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

![Chart showing consumption of vitamin C, polyphenols, and other nutrients](chart.png)

- Vitamin C: 90 mg/day
- Polyphenols: 1200 mg/day
- Carotenoids: 5 mg/day
- Vitamin E: 12 mg/day

![Phytochemicals](phytochemicals.png)

(Pérez-Jiménez J et al 2011)
Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels

Claire M. Williams,1, Manal Abd El Mohsen,1, David Vauzour,2, Catarina Rendeiro,2, Laurie T. Butler,1, Judi A. Ellis,1, Matthew Whiteman,3, Jeremy P.E. Spencer


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

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

Flavanone-rich orange juice is beneficial for cognitive function in healthy older adults with no significant effects on mood or blood pressure
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
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


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

Adam M Brickman1,2,8, Usman A Khan1,3,8, Frank A Provenzano1,2,8, Lok-Kin Yeung1,2, Wendy Suzuki1, Hagen Schroeter4, Melanie Wall5,6, Richard P Sloan5,6, and Scott A Small1,2,3,7

Diary Polyphenols and Neurological Functions

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)
- BBB dysfunction, a key contributor 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.

(Palmer et al., 2011; Montagne et al., 2015)
Dysfunction of Blood-Brain Barrier

- Tethering/Rolling
- Activation
- Adhesion
- Transmigration

**Basement Membrane**

**Perivascular Space**

**Endothelium:** Selectins, Carbohydrate ligands, Chemokines

**Leukocytes:** G-protein coupled receptors

**Adhesion molecules:** Integrins

**Chemokines:** G-protein coupled receptors

**MMPs**

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

Dilek Coban¹,², Dragan Milenkovic¹,², Audrey Chanet¹,², Jamila Khallou-Laschet³, Linde Sabbe⁴, Ajay Palagani⁵, Wim Vanden Berghe⁶, Andrzej Mazur¹,² and Christine Morand¹,²

POLYPHENOLS AND ENDOTHELIAL CELL FUNCTION - NUTRIGENOMIC EFFECTS -

Krga, Milenkovic et al., 2012, Food and Function
CELL FUNCTION REGULATION

Adapted from: Barallobre-Barreiro et al., Rev Esp Cardiol. 2013

- Epigenetics
- Allelic variants
- Pseudo genes

- micro RNA
- long-non coding RNA
- mRNA stability
- RNA editing

- Post-translational modifications
- Degradation
- Phosphorylation

Complexity
Adapted from Geschwind & Konopka, Nature 2009
DNA TRANSCRIPTION

« junk DNA » - 98% of DNA

RNA – 2% of DNA
- 80% of DNA transcripted

non-coding RNAs
80% of DNA transcripted

Uchida et al., Circ Res. 2015

DNA TRANSCRIPTION

Small non-coding RNAs
"microRNAs"
- <200nt
- transcribed by RNA Polymerase II
- endogenously processed by endonucleases
- well conserved

> 2,000 microRNAs

Long non-coding RNAs
"IncRNAs"
- >200nt
- transcribed by RNA Polymerase II
- mostly 5’-cap, polyadenylated (in part), spliced
- poorly conserved

> 30,000 IncRNAs
LncRNAs regulate gene expression through diverse mechanisms.

- Regulate translation
- Recruit/regulate TF/Chromatin complexes
- MicroRNA sponge
- Transcription complex
- DNA
- mRNA
- lncRNA

- Sequestration transport
- Organelle formation
- Co-activation
- Regulate mRNA stability, splicing, degradation

Paralkar et al., Blood. 2013
Non-coding RNA in neural function, disease, and aging

**Kirk Szafirski**†, Karan J. Abraham †† and Karim Mekkali†‡‡

<table>
<thead>
<tr>
<th>Transcript/protein</th>
<th>Diseases implicated</th>
<th>Proposed mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUS/TLS</td>
<td>ALS, FTLD</td>
<td>Indirect Drosha interactions regulates various miRNAs</td>
</tr>
<tr>
<td>ATX2</td>
<td>ALS, SCA2, PSP</td>
<td>Interactions with RISC components regulates long-term habituation</td>
</tr>
<tr>
<td>DGCPR8</td>
<td>Prader–Willi syndrome</td>
<td>Microprocessor, can process miRNAs and snoRNAs</td>
</tr>
<tr>
<td>nT210</td>
<td>N/A. Neural cell death observed with mutation</td>
<td>tRNA isodecoder necessary for the production of GTPBP2</td>
</tr>
<tr>
<td>CLP1</td>
<td>PCH 10</td>
<td>Necessary for maturation of tRNAs</td>
</tr>
<tr>
<td>BACE1AS</td>
<td>AD</td>
<td>Stabilization of BACE1 which ultimately results in amyloid beta accumulation</td>
</tr>
<tr>
<td>ATXN8AS</td>
<td>SCA8</td>
<td>May reduce the amount of KHL1, may produce toxic dipeptidyl or sequester RNA binding proteins</td>
</tr>
<tr>
<td>UCHL1-AS</td>
<td>AD, PD</td>
<td>Regulates translation of neuroprotective protein UCHL1</td>
</tr>
<tr>
<td>NEAT1</td>
<td>ALS, HD, aging</td>
<td>Produces paraspeckle bodies which storage RNAs, potentially for release during stress</td>
</tr>
<tr>
<td>C9orf72</td>
<td>ALS</td>
<td>Abortive transcripts sequester RNA binding proteins and produce toxic dipeptides</td>
</tr>
<tr>
<td>SETX</td>
<td>AOA2, ALS</td>
<td>RNA–DNA helicase prevents R-loop formation</td>
</tr>
<tr>
<td>Pbp1</td>
<td>ALS, SCA2, PSP</td>
<td>Interacts with RNAs to prevent RNA–DNA hybrids, maintains genomic stability, and cellular lifespan</td>
</tr>
</tbody>
</table>

**Salta et al., Lancet Neurol 2012**

The link between long noncoding RNAs and depression

Xiao Huang ††, Yan-li Luo †, Yue-shi Mao †‡, Jian-lin Ji †‡,†

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

<table>
<thead>
<tr>
<th>LncRNA</th>
<th>Type/localization</th>
<th>Putative function in SMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATG9B</td>
<td>NAT-lncRNA/nuclear+cytoplasmatic</td>
<td>↓ NOS3 following hypoxia</td>
</tr>
<tr>
<td>TIE1-AS</td>
<td>NAT-lncRNA/cytoplasmic</td>
<td>Regulation of EC contact junctions</td>
</tr>
<tr>
<td>NRON</td>
<td>Intr-lncRNA/cytoplasmic</td>
<td>↓ EC proliferation/↓ migration</td>
</tr>
<tr>
<td>SENCr</td>
<td>antisense oh-lncRNA/cytoplasmic</td>
<td>?</td>
</tr>
<tr>
<td>MALAT1</td>
<td>LincRNA/nuclear</td>
<td>Proper angiogenesis</td>
</tr>
<tr>
<td>TGFB2-ot1</td>
<td>sense cl-lncRNA/?</td>
<td>ceRNA regulating autophagy</td>
</tr>
<tr>
<td>MIAT</td>
<td>LincRNA/nuclear</td>
<td>ceRNA regulating EC function</td>
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<tr>
<td>ASncmtRNA-2</td>
<td>Stem loop/nucleus</td>
<td>Replicative senescence</td>
</tr>
<tr>
<td>AGAP2-AS1</td>
<td>NAT-lncRNA/cytoplasm</td>
<td>Proper angiogenesis</td>
</tr>
<tr>
<td>HIF1A-AS1</td>
<td>NAT-lncRNA/?</td>
<td>↑ EC apoptosis/↑ EC proliferation</td>
</tr>
<tr>
<td>DLL4-AS</td>
<td>NAT-lncRNA/cytoplasm</td>
<td>↑ EC proliferation/↑ EC migration</td>
</tr>
</tbody>
</table>

Miano et al., Cell. Mol. Life Sci, 2015
### Epigallocatechin gallate

![Epigallocatechin gallate molecule]

### Dietary Polyphenols and LncRNA

<table>
<thead>
<tr>
<th>IncRNAs</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>N342928</td>
<td>↓</td>
</tr>
<tr>
<td>NONHSAT015959</td>
<td>↓</td>
</tr>
<tr>
<td>N333444</td>
<td>↓</td>
</tr>
<tr>
<td>AT088005</td>
<td>↓</td>
</tr>
<tr>
<td>TM4SF4-2</td>
<td>↓</td>
</tr>
<tr>
<td>AT009251</td>
<td>↓</td>
</tr>
<tr>
<td>AT008445</td>
<td>↓</td>
</tr>
<tr>
<td>AT068602</td>
<td>↓</td>
</tr>
<tr>
<td>AT020294</td>
<td>↓</td>
</tr>
<tr>
<td>AT122449</td>
<td>↓</td>
</tr>
<tr>
<td>AT068591</td>
<td>↓</td>
</tr>
<tr>
<td>PHF17-1</td>
<td>↓</td>
</tr>
<tr>
<td>AT098264</td>
<td>↓</td>
</tr>
<tr>
<td>n382512</td>
<td>↓</td>
</tr>
<tr>
<td>n409611</td>
<td>↓</td>
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<tr>
<td>DCLK3-3</td>
<td>↓</td>
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<tr>
<td>AT101123</td>
<td>↓</td>
</tr>
<tr>
<td>ISLR2-3</td>
<td>↓</td>
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<tr>
<td>AT102202</td>
<td>↑</td>
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<tr>
<td>AT115872</td>
<td>↑</td>
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<td>AT017383</td>
<td>↑</td>
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<td>AT004532</td>
<td>↑</td>
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<tr>
<td>AT027943</td>
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<td>ITGB2-3</td>
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<tr>
<td>AT078273</td>
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<td>AT016514</td>
<td>↑</td>
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<td>AT097214</td>
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</tr>
<tr>
<td>FAM75A3-1</td>
<td>↑</td>
</tr>
<tr>
<td>FAM75A3-2</td>
<td>↑</td>
</tr>
</tbody>
</table>

EGCG may potentially regulate gene expression through IncRNAs:

- Cholesterol synthesis
- Lipid droplets formation
- Cell replication

Liu et al., CMJ 2015
- 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

miRNA

Cell development
Cell differentiation
Apoptosis
Cell proliferation

Cancer
Neurodegenerative disease
Metabolic syndrome
Cardiovascular diseases

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

- 11 groups
  - C57
  - ApoE/-
  - 9 different polyphenols (nutritional doses)
- N=4
- 2 weeks

miRNA extraction

Microarray hybridization

Image acquisition

Image analysis

Statistical analysis

miRNA analysis

Database analysis

Prediction of targeted mRNA
Pathways analysis and clustering

miRNA

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miRNA
Differentially expressed miRNA in response to various polyphenol supplementation

Polyphenols, at nutritional doses, regulate expression of miRNA

Milenkovic et al., PloSONE 2012
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

Exposure of HUVECs to flavanol metabolites:
- 4’Methyl Epicatechin
- 4’Methyl Epicatechin 7 Glucuronide
- Epicatechin-4’-Sulfate

Induction of inflammatory stress

Monocyte/HUVEC co-incubation

+TNFα (0.1ng/ml)

Fixation and labelling

RNA extraction

Protein extraction

miRNA extraction

Transwell assay

Monocytes adhesion to endothelial cells

Counting of attached monocytes

Monocytes transendothelial migration
**FLAVANOLS AND miRNA EXPRESSION IN ENDOTHELIAL CELLS**

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

![Diagram of miRNA processing](image-url)
ROLE OF IDENTIFIED miRNAs

- Cell adhesion
- Atherosclerosis
- Endothelial function
- Inflammation
- Angiogenesis
- Cellular senescence
- Cancer
- Cellular proliferation
- Cell invasion
TARGET GENES OF DIFFERENTIALLY EXPRESSED miRNAs

hsa-let-7a
hsa-let-7f
hsa-miR-10a
hsa-miR-10b
hsa-miR-1246
hsa-miR-1290
hsa-miR-133b
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-222
hsa-miR-29b-1*
hsa-miR-30a*
hsa-miR-30c
hsa-miR-30e*
hsa-miR-320a
hsa-miR-320b
hsa-miR-320d
hsa-miR-361-5p
hsa-miR-365
hsa-miR-369-5p
hsa-miR-455-5p
hsa-miR-543
hsa-miR-574-5p
hsa-miR-575
hsa-miR-584
hsa-miR-638
hsa-miR-765-5p
hsa-miR-98
TARGET GENES OF DIFFERENTIALLY EXPRESSED miRNAs
TARGET GENES OF DIFFERENTIALLY EXPRESSED miRNAs
ROLE OF TARGET GENES OF DIFFERENTIALLY EXPRESSED miRNAs

Gene network analysis

Cellular pathway analysis
IMPACT OF miRNAs ON mRNA AND PROTEINS OF TARGET GENES

mRNA stability?

Protein synthesis
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 -
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- 4’Methyl Epicatechin 7 Glucuronide
- Epicatechin-4’-Sulfate

Induction of inflammatory stress

Signaling pathway analysis
Western-blotting

Protein extraction

miRNA expression
transcriptomics

miRNA extraction
transcriptomics

Epigenetics
global methylation

Fixation and labelling
Monocytes adhesion to endothelial cells
Counting of attached monocytes

Transwell assay
Monocytes transendothelial migration

RNA extraction
Nutrigenomics transcriptomics

RNA extraction

HUVECs
(Human Umbilical Vein Endothelial Cells)

U937

+TNFα (0.1ng/ml)

Monocyte/HUVEC co-incubation

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

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

- monocyte adhesion to endothelial cells
- transendothelial migration

Claude et al., MNFR (2014)
IMPACT OF miRNAs ON mRNA OF TARGET GENES
- SYSTEMS BIOLOGY -
IMPACT OF miRNAs ON mRNA OF TARGET GENES
- SYSTEMS BIOLOGY -
IMPACT OF miRNAs ON mRNA OF TARGET GENES - SYSTEMS BIOLOGY -
FLAVANOLS AND MONOCYTE TO ENDOTHELIAL CELL ADHESION

Monocyte adhesion

![Diagram showing monocyte adhesion](image)

**Graph:**
- **X-axis:** TNFα
- **Y-axis:** Monocyte Adhesion (Ratio U937 / HUVEC)
- Comparison between - and + for Flavanol mixture

**Notes:**
- The graph illustrates the effect of TNFα and Flavanol mixture on monocyte adhesion to endothelial cells.
In-vitro model of transendothelial migration using transwells

Monocytes + MCP-1 (50ng/ml)

FLAVANOLS AND TRANSENDOTHELIAL MIGRATION
- 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
www.ilsi.eu

Thank you for attention