Neuroblastoma is a devastating pediatric cancer and most patients older than 18 months present with multi-organ metastatic disease. High grade or recurrent disease is refractory to treatment with chemotherapy and almost uniformly fatal. Glucogen Synthase Kinase-3 (GSK-3) was recently identified as a potential therapeutic target in human neuroblastoma. Because GSK-3 has been shown to be a positive regulator of NF-κB-mediated survival and chemoresistance in cancer cells, we hypothesize that the inhibition of GSK-3 may have therapeutic effects in neuroblastoma. Using chemically distinct GSK-3 inhibitors (AR-A014148, TDF22 and 9-ING-41), we found that the inhibition of GSK-3 led to a decrease in viability of neuroblastoma cell lines. Our novel, proprietary GSK-3 inhibitor 9-ING-41 was identified as being most potent. We observed that inhibition of GSK-3 results in decreased expression of the antiapoptotic molecule XIAP (NF-κB) and a subsequent decrease in XIAP mRNA and protein in human neuroblastoma cell apoptosis. Our xenograft in vivo studies show that the combination of imatinib (CPT-11) and 9-ING-41 leads to regression of subcutaneous (SK- N-DZ) neuroblastoma xenograft tumors at doses of imatinib that are below the maximum tolerated dose whereas imatinib or 9-ING-41 monotherapy had little or only modest effects on tumor growth. Our results suggest that the inhibition of GSK-3 is a promising new approach for the treatment of neuroblastoma, especially when combined with imatinib cytotoxic therapy.

Using immunohistochemical staining, we found that GSK-3 is highly expressed in human neuroblastomas and in subcutaneous xenograft tumors established from SK-N-BE2 and SK-N-DZ neuroblastoma cell lines. Using a cell viability assay, we found that the GSK-3 of our novel GSK-3 inhibitor 9-ING-41 (100 nM) for inhibiting the growth of SK-N- BE2 and SK-N-DZ neuroblastoma cells is more than 80 times lower than the GSK-3 of other GSK-3 inhibitors, namely AR-A014148 and TDF22. Our results show that 9-ING-41 inhibits GSK-3, as shown by downregulation of phospho-glycogen synthase (phospho-GS) expression, leading to a decrease in expression of NF-κB target XIAP (anti-apoptotic protein) and leading to significant apoptosis in neuroblastoma cells in vitro as shown by PARP cleavage, an apoptosis marker (Fig. 1, lower panel).

Neuroblastoma is a devastating pediatric cancer and most patients older than 18 months present with multi-organ metastatic disease. High grade or recurrent disease is refractory to treatment with chemotherapy and almost uniformly fatal. Thus, neuroblastoma represents a significant unmet medical need and the identification of new therapeutic agents is urgently needed for the effective treatment of neuroblastoma to improve clinical outcomes. NF-κB activation is known to promote human cancer progression, metastasis, and chemoresistance (5, 23). Glycogen Synthase Kinase-3 (GSK-3), a serine/threonine protein kinase, is an essential positive regulator of NF-κB transcription activity (5). Our previous studies showed that GSK-3 is a positive regulator of NF-κB-mediated survival and chemoresistance of cancer cells (16). It has been shown that treatment of neuroblastoma cells with doxorubicin or etoposide resulted in enhanced NF-κB transcriptional activity in an dose-dependent manner (16). Our previous studies have demonstrated that the inhibition of GSK-3 decreases cancer cell survival via suppression of the NF-κB-mediated expression of BCL-2 and XIAP (4, 5). Moreover, we have demonstrated that AR-A014148, a toboxos GSK-3 inhibitor, enhanced the antineoplastic effect of doxorubicin and synergistically decreased the viability of renal cancer cells (8). Similarly, AR-A014148 was shown to sensitize pancreatic cancer to gemcitabine (11). Finally, a number of published studies identified GSK-3 as a new therapeutic target in human neuroblastoma (12-13). Hypophosphorylation of GSK-3β, a positive regulator of NF-κB activity, overcomes NF-κB-mediated chemoresistance and decreases the effect of conventional chemotherapies in human neuroblastomas.

Results (1)

Figure 1. Inhibition of GSK-3 suppresses XIAP expression and leads to apoptosis in neuroblastoma cells. a) Immunohistochemical analysis and immunoblot analysis of human neuroblastoma cells were treated with 9-ING-41 (100 nM) for 48 h. Expression of XIAP protein was measured using a polyclonal antibody to XIAP (A) and a monoclonal antibody to XIAP (B). b) PARP cleavage, an apoptosis marker (Fig. 1, lower panel).

Results (2)

Figure 2. Treatment with CPT-11+9-ING-41 leads to an increased apoptosis and a partial regression of SK-N-DZ xenograft tumors. SK-N-DZ neuroblastoma cells were inoculated subcutaneously (paw) to 20-week-old male nude mice (1 tumor per mouse). Tumors were size measured and mice were randomized into 4 treatment groups: control (DMSO, n=6 mice); CPT-11 (5 mg/kg, n=6 mice); 9-ING-41 (100 nM, n=6 mice); CPT-11+9-ING-41 (5 mg/kg, 100 nM, n=6 mice). On Day 22, mice were sacrificed when tumors grew more than 5 times the original starting volume and the weight of resected tumors was measured. Cutoff for tumor weight was 300 g. 3. Representative pictures of GS phosphorylated (phospho-GS) tumors from each group of animals. The percentage of apoptosis was measured by TUNEL staining. Column, means+s.e.; B, Representative picture of TUNEL staining of SK-N-DZ neuroblastoma tumors treated as indicated.