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HDAC activity inhibition abolishes pluripotency, proliferation and survival pathways leading Ewing sarcoma cells to a differentiated state

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Ewing sarcoma (ES) is an aggressive pediatric small round cell tumor that shows neuroectodermal features and stem-cell like phenotype. Survival rate in the metastatic or recurrent disease setting remains dramatically low at 20% and it is compromised as a result of a lack of appropriated treatments. Aside few somatic mutations, it has been found aberrant regulations in the epigenomic landscape of pediatric tumors. Histone deacetylase inhibitors (HDIs) are being tested as a promising new class of antineoplastic agents and it has been shown to induce growth arrest, differentiation and apoptosis in different types of cancer. In this study, we evaluated the effect of HDAC activity inhibition, using sodium butyrate (NaB), on the cell growth, proliferation, differentiation and pluripotency status of ES cells. To examine the effect of NaB on ES cell growth, we treated SK-ES1 and RD-ES cell lines for 72H and measured cell viability by PI uptake in a flow cytometer. NaB treatment significantly reduced cell viability and also altered cell cycle distribution of ES cells, with an accumulation in Go/G1 phase, measured by DNA content with PI staining. These results show that NaB promotes an arrest in ES cell growth. To evaluate the effect of NaB on the differentiation of ES cells we evaluated gene expression of key proteins which control differentiation, pluripotency, cell proliferation and survival pathways, by immunoblot analysis. HDAC activity inhibition by NaB drastically reduced protein levels of c-MYC, nanog, Klf4, pERK, pAKT, pTrkB, but increased protein level of the neuronal marker β-III tubulin. Also, to evaluate the effect of NaB on ES tumorsphere formation ability, we plated ES cells in a serum-free stem cell inductor media, and treated with NaB for 7 days. HDAC activity inhibition significantly impaired ES tumorsphere formation ability, decreasing number and size of ES tumorspheres after treatment. These results may suggest that targeting HDAC activity by NaB may reprogram ES cells to a more differentiated state. Future experiments to investigate transcriptome profile, by RNA-seq, of Ewing sarcoma upon HDAC activity inhibition will be important to understand which molecular mechanisms control differentiation in Ewing sarcoma. In conclusion, these results suggest that HDAC activity inhibition may be a good strategy to reprogram Ewing sarcoma cells to a more differentiated state, which may have an important impact for therapeutic application in ES treatment. Unitermos: Ewing Sarcoma