Disruption of DNA Damage Signaling: A Novel Therapy for Head and Neck Cancer

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INTRODUCTION

Radiation, with or without chemotherapy, is commonly used in the treatment of advanced head and neck cancer. These therapies are associated with a significant risk of treatment-related toxicity.

Human cells accumulate DNA single-strand breaks (SSBs) during the process of DNA replication. The repair of this physiologic DNA damage occurs approximately 10^5 times in each cell per day. PARP-1 is a nuclear protein critical to this process. PARP-1 inhibition results in the accumulation of SSBs, triggering an alternative pathway which converts SSBs to double-strand breaks (DSBs) to avoid cell death. These DSBs are repaired by a backup mechanism, the Mre11/Rad50/Nbs1 (MRN) complex, through the process of homologous recombination (HR).

We have previously used a novel adenoviral vector, Ad-Nbs1, to disrupt the function of Nbs1, a key component of the MRN complex that is involved in the initial detection of DNA DSB damage and downstream signaling. As a monotherapy, PARP inhibitors (PARPI) are not lethal to cancer cells due to intact MRN-mediated DSB repair. But, by simultaneously disrupting PARP-1 SSB repair function and MRN-mediated DSB repair, cancer cells have no reliable method of preventing the accumulation of DNA damage (Figure 1).

We hypothesize that disruption of Nbs1 function, combined with PARP-1 inhibition, will produce a marked cytotoxic effect in cancer cells without the need for chemotherapy or ionization radiation.

METHODS

Cell lines. The human head and neck squamous cell carcinoma cell lines JHU012 and JHU022 were used in this study.

PARP-1 inhibitor. The prototype PARP-1 inhibitor, GPI-15427, was provided by MGI Pharma (Baltimore, MD). The IC50 dose was used for all experiments (8 µM for JHU012; 15 µM for JHU022).

Construction of the mutant Nbs1 adenoviral vector (Ad-Nbs1). The cDNA for the 300 amino acids from the C-terminal of wild-type Nbs1 protein, carrying intact targets for the PARP inhibitor and adenovirus vector, respectively. Determination of baseline PARP-1 and Nbs1 expression. Cell lines were screened for PARP-1 and Nbs1 protein expression using Western blot (Figure 2).

Quantifying DNA double-strand break damage. The neutral comet assay was used on JHU012 cells divided into 6 treatment groups: Control, Ad-GFP, Ad-Nbs1, PARP-, Ad-GFP + PARP-, Ad-Nbs1 + PARP-.

Adenovirus (Ad-GFP or Ad-Nbs1) was administered at an MOI of 10. The PARP inhibitor (PARP-) was used at the IC50 dose. Images were captured digitally and analyzed with software to determine the mean tail moment (MTM), an indicator of DNA DSB quantity.

Cell growth and survival. A consecutive 5-day MTT cell proliferation assay was performed on JHU012 and JHU022 cells in 96 well plates according to the above treatment groups. Cells were plated at a density of 2x10^3/well and transfected at an MOI of 10. The PARP inhibitor was administered at the IC50 dose. Cell density was determined using a microplate reader.

RESULTS

Figure 3. (A) Schematic diagram of a “comet” produced by the neutral comet assay. (B) Representative images of JHU012 cancer cells subject to neutral comet assay. Tail moment is greatest in the Ad-Nbs1 + PARP- group indicating increased DNA DSB damage in these cells.

Figure 4. Averaging tail moment for 30-50 cells within each treatment group results in the “mean tail moment”, a correlate of DNA DSB damage. There was an exponential increase in DNA DSB damage in the dual disruption (Ad-Nbs1 + PARP-) group versus all other groups (p<0.001). Dual disruption, therefore, induces significant DNA damage.

Figure 5. Cell growth assays were conducted in (A) JHU012 and (B) JHU022 head and neck cancer cell lines. Cell proliferation was measured for 5 consecutive days. Dual disruption (Ad-Nbs1 + PARP-) of MRN and PARP function induced significant suppression of cell growth from 48 hours onwards versus all other groups (p<0.001) in both cell lines. This dramatic cytotoxic effect was maintained throughout the 5 day duration of the assay.

CONCLUSIONS

• Disruption of MRN-mediated DNA repair via impairment of Nbs1 damage signaling produces a potent anti-tumor effect when combined with PARP-1 inhibition. This increased cytotoxicity is associated with a significant and lethal increase in DNA damage in cancer cells.

• This novel treatment approach bypasses the need for chemotherapy or radiation while inducing a comparable in vitro cytotoxic effect.

• Ongoing in vivo experiments suggest that by inducing DSB damage signaling deficiency in tumor cells using Ad-Nbs1 and then administering a PARP inhibitor systemically, we can induce tumor-targeted cell death without the need for any chemotherapy or radiation.

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