Targeting of Survivin Inhibits Neuroblastoma Cell Proliferation

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INTRODUCTION

High-risk neuroblastoma (NB) is an aggressive pediatric tumor which develops from the extracranial sympathetic nervous system and accounts for almost 15% of all childhood cancer-related deaths. Survivin is known to be involved in controlling cell division and apoptosis and belongs to the inhibitor of apoptosis protein family. Oncogenic activation of survivin has been reported in different cancers including NB. In the present study, we analyzed genomic datasets of 1135 NB patients and found that high expression of survivin coding gene BIRC5 strongly correlates with poor overall and event-free survival of NB patients, and more aggressive tumors have significantly higher BIRC5 levels. To understand and evaluate the biological effects of survivin inhibition on NB growth, five experiments were performed. The cell viability assay (MTT) results from six cell lines: 3 MYCN-non-amplified cell lines (SK-N-SY, SK-N-AS, CHLA-225), and 3 MYCN-amplified cell lines (NGP, LAN-5, IMR-32) demonstrated the inversed proportional relationship between increased dose, and tumor growth. Clonogenic and Spheroid experiments further confirmed a noteworthy reduction of colony formation and reduced tumor size respectively, with increased dose. Utilizing Annexin V apoptosis assays and Click-IT EdU cell proliferation assays, the effect on Cell Cycle and Apoptosis were assessed, which revealed that in comparison to control, increased dose promoted apoptosis and prevented the advancement of the cell cycle.

OBJECTIVES

- To evaluate the effects of a small molecule inhibitor of survivin.
- To determine in vitro effects of survivin inhibition on NB cell proliferation, apoptosis, and cell cycle.
- To identify a novel therapeutic approach to treat high-risk neuroblastoma.

METHODS

- **R2 Genomic Analyses:** Neuroblastoma patient datasets of Kcoc (N=649), Versteeg (N=88), and SEQC (N=498) were analyzed using R2 Genomic Analysis and Visualization Platform. These datasets were analyzed for overall survival correlation and gene expression.
- **Cell Culture:** The human neuroblastoma MYCN-non-amplified (SK-N-SY, SK-N-AS, CHLA-225), and MYCN-amplified (NGP, LAN-5, IMR-32) cell lines were cultured in a 5% CO2 atmosphere with 95% humidity at 37°C in RPMI-1640 supplemented media.
- **Viability Assay:** Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay after 72 hours incubation.
- **Spheroidal Assay:** Spherical assay was performed using corning 3D-spheroidal plates, and Biotium live/dead kit.
- **Clonogenic Assay:** Colony formation was evaluated using 0.5% crystal violet staining.
- **Apoptosis and Cell Cycle Assay:** Apoptosis and cell cycle assays were performed using Annexin V apoptosis assay kit and Click-IT EdU cell proliferation assay in the Attune NXT Flow cytometer.
- **Statistical Analysis:** All experimental data was obtained using three biological replicates for each of the two technical replicates done.

RESULTS

**Figure 1. BIRC5 promotes NB progression.**
(A, B, C) Kaplan-Meier curves show the overall probability of NB survival in comparison with BIRC5 expression: A. 649 patients in Kcoc dataset. B. 498 patients in SEQC dataset. C. 66 patients in the Versteeg dataset. (D, E, F) ROC analysis showing correlation of BIRC5 expression levels in neuroblastoma disease stages. (D) ROC curve. (E) AUC. (F) ROC curve of BIRC3 expression for each cell line. (G) Statistical analysis of BIRC5 expression in MYCN amplified and non-amplified cell lines. (H) Statistical analysis of BIRC5 expression in MYCN amplified and non-amplified cell lines.

**Figure 2. BIRC5 inhibitor prevents NB cell proliferation.**
(A) Representative Flow cytometer images and graphical representation of % Apoptosis in SH-SY5Y. (B, C) Representative Flow cytometer images and graphical representation of cell cycle analysis performed using Actinomycin cell cycle assay kit in B. SH-SY5Y, and C. NGP cell lines.

**Figure 3. BIRC5 inhibitor prevents NB 3D spheroidal growth.**
(A) Representative images of low and high magnification of BIRC5 inhibitor treated and untreated cell lines. (B, C) Representative images of low and high magnification of BIRC5 inhibitor treated and untreated cell lines.

**CONCLUSION/ FUTURE WORK

- Survivin inhibition significantly inhibits NB cell proliferation, colony growth, blocks cell cycle progression, induce apoptosis, and inhibit 3D spheroid tumor formation and growth in a dose-dependent manner.
- Overall, our data highlights the importance of survivin as a target in NB, and the potential of survivin inhibition as a novel therapeutic approach for NB.
- In future efforts, we will combine the survivin inhibitor with chemotherapy drugs to develop a less-toxic and more-effective therapeutic approach for NB treatment.

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