



Tel Aviv, Museum of Natural History
December 2nd 2019

Healthy Life and Longevity

Circulating cell-free DNA and microRNAs

MIRIAM CAPRI

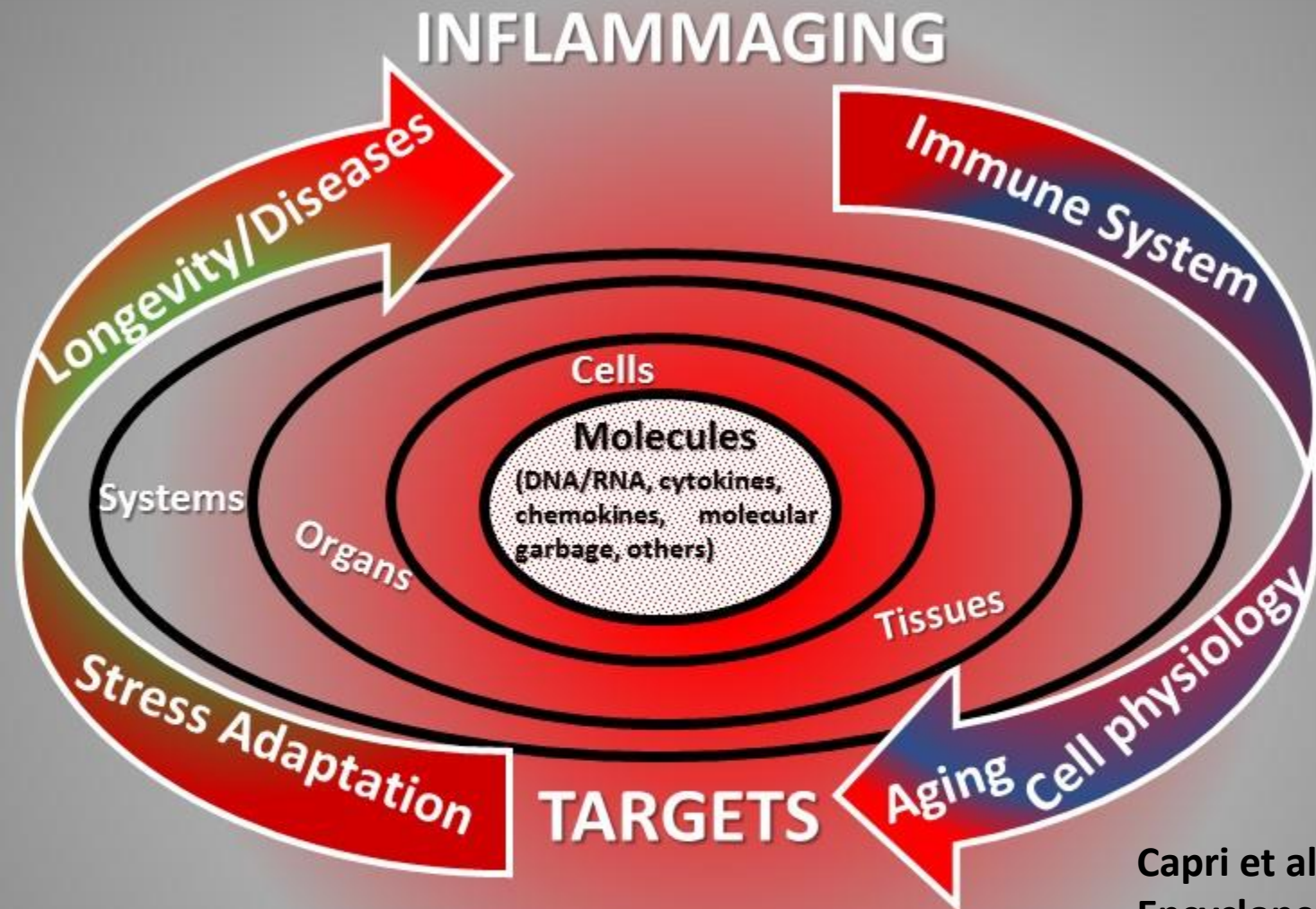
DIMES – Department of Experimental, Diagnostic and Specialty Medicine



ALMA MATER STUDIORUM
Università di Bologna, Italy

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA

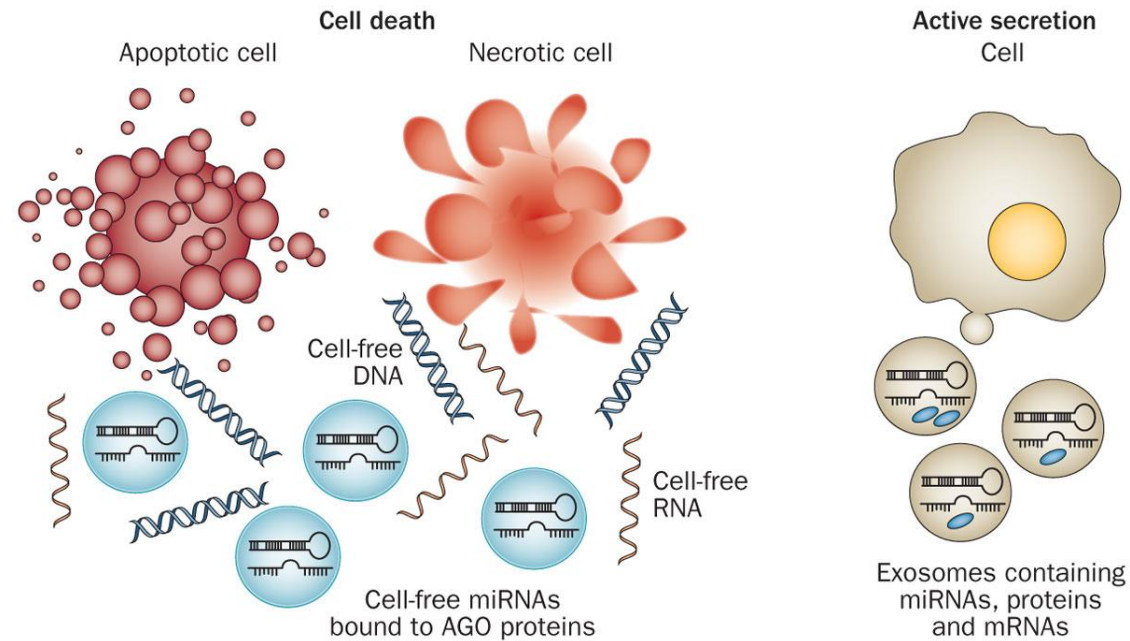
IL PRESENTE MATERIALE È RISERVATO AL PERSONALE DELL'UNIVERSITÀ DI BOLOGNA E NON PUÒ ESSERE UTILIZZATO AI TERMINI DI LEGGE DA ALTRE PERSONE O PER FINI NON ISTITUZIONALI



PROPAGATION PHENOMENA

Capri et al., 2019 –
Encyclopedia of
Biomedical
Gerontology- Edited by
Suresh Rattan (Elsevier)

Release of Cell-free DNA and miRNAs from cells into the blood circulation



Schwarzenbach, H. *et al.* (2014) Clinical relevance of circulating cell-free microRNAs in cancer
Nat. Rev. Clin. Oncol. doi:10.1038/nrclinonc.2014.5

Received: 31 May 2018 | Revised: 12 October 2018

DOI: 10.1111/ace1.12890

ORIGINAL PAPER

WILEY

Aging Cell



Cell-free DNA as a biomarker of aging

Yee Voan Teo¹ | Miriam Capri^{2,3} | Cristina Morsiani³ | Grazia Pizza^{3,4} |
Ana Maria Caetano Faria⁵ | Claudio Franceschi⁶ | Nicola Neretti^{1,7}

Feasibility study on 12 volunteers:

- Three healthy YOUNG 25 y ± 0.5; **Y**
 - Three healthy OLD 71 y ± 1.6; **O**
 - Six 101.8 y ± 1.1; 3 in very good health conditions-HC- (SMMSE ≥ 24, ability to walk) and 3 in unhealthy conditions UHC- (not able to perform SMMSE, bedridden, they died about three years after).
- ✓ **F:M = 2:1** (except for unhealthy centenarians who consisted of all females)

Biological questions:

- Did they differ in term of quantity of cf-DNA?

➤ No, they didn't.

Y (7.9 ng/mL \pm 2 SD);

O (7 ng/mL \pm 0.8);

H (8.4 ng/mL \pm 0.8);

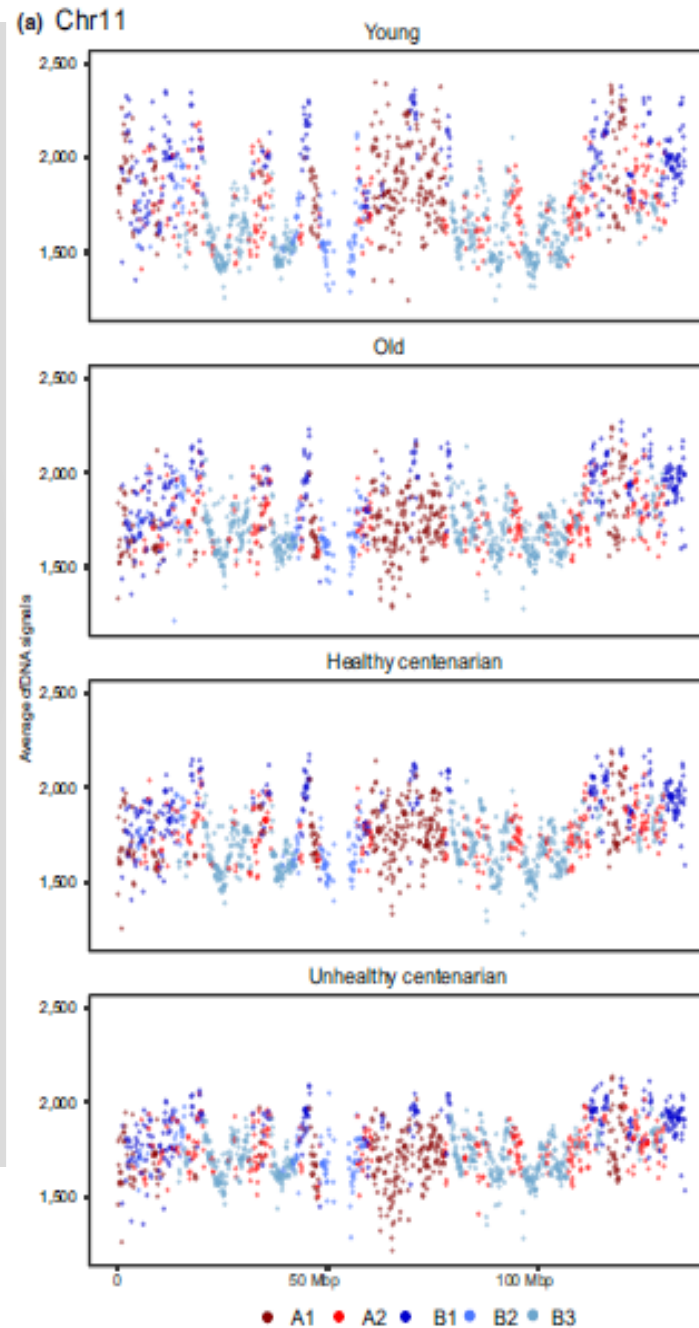
UHC (7.7 ng/mL \pm 1.3).

- Did they differ in term of cf-DNA sequences, in particular Healthy and Unhealthy 100+?

➤ Yes, they did.

- Identified an enrichment of 166-175 bp fragments, which corresponds to the length of a chromosome. Where do they are from? => a bioinformatic approach (MNase-seq).

- Identified 5 subcompartments (A1, A2, B1, B2, B3) using Hi-C data-seq associated with distinct histone modifications.



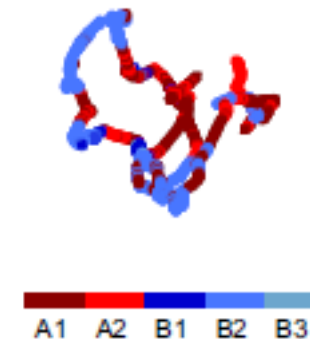
A1- A2 euchromatic regions that are gene rich

B1- facultative heterochromatic regions

B2- enriched at the **nuclear lamina and nucleolus-associated domains (NADs)**

B3- also enriched at the **nuclear lamina domain** but not at NADs.

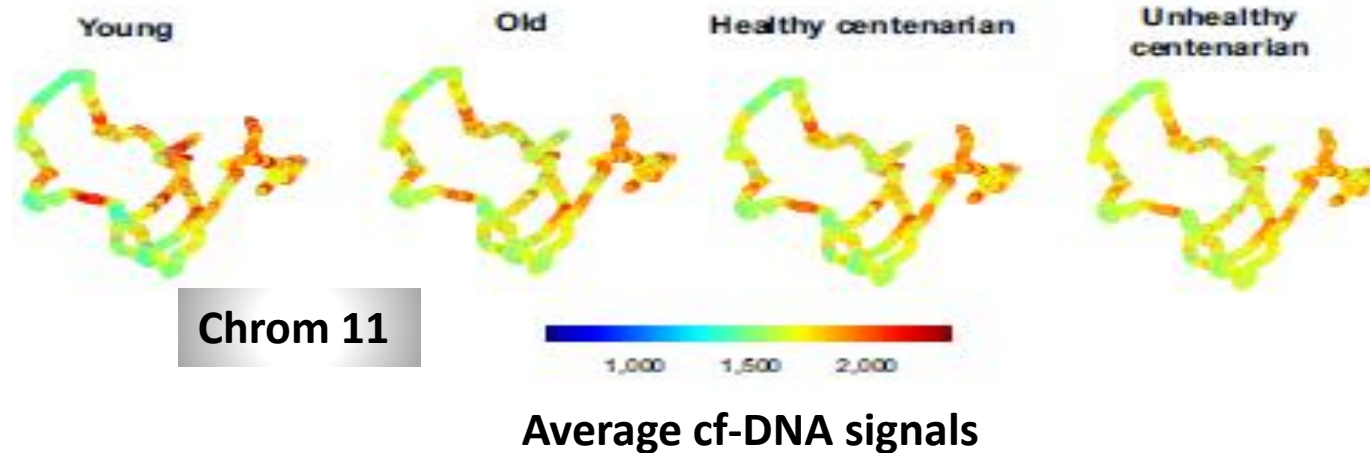
(c) Chr11



Cf-DNA signal average in the different subcompartments along all chromosomes

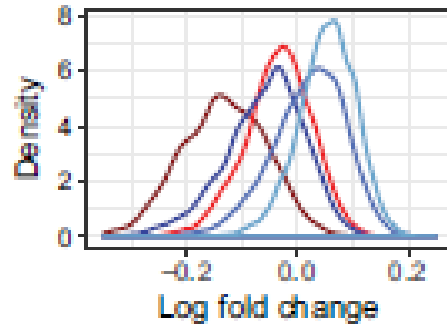
(d)

Age group	cfDNA signals	Variance	P-value (relative to Young)
Young	B1>A1>A2>B2>B3	68418.82	-
Old	B1>B2>A2>A1>B3	41924.42	$< 2.2 \times 10^{-16}$
Healthy centenarian	B1>A1>B2>A2>B3	39290.87	$< 2.2 \times 10^{-16}$
Unhealthy centenarian	B1>B2>A2>B3>A1	33982.7	$< 2.2 \times 10^{-16}$



COMPARISONS OF ALL GROUPS INSIDE THE DIFFERENT REGIONS

(e) All chr Old - Young

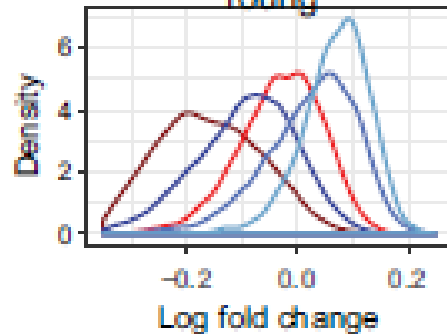


— A1 — A2 — B1
— B2 — B3

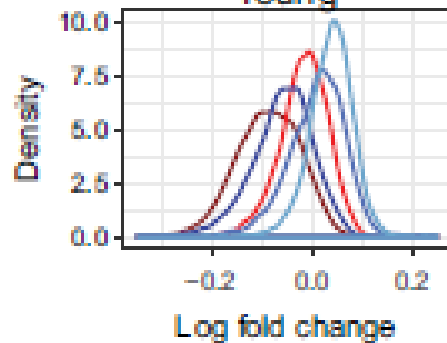
All groups vs Y group (in the picture):

- Increase of **B2** and **B3**;
- Decrease of **A1**, **A2** and **B1** in O and UHC groups (also confirmed in HC except A2).

Unhealthy centenarian - Young



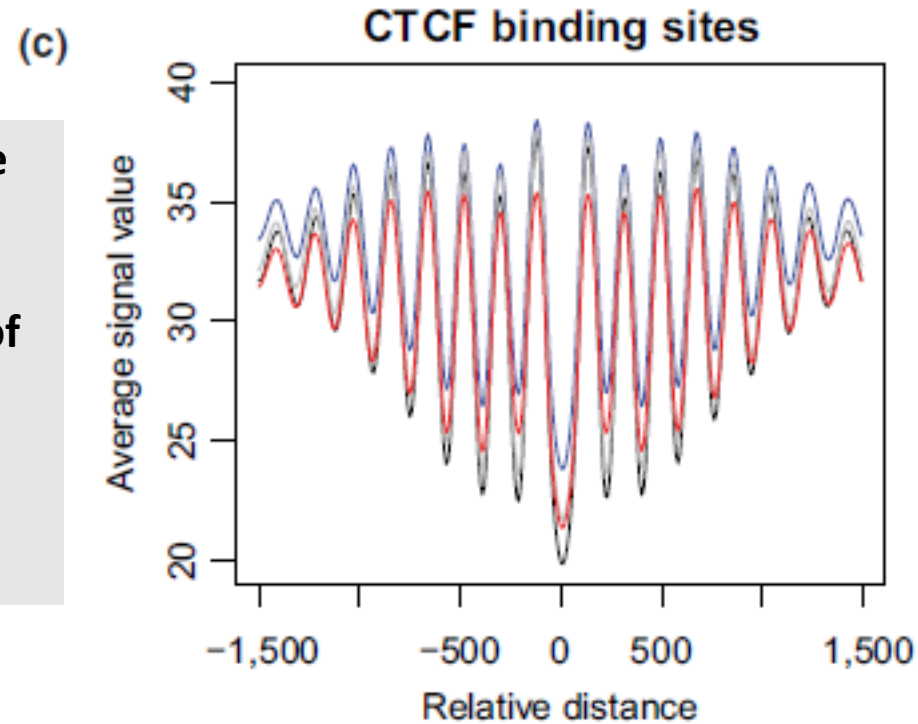
Healthy centenarian - Young



- Young subjects are similar to healthy centenarians
- Old subjects are similar to unhealthy centenarians
- **Redistribution of cf-DNA signals from heterochromatin regions to euchromatic regions in old age and unhealthy conditions**

WHAT RELEVANT SEQUENCES in cf-DNA?

- Expected structure characterizing Nucleosome-depleted regions of DNA (**NDRs**);
- Comparison with GM12878ENCODE ChIP-seq data



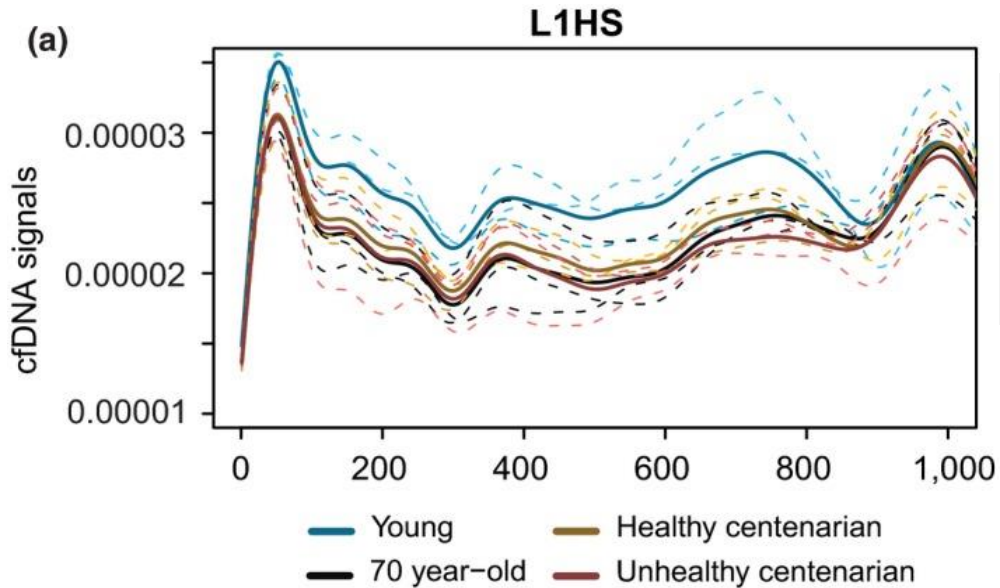
- CCCTC-binding factor (CTCF): NDR flanked by **well-positioned nucleosomes** and **these signals attenuate with age**.
- CTCF has been shown to play roles in mediating the formation of chromatin loops.

— Young — Old — Healthy centenarian — Unhealthy centenarian

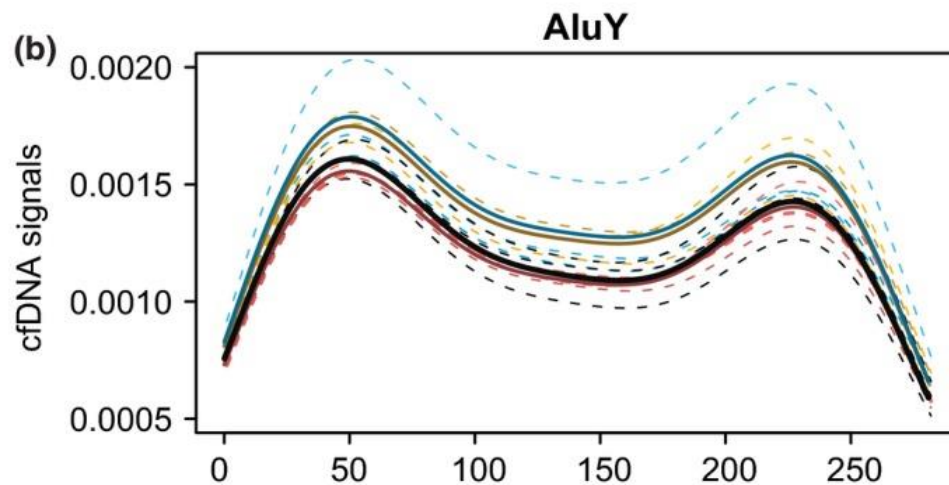
Major differences between Y and UHC

WHAT GENE-RELATED SEQUENCES in cf-DNA?

Transposable elements: L1HS and AluY

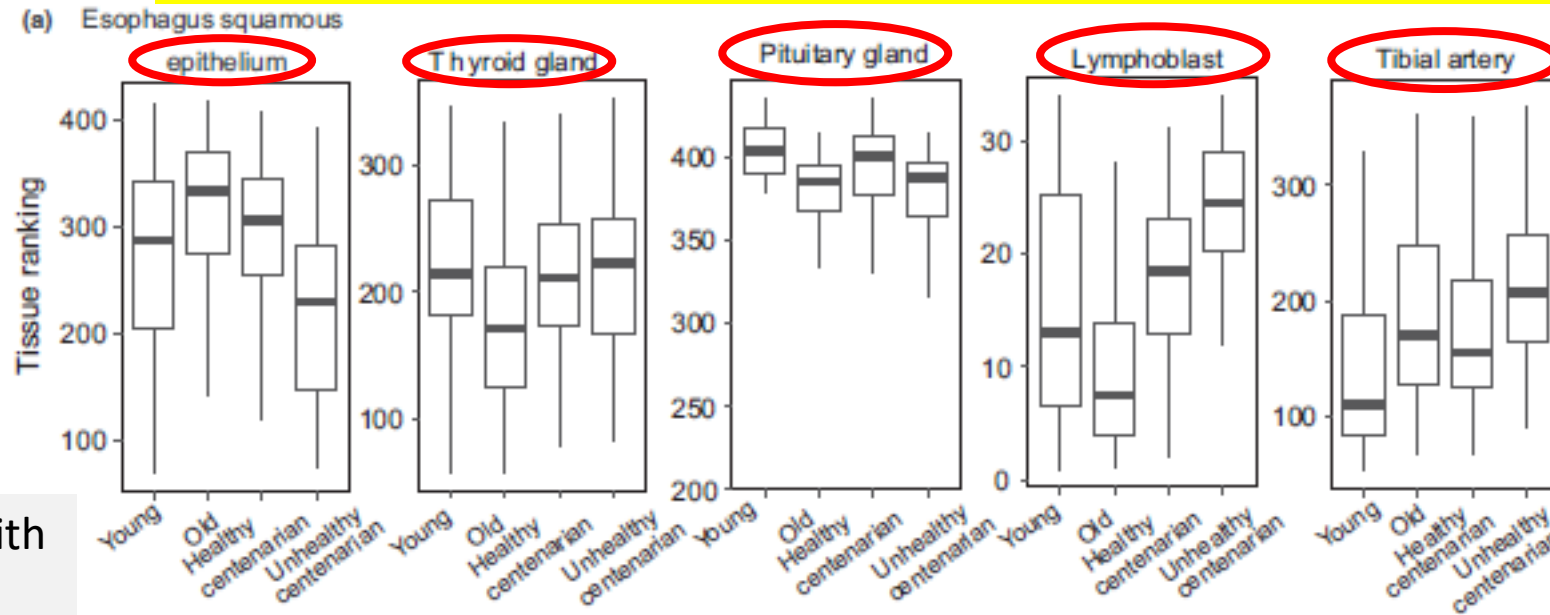


Decreased (first 668 bp of 5'UTR) cf-DNA signals from **L1HS** promoter and enhancer in **UHC** and **O**.



Decreased cf-DNA signals from **AluY** in **UHC** and **O**.

WHERE DOES cf-DNA COME FROM?



Correlations with corresponding signal intensities in **Genotyping Tissue Expression Projects (GTEx)**

	Old - Young	Healthy centenarian - Young	Unhealthy centenarian - Young
Esophagus squamous epithelium	Decreased rank (47)	-	Increased rank (57)
Thyroid gland	Increased rank (43.5)	-	-
Pituitary gland	Increased rank (19)	-	Increased rank (16.5)
Lymphoblast	Increased rank (5.5)	-	Decreased rank (11.5)
Tibial artery	Decreased rank (60)	Decreased rank (45)	Decreased rank (97)

	Healthy centenarian - Unhealthy centenarian	Old - Unhealthy centenarian	Healthy centenarian - Old
Esophagus squamous epithelium	Decreased rank (76)	Decreased rank (104)	-
Thyroid gland	-	Increased rank (52)	Decreased rank (40)
Pituitary gland	-	-	-
Lymphoblast	Increased rank (6)	Increased rank (17)	Decreased rank (11)
Tibial artery	Increased rank (52)	Increased rank (37)	-

$p < 0.05$; Kruskal-Wallis Test

CONCLUSIONS (I)

- ✓ Circulating **cf-DNA profile changes with age** (increase of euchromatin regions).
- ✓ **Nucleosome sequences** at several genomic locations (CCTF-binding site and retrotransposon elements) have been identified and their **signals decrease in old age/unhealthy longevity**.
- ✓ Data of cf-DNA-seq pave the way for identifying the organs/tissues where cf-DNA comes from and likely characterise new biomarkers of healthy/unhealthy trajectories.



ELSEVIER

Contents lists available at ScienceDirect

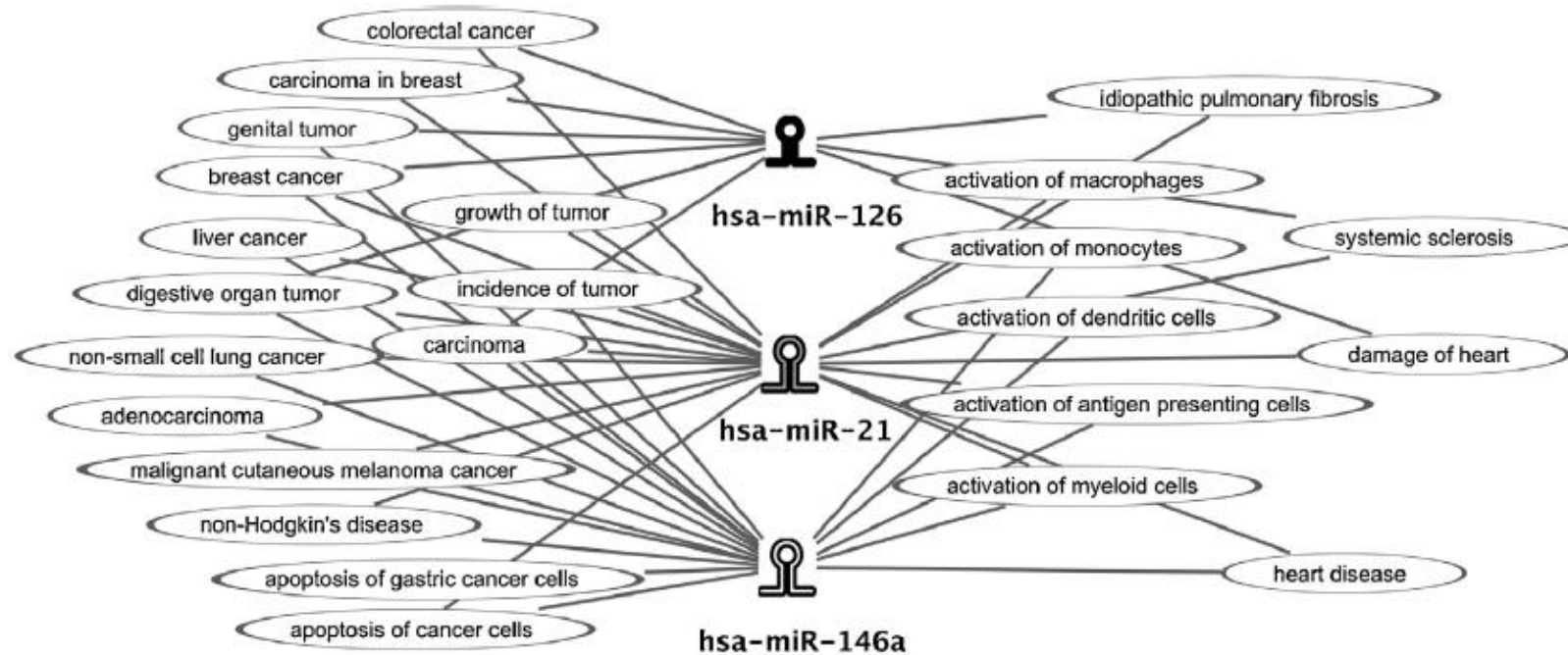
Ageing Research Reviews

journal homepage: www.elsevier.com/locate/arr

Review

MicroRNAs linking inflamm-aging, cellular senescence and cancer

Fabiola Olivieri^{a,b}, Maria Rita Rippo^a, Vladia Monsurrò^c, Stefano Salvioli^{d,e},
 Miriam Capri^{d,e}, Antonio Domenico Procopio^{a,b}, Claudio Franceschi^{d,e,*}



THE DEEP SEQUENCING OF CIRCULATING small-RNAs: the same cohort of 12 volunteers

Subjects	Total reads	Aligned reads	Precursor miR reads	Mature miR Reads
Y1	1,6E+07	6,0E+06	4,5E+03	5,1E+06
Y2	1,2E+07	6,2E+06	3,2E+03	5,8E+06
Y3	7,7E+06	1,3E+06	1,6E+03	9,3E+05
O1	2,1E+07	9,4E+06	5,7E+03	8,4E+06
O2	1,1E+07	3,4E+06	3,2E+03	2,8E+06
O3	1,4E+07	5,6E+06	9,0E+03	4,5E+06
HC1	7,0E+06	2,7E+06	1,9E+03	2,3E+06
HC2	3,9E+06	1,2E+06	8,6E+02	1,0E+06
HC3	4,1E+06	1,5E+06	1,1E+03	1,2E+06
UHC1	5,1E+06	8,3E+05	3,8E+02	6,4E+05
UHC2	4,5E+06	9,9E+05	7,4E+02	7,8E+05
UHC3	4,6E+06	7,2E+05	1,2E+03	4,6E+05

NextSeq500 (Illumina platform/Exiqon).

Quality control: Q-score>30; 99.9% accuracy.

Mapping on miRBase 2.0

*Manuscript in
preparation;
Morsiani et al.,*

18 Significant deep-sequenced miRs with age

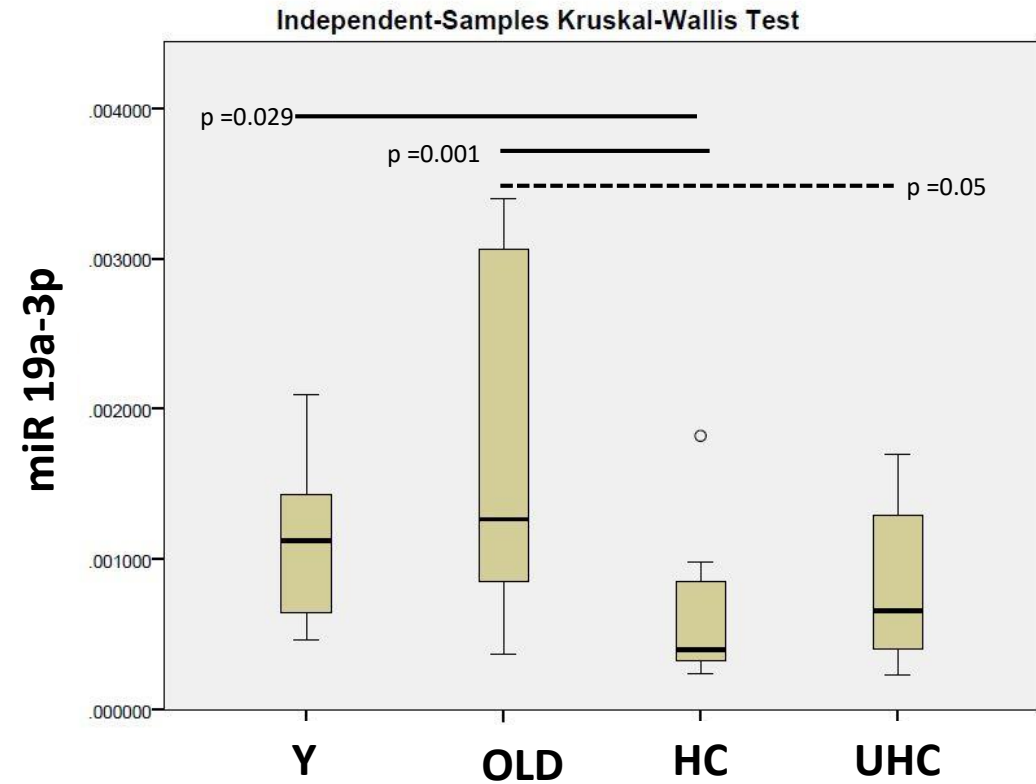
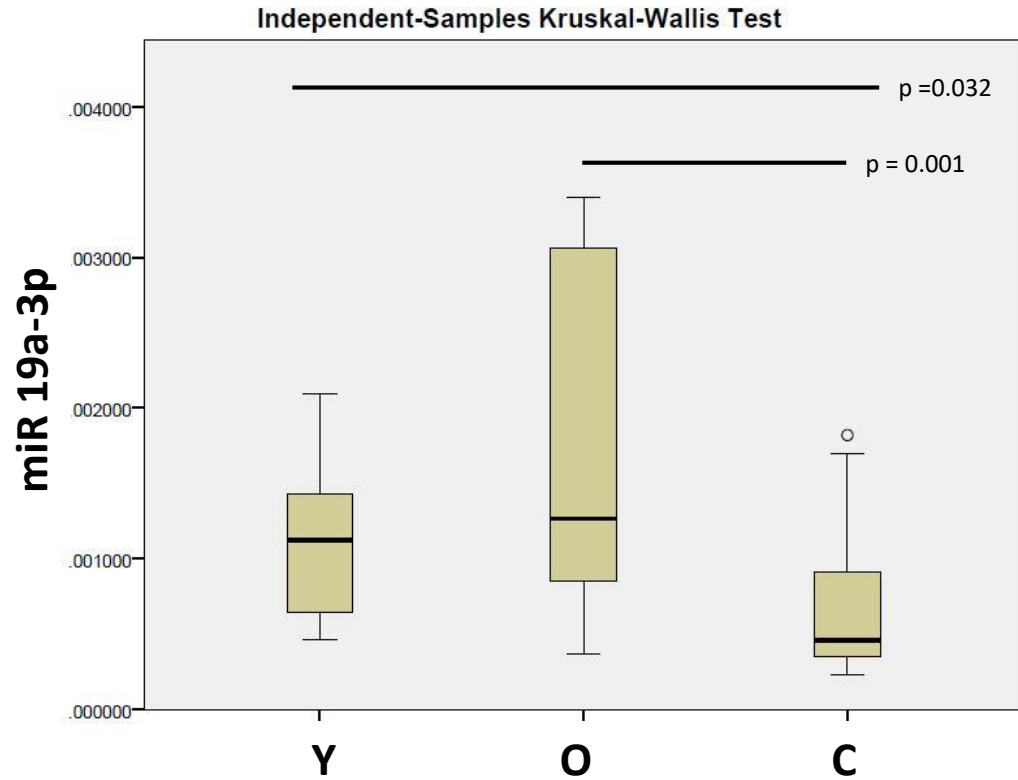
miR	Young vs Old			Young vs cent			Old vs cent		
	logFC	logCPM	PValue	logFC	logCPM	PValue	logFC	logCPM	PValue
hsa-miR-106b-5p	-0.0311	8.3847	0.9634	-1.9951	8.3847	0.0002	-1.9641	8.3847	0.0003
hsa-miR-122-5p	1.0975	10.8101	0.0595	-0.7437	10.101	0.1163	-1.8412	10.8101	0.0001
hsa-miR-133a-3p	-1.5420	5.4577	0.0324	-1.1319	5.4577	0.0487	0.4092	5.4577	0.5639
hsa-miR-144-5p	-0.5618	7.1328	0.3424	-1.9516	7.1328	0.0001	-1.3900	7.1328	0.0043
hsa-miR-15b-5p	-0.6828	7.5036	0.2543	-1.4883	7.5036	0.0026	-0.8056	7.5036	0.1032
hsa-miR-16-5p	-0.7083	15.1046	0.3189	-1.9051	15.1046	0.0010	-1.1968	15.1046	0.0380
hsa-miR-182-5p	0.1295	8.9882	0.8019	-1.1937	8.9882	0.0053	-1.3232	8.9882	0.0019
hsa-miR-185-5p	0.4239	10.6919	0.4907	-1.5944	10.6919	0.0016	-2.0183	10.6919	0.0001
hsa-miR-186-5p	0.2303	10.9363	0.6683	-1.1006	10.9363	0.0132	-1.3309	10.9363	0.0027
hsa-miR-1908-5p	0.6625	8.3791	0.1675	1.4024	8.3791	0.0017	0.7400	8.3791	0.0886
hsa-miR-192-5p	0.3553	9.9912	0.5382	-1.2916	9.9912	0.0067	-1.6469	9.9912	0.0005
hsa-miR-19a-3p	0.7032	6.7436	0.4410	-0.8372	6.7436	0.2571	-1.5404	6.7436	0.0376
hsa-miR-19b-3p	0.5460	8.3340	0.5427	-1.4363	8.3340	0.0478	-1.9823	8.3340	0.0065
hsa-miR-20b-5p	-0.2778	6.7998	0.6658	-1.7899	6.7998	0.0006	-1.5122	6.7998	0.0039
hsa-miR-224-5p	1.6153	4.8278	0.0730	1.6481	4.8278	0.0429	0.0354	4.8278	0.9993
hsa-miR-3138	0.8065	4.2355	0.1851	1.8036	4.2355	0.0018	0.9996	4.2355	0.0669
hsa-miR-451a	-0.4854	15.7944	0.4902	-2.5304	15.7944	0.0001	-2.0451	15.7944	0.0004
hsa-miR-7-5p	-0.3488	6.8461	0.4976	-1.4227	6.8461	0.0009	-1.0740	6.8461	0.0121

7 Significant deep-sequenced miRs in Healthy/Unhealthy longevity

miR	Healthy vs Unhealthy Centenarians		
	logFC	logCPM	PValue
hsa-miR-19a-3p	2.9109	6.1878	0.0008
hsa-miR-19b-3p	2.8257	7.3973	0.0008
hsa-miR-221-5p	2.1594	4.8648	0.0053
hsa-miR-145-5p	2.2146	8.2412	0.0064
hsa-miR-10b-5p	2.0145	10.6144	0.0076
hsa-miR-4433b-3p	2.4683	6.7413	0.0104
hsa-miR-206	2.0291	5.2636	0.0327

Validation in an enlarged cohort (66 subjects) by RT-qPCR

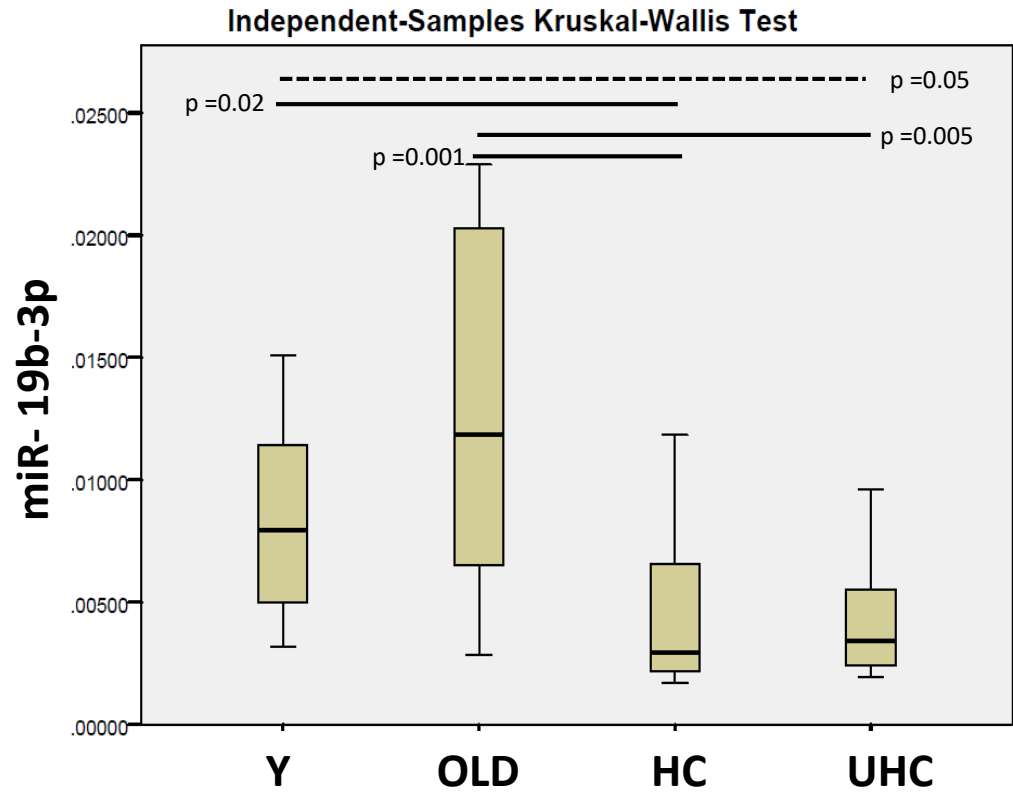
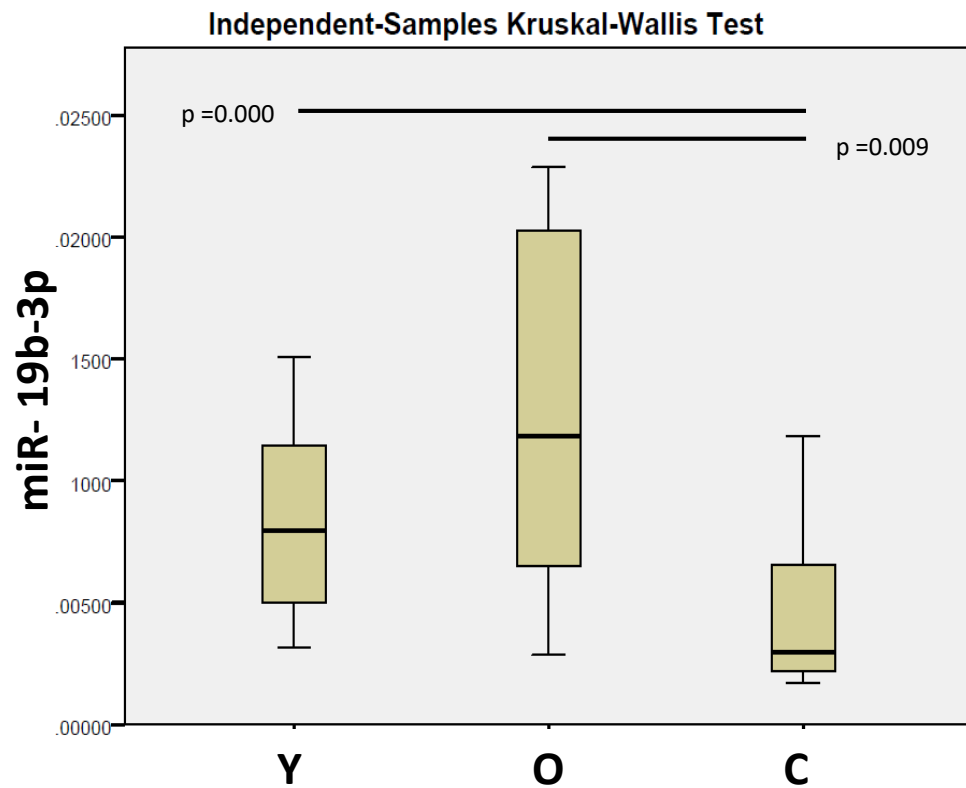
miR-19a-3p



Y = 16 subjects
O = 16
C = 17
HC = 11
UHC = 6

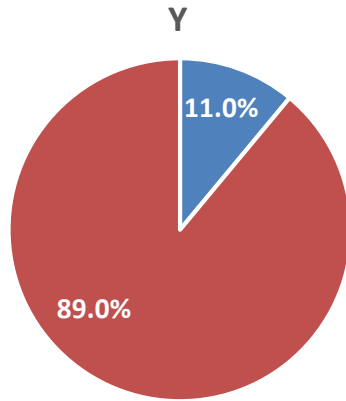
Validation in an enlarged cohort (66 subjects) by RT-qPCR

miR-19b-3p

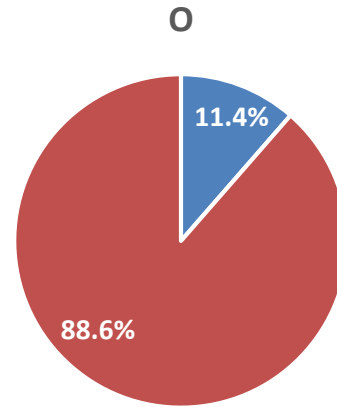


Y = 16 subjects
O = 16
C = 17
HC = 11
UHC = 6

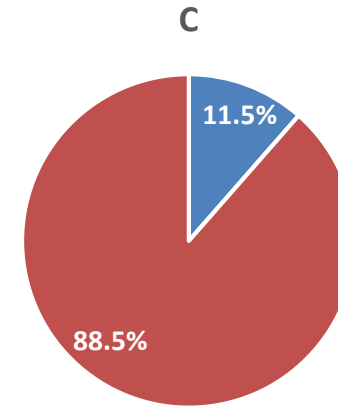
miR-19a-3p ISOMIRs: Expression (%) of the exact sequence vs other sequences



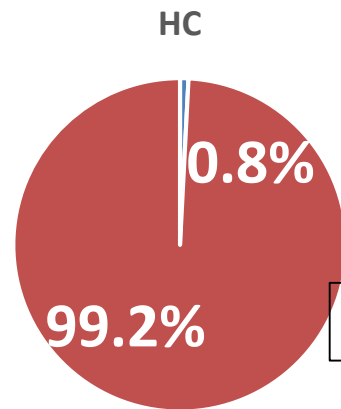
■ base seq ■ others seq



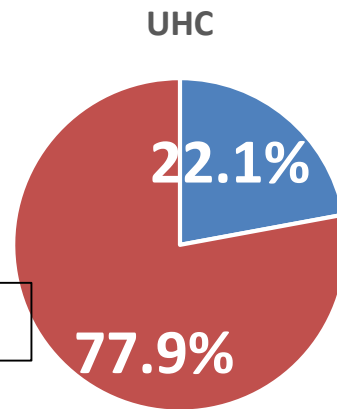
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■ base seq ■ others seq



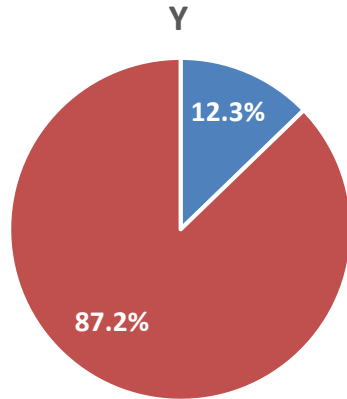
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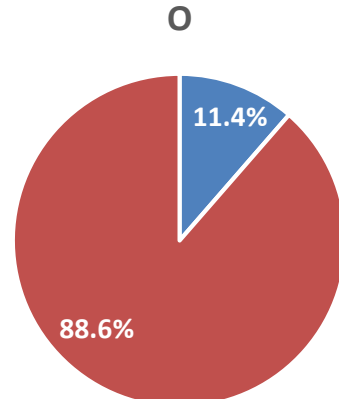
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*
p = 0.012

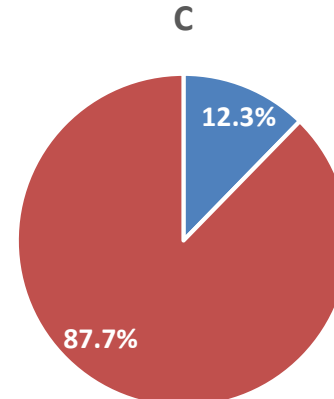
miR-19b-3p ISOMIRs: Expression (%) of the exact sequence vs other sequences



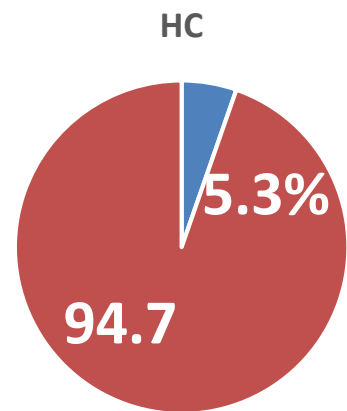
■ base seq ■ others seq



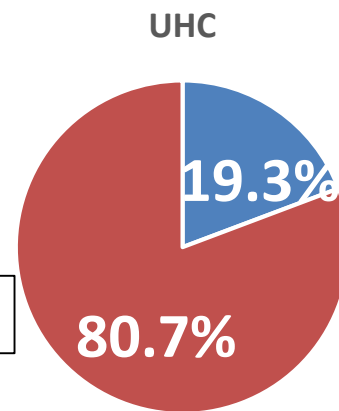
■ base seq ■ others seq



■ base seq ■ others seq



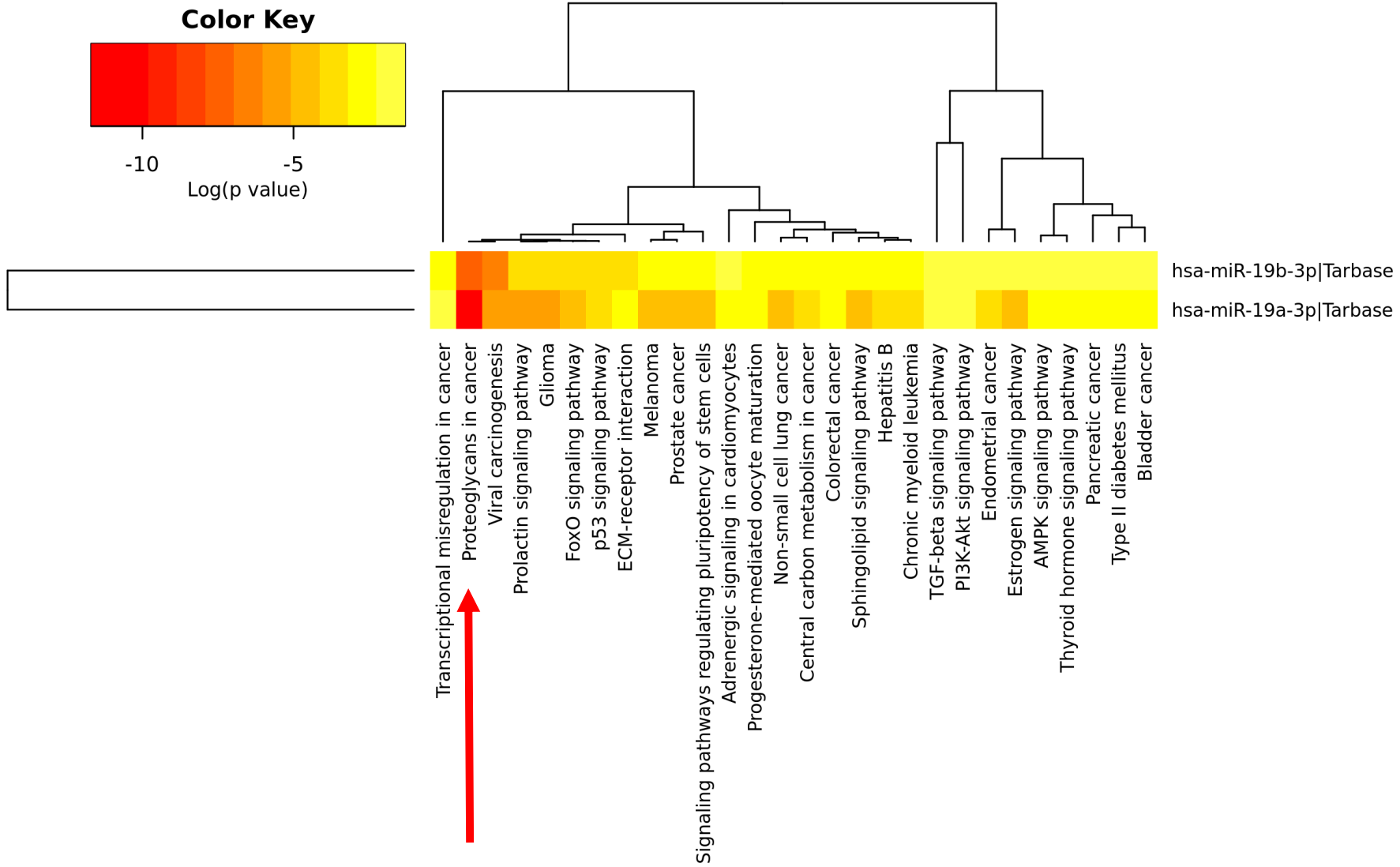
■ base seq ■ others seq



■ base seq ■ others seq

—
p = n.s.

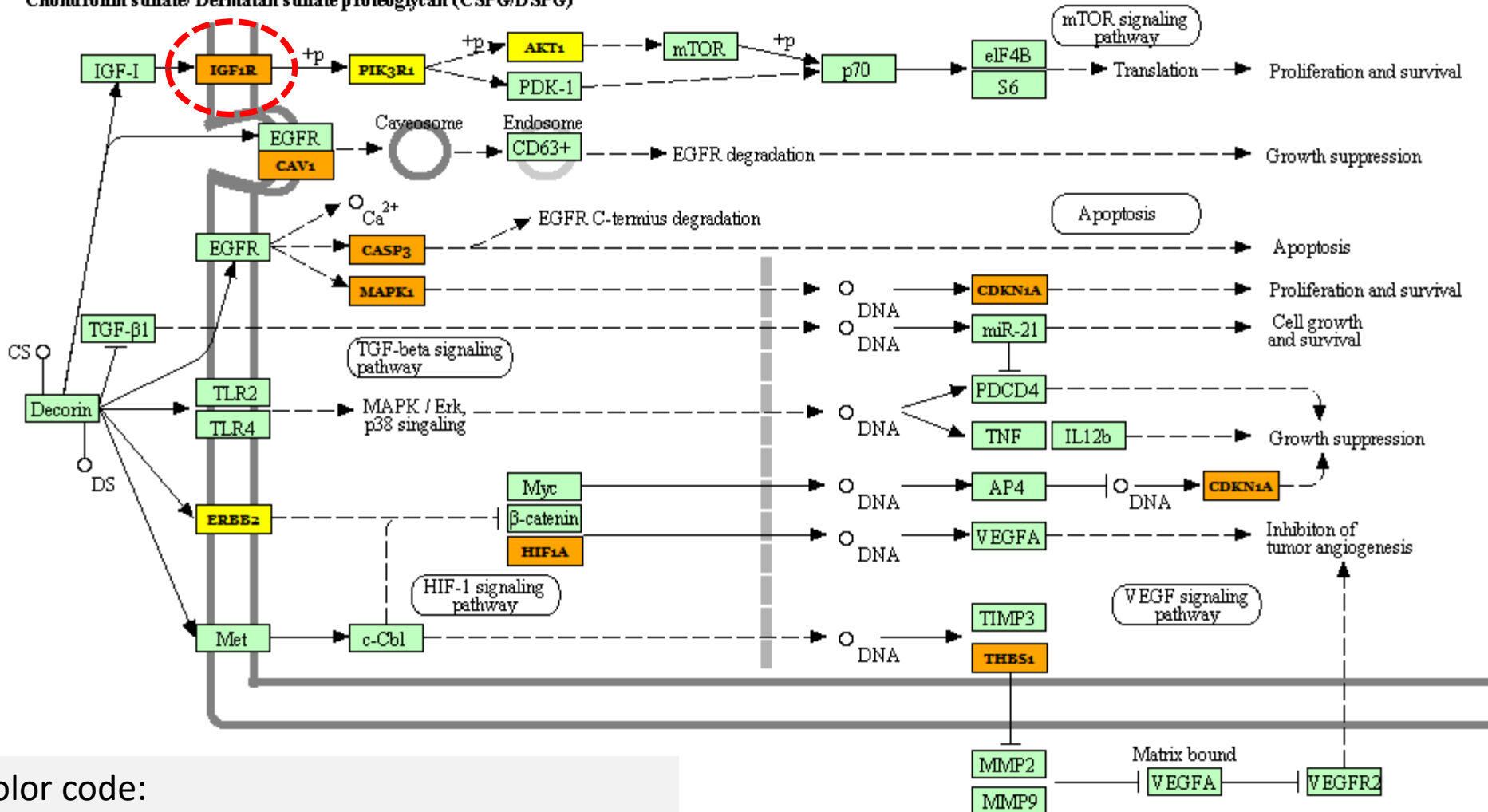
KEGG pathways considering the intersection of miR-19a-3p and miR-19b-3p validated targets



KEGG pathway: Proteoglycans in cancer (44 genes)

p < 1e-325

Chondroitin sulfate/ Dermatan sulfate proteoglycan (CSPG/DSPG)



Color code:

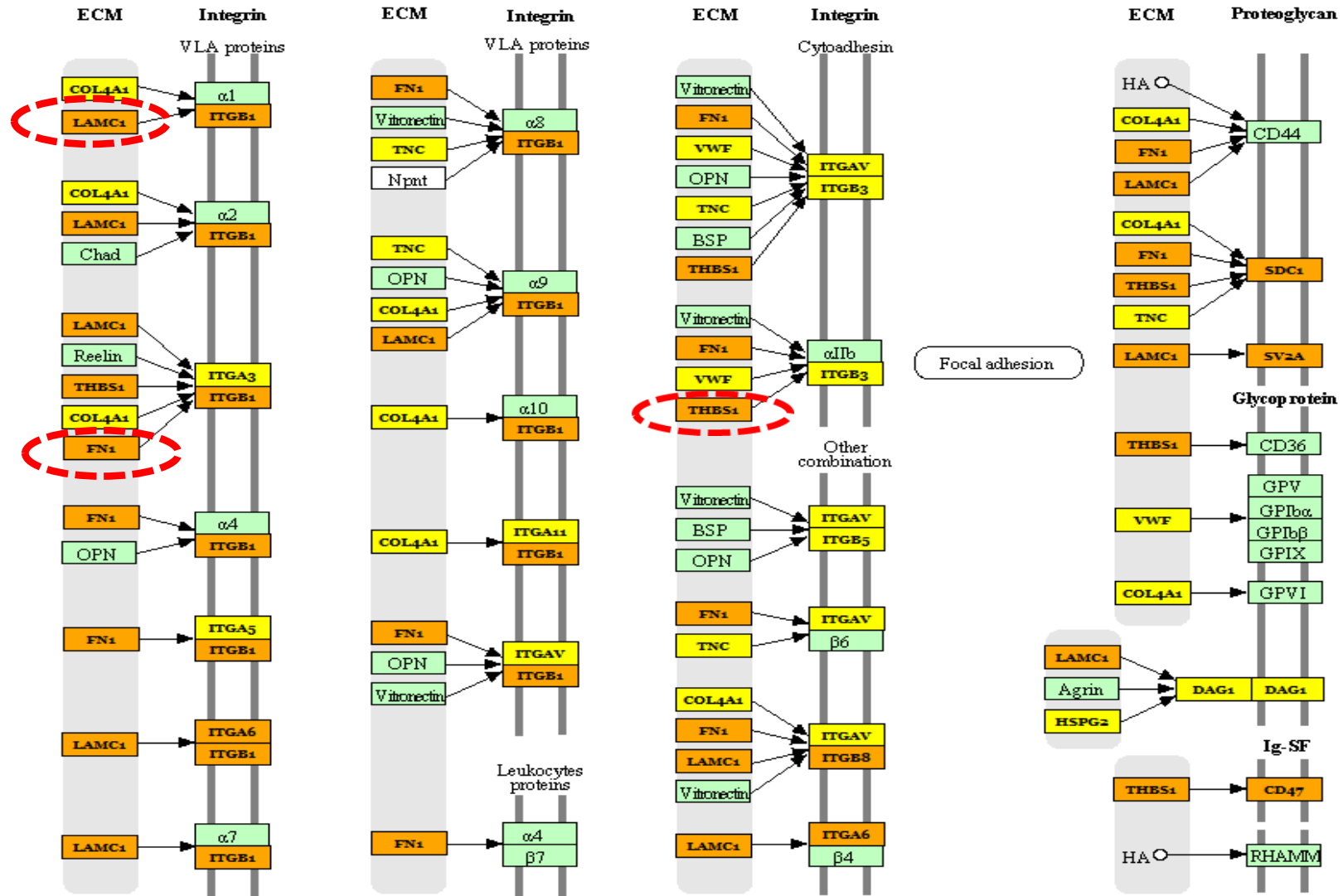
Orange: target of both miRs

Yellow: target of one out of two miRs

KEGG pathway: Intersection of miR-19a-3p; -19b-3p; -221-5p and -145-5p validated targets

ECM-RECEPTOR INTERACTION

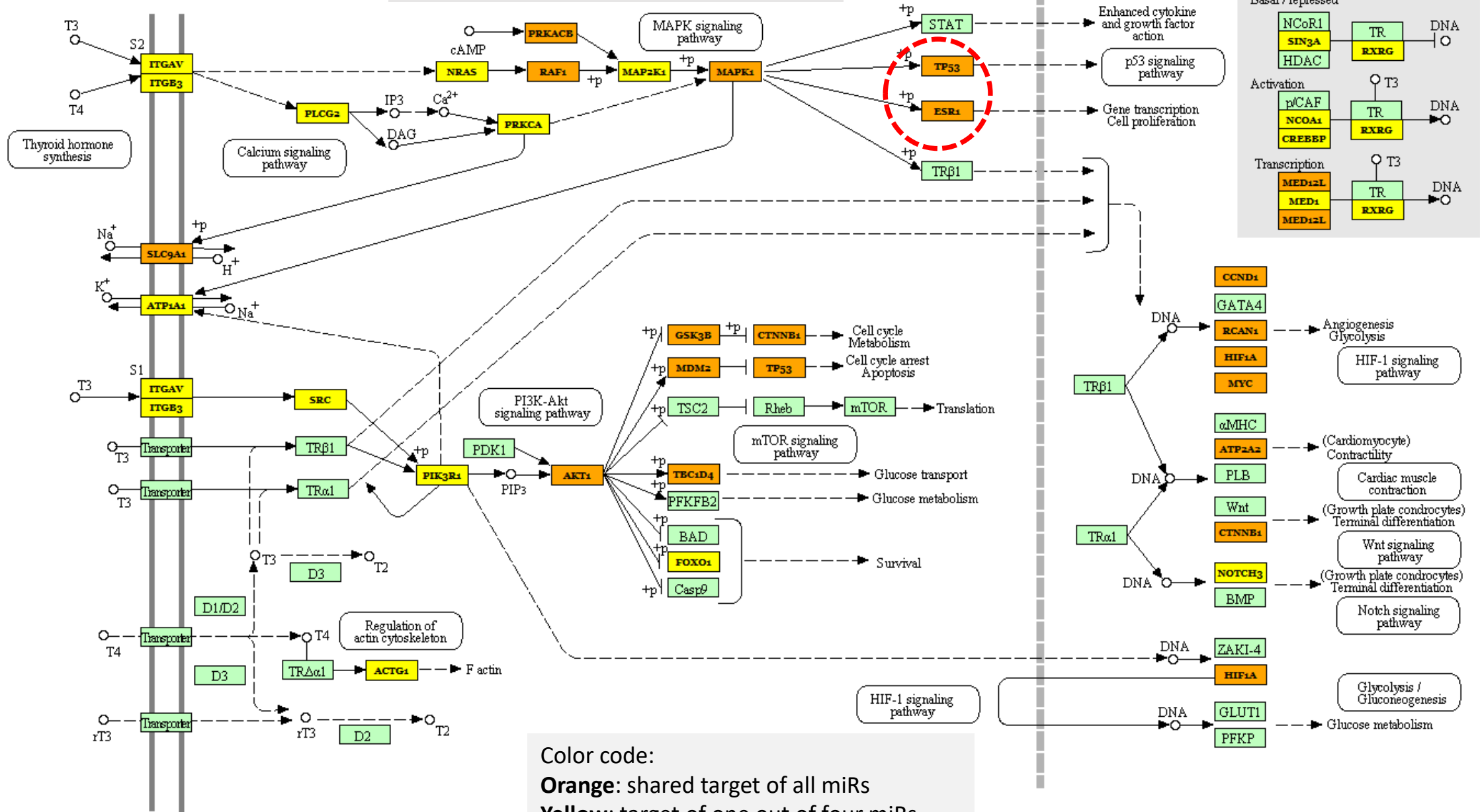
$p < 1e-325$ (30 genes)



Color code:
Orange: shared target of all miRNAs
Yellow: target of one out of four miRNAs

THYROID HORMONE SIGNALING PATHWAY

$p < 1.192673e-05$ (47 genes)



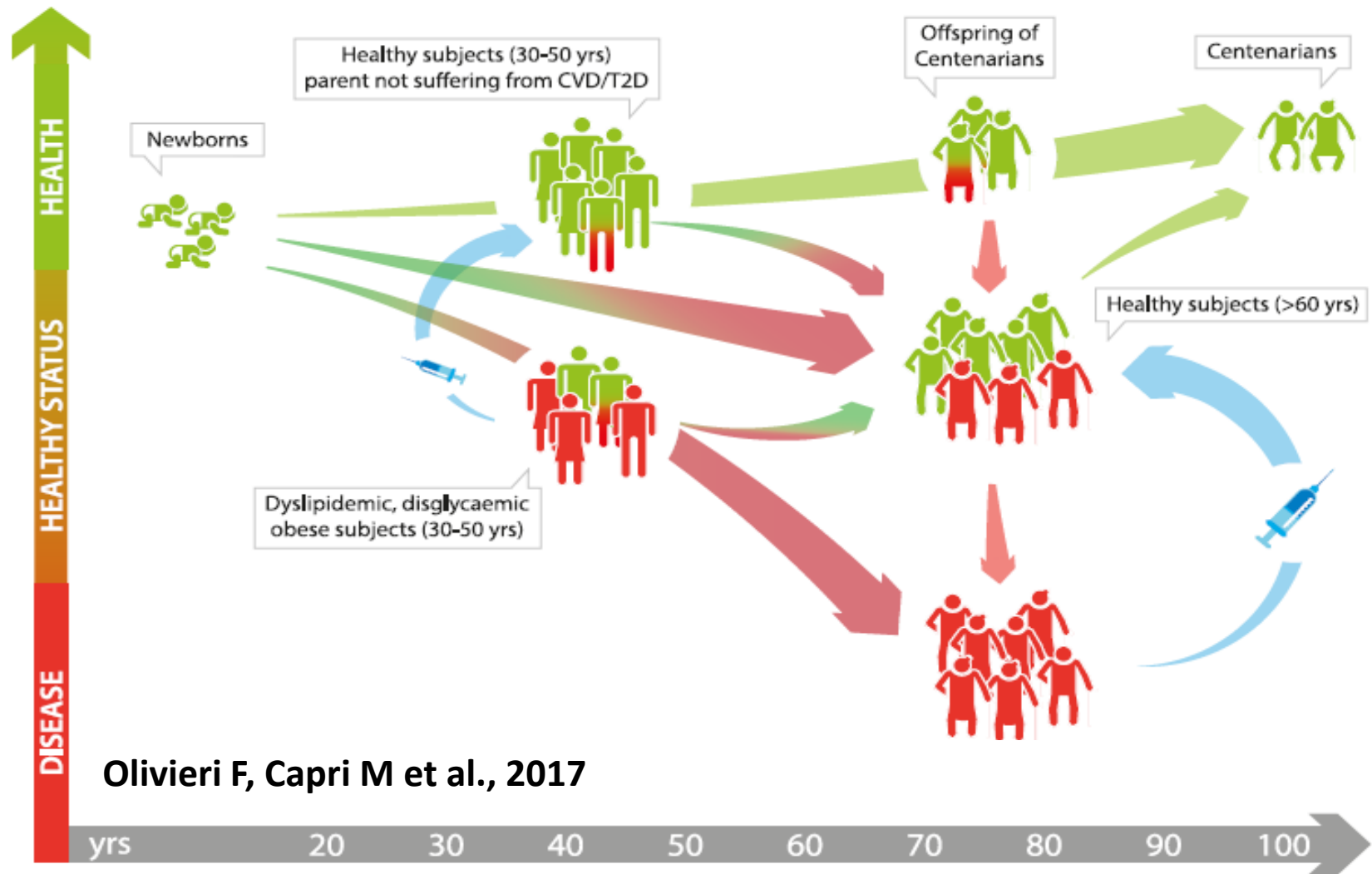
CONCLUSIONS (II)

- ✓ **miR-19a-3p** and **miR-19b-3p** change with age/longevity and are involved in the PROTEOGLYCANS IN CANCER pathway (KEGG) in particular **IGF1R** is a target of both;
- ✓ Small-RNA deep sequencing between **HC** and **UHC** identify seven miRs and their targets (shared by 4 out of 7 miRs) converge on **Extracellular-Matrix interaction** and **Thyroid Hormone signalling pathways** => convergence with cf-DNA-seq data;
- ✓ **Isomirs-19a/19b increase in HC.**

NEW PERSPECTIVE

- **Do isomirs explain epigenetic adaptation phenomena? Do they represent a possible molecular advantage? => association with ER –stress (Mesitov et al., 2017); induced by IFN-type I (Nejad et al., 2018).**
- **THE INCREASE OF CIRCULATING ISOMIRs IN HEALTHY LONGEVITY MAY BE THE RESULT OF THE INDIVIDUAL IMMUNOBIOGRAPHY**

Circulating cf-DNA and miRs/IsomiRs for healthy and unhealthy trajectory prediction?



Olivieri F, Capri M et al., 2017

MiR-TEAM at ALMA MATER STUDIORUM

Current projects:

- ❖ Aging/longevity
- ❖ Carotid disease
- ❖ Liver transplant and old donors
- ❖ Myopathies
- ❖ Myelofibrosis syndromes
- ❖ Inflammation associated with human spaceflight



Miriam Capri

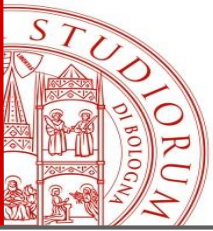


Cristina Morsiani



Salvatore Collura

Thank you very much for your attention!



International Collaboration with:

- DLR- Institute of Aerospace Medicine-DE
- University of Konstanz- DE
- Karolinska Institutet- SW
- University College London-UK
- University of Nottingham-UK
- University of Leiden- NL
- University of Bordeaux-FR
- NIHS/Nestec- CH
- University of Wien- AU
- University of Jyväskylä-FI

- Buck Institute- CA, USA
- Stanford University- CA, USA
- University of Los Angeles-CA, USA
- Brown University- RI, USA
- Albert Einstein Institute- NY-USA
- NIH-NIA-National Institute of Health-MD, USA

- BGI- Honk Kong
- Universidade Federal de Minas Gerais, Brasil

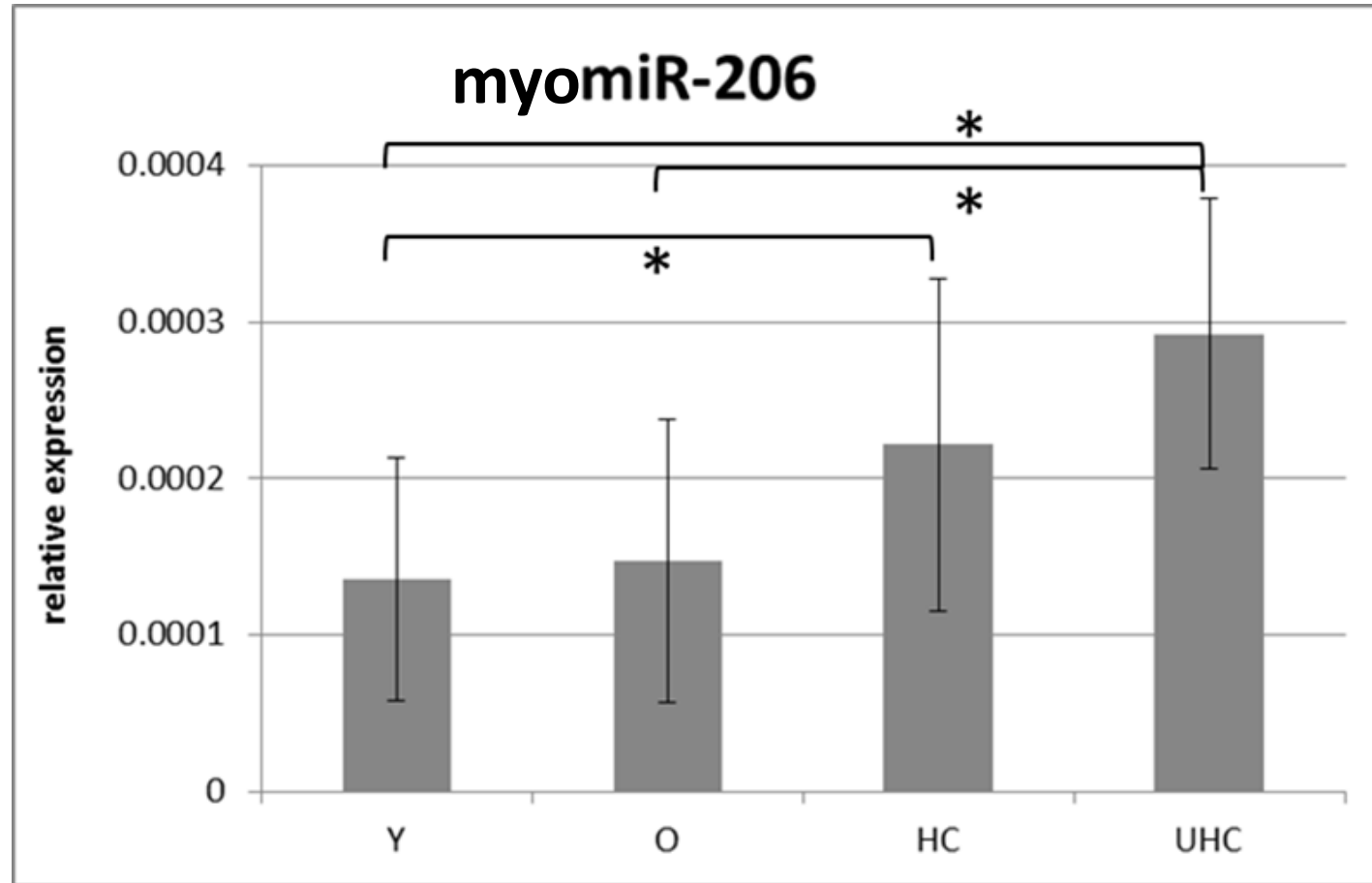


Haemato-biochemical Parameters

		WBC	MCHC	RDWCV	ALB	PROT	CRP
HC	mean	5.65	31.82	14.68	3.82	7.10	3.41
	sd	0.92	0.78	0.99	0.58	0.48	4.41
UHC	mean	7.55	30.43	16.30	3.25	6.46	11.57
	sd	2.41	1.32	2.23	0.23	0.36	10.18
	p value	0.039	0.019	0.063	0.039	0.028	0.041

WBC: white blood cell count (4.8-8.5 x1000/ μ l). **MCHC:** mean corpuscular hemoglobin concentration (33-38 gr/dl). **RDWCV:** red blood cell distribution (11.5-14.5 %). **ALB:** albumin (3.5-5.2 gr/dl). **PROT:** total protein (6.2-8 gr/dl). **CRP:** C-reactive protein (max 6 mg/l).

microRNAs from skeletal muscle



Relative expression of miR-206.
RT-qPCR in plasma samples of **15 healthy young donors** (average 30 years old), **16 healthy old donors** (average 71 years old), **16 centenarians of which 10 healthy and 6 unhealthy** (average 101 years). miR-206 was evaluated in young (Y), old (O), healthy (HC) and unhealthy centenarians (UHC). Data are reported as **mean values ± standard deviation**. Data were analyzed with Kruskal-Wallis test: * = $p \leq 0.05$.

CENTENARIANS IN ITALY (100+): 14,456; M = 2,324; F= 12,132

