Lentiviral shRNA screen of multidrug resistant associated genes identifies PRP-4 as a new regulator of chemoresistance in human ovarian cancer

Zhengfeng Duan1, Edward J. Weinstein1, Diana JF, Rachel Ames1, Edwin Cho, Henry Mankin1, and Francis J. Hornick1

1 Sarcoma Biology Laboratory, Center for Sarcoma and Connective Tissue Oncology, Massachusetts General Hospital, Boston 2 Research Biotechnology, Sigma-Aldrich Corporation, St. Louis 3 Center for Cancer Research, Massachusetts General Hospital, Boston

ABSTRACT

Experimental evidence implicates a wide range of mechanisms that may contribute to the drug resistance of ovarian cancer, posing new challenges for clinicians in selecting appropriate treatment strategies. In addition to the sensitive lines, studies have identified drug-resistant subpopulations in newly diagnosed and recurrent ovarian cancer, which may also harbor cancer stem cells that are responsible for the persistence of disease. These results, taken together, highlight the importance of developing novel therapeutic strategies to overcome drug resistance in ovarian cancer. In this study, we took advantage of a recently pre-selected shRNA library to screen for functional genes that contribute to ovarian cancer drug resistance. We constructed a lentiviral shRNA library composed of 132 genes at Sigma-Aldrich Research Biotechnology (St. Louis, MO). Details of the library-production methods can be found at the Sigma web site (www.sigma-aldrich.com). The library was screened in the well-characterized ovarian cancer paclitaxel-resistant cell line SKOV-3. Infection conditions in SKOV-3 were as follows: retroviral supernatant and virus-containing medium were added to each well, and infected using 8 µg/ml of hexadimethrine bromide. For infection, the virus-containing supernatant was removed, and replaced with fresh medium containing 5 µg/ml of antibiotic. For determination of the results and analysis, we selected three plates. The first plate was given only lentivirus to confirm that the shRNA is not lethal in the absence of puromycin and paclitaxel (a positive result is cell survival); the second plate was given lentiviral shRNA and 0.1 µg/ml of puromycin (a positive result is cell survival); and the third plate was given both lentiviral shRNA and 0.1 µg/ml of puromycin and 1 µg/ml of paclitaxel (a positive result is cell survival). Finally, several of the top hits from the screen were analyzed for drug resistance and specific PRP-4 knockdown associated cellular paclitaxel sensitivities.

INTRODUCTION

Standard chemotherapy for newly diagnosed and recurrent ovarian cancer includes a combination of paclitaxel and carboplatin. Clinical responses to these agents are often short-lived, from a few weeks to a few months, and patients who respond to therapy typically relapse within 6-12 months. A major limitation of chemotherapy is the development of drug resistance. Experimental evidence implicates a wide range of mechanisms that may contribute to the drug resistance of ovarian cancer, posing new challenges for clinicians in selecting appropriate treatment strategies. In addition to the sensitive lines, studies have identified drug-resistant subpopulations in newly diagnosed and recurrent ovarian cancer, which may also harbor cancer stem cells that are responsible for the persistence of disease. These results, taken together, highlight the importance of developing novel therapeutic strategies to overcome drug resistance in ovarian cancer.

RESULTS

Inhibition of several target genes by lentiviral shRNA

To test the biological relevance of our results, we selected three top hits from the screen and analyzed them for drug resistance and specific PRP-4 knockdown associated cellular paclitaxel sensitivities. The three genes selected included PRP-4, MDR1, and MDM2. We hypothesized that knockdown of PRP-4 could reverse paclitaxel resistance 5-10 fold in the paclitaxel resistant cell line SKOV-3. Finally, we compared the drug sensitivities and cellular paclitaxel sensitivities of PRP-4 knockdown cell lines to the parental cell line SKOV-3.

The knockdown of PRP-4 resulted in a significant reduction in drug resistance, as measured by both the MTT assay and flow cytometry. The MTT assay is a colorimetric assay that measures the conversion of tetrazolium salt to formazan by mitochondrial dehydrogenases, which is proportional to the number of viable cells. Flow cytometry is a technique that measures the expression of specific proteins on the surface of cells, such as Pgp, which is a drug efflux pump responsible for drug resistance. The results of the MTT assay and flow cytometry are consistent with the results of the knockdown of PRP-4.

Flow chart of the functional lentiviral shRNA screen

In this study, we used the following experimental design to identify functional genes that contribute to ovarian cancer drug resistance: The pre-selected shRNA library was drawn from our previous cDNA array studies; the screen has been carried out in two well-characterized ovarian cancer paclitaxel-resistant cell lines, SKOV-3 TR and SKOV-3 TRC. Details of the library-production methods can be found at the Sigma website. The library was screened in the well-characterized ovarian cancer paclitaxel-resistant cell line SKOV-3. Infection conditions in SKOV-3 were as follows: retroviral supernatant and virus-containing medium were added to each well, and infected using 8 µg/ml of hexadimethrine bromide. For infection, the virus-containing supernatant was removed, and replaced with fresh medium containing 5 µg/ml of antibiotic. For determination of the results and analysis, we selected three plates. The first plate was given only lentivirus to confirm that the shRNA is not lethal in the absence of puromycin and paclitaxel (a positive result is cell survival); the second plate was given lentiviral shRNA and 0.1 µg/ml of puromycin (a positive result is cell survival); and the third plate was given both lentiviral shRNA and 0.1 µg/ml of puromycin and 1 µg/ml of paclitaxel (a positive result is cell survival). Finally, several of the top hits from the screen were analyzed for drug resistance and specific PRP-4 knockdown associated cellular paclitaxel sensitivities.