

## Post-translational modifications of Hsp60 and its extracellular release via exosomes are induced by the histone deacetylase inhibitor (HDACi) SAHA in the mucoepidermoid tumor H292 cells

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The chaperonin Hsp60 has multiple functions, among which that of supporting the growth of some type of tumours. HDACi (histone-deacetylase inhibitors) are drugs that regulate gene expression via modulation of epigenetic mechanisms, and induce tumor-cell death. Here, we show that in the tumor cells H292 the HDACi SAHA decreases the intracellular level of Hsp60 and promotes its extracellular trafficking by exosomal vesicles. SAHA caused a time- and dose-dependent decrease in cell viability with a G/2M cell-cycle arrest at 24 h and cell death at 48 h. These effects were accompanied by production of reactive oxygen species and mitochondrial membrane-potential dissipation. The marked decrease in Hsp60 level in SAHA-treated cells was not related to proteasomal degradation since it was not affected by the addition of the proteasome inhibitor MG132. Moreover, the analysis of post-translational modifications of Hsp60 revealed that SAHA treatment induced a modest reduction in the ubiquitination of the protein, with no effect on its acetylation state, but did cause a marked increase in tyrosine-nitrated Hsp60. This effect was related to oxidative stress since it was prevented by the anti-oxidant N-acetylcysteine. Most importantly, we showed for the first time that SAHA markedly increases extracellular Hsp60 export via exosomes, which might explain the concomitant decrease of the intracellular chaperonin. Our results suggest that SAHA modifies Hsp60 by nitration and stimulates its extracellular export via exosomes. Since Hsp60-bearing exosomes have been implicated in effective anti-tumour responses, and since elevated intracellular levels of Hsp60 have been related to the arrest of tumour-cell death, our data offer clues to explore what might be as yet uncharacterized mechanisms by which SAHA works as antitumor drug.

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### Keywords

Histone deacetylase inhibitor; Hsp60; nitration; exosomes.