Cancer and H2O2

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Generation of Hydrogen Peroxide in Cancer Cells: Advancing Therapeutic Approaches for Cancer Treatment 2024

Simple Summary: Cancer cells grow and divide so rapidly that they are literally engaged in a metabolic marathon—leading to the formation of high levels of reactive oxygen species (ROS). The altered redox balance in cancer cells offers therapeutic opportunities for targeting cancer. Some ROS-targeting strategies attempt to enhance ROS production to inflict lethal cell damage or trigger apoptosis, while other anticancer agents inhibit enzymes that are essential to maintain the redox potential of the cell. One type of ROS that shows potential to be utilized as a treatment mechanism is hydrogen peroxide (H_2O_2), which can be generated by numerous mechanisms, including metal-based prodrugs, photodynamic therapy, enzymes, nanoparticles, chemical modulators, and various combinations thereof. This review focuses on various chemical agents that induce H_2O_2-mediated cancer cell death, including their mechanism of function, preclinical and clinical studies, recent advancements, and potential applications as a new and exciting avenue for targeted and efficacious cancer treatment.

One such approach involves manipulation of reactive oxygen species (ROS), due to the fact that cancer cells are known to exhibit increased intrinsic oxidative stress compared to normal cells [1 –5]. This intrinsic feature of cancer paves the way for the development of tumor-selective therapeutic strategies [3 ,6– 9]. This review specifically focuses on strategies to selectively modulate the most stable ROS, hydrogen peroxide (H_2O_2), in cancer cells to achieve therapeutic effects

4.1. **Phenol and Polyphenol** Analogues

Among various naturally occurring and synthesized compounds, structures with multiple phenolic groups facilitate H_2O_2 generation either via repeated steps of autooxidation in the presence of molecular oxygen (O_2) [40, 41], or via redox cycling that involves NAD(P)H (Figures 5 and 6). Autooxidation is often a slow reaction due to the lower redox potential of $O_2/O_2^{\bullet-}$ and produces H_2O_2 through dismutation. During the autooxidation process, phenols are oxidized to semiquinone, which rapidly transforms into quinone, while O_2 is reduced to a superoxide radical ($O_2^{\bullet-}$) that undergoes dismutation to generate H_2O_2 at the same time (Figure 6).

Compounds that feature polyphenolic (hydroxyl) groups include a wide range of compounds such as **flavonoids, hydroxytyrosol, propyl gallate, hydroxycinnamic acids,** etc.

4.1.1. **Flavonoids**

Flavonoids are a diverse set of polyphenolic compounds found in plant-based foods and beverages. They have been extensively explored for their vast range of pharmacological properties such as antibacterial, antimutagenic, antiresorptive, antioxidative, and anticancer effects [42].

There are five subclasses of flavonoids: flavan-3-ols (such as catechins and gallocatechins), flavones (such as apigenin, luteolin, and baicalein), flavonols (such as kaempferol, quercetin, and myricetin), flavanones (such as naringenin and carthamidin), and anthocyanins (such as delphinidine). These subclasses vary in their structural arrangements of hydroxyl and methoxy groups, and also in their ring conjugations (Figure 7). They have been found to act as prooxidants, and a wide variety of flavonoids (such as catechins [43 – 46], baicalein, quercetin, morin, myricetin [47– 49], and wogonin [50 – 52]) have been reported to produce high level of H_2O_2. These flavonoids selectively kill malignant cells via **H_2O_2** mediated processes and interactions with cellular functions that lead to apoptosis, such as enhanced hydroxyl radical formation via the Fenton reaction, which causes DNA, protein, and cell membrane damage [43 – 45]. The production of H_2O_2 has been observed in flavonoid-treated media as well as in cell cultures. Their ability to generate ROS can be influenced by the presence and location of hydroxyl groups [53]. In order to harness their therapeutic benefits while avoiding unintended harmful effects, it is essential to attain a better understanding of their prooxidant activities.

4.1.2. **Hydroxycinnamic Acid**

Caffeic acid (CA) and rosmarinic acid (RA) are hydroxycinnamic acid (HCA) analogues containing phenol moiety and are found in various dietary sources, including green tea, red wine, fruits, vegetables, and coffee, as well as in medicinal plants such as rosemary and salvia. Their extensive range of properties encompass anticancer, antioxidant, antiproliferative, and anti-inflammatory effects [66 ,67]. CA showed pro-oxidant potential due to its ability to interact with metals like copper, inducing lipid peroxidation and causing DNA damage within tumor cells through either oxidation or covalent adduct formation [68–70]

4.1.3. **Hydroxytyrosol**

Hydroxytyrosol (HT), abundantly present in **olives**, has demonstrated anticancer properties in vitro [74 ,75]. Sun et al. demonstrated that HT exhibited antiproliferative and pro-apoptotic effects in cancer cells through **H_2O_2** generation [75, 76].

4.1.4. **Propyl Gallate**

Propyl gallate (PG), chemically known as propyl-3,4,5-trihydroxybenzoate, is widely present in processed food and cosmetics, hair products, and lubricants [82 – 85]. This versatile compound boasts various biological properties, including potential antitumor effects. PG alone demonstrated antioxidative and cytoprotective properties against cellular damage and gained a pro-oxidative property in combination with copper (II) [86].

4.2. **Compounds with Quinone Moieties**

A wide range of quinone-containing compounds showed anticancer, antimicrobial and antiparasitic effects, such as naphthoquinones, aziridinylquinones, anthracyclines, indolequinones (i.e., mitomycins), aminoquinones (i.e., streptonigrins) and certain vitamins (Figure 11). H_2O_2 generation induced by these compounds is one of the possible mechanisms for their function. Quinones can induce H_2O_2 production in cells via autooxidationand redox cycling mechanisms.

4.2.1. **Naphthoquinones**

Many naturally occurring naphthoquinones and their derivatives showed cytotoxicity, which has been investigated for the development of anticancer drugs, such as menadione (2-methyl-1,4-naphthoquinone, also termed vitamin K3), plumbagin, and juglone (Figure 13). Their toxic effects on cells are mostly caused by ROS species including **H_2O_2** generated through redox cycling [88, 89].

4.2.2. **Hydroxyl Naphthoquinon**e

Plumbagin and juglone are hydroxyl naphthoquinone derivatives found in various plants, such as plants of the Plumbago genus and black walnuts.

Their cytotoxicity is caused by two possible mechanisms: redox cycling and reaction with GSH, which both result in generation of the corresponding semiquinone radical and $O_2^{\bullet-}$, leading to DNA damage and oxidative stress [92 –95].

4.2.3. **1,2-Naphthoquinone**

β-Lapachone (β-Lap), a 1,2-naphthoquinone natural product isolated from the lapacho tree, is a potent anticancer drug that has been advanced into clinical trials based on its tumor-selective cytotoxic properties [99]

Chau et al. reported that human leukemia cells treated with β-Lap had a substantial increase in intracellular H_2O_2, especially the ones with lower levels of GSH, including HL-60, U937, and Molt-4 [101]. The generated ROS have been linked to different pathways to apoptosis.

4.2.4. **Anthracyclines**

Some naphthoquinone-containing compounds, such as anthracyclines, are FDA-approved anticancer agents. Anthracyclines are among the most effective anticancer drugs ever developed. Doxorubicin (DOX) and daunorubicin (DNR) were the first anthracyclines that were isolated from Streptomyces peucetius bacteria (Figure 15) [104], and are commonly used for the treatment of both hematologic and solid tumors, such as breast cancer, childhood solid tumors, soft tissue sarcomas, aggressive lymphomas,and acute leukemias.

The quinone moiety in ring C undergoes one-electron reduction to form a semiquinone that quickly regenerates its parent quinone by reducing O_2 to $O_2^{\bullet-}$ and H_2O_2.

4.2.5. **Aziridinylquinones**
Aziridinylquinones, such as carbazilquinone, diaziquone (AZQ), BZQ, triaziquone, and apaziquone, have a unique structural composition with an aziridine ring attached to a quinone group (Figure 17). These compounds possess the ability to alkylate DNA and generate ROS, both of which contribute to their cytotoxic effects [111 , 112].
4.2.6. **Indolequinones**
Many naturally occurring indolequinone analogues, such as mitomycins (Figure 19), have shown potent anticancer properties [114]. They were recognized as prodrugs which undergo bioreduction in vivo to form irreversible bis-alkylation of DNA. The reduction of mitomycin initiates a reduction–oxidation cycle, which generates **H2O2** as a byproduct [115].
4.2.7. **Aminoquinones**
Streptonigrin and its derivatives contain aminoquinone moieties (Figure 21). They were isolated from Streptomyces flocculus and exhibit potent antitumor and antibiotic effects. Streptonigrin interacts with oxygen to generate superoxide radicals that undergo dismutation, producing **H2O2** (Figure 12) [119].
4.3. **Vitamin C**
Vitamin C (also known as ascorbic acid or ascorbate) is an essential vitamin in the body's daily function. It allows for the biosynthesis of collagen and various neurotransmitters, is involved in protein metabolism, and strengthens the body's immune system. In recent years, ascorbic acid has been shown to have selective anticancer properties at millimolar (mM) concentrations, with such an anticancer effect demonstrated both in vitro
and in vivo [123 –126]. The main mechanism through which vitamin C kills tumor cells is by formation of H2O2 [124 , 127].
At the beginning of this process, the ionized vitamin C is transformed into ascorbate radical by losing one electron (Figure 22). This electron then reduces a protein-centered metal, such as Fe3+ to Fe2+. The created Fe 2+ then donates an electron to O2, forming O2●− that is subsequently dismutated to form H 2O2 and O2. The created H 2O2 can cause damage to DNA, lipids, and proteins, inducing cancer cell death. Notably, these concentrations of Vitamin C are not enough to kill healthy, non-cancerous cells due to the high level of plasma catalase and/or GSH peroxidase that inhibit the redox reaction or destroy any formed H2O2 molecules, thus making cancer treatment via vitamin C even more appealing due to its selective nature
Another possible avenue that could be taken with vitamin C cancer treatment is using it in conjunction with other therapeutics, such as vitamin K3, triethylenetetramine, or other H2O2-responsive chemotherapeutic drugs (i.e., camptothecin) to achieve synergistic anticancer effect while minimizing unwanted side effects [133–135
4.4. **Metal, Metal Oxides, and Metal Peroxides**
Metals play an essential role in biological systems and human health. Many enzymatic reactions require metals for their catalytic action [136]. Essential metals such as calcium, sodium, potassium, magnesium, and transition metals iron, copper, and zinc are vital as well. Deficiency or excess of these metals can cause various diseases including cancer [137]
Although the molecular mechanism is not completely understood, their potential to generate ROS and alter cellular redox status is considered significant in metal-induced carcinogenesis [138]. On the other hand, many metals, metal complexes, or metal peroxides have gained significant attention in cancer treatment, which has been highlighted in several reviews [139 – 141]. Some metal oxides and peroxides are reported to enhance H2O2 production, which is one of the possible mechanisms for their anticancer efficacy and selectivity [141].
Many metal peroxides, such as MgO2, CaO2 can react with H2O to produce H2O2. Such a reaction is facilitated under acidic conditions
The popularity of ZnO has also risen due to being safe and efficient delivery [147 ,148], and being categorized as "generally recognized as safe" (GRAS) by the U.S. FDA (21CFR182.8991). Its functionality as an antibacterial and anticancer agent primarily relies on its ability to generate ROS [147 ,149].
Among various metal peroxides, CaO2 shows the most promise due to its biocompatibility and potential for use in cancer treatments like calcium overload therapy and treatment of bone-related cancers. In catalytic medicine, H2O2 can be utilized to generate large amounts of hydroxyl radicals through a Fenton-like reaction.
5. **Conclusions**
Redox adaptations among cancer cells have been characterized by their heightened oxidative stress thresholds as well as their increased intracellular antioxidant defenses. To push cancer cells beyond their cytotoxic threshold necessitates approaches that disrupt these adaptations. One such approach is the manipulation of ROS, particularly H2O2.

https://pubmed.ncbi.nlm.nih.gov/34138092/
Recent Advances in Tumor Microenvironment Hydrogen Peroxide-Responsive Materials for Cancer Photodynamic Therapy 2020
High concentration of hydrogen peroxide (H_2O_2), one of the hallmarks of TME (tumor microenvironment) , has been recognized as a double-edged sword, posing both challenges, and opportunities for cancer therapy.

https://pubmed.ncbi.nlm.nih.gov/17150302/
Dual role of hydrogen peroxide in cancer: possible relevance to cancer chemoprevention and therapy 2007
Accumulating evidence suggests that hydrogen peroxide (H(2)O(2)) plays an important role in cancer development. Experimental data have shown that cancer cells produce high amounts of H(2)O(2). An increase in the cellular levels of H(2)O(2) has been linked to several key alterations in cancer, including DNA alterations, cell proliferation, apoptosis resistance, metastasis, angiogenesis and hypoxia-inducible factor 1 (HIF-1) activation. It has also been observed that the malignant phenotype of cancer cells can be reversed just by decreasing the cellular levels of H(2)O(2). On the other hand, there is evidence that H(2)O(2) can induce apoptosis in cancer cells selectively and that the activity of several anticancer drugs commonly used in the clinic is mediated, at least in part, by H(2)O(2). The present report discusses that the high levels of H(2)O(2) commonly observed in cancer cells may be essential for cancer development; these high levels, however, seem almost incompatible with cell survival and may make cancer cells more susceptible to H(2)O(2)-induced cell death than normal cells. An understanding of this dual role of H(2)O(2) in cancer might be exploited for the development of cancer chemopreventive and therapeutic strategies.

https://onlinelibrary.wiley.com/doi/10.1155/2016/1908164
Molecular and Cellular Effects of Hydrogen Peroxide on Human Lung Cancer Cells: Potential Therapeutic Implications 2016
Lung cancer has a very high mortality-to-incidence ratio, representing one of the main causes of cancer mortality worldwide. Therefore, new treatment strategies are urgently needed. Several diseases including lung cancer have been associated with the action of reactive oxygen species (ROS) from which hydrogen peroxide (H_2O_2) is one of the most studied. Despite the fact that H_2O_2 may have opposite effects on cell proliferation depending on the concentration and cell type, it triggers several antiproliferative responses. H_2O_2 produces both nuclear and mitochondrial DNA lesions, increases the expression of cell adhesion molecules, and increases p53 activity and other transcription factors orchestrating cancer cell death. In addition, H_2O_2 facilitates the endocytosis of oligonucleotides, affects membrane proteins, induces calcium release, and decreases cancer cell migration and invasion. Furthermore, the MAPK pathway and the expression of genes related to inflammation including interleukins, TNF-α, and NF-κB are also affected by H_2O_2. Herein, we will summarize the main effects of hydrogen peroxide on human lung cancer leading to suggesting it as a potential therapeutic tool to fight this disease. Because of the multimechanistic nature of this molecule, novel therapeutic approaches for lung cancer based on the use of H_2O_2 may help to decrease the mortality from this malignancy.

https://cancerci.biomedcentral.com/articles/10.1186/1475-2867-13-123

Hydrogen peroxide (H2O2) induces leukemic but not normal hematopoietic cell death in a dose-dependent manner 2013
Over the last few years, studies have suggested that oxidative stress plays a role in the regulation of hematopoietic cell homeostasis. In particular, the effects of hydrogen peroxide (H_2O_2) range from hematopoietic cell proliferation to cell death, depending on its concentration in the intracellular milieu. In this work, we evaluated the effects of an oxidative environment on normal and leukemic hematopoietic cells by stimulating normal human (umbilical cord blood) and murine (bone marrow) hematopoietic cells, as well as human myeloid leukemic cells (HL-60 lineage), upon H_2O_2 stimulus. Total cell populations and primitive subsets were evaluated for each cell type. H_2O_2 stimulus induces HL-60 cell death, whereas the viability of human and murine normal cells was not affected. The effects of H_2O_2 stimulus on hematopoietic stem/progenitor cell subsets were examined and the normal primitive cells were found to be unaffected; however, the percentage of leukemic stem cells (LSC) increased in response to H_2O_2, while clonogenic ability of these cells to generate myeloid clones was inhibited. In addition, H_2O_2 stimulus caused a decrease in the levels of p-AKT in HL-60 cells, which most likely mediates the observed decrease of viability. In summary, we found that at low concentrations, H_2O_2 preferentially affects both the LSC subset and total HL-60 cells without damage normal cells.

https://pubmed.ncbi.nlm.nih.gov/29956787/
Hydrogen peroxide inhibits the growth of lung cancer cells via the induction of cell death and G1-phase arrest 2018
In conclusion, H2O2 inhibited the growth of Calu-6 and A549 lung cancer cells through cell death and G1-phase arrest. H2O2-induced cell death resulted from necrosis, as well as caspase-dependent apoptosis.

https://pubmed.ncbi.nlm.nih.gov/23381883/
H_2O_2 inhibits the growth of human pulmonary fibroblast cells by inducing cell death, GSH depletion and G1 phase arrest 2013
In the present study, the effect of exogenous H2O2 on cell growth and death was evaluated in normal human pulmonary fibroblast (HPF) cells with respect to reactive oxygen species (ROS) and glutathione (GSH) levels.
H2O2 also induced GSH depletion in HPF cells at 24 h and decreased GSH levels after only 25 min. In conclusion, H2O2 inhibited the growth of HPF cells via apoptosis and/or necrosis as well as G1 phase arrest, which was accompanied by an intracellular increase in ROS levels and the depletion of GSH.

https://onlinelibrary.wiley.com/doi/10.1002/anie.202213703
Mass Spectrometry Reveals High Levels of Hydrogen Peroxide in Pancreatic Cancer Cells 2023
By using mass spectrometry and biological techniques, we found that H2O2 is dysregulated in PDAC, further validating that the main reasons for H2O2 enrichment in PDAC cells may be that oncogenic KRAS mutation promotes H2O2 production and silences CAT levels in PDAC. Furthermore, we identified that H2O2 affects the cells' metabolic profile and promotes the survival of PDAC cells.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3180186/
Hydrogen peroxide fuels aging, inflammation, cancer metabolism and metastasis 2011
For example, cancer patients (such as those with breast and lung tumors) can be distinguished from normal controls, based on the detection of hydrogen peroxide in their exhaled breath.110–112 Consistent with the idea that hydrogen peroxide may also originate from the tumor stroma, patients with interstitial pulmonary fibrosis show increased levels of hydrogen peroxide in their exhaled breath.113

https://pubmed.ncbi.nlm.nih.gov/35564184/
Recent Advances in Electrochemical Sensing of Hydrogen Peroxide (H2O2) Released from Cancer Cells 2022
Cancerous cells are well known for their substantial amounts of hydrogen peroxide (H_2O_2). The common methods for the detection of H_2O_2 include colorimetry, titration, chromatography, spectrophotometry, fluorimetry, and chemiluminescence. These methods commonly lack selectivity, sensitivity, and reproducibility and have prolonged analytical time. New biosensors are reported to circumvent these obstacles. The production of detectable amounts of H_2O_2 by cancerous cells has promoted the use of bio- and electrochemical sensors because of their high sensitivity, selectivity, robustness, and miniaturized point-of-care cancer diagnostics. Thus, this review will emphasize the principles, analytical parameters, advantages, and disadvantages of the latest electrochemical biosensors in the detection of H_2O_2. It will provide a summary of the latest technological advancements of biosensors based on potentiometric, impedimetric, amperometric, and voltammetric H_2O_2 detection. Moreover, it will critically describe the classification of biosensors based on the material, nature, conjugation, and carbon-nanocomposite electrodes for rapid and effective detection of H_2O_2, which can be useful in the early detection of cancerous cells.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7268291/
33% hydrogen peroxide as a Neoadjuvant treatment in the surgical excision of non-melanoma skin cancers: a case series 2020
Hydrogen peroxide (H_2O_2) is a product of respiration in mitochondria and an important oxidizing agent in biological systems. Previous investigations have shown the efficacy of H_2O_2 in treating skin conditions such as seborrheic keratosis and actinic keratosis. In an area like the face, reconstruction of excision defects and ultimately aesthetic outcomes are of utmost importance. Hydrogen peroxide may represent a simple yet effective method at shrinking non-melanoma skin cancers (NMSC) of the head and neck before they are excised
We have demonstrated a significant reduction in the size of multiple lesions after application of 33% hydrogen peroxide, simplifying definitive excision and reconstruction. Hydrogen peroxide demonstrated an ability to successfully treat non-melanoma skin cancers as well.
Non-melanoma skin cancers (NMSC) are the most commonly occurring cancers worldwide.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7731207/
Use of H2O2 to Cause Oxidative Stress, the Catalase Issue 2020
Addition of hydrogen peroxide (H_2O_2) is a method commonly used to trigger cellular oxidative stress. However, the doses used (often hundreds of micromolar) are disproportionally high with regard to physiological oxygen concentration (low micromolar). In this study using polarographic measurement of oxygen concentration in cellular suspensions we show that H_2O_2 addition results in O_2 release as expected from catalase reaction. This reaction is fast enough to, within seconds, decrease drastically H_2O_2 concentration and to annihilate it within a few minutes. Firstly, this is likely to explain why recording of oxidative damage requires the high concentrations found in the literature. Secondly, it illustrates the potency of intracellular antioxidant (H_2O_2) defense. Thirdly, it complicates the interpretation of experiments as subsequent observations might result from high/transient H_2O_2 exposure and/or from the diverse possible consequences of the O_2 release.

https://pubmed.ncbi.nlm.nih.gov/31956964/
Potential implications of hydrogen peroxide in the pathogenesis and therapeutic strategies of gliomas 2020
Glioma is the most common type of primary brain tumor, and it has a high mortality rate. Currently, there are only a few therapeutic approaches for gliomas, and their effects are unsatisfactory. Therefore, uncovering the pathogenesis and exploring more therapeutic strategies for the treatment of gliomas are urgently needed to overcome the ongoing challenges. Cellular redox imbalance has been shown to be associated with the initiation and progression of gliomas. Among reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) is considered the most suitable for redox signaling and is a potential candidate as a key molecule that

determines the fate of cancer cells. In this review, we discuss the potential cellular and molecular roles of H_2O_2 in gliomagenesis and explore the potential implications of H_2O_2 in radiotherapy and chemotherapy and in the ongoing challenges of current glioma treatment. Moreover, we evaluate H_2O_2 as a potential redox sensor and potential driver molecule of nanocatalytic therapeutic strategies for glioma treatment.