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Methylation and epigenetic modification by 5' azacytidine and valproic acid treatment increase stemness attributes in bone sarcoma cell lines

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Bone sarcoma is an aggressive malignancy with high mortality rate. Despite recent advances, the prognosis is still extremely poor. Bone sarcomas contain a small cell population with stem cell like properties, referred to as cancer stem cells (CSCs) expressing CD133 (Tirino et al, 2009; 2011). The biological relevance and regulatory mechanism of CD133 expression are not yet understood. The aim of this study is to elucidate mechanisms regulating aberrant expression of CD133 and stemness phenotype. Saos-2, MG63 and BS15 cell lines were treated with 0,5 mM valproic acid (VPA) and 3μM 5'azacytidine (5-AZA) for 48 hours alone and in combination. CD133 and stemness markers expression including OCT4, Sox2 and Nanog were 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 cell number. After treatment with 5-AZA or VPA, the expression level of CD133 mRNA as well as of protein was significantly increased in all three cell lines. Also OCT4, Sox2 and Nanog, stemness markers, and vimentin, mesenchymal marker resulted to be upregulated after treatment by real time-PCR. On the contrary, the expression level of osteocalcin remained similar before and after treatment. Interestingly, combined treatment with 5-AZA and VPA induced an increase of CD133 expression in a synergistic manner in all three cell lines. In addition, sarcospheres formation rate was increased after drug treatment compared to untreated cells. Also in this case, the drug combination lead to synergistic increase of formation rate of spheres. In conclusion, our results indicate that DNA methylation is an important determinant of CD133 and stemness profile in human bone sarcomas and this mechanism may be associated with histone deacetilase inhibition.

References

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Keywords

CSCs, CD133, osteosarcomas, sarcospheres, methylation, histone deacetilase inhibitor.