The anticancer effects of Supinoxin™ (RX-5902) in triple-negative breast cancer MDA-MB-231 through phosphorylated p68 on Tyr593

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ABSTRACT

Several studies have indicated that the DEAD box RNA helicase DDX5/p68 plays several important roles in cancer (1, 2). In particular, p68 that is phosphorylated on Tyr593 has been shown to be associated with cell transformation, epithelial mesenchymal transition (EMT) and cell migration (3). Therefore, phosphorylated p68 (p-p68) may be a promising target for novel anti-cancer therapeutics. We previously reported that 1-(3,5-dimethoxyphenyl)-4-[(6-fluoro-2-methoxyquinolin-3-yl)-amino]carbonyl piperazine (RX-5902, Supinoxin™) inhibits the growth of cancer cells at low nanomolar concentrations by interacting with p-p68 on Tyr593, interfering with the p-p68-β-catenin signaling pathway (4). In this study, we sought to determine whether p-p68 on Tyr593 plays a key role in RX-5902’s ability to inhibit cancer cell growth by knocking down p68. p68-siRNA efficiently down-regulated the expression of phosphorylated p68 on Tyr593 as well as p68 in the triple-negative (TN) breast cancer cell line, MDA-MB-231. Exposure of p68-siRNA-transfected cells to the IC50 concentration of RX-5902 protected MDA-MB-231 cells from the cytotoxic effects of RX-5902, indicating the p-p68 on Tyr593 is a key molecule for RX-5902 cytotoxic effects. We also examined the tumor growth inhibition (TGI) of RX-5902 in the human TN-breast tumor (MDA-MB-231) xenograft mouse model. Not only did RX-5902 demonstrate potent efficacy in this model but also oral administration with RX-5902 resulted in dose-dependent TGI and extended the overall survival of these animals. Oral administration of 160, 320 and 600 mg/kg of RX-5902 showed 44%, 65% and 83% TGI, respectively, whereas 5mg/kg of Abraxane(iv) showed only 50% TGI at day 29. Further studies demonstrated the inhibitory effects of RX-5902 on cellular motility in MDA-MB-231 in wound healing assays, suggesting the potential function of phosphorylated p68 on Tyr593 in cell migration (5). These data support the potential therapeutic activity of RX-5902 in triple negative breast cancers. A Phase 1 study of RX-5902 on relapse/refractory solid tumors is ongoing.

MATERIALS & METHODS

siRNA transfections: The siRNA transfections were performed using 20nm siRNA (Dharmacon) and Lipofectamine® RNAiMAX Transfection Reagent according to the manufacturer’s protocols. p68: AAGUCUAAUGUGGAGCGAC, NS (non-specific control): CAGUCGCGUUUGCGACUGG

In Vivo Tumor Studies: The efficacy of RX-5902 (oral doses of 160, 320, and 600 mg/kg; given once a week [QWK] for 3 weeks) was examined in human TN-breast cancer MDA-MB-231 xenograft models, grown subcutaneously in athymic nude mice. RX-5902 or vehicle treatments were initiated when established tumors reached an average size of ~100 mm3. The tumor volumes were measured twice weekly throughout the study duration, and efficacy was calculated based on the percentage inhibition of tumor volume.

Migration assay: Cells were treated with RX-5902 for 16h and grown to confluence. ‘Scratch wounds’ were created and migration was measured in 1% FBS-DMEM to minimize proliferation using the Incucyte live-cell imager.

RESULTS

p68 downregulation prevented MDA-MB-231 from RX-5902 cytotoxicity

Un-tfx = un-transfected

si-NS = transfected with non-specific siRNA

si-p68 = transfected with p68 siRNA

RX-5902 inhibited tumor growth in a dose-dependent manner without body weight changes in MDA-MB-231 xenograft model

<table>
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<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>TGI</th>
<th>%TGD</th>
<th>PR</th>
<th>CR</th>
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RX-5902 inhibited MDA-MB-231 cell motility

The changes in epithelial-mesenchymal transition related genes are still under investigation

REFERENCES


CONCLUSION/DISCUSSION

1. p-p68 plays a key role in RX-5902-induced cytotoxicity.
3. RX-5902 shows the inhibitory effects on cellular motility in MDA-MB-231, suggesting the potential function of p-p68 in cell migration.
4. A Phase 1 study of RX-5902 on relapse/refractory solid tumors is ongoing (NCT02003092).

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