Mechanistic study of a new 4-(3, 5-dimethoxyphenyl)-N-(7-fluoro-3-methoxyquinoxalin-2-yl)piperazine-1-carboxamide compound (RX-5902)

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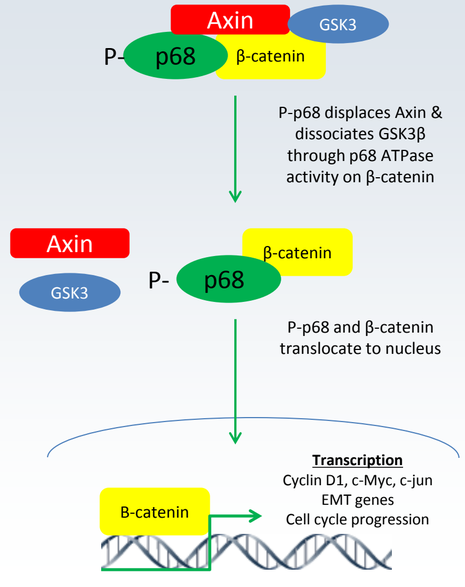
ABSTRACT # 5507

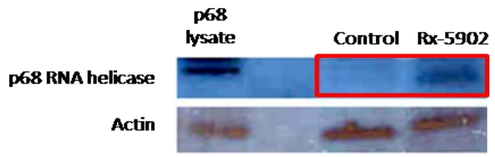
In our previous study, we showed that a novel compound 1-(3,5-dimethoxyphenyl)-4-[(6-fluoro-2-methoxyquinoxalin-3-yl) aminocarbonyl] piperazine (RX-5902) induces apoptosis of cancer cells by downregulation of Bcl-2 protein levels and causing G2/M cell cycle arrest. We observed also a synergistic effect in growth inhibition when combined with known anticancer agents in cancer cells and potent anti-growth activity in drug-resistant cancer cells as well as anti-proliferation of cancer cells at IC_{50} values of low nanomolar concentrations. Oral administration of the compound led to inhibition of tumor growth and enhanced survival in several tumor xenograft models. Our recent studies indicated that the compound interacts with p68 RNA helicase (also known as DDX5), a member of the DEAD box family of RNA helicases. p68 has been demonstrated to play a vital role in cell proliferation and tumor/cancer progression. Our studies showed that the compound did not exert its anti-cancer effects by inhibition of RNA unwinding by p68. Instead, the compound completely abrogated the β-catenin stimulated ATPase activity of p68 with an IC_{50} of 61 nM. More extensive studies are ongoing to validate and further characterize our interesting discovery.

BACKGROUND



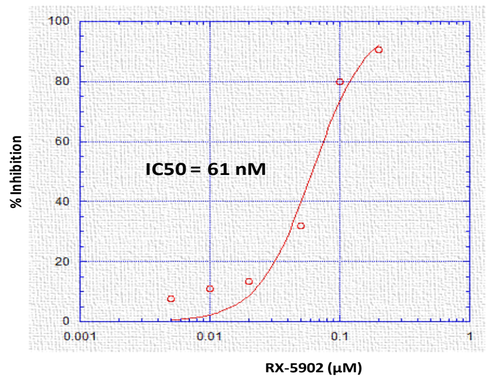
RESULTS

Binding to p68 RNA Helicase



p68 RNA helicase: Present only in RX-5902-treated sample using enriched p68 RNA helicase extract

Dose-dependent Inhibition of ATPase Activity of p68 RNA Helicase



RX-5902 inhibits ATPase activity of p68 on β-catenin

MATERIALS & METHODS

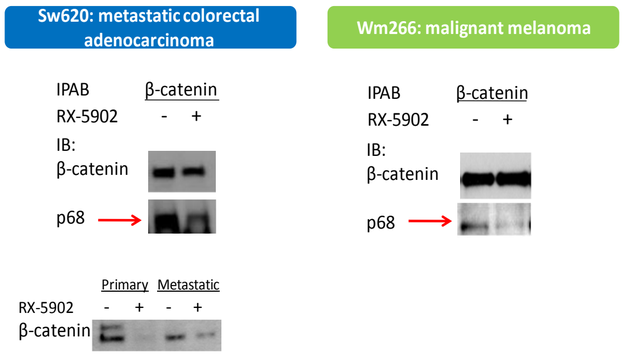
RX-5902 binding to p68 RNA helicase: Whole cell lysate from p68-transfected HEK293T cells incubated with or without RX-5902, eluted and analyzed after binding affinity assay. Samples were eluted and analyzed by western blotting.

In vitro ATPase activity of p68 on β-catenin: Release of inorganic phosphate was measured during ATP hydrolysis using a direct colorimetric assay. ATPase activity was measured in the presence of 1ug β-catenin, ATP and recombinant p68 RNA helicase, in the presence or absence of RX-5902 at the indicated concentrations.

RNA-dependent ATPase activity of p68: Release of inorganic phosphate was measured during ATP hydrolysis using a direct colorimetric assay. ATPase activity was measured in the presence of 2ug total RNA, ATP and recombinant p68 RNA helicase, in the presence or absence of RX-5902 at the indicated concentrations.

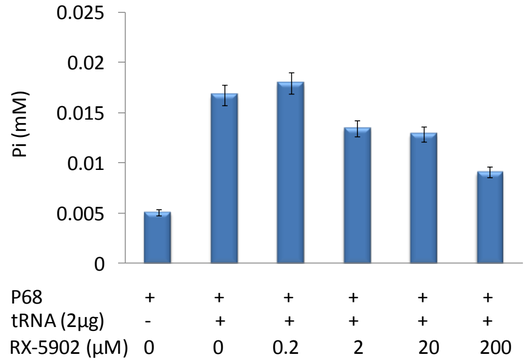
IP and Weasern blot: Cells were treated with RX-5902 & processed for IP and western blotting.

Effect of RX-5902 on the Interaction between p68 and β-catenin In Cancer Cells



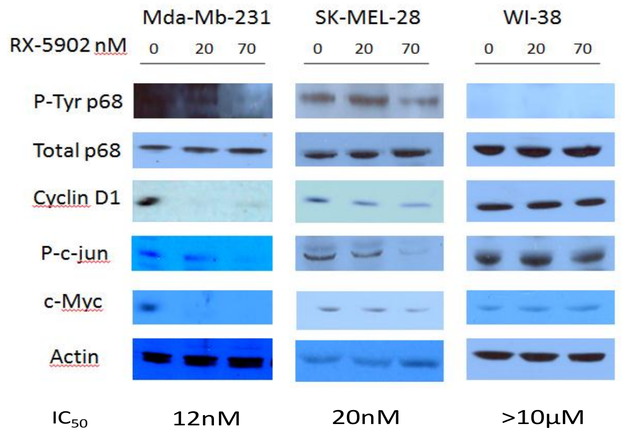
RX-5902 decreases p68 and β-catenin interaction in cancer cells

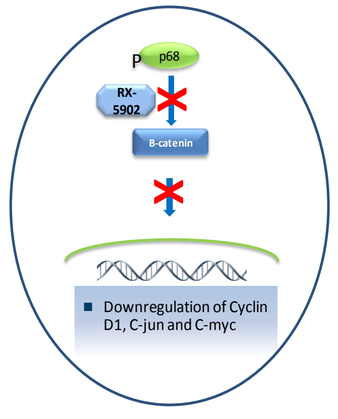
No Effects on RNA-dependent ATPase Activity of p68 RNA Helicase



No inhibition against normal p68 RNA unwinding activity

Effect of RX-5902 on Downstream Genes of p68 – β-catenin In Cancer





RX-5902 downregulated the downstream genes of p68-beta catenin interaction only in cancer cells, but not in normal cells

CONCLUSION/DISCUSSION

RX-5902 has a unique mechanism of action. Protein binding studies indicate RX-5902 binds to p68 RNA helicase (DDX5). p68 RNA helicase is known to play a vital role in cell proliferation, initiation of gene transcription and has been strongly implicated in tumor/cancer progression. Also, p68 expression may be a relevant biomarker as it has been shown that increased p68 expression is correlated with tumor progression and metastasis. Our preliminary studies indicate that RX-5902 binds to p68 and inhibits its enzymatic ATPase activity in the presence of its known biological substrate such as β-catenin.

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