ROS

RESEARCH ARTICLE

Catalase Deficiency Compromises Survival in Extracellular Matrix-Detached SKOV3 Ovarian Cancer Cells

Cassandra L. Libbing, Kassidy M. Jungles, Ellen Rabaut, and Calli A. Davison-Versagli

Department of Biology, Saint Mary's College, Notre Dame, IN 46556, USA

Correspondence: cversagli@saintmarys.edu (C.A.D-V.)

Libbing CL et al. Reactive Oxygen Species 7(20):121–128, 2019; ©2019 Cell Med Press
http://dx.doi.org/10.20455/ros.2019.821
(Received: November 29, 2018; Revised: December 6, 2018; Accepted: December 6, 2018)

ABSTRACT | For epithelial ovarian cancer cells to survive during the metastatic cascade, cells must be able to evade anoikis, a caspase-dependent cell death mechanism initiated by extracellular matrix (ECM) detachment. However, many of the details behind this phenomenon have yet to be unveiled. Here, we examined the role of the antioxidant enzyme, catalase, in the survival and proliferation of anchorage-independent SKOV3 ovarian cancer cells. Catalase deficiency severely compromises cell viability and anchorage-independent growth in ECM-detached SKOV3 cells. Notably, cell viability and proliferation were unaffected in ECM-attached catalase-deficient SKOV3 cells. In aggregate, we discovered that catalase plays a prominent role in protection from ECM-detachment-induced cell death in SKOV3 cells. Furthermore, these findings imply that catalase may be an effective therapeutic target for epithelial ovarian cancer cells that survive the ECM-bereft metastatic cascade.

KEYWORDS | Catalase; Extracellular matrix detachment; Metastasis; Ovarian cancer; SKOV3 ovarian cancer cell

ABBREVIATIONS | ECM, extracellular matrix; ROS, reactive oxygen species; shRNA, short hairpin RNA; SNP, single nucleotide polymorphism

CONTENTS

1. INTRODUCTION

Ovarian cancer, the deadliest form of gynecological cancer, is anticipated to claim approximately 14,000 lives alone in the United States in 2018 [1, 2]. Epithelial ovarian carcinomas, the most common form of ovarian cancer, are most often diagnosed after the cancer cells have already spread, or metastasized, throughout the abdominal cavity (stage III) or distant sites (stage IV); furthermore, these individuals diagnosed with stage III or stage IV epithelial ovarian cancer have a 5-year relative survival rate of 41% and 20%, respectively [1, 3]. This low 5-year relative survival rate highlights the need for better therapeutic options to treat metastatic ovarian cancer.

During the metastatic cascade, tumor cells are shed from the primary ovarian tumor and are transported by peritoneal fluid to other areas throughout the abdominal cavity [4]. To successfully survive, metastatic cancer cells must survive and combat extracellular matrix (ECM) detachment-induced effects, including anoikis, a caspase-mediated cell death program initiated in ECM-detached cells [5, 6]. Notably, cancer cells must also contend with anoikis-independent mechanisms, including increased levels of reactive oxygen species (ROS) [6]. The antioxidant enzyme, catalase is a ubiquitous housekeeping oxidoreductase responsible for the breakdown of hydrogen peroxide into water and oxygen [7]. It seems plausible to speculate that antioxidant enzymes, specifically catalase, play a pivotal role in the survival of ECM-independent metastatic ovarian cancer cells and antioxidant deficiency abrogates their survival.

The goal of this study was to determine the role of catalase in the growth of anchorage-independent SKOV3 cells, an epithelial carcinoma cell line. Here, we discovered catalase specifically protects ECM-detached SKOV3 cells from cell death, and catalase deficiency compromises survival of anchorage-independent growth through an increase in cell death. These data identify catalase as a novel target that could be utilized for the development of chemotherapeutic approaches to stage III and IV ovarian cancer.

2. MATERIALS AND METHODS

2.1. Cell Culture

The SKOV3 cells (ATCC, Manassas, VA, USA), an ovarian adenocarcinoma cell line, and derivatives were cultured in McCoy's media (Gibco, Waltham, MA, USA), 10% fetal bovine serum (FBS) (Gibco), and 1% penicillin/streptomycin (Gibco). The cells were maintained in a 5% CO_2 incubator at 37ºC.

2.2. shRNA

MISSION short hairpin RNA (shRNA) construct against catalase in the puromycin-resistant PLKO.4 vector along with an empty vector control were from Sigma-Aldrich (St. Louis, MO, USA) and utilized as described [6]. Briefly, target DNA (0.5 μg) with a pCMV-D8.9 packaging vector (0.5 μg) and VSV-G envelope vector (60 ng) were transfected into HEK293T cells (ATCC) using Lipofectamine 2000 and PLUS reagent (Gibco). The virus was collected at 24 and 48 h, filtered, and used for transduction of SKOV3 cells using 8 μg/ml polybrene. We selected for a catalase-deficient SKOV3 cells and PLKO.4 parental control cells using 2 μg/ml puromycin (Invivogen, San Diego, CA, USA).

2.3. Catalase Re-Expression

A total of 0.75 μg of PLNCX-neomycin-catalase vector encoding catalase (PLNCX-KANL) or PLNCX control was transfected with the packaging vector pCLAmpho (0.75 μg) using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) into HEK293T cells (ATCC, Manassas, VA). Virus was collected at 48 and 72 h after transfection, filtered, and used for transduction of SKOV3 shRNA Catalase (Catalase deficient) cells. Cells containing the PLNCX-KANL were selected using 300 μg/ml G418 (Thermo Fisher, Waltham, MA, USA). Catalase re-expression was confirmed through western blotting.

2.4. Western Blotting

The cells were lysed in 1% NP40 (Thermo Fisher) with aprotinin and leupeptin (Gibco), and the resulting samples were normalized using a BCA Assay (Pierce Biotechnology, Rockford, IL, USA). The samples were heated at 95ºC for 5 min before being loaded and electrophoresed at 150 V for 1 h. The electrophoresis gel was transferred to PVDF membrane for 1 h at 100 V. The membrane was washed in a 1× Tris-buffered saline and 0.05% Tween 20 (1× TBST) (Bio-Rad Laboratories, Hercules, CA, USA) plus 5% milk blocking buffer for 1 h. Primary antibodies against catalase (rabbit) and β-actin (mouse) (Proteintech, Rosemont, IL, USA) were used at a 1:1000 dilution in 1× TBST plus 5% milk. The β-actin served as a control. The membrane was incubated in the secondary antibodies, goat anti-mouse and goat anti-rabbit (Proteintech), before the membrane was washed with 1× TBST three times for 15 min each. The membrane was then developed using a chemiluminescence-based assay kit (Pierce Biotechnology).

2.5. Soft Agar Assays

SKOV3 parental cells and SKOV3 catalase-deficient cells were plated in 0.5% low-melt agarose (Sigma-Aldrich) with McCoy's growth media between 15,000–20,000 cells per well in a 6-well plate in triplicate. The soft agar plates were maintained for 19-21 days, with media replacement every other day. Plates were stained with *p*-iodonitrotetrazolium violet (INT-Violet) (Sigma-Aldrich) and imaged. Colonies were counted using ImageJ (NIH, Bethesda, MD, USA). Each soft agar experiment was repeated for a minimum of three times.

2.6. Cell Proliferation and Cell Viability Assays

To measure cell viability and proliferation, 50,000 SKOV3 parental cells and SKOV3 catalase-deficient cells were plated in 1 ml of media in triplicate on regular or poly-HEMA-coated 6-well plates in triplicate. After 24, 48, and 72 h, cells were collected and stained with trypan blue (Hyclone, Logan, UT, USA). The total cells and total dead cells (dead cells stained purple) were then counted using a hemocytometer under light microscopy. This experiment was completed three times.

2.7. Statistical Analysis

Error bars represent standard error of the mean (SEM) and statistical significance was determined by utilizing a two-tailed t-test with $p < 0.05$.

3. RESULTS

3.1. Catalase Deficiency Abrogates Survival of Anchorage-Independent SKOV3 Cells

To determine the importance of catalase in the survival in ECM-detached SKOV3 cells, we used lentiviral-mediated delivery of shRNA to generate a SKOV3 cell line that was deficient in catalase expression. Catalase deficiency was confirmed through western blotting (**Figure 1A**). We then sought to determine whether catalase deficiency affected anchorage-independent growth in soft agar assays. Using these cells, we discovered that catalase elimination resulted in abrogated anchorage-independent growth in soft agar (**Figure 1B** and **1C**).

3.2. Decreased Catalase Expression Compromises Cell Viability in ECM-Detached SKOV3 Cells

Changes in growth in soft agar assays can be a result of changes in proliferation or cell viability. To understand if abrogated cell proliferation and/or cell viability was responsible for decreased colony formation in soft agar observed in catalase-deficient SKOV3 cells, catalase-deficient SKOV3 cells and SKOV3 parental cells were cultured on non-adherent/poly-HEMA-coated plates, and total cells and total viable cells were determined using trypan blue and a hemocytometer. Catalase-deficient cells displayed significant decrease in cell viability at 48 and 72 h compared to SKOV3 parental cells (**Figure 2A**), while no changes in proliferation were observed between catalase-deficient and SKOV3 parental cells (**Figure 2B**).

3.3. Attached Catalase-Deficient SKOV3 Cells Maintain Cell Viability and Proliferation

Given that catalase elimination was shown to affect cell viability rather than proliferation in ECM detachment, we were curious if decreased catalase expression affected ECM-attached SKOV3 cells. Catalase-

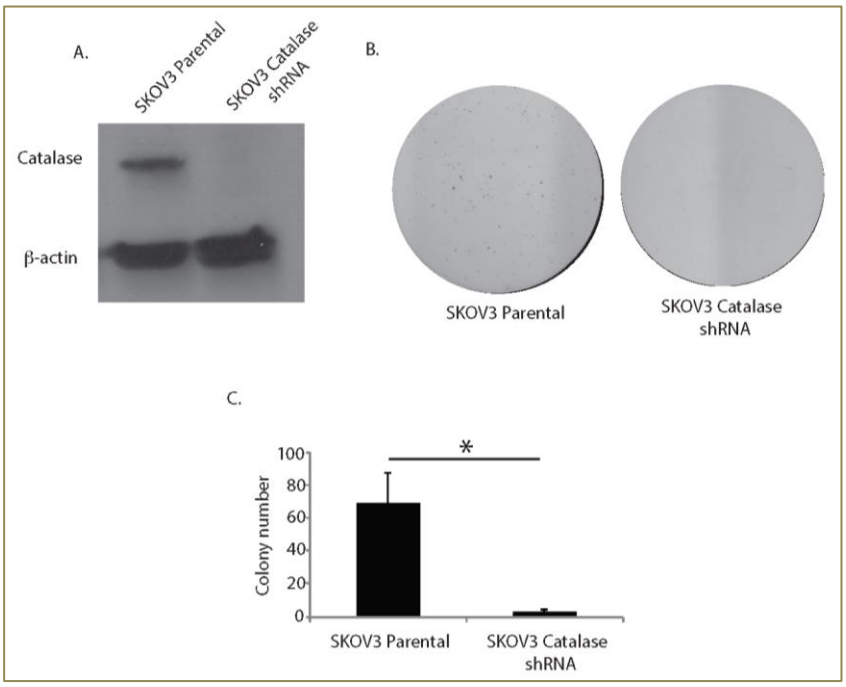

FIGURE 1. Stable suppression of catalase by shRNA in SKOV3 ovarian cancer cells abrogates anchorage-independent growth in soft agar. Stable catalase-deficient SKOV3 cells were engineered using lentiviral transduction of shRNA. Immunoblotting (A) for catalase and β-actin (loading control) was used to confirm the success of transduction. The indicated cells were grown in soft agar for 19–20 days and stained with INT-violet and counted using Image J. Representative images are shown in (B) and quantitation of colonies is shown in (C). *, $p < 0.05$.

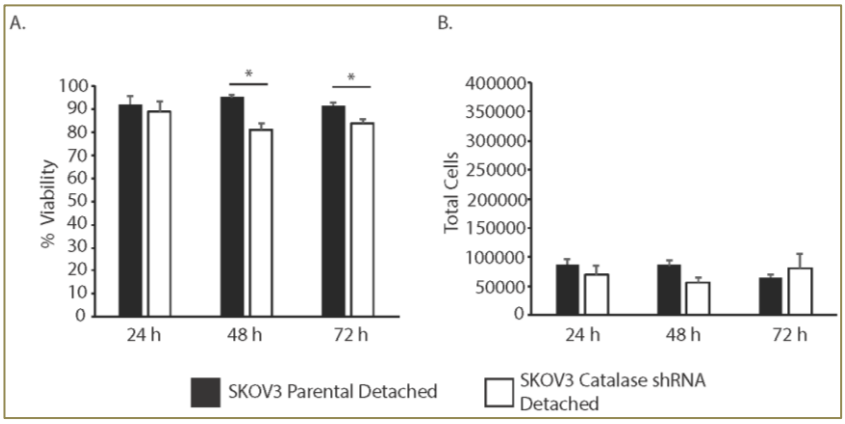

FIGURE 2. Catalase deficiency alters cell viability but does not alter proliferation in ECM-detached SKOV3 cells. SKOV3 cells were grown in ECM detachment using poly-HEMA-coated plates. Cells were stained with trypan blue and counted at the indicated time points using a hemocytometer. From these counts, differences in cell viability (A) and proliferation (B) were determined between ECM-detached SKOV3 parental and ECM-detached SKOV3 catalase shRNA cells. *, $p < 0.05$.

knockdown SKOV3 cells and parental SKOV3 cells were grown on adherent plates and total cells and total viable cells were counted at 24, 48, and 72 h. No changes in cell viability (**Figure 3A**) or proliferation (**Figure 3B**) were seen, suggesting that catalase activity only becomes crucial in the survival of ECM-detached SKOV3 cells.

3.4. Catalase Is Critical in the Preservation of Anchorage-Independent Growth in SKOV3 Cells

To confirm that loss of anchorage-independent growth in SKOV3 cells was due to catalase elimination, catalase was stably re-expressed in SKOV3 catalase deficient cells through retroviral delivery of a plasmid encoding for catalase. Catalase re-expression was confirmed through western blotting (**Figure 4A**). SKOV3 parental cells, SKOV3 catalase shRNA cells, and SKOV3 catalase shRNA catalase re-expression cells were grown in soft agar. SKOV3 catalase shRNA cells continued to display diminished colony formation in soft agar compared to SKOV3 parental cells; however, colony formation in soft agar was rescued through catalase re-expression (**Figure 4B** and **4C**). In aggregate, these data confirmed that anchorage-independent growth is inhibited due to catalase deficiency in SKOV3 cells, thus suggesting that catalase plays a pivotal role in the growth of ECM-detached SKOV3 ovarian cancer cells.

4. DISCUSSION

We found that catalase protects and promotes survival of ECM-detached SKOV3 cells. Furthermore, catalase deficiency severely abrogates growth and viability in ECM-detached SKOV3 cells. Re-expression of catalase in catalase-deficient SKOV3 cells rescued cell survival and growth of anchorage-independent SKOV3 cells, which further confirmed that catalase plays a critical role in the viability and growth in anchorage-independent SKOV3 cells. Moreover, these data described present a unique role for catalase that is specific to ECM-detached SKOV3 cells only as catalase deficiency in ECM-attached cells displayed no change in proliferation, viability, or colony formation in soft agar (**Figure 5**).

The identification of catalase as a key player in ECM-detached SKOV3 cells adds to a growing body of literature implicating antioxidant enzymes in chemotherapy resistance and as potential tumor markers; however, few reports exist in understanding the role of antioxidant enzymes in completing the

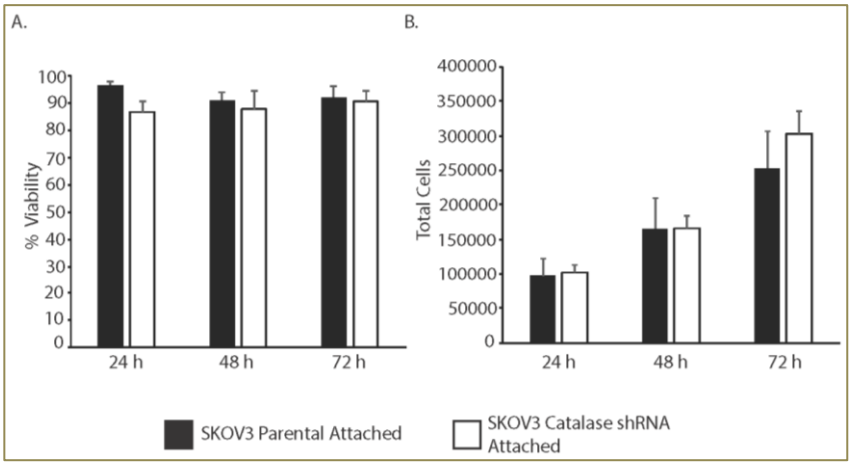

FIGURE 3. The effect of catalase suppression on cell viability and proliferation in ECM-attached SKOV3 cells. SKOV3 cells were grown on 6-well plates. Cells were stained with trypan blue and counted at the indicated time points using a hemocytometer. From these counts, cell viability (A) and proliferation (B) were determined between ECM-attached SKOV3 parental and ECM-attached SKOV3 Catalase shRNA cells.

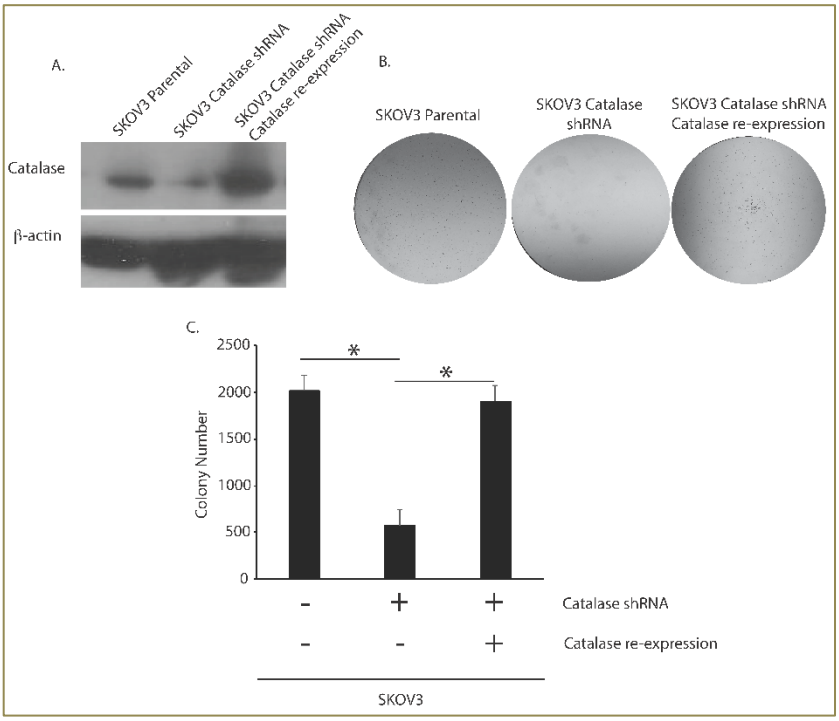

FIGURE 4. Catalase re-expression rescues anchorage-independent growth in SKOV3 cells. Catalase was re-expressed in SKOV3 catalase shRNA cells using retroviral transduction. Western blotting (A) for catalase and β-actin (loading control) confirmed the success of the transduction. The indicated cells were grown in soft agar for 21 days and stained with INT-violet and counted using ImageJ. Representative images are shown in (B) and quantitation of colony number is shown in (C). *, $p < 0.05$.

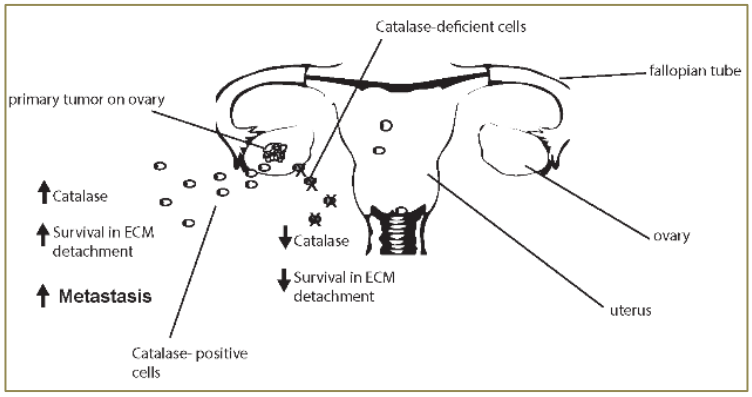

FIGURE 5. Model for catalase deficiency in ECM-detached ovarian cancer cells. During metastasis, ovarian cancer cells shed from the primary ovarian tumor and are transported by peritoneal fluid to other areas throughout the abdominal cavity [3]. Evasion of ECM-detachment induced cell death is crucial in the successful metastasis of ovarian cancer cells [4, 5]. Elimination of catalase in ECM-detached SKOV3 cells severely compromises survival, thus decreasing metastatic ability.

ECM-bereft metastatic cascade [2, 8–17]. Previously, we reported that antioxidant enzymes were critical in maintaining metabolism and thus survival of ECM-detached metastatic breast cancer cells. Most recently, manganese superoxide dismutase was implicated in the survival of ECM-detached nasopharyngeal cells [18]. We are first to report that catalase plays a critical role in the survival of ECM-detached SKOV3 cells. This presents a potentially unique opportunity in the development of a drug that solely targets ECM-detached ovarian cancer cells found in stage III and stage IV ovarian cancer patients.

Recent evidence has identified single nucleotide polymorphisms (SNP) in catalase associated with increased likelihood of death from ovarian cancer. This underscores the importance of further investigation of this mechanism and utility for a therapeutic [19]. Additional studies examining the relationship between this SNP in catalase and survival in ECM-detached cancer cells are warranted to better understand and develop personalized novel therapeutic strategies.

Collectively, it is plausible that the reduction of catalase could be an attractive chemotherapeutic target strategy for specific elimination of ECM-detached metastatic epithelial ovarian cancer cells. The low five-year survival rate displayed by stage III and stage IV patients presents a formidable challenge to develop improved and affordable therapies to treat the majority of patients that are diagnosed with epithelial ovarian cancer at these stages [1, 3]. Future experiments aimed at assessing the efficacy of targeting catalase will be critical to the development of therapeutics that are designed to eliminate stage III and stage IV epithelial ovarian cancer.

5. CONCLUSION

We discovered that catalase deficiency abrogates growth of ECM-detached SKOV3 ovarian cancer cells. Specifically, colony formation is decreased in soft agar as a result of decreased cell viability in SKOV3 catalase-deficient cells. Re-expression of catalase rescued colony formation in soft agar. Catalase deficiency in ECM-attached cells show no change in cell viability or proliferation. Thus, catalase is an attractive target for ECM-detached metastatic ovarian cancer cells and should be further explored.

ACKNOWLEDGMENTS

This project was funded through the Maryjeanne Burke SISTAR Grant for pre-tenure faculty through Saint Mary's College awarded to C.A.D.V. and C.L.L. We would like to thank Dr. Zachary Schafer and the entire Schafer Lab for allowing the use of their lab space while our space was under construction, and Stanley Terlecky (Wayne State University, Detroit, MI, USA) for the gift of the catalase plasmids. The authors declare no conflicts of interest.

REFERENCES

1. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018. *CA Cancer J Clin* 2018; 68(4):284–96. doi: 10.3322/caac.21456.
2. Joo WD, Kim JH, Lee SH, Kim DY, Suh DS, Kim YM, et al. The relationship between expression of 1-cys peroxiredoxin and resistance to cisplatin in epithelial ovarian cancer cell lines. *Korean J Obstet Gynecol* 2006; 49(10):2137–47.
3. Siegel RL, Miller KD, Jemal A. cancer statistics, 2018. *CA Cancer J Clin* 2018; 68(1):7–30. doi: 10.3322/caac.21442.
4. Langyel E. Ovarian cancer development and metastasis. *Am J Pathol* 2010; 177(3):1053–64. doi: 10.2353/ajpath.2010.100105.
5. Cai Q, Yan L, Xu Y. Anoikis resistance is a critical feature of highly aggressive ovarian cancer cells. *Oncogene* 2015; 34(25):3315–24. doi: 10.1038/onc.2014.264.
6. Davison CA, Durbin SM, Thau MR, Zellmer VR, Chapman SE, Diener J, et al. Antioxidant enzymes mediate survival of breast cancer cells deprived of extracellular matrix. *Cancer Res* 2013; 73(12):3704–15. doi: 10.1158/0008-5472.can-12-2482.
7. Glorieux C, Calderon PB. Catalase, a remarkable enzyme: targeting the oldest antioxidant enzyme to find a new cancer treatment approach. *Biol Chem* 2017; 398(10):1095–108. doi: 10.1515/hsz-2017-0131.
8. Buurma V, Freeman B, Adzenyah A, Pettit G, Heinen F, Muhlbauer J, et al. Peroxiredoxin induction in breast cancer. *Cancer Res* 2015; 75. doi: 10.1158/1538-7445.am2015-1245.
9. Goncalves K, Sullivan K, Phelan S. Differential

expression and function of peroxiredoxin 1 and peroxiredoxin 6 in cancerous MCF-7 and noncancerous MCF-10A breast epithelial cells. *Cancer Investigation* 2012; 30(1):38–47. doi: 10.3109/07357907.2011.629382.

10. Hoskins E, Hood B, Sun M, Krivak T, Conrads T, Edwards R. Detection of the tissue-derived biomarker peroxiredoxin 1 in serum of patients with ovarian cancer: a biomarker feasibility study. *Gynecol Oncol* 2011; 121(1):S47–S. doi: 10.1016/j.ygyno.2010.12.114.
11. Hoskins ER, Hood BL, Sun M, Krivak TC, Edwards RP, Conrads TP. Proteomic analysis of ovarian cancer proximal fluids: validation of elevated peroxiredoxin 1 in patient peripheral circulation. *Plos One* 2011; 6(9). doi: 10.1371/journal.pone.0025056.
12. Kalinina E, Chernov N, Berezov T, Shtil A, Glasunova V, Sukhanov V. Role of thioredoxin, thioredoxin reductase and peroxiredoxin in redox-dependent mechanism of development of multidrug resistance in cancer cells. *FEBS J* 2013; 280:214.
13. Kalinina EV, Berezov TT, Shtil AA, Chernov NN, Glazunova VA, Novichkova MD, et al. Expression of peroxiredoxin 1, 2, 3, and 6 genes in cancer cells during drug resistance formation. *Bull Exp Biol Med* 2012; 153(6):879–82. doi: 10.1007/s10517-012-1849-7.
14. McDonald C, Muhlbauer J, Perlmutter G, Taparra K, Phelan SA. Peroxiredoxin proteins protect MCF-7 breast cancer cells from doxorubicin-induced toxicity. *Int J Oncol* 2014; 45(1):219–26. doi: 10.3892/ijo.2014.2398.
15. Muhlbauer J, Perlmutter G, McDonald C, Cintineo H, Aiello C, Phelan SA. Elevated peroxiredoxin expression in breast cancer and its protective role in doxorubicin-resistance. *Cancer Res* 2014; 74(19). doi: 10.1158/1538-7445.am2014-2262.
16. Pak JH, Choi WH, Lee HM, Joo W-D, Kim J-H, Kim Y-T, et al. Peroxiredoxin 6 overexpression attenuates cisplatin-induced apoptosis in human ovarian cancer cells. *Cancer Investigation* 2011; 29(1):21–8. doi: 10.3109/07357907.2010.535056.
17. Ren P, Ye H, Dai L, Liu M, Liu X, Chai Y, et al. Peroxiredoxin 1 is a tumor-associated antigen in esophageal squamous cell carcinoma. *Oncol Rep* 2013; 30(5):2297–303. doi: 10.3892/or.2013.2714.
18. Li S, Mao Y, Zhou T, Luo C, Xie J, Qi W, et al. Manganese superoxide dismutase mediates anoikis resistance and tumor metastasis in nasopharyngeal carcinoma. *Oncotarget* 2016; 7(22):32408–20. doi: 10.18632/oncotarget.8717.
19. Belotte J, Fletcher NM, Saed MG, Abusamaan MS, Dyson G, Diamond MP, et al. A Single nucleotide polymorphism in catalase is strongly associated with ovarian cancer survival. *Plos One* 2015; 10(8). doi: 10.1371/journal.pone.0135739.