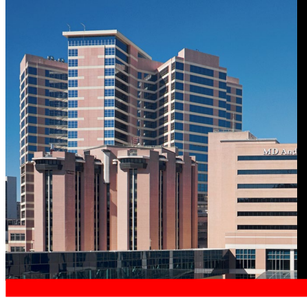

Preclinical Evaluation of APR-246, a p53 reactivator in Head and Neck Squamous Cell Carcinoma

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Introduction

Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most common cancer type and has an annual incidence of over 40,000 new cases in the US, and over 500,000 worldwide (1). The cure rate for patients with advanced HNSCC remains in the 25-40% range due to resistance to standard therapy, primarily consisting of platinum-based chemoradiation therapy (2). Mutation of the *TP*53 is the most common aberration in head and neck cancer and often associated poor survival and treatment resistance (3). Therefore, novel therapeutic approaches to overcome this resistance are urgently needed. As WT p53 protein is a known potent inducer of apoptosis and senescence when expressed in tumor cells, reactivation of some level of WT function in mutant TP53 bearing cells is an attractive therapeutic strategy. APR-246, a methylated PRIMA-1 analog, is a novel, first-in-class, small molecule that selectively induces apoptosis in TP53 mutant cancer cells. Mechanistically, APR-246 is spontaneously converted into the active form methylene quinuclidinone (MQ), and covalently capable of binding to cysteine residues in mutant p53 thereby producing a thermodynamically stable protein and shifting equilibrium toward a functional conformation (4-6).

Purpose

The goal of this study was to test the effect of APR-246 on various HNSCC cell lines with different *TP*53 mutational status as a single agent and in combination with cisplatin-based therapy and to examine the mechanism of action of APR-246 in these cell lines.

Methods

Clonogenic survival assays were used to examine in vitro sensitivity of HNSCC cell lines harboring a variety of TP53 mutations to APR-246. Western blotting was performed to dissect molecular mechanisms. Briefly, HNSCC cells were seeded at predetermined densities and treated with various doses of APR-246 and/or cisplatin for clonogenic survival assays. For western blotting, cells were treated with a dose of the drug that is determined based on the IC50 of APR-246 and harvested at different time points. Controls were treated with DMSO. The Talalay-Chow Combination Index and Isobologram method was used to evaluate the drug synergistic interactions in the cells tested. Immunostaining with Pab240 (recognizes mutant p53) and Pab1640 (recognizes wildtype p53) antibodies were used to test for the proper conformational changes and reactivation of p53 in the mutant p53 HNSCC cells.

Acknowledgements

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Results

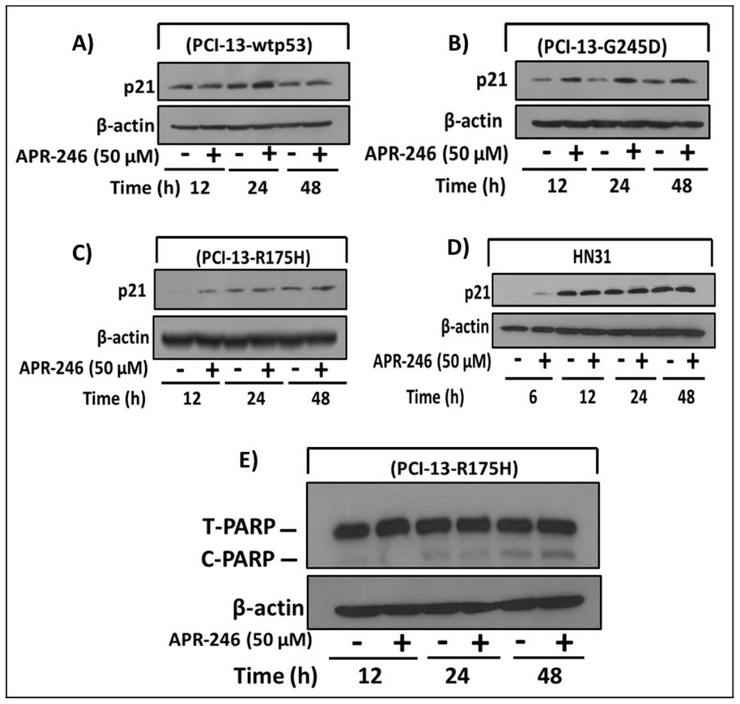


Figure 1. APR-246 induces p21 in HNSCC mutant p53 cell lines. The cell lines were treated with APR-246 dose at different time points as indicated. Protein lysates were collected and subjected to western blot analysis with appropriate antibodies. No induction of p21 was observed in the wtp53 (A). APR-246 treatment resulted in significant p21 (primary downstream target of p53) induction within 6 hours following treatment with the drug in the mutant p53 cell lines (B-D), indicating reactivation of p53. Addition of the APR-246 as single agent caused apoptotic cell death as determined by PARP-1 cleavage (E).

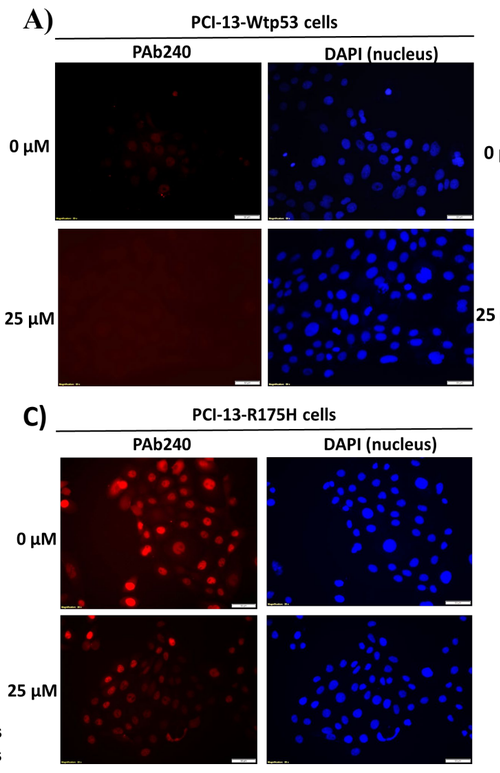
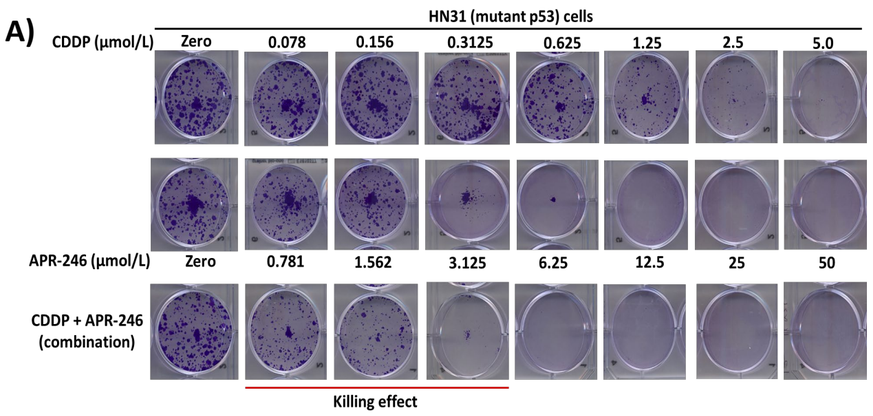


Figure 2. APR-246 induces conformational changes in HNSCC mutant *TP*53 cell lines. Isogenic HNSCC cell lines with wildtype p53 and different p53 mutation were treated with APR-246 for 24 hours and stained with the PAb240 antibody (recognizes mutant p53 protein) as indicated. A) Image representative of immunostaining of the PAb240 in the wild type p53 cells (negative control). B-C) PAb240 immunostaining was decreased in the mutant cell lines following treatment with the APR-246, indicating changes from mutant to wild type p53 structure. These results suggest reactivation of wildtype p53 in the mutant p53 cell lines tested.



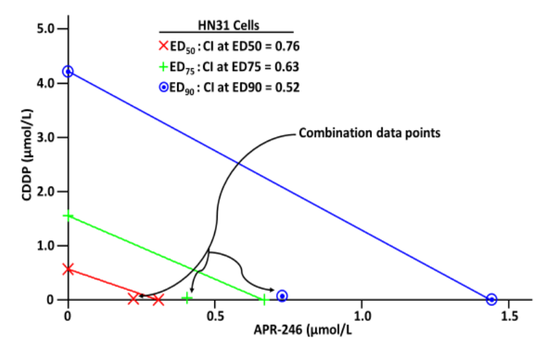


Figure 3 (cont). APR-246 synergizes with cisplatin *in vitro* in HNSCC cells expressing mutant p53. Results showed that APR-246 decreased cell survival of these mutant HNSCC cells and synergized with cisplatin *in vitro*. The combination index therapy determined by Talalay-Chow method is shown in D. CI <1.0 indicates synergism, CI = 1, indicates additivity, and CI > 1.0 indicates antagonism. Addition of APR-246 to cisplatin resulted in CI = 0.76 at the effective dose (ED50) that killed 50% of the cells following treatment with the two drugs. Drugs were used at constant ratio of 1:10 as indicated.

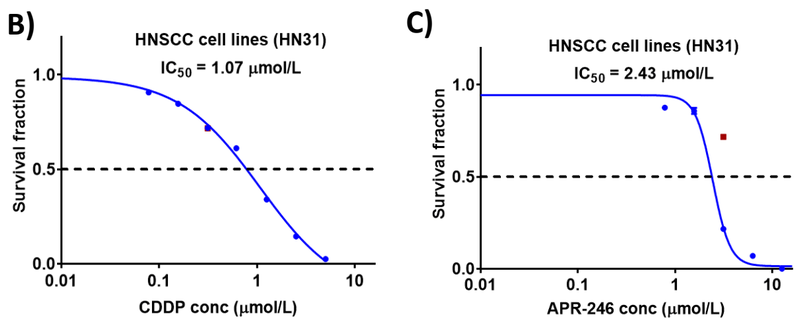


Figure 3. Clonogenic survival assay showing single agent activity based on IC_{50} for cisplatin (CDDP) and APR-246 in HNSCC cells. HNSCC cell line (HN31) was treated with APR-246 (0-50 μmol/L) alone, in combination with cisplatin (0-5 μmol/L) and subjected to clonogenic survival analysis. Representative images of clonogenic survival are shown in A. We used the human HN31 p53 mutant cell line to explore the degree of sensitivity to cisplatin and APR-246 *in vitro*. We calculated dose-response curves and the cell line tested was resistant to cisplatin in agreement with the published IC_{50} (B). The cells seemed to display some degree of sensitivity to APR-246 with an IC_{50} value of 2.43 μmol/L (C), compared to other tumor types.

Conclusions

- APR-246 reactivates the HNSCC expressing p53 mutations in vitro through induction of conformational changes.
- APR-246 displays single agent activity and sensitizes the HNSCC harboring p53 mutation to cisplatin.
- APR-246 enhances cell killing through induction of apoptosis in HNSCC.