DNA Damage Response and Ferroptosis

Subjects: Biology

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Ferroptosis is a novel form of iron-dependent cell death characterized by lipid peroxidation. While the importance and disease relevance of ferroptosis are gaining recognition, much remains unknown about its interaction with other biological processes and pathways. Recently, several studies have identified intricate and complicated interplay between ferroptosis, ionizing radiation (IR), ATM (ataxia–telangiectasia mutated)/ATR (ATM and Rad3-related), and tumor suppressor p53, which signifies the participation of the DNA damage response (DDR) in iron-related cell death. DDR is an evolutionarily conserved response triggered by various DNA insults to attenuate proliferation, enable DNA repairs, and dispose of cells with damaged DNA to maintain genome integrity. Deficiency in proper DDR in many genetic disorders or tumors also highlights the importance of this pathway.

ferroptosis DNA damage ATM ATR p53 MDM2 MDMX

1. Ferroptosis—Biological Processes and Genetic Determinants

Programmed cell death (PCD) plays a critical role in tissue homeostasis and many pathological conditions. Recently, ferroptosis was identified as a new form of PCD, with distinct biochemical, morphological, and cellular features [1]. Ferroptosis is characterized by iron-dependency, extensive lipid peroxidation, and plasma membrane damage [1][2]. Ferroptosis was first discovered by the mechanistic investigation of erastin-induced cell death [3]. Since then, significant progress has been made in the understanding of the biological processes of ferroptotic death and genetic determinants that promote or limit ferroptosis [1][2]. These genetic determinants typically affect lipid peroxidation and protection mechanisms by regulating the levels of cysteine, glutathione, iron, or lipids.

Most mammalian cells have developed systems to neutralize lipid peroxidation and prevent ferroptosis. Genetic and chemical studies have pinpointed glutathione peroxidase (GPX4) as the critical regulator of this form of cell death [4]. Therefore, various GPX4 inhibitors can directly trigger ferroptosis [3]. In addition, because GPX4 relies on glutathione (GSH) and NADPH (dihydronicotinamide adenine dinucleotide phosphate) as cofactors, the depletion of GSH or NADPH can interfere with GPX4 function and induce ferroptosis.

Cysteine is the limiting component of GSH that is imported via xCT from extracellular space in the form of cystine (a dimeric form of cysteine). xCT (encoded by *SLC7A11*) is the transmembrane antitransporter that mediates the cystine import through the export of glutamate. Therefore, xCT inhibitors (e.g., erastin or sulfasalazine) would deplete cysteine and GSH and trigger ferroptosis [3]. Similarly, when each amino acid is removed in several nutria-

genetic screens, cystine emerged as indispensable for multiple cancer types, including triple-negative breast cancer [5], renal cell carcinoma [6], and nonsmall cell lung cell carcinoma [7].

NADPH is an essential cofactor for redox balance, and it participates in GSH regeneration. Therefore, the levels of NADPH in various cancer cells also predict ferroptosis sensitivity [8]. This idea is further corroborated by our recent finding of MESH1 as the first mammalian NADPH phosphatase, which is induced during ferroptosis to deplete NADPH [9]. Additionally, nuclear factor erythroid 2-related factor 2 (NRF2), the master transcriptional regulator of antioxidant stresses, can protect ferroptosis by inducing various components for GSH synthesis [10][11].

Other than GPX4, cell density and contact have recently been found to be robust determinants of ferroptosis in several different cancer cell lines via regulation of the Hippo effectors, YAP or TAZ, linking various mechanical stimuli, environmental cues, and metabolic status to ferroptosis [12][13][14][15]. Although GPX4 is crucial for protecting against ferroptosis, two studies have identified another independent system composed of FSP1–CoQ_{10} [16][17]. The myristoylation of FSP1 (ferroptosis-suppressing protein 1) allows its movement to the plasma membrane. FSP1 catalyzes the formation of reduced coenzyme Q10 (CoQ_{10}), which serves as a radical-trapping antioxidant to neutralize lipid peroxidation and reactive oxygen species (ROS) and peroxidation. This novel mechanism of ferroptosis protection may provide additional insights and therapeutic opportunities.

As the name implied, ferroptosis is an iron-dependent process since iron chelators robustly block ferroptosis [3]. Mammalian cells with high iron contents, such as erythrocyte-ingested macrophages [18] and hemochromatosis hepatocytes [19], are highly sensitive to ferroptosis. Iron has been speculated to drive the Fenton reaction and generate hydrogen peroxide to cause lipid peroxidation [20]. Regardless, ferroptosis can also be regulated by the levels and activities of these iron-related proteins. However, the role of iron in ferroptosis remains poorly understood.

2. Canonical Functions of DNA Damage Responses (DDRs)—The Role of ATR, ATM, p53, and MDM2

The integrity of the genome is essential for the faithful transfer of genetic materials to the progeny. However, certain endogenous levels of mutational changes are also crucial to enable genetic variations, adaptation, and evolution. Genomic DNA can be damaged from multiple exogenous and endogenous sources. The types of damage include replication errors, chemical-induced adducts, and crosslinks, ultraviolet light (UV)-induced injury, and single-strand (SSB) or double-strand (DSB) breaks created by ionizing radiation or chemical reactions [21][22]. To cope with these types of DNA damage and maintain genome integrity, DDR has been developed in all organisms to detect and sense DNA damage, transmit the signals to the appropriate effectors, and repair various forms of DNA damage and genomic insults. When DNA damage is not correctly repaired, DDR will trigger apoptosis and cell death programs to eliminate the unrepaired cells. In mammalian cells, the central sensors and upstream DDR kinases include the ATM (ataxia–telangiectasia mutated), ATR (ATM and Rad3-related), and DNA–PKs (DNA dependent protein kinase). These DDR kinases regulate the levels and activities of various effector

proteins [23], including the guardian of genome integrity, p53, and its critical negative regulators, MDM2 (mouse double minute 2) and MDMX (murine double minute X).

2.1. ATM and ATR—The Kinases Sensing DNA Damages

ATM and ATR are two large serine/threonine kinases in the phosphatidylinositol-3-kinase-like kinase family (PIKK) [24][25]. ATM is mutated in ataxia–telangiectasia (AT; OMIM #208900) patients from all complementation groups, indicating that it is probably the single gene responsible for AT [26]. Ataxia–telangiectasia is a rare human autosomal recessive disorder. The affected AT individuals are immunodeficient, radiosensitive, and predisposed to the development of cancer [26]. Consistent with the role of ATM in DDR, AT cells have abnormal cell-cycle arrests and hypersensitivity to ionizing radiation. In contrast, ATR is identified based on its sequence homology to ATM, featuring its ability to prevent abnormal cell division or aneuploidy when activated during DNA damage [27].

Consistent with these genetic data, ATM and ATR are crucial for DDR and maintenance of genome stability. During DDR, ATM and ATR are activated within seconds and mediate the phosphorylation of hundreds of proteins at the Ser/Thr-Glu motifs [28][29][30]. Although structurally and functionally similar, ATM and ATR are triggered by distinct forms of DNA damage. The main trigger for ATM is DNA double-strand breaks. In contrast, ATR responds to a much broader spectrum of DNA damage, including single-strand DNA (ssDNA), stalled replication forks, and other replication stresses. In the absence of DNA damage, ATM exists as an inactive homodimer [31]. Upon DSB, ATM is recruited by the MRE11–RAD50–NBS1 complex (MRN complex) [32] on DSB lesion sites and becomes activated to phosphorylate its downstream effectors [33]. Downstream effectors of ATM are composed of hundreds of proteins, including Chk2, KAP1, RNF20, RNAP1/2, to mediate the p53 phosphorylation, epigenetic DNA repair, chromatin remodeling, and transcription inhibition, respectively [34][35]. Noteworthily, ATM itself can also activate p53 and its stability regulators, MDMX and MDM2 [36][37], to safeguard the genome from DSB damage.

On the other hand, the activation of ATR is a multistep process. Under replication stresses, ATR and its partner protein, ATRIP (ATR-interacting protein), are recruited by ssDNA-binding protein complexes and replication protein A (RPA) associated with the extended tracts of ssDNA [38][39]. Besides recruitment by RPA, ATR also interacts with TopBP1 and ETAA1 to achieve optimal activation and kinase activity [40][41]. Activated ATR phosphorylates a distinct set of downstream substrates, including Chk1 and CDC25A, to prevent premature mitosis and increase DNA repair for cell survival [42]. Although ATR and ATM kinases have some functional redundancies and interconnect with each other in the DDR pathways, they have distinct substrates and downstream functions. For example, ATR mainly activates the Fanconi anemia (FA) pathway to promote the repair of DNA interstrand crosslinks [43]. In contrast, ATM, but not ATR, regulates the development of the central nervous system, thus explaining the neurodegenerative and other neurological phenotypes in AT patients with defective ATM protein [44].

2.2. p53—The Guardian of the Mammalian Genome

One of the most critical targets of ATM/ATR in the DDR pathway is p53, often referred to as the guardian of the mammalian genome. ATM is the predominant upstream regulator of p53 through the regulation of Chk2 and MDM2/MDMx. While ATR is not required for p53 activation, ATR synergizes with p53 to ensure a successful

replication checkpoint [45]. ATM/ATR defects lead to a significant delay in p53 upregulation in response to ionizing radiation or other types of DNA damage. p53 is one of the most critical tumor suppressor genes, and it is crucial for preventing cancer formation in vertebrates. As such, p53 mutations or polymorphisms are responsible for a significant portion of human cancer [46]. First, the inheritance of a p53 mutation leads to Li–Fraumeni syndrome (LFS, OMIM #151623), characterized by various early-onset cancers, such as breast, brain, and adrenal cancer and sarcomas. Secondly, somatic mutations in p53 are found in ~50% of all human cancers, including more than 90% of ovarian and uterine carcinoma. Additionally, the coding and noncoding regions of p53 contain at least hundreds of single nucleotide polymorphisms (SNPs; germline variants), which may alter p53 functions with significant effects on cancer susceptibility, progression, and response to various therapeutics [47]. Functionally, p53 is a transcription factor that elicits antitumorigenesis effects such as cell senescence, cell-cycle arrest, and cell death by affecting the expression of its target genes (e.g., p21, Puma, Bax) [48].

2.3. MDM2/MDMX—The Main Brake that Restrains p53

Given its essential role and dramatic effects on cellular phenotypes, p53 is elaborately regulated by MDM2/MDMX through several mechanisms [49]. In nonstressed cells, p53 is continuously translated but rapidly ubiquitinated by MDM2/MDMX and degraded by proteasome on the protein level [50][51][52][53]. MDM2 also inhibits p53 by other means, binding to the transactivation domain of p53 to curb its transcription activity [54][55]. Furthermore, MDM2 interferes with the nuclear localization signal of p53 and prevents nuclear translocation [56]. Upon DNA damage, p53 is activated by ATM and ATR by multiple mechanisms to enhance its protein stability and transcription capacity [57]. ATM phosphorylates MDM2 to compromise its ligase activity and p53 ubiquitination [58][59], while ATR attenuates p53 nuclear export by phosphorylation of MDM2 at S407 [60]. Collectively, this ATR/ATM–MDM2/MDMX–p53 axis is essential for the DNA damage-induced p53 stabilization and transcriptional control to trigger cell-cycle arrest, DNA repair, and apoptosis events as part of canonical DDR phenotypic responses.

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