Carbamoylating chemoresistance induced by cobalt pretreatment in C6 glioma cells: putative roles of hypoxia-inducible factor-1.

許重義

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Abstract

We tested whether pretreatment of reagents known to induce hypoxia-inducible factor-1 (HIF-1) may confer chemoresistance against cytotoxicity of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) to rat C6 glioma cells. We also studied which cytotoxic mechanism(s) of chloroethylnitrosoureas could be neutralized by cobalt preconditioning.

Preconditioning of rat C6 glioma cells with cobalt chloride (300 μM, 2 h) induced HIF-1 binding activity based on electrophoretic mobility shift assay (EMSA). Results from Western blotting confirmed a heightened HIF-1α level upon cobalt chloride exposure (300–400 μM, 2 h). Cobalt chloride (300 μM) pretreatment for 2 h substantially neutralized BCNU toxicity, leading to increases in glioma cell survival based on MTT assay. In addition, pre-exposure of C6 cells with desferrioxamine (DFO; 400 μM, 3 h), an iron chelator known to activate HIF-1, also induced HIF-1 binding and rendered the glioma cells resistant to cytotoxicity of BCNU.

Pre-incubation with cobalt chloride abolished the cytotoxicity of several carbamoylating agents including 2-chloroethyl isocyanate and cyclohexyl isocyanate, the respective carbamoylating metabolites of BCNU and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. The protective effect of cobalt exposure, however, was not observed when cells were challenged with alkylating agents including temozolomide.

Cadmium chloride (50 μM) effectively reversed cobalt-induced HIF-1 activation. Correspondingly, cadmium chloride suppressed carbamoylating chemoresistance mediated by cobalt chloride pretreatment. Furthermore, both double-stranded oligodeoxynucleotide (ODN) decoy with HIF-1 cognate sequence and antisense phosphorothioate ODNs against HIF-1α partially abolished the carbamoylating chemoresistance associated with cobalt preconditioning.

Our results suggest that cobalt- or DFO-preconditioning may enhance glioma carbamoylating chemoresistance that is dependent, at least in part, on induction of HIF-1.