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Sensitization of neuroblastoma to apoptosis mediated by oncogene-specific antisense peptide nucleic acid oligomers

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Apoptosis, or programmed cell death, is a major pathway by which chemotherapeutic drugs kill tumor cells. The B-cell lymphoma/leukemia-2 (bcl-2) and FLICE-inhibitory (FLIP) cellular oncogenes promote tumor cell survival, by blocking apoptotic signals or mechanisms of action. The objective of the present studies was to determine whether antisense peptide nucleic acid (PNA) oligomers targeted to bcl-2 and FLIP sensitize resistant neuroblastoma cells to Fas-mediated apoptosis. The Fas receptor pathway has been shown to be an important mediator of drug-induced apoptosis, and the development of drug resistance has been linked to deficient activation of Fas. IMR-32 neuroblastoma cells overexpress the oncoprotein bcl-2, which inhibits mitochondrial activation of apoptosis. These cells also overexpress the caspase-8 inhibitor FLIP, which blocks poly(ADP-ribose) polymerase cleavage and apoptotic DNA degradation. SH-SY5Y neuroblastoma cells express lower levels of bcl-2 protein and have minimal caspase-8 expression. Using lipofectamine PLUS as a delivery vehicle, the two cell lines were treated with anti-bcl-2 or anti-FLIP PNAs, a combination of the two, or a negative control PNA not complementary to any known mammalian DNA or RNA sequence. After PNA treatment, IMR-32 and SH-SY5Y cells were incubated with anti-Fas monoclonal antibody CH11. Cell death was measured by the MTT colorimetric assay, and apoptosis was evaluated by the TUNEL method and nucleosome ELISA. The results of these studies allowed assessment of differential sensitization of these two neuroblastoma cell lines to Fas-mediated apoptosis induced by antisense PNAs.