Mini-review

Dual role of hydrogen peroxide in cancer: Possible relevance to cancer chemoprevention and therapy

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Abstract

Accumulating evidence suggests that hydrogen peroxide (H_2O_2) plays an important role in cancer development. Experimental data have shown that cancer cells produce high amounts of H_2O_2. An increase in the cellular levels of H_2O_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_2O_2. On the other hand, there is evidence that H_2O_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_2O_2. The present report discusses that the high levels of H_2O_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_2O_2-induced cell death than normal cells. An understanding of this dual role of H_2O_2 in cancer might be exploited for the development of cancer chemopreventive and therapeutic strategies.

1. Introduction

Reactive oxygen species (ROS) are generated by all aerobic organisms and their production seems to be needed for signal-transduction pathways that regulate multiple physiological processes. Excessive amounts of ROS, however, can start toxic and lethal chain reactions, which oxidize and disable structures that are required for cellular integrity and survival. ROS are generated in multiple compartments and by multiple enzymes within the cell. Important contributions include proteins within the plasma membrane, such as the growing family of NADPH oxidases; lipid metabolism within the peroxisomes; as well as the activity of various cytosolic enzymes such as cyclooxygenases. Although all these sources contribute to the overall ROS production, the vast majority of cellular ROS can be traced back to the mitochondria [1–4].

Most of the energy that our cells need to live depends on a mitochondrial process that requires oxygen (O_2). In this process, called oxidative phosphorylation, ATP generation is coupled with

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a reaction in which O_2 is reduced to H_2O. Under certain conditions, O_2 can also be reduced to H_2O via the ROS superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) [2,5]. It is recognized that the cellular production of $O_2^{\cdot-}$ and H_2O_2 favors the formation of other reactive oxygen and nitrogen species – such as hydroxyl radical ($OH^{\cdot}$) and peroxynitrite ($ONOO^-$) – and that an excessive production of these species causes oxidative stress and may play an important role in carcinogenesis [6]. It is not clear, however, which species is directly responsible for each of the biological activities in which ROS have been implicated. For instance, several studies have demonstrated that the overexpression of the enzymes superoxide dismutases (SOD) in tumor cells can reduce tumor cell growth, metastasis and other malignant features of cancer cells [7–12]. Since these enzymes catalyze the conversion of $O_2^{\cdot-}$ to H_2O_2, the anticancer effects induced by SOD overexpression may be mediated by a decrease in the cellular levels of $O_2^{\cdot-}$ or by an increase in the cellular concentrations of H_2O_2. Experimental data suggest that the anticancer effects produced by overexpression of manganese SOD can be reverted by overexpression of two enzymes involved in H_2O_2 catabolism, catalase and glutathione peroxidase; this supports that the anticancer effects induced by SOD overexpression are mediated by an increase in H_2O_2 [13,14].

The present report discusses evidence that suggests that an increase in the cellular levels of H_2O_2 may play, directly or indirectly, a key role in malignant transformation, but can also sensitize cancer cells to H_2O_2-induced cell death. An understanding of this dual role of H_2O_2 in cancer might be exploited for the development of cancer chemopreventive and therapeutic strategies.

2. Key role of hydrogen peroxide in carcinogenesis

Many researchers consider that cancer is a genetic disease caused by the acquisition of multiple mutations in genes that control cell proliferation, cell death and genomic instability [15]. This hypothesis – called the somatic mutation theory of cancer – has been the prevalent paradigm to explain the process of carcinogenesis in the last several decades. There is growing experimental evidence, however, that contradicts or cannot be explained by this hypothesis, and other theories are being developed or revisited [16–23]. It is currently accepted – even by those who challenge the somatic mutation theory

of cancer – that cells must develop several acquired capabilities in order to become a malignant cancer: increased cell proliferation (caused, in part, by resistance to growth inhibition and independence from mitogenic stimulation), apoptosis resistance, cellular immortalization, increased angiogenesis and invasion/metastasis. Besides, it is considered that genetic instability is a key event that enables the acquisition of these capabilities [24,25].

Accumulating experimental data suggest that an increase in the cellular concentrations of H_2O_2 can explain all these hallmarks of cancer. It is known that H_2O_2 is associated with DNA damage, mutations and genetic instability [26–31]; H_2O_2-induced DNA damage seems to be mediated by $OH^{\cdot}$ generated from H_2O_2 by the Fenton reaction [26,30,31]. Several studies have also demonstrated that H_2O_2 can induce cell proliferation [2,32,33], apoptosis resistance [34,35], increased angiogenesis [36,37] and invasion and metastasis [33,38,39]. Indeed, these studies showed that an increase in the levels of H_2O_2-detoxifying enzymes could reduce cell proliferation, promote apoptosis, and inhibit invasion, metastasis and angiogenesis. The activation of hypoxia-inducible factor 1 (HIF-1) by H_2O_2 can contribute to explain these hallmarks of cancer. There is evidence that the most important oncogenes and tumor-suppressor gene pathways may culminate in HIF-1 activation [15] and that HIF-1 activation plays an important role in apoptosis resistance, invasion/metastasis, angiogenesis and immortalization [5,40–43]. It is not surprising, therefore, that HIF-1 overexpression is observed in many human cancers and has been associated with increased patient mortality [40,41,44]. Interestingly, recent research has established that an increase in the cellular concentrations of H_2O_2 can activate HIF-1, and that overexpression of the H_2O_2-detoxifying enzyme catalase prevents the activation of HIF-1 induced by different stimuli [5,45–49].

The key role of H_2O_2 in carcinogenesis is supported by experimental data that have shown that cancer cells commonly have increased levels of H_2O_2 [2,50–52]. For instance, Szatrowski and Nathan reported that several tumor cell lines, representing a variety of tissue types, constitutively produced large amounts of H_2O_2. They observed that the cumulative amount of H_2O_2 produced after 4 h by these tumor cells was comparable to the amount of H_2O_2 produced by similar numbers of phorbol ester-triggered neutrophils [50]. It has also

been demonstrated that H_2O_2 can induce malignant transformation [53–56] and that the expression of the H_2O_2-detoxifying enzymes catalase or glutathione peroxidase in cancer cells can reverse their malignant phenotype [55,57,58]. For instance, expression of the ROS generation system Nox1 in normal NIH3T3 fibroblasts resulted in cells with malignant characteristics that produced tumors in athymic mice. These transformed cells showed a 10-fold increase in H_2O_2 levels. When human catalase was expressed in these transformed cells, H_2O_2 concentration decreased, and the cells reverted to a normal appearance, the growth rate normalized, and cells no longer produced tumors in athymic mice [55].

In short, it seems that cancer cells produce high amounts of H_2O_2, and high cellular levels of this ROS have been associated, directly or indirectly, with all the hallmarks of cancer. Furthermore, H_2O_2 can produce cell malignant transformation, and expression of H_2O_2-detoxifying enzymes can reverse the malignant phenotype of cancer cells. This suggests that H_2O_2 plays an essential role in carcinogenesis.

3. Selective killing of cancer cells by hydrogen peroxide

As discussed above, there is evidence that H_2O_2 may have an important function in cancer development. However, there is also compelling evidence that have shown that increasing the cellular levels of H_2O_2 may be an efficient way of killing cancer cells. Fig. 1 represents that different concentrations of H_2O_2 can produce different cellular effects; this may contribute to explain apparently controversial studies that have shown, for instance, that H_2O_2 can both produce apoptosis resistance [35] and be an efficient inductor of apoptosis in cancer cells [59,60].

Numerous reports have demonstrated that H_2O_2 can induce cell death in cancer cells. It has been observed that a significant increase in the intracellular H_2O_2 production and downstream acidification provides an environment conducive for apoptotic cell death in tumor cells [59–61]. Recent data support that increasing the cellular levels of H_2O_2 by using H_2O_2-generating drugs may be an efficient way of killing cancer cells. Thus, the anticancer effect of various chemotherapeutic agents currently used in the clinic (e.g., paclitaxel, cisplatin, arsenic trioxide, etoposide, doxorubicin) is mediated, at least in part, by an increase in the cellular levels of H_2O_2 [62–72].

There is experimental evidence that cancer cells are more susceptible to H_2O_2-induced cell death than normal cells [73–76]. Using several cancer and normal cell lines, Chen et al. observed that pharmacologic ascorbic acid concentrations selectively killed cancer cells; this effect was mediated by H_2O_2. They showed, for instance, that a concentration of 50 µM of H_2O_2 induced more percentage of cell death in Burkitt's lymphoma cells than 250 µM in normal lymphocytes and normal monocytes [74].

It is not clear why specific concentrations of H_2O_2 can kill cancer cells selectively. It has been proposed that, in normal cells, ROS are at low levels, originate from NADPH oxidase and the concentration of H_2O_2 is regulated by the glutathione system. By contrast, in tumor cells, high levels of ROS close to the threshold of cytotoxicity are produced through the mitochondrial respiratory chain, and H_2O_2 concentration is controlled by catalase [77]. On the other hand, Chen et al. found no correlation between H_2O_2-mediated selective cell death of cancer cells and intracellular glutathione concentrations, catalase activity, or glutathione

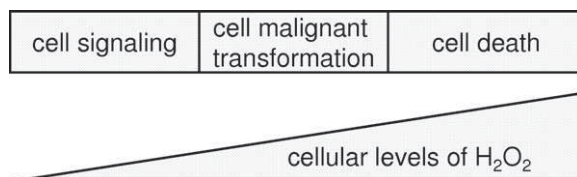Fig. 1. Different cellular effects by different cellular levels of H_2O_2. Low levels of H_2O_2 have a physiological role in cell signaling. A constitutive increase in the cellular levels of H_2O_2 has been associated with the carcinogenesis process. Higher levels of H_2O_2 can produce cell death.
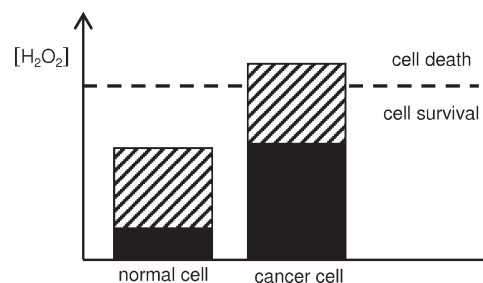

Fig. 2. Selective killing of cancer cells by H_2O_2. There is evidence that cancer cells have higher levels of H_2O_2 than normal cells [2,50–52] (represented in black) and that there is a threshold of H_2O_2 above which cells cannot survive. This might explain why specific concentrations of H_2O_2 (represented in striped black) can produce selective death of cancer cells.

peroxidase activity [74]. A possible explanation to the high susceptibility of cancer cells to H_2O_2 is represented in Fig. 2.

4. Relevance to cancer therapy and cancer chemoprevention

The possible use of H_2O_2 in cancer therapy has been controversial over the years. In 1957, it was reported that 50–60% of rats implanted with the Walker 256 adenocarcinoma were cured by simply replacing their drinking water with dilute solutions of H_2O_2 [78]. One year later, however, no anticancer effect was found when H_2O_2 was given to rats using comparable experimental conditions [79]. In 1981, it was reported that the use of an H_2O_2-generating system could deliver H_2O_2 to sites of malignancy and produce anticancer effects in mice, with little toxicity to the host [80]. Because this and other reports suggested that H_2O_2 might be useful in cancer therapy, many individuals looked for therapies with H_2O_2 for cancer management. In 1993, the American Cancer Society studied the available literature and found no evidence that treatment with H_2O_2 was safe or resulted in objective benefit in the treatment of cancer [81].

It is now accepted that the direct administration of H_2O_2 to cancer patients is not an appropriate therapeutic strategy [81]. However, as discussed before, there is now convincing evidence that supports that increasing the cellular levels of H_2O_2 by using H_2O_2-generating systems may be an efficient way of killing cancer cells. For instance, recent data have shown that the generation of H_2O_2 by using high-dose intravenous vitamin C therapy may be useful in the treatment of cancer [74,82]. It has also been proposed that the identification of compounds that trigger a significant increase in intracellular H_2O_2 and their use in conjunction with chemotherapy agents could be an attractive strategy to enhance the sensitivity of tumor cells to drug therapy [83]. Therefore, there is evidence that supports that increasing the cellular levels of H_2O_2 by using H_2O_2-generating systems may be a key strategy for the development of clinically useful anticancer strategies.

Cancer therapy has not managed to decrease cancer mortality in the last three decades; this suggests that we need new strategies to control a disease that kills over 6 million people worldwide every year [84,85]. It is accepted that cancer chemoprevention – the use of chemicals to prevent, stop or reverse the process of carcinogenesis – is an essential approach to controlling cancer, yet the clinical usefulness of this strategy is very limited [86,87]. Successful implementation of cancer chemoprevention depends on a mechanistic understanding of the carcinogenesis process [86]. Our knowledge about this process is still limited and may therefore be preventing cancer chemoprevention from becoming a widely used anticancer tool. The present report discusses that an excessive cellular production of H_2O_2 may be a key event in cancer development; cancer chemoprevention may therefore be achieved by using chemicals to prevent or reduce excessive cellular levels of this oxidant.

It is known that most cancer chemopreventive agents have antioxidant properties. It is important to note, however, that many of these agents become prooxidants at relatively high concentrations. This means that these agents may reduce or increase the cellular levels of H_2O_2 depending on the concentration at which they are used, and suggests that different concentrations of these agents may produce chemopreventive or chemotherapeutic effects. Indeed, it is recognized that antioxidant/prooxidant agents, such as curcumin, epigallocatechin gallate, beta-carotene, sulforaphane, capsaicin or vitamin C, are potential cancer chemopreventive and chemotherapeutic agents [88–95]. What is not acknowledged, however, is that these agents can also produce carcinogenic effects. Fig. 3 represents that an antioxidant/prooxidant agent can produce
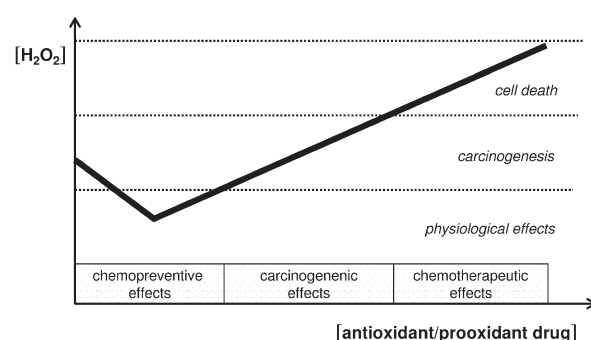
Fig. 3. Antioxidant/prooxidant drugs as potential cancer chemopreventive, carcinogenic and chemotherapeutic agents. At low concentrations, these drugs may act as cancer chemopreventive agents by reducing or keeping the cellular levels of H_2O_2 within the physiological levels. At higher concentrations, these drugs may increase the levels of H_2O_2 and produce carcinogenic effects. At concentrations that result in levels of H_2O_2 that cannot be counterbalanced by the cellular antioxidant systems, these drugs can produce cell death and may act as chemotherapeutic agents.

chemopreventive, carcinogenic and chemotherapeutic effects mainly depending on its concentration. This is in accordance with experimental data that suggest that the antioxidant/prooxidant agent vitamin C produces cancer chemopreventive, carcinogenic and chemotherapeutic effects [74,82,96–98].

The prooxidant effect of cancer chemopreventive agents might increase the cellular H_2O_2 levels and produce carcinogenic effects in people receiving these agents. In three major cancer chemoprevention clinical trials, high doses of beta-carotene – an antioxidant chemically related to vitamin A – were given to people in an attempt to prevent lung cancer. Two studies found beta-carotene supplements to be associated with a higher risk of lung cancer in cigarette smokers, and a third found neither benefit nor harm from beta-carotene supplements [99,100]. The model proposed in Fig. 3 may contribute to explain these disappointing results, as these trials used high concentrations of beta-carotene and this agent has known antioxidant/prooxidant properties [89,90]. Current cancer chemopreventive clinical trials are testing antioxidant/prooxidant agents (e.g., vitamin E) at concentrations that may also produce carcinogenic effects [101–103]. It is the author opinion that the use of relatively high doses of antioxidant/prooxidant agents in cancer chemoprevention may camouflage their possible efficiency and also produce carcinogenic effects.

It has been observed that some antioxidants such as *N*-acetylcysteine (NAC) can decrease the cellular levels of H_2O_2 and yet increase the proliferation of several cancer cell lines [62,83]. One possible explanation is that, in these cancer cells, the levels of H_2O_2 may be above the toxic threshold and may therefore induce antiproliferative effects; the reduction of these H_2O_2 levels by antioxidants would stimulate cell proliferation. These observations might explain the detrimental effect of NAC in patients with head and neck cancer or lung cancer supplemented with this antioxidant [104].

5. Conclusions

Evidence suggests that an increase in the cellular levels of H_2O_2 may be an important event in cancer development. Cancer chemoprevention might therefore be achieved by using any chemical capable of reducing or preventing excessive cellular levels of H_2O_2. On the other hand, it seems that the high levels of H_2O_2 commonly observed in cancer cells are almost incompatible with cell survival and make these cells more susceptible to H_2O_2-induced cell death than normal cells. Any chemical or strategy capable of increasing the cellular levels of H_2O_2 sufficiently may therefore produce selective killing of cancer cells and be therapeutically useful. Finally, it is important to note that the use of a drug with antioxidant/prooxidant properties can result in a decrease or increase in the cellular levels of H_2O_2 mainly depending on the concentration at which this drug reaches the cell. This factor should be considered carefully, as it can determine that the drug produces cancer chemopreventive, chemotherapeutic or carcinogenic effects.

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