Targeting GSK-3: a new approach for the treatment of neuroblastoma

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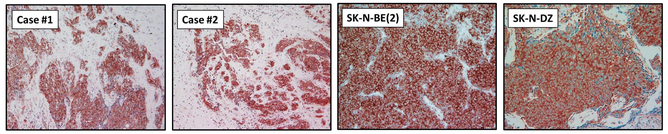
Abstract

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. Glycogen Synthase Kinase-3 (GSK-3) was recently identified as a potential therapeutic target in human neuroblastoma. Because GSK-3β 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-A014418, TDZD8 and 9-ING-41), we found that pharmacological inhibition of GSK-3 led to a decrease in viability of neuroblastoma cells. However, our novel and 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 target gene) and a subsequent increase in neuroblastoma cell apoptosis. Our xenograft in vivo studies show that the combination of irinotecan (CPT-11) and 9-ING-41 leads to regression of subcutaneous SK-N-DZ neuroblastoma xenograft tumors at doses of irinotecan that are below the maximum tolerated dose whereas irinotecan 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 irinotecan cytotoxic therapy

Results (1)



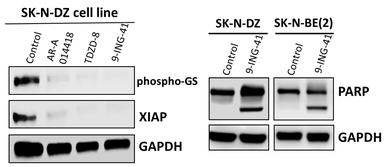


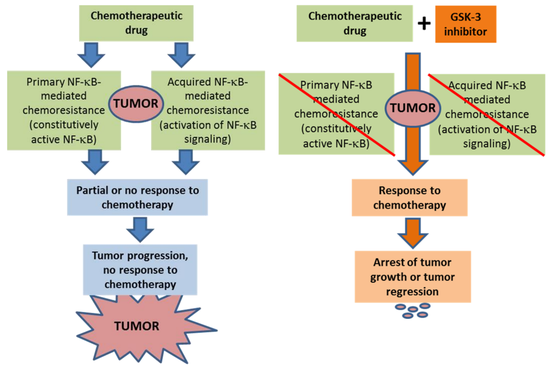
Figure 1. Inhibition of GSK-3 suppreses XIAP expression and leads to apoptosis in neuroblastoma cells. *Upper panel*, immunohistochemical analysis of GSK-3β expression and localization in human neuroblastoma tumors obtained from cancer patients (Case #1 and Case #2) and in xenograft tumors established from SK-N-BE(2) and SK-N-DZ neuroblastoma cell lines. *Lower panel*, neuroblastoma cells were treated with 5 μM AR-A014418, 5 μM TDZD-8 and 0.1 μM (lower left panel) or 1 μM (lower right panel) of 9-ING-41 for 48 hours. 30 μg of proteins (whole cell lysate) was separated by SDS-PAGE, transferred to PVDF membrane, and immunoblotted as indicated.

Using immunohistochemical staining, we found that GSK-3β is highly expressed in human neuroblastomas and in subcutaneous xenograft tumors established from SK-N-BE(2) and SK-N-DZ neuroblastoma cell lines (Fig. 1, upper panel). Using a cell viability assay, we found that the GI_{50} of our novel GSK-3 inhibitor 9-ING-41 (100 nM) for inhibiting the growth of SK-N-DZ and SK-N-BE(2) neuroblastoma cells is more than 80 times lower than the GI_{50} of other GSK-3 inhibitors, namely AR-A014418 and TDZD-8. Our results show that 9-ING-41 inhibits GSK-3, as shown by downregulation of phospho-glycogen synthase (phospho-GS) expression, leading to a decreased expression of NF-κB target XIAP (anti-apoptotic protein), leading to significant apoptosis in neuroblastoma cells *in vitro* as shown by PARP cleavage, an apoptosis marker (Fig. 1, lower panel).

Introduction

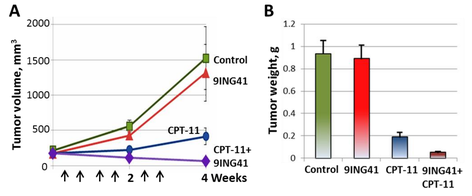
Neuroblastoma is a devastating pediatric cancer and most patients older than 18 months present with multi-organ metastatic disease. High grade or recurrent disease is refractory to treatment with chemotherapy and almost uniformly fatal. Thus, neuroblastoma represents a significant unmet medical need and the identification of new therapeutic agents is urgently needed for the effective treatment of neuroblastoma to improve clinical outcomes.

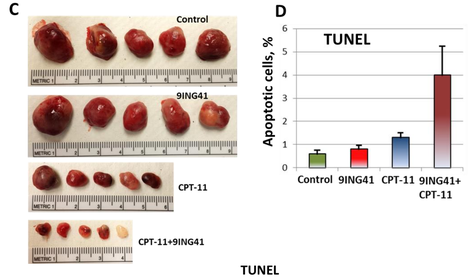
NF-κB activation is known to promote human cancer progression, metastasis, and chemoresistance (1, 2). Glycogen Synthase Kinase-3β (GSK-3β), a serine/threonine protein kinase, is an essential positive regulator of NF-κB transcriptional activity (3). Our previous studies showed that GSK-3β is a positive regulator of NF-κB-mediated survival and chemoresistance of cancer cells (4-9). It has been shown that treatment of neuroblastoma cells with doxorubicin or etoposide resulted in enhanced NF-κB transcriptional activity in a dose-dependent manner (10). 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 (4-9). Moreover, we have demonstrated that AR-A014418, a toolbox GSK-3 inhibitor, enhanced the anticancer effect of docetaxel and synergistically decreased the viability of renal cancer cells (8). Similarly, AR-A014418 was shown to sensitize pancreatic cancer cells to gemcitabine (11). Finally, a number of published studies identified GSK-3β as a new therapeutic target in human neuroblastoma (12-13).



Hypothesis: inhibition of GSK-3β, a positive regulator of NF-κB activity, overcomes NF-κB-mediated chemoresistance and thereby potentiates the effect of conventional chemodrugs in human neuroblastoma.

Results (2)





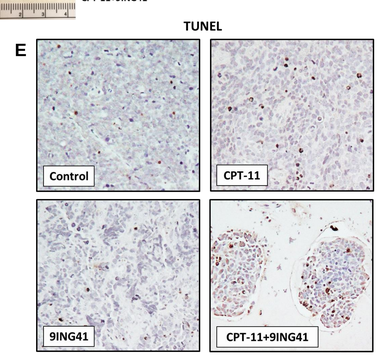


Figure 2. Treatment with CPT-11+9ING41 leads to an increased apoptosis and a partial regression of SK-N-DZ xenograft tumors. SK-N-DZ neuroblastoma cells were inoculated subcutaneously (subQ) to 20 nude mice (1 tumor per mouse). Tumors were size matched and mice were randomized into 4 treatment groups: control (DMSO; n=5 mice), CPT-11 (5 mg/kg, n=5 mice), 9ING41 (70 mg/kg, n=5 mice) and Irinotecan+9ING41 (n=5 mice). ***A***, Vehicle (20 μL DMSO) or drugs were injected as shown by arrows. Points, mean tumor volume; bars, SE. ***B***, Mice were sacrificed when tumors grew to more than 5 times the original starting volume and the weight of resected tumors was measured. Columns, mean tumor weight; bars, SE. ***C***, Representative pictures of GBM PDX subQ tumors from each group of animals. ***D***, The percentage of apoptotic cells was determined by TUNEL staining. Columns, mean; bars, SE. ***E***, Representative pictures of TUNEL staining of SK-N-DZ neuroblastoma xenograft tumors treated as indicated.

Conclusion

Our results suggest that the inhibition of GSK-3 is a promising new therapeutic approach to enhance chemosensitivity of neuroblastoma to the antitumor effect of irinotecan.

Materials and Methods

Human neuroblastoma cell lines SK-N-DZ and SK-N-BE(2) were purchased from ATCC. GSK-3 inhibitors AR-A014418 and TDZD-8 and other chemical reagents have been purchased from Sigma-Aldrich. GSK-3β, GAPDH, PARP, phospho-glycogen synthase, Ser641 antibodies were purchased from Cell Signaling Technologies. XIAP antibody has been purchased from BD Biosciences. Cell viability was examined using a colorimetric MTS assay, the CellTiter 96 assay (Promega), according to the manufacturer's protocol. IHC staining was performed with Dako Envision+/HRP kit according to the recommended manufacturer procedure.

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