cancer loci

Table 2. Examples of miRs located in minimal deleted regions, minimal amplified regions, and breakpoint regions involved in human cancers

Chromosome	Location (defining markers)	Size, Mb	miR	Hystotype	Known OG/TS
3p21.1–21.2-D	ARP-DRR1	7	*let-7g/miR-135–1*	Lung, breast cancer	—
3p21.3(AP20)-D	GOLGA4-VILL	0.75	*miR-26a*	Epithelial cancer	—
3p23–21.31(MDR2)-D	D3S1768-D3S1767	12.32	*miR-26a; miR-138–1*	Nasopharyngeal cancer	—
5q32-D	ADRB2-ATX1	2.92	*miR-145/miR-143*	Myelodysplastic syndrome	—
9q22.3-D	D9S280-D9S1809	1.46	*miR-24–1/mir-27b/miR-23b; let-7a-1/let-7f-1/let-7d*	Urothelial cancer	PTC, FANCC
9q33-D	D9S1826-D9S158	0.4	*miR-123*	NSCLC	—
11q23-q24-D	D11S927-D11S1347	1.994	*miR-34a-1/miR-34a-2*	Breast, lung cancer	PPP2R1B
11q23-q24-D	D11S1345-D11S1328	1.725	*miR-125b-1/let-7a-2/miR-100*	Breast, lung, ovary, cervix cancer	—
13q14.3-D	D13S272-D13S25	0.54	*miR-15a/miR-16a*	B-CLL	—
13q32–33-A	stSG15303-stSG31624	7.15	*miR-17/miR-18/miR-19a/miR-20/ miR-19b-1/miR-92–1*	Follicular lymphoma	—
17p13.3-D	D17S1866-D17S1574	1.899	*miR-22; miR-132; miR-212*	HCC	—
17p13.3-D	ENO3-TP53	2.275	*miR-195*	Lung cancer	TP53
17q22-t(8;17)	miR-142s/c-MYC		*miR-142s; miR-142as*	Prolymphocytic leukemia	c-MYC
17q23-A	CLTC-PPM1D	0.97	*miR-21*	Neuroblastoma	—
20q13-A	FLJ33887-ZNF217	0.55	*miR-297–3*	Colon cancer	—
21q11.1-D	D21S1911-ANA	2.84	*miR-99a/let-7c/miR-125b*	Lung cancer	—

D, deleted region; A, amplified region; NSCLC, non-small-cell lung cancer; HCC, hepatocellular carcinoma; PTC, patched homolog (*Drosophila*); FANCC, Fanconi anemia, complementation group C; PPP2R1B, protein phosphatase 2, regulatory subunit A (PR 65), β isoform, miRs in a cluster are separated by a slash. For references, see Table 6.

Calin, G.A., *et al.,* 2004. *PNAS* 101:2999-3004

MicroRNAs commonly associated with human cancer

miRNA	Gene Loci	Cancer association	Function*	References
miR15, miR-16	chromosome 13q14	Frequently deleted/downregulated in B-cell chronic lymphocytic leukemia. Negatively regulates the anti-apoptotic gene, BCL2.	TS	Calin, 2002 Cimmino, 2005
miR-143, miR-145	chromosome 5q3233	Decreased abundance in colorectal cancer. Down-regulated in breast, prostate, cervical, and lymphoid cancer celllines. miR-145 decreased in breast cancer.	TS	Michael, 2003 Iorio, 2005
miR-21	chromosome 17q23.2	Antiapoptotic factor. Upregulated in glioblastomas and breast cancer.	OG	Chan, 2005 Ciafre, 2005 Iorio, 2005
let-7	multiple loci	Negatively regulates the Ras oncogene. Directs cell proliferation, differentiation. Decreased abundance in lung cancer.	TS	Johnson, 2005 Takamizawa, 2004
miR-142	chromosome 17q22	t(8,17) translocation that places the MYC oncogene downstream of the *mir-142* hairpin resulting in an aggressive B cell leukemia due to MYC over-expression.	N/A	Lagos-Quintana, 2002
BIC/miR-155	chromosome 21q21	Upregulated in pediatric Burkitt's lymphomas, Hodgkins, primary mediastinal and diffuse large B cell lymphomas. Upregulated in human breast cancer.	OG	Eis, 2005 Kluiver, 2005 van den Berg, 2003 Metzler, 2003 Iorio, 2005
miR-17-19b cluster	chromosome 13q3132	Upregulated by the c-Myc oncogene Negatively modulates E2F1 oncogene. Loss-of-heterozygosity of cluste in hepatocellular carcinoma. Over-expressed in B-cell lymphomas.	TS/ OG	He, 2005 O'Donnell, 2005

*Abbreviations: TS, tumor-suppressor gene; OG oncogene; N/A, not applicable

courtesy of F. Slack

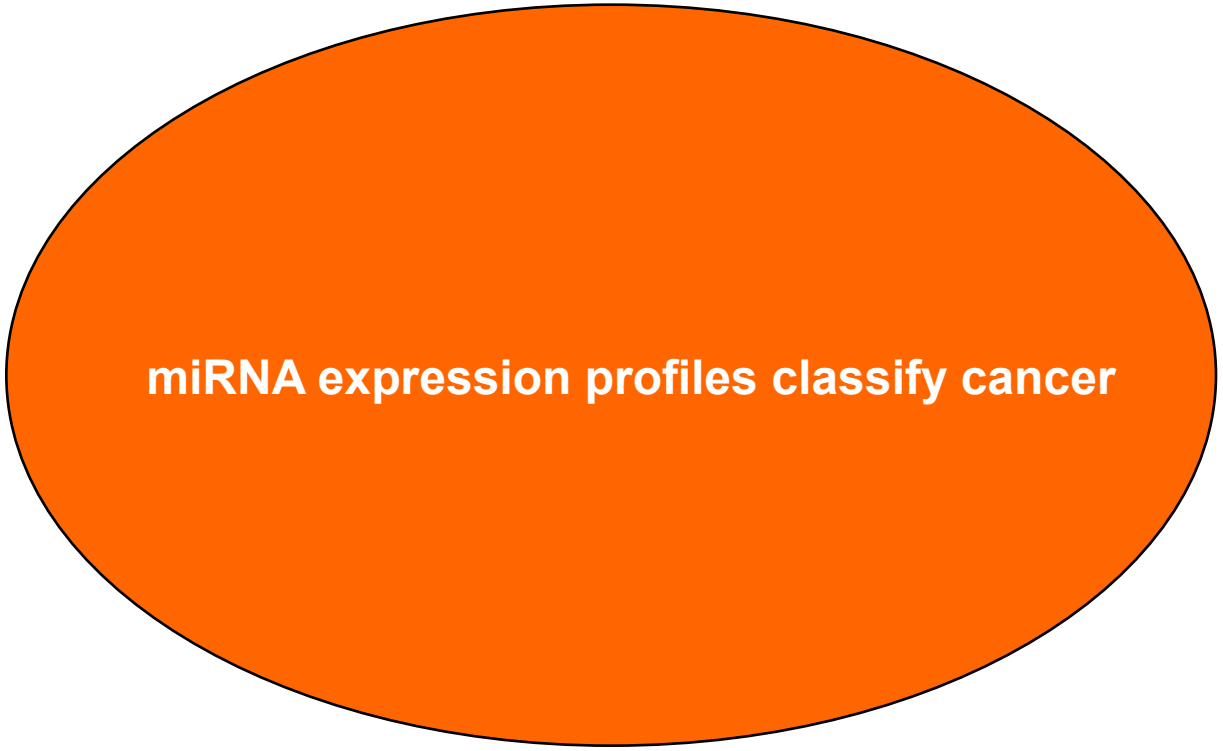
miRNAs expression profiles clasify tumors

miRNAs are associated with disease prognosis

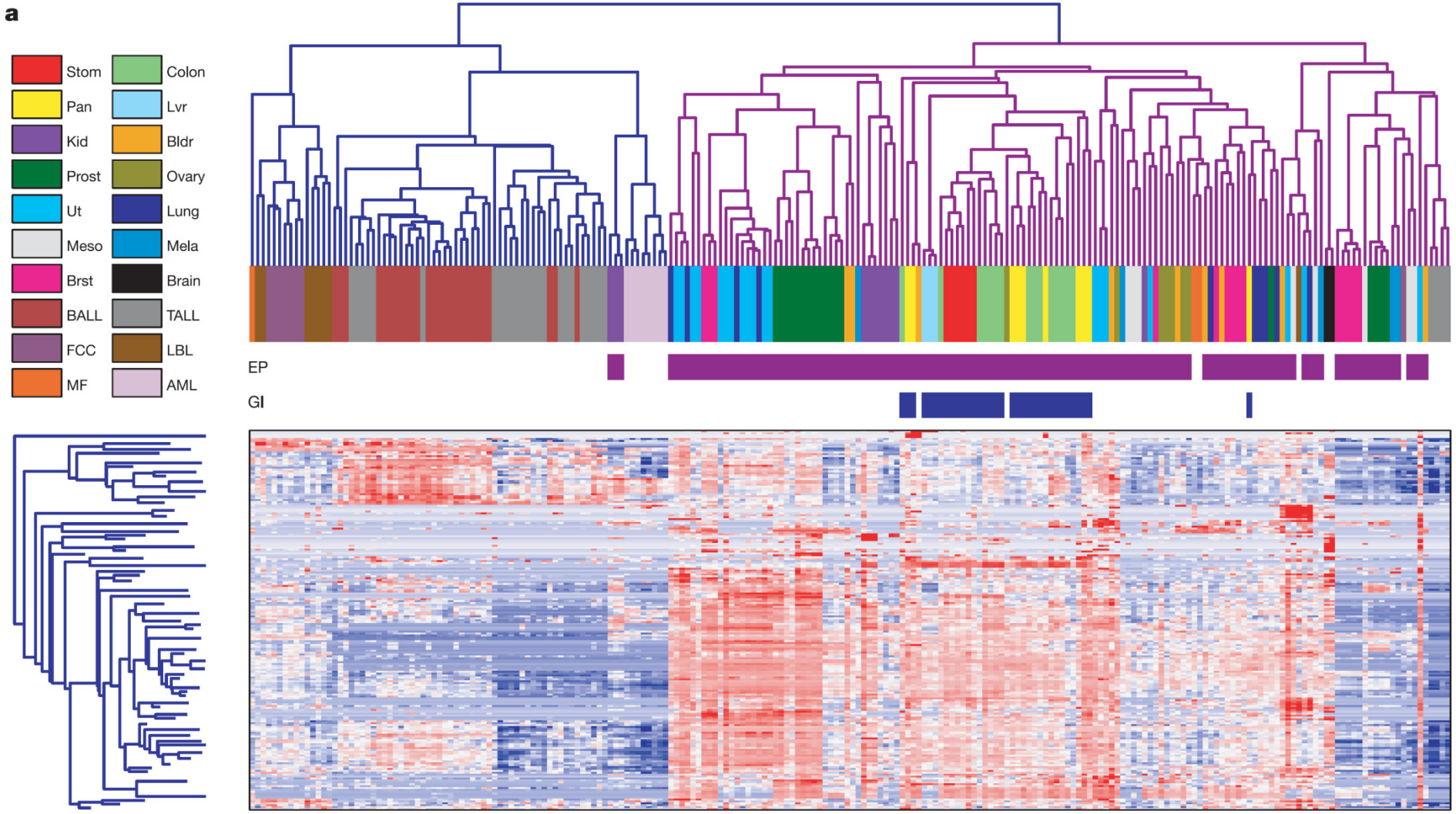
miRNAs are tumor-supressors and proto-oncogenes

miRNAs and microRNA binding sites are mutated in cancer

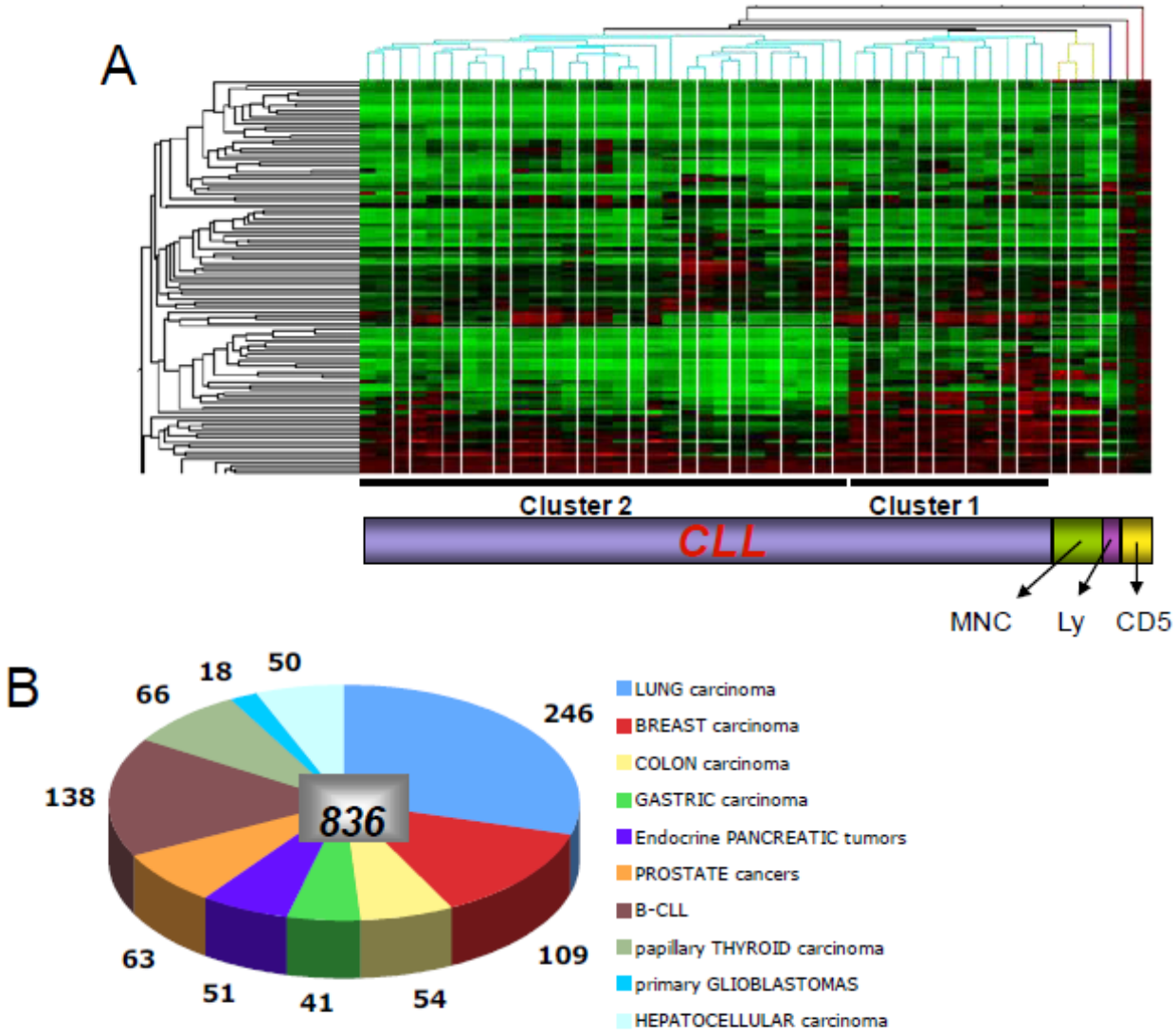
miRNAs as drugs and targets in oncology


miRNA expression profiles classify cancer

microRNA expression can discriminate tumor types



Lu et al. Nature 435: 834, 2005



(Calin et al, PNAS, 2004; Esquela-Kerscher & Slack, Nat Rev Cancer 2006; Calin & Croce, Nat Rev Cancer 2006)

courtesy of G. Calin

A microRNA expression signature of human solid tumors defines cancer gene targets

Stefano Volinia*†‡, George A. Calin*‡, Chang-Gong Liu*, Stefan Ambs§, Amelia Cimmino*, Fabio Petrocca*, Rosa Visone*, Marilena Iorio*, Claudia Roldo*, Manuela Ferracin¶, Robyn L. Prueitt§, Nozumu Yanaihara§, Giovanni Lanza¶, Aldo Scarpa‖, Andrea Vecchione**, Massimo Negrini¶, Curtis C. Harris§, and Carlo M. Croce*††

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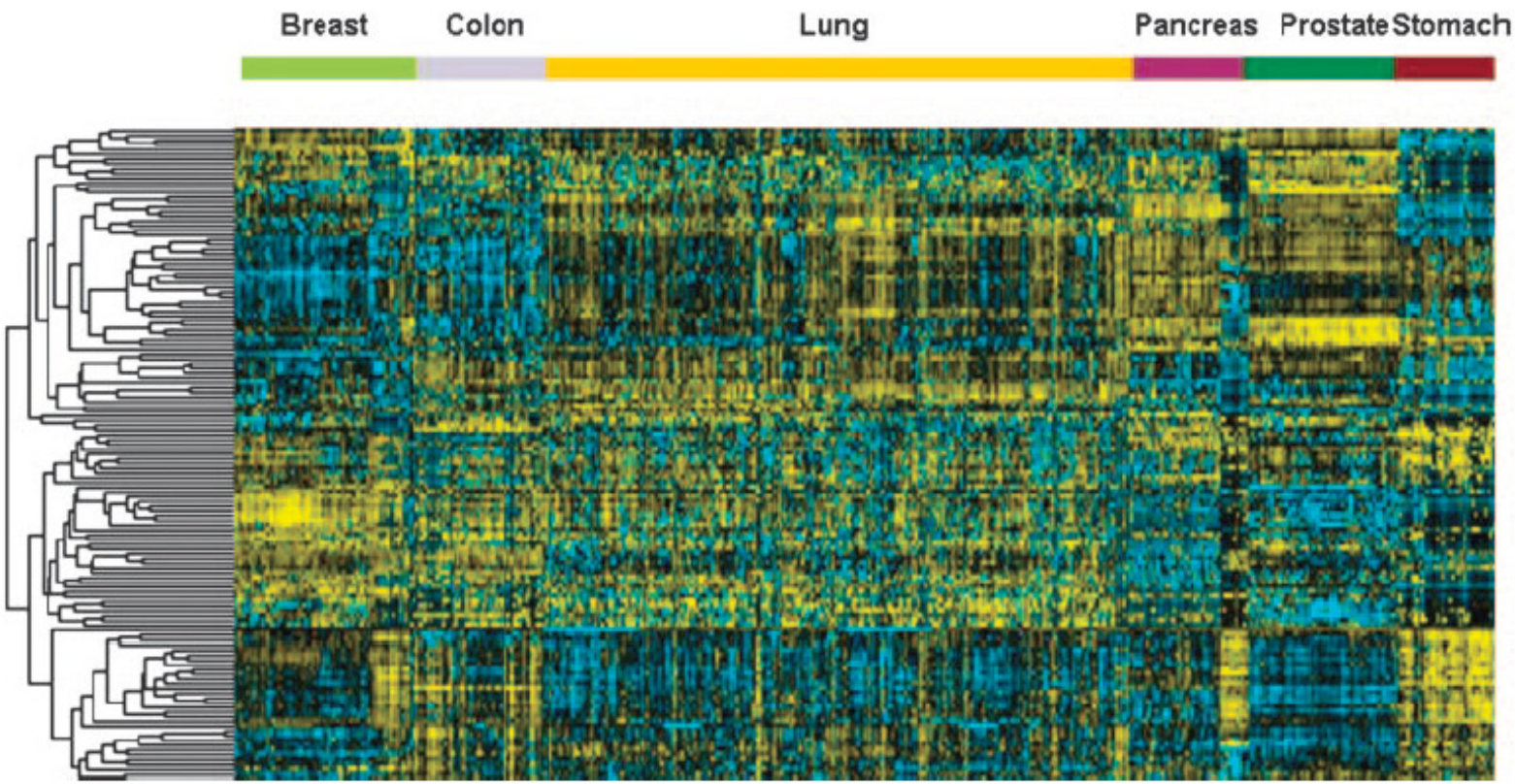


Table 2. The miRNAs shared by the signatures of the six solid cancers

miR	*N*	Tumor type
miR-21	6	Breast, colon, lung, pancreas, prostate, stomach
miR-17-5p	5	Breast, colon, lung, pancreas, prostate
miR-191	5	Colon, lung, pancreas, prostate, stomach
miR-29b-2	4	Breast, colon, pancreas, prostate
miR-223	4	Colon, pancreas, prostate, stomach
miR-128b	3	Colon, lung, pancreas
miR-199a-1	3	Lung, pancreas, prostate
miR-24-1	3	Colon, pancreas, stomach
miR-24-2	3	Colon, pancreas, stomach
miR-146	3	Breast, pancreas, prostate
miR-155	3	Breast, colon, lung
miR-181b-1	3	Breast, pancreas, prostate
miR-20a	3	Colon, pancreas, prostate
miR-107	3	Colon, pancreas, stomach
miR-32	3	Colon, pancreas, prostate
miR-92-2	3	Pancreas, prostate, stomach
miR-214	3	Pancreas, prostate, stomach
miR-30c	3	Colon, pancreas, prostate
miR-25	3	Pancreas, prostate, stomach
miR-221	3	Colon, pancreas, stomach
miR-106a	3	Colon, pancreas, prostate

The list includes 21 commonly up-regulated microRNAs in 3 or more (*N*) types of solid cancers (P value $= 2.5 \times 10^{-3}$).

miRNAs are associated with disease prognosis

A unique miRNA signature is associated with lung cancer prognosis

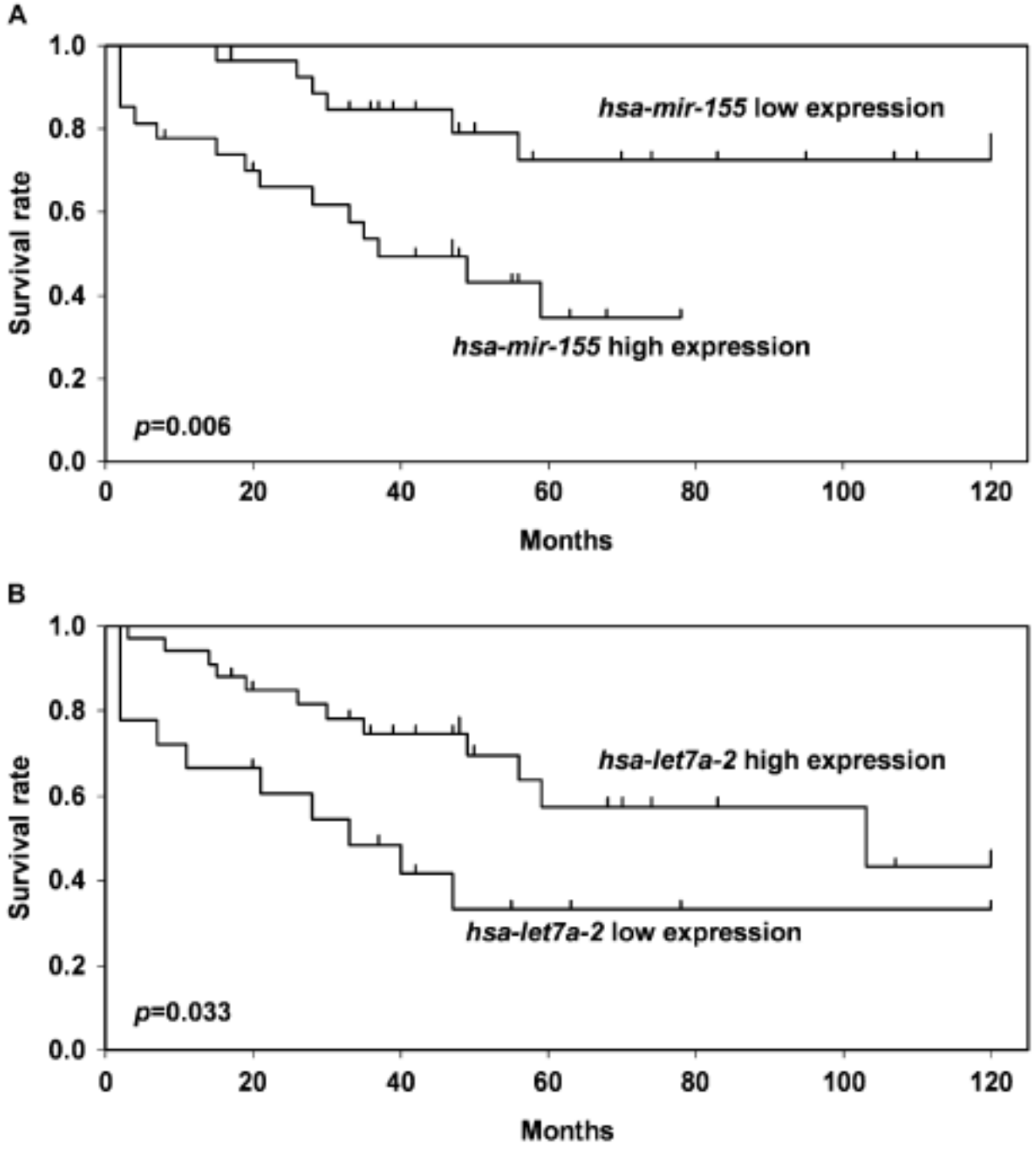
Table 5. Postoperative survival of patients with lung adenocarcinoma in relation to clinicopathological characteristics and miRNA expression analyzed by microarray analysis

Variable	Subset	Hazard ratio (95% confidence interval)	p
Univariate analysis (n = 65)			
Age	age ≥ 67/age < 67	1.41 (0.67–3.06)	0.348
Sex	male/female	1.36 (0.64–2.93)	0.413
Stage	II–IV/I	2.51 (1.29–6.82)	0.010
Smoking history	current/former	1.32 (0.63–2.79)	0.456
hsa-mir-155 (n = 55)	high/low	3.42 (1.42–8.19)	0.006
hsa-let-7a-2 (n = 52)	low/high	2.35 (1.08–6.86)	0.033
Multivariate analysis (n = 55)[a,b]			
Age	age ≥ 67/age < 67	1.92 (0.71–5.17)	0.195
Sex	male/female	1.23 (0.47–3.22)	0.669
Stage	II–IV/I	3.27 (1.31–8.37)	0.013
Smoking history	current/former	1.49 (0.51–4.34)	0.457
hsa-mir-155	high/low	3.03 (1.13–8.14)	0.027

[a]Multivariate analysis, Cox proportional hazard regression model.
[b]*hsa-let-7a-2* low/high was not statistically significant (p = 0.129).

(Yanaihara et al, Cancer Cell, 2006)

courtesy of G. Calin

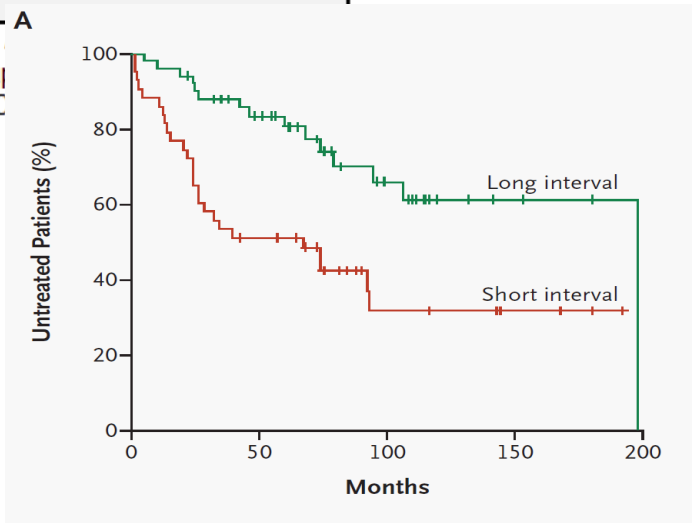

A
1.0
0.8
0.6
0.4
0.2
0.0
Survival rate
hsa-mir-155 low expression
hsa-mir-155 high expression
p=0.006
0
20
40
60
80
100
120
Months
B
1.0
0.8
0.6
0.4
0.2
0.0
Survival rate
hsa-let7a-2 high expression
hsa-let7a-2 low expression
p=0.033
0
20
40
60
80
100
120
Months

A unique miRNA signature is associated with CLL prognosis

Nr. Crt.	Component	Map	P value	Aggressive CLL **	Observation***
1	***miR-15a***	13q14.3	0,018	high	cluster 15a/16-1 del CLL & Prostate ca.
2	***miR-195***	17p13	0,017	high	del HCC
3	***miR-221***	Xp11.3	0,010	high	cluster 221/222
4	***miR-23b***	9q22.1	0,009	high	cluster 24-1/23b FRA 9D; del Urothelial ca.
5	***miR-155***	21q21	0,009	high	amp child Burkitt's lymphoma
6	***miR-223***	Xq12-13.3	0,007	low	normally expression restricted to myeloid lineage
7	***miR-29a-2***	7q32	0,004	low	cluster 29a-2/29b-1 FRA7H; del Prostate ca.
8	***miR-24-1***	9q22.1	0,003	high	cluster 24-1/23b FRA 9D; del Urothelial ca.
9	***miR-29b-2***	1q32.2-32.3	0,0007	low	
10	***miR-146***	5q34	0,0007	high	
11	***miR-16-1***	13q14.3	0,0004	high	cluster 15a/16-1 del CLL, prostate ca.
12	***miR-16-2***	3q26.1	0,0003	high	identical miR-16-1
13	***miR-29c***	1q32.2-32.3	0,0002	low	

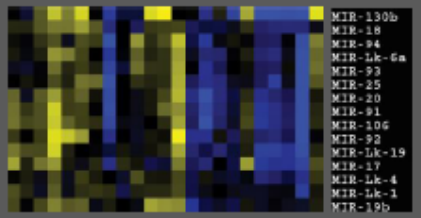
Note: * - All the members of the signature are mature microRNAs; ** - represented by group 1 includes patients with IgVh unmutated and Zap predictors of poor prognosis. *** - FRA = fragile site; del = deletion; HC carcinoma; ca. = carcinoma.

(Calin et al, N Engl J Med, 2005)



miRNAs are tumor-supressors and proto-oncogenes

A polycistronic cluster of microRNAs are overexpressed in cancer



Proliferation

Angiogenesis

Apoptosis

Invasiveness

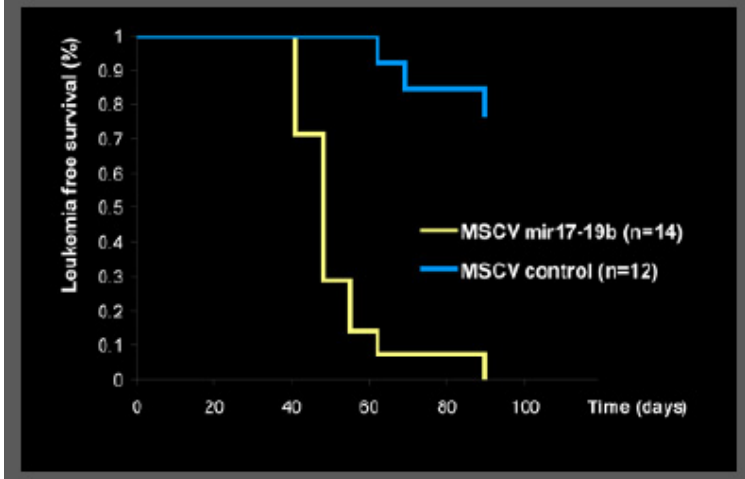
miR-17 miR-18 miR-19a miR-20 miR-19b miR-92

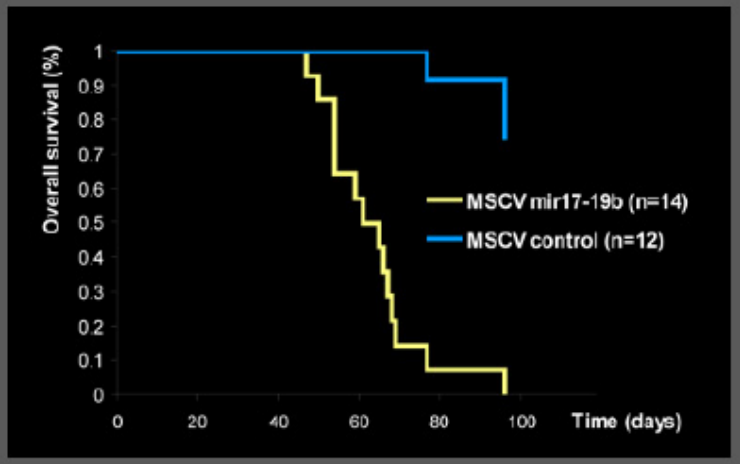
Ch13-ORF25

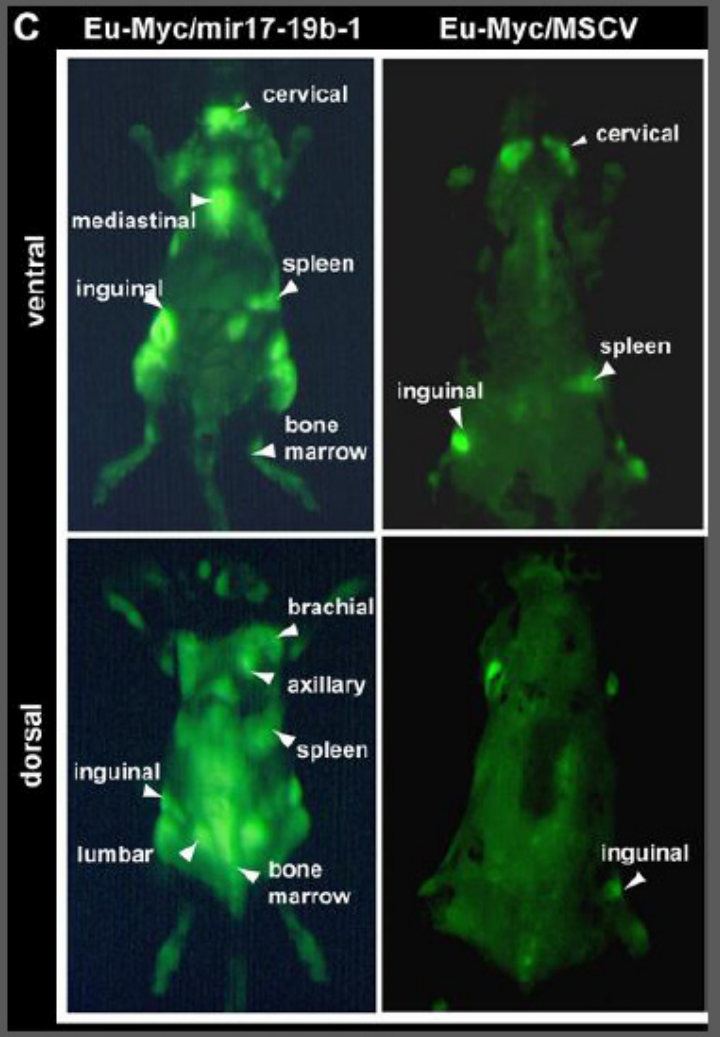
OncomiR-1 (Oncogenic microRNA-1)

courtesy of S. Hammond

OncomiR-1 expression accelerates lymphoma development

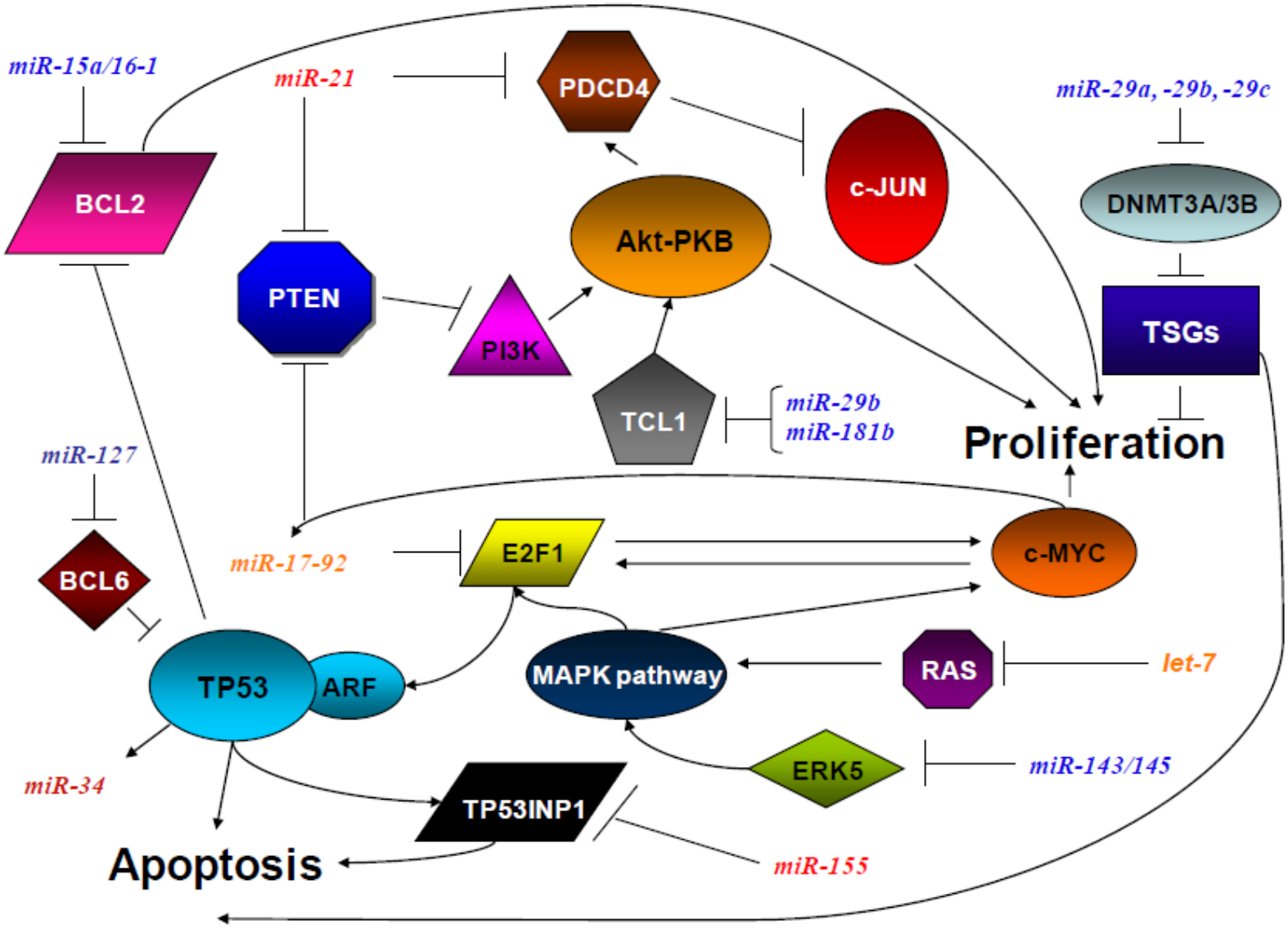






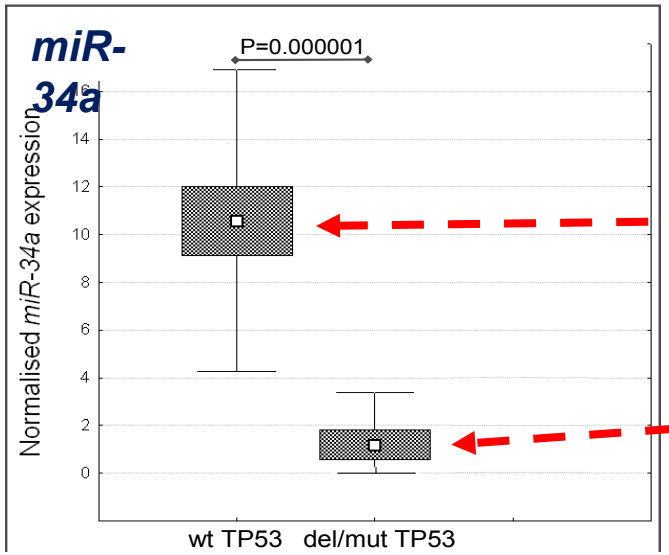
He et al, *Nature* 435(7043): 828-833 (2005)

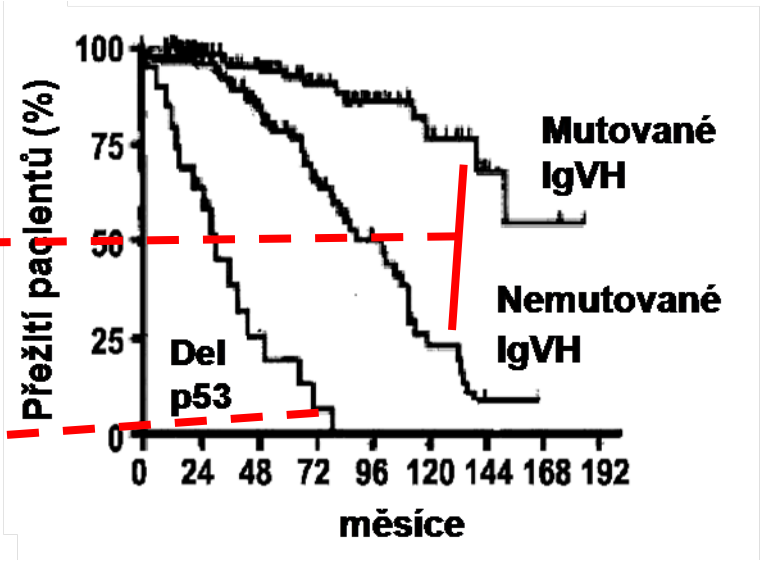
courtesy of S. Hammond

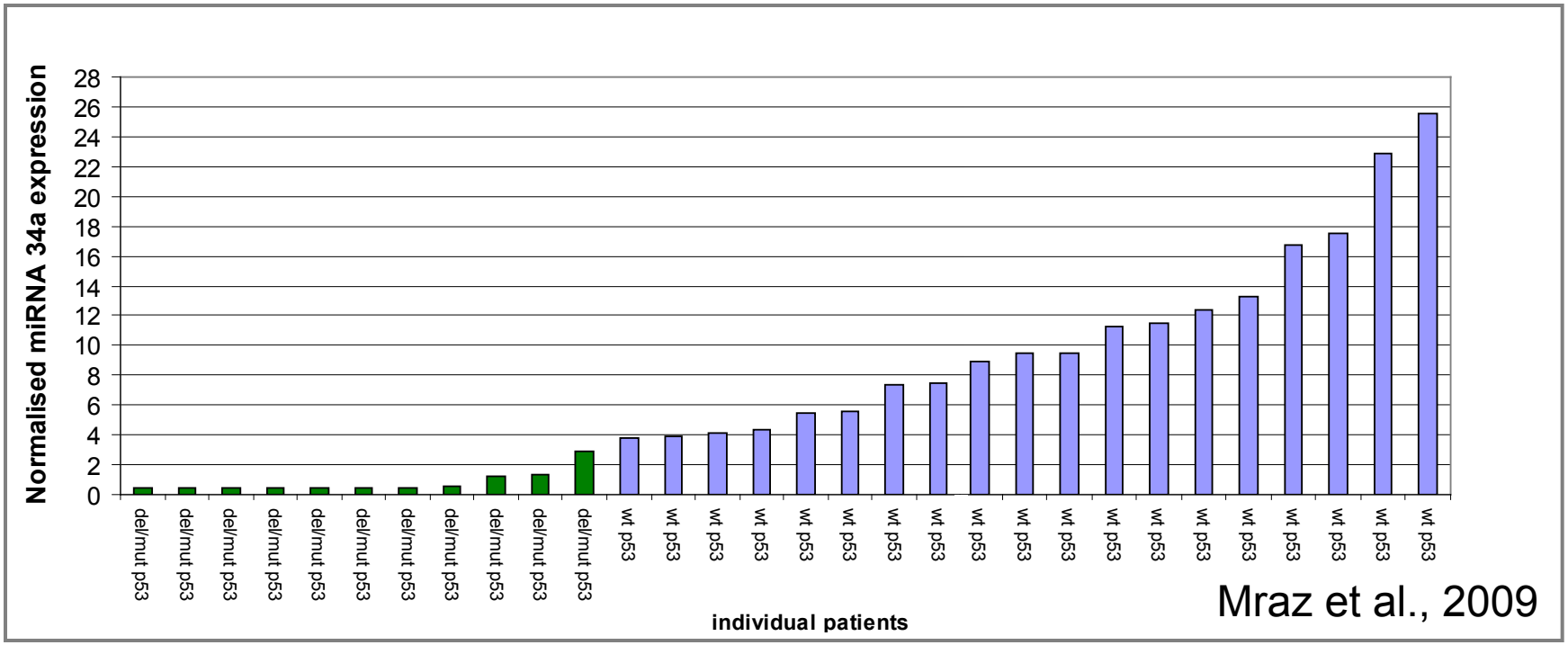


courtesy of G. Calin

Pacienti s funkčním p53 mají ~ 10 násobně vyšší expresi *miR-34a* (p=0,000001)

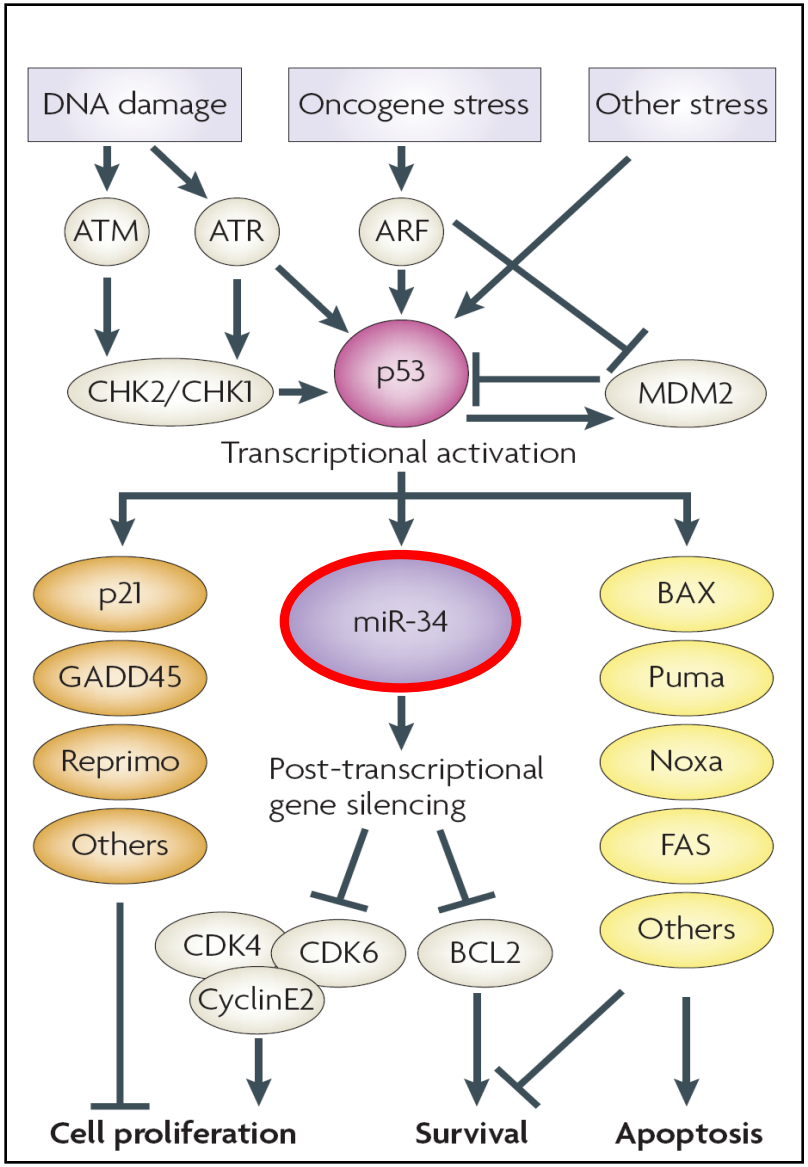






Mraz et al., 2009

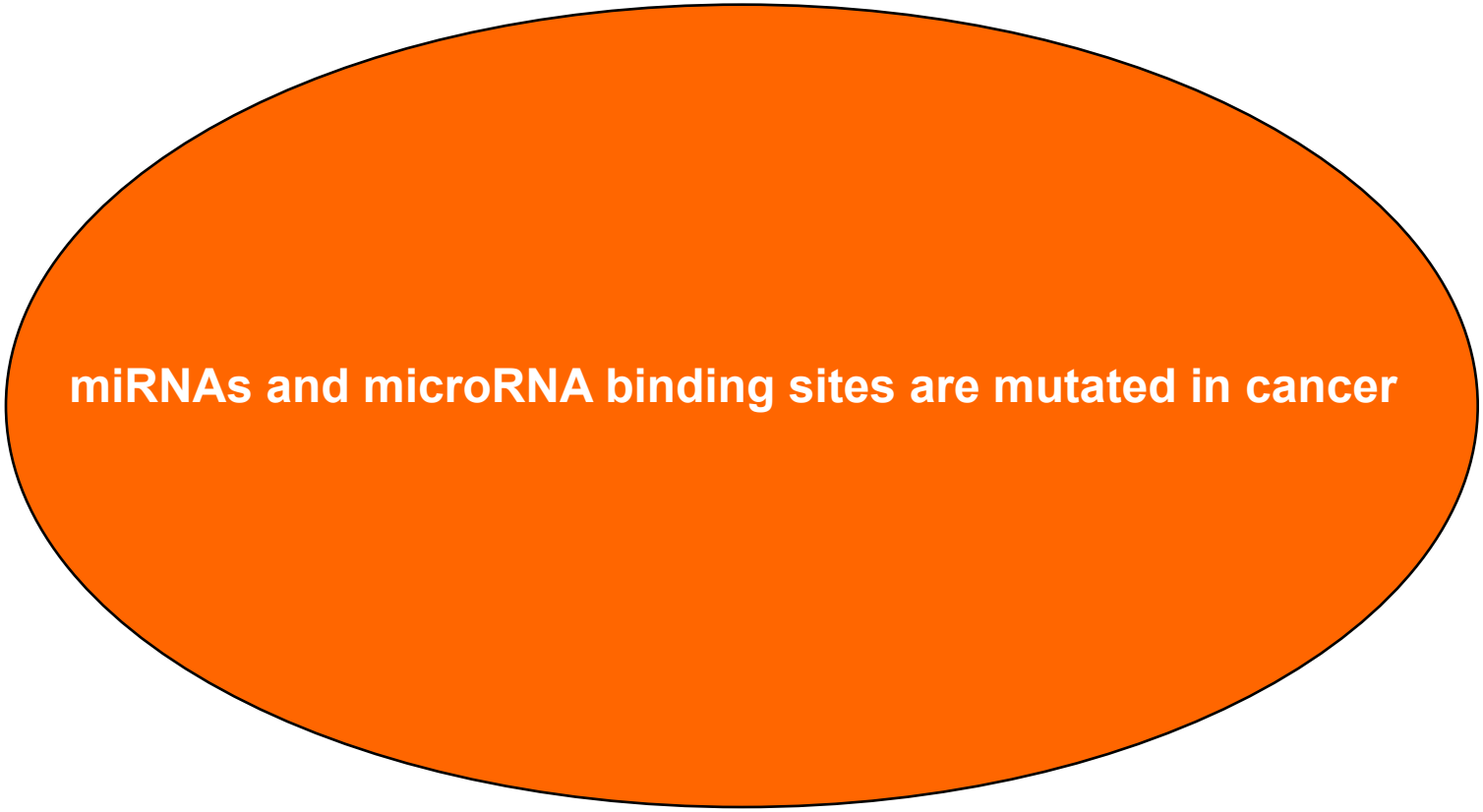
miR-34a a regulace apoptózy



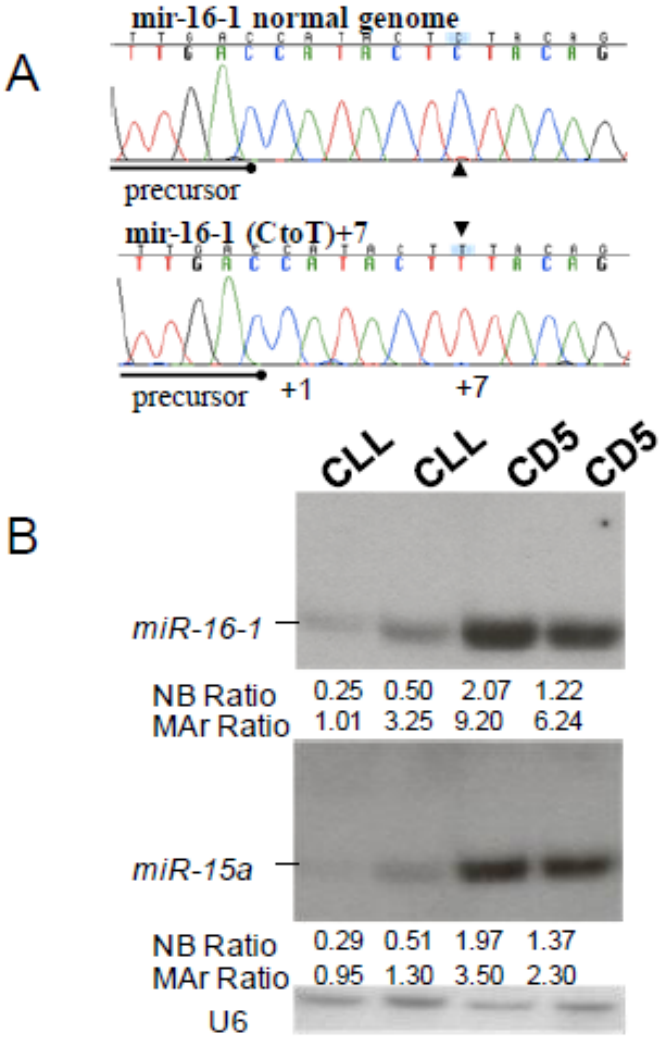
- ***He et al., NATURE 2007*:**
  miR-34a je transkripčně aktivovaná proteinem p53 (studium buněčných linií)

- ***Bommer et al., CURR. BIOL. 2007*:**
  miR-34a ovlivňuje BCL2 (studium buněčných linií)

- **miR-34a je abnormálně exprimována u CLL pacientů s abnormalitou p53 (Mraz et al., 2009; Mraz et al., 2009)**
  - **první potvrzení významu miR-34a přímo in vivo u pacientů**
  - **je známa úloha BCL2 v patogenezi CLL, možný význam deregulace miR-34a**


miRNAs and microRNA binding sites are mutated in cancer

Germline abnormalities in miR15-16 are associated with CLL and breast cancer



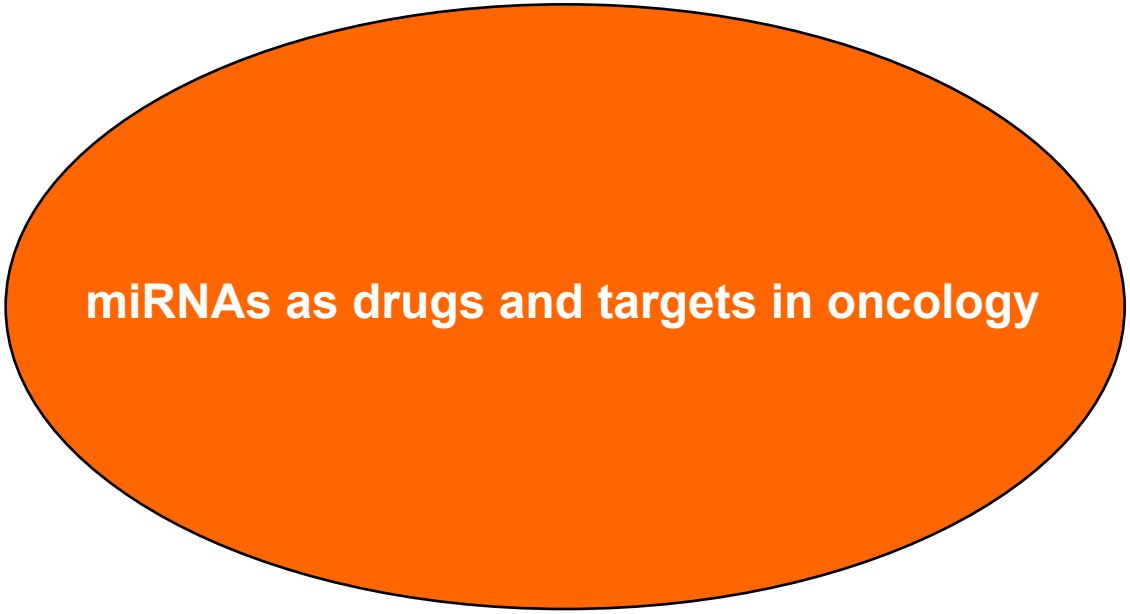
(Calin et al, N Engl J Med, 2005; Raveche et al, Blood 2007)

courtesy of G. Calin

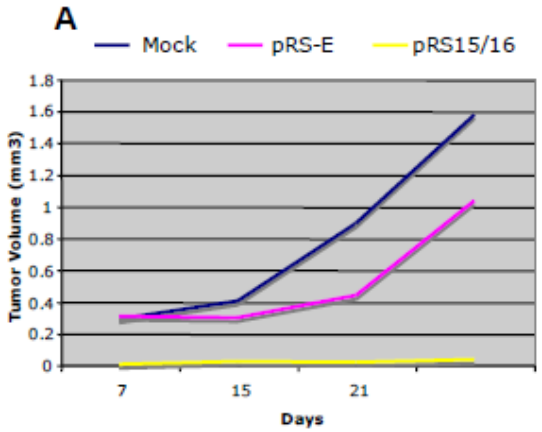
Table 4. A catalog of sequence variations in microRNAs and ultraconserved genes - DNA sequence variations with functional effects in ncRNAs.

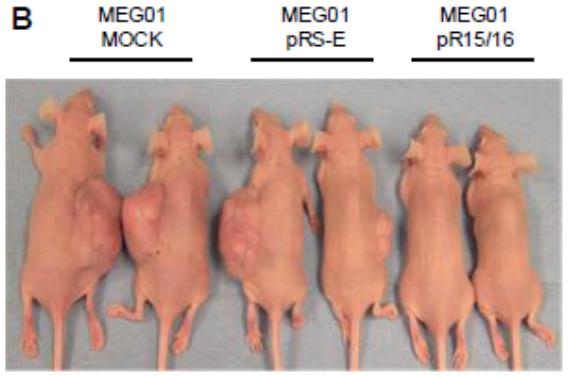
miRNA	Variation	Location	Type of cancer	Functional consequences	Reference
let-7e	(GtoA) + 19 nt	3' of miRNA (pri-miRNA)	Human cancers	Decreased expression of mature miRNA	(38)
miR-15a/16-1 cluster	Human germ-line (CtoT) +7nt ; NZB specific (AtoT)+6nt	3' of miRNA (pri-miRNA)	Sporadic and familial CLL; B-lymphoproliferative disease in mice	Decreased expression of mature miRNA and failure to decrease BCL2 protein levels ; Decreased levels of miR-16 expression in lymhoproliferative tissues	(14); (15)
miR-17	C/T	Pri-miRNA	Breast Cancer	Conformational changes in the predicted secondary structures with consequently alteration of the mature miR-17	(46)
miR-30c-1	G/A	Pre-miRNA	Breast Cancer	Conformational changes in the predicted secondary structures with consequently alteration of the mature miR-30c-1	(46)
miR-125a	SNP	Mature miR position 8	Not reported	Alteration of pri-miRNA processing	(35)
miR-146	rs2910164 G/C	Precursor	Papillary thyroid carcinoma predisposition; Hepatocellular Carcinoma; Familial breast/ovarian cancer	Decreased expression of mature miRNA; G-allelic miR-146a precursor increased production of mature miR-146a compared with C-allelic one	(40); (43); (44)
*miR-146a**	rs2910164 G/C	Precursor	Thyroid Cancer;	Epistasis through the production of additional mature miRs: miR-146a*G and miR-146a*C;	(45);
mir-196a	rs11614913 CC	Mature miR position 12	Non-small cell lung cancer	Decreased expression of mature miRNA	(37)
miR-196a2	rs11614913 CC ; rs11614913 C/T	Pre-miRNA	Lung Cancer ; Esophageal Cancer	Might affect mature miR-196a expression and target mRNA-binding activity ; Affect esophageal cancer risk	(47) (42)

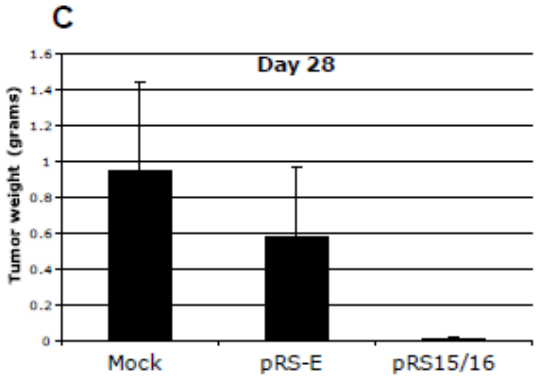
Wojcik et al., 2009


miRNAs as drugs and targets in oncology

miR-16 & miR-15 based Gene Therapy in CLL







Calin et al., 2008

Summary

MicroRNAs are involved in the regulation of „everything“

MicroRNAs are abnormaly expressed in cancer, which makes them also attractive as prognostic markers

Deregulation of microRNAs may be directly responsible for disease pathogenesis (miR-15-16 – deletion 13q14)

MicroRNAs are important proto-oncogenes (miR-155, miR-17-92)

MicroRNAs can work as tumor supressors (let-7, miR-34a)

MicroRNAs represent a potential therapeutic target

Thank you for your attention

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