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Gene Therapy: Targeting Apoptotic Pathways with TRAIL

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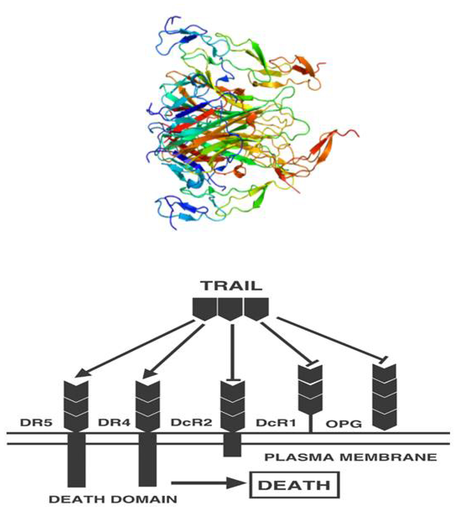
Introduction

Cancer is the name given to a broad range of diseases in which abnormal cells grow uncontrollably, forming masses of tissue known as malignant tumors, and invade nearby organs and tissues through metastasis. The paradigm for cancer treatment has evolved over the past few decades from relatively non-specific methods, such as chemotherapy, radiation therapy, and surgery, to new anti-cancer agents and delivery methods, that will selectively target cancer cells, without sacrificing healthy tissue (1). While chemotherapeutic drugs remain the backbone of current cancer treatments, an increased understanding of cancer pathogenesis and molecule pathways critical to tumor growth has given rise to new treatment options, including targeted agents. The focus of targeted approaches is the inhibition of molecular pathways crucial for tumor growth and maintenance. It is apparent that the future of targeted therapies is contingent on a thorough understanding of the molecular signal transduction pathways triggered by anti-cancer genes to induce apoptosis in cancer cells, as well as an appropriate use of diagnostics, as both a prescreening tool, and to recognize early sign of either drug response or acquired resistance. The targeted approach to cancer treatment offers ample hope to improving treatment for cancers that are otherwise therapy resistant.

The objective of my research is to test novel combination treatment involved in the induction of apoptosis in cancer cells, triggered by the anti-cancer ligand, TRAIL (tumor necrosis factor related apoptosis inducing ligand). We analyzed the induction of apoptosis after a combinatorial approach involving TRAIL which antitumor chemotherapeutic agents, to sensitize cancer cells to TRAIL–mediated cytotoxicity, thereby enhancing anti-cancer efficacy.

TRAIL is a member of the tumor necrosis factor (TNF) family of ligands capable of initiating apoptosis through engagement of its death receptors (2). TRAIL has been shown to selectively induce apoptosis in a variety of tumor and transformed cells without any toxicity to normal cells (3). The approach will be to deliver the gene necessary for TRAIL expression, via adenovirus vector. The sigma-1 receptor antagonists Rimcazole, and BD1047 will be utilized at various concentrations, to examine their apoptotic inducing effect on the sigma-1 receptor antagonists to sensitize cancer cells to TRAIL–mediated cytotoxicity. Elucidating the cancer cell-specific activity of TRAIL will empower prediction, analysis, and amelioration of potentially harmful side effects, and improve the effectiveness of potential cancer therapies utilizing TRAIL.



Experimental design

Cell Plating DMEM was warmed to 37^{0}C, cells were plated in 96-well dishes (1x10^3 cells/well). The medium was removed with a micropipette and cells were washed with 10-mL of PBS; medium was then removed. 1-mL of trypsin was introduced to detach cells from surface and warming in incubator was done for 5 minutes. Trypsin was deactivated with 6-10-mL of DMEM with FBS, and was mixed with micropipette 10 times. 5-mL of DMEM was later added to a conical tube and cells were transferred. After removing 10-µL, the sample was placed in Neubauer ruling for cell counting. Cells were counted in a 4-quadrant manner while average was multiplied by 10^4 per mL. 2.3-mL of stock solution was then added into a separate conical tube while addition of 17.7-mL DMEM with FBS was done sequentially. Solution was transfer into a 96-well plates; 100-µL per well and then placed in an incubator.

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.

Results and Conclusions

Gene therapy for cancer treatment is becoming a promising strategy for curing cancer. However, the development and improvement of effective gene therapy for cancer requires understanding the molecular mechanisms involved in anti-cancer agents such as TRAIL. TRAIL have been identified as showing a remarkable tumor-specific cytotoxic activity without any adverse effect in normal cells. Thus, we propose of the present study is, therefore, to improve gene therapy with the anti-cancer agent TRAIL which is already being used in clinical trials in various cancers.

Expression of TRAIL by an adenoviral expression system (Ad.TRAIL) induces growth suppression human breast cancer cell. Initial studies determined if infection with an adenovirus expressing TRAIL protein (Ad.TRAIL) produced growth suppression (loss of viability) in breast tumor cells. Exposing MCF-7 cells to increasing concentration of the Ad.TRAIL resulted in a dose-dependent increase in cell death **(Figure 1).**

BD 1047 and Rimcazole, two of Sigma-1-receptor antagonists, decreased viability in breast cancer cells. We next determined the concentration of BD1047 and Rimcazole that produced growth suppression in breast tumor cells. BD1047 as well as Rimcazole decreased proliferation significantly after 72 h **(Figure 2).**

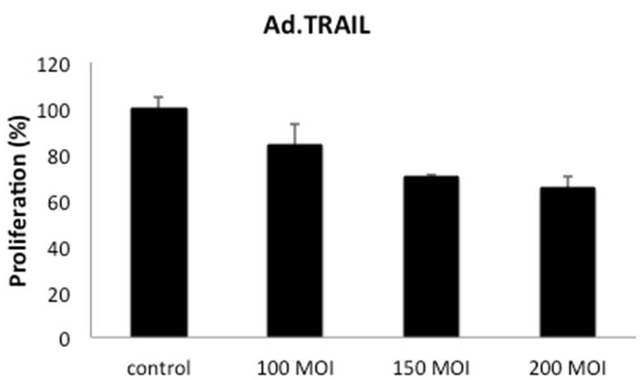


Figure 1. Ad.TRAIL decreased viability in breast cancer cells. Cells were infected with different pfu/cell of Ad.vector (control) or Ad.TRAIL for 72h. Cell viability was determined by MTT assay. MTT absorbance of untreated control cells was set at 1 to determine relative number of viable cells.

Results and Conclusions

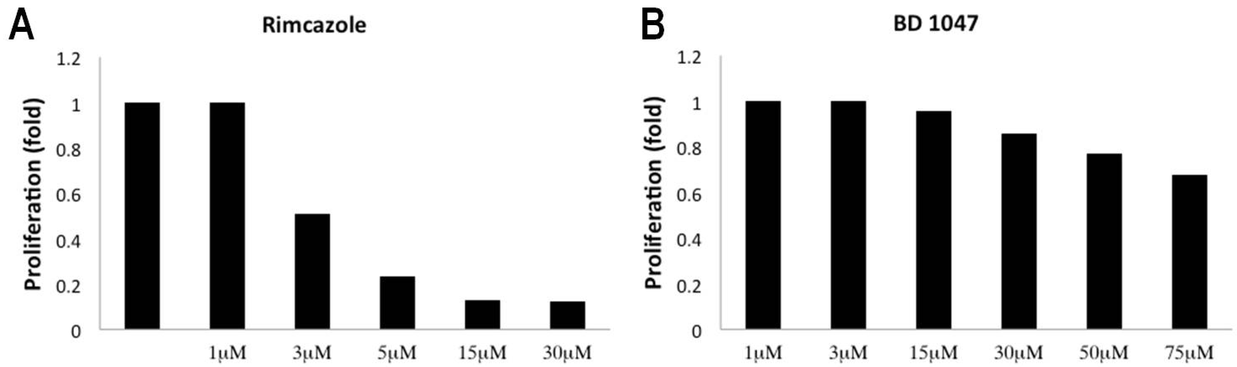


Figure 2. Rimcazole as well as BD1047 decreased viability in breast cancer cells. A representative cytotoxicity assay of a tumor cell line (MCF-7) grown in high serum (10% FCS) and exposed to a range of concentrations of the sigma 1 receptor antagonists Rimcazole (A) and BD1047 (B) over a 72-h time course. 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.

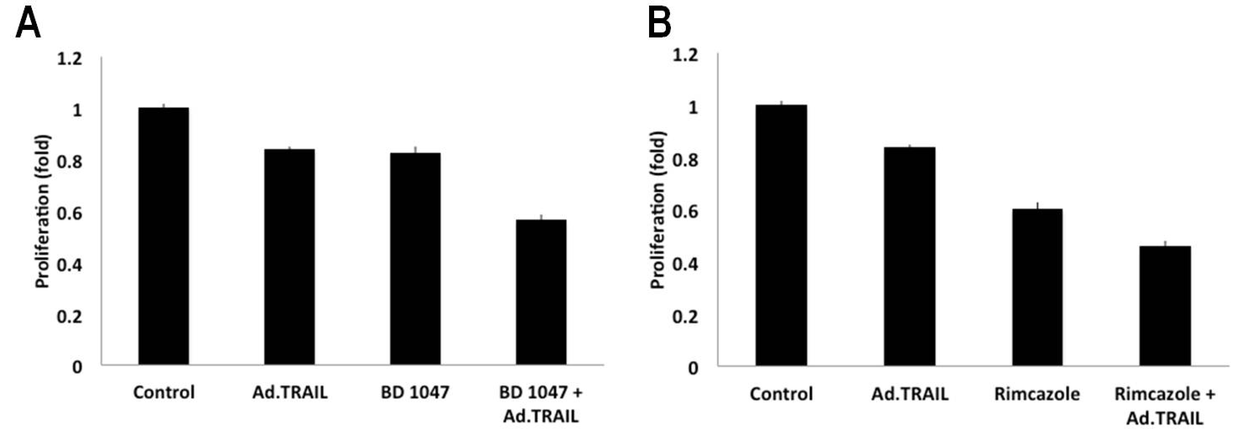


Figure 3. Combination of Ad.TRAIL with sigma 1 receptor antagonist inhibits in vitro growth of MCF-7 breast cancer cells. Viability of MCF-7 breast cancer cells was measured by MTT assays 3 days post-treatment. Cells were treated with sigma 1 receptor antagonists (Rimcazole and BD1074) after 2 hours to the indicated Ad infection.

Combinational treatment with Ad.TRAIL and sigma 1 Receptor antagonist induces growth inhibition in breast cancer cells. The importance of TRAIL as a potential therapeutic agent became obvious when it was demonstrated to be selectively toxic to transformed and tumor cells but not to the majority of normal cells. These observations were confirmed in a number of in vivo studies where TRAIL was effective at reducing solid tumor growth and inducing regression of tumor cell xenografts in the absence of the systemic toxicity observed with anti-Fas/CD95 or TNF treatment. We employed MCF-7 to investigate growth inhibitory properties of combinational treatment with Ad.TRAIL and Rimcazole as well as BD1047. A non-toxic concentration of Rimcazole (1µM) and BD1047 (50µM) were chosen, which might be clinically achievable in patients, to evaluate a combinatorial effect of Rimcazole as well as BD1047 and Ad.TRAIL. In a 3-day assay, Rimcazole, BD1047 or Ad.TRAIL alone had no discernible or minor effect on breast cancer cells while their combination significantly inhibited growth of MCF-7 in an additive fashion **(Figure 3)**.

Reference

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