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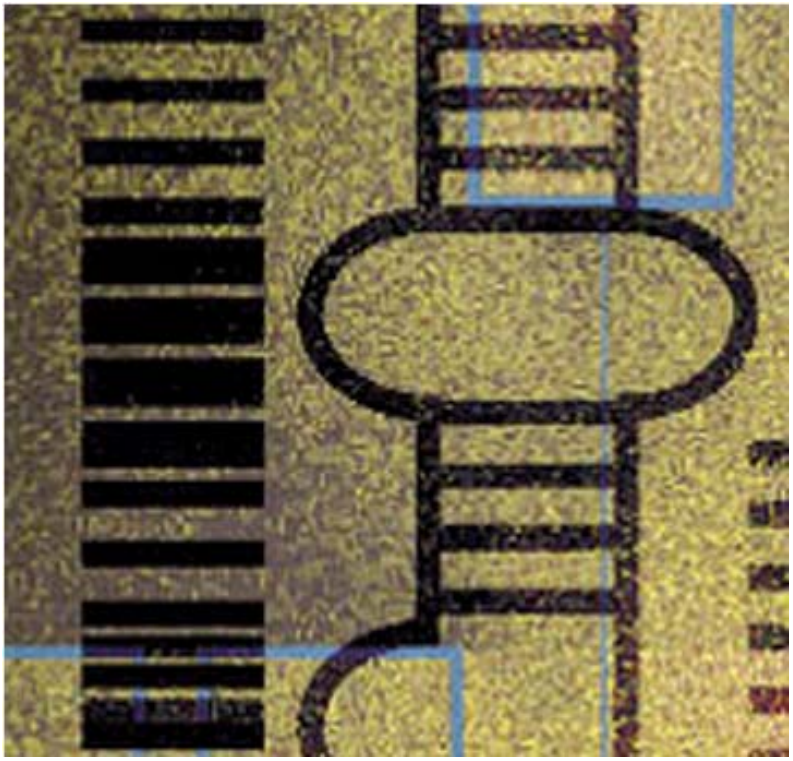
Los microRNAs como genes de susceptibilidad en cáncer

por
José Fernández Piqueras
Catedrático de Genética
Universidad Autónoma de Madrid
Centro de Biología Molecular Severo Ochoa CSIC-UAM



'All seeing, all controlling', by Claudia Bentley

The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

Photo: Stanford

Andrew Z. Fire

1/2 of the prize

USA

Stanford University
School of Medicine
Stanford, CA, USA

b. 1959

Photo: UMASS

Craig C. Mello

1/2 of the prize

USA

University of
Massachusetts Medical
School
Worcester, MA, USA;
Howard Hughes Medical
Institute

b. 1960

La interferencia mediada por RNA" (**RNAi**) una nueva vía de silenciamiento ejecutado por pequeñas moléculas de RNA no codificante de cadena doble (Fire et al., 1998).

HAY TRES TIPOS DE MOLÉCULAS RNAi

1

2

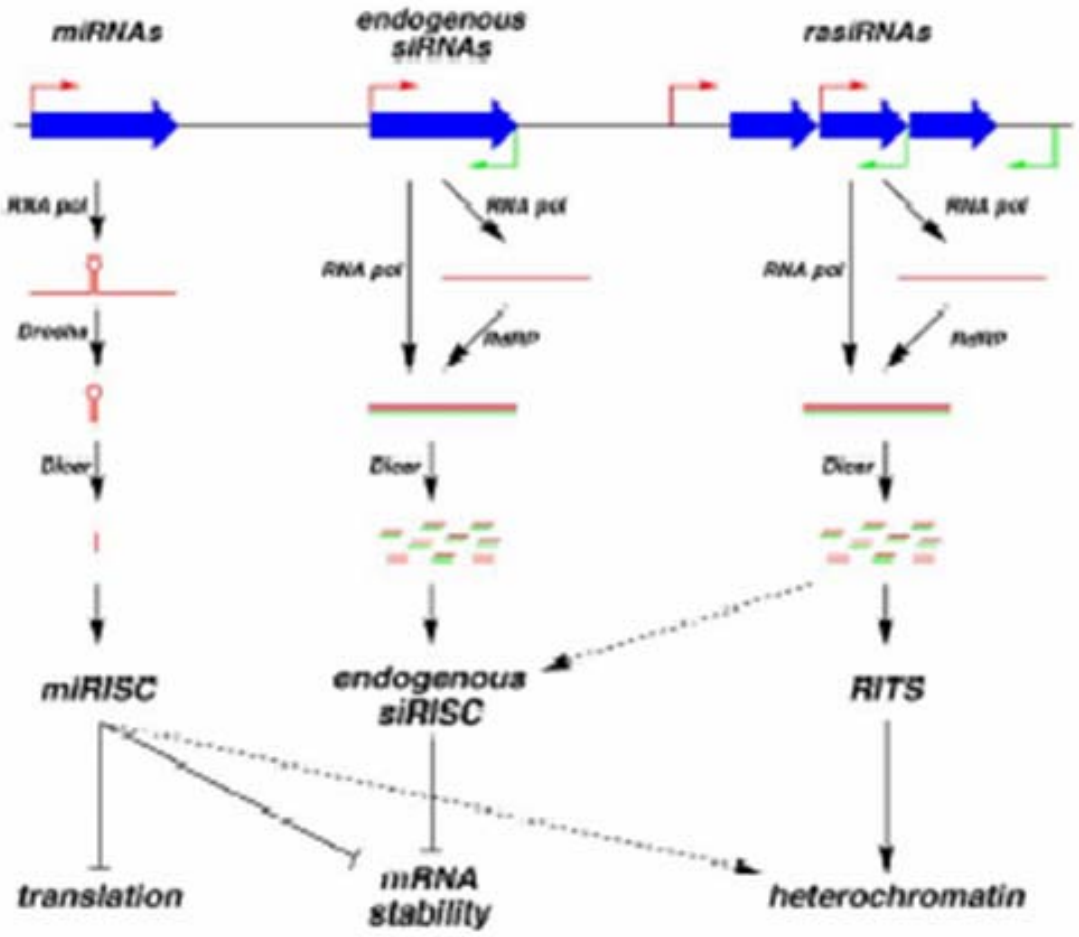
3

Table 1 | **Common ncRNAs and their functions**

ncRNA type	Description	Function
RNase P	~400 nt long	Cleaves tRNA precursors to result in mature 5′ ends; as a catalytic RNA (ribozyme) in bacteria, for example, cleaves tRNA precursors under high monovalent salt conditions in the absence of a protein
miRNA	Small, 21–23 nt long ssRNA	Targets mRNAs for cleavage (in plants) or translation inhibition (in mammals)
siRNA	21–23 nt long ssRNA	Targets mRNAs for cleavage
rasiRNA	Small, 21–23 nt long ssRNA	Involved in repeat silencing
snoRNA	~50–200 nt long, structured RNA that is localized to the nucleolus	Specifies modification of rRNAs, snRNAs or tRNAs (in Archaea only); C/D box snoRNAs specify 2′-O-methylation of the ribose of a target RNA, H/ACA box snoRNAs specify pseudouridylation
gRNA	Small, ~60 nt long ssRNA, containing a poly U tract at its 3′ end (from 5–20 U residues)	Guides U insertions or deletions within mitochondrial pre-mRNAs of certain protozoan organisms, for example, trypanosomes
snRNA	Structured; ~100–300 nt long (in humans)	Guides splicing of pre-mRNAs (for example, U1, U2, U4, U5 and U6 snRNAs)
rRNA	Highly structured; sized between ~120 (5S rRNA) and several thousand nucleotides (18S, 28S rRNAs)	As part of the ribosome it catalyses peptide bond formation (for large rRNA only)
Xist RNA	~17 kb long RNA, which is transcribed from the X chromosome	Involved in X-chromosome inactivation and dosage compensation
tRNA	Highly structured, sized between ~70 and 95 nt	RNA adapter molecules for amino acids; guides amino acids to the ribosome in an mRNA-dependent mode
SRP RNA	Has a rod-like structure, sized ~300 nt (in humans)	Part of the SRP, a ribonucleo-protein complex that is involved in targeting specific proteins to the endoplasmic reticulum for subsequent secretion
6S RNA	Highly structured RNA (~180 nt long in *Escherichia coli*), which forms a single hairpin that is found in bacteria	Binds to the σ^{70} factor of the RNA polymerase complex, thereby regulating transcription of σ^{70} promoters

gRNA, classical guide RNA; miRNA, microRNA; ncRNA, non-protein-coding RNA; rasiRNA, repeat-associated siRNA; rRNA, ribosomal RNA; siRNA, small interfering RNA; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; SRP, signal recognition particle; Xist, X-(inactive)-specific transcript.

MECANISMOS MOLECULARES DEL SILENCIAMIENTO



Cell, Vol. 122, 9–12, July 15, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.06.030

Silence from within:
Endogenous siRNAs and miRNAs

Erik J. Sontheimer* and Richard W. Carthew
Department of Biochemistry, Molecular Biology & Cell Biology
Northwestern University
2205 Tech Drive
Evanston, Illinois 60208

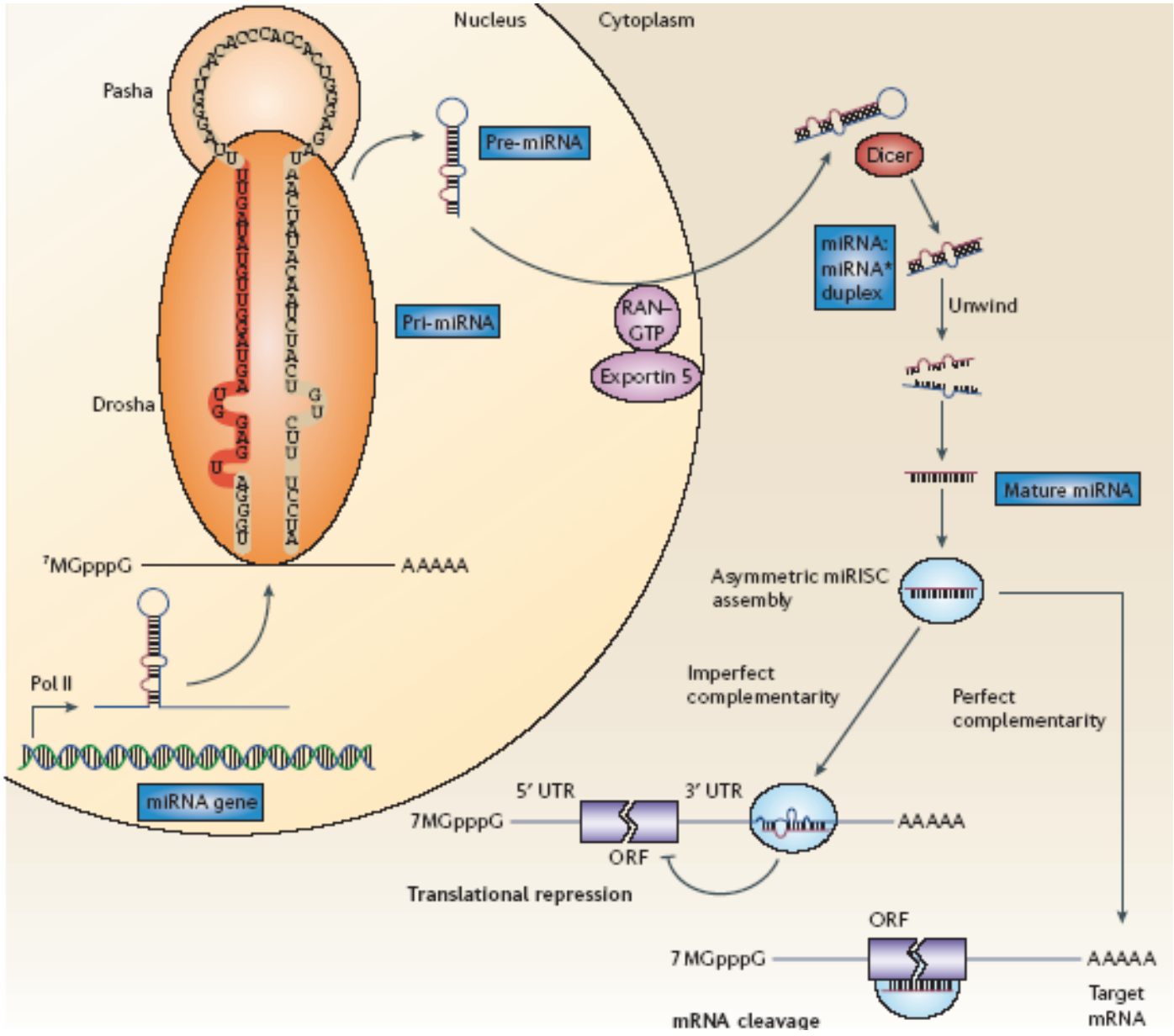
-Se conocen varios centenares de microRNAs humanos y se estima que debe haber en torno al millar (4% del número total de genes)

-Más del 50% de los microRNA humanos están en intrones de genes codificantes o no codificantes aunque podrían tener sus propios promotores

-El resto corresponden a exones de genes no-codificantes

-Se agrupan en familias (por el parecido de su secuencia) y pueden estar aislados o agrupados (“clusters”). El cromosoma 19 tiene 54 microRNAs.

microARNs


Nucleus
Cytoplasm
Pasha
Pre-miRNA
Dicer
miRNA:miRNA* duplex
Unwind
Pri-miRNA
RAN–GTP
Exportin 5
Drosha
Mature miRNA
7MGpppG
AAAAA
Asymmetric miRISC assembly
Pol II
Imperfect complementarity
Perfect complementarity
miRNA gene
5′ UTR
3′ UTR
7MGpppG
AAAAA
ORF
Translational repression
ORF
7MGpppG
AAAAA
Target mRNA
mRNA cleavage

LA **RNAi** ES:

1.-Importante para el desarrollo y la fisiología celular y tisular de los organismos

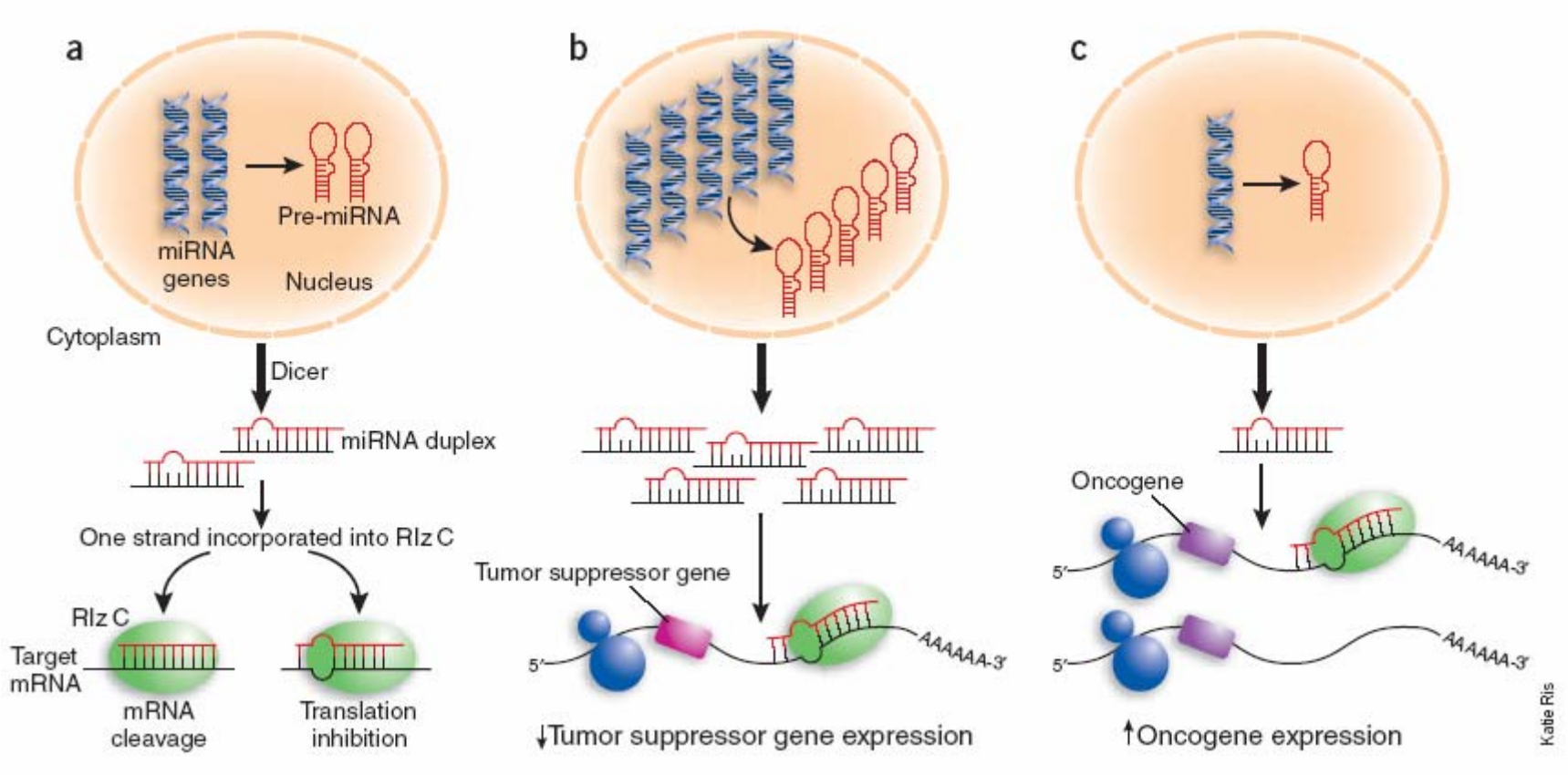
2.- Un sistema de protección frente a infecciones virales, especialmente en plantas e invertebrados

3.- Contribuye decisivamente a la estabilidad genómica participando en la organización de la cromatina y "sujetando" elementos móviles

4.- Una herramienta experimental de silenciamiento génico (*knock-down*) para clarificar la fisiología celular del gen y como procedimiento diagnóstico y terapéutico.

-Las predicciones bioinformáticas vaticinan que los microRNA podrían controlar la tercera parte del acerbo total de genes de cualquier mamífero, incluida la especie humana.

-Puesto que los microRNAs son capaces de controlar aspectos fundamentales del desarrollo (Plasterk, 2006), la apoptosis (Jovanovic & Hengarter, 2006) y el ciclo de división celular, la hipótesis de su implicación en cáncer no pareció una propuesta descabellada (Caldas & Brenton, 2005; Chen, 2005).


a
b
c
Pre-miRNA
miRNA genes
Nucleus
Cytoplasm
Dicer
miRNA duplex
One strand incorporated into RIz C
RIz C
Target mRNA
mRNA cleavage
Translation inhibition
Tumor suppressor gene
5′
AAAAAA-3′
↓Tumor suppressor gene expression
Oncogene
↑Oncogene expression
Katie Ris

Sizing up miRNAs as cancer genes

Carlos Caldas & James D Brenton

VOLUME 11 | NUMBER 7 | JULY 2005 NATURE MEDICINE

Findings over the last year or so have built the case that microRNAs might contribute to cancer. Three studies now definitively show this to be the case and also suggest that these small RNAs could be used to categorize tumors.

MicroRNAs (miRNAs), a family of mature noncoding small RNAs 21–25 nucleotides in length, negatively regulate the expression of protein-encoding genes. Therefore, it is not surprising that studies directly implicating miRNAs in cancer are emerging, because cancers ultimately arise because of disrupted gene expression. Such findings are epitomized by three reports in a recent issue of *Nature*[1–3]. The new work describes miRNAs with oncogenic and tumor suppressor activity and unveils a new molecular taxonomy of human cancers based on miRNA profiling.

miRNAs are processed sequentially from primary miRNA (pri-miRNA) precursor transcripts, and regulate gene expression at the post-transcriptional level[4–6]. They work either

The founding miRNA family members (*lin-4* and *let-7*) were identified as loss-of-function mutations in *C. elegans* that cause defects in developmental timing[7,8]. *lin-4* and *let-7* encode noncoding RNAs 21–22 nucleotides in length that are complementary to conserved sites in the 3′ untranslated region of their target genes.

Subsequently, miRNAs were found to be an abundant class of transcripts in all metazoans. Bioinformatic approaches identified 200–255 human miRNA genes, but more recent work has predicted the number to be closer to 1,000 (ref. 9). This makes the miRNA genes one of the most abundant classes of regulatory genes in mammals.

MicroRNAs as Oncogenes and Tumor Suppressors

Chang-Zheng Chen, Ph.D.

Related article, page 1793

Recent discoveries related to microRNAs, RNA interference, small interfering RNAs (siRNAs), and small modulatory RNAs have revealed a new class of mechanisms of gene regulation that are mediated by small, noncoding RNAs. Among these small RNAs are the microRNAs. These are thought to control gene expression at the post-transcriptional level by degrading or repressing target messenger RNAs (mRNAs). Surprisingly simple and elegant, microRNA-mediated gene regulation is guided by the base-pairing rules of Watson and Crick.

MicroRNAs are individually encoded by their own set of genes and are an integral component of the genetic program. Some are located in noncoding regions of the genome, whereas others occur in the introns (noncoding regions) of protein-coding genes. Furthermore, as shown in the figure, a complex set of proteins is required for the formation and function of microRNAs. Many of these proteins, as well as the microRNAs themselves, are found in a wide range of animal species and are important for development.

There is accumulating evidence that microRNA-mediated gene regulation has a broad impact on gene expression. First, microRNA genes constitute about 1 to 5 percent of the predicted genes in worms, mice, and humans — there may be as many as 1000 microRNA genes in the human genome. Second, microRNAs are expressed at high levels in animal

1.-En los tres últimos años se han acumulado evidencias de la implicación de microRNAs en el desarrollo de la práctica totalidad de los cánceres conocidos, actuando a través de conocidos oncogenes y genes supresores (Esquela-Kerscher & Snack, 2006; Blenkiron & Miska, 2007).

2.-En otros casos, es el propio oncogen o el gene supresor (al ser un factor de transcripción) el que induce la expresión de un microRNA: *c-Myc* :O´Donnel et al 2005; *Tp53*: Corney et al., 2007; He et al 2007; Hermeking, 2007).

3.-Los equipos de Robert Weinberg y Joan Massagué han demostrado la implicación de microRNAs en los procesos de invasión y metástasis en cáncer de mama (Ma et al., 2007; Tavazoie et al., 2008).

1.- MicroRNAs que controlan oncogenes y genes supresores

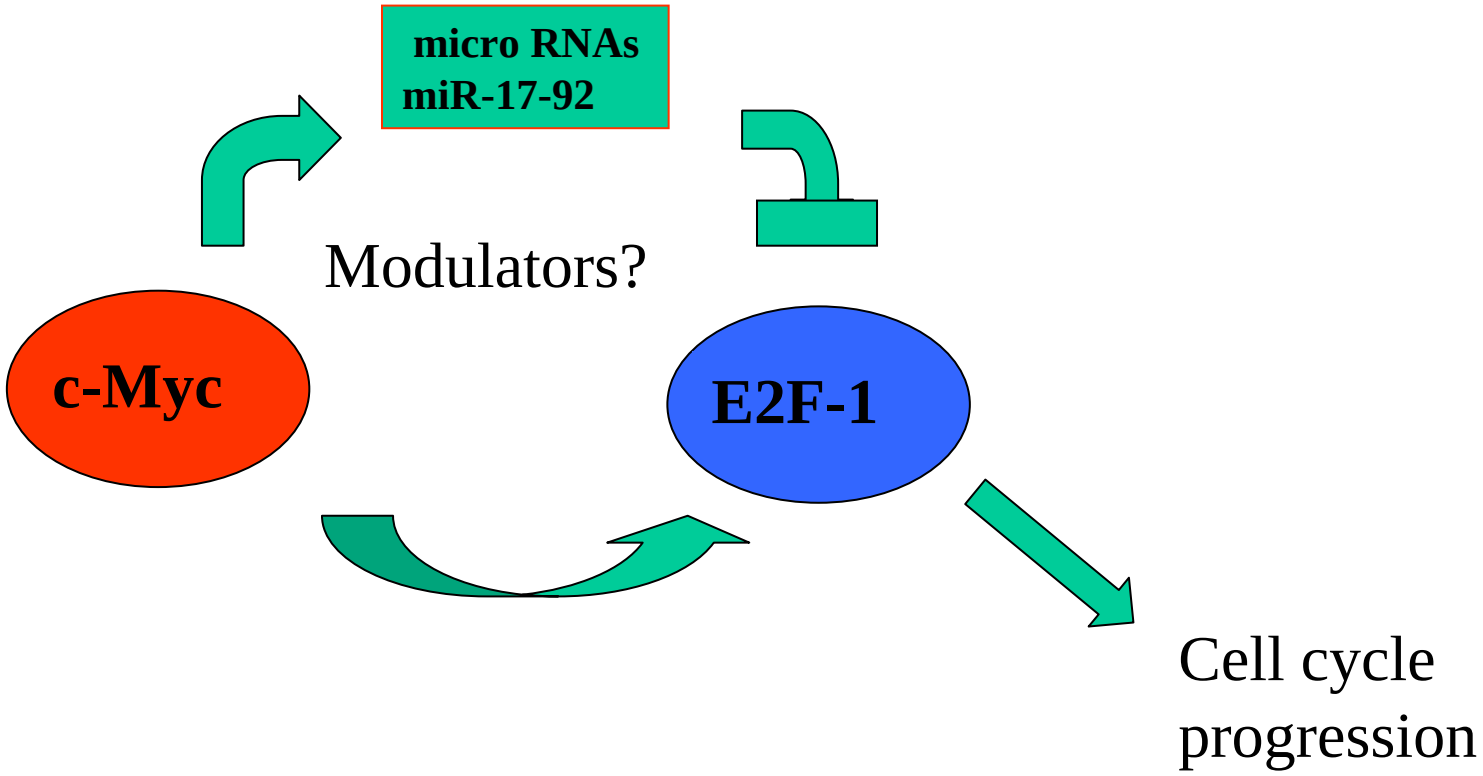
Table 1. miRNAs that may regulate cancer-related genes

Gene	Gene summary	Cell function	miRNAs	References
AIB1	Amplified in breast cancer 1		miR-17-5p	(62)
AT1R	Angiotensin receptor 1	Angiogenesis	miR-155	(105)
BCL2	B-cell CLL/lymphoma 2	Apoptosis	miR-21	(57,58)
			miR-15a	
			miR-16-1	
BCL6	B-cell CLL/lymphoma 6	Cell cycle	miR-127	(56)
E2F1	E2F transcription factor 1	Cell cycle	miR-17-5p	(65)
		Apoptosis	miR-20a	
E2F3	E2F transcription factor 3	Cell cycle	miR-34a	(54)
FOS	FBJ osteosarcoma virus oncogene homolog		miR-7b	(51)
HMGA2	High mobility group AT-hook 2		*let-7* family	(53)
				(52)
HOXB8	Homeobox B8		miR-196a	(106)
KIT	KIT oncogene		miR-221	(59,60)
			miR-222	(61)
			miR-146	
LATS2	Large tumour suppressor homolog	Cell cycle	miR-372	(48)
			miR-373	
NFI-A	Nuclear factor I/A		miR-107	(107,108)
			miR-233	
PLAG1	Pleomorphic adenoma gene 1		miR-26a	(50)
PTEN	Phosphatase and tensin homolog	Cell cycle	miR-21	(49)
		Cell migration		
RAS	Harvey rat sarcoma viral oncogene homolog		*let-7* family	(30)
RB	Retinoblastoma	Cell cycle	miR-106a	(50)
TCL1	T-cell leukaemia/lymphoma 1A	Apoptosis	miR-29	(55)
			miR-181	
TGFBR2	Transforming growth factor-beta receptor 1		miR-20a	(50)
TSP1	Thrombospondin 1	Angiogenesis	miR-17-92	(69)

Italics indicates human oncogenes. Bold indicates human tumour suppressors. Only predicted targets for which there is some experimental verification are listed.

2.- MicroRNAs inducidos por oncogenes o genes supresores

The *miR-17-92* cluster or polycistron is highly expressed in malignant lymphomas containing amplified 13q31-32 and determines increasing oncogenenic effect by *c-Myc in HSCs*.



O´Donnel et al 2005. Nature 435: 839

NATURE GENETICS VOLUME 40 | NUMBER 1 | JANUARY 2008

Widespread microRNA repression by Myc contributes to tumorigenesis

Tsung-Cheng Chang[1,7], Duonan Yu[2,7], Yun-Sil Lee[1], Erik A Wentzel[1], Dan E Arking[1,3], Kristin M West[1], Chi V Dang[3,4], Andrei Thomas-Tikhonenko[2] & Joshua T Mendell[1,5,6]

The c-Myc oncogenic transcription factor (Myc) is pathologically activated in many human malignancies. Myc is known to directly upregulate a pro-tumorigenic group of microRNAs (miRNAs) known as the miR-17–92 cluster. Through the analysis of human and mouse models of B cell lymphoma, we show here that Myc regulates a much broader set of miRNAs than previously anticipated. Unexpectedly, the predominant consequence of activation of Myc is widespread repression of miRNA expression. Chromatin immunoprecipitation reveals that much of this repression is likely to be a direct result of Myc binding to miRNA promoters. We further show that enforced expression of repressed miRNAs diminishes the tumorigenic potential of lymphoma cells. These results demonstrate that extensive reprogramming of the miRNA transcriptome by Myc contributes to tumorigenesis.

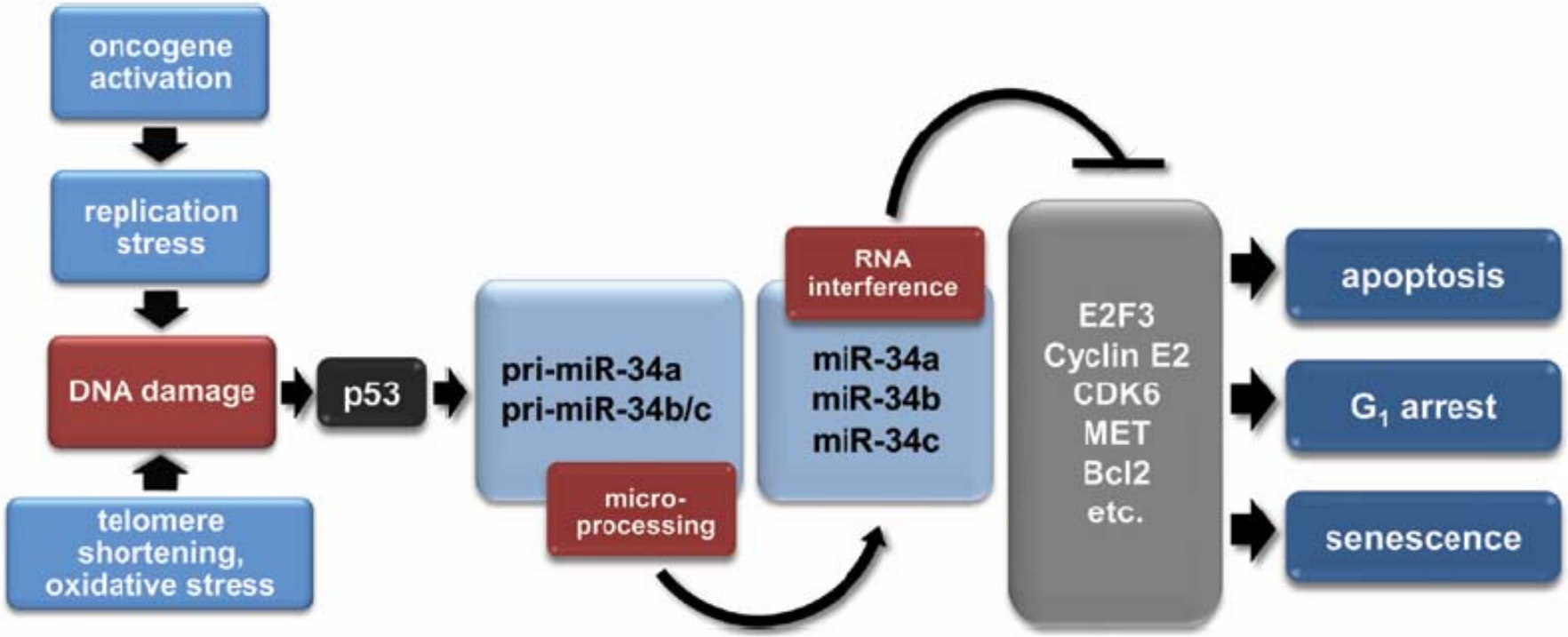
Cell PRESS

Cancer Cell
Minireview

p53 Enters the MicroRNA World

Heiko Hermeking[1,*]
[1]Molecular Oncology, Max-Planck-Institute of Biochemistry, Am Klopferspitz 18, D-82152 Martinsried, Germany
*Correspondence: herme@biochem.mpg.de
DOI 10.1016/j.ccr.2007.10.028

Recently, microRNAs, which are regulated by the transcription factor encoded by the tumor suppressor gene *p53*, were identified independently by seven groups. Their studies highlight the microRNAs *miR-34a* and *miR-34b/c* as direct, conserved p53 target genes that presumably mediate induction of apoptosis, cell cycle arrest, and senescence by p53. Since these microRNAs may regulate the levels of hundreds of different proteins, these findings add a new, challenging layer of complexity to the p53 network. The initial evidence suggesting that *miR-34* genes are central mediators of p53 function is summarized here.


oncogene activation
replication stress
DNA damage
telomere shortening, oxidative stress
p53
pri-miR-34a
pri-miR-34b/c
micro-processing
RNA interference
miR-34a
miR-34b
miR-34c
E2F3
Cyclin E2
CDK6
MET
Bcl2
etc.
apoptosis
G_1 arrest
senescence

3.- Implicación de microRNAs en los procesos de invasión y metástasis

Vol 451|10 January 2008|doi:10.1038/nature06487 nature

ARTICLES

Endogenous human microRNAs that suppress breast cancer metastasis

Sohail F. Tavazoie[1,2], Claudio Alarcón[1], Thordur Oskarsson[1], David Padua[1], Qiongqing Wang[1], Paula D. Bos[1], William L. Gerald[3] & Joan Massagué[1]

A search for general regulators of cancer metastasis has yielded a set of microRNAs for which expression is specifically lost as human breast cancer cells develop metastatic potential. Here we show that restoring the expression of these microRNAs in malignant cells suppresses lung and bone metastasis by human cancer cells *in vivo*. Of these microRNAs, miR-126 restoration reduces overall tumour growth and proliferation, whereas miR-335 inhibits metastatic cell invasion. miR-335 regulates a set of genes whose collective expression in a large cohort of human tumours is associated with risk of distal metastasis. miR-335 suppresses metastasis and migration through targeting of the progenitor cell transcription factor *SOX4* and extracellular matrix component tenascin C. Expression of miR-126 and miR-335 is lost in the majority of primary breast tumours from patients who relapse, and the loss of expression of either microRNA is associated with poor distal metastasis-free survival. miR-335 and miR-126 are thus identified as metastasis suppressor microRNAs in human breast cancer.

MicroRNAs y “Cancer Stem Cells”

let-7 Regulates Self Renewal and Tumorigenicity of Breast Cancer Cells

Fengyan Yu,[1,2] Herui Yao,[1] Pengcheng Zhu,[2] Xiaoqin Zhang,[1] Qiuhui Pan,[1] Chang Gong,[1] Yijun Huang,[3] Xiaoqu Hu,[1] Fengxi Su,[1] Judy Lieberman,[2,*] and Erwei Song[1,*]
[1]Department of Breast Surgery, No. 2 Affiliated Hospital, Sun-Yat-Sen University, Guangzhou 510120, People's Republic of China
[2]Immune Disease Institute, Harvard Medical School, Boston, MA 02115, USA
[3]Department of Pharmacology, Zhongshan School of Medicine, Sun-Yat-Sen University, Guangzhou, 510089, People's Republic of China
*Correspondence: lieberman@cbr.med.harvard.edu (J.L.), songew@mail.sysu.edu.cn (E.S.)
DOI 10.10 Cell *131*, 1109–1123, December 14, 2007 ©2007 Elsevier Inc. 1109

SUMMARY

Cancers may arise from rare self-renewing tumor-initiating cells (T-IC). However, how T-IC self renewal, multipotent differentiation, and tumorigenicity are maintained remains obscure. Because miRNAs can regulate cell-fate decisions, we compared miRNA expression in self-renewing and differentiated cells from breast cancer lines and in breast T-IC (BT-IC) and non-BT-IC from 1° breast cancers. *let-7* miRNAs were markedly reduced in BT-IC and increased with differentiation. Infecting BT-IC with *let-7*-lentivirus reduced proliferation, mammosphere formation, and the proportion of undifferentiated cells in vitro and tumor formation and metastasis in NOD/SCID mice, while antagonizing *let-7* by antisense oligonucleotides enhanced in vitro self renewal of non-T-IC. Increased *let-7* paralleled reduced H-RAS and HMGA2, known *let-7* targets. Silencing *H-RAS* in a BT-IC-enriched cell line reduced self renewal but had no effect on differentiation, while silencing *HMGA2* enhanced differentiation but did not affect self renewal. Therefore *let-7* regulates multiple BT-IC stem cell-like properties by silencing more than one target.

genic in immunodeficient mice. According to the hypothesis, T-IC are not only the source of the tumor but also may be responsible for tumor progression (Dalerba et al., 2007), metastasis (Wicha, 2006), resistance to therapy, and subsequent tumor recurrence (Al-Hajj, 2007). Breast T-IC (BT-IC) can be enriched by sorting for $CD44^{+}CD24^{-/low}$ cells (Al-Hajj et al., 2003), by selecting for side-population (SP) cells that efflux Hoechst dyes (Patrawala et al., 2005), or by isolating spherical clusters of self-replicating cells (“mammospheres”) from suspension cultures (Ponti et al., 2005). However, these methods purify both T-IC and some EPC (Al-Hajj et al., 2003; Ponti et al., 2005).

Since miRNAs regulate differentiation and can function as either tumor suppressors or oncogenes to regulate tumor development and prognosis (Lu et al., 2005), we looked at whether differences in miRNA expression might distinguish BT-IC/EPC from their more differentiated progeny. miRNAs are known to contribute to preserving stemness of embryonic stem (ES) cells, because ES cells deficient in miRNA processing cannot be maintained (Shcherbata et al., 2006). Previous studies have shown an overall reduction in miRNA expression in embryonic or tissue stem cells (Croce and Calin, 2005), and changes in specific miRNAs have been associated with ES cell self renewal and differentiation (Shcherbata et al., 2006). Moreover, miRNA-expression profiling can help characterize the stage, subtype, and prognosis of some cancers (Lu et al., 2005).

MicroRNAs virales y cáncer

nature Vol 450|13 December 2007|doi:10.1038/nature05992

LETTERS

A viral microRNA functions as an orthologue of cellular miR-155

Eva Gottwein[1], Neelanjan Mukherjee[2], Christoph Sachse[4], Corina Frenzel[4], William H. Majoros[5], Jen-Tsan A. Chi[1,5], Ravi Braich[7], Muthiah Manoharan[7], Jürgen Soutschek[7], Uwe Ohler[3,5,6] & Bryan R. Cullen[1]

All metazoan eukaryotes express microRNAs (miRNAs), roughly 22-nucleotide regulatory RNAs that can repress the expression of messenger RNAs bearing complementary sequences[1]. Several DNA viruses also express miRNAs in infected cells, suggesting a role in viral replication and pathogenesis[2]. Although specific viral miRNAs have been shown to autoregulate viral mRNAs[3,4] or downregulate cellular mRNAs[5,6], the function of most viral miRNAs remains unknown. Here we report that the miR-K12-11 miRNA encoded by Kaposi's-sarcoma-associated herpes virus (KSHV) shows significant homology to cellular miR-155, including the entire miRNA 'seed' region[7]. Using a range of assays, we show that expression of physiological levels of miR-K12-11 or miR-155 results in the downregulation of an extensive set of common mRNA targets, including genes with known roles in cell growth regulation. Our findings indicate that viral miR-K12-11 functions as an orthologue of cellular miR-155 and probably evolved to exploit a pre-existing gene regulatory pathway in B cells. Moreover, the known aetiological role of miR-155 in B-cell transformation[8–10] suggests that miR-K12-11 may contribute to the induction of KSHV-positive B-cell tumours in infected patients.

Inspection of mature KSHV miR-K12-11 and cellular miR-155 reveals significant homology, including the entire seed region that is often critical for mRNA target recognition[7]; that is, nucleotides 2–8 (Fig. 1a). miR-155, the product of the *bic* gene[10], is overexpressed in several types of B-cell lymphoma, and its transgenic expression in mice causes B-cell malignancies[9]. miR-155 expression is induced in activated B cells, T cells and macrophages[11–13], and miR-155 knockout mice have impaired immune functions[14,15]. Given the emerging importance of miR-155 in cancer and B-cell function, we asked whether miR-K12-11 functions as an orthologue of miR-155.

We first prepared transductants of the KSHV-negative human B-cell line BJAB expressing physiological levels of miR-K12-11. A miR-K12-11 expression cassette was placed 3′ to the *AcGFP* (a variant of green fluorescent protein cloned from *Aequorea coerelescens*) open reading frame (ORF) present in the lentiviral vector pNL-SIN-CMV-AcGFP (Fig. 1b). Cells transduced with this vector express transcripts that function as *AcGFP* mRNAs and as primary miRNAs (pri-miRNAs)[16]. Transduced BJAB cells were sorted to generate pools expressing only AcGFP or expressing AcGFP and miR-K12-11. Expression of miR-K12-11 was confirmed by primer extension (Fig. 1c, lanes 1–8, and Supplementary Fig. 2a, b) and by knockdown of an indicator bearing perfectly complementary sites[17] (Supplementary Fig. 2c–e). The level of expression and activity of miR-K12-11 in transduced BJAB cells was comparable to that observed in the B-cell line BC-1 (Fig. 1c, lane 15, and Supplementary Fig. 2), which is latently infected with KSHV[18].

Cytoplasmic RNA was isolated from BJAB cells and analysed on microarrays in three independent experiments. Expression of

MicroRNAs asociados con cánceres humanos I

miRNA	Gene loci	Cancer association	Function	References
miR-15a, miR-16-1	Chromosome 13q14	Frequently deleted or downregulated in B-cell chronic lymphocytic leukemia; negatively regulates the anti-apoptotic gene *BCL2*	TS	93,94
miR-143, miR-145	Chromosome 5q32–33	Decreased abundance in colorectal cancer; downregulated in breast, prostate, cervical and lymphoid cancer cell lines; miR-145 is decreased in breast cancer	TS	84,97
miR-21	Chromosome 17q23.2	Anti-apoptotic factor; upregulated in glioblastomas and breast cancer	OG	84,100,101
let-7 family members	Multiple loci	Negatively regulate the Ras oncogenes; direct cell proliferation and differentiation; decreased abundance in lung cancer	TS	81,82

MicroRNAs asociados con cánceres humanos II

miR-142	Chromosome 17q22	A t(8;17) translocation that places the *MYC* oncogene downstream of the *mir-142* hairpin, resulting in an aggressive B-cell leukemia that is due to *MYC* overexpression	N/A	41
BIC/miR-155	Chromosome 21q21	Upregulated in paediatric Burkitt, Hodgkin, primary mediastinal and diffuse large-B-cell lymphomas; upregulated in human breast cancer	OG	84,105–108
miR-17–106 cluster	Chromosome 13q31–32	Upregulated by MYC; negatively modulates the *E2F1* oncogene; loss of heterozygosity of this cluster is found in hepatocellular carcinoma; overexpressed in B-cell lymphomas	TS/OG	109,110

N/A, not applicable; OG, oncogene; TS, tumour suppressor.

Alteraciones de los microRNAs en cáncer

Aunque hay algunos **sobre-expresados** en cáncer, el patrón global preponderante es el de la **reducción** en sus niveles de expresión (Lu et al., 2005. Nature 435):

-***Bloqueos en la primera etapa de su procesamiento*** (Thomson et al., 2006 Genes Dev)
-***Deleciones*** (Calin et al., 2004. PNAS)
-***Mutaciones*** (Calin et al., 2005. NEJM)
-***Represión transcripcional directa*** (Chang et al., 2008. Nature Genet)
-***Mecanismos Epigenéticos y combinados*** (nuestra contribución)

Genetic and Epigenetic Silencing of microRNA-203 Enhances ABL1 and BCR-ABL1 Oncogene Expression

María J. Bueno,[1,2] Ignacio Pérez de Castro,[1] Marta Gómez de Cedrón,[1] Javier Santos,[2] George A. Calin,[3] Juan C. Cigudosa,[4] Carlo M. Croce,[3] José Fernández-Piqueras[2] and Marcos Malumbres[1]*

[1] Cell Division and Cancer Group, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid; [2] Centro de Biología Molecular Severo Ochoa CSIC-Universidad Autónoma de Madrid (UAM) and Dept.de Biología, UAM, Madrid; [3]The Ohio State University, Comprehensive Cancer Center, Columbus, OH; and [4]Molecular Cytogenetics Group, CNIO, Madrid.

Cancer Cell (in revision)

Los“perfiles” genómicos de expresión de microRNAs como “firmas genéticas” para la clasificación, el diagnóstico, la estadificación, el pronóstico y la respuesta al tratamiento de los diferentes tipos de cánceres

MicroRNA signatures in human cancers

Nature Rev Cancer vol 6 November 2006

George A. Calin and Carlo M. Croce

Abstract | MicroRNA (miRNA) alterations are involved in the initiation and progression of human cancer. The causes of the widespread differential expression of miRNA genes in malignant compared with normal cells can be explained by the location of these genes in cancer-associated genomic regions, by epigenetic mechanisms and by alterations in the miRNA processing machinery. MiRNA-expression profiling of human tumours has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment. In addition, profiling has been exploited to identify miRNA genes that might represent downstream targets of activated oncogenic pathways, or that target protein-coding genes involved in cancer.

nature Vol 435|9 June 2005|doi:10.1038/nature03702

LETTERS

MicroRNA expression profiles classify human cancers

Jun Lu[1,4]*, Gad Getz[1]*, Eric A. Miska[2]*†, Ezequiel Alvarez-Saavedra[2], Justin Lamb[1], David Peck[1], Alejandro Sweet-Cordero[3,4], Benjamin L. Ebert[1,4], Raymond H. Mak[1,4], Adolfo A. Ferrando[4], James R. Downing[5], Tyler Jacks[2,3], H. Robert Horvitz[2] & Todd R. Golub[1,4,6]

Recent work has revealed the existence of a class of small non-coding RNA species, known as microRNAs (miRNAs), which have critical functions across various biological processes[1,2]. Here we use a new, bead-based flow cytometric miRNA expression profiling method to present a systematic expression analysis of 217 mammalian miRNAs from 334 samples, including multiple human cancers. The miRNA profiles are surprisingly informative, reflecting the developmental lineage and differentiation state of the tumours. We observe a general downregulation of miRNAs in tumours compared with normal tissues. Furthermore, we were able to successfully classify poorly differentiated tumours using miRNA expression profiles, whereas messenger RNA profiles were highly inaccurate when applied to the same samples. These findings highlight the potential of miRNA profiling in cancer diagnosis.

5′-phosphate and the 3′-hydroxyl groups of miRNAs[13], reverse-transcribed miRNAs were (1) amplified by polymerase chain reaction (PCR) using a common biotinylated primer, (2) hybridized to the capture beads, and (3) stained with streptavidin-phycoerythrin. The beads were then analysed using a flow cytometer capable of measuring bead colour (denoting miRNA identity) and phycoerythrin intensity (denoting miRNA abundance) (see Supplementary Fig. 1).

Bead-based hybridization has the theoretical advantage that it might more closely approximate hybridization in solution, and as such, we might expect the specificity to be superior to glass microarray hybridization. Indeed, a spiking experiment involving 11 related sequences showed increased specificity of bead-based detection compared with microarray-based detection, even for single base-pair mismatches (Fig. 1a, b). In addition, the bead method

Table 1 | **Facts about microRNA-expression profiling in human cancers**

Cancer type*	MiRNA profiling data	Significance	Refs
Chronic lymphocytic leukaemia	A unique signature of 13 genes associated with prognostic factors (ZAP70 and IgVH mutation status) and progression (time from diagnosis to therapy)	MiRNAs as diagnostic markers (the identification of two categories of patients)	49,35
Lung adenocarcinoma	Molecular signatures that differ with tumour histology; miRNA profiles correlated with survival (*miR-155* and *let-7*)	MiRNAs as prognostic and diagnostic markers	53
Breast carcinoma	MiRNA expression correlates with specific pathological features	MiRNAs as prognostic markers	50
Endocrine pancreatic tumours	A signature that distinguishes endocrine from acinar tumours; the overexpression of *miR-21* is strongly associated with both a high Ki67 proliferation index and the presence of liver metastases	MiRNAs as diagnostic and prognostic markers	54
Hepatocellular carcinoma	MiRNA expression correlated with differentiation	MiRNAs as prognostic markers	52
Papillary thyroid carcinoma	MiRNA upregulation (for example, *miR-221* and *miR-222*) in tumoral cells and normal cells adjacent to tumours, but not in normal thyroids without cancers	MiRNAs probably involved in cancer initiation	37 114
Glioblastoma	A specific signature compared with normal tissues	MiRNAs as diagnostic markers	51
Human cancers	MiRNA-expression profiles accurately classify cancers; an miRNA classifier classes poorly differentiated samples better than a messenger RNA classifier	MiRNAs as diagnostic markers	41
Human solid cancers	Common signature for distinct types of solid carcinomas	Specific miRNAs are involved in common molecular pathways	47

*Only data from microarray studies reporting results on human primary tumours were included in this table. IgV_H, immunoglobulin heavy-chain variable-region, MiRNA, microRNA. ZAP70, 70 kDa zeta-associated protein.

Las estrategias “Knock-down”

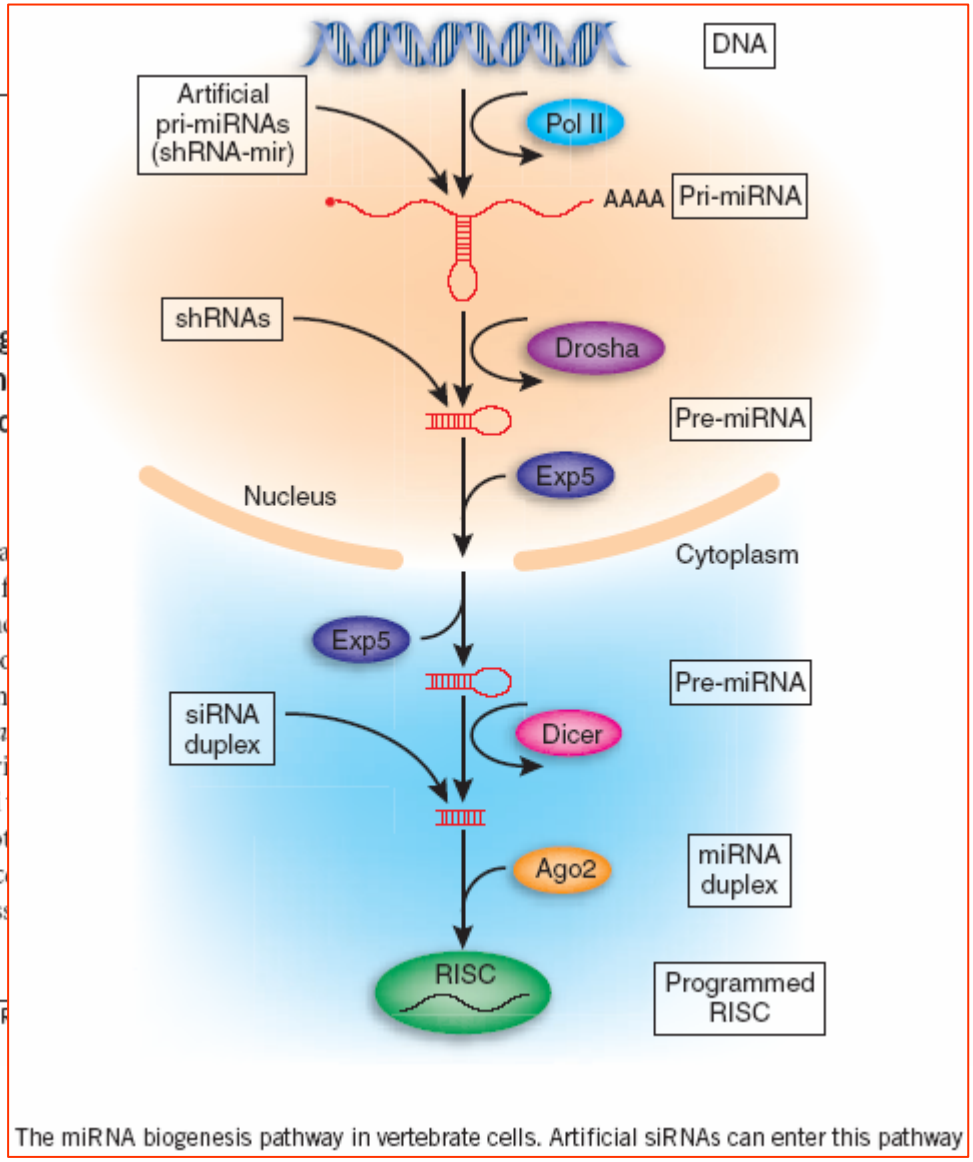
RNAi the natural way

Bryan R Cullen

Although RNA interference has become a useful g genes remains a challenge, especially *in vivo*. A n interfering RNAs, not only seems to be more effec RNA interference in culture and *in vivo*.

The discovery that genes can be turned off post-transcriptionally by RNA interference (RNAi), made first in nematodes and then extended to all multicellular eukaryotes, raised the possibility that vertebra tractable as f RNAi has fa in culture, c cally challen Dickins *et a* issue, descri allow RNAi allowing not vertebrate c gene expres

Bryan R. Cullen is in the Center for Virology and Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710, USA.
e-mail: culle002@mc.duke.edu



The miRNA biogenesis pathway in vertebrate cells. Artificial siRNAs can enter this pathway

Table 1 | **Delivery methods for RNA-interference-based therapeutics**

Method	Nucleic acid delivered	Advantages	Disadvantages
Non-viral delivery			
Cholesterol	siRNA	Systemic delivery, stable	Non-selective delivery
SNALP	siRNA	Systemic delivery, highly stable	Non-selective delivery
Fab	siRNA	Receptor-specific delivery	Relatively complex formulation
Aptamer	siRNA	Receptor-specific delivery	Large-scale sequence screening required
Nanoparticle	siRNA	Receptor-specific, self-assembling	Sophisticated preparation required
Viral delivery			
Lentivirus	RNA (shRNA produced)	Stable expression, transduces non-dividing cells	Gene-disruption risk, localized delivery
Adenovirus	dsDNA (shRNA produced)	Episomal, no insertional mutagenesis	Immunogenic, dose-dependent hepatotoxicity
AAV	ssDNA/dsDNA (shRNA produced)	Episomal, low genomic integration	Immunogenic, small vector capacity

AAV, adeno-associated virus; dsDNA, double-stranded DNA; Fab, heavy-chain antibody fragment; shRNA, short hairpin RNA; siRNA, small interfering RNA; SNALP, stable nucleic acid–lipid particle; ssDNA, single-stranded DNA.

Table 2 | **Development of RNA-interference-based therapies**

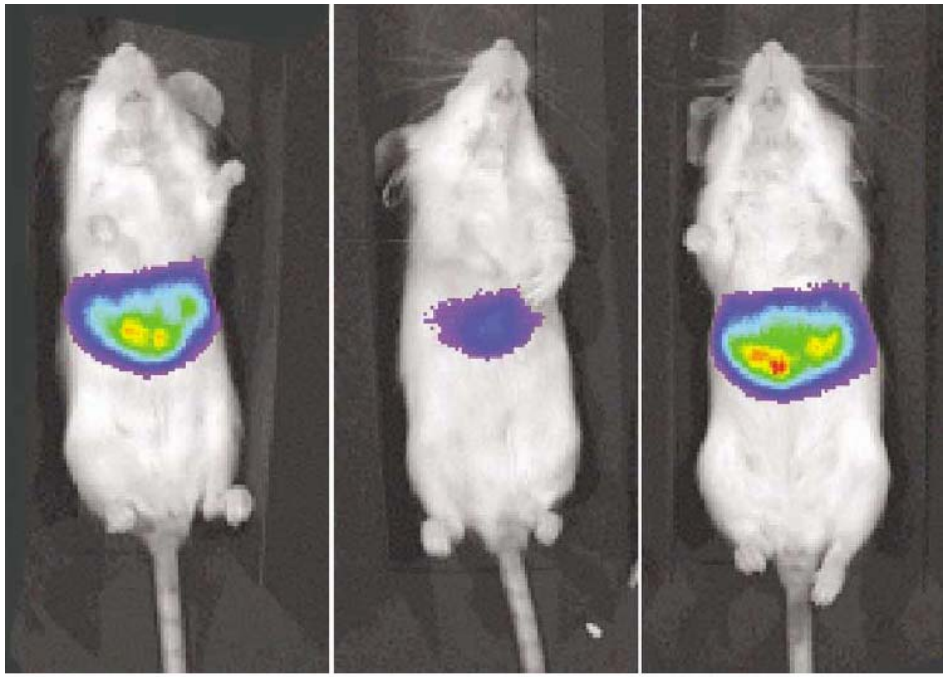
Disease	Stage	RNAi reagent	Delivery	Company/Institution
Ocular diseases				
AMD	Preclinical stage	siRNA	Direct intravitreal injection	Quark Biotech
	Clinical trial phase I	siRNA	Direct intravitreal injection	Sirna
	Clinical trial phase II	siRNA	Direct intravitreal injection	Acuity
Viral infections				
Hepatitis B and C	Preclinical stage	shRNA	Liganded nanoparticle	Nucleonics/Intradigm
RSV	Clinical trial phase I	siRNA	Aerosol	Alnylam
HIV	Clinical trial phase I (scheduled for 2007)	shRNA	Lentivirus	Benitec/City of Hope
Cancer				
Hepatic cancer	Preclinical stage	siRNA	Liganded nanoparticle	Calando
Solid tumour cancers	Preclinical stage	siRNA	Liganded nanoparticle	Intradigm
Other disease types				
ALS	Preclinical stage	siRNA	N/A	CytRx
Inflammatory diseases	Preclinical stage	siRNA	Peptide	Nastech

ALS, amyotrophic lateral sclerosis; AMD, age-related macular degeneration; RNAi, RNA interference; RSV, respiratory syncytial virus; shRNA, short hairpin RNA; siRNA, small interfering RNA.

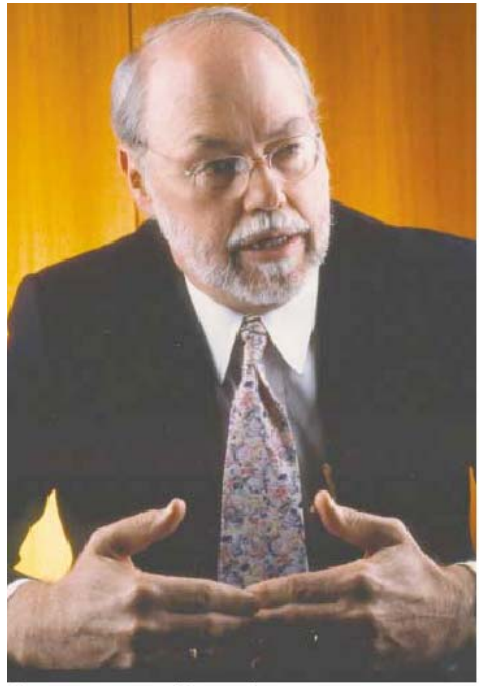
Las estrategias “Knock-down”:

RNAi to the rescue? By *Erika Check*, Nature 4 Sep 2003

Little helpers: Mark Kay hopes to use harmless viruses to deliver RNAi therapy to patients.

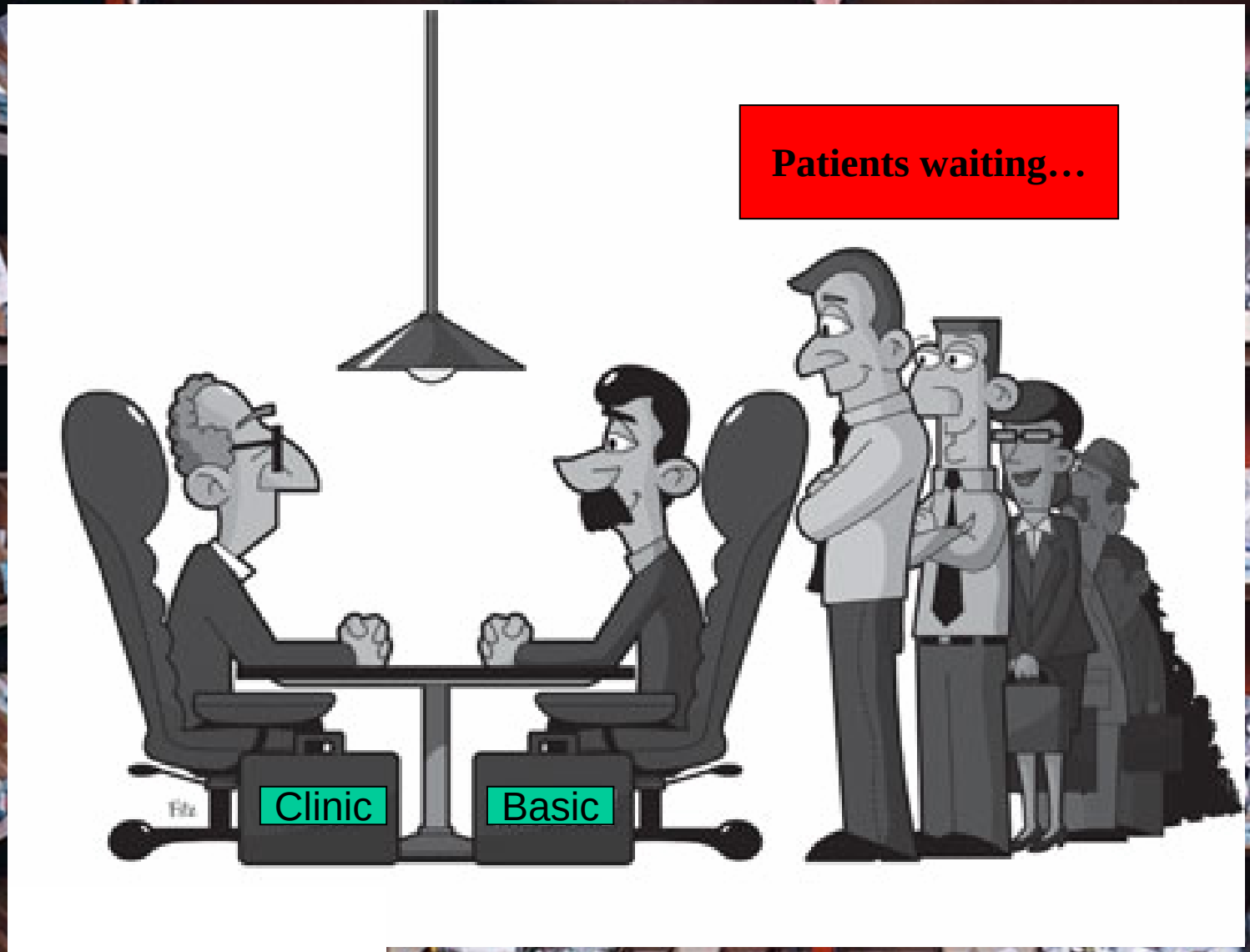


In mice containing a glowing version of a hepatitis C gene (left), a small interfering RNA (siRNA) against the gene reduces liver fluorescence (middle), but an unrelated copy of the siRNA (right) does not.



Going to market: Phillip Sharp is one of several RNAi researchers to form start-up biotech firms.

La investigación traslacional:



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