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

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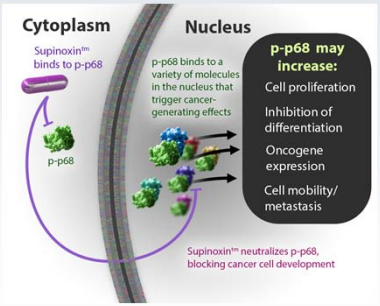
San Antonio Breast Cancer Symposium – December 8-12, 2015

ABSTRACT (P5-03-13)

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-methoxyquinoxalin-3-yl) aminocarbonyl] 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 5 mg/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.

BACKGROUND

We previously reported that RX-5902 showed potent inhibition of cancer cell growth and induction of apoptosis, with IC_{50} between 10 and 20 nM (6). We also reported that RX-5902 interacted with Y593 p-p68 RNA helicase and resulted in inhibition of β-catenin signaling pathway (4). Since p-p68 is involved in several signaling pathways, it is possible RX-5902 may have other anti-cancer effects.



Aims of this study:

1. Determine whether phosphorylated p68 on Tyr593 plays a key role in RX-5902's ability to inhibit TN-breast cell growth.
2. Examine the tumor growth inhibition of RX-5902 in TN-breast tumor xenograft.
3. Address whether RX-5902 has other anti-cancer effects such as inhibition of metastasis.

MATERIALS & METHODS

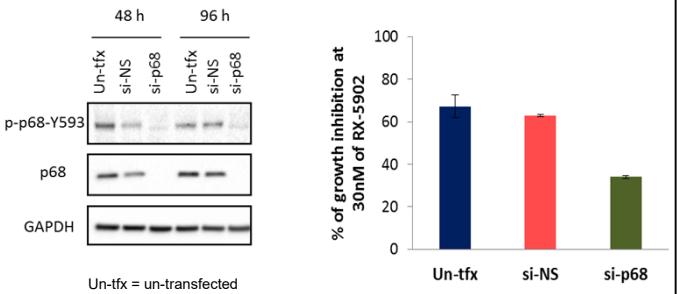
siRNA transfections: The siRNA transfections were performed using 20nM siRNA (Dharmacon) and Lipofectamine® RNAiMAX Transfection Reagent according to the manufacturer's protocols. p68: AACUCUAAUGUGGAGUGCGAC, NS (non-specific control): CAGUCGCGUUUGCGACUGG

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 mm^3. 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.

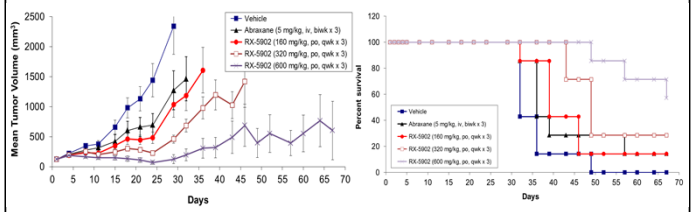
RESULTS

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

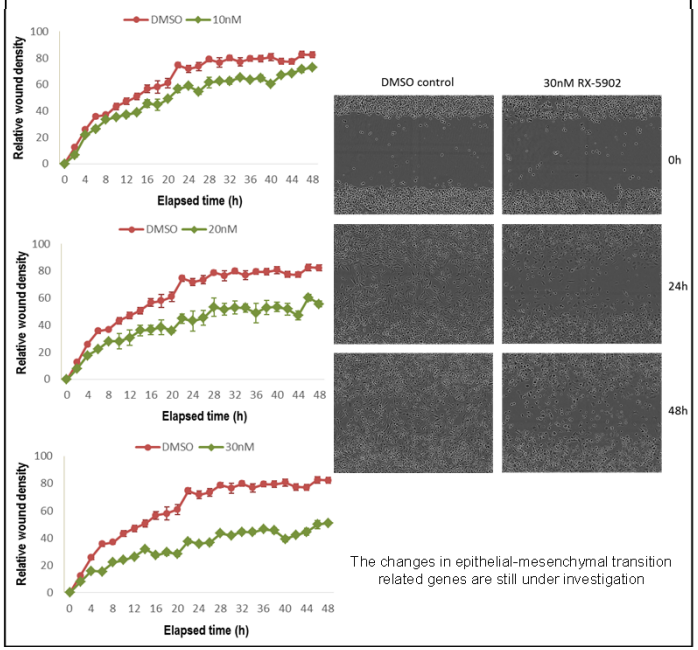
RX-5902 inhibited tumor growth in a dose-dependent manner without body weight changes in MDA-MB-231 xenograft model



Treatment	Dose (mg/kg)	%TGI	%TGD	PR	CR
Vehicle	-	-	-	0	0
Abraxane	5	50^{ns}	27^{ns}	0	1
RX-5902	160	44^{ns}	39^{ns}	0	0
RX-5902	320	65^{ns}	82**	0	1
RX-5902	600	83**	165***	2	2

%TGI = percent tumor growth inhibition; %TGD = percent tumor growth delay
ns = not significant, ** = P ≤ 0.01, *** = P ≤ 0.001, compared to vehicle
PR = partial regression; CR = complete regression

RX-5902 inhibited MDA-MB-231 cell motility



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

CONCLUSION/DISCUSSION

1. **p-p68 plays a key role in RX-5902-induced cytotoxicity.**
2. **RX-5902 significantly inhibits tumor growth in human TN-breast tumor MDA-MB-231 xenograft mouse model in a dose-dependent manner.**
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).**

REFERENCES

1. Fuller-Pace, RNA Biology, 10, 121–132 (2013).
2. Dai et al. Journal of Experimental & Clinical Cancer Research, 33, 64-71 (2014).
3. Yang et al., Cell, 127, 139–155 (2006).
4. Kost et al., Journal of Cellular Biochemistry, 15, 1595-1601 (2015).
5. Remenyi et al, American Association for Cancer Research, Abstract No:3577 (2015).
6. Lee et al., Bioorg Med Chem, 18, 7966-7974 (2010).

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