Electronic Journal of General Medicine
2025, 22(6), em695
e-ISSN: 2516-3507
https://www.ejgm.co.uk/

MODESTUM

Original Article

OPEN ACCESS

Exploring P62/SQSTM1 and Nrf2 as predictors of chemoresistance in ovarian cancer: An *in vitro* and clinical analysis

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Citation: Alsamman K, Owaidah AY, Azam F, Mashhour M, Alwosaibai K, Aldossary GA, Althagafi MH, Ghazal NA, El-Masry OS. Exploring P62/SQSTM1 and Nrf2 as predictors of chemoresistance in ovarian cancer: An *in vitro* and clinical analysis. Electron J Gen Med. 2025;22(6):em695. https://doi.org/10.29333/ejgm/17252

ARTICLE INFO

Received: 09 Apr. 2025

Accepted: 21 Sep. 2025

ABSTRACT

Ovarian cancer (OC) remains a major cause of female cancer mortality, with chemoresistance impeding effective treatment. This study investigated the expression of p62/SQSTM1 and Nrf2 in chemoresistant versus chemosensitive OC cell lines and patient tissues. Using quantitative reverse transcription PCR, Western blotting, and immunohistochemistry, we found co-upregulation of p62 and Nrf2 proteins in chemoresistant samples. Proliferation assays revealed that higher p62 expression correlated with reduced cisplatin sensitivity. Sanger sequencing detected synonymous p62 variants in exon 6, with no functional impact. Immunohistochemical scoring showed significantly higher p62 and Nrf2 levels in chemoresistant patients compared to remission and sensitive groups. A strong correlation was observed between p62 expression and cisplatin IC50 values. These findings support the role of the p62/Nrf2 axis in OC chemoresistance and highlight their potential as predictive biomarkers and therapeutic targets. Future studies are warranted to explore targeted interventions that disrupt this pathway to enhance treatment response in OC.

Keywords: p62, Nrf2, ovarian cancer, chemoresistance, autophagy

INTRODUCTION

Ovarian cancer (OC) ranks as the seventh most prevalent cancer among women worldwide and remains the most lethal gynecological malignancy globally. Although breast cancer is more common, OC is three times more lethal, accounting for approximately 4.3% of all cancer-related deaths among women [1]. Recent global cancer statistics indicate that the incidence of OC varies significantly across regions, with higher rates reported in developed countries such as the USA and Europe compared to lower-income countries in Africa and Southeast Asia [2, 3]. In Saudi Arabia, the incidence of OC has increased by 5% over a seven-year period, reflecting a concerning upward trend [4].

OC is a highly heterogeneous disease due to the complex anatomical nature of the ovary. While epithelial ovarian tumors constitute the majority of OC cases, other types such as germ cell and sex cord-stromal tumors also contribute to the disease burden. Epithelial tumors are further classified into subtypes such as serous, mucinous, endometrioid, and clear cell tumors, with serous carcinomas being the most prevalent. Although mucinous tumors represent only 3% of epithelial OC cases, their distinct molecular characteristics have garnered significant research interest [5, 6]. Recent studies propose dual classifications of epithelial OC based on tumor differentiation: low-grade (type I) and high-grade (type II) tumors, which differ in genetic mutations and clinical outcomes [7]. For brevity, the detailed subtypes are referenced rather than described in full.

Despite advances in diagnostics and therapeutics, chemoresistance remains a major obstacle in the effective treatment of OC, particularly in cases treated with platinum-based chemotherapy such as cisplatin. Chemoresistant OC patients exhibit significantly worse prognosis compared to those with chemosensitive tumors. For this study, patients were categorized into three groups based on their response to chemotherapy: sensitivity, remission, and resistance. “Sensitive” refers to patients whose tumors respond completely to chemotherapy, “remission” denotes those with partial responses or stable disease, and “resistant” indicates tumors that exhibit progressive disease despite treatment [8, 9].

Among the molecular mechanisms underlying chemoresistance, the crosstalk between p62/SQSTM1 and erythroid 2-related factor 2 (Nrf2) has emerged as a pivotal player. p62 is a multifunctional protein involved in autophagy, oxidative stress response, and signal transduction. It has been reported to mediate oncogenic signaling pathways, including the activation of nuclear factor Nrf2, a key regulator of cellular defense mechanisms against oxidative stress [8, 9]. Accumulating evidence suggests that the dysregulation of the p62-Nrf2 axis promotes tumor survival and drug resistance by

enhancing cellular antioxidant capacity and metabolic adaptability [10]. In line with this, a recent study reported that mTOR mediates elevated expression of p62 and activates p62-Nrf2 pathway in response to chemotherapy promoting chemoresistance and survival of OC [11]. In addition, another recent study reported the potential therapeutic efficacy of Nrf2 and p62 downregulation and modulation of autophagy in OC [12]. Likewise, it was recently reported that modulation of oxidative stress via Nrf2-FTH1 signaling improved chemosensitivity in OC. Moreover, the authors reported that abnormal gene expression profile of Nrf2 and FTH1 has a significant prognostic use [13].

This study aims to investigate the expression profiles of p62 and Nrf2 in chemoresistant and chemosensitive OC cell lines and clinical samples, with a specific focus on their correlation with cisplatin sensitivity. By exploring the potential of these proteins as biomarkers and therapeutic targets, we hope to contribute to the development of novel strategies for overcoming chemoresistance in OC patients.

PATIENTS AND METHODS

Study Design and Ethical Approval

This is a cross-sectional, laboratory-based study conducted after obtaining ethical approval from the Institutional Review Board at King Fahd Specialist Hospital, Dammam, Saudi Arabia (ONC0331). Formalin-fixed, paraffin-embedded (FFPE) tissue samples from OC patients and seven OC cell lines were used. Patients were categorized as sensitive, remission, or resistant based on their response to chemotherapy, as previously defined.

Cell Lines and Culture Conditions

Seven OC cell lines (PEO1, PEO4, PEO6, PEO1R, OVCAR3, OVCAR4, and SKOV3) were obtained from Prof. Yusuf Deeni (department of microbiology and biotechnology, University of Abertay, UK). Cell culture conditions were standardized using RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics (penicillin/streptomycin). Cells were maintained at 37 °C in a 5% CO_2 humid atmosphere. Sub-culturing was performed at 80-90% confluence [8, 14].

RNA Extraction and Quantitative Reverse Transcription PCR

Total RNA was extracted using the Qiagen RNeasy Mini Kit (cat. #74104) following the manufacturer's protocol. Quantitative reverse transcription PCR (qRT-PCR) was conducted to quantify p62 and Nrf2 expression relative to GAPDH using specific primer pairs listed in **Table A1** in **Appendix A**. Gene expression was calculated using the 2-ΔΔCT method as previously described [15].

Western Blotting Analysis

Proteins were extracted from OC cells PEO1, PEO4, PEO6, PEO1R, OVCAR3, OVCAR4 and SKOV3 using RIPA lysis buffer (cat. # 89900, Thermo-Fisher, USA) supplemented with protease and phosphatase inhibitors (cat. # 78420 and 78430, respectively, Thermo Fisher, USA). Protein concentrations were quantified using a BCA kit (cat. # 23225, Thermo-Fisher, USA). Western blotting was performed to analyze p62 and Nrf2 expressions, normalized to GAPDH, as described previously [8, 16]. Antibodies used are listed in **Table A2** in **Appendix A**.

Cell Proliferation and Cisplatin Sensitivity Assay

Cell proliferation was assessed using the CCK-8 assay (cat. # CCK-M-002-1000, Molequle-On, Auckland, New Zealand). Cells were treated with cisplatin (25-300 μM) for 48 hours, and IC50 values were calculated from dose-response curves generated using Excel regression analysis. This method has been validated in prior studies [8, 14].

Sanger Sequencing of p62 Exons

Exons 2-8 of p62 were amplified using the list of primers in **Table A3** in **Appendix A** (exon 1 was not sequenced due to technical problems). PCR products were then purified according to the instructions of the QIAquick PCR Purification Kit (cat. # 28104, Qiagen, Germany). Another PCR reaction was then performed according to the well-established Sanger's principle, the chain termination approach, followed by analysis of the amplified fragments by capillary electrophoresis. The sequence results were then presented as chromatograms in which each peak represents a nucleotide in the target DNA (p62 exons in this case).

Immunohistochemical Staining of p62 and Nrf2

FFPE tissue sections were stained with primary antibodies against p62 and Nrf2 (**Table A2** in **Appendix A**) using a DAB substrate kit (cat. #SK4105, Vectorlabs, USA). The antigen retrieval process, blocking, and staining protocols followed standard procedures that were modified from the protocol described by Victor Chi and colleagues [17]. IHC scoring was performed by two independent pathologists based on intensity and coverage area. All antibodies used are listed in **Table A2** in **Appendix A**.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 9.0 or Microsoft Office Excel spreadsheets data analysis tool. Cell proliferation data were presented as mean ± SEM, and statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparisons test. IC50 was calculated from the regression line equation of dose response curves using Microsoft Excel spreadsheets. A regression analysis was performed in Excel spreadsheets to estimate the correlation between cisplatin IC50s and RNA and protein expression levels of p62 and Nrf2. p value > 0.05 was considered statistically significant.

RESULTS

Expression Level of p62 and Nrf2 in OC Cell Lines

qRT-PCR results showed that the levels of p62 were highest in OVCAR3 and 4, PEO1R and SKOV3, in order. PEO4 and PEO6 cells have a similar level of p62, whilst the level of expression in PEO1 was slightly higher than that in PEO6. Nrf2 expression pattern was almost similar in PEO6, PEO1 and PEO1R. The expression level in PEO4 was slightly higher than PEO6. In addition, the level in OVCAR3 and 4 was lower than that in PEO6 (**Figure 1**), which was unlike the pattern of expression observed for p62 and this will be discussed later in the discussion section.

Quantitative PCR results showed overexpression of p62 in chemoresistant OC cells when PEO6 was used as the control cell line. Highest levels of p62 expression were observed in

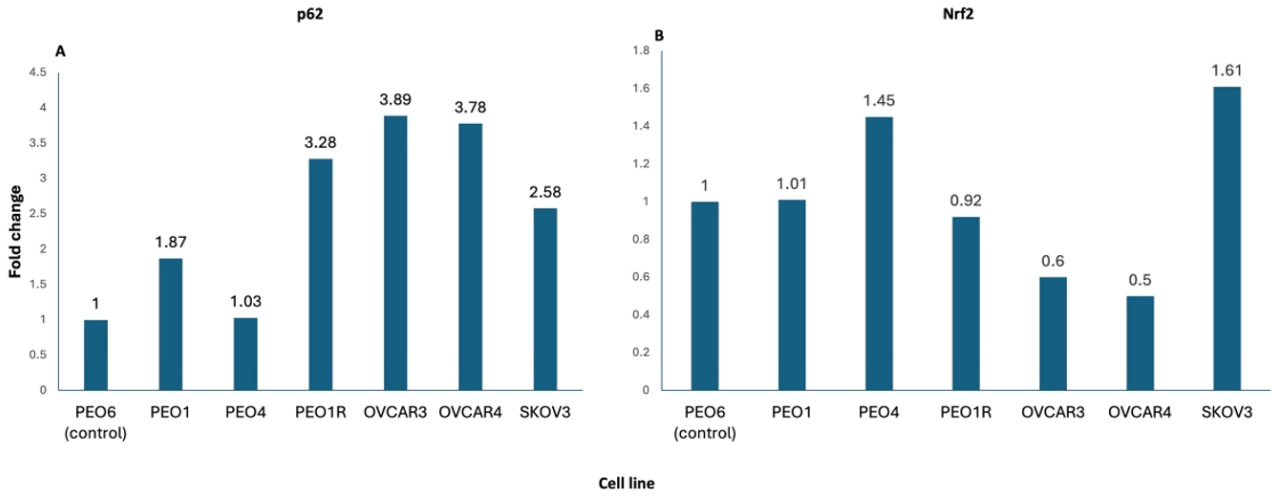

Figure 1. Gene expression profile of p62 and Nrf2 in OC cell lines (Source: Authors' own elaboration)

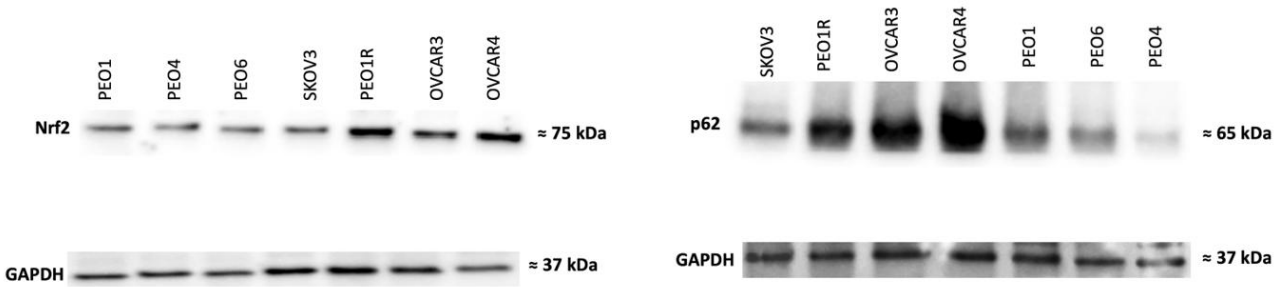

Figure 2. Western blotting of p62 and Nrf2 in OC cell lines (Source: Authors' own elaboration)

OVCAR3 and 4, PEO1R and SKOV3, in order (part A in **Figure 1**). Results of Nrf2 gene expression showed higher levels in SKOV3 and PEO4 than the control cell line, PEO6 (part B in **Figure 1**).

p62 and Nrf2 Protein Expression Level in OC Cell Lines

Western blotting showed higher levels of p62 in OVCAR3 and 4, PEO1R and SKOV3 cells than in PEO1, PEO4 and PEO6. The results also revealed a similar profile of high Nrf2 levels in OVCAR3 and 4 and PEO1R; however, SKOV3 cells expressed lower Nrf2 protein levels like PEO1, PEO4 and PEO6. GAPDH protein level was consistent in all cell types as shown in the representative plot in **Figure 2**.

Results showed overexpression of p62 in PEO1R, OVCAR3 and OVCAR4 cells in comparison to other cell types. Similarly, Nrf2 level was higher in the same cell lines when compared to other cell types. GAPDH was used as a loading control and its level was similar in all cell lines.

Cell Proliferation Assay Results

OC cell lines (PEO1, PEO4, PEO6, PEO1R, OVCAR3, OVCAR4, and SKOV3) with different levels of p62 were incubated with cisplatin (25-300 µM) for 48 hours. The results showed that proliferation of cells expressing lower levels of p62 (PEO1, PEO4, PEO6, and SKOV3) was significantly inhibited at low concentration of cisplatin ($p < 0.001$). On the other hand, cells expressing higher levels of p62 (OVCAR3, OVCAR4, and PEO1R) showed a higher proliferation rates at low concentration of cisplatin, although the inhibition of cell proliferation was also significant at different concentrations of the drug ($p < 0.001$) (**Figure 3**).

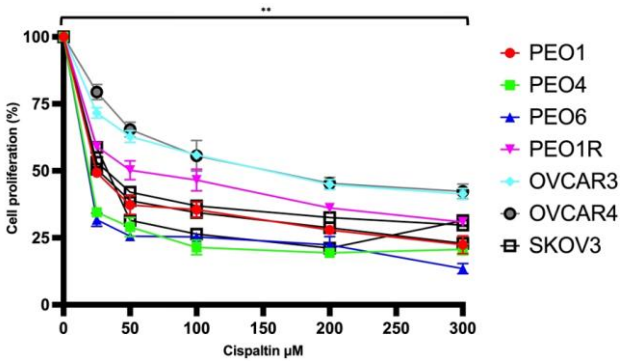

Figure 3. The anti-proliferative effect of cisplatin on OC cell lines with different chemosensitivity profile (Source: Authors' own elaboration)

We have further estimated the IC50 of cisplatin in OC cell lines from the regression line equation of cell proliferation curves (**Figure 4**). IC50s in different OC cell lines are presented in **Table A4** in **Appendix A** showing that cells expressing higher levels of p62 and Nrf2 (OVCAR3, OVCAR 4, and PEO1R) have higher IC50s than other cells with lower protein levels of p62 and Nrf2 (PEO1, PEO4, PEO6, and SKOV3).

Figure 3 shows that increasing doses of cisplatin have significantly reduced proliferation of OC cells. Although this influence was significant for all cell types ($p < 0.001$), OVCAR3 and 4 and PEO1R cells that express higher levels of p62 and Nrf2 exhibited a more resistive pattern than other cell lines. Results are presented as mean ± SEM of 4 independent experiments (** = [$p < 0.001$]).

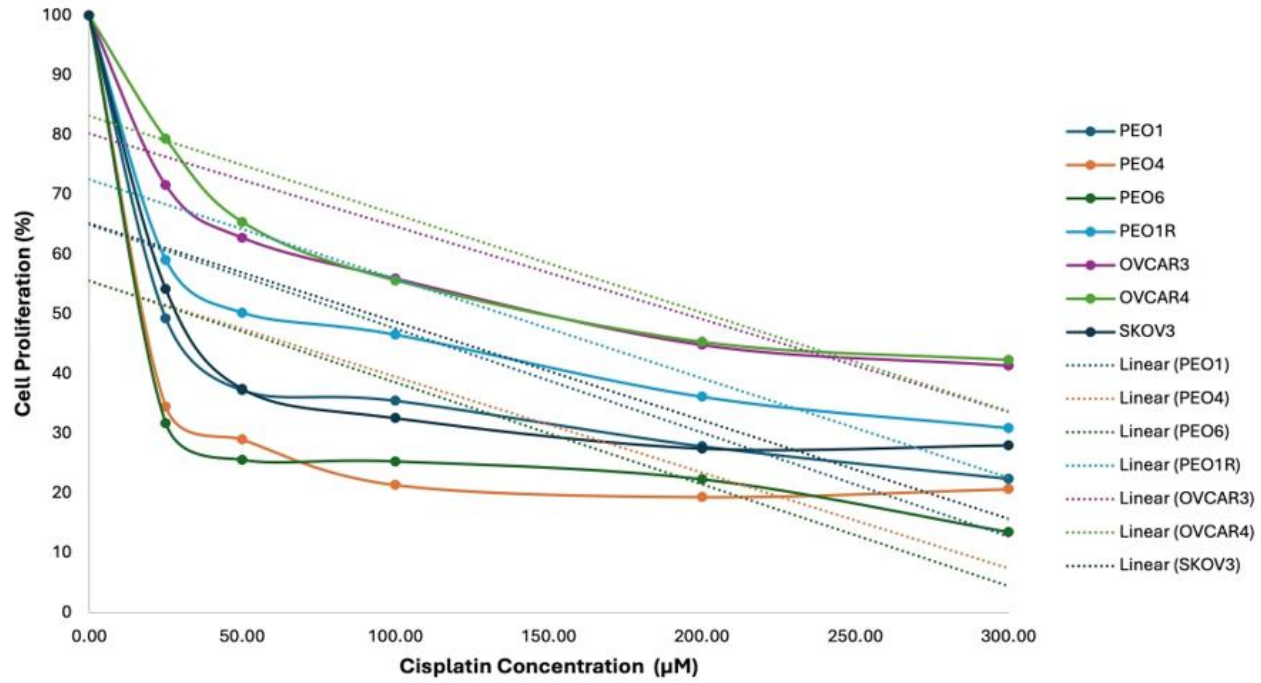

Figure 4. Linear regression graph used for calculation of IC50s of cisplatin in OC cells (Source: Authors' own elaboration)

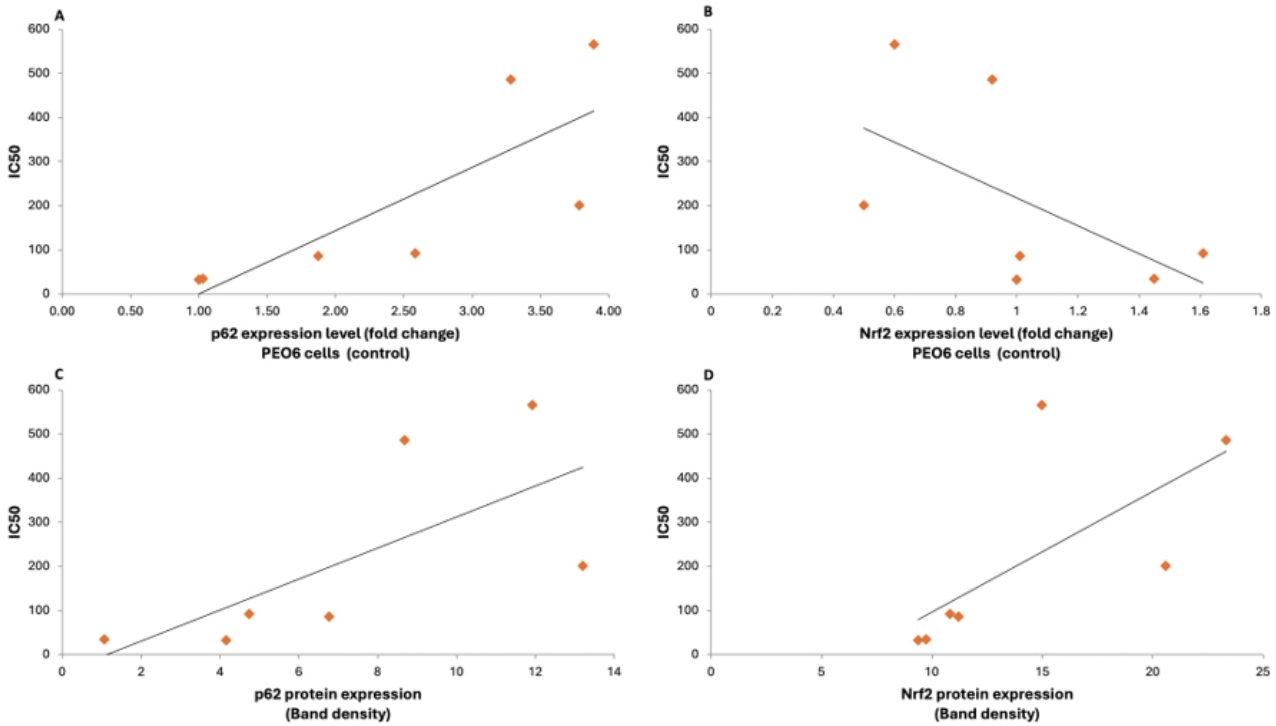

Figure 5. Regression plots showing the correlation relationship between IC50s and p62 and Nrf2 RNA and protein expression (Source: Authors' own elaboration)

The linear trendlines for dose response curves were plotted and the IC50s of cisplatin were calculated using the trendline equation $Y = mX + C$. The IC50 of cisplatin is the X value when Y = 50%.

Correlation Between p62 and Nrf2 Expression and Cisplatin IC50s

A regression analysis was also performed to estimate the predicted values of cisplatin IC50s from RNA and protein expression levels of p62 and Nrf2 in OC cells. The values of correlation coefficient and regression plots are presented in **Figure 5** and **Table 1** showing a strong, positive correlation between p62 RNA expression and IC50s (R = 0.79), which was statistically significant (p = 0.03). However, the correlation was weaker for Nrf2 RNA expression. Likewise, the correlation was quite strong, positive between protein expression of p62 and Nrf2 (band densities of Western blotting were estimated using Image J software) and IC50s; however, this relationship was not statistically significant (**Table 1**).

Table 1. Correlation coefficient and significance level of the regression relationship between p62 and Nrf2 expression and IC50s of cisplatin in OC cells

Cell line	IC50	p62 expressionlevel (fold change)	Nrf2 expression level (fold change)	p62 protein expression (band density)	Nrf2 protein expression (band density)
PEO1	85.96	1.87	1.01	6.77	11.21
PEO4	34.48	1.03	1.45	1.07	9.73
PE06 control	32.83	1	1	4.16	9.37
PE01R	485.9	3.28	0.92	8.68	23.32
OVCR3	565.8	3.89	0.6	11.92	14.97
OVCR4	201.4	3.78	0.5	13.2	20.58
SKOV3	91.95	2.58	1.61	4.74	10.82
R		0.79	0.58	0.69	0.69
p-value		0.03*	0.172	0.08	0.08

Note. R: Correlation cofficient & *Statistically significant

Table 2. Clinical characteristics of patients

PN	General cluster	Subclass 1	Subclass 2	Result of chemotherapy
Resistance group				
1	Epithelial	Not specified	Adenocarcinoma	Resistant to gem/carbo
2		Serous		Resistant to gem/carbo and died
3				Resistant to gem/carbo and died
4				Resistant to gem/carbo and died
5				Resistant to gem/carbo
6				Resistant to taxol/carbo and died after 5 months
7			Not specified	Resistant to new chemo
8			Not specified	Resistant to gem/carbo
Remission group				
1	Non-epithelial	Germ line	Dysgerminoma	Complete remission (taxol-carbo)
2	Epithelial	Serous	Adenocarcinoma	
3		Endometroid		
4	Non-epithelial	Germ line	Sertoli lydeg	
5			dysgerminoma	
6			Yolk sac	
Sensitive group				
1	Epithelial	Endometroid	Adenocarcinoma	Sensitive/new treatment (taxol-carbo/gem-carbo)
2	Not specified	Not specified	Not specified	Sensitive same chemo (taxol/taxol-carbo)
3	Non-epithelial	Germ line	Teratoma	Sensitive new chemo (taxol/taxol-carbo)
4	Epithelial	Serous	Adenocarcinoma	Sensitive same chemo (taxol/taxol-carbo) died
5				Sensitive same chemo (taxol/taxol-carbo) died
6				Sensitive same chemo (taxol/carbo)
7		Endometroid		Sensitive same chemo (taxol/carbo)
8		Serous		Sensitive same chemo (taxol/carbo)

Note. PN: Patient number; Gem/Carbo: Gemcitabine and carboplatin; & Taxol/carbo: Paclitaxel and carboplatin

Plot A and plot B in **Figure 5** show the relationship between RNA expression level of p62 and Nrf2, respectively and IC50 of cisplatin in OC cells. Plot C and plot D explain the relationship of p62 and Nrf2 protein expression with IC50 of cisplatin. The plots show a moderately strong correlation between expression level of p62 and Nrf2 and IC50s in OC cells except for the RNA expression level of Nrf2, which exhibited a weaker correlation with IC50.

Sanger Sequencing of p62 in OC Cell Lines

The results of Sanger's sequencing of p62 exons are summarized in **Table A5** in **Appendix A**. There were no variants reported in exons 2, 3, 4, 5, 7, and 8, while exon 1 was not done due to technical issues in amplification. Similar variants were found between exon 5 and exon 6, exon 6 and between exon 7 and exon 8 in PEO1, PEO1R, PEO4, and PEO6 cells, while no changes were found in p62 gene in OVCAR3 and 4 and SKOV3 cells. In exon 6, two synonymous variants were detected and were previously reported with references to rs4935 and rs4797 with no amino acid change in the protein sequence (SQSTM1 (NM_003900.4; genomic position in base pairs according to hg19 (GRCh37)). The clinical significance of these variants in the coding sequence of p62 is benign (data was verified at https://www.ncbi.nlm.nih.gov/snp).

Patients' Characteristics and Immunohistochemical Staining Scores of p62 and Nrf2 in Patients' Specimens

Patients' clinical characteristics

The clinical characteristics of the study population included OC main class, subtypes of OC, treatment history and outcome of the treatment are outlined in **Table 2**. Percentages of OC classes and subclasses are summarized in **Figure B1** in **Appendix B**. According to the outcome of treatment, patients were divided into 3 groups: sensitive, remission, and resistant. Treatment regimens were gemcitabine carboplatin (gem/carbo), paclitaxel and carboplatin (taxol/carbo), taxol/carbo then shifting to gem/carbo, or taxol then shifting to taxol/carbo. The patients in the remission group who achieved complete remission were treated primarily with taxol/carbo. Those patients in the sensitive group were either sensitive to the same treatment (taxol/taxol-carbo) or were sensitive to a new treatment (taxol-carbo/gem-carbo). In the resistant

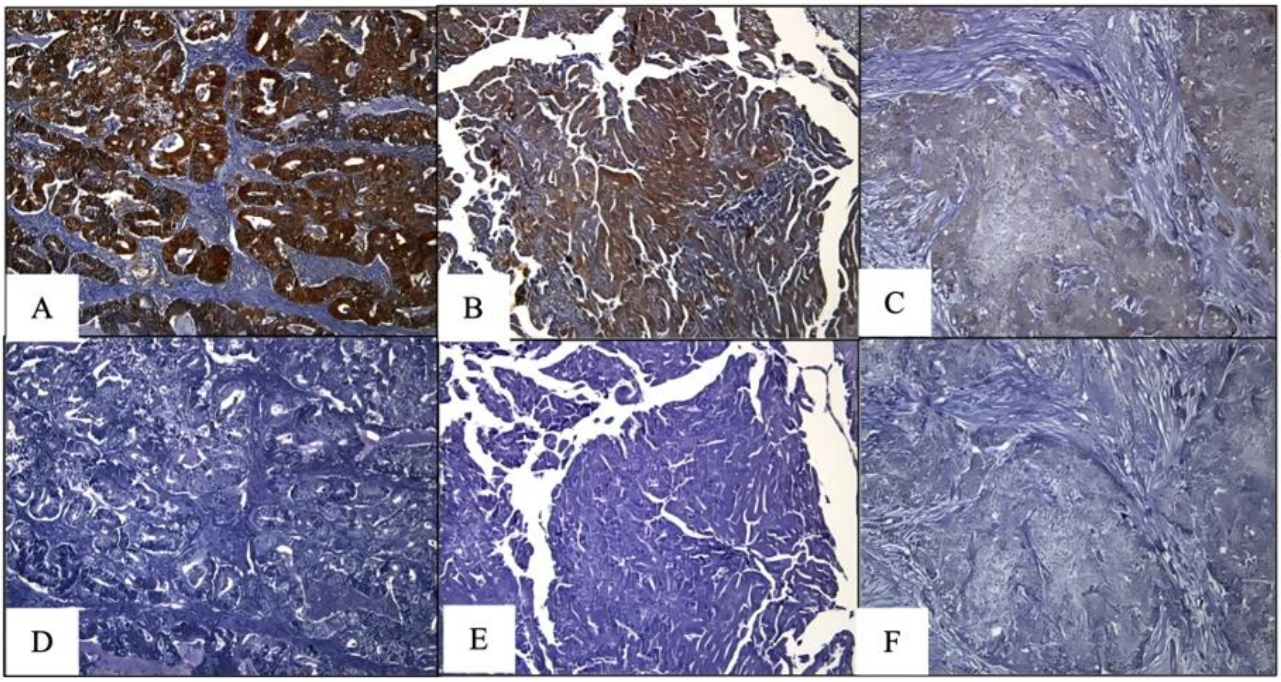

Figure 6. Representative immunohistochemical staining intensity of p62 and Nrf2 in OC patient tissue samples (Source: Authors' own elaboration)

group, 50% out of 8 patients died out, 3 of them were resistant to gem/carbo and 1 was resistant to taxol/carbo.

Immunohistochemical scoring of p62 and Nrf2

Figure 6 depicts immunohistochemical staining and scoring of p62 and Nrf2 in patients' sections, **Figure 6** is representative for the intensity scores of p62 and Nrf2 in all study groups. Dense brownish color indicates a positivity score of +3 with 85% area of coverage, moderate brownish staining indicates a +2 score with 50% area of coverage, and a mild brownish color indicates a weak positivity of +1 and 50% area of coverage. Brownish staining is almost absent in the control sections.

Comparison between the intensity scores of p62 and Nrf2 between patients' groups indicated a significant difference between the groups with higher score sums recorded in the resistant group than the remission and sensitive groups ($p = 0.0002$). There was no statistical difference between the scores of p62 and Nrf2 in all groups suggesting that the pattern of their expression is similar in patients, increasing together and decreasing together at protein level (**Figure B2** in **Appendix B**).

Panels A-C show positive staining intensities of p62 and Nrf2, with dense brownish staining representing a +3 score and 85% coverage (A), moderate staining corresponding to a +2 score and 50% coverage (B), and mild staining as a +1 score with 50% coverage (C). Panels D-F show control sections with negligible or no staining. **Figure B2** in **Appendix B** highlights the variations in staining intensity among study groups and is representative of the intensity scoring across the patient samples.

DISCUSSION

OC remains a significant clinical challenge due to the high rates of chemoresistance, particularly in cases treated with platinum-based chemotherapy. Our study investigated the role of p62 and Nrf2 in chemoresistance, with a focus on their expression profiles in chemoresistant and chemosensitive OC cell lines and patients' samples. The use of OC cell lines in conjunction with patients' samples was to investigate cisplatin IC50s in chemosensitive (PEO1, PEO4, and PEO6) and chemoresistance (PEO1R, OVCAR3, OVCAR4, and SKOV3) OC cells, and to assess the correlation between IC50s and p62 and Nrf2 co-expression profile. Whilst pathological samples were used to confirm the profile of p62 and Nrf2 co-expression in chemoresistant patients who were treated with platinum-based drugs in comparison to remission and sensitive groups. Here, we confirm our findings within the broader scientific literature and explore the implications of the p62/Nrf2 axis in chemoresistance and oxidative stress. The results showed a differential expression of p62 and Nrf2 in OC cells (**Figure 1** and **Figure 2**), with higher protein levels of both proteins observed in cells with resistant phenotype. These resistant cells showed higher IC50s of cisplatin (**Figure 3** and **Figure 4**). Moreover, p62 and Nrf2 expressions were significantly higher in chemoresistant OC patients (**Figure 6** and **Figure B2** in **Appendix B**). In this context, positive correlation was found between p62 and Nrf2 expression and IC50s, except for Nrf2 RNA expression, where correlation was moderately positive as presented in **Table 1** and **Figure 5**.

In concordance with the findings of this study, p62 was reported as a potential prognostic marker for OC and that p62-dependent autophagy could be a possible therapeutic target for metastasis prevention and reversion of drug resistance [18]. However, unlike our findings, the authors reported lower expression of p62 in multidrug resistant cell lines than their sensitive parental cells [18]. Additionally, in contrast to the results of our study, p62 was found to mediate overexpression of caspase-8, which provides pro-apoptotic effect in OC cell lines, SKOV3 and A2780 [19]. However, the authors have also reported that p62 could exert pro-survival or pro-apoptotic actions depending on its downstream effectors [19]. In this study, we showed that p62 and Nrf2 are co-upregulated at

protein level in OC chemoresistant cells as well as tissues of chemoresistant OC patients. Upregulation of Nrf2 is directly associated with chemoresistance by helping cancer cells to overcome oxidative insult that is induced by platinum-based drugs [20]. Our findings of elevated p62 levels in chemoresistant OC cells align with the study in [21], which demonstrated that p62 interacts with keap1, leading to the activation of Nrf2 and subsequent upregulation of antioxidant defenses. This mechanism mitigates oxidative damage and enhances cellular survival under stress conditions, which may contribute to the development of chemoresistance. These observations underscore the critical role of the p62-keap1-Nrf2 axis in protecting cancer cells from chemotherapy-induced oxidative stress, supporting the hypothesis that p62-mediated activation of Nrf2 promotes tumor resilience [21]. In the context of linking autophagy, p62 and Nrf2 antioxidant activity in OC, it was reported that the overexpression of the autophagy-related enzyme UBE2E2 in OC tissues in comparison to normal tissues. Overexpression of UBE2E2 was found to be associated with accumulation of p62 and activation of Nrf2 antioxidant response element (ARE) leading to elevation of snail by rescuing it from ubiquitin-mediated degradation. Snail promotes epithelial mesenchymal transition, which enhances metastasis and invasiveness of OC [22]. In line with this, it was recently reviewed that activation of autophagy, which is promoted by p62, inhibits apoptosis due to degradation of misfolded proteins and elimination of damaged organelles confirming important involvement of p62 in carcinogenesis and chemotherapeutic resistance [23]. Therefore, inhibition of autophagy was proposed as a mechanism of ursolic acid against OC, which was indicated by elevated levels of p62 and lowered levels of beclin1 and LC3 [24].

In this context, it was reported that p62 binds to keap-1 and protects Nrf2 from degradation resulting in the presence of high levels of Nrf2 in cancer cells, which promotes p62-mediated chemoresistance [25]. Additionally, it was recently reported that CEBPB promotes OC progression by upregulating Nrf2, thereby enhancing the antioxidant defenses in OC cells as demonstrated in SKOV3 and A2780 OC cell lines [26]. The therapeutic potential of modulating the keap1-Nrf2 pathway is highlighted by [27], which showed that enhancing Nrf2 degradation through keap1-mediated ubiquitination increased oxidative stress and inhibited ovarian tumor growth. This study provides compelling evidence that disrupting the keap1-Nrf2 interaction can effectively counteract the antioxidant defenses of cancer cells, leading to tumor suppression. Incorporating such strategies in combination with existing chemotherapeutic regimens could offer a novel approach to overcoming chemoresistance in OC [27]. In support of this notion, a recent study demonstrated that inhibition of PERK/Nrf2 pathway through suppression of PERK/Nrf2 phosphorylation helped to sensitize cisplatin-resistant A2780 OC cells and triggered cell death [28].

Our data also aligned with [29], which demonstrated that p62/SQSTM1 contributes to cisplatin resistance in OC cells by interacting with keap1 and stabilizing Nrf2. The study reported that this interaction activates AREs, enhancing the cells' ability to counteract oxidative damage induced by cisplatin. These findings further substantiate the role of the p62-keap1-Nrf2 pathway as a central player in chemoresistance and highlight its potential as a therapeutic target. Notably, our study also observed a lack of correlation between Nrf2 RNA expression and IC50 values, suggesting that the chemoresistance conferred by Nrf2 may primarily occur at the post-transcriptional level, consistent with the observations of [29]. The co-upregulation of p62 and Nrf2 was evident at protein level, but not at the RNA level, which was also reported by another team supporting our findings in this study [30].

Having introduced the multifaceted role of p62 in cancer, it was recently reported that knocking out p62 has delayed carcinogenicity in experimental animals [31]. Moreover, an interaction between p53 and p62 was reported and found to sensitize OC cells to cisplatin [32]. This supports the anti-oncogenic properties of p62 and contradicts our observation that p62 is overexpressed along with Nrf2 in chemoresistant OC cells and patients. On the other hand, Yu and colleagues have reported that p62 is overexpressed in cisplatin-resistant SKOV3/DDP OC cells, and knocking down p62 in these cells restored their sensitivity to the anti-proliferative action of cisplatin [33]. This supports the findings of our study and the notion that p62 could be pro-oncogenic in OC. In a similar context and in agreement with our results, co-involvement of p62 and Nrf2 in cancer cell resistance to anti-cancer agents was also reported in a cell line model of glioblastoma and breast cancer in a study that reported upregulation of both proteins together in response to treatment with Zn(II)–curcumin complex [30]. It is also noteworthy that Nrf2 has a multifaceted action in cancer being a mediator of pro-oncogenic as well as anti-oncogenic events; however, the pro-oncogenic actions outweigh the anti-oncogenic influences according to a recent article [34]. This information, taken together indicate that the role of p62/Nrf2 axis in cancer is controversial and should be subject to clinical evaluation of individual cases.

CONCLUSIONS

The findings of the present study and other published studies supported the interaction between p62 and Nrf2 and their co-involvement as partner proteins in different cellular pathways in OC as well as other human cancers. The controversy of p62 and Nrf2 in cancer supported by the multifaceted roles of both proteins in different types of human neoplasms casts a beam of light on the importance of evidence-based medicine and meticulous screening of individual cases for decisive clinical intervention. Our study highlights the critical role of the p62/Nrf2 pathway in mediating chemoresistance in OC. By integrating findings from recent studies, we propose that targeting the p62-keap1-Nrf2 axis holds a significant promise as a therapeutic approach to overcoming chemoresistance. Further investigations into the mechanistic underpinnings of this pathway are warranted to inform the development of targeted therapies.

Author contributions: KA & OSE: conceptualization, funding acquisition, data analysis, writing - original draft; **FA, MM, & KA:** clinical specimens and data; **AYO, GAA, MHA, & NAG:** data curation and analysis, writing – review & editing. All authors have agreed with the results and conclusions.

Funding: This study was supported by the Deanship of Scientific Research at Imam Abdulrahman Bin Faisal University (grant numbers 2016-291-CAMS, 2018-075-CAMS).

Acknowledgments: The authors would like to thank Prof. Yusuf Deeni for providing the cell lines that were used in this study. The authors would also like to thank Dr. Chittibabu Vatte for helping with Sanger's sequencing and Afnan Al-Shuail and Sarah Al-Shungaity for helping with immunohistochemistry staining.

Ethical statement: The authors stated that the study was approved by the Institutional Review Board at King Fahd Specialist Hospital, Dammam, Kingdom of Saudi Arabia on 23 October 2018 with approval number ONC0331. Written informed consents were obtained from the participants.

AI statement: The authors stated that no generative artificial intelligence (AI) or AI-based tools were used in the writing, data analysis, or preparation of this manuscript. All parts of the research, including study design, data collection, analysis, and manuscript drafting, were carried out solely by the authors.

Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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APPENDIX A

Table A1. Primer pairs used for quantification of p62 and Nrf-2 gene expression by q-RT-PCR

Gene	Forward primer	Reverse primer
P62	CGACAGAGGGGGAGGACTTTA	AGTTTCCTGGTGGACCCATTT
Nrf-2	CAGCGACGGAAAGAGTATGA	TGGGCAACCTGGGAGTAG
GAPDH	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC

Table A2. Immunohistochemistry primary, isotype controls, and secondary antibodies

No	Antibody	Source and catalogue number
1	Anti-SQSTM1/p62 mouse monoclonal antibody	Abcam, UK (Ab56416)
2	Mouse IgG2a (b12/8)	Abcam, UK Isotype control (ab91361)
3	Anti-Nrf2 polyclonal rabbit antibody	Abcam, UK (Ab137550)
4	Rabbit IgG polyclonal	Abcam, UK Isotype control (Ab37415)
5	HRP Horse Anti-Mouse IgG	Vectorlabs, USA (MP-7402)
6	HRP Horse Anti-Rabbit IgG	Vectorlabs, USA (MP-7401)

Table A3. Primer pairs used for amplification of p62 exons for Sanger sequencing

Exon	Forward primer	Reverse primer
2	GTCTTGCCTCTCACTCCTGC	CCACACCTGGCCTATGTCTC
3	GGATTCCATGCTGGAGAGCAG	TTCACCTTCCGGAGCCAG
4	ACTTGTGTAGCGTCTGCGAG	TTGTAGGGCACCAGGAAGGT
5	CACAGGGACCTTGGCAAGAA	TGAGGCAACAAATCCTCACCA
6	TCTGTAGTCTCCACAGGCCA	CTGCAGAGGTGCTGAGGATG
7	CCCTGCAGCCTTAACTGCAC	TGTCGCTGAAATCAGAGGAGG
8	CCAAGGCAGCAGGGTATGTG	TGGCTTCTTGCACCCTAACC

Table A4. IC50s of cisplatin in OC cell lines

Cell line	PEO1	PEO4	PEO6	PEO1R	OVCAR3	OVCAR4	SKOV3
IC50 (μM)	85.96	34.48	32.83	485.9	565.8	201.4	91.95

Table A5. Gene variants detected by Sanger's sequencing in p62 in OC cell lines

Cell line	Between exon 5 and exon6	Exon 6		Between exon 6 and exon 7
PEO1	g.179260009 G>A	g.179260153C>T p. D292D (rs4935)	g.179260213G>A p.R312R (rs4797)	g.179260494 G>A
PEO1R				
PEO4				
PEO6				

APPENDIX B

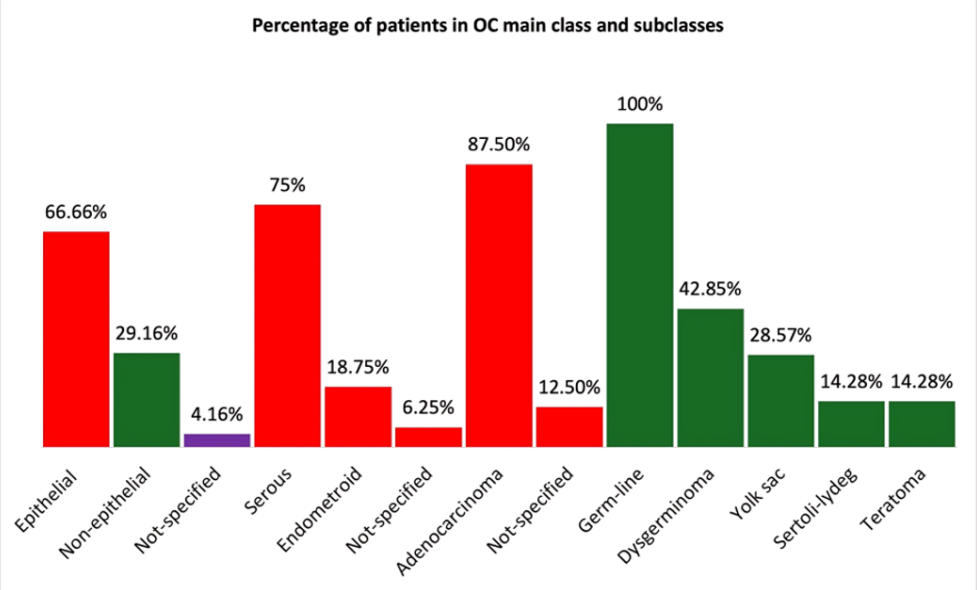

Figure B1. Distribution of patients in OC main class and subclasses (patients' distribution showed that majority of participants had epithelial OC, and most of these patients had serous sub-type and most of them had adenocarcinoma; 100% of non-epithelial OC patients had germ-line cancer with a majority of dysgerminoma subclass; & the values are percentages out of the number of all participants regardless the treatment outcome) (Source: Authors' own elaboration)

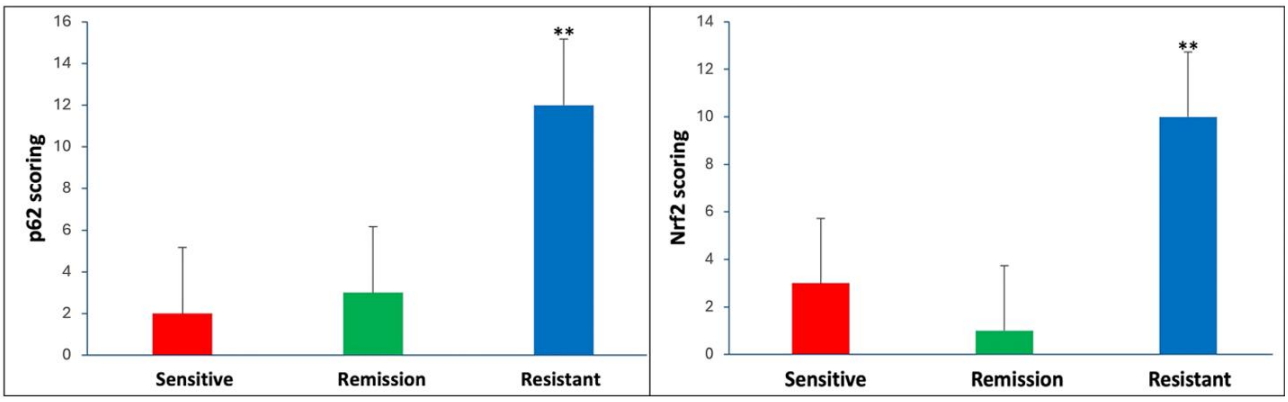

Figure B2. Scoring of p62 and Nrf2 staining in OC patients' specimens (the results showed a significant difference between the sums of staining scores between groups with obviously higher scores in the resistant group; a similar profile was reported for p62 and Nrf2 suggesting a concomitant elevation of both proteins; & **$p < 0.001$) (Source: Authors' own elaboration)