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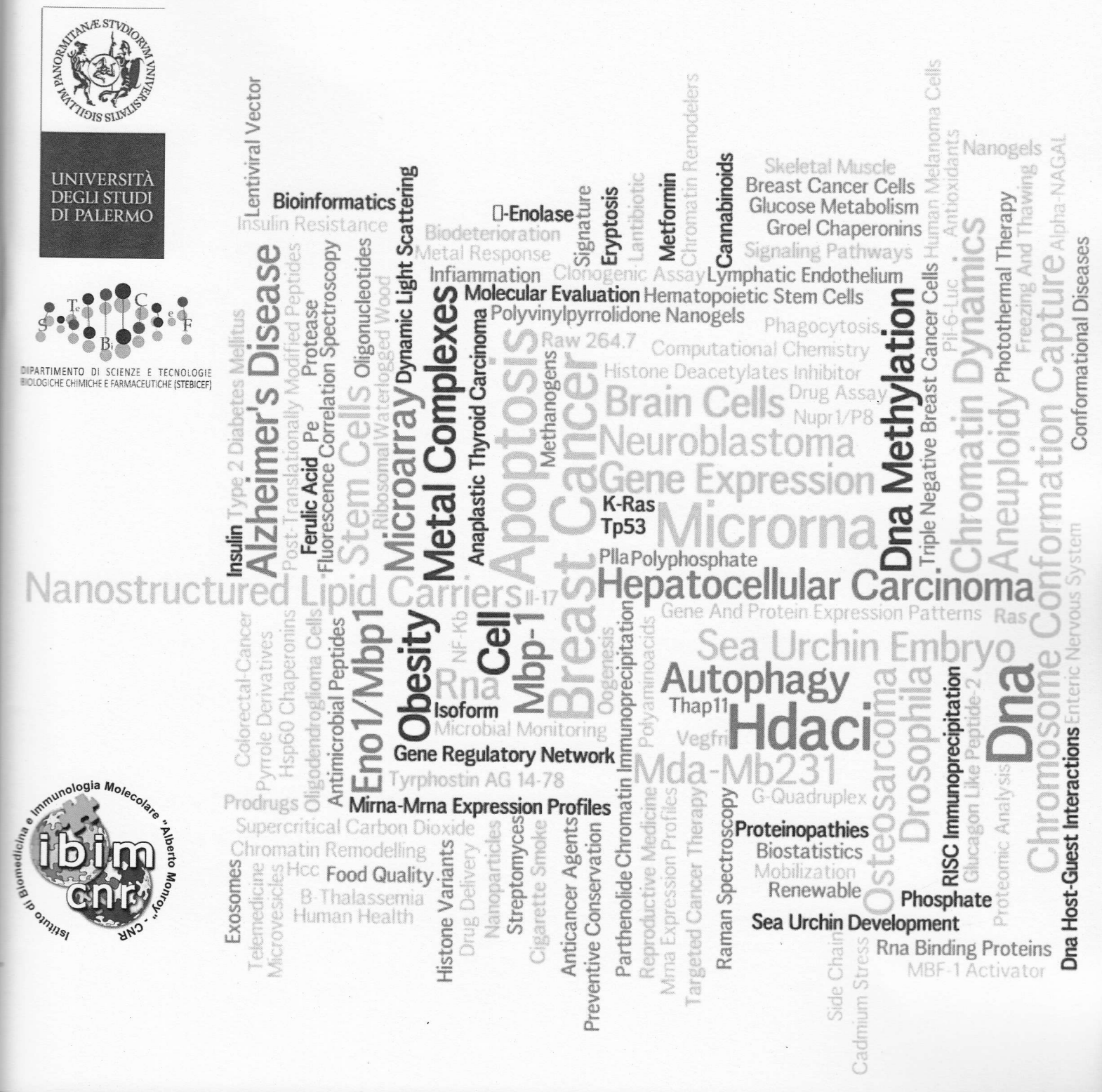
DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)



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Gene and Protein Signatures Associated to Treatment of MDA-MB231 Breast Cancer cells with JAHA , a novel Histone Deacetylases Inhibitor

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Keywords: **HDACi, Differential Display PCR, Real Time PCR, Proteomic analysis, gene and protein expression patterns**

Jay Amin hydroxamic acid (JAHA) [1] is a novel metal-based SAHA analogue synthesized in vitro, that shows significant cytotoxic activity [2] on MDA-MB231 breast cancer cells. To identify protein signatures associated to its cytotoxic activity, we utilized a proteomic approach to reveal protein expression changes after 18, 24 and 48 h of exposure. The protein identification was performed by mass spectrometry, and a total of eleven differentially-expressed proteins were visualized. Subsequently, Differential Display (DD) gene expression analysis was used to identify gene signatures in MDA-MB231 human breast cancer cell line after exposure to JAHA. We found two genes, Rad50 (DNA repair protein) and NTRK2 (neurotrophic tyrosine kinase, receptor, type 2), upregulated in treated cell preparations, and five genes that encode for Protein kinase C epsilon type, Protein kinase C iota type, Ergic (ER-Golgi intermediated compartiment 2KDa protein), MED25 (Mediator of RNA Polymerase II transcription subunit25) and Brefeldine A-inhibited guanine nucleotide-exchange protein 3 that were significantly down-regulated after treatment with JAHA.The result obtained by DD-PCR will be confirmed by Real Time PCR analysis. Further study will be required to compare the reported signature pattern with that obtained after exposure of MDA-MB231 cells with the parental molecule SAHA, and to understand the biological implications of the expression changes found.

[1] Spencer et al. (2011). *ACS Med Chem Lett*. **2**, 358-62.
[2] Librizzi et al. (2012).*Chem. Res. Toxicol*. **25**, 2608-16.

The effect of the HDACi JAHA on DNA Methylation of breast cancer cells by down-regulating DNMT1 through ERK signaling

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Keywords: **HDACi, DNA Methylation, DNMT1 level, ERK signaling, MDA-MB231 breast cancer cells**

Methylation of CpG repeats in the upstream/promoter regions of genes is an established mechanism of gene silencing in many cell types. DNA methylation results in the recruitment of histone deacetylases (HDACs) to promoter regions, thereby repressing expression of genes. General inhibitors of class I and II HDACs (HDACi), suppress the growth of cancer cells in vitro and in vivo. In this study, we investigated the effect of JAHA [1], a novel HDACi, on the intracellular signaling pathways of MDA-MB231 breast cancer cells. Concerning the MEK pathway JAHA repressed MAP kinase (ERK) activation after 18 h until 30 h of the treatment, and also down-regulated DNA (cytosine-5-)-methyltransferase 1 (DNMT1), a downstream ERK target, already at 18h with an increase up to 48 h. To check the occurrence of changes in the extent of global DNA methylation, genomic DNA was submitted to MeSAP (Methylation Sensitive Restriction Arbitrarily-Primed) PCR [2] using Afa and then HpaII enzymes followed by PCR amplification with an arbitrary primer binding preferentially to guanine and cytysine (GC)-rich regions of DNA, including CpG islands. Preliminary indications suggest the ability of JAHA to induce hypomethylation patterns in tumoral breast cancer cells after 30 h of the treatment. Collectively, these data demonstrate that the HDACi JAHA, by inhibiting ERK activity, regulates DNMT1 expression and ultimately DNA methylation.

[1] Spencer J. et al. (2011). *ACS Med Chem Lett*. **2**, 358-62.
[2] Naselli F. et al. (2014).*Gene* **536**: 29-39