AST AMERICAN SOCIETY OF TRANSPLANTATION

Epigenetics- *Does miRNA Ask the Right Question?*

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CEOT CUTTING EDGE of **TRANSPLANTATION**

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NO SIZE FITS ALL: *Uncovering the Potential of Personalized Transplantation*

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Disclosure

I have no financial relationships with commercial interests to disclose

AND

My presentation does/does not include discussion of off-label or investigational use.

Learning Objectives

- Definition of Epigenetics
- Epigenetics regulation of T cell functions - Mechanisms
- DNA methylation
- Histone modification
- Non coding genes and their role in immune modulation
- MicroRNA mediated regulation of immune responses
- Exosomes: regulation of immune function via microRNA

Epigenetics

- Coined by Conrad H. Waddington in 1942
- ***Simple Definition:***
- Epigenetics (Epi - above, over, in addition to) is the study of chromatin modification that affect gene expression without altering the DNA or RNA sequence.
- ***Recent definitions:***
- "mechanisms of temporal and spatial control of gene activity during the development of complex organisms"
- "Mitotically heritable changes in gene function that cannot be explained by changes in DNA sequences"
- "The structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states"

Chromatin Organization

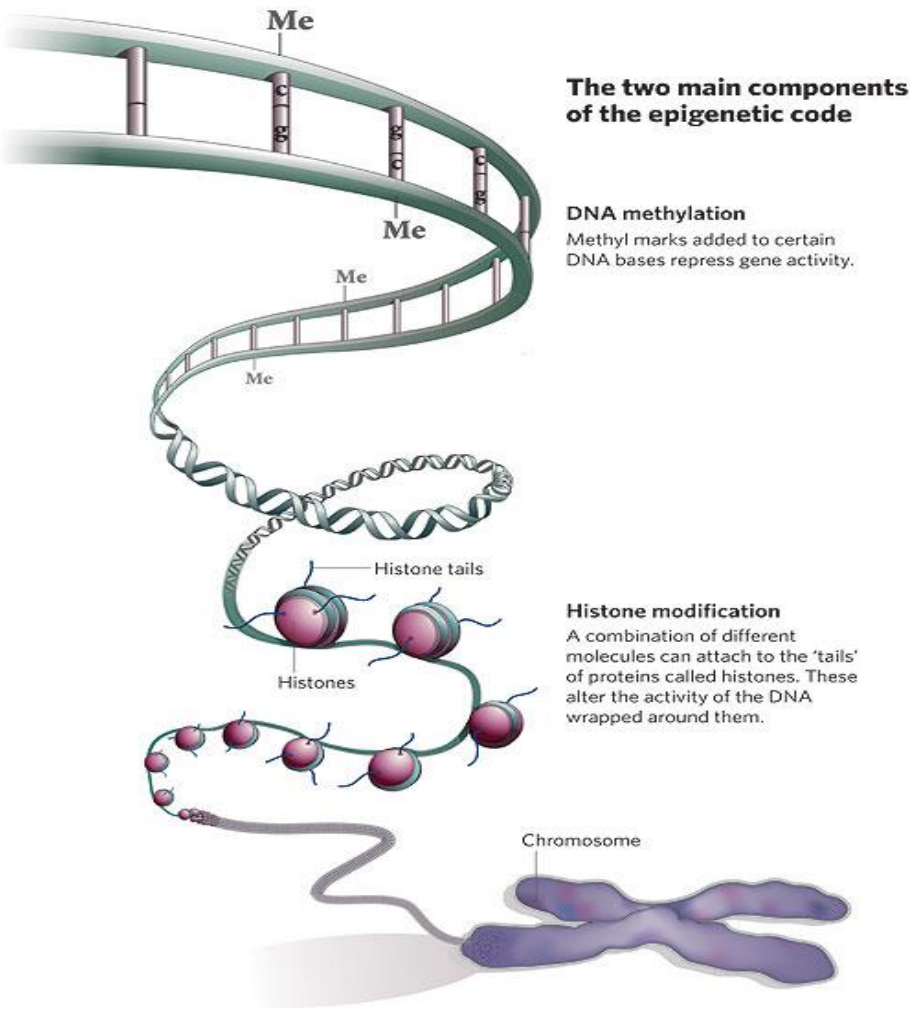
Chromatin is made up of fundamental unit called nucleosome.

Nucleosome Structure Image – Lodish et. al., Molecular cell biology Fourth Edition 2000

Nucleosome consists of 146 bp of DNA wrapped around octamers of histone proteins– H2A, H2B, H3 and H4.

Histones can be altered by different processes such as methylation, acetylation, phosphorylation, etc. These post-transcriptional modifications alter chromatin condensation, resulting in activation or inactivation of gene expression.

Major Epigenetic Mechanisms



DNA methylation

Addition of methyl groups to cytosine affects transcriptional repression.

Histone modification

Covalent modifications of histones which are involved in chromatin reorganization and regulation of transcription. Histone methylation affects transcriptional repression or activation.

Qiu J. Nature, 441: 143,2006

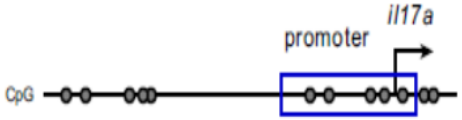
DNA methylation

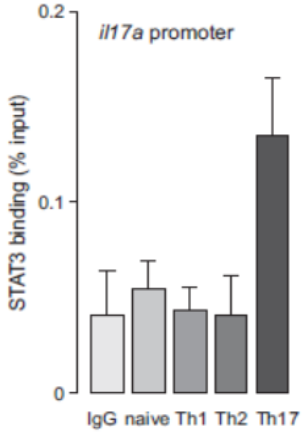
(keeping unwanted genes turned off)

Modification	Writer	Eraser	Reader	Function
Methyl-CpG	DNMT*	TET*	MBD coR*	silencing
5hm-CpG*	TET	DNA repair	repair, coR	demethylation

*DNMT = DNA methyltransferase,
TET = ten eleven translocation methylcytosine dioxygenase,
MBD = methyl binding domain,
coR = co-repressor complex,
5hm-CpG = 5-hydroxymethylcytosine Cytosine phosphodiester Guanine

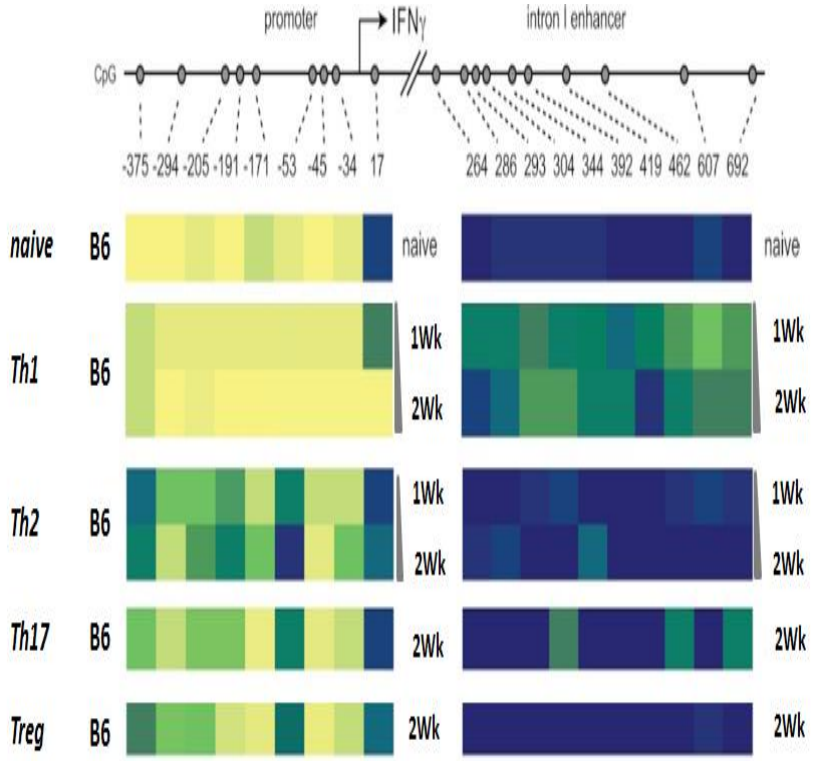
DNA Methylation regulates transcription at the IL17 locus





Thomas et al, The JBC 287 : 25049, 2012

DNA Methylation required for T helper cell lineage



Imprints T helper lineage choice

Thomas et al, The JBC 287:22900, 2012

Methyl Transferases (G9a and SUV39H1) Control T Helper cells Lineage

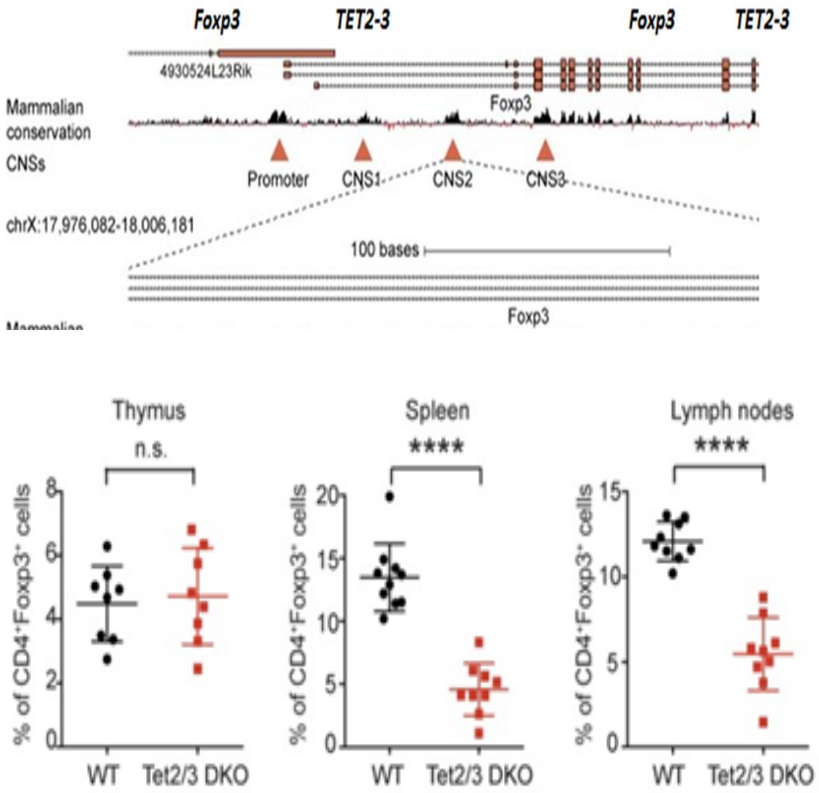
Histone methyltransferase G9a is necessary for Th2 lineage commitment. Cytokine IL4, IL5 and IL13 Production.

Lenhertz et al JEM 207: 915, 2010

Suv39h1-H3K9 me3 and HP1α silencing pathway control Th2 lineage stability.

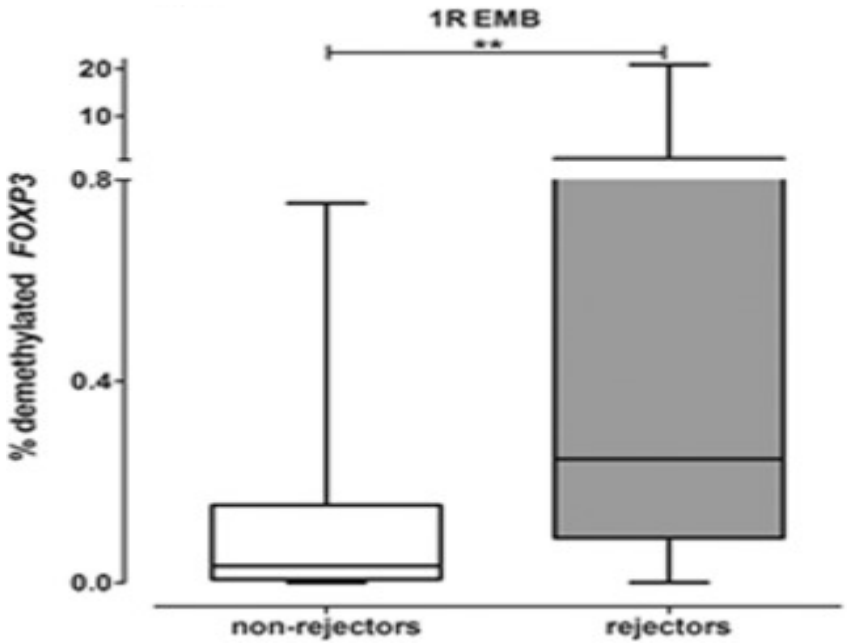
Allan et al, Nature 847: 249, 2012

TET is required for Foxp3 stability and T Reg efficacy



Yue X et al, JEM 20151438, 2016

Clinical Potential of DNA methylation in organ transplantation



Demethylated *FOXP3* was significantly higher in the Grade 1R endomyocardial biopsies collected before rejection compared with the Grade 1R biopsies.

Peters FS. et al., J Heart Lung Transplant., 2016; 35:843-50.

Histone modification

(signaling and recruitment from the gene up)

Modification	Writer	Eraser	Reader	Function
Methyl-CpG	DNMT1/3	TET	MBD coR	silencing
H3K9/27-methyl	SUV/G9a/EZH2	JMJ/UTX	HP1, PRC1*	silencing
H3/4-acetyl	HATs*	HDACs	Bromodomain*	permission
H3k4-methyl	MLL/SET	JARID	Chromodomain*	permission

*HAT = histone acetyltransferase
bromodomain = components of RNApol2 (TAF1)
chromodomain = components of RNApol2 (TFIID)
Swi/Snf chromatin remodeling (CHD1) complexes
HP1 = heterochromatin protein 1
PRC1 = policomb repressive complex 1

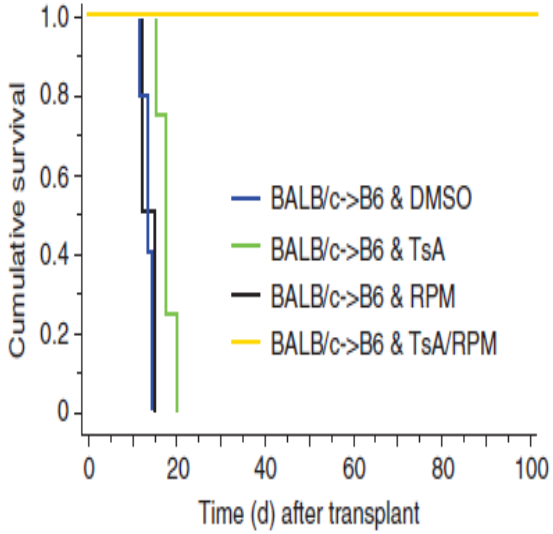
Histone deacetylase inhibitor enhances Foxp3+ T-Reg function

Class	Isoform	Domain structure	Location	Effect of HDAC targeting	Therapeutic potential in transplantation
I	HDAC1		N	Impaired Treg	Not suitable
	HDAC2		N	Improved Treg, potential IRI benefit	Lack of specific inhibitor
	HDAC3		N,C	Deletion impairs Treg, autoimmune phenotype	Not suitable
	HDAC8		N	Impaired Treg	Not suitable
IIa	HDAC4		N,C	Impaired Treg	Not suitable
	HDAC5		N,C	Impaired Treg, failure to maintain allograft	Not suitable
	HDAC7		N,C	Improved Treg	Lack of specific inhibitor
	HDAC9		N,C	Improved Treg, prolonged allograft survival	Lack of specific inhibitor
IIb	HDAC6		N,C	Improved Treg, prolonged allograft survival	Specific inhibitor, tissue selective ★
	HDAC10		N,C	Improved Treg, prolonged allograft survival	Lack of specific inhibitor
III	SIRT1		N	Improved Treg, prolonged allograft survival	Proinflammatory in myeloid cells
	SIRT2		C	No effect observed in vitro	Not suitable
	SIRT3		M	Impaired Treg, failure to maintain allograft	Not suitable
	SIRT4		M	Unknown	Unknown
	SIRT5		M	Unknown	Unknown
	SIRT6		N	Unknown	Unknown
	SIRT7		N	Unknown	Unknown
IV	HDAC11		N	Improved Treg, prolonged allograft survival	Specific inhibitor available

Wang L. et al, AJT 18:1596,2018

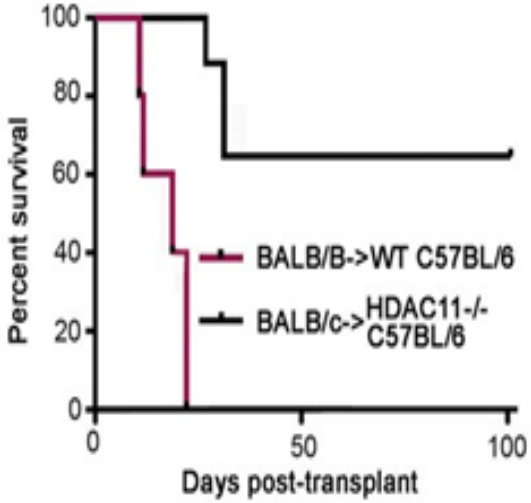
Histone deacetylase inhibition results in long term acceptance of allogenic islets and cardiac allografts

Islet transplant



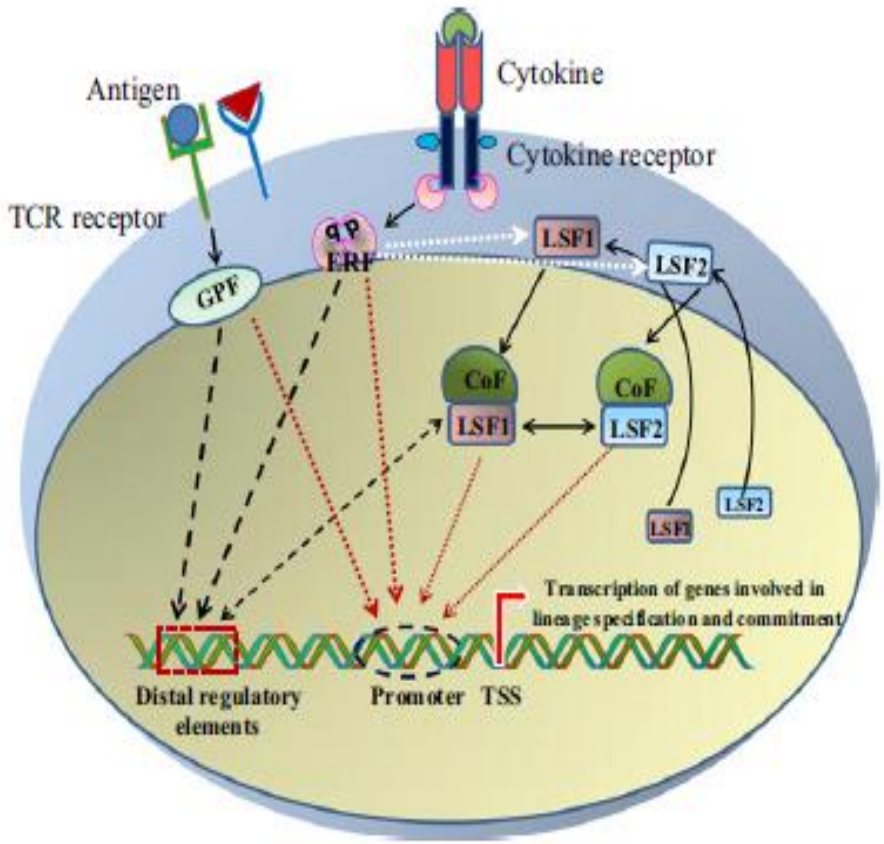
Tao R. et al, Nat. Med. 13:1299, 2007

Cardiac transplant



Huang J. et al, Sci. Reports 7:8626, 2017

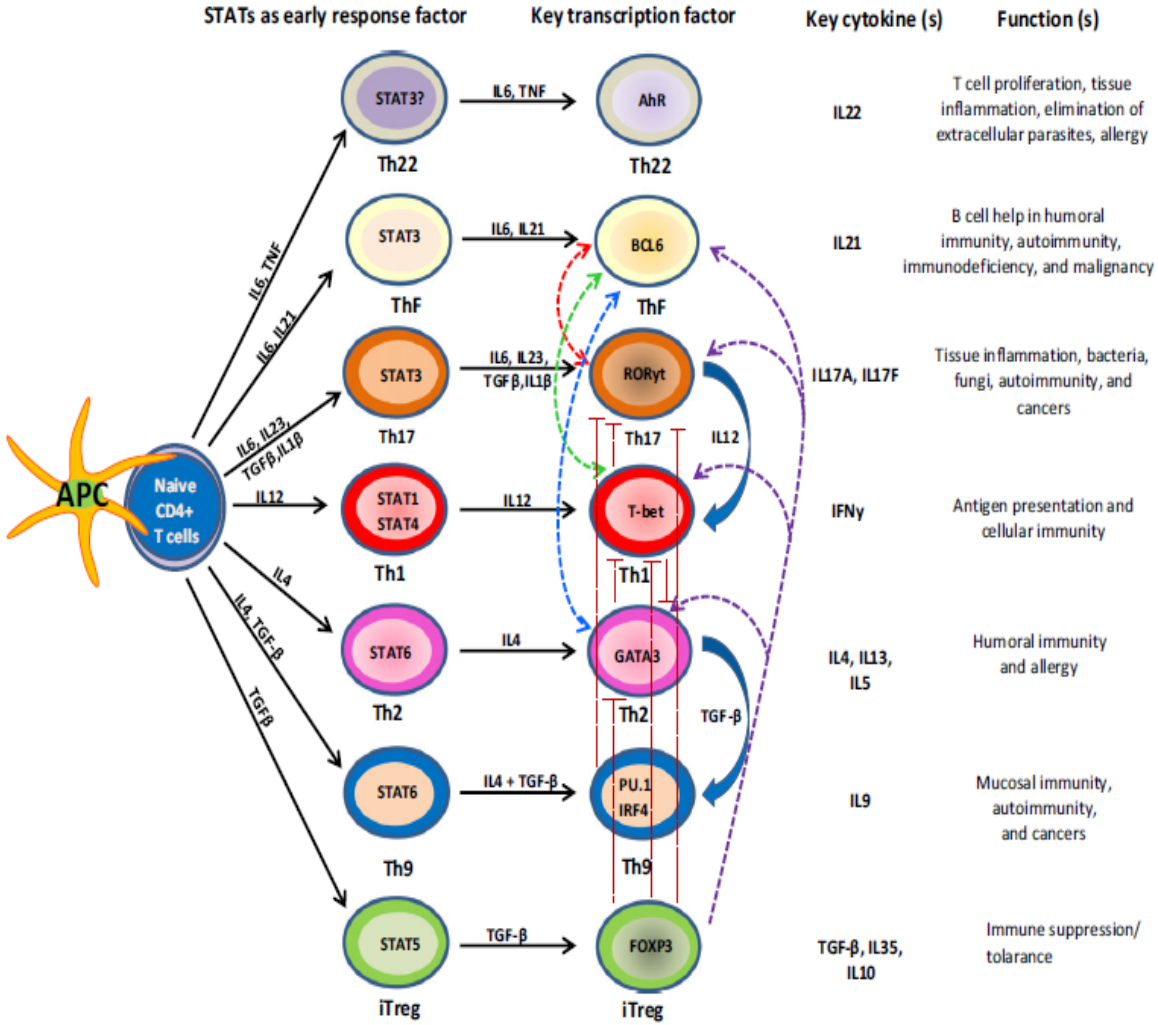
Transcription factors induce T cell lineage genes



- Antigens and cytokines are extracellular signals received by T cells through TCRs.
- Ligation of antigens to TCRs activate NF-kB, NFAT and AP-1.
- Cytokines binding to cognate cytokine receptors activate STATs.
- These transcription factors independently and synergistically regulate chromatin state and expression of a lineage specifying factors (ISFs).
- ISFs and other co–factors further bind to the pre-existing chromatin landscape to regulate transcription of genes involved in lineage specific gene expression program.

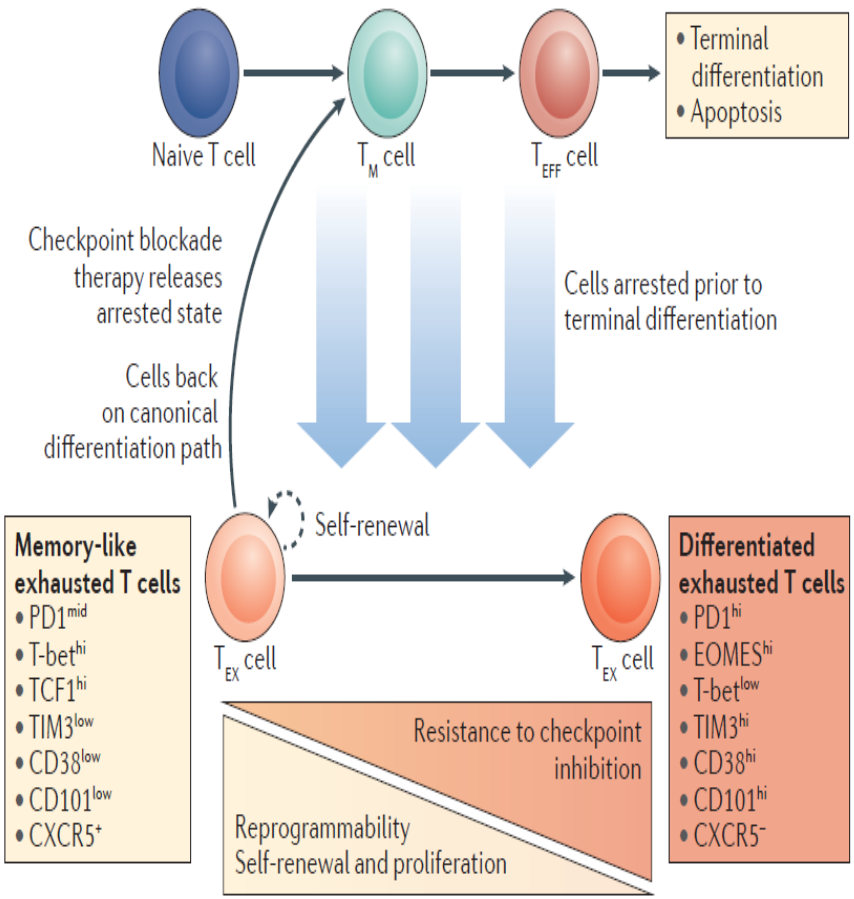
Tripathi S. et al, Immuno. Rev.261:62, 2014

CD4+ T helper cell subset and their plasticity



Tripathi S. et al, Immuno. Rev.261:62, 2014

Epigenetic control of CD8+ T cell differentiation



CXCR5, CXC-chemokine receptor 5; EOMES, eomesodermin homologue; PD1, programmed cell death protein 1; TCF1, transcription factor 1; TIM3, T cell immunoglobulin mucin receptor 3; T_M, memory T; T_{EFF} effector T.

- The exhausted state is heterogenous (with subset of exhausted T (T_{EX}) cells, exhibiting memory like phenotypes and characterized by specific cell surface markers).
- Differentiated T_{EX} cells have their own unique cell surface marker expression.
- Canonical T_{EX} cell differentiation at which cells become arrested may determine their T cell phenotype.
- Memory Like T_{EX} cells appear to be more responsive to checkpoint inhibitor therapy, which releases arrested cells, returning them to he canonical differentiation path. This would ultimately result in terminal differentiation and apoptosis of T_{EX} cells that had reversed the arrested state.

Henning AN. et al, Nat. Rev. 18:340, 2018

Biogenesis and release of miRNA

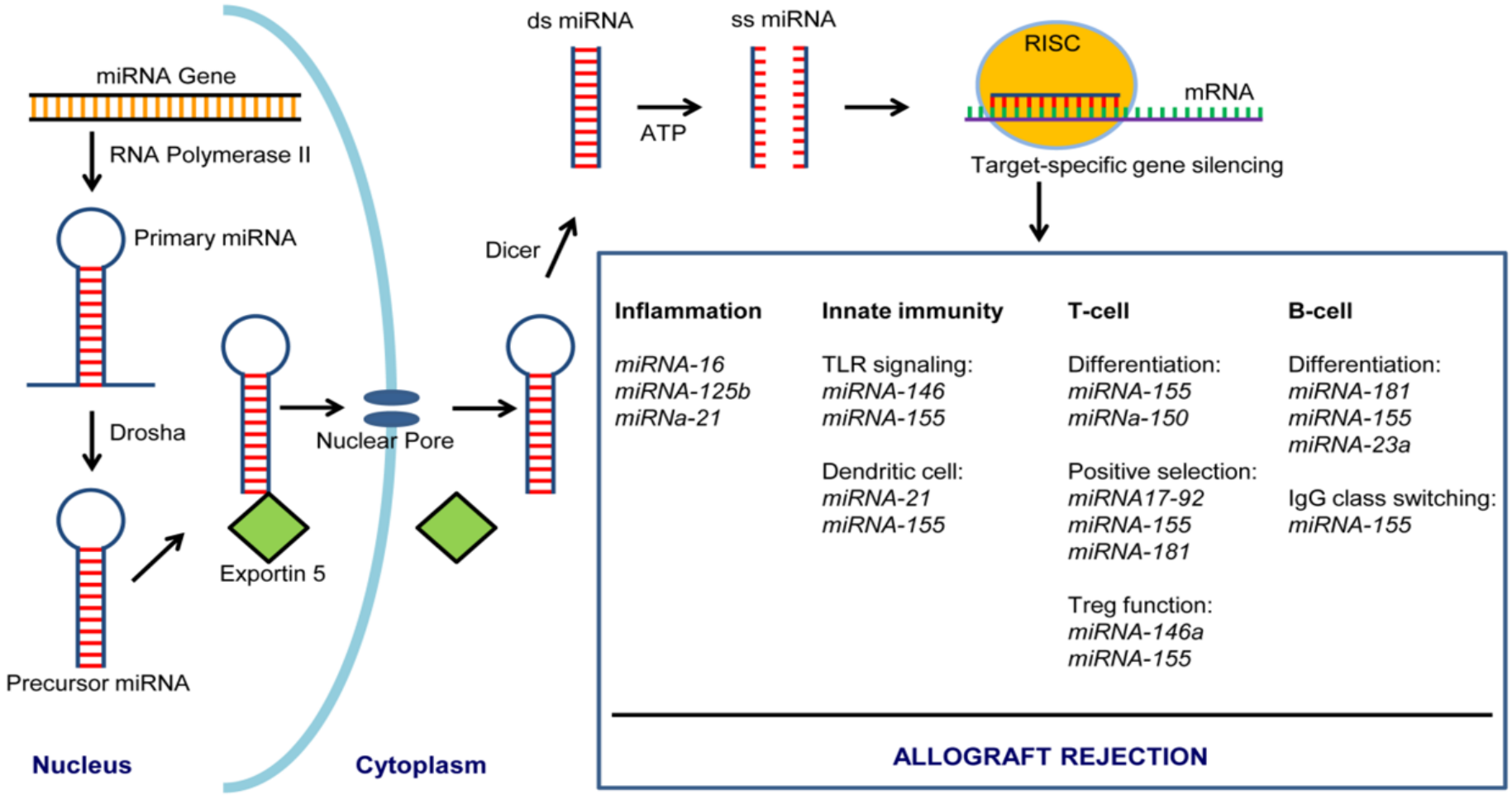
Transcription
Pri-microRNA
Drosha
DGCR8
Pre-microRNA
Exportin 5
Ran-GTP
Nucleus
Cytoplasm
Dicer
TRBP
microRNA duplex
Unwind
Degradation
Translational Repression
mRNA Degradation
RISC
RISC
Target mRNA
One strand of mature microRNA gets incorporated into RISC

Packed in

Ago2
PROTEIN COMPLEX
HDL
LIPOPROTEINS
MICROVESICLE
EXOSOMES

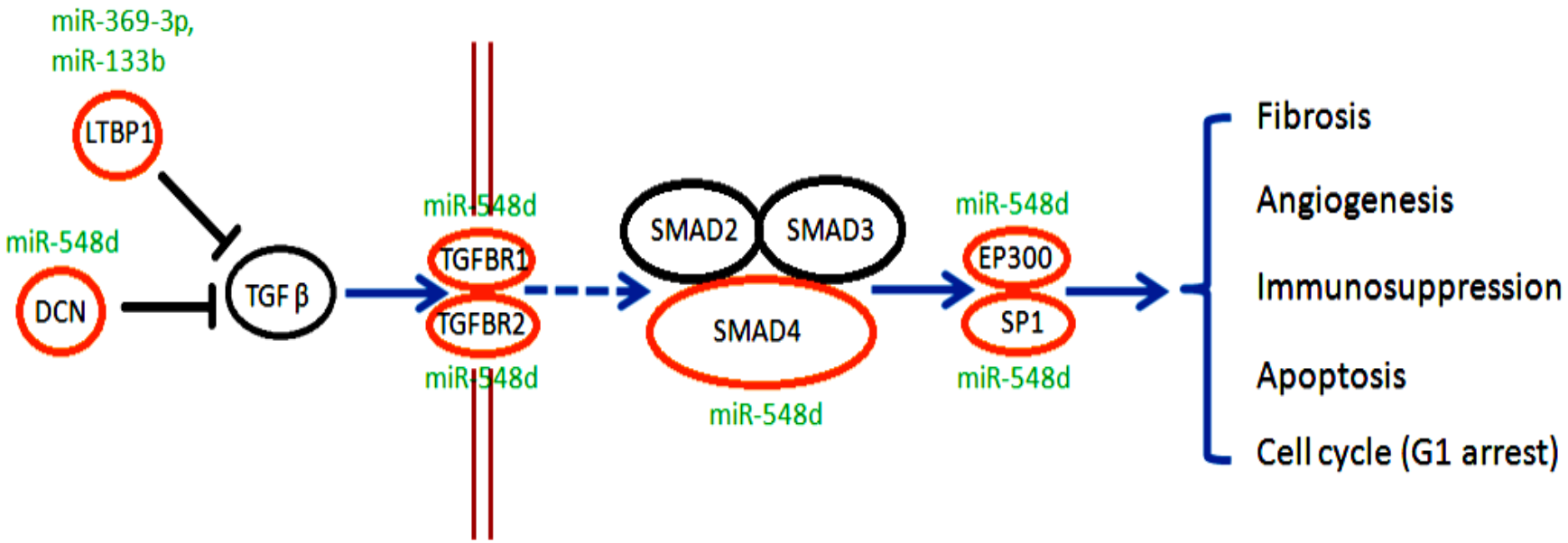
Mas VR. et al., Am J Transplant 13: 11,2013

Significance of microRNA in transplant



Sarma NJ. et al. Exp Mol Pathol. 93: 378,2012

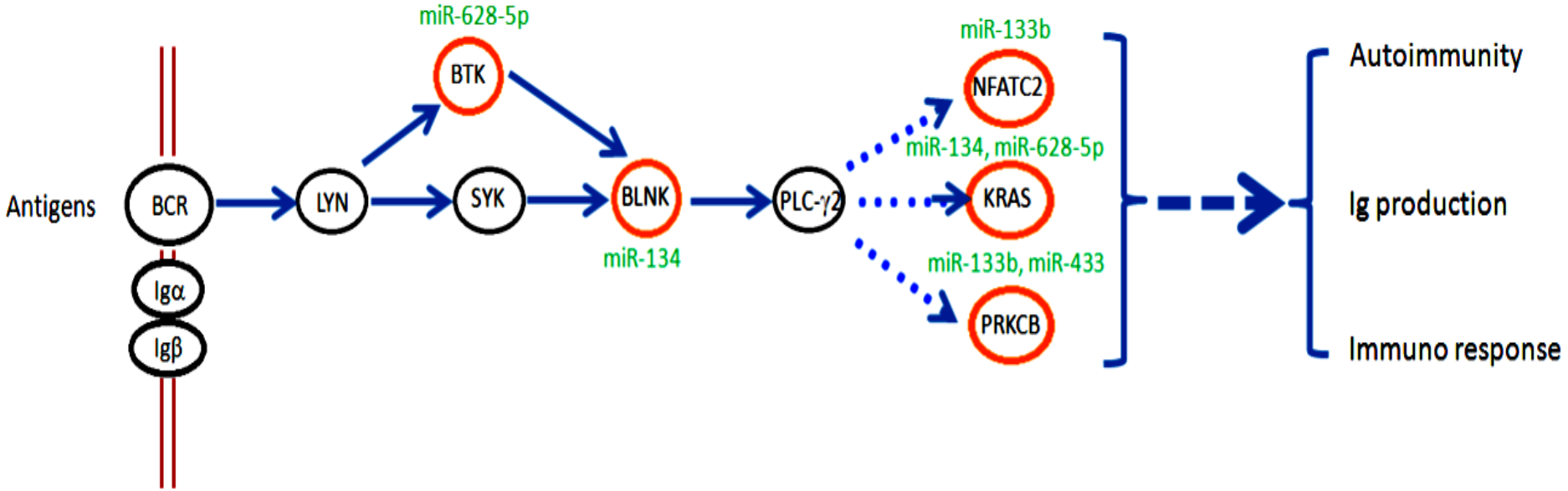
miR-369-3p, miR-548d, and miR-133b:
Dysregulation of TGF beta signal pathway



Xu Z. et al., AJT 15: 1933, 2015
Ogata et al., Biosystems. 47:119,1998.

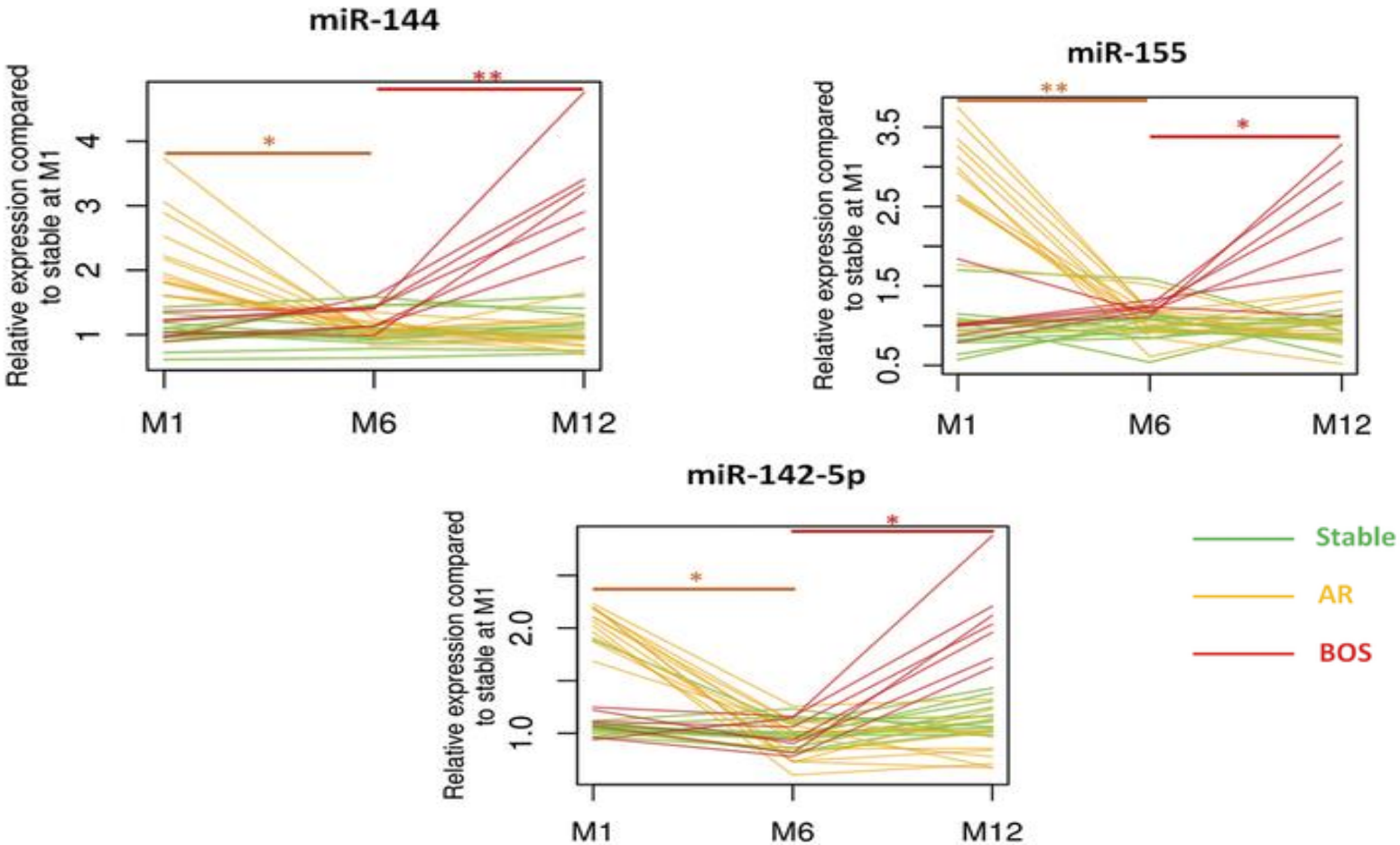
miR-628-5p, miR-133b, miR-134, miR-433:

Dysregulation of B cell activation signal pathway



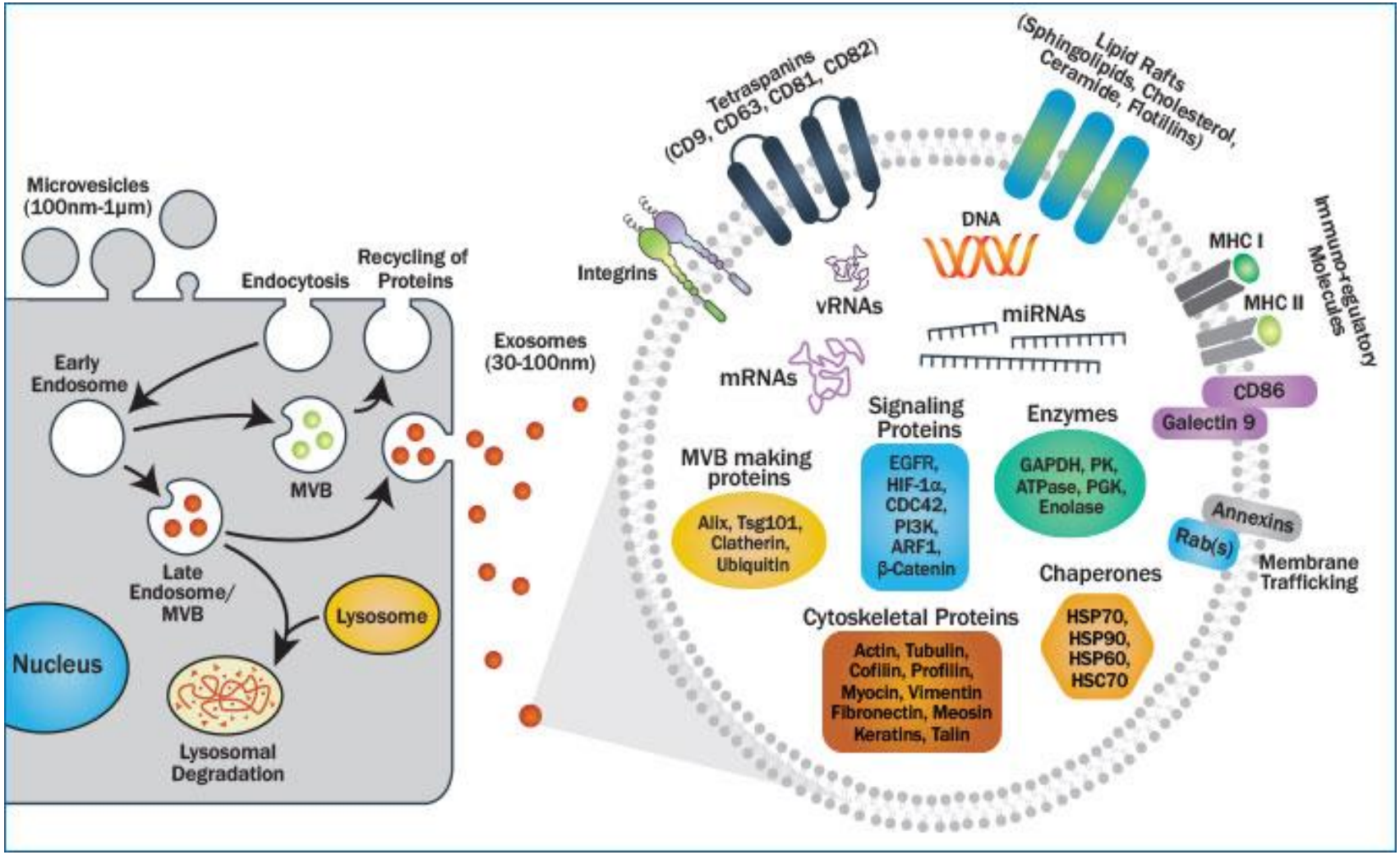
Xu Z. et al., AJT 15: 1933,2015
Ogata et al., Biosystems. 47:119,1998.

Upregulation of miR-144, miR-142-5p, and miR-155 in sera: Acute and chronic rejection following pediatric lung transplantation



Xu Z. et al., Transplantation 101:2461,2017

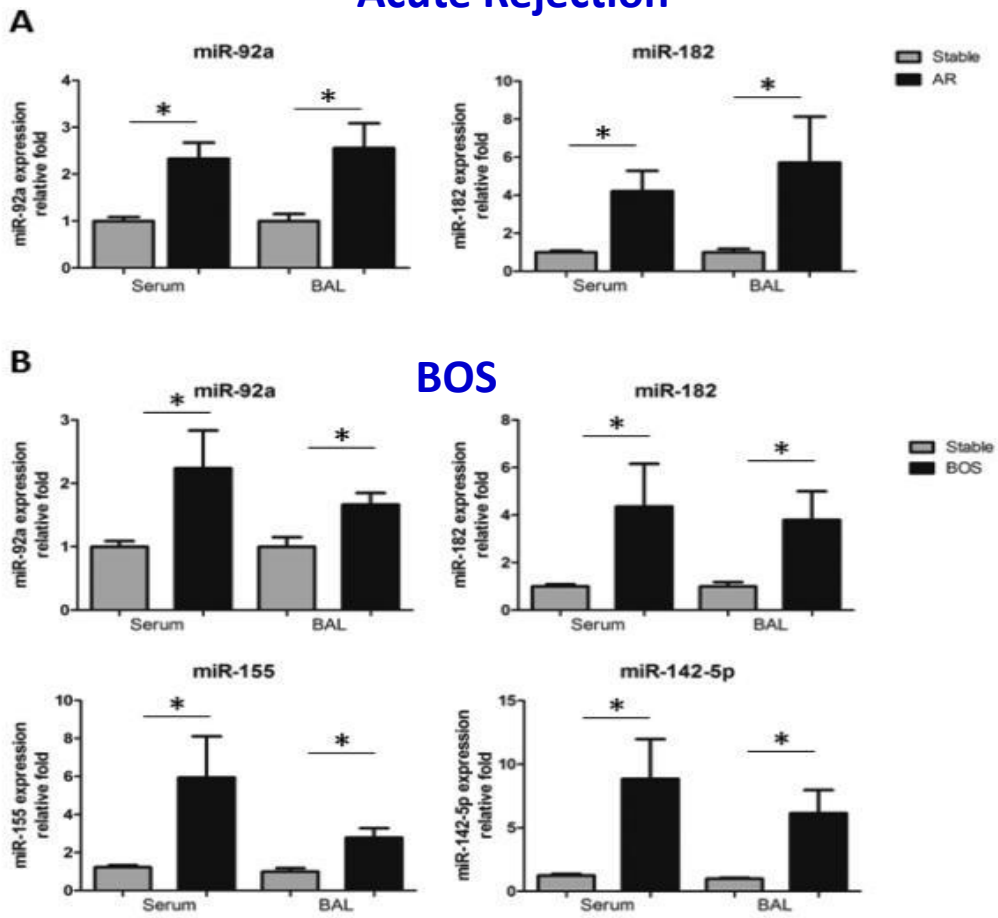
Exosomes and miRNA



https://www.novusbio.com/research-areas/cell-biology/Exosome-research-tools

Exosomes from lung transplant recipients with acute and chronic rejection contain distinct miRNA

Acute Rejection



Gunasekaran M. et al., AJT 17: 474,2017

Summary

- Epigenetics play a role in modification of transcriptome.
- DNA methylation, histone modification and chromatin architecture are major epigenetic mechanisms that regulate T cell differentiation and function.
- MicroRNAs are playing a major role in allograft rejection. Serum exosomes contain different miRNA involved in immune regulation.
- Detailed understanding of the epigenetics underlying T cell differentiation and function will enable enormous therapeutic possibilities to prevent allograft rejection.