SOCIETÀ ITALIANA DI TOSSICOLOGIA

SITOX

1972

21° Congresso Nazionale

Società Italiana di Tossicologia

Pericolo, rischio e rapporto rischio-beneficio

BOLOGNA

20-22 Febbraio 2023

www.sitox.org

Effetti dell'esposizione a concentrazioni subtossiche di atrazina, cipermetrina e vinclozolin sulla segnalazione PI3K/Akt/mTOR mediata da microRNA in cellule SH-SY5Y

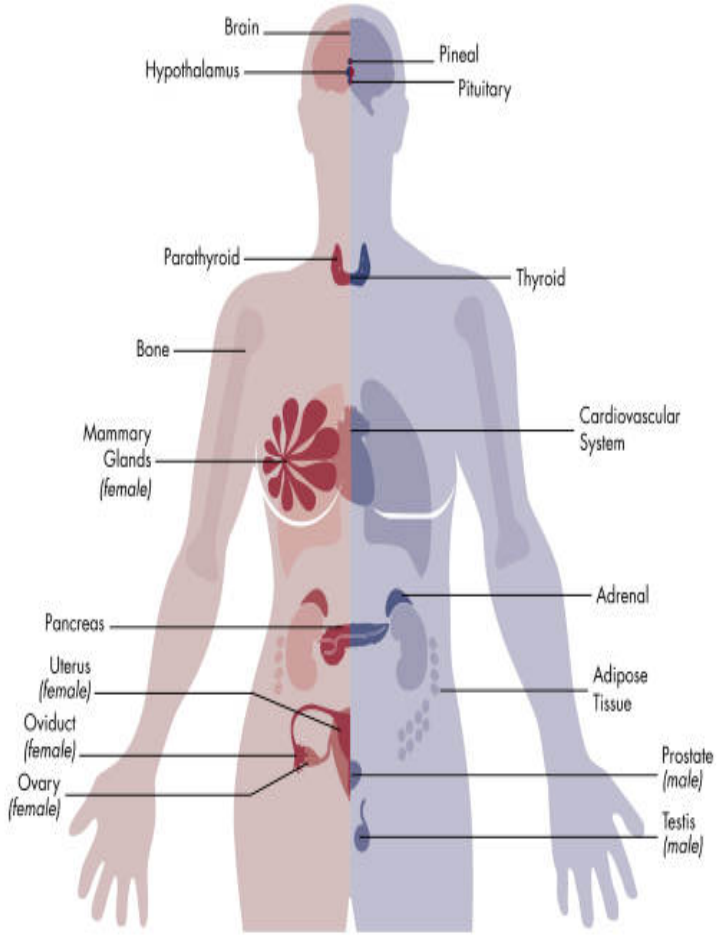
Agnese Graziosi, Giulia Sita, Camilla Corrieri, Patrizia Hrelia, Fabiana Morroni

Endocrine disruptors (EDCs)

Gli EDCs possono mimare, bloccare o interferire con il **sistema ormonale endogeno**.

L'esposizione può avvenire **ovunque**, dall'aria che respiriamo, al cibo che mangiamo, all'acqua che beviamo..

Gli EDCs possono entrare nel nostro corpo anche attraverso la cute e passare dalla madre al feto attraverso la **placenta** o dalla madre al neonate attraverso **l'allattamento**.



COMMON EDCS

Some common EDCs and their uses include the following:

PESTICIDES
Example EDCs: DDT, Chlorpyrifos, Atrazine, 2,4-D, Glyphosate

CHILDREN'S PRODUCTS
Example EDCs: Lead, Phthalates, Cadmium

INDUSTRIAL SOLVENTS OR LUBRICANTS AND THEIR BYPRODUCTS
Example EDCs: PCBs and Dioxins

PLASTICS AND FOOD STORAGE MATERIALS
Example EDCs: BPA, Phthalates, Phenol

ELECTRONICS AND BUILDING MATERIALS
Example EDCs: Brominated Flame Retardants, PCBs

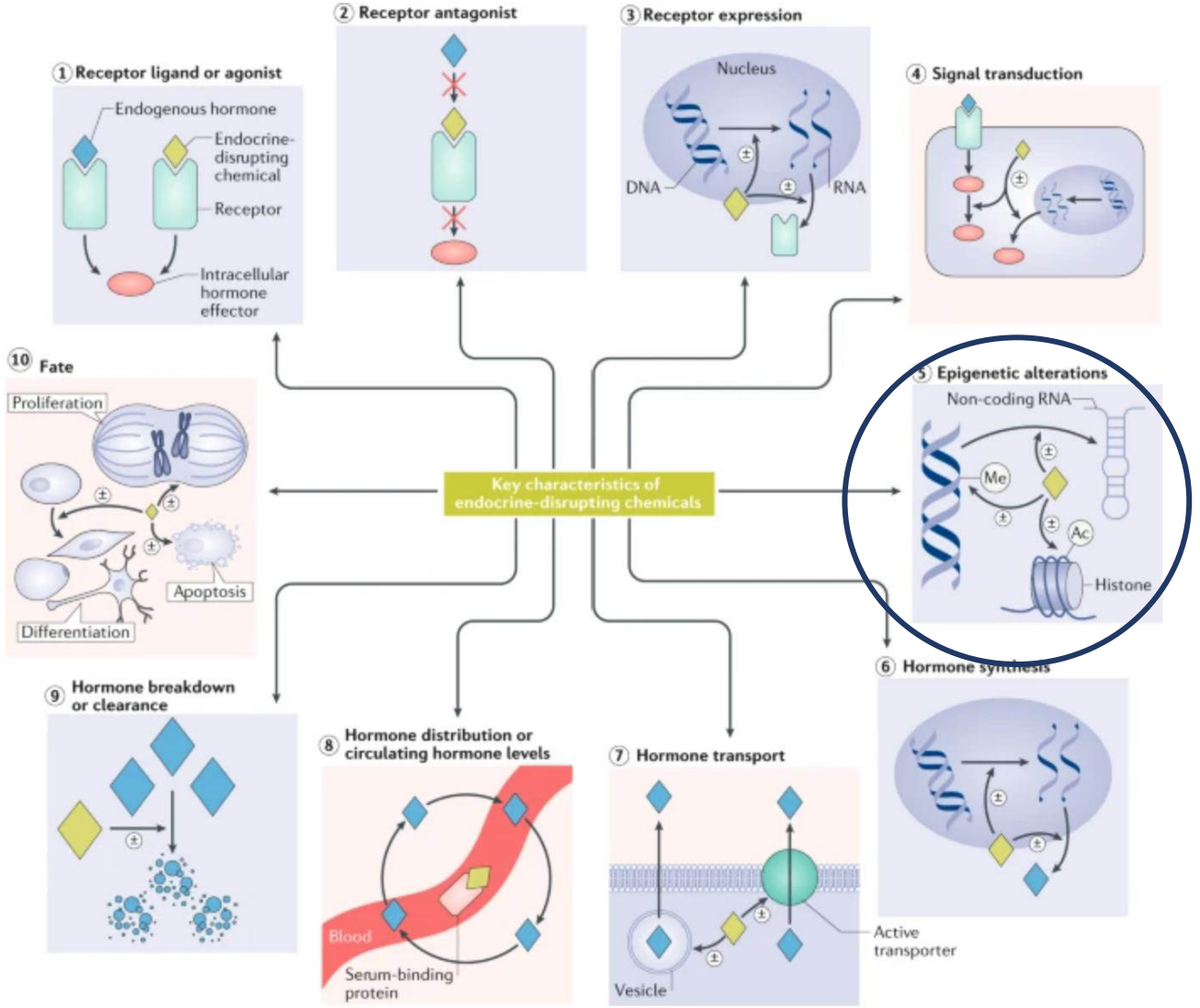
PERSONAL CARE PRODUCTS, MEDICAL TUBING
Example EDCs: Phthalates, Parabens, UV Filters

ANTI-BACTERIALS
Example EDCs: Triclosan

TEXTILES, CLOTHING
Example EDCs: Perfluorochemicals

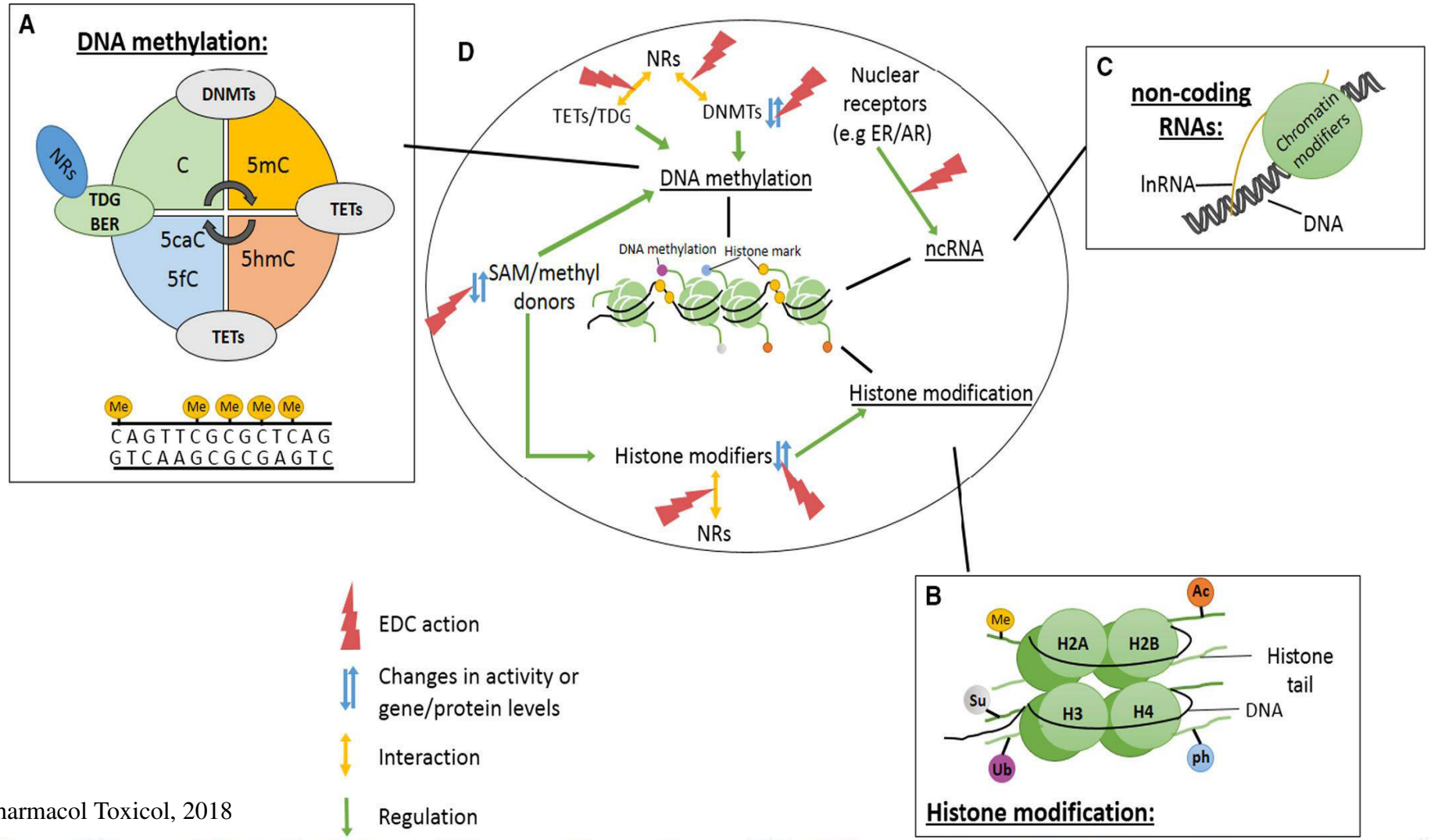
Effetti degli EDCs

Gli EDCs generalmente interferiscono con il sistema endocrino mimando o interferendo con gli ormoni endogeni o i loro recettori, causando alterazioni nell'omeostasi corporea.



M.A. Merril, Nat Rev Endocrinol, 2020

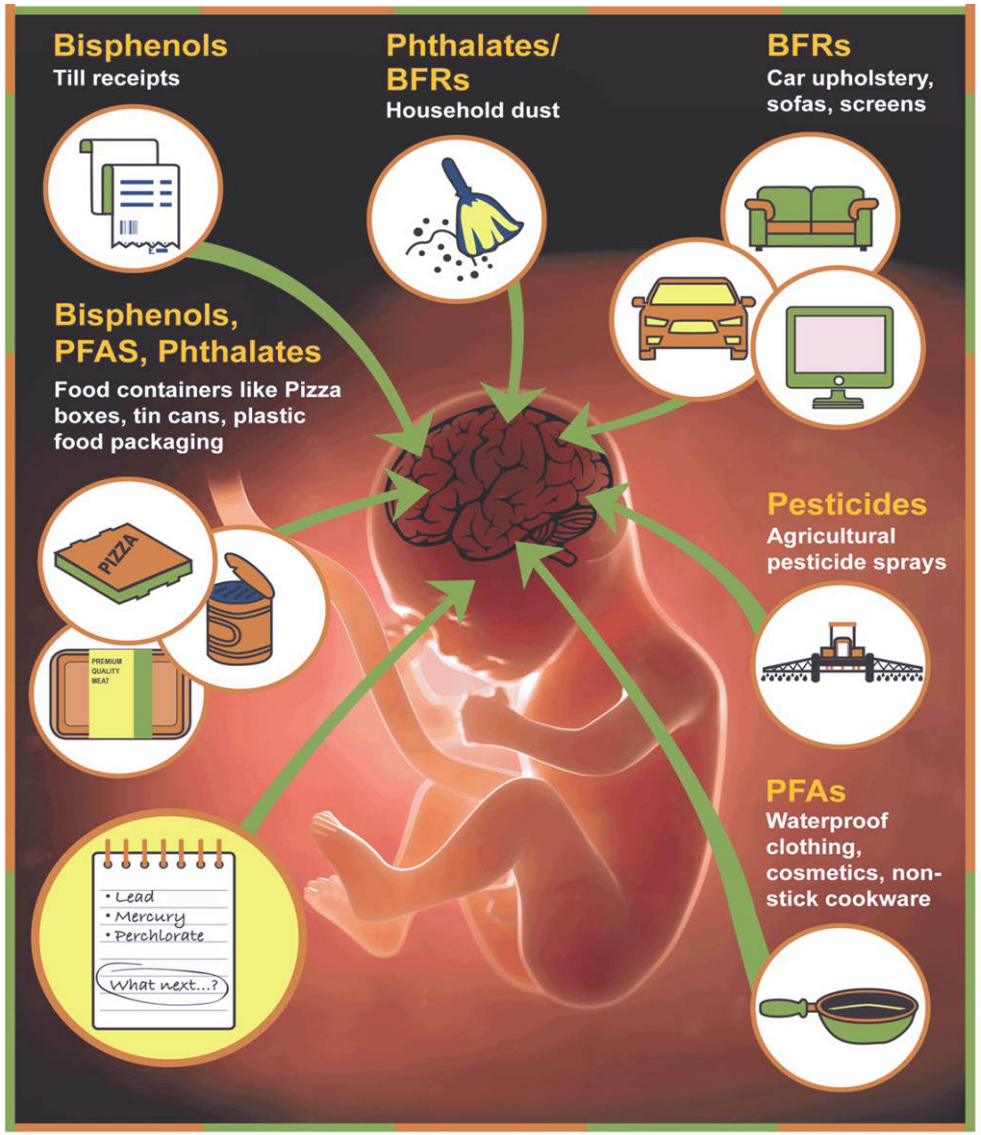
Potenziali meccanismi di alterazione epigenetica indotta da EDCs



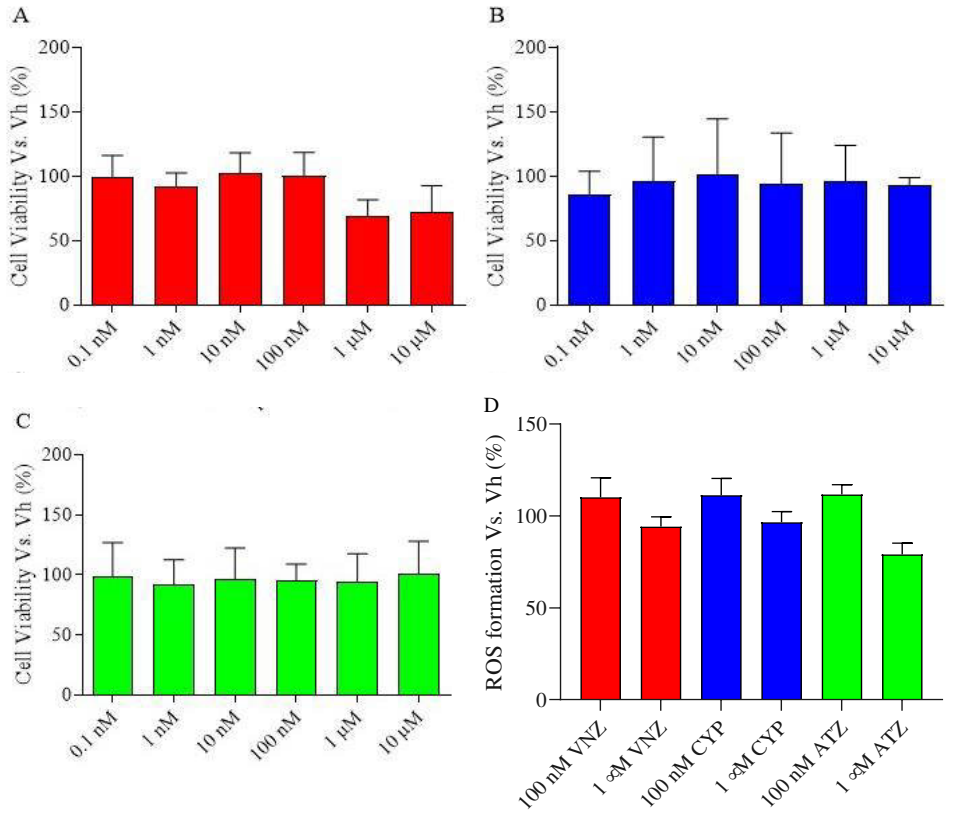
A.Ghvanini, Basic Clin Pharmacol Toxicol, 2018

Scopo della ricerca

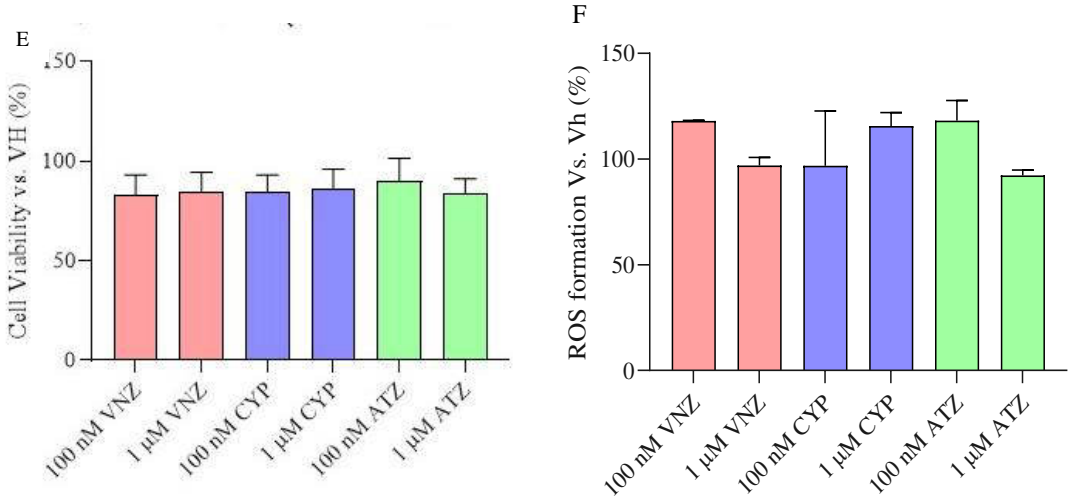
Identificare i **miRNA differenzialmente** espressi nelle cellule SH-SY5Y esposte a concentrazioni subtossiche di atrazina (ATZ), cipermetrina (CYP) e vinclozolina (VNZ) e determinare le potenziali funzioni dei miRNA differenzialmente espressi e le **possibili vie che mediano la loro neurotossicità.**



Vitalità cellulare e formazione di ROS in cellule SH-SY5Y NON differenziate



Vitalità cellulare e formazione di ROS in cellule SH-SY5Y differenziate



Data are expressed as fold increases of the percentage of cell viability or ROS formation versus the vehicle group and reported as mean ± SD of three independent experiments (One-way ANOVA, post hoc test Dunnet)

Graziosi A, Sita G, Int J Mol Sci,2022

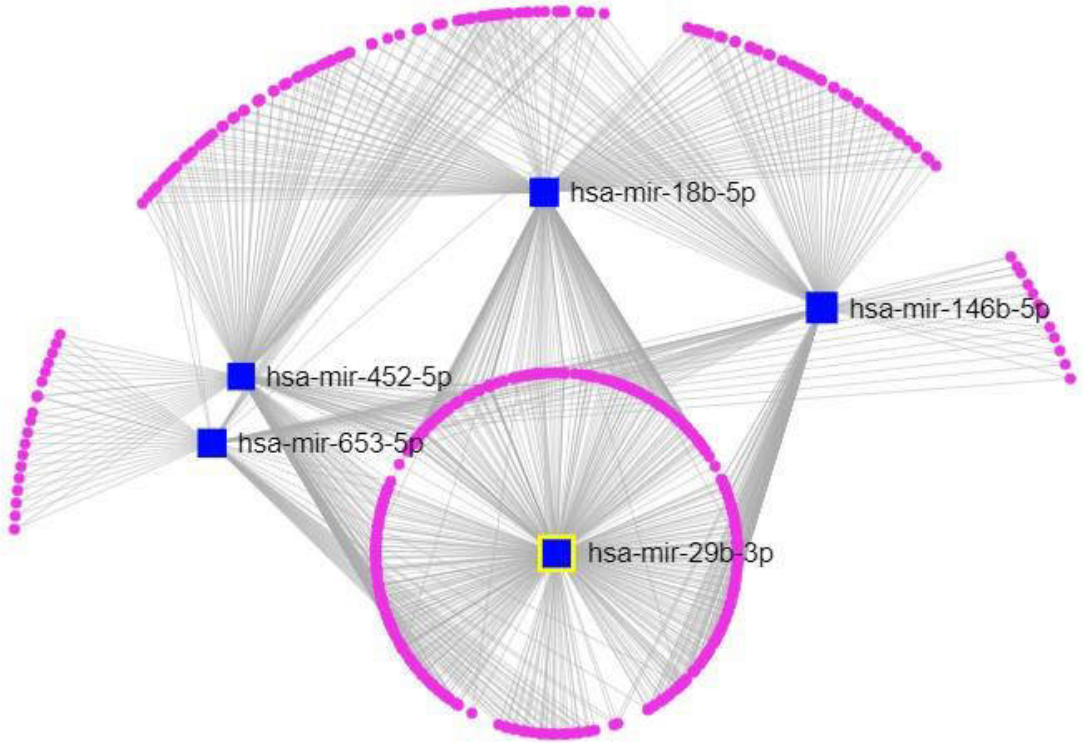
Alterazioni del profilo dei miRNA in cellule SH-SY5Y differenziate

qRT-PCR validation of miRNA expression in undifferentiated and differentiated SH-SY5Y cells. Quantitative analysis was performed by the $2^{-\Delta\Delta Ct}$ method and vehicle samples were considered as the calibrator of the experiment

Undifferentiated cells						
Target name	ATZ 100 nM	ATZ 1 µM	CYP 100 nM	CYP 1 µM	VNZ 100 nM	VNZ 1 µM
miR-18b-5p	1.88	1.26	1.28	1.13	1.70	1.74
miR-29b-3p	1.21	0.63	1.53	0.95	1.49	0.78
miR-146b-5p	1.28	1.22	1.47	1.18	1.99	0.93
miR-452-5p	1.94	1.24	1.41	1.45	1.46	1.90
miR-653-5p	1.12	0.80	1.17	1.16	0.95	0.91
Differentiated cells						
Target name	ATZ 100 nM	ATZ 1 µM	CYP 100 nM	CYP 1 µM	VNZ 100 nM	VNZ 1 µM
miR-18b-5p	0.30*	0.65	0.95	0.67	0.26*	0.32*
miR-29b-3p	0.42*	0.92	0.65	0.87	0.38*	0.70
miR-146b-5p	0.49*	0.56	1.06	1.32	1.51	0.97
miR-452-5p	0.55	1.09	0.81	0.86	0.57	0.25*
miR-653-5p	0.41*	0.59	1.15	1.07	0.26*	0.32*

*significantly deregulated miRNAs.

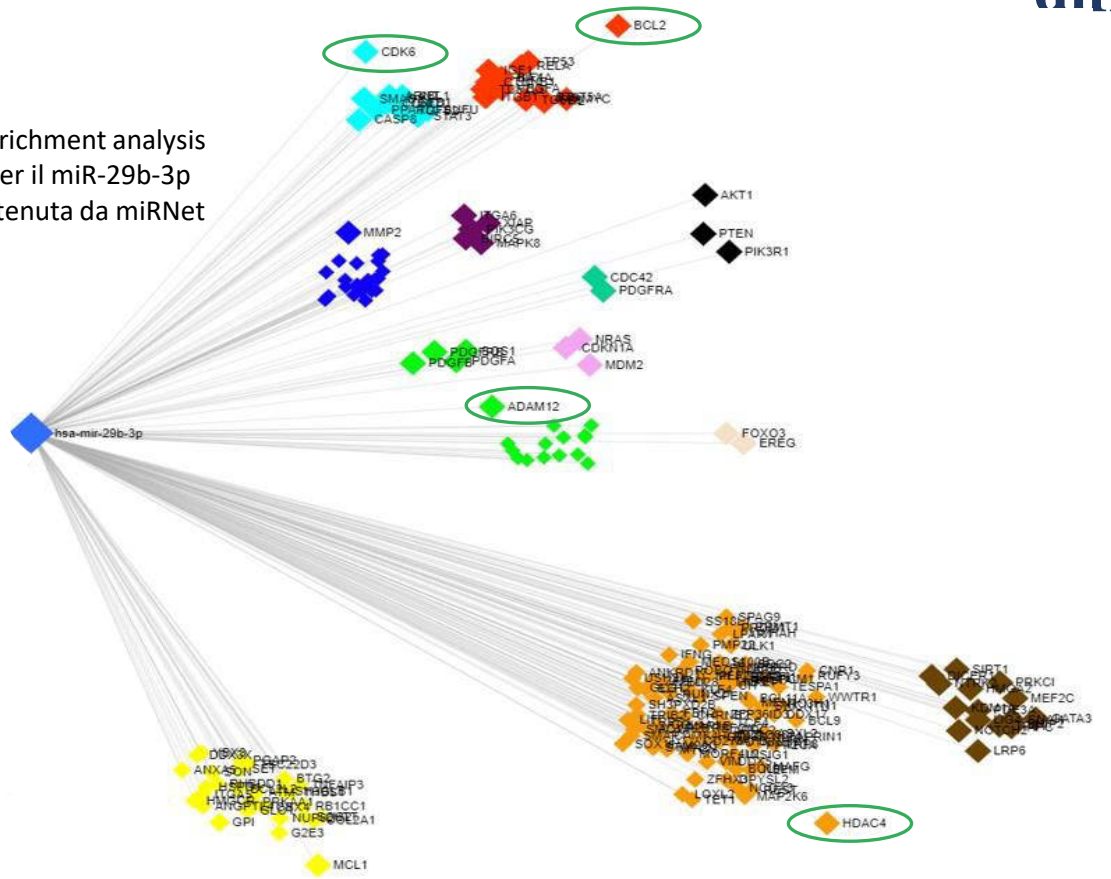
Graziosi A, Sita G, Int J Mol Sci,2022



Network gene analysis of the differentially expressed miRNA

Concentrazioni subtossiche di VNZ sottoregolano il miR-29b-3p contribuendo all'aumento dell'espressione di ADAM12 e CDK6 nelle cellule SH-SY5Y differenziate

Enrichment analysis per il miR-29b-3p ottenuta da miRNet



Green nodes: signaling by EGFR; **orange nodes**: regulation of cell differentiation; **light blue nodes**: pathway in cancer and regulation of cell differentiation; **red nodes**: pathway in cancer, regulation of cell differentiation and negative regulation of apoptotic processing.

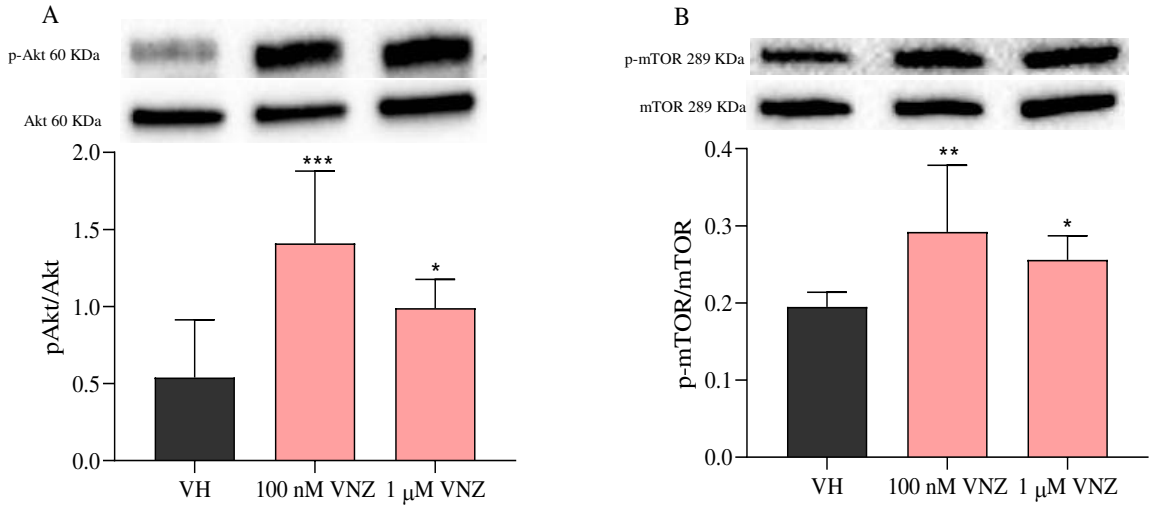
qRT-PCR validation of gene expression in differentiated SH-SY5Y cells, treated with ATZ, CYP and VNZ. Quantitative analysis was performed by the $2^{-\Delta\Delta Ct}$ method and vehicle samples were considered as the calibrator of the experiment

	ADAM12	Bcl2	CDK6	HDAC4
ATZ 100 nM	1.20	1.31	1.28	1.12
CYP 100 nM	1.43	1.56	1.98	1.30
VNZ 100 nM	2.11*	1.99	2.13*	1.46
ATZ 1 μM	1.38	1.13	1.58	1.32
CYP 1 μM	1.34	1.05	1.74	1.46
VNZ 1 μM	2.58*	1.60	2.97*	1.94

*significantly deregulated genes.

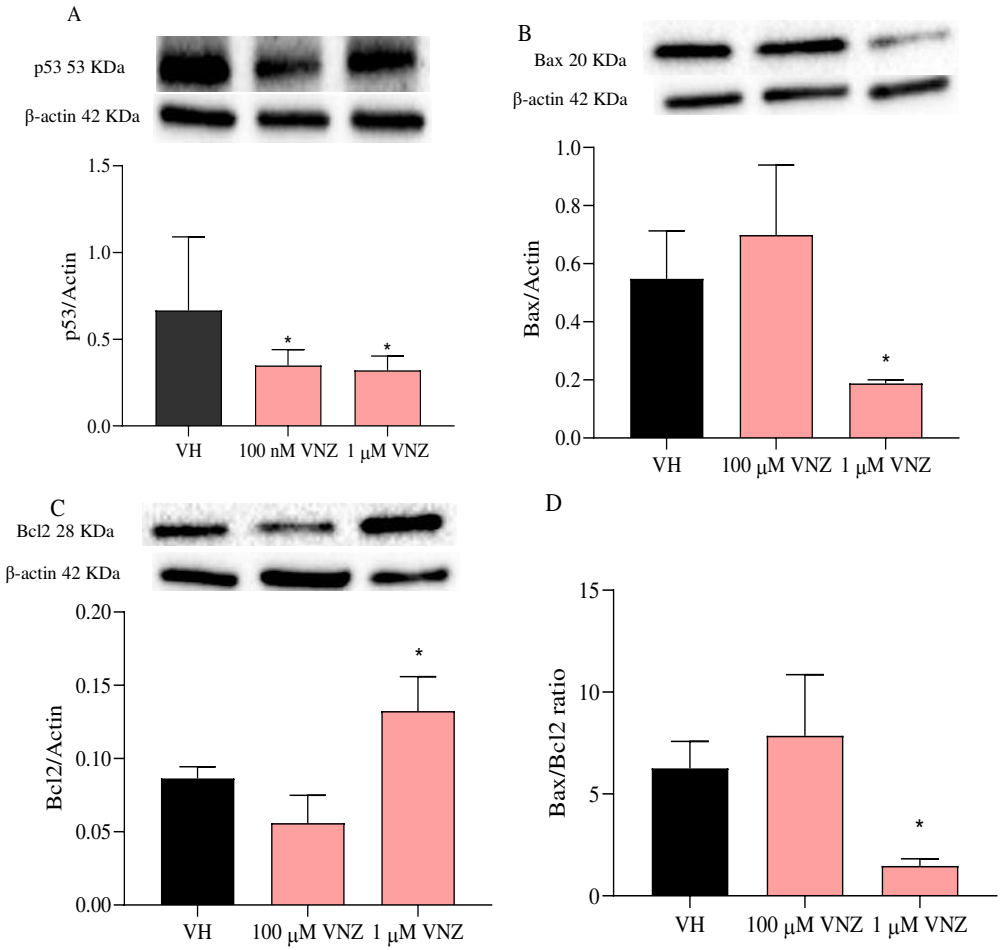
Graziosi A, Sita G, Int J Mol Sci,2022

Concentrazioni subtossiche di VNZ attivano la via PI3K/Akt/mTOR e sopprimono l'attivazione di p53 nelle cellule SH-SY5Y differenziate



Data are expressed as the ratio between the phosphorylated form and the total protein expression and reported as mean ± SD of three independent experiments (A: *p < 0.05 and *** p < 0.001 vs. VH; B: *p < 0.05 and **p < 0.01 vs. VH. One-way ANOVA, post hoc test Dunnett)

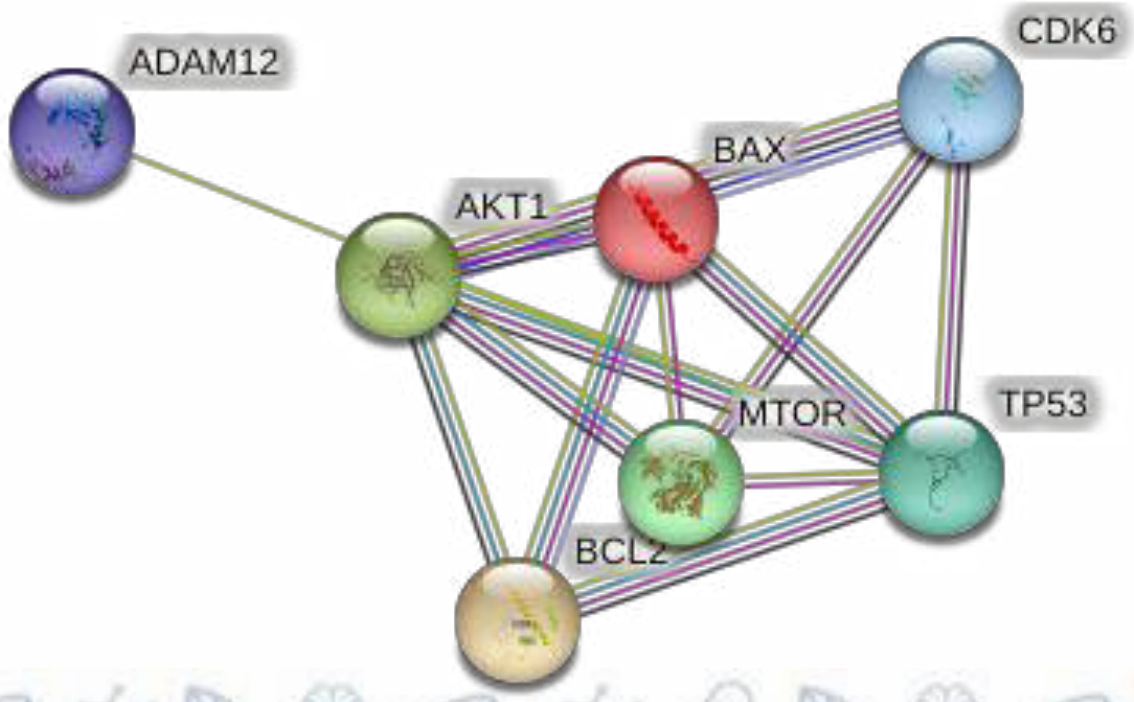
Graziosi A, Sita G, Int J Mol Sci,2022



Data are expressed as the ratio between the protein of interest and β-actin expression and reported as mean ± SD of three independent experiments (A: *p < 0.05 vs. VH; B: *p < 0.05 vs. VH; C: *p < 0.05 vs. VH; D: *p < 0.05 vs. VH. One-way ANOVA, post hoc test Dunnett).

Conclusioni

La downregulation del miR-29b-3p mediata da VNZ è in grado di modulare l'espressione della via PI3K/Akt/mTOR che regola che regola la proliferazione cellulare, la differenziazione, l'autofagia e l'apoptosi.



Conclusioni

Le modificazioni epigenetiche, che regolano il destino e l'identità cellulare, sono un processo plastico e dinamico e la comprensione dei meccanismi epigenetici, in una prospettiva a lungo termine, potrebbe permettere una più profonda comprensione degli effetti avversi associati all'esposizione agli EDCs.

Ringraziamenti

UNIBO
Prof.ssa Fabiana Morroni
Prof.ssa Patrizia Hrelia
Dott.ssa Giulia Sita
Dott.ssa Camilla Corrieri

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

UNINA
Prof.ssa Roberta d'Emmanuele di Villa Bianca
Prof.ssa Raffaella Sorrentino
Dott.ssa Emma Mitidieri

UNIVERSITÀ DEGLI STUDI DI NAPOLI
FEDERICO II

Progetto PRIN 2017 (Prot. 2017MLC3NF) e Fondazione del Monte di Bologna e Ravenna (Prot. 1355bis/2021