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REVIEW ARTICLE

INTERCONNECTION BETWEEN OXIDATIVE STRESS AND CANCER: METHODOLOGIES FOR DETECTING THE REACTIVE OXYGEN SPECIES

Yoghinni Manogaran[1], Neeraj Kumar Fuloria[1], Sundram Karupiah[1], Veerasamy Ravichandran[1], Venugopal Balakrishnan[2] and Shivkanya Fuloria[1]*

[1]*Faculty of Pharmacy, AIMST University, Bedong 08100, Kedah, Malaysia*
[2]*Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Pulau Pinang, Malaysia*

**E-mail*: shivkanya_fuloria@aimst.edu.my
Tel.: +60 14-303 4057.

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Reactive oxygen species (ROS) and antioxidants offer both favorable and detrimental effects over cancer. The property of ROS to display different functions in different concentrations creates the need to understand the relation between cancer, antioxidants and ROS, and the methods to detect ROS. This study focusses on sources, types, tumorigenic and tumor prevention effects of ROS; types, tumorigenic and tumor prevention effects of antioxidants; detoxification of ROS; and various methodologies to detect ROS. Study concludes that improvement of genetic methods, enzymes pathways and scaling-up of cancer models profiling, use of omics will support in better understanding of complexity of ROS and antioxidant pathways. Though ROS detection methods are developing at a fast pace, further improvement is needed to counter the drawbacks of current methods.

Key words: ROS, Antioxidants, Cancer progression, Tumor suppression, Oxidative stress.

INTRODUCTION

Cellular aerobic reactions produce reactive oxygen species (ROS) that regulate signaling, differentiation, and cell survival [1]. Cancer cells (CCs) exhibit elevated ROS, causing damage, genome instability, and tumorigenesis. They balance ROS via antioxidants to prevent oxidative stress and promote survival. Altered redox states in cancer cells make them sensitive to ROS modulation, suggesting therapeutic potential in targeting ROS levels for cancer treatment [2].

To counter ROS and maintain redox balance, CCs enhance antioxidant capacity, balancing increased ROS production and scavenging. Their sensitivity to redox changes highlights ROS modulation as a potential cancer therapy. Hence present review intents to highlight the relation between ROS, antioxidants, and cancer & methods for detecting ROS.

ROS sources and types

Superoxides

Electrons reacting with O_2 during mitochondrial processes generate $O_2^{\cdot-}$ species. In cancer cells, NOX1-derived $O_2^{\cdot-}$ stimulates proliferation and signaling, while catalase (CAT) and superoxide dismutase (SOD) offer protection. Inhibiting SOD triggers apoptotic pathways via peroxynitrite ($ONOO^-$), which damages proteins, DNA, and lipids. $ONOO^-$ forms radicals like NO_2 and $^{\cdot}OH$, inducing apoptosis through lipid peroxidation and oxidative stress [3,4].

Hydrogen peroxides (H_2O_2)

Superoxide dismutase converts O_2^- into long-

lived H_2O_2, also generated by Ero1 during oxidative protein folding. Aquaporins transport H_2O_2, enabling distant effects. H_2O_2 aids HOCl synthesis, forming hydroxyl radicals via Fenton chemistry, crucial for malignant cell elimination. Low H_2O_2 levels regulate signaling; high levels cause oxidative stress and protein oxidation [5,6].

Hydroxy radicals (˙OH)

Hydroxyl radicals (˙OH) are produced through the Fenton reaction or iron reduction processes [7].

Lipid peroxides (LP)

The ˙OH radical induces lipid peroxidation in polyunsaturated fatty acids, generating lipid radicals and peroxides. Excessive LP disrupts membrane integrity and links to ferroptosis, an iron-dependent form of cell death [8].

ROS protumorigenic activity

Radiation generates ROS, linked to tumor initiation and promotion. ROS oxidize nucleic acids, causing DNA damage and mutagenesis. They alter proteins, with low oxidation aiding signaling via reversible modifications like sulfenic acid, while high oxidation irreversibly impairs functions. Protective mechanisms, including KEAP1 activation, glutathionylation, and adapt cells to ROS. Effects depend on ROS location, amount, and duration [9]. **Figure 1** represents relation between oxidative stress, ROS/RCS and cancer.

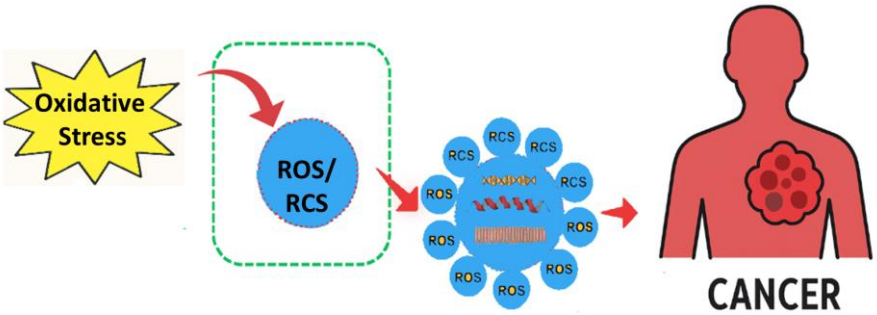

Fig. 1. Oxidative stress, ROS/RCS and Cancer

ROS antitumorigenic activity

Excess ROS trigger cell cycle arrest, senescence, or cancer cell death. Malignant cells utilize intercellular HOCl or ˙NO/$ONOO^-$ signaling for selective apoptosis. NOX1 produces extracellular $O_2^{\cdot-}$, forming H_2O_2 and HOCl, or reacting with ˙NO to form $ONOO^-$, generating ˙OH radicals. Insufficient antioxidants activate caspase-mediated apoptosis, highlighting ROS-driven pathways for transformed cell elimination [10]. Membrane-associated catalase (MAC) prevents HOCl and ˙NO/$ONOO^-$ apoptosis pathways, enabling tumor survival; MAC inactivation restores apoptosis [11]. Singlet delta O_2 production inactivates MAC, reactivating ROS/RNS-driven apoptosis. Cold atmospheric plasma (CAP) and plasma-activated medium (PAM) exhibit antitumor effects by transferring CAP-derived ROS/RNS to liquid media. Key components like H_2O_2 and NO_2^- synergize, forming $ONOO^-$ with potent biological effects. CAP and PAM demonstrate in vitro, in vivo antitumor activity with minimal clinical side effects [11,12]. CAP and PAM primarily target malignant cells, though some studies report non-selective effects. Standardizing dose and composition may clarify the selective antitumor mechanisms, which are still under investigation. Aquaporins enhance tumor cell H_2O_2 influx, enabling apoptosis via Fenton reaction. Tumor cells have low cholesterol, aiding selective CAP and PAM action by promoting ROS entry. Both models highlight H_2O_2 as a key effector. Tumor progression involves resistance to H_2O_2, driven by MAC expression, which protects cells from oxidative damage by decomposing $ONOO^-$ and oxidizing ˙NO [13]. Non-malignant and early-stage tumor cells are more sensitive to H_2O_2-induced apoptosis due to lower MAC expression. Tumor cells express less catalase, weakening their protective function against oxidative stress. A study explored CAP and PAM selectivity on tumor cells, revealing tumor cells express NOX1, SOD, and MAC, while non-malignant cells lack MAC. CAP-derived singlet oxygen ($^1O_2$) inactivates MAC, allowing H_2O_2 or $ONOO^-$ to produce secondary $^1O_2$, triggering apoptosis-inducing ROS pathways. Low levels of $^1O_2$ interact with tumor cell surfaces, inducing substantial responses. The reaction between

H_2O_2, $ONOO^-$, and residual H_2O_2 forms $^1O_2$, which inactivates catalase (CAT). Tumor cells, through aquaporin-mediated H_2O_2 influx, deplete GSH, facilitating apoptosis via HOCl signaling and lipid peroxidation. Inhibition of aquaporins impedes PAM-induced apoptosis [13,14]. ROS activation of ASK1/p38 and ASK1/JNK pathways triggers anti-apoptotic factors, promoting cancer cell (CC) survival. Deactivation of JNK and p38 pathways in tumors suggests their role in CC death. Excess ROS levels prevent metastasis by inhibiting CC proliferation, but metabolic transformation increases antioxidant properties to support anchorage-independent growth, making ROS-alleviating therapies promising for metastasis suppression [15].

Endogenous antioxidants (EnAOs)

Endogenous antioxidants (EnAO), such as SOD, CAT, and GPx, are crucial for eliminating ROS and protecting cells from oxidative damage. SOD converts $O_2^{\cdot}-$ into H_2O_2, while CAT and GPx reduce H_2O_2 to water and oxygen, preventing further harm. The SOD family includes three types: SOD1, SOD2, and SOD3, each with distinct roles in regulating oxidative stress, cancer development, and metabolism. CAT, which is abundant in all tissues, plays a vital role in maintaining cellular health by limiting H_2O_2 concentrations. A deficiency in CAT can lead to oxidative damage and increase cancer susceptibility [16, 17]. Glutathione (GSH), the most abundant endogenous antioxidant, detoxifies ROS in cancer cells. GSH is biosynthesized in two steps: glutamate and cysteine form a dipeptide catalyzed by glutamate cysteine ligase, followed by GSH synthetase adding glycine to form GSH. Enzymes like GSH S-transferase (GST) and GSH peroxidase (GPX) use GSH to eliminate ROS. Sulfaredoxin (SRX) and thioredoxin (TXN) also regenerate peroxiredoxins (PRDX) to neutralize H_2O_2. Both systems rely on NADPH to regenerate their active forms, buffering oxidative stress (OS) [18]. EnAOs play roles in tumorigenesis, preventing ROS-induced DNA damage, though their direct role in tumor initiation prevention remains unclear.

Tumor prevention by EnAOs

ROS detoxification by EnAOs protects against oncogenesis. GST, GPX, and SOD enzymes prevent tumor initiation in skin, liver, colon, and mitochondria by reducing carcinogens, ROS, and DNA damage [19]. The TXN system suppresses tumors by reducing DNA damage and activating PTEN. PRDX1/PRDX6 prevent skin and liver cancer, while TXN-GSH synergy protects against carcinogen-induced liver malignancy [20]. Oxidized DNA promotes tumor initiation, while OGG1 loss triggers lung tumors, and $Gpx2^{-/-}$ mice show colorectal cancer resistance. EnAOs prevent tumors but may also promote initiation, as seen in $Srx^{-/-}$ mice [21, 22]. The complex role of ROS and antioxidant proteins in cancer necessitates further research to determine if enhancing tumor-suppressing antioxidant systems can prevent cancer without unintended promotion.

Tumor progression by EnAOs

During cellular transformation, increased mitochondrial metabolism and protein translation elevate ROS levels, necessitating antioxidants for redox balance. GSH prevents DNA damage and maintains protein homeostasis, while insufficient GSH promotes aggressive malignancy. GPX and GST enzymes utilize GSH in tumor progression, with GST metabolizing chemotherapy and activating oncogene signaling, while GPX4 inhibits lipid peroxidation and ferroptosis. Ferroptosis, including GSH-independent pathways using ubiquinone (CoQ10), targets therapy-resistant cancers. TXN components, like TXN and TXNRD1, promote tumor growth, while PRDX1 and PRDX4 expression supports tumor survival. PRDX6 overexpression accelerates cancer progression, highlighting the dual roles of antioxidant systems in tumor regulation [23]. O_2^- metabolism drives tumor progression, with SOD1 inhibition blocking lung tumorigenesis. Targeting TXN or SOD1 disrupts lung CC survival. EnAOs support tumors, but redundancy in AO systems complicates cancer treatments. NADPH regeneration via the pentose phosphate pathway (PPP) and G6PD is critical in tumor suppression. G6PD deficiency lowers colorectal cancer risk, warranting deeper studies into GSH/TXN, NADPH, and ROS interplay.

Exogenous antioxidants (ExAOs)

Exogenous antioxidants (ExAOs) include polyphenols, vitamins, and AO minerals. Polyphenols, abundant in fruits, vegetables, tea, and wine, protect against cancer. Key vitamins include C, E, and K, along with carotenoids like β-carotene and lycopene, which neutralize radicals and prevent oxidative stress. Vitamin E protects membranes, while vitamin K reduces GSH

depletion, highlighting diverse AO roles in combating oxidative damage. Selenium supports GPX, thioredoxin reductase, and SOD, while zinc prevents lipid peroxidation and protects membranes [26]. ExAOs aid cancer treatments by reducing ROS effects and enhancing radiotherapy outcomes. Vitamins C and E improve radiotherapy side effects, while curcumin and catechins show synergistic antitumor effects in various cancers. Melatonin protects against oxidative damage during radiotherapy and is non-toxic at high doses, though its optimal human dosage for radiotherapy requires further investigation [24,25]. Chemotherapy induces oxidative stress (OS) via redox disruption and antioxidant (AO) reduction. Agents like meroxest, jadomycin, and anthracyclines increase ROS to induce apoptosis, with doxorubicin causing DNA damage in breast tumors [27]. However, ROS elevation contributes to cardiotoxicity. Antioxidants like vitamin E and N-acetyl cysteine (NAC) mitigate doxorubicin's cardiotoxic effects [28]. High-dose vitamin C, however, may reduce chemotherapy efficacy, as shown in leukemia and lymphoma studies [29]. Understanding AO-chemotherapy interactions is crucial to balance antitumor activity and mitigate adverse effects like cardiotoxicity, ensuring effective and safer cancer treatments. Combined chemotherapy with doxorubicin, cyclophosphamide, and 5-fluorouracil reduces antioxidant levels, causing lipid peroxidation [30]. Tannins reduced doxorubicin-induced cardiotoxicity [31]. Epigallocatechin-3-gallate enhanced doxorubicin's effects in hepatocellular carcinoma, while paclitaxel and docetaxel induce ROS and oxidative stress, reducing antioxidant potential and activating apoptotic pathways in cancer cells [32, 33]. Cisplatin, a heavy metal used in treating solid tumors, induces oxidative stress (OS) and side effects, including apoptosis via Bax, p53, p21, and caspase activation. Quercetin enhances cisplatin's proapoptotic effects at high doses and reduces ROS-induced damage at low doses, increasing SOD levels [34]. N-acetylcysteine (NAC) reverses cisplatin's cytotoxicity and proapoptotic effects in ovarian carcinoma, glioblastoma, and fibroblast cells, with timing of administration influencing its efficacy [35]. Clinical studies show that NAC also protects against cisplatin-induced ototoxicity in children [36]. Melatonin, with high antioxidant activity, is used as an adjuvant in chemotherapy. It improves tumor regression and survival in cancer patients but may not increase survival in advanced lung carcinoma despite reducing side effects [37]. NAC inhibits Hif1a stabilization and affects tumors but may promote cancer progression and metastasis in melanoma and lung models, with unclear redox effects. Vitamin E has antitumor potential, but studies suggest it may promote lung tumors and melanoma progression while preventing lipid oxidation and ferroptosis. Exogenous antioxidants like vitamin C can autoxidize, increasing oxidative stress, and NAC produces hydrogen sulfide, influencing metabolic pathways. Careful evaluation of their effects on tumors is necessary [38-40].

Detoxification of ROS in cancer

NRF2, an antioxidant transcription factor, regulates GSH and TXN pathways. Its activation by oxidative stress can promote cancer through impaired KEAP1 interaction, influencing tumor progression in various stages. NRF2 accumulation in cancer, especially lung cancer, results from mutations in NRF2 and KEAP1, impairing degradation. This activates antioxidant pathways and reduces ROS. NRF2 impacts tumorigenesis by regulating metabolic processes, including PPP and serine biogenesis, and promotes tumor growth by decreasing oxidative damage.

Studies suggest NRF2 activation aids in tumor progression through various metabolic and AO defense pathways. The role of ROS in metastasis is complex. NRF2 activation can promote metastasis by stabilizing BACH1, affecting glycolysis and metastasis in pancreatic cancer models. KEAP1 deletion and NRF2 activation influence tumor progression differently depending on tissue type, ROS effects, and genetic context. Further studies are needed to clarify how KEAP1 mutations and NRF2 levels impact tumor growth and progression [41].

ROS detection methods

ROS plays a key role in cell proliferation, differentiation, and apoptosis. Different concentrations of ROS induce various effects, with several methods available to measure ROS levels, including spectrometry and fluorescent probes.

Fluorescent chemicals

Fluorescent chemical-based ROS detection uses probes like dihydroethidium and Amplex Red, which become fluorescent after oxidation by ROS, helping assess oxidative states and radical production in cells [42].

Dihydroethidium (DHE)
DHE detects ROS by oxidation, producing fluorescent 2-OH-E+ and E+, but it cannot precisely quantify $O2^{\cdot-}$. For accuracy, HPLC or LC-MS is used to distinguish products. Mito-SOX, a mitochondrial $O2^{\cdot-}$ indicator, also detects oxidative stress but faces interference from other radicals. Chromatographic separation is needed for reliable $O2^{\cdot-}$ detection [42].

Dichlorodihydro fluorescein diacetate (DCFHDA)
DCFHDA measures intracellular redox states by entering cells, undergoing hydrolysis into DCFH, and then oxidation into fluorescent DCF. Its fluorescence correlates with oxidative stress (OS) levels, but variations in cell permeability and esterase activity can affect results. While DCFHDA detects ROS, including H_2O_2, $O2^{\cdot-}$, and NO, its sensitivity to other radicals like $ONOO^-$ complicates interpretations. Additionally, interference from radicals like NO_2^- and per-oxidases can affect measurements. Despite these challenges, DCFHDA is useful for detecting OS changes, though careful control group adjustments and data analysis are essential for accurate results [42].

Amplex Red (AR)
Amplex Red (AR) is a sensitive probe for measuring extracellular H_2O_2 with a detection limit of 5 pmol. It reacts with H_2O_2 via HRP, producing fluorescent resorufin. While AR is useful for detecting mitochondrial-generated H_2O_2, its selectivity is challenged by interference from peroxynitrite, NADPH, GSH, and membrane permeability, complicating intracellular measurements. AR is better suited for cell-free systems [43].

Fluorescent protein
Fluorescent protein-based redox (FPBR) probes use recombinant proteins to detect redox changes in cells. They target specific organelles, allowing real-time detection of cellular radicals without cell permeation [44].

Redox-sensitive green fluorescent Protein (roGFP)
roGFP probes detect redox changes through fluorescence intensity variation [44]. The roGFP1 and roGFP2 are the two analogues of roGFP. These etect dynamic redox changes by indicating dithiol/disulfide conversion due to ROS accumulation. Suitable for monitoring long-term redox shifts in reduced environments, sensitive to pH variations [45]. These include:

- *roGFP1-iX:* Modified version of roGFP1 for oxidizing environments, offering fast reaction speed and low pH sensitivity, ideal for monitoring redox in the endoplasmic reticulum [46].
- *roGFP1-iL and roGFP1-RX:* roGFP1-iL detects redox changes in the ER with high sensitivity. roGFP1-RX has improved speed and dynamic range for redox monitoring by incorporating positively charged amino acids [43].
- *Grx1-roGFP2-iL and roGFP2-Orp1:* Grx1-roGFP2-iL monitors redox in ER/cytosol with high specificity for GSH. roGFP2-Orp1 is H_2O_2-specific, ideal for detecting H_2O_2 levels in mitochondria and cytosol, stable across pH ranges [42].

Redox-sensitive yellow fluorescent protein (rxYFP)
rxYFP, derived from yellow-shifted GFP, senses dynamic redox changes by forming reversible disulfide bonds. It detects GSH, GSSG, thiol, and disulfide variations in various cellular regions like cytosol and mitochondria [42]. These include:

- *rxYFP-Grx1P:* This modified probe combines yeast glutaredoxin-1 (Grx1p) with rxYFP, making it highly specific for intracellular GSH redox potential compared to H_2O_2, hydroxyethyl disulfide, or cysteine [42].
- *rxYFP 3R:* By adding three positively charged residues (200R/204R/227R), rxYFP 3R achieves 13 times higher GSH reactivity than rxYFP, with enhanced specificity for 2GSH and GSSG, though unsuitable for ratiometric quantification [43].

HyPer
HyPer is a cpYFP-OxyR recombinant protein engineered for H_2O_2 detection. It forms a disulfide bond between Cys208 and Cys199 upon H_2O_2 interaction, altering its conformation and fluorescence. Applicable for studying H_2O_2 dynamics in various organisms, it exhibits high sensitivity and rapid response to H_2O_2. Its reduction is mediated by intracellular GSH, enabling equilibrium analysis between GSH and H_2O_2 [47]. These include:

- *HyPer-2 and HyPer-3:* HyPer-2 (mutation A406V) is more stable, while HyPer-3 (mutation H34Y) reacts faster to H_2O_2. Both have broader dynamic ranges than HyPer and are used in fluorescence lifetime imaging of H_2O_2, with pH sensitivity being a critical consideration [43, 48].

Circularly permuted green fluorescent protein (cpYFP)

Originally for calcium detection, cpYFP detects O_2^- via ratiometric fluorescence. Mitochondrial targeting enhances its subcellular specificity for ROS. However, cpYFP fluorescence is pH-sensitive, necessitating caution during mitochondrial $O_2^{\cdot-}$[49].

HyPerRed (rxRFP)

HyPerRed, a red fluorescent probe, detects H_2O_2 via disulfide bond formation (Cys208-Cys199). While sensitive to H_2O_2 and pH changes, it requires adjacent controls like HyPer-C199S for accuracy [47]. These include:

- *TrxRFP1 and cpRFP:* Redox-RFP probes like TrxRFP1 and cpRFP monitor thioredoxin and thiol/disulfide redox dynamics in mammalian cells. While aiding redox state analysis, limitations include slow reaction rates, sensitivity variations, and complex construction. Accurate analysis requires calibration, microscopy, or flow cytometry for fluorescence imaging, yet these methods have limitations in throughput and cellular-level detail [43].

Chemiluminescence analysis (CLA)

CLA probes detect O_2^- via photon emission without excitation [50]. These include:

- *Lucigenin:* Lucigenin (LC2+) detects O_2^- in cells and enzymes but has limitations, including luminescence interference from reductase-generated $LC^{\cdot+}$ and low reactivity, reducing its sensitivity for low-level O_2^- detection [51].
- *Luminol (LH):* Luminol detects O_2^- and various free radicals ($^{\cdot}OH$, H_2O_2, $ONOO^-$) but faces limitations, including low specificity, inefficient reaction at neutral pH, and interference from antioxidants. Emitting at 400 nm, it suffers from biomolecular auto-absorption. Despite complexities, luminol indicates O_2^- presence without excluding other radicals [50].
- *Luminol analogue (L012):* L012, a luminol analogue, detects O_2^- with high sensitivity but forms non-specific intermediates, causing false results with H_2O_2. Chemiluminescence enables rapid, sensitive O_2^- detection, even at low levels [52].

Electro-chemical biosensing (ECB)

Electro-chemical biosensors (ECB) utilize polyaniline-sulfonic acid and cytochrome-c layers on gold electrodes for sensitive O_2^- quantification. ECB measures real-time O_2^- dynamics *in vitro/in vivo*, but protein coating availability limits efficiency. The layered immobilization enhances cytochrome-c selectivity and detection sensitivity compared to mono-layers [42].

Chromatographic analysis

Chromatography, including Liquid Chromatography (LC), GC, UPLC, and HPLC, is widely used for oxidative stress (OS) analysis, often coupled with mass spectrometry (e.g. UPLC-MS, GC-MS). It detects $^{\cdot}OH$ radicals by stabilizing them with reagents like salicylic acid. Though sensitive and efficient, complex processes limit broader application [53, 54].

Spectro-photometric analysis

Spectrometric analysis detects ROS through absorbance difference and assay methods [42]. These include:

- *Cytochrome c reduction assay:* Cytochrome c reduction (CCR) assays detect O_2^- by measuring absorbance at 550 nm. However, CCR's specificity is limited, as enzymes like GSH and ascorbate can reduce cytochrome c, interfering with O_2^- detection. Inhibitors or scavengers, like urate or catalase, prevent reoxidation and improve specificity for O_2^- detection [42, 55].
- *Nitro blue tetrazolium (NBT) assay:* Nitro blue tetrazolium (NBT) detects O_2^- by forming diformazan with absorbance at 620 nm. NBT detects radicals from monocytes/macrophages but lacks specificity due to susceptibility to intracellular reductase [56].
- *Aconitase inactivation assay:* Aconitase activity is inactivated by O_2^- through the reversible loss of Fe from its 4Fe–4S cluster, affecting absorbance at 240 and 340 nm. This allows monitoring O_2^- generation, especially in cells like fibroblasts and macrophages. However, other oxidants like NO, O_2, and H_2O_2 may also inactivate aconitase, requiring the use of specific inhibitors to ensure accurate detection of O_2^- [57].
- *Boronates assay:* Synthetic boronates react with $ONOO^-$ and H_2O_2, generating stable phenolic products for monitoring these radicals in cells. Fluorophore-conjugated boronates, like PF1, PC1, and PG1, offer sensitive and selective H_2O_2 detection. Combined probes, such as MitoPY1, allow for targeted fluorescence imaging, generating a fluorescent signal at 528 nm upon reaction with H_2O_2, enhancing detection accuracy [58].

- *Diaminobenzidine (DAB) assay:* Diaminobenzidine (DAB) reacts with H_2O_2 to form a brown precipitate for ROS detection. It is stable, insoluble, cost-effective, and easy to use, making it ideal for subcellular ROS imaging [59].

Electron Spin Resonance Assay

Electron spin resonance (ESR) detects oxygen free radicals using spin traps for stability. The DMPO probe forms adduct with $^{\cdot}OH$ and O_2^{-} detected by ESR. While effective, DMPO-based detection can be affected by reducing agents, aqueous conditions, and short adduct lifespans. Transition metals and reactions with other radicals may also hinder accurate interpretation in cells or tissues [60]. PP-H, CAT1-H, and CP-H are cyclic hydroxyl-amines used in ESR assays, providing stable radicals for detection. These probes vary in membrane permeability and lipophilicity, enabling differentiation of O_2^{-} sources. Temperature, pH, and liquid environment affect ESR accuracy, making careful control essential for reliable radical detection in various systems [42].

Various methods can detect reactive oxygen species levels, each having its utility depending on experimental needs. Fluorescent and luminescent probes offer high sensitivity, detecting oxygen radicals qualitatively and semi-quantitatively. They can detect short-lived radicals, though regional ROS generation complicates measurements. Specialized probes like MitoTracker, Dihydroethidium (DHE), and HyPer proteins enable localized detection in organelles. Fluorescent proteins and methods like cytochrome c and DHE assays provide quantitative and qualitative detection of ROS, including NADPH oxidase-generated O_2^{-}. While ROS detection methods are advancing, minimizing their limitations remains a challenge [43].

CONCLUSION

Understanding the dual effects of ROS and antioxidants on tumor processes is essential. ROS pools have distinct actions, such as NADPH oxidase promoting cell proliferation and TIGAR protecting from ROS. Advancements in technology, like genetic screening and omics, are crucial for unravelling antioxidant pathways and ROS complexities in cancer models.

This review discusses various ROS detection methods, including spectrometry, probes, and genetic encoding assays. It emphasizes combining methods for qualitative and quantitative analysis, identifying specific radicals, and overcoming challenges like short ROS lifespans and low concentrations. Further development is needed to address the limitations of current detection methods.

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