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 surgical, 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 this lethal disease. Gene therapy has gained more popularity over the past years, and its disease gene specific targeting is what it makes its unique. 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 now reveal that a p53 in combination with Rimcazole have a synergistic effect in growth suppression that occurs independent of p53 status. Furthermore, this combination decreased Sigma 1 Receptor protein expression, and mediated apoptosis through p38 MAPK-dependent pathway. Since both Rimcazole and p53 are being evaluated in clinical trials, combining a dietary agent and a virally delivered therapeutic anti-cancer molecule provide an innovative approach for potentially treating human breast cancer.

Introduction

The transcriptional factor p53, one of the most important tumor suppressors, protects normal cell growth and initiates malignant cell death. p53 can be activated by a variety of cytotoxic stresses, such as DNA damage induced by ionic irradiation and chemicals, activation or mutation of oncogenes, hypoxia and virus infection. In unstressed cells, p53 level and activity is strictly controlled especially by the ubiquitin E3 ligase MDM2, which binds p53 directly and continuously mediates p53 ubiquitination and proteasomal degradation (1). When cells suffer toxic stresses, p53 is activated. Through both transcription-dependent and-independent mechanisms, p53 induces cell growth arrest, DNA repair, senescence and apoptosis (2). Adenovirus-mediated p53 gene (Ad.p53) therapy has been developed, in order to restore wild-type p53 function in p53-inactivated tumors, as a promising antitumor strategy in preclinical experiments and clinical studies. The clinical studies had various cancers of replication-deficient adenovirus vectors that carry the wild-type p53 gene (Ad.p53; Advexin, Gendicine and SCH-58500), and the preclinical experiments had conditional replicating adenovirus vectors, expressing the wild-type p53 gene (CRAd-p53; AdDelta24-p53, SG600-p53, OBP-702). These pre- and clinical trials have shown promising results, but further experiments for enhancing the p53-mediated cell death signaling pathway, would provide novel insights into the improvement of clinical outcome in p53-based cancer gene therapy (3).

Sigma 1 Receptor (Sig-1R) is a ligand-regulated protein chaperone subject of an evolving research area that could lead to therapeutic developments for many diseases. Sig-1R has also been shown to modulate endothelial cell proliferation and can control angiogenesis, which makes it a promising target for oncology applications. Sig-1R agonists promote cellular survival by preventing oxidative stress caused by ischemia, diabetes, inflammation, and amyloid toxicity. Conversely, antagonists of the Sig-1R inhibit tumor cell survival and induce apoptosis. Furthermore, systemic administration of sigma antagonists significantly inhibits the growth of mammary carcinoma xenografts, prostate tumors, and lung carcinoma in the absence of side effects. On the other hand, several normal cell types such as fibroblasts, epithelial cells, and even sigma 1 receptor-rich neurons are resistant to the apoptotic effects of sigma antagonists (4,5).

Materials and Methods

Cell culture and reagents. Human breast MCF7 cell line was obtained from American Type Culture Collection (ATCC). Rimcazole was purchased from Fisher Scientific

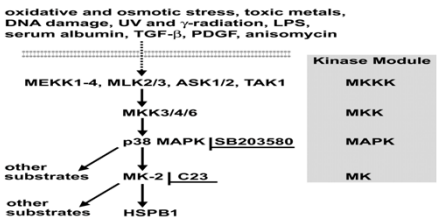
MTT Assay. Cells were plated in 96 well dishes (1x 10^3 cells/well) in DMEM containing 10%FBS and allowed to attach for 12 h prior to treatments. Inhibitors were added 4 h after infection with adenovirus. Cells growth and viable cell numbers were monitored by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) staining as described (4).

Western Blot Analysis Cell lines 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, phospho-p38MAPK, and β-actin.

Previous Results

Combinational treatment with Ad.p53 and Rimcazole induces growth inhibition in breast cancer cells. Ad.p53 and Rimcazole were used individually or in combination on T47D (p53 mutant), and MCF-7 (p53 wild type) human breast cancer cell lines. A non-toxic concentration of Rimcazole (0.2 µM) was chosen, which might be clinically achievable in patients, to evaluate a combinatorial effect of Rimcazole and Ad.p53. In a 5-day assay, Rimcazole or Ad.p53 alone had no discernible or minor effect on breast cancer cells while their combination significantly inhibited growth of breast cancer cells (Figure 1). Therefore, the combination of Ad.p53 and Rimcazole, at sub-optimal apoptosis-inducing concentrations synergistically enhanced growth inhibition and apoptosis induction over that observed with either agent alone independently from their p53 status.

Sigma 1 Receptor (Sig-1R) down-Regulation is promoted by the Combination of Ad.p53 plus Rimcazole. Studies were performed to define potential target genes associated with apoptosis that might be regulated by p53 + Rimcazole, thereby resulting in selective toxicity in breast cancer cells. Sig-1R protein was not down regulated by Ad.p53 or Rimcazole alone. However, the combination of Ad.p53 + Rimcazole significantly reduced Sig-1R protein in all cells (Figure 2).



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 caused p53 + Rimcazole mediated apoptosis in Breast cancer cells. To test this hypothesis Ad.p53 was used to treat MCF-7 and T47D in the presence or absence of ectopic expression of Sig-1R, and measured cell viability and induction of apoptosis by MTT. Ectopic expression of Sig-1R inhibited Ad.p53 + Rimcazole-mediated killing in MCF-7 and T47D cells (Figure 1). Taken 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 activation or GSK3β inhibition might also play a role in Ad.p53 plus Rimcazole-induced killing in Breast cancer cells. MCF-7 cells were uninfected or infected with Ad.p53 in combination with Rimcazole and analyzed by SDS-PAGE followed by Western blotting with anti-phospho-p38 MAPK, anti-p38 MAPK, anti-phospho-GSK3β, β-actin 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 (Figure 3). 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 override resistance to standard chemotherapy in the clinical setting.

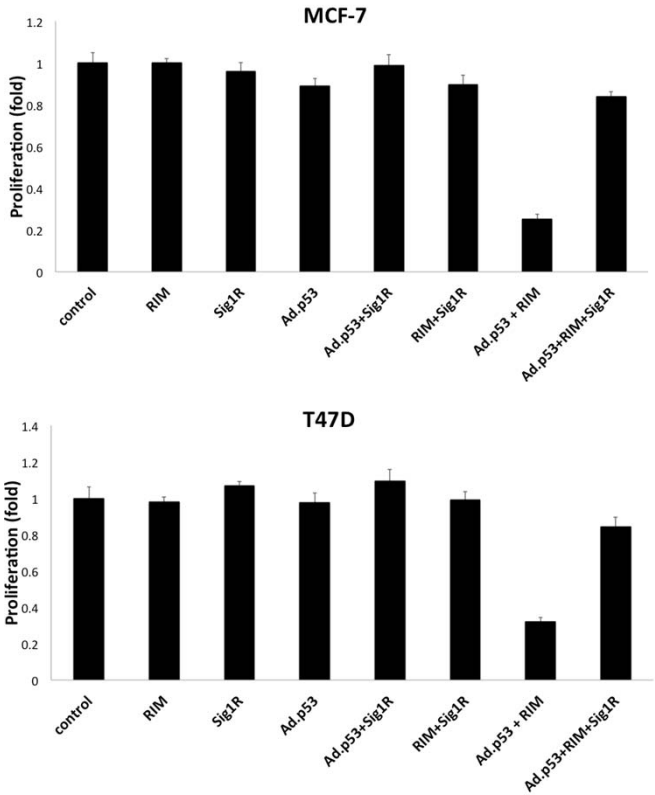


Figure 1. Ectopic expression of Sigma 1 Receptor inhibits the sensitivity of Ad.p53 and Rimcazole combinational treatment. MCF-7 and T47D cells were infected with Ad.vector (control), Ad.p53 at lower doses and Rimcazole, with or without ectopic expression of Sigma 1 Receptor (Sig1R). MTT assays was measured 5 days after treatment. An average of three independent experiments is shown ± SD.

Results and Conclusions

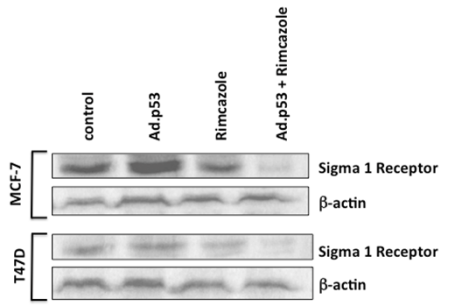


Figure 2. Effect of the combination of Ad.p53 and Rimcazole on Sigma 1 Receptor protein expression and downstream signaling. MCF-7 and T47D cells were infected with either Ad.vector (control) or Ad.p53 and either untreated or treated with the indicated concentrations of Rimcazole. Two hours after infection cells were treated with Rimcazole. Western Blot analysis was performed with antibodies for Sigma 1 receptor, and β-actin.

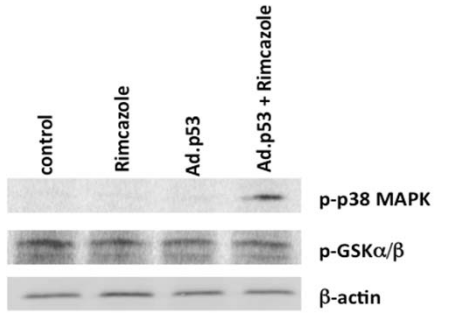


Figure 3. Combination treatment of Ad p53 + Rimcazole activate p38 MAPK. MCF-7 cells were infected with either Ad.vector (control) or Ad.p53 and either untreated or treated with the indicated concentrations of Rimcazole. Two hours after infection cells were treated with Rimcazole. Western Blot analysis was performed with antibodies for phospho-p38 MAPK, phospho-GSKα/β and β-actin.

Reference

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