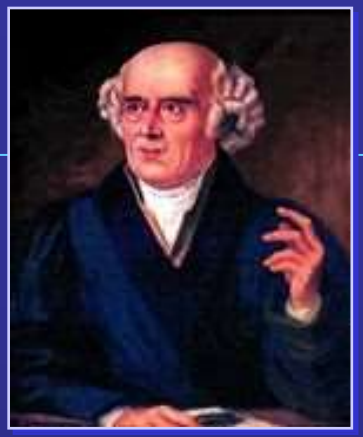
Paolo Bellavite
(Verona, I)

HOMEOPATHY AND GENE EXPRESSION

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

Organon, Par. 16

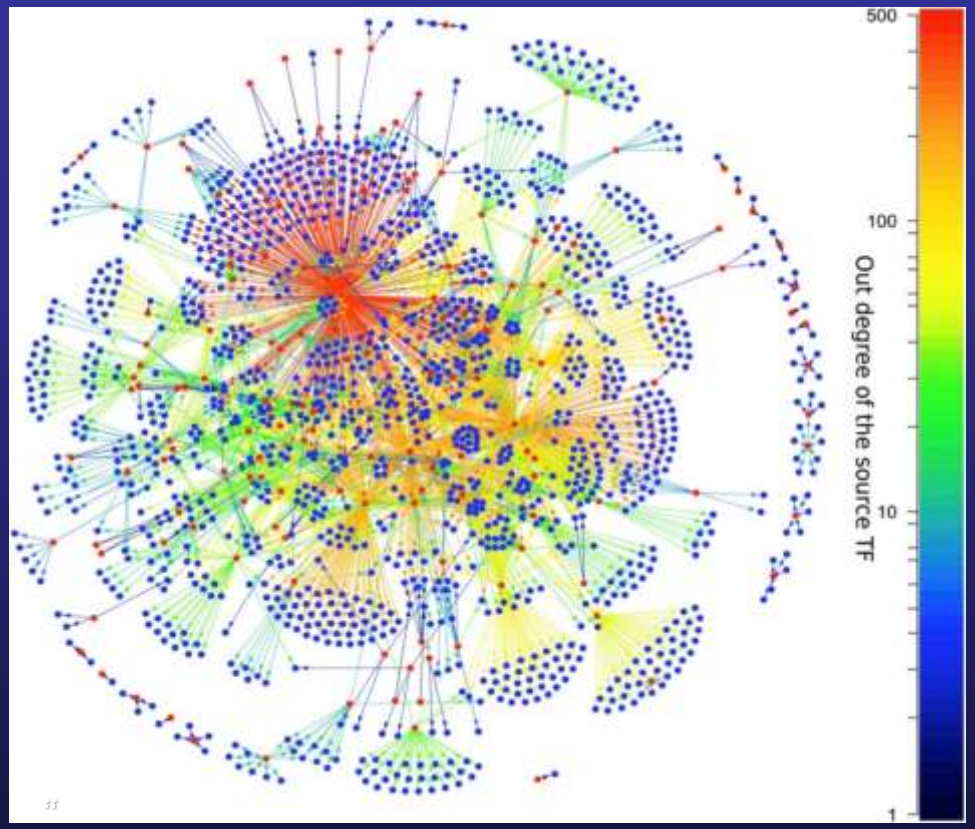


C.F.S. Hahnemann
(1755-1843)

"It is only by their dynamic action on the vital force that remedies are able to re-establish and do actually re-establish health and vital harmony"

«*Dynamis*»: «Energy», «Movement», «Evolution»
«*Vital force*»: Dynamic equilibrium of self-organized physiologic attractors in the energy landscape

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)

See also *Cell sensitivity, non-linearity and inverse effects*, by P. Bellavite et al., Homeopathy 2015 Apr;104(2):139-60. doi: 10.1016/j.homp.2015.02.002

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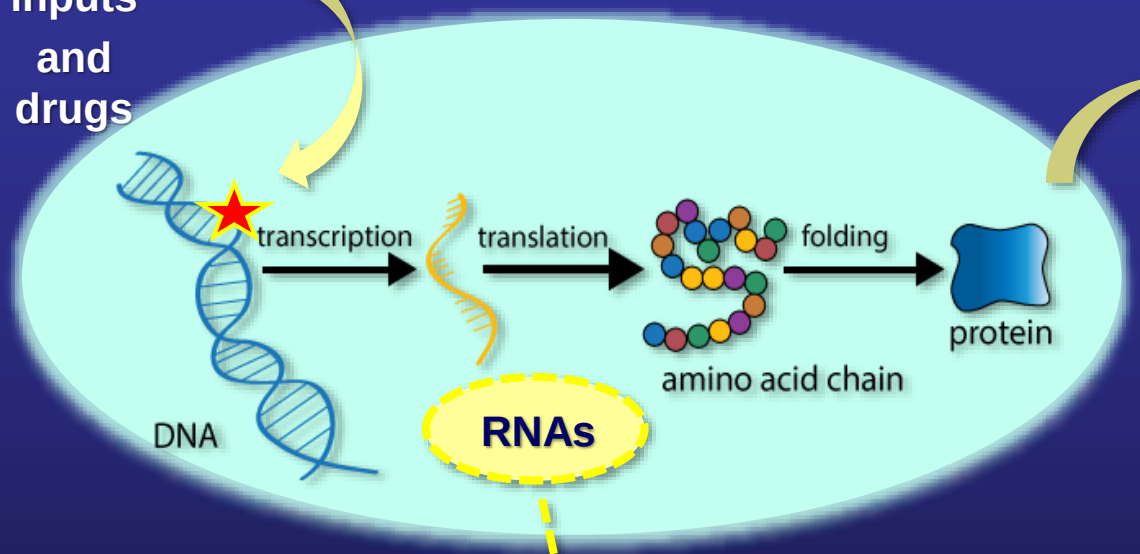
Analysis of cell responses through the transcriptome

Environmental inputs and drugs

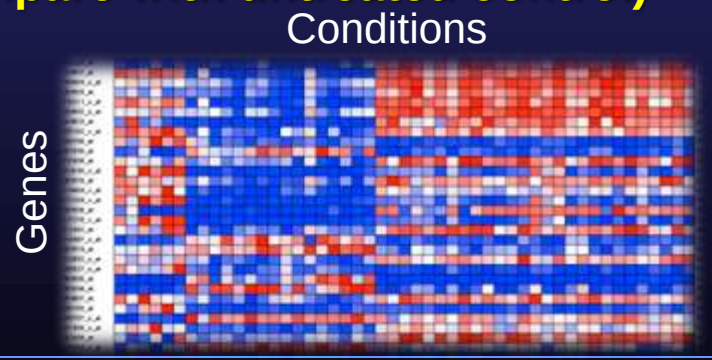


Phenotype

Metabolic activity

Differential gene expression

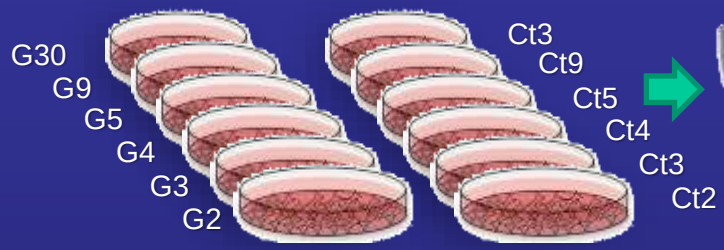
(Compare with untreated control)



- **cDNA Microarrays**
- **RNA-sequerncig**
- **RT-qPCR**

Microarray analysis of gene expression changes in human neurocytes

24h



Gelsemium s. dilutions (G)

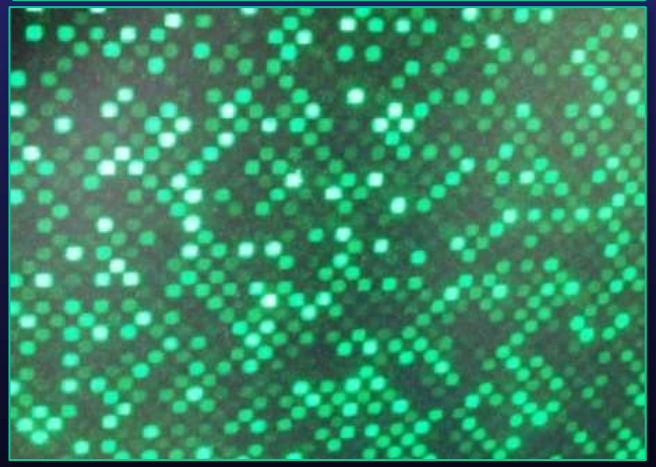
Control (solvent) Dilutions (Ct)

RNA →cDNA

12-samples microarray chip

4 independent replicate experiments

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



Scanning and data analysis

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Mini-review

Gelsemium

Arnica

Drosera

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; Olioso 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**	**(Olioso et al. 2016 Marzotto et al, 2016)**
Drosera rotundifolia	3D-6D	16HBE bronchial cells	**↓ 25 genes** **↑ 44 genes, epitelial cell proliferation**	**(Arruda et al. 2021)**

LOOKING FORWARD: HOMEOGENOMICS AND HOMEOGENETICS

REVIEW ARTICLE

The contribution of homeogenomic and homeogenetic studies in the support of the practice of Homoeopathy

Peter H. Kay[1*], Anisur R. Khuda-Bukhsh[2]

A.R. Khuda-Bukhsh

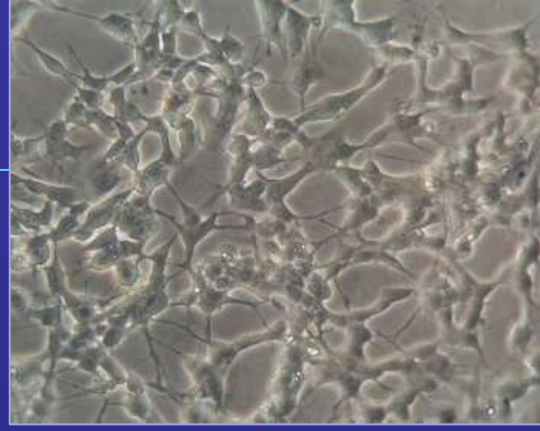
ABSTRACT

Almost two decades ago, it was postulated that homoeopathic remedies could deliver their benefits by interacting with the genetic blueprint. Over the years, the results of many homeogenomic gene expression studies have confirmed this postulate. The results of homeogenomic studies have begun to recognize which of the estimated 25,000 human genes are targeted by different homoeopathic remedies and how the expression profiles of these targeted genes are rearranged.

Indian J. Res.Homeopathy 2016, 10: 101-107

First report of Gelsemium S. *in vitro*

(GIRI 2012)



Int J High Dilution Res 2012; 11(40):144-146
Proceedings of the XXVI GIRI Symposium; 2012 Sep20-22; Florence (Italy)

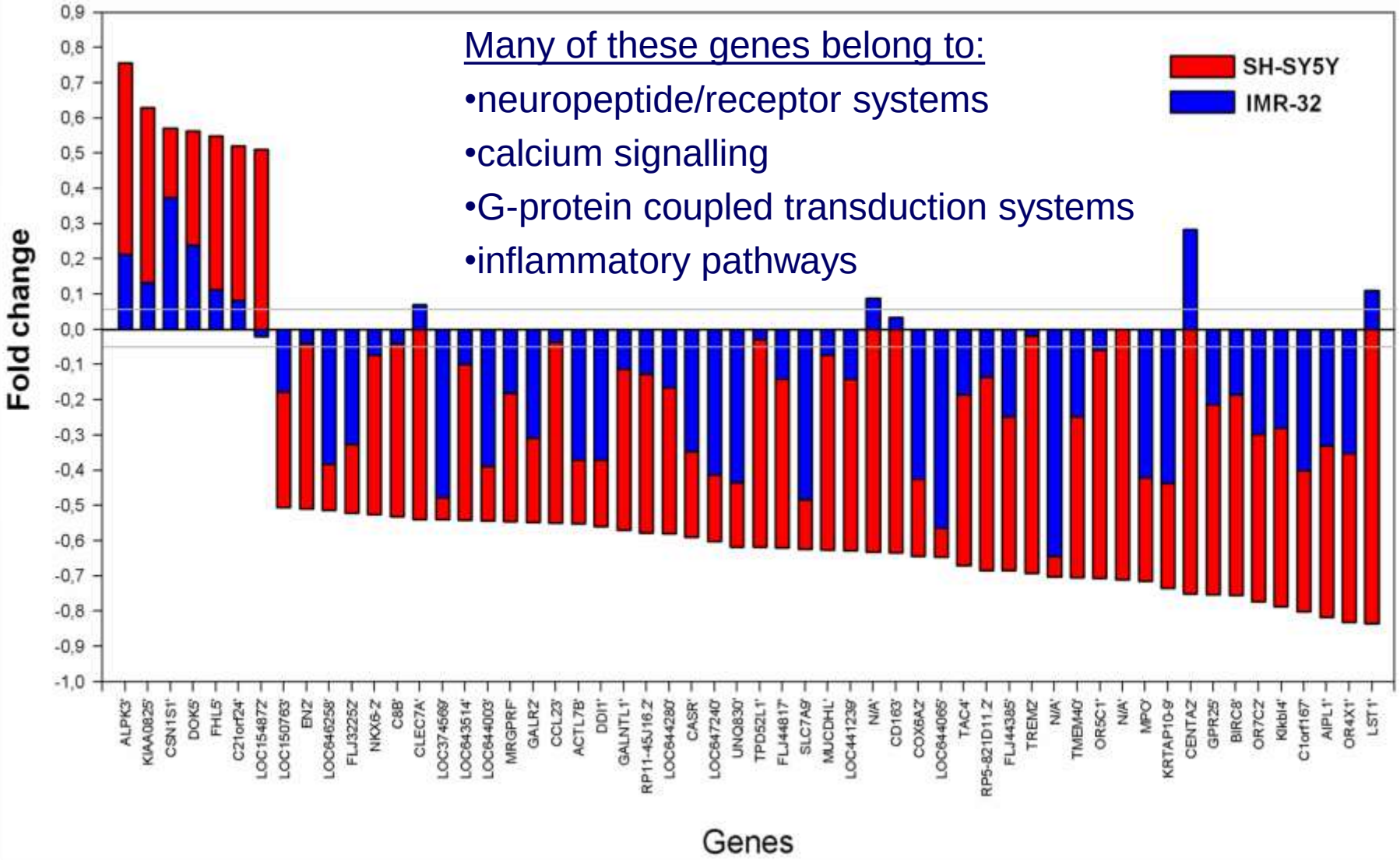
Oral Section

Cellular and transcriptional responses of SH-SY5Y human neurocytes following in vitro exposure to *Gelsemium sempervirens*

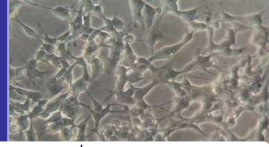
Paolo Bellavite[1], Marta Marzotto[1], Debora Olioso[1], Mirko Cristofoletti[1], Maurizio Brizzi[2], Paola Tononi[3], Ilaria Pierpaola Dal Prà[4], Ubaldo Armato[4]

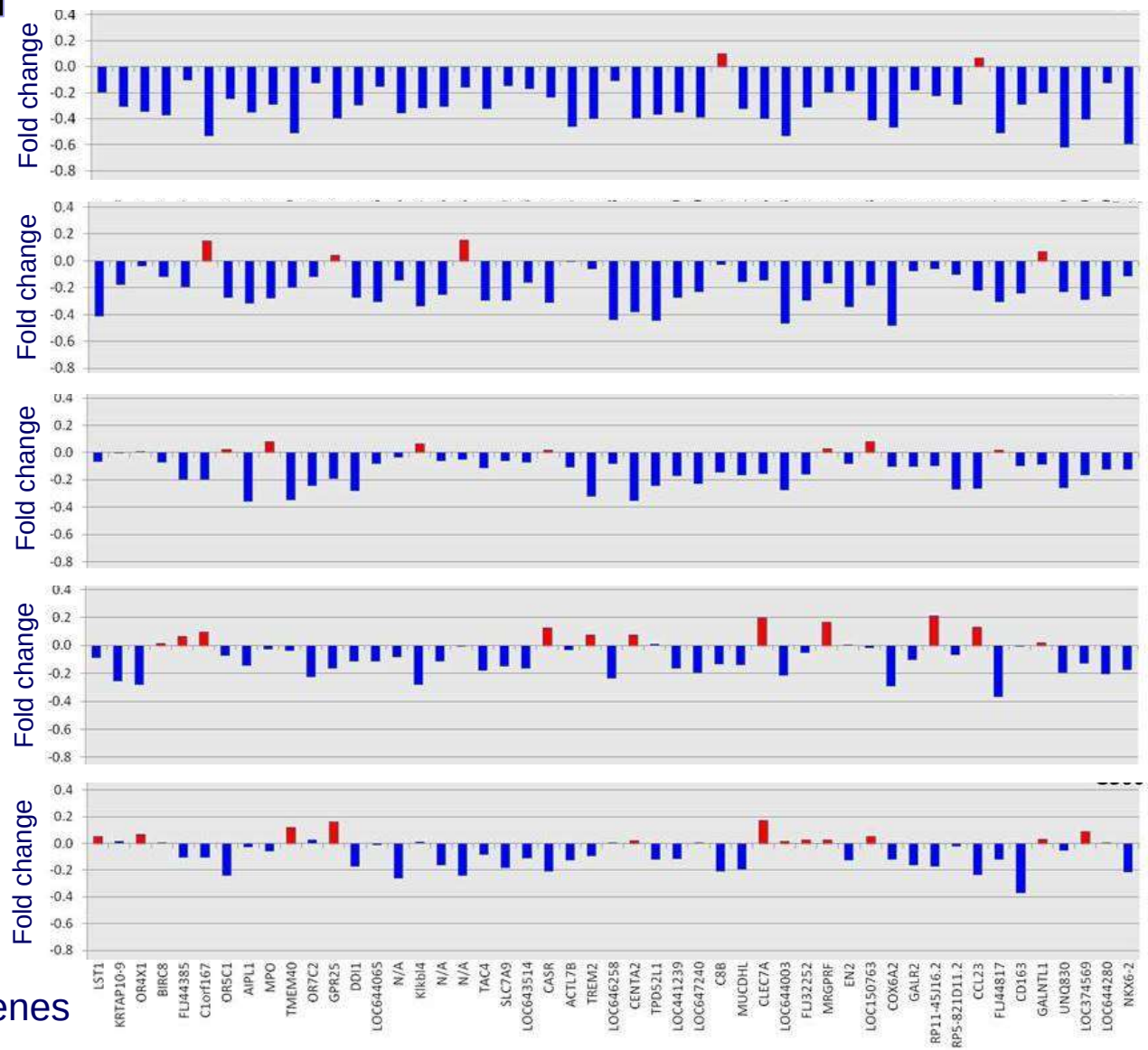
[1] Department of Pathology and Diagnostics, University of Verona, Verona, Italy
[2] Department of Statistical Sciences, University of Bologna, Bologna, Italy
[3] Department of Biotechnology, University of Verona, Verona, Italy
[4] Department of Life and Reproduction Sciences, University of Verona, Verona, Italy

Up (left) and down (right) regulated genes in two cell lines exposed to *Gelsemium s.* 2C



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



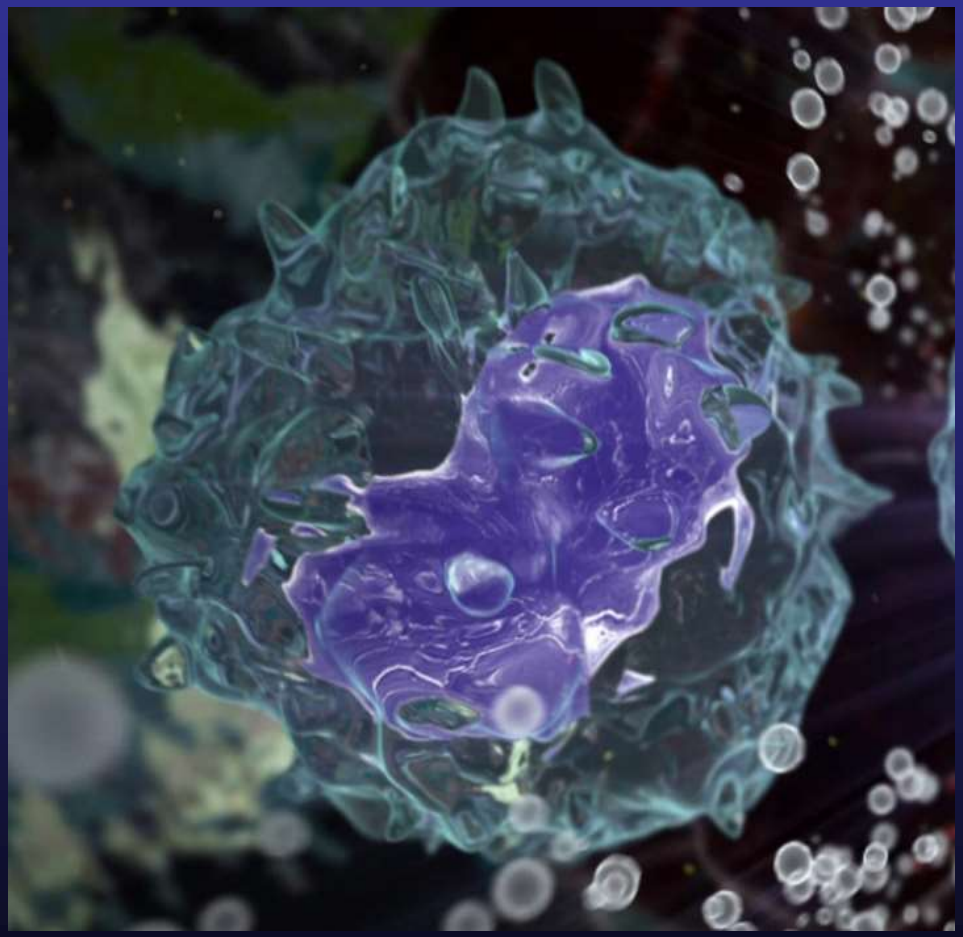


	N. Genes UP/DOWN	P (Fisher)
Gels 3c /Placebo 3c	2↑ 47↓	<0.001
Gels 4c /Placebo 4c	3↑ 42↓	<0.001
Gels 5c /Placebo 5c	3↑ 38↓	<0.001
Gels 9c /Placebo 9c	9↑ 30↓	<0.01
Gels 30c /Placebo 30c	7↑ 27↓	<0.01

Genes

Evaluation of *Arnica montana* dilutions on gene expression in a human macrophage cell line

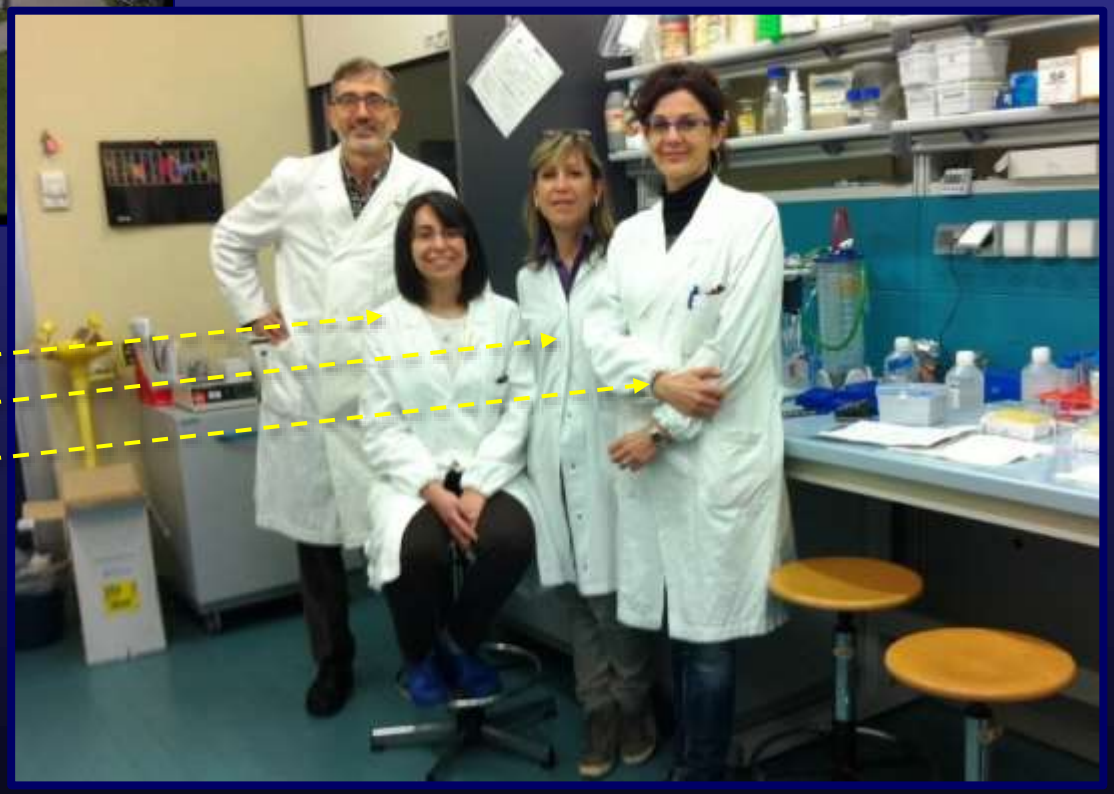
(Verona's group 2015-2020)



Verona's Homeopathic Research Group

We thank for research grants:

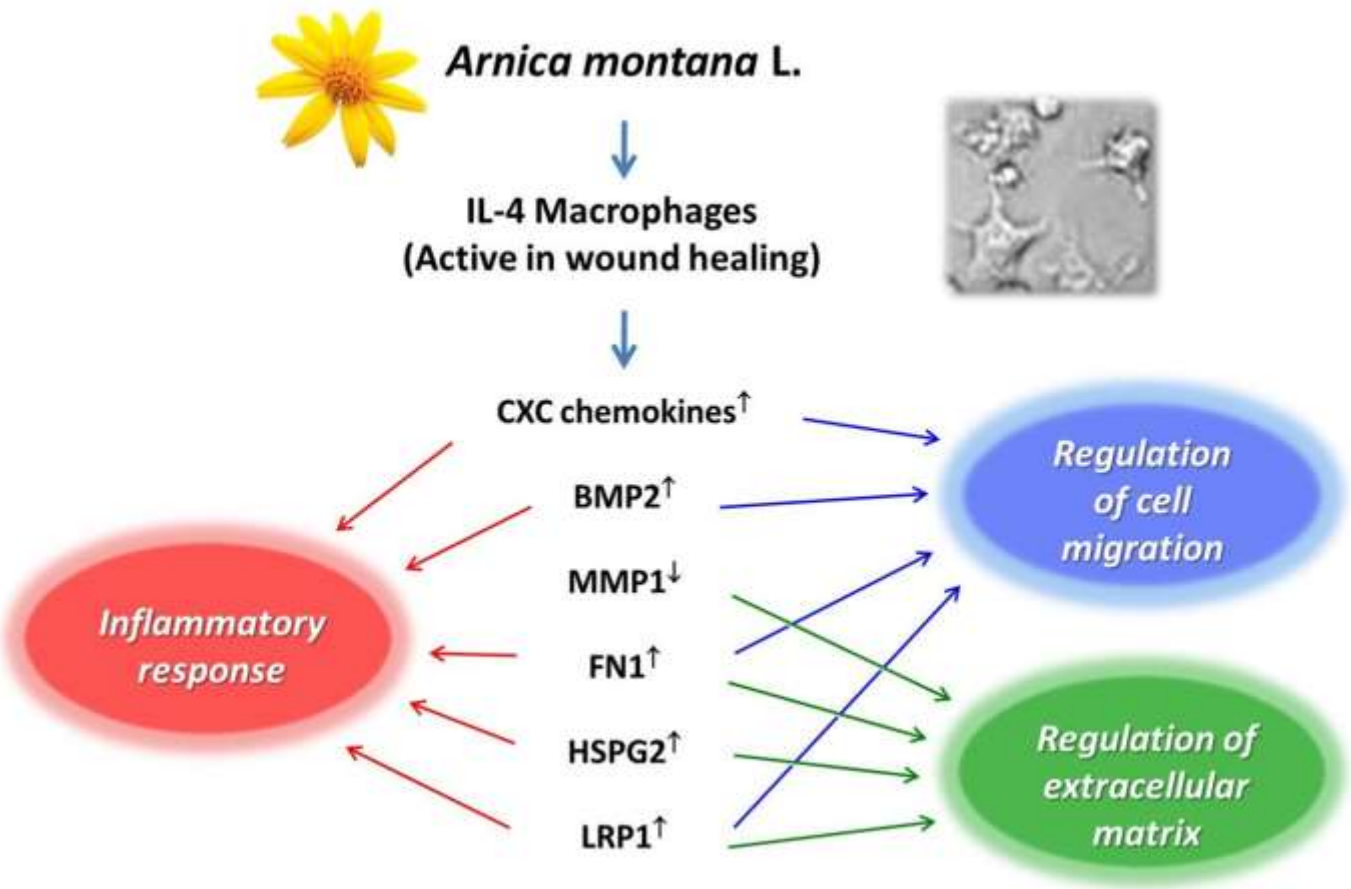
- Laboratoires Boiron
- Standard Homeopathic/Hyland's
- Italian Research Ministry



Paolo Bellavite
Clara Bonafini
Marta Marzotto
Debora Olioso
(y. 2015)

Others: Andrea Signorini, Anita Conforti, Elisabetta Zanolin, Elisabetta Moratti, Beatrice Andreoli, Fabio Arruda e Silva

FUNCTIONS OF THE GENES MODIFIED BY ARNICA IN MACROPHAGES



BMP-2: Bone Morphogenetic Protein; **MMP1**: Matrix Metallopeptidase 1; **FN1**: Fibronectin; **HSPG2**: heparan sulfate proteoglycan 2; LRP1: Low density lipoprotein receptor-related protein 1

Validation and confirmation of previous findings

Fibronectin Gene Up-regulation by *Arnica montana* in Human Macrophages: Validation by Real-Time Polymerase Chain Reaction Assay

Marta Marzotto[1] Fabio Arruda-Silva[1] Paolo Bellavite[1]

[1] Department of Medicine, University of Verona, Verona, Italy

Homeopathy 2020;109:140–145.

Address for correspondence Paolo Bellavite, MD, Department of Medicine, University of Verona, Strada Le Grazie 8, 37134 Verona, Italy (e-mail: paolo.bellavite@univr.it).

*The results support evidence that the extracellular matrix is a potential therapeutic target of **Arnica m.**, with positive effects on cell adhesion and migration during tissue development and healing.*

Scientific Reports | (2021) 11:2356 | https://doi.org/10.1038/s41598-021-81843-y nature portfolio

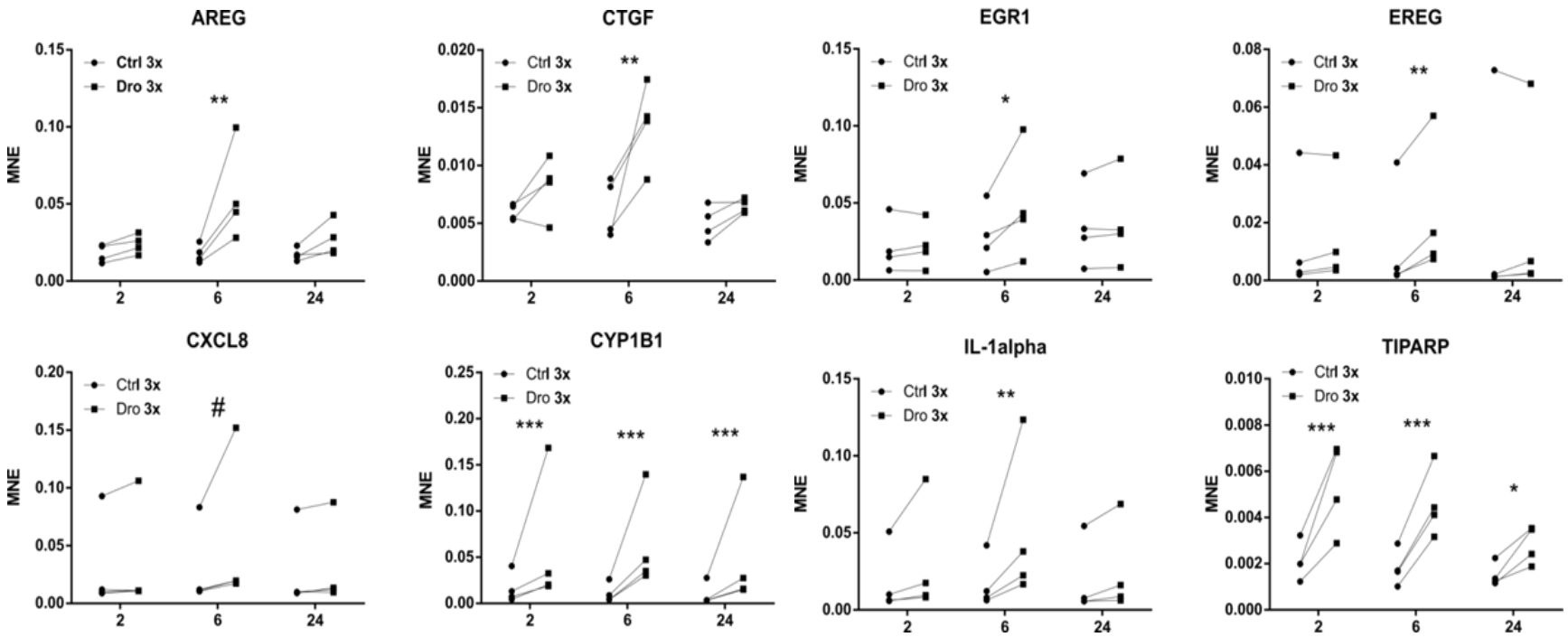
scientific reports

OPEN

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

Fabio Arruda-Silva, Paolo Bellavite & Marta Marzotto

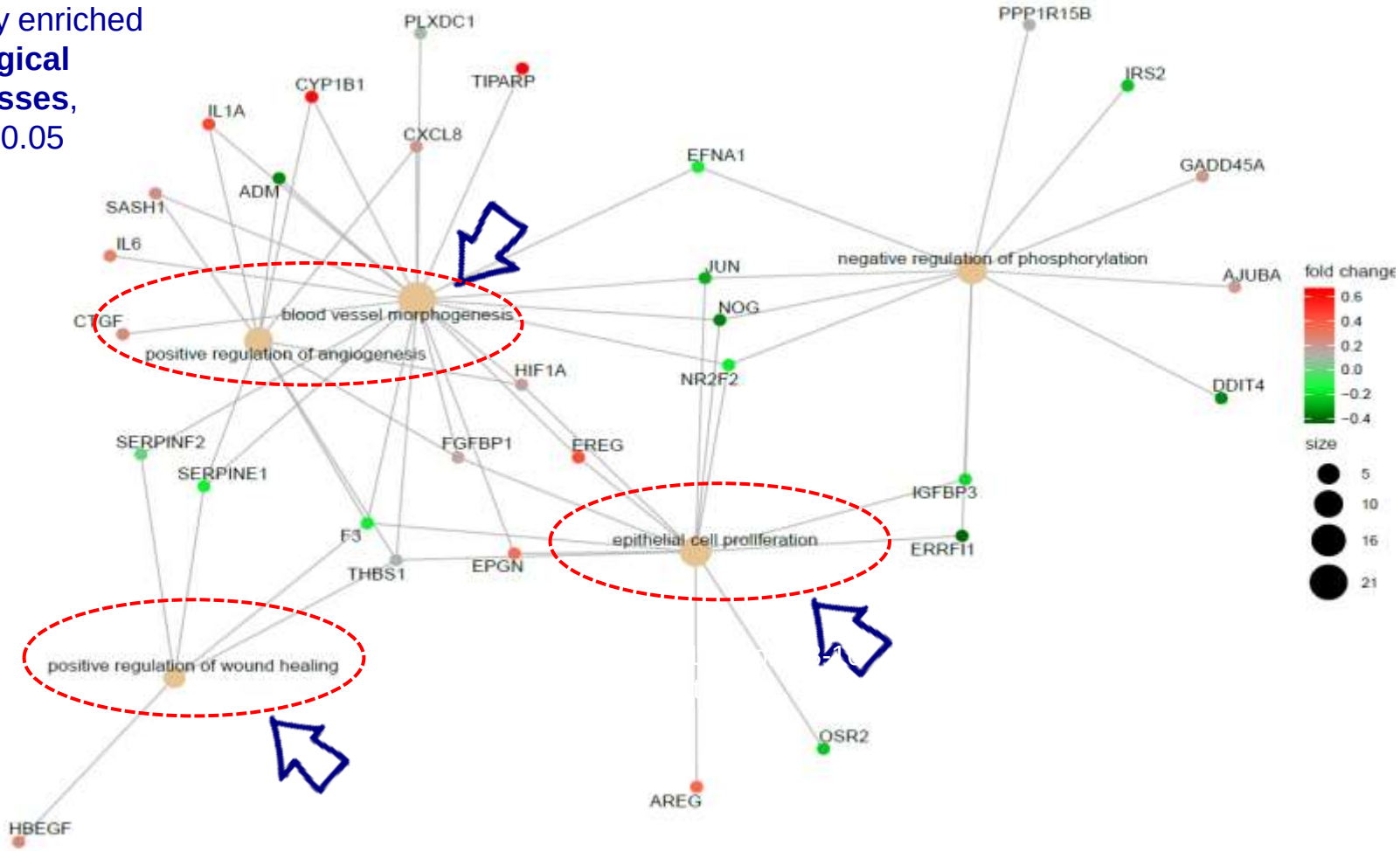
Gene expression in 16HBE bronchial cells with *D. rotundifolia* 3x



Arruda e Silva et al. 2021 Scientific Reports

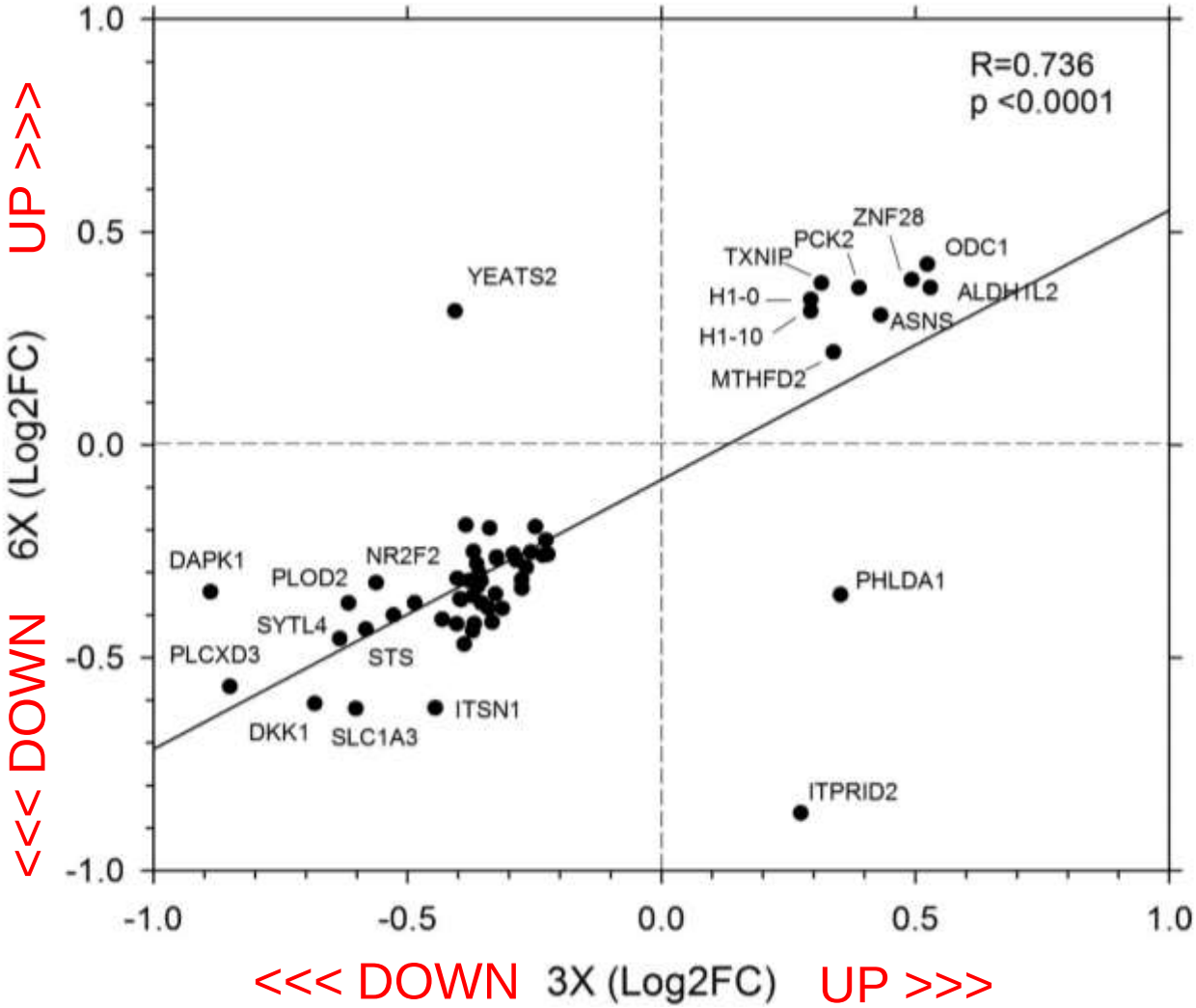
Gene regulatory networks of bronchial cells affected by Drosera 3X

The 5 most significantly enriched **Biological Processes**, padj<0.05

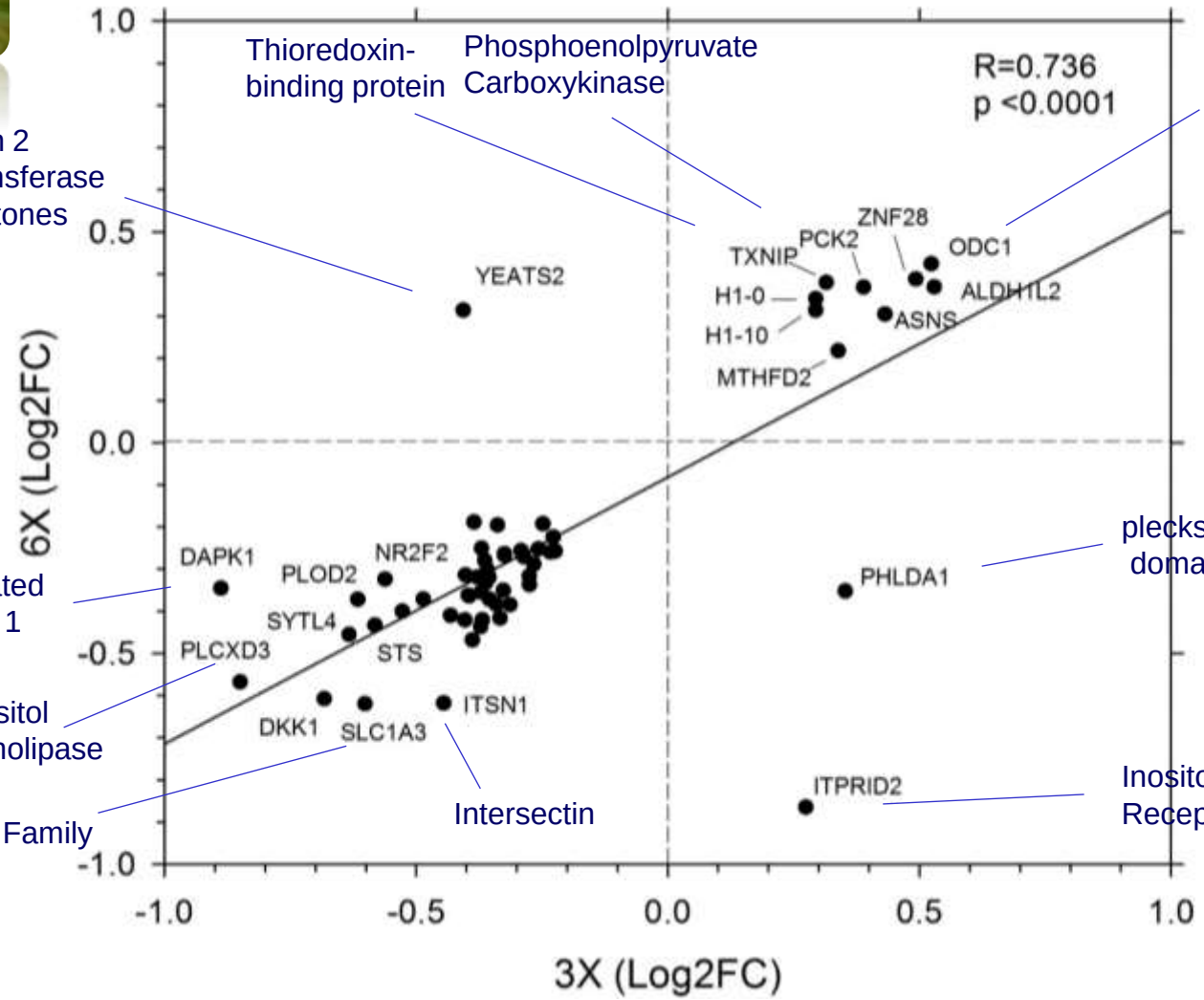


Arruda e Silva et al. 2021 Scientific Reports

Significant changes with Drosera 3X AND 6X



Significant changes with Drosera 3X AND 6X



Thioredoxin-binding protein

Phosphoenolpyruvate Carboxykinase

Ornithine decarboxylase

YEATSProtein 2 with acetyltransferase activity on histones

pleckstrin homology like domain family A1:

Death-associated protein kinase 1

Phosphatidylinositol Specific Phospholipase C X Domain

Solute Carrier Family

Intersectin

Inositol 1,4,5-Trisphosphate Receptor interacting domain

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

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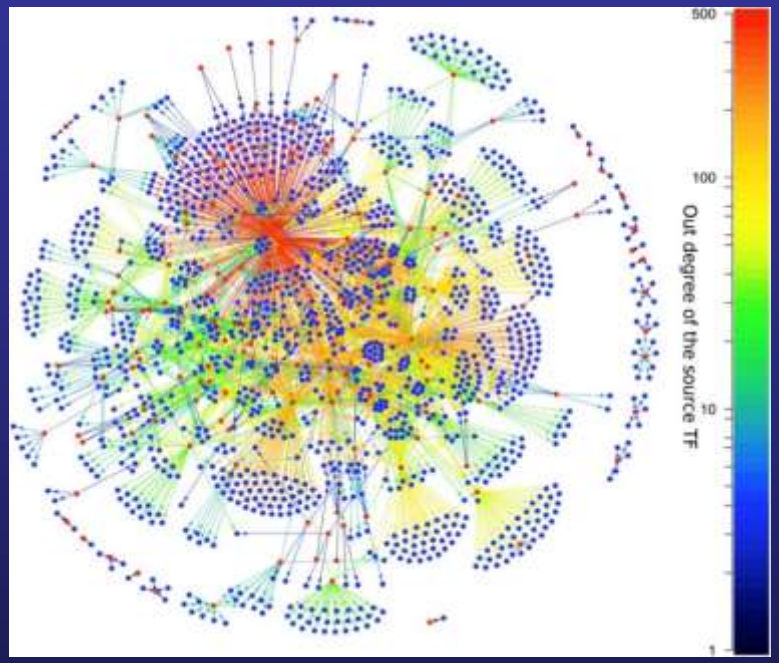
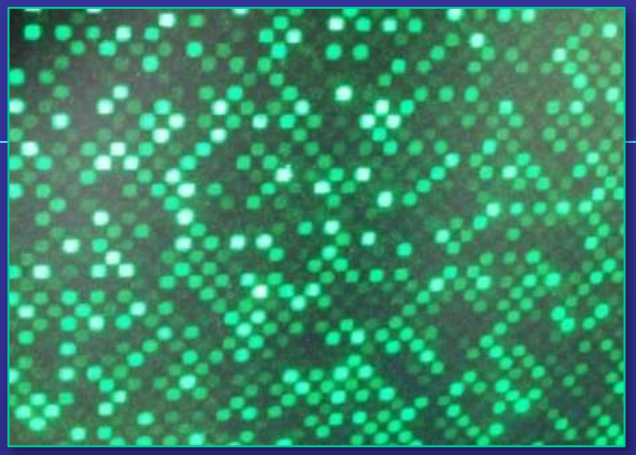
High sensitivity of gene networks



Cells respond to homeopathic dilutions with the expression of dozens of genes

Highest changes “ vitro” observed using low dilutions
(e.g. Arnica 2C, Gelsemium 2C, Drosera 3X)

Detectable activity remains even at higher dilutions
(e.g. Arnica 15C, Gelsemium 30C, Drosera 6X)



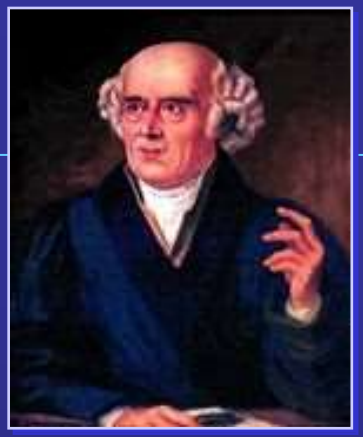
Problems with high-throughput technologies

- High sensitivity means also high variability and difficult reproducibility → need of many replicates

- Cost of laboratory instruments, reagents and working time

- Not easily translated to clinical applications

Organon, Par. 278



C.F.S. Hahnemann
(1755-1843)

"How small must be the dose of each individual medicine, to effect the best cure?

This is not the work of theoretical speculation.

Pure experiment, careful observation of the sensitiveness of each patient, and accurate experience can alone determine this in each individual case"

THANKS FOR YOUR ATTENTION!