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MicroRNA and Stem Cell Differentiation

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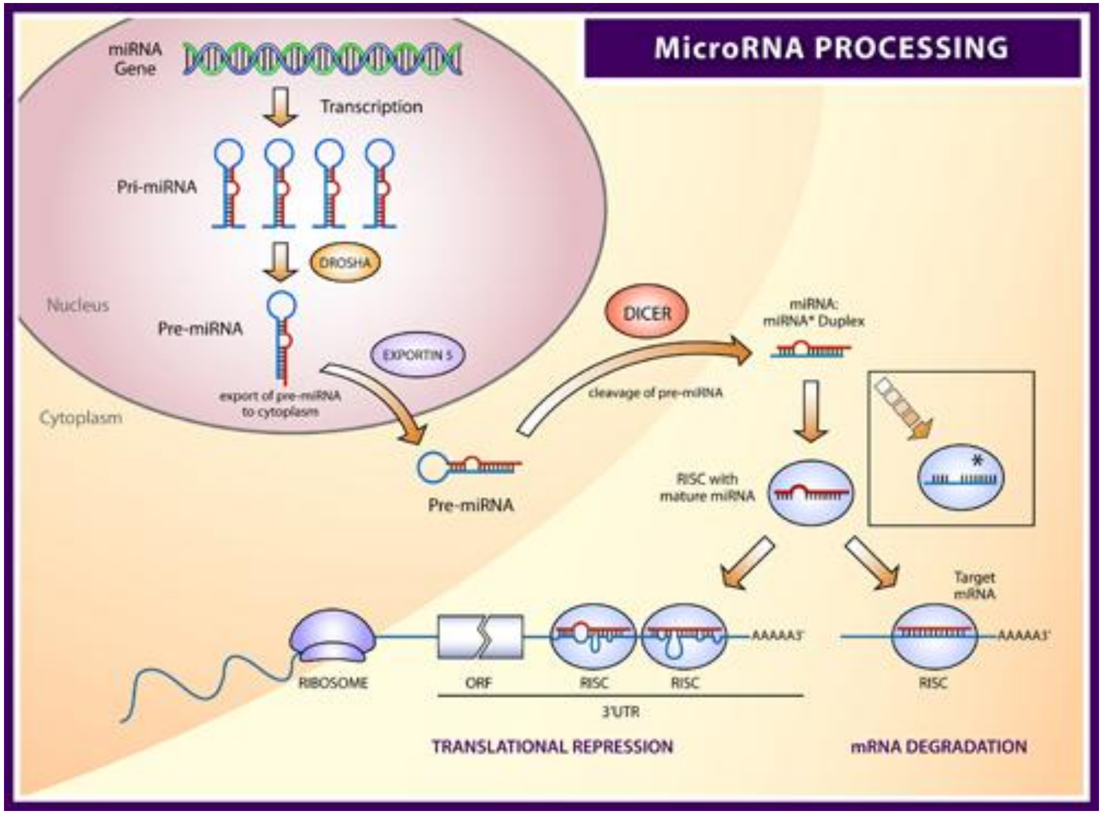
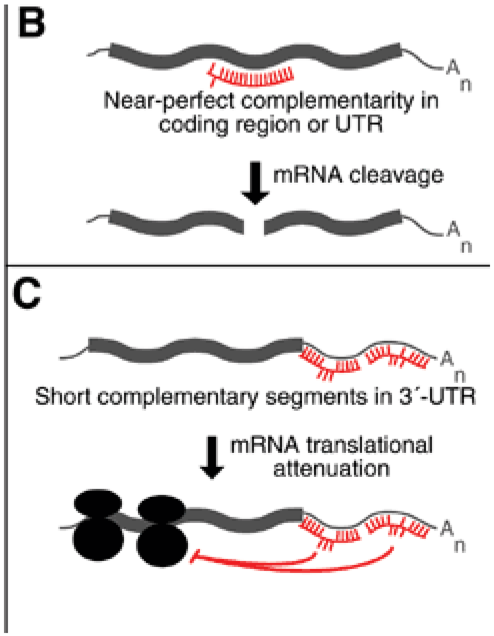
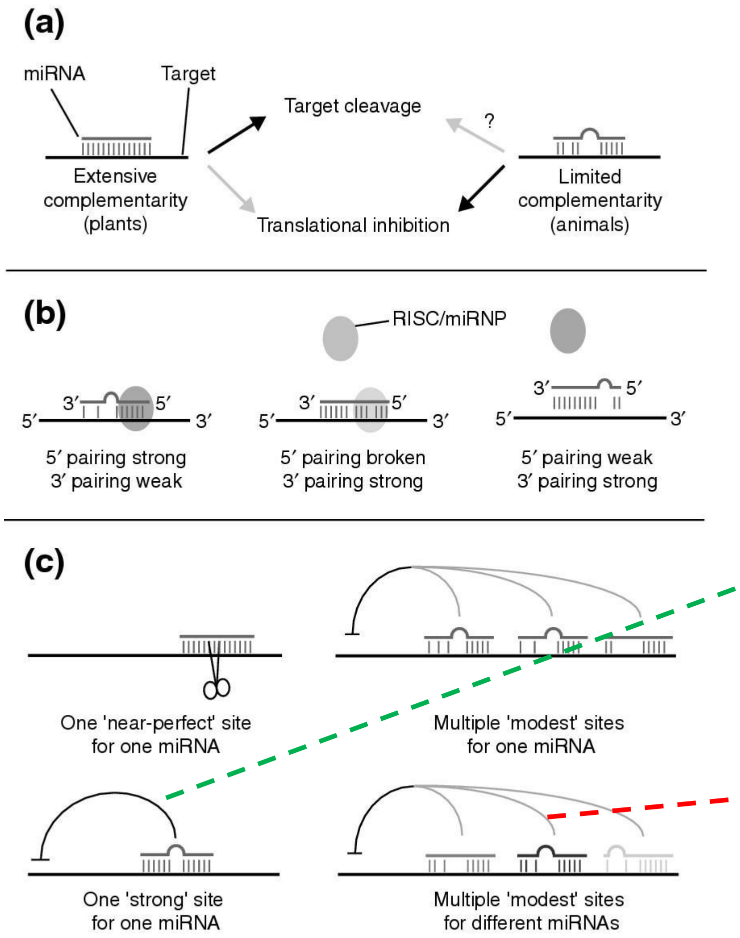
The processing of microRNA from gene to RISC complex





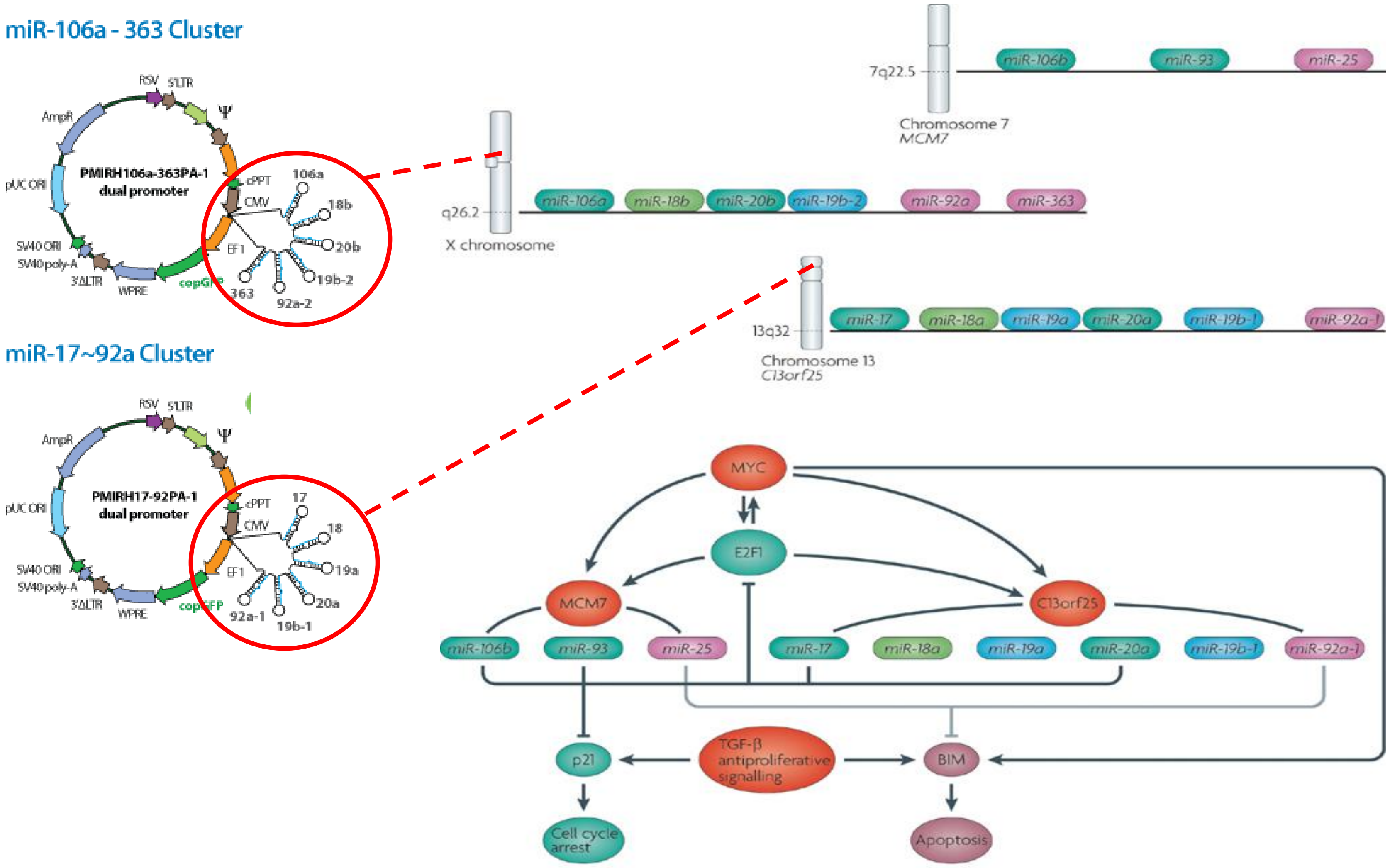
To suppress translation of a transcript; one or more microRNA species?



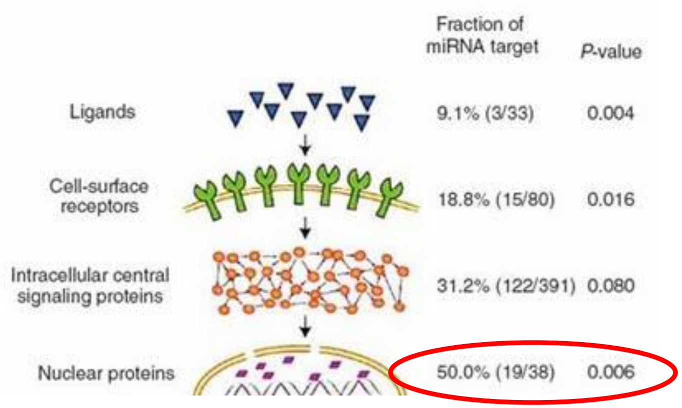
There are two "concepts" advocating the need for microRNAs to control gene expression:

Some people assert that ***only one microRNA*** is necessary and sufficient to alter gene expression/cell phenotype, while others claim that ***5-6 microRNA species*** are necessary to do the same job

Some microRNAs are located in clusters outside/within genes on given chromosomes and may be organized in hierarchical regulatory sequences or loops encompassing microRNAs, TFs and functional genes

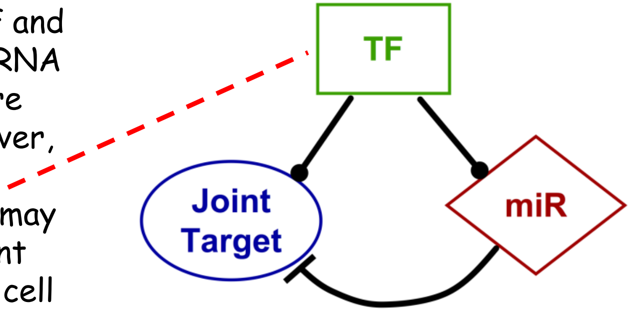


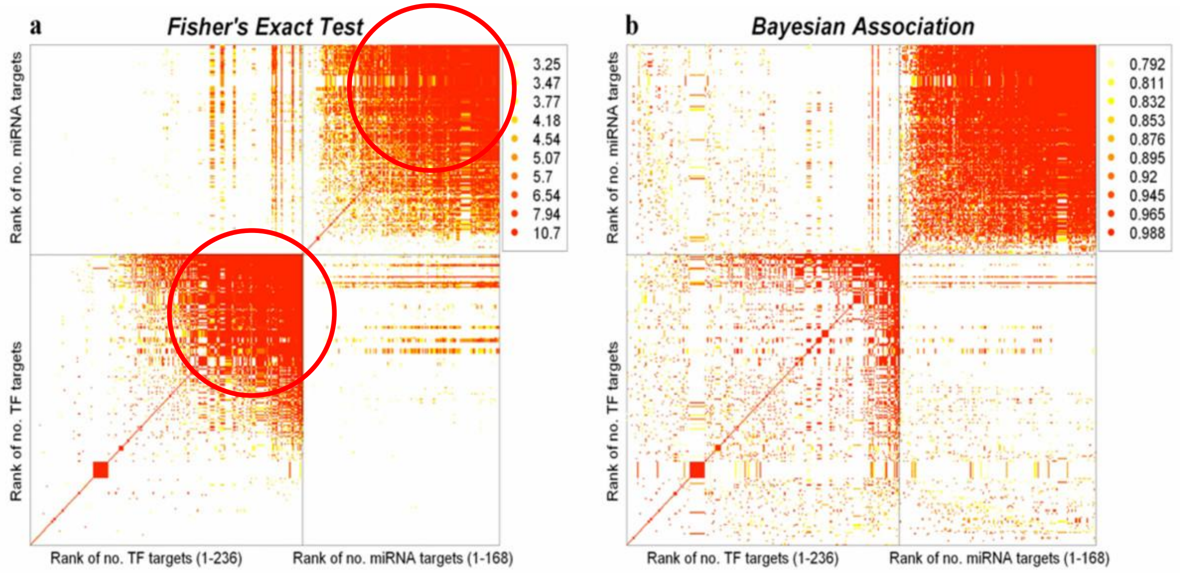
The interrelationship between microRNAs, transcription factors (TFs) and target (functional) genes



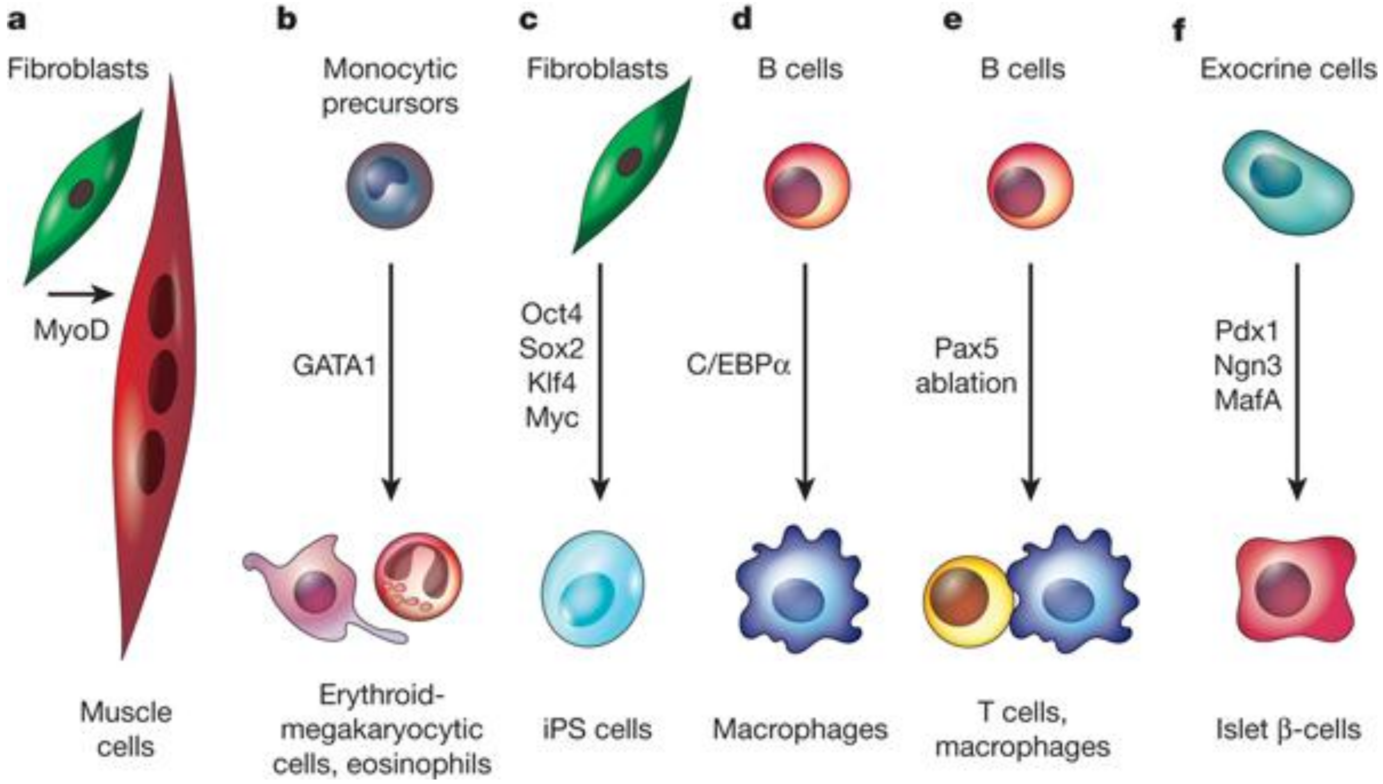
		Fraction of miRNA target	P-value
Ligands		9.1% (3/33)	0.004
Cell-surface receptors		18.8% (15/80)	0.016
Intracellular central signaling proteins		31.2% (122/391)	0.080
Nuclear proteins		50.0% (19/38)	0.006

Putatively, TF-TF and microRNA-microRNA interactions are preferred, however, searches for *regulatory loops* may reveal important determinants of cell phenotype



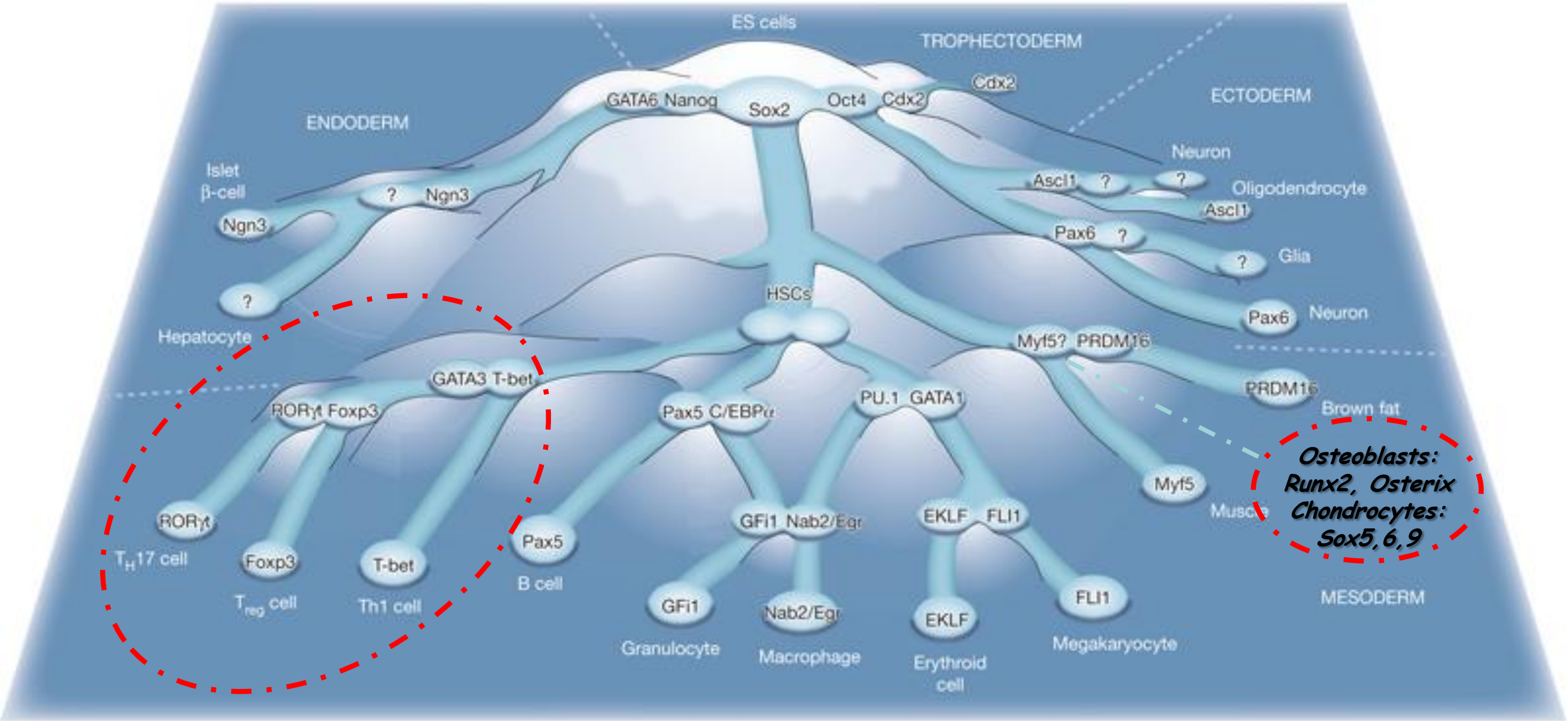


Examples of transcription factor overexpression or ablation experiments that result in cell fate changes



Thomas Graf & Tariq Enver *Nature* **462**, 587-594 (2009)

Transcription factor cross-antagonisms in a cascading landscape of unstable and stable cell states

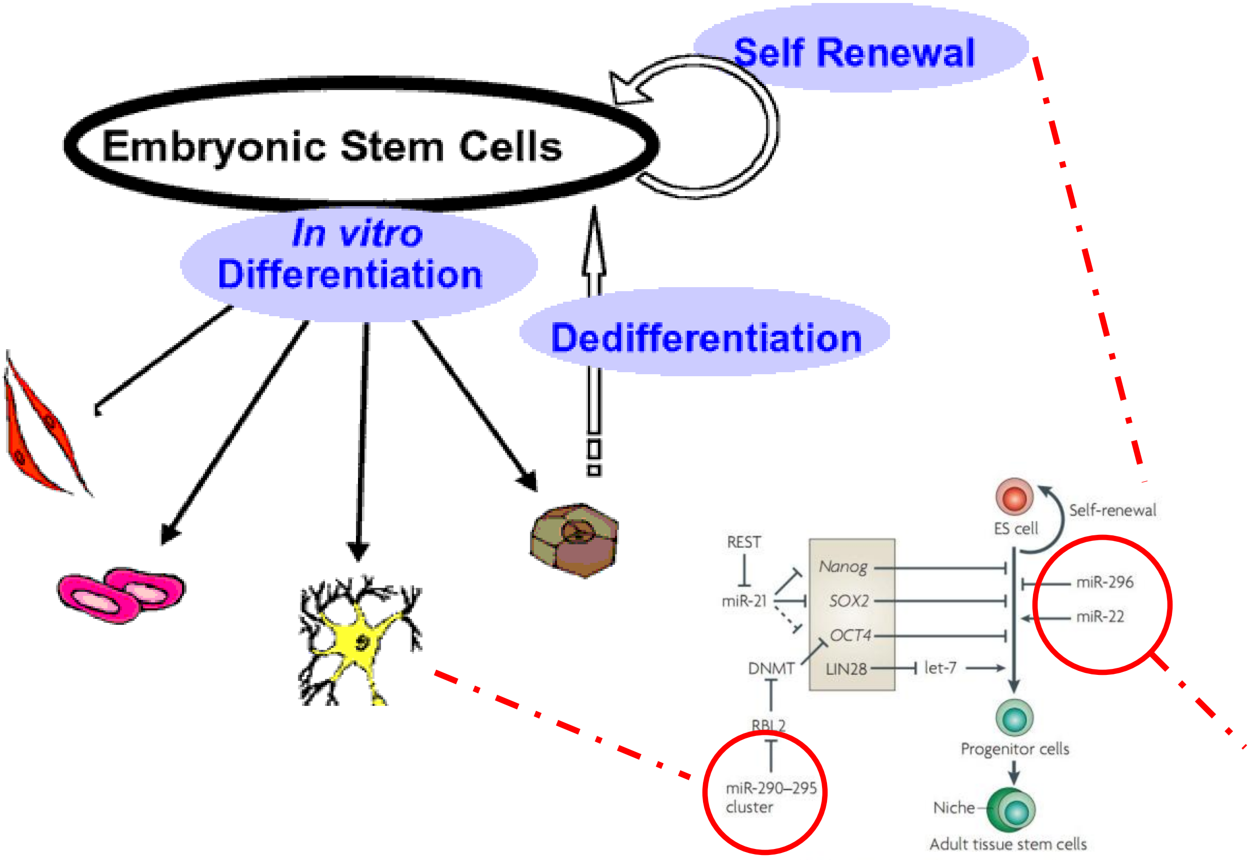


Osteoblasts:
Runx2, Osterix
Chondrocytes:
Sox5,6,9

Thomas Graf & Tariq Enver *Nature* **462**, 587-594 (2009)

nature

Manipulering av stamceller med gener (som er viktig for selvfornyelse) og mikroRNA



Self Renewal

Embryonic Stem Cells

***In vitro* Differentiation**

Dedifferentiation

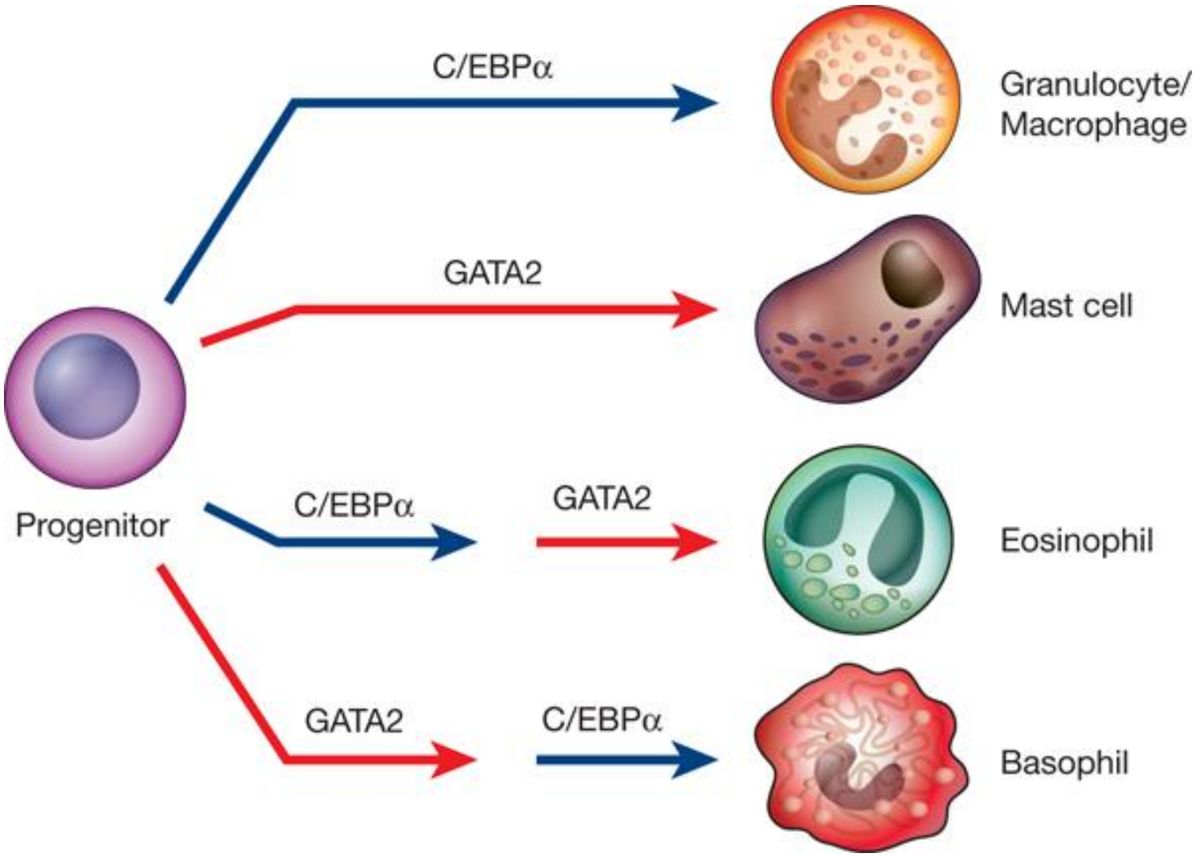
RNA regulation of embryonic stem cells.

- *Man kan dedifferensiere benceller og bruskceller ved å la dem gro i en 2D-struktur i Petri-skåler, eller introdusere (overuttrykke) gener som sørger for selv-fornyelse av stamceller.*

- *Eller man kan også manipulere med cellenes konsentrasjoner av såkalt mikroRNA*

Jan O. Gordeladze, professor, dr. philos, Avd. for Biokjemi, Institutt for Medisinske Basalfag, UiO

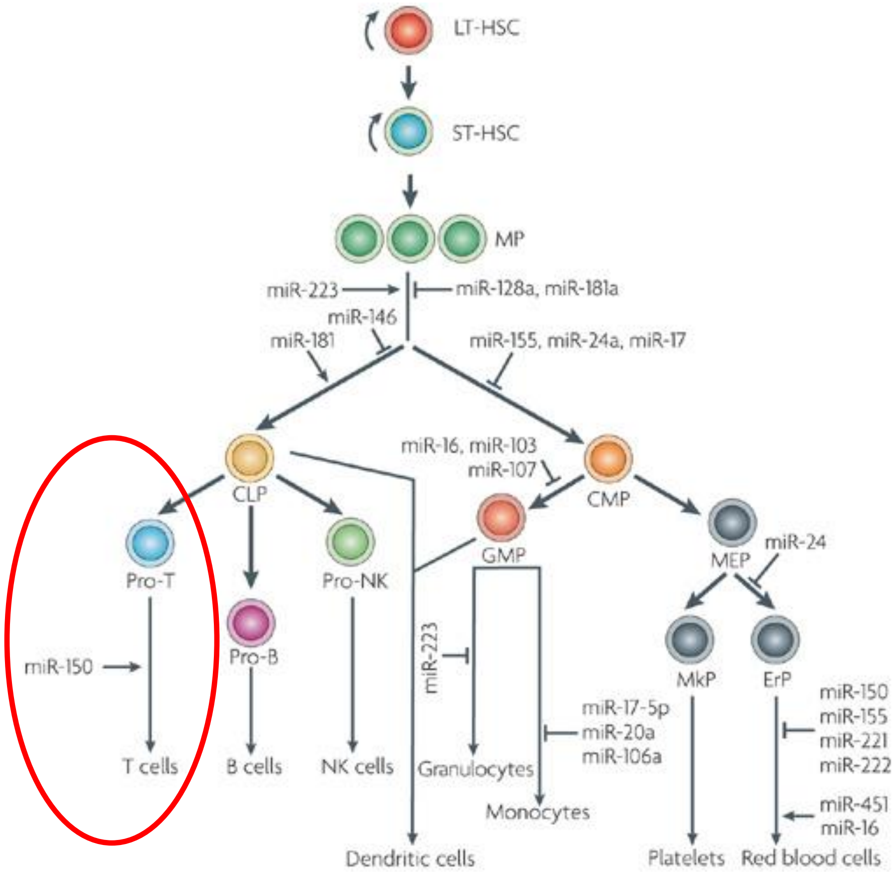
Timing of transcription factor expression and lineage outcome



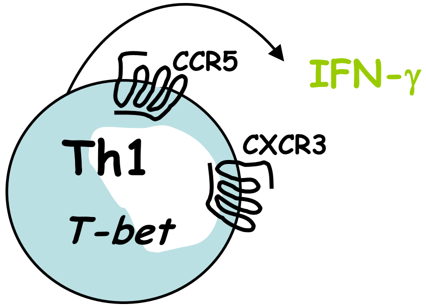
Thomas Graf & Tariq Enver *Nature* **462**, 587-594 (2009)

MicroRNA species shown to be involved in hematopoietic stem cell differentiation

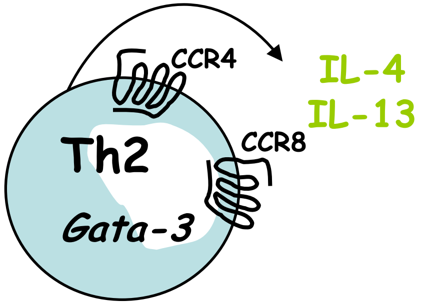
Little is known of
the microRNAs
responsible for the
differentiation and
plasticity of naive
T cells



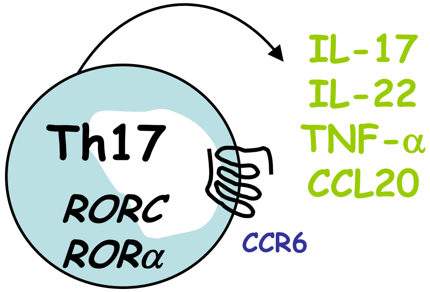
Transcription factors involved in the differentiation of Th-cells from naïve T-cells (literature survey, 2009)



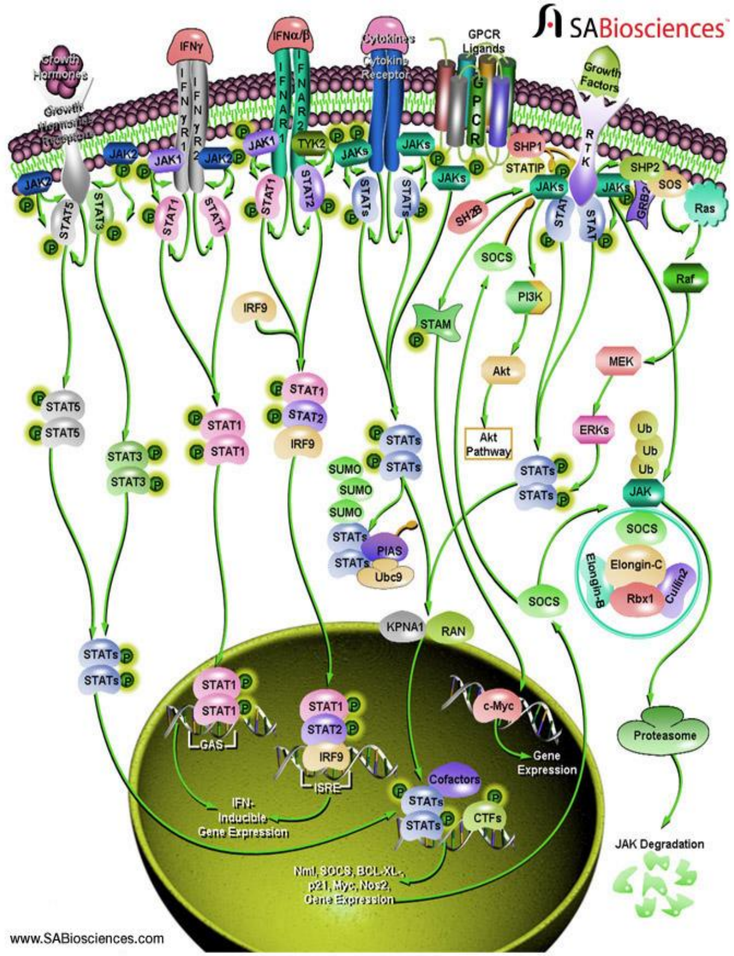
T-bet (TBX21), STAT1, STAT4, IRF1, NFATc1, Runx3

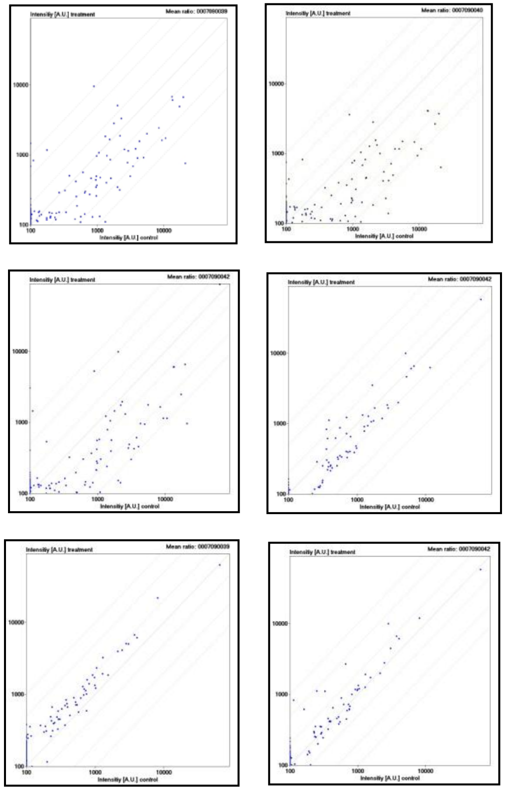


GATA-3, STAT6, c-Maf, c-Jun, NFATc1



RORα (RORA), RORγ (RORC), STAT3, STAT4, IRF4, Act-1, Foxp3, Runx1



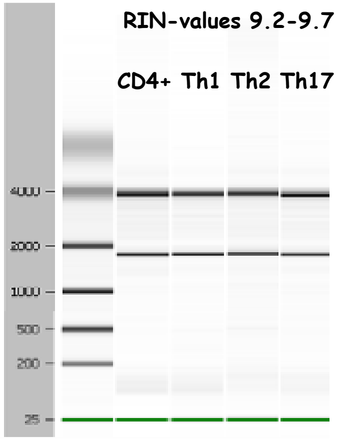
Micro-RNA species	*a) Naive T vs Th (1, 2, 17) ratio*	*Micro-RNA species*	*b) Th17 vs Th1 and Th2 ratio*
150	26.8	923	2.78
20a	9.25	638	2.71
30d	9.04	663	3.09
17	8.93		
19b	7.91		
26a	7.64		
106a	6.79		
20b	6.73		
Let-7g	6.52		
Let-7a	5.28		
16	5.13		
19a	4.94		
768-3p	4.93		
142-5p	4.56		
146b-5p	4.43		
155	3.24		
923	0.32		
638	0.12	*Miltenyi Biotech, France*	
663	0.057		

Relative expression of micro-RNA species in:

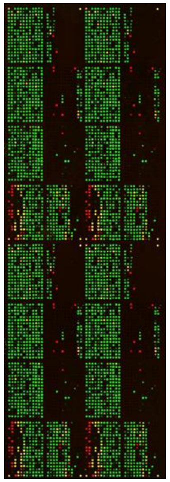
a) Activated naive T (CD4+) cells vs the average for activated Th1, Th2 and Th17 cells

b) Activated Th17 cells vs the average for activated Th1 and Th2 cells

RNA-solation by *mirVana®*



RIN-values 9.2-9.7



Question 3

"We would like to know which of the genes, putatively being targeted by the above mentioned microRNAs will have two or more of the subject microRNAs "in common"

Directory: "Common Targets"

I used 3 sets of parameters to find putative target genes: "Stringent", "medium" and "large". Genes are identified by their transcript identifier (from Ensembl). That explains multiple gene occurrences in lists. Lists are ordered by scores, and can be explored using HTML file format.

Stringent list: 57 targeted genes

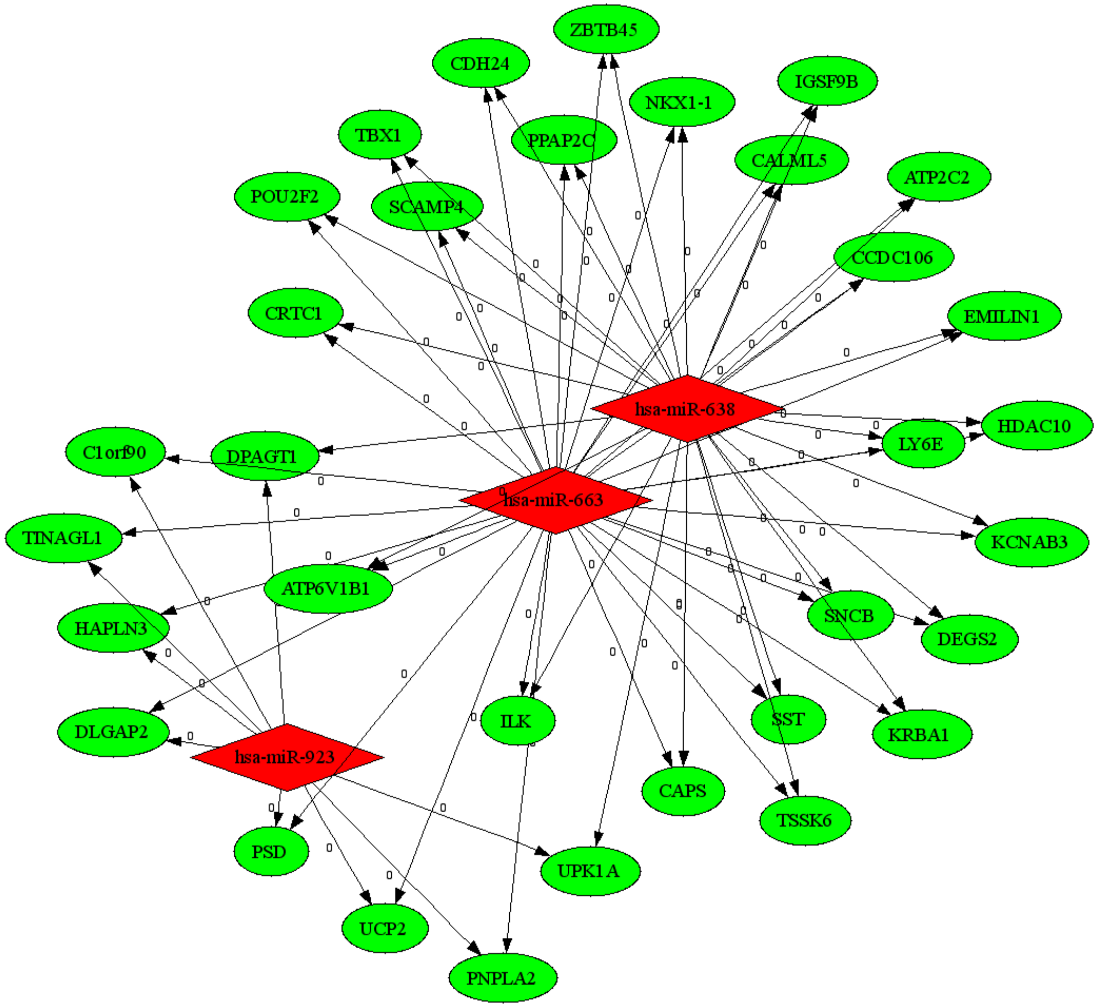
Score>=18, p-value<=0.001, number of miRNAs on targeted genes >= 2

Medium list: 247 targeted genes

Score>=17, p-value<=0.001, number of miRNAs on targeted genes >= 2

Large list: 620 targeted genes

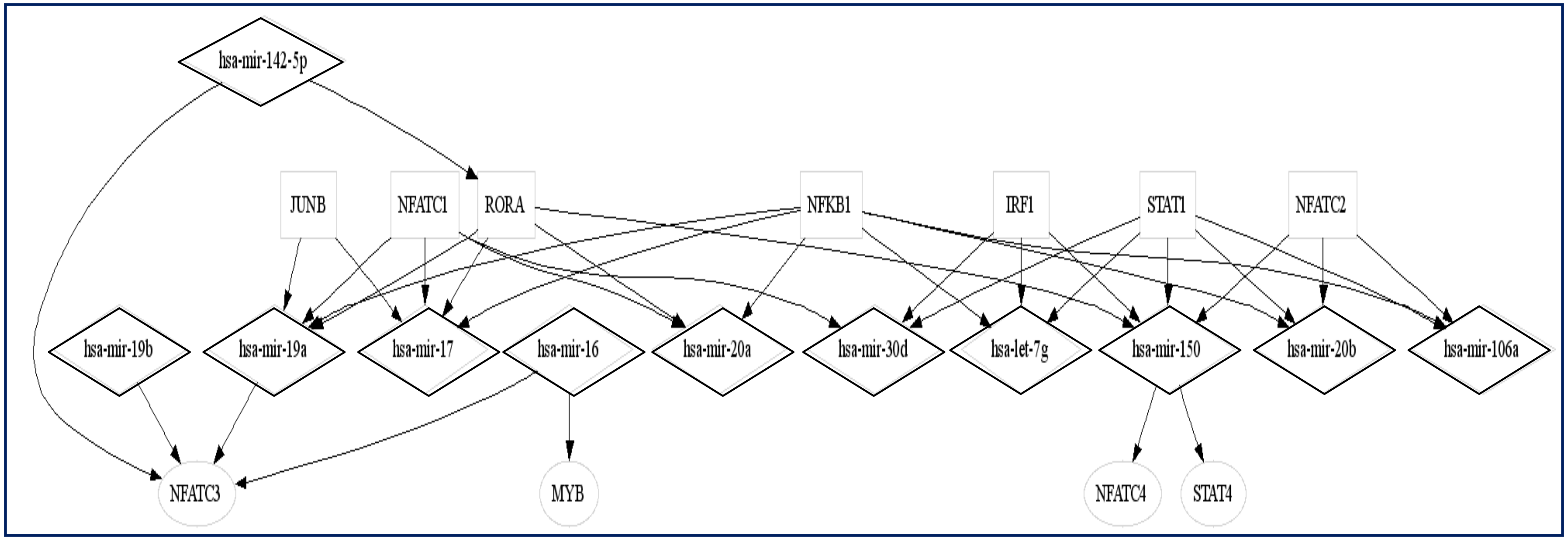
Score>=17, p-value<=0.01, number of miRNAs on targeted genes >= 2



Question 1, addressed by using the Mir@nt@n database

"We would like to see which microRNAs may target two or more of the transcription factors from the […] complete list"

* Directory: "TF/ListComplete"
2 graphs were generated (Hierarchical and Organic views). TFs found to be targeted by miRNAs: RORA, STAT4, NFATc4, NFATc3 and MYB.



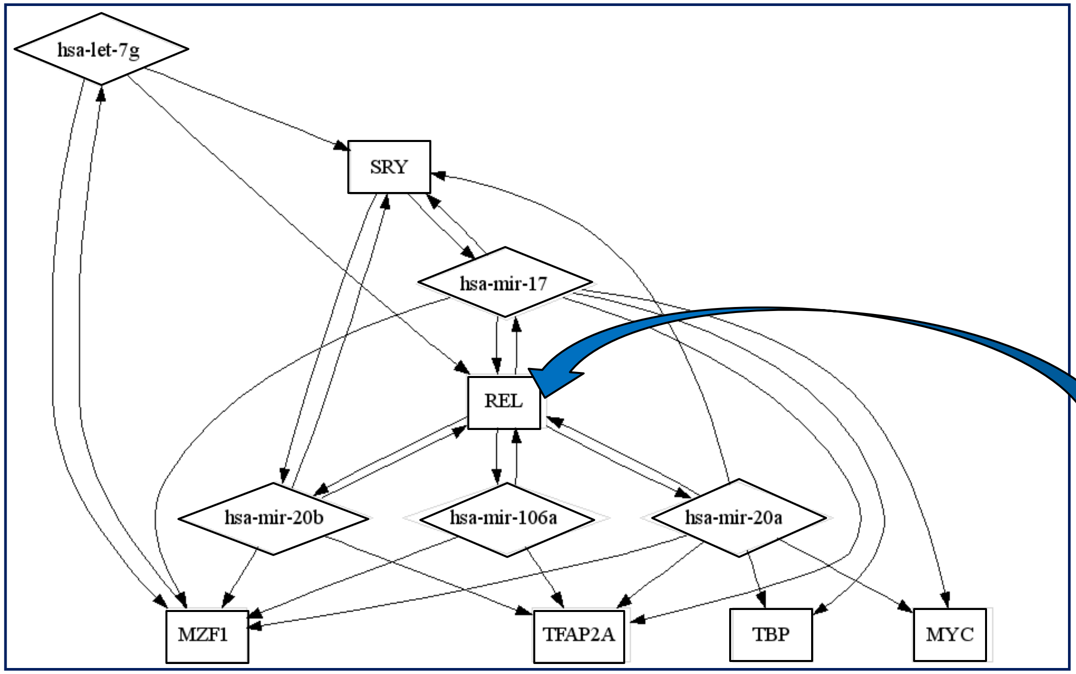
* T-bet (TBX21), STAT1, STAT3, STAT4, STAT6, IRF1, NFATc1, NFATc2, NFATc3, NFATc4, NFATc5, GATA3, c-maf, c-Jun, JunB, RORalpha (RORA), RORgamma (RORC), IRF4, Act-1, Runx1, Runx3, NFkappaB, IkappaB, AP-1, MYB, TOX, Notch, MAML1, p50, p65, Th-POK, Twist

Question 2, addressed by using the Mir@nt@n database

"Can we identify feedback loops using the input microRNA list?"

Directory: "TF/FeedbackLoop"

This question can be answered in one click! Feedback loop is defined as a couple of TF and miRNA that regulate each other. A hierarchical graph was generated and includes 6 TFs and 5 miRNAs.

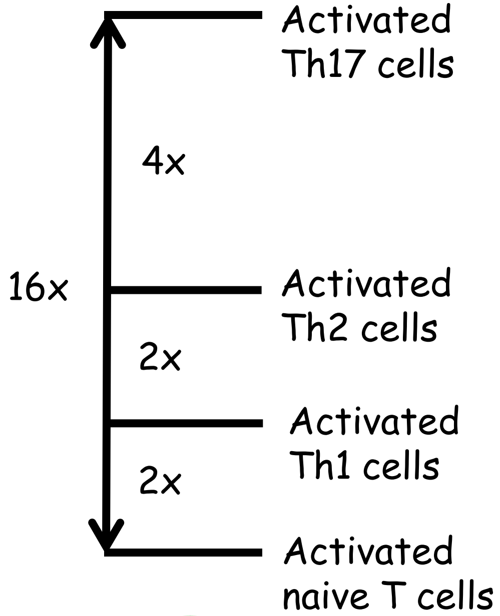


Names of genes involved in feedback loops:

SRY: Testis determining factor
REL: C-rel proto-oncogene protein
MZF1: Myeloid zinc finger 1 (MZF-1)
FAP2A: Transcription factor AP-2
TBP: TATA-box binding protein
MYC: Myc proto-oncogene (transcription factor p64)

REL is heavily involved in lymphocyte proliferation, but also important for T cell function. It interacts with IRF1 and IRF4, as well as the NFκB family of TFs

Relative levels of miRNAs 663, 638 and 923 between T cell species



Activated Th17 cells

4x

16x

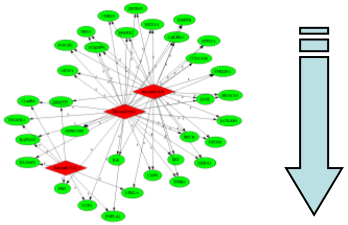
Activated Th2 cells

2x

Activated Th1 cells

2x

Activated naive T cells



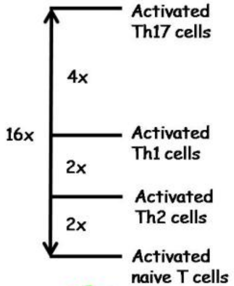
Hypothesis: May these microRNAs determine the polarity/plasticity of activated Th cells solely by endogenous levels?

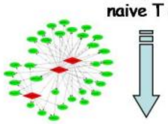
List of genes targeted by microRNAs 663, 638 and 923

C1orf90: Chromosome 1 open reading frame 90
EMILIN1: Elastin microfibril interfacer 1
DPAGT1: N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transf.)
HDAC10: Histone deacetylase 10
IGSF9B: Immunoglobulin superfamily, member 9B
TINAGL1: Tubulointerstitial nephritis antigen-like 1
ATP6V1B1: ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B1
CDH24: Cadherin-like 24
CALML5: Calmodulin-like 5
SNCB: Synuclein, beta
PPAP2C: Phosphatidic acid phosphatase type 2C
CAPS: Calcyphosine
PNPLA2: Patatin-like phospholipase domain containing 2
ZBTB455: Zinc finger and BTB domain containing 45
ATP2C2: ATPase, Ca^{2+} transporting, type 2C, member 2
SST: Somatostatin
ILK: Integrin-linked kinase-2
SCAMP4: Secretory carrier membrane protein 4
DLGAP2: Discs, large (Drosophila) homolog-associated protein 2
NKX1-1: NK1 homeobox 1
POU2F2: POU class 2 homeobox 2
CRTC1: CREB regulated transcription coactivator 1
TBX1: T-box 1
UCP2: Uncoupling protein 2 (mitochondrial, proton carrier)
LY6E: Lymphocyte antigen 6 complex, locus E
UPK1A: Uroplakin 1A
KCNAB3: Potassium voltage-gated channel, beta member 3
HAPLN3: Hyaluronan and proteoglycan link protein 3
KRBA1: KRAB-A domain containing 1
TSSK6: Testis-specific serine kinase 6
DEGS2: Degenerative spermatocyte homolog 2, lipid desaturase
PSD: Pleckstrin and Sec7 domain containing
CCDC106: Coiled-coil domain containing 106

Expression of Th cell "specific" TF s (mRNA) in cells transfected with various amounts of premir-638 or antagomir-638

Relative levels of miRNAs 663, 638 and 923 between T cell species





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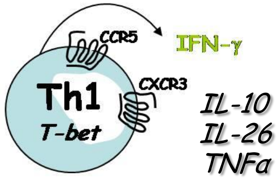
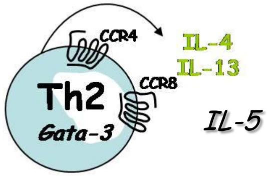
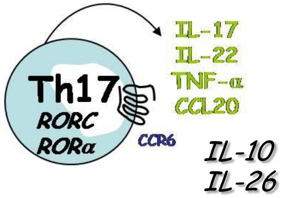
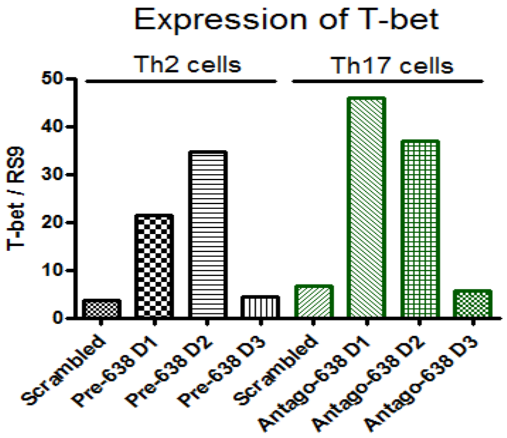
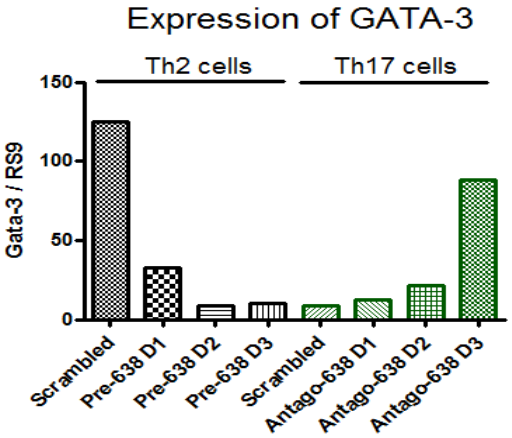
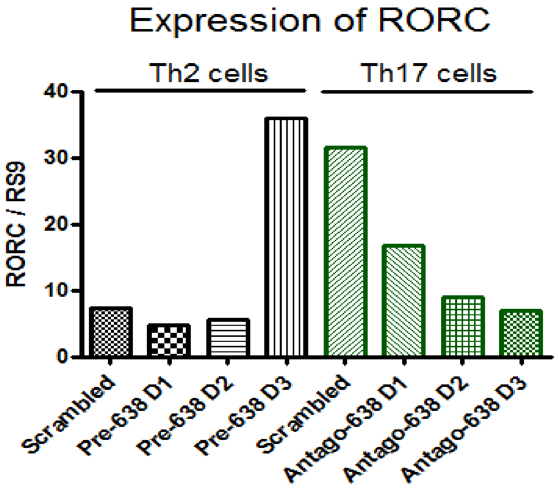






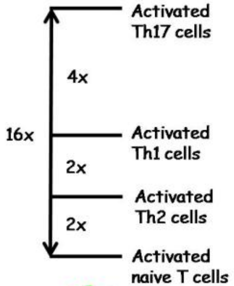




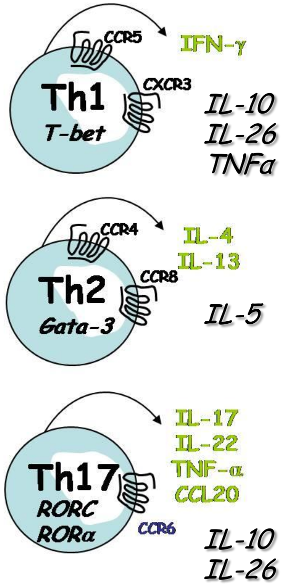


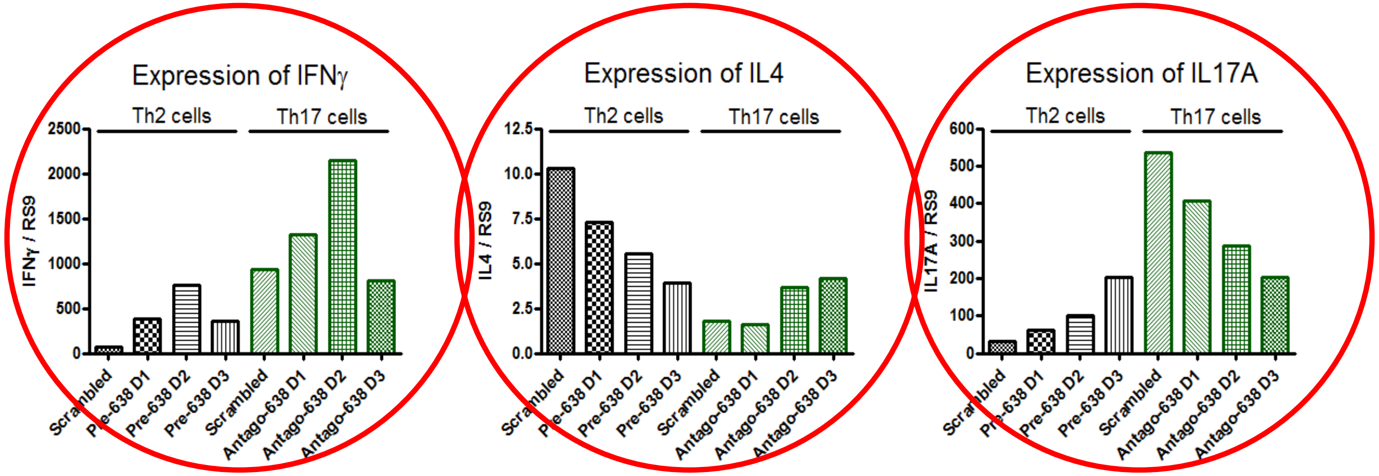
Expression of Th cell "specific" cytokines (mRNA) in cells transfected with various amounts of premir-638 or antagomir-638

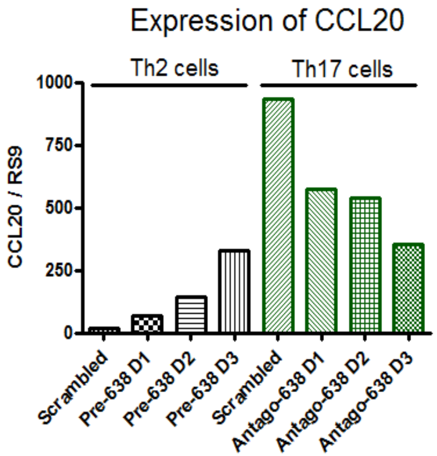
Relative levels of miRNAs 663, 638 and 923 between T cell species

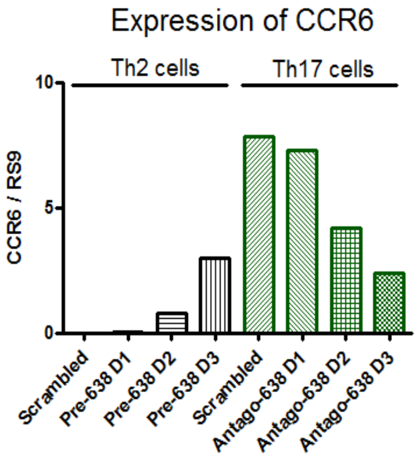


Hypothesis: May these microRNAs determine the polarity/plasticity of activated Th cells solely by endogenous levels?



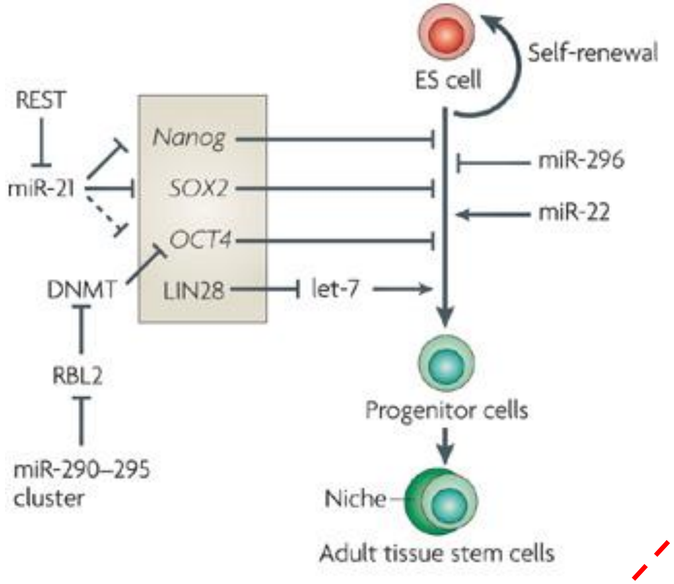






MicroRNAs are heavily involved in self-renewal and differentiation of stem cells

Published microRNAs involved in embryonic stem cell renewal and differentiation



hematopoietic stem cells
(HSCs)

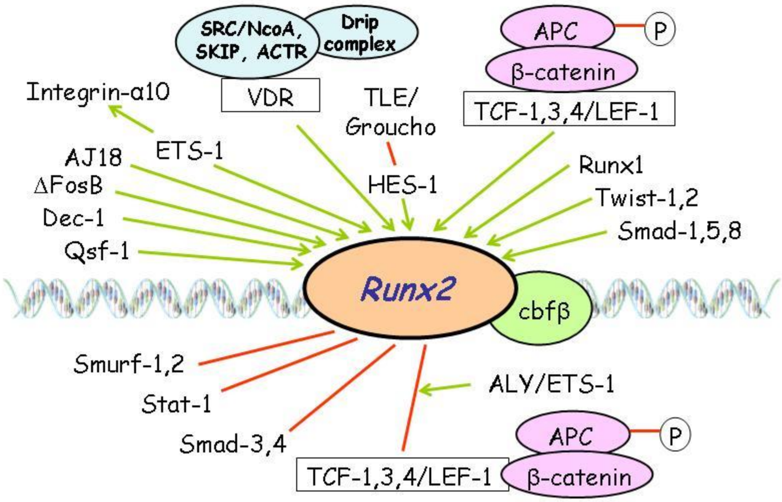
In silico search for microRNA species targeting transcripts of family members of evolutionally conserved and developmental prominent genes (Wnt-, TGFβ-, SHH- Notch- and Homeobox-related) shown to be important for the self-renewal and/or pluripotency of *hematopoietic stem cells* (HSCs)

Gene	***Micro-RNA (according to MiRNA Viewer and PicTar)***
Lef1	22, 24, 26ab, 34abc, 93, 145, 149, 193, 302abcd, 320, 370, 372, 373
BMP4	206, 337
NIK = MAP3K14	17-5p, 19ab, 20, 27ab, 93, 106ab, 130ab, 155, 204, 211, 214, 301, 302abcd, 326, 331, 345, 370, 372, 373
SMO	326, 346, 370
Notch1	15a, 15b, 32, 34abc, 125a, 125b, 139, 195, 223
Hoxa9	Let-7abcefgi, 19b, 26ab, 32, 96, 98, 99, 101, 126, 128ab, 139, 144, 145, 147, 182, 186, 196ab, 199, 205, 301

Many of the microRNAs listed immediately above, like *microRNAs 17-5p, 22, 24, 34ac, 125ab, 128b, 149, 193, 326 and 337* are putatively targeting transcription factors APC, ATF4, Dlx5, ETS-1, HES-1, LEF-1, NFATc1, Sp3, Sp7 (osterix), RNF11, Runx2/cbfa1, Satb2, TAZ, and VDR involved in *osteoblastogenesis*!

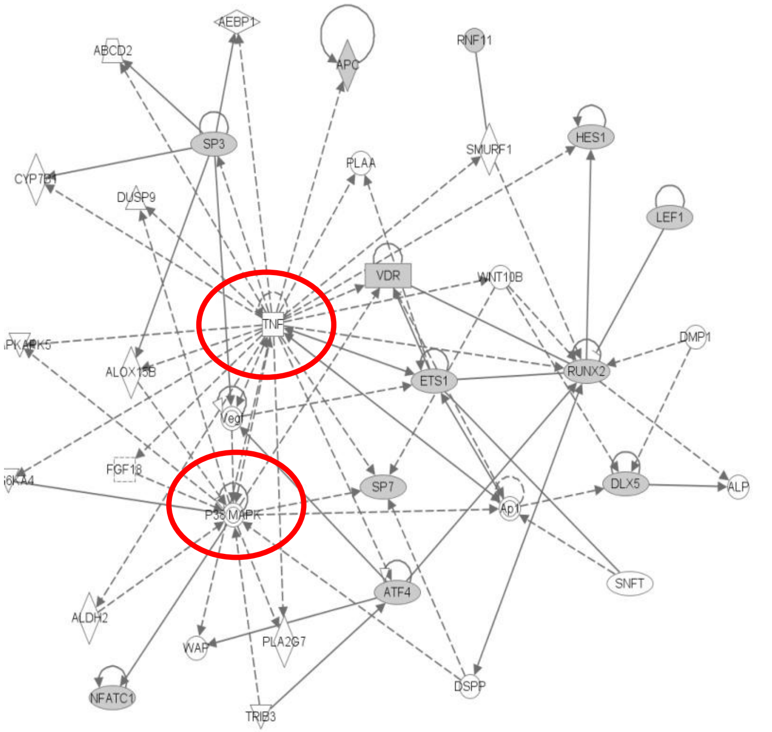
Strategy to ensure blockage of osteogenic differentiation in chondrocytes engineered from hMSCs for cartilage replacement

Focus on *transcription modulators* known to be important for the differentiation of osteoblasts



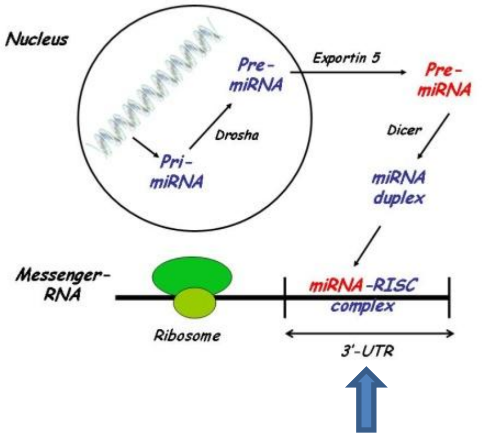
Selected target transcripts:
APC, ATF4, Dlx5, ETS-1, HES-1, LEF-1, NFATc1, Sp3, Sp7 (osterix), RNF11, Runx2/cbfa1, Satb2, TAZ, and VDR

Interrelations between the transcriptional modulators and other genes (the Ingenuity algorithm): *confined to osteoblasts* ($p < 5 \cdot 10^{-13}$)



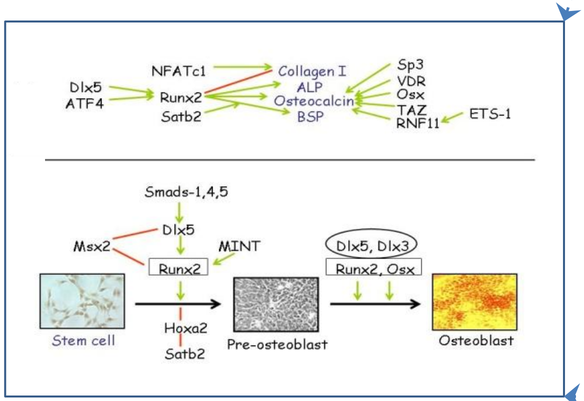
Key junctions: *TNFα* and *p38 MAPK*

Search for putative microRNA species targeting the selected transcriptional modulators



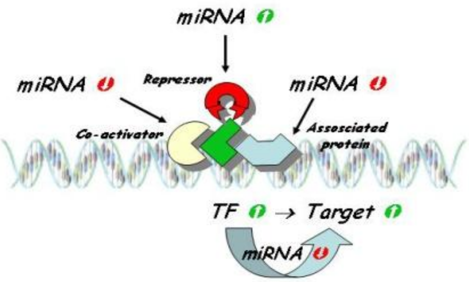
Concept: look for microRNAs *targeting two or more* transcriptional modulators specific for osteoblasts

MiRNA species (ranked by number of hits)	Predicted osteoblast (OB) gene targets	Tentative effect on OB development and function
296	APC, HES-1, NFATc1, Osterix, Runx2, Satb2	**Precommitment and differentiation**
34c	APC, ETS-1, Sp3, Satb2, Taz, VDR	**Precommitment and differentiation**
34a	APC, ETS-1, LEF-1, Satb2, VDR	**Precommitment and differentiation**
124a	Dlx5, ETS-1, RNF11, Sp3, VDR	**Precommitment and differentiation**
125a	ETS-1, HES-1, Osterix, Satb2, VDR	**Precommitment and differentiation**
125b	ETS-1, HES-1, Osterix, Satb2, VDR	**Precommitment and differentiation**
328	APC, ETS-1, Osterix, Runx2, VDR	**Differentiation**
449	RNF11, Satb2, Sp3, TAZ, VDR	**Precommitment and differentiation**
128b	APC, LEF-1, NFATc1, Satb2	**Precommitment and differentiation**
339	ETS-1, Osterix, RNF11, VDR	**Differentiation**
16	APC, ETS-1, Satb2	**Precommitment and differentiation**
22	APC, LEF-1, Satb2	**Precommitment and differentiation**
331	APC, Osterix, RNF11	**Differentiation**
337	ETS-1, Osterix, VDR	**Differentiation**
338	APC, ETS-1, Sp3	**Differentiation**
17-3p	ETS-1, Satb2, VDR	**Precommitment and differentiation**
24, 149	APC, LEF-1, RNF11	**Differentiation**
193	APC, ETS-1, LEF-1	**Differentiation**
328	APC, Runx2, Osterix	**Differentiation**

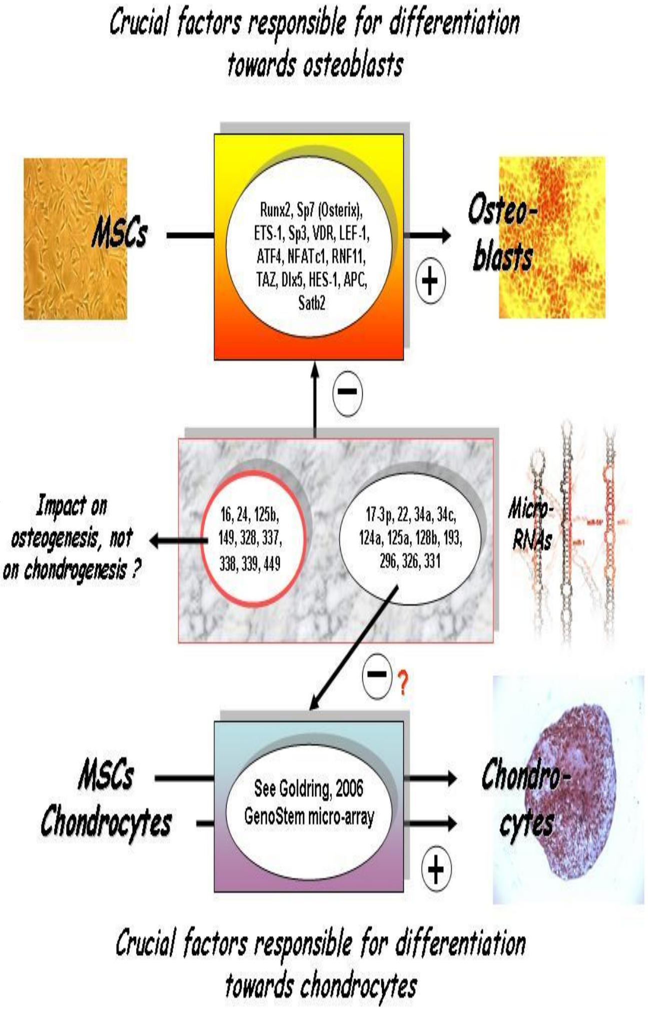


Search for possible detrimental effects of selected microRNA species on chondrogenesis

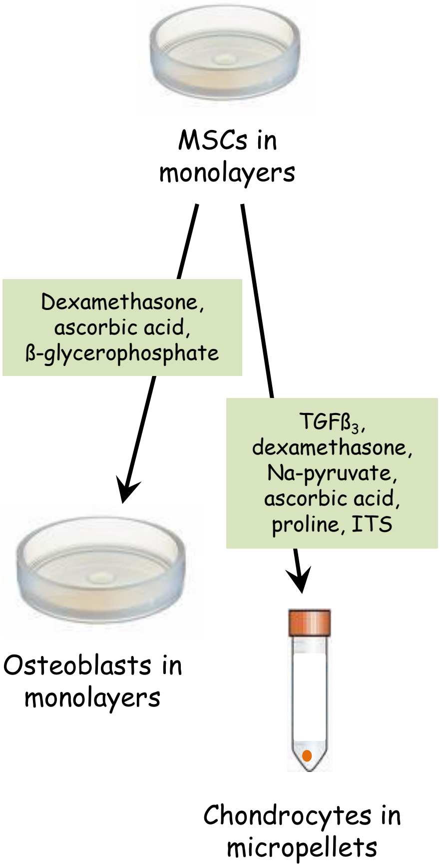
Watch out for microRNAs putatively affecting repressors of osteoblastic transcriptional modulators and microRNAs negatively affecting chondrogenesis



Names of genes (MSC condensation and differentiation; chondrogenesis and terminal differentiation) **Goldring et al;, 2006**	Aggrecan, Coll10a1, Coll11a1, Coll11a2, Coll2a1, Coll9a1, Fibronectin type 3, Hyaluronan, N-cadherin, Thrombospindin-2, ALK-2, ALK-3, ALK-6, BMPR1A, BMPR1B, CD-RAP, Chordin, FGF-10, FGF-8, FGRF1, FGRF2, FGRF3, Flk-1, GADD45β, Gli1, Gli2, Gli3, Hoxd11, Hoxd13, L-Sox5, Noggin, Npn1, Npn2, Ptc1, Smad1, Smad4, Smad5, Smad8, Smo, Sox6, Sox9, Tak1, Wnt3a, Wnt7a		
GenoStem transcriptome analysis (chondrocytes from cartilage incubated with TGFβ$_3$ *in vitro*)	**Genes related to transcription**	**Genes related to signalling systems**	**Genes related to matrix/anchoring proteins**
Early up-regulated genes (more than 2-fold on day 1)	Foxo3A, BHLHB2, MXI1, Sox9, Notch3, CEBPD	Wnt5A, STK24, TGFβ1, VEGF, ARL7, THY1, P311, FGF2, IL6, PTP4A1, PARG1, FZD2, NMB, STC1, PENK	CD44, Coll7a1, SPP1, NID, DPT, FN(1)
Early down-regulated genes (more than 2-fold on day 1)	Foxo1A, ID3, SMURF2, RYBP, HMGIY	DKK1, SPRY4, GADD45β, TNFRSF1, YWAH, PDE8A, PTPRC, MKP-L, NDRG1	PRELP, COMP, Coll1a1, ITGA10, MGP



MicroRNA microarray differential display analysis of osteoblasts and chondrocytes differentiated from hMSCs for 3 days



MSCs in monolayers

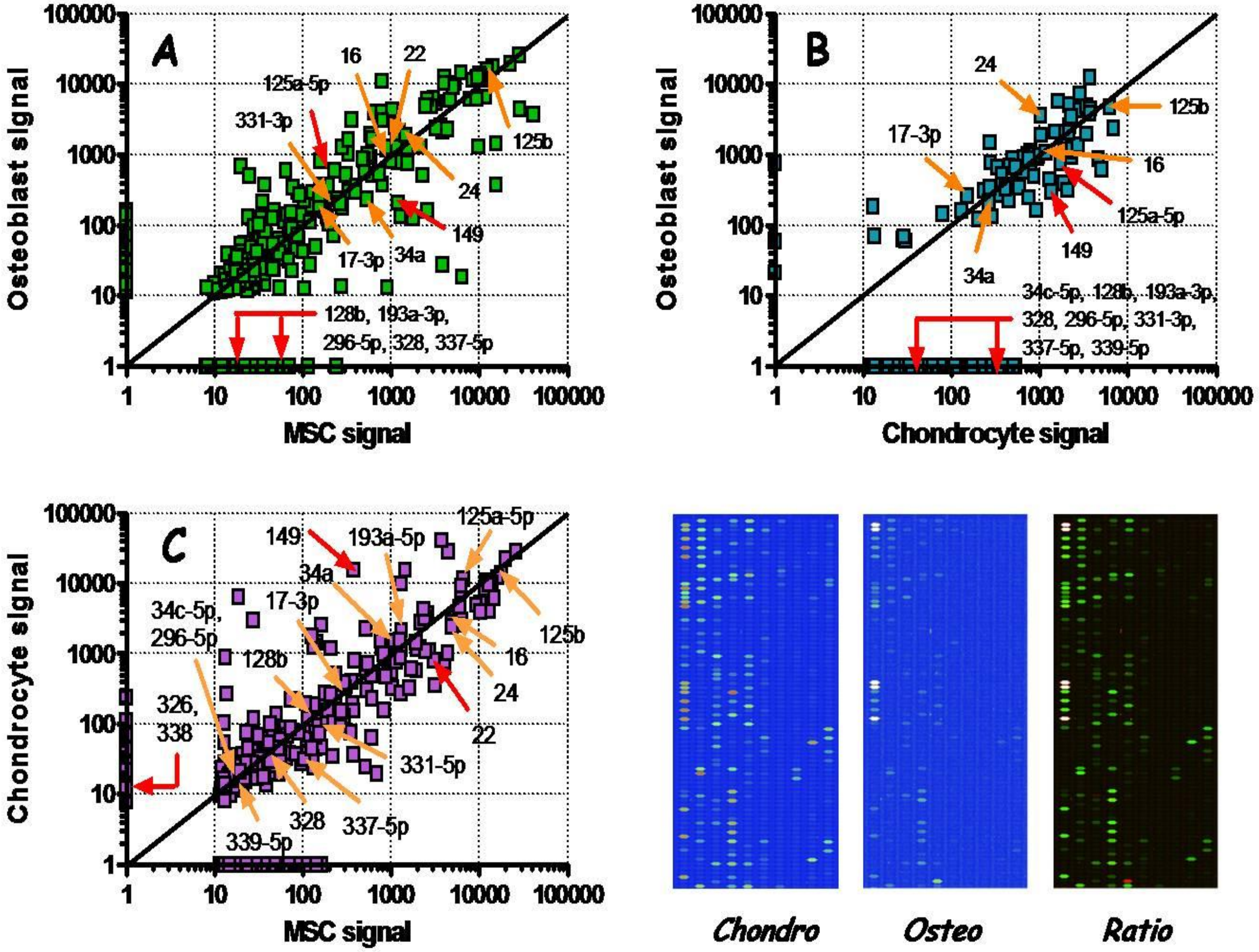
Dexamethasone,
ascorbic acid,
ß-glycerophosphate

TGFß$_3$,
dexamethasone,
Na-pyruvate,
ascorbic acid,
proline, ITS

Osteoblasts in monolayers

Chondrocytes in micropellets

Isolation of total RNA using the *mir*Vana® kit



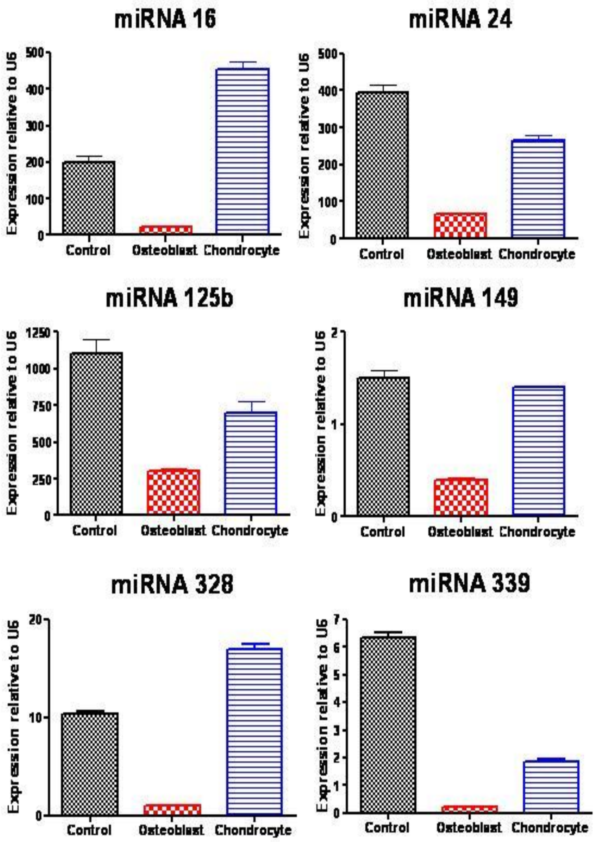
Comparison between the *in silico* search for putative microRNA species and the microRNA microarray analyses

Human miRNAs	Log2 [chondro/osteo] ($p < 0.01$)	Predicted microRNAs	Number of putative targets
34c-5p	Absent in osteo	34c	6
128b	Absent in osteo	128b	4
193a-3p	Absent in osteo	193a	3
328	Absent in osteo	328	3
296-5p	Absent in osteo	296	6
331-3p	Absent in osteo	331	3
337-5p	Absent in osteo	337	3
339-5p	Absent in osteo	339	4
671-5p	5.69		
24-2	4.04	24	3
212	3.68		
26b	3.50		
663	2.98		
29b	2.81		
29c	2.72		
149	2.42	149	3
148a	2.41	148b	1
638	2.38		
15a	2.31	15a	1
923	2.31		
411	2.23		
376c	2.19		
574-3p	2.17		

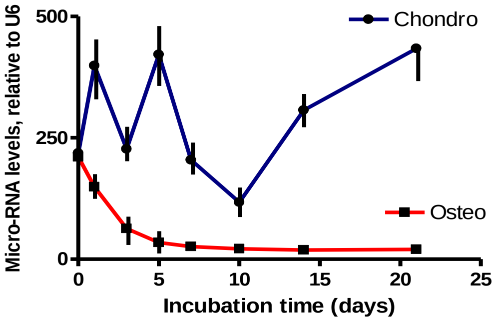
Conclusion: 16 predicted out of 36 analysed microRNA species in common, including miRNAs 149, 328, 337, and 339, putatively not perturbing chondrogenesis

Human miRNAs	Log2 [chondro/osteo] ($p < 0.01$)	Predicted microRNAs	Number of putative targets
99a	2.17		
575	1.62		
1231	1.61		
21	1.60		
Let-7g	1.49	Let-7c	1
494	1.37		
214	1.26	214	1
27b	1.19		
125a-5p	1.10	125a	5
27a	1.03		
199a-3p	0.94	199a	1
100	0.94		
29a	0.91		
		34a	5
		124a	5
		125b	5
		326	5
		449	5
		16	3
		17-3p	3
		22	3
		338	3
		18, 30e-3p, 31, 34b, 103, 107, 128a, 133a, 133b, 205, 330, 365, 368, 370, 422a, 424	1-2

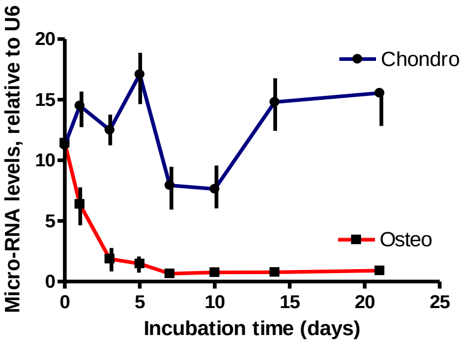
Profile of the microRNA species 16, 24, 125b, 149, 328, and 339 during osteogenic and chondrogenic differentiation from hMSCs for 5 days (left) and up to 21 days (right)



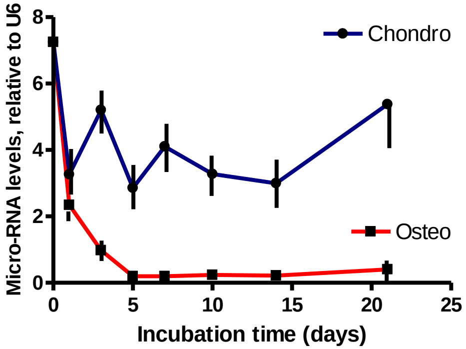
Time-course of *mir-16* expression in hMSCs (P17, PMP7 and P23) differentiated into Chondrocytes or Osteoblasts



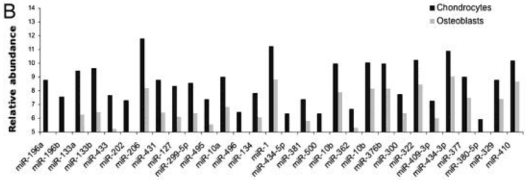
Time-course of *mir-328* expression in hMSCs (P17, MP7 and P23) differentiated into Chondrocytes or Osteoblasts



Time-course of *mir-339* expression in hMSCs (P17, MP7 and P23) differentiated into Chondrocytes or Osteoblasts

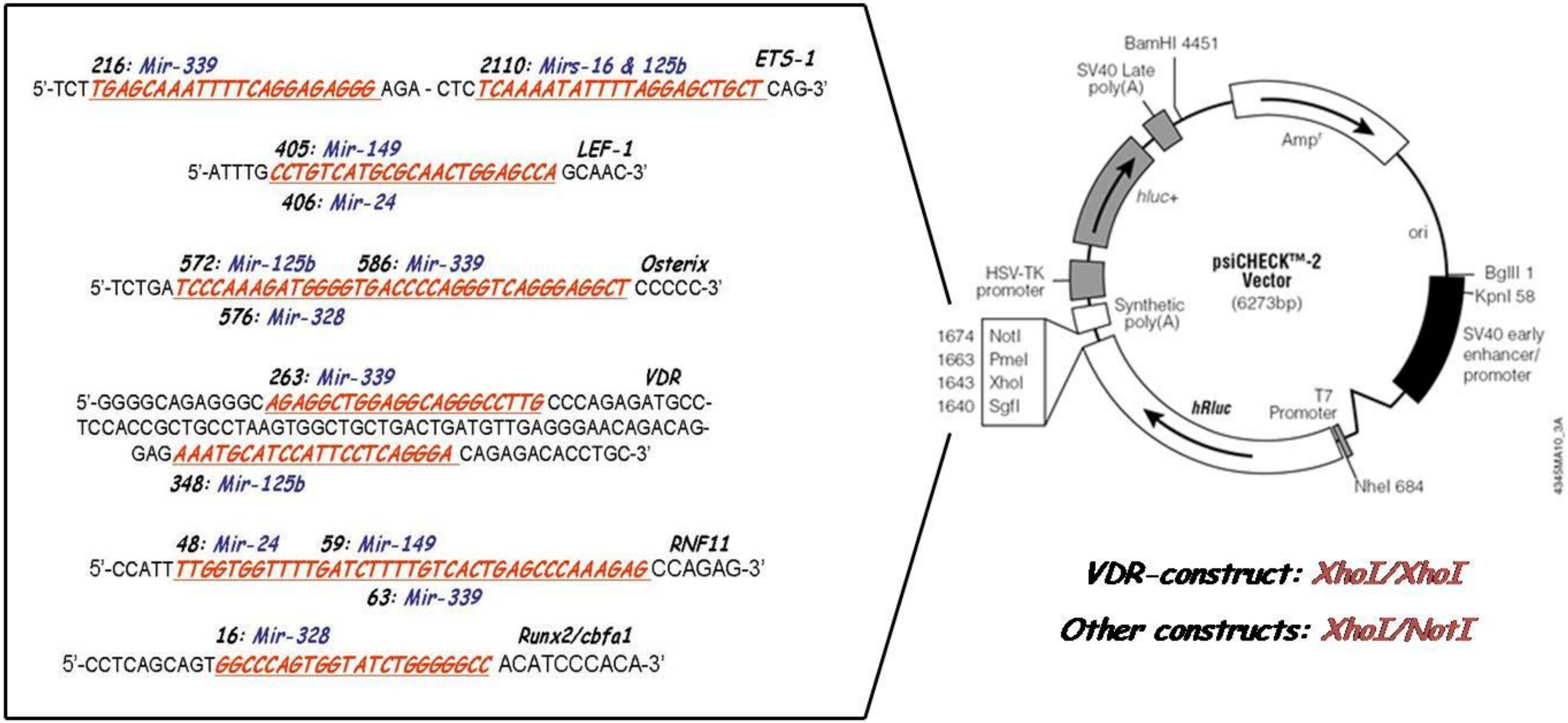


The subject microRNAs are maintained in differentiating chondrocytes, but strongly down-rgulated in differentiating osteoblasts – *"all or none" effect*



Dicer-dependent pathways regulate chondrocyte proliferation and differentiation
Tatsuya Kobayashi et al., 2009, PNAS

psiCECK2 reporter constructs containing parts (from 473 to 2010 bases) of XhoI/XhoI or XhoI/NotI digests of PCR amplified 3'-UTR sequences



216: Mir-339 *2110: Mirs-16 & 125b* ***ETS-1***
5'-TCT*TGAGCAAATTTTCAGGAGAGGG* AGA - CTC*TCAAAATATTTTAGGAGCTGCT* CAG-3'

405: Mir-149 ***LEF-1***
5'-ATTTG*CCTGTCATGCGCAACTGGAGCCA* GCAAC-3'
406: Mir-24

572: Mir-125b *586: Mir-339* ***Osterix***
5'-TCTGA*TCCCAAAGATGGGGTGACCCCAGGGTCAGGGAGGCT* CCCCC-3'
576: Mir-328

263: Mir-339 ***VDR***
5'-GGGGCAGAGGGC*AGAGGCTGGAGGCAGGGCCTTG* CCCAGAGATGCC-
TCCACCGCTGCCTAAGTGGCTGCTGACTGATGTTGAGGGAACAGACAG-
GAG*AAATGCATCCATTCCTCAGGGA* CAGAGACACCTGC-3'
348: Mir-125b

48: Mir-24 *59: Mir-149* ***RNF11***
5'-CCATT*TTGGTGGTTTTGATCTTTTGTCACTGAGCCCAAAGAG* CCAGAG-3'
63: Mir-339

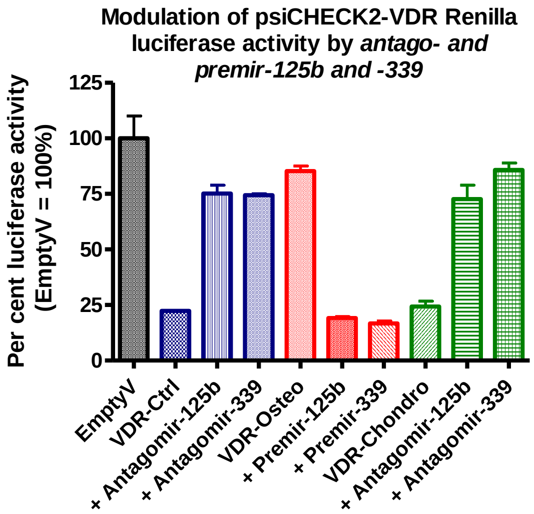
16: Mir-328 ***Runx2/cbfa1***
5'-CCTCAGCAGT*GGCCCAGTGGTATCTGGGGGCC* ACATCCCACA-3'

VDR-construct: XhoI/XhoI

Other constructs: XhoI/NotI

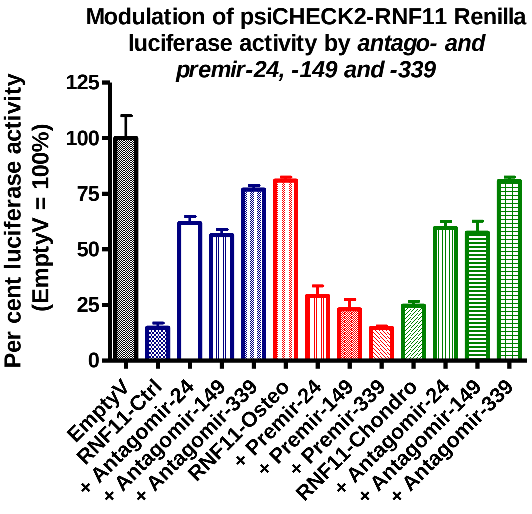
Each construct contains at least one putative target sequence for the osteoblast/chondrocyte signature microRNA species
16, 24, 125b, 149, 328, and 339

Effect of pre-miRNAs and antago-miRNAs on the luciferase activity of the psiCHECK2 constructs in osteoblasts and chondrocytes differentiated from hMSCs for 3 days (cont.)



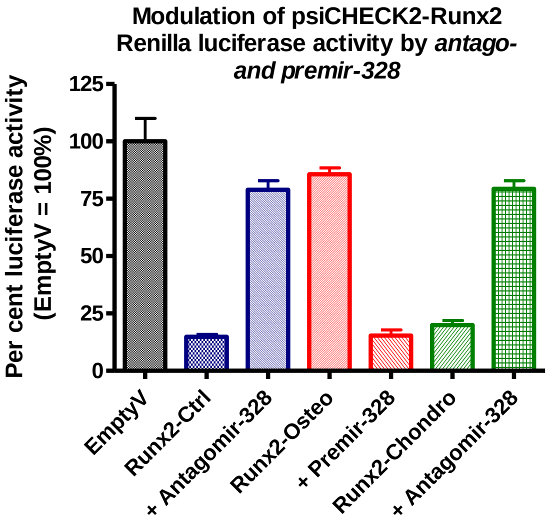
Modulation of psiCHECK2-VDR Renilla luciferase activity by *antago- and premir-125b and -339*

MicroRNAs 125b and 339 seem to be equally potent as to their impact on the VDR transcript



Modulation of psiCHECK2-RNF11 Renilla luciferase activity by *antago- and premir-24, -149 and -339*

MicroRNA 339 seems to be more potent as to its impact on the RNF-11 transcript than miRNAs 24 and 149



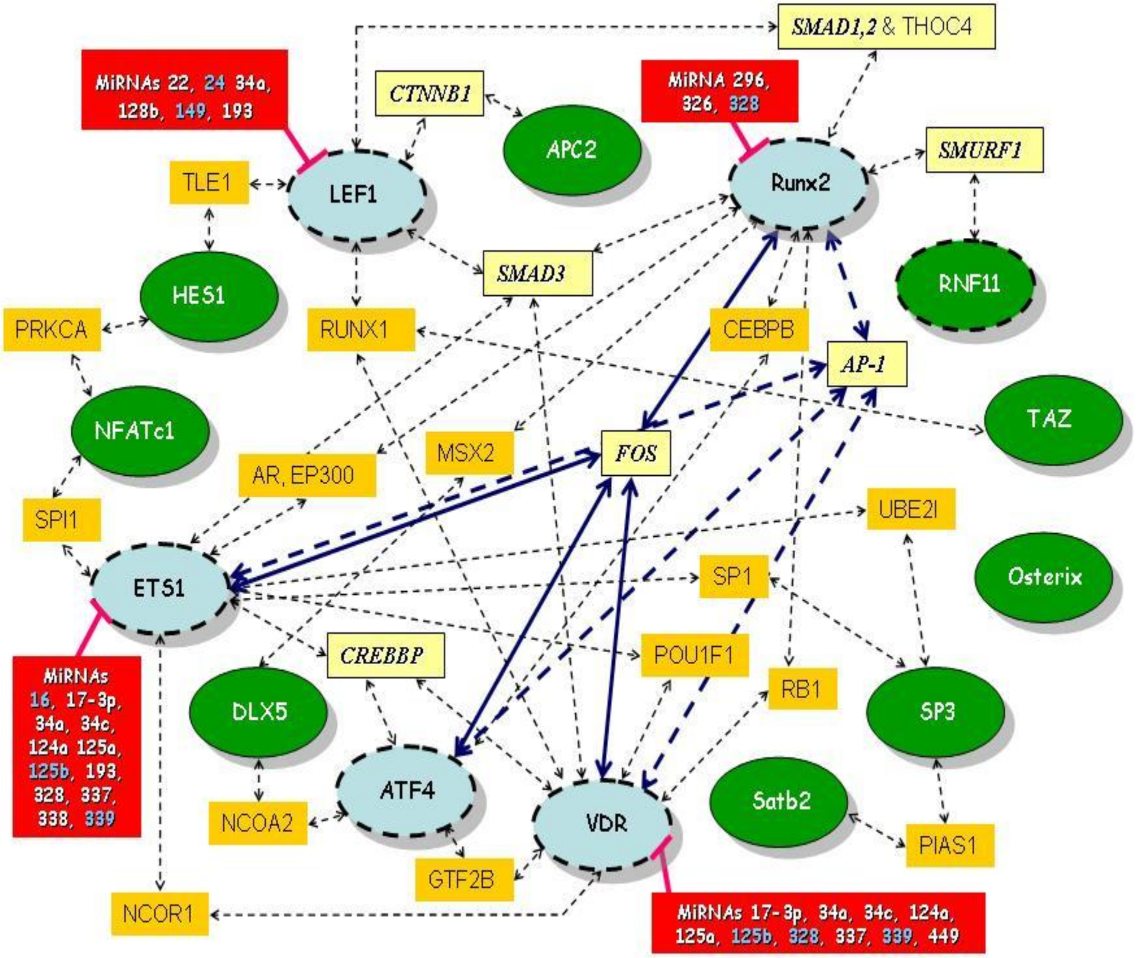
Modulation of psiCHECK2-Runx2 Renilla luciferase activity by *antago- and premir-328*

MicroRNA 328 seems to be as potent as its impact on the Runx2 transcript as 339 on the RNF-11 transcript

The transcriptional modulators specific for osteoblasts closely interact with many signalling system molecules

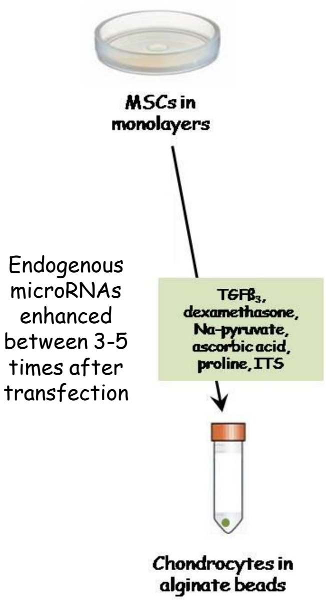
Proteins interacting with at least two of the 14 transcription modulators (according to the«Pina» algorithm) important for osteoblastogenesis:

PRKCA: Protein kinase C alpha type (PKCα)
SPI1: hematopoietic transcription factor PU.1
TLE1: Transducin-like enhancer protein 1 (ESG1)
NCOR1: Nuclear receptor corepressor 1 (N-CoR1)
RUNX1: Runt-related transcription factor 1
AR: Androgen receptor (DHT receptor)
EP300: Histone acetyltransferase p300 (p300 HAT)
NCOA2: Nuclear receptor coactivator 2 (NCoA-2)
CTNNB1: Catenin β1
SMAD3: TGFβ-signaling protein 3
MSX2: Homeobox protein MSX-2 (Hox-8)
CREBBP: CREB-binding protein
GTF2B: Transcription initiation factor IIB
FOS: Proto-oncogene protein c-fos
CEBPB: CCAAT/enhancer-binding protein beta (C/EBPβ)
SP1: Transcription factor Sp1
POU1F1: Pituitary-specific positive TF factor 1 (Pit-1)
SMAD1: TGFβ-signaling protein 1
SMAD2: TGFβ-signaling protein 2
THOC4: THO complex subunit 4 (incl. AML1& LEF1)
SMURF1: SMAD ubiquitination regulatory factor 1
AP-1: Adaptor protein complex AP-1
UBE2I: Ubiquitin-conjugating enzyme E2 I
RB1: Retinoblastoma-associated protein (pRb)
PIAS1: Protein inhibitor of activated STAT protein 1



The chondrocyte differentiating potential of the microRNAs shown to block osteoblastogenesis and facilitate chondrogenesis

Complete Ambion®
transfection kit and
protocol.
Transfection was
performed every 4
days until day 21



MSCs in monolayers

Endogenous microRNAs enhanced between 3-5 times after transfection

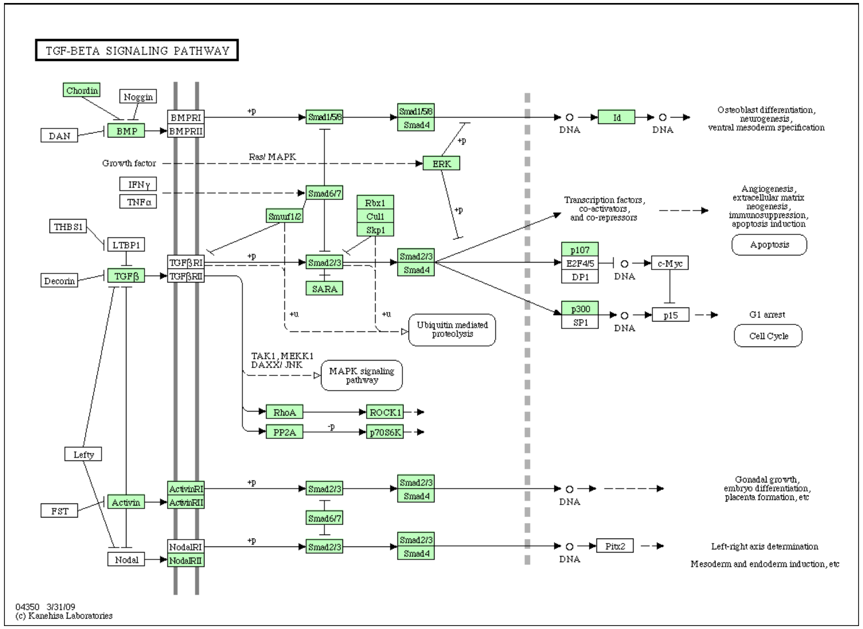
TGFß$_3$, dexamethasone, Na-pyruvate, ascorbic acid, proline, ITS

Chondrocytes in alginate beads

End point measures:
RT-PCR of marker genes (all values expressed relative to controls = TGFß$_3$ = 100%)

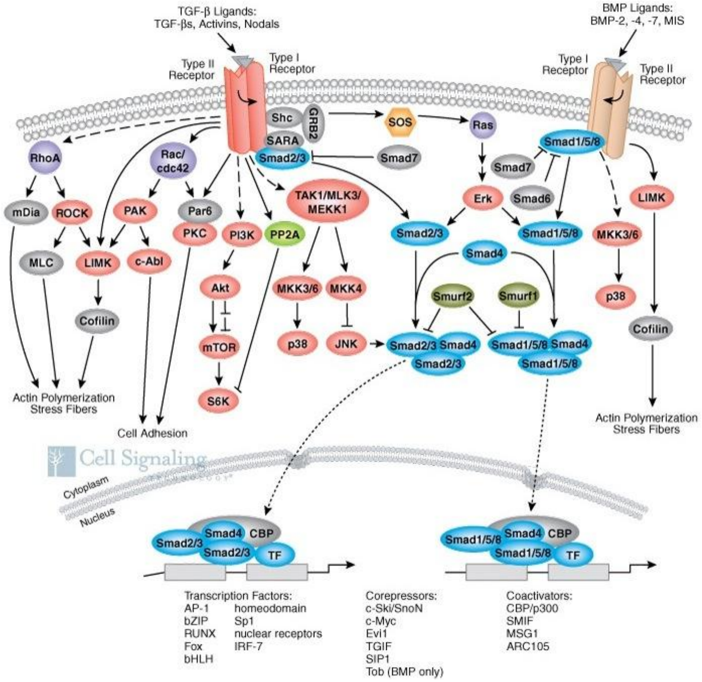
Markers	RT-PCR (%) of gene transcripts, GAG/DNA-ratio, and Clinical score (histology, distance between cells, immunihistochemistry)					
Sox9	100	11	23	55	23	63
Wnt5	100	7.6	18	58	16	74
GAG/DNA	100	8.3	21	65	28	62
Clin. Score	100	6.8	26	66	21	66
Aggrecan	100	13	19	55	24	68
Collagen 2a	100	5.1	18	49	16	73
Collagen 10a	100	3.6	24	47	21	61
Cell manipulation by						
TGFß$_3$	+					
Premirs 16&125b			+			
Premirs 24&149				+		
Premirs 328&339					+	
All premirs						+

Conclusion: The microRNA species are not able to substitute completely for TGFß$_3$ (with the exception of miRNAs 24&149) in achieving typical chondrocyte differentiation from MSCs

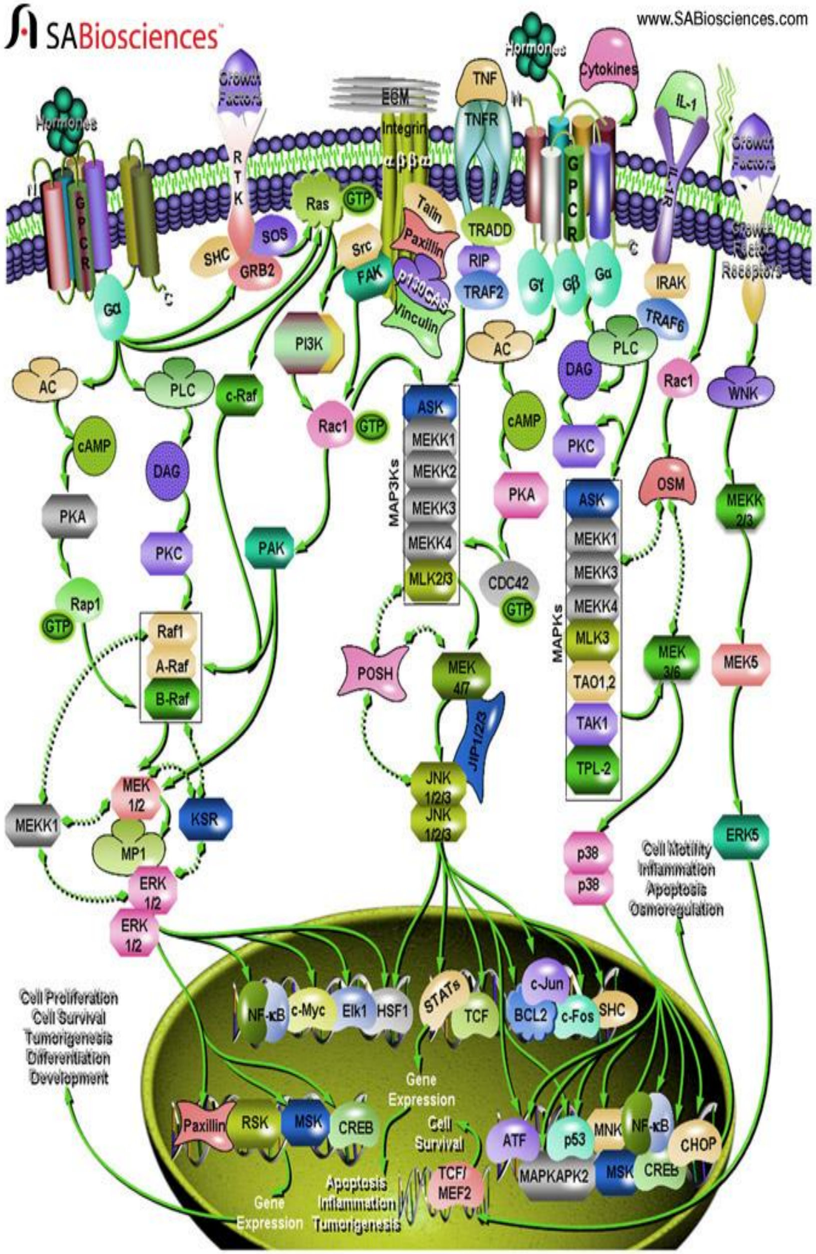


MicroRNAs of the osteo-chondro signature may heavily interfere with antagonists of the chondrocyte differentiation from MSCs

Gene name	***Transcript targeted by***
Receptor antagonists	
Chordin (CHRD): BMP	24, 125b, 149, 328
Noggin (NOG): BMP	16, 149
THBS1: TGFβ	16, 328
Decorin (DCN): TGFβ	24, 339
TF antagonists	
Smad6: BMP/TGFβ	16, 149
Smad7: BMP/TGFβ	16
Smurf1: TGFβ	16, 125b
Smurf2: TGFβ	16
MAPK14 (p38-MAPK)	24, 125b, 149, 328, 339
Rbx1: vs Smad 2/3 only	16, 149
Cul1: vs Smad 2/3 only	125b
Skp1: vs Smad 2/3 only	125b
Co-repressors of TFBEs	
c-ski/snoN (SKI)	16, 339
c-myc (MYC)	
EvI1	24, 328
TGIF	24, 149
SIP1	16, 125b
Tob: BMP only	16, 149



In silico searches using the Sanger, Viewer, PicTar, Segal and Sloan-Kettering databases



The microRNAs of the osteo-chondro signature are putatively heavily involved in the regulation of the TNFa pathway (i.e. "taking out" its inhibitory impact)

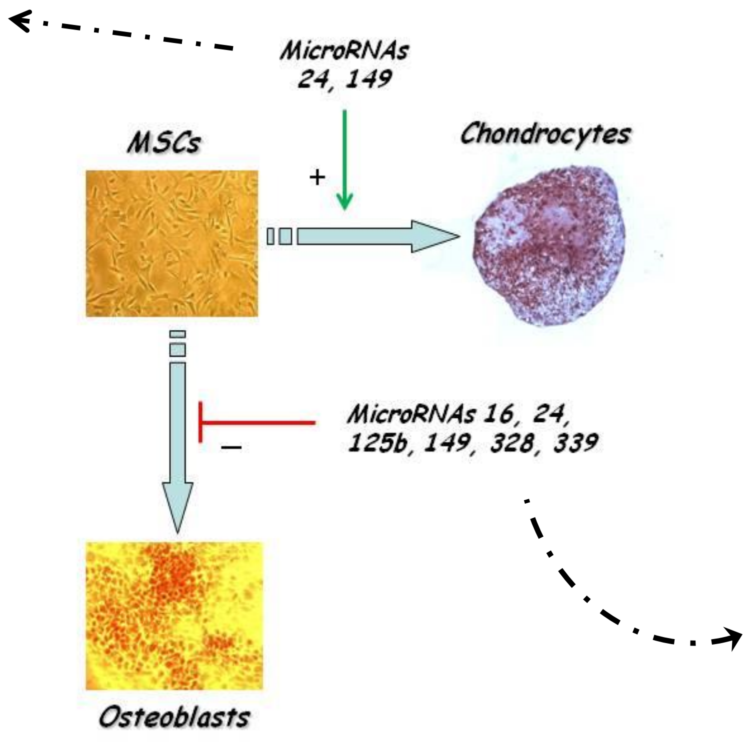
Gene names	*Transcripts targeted by*
Post receptor level	
TRADD	149
RIP = RALBP1	125b
TRAF2	328
ASK = DBF4	
MEKK1 = MAP3K1	16, 24, 125b, 328
MEKK2 = MAP3K2	24
MEKK3 = MAP3K3	16, 24, 125b
MEKK4 = MAP3K4	16, 24
MLK2 = MAP3K10	125b, 328, 339
MLK3 = MAP3K11	125b, 149, 328
MEK4 = MAP2K4	16, 339
MEK7	
JNK1 = MAPK8	24
JNK2 = MAPK9	16, 125b
JNK3 = MAPK10	125b

In silico searches using the Sanger, Viewer, PicTar, Segal and Sloan-Kettering databases

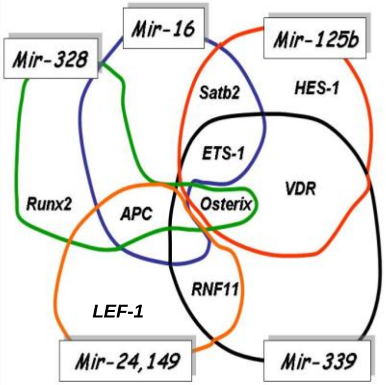
Model for how the microRNA signature affects differentiation of osteoblasts and chondrocytes from hMSCs

MiRNA 149, may serve as switch (since it targets ATF3, which activates Runx2 and inhibits Sox9) between the osteoblast and the chondrocyte phenotypes depending on its endogenous levels and cooperation with other, unidentified, microRNAs

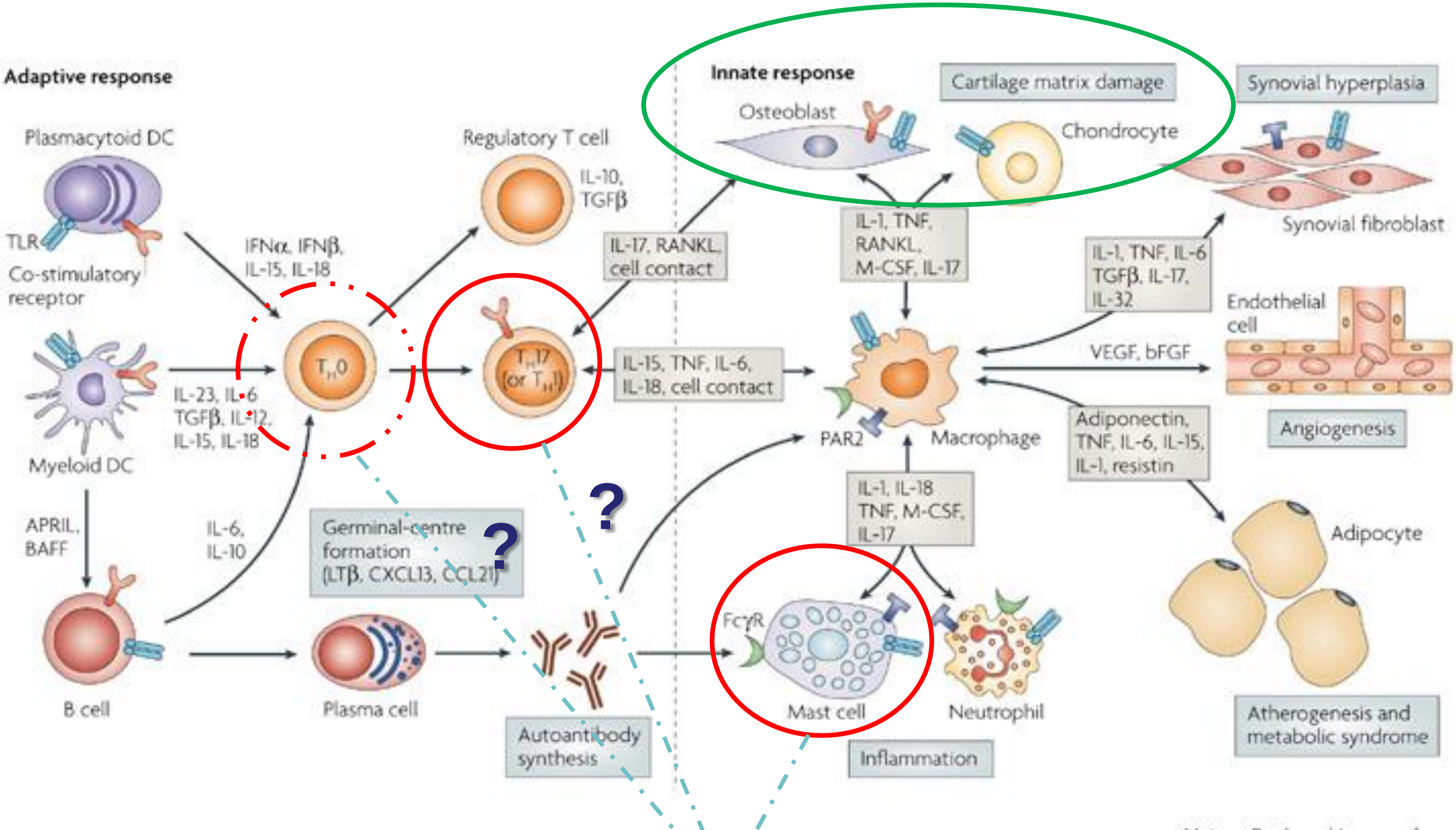
MiRNAs 24 and 149 are putatively interfering with gene transcripts like: PIAS1 (repressing Sox9 through SUMOylation), Stat6 (Sox9 inhibitor), SP1 (inhibitor of CEBPA interacting with Sox9), and PPPIRI6B (TGFß-inhibiting membrane associed protein = protein phosphatase 1 inhibitory subunit 6B) etc.



6 microRNA species specifically block osteoblastogenesis, thereby promoting chondrogenesis, by targeting at least 9 transcriptional modulators:



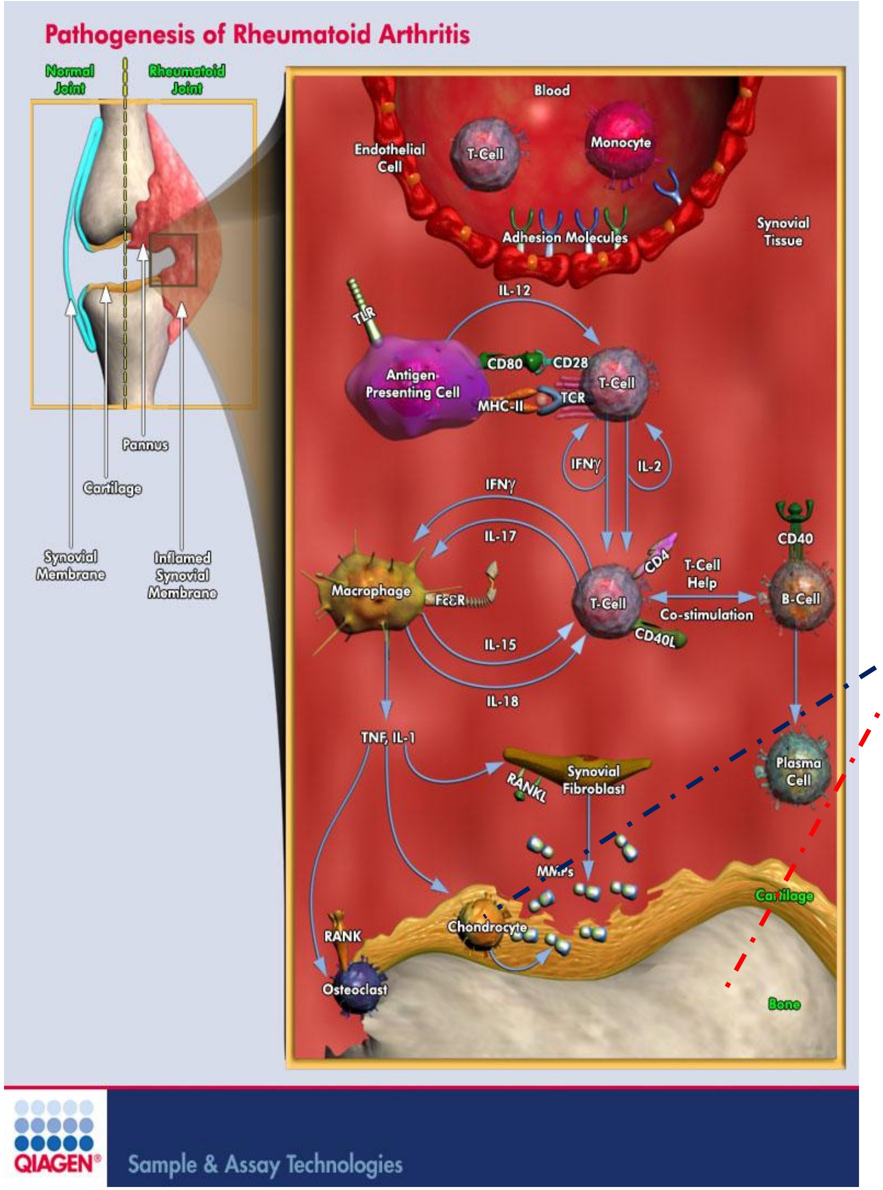
Cells involved in inflammation (e.g. rheumatoid arthritis)



Nature Reviews | Immunology

Shedding exosomes containing a plethora of microRNAs

Possible interactions between microRNA-presenting compartments in rheumatic disease



MicroRNAs increased in whole blood from RA-patients:

144, 142-3p, 32, 19a, 340, 7, 101, 142-5p, 19b, 96, 29bc, 424, 125b,

Some microRNAs found in exosomes from mast cells:

451, 10a, 450, 150, 296, 341, 15ab, 24, 20a, ***222***, 324-3p, 23ab, ***21***, ***184***, 500, 29a, 329, ***26a***, 30c, 326, 433,18, 16, 207, 129-5p, 146b, 17-5p, 142-3p, 142-5p, 183, 191, 96, 106b, 291ab, 107, 290, 351, 182, 27b, 468, 300, 470, ***let-7b***, 370, 298, 185, 503

MicroRNAs produced in large amounts in activated Th 17 cells:

21, 22, 638, 663, 34a, 923

↓

Potential detrimental microRNAs affecting chondrocytes: 26a, 222, 184 and osteoblasts: 21, 22,663, 638, 923, 34a

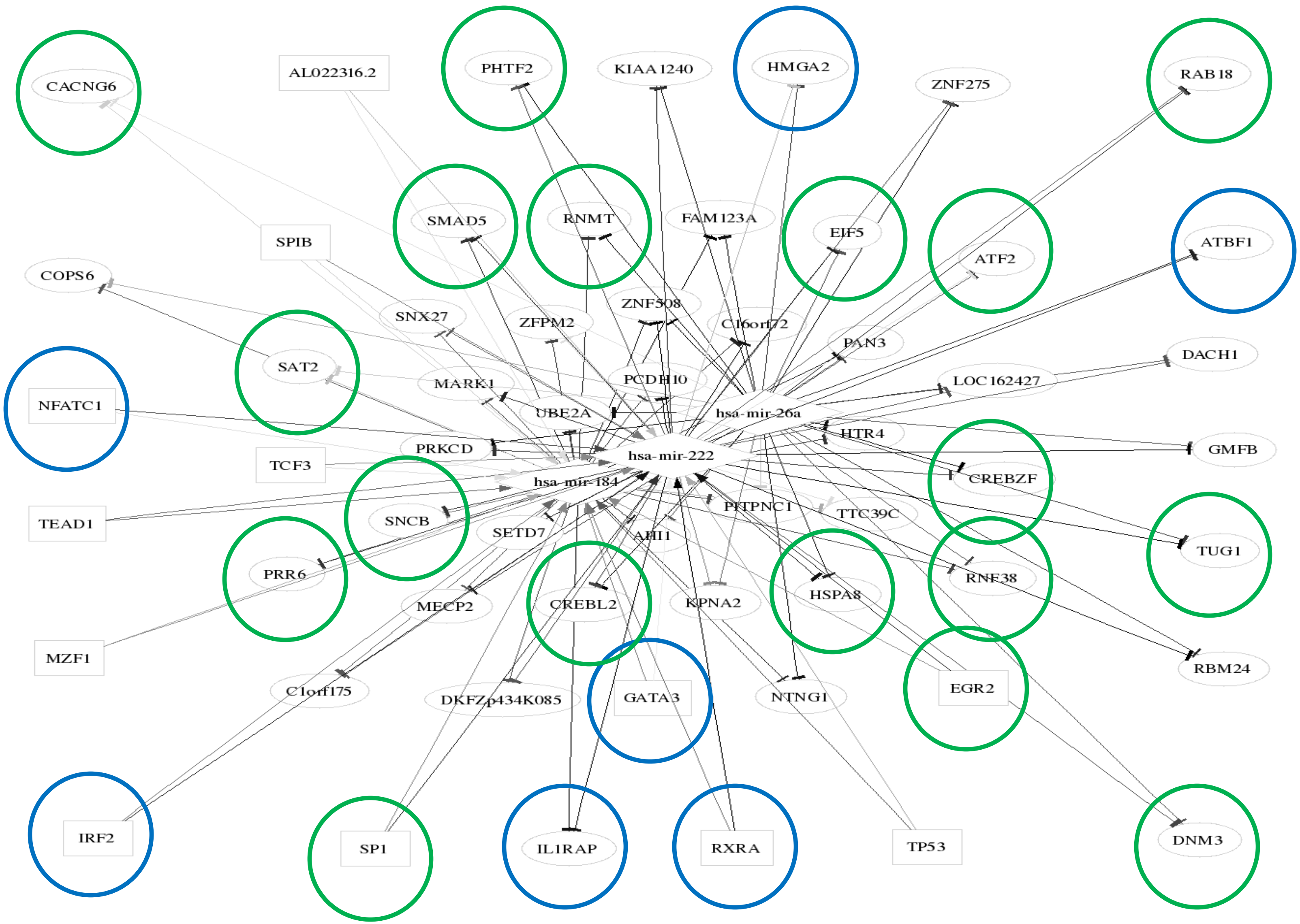
↑

MicroRNAs produced in small amounts in differentiated chondrocytes: ***26a***, ***222***, 145, 143, ***184***

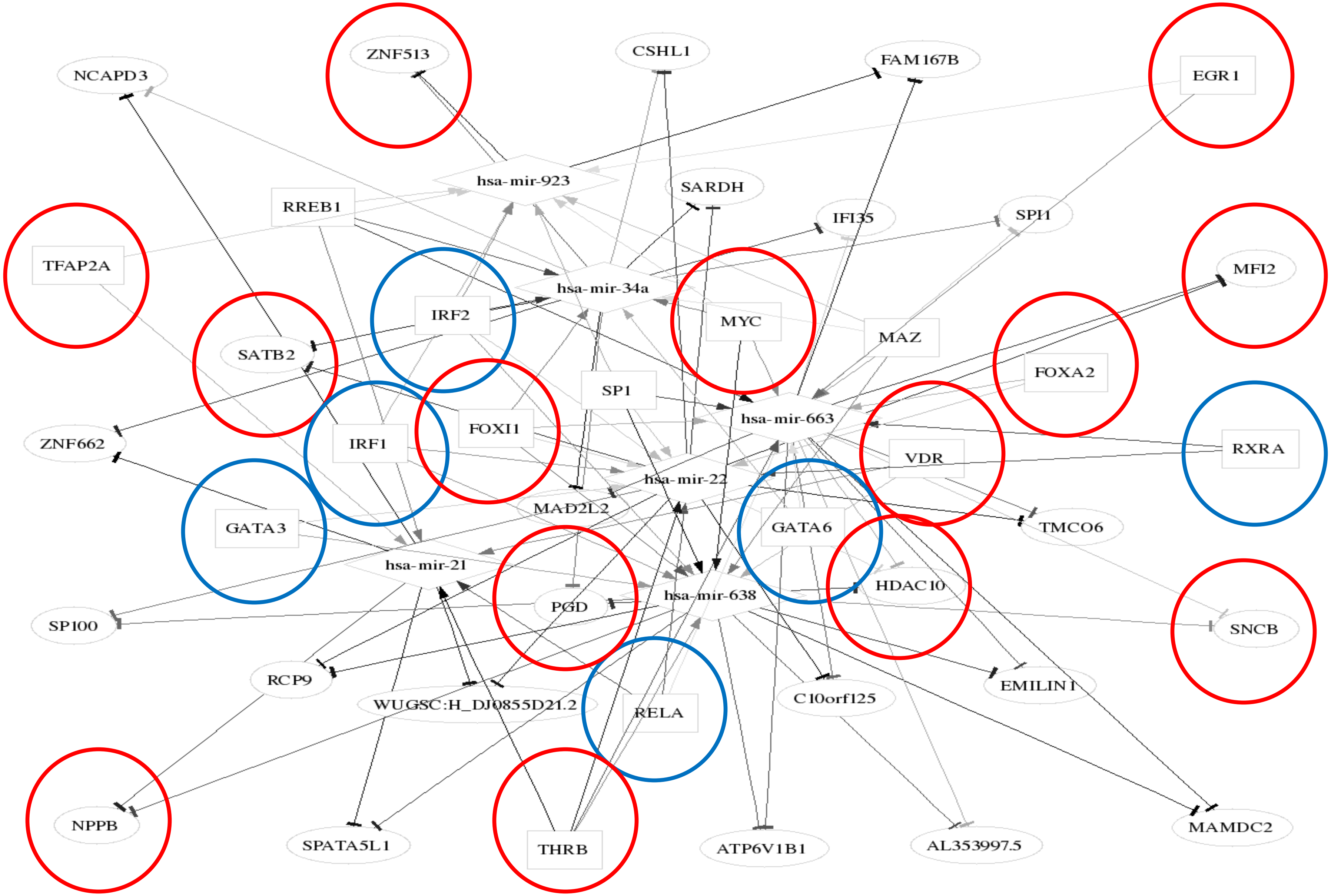
MicroRNAs produced in small amounts in differentiated osteoblasts:

34c-5p, 128b, ***34a***, 193a-3p, 328, 296-5p, 331-3p, 337-5p, 339-59, 671-59, 24, 26b, ***663***, 29bc, 149, 148a, ***638***, 15a, ***923***, 411, 376c, 574-3p, 125ab, 99a, 575, ***21***, 494, 214, 27ab, 199a-3p, ***22***, 100, 29a

Mir@nt@n algorithm: Interaction between microRNAs 26a, 222, and 184, transcription factors and target genes



Putative interrelation between microRNAs 21, 22, 34a, 638, 663, 923 (Mir@nt@n algorithm), transcription factors and target genes



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CENTRE HOSPITALIER UNIVERSITAIRE

To be commended for their scientific and technical support and never-ceasing enthusiasm:
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&
Université Montpellier 1, enabling me to work within the U844 as a guest professor for 3 years

Thank you for your attention!