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Interactions of ROS, Antioxidants, and Ferroptosis through Cancer: A Review

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Abstract

The field of oncology is rapidly growing in search of various therapies for cancer, which calls for further study of cellular processes and molecules. ROS, or reactive oxygen species, are necessary throughout a cell's metabolic processes; high reactivity at high concentrations could threaten cells, possibly allowing for beneficial use against cancers. Conversely, antioxidants regularly neutralize ROS in cells and manage lipid peroxide. Depleting certain antioxidants may lead to ferroptosis, which may also target cancer cells. It has been found that antioxidants can protect healthy cells from ROS treatment while cancer cells suffer damage. However, antioxidants may also diminish the effects of chemotherapy/ROS treatment, increase the risk of cancer, or have no effect at all, so additional work is needed to understand the balance and specific molecules necessary. The metabolic pathways generating ROS, involvement of ROS in cancer, and interplay among antioxidants, ferroptosis, and reactive oxygen species are evaluated in this review.

Keywords: Reactive oxygen species (ROS), Antioxidants, Ferroptosis, Lipid peroxidase, Cancer therapy

1. Introduction

The field of oncology is rapidly growing in search of various therapies for cancer, which calls for further study of cellular processes and molecules. Cancer, the major cause of death globally, is described by the World Health Organization (WHO) to be the abnormal, rapid replication of cells that may spread toward and disrupt other areas or body organs (De Leo et al., 2021). ROS are the most reactive molecules that are developed by normal cellular metabolism. These molecules are believed to play a vital role in the development and progression of cancer but require a certain balance since they can also damage cells (Aggarwal et al., 2019). The cellular processes that create ROS such as the electron transport chain (ETC) within mitochondria and the phagocytosis procedure are observed in both unicellular and multicellular cells (Hamanaka and Chandel, 2010; Johansson et al., 2016).

In cancer, ROS can stimulate angiogenesis and activate cell signaling pathways in cancer cells to support tumor cell growth and survival (Hamanaka and Chandel, 2010). However, high levels of ROS can also lead to DNA damage and cell death (Auten and Davis, 2009). Ferroptosis is a recently discovered form of cell death triggered by high levels of ROS (Liu et al., 2021). It is characterized by iron accumulation and lipid peroxidation, leading to cell membrane damage and cell death. Antioxidants are molecules that can neutralize ROS and prevent cell damage (He et al., 2017). They play a key role in protecting cells from the harmful effects of ROS. However, in cancer, high levels of antioxidants may also protect cancer cells from cell death (*Antioxidants accelerate the growth and invasiveness of tumors in mice*, 2015). Suppose high amounts of ROS were given to cancer cells with low amounts of antioxidants to counter them, these cancer cells could be killed off in a possibly therapeutic manner. In this review article, we examine

Vol. 2024 (11) 447 – 453
ISSN 2688-3651 [online]

the metabolic pathways that generate ROS and their involvement in cancer, including the topics of ferroptosis and antioxidants.

2. Reactive Oxygen Species

ROS, or reactive oxygen species, are produced through various metabolic pathways, including the electron transport chain in the mitochondria and the process of phagocytosis (Raimondi et al., 2020; Segal, 2008). These pathways are essential for the normal functioning of cells, but can also lead to the production of ROS. High levels of ROS can cause damage to cells by binding and altering the structure of lipids, proteins, and DNA, leading to cell dysfunction or death (Shields et al., 2021). ROS molecules include superoxide anions, hydrogen peroxide, and hydroxyl radicals.

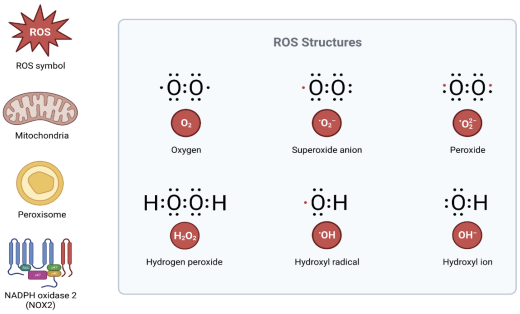

Figure 1. Structures of Reactive Oxygen Species Examples. The figure shows the Lewis Dot structures of six different types of reactive oxygen species and where they are used/made. The lone electrons, electronegative oxygens, and polarity of these molecules show their potential for high reactivity with other substances. Reprinted from "Sources of Reactive Oxygen Species (ROS) with Cell Background", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates

Pathways such as phagocytosis and the electron transport chain are essential for the normal functioning of cells but can also lead to ROS production (Black et al., 2021). The electron transport chain in mitochondria generates healthy cell energy through oxidative phosphorylation. During this process, electrons coming from the initial stages of cellular respiration are transferred through a protein complexes series, ultimately producing ATP. However, a small fraction of electrons can leak out of the electron transport chain and react with the electron acceptor oxygen to form ROS, such as superoxide anions (Raimondi et al, 2020). This process is also called Mitochondrial ROS (mtROS) production (Raimondi et al., 2020).

Phagocytosis, the process in which immune cell subsets (such as neutrophils, macrophages, etc.) engulf as well as degrade various foreign particles, could also lead to ROS production. One of the major players in this procedure is an enzymatic group called NADPH oxidases, which are used in certain ways in cancer cells, such as driving various signaling pathways. During phagocytosis, they create ROS to break down the engulfed particles. These enzymes could use NADPH as a cofactor to develop superoxide anions, which then can be converted to other ROS, (i.e., hydrogen peroxide) by the enzyme named superoxide dismutase (SOD). This whole mechanism is also called the Phagocytic ROS (phag ROS) production process (Segal, 2008).

One way ROS can be damaging to cells is through the propagation of free radicals. ROS are free radical molecules with unpaired valence electrons, making them highly reactive (Lobo et al., 2010; Shields et al., 2021). The superoxide anion and hydroxyl radical are examples of free radicals. These molecules could disrupt cells by reacting with or changing the structure of essential biomolecules such as lipids, proteins, and DNA (Lobo et al., 2010; Shields et al., 2021).

Other enzymes and proteins that play a role in the cellular response to ROS include superoxide dismutases, catalases, and glutathione peroxidases. The enzyme superoxide dismutase (SOD) converts superoxide anions into hydrogen peroxide, which can then be neutralized by other antioxidants (Snezhkina et al., 2019). Catalase, another enzyme, converts hydrogen peroxide into water and oxygen. The protein Bcl-2 can inhibit the production of ROS by inhibiting the release of cytochrome c, a protein involved in cellular respiration, from the mitochondria (Snezhkina et al., 2019).

Cells with high levels of ROS and low levels of substances to neutralize them are considered to have oxidative stress. A study by Singh & Manna (2022) has explored the role of ROS in the context of cancer with different findings that indicate that oxidative stress is in sync with cancer progression and development as it facilitates DNA mutation, cell proliferation, DNA damage, and genomic instability. Ushio-Fukai and Nakamura (2008) found that ROS in cancer

Vol. 2024 (11) 447 – 453
ISSN 2688-3651 [online]

cells trigger cell signaling pathways and promote angiogenesis, cell growth, and survival. ROS overproduction and decreased levels or inactivation of antioxidant equipment can form oxidative stress within cancer cells. However, high levels of ROS can also lead to DNA damage and cell death or apoptosis. For example, superoxide generation through the Rac-1/NADPH oxidase pathway may induce signals that promote apoptosis (Chung et al., 2003). Since ROS has the potential to both help and harm cancer cells, understanding the enzymes, proteins, and small molecules that regulate ROS production and the cellular response to it will be crucial in the development of new cancer therapies. It's important to note that the body produces some ROS as part of normal physiological processes. Still, the overproduction of ROS or dysfunction in antioxidant defense mechanisms can drive cancer and other diseases.

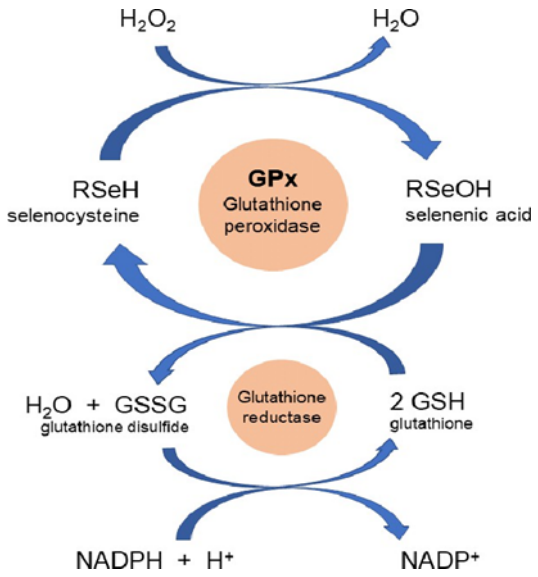

Figure 2. Reduction of Hydrogen Peroxide through Glutathione Peroxidase. Reduced monomeric glutathione is denoted by GSH, and glutathione disulfide is denoted by GS-SG. The mechanism entails the oxidation of a selenocysteine residue selenol by hydrogen peroxide. The derivative with a selenenic acid (RSeOH) group is produced using this procedure. GSH then undergoes a two-stage process not shown in the figure by first producing GS-SeR and water. The GS-SeR intermediate then reduces selenic acid to make selenol, and a second GSH molecule helps create GS-SG as a by-product (Lubos et al., 2011).

3. Antioxidants

Antioxidants are substances that prevent or slow oxidative damage to cells by neutralizing ROS (He et al., 2017). Antioxidants play a crucial role in maintaining cellular homeostasis by counteracting the harmful effects of ROS. Antioxidants are classified into two categories: endogenous and exogenous (He et al., 2017). Endogenous antioxidants are produced by the body, including enzymes such as superoxide dismutase and glutathione peroxidase (*Endogenous And Exogenous Antioxidants Benefits*, 2018). Exogenous antioxidants are obtained from dietary sources, such as vitamin C, vitamin E, and beta-carotene (*Endogenous And Exogenous Antioxidants Benefits*, 2018).

The process by which antioxidants counteract ROS can take many forms but is commonly understood to neutralize them by donating electrons to ROS (*Free radicals vs reactive oxygen species*, 2021). He et al. (2017) explain various ways in which antioxidants play a role in regulating ROS. Antioxidants such as vitamin E and beta-carotene can interrupt the chain reaction of free radicals by reacting with them and preventing further damage. Antioxidants like vitamin C can regenerate other antioxidants that have been oxidized by ROS, allowing them to continue their protective role. Other antioxidants such as flavonoids can bind to metal ions that catalyze the production of ROS, preventing their generation. Certain indirect antioxidants like curcumin and resveratrol can cause endogenous antioxidant enzyme expression, such as glutathione peroxidase and superoxide dismutase, to enhance the cellular defense against ROS.

Antioxidants have been proposed as potential cancer prevention and treatment agents, as they can neutralize ROS and prevent DNA damage. However, the relationship between antioxidants and cancer is complex and poorly understood. Multiple research studies have explored the association between antioxidants and cancer. A few studies found that elevated concentrations of endogenous and exogenous antioxidants can decrease cancer risks. For example, in a study of 35,000 females, it was demonstrated that increased vitamin C levels had reduced the risk of breast cancer (Yun et al., 2015).

However, other studies have found no association between antioxidant intake and cancer risk. A systematic review of 34 studies found no association between beta-carotene intake and prostate cancer risk (Wang et al., 2015). Similarly, a dose-response meta-analysis found little association between vitamin C intake and breast cancer risk (Luo et al., 2014).

Vol. 2024 (11) 447 – 453
ISSN 2688-3651 [online]

There were also studies where antioxidants proved to promote cancer development. For example, in a research study of 36,000 men, it was demonstrated that those who took vitamin E supplements had a higher risk of prostate cancer (Skerrett, 2014). Meanwhile, research on 73,000 women revealed that those who took vitamin E supplements had a higher lung cancer risk (Wu et al., 2014).

Antioxidants have also been proposed as potential adjunct therapies for cancer treatment. Radiation therapy and chemotherapy generate ROS as a mechanism of action, leading to DNA damage and cancer cell death. However, ROS can also damage normal cells, leading to side effects of treatment. Antioxidants have been suggested as a way to protect normal cells from ROS damage during cancer treatment, potentially reducing side effects (Singh et al., 2018).

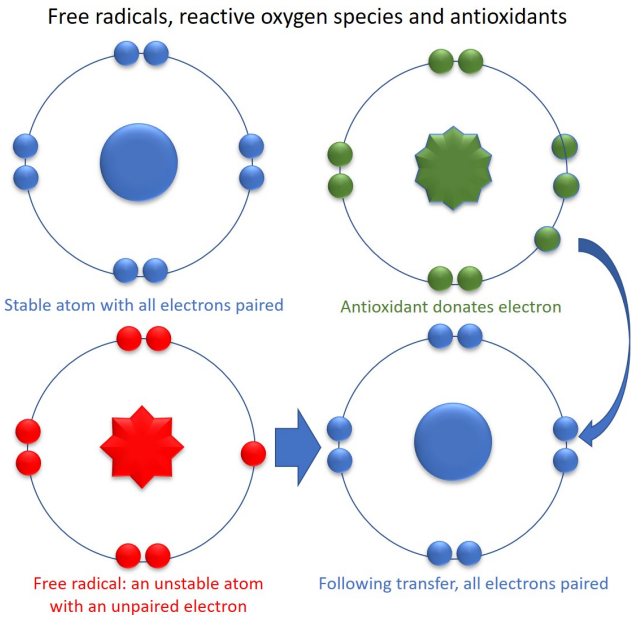

Figure 3. Antioxidant Neutralizing a Free Radical (*Free Radicals Vs Reactive Oxygen Species*, 2021).

However, some studies have suggested that antioxidants may also protect cancer cells from the effects of treatment, reducing its effectiveness. Namely, a study of breast cancer patients found that those who took antioxidant supplements during chemotherapy had a lower response rate and lower survival rates compared to those who did not take supplements (Ambrosone et al., 2020).

4. Ferroptosis

Ferroptosis is a distinct form of cell death dependent on iron that is morphologically, biochemically, and genetically different from other forms of programmed cell death, such as apoptosis and necroptosis (Čepelak et al., 2020). It was first found by Dixon et al. (2012) who recognized a small molecule known as erastin that induced cell death selectively in a panel of cancer cell lines. Erastin reduces the intracellular glutathione antioxidant levels by suppressing the cysteine-glutamate antiporter system (system Xc-) and deactivating glutathione peroxidase 4 (GPX4), which increases the development of lipid peroxides and iron-dependent ROS, consequently inducing ferroptosis since cysteine is needed for the glutathione (GSH) synthesis (Zhao et al., 2021). Yang et al. (2014) revealed that erastin-induced cell death was based on the activity of lipid peroxidation enzyme 15-lipoxygenase (15-LOX) and was presented by lipid peroxide accumulation within the cell membrane. Since then, other associated molecules have been believed to induce ferroptosis, including sulfasalazine, RSL3, and artesunate, among others.

Ferroptosis greatly influences different pathological conditions, such as cancer. It is believed that ferroptosis could offer promising therapeutic intervention for cancer management since cancer cells may have more susceptibility to ferroptosis compared to healthy cells due to their altered metabolic pathways (Yang et al., 2014). For instance, several oncogenes such as MYC, RAS, and BCL-2 are considered to support the growth of tumor cells through ferroptosis suppression (Yang et al., 2014). Similarly, Nengroo et al. (2022) explored that oncogenes such as TP53's mutation could change cell susceptibility to ferroptosis. Antioxidants also stop ROS-induced ferroptosis in normal and cancerous cells, which reduces the effectiveness of this therapy.

Furthermore, taking too many antioxidants can throw off the redox balance of cells, which can have detrimental effects, including lowered immune responses and increased toxicity. Additionally, it has been investigated if tissue damage and organ dysfunction might result from noncancerous cells experiencing ferroptosis. Therefore, to minimize

Vol. 2024 (11) 447 – 453
ISSN 2688-3651 [online]

side effects and optimize therapeutic benefits, the right dosage and tailored distribution are essential (Guan et al., 2021).

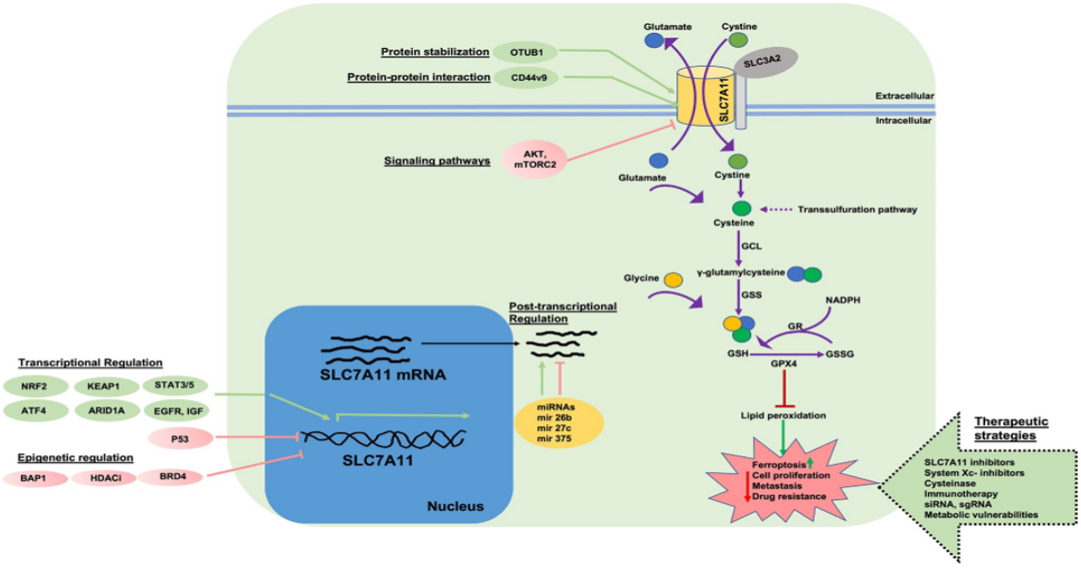

Figure 4. Function, Regulation, and Targeting of SLC7A11. The light chain component SLC7A11 and heavy chain subunit SLC3A2 form the cystine/glutamate antiporter system Xc-, which imports extracellular cystine to produce cysteine, a key precursor in glutathione (GSH) synthesis. GSH acts as a cofactor for GPX4, protecting cells from lipid peroxidation and ferroptosis (promoting cell proliferation, survival, and drug resistance), while SLC7A11 is regulated at multiple levels, including transcriptional activation by factors like ATF4 and NRF2, post-transcriptional control by miRNAs, and post-translational stabilization through protein-protein interactions (Jyotsana et al., 2022).

5. Conclusion

The increasing understanding of the interplay between ROS, ferroptosis, and antioxidants has led to the development of novel cancer therapies. These therapies target ROS and ferroptosis, promoting cancer cell death and inhibiting tumor growth. Some promising approaches include using pro-oxidant agents to increase ROS levels and induce ferroptosis in cancer cells. Other methods involve using small molecules that inhibit antioxidant enzymes or promote lipid peroxidation, leading to ferroptosis.

However, the results with the details above show no evident similarities between the results, the cancer types, and the type of antioxidant used. The clinical application of these novel therapies requires further investigation and optimization. The potential side effects of these therapies on normal cells and tissues need to be carefully evaluated, and the optimal dosing and combination with other therapies need to be determined. Additionally, the impact of these therapies on the tumor microenvironment, including the immune system, requires further investigation.

In conclusion, the interplay between ROS, ferroptosis, and antioxidants in cancer is a promising area of research that has the potential to lead to novel cancer therapies. The development of these therapies requires further investigation and optimization, but their potential to improve cancer treatment outcomes is significant.

References

Aggarwal, V. et al. (2019). Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. *Biomolecules, 9*(11), 735. https://doi.org/10.3390/biom9110735

Vol. 2024 (11) 447 – 453
ISSN 2688-3651 [online]

Ambrosone, C. B., et al. (2020). Dietary supplement use during chemotherapy and survival outcomes of patients with breast cancer enrolled in a cooperative group clinical trial (SWOG S0221). *Journal of Clinical Oncology, 38*(8), 804–814. https://doi.org/10.1200/jco.19.01203

Antioxidants accelerate the growth and invasiveness of tumours in mice. (2015, November 12). National Cancer Institute. https://www.cancer.gov/news-events/cancer-currents-blog/2015/antioxidants-metastasis

Auten, R. L., & Davis, J. M. (2009). Oxygen toxicity and reactive oxygen species: The devil is in the details. *Pediatric Research, 66*(2), 121–127. https://doi.org/10.1203/pdr.0b013e3181a9eafb

Black, B., Lee, C., Horianopoulos, L. C., Jung, W. H., & Kronstad, J. W. (2021). Respiring to infect: Emerging links between mitochondria, the electron transport chain, and fungal pathogenesis. *PLoS pathogens*, *17*(7), e1009661. https://doi.org/10.1371/journal.ppat.1009661

Business Bliss FZE. (2023, November 6). *Endogenous And Exogenous Antioxidants Benefits*. https://us.ukessays.com/essays/biology/using-endogenous-and-exogenous-antioxidants-biology-essay.php? vref=1

Čepelak, I., Dodig, S., & Dodig, D. Č. (2020). Ferroptosis: regulated cell death. *Archives of Industrial Hygiene and Toxicology*, *71*(2), 99-109. https://sciendo.com/article/10.2478/aiht-2020-71-3366

Chung, Y. M., Bae, Y. S., & Lee, S. Y. (2003). Molecular ordering of ROS production, mitochondrial changes, and caspase activation during sodium salicylate-induced apoptosis. Free radical biology & medicine, 34(4), 434–442. https://doi.org/10.1016/s0891-5849(02)01301-1

De Leo, A., et al. (2021). What is new on ovarian carcinoma: integrated morphologic and molecular analysis following the new 2020 World Health Organization classification of female genital tumours. *Diagnostics*, *11*(4), 697. https://www.mdpi.com/2075-4418/11/4/697

Dixon, S. J., et al. (2012). Ferroptosis: an Iron-Dependent form of nonapoptotic cell death. *Cell, 149*(5), 1060–1072. https://doi.org/10.1016/j.cell.2012.03.042

Free radicals vs reactive oxygen species. (2021, August 30). Cell Guidance Systems. https://www.cellgs.com/blog/free-radicals-vs-reactive-oxygen-species-whats-the-difference.html

Guan, Q., Zhou, L. L., & Dong, Y. B. (2021). Ferroptosis in cancer therapeutics: a materials chemistry perspective. Journal of Materials Chemistry B, 9(43), 8906-8936. https://pubs.rsc.org/en/content/articlelanding/2021/tb/d1tb01654g/unauth

Hamanaka, R. B., & Chandel, N. S. (2010). Mitochondrial reactive oxygen species regulate cellular signalling and dictate biological outcomes. *Trends in biochemical sciences, 35*(9), 505–513. https://doi.org/10.1016/j.tibs.2010.04.002

He, L., et al. (2017). Antioxidants maintain cellular redox homeostasis by the elimination of reactive oxygen species. *Cellular Physiology and Biochemistry, 44*(2), 532–553. https://doi.org/10.1159/000485089

Johansson, Å., et al. (2016). Impaired phagocytosis and reactive oxygen species production in phagocytes are associated with systemic vasculitis. *Arthritis Research & Therapy, 18*(1).

Jyotsana, N., Ta, K. T., & DelGiorno, K. E. (2022). The role of cystine/glutamate antiporter SLC7A11/xCT in the pathophysiology of Cancer. *Frontiers in Oncology*, *12*, 858462. https://doi.org/10.3389/fonc.2022.858462

Klein, E. A., et al. (2011). Vitamin E and the risk of prostate cancer. *JAMA, 306*(14), 1549. https://doi.org/10.1001/jama.2011.1437

Liu, J., Kang, R., & Tang, D. (2021). Signalling pathways and defence mechanisms of ferroptosis. *The FEBS Journal, 289*(22), 7038–7050. https://doi.org/10.1111/febs.16059

Vol. 2024 (11) 447 – 453
ISSN 2688-3651 [online]

Lobo, V., et al. (2010). Free radicals, antioxidants and functional foods: Impact on human health.

Lubos, E., Loscalzo, J., & Handy, D. E. (2011). Glutathione peroxidase-1 in Health and Disease: From molecular mechanisms to therapeutic opportunities. *Antioxidants & Redox Signaling, 15*(7), 1957–1997. https://doi.org/10.1089/ars.2010.3586

Luo, J., Shen, L., & Zheng, D. (2014). Association between vitamin C intake and lung cancer: a dose-response meta-analysis. *Scientific Reports, 4*(1). https://doi.org/10.1038/srep06161

Nengroo, M. A., Sinha, A., & Datta, D. (2022). Iron Vulnerability of Cancer Stem Cells: Role of ROS and Beyond. In Handbook of Oxidative Stress in Cancer: Therapeutic Aspects (pp. 2509-2537). Singapore: Springer Nature Singapore. https://link.springer.com/referenceworkentry/10.1007/978-981-16-5422-0_235

Raimondi, V., Ciccarese, F., & Ciminale, V. (2019). Oncogenic pathways and the electron transport chain: a dangerous liaison. *British Journal of Cancer, 122*(2), 168–181. https://doi.org/10.1038/s41416-019-0651-y

Segal, A. W. (2008). The function of the NADPH oxidase of phagocytes and its relationship to other NOXs in plants, invertebrates, and mammals. *The International Journal of Biochemistry & Cell Biology, 40*(4), 604–618. https://doi.org/10.1016/j.biocel.2007.10.003

Shields, H. J., Traa, A., & Van Raamsdonk, J. M. (2021). Beneficial and detrimental effects of reactive oxygen species on lifespan: A comprehensive review of comparative and experimental studies. *Frontiers in Cell and Developmental Biology, 9*. https://doi.org/10.3389/fcell.2021.628157

Singh, K., et al. (2018). Antioxidants as precision weapons in the war against cancer chemotherapy-induced toxicity – Exploring the armoury of obscurity. *Saudi Pharmaceutical Journal, 26*(2), 177–190. https://doi.org/10.1016/j.jsps.2017.12.013

Singh, R., & Manna, P. P. (2022). Reactive oxygen species in cancer progression and its role in therapeutics. *Exploration of Medicine*, *3*(1), 43-57. https://doi.org/10.37349/emed.2022.00073

Skerrett, P. (2014, February 28). *Selenium and vitamin E supplements increase prostate cancer risk*. Harvard Health.

Snezhkina, A. V., et al. (2019). ROS generation and antioxidant defence systems in normal and malignant cells. *Oxidative Medicine and Cellular Longevity, 2019*, 1–17. https://doi.org/10.1155/2019/6175804

Sources of Reactive Oxygen Species (ROS) with Cell Background, by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates

Ushio-Fukai, M., & Nakamura, Y. (2008). Reactive oxygen species and angiogenesis: NADPH oxidase as a target for cancer therapy. *Cancer Letters, 266*(1), 37–52. https://doi.org/10.1016/j.canlet.2008.02.044

Wang, Y., et al. (2015). Effect of carotene and lycopene on the risk of prostate Cancer: A Systematic Review and Dose-Response Meta-Analysis of Observational Studies. *PLOS ONE, 10*(9), e0137427. https://doi.org/10.1371/journal.pone.0137427

Wu, Q., et al. (2014). Vitamin E intake and lung cancer risk among female nonsmokers: A Shanghai Women's Health Study report. *International Journal of Cancer, 136*(3), 610–617. https://doi.org/10.1002/ijc.29016

Yang, W. S., et al. (2014). Regulation of ferroptotic cancer cell death by GPX4. *Cell, 156*(1–2), 317–331. https://doi.org/10.1016/j.cell.2013.12.010

Yun, J., et al. (2015). Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science, 350*(6266), 1391–1396. https://doi.org/10.1126/science.aaa5004

Zhao, J., et al. (2021). Erastin-induced ferroptosis causes physiological and pathological changes in the healthy tissues of mice. Molecular Medicine Reports, 24(4). https://doi.org/10.3892/mmr.2021.12352