ROS-Based Cancer Radiotherapy

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Abstract Reactive oxygen species (ROS) in cancer cells play a crucial role in metabolic reprogramming, altering tumor microenvironment, regulating cell death, repairing DNA and other physiological functions of living organisms. The unique features of ROS, which underlie the mechanisms indispensable for the aging, fitness, or growth of cells, have opened new route for researchers to take all benefits of these potential species in order to potentiate treatment efficacy and boost medical advances. Radiation therapy (RT) as a common method of cancer treatment, destroys malignant tumors and cells through both direct and indirect mechanisms. In the context of indirect mechanism, radiation could induce the generation of ROS and free radicals, resulting in the induction of cellular stress, injuring biomolecules, and ultimately altering cellular signaling pathways. Accordingly, adjusting ROS generation and elimination in favor of killing cancer cells without impairing normal cells

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hold promising approach in achieving favorable results in cancer radiotherapy. Over the past few years, nanotechnology-based materials have driven notable progress in medical and biological fields, a large number of nanomaterials with unique ROS-regulating features or nanomaterial-induced ROS formation have been exploited for its potential in modulating the tumor microenvironment and in more particular cancer cells, which contributes to the emergence of a new therapeutic modality. In order to use ROS as a potent weapon in cancer therapy, we need to eludicate its corresponding biology and chemistry as well. Herein, this chapter summarized some recent advances in ROS-based RT in detail to harness the innate powers of ROS for effective tumor therapy. Our demonstration on this emerging field will be very useful to further development of ROS-based fundamental researches and clinical applications in favor of mitigating the burden of cancer treatment.

Keywords Radiotherapy · ROS · Nanomaterials · Cancer · Tumor therapy

1 Introduction

Since late 90s, chemistry has been tightly linked with biology which led to role verification of chemical molecules in biological mechanisms. This matter along with endeavors to discover Reactive Oxygen Species (ROS) and their redox chemistry over last 50 years, have opened new doors toward development of new therapeutic agents and approaches (Fig. 1). Free radicals are molecules with one or more unpaired electrons. Incomplete reduction of oxygen (O_2) mainly forms reactive chemical species in the cell [1]: (bold ones are the major ROS products).

I. Radical ROS: **Superoxide anion ($O_2^{\cdot -}$)**, **hydroxyl radical (·OH)**, nitric oxide (NO·), peroxyl radicals (ROO·) and peroxyl radicals (RO·), organic radicals (R·), alkoxyl radicals (RO·), sulfonyl radicals (ROS·), thiyl peroxyl radicals (RSOO·), thiyl radicals (RS·), and disulfides (RSSR)
II. Non-radical ROS: **hydrogen peroxide (H_2O_2)**, **singlet oxygen ($^1O_2$)**, organic hydroperoxides (ROOH), hypochlorous acid (HOCl), hypobromous acid (HOBr), and ozone (O_3) [2–5], organic hydroperoxides (ROOH), peroxynitrite (ONO^-), nitrosoperoxycarbonate anion ($O{=}NOOCO_2^-$), dinitrogen dioxide (N_2O_2), nitronium (NO_2^+), nitrocarbonate anion ($O_2NOCO_2^-$), and highly reactive carbonyl compounds derived from lipid or carbohydrate.

ROS is generated inside the cell through oxidative phosphorylation of mitochondria or cell interaction with exogenous-sourced materials such as xenobiotics. Electron transport chain (ETC) of mitochondria and nicotinamide adenine dinucleotide phosphate oxidases (NOXs) are the major sources of endogenous ROS production. Superoxide major source is endoplasmic reticulum and mitochondria in which respiratory chain is placed. Leakage of ~2% electrons from mitochondria chain carriers and joining with O_2 develop superoxide [6]. Genomic stability and cellular macromolecular function is properly preserved from random radical damages by an arranged complex antioxidant system; prominently by GSH and SOD system. Super Oxide

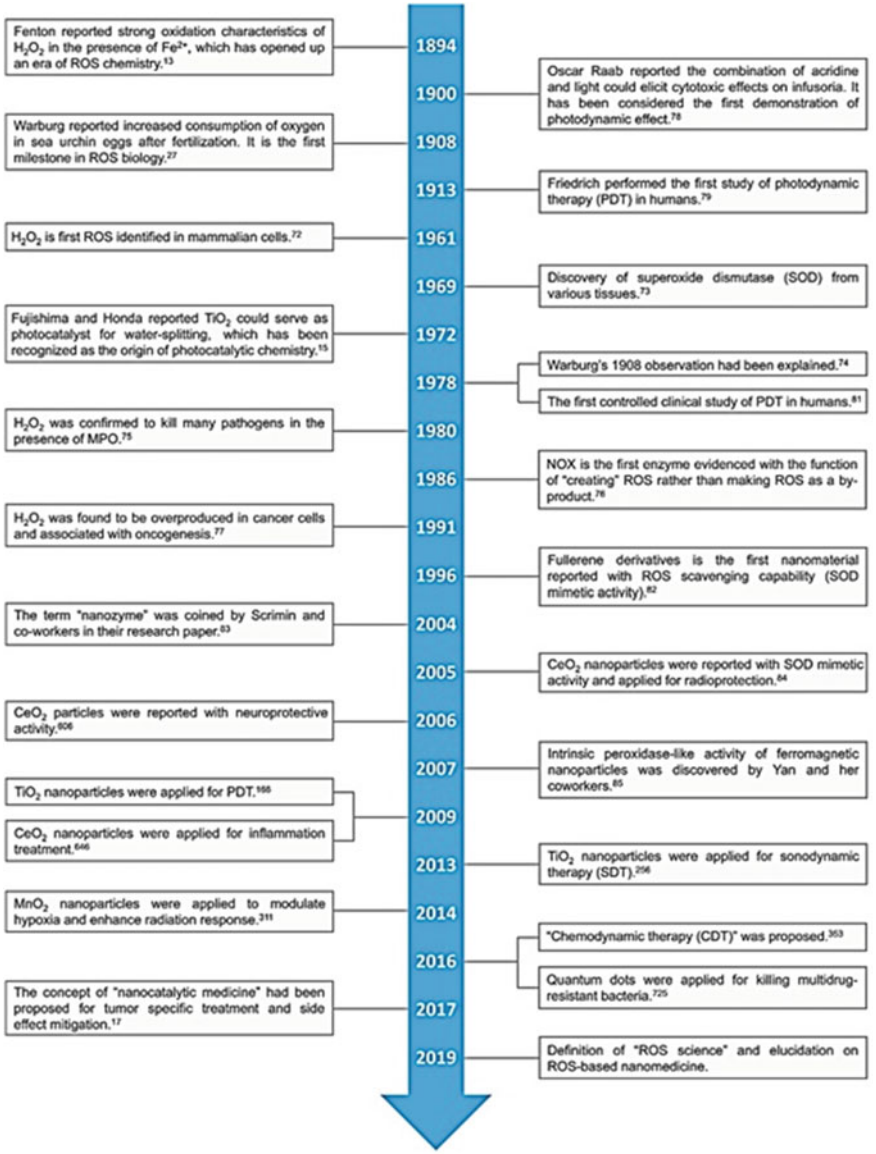

Fig. 1 Historical trend of ROS science at a glance. Reproduced with permission from Ref. [9]. Copyright 2019, with permission from ACS

Dismutase (SOD) enzyme is evolved for scavenging superoxide specifically. Important role of ROS in controlling body functions is undeniable. It involves both in cell proliferation and differentiation mechanisms [7]. Oxidative stress (which is known as a major problem in various diseases) occurs if excess amount of ROS production, more than cell antioxidant capacity, happens or if cell antioxidant storage is decreased. Cell should be able to defense against extra production of ROS because if oxidative stress occurs, proteins, lipids and nucleic acids may be damaged and subsequently involve in diabetes (i/ii), atherosclerosis, neurodegeneration, aging and carcinogenesis physiopathology [8]. ROS are involved in diseases' mechanisms both by damaging macro molecules and signaling alternation; for example by activating genes responsible for tumor metastasis.

- ROS are *produced* endogenously:
  - by the lack of oxygen, metabolic defects, physical agents (UV, heat, X-ray, γ-ray)
  - in endoplasmic reticulum (ER), mitochondria and peroxisomes where fatty acid ß-oxidation and electron transportation are conducted.
  - by enzymatic reactions: **lipoxygenase enzymes**, cyclooxygenase enzymes, **NADPH oxidases (NOX)** [10], **xanthine oxidase**, **cytochrome P450** and redox metals like Fe^{2+} and Cu^{2+} in Fenton reaction.

- ROS are *eliminated* by antioxidants inside the cell by:
  - Antioxidative enzymes: superoxide dismutase (SOD), catalase (CAT), **peroxiredoxins (PRXs)**, thioredoxine, **glutathione peroxidases (GPXs)**, glutathione reductase (GR).

Nuclear factor erythroid 2-related factor 2 (NRF2) (transcription factor which provokes antioxidant response in cancer cells), glutathione (GSH), NADPH, **vitamin C, and vitamin E** and many other antioxidant compounds are part of our food regimen components (e.g. selenium, etc.) [11]. Bold ones participate closely in redox system. SOD exists in cytoplasm, mitochondria and extra cellular matrix (ECM) with different bounded metal ions (Zn/Cu, Mn) [12].

Diseases associated with ROS toxicity and associated signaling have been investigated for either prevention or therapy. Innovative therapeutics, regarding disease pathogenesis and ROS regulation role can be developed. Although reducing ROS level is considered to be an approach for disease prevention or control like in neurodegenerative diseases and cancer therapy, the vice versa can be another useful approach for designing novel therapeutic agents. Looking from another perspective, molecules involved in ROS signaling pathways can be targeted for prevention or treatment of related diseases [13, 14]. Cancer, a deadly disease worldwide, is caused by tumorigenesis. Dysregulation of proliferation and apoptosis by ROS regulators lead to tumorigenesis. Oxidative stress involves closely in cancer pathophysiology which is caused by high level of ROS. Excessive concentration of ROS may damage pivotal macromolecules such as DNA and proteins. Cancer radiotherapy is being used as

adjuvant or monotherapy depending specially on tumor radiosensitivity. Basically, ionizing radiations such as high-energy photons (for instance X and γ ray) and particle photons (alpha–beta, electron-proton-neutron particles) are used depending on clinical necessity to interact with intracellular components and cause damage to macromolecules especially DNA, directly (cell proliferation in tumor cells will be stopped and necrosis or apoptosis may happen by damaging DNA) or indirectly by inducing ROS production as main product of water radiolysis [15]. Further, chemical reactions cause biomolecular damage which leads to structural damage, directing cell elimination. Meanwhile solid tumors with hypoxic conditions (oxygen itself acts as a radiosensitizer and fixes the induced damage) manifest more resistance to radiotherapy, thus require higher dose of radiation for treatment. Radiosensitizers can be implemented to overcome this issue with different mechanisms and attenuate subsequent side effects [16]. For oxygen concentration less than 1%, cancer cells begin to resist (mild hypoxia), and at 0.01%, they fully resist to ionizing radiation. Hypoxia intensity, cell survival and repopulation after RT within 6–7 weeks and tumor radioresistance are the three main key factors determining RT outcome [17, 18]. ROS are able to regulate specific signaling pathways involved in tumorigenesis. Specific pathways are altered in various types of cancer cells and regulate cell proliferation/growth, glucose metabolism, protein synthesis, differentiation and inflammation. It's a challenging to control ROS concentration in favor of medical purposes, considering their unstable nature. Additionally, ROS-related therapies could initiate unwanted biological reactions which makes this approach more peculiar [9]. Due to recent advancement in nanotechnology, various nanomaterials have been synthesized with the ability to regulate ROS concentration in biological environments through manipulating chemical reactions associated with ROS. New therapeutic advancements can be made considering the effect of nanomaterials on biochemistry of the living cell. Biochemical regulation of ROS modifying nanomaterials could direct us into manipulating ROS production, transformation and decrement-associated processes in order to achieve therapeutic effect. In this regard, forecasting innate ROS-regulating properties of these nanomaterials is of essence which affect ROS dynamic behavior. ROS chemistry and biology are required to be studied to design new therapeutic protocols by means of novel nano-scale materials; which can be fabricated to aim ROS production/decrement [9].

In addition to cancer, ROS can be employed for a variety of therapeutic purposes including cardiovascular diseases [19] (e.g., myocardial infarction), inflammatory diseases (such as periodontal disease [20]and intestinal inflammation)), brain diseases (such as neuroprotective therapy, PD and treatment and ischemic stroke), bacterial infection and myopia [21, 22].

2 ROS Chemistry

Chemistry of ROS has been studied for more than 50 years. The dawn of ROS chemistry investigations was the time that strong oxidation characteristics of H_2O_2 in Fe^{2+} presence was reported in 1894 (Fenton reaction) [23]. Since the beginning,

chemical properties of ROS have been investigated in different environments; nature, biology and matters. ROS are synthetized in various compartments in the nature with different mechanisms. For instance, in higher altitudes of troposphere, ·OH radicals are produced by photolysis of O_3 gas [24]. Materials can control ROS production by catalytic reactions; these reactions convert external energy to internal energy of ROS' chemical bonds. In 1972, semiconducting TiO_2 was found to be play photocatalyst role in water electrolysis [25]. Further, ·OH-involving processes were recognized in which ·OH was a photocatalyst [26]. The study of ROS generating catalysts is of importance as they can be employed for medical purposes to treat diseases such as cancer [27]. Mechanisms underlying ROS production by means of various materials will be discussed further. ROS are also produced in viable cells in different subcellular sections, especially in mitochondria. Some electrons can escape from mitochondrial respiratory chain, react with O_2 and produce $O_2^{\cdot -}$ (primary ROS) which can be further changed into H_2O_2, ·OH, ClO^-, etc. (secondary ROS) by different catalytic reactions [28]. In some types of cancer cells, ROS are produced in proliferated cell membranes by NADPH oxidase (NOX) complexes activation. Studies have shown NOX oxidase impact on cancer hall marks such as metastasis, cell survival, uncontrolled growth and angiogenesis [29]. Endoplasmic reticulum (ER) is another organelle which produces $H_2O_2$by O_2 oxidation through Flavoenzyme ERO1. This phenomenon causes a tense flux in subcellular level. Some enzymes like xanthine oxidase (XO), lipoxygenases (LOX) and cyclooxygenases (COX) and several metal ions such as Fe^{2+} and Cu^{2+} could participate in catalytic ROS production as well [11]. ROS properties are high instability and therefore high reactivity in liquid media; this results in several reactions such as reduction, oxidation or dismutation. Depending on reaction mechanism, radicals (such as $O_2\cdot^-$ and ·OH) or neuter (such as H_2O_2) oxidant molecules can be produced. Reactivity of these molecules differ from each other. Distinguishing between ROS species, based on oxidative strength is crucial, so we can comprehend the mechanisms better; for instance it enables us to anticipate which kind of ROS species take part in a specific reaction. The oxidizing capabilities of one-electron ROS radicals in a reaction can be evaluated on the basis of one-electron reduction potential as the energy necessary for activation is relatively low [30]. Activation energy is a practical tool to predict a reaction possibility to happen. For instance, in case of ROS-associated reactions, standard activation energy required to convert $O_2\cdot^-$ to H_2O_2 is lower than that of H_2O_2 to ·OH; so, former reaction is more favorable in pH = 7. In comparison, activation energy of neuter molecules of ROS (non-radical ROS) in association with kinetic factors determines their oxidative reactivity where the former is more dominant. Higher activation energy correlates with lower reaction rate [30]. In a biological setting, ROS reactivity establishes which reaction they participate in. ROS intercedes various types of redox reactions with macromolecules, right after their production. ROS can modify biomolecules in a sequence-specific or temporal manner [31, 32]. As the oxidizing strength declines, the power of specific species to discriminate between different reactions declines; so $O_2^{\cdot -}$ or H_2O_2 are capable of targeting specific biological molecules while ·OH and $^1O_2$ are not. Such a preference was first observed in E.coli in which superoxide specific reactivity with Fe-S clusters was identified. Further release of H_2O_2 and

Fe^{2+} is responsible for superoxide mediated toxicity as it leads to ·OH generation by Fenton reaction [1, 33, 34]. Other observation in E.coli provided the mechanism of high reactivity of H_2O_2 with Cys residues of macromolecules to manipulate cellular signaling pathway [35]. $^1O_2$ biochemistry has been rarely investigated as it commonly is produced by exogenous reactions rather than biological reactions. The ROS reactions has been assumed to be conducted in a homogenous media, therefore steric hindrance has not been contemplated and is ignored in above discussion. Moreover, all unit components of an organism such as organelles and cells, and in whole the organism itself are systems which have the following characteristics: heterogeneous, dynamic architecture relating to time and space. As ROS are naturally transitory and reactive, redox reactions take place in the site they are produced and subsequently localized. ROS can barely move farther than the cell diameter, except in the case of H_2O_2 which can move approximately for 1.5 mm [30]. Hence, in biology context, ROS should be compartmentalized considering ROS-involved reactions. Theoretically, some redox reactions may happen but taking time and space restrictions into account, they will not be practically probable in a physiological environment [30]. In physiochemical environment, reaction characteristics that determine ROS biochemical reactions are as follows; reactivity, specificity, and the ability to diffuse. Study on the ROS chemistry can lead us to clearly understand complicated chemical processes and develop tools in order to investigate ROS biology (for instance its role in oxidative stress and pathology).

3 ROS Biology—ROS Modulation to Radiotherapy Efficacy Improvement

Physiological roles of ROS has been a subject of study since 1908 when, for the first time, the role of H_2O_2 was discovered [11, 36]. There are controversial data on the role of ROS as intracellular signaling molecules. ·OH and $^1O_2$ cannot contribute in intracellular signaling by redox pathway as they are localized due to their more relatively transient nature and less ability to travel farther than the site of production. $O_2^{\cdot -}$ cannot penetrate cell membrane while is unstable thus, is not a strong candidate for cell signaling. H_2O_2 can be a potent candidate with the opposite characteristics of above-mentioned molecules along with its high specificity and more capability to travel longer [1]. In 1981, insulin potency for promoting tumor growth was demonstrated; the underlying mechanism was elevating intracellular H_2O_2 level [37]. In 2003, it was observed that ROS production by NOX can facilitate plant growth by further activation of Ca^{2+} channels [38]. Then, it was discovered that H_2O_2 has a regulatory role in cell migration by modulating cytoskeleton structure in which the mechanism was NOX-derived H_2O_2 production [39]. As investigations followed, H_2O_2 concentration gradient in healthy cells near the wound was found to be associated in migration of leukocytes to the injured tissue [40]. Intracellular crosstalk mediated by ROS can help us improve our comprehension about

other areas of science such as circadian cycle and cell biology. In circadian cycle studies, red blood cells (RBCs) has been discovered to have a significant role in circadian regulation by redox reactions of peroxiredoxin proteins in a 24-h cycle which may continue for several days [41]. Despite molecular structure and reaction ambiguity, it is clear that ROS contribute in circadian regulation; it was detected by observing fluctuations of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) in relation to each other [32]. Phagocytosis mechanism depends on superoxide generation as leucocytse are not able to kill infectious organism without this reactive molecule [42, 43]. As such, glial cells are able to protect CNS from infectious diseases, but higher than necessity production of radicals leads to radical induced damages [44]. ROS role in regulation of cell signaling is accomplished by interacting with signaling molecules thereby, regulating cell processes. In an intracellular cross talk, H_2O_2 mostly targets reactive cysteine residues for reversible oxidization. It is noteworthy that cysteine amino acid is part of a regulation system for numerous physiological events [45].

In brief, ROS regulates signaling pathway by modifying redox state of Cys (Cystein) residues on proteins which leads to function and/or structure alternation of aforementioned proteins. Detailed pathways in which ROS affects signaling are reviewed by Ray et al. [46].

Several enzymes like ribonucleotide reductase and cytochrome 450 use iron for their function. Excess amount of free iron involves in Fenton reaction and develops ROS and causes oxidative stress. Thus, maintaining normal function of cell and prevention of iron induced oxidative stress are owed to iron balance. Iron can express genes which regulate its storage and transportation in post transcriptional level. ROS can regulate iron regulation. Cellular iron hemostasis (IRE–IRP regulatory system) is regulated by both intra cellular iron and ROS to prevent cell from undergoing iron-induced oxidative stress [46].

ROS need to be neutralized to maintain redox homeostasis. That is of importance, as overwhelming antioxidant capacity can lead to pathological events like aging and cancer. So, with regard to their role as intracellular messengers in moderate concentration, ROS production and elimination should be balanced as excessive amounts can cause cell death [47].

Anti-oxidant system retains cell's redox balance. Upon exposure of normal cell to continuous exogenous stimuli and activation of endogenous oncogene, the redox balance fails to be maintained and normal cell turns to a cancer cell. Nascent cancer cell's ROS level should adapt with redox system to survive. High level of ROS and defective antioxidant defense system of cell make it (more than normal cell) susceptible to ROS modulation. While the same concentration of ROS makes cancer cells undergo apoptosis, normal cells can tolerate the condition [48].

Normal cells detoxify themselves from radicals by molecules or enzymatic reaction as shown in Fig. 2:

- Superoxide dismutase: a group of metalloenzymes which convert superoxide to oxygen and hydrogen peroxide and regulate biology processes specifically.

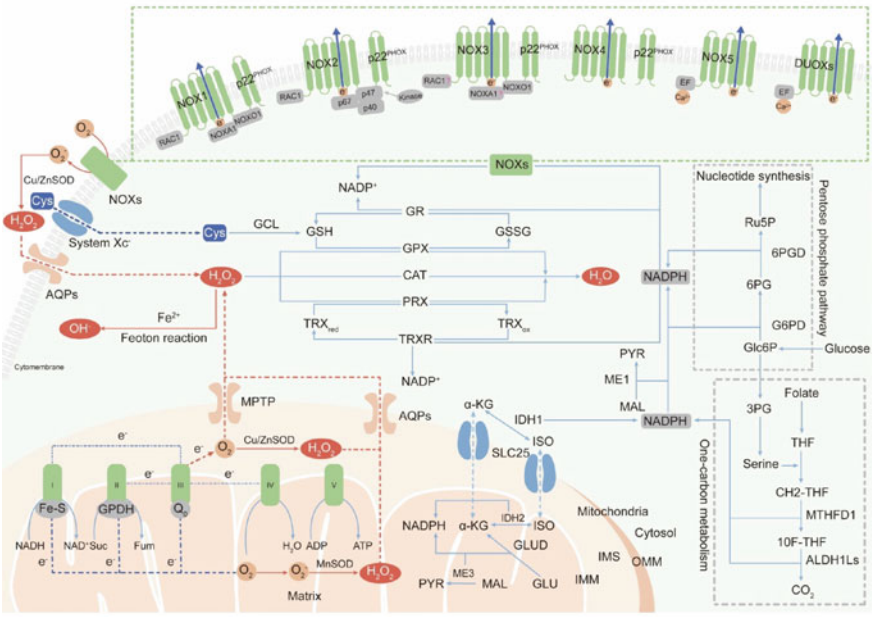

Fig. 2 Intracellular primary ROS generation and elimination mechanisms. Reproduced with permission from Ref. [52], with the permission of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). Copyright 2021, Ivyspring

- Catalase, which converts hydrogen peroxide to water and oxygen, and it mostly resides in cytosol and peroxisomes in eukaryotes [49, 50].
- Glutathione system consists of glutathione, glutathione reductase, glutathione peroxidases (GPX) and glutathione S-transferases (GST). Glutathione shields the cell from oxidative stress by transforming disulfide bonds of proteins to cysteine and changes into GSSG (oxidized form) which further is converted to GSH (reduced form) by the involvement of glutathione reductase. Glutathione transferase facilitates the covalent bond construction between S atom of GSH and the electrophile compounds [47, 51].

Investigations have exhibited that aging is a result of oxidative stress which itself is caused by ROS over production [53, 54]. Although ROS production as long as its amount is lower than cell capability to overcome oxidants, seems to elevate lifespan of living creatures; as manifested recently in case of drosophila, low amounts of oxidants leverage its life time [55]. Different levels of ROS lead to different effects:

- Basal level of ROS maintains normal cell hemostasis.
- Continuous and low level of ROS is associated with cell mitosis and elevated gene instability which induces tumor initiation and progression [56].
- Cell cycle arrest (temporary or permanent) and differentiation are related to moderate concentration of ROS [57].

- Macromolecule damage and induced apoptosis, necrosis and ferroptosis are related to acute elevation of ROS concentration [58, 59].

As radiation passes through cells, induced ROS causes unrepairable damage to gene stability and causes death. Of note, cancer cells are not efficient as normal cells to repair radiotherapy-induced damage. Subsequently, killing cells is relatively differential [60]. Many strategies in cancer therapy focused on increasing intracellular ROS level to promote irreversible damage and trigger apoptosis of tumor cells [59]. Regulating intracellular ROS level to efficiently kill cancer cell is a fundamental approach to treat cancer and decrease radiotherapy side effect. Radiation affects cancer prognosis which is dependent on ROS regulation directly or indirectly [59]. Cells can protect themselves from oxidative stress by activation of autophagy program. This is achieved by phosphorylation of proteins involved in cell survival/death/proliferation and DNA repair [61]. Elevated ROS level has been identified in many kinds of cancer cells afterwards, but the correlation between ROS overproduction and its tumorigenesis ability is still controversial. Several factors related to cancer cells and tumor environment facilitate ROS overproduction and influence cellular equilibrium to control radical species. Conversely, some strong evidence propose that raising levels of ROS provokes presence of cancer hallmarks. When provided level of ROS is medium, they involve in pro-oncogenic processes or boost DNA mutation and as a result, assist tumorigenesis [48]. High amounts of ROS are produced in cancer cells from different sources; high metabolism, peroxisome activity and mitochondrial dysfunction, mainly. H_2O_2 is the second messenger as it passes easily from membrane aquaporine-8. Besides, cytokines like IL-1 and growth factors like EGF provoke ROS production in tumor cells. Incidence of several cancers depends on generation of superoxide radical [62–64]. Inflammation has an essential role in progression of tumor [65–67]. Inflammation-activated macrophage produces nitric oxide that converts superoxide to peroxinitrite radical which can contribute to apoptosis of tumor cells similar to hydroxyl radicals. Macrophages and neutrophils stimulate ROS production inside cancer cells and the burst generation of superoxide, which changes into hydrogen peroxide and kills tumor cells [43, 68]. This contradictory role of ROS in cancer cells, in which ROS level is raised and detoxified simultaneously, implies the existing ROS balance inside cancer cells [51]. Redox hemostasis is regulated by oxidative stress sensitive transcription factors which include Nrf2/Keap1 complex (Fig. 3), nuclear factor-κB (NF-κB), p53, and hypoxia inducible factor 1 (HIF-1). After sensing oxidative stress, Nrf2/Keap1 activates downstream antioxidant elements such as glutathione-S-transferases (GST), PRXs, GPXs, and CAT and NAD (P)H: quinone oxidoreductase (NQO1). Other transcription factors rise ROS scavenger enzymes (such as GSH and SOD), inhibit cell death associated factors and activate survival factors (such as B-cell lymphoma-2 (Bcl-2) and myeloid cell leukaemia-1 (Mcl-1)) [47, 69, 70].

Following oxidative stress, ROS regulate specific tumor cell signaling pathways which determine cell fate (Fig. 4).

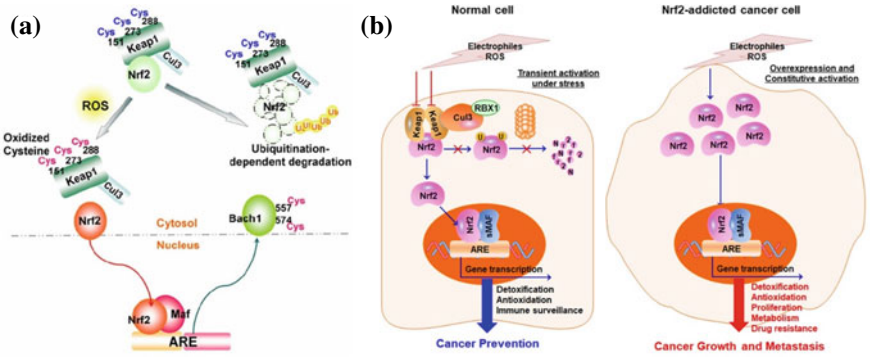

Fig. 3 **a)** Redox regulation of the Nrf2–ARE pathway. Reproduced with permission from Ref. [46]. Copyright 2012, with permission from Elsevier. **b)** Dual roles of Keap1-Nrf2 signaling pathway studied in pancreatic cancer. Reproduced with permission from Ref. [71], with the permission of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). Copyright 2019, BMC

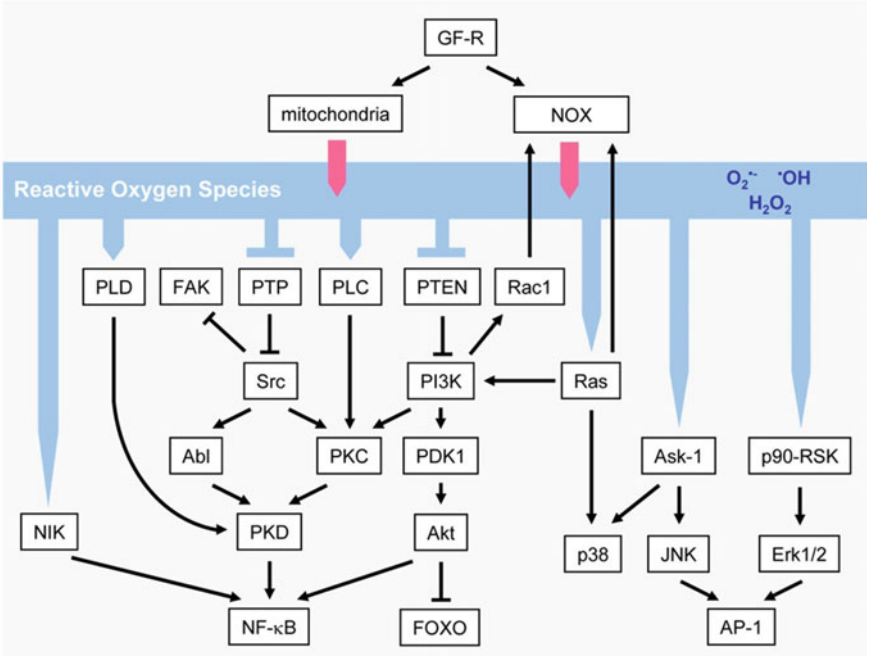

Fig. 4 ROS-mediated cell signaling pathways. Reproduced with permission from Ref. [51]. Copyright 2010, with permission from Taylor and Francis

1. MAPK/Erk 1/2: Activation of this pathway by growth factors leads to cell proliferation. The key role of this pathway activation by ROS increment is well established in proliferation of tumor cells, motility and cell growing without adhesion (anchorage-independent) [72–74]. ROS ability to regulate this pathway is due to their impact on upstream activator/kinases of Erk 1/2 [73, 75]. As demonstrated in ovarian cancer cells, the suppression of negative regulators mediated by elevated endogenous ROS leaded to increased activation of this pathway [73, 75]. ROS effect on cell survival is dependent on cell type and Erk1/2 signaling role, activated by ROS, is evident [73, 75–77]. In breast cancer cell, decreased ROS concentration results in higher apoptosis rate while in human pancreatic and glioma tumor cells, elevated level of ROS concentration is responsible for cell death [78, 79]. MAPK (mitogen-activated protein kinase)- Erk1/2 (extracellular regulated kinase 1/2).
2. PI3K/Akt: PIK3, a key signaling pathway that determines cell survival or proliferation, following hormone secretion, growth factor and cytokine effects, is regulated by ROS (Fig. 5). Cell survival is influenced by phosphorylation of Akt substrates as found in ovarian, breast and human pancreatic cell lines. ROS production activates Akt and upstream kinases including PDK-1 (3′-phosphoinositide-dependent kinase-1) and mTOR which control (increase) Akt activity along with PTEN as a phosphatase (decreased activity) [80–82]. PTEN

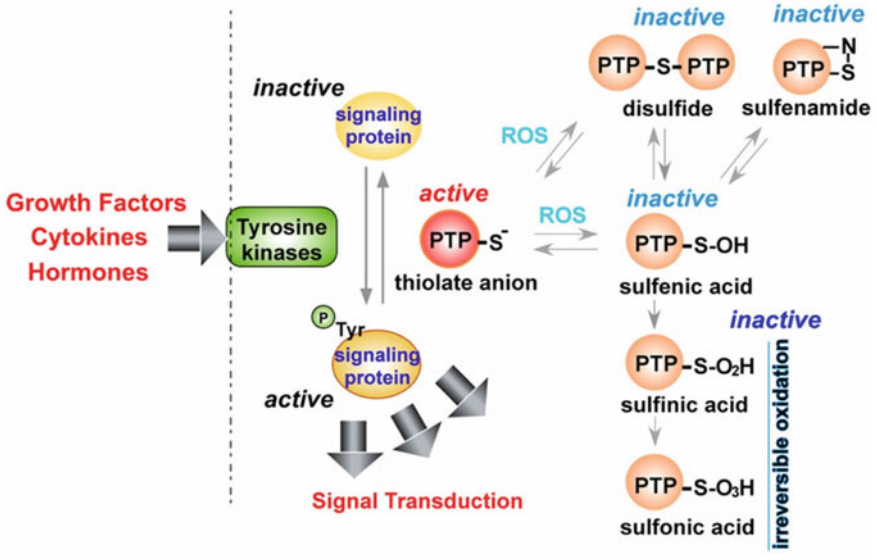

Fig. 5 ROS-mediated protein tyrosine phosphatase inactivation. Protein tyrosine phosphatases (PTP) can reverse phosphorylation; ROS inactivates oxidation of catalytic cysteine residues by PTP, eventually resulting in formation of relatively irreversible sulfinic ($-SO_2H$) or sulfonic acid ($-SO_3H$). Reproduced with permission from Ref. [46]. Copyright 2012, with permission from Elsevier

loss (phosphatase and tensin homolog delete on chromosome 10) leads to constant activation of Akt by enhancing ROS production as well as inducing upstream activating kinases by oxidative stress as basal level of superoxide and H_2O_2 increases due to depleted expression of several antioxidant enzymes including Cu/Zn-SOD and peroxiredoxins.

3. IKK/NF-κB: Increased activity of NF-κB transcription factor following increased cellular oxidative stress and its essential role is discovered in survival, cycle regulation, adhesion and drug resistance of tumor cells [83–86]. Low doses of hydrogen peroxide are able to activate NF-κB (redox-regulation) [87]. Cytokines like TNF-α and IL-1 induce translocation of NF-κB and consequently anti-apoptotic and anti-inflammatory genes will be expressed [88, 89]. As observed in MCF-7 breast cancer (following TNF-α and IL-1ß treatment) and oral squamous carcinoma cells (following SOD silencing), increased ROS concentration and activated NF-κB resulted in cell proliferation and increasing NIK/NF-κB activity, respectively [90, 91].

Specific role of ROS in tumorigenesis and cancer cells are categorized as follows:

1. **Proliferation**: In various cancer cell types, low doses of superoxide and hydrogen peroxide are capable of stimulating cell proliferation [51, 92]. For instance, increased mitochondrial ROS in breast cancer cells, either by estrogen translocation or decreased MnSOD activity and hydrogen peroxide attenuation simultaneously, directs the cell toward proliferation. Therefore, the role of mitochondrial induced tumor growth is well-known [93]. Upregulated level of cyclins' mRNA by ROS indicates that loss of cell redox balance of cell cycle in normal cell like MCF-10A causes abnormal proliferation which can be treated by NAC antioxidant and as a result postpone G1 to S phase progression [94, 95]. As mentioned before, it has been shown that antioxidants can halt proliferation in vivo and in vitro in pancreatic cancer cell line and ATM knocked-out mice, respectively (Fig. 6) [96–98]. Taken together, ROS positively regulate tumor proliferation by modifying crucial proteins that control cell cycle [46].
2. **Cell survival and apoptosis**: Off-balanced intracellular ROS and incremental oxidative stress in mitochondria predispose the process of senescence, cell cycle arrest and apoptosis (including Rac-1/NADPH oxidase pathway) [99, 100]. Chemotherapy, antioxidant deprivation and immune system are capable of inducing of ROS overproduction. Bcl-2 and Bcl-XL have the ability to disaffect ROS-induced apoptosis in the cell [100–102]. Mitochondrial H_2O_2 and NO release eventually, downregulating the production of these two anti-apoptotic proteins [99, 103]. Furthermore, alternation of Bax/Bcl-2 complex conformation dissipates integrity of mitochondrial membrane [104–106]. ROS elevation affects JNK, p38, Ask-1, forkhead transcription factors and p66Shc in the process of apoptosis induction (Fig. 7) [107, 108]. It is hypothesized that continuous oxidative stress status may result in selection of p53 deficient colony of cells which are resistant to apoptosis [51]. ROS production is also induced by death receptors like TNF receptor I, mediating several pathways. Of note, anti-apoptotic signals are also involved after oxidative stress is induced by TNFα.

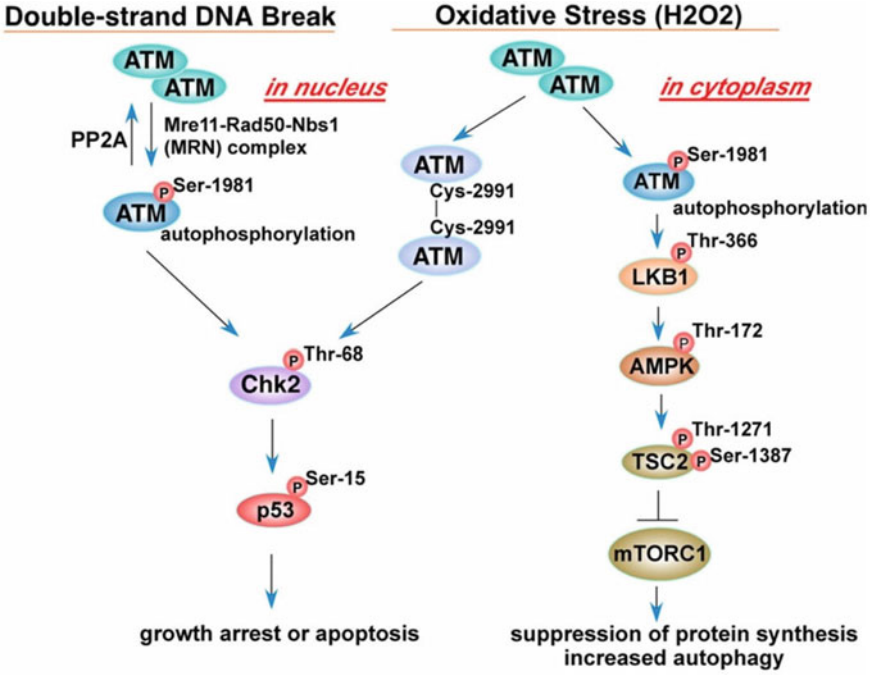

Fig. 6 ATM signaling following oxidative stress and double-strand DNA breaks. Reproduced with permission from Ref. [46]. Copyright 2012, with permission from Elsevier

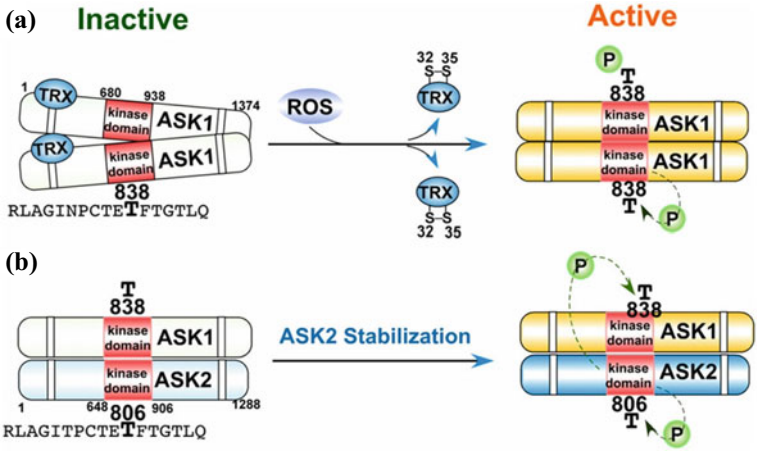

Fig. 7 ASK kinases activation in response to oxidative stress. **a)** Oxidation of thioredoxin (TRX) and its subsequent dissociation. **b)** Hetero-oligomerization of ASK1 and ASK2. Reproduced with permission from Ref. [46]. Copyright 2012, with permission from Elsevier

MnSOD and catalase are expressed after NF-κB pathway involvement [109, 110]. Inconsistently, low levels of oxidative stress and increased generation of mitochondrial ROS develop signaling pathways associated with cell survival and upregulate the generation of antioxidants and anti-apoptotic proteins (such as Akt which is activated by ROS, respectively [51, 111–113]. The elimination of related pathway matters in sensitizing tumor cells to ROS-induced cell death [114–117].

3. **Metastasis**: Higher concentration of intracellular ROS level is detected in metastatic and motile subpopulations of non-or low- motile MCF-7 cell line [118]. Also, a decreased level of MnSOD activity was detected in metastatic breast and highly invasive pancreatic cancer [119–121]. This implies that a crucial part of metastatic process relies on intracellular redox status. Metastatic processes include lower cell adhesion to extracellular matrix, elevated potential for migration and invasion, independency of anchorage for cell survival and eventually intra-vasation. In contrast, mitosis of normal cells require to be anchored to extra cellular matrix (ECM) and similar to tumor cells, mediation of ROS is required; increased mitochondrial ROS increases normal cell adhesion to ECM and proliferation [122, 123]. In the case of a normal cell detaching from ECM, anoikis will be triggered and cell life will be terminated while tumor cells are immune to this effect and are able to form new colonies distant from initial site of tumorigenesis. Tumor cells' independency of anchorage signals to proliferate and terminating apoptosis signals after losing contact with ECM owes most likely to increased level of intracellular ROS generation [124, 125]. Other processes ROS involves in to direct metastasis include epithelial-mesenchymal transition of cell membrane to degrade basal membrane protein composition, DNA damage and instability of genome, actin reorganization and cell shape change, increased cell migration, induction and maintenance of tumorigenic state of cell, ECM degradation and reorganization, losing cell–cell adhesion and probably increased permeability of vasculature. It is noteworthy that mitochondrial ROS are capable of enhancing cancer cell metastasis by contributing to tumor progression [51, 126, 127].
4. **Tumor progression**: As tumor grows, limited oxygen supply happens and activates the transcription of several transcriptional factors including HIF-1 where its expression is suppressed by MnSOD under hypoxic condition [128–130]. This implies the involvement of superoxide and H_2O_2 in the accumulation of HIF-1α [131]. As HIF-1 regulates genes related to glycolysis and halts mitochondrial respiration, tumor cell energy metabolism shifts to anaerobic glycolysis by transactivation of glucose transporter (GLUT-1) and lactate dehydrogenase (LDH). As a consequent of inhibiting mitochondrial activity, H_2O_2 production decreases and the survival rate of oxygen-deprived cells enhances [132]. Thus, tumor cells' metabolism is shifted to anaerobic after oncogenic transformation assuming even normal oxygen supply [130, 133]. Ultimately, this adaption to hypoxia invokes the expression of genes related to metastasis which breeds tumor malignancy and subsequent poor prognosis. Furthermost, HIF-1 prevents intracellular acidic condition (increased formation of lactate and CO_2)

and both molecules favor cell motility with degradation of extra cellular matrix [134, 135].

5. **Angiogenesis**: As tumor grows, oxygen and nutrition need are increases [136, 137]. New blood vessels should develop to accomplish this. Oxygen and nutrition shortage induce expression of intracellular ROS and VEGF (vesicular epithelial growth factor), increasing the intracellular level of ROS [138–141]. Chaotic blood flow in newly formed micro-vasculature assists oxidative stress condition by hypoxia and re-oxygenation fluctuation which augments ROS concentration [142]. ROS elevation provokes angiogenesis with upregulating HIF-1 which initiates gene expression of angiogenesis mediators (growth factors) especially, VEGF [143, 144]. Similarly, intracellular ROS decrease caused by mitochondrial suppressors or glutathione peroxidase decreases HIF-1 induction and VEGF expression in cancer cells [135]. ROS-induced matrix metalloproteinase formation corporates in angiogenesis as well [145]. Additionally, ROS can trigger vasodilation and increases blood supply through heme oxygenase-1 activation leading to formation of CO and NO [146].
6. **Redox status regulation of cancer stem cells (CSC)**: Cancer stem cells are responsible for recurrence initiation of treated tumor with radio- or chemotherapy because CSCs can resist to radiotherapy-induced damage or chemotherapy drugs oppositely to their more mature progeny [147, 148]. Increased HIF-1α inside CSCs causes poor prognosis and radiotherapy resistance. As reviewed earlier, normal cells transform to cancer cells through genome instability, enhanced motility, anchorage independency to grow and oncogenic growth. The level of ROS in CSCs is lower that of in normal cells and more mature tumor cells. This feature of stem cells are due to higher anti-oxidant capacity, anti-apoptotic proteins (such as Bcl-2), DNA repair enzymes and increased expression of ROS scavenging enzymes, especially those involved in glutathione synthesis which induce a moderate level of ROS inside the cell [47, 149]. ROS scavenger depletion leads to lower colony forming ability of CSCs and increased radio sensitivity [149]. By continuous exposure to low level of oxygen, CSCs undergo epithelial to mesenchymal transition (EMT) and make the tumor invasive and metastatic. Ionizing radiation will cause cell death by means of ROS production, but cancer stem cells are immune to this effect due to high anti-oxidant expression [150, 151]. For instance, pharmacological reduction of GSH in epithelial cancer stem cells leads to attenuated growth and enhanced radio sensitization [149]. Characteristics lower DNA damage (double strand and single strand DNA break) in the population (higher the stem cell count, lower the total damage will be) following radiotherapy and the survival chance rises. Due to self-renewal and differentiation capability of CSCs, they are more inclined to form resistant, aggressive and heterogeneous tumors, especially when tumor is not vascularized enough to deliver oxygen [152]. Thus, resistance of stem cells to chemotherapy drugs, which target redox system and elevate intracellular ROS level, may occur. In order to reduce recurrence following conventional treatments, stem cell special redox state should be considered (Fig. 8) [51].

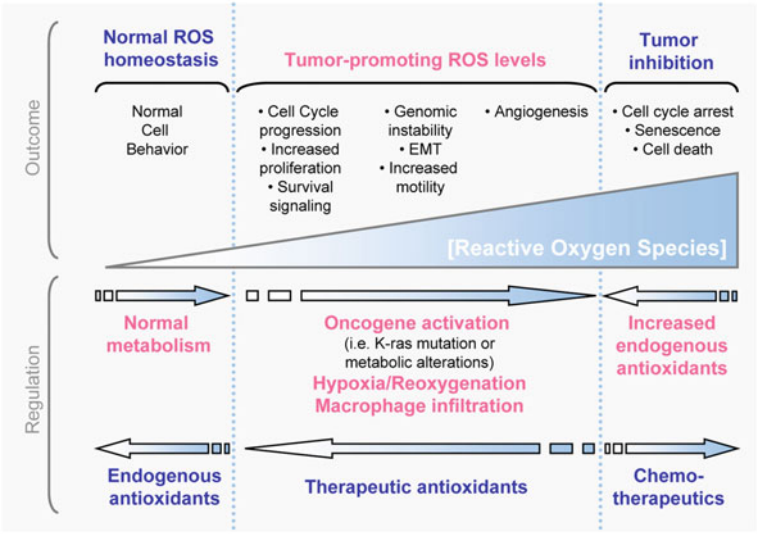

Fig. 8 Cellular ROS regulation and the corresponding response in different ROS levels. Reproduced with permission from Ref. [51]. Copyright 2010, with permission from Taylor and Francis

Oxygen is a crucial enhancer of radiotherapy efficacy for fixing the damage and subsequently killing cancer cell. Increasing oxygen in tumor site is possible by following approaches. Although implementation of hyperbaric oxygen elevates oxygen level directly, in some cases causes complications as well [153, 154]. Also, hydrogen peroxide can be used intra-tumorally and increase oxygen production to boost irradiation efficacy [155]. Hemoglobin level or oxygen binding modification could be another useful approach seemingly beneficial to the patient [156–158]. **DNA damage fixation**, **cell death programing**, CSC features and tumor microenvironment condition significantly impact radiotherapy outcome in which the bold ones are the most influenced by cell redox condition.

ROS balance can be altered in favor of cancer therapy by affecting involving pathways (Figs. 9 and 10). These pathways can precede to initiate cell apoptosis and autophagy. This phenomenon results in altering ROS balance in favor of apoptosis and consequently killing the cancer cell. Other pathways can affect inflammation responses and iron hemostasis in cancer therapy. Changing ROS concentration, production and elimination should be carried out by xenobiotics and materials implementation. Next section will focus on nanomaterials which are promising tools for ROS production/elimination interventions.

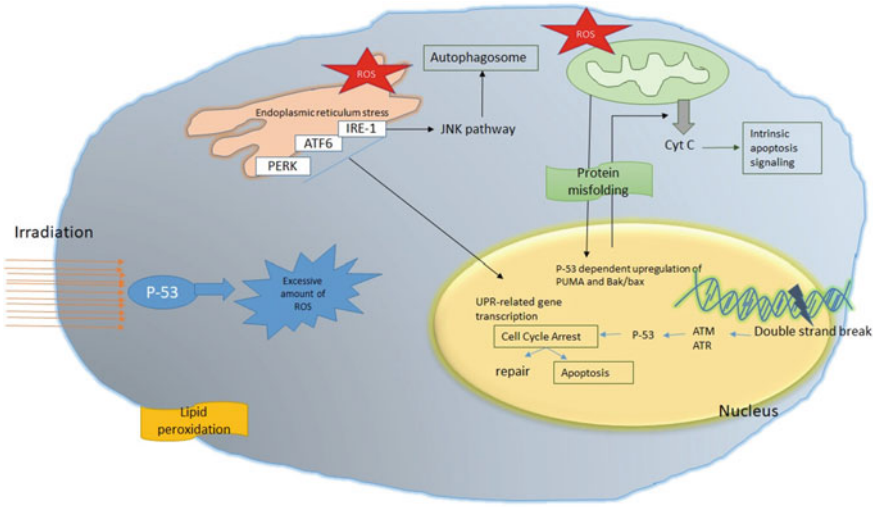

Fig. 9 ROS-induced events following irradiation

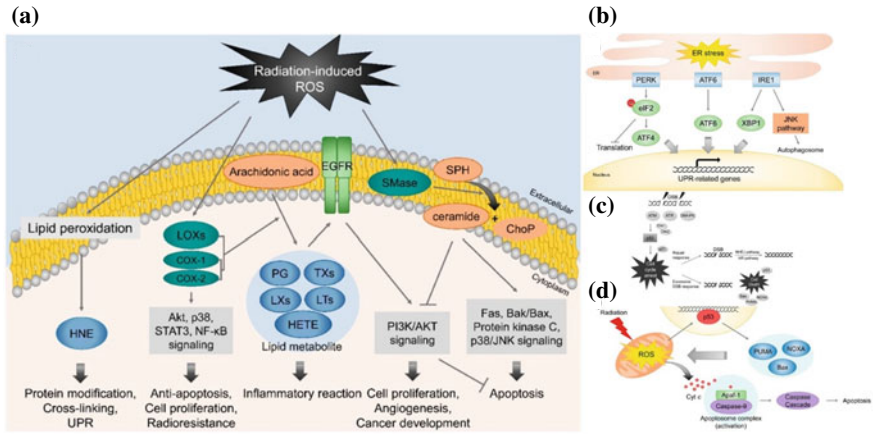

Fig. 10 Detailed cell responses to radiation. **a** lipid peroxidation and ceramide signaling, **b** ER stress, **c** Double strand break and **d** Mitochondrial response induced by radiation. Reproduced with permission from Ref. [159] with the permission of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). Copyright 2019, MDPI

4 ROS Nanotechnology

Various underlying pathological mechanisms are connected to ROS generation more than cell capacity to handle. In recent era, numerous nanomaterials have been synthesized that can be both multifunctional and ROS-regulating at the same time, owing to nanotechnology. The utilization of functional nanomaterials has been widely

expanded over the past few years [160–166]. Nanomaterials are capable of affecting biology of cell by generation, transition or depletion of ROS levels; thereby they can apply their therapeutic effects by regulating cellular redox hemostasis. Thus, ROS regulating nanomaterials can be counted as therapeutic modalities with unique chemistry to intervene pathological abnormalities [2].

Temporo-spatial characteristics of nanomaterials are determined by two innate features: Biophysics and biomedical features.

- Biophysical features include thermodynamics, 2D surface topography, 3D stereoscopic geometry etc.
- Biochemical features include interfacial reactivity, enzymatic interaction etc.
- ROS research in nanotechnology should consider nanomaterials' chemistry (ROS production, transition and depletion) and biology (bioavailability, biodegradability, and biocompatibility) (Fig. 11).

There are two different methods for nanomaterial synthesis: Top-down (making bulk materials smaller to reach a nanoscale) and bottom-top (reaching nanoscale from atoms' angstrom scale dimension). These two methods make it possible to synthesize nanomaterials with distinct dimensions.

Dimensions of nanomaterial	Nanomaterial specific name
0	Nanoparticle
1	Nanowires
2	Nanosheets
3	Nanoformulations

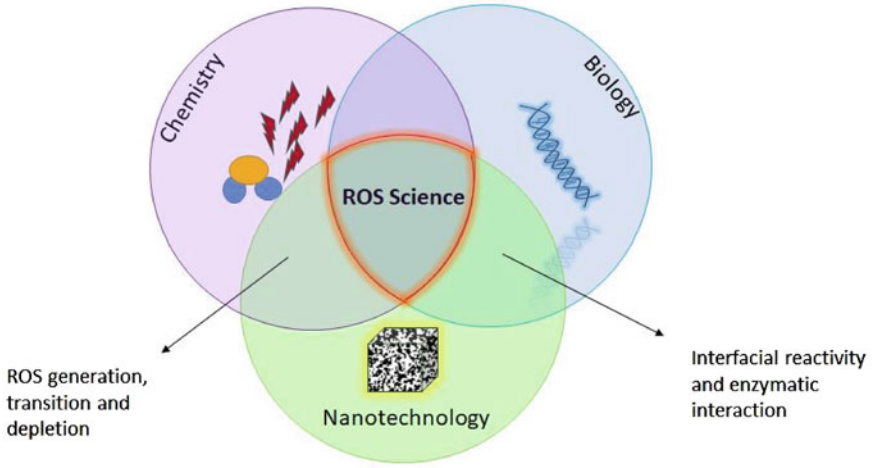

Fig. 11 Nanotechnology interface with biology and chemistry

Why nano-scale? Not micro? For designing ROS-based therapeutic agents, size impact and surface area to volume ratio are of importance.

$$\text{Surface Area (SA)/Volume} = \text{Specific SA}$$

Nanomaterials are equipped with unique features because of their nanoscale size. For instance, for inorganic nanoparticles, when the size is less than 20–30 nm, crystallographic properties regulate kinetics of interfacial reactions including kinetics of ROS reactions which occur at the surface of nanoparticles [167]. With nanotechnology, large specific surface area (SA) and small volume are useful features that come in the territory. Large specific surface area creates plenty of anchoring sites available for ambient reactive species which improves the chemical reactivity. In addition, small volume provides easier tissue penetration and cellular uptake and possibility of intracellular transportation of these nanosystems. As investigated before, size of ROS based nanomaterials determines their physiochemical role and consequently regulates biological responses [168]. To acquire the best ROS-regulating properties and also achieving better in vivo therapeutic effect, extrinsic (e.g. particle size, volume and specific SA) and intrinsic factors (physiochemical features) concerning nanomaterial performance should be considered given that nanomaterials have acquired magnificent in vivo ROS regulating behavior for therapeutic applications due to aforementioned characteristics. We will discuss nanomaterial modulation from the chemical and biological perspectives.

In chemical view, due to recent advancements, reaction characteristics of nanomaterials can be modulated feasibly. This can be achieved by:

- Precise topographic modulation by increasing quantity of localized action sites on nanomaterial surface
- Combining nanomaterials with other functional materials; this integration enhances nanoparticle activity, selectivity and versatility in biological systems.

Developing ROS based nanomaterials with ROS generating/scavenging ability effectively modulates reactions to further benefit therapeutic results. As an example, heterogeneous nanocomposite catalysts can intervene effectively in cancer therapy by in situ ROS production through catalytic enzymatic reactions [169, 170]. In biological view, comprehending systematic effect of the materials is mandatory. Due to nanomaterials' large SA, strong adsorption of biomolecules such as DNA, proteins and phospholipids occurs to stabilize surface area by attenuating excessive surface energy [171]. These interactions can affect how the cell and the nanoparticle affect each other which further influence how cellular substructure and cellular biochemistry can change [172]. Thus, in ROS research field, nanomaterial's biological systematic effect should be examined to achieve a better therapeutic result. Taking TiO_2 as an example, it was revealed that size determines physiochemical properties, ROS-induced toxicity, shape, efficacy and subsequently its biological effect, as studied theoretically [173]. In this context, quantitative optimal parameters should be considered to be evaluated extensively along with nanomedicine efficacy evaluation.

In a unique pathological region, investigating nanotechnology and biology interaction may enhance the outcome of ROS-based nano-therapeutics significantly. For instance, pores of tumor blood vessels enhances nanomedicine delivery of smaller nanoparticles (~12 nm). Notably, hydrodynamic diameter and steric hindrance are responsible for difficult entrance of larger nanoparticles (125 nm) into tumors [172]. Thus, for treating cancer, nanomaterials should be designed small enough to acquire the capability of transportation (within vessels), accumulation (inside the tumor) and excretion (from kidney) [174]. To achieve improved ROS-regulating efficacy and enhanced therapeutic outcome, integrating chemistry and biology at nanoscale is essential for designing ROS-based nanomedicines.

In cancer treatment, ROS-based nanoplatforms can push forward redox regulating therapeutic modalities such as RT [2].

(1) **ROS-based nanomaterial chemistry**: It is essential to elucidate nanomaterial chemistry to determine biological behavior and in vivo chemical features. Unique chemical characteristics of representative materials that involve in cell redox status will be overviewed (such as nanozyms, photosensitizers, radiosensitizers). In this context, utilizing ROS detectors as developing tools can improve our understanding of chemistry of nanomaterials in which provided biomedical assays respond quantitatively to ROS level changes [175, 176].

1.1. **ROS-generating nanomaterials**: Excessive ROS generation in tumor is considered a general therapeutic approach in which intracellular redox status upregulation occurs by exo-/endogenous interventions [48]. ROS-generating nanosystems utilize their intrinsic chemistry to extract and transit energy of exogenous sources into ROS internal chemical energy. Similar to radiotherapy, which employs ionizing radiation, photodynamic (PDT), sonodynamic (SDT) and chemodynamic therapy (CDT) employ optical, mechanical and chemical energies, respectively, to evoke remote-controlled ROS generation in site of pathology.

1.2. ROS-scavenging nanomaterials: Various materials have enzyme mimicking activity (such as SOD-CAT). These nanoenzymes down-regulate deviant amount of ROS to level which is compatible with cell function [177]. Fullerene derivatives are counted as a great achievement in nanozyms. They behave like SOD and electrostatically stabilize superoxide with their electron deficient regions. Fullerene derivatives are extensively exploited as antioxidants due to their unique ROS detoxifying capability [178]. Next eye catching improvement in nanozyme field was discovering SOD and CAT mimicking activity of inorganic nanomaterials, such as CeO_2 nanoparticles [179–181]. Mixed valance state of cerium (Ce^{3+} and Ce^{4+}) and presence of spare oxygen vacancies are the reasons behind CeO_2 nanoparticle capability of efficiently scavenging superoxide and H_2O_2 [182, 183]. Thus, it is clear that cerium nanoparticle has a vital role in radiation protection, despite their role in cancer cell sensitization to RT [184, 185]. Next generation of ROS

scavengers are inorganic nanomaterials with intrinsic catalytic activity such as Pt, NiO and Prussian blue (PB) nanoparticles [186–188].

(2) **ROS-based cancer therapy-nanomaterials**: Cancer cell retains its high reproducibility by activating potent endogenous antioxidant defense system equal to induced oxidative damage. In new steady state of cancer cell redox hemostasis with upregulated antioxidant and ROS level, total ROS concentration is remained below toxic level. This mechanism of adaption to new redox status makes the cell resistant to excessive ROS-induced toxicity and the cell can escape from high oxidative stress damages [47]. Therefore, if oxidative stress is further intensified, endogenous antioxidant system cannot maintain cellular redox hemostasis and cancer cell becomes much more vulnerable. Thus, to disrupt cancer cell redox adaption, ROS-generating nanomedicine can overwhelm cell antioxidant system by excessive ROS production, beyond cell tolerance. This highly qualified and feasible approach for cancer therapy is based on cancer redox biology and employed ROS-based nanomaterials. Another complementary and feasible approach is exploiting elevated intracellular ROS level to activate ROS-sensitive nanomaterials for releasing drug, on site of neoplasm. On-demand drug release can be considered beside aforementioned approaches [2]. Oxidative stress and aerobic glycolysis are two main characteristics of cancer cell that can be utilized for designing therapeutic strategies [47].

(3) **Radiotherapy efficacy enhancement**

3.1. **Radiotherapy physiochemical basis**: RT, as a mainstream therapeutic modality for cancer, utilizes ionizing radiation to suppress tumor progression by causing water ionization and subsequently elevating ROS level [189–191]. The source of radiation can be external (such as high-energy X-ray which radiates on pathological site), or internal (such as sources from radioisotopes like ^{131}I, that are already located in pathological site by systematic administration and on-site tumor accumulation) [192]. These two techniques are named External Beam Radiotherapy (EBRT) and Internal Radioisotope Therapy (IRT), respectively. Nevertheless, EBRT is the most common RT (which is discussed here assuming X-ray is used), and IRT is the competent therapeutic way for metastatic tumors [193]. Considering high energy X-ray is the most commonly used radiation for RT, specific physical processes during nanomaterial and X-ray interaction lead to Auger electron, Compton electron and photoelectron generation which react with surrounding H_2O and produce ROS to induce cell death. Although X-ray therapeutic efficacy is compromised because of low mass energy absorption coefficient (LEAC) of tumors, increased dose leads to serious side effects [191, 194]. ROS-based nanomaterials enhance therapeutic result of X-ray-based RT which will be discussed further along with existing approaches.

3.2. **Tumor Radiosensitization**: Lack of oxygen in tumor cells necessitates means to fix the damage. Radiosensitizers can fix radiotherapy-induced damage and by lowering required radiotherapy dosage, thereby minimizing potential side effects. Radiosensitizers enhance intratumoral ROS generation under X-ray irradiation and improve RT efficacy. Organic molecules like porphyrins are known as radiosensetizers, but their main drawback is rapid degradation and wide distribution which ends up with limited therapeutic outcomes [195]. That is where nanotechnology can propose strategies to modify material properties. The mechanisms involved in boosting tumor radiotherapy by radiosensitizers include:

1. Reducing intracellular radioprotectors-enhancing biomolecular damage fixation
2. Cytotoxic material production subsequent to radiosensitizer radiolysis to free radicals (preferentially in hypoxic cells)
3. Inhibition of biomolecular damage repair-suppressing cellular radioprotectors and oxidoreductases
4. Incorporation of thymine analogs into DNA strand
5. Mimics of oxygen
6. Chemicals modulating vital pathways; Apoptosis, DNA repair, metastasis and protein degradation [196].

Radiosensitizers might involve in production of ROS and affect subsequent signaling pathways. Considerably, specific chemical radiosensitizers are capable of regulating important cellular pathways which can boost RT impact on tumor cells. Nano radio sensitizers in some cases are able to elevate ROS production through various mechanisms. Nanoscale radiosensitizers are divided into two categories based on chemical and action nature:

1. Containing high Z (atomic number) elements: these materials attenuate X-ray strongly and focus the energy inside the tumor cell.
2. NO-generators: when requested, they release NO (radiosensitizer) in response to X-ray exposure [196].

Energy conversion of X-ray after interaction with nanomaterial is attributed to the Z of atoms which nanomaterial is composed of. With increased Z, capacity of photoelectric absorbtion and electron ejection (Auger and photoelectron) is intensified and ROS generation is enhanced which is followed by improved RT outcome. Efficient inorganic nanoradiosensitizers including Au, Bi_2Se_3, TaO_x, W-based materials, Hf^{4+} containing MOF and UCNPs, can serve radiotherapy by focusing ionizing radiation energy [197–202]. For instance, ultrathin Bi_2Se_3 nanosheets (2 nm by 30 nm) decorated with RGD peptide and chitosan, were evaluated as a strong theranostic agent and it exhibited high stability and enhanced HELA cells sensitivity to RT. RGD was used as targeting agent and the nanosheet could inhibit TrXR activity and activate ROS-ralated signaling pathway. Further, release of Si could reduce liver, lung and

prostate cancer occurrence. Considering Bi_2O_3-coated mesoporous silica nanoparticle (BMSN) conjugation with radiotherapy, tumor growth was suppressed tremendously in comparison to radiotherapy alone. Notably, this pH-responsive nanoparticle could encapsulate high load of doxorubicin and develop a significant therapeutic consequence against multi drug resistant cancer cells versus using doxorubicin alone. Thus, BMSN is considered a multifunctional nanosystem with high efficiency for tumor eradication in simultaneous chemo- and radio-therapy [198]. Controlled drug release and RT can synergistically develop this enhanced therapeutic outcome against multi drug resistant cancer cells, showing that RT plays a complementary role for conventional chemotherapy in removing therapy limitations. To overcome radioresistance which is responsible for treatment failure, Gd-containing polyoxometalates-conjugated chitosan nanosphere (GdW10@CS) with HIF-1α siRNA (to prevent DNA damage reconstruction) was fabricated for tumor hypoxia attenuation and response induction. The significant radiosensitization of hypoxic tumor due to high atomic number of Gd and W elements, W6+-mediated GSH oxidization and as a result sufficient level of ROS production was observed [200]. X-ray energy transfers to nanomaterial containing high Z atoms and then to semiconductor. Ejected electron from nanomaterial can interact with adjacent semiconductors (at the interface) as well as surrounding tissue. This interaction facile hole and electron motility and consequently electron generation to amplify ROS production efficiency. Enhancing RT outcome is possible by coupling nanomaterials (containing high Z atoms) and nano semiconductors. Au@TiO_2 anisotropic nanostructure was first proposed for radiosensetization and the synergistic therapeutic outcome in contrast to other Au nanoparticles suggested the coupling at the interface, the main reason. Tumor model depicted suppression in growth and survival improvement. High energy electrons emitted from Au, generate ROS inside the cancer cell and low-energy electrons are responsible for electric coupling and further ROS generation [197]. The most important gasotransmitter with several pathophysiological functions, NO, is another important tumor radiosensetizer in high levels which efficiently generate ROS and react to produce peroxynitrite (ONOO–) that is a biocide and highly reactive molecule [203]. X-ray-responsive NO-releasing nanosystems can kill cancer cells directly in synergistic therapy when demanded. On-demand release of NO of these nanosystems provide less side effects, and enhanced RT outcome. In 2015, X-ray responsive nano theranostic system (PEG-USMSs-SNO) was architected to release NO in a dose-controlled manner in response to X-ray irrediation after breaking the bond S–N (even in RT of deep solid tumors) [204]. Previously in 2008, nitrite was proven to be converted to bioactive NO under specific condition in tumor cell [205]. Then in 2017, remotely triggering NO release from nitro imidazole (nitro imidazole-Au-CPP-PEG NP) upon responding to exogenous stimuli (X-ray) proved to enhance hypoxic cancer cell sensitivity, in vitro [206]. These nanosystems are highly desirable for enhancing RT efficacy.

3.3. **Tumor Microenvironment (TME) Modulation**

Radioresistance is a great clinical concern for radiotherapy of solid tumors with hypoxic nature. Under hypoxic condition, thiol compounds neutralize produced DNA

radical and prevent its cytotoxic effect and cell apoptosis. Furthermore, VEGF expression induced by HIF-1 activation (Hypoxia hallmark) compensates for its pleiotropic effect on tumor cell and cell apoptosis which is not desired in RT. Thus, TME should be re-oxygenated to conquer hypoxic-induced radioresistance [128]. Three categories of nanosystems are synthetized to attenuate hypoxic condition of tumors:

i) O_2 generating nanomaterials: These nanosystems convert endogenous H_2O_2 of tumor cell to O_2 and reduce hypoxia. Nanoshells of CAT-loaded TaO_x enhances RT therapeutic effect as this bioreactor can focus radiation energy inside the cell by high atomic number of Ta, CAT can generate O_2 and attenuate hypoxia, and mesoporous architecture guarantees high level of enzyme activity [128]. MnO_2 nanoparticles like albumin coated MnO_2 nanoparticles react with H_2O_2 and H^+ which consequently produce O_2 (by 45%) and increase cell pH. This nanosystem has considered hypoxia, ROS production and low pH interplay effect on outcome of treatment with considering Mn reactivity for peroxide and downregulation of two major factors in cancer progression, HIF-1alpha and VEGF expression. RT and nanoparticle combination therapy inhibited the growth of breast tumor, increased dsDNA breakage and cancer cell death compared to radiotherapy alone [207]. Notably, complete HIF-1 degradation which is dependent on O_2, is possible by re-oxygenation and remaining HIF-1 function inhibition (by acriflavine or other hydrophilic cationic drugs) simultaneously. HIF inhibitor@MnO_2 ROS-responsive nano materials release Mn^{2+} from MnO_2 for MRI and activate tumor immune response (T-cell) which leads to efficient inhibit the tumor growth. The risk of metastasis is lowered when VEGF and MMP-9 expressions are decreased [208]. Further, Au@MnO_2 core–shell nanoparticle with PEG coating was developed. Au, in the core, interacted with X-ray to produce charged particles (radiosensitizer) and MnO_2, in the shell, reacted with H_2O_2 to reduce hypoxia-associated radioresistance. The nanoparticle could effectively radiosensitize hypoxic cancer cell to RT in vitro and in vitro with no obvious side effect [209].

ii) O_2 Delivering Nanomaterial: High capacity of these nanosystems for oxygen loading modulates TME. Nanosystems have used oxygen transporters such as perfluorocarbon (PFC) (commonly used as artificial blood), hemoglobin and oxygen microbubbles. Erythrocyte membrane-PFC@PLGA (PFC is located in the core) nanoparticle plays the role as artificial RBC and transfers oxygen to tumor site while the RBC coating membrane increases blood circulation time. Nanoscale size of these nanoparticles enables them to penetrate into tumor tissue properly, in contrast to natural micro-scaled RBCs, and greatly compensates for tumor hypoxia after intravenous injection to refine RT efficacy. In addition to RT, this nanosystem can potentially improve therapeutic outcome of other modalities such as PDT and SDT while the radioresistance is due to tumor hypoxia [210]. Oxygen delivery with hemoglobin or oxygen micro-bubble nanocomposites are comparable with PFC-containing nanomaterials in modulating TME and improving RT. Hemoglobin containing liposome (10 ml/kg) administered in mice 30 min before radiation lowered HIF-1α

accumulation in treated tumor and enhanced RT efficacy [211]. Independent of hemoglobin transport, oxygen was delivered to not-vascularized regions of solid tumor by ultrasound-sensitive oxygen loaded microbubbles, tripling radiosensitization when administered immediately before RT exposure [212].

iii) Photothermal therapy (PTT) + Radiotherapy (RT): As blood flow increases, oxygen delivery rises. Photothermal agents, in response to light, produce mild heat which leads to higher blood flow and higher intratumoral O_2 level to vanquish radioresistance and sensitize the tumor. For instance, 2D MoS_2-Bi_2S_3 nanosheet was synthesized for RT/PTT synergistic therapy and tumor eradication. MnO_2 nanosheet, a famous transition metal dichalcogenide (TMD), performs its role in PTT and Bi_2O_3 role is radiosensitizing due to extensive ability to attenuate X-ray. This theranostic nanosystem is also suitable for tumor imaging (photo-acoustic and computed tomography) and diagnosis [213]. Above discussion declared the role of nanosystems in ameliorating radioresistance caused by hypoxia.

3.4. **Normal tissue radioprotection**

Unfavorable RT side effects including activity alternation of apoptotic proteins, mitosis inhibition, mitochondria dysfunction and cell hemostasis dysregulation, are the results of unavoidable ROS production in adjacent healthy cells [184]. Although cellular antioxidant system defends against oxidative stress, the counterbalance is not successful to encounter all radiation-induced cell damages. Consequently, side effects such as vomiting, nausea and hair loss can occur which emphasize the urgency of considering applicable strategies for radioprotection. Diverse science of nanotechnology proposes three categories of efficient radioprotective nanoplatforms:

1. **Inorganic nano enzymes**

   CeO_2 nanoparticles with enzyme mimicking (SOD, CAT and oxidase) activity scavenge excessive ROS sequentially (superoxide to H_2O_2 to H_2O) by reverse binding to oxygen atoms in the molecules and alternating between reduced (3+) and oxidized (4+) form of Ce [181, 214, 215]. Its unique anti-oxidative feature has been investigated intensely in radioprotection. In 2005, radioprotective effect of vacancy-engineered CeO_2 nanoparticles on normal human breast cell was examined (99% protection) while no protection was seen on MCF-7 cell line, probably due to transmembrane pH difference [216]. This feature is beneficial because it does not compromise RT outcome as it depends on high ROS level while radioprotectors decline ROS concentration. Radioprotection of CeO_2 nanoparticles over normal gastrointestinal epithelium and lung fibroblast cells was further demonstrated [184, 217].

2. **Inorganic electro-catalysts**

   Inorganic nanomaterials with ROS scavenging properties reduce oxygen efficiently, and that are used for excessive ROS depletion in RT. Cys-MoS_2 quantum dots (below 5 nm in size) reported to exhibit radioprotection by increasing

survival percentage, cell viability and DNA recovery with striking properties including low toxicity, effective clearance from kidney and their role as radical scavenger which could be credited to excellent H_2O_2 reduction (electrocatalytic properties) and enabling electron transfer. High renal excretion (80%) after 24 h of administration and biocompatibility of these dots, make them appropriate for radioprotection applications [218]. WS_2 quantum dots presented DNA and hematopoietic system protection by neutralizing overproduced ROS and exhibited high renal clearance (80% in 24 h) due to their ultra-small size, resembling MoS_2 dots. Blue photo luminescent properties and scavenging ROS from whole biological system are other considerable advantages of these dots .With decreased size, extraordinary catalytic properties of these quantum dots make them favorable for medical purposes [219]. PVP-protected PtPdRh hollow nanocubes adsorb ROS in low energy active sites of pores and reduce oxygen in vivo. This modified electronic structure with catalytic activity and hollow structure bestow large surface area and more reachable active sites on H_2O_2 compared to solid counterparts. Galvanic replacement of Pt lattice with Rh and Pd leaded to synthesis of Pt-based alloys with better catalytic characteristics. This tertiary alloy breaks superoxide bond on the surface and produce oxygen through radioprotection. Bone marrow cells containing nucleus showed increased SOD activity, lowered DNA damage and malondialdehyde (MDA) level which indicates Pt-based alloys mimic SOD, CAT and peroxidase activity [220]. More catalysts with Oxygen Reduction Reaction (ORR) are expected to be in the pipeline for protection against RT-induced oxidative damage.

3. **Organic nanomaterials**

As biodegradable ROS scavengers' representative, melanin nanoparticle has a pleiotropic effect in neutralizing excessive ROS. Combination of physical (shielding) and chemical (scavenging) properties of melanin are responsible for its effects as first realized that melanized fungal response to high dose of radiation was enhanced growth [221]. A generated Compton recoil electron slowly loses energy while passing through melanin, until it reaches a low enough energy that it can be trapped by stable free radicals of the pigment. Controlled dissipation of high-energy recoil electrons by melanin inhibits secondary ionizations and the generation of damaging free radical species [222]. Treatment of Balb/C mice with melanin nanoparticles pre- or post- irradiation provided protection to hematopoietic tissues and enhanced the survival (40% in post radiation treatment) [223]. Considering double sword edge effect of ROS, radioprotector overdose may result in attenuation of RT outcome by encountering excessive ROS cell toxicity in tumor cells. Therefore, investigations on involved mechanisms are still necessary. Wise inspection of discussed strategies—**radio protection/sensitization and TME modulation**—is of importance to develop conventional RT further and overcome limitations of this therapeutic modality in clinic.

5 RT Synergic Therapy

Tumor cell diversity and complexity make it laborious to contend with cancer, despite development of redox-based modalities (RT, PDT, CDT, CDR, and SDT) which may still not be capable of reaching considered therapeutic goal when applied alone due to probable existence of specific resistant cancer cell subpopulations. The shift from monotherapy to multiple modality synergic therapy is the current approach for improving treatment outcome [224]. Coalescence of strategies based on nanomaterials and their responsiveness to stimulus should be designed to improve the synergistic therapeutic effect of monotherapies such as RT, PDT and immunotherapy. Ineffective treatment of solid hypoxic tumors and distant metastasis from tumor location hinder broader RT application. The solution to this obstacles is application of nanotechnology in integrating RT with other therapeutic modalities for alleviating side effects while enhancing efficacy. Three strategies in synergic therapy with RT are considered:

i) RT+ Chemotherapy: To surmount radioresistant malignancies and improve cancer therapeutic efficacy, chemotherapy was implemented besides RT. By inhibiting DNA damage repair and changing cell phenotype from radioresistant to radiosensitive, chemotherapy collaboration with RT has been estimated to improve cancer therapy significantly. Cisplatin-loaded UCNP@SiO_2 nanoparticles (up-converting core and porous silica shell) were employed for RT + chemo/synergic therapy in which cisplatin plays two roles, a radio sensitizer and a chemotherapeutic drug. In vivo experiment on Balb/C nude mice bearing HeLa tumor demonstrated a higher therapeutic effect when this rattle-structured nanotheranostic system was utilized besides RT rather than employing one therapeutic strategy. UCNP favors dual (magnetic—luminescent) mode imaging in addition to delivering cisplatin which is more effective than cisplatin alone as radiosensitizer [225]. Hollow TaO_x (tantalum oxide)-based theranostic platform was designed to deliver chemotherapeutic drug and enhance RT effect simoultanously. 7-ethyl-10-hydroxy-camptothecin (SN38) was efficiently loaded in mesoporous shell and large cavities of its hollow structure, and Ta with high atomic number was used to attenuate X-ray radiation. PEG-modified TaO_x nanoshells by intrinsic binding tendency with metal ions upon mixing qualify for single photon emission computed tomography (PET) imaging and magnetic resonance imaging (MRI) which enable locating the tumor and tracking its distribution in biosystems. With SN38 inducing cell cycle arrest into a radiosensitive phase, and Ta depositing X-ray energy inside the tumor cell, this nanocomposite (TaOx-PEG@SN-38) unveiled synergistic therapeutic effect, in vivo and in vitro [199]. Thus, chemo/RT corporation can improve cancer therapeutic efficacy as shown by mitomycin C-SiO_2@UCNP nanotheranostic system design

which could efficiently treat multi drug resistance cancer cell efficiently both in vivo and in vitro [226].

Recently, nitrosylated tubulin targeted DM-1 loaded in PLGA-b-PEG nanoparticles showed enhanced drug delivery to tumor via increased permeability and retention effect. By increased oxidative stress as a result of tumor irradiation, S-N bond cleavage occurs which leads to the release of NO and DM-1. DM-1 inhibits cell polymerization of microtubules and arrests the cell cycle at G2/M which is relatively more radiosensitive, and simultaneously NO forms toxic radicals which both contribute in tumor suppression. Synergistically enhanced RT outcome in head and neck cancer was confirmed by in vivo and in vitro experiments (H1299 tumor-bearing mouse and clonogenic assay). The promising results of DM1-NO PLGA nanoparticles demonstrated enhanced RT outcome for NSCLC (non-small cell lung cancer) and potentially other types of cancer. It is noteworthy that DM-1 toxicity was suppressed by nitrosylation and encapsulation [227].

ii) RT + PTT: To diminish hypoxia-induced radioresistance by modulation of tumor environment, mild hyperthermia can be induced during PTT which boosts blood flow of tumor tissue and consequently mitigates hypoxia. For instance, PVP-Bi_2Se_3@selenocysteine nanotheranostic system was proposed for enhancing RT therapeutic outcome and shielding healthy tissue from radiation by enhanced generation of ROS and high capability of absorbing NIR laser beam. Bi_2Se_3 strongly absorbed and performed photothermal conversion and subsequently modulated hypoxia and provided a complementary effect during RT. No considerable in vivo or in vitro toxicity was observed, demonstrating its high biocompatibility. In vivo release of selenium to the blood flow protects whole body from irradiation and enhance immune responses [228]. Another proposed nanotheranostic system with the same mechanism of action used CuS-modified mesoporous organosilica nanoparticles to specifically deliver O_2-saturated perfluoropentane (PFC) to tumor site which is gasified by hyperthermia, which was induced by NIR laser. Also, this nanotheranostic system was ^{64}Cu-labeled which enabled multimodality imaging of tumor while oxygenating the area to increase radiosensitivity [229]. In the recent decade, enormous investigations have been performed on nanosystems with capability of simultaneous X-ray attenuation and photothermal conversion. First, a core/satellite theranostic platform based on silica@UCNP decorated with CuS ultra-small nanoparticles was synthetized to absorb NIR energy and transform it to local heat (by photothermal feature of CuS) and to enhance the radiation dose around nanoparticles to radiosensitize the tumor cell (by high Z elements such as Gd, Er and Yb in UCNP). The synergistic effect of combining PTT and RT was confirmed when administered intratumorally, and a distinct therapeutic effect was observed when administered intravenously. Eradication of tumor was observed within 120 days by integrating PTT and enhanced RT in this

powerful and biodegradable platform [202]. MnSe@Bi_2O_3 core/shell was fabricated to overcome RT resistance by combining RT/PTT and implementing a synergistic effect. Bi_2O_3 is capable of absorbing X-ray strongly and concentrate its energy locally while NIR-triggered PTT favors hypoxia attenuation [230]. Another effective theranostic platform, BiOI@Bi_2S_3@BSA had the capability of concurrent application of RT/PDT/PTT synergistically. Bismuth Oxyiodide (BiOI) plays two roles simultaneously, as a radiosensitizer (containing high Z elements such as Bi and I) and as a photosensitizer (upon X-ray irradiation, this photocatalytic semiconductor forms electron-hole pair and generates ROS which causes X-ray excited PDT). Bi_2S_3 coating presents increased ROS generation (by forming a heteroconjunction structure at the interface of dissimilar BiOI semiconductor to decrease recombination of electron-hole and further enhance the electron-hole generation) along with NIR photothermal conversion for photothermal tumor ablation where the phenomenon also aids improved oxygen level of TME by increasing local blood flow and enhance RT outcome. Synergistic RT/PDT/PTT therapy demonstrated more significant effect than RT or PTT or PDT alone based on in vivo tumor ablation experiments. Such corporation among these modalities can be a promising approach for cancer therapy in the future [194].

iii) RT+ Immunotherapy: Clinical studies have revealed the role of RT in provoking crucial responses locally (in site of treatment) and remotely which is called abscopal effect. Immune system's major role in abscopal effect is elucidated which provides a way for integrating RT and immunotherapy for synergistic therapeutic strategies [231]. Inflammatory response-induced dendritic cell (DC) maturation and upregulation of chemokine receptors occur after RT exposure. Upon tumor antigen processing by DCs, they migrate to lymphatic nodes to express tumor antigen peptide and present it to $CD8^+$ cytotoxic T lymphocytes (CTL) which further reside in tumor and kill (phagocytosis) tumor cells, mediated by the proteins expressed on the surface of plasma membrane [232].

Immunomodulatory effect of RT by altering microenvironment of irradiation site, when administered alone, rarely leads to rejection of tumor systematically, while the RT-augmented immunotherapy enhances the therapeutic effect. At a glance, tumor may prevent immunization, cause wrong immune response or enable accumulation/expansion of T regulator (preventing cytotoxic T lymphocyte function) in tumor location. Effector T cell can undergo anergy if PD-1 receptors connect with specific surface molecules of tumor cells such as PD-L1 and PD-L2. MHC I molecules or target tumor antigen expression may be downregulated to prevent effector T cell response as well. Molecules with immunosuppressive function which are released from tumors such as indole amine 2,3-dioxygenase (IDO) enzyme (by consuming tryptophan) are capable of limiting T effector. Similarly, adenosine which is produced as a result of TME, hypoxia inhibits T cell function. Hypoxia emphasizes the presence of regulator T cells by producing CCL28. As a conclusion, several factors inhibit effector T cell function to avoid systemic response against tumor tissue.

This is where immunotherapy is employed for provoking immune responses and preventing tumor escape from immune attack as a result of immunosuppression [233–236]. High IDO expression, which is responsible for immune tolerance and poor prognosis of various types of cancer cells, can be inhibited by small molecules that has displayed a moderate effect in anticancer monotherapy [237]. In 2018, aiming to combine RT and immunotherapy for systematic tumor rejection after local radiation exposure, two engineered nano-metal organic framework (MOFs) were designed. After irradiation of X-ray at low doses, the crystalline structure of 5,15-di(p-benzoato)-porphyrin-Hf (DBP-Hf) and 5,10,15,20-tetra(p-benzoato)-porphyrin-Hf (TBP-Hf) acted as strong photosensitizers which produced ROS, especially $^1O_2$, killing malignant cells efficiently and enhancing the presentation of tumor specific antigens to T cells by stimulating immune system [238]. PDT might produce acute inflammation and subsequently attract leukocytes to the treated location, increase immunogenicity of dead tumor cells by exposing or creating new tumor antigens and inducing heat shock protein (HSP) expression which consequently increase antigen presentation to cytotoxic T cells. DC migration and maturation may be promoted by pro-inflammatory effect of PDT. According to in vivo tumor models, PDT has produced long lasting immunity memory [149]. Hf clusters with strong X-ray absorption favors RT with production of hydroxyl radicals which excite porphyrine- based photosensetizers for treatment of remote tumors. In vivo and in vitro eradication of various types of tumors were observed by employing nano-MOFs. Loading of small molecules (IDO inhibitors) in porous structure of DBP-Hf demonstrated enhanced therapeutic efficacy (including checkpoint blockade) and 100 % abscopal effect, rejecting both irradiated and non-radiated, treated and non-treated tumors in breast and colorectal mouse cancer models. In this nanoplatform, RT, PDT and immunotherapy were integrated for systematic and local tumor treatment [201]. Considering intrinsic immunomodulatory effect of RT and PDT, complementary role of these three modalities is beneficial for treating metastatic tumors. Again in 2018, immunomodulatory effect of IRT was combined with immunotherapeutic systemic checkpoint blockade by anti CTLA-4 antibody. Catalase was labeled with ^{131}I (which is employed as a source for internal excitation) to decompose overproduced H_2O_2 to O_2, relieve hypoxia and subsequently improve IRT efficacy with low doses of radioisotope. Intratumoral injection of ^{131}I-CAT/sodium alginate enabled tumor eradication and caused hypoxia elimination for a long period of time. Upon intratumoral injection, endogenous Ca^{2+} binds with alginate polysaccharide and forms hydrogel which causes ^{131}I-CAT fixation within the tumor and inhibits excessive radioisotope leakage to healthy tissues. CpG oligonucleotide role is stimulating intratumoral tumor specific antigen generation which leads to strong immune response. Checkpoint blockade and immunostimulation circumvent metastasis and tumor recurrence in advanced-stage patients [239]. RT and immunotherapy combination is a promising synergistic approach for promoting long term immunity

against tumor recurrence and treating hypoxic solid tumors along with remote metastatic cells when RT is inefficient as monotherapy.

Other approaches include:

I. Recently, platelets loaded with Au-hemoglobin complex (Au-Hb@ Plot) were fabricated to alleviate tumor hypoxia and penetrate deeply into tumor (due to small size). This nanosystem can be activated by cancer cells, where hemoglobin carries oxygen and Au potentiates tumor sensitivity to X-ray radiation. The enhanced in vivo RT therapeutic outcome was observed in tumor bearing mouse under low dose of radiation which was confirmed by in vitro experiments [240].

II. Full-process radiosensitizing hafnium-based nanoscale metal–organic frameworks (Hf-nMOFs) with uniform dispersion of Fe^{3+} ions were constructed to improve in vivo radiotherapy outcome by fenton reaction (due to Fe^{3+} presence) and X-ray energy conversion (due to Hf^{4+} presence) [241].

III. Other radiations can benefit radiotherapy efficacy by activating radioisotopes in the site of tumor. For instance, neutron irradiation can activate ^{152}Sm-filledcarbon nanocapsules inside the biological system after intravenous administration and turn it into ^{153}Sm radioactive form. This approach reduces nuclear waste, eliminates the need for nuclear facilities for nanoparticle preparation, increases stability of loaded in vivo radioactive content and can be used for imaging and RT simultaneously [242].

6 Challenges and Conclusion

Below a certain threshold, ROS assist cell survival but when elevated above cell antioxidant capacity, cell dies due to intolerance. Such balance is maintained in normal and tumor cells, but the level of toleration varies for different types of cells. Normal cells change into cancer cells when oncogenes are activated and cell redox hemostasis is in unbalanced state. ROS affect radiotherapy efficacy in both direct and indirect ways. Direct effect majorly results in lethal DNA damage, and indirect effects include cell death regulation, DNA damage repair, cancer stem cell (CSC) characteristics and tumor microenvironment (TME) modulation. Radiotherapy can be utilized based on tumor stage and type for curative or palliative purposes, and the underlying mechanism is increasing reactive species (ROS) inside the cell to damage macromolecules and shift the signaling pathways. Hypoxia is well-known for its association with radioresistance in which oxygen plays an important role in promoting induced cell damage. Low oxygen level and high antioxidant capacity are two major obstacles responsible for radiotherapy resistance which is a reason for poor prognosis of cancer. Radiosensitizers should be employed for enhancing cell sensitivity, and radioprotectors should be used for their ability to protect healthy tissue from radiation-induced damages. By using these tools, ROS concentration and

signaling pathways can be altered in favor of producing more ROS in tumor cells and lowering the ROS level in healthy cells. Despite engineering smart nanoplatforms to enhance/deplete ROS generation, modulating tumor microenvironment and synergistic therapy, ROS-based cancer therapeutics have been restricted to academic research, while there is an urge for more efficient cancer therapeutics, noting the severe cancer status across the world. So it is essential to:

- Acquire more advanced comprehension of intrinsic features of modalities which are based on ROS
- Determine the mechanism and extent of synergistic therapeutic effect in contrast to employing monotherapy [243].

Unique characteristics of ROS in biological mechanisms have been utilized for medical purposes. ROS regulating nanomaterials can be manipulated to direct temporospatial dynamic behaviors of these chemical species to develop advanced in vivo therapeutic approaches. Nanocatalytic medicine refers to catalytic nature of nanosystems in ROS-involving therapeutic approaches where nanomaterials attenuate energy barriers of ROS-related reactions for initiating therapeutic effects via ROS regulation. Exogenous stimuli can induce or accelerate these catalytic reactions in which therapeutic processes generally can be categorized as nanocatalytic medicine. Despite advancements in ROS science, the is in initial stages, and with development of advanced approaches, ROS-related biomedical research area will emerge. For development of ROS-based therapeutic materials, there are still scientific/technological issues remaining to be investigated:

1. **Biosafety:** In vivo application of ROS-generating nanomaterials with cytotoxic chemical feature may be associated with surrounding normal tissue impairment and degradability issue which causes continuous ROS generation and consequently oxidative damage [244, 245]. Precise characterization of ROS behavior and safety assessment of nanomaterials by developing cutting edge tools are steps needed to be taken in order to balance the benefit of ROS generating nanomaterials and their side effect [246].
2. **Chemical mechanism:** Computational chemistry has provided tools for prediction/exploration of chemical reactions including mechanisms of ROS-related reactions such as ROS generation and interaction with biomolecules which affect progression of disease pathology. Most investigations lack mechanism exploration of nanomaterial therapeutic processes [247]. Computational chemistry can come in handy in ROS investigations to unveil chemical role of ROS in therapeutic processes [220, 248]. Consequently, redox active nanomaterials can be discovered and fabricated noting the emergence of cheminformatics which can contribute greatly in ROS science and exploration of related molecular mechanisms.
3. **Therapeutic concept:** Precise fabrication of nanomaterials with unique compositions and structures for fulfilling the individual demands, is the opening of a new era [249–251]. Immunotherapy is considered a personalized therapeutic

modality for cancer. Thus, integrating immunotherapy with ROS-based modalities is of importance. For instance, combination of RT/PDT with immunotherapy leads to super additive therapeutic effect, given that PDT and RT initiate immunoregulatory responses. Immunoregulatory effect of cutting-edge therapeutic modalities such as SDT and CDT have to be investigated to answer the query whether these modalities are competent for synergic therapy and enhancing cancer therapeutic outcome.

4. **Catalytic efficiency:** Material's capability to scavenge/produce ROS determines its final therapeutic performance. The common focus is still synthesis and design to achieve significant redox-regulation in nanomaterials. Inorganic nanozyms has managed intracellular ROS concentration but their in vivo catalytic efficiency should be further improved due to strong demand for decreasing drug dosage as much as possible. Significant improvement of catalytic chemistry owes to recent advances which can be utilized for in vivo ROS regulation such as atomic catalytic modalities. In 2015, a pioneering work demonstrated catalytic oxidization of benzene by single iron atom catalysis of H_2O_2 which was confined in graphene matrix [252].

Clinical translation of these engineered nanomaterials is the last concern after addressing aforementioned design issues. Most FDA-approved nanomaterials are organic where certain types have been chosen for loading specific APIs (active pharmaceutical ingredients) for chemo/immuno/gene therapies but ROS-based modalities are not included. A clinical trial for RT enhancement of adult soft tissue carcinoma is ongoing phase I which involves one-time intratumoral implementation of hafnium oxide nanoparticles [253].

Clinical translation of ROS-based nanosystems may be hindered by several issues:

1. Increasing number of fabricated ROS-regulating nanomaterials claimed to be effective may cause difficulties in choosing optimized platform for clinical trials.
2. Generated ROS dose should be identified and managed, because ROS as double edge sword, can guide cells toward therapeutic or pathological effect.
3. Safety and efficacy of each component are hard to be assessed as design of nanomedicines has shifted to co-delivery of therapeutic agents to achieve multifunctional and efficient ROS-regulating nanomaterials.
4. Although preclinical studies provide therapeutic results in animal models, they lack mechanistic understanding of nanomedicine interaction with in vivo environment. Considering much different texture and physiological response between human and animal models, more strict evaluations of safety and efficacy are necessary before nanomedicine administration to patient's body.

Considering the efforts made so far, further acceleration to clinical translation by meeting aforementioned stringent requirements and patient specific approaches should be given attention to. In this regard, cancer nanomedicines should address problems associated with tumor heterogeneity. For instance, ROS-generating nanomedicines that respond to exogenous stimuli (RT, SDT and PDT) can be employed for localized treatment of skin malignancies whereas nanomedicines

which respond to tumor microenvironment should be utilized for treating deep tumors systematically. Optimized platform can be selected based on the unique chemistry of the nanomaterial and the stage of specific type of cancer. To determine appropriate dose and administration route, feasible diagnostic tools should be developed to monitor ROS level in pathological site in a real-time manner. Optimal clinical outcome can be achieved if these goals are achieved. To facilitate the clinical translation of nanomedicines, designs need to be simplified rather than getting more sophisticated to avoid potential biosafety issues. In this context, deep investigation of underlying mechanisms of long term biological effects induced by ROS-based nanomedicines should be undertaken (in both animal and human body) which necessitates close collaboration of university, hospital and industry with each other.

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