

**RNA interference:
una nuova prospettiva terapeutica**

DNA non codificante = DNA spazzatura

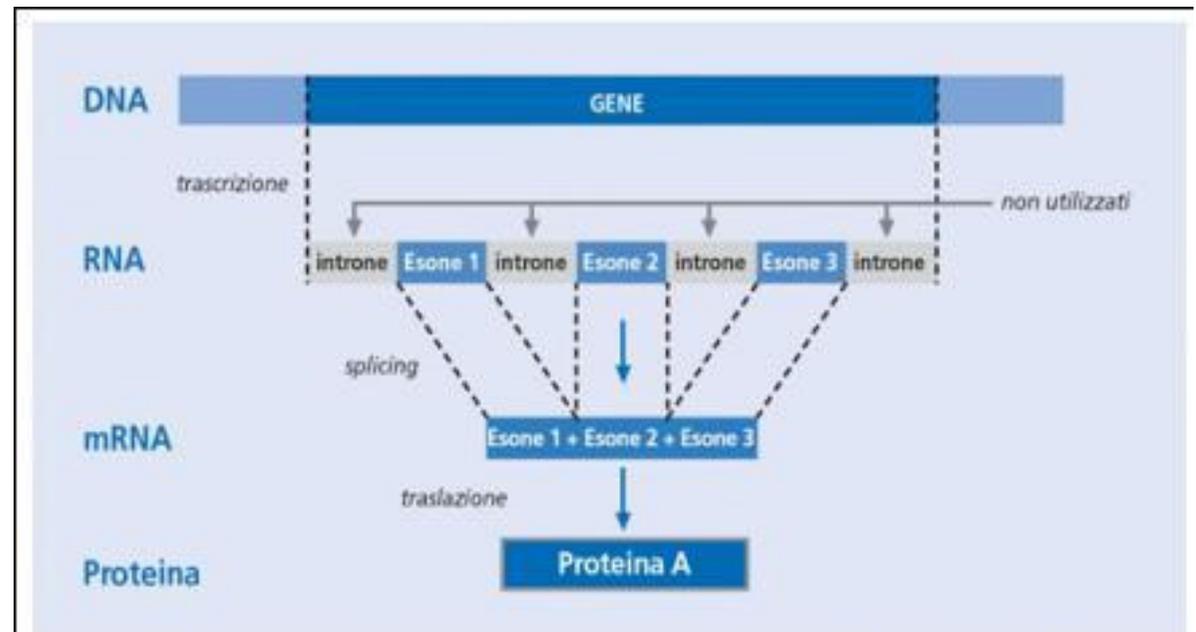
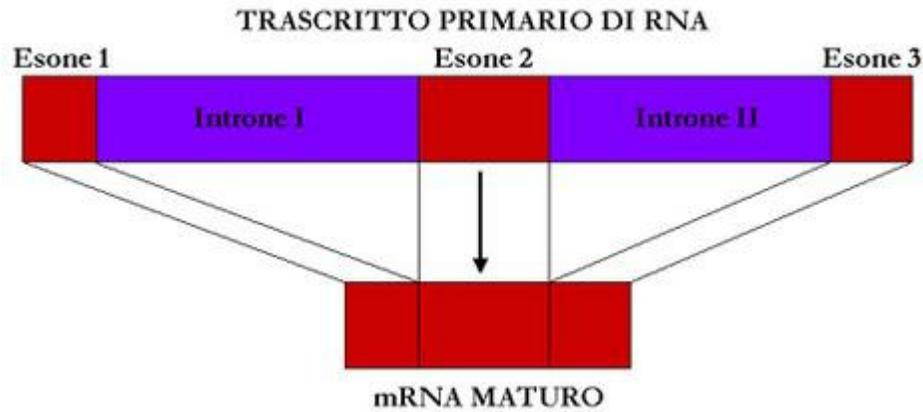
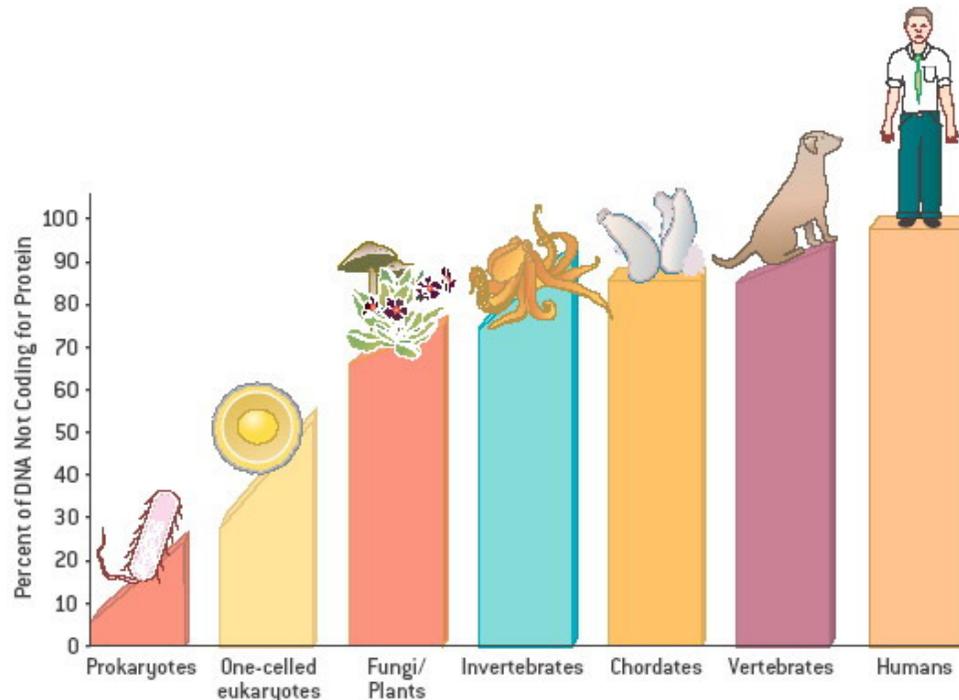
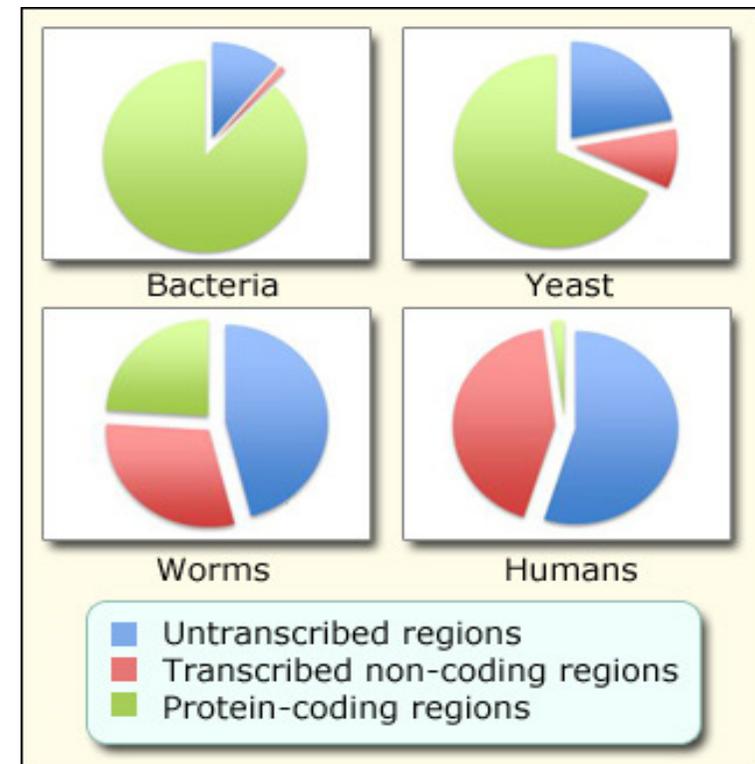


Figura 1. Composizione dell'mRNA a partire dagli esoni (modificata da Pasternak, 2004).

DNA non codificante aumenta con la complessità degli organismi



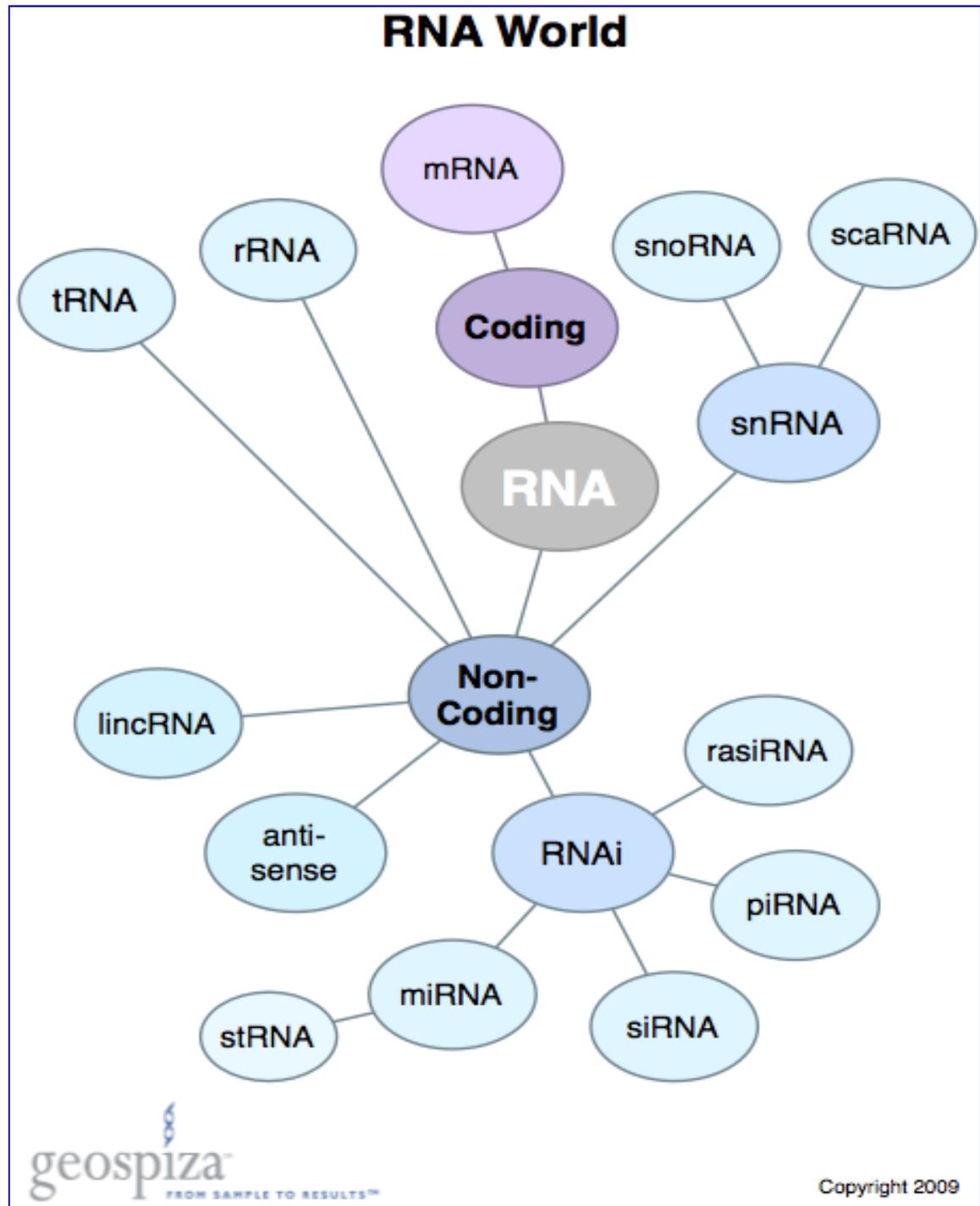
NONPROTEIN-CODING SEQUENCES make up only a small fraction of the DNA of prokaryotes. Among eukaryotes, as their complexity increases, generally so, too, does the proportion of their DNA that does not code for protein. The noncoding sequences have been considered junk, but perhaps it actually helps to explain organisms' complexity.



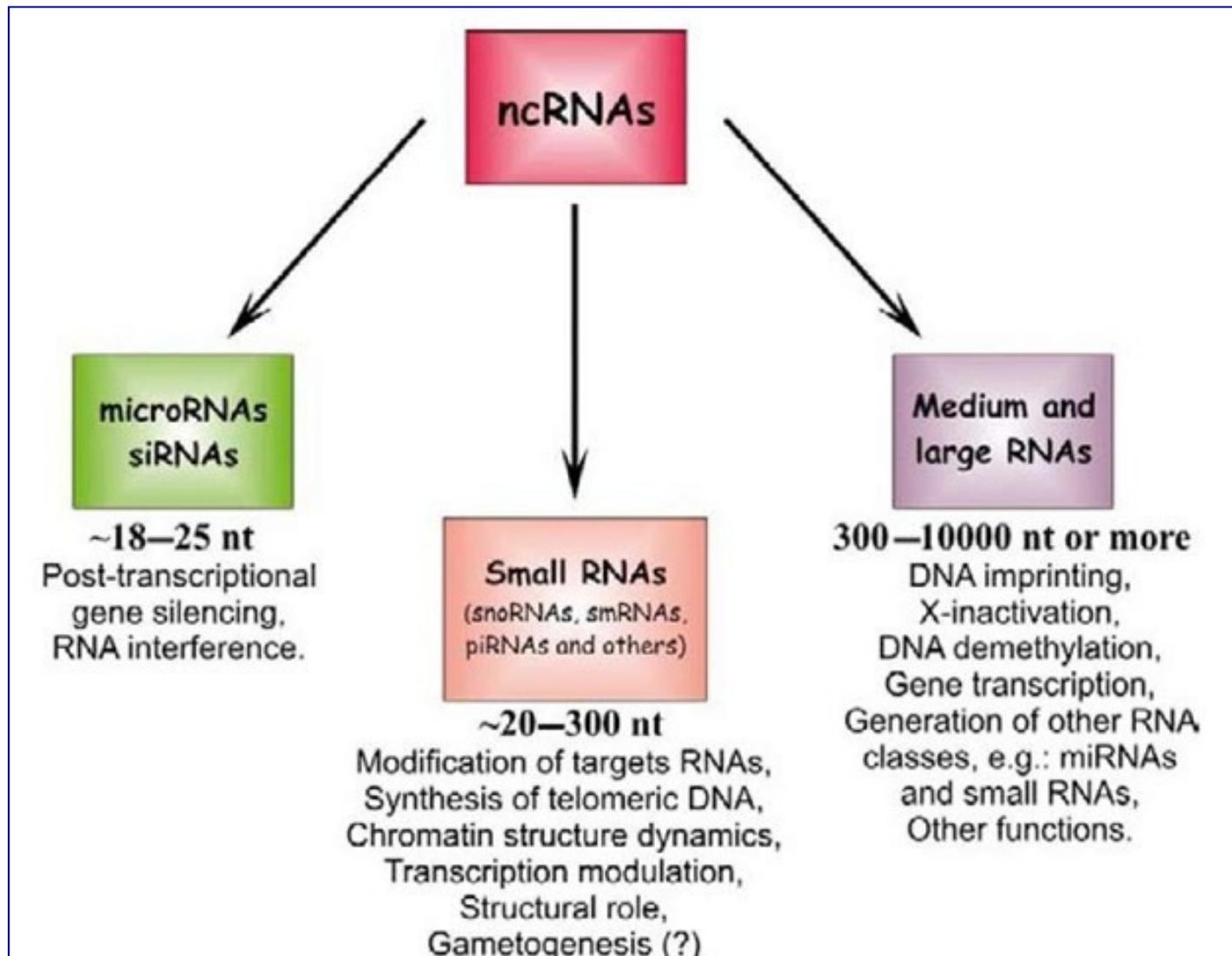
Contrordine, il Dna "spazzatura" serve

- Più del 90% dell'intero genoma è composto da sequenze che non esprimono proteine, e per questo motivo è stato considerato per lungo tempo privo di qualsiasi funzione.
- Dna spazzatura considerato come una sorta di relitto dell'evoluzione
- Il progetto internazionale Encode ha permesso di attribuire una funzione al DNA non codificante
- Il DNA non codificante funge da interruttore dell'espressione genica, modulandone la trascrizione, ma anche la traduzione.

NUOVE FORME DI RNA



CLASSIFICAZIONE DEI NON-CODING RNAs



INTERFERENZA DA RNA (RNA interference)

Fenomeno per cui l'inserimento di un RNA a doppia catena (dsRNA, double-stranded RNA) in una cellula causa la degradazione di un mRNA complementare.



SILENZIAMENTO GENICO

E' uno strumento per i seguenti studi:

- funzione di un determinato gene**
- identificazione e conferma di target biologici**
- generazione di modelli knockout**

Fenomeno osservato in differenti organismi (da protozoi a mammiferi), conservatosi durante l'evoluzione come meccanismo di difesa contro i virus.

RNAi – Cenni storici

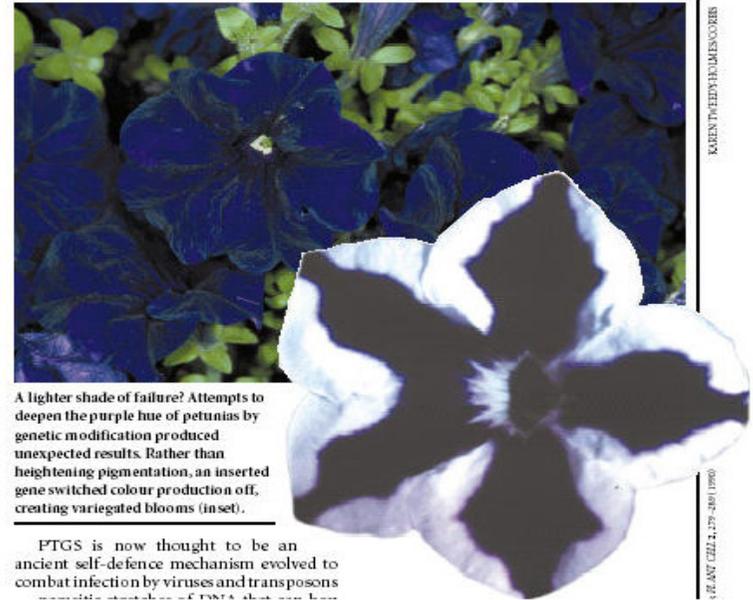
- Piante

Anni '90, un'osservazione sorprendente

- Obiettivo: aumentare la pigmentazione viola nelle petunie utilizzando un sistema d'espressione
- Risultato: colorazione variegata o completamente bianca



Al fenomeno è stato dato il nome di 'silenziamento genico post trascrizionale'



RNAi – Cenni storici

- Vermi (*Caenorhabditis elegans*)

1998

- Fire & Mello iniettarono RNA senso, RNA antisenso e dsRNA in *C. elegans* (Fire A et al. *Nature*; 391: 806-811)
- Prima evidenza che l'RNA a doppio filamento (dsRNA, double stranded RNA) causava il silenziamento genico in maniera sequenza-specifica.

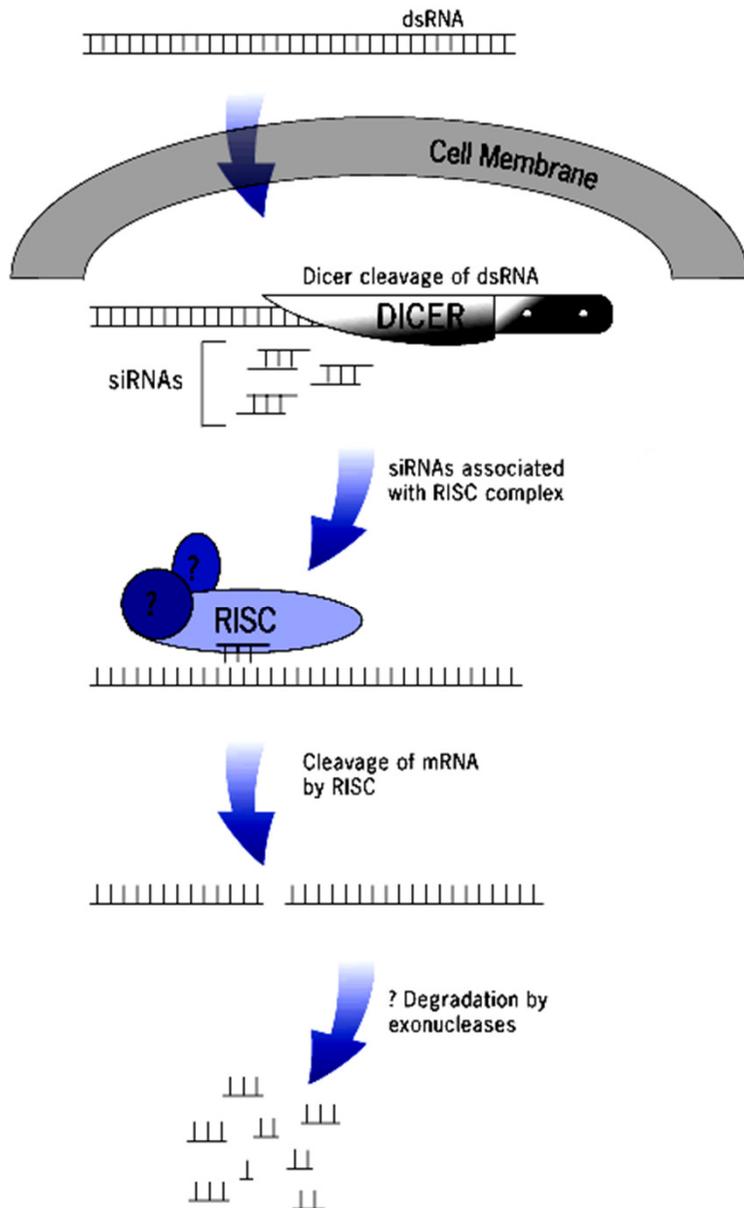


Questa importantissima osservazione portò a coniare il termine “RNA interference”



Premio Nobel 2006

Meccanismo biochimico dell'RNAi



Il dsRNA viene introdotto nella cellula

DICER digerisce il dsRNA frammenti da ~21bp (short interfering RNAs → siRNAs)

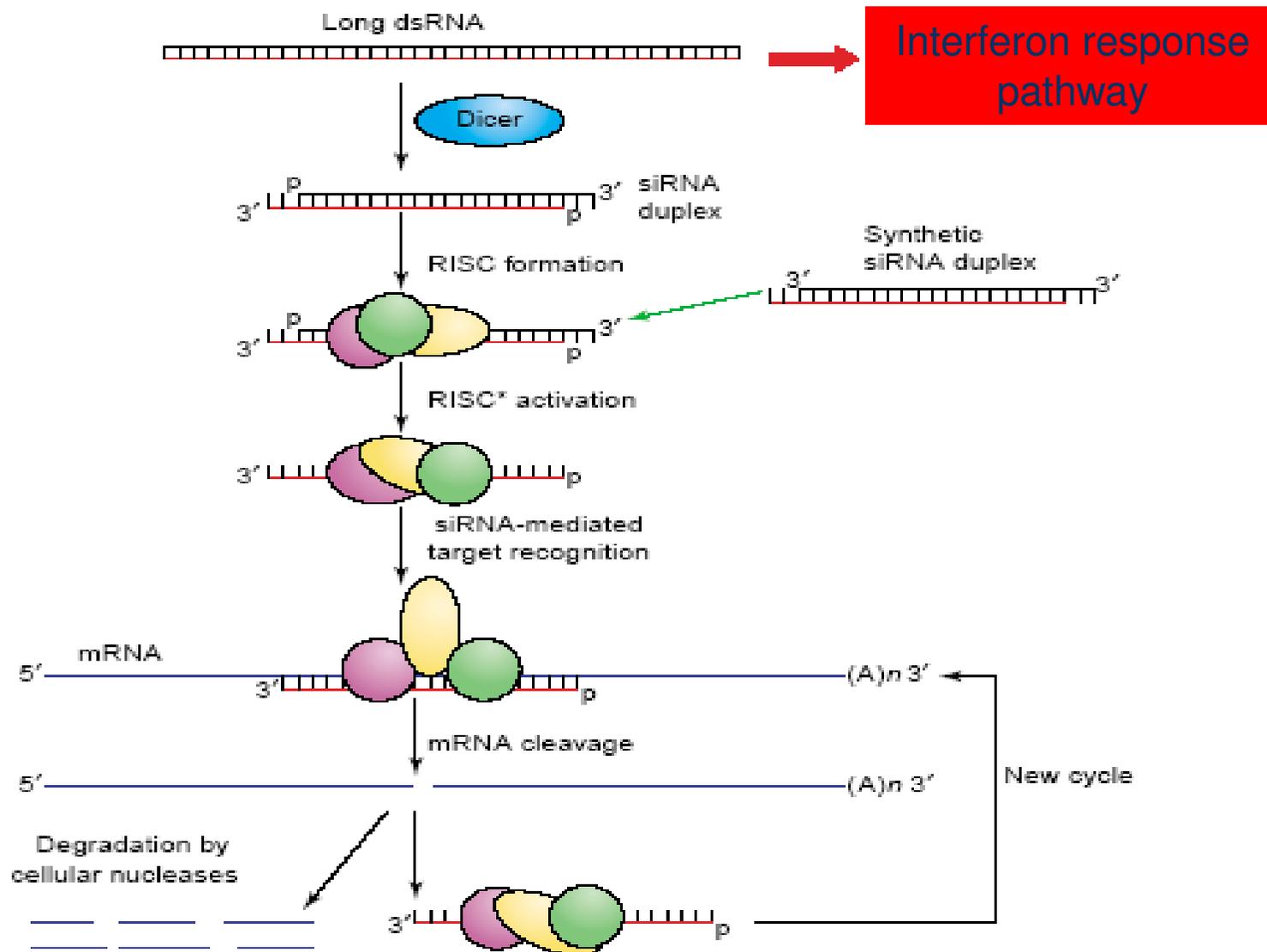
Gli siRNA subiscono separazione delle eliche e vengono integrati nel RISC (RNA Induced Silencing Complex)

La strand antisenso dell'siRNA si lega all'mRNA complementare (target)

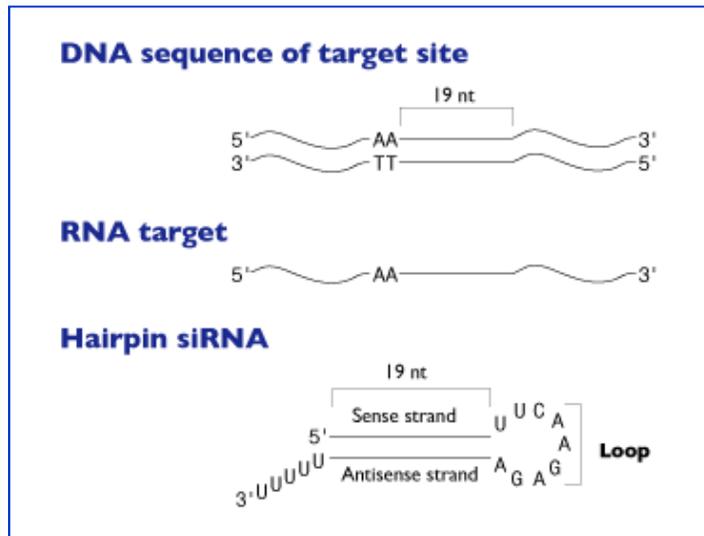
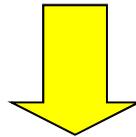
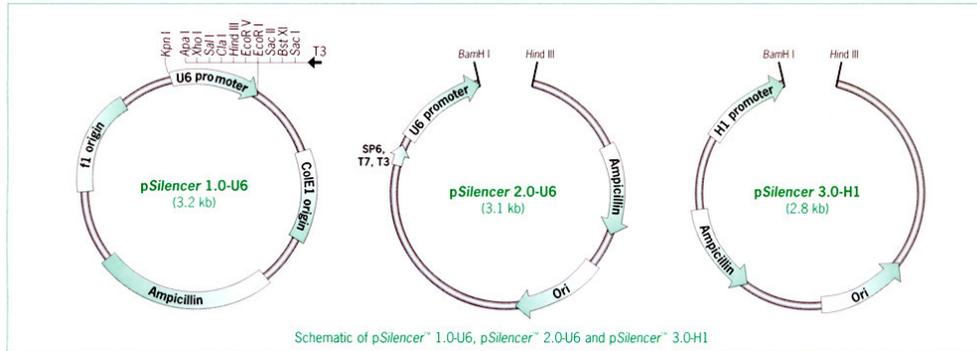
L'attività nucleasica (*Slicer*) di RISC degrada l' mRNA

Le ribonucleasi cellulari completano la degradazione dell'mRNA target

RNA interference by siRNA



Silenziamento tramite vettore d'espressione



- Una sequenza stampo per un “hairpin siRNA” viene clonata in un opportuno vettore.
- Trasfezione stabile nella linea cellulare di cui si vuol silenziare il gene target dell’RNAi.
- Produzione di siRNA nelle cellule trasfettate.
- Silenziamento a lungo termine del gene target.

siRNA PROFILING TECHNOLOGIES

Disegno di siRNA

- **il siRNA possiede un gruppo UU al terminale 3'**
- **19 nucleotidi**
- **un contenuto in G/C < 50% è preferibile.**

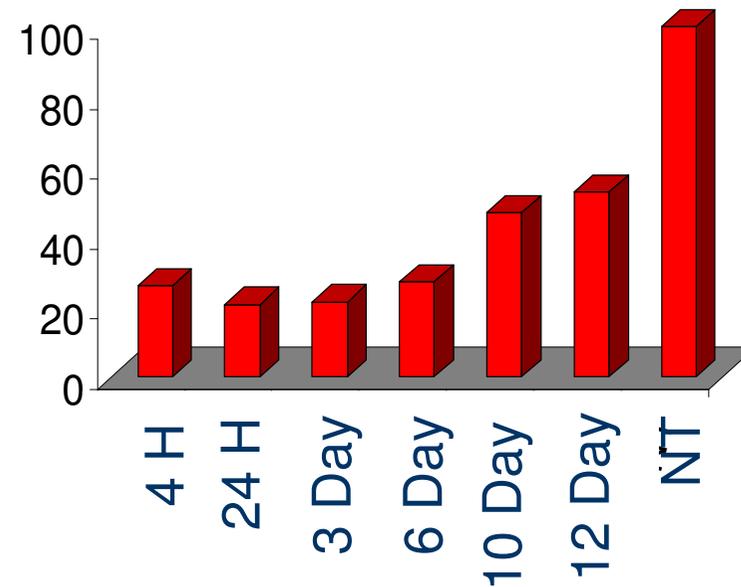
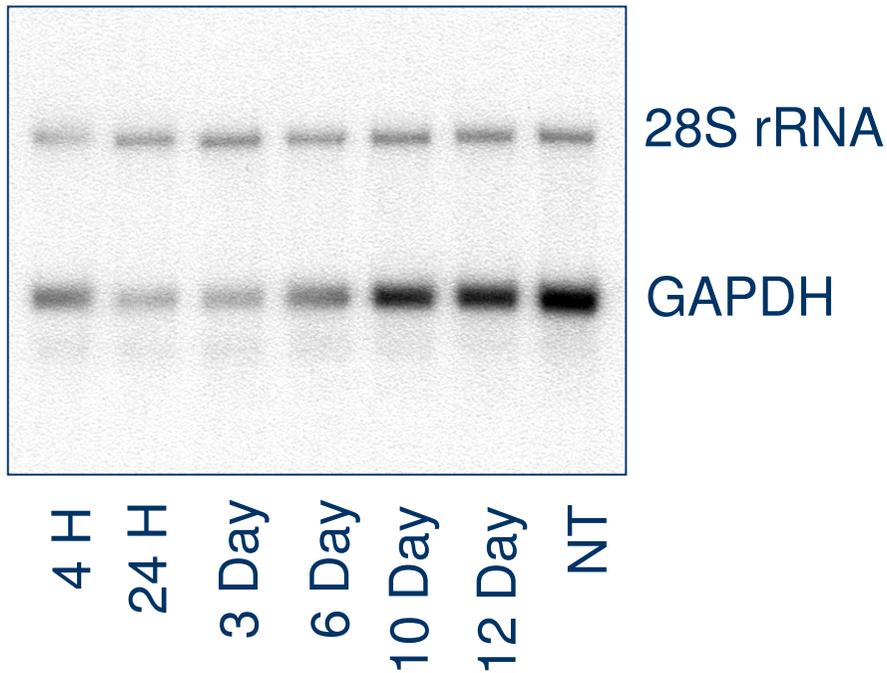
Algoritmo di nuova generazione

- **Tm dell'siRNA**
- **Effetti della posizione nucleotidica**
- **Contenuto nucleotidico dei 3' overhangs**
- **Distribuzione nucleotidica**
- **Controllo della specificità**

Duration of transient silencing by siRNAs: mRNA Levels

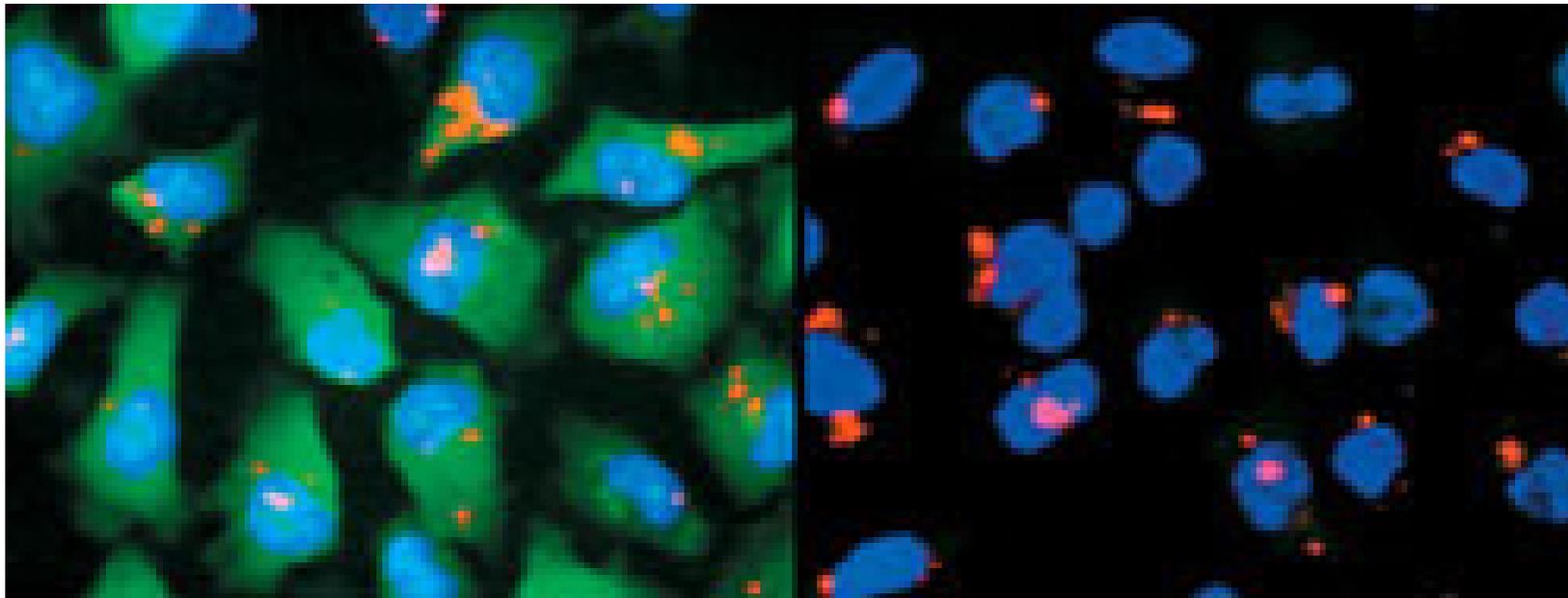
Down-regulation of GAPDH gene expression in HeLa cells

Northern Blot Analysis



CELLULE HeLa

In rosso: siRNA marcato
In blu: nuclei
In verde la proteina GAPDH



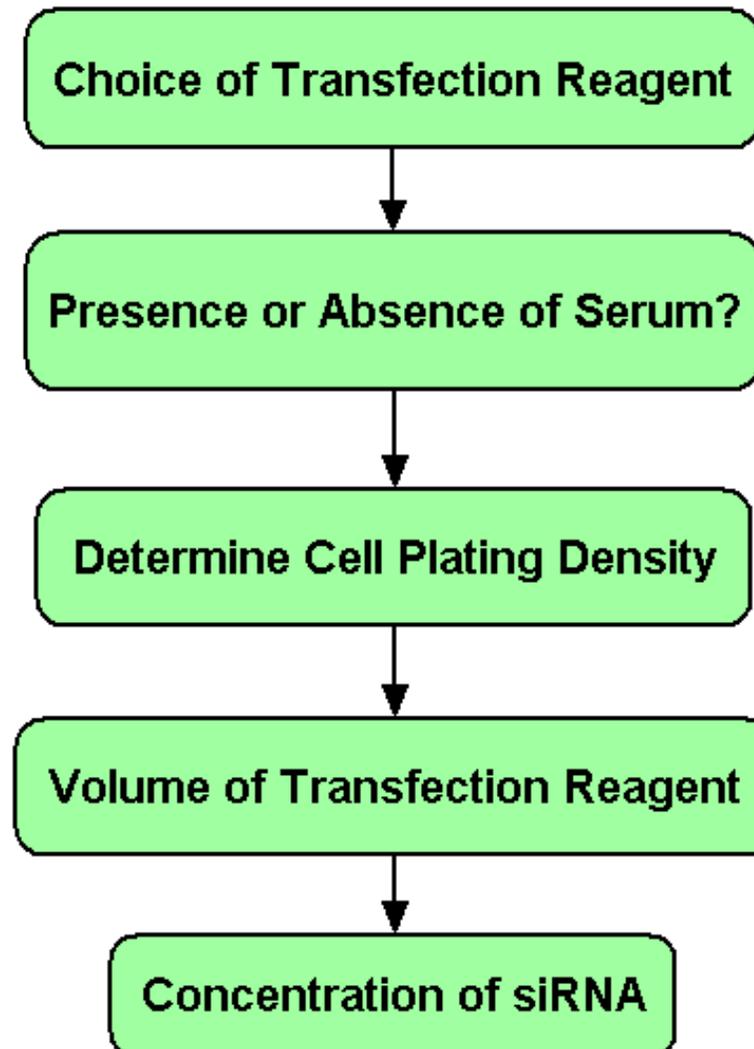
si RNA non specifico

si RNA contro GAPDH

Trattamento: 48h

Transfection of siRNAs

These transfection conditions must be optimized for every cell line.



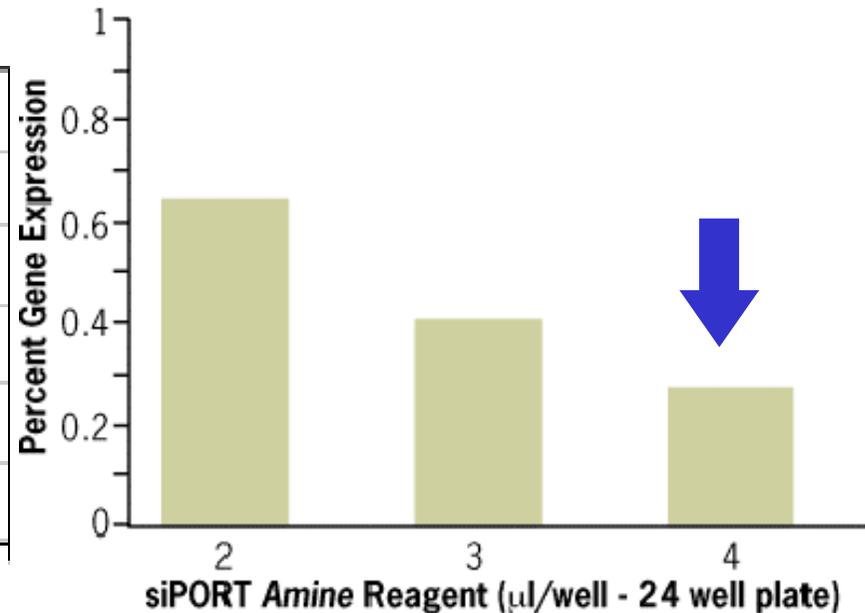
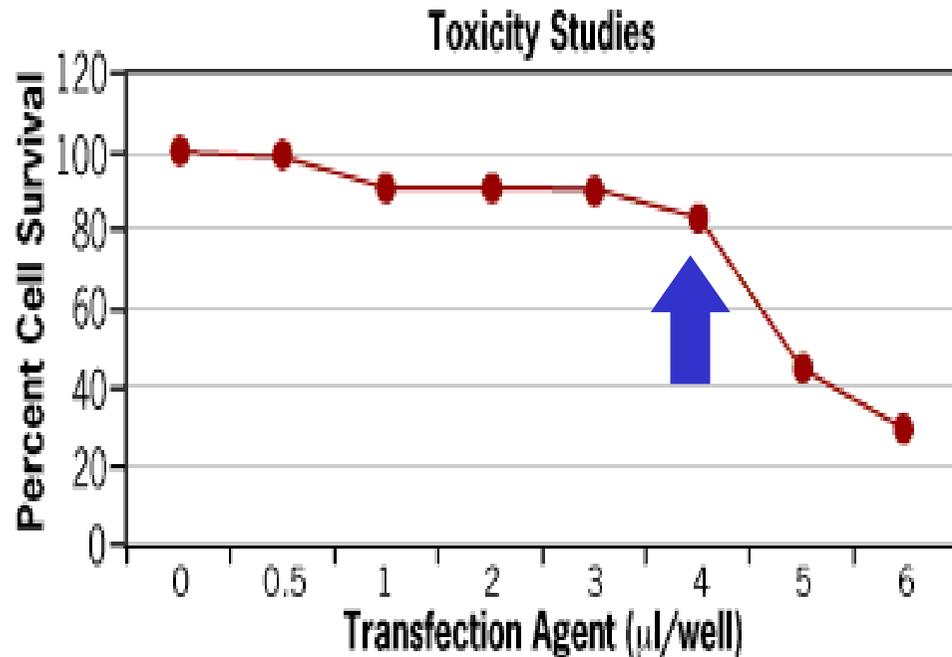
Transfection of siRNAs

Choice of Transfection Reagent

Determining optimal amount of transfection reagent

No cytotoxic

Effective

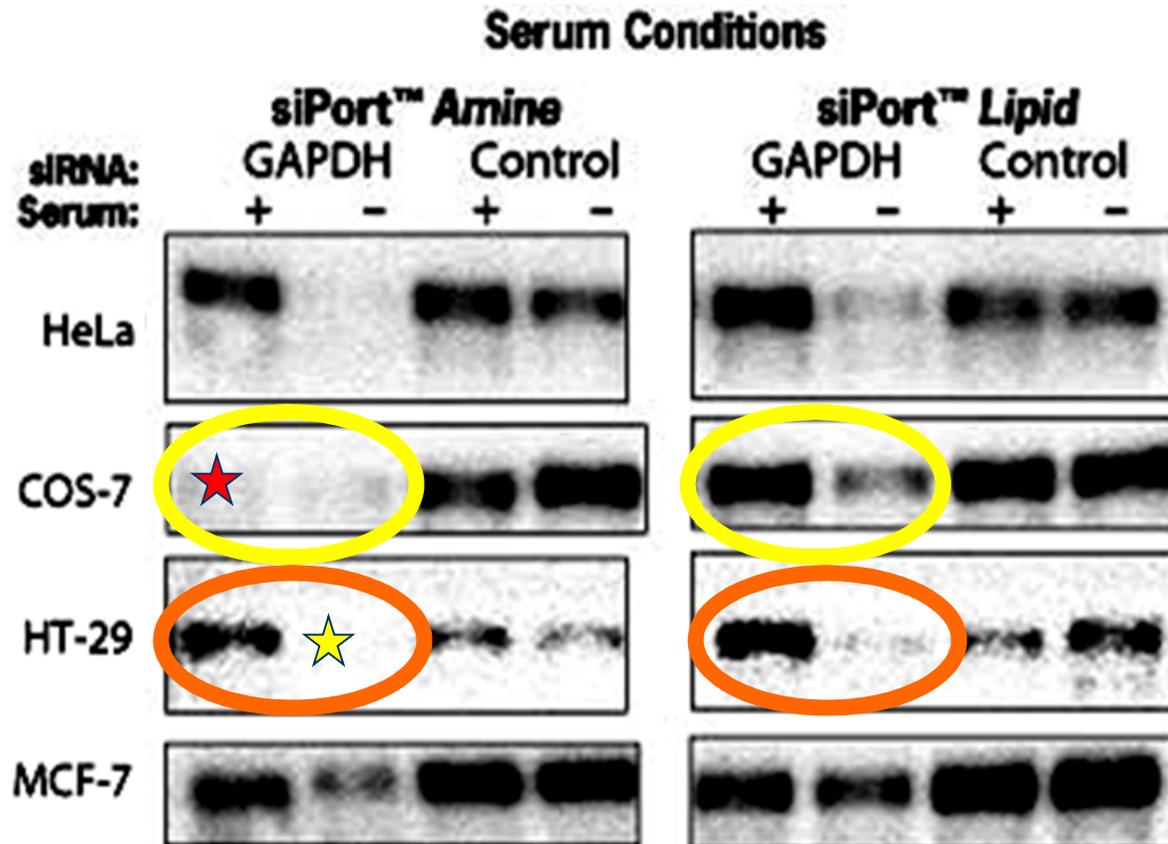


- COS-7 cells grown at 30K/well
- Transfected with GAPDH siRNA (10nM) after 24 hrs
- Real-time RT-PCR: GAPDH/18S rRNA (after 48 hrs)

Transfection of siRNAs

Presence or Absence of Serum?

Effect of serum on siRNA transfection

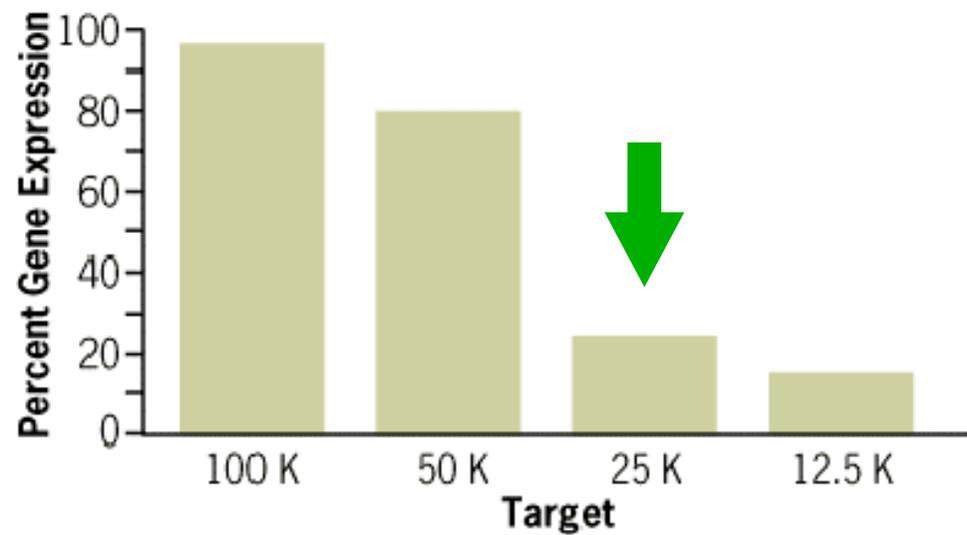


Transfection of siRNAs

Determine Cell Plating Density

Optimal cell plating density

- COS-7 cells/well (24 well plate)
- GAPDH siRNA
- Real-time RT-PCR- looked at ratio of GAPDH/18S RNA after 48 hrs



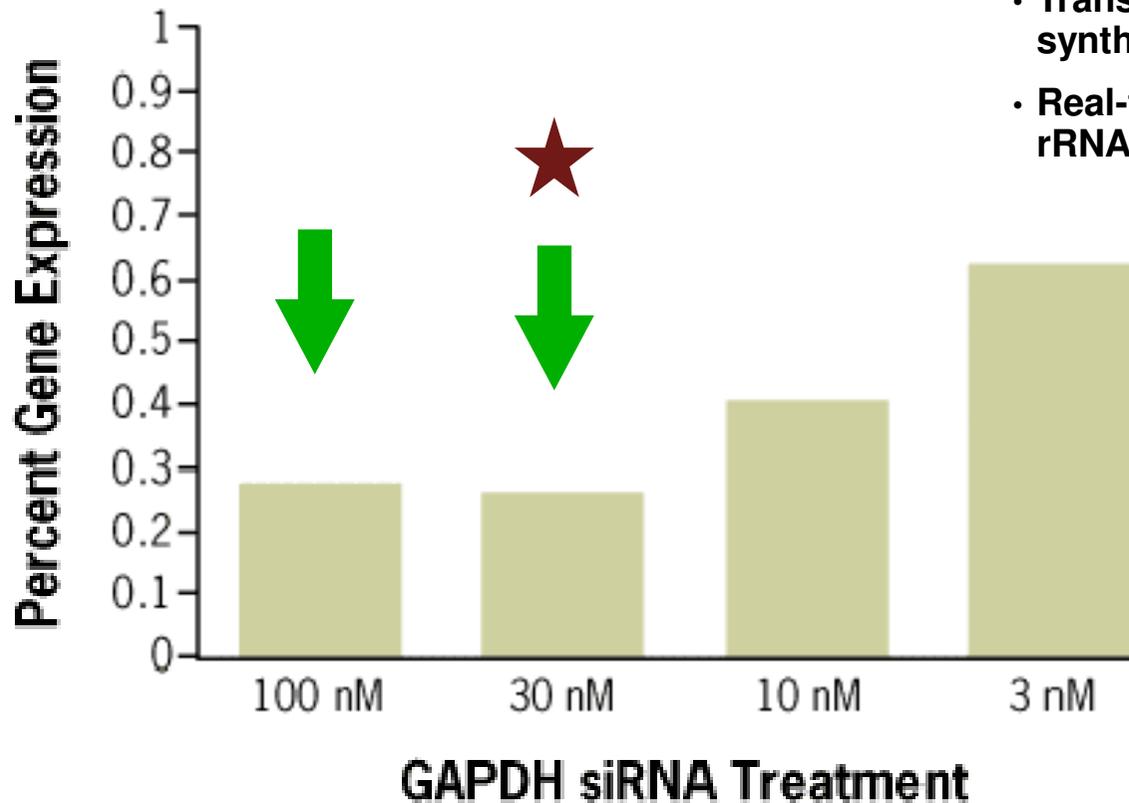
Transfection of siRNAs

Concentration of siRNA

Determining optimal amount of siRNA

lowest concentration of siRNA possible to minimize off-target effects

- COS-7 cells grown at 30K/well
- Transfected with chemically synthesized GAPDH siRNA
- Real-time RT-PCR: GAPDH/18S rRNA



Preventing Off-Target Effects

Overabundance of the siRNA activates the interferon pathway, as antiviral response



Low concentrations (~5-30nM) of single siRNA minimizes:

- **chances of off-target effect**
- **induction of interferon response**

It is currently preferable to use **ONE** highly potent siRNA than a **MIXTURE** of siRNAs that raise overall siRNA conc.

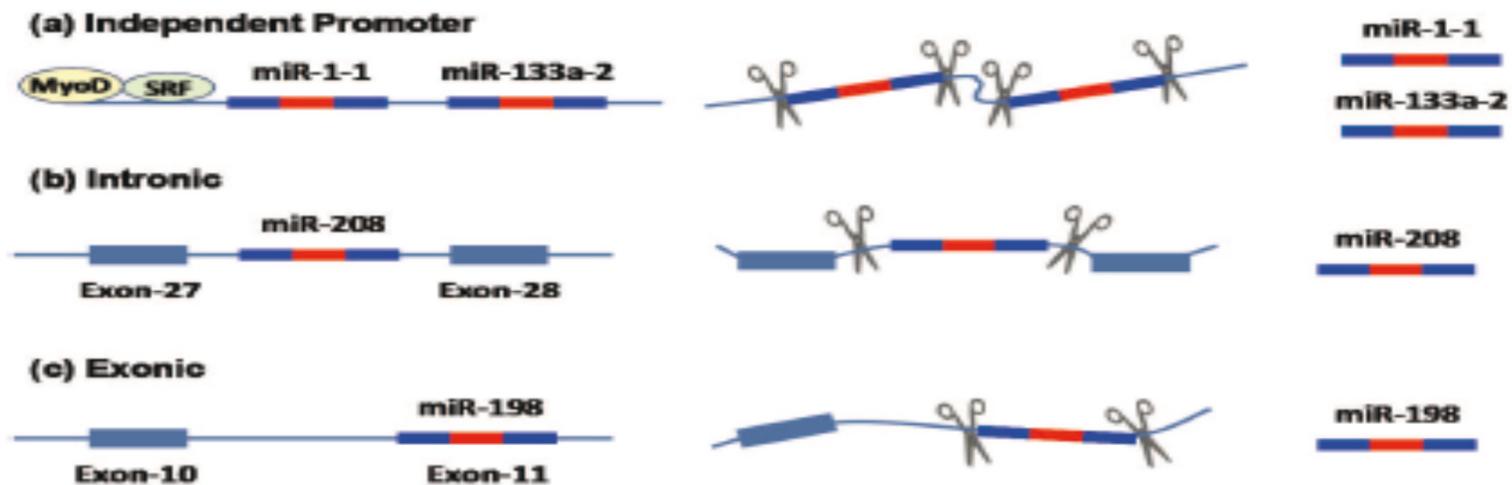


Verify specificity of RNAi effect by testing independent siRNAs to the same target

RNA interference by miRNA (miR)

MicroRNAs

❖ Tutti i miRNA sono piccoli RNA endogeni, non codificanti (derivanti da regioni introniche, ma non solo) in genere costituiti da 22 nucleotidi

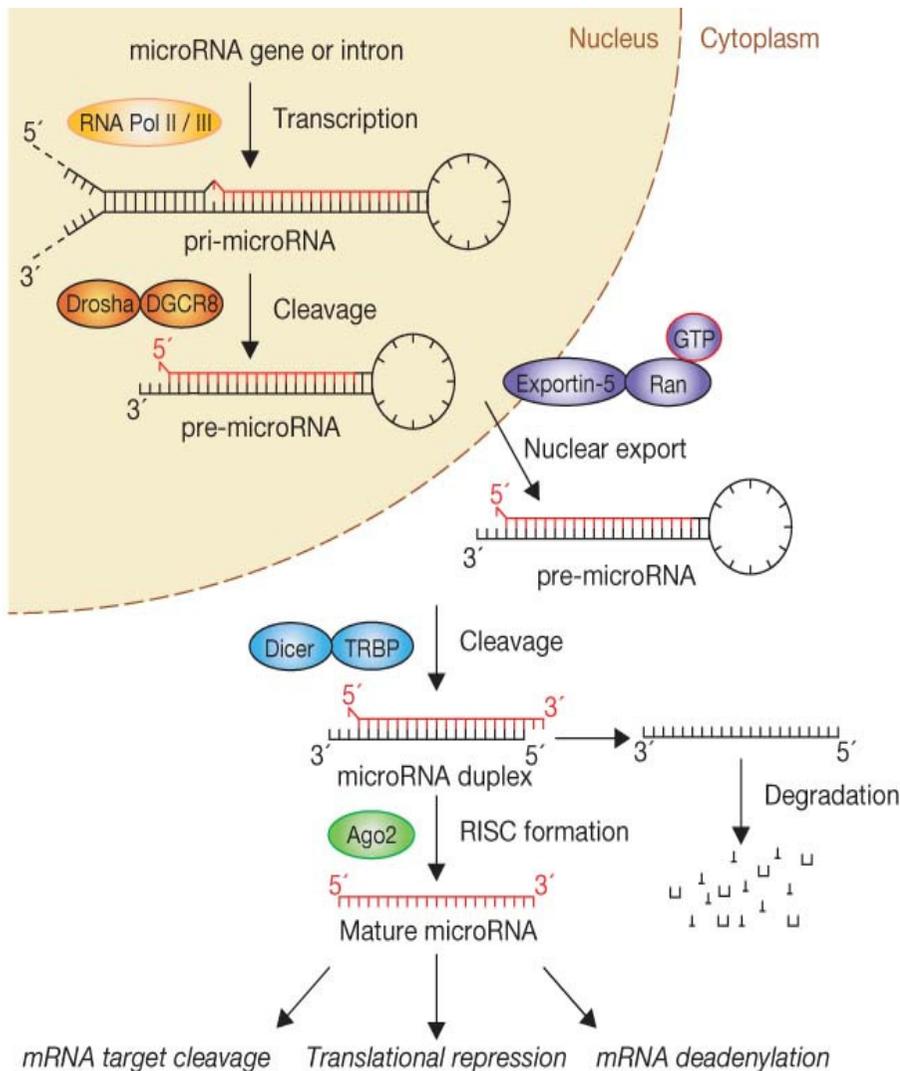


- Intergenic miRNA genes: either monocistronic or polycistronic with a common promoter
- Intronic miRNA genes: present in the introns of protein coding or noncoding genes, can also be in clusters, transcribed by the host gene promoter
- Exonic miRNAs genes: rare and often overlap an exon and an intron of a noncoding gene
- miRNAs can be transcribed from the negative strand within or near a protein coding gene

MicroRNAs

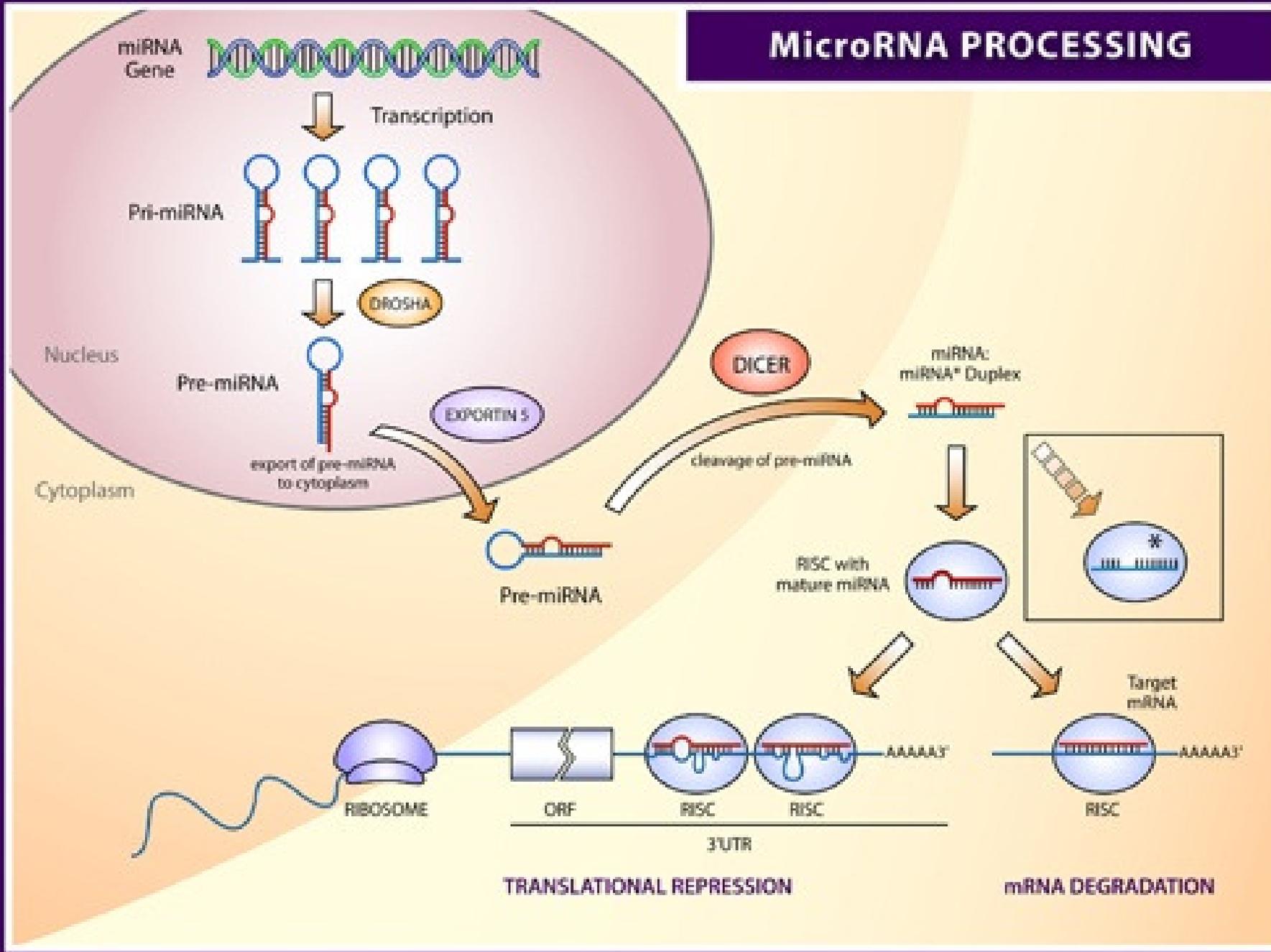
- ❖ Tutti i precursori dei miRNA (pre-miRNA) hanno una ben predicibile e molto stabile struttura a hairpin (ripiegamento e ansa).
- ❖ Molti miRNA sono conservati nelle varie specie, anche se molto distanti dal punto di vista evolutivo.
- ❖ Ogni miRNA può controllare l'espressione di molti geni. Si pensa che l'espressione di circa 2/3 dei geni umani è regolata da miRNA.
- ❖ Sono espressi in alto numero di copie
- ❖ Esistono decine di migliaia di miRNA maturi, nell'uomo sono stati identificati circa 1000 miRNAs differenti
- ❖ L'espressione è tessuto-specifica.

The miRNA processing pathway has long been viewed as universal to all mammalian miRNAs.



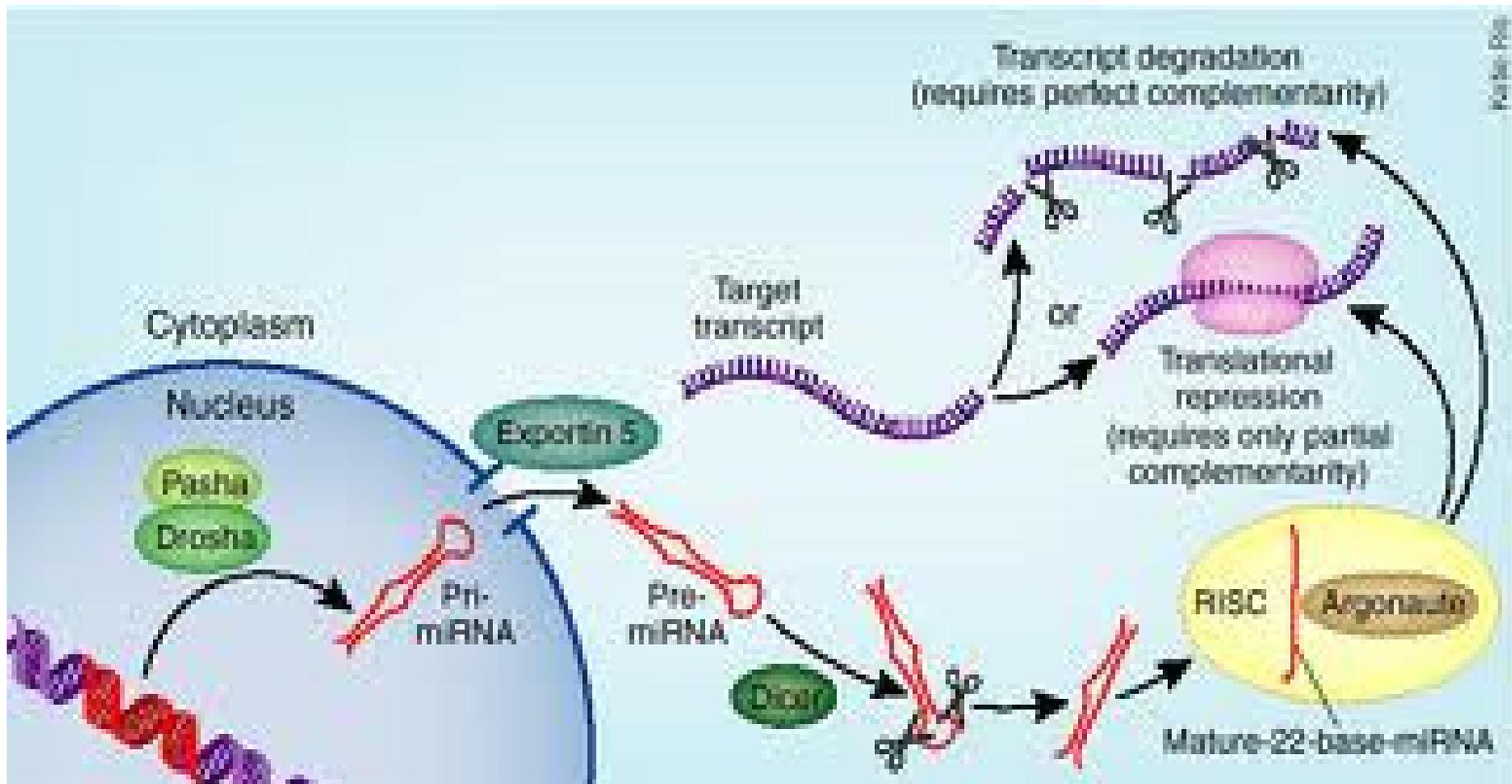
- Production of the primary miRNA transcript (pri-miRNA) by RNA polymerase II or III
- Cleavage of the pri-miRNA by the microprocessor complex Drosha–DGCR8 (Pasha) in the nucleus (pre-miRNA).
- Pre-miRNA, is exported from the nucleus by Exportin-5–Ran-GTP.
- In the cytoplasm, the RNase Dicer in complex with the double-stranded RNA-binding protein TRBP cleaves the pre-miRNA hairpin to its mature length.
- The functional strand of the mature miRNA is loaded together with Argonaute (Ago2) proteins into the RNA-induced silencing complex (RISC)
- The complex RISC silences target mRNAs through mRNA cleavage, translational repression or deadenylation, whereas the passenger strand (black) is degraded.

MicroRNA PROCESSING



MECCANISMO DI AZIONE DEI MicroRNAs

LEGANO MOLECOLE DI mRNA COMPLEMENTARE E AGISCONO COME REGOLATORI NEGATIVI DELLA TRADUZIONE.

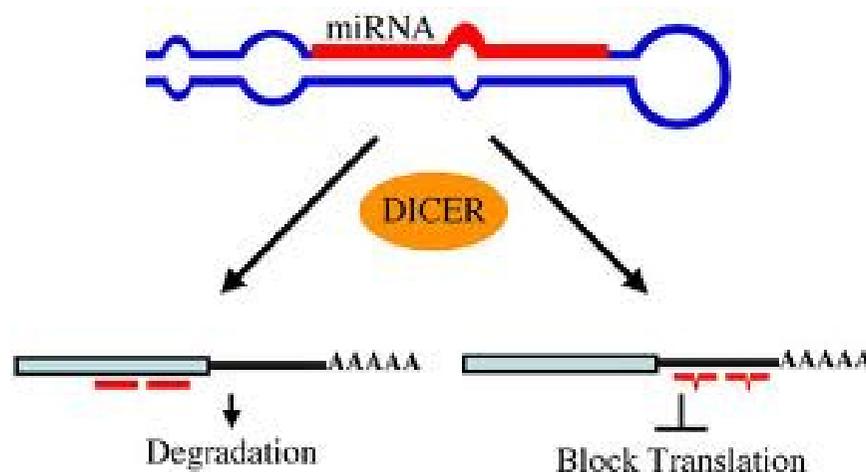


MECCANISMO DI AZIONE DEI MicroRNAs

I miRNA operano in base a uno dei due seguenti meccanismi:

✓ APPAIAMENTO COMPLETO → RNA messaggero DEGRADATO
(nelle piante)

✓ APPAIAMENTO PARZIALE → RNA messaggero INTATTO,
ma traduzione repressa
(prevalentemente negli animali)



miRNA and siRNA comparison

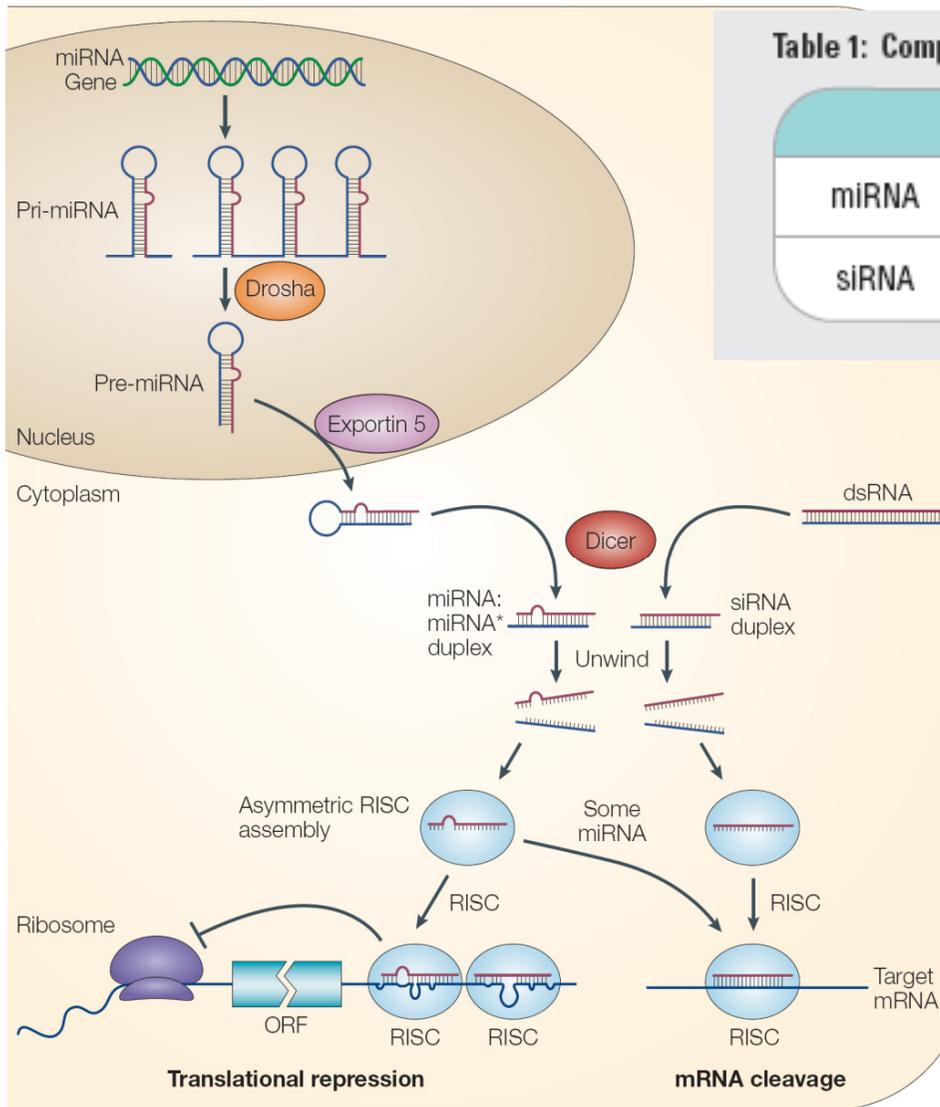


Table 1: Comparison of miRNA and siRNA.

	Length	Where Found?	Target Recog	Mechanism
miRNA	19-25 nt	Endogenous	Imperfect Match	Translational Repression
siRNA	19-21 nt	Exogenous	Exact Match	mRNA Cleavage

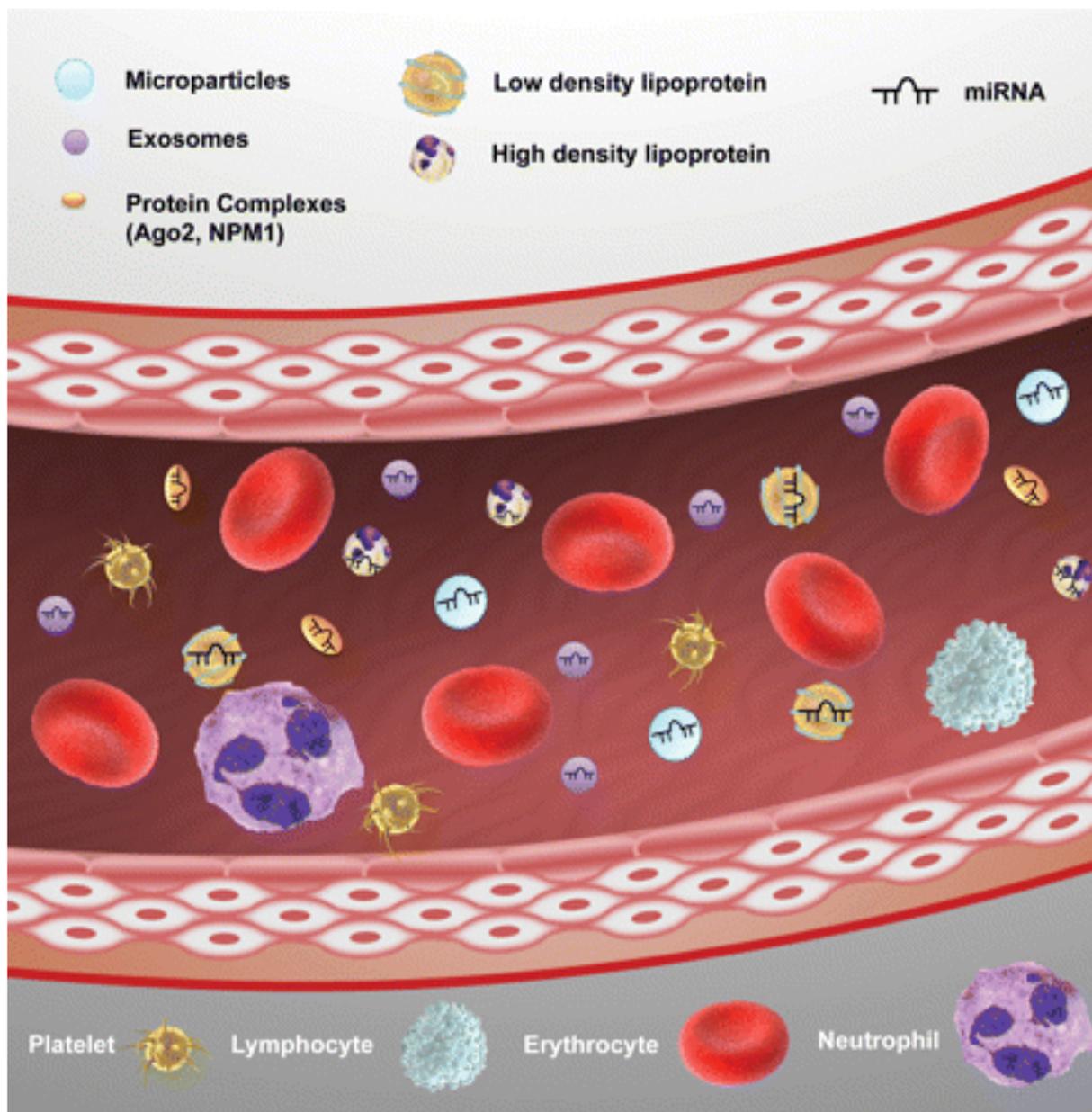
ORIGINE: endogena (miRNA) vs esogena (siRNA)

MECCANISMO D'AZIONE:

•**siRNAs** richiedono un perfetto matching con l'mRNA target, che comporta la degradazione dell'mRNA.

•**miRNAs** non necessitano di un matching perfetto per indurre l'inibizione della traduzione dell'mRNA target

I miRNAs circolanti



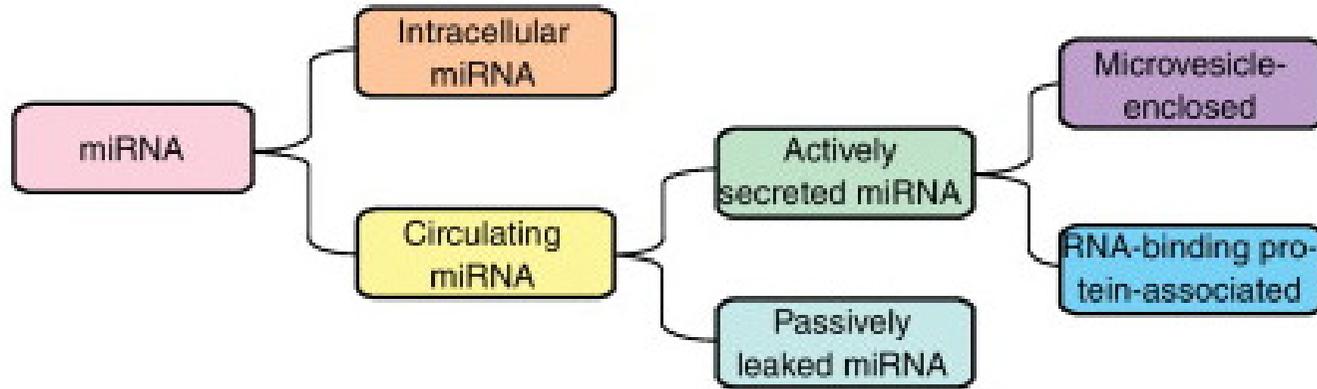
I miRNAs si sono rivelati estremamente stabili nei fluidi biologici

Il loro profilo di espressione spesso correla con uno specifico stato patologico



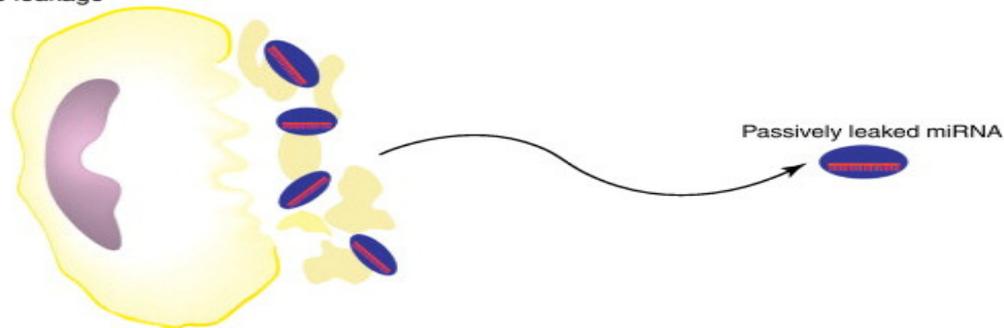
Analisi dei miRNA circolanti come biomarkers precoci di varie patologie

Stabilità dei miRNAs circolanti

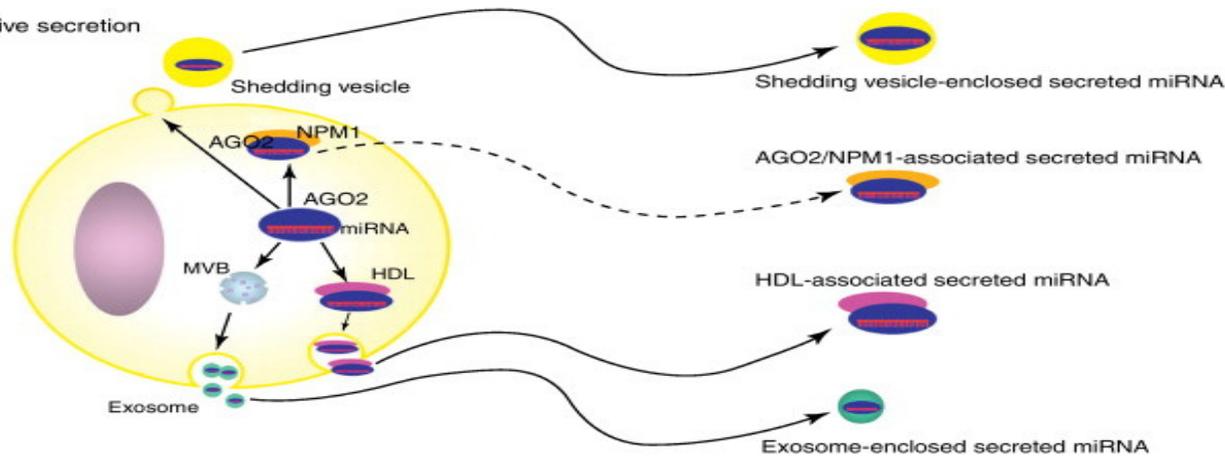


TRENDS in Cell Biology

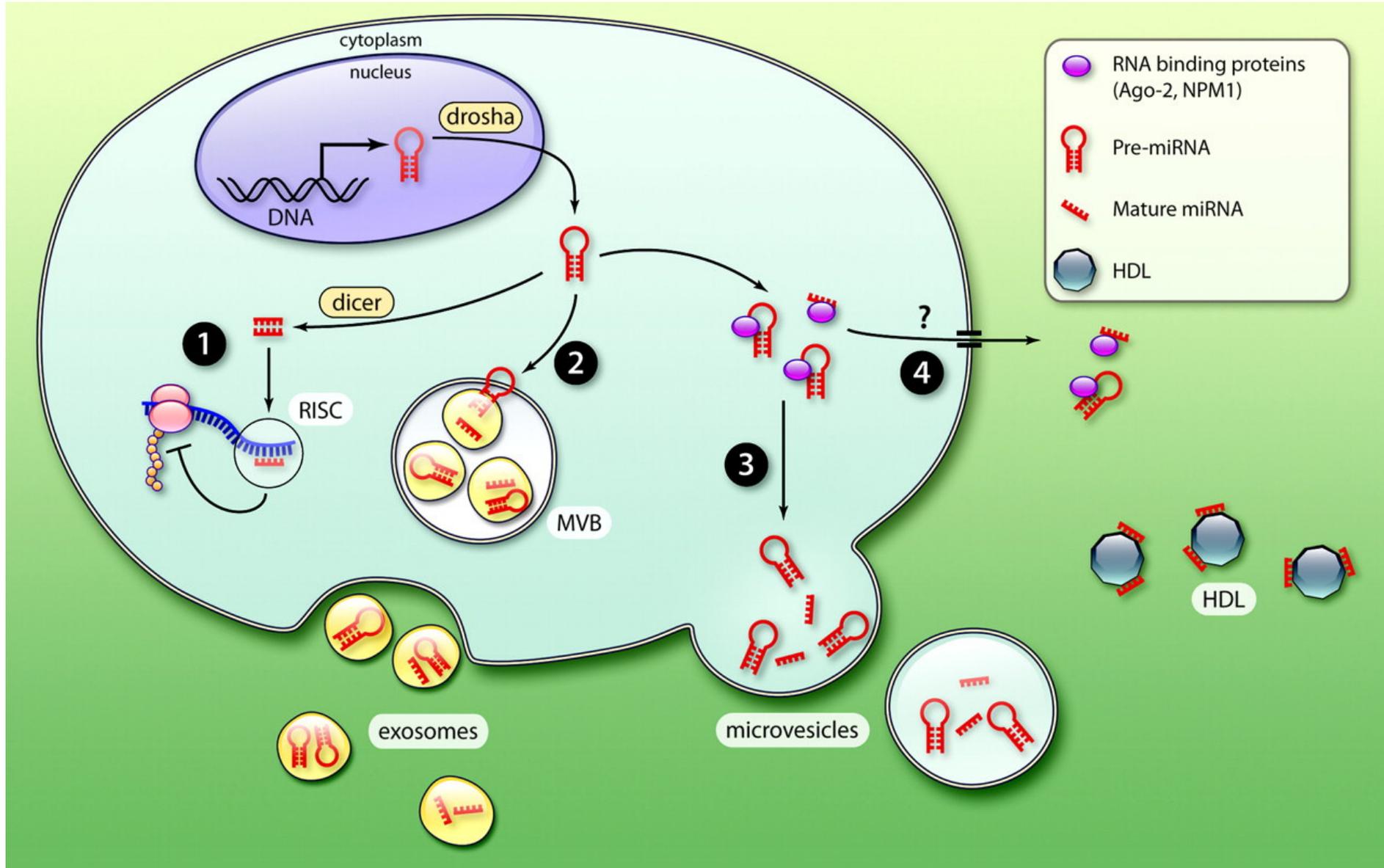
Passive leakage



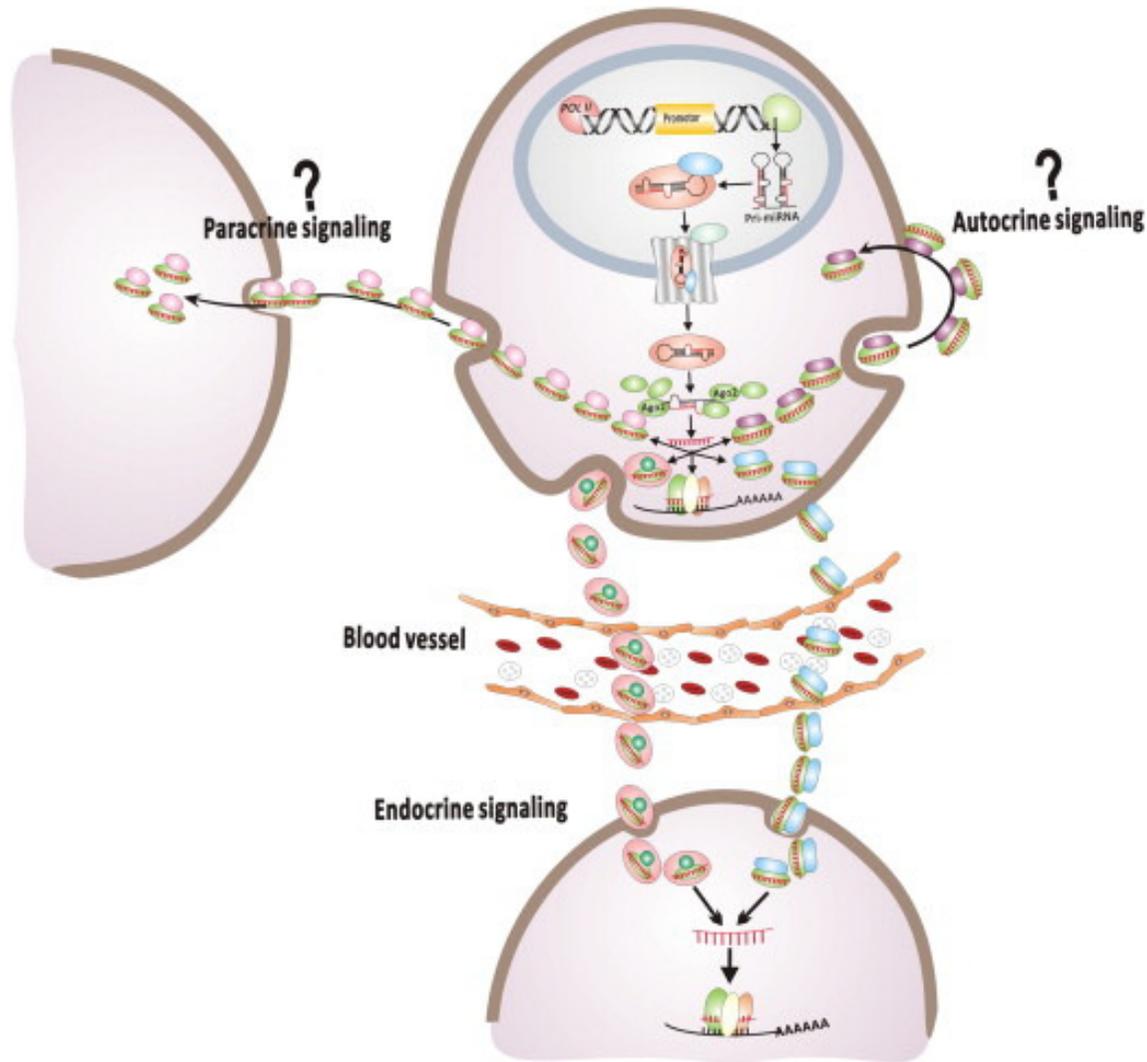
Active secretion



Carriers dei miRNAs



I miRNAs extracellulari: una nuova forma di comunicazione intercellulare

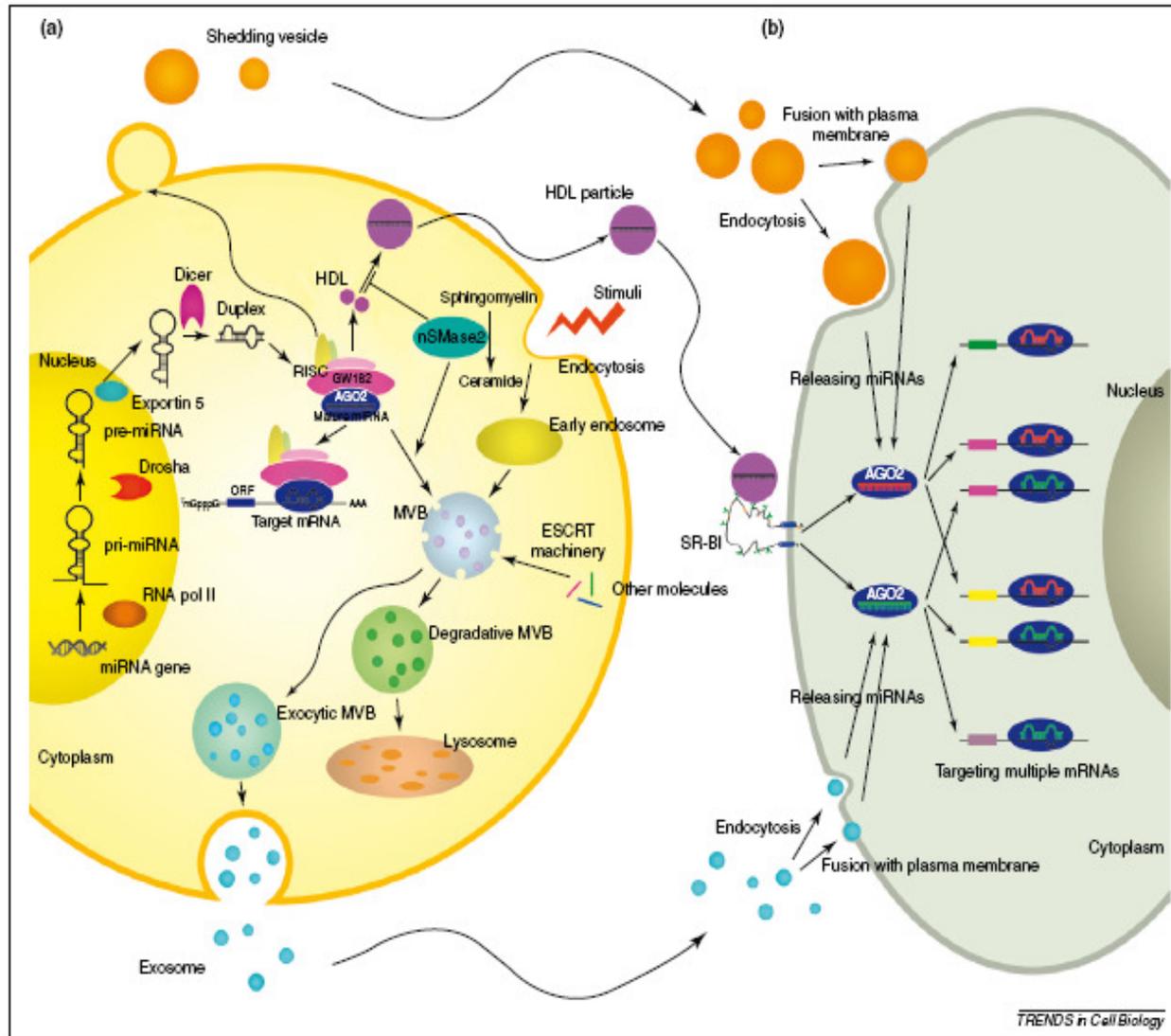


EFFETTO ENDOCRINO

EFFETTO AUTOCRINO

EFFETTO PARACRINO

Uptake dei miRNAs extracellulari da parte della cellula target



Endocitosi, fagocitosi e fusione diretta con la membrana rappresentano i meccanismi di ingresso dei miRNAs associati a vescicole (esosomi e 'shedding vesicles')

L'ingresso recettore-mediato è riservato ai miRNA associati a proteine. Es. HDL-miRNAs si servono del recettore SR-BI.

Table 1. The potential roles of secreted miRNAs in various physiological and pathological processes

Secreted miRNA	Transport system	Donor cell	Target cell	Target gene	Biological function of secreted miRNA
let-7, miR-1, miR-15, miR-16, miR-181 and miR-375	Exosome	Mast cell line (MC9 and HMC-1), primary bone marrow-derived mast cell			
miR-150	Microvesicle	Human monocytic cell line (THP-1)	Human microvascular endothelial cell line (HMEC-1)	<i>c-Myb</i>	Promote cell migration
miR-335	Exosome	Human Jurkat-derived T-cell line (J77 cell expressing miR-335)	Human lymphoblastoid B cell line (Raji)	<i>SOX4</i>	Regulate immune synapsis
miR-16, miR-21, miR-143, miR-146a and miR-155	Exosome	Human embryonic kidney cell line (HEK293)			
miR-290, miR-291-3p, miR-292-3p, miR-294 and miR-295	Microvesicle	Mouse embryonic stem cell	Mouse embryonic fibroblast		
miR-21, miR-99a, miR-100 and miR-223	Microvesicle	Human bone marrow derived mesenchymal stem cell, liver resident stem cell	Murine tubular epithelial cell	<i>PTEN, cyclin D1 and Bcl-2</i>	
miR-223, miR-484, miR-191, miR-146a, miR-16, miR-26a, miR-222, miR-24, miR-126 and miR-32	Microvesicle	Peripheral blood cell			
let-7b, miR-103, miR-148a, miR-16, miR-27a, miR-146b and miR-222	Microvesicle	Mouse preadipocyte cell line (3T3-L1), primary rat adipocyte			
miR-21	Microvesicle	Primary human glioblastoma cell	Human brain microvascular endothelial cell (HBMVEC)		
EBV-miRNA	Exosome	EBV-transformed B lymphoblastoid cell	Monocyte-derived dendritic cell	<i>CXCL11 and LMP1</i>	Repress immunostimulatory gene
EBV-miRNA	Exosome	EBV-positive nasopharyngeal carcinoma cell line (C666)	Human umbilical vein endothelial cell (HUVEC)		
miR-517a	Exosome	Human chorionic villi (especially syncytiotrophoblast), human trophoblast cell line (BeWo)			
miR-101, miR-125b, miR-150, miR-223, miR-24 and miR-93	Microvesicle	Bovine milk			
miR-223	HDL particle	Human plasma	Hepatocyte (Huh7)	<i>RhoB and EFNA1</i>	

Elenco dei principali miRNA circolanti coinvolti in differenti processi fisiologici/patologici

I miRNAs extracellulari: una nuova forma di comunicazione madre-feto

Elevati livelli di esosomi contenenti miRNAs specifici della placenta sono stati osservati all'interfaccia madre-feto

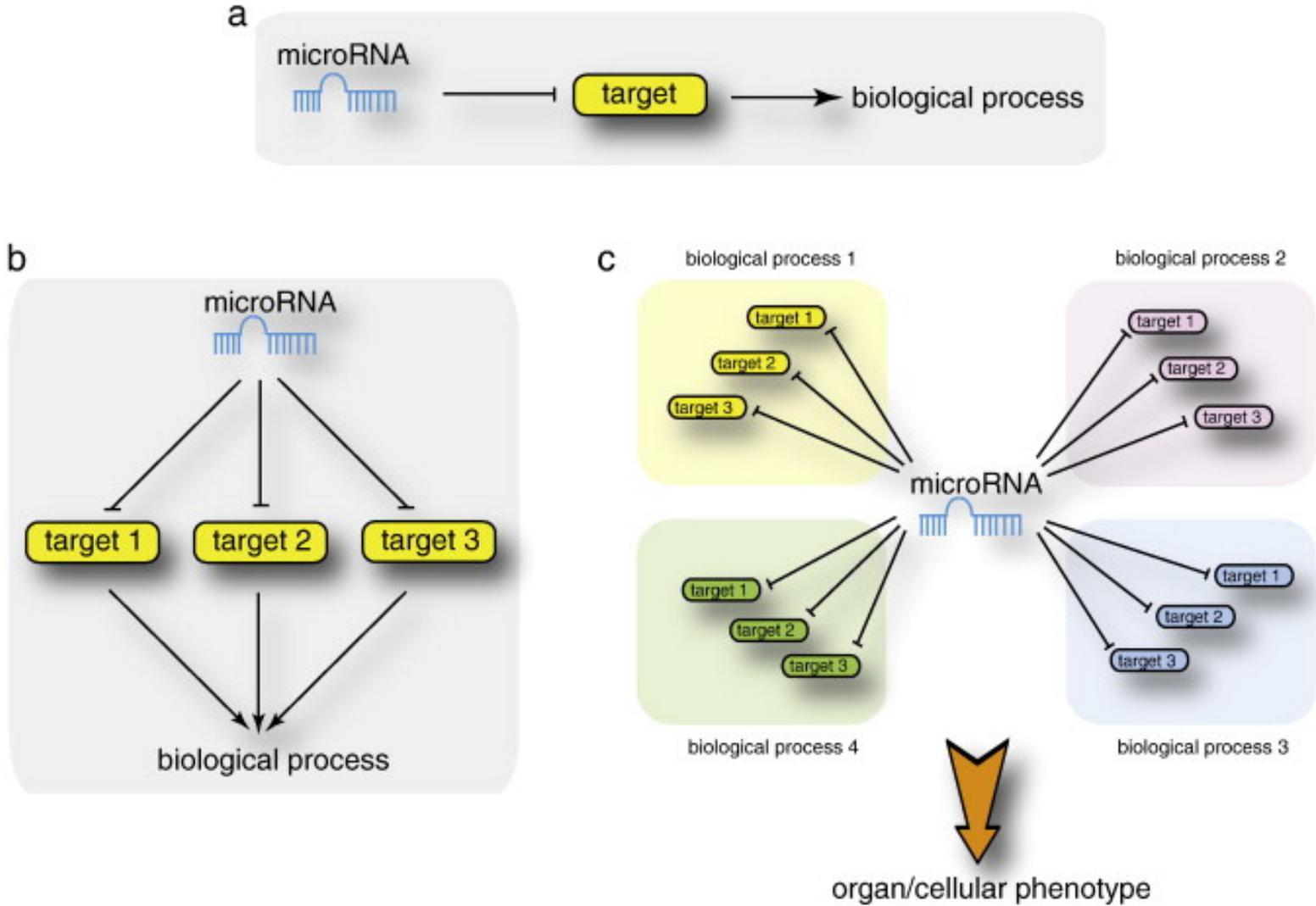
Alcuni miRNAs correlati con le funzioni immunologiche sono stati osservati in microvescicole derivanti da latte bovino

Alcuni miRNAs correlati con le funzioni immunologiche sono stati osservati anche nel colostro

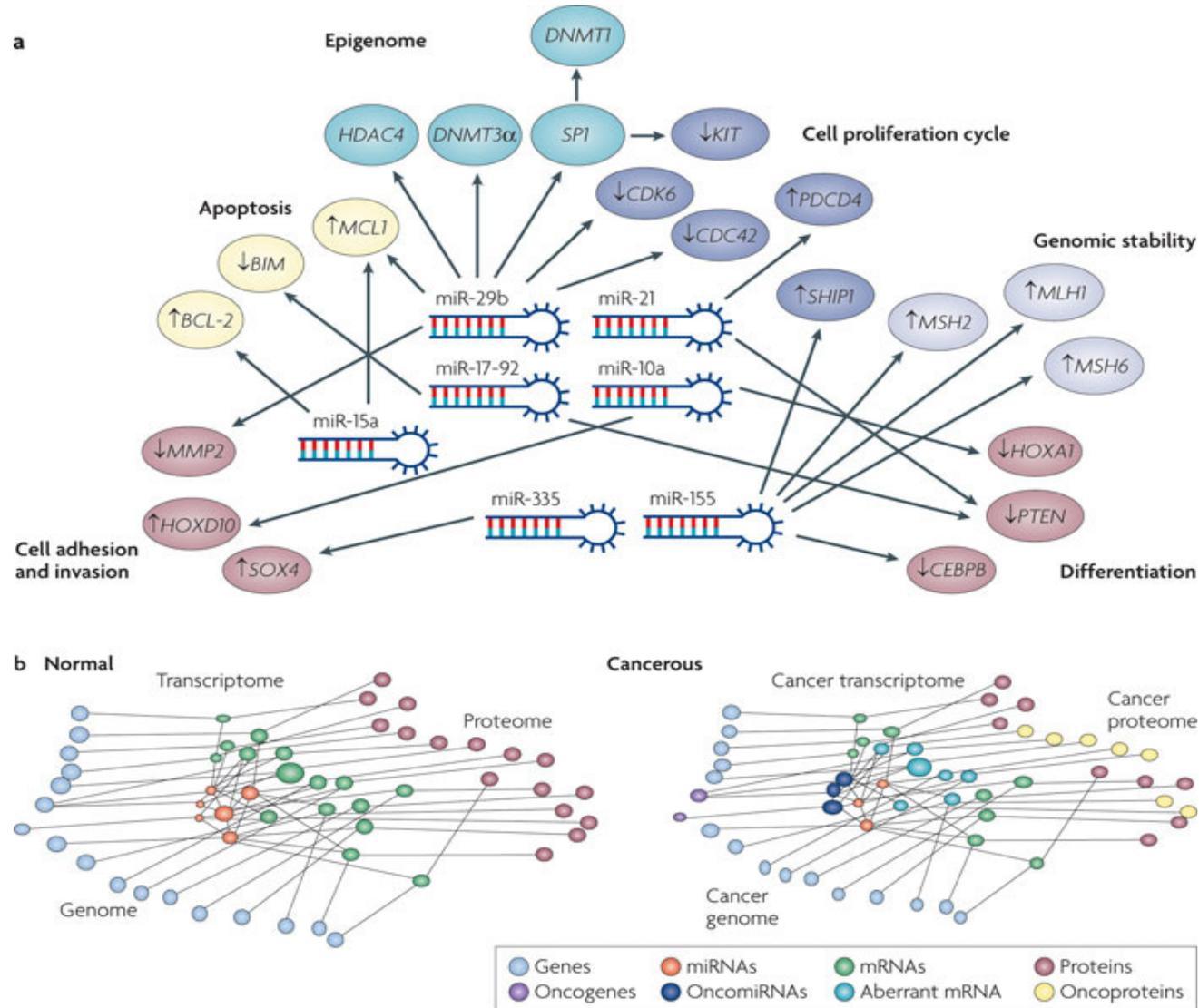


Ruolo dei miRNAs nell'adattamento immunologico della madre verso il feto

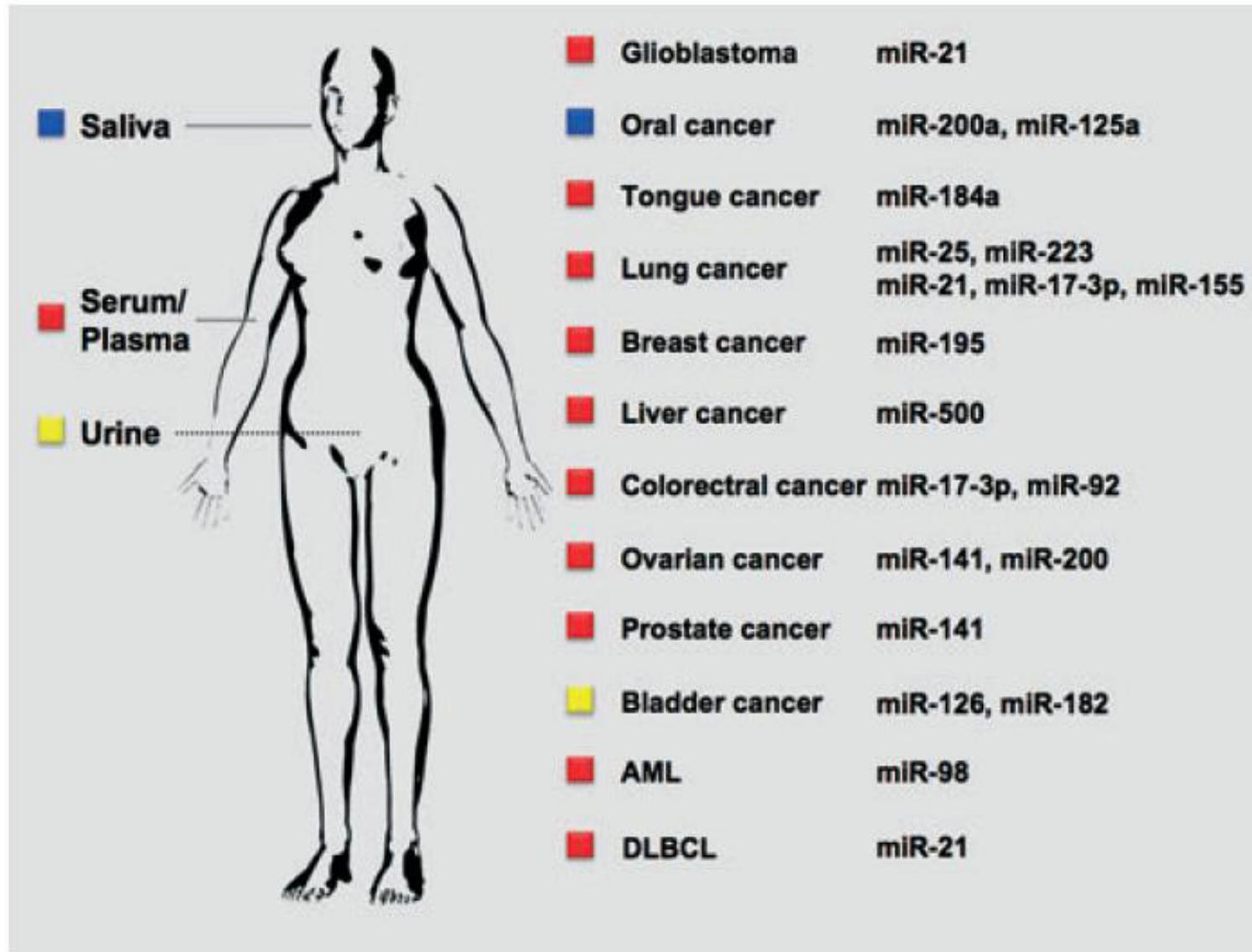
I miRNAs costituiscono una complessa rete biologica



Network trascrittoma-microRNA nel cancro

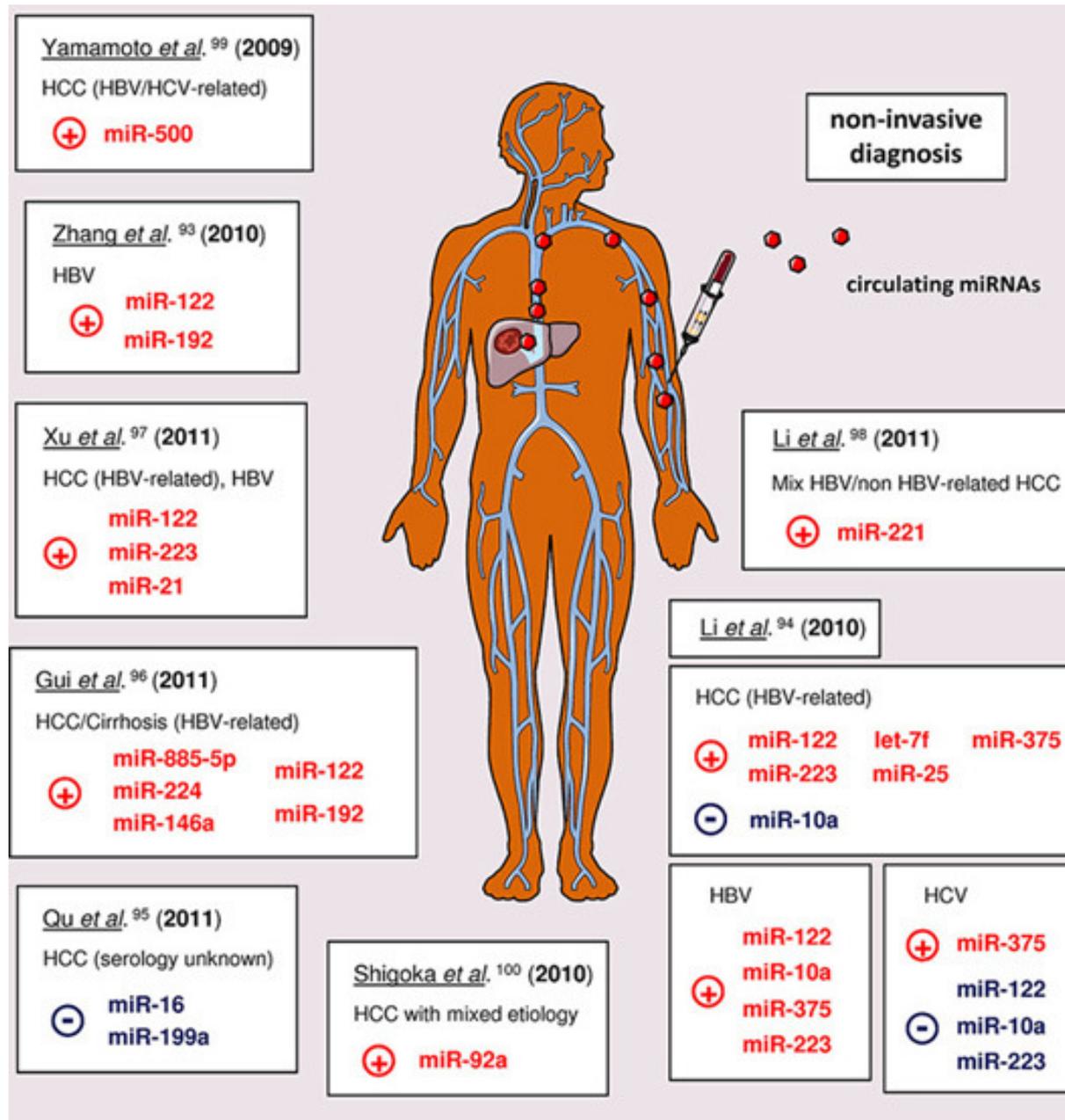


Circulating microRNAs associated with cancer



Good candidates for non-invasive diagnostic markers for cancer

Circulating miRNAs as biomarkers for hepatocellular carcinoma (HCC) diagnosis



I miRNAs come biomarker precoci del tumore al polmone

Table 1. Top 10 miRNAs deregulated between tumor and normal lung tissue (class comparison analysis)

miRNAs deregulated ($P < 0.001$)	Tumor vs. normal tissues	
	Direction	Fold change
mir-7-2-prec	Up	1.3
mir-126	Down	0.4
mir-200b	Up	1.3
mir-210	Up	3
mir-219-1	Up	1.6
mir-21	Up	2.9
mir-324-5p	Up	1.3
mir-451	Down	0.5
mir-486-5p	Down	0.5
mir-30a	Down	0.6

La mappatura di espressione dei miRNAs nel tumore al polmone permette:

- Distinzione di un tessuto normale da quello neoplastico
- Conoscere la velocità di crescita tumorale
- Predire l'insorgenza della patologia neoplastica

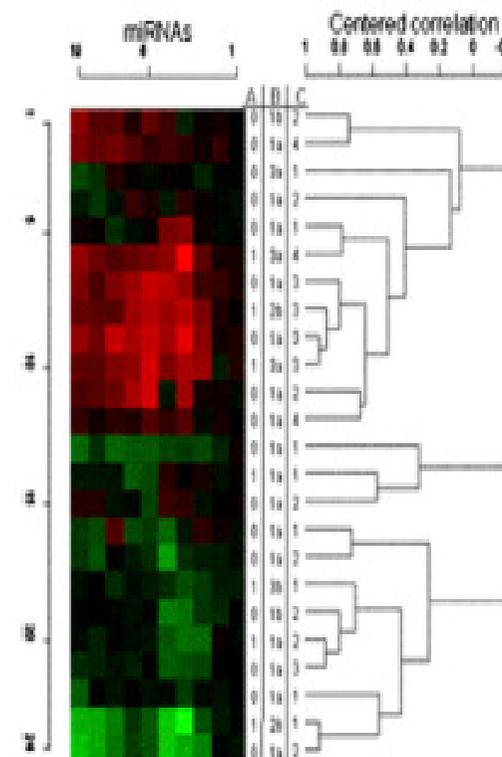
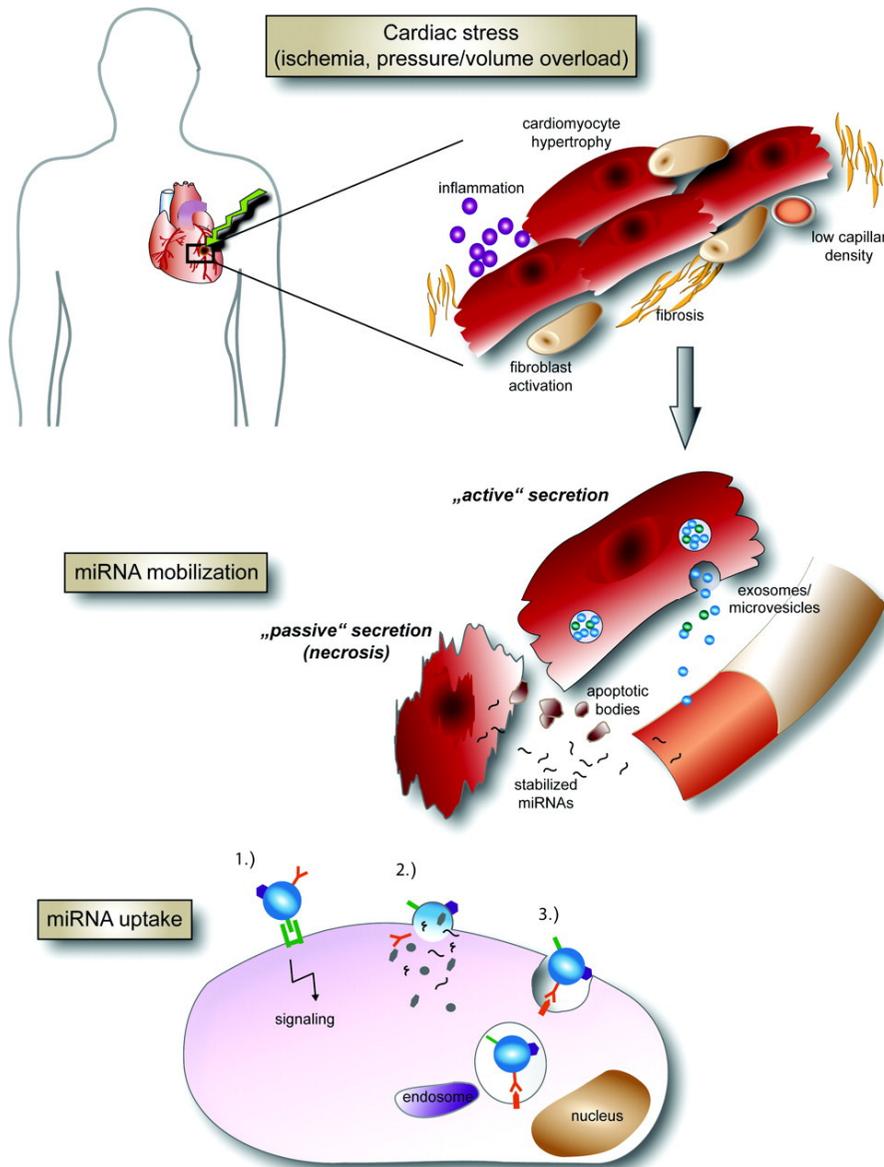


Fig. 2. Clustering analysis on 24 normal lung tissue samples using miRNAs differentially expressed between patients with tumors detected in the first 2 y and those of later years of screening. Clinical status of the patient (0 = alive, 1 = dead), tumor stage, and year of tumor detection are reported in columns A, B, and C, respectively.

I miRNAs come biomarker precoci delle patologie cardiovascolari

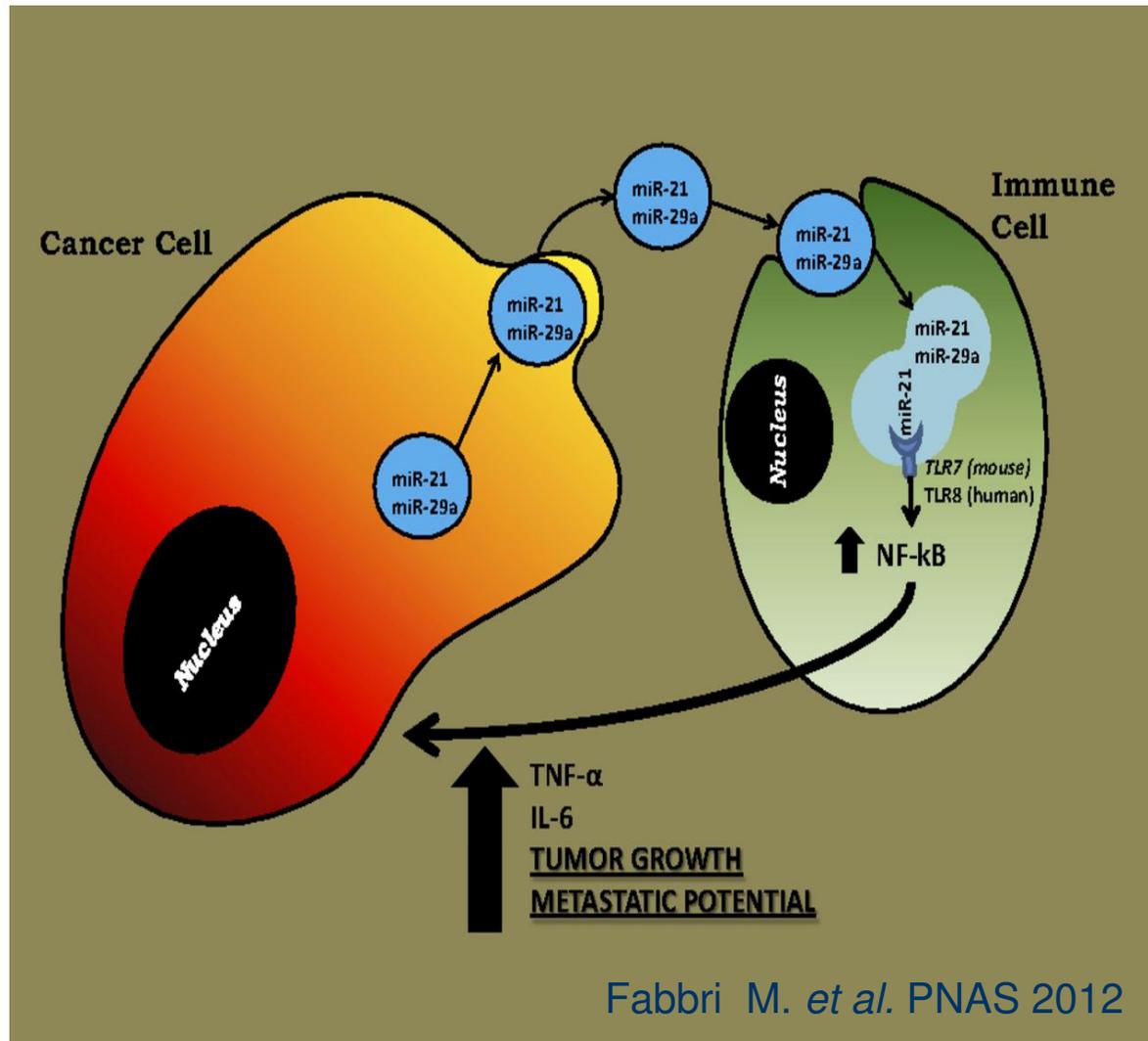


Lo stress cardiaco può favorire la mobilizzazione dei miRNAs attraverso meccanismi di secrezione attiva o passiva.

L'uptake dei miRNAs circolanti da parte della cellula target può essere mediata da recettore (1), oppure gli esosomi possono fondersi con la membrana della cellula target (2,3).

I miRNAs extracellulari come ligandi dei TLR

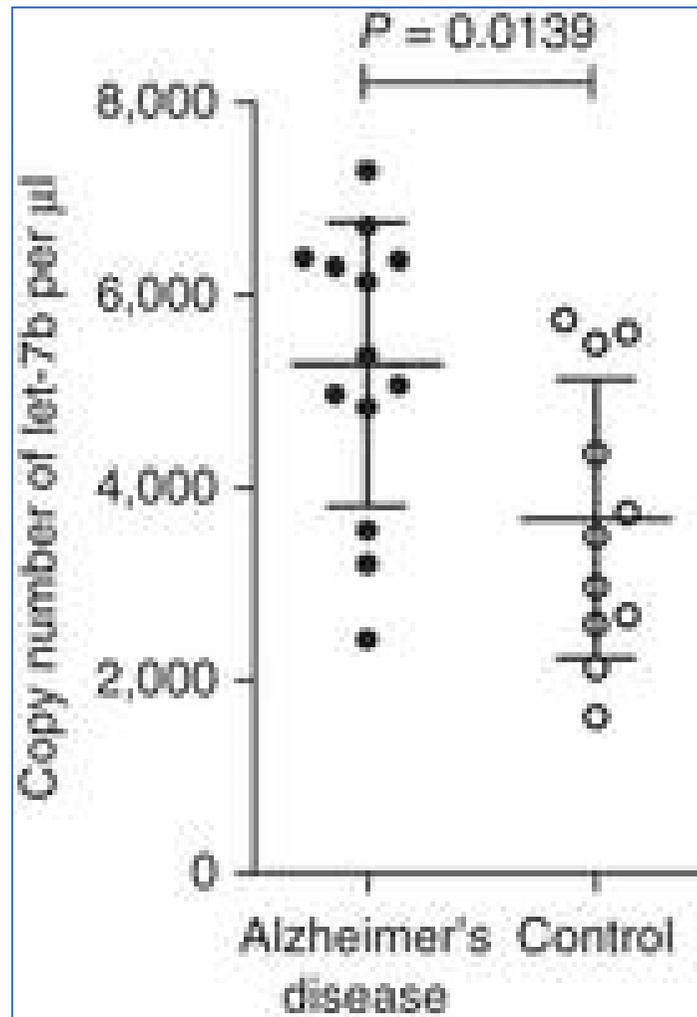
E' stato dimostrato che alcuni miRNAs, come miR-21 e miR-29a, legandosi rispettivamente ai TLR7 (murino) e TLR8 (umano), inducono un processo infiammatorio che favorisce la progressione tumorale.



miR-21 e miR-29a funzionano come molecole di segnale attraverso i TLR7 e TLR8

I miRNAs extracellulari come ligandi dei TLR

Nel liquido cerebrospinale dei malati di Alzheimer's è stata dimostrata una maggiore espressione del miRNA let-7b, che legandosi al TLR7 induce un processo neurodegenerativo



L'introduzione intratecale di let-7b in topi wild-type induce neurodegenerazione

I topi TLR7 KO risultano resistenti alla neurodegenerazione indotta da let-7b

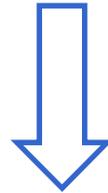


Il miRNA let-7b funziona come molecola di segnale attraverso il TLR7

miRNA e CANCRO

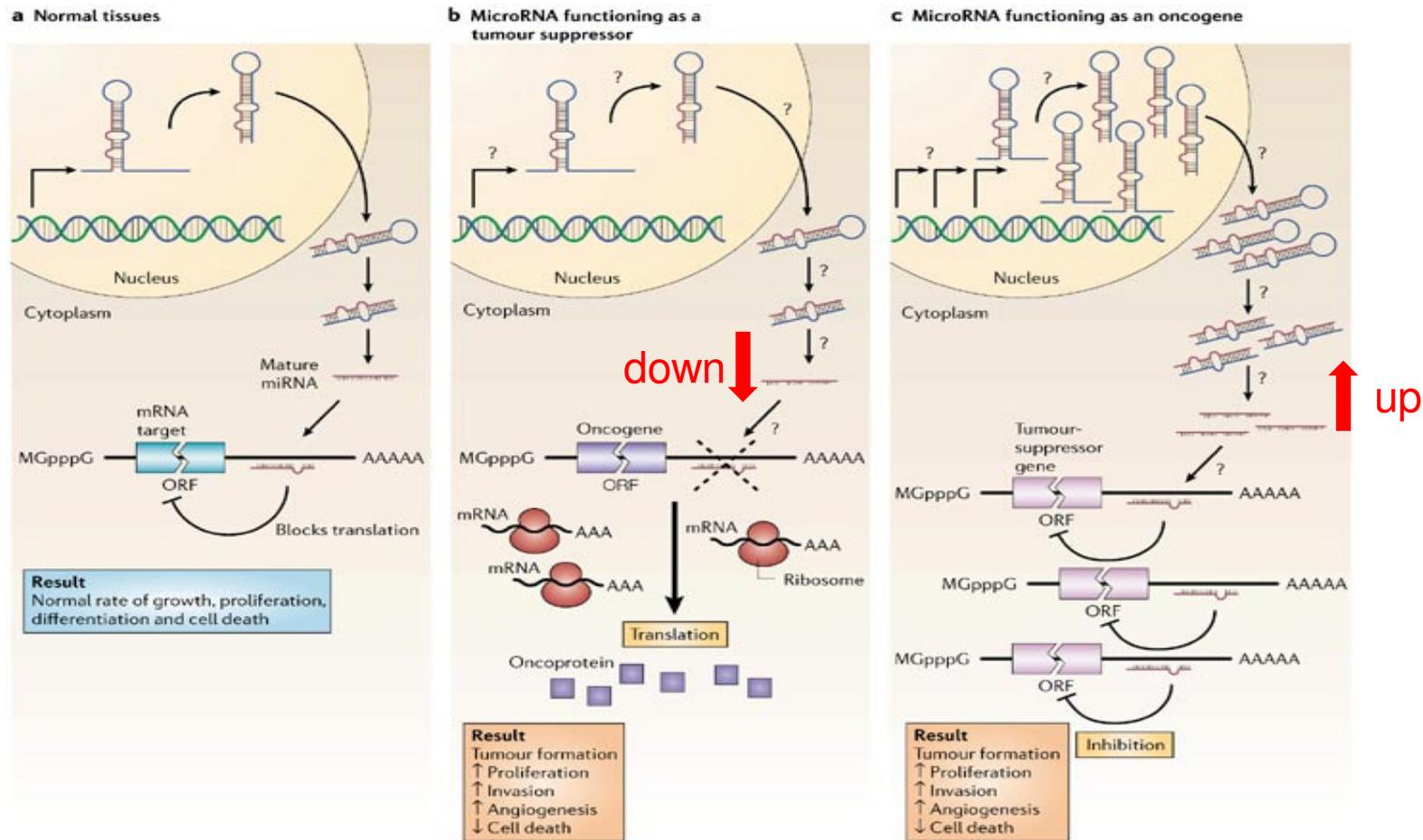
Circa il 50% dei miRNAs sono localizzati in regioni genomiche associate a tumori.

Espressione aberrante dei miRNAs in diversi tipi di tumore



Oncogeni

Tumour suppressor genes



a | In normal tissues, proper microRNA (miRNA) transcription, processing and binding to complementary sequences on the target mRNA results in the repression of target-gene expression through a block in protein translation or altered mRNA stability (not shown). The overall result is normal rates of cellular growth, proliferation, differentiation and cell death. b | The reduction or deletion of a miRNA that functions as a tumour suppressor leads to tumour formation. A reduction in or elimination of mature miRNA levels can occur because of defects at any stage of miRNA biogenesis (indicated by question marks) and ultimately leads to the inappropriate expression of the miRNA-target oncoprotein (purple squares). The overall outcome might involve increased proliferation, invasiveness or angiogenesis, decreased levels of apoptosis, or undifferentiated or de-differentiated tissue, ultimately leading to tumour formation. c | The amplification or overexpression of a miRNA that has an oncogenic role would also result in tumour formation. In this situation, increased amounts of a miRNA, which might be produced at inappropriate times or in the wrong tissues, would eliminate the expression of a miRNA-target tumour-suppressor gene (pink) and lead to cancer progression. Increased levels of mature miRNA might occur because of amplification of the miRNA gene, a constitutively active promoter, increased efficiency in miRNA processing or increased stability of the miRNA (indicated by question marks). ORF, open reading frame.

Emerging miRNAs in cancer biology

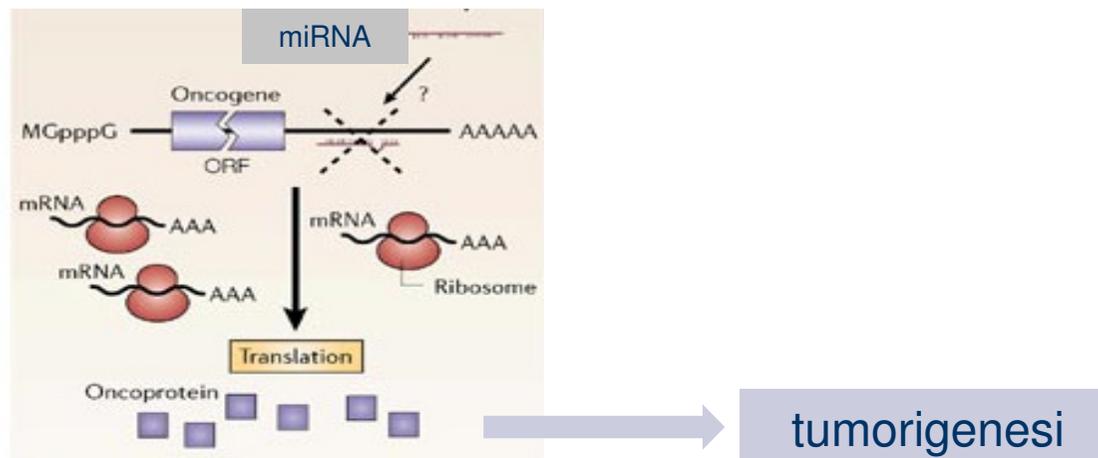
Let-7

Funziona come tumour suppressor

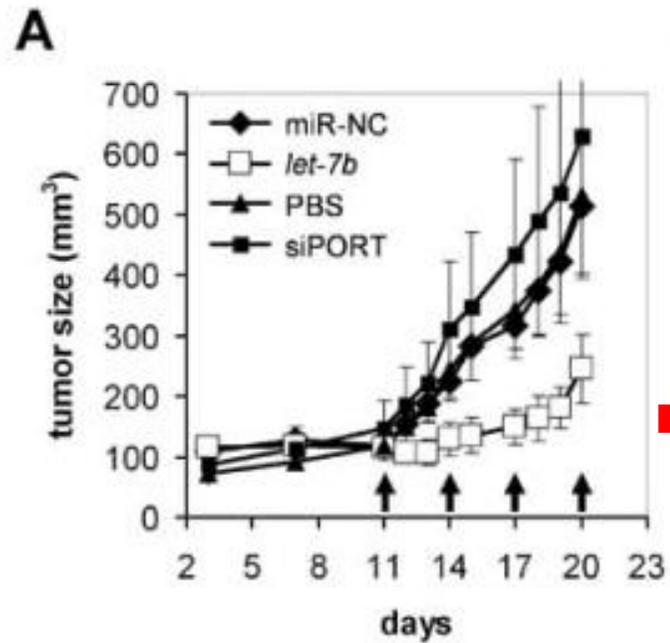
Localizzato su differenti regioni cromosomiche (9q22.3, 21p11.1, and 3p21.1-p21.2)

Principali geni target: HMGA2 (high mobility group AT-hook 2) and RAS (rat sarcoma).

La sua espressione diminuisce in molti tipi di tumore

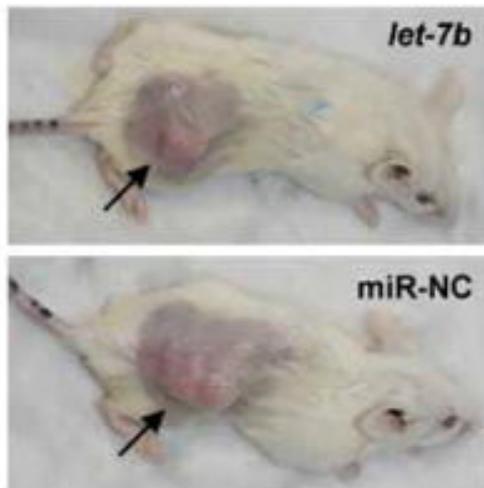


Delivery in vivo di let-7b in un modello sperimentale di tumore al polmone



Il trattamento con let-7b inibisce la crescita tumorale

B



miR-34

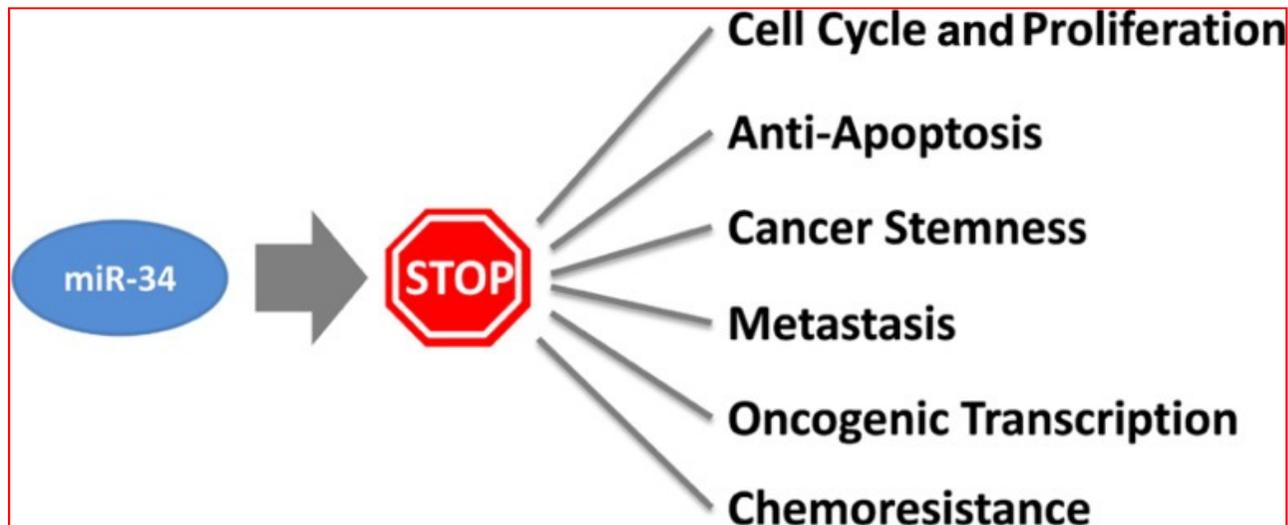
Famiglia di più membri: miR-34a, b, c. Condividono un'omologia di sequenza dell'82-86%.

I membri della famiglia miR-34 risultano identici nella “seed region,” una sequenza di 8 nucleotidi all'estremità 5', che è responsabile della selezione degli mRNA targets.

Localizzato sui cromosomi 1 (34a) e 11 (34b e c)

La sua espressione diminuisce in molti tipi di tumore: al polmone, all'ovaio, al colon-retto, alla prostata e glioma.

Cancer processes counteracted by miR-34



TUMOUR SUPPRESSOR

Attività terapeutica di miR-34 in differenti modelli sperimentali di cancro

Table 3 | Therapeutic activity of miR-34a in animal models of cancer.

Cancer type	Mouse tumor model	Effects (TGI)*	Reference
Non-small cell lung cancer	s.c. H460 xenograft	~78% TGI	(Wiggins et al., 2010)
	s.c. A549 xenograft	~62% TGI	(Wiggins et al., 2010)
	KRAS G12D GEMM	~60% TGI	(Trang et al., 2011)
Prostate cancer	o.t. PC3 xenograft	~50% TGI	(Liu et al., 2011)
	o.t. LAPC9 xenograft	~20% TGI	(Liu et al., 2011)
		~83% TGI on lung metastasis	(Liu et al., 2011)
Melanoma	metastatic B16 xenograft	~50% TGI	(Chen et al., 2010)
Pancreatic cancer	s.c. MiaPaca-2 xenograft	~62% TGI	(Pramanik et al., 2011)
	o.t. MiaPaca-2 xenograft	~68% TGI	(Pramanik et al., 2011)
Lymphoma	s.c. U2932 xenograft	~76% TGI	(Craig et al., 2012)

*Effects are expressed as percentage TGI in miR-34a-treated animals compared to controls. 100% TGI indicates a complete block of tumor development. TGI was determined by either caliper measurements, tumor area, tumor weight, ultra sound, or luminescence of luc-expressing tumor cells. s.c., subcutaneous; o.t., orthotopic; TGI, tumor growth inhibition.

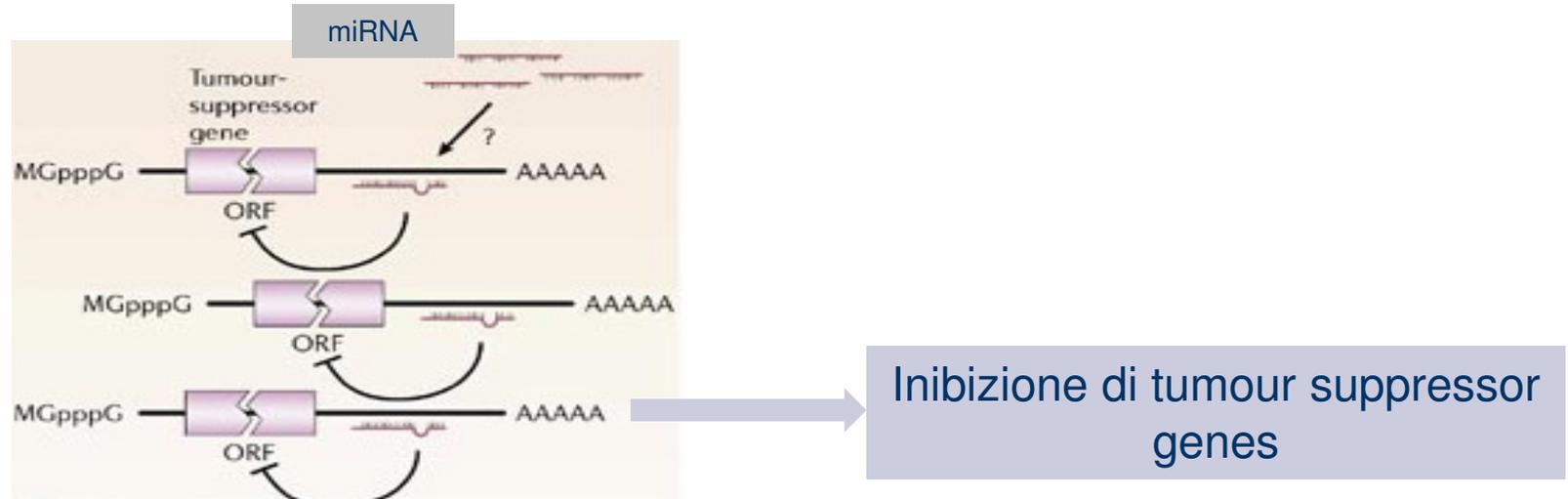
TGI = Tumour Growth Inhibition

TGI = 100%
blocco completo della crescita tumorale

TGI = 0
Nessuna inibizione

miR-21

Funziona come oncogene



Localizzato sul cromosoma 17

Principali geni target: TPM1 (tropomiosina) and PDCD4 (programmed cell death protein 4), and PTEN (phosphatase and tensin homolog)

Regola i principali meccanismi di tumorigenesi: proliferazione cellulare, migrazione e invasione cellulare.

Over-espresso in molti tipi di tumore: al polmone, al seno, al colon-retto.

miRNA e risposta ai chemioterapici

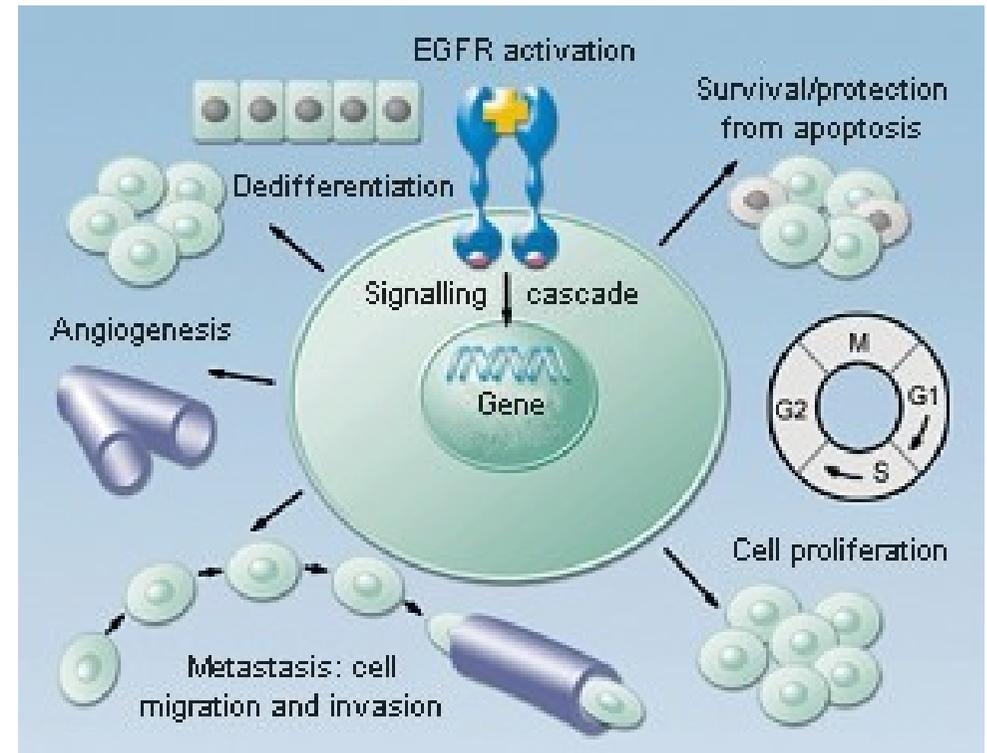
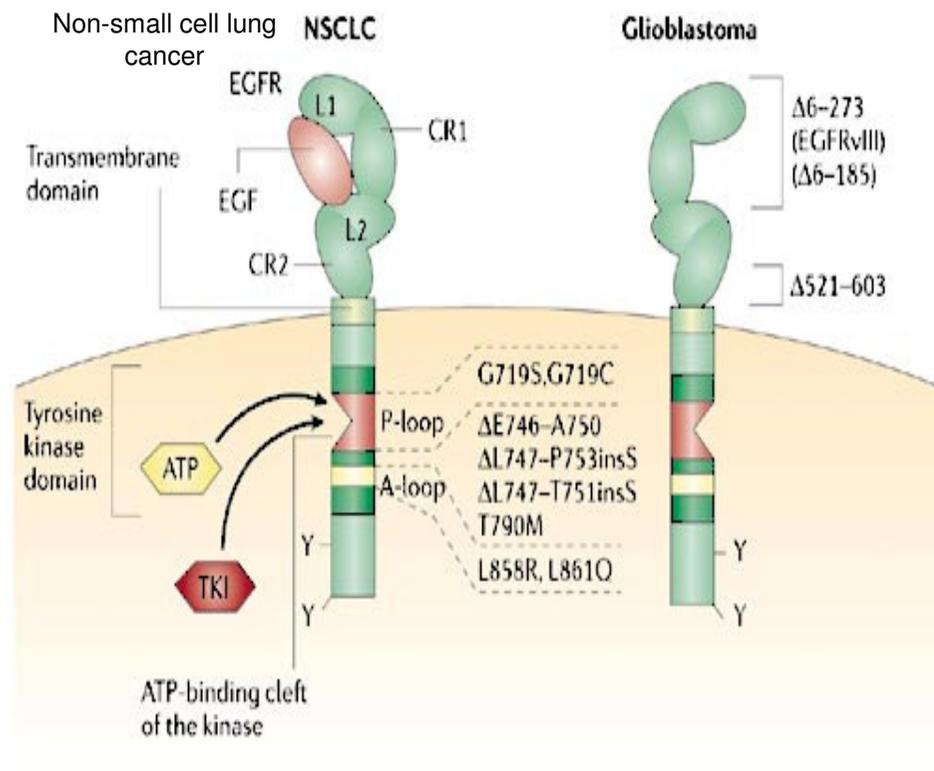
L'overespressione o il silenziamento di alcuni miRNA aumentano la sensibilità del tumore al chemioterapico

OSSERVAZIONI SPERIMENTALI:

- Il silenziamento di miR-21 rende il cancro al polmone più sensibile ai tradizionali chemioterapici.
- Elevati livelli ematici di miR-21 correlano con la chemioresistenza sia nel tumore pancreatico che al polmone.
- Il silenziamento di miR-21 aumenta la sensibilità delle cellule tumorali agli inibitori tirosino-chinasici.

RUOLO DI EGFR NELLA TUMORIGENESI

Mutations in the *EGFR* Gene



miR-21 tended to be overexpressed in nonsmoking lung cancer patients with EGFR mutations and that *in vitro* antisense targeting of miR-21 sensitized cancer cells to an EGFR-tyrosine kinase inhibitors (*Seike et al., PNAS 2009*)

Silenziamento di miR-21 (funzionante come oncogene) per aumentare la sensibilità al farmaco antitumorale

miRNA e risposta ai chemioterapici

Table 2 | Effects of miR-34a in combination with conventional therapies.

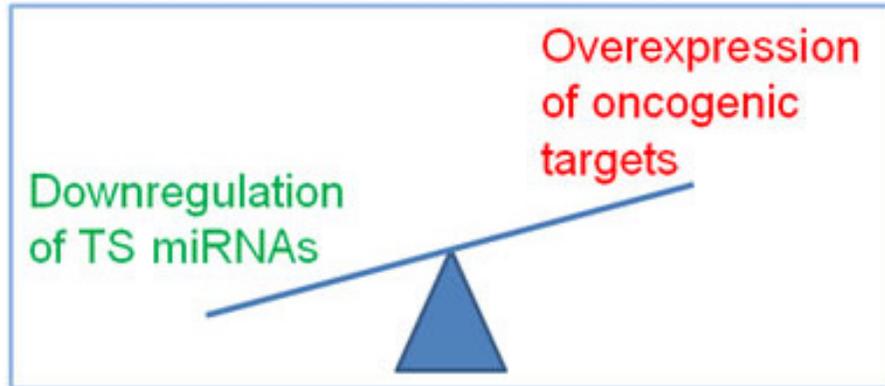
Cancer therapy	Cancer type	Effects of the miR-34/chemo combination	Reference
Camptothecin	Prostate	Reduced cell viability	(Fujita et al., 2008)
Paclitaxel	Prostate	Attenuates chemoresistance	(Kojima et al., 2010)
5-Fluorouracil	Colon	Attenuates chemoresistance	(Akao et al., 2010)
Mitomycin C	Brain	~2-fold lower IC50 than chemo alone	(Weeraratne et al., 2010)
Cisplatin	Brain	~2-fold lower IC50 than chemo alone	(Weeraratne et al., 2010)
	Bladder	Reduced clonogenic potential; increased senescence	(Vinall et al., 2011)
	Pancreas	2.7-fold lower IC50 than chemo alone	(Ji et al., 2009)
	Stomach*	1.8-fold lower IC50 than chemo alone	(Ji et al., 2008)
Docetaxel	Pancreas	3.2-fold lower IC50 than chemo alone	(Ji et al., 2009)
	Stomach*	2.4-fold lower IC50 than chemo alone	(Ji et al., 2008)
Gemcitabine	Pancreas	2.6-fold lower IC50 than chemo alone	(Ji et al., 2009)
	Stomach*	2.6-fold lower IC50 than chemo alone	(Ji et al., 2008)
Doxorubicin	Stomach*	2.0-fold lower IC50 than chemo alone	(Ji et al., 2008)

*Values were generated in cancer cells with high BCL2 levels.

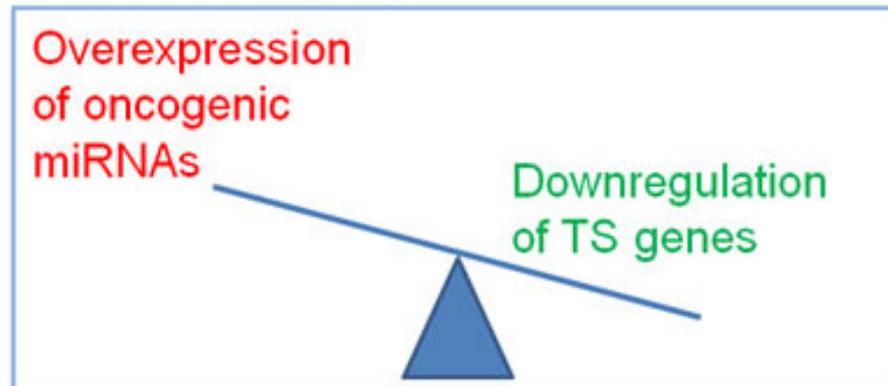
Overespressione di miR-34 (funzionante come tumour suppressor) per aumentare la sensibilità al farmaco antitumorale

DUAL ROLE OF miRNA IN CANCER

miRNA as Tumor Suppressor



miRNA as Oncogene



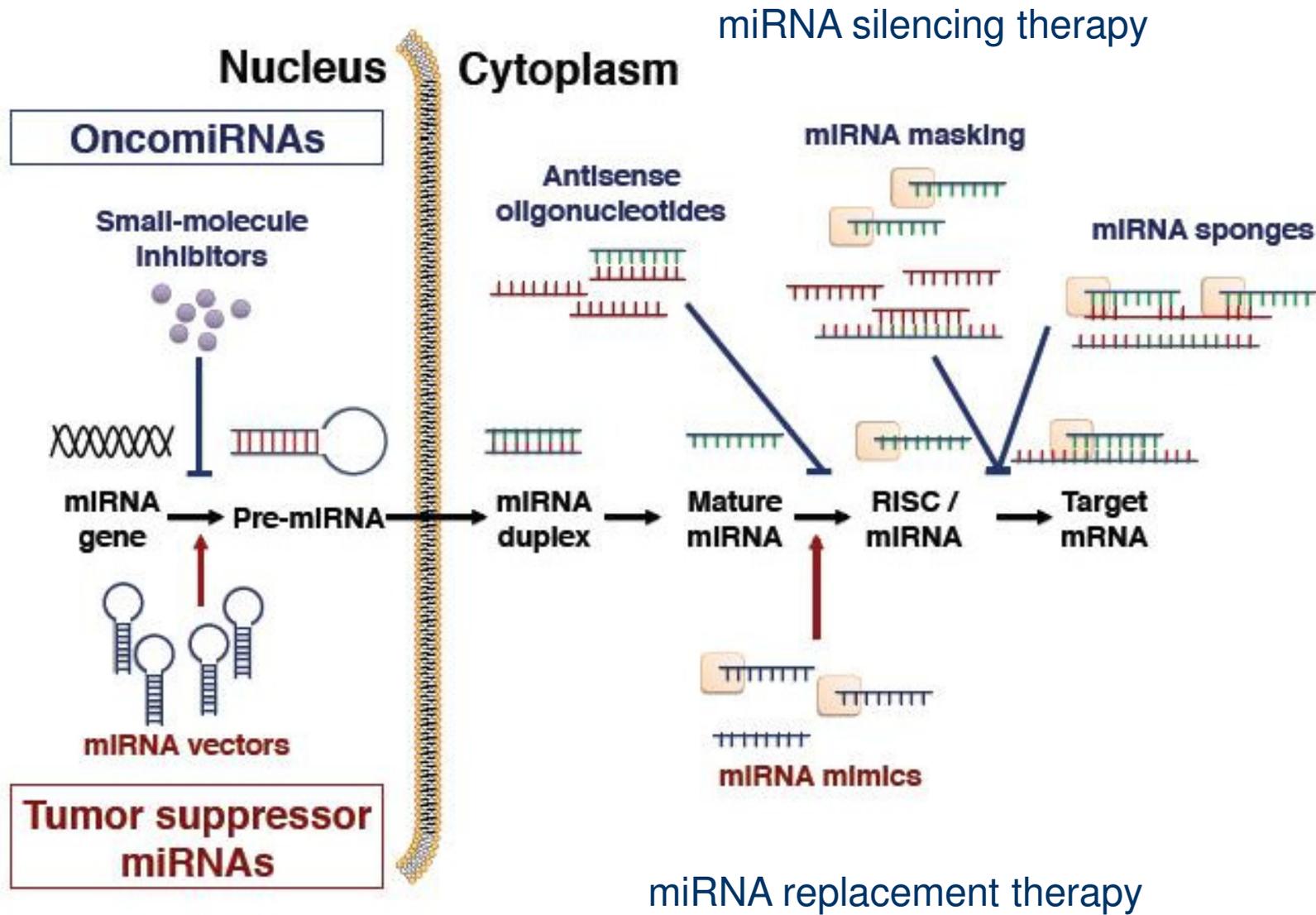
- ↑ Angiogenesis
- ↑ Proliferation
- ↑ Invasion
- ↓ Apoptosis

miRNA
"replacement"
therapy

Cancer initiation
& progression

miRNA
"knockdown"
therapy

miRNA-based therapeutical strategies in cancer



miRNAs perspectives

miRNAs as tumor markers

- Differentiate cancer from normal tissue
- Stratify into tumor subgroups
- Prognostication
- Monitor response to therapy
- Assess tumor load/minimal residual disease

miRNAs as
cancer
biomarkers

miRNAs as
therapeutic
targets

miRNAs as therapeutic agents or targets

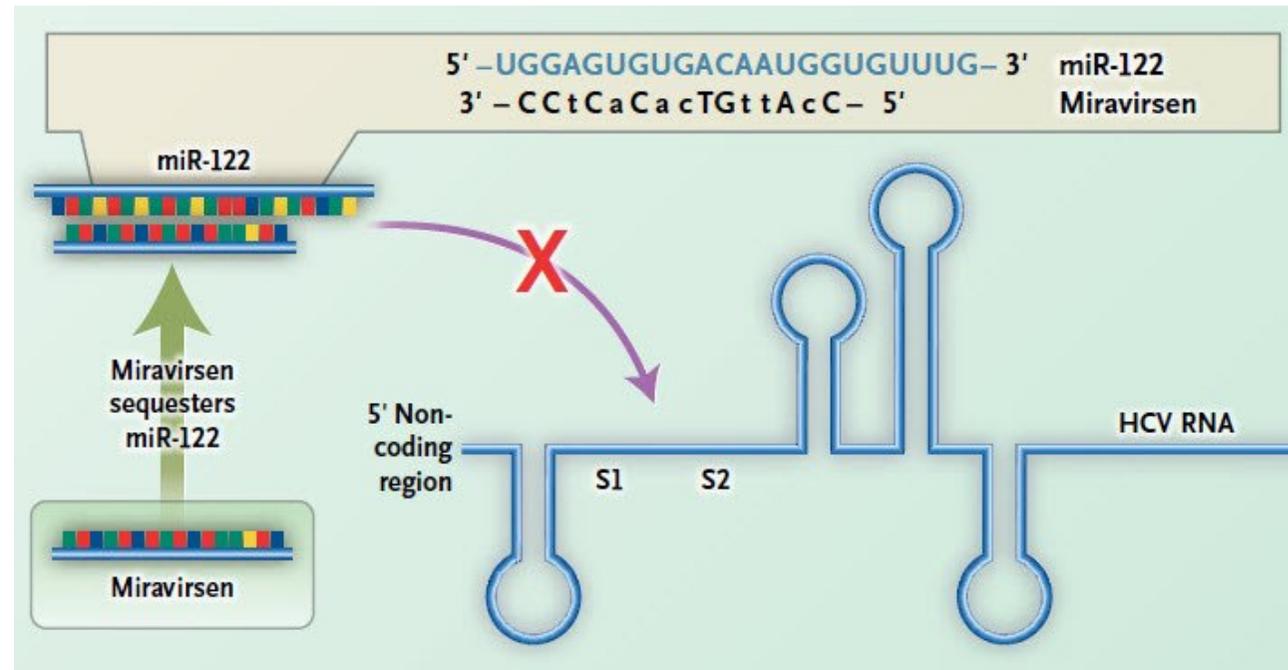
- miRNA knockdown strategy (*miR-21*)
- miRNA replacement therapy (*Let-7*)
- Alter responsiveness of tumors to standard therapy (*miR-34a*)

Synthetic RNAi-based Drugs in clinical trials

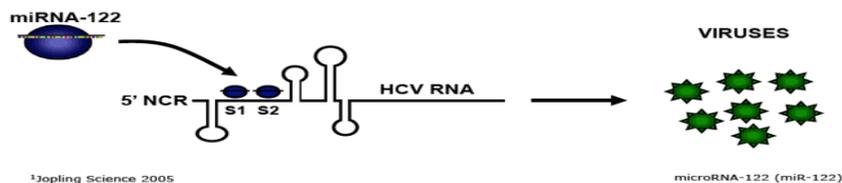
Drug	Target (cell role)	Chemistry / formulation	Route	Disease	Phase	Status/completion	Company/ collaborator	Refs
PF-04523655 (PF-655)	RTP801 / (hypoxia-inducible)	Naked siRNA, O-methylated	IVT	AMD, DME	II	Completed/2013	Quark / Pfizer	16, 18
QPI-1002* ¹ (ISNP)	p53 (apoptotic)	Naked siRNA, O-methylated	IV	AKI DGF	II III	Recruiting / 2018* ² Recruiting / 2019* ²	Quark	40
QPI-1007	CASP2 (apoptotic)	Naked siRNA, O-methylated; changes in sense strand	IVT	NAION Glaucoma	II / III II	Recruiting / 2019* ² Completed / 2015	Quark	52
TKM-080301 (TKM-PLK1)	PLK1 (kinase)	siRNA / SNALP	IV	Solid tumors, HCC, NET, ACC, lymphoma	I / II	Completed / 2015	Arbutus	59–62
Atu027	PKN3 (kinase)	siRNA / LIPOPLEX	IV	Pancreatic cancer	I / II	Completed / 2016	Silence / Granzer, FGK	67, 70
SYL040012 (Bamosiran)	ADRB2 (β 2 receptor)	Naked siRNA	Eye drops	Ocular hypertension, glaucoma	II	Completed / 2013; 2016	Sylentis	82–85
SYL1001	TRPV1 (nociceptor)	Naked siRNA	Eye drops	Ocular pain in Dry Eye Syndrome	II	Completed / 2016	Sylentis	92, 93
Patisiran (ALN-TTR02)	TTR (amyloidogenic)	siRNA / Lipid particle, ApoE	IV	TTR-mediated Amyloidosis	III	Active / 2017* ²	Alynham	99, 101, 103, 104
siG12D-LODER	KRAS (oncogene, GTPase)	siRNA / Miniature PLGA device	Intra-tumoral	Pancreatic cancer* ³	II	Active, not yet recruiting / 2020* ²	Silenseed	112
Miravirsen	miR-122 (microRNA)	AntimiR, antisense oligodeoxynucleotide, LNA, PS	SC	Hepatitis C infection	II	Complete / 2011	Santaris	119, 126–128

ACC adrenocortical carcinoma; ADRB2 adrenoceptor beta 2; AKI acute kidney injury after cardiac surgery; AMD Age-Related Macular Degeneration; ApoE apolipoprotein E; CASP2 Caspase 2; DGF Delayed Graft Function in kidney transplantation; DME Diabetic Macular Edema; HCC hepatocellular carcinoma; IV intravenous injection; IVT intravitreal injection; KRAS Kirsten rat sarcoma viral oncogene homolog; LNA locked nucleic acid; miR-122 microRNA 122; NAION Acute Non-Arteritic Anterior Ischemic Optic Neuropathy; NET neuroendocrine tumors; PKN3 protein kinase N3; PLK1 Polo-like kinase-1; PS phosphorothioated; SC subcutaneous injection; SNALP stable-nucleic-acid-lipid particles; TRPV1 transient receptor potential vanilloid 1; TTR transthyretin. *1- drug licensed to Novartis; *2- estimated completion data; *3- siG12D-LODERs combined with chemotherapy treatment (Gemcitabine + nab-Paclitaxel). Company names: Quark Pharmaceuticals; Pfizer; Arbutus Biopharma Corporation; Silence Therapeutics GmbH; Granzer Regulatory Consulting and Services; FGK Clinical Research GmbH; Sylentis, S.A.; Alynham Pharmaceuticals; Silenseed Ltd.; Santaris Pharma A/S

The first miRNA-targeted drug, miravirsin



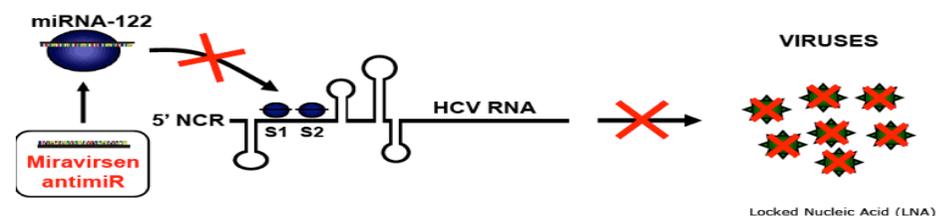
LNA= Locked nucleic acid
antimiR-122 ←



¹Jopling Science 2005

Miravirsin Mode of Action

- Miravirsin is a LNA modified phosphorothioate anti-sense oligonucleotide targeting and blocking miR-122
 - First drug to exploit a microRNA target for therapeutic use
 - As a host targeting agent miravirsin poses a high barrier to resistance
 - Miravirsin should work in all HCV genotypes because miR-122 binding sites are conserved



Rationale for Clinical Use

The first miRNA-targeted drug, miravirsen

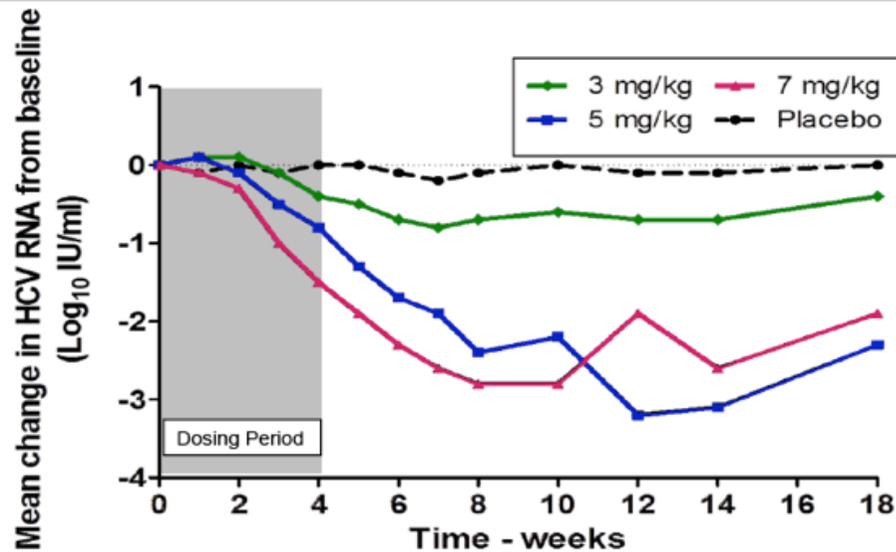


Phase IIa clinical trial

RNA Medicines for the 21st Century

HCV RNA Decline from Baseline

SPC3649-203 – All Treatment Groups (through 18 weeks)



Dose Group	Mean Maximum HCV RNA decline IU/mL (SEM) [without SOC]	p value MIR vs placebo
placebo	-0.5 (0.3)	-
3 mg/Kg	-1.2 (0.6)	0.013
5 mg/Kg	-2.9 (1.7)	0.003
7 mg/Kg	-3.0 (1.6)	0.002

Miravirsen- the 1st miRNA targeted drug

Treatment of HCV Infection by Targeting MicroRNA

This article was published on March 27,
2013, at NEJM.org.

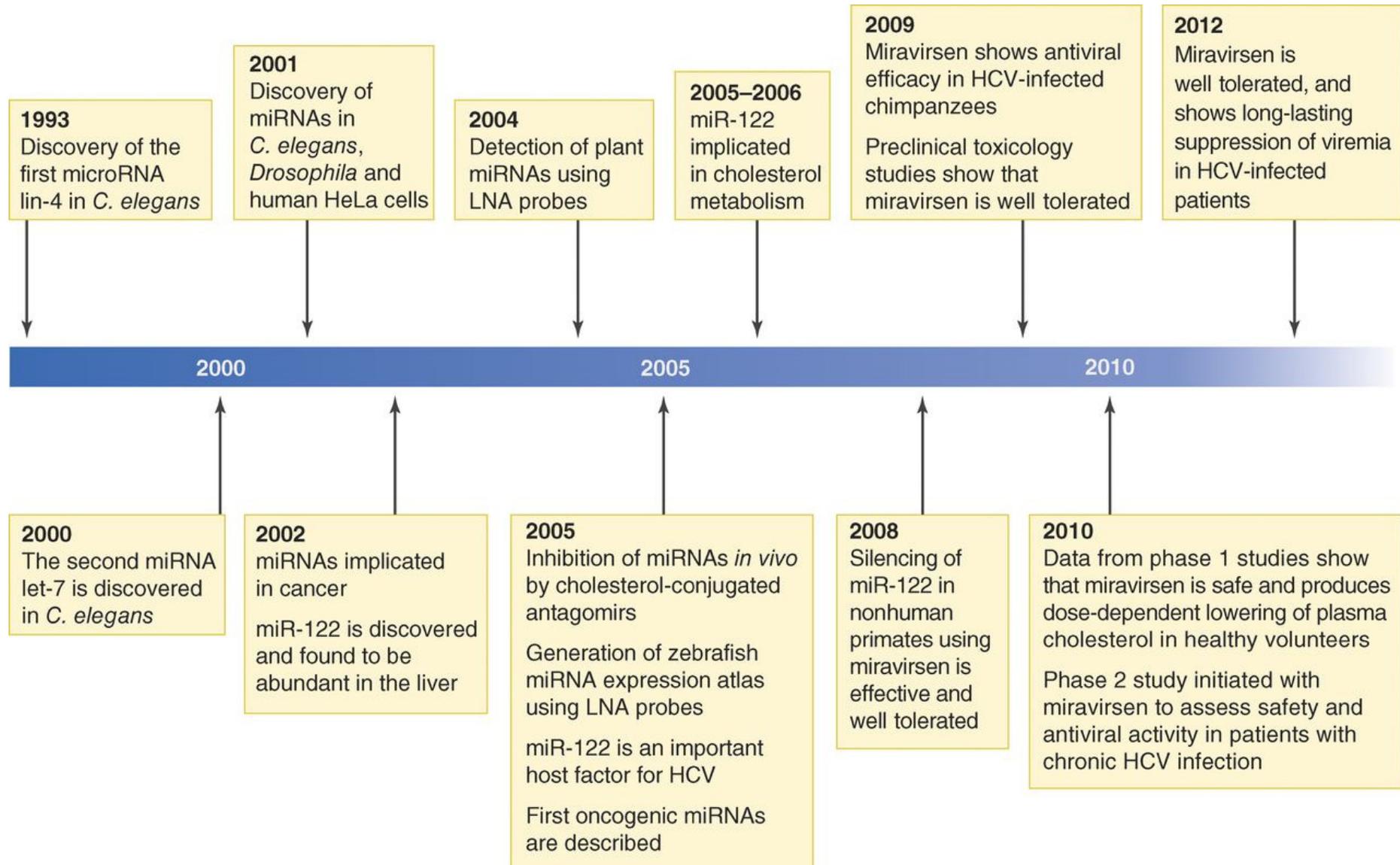
Harry L.A. Janssen, M.D., Ph.D., Hendrik W. Reesink, M.D., Ph.D., Eric J. Lawitz, M.D.,
Stefan Zeuzem, M.D., Maribel Rodriguez-Torres, M.D., Keyur Patel, M.D.,
Adriaan J. van der Meer, M.D., Amy K. Patick, Ph.D., Alice Chen, B.A.,
Yi Zhou, Ph.D., Robert Persson, Ph.D., Barney D. King, M.D.,
Sakari Kauppinen, Ph.D., Arthur A. Levin, Ph.D., and Michael R. Hodges, M.D.

ABSTRACT

- ✓ **First drug to exploit miRNA for therapeutic use**
- ✓ **As a host targeting agent miravirsen poses a high barrier to resistance**
- ✓ **Can work in all HCV genotypes because miR-122 binding sites are conserved**
- ✓ **Has successfully completed Phase II clinical trial**

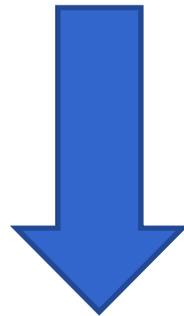
(Janssen *et al.*, 2013)

The first miRNA-targeted drug, miravirsen



Ci mangiamo un po' di RNA?

- miRNA esogeni di origine vegetale sono stati ritrovati nel siero di umani ed altri mammiferi
- MIR168a è un miRNA molto abbondante nel riso ed è stato ritrovato nel siero di volontari sani cinesi
- Nel topo MIR168 inibisce l'espressione di una proteina adattatrice del recettore per LDL, inibendo il processo di rimozione delle LDL dal plasma murino.

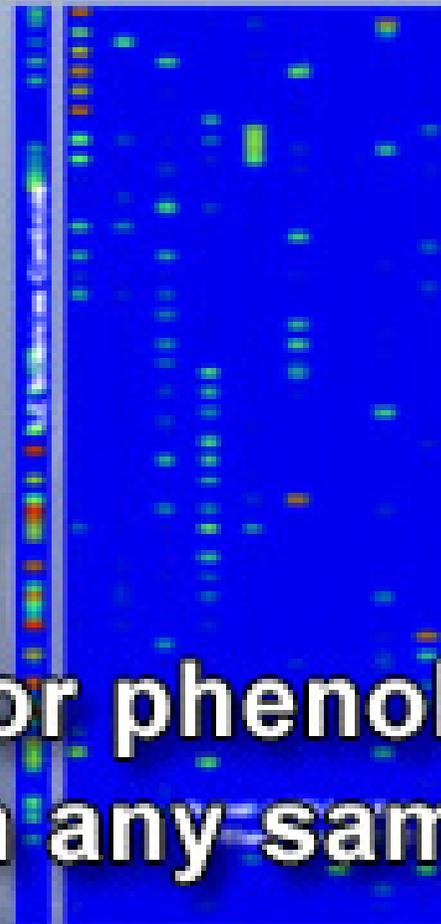
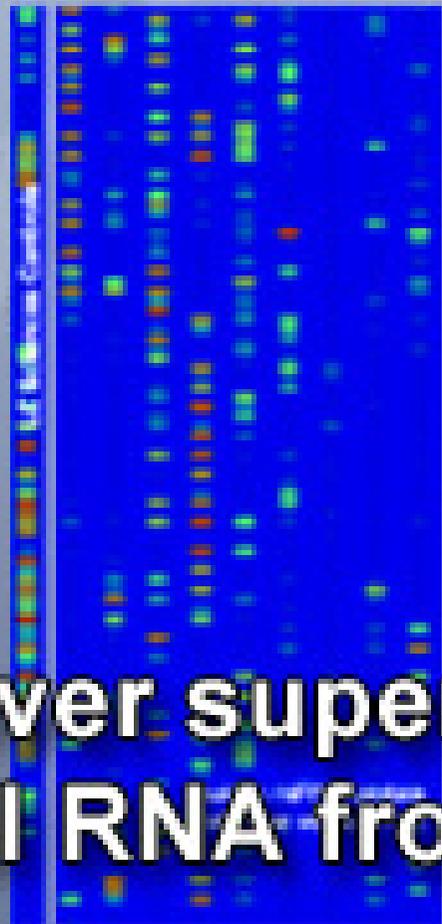


miRNA di origine vegetale, ingeriti con la dieta, potrebbero modulare l'espressione dei nostri geni (Zhang L. et al. 2012, Nature Cell Research)

Don't Miss Any RNA

NORGEN
BIOTEK  CORP

OTHERS



Recover superior phenol-free
total RNA from any sample