Induced apoptosis of neuroblastoma by treatment with anti-oncogene peptide nucleic acids

Neuroblastoma is a cancerous tumor found in the nervous system of young children. These cells overexpress bcl-2 and FLIP proteins, which both prevent apoptosis. The bcl-2 protein inhibits the pro-apoptotic protein bax, and the FLIP protein inhibits caspase-8, an enzyme that mediates apoptosis. Antisense PNAs, or peptide nucleic acids, which are similar to DNA molecules, may have potential to induce apoptosis in these cells, by inhibiting bcl-2 and FLIP gene expression. The objective of this study was to determine whether these neuroblastoma cells could be induced to undergo apoptosis when treated with anti-oncogene PNAs. The effect of anti-bcl-2 and anti-FLIP PNAs on IMR-32 and SH-SY5Y neuroblastoma cells was tested. The cells were treated with each of the PNAs separately, a combination of the two, and with nonsense PNA as a negative control. Using Western blots, the protein expression after treatment was analyzed. Then the anti-Fas antibody CH11 was added, and the cells’ ability to undergo apoptosis was analyzed by TUNEL assays and nucleosome ELISA. It was found that anti-bcl-2 PNA, in IMR-32 cells only, resulted in decreased protein expression and in apoptosis through the Fas receptor pathway. Failure of anti-FLIP PNA to induce apoptosis suggested that the anti-bcl-2 PNA acted by a caspase-8-independent mechanism, perhaps involving protein kinase C, MAPK, the Traf/TRAIL system, or other mitochondrial pathways. Because the Fas receptor mediates drug-induced apoptosis, treatment with anti-oncogene PNAs may cause resistant neuroblastoma cells to become susceptible to apoptosis during chemotherapy.