

Eukaryotic DNA methyltransferase Dnmt3a as one of the cellular targets of novel anticancer DNA-intercalating drug curaxin CBL0137

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Various anticancer drugs exert antitumor activity by interacting with DNA via mechanisms such as intercalation into DNA, groove binding and covalent modifications of heterocyclic bases thus causing cytotoxicity. Recently characterized curaxin CBL0137 is a member of a new class of potent carbazole-derived anticancer compounds that combines two of these DNA binding modes: the planar carbazole core intercalates between the base pairs of DNA with symmetrical side chains protruding into the major groove, while the positively charged nitrogen-containing chain fills the minor groove. CBL0137 binding was shown to cause nucleosome disassembly and spatial genome organization changes in cancer cells. We investigated the impact of CBL0137 on the key epigenetic process of DNA methylation by *de novo* murine DNA methyltransferase Dnmt3a and compared it to that of the other DNA intercalator doxorubicin. CBL0137 binding to DNA saturated at ~one molecule/bp, which was demonstrated by fluorescence polarization using specially designed fluorescently labelled oligonucleotide substrates. Binding of CBL0137 to Dnmt3a-CD was not observed. CBL0137 significantly inhibited *in vitro* DNA methylation by Dnmt3a at low micromolar concentrations (IC_{50} 9 ± 3 to 3 ± 2 μ M) and reduced the binding affinity of Dnmt3a to its DNA target, causing up to four-fold increase in the K_d of the DNA-enzyme complex. Doxorubicin acted as a significantly weaker inhibitor of DNA methylation. Therefore, decreased methylation efficiency in the presence of CBL0137 can be attributed mostly to blocking of the DNA minor groove by the side chain of curaxin. Given that many cancer cells exhibit hypermethylation of tumor suppressor genes by Dnmt3a and/or express mutated Dnmt3a variants, the obtained results allow one to consider Dnmt3a as one of the cellular targets of DNA-intercalating anticancer agents. This work was supported by RFBR grants 18-34-00364 and 19-04-00533.