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

Mabel Marte Taveras1 and Dr. Moira Sauane2
CUNY Hostos Community College, 500 Grand Concourse, Bronx, New York 10451
CUNY Lehman College, Bronx, New York 10468

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 250,000 women will be diagnosed with breast cancer annually in the United States. While breast cancer can occur at any age, more than 35,000 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 recently reported that Ad.p53 in combination with Rimcazole have a synergistic effect in growth suppression and apoptosis.

Results and Conclusions

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 IC50 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 1.5 to 38 mM. In the study, water-soluble Rimcazole was used.

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

Combinational treatment with Ad.p53 and Rimcazole induces growth inhibition in breast cancer cells. P53 is the unique ability to inhibit apoptosis in diverse 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 adenoviruses 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. Detecting the precise molecular mechanisms by which cancer-therapeutic agents selectively kill cancer cells is the main basis of such concepts that is needed to cure cancer.

P53 protein has been associated with 50% of cancer cases. It is a protein that is found at the crossroad of a network signaling pathways essential for cell growth regulation and apoptosis induced by genotoxic stresses (2). In normal untransformed cell, p53 is down-regulated by several binding proteins by ubiquitin (proteasomal) and non-genotoxic or non-genotoxic stress, activation can be by two steps: primarily, p53 protein level is augmented as the inhibition of its interaction with mdm2 and the other negative regulators. Over translation of p53 RPAs is a complementary that will also prevent p53 accumulation. Second, a series of modulator (kinases, acetylases) will activate p53 as transcriptional activity. The mechanism of p53 begins with a transcription via stress signals after which there is an upstream of the medications that detect and interpret the apoptosis signals. The p53 then follows the normal regulation of p53 through its intrinsic interaction with several proteins that modulate its stability, these then results into the downstream event, namely 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-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 Sigma-1 receptor, to target the unique ability of p53 to selectively kill cancer cells and to the combination of Ad.p53 and Rimcazole, at sub-optimal apoptosis-inducing concentrations synergistically enhanced growth inhibition and apoptosis induction over those observed with either agent alone. These experiments shows that in combination with Ad.p53, mediated molecular therapy could be promising strategies to treat cancer and could serve as a basis for guiding p53-based combinatorial treatment designed in future preclinical and clinical trials.

Introduction

Cancer is generally known as the uncontrollable growth of abnormal cells and it is a worldwide health problem. 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 radiotherapy (1), while surgery on the other hand can be biologically and psychologically devastating. It is highly imperative to develop anticancer therapeutic agents and strategies for cancer treatment and diagnosis to fight cancerous cells. The next generations of cancer therapies are aimed at 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 adenoviruses 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. Detecting the precise molecular mechanisms by which cancer-therapeutic agents selectively kill cancer cells is the main basis of such concepts that is needed to cure cancer.

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Discussion

Sigma-1 receptor antagonists (Sigma-1 RAs) are considered to be potential chemosensitizers for combination chemotherapy. We have previously shown that Sigma-1 RA Rimcazole inhibited viability and induced apoptosis in breast cancer cells. Furthermore, Sigma-1 RA Rimcazole sensitized triple-negative breast cancer cells to an anti-cancer agent. In the present study, we investigated the effects of Rimcazole in combination with Ad.p53 in breast cancer cells. To our knowledge, this is the first study to investigate the effects of Rimcazole in combination with Ad.p53 in breast cancer cells.

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 antagonist 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. Untreated control cells was set at 1 to determine relative number of viable cells.

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