Combination Efficacy of Doxorubicin and Adenoviral Methioninase Gene Therapy with Prodrug Selenomethionine

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Abstract. We have previously demonstrated an enzyme activation prodrug gene therapy strategy using the methionine α,γ-lyase gene (MET) cloned from Pseudomonas putida, in combination with selenomethionine (SeMET) as a prodrug. MET gene transfer via a recombinant adenovirus (Ad-MET) converts the physiologic compound SeMET to highly toxic methyselenol. In this study, we have developed a combination therapy approach using Ad-MET/SeMET gene therapy and doxorubicin (DOX). The combination significantly delayed the growth of H460, an aggressively-growing human lung cancer cell line, in nude mice. H460 cells were injected intra-dermally in nude mice. Tumor-bearing mice were divided into 12 groups [Control (Ctrl), DOX, SeMET, SeMET+DOX, Ad-Ctrl, Ad-Ctrl+SeMET, Ad-Ctrl+DOX, Ad-Ctrl+SeMET+DOX, Ad-MET, Ad-MET+DOX, Ad-MET+SeMET, and Ad-MET+SeMET+DOX]. DOX (2 mg/kg body weight) was given intra-peritoneally twice at 7-day intervals. SeMET (1 μM/mouse) was given by intra-tumor injection everyday, starting the following day after transfection with adenovirus. Tumor growth in the untreated group showed a 10-fold increase in tumor volume after two weeks. In contrast, the increase was only 2.5-fold in the DOX+Ad-MET/SeMET group. The treatment with DOX alone at the low-dose used showed no effect compared to the control group. There was a 5.8-fold increase in tumor volume in mice treated with Ad-MET/SeMET gene therapy alone. The tumor doubling-time was increased to approximately 10 days with the combination therapy of Ad-MET+SeMET+DOX as opposed to 2-3 days in all other treatment groups.

Adenoviral methioninase (Ad-MET)/selenomethionine (SeMET) prodrug-enzyme gene therapy induces apoptosis in the tumor cells in vitro as well as in vivo (1). Selenomethionine is a Se analogue of methionine and is relatively non-toxic (9, 10).

Methyselenol is produced as a result of activity of the MET gene product, methionine α,γ-lyase (methioninase), on SeMET (2). Methyselenol induces oxidative stress in the cells (1, 3, 4), which leads to mitochondrial permeability transition (MPT) activation and loss of mitochondrial potential (5, 6). This mitochondrial damage results in cyt-c release into the cytosol, which then activates the caspase cascade (7) and induces apoptosis (8).

The MET gene product methioninase α,γ-lyase has anti-tumor activity in vitro as well as in vivo (11-14). This anti-tumor activity is attributed to cellular methionine depletion, since a majority of tumors have an increased dependence on methionine for cell proliferation (15-20).

When tumor cells are transfected with Ad-MET and are subsequently treated with SeMET, the toxicity of SeMET increases 100-1000 fold in vitro (1). A substantial reduction in ascites tumor growth was seen in vivo as well with this novel prodrug enzyme-gene therapy.

Doxorubicin is commonly used for the treatment of various cancers including breast, bladder and lung (21). Various mechanisms have been proposed for its mechanism of action such as metal chelation (22, 23), DNA binding (24, 25), and, importantly, free oxygen radical formation (26-29). Different mechanisms are active in different types of tumor cells. Since both DOX and Ad-MET/SeMET therapy produce oxidative stress in tumor cells, synergy is possible. To explore this possibility, an in vivo study was done on the H460, a human lung cancer cell line that grows aggressively in nude mice (30).

Materials and Methods

Recombinant adenoviruses. The construction and propagation of recombinant adenovirus Ad-MET, in which the MET gene is driven by the CMV-5 promoter and enhancer, has been described (14).

Doxorubicin (Sigma, St. Louis, MO) was prepared as 250 mg/ml solution and used in final dosage of 2 mg/kg body weight as an i.p. injection. SeMET (Sigma, St. Louis, MO) was prepared as a 20 mM solution in PBS, and 50 μl was injected intra-tumorally. Fifty μl PBS was used in appropriate control groups for intra-tumor injections. Ad-MET and Ad-Ctrl were administered in 50 μl intra-tumor injections containing 3 × 10^5 PFU virus.

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In vivo study protocol. $1 \times 10^7$ H460 human lung cancer cells were injected i.d. in 200 male NCr nude mice. As per pre-determined criteria, mice were included in the study on the day the tumor volume reached 225-325 mm$^3$. Mice were divided randomly into 12 groups with 8 mice per group. Study groups included: Ctrl, SeMET, DOX, SeMET+DOX, Ad-Ctrl, Ad-Ctrl+SeMET, Ad-Ctrl+DOX, Ad-Ctrl+SeMET+DOX, Ad-MET, Ad-MET+DOX, Ad-MET+SeMET, and Ad-MET+SeMET+DOX. Ad-MET and Ad-Ctrl were injected on days 0, 4, 8, and 12; DOX on days 1 and 8; and SeMET/PBS injected daily starting from day 1. Tumor volume was measured everyday. For comparison of the groups, average percent-relative-volume of each tumor was plotted together against time. Percent-relative-volume was calculated as $\frac{V_{t}}{V_{0}} \times 100$, where $V_{t}$ = volume of the tumor on a given day and $V_{0}$ = volume of the tumor on day zero when the study was started. Body weight of each mouse was measured on days 0, 4, 8, 12, and at the end of the study to determine the toxicity of the therapy. Average body weight of mice in each treatment group was plotted against the days of treatment.

Results and Discussion

The study consisted of 12 groups of NCr nude mice with 8 mice in each group (See Materials and Methods for details). Out of these, one group was the study group (Ad-MET/SeMET+DOX) and 11 control groups which were all other possible combinations of the drugs or vehicles used in the combination therapy.

The average percent relative volume of each mouse was plotted against time (Figure 1A). Whereas the tumor growth in the untreated group showed a 10-fold increase in the tumor volume after 2 weeks, the increase was only 2.5-fold in the DOX+Ad-MET/SeMET group. The treatment with DOX alone at the low-dose used showed no difference as compared to the control group. There was a 5.8-fold increase in tumor volume in mice treated with Ad-MET/SeMET gene therapy alone. The tumor doubling-time was increased to approximately 10 days with the combination therapy of Ad-MET+SeMET+DOX as opposed to 2-3 days in all other treatment groups.

The comparison of individual mice in Figure 1B shows the efficacy of the combination therapy of Ad-MET/SeMET and DOX against the untreated control. Whereas the tumor volumes increased 8-12 fold in 2 weeks in the untreated
Figure 1B.

Figure 1C.
Figure 1D.

Figure 1E.
Figure III.

Figure II.
Figure 1J.

Figure 2. Means body weight measurement of experimental mice.
group, tumor growth was only 1 to 2.5-fold in the mice treated with the combination of Ad-MET/SeMET+DOX.

SeMET, DOX, or Ad-MET individually were unable to inhibit the tumor growth at the low doses that they were used (Figures 1 C-E).

Similarly, the combination of Ad-MET+DOX and the combination of SeMET+DOX with Ad-Ctrl instead of Ad-MET, were also ineffective when compared with the Ad-MET/SeMET+DOX combination (Figures 1 F, G).

Ad-MET/SeMET gene therapy alone did show efficacy when compared to the untreated control (Figure 1 H). The tumor growth was about 3 to 8-fold in the individual mice at the end of 2 weeks as opposed to 8 to 10-fold in the untreated group.

A comparison of Ad-MET/SeMET with Ad-MET/ SeMET+DOX indicates that the addition of DOX to Ad- MET/SeMET provides a stronger efficacy against the H460 tumor (Figure 1 I).

DOX alone at the low-dose used did not show efficacy compared to the untreated control (Figure 1 J), suggesting that the combination of DOX and Ad-MET/SeMET gene therapy is synergistic. The average body weight of mice in each group is shown (Figure 2) indicating lack of toxicity. The Ad-MET/SeMET+DOX combination should be further evaluated in other models for general efficacy.

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