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Selenium and Triple Negative Breast Cancer

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Abstract

Background. The deadliest, most dangerous subtype of breast cancer is triple-negative, which lacks treatment targets and accounts for 30% of all breast cancer-related deaths worldwide. TNBC is characterized by the expression of no estrogen, progesterone, or human epidermal growth factor 2 receptors. This suggests that new treatment modalities with fewer adverse effects are required. **Objective.** The aim of the present study was to investigate the therapeutic potential of selenium compounds as an adjuvant therapy for Triple Negative Breast Cancer (TNBC), either on their own or in conjunction with nutritional supplements and chemotherapy medications. **Methods.** Using the keywords "selenium" and "triple negative breast cancer", a thorough search was conducted in the PubMed database, yielding 23 articles. The following factors were taken into consideration for inclusion: studies using TNBC cell culture lines or in vivo tumors/specimens; full-text articles from the PubMed database; studies published in the English language; experiments with statistically significant results; and selenium used alone or in combination with other antioxidants or chemotherapy. This led to the evaluation of 13 articles in this review. **Results.** The results show that selenium therapy increased the anti-cancer drug's effects and produced tumor cytotoxicity, while reducing the cellular features of the cancer (hyperproliferation, growth, and metastasis). **Discussion.** This study evaluated the various selenium compounds tested, the cell lines and model organisms used, the assays performed, and the cellular pathways affected. **Conclusion.** Examining the possible benefits of selenium in TNBC treatment highlights the need for more studies to confirm selenium compounds as viable co-therapeutic agents.

Key Words: Selenium ▪ Triple Negative Breast Cancer ▪ Antioxidants ▪ Oncology.

Introduction

Breast cancer ranks among the top causes of cancer-related fatalities worldwide (about 600,000 deaths yearly) (1). Triple negative breast cancer (TNBC) is a highly aggressive subtype, due to the fact that human epidermal growth factor receptor 2 (HER2), progesterone (PR), and estrogen (ER) receptors are not expressed molecularly. TNBC is characterized by significant invasiveness, a high potential for metastasis, and an unfavorable prognosis.

Due to the lack of therapeutic targets (ER, PR, HER2), treatment options for TNBC are limited (2). According to studies, selenium (Se) may be used as a successful treatment for TNBC. Se components, in particular, have the potential to be used in both organic and inorganic forms, as well as conjugated with other antioxidants or conventional chemotherapeutic agents. They have the potential to significantly inhibit tumor growth (2-8), and improve the efficacy of chemotherapeutic agents by influencing multiple molecular pathways involved in cancer cells, while posing minimal toxicity to healthy cells.

This narrative review aims to investigate the potential therapeutic applications of selenium compounds as a customized treatment for TNBC,

either in isolation or in combination with dietary supplements and chemotherapy.

Methods

This review's objective is to evaluate selenium and its constituents' potential as treatments for TNBC. A comprehensive search for the terms "selenium" and "triple negative breast cancer" was done on the PubMed database in August 2023. This search turned up a total of 23 articles that were published between the years 2012 and 2023.

The papers were assessed using the following criteria: (a) use of selenium alone, or in combination with other antioxidants or chemotherapy; (b) research employing in vivo specimens or TNBC cell culture lines; (c) experimental research yielding results that are statistically significant; (d) full-text publications that can be found in the PubMed database; (e) research articles that are available in English. Thirteen articles remained after elimination of others from consideration for this review on the basis of these criteria.

Results

It was found that selenium compounds augment the effects of anticancer medications and induce notable morphological changes in tumor models, which leads to a decrease in tumor development (2-8). The cells displayed apoptotic body formation, along with enlargement, shrinkage, increased granularity, and rupture of the cytoplasmic membrane (1, 8, 9, 10, 11). Increased oxidative stress, superoxide, and ROS production were also noted (1, 3, 8, 10). The tumor models showed reduced growth, viability, and proliferation of cells, which enhanced cytotoxicity against TNBC cells and resulted in cell cycle arrest (2, 5, 7, 12, 13). Numerous investigations, executed at various periods of the experimental process, revealed increased autophagy at first, followed by necrosis, and apoptosis (1, 3-11, 13). Moreover, tumoral metastasis was decreased by treatment with selenium compounds, either by themselves or in combination with anticancer therapy (1-3, 6, 11-13). This is an highly noteworthy result, especially in patients of TNBC with extensive metastases. The results that were reported were dependent on the treatment's dosage and/or duration (1-12). Furthermore, while most research showed little to no negative effects, a few detected cytotoxicity in normal epithelial cells when using selenium compounds alone (7, 8, 11).

Discussion

In the current study, we assessed papers that employed different kinds of selenium compounds. Specifically, some studies used different selenium compounds or derivatives alone, while the remaining studies used selenium compounds in combination with different chemotherapeutic medications and/or supplements. To be more precise, the anticancer medications most often utilized in the second group of research included the chemotherapeutic medications Trastuzumab, Bevacizumab (Avastin), Doxorubicin (Adriamycin), and Paclitaxel (Taxol), as well as the reverse transcriptase inhibitor AZT (azidothymidine). The selenium compounds used in this category of studies were: Se-modified Bolton-Hunter reagent in combination with Trastuzumab and Bevacizumab (1), fish oil with Se (FO/Se) in combination with Doxorubicin (2), Se with AZT resulting in three drugs (S1072, S1073, S1079) (9), Se with EDA/DHA alone or combined with Taxol, Adriamycin and Avastin (3), methylselenic acid (MSA) with Paclitaxel (4), Se-containing polysaccharides from *Pyracantha fortuneana* (Se-PFPs) in combination with Doxorubicin (5), supplementary marine-based FO and Se yeast (FO/SE) in combination with Avastin (6). In the first category of studies, Se was utilized in the form of: selenoesters EDA-71 (Se-(2-oxopropyl)4-chlorobenzoselenate) and E-NS-4 (Se-cyanomethyl 4-chlorobenzoselenate) (7), selenofolate (conjunction of folic acid and redox selenium adduct) (10), seleno-purine SLLN-15 (4-selenomorpholinophenyl and tetrahydroselenophene- substituted diamino-purines) (12), selenomethionine (organic), sodium selenate and sodium selenite (inorganic), ebselen and diphenyl diselenide (synthetic organoselenium

compounds) (11), Se yeast, methylselenic acid (MSA) and methylselenocysteine (MSC) (8), Se-containing polysaccharides from *Pyracantha fortuneana* (13), and Benzimidazole-Containing Selenadiazole Derivatives (BSeDs: 1a, 1b, 1c, 1d) (13). The compounds mentioned above, along with their chemical formulas, dosages, and effects on TNBC viability, proliferation and metastatic potential are summarized in Table 1.

Table 1. Effects of Selenium on TNBC Viability

Authors	Selenium compound used	Chemical formula	Dosage	Effects on TNBC cells
Khandelwal, Soni et al. (1)	Selenotrastuzumab (Se-TZ) and Selenobevacizumab (Se-BV)	Conjuction of Trastuzumab and Bevacizumab with selenium-modified Bolton-Hunter reagent (redox active and toxic form of selenium)	2 µg Se as Se-TZ or Se-BV	1) Inhibition of cell proliferation by generating superoxide and other ROS (resulting from oxidation of glutathione and other thiols); 2) Cell swelling and shrinking; 3) Induced apoptosis by reducing ΔψM and increasing superoxide generation; 4) Reduced chance of metastasis.
Guo, Chih-Hung et al. (2)	Doxorubicin with FO/Se	Combination of Fish oil (FO) containing $C_{22}H_{32}O_2$ (docosahexaenoic acid, DHA, 22:6 n3) and $C_{20}H_{30}O_2$ (eicosapentaenoic acid, EPA, 20:5 n3), and Se with Doxorubocin	5 mg/kg doxorubicin together with 0.4 g of FO/Se in low, medium, and high concentrations (8.8 mg/2.7 µg/g, 16.9 mg/4.0 µg/g, and 19.0 mg/6.7 µg/g, respectively)	1) Reduced tumor growth; 2) Fewer metastases detected; 3) Alteration of tumor cytoplasmatic signaling pathway; 4) Reduction of the expression of tumor oncogenes and proteins; 5) Alteration of immune checkpoints; 6) Decreased cell proliferation, cell cycles and cancer stemness.
Guo, Chih-Hung et al (3)	Nutritional Supplement (NS) containing Se and EPA/DHA used alone or in combination with Taxol (Tax), Adriamycin (Adr), and Avastin (Ava)	Se yeast along with $C_{22}H_{32}O_2$ (docosahexaenoic acid, DHA, 22:6 n3) and $C_{20}H_{30}O_2$ (eicosapentaenoic acid, EPA, 20:5 n3), combined with Tax, Adr, and Ava	TB-Tax-NS group; Tax (5 mg/kg every 4 days) and NS (0.4 mg) by oral gavage twice a day TB-Adr-NS group; Adr (2 mg/kg every 4 days) and NS (0.4 mg) by oral gavage twice a day TB-Ava-NS group; Ava (5 mg/kg every 4 days) and NS (0.4 mg) by oral gavage twice a day for 25 days	1) Reduction of tumor weights and sizes; 2) Reduction of metastatic potential; 3) Alteration of tumor immune micro-environment; 4) Increase of oxidative stress; 5) Suppression of angiogenesis and cancer stem cells; 6) Induction of apoptotic tumor responses.
Qi, Yanfeng et al. (4)	MSA and Paclitaxel	CH_3SeO_2H (Methylseleninic acid, MSA) combined with Paclitaxel	In vitro; MSA (2.5, 3.2, 4 µM) and Paclitaxel (10, 20, 40 nM) in various combinations. In vivo; 3 mg MSA per kg body with 10 mg/kg Paclitaxel.	1) Enhanced inhibition of tumor growth (reduction of tumor weight); 2) Significant decrease in tumor cell proliferation; 3) Induction of apoptosis; 4) Inhibition of tumor re-growth after termination of treatment.
Yuan, Chengfu et al. (5)	1) Se-containing polysaccharides from Pyracantha fortuneana (Se-PFPs); 2) Se-PFPs in combination with doxorubicin	Heteropolysaccharides composed of xylose, arabinose, fucose, mannose, ribose, rhamnose, glucuronic acid, galacturonic acid, glucose, and galactose, along with uronic acid and Se	Escalating concentrations of Se-PFPs; 0, 50, 100, 200 and 400 µg/ml.	1) Inhibition of cell growth in MDA-MB-231 cells, caused by arrest at G2/M phases of cell cycle; 2) Induction of apoptosis; 3) Inhibition of tumor growth in treated mice (decrease in tumor volume and weight); 4) Enhanced effect of Se-PFPs on the sensitivity of MDA-MB-231 cells to doxorubicin.

Continuation of Table 1. Effects of Selenium on TNBC Viability

Authors	Selenium compound used	Chemical formula	Dosage	Effects on TNBC cells
Guo, Chih-Hung et al. (6)	FO/SE with Avastin	Supplemental marine-based fish oil (FO) containing omega-3 fatty acids such as $C_{22}H_{32}O_2$ (docosahexaenoic acid, DHA, 22:6 n3) and $C_{20}H_{30}O_2$ (eicosapentaenoic acid, EPA, 20:5 n3), along with Se yeast (FO/SE) in combination with Avastin (bevacizumab)	Concentrations of EPA, DHA, and elemental Se, respectively in low, medium, and high doses of FO/Se supplements; low; 5.1 mg, 3.7 mg, and 2.7 μg/g medium; 9.1 mg, 6.9 mg, and 4.0 μg high; 10.7 mg/g, 8.3 mg, and 6.7 μg/g 5 mg/kg of the Avastin (once every four days) and 0.4 g of low, medium, and high concentrations of EPA/DHA/Se supplements twice a day for 25 days	Enhanced efficacy of Avastin in 4T1 tumor cells (CRL-2539): 1) Markedly decreased tumor size; 2) Inhibition of EMT (epithelial-to-mesenchymal transition);3) Inhibition of metastasis; 4) Further induction of apoptosis.
Radomska, Dominika et al. (7)	Novel selenoesters EDA-71 and E-NS-4	EDA-71; Se-(2-oxopropyl) 4-chlorobenzoselenoate E-NS-4; Se-cyanomethyl 4-chlorobenzoselenoate	Escalating concentrations of both compounds; 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 μM	1) High cytotoxicity and growth suppression 2) Increased Apoptosis and necrosis 3) Induction of Autophagy 4) Induction of cell cycle arrest (all the above were more significant in MDA-MB-231 cells by using EDA-71)
Guo, Chih-Hung et al. (8)	1) Se yeast 2) Methylseleninic acid (MSA) 3) Methylselenocysteine (MSC)	Se yeast CH_3SeO_2H (methylseleninic acid, MSA) $C_4H_9NO_2Se$ (methylselenocysteine, MSC)	Se yeast (100, 750, and 1500 ng Se/mL) Methylseleninic acid (1500 ng Se/mL) Methylselenocysteine (1500 ng Se/mL)	1) Inhibition of tumor growth; 2) Increased superoxide production and antioxidant enzyme (SOD, GPx) activities; 3) Induction of early and late apoptosis; 4) Loss of mitochondrial membrane potential; 5) Nuclear morphological changes (formation of apoptotic bodies).
Wagner, Mônica Silveira et al. (9)	Seleno-AZT derivatives	Se combined with $C_{10}H_{13}N_5O_4$ (Azido-3´-deoxythymidine, AZT), a nucleoside reverse transcriptase inhibitor resulting in 3 derivatives; S1072, S1073, S1079	Medium containing derivatives S1072, S1073, S1079, and commercial AZT at concentrations 50 and 100 μM	1) Selective decrease in cell proliferation (especially in TNBC cell line); 2) Apoptotic morphology (=loss of attachment to other cells and ECM + having a rounder shape); 3) Cell death rate of a 35% in TNBC line.
Khandelwal, Soni et al.(10)	Selenofolate	2-selenocyan folate (conjuction of folic acid and 2-selenocyanoethanol)	Selenofolate 100 μM (8 μg Se)	1) Morphological changes such as shrinkage, swelling and cell membrane disruption. 2) Loss of mitochondrial membrane potential 3) Apoptosis induction through superoxide generation

Continuation of Table 1. Effects of Selenium on TNBC Viability

Authors	Selenium compound used	Chemical formula	Dosage	Effects on TNBC cells
da Costa, Nayara Souza et al. (11)	Selenomethionine (organic), sodium selenate and sodium selenite (inorganic), ebselen and diphenyl diselenide (synthetic organoselenium compounds)	$C_5H_{11}NO_2Se$ (selenomethionine) Na_2SeO_4 (sodium selenate) Na_2SeO_3 (sodium selenite) $C_{13}H_9NOSe$ (ebselen) $C_{12}H_{10}Se_2$ (diphenyl diselenide)	1, 10, 50, and 100 μM for 48 h	1) Decreased cell viability of MDA-MB-231 by selenate, selenite (100μM), ebselen and diphenyl diselenide (100μM); 2) Decreased cell viability of BT-549 by selenite (100μM), ebselen and diphenyl diselenide (50+100μM); 3) Reduced cell size in BT-549 by diphenyl diselenide at all concentrations; 4) Increased cell granularity by selenate (50+100 μM); 5) Increased number of apoptotic/ necrotic cells in TNBC cell lines by selenite (100μM), ebselen (100 μM); 6) Increased number of apoptotic/necrotic cells only in BT-549 by diphenyl diselenide (50+100μM); 7) Less colonies were formed in all cell lines by diphenyl diselenide; 8) Complete inhibition of colony formation in all cell lines by selenite (10-100μM); 9) Suppression of cell migration in BT-549 by diphenyl diselenide (1 μM).
Chang, Chia-Hao et al. (12)	Seleno-purine SLLN-15	$C_{19}H_{23}N_7Se_2$; (S)-N2-(4-selenomorpholinophenyl)-N6-(tetrahydroselenophen-3-yl)-9H-purine-2,6-diamine	In vivo; 30 mg/kg SLLN-15, 3 times a week for 40 days In vitro; 10 μM SLLN-15 for 24 h	1) Inhibition of cell growth in vitro and in vivo 2) Decreased number of metastases and TNBC cell progression 3) Stimulation of autophagy 4) G2/M cell cycle arrest
Liang, Yuanwei et al. (13)	Benzimidazole-Containing Selenadiazole Derivatives (BSeDs): 1a, 1b, 1c, 1d	1a; 5-(1H-benzo[d] imidazol-2-yl)benzo[c] [1,2,5]selenadiazole 1b; 5-(6-methyl-1H-benzo[d]imidazol-2-yl)benzo[c][1,2,5] selenadiazole 1c; 5-(6-chloro-1H-benzo[d]imidazol-2-yl)benzo[c][1,2,5] selenadiazole 1d; 5-(6-bromo-1H-benzo[d]imidazol-2-yl)benzo[c][1,2,5] selenadiazole	1b (0.5 and 1.0 μM), 1c (0.5, 1.0, and 2.1 μM), 1d (0.5, 1.0, and 1.3 μM)	1) Inhibition of cancer cell growth through cell-cycle arrest (increased sub-G1 and G2/M cell population and enhanced accumulation of cells in G0/G1 phase) and apoptosis (both early- and late-phase); 2) Inhibition of cancer cell migration.

Both in vitro and in vivo experiments were used in the studies evaluated for this review. The in vitro tests employed the following cell lines: HME50-5E (1, 10), MCF-10A (7, 11), CHO (9), HMEC (8), which are normal epithelial cell lines, and MDA-MB-231 (1, 4, 5, 7-9, 11-13), MDA-MB468 (1, 10, 12), BT-549 (4, 11), BT20 (12), which are TNBC cell models. Mice were the model organism used in all of the in vivo investigations. More precisely, MDA-MB-231 triple negative human

tumor cells were implanted into Severe Combined Immunodeficient (SCID) mice (4, 12), nude animals bearing MDA-MB-231 human cells (5), BALB/c mice implanted with 4T1 human TNBC cells, and mice bearing 4T1 human TNBC cells (2, 3, 6).

We also looked at the instruments and assays that each study used. More analytical methods were used to assess cell viability, including the Trypan blue cell exclusion method, the MTT assay (1, 4, 10), the XTT cell viability assay (5, 10, 12, 13), the LIVE/DEAD viability assay with fluorescence microscopy (9), and sulforhodamine B (SRB) (4). Additionally, the MTT assay (1), Brdu-labeling assay (4, 12), and [3H]-Thymidine incorporation assay were used to measure cell proliferation (7). The following techniques were employed to detect apoptosis: annexin V staining (1, 7-11, 13), propidium iodide (PI) staining (7, 8, 13), and other flow cytometry assays (7-9, 13), along with other apoptosis assays (4, 5, 8). In addition, phase contrast microscopy (1), photographic evaluation (10), flow cytometry (11), nuclei staining with DAPI, and fluorescence microscopy were used to evaluate the morphological alterations in the cells (8). Finally, target protein concentrations were assessed via flow cytometry and Western blotting (1, 2, 3-7, 10, 13).

Changes in mitochondrial membrane potential were detected by flow cytometry (1, 7, 8), whereas RNA levels and gene transcription were evaluated by using cDNA (2, 9) and real-time quantitative PCR (2, 6, 9). In regard to cytotoxic activity on cells, MTT assay (7, 9) and LDH assay (5) were most commonly performed. Superoxide generation was measured using lucigenin-amplified chemiluminescence (8, 10), dihydroethidium (10), and oxidation-sensitive fluorescent probe H2DCF (13).

In order to determine the distribution of the cell cycle, cell cycle analysis and flow cytometry were utilized (4, 5, 7, 13). Using the autophagy assay (7) and immunohistochemistry with the autophagy markers MAD1LC3B/LC3B, ATG12, and H+E staining, the quantity of autophagosomes and autolysosomes was estimated. The wound healing migration assay, the cell migration assay, and the colony formation assay were used to assess cell migration (11). Lastly, the colony formation test was used to assess the proliferation of the tumor cells, while flow cytometry was utilized to identify the tumor necrosis using 7-aminoactinomycin D (11) and annexin V-FITC/PI (8).

Cellular Pathways and Structures Affected

The bulk of the research evaluated for this review describes potential signaling pathways, and cellular structures or molecules impacted by using selenium compounds as a therapeutic approach against TNBC cell lines (Table 2). EGFR, FGFR (2, 3, 6), VEGF, phospho-VEGFR2, PDGFR2, TGF-β, and TGF-βR2 (6), as well as variations in VEGFA levels were the first proteins of the growth factor family to show changes in levels. Moreover, elevated p53 (2, 5) and its phosphorylated version (3, 13) levels were observed.

Furthermore, the PI3K-AKT-mTOR pathway was downregulated (2, 3, 6, 13), with higher levels of TSC1/2 (2, 6), whereas the AURKA-AKT-mTOR autophagy pathway was activated (12). The Ras-Raf-MEK-ERK pathway was downregulated (2, 3, 6, 13), the transcription factors c-jun and c-fos were decreased (2, 6), and levels of JNK protein were increased (13). The deactivation of the JAK2/STAT3 signaling (2, 3, 6), as well as the reduction of p-c-Src were detected (2). In addition, the levels of active NF-κB (2, 7), c-Myc (2), HIF-1α (2, 3, 6), and HIF-2α (2, 6) appeared reduced. Regarding the caspase proteins and PARP signaling, the intrinsic apoptosis pathway was induced by the increased levels of caspase 9 (5, 7), and the extrinsic apoptosis pathway appeared activated by the rise of the caspase-3/-8/-10 levels (3, 5-7, 9). The common apoptosis pathway was also activated by the elevated levels of caspase 3/7 (4, 7), along with increased PARP expression (4), and the image of cleaved DARP-1 (6). Additionally, co-signal molecules in T-cell activation were affected as follows: decreased PD-L1 (2, 3, 6), CTLA-4, Foxp3 (2), PD-1 in mammary glands (3), and an augmentation of PD-1 (2, 3) and IL-2 levels (2). The levels of molecules of cell cycle checkpoints (cyclins and CDKs) were also affected: cyclin E (2, 6, 7), cyclin D1 (2, 6), cyclin B1 (5), CDK2 (6),

Table 2. Selenium Compounds and Pathways Affected

Authors	Selenium compound used	Cellular Pathways and structures affected
Khandelwal, Soni et al. (1)	Selenotrastuzumab (Se-TZ) and Selenobevacizumab (Se-BV)	1) Se-TZ and Se-BV: Apoptosis induced by effect on cell membranes and mitochondrial activity (mechanism unknown); 2) Selenite: Cleaved β-actin bands in MDA-MB-468 cells and HME50-5E cells; 3) Se-BV: Cell death in TNBC cells but not HME-50-5E cells when the cell lines exhibited similar levels of VEGFA.
Guo, Chih-Hung et al. (2)	Doxorubicin with FO/Se	1) Lower GPR-40 mRNA levels and higher expression of all selenoproteins,; 2) Decreased expression of membrane EGFR and FGFR; 3) Higher mRNA levels of PTEN and decreased levels of p-PI3K, p-Akt, p-mTOR, p-4EBP1 and p70S6K; 4) Increased expression of TSC1 and TSC2; 5) Decreased levels of Ras, p-Raf1, p-MEK, and p-ERK1/2 proteins; 6) Lower levels of p-c-Src, p-JAK2 and p-STAT3 proteins; 7) Decreased tumor mRNA expression of c-Jun and c-Fos; 8) Lower protein levels of c-Myc and p-NF-κB p65 and lower levels of tumor HIF-1α and HIF-2α protein; 9) Increased tumor levels of p-P53 and lower levels of Ki-67; 10) Decreased mRNA levels of PD-L1, CTLA-4, and Foxp3 and higher mRNA levels of PD-1 and IL-2; 11) Lower expression of tumor PD-L1, CTLA-4, Foxp3, and CD86 and increased expression levels of PD-1, NKp46, and IL-2 proteins; 12) Lower mRNA levels of cyclin E and decreased expression of cyclin D1, CDK4, and CDK6; 13) Lower expression levels of CD24 and CD29.
Guo, Chih-Hung et al. (3)	Nutritional Supplement (NS) containing Se and EPA/DHA used alone or in combination with Taxol (Tax), Adriamycin (Adr), and Avastin (Ava)	1) Increased levels of MDA (oxidative stress biomarker) ; 2) Decreased plasma concentrations of IL-1β, IL-6, IL-10, TNF-α, and VEGF, and increased IFN-γ and IL-2; 3) Decreased expression of tumor HIF1-α; 4) Lower levels of tumor VEGF and decreased CD31, MMP-9, CD24 and CD29 expression; 5) Decreased tumor levels of HSP-70, HSP-90, AXL and p-AXL; 6) Increased levels of phosphorylated p53, cleaved caspase-3 and cytosolic cytochrome c and decreased Bcl-2 and mitochondrial cytochrome c expression; 7) Reduction of PD-L1 (through deactivation of AKT/ERK and JAK2/STAT3 signaling) and augmentation of PD-1 tumor levels; 8) Reduction of PD-L1 and PD-1 levels in mammary glands.
Qi, Yanfeng et al. (4)	MSA and Paclitaxel	1) Further decreased fraction of cells in G0/G1 phase; 2) Further increased G2/M arrest (cause of extensive apoptosis); 3) Activation of the caspase-PARP pathway (enhanced activation of caspase-3, caspase-7 and PARP).
Yuan, Chengfu et al. (5)	1) Se-containing polysaccharides from Pyracantha fortuneana (Se-PFPs); 2) Se-PFPs in combination with doxorubicin	1) Reduction of p-H3 levels in MDA-MB-231 cells; 2) Decreased levels of CDC25C, CDC2 and Cyclin B1; 3) Increase in the activities of caspases 3 and 9; 4) Increases in the protein levels of p53, Bax, Puma and Noxa; 5) Decreased levels of Bcl-2 and, in turn, increased Bax/Bcl-2 ratio; 6) Increased cytochrome C levels (apoptosis via the p53-mediated cytochrome c-caspase pathway).
Guo, Chih-Hung et al. (6)	FO/SE with Avastin	1) Increased SEPW1 in tumor tissues at all FO/Se concentrations and SPEN1 at medium and high doses of FO/se; 2) Lower levels of HSP90, HIF-1α and HIF-2α expression; 3) Lower cyclooxygenase (COX-2), superoxide dismutase (SOD-1) and metalloprotease-9 (MMP-9) levels; 4) Decrease in VEGF+ phospho-VEGFR2, EGFR, FGFR PDGFR2, TGFβ+ TGFβR2 levels; 5) Reduction of growth arest-specific-6 (Gas6) and AXL phosphorylation levels; 6) Lower levels of CXCL12, CXCR4+ CXCR7 (chemokines); 7) Decreased levels of Wnt3α/5α FZD7; 8) Reduction of PI3K, PTEN, AKT, mTOR, phosphor-p70s6K, phospho-4EBP1 and increased in TSC1 and TSC2 expression; 9) Lower levels of Ras, phospho-Raf1, phospho-MEK, phospho-ERK1/2 and higher levels of LKB-1, phospho-AMPK expression; 10) Reduction of phospho-smad2/3, smad 4 and TMEPAI at high concentrations of FO/Se; 11) Lower levels of phospho-c-Src+ phospho-JAK2, STAT3, phospho-GSK-3β, p-S552-β-catenin, p-S33-37-Y41-β-catenin and increase in GSK-3β; 12) Decrease in SNAIL, SLUG (EMT-activated transcription factors), cyclinD1,E, CDK-2/-4/-6 levels; 13) Increased cleaved-caspase-3/-8, phospho-Bcl-2, cleaved-DARP-1 and decreased cofilin-1 (CFL-1);14) Decreased CSC rkermas (CD29/24/44 and CXCR2).
Radomska, Dominika et al. (7)	Novel selenoesters EDA-71 and E-NS-4	1) Increased activation of caspases 8+10 (extrinsic apoptosis pathway); 2) Decreased Mitochondrial Potential (intrinsic apoptosis pathway); 3) Reduction of NF-κB active form (intrinsic apoptosis pathway); 4) Higher levels of caspase 9 (intrinsic apoptosis pathway); 5) Elevation of the caspase 3/7 active form levels (executive common phase of apoptosis); 6) Lower mTOR levels (autophagy pathway); 7) Increased cyclin A2 activity and decreased E1 activity (cell cycle arrest in S phase).

Continuation of Table 2. Selenium Compounds and Pathways Affected

Authors	Selenium compound used	Cellular Pathways and structures affected
Guo, Chih-Hung et al. (8)	1) Se yeast 2) Methylseleninic acid (MSA) 3) Methylselenocysteine (MSC)	-
Wagner, Mônica Silveira et al. (9)	Seleno-AZT derivatives	Mainly affected pathway seems to be the extrinsic apoptosis pathway because of the significant increase in caspases 3 and 8 gene expression (especially by using the S1072 drug)
Khandelwal, Soni et al.(10)	Selenofolate	1) Glutathione triggered superoxide generation; 2) Cellular targets= reduced GSH/other thiols and cysteines of mitochondrial potential; 3) Internalization through FRA (folic acid receptors that are overexpressed in TNBC).
da Costa, Nayara Souza et al. (11)	Selenomethionine (organic), sodium selenate and sodium selenite (inorganic), ebselen and diphenyl diselenide (synthetic organoselenium compounds)	-
Chang, Chia-Hao et al. (12)	Seleno-purine SLLN-15	1) AURKA- AKT- mTOR activation for indication of autophagy; 2) Inhibition of phosphorylation of AURKC; 3) Decreased expression of AURKA+ AURAKB resulting from the proteasome pathway.
Liang, Yuanwei et al. (13)	Benzimidazole-Containing Selenadiazole Derivatives (BSeDs): 1a, 1b, 1c, 1d	1) Induction of intracellular ROS generation; 2) Increased expression levels of P-histone; 3) Increased expression levels of P-ATM, causing the activation of P-BRCA1, which results in the upregulation of P-p53; 4) Downregulation of Bcl-2 and Bcl-xl expression; 5) Upregulation of Puma and Noxa expression; 6) Downregulation of MDM2 expression; 7) Upregulated phosphorylation of pro-apoptotic kinases p38 and JNK (MAPK family members); 8) Suppressed phosphorylation of anti-apoptotic kinase AKT; 9) Suppressed phosphorylation of anti-apoptotic kinase ERK (MAPK family member).

CDK4/6 (2, 6), and CDC25C/2 (5) were reduced, in contrast to the increase in the levels of cyclin A2 (7). This indicates cell cycle arrest in the S phase. Changes in mitochondrial potential and enzymes were also detected: loss of mitochondrial potential (1, 7, 8, 10) and reduction of mitochondrial cytochrome C enzyme (3, 5). Anti-apoptotic and pro-apoptotic protein levels were also impacted: Bcl-2 appeared decreased (3, 5, 13) as well as Bcl-xl (13) in contrast to increased phospho-Bcl-2 (6), Bax (5), Puma, and Noxa (5, 13). Furthermore, IL-2 (2, 3) and IFN-γ (3) were increased, whereas IL-1β, IL-6, IL-10, and TNF-α were deceased (3). Lastly, the heat-shock proteins HSP-90 (3, 6) and HSP-70 (3) appeared to decrease.

In terms of overall TNBC tumoral model findings, all of the trials that were assessed showed that selenium compounds were effective. More precisely, the effects of anticancer medications were increased when selenium compounds were added (1-6, 9). Additionally, it was evident that the use of selenium compounds, either alone or in conjunction with anticancer therapy, resulted in significant morphological alterations in the tumoral models under investigation, by slowing tumor growth (tumor weights and sizes) (2-8).

Regarding the morphology and morphological alterations, the cells showed signs of swelling and shrinkage, along with increased granularity, breakdown of the cytoplasmic membrane, and

apoptotic body formation (1, 8-11). Furthermore, an increase in oxidative stress, as well as the production of superoxide and ROS were noted (1, 3, 8, 10). Regarding the tumor models' cellular activity, it was found that TNBC cells were subjected to increased cytotoxicity and that cell growth (5, 12, 13), viability (7, 11), and proliferation were all markedly suppressed (1, 2, 4, 9).

Additionally, cell cycle arrest was found, usually in the G2/M phase (2, 5, 7, 12, 13). Numerous studies that were reviewed showed a significantly higher induction of necrosis and apoptosis by changes in mitochondrial membrane potential, ROS production, and cell cycle arrest (1, 3-11, 13). The induction of autophagy induced by selenium compounds in several experiments was an intriguing finding (7, 12).

Ultimately, administration of selenium compounds either alone or in conjunction with anticancer therapies, led to a reduction in the potential for tumoral metastasis through the suppression of angiogenesis, EMT (epithelial to mesenchymal transition), colony formation, and cell progression (1, 2, 3, 6, 11-13). TNBC appears to be very metastatic, so this result is of great importance. These outcomes were dependent on either time or dose (1-12).

Most of the investigations also assessed the adverse effects of selenium compound treatment on normal epithelial cells. Some research that examined the utilization of selenium compounds alone revealed cytotoxicity on normal epithelium (7, 8, 11). However, the majority of the other investigations had negligible to no negative effects (1, 4, 5, 8-10, 13).

Conclusion

Since the biomarkers ER, PR, and HER2 are not expressed in TNBC, target therapy is ineffective, and the cancer continues to be one of the most aggressive forms of breast cancer. The aforementioned research shows impressive outcomes when using selenium compounds alone, in combination with nutritional supplements, or as a co-therapeutic agent with common chemotherapy medications such as trastuzumab, bevacizumab, doxorubicin, and paclitaxel. Treatment with selenium compounds inhibited tumor development, caused widespread cell death, and decreased metastatic potential, with minimal damage to normal epithelial cells of in vitro and in vivo TNBC models, according to the publications reviewed in this study. These encouraging findings point to the necessity of additional investigation and assessment, as well as the potential of selenium compounds moving forward in clinical trials as a promising adjunctive treatment for TNBC in the future.

What Is Already Known on This Topic:

Breast cancer is the most frequent type of cancer in women and one of the leading causes of cancer-related deaths globally, accounting for over 600,000 deaths annually. TNBC is a particularly aggressive subtype of the disease due to the absence of molecular expression of human epidermal growth factor receptor 2 (HER2), progesterone (PR), or estrogen (ER) receptors. The characteristics of TNBC include a high risk of metastasis, considerable invasiveness, and a dismal prognosis. Since ER, PR, and HER2 are not therapeutic targets, there are limited treatment options for TNBC. Selenium (Se) has been successfully utilized as a treatment for TNBC, according to studies.

What This Study Adds:

The study presents the remarkable results when selenium compounds were used as a co-therapeutic agent with common chemotherapy drugs such as paclitaxel, bevacizumab, doxorubicin, and trastuzumab, or in isolation with dietary supplements. The papers evaluated for this review indicate that treatment with selenium compounds resulted in a reduction in the potential for metastasis, extensive cell death, and suppression of tumor growth, while causing little harm to the normal epithelial cells of TNBC models, both in vitro and in vivo.

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References

1. Khandelwal S, Boylan M, Spallholz JE, Gollahon L. Cytotoxicity of Selenium Immunoconjugates against Triple Negative Breast Cancer Cells. Int J Mol Sci. 2018;19(11):3352. doi: 10.3390/ijms19113352.
2. Guo CH, Shih MY, Chung CH, Lin YC, Fan CT, Peng CL, et al. Fish Oil and Selenium with Doxorubicin

Modulates Expression of Fatty Acid Receptors and Selenoproteins, and Targets Multiple Anti-Cancer Signaling in Triple-negative Breast Cancer Tumors. Int J Med Sci. 2022;19(14):2044-57. doi: 10.7150/ijms.75848.

3. Guo CH, Hsia S, Chung CH, Lin YC, Shih MY, Chen PC, et al. Nutritional supplements in combination with chemotherapy or targeted therapy reduces tumor progression in mice bearing triple-negative breast cancer. J Nutr Biochem. 2021;87:108504. doi: 10.1016/j.jnutbio.2020.108504. Epub 2020 Sep 19.
4. Qi Y, Fu X, Xiong Z, Zhang H, Hill SM, Rowan BG, et al. Methylseleninic acid enhances paclitaxel efficacy for the treatment of triple-negative breast cancer. PLoS One. 2012;7(2):e31539. doi: 10.1371/journal.pone.0031539. Epub 2012 Feb 14.
5. Yuan C, Wang C, Wang J, Kumar V, Anwar F, Xiao F, et al. Inhibition on the growth of human MDA-MB-231 breast cancer cells in vitro and tumor growth in a mouse xenograft model by Se-containing polysaccharides from Pyracantha fortuneana. Nutr Res. 2016;36(11):1243-54. doi: 10.1016/j.nutres.2016.09.012. Epub 2016 Oct 2.
6. Guo CH, Hsia S, Chung CH, Lin YC, Shih MY, Chen PC, et al. Combination of Fish Oil and Selenium Enhances Anticancer Efficacy and Targets Multiple Signaling Pathways in Anti-VEGF Agent Treated-TNBC Tumor-Bearing Mice. Mar Drugs. 2021;19(4):193. doi: 10.3390/md19040193.
7. Radomska D, Czarnomysy R, Szymanowska A, Radomski D, Domínguez-Álvarez E, Bielawska A, et al. Novel Selenoesters as a Potential Tool in Triple-Negative Breast Cancer Treatment. Cancers (Basel). 2022;14(17):4304. doi: 10.3390/cancers14174304.
8. Guo CH, Hsia S, Shih MY, Hsieh FC, Chen PC. Effects of Selenium Yeast on Oxidative Stress, Growth Inhibition, and Apoptosis in Human Breast Cancer Cells. Int J Med Sci. 2015;12(9):748-58. doi: 10.7150/ijms.12177.
9. Wagner MS, Schultze E, Oliveira TL, de Leon PMM, Thurow HS, Campos VF, et al. Revitalizing the AZT Through of the Selenium: An Approach in Human Triple Negative Breast Cancer Cell Line. Front Oncol. 2018;8:525. doi: 10.3389/fonc.2018.00525.
10. Khandelwal S, Boylan M, Kirsch G, Spallholz JE, Gollahon LS. Investigating the Potential of Conjugated Selenium Redox Folic Acid as a Treatment for Triple Negative Breast Cancer. Antioxidants (Basel). 2020;9(2):138. doi: 10.3390/antiox9020138.
11. da Costa NS, Lima LS, Oliveira FAM, Galiciolli MEA, Manzano MI, Garlet QI, et al. Antiproliferative Effect of Inorganic and Organic Selenium Compounds in Breast Cell Lines. Biomedicines. 2023;11(5):1346. doi: 10.3390/biomedicines11051346.
12. Chang CH, Bijian K, Wernic D, Su J, da Silva SD, Yu H, et al. A novel orally available seleno-purine molecule suppresses triple-negative breast cancer cell proliferation and progression to metastasis by inducing cytostatic autophagy. Autophagy. 2019;15(8):1376-90. doi: 10.1080/15548627.2019.1582951. Epub 2019 Mar 1.
13. Liang Y, Zhou Y, Deng S, Chen T. Microwave-Assisted Syntheses of Benzimidazole-Containing Selenadiazole Derivatives That Induce Cell-Cycle Arrest and Apoptosis in Human Breast Cancer Cells by Activation of the ROS/AKT Pathway. ChemMedChem. 2016;11(20):2339-46. doi: 10.1002/cmdc.201600261. Epub 2016 Sep 28.