V10: microRNAs and cancer

What are microRNAs?

…

How can one identify microRNAs?

What is the function of microRNAs?

How are microRNAs related to cancerogenesis?

Can one use microRNAs as biomarkers for cancerogenesis?

RNA world

short name	full name	function	oligomerization
mRNA, rRNA, tRNA,	you know them well ...		Single-stranded
snRNA	small nuclear RNA	splicing and other functions	
snoRNA	small nucleolar RNA	nucleotide modification of RNAs	
Long ncRNA	Long noncoding RNA	various	
miRNA	microRNA	gene regulation	single-stranded
siRNA	small interfering RNA	gene regulation	double-stranded

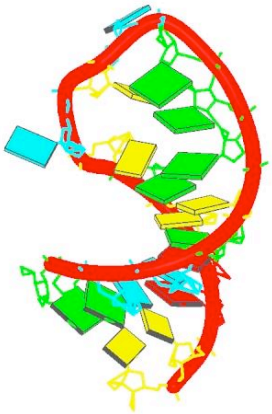
RNA structure

Also single stranded RNA molecules frequently adopt a specific **tertiary structure**.

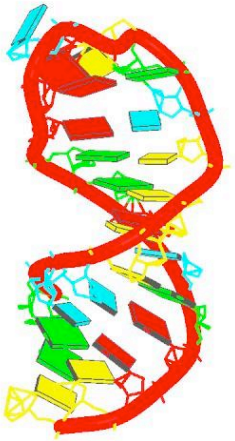
The scaffold for this structure is provided by secondary structural elements which are **H-bonds** within the molecule.

This leads to several recognizable "domains" of secondary structure like **hairpin loops**, **bulges** and **internal loops**.

RNA hairpin 2RLU



Stem loop 1NZ1



www.rcsb.org

snRNAs

Small nuclear RNA (**snRNA**) are found within the nucleus of eukaryotic cells.

They are transcribed by RNA polymerase II or RNA polymerase III and are involved in a variety of important processes such as
- RNA splicing,
- regulation of transcription factors or RNA polymerase II, and
- maintaining the telomeres.

They are always associated with specific proteins. The complexes are referred to as small nuclear ribonucleoproteins (**snRNP**) or sometimes as **snurps**.

www.wikipedia.org

snoRNAs

A large group of snRNAs are known as small nucleolar RNAs (**snoRNAs**).

These are small RNA molecules that play an essential role in **RNA biogenesis** and guide chemical modifications of rRNAs, tRNAs and snRNAs.

They are located in the nucleolus and the cajal bodies of eukaryotic cells.

www.wikipedia.org

siRNAs

Small interfering RNA (**siRNA**), sometimes known as **short interfering RNA** or silencing RNA, is a class of

- **double-stranded RNA molecules**,
- that are 20-25 nucleotides in length (often precisely 21 nt) and play a variety of roles in biology.

Most notably, siRNA is involved in the RNA interference (RNAi) pathway, where it interferes with the expression of a specific gene.

In addition to their role in the RNAi pathway, siRNAs also act in RNAi-related pathways, e.g., as an antiviral mechanism or in shaping the chromatin structure of a genome.

www.wikipedia.org

miRNAs

RNA interference may involve siRNAs or miRNAs.

Nobel prize in Physiology or Medicine **2006**
for their discovery of RNAi in *C. elegans*.


Andrew Fire

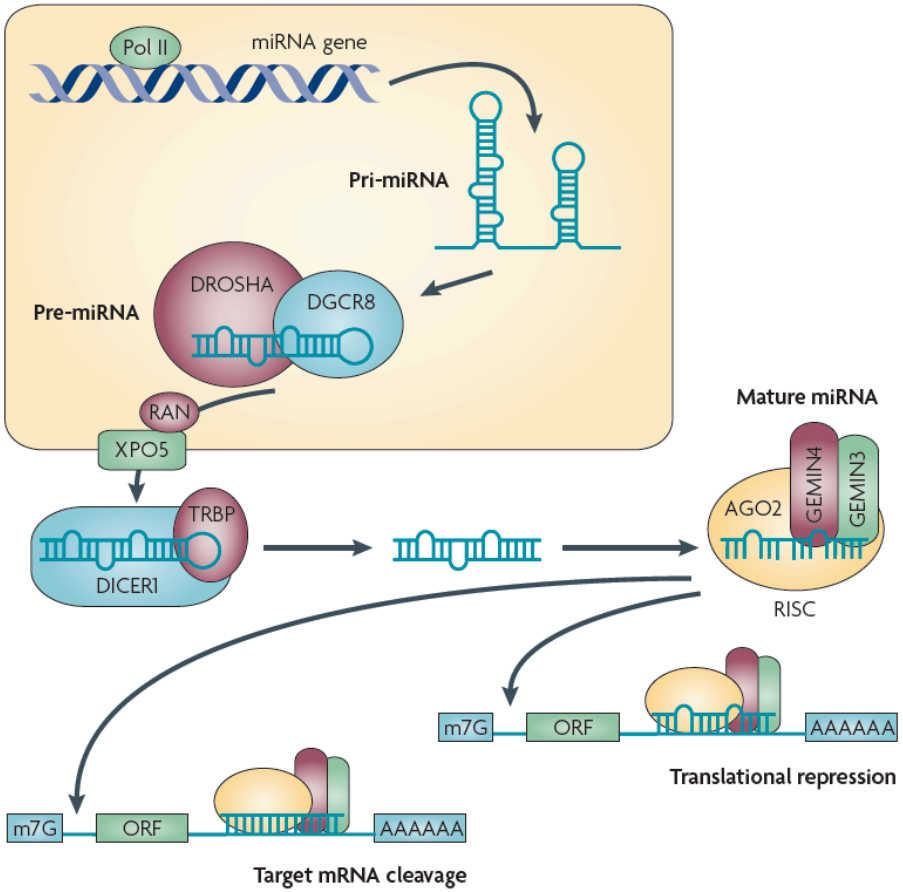

Craig Mello

microRNAs (miRNA) are **single-stranded RNA** molecules of 21-23 nucleotides in length, which regulate gene expression.

Remember: miRNAs are encoded by DNA but not translated into protein (non-coding RNA).

www.wikipedia.org

Overview of the miRNA network



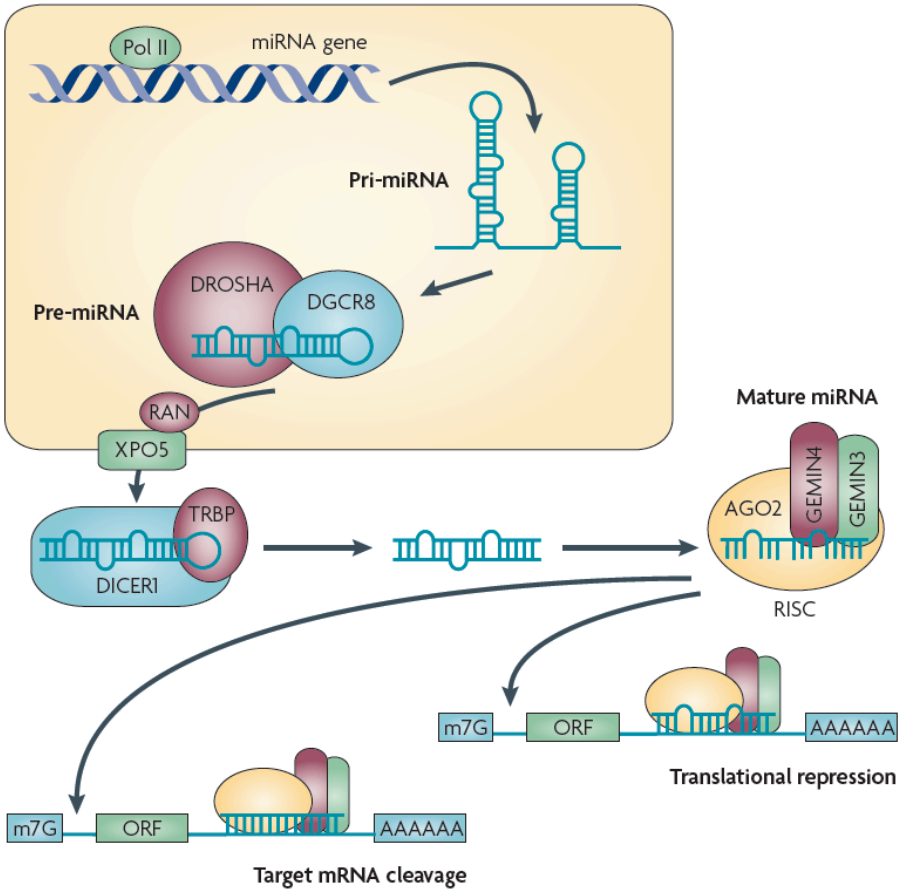
RNA polymerase II (Pol II) produces a 500–3,000 nucleotide transcript, called the primary microRNA **(pri-miRNA)**.

This is then cropped to form a **pre-miRNA** hairpin by a multi-protein complex that includes **DROSHA** (~60–100 nucleotides).

AA, poly A tail;
m7G, 7-methylguanosine cap;
ORF, open reading frame.

Ryan et al. Nature Rev. Cancer (2010) 10, 389

Overview of the miRNA network



This double-stranded hairpin structure is **exported** from the nucleus by RAN GTPase and exportin 5 (XPO5).

Finally, the pre-miRNA is cleaved by **DICER1** to produce two miRNA strands, a mature miRNA sequence, approximately 20 nt in length, and its short-lived complementary sequence, which is denoted miR.

AA, poly A tail;
m7G, 7-methylguanosine cap;
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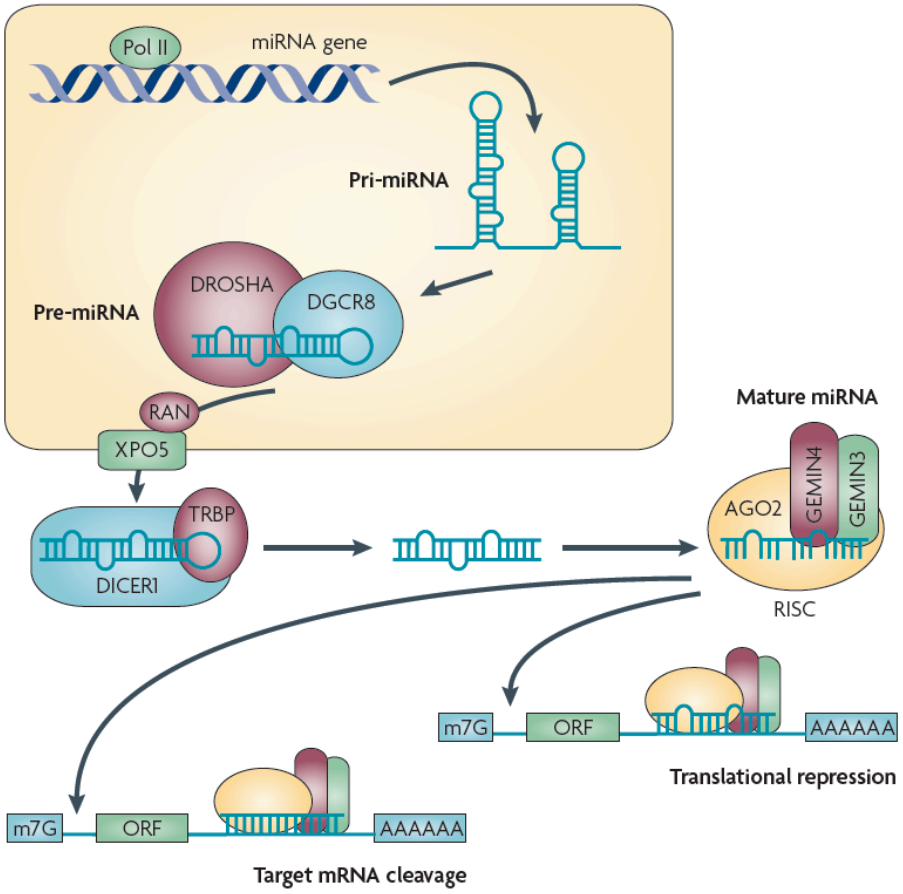
Overview of the miRNA network



The thermodynamic stability of the miRNA duplex termini and the identity of the nucleotides in the 3′ overhang determines which of the strands is incorporated into the RNA-inducing silencing complex (**RISC**).

The single stranded miRNA is incorporated into RISC.

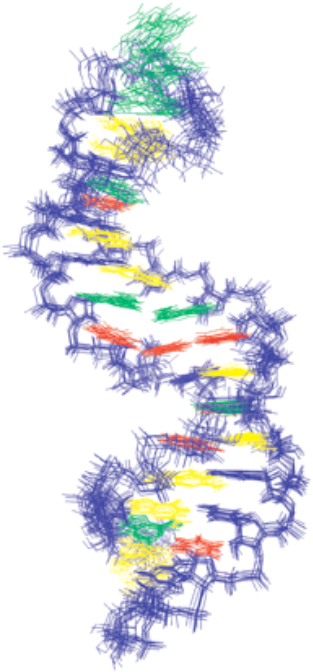
This complex then targets it e.g. to the target 3′ untranslated region of a mRNA sequence to facilitate repression and cleavage.

AA, poly A tail;
m7G, 7-methylguanosine cap;
ORF, open reading frame.

Ryan et al. Nature Rev. Cancer (2010) 10, 389

miRNAs

Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules.



solution NMR-structure of *let-7* miRNA:*lin-41* mRNA complex from *C. elegans*
Cevec et al. *Nucl. Acids Res. (2008) 36: 2330.*

The main function of miRNAs is to down-regulate gene expression of their target mRNAs.

miRNAs typically have incomplete base pairing to a target and inhibit the translation of many different mRNAs with similar sequences.

In contrast, siRNAs typically base-pair perfectly and induce mRNA cleavage only in a single, specific target.

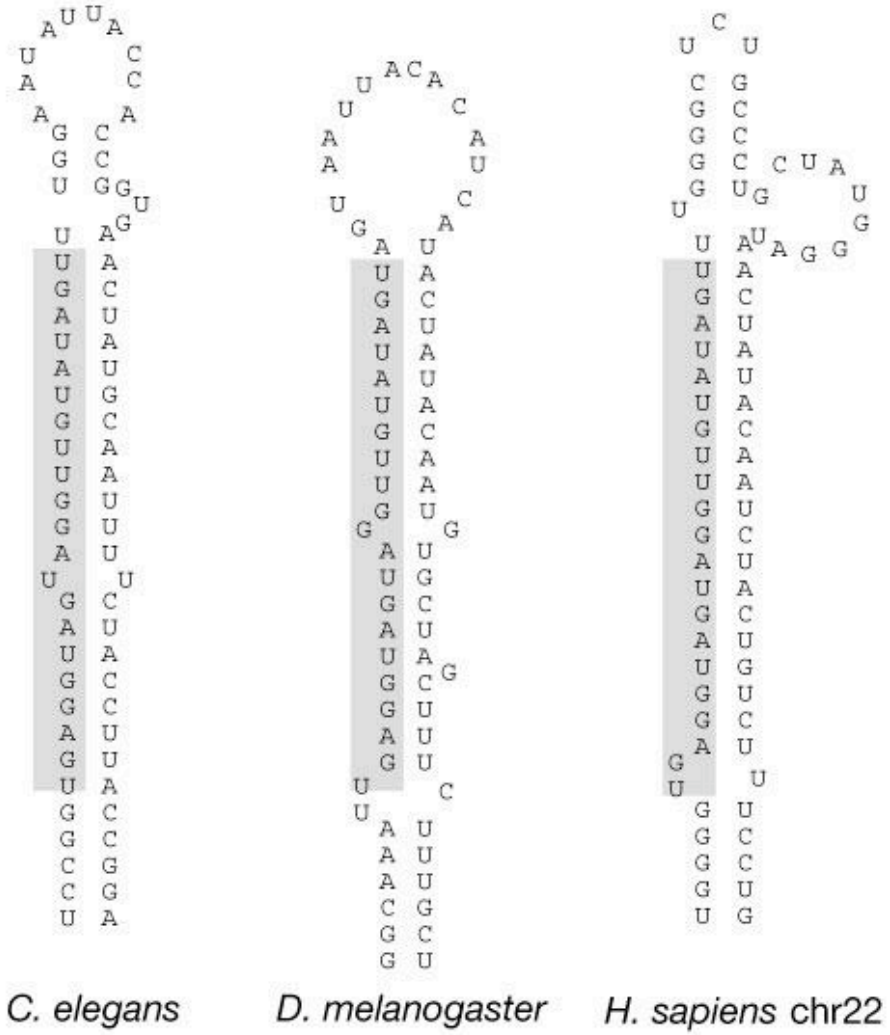
www.wikipedia.org

discovery of let7

The first two known microRNAs, lin-4 and let-7, were originally discovered in the nematode *C. elegans.*

They control the timing of stem-cell division and differentiation.
let-7 was subsequently found as the first known human miRNA.

let-7 and its family members are **highly conserved** across species in sequence and function.
Misregulation of let-7 leads to a less differentiated cellular state and the development of cell-based diseases such as cancer.



Pasquinelli et al. Nature (2000) 408, 86
www.wikipedia.org

Action of let7

Let-7 is a direct regulator of *RAS* expression in human cells.

All the three *RAS* genes in human, *K-, N-*, and *H-*, have the predicted *let-7* binding sequences in their 3'UTRs.

In lung cancer patient samples, expression of *RAS* and *let-7* showed a reciprocal pattern, which has low *let-7* and high *RAS* in cancerous cells, and high *let-7* and low *RAS* in normal cells.

Another oncogene, *high mobility group A2* (*HMGA2*), has also been identified as a target of *let-7*.

Let-7 directly inhibits *HMGA2* by binding to its 3'UTR. Removal of *let-7* binding site by 3'UTR deletion cause overexpression of *HMGA2* and formation of tumor.

MYC is also considered as a oncogenic target of *let-7*.

www.wikipedia.org

miRNA discovery

miRNA discovery approaches, both biological and bioinformatics, have now yielded many thousands of miRNAs.

This process continues with new miRNA appearing daily in various databases and compiled officially as the **miRBase** (http://www.mirbase.org/).

miRBase is the primary online repository for published miRNA sequence and annotation (stored in miRBase database).

Each entry in the database represents a predicted hairpin portion of a miRNA transcript with information on the location and sequence of the mature miRNA sequence

Liu et al. Brief Bioinf. (2012) doi: 10.1093/bib/bbs075

Bioinformatics prediction of miRNAs

With bioinformatic methods, putative miRNAs are first predicted in genome sequences based on the **structural features** of miRNA.

These algorithms essentially **identify hairpin structures** in non-coding and non-repetitive regions of the genome that are characteristic of miRNA precursor sequences.

The candidate miRNAs are then **filtered** by their evolutionary **conservation** in different species.

Known miRNA precursors play important roles in searching algorithms because structures of known miRNA are used to train the learning processes to discriminate between true predictions and false positives.

Many algorithms, for example, miRScan, miRSeeker, miRank, miRDeep, miRDeep2 and miRanalyzer, have been proposed.

Liu et al. Brief Bioinf. (2012) doi: 10.1093/bib/bbs075

Bioinformatics of miRNA prediction

miRNAs target mRNAs through complementary base pairing, in either complete or incomplete fashion.

It has been generally believed that miRNAs bind to the 3'-UTRs of the target transcripts in at least one of two classes of binding patterns.

One class of target sites has perfect Watson–Crick complementarity to the 5'-end of the miRNAs, referred as 'seed region', which positions at 2–7 of miRNAs.

When bound in this way, miRNAs suppress their targets without requiring significant further base pairings at the 3'-end of the miRNAs.

Liu et al. Brief Bioinf. (2012) doi: 10.1093/bib/bbs075

Bioinformatics of miRNA prediction

On the contrary, the second class of target sites has imperfect complementary base pairing at the 5'-end of the miRNAs, but it is compensated via additional base pairings in the 3'-end of the miRNAs.

The multiple-to-multiple relations between miRNAs and mRNAs lead to complex miRNA regulatory mechanisms.

Liu et al. Brief Bioinf. (2012) doi: 10.1093/bib/bbs075

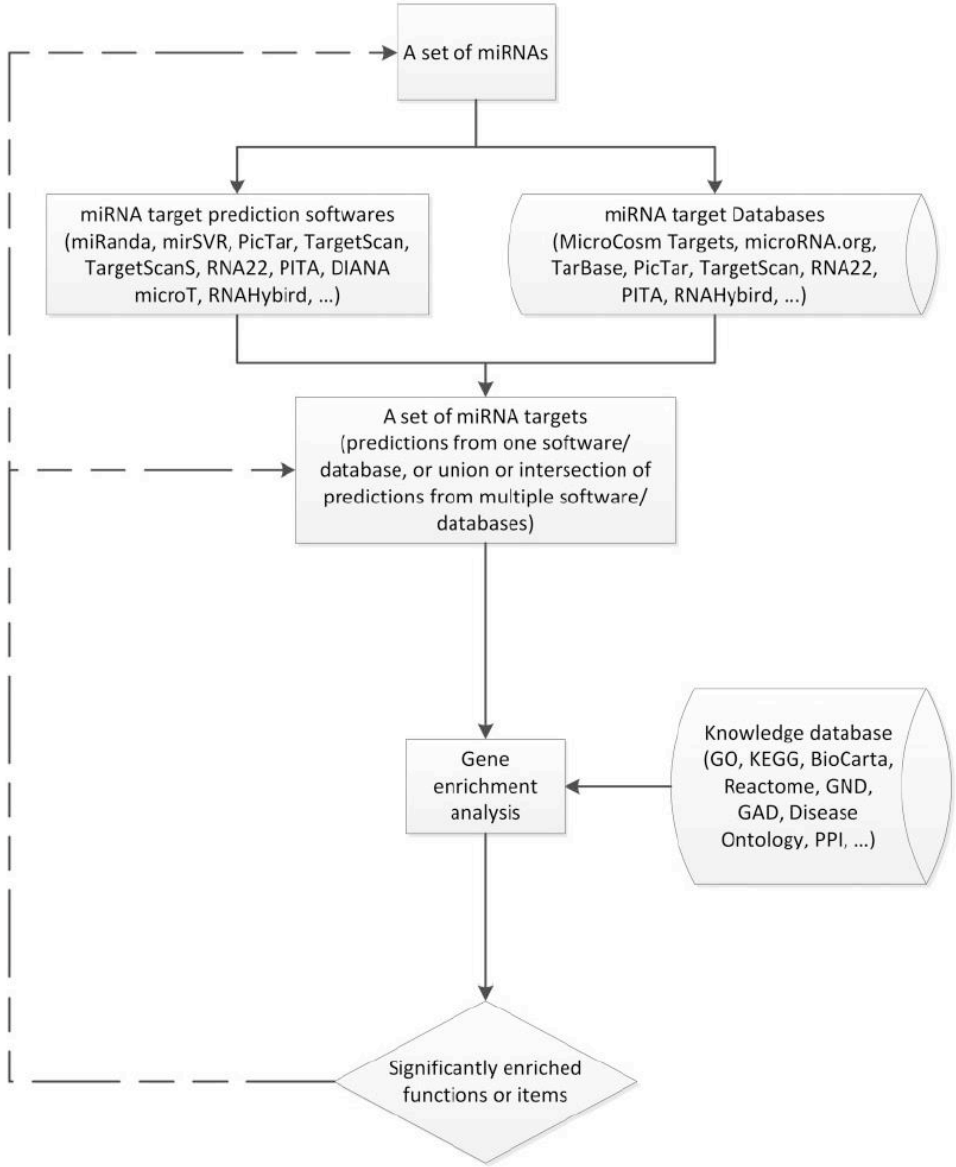
miRNA-target prediction algorithms

Table I: miRNA-target prediction algorithm

Algorithm	Regions scanned	Species conservation	Species	Brief description of the prediction method
miRanda	3′-UTR	Yes	Human, mouse, rat, fly and worm	Predict targets based on rules: (i) sequence complementarity, (ii) binding energy and (iii) evolutionary conservation.
mirSVR	No restriction	Yes	Human, mouse, rat, fly and worm	To score and rank miRanda-predicted miRNA-target sites with a supervised vector regression (SVR) model for features including secondary structure accessibility of the site and conservation.
PicTar	3′-UTR	Yes	Vertebrates, fly and worm	Filter alignments according to the thermodynamic stability, then score and rank the predicted target by hidden Markov model maximum-likelihood fit approach.
TargetScan	8mer and 7mer sites, and open reading frames	Yes	Human, mouse, rate, dog and chicken	Predict targets by searching for the presence of conserved 8mer and 7mer sites that match the seed region. Predictions are ranked by a combinatorial score based on site number, site type and site context.
TargetScanS	3′-UTR	Yes	Human, mouse, rate, dog and chicken	Predict targets that have a conserved 6 nt seed match flanked by either a m8 match or a tlA anchor.
RNA22	No restriction	No restriction	Any	Use the patterns discovered from the known mature miRNAs for predicting candidate miRNA-target sites in a sequence.
PITA	3′-UTR	Yes	Human, mouse, worm and fly	Predict miRNA targets using a non-parameter model that computes the difference between the free energy gained from the formation of the miRNA-target duplex and the energetic cost of unpairing the target to make it accessible to the miRNA.
RNAhybird	3′-UTR and coding sequence	No restriction	Any	A tool to identify mRNA secondary structure and energetically favourable hybridization between miRNA and target mRNA.
DIANA-microT	3′-UTR and CDS	No restriction	Human and mouse	The fifth version of microT algorithm which is specifically trained on a positive and negative set of miRNA recognition elements located in both the 3′-UTR and CDS region. The conserved and non-conserved miRNA recognition elements are combined into a final prediction score.

Liu et al. Brief Bioinf. (2012) doi: 10.1093/bib/bbs075

Predicting miRNA function based on target genes

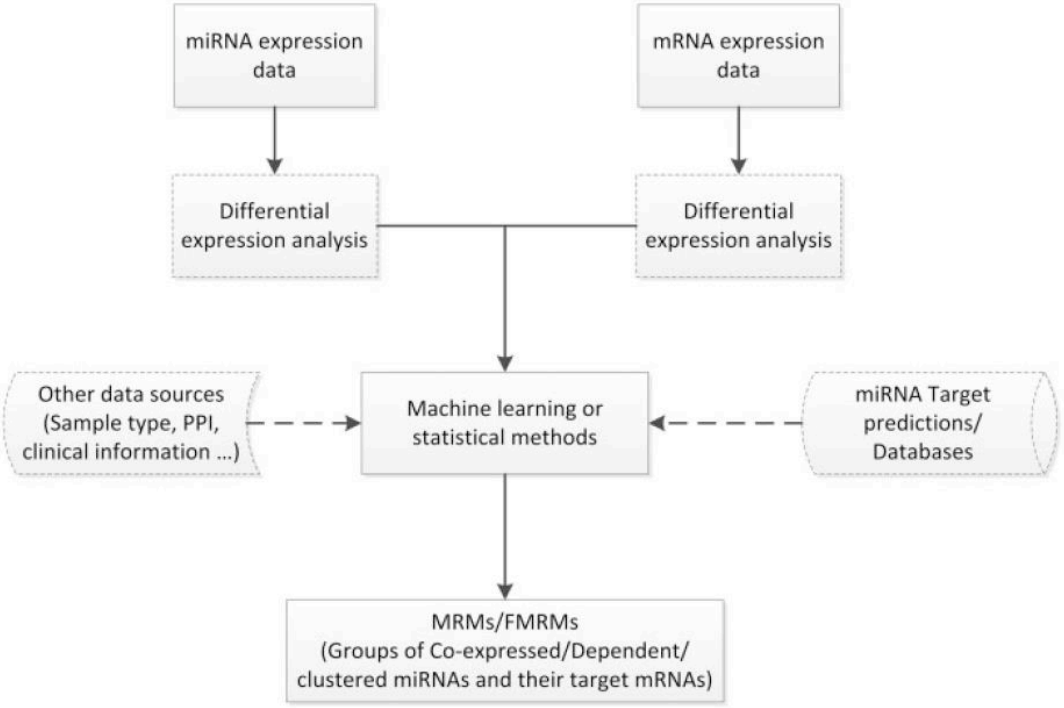


The most straight-forward approach for miRNA functional annotation is through **functional enrichment analysis** using the miRNA-target genes.

This approach assumes that miRNAs have similar functions as their target genes.

Liu et al. Brief Bioinf. (2012) doi: 10.1093/bib/bbs075

Predicting miRNA function based on correlated expression



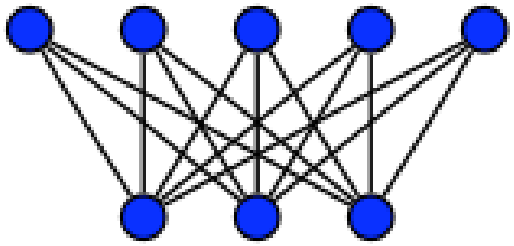
miRNA functional annotation heavily relies on the miRNA-target prediction.

In the last few years, many studies have been conducted to infer the miRNA regulatory mechanisms by incorporating target prediction with other genomics data, such as the expression profiles of miRNAs and mRNAs.

Liu et al. Brief Bioinf. (2012) doi: 10.1093/bib/bbs075

Discovering MRMs

A MRM (group of co-expressed miRNAs and mRNAs) may be defined as a special bipartite graph, named **biclique**, where Two sets of nodes are connected by edges.



Every node of the first set representing miRNA is connected to every node of the second set representing mRNAs.

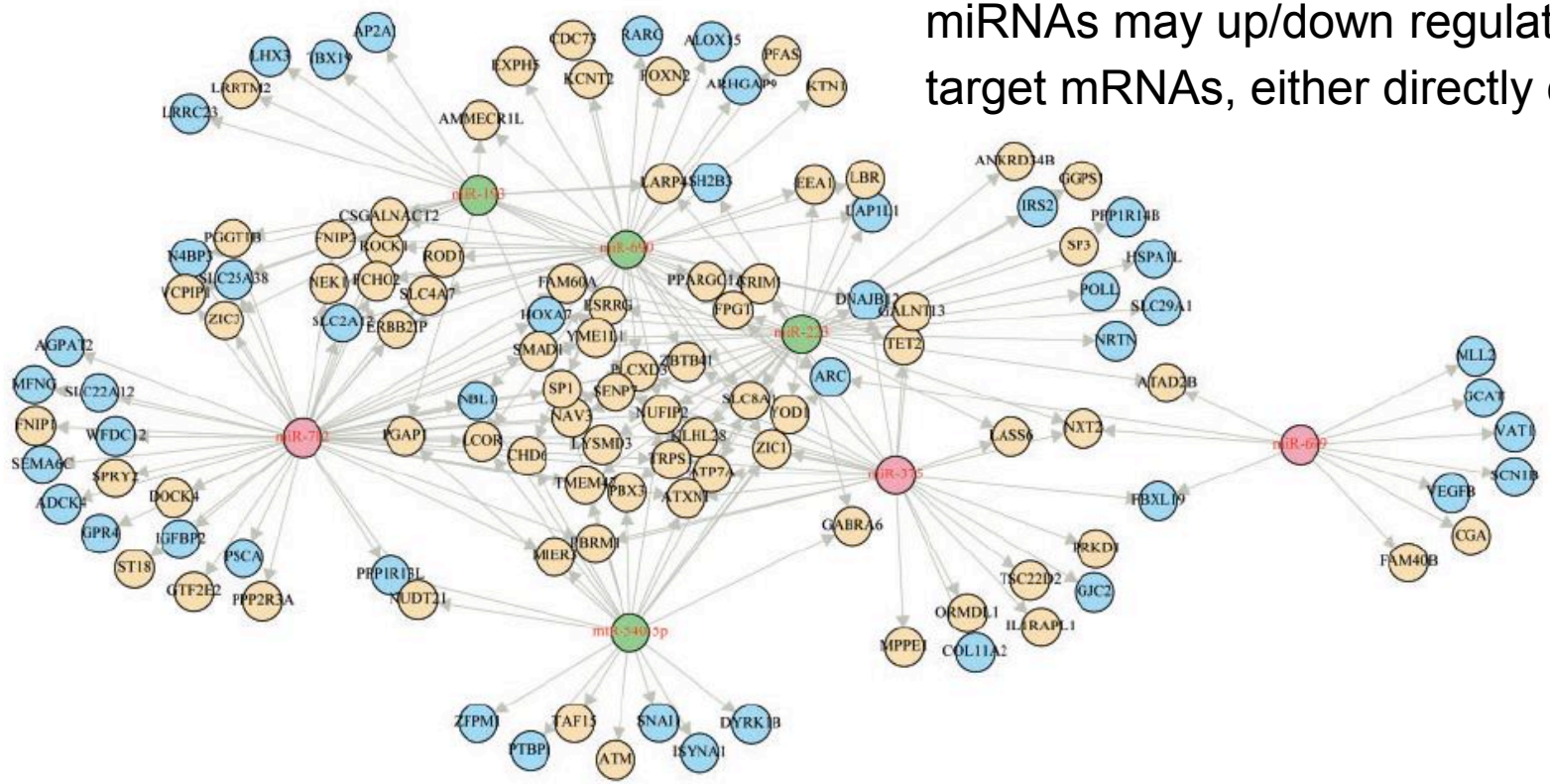
The weights of edges correspond to the miRNA–mRNA binding strength inferred from target prediction algorithms

Most of the integrative methods of MRM discovery are based on the assumption that miRNA negatively regulate their target mRNAs to the effect that an inverse relationship should exist between the expression of a specific miRNA and its targets.

Liu et al. Brief Bioinf. (2012) doi: 10.1093/bib/bbs075

miRNA-mRNA network

A FMRM identified from analysis of **schizophrenia** patients. It shows that miRNAs may up/down regulate their target mRNAs, either directly or indirectly.



Up-regulated miRNAs are coloured in red and down-regulated miRNAs are coloured in green. Up-regulated mRNAs are coloured in yellow, while down-regulated mRNAs are coloured in blue.

Liu et al. Brief Bioinf. (2012) doi: 10.1093/bib/bbs075

SNPs in miRNA may lead to diseases

miRNAs can have dual oncogenic and tumour suppressive roles in cancer depending on the cell type and pattern of gene expression.

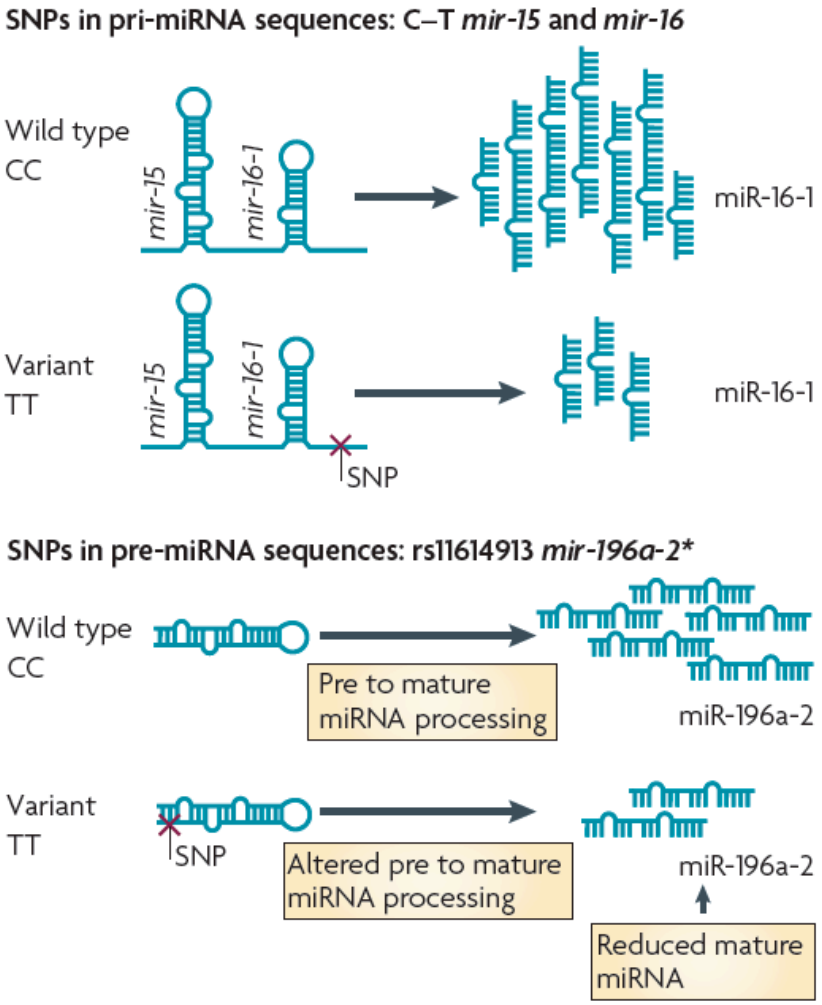
Approximately 50% of all annotated human miRNA genes are located in fragile sites or areas of the genome that are associated with cancer.

E.g. Abelson *et al.* found that a mutation in the miR-189 binding site of *SLITRK1* was associated with Tourette's syndrome.

SNPs in miRNA genes are thought to affect function in one of three ways:
(1) through the transcription of the primary transcript;
(2) through pri-miRNA and pre-miRNA processing; and
(3) through effects on miRNA–mRNA interactions

Volinia et al. PNAS (2013) 110, 7413

SNPs in pri-miRNA and pre-miRNA sequences

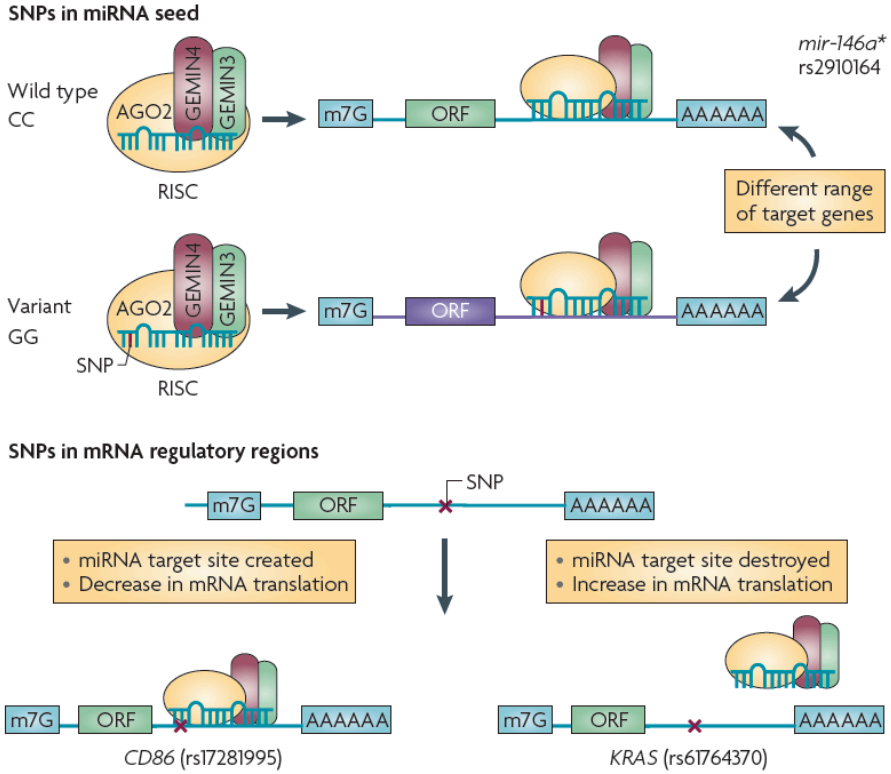


SNPs can occur in the pri-miRNA and pre-miRNA strands and are likely to affect miRNA processing and subsequent mature miRNA levels.

Such SNPs can lead to either an increase or decrease in processing.

Ryan et al. Nature Rev. Cancer (2010) 10, 389

SNPs in miRNA seed and regulatory regions



SNPs in mature microRNAs (miRNAs) within the seed sequence can strengthen or reduce binding between the miRNA and its mRNA target.

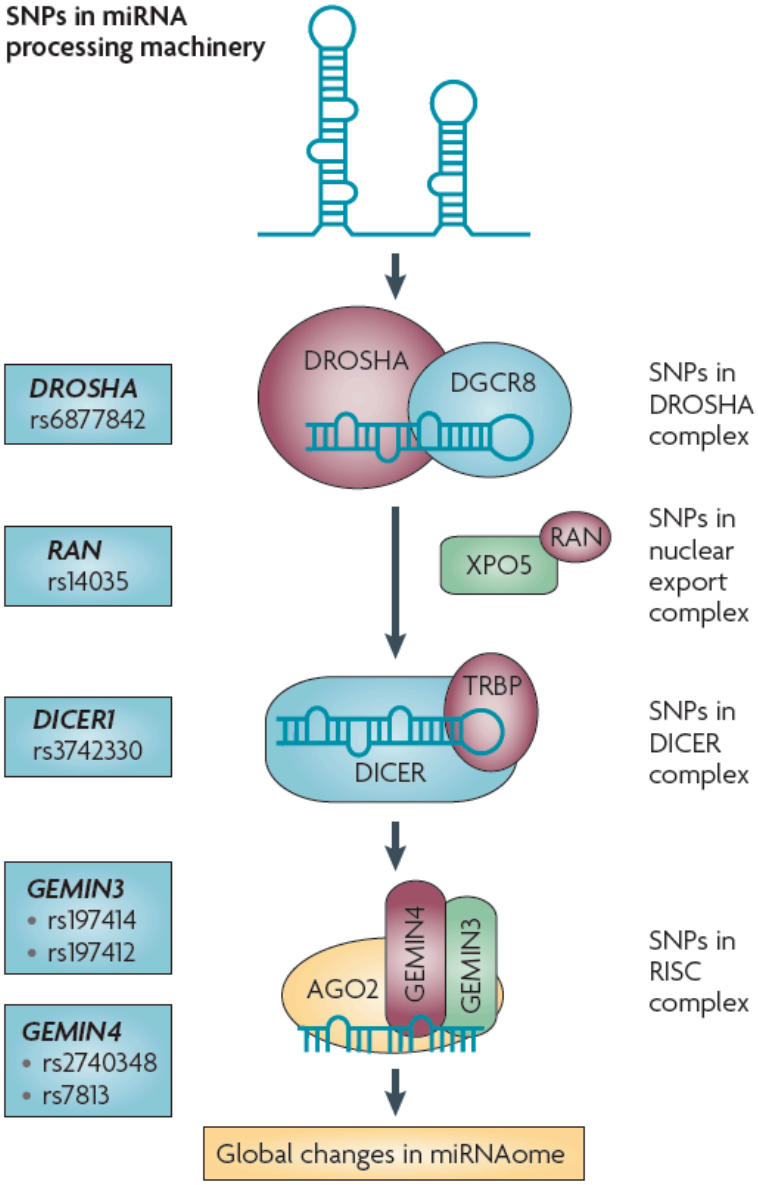
Moreover, such SNPs can create or destroy target binding sites, as is the case for mir-146a*.

SNPs located within the 3′ untranslated region miRNA binding sites function analogously to seed region SNPs and modulate the miRNA–mRNA interaction.

They can create or destroy miRNA binding sites and affect subsequent mRNA translation.

Ryan et al. Nature Rev. Cancer (2010) 10, 389

SnPs in miRNA processing machinery



SNPs can also occur within the processing machinery.

These SNPs are likely to affect the microRNAome (miRNAome) as a whole, possibly leading to the overall suppression of miRNA output.

In addition, SNPs in cofactors of miRNA processing, such as p53, may indirectly affect miRNA maturation.

Ryan et al. Nature Rev. Cancer (2010) 10, 389

microRNAs as biomarkers for cancer

miRNAs can be used for sensitive classification of cancer risks or cancer progression (e.g. 95%), see research in HP Lenhof's group.

Various companies market such tools.

www.exiqon.com

Summary

The discovery of microRNAs has led to an additional layer of complexity in understanding cellular networks.

Prediction of miRNA-mRNA networks is challenging due to the often non-perfect base matching of miRNAs to their targets.

Individual SNPs may alter network properties, and may be associated with cancerogenesis.

microRNAs can be exploited as sensitive biomarkers.

Volinia et al. PNAS (2013) 110, 7413