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NRF2 promotes survival following exposure to ionizing radiation

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ABSTRACT

NRF2 is a transcription factor that promotes antioxidant and drug-metabolizing gene expression. It also regulates the transcription of genes involved in carbohydrate and lipid metabolism, NADPH regeneration, and heme and iron metabolism, as well as proteasome metabolism. Emerging research has identified NRF2 as a critical factor for promoting survival of mammalian cells subjected to ionizing radiation. At a mechanistic level, NRF2 promotes the repair of DNA damage and drives detoxification of superoxide that is generated hours to days after irradiation. This review summarizes research in these areas and discusses targeting of NRF2 in radiation-resistant cancer and NRF2's role in mitigating acute radiation syndrome.

Contents

Introduction

Electrophiles and reactive oxygen intermediates, as well as reactive nitrogen species, can contribute significantly to the etiology of many chronic human diseases. This knowledge has driven a major research effort that focused on providing mechanistic insight and guidance for the development of redox-based therapeutic strategies. The effort, however, was hampered by the structural diversity of electrophilic and oxidative compounds. It took the pioneering work of Talalay and colleagues [1–3] and Pickett and associates [4,5], as well as research from Violet Daniel's laboratory [6,7], to provide a fundamental molecular framework that ultimately was used to explain how a thiol-based protein sensor distinguished between

Abbreviations: ARE, antioxidant response element; ARS, acute radiation syndrome; ATM, ataxia telangiectasia mutated; BRCA1, breast cancer type 1 susceptibility protein; ChIP, chromatin immunoprecipitation; CnC, cap 'n' collar family of basic leucine zipper transcription factors; DSB, double strand break; HR, homologous recombination; Keap1, kelch-like ECH-associated protein 1; LD_{50}, lethal dose for 50% of a population; MEF, mouse embryo fibroblast; NADPH, nicotinamide adenine dinucleotide phosphate; NHEJ, nonhomologous end joining; NRF2, human nuclear factor (erythroid-derived 2)-like 2 protein; Nrf2, mouse nuclear factor (erythroid-derived 2)-like 2 protein; OGG1, 8-oxoguanine DNA glycosylase; •OH, hydroxyl radical; PARP1, poly(ADP-ribose) polymerase 1; RNAi, RNA interference; ROS, reactive oxygen species; RPA, replication protein A.

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different types of chemistries and translated the information into physiological responses. The sensor is kelch-like ECH-associated protein 1 (Keap1), originally characterized by Itoh et al. [8]. The murine protein contains 25 and the human contains 27 cysteine residues that function as redox sensors capable of integrating diverse chemistries [9], including radiolytically generated hydroxyl radical (●OH) and hydrogen peroxide [10], into a common signal: activation of nuclear factor erythroid 2-like 2, or NRF2 (reviewed in [11]).

NRF2 (HGNC:7782), encoded by *NFE2L2*, is a member of the cap 'n' collar (CnC) family of basic leucine zipper transcription factors [12] that are conserved in mammals [13], birds [8], fish [14], insects [15], and worms [16], but not expressed in plants and fungi [13]. The family is composed of the transcription factors SKN-1, NRF1, NRF2, NFE2, NRF3, CncC, BACH1, and BACH2 [13]. These proteins are characterized by a leucine zipper protein–protein dimerization domain as well as CnC and abasic domains that confer DNA binding activity [17]. An NMR solution structure of the DNA binding domain may be found at http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=105542.

Nrf2 was originally identified as a key regulator of canonical antioxidant and drug-metabolizing gene expression [18,19]. Nrf2 heterodimerization with MAF-G [20] or the JUN CnC-bZIP factor [21] licenses binding to *cis* antioxidant response elements (AREs) located in proximal promoters of Nrf2 target genes that now number over 500 [20], including those involved in carbohydrate and lipid metabolism, NADPH regeneration, and heme and iron metabolism, as well as proteasome metabolism [19,22]. The functional ARE has recently been defined as TMANNRTGACTCAGCRWWWW, where M = A or C, R= A or G, and W = A or T [20]. Emerging research now shows that Nrf2-mediated transcription can protect cells and tissues from the pathogenic consequences of hydroxyl radicals that are directly generated by ionizing radiation as well as the hydrogen peroxide and superoxide that are generated as a secondary consequence of irradiation.

NRF2 promotes survival of irradiated cells

Preclinical cell culture models have been used to address the question of whether Nrf2 impacts survival of irradiated cells. Keap1$^{-/-}$ mouse embryo fibroblasts (MEFs) constitutively overexpress Nrf2 and Nrf2 target genes and are characterized by low levels of intracellular reactive oxygen species (ROS) and a radiation-resistant phenotype compared to wild-type MEFs [23]. The generalized term ROS is used in this review when the initial oxidizing species were not identified in the cited papers [24] and has been defined by C. Winterbourn as "those initial species generated by oxygen reduction (eg, superoxide) as well as all secondary reactive products. The definition includes overlapping reactive nitrogen species" [25]. Relative to wild-type MEFs, Nrf2$^{-/-}$ MEFs express high levels of intracellular ROS and are intrinsically radiosensitive [23,26]. Activation of Nrf2/NRF2 signaling due to electrophilic adduction of Keap1 or a deficiency in the expression/function of Keap1 has been shown to lower intracellular ROS and confer radioresistance in fibroblasts [27], bronchial and breast epithelial cells [28], DU145 prostate cells [29], squamous cell lung cancer [30], and glioblastoma cells [31]. RNA interference (RNAi) or pharmacological targeting of NRF2 in DU145 prostate cancer cells [29,32], non-small-cell lung cancer A549, H460, or H1299 cells [23,33], or gliobastoma cells [31] elevates ROS and produces a corresponding radiosensitive phenotype. Taken all together, these investigations support a hypothesis that NRF2 promotes a prosurvival response in irradiated cells.

Molecular effects of ionizing radiation

Initial events

The term ionizing radiation describes a photon or particle with sufficient energy to displace orbital electrons from atoms, thereby yielding ions and ionized electrons [34]. Coulomb interactions occur between ionized charged particles (e.g., an electron) moving through a medium such as a cell and the orbital electrons of the constituent atoms. These interactions result in a transfer of kinetic energy from the ionized charged particles to the electrons in the medium [35] and are quantified as absorbed dose (D), which is defined as the absorption of energy in a medium of known mass by ionizing particles [35]. The units of D are Gy (the SI unit; 1 Gy = 1 J/kg) or rad, which is equal to 0.01 Gy. In the case of X- or γ-irradiation, 70% of photons traversing a cell interact with water molecules that ultimately decompose into hydroxyl radicals (●OH), hydrogen radicals (●H), hydrogen peroxide, superoxide, and solvated electrons (e^-_{aq}) [36]. The hydroxyl radical can react at diffusion-controlled rates with all four purine and pyrimidine bases, as well as 2-deoxyribose. However, neither superoxide nor hydrogen peroxide reacts significantly with DNA bases or 2-deoxyribose [37] and as discussed below, radiation-induced damage to DNA is a critical event. Thus, the initial reactions relevant to this review can be described as follows [38–40].

$$\rightsquigarrow H_2O^{+\bullet} + e^-_{aq} \quad (1)$$

$$\rightsquigarrow H_2O^* \quad (2)$$

$$H_2O^{+\bullet} + H_2O \rightarrow H_3O + \bullet OH \quad (3)$$

$$H_2O^* \rightarrow \bullet OH + \bullet H \quad (4)$$

The radical cation in Eq. (1) ($H_2O^{+\bullet}$) can donate a proton to a nearby water molecule within 10^{-14} s to yield H_3O and the hydroxyl radical (●OH), Eq. (3) while H_2O^* (Eq. (2)) can decompose into ●OH + ●H (Eq. (4)). These reactions are complete on a time scale of milliseconds.

G-value is a term used to quantify the chemical effects of ionizing radiation. The term was originally defined as the number of molecules transformed, produced, destroyed, or changed per 100 eV of energy absorbed. In SI units the G-value is assigned a value of moles per Joule. G-values are energy dependent. For example, cobalt 60 emits two monoenergetic photons of 1.17 and 1.34 MeV. The G-value for the hydroxyl radical was calculated to be 2.74 molecules formed per 100 eV absorbed [38–40]. The $LD_{50/60}$ is a term to describe the mean lethal dose that will produce 50% mortality in a population over 60 days. For humans the $LD_{50/60}$ is approximately 4 Gy [34]. The G-values discussed above allow one to calculate the concentration of hydroxyl radicals generated by a given dose of radiation. For example, 4 Gy will generate approximately 20.4 μmol of hydroxyl radicals in a person who weighs 190 pounds.

Radiation-induced damage to DNA

While all cellular macromolecules are susceptible to attack by ●OH, damage to DNA represents a critical injury with pluripotent consequences: cell death, tissue injury, and disease. Hydroxyl radicals can abstract a hydrogen atom from the methyl group of thymine and from each carbon atom of the 2-deoxyribose moiety [41,42]. Hydroxyl radicals are able to add to the double bonds in DNA bases to generate hydroxyl DNA base radicals [41]. These and other reactions can result in apurinic/apyrimidinic (AP) sites, single strand breaks [42], double

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