Interplay Between Reactive Oxygen Species and Key Players in the DNA Damage Response Signaling Network

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Abstract

Reactive oxygen species (ROS) generated as by-products of aerobic metabolism or induced by exogenous agents such as radiation, chemicals, and drugs can cause extensive damage to cellular macromolecules, primarily DNA. In particular, ROS-induced DNA double-strand breaks (DSBs) are the most lethal lesions that induce mutations with consequent genomic instability and increased susceptibility to cancer. Eukaryotic cells have evolved a complex network of kinase-driven signaling systems referred to as the DNA damage response (DDR) to counter the deleterious effects of DNA damage. Based on the mode of action, the DDR proteins are classified as sensors, transducers, and effectors,

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although their functions are often overlapping. The DDR involves a cascade of events initiated by recognition of DNA damage, followed by signal transduction to downstream effectors that exert an appropriate response such as cell cycle arrest to facilitate DNA repair, or induce apoptosis if the damage is extensive. Inactivation of DDR, a hallmark of cancer, has been exploited for therapeutic intervention. This chapter provides a bird's eye view on the interplay between ROS and the vast array of molecules that orchestrate the DDR such as ATM, H2AX, 53BP1, and transcription factors and the impact on cell fate decision and oncogenic transformation.

Keywords

Ataxia-telangiectasia-mutated (ATM) kinase · DNA double-strand breaks · H2AX · HMGB1 · MRN complex · Nuclear factor (erythroid-derived 2)-like factor 2 · NF-κB · p53-binding protein 1

Introduction

Cellular DNA is vulnerable to damage by environmental chemicals, radiation, biological agents, drugs, and endogenous metabolites that can adversely affect genomic stability. In particular, reactive oxygen species (ROS) formed as by-products of aerobic metabolism are a major source of DNA damage. ROS induce various types of DNA damage including base modifications, adducts, base-free sites, deletions, frameshifts, intra- and inter-strand crosslinks, DNA-protein crosslinks, single-strand breaks (SSBs), double-strand breaks (DSBs), and oxidized bases. Oxidative DNA damage that forms approximately 10^4 lesions/cell/day is an inevitable consequence of ROS metabolism (Klaunig 2018; Roos et al. 2016). One of the most frequent ROS-induced oxidative base lesions is 8-hydroxy-2′-deoxyguanosine (8-OHdG) that can cause GC to TA transversions resulting in replication errors (Salehi et al. 2018). DNA DSBs are among the most deleterious lesions that are capable of causing mutations leading to activation of oncogenes and inactivation of tumor suppressor genes resulting in altered expression and/or functions of proteins with consequent increased susceptibility to cancer (Krenning et al. 2019; Vítor et al. 2020).

Eukaryotic cells have, however, evolved a sophisticated network of signal transduction mechanisms, collectively referred to as the *DNA damage response (DDR)* to overcome the consequences of DNA damage to genomic integrity. The DDR encompasses a complex network of molecules and signaling pathways that can be classified as *"sensors"* that recognize DNA damage, *"transducers"* that transmit the signal to "*effectors,"* which mediate cellular responses such as cell cycle arrest, apoptosis, or repair. Following detection of the DNA lesion, the cell cycle is temporarily arrested to allow time for repair, or promote senescence, or induce cell death by apoptosis if the damage is irreparable. Cell fate decision depends on the mode and severity of DNA damage. Figure 1 presents an overview of the DDR. Inactivation of DDR that causes accumulation of mutations has been recognized as a

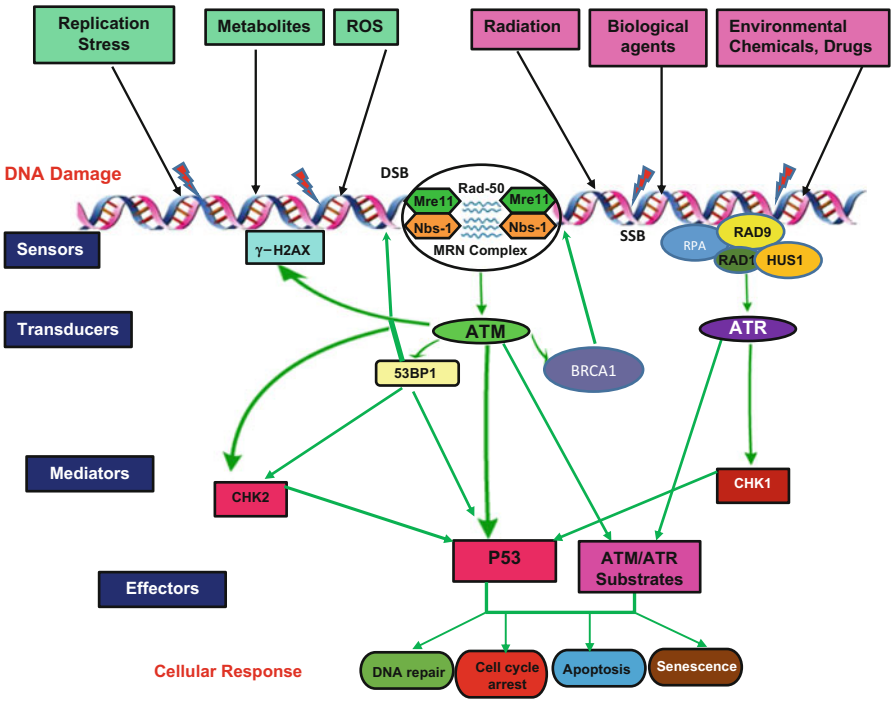

Fig. 1 Schematic representation of the DDR. The DDR involves a cascade of events initiated by recognition of DNA damage, followed by signal transduction to downstream effectors that exert an appropriate cellular response such as cell cycle arrest to facilitate DNA repair, or promote senescence, or induce apoptosis if the damage is irreparable. Based on the mode of action, the DDR proteins are classified as sensors, transducers, mediators, and effectors, although their functions are often overlapping. The first step in DDR is the detection of DNA damage induced by a wide variety of exogenous and endogenous agents. The MRN complex composed of MRE11, RAD50, and NBS1 is primarily involved in recognizing DNA DSBs and activates the ATM pathway, whereas RPA and RAD9/RAD1/HUS1 (9-1-1) function as SSB sensors to activate the ATR pathway. ATM and ATR, central players of the DDR network, phosphorylate various molecules to initiate downstream signaling events that determine cell fate

hallmark of cancer (Davalli et al. 2018; Mirza-Aghazadeh-Attari et al. 2019; Vítor et al. 2020).

Of late, ROS have gained increasing attention as central players in the pathophysiology of DDR as well as in oncogenic transformation. ROS belong to a family of short-lived free radicals that contain an atom or molecule with one or more unpaired electrons. These include oxygen radicals, such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), peroxyl radical ($RO_2^{\bullet}$), and alkoxyl radical ($RO^{\bullet}$), as well as nonradicals such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and ozone (O_3). ROS perform an array of physiological functions that range from phagocytosis, electron transport chain, peroxisomal β-oxidation, protein folding, and cellular signaling (Zhang et al. 2016). ROS signaling has been implicated in cellular response to radiotherapy and

chemotherapy by influencing cell survival or cell death pathways. Most importantly, ROS play a pivotal role in modulating DDR (Moloney and Cotter 2018; Srinivas et al. 2019).

This chapter focuses on the effect of ROS on key molecules and pathways involved in the DDR. Components of the DDR signaling system such as the MRN complex that detects DNA DSBs, ataxia-telangiectasia-mutated (ATM) kinase, the master regulator of DDR, the histone variant H2AX, a sensitive indicator of DNA damage, the p53-binding protein1 (53BP1), and transcription factors that orchestrate the DDR are discussed in the context of their interactions with ROS. The impact of the complex interplay between ROS and DDR on the opposing processes of cell proliferation and cell death by apoptosis as well as on cancer therapeutics is described.

Components of the DDR Signaling System

The MRN Complex

The first step in DDR is the detection of DNA damage by "sensors" that initiate a cascade of events culminating in an effector response based on the severity of the lesions. ROS-induced oxidative stress has been documented to influence the expression and activation of DDR sensors. Conversely, DDR sensor proteins also exert significant changes in ROS levels. The MRN complex composed of MRE11, RAD50, and NBS1 is primarily involved in recognizing DSBs and activates the ATM pathway, whereas replication protein A (RPA) and RAD9/RAD1/HUS1 (9-1-1) function as SSB sensors to activate the ATR pathway (Lavin et al. 2015; Paull 2015).

The MRN complex plays a pivotal role in processing DSBs prior to repair. Both MRE11 and NBS1 (also known as NBN or nibrin) are involved in the initial detection of DSBs, while RAD50 tethers the two linear DNA molecules together. The MRN complex rapidly locates to the sites of DNA DSBs where it functions to recruit and activate ATM, which in turn phosphorylates a myriad of substrates including the components of the MRN complex (Lavin et al. 2015).

Phosphorylation of Mre11 by ATM at two adjacent sites (S676 and S678) initiates signaling to regulate cell cycle progression as well as end resection during homologous recombination (HR) repair. It also serves as a signal for Mre11 to dissociate from DNA (Lavin et al. 2015). ATM phosphorylates Nbs1 on two sites (S278 and S343), and Rad50 at a single site (S635) in response to DNA DSB. While phosphorylated NbS1 is required for signaling to the G2/M checkpoint, phosphorylated Rad50 is essential for signaling to the S phase checkpoint, mediating DNA repair and protecting against radiation damage as well as for cell survival (Lavin et al. 2015). ATR also phosphorylates Rad50 at the same site (S635) in response to DNA replication stress. Phosphorylated Rad50 stimulates ATR downstream signaling and DNA replication fork restart. Phosphorylated Rad50 is essential for cell cycle control through Chk1 (Gatei et al. 2011).

A rapid increase in the expression of Mre11 was observed in A549 lung cancer cells treated with H_2O_2, an oxidant and DNA damaging agent that correlated with the activation of ATM (Zhao et al. 2008). Mutations of the *NBN* gene that encodes nibrin cause Nijmegen breakage syndrome (NBS), an autosomal recessive disease that predisposes to cancer. Lack of functional nibrin protein in conditional null mice as well as in NBS patient cells was found to cause increased ROS levels and oxidative stress. Krenzlin et al. (2012) demonstrated that DSB repair deficiency causes perturbations in cellular metabolism, compromised antioxidant status leading to accumulation of ROS, and further DNA lesions, thereby enhancing the potential of developing malignancy.

ATM/ATR

ATM and ATR, members of the phosphatidylinositol 3-kinase–related kinase (PIKKs) family and central players of the DDR network, are the two most important DDR transducers that interact with various effector molecules to initiate downstream signaling events that determine cell fate. The transducer kinases Chk1 activated by ATR, and Chk2 activated by ATM phosphorylate and regulate proteins involved in DDR. Overproduction of ROS is documented to activate both ATM/Chk2 and ATR/Chk1 axes (Lavin et al. 2015; Menolfi and Zha 2020; Paull 2015; Srinivas et al. 2019;). Choi and Chung (2020) provide an overview of the elaborate crosstalk between oxidative stress response and DNA damage checkpoint signaling by orthologs of ATM and ATR in *Saccharomyces cerevisiae*.

ATM is activated in response to DNA DSBs that are recognized by the MRN complex (Paull 2015). ATM phosphorylates H2AX at S139 followed by recruitment of several DDR components such as BRCA1 and 53BP1. ATM phosphorylates and activates the transcription factor p53 both directly as well as indirectly via activation of CHK2 leading to cell cycle arrest at G1/S phase and induction of apoptotic cell death. ATR, on the other hand, activated by replication stress–induced SSB which is recognized by RPA activates and phosphorylates its downstream target CHK1. Activated CHK1 phosphorylates and inactivates CDC25A and CDC25C that catalyze dephosphorylation and activation of CDK2 and CDK1, respectively. These events arrest the cell cycle at G1/s and G2/M phase. WEE1 directly inhibits the activities of CDKs, thereby regulating S phase and M phase entry. This chapter lays more emphasis on ATM because DSBs are highly toxic lesions.

ATM is mutated in ataxia-telangiectasia (A-T), a rare, autosomal recessive neurodegenerative disorder that affects children. A-T is characterized by progressive cerebellar degeneration, immunodeficiency, genomic instability, radiation sensitivity, and lymphoid malignancies. ATM is predominantly present in the nucleus as an inactive dimer that undergoes monomerization upon activation in response to DSB. Activation of ATM is associated with autophosphorylation at S1981 within the highly conserved FRAP-ATM TRRAP domain. Interaction with the MRN complex is essential for ATM activation. Following activation, ATM phosphorylates several hundred proteins in response to DNA DSB including the components of the MRN

complex, histone H2AX, checkpoint kinase 2 (CHK2), p53, and KRAB-associated protein 1 (KAP1) (Paull 2015). Phosphorylation occurs on consensus sites (S/TQ) as well as on nonconsensus sites. The proteins phosphorylated by ATM function in signaling pathways that regulate the cell cycle, apoptosis, DNA repair, transcription, and protein synthesis. ATM-driven phosphorylations influence protein–protein interactions, protein–DNA interactions, chromatin structure, and collectively safeguard genome integrity and minimize pathological changes. Although ATM acts on over 700 different substrates, phosphorylation of the components of the MRN complex plays a crucial role in mediating the downstream signaling events that determine cell fate (Lavin et al. 2015).

Using separation-of-function mutations, Lee et al. (2018) identified two pathways of ATM activation. The DSB-induced pathway that operates via the MRN complex regulates cell cycle and DNA repair, whereas the oxidative pathway controls ROS levels and protein homeostasis. Deficiency of the DSB pathway impacted cell viability, checkpoint activation, and DNA end resection in response to DNA damage. On the other hand, although loss of ATM activation by the oxidative pathway did not significantly influence the outcome of DNA damage, it prevented checkpoint responses and induced mitochondrial dysfunction and generated protein aggregation.

ATM has been suggested to function as a sensor of ROS and/or oxidative damage to cellular macromolecules. ATM deficiency is associated with elevated ROS levels. Increased oxidative damage to lipids and DNA associated with low plasma antioxidant levels have been recorded in A-T patients (Reichenbach et al. 1999). A-T fibroblasts exhibited increased sensitivity to hydrogen peroxide and nitric oxide donors. In ATM-deficient mice, there was no evidence of neurodegeneration or apoptosis, but Purkinje cells displayed reduced survival and oxidative stress as evidenced by elevated levels of heme oxygenase-1 (HO-1) (Chen et al. 2003). Antioxidants such as isoindoline nitroxide prevented Purkinje cell death and enhanced survival (Gueven et al. 2006).

Oxidative stress is documented to activate ATM by dimerization via formation of disulfide bonds in contrast to DNA DSB that activates ATM by monomerization of the dimeric protein. In particular, the disulfide at C2991 is crucial as mutation of this residue blocks ROS-mediated ATM activation (Guo et al. 2010). Unlike DNA DSB–induced ATM activation, oxidative stress–induced ATM activation does not phosphorylate H2AX and KAP1 (Lee et al. 2018), presumably because ATM is not recruited to DSB sites where these proteins are located. These findings suggest that ATM phosphorylates specific proteins and downstream effector pathways based on the type of cellular stress. ROS-induced ATM was found to be diffusely distributed in the nucleus unlike DSB, which localize ATM to the sites of DNA damage. Furthermore, ROS-induced ATM activation is prevented by the antioxidant N-acetylcysteine (Guo et al. 2010).

In addition to the nucleus, ATM is localized in cytoplasmic organelles such as the mitochondria and peroxisomes where ROS are generated (Valentin-Vega and Kastan 2012; Zhang et al. 2015). In the peroxisomes, ATM is bound to PEX5, an import receptor. In presence of ROS, ATM phosphorylates PEX5 at S141, followed by ubiquitination that directs the autophagosome to peroxisomes eventually culminating

in pexophagy (Zhang et al. 2015). ROS has been demonstrated to activate ATM in the cytoplasm, a process that is independent of MRN and DNA DSB indicating that the components of the MRN complex will not be phosphorylated by ATM under these conditions. Consequently, a full-fledged DDR does not occur during ATM activation by low levels of ROS (Paull 2015). The response of ATM to oxidative damage in the different subcellular organelles is, however, not clear.

H2AX

The histone variant H2AX, a critical protein that acts as a sensitive indicator of DNA damage, is essential for DDR efficiency and maintenance of genomic integrity. One of the earliest and critical events in response to DSBs is the phosphorylation of H2AX at Ser139 by ATM to generate γH2AX. H2AX is also phosphorylated by ATR in response to SSBs and DNA-dependent protein kinases (DNA-PK) under hypertonic conditions and during apoptosis. Phosphorylation of H2AX in response to DNA damage is followed by recruitment and accumulation of a large number of DDR proteins at the sites of DSBs, thereby amplifying the DNA damage signal (Podhorecka et al. 2010; Srinivas et al. 2019).

DNA damage was shown to induce ROS through H2AX that is mediated via Nox1 and Rac1 GTPase. H2AX in turn activates Rac1GTPase and Nox1 via sequestration of the adapter protein 14-3-3 zeta resulting in increased intracellular ROS levels. Phosphorylation of H2AX as well as regulation of ROS by H2AX may be critical determinants of cell survival or cell death after DNA damage. Regulation of ROS by H2AX may be one mechanism to determine cell survival or apoptosis after DNA damage (Kang et al. 2012). Chronic elevation of ROS causes degradation of H2AX with decrease in γH2AX leading to improved chemosensitivity (Gruosso et al. 2016). On the other hand, an acute increase in ROS levels activates γH2AX and DDR signaling that could be responsible for blunting treatment response to chemotherapy and radiation (Lee et al. 2015; Li et al. 2006). H2AX, a reliable biomarker for DSB, apoptosis, prognosis as well as treatment response, is thus sensitive to ROS levels. Figure 2 illustrates ROS induction through H2AX.

53BP1

p53-binding protein1 (53BP1), a binding partner of p53, is one of the important components of the DDR (Zhang et al. 2017). 53BP1 is a large protein of 200 kDa containing 1972 amino acids encoded by the TP53BP1 gene located on chromosome 15 (15q15.3). The protein structure is characterized by two BRCA1 carboxy-terminal (BRCT) repeats, tandem Tudor domains, a glycine/arginine-rich region, and two dynein 8 kDa light chain (LC8) binding sites. There are a number of Ser/Thr-Gln (S/TQ) and Ser/Thr-Pro (S/TP) phosphorylation sites that are targets of ATM kinase. Phosphorylation of 53BP1 at Ser 25 is required for CHK2 phosphorylation, whereas phosphorylation at Ser 1219 has a role in G2/M-phase arrest following ionizing radiation (Mirza-Aghazadeh-Attari et al. 2019).

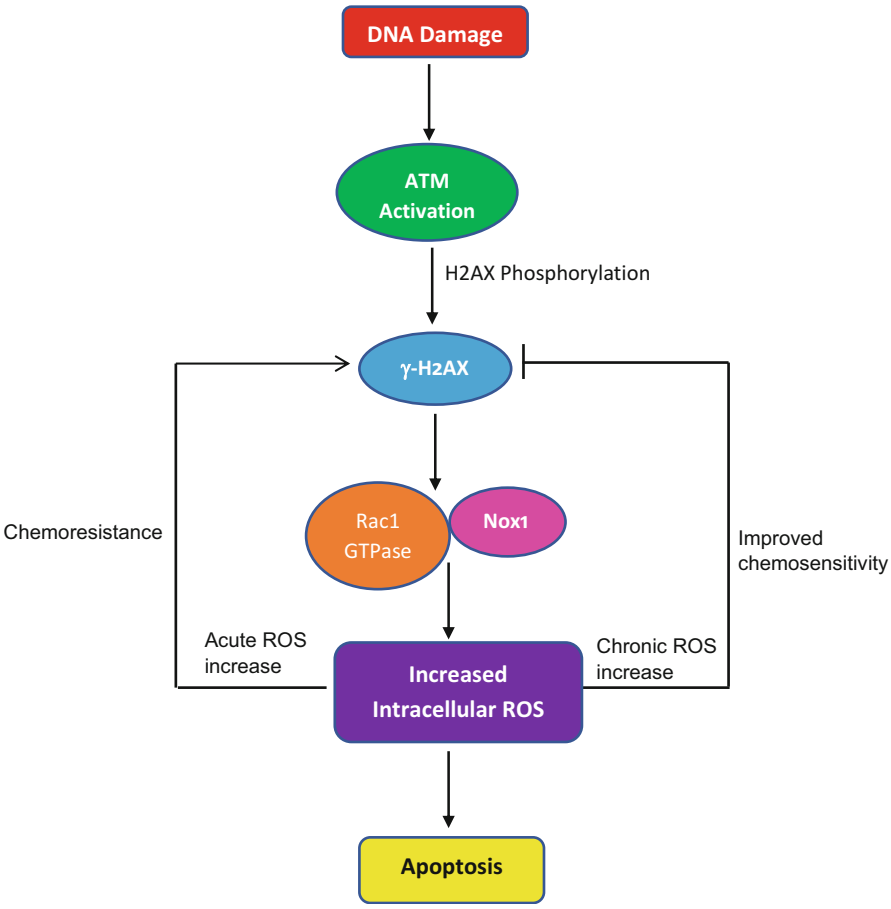

Fig. 2 ROS induction by H2AX. Following DNA damage, H2AX undergoes phosphorylation by ATP. H2AX in turn activates Rac1GTPase and Nox1 resulting in increased intracellular ROS levels and cell death. Chronic ROS elevation causes decrease in γH2AX leading to improved chemosensitivity, whereas an acute increase activates γH2AX and DDR signaling that could cause therapy resistance

53BP1 is found localized in the nucleoplasm. Nucleoplasmic bulk 53BP1 prevents RAP1-interacting factor1 (RIF1) from binding to undamaged DNA. Following DNA damage, 53BP1 binds to the damage site, to which it recruits RIF1 to inhibit end resection, while the bulk 53BP1 is degraded. ATM-mediated phosphorylation of H2AX leads to the formation of K63-linked polyubiquitin chains on histones at DSBs with localization of repair mediators such as 53BP1 to the DNA damage sites. Several deubiquitylating enzymes (DUBs) as well as the structural protein NuMa inhibit 53BP1 accumulation at DNA breaks (Sato et al. 2012; von Morgen et al. 2018).

53BP1 functions both as a sensor and mediator of DNA damage and interacts with several molecules in the DDR network functioning as a recruitment platform for other DSB-responsive proteins. Interaction between the BRCT tandem repeats of 53BP1 and the MRN complex was found to promote ATM activation (Lee et al. 2010). 53BP1 mediates changes in chromatin structure to stimulate ATM activation and induces T68 phosphorylation of Chk2. Most importantly, p53, which plays a central role in cell fate decision, is dependent on 53BP1 (Cuella-Martin et al. 2016). Interaction of p53 with 53BP1 was found to induce cell cycle arrest. 53BP1 also plays a decisive role in the endpoints of DDR. It promotes nonhomologous end-joining (NHEJ)-mediated DSB repair as well as microhomology-mediated end-joining (MMEJ) but prevents HR (Gupta et al. 2014). 53BP1 has also been reported to increase the expression of proapoptotic molecules such as BAX and downregulate the expression of antiapoptotic BCL2 (Hong et al. 2012).

Activation of ATM associated with formation of 53BP1-foci was demonstrated during oxidative stress–induced DDR (Bhatia et al. 2018; Benkafadar et al. 2019). Malignant ascites generated by serous ovarian tumors was found to induce oxidative damage to DNA as illustrated by increased 53BP1 (Pakuła et al. 2018). Activation of 53BP1 UV stress response pathway was observed during oxidative stress induced by voriconozole, which promotes cutaneous squamous cell carcinoma (Lee et al. 2020). Aberrant expression of 53BP1 impairs DNA damage checkpoints and DNA repair and contributes to tumor development. Low levels of 53BP1 were found to correlate with tumor progression, metastasis, and lower survival (Zhu et al. 2014). Mirman and Lange (2020) suggested that 53BP1 functions as a DSB escort that guards against illegitimate and potentially tumorigenic recombination.

DDR and Redox-Sensitive Transcription Factors

At high concentrations, ROS induce cell death, whereas at moderate concentrations, ROS activate various protein kinases such as mitogen-activated protein kinase/extracellular signal-regulated protein kinases 1/2 (MAPK/ERK1/2), p38, c-Jun N-terminal kinase (JNK), and phosphoinositide-3-kinase/protein kinase B(PI3K/Akt). These kinases phosphorylate several substrates and regulate the activity of redox-sensitive transcription factors such as p53, NF-κB, and Nrf2 that orchestrate the DDR. This leads to cell proliferation, apoptosis evasion, angiogenesis, and metastasis, key hallmarks of neoplastic transformation. The influence of these transcription factors on DDR and modulation of their activities by ROS are summarized.

p53

p53, a master tumor suppressor and transcription factor that binds to more than 4000 sites in the genome to regulate the expression of over 500 genes, exerts a significant influence on DDR (Simabuco et al. 2018). Following DNA damage, p53 is stabilized

and regulates the transcription of genes involved in cell survival and cell death. p53 is phosphorylated by ATM kinase at multiple Ser/Thr residues, predominantly Ser15, Ser20, and Ser46. ATM-catalyzed p53 phosphorylation stabilizes p53 and facilitates its interaction with its transcriptional cofactors leading to activation of target genes and cellular responses such as cell cycle arrest, DNA repair, apoptosis, and senescence. While phosphorylation at Ser15 and Ser20 leads to cell cycle arrest and DNA repair, phosphorylation at Ser46 induces apoptosis. Homeodomain-interacting protein kinase 2 (HIPK2), a serine/threonine protein kinase that mediates phosphorylation of p53 by ATM, has been linked to the transcriptional activity of p53 as well as the expression of proapoptotic genes such as BAX and PUMA (Hofmann et al. 2013). p53 exerts a major influence on both the intrinsic and extrinsic pathways of apoptosis (Roos et al. 2016).

p53, a redox protein containing clusters of cysteine residues, is a target of ROS that can reciprocally regulate ROS. Activation of p53 occurs in response to ROS-induced DNA damage. Conversely, p53 has also been reported to increase the expression of genes that induce ROS and cell death such as p53-induced genes (PIGs) (Maillet and Pervaiz 2012). However, the effect of ROS on cell fate depends on the status of p53 that in turn depends on the intensity of ROS accumulation (Fig. 3). p53 plays a protective role in response to lower ROS intensity by activating antioxidant genes including manganese superoxide dismutase (MnSOD) and glutathione peroxidase1 (GPx1), whereas under conditions of higher ROS intensity, p53 downregulates antioxidant proteins and switches on the expression of prooxidant PUMA and PIGs, thereby tipping the balance towards cell death (Humpton and Vousden 2016).

NF-κB

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a transcription factor that regulates a plethora of diverse cellular processes such as cell survival, proliferation, apoptosis, inflammation, chemoresistance, and radioresistance, is induced both by DNA damage as well as oxidative stress. In resting cells, NF-κB is present as an inactive heterodimer of p50 and p65 subunits complexed with inhibitor of κB (IκB) in the cytoplasm. Activation of NF-κB documented in a wide variety of malignant neoplasms involves phosphorylation and proteasomal degradation of IκB followed by nuclear translocation of the NF-κB heterodimer and transactivation of a panel of genes associated with acquisition of cancer hallmarks (Fusella et al. 2020).

ATM plays a critical role in NF-κB activation in response to genotoxic stress. Following detection of DNA DSBs, nuclear translocation of ATM occurs, leading to stimulation of the ubiquitin ligase activity of tumor necrosis factor receptor–associated factor 6 (TRAF6) or X-linked inhibitor of apoptosis protein. This causes assembly of signalosomes, which contain transforming growth factor β-activated kinase 1 (TAK1) that eventually culminates in NF-κB activation and induction of a pro-proliferative and antiapoptotic program (Dunphy et al. 2018). ATM-induced NF-κB activation has also been reported to be mediated via phosphorylation of

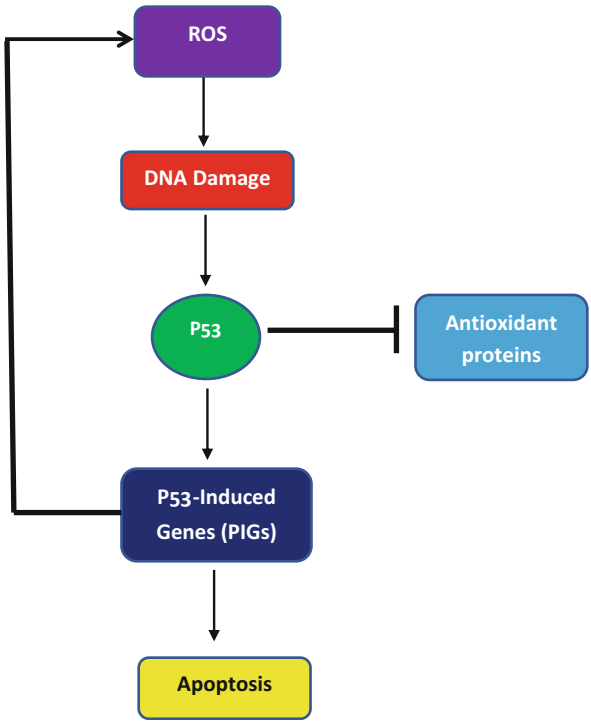

Fig. 3 Interplay between ROS and p53. p53, a target of ROS, reciprocally regulates ROS. p53 activated in response to ROS-induced DNA damage increases the expression of p53-induced genes (PIGs) that stimulate ROS and apoptosis. Under conditions of high ROS intensity, p53 downregulates antioxidant proteins and switches on the expression of prooxidant PUMA and PIGs, thereby tipping the balance towards cell death

NEMO at Lys85 by ATM with subsequent ubiquitination, and export of the NEMO-ATM complex to the cytosol followed by binding to and activation of IKKα (Li et al. 2001). These findings underscore the cytosolic functions of ATM in response to DSBs.

The effect of ROS on NF-κB signaling is rather complex with reports in literature indicating both activation as well as repression. Furthermore, the interaction between NF-κB and ROS is cell-type specific and depends on subcellular localization. ROS have been demonstrated to exert inhibitory effects on nuclear NF-κB activity and stimulatory effects in the cytoplasm (Morgan and Liu 2011; Nakajima and Kitamura 2013). ROS is also known to influence the DNA binding activity of NF-κB both by direct and indirect mechanisms. Oxidation of a specific cysteine residue (Cys-62) in the Rel-homology domain (RHD) of the p50 subunit inhibits DNA binding. Phosphorylation of RelA on Ser 276 essential for its interaction with CBP/300 as well as for expression of certain NF-κB-dependent genes is believed to be regulated by ROS (Morgan and Liu 2011).

NFE2L2

Nuclear factor (erythroid-derived 2)-like factor 2 (NFE2L2, formerly known as NRF2), is a cap "n" collar transcription factor that binds to the antioxidant response

element (ARE) to regulate the expression of genes involved in diverse cellular processes including xenobiotic metabolism, redox homeostasis, DNA repair, apoptosis, autophagy, and inflammation (Ma et al. 2018; Tu et al. 2019). NRF2 contains seven NRF2-epichlorhydrin (ECH) homology domains, Neh1–Neh7. Neh1 contains the DNA-binding motif and together with Neh6 stabilizes NRF2. Neh2 is necessary for regulation of NRF2 by Keap1, while Neh3-5 promotes the transactivation of Nrf2 target genes (Niture et al. 2014).

In normal conditions, NFE2L2 is present in the cytoplasm bound to its inhibitor Kelch-like epichlorhydrin (ECH)-associated protein-1 (Keap1). Keap-1 is a cysteine-rich zinc-finger protein that catalyzes polyubiquitination and degradation of NFE2L2. Keap1 consists of a BTB (broad complex/tramtrack/bric-a-brac) domain and a Kelch domain separated by an intervening region (IVR). Keap1 binds to NFE2L2 via the Kelch domain. A specific cysteine residue (Cys151) in the BTB domain is involved in NFE2L2 ubiquitination mediated by a Cul3-based ubiquitin E3 ligase. Cysteine residues in the IVR function as sensors for ROS. Under conditions of oxidative stress, Cys151, Cys 273, and Cys 288 undergo thiol modifications that lead to inactivation of Keap1, followed by stabilization and nuclear translocation of NFE2L2 resulting in transactivation of cytoprotective genes (Taguchi et al. 2011).

The NFE2L2/Keap-1 signaling plays a pivotal role in DDR. In silico analysis revealed that ATM may be a relevant NFE2L2 kinase (Ma et al. 2018). Kim et al. (2012) reported that activation of NRF2 increased DNA damage signaling by enhancing nuclear accumulation of BRCA1 and RAD51 as well as the expression of 53BP1 in irradiated human colonic epithelial cells. Wang and Konishi (2019) demonstrated that cytoplasmic irradiation of normal human lung fibroblast WI-38 cells induced nuclear localization of NRF2 associated with the expression of the NRF2 target protein heme oxygenase-1. While tert-butylhydroquinone activated NRF2 with decrease in cytoplasmic irradiation-induced DSBs, mitochondrial division inhibitor-1 inhibited cytoplasmic irradiation-induced NRF2 activation and enhanced DSB formation. Decreased NRF2 activity leads to an increase in intracellular ROS resulting in damage to cellular macromolecules and apoptosis, whereas high NRF2 activity confers resistance to cellular stress that contributes to tumorigenesis and therapy resistance (Cloer et al. 2019).

ROS at the Crossroads of Cell Proliferation and Apoptosis

Cell cycle arrest that prevents the proliferation of cells which have sustained DNA damage is an important component of DDR. During cell cycle progression, signaling pathways activate checkpoint proteins in response to DNA damage to prevent perpetuation of damaged DNA and maintain genomic integrity. ROS promote cell cycle arrest by activating the cell cycle checkpoints Chk1 and Chk2 as well as by direct inhibitory effects on the Cdc25 family of protein phosphatases. Periodic oscillations in the cellular redox status regulate cell cycle progression (Davalli et al. 2018). A loss in redox control can result in aberrant proliferation, a hallmark of cancer. A dichotomous relationship was observed between ROS and the mitotic kinases, polo-like kinase1 (PLK1), and AURORA-A that mediate mitotic entry and

recovery from G2/M arrest. Oxidative stress increases PLK1 in a p53-dependent manner while maintaining a G2/M arrest, whereas Aurora kinase A was inhibited (Srinivas et al. 2019; Ward and Hudson 2014).

Activation of oncogenes is known to increase ROS production leading to replication stress. ROS reduce replication fork progression by oxidation of dNTPs and by dissociation of peroxiredoxin2 (PRDX2) oligomers that forms the replisome-associated ROS sensor by binding to TIMELESS, a fork accelerator. Elevated ROS can result in breakdown of replication forks at fragile sites in the genome resulting in over- or under-replicated DNA and genomic instability (Kotsantis et al. 2018; Zeman and Cimprich 2014).

Oncogenic RAS-induced ROS generated in a Rac1-NADPH oxidase (NOX4)-dependent manner function as mitogenic signaling molecules to fuel aberrant cell proliferation. On the other hand, ROS can also cause oxidative damage to DNA thereby activating the DDR and arresting cell proliferation. Ogrunc et al. (2014) proposed a model to reconcile the apparent contradictory roles of ROS. They demonstrated that ROS accumulates in a RAC1-NOX4-dependent manner upon activation of oncogenic HRasVI2 leading to hyperproliferation, DDR activation, and proliferation arrest. Upon activation of oncogenic RAS, RAC1 and NOX4 are recruited with accumulation of mitogenic ROS that drives cellular hyperproliferation resulting in DDR activation and cellular senescence. Inactivation of DDR enables oncogene-induced senescence (OIS) bypass, facilitating proliferation and transformation.

The relationship between ROS and apoptosis continues to be enigmatic. Enhanced ROS generation documented in various malignancies produces dichotomous effects (Moloney and Cotter 2018). ROS can activate pro-tumorigenic signaling, with consequent cell proliferation, evasion of apoptosis, DNA damage, and genetic instability. Paradoxically, ROS are also capable of inducing apoptosis, a mechanism that prevents tumor cell development. Several anticancer agents have been reported to induce tumor cell death by apoptosis and autophagy via ROS generation (Aggarwal et al. 2019). Conflicting reports on the role of apoptosis in cancer initiation and development have further compounded the involvement of ROS. Contrary to the widely accepted tenet of apoptosis evasion as a hallmark of cancer, several clinical studies have demonstrated a correlation between apoptosis and poor prognosis due to the phenomenon of apoptosis-induced compensatory proliferation (AiP) of surviving cells (Diwanji and Bergmann 2019). Recent studies reveal the involvement of ROS in AiP. Accumulating evidence indicates that the levels of ROS determine cell fate outcome. At low levels, ROS modulate transcription factor activities, whereas at higher concentrations they induce oxidative DNA damage and cell death (Aggarwal et al. 2019).

Therapeutic Implications of DDR-ROS Interplay

The altered redox balance of tumor cells relative to their normal counterparts renders them amenable to targeted therapy by ROS manipulation (Davalli et al. 2018). ROS production has been implicated in the therapeutic efficacy of several chemotherapeutic agents and ionizing radiation (Srinivas et al. 2019). However, cellular

mechanisms offer resistance to chemotherapy and radiotherapy including the host antioxidant defense systems. Prooxidant strategies such as RNA interference–induced GSH depletion have been demonstrated to improve sensitivity to chemotherapy and radiation therapy (Ju et al. 2015). ROS is documented to play an important role in immunogenic cell death (ICD) that is increasingly implicated as a key mechanism of tumor cell death induced by chemotherapy and radiation. ICD involves release of damage-associated molecular patterns (DAMPs) such as high-mobility group box1 (HMGB1) proteins and calreticulin that are recognized by pattern recognition receptors (PRRs) leading to recruitment and activation of antigen presenting cells (APCs), priming of adaptive immunity and T-cell-mediated killing of resistant cancer cells. ROS overproduction causes autophagy leading to ICD evasion (Krysko et al. 2012).

HMGB1 is a redox-sensitive nonhistone chromatin-binding protein whose functions vary based on its localization. HMGB1 when present intracellularly in the nucleus binds to DNA and regulates transcription, whereas extracellular HMGB1 functions as a proinflammatory cytokine (Janko et al. 2014). Recently, Ayoub et al. (2019) provided evidence to show the involvement of extracellular HMGB1 in bladder cancer radioresistance via modulation of the tumor immune microenvironment and by promoting DNA damage repair and autophagy. The combination of radiotherapy and HMGB1 inhibition significantly shifted the tumor immune landscape towards an antitumoral response.

Distress signals such as oxidative stress induce release of HMGB1. HMGB1 functions as a redox sensor and activates autophagy during oxidative stress. On the other hand, several studies have also demonstrated an increase in ROS levels as a consequence of HMGB1 release (Janko et al. 2014). Three conserved cysteine residues, Cys23, Cys45, and Cys106, that are sensitive to oxidation are essential for the activity and functions of HMGB1. Under mild oxidative conditions, Cys106 is found in the reduced state, whereas Cys23 and Cys45 are linked by an intramolecular disulfide bond. This enables cytokine release. Oxidation of all three cysteine residues in HMGB1 (Cys23, Cys45, and Cys106) by ROS enhances apoptosis as well as chemosensitivity, whereas complete reduction of cysteines to thiols inhibits cytokine release and stimulates chemoresistance via induction of autophagy. Complete oxidation of cysteines to sulfonates abolishes HMGB1 activity resulting in resolution of inflammation (Janko et al. 2014).

Recently, Brinkman et al. (2021) reviewed the various drugs and natural products known to disrupt cellular responses to DNA damage that offer potential for repurposing as chemo- and/or radiosensitizers. Interestingly, these include compounds recognized to exhibit potent antioxidant activity.

Conclusions

DNA DSBs induced by various agents including ROS are sensed by the MRN complex that recruits and activates ATM, a keystone DDR transducer. ATM in turn phosphorylates a multitude of effector molecules that trigger downstream signaling

events, which eventually determine cell fate. Prominent substrates of ATM include H2AX and 53BP1 that recruit other DSB-responsive proteins thereby amplifying the DNA damage signal. Additionally, ATM phosphorylates several redox-sensitive transcription factors such as p53, NF-κB, and Nrf2 that play crucial roles in DDR signaling. The role of ROS in DDR is paradoxical and dependent on cell type, context, and concentrations. Several components of DDR signaling are targets of ROS and reciprocally regulate ROS levels. ROS are recognized to stimulate oncogenic signaling leading to cell proliferation, apoptosis avoidance, DNA damage, and genetic instability. On the other hand, ROS have also been reported to exert modulatory effects on transducers and effectors of DDR, arrest the cell cycle, and induce apoptosis. Despite their enigmatic role in DDR, manipulation of ROS offers immense scope for therapeutic intervention in cancer. However, complete understanding of the complex interplay between ROS and proteins that regulate the entire gamut of the DDR signaling network as well as on the opposing pathways of cell proliferation and cell death in different contexts is warranted.

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