

## Valproic acid and 5-azacytidine promote an increase of stemness phenotype in human osteosarcomas

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Osteosarcoma (OS) is the most common pediatric tumour of bone in the world. It leads to local invasion and early metastasis to lung. The prognosis is poor with the 5-year survival rate of 65% for patients with primary tumour and 20% for patients with metastasis. OS contain a small cell population with stem cell like properties, referred to as cancer stem cells characterized by CD133 expression [1,2]. Recent studies have implicated epigenetic alterations, including DNA methylation and histone modifications, as prominent factors that contribute to the phenotype of cancer progenitor or stem cells. Here, we examined the epigenetic effects of the HDAC inhibitor valproic acid (VPA) and demethylating agent, 5-azacytidine (DAC), on the stemness phenotype in osteosarcoma cell lines. Saos-2 and MG63 cell lines were treated with 0,5 mM VPA and 3 $\mu$ M DAC for 48 hours alone, and in combination. CD133 expression as well as stemness markers expression including OCT4, Sox2 and Nanog was analyzed by flow cytometry and real-time PCR. Vimentin and osteocalcin levels were also tested. Sarcospheres formation rate was assessed as spheres number/seed single cells number. Specific histone modifications including H3-trymethyl-k9, H3-acetyl-k9, H3-trymethyl-k27 and global methylation were analysed by flow cytometry and immunofluorescence. Moreover, soft agar and invasion assays were performed. Our findings indicated that DAC or VPA and their combination induced an increase of stemness characteristics of OS cells in terms of high expression of CD133, OCT4, Sox2 and Nanog and high sarcospheres-forming efficiency. Interestingly, combined treatment with DAC and VPA induced an increase of CD133 expression in a synergistic manner in all cell lines. Vimentin resulted up-regulated after treatment, whereas, the level of osteocalcin remained similar before and after treatment. Furthermore, soft agar assay revealed major colony-forming efficiency in treated cells compared to untreated cells, whereas invasion potential decreased after drug treatment. Interestingly, histone modification analyses correlated with those typical of embryonic stemness with an increase of H3-acetyl-k9 and decrease of H3-trymethyl-k9, and H3-trymethyl-k27 after drug treatment as well as global methylation decreased in treated cells. In conclusion, DAC and VPA induced an increase of stemness associated to an decrease of global methylation, increase of acetylation and decrease of H3-trymethyl-k9, and H3-trymethyl-k27.

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### References

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### Keywords

Cancer stem cells; osteosarcomas; sarcospheres; methylation; histone deacetylase inhibitor.