

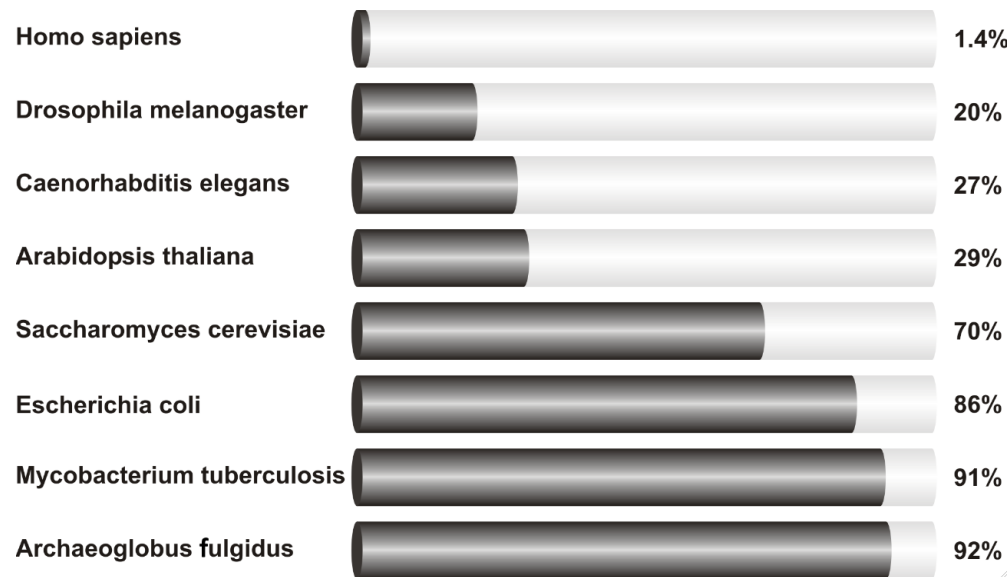
Svět nekódujících RNA

kódující geny představují méně než 2% celkové sekvence genomu

vs.

minimálně 90% lidského genomu je aktivně transkribováno

čím více je organizmus komplexní, tím více obsahuje nekódujících RNA



Recent evidence suggests that the non-coding RNAs (ncRNAs) may play major biological roles in cellular development, physiology and pathologies. NcRNAs could be grouped into two major classes based on the transcript size: small ncRNAs and long ncRNAs.



REVIEW

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Novel classes of non-coding RNAs and cancer

Jiri Sana^{1,2}, Petra Faltejskova^{1,2}, Marek Svoboda¹ and Ondrej Slaby^{1,2,3*}

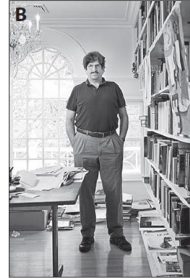
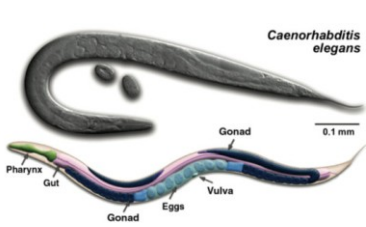
Nové třídy nekódujících RNA

Table 1: Types of recently discovered human non-coding RNAs.

	Class	Symbol	Characteristic	Disease / biological function associations
Small	MicroRNAs	miRNAs	18-25 nt; account 1-2% of the human genome; control the 50% of protein-coding genes; guide suppression of translation; Drosha and Dicer dependent small ncRNAs	Initiation of various disorders including many, if not all, cancers / regulation of proliferation, differentiation, and apoptosis; involved in human development
	all interfering RNAs	siRNAs	19-23 nt; made by Dicer processing; guide sequence specific degradation of target mRNA;	great potential in diseases treatment / posttranscriptional gene silencing mainly through RISC degradation mechanism; defence against pathogenic nucleic acids
	si-interacting RNAs	piRNAs	26-30 nt; bind Piwi proteins; Dicer independent; exist in genome clusters; principally restricted to the germline and somatic cells bordering the germline	relationship between piRNAs and diseases has not yet been discovered / involved in germ cell development, stem self-renewal, and retrotransposon silencing
	sall nucleolar RNAs	snoRNAs	60-300 nt; enriched in the nucleolus; in vertebrate are excised from pre-mRNA introns; bind snoRNP proteins	association with development of some cancers / important function in the maturation of other non-coding RNAs, above all, rRNAs and srRNAs; miRNA-like snoRNAs regulate mRNAs
	oter-associated small RNAs	PASRs	20-200 nt; modified 5' (capped) ends; coincide with the transcriptional start sites of protein- and non-coding genes; made from transcription of short capped transcripts	relationship with diseases has not yet been discovered / involved in the regulation of the transcription of protein-coding genes by targeting epigenetic silencing complexes
	Transcription Initiation RNAs	tRNAs	- 18 nt; have the highest density just downstream of transcriptional start sites; show patterns of positional conservation; preferentially located in GC-rich promoters	
	Centromere repeat associated small interfering RNAs	crasiRNAs	34-42 nt; processed from long dsRNAs;	relationship between crasiRNAs and diseases has not yet been discovered / involved in the recruitment of heterochromatin and/or centromeric proteins
	Telomere-specific small RNAs	tel-sRNAs	- 24 nt; Dicer independent; 2'-O-methylated at the 3' terminus; evolutionarily conserved from protozoa to mammals; have not been described in human up to now	relationship between tel-sRNAs and diseases has not yet been discovered / epigenetic regulation
	Pyknonas		subset of patterns of variable length; form mosaics in untranslated and protein-coding regions; more frequently in 3' UTR	expected association with cancer biology / possible link with posttranscriptional silencing of genes, mainly involved in cell communication, regulation of transcription, signaling, transport, etc.
	Long non-coding RNAs	Long intergenic noncoding RNAs	lincRNAs	ranging from several hundreds to tens of thousands nts; lie within the genomic intervals between two genes; transcriptional cis-regulation of neighbouring genes
Long intronic noncoding RNAs			lie within the introns; evolutionary conserved; tissue and subcellular expression specified	aberrantly expressed in human cancers / possible link with posttranscriptional gene silencing
Telomere-associated ncRNAs		TERRAs	100 bp - >9 kb; conserved among eukaryotes; synthesized from C-rich strand; polyadenylated; form inter-molecular G-quadruplex structure with single-stranded telomeric DNA	possible impact on telomere-associated diseases including many cancers / neovative regulation of telomere length and activity through inhibition of telomerase
Long ncRNAs with dual functions			both protein-coding and functionally regulatory RNA capacity	deregulation has been described in breast and ovarian tumors / modulate gene expression through diverse mechanisms
Pseudogene RNAs			code for a protein; potential to regulate their protein-coding cousin; made through retrotrans-position; tissue specific	and cancer progression / regulation of tumor suppressors and oncogenes by acting as microRNA decoys
	Transcribed-ultraconserved regions	T-UCRs	longer than 200 bp; absolutely conserved between orthologous regions of human, rat, and mouse; located in both intra- and intergenic regions	expression is often altered in some cancers; possible involvement in tumorigenesis / antisense inhibitors for protein-coding genes or other ncRNAs

HISTORIE

1993 Ambros, Ruvkun – objev miRNA lin-4



1998 Fire, Mello – popis RNA interference

1999 Tuschl, Zamore, Bartel, Sharp
-RNAi 21-23 fragmenty



2006 Nobel Prize winners
Andrew Fire and Craig Mello for their discovery of the RNAi mechanism.

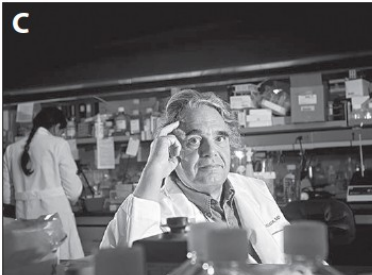
2000-2001 Hannon
-Ago2, Dicer

Nature, 1998

Cell, 1993

2002 Zamore

RNAi a miRNA sdílejí svoji efektorovou dráhu



2002 Croce, Calin miR-15, miR-16 u CLL

2004 Croce

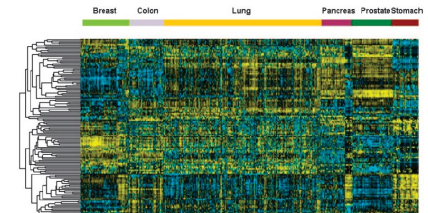
50% miRNA genů na fragilních částech chromozomů

2006 Croce

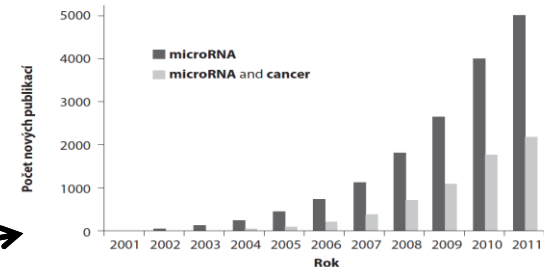
Deregulace miRNA v nádorové tkáni

...

2007 První originální práce na téma miRNA v onkologii v ČR 😊



PNAS, 2004



Obr. 1.2. Počet nových publikací v databázi medicínských informací PubMed při zadání klíčového slova „microRNA“ nebo kombinace „microRNA“ a „cancer“.





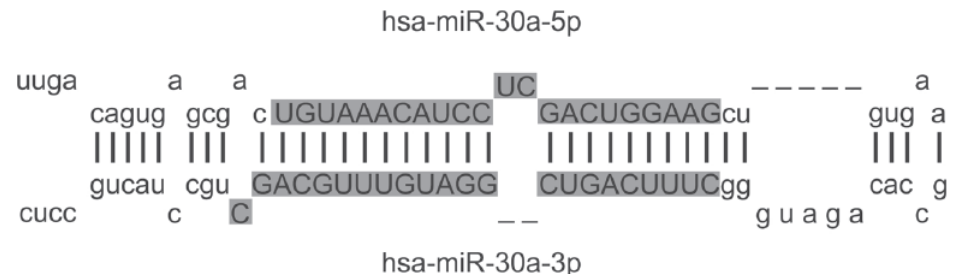
Victor Ambros
2012, CSHL

Definice mikroRNA

MikroRNA je definována jako krátká, 21–23 nukleotidů dlouhá jednořetězcová RNA, která vzniká z dlouhého primárního transkriptu (pri-miRNA) a vlásenkové prekurzorové struktury (pre-miRNA) účinkem ribonukleáz v jádře a cytoplazmě a jejíž hlavní funkcí je post-transkripční regulace genové exprese.

Kritéria:

- 1) krátká RNA musí být jednoznačně detekovatelná pomocí Northern blotu, RT-PCR nebo jiné standardní metody umožňující detekci RNA,
- 2) krátká RNA se musí vyskytovat v kmenové části vlásenkové, asi 70 nukleotidů dlouhé prekurzorové struktury,
- 3) sekvence krátké RNA a jejího prekurzoru musí být fylogeneticky konzervovaná (s výjimkou druhově specifických miRNA),
- 4) inhibice klíčových ribonukleáz v biogenezi miRNA musí vést k poklesu hladin krátké RNA a k akumulaci její prekurzorové struktury.



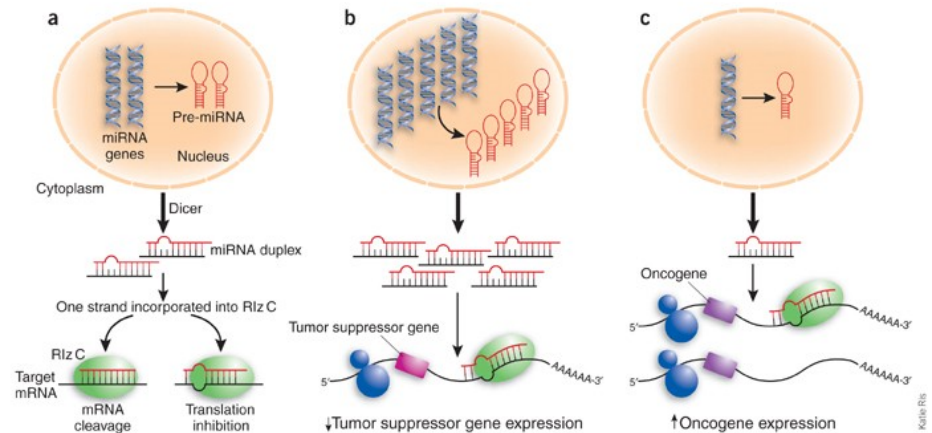
Obr. 1.5. Ukázka prekurzorové vlásenkové struktury mikroRNA.

Základní fakta o mikroRNA

- miRNA were firstly described by Ambros et al. (1993) in *C. elegans* (lin-4)
- Approx. 3% predicted human genes are miRNA genes
- 1390 annotated human microRNAs* (1000 -2000 predicted)
- miRNAs have potential to regulate up to one half of genes in human genome
- Phylogenetically well conserved
- Several miRNAs are encoded by more than one gene
- miRNA genes frequently form clusters and families (eg. cluster miR-17)
- miRNA genes are mainly localized in intergenic regions and in introns

miRNA family members can be very similar eg let-7 fam

mmu-let-7a:	UGAGGUAGUAGGUUGUAUAGUU
mmu-let-7b:	UGAGGUAGUAGGUUGUGUGGUU
mmu-let-7c:	UGAGGUAGUAGGUUGUAUGGUU
mmu-let-7d:	AGAGGUAGUAGGUUGCAUAGUU
mmu-let-7e:	UGAGGUAGGAGGUUGUAUAGUU
mmu-let-7f:	UGAGGUAGUAGAUUGUAUAGUU
mmu-let-7g:	UGAGGUAGUAGUUUGUACAGUU
mmu-let-7i:	UGAGGUAGUAGUUUGUGCUGUU

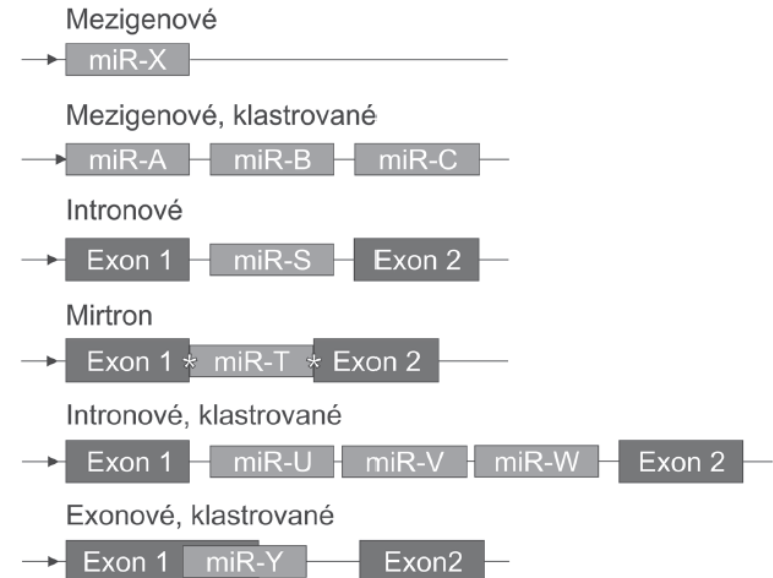


Struktura a distribuce genů pro mikroRNA v lidském genomu

- Geny pro miRNA jsou rozmístěny na všech lidských chromozomech s výjimkou chromozomu Y.
- Přibližně 50 % miRNA se nachází v podobě klastrů (u člověka 55 klastrů), které jsou přepisovány jako polycistronní primární transkripty.

Dle vztahu ke známým a definovaným transkripčním jednotkám dělíme na

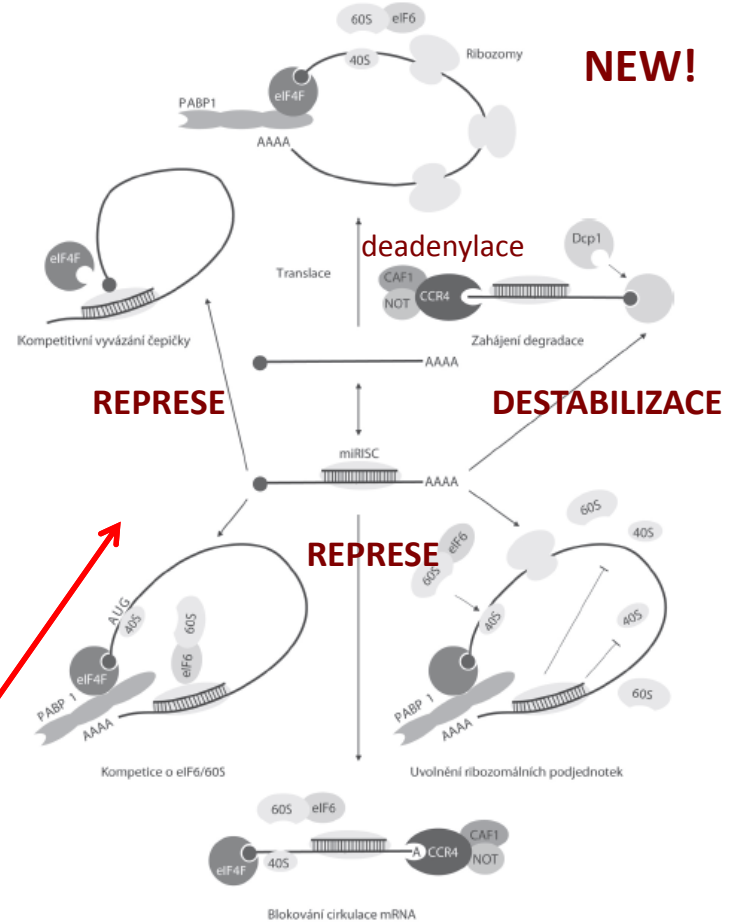
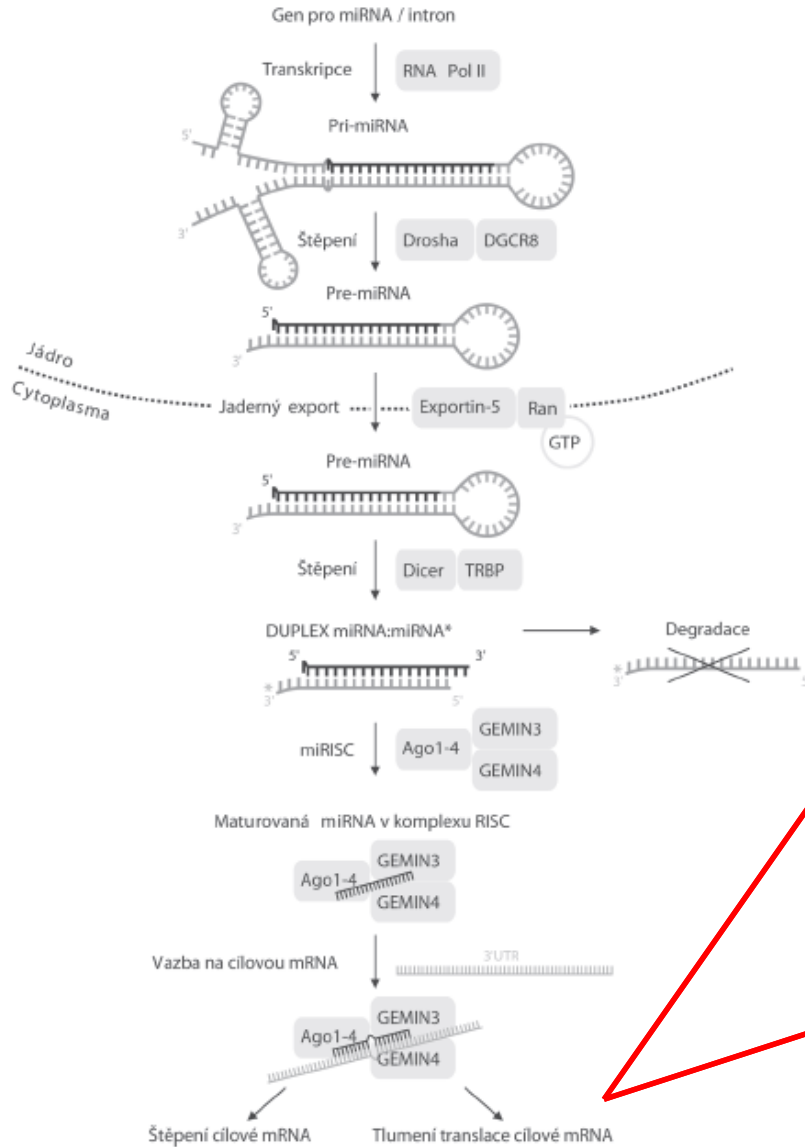
- Mezigenové miRNA (přibližně 50 %)
- Intronové miRNA (přibližně 40 %)
- miRtrony
- Exonové miRNA



Obr. 1.3. Genomová lokalizace genů pro mikroRNA.

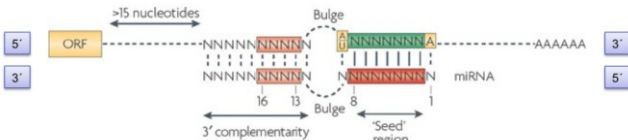
Kanonický model biogeneze a funkce mikroRNA

NEW!



DESTABILIZACE mRNA!!!

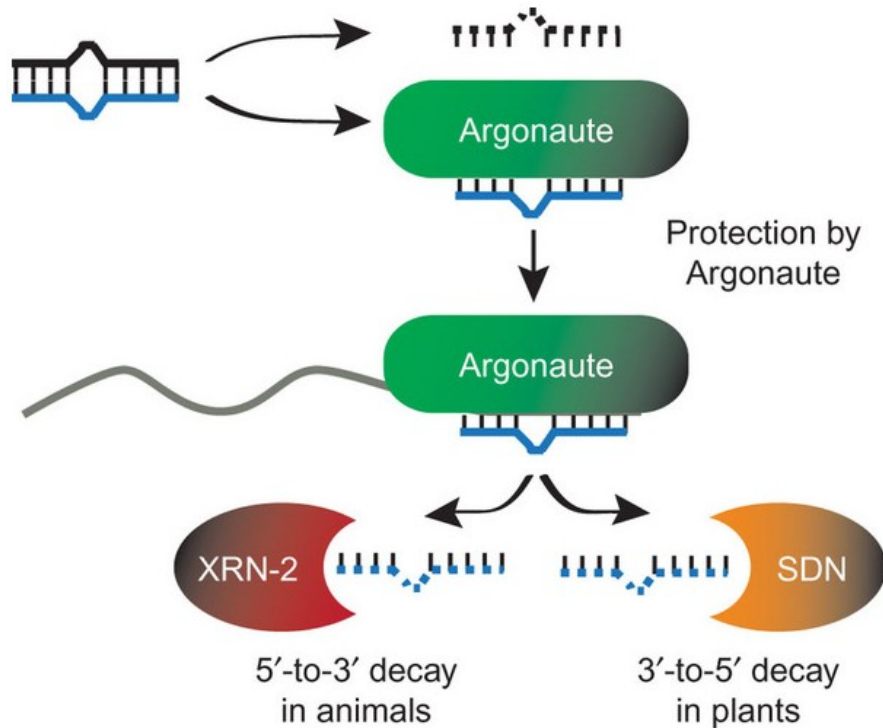
Pokles hladiny proteinových produktů cílových genů je ve více než 80% způsoben poklesem hladin mRNA v důsledku jejich destabilizace a nikoliv translační represí. (využití RPF)



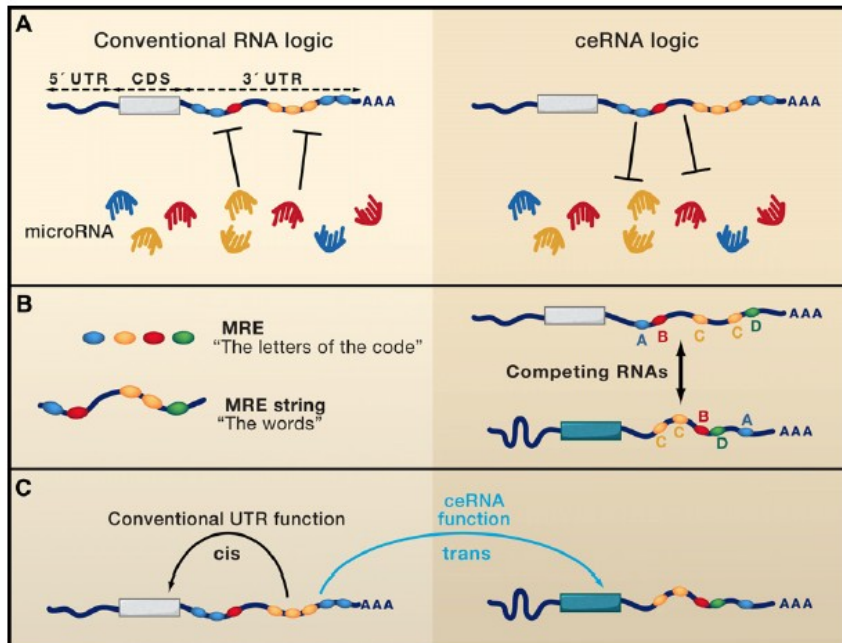
Biologický poločas a rozpad mikroRNA

MiRNA obecně představují vysoce stabilní molekuly, poločas miRNA v buněčných liniích nebo orgánech dosahuje mnoho hodin nebo dokonce i dnů!

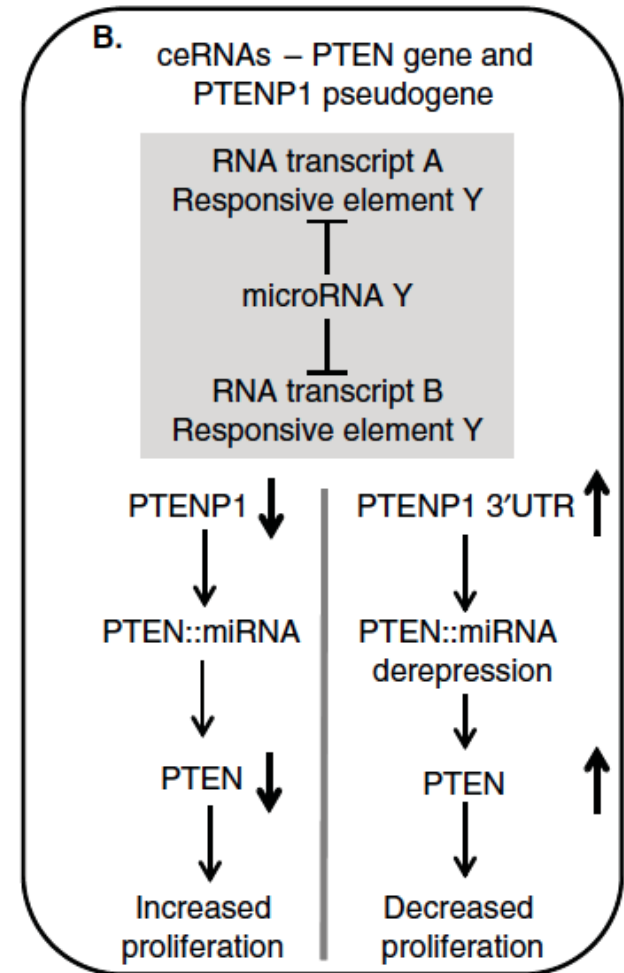
MiRNA mohou být regulované například blokováním jejich vazebných míst na mRNA pomocí RNA-vazebných proteinů. Volné miRNA jsou méně stabilní



Opačné vnímání regulačního mechanismu miRNA → mRNA, kdy tzv. „competing endogenous RNA“ (ceRNA) regulují hladinu daného transkriptu tak, že soutěží o vazbu miRNA, která má schopnost daný transkript post-transkripčně regulovat. Všechny transkripty, které mají vazebná místa pro jednu miRNA, a tak možnost soutěžit její vazbu a vzájemně ko-regulovat svou expresi, potom vytvářejí komplexní síť (ceRNA network).



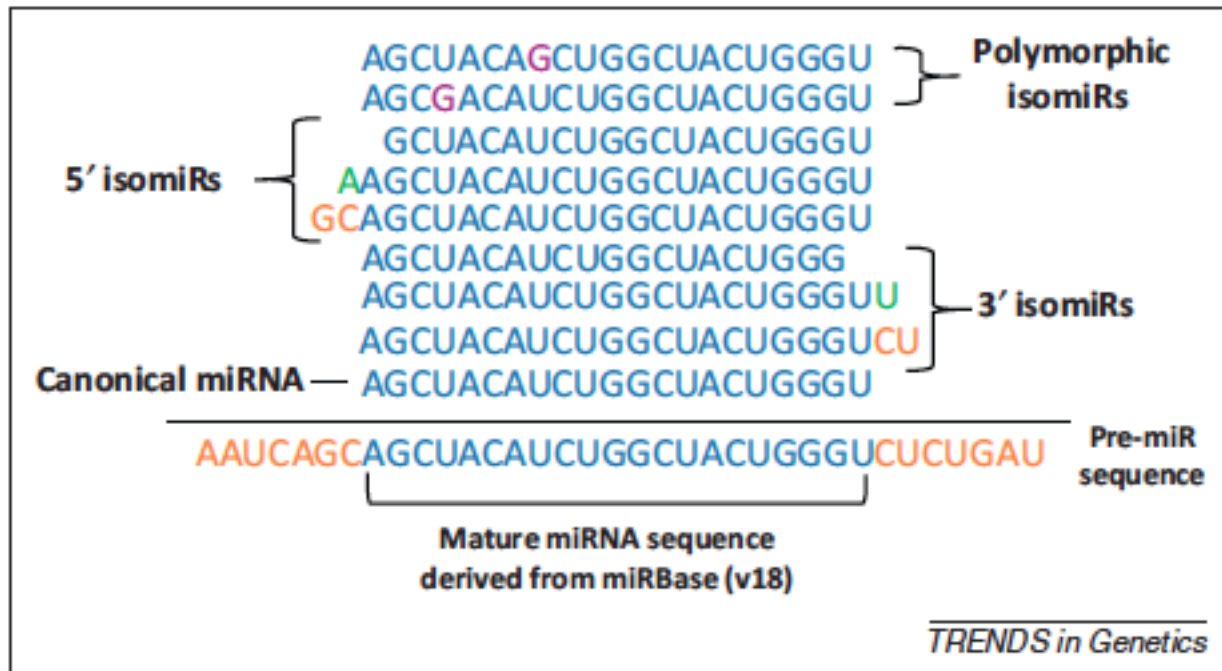
Salmena, Pandolfi, Cell, 2011



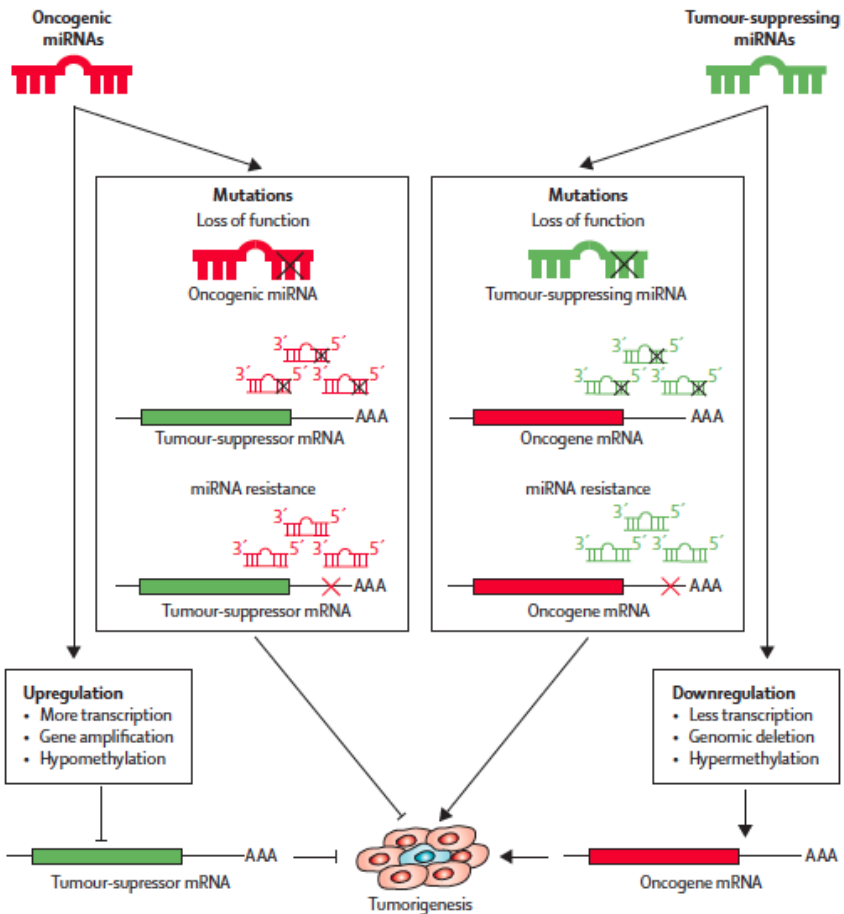
Alemeida, Calin, Expert Opin. Biol. Ther., 2012

- Poprvé popsány v roce 2008 na základě výsledků sekvenačních studií (Morin et al., 2008, Genome Res)
- Jedna oblast kódující miRNA může vést ke vzniku mnoha rozdílných izomiRs.
- Zastoupení jednotlivých izomiRs je tkáňově specifické a může se měnit v rámci odpovědi na různé biologické signály.
- Vznik 5' a 3' izomiRs je podmíněn štěpením Droshou a Dicerem nebo účinkem transferáz.
- Polymorfní izomiRs mohou vznikat v důsledku SNP (vzácné) nebo editací mikroRNA (A-I).

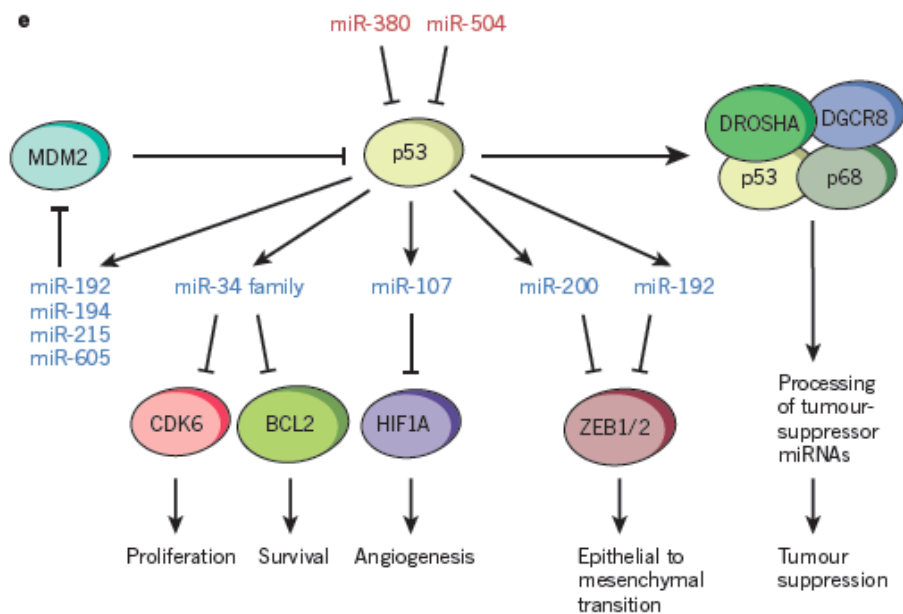
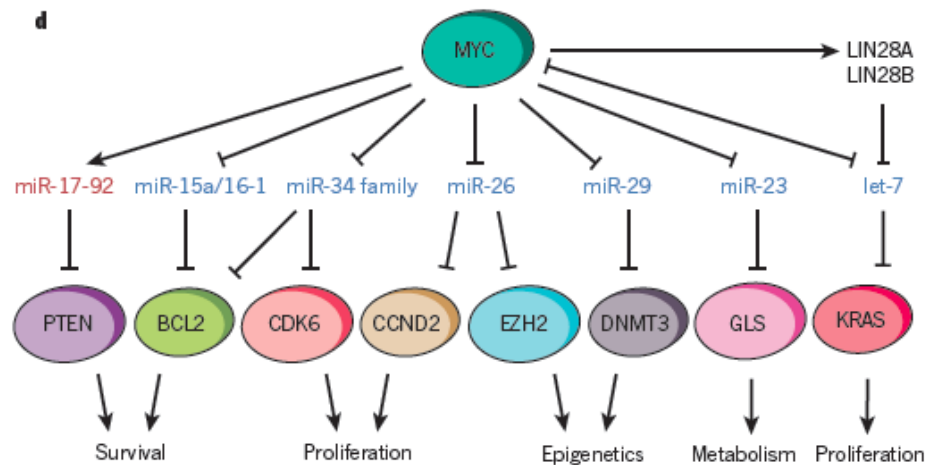
Funkční dopady: specificita pro cílové mRNA, Ago loading, biologický poločas



MikroRNA jako onkogeny nebo nádorové supresory

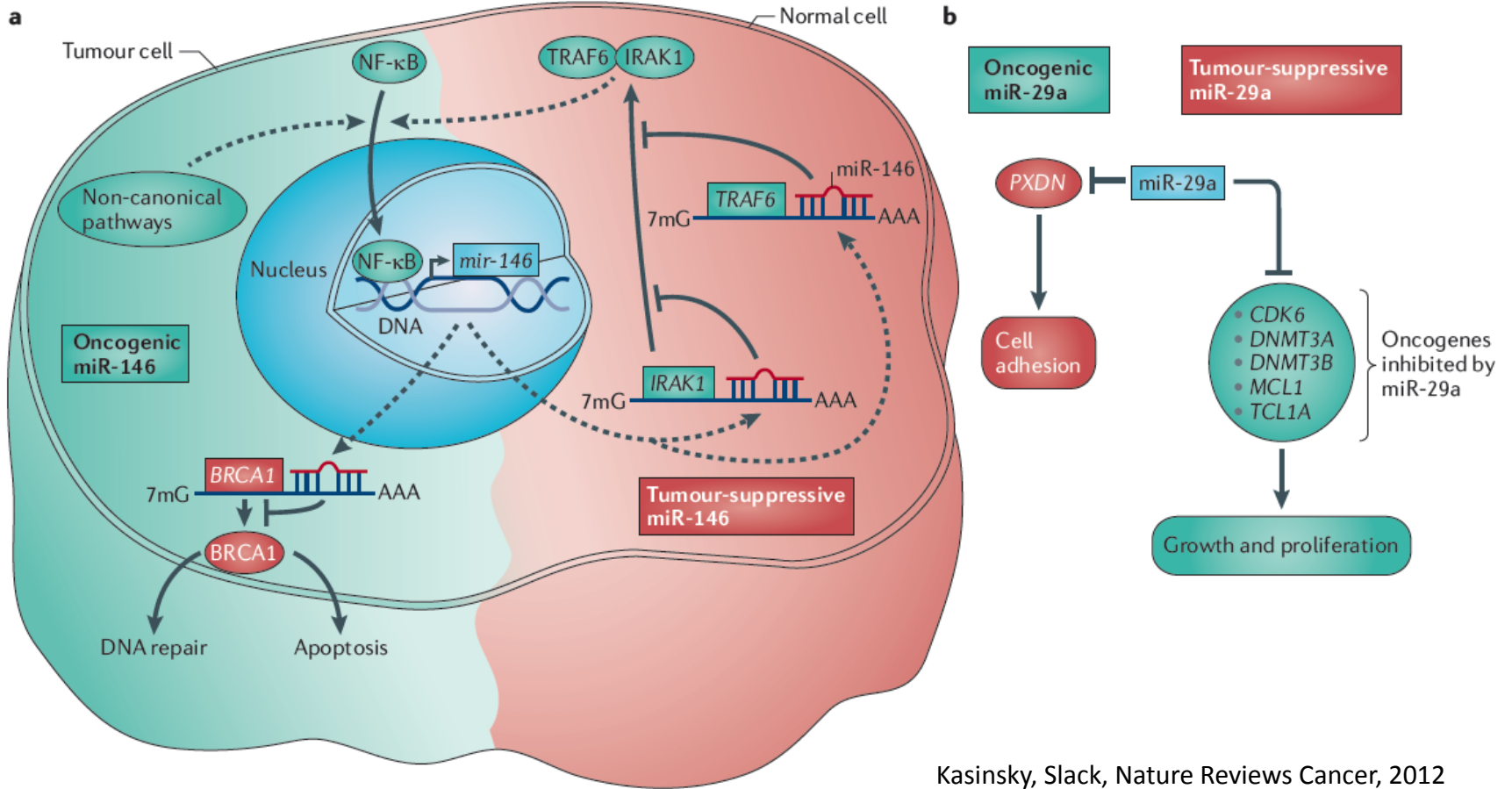


Kong et al, Lancet Oncology 2012



Lujambio, Nature 2012

MikroRNA jako onkogeny nebo TS v závislosti na kontextu



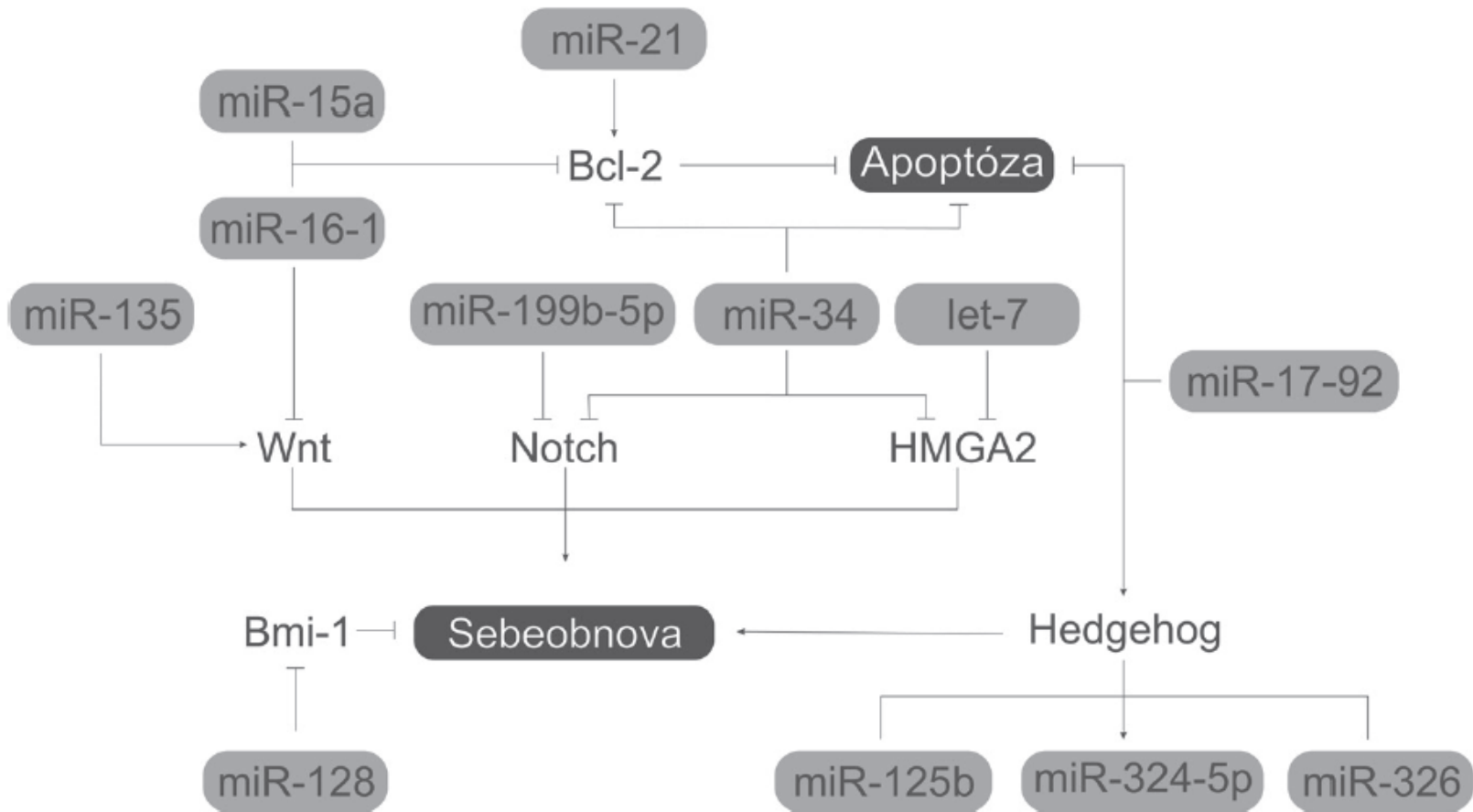
Kasinsky, Slack, Nature Reviews Cancer, 2012

MikroRNA v patogenezi nádorových onemocnění

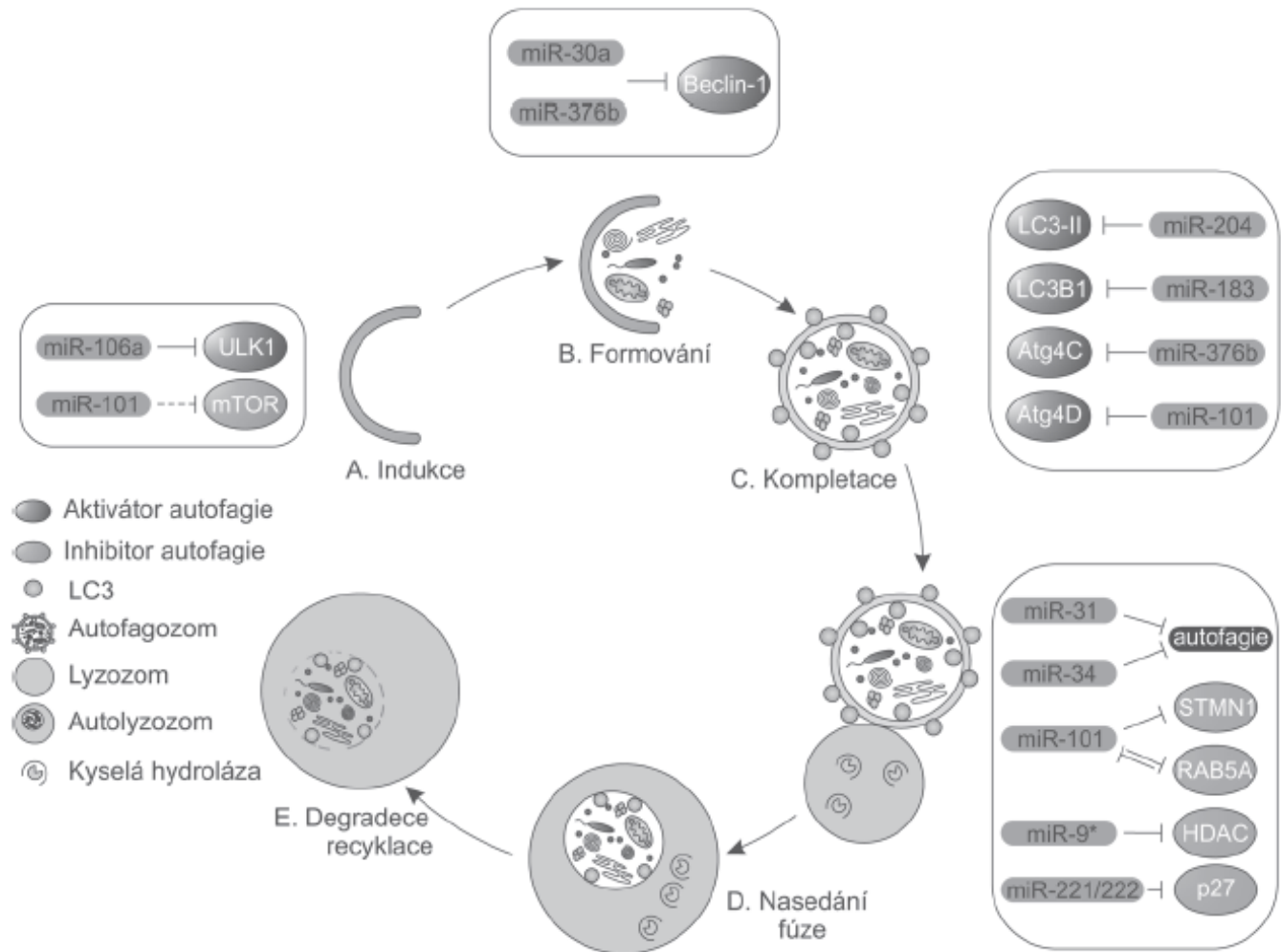
MikroRNA jsou zapojeny do regulace všech šesti klasických a čtyř nových znaků maligního nádoru dle Weinberga a Hanahana. (Hallmarks of Cancer: The Next Generation, Cell, 2011)

Znaky maligní transformace	MikroRNA
soběstačnost v produkci růstových signálů	let-7, miR-7, miR-21, miR-34b/c, miR-125a/b, miR-126, miR-128, miR-143, miR-145, miR-199, miR-331
necitlivost k signálům zastavujícím buněčný cyklus	klastr miR-17-92 a miR-106b-25, miR-34a, miR-221, miR-222
poškozená apoptóza	miR-15, miR-16, miR-21, miR-29, rodina miR-34, miR-125b, miR-133, miR-145, miR-221, miR-222, miR-605
neomezený replikační potenciál	rodina miR-34, miR-138, miR-290, miR-372, miR-373
angiogeneze	miR-23, miR-24, miR-26, miR-27, miR-103, miR-107, miR-126, miR-181, miR-210, miR-213, miR-221, miR-222, miR-296
invazivita a metastazování	rodina miR-9, miR-10b, miR-21, miR-31, miR-122, miR-146a, miR-148a/b, miR-155, rodina miR-200, miR-210, miR-373, miR-520c
narušený energetický metabolismus	miR-23a/b, miR-122, miR-375
únik imunitnímu systému	miR-17-5p, miR-20a, miR-93, miR-106b, miR-372, miR-373 a miR-520c, miR-155, hcmv-miR-UL112
genomová nestabilita	klastr miR-17-92, miR-15, miR-16, let-7
nádorový zánět	miR-9, klastr miR-17-92, miR-21, miR-101, miR-146a, miR-192

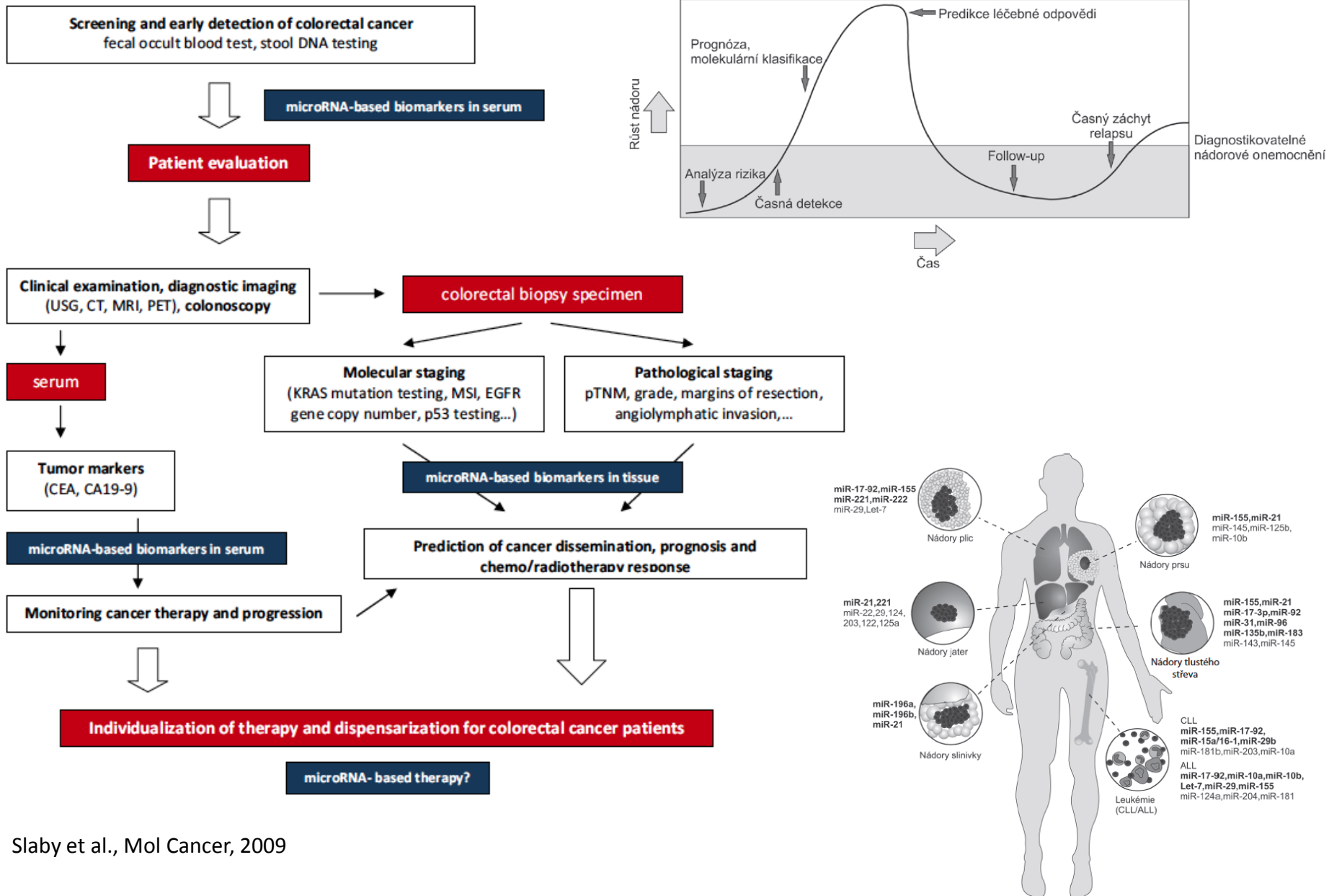
ch buněk.



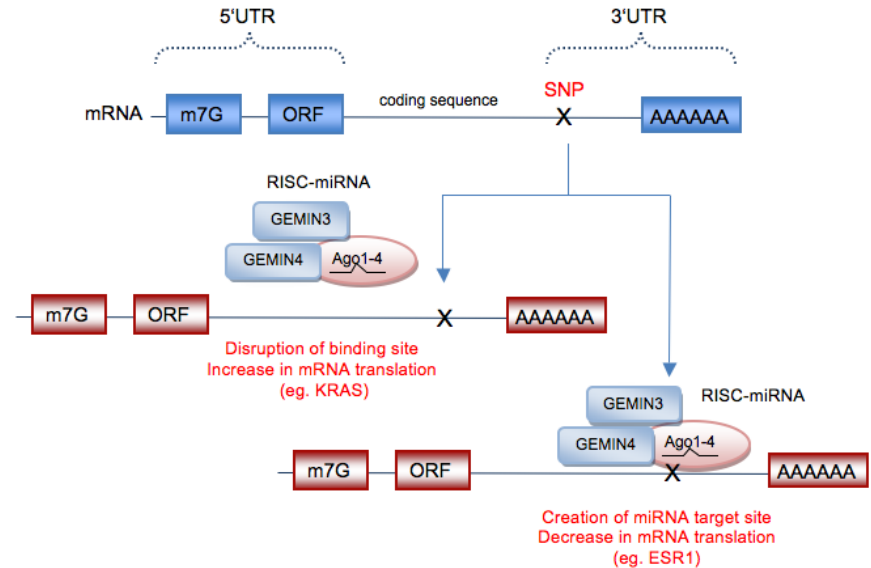
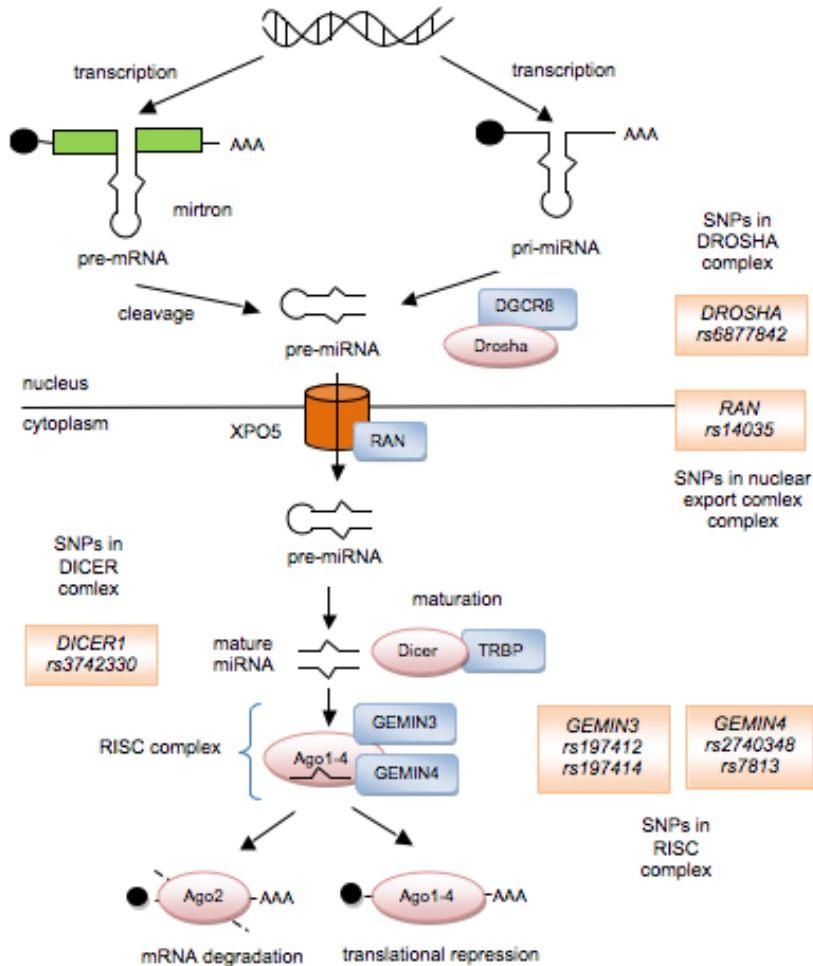
ch procesů autofagie, jako jsou signalizace mTOR, skládání komplexu ULK1, interakce proteinu Beclin-1 nebo ATG4 signalizace.



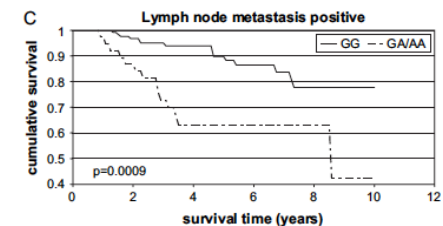
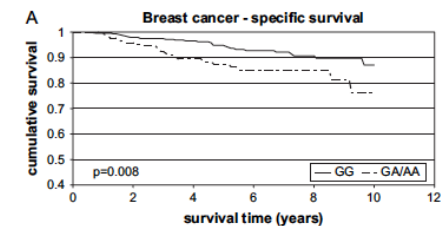
MikroRNA jako biomarkery



SNP a mikroRNA, rizikové faktory



Slaby et al., J Cell Mol Med, 2011



1. SNPs v biogenetické dráze miRNA
2. SNPs v sekvenci pri, pre a maturované miRNA
3. SNPs ve vazebných oblastech pro miRNA

G/A ITGB4 (miR-34a) Brendle et al, Carcinogenesis 2009

SNP a mikroRNA

SNP ve vazebných místech pro miRNA regulované chemoprotektanty

NEW!

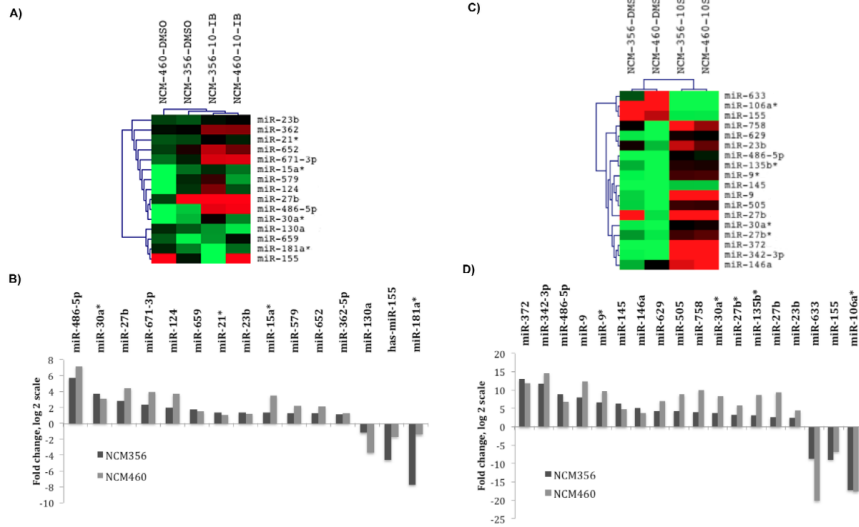


Table 1 MiRNAs affected by isothiocyanates treatment.

miRNA	ITCs	Tumor tissue	Association with cancer	Chromosome localization	Validated targets	References
miR-27b	up	down/up	NBL, glioma	9q22.32	PPARG, MEF2C, MMP13	25, 40, 41
miR-23b	up	down/up	CRC, HCC, RCC	9q22.32	SMAD3, FZD7, MAP3K1	21-24, 42
miR-155	down	up	CRC, BC, NSCLC, PC, EC, see Tili et al. 2009	21q21.3	SHIP1, FOXO3A, SOCS1, CKAP5, STAB1, SMAD5, BMPs	26-30

NBL neuroblastoma, CRC colorectal cancer, HCC hepatocellular carcinoma, RCC renal cell carcinoma, BC breast cancer, NSCLC non-small cell lung cancer, PC pancreatic cancer, EC endometrial cancer, PPARG peroxisome proliferator-activated receptor gamma, MEF2C myocyte enhancer factor 2C, MMP13 matrix metalloproteinase 13, SMAD3 SMAD family member 3, FZD7 frizzled family receptor 7, MAP3K1 mitogen-activated protein kinase kinase 1, SHIP1 phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1, FOXO3 forkhead box O3, SOCS1 suppressor of cytokine signaling 1, CKAP5 cytoskeleton associated protein 5, STAB1 stabilin 1, SMAD5 SMAD family member 5, BMPs bone morphogenetic proteins

Table 2 In silico identified SNPs in ITC-related miRNA binding sites and flanking regions.

SNP	Symbol	Gene name	Association with cancer*	Variation	MAF**	Targeting miRNA
SNPs in miRNA binding regions						
rs5186	AGTR1	angiotensin II receptor, type 1 tumor necrosis factor, alpha-induced	17	A/C	0.25	miR-155
rs710100	TNFAIP2	protein 2	15	A/G	0.29	miR-155
rs411103	PRKCB	protein kinase C, beta	90	A/T	0.33	miR-27b
SNPs in flanking regions of miRNA binding sites						
rs12009	HSPA9	heat shock 70kDa protein 5	20	A/G	0.48	miR-155
rs700082	RABGAP1	RAB GTPase activating protein 1	2	G/C	0.12	miR-155
rs1057035	DICER1	dicer 1, ribonuclease type III	96	C/T	0.36	miR-23b
rs10404	ADAM19	ADAM metalloproteinase domain 19 von Willebrand factor A domain	17	T/C	0.23	miR-23b
rs1939861	VWA5A	containing 5A	6	C/T	0.38	miR-27b
rs3757261	SIRT5	sirtuin 5	5	T/C	0.24	miR-27b

*The total number of hits in Pubmed when gene symbol is combined with keyword cancer

**Minor allele frequency in Caucasians

Table 3 Logistic regression analysis for the selected SNPs in the miRNA target sequences and their flanking regions.

Gene	SNP	Controls ^a		Cases ^a		-/+ versus +/+		OR ^b	95% CI	P-value	-/- versus +/+		P-value	P-value
		+/+	-/+	+/+	-/+	OR ^b	95% CI							
AGTR1	rs5186	125	73	14	104	78	15	1.25	(0.83, 1.90)	0.313	1.37	(0.62, 3.00)	0.42	0.239
TNFAIP2	rs710100	100	89	23	85	86	26	1.15	(0.76, 1.75)	0.507	1.33	(0.70, 2.53)	0.399	0.516
PRKCB	rs411103	77	108	27	75	97	25	0.96	(0.63, 1.47)	0.869	0.99	(0.52, 1.87)	0.947	0.841
HSPA9	rs12009	63	108	32	60	100	38	0.94	(0.60, 1.48)	0.831	1.19	(0.66, 2.17)	0.603	0.659
RABGAP1	rs700082	166	36	1	158	37	2	1.13	(0.68, 1.90)	0.73	2.76	(0.24, 32.06)	0.395	0.458
ADAM19	rs10404	91	32	96	82	20	17.5	0.49	(0.49, 1.15)	0.217	0.49	(0.25, 0.95)	0.036	0.183
VWA5A	rs1939861	114	74	15	107	72	19	1.13	(0.74, 1.73)	0.54	1.45	(0.69, 3.04)	0.351	0.312
SIRT5	rs3757261	79	93	31	91	80	27	0.74	(0.48, 1.14)	0.213	0.78	(0.42, 1.43)	0.402	0.169
		116	81	6	117	75	6	0.95	(0.63, 1.44)	0.839	0.92	(0.28, 2.98)	0.789	0.798

^aNumbers may not add up to 100% of subjects due to genotyping failure. All samples that did not give a reliable result in the first round of genotyping were resubmitted to up to two additional rounds of genotyping. Data points that were still not filled after this procedure were left blank.

^bORs were adjusted for sex and age. Only results for the codominant model are shown. Statistically significant results (P < 0.05) are shown in bold.

Evaluation of SNPs in miR-196-a2, miR-27a and miR-146a as risk factors of colorectal cancer

Renata Hezova, Alena Kovarikova, Julie Bienertova-Vasku, Milana Sachlova, Martina Redova, Anna Vasku, Marek Svoboda, Lenka Radova, Igor Kiss, Rostislav Vyzula, Ondrej Slaby

Table 1 Logistic regression analysis of genotype frequencies of single nucleotide polymorphisms rs11614913, rs895819 and rs2910164 in colorectal cancer cases and controls in the Czech population

	Control		CRC		OR ¹	95% CI	P value
	n	%	n	%			
miR-27a	A/A	93	43.87	88	44.67	1	0.996 ²
	A/G	94	44.34	86	43.65	0.98	(0.64-1.49)
	G/G	25	11.79	23	11.68	1.04	(0.54-1.98)
	AG + GG vs AA					1.01	(0.68-1.51)
	[G] vs [A]					0.999	(0.71-1.39)
miR-146a	Trend	212		197		0.99	(0.8-1.22)
	G/G	124	58.49	115	58.38	1	0.761 ²
	C/G	79	37.26	70	35.53	0.93	(0.61-1.41)
	C/C	9	4.25	12	6.09	1.31	(0.52-3.27)
	CG + CC vs GG					1.03	(0.69-1.54)
miR-196-a2	[C] vs [G]					1.37	(0.56-3.33)
	Trend	212		197		0.97	(0.79-1.19)
	C/C	87	41.04	82	41.62	1	0.6098 ²
	C/T	103	48.58	89	45.18	0.95	(0.62-1.45)
	T/T	22	10.38	26	13.2	1.32	(0.69-2.54)
miR-196-a2	CT + TT vs CC					1.01	(0.68-1.51)
	[T] vs [C]					1.04	(0.75-1.45)
	Trend	212		197		1.08	(0.8-1.46)

P-values are calculated according to Wald's test. ²P-values according to LR-test; ¹Age and sex adjusted; CRC: Colorectal cancer; OR: Odds ratio; CI: Confidence interval.

Cirkulující mikroRNA jsou přítomny ve dvanácti typech tělních tekutin

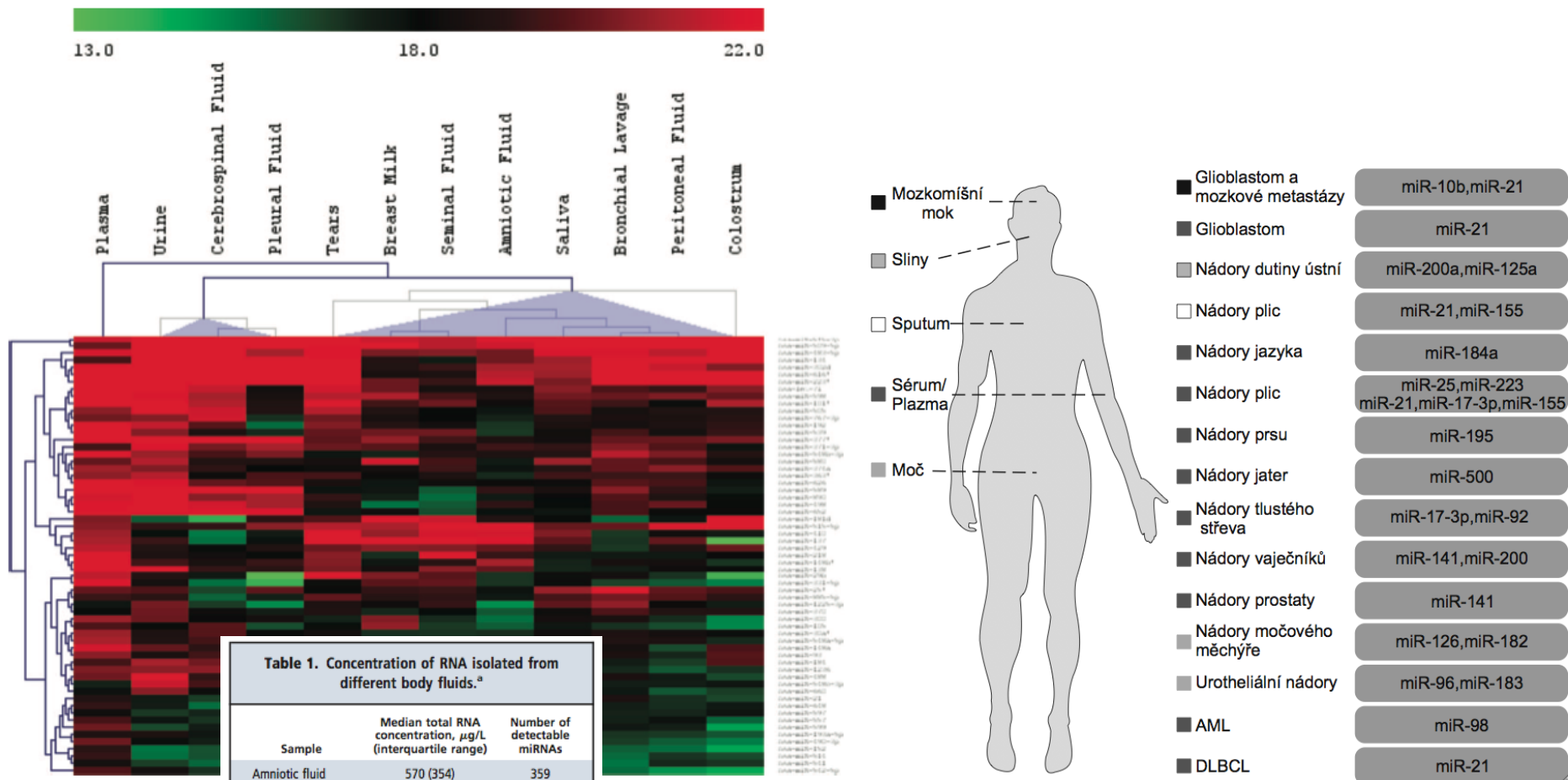


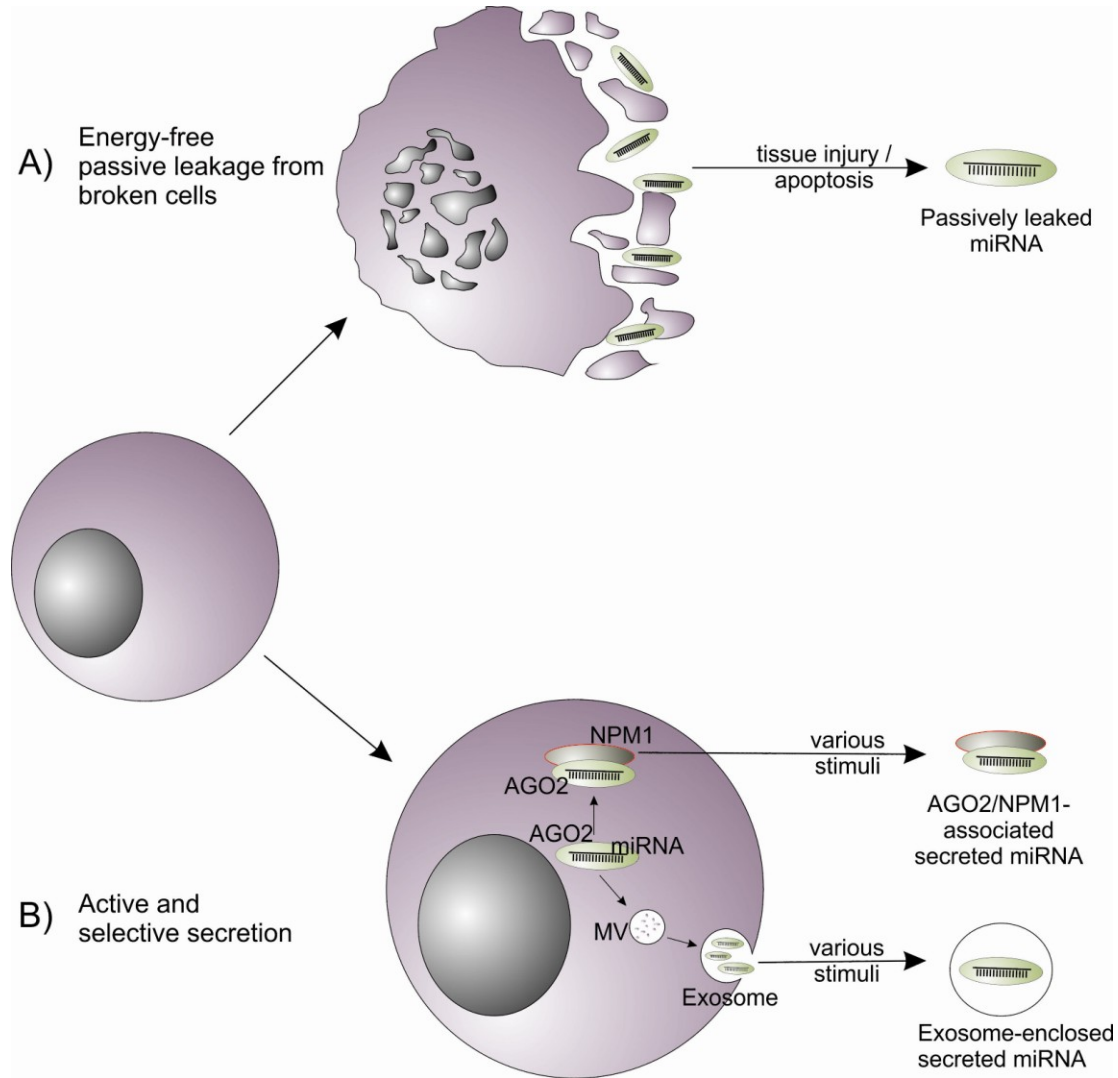
Table 1. Concentration of RNA isolated from different body fluids.^a

Sample	Median total RNA concentration, $\mu\text{g/L}$ (interquartile range)	Number of detectable miRNAs
Amniotic fluid	570 (354)	359
Breast milk	47 240 (73 180)	429
Bronchial lavage	1128 (886)	260
Cerebrospinal fluid	111 (66)	212
Colostrum	585 (NA)	386
Peritoneal fluid	775 (345)	397
Plasma	308 (104)	349
Pleural fluid	470 (190)	210
Saliva	1945 (2495)	458
Seminal fluid	17 770 (7673)	436
Tears	564 (631)	320
Urine	94 (129)	204

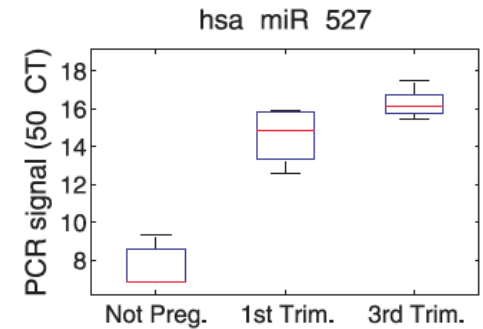
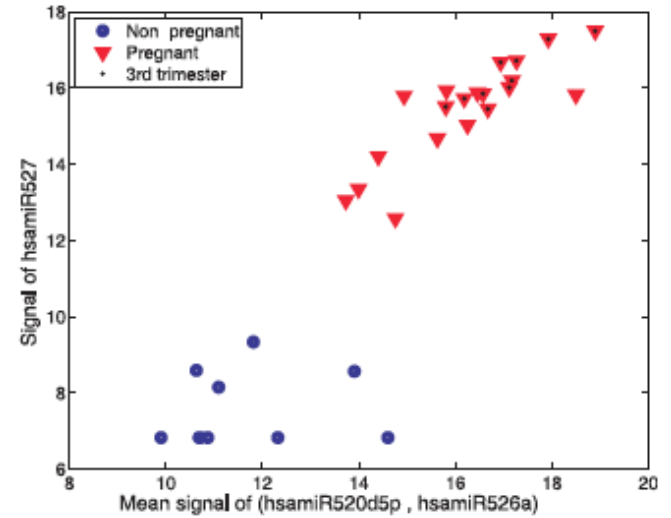
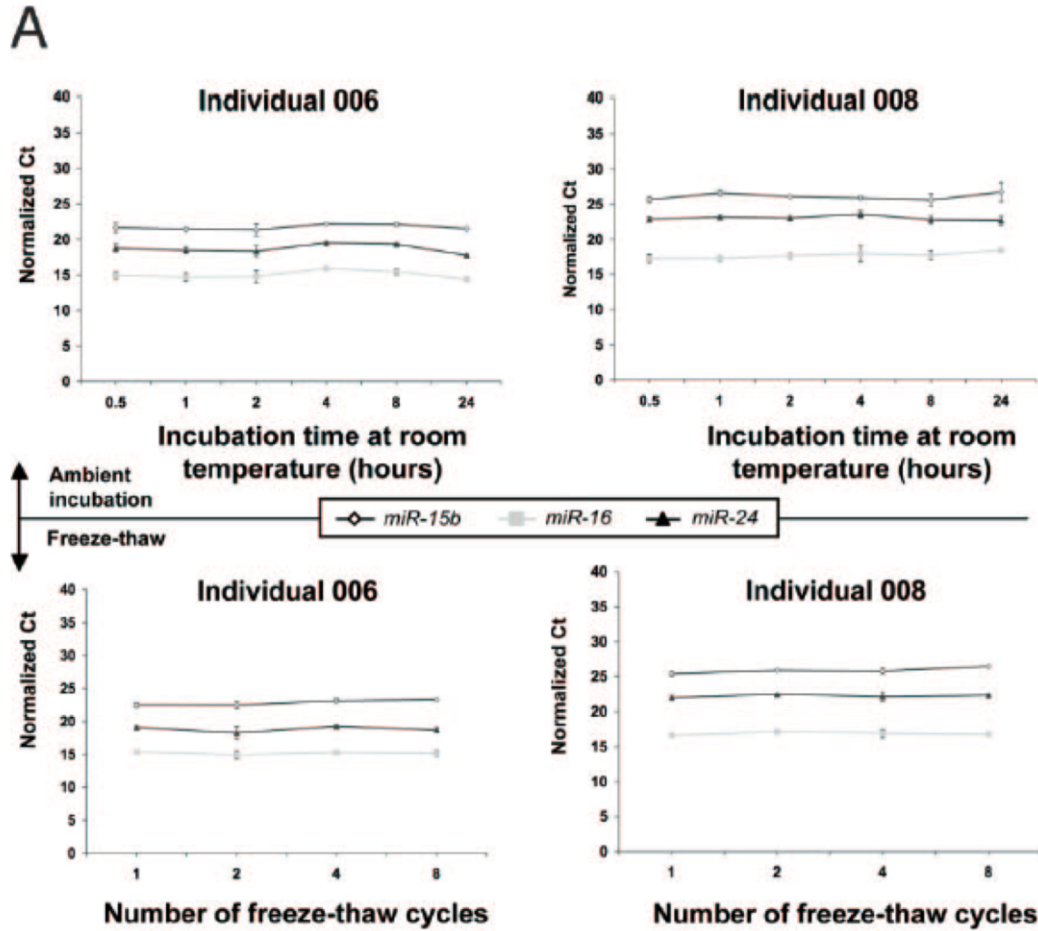
^a As estimated by the Agilent 2100 Bioanalyzer using the RNA 6000 Pico Total RNA chip, median concentration across all 5 samples except colostrum. The number of detected miRNAs in each body fluid is based on the number of miRNA species with a level of >80% of the global mean.

Weber et al., Clin Chem, 2010

Původ cirkulujících mikroRNA



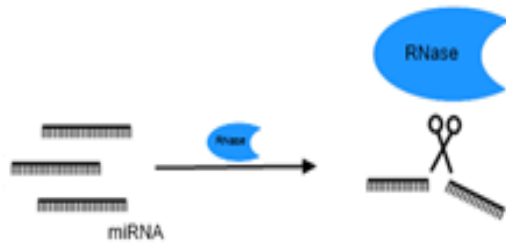
Stabilita cirkulujících mikroRNA



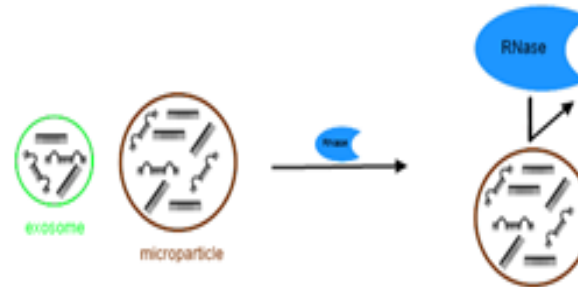
Gilad et al., 2008

Příčina stability cirkulujících mikroRNA

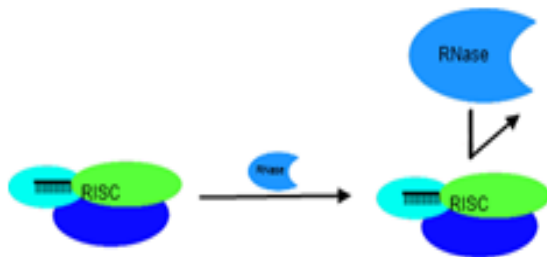
A. Naked miRNAs



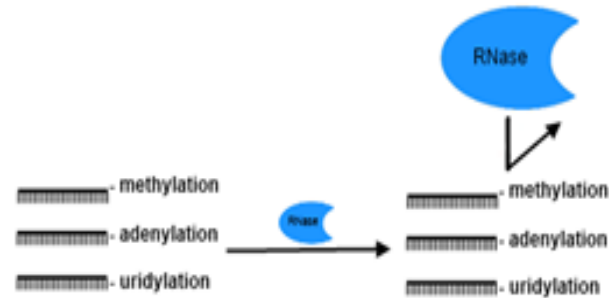
B. MiRNAs in microvesicles



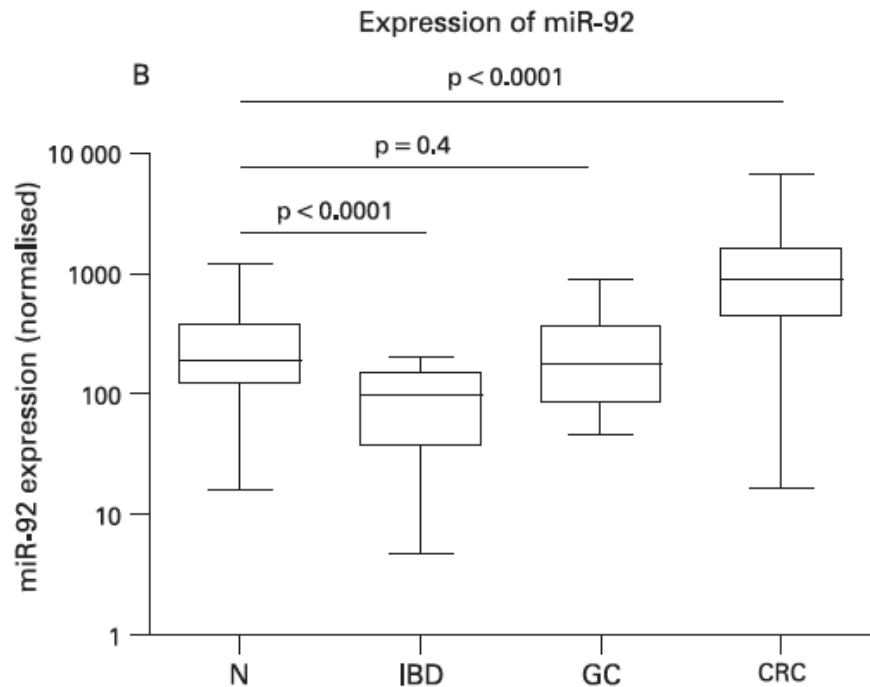
C. MiRNAs as a part of RISC



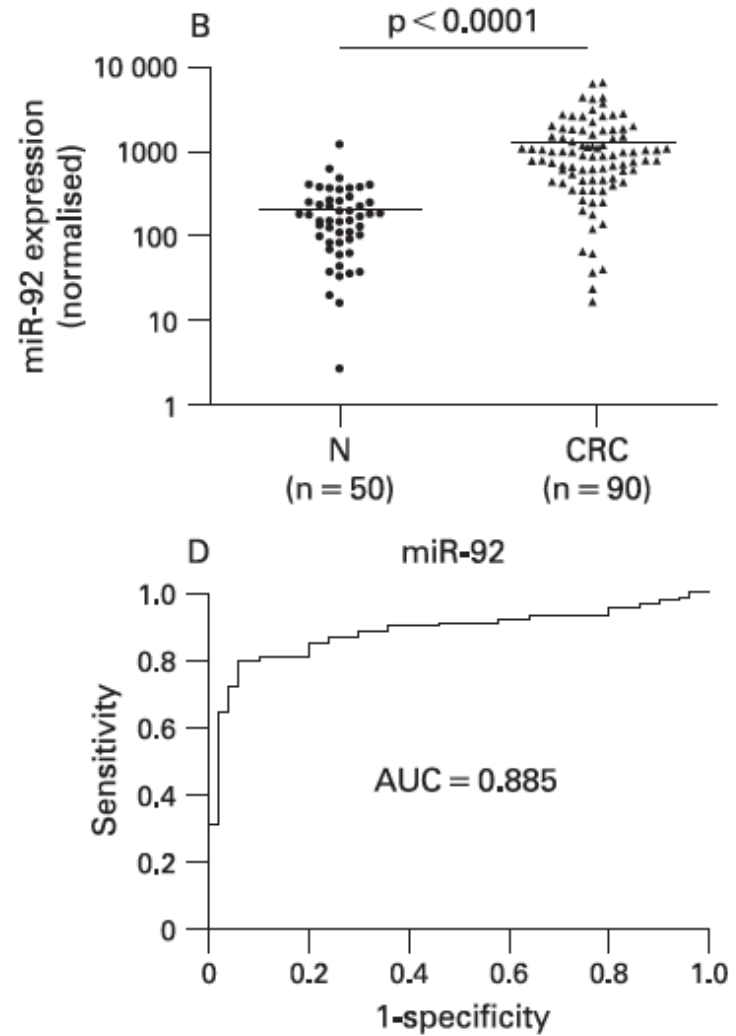
D. Modifications of MiRNAs (methylation, adenylation, uridylation)



Sérové mikroRNA jako diagnostické biomarkery u CRC



89% senzitivita
70% specificita

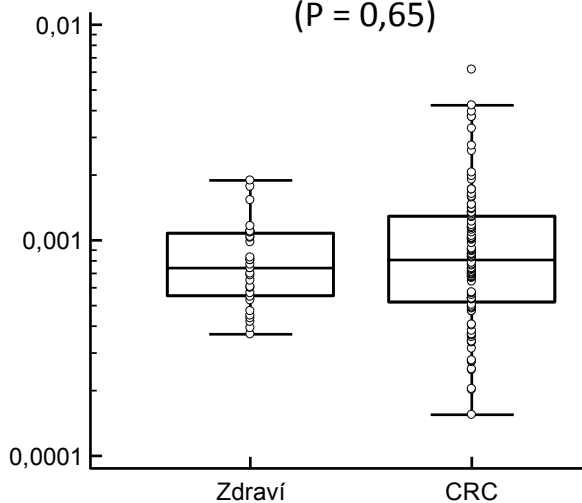


ALE.....

Sérové mikroRNA jako diagnostické biomarkery u CRC

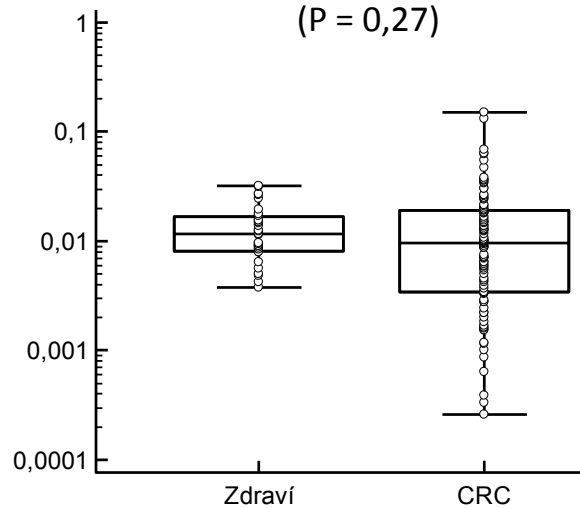
miR-17-3p

(P = 0,65)



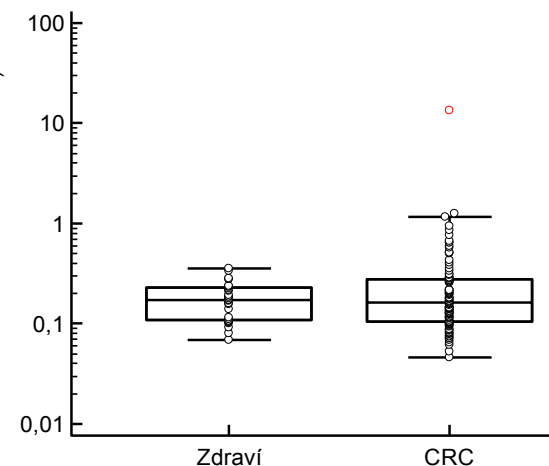
miR-29a

(P = 0,27)



miR-92a

(P = 0,90)



- **miR-17-3p, miR-29a, miR-92a, miR-135b**
- **endogenní kontrola: miR-16**
- 100 pacientů, 30 zdravých dárců

ČESKÁ REPUBLIKA

MicroRNAs in serum: diagnostic biomarkers of renal cell carcinoma

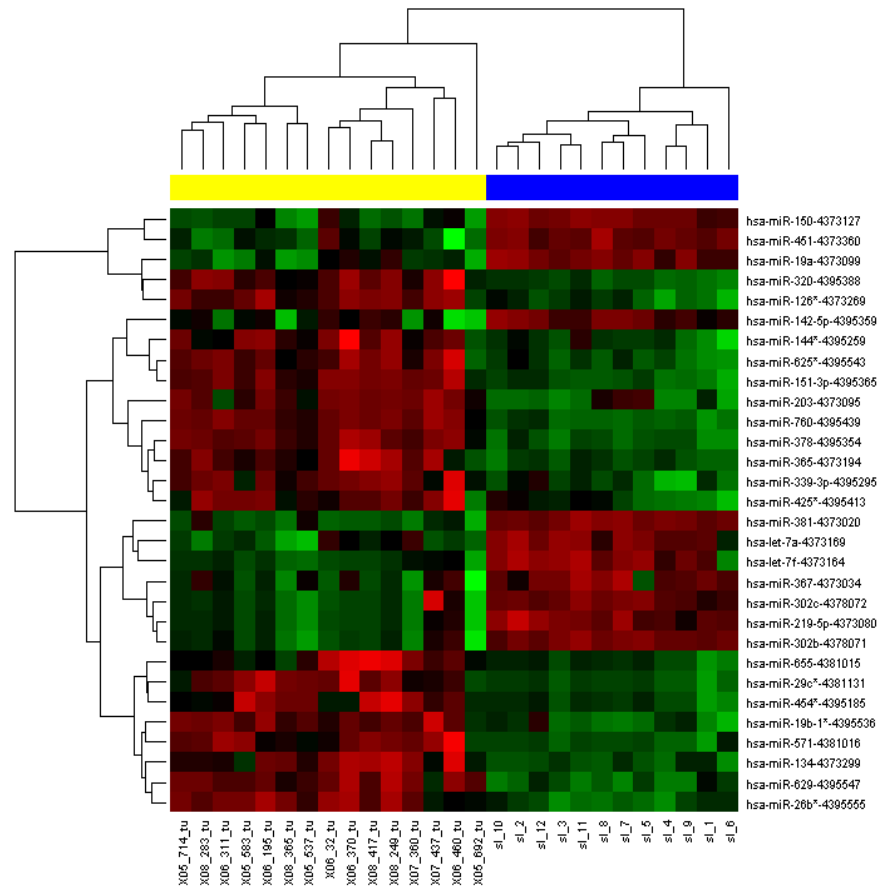
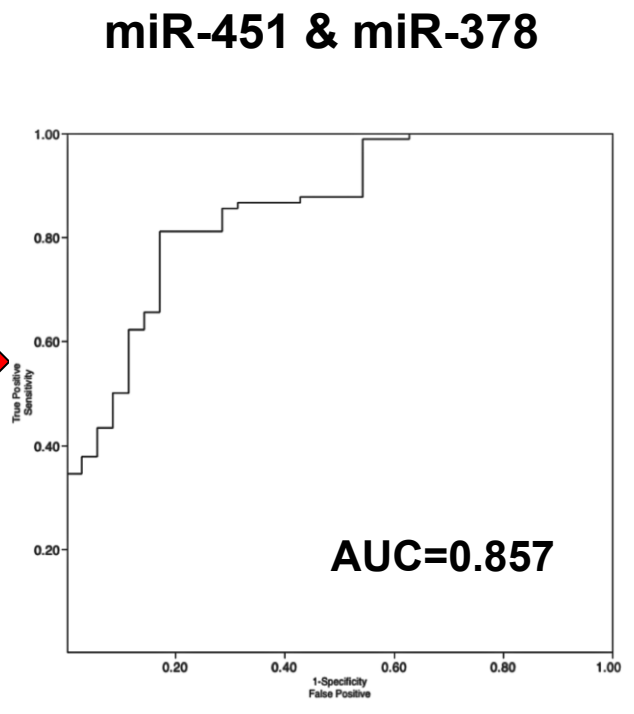
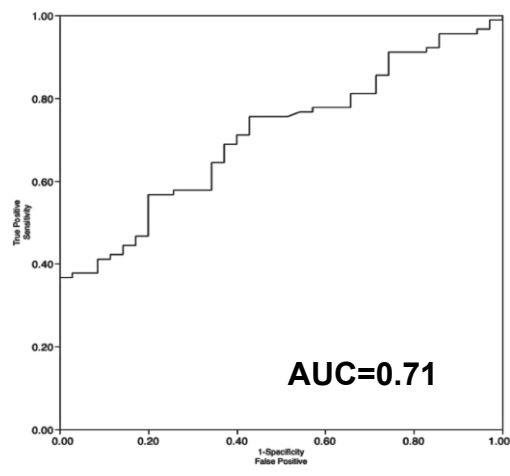
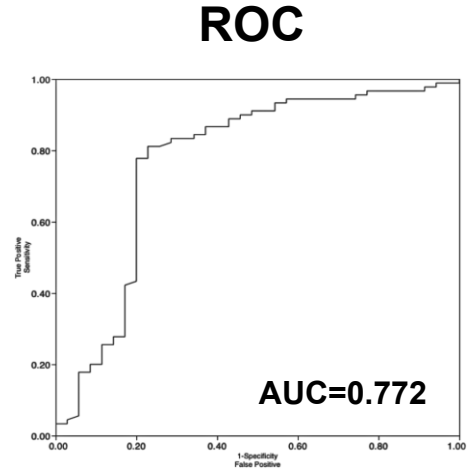
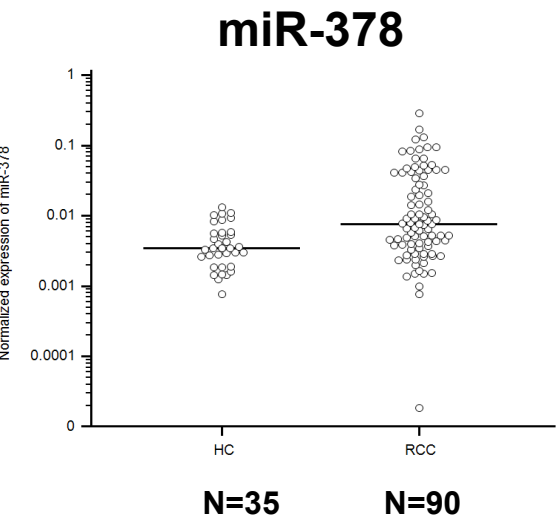
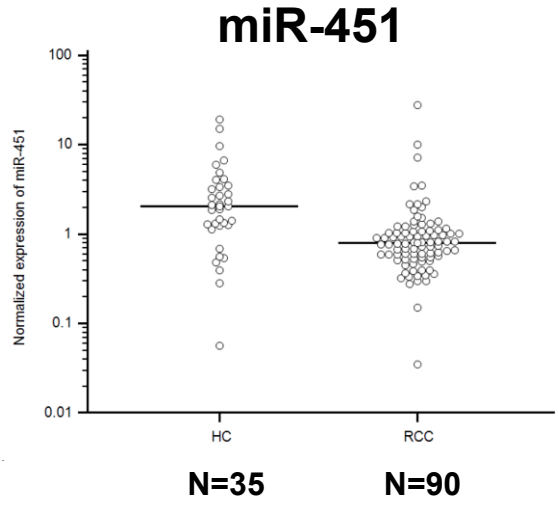


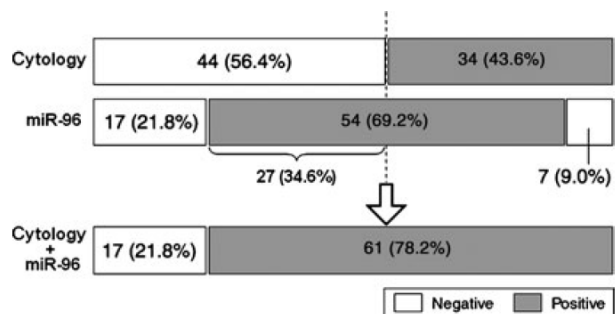
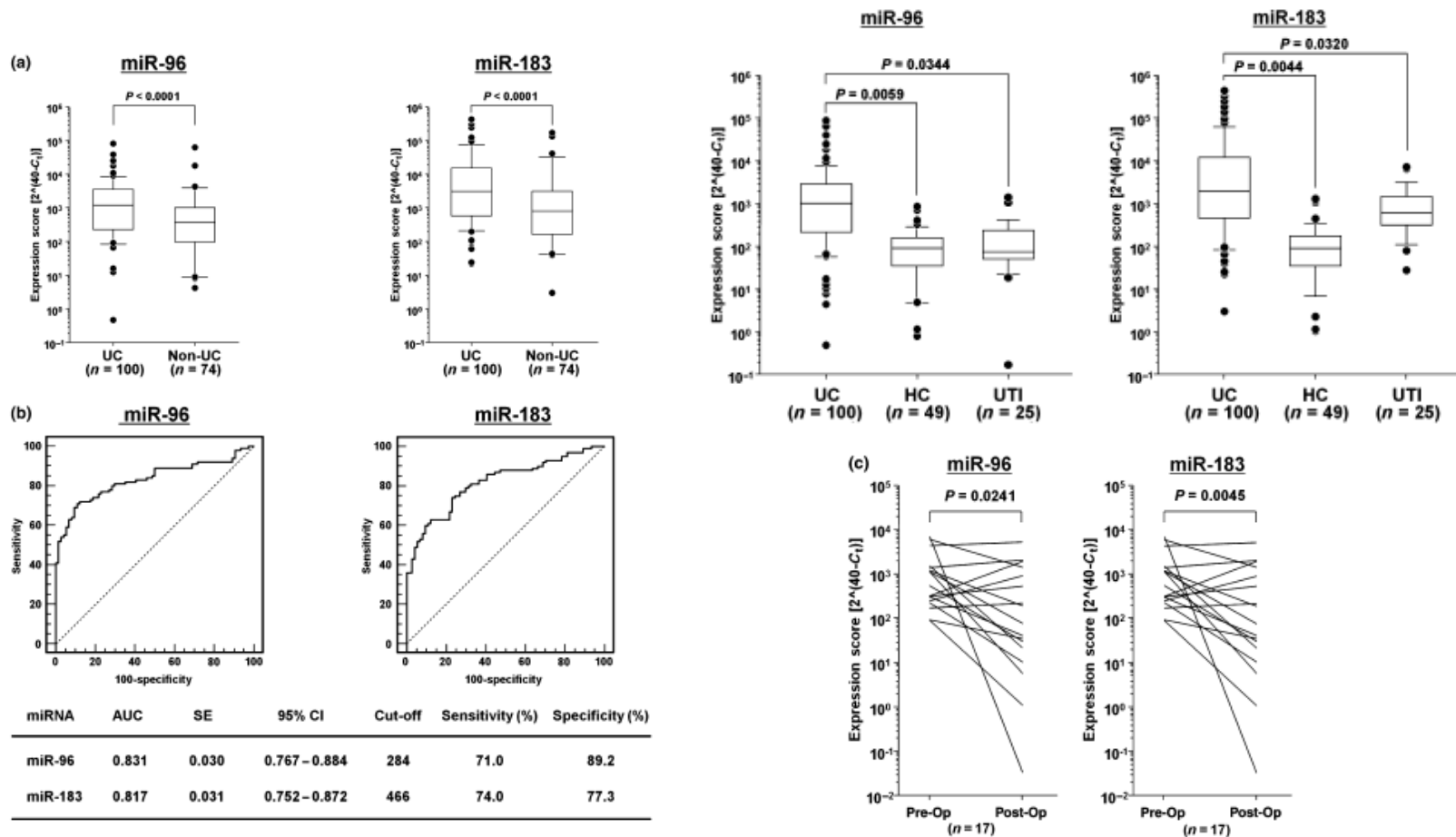
Figure 1. Serum of healthy controls and RCC patients clustered according to differentially expressed miRNAs (yellow color indicate serum samples of RCC patients, blue healthy controls, LIMMA method, $p < 0.0001$).

MicroRNAs in serum: diagnostic biomarkers of renal cell carcinoma



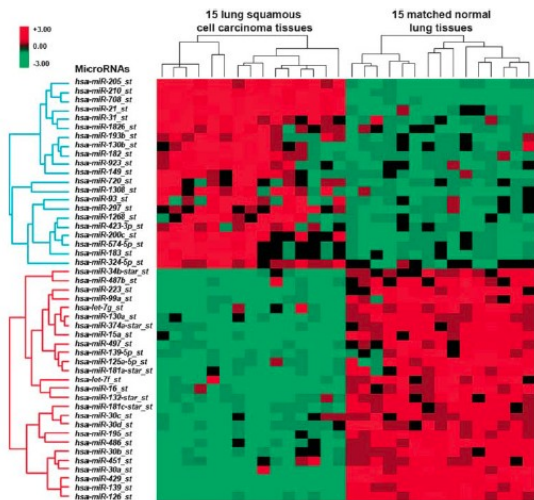
Sensitivity=81%
Specificity=83%

Močové mikroRNA jako diagnostické biomarkery uroteliálního karcinomu

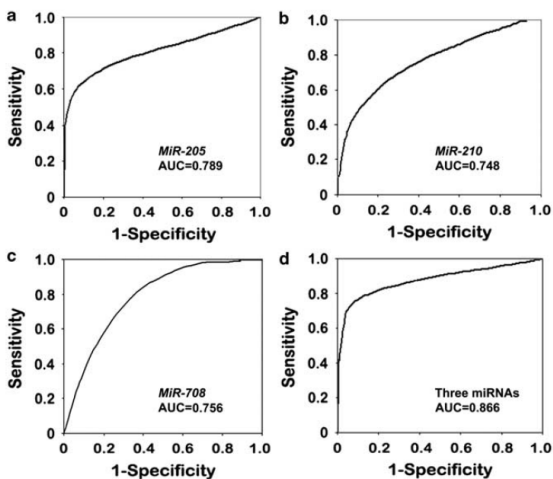


Yamada et al. Cancer Science, 2010

MikroRNA jako diagnostické biomarkery NSCLC ve sputu



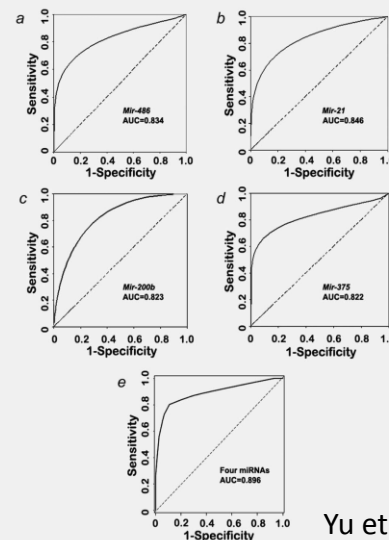
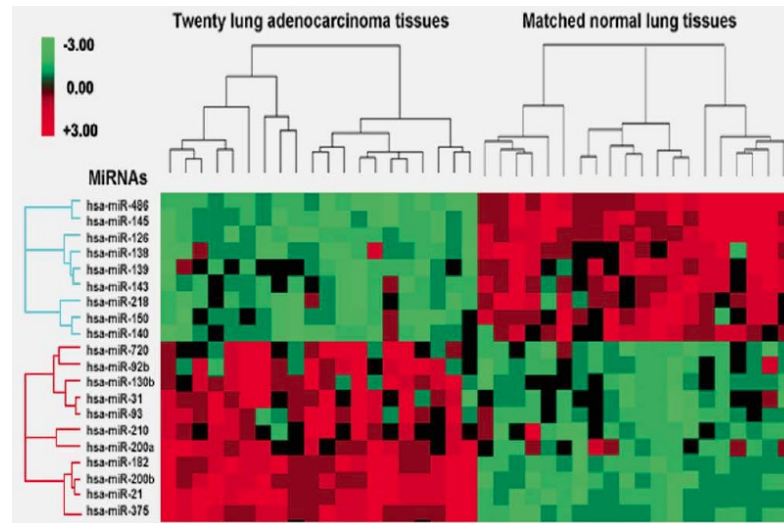
Xing et al, Mod Pat, 2010



SPINOCELULÁRNÍ KARCINOM

	Sensitivity, %	Specificity, %
Cases with all stages	72 (48/67)*	95 (52/55)
Cases with different stages		
I	71 (10/14)*	95 (52/55)
II	69 (11/16)*	95 (52/55)
III	74 (14/19)*	95 (52/55)
IV	72 (13/18)*	95 (52/55)

ADENOKARCINOM



Yu et al, IJC, 2010

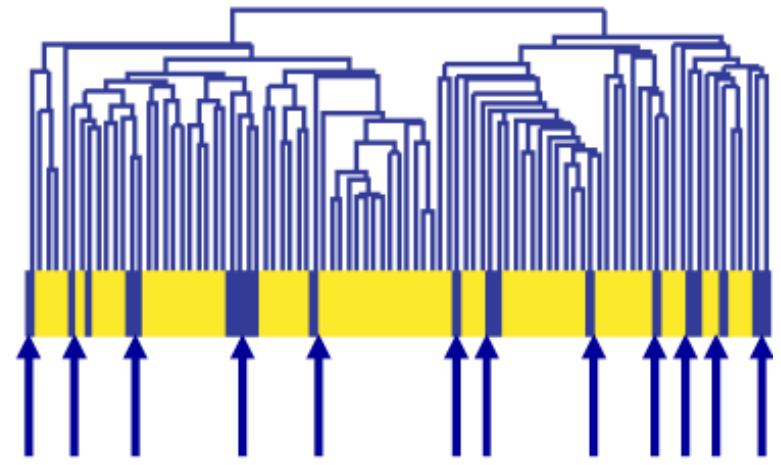
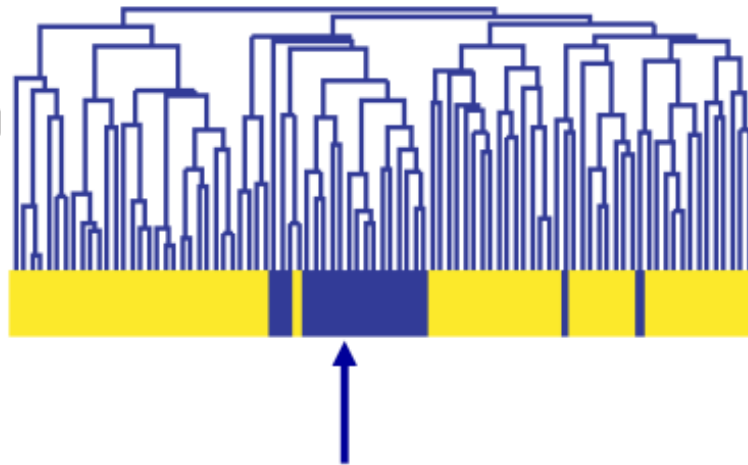
MikroRNA jako tkáňové biomarkery

· microRNA profiles appear superior to mRNA for cancer classification

miRNA

mRNA

■ GI
■ Non-GI

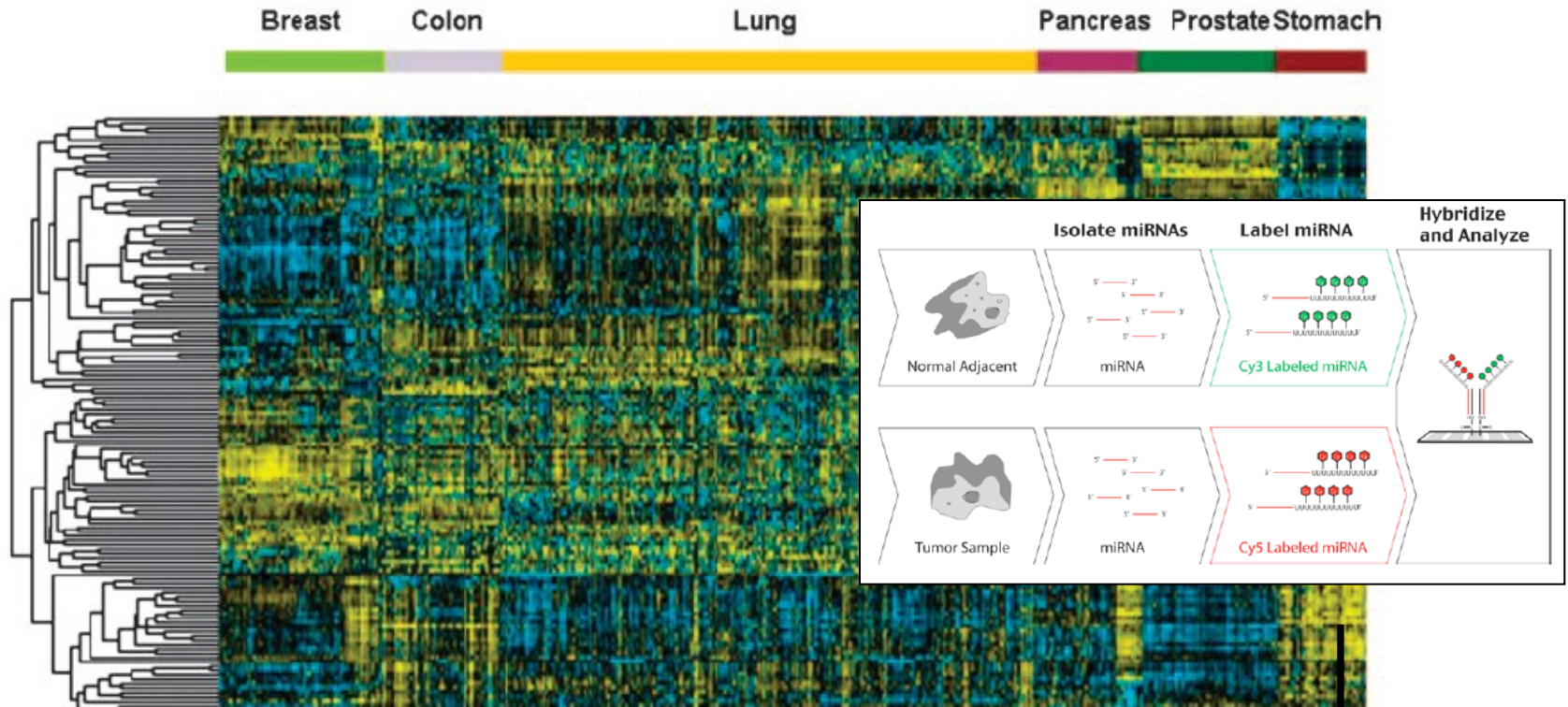


miRNA profiles cluster the GI samples well (GI, gastrointestinal origin)

mRNAs do not cluster the GI-samples very well

MikroRNA jako tkáňové biomarkery

Rozdílné expresní profily mikroRNA mezi nádorovou a nenádorovou tkání

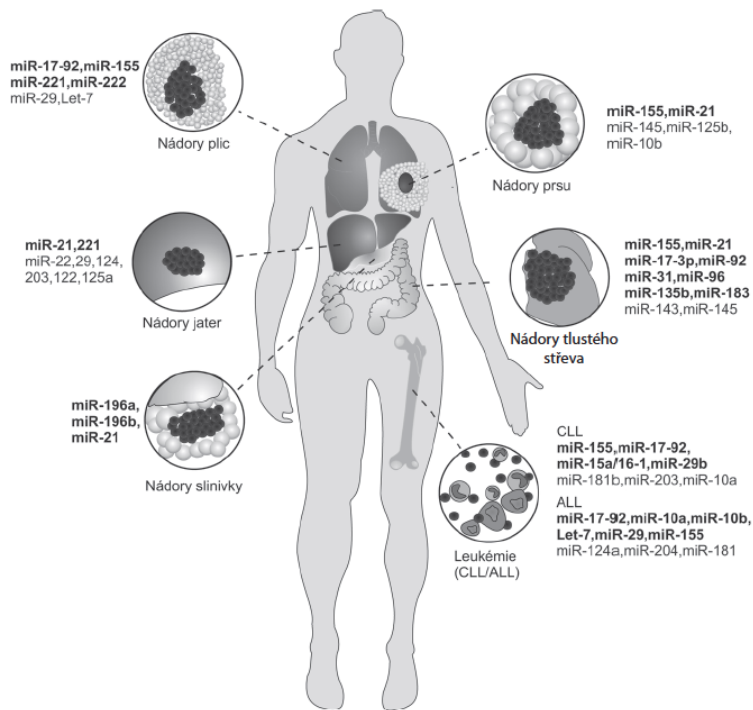


Volinia et al., PNAS, 2006

HCL analýza 540 vzorků nádorových a paralelních nenádorových tkání šesti typů solidních nádorů.

Žlutá znamená zvýšenou a modrá sníženou expresi v tumoru

MikroRNA jako tkáňové biomarkery spojené se známými histologickými a molekulárními podtypy nádorových onemocnění



Tab. 4.1. Příklady mikroRNA asociovaných se známými histologickými a molekulárními podtypy nádorových onemocnění. Doplněno podle [1].

Nádorové onemocnění	MikroRNA	Literatura
Mamární karcinom		
ER status	miR-26a/b, rodina miR-30, miR-29b, miR-155, miR-342, miR-206, miR-191	[12–14]
PR status	let-7c, miR-29b, miR-26a, rodina miR-30, miR-520g	[15,16]
HER2/neu status	miR-520d, miR-181c, miR-302c, miR-376b, miR-30e	[12,15]
Karcinom plic		
dlaždicobuněčný vs. adenokarcinom	miR-205	[17]
malobuněčný vs. nemalobuněčný karcinom	miR-17-5p, miR-22, miR-24, miR-31	[18]
mutace EGFR/KRAS	miR-155, miR-25, miR-495, let-7g	[19]
Kolorektální karcinom		
mutace KRAS	miR-127-3p, miR-92a, miR-486-3p, miR-378	[24]
Karcinom žaludku		
difuzní vs. intestinální karcinom	miR-29b/c, rodina miR-30, miR-135a/b	[20]
Karcinom endometria		
endometroidní vs. serózní papilární karcinom	miR-19a/b, miR-30e-5p, miR-101, miR-452, miR-382, miR-15a, miR-29c	[21]
Rendílní karcinom		
světlobuněčný vs. papilární karcinom	miR-424, miR-203, miR-31, miR-126	[22]
onkocytom vs. chromofobní karcinom	miR-200c, miR-139-5p	[22]
Maligní melanom		
s mutací BRAF V600E	miR-193a, miR-338, miR-565	[23]
Akutní myeloidní leukemie		
t(15;17)	miR-382, miR-134, miR-376a, miR-127, miR-299-5p, miR-323	[25]
t(8;21) nebo inv(16)	let-7b/c, miR-127	[25]
mutace NPM1b	miR-10a/b, let-7, miR-29, miR-204, miR-128a, miR-196a/b	[25]
s FLT3 ITD	miR-15	[25]
Chronická lymfatická leukemie		
hladiny ZAP-70 a status IgVH	miR-15a, miR-195, miR-221, miR-155, miR-23b	[26,27]

MikroRNA jako tkáňové biomarkery

mikroRNA nepodléhají degradačním procesům během formalínové fixace a archivace v parafinových blocích jako je tomu u mRNA

→ ARCHIVY FFPE MATERIÁLU NA PATOLOGÍCH JE TAK MOŽNÉ VYUŽÍVAT PRO ROZSÁHLE RETROSPEKTIVNÍ STUDIE

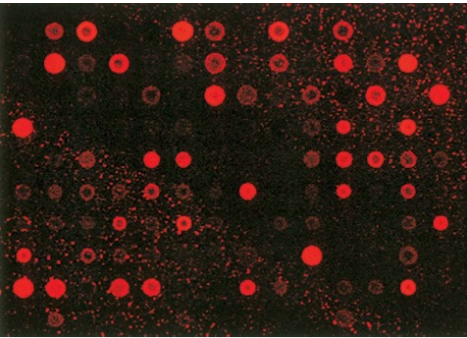
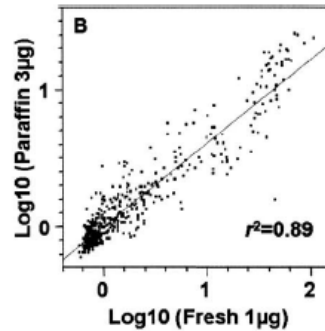
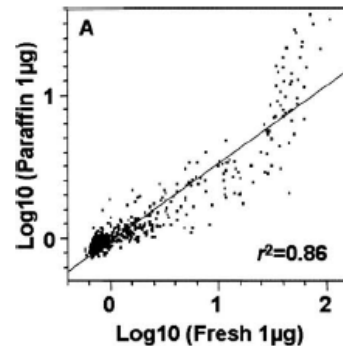


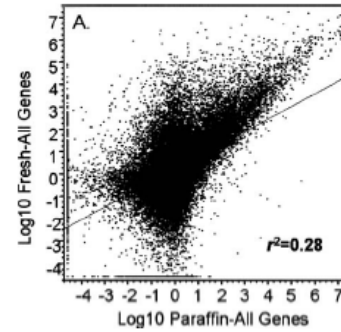
FIGURE 7. Representative miRNA array image from a 10-yr-old FFPE colon cancer sample.



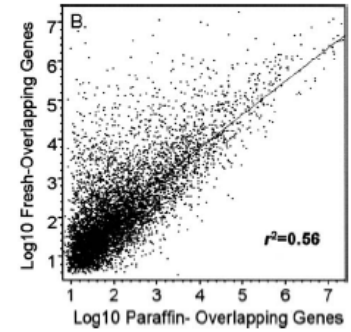
mikroRNA z FFPE



versus



mRNA z FFPE



Yaguang Xi, RNA, 2007, 13:1-7

Table 2 Consistently reported up-regulated miRNAs (n = 26) in profiling studies (lung cancer tissue *versus* normal)

miRNA name*	No. of studies with same direction (reference)	No. of tissue samples tested	Subset of studies with fold change		
			No. of studies	No. of tissue samples tested	Mean fold Range change
miR-210	9 (19,22,24,25,26,27,29,30,32)	796	6	449	2.65 1.51 - 5.10
miR-21	7 (19,21,25,28,29,30,32)	448	6	240	4.39 1.74 - 13.60
miR-182	6 (22,24,26,27,28,32)	496	4	357	6.34 1.85 - 19.00
miR-31	6 (21,22,26,27,29,32)	425	5	357	2.89 1.58 - 4.80
miR-205	5 (26,27,28,29,30)	417	3	141	23.20 2.99 - 54.30
miR-200b	5 (19,25,26,28,32)	262	4	194	3.69 1.30 - 9.80
miR-183	4 (22,24,27,28)	388	3	317	3.94 2.11 - 11.00
miR-203	3 (24,26,30)	347	0	-	- -
miR-196a	3 (22,27,28)	317	3	317	37.50 2.10 - 101.80
miR-708	3 (22,27,29)	301	3	301	3.20 1.85 - 5.50
miR-92b	3 (27,28,32)	151	3	151	3.71 1.54 - 6.80
miR-193b	3 (21,26,27)	149	2	81	4.68 2.56 - 6.80
miR-106a	2 (24,30)	279	0	-	- -
miR-21*	2 (22,27)	271	2	271	2.23 2.16 - 2.30
miR-135b	2 (21,22)	222	2	222	2.29 2.28 - 2.31
miR-96	2 (22,23)	218	2	218	171.56 2.30 - 340.81
miR-17-5p	2 (24,27)	136	1	65	3.80 -
miR-20b	2 (24,28)	117	1	46	5.70 -
miR-18a	2 (26,28)	114	1	46	7.80 -
miR-200a	2 (24,32)	111	1	40	1.86 -
miR-93	2 (24,32)	111	1	40	1.68 -
miR-130b	2 (26,32)	108	1	40	1.57 -
miR-200c	2 (24,29)	101	1	30	1.66 -
miR-375	2 (28,32)	86	2	86	5.35 2.89 - 7.80
miR-20a	2 (20,24)	83	0	-	- -
miR-18b	2 (20,26)	80	0	-	- -

* The asterisk is part of the miRNA nomenclature system and is not linked to any footnote specific to this table.

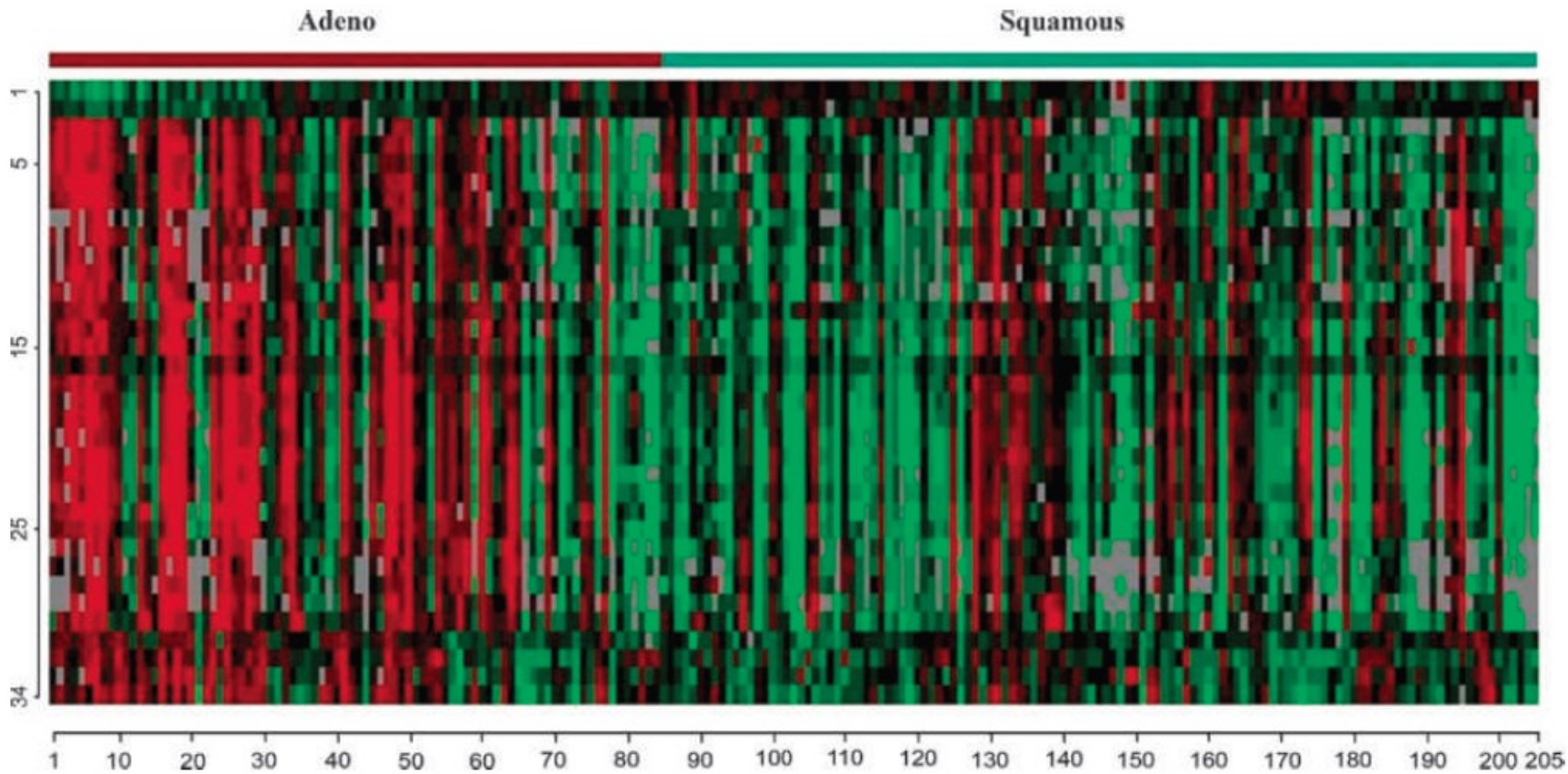
Meta-analýza mikroRNA down-regulovaných v tkáni NSCLC

Table 3 Consistently reported down-regulated miRNAs (*n* = 28) in profiling studies (lung cancer tissue *versus* normal)

miRNA name ^a	No. of studies with same direction (reference)	Total number of tissue samples tested	Subset of studies with fold change			
			No. of studies	Total number of tissue samples tested	Mean fold change	Range
miR-126	10 (19,21,25,26,27,28,29,30,31,32)	587	8	311	0.33	0.00 - 0.69
miR-30a	8 (19,21,25,26,27,28,29,31)	339	7	271	0.36	0.04 - 0.61
miR-451	6 (19,21,25,27,28,29)	265	6	265	0.37	0.01 - 0.53
miR-486-5p	5 (19,22,26,27,28)	437	4	369	0.39	0.13 - 0.53
miR-30d	5 (21,25,28,29,31)	154	5	154	0.34	0.08 - 0.57
miR-145	4 (26,28,30,32)	362	2	86	0.23	0.09 - 0.38
miR-143	4 (21,28,30,32)	310	3	102	0.33	0.13 - 0.59
miR-139-3p	3 (22,27,29)	301	3	301	0.33	0.40 - 0.64
miR-126*	3 (21,25,30)	280	2	72	0.33	0.20 - 0.45
miR-140-3p	3 (26,27,28)	179	2	111	0.29	0.17 - 0.42
miR-138	3 (25,26,32)	164	2	96	0.64	0.56 - 0.72
miR-30b	3 (25,28,29)	132	3	132	0.41	0.11 - 0.58
miR-486	3 (25,29,32)	126	3	126	0.44	0.34 - 0.53
miR-101	3 (21,27,31)	87	3	87	0.34	0.24 - 0.48
miR-125a	2 (24,30)	279	0	-	-	-
miR-198	2 (27,30)	273	1	65	0.25	-
miR-144*	2 (22,27)	271	2	271	0.31	0.14 - 0.48
miR-140	2 (30,32)	248	1	40	0.66	-
miR-218	2 (22,32)	246	2	246	0.61	0.60 - 0.62
miR-32	2 (20,30)	220	0	-	-	-
miR-338-3p	2 (26,27)	133	1	65	0.20	-
miR-99a	2 (27,28)	111	2	111	0.31	0.20 - 0.42
miR-195	2 (26,29)	98	1	30	0.53	-
miR-497	2 (26,29)	98	1	30	0.66	-
miR-30c	2 (25,29)	86	2	86	0.58	0.54 - 0.61
miR-130a	2 (21,27)	81	2	81	0.46	0.45 - 0.46
miR-16	2 (28,29)	76	2	76	0.37	0.18 - 0.57
miR-139	2 (29,32)	70	2	70	0.53	0.49 - 0.58

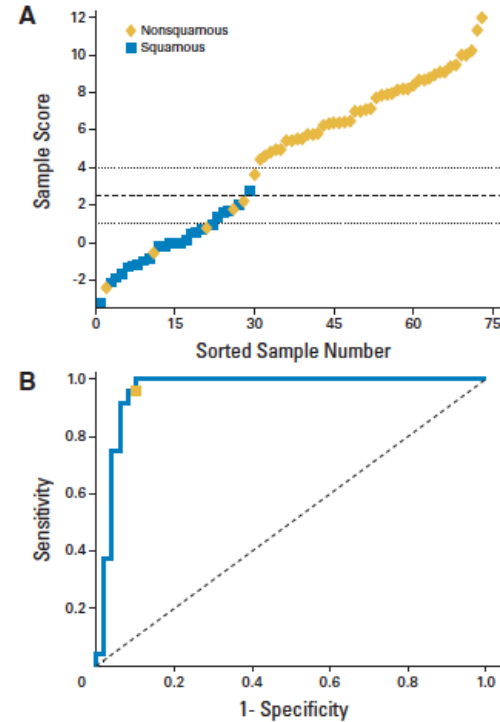
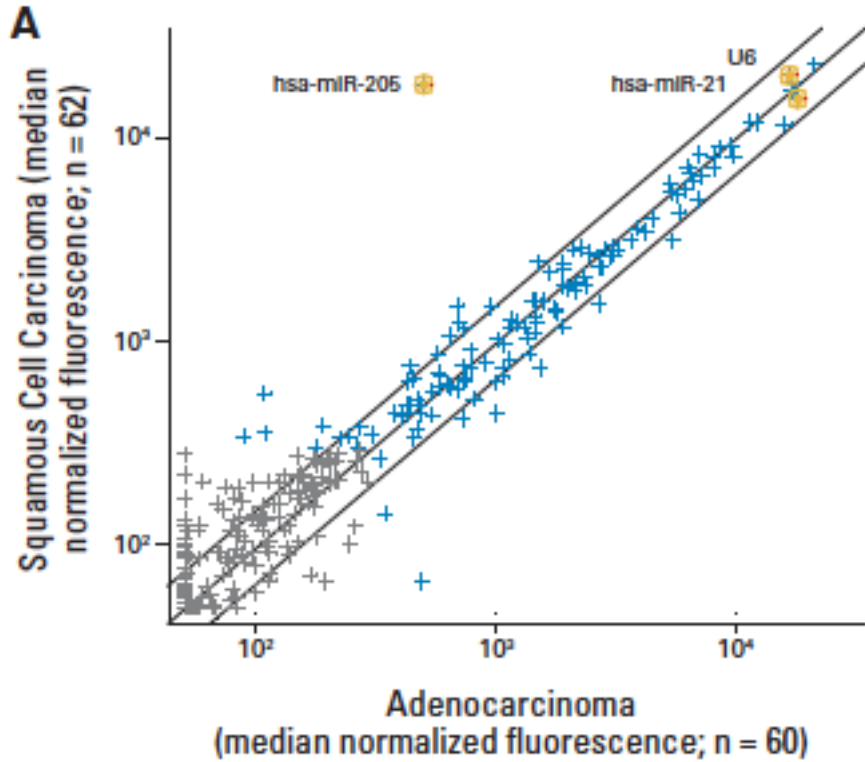
^a The asterisk is part of the miRNA nomenclature system and is not linked to any footnote specific to this table.

MikroRNA asociované s různými histologickými podtypy NSCLC



Landi et al, Clin Cancer Res, 2010

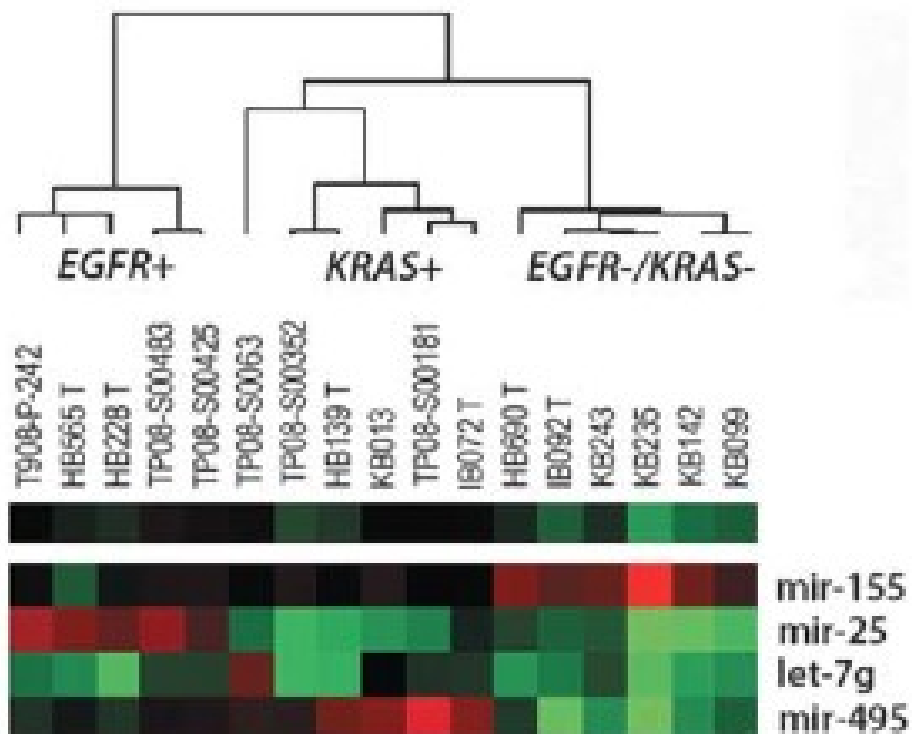
MikroRNA asociované s různými histologickými podtypy NSCLC



Lebanony et al, JCO, 2009

miR-205
SENZITIVITA 96%
SPECIFICITA 90%

MikroRNA asociované s mutačným stavem KRAS/EGFR u pacientů s NSCLC



Dacic et al, Modern Pathology, 2010

miR-21: new diagnostic biomarker of colorectal cancer

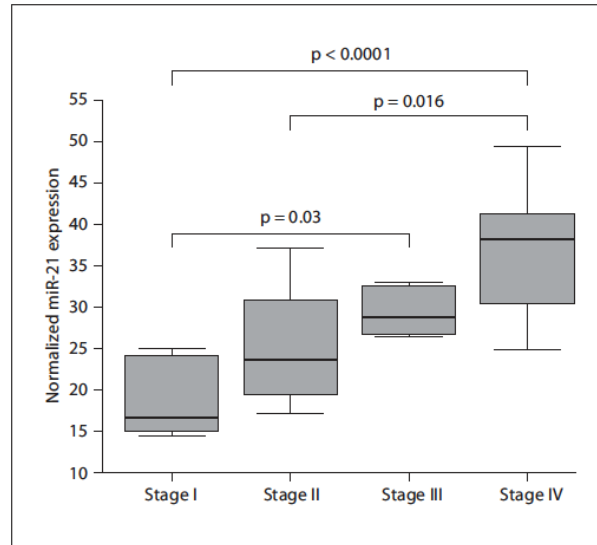
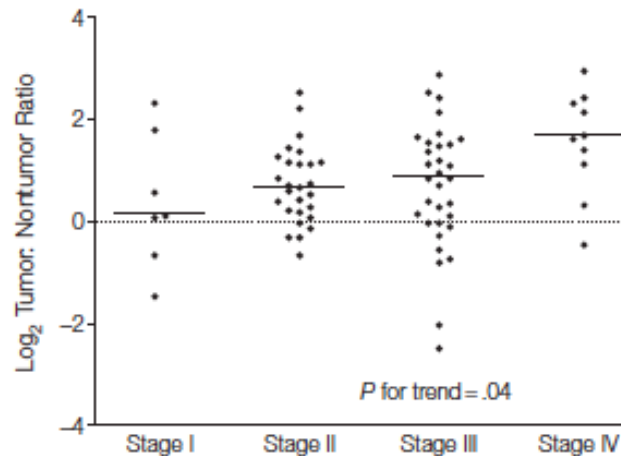


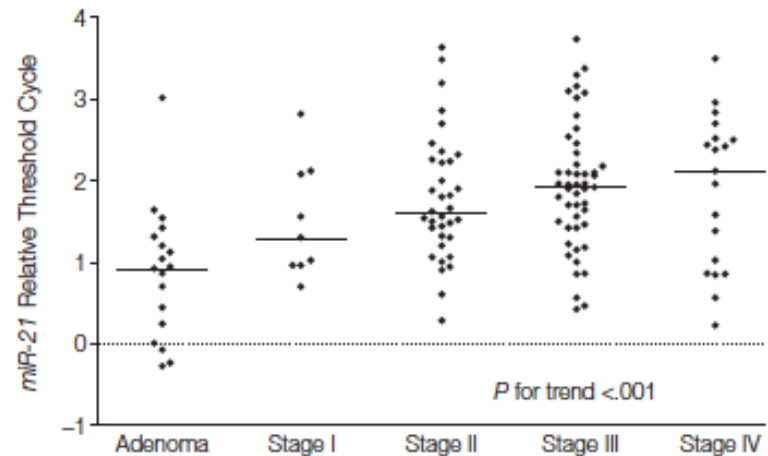
Fig. 1. Correlation between clinical stage and normalized expression of miR-21 in patients with CRC.

Slaby et al, Oncology, 2007 ☺

A Maryland test cohort (microarray data)

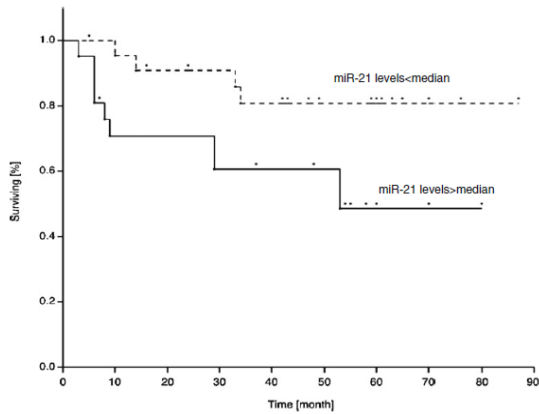


B Hong Kong validation (quantitative reverse transcription polymerase chain reaction data)

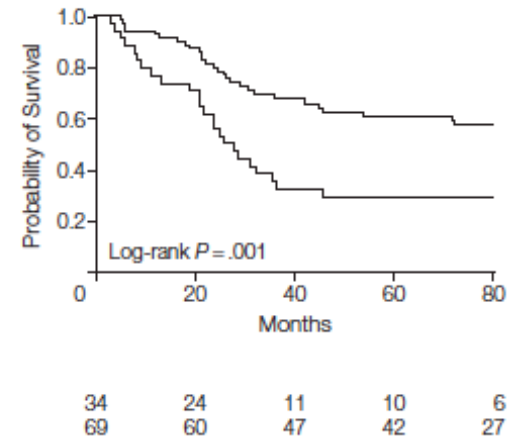
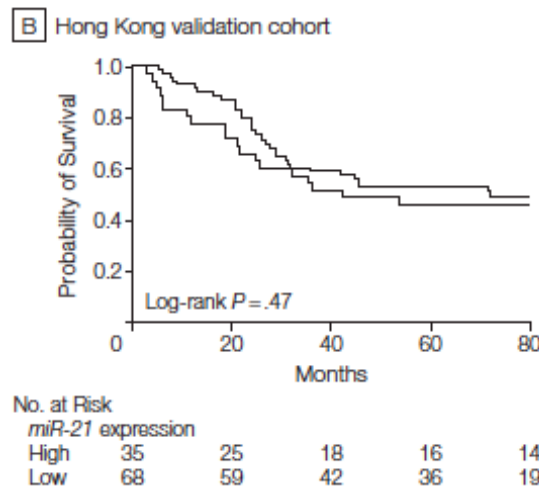
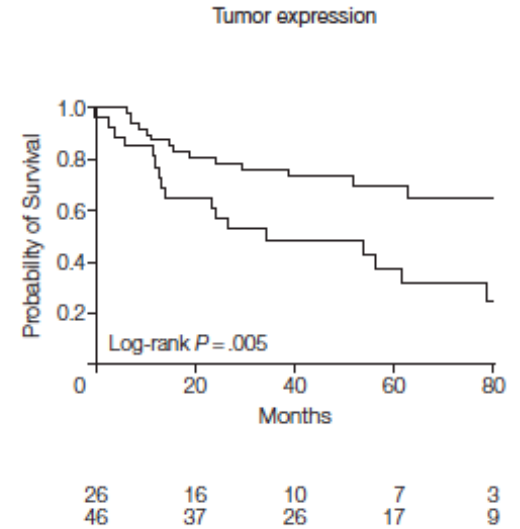
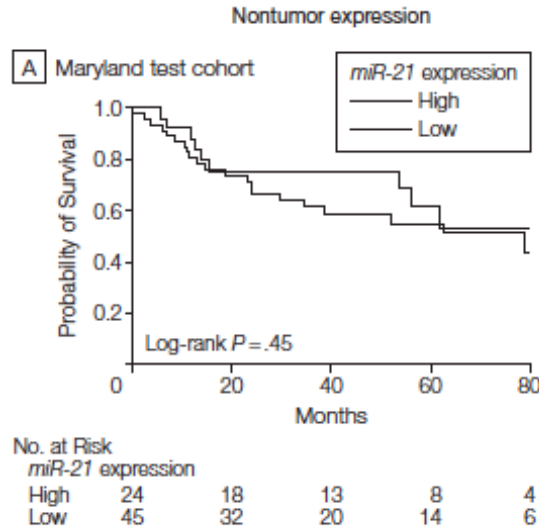


Schetter et al, JAMA, 2008

miR-21: prognostic and predictive marker of colorectal cancer

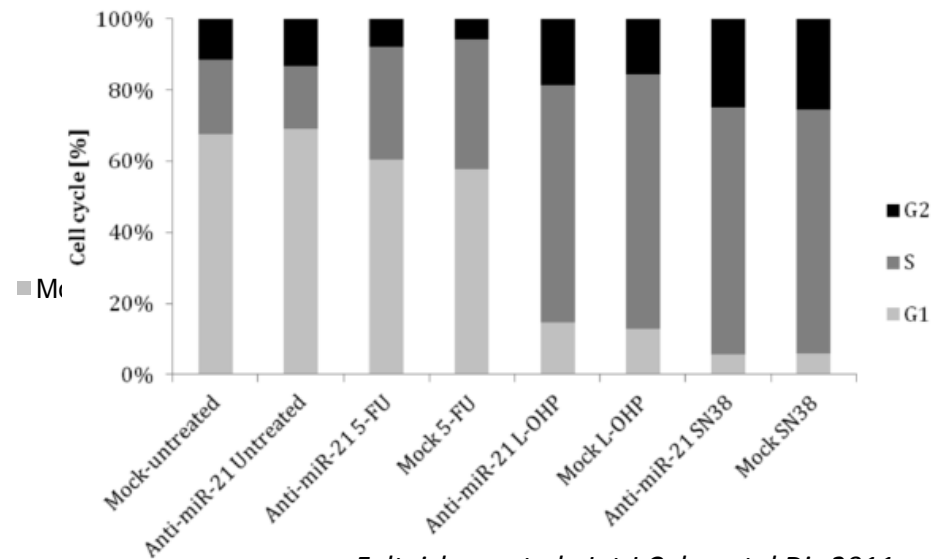
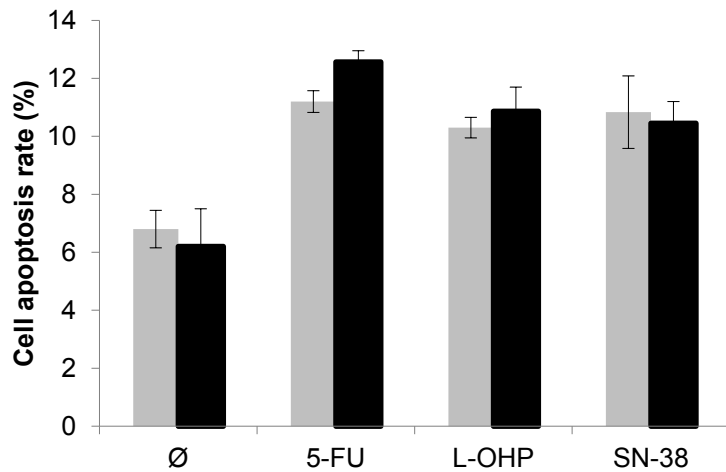
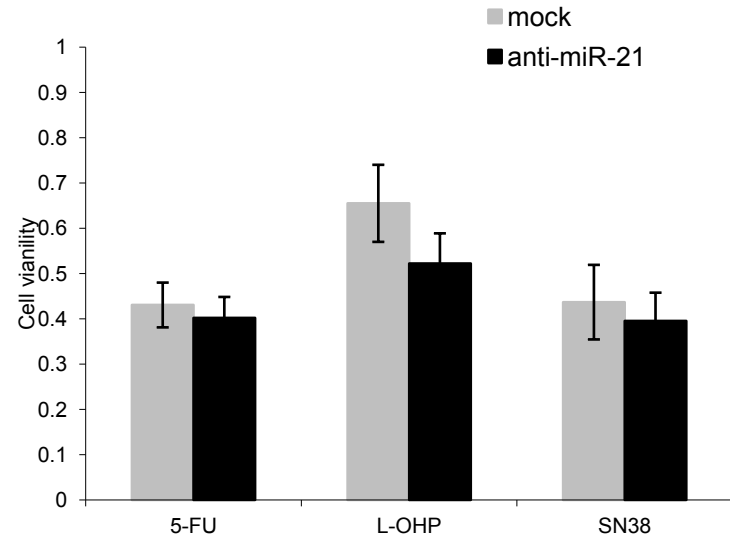
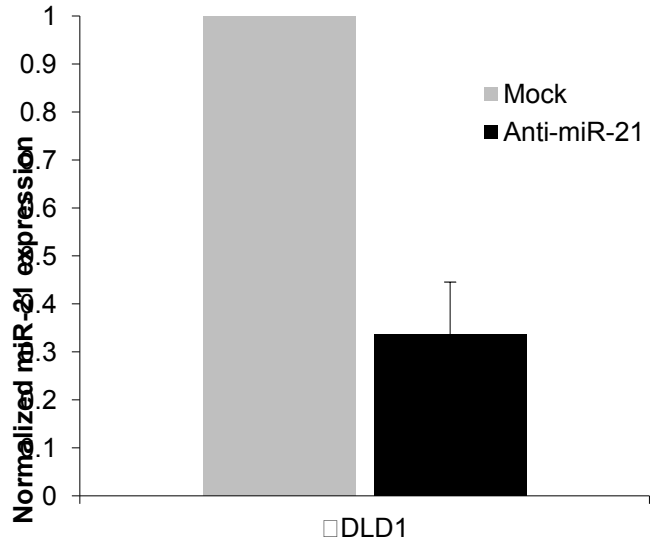


Faltejsova et al, IJCD, 2012



Schetter et al, JAMA, 2008

Analysis of miR-21 inhibition of colon cancer cells DLD1



Analysis of miR-21 inhibition of colon cancer cells DLD1

Scratch wound healing assay – inhibition of DLD1 cells migration

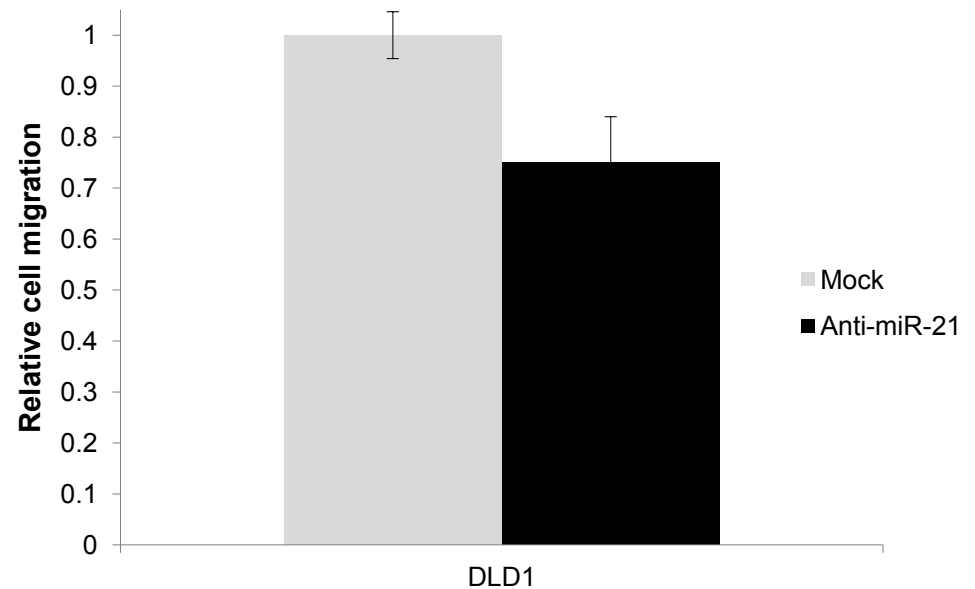
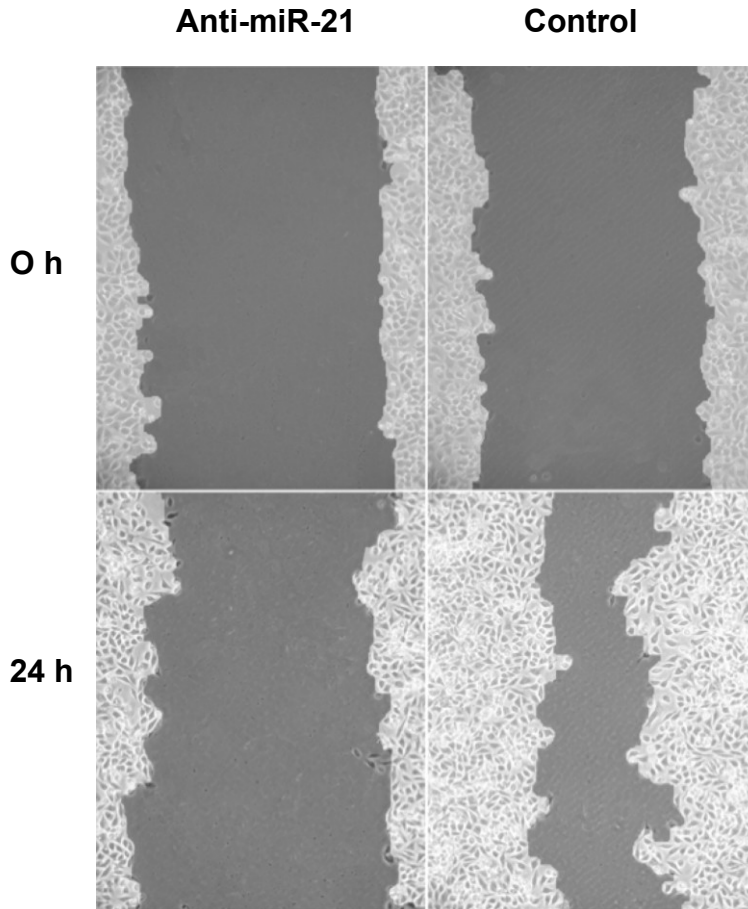
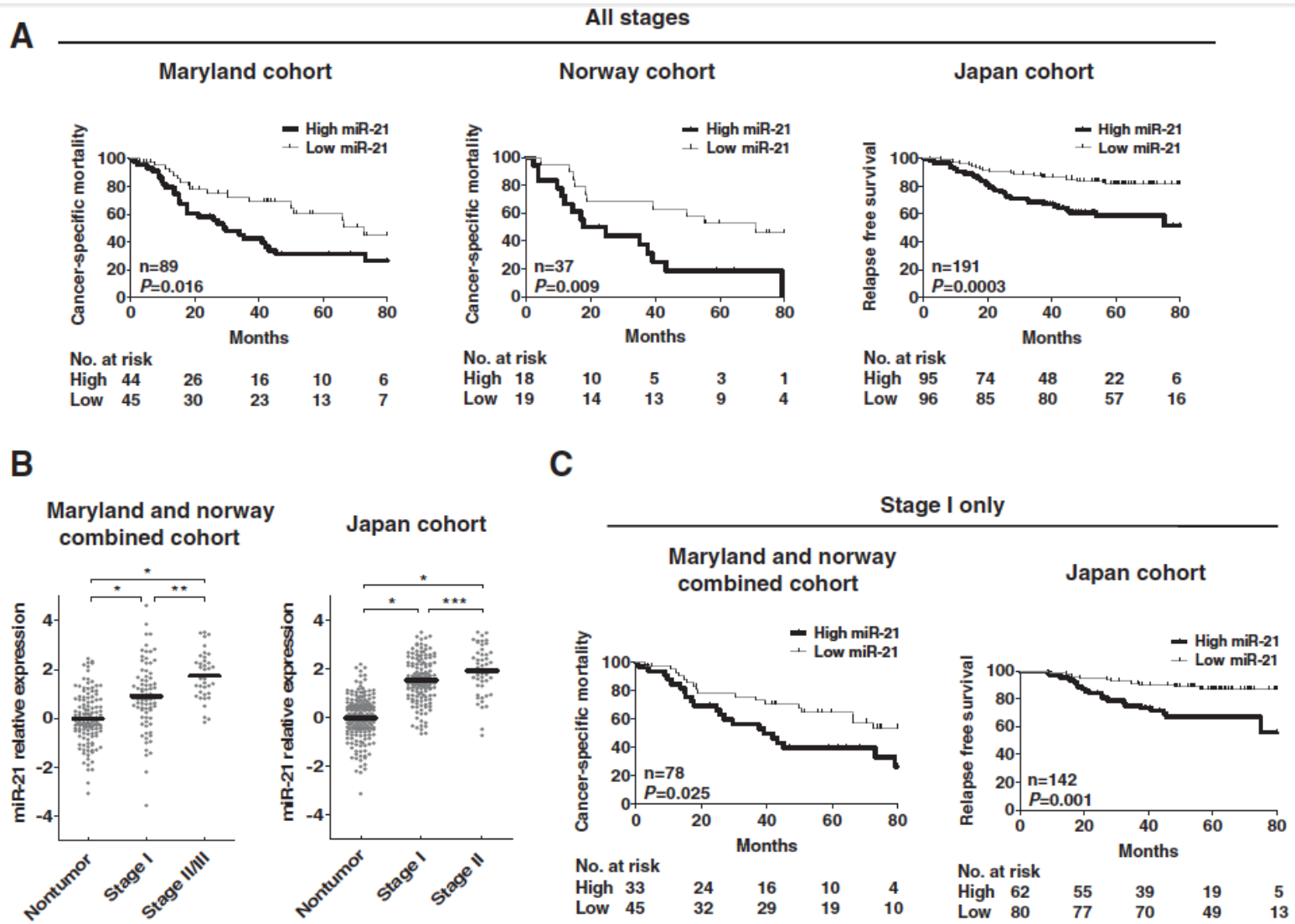


Figure 10 Relative cell motility (two-tailed t-test, $p=0.016$)

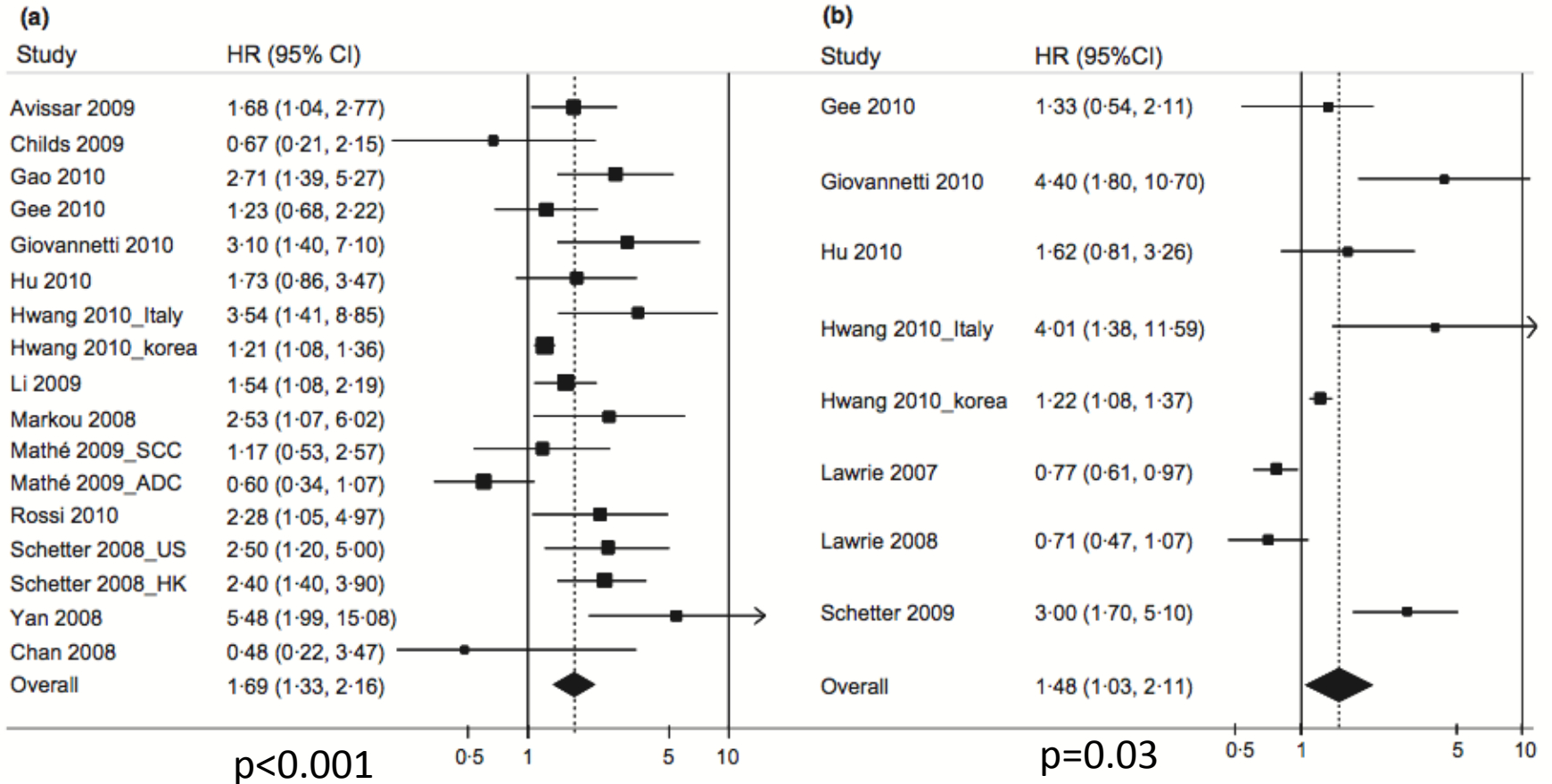
Asociace miR-21 s klinickým stádiem a prognózou u pacientů s NSCLC



Asociace miR-21 s klinickým stádiem a prognózou u pacientů s NSCLC

	Combined Maryland/ Norway cohort		Japan cohort (<i>n</i> = 191)	
	<i>(n</i> = 126; cancer-specific mortality)		<i>(relapse-free survival)</i>	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Univariate analysis				
miR-21 (high vs. low) ^a	2.31 (1.42–3.76)	0.001	2.82 (1.57–5.07)	<0.0005
Stage (II, III, vs. I) ^b	2.35 (1.46–3.80)	<0.0005	3.17 (1.82–5.52)	<0.0005
Sex (M vs. F)	1.09 (0.68–1.73)	0.734	1.29 (0.75–2.32)	0.351
Age (≥65 vs. <65)	1.42 (0.89–2.28)	0.142	1.58 (0.91–2.77)	0.106
Ever smoke (ever vs. never) ^c	0.94 (0.48–1.83)	0.847	1.34 (0.78–2.32)	0.287
Pack years (≥20 vs. <20)	1.53 (0.85–2.78)	0.155	1.60 (0.92–2.79)	0.098
Multivariate analysis, final model ^d				
miR-21 (high vs. low) ^a	2.25 (1.32–3.82)	0.003	2.66 (1.47–4.83)	0.001
Stage (II, III, vs. I) ^b	2.37 (1.42–3.95)	0.001	2.78 (1.57–4.94)	<0.0005
Age (≥65 vs. <65)	1.02 (0.68–1.53)	0.278	1.08 (0.68–1.71)	0.688
Pack years (≥20 vs. <20)	2.00 (1.07–3.72)	0.028	1.50 (0.85–2.65)	0.160

Meta-analýza prognostické role miR-21 u pacientů s nádorovým onemocněním



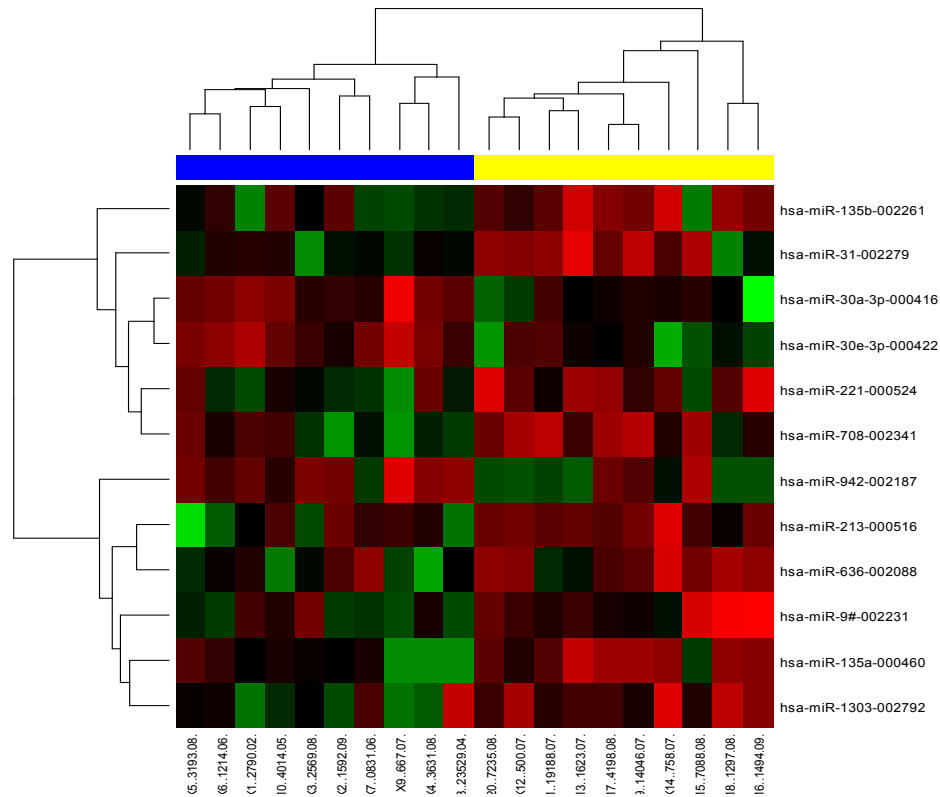
Meta-analýza 43 studií u různých typů nádorových onemocnění

Table 4. MicroRNAs selected most frequently in classifiers compared with expected frequency*

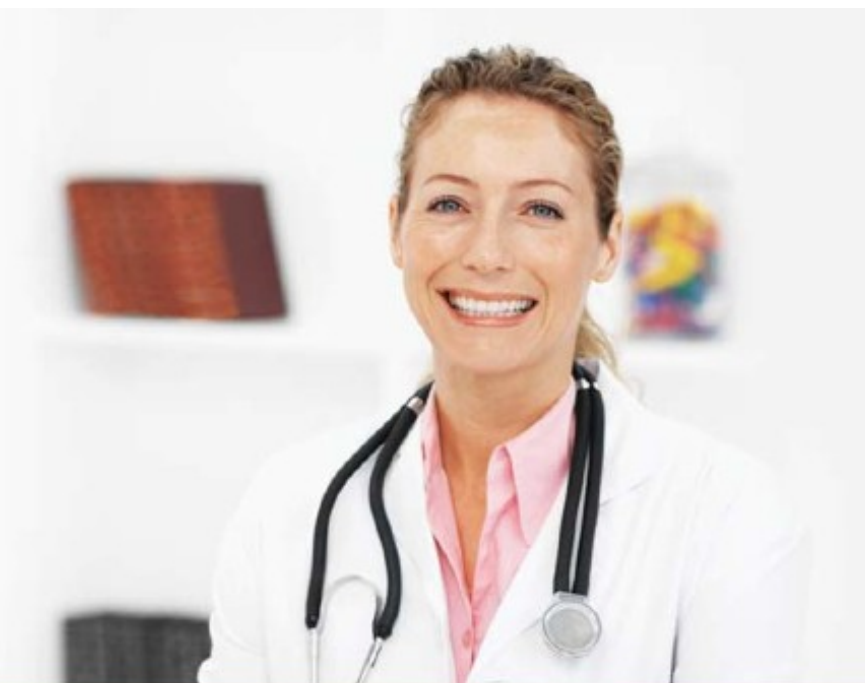
MiR	No. of times assessed, n_{pi} (%)†	No. of times selected in classifier, n_{ci} (%)‡	P§	Cancer type
All miRs in patients with poor outcome				
let-7	244 (2.46)	16 (6.23)	<.001	Gastric, HCC, leukemia, lung, melanoma, NHL, ovarian
21	34 (0.34)	5 (1.95)	.0018	Astrocytoma, colon, lung, ovarian
100	26 (0.26)	4 (1.56)	.0043	Gastric, HCC, lung
125	71 (0.72)	6 (2.33)	.0084	HCC, gastric, neuroblastoma
MiRs with increased expression in patients with poor outcome				
21	34 (0.34)	5 (4.72)	<.001	Astrocytoma, colon, lung, ovarian
20	47 (0.47)	4 (3.77)	.0046	Colon, leukemia, NHL
155	26 (0.26)	3 (2.83)	.0063	Lung, melanoma
193	55 (0.56)	4 (3.77)	.0078	Melanoma
MiRs with decreased expression in patients with poor outcome				
let-7	244 (2.46)	7 (9.59)	.0017	Gastric, lung, HCC, ovarian
30	135 (1.36)	5 (6.85)	.0028	Astrocytoma, HCC, lung
29	94 (0.95)	4 (5.48)	.0046	Endometrial, NHL
7039	1 (0.01)	1 (1.37)	.0073	Ovarian

MikroRNA jako prediktivní tkáňové biomarkery

Predikce léčebné odpovědi u pacientů s CRC wtKRAS léčených anti-EGFR terapií (cetuximab)



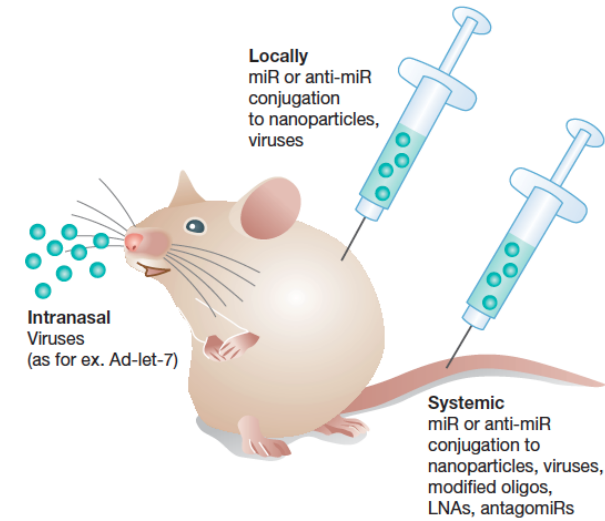
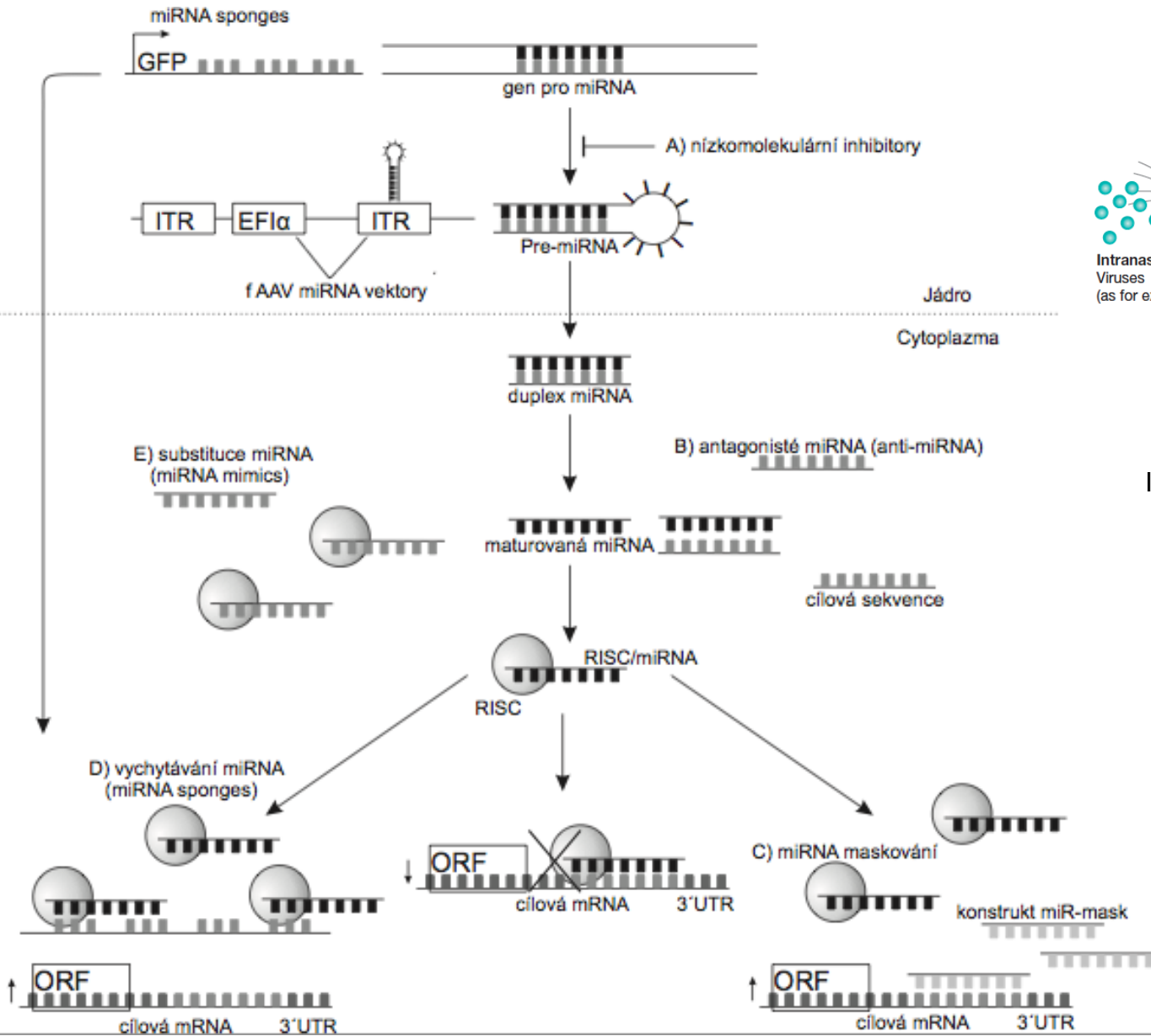
↑ miR-135b, miR-31, miR-135a
↓ miR-30a-3p, miR-30e-3p



miRview™ mets: Unsolving the Mystery of Cancer of Unknown Primary (CUP)

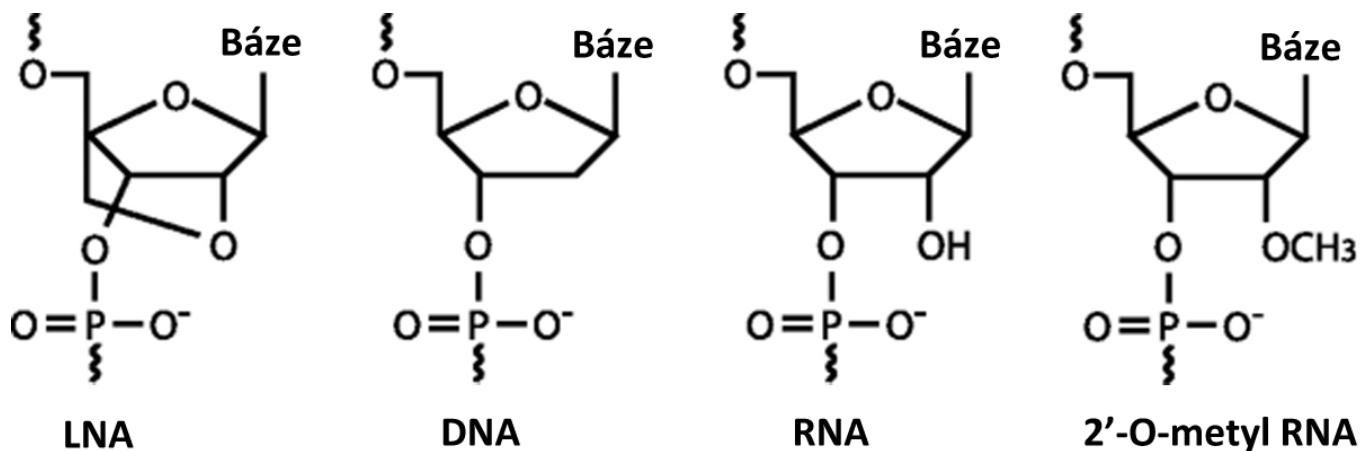
Identifying the primary origin of a metastasis

MikroRNA jako terapeutické cíle



lorio et al., EMBO Mol Med, 2012

MikroRNA jako terapeutické cíle



5' - x-ACCAAUGUGCAGACUACUGt

3' - AUUGGUUACACGUCUGAUGACAp

Legenda

- p = fosfát
- x = C3-mezerník (spacer)
- AGCU = 2'OMe RNA
- AGCU = RNA
- agct = DNA

Vedoucí řetězec ←

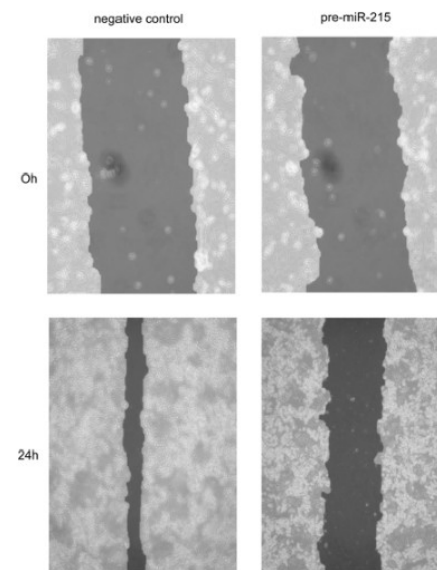
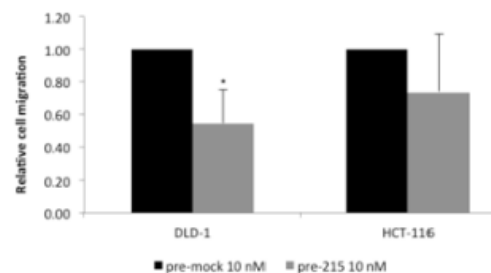
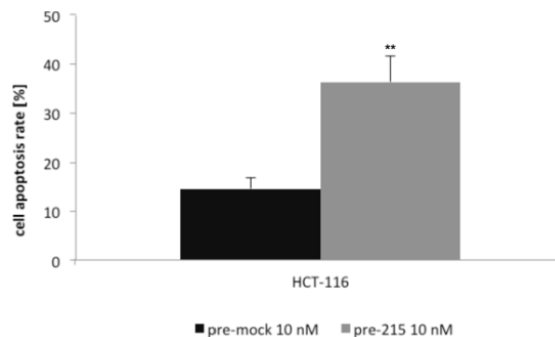
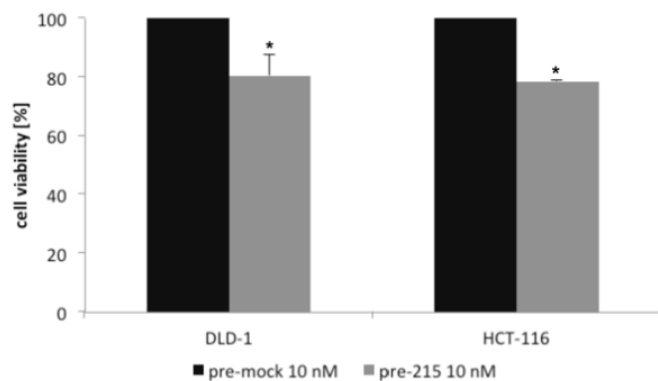
„Passenger” řetězec ←

In vitro functional analysis of highly deregulated microRNAs in colorectal cancer

Table 3 Summarized results of *in vitro* analyses (observed effects of particular pre- and anti-miR oligonucleotides)

	pre-miR-378	pre-miR-422a	pre-miR-375	pre-miR-215	anti-miR-135b
HCT-116 cells					
Apoptosis	–	–	–	Increased apoptosis $P = 0.005$	–
Cell cycle	G1/S increased $P = 0.005$	G1/S increased $P = 0.01$	G1/S increased $P = 0.01$	G1/S increased $P = 0.01$	–
MTT	Trend ($P > 0.05$)	–	Decreased viability $P = 0.005$	Decreased viability $P = 0.05$	Decreased viability $P = 0.05$
SWA*	–	Trend ($P > 0.05$)	–	Trend ($P > 0.05$)	–
DLD-1 cells					
Apoptosis	–	–	–	–	–
Cell cycle	–	–	–	–	–
MTT	Trend ($P > 0.05$)	–	Trend ($P > 0.05$)	Decreased viability $P = 0.05$	Decreased viability $P = 0.005$
SWA	–	Trend ($P > 0.05$)	–	Decreased viability $P = 0.05$	–

SWA, scratch wound assay.



MikroRNA jako terapeutické cíle

Seznam mikroRNA které jsou testovány jako potenciální terapeutické cíle

MikroRNA	Indikace	Společnost	Validace cíle	Optimalizace vůdčí struktury	Pre-klinické testování	Fáze 1	Fáze 2
Inhibiční strategie							
miR-122*	hepatitida typu C	Santaris Pharma	X	X	X	X	X
miR-21	multiformní glioblastom	Regulus Therapeutics	X	X	X		
miR-21	hepatocelulární karcinom	Regulus Therapeutics	X	X	X		
miR-21	renální fibróza	Regulus Therapeutics	X	X	X		
miR-33a/b	ateroskleróza	Regulus Therapeutics	X	X	X		
miR-208/miR-499	chronické srdeční selhání	miRagen Therapeutics	X	X	X		
miR-15/miR-195	remodelace po infarktu myokardu	miRagen Therapeutics	X	X	X		
miR-451	polycythemia vera	miRagen Therapeutics	X	X	X		
Substituční strategie							
miR-34	hepatocelulární karcinom fáze 1 – první čtvrtletí 2013	miRNA therapeutics	X	X	X		
miR-34	nemalobuněčný karcinom plic	miRNA therapeutics	X	X	X		
miR-34	karcinom prostaty	miRNA therapeutics	X	X	X		
let-7	nemalobuněčný karcinom plic	miRNA therapeutics	X	X	X		
miR-29	srdeční fibróza	mirage Therapeutics	X	X			

Miravirsen (SPC3649)

miR-122

Hepatitis C



Miravirsen (SPC3649)
A New Treatment Targeting Hepatitis C

MicroRNAs as therapeutic targets

Miravirsen, anti-miR-122

Léčivou látkou je nový LNA-antimiR™ oligonukleotid s přiřazeným mezinárodním nechráněným názvem (INN) miravirsen sodium. Léčivá látka je vyráběna jako sodná sůl. Miravirsen sodium je hygroskopický, bílý až lehce nažloutlý prášek rozpustný ve vodě. Sumární vzorec a molekulová hmotnost jsou uvedeny níže.

Sumární vzorec (protonovaná forma): $C_{156}H_{181}N_{49}Na_{14}O_{83}P_{14}S_{14}$
($C_{156}H_{195}N_{49}O_{83}P_{14}S_{14}$)

Molekulová hmotnost (protonovaná forma): 5,274 8 g/mol (4,967 g/mol)

Název molekuly: SPC3649

INN: miravirsen sodium

Miravirsen je 15nukleotidový (15-mer) fosforothioatový oligonukleotid, tvořený -D-oxy-LNA a DNA monomery. Molekula miravirsenu obsahuje osm nukleotidů s LNA modifikací a sedm DNA nukleotidů uspořádaných v následující sekvenci:

5'- mC c A t t G T c a mC am C t mC mC -3'

Velká písmena označují nukleotidy s LNA modifikací (mC značí LNA-5-methylcytidin) a malými písmeny jsou označeny nukleotidy DNA. LNA modifikace významně zvyšuje vazebnou afinitu molekuly k cílové miR-122 a také její biologický poločas. Všechny vazby mezi nukleotidy u miravirsenu, celkem 14, mají charakter fosforotioátu, což zlepšuje farmakokinetické vlastnosti oligonukleotidu.

Delivering on the promise of RNA medicines today

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