Role of p38 MAPK signaling pathway and Sigma 1 Receptor on induction of Apoptosis in response to Ad.p53 and Rimcazole in Breast Cancer Cells

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Abstract

In 2014, there will be an estimated 1,665,540 new cancer cases diagnosed and 585,720 cancer deaths in the US. Cancer remains the second most common cause of death in the US, accounting for nearly 1 of every 4 deaths. Current therapeutic regimens such as surgery, or chemotherapy provide little benefit in extending the lifespan of the patient. This alarming data calls for the development of enhanced single or combinatorial therapies to reduce the further development of the lethal diseases. Gene therapy has gained more popularity over the past few years, and as with most treatment modalities, particular subsets of tumor cells might be inherently resistant to those anti-cancer genes, or they might acquire resistance because of repeated exposure to them. We have noticed that a p33R combination with Rimcazole has a synergistic effect in growth suppression that occurs independent of p53 status. Furthermore, this combination decreased Sigma 1 Receptor protein expression, and modulated apoptosis through p38 MAPK-dependent pathway. Since both Rimcazole and p33R are being evaluated in clinical trials, combining a dietary agent and a vinylylated therapeutic protein to provide an innovative approach for potentially treating human breast cancer.

Results and Conclusions

Ectopic expression of Sig-1R inhibits p53 + Rimcazole-mediated apoptosis in Breast cancer cells. The previous results led us to examine the possibility that down-regulation of Sig-1R expression might cause p53 + Rimcazole mediated apoptosis in Breast cancer cells. To test the hypothesis, Ad.p53 was used to transfect MCF-7 and T47D in the presence or absence of ectopic expression of Sig-1R, and measured caspase activity and induction of apoptosis by MTT. Ectopic expression of Sig-1R inhibited Ad.p53 + Rimcazole-mediated killing in MCF-7 and T47D cells (Figure 6). Together, these data suggest that Ad.p53 + Rimcazole-induced cell death in cancer cells would be achieved by down-regulation of Sig-1R, and is therefore inhibited by ectopic expression of Sig-1R.

Combinational treatment with Ad.p53 and Rimcazole activates p38 MAPK in breast cancer cells. Mechanisms by which Ad.p53 + Rimcazole down-regulates Sig-1R protein levels could involve activation of (p38 MAPK), and GSK3β inhibition. Based on this consideration, we determined if p38 MAPK inhibitors or GSK3β inhibitors might also play a role in Ad.p53 + Rimcazole-mediated killing in Breast cancer cells. MCF-7 cells were untreated or infected with Ad.p53 in combination with Rimcazole and analyzed by SDS-PAGE. Followed by Western blotting with anti-p38 MAPK, and anti-phospho-GSK3β antibodies. Treatment with Ad.p53 in combination with Rimcazole promoted p38 MAPK phosphorylation in MCF-7 cells, whereas it did not affect total p38 MAPK, neither GSK3β activity. Interestingly, there is a negative correlation between the basal levels of Sig-1R protein expression and sensitivity to standard chemotherapeutic drugs. Based on this consideration, it is possible that this combination would overcome resistance to standard chemotherapy in the clinical setting.

Introduction

The transcriptional factor p53, one of the most important tumor suppressors, protects normal growth and initiates malignant cell death. p53 is activated by a variety of cytotoxic stimuli, such as DNA damage induced by ionizing irradiation and chemotherapeutic, activation or modulation of cytokines, hypoxia and virus infection. In unstressed cells, p53 level and activity is strictly controlled especially by the ubiquitin E3 ligase MDM2, which binds p53 and targets it for proteosomal degradation (1). Cancer cells expressing the wild-type p53 gene (CRAd-p53; AdDelta24-p53, SG600-p53, OBP-702). These pre- and clinical trials have demonstrated that p53 exerts a profound influence on the improvement of the clinical outcome in p53-based cancer therapy (3).

Sigma 1 Receptor (Sig-1R) is upregulated in prostate cancer and breast cancer cells. Our current study unveils the synergistic effect of p53 in combination with Rimcazole. These two individually have the ability of inducing apoptosis in diverse cancer cells without harming normal cells or tissues. However, as with most treatment modalities, particular subsets of tumor cells might be inherently resistant to those anti-cancer genes, or they might acquire resistance because of repeated exposure to them. We have noticed that a p33R combination with Rimcazole has a synergistic effect in growth suppression that occurs independent of p53 status. Furthermore, this combination decreased Sigma 1 Receptor protein expression, and modulated apoptosis through p38 MAPK-dependent pathway. Since both Rimcazole and p33R are being evaluated in clinical trials, combining a dietary agent and a vinylylated therapeutic protein to provide an innovative approach for potentially treating human breast cancer.

Materials and Methods

Cell culture and reagents. Human breast MCF-7 cell line was obtained from American Type Culture Collection (ATCC). Rimcazole was purchased from Fisher Scientific. MTT Assay. Cells were plated in 96 well plates (1 x 104 cells) in DMEM containing 10%FBS and allowed to attach for 12 h prior to treatments. Treatments were added 4h after infection with adenovirus. Cells growth and viable cell numbers were monitored by 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) staining as described (4). Western Blot Analysis. Cells were grown on 10cm plates and protein extracts were prepared with RIPA buffer containing a cocktail of protease inhibitors. A total of 50 µg of protein was applied to 12% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were probed with polyclonal or monoclonal Antibodies to Sigma 1 Receptor, p38 MAPK, and α-actin.

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References


Figure 1. Ectopic expression of Sigma 1 Receptor inhibits the sensitivity of Ad.p53 and Rimcazole combinational treatment. MCF-7 and T47D cells were infected with Ad-vector (control), Ad.p53 all lower doses and Rimcazole, with or without ectopic expression of Sigma 1 Receptor (Sig-1R). MTT assays was measured 5 days after treatment. An average of three independent experiments is shown ± SD.