Gene Therapy: Strategies to enhance the anti-cancer activity of p53

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Abstract

Breast cancer is the most common cancer diagnosed in the United States, after skin cancer. It is the second leading cause of cancer deaths in women today, after lung cancer. According to the American Cancer Society, more than 230,000 women will be diagnosed with breast cancer annually in the United States, and more than 39,000 will die from the disease. These dire statistics necessitate the development of enhanced single or combinatorial therapies to decrease the pathogenesis of this invariably fatal disease. We now reveal that a p53 in combination with Rimcazole have a synergistic effect in growth suppression and apoptosis. Furthermore, this combination decreased Sigma 1 Receptor protein expression, promoting apoptosis induction of breast cancer cells . 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. This combinatorial regimen specifically induced *in vitro* apoptosis in breast cancer cells. These provocative findings suggest that this combinatorial strategy might provide a platform for developing effective treatments for therapy-resistant cancers.

Introduction

Cancer is generally known as the uncontrolled growth of abnormal cells and it is a worldwide health problem. Treatment of cancer can be more detrimental than the effect of the disease itself. The ability of tumor to become resistant and the toxicity they establish in normal cells are major obstacles for the conventional treatment of cancer; chemo – and radiation – therapy (1), while surgery on the other hand can be biologically and psychologically damaging. It is highly imperative to develop anti-cancer therapeutic agents and strategies for cancer treatment and diagnosis to fight cancerous cells. The next generations of cancer therapies are aimed to specifically target cancer cells without causing any damaging effect on neighboring normal cells. One such approach is known as gene therapy, and involves inserting genes into individual cancer cells to manipulate or selectively destroy cancer cells using adenovirus as the vector. In order to develop an effective gene therapy to treat cancer, more knowledge need to be acquired about the individual molecules that inhibit tumor growth. Discovering the precise molecular mechanisms by which cancer-therapeutic agents selectively kill cancer cells is the main basis of such conquest which is needed to cure cancer.

P53 protein loss has been associated with 50% of cancer cases. It is a protein that is found at the crossroad of a network of signaling pathways essential for cell growth regulation and apoptosis induced by genotoxic stresses (2). In normal unstressed cell, p53 is down-regulated by several binding proteins by ubiquitin / proteasome pathway. After genotoxic or non genotoxic stress, activation can be of two steps: primarily, p53 protein level is augmented via the inhibition of its interaction with mdm2 and the other negative regulators. Over translation of p53 RNA is a complementary that will also ensure p53 accumulation. Second, a series of modulator (kinases, acetylases) will activate p53 transcriptional activity. The mechanism of p53 begins with activation via stress signals after which there is an upstream of the mediators that detect and interpret the upstream signals. This is then followed by the core regulation of p53 through its interaction with several proteins that modulate its stability, these then results into the downstream event, mainly transcriptional activation or protein interactions. After the series of event, growth arrest, apoptosis or DNA repair is carried out.

Gene therapy utilizing tumor suppressor genes (such as p53) has been tested in a number of cancers in both preclinical models and clinical trials. Despite the ability of anti-cancer p53 to significantly inhibit tumor growth in preclinical models, it has met limited clinical success. This is due, in part, to the fact that a substantial fraction of tumors become resistant to the mono-therapy. The objective of my research will be combining the tumor suppressor gene p53 and the specific drug Rimcazole, an antagonist of the sigma 1 receptor to test their effectiveness over single gene therapy. Therefore, we will focus on defining appropriate combinations of pharmacological agents that can improve the anti-cancer efficacy of p53, based on the mechanism of action of these molecules. We will test if specific combination treatments interact in a greater than additive fashion to kill a diverse array of tumor cell types. Furthermore, we will determine if the degradation of sigma 1 receptor due to its connection with Bcl-2 is the cause of the synergistic effect followed by the combination of p53 and Rimcazole.

Experimental design

Cells and culture conditions MCF-7 was grown in DMEM with 10% fetal bovine serum, 2 mM l-glutamine, 50 U/ml penicillin/streptomycin and 1 mM sodium pyruvate. Rimcazole, SK(+)10047 and BD1047 were purchased from Fisher Scientific.

MTT Assay A colorimetric assay that measures the reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria, where it is reduced to an insoluble, dark purple colored formazan product. The cells are then dissolved with an organic solvent and the released. The dissolved formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. MTT Assay Solution: The dilution of 1 part MTT (4-mL) to 10 parts DMEM (36-mL); 100-µL per well X 96 wells X 4 was done which was accompanied with 4 hours of incubation. Buffer was added to solution after 4 hours of incubation and was left in the incubator overnight. The formazan dye was dissolved in a micro plate. Quantification of the dye was done with an ELIZA plate reader. The absorbance directly correlates with the cell number.

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 MAbs to Sigma 1 Receptor, and β-actin.

Results and Conclusions

Rimcazole, an antagonist of Sigma-1-receptor, decreased viability in breast cancer cells. We determined the concentration of Rimcazole that produced growth suppression in breast tumor cells. Rimcazole decreased proliferation significantly after 72 h (Figure 1A). In an analysis conducted by the National Cancer Institute, Rimcazole's IC_{50} (GI50) values (the concentration of drug required to produce a 50% reduction in growth over 72 h) across a panel of tumor cell lines (from the NCI60 panel) ranged from 1.9 to 38 mM. In this study, water-soluble Rimcazole was used.

Ad.p53 decreased viability in breast cancer cells. Initial studies determined if infection with an adenovirus expressing p53 protein (Ad.p53) produced growth suppression (loss of viability) in breast tumor cells. Exposing MCF-7 cells to increasing concentration of the Ad.p53 resulted in a dose-dependent increase in cell death (Figure 1B).

Combinational treatment with Ad.p53 and Rimcazole induces growth inhibition in breast cancer cells. P53 has the unique 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 these anti-cancer genes. In these contexts, should resistance to mono-therapy with p53 occur, combinatorial therapies with each anti-cancer gene might provide a viable path for potentially curing patients of primary and metastatic cancer. We employed MCF-7 to investigate growth inhibitory properties of combinational treatment with Ad.p53 and Rimcazole. A non-toxic concentration of Rimcazole (1mM) was chosen, which might be clinically achievable in patients, to evaluate a combinatorial effect of Rimcazole and Ad.p53. In a 3-day assay, Rimcazole or Ad.p53 alone had no discernible or minor effect on breast cancer cells while their combination significantly inhibited growth of MCF-7 (Figure 2). Furthermore, Rimcazole or Ad.p53 alone had no effect on Sigma 1 receptor protein expression while their combination significantly inhibited Sigma 1 Receptor protein (Figure 3). 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. These experiments shows that Rimcazole in combination with p53-mediated molecular therapy could be promising strategies to treat cancers and they could serve as a basis for guiding p53-based combination treatment designs in future preclinical and clinical trials.

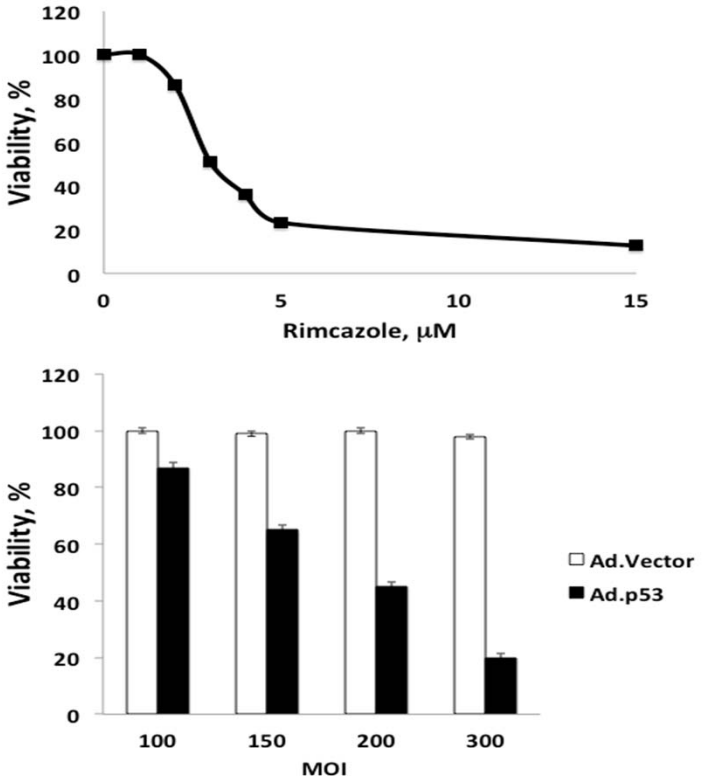

Figure 1. (A) **Rimcazole decreased viability in breast cancer cells.** A representative cytotoxicity assay of a tumor cell line (MCF-7) grown in high serum (10% FCS) and exposed to a range of concentrations of the sigma 1 receptor antagonists Rimcazole over a 72-h time course. Changes in cell viability were measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. Each data set was obtained from a representative experiment performed at least three times. Data points represent mean values (±SD) from wells in quadruplicate. (B) **Ad.p53 decreased viability in breast cancer cells.** Cells were infected with different pfu/cell of Ad.vector (control) or Ad.p53 for 72h. Cell viability was determined by MTT assay. MTT absorbance of untreated control cells was set at 1 to determine relative number of viable cells.

Results and Conclusions

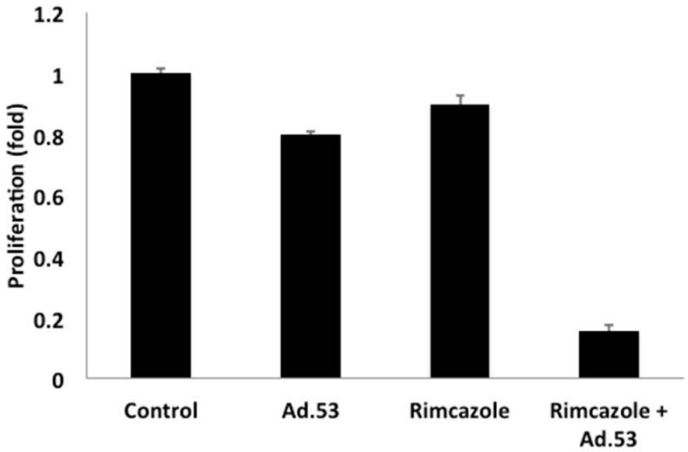

Figure 2. Combination of Ad.p53 with sigma 1 receptor antagonist (Rimcazole) efficiently inhibits in vitro growth of breast cancer cell line. MCF-7 cells was infected with Ad.vector (control), Ad.p53 at lower doses, with or without Rimcazole. Cells were treated with Rimcazole after 2 hours to the indicated Ad infection. MTT assays was measured 3 days after treatment. An average of three independent experiments is shown ± SD. .

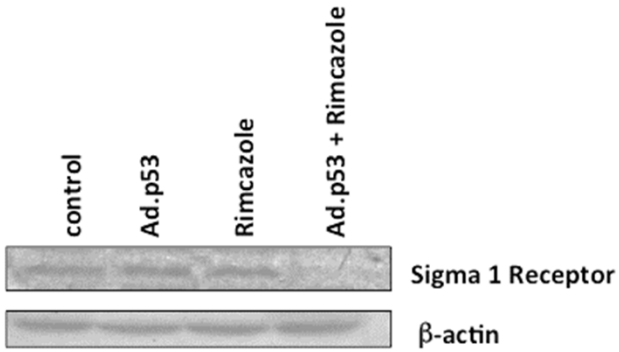

Figure 3. **Effect of the combination of Ad.p53 and Rimcazole on Sigma 1 Receptor protein expression and downstream signaling.** MCF-7 cells were infected with either Ad.vec or Ad.p53 and either untreated or treated with the indicated concentrations of Rimcazole. Two hours after infection cells were treated with Rimcazole. Western Blot analysis was performed with antibodies for Sigma 1 receptor, and β-actin.

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