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Review Article

Concerted action of Nrf2-ARE pathway, MRN complex, HMGB1 and inflammatory cytokines - Implication in modification of radiation damage

Anuranjani, Madhu Bala*

[a]Radiation Biology Department, Institute of Nuclear Medicine and Allied Sciences, Brig SK Mazumdar Marg, Delhi -110054, India

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ABSTRACT

Whole body exposure to low linear energy transfer (LET) ionizing radiations (IRs) damages vital intracellular bio-molecules leading to multiple cellular and tissue injuries as well as pathophysiologies such as inflammation, immunosuppression etc. Nearly 70% of damage is caused indirectly by radiolysis of intracellular water leading to formation of reactive oxygen species (ROS) and free radicals and producing a state of oxidative stress. The damage is also caused by direct ionization of biomolecules. The type of radiation injuries is dependent on the absorbed radiation dose. Sub-lethal IR dose produces more of DNA base damages, whereas higher doses produce more DNA single strand break (SSBs), and double strand breaks (DSBs). The Nrf2-ARE pathway is an important oxidative stress regulating pathway. The DNA DSBs repair regulated by MRN complex, immunomodulation and inflammation regulated by HMGB1 and various types of cytokines are some of the key pathways which interact with each other in a complex manner and modify the radiation response. Because the majority of radiation damage is via oxidative stress, it is essential to gain in depth understanding of the mechanisms of Nrf2-ARE pathway and understand its interactions with MRN complex, HMGB1 and cytokines to increase our understanding on the radiation responses. Such information is of tremendous help in development of medical radiation countermeasures, radioprotective drugs and therapeutics. Till date no approved and safe countermeasure is available for human use. This study reviews the Nrf2-ARE pathway and its crosstalk with MRN-complex, HMGB1 and cytokines (TNF-α, IL-6, IFN-γ etc.). An attempt is also made to review the modification of some of these pathways in presence of selected antioxidant radioprotective compounds or herbal extracts.

Introduction

Ionizing radiation (IR) is being increasingly used in medicine for diagnosis and therapy, industry and warfare. The uncontrolled exposure of normal biological systems to IR causes various unwanted biological effects, which are often dependent on IR dose. To counter such effects there is an imminent need for development of medical radiation countermeasures. After the World War II, a large number of chemical agents were reported to have radio-protective properties in pre-clinical studies, yet none was found suitable for human use. Development of a safe, protective and effective medical radiation countermeasure, therefore, remains a global challenge till date. Understanding more about the mechanisms of radiation injuries and interactions between various IR induced pathways, is very important

Abbreviations: IR, ionizing radiation; LET, linear energy transfer; ROS, reactive oxygen species; DSB, double strands break; DDR, DNA damage response; SOD, superoxide dismutase; SSBs, single strand DNA breaks; ·OH, hydroxyl radical; ARE, antioxidant response element; GPx, glutathione peroxidase; GSH, glutathione (reduced); MRN, Mre11, Rad50 and Nbs1 subunits; NADPH, nicotinamide adenine dinucleotide phosphate; NES, nuclear export sequence; Keap1, Kelch like ECH associated protein 1; DGR, double glycine repeats; PKC, protein kinase C; GSK-3β, glycogen synthase kinase 3 beta; t-BHQ, tert butyl hydroquinone; Bcl-2, B cell lymphoma-2 protein; NHEJ, non-homologous end joining; HR, homologous recombination; RIF, radiation induced foci; ATM, ataxia telangiectasia mutagenesis; Chk-2, checkpoint kinase-2 protein; HMGB1, high mobility group Box 1; RAGE, receptor for advance glycation end products; MRP, multidrug resistance protein; GM-CSF, granulocytes macrophages colony stimulating factor; TRAIL, TNF related apoptosis inducing ligand; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; TWEAK, tumour necrosis factor weak inducer of apoptosis; AP1, activator protein-1; RNS, reactive nitrogen species; NLS, nuclear localization sequence; DAMP, death associated molecular pattern; bFGF, basal fibroblast growth factor; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells; FGF2, fibroblast growth factor-2; CBP, CREB-binding protein; MDA, malondialdehyde; MIP, macrophages inflammatory proteins.

* Correspondence to: Dr Madhu Bala, Head, Radiation Biology Department, Institute of Nuclear Medicine and Allied Sciences, Defence Research Development Organisation (Grant Sponser), Brig SK Mazumdar Marg, Delhi -110054, India. Phone: +91 11 23970081.

E-mail address: bala44@gmail.com (M. Bala).

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to meet these challenges. Total body exposure to IR results in multiple inter- or intra-cellular lesions. The damage caused by IR is primarily by two mechanisms. The energy may be directly deposited into the biomolecules (nucleic acids, proteins, lipids etc.) resulting in disruption of their chemical bonds. On the other hand, energy may cause radiolysis of intracellular water molecules leading to production of a flux of multiple reactive oxygen species (ROS) and free radicals which damage the cellular biomolecules. The high LET IR (e.g. α-particles, heavy ions) causes most of the damage by direct deposition of energy into the biomolecules. The low LET IR (e.g. X-rays, γ-rays) causes most of the damages by ionization of intracellular water molecules. The cell generally has 70–80% water content. Therefore, the low LET IR causes 70–80% of damage by generating ROS and free radicals. Superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($\cdot$OH), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are some of the reactive oxygen species which cause multiple lesions such as oxidation of membrane lipids, amino acids, modification of thiols and DNA damage. The low LET IR is deeply penetrating radiations and therefore, pose a greater challenge. The majority of DNA damage caused by sub-lethal doses of low LET IR is the DNA base modification and base damage. At higher doses, besides DNA base modification and base damage, more complex DNA lesions such as DNA single strand breaks (SSBs), double strand breaks (DSBs) and other clustered DNA lesions are observed [1]. In general, the exposure of cultured mammalian cells to 1 Gy of IR can generate approximately 20–40 DSBs as well as approximately 10^3 SSBs and base damages in DNA [2]. IR injuries to lipids and proteins result in membrane damage and cell disruption, leading to inflammation and immunomodulation. Altered levels of various cytokines such as TNF-α [3], IL-6, IFN-γ as well as of damage associated molecular pattern such as HMGB1 are reported [4].

Under normal physiological conditions, the intracellular ROS are generally produced as a by-product of mitochondrial electron transport chain and a delicate balance is maintained between the level of oxidative species generated and the inherent antioxidant defence mechanisms resulting in a state of 'redox homeostasis'. The physiologically normal level of ROS helps in cellular growth and regulating multiple signalling pathways [5]. The increased flux of ROS, after exposure to low LET IR, disturbs the normal redox homeostasis to favour a state of "oxidative stress", where the intracellular concentration of ROS far exceeds the balancing capacity of antioxidant defence mechanisms [6]. The IR induced oxidative stress leads to increased apoptosis [7,8] as well as multiple pathologies, such as necrosis [9], inflammation, aging [10,11], ischemic injuries, neurodegenerative diseases, rheumatoid arthritis, and cancer [12]. The translesional synthesis of nucleic acids, disturbances in cell cycle progression, accumulation of unrepaired damage, necrosis mediated activation of inflammatory pathways etc., are some of the underlying mechanisms of these pathologies. To maintain the intracellular state of redox homeostasis, the intracellular antioxidants act as first line of defence. Several antioxidant enzymes such as superoxide dismutase (SOD), catalase, heme oxygenase (HO-1), NAD(P)H oxidoreductase quinone-1 (NQO-1), glutathione (GSH), glutathione S-transferase subunit (GSTs), and glutathione peroxidase (GPx) act as primary scavengers of ROS and free radicals [13]. Certain non-enzymatic chemical and biochemical antioxidant species (ascorbic acid, alpha tocopherol, polyphenols etc.) act to neutralize the ROS by donating the electrons. The ascorbic acid (vitamin C) is a natural water soluble essential micronutrient. It primarily scavenges ROS and their derivatives, to protect the oxidation of intracellular macromolecules [14]. The α-tocopherol (vitamin E) is an important lipid soluble vitamin and another strong antioxidant, which acts as a neutralizing agent for peroxyl radicals to form lipid peroxides and tocopheryl radicals. Fig. 1 schematically represents these events.

Polyphenols are the dietary supplements which act as scavenger of ROS, enzyme modulator and metal chelators [15–17]. The common phenolic components are resveratrol, gallic acid, anthocyanin, catechin, myricetin, quercetin etc. At molecular level the role of the transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2), is well recognized in modulating antioxidant response. The mechanisms underlying the Nrf2-antioxidant response element (ARE) pathway are being actively investigated. It is very important to understand the Nrf2-ARE pathway and its interactions with the various other molecular pathways that are affected by IR. This study reviews the crosstalk between the Nrf2-ARE pathways, MRN-complex regulated DNA damage repair pathway, HMGB1 and cytokine mediated inflammation and immune pathways. An attempt is also made to review the modification of these pathways by selected radiomodifying plant extracts and antioxidant compounds.

Keap1-Nrf2-ARE pathway

The transcription factor Nrf2, also known as heme binding protein 1 (HEBP1), is a potent transcriptional activator and plays a central role in inducing the expression of many cytoprotective genes in response to oxidative and electrophilic stress. Nrf2 is encoded by the *Nfe2l2* gene located on chromosome 2 in humans. Theoretically, the molecular weight of Nrf2 is 66 kDa [18]. Nrf2 was first identified in *Drosophila cap 'n' collar* protein form, where this gene is required for labial and mandibular development [19]. Using the tandom repeats of nuclear factor like erythroid factor-2 (NF-E2)/activator protein-1 (AP1) of the β-globulin locus as a recognition site probe, two NF-E2 related proteins, Nrf1 [20] and Nrf2 [21], were identified. Nrf2 contained leucine zipper motif and had N-terminal acidic domain (rich in glutamic and aspartic acid), which could potentially function as an acidic transactivation domain. The homologous recombination mutational study on Nrf1 disrupted mice showed lethality due to anaemic condition of erythroid cells and fatal liver abnormalities. Nrf1 was, therefore, proposed to be essential for development because of its direct role in erythropoiesis. In the absence of adequate studies with Nrf2, the early reports suggested that Nrf2 was dispensable for growth and development [22]. However, later studies proposed that Nrf2 played a key role in regulating the expression of cytoprotective genes under xenobiotic stress [23]. A common binding motif of Nrf1 and Nrf2 on to hARE sequence driven NQO-1 gene was reported. Subsequent studies demonstrated that expression of GSTs, NQO-1 enzymes were markedly reduced in liver and intestine of mice which had disrupted Nrf2 gene [24]. Such animals showed extreme sensitivity towards oxidative stress which explained the critical role of Nrf2 in cell survival and growth.

Regulation of Nrf2-ARE pathway

Nrf2, in association with small Maf and Jun protein family, forms an upstream transcriptional complex [25–27]. This heterodimer state of Nrf2 binds to the ARE sequence of DNA and regulates ARE-driven genes that encode for detoxification enzymes as well as antioxidant proteins to augment the cellular first line defense system against oxidative stress [28,29]. ARE was initially identified as electrophilic response element (EpRE) in the promoter region of the mouse GSTa1 gene [30]. ARE in association with Nrf2 activates transcription of many downstream genes such as NQO-1, GST, UDP-glucosyl transferase 1-Ab, glutamate cysteine ligase (Gclc), HO-1, thioredoxin reductase-1 (TXNRD1), thioredoxin, and ferratin-12.

Under normal redox conditions, Nrf2 is present in the cell cytoplasm and promotes only basal level expression of cytoprotective enzymes. Nrf2 acts as a transcription activator when transferred inside the nucleus. The Nrf2 has two kinds of binding partners, (a) a cytoplasm repressor Kelch like ECH associated protein 1 (Keap1) which strictly regulates Nrf2 stabilization in cell cytoplasm under a normal redox state [24], and (b) ARE sequence, which is an upstream binding enhancer element of cytoprotective genes present in the nucleus [31]. The Keap1 acts as a cytoplasmic repressor of Nrf2.