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The impact of mitochondrial functionality on oxidative stress in human cancer cells: a Systematic Review

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ABSTRACT
This systematic review aimed to evaluate research done on the impact of mitochondrial functionality on oxidative stress in human cancer cells with three objectives. The objectives were related to observing temporal trends in research on this topic, evaluating the cause of oxidative stress in human cancer cells and reviewing the progress in research on interventions and therapies. Papers were identified from Google Scholar using search terms for each objective. The identified papers were screened through the PRISMA flow process to obtain 10 papers each for each objective.
This review showed that the number of papers increased by the year and the USA and China dominated in this respect. Oxidative stress due to mitochondrial malfunctions and certain allied factors were identified as the main causes of human cancer. Many drugs are used for cancer therapy, but without adequate evidence and involve many risks. Many new drugs are at various trial stages. Nano-technology-based targeted drug delivery systems promise to be a promising method of effective cancer therapy with minimum risk. This may be a future research area along with the development of novel drugs.
The limitations of using a single database, papers in English only and limiting the number of papers to 10 for each objective are acknowledged.

Keywords: Mitochondrial functions, Oxidative Stress, Human cancer cells, Reactive Oxygen Species (ROS).

INTRODUCTION

A description of mitochondria and their relationship with health was provided by Harrington, et al. (2023). Mitochondria are organelles essential for sustaining cellular bioenergetics through ATP production. Apart from oxidative phosphorylation, mitochondria helpsynthesise metabolic precursors, regulate calcium, generate reactive oxygen species, immune signalling, and facilitate apoptosis. Thus, mitochondria are critical for cellular metabolism and maintaining homeostasis. The glucose metabolism pathways and oxygen production in mitochondria are presented in Fig 1 from Sosa, et al. (2012). In the presence of oxygen, glucose is converted into pyruvate, which is then utilised by mitochondria for respiration and ATP production. Without oxygen, glucose may enter the pentose phosphate pathway (PPP) or be transformed into lactate, but its byproducts do not reach the mitochondria. Under normal physiological conditions, mitochondria are the primary source of reactive oxygen species (ROS). During this process, superoxide is produced and quickly converted into hydrogen peroxide (H2O2) by the antioxidant enzyme manganese superoxide dismutase (MnSOD), aiding in the defence against oxidative stress. Glycolysis through the PPP pathway is the typical response when cellular oxygen levels are low.

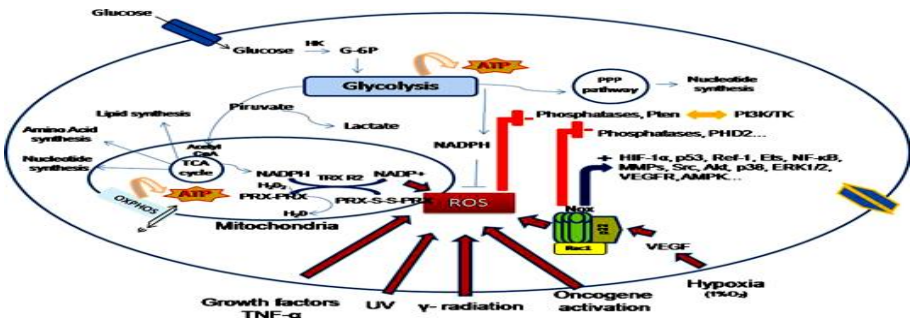


Figure 1: Glucose pathways and oxygen production in mitochondria (Sosa, et al., 2012).

Fig 2 from Sosa, et al. (2012) shows the tumour microenvironment significantly influencing angiogenesis and tumour development. Cancer cells release reactive oxygen species (ROS) that affect nearby cancer-associated fibroblasts (CAFs), leading to oxidative stress and the activation of key transcription factors and signalling proteinswhich promote angiogenesis. CAFs produce matrix metalloproteinases (MMPs) and cytokines that support tumour growth and suppress the immune response. Additionally, senescent cells release cytokines that aid tumour development. Within the immune system, cytotoxic CD8+ and CD4+ T cells, along with their cytokine IFN-γ, act as anti-tumour agents, while macrophages associated with cancer release various cytokines (e.g., IL-6, TNF) that generally enhance tumour growth.

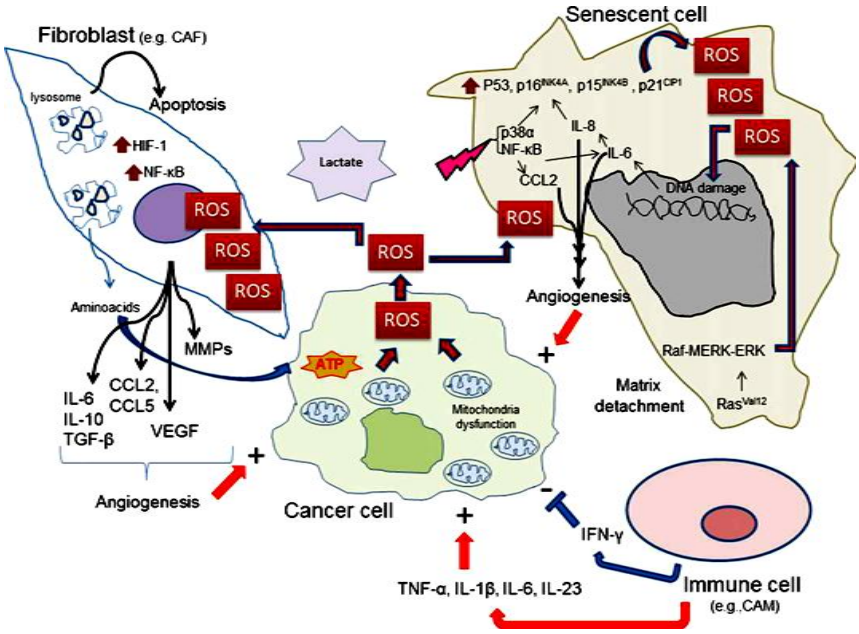


Figure 2: Tumour environment biochemistry (Sosa, et al., 2012).

Mitochondrial metabolism, cellular bioenergetics, mitochondrial dynamics, autophagy, damage-associated molecular patterns and pathways of mitochondria-mediated cell death are linked to disease development. Consequently, mitochondrial pathways could serve as promising therapeutic targets for improving human health. As shown in Fig 3, (A) Normal mitochondria produce ATP via an active electron transport chain generating energy by oxidizing acetyl-CoA during the Krebs cycle and ensuring mitochondrial integrity through dynamic processes like fission and fusion. (B) The loss of mitochondrial bioenergetics is linked to various diseases. Mitochondrial reactive oxygen species (mtROS) can activate cyclophilin D, leading to necrosis and further mitochondrial dysfunction. Various mitochondrial processes are associated with the initiation and development of cancer (Harrington, et al., 2023).

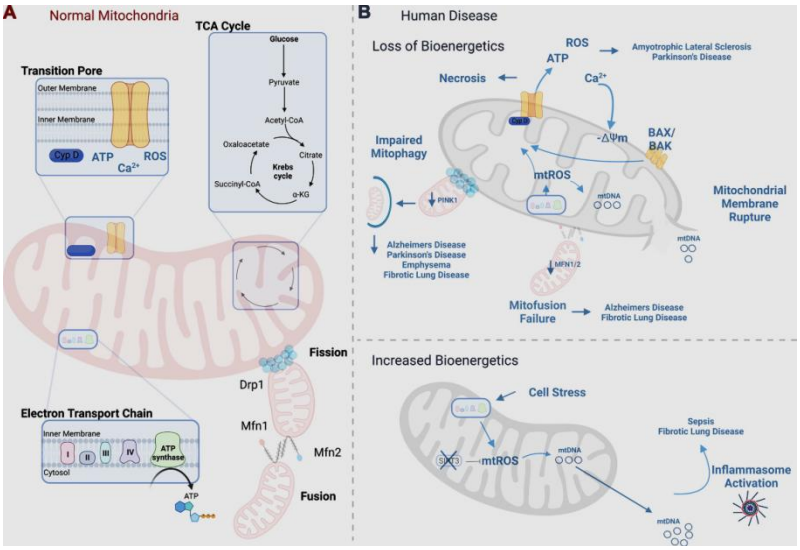


Figure 3: Mitochondrial bioenergetics in normal and human disease conditions (Harrington, et al., 2023)

Although oxygen is a vital necessity for health, excess oxygen can lead to oxidative stress and related diseases. Hypoxia (oxidative stress) occurs when there is an insufficient oxygen supply condition andhyperoxia occurs when oxygen is in oversupply. Hypoxia can cause breast, head, neck, and cervical cancers (Bae, et al., 2024). Cancer cells demand higher levels of oxygen forfaster metabolism leading to a faster multiplication rate. Many cancer treatments rely on interventions aimed at developing resistance against oxygen accumulation.

The above description of mitochondrial functions, glucose metabolism and tumour biochemistry prompts a review of the research works done on the impact of mitochondrial functionality on oxidative stress in cancer cells. A systematic review of the relevant literature was conducted with the following aim and research objectives.

The aim and research objectives

Aim

To evaluate research done on the impact of mitochondrial functionality on oxidative stress in human cancer cells.

Research objectives

The research objectives are:

a) To observe temporal trends in the research on the impact of mitochondrial functionality on oxidative stress in human cancer cells.
b) To evaluate the causes of oxidative stress in human cancer cells related to mitochondrial functionality.
c) To review the research done oninterventions to reduce oxidative stress due to the impact of mitochondrial functionality.

The detailed methodology to achieve these objectives is described below.

METHODOLOGY

Searching and identification of papers for possible inclusion

Google Scholar web pages were searchedto identify papers for this review. The search terms used were related to the research objectives as given in Table 1.

Objective	Search terms
To observe temporal trends in the research on the impact of mitochondrial functionality on oxidative stress in human cancer cells.	Historical trends in research on the impact of mitochondrial functionality on oxidative stress in cancer cells. A chart on years versus frequency of papers will be done from the list of papers selected.
To evaluate the causes of oxidative stress in human cancer cells related to mitochondrial functionality.	Epidemiological factors, biochemical factors, micro-environment in cancer cells,
To review the research done on interventions to reduce oxidative stress due to the impact of mitochondrial functionality.	Interventions, therapy, prevention

The papers identified using the above search terms were screened and selected repeatedly according to the inclusion and exclusion criteria given in Table 2 through a PRISMA flow process (Appended) to select 30 important papers, 10 each for each objective.

Table 2. Inclusion and exclusion criteria for selection of papers.

Inclusion criteria	Exclusion criteria	Remarks
Papers in English	Other languages	Although translations are possible, some distortions may occur in the translations.
Published during 2020-2025 to reflect the recent trends.	Earlier papers	If some earlier paperscontain some useful concepts or frameworks cited in a selected paper, they will be cross-referred.
Full texts	Abstracts	However, if abstracts contain useful information, they may be used to strengthen the points, but not included in the Excel spreadsheet.
Research papers, reports, reviews	Books, book sections, dissertations, comments on papers,	If the full book is available and editorials contain important aspects, they may be included.

	editorials	
Only papers related to cancer in human beings	Studies on animals.	This is aimed at reducing the complexity of matching the results on human and animal cells.

The data from the finally selectedpapers were tabulated in an Excel spreadsheet with the reference details, aim, method, findings, limitations, and quality assessments using the following variables:

Citations per year
The number of citations is available for most papers in Google Scholar. However, the years of their publications are different. To compare the quality of papers based on the number of citations the total number of citations was divided by the number of years from publication to 2024. Thus, the average number of citations per year was used as a quality measure.

Adequacy of evidence assessment
Whether the evidence presented is adequate to reach the stated conclusion was qualitatively assessed using 1 (lowest) to 5 (highest) levels of adequacy.

Risk of Bias (RoB)
The National Toxicology Program's Office of Health Assessment and Translation (NTP OHAT) risk of bias (RoB) tool was used. This tool assesses RoB based on the criteria to select study participants, confounding, measurement of exposure and outcomes, follow-up of study participants, adequacy of the reporting of outcomes, and pre-specification of study analysis/study protocol. RoB was rated as 1 (lowest) to 5 (highest).

GRADE
Certainty of evidence was evaluated by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework. This was assessed as 1(lowest) to 5 (highest).
CCAT
Crowe Critical Appraisal Tool (CCAT; version 1.4) was used as a quality index of papers. CCAT consists of preliminaries, introduction, design, sampling, data collection, ethical matters, results, and discussion as the assessment items. The range of scores is 0 (lowest quality) to 5 (highest quality).

Overall quality
This score was obtained by adding the scores of mean citations per year, adequacy of evidence, GRADE and CCAT, subtracting the risk of bias from it, and then dividing the net sum by 5. That is-
Overall quality = (Citations +Evidence adequacy+GRADE+CCAT-RoB)/5.

Synthesis of literature
The data collected in the Excel spreadsheet were used for some quantitative synthesis across the papers to discover general trends. The detailed discussions were based on these and other qualitative aspects. Similar findings across different papers were pooled together and differentiated from contradictory findings to achieve the desired level of synthesis.In the results section, each paper is described in detail cross-referring to earlier concepts or frameworks if relevant. The PRISMA flow diagram is appended.

RESULTS
1. To observe temporal trends in the research on the impact of mitochondrial functionality on oxygen stress in human cancer cells.
Search term: Historical research trends on the impact of mitochondrial functionality on oxidative stress in human cancer cells.
A steady increase in papers over the years and the dominance of the USA and China in this field of research were observed by Song, et al. (2023), Jiang, et al. (2024), Chen, et al. (2024), Ji, et al. (2024), Qi, et al. (2022),Wan, et al. (2024) and Hu, et al. (2022). Most of these papers selected papers from the Web of Science Core Collection (WoSCC) and used VOSviewer and CiteSpace for bibliometric analyses.
Wang, et al. (2020)noted thatthe earliest evidence of mitochondria dates back to the 1840s, but the term "mitochondria" was coined in the 1890s when their structure was identified during spermatogenesis. In the 1920s, Otto Warburg identified the deregulation of cellular energetics as a potential cause of cancer. While normal cells metabolise glucose using mitochondria in oxygen-rich environments, they resort to glycolysis and convert glucose to lactate under anaerobic conditions. Cancer cells, however, continue to rely on glycolysis even in the presence of oxygen, prompting further research into the biochemical mechanisms of mitochondrial

function.Additionally, a diagram was givenbyJiang, et al. (2024) explaining the research area focus each year as shown in Fig 4.

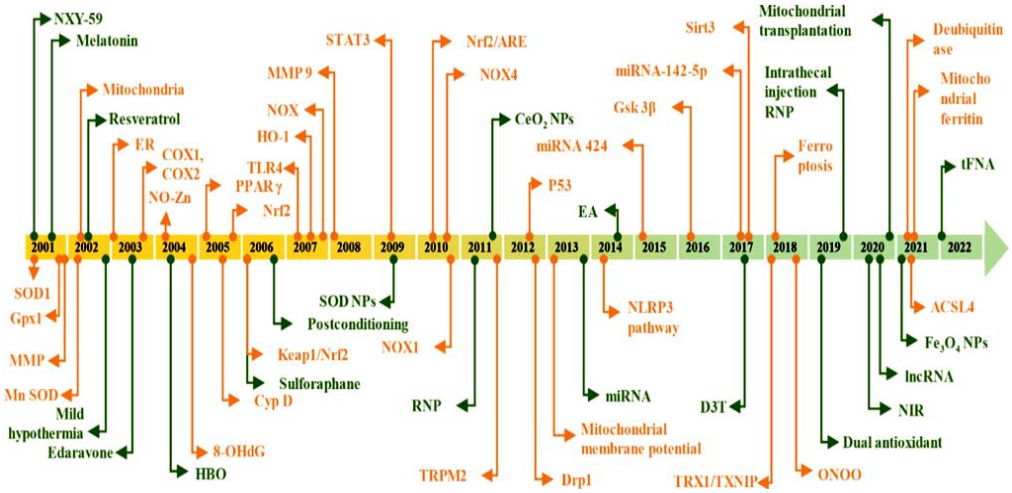


Figure 4: Historical trend of research topics in oxidative stress and ischemic stroke (Jiang, et al., 2024).

Pang, et al. (2023)usedthe Number of Papers (NP) to measure production capacity, while the Number of Citations (NC) serves as an indicator of a journal's impact factor. When a scholar publishes H articles, and each of those articles is cited at least H times, the scholar's H-index is H. This index merges productivity and influence by establishing a link between NP and NC. A similar index (citations per year) for quality assessment of the papers has been used in this review.The USA leads in terms of both the number of articles published and the highest NC and H-index, followed by China and Italy. The Global Citation Score trend across countries is shown in Fig 5.

Figure 5: G Score of reviewed papers by countries (Pang, et al., 2023).

In the bibliometric analysis by Hu, et al. (2022) Science Citation Index Expanded (SCIE) within the Web of Science Core Collection (WoSCC) was used.The mean number of citations per paper was 28.1 and the total H-index was 63. For mitochondrial-related research in the ALI/ARDS field, 4574 authors affiliated with 1217 institutions were published in 346 journals worldwide from 61 countries or regions.The primary research areas

weremtDNA, mitophagy, and apoptosis, with mitochondrial transfer being of increased recent attention.The research hotspots shifted from potential treatments and mitochondrial DNA (mtDNA) to endothelial cells and mesenchymal stromal cells (MSC).

Oxidative stress was one main area of research in the above reviews. The points relevant to this review are the relationships of oxidative stress (Lin, et al., 2021) on human breast cancer and the biochemical mechanisms (Liu, et al., 2020) on colorectal cancer. In another bibliometric review, Xing, et al. (2024) used a method similar to Song, et al. (2023) and obtained similar results. However, this paper specifically dealt with tumour microenvironment (TME) and immune response.

2. To evaluate the causes of oxidative stress in human cancer cells related to mitochondrial functionality.

Search terms: Epidemiological factors, biochemical factors, micro-environment in cancer cells (epidemiological and biochemical factors related to oxidative stress in microenvironment of human cancer cells).

The imbalance between prooxidants and antioxidants, resulting in excessive production of reactive oxygen and nitrogen species (ROS/RNS), leads to oxidative stress, which is linked to cancer development. High levels of ROS can damage cellular components like proteins, lipids, and DNA, potentially initiating cancer and producing 8-hydroxydeoxyguanosine (8-OH-G), a marker for carcinogenesis. The role of antioxidants in cancer therapy is controversial due to their unpredictable interactions with chemotherapy drugs. While antioxidants can neutralize ROS and mitigate their harmful effects, excessive long-term supplementation may increase cancer risk. This dual role of antioxidants is noted in various cancers, including colorectal and breast cancers, with some research suggesting the effectiveness of endogenous and exogenous antioxidants in cancer prevention. Recent advancements in nanotechnology also show promise for delivering bioactive compounds directly to tumour tissues for enhanced treatment efficacy(Zahra, et al., 2021).

The oxidative stress is exploited to develop therapeutic strategies that control tumour growth by modulating the oxidative stress in tumour cells. A review of the recent progress in the impact of ROS on cancer cells and various immune cells in the tumour microenvironment(TME) byAboelella, et al. (2021) showed some promising and emerging trends of ROS-modulating strategies that can combine with cancer immunotherapies to achieve enhanced antitumor effects. A diagram of the interactions between cells in a tumour microenvironment leading to changes in redox status is shown in Fig 6.

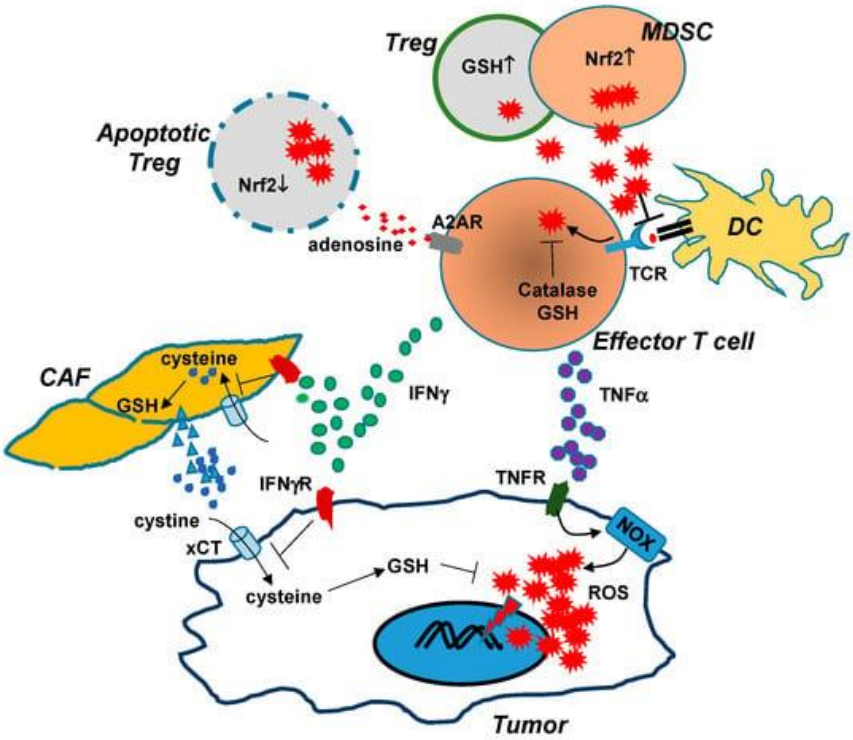


Figure 6: interactions between cells in TME leading to redox status changes (Aboelella, et al., 2021).

A diagram ofthe hypothetical mechanisms by which certain pro-oxidants enhance the efficacy of cancer immunotherapies is shown in Fig 7.

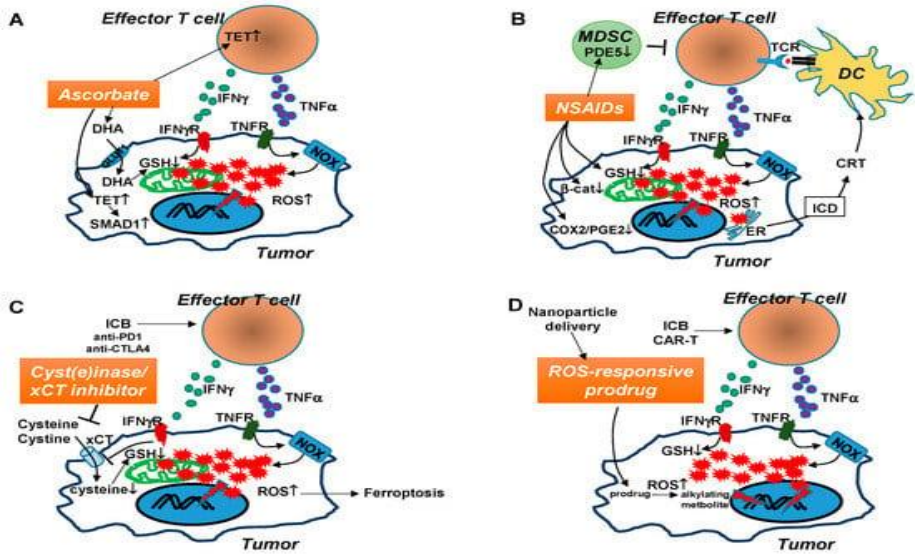


Figure 7: A diagram explaining some hypothetical mechanisms of oxidants enhancing cancer immunotherapies (Aboelella, et al., 2021).

Zińczuk, et al. (2021) evaluatedthe total antioxidant capacity, nitrosative stress, and levels of protein/DNA oxidation and glycoxidation products in 30 colorectal cancer patientsconcerning histopathological features of the tumour microenvironment, such as inflammatory infiltration and tumour budding. Comparisons were made between tumours located on the right and left sides of the colon, as well as with normal mucosa. Certain chemical levels were found to be varied between the left and right-side tumours, suggesting an association of colorectal cancer with impaired antioxidant defence and increased oxidative and nitrosative damage to proteins and DNA. These parameters may help assess tumour progression and differentiation, as redox indicators appear to be linked to the tumour's histological type, influencing factors like tumour invasion depth, lymph node and distant metastasis, vascular and neural invasion, inflammatory infiltration, and tumour budding.

Based on a review of the literature,Wigner, et al. (2021) proposed a model for the mutual interaction of oxidative stress, inflammation and angiogenesis in the development of bladder cancer, as shown in Fig 8.Oxidative stress maintains an inflammatory microenvironment topromote an increased proliferation of cancer cells. Cytokines further increase ROS production. Thisleads to a vicious circle phenomenon. In angiogenesis, both oxidative stress and inflammation are involved in the activation of pro-angiogenesis factors and the inhibition of anti-angiogenesis compounds. The JAK-STAT-3 pathway is related tothe malignant transformation of urothelial cells. Therefore, the progression of bladder cancer may involve both ROS and proinflammatory cytokines.

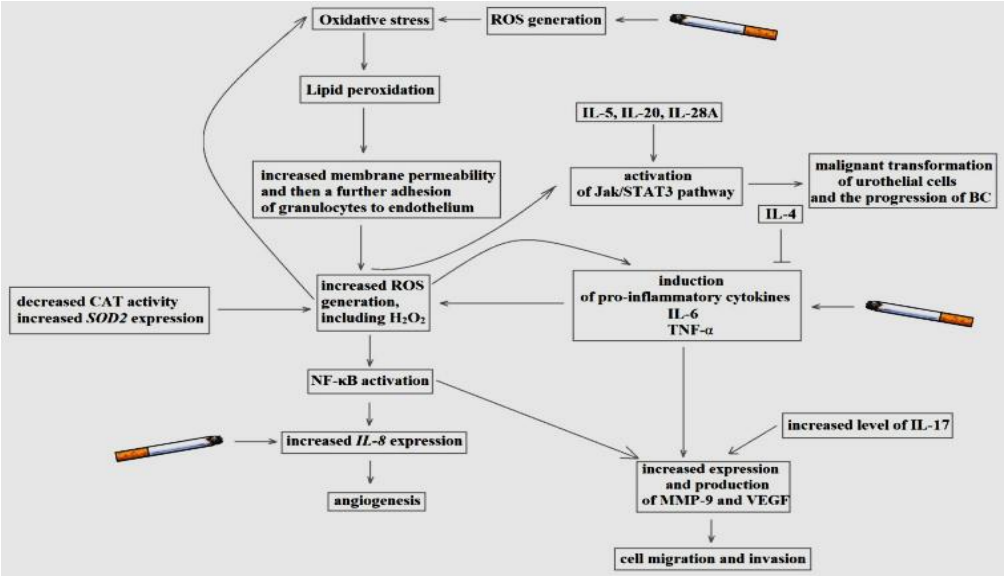


Figure 8: Mutual interaction of oxidative stress, inflammation and angiogenesis in bladder cancer development (Wigner, et al., 2021).

A review byAceto, et al. (2020) concentrated on howadenomatous polyps develop through the interplay of renewal signalling in colon epithelium and the generation of reactive oxygen species (ROS). Key risk factors for colorectal cancer (CRC) include older age, male sex, Western and African American ethnicity, exposure to organic pollutants and pesticides, heavy metals (like arsenic and cadmium), antibiotics, food additives, processed red meat, high saturated fats, stress, sedentary lifestyle, obesity, smoking, alcohol consumption, and changes in gut microbiota. The authors suggest that oxidative stress could promote the formation of colorectal adenomas by influencing Wnt signalling pathways and the DNA damage response. Understanding the pathophysiological mechanisms that link Wnt signalling, microbiota imbalances, oxidative stress, and DNA damage could enhance knowledge about the development of CRC. This knowledge can inform new prevention strategies and innovative treatments for colorectal tumours.

Many studies of the health risks of low-dose radiation, particularly after the Fukushima Daiichi nuclear accident indicate that cancer risks from exposure below ~100 mSv are mostly negligible. However, when the antioxidant response is disrupted, it can lead to chronic oxidative stress, particularly in cancer-associated fibroblasts, which may promote carcinogenesis. Recent studies suggest that radiation impacts not only cancer cells but also surrounding stromal cells. Further research is necessary to explore the interactions between tumour cells and stromal fibroblasts to better understand the implications of low-dose radiation exposure(Shimura, 2021). A diagram of mitochondrial ROS-mediated tumour microenvironment formation due to radiation-related cancer is shown in Fig 9.

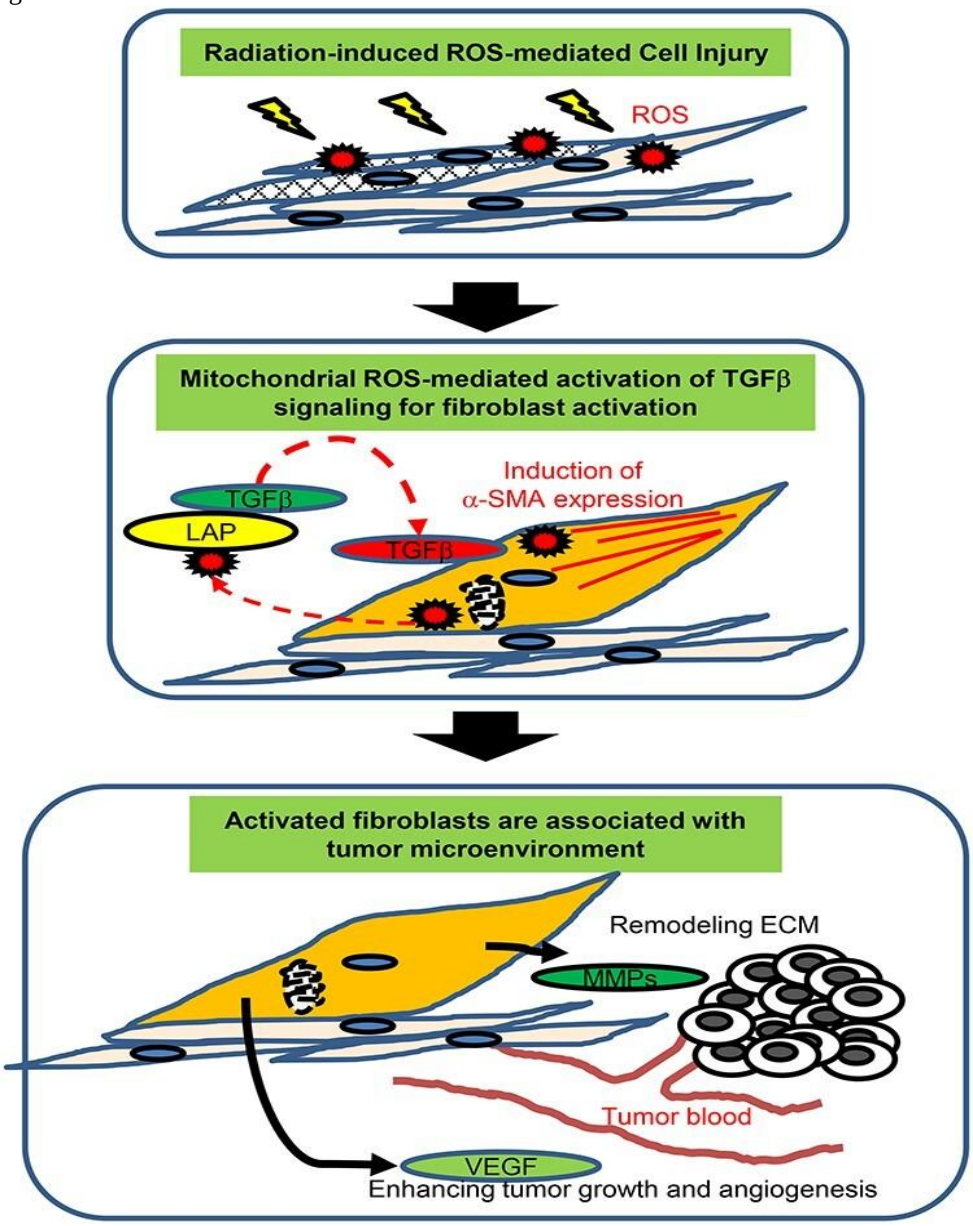


Figure 9: Mitochondrial ROS-mediated tumour microenvironment formation due to radiation-related cancer (Shimura, 2021).

Dharshini, et al. (2023)found that oxidative stress and inflammation contribute to several cancer processes like cellular proliferation, differentiation, angiogenesis, migration, invasion, metabolic alterations, and resistance to programmed cell death. An imbalance between ROS and antioxidants leads to oxidative stressdamaging essential macromolecules and disrupting biological signalling pathways. Extended oxidative stress results in inflammation through the activation of transcription factors which modify the expression of numerous other genes and proteins, including growth factors, tumour-suppressor genes, oncogenes, and pro-inflammatory cytokines, ultimately aiding in the survival of cancer cells.

Prolonged oxidative stress (OS) may contribute to the onset of different types of cancers. The review by Bardelčíková, et al. (2023) examined the role of oxidative stress in inflammation related to colorectal cancer (CRC). Cancer could arise from extended exposure to various carcinogenic influences, particularly inflammation.While various endogenous and exogenous antioxidant molecules may effectively decrease oxidative stress in the intestines, definite proof for antioxidant therapy leading to a cure is lacking. Hence, anti-inflammatory drugs or substances with an antioxidant effect are used for the treatment and prevention of cancer.

Under normal conditions, free radicals are removed from the body by antioxidant processes. Disruption of these processes leads to their excess accumulation and the development of various illnesses. A review byChaudhary, et al. (2023) showed that antioxidants of various types can be used to mitigate this problem. They help to supplement the antioxidant defence mechanism internally. A summarised scheme of ROS generation in mitochondria is shown in Fig 9.

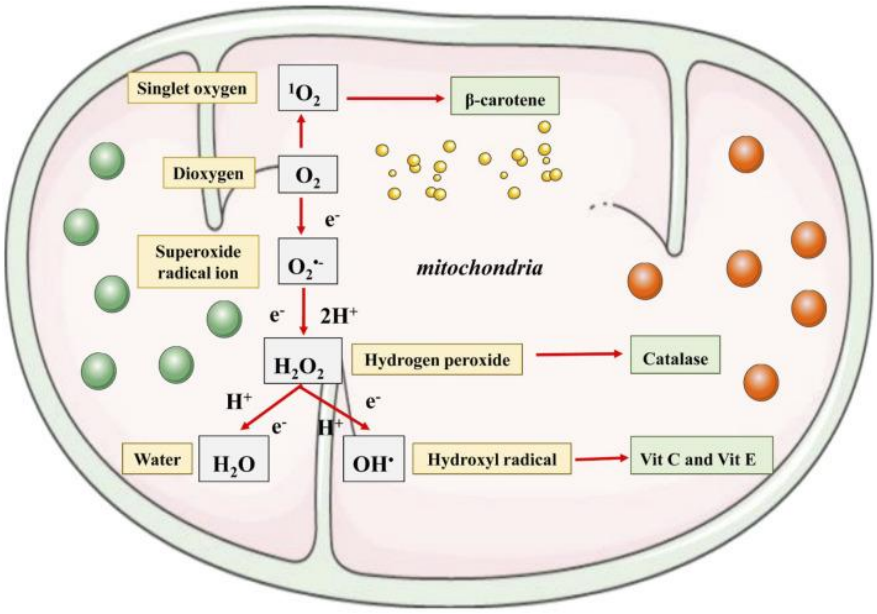
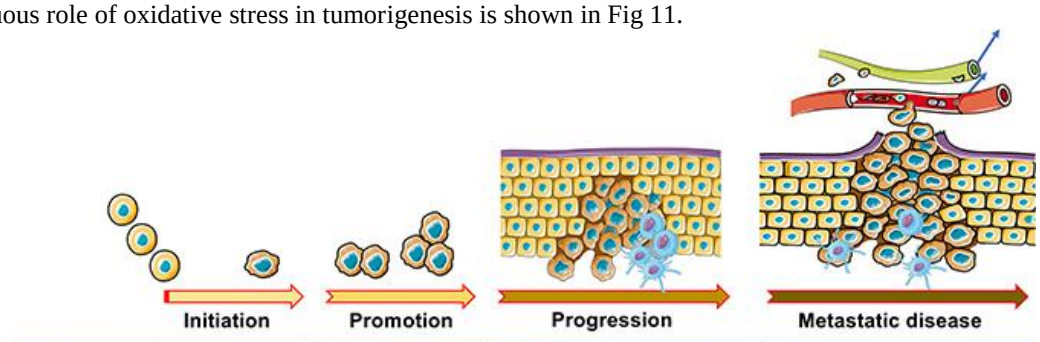


Figure 10: A summarised scheme of ROS generation in mitochondria (Chaudhary, et al., 2023).

Hepatocellular carcinoma (HCC) is a severe cancer often linked to chronic liver diseases, and it can arise from noncirrhotic non-alcoholic steatohepatitis (NASH), a condition associated with obesity, hyperlipidaemia, insulin resistance, and type 2 diabetes. Gutiérrez-Cuevas, et al. (2023)reviewed the molecular aspects of the HCC-NASH relationship, focusing on metabolic changes, genetic and epigenetic factors, and current therapeutic strategies. They highlighted the significant role of mitochondrial dysfunction in the transition from NASH to HCC, characterised by metabolic stress, ROS production, lipid peroxidation, apoptosis, and megamitochondria formation. They identified eleven mitochondrial gene signatures (MTGs-OS) strongly linked to the progression of pancreatic cancer (PC) and pancreatic neuroendocrine tumours (PNET), their biological functions and prognostic significance. They established a new protocol for evaluating prognosis and tailoring treatments for patients with pancreatic cancer. The authors noted many advantages and disadvantages of pharmacological treatments aimed at reducing steatosis and glucose levels in NASH and HCC-NASH.

In a similar study, Cui, et al. (2023)explored mitochondrial genes associated with oxidative stress (MTGs-OS) in pancreatic cancer (PC) and pancreatic neuroendocrine tumours (PNET). The authors analysed expression patterns, prognostic implications, mutation data, methylation levels, and regulatory pathway interactions to understand the role of MTGs-OS in these cancers. They categorised 930 PC patients and 226 PNET patients into three clusters based on MTGs-OS expression and scores. Using LASSO regression analysis, a new prognostic model for PC was developed. To validate the expression levels of the identified model genes, quantitative real-time PCR (qRT-PCR) experiments were conducted. Ultimately, eleven MTGs-OS were identified as linked to

the progression of PC and PNET, revealing their biological functions and prognostic significance. The authors established a novel protocol for prognostic assessment and personalized treatment strategies for PC patients.
Hayes, et al. (2020)examined the complex role of ROS in cancer biology. They illustrate how ROS can either promote tumour formation and cancer cell growth or induce cell death, depending on their concentration. During the initiation of cancer, genetic changes enable cells to thrive in high ROS environments by activating antioxidant transcription factors and increasing NADPH levels via the PPP. As cancer advances, tumour cells adapt further to oxidative stress by modifying sulphur metabolism and enhancing NADPH production through mechanisms like AMPK activation and pathways linked to glutamine and folate metabolism. The authors also discuss various ROS and RNS sources and the roles of antioxidant defences and cysteine in redox regulation.An ambiguous role of oxidative stress in tumorigenesis is shown in Fig 11.



	Initiation	Promotion	Progression	Metastatic disease
Disease stage	Acquisition of genetic changes sufficient for autonomous cell growth	Proliferation of preneoplastic nodules that have acquired autonomous growth	Local invasion of tumor cells into adjacent normal tissue, with anchorage independent growth, and interaction with stromal cells and also immune cells	Dispersal of tumor cells via the bloodstream in order to colonise distant organs
Effect of oxidative stress on the different stages of cancer	↑↑ +ve $O_2^{\bullet-}$, H_2O_2, $HO^{\bullet}$ and $ONOO^-$ are genotoxic	↑↑ +ve H_2O_2 employed as a signaling molecule that mediates cell proliferation	↑ and ↓ +/-ve H_2O_2 likely supports EMT and the pro-tumorigenic actions of tumor-associated macrophages, but $O_2^{\bullet-}$ and $ONOO^-$ used by immune cells to kill tumor cells	↓↓ -ve High O_2 levels in circulation trigger apoptosis in certain types of tumor cells (n.b., both +ve and -ve effects of antioxidants on different forms of metastatic disease)

Figure 11:An ambiguous role of oxidative stress in tumorigenesis (Hayes, et al., 2020).

The mechanisms of glucose metabolism under normal and oxidative stress conditions differ significantly. Under normal conditions (A), glucose undergoes glycolysis to form pyruvate and then acetyl-CoA for the TCA cycle, while G6PD activity is inhibited by NADPH, resulting in minimal pentose phosphate pathway (PPP) utilisation. During acute oxidative stress (B), NADPH's feedback inhibition on G6PD decreases significantly (1), leading to the oxidation of cysteine residues in key proteins like GAPDH (2), ATM (3), and electron transport chain complexes (4). This results in reduced glycolysis and increased PPP activity. The oxidation of cysteine in PTEN (5) activates PKB/Aktenhancing cellular survival.

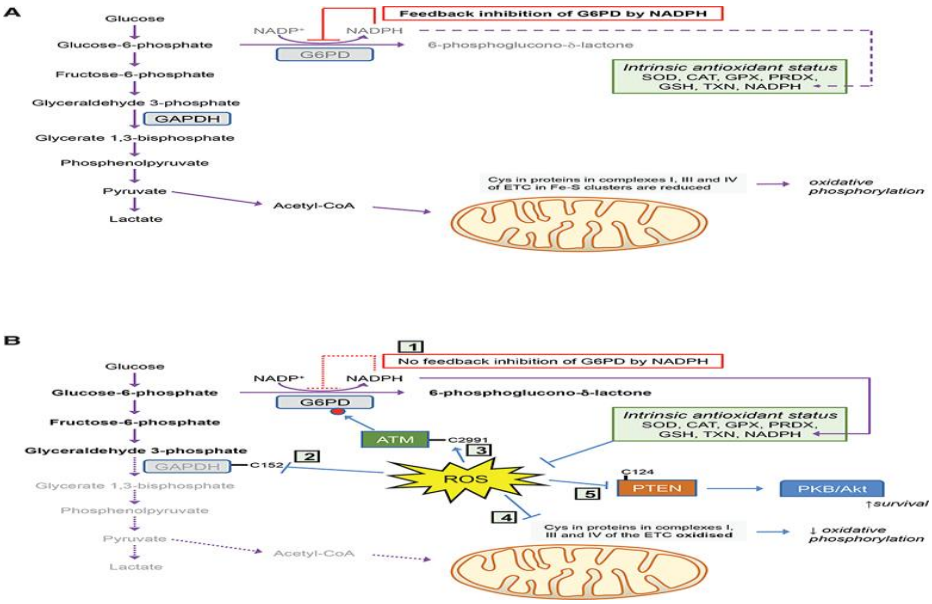


Figure 12: Biochemical mechanisms (A) under normal and (B) under oxidative stress (Hayes, et al., 2020).

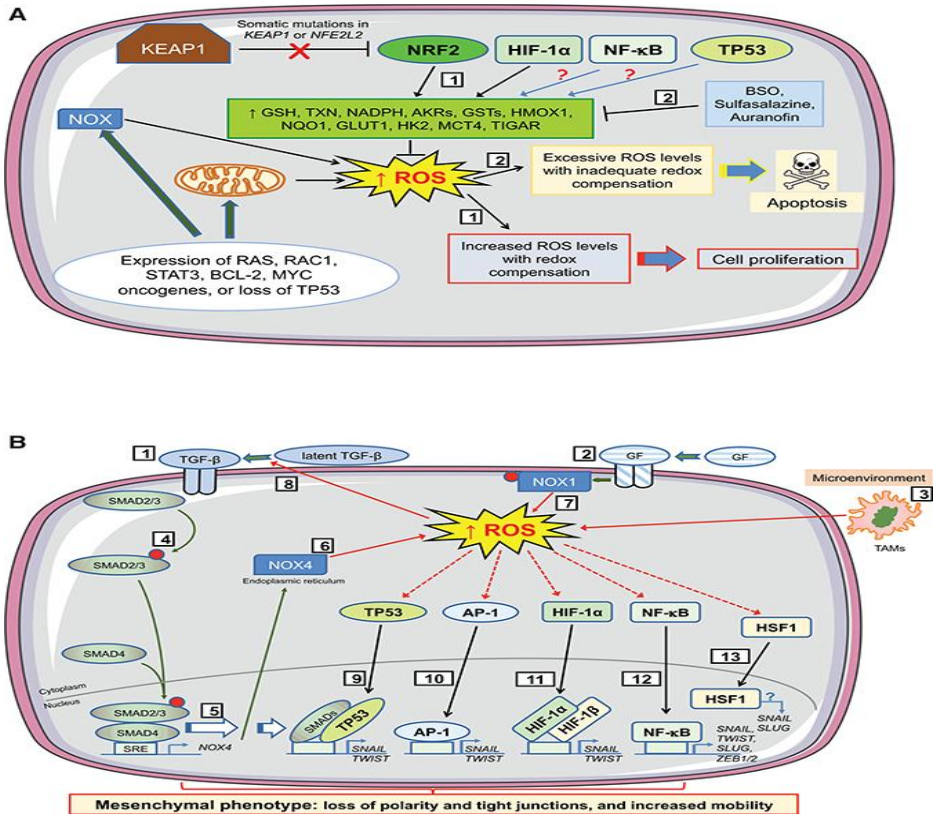


Figure 13: Mechanisms involved in the early stages and its progression during tumorigenesis (Hayes, et al., 2020).

Arfin, et al. (2021)showed how both pro- and anti-tumorigenic ROS signalling pathways can be targeted in cancer therapy. Key intracellular ROS sources include mitochondria, peroxisomes, ER stress, NADPH oxidase, metabolising enzymes, and external sources like radiation and xenobiotics. The drugs that regulate ROS for autophagy or apoptosiswere tabulated without their regulatory approval status.

In a review,Sule, et al. (2022)noted that pesticides increase NADPH oxidases (NOXs) and superoxide ($O_2^{\bullet-}$) levelsleading to an increase in ROS signalling in the cell(See Fig 14). Besides, pesticides can induce NOXs and $O_2^{\bullet-}$ to increase ROS leading to mitochondrial dysfunction and activating the mitochondrial apoptosis pathway. The **.**OH may also cause mitochondrial stress. This stress can be reducedby the activation ofthe body's immune systems including Vitamin C and E, minerals and other natural substances.

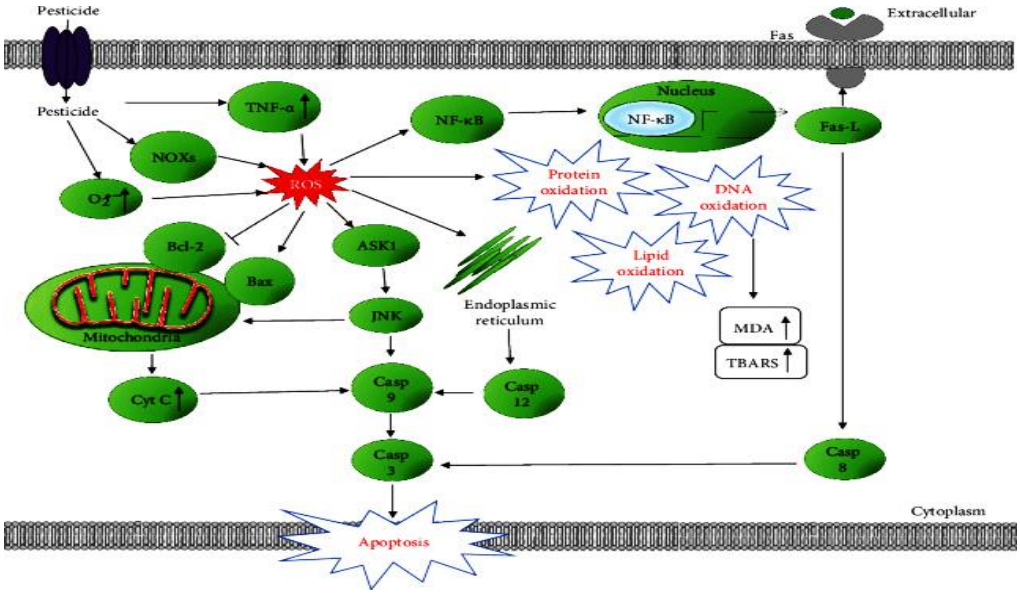


Figure 14: Signalling pathways in pesticide-induced reactive oxygen species(Sule, et al., 2022).

Various biochemicals involved in oxidation and oxidation stress were reviewed by Juan, et al. (2021). The redox processes in the mitochondrial matrix are shown in Fig 15. Reactive species ROS and RNS formed in the mitochondrial matrix by the Haber–Weiss reaction (**A**), the Fenton reaction (**B**) or by the decomposition of peroxynitrite (**C**). How ROS causes cancer through its DNA base oxidation, lipid peroxidation and protein carbonylation, respectively is shown in Fig 16. Each of the reactions of these compounds has been described in detail.

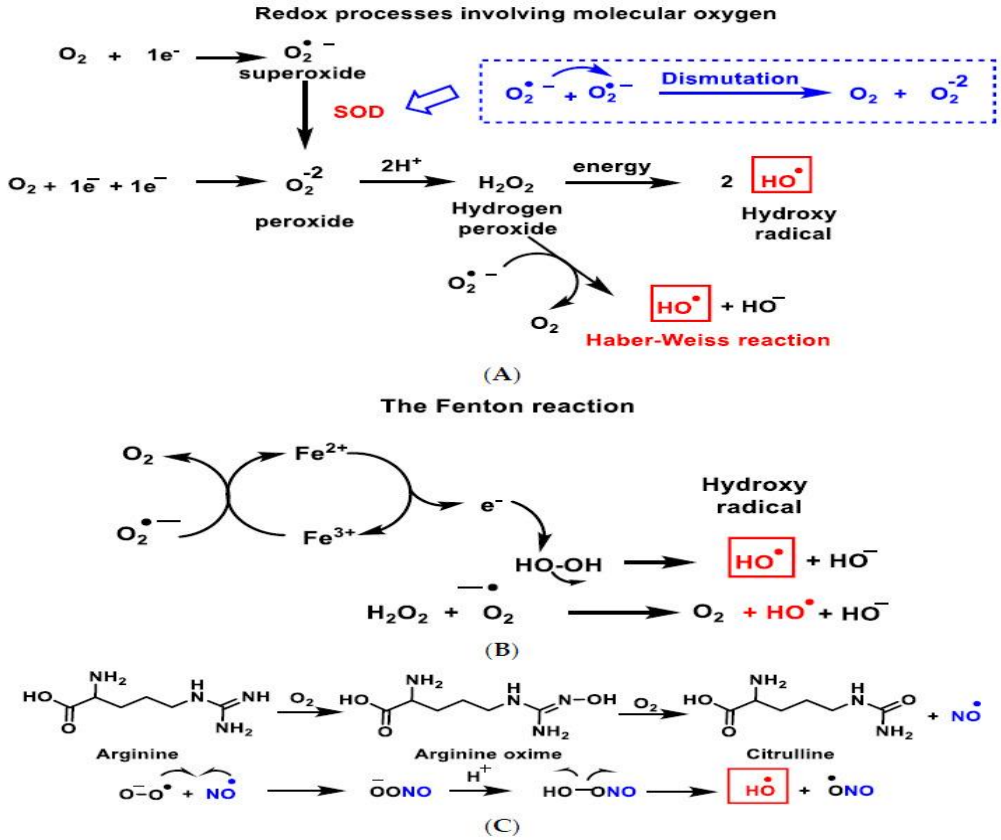


Figure 15: The redox processes in the mitochondrial matrix (Juan, et al., 2021).

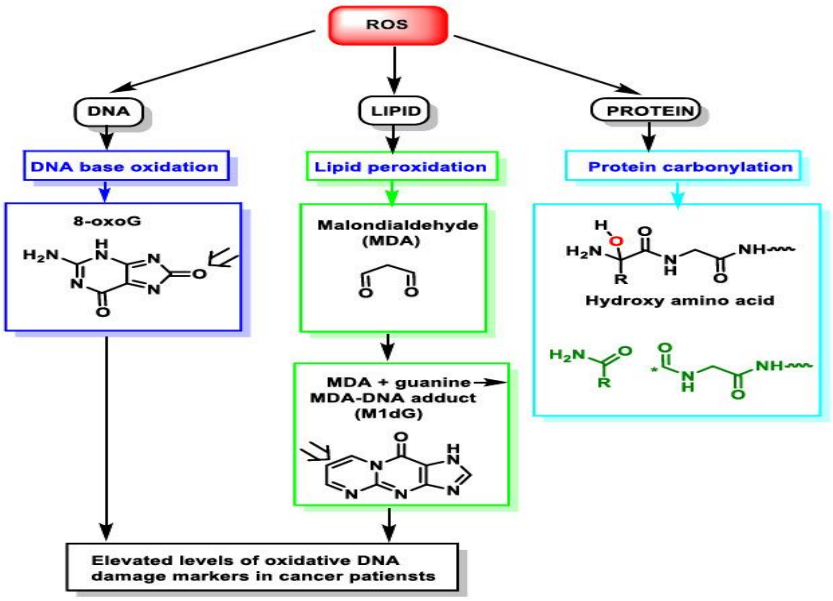


Figure 16: ROS action leads to DNA base oxidation, lipid peroxidation and protein carbonylation(Juan, et al., 2021).

The review by Vo, et al. (2024) highlighted the discovery of cuproptosis offering a novel approach to cancer therapy by exploiting the pathophysiological role of copper. The predominant mechanisms of cuproptosis for therapeutic purposes are intricately linked to apoptosis, ferroptosis and pyroptosisthrough ROS-induced signalling pathways and cellular events. Such crosstalk between different forms of regulated cell death may trigger a series of cell death events, thereby overcoming the cell death resistance of cancer cells (Fig 17).

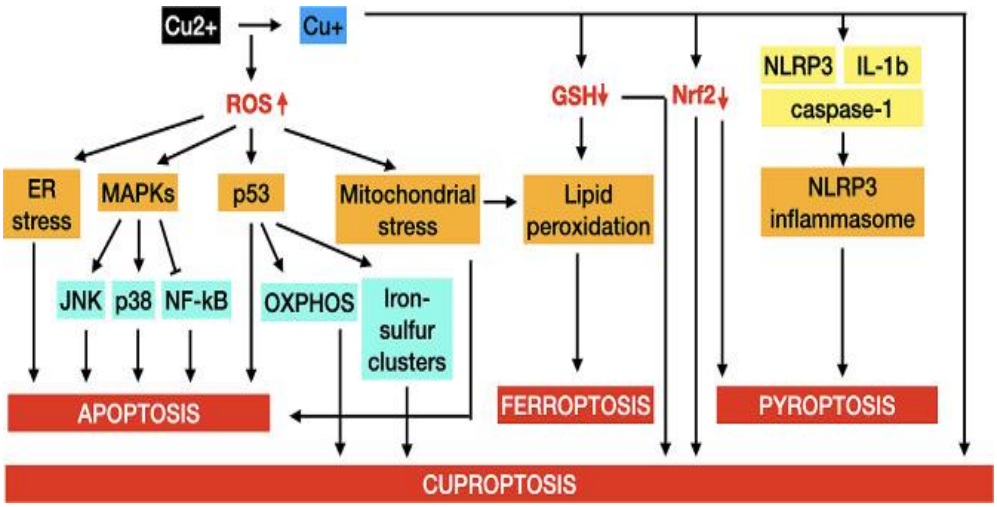


Figure 17:The crosstalk between cuproptosis and other regulated cell death pathways (Vo, et al., 2024).

Various causes of cancers are redox reactions leading to free radicals (ROS, RNS) in the first stage, oxidative stress in the second stage and the removal of biomolecules by enzyme reactions in the third stage(Jomova, et al., 2023).

3. To review the research done on interventions to reduce oxidative stress due to the impact of mitochondrial functionality.

Search terms: Interventions, therapy and prevention to reduce oxidative stress due to mitochondrial functions.

A review by Vassalle, et al. (2020)showed that the assessment of circulating oxidative stress(OxS)-related biomarkers may give several advantages like easy collection and assay bioavailability, low cost, and scope for widespread use. Its clinical application is affected by the extremely highly variable and dynamic nature of the pathophysiological mechanisms underlying oxidative stress. Antioxidant supplementation planning is affected by disagreements regarding the type of antioxidant, whether to use a single or a multi-marker approach, at which dosage, treatment time, etc. Its determinants are the antioxidant type and dosage, time of antioxidant supplementation/therapy, the background and status of the patient, and type of cancer and concomitant antitumoral therapy. Promising approaches like nanotechnology-based drug delivery systems, gene therapy and anti-mRNA are emerging.

Forman and Zhang (2021)noted that although many antioxidants are used for cancer therapy, many of them lack adequate evidence. New drugs in various stages of clinical trials give disappointing results. Limitations of these therapies include reduced effectiveness of in vivo concentrations, negligible scavenging effects of small molecules, patient age and unforeseen risks. The need for a better understanding of the disease and drug action mechanisms is stressed. Specifically, Mitochondria-targeted antioxidant (MTA) delivery systems are better than conventional ones. New MTA drugs for cancer were mostly tested on mice or cultured cells(Jiang, et al., 2020).

In a review,Chen, et al. (2022) observed that the anti-cancer effects of several mitochondria-targeting agents need further testing in breast cancers.There is scope fordeveloping drugs based on the crosstalk mechanism between mito-epigenetics and cancer-associated mtDNA mutations against breast cancers.

Azmanovaand Pitto-Barry (2022)noted that anticancer metal complexes (like platinum, gold, ruthenium, and osmium) interact with the redox homeostasis of cells leading to a few complexes in clinical use or human clinical trials. The mechanism of action of only cisplatin is well understood. New anticancer metallodrugscan be developed with more research in this area.

A dual strategy by regulating redox status (pro-oxidant/antioxidant) was discussed by Jiang, et al. (2023)in a review. Pro-oxidant therapy has a great anti-cancer capability due to its higher oxidant accumulation within cancer cells.Antioxidant therapy restores redox homeostasis and hence, fails in several clinical practices. Targeting the redox vulnerability of cancer cells by pro-oxidants which can generate excess ROS has emerged as a good anti-cancer strategy. However, it has many adverse effects due to the indiscriminate attacks of uncontrolled drug-induced OS on normal tissues and the drug-tolerant capacity of certain cancer cells greatly limits their further applications. Nano technology-based targeted drug delivery system is a promising therapy

approach. Targeted strategies to maximise the efficacy with minimum side effects of pro-oxidant anti-cancer therapyare shown in Fig 18.

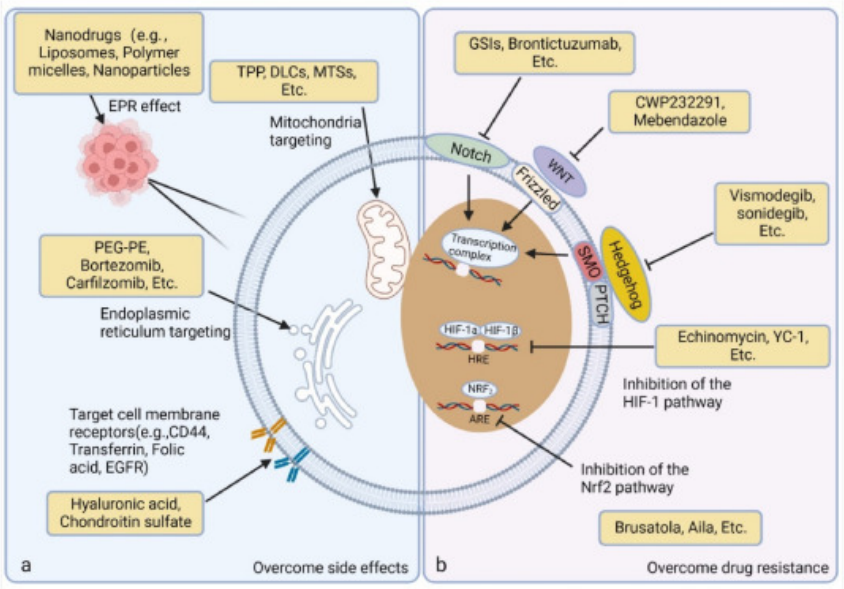


Figure 18: Targeting strategies to maximise efficacy with minimum side effects in pro-oxidant anti-cancer therapy (Jiang, et al., 2023).

According toAn, et al. (2024)oxidative cell death provides scope for the development of anti-cancer drugs. Specifically, targeting key antioxidant proteins, such as SLC7A11, GCLC, GPX4, TXN, and TXNRD has great scope for inducing oxidative cell death in cancer cells. However, there arechallenges of ROS regulation complexity, resistance mechanisms and clinical transitions.

Pancreatic cancer reprograms cellular energy metabolism to facilitate its rapid growth, invasiveness, and cancerous cell survival. Rectification of metabolic dysfunction is essential in therapeutic cancer targeting. Isoliquiritigenin (ISL) is a chalcone obtained from the plant *Glycyrrhizaglabra* asa powdered root liquorice. ISL is a natural antioxidant with diversified functions including redox regulation in cells and hence can be used for pancreatic cancer therapy (Zhang, et al., 2022).

Chemo and radiation resistance are the main obstacles to cancer treatment with both conventional and targeted therapy. Metabolic remodellingbeyond specific molecular alterations, including redox status control, can lead to targeted therapy and cancer cell survival. Chemo-resistant cancer cells become highly adapted to intrinsic or drug-induced oxidative stress by upregulating their antioxidant systems. The transcription factor, Nrf2 plays an important role in these mechanisms(Barrera, et al., 2021).

DISCUSSION

Some papers mentioned the limitations of using a single database and papers in English. None of the qualitative reviews mentioned any limitations.Some quantitative trends are given below.

Number of papers per year (Fig 19)

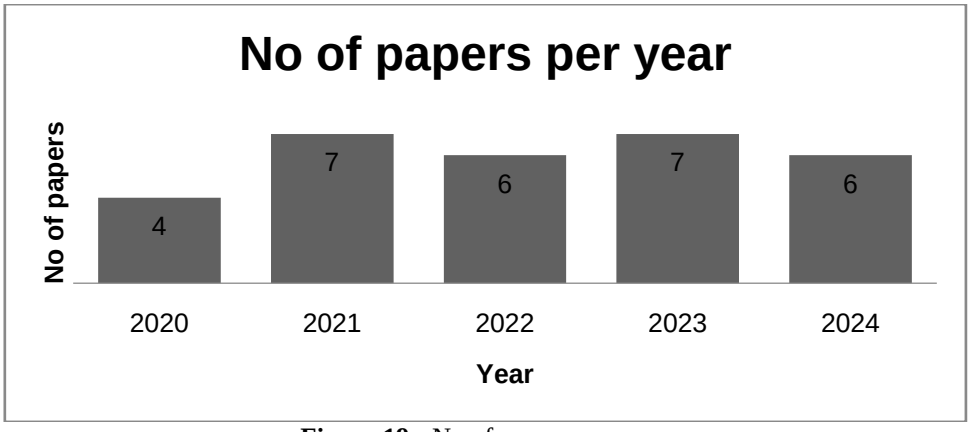


Figure 19: No of papers per year.

The number of papers was 6-7 in all years except only 4 in 2020.
Method (Fig 20)

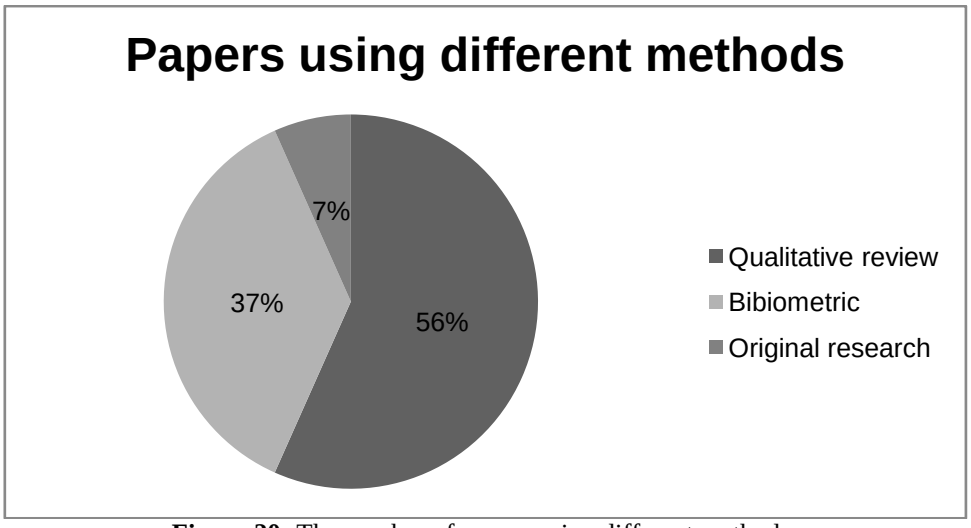


Figure 20: The number of papers using different methods.

More than half were qualitative reviews followed by bibliometric reviews. Only 7% were original research.
Citations per year
There were 10 papers with less than 5 citations, 15 with 5 to 50 citations and five with above 50 citations per year.
Evidence adequacy, GRADE and CCAT
These positive aspects of quality ranged between 4 and 4.9.
Risk of bias
This negative quality aspect ranged between 2.5 and 3.5, lower value related to lower RoB.
Overall quality
The formula used for the calculation of this parameter is given in the Methodology section.A higher number of citations produced higher values. Although most values were in the range of 15 and 40, very high values of 316 (Chaudhary et al. 2023), 631 (Forman & Zhang, 2021) and 104 (Chen et al. 2022) were also obtained.
The nuanced interplay between mitochondrial dysfunction and oxidative stress in cancer cells not only highlights the complexity of cancer pathophysiology, but also opens avenues for targeted therapeutic interventions. As noted by several studies, mitochondrial functionality is instrumental in maintaining cellular homeostasis and energy production through ATP (Harrington et al., 2023). In cancer cells, however, this functionality is often compromised, leading to excessive production of reactive oxygen species (ROS) and subsequent oxidative stress, a precursor to cancer progression (Sosa et al., 2012; Hayes et al., 2020).
The research trends identified in this systematic review underscore a significant increase in studies focusing on these mechanisms, especially in powerhouse research regions like the USA and China (Song et al., 2023; Jiang et al., 2024). This surge indicates a growing recognition of the central role mitochondrial dysfunction plays in cancer biology. The ongoing challenge is developing effective therapeutic strategies that target these mitochondrial pathways without causing undue harm to normal cellular processes.
Emerging therapies aim to modulate oxidative stress in cancer cells, either by enhancing the cell's natural antioxidant defenses or by increasing oxidative stress to cytotoxic levels selectively within cancer cells (Lin et al., 2021; Zahra et al., 2021). Nanotechnology-based drug delivery systems exemplify the innovative approach to targeting cancer at the molecular level, promising improved efficacy and reduced side effects (Jiang et al., 2023). These systems leverage the abnormal metabolic and oxidative characteristics of cancer cells, a strategy that shows significant promise for future clinical applications (Chen et al., 2022).
However, current treatments face limitations such as varying efficacy and safety profiles, highlighting an urgent need for comprehensive clinical trials to validate these findings (Forman & Zhang, 2021; Chen et al., 2022). Furthermore, a more profound understanding of mitochondrial biogenesis and its regulation could potentially lead to novel therapeutic targets, advancing current treatment modalities (Hayes et al., 2020).

CONCLUSIONS

This systematic review highlights the crucial role of mitochondrial functionality and oxidative stress in the pathogenesis of cancer, underscoring a prevalent theme in recent research. As documented in the results, the manipulation of oxidative balance via mitochondrial pathways is rapidly gaining traction as a therapeutic target,

an insight reinforced by studies demonstrating a direct correlation between mitochondrial dysfunction and cancer progression (Sosa et al., 2012; Wang et al., 2020).

One of the main conclusions from this review is the potential for mitochondrial-targeted therapies to manage oxidative stress in cancer cells effectively. Innovative approaches such as nanotechnology-based drug delivery systems offer considerable promise, suggesting that these methods could achieve more precise targeting with reduced systemic toxicity (Jiang et al., 2023; Zahra et al., 2021). Such strategies could represent a shift toward more personalized and effective cancer therapies. However, despite the potential, it is crucial to note that many existing therapies lack robust clinical evidence, necessitating further exploration through comprehensive and large-scale clinical trials (Forman & Zhang, 2021; Chen et al., 2022).

The global trend in research output, with the USA and China leading in the number of studies, indicates a concentrated interest in these regions, likely due to their significant investment in biomedical research (Song et al., 2023; Pang et al., 2023). This underscores an opportunity for broader international collaboration to further investigate and refine therapeutic modalities.

The challenges identified include the complex nature of ROS regulation and the risk of inducing oxidative damage in healthy tissues—a consideration that must guide cautious therapeutic application (Bae et al., 2024; Hayes et al., 2020). Studies focusing on the dual role of antioxidants in cancer further complicate this landscape, as their interactions with chemotherapy drugs can induce either protective or harmful effects (Zahra et al., 2021; Dharshini et al., 2023).

While the systematic review delineates the potential for modifying mitochondrial functionality to manage oxidative stress in cancer, it also calls for more detailed mechanistic studies to unravel the intricate regulatory networks involved. This understanding will be crucial for optimizing therapeutic approaches and minimizing adverse effects, paving the way for more efficient and precise oncology practices. Moving forward, integrative research efforts across disciplines will be essential to harness the full potential of these emerging therapies and improve patient outcomes.

Limitations

The limitations of a single database, English papers and targeting a fixed number of papers are acknowledged.

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APPENDIX - PRISMA Flowchart