Elimination of Peroxiredoxin 1 Abrogates Proliferation and Viability in Extracellular Matrix-Detached SKOV3 Ovarian Cancer Cells

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ABSTRACT | Epithelial ovarian carcinoma has a relatively low survival rate due to the fact that this disease is often diagnosed after it has already progressed to stage III or stage IV, thus making the disease more difficult to treat. In order to successfully metastasize to these regional and distant sites, tumor cells must be able to overcome extracellular matrix (ECM)-detachment-induced effects, including combatting increased reactive oxygen species (ROS). Here, we investigate peroxiredoxin 1 (PRDX1) and find that PRDX1 plays an important role in proliferation in ECM-attached and ECM-detached SKOV3 cells, an epithelial ovarian carcinoma cell line. Furthermore, we find that PRDX1 deficiency in SKOV3 cells promotes a substantial decrease in cell viability in ECM detachment contributing to abrogated cell growth in anchorage independence. Thus, our study finds that PRDX1 deficiency uniquely compromises cell viability in ECM-detached cells, suggesting that PRDX1 could be a potential therapeutic target for late-stage epithelial ovarian carcinoma.

KEYWORDS | Extracellular matrix; Metastasis; Ovarian cancer; Peroxiredoxin 1; SKOV3 cells

ABBREVIATIONS | ECM, extracellular matrix; PRDX1, peroxiredoxin 1; ROS, reactive oxygen species; shRNA, short hairpin RNA

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1. INTRODUCTION

Ovarian cancer is the fifth-leading cause of cancer death among women, where only 47% of women survive 5 years after diagnosis [1]. This low survival rate is a result of poor early detection strategies for ovarian cancer, thus driving late stage diagnoses of the disease and leaving fewer treatment options [1, 2]. Specifically, epithelial ovarian carcinomas, which account for 90% of all ovarian cancer cases, are most commonly diagnosed at stage III and stage IV with a 5-year survival rate of 41% and 20%, respectively [3]. These high death rates associated with late stage diagnoses underscore the need for improved therapeutic options for these patients.

Epithelial ovarian carcinoma cells metastasize (spread) from the primary tumor to regional and distant sites by traveling in the peritoneal fluid, bloodstream, and lymphatics [2]. To accomplish this feat, it is well recognized that cancer cells must be able to overcome extracellular matrix (ECM) detached-inducement effects to successfully metastasize, including contending with increased reactive oxygen species (ROS) [4–6]. ROS are reactive chemicals that impact many activities within a cell, including inducing programmed cell death [5, 7]. Previous studies in metastatic breast cancer have found that metastatic ECM-detached breast cancer cells utilize enzymes, termed antioxidant enzymes, to eliminate ROS and thrive [5]. Most recently, we reported that the elimination of catalase severely abrogates the survival of ECM-detached ovarian carcinoma cells [8]. These studies suggest that antioxidant enzymes may be viable therapeutic targets for metastatic cancers, including metastatic epithelial ovarian carcinoma; however, many antioxidant enzymes and their role in epithelial ovarian carcinoma progression have yet to be studied. Chief amongst these antioxidant enzymes is peroxiredoxin 1 (PRDX1), an antioxidant enzyme known to eliminate ROS and to have increased expression in ovarian cancer [9]. While recent studies have begun to unravel the potential of PRDX1 as a diagnostic marker for ovarian cancer [9, 10], the importance of PRDX1 in the viability and proliferation, specifically of ECM-detached epithelial ovarian carcinoma cells, has yet to be unveiled.

The purpose of this study is to elucidate the role of PRDX1 in ECM-attached and ECM-detached SKOV3 cells, an epithelial carcinoma cell line. Here, we identify PRDX1 deficiency diminishes growth of anchorage-independent SKOV3 cells. While PRDX1 deficiency controls proliferation in ECM-attached and ECM-detached SKOV3 cells, our data indicate that elimination of PRDX1 severely compromises cell viability uniquely in ECM-detached SKOV3 cells. Thus, our study represents a novel paradigm whereby PRDX1 plays a special role in the viability of ECM-detached ovarian cancer cells, a phenomenon not seen in ECM-attached cells. These data find PRDX1 as a potential therapeutic target to treat metastatic ovarian cancer.

2. MATERIALS AND METHODS

2.1. Cell Culture

SKOV3 cells were maintained as described previously [8]. Briefly, SKOV3 cells, an ovarian adenocarcinoma cell line, were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and were maintained in McCoy’s 5A medium modified with L-glutamine and sodium bicarbonate (Sigma-Aldrich, St. Louis, MO, USA), 10% fetal bovine serum (FBS) (Alkali Scientific, Ocala, FL, USA), and 1% penicillin/streptomycin (GIBCO, Waltham, MA, USA). HEK293T cells (ATCC) were maintained in DMEM (GIBCO), 10% FBS (Alkali Scientific), and 1% penicillin/streptomycin (GIBCO). Both cell lines were grown at 37°C and 5% CO2.

2.2. Short Hairpin RNA (shRNA)

SKOV3 cells were engineered to be deficient in PRDX1 using shRNA. MISSION shRNA targeting PRDX1 in the puromycin-resistant pLKO.4 vector
along with an empty vector control were purchased from Sigma-Aldrich and stable SKOV3 PRDX1 knockout cells along with control SKOV3 cells containing empty vector pLKO.4 (SKOV3 parental) were engineered through lentiviral transduction as described previously [8]. To produce lentivirus, a total of 0.5 µg of target DNA, 0.5 µg of pCMV-D8.9 packaging vector, and 60 ng VSV-G envelope were transfected into HEK293T cells using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) and PLUS reagent (Invitrogen). Virus was then collected at 24 and 48 h post-transfection and filtered through a 0.45 µm filter (Millipore, St. Louis, MO, USA). Resulting lentivirus along with 8 µg/ml of polybrene were used for transduction of SKOV3 cells, and stable populations of PRDX1-deficient cells and SKOV3 parental cells were selected using 2 µg/ml puromycin (Invitrogen, San Diego, CA, USA).

2.3. Cell Proliferation and Cell Viability Assays

This experiment was completed as previously described [8]. Briefly, to measure cell proliferation and viability, cells were plated at a density of 100,000 per well of SKOV3 parental and SKOV3 PRDX1-deficient cells in triplicate on a regular or polyHEMA-coated 6-well plates, and this process was repeated three times. After 24, 48, and 72 h, cells were collected, stained with trypan blue (Hyclone, Logan, UT, USA), and total cells and dead cells were counted using a hemocytometer under light microscopy.

2.4. Soft Agar Assay

Soft agar assay was completed as previously described [8]. A total of 20,000 SKOV3 parental cells and SKOV3 PRDX1-deficient cells were added to 1.5 ml of McCoy’s media plus 0.4% low-melt agarose (Sigma-Aldrich) and plated on top of a 2 ml bed of 0.5% low-melt agarose/media mixture in a 6-well plate. For each experiment, three wells were plated for both SKOV3 parental cells and SKOV3 PRDX1-deficient cells. Cells were given 1 ml of media every two days for 21 days and maintained at 37°C and 5% CO2. Living colonies were then stained with p-iodonitrotetrazolium violet (INT) (Sigma-Aldrich), imaged, and counted using ImageJ (NIH, Bethesda, MD, USA). In total, 14 individual experiments of triplicate-plated SKOV3 parental and SKOV3 PRDX1-deficient cells were completed and analyzed.

2.5. Western Blotting

To confirm successful elimination of PRDX1, western blotting was performed as described previously [8]. Cells were harvested and lysed in 1% Nonidet-40 (Thermo Fisher, Waltham, MA, USA) with aprotinin (1 µg/ml) (GIBCO) and leupeptin (5 µg/ml) (GIBCO). After spinning for 30 min at 4°C, lysates were normalized using a BCA assay (Pierce, Rockford, IL, USA). The samples were boiled at 95°C for 10 min, run on a 12% SDS-PAGE gel (BioRad, Hercules, CA, USA), transferred to PVDF membrane (Millipore), and membrane was blocked in 5% milk in 1x Tris-buffered saline and 0.05% Tween 20 (1x TBST) (BioRad). Primary antibodies PRDX1 rabbit polyclonal antibody (#15816-1-AP, Proteintech, Rosemont, IL, USA) and β-actin mouse monoclonal antibody (#66009-1-Ig, Proteintech) were used at concentrations of 140 pg/µl and 85 pg/µl, respectively in 5% milk and 1x TBST. Goat anti-rabbit IgG (H + L), HRP conjugated (#SA00001-2, Proteintech) and goat anti-mouse IgG (H + L), HRP conjugated (#SA00001-1, Proteintech) secondary antibodies were used at a dilution of 1:1500 in 5% milk and 1x TBST. The membrane was then developed through enhanced chemiluminescence using a Pierce ECL Western Blotting Substrate kit (Pierce).

2.6. Statistical Analysis

All data are presented as a mean ± standard error of the mean (SEM). For soft agar experiments, vertical bars represent the mean from 14 independent experiments. For proliferation and viability assays, vertical bars represent the mean from three independent experiments. Statistical analysis of the data was performed by two-tailed t-test, and a p value ≤ 0.05 was considered statistically significant.

3. RESULTS

3.1. Decreased PRDX1 Expression Compromises Growth of Anchorage-Independent SKOV3 Cells

To extend our previous studies that revealed that decreased expression of the antioxidant enzyme cata-
lase severely abrogated anchorage-independent growth of breast cancer cell lines and the epithelial ovarian carcinoma cell line SKOV3 [5, 8], we investigated whether elimination of PRDX1 could similarly decrease anchorage-independent growth of SKOV3 cells. To address this matter, PRDX1 expression was eliminated in SKOV3 cells using shRNA techniques, and successful PRDX1 elimination was confirmed through western blotting (Figure 1A). We found that PRDX1 knockdown in SKOV3 cells...
cells substantially abrogated anchorage-independent growth in soft agar (Figure 1B and 1C). Thus, these data suggest that PRDX1 plays an important role in the growth of ECM-detached SKOV3 cells.
3.2. PRDX1 Elimination Compromises Proliferation but not Cell Viability in ECM Attached SKOV3 Cells

Given that PRDX1 elimination was shown to compromise growth of ECM-detached SKOV3 cells in soft agar, we were curious if decreased PRDX1 expression affected ECM-attached SKOV3 cells. SKOV3 parental cells alongside SKOV3 PRDX1-deficient cells were grown on adherent plates for 24, 48, and 72 h before being analyzed for changes in cell proliferation and viability. ECM-attached SKOV3 PRDX1-deficient cells showed a significant decrease in proliferation at 72 h (Figure 2A). However, no changes in cell viability were seen between attached SKOV3 parental and attached SKOV3 PRDX1-deficient cells at any of the time points (Figure 2B). These data suggest that PRDX1 is important in maintaining proliferation of ECM-attached SKOV3 cells but is not critical in maintaining cell viability in ECM-attached cells.

**FIGURE 3.** PRDX1 suppression compromises cell proliferation in ECM-detached SKOV3 cells. SKOV3 parental and SKOV3 PRDX1-deficient cells were grown in ECM attachment on six well plates as well as in ECM detachment using non-adherent poly-HEMA-coated 6 well plates. At the indicated time points, cells were stained with trypan blue, counted using a hemocytometer, and cell proliferation was determined for ECM-attached SKOV3 parental cells, ECM-attached SKOV3 PRDX1-deficient cells, ECM-detached SKOV3 parental cells, and ECM-detached SKOV3 PRDX1-deficient cells. Values are expressed as mean ± SEM. Significance was determined by a two-tailed t-test where p < 0.05. Letters indicate p < 0.05 for specific labeled comparison at specific time point.
3.3. ECM-Detached PRDX1-Deficient SKOV3 Cells Display Abrogated Proliferation and Cell Viability

Changes in anchorage-independent growth seen in soft agar can be due to alterations in proliferation or cell viability. Given that PRDX1 deficiency affected proliferation and not viability in ECM-attached SKOV3 cells, we were interested if PRDX1 deficiency affected proliferation and/or cell viability specifically in ECM-detached SKOV3 cells compared to ECM-detached SKOV3 parental cells. To determine if abrogated cell proliferation and/or cell viability was critical for growth of ECM-detached SKOV3 cells, SKOV3 parental cells and SKOV3 PRDX1-deficient cells were plated on normal plates as a control and on non-adherent/poly-HEMA-coated plates to specifically measure cell proliferation and viability for ECM-detached SKOV3 cells. Cells grown on normal or non-adherent plates were cultured for 24, 48, and 72 h before dead cells were stained with trypan blue. Total cells and total viable cells were counted for all wells at indicated time points using a hemocytometer. As expected, control ECM-attached SKOV3 parental cells showed a significant decrease in proliferation after 72 h (Figure 3) with no change in cell viability at any time point (Figure 4) compared to ECM-detached SKOV3 parental cells, further confirming our earlier results (Figure 2A and 2B). However, ECM-detached SKOV3 PRDX1-knockdown cells showed decreased proliferation earlier at the 48 and 72 h time points (Figure 3) and significant decreased cell viability at 72 h (Figure 4) when compared to ECM-detached parental cells suggesting that PRDX1 plays a unique role in maintaining cell viability in ECM-detached SKOV3 cells. In aggregate, these data suggest that PRDX1 deficiency uniquely impacts cell viability of ECM-detached SKOV3 cells only, and that PRDX1 deficiency impacts proliferation earlier and more greatly in ECM-detached SKOV3 cells compared to ECM-attached SKOV3 cells.

FIGURE 4. Elimination of PRDX1 abrogates cell viability in ECM-detached SKOV3 cells. Both SKOV3 parental and SKOV3 PRDX1-deficient cells were grown in ECM-attachment and ECM-detachment, stained with trypan blue at 24, 48, and 72 h, and cell viability was determined for ECM-attached SKOV3 parental cells, ECM-attached SKOV3 PRDX1-deficient cells, ECM-detached SKOV3 parental cells, and ECM-detached PRDX1-deficient cells. Values are expressed as mean ± SEM. Statistical analysis was performed using a two-tailed t-test where p < 0.05 was considered significant. *, p < 0.05.
4. DISCUSSION

We found that PRDX1 promotes cell growth in anchorage-independent SKOV3 cells. Furthermore, we find that PRDX1 deficiency severely compromises proliferation and viability in ECM-detached SKOV3 cells. This suggests that PRDX1 plays an important role in maintaining proliferation and viability in ECM detachment, which is critical in order to progress through the ECM- bereft metastatic cascade. Interestingly, PRDX1 does play a protective role in proliferation of ECM-attached cells, suggesting that PRDX1 also plays a role at the primary tumor in addition to metastasis. Most importantly, these data present a special responsibility for PRDX1 in protecting cell viability of ECM-detached cells (Figure 5).

PRDX1 belongs to a family of antioxidant enzymes that are responsible for reducing ROS, specifically hydrogen peroxide and alkyl hydroperoxides [11]. While ROS appear to play different roles during tumor formation, angiogenesis, and tumor pro-
gression, and most recently, it has been discovered that ECM-detached metastatic cancer cells must overcome increases in ROS in order to survive [5, 6, 12, 13]. Thus, understanding the role of different antioxidant enzymes in combatting ECM-detachment-induced ROS is important and could lead to new therapeutic approaches for treating ECM-detached metastatic cancer cells, specifically metastatic epithelial ovarian cancer cells. Interestingly, PRDX1, a ubiquitously expressed enzyme, has been found to be increased in the peripheral circulation and serum of ovarian cancer patients [9, 10]. In addition, PRDX1 has been connected with poor prognosis and has been proposed as a prognostic biomarker in not only ovarian cancer [9–11] but also esophageal squamous cell carcinoma [14, 15], hepatocellular carcinoma [16], and hilar cholangiocarcinoma [17]. Furthermore, the identification of PRDX1 as a mediator of proliferation in SKOV3 cells in ECM attachment adds to the growing body of literature suggesting that PRDX1 can modulate distinct proliferative functions in cancer cells [18–20]. However, for the first time (to our knowledge), our studies suggest that PRDX1 plays a unique role in maintaining cell viability that is unique to ECM-detached SKOV3 cells and could promote epithelial ovarian carcinoma metastasis specifically.

Most recently, our lab discovered that the hydrogen peroxide-eradicating enzyme, catalase, plays a protective role in the anchorage-independent growth of SKOV3 cells, and catalase deficiency severely compromised cell viability in ECM-detached SKOV3 cells [8]. Furthermore, we found that elimination of catalase did not affect cell proliferation in ECM-attached or ECM-detached SKOV3 cells [8]. While both catalase and PRDX1 are ubiquitously expressed and have a similar role in eliminating hydrogen peroxide, their complex relationship and the different mechanisms that they impact have yet to be fully elucidated [21]. Our studies with catalase and PRDX1 suggest that catalase and PRDX1 may play unique roles in ECM-attached and ECM-detached SKOV3 cells. Specifically, our data suggest that while both catalase and PRDX1 play a role in maintaining cell viability in ECM-detached SKOV3 cells, PRDX1 also appears to be important in maintaining proliferation in ECM-attached and ECM-detached SKOV3 cells. These data suggest that additional studies examining other antioxidant enzymes are warranted to better understand the unique roles of each enzyme and better ascertain the best potential therapeutic targets to eliminate metastatic ovarian cancer cells.

Collectively, it is conceivable that the reduction of PRDX1 could be a novel therapeutic option to compromise cell viability and proliferation of ECM-detached metastatic epithelial ovarian cancer cells. Our findings are consistent with data suggesting that PRDX1 activity can mediate the sensitivity of cancer cells to targeted therapies and further support future work in development of PRDX1 antagonists [22, 23]. Given poor diagnostics for ovarian cancer, patients are often diagnosed after the disease has already progressed to stage III or stage IV, resulting in low 5-year survival rates. It is critical to continue to investigate the utility of targeting PRDX1 in the quest to design affordable and effective treatments directed for individuals that present with stage III and stage IV epithelial ovarian cancer.

5. CONCLUSION

Here, we discover that PRDX1 deficiency compromises growth of anchorage-independent SKOV3 epithelial ovarian carcinoma cells. Specifically, elimination of PRDX1 resulted in decreased colony formation in soft agar. PRDX1 deficiency results in decreased proliferation in ECM-attached and ECM-detached SKOV3 cells. Furthermore, PRDX1-deficient SKOV3 cells show compromised cell viability in ECM detachment only, suggesting that PRDX1 protects against cell death uniquely in ECM-detached cells. Thus, PRDX1 could be an attractive target for eliminating epithelial ovarian carcinoma cells progressing through the ECM-bereft metastatic cascade and should be further investigated.

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