Abstract

The enhanced expression of histone deacetylases (HDACs) in a variety of malignancies drew attention to investigate a new category of anti-cancer drugs that are based on the inhibition of those enzymes. Valproic acid (VPA) is a well-known antiepileptic drug that exhibits antitumor activities through inhibition of HDACs class I and IIa. Cancer stem cells (CSCs) have been recognized to drive the tumor growth and progression hence; attention has been given to target this small subpopulation of CSCs rather than the whole bulk tumor cells. CD133 is considered to be a CSC marker in several tumors and its transcription is strongly influenced by epigenetic changes that will be altered upon administration of histone deacetylase inhibitors (HDACi) in cancer treatment. Therefore, we evaluated the epigenetic and cytotoxic effects of treatment with 1 mM VPA in combination with other chemotherapeutics and its influence on the expression of CD133 in human neuroblastoma (NB) cell lines.

Our results revealed that addition of VPA to DNA-damaging chemotherapeutics induced a synergistic anti-tumor effect that was associated with caspase-3 dependent induction of apoptosis in UKF-NB-4 cells. This synergism was related to the increase of the acetylation status of histones H3 and H4 and was only produced either by simultaneous treatment with both drugs or when the cells were pretreated with DNA-damaging chemotherapeutics before their exposure to VPA. On the other hand, our results showed that VPA induced CD133 expression that was dependent on increased acetylation of histones H3 and H4. On treatment with VPA and cytostatics, CD133+ cells were mainly detected in the proliferative phases of the cell cycle and they showed less activated caspase-3 compared to CD133− cells. UKF-NB-3 cells which express CD133 displayed higher colony and neurosphere formation capacities when treated with VPA, unlike IMR-32 cells which lack the CD133 protein. Induction of CD133 in UKF-NB-3 was associated with increased expression of phosphorylated Akt and pluripotency transcription factors (Oct4, Sox2 and Nanog). VPA did not induce CD133 expression in cell lines with methylated P1 and and P3 promoters, unless they were pretreated with demethylating agent 5-aza-2'-deoxycytidine. In conclusion, VPA potentiates the cytotoxicity of DNA-damaging chemotherapeutics in NB that is conditioned by the sequence of drugs administrated. CD133 expression in NB can be regulated by histone acetylation and/or methylation of its CpG promoters hence influenced by VPA therapy. VPA can induce CD133+ cells which display high proliferation potential and low sensitivity to cytostatics in NB. Even though these results confirm the potentiating cytotoxic effect of VPA in cancer therapy; they give new insight into a possible limitation to use VPA in some types of cancer which require caution before its use in clinical application.