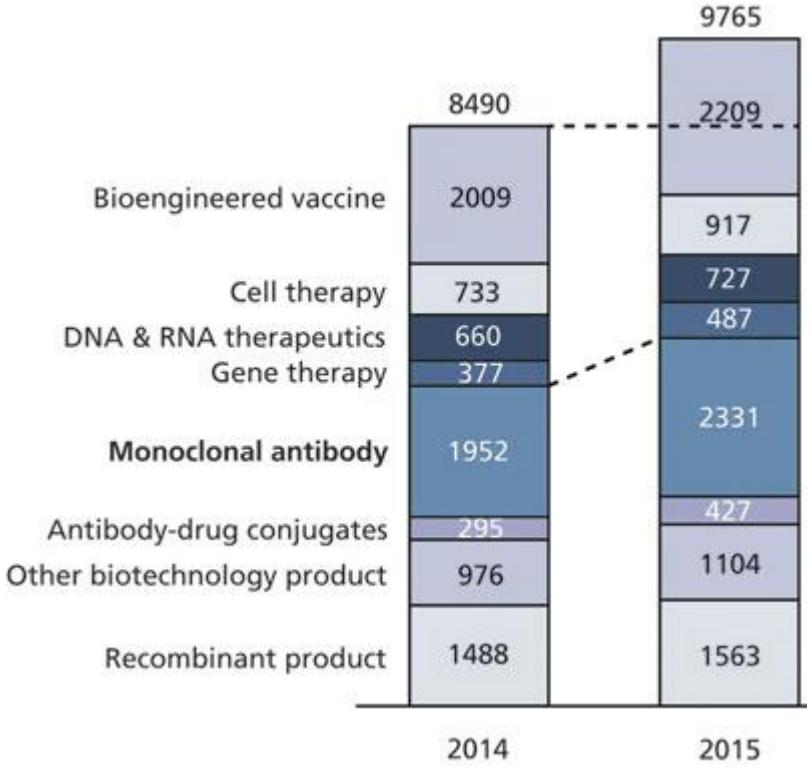
RNA world prospects for biomedicine

Dmitry V.Sosin

Some information about money and RNA

In 2014, the monoclonal antibodies market had the highest growth rate (19%) for the number of new molecules in the pipeline. DNA and RNA therapeutics were not far behind, achieving 12% year-over-year growth. Industry analytics data suggest that the RNA-based therapeutics market will reach $1.2 billion by 2020.

more than 700 nucleic acid-based therapeutics (DNA and RNA) in the pipeline and more than 60% of the nucleic acid-based therapeutic pipeline is in preclinical development (35% of such pipeline is focused on oncology)

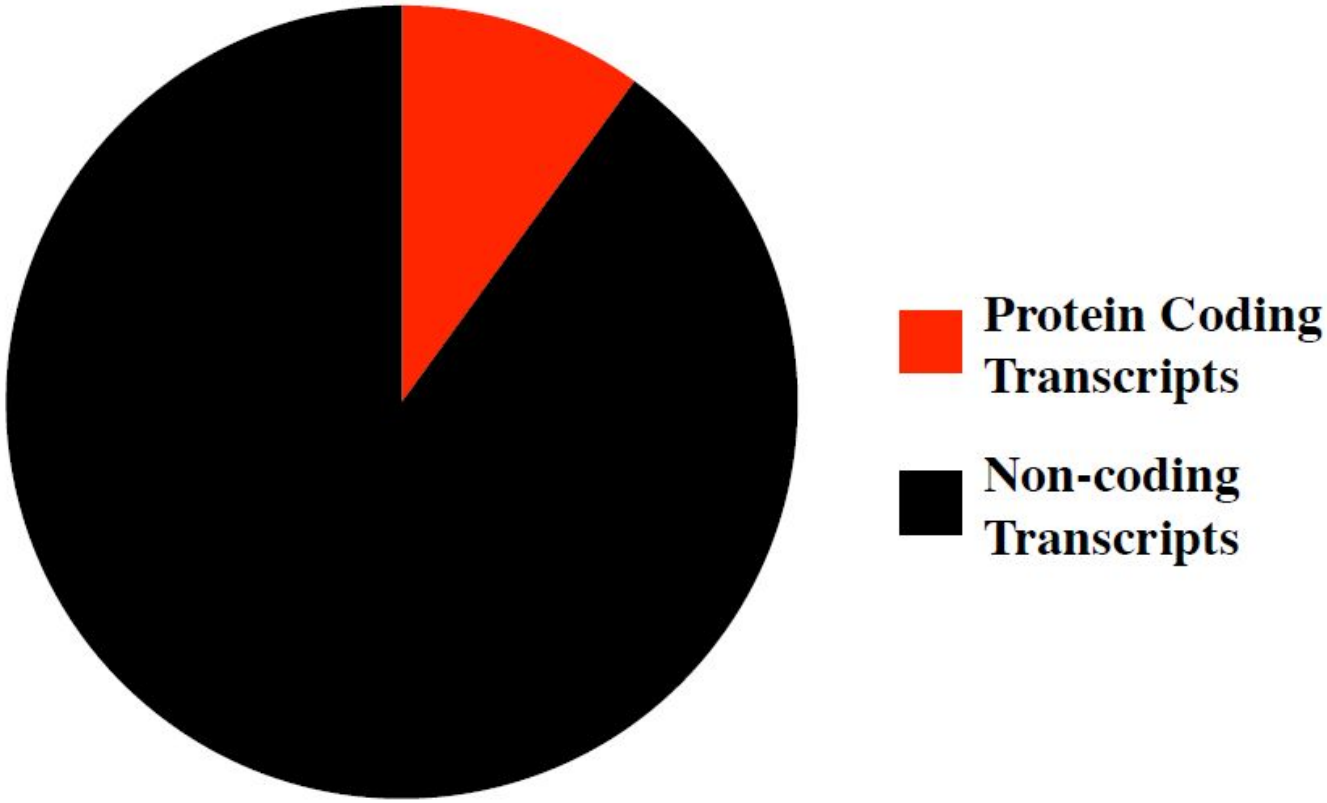


2015 research and development (R&D) biotech pipeline

Several companies (approximately 160) and many academic institutes (approximately 65) are developing RNA-based therapeutics and vaccines

siRNA	miRNA	mRNA
Kyowa Hakko Kirin	Andes Biotechnologies	CureVac
Silence Therapeutics	Mirna Therapeutics	Biontech RNA Pharmaceuticals
Debiopharm	miRagen Therapeutics	Boehringer Ingelheim
Marina Biotech	Marina Biotech	Johnson & Johnson
Ipsen	Moderna Therapeutics	Ludwig Institute for Cancer Research
Alnylam Pharmaceuticals	Alnylam Pharmaceuticals	BioNTech
Sanofi Pasteur	Sanofi Pasteur	Sanofi Pasteur
Tekmira Pharmaceuticals	Tekmira Pharmaceuticals	
NanoCarrier	Regulus Therapeutics	
Dicerna Pharmaceuticals	Biogen Idec	
BioCancell Therapeutics	GlaxoSmithKline	
Samyang Group	AstraZeneca	
Silenseed	Ionis Pharmaceuticals	
siRNAsense	Les Laboratoires Servier	
Reference Biolabs	Celsion	
Avena Therapeutics	Rosetta Genomics	
Lipella Pharmaceuticals	Santaris Pharma	
Arrowhead Research	Shire	
	InteRNA Technologies	
	Alexion Pharmaceuticals	
	t2cure	
	Rigontec	
	Microlin Bio	

Non-coding transcripts constitute a large fraction of the mammalian transcriptome

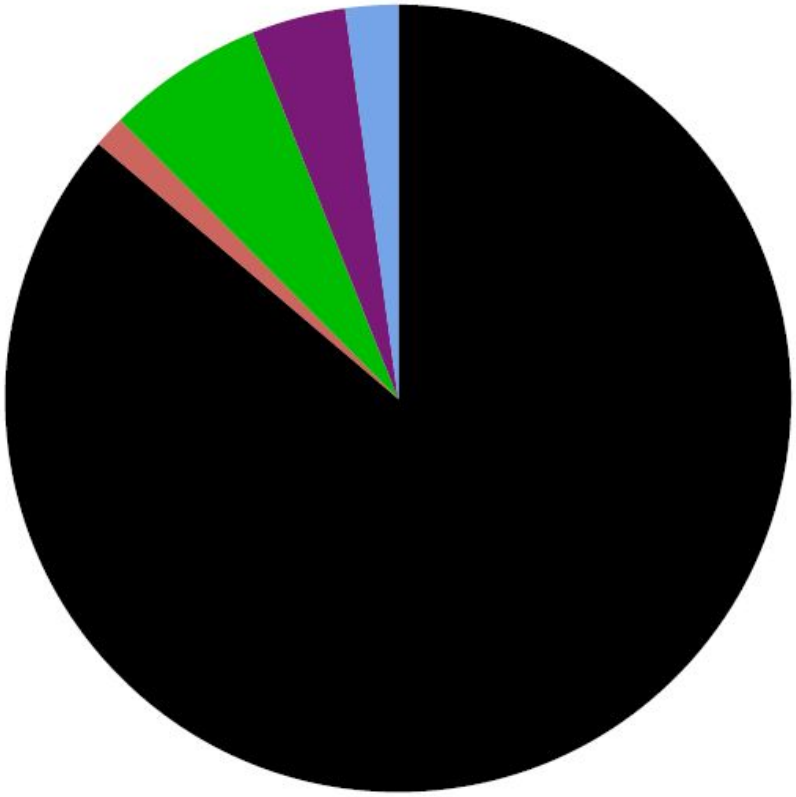


The GENCODE Project Consortium (http://www.gencodegenes.org/) and FANTOM5 (Chung-Chau Hon et al., Nature (2017) 543, 199–204)

As metazoans evolved the number of encoded proteins remained roughly constant whilst the genome size exploded

	Genome size bp (millions)	Number of encoded proteins	Number of chromosome
Drosophila melanogaster	168	13,781	4
C elegans	100	20,424	6
Zebrafish	1505	19,929	25
Xenopus tropicalis	1700	~21,000	10
Chicken	1050	14,923	39
Mouse	3420	22,085	20
Homo sapiens	3279	21,077	23
Arabidopsis	134	27,416	5

The composition of non-coding transcripts in the mammalian transcriptome



Huttenhofer et al.

(FANTOM5 identified 27,919 long ncRNAs and ~78% of lncRNAs were characterized as tissue-specific, as opposed by only ~19% of mRNAs)

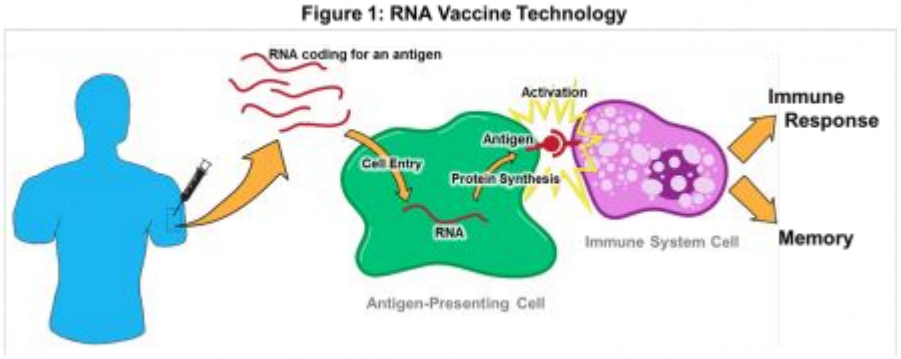
Therapeutic RNAs

- small-interfering (siRNAs)
- microRNAs (miRNAs)
- antisense oligonucleotides (ASOs)
- synthetic mRNAs
- CRISPR–Cas9
- LncRNAs

amers

mRNA-based therapy

- DNA→mRNA→protein
- mRNA technologies are mostly used in vaccines or gene therapy
  - mRNA is translated to protein, which can ultimately replace a missing protein (therapy)
  - mRNA induce an immune response



  - dendritic cells (antigen-presenting cells) take-up, process, and encode the target antigen, which in turn induces an immune response
  - Typically, mRNA vaccines are produced by *in-vitro* synthesis through an enzymatic process
  - synthetic process can be tightly controlled, resulting in a quality and predictable product profile
  - mRNA can be easily tailored to offer a specific immunogenic profile and pharmacokinetics (self-amplifying mRNA)
  - mRNA's stability and antigenic properties can be easily manipulated by changing codon or modifying base pairs
  - mRNA can be delivered as naked mRNA; immobilized on particles or in liposome nanoparticle

(Novartis and Synthetic Genomics 2013 Influenza A virus subtype H7N9 (avian influenza virus) in China, about 40 % have died)

Novartis vaccine to protect farm-raised salmon from the infectious hematopoietic necrosis (IHN) virus

The only barrier was having enzymes sufficiently pure to make products that could be put into humans

Funding for RNA vaccines

Company	Date of funding, source	Terms
BioNTech	November 2015, Sanofi	$60 million upfront and near-term milestones for co-development and co-commercialization of mRNA cancer immunotherapies
	September 2016, Genentech	$310 million upfront & near-term payments, 50:50 cost and profit share to develop novel mRNA-based, individualized cancer vaccines IVAC Mutanome
	May 2016, Bayer	mRNA vaccines and therapeutics specifically for animal health applications
CureVac	November 2011, Sanofi with DARPA funding	$33.1 million to co-develop RNA vaccines for infectious diseases using RNActive technology. Terms with Sanofi undisclosed
	November 2011, Sanofi	Option to exclusive license to mRNA vaccine to undisclosed pathogen, terms undisclosed
	October 2013, Johnson & Johnson	Partnership to develop influenza RNA vaccine, terms undisclosed
	March 2015, Gates Foundation	$52 million equity investment to construct GMP production facility with additional funding for prophylactic vaccines for infectious diseases
	September 2015, International AIDS Vaccine Initiative	Combine HIV trimer construct with RNAactive technology, terms not disclosed
	September 2014, Boehringer Ingelheim	€35 million ($45M) upfront payment and €430 million ($563M) milestone payments for exclusive global license for CV9202 in NSCLC
Infectious Disease Research Institute (Seattle)	October 2016, NIH	$500,000 for Zika mRNA vaccine
Moderna	October 2013, DARPA	$25 million, emerging infectious disease and engineered biological threats (including Chikungunya)
	January 2015, Merck	$50 million up front for partnership to discover, develop and commercialize five mRNA vaccines against four undisclosed viruses
	January 2016, Gates Foundation	Up to $100 million infectious diseases
	July 2016, BARDA	$125 million, Zika
Synthetic Genomics	January 2017, Johnson & Johnson	Partnership to develop RNA-based therapies for cancer and infectious diseases; terms not disclosed

RNA vaccines in clinical trials

Company	Drug	Indication	Stage of development
BioNTech	Lipo-MERIT	Melanoma	Phase 1
BioNTech	IVAC mutanome	Melanoma	Phase 1
BioNTech	TNBC-MERIT	Triple-negative breast cancer	Phase 1
CureVac	CV9104	Prostate cancer	Phase 1, phase 2
CureVac	CV9202 plus radiation	NSCLC	Phase 1
CureVac	CV7201	Rabies	Phase 1
CureVac	CV8102	RSV, HIV, rabies	Phase 1 rabies
CureVac	CV9103	Prostate cancer	Complete
Moderna	mRNA 1851	Influenza H10	Phase 1
Moderna (DARPA funded)	mRNA1388	Chikungunya	Preclinical
Moderna (BARDA funded)	mRNA 1325	Zika	Phase 1/2
Moderna	mRNA1440	Influenza H7	Phase 1
Moderna and Merck (Kenilworth, New Jersey)	mRNA 4157	Cancer (personalized)	Preclinical
Moderna	mRNA-1647	CMV	Preclinical
Moderna	mRNA-1653	HMPV/PIV3	Preclinical
Argos	AGS-003	RCC	Phase 3
Argos	AGS-003	NSCLC	Phase 2
Argos	AGS-0004	HIV/AIDS	Phase 2
GSK and Vaccine Research Center at NIH	Self-amplifying RNA vaccine	Zika	Preclinical

PPD, Pharmaceutical Product Development; NSCLC non-small-cell lung cancer; RSV, respiratory syncytial virus; CMV, cytomegalovirus. HMPV/PIV3, metapneumovirus (hMPV) and parainfluenzavirus type 3 (PIV-3).

Laura DeFrancesco, Nature Biotechnology 35, 193–197 (2017)

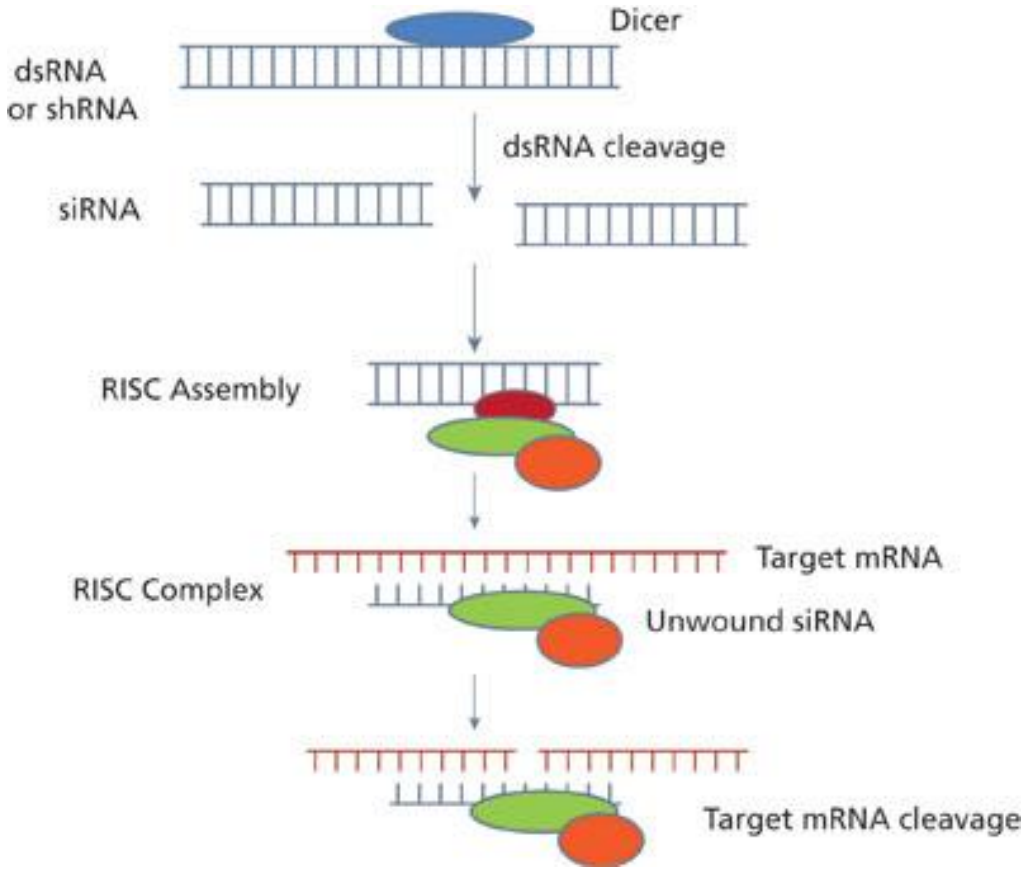
siRNA

- It has been reported that synthetic siRNA is able to knock down targets in various diseases *in vivo*, including hepatitis B, human papilloma virus, ovarian cancer, bone cancer, hypercholesterolemia, and liver cirrhosis

- Only a few molecules of siRNA per cell are required to produce effective gene silencing

- siRNAs are most commonly delivered into cells using microinjection or a transfection agent. Many companies now offer siRNA-delivering reagents to simplify this process

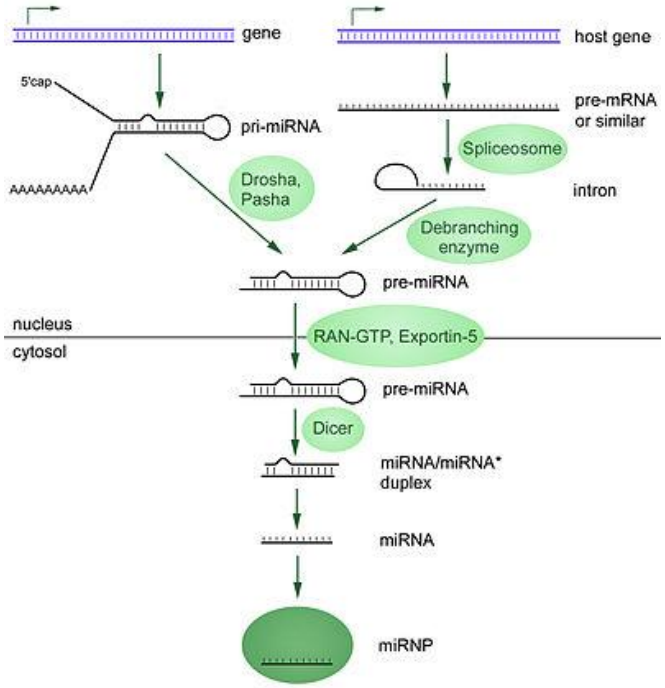
RNA-based technologies for biomedicine. RNA-interference (RNAi)



- RNAi utilizes a “dicer” enzyme to cut dsRNA into 21 oligonucleotide segments, called siRNA
- siRNAs can bind to a Argonaute proteins of two classes: Ago and Piwi.
- Ago proteins bind to siRNAs or miRNAs, while Piwi proteins bind to Piwi-interacting RNA (piRNA) and are used to silence mobile genetic elements.
- The siRNA, miRNA, or piRNA complex bound to the Argonaute protein is called the RNA-induced silencing complex (RISC)
- one strand of the dsRNA is removed and the remaining strand binds to and directs the degradation of the complementary RNA target sequence

miRNA

- functions in RNA silencing and post-transcriptional regulation of gene expression
- Approximately 60% of genes in the human genome are regulated by miRNA
- 40% of miRNA genes lie in the introns or exons of other genes



- Cleavage of the mRNA strand into two pieces,
- Destabilization of the mRNA through shortening of its poly(A) tail, and
- Less efficient translation of the mRNA into proteins by ribosomes

relationships between miRNA dysregulation and human disease
http://www.mir2disease.org/

Inherited diseases

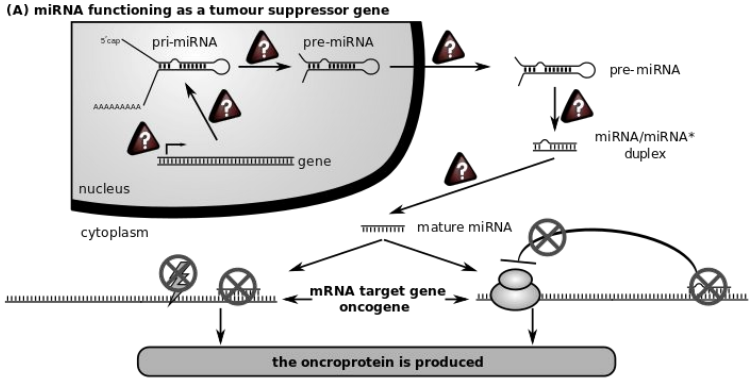
- A mutation in the seed region of miR-96, causes hereditary progressive hearing loss
- A mutation in the seed region of miR-184, causes hereditary keratoconus with anterior polar cataract.
- Deletion of the miR-17~92 cluster, causes skeletal and growth defects.

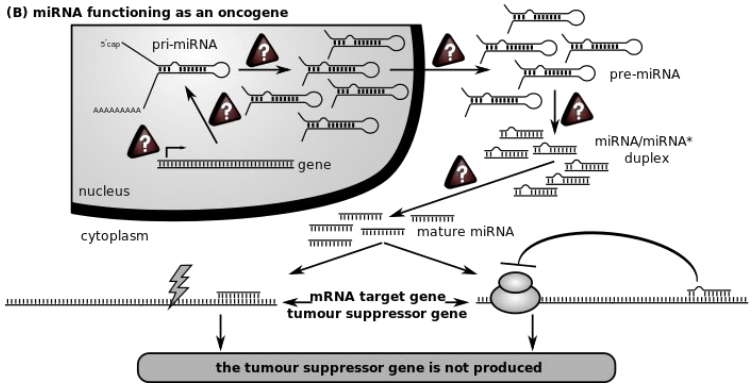
Heart disease

Kidney disease

Nervous system (alcoholism, obesity)

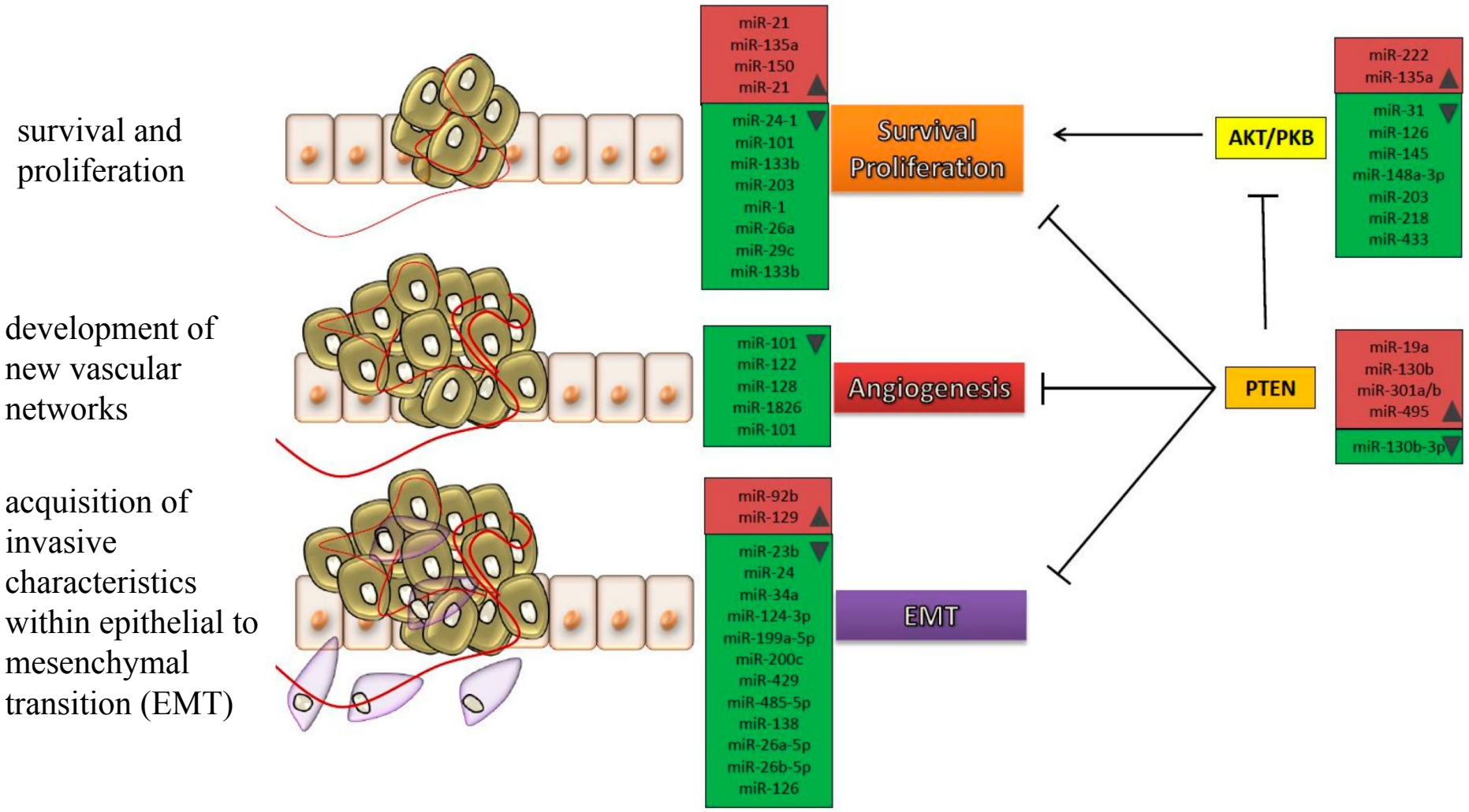
Cancer





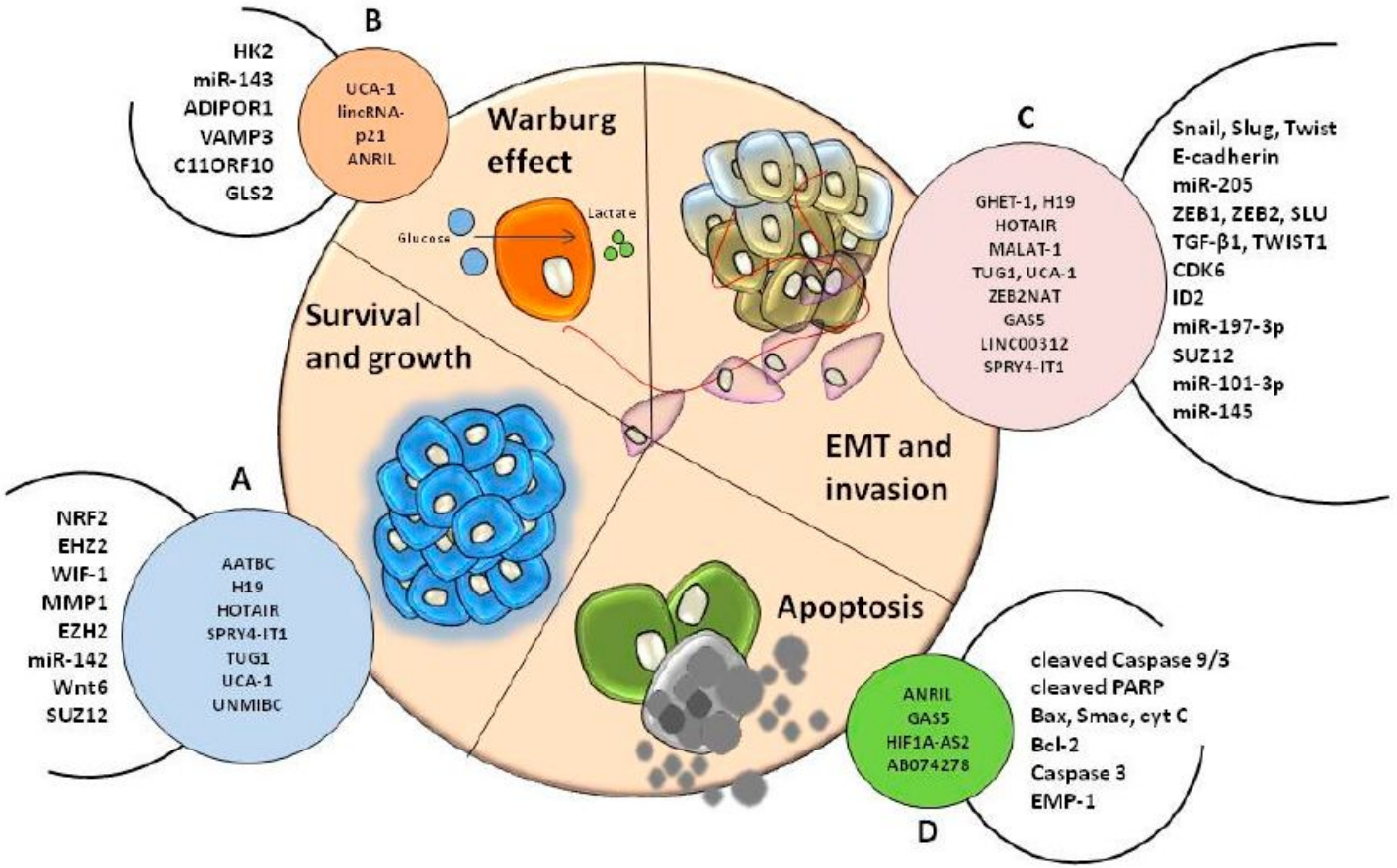
Processes affected by miRNAs in patients with bladder cancer.

In red are represented the upregulated microRNAs and in green those downregulated in bladder cancer; the arrow represents promotion of a process, and the T bar represents suppression of a process.



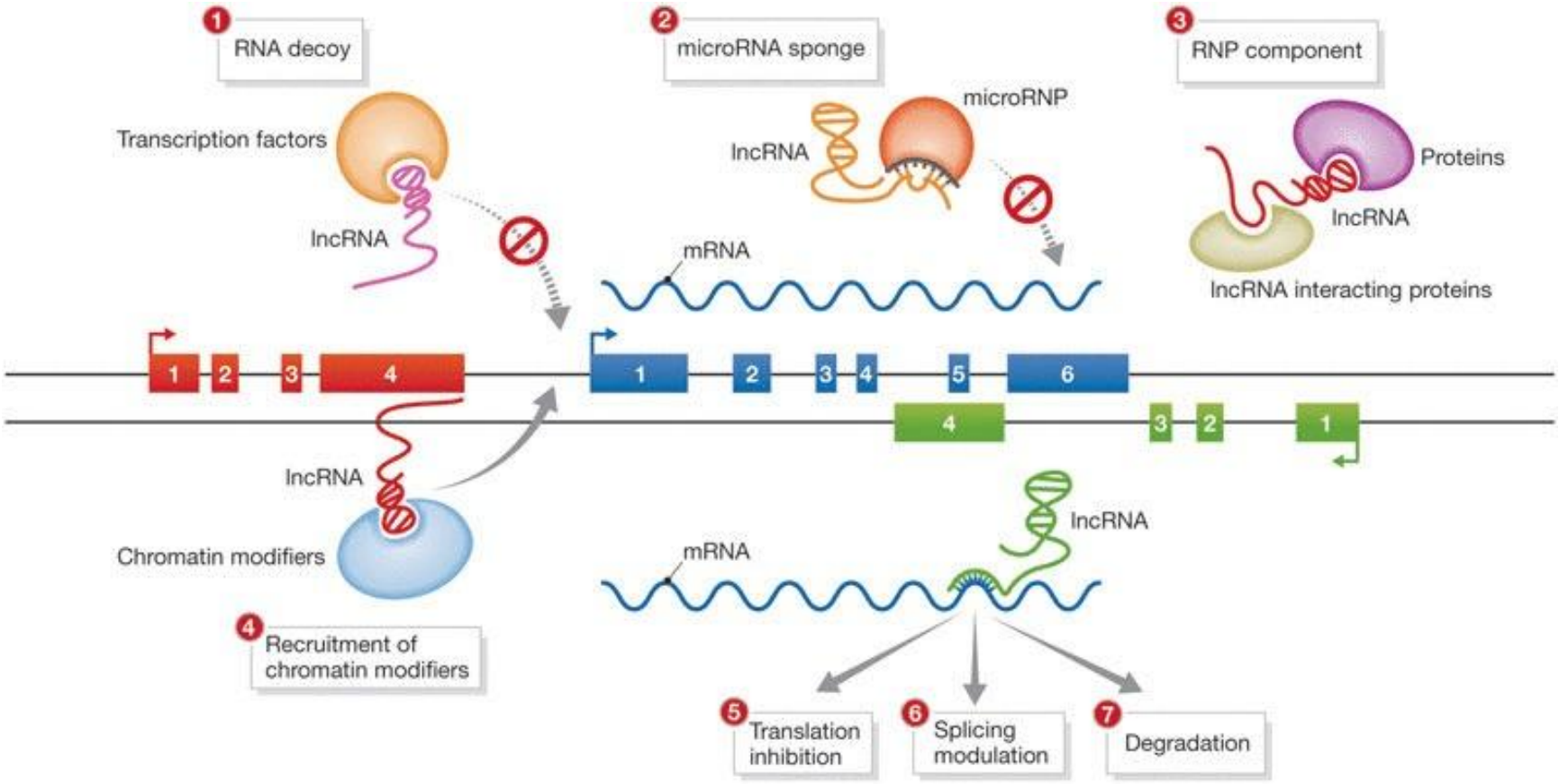
Pop-Bica C et al., Int J Mol Sci. 2017 18(7)

The involvement of lncRNAs in different processes associated with the hallmarks of cancer in bladder malignancies



Pop-Bica C et al., Int J Mol Sci. 2017 18(7)

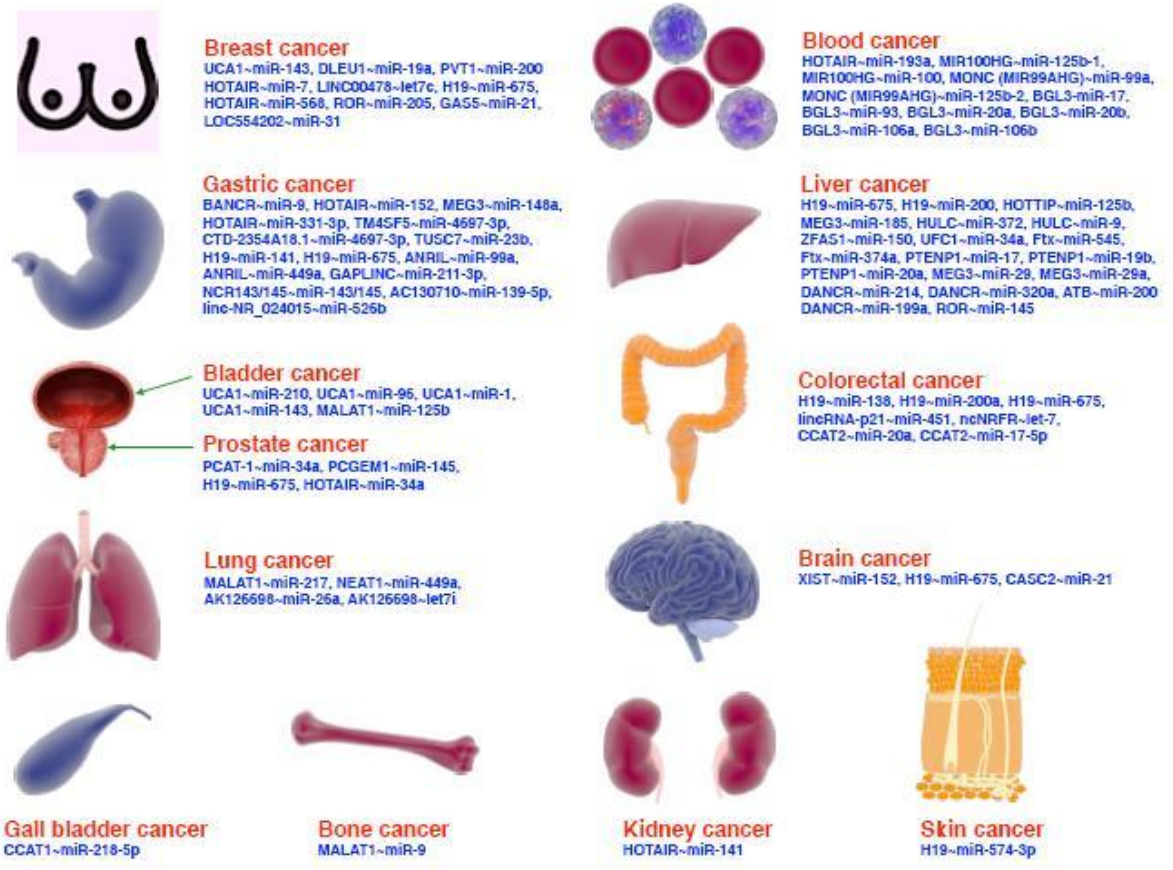
Mechanisms of lncRNA function



LncRNAs are involved in several important biological processes

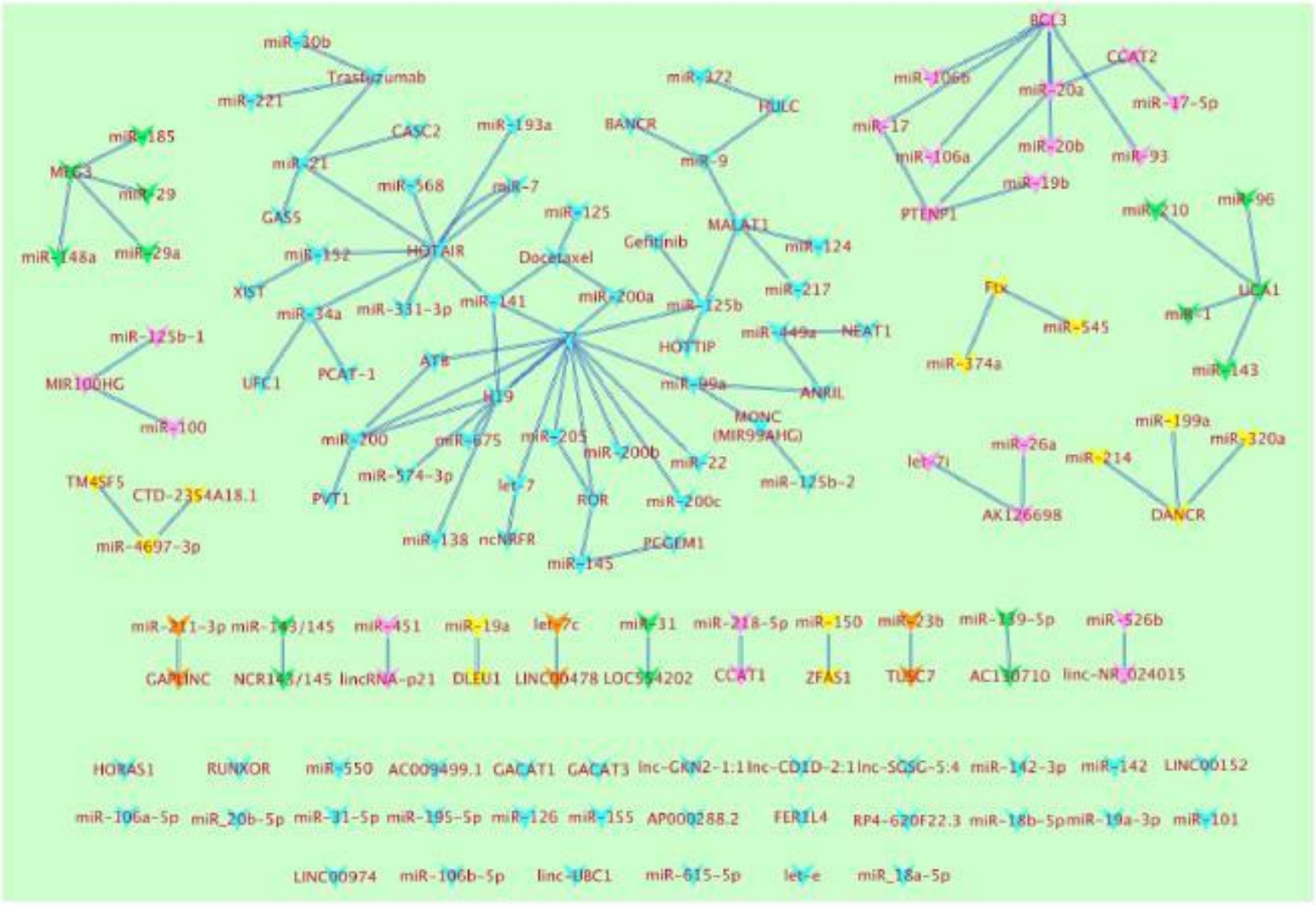
- X chromosome inactivation: Xist
- Epigenetic modification: HOTAIR
- Enhancers for neighboring genes: ncRNA-7a
- Genomic imprinting: H19, Air
- p53 signaling pathway: lincRNA-p21
- Oncogenic trasformation
- To be discovered

Current knowledge of interactions between lncRNAs and miRNAs in malignancies affecting various organs is summarized in the figure. The ~ sign denotes an interaction between the particular lncRNA and miRNA.



AIMS Molecular Science, 2016, 3(2): 104-124. doi: 10.3934/molsci.2016.2.104

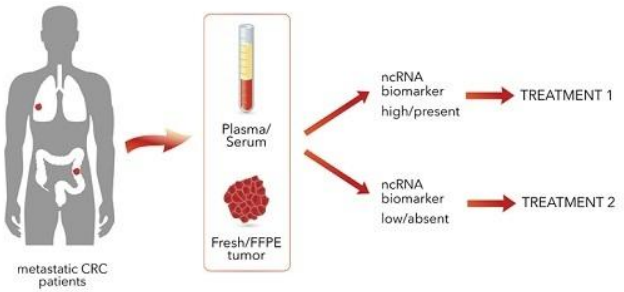
Regulatory cancer network of lncRNA-miRNA interactions. A regulatory cancer network of lncRNA-miRNA interactions is presented wherein each noncoding RNA (ncRNA) is represented as a “node” and interacting ncRNAs are joined by “edges”. Nodes interacting within the same subset or subgraph of the regulatory network retain the same color in the figure. Nodes listed in the bottom panel without any edges represent lncRNAs or miRNAs that have been implicated in cancer but whose interacting partners are yet to be identified. A question mark (?) as a node label indicates that the node identity is uncharacterized. The figure was generated using Cytoscape 3.3



Summary of ncRNAs that are response-to-therapy predictors in CRC

Prognostic ncRNA *	Tissue	Effect
Conventional cytostatics		
miR-21-5p	Tumor	Upregulation increases resistance to 5-FU
miR-129-5p	Tumor	Downregulation increases resistance to 5-FU
miR-429	Tumor	Upregulation increases resistance to 5-FU
miR-577	Tumor	Upregulation increases resistance to 5-FU
miR-1915-3p	Tumor	Downregulation increases resistance to chemotherapy
miR-122-5p	Tumor	Downregulation increases resistance to 5-FU
miR-21-5p	Tumor	Upregulation decreases resistance to nCRT
miR-153-3p	Tumor	Upregulation increases resistance to oxaliplatin
miR-19a-3p	Tumor	Upregulation increases resistance to FOLFOX therapy
miR-106a-5p, miR-130b-3p, miR-484	Plasma/serum	Upregulation increases resistance to FOLFOX therapy
miR-20a-5p, miR-130, miR-145-5p, miR-216a-5p and miR-372-3p	Plasma/serum	Response prediction to 5-FU-based adjuvant therapy
TUG1	Tumor	Upregulation increases resistance to methotrexate
MALAT1	Tumor	Upregulation increases resistance to FOLFOX
UCA1	Tumor	Upregulation increases resistance to 5-FU
Anti-VEGF antibodies		
miR-126-3p	Plasma/serum	Upregulation increases resistance to CT and bevacizumab
miR-664-3p	Tumor	Upregulation increases sensitivity to CT and bevacizumab
miR-455-5p	Tumor	Downregulation increases sensitivity to CT and bevacizumab
Anti-EGFR antibodies		
miR-99a-5p/let-7c/ miR-125b-5p	Tumor	Response prediction to EGFR target-therapies
miR-31-3p and miR-31-5p	Tumor	Response prediction to cetuximab but not panitumumab
miR-31-5p	Tumor	Upregulation increases resistance to EGFR target-therapies
miR-181a-5p	Tumor	Downregulation increases resistance to EGFR target-therapies
miR-143-3p	Tumor	Upregulation increases sensitivity to cetuximab
miR-145-5p	Tumor	Upregulation increases sensitivity to cetuximab

* The nomenclature of mature miRNAs has been updated to the latest miRBase release (v.21).



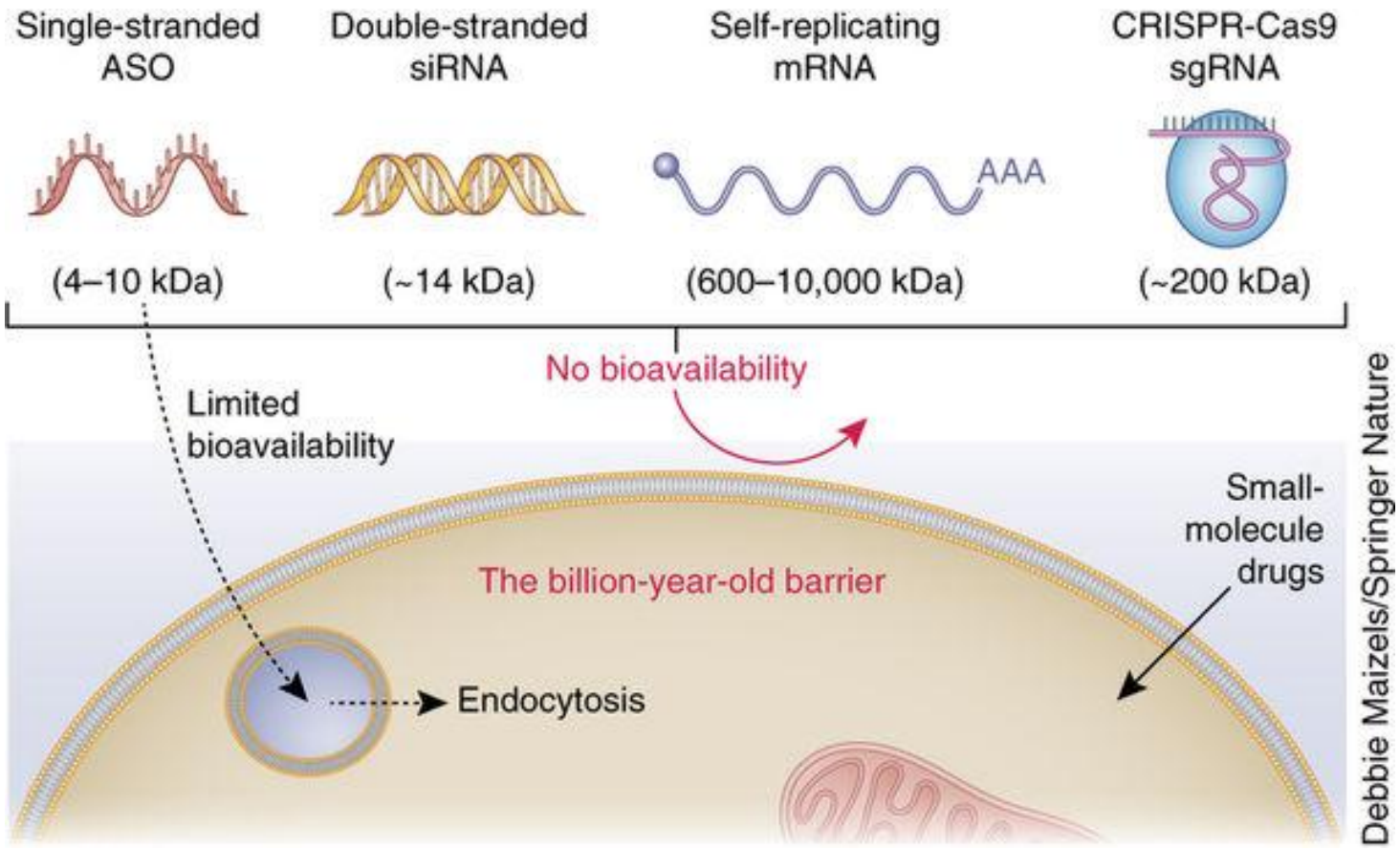
RNA-interference (RNAi) steps into biomedicine

- 1998 Andrew Fire and Craig Mello first demonstrated RNAi in C. elegans
- 2001 Thomas Tuschl demonstrated potent and specific RNAi silencing in mammalian cells
- Around 2005 Major pharmaceutical companies invested several billion dollars in RNAi therapeutics
- 2006 Nobel Prize in Physiology or Medicine
- 2009 era of disappointments, doubts and despair (OPKO Health shut down its Phase 3 trial of an RNAi treatment for wet macular degeneration)
- 2010 Roche, which had invested about $500 million in RNAi, shut down its internal research program (the same for Pfizer, Abbott and Merck
- 2014 The Second Coming of RNAi

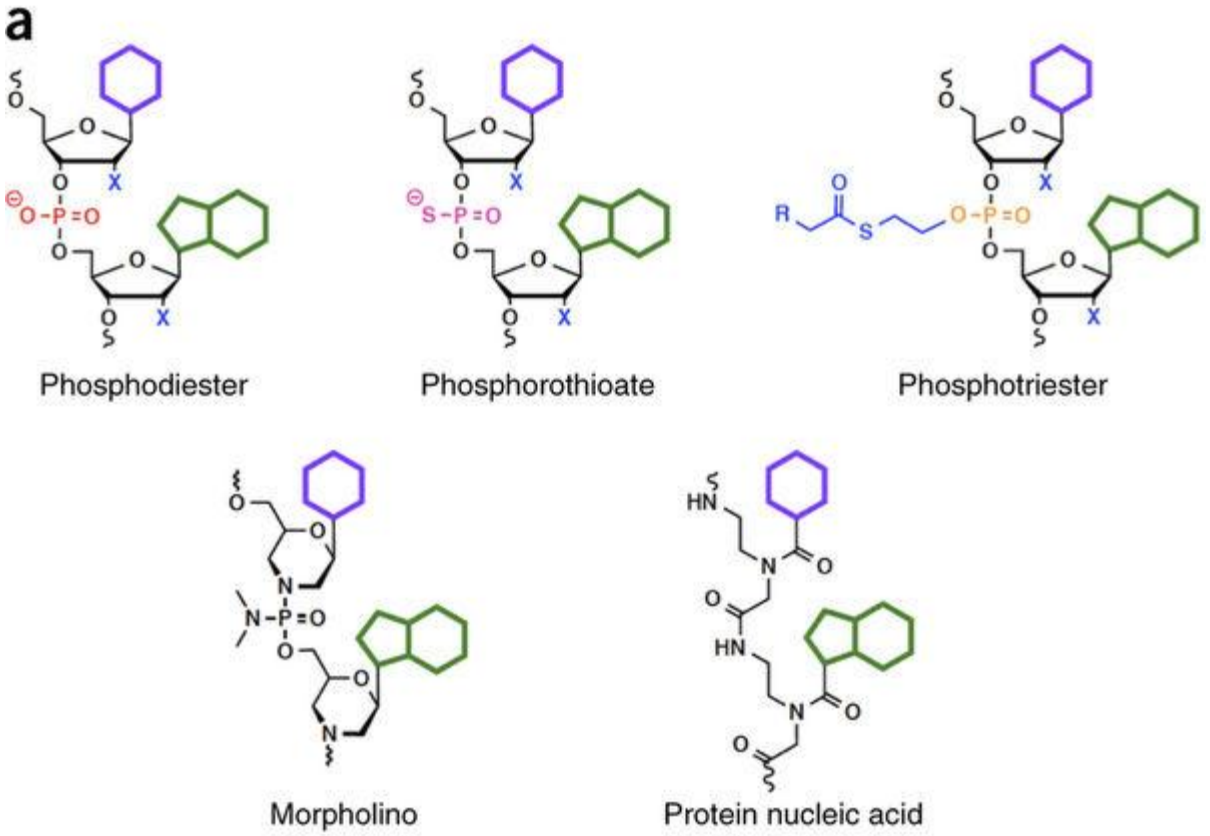
Challenges erected by evolutionary barriers to RNA therapeutic delivery

Feature	Challenge for delivery
Oligonucleotide size and charge	Too large or too charged to passively diffuse across the lipid bilayer
RNase susceptibility	Rapid degradation by blood and tissue RNases.
Reticuloendothelial system	Rapid clearance from the blood by the kidneys and liver scavenger receptors
Immunogenicity	Oligonucleotides activate extracellular and intracellular innate immune responses
Endocytosis	Oligonucleotides are taken up, but trapped inside endosomes

The four-billion-year-old lipid bilayer protects cells from invading RNAs



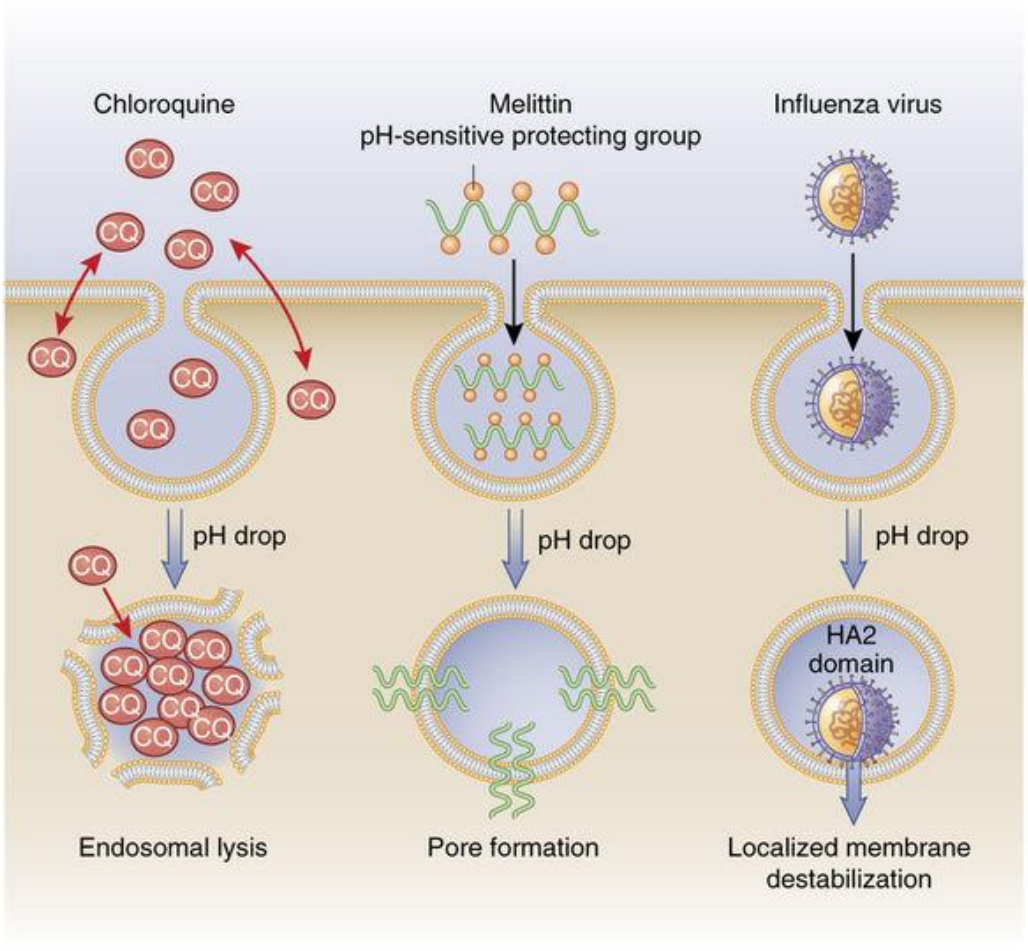
Native RNA and RNA-based therapies are vulnerable to degradation from the ribonucleases



Common ASO and siRNA modifications

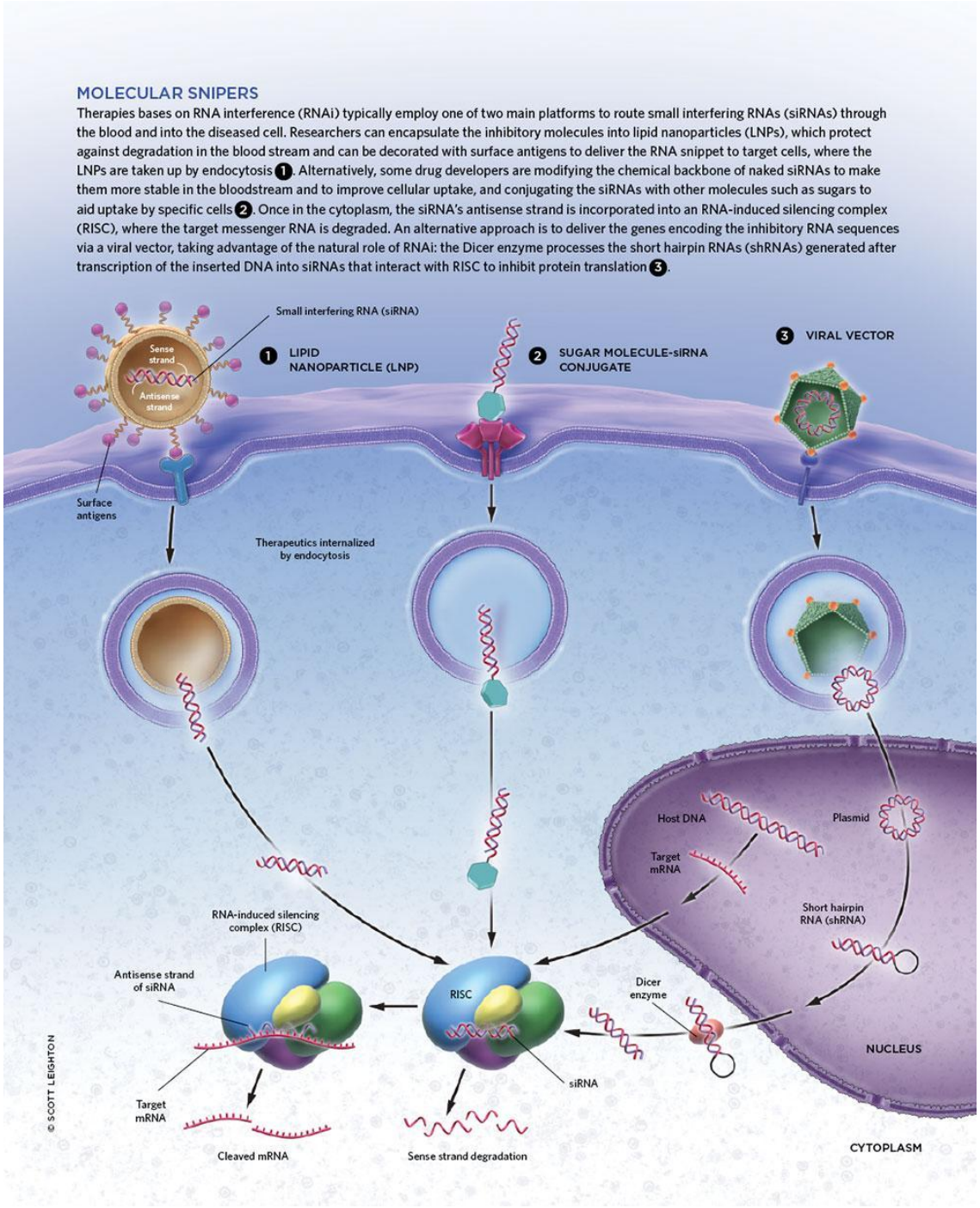
- hardening the RNA against enzymatic attacks
- increase target affinity
- decrease undesired immunogenicity
- improve overall efficacy

Endosomal escape agents



Dynamic Polyconjugates (DPCs), is a siRNA bound to an endosomolytic polymer backbone via a disulfide bond111

Strategies for the delivery of RNA-based therapeutics



- Stable nucleic acid lipid particles (SNALPs from Tekmira Pharma (LNP technology))
- Smarticles technology from Marina Biotech (negatively charged Smarticles avoid the often seen toxic effects of positively charged lipids at physiological pH)
- PLGA nanoparticles
- Alnylam Pharmaceuticals (*N*-Acetylgalactosamine (GalNac) improve the delivery of siRNA)
- The co-injection therapy was shown to increase the efficacy of siRNA-cholesterol 500-fold with a 90% knockdown (Arrowhead Research)

Manufacturing RNA-based biopharmaceuticals

- RNA-based biopharmaceuticals are inherently susceptible to endonucleases
- special handling is required for production and purification
- mRNA purification (post-chemical synthesis)
  - concentration precipitation
  - extraction
  - chromatographic methods

First ever RNA-based gene-silencing drug approved by FDA

hereditary transthyretin-mediated amyloidosis (hATTR)

US$450,000 per year for a single patient

disrupts the RNA mechanism producing transthyretin, a protein that promotes an accumulation of amyloid deposits in the body

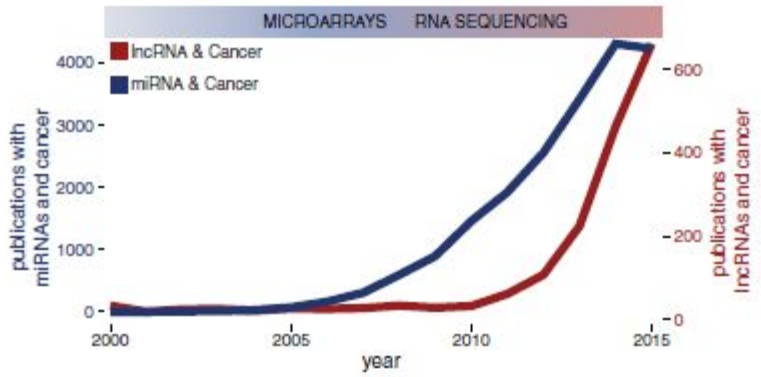


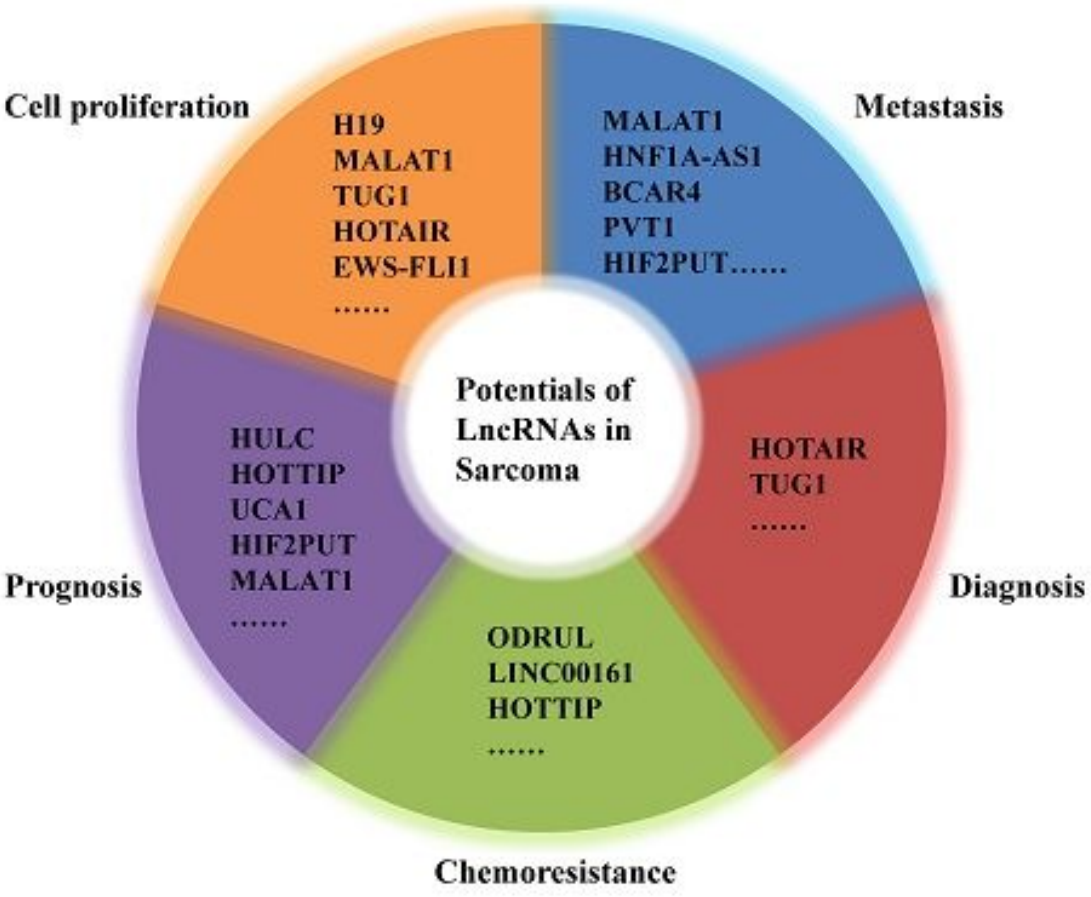
Fig. 1 Interest for lncRNAs (*red*) in the cancer scientific community compared to miRNAs (*blue*). The y-axis represents the number of publications and the x-axis represents time. Data was obtained by searching Pubmed for 'lncRNA cancer' or 'miRNA cancer'. Data from 2016 was not used in the graph. Publications with terms 'miRNA' and 'cancer' plateau in 2015

Какие разделы добавить:
(конкретные примеры)
https://nplus1.ru/news/2017/10/05/gold-crispr
https://nplus1.ru/news/2017/10/06/Cas13-vs-interference

Table 2. Differential expression between the tumor (T), the stroma (S), and the normal tissue (N) compartment (fold change, *p*-values were calculated using a Wilcoxon signed-rank test).

Differential Expression in Liver and Lung Metastases							
miRNA	**Tissue**	**S vs. N**	***p*-Value**	**T vs. N**	***p*-Value**	**S vs. T**	***p*-Value**
miR-125b	liver	3.417	0.438	0.070	<0.0001	211.500	<0.0001
	lung	7.305	0.0078	0.206	<0.0001	43.990	<0.0001
miR-145	liver	4.048	0.533	0.095	<0.0001	223.100	<0.0001
	lung	1.848	0.3465	0.203	<0.0001	14.560	<0.0001
miR-199a-3p	liver	6.593	0.07	0.249	<0.0001	624.400	<0.0001
	lung	4.700	0.0296	0.345	<0.0001	14.480	<0.0001
miR-199a-5p	liver	9.137	0.0803	0.071	<0.0001	510.900	<0.0001
	lung	9.143	0.0004	0.649	0.0016	25.560	<0.0001
miR-429	liver	14.610	0.0065	46.150	0.0009	0.25	0.0066
	lung	9.418	0.0163	80.890	<0.0001	0.178	<0.0001
miR-127-3p	liver	1.310	0.0658	0.101	<0.0001	61.180	<0.0001
	lung	4.907	0.0149	0.702	0.0023	10.950	<0.0001
miR-19b	liver	0.278	<0.0001	0.291	<0.0001	2.569	0.2699
	lung	1.944	0.0006	2.326	0.0007	1.475	0.0383
miR-194	liver	0.349	<0.0001	3.103	0.7265	0.466	<0.0001
	lung	168.500	<0.0001	714.900	<0.0001	0.272	<0.0001
miR-215b	liver	0.167	<0.0001	1.147	0.0372	0.392	<0.0001
	lung	58.310	0.0028	319.100	<0.0001	0.095	<0.0001
miR-192	liver	0.030	<0.0001	0.306	<0.0001	0,255	<0.0001
	lung	33.480	0.0012	192.100	<0.0001	0,198	<0.0001
miR-21	liver	13.900	0.0006	2.872	0.2296	33.680	<0.0001
	lung	11.880	0.0001	5.653	0.0025	4.557	0.0045

Int. J. Mol. Sci. **2016**, *17*(10), 1755; doi:10.3390/ijms17101755


Cell proliferation
H19
MALAT1
TUG1
HOTAIR
EWS-FLI1
.....
Metastasis
MALAT1
HNF1A-AS1
BCAR4
PVT1
HIF2PUT.....
Potentials of LncRNAs in Sarcoma
Prognosis
HULC
HOTTIP
UCA1
HIF2PUT
MALAT1
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Diagnosis
HOTAIR
TUG1
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Chemoresistance
ODRUL
LINC00161
HOTTIP
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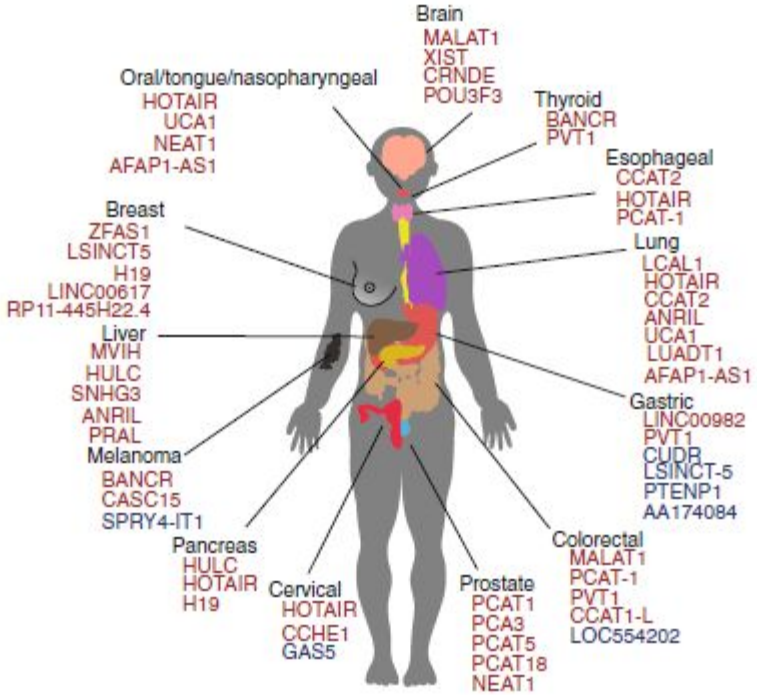
Brain
MALAT1
XIST
CRNDE
POU3F3
Oral/tongue/nasopharyngeal
HOTAIR
UCA1
NEAT1
AFAP1-AS1
Thyroid
BANCR
PVT1
Esophageal
CCAT2
HOTAIR
PCAT-1
Breast
ZFAS1
LSINCT5
H19
LINC00617
RP11-445H22.4
Lung
LCAL1
HOTAIR
CCAT2
ANRIL
UCA1
LUADT1
AFAP1-AS1
Liver
MVIH
HULC
SNHG3
ANRIL
PRAL
Gastric
LINC00982
PVT1
CUDR
LSINCT-5
PTENP1
AA174084
Melanoma
BANCR
CASC15
SPRY4-IT1
Pancreas
HULC
HOTAIR
H19
Cervical
HOTAIR
CCHE1
GAS5
Prostate
PCAT1
PCA3
PCAT5
PCAT18
NEAT1
Colorectal
MALAT1
PCAT-1
PVT1
CCAT1-L
LOC554202