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Long Non-Coding RNAs and miRNA: potential targets of polyphenols underlying their vascular health

Dragan Milenkovic

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Long Non-Coding RNAs and miRNA: potential targets of polyphenols underlying their vascular health

Dr Dragan MILENKOVIC

French Institute for Agricultural Research - INRA

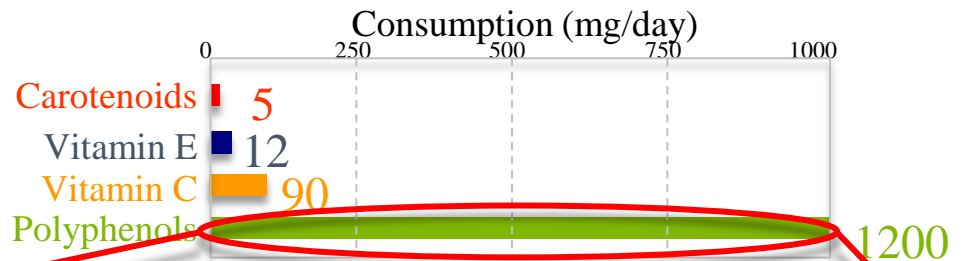
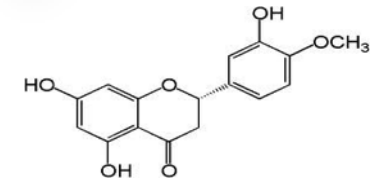
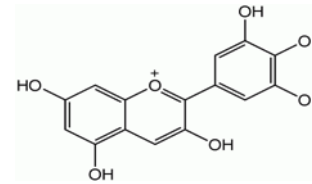
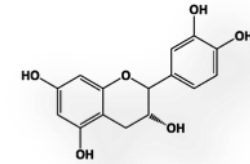
Human Nutrition Unit

Clermont-Ferrand, France

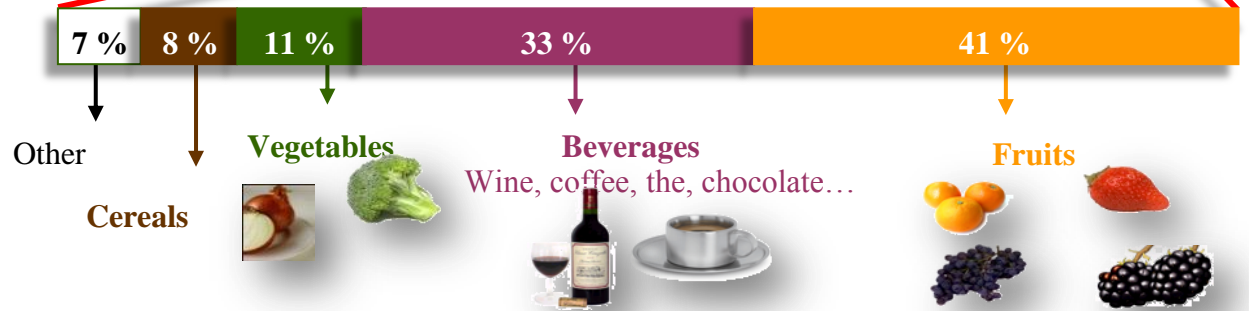


DIETARY POLYPHENOLS

- Phytomicronutrients
- Confer organoleptic properties to plant foods
- Most abundant dietary antioxidants



(Pérez-Jiménez J et al 2011)

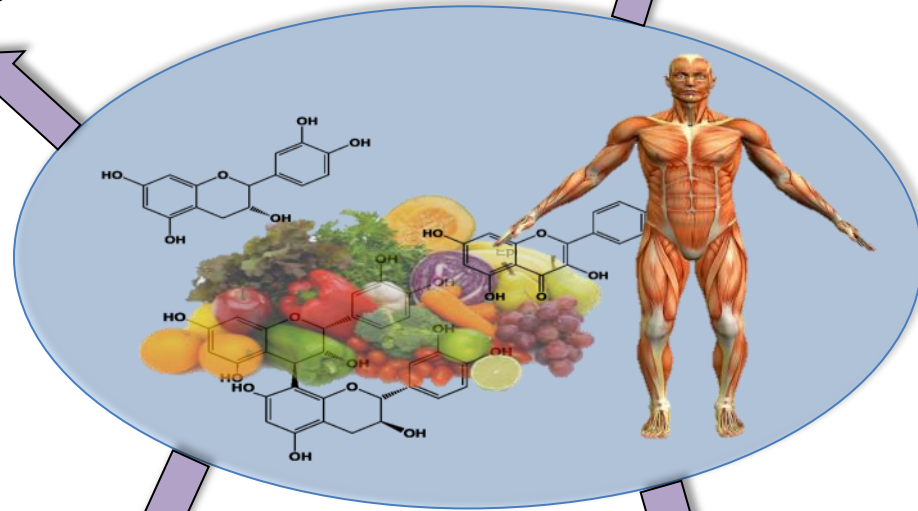
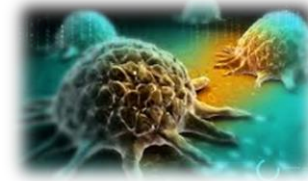


DIETARY POLYPHENOLS AND HEALTH

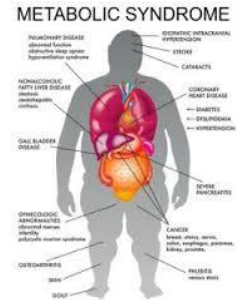


Cardiovascular diseases

Cancer



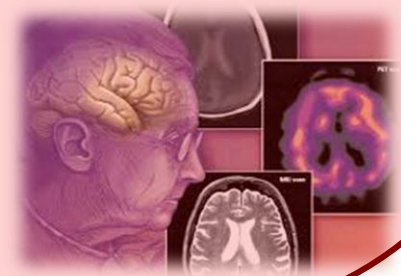
Metabolic syndrome



T2 Diabetes



Neurodegenerative Diseases



DIETARY POLYPHENOLS AND NEUROLOGICAL FUNCTIONS



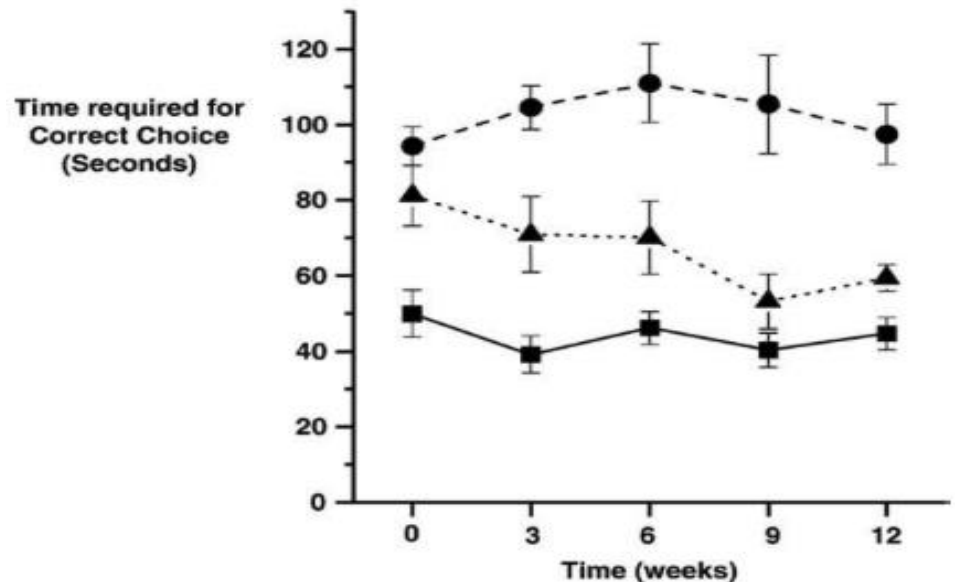
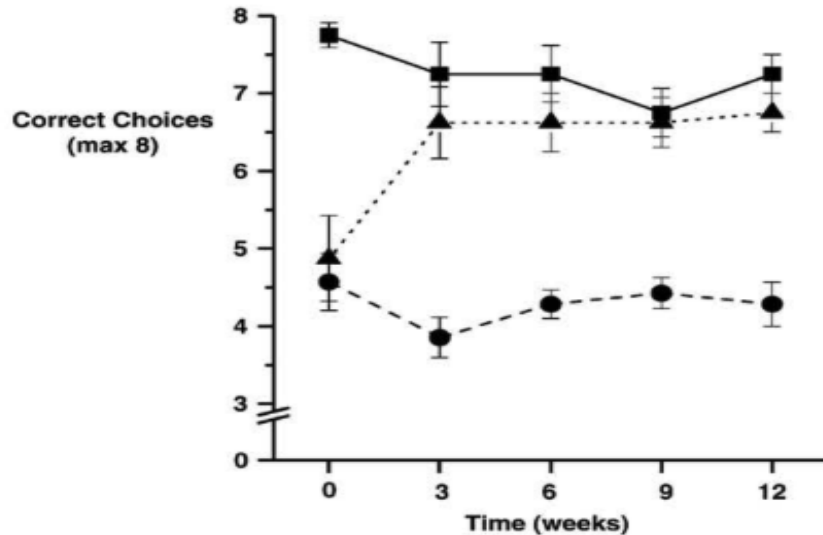
Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels



Claire M. Williams^{a,1}, Manal Abd El Mohsen^{b,1}, David Vauzour^b, Catarina Rendeiro^b, Laurie T. Butler^a, Judi A. Ellis^a, Matthew Whiteman^c, Jeremy P.E. Spencer^{b,*}

Free Radical Biology & Medicine 45 (2008) 295–305

- : “young” 9-month-old rats
- : “old” 21-month-old rats
- ▲ : “old” 21-month-old rats supplemented with a 2% BB diet



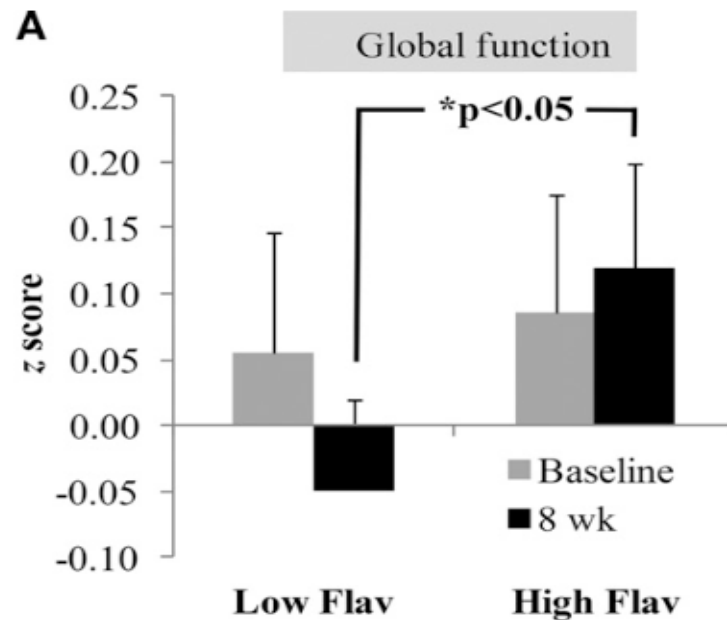
Blueberry diet improves the performance of aged animals in spatial working memory tasks

DIETARY POLYPHENOLS AND NEUROLOGICAL FUNCTIONS

Chronic consumption of flavanone-rich orange juice is associated with cognitive benefits: an 8-wk, randomized, double-blind, placebo-controlled trial in healthy older adults¹⁻³

Rebecca J Kean, Daniel J Lamport, Georgina F Dodd, Jayne E Freeman, Claire M Williams, Judi A Ellis, Laurie T Butler, and Jeremy PE Spencer

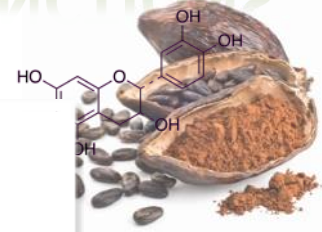
Am J Clin Nutr 2015;101:506-14. Printed in USA. © 2015 American Society for Nutrition



Flavanone-rich orange juice is beneficial for cognitive function in healthy older adults

with no significant effects on mood or blood pressure

DIETARY POLYPHENOLS AND NEUROLOGICAL FUNCTIONS

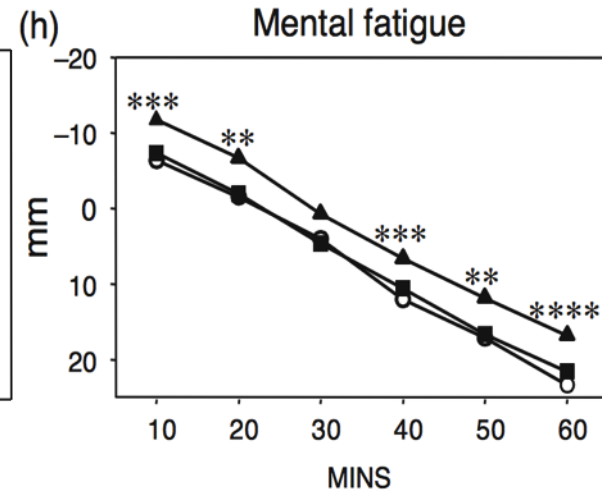
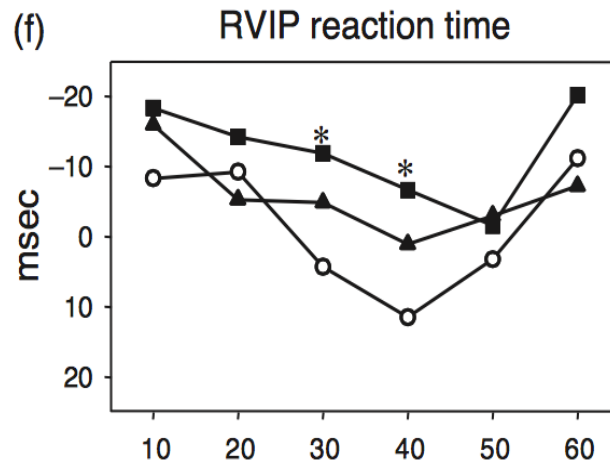
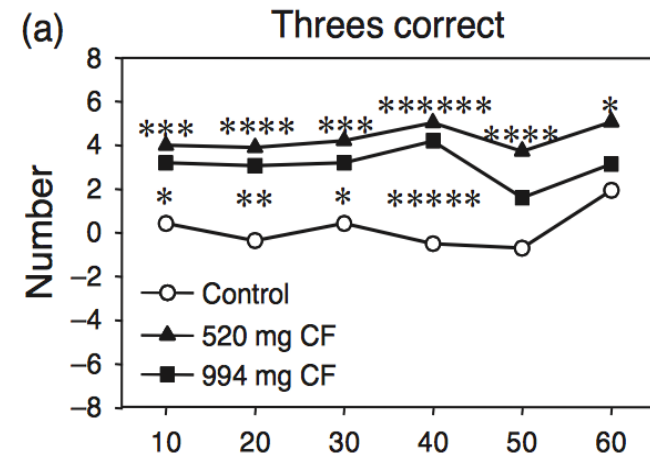


Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort

Andrew B Scholey¹, Stephen J French², Penelope J Morris²,
David O Kennedy³, Anthea L Milne³ and Crystal F Haskell³

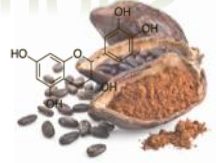
Journal of Psychopharmacology 2010 24(10) 1505–1514

Cognitive Demand Battery



Cocoa flavanols beneficially affect cognitive function regarding performance and mood during highly effortful cognitive processing

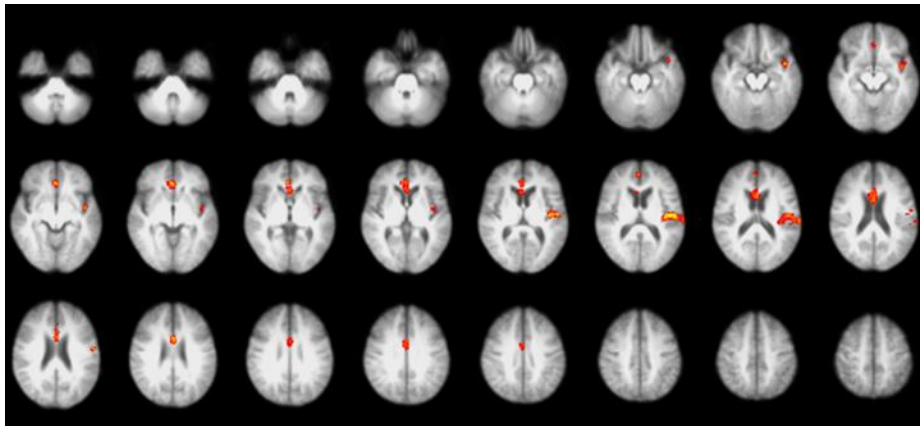
DIETARY POLYPHENOLS AND NEUROLOGICAL FUNCTIONS



The effect of flavanol-rich cocoa on cerebral perfusion in healthy older adults during conscious resting state: a placebo controlled, crossover, acute trial

Daniel J. Lamport¹ · Deepa Pal¹ · Christina Moutsiana¹ · David T. Field¹ ·
Claire M. Williams¹ · Jeremy P. E. Spencer² · Laurie T. Butler¹

Psychopharmacology (2015) 232:3227–3234

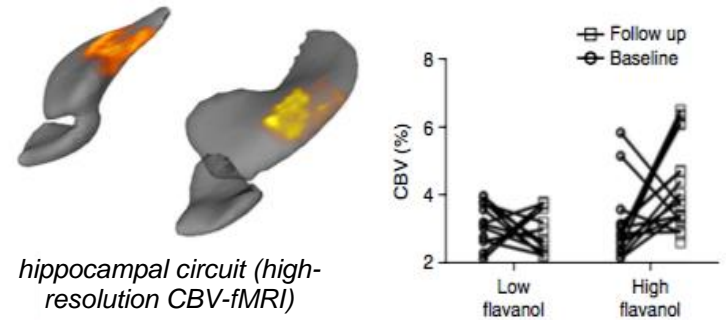


Increase in blood flow in brain 2h post-consumption of the high flavanol drink

nature neuroscience 26 October 2014; doi:10.1038/nn.3850

Enhancing dentate gyrus function with dietary flavanols improves cognition in older adults

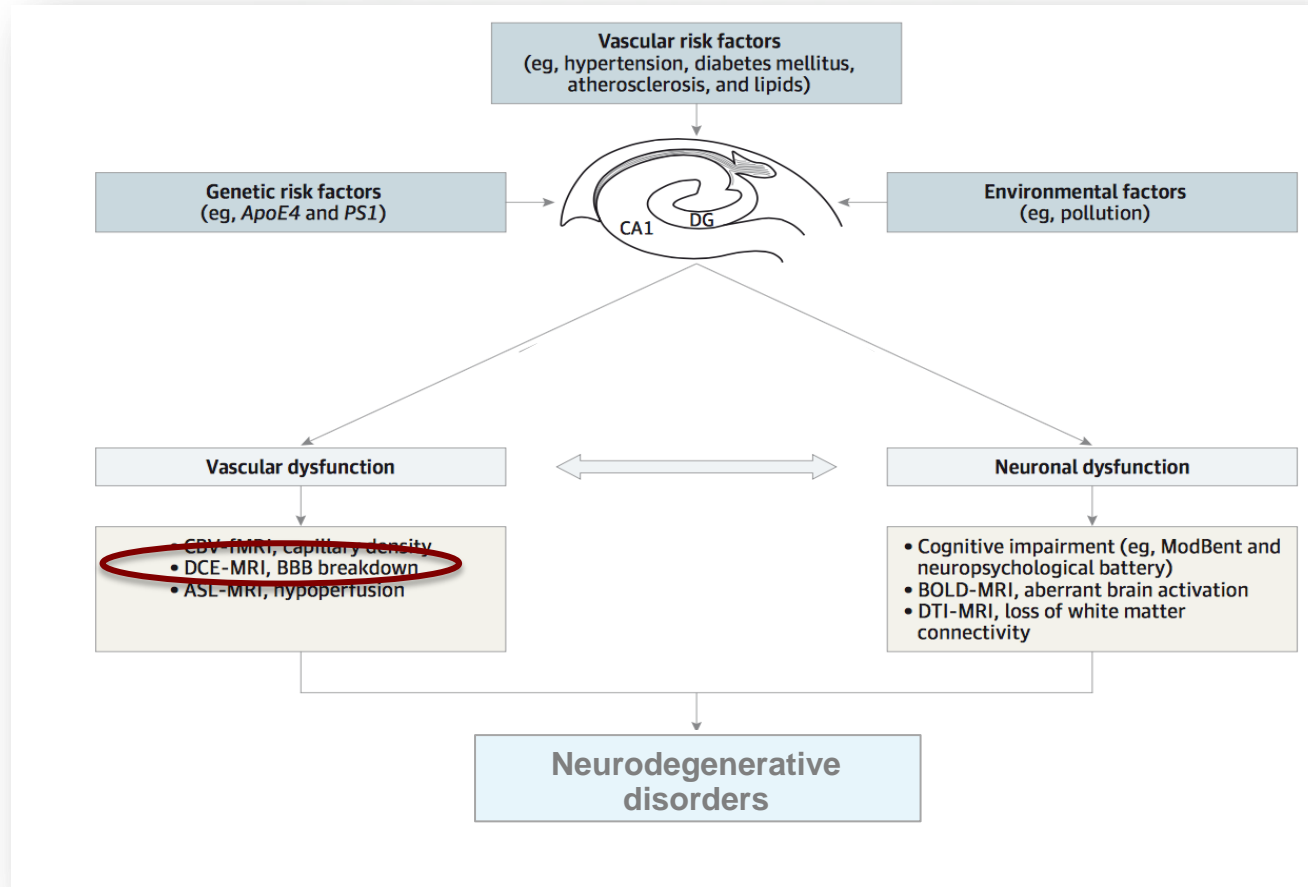
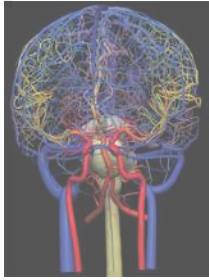
Adam M Brickman^{1,2,8}, Usman A Khan^{1,2,8}, Frank A Provenzano^{1,2,8}, Lok-Kin Yeung^{1,2}, Wendy Suzuki³, Hagen Schroeter⁴, Melanie Wall^{5,6}, Richard P Sloan^{5,6} & Scott A Small^{1,2,5,7}



The dentate gyrus (DG) is a region in the hippocampal formation whose function declines with age and is therefore considered as source of age-related memory decline.

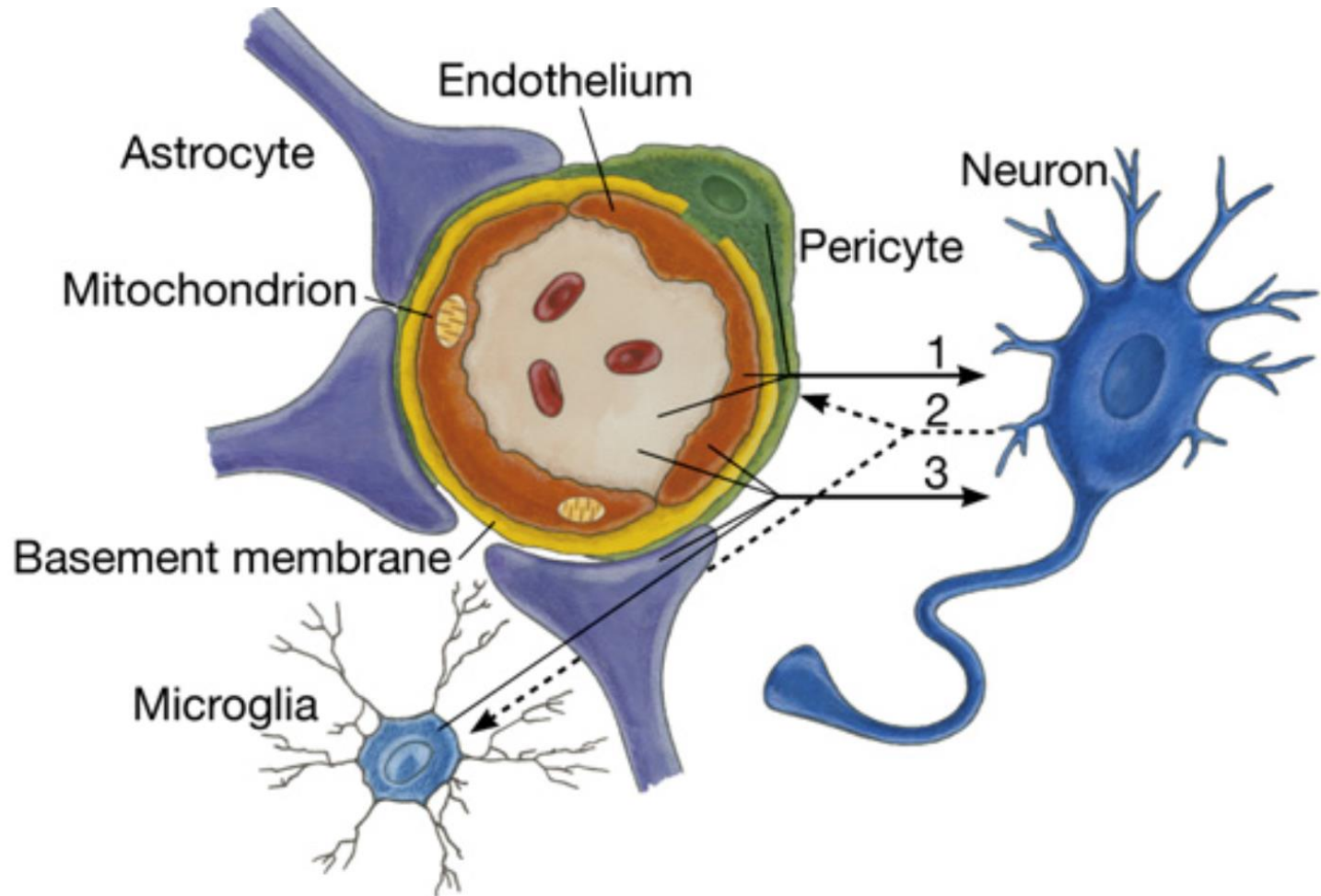
A high-flavanol intervention enhances DG function (cerebral blood volume and cognitive function)

ORIGINS OF NEURODEGENERATIVE DISORDERS: BLOOD-BRAIN-BARRIER

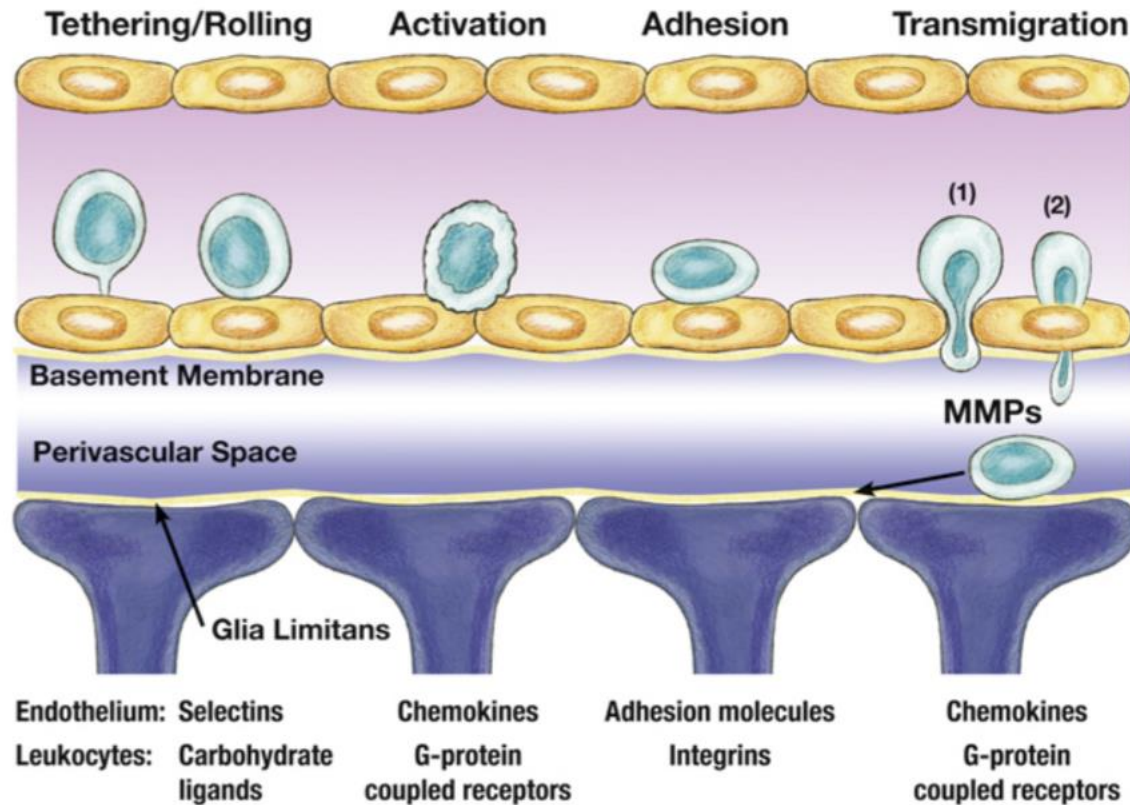


- BBB dysfunction, a key contributors to the pathogenesis of neurodegenerative disorders
- BBB breakdown is an early event in the aging human brain that begins in the hippocampus and may contribute to cognitive impairment.

STRUCTURE OF BLOOD-BRAIN-BARRIER - NEUROVASCULAR UNIT -



DYSFUNCTION OF BLOOD-BRAIN-BARRIER

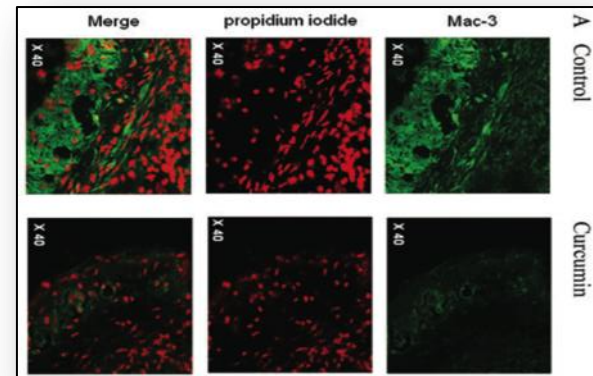
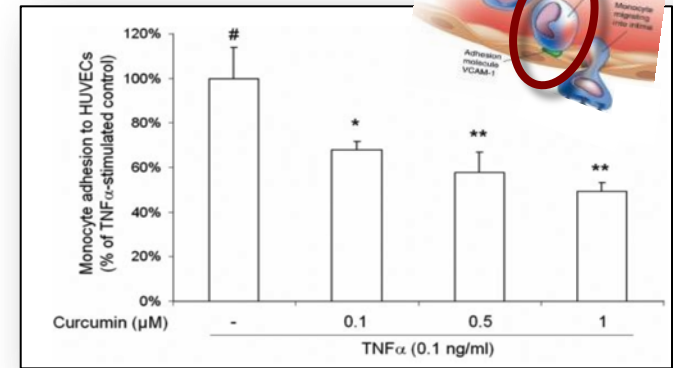
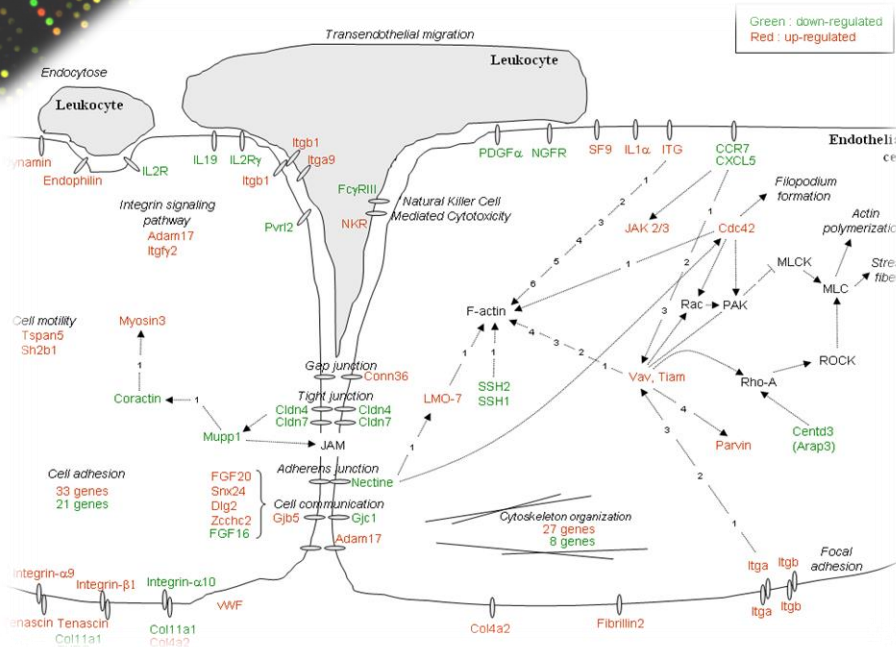


POLYPHENOLS AND ENDOTHELIAL CELL FUNCTION

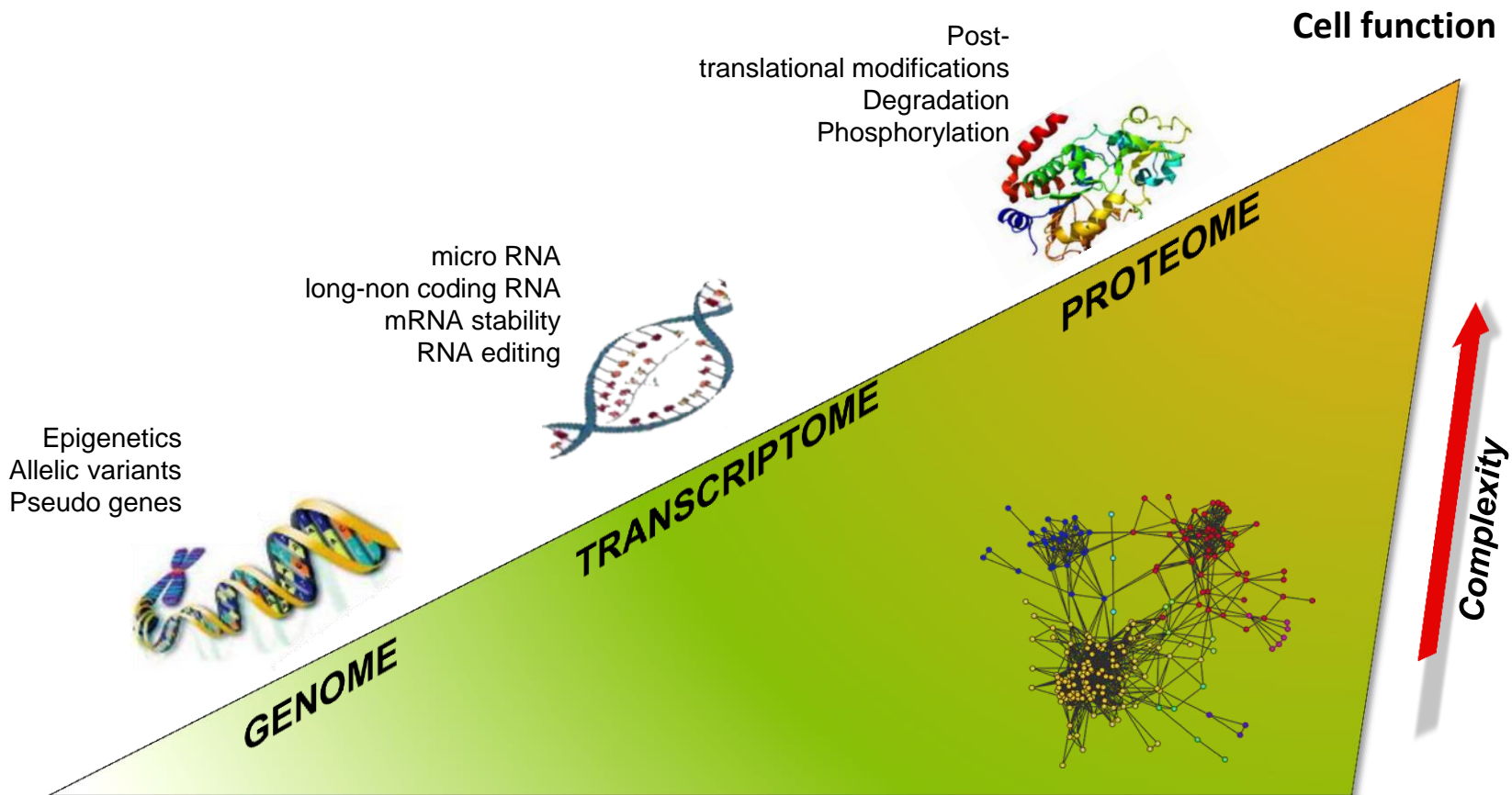
Dietary curcumin inhibits atherosclerosis by affecting the expression of genes involved in leukocyte adhesion and transendothelial migration

Dilek Coban^{1,2}, Dragan Milenkovic^{1,2}, Audrey Chanet^{1,2}, Jamila Khallou-Laschet³, Linde Sabbe⁴, Ajay Palagani⁴, Wim Vanden Berghe^{4*}, Andrzej Mazur^{1,2} and Christine Morand^{1,2}

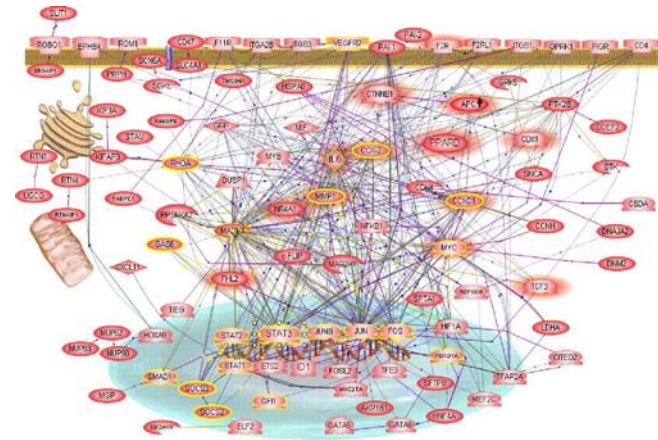
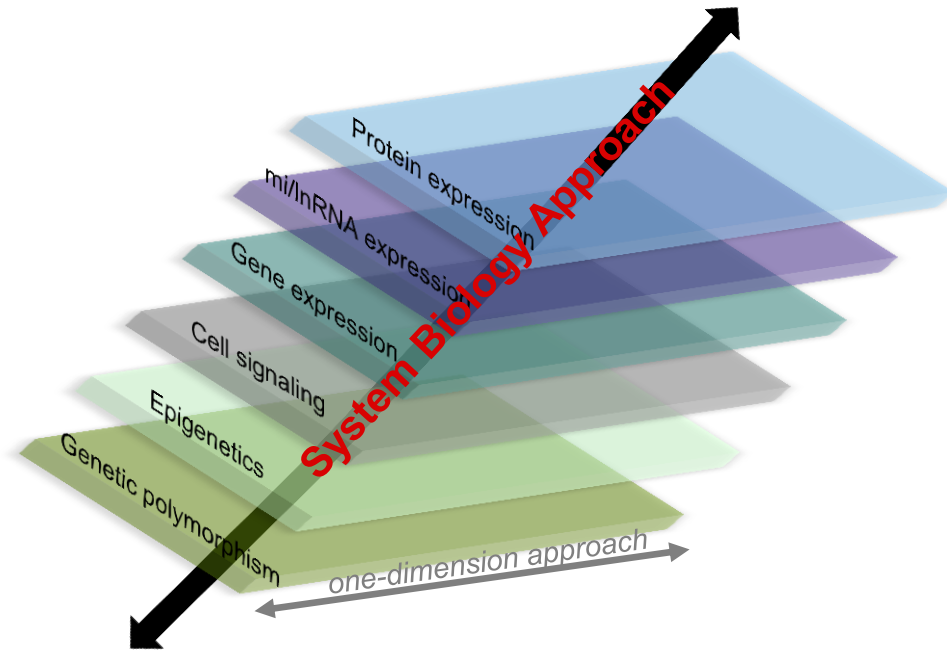
Mol. Nutr. Food Res. 2012, 00, 1–12



CELL FUNCTION REGULATION

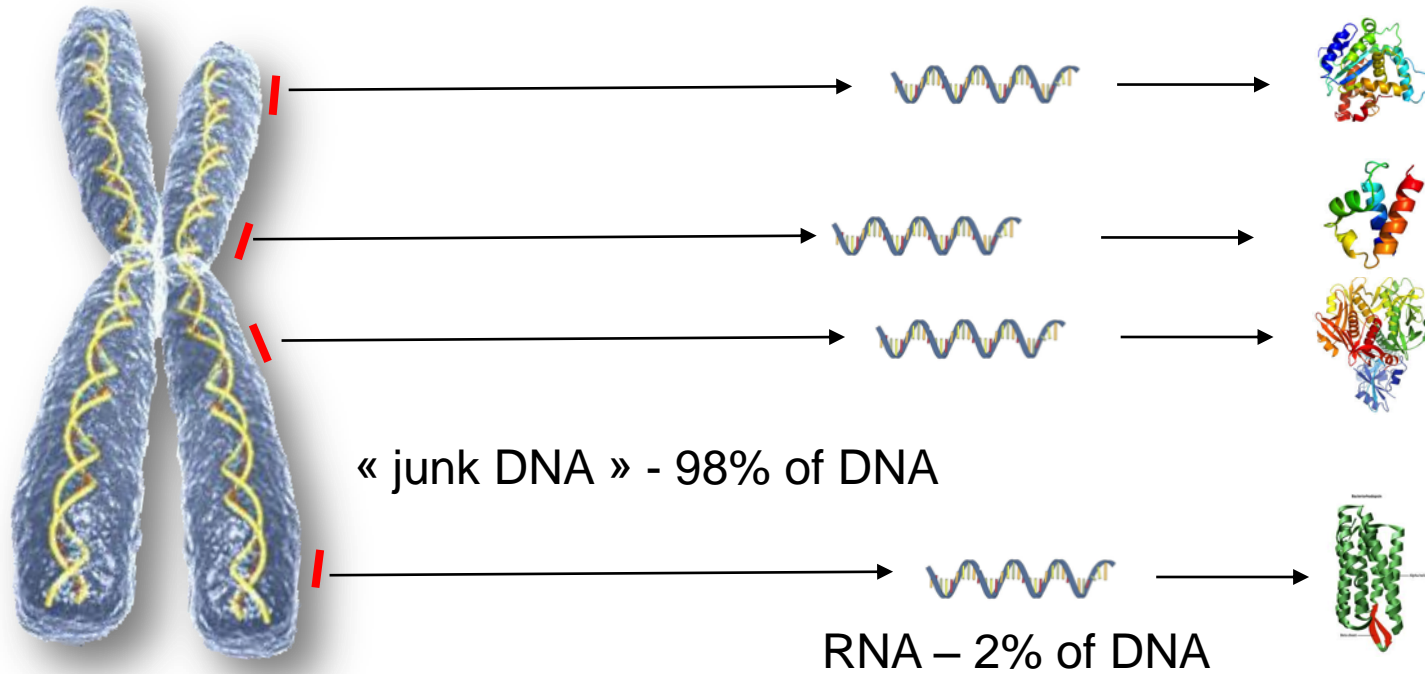


SYSTEMS BIOLOGY



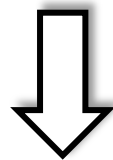
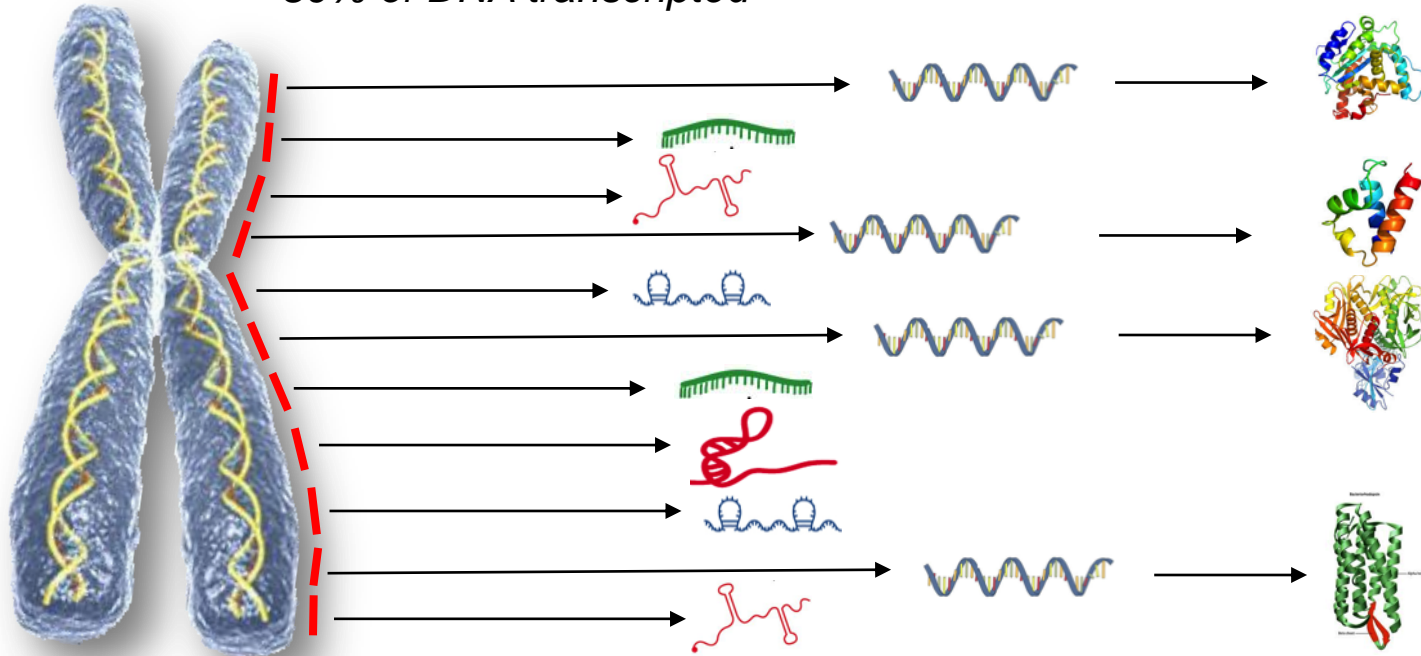
Adapted from Geschwind & Konopka, Nature 2009

DNA TRANSCRIPTION



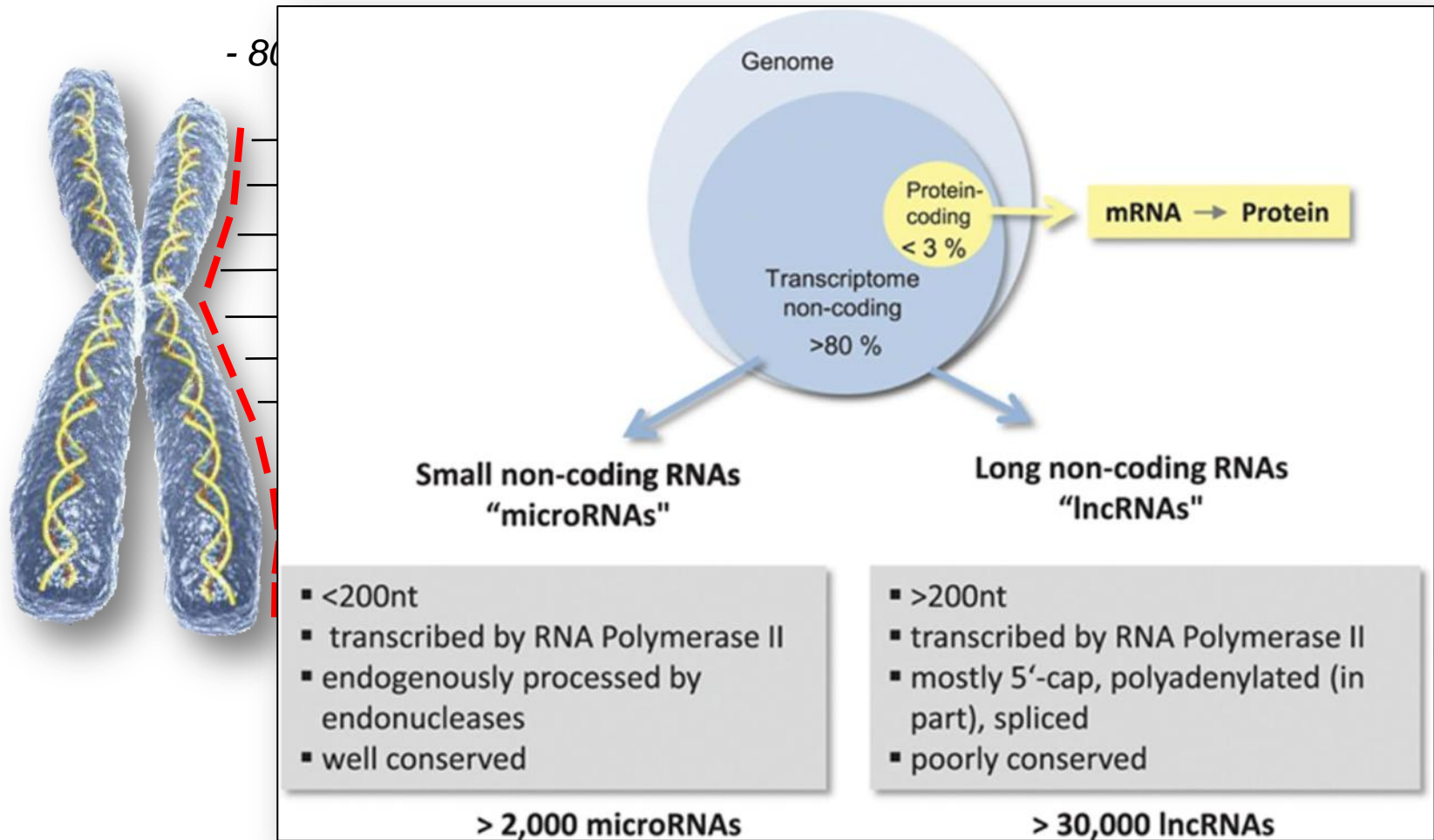
DNA TRANSCRIPTION

- 80% of DNA transcribed

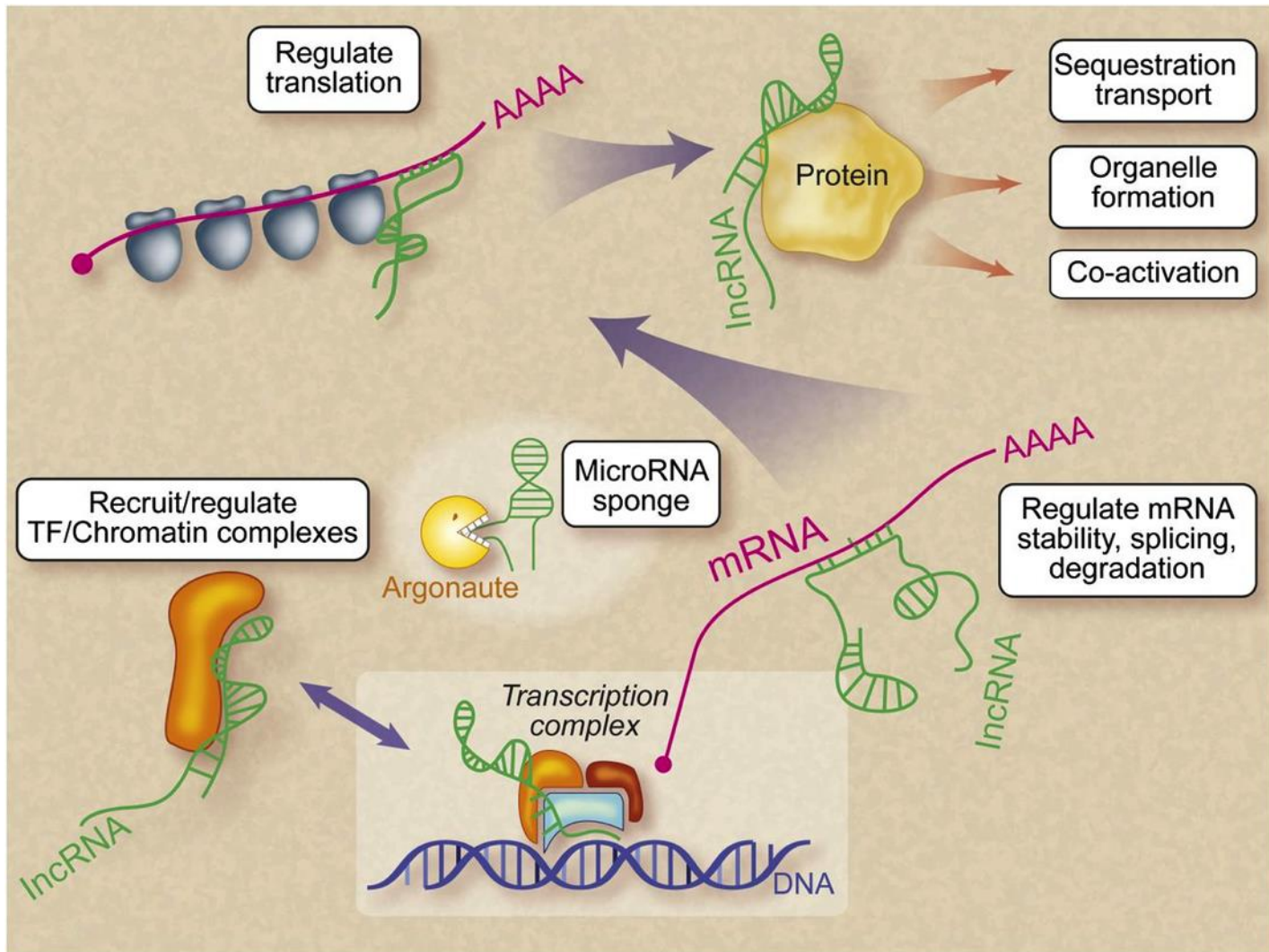


non-coding RNAs

DNA TRANSCRIPTION



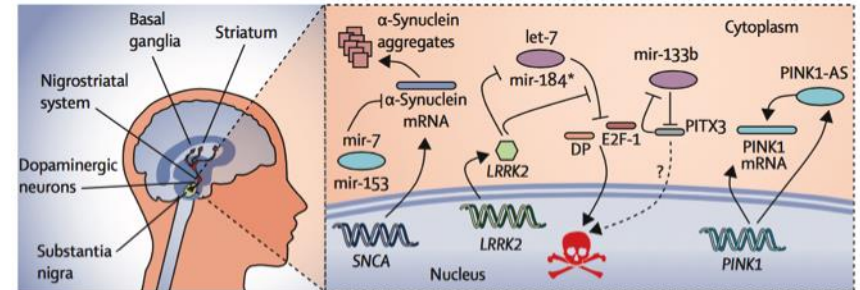
lncRNAs REGULATE GENE EXPRESSION THROUGH DIVERSE MECHANISMS



LncRNAs AND NEURODEGENERATIVE DISEASES

Non-coding RNA in neural function, disease, and aging

Kirk Szafranski^{1†}, Karan J. Abraham^{1†} and Karim Mekhail^{1,2*}

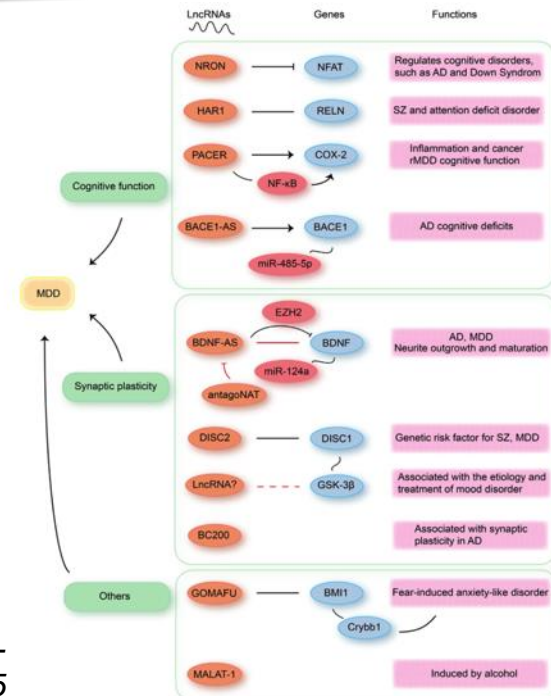


Salta et al., *Lancet Neurol* 2012

Transcript/protein	Diseases implicated	Proposed mechanism
<u>FUS/TL5</u>	ALS, FTLT	Indirect Drosha interactions regulates various miRNAs
<u>ATX2</u>	ALS, SCA2, PSP	Interactions with RISC components regulates long-term habituation
<u>DGCR8</u>	Prader-Willi syndrome	Microprocessor, can process miRNAs and snoRNAs
<i>n-Tr20</i>	N/A. Neural cell death observed with mutation	tRNA isodecoder necessary for the production of GTPBP2
<u>CLP1</u>	PCH 10	Necessary for maturation of tRNAs
<u>BACE1AS</u>	AD	Stabilization of <i>BACE1</i> which ultimately results in amyloid beta accumulation
<u>ATXN8OS</u>	SCA8	May reduce the amount of KLHL1, may produce toxic dipeptides or sequester RNA binding proteins
<u>UCHL1-AS</u>	AD, PD	Regulates translation of neuroprotective protein UCHL1
<u>NEAT1</u>	ALS, HD, aging	Produces paraspeckle bodies which storage RNAs, potentially for release during stress
<u>C9ORF72</u>	ALS	Abortive transcripts sequester RNA binding proteins and produce toxic dipeptides
<u>SETX</u>	AOA2, ALS	RNA-DNA helicase prevents R-loop formation
<u>Pbp1</u>	ALS, SCA2, PSP	Interacts with RNAs to prevent RNA-DNA hybrids, maintains genomic stability, and cellular lifespan

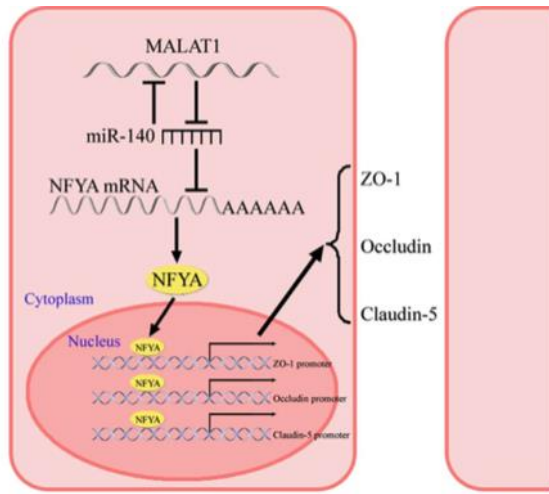
The link between long noncoding RNAs and depression

Xiao Huang^a, Yan-li Luo^b, Yue-shi Mao^{c,*1}, Jian-lin Ji^{a,*1}



Huang et al., *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 2015

LONG NON-CODING RNA REGULATE ENDOTHELIAL CELL FUNCTION AND BARRIER

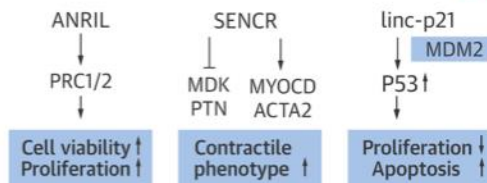
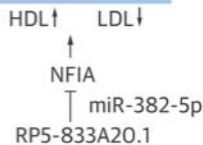


MA et al., ABB 2015

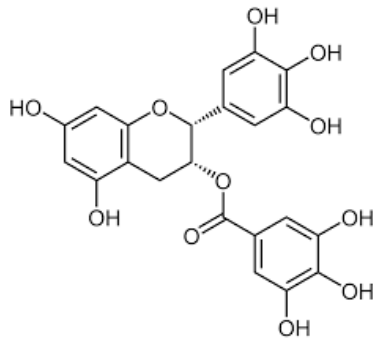
LncRNA	Type/localization	Putative function in SMC
<i>ATG9B</i>	NAT-lncRNA/nuclear+cytoplasmic	↓ <i>NOS3</i> following hypoxia
<i>TIE1-AS</i>	NAT-lncRNA/cytoplasmic	Regulation of EC contact junctions
<i>NRON</i>	Intr-lncRNA/cytoplasmic	↓ EC proliferation/↓ migration
<i>SENCR</i>	antisense oh-lncRNA/cytoplasmic	?
<i>MALAT1</i>	LincRNA/nuclear	Proper angiogenesis
<i>TGFB2-ot1</i>	sense ol-lncRNA/?	ceRNA regulating autophagy
<i>MIAT</i>	LincRNA/nuclear	ceRNA regulating EC function
<i>ASncmtRNA-2</i>	Stem loop/nucleus	Replicative senescence
<i>AGAP2-ASI</i>	NAT-lncRNA/cytoplasm	Proper angiogenesis
<i>HIF1A-ASI</i>	NAT-lncRNA/?	↑ EC apoptosis/↓ EC proliferation
<i>DLL4-AS</i>	NAT-lncRNA/cytoplasm	↑ EC proliferation/↑ EC migration

Miano et al., Cell. Mol. Life Sci, 2015

Lipid homeostasis

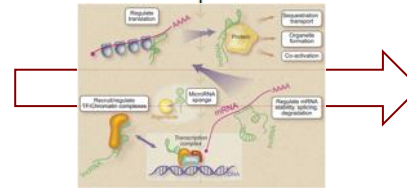


DIETARY POLYPHENOLS AND LncRNA



Epigallocatechin gallate

IncrNAs	Change
N342928	↓
NONHSAT015959	↓
N333444	↓
AT088005	↓
TM4SF4-2	↓
AT009251	↓
AT008445	↓
AT068602	↓
AT020294	↓
AT122449	↓
AT068591	↓
PHF17-1	↓
AT098264	↓
n382512	↓
n409611	↓
DCLK3-3	↓
AT101123	↓
ISLR2-3	↓
AT102202	↓
AT115872	↓
AT017383	↓
AT004532	↓
AT027943	↓
ITGB2-3	↓
AT078273	↓
AT016514	↓
AT097214	↓
FAM75A3-1	↓
FAM75A3-2	↓

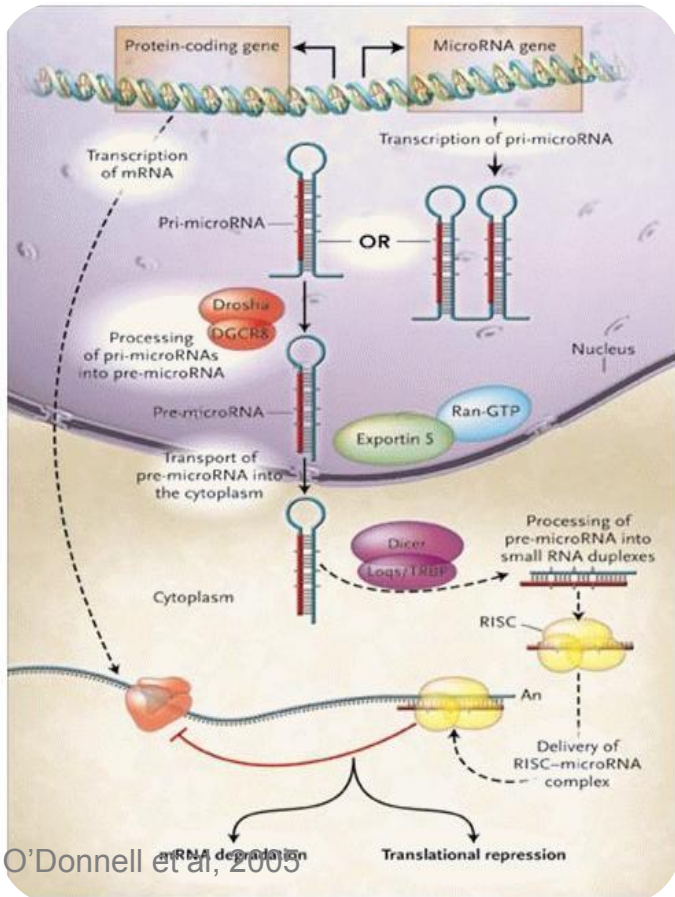


- Cholesterol synthesis
- Lipid droplets formation
- Cell replication

EGCG may potentially regulate gene expression through by IncRNAs

MICRORNAs

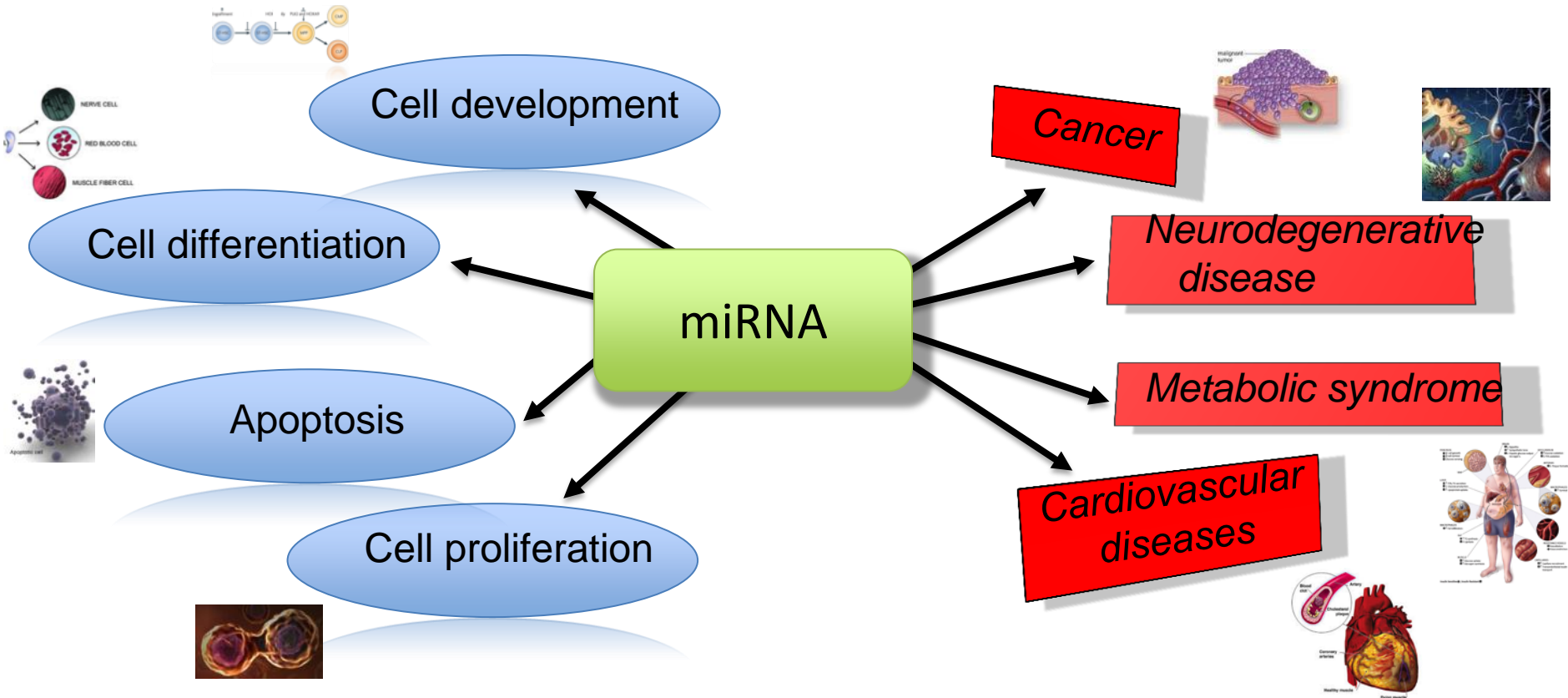
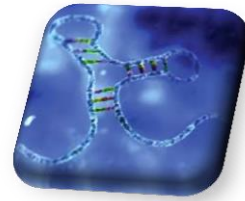
- MicroRNAs (miRNAs) are short non-coding RNA that act as negative regulators of gene expression and protein synthesis in eukaryotic organisms.



Each miRNA can regulate the translation of multiple genes and many genes can be regulated by multiple miRNAs.

miRNAs control the post-transcriptional regulation of over 50% of genes

IMPACT OF miRNA ON CELL PROCESSES AND DISEASE DEVELOPMENT



EFFECT OF POLYPHENOLS ON miRNAs EXPRESSION IN-VIVO - EXPERIMENTAL APPROACH -



miRNA extraction



- 11 groups

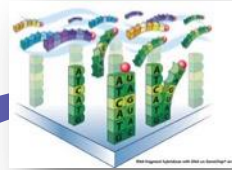
* C57

* ApoE-/-

* 9 different polyphenols (nutritional doses)

- N=4

- 2 weeks



Microarray hybridization



Image acquisition

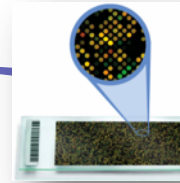
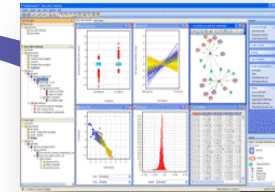
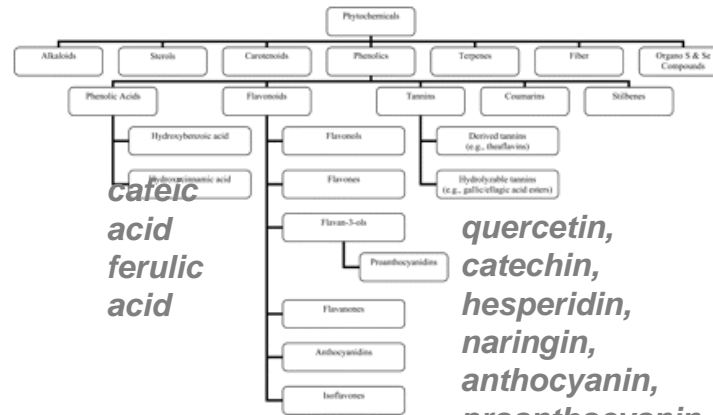


Image analysis



Statistical analysis

miRNA

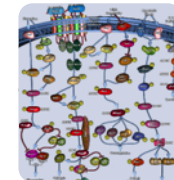


*caffeic acid
ferulic acid*

*quercetin,
catechin,
hesperidin,
naringin,
anthocyanin,
proanthocyanin,
curcumin*

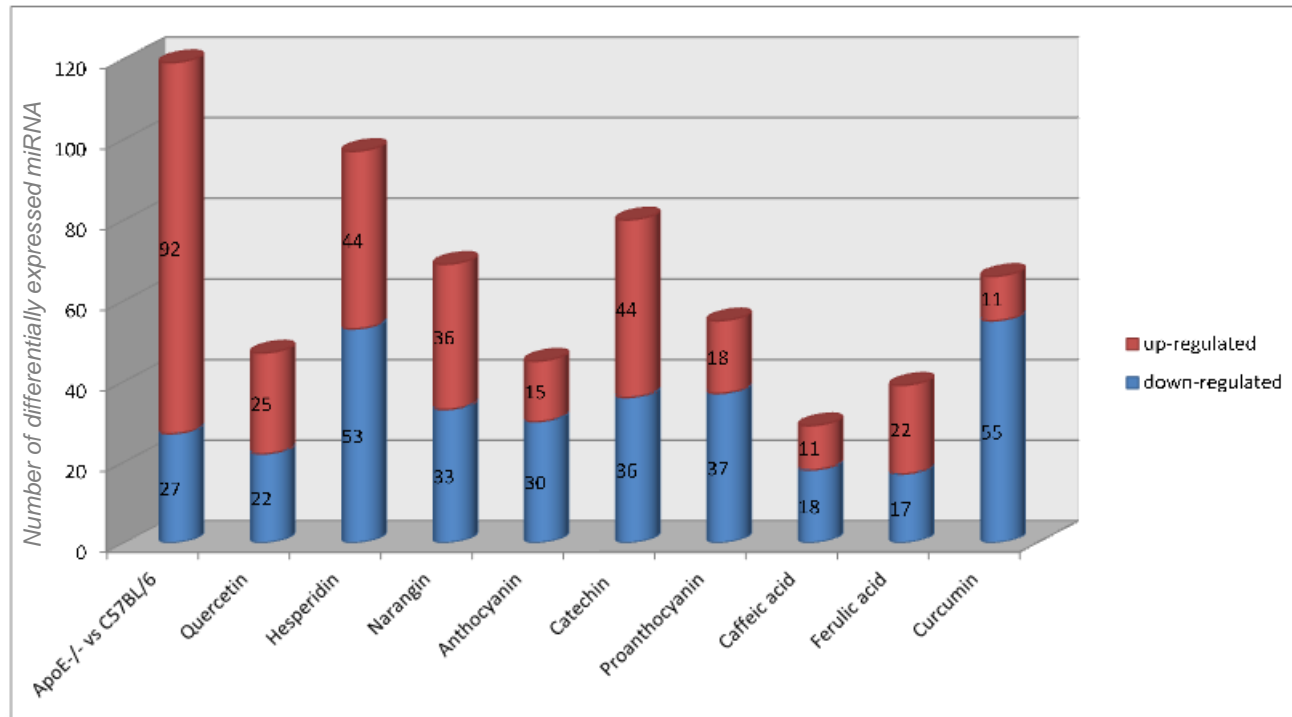
Prediction of targeted mRNA

Pathways analysis and clustering



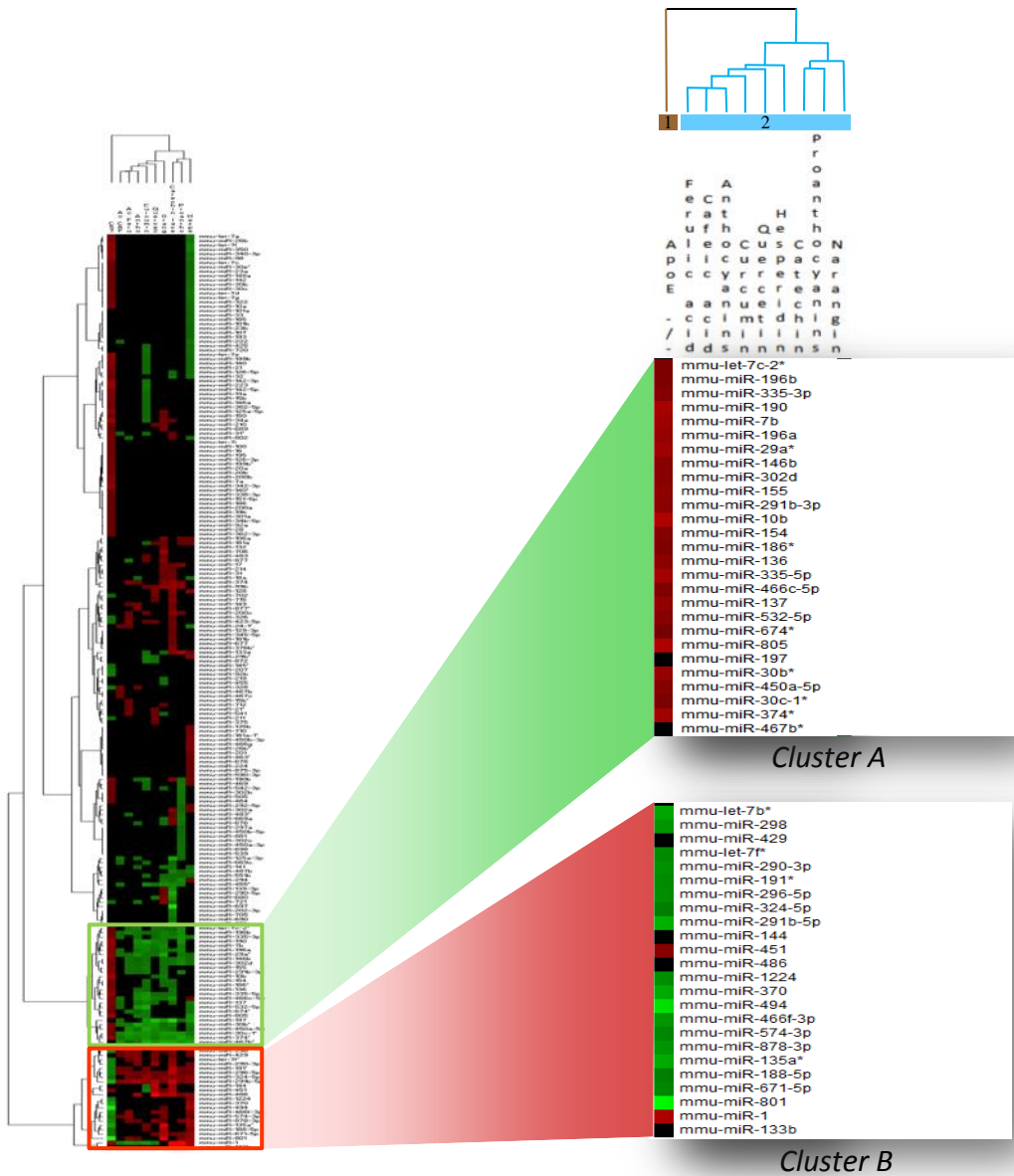
Database analysis

DIFFERENTIALLY EXPRESSED miRNA IN RESPONSE TO VARIOUS POLYPHENOL SUPPLEMENTATION



Polyphenols, at nutritional doses, regulate expression of miRNA

HIERARCHICAL CLUSTERING OF miRNA EXPRESSION PROFILES



FLAVANOLS AND miRNA EXPRESSION IN ENDOTHELIAL CELLS

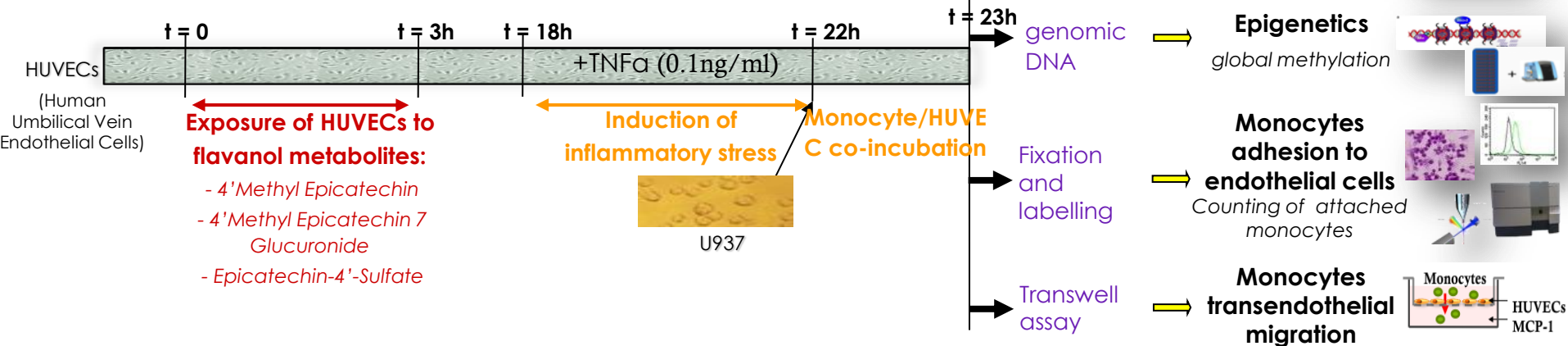
EXPERIMENTAL APPROACH

Flavanol plasma metabolites:

- 4'Methyl Epicatechin (4'MEC)
- 4'Methyl Epicatechin 7 Glucuronide (4'MEC7G)
- Epicatechin 4'Sulfate (EC4'S)

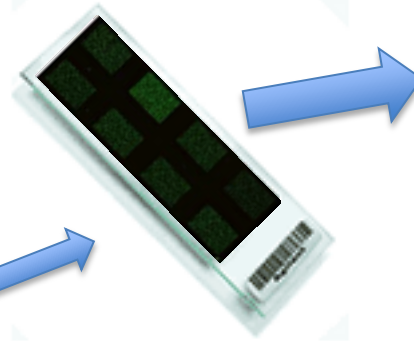
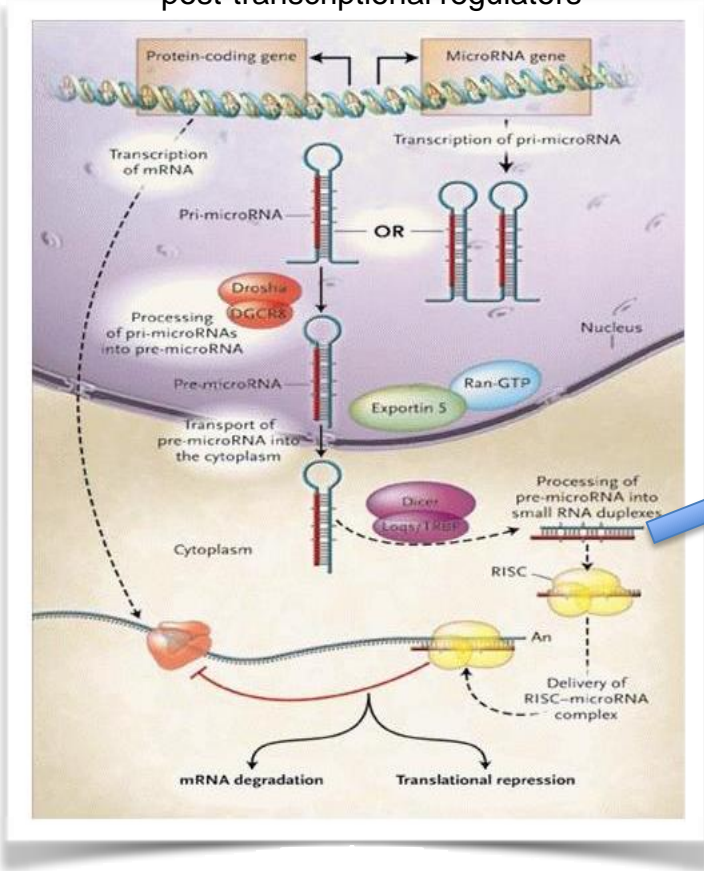
Flavanol metabolites

- Working concentrations : 0.1 to 2 μ M

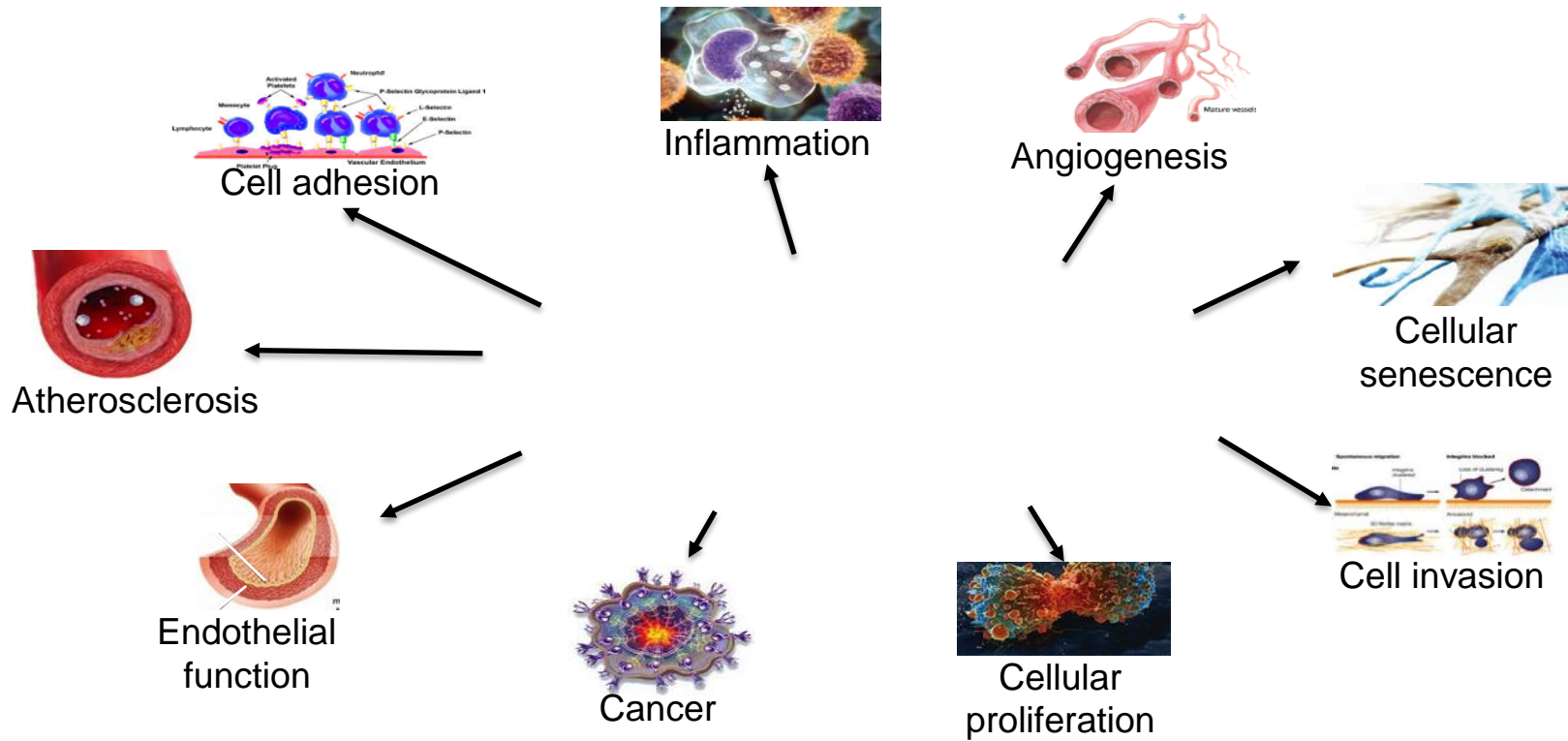


FLAVANOLS AND miRNA EXPRESSION IN ENDOTHELIAL CELLS

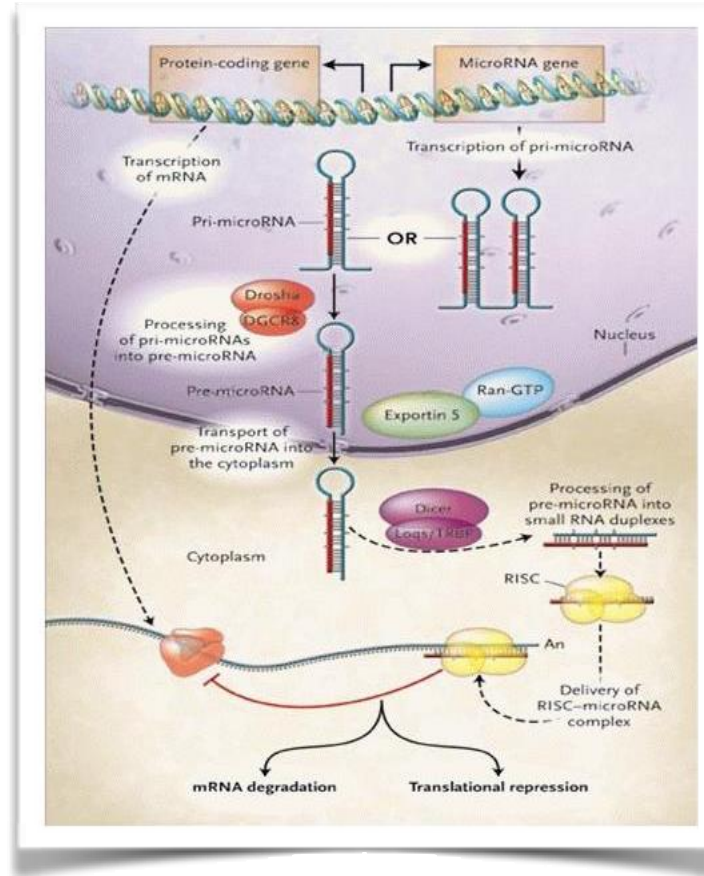
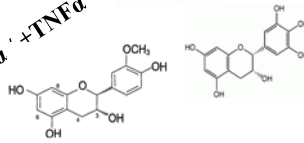
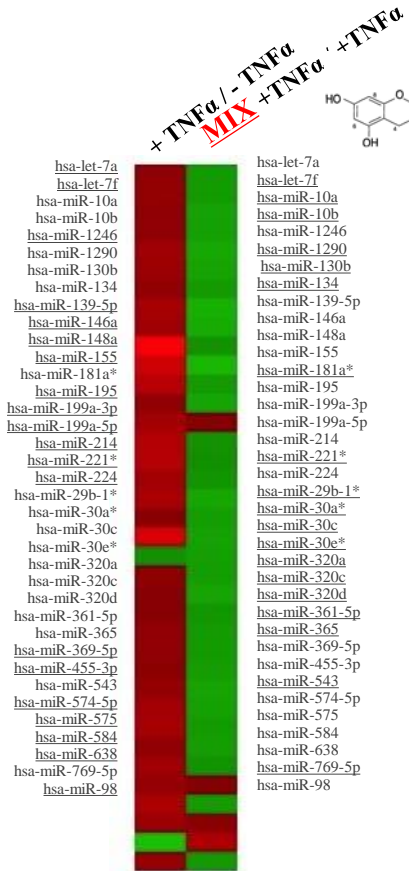
miRNA: small, non-coding RNA that are post-transcriptional regulators



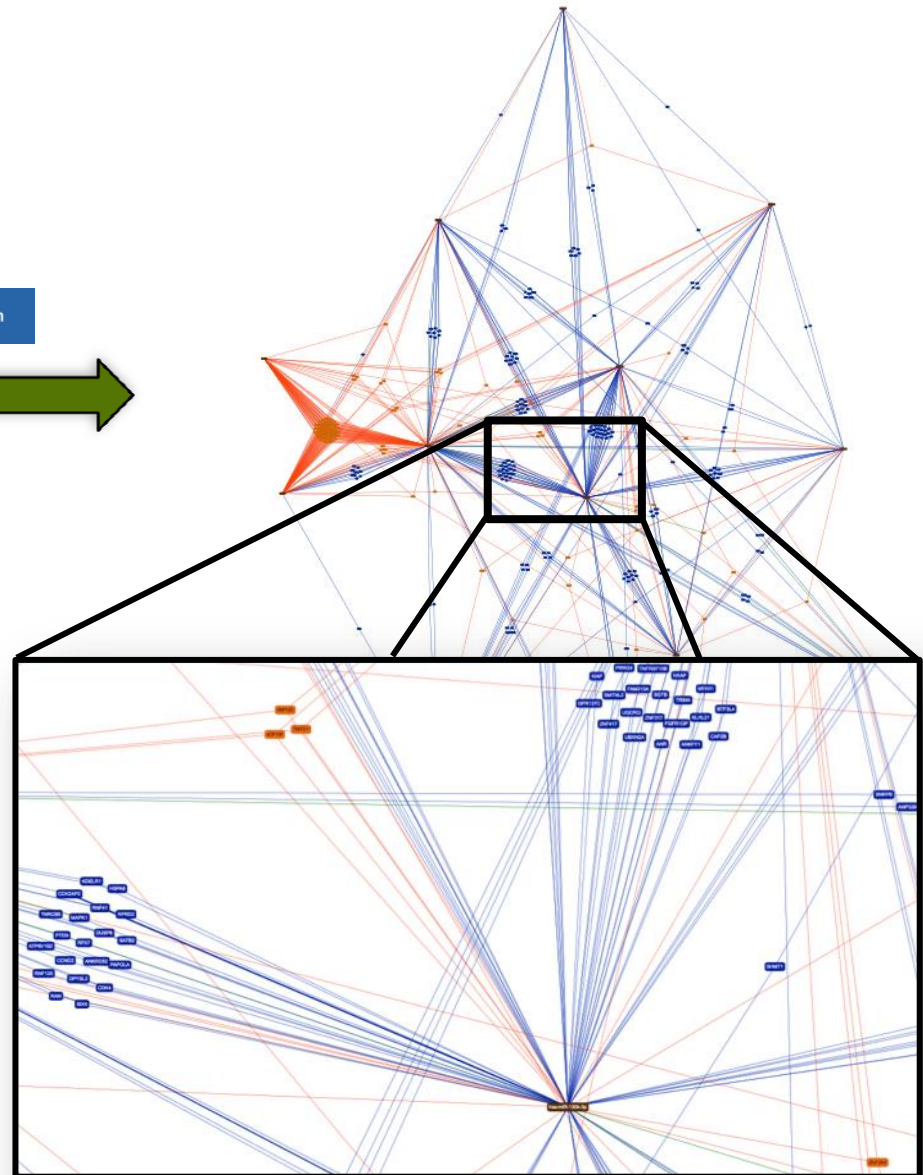
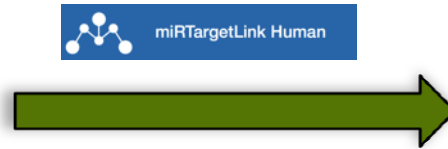
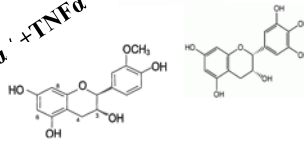
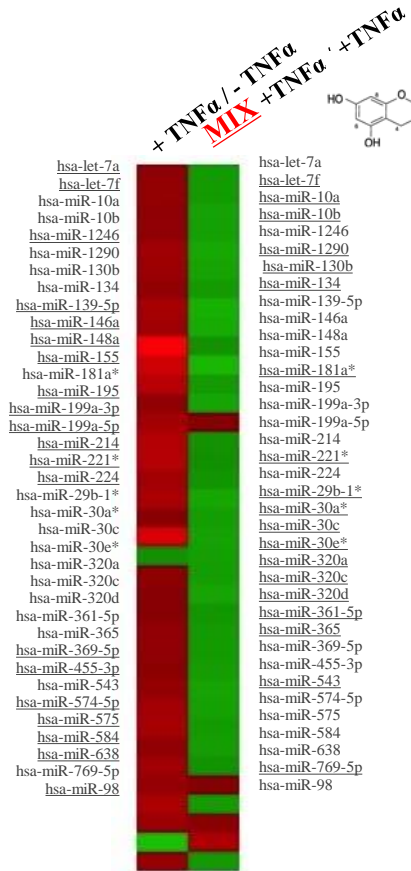
ROLE OF IDENTIFIED miRNAs



TARGET GENES OF DIFFERENTIALLY EXPRESSED miRNAs

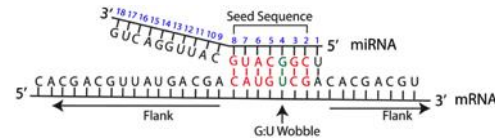
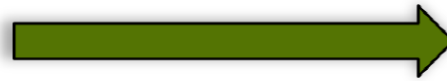
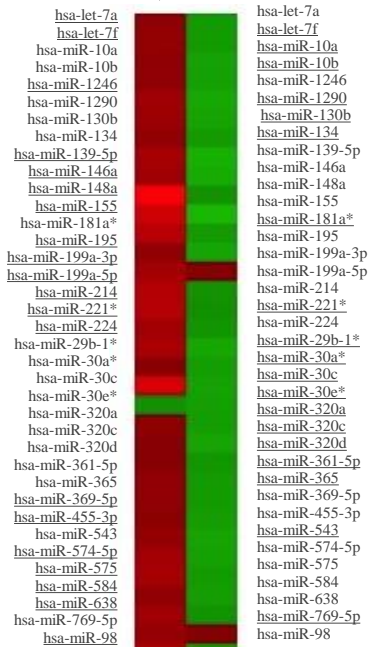
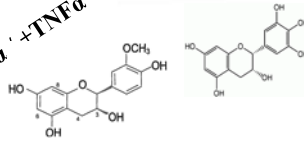


TARGET GENES OF DIFFERENTIALLY EXPRESSED miRNAs



TARGET GENES OF DIFFERENTIALLY EXPRESSED miRNAs

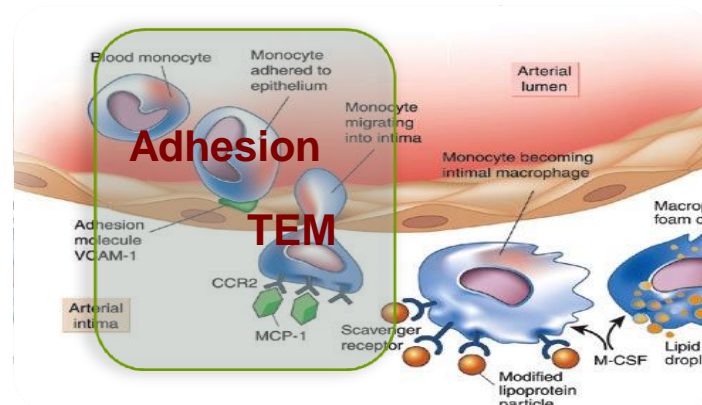
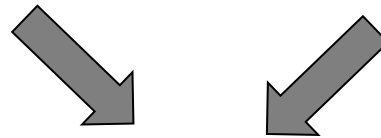
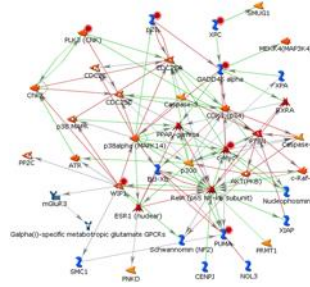
+ TNF α / - TNF α
MIX + TNF α - TNF α



ROLE OF TARGET GENES OF DIFFERENTIALLY EXPRESSED miRNAs

Cellular pathway analysis

Gene network analysis



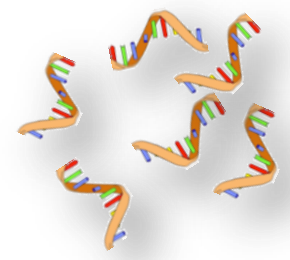
IMPACT OF miRNAs ON mRNA AND PROTEINS OF TARGET GENES

MIX +TNF α / +TNF α

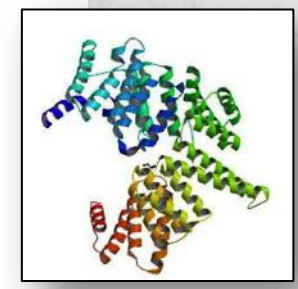
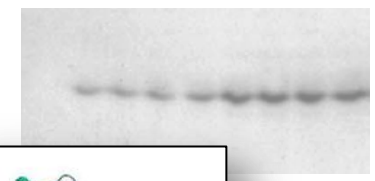
- hsa-let-7a
- hsa-let-7f
- hsa-miR-10a
- hsa-miR-10b
- hsa-miR-1246
- hsa-miR-1290
- hsa-miR-130b
- hsa-miR-134
- hsa-miR-139-5p
- hsa-miR-146a
- hsa-miR-148a
- hsa-miR-155
- hsa-miR-181a*
- hsa-miR-195
- hsa-miR-199a-3p
- hsa-miR-199a-5p
- hsa-miR-214
- hsa-miR-221*
- hsa-miR-224
- hsa-miR-29b-1*
- hsa-miR-30a*
- hsa-miR-30c
- hsa-miR-30e*
- hsa-miR-320a
- hsa-miR-320c
- hsa-miR-320d
- hsa-miR-361-5p
- hsa-miR-365
- hsa-miR-369-5p
- hsa-miR-455-3p
- hsa-miR-543
- hsa-miR-574-5p
- hsa-miR-575
- hsa-miR-584
- hsa-miR-638
- hsa-miR-769-5p
- hsa-miR-98



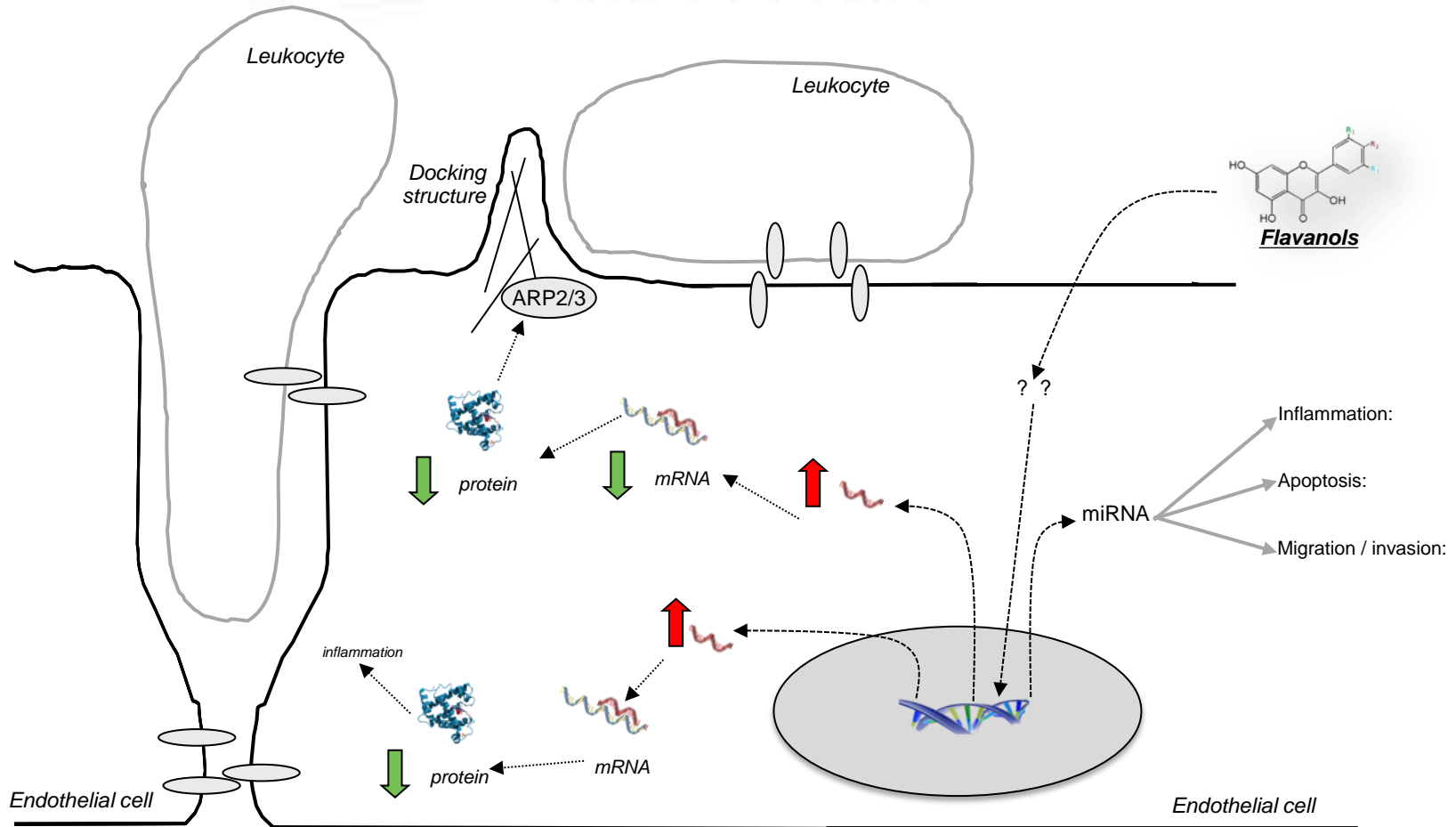
mRNA stability ?



Protein synthesis



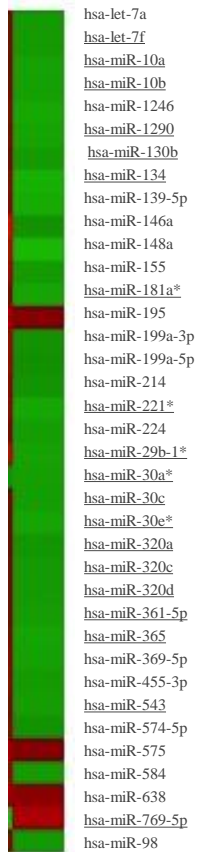
IMPACT OF miRNAs ON mRNA AND PROTEINS OF TARGET GENES



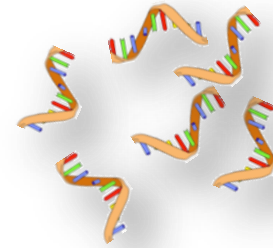
miARN decrease protein expression of gene by inhibiting translation and that of gene by decreasing mRNA level

IMPACT OF miRNAs ON mRNA OF TARGET GENES - SYSTEMS BIOLOGY -

MIX +TNF α / +TNF α

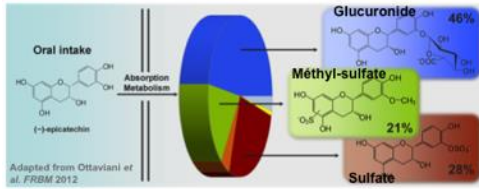


mRNA stability ?



IMPACT OF miRNAs ON mRNA OF TARGET GENES

- SYSTEMS BIOLOGY -

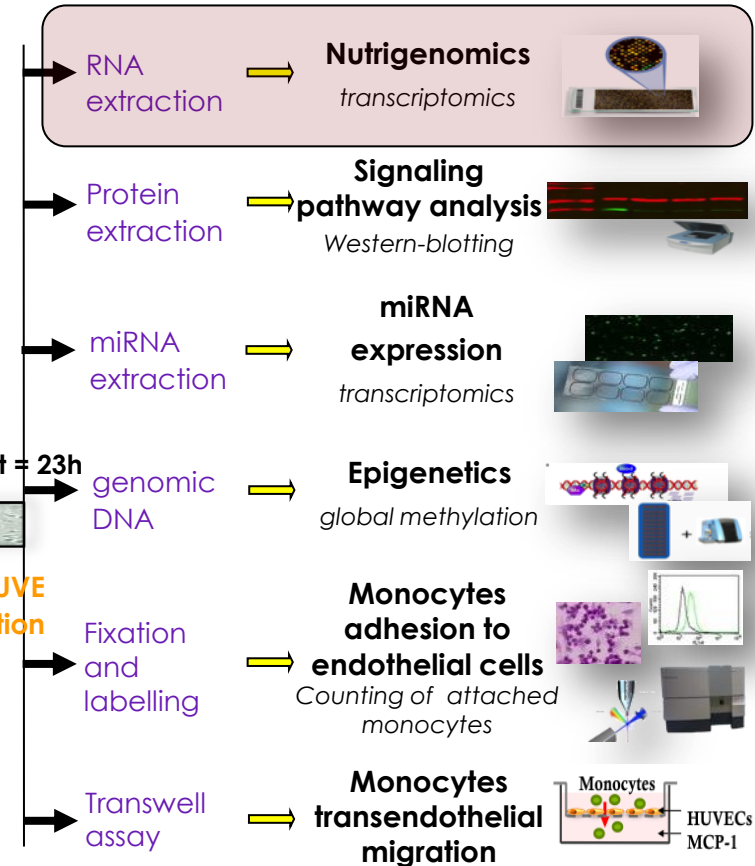
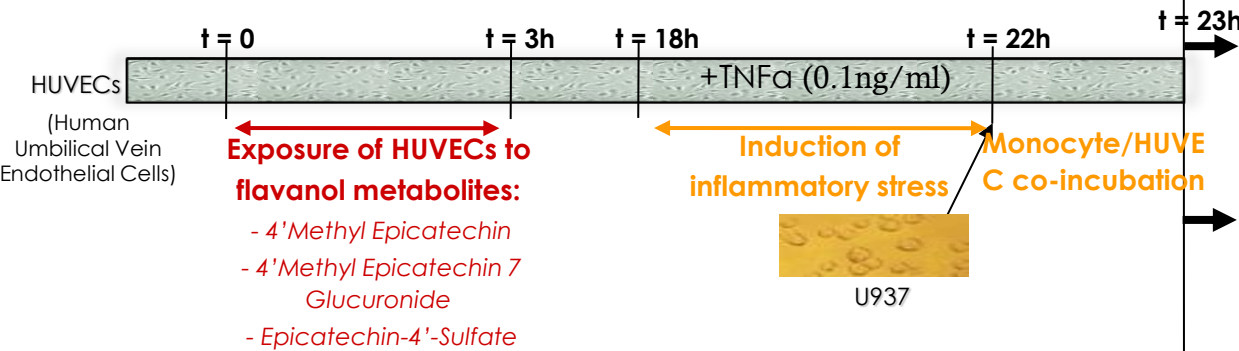


Flavanol plasma metabolites:

- 4'Methyl Epicatechin (4'MEC)
- 4'Methyl Epicatechin 7 Glucuronide (4'MEC7G)
- Epicatechin 4'Sulfate (EC4'S)

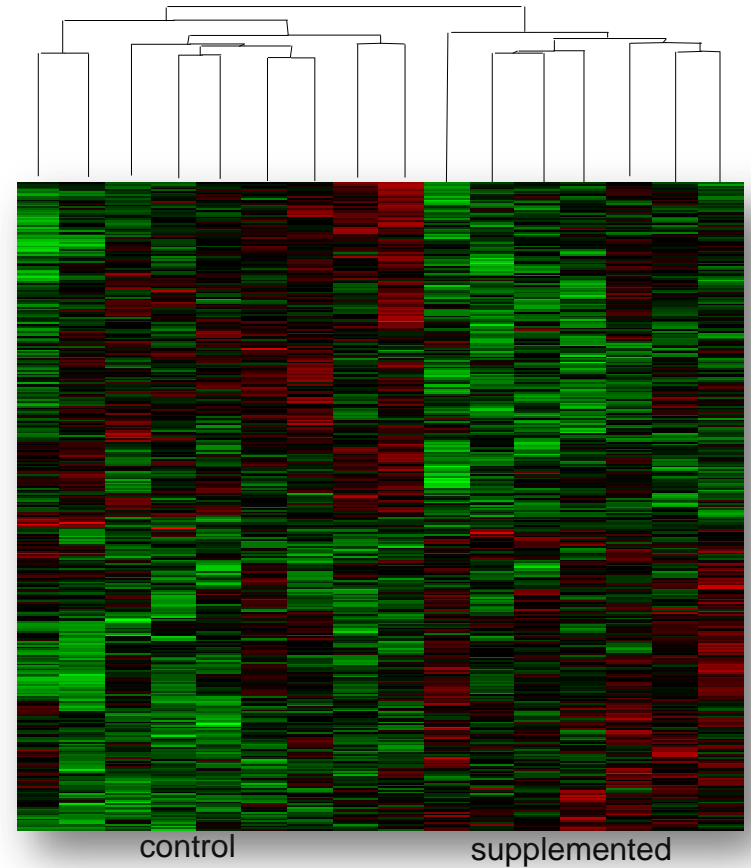
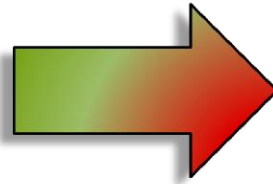
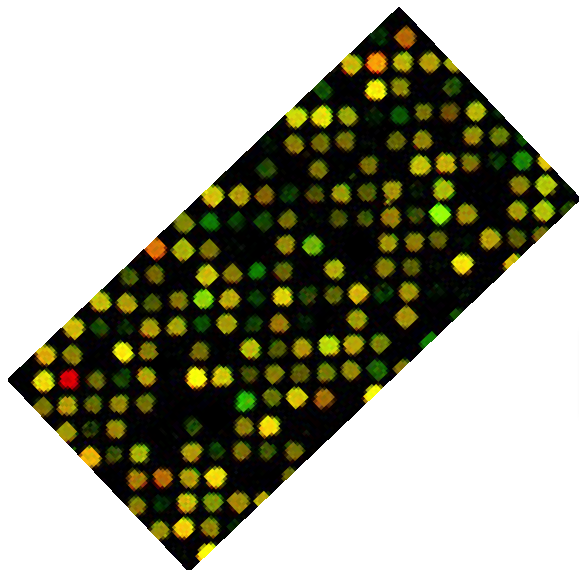
Flavanol metabolites

- Working concentrations : 0.1 to 2 μM



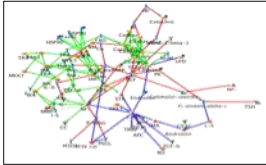
IMPACT OF miRNAs ON mRNA OF TARGET GENES

- SYSTEMS BIOLOGY -

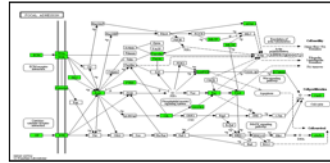


IMPACT OF miRNAs ON mRNA OF TARGET GENES - SYSTEMS BIOLOGY -

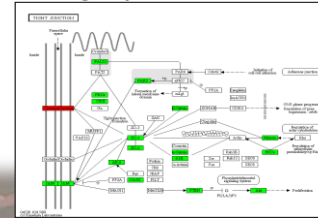
Leukocyte extravasation



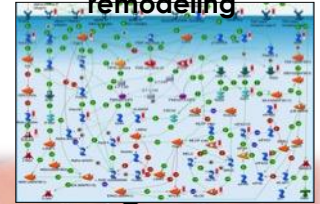
Focal adhesion



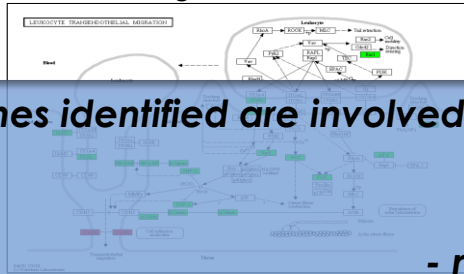
Tight junction



Cytoskeleton remodeling



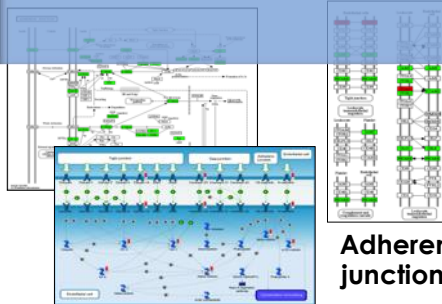
Leukocyte transendothelial migration



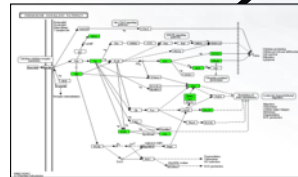
Genes identified are involved in the cell adhesion, cytoskeleton organization, focal adhesion possibly affecting:

- monocyte adhesion to endothelial cells
- transendothelial migration

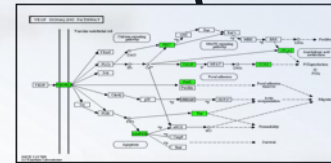
Adherent junctions



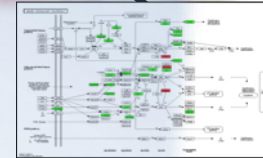
Chemokine pathway



VEGF pathway

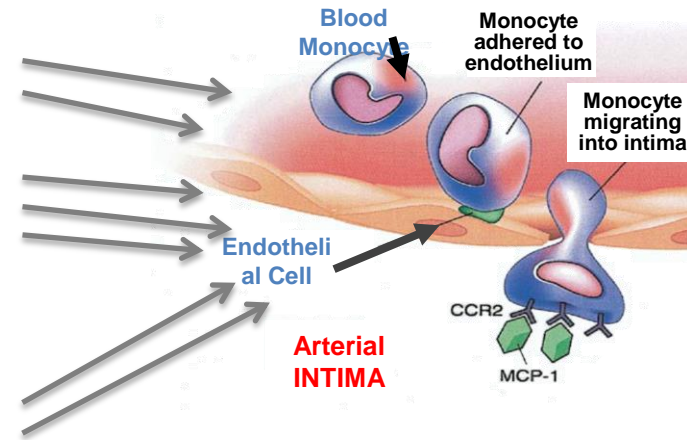
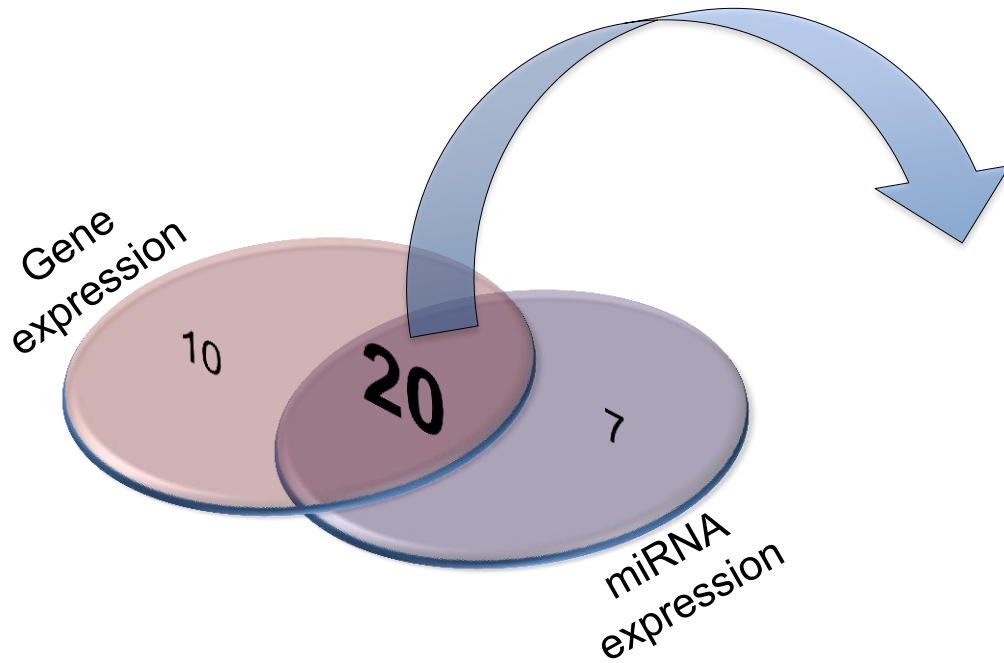


MAPK pathway

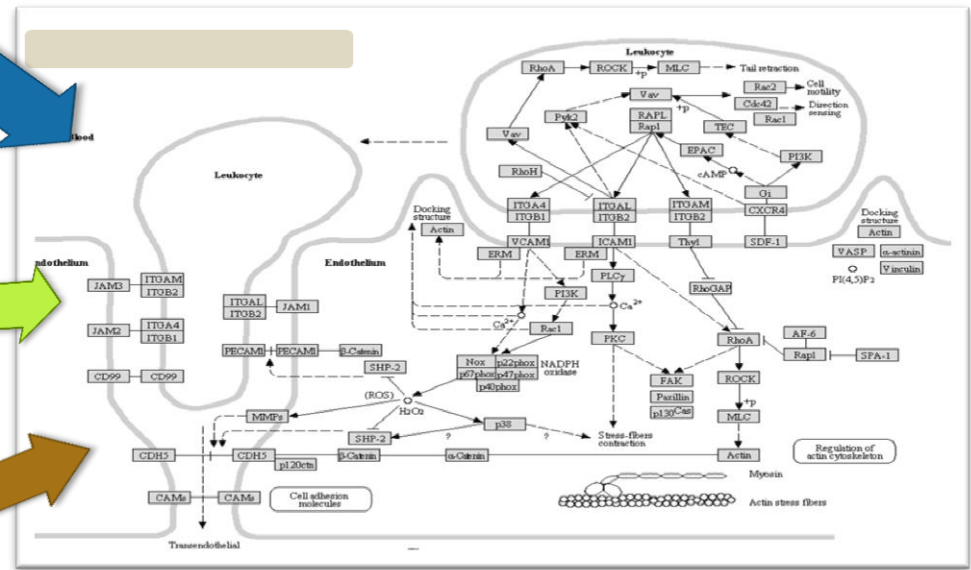
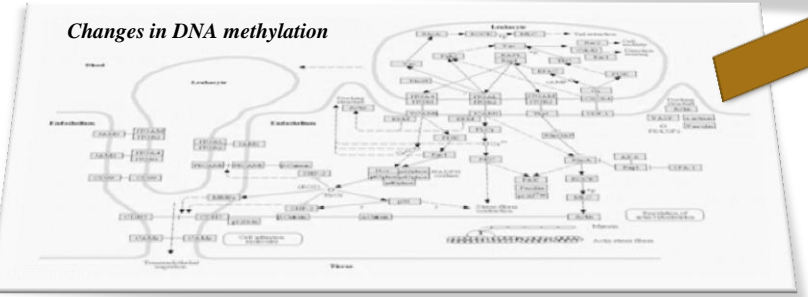
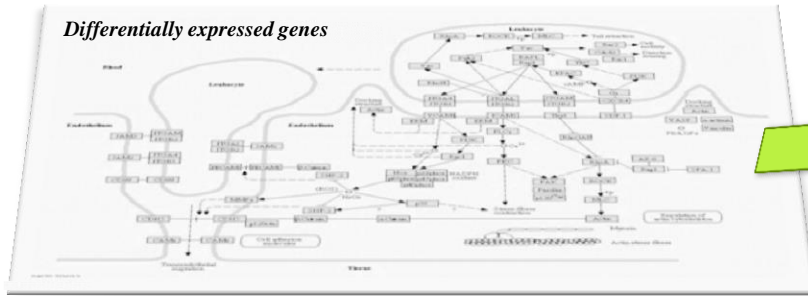
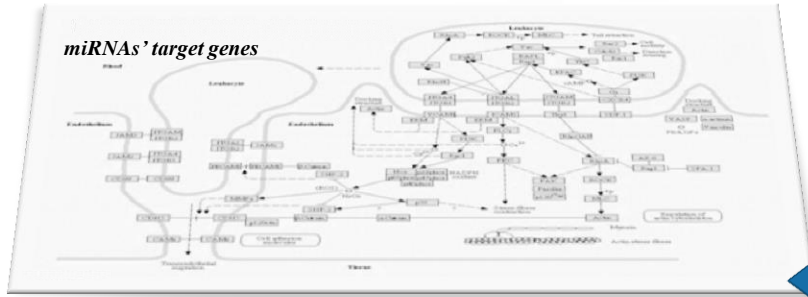


IMPACT OF miRNAs ON mRNA OF TARGET GENES

- SYSTEMS BIOLOGY -



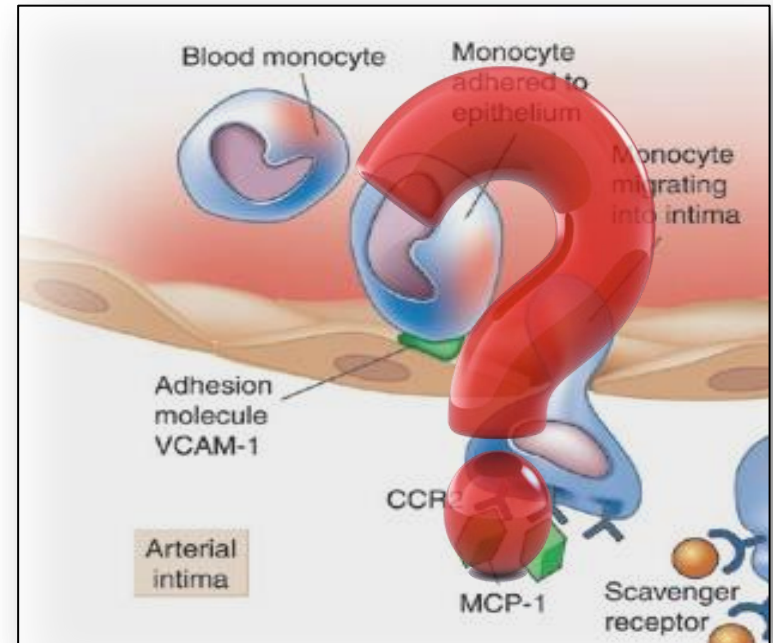
IMPACT OF miRNAs ON mRNA OF TARGET GENES - SYSTEMS BIOLOGY -



- Changes in gene expression
- ◆ Changes in DNA methylation
- Target genes of differentially expressed miRNAs

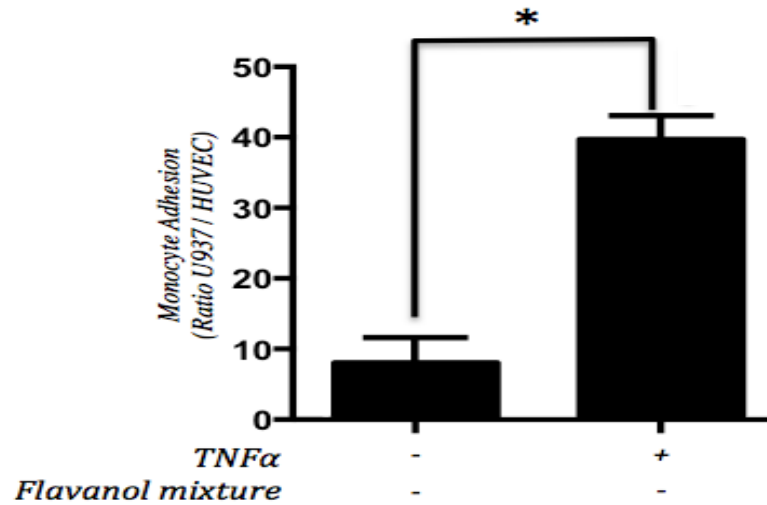
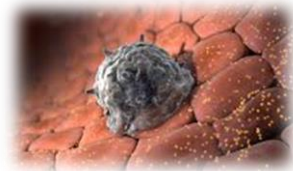
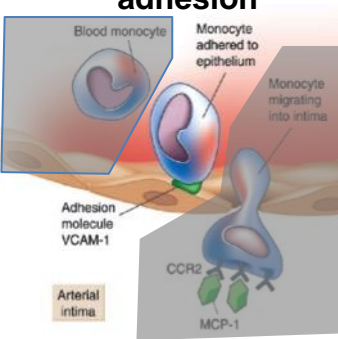
IMPACT OF miRNAs ON mRNA OF TARGET GENES

- SYSTEMS BIOLOGY -



FLAVANOLS AND MONOCYTE TO ENDOTHELIAL CELL ADHESION

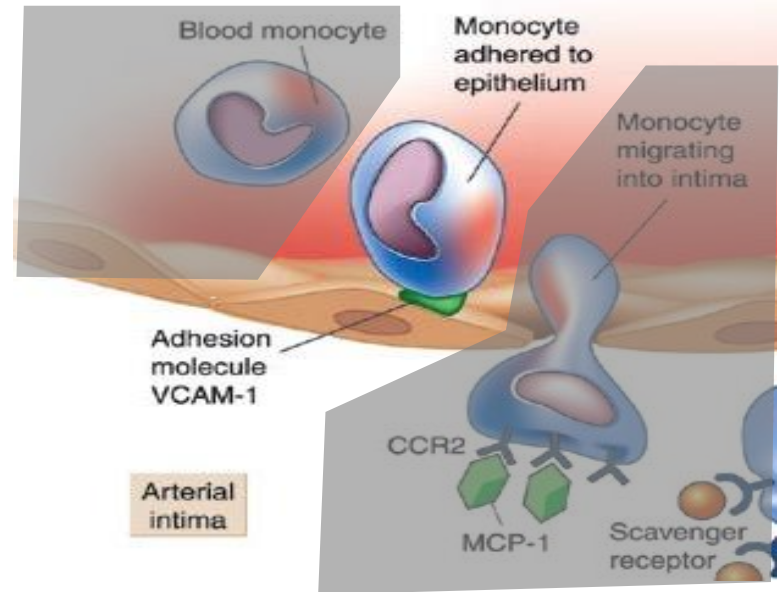
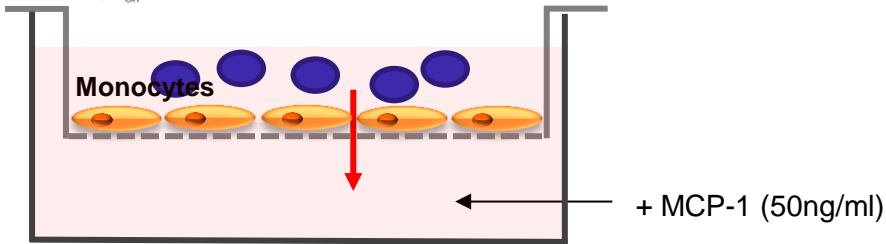
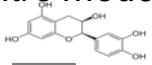
Monocyte adhesion



FLAVANOLS AND TRANSENDOTHELIAL MIGRATION



In-vitro model of transendothelial migration using transwells





THE

TAKE-HOME MESSAGE

- Non-coding RNA are new important cell regulator



- Play important role in regulation of aging and brain function



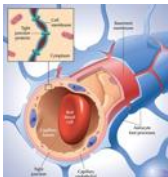
- Can be modulated by dietary nutrients, ex. plant food bioactives



modulate endothelial cell function and BBB → *improve brain function*



- Further studies are needed to identify their role and impact of nutrition in prevention of age-related diseases





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Thank you for attention

