Histone Deacetylase 4 promotes Ubiquitin-dependent proteosomal degradation of Sp3 in SH-SY5Y cells treated with Di(2-ethylhexyl) phthalate (DEHP), determining neuronal death.

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Phthalates, phthalic acid esters (DEHP), are widely used, as plasticizers to produce polymeric materials, in industrial production of plastics and daily consumable products. Animal studies have shown that DEHP may cause toxic effects in rat brain. In humans, exposure to DEHP has been found associated to the autism spectrum disorders (ASDs). In the present study, chronic exposure to DEHP (0.1-100 μM) caused a dose-dependent cell death via the activation of caspase-3, in neuroblastoma cells SH-SY5Y. Intriguingly, this harmful effect was prevented by the pan- histone deacetylase (HDAC) inhibitor Trichostatin A (TSA), by the HDAC class II inhibitor MC1568, but not by the HDAC class I inhibitor MS275. Furthermore DEHP reduced specificity protein 3 (Sp3) gene product expression, but not Sp3 mRNA, after 24 and 48 hours of exposure and protein reduction was prevented by pre-treatment with MC1568 , thus suggesting an involvement of HDAC II in causing this effect. Then we investigated the possible relationship between DEHP-induced neuronal death and the post-translational mechanisms responsible for the down-regulation of Sp3.Interestingly DEHP-induced Sp3 reduction was associated to its deacetylation and polyubiquitination. Coimmunoprecipitation studies showed that Sp3 physically interacted with HDAC4 after 12 hours of exposure to DEHP and HDAC4 inhibition by antisense oligodeoxynucleotide reverted the DEHP-induced degradation of Sp3. Notably Sp3 overexpression was able to counteract the detrimental effect induced by DEHP. Taken together, these results suggest that DEHP exerts its toxic effect by inducing deacetylation of Sp3 via HDAC4 and afterwards Sp3-polyubiquitination.