Epigenetic Nanotherapy

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ABSTRACT

Epigenetic alterations such as aberrant DNA methylation and histone modifications contribute substantially to both the cause and maintenance of drug resistance. We show that the sequential treatment of doxorubicin resistant breast cancer cells (MCF-7/Adr), first with an epigenetic drug, decitabine (DAC), and then with doxorubicin (Dox), induces a highly synergistic effect, thus reducing the IC50 of doxorubicin by several thousand folds. Further, we demonstrate DAC-loaded nanogel is more effective than DAC in solution in overcoming drug resistance in MCF-7/Adr, DAC-resistant melanoma cells, and leukemia cells. DAC-loaded nanogel sustained the depletion DNA methyltransferase1 (DNMT1) that promotes methylation of DNA, prolonged cell arrest in the G2/M cell-cycle phase, and significantly enhanced antiproliferative effect of DAC.

INTRODUCTION

Epigenetic changes lead to silencing of tumor suppressor genes involved in key DNA damage-response pathways, making drug-resistant cancer cells nonresponsive to conventional anticancer drug therapies. Since epigenetic modifications do not mutate the DNA sequence of a gene, strategies to reverse epigenetic abnormalities are considered useful in reversing drug resistance in cancer therapy. Certain compounds, such as 5-aza-2’-deoxycytidine (decitabine, DAC), a DNA hypomethylating agent, and suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, can reactivate the expression of transcriptionally silenced genes. Our hypothesis is that treating drug-resistant cells with epigenetic drugs could restore sensitivity to Dox by reactivating previously silenced genes and could thereby overcome drug resistance. Since DAC is unstable, hence its effect is transient that limits the therapeutic potential for DAC for treating solid tumors. To address this issue, we encapsulated DAC in NIPAM-based nanogels.

EXPERIMENTAL METHODS

We first tested the efficacy of pretreatment of DAC and SAHA in MCF-7/Adr in time- and dose-response studies for each drug’s ability to sensitize cells to the cytotoxic effect of doxorubicin. To understand the mechanism of efficacy, we analyzed treated cells for cell-cycle and microarray analyses.

DAC-loaded nanogels were tested for efficacy in MCF-7/Adr, and in DAC-resistant melanoma (B16 res) and leukemia cell (THP1) lines. To understand the mechanism of efficacy, we determined DNMT1 depletion in cells treated with DAC-loaded nanogels compared to DAC in solution treated cells.

RESULTS AND DISCUSSION

We show that the sequential treatment of MCF-7/Adr cells, first with DAC and then with Dox, induces a highly synergistic effect, thus reducing the IC50 of Dox by several thousand folds (Fig 1) (1). The sequential treatment caused over 90% resistant cells to undergo G2/M cell-cycle arrest, determined to be due to upregulation of p21WAF1/CIP1 expression, which is responsible for cell-cycle regulation. The induction of p21WAF1/CIP1 correlated well with the depletion of DNMT1, an enzyme that promotes methylation of DNA, suggesting that the p21WAF1/CIP1 gene may have been methylated and hence is inactive in resistant cells. Microarray analysis shows expression of several tumor suppressor genes and downregulation of tumor promoter genes in sequentially treated resistant cells. Sequential treatment was found to be significantly more
Fig 1. Synergistic effect of sequential treatment DAC and Dox in MCF-7/Adr cells. a) Effect of DAC or Dox alone treatment and sequential treatment with different doses of DAC and Dox. b) Comparison of sequential and simultaneous treatment of DAC and Dox. c) Combination index (CI) for sequential and simultaneous treatments CI Value: < 1 Synergistic; = 1 Additive; and > 1 Antagonistic.

Fig 2. Analysis of DNMT1 depletion in MCF-7/Adr cells treated with DAC solution or DAC nanogel. Actin was used as the loading control.

CONCLUSIONS
Sequential treatment of an epigenetic drug in combination with doxorubicin induces a highly synergistic effect that overcomes Dox resistance in breast cancer cells. Our data also suggest that effective delivery of DAC and prolonged DNMT1 depletion are critical to overcoming drug resistance.

REFERENCES

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