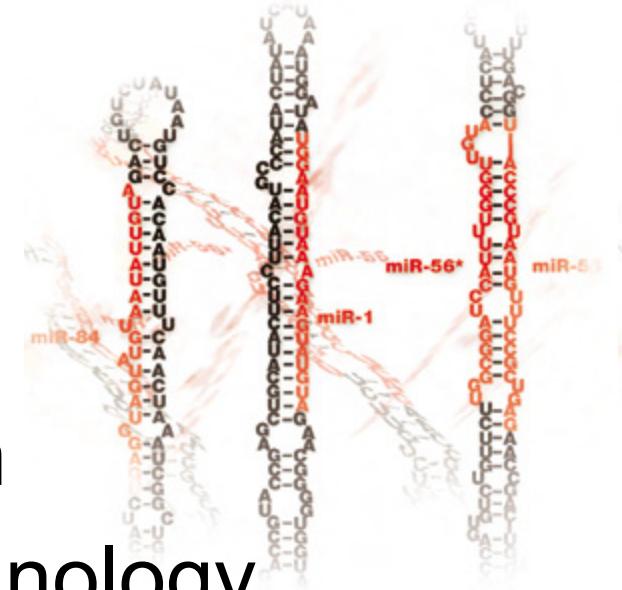
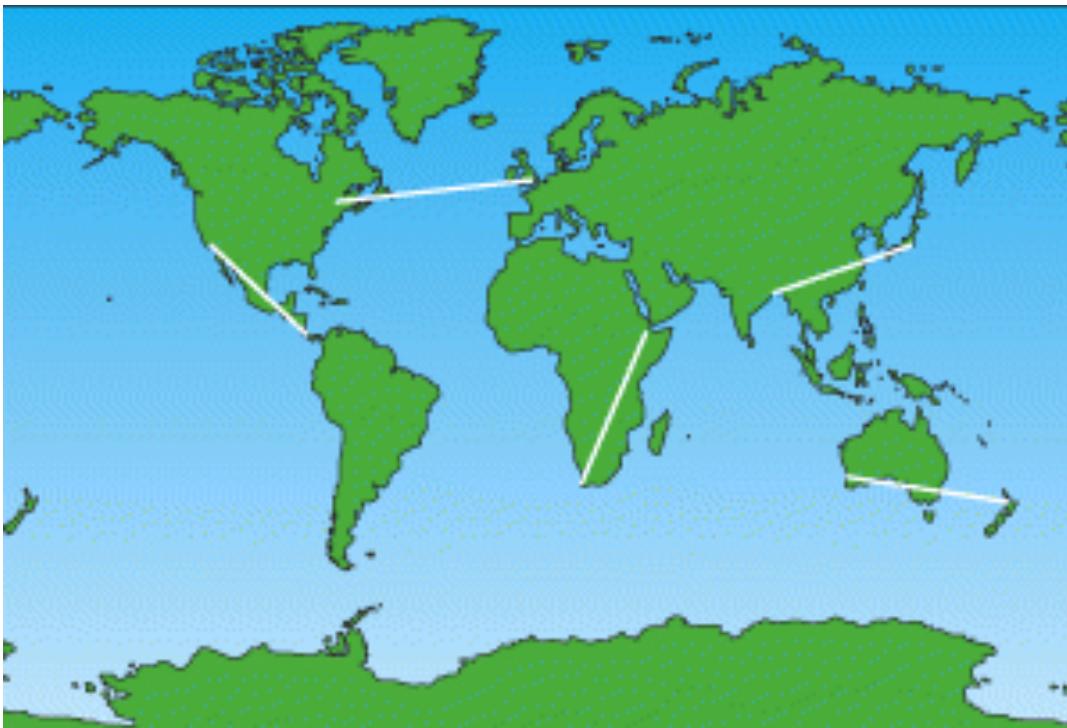


Role of Micro RNAs in Cancer

D. Karunagaran
Department of Biotechnology
IIT Madras, Chennai



Human Genome Project



- 3 billion chemical base pairs
- Approx. 30 thousand human genes
- "Genome"- Brown TA ed., Bios Scientific Publishers 2002



What does the draft human genome sequence tell us?

How It's Arranged

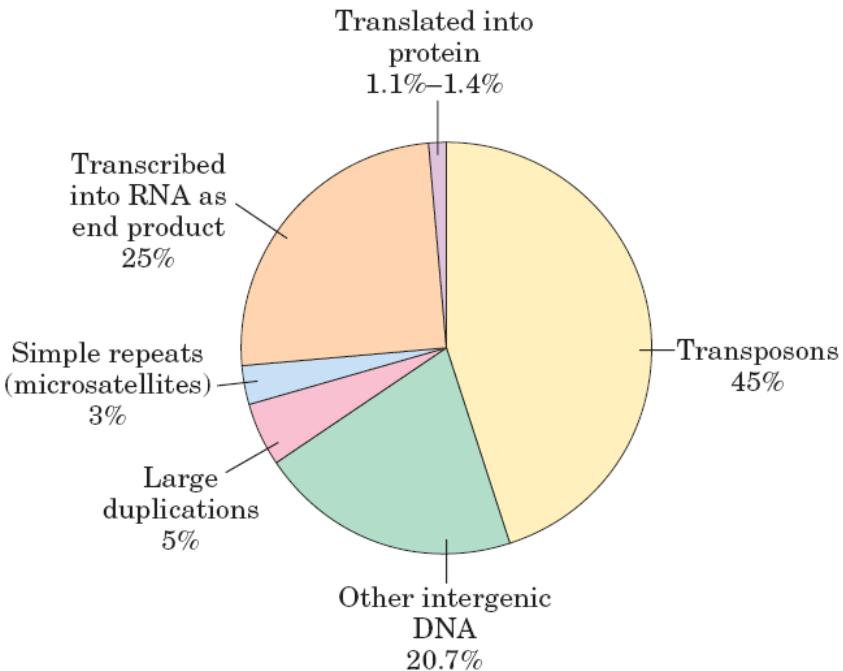
- gene-dense "urban centers" are predominantly composed of **G and C**.
- gene-poor "deserts" are rich **A and T**
- Genes concentrated in random areas with many noncoding DNA in between
- Stretches of up to 30,000 C and G bases often occur **between** the **genes** and the "junk DNA" These **CpG islands** are believed to help regulate gene activity.

RECENT TRENDS

The Human Genome Project

The Human Genome Project marks the culmination of twentieth-century biology and promises a vastly changed scientific landscape for the new century. The human genome is only part of the story, as the genomes of many other species are also being (or have been) sequenced

Snapshot of the human genome.



Genomic sequencing timeline.

Junk-DNA: Role in evolution?

- Human genes vs. Genes of chimpanzee:
Difference is only 0,1%
(difference in approximately 20 genes only)

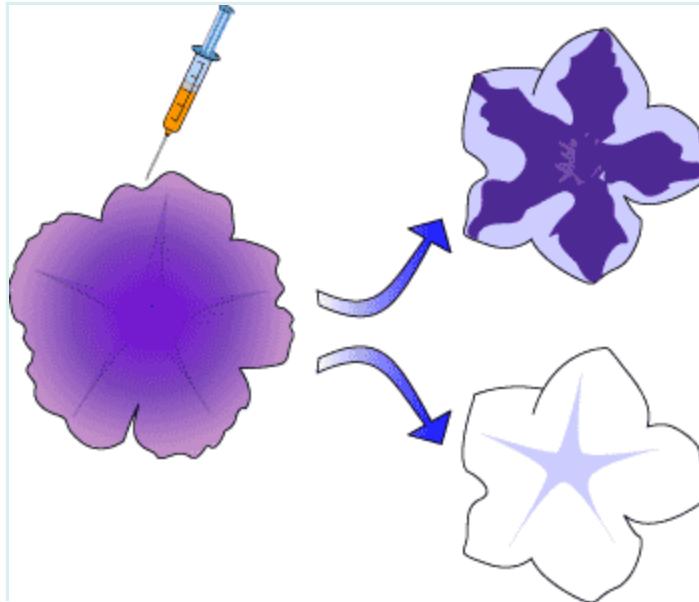
Difference in non-coding regions: 40x more
(4%)

– it can make the difference between humans and chimpanzee)

Junk-DNA: Role in gene-silencing

- Post-transcriptional silencing of active genes:
 - by complementary micro-RNA- s (miRNA) and small interfering RNA-s (siRNA) (21-23 bp)
 - miRNA- can bind complementary mRNA and stop transcription
 - (Nobel prize in Medical Physiology, 2006: Fire A.Z and Mello C.C.)
-



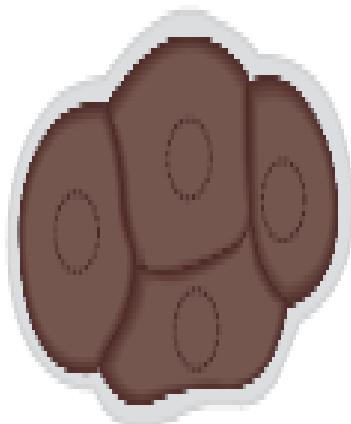


1990 Rich Jorgensen at the University of Arizona

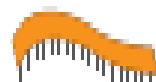
Researchers were trying to deepen the purple color of the flowers by injecting the gene responsible into the petunias but were surprised at the result. Instead of a darker flower, the petunias were either variegated or completely white!

This phenomenon was termed co-suppression, since both the expression of the existing gene (the initial purple colour), and the introduced gene (to deepen the purple) were suppressed. Co-suppression has since been found in many other plant species and also in fungi. It is now known that double stranded RNA is responsible for this effect.

Uninjected



Antisense RNA

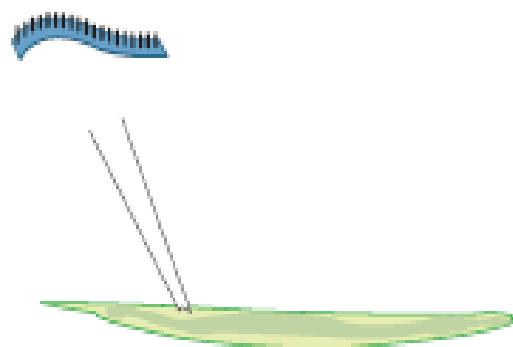


Double-stranded RNA

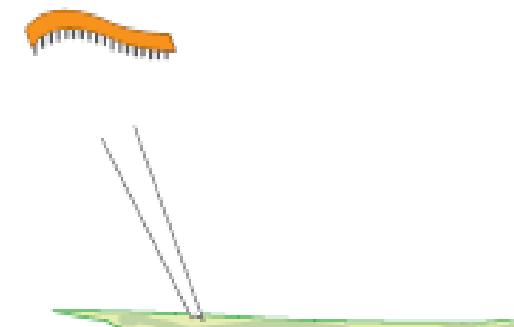


C. elegans embryos

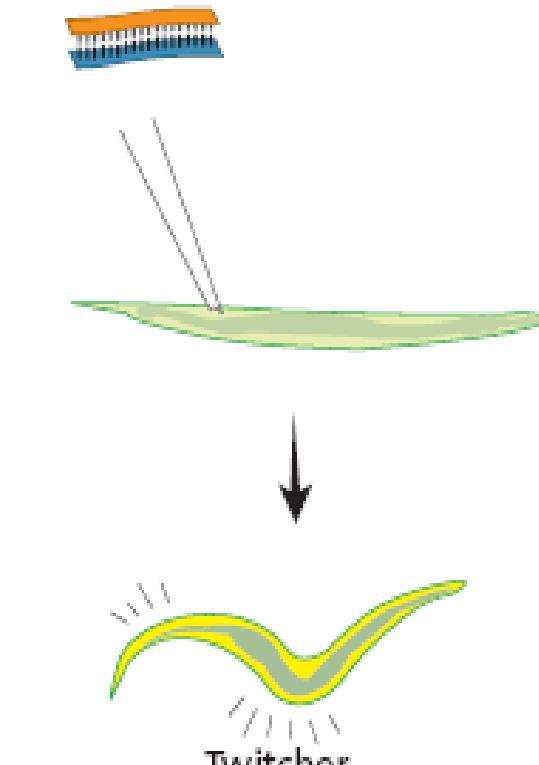
Sense RNA



Antisense RNA



Double-stranded RNA



Wild type

Wild type

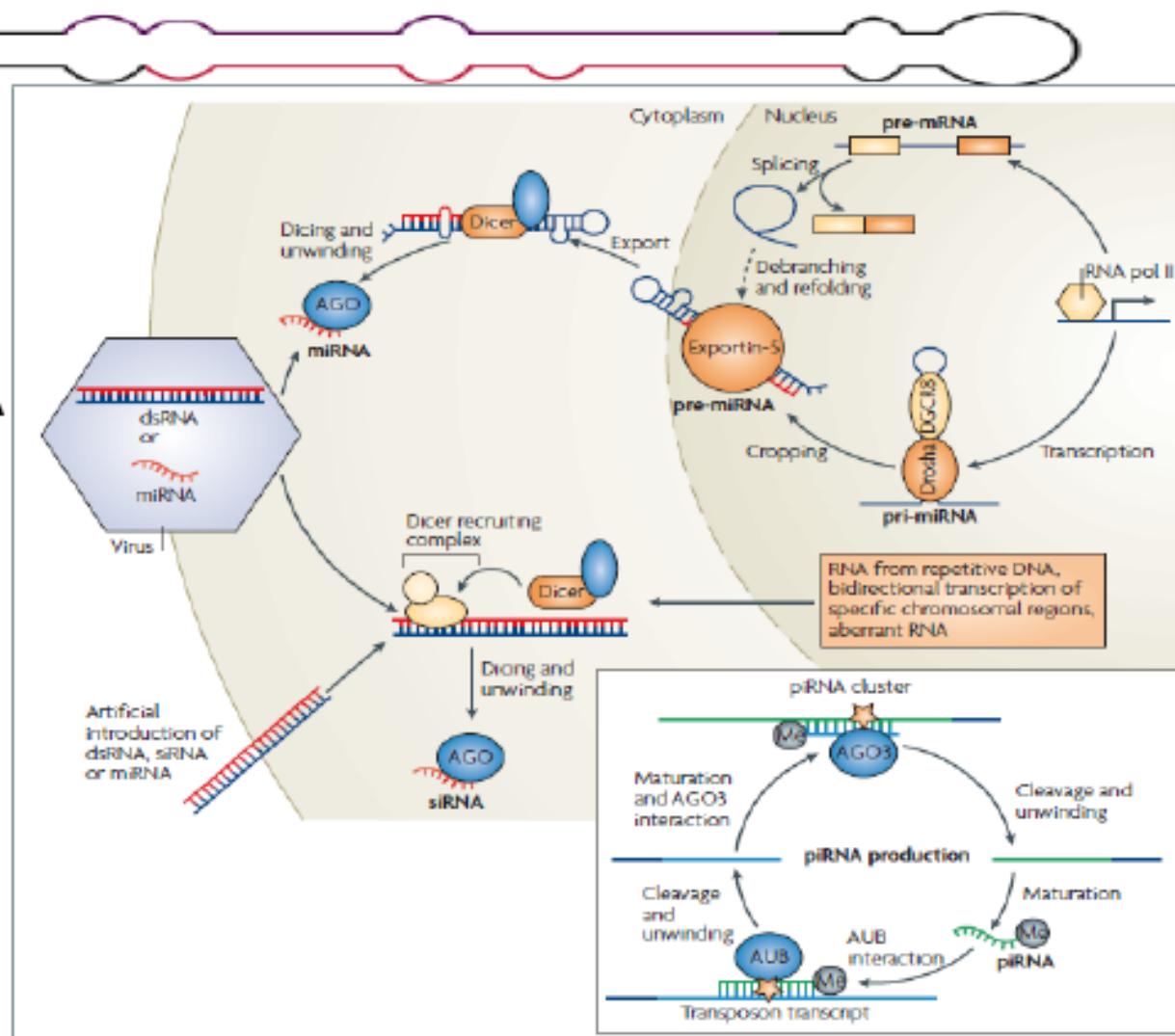
Twitcher

C. elegans

Figure 1. Phenotypic effect after injection of single-stranded or double-stranded *unc-22* RNA into the gonad of *C. elegans*. The *unc-22* gene encodes a myofilament protein. Decrease in *unc-22* activity is known to produce severe twitching movements. Injected double-stranded RNA, but not single-stranded RNA, induced the twitching phenotype in the progeny.

Small and non-coding RNAs

- miRNA
- siRNA
- snoRNA
- piRNA



Specifically, lin-4 was responsible for degradation of the protein LIN-14; mutant worms deficient in lin-4 function had high levels of LIN-14 and displayed developmental problems.

In 1993, Ambros and colleagues found that lin-4, unexpectedly, did not encode a regulatory protein. Instead, it gave rise to several small RNA molecules, 22 and 61 nucleotides in length, which Ambros called lin-4S short and lin-4L long. Sequence analysis showed that lin-4S was part of lin-4L: lin-4L was predicted to form a stem-loop structure, with lin-4S contained in one of the arms, the 5' arm. Furthermore, lin-4S was partially complementary to several sequences in the 3' untranslated region of the messenger RNA encoding the LIN-14 protein. Ambros and colleagues hypothesized that lin-4 could regulate LIN-14 through binding of lin-4S to these sequences in the lin-14 transcript in a type of antisense RNA mechanism.

In 2000, another *C. elegans* small RNA regulatory molecule, let-7, was characterized and found to be conserved in many species, including vertebrates. These discoveries confirmed that Ambros had in fact discovered a class of small RNAs with conserved sequence and functions. These molecules are now known as microRNAs.

Uncovered from the forgotten landscape of ‘dark genomic matter’, microRNAs (miRNAs) have become rising stars in cancer genetics.

miRNAs are small evolutionarily conserved non-coding RNAs of 18–25 nucleotides in length that act as expression regulators of genes involved in fundamental cell processes, such as development, differentiation, proliferation, survival and death

miRNAs are mostly transcribed from intragenic or intergenic regions by RNA polymerase II into primary transcripts of variable length (usually between 1 kb and 3 kb), called pri-miRNAs

The primary transcripts undergo further processing by the ribonucleases Drosha and DiGeorge syndrome critical region gene 8 (DGCR8) complex in the nucleus, thereby resulting in a hairpin intermediate of about 70–100 nucleotides, called pre-miRNA

The specificity of miRNA targeting is defined by Watson–Crick complementarities between positions 2 to 8 from the 5' miRNA (also known as the seed), with the 3' untranslated region (UTR) of their target mRNAs

When miRNA and its target mRNA sequence show perfect complementarities, the RISC induces mRNA degradation. Should an imperfect miRNA–mRNA target pairing occur, translation into a protein is blocked

the net result is a decrease in the amount of the proteins encoded by the mRNA targets

Each miRNA has the potential to target a large number of genes (on average about 500 for each miRNA family)

Conversely, an estimated 60% of the mRNAs have one or more evolutionarily conserved sequences that are predicted to interact with miRNAs

Bioinformatical analysis predicts that the 3' UTR of a single gene is frequently targeted by several different miRNAs

The pre-miRNA is then transported out of the nucleus to the cytoplasm by **exportin 5**

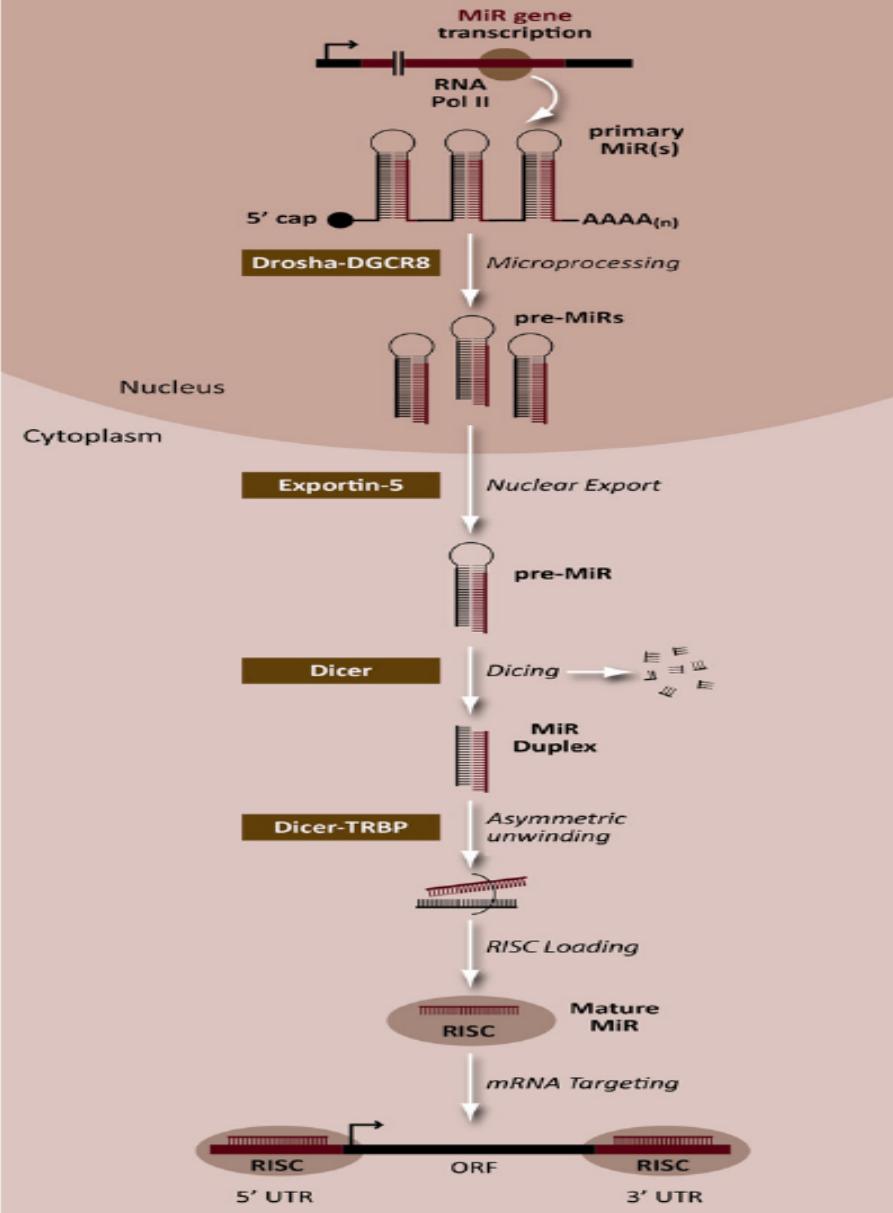
In the cytoplasm, the pre-miRNA is processed by another ribonuclease, Dicer, into a mature double-stranded **miRNA of variable length (~18–25 nucleotides)**

After strand separation, the **guide strand or mature miRNA** is incorporated into an RNA-induced silencing complex (RISC), whereas the **passenger strand**, denoted with a star (miRNA*) is commonly degraded

The RISC is the effector complex of the miRNA pathway and is comprised of miRNA, argonaute proteins (**argonaute 1– argonaute 4**) and other protein factors

Argonaute proteins have a crucial role in miRNA biogenesis, maturation and miRNA effector functions. The mature strand is important for target recognition and for the incorporation of specific target mRNAs into the RISC

MiR Biogenesis



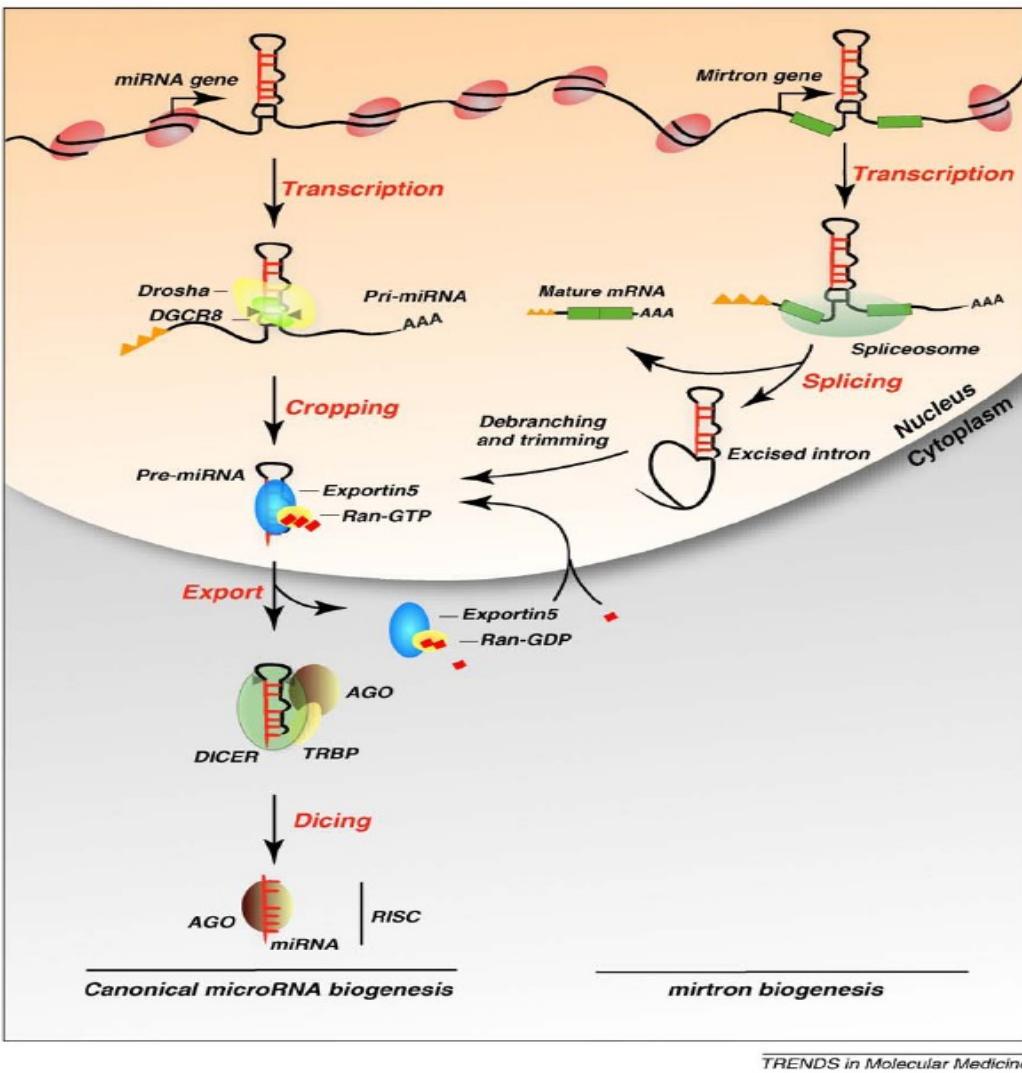
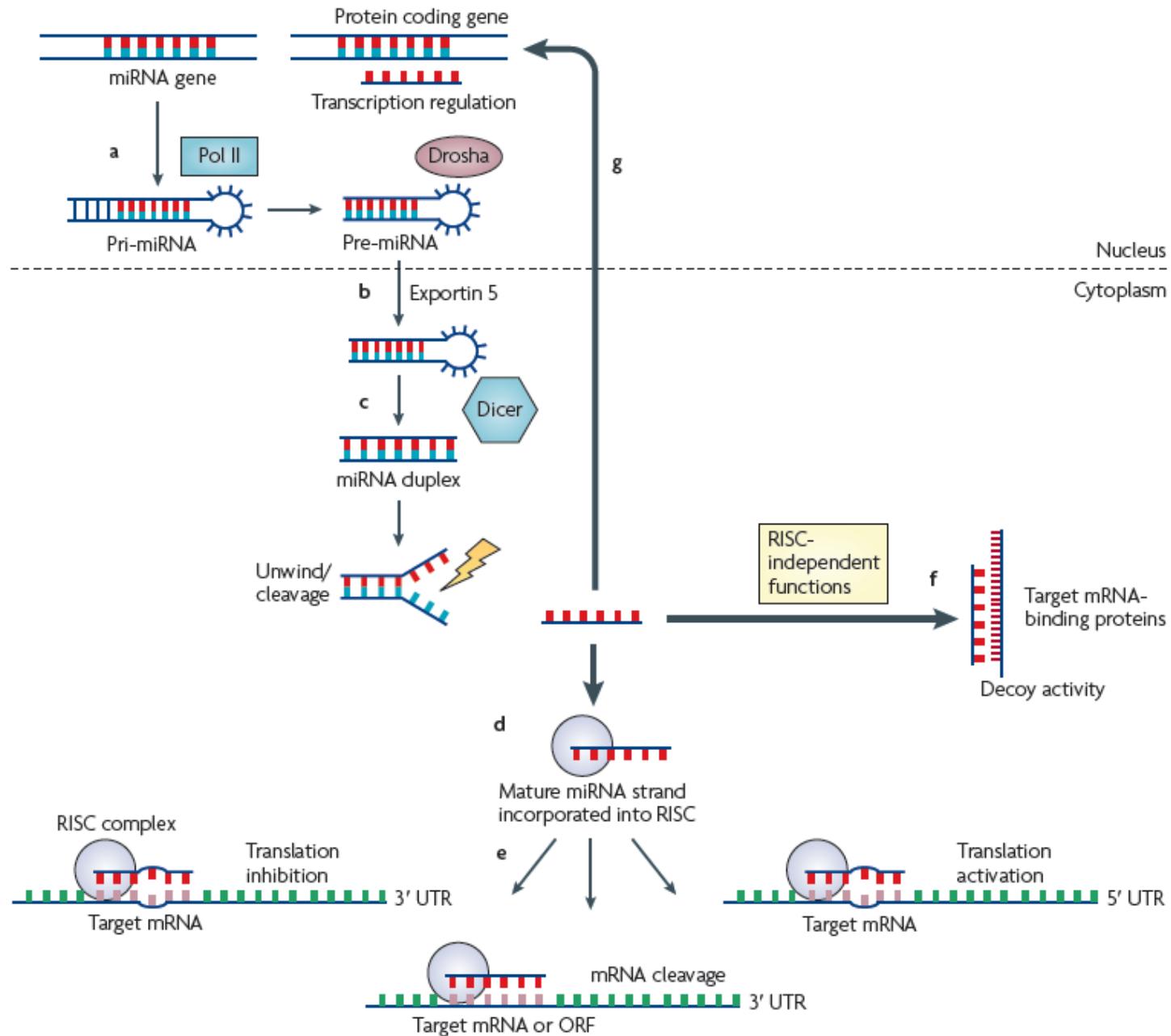


Figure 1. miRNA biogenesis. Canonical miRNAs are transcribed by RNA polymerase II to generate the primary transcript (pri-miRNAs), a long capped and polyadenylated RNA with a hairpin-shaped structure (left). Cropping is the first step in the maturation mediated by the microprocessor complex, essentially composed of the RNase III enzyme Drosha and the molecular anchor Di George syndrome critical region 8 (DGCR8), and produces a ~65 nt hairpin RNA called a precursor-miRNA (pre-miRNA). Pre-miRNA has a short stem with a 2–3 nt overhang, which is recognized by the Exportin-5-Ran-GTP complex. After export from the nucleus, the pre-miRNA is subjected to the dicing step operated by Dicer with its partner TRBP and Argonaut proteins 1–4 (AGO). This processing step creates the final duplex and allows the formation of the RNA-induced silencing complex (RISC), which mediates miRNA activity. This multistep process is used for independent miRNA transcription units or intronic miRNAs. In flies and mammals, some miRNAs called mirtrons (right and Box 2) are located in short introns and bypass the microprocessor complex-dependent step. After the splicing and production of the mature mRNA, the excised intron is debranched and trimmed to produce the pre-miRNA, which follows the canonical pathway for miRNA biogenesis beginning at the export step.

TRENDS in Molecular Medicine



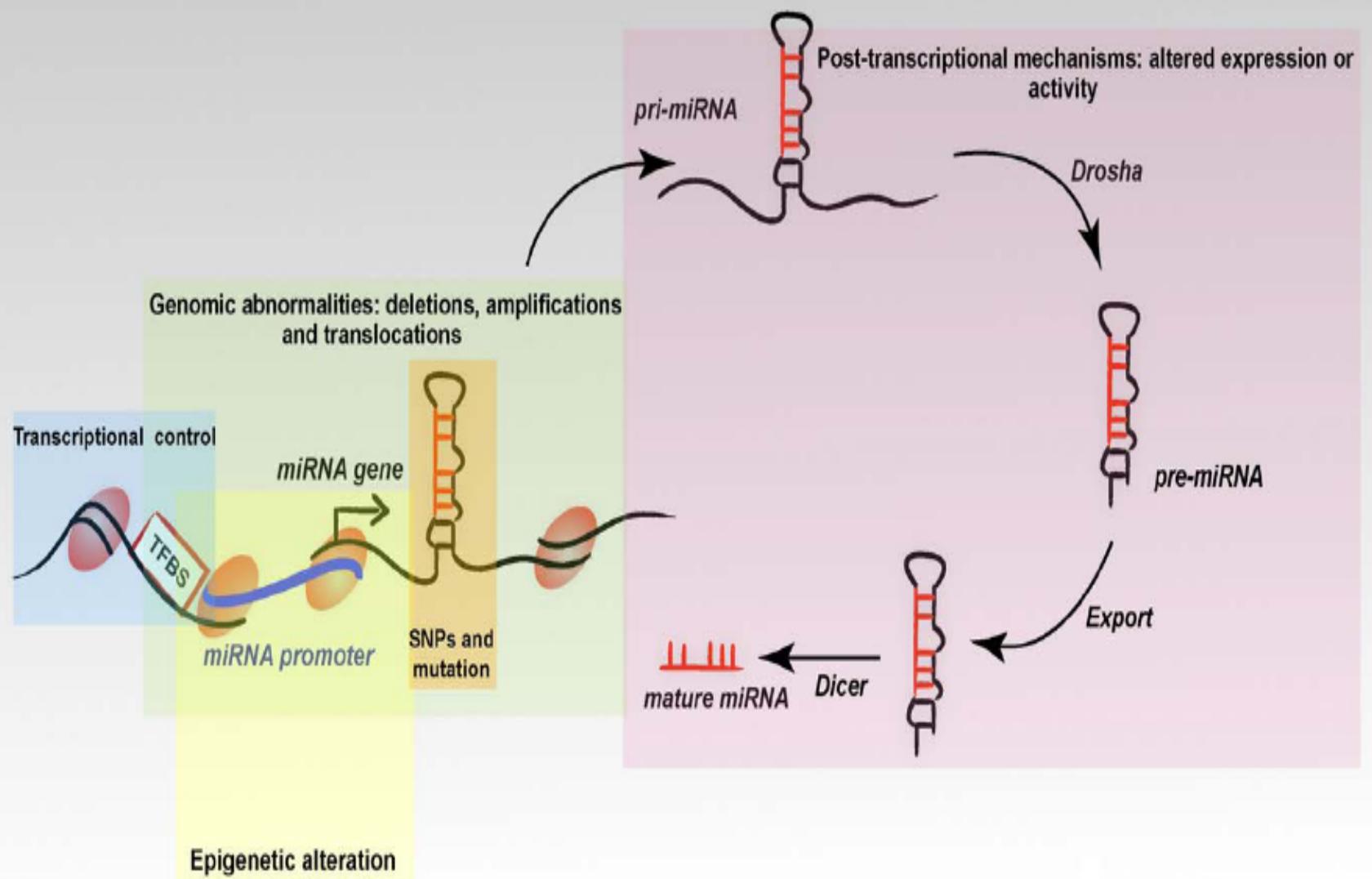
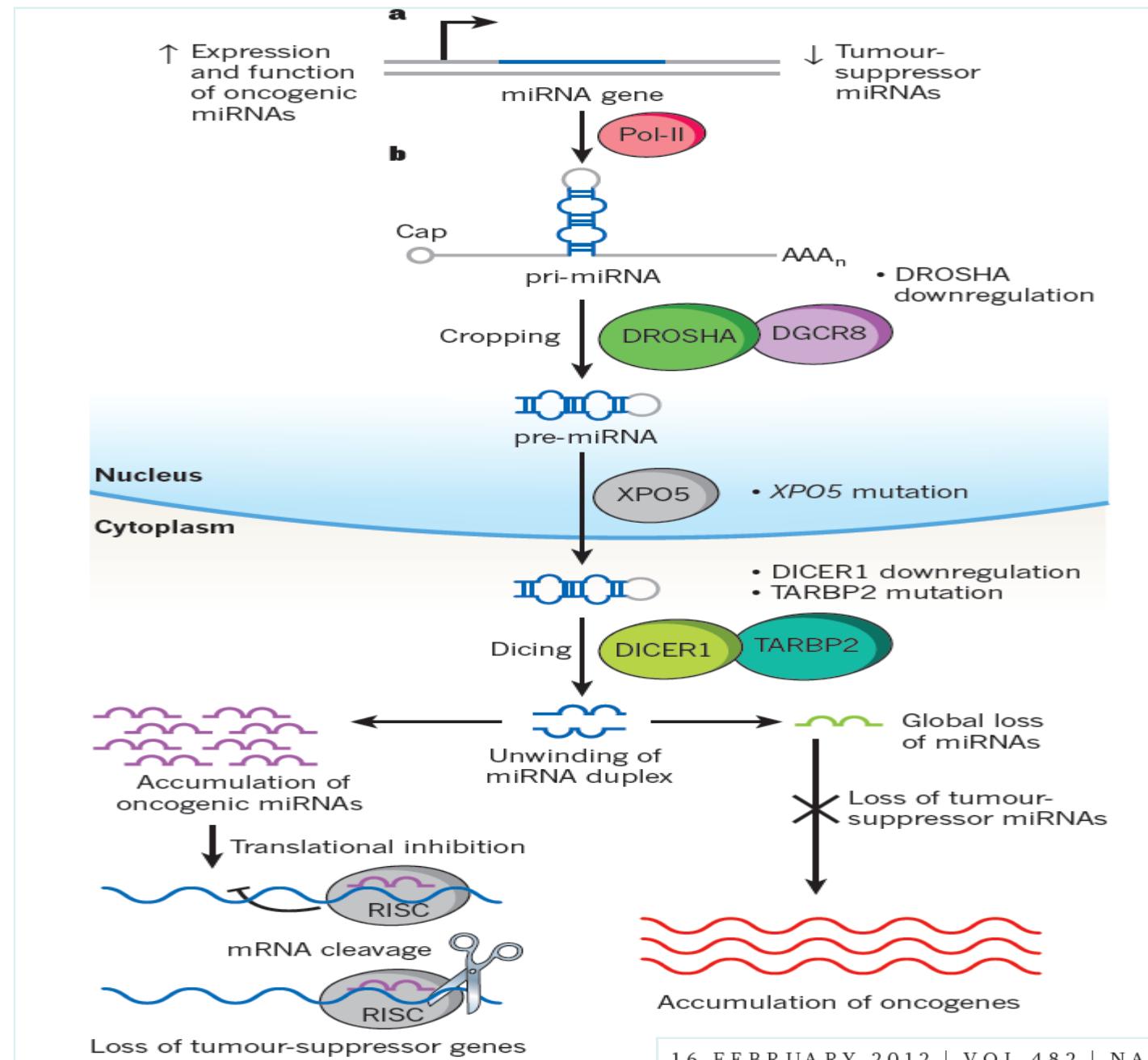
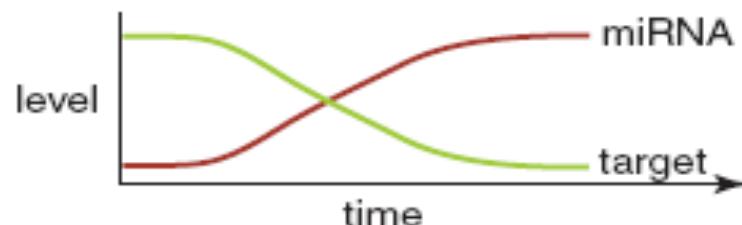


Figure 2. Mechanisms of miRNA dysregulation. MiRNA gene expression is a multistep, tightly regulated process and any alteration can contribute to miRNA dysregulation and neoplastic transformation. These mechanisms of miRNA regulation can be differentiated into 1) mechanisms targeting miRNA genes, including genomic alterations (deletions, amplifications or translocations), epigenetics changes (methylation and histone modification), polymorphisms or mutations and transcriptional alterations; and 2) mechanisms modulating the activity of the multistep processing enzymes (Drosha, DGCR8, Exportin-5, Dicer and TRBP).

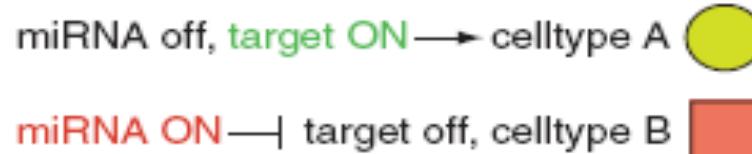
Mechanisms of miRNA perturbation in cancer.



A Temporal switch (*lin-4*, *let-7*)



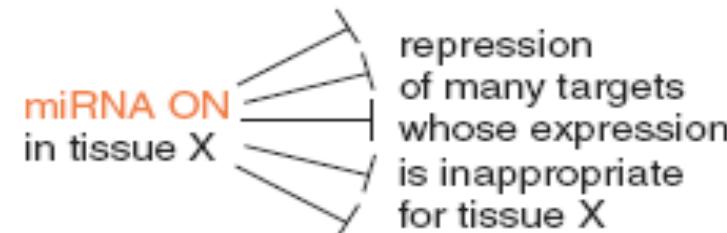
B Spatial switch (*miR-273*, *lsy-6*)



C Fine-tuning expression



D Tissue identity (*miR-1?*, *miR-124?*)



Current Biology

Figure 2. Biological rationales of miRNA-mediated regulation.

Many genetically characterized miRNAs are switches that strongly repress one or a few target genes at a particular time (A) or place (B) during development. Not shown are potential metabolic switches, such as *miR-375* or *miR-277*, which may dynamically regulate target genes in response to changing metabolic demands. Other miRNAs may regulate tens or hundreds of target genes. Some may function as general repressors of genes whose overexpression is undesirable or as fine-tuners of expression (C). Other miRNAs may specifically enforce the identity of a tissue by repressing genes whose expression in that particular tissue is detrimental (D). Note that a given miRNA may have a spectrum of targets which could collectively fall into several of these categories.

NEWS & VIEWS

GENE-EXPRESSION FORUM

Decoy for microRNAs

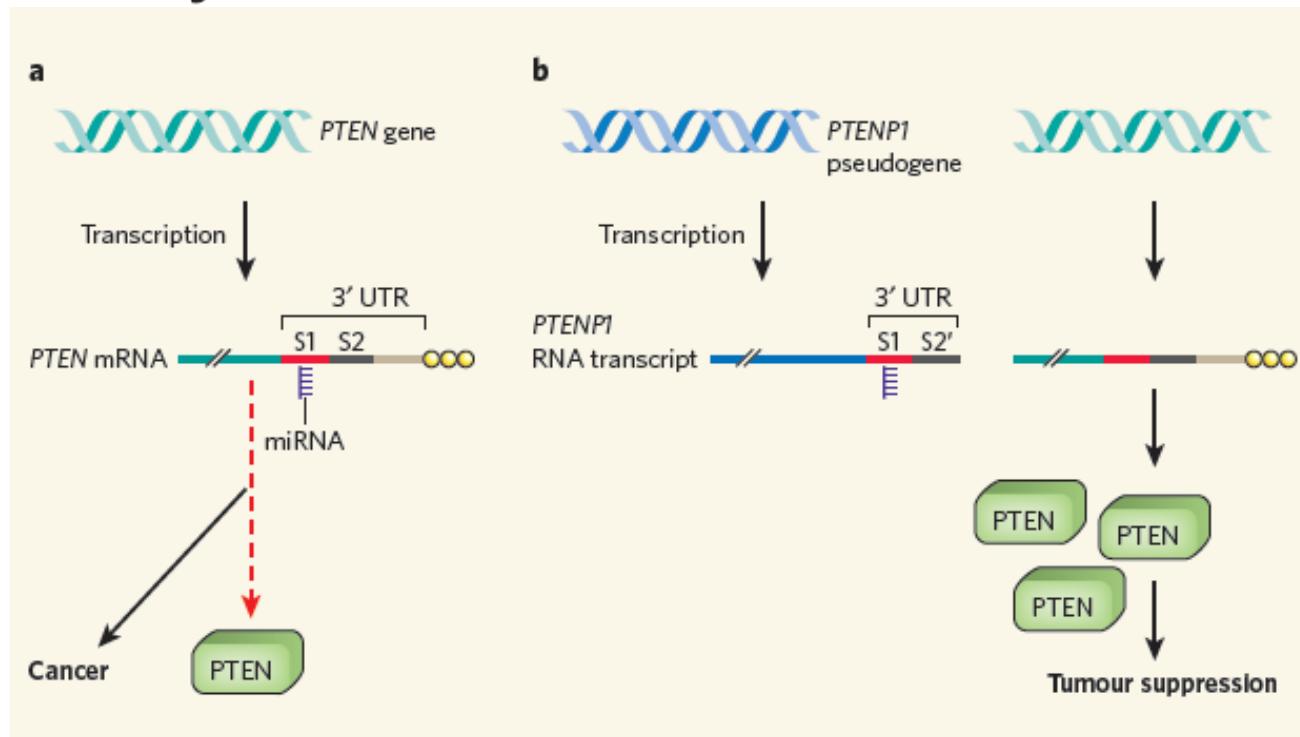
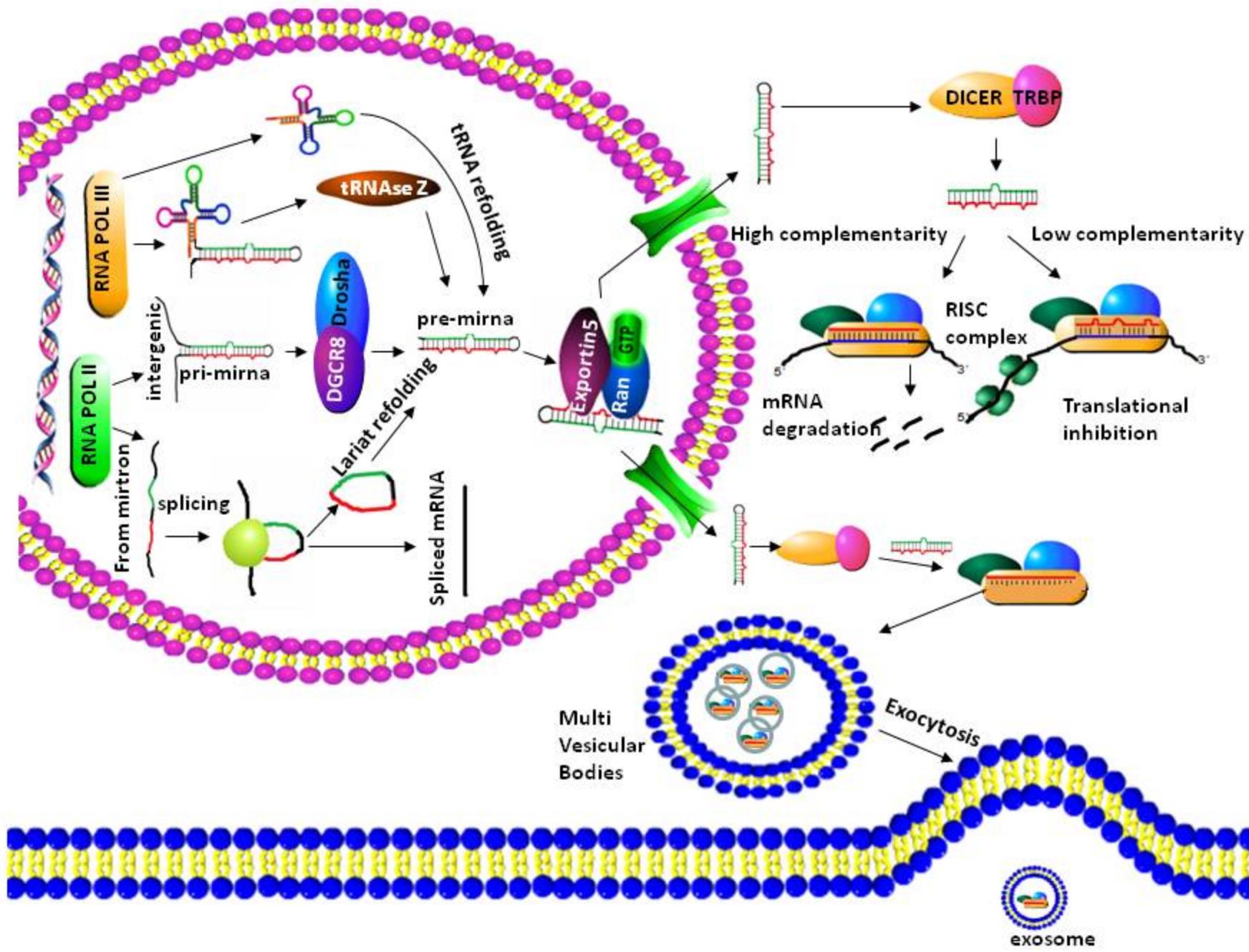


Figure 1 | Evolutionary relatives cooperate. **a**, Expression of the tumour-suppressor gene *PTEN* is downregulated by miRNA sequences that bind to the S1 portion of the 3' untranslated region (3' UTR) of its mRNA. **b**, Poliseno *et al.*¹ show that the RNA transcript of the *PTENP1* pseudogene, which has a similar — albeit shorter — 3' UTR to that of *PTEN* mRNA, binds to the same miRNAs, allowing expression of the *PTEN* protein. The outcome is suppression of cancer-cell growth.



Some miRNAs have been shown to bind to the open reading frame or to the 5' UTR of the target genes and, in some cases, they have been shown to activate rather than to inhibit gene expression

miRNAs can bind to ribonucleoproteins in a seed sequence and a RISC-independent manner and then interfere with their RNA binding functions (decoy activity).

miRNAs can also regulate gene expression at the transcriptional level by binding directly to the DNA



Figure 1 | MicroRNA genes map to chromosomal regions that are involved in alterations in human cancer.

miRNA in cancer

Tumor suppressors

- Usually down-regulated in malignant cells – validated targets include anti-apoptotic genes
- *let-7* targets oncogenes *KRAS*, *NRAS* and *HMGAT2* -> low expression in lung tumors
- miR-15a and miR-16-1 target Bcl-2
miR-15 and 16 at chr 13q14 -> frequently deleted in CLL
- miR-29 family targets Mcl-1, Tcf-1 , indirect induction of p53
- p53-inducible miR-34a (1p36) is commonly deleted in neuroblastomas – targets SIRT1, Bcl-2 → induces p53

oncomirs

- Up-regulated in tumors – validated targets include anti-proliferative/ cell cycle check point proteins
- mir-17-92 cluster on chr 13 frequently amplified in B-cell lymphoma
- miR-155 upregulated in diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma and Hodgkin's lymphoma

a

- Homozygous deletion
 - Heterozygous deletion and mutation
 - Mutations and polymorphism
 - Promoter hypermethylation
 - Histone deacetylation
- miRNA processing
- Transcription repression
 - Drosha/Dicer loss
 - RISC interface



Tumour suppressor miRNAs

- miR-15a–miR-16-1
miR-29a, miR-29b and miR-29c
let-7



- BCL-2*
MCL1, TCL1, CDK6, DNMT3α
RAS

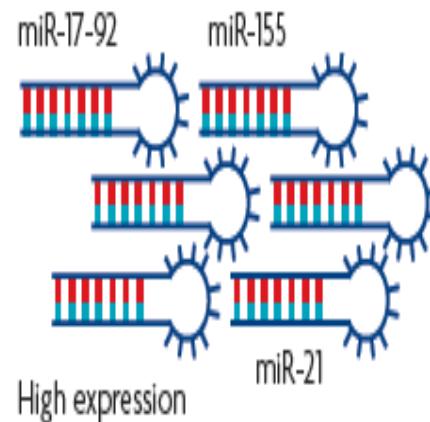
Oncogene mRNAs

- ↑Proliferation
↑Angiogenesis
↓Apoptosis
↑Invasion
↑Genetic instability

b

- Amplification
- Translocation
- Mutations/polymorphism
- Transcription activation

OncomiRNAs



- SHIP1, CEBPB*
PTEN, PDCD4
BIM

Tumour suppressor mRNAs

SUMMARY POINTS

1. Half of the known miRNAs are located inside or close to fragile sites and in minimal regions of loss of heterozygosity, minimal regions of amplification, and common break-points associated with cancer.
2. The loss of function of a miRNA could be due to several mechanisms, including genomic deletion, mutation, epigenetic silencing, and/or miRNA processing alterations.
3. MiRNAs can act as oncogenes or tumor suppressors depending on the tissue and the expression of their targets.
4. MiRNAs with a tumor suppressor function frequently have more than one genomic locus. This could be a natural defense against cancer, preserving tumor suppressor miRNA levels in the event of loss or mutation of one locus.

Table 1 | Key microRNAs involved in cancer

MicroRNA	Function	Genomic location	Mechanism	Targets	Cancer type	Mouse models	Clinical application
miR-17-92 cluster	Oncogene	13q22	Amplification and transcriptional activation	BIM, PTEN, CDKN1A and PRKAA1	Lymphoma, lung, breast, stomach, colon and pancreatic cancer	Cooperates with MYC to produce lymphoma. Overexpression induces lymphoproliferative disease	Inhibition and detection
miR-155	Oncogene	21q21	Transcriptional activation	SHIP1 and CEBPB	Chronic lymphocytic leukaemia, lymphoma, lung, breast and colon cancer	Overexpression induces pre-B-cell lymphoma and leukaemia	Inhibition and detection
miR-21	Oncogene	17q23	Transcriptional activation	PTEN, PDCD4 and TPM1	Chronic lymphocytic leukaemia, acute myeloid leukaemia, glioblastoma, pancreatic, breast, lung, prostate, colon and stomach cancer	Overexpression induces lymphoma	Inhibition and detection
miR-15a/16-1	Tumour suppressor	13q31	Deletion, mutation and transcriptional repression	BCL2 and MCL1	Chronic lymphocytic leukaemia, prostate cancer and pituitary adenomas	Deletion causes chronic lymphocytic leukaemia	Expression with mimics and viral vectors
let-7 family	Tumour suppressor	11 copies (multiple locations)	Transcriptional repression	KRAS, MYC and HMGA2	Lung, colon, stomach, ovarian and breast cancer	Overexpression suppresses lung cancer	Expression with mimics and viral vectors
miR-34 family	Tumour suppressor	1p36 and 11q23	Epigenetic silencing, transcriptional repression and deletion	CDK4, CDK6, MYC and MET	Colon, lung, breast, kidney, bladder cancer, neuroblastoma and melanoma	No published studies	Expression with mimics and viral vectors
miR-29 family	Oncogene	7q32 and 1q30	Transcriptional activation	ZFP36	Breast cancer and indolent chronic lymphocytic leukaemia	Overexpression induces chronic lymphocytic leukaemia	No published studies
	Tumour suppressor		Deletion and transcriptional repression	DNMTs	Acute myeloid leukaemia, aggressive chronic lymphocytic leukaemia and lung cancer		No published studies

BCL2, B-cell lymphoma protein-2; BIM, BCL-2-interacting mediator of cell death; CDKN1A, cyclin-dependent kinase inhibitor 1A; CEBPB, CCAAT/enhancer binding protein β ; HMGA2, high mobility group AT-hook 2; CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6; DNMT, DNA methyltransferase; MCL1, myeloid cell leukaemia sequence 1; PTEN, phosphatase and tensin homologue; PRKAA1, protein kinase, AMP-activated, alpha 1 catalytic subunit; PDCD4, programmed cell death 4; SHIP1, Src homology 2 domain-containing inositol 5-phosphatase 1; TPM1, tropomyosin 1; ZFP36, zinc finger protein 36.

Table 3 – Effect of up-/downregulation of different miRNAs on sensitivity to chemotherapy. The table presents the findings indicating a negative correlation between expression of miRNAs and effect on sensitivity to chemotherapy.

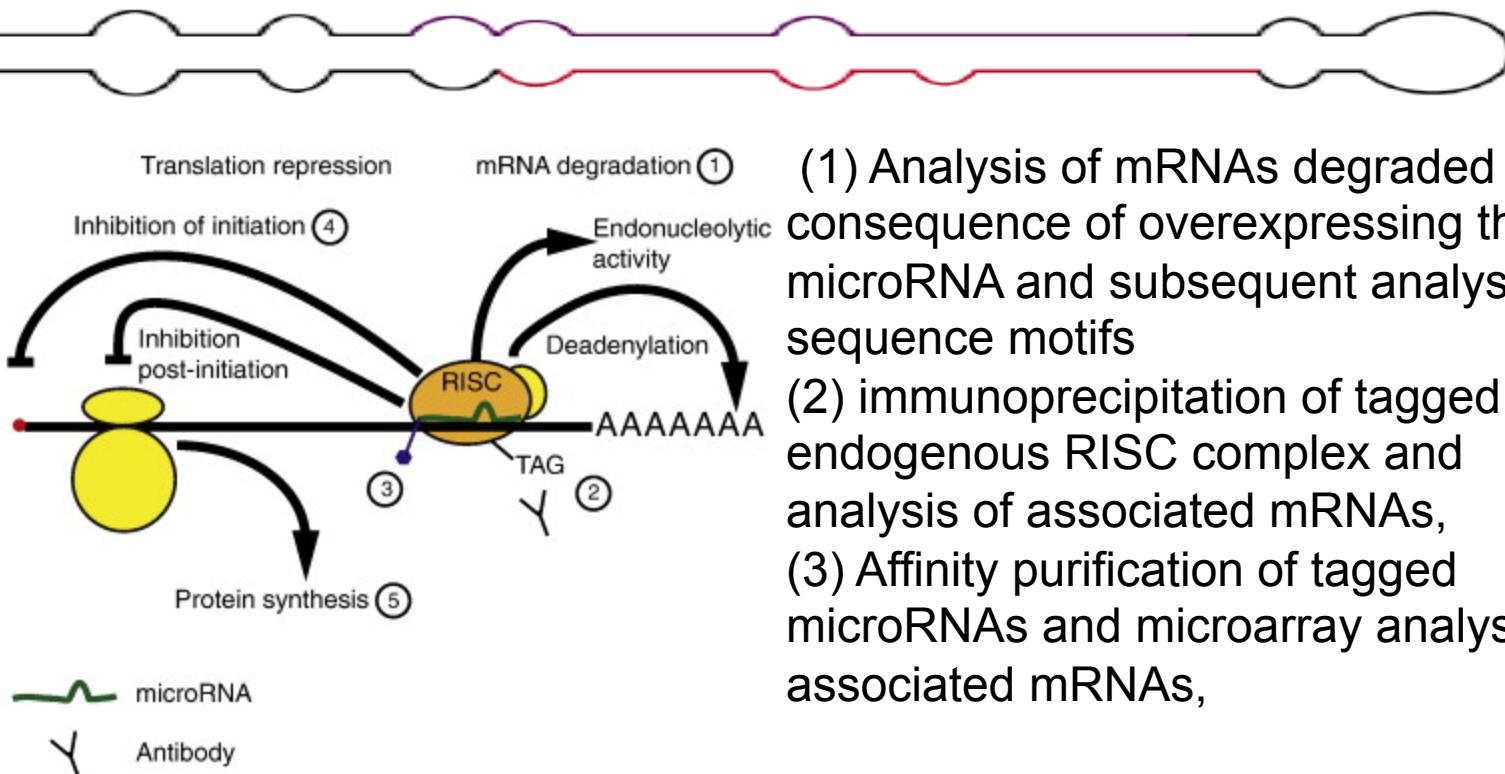
MicroRNA	Ectopic up-/downregulation	Chemotherapeutic agent	Effect on sensitivity to chemotherapy	Cancer type	Author
Dicer ^a	↓	Cisplatin	↑	Breast cancer	Bu et al. ⁵⁶
miR-17-5p	↓	Doxycycline	↑	Neuroblastoma	Fontana et al. ⁵⁷
miR-18	↑	–	↓ (Repressed glucocorticoid-receptor)	–	Vreugdenhil et al. ⁷²
miR-124a	↑	–	↓ (Repressed glucocorticoid-receptor)	–	Vreugdenhil et al. ⁷²
miR-21	↓	Topotecan	↑	Breast cancer	Si et al. ⁴⁵
miR-21	↓	Gemcitabine	↑	Malignant cholangiocytes	Meng et al. ⁴⁴
miR-21	↓	NSC 621888, NSC 622700, NSC 670550, NSC 122750	↑	Lung cancer	Blower et al. ⁵⁴
miR-21	↓	NSC 670550, NSC 122750	↑	Glioblastoma	Blower et al. ⁵⁴
miR-27b	↓	–	↑ (CYP1B1)	Breast cancer	Tsuchiya et al. ⁶⁵
miR-98	↑	Cisplatin, doxorubicine	↓	Head and neck squamous cell carcinoma	Hebert et al. ⁵³
miR-200b	↓	Gemcitabine	↑	Malignant cholangiocytes	Meng et al. ⁴⁴
miR-206	↑	–	↓ (repressed oestrogen receptor α)	Breast cancer	Kondo et al. ⁷⁴
miR-214	↓	Cisplatin	↑	Ovarian cancer	Yang et al. ⁶¹
miR-221 and/or miR-222	↑	–	↓ (repressed oestrogen receptor α)	Breast cancer	Zhao et al. ⁷³
miR-221 and/or miR-222	↓	Tamoxifen	↑	Breast cancer	Zhao et al. ⁷³
miR-222/miR-222	↑	Tamoxifen	↓	Breast cancer	Miller et al. ³⁴
Let-7i	↓	NSC 670550	↑	Lung cancer	Blower et al. ⁵⁴
Let-7a	↓	Gemcitabine, 5-fluorouracil, camptothecin	↑	IL-6-overexpressing malignant cholangiocytes	Meng et al. ⁴⁷
Let-7a	↑	Interferon-gamma, doxorubicine, paclitaxel	↓	Squamous cell carcinoma, hepatocellular carcinoma	Tsang and Kwok ⁶³
BART cluster 1 miRNAs	↑	Cisplatin	↓	LMP1-expressing EBV-positive epithelial cell line	Lo et al. ⁶²

^a miRNA processing/function enzyme.

Table 4 – Effect of up-/downregulation of different miRNAs on sensitivity to radiotherapy.

MicroRNA	Ectopic up-/downregulation	Effect on sensitivity to radiotherapy	Cancer type	Author
miR-521	↑	↑	Prostate cancer	Josson et al. ³⁹
Let-7g	↑	↓	Lung cancer	Weidhaas et al. ³⁸
Let-7a	↑	↑	Lung cancer, C. elegans	Weidhaas et al. ³⁸
Let-7b	↑	↑	Lung cancer, C. elegans	Weidhaas et al. ³⁸

Experimental identification of microRNA targets



(1) Analysis of mRNAs degraded as a consequence of overexpressing the microRNA and subsequent analysis of sequence motifs
(2) immunoprecipitation of tagged or endogenous RISC complex and analysis of associated mRNAs,
(3) Affinity purification of tagged microRNAs and microarray analysis of associated mRNAs,

(4) by using the observation that some microRNA targets move in the polysomal distribution upon microRNA targeting and analyzing differences in polysomal associated mRNAs with and without the microRNA,
(5) analyzing protein production following labeling of proteins and mass spectrometry.

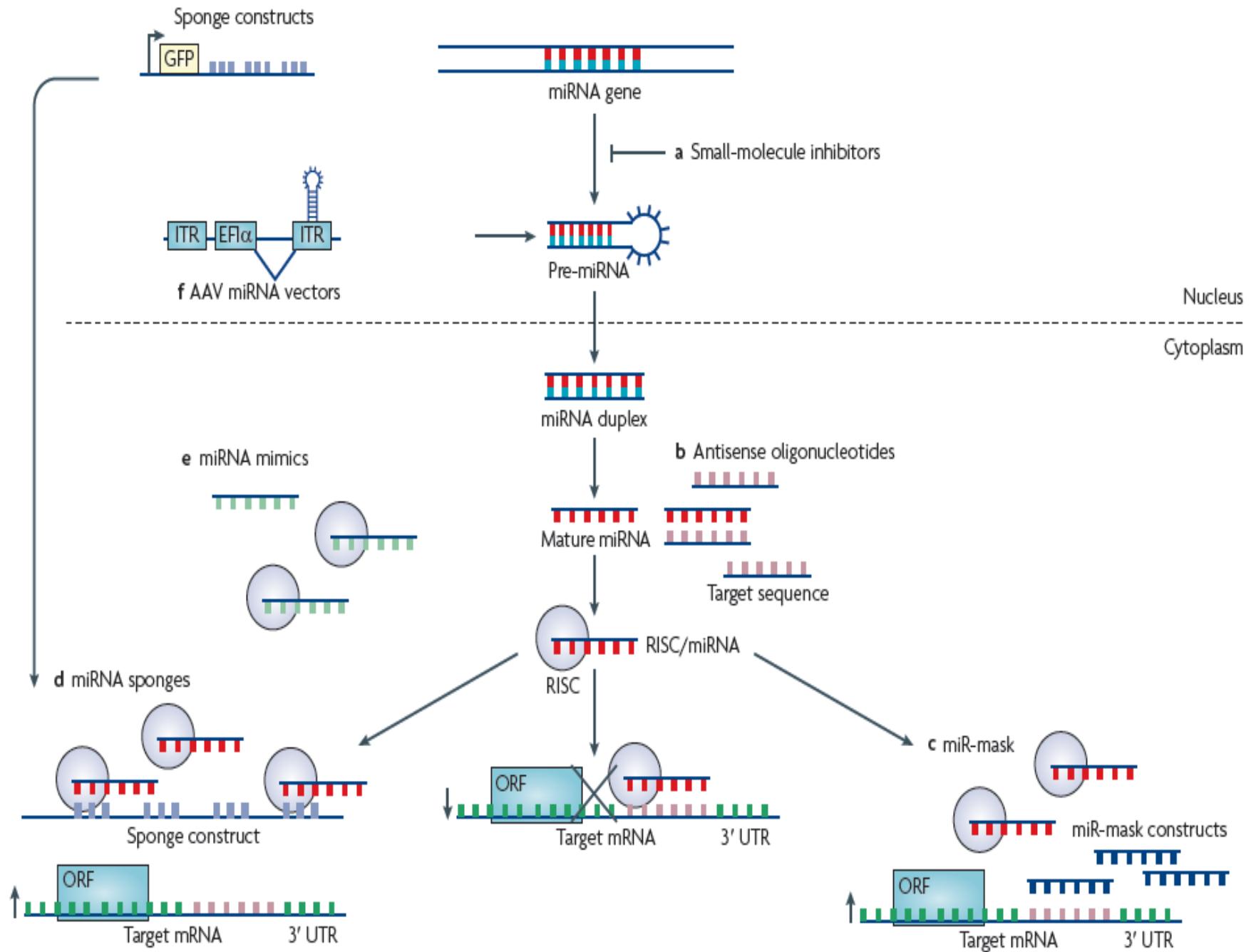
Target Prediction Programs

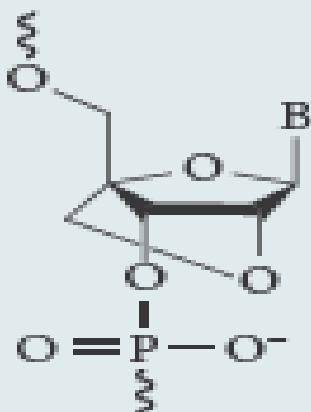
Method	Type of Method	Ref	Method Availability
Stark et. al	Complementary	(Stark et. al., 2003)	Online search
miRanda	Complementary	(John et al., 2004)	Download
miRanda MiRBase	Complementary	(Enright et al., 2003)	Online search
Target Scan	Seed Complementary	(Lewis et al., 2005)	Online search
DIANA microT	Thermodynamics	(Kirakidou et al., 2004)	Download
PicTar	Thermodynamics	(Krek et al., 2005)	N/A
RNAHybrid	Thermodynamics & Statistical model	(Rehmsmeier et al., 2004)	Download
miRGen++	Baynesian Inference	(Huang et al., 2007b)	Mathlab Code
MiTarget	Support Vector Machine	(Kim et al. 2006)	Online search
MiRtaget2	Support Vector Machine	(Wang and El Naqa, 2008)	Online search
TarBase	Experimentally Validated Targets	(Sethupathy et al., 2006)	N/A

Rules of Target Prediction

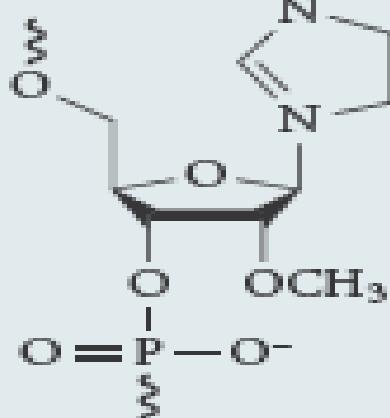
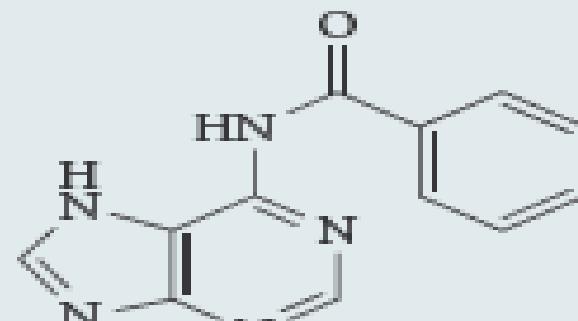


- Sequence complementarities – nucleus or seed region
 - 5' dominant site and 3' compensatory site
 - Six or seven nucleotides starting at 1st or 2nd position of 5' end
- Conservation of target site across species
- Free energy changes of miRNA-mRNA interaction – computational or quantitative luciferase assay (Kertesz et al., Nat. Genet. 2007)
- Correlation of miRNA and gene expression profiles
- AU-rich nucleotide composition near the site
- Secondary structure of the miRNA-mRNA complex – affects binding of protein complexes
- Proximity to sites on mRNA that are targeted by co-expressed miRNA
- Positioning from the stop codon
- Positioning away from the centre of long UTRs

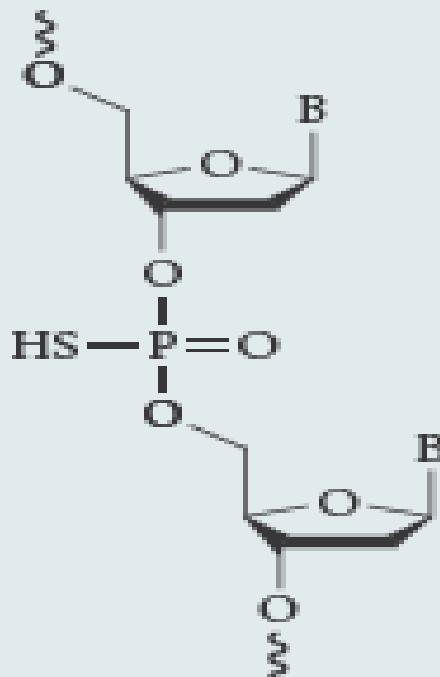




LNA



2'-O-Methyl



Phosphorothioate

Figure 5 | Common oligonucleotide modification structures. The three most common oligonucleotide modification structures are shown; locked nucleic acid (LNA), 2'-O-methyl and phosphorothiolate^{101,107}. B, base.

miRNA Therapeutics



Requirements

- Superior affinity of modified oligos
- Mismatch discrimination and lower off-target effects
- Low toxicity
- Increased metabolic stability

Delivery Strategies

- Complexing or covalently linking to delivery lipids/proteins
- Cationic liposome / cholesterol
- Phage packaging
- Receptor binding RNA aptamers
- Antibody-protamine complex

- Long lasting non-toxic silencing of miR-122 by intravenously injected antagonirs in mice (Krutzfeldt *et al.*, *Nature*, 2005)
- Systemic administration of miR-26a in a mouse model of HCC using adeno-associated virus (AAV) (Kota *et al.*, *Cell*, 2009)
- miRNA Delivery to Leukemia Cells Through TfR-Targeted Liposomes – Ohio State Univ ; unpublished

Table 2 | Therapeutic use of miRNAs and antagonists *in vivo*

miRNA [†]	Delivery method	Model used	Phenotypes	Refs
let-7	Intranasal delivery of viral particles	Kras ^{G12D/+} autochthonous NSCLC mouse	Suppression of lung tumour initiation when delivered at the same time as transgene activation	145, 146
	Intratumoral injection of lipid-based mimetic	Subcutaneous H460 NSCLC xenografts	Interfered with further tumour growth	62
	Intranasal delivery of viral particles	Kras ^{G12D/+} autochthonous NSCLC mouse	Reduced burden of tumours that were allowed to preform 10 weeks before let-7 therapy	62
	Systemic delivery of lipid-based mimetic	Kras ^{G12D/+} autochthonous NSCLC mouse	Reduced burden of tumours that were allowed to preform 10 weeks before let-7 therapy	90
	Transfected into cells before transplantation	Subcutaneous human U251 or U87 glioblastoma cells	Reduced tumour formation	91
	Transduced into cells before transplantation	Chemotherapy-resistant breast tumour initiating cells	Reduced tumour formation and metastasis	92
miR-143 and miR-145	Transduced into cells before transplantation	Subcutaneous MiaPaCa2 and Panc1 PDAC xenografts	Unable to form tumours	94
	Systemic delivery of lipid-based expression vectors	Subcutaneous MiaPaCa2 PDAC xenografts	Inhibited growth	96
	Systemic delivery of lipid-based expression vectors	Orthotopic MiaPaCa2 PDAC xenografts	Inhibited growth	96
miR-143	Systemic delivery of anti-miR-143	p21-HBx HCC mouse	Inhibited primary tumour and local and distant metastatic growth	100
miR-34	Intratumoral delivery of lipid-based mimetic	Subcutaneous H460 NSCLC xenografts	Inhibited growth	106
	Systemic delivery of lipid-based mimetic	Subcutaneous H460 and A549 NSCLC xenografts	Inhibited growth	106
	Systemic delivery of lipid-based mimetic	Kras ^{G12D/+} autochthonous NSCLC mouse	Reduced burden of tumours that were allowed to preform 10 weeks before miR-34 therapy	90
	Transfected oligonucleotides into cells before transplantation	Subcutaneous prostate cancer xenografts (multiple cell lines)	Reduced tumour incidence	107
	Transduced cells with virus encoding precursor (pre)-miR-34 before transplantation	Subcutaneous prostate cancer xenografts (multiple cell lines)	Reduced tumour incidence	107
	Intratumoral injection of lipid-based mimetic	Subcutaneous PPC1 prostate cancer xenografts	Inhibited growth	107
	Systemic delivery of lipid-based mimetic	Orthotopic PC3 prostate cancer xenografts	Reduced tumour burden	107
	Systemic delivery of lipid-based mimetic	Orthotopic LAPC9 prostate cancer xenografts	Reduced lung metastasis without affecting primary tumour growth; extended survival	107
miR-122	Transduced into cells before transplantation	Orthotopic SKHEP1 HCC xenografts	Inhibited growth	96
	Systemic delivery of nucleic acid antagonist-miR-122	HCV-infected non-human primates	Reduced tumorigenesis, angiogenesis and intrahepatic metastasis	111
	Systemic delivery of lysine-lipid nanoparticle antagonist-miR-122	C57BL/6J mice	Suppression of HCV viraemia and improved liver pathology	112
	Systemic delivery of antagonist-miR-122	C57BL/6J mice	Decreased plasma cholesterol levels	113
miR-26a	Systemic delivery of adeno-associated virus	C57BL/6J mice	Decreased plasma cholesterol levels	114
miR-10b	Systemic delivery of antagonist-miR-10b	Implantation of 4T1 breast cancer cells into the mammary fat pad	Prevented metastasis with no effect on the primary tumour	84

[†]miRNAs are presented in the order in which they are discussed in the main text. miRNAs for which there is therapeutic evidence in endogenously occurring tumours or orthotopic implants are included. In some instances xenograft models are included but only as supporting evidence. HCC, hepatocellular carcinoma; HCV, hepatitis C virus; miRNA, microRNA; NSCLC, non-small-cell lung cancer; PDAC, pancreatic ductal adenocarcinoma.

Table 1 miRNA therapeutics in commercial development

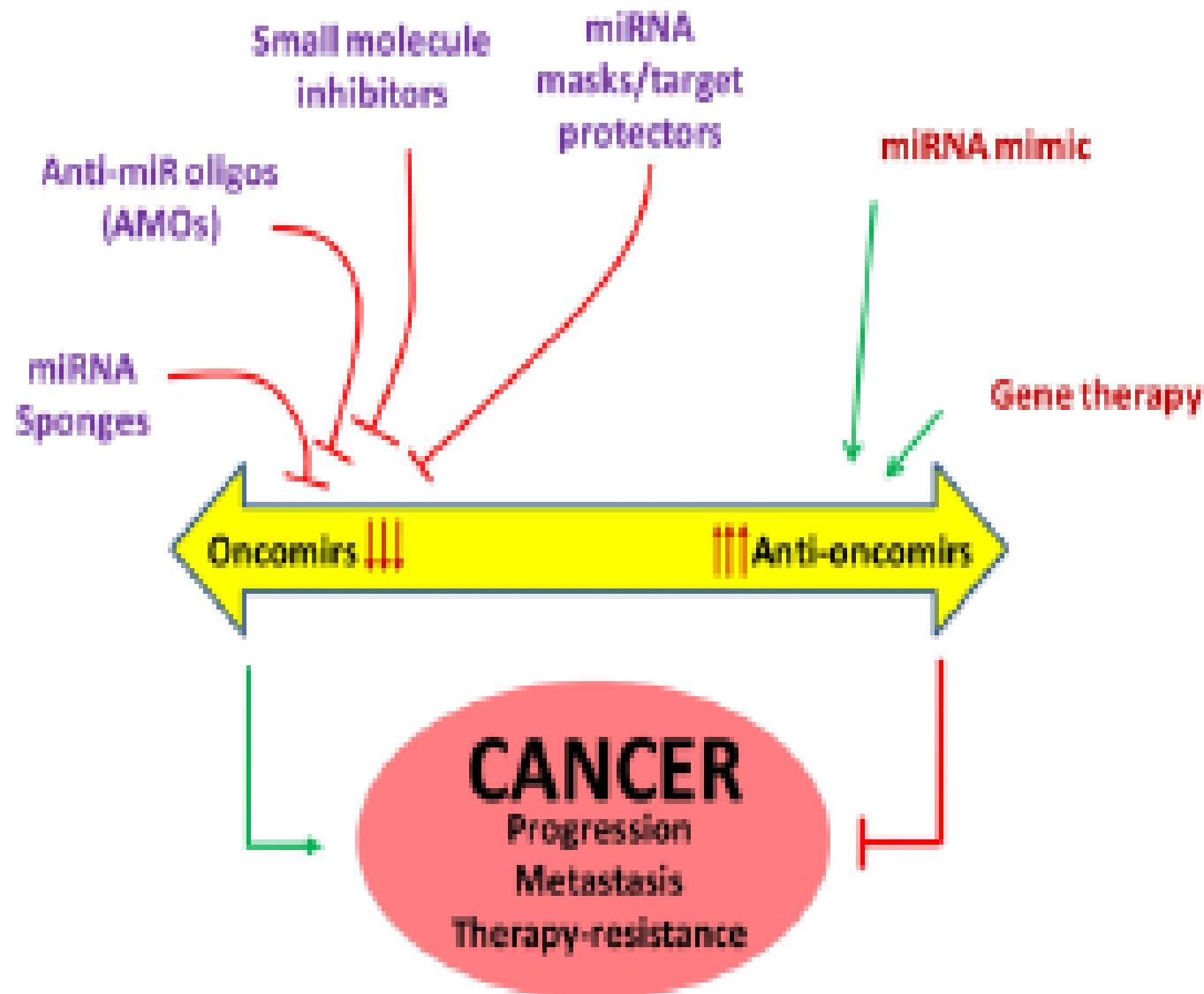
Company	Diseases	Chemistries	Stage	References
Regulus Therapeutics	Immunoinflammatory, cardiovascular, metabolic disease, oncology, fibrosis and hepatitis C infection	miRNA inhibitors using 2'-methoxyethyl, 2'-fluoro RNA, bicyclic ribose modifications	Pre-clinical	Krützfeldt <i>et al.</i> ³⁷ ; Krützfeldt <i>et al.</i> ³⁸ ; Esau <i>et al.</i> ⁴⁰ ; http://www.regulusrx.com
Santaris Pharma A/S	Cancer and inflammatory diseases, hepatitis C infection	miRNA inhibitors using locked nucleic acid chemistry	miR-122 inhibitor: phase I completed, phase II initiated	Ørom <i>et al.</i> ⁴¹ ; Elmén <i>et al.</i> ⁴² ; http://www.santarisperapeutics.com
miRagen Therapeutics	Cardiovascular and muscle diseases	miRNA inhibition and replacement	Pre-clinical	http://www.miragentherapeutics.com
Mirna Therapeutics	Non-small cell lung cancer and prostate cancer	miRNA replacement using siRNAs	Pre-clinical	http://www.mirnatherapeutics.com

Abbreviations: miRNA, microRNA; siRNA, small interfering RNA.

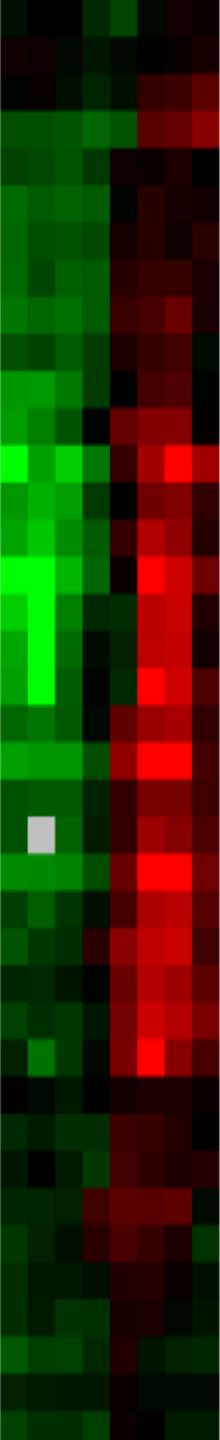
Table 1. Cancer subtypes that can be distinguished by microRNA or miRNA profiles

Cancer type	miRNAs ^a	Ref.
Breast		
ER status	miR-26a/b, miR-30 family, miR-29b, miR-155, miR-342, miR-206, miR-191	[38–40,42]
PR status	let-7c, miR-29b, miR-26a, miR-30 family, miR-520g	[41,42]
HER2/neu status	miR-520d, miR-181c, miR-302c, miR-376b, miR-30e	[38,41]
Lung		
Squamous vs non-squamous cell	miR-205	[33]
Small cell vs non-small cell	miR-17-5p, miR-22, miR-24, miR-31	[32]
Gastric		
Diffuse vs intestinal	miR-29b/c, miR-30 family, miR-135a/b	[35]
Endometrial		
Endometrioid vs uterine papillary	miR-19a/b, miR-30e-5p, miR-101, miR-452, miR-382, miR-15a, miR-29c	[37]
Renal		
Clear cell vs papillary	miR-424, miR-203, miR-31, miR-126	[34,36]
Oncocytoma vs chromophobe	miR-200c, miR-139-5p	[36]
Myeloma		
with t(14;16)	miR-1, miR-133a	[60]
with t(4;14)	miR-203, miR-155, miR-375	[60]
with t(11;14)	miR-125a, miR-650, miR-184	[60]
Acute myeloid leukemia		
with t(15;17)	miR-382, miR-134, miR-376a, miR-127, miR-299-5p, miR-323	[52]
with t(8;21) or inv(16)	let-7b/c, miR-127	[52]
with <i>NPM1</i> ^b mutations	miR-10a/b, let-7, miR-29, miR-204, miR-128a, miR-196a/b	[51,52]
with <i>FLT3</i> ITD	miR-155	[51,52,54]
Chronic lymphocytic leukemia		
ZAP-70 levels and IgVH status	miR-15a, miR-195, miR-221, miR-155, miR-23b	[50]
Melanoma		
with BRAF V600E	miR-193a, miR-338, miR-565	[56]

^aNot all distinguishing miRNAs are represented in this table.^bnucleophosmin 1.



Techniques used in miRNA work



miRNA expression profiling

- microarray
- qRT-PCR

miRNA over expression

- oligos
- Expression vectors

miRNA silencing

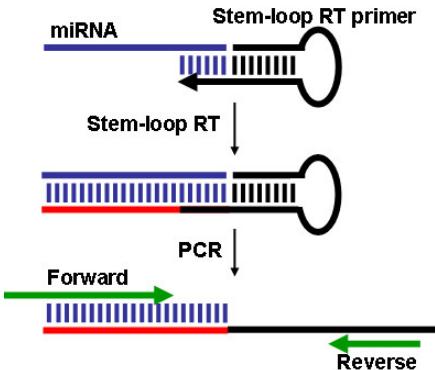
- sponges
- AMOs
- ASOs

Target Prediction

Computational

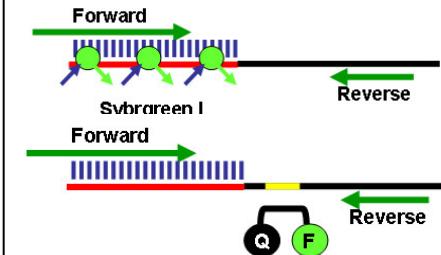
RNA isolation and RT

- mRNA to cDNA
- miRNA to cDNA (stem loop RT)



miRNA detection and Quantification

- qRT PCR



- Northern Blot

Target Validation

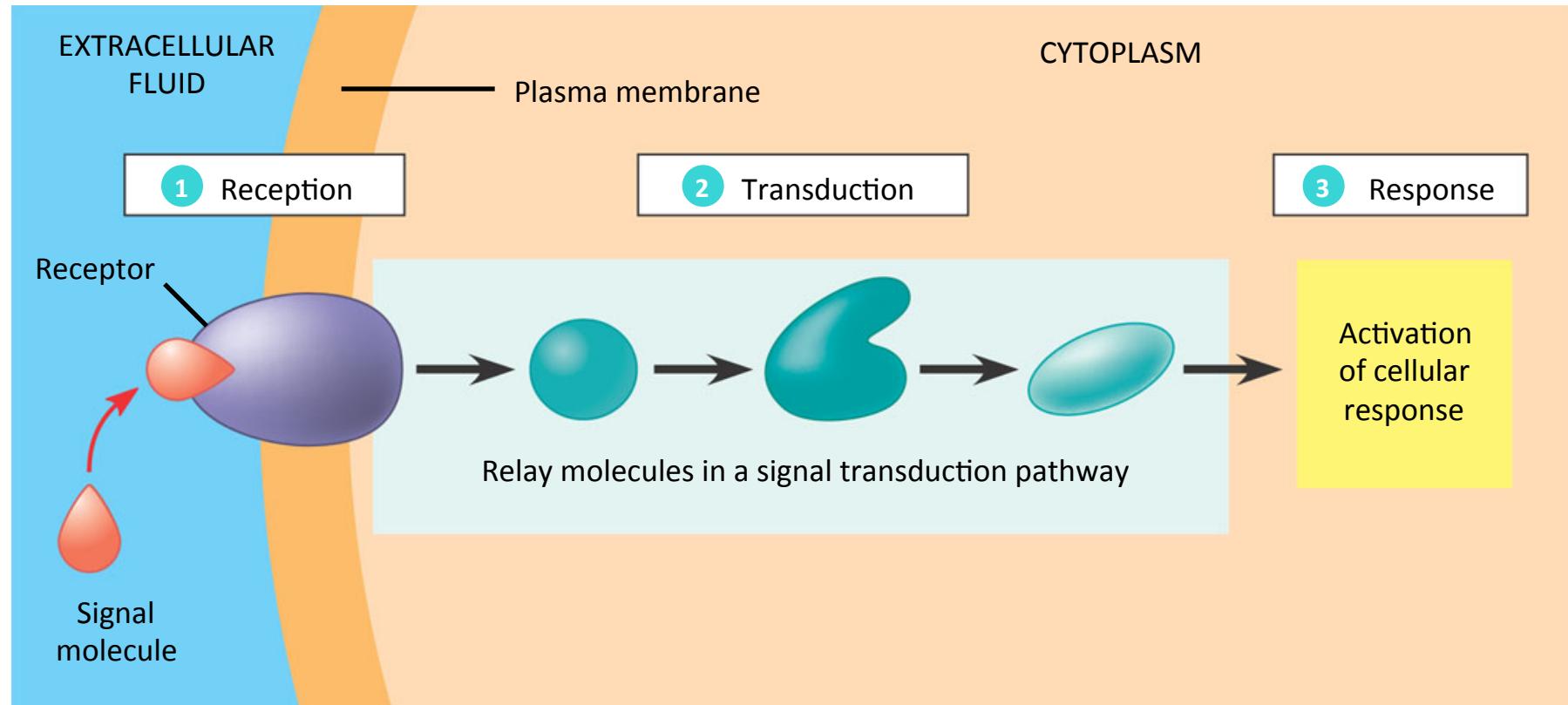
- 3'UTR Luciferase
- Real Time PCR
- Western Blot



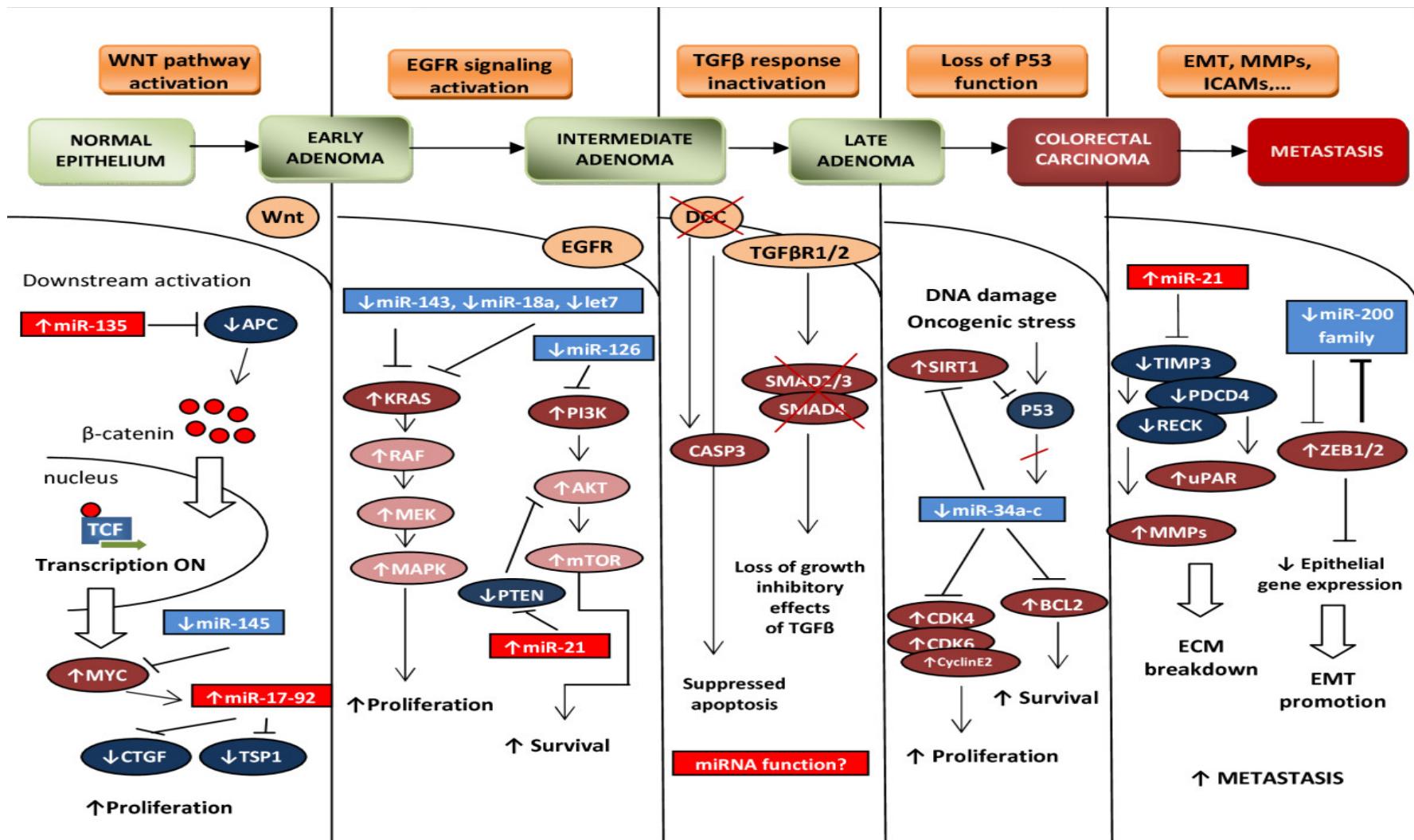
pMir- Report-Luc

- 3' UTR
- Luciferase

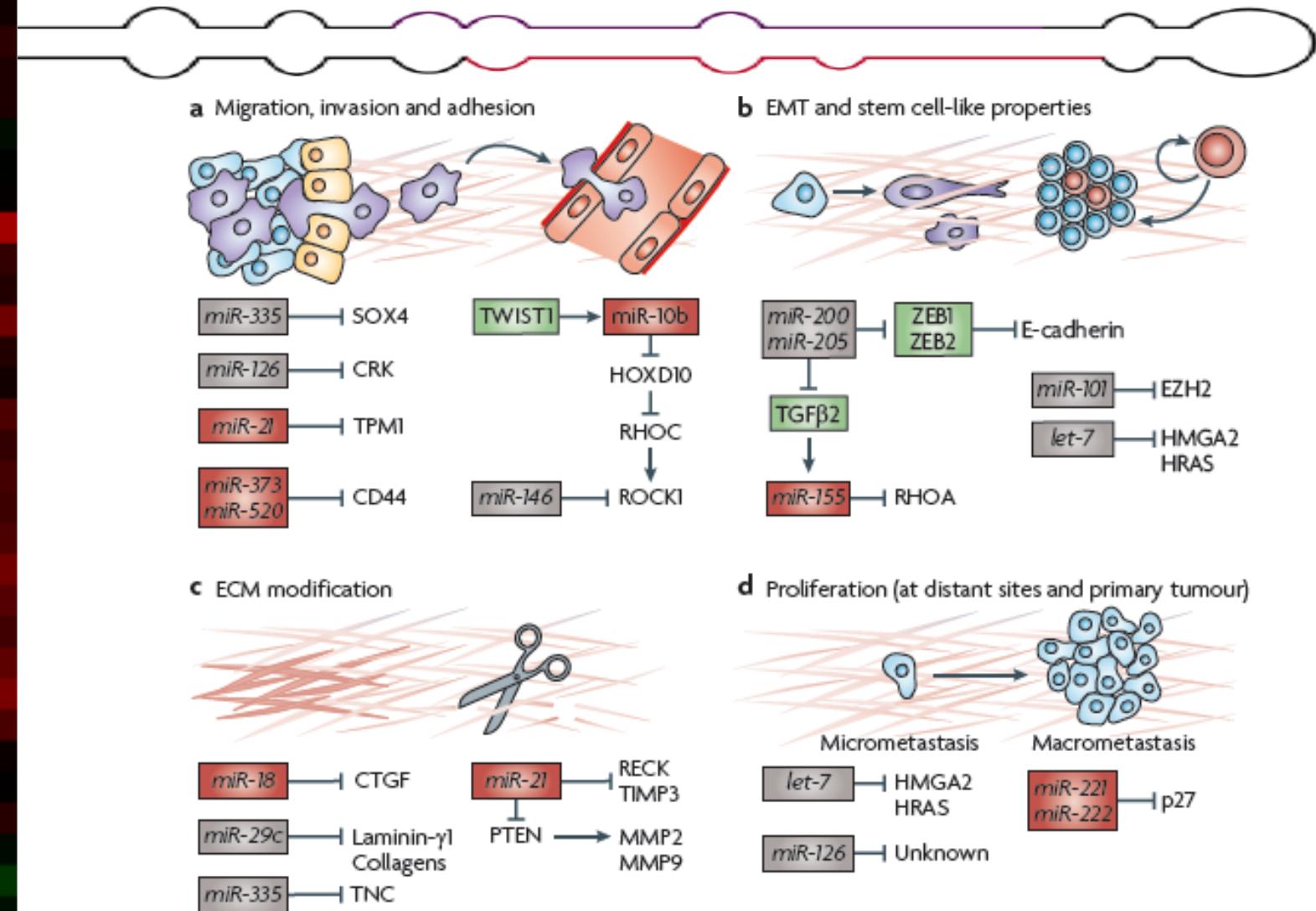
OVERVIEW OF CELL SIGNALING



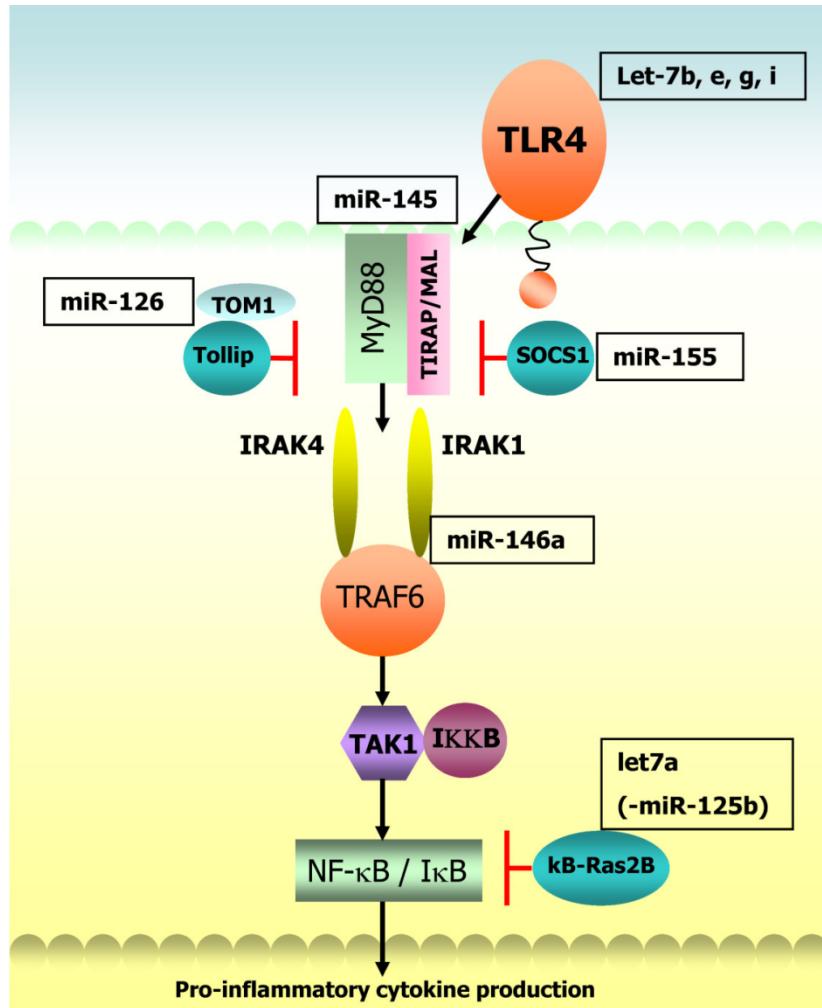
MicroRNAs' involvement in Vogelstein's model of colorectal cancer pathogenesis



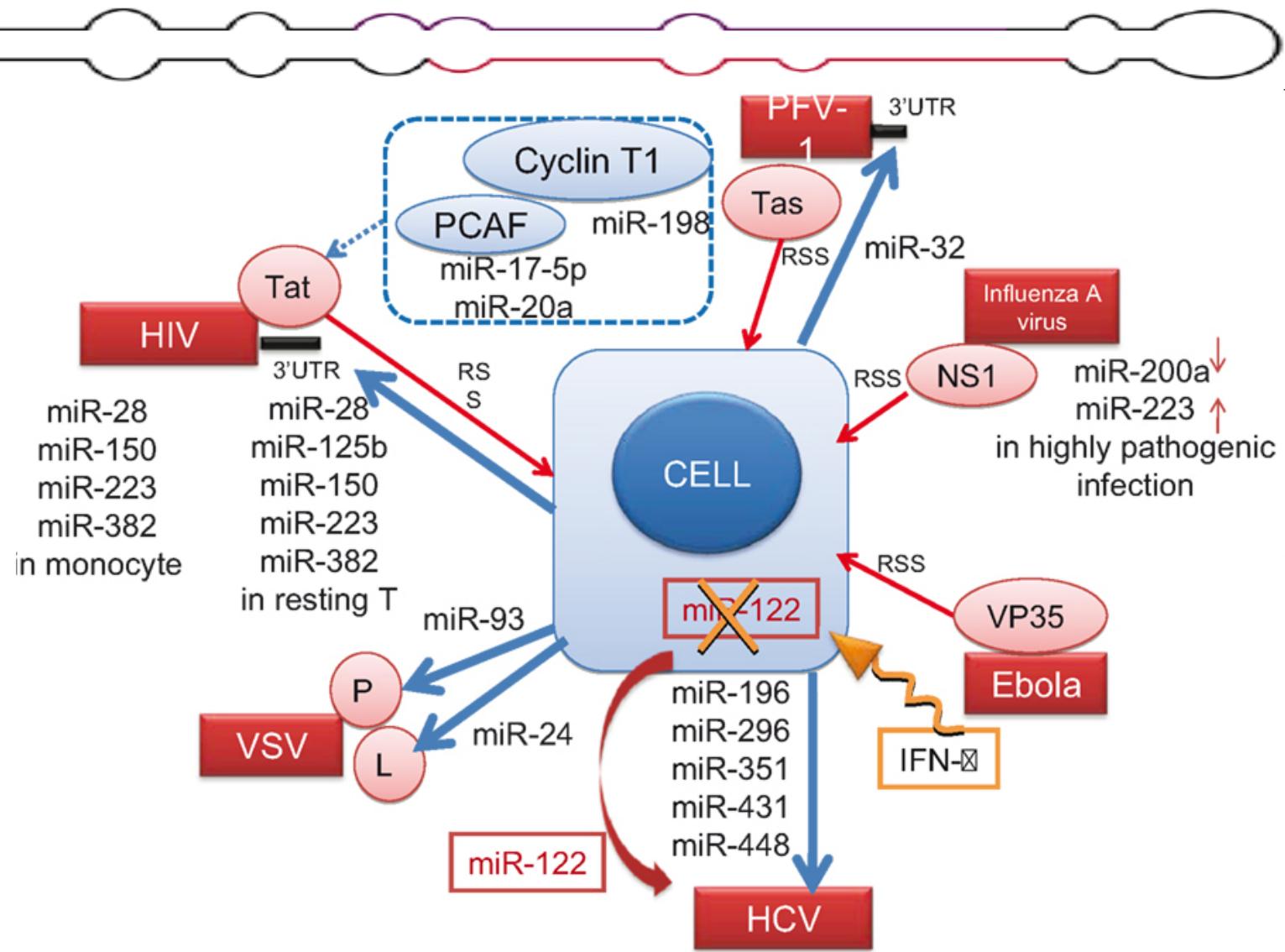
Involvement of miRNAs in tumor metastasis



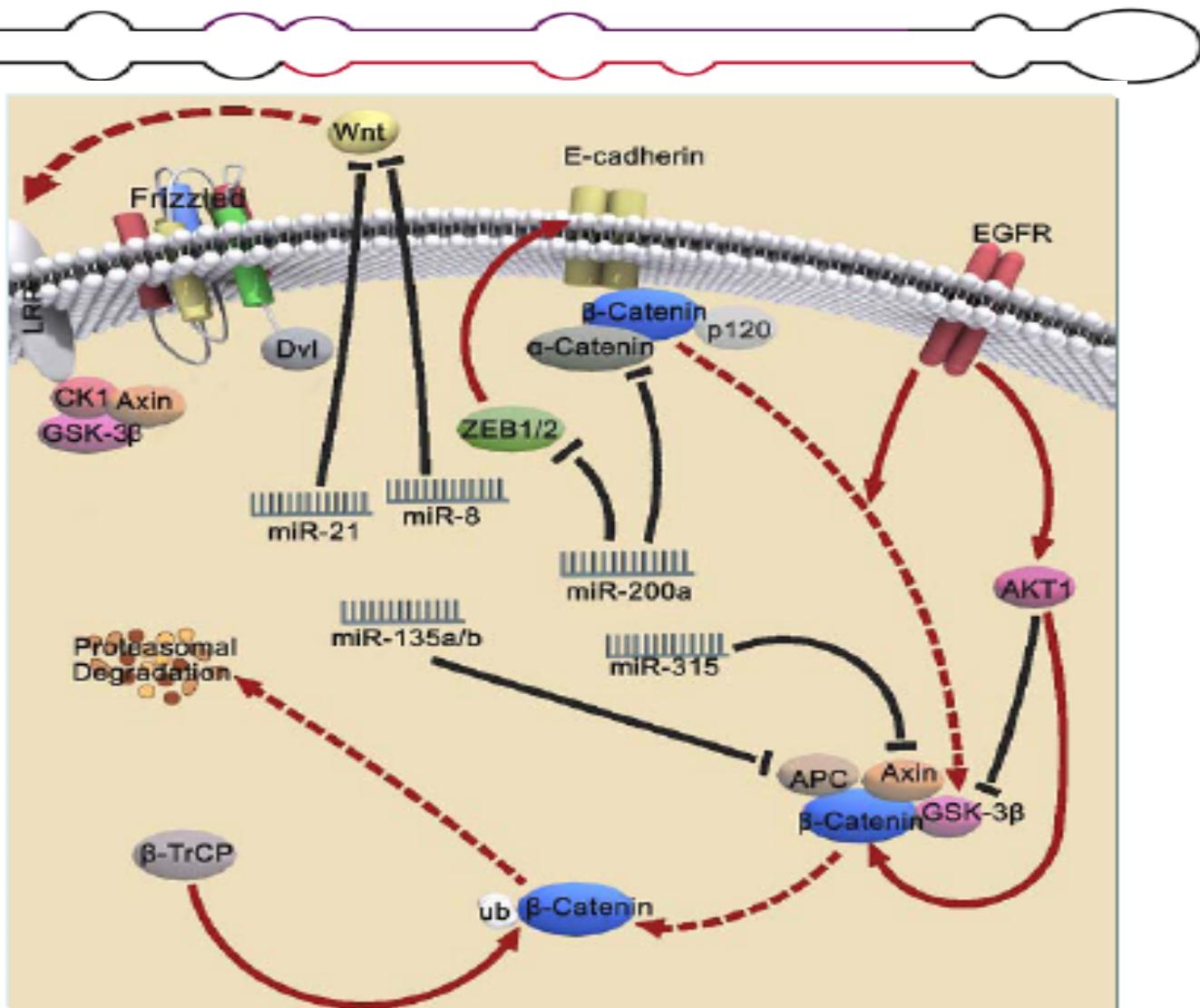
miRNA and inflammation



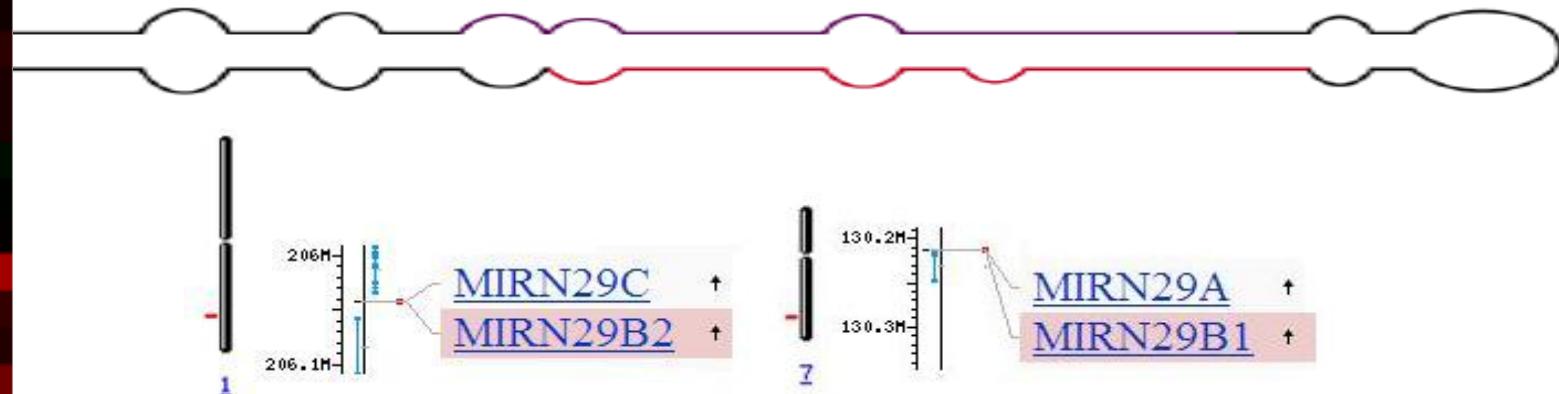
miRNA and viral infection



Involvement of miRNAs in Wnt Signaling



miR-29abc



miR-29a : 5'- uagcaccaucugaaaucgguaua -3'
miR-29b : 5'- uagcaccauuugaaaucaguguu -3'
miR-29c : 5'- uagcaccauuugaaaucggua -3'

- miR-29c/b2 transcript on chromosome 1
- miR-29a/b1 transcript resides inside the common fragile site FRA7H (chromosome 7). In RC-K8 cell which show this fragile site involvement, miR-29b1 is downregulated not miR-29a.
(Schneider *et al.*, *Leukemia*, 2008)
- miR-29b is localized to the nucleus; it has a short half life compared to miR-29a and miR-29c and it is stabilized during mitosis.
-Hwang *et al.*, *Science*, 2007)

Predicted targets of miR-29 in Wnt Signaling (TargetScan 5.1)



Gene symbol	Gene name	Function
HBP1	HMG-box transcription factor 1	Repressor
BCL9L	B-cell CLL/lymphoma 9-like (B9L)	Activator
CTNNBIP1	catenin, beta interacting protein 1 (ICAT)	Repressor
FZD5	frizzled homolog 5	Activator
LRP6	low density lipoprotein receptor-related protein 6	Activator
FZD4	frizzled homolog 4	Activator
PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (p85alpha)	Activator

Summary and Conclusions

- miR-29b suppresses Wnt-3a or lithium chloride-induced activation of Wnt signalling in HEK293 cells.
- miR -29b significantly reduces TCF7L2(TCF4) levels in HEK293 cells.
- mRNA levels of the Wnt signalling target genes CyclinD1 and c-Myc are down-regulated by miR-29b in HCT116WT cells.
- miR-29b negatively regulates Wnt signalling in HCT116wt, HCT116 p53-/- and SW480 cells
- miR-29b had no significant effect on HBP1 3' UTR and BCL9L 3' UTR luciferase.

miR-22



miR-22:

5'- AAGCUGCCAGUUGAAGAACUGU -3'

- The microRNA hsa-miR-22 is processed from the second exon of a non-coding transcriptional unit in chromosome 17 (Rodriguez *et al.*, *Genome Research*, 2004)
- It has been termed a “putative tumor suppressor” (Gaur *et al.*, *Cancer Res.*, 2007) and is known to target Estrogen Receptor α (ESR1) and inhibits ER α-dependent proliferation in breast cancer cells (Pandey and Picard, *Mol. Cell Biol.*, 2009)
- It suppresses the oncogene EVI1 in ovarian cancer cells (Nagaraja *et al.*, *Mol. Endocrinol.*, 2009)
- Curcumin is known to upregulate miR-22 expression (Sun *et al.*, *Mol. Cancer Ther.*, 2008) and c-Myc, a transcriptional target of Wnt signaling downregulates miR-22 expression (Chang *et al.*, *Nat. Genet.*, 2008)

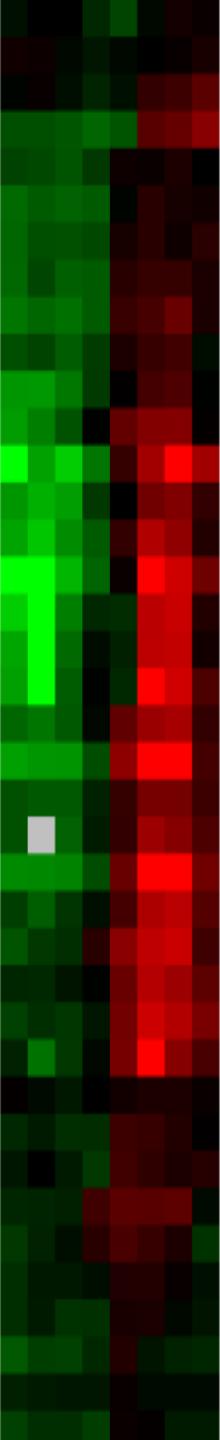
Predicted targets of miR-22 in Wnt Signaling (TargetScan 5.1)



Gene symbol	Gene name	Function
EP300 (p300)	E1A binding protein p300 /histone acetyltransferase p300	Activator
BCL9L	B-cell CLL/lymphoma 9-like (B9L)	Activator
AKT3	protein kinase B gamma	Activator
TP53 (p53)	tumor protein p53	Repressor
TP53INP1	tumor protein p53 inducible nuclear protein 1	Repressor

Summary and Conclusions

- miR-22 suppresses lithium chloride-induced activation of Wnt signalling in HEK293 cells.
- mRNA levels of the Wnt signalling target genes CyclinD1, c-Myc and TCF7L2 are down-regulated by miR-22 in HEK293 cells.
- miR-22 down-regulates Cyclin D1 promoter luciferase activity
- BCL9L is a direct target of miR-22. EP300 is not targeted by miR-22 even though the putative miR-22 binding sites are highly conserved
- miR-22 represses its own repressor cMyc



miRNAs as Biomarkers

- 
- De-regulated under different physiological conditions
 - Stable even in formalin-fixed and paraffin-embedded tissue samples and can be efficiently extracted and evaluated
 - Secreted from the cell through micro-vesicles
 - Detectable in body fluids and potential for non-invasive detection

Wnt signaling – PI3K signaling – P53

