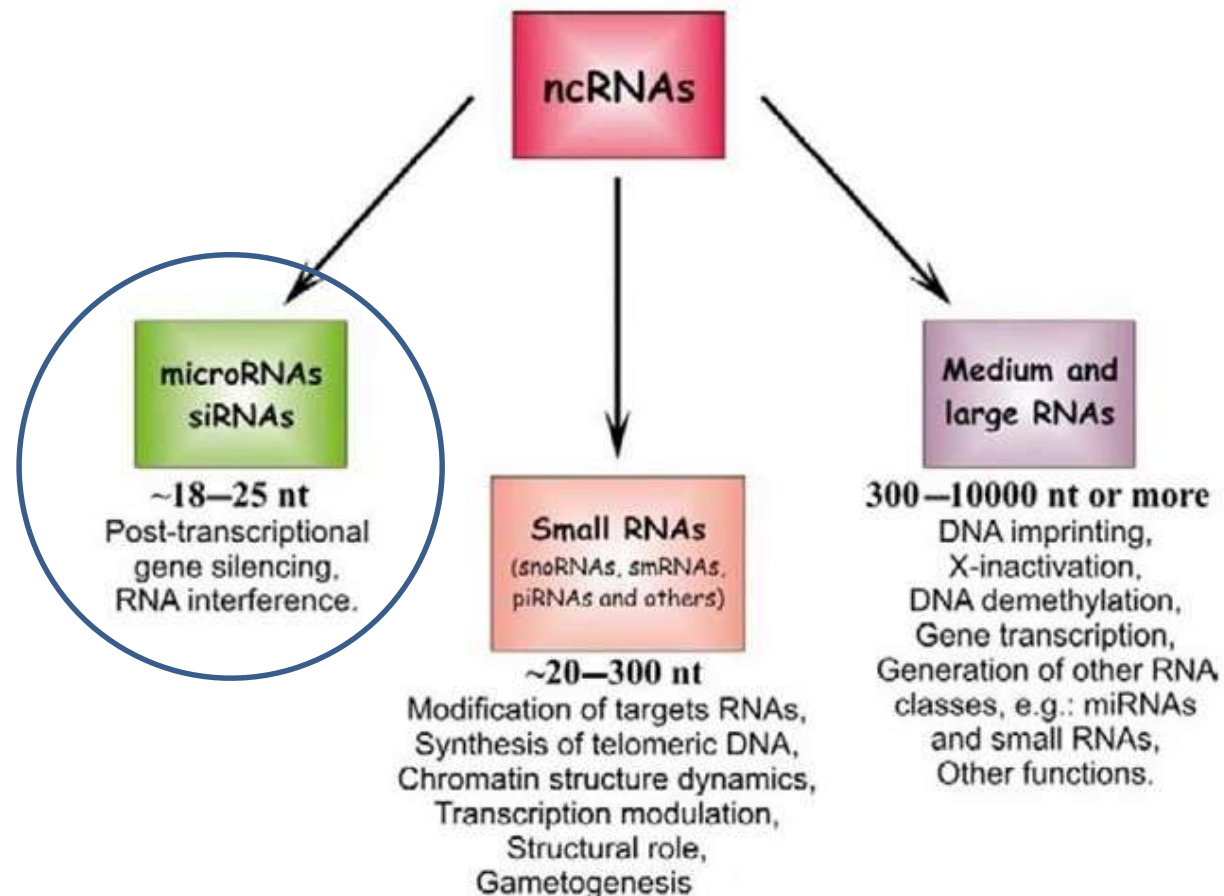


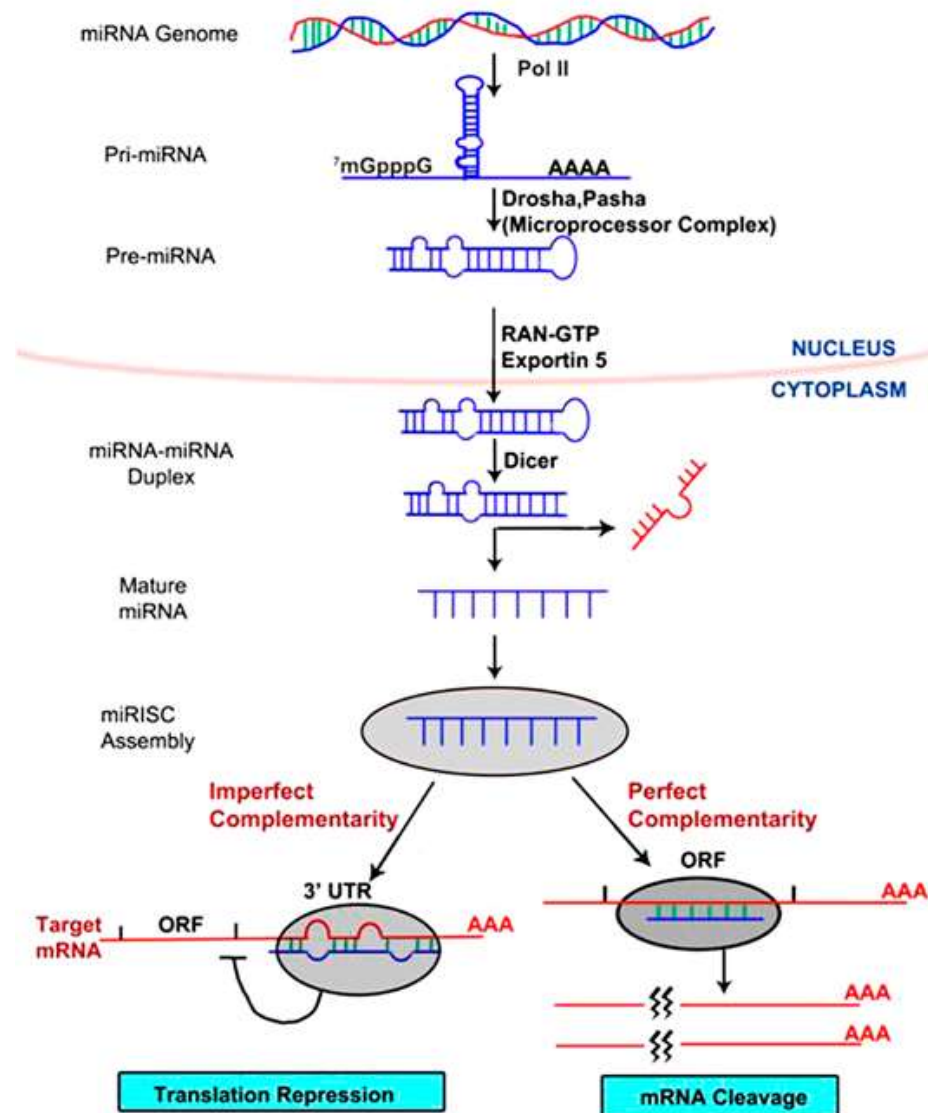
**Pharmacoepigeneretics:  
an element of personalized  
therapy?**

# RNA non codificante

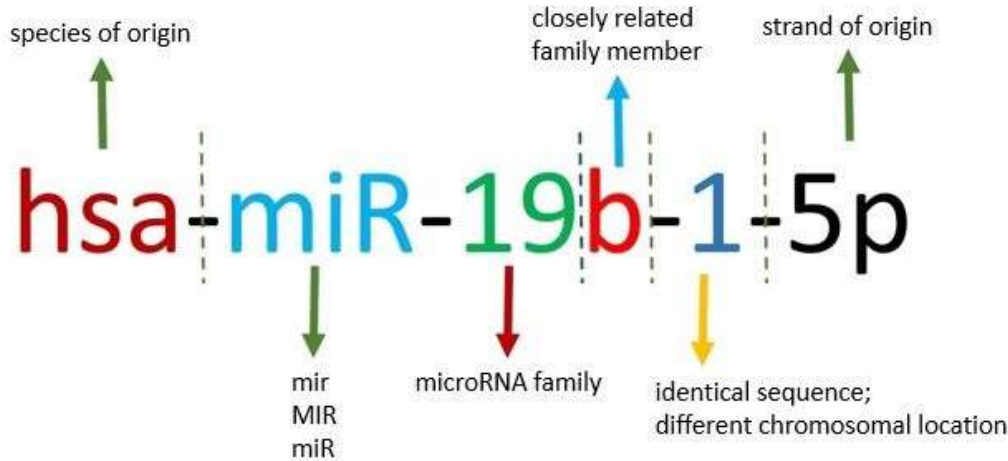
Non-coding RNA is an RNA that functions without being translated to a protein.



# micro-RNA

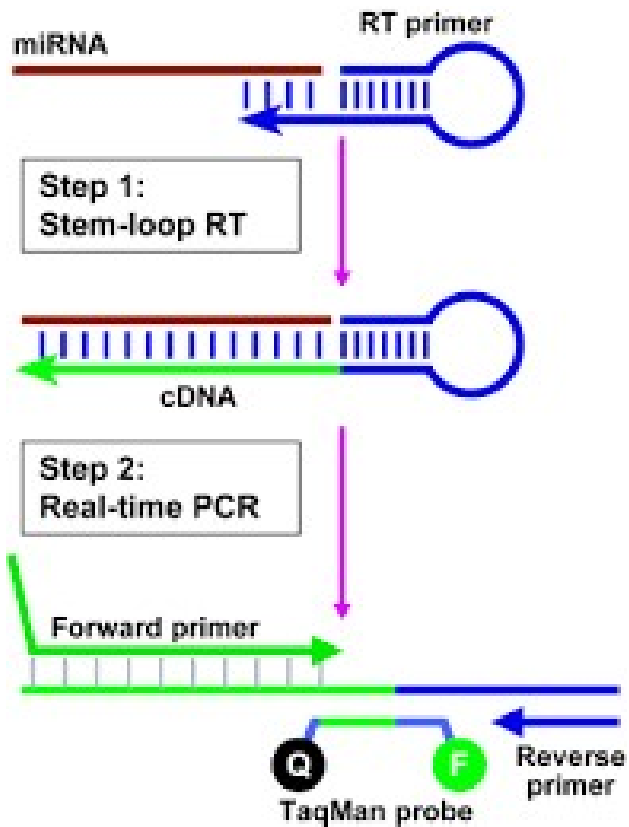


# Nomenclature of microRNA



- According to standard nomenclature system, name of any MicroRNA is written as mir-123.
- miR = MicroRNA (mature form).
- mir = Precursor MicroRNA.
- Number indicates order of discovery.
- Annotated with an additional lower case letter e.g.- miR-123a & miR-123b, if deference in only one or two nucleotides.

## microRNA detection methods



- qRT-PCR;
- TaqMan miRNA array;
- Microarray;
- NGS.

# microRNA database

## <http://www.mirbase.org/>

**miRBase: the microRNA database**

miRBase provides the following services:

- The [miRBase database](#) is a searchable database of published miRNA sequences and annotation. Each entry in the miRBase Sequence database represents a predicted hairpin portion of a miRNA transcript (termed mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR). Both hairpin and mature sequences are available for [searching](#) and [browsing](#), and entries can also be retrieved by name, keyword, references and annotation. All sequence and annotation data are also [available for download](#).
- The [miRBase Registry](#) provides miRNA gene hunters with unique names for novel miRNA genes prior to publication of results. Visit the [help pages](#) for more information about the naming service.

To receive email notification of data updates and feature changes please subscribe to the [miRBase announcements mailing list](#). Any queries about the website or naming service should be directed at [mirbase@manchester.ac.uk](mailto:mirbase@manchester.ac.uk).

miRBase is managed by the [Griffiths-Jones lab](#) at the [Faculty of Biology, Medicine and Health, University of Manchester](#) with funding from the [BBSRC](#). miRBase was previously hosted and supported by the [Wellcome Trust Sanger Institute](#).

**References**

**If you make use of the data presented here, please cite the following articles in addition to the primary data sources:**

[miRBase: from microRNA sequences to function.](#)  
Kozomara A, Birgaoanu M, Griffiths-Jones S.  
*Nucleic Acids Res* 2010; 38:D155-D162

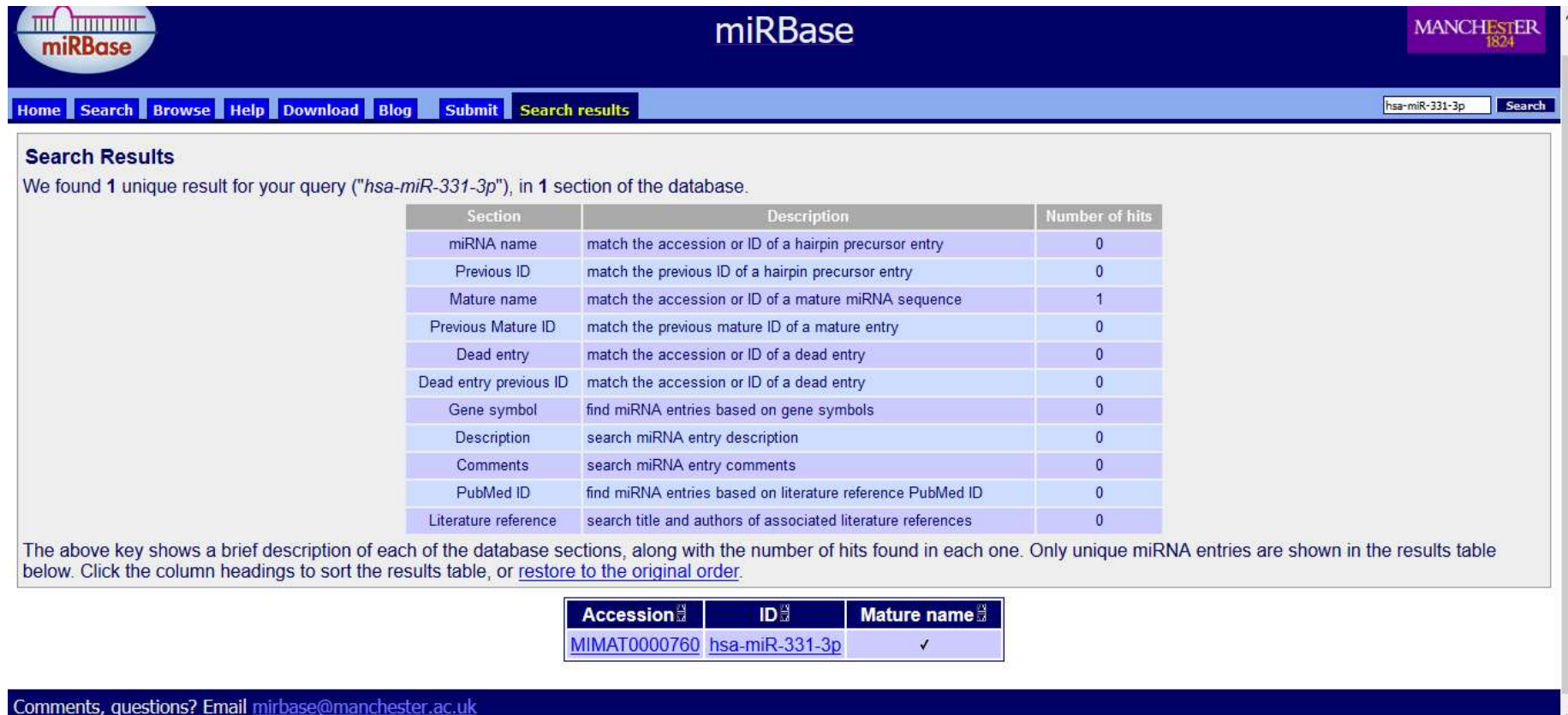
miRNA count: 38589 entries  
Release 22.1

Search by miRNA name or keyword  
Go Example

Download published miRNA data  
Download page

# microRNA database

## <http://www.mirbase.org/>



The screenshot shows the miRBase website interface. At the top, there is a navigation bar with links for Home, Search, Browse, Help, Download, Blog, Submit, and Search results. The search bar contains the query "hsa-miR-331-3p". Below the navigation bar, the search results are displayed. A message states: "We found 1 unique result for your query ('*hsa-miR-331-3p*'), in 1 section of the database." A table lists the search sections and the number of hits for each. The 'Mature name' section has 1 hit. Below the table, a key explains the columns: Accession (MIMAT0000760), ID (hsa-miR-331-3p), and Mature name (checked).

**miRBase** MANCHESTER 1824

Home Search Browse Help Download Blog Submit **Search results** hsa-miR-331-3p Search

### Search Results

We found 1 unique result for your query ("*hsa-miR-331-3p*"), in 1 section of the database.

Section	Description	Number of hits
miRNA name	match the accession or ID of a hairpin precursor entry	0
Previous ID	match the previous ID of a hairpin precursor entry	0
Mature name	match the accession or ID of a mature miRNA sequence	1
Previous Mature ID	match the previous mature ID of a mature entry	0
Dead entry	match the accession or ID of a dead entry	0
Dead entry previous ID	match the accession or ID of a dead entry	0
Gene symbol	find miRNA entries based on gene symbols	0
Description	search miRNA entry description	0
Comments	search miRNA entry comments	0
PubMed ID	find miRNA entries based on literature reference PubMed ID	0
Literature reference	search title and authors of associated literature references	0

The above key shows a brief description of each of the database sections, along with the number of hits found in each one. Only unique miRNA entries are shown in the results table below. Click the column headings to sort the results table, or [restore to the original order](#).

Accession	ID	Mature name
<a href="#">MIMAT0000760</a>	<a href="#">hsa-miR-331-3p</a>	✓

Comments, questions? Email [mirbase@manchester.ac.uk](mailto:mirbase@manchester.ac.uk)

# microRNA database

## <http://www.mirbase.org/>



miRBase

MANCHESTER 1834

Home Search Browse Help Download Blog Submit

Mature sequence hsa-miR-331-3p

**Accession number** MIMAT0000760

**ID** hsa-miR-331-3p

**Previous IDs** hsa-miR-331

**Stem-Loop** [hsa-mir-331](#)

**Sequence** [gcccucugggcccuaucuuagaa](#)  
[Get sequence](#)

**Deep sequencing** [118219](#) reads, 152 experiments

**Database links** RNAcentral: [URS00003DDE27\\_9606](#)

**Predicted targets** TargetMiner: [hsa-miR-331-3p](#)  
TargetScanVert: [hsa-miR-331-3p](#)  
miRDB: [hsa-miR-331-3p](#)  
microna.org: [hsa-miR-331-3p](#)

**QuickGO function**

Qualifier	GO term	Evidence	Notes	Reference
part_of	<a href="#">GO:0005615</a> extracellular space	<a href="#">ECO:0007005</a> high throughput direct assay evidence used in manual assertion	undefined <a href="#">UBERON:0001969</a>	<a href="#">PMID:26646931</a>

[Records 1-1 of 1] [\[QuickGO full record\]](#)

**References**

- PMID: [14691248](#)  
"Identification of many microRNAs that copurify with polyribosomes in mammalian neurons"  
Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM, Ruvkun G  
Proc Natl Acad Sci U S A. 101:360-365(2004).
- PMID: [15634332](#)  
"New human and mouse microRNA genes found by homology search"  
Weber MJ



# microRNA database

## <http://mirdb.org/>



[Target Search](#)

[Target Mining](#)

[Custom Prediction](#)

[FuncMir Collection](#)

[Data Download](#)

[Statistics](#)

[Help | FAQ](#)

[Comments](#)

[Citation | Policy](#)

Choose one of the following search options:

### Search by miRNA name

Human

### Search by gene target

Human  Gene Symbol

miRDB is an online database for miRNA target prediction and functional annotations. All the targets in miRDB were predicted by a bioinformatics tool, MirTarget, which was developed by analyzing thousands of miRNA-target interactions from high-throughput sequencing experiments. Common features associated with miRNA target binding have been identified and used to predict miRNA targets with machine learning methods. miRDB hosts predicted miRNA targets in five species: human, mouse, rat, dog and chicken. As a recent update, users may provide their own sequences for customized target prediction. In addition, through combined computational analyses and literature mining, functionally active miRNAs in humans and mice were identified. These miRNAs, as well as associated functional annotations, are presented in the FuncMir Collection in miRDB.

### References:

- Nathan Wong and Xiaowei Wang (2015) miRDB: an online resource for microRNA target prediction and functional annotations. *Nucleic Acids Research*. [43\(D1\):D146-152](#).
- Xiaowei Wang (2016) Improving microRNA target prediction by modeling with unambiguously identified microRNA-target pairs from CLIP-Ligation studies. *Bioinformatics*. [32\(9\):1316-1322](#).

# microRNA database

<http://mirdb.org/>



There are 411 predicted targets for hsa-miR-331-3p in miRDB.

Target Detail	Target Rank	Target Score	miRNA Name	Gene Symbol	Gene Description
<a href="#">Details</a>	1	99	hsa-miR-331-3p	<a href="#">NRP2</a>	neuropilin 2
<a href="#">Details</a>	2	96	hsa-miR-331-3p	<a href="#">PTPN2</a>	protein tyrosine phosphatase, non-receptor type 2
<a href="#">Details</a>	3	96	hsa-miR-331-3p	<a href="#">ZBTB2</a>	zinc finger and BTB domain containing 2
<a href="#">Details</a>	4	96	hsa-miR-331-3p	<a href="#">PHLPP1</a>	PH domain and leucine rich repeat protein phosphatase 1
<a href="#">Details</a>	5	94	hsa-miR-331-3p	<a href="#">CPSF2</a>	cleavage and polyadenylation specific factor 2
<a href="#">Details</a>	6	94	hsa-miR-331-3p	<a href="#">ZNF652</a>	zinc finger protein 652
<a href="#">Details</a>	7	93	hsa-miR-331-3p	<a href="#">DCLRE1B</a>	DNA cross-link repair 1B
<a href="#">Details</a>	8	93	hsa-miR-331-3p	<a href="#">TSPAN18</a>	tetraspanin 18
<a href="#">Details</a>	9	92	hsa-miR-331-3p	<a href="#">SLAMF9</a>	SLAM family member 9
<a href="#">Details</a>	10	92	hsa-miR-331-3p	<a href="#">SEMA7A</a>	semaphorin 7A (John Milton Hagen blood group)
<a href="#">Details</a>	11	92	hsa-miR-331-3p	<a href="#">BAIAP2</a>	BAI1 associated protein 2
<a href="#">Details</a>	12	91	hsa-miR-331-3p	<a href="#">CNTNAP4</a>	contactin associated protein like 4
<a href="#">Details</a>	13	90	hsa-miR-331-3p	<a href="#">FBLN7</a>	fibulin 7
<a href="#">Details</a>	14	90	hsa-miR-331-3p	<a href="#">CDC42EP4</a>	CDC42 effector protein 4
<a href="#">Details</a>	15	90	hsa-miR-331-3p	<a href="#">ARHGEF37</a>	Rho guanine nucleotide exchange factor 37
<a href="#">Details</a>	16	90	hsa-miR-331-3p	<a href="#">XPO7</a>	exportin 7
<a href="#">Details</a>	17	88	hsa-miR-331-3p	<a href="#">DUSP5</a>	dual specificity phosphatase 5
<a href="#">Details</a>	18	88	hsa-miR-331-3p	<a href="#">APBA1</a>	amyloid beta precursor protein binding family A member 1
<a href="#">Details</a>	19	88	hsa-miR-331-3p	<a href="#">UBL3</a>	ubiquitin like 3
<a href="#">Details</a>	20	87	hsa-miR-331-3p	<a href="#">TGFBR1</a>	transforming growth factor beta receptor 1
<a href="#">Details</a>	21	87	hsa-miR-331-3p	<a href="#">ZMYM4</a>	zinc finger MYM-type containing 4
<a href="#">Details</a>	22	87	hsa-miR-331-3p	<a href="#">SARM1</a>	sterile alpha and TIR motif containing 1

# microRNA database

<http://mirtarbase.mbc.nctu.edu.tw/php/index.php>

Search... Search Example

## miRTarBase

Home Search Browse Statistics Help Download Contact Us

### miRTarBase: the experimentally validated microRNA-target interactions database

As a database, miRTarBase has accumulated more than three hundred and sixty thousand miRNA-target interactions (MTIs), which are collected by manually surveying pertinent literature after NLP of the text systematically to filter research articles related to functional studies of miRNAs. Generally, the collected MTIs are validated experimentally by reporter assay, western blot, microarray and next-generation sequencing experiments. While containing the largest amount of validated MTIs, the miRTarBase provides the most updated collection by comparing with other similar, previously developed databases.

### Major improvements

Features	miRTarBase 6.0	miRTarBase 7.0
Release date	2015/09/15	2017/09/15
Known miRNA entry	miRBase v20	miRBase v21
Known Gene entry	Entrez 2015	Entrez 2017
Species	18	23
Curated articles	4,966	8,510
miRNAs	3,786	4,076
Target genes	22,563	23,054
CLIP-seq datasets	138	231
Curated miRNA-target interactions	366,181	422,517
Text-mining technique to prescreen literature	NLP	Enhanced NLP

### Current curation

Release 7.0: Sept. 15, 2017

Number of articles: 8,510

Number of species: 23

Number of target genes: 23,054

Number of miRNAs: 4,076

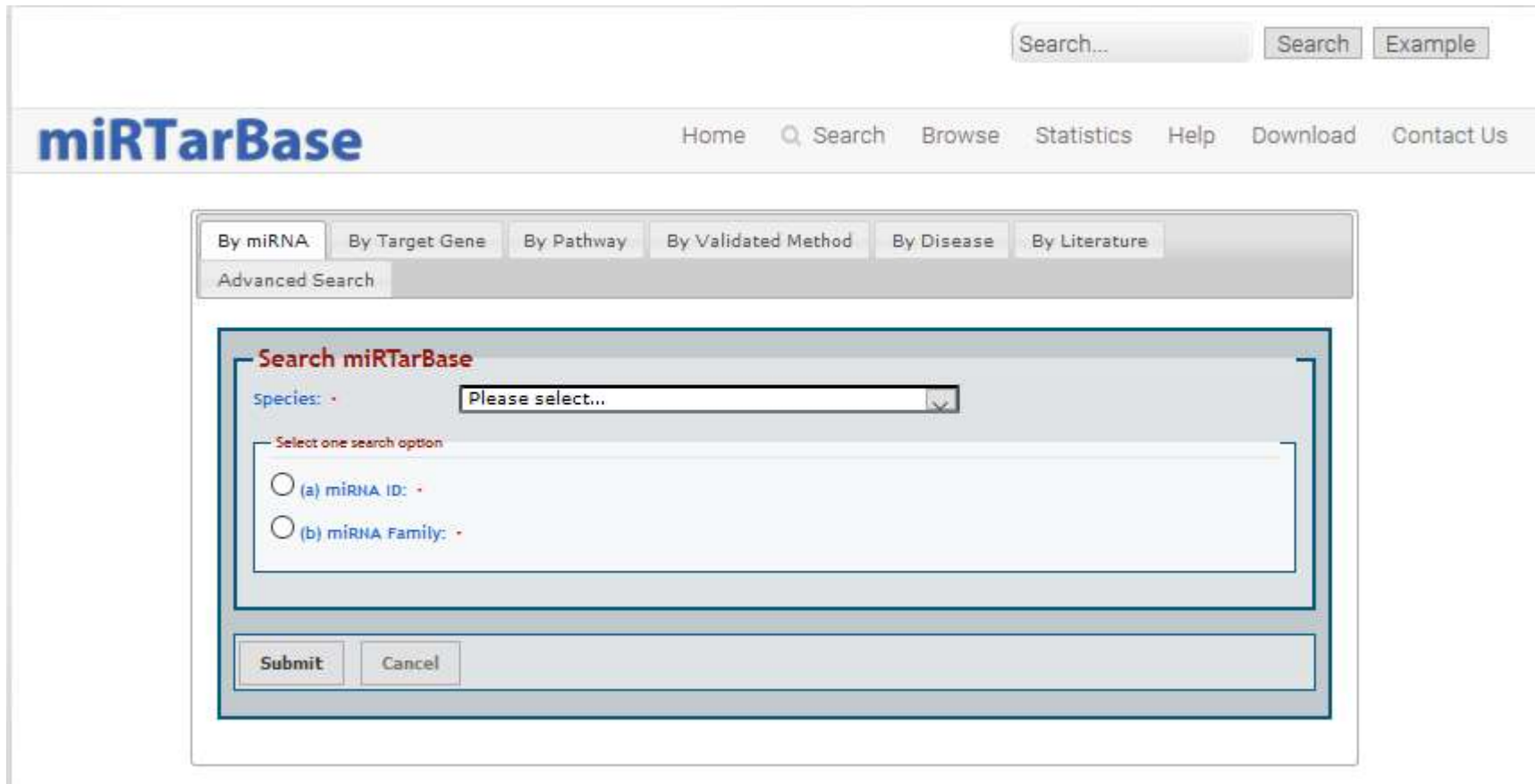
Number of miRNA-target interactions: 422,517

### MicroRNA resources from ISBLAB

miRTar - An integrated web server for identifying miRNA target interactions

## microRNA database

<http://mirtarbase.mbc.nctu.edu.tw/php/index.php>



The image shows the miRTarBase search interface. At the top right, there is a search bar with the text "Search..." and two buttons: "Search" and "Example". Below this is a navigation bar with the miRTarBase logo and links for Home, Search, Browse, Statistics, Help, Download, and Contact Us. The main search area is titled "Advanced Search" and contains several tabs: "By miRNA", "By Target Gene", "By Pathway", "By Validated Method", "By Disease", and "By Literature". The "By miRNA" tab is selected. Below the tabs is a section titled "Search miRTarBase" with a "species:" label and a dropdown menu showing "Please select...". Underneath, there is a section titled "Select one search option" with two radio buttons: "(a) miRNA ID:" and "(b) miRNA Family:". At the bottom of the search area are "Submit" and "Cancel" buttons.

# microRNA database

<http://mirtarbase.mbc.nctu.edu.tw/php/index.php>

**miRTarBase**

Home Search Browse Statistics Help Download Contact Us

Page of 15 < Prev 1 2 ... 15 Next >

Pulsed stable isotope labeling by amino acids in cell culture (pSILAC)

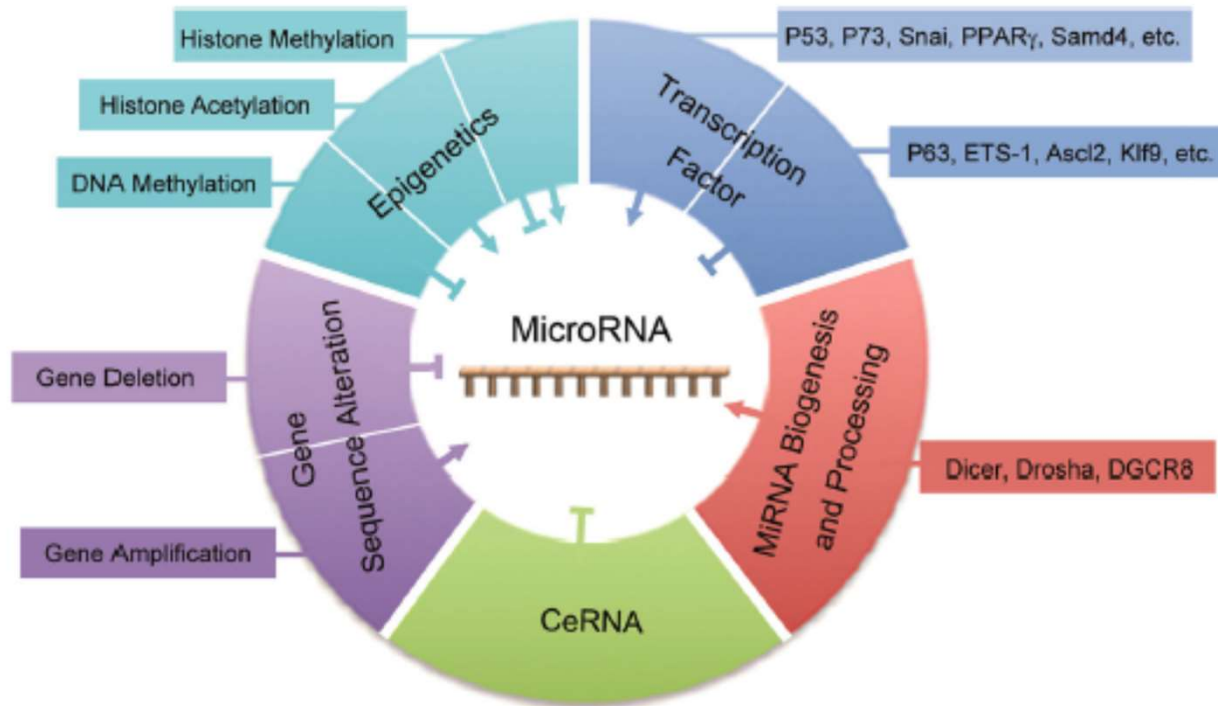
ID	Species (miRNA)	Species (Target)	miRNA	Target	Validation methods								Sum	# of papers
					Strong evidence			Less strong evidence						
					Reporter assay	Western blot	qPCR	Microarray	NGS	pSILAC	Other			
MIRT002222	Rattus norvegicus	Rattus norvegicus	no-miR-331-3p	Fgf16				✓			✓	2	1	
MIRT005805	Homo sapiens	Homo sapiens	hsa-miR-331-3p	ERBB2	✓	✓	✓	✓			✓	5	5	
MIRT006364	Homo sapiens	Homo sapiens	hsa-miR-331-3p	FHIT	✓							1	1	
MIRT006506	Homo sapiens	Homo sapiens	hsa-miR-331-3p	E2F1	✓	✓						2	1	
MIRT006887	Homo sapiens	Homo sapiens	hsa-miR-331-3p	DOHH	✓	✓	✓		✓			4	2	
MIRT019230	Homo sapiens	Homo sapiens	hsa-miR-331-3p	WDR60					✓			1	1	
MIRT019231	Homo sapiens	Homo sapiens	hsa-miR-331-3p	RNF7					✓			1	1	
MIRT019232	Homo sapiens	Homo sapiens	hsa-miR-331-3p	ARLBA					✓			1	1	

# MicroRNAs in the Control of Drug Metabolism and Transport

Some P450 drug-metabolizing enzymes and ABC transporters shown to be targeted by noncoding miRNAs

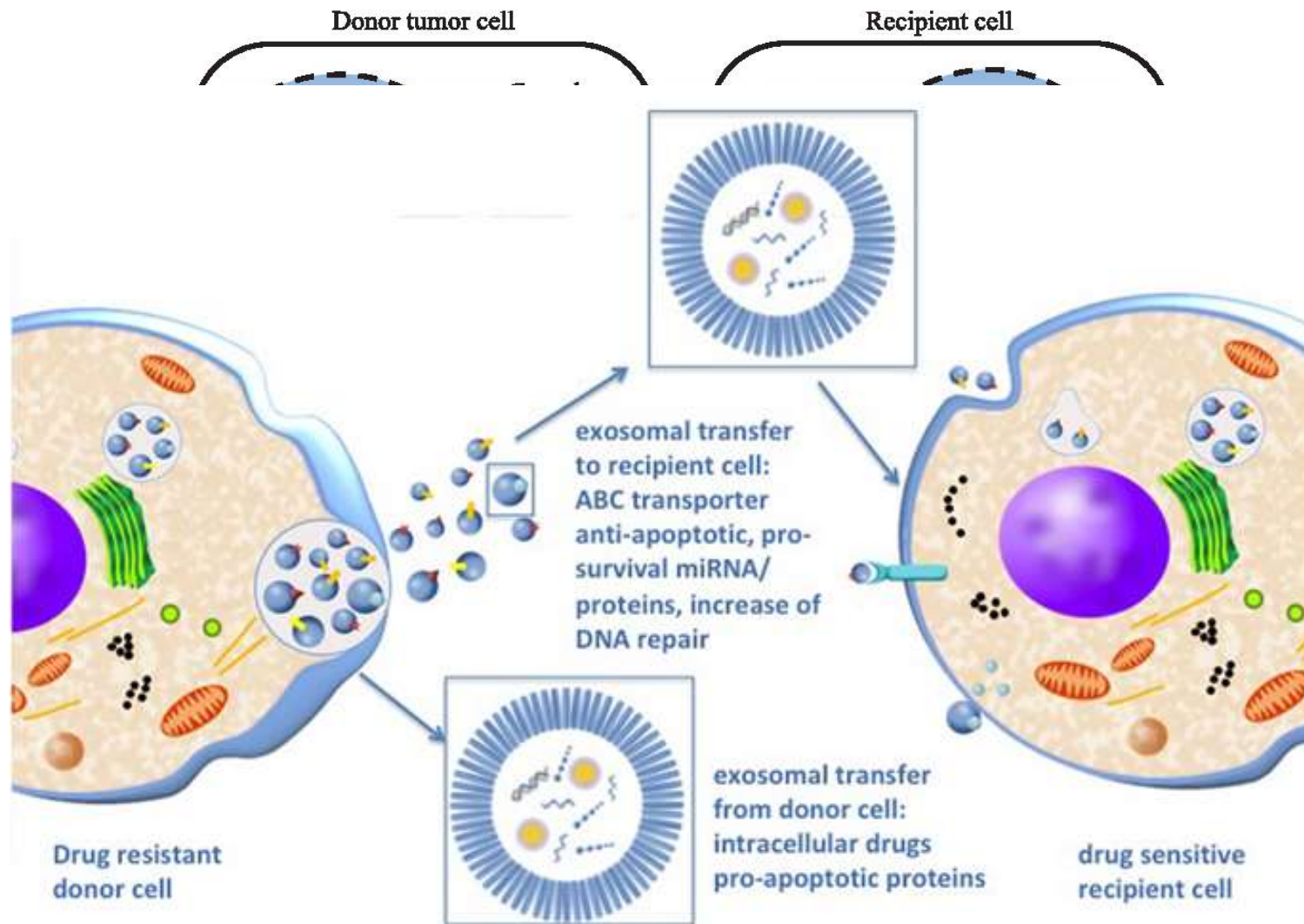
		MicroRNA	Reference
Enzymes	CYP1B1	miR-27b	Tsuchiya et al., 2006
	CYP2E1	miR-378	Mohri et al., 2010
	CYP3A4	miR-27b, mmu-miR-298	Pan et al., 2009a
Transporters	ABCB1	miR-451	Kovalchuk et al., 2008
		miR-27a	Zhu et al., 2008
	ABCG2	miR-520h	Liao et al., 2008; Wang et al., 2010; Li et al., 2011
		miR-519c	To et al., 2008; To et al., 2009; Li et al., 2011
		miR-328	Pan et al., 2009b; Li et al., 2011
	ABCC1	miR-134	Guo et al., 2010
		miR-326	Liang et al., 2010
		miR-199a, miR-199b, miR-296	Borel et al., 2012
		miR-1291	Pan et al., 2013
	ABCC2	miR-379	Haenisch et al., 2011
	ABCC3	miR-9-3p	Jeon et al., 2011
	ABCC4	miR-125a, miR-125b	Borel et al., 2012
ABCC5	miR-101, miR-125a, Let-7a	Borel et al., 2012	
	miR-128	Zhu et al., 2011	
ABCC6	miR-9-3p	Jeon et al., 2011	

# Deregulated expression of microRNAs



The mechanisms of deregulated expression of microRNAs. Different mechanisms can promote or/and inhibit the expression of miRNA

# Exosomes-derived microRNA and drug resistance



 miR-binding Protein





# Circulating microRNA

## cerebrospinal fluid

PCNSL miR-19, miR-21, miR-92a [33]  
 Glioblastoma miR-21 [34]

## pleural effusion

Lung cancer miR-198 [35]  
 Lung cancer miR-22, miR-134, miR-185 [36]  
 Malignant pleural mesothelioma  
 let-7a, miR-125a-5p, miR-320, miR-484 [37]

## ascites

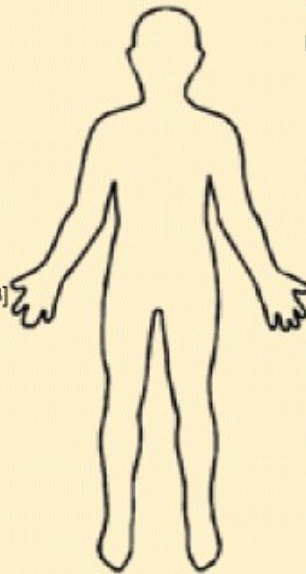
Ovarian cancer miR-21, miR-23b, miR-29a [38]  
 Serosa-invasive gastric cancer  
 miR-21, miR-1225-5p [39]

## vaginal discharge

Cervical cancer miR-21, miR-146a [40]

## urine

Ovarian cancer miR-30-5p [41]  
 Bladder cancer miR-106b [42]  
 Bladder cancer miR-99a, miR-125b [43]  
 Bladder cancer miR-155, miR-21, miR-125b, miR-451 [44]  
 Prostate cancer miR-484, miR-1825 [45]



## saliva

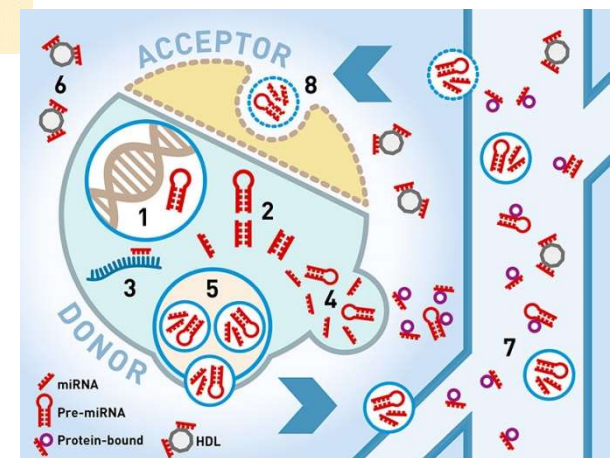
Oral cancer miR-125a, miR-200a [46]  
 Esophageal cancer miR-10b-3p, miR-21,  
 miR-144, miR-451 [47]  
 Pancreatic cancer miR-940, miR-3679-5p [48]

## breast milk

miR-335-3p, miR-26a-2-3p, miR-181d [49]

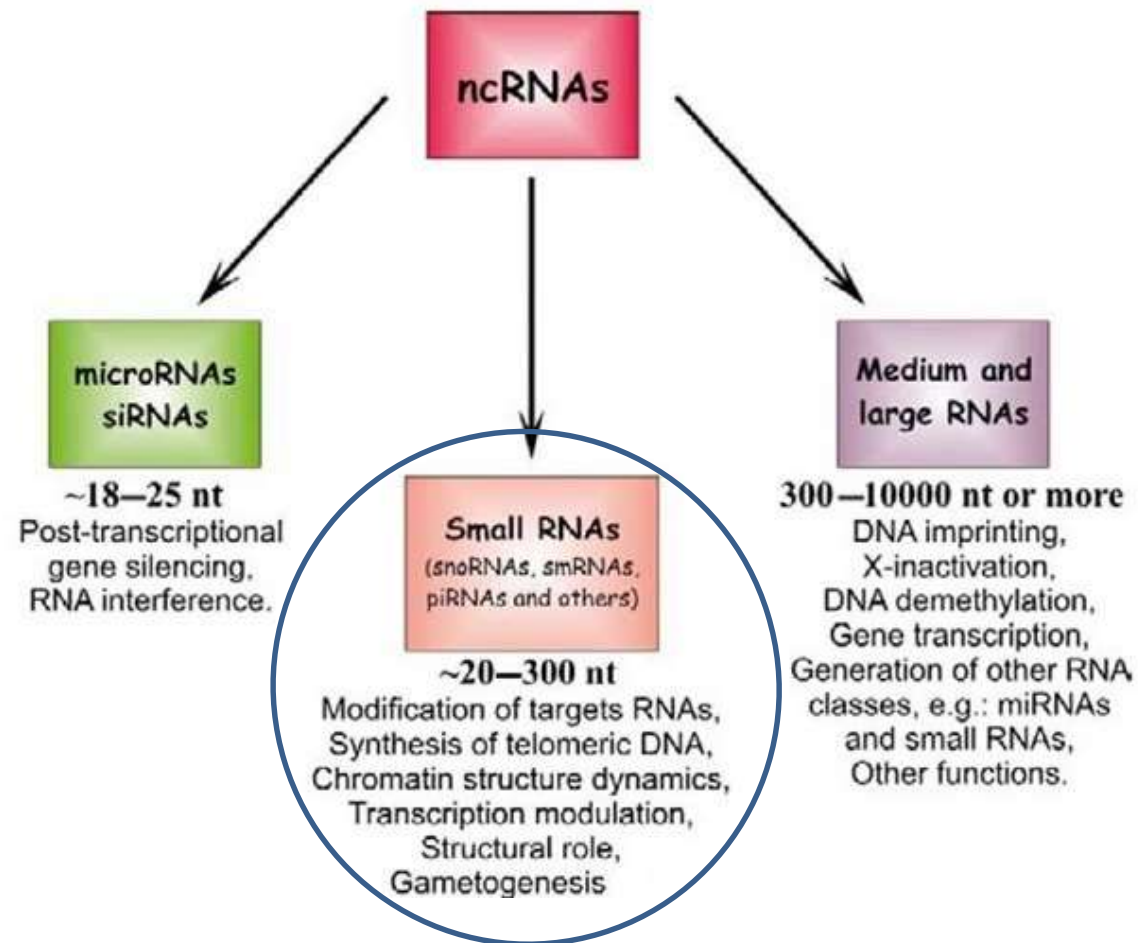
## blood (serum/plasma)

Ovarian cancer miR-21, miR-141, miR-200a,  
 miR-200b, miR-200c, miR-203, miR-205,  
 miR-214 [50]  
 Gastric cancer miR-1, miR-20a, miR-27a,  
 miR-34, miR-423-5p [51]  
 Malignant melanoma miR-221 [52]  
 Cervical cancer miR-218 [53]  
 Thyroid cancer let-7e, miR-151-5p, and miR-222 [54]  
 Renal cell cancer miR-378, miR-451 [55]  
 Colorectal cancer miR-7, miR-93, miR-409-3p [56]  
 Breast cancer miR-148-3p, miR-652-3p [57]  
 NSCLC miR-21, miR-148a, miR-148b, miR-152 [58]



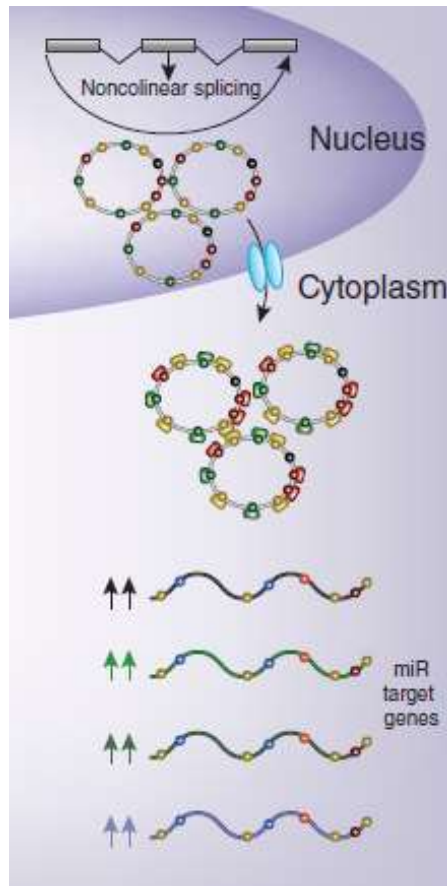
# RNA non codificante

Non-coding RNA is an RNA that functions without being translated to a protein.

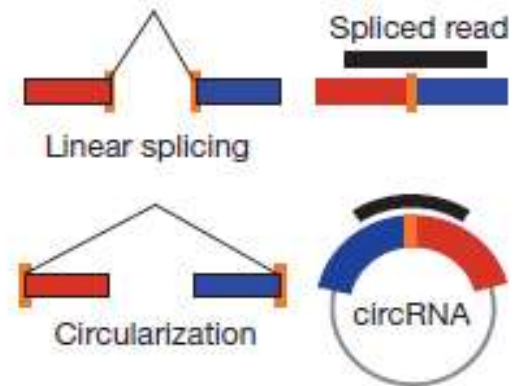


# circRNAs

- Covalently circularized RNA loops
- Stable in cells and long in half-lives
- Multiple miRNA binding sites as miRNA sponges



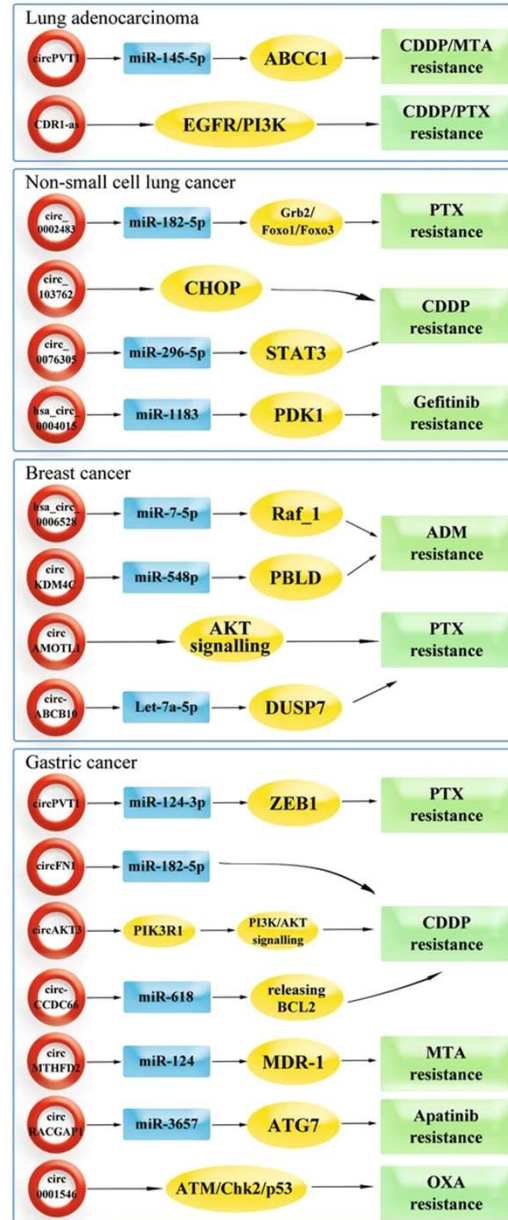
Nature Structural & Molecular Biology (2013), 20:5, 541-3



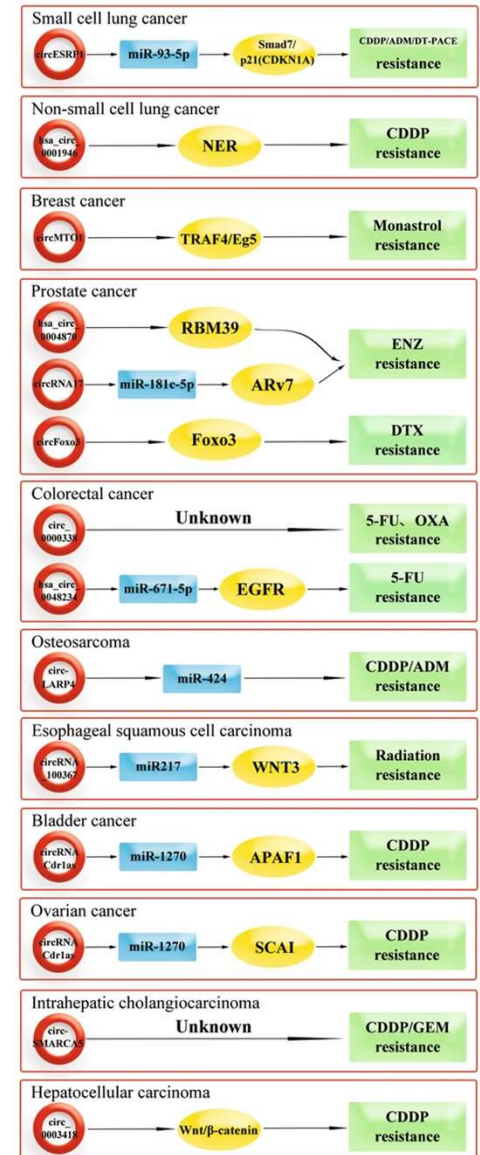
Nature (2013) 495, 333-343



## CircRNAs promote drug resistance



## CircRNAs inhibit drug resistance

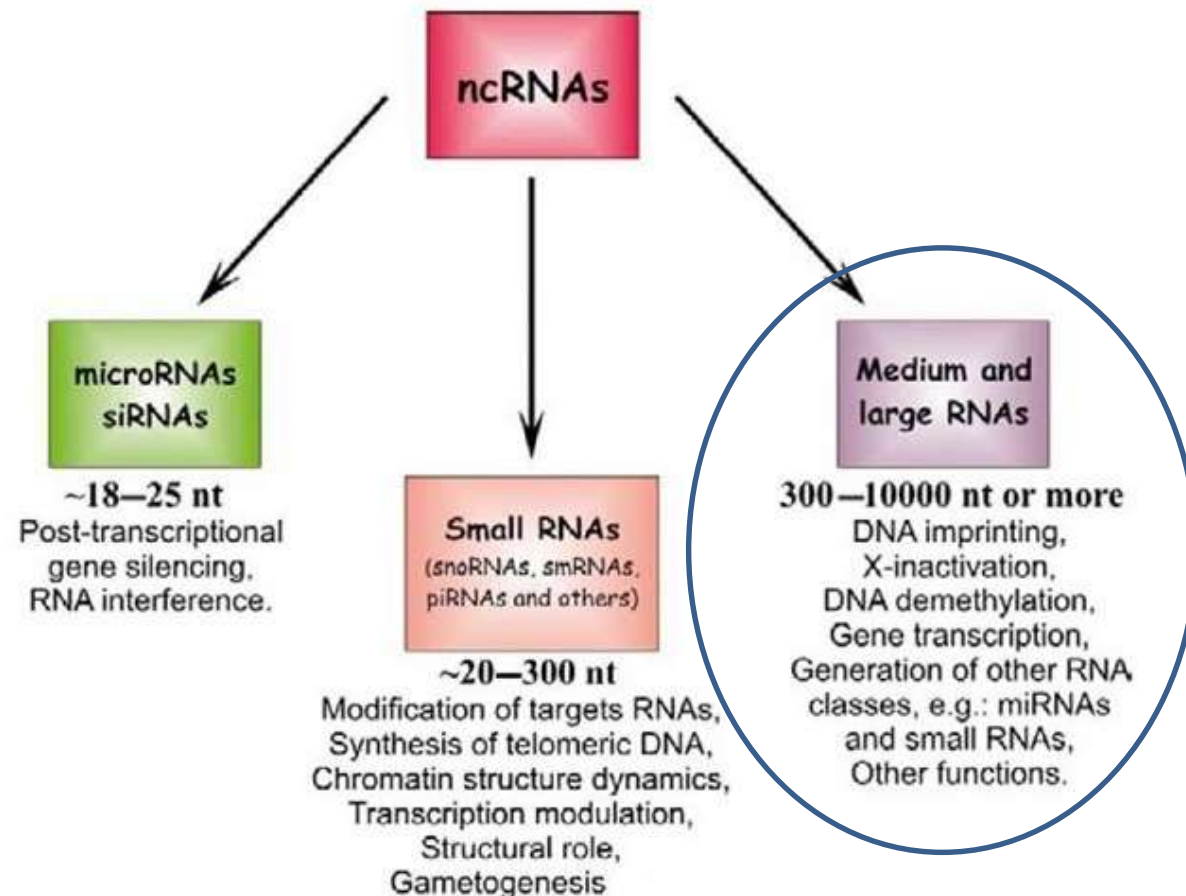


Review | Open Access | Published: 05 August 2020

## CircRNAs: biogenesis, functions, and role in drug-resistant Tumours

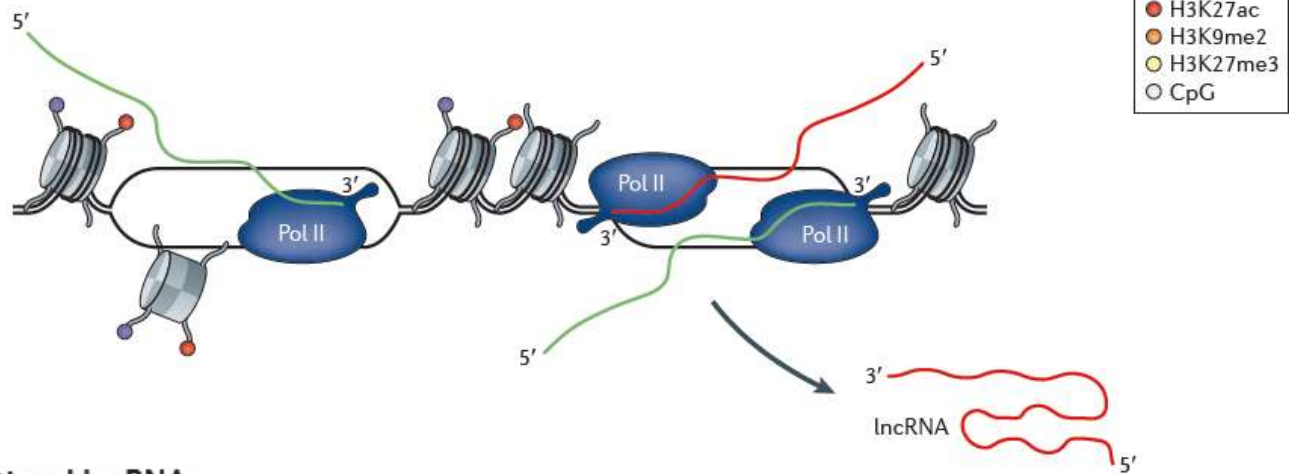
# What is non-coding RNA ?

Non-coding RNA is an RNA that functions without being translated to a protein.

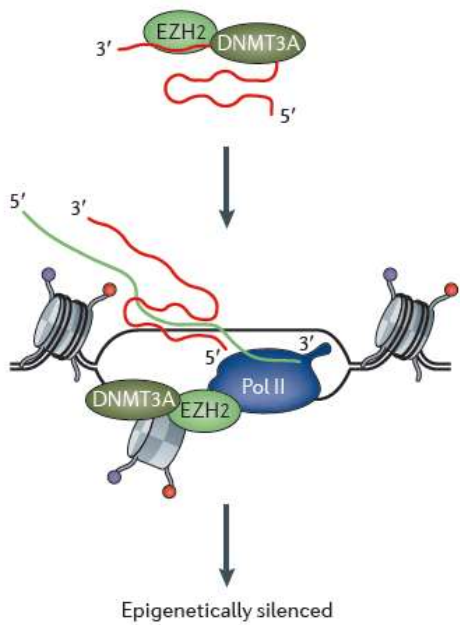


# lncRNA

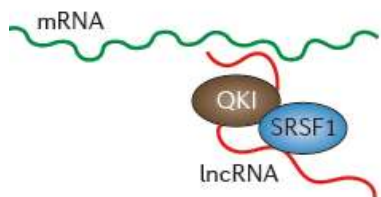
## Transcription of lncRNAs



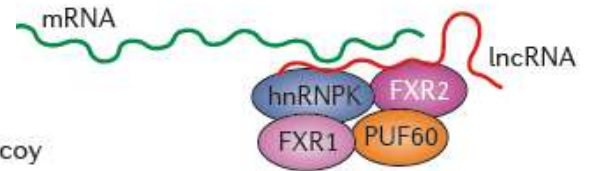
## Nuclear and cytoplasmic functional lncRNAs



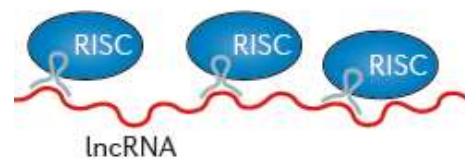
### lncRNA as splicing regulator



### lncRNA as translational regulator



### lncRNA as miRNA decoy



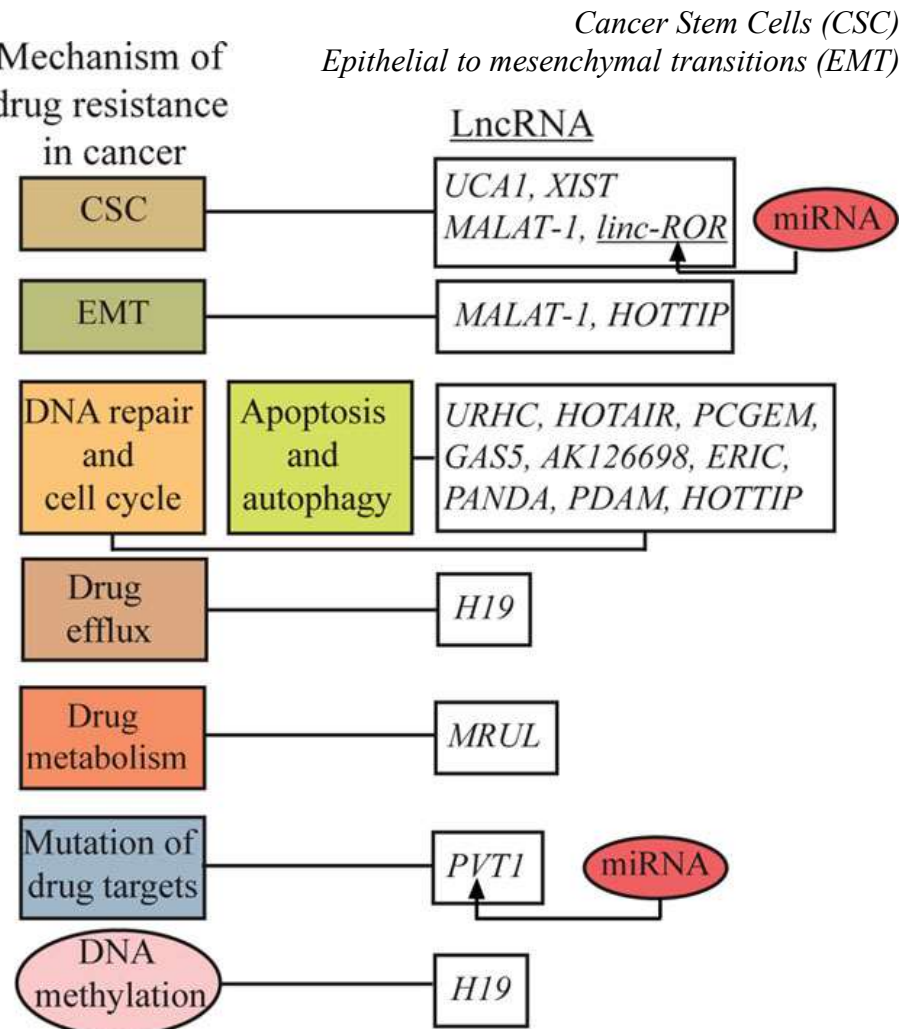
# lncRNA

## LncRNAs and drugs affecting their expression in cancers

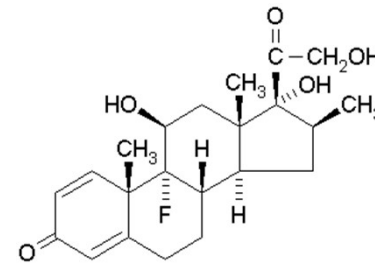
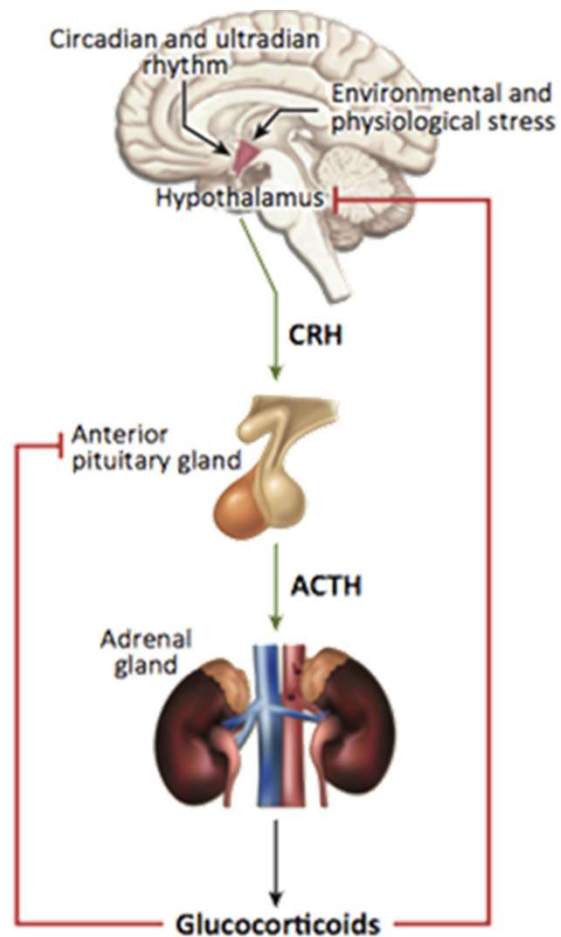
LncRNA	Cancer	Drug
<i>UCA1</i>	Bladder	?
<i>linc-ROR</i>	Hepatocellular carcinoma	Sorafenib and doxorubicin
<i>XIST</i>	Ovarian, breast	Cisplatin, abexinostat
<i>MALAT-1</i>	Pancreas	Gemcitabine
<i>URHC</i>	Hepatocellular carcinoma	PD98059
<i>HOTAIR</i>	Lung	Cisplatin
<i>PCGEM1</i>	Prostate	Doxorubicin
<i>GAS5</i>	Lung	Gefitinib
<i>AK126698</i>	Lung	Cisplatin
<i>ERIC</i>	Bone osteosarcoma	Etoposide
<i>PANDA</i>	Breast	Doxorubicin
<i>PDAM</i>	Oligodendroglial	Cisplatin
<i>HOTTIP</i>	Pancreas	Gemcitabine
<i>rRNA</i>		Mitoxantrone
<i>H19</i>	Hepatocellular carcinoma cells	Paclitaxel, doxorubicin, etoposide, and vincristine
<i>MRUL</i>	Gastric	Doxorubicin and vincristine
<i>ARA</i>	Breast, hepatocellular carcinoma cells	Doxorubicin
<i>PVT1</i>	Pancreas, gastric cancer	Gemcitabine, paclitaxel
<i>BCAR4</i>	Breast	Tamoxifen

*Urothelial Carcinoma-Associated 1 (UCA1)*  
*Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT-1)*

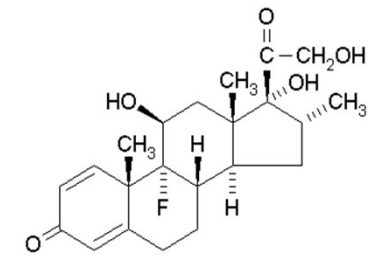
## Mechanism of drug resistance in cancer



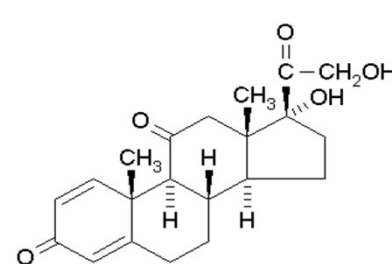
# Pharmacoeigenetics of Glucocorticoids



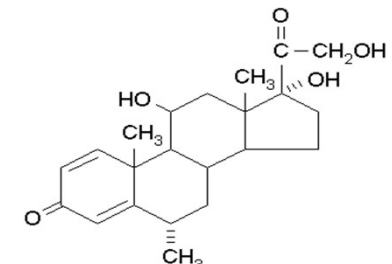
BETAMETHASONE



DEXAMETHASONE



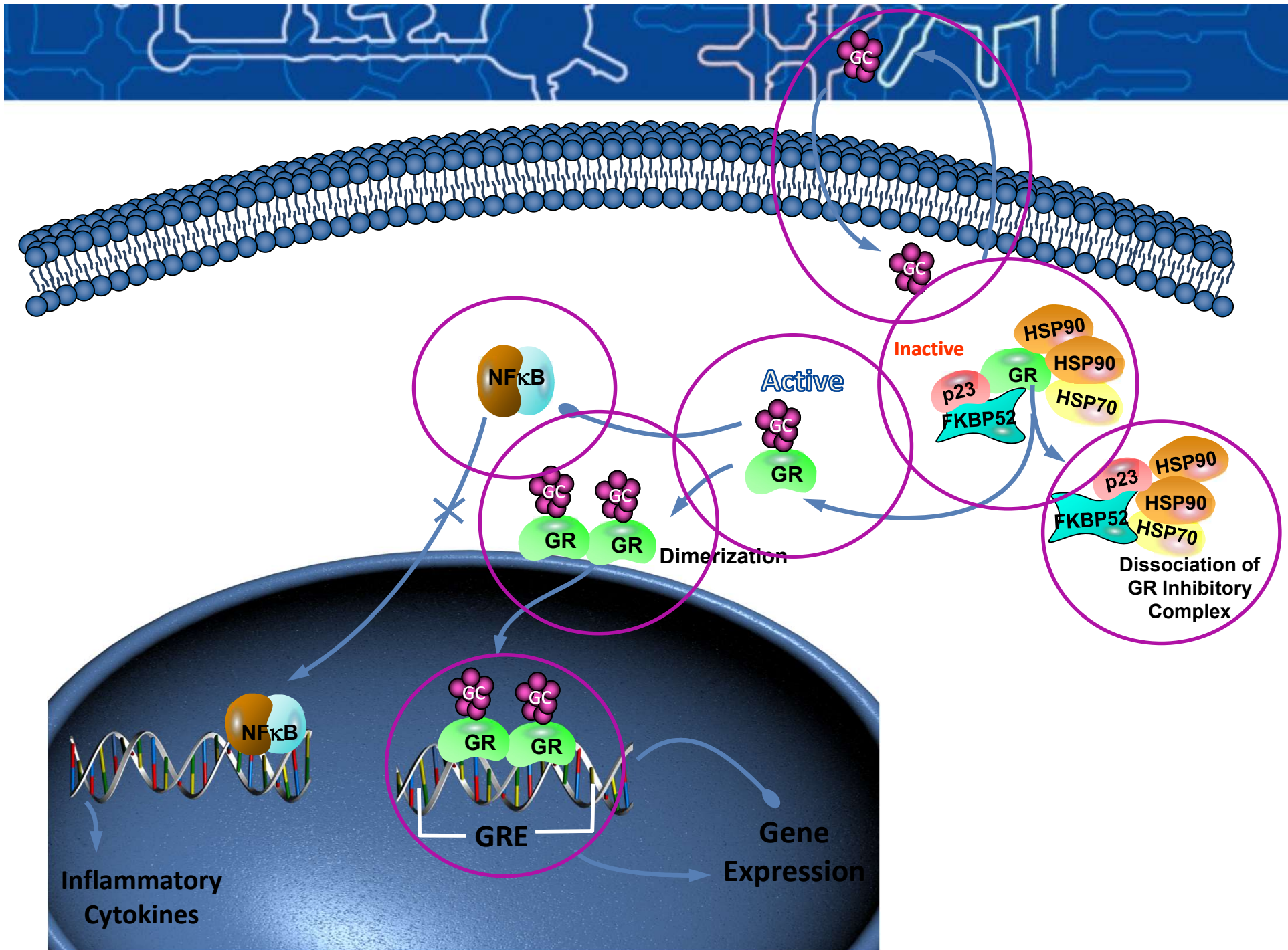
PREDNISONE



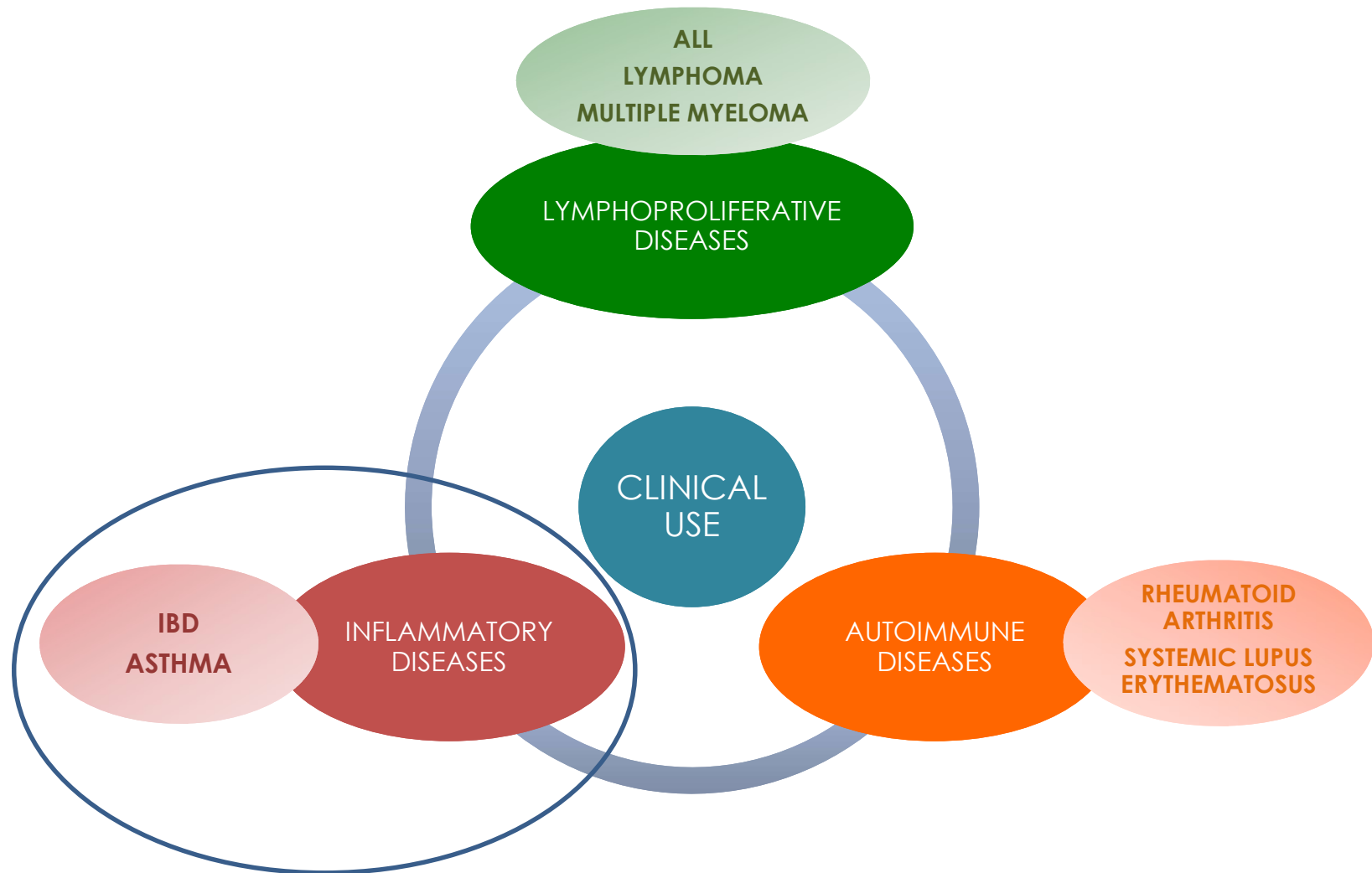
METHYLPREDNISOLONE

(Kadmiel & Cidlowski, 2013)



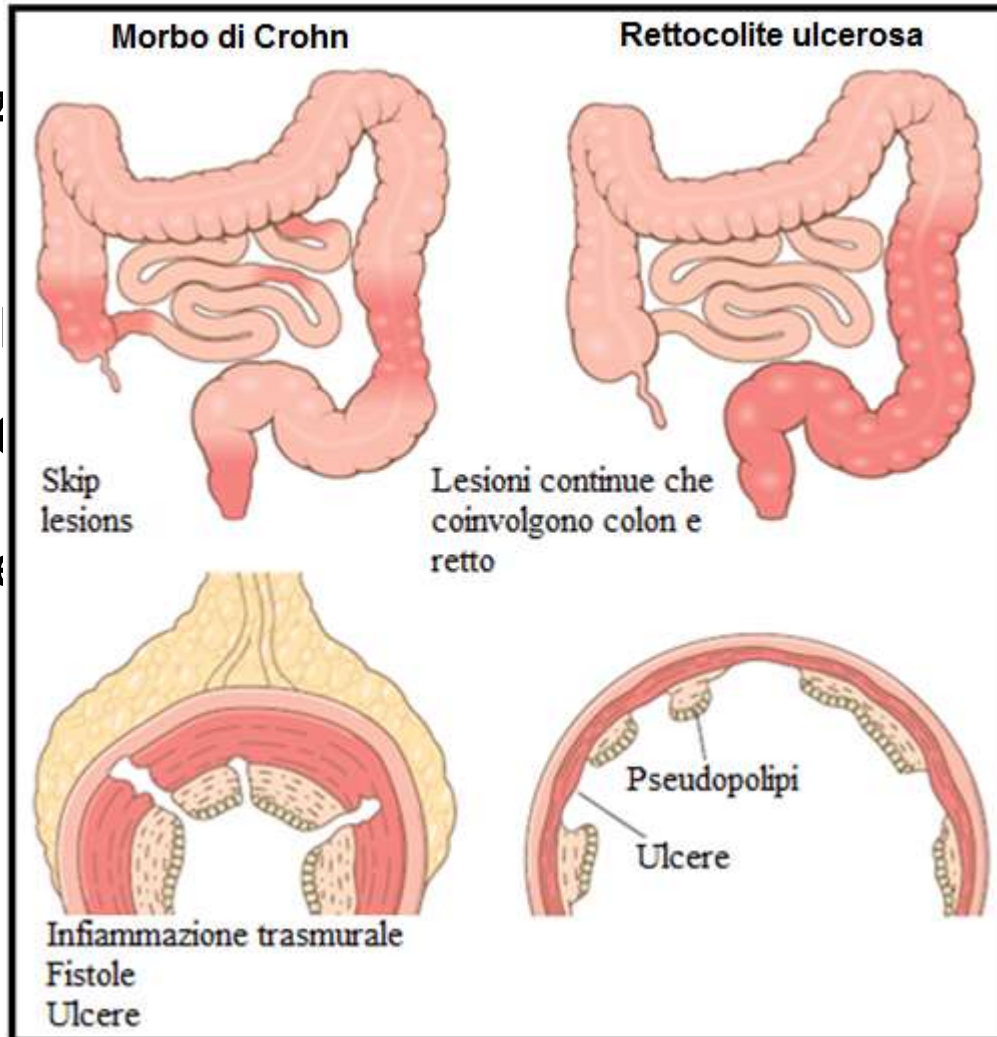
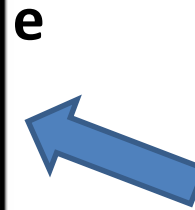


# GLUCOCORTICOIDS



# IBD

- **Infiammaz**
- **Due princ**
- **Morbo di C**
- **Rettocolite**



e

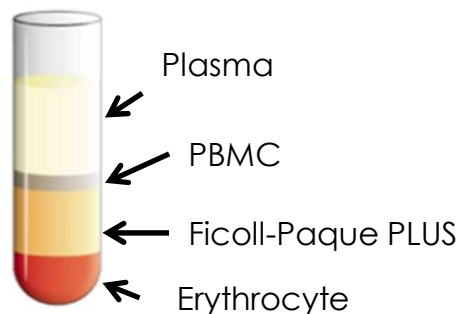
# EPIGENETIC PREDICTORS OF GLUCOCORTICOID RESPONSE IN CHILDREN WITH IBD

The research project was supported by Italian Ministry of Health, No. 44/GR-2010-2300447








**Correlation**  
between miRNAs expression and the clinical response to GC therapy

# CANDIDATE PREDICTORS OF GLUCOCORTICOID RESPONSE IN CHILDREN WITH IBD



Article

## High-Throughput Sequencing of microRNAs in Glucocorticoid Sensitive Paediatric Inflammatory Bowel Disease Patients

Sara De Iudicibus<sup>1,†</sup>, Marianna Lucafò<sup>2,†</sup> , Nicola Vitulo<sup>3</sup>, Stefano Martellosi<sup>1</sup>, Rosanna Zimbello<sup>4</sup>, Fabio De Pascale<sup>4</sup>, Claudio Forcato<sup>4</sup>, Samuele Naviglio<sup>5</sup> , Alessia Di Silvestre<sup>5</sup>, Marco Gerdol<sup>6</sup> , Gabriele Stocco<sup>6</sup> , Giorgio Valle<sup>4</sup>, Alessandro Ventura<sup>1,2</sup>, Matteo Bramuzzo<sup>1,\*</sup>  and Giuliana Decorti<sup>1,2</sup>

**T4 vs T0**

Table 1. Differentially expressed miRNAs.

Upregulated miRNAs	FC	FDR Corrected P-Value	Downregulated miRNAs	FC	FDR Corrected p-Value
hsa-miR-451a * [13]	4.16	$1.66 \times 10^{-6}$			
hsa-miR-144-3p * [14,15]	4.44	$1.04 \times 10^{-5}$			
hsa-miR-96-5p * [13,14]	2.96	$6.38 \times 10^{-3}$			
hsa-miR-29b-3p * [13]	2.89	0.026			
hsa-miR-142-3p * [14]	2.21	0.026			
hsa-miR-873-5p	3.36	0.026			
hsa-miR-29c-3p * [16,17]	3.37	0.037			
hsa-miR-29a-3p * [13]	2.72	0.041	hsa-miR-7109-3p	-4.62	0.044
hsa-miR-363-3p	2.31	0.041	hsa-miR-654-5p	-2.27	0.049
hsa-miR-141-3p	2.59	0.041			
hsa-miR-548ak	3.11	0.042			
hsa-let-7g-3p* [18]	2.44	0.042			
hsa-miR-4772-5p	2.70	0.047			
hsa-miR-106a-3p	3.52	0.047			
hsa-miR-31-3p	3.36	0.049			
hsa-miR-146b-5p * [19]	2.27	0.049			

Fold changes (FC) for each miRNA regulated by glucocorticoids (GCs); \* Linked to GC regulation in the literature. FDR, False Discovery Rate.

# EPIGENETIC PREDICTORS OF GLUCOCORTICOID RESPONSE IN CHILDREN WITH IBD

Validation of Selected Differentially Expressed miRNAs by qRT-PCR

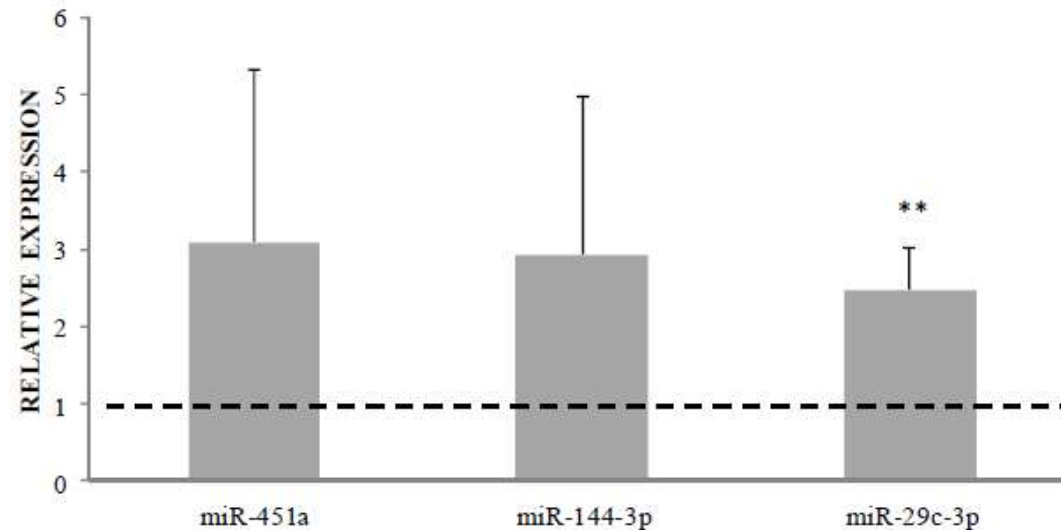


Figure 2. Relative expression of miR-451a, miR-144-3p, and miR-29c-3p (calculated as  $2^{-\Delta\Delta C_t}$  T4 vs. T0). Values > 1 (dotted line) indicate upregulation, values < 1 indicate downregulation. Parametric *t*-test  $\Delta C_t$  T0 vs. T4, \*\*  $p < 0.01$ .

Table 2. Glucocorticoid responsive element (GRE) sites predicted on miRNA promoter regions.

miRNA	pGRE	Start	End	Strand	Chrom	Expression
hsa-miR-363	GTGATAATGTGTGCTT	133303695	133303710	-	chrX	Up
hsa-miR-96	AGGACAAAGAGTCCTC	129416083	129416098	-	chr7	Up
hsa-miR-142	CTCACCTTCAGTTCTG	58331606	58331621	+	Chr17	Up
hsa-miR-142	CTGTCACTCTGTCTC	58332656	58332671	-	Chr17	Up

GC-sensitive miRNAs presenting positive GREs (pGRE).

# CANDIDATE PREDICTORS OF GLUCOCORTICOID RESPONSE IN CHILDREN WITH IBD

T0 PRvsPS			
UP	FC	DOWN	FC
hsa-miR-1180-3p	7,96	hsa-miR-100-5p	43,95
hsa-miR-3591-3p	11,2	hsa-miR-1227-5p	2093,27
		hsa-miR-1255a	45,07
		hsa-miR-1271-5p	17,98
		hsa-miR-24-2-5p	15
		hsa-miR-25-5p	19,44
		hsa-miR-3065-5p	22,25
		hsa-miR-31-3p	25,65
		hsa-miR-3196	38,62
		hsa-miR-3656	19,63
		hsa-miR-3960	150,33
		hsa-miR-4443	47,81
		hsa-miR-4772-3p	20,55
		hsa-miR-5586-3p	17,04
		hsa-miR-6075	4465,59
		hsa-miR-6087	25,93
		hsa-miR-618	26,38
		hsa-miR-876-5p	19,32



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SCIENTIFIC REPORTS

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**MiR-1180 promotes apoptotic resistance to human hepatocellular carcinoma via activation of NF- $\kappa$ B signaling pathway**

Received: 00 July 2015  
Accepted: 03 February 2016  
Published: 01 March 2016

Guosheng Tan<sup>1,2</sup>, Linwei Wu<sup>2,3</sup>, Jinfu Tan<sup>2</sup>, Bing Zhang<sup>4</sup>, William Chi-shing Tai<sup>5,6</sup>, Shiqiu Xiong<sup>7</sup>, Wei Chen<sup>1</sup>, Jianyong Yang<sup>1</sup> & Heping Li<sup>1,8</sup>

T4 PRvsPS			
UP	FC	DOWN	FC
hsa-miR-1180-3p	6,48	hsa-miR-1197	10,19
hsa-miR-4732-5p	5	hsa-miR-1227-5p	434,07
		hsa-miR-154-3p	10,26
		hsa-miR-4443	64,78
		hsa-miR-4523	11,82
		hsa-miR-6075	1561,21
		hsa-miR-6087	20,31
		hsa-miR-876-5p	25,93

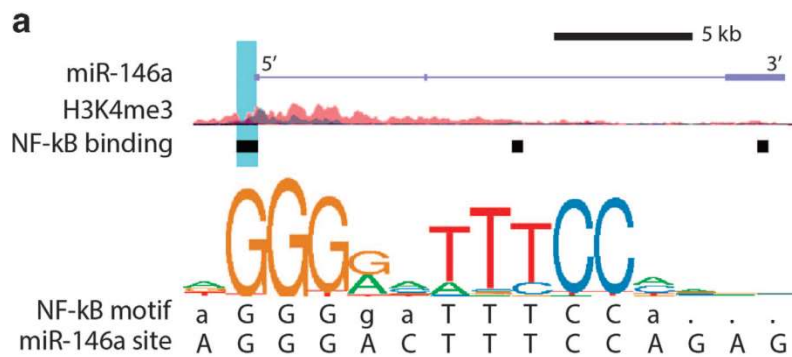
# CANDIDATE PREDICTORS OF GLUCOCORTICOID RESPONSE IN CHILDREN WITH IBD

Clin Transl Gastroenterol. 2016 Sep 15;7(9):e192. doi: 10.1038/ctg.2016.49.

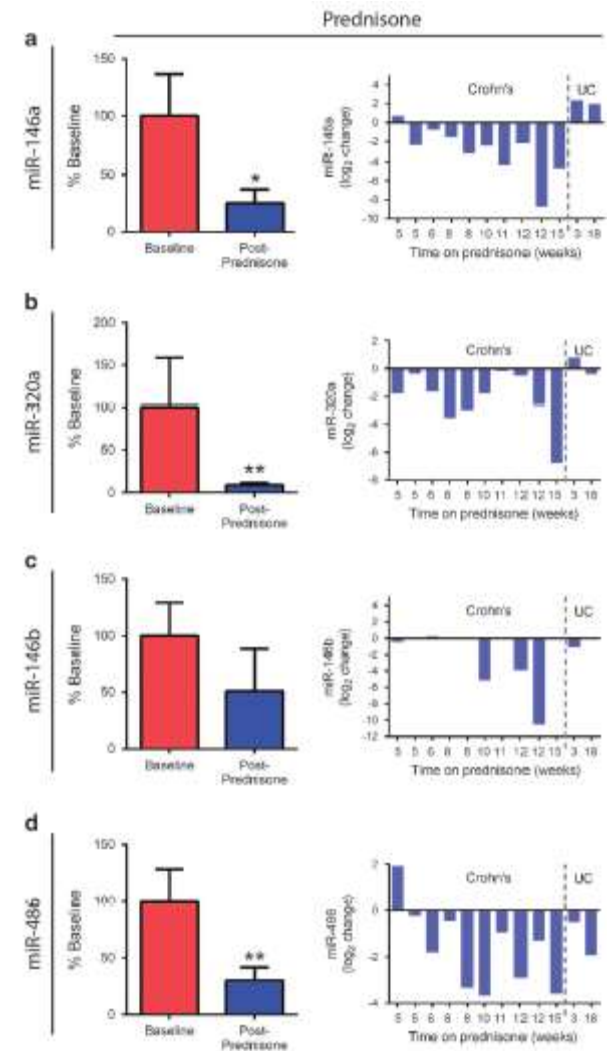
## Identification of Pathway-Specific Serum Biomarkers of Response to Glucocorticoid and Infliximab Treatment in Children with Inflammatory Bowel Disease.

Heier CR<sup>1</sup>, Fiorillo AA<sup>1</sup>, Chaisson E<sup>2</sup>, Gordish-Dressman H<sup>1,3</sup>, Hathout Y<sup>1,3</sup>, Damsker JM<sup>1,4</sup>, Hoffman EP<sup>1,3,4</sup>, Conklin LS<sup>1,2</sup>.

### Bioinformatic analysis of gene regulation pathways

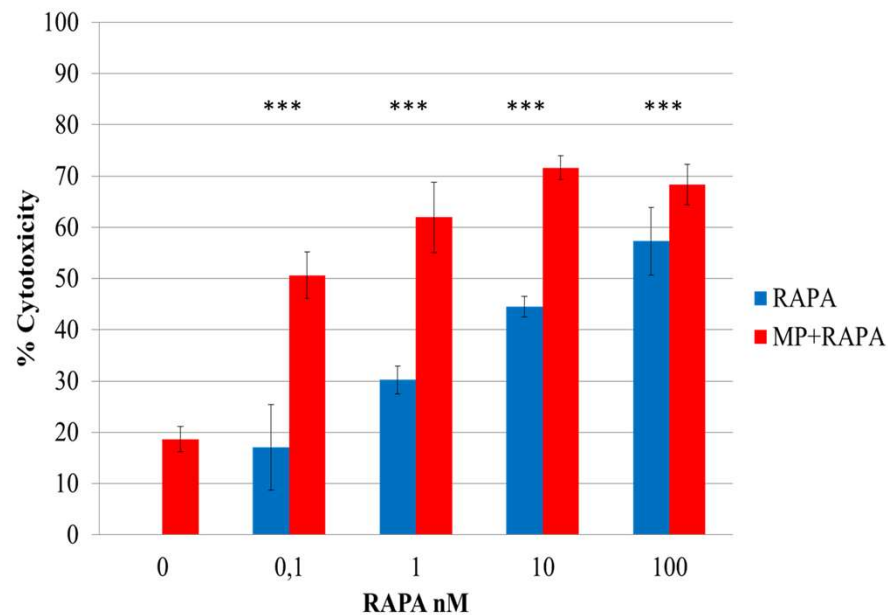


Molecular marker	Expression Regulated by	Source	Drug changed	Up or Down
miR-146a	NF-kB	Wang 2012	Both	Down
miR-146b	NF-kB	Wang 2012	Both	Down
miR-320a	NF-kB and GR	Wang 2012	Both	Down
miR-486	GR	Wang 2012	Prednisone	Down





# DIFFERENTIAL EXPRESSION OF miRNAs IN RAPAMYCIN-INDUCED REVERSION OF GC RESISTANCE



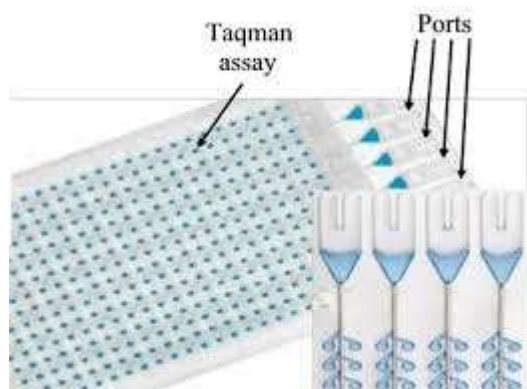
**Fig. 1** Cells were exposed for 72 h to MP at 20  $\mu\text{g}/\text{ml}$  and/or different concentrations of RAPA, and cell proliferation was evaluated by MTT assay. Two-way ANOVA ( $P < 0.0001$ ) and Bonferroni post-test \*\*\*  $p\text{-value} < 0.001$ .

miR-331-3p is involved in glucocorticoid resistance reversion by rapamycin through suppression of the MAPK signaling pathway

# DIFFERENTIAL EXPRESSION OF miRNAs IN RAPAMYCIN-INDUCED REVERSION OF GC RESISTANCE

## Differentially expressed miRNAs.

The expression analysis identified 70, 99 and 96 miRNAs that were differentially expressed after treatment with MP, RAPA and MP+RAPA, respectively.



TaqMan® Array MicroRNA Cards

miRNA up e downregolati selettivamente dai diversi trattamenti farmacologici

	UP	DOWN
<b>MP</b>	hsa-miR-200b-3p	hsa-miR-181c-5p hsa-miR-192-5p hsa-miR-324-3p hsa-miR-361-5p hsa-miR-455-5p hsa-miR-576-3p
<b>RAPA</b>	hsa-miR-140-3p hsa-miR-26b-5p hsa-miR-28-5p hsa-miR-324-5p hsa-miR-454-3p	hsa-miR-142-5p hsa-miR-365a-3p hsa-miR-455-3p hsa-miR-501-5p
<b>MP+RAPA</b>	hsa-miR-30b-5p hsa-miR-30c-5p hsa-miR-331-3p hsa-miR-345-5p hsa-miR-744-5p	hsa-miR-19a-3p hsa-miR-886-3p hsa-miR-886-5p

# DIFFERENTIAL EXPRESSION OF miRNAs IN RAPAMYCIN-INDUCED REVERSION OF GC RESISTANCE

**DIANA miRPath v.2.0**: investigating the combinatorial effect of microRNAs in pathways

<http://snf-515788.vm.oceanos.gnet.gr/>

The screenshot displays the DIANA miRPath v.3 web application interface within a browser window. The browser's address bar shows the URL [snf-515788.vm.oceanos.gnet.gr](http://snf-515788.vm.oceanos.gnet.gr/). The page features a red header with the text "DIANA TOOLS" and logos for the University of Thessaly, IMIS Athens IC, and BSRC AL Fleming. Below the header, there are navigation buttons for "HOME" and "SOFTWARE".

On the left side, there is a sidebar with a "Software » mirPath v.3" section. It includes a login form with fields for "Username \*" and "Password \*", a "Remember me next time" checkbox, and a "Login" button. Below the login form, there is a link for "Forgot your password?" and a section for "Available features for registered users" which lists "Download databases", "History", and "Bookmarks". A note states "Login is not required to access the site!".

The main content area is titled "mirPath v.3" and includes a "Please cite:" section with the following text: "Vlachos, Ioannis S., Konstantinos Zagganas, Maria D. Paraskevopoulou, Georgios Georgakilas, Dimitra Karagkouni, Thanasis Vergoulis, Theodore Dalamagas, and Artemis G. Hatzigeorgiou. "DIANA-miRPath v3. 0: deciphering microRNA function with experimental support." Nucleic acids research (2015): gkv403." Below this, there is a "New search" section with tabs for "KEGG analysis" and "GO analysis". The "KEGG analysis" tab is active, showing a "Species:" dropdown menu set to "Human", a "Gene filter:" dropdown menu set to "determine genes (optional)", and an "Add miRNAs:" input field. There is also a "TarBase v7.0" dropdown menu and a link to "upload a file". A "Reverse Search" link is visible in the top right of the search area, and a "Run example" link is at the bottom right.

At the bottom of the main content area, there is a paragraph of text: "DIANA-miRPath is a miRNA pathway analysis web-server, providing accurate statistics, while being able to accommodate advanced pipelines. mirPath can utilize predicted miRNA targets (in CDS or 3'-UTR regions) provided by the DIANA-microT-CDS algorithm or even experimentally validated miRNA interactions derived from DIANA-TarBase. These interactions (predicted and/or validated) can be subsequently combined with sophisticated merging and meta-analysis algorithms."

The browser's taskbar at the bottom shows the system tray with the date and time "13:33 12/12/2017" and various system icons.

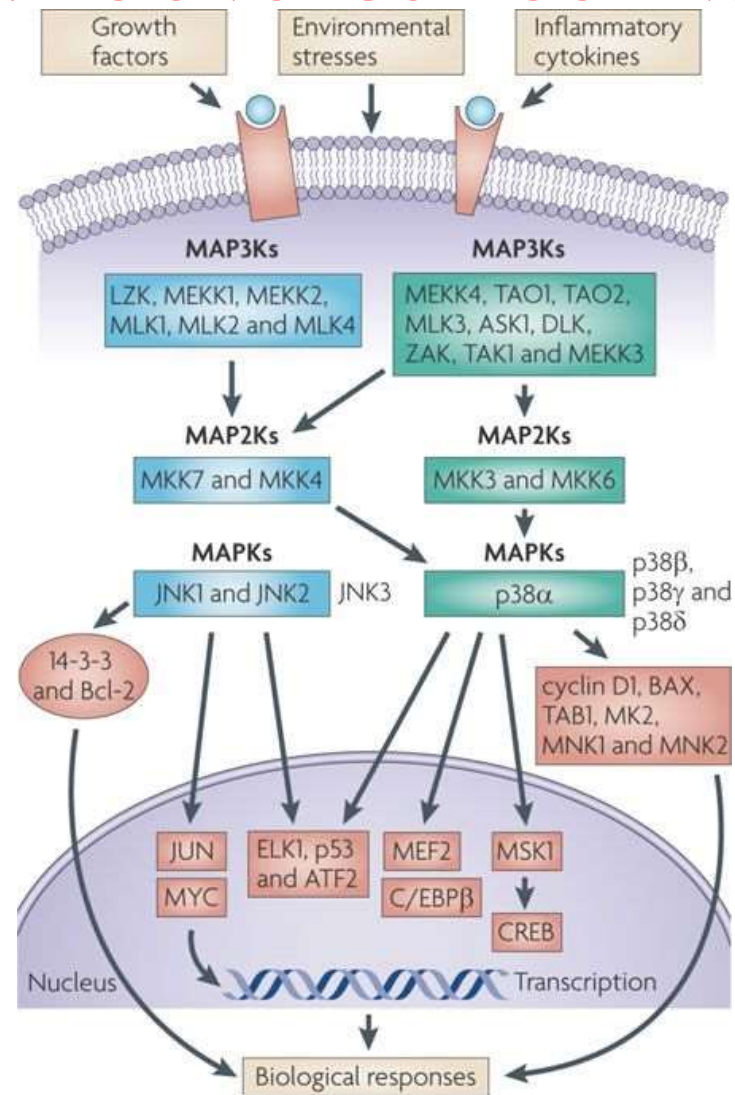
# DIFFERENTIAL EXPRESSION OF miRNAs IN RAPAMYCIN-INDUCED REVERSION OF GC RESISTANCE

Pathways	UPREGULATED miRNAs								
	MP			RAPA			MP+RAPA		
	p-Value	Gene	miRNA	p-Value	Gene	miRNA	p-Value	Gene	miRNA
ECM-receptor interaction	5.067 e-27	26	14	1.611 e-27	30	20	1.674 e-21	36	21
Biotin metabolism	7.131 e-5	2	2	0.0009	2	3	0.002	2	3
Vitamin B6 metabolism	0.00057	4	4	0.0294	3	3	0.0416	3	4
PI3K-Akt signaling pathway	0.00687	96	29	0.0009	124	44	0.0025	130	48
p53 signaling pathway	0.0086	24	18	0.0071	30	27	0.00271	33	31
Protein digestion and absorption	0.02	31	15	-	-	-	-	-	-
Ras signaling pathway	0.0201	64	26	0.003	85	39	0.027	83	42
Glycosaminoglycan biosynthesis	0.025	6	5	-	-	-	0.0065	11	11
Tight junction	0.028	41	21	-	-	-	-	-	-
Estrogen signaling pathway	0.0291	29	16	-	-	-	-	-	-
Neurotrophin signaling pathway	0.042	36	22	0.0294	47	31	0.0053	53	34
Fatty acid biosynthesis	-	-	-	1.898 e-13	4	4	3.734 e-12	4	5
Prion diseases	-	-	-	5.169	9	12	-	-	-
Bacterial invasion of epithelial cells	-	-	-	0.0052	34	28	-	-	-
Ubiquitin mediated proteolysis	-	-	-	0.0071	55	33	0.0146	57	37
MAPK signaling pathway	-	-	-	-	-	-	0.00576	98	41
Proteoglycans in cancer	0.019	74	39	-	-	-	-	-	-

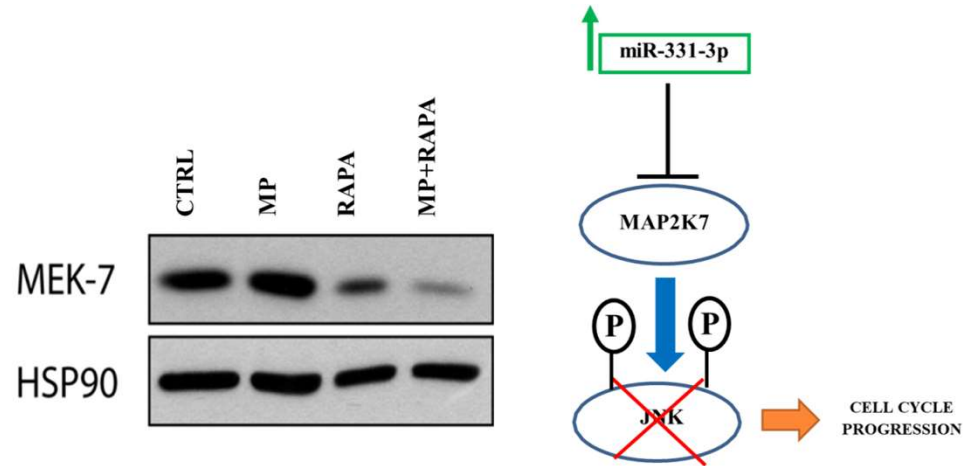
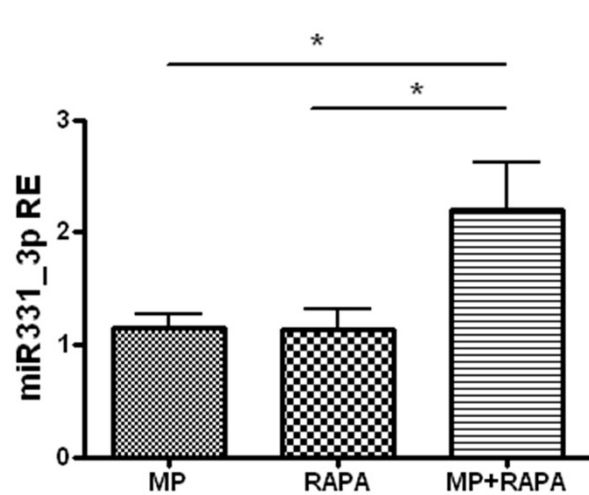
Pathways	DOWNREGULATED miRNAs								
	MP			RAPA			MP+RAPA		
	p-Value	Gene	miRNA	p-Value	Gene	miRNA	p-Value	Gene	miRNA
Fatty acid biosynthesis	3.31 e-26	4	3	1.123 e-23	3	2	7.532 e-21	3	1
Fatty acid metabolism	2.069 e-8	11	9	0.00064	9	8	0.007	9	6
Proteoglycans in cancer	3.569 e-5	40	16	0.00074	37	20	0.0019	35	15
Thyroid hormone signaling pathway	0.0015	20	15	-	-	-	-	-	-
Path regulat pluripot of stem cells	0.005	30	15	0.03099	30	20	-	-	-
Thyroid hormone synthesis	0.0075	12	8	-	-	-	-	-	-
Glycosphingolipid biosynthesis	0.0106	5	3	0.0248	6	7	-	-	-
N-Glycan biosynthesis	0.0314	10	9	0.0248	12	9	0.03	10	8
PI3K-Akt signaling pathway	-	-	-	0.0248	67	22	-	-	-
Acute myeloid leukemia	-	-	-	0.0309	15	14	-	-	-
ErbB signaling pathway	-	-	-	-	-	-	0.014	22	14

miR-331-3p

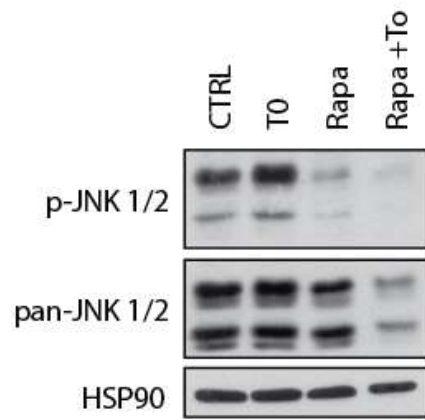
# DIFFERENTIAL EXPRESSION OF miRNAs IN RAPAMYCIN-INDUCED REVERSION OF GC RESISTANCE



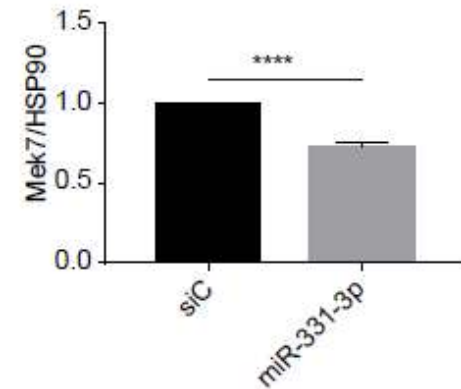
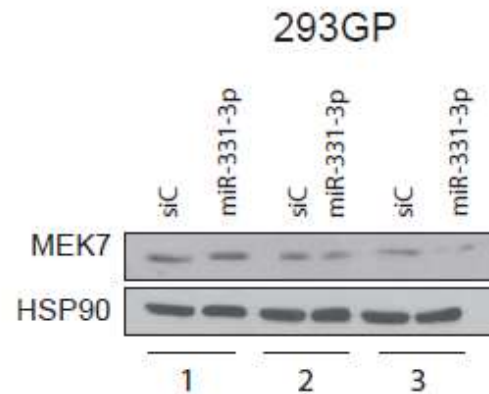
# DIFFERENTIAL EXPRESSION OF miRNAs IN RAPAMYCIN-INDUCED REVERSION OF GC RESISTANCE



**Validation of MAP2K7 as a target of miR-331-3p**

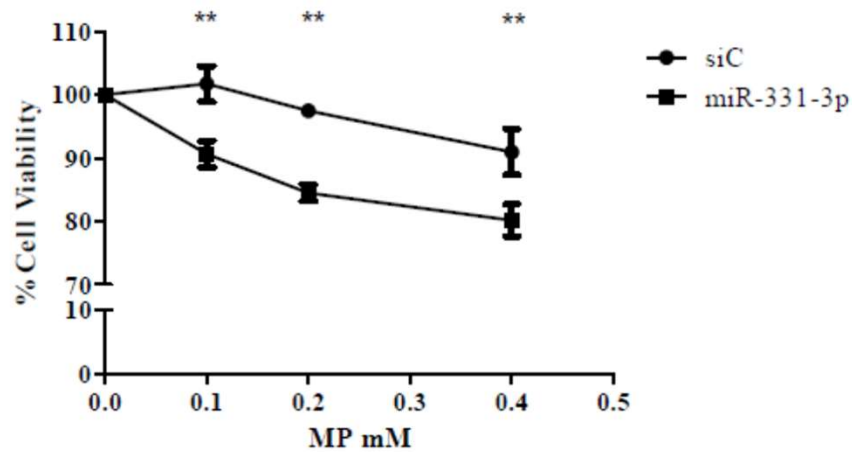


**GC resistance reversion by RAPA through suppression of the JNK protein**



# DIFFERENTIAL EXPRESSION OF miRNAs IN RAPAMYCIN-INDUCED REVERSION OF GC RESISTANCE

miR-331-3p is involved in glucocorticoid resistance reversion by rapamycin through suppression of the MAPK signaling pathway



triplicate. C) Effect of MP on 293-T cell viability in cells transfected with miR-331-3p mimic (miR-331-3p) and control (siC). Two-way ANOVA  $p < 0.0001$  and Bonferroni post-test  $**p < 0.01$ . The data are reported as means  $\pm$  SE of three independent experiments

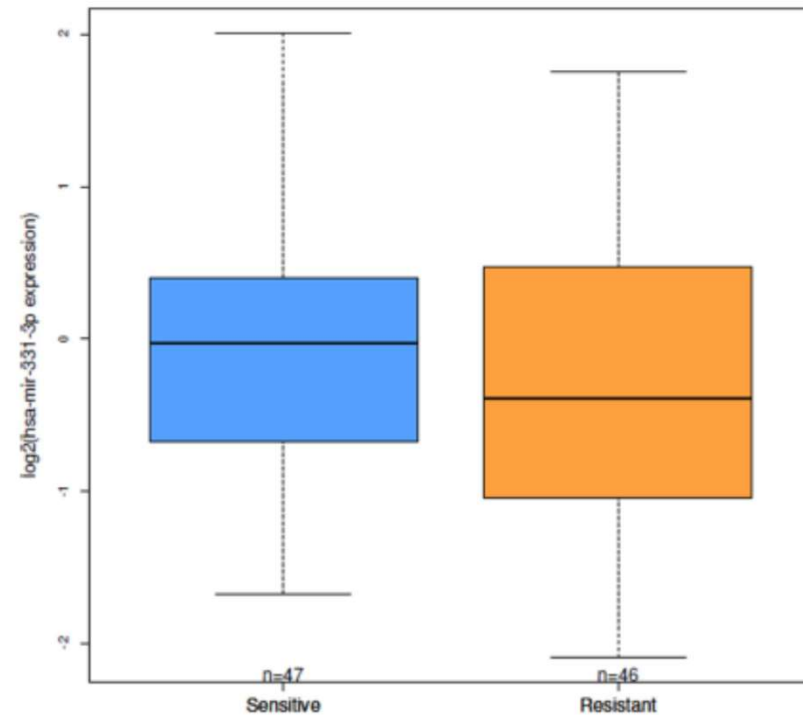
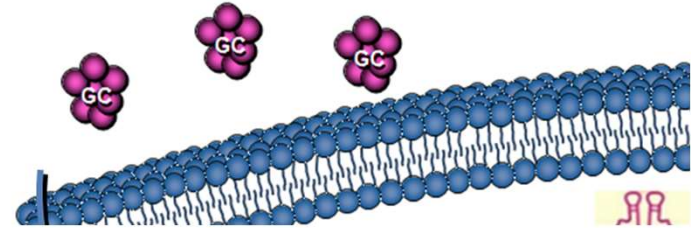
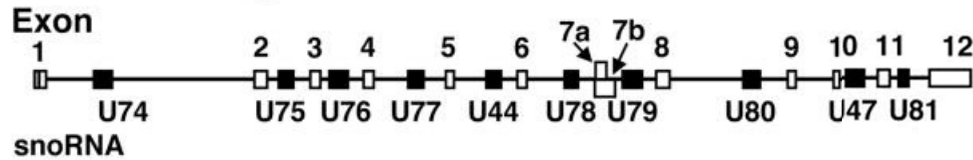


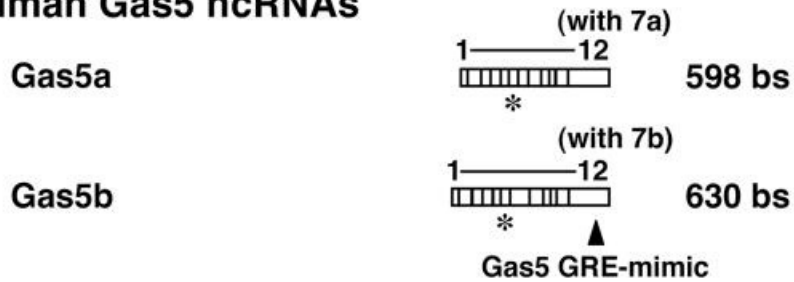
Fig. 7 Expression level of miR-331-3p in leukemia cells derived from patients sensitive or resistant to GCs. Linear model  $*p < 0.05$

# Growth arrest-specific 5 (GAS5)

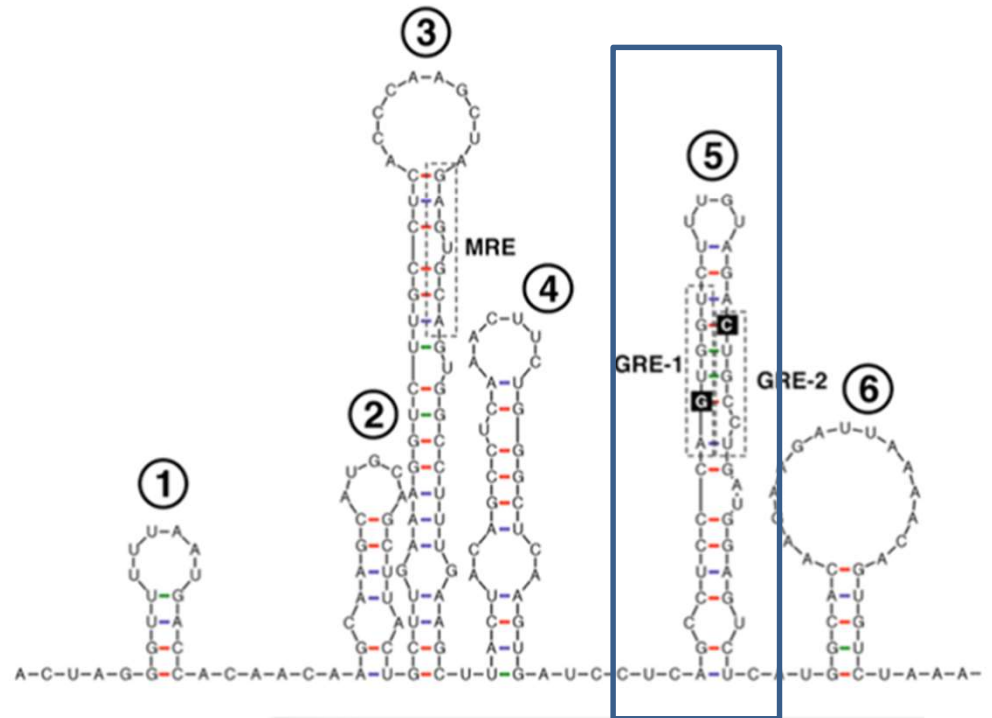
## Human Gas5 gene



## Human Gas5 ncRNAs



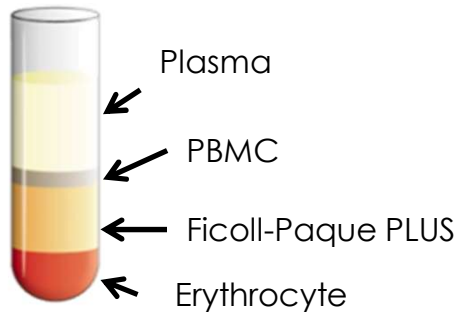
(Kino et al., *Sci Signal.* 2010)



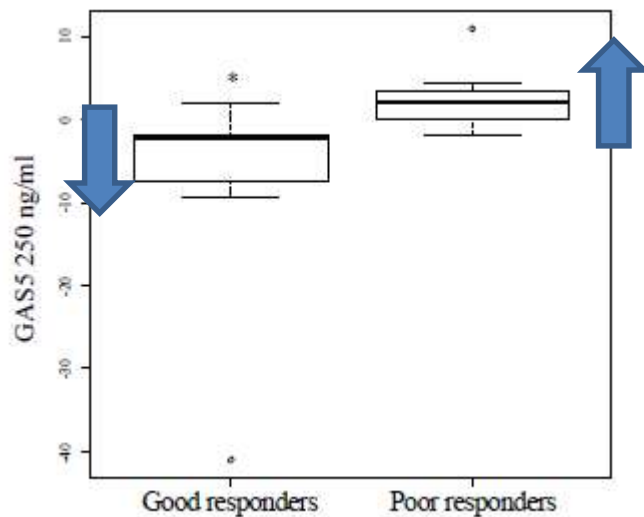
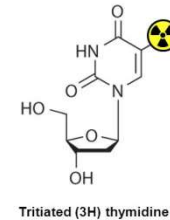


# ROLE OF GAS5 IN GC RESPONSE

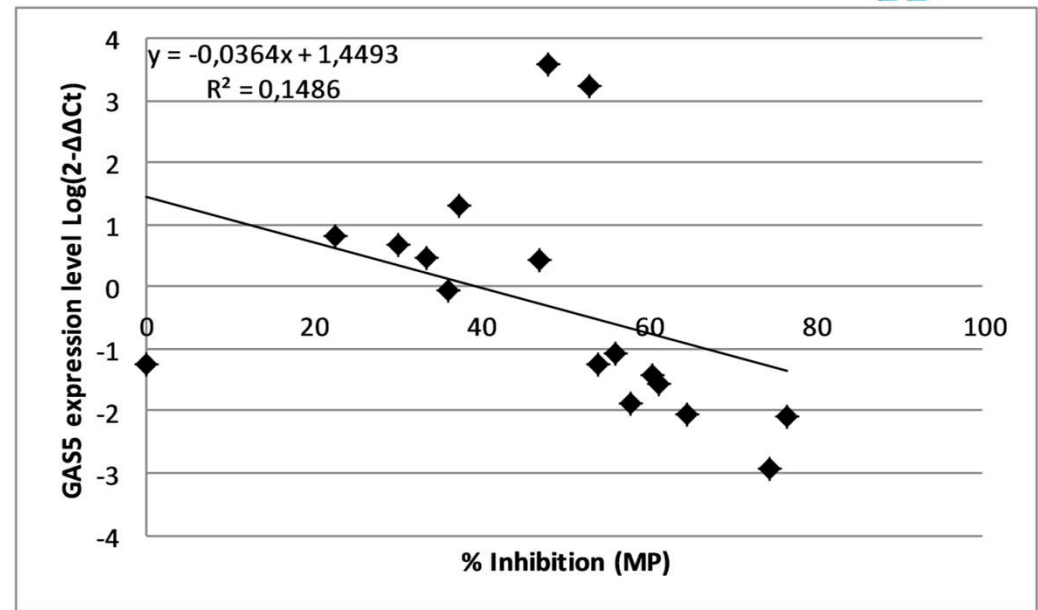
Samples from blood donors



Pharmacodynamic test: inhibition of in vitro proliferation by the test of incorporation of [<sup>3</sup>H]-thymidine.

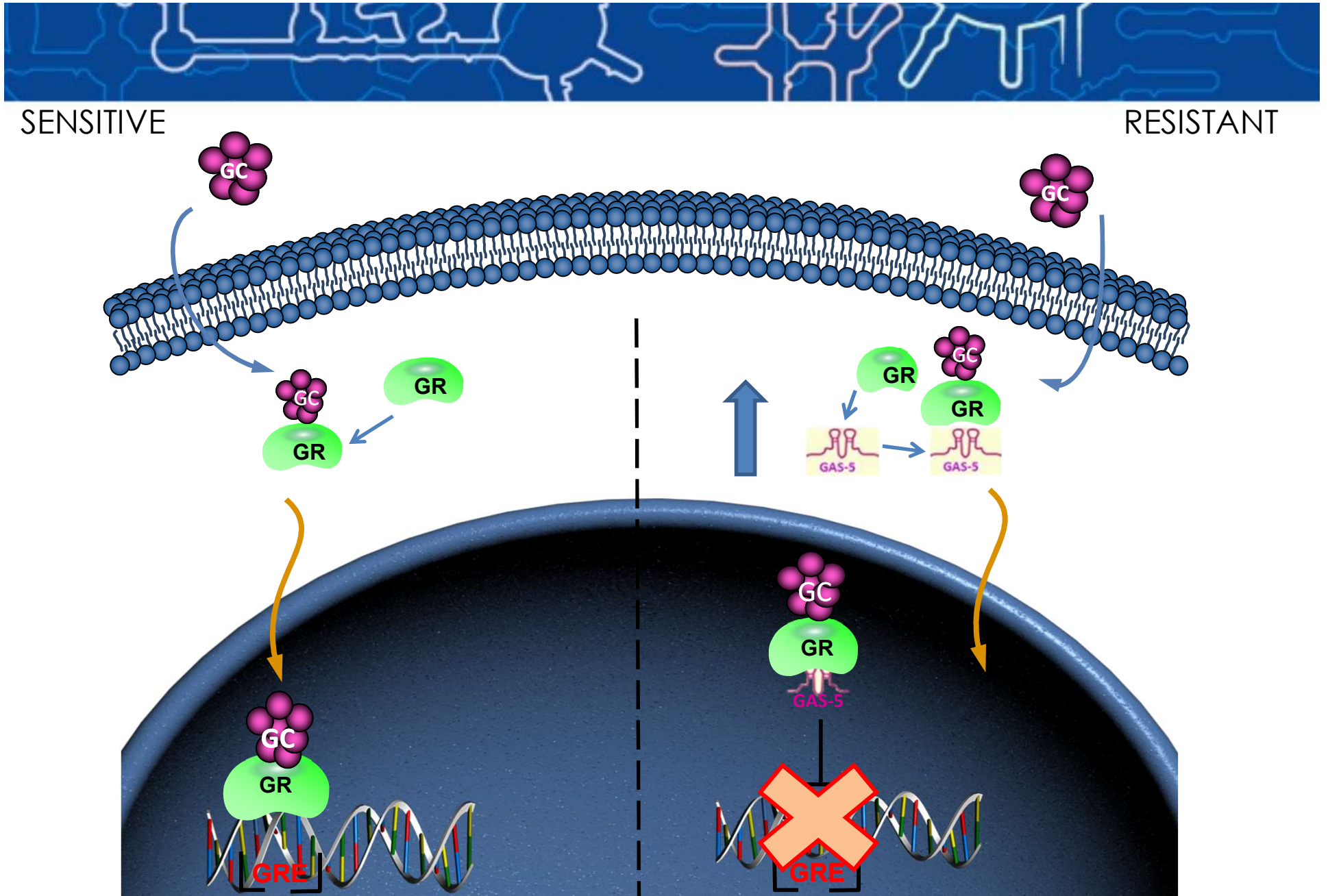


Lucafò M. et al., *Curr Mol Med* 2015

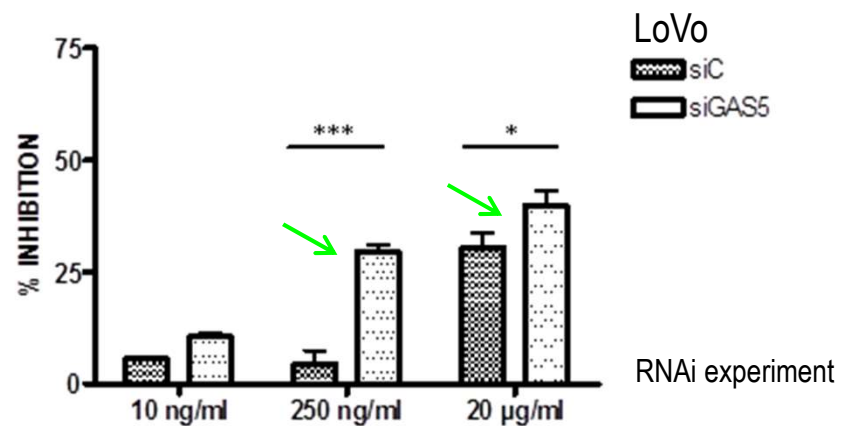
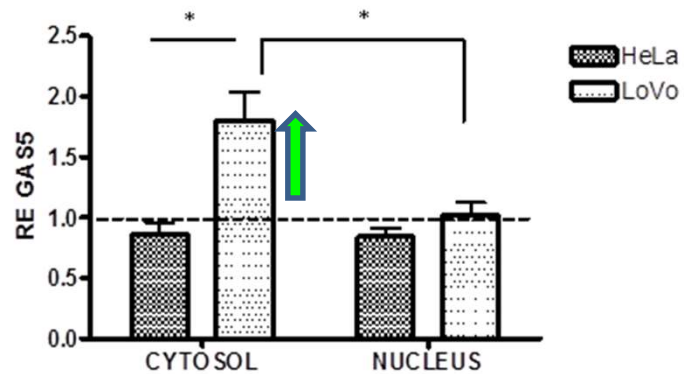
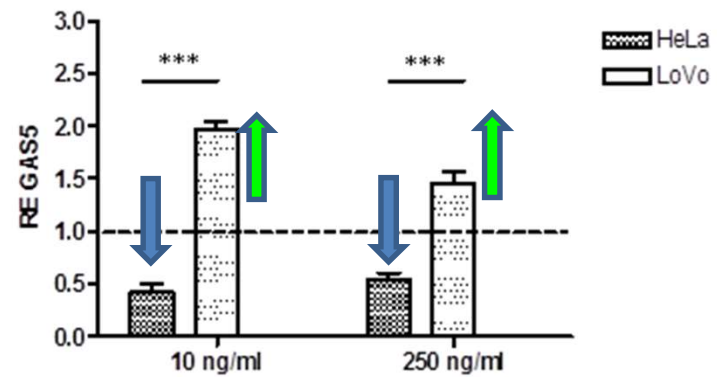
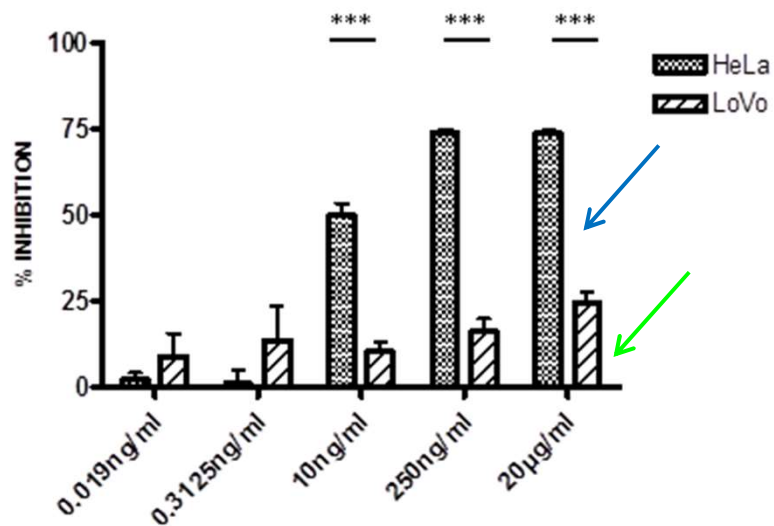


Spearman rho= -0,73; P value= 0.0009

Lucafò M. et al., *Clin and Exp Pharm and Phys* 2016

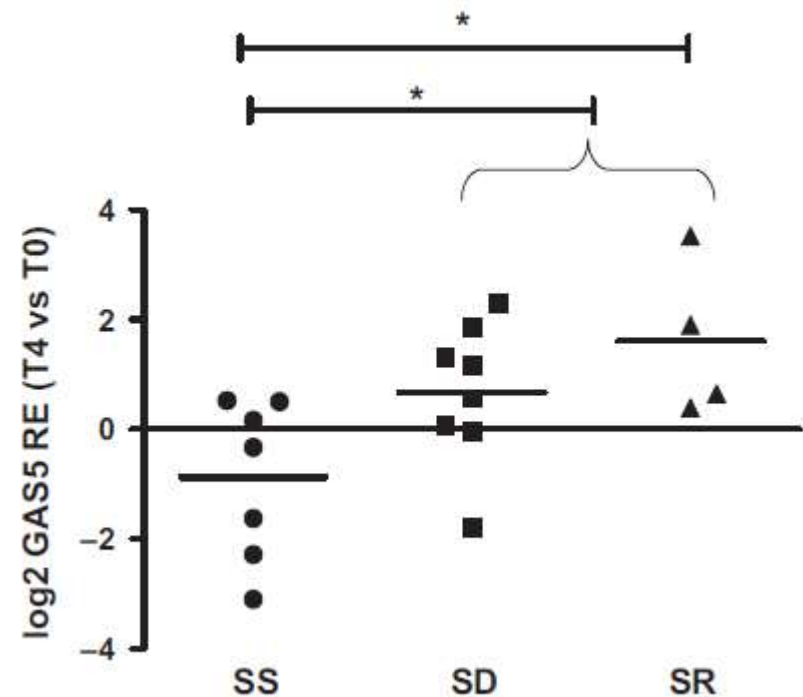
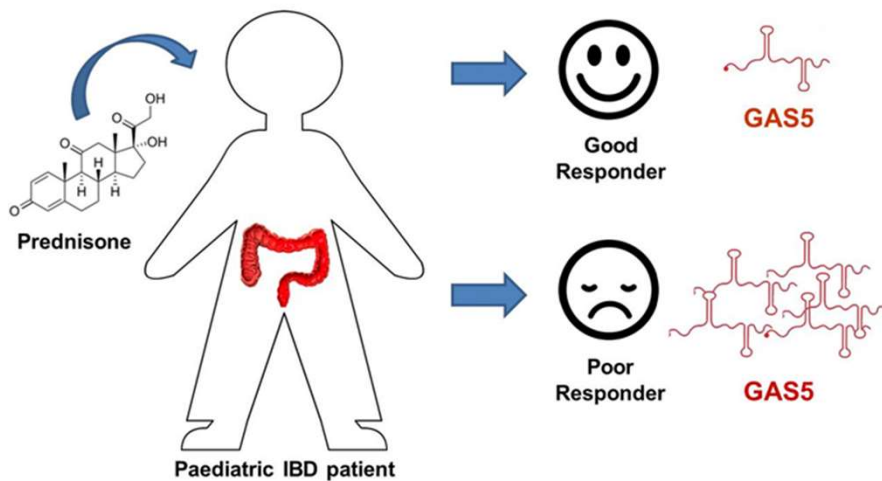
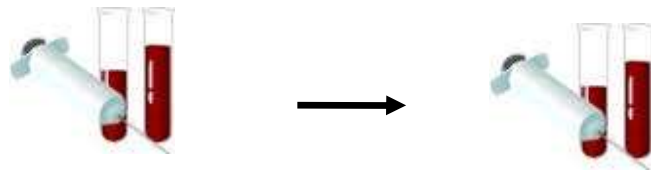


# ROLE OF GAS5 IN GC RESPONSE

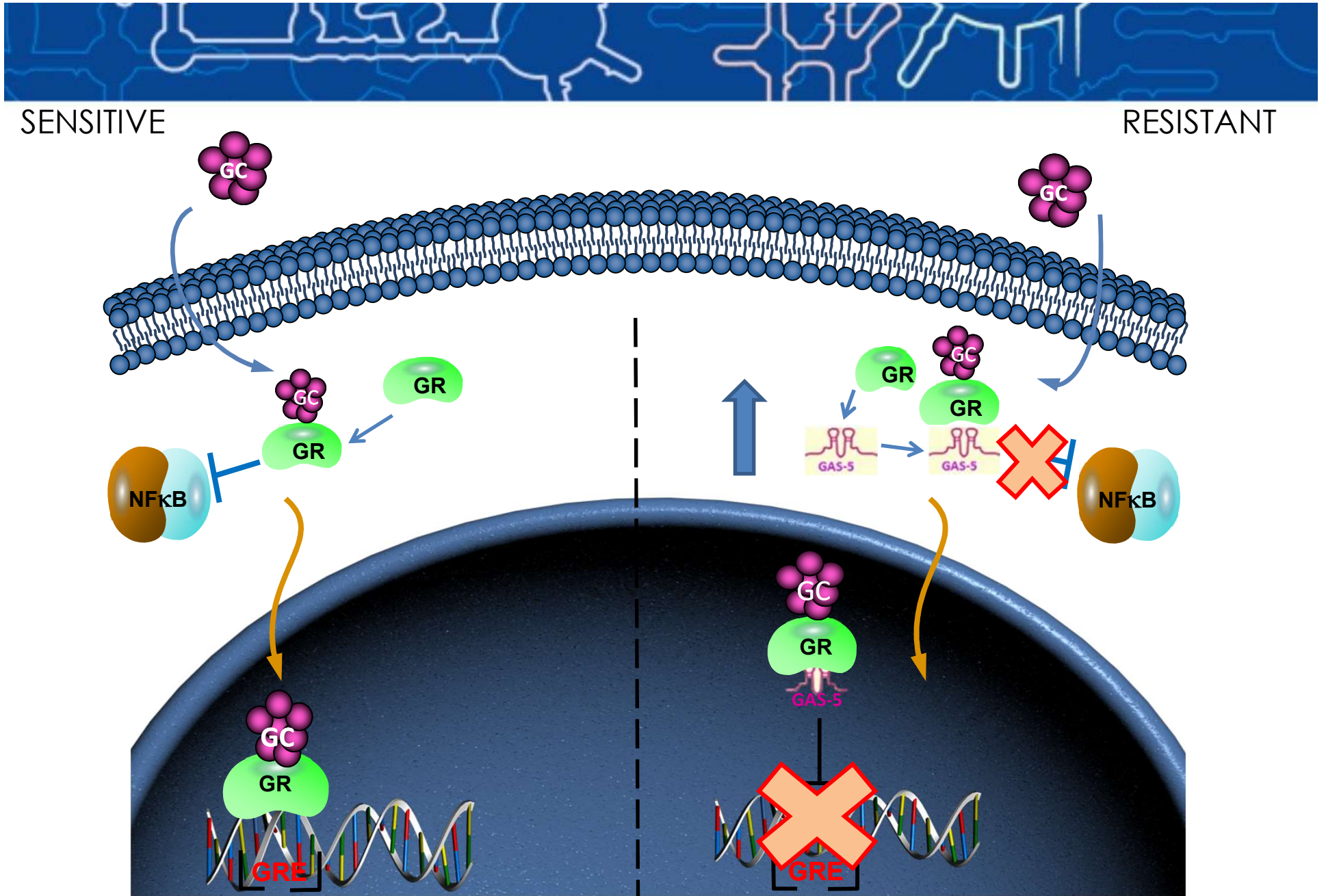


# ROLE OF GAS5 IN GC RESPONSE IN CHILDREN WITH IBD

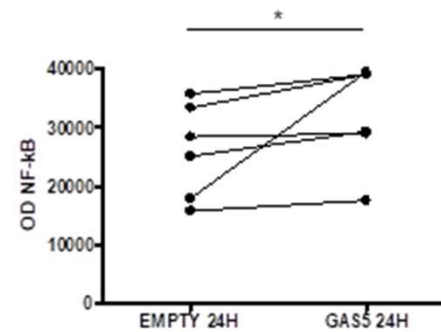
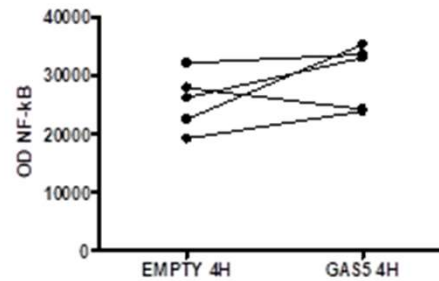
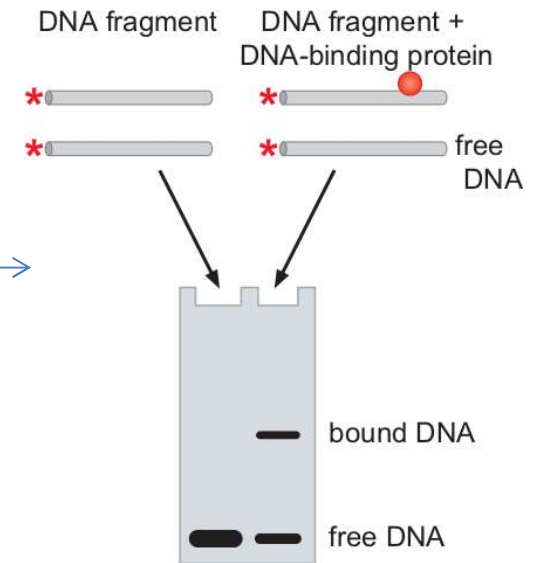
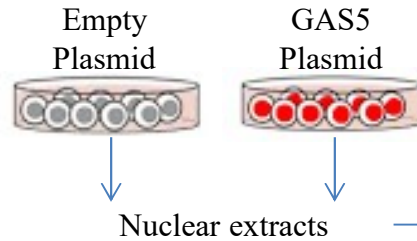
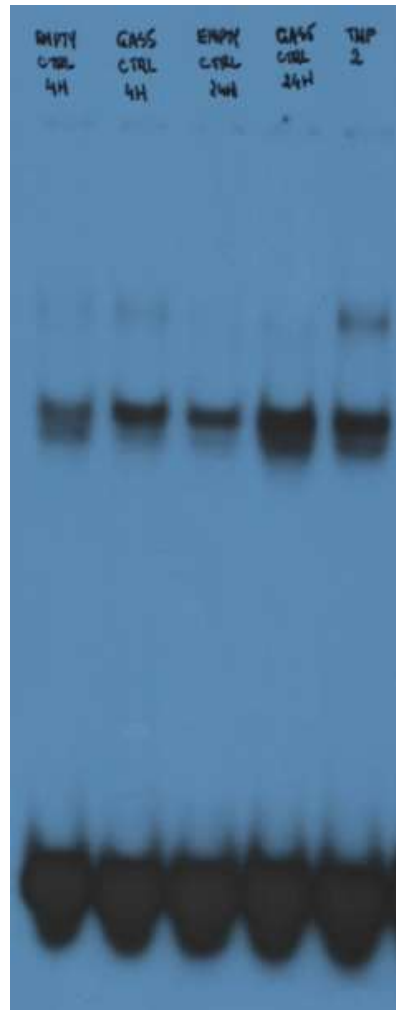
DIAGNOSIS (T0) → 30 DAYS OF TREATMENT WITH GC (T4)



Lucafò M. et al., *Basic & Clinical Pharmacology & Toxicology* 2017

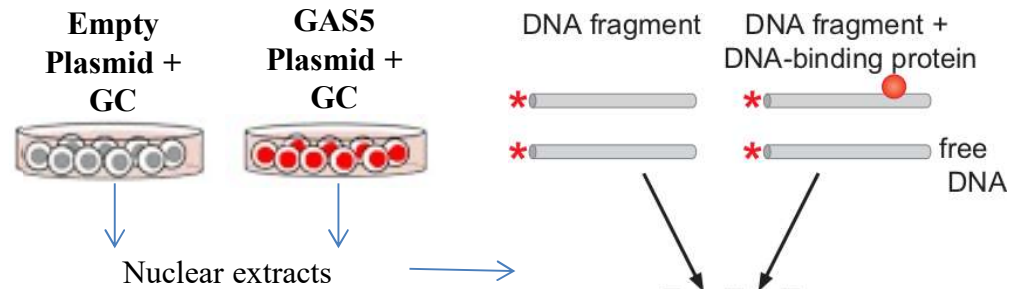
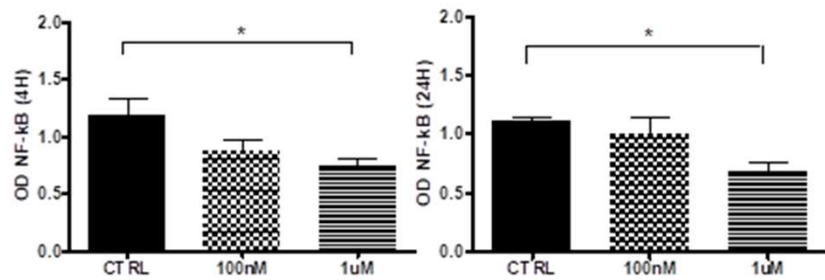


# ROLE OF GAS5 on NF- $\kappa$ B activity by EMSA

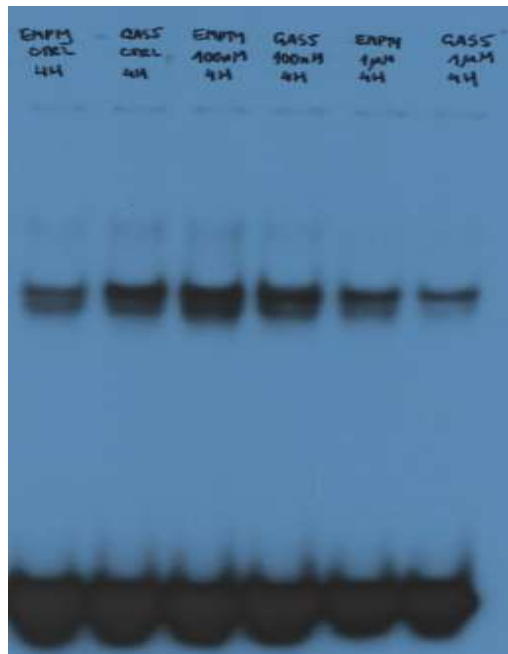


NF- $\kappa$ B EMSA analyses in HeLa cells transfected with empty pcDNA3.1 (EMPTY CTRL) and pcDNA3.1\_GAS5 (GAS5 CTRL), after 4 (lanes 1, 2) and 24 h (lanes 3, 4) from transfection or treated with TNF- $\alpha$  (lane 5).

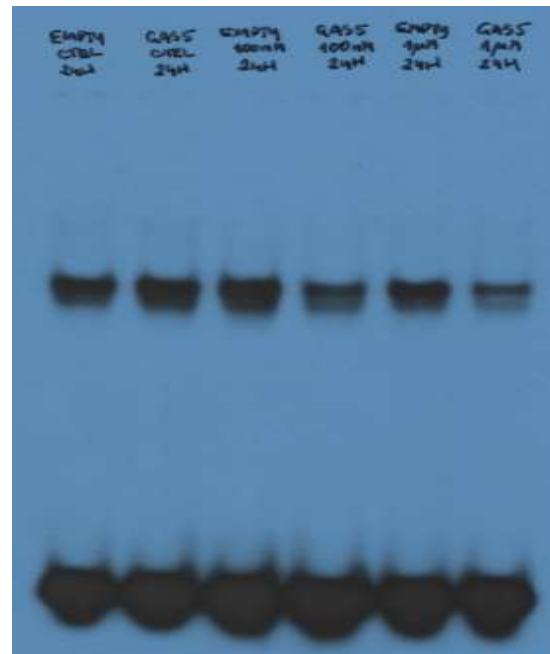
# ROLE OF GAS5 on NF- $\kappa$ B activity by EMSA



A  
4H



B  
24H

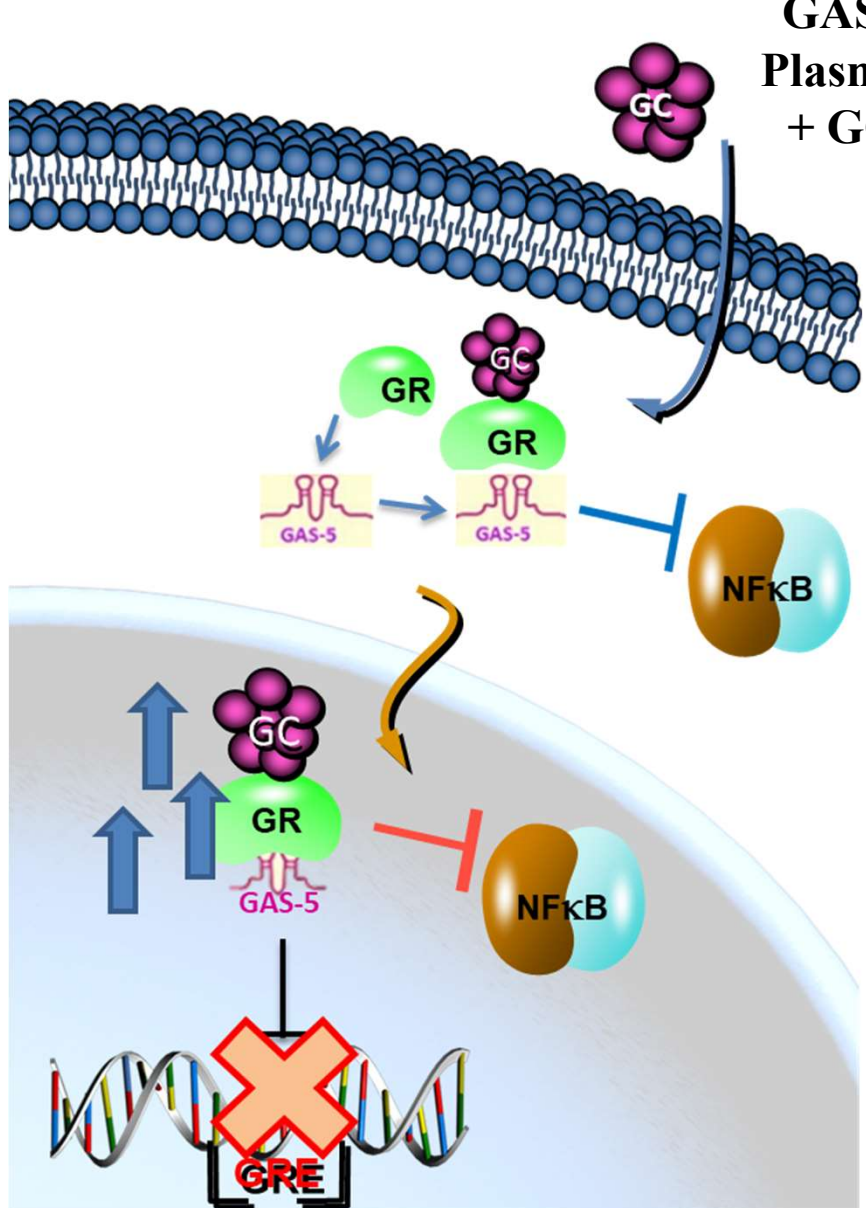
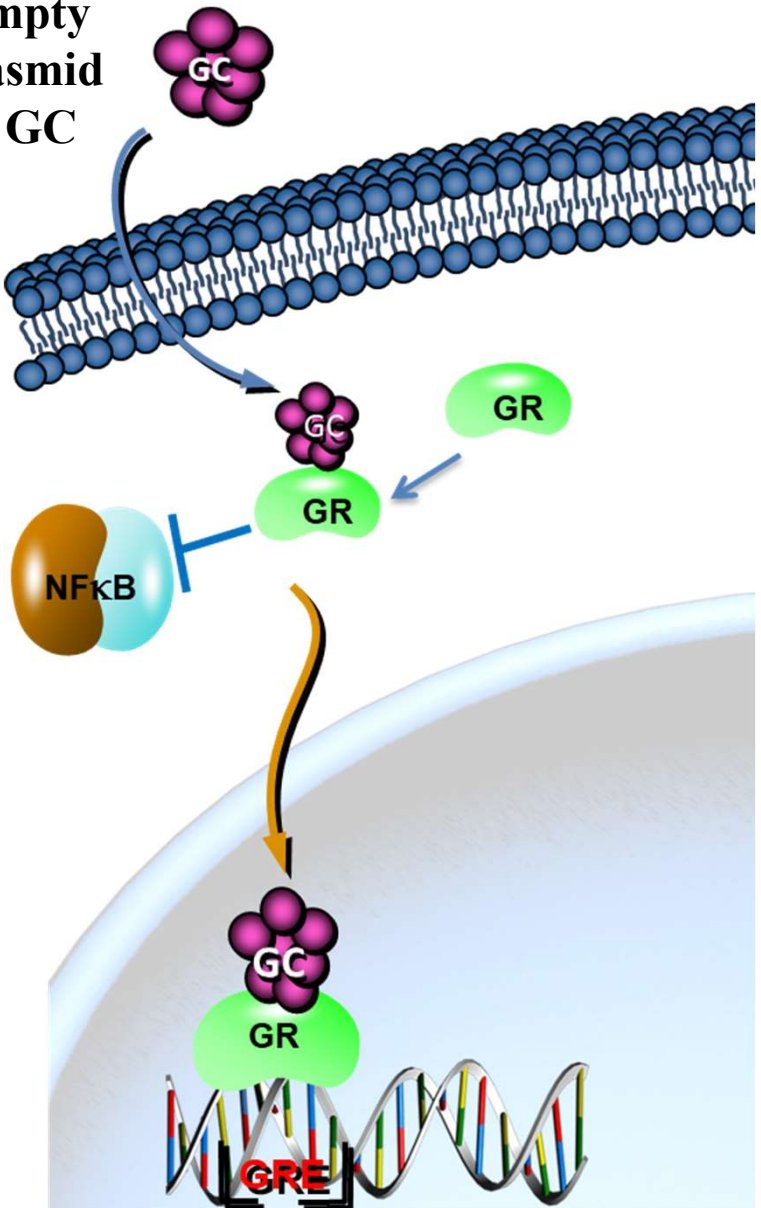


NF- $\kappa$ B EMSA analyses in HeLa cells transfected with empty pcDNA3.1 (EMPTY) and pcDNA3.1\_GAS5 (GAS5), treated with DEXA 100 nM (lanes A and B 3, 4) and 1  $\mu$ M (lanes A and B 5, 6) and untreated (CTRL; lanes A and B 1, 2) after 4 (A) or 24 h (B).



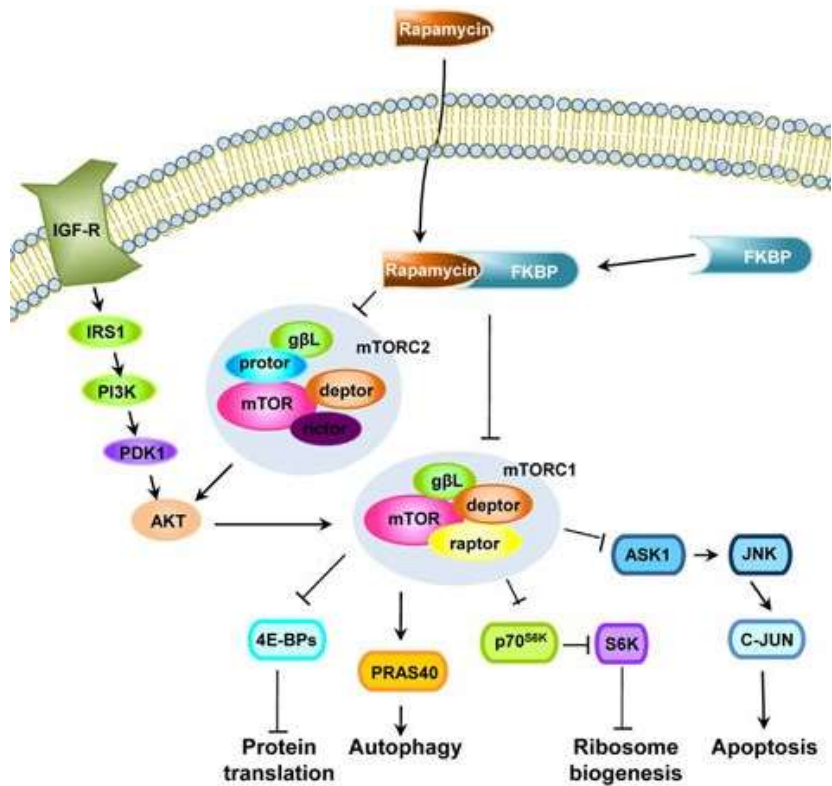
Empty  
Plasmid  
+ GC

GAS5  
Plasmid  
+ GC

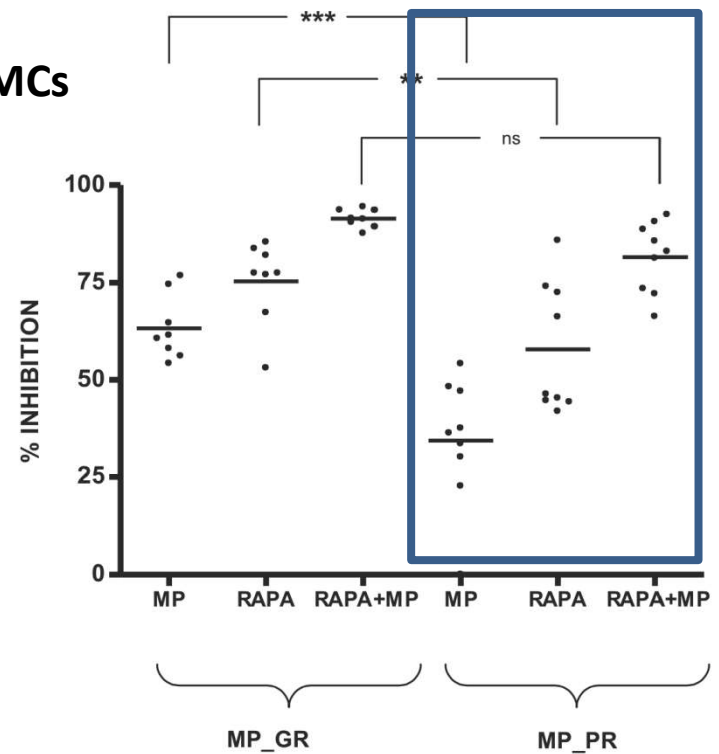




# DIFFERENTIAL EXPRESSION OF GAS5 IN RAPAMYCIN-INDUCED REVERSION OF GC RESISTANCE



PBMCs



MPvsRAPA pvalue > 0.05  
 MPvsRAPA+MP pvalue < 0.001  
 RAPAvsRAPA+MP pvalue < 0.05

MPvsRAPA pvalue < 0.001  
 MPvsRAPA+MP pvalue < 0.001  
 RAPAvsRAPA+MP pvalue < 0.001

Lucafò M. et al., *Clin and Exp Pharm and Phys* 2016