



ECH

Spring Meeting

Bergamo 25-27 April 2024

HOMEOPATHY AND GENE EXPRESSION

**Paolo Bellavite
(Verona, I)**



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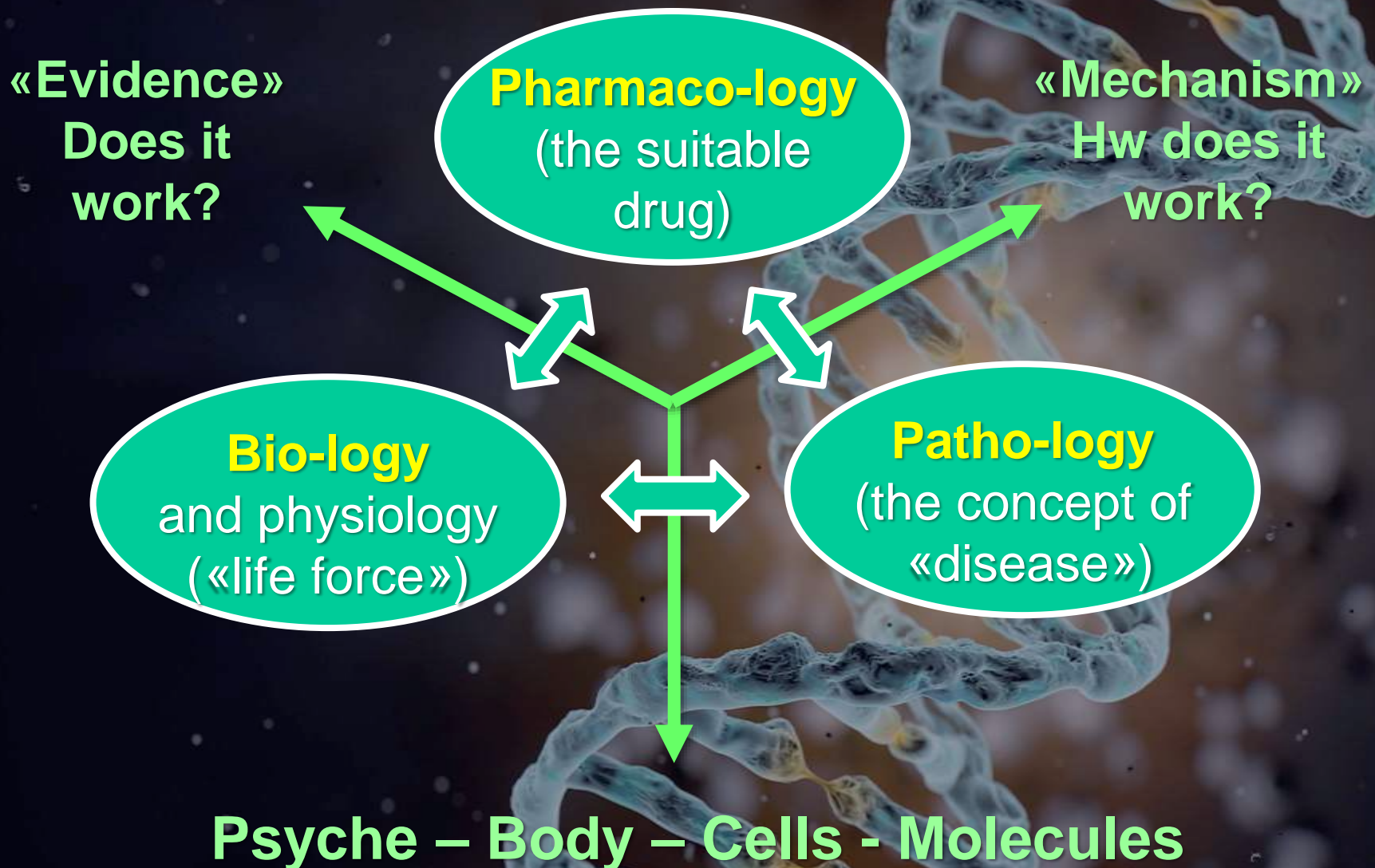
1. Introduction

2. Methods

3. Results

4. Discussion

THE «CORE» OF (ANY) MEDICAL RATIONALITY



THE DYNAMIC DIMENSION OF LIFE AND THE ROLE OF MOLECULAR BIOLOGY



Molecular biology (i.e. the study of information coded in nucleic acids and expressed as proteins) is a modern way to investigate biology, pathology and the action of drugs at a basic level.

Could **gene expression** (i.e. the study of transcriptomics) help to investigate also the action mechanism of homeopathic drugs?

Psychological stress (giving a lecture and performing difficult calculations) modifies gene expression in blood

Original Research

Genome-Wide mRNA Expression Analysis of Acute Psychological Stress Responses

Jeongok G. Logan RN MSN PhD, Sijung Yun MS PhD, Bethany A. Teachman MS PhD, Yongde Bao MS PhD, Emily Farber MS, Charles R. Farber MS PhD

ABSTRACT

INTRODUCTION Most previous studies have examined the effects of acute psychological stress in humans based on select gene panels. The genomic approach may help identify novel genes that underline biological mechanisms of acute psychological stress responses.

OBJECTIVE This exploratory study aimed to investigate genome-wide transcriptional activity changes in response to acute psychological stress.

METHODS The sample included 40 healthy women (mean age 31.4 ± 11.6 years). Twenty-two participants had a stress experience induced by the Trier Social Stress Test (experimental group) and 18 did not (control group). Psychological stress levels and hemodynamic changes were assessed before and after the Trier Social Stress Test. Peripheral blood samples obtained before and after the Trier Social Stress Test were processed for mRNA sequencing.

RESULTS Psychological and hemodynamic stress parameters indicated that the Trier Social Stress Test induced moderate

levels of stress in the experimental group. Six genes (HCG26, HCP5, HLA-F, HLA-F-AS1, LOC1019287, and SLC22A16) were up-regulated, and five genes (CA1, FBXO9, SNCA, STRADB, and TRMT12) were down-regulated among those who experienced stress induction, compared with the control group. Nine genes of eleven were linked to endocrine system disorders, neurological disease, and organismal injury and abnormalities.

CONCLUSION Of the genes identified in this study, HCP5, SLC22A16, and SNCA genes have previously been proposed as therapeutic targets for cancer and Parkinson disease. Further studies are needed to examine pathological mechanisms through which these genes mediate effects of psychological stress on adverse health outcomes. Such studies may ultimately identify therapeutic targets that enhance biological resilience to adverse effects of psychological stress.

KEYWORDS Stress, psychological; sequence analysis, RNA; psychological tests; US

Psychological stress (giving a lecture and performing difficult calculations) modifies gene expression in blood

Table 3: Up-regulated genes following stress induction in experimental group compared with control group

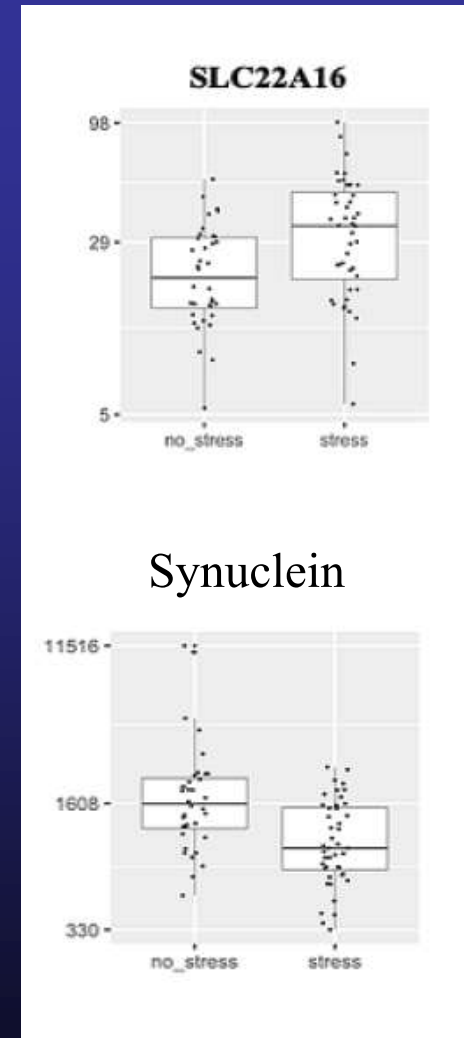
Gene symbol	Gene name	log2 (Fold-change)	Adjusted p Value (FDR)
HCG26	Human leukocyte antigen complex group 26	0.36	0.001
HCP5	HLA complex P5	0.31	0.043
HLA-F	Major histocompatibility complex class I, F	0.29	0.049
HLA-F-AS1	HLA-F antisense RNA 1	0.33	0.006
LOC1019287	Undefined	0.82	0.001
SLC22A16	Solute carrier family 22 member 16	0.78	0.041

FDR: false discovery rate; HLA: Human Leukocyte Antigen

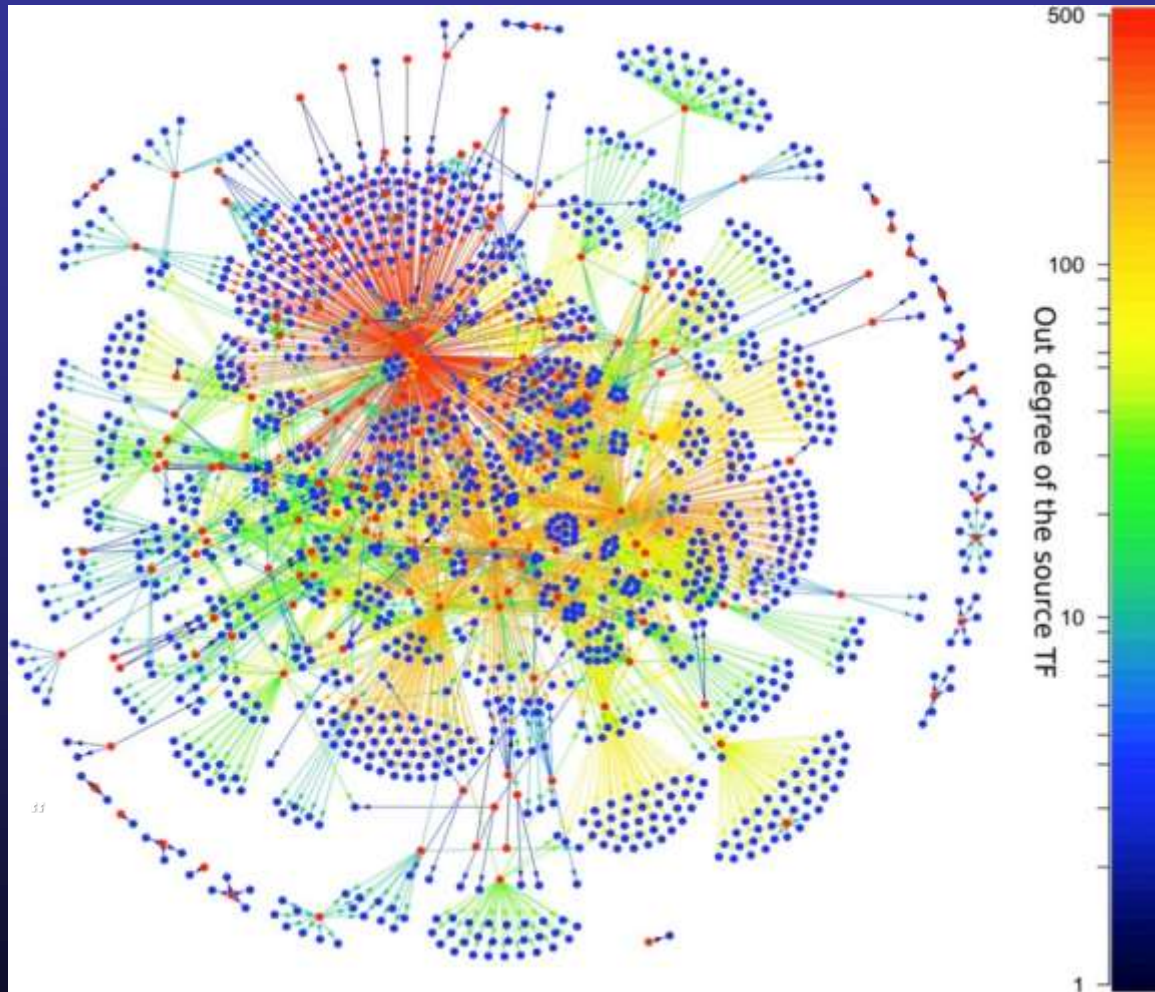
Table 4: Down-regulated genes after stress induction in experimental group compared with control group

Gene symbol	Gene name	log2 (Fold-change)	Adjusted p Value (FDR)
CA1	Carbonic anhydrase1	-1.08	0.001
FBXO9	F-box protein 9	-0.27	0.004
SNCA	Synuclein alpha	-0.86	0.048
STRADB	STE20 related adaptor beta	-0.71	0.043
TRMT12	tRNA methyltransferase 12 homolog	-0.28	0.030

FDR: False discovery rate



Working hypothesis: gene networks as targets of homeodrugs



Complexity, flexibility
and chaotic
behaviors



High sensitivity to
small perturbations



Very suitable for
exploratory research
of targets
(High throughput
transcriptomics)



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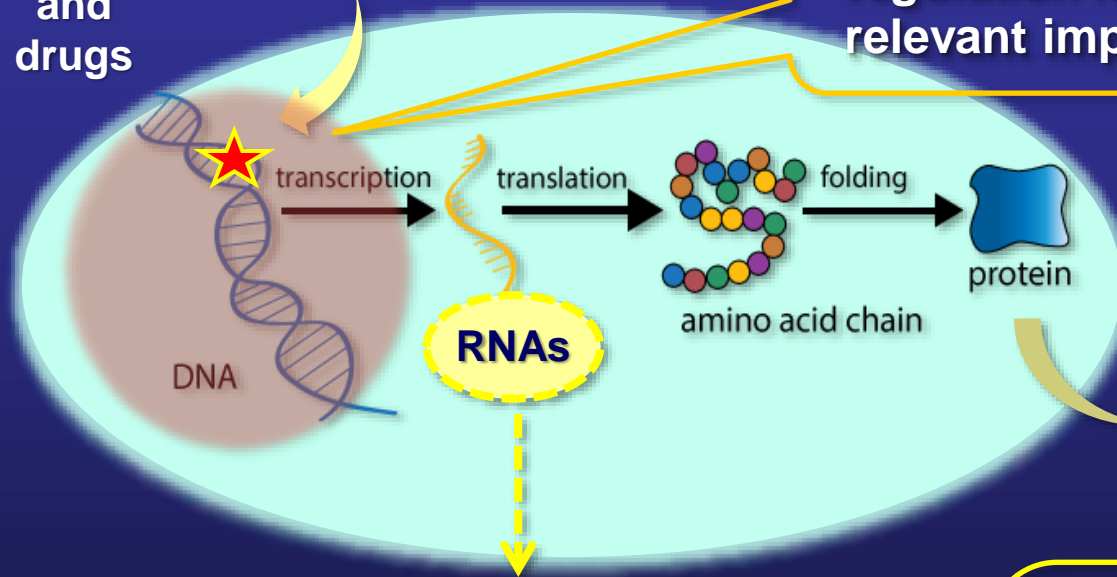
3. Results

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Analysis of cell responses through the transcriptome

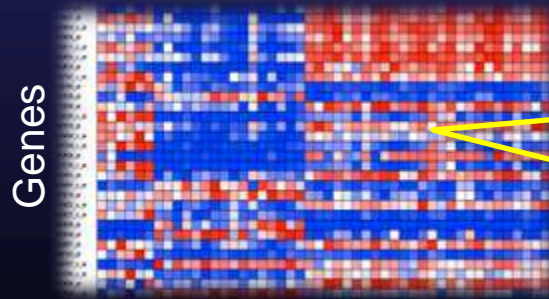
Environmental inputs and drugs

• Small changes in the gene regulation networks have a relevant impact in the cell



Phenotype
Metabolic activity

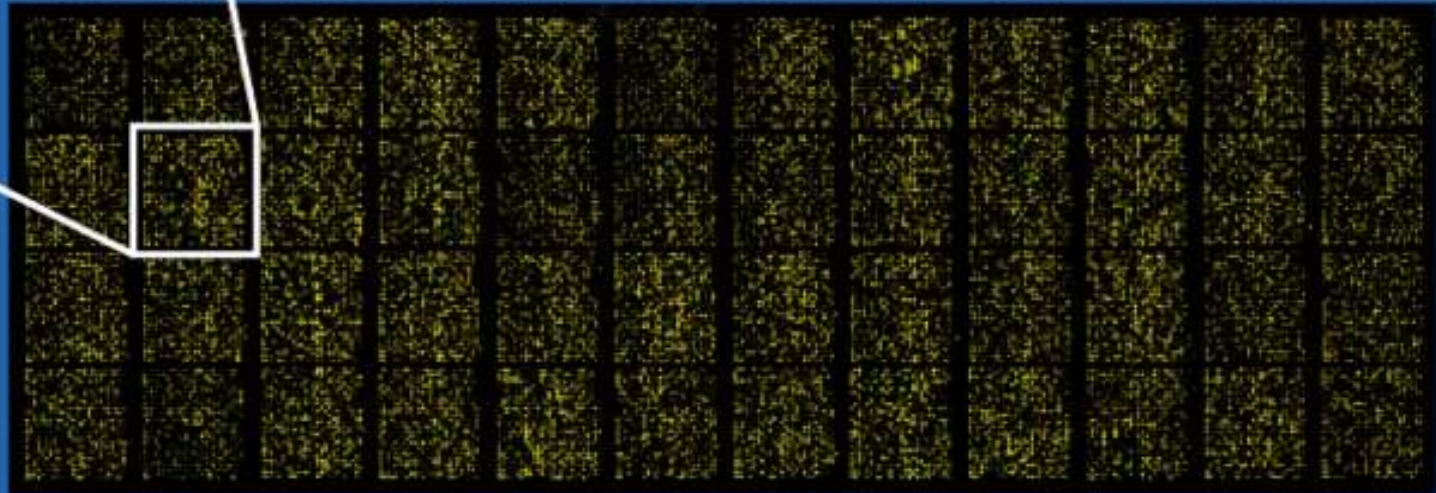
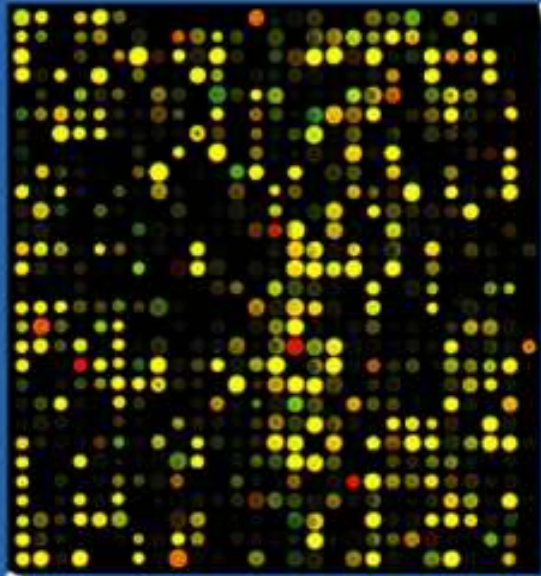
Differential gene expression
Conditions



- ✓ Quantification of the RNA expression levels of all the genes (microarray, RNA-seq, Rt-qPCR)
- ✓ Compare to control

Microarray

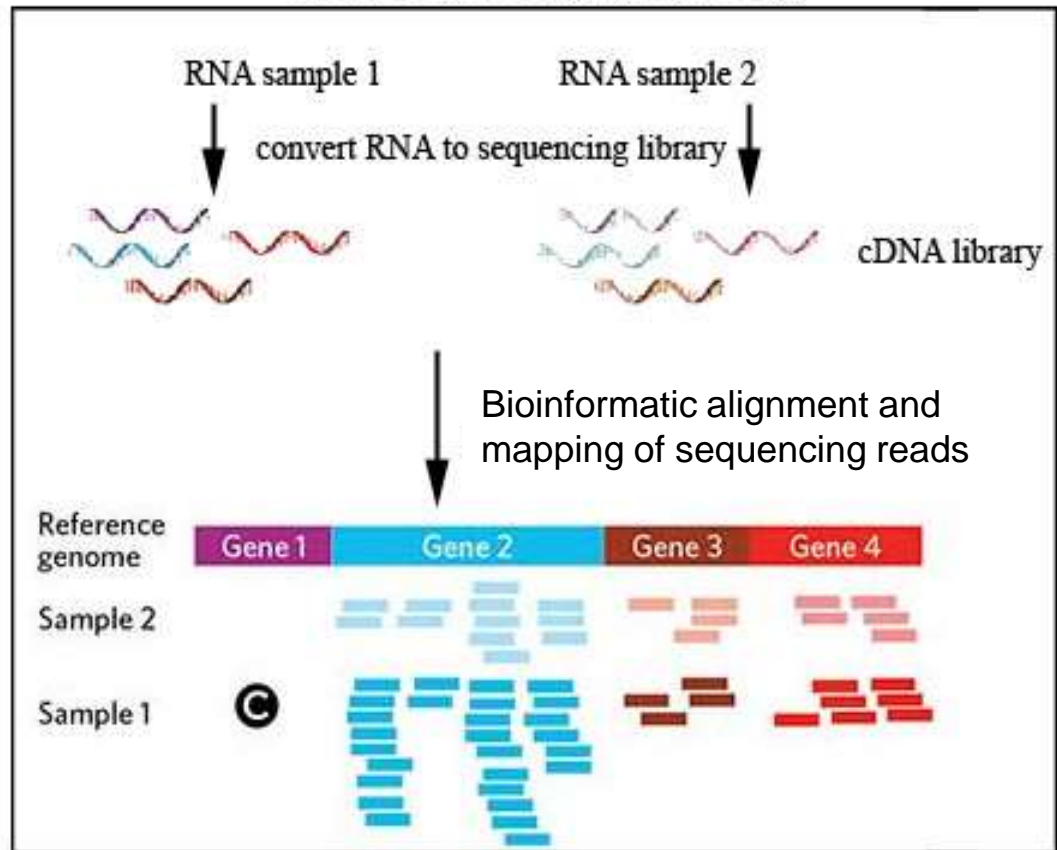
- 1) Known DNA «Probes» spotted on the microchip
- 2) Hybridization with fluorescent cDNA derived from sample mRNA by reverse transcription
- 3) Fluorescence detection of each spot to determine relative abundance of nucleic acid sequences in the target.



RNAseq



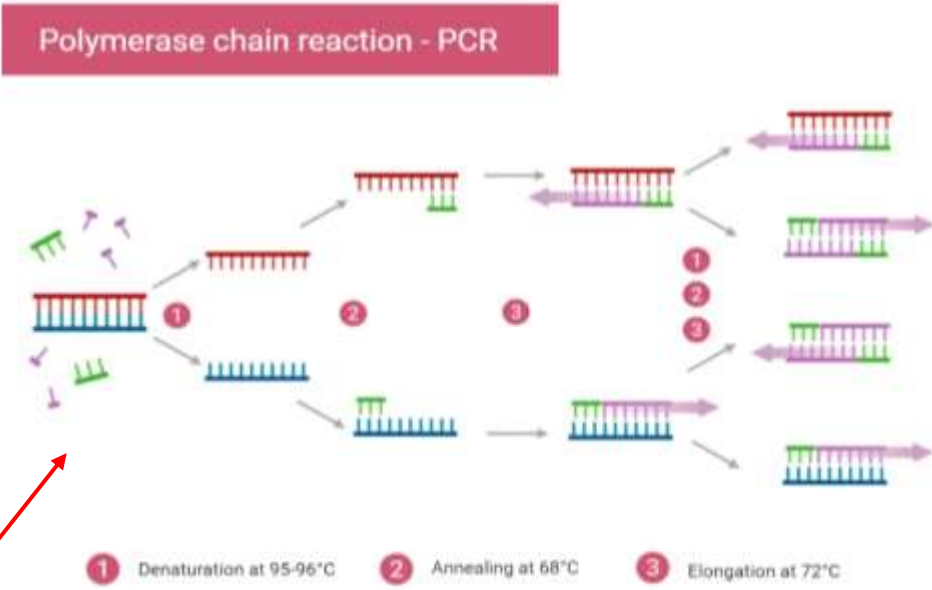
RNA Sequencing (RNA-Seq)



High sensitivity
High dynamic range
Novel transcripts sequences identified
structural variation & alternative splicing revealed
unlimited sample comparisons

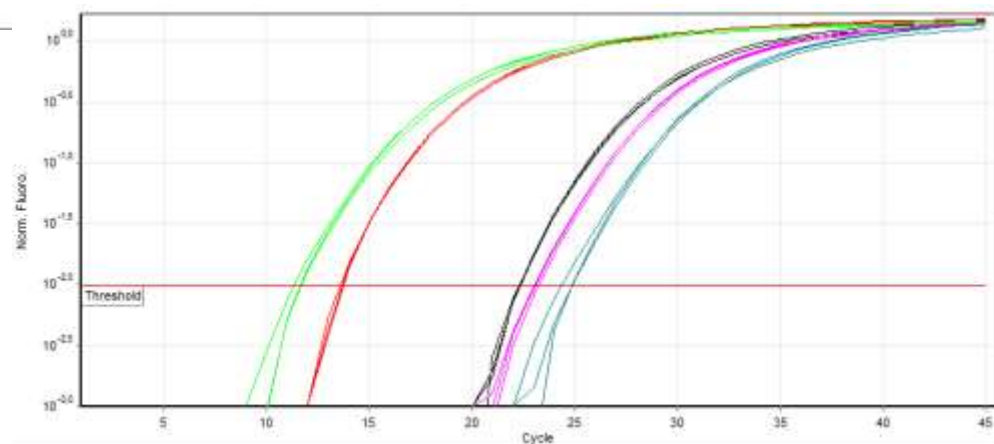
Sequencing Reads
=
expression levels

Real Time PCR /Quantitative PCR (qPCR)



mRNA → cDNA

Cell lysate



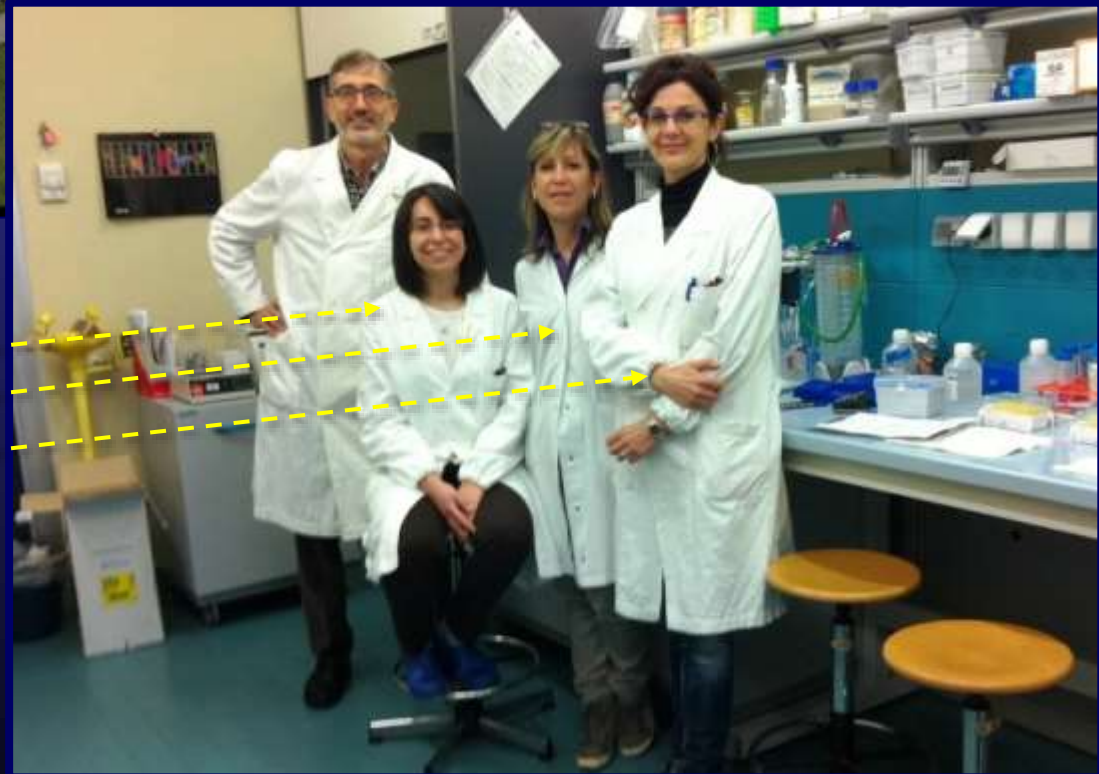
Verona's «Homeopathic gene expression» Group (2010-2021)



We thank for research grants:

- Laboratoires Boiron
- Standard Homeopathic/Hyland's
- Italian Research Ministry

Paolo Bellavite
Clara Bonafini
Marta Marzotto
Debora Oliosio



Others (not in figure): Fabio Arruda e Silva

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Gelsemium s.



Arnica m.



Drosera r.



Mini-review

Gelsemium showed an anxiolytic-like effect on laboratory mice

Psychopharmacology (2010) 210:533–545
DOI 10.1007/s00213-010-1855-2

2010

ORIGINAL INVESTIGATION

Dose-effect study of *Gelsemium sempervirens* in high dilutions on anxiety-related responses in mice

Paolo Magnani • Anita Conforti • Elisabetta Zanolin •
Marta Marzotto • Paolo Bellavite



Research Article

2012

Testing Homeopathy in Mouse Emotional Response Models: Pooled Data Analysis of Two Series of Studies

Paolo Bellavite,¹ Anita Conforti,² Marta Marzotto,¹ Paolo Magnani,¹ Mirko Cristofolletti,¹
Debora Oliosio,¹ and Maria Elisabetta Zanolin²

¹Department of Pathology and Diagnostics, University of Verona, 37134 Verona, Italy

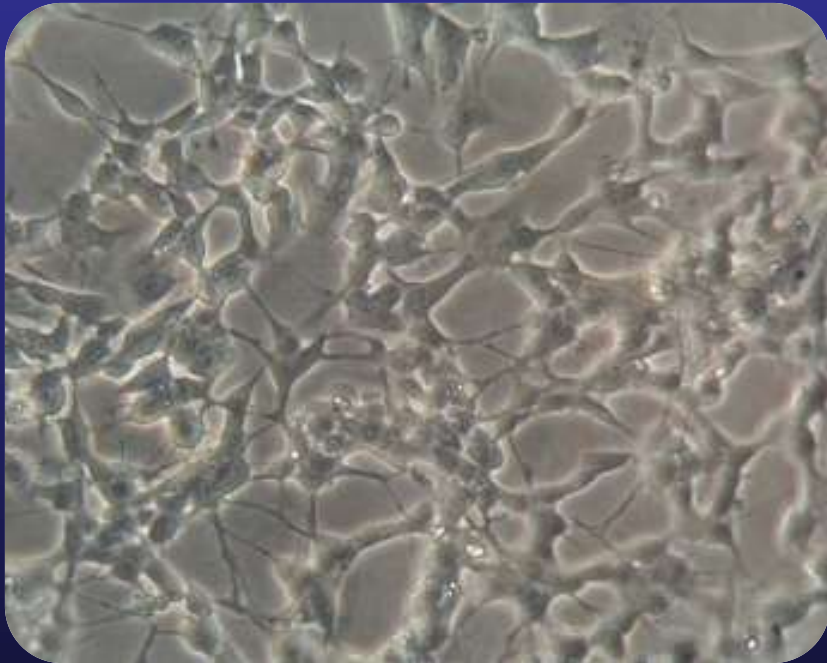
²Department of Public Health and Community Medicine, University of Verona, 37134 Verona, Italy



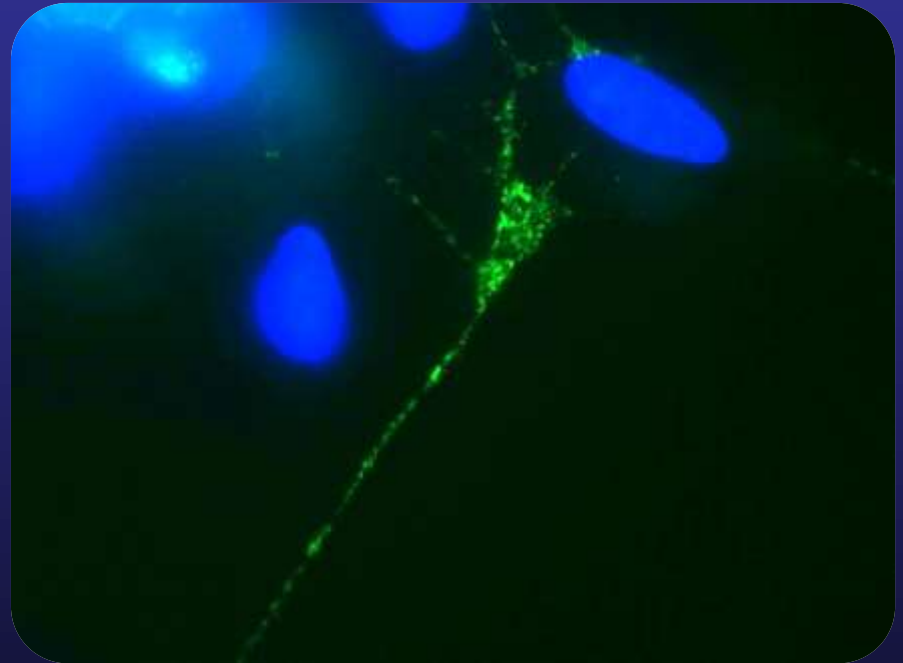
Gelsemium s. in a neuronal model

(Verona research group 2010-2014)

SH-SY5Y neurocytes-human neuroblastoma cells

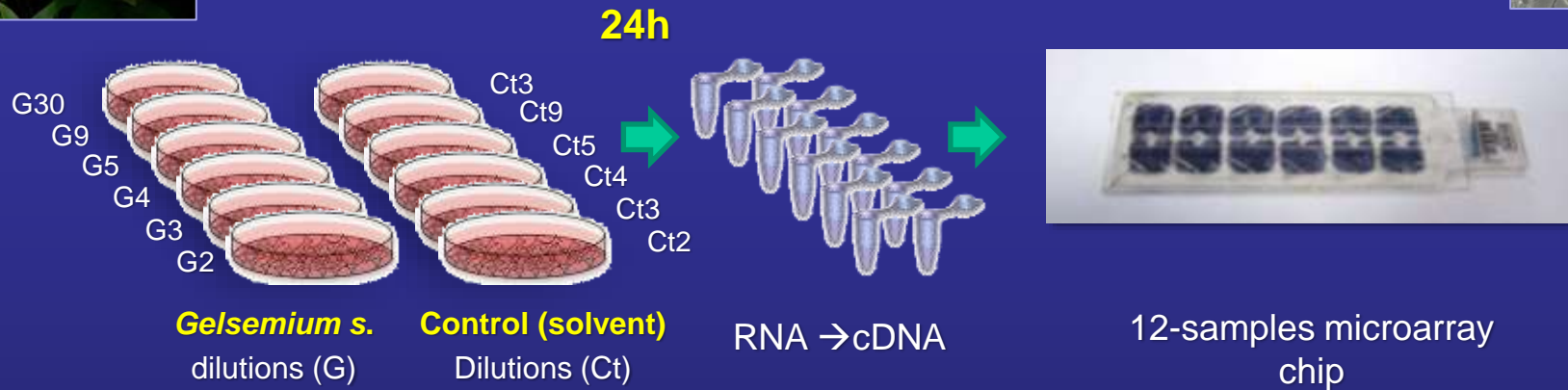
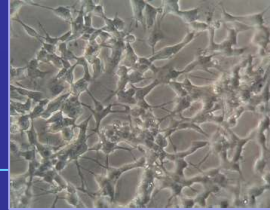


Inverted microscope image



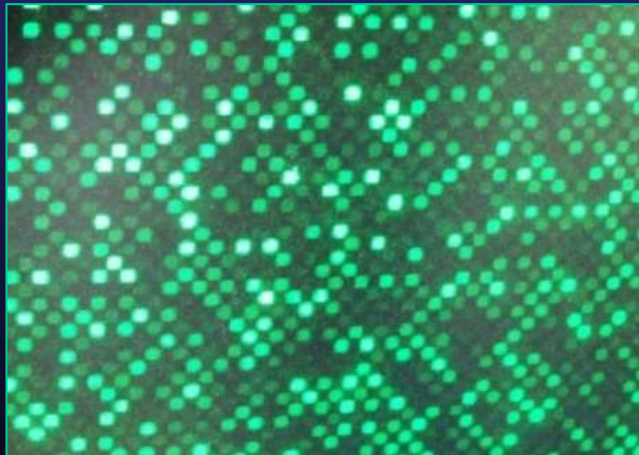
Confocal immunofluorescent
image

Microarray analysis of gene expression changes in human neurocytes



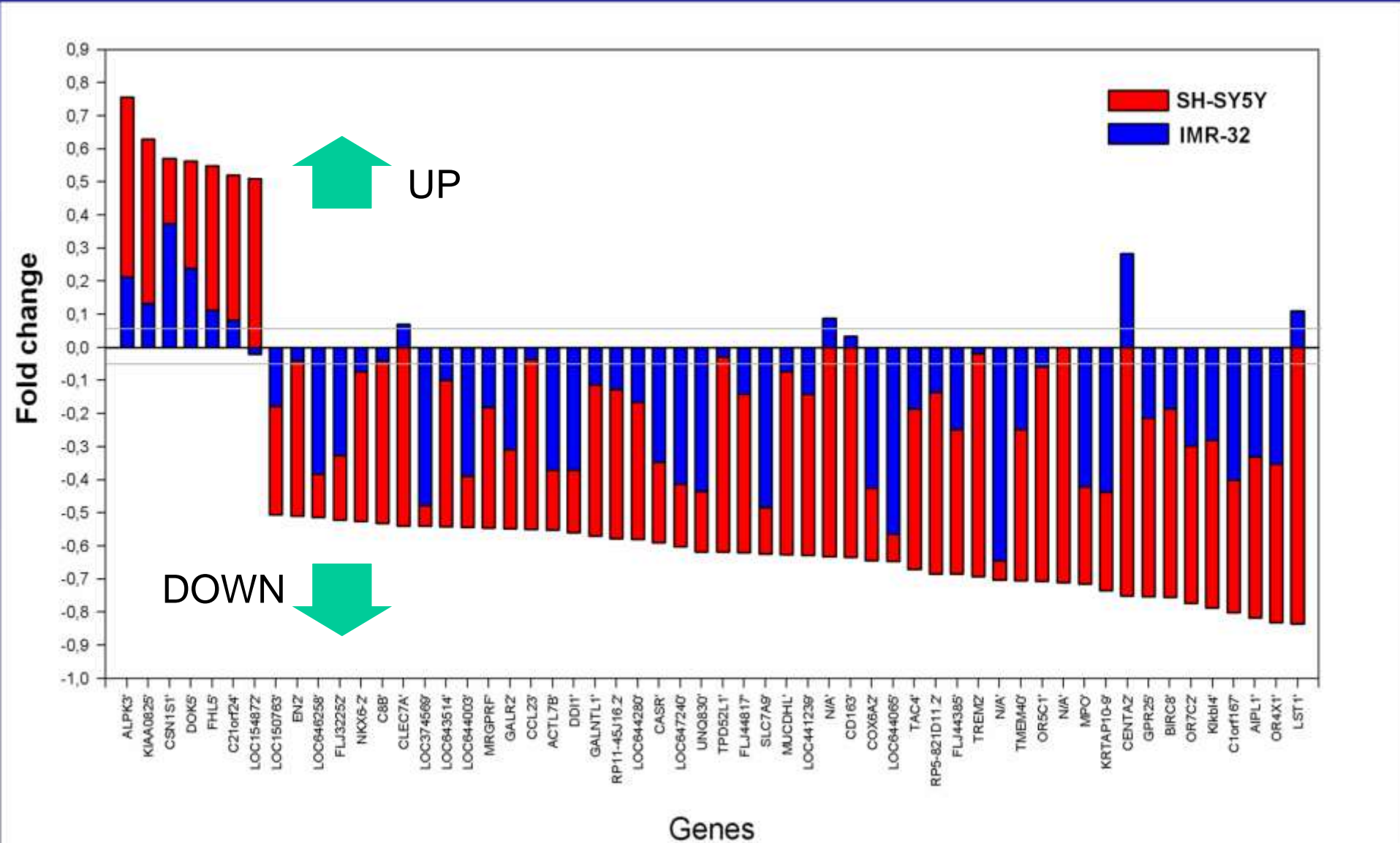
4 independent replicate experiments

- NimbleGen chip
- Entire human transcriptome (45034 genes!)



Scanning and data analysis

Differentially expressed genes in two neurocyte lines exposed to *Gelsemium s. 2C*



Significant changes of 56 genes induced by Gels 2CH (BMC-Complementary Alternative Medicine – 2014)

Many of these genes belong to:

- neuropeptide/receptor systems
- calcium signalling
- G-protein coupled transduction systems
- inflammatory pathways

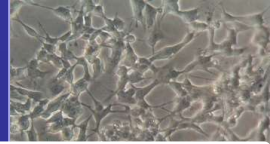
DOWN

UP

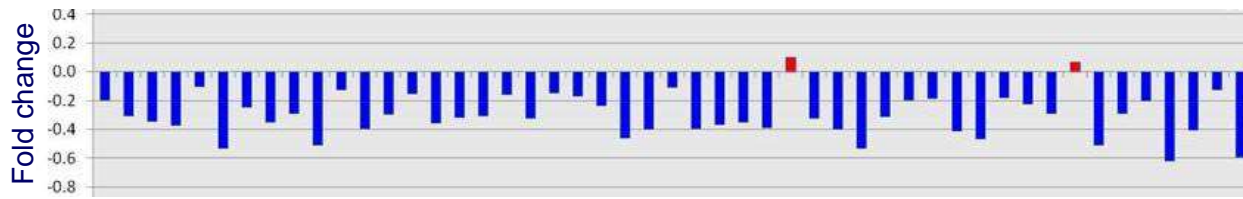
Gene ID	Transcript ID	Symbol	Log2 fold change	p ¹	Description	
7940	AF000424	LST1	-0.84	± 0.14	0.04	leukocyte specific transcript 1
390113	NM_001004726	OR4X1	-0.83	± 0.06	0.01	olfactory receptor, family 4, subfamily X, member 1
23746	AJ830742	AIP1L1	-0.82	± 0.16	0.04	aryl hydrocarbon receptor interacting protein-like 1
284498	AL833920	C1orf167	-0.80	± 0.17	0.05	chromosome 1 open reading frame 167
221191	AK058068	Kikbl4	-0.79	± 0.12	0.04	plasma kallikrein-like protein 4
26658	NM_012377	OR7C2	-0.77	± 0.07	0.01	olfactory receptor, family 7, subfamily C, member 2
112401	BC039318	BIRC8	-0.76	± 0.11	0.00	baculoviral IAP repeat-containing 8
2848	NM_005298	GPR25	-0.75	± 0.15	0.02	G protein-coupled receptor 25
55803	NM_018404	ADAP2	-0.75	± 0.11	0.02	ArfGAP with dual PH domains 2
386676	NM_198690	KRTAP10-9	-0.73	± 0.12	0.04	keratin associated protein 10-9
4353	X04876	MPO	-0.72	± 0.15	0.04	Myeloperoxidase
N/A	AY358413	N/A	-0.71	± 0.18	0.02	Homo sapiens clone DNA59853 trypsin inhibitor
392391	NM_001001923	OR5C1	-0.71	± 0.05	0.04	olfactory receptor, family 5, subfamily C, member 1
N/A	AK094115	N/A	-0.70	± 0.11	0.04	Homo sapiens cDNA FLJ36796 fis, clone ADRGL2006817
55287	BC020658	TMEM40	-0.70	± 0.15	0.02	transmembrane protein 40
54209	NM_018965	TREM2	-0.69	± 0.10	0.02	triggering receptor expressed on myeloid cells 2
150365	AK097834	RP5-821D11.2	-0.68	± 0.17	0.02	similar to mouse meiosis defective 1 gene
400934	NM_207478	FLJ44385	-0.68	± 0.09	0.04	FLJ44385 protein
255061	NM_170685	TAC4	-0.67	± 0.14	0.01	tachykinin 4 (hemokinin)
644065	XM_931993	LOC644065	-0.65	± 0.23	0.04	hypothetical protein LOC644065
1339	NM_005205	COX6A2	-0.64	± 0.17	0.01	cytochrome c oxidase subunit VIa polypeptide 2
N/A	AK128093	N/A	-0.63	± 0.09	0.04	Homo sapiens cDNA FLJ46214 fis, clone TEST14012623.
53841	AY358368	CDHR5	-0.63	± 0.11	0.04	mucin-like protocadherin
9332	NM_004244	CD163	-0.63	± 0.18	0.03	CD163 molecule
441239	XM_499305	LOC441239	-0.63	± 0.22	0.05	hypothetical gene supported by BC063653
7164	NM_001003397	TPD52L1	-0.62	± 0.09	0.02	tumor protein D52-like 1
11136	NM_014270	SLC7A9	-0.62	± 0.09	0.04	solute carrier family 7 member 9
389084	NM_206895	UNQ830	-0.62	± 0.11	0.04	ASCL830
400224	XM_375090	FLJ44817	-0.62	± 0.20	0.04	similar to pleckstrin homology domain protein (5V327)
647240	XM_934559	LOC647240	-0.60	± 0.06	0.00	hypothetical protein LOC647240
846	BC104999	CASR	-0.59	± 0.06	0.00	calcium-sensing receptor
116123	NM_138784	RP11-45J16.2	-0.58	± 0.09	0.04	flavin-containing monooxygenase pseudogene
644280	XM_497769	LOC644280	-0.58	± 0.06	0.05	hypothetical protein LOC644280
57452	AB032956	GALNTL1	-0.57	± 0.17	0.05	alpha-D-galactosamine N-acetylgalactosaminyltransferase
414301	NM_001001711	DDI1	-0.56	± 0.11	0.04	DDI1, DNA-damage inducible 1, homolog 1 (<i>S. cerevisiae</i>)
116535	BC016964	MRGPRF	-0.55	± 0.17	0.01	MAS-related GPR, member F
8811	NM_003857	GALR2	-0.55	± 0.07	0.04	galanin receptor 2
10880	NM_006686	ACTL7B	-0.55	± 0.12	0.04	actin-like 7B
6368	NM_145898	CCL23	-0.55	± 0.11	0.05	chemokine (C-C motif) ligand 23
64581	BC071746	CLEC7A	-0.54	± 0.08	0.04	C-type lectin domain family 7, member A
644003	XM_927256	LOC644003	-0.54	± 0.11	0.04	similar to Mucin-2 precursor (Intestinal mucin 2)
643514	XM_931594	LOC643514	-0.54	± 0.10	0.03	hypothetical protein LOC643514
374569	XM_935431	LOC374569	-0.54	± 0.07	0.04	Similar to Lysophospholipase
84504	BC101635	NKX6-2	-0.53	± 0.13	0.03	NK6 transcription factor related, locus 2 (<i>Drosophila</i>)
732	NM_000066	C8B	-0.53	± 0.06	0.05	complement component 8, beta polypeptide
146336	NM_182510	FLJ32252	-0.52	± 0.03	0.01	hypothetical protein FLJ32252
150763	BC042847	LOC150763	-0.51	± 0.10	0.04	hypothetical protein LOC150763
2020	NM_001427	EN2	-0.51	± 0.08	0.04	engrailed homolog 2
646258	XM_929203	LOC646258	-0.51	± 0.11	0.04	hypothetical protein LOC646258
154872	NM_001024603	LOC154872	0.51	± 0.10	0.03	hypothetical LOC154872
400866	NM_001001789	C21orf24	0.52	± 0.12	0.05	chromosome 21 open reading frame 24
9457	NM_020482	FHL5	0.55	± 0.19	0.04	four and a half LIM domains 5
55816	NM_018431	DOK5	0.56	± 0.04	0.03	docking protein 5
1446	NM_001890	CSN1S1	0.57	± 0.09	0.04	casein alpha s1
285600	AK130941	KIAA0825	0.63	± 0.06	0.01	KIAA0825 protein
57538	NM_020778	ALPK3	0.76	± 0.10	0.01	alpha-kinase 3



Effects of *Gelsemium* increasing dilutions/dynamizations on the expression of 49 Gels C2-down-regulated genes



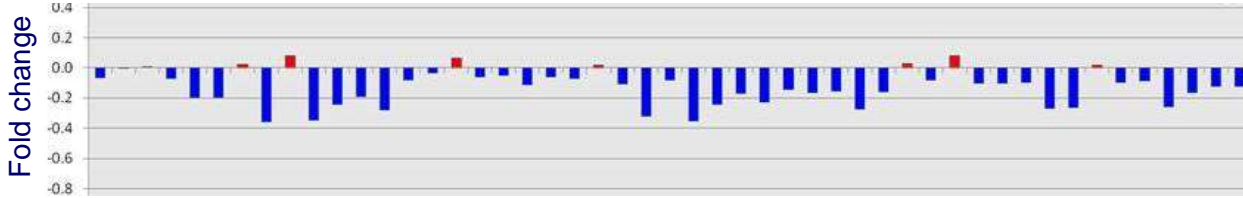
Gels 3c
/Placebo 3c



Gels 4c
/Placebo 4c



Gels 5c
/Placebo 5c



Gels 9c
/Placebo 9c



Gels 30c
/Placebo 30c



Genes

LST1
KRTAP10-9
OR4X1
BIRC8
FUJ44385
C1orf167
OR5C1
AIP1
MPO
TMEM40
OR7C2
GPR25
DDI1
LOC644065
N/A
KIB14
N/A
N/A
TACA
SLC7A9
LOC643514
CASR
ACTL7B
TREM2
LOC646258
CENTA2
TPD52L1
LOC441239
LOC647240
C8B
MUCDH1
CLEC7A
LOC644003
FUJ3252
MRGPRF
EN2
LOC150763
COX6A2
GALR2
RP11-45J16.2
RPS-82J11.2
CCL23
FUJ44817
CD163
GALNT1
UNC830
LOC374569
LOC644280
NKX6-2

N. Genes
UP/DOWN

2↑
47↓

<0.001

[Gelsemine]
= 1.3 x 10⁵
mol/cell

3↑
42↓

<0.001

[Gelsemine]
= 1300
mol/cell

3↑
38↓

<0.001

[Gelsemine]
= 13
mol/cell

9↑
30↓

<0.01

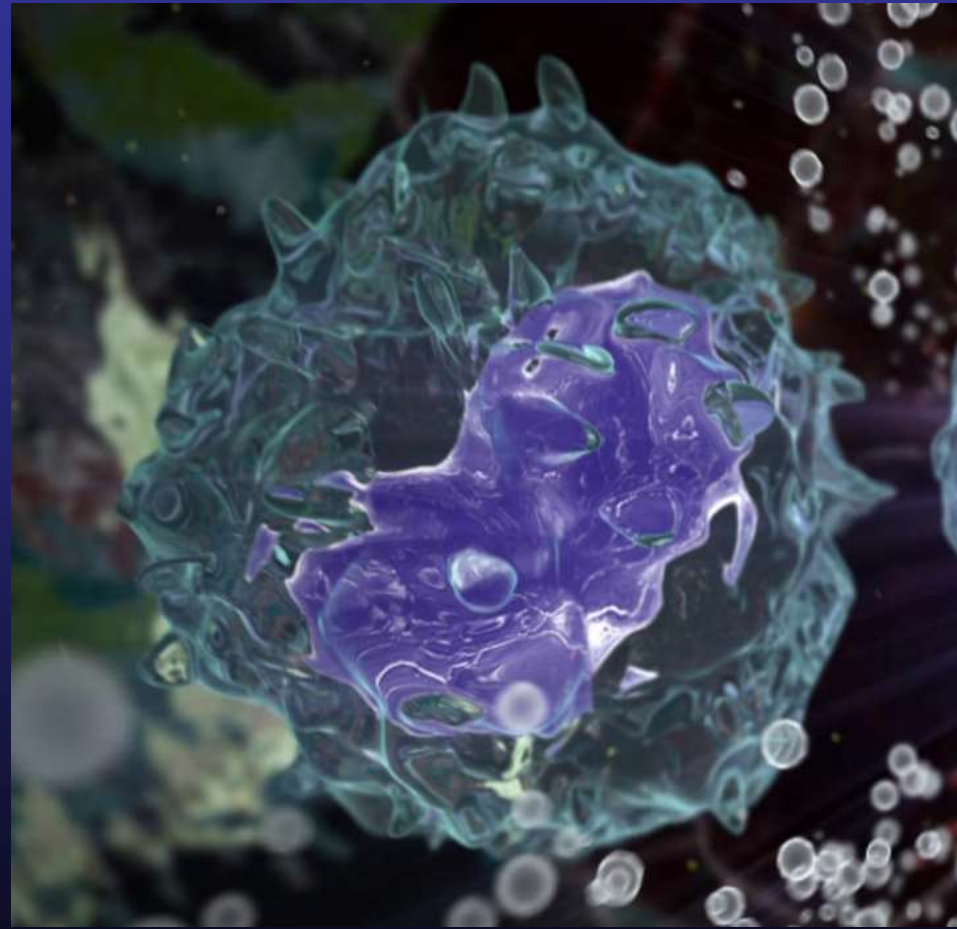
[Gelsemine]
< 1
mol/cell

7↑
27↓

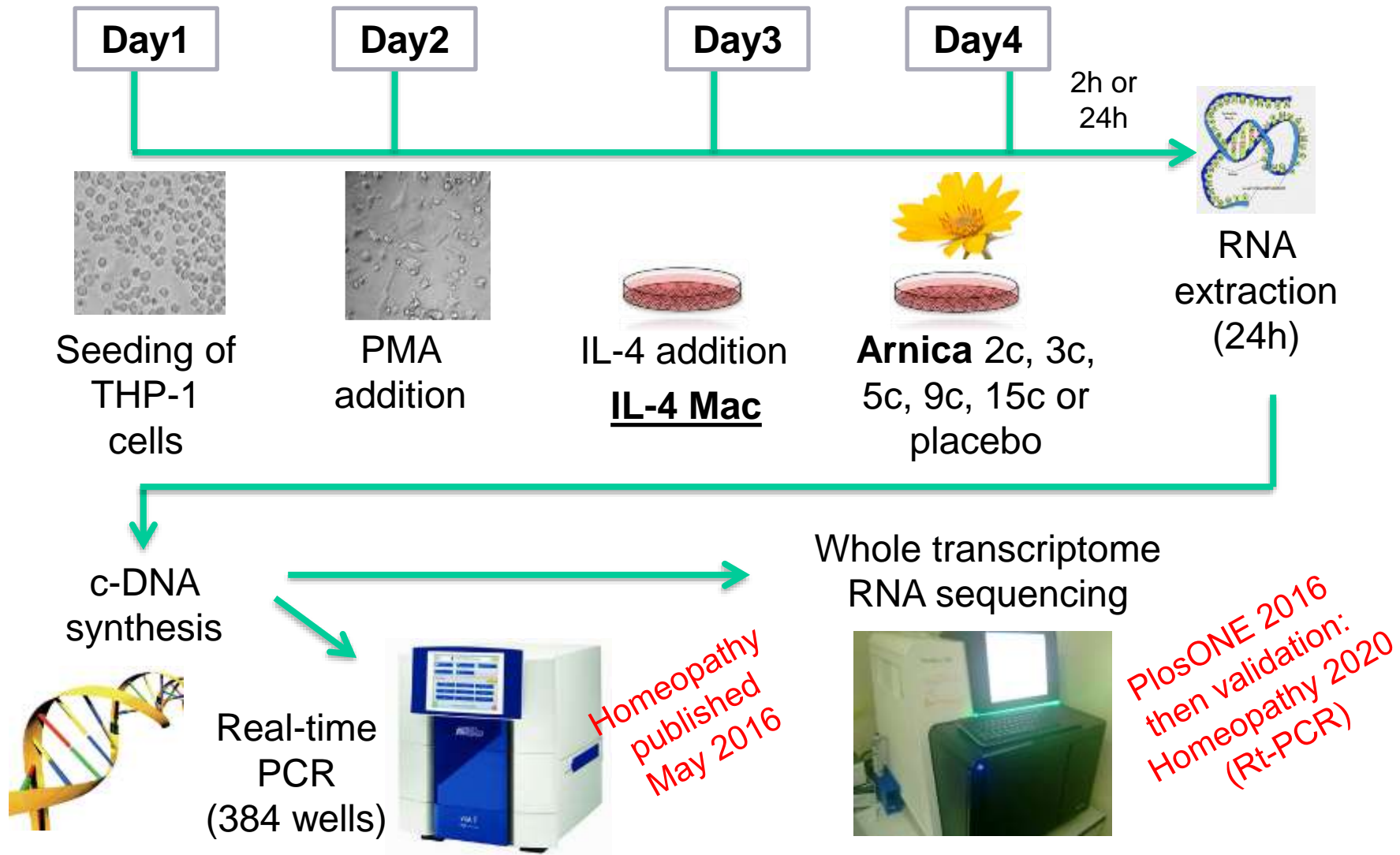
<0.01

[Gelsemine]
= 0
mol/cell

Evaluation of *Arnica montana* dilutions on gene expression in a human macrophage cell line (Verona's group 2015-2020)

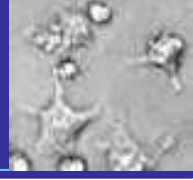


Macrophages treatment with *Arnica m.* and gene expression (Verona group 2015-2020)





Up-regulated genes of THP-1 macrophages treated for 24h with *Arnica m. 2c* (n=5). RNA-seq study



Effect	HGC Nomenclature		Mean RPKM	Log ₂ Fold Change			Description
	Symbol	ID		Mean	SE	P value	
Up-reg	CR1	2334	1.8	0.3	0.07	0.0225	complement component (3b/4b) receptor 1
	LRP1	6692	21.5	0.29	0.05	< 0.0001	low density lipoprotein receptor-related protein 1
	FN1	3778	467.9	0.27	0.05	0.0007	fibronectin 1
	FBN2	3604	3.3	0.27	0.09	0.0418	fibrillin 2
	HSPG2	5273	10.4	0.25	0.09	0.0332	heparan sulfate proteoglycan 2
	KMT2D	7133	6.9	0.24	0.06	0.0037	lysine (K)-specific methyltransferase 2D
	MACF1	13664	2.7	0.24	0.08	0.0379	microtubule-actin crosslinking factor 1

Fibronectin

MATBIO-1277;

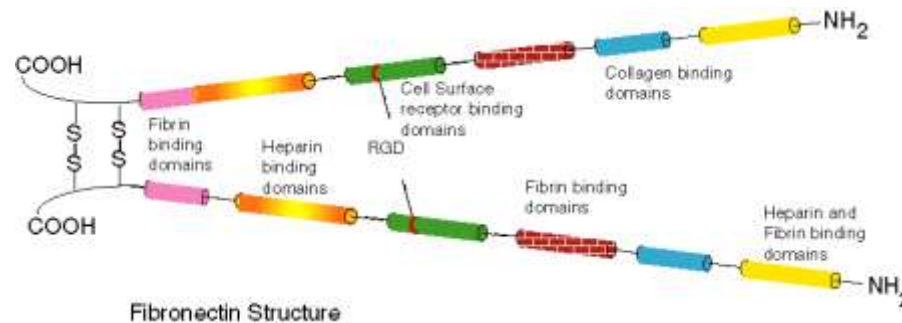
Matrix Biol. Aug. 2016. doi: 10.1016/j.matbio.2016.07.011.



Fibronectin, the extracellular glue

Alicia J. Zollinger and Michael L. Smith

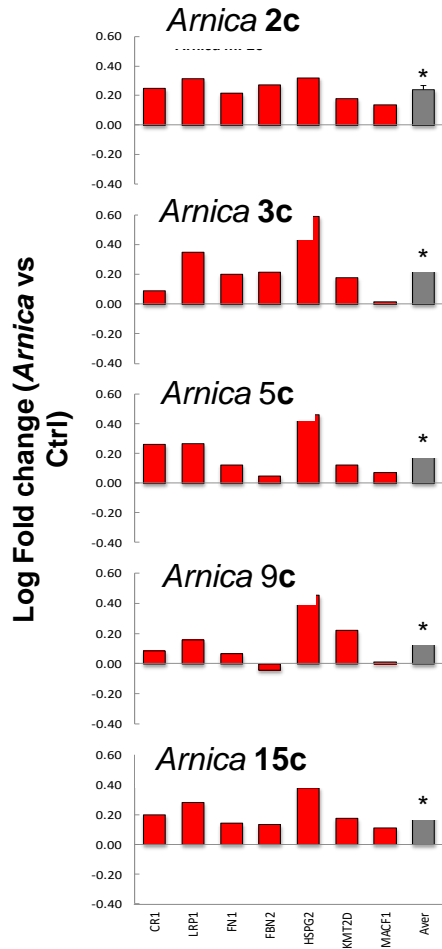
Department of Biomedical Engineering, Boston University, Boston, MA



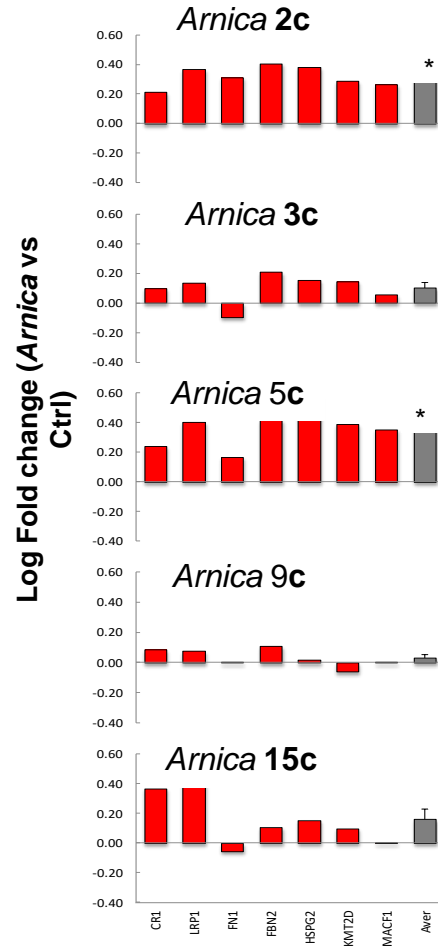
Effect of *Arnica m.* on macrophages pre-treated with different agents



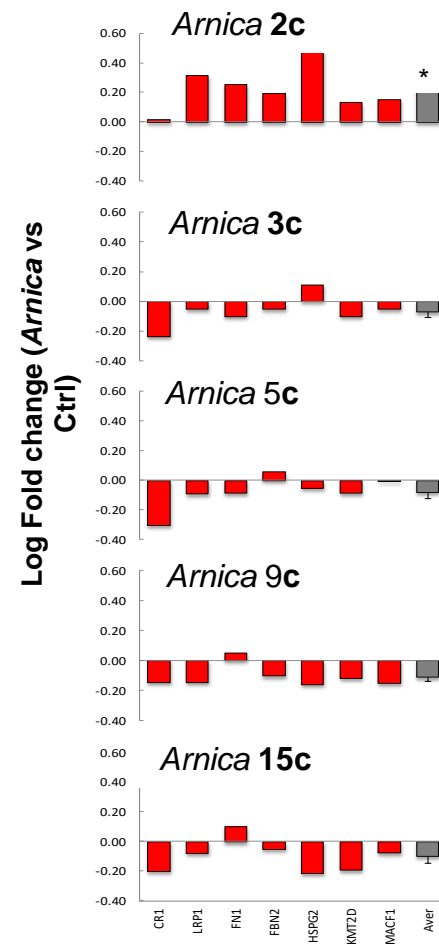
A Interleukin-4



B Endotoxin (LPS)



C Untreated (Resting-Mφ)



pool of 5 exp

scientific reports



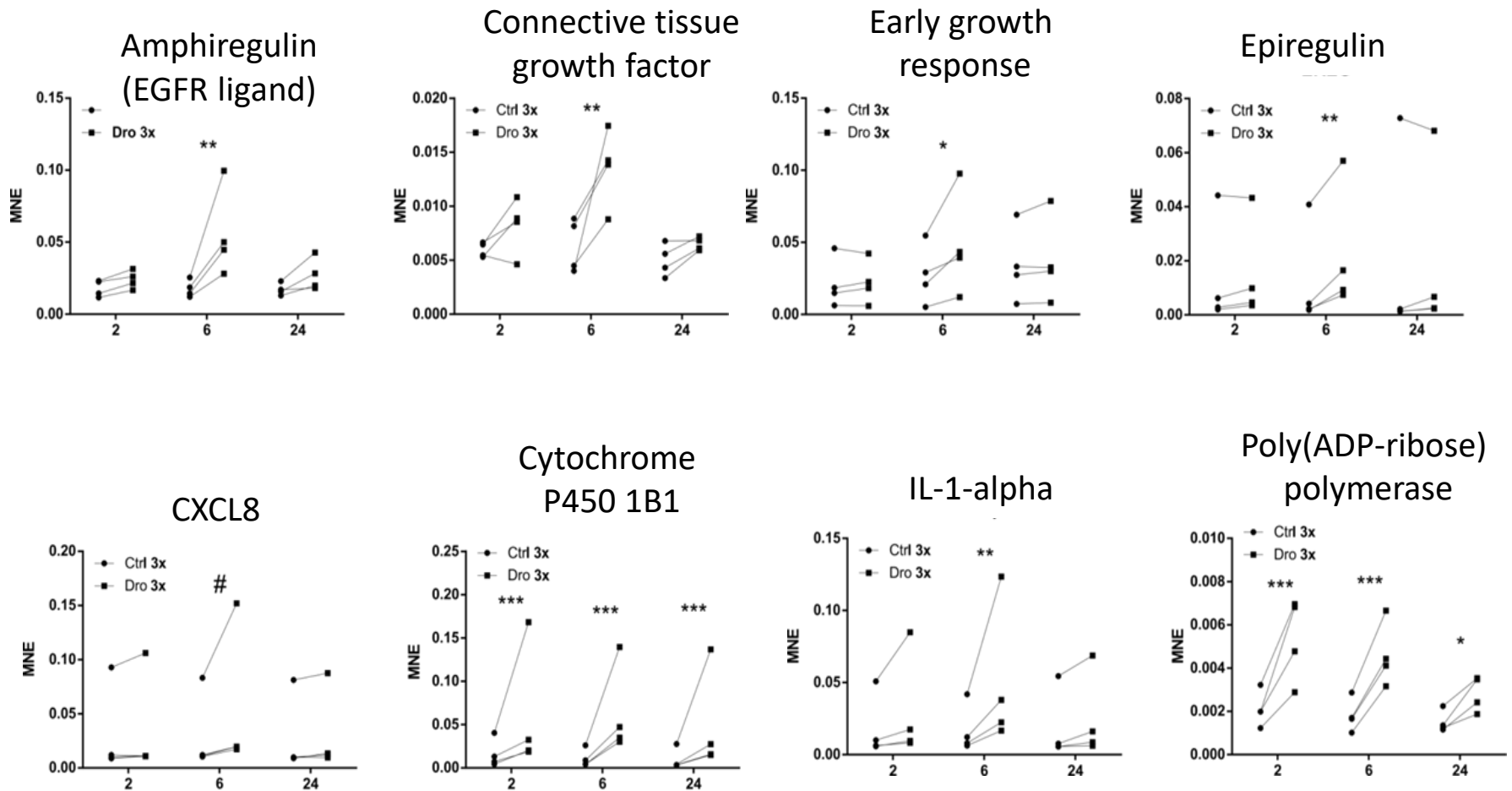
OPEN

Low-dose *Drosera rotundifolia* induces gene expression changes in 16HBE human bronchial epithelial cells

Fabio Arruda-Silva[✉], Paolo Bellavite & Marta Marzotto



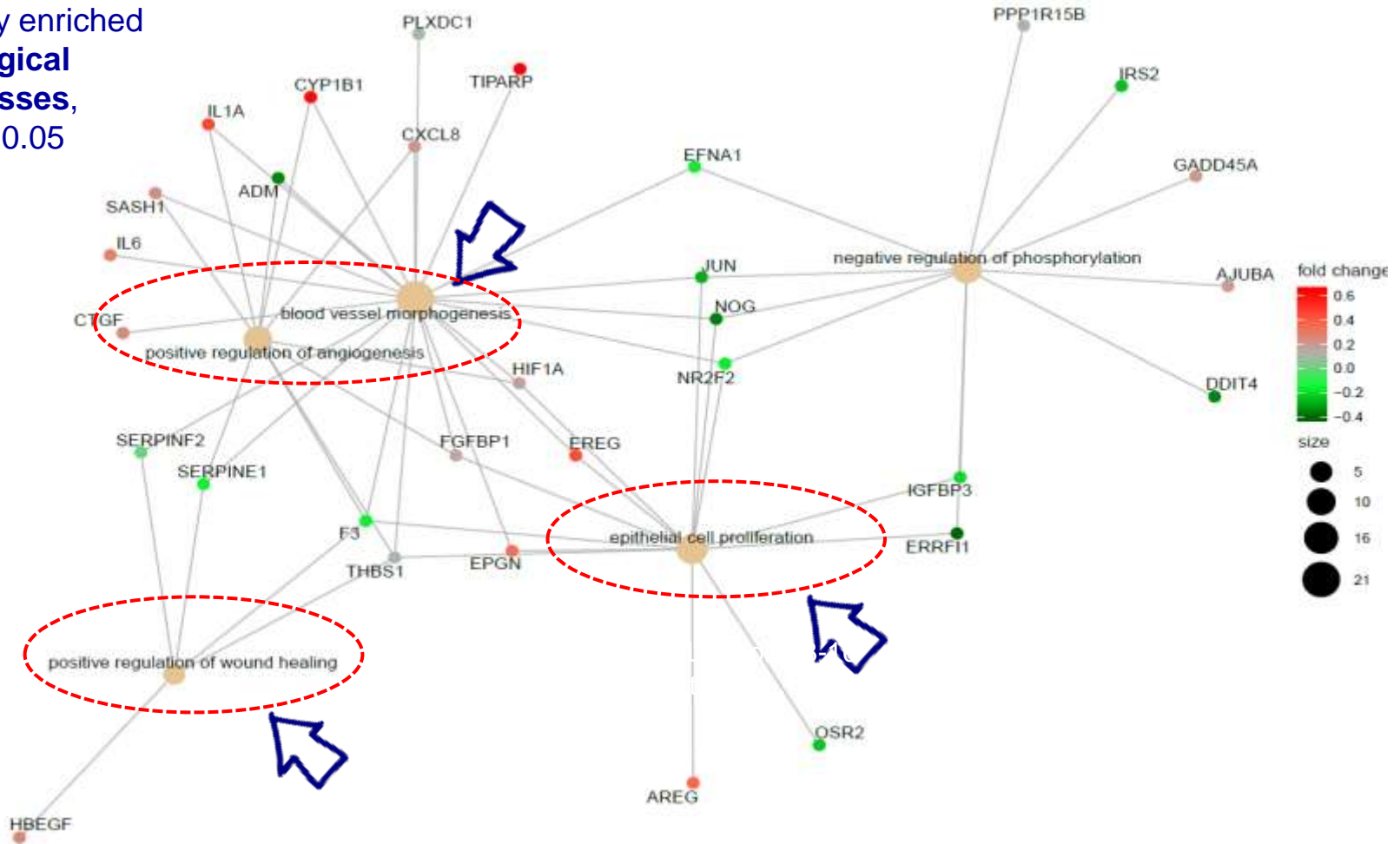
Gene expression in 16HBE bronchial cells with *D. rotundifolia* 3x



Arruda e Silva et al. 2021 Scientific Reports

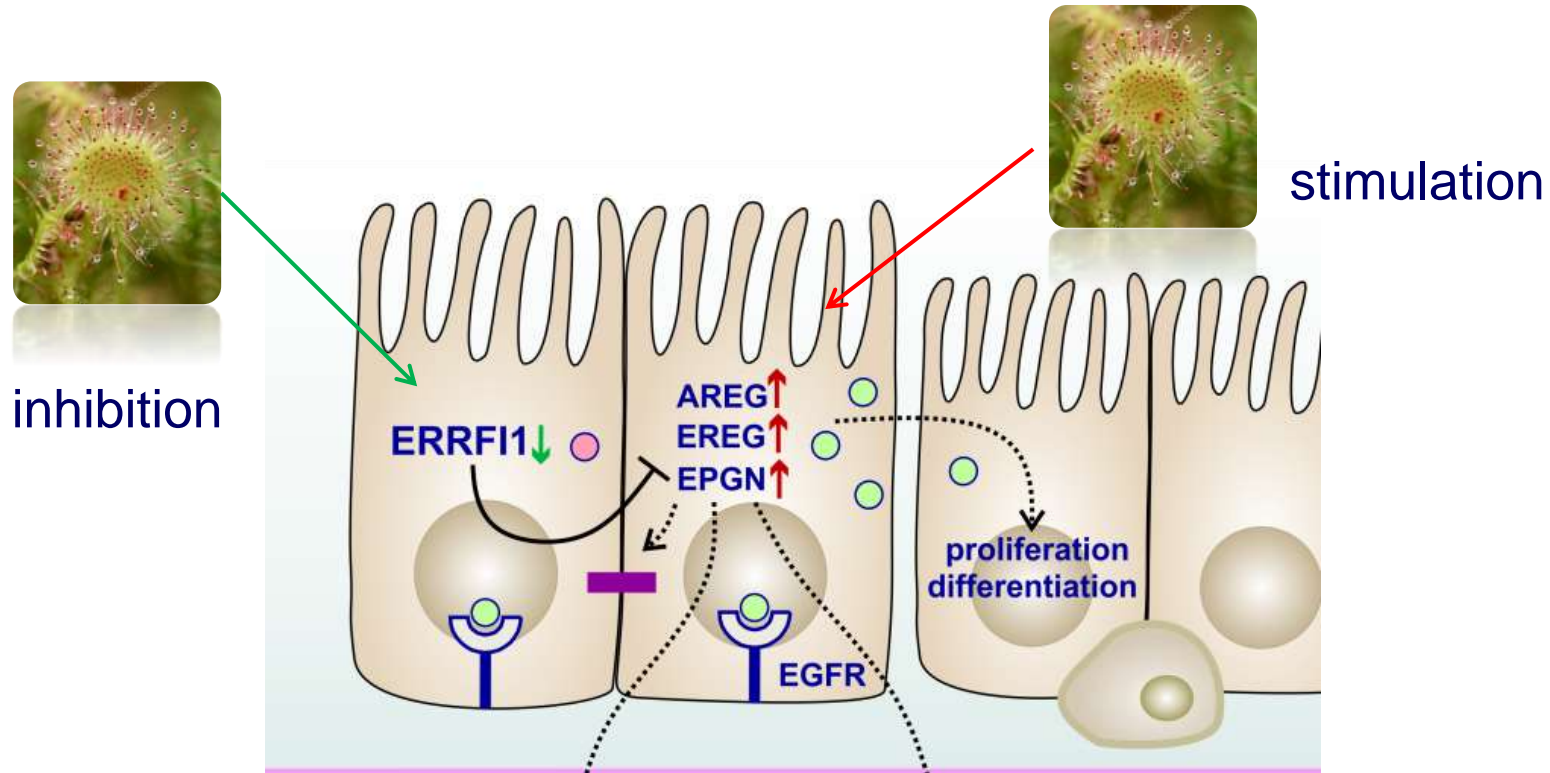
Gene regulatory networks of bronchial cells affected by Drosera 3X

The 5 most significantly enriched Biological Processes, padj<0.05



Arruda e Silva et al. 2021 Scientific Reports

Drosera modulates the mechanisms of epithelial tissue healing



Improves the healing of bronchial mucosa

Gene Ontology analysis of DEGs in cells treated with 3x and 6x. BP:
Biological process; MF: Molecular function

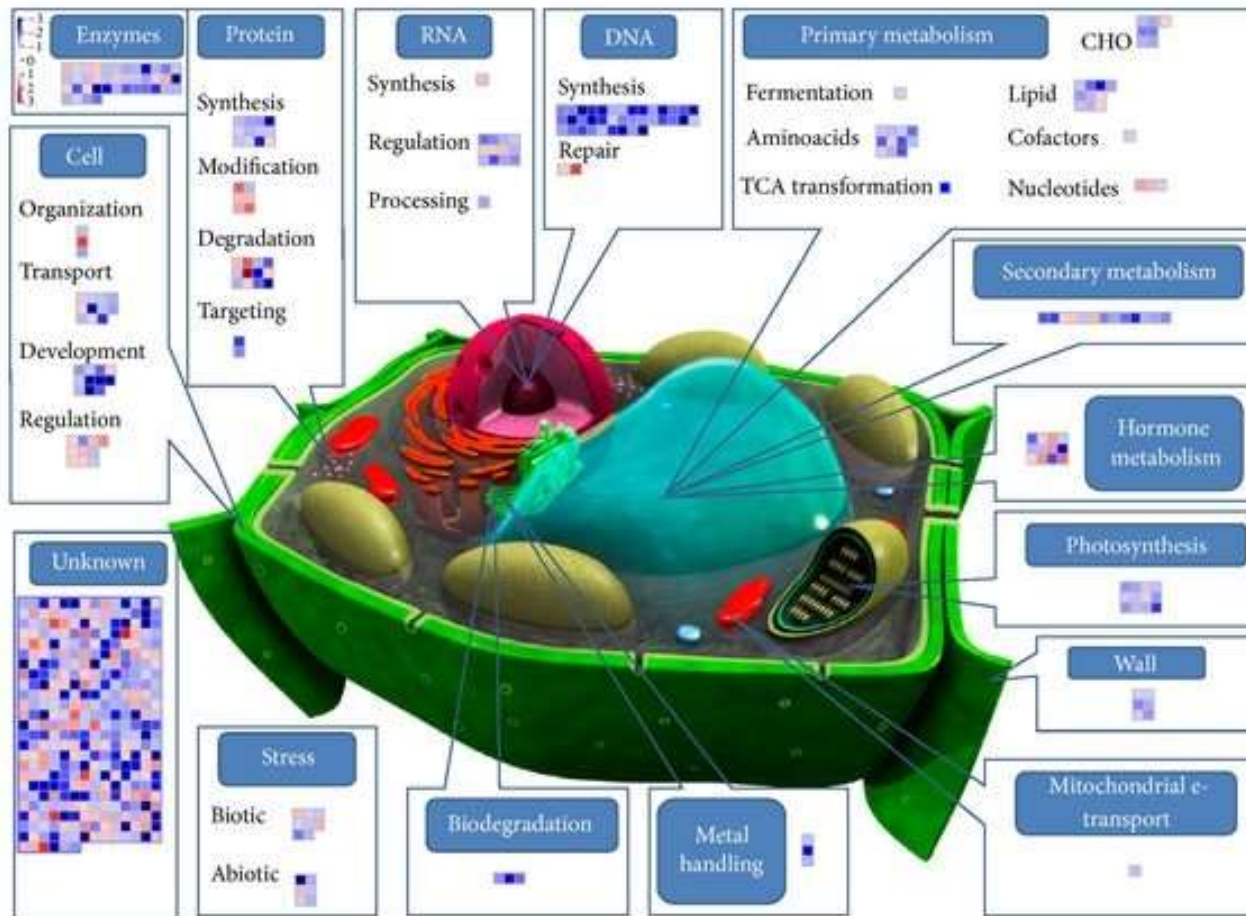
Source	Dilution	Term name	Term ID	Adjusted p	N. genes
GO:BP	3x	response to hypoxia	GO:0001666	2.13E+09	28
GO:BP	3x	blood vessel morphogenesis	GO:0048514	2.34E+09	40
GO:BP	3x	regulation of cell adhesion	GO:0030155	0.00004	37
GO:BP	3x	epithelial cell proliferation	GO:0050673	0.0017	25
GO:MF	3x	cell adhesion molecule binding	GO:0050839	9.48E+07	34
GO:MF	3x	enzyme regulator activity	GO:0030234	0.0003	47
GO:MF	3x	growth factor activity	GO:0008083	0.016	12
GO:MF	6x	GTPase regulator activity	GO:0030695	0.021	14

Evidence of Homeopathy and gene expression

Test compound	Potencies	Cell type	Effect	REF
Canova complex	11D-12D	Mouse macrophages	↑ expression of 45 genes ↑ expression of 102 genes	(Oliveira et al. 2008)
Carcinosinum	MT, 30C, 200C	DLA cells	↑ specific gene expression (p53 pro-apoptotic)	(Sunila et al. 2009)
Arsenicum alb.	30C	Saccharomyces cerevisiae, E. coli	↑ Resistance to arsenicum toxicity ↓ expression of specific genes (apoptotic, stress response proteins)	(Das et al. 2011; De et al. 2012 of Khuda-B.group)
Carcin., Ruta, Hydrastis, Thuja	200C	DLA cells	↑ Apoptosis, ↓ Gene expression (whole genome analysis)	(Preethi et al. 2012)
Gelsemium s.	2C, 3C, 5C, 9C, 30C	Human neurocytes SHSY5Y	7 genes ↑ 49 genes ↓ expression (whole genome analysis) ↓ gene expression (RT-Array, 2C)	(Marzotto et al. 2014; Oliosio et al. 2014)
Apis mellifica	3C, 5C, 7C, 15C, 30C	Human prostate RWPE-1	↑↓ expression of different groups of genes (whole genome analysis)	(Bigagli et al. 2014, 2016)
Rhus tox.	30X	Primary cultured chondrocytes	↑ gene expression (COX-2), ↓ specific gene expression (collagen II; de-differentiation role)	(Huh et al. 2013)
Arsenicum alb.	45X	Arsenic-intoxicated wheat seeds	↑ Germination ↓ Gene expression levels	(Marotti et al. 2014)
Condurango	30C	H460-non-small-cell lung cancer cells	↓ expression of specific genes (apoptotic), ↑ Apoptosis, oxidative stress, mitochondrial depolarization	(Sikdar et al. 2014)
Arnica montana	2C, 3C, 5C, 9C, 15C	THP-1 macrophages	↓ expression of TNF, IL1B, CCL5, MMP1 ↑ expression of CXC chemokines and BMP2 (in IL-4 differentiated cells) and Fibronectin	(Oliosio et al. 2016 Marzotto et al, 2016)
Drosera rotundifolia	3D-6D	16HBE bronchial cells	↓ 25 genes ↑ 44 genes, epithelial cell proliferation	(Arruda et al. 2021)

Transcriptome Profiling of Wheat Seedlings following Treatment with Ultrahigh Diluted Arsenic Trioxide (45X)

Marotti et al. Evid Based Complement Alternat Med . 2014:2014:851263. doi: 10.1155/2014/851263. Epub 2014 Nov 27



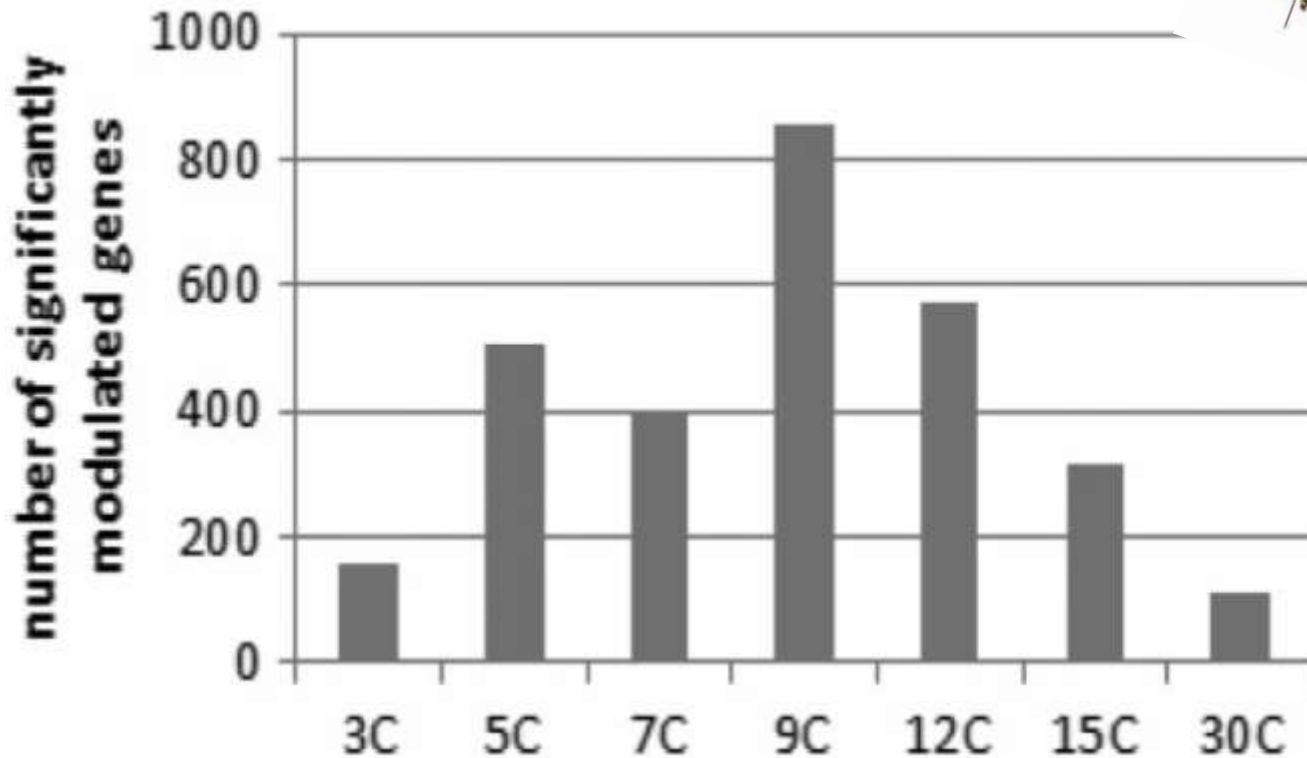


Effects of Extreme Dilutions of *Apis mellifica* Preparations on Gene Expression Profiles of Human Cells

Elisabetta Bigagli¹, Cristina Luceri¹, Andrea Dei², Simonetta Bernardini³, and Piero Dolara¹

E. Bigagli

Apis vs ethanol:water



Number of genes significantly up- or downregulated comparing cells exposed to *Apis mellifica* preparations versus cells exposed to the reference ethanol:water solutions (Apis vs ethanol–water).

Dose-Response:
An International Journal
January-March 2016:1-7



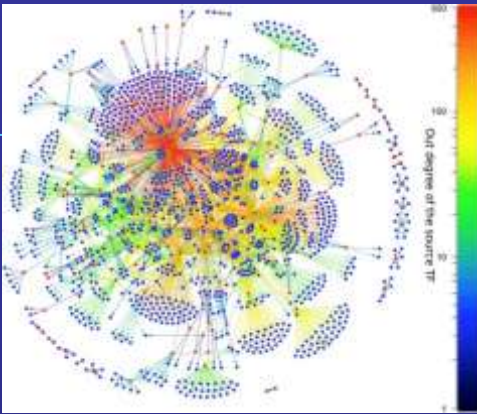
ECH

Spring Meeting

Bergamo 25-27 April 2024

HOMEOPATHY AND GENE EXPRESSION

1. Introduction
2. Methods
3. Results
- 4. Discussion**

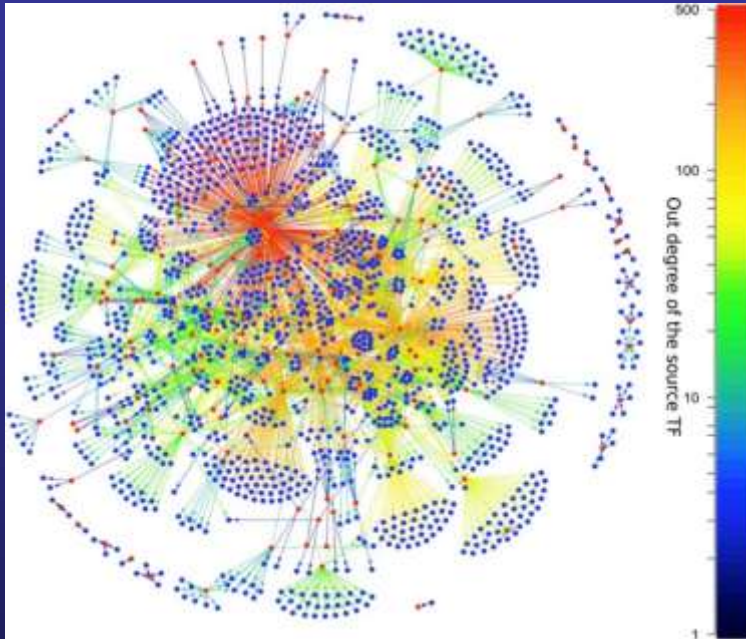


Synthesis of major achievements

- **Cell cultures respond to homeopathic dilutions** with the expression of dozens of genes, which are organized in functional networks (e.g. *Drosera*)
- Highest changes “in vitro” have been observed using **low dilutions (low doses, in the ponderal range)** (e.g. *Gelsemium* 2C, *Arnica* 2C, *Drosera* 3X)
- Detectable activity remains even at **high dilutions/dynamizations** (e.g. *Arnica* 15C*, *Gelsemium* 9C and 30C*, *Drosera* 6X)
- Among the differentially expressed genes there are some whose **function is coherent with the mechanism of action of the remedy**: *Gelsemium*: genes that reduce the excitability of neurons; *Arnica*: increase in chemokines and above all fibronectin, the intercellular “glue”; *Drosera*: genes that are correlated with the proliferation of bronchial cells and epithelial repair.

* beyond Avogadro molecular threshold

High sensitivity of gene networks

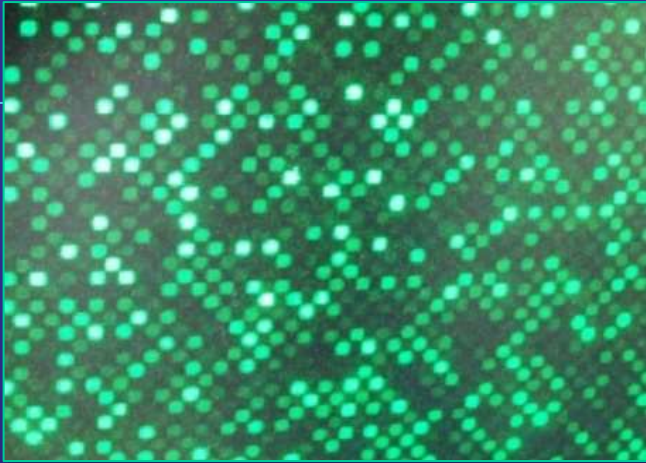


Detectable activity remains even at high dilutions

Poor Avogadro!



NOTE: In these systems high dilutions/dynamizations have less activity than low dilutions/ponderal doses. This suggests that homeopathic “potency” depends on the “potency” of the living organism, not of the remedy.

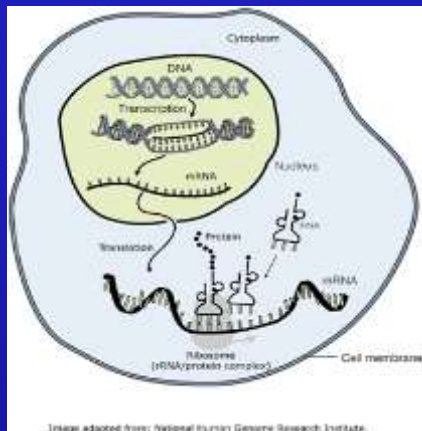


Limitations of basic research on transcriptomics

- High sensitivity means also high variability and difficult reproducibility
- High cost of laboratory instruments, reagents and working time
- Many (with drosera 6x most) of newly identified DEGS have no a clearly defined meaning yet
- Theoretical advances not easily applicable to clinical homeopathy

LOOKING FORWARD: HOMEOPENOMICS AND HOMEOPENETICS

Homeopenomics: Effects of homeopathic remedies on gene expression



- Cell and tissue targets
- «Provings»?

Homeopenetics: Individual genetic sensitivity to specific homeodrugs



- Whole genome sequencing
- Group classifications («constitutions», «miasms»?)



**THANKS FOR
YOUR ATTENTION!**