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Survivin-targeted siRNA sensitizes retinoblastoma primary cells to Melphalan and Carboplatin

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PURPOSE

Survivin is overexpressed in many cancer types and play a relevant role in cancer resistance by inhibiting activation of effector caspases.

In retinoblastoma (RB), refractory cases could rapidly evolve to brain metastasis.

Here we propose the delivery of survivin targeting siRNA by **pH-sensitive LNP** and evaluate its impact on immortalized (Y79) and primary retinoblastoma cells.

OBJECTIVES

1. To screen the ability of pH-sensitive LNP to downregulate survivin on different cancer cell lines;
2. To confirm survivin as a specific cancer target in a retinoblastoma (RB) cell line model (Y79);
3. To evaluate survivin downregulation effects on Y79 cells regarding cell proliferation and cell cycle;
4. To measure the impact of survivin downregulation prior Carboplatin (CBDA) and Melphalan (MELPH) treatment in Y79 and primary RB cells.

METHODS

qPCR: Cells were transfected with 40 nM (panel of cancer cells) or 20 nM (Y79) of siRNA-targeted survivin with pH-sensitive liposomes or Lipofectamine RNAiMAX. Cells were analyzed after 48 hours.

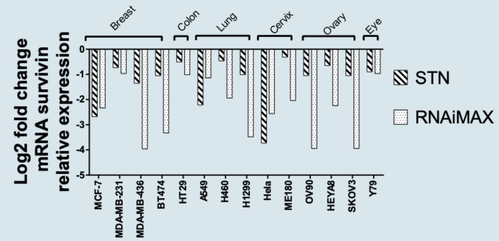
Western Blot: Cells were transfected with 20 nM siRNA-targeted survivin for 48 hours before analysis. 40 ug of total proteins were loaded per lane. When treated with CBDA, Y79 cells were transfected at day 0, drug added at day 2 and WB performed at day 4.

Viability assay: Y79 cells were transfected at day 0, drugs added at day 2 and Resazurin-based viability assay executed at day 4.

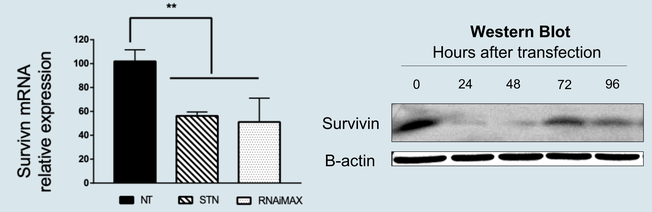
Synergism: Synergism was calculated using COMPUSYN for conditions where combined treatment values were statistically different than for drug only and the effect on cell viability was higher than 50 %. Combination index (CI) are above each concentration. CI < 1 indicates synergism.

RESULTS

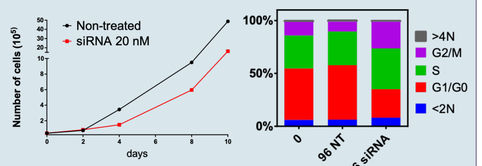
pH sensitive LNP silenced survivin in a cell-type dependent fashion



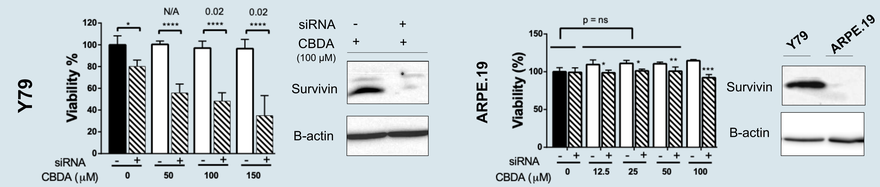
In Y79, LNP silenced survivin to an extent comparable to Lipofectamine. Western blotting confirmed protein depletion for up to 48 hours following transfection



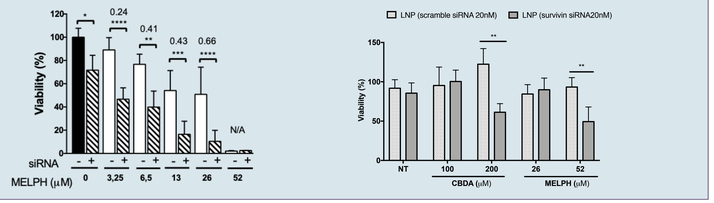
Survivin silencing delayed Y79 proliferation and arrested cells in G2/M phase



Survivin silencing synergistically improved Carboplatin (CBDA) cytotoxicity in Y79 cells (left), but not in a non-cancerous ARPE.19 cells (right)



Survivin silencing improved Carboplatin and Melphalan and cytotoxicity in immortalized (Y79, Left)) and primary RB cells (right)



CONCLUSIONS

pH-sensitive liposomes efficiently delivered and silenced survivin in a panel of cancer cell lines. In Y79 cells, survivin silencing was observed for up to 48 hours.

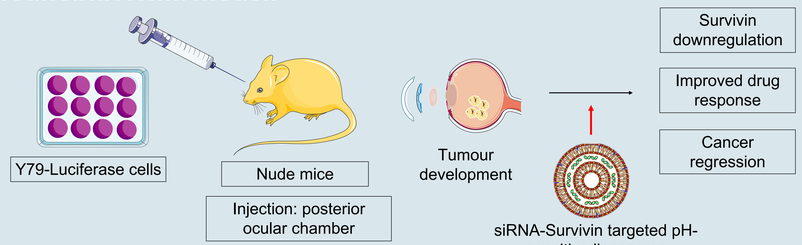
Survivin is expressed in retinoblastoma cells (Y79) but not in healthy retinal pigment endothelium cells (ARPE.19).

Survivin silencing delayed Y79 cells proliferation and arrested cells in G2/M phase

Survivin silencing synergistically improved carboplatin and melphalan's cytotoxicity in Y79 and primary RB cells

PERSPECTIVES

The high efficiency of the pH-sensitive liposomes in delivering siRNA and specifically survivin expression in cancer cells encourage us to perform in vivo experimentation in a murine retinoblastoma model.



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