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Review article

Regulators in the DNA damage response

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ABSTRACT

Maintenance of genome integrity is essential for the proper function of all cells and organisms. In response to both endogenous and exogenous DNA damaging agents, mammalian cells have evolved a delicate system to sense DNA damage, stop cell cycle progression, modulate cell metabolism, repair damaged DNA, and induce programmed cell death if the damage is too severe. This coordinated global signaling network, namely the DNA damage response (DDR), ensures the genome stability under DNA damaging stress. A variety of regulators have been shown to modulate the activity and levels of key proteins in the DDR, including kinases, phosphatases, ubiquitin ligases, deubiquitinases, and other protein modifying enzymes. Epigenetic regulators, particularly microRNAs and long noncoding RNAs, have been emerging as an important payer of regulation in addition to canonical DNA damage signaling proteins. In this review, we will discuss the functional interaction between the regulators and their targets in the DDR.

Contents

1. Introduction

Maintenance of genome integrity is essential to prevent development of diseases associated with genomic instability including cancer, development defects, infertility, immune deficiency and neurodegenerative disorders [1,2]. The integrity of cellular DNA is constantly challenged by a variety of environmental and endogenous genotoxic insults, such as ultraviolet (UV) in sunlight or ionizing radiation (IR), numerous chemotherapeutic agents, as well as by-products of normal cell metabolism, notably reactive oxygen species (ROS) [3,4]. In addition, numerous studies from cell culture, animal models and clinical specimens showed that activation of oncogenes as well as loss of tumor suppressors

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also contribute to DNA replication stress, DNA damage and genomic instability [5,6]. To protect genome integrity after DNA damage, cells have evolved a highly coordinated cellular system to sense and counteract these threats, generally named the DNA damage response (DDR). In fact, cells have developed a number of machineries to detect and repair the various types of damage that can occur to DNA. Different DNA lesions are repaired by distinct pathways [4]. Double-stranded breaks (DSBs) can be repaired through homologous recombination (HR) or through non-homologous end joining (NHEJ), an error-prone DNA joining mechanism that leads to mutations. UV-induced DNA lesions, and other bulky DNA adducts are repaired by nucleotide excision repair (NER). Individual or short-patch base lesions are repaired by base-excision repair (BER) and DNA base mismatches are corrected by mismatch repair (MMR), while the Fanconi anemia (FA) pathway repairs DNA crosslinks [7]. Of the many types of DNA lesions, the most harmful one is the DSB. Failure to repair DSBs may lead to chromosome breaks or rearrangements, mutations, cell death or cancer.

In general, all the DDR pathways encompass a similar set of tightly regulated steps: initial detection of DNA damage, recruitment of DNA repair factors to the damage site and the final repair of DNA lesions [1]. Accordingly, all these components in the signaling pathways can be functionally categorized into sensors of damage, and signal transducers and effectors, which are organized in a hierarchical manner and communicate with each other. The whole DDR process is tightly controlled by post-translational modifications (PTM), including phosphorylation, ubiquitination, sumoylation, methylation, acetylation and others [8,9]. PTM has been shown to play a pivotal role in the DDR, which involves recruiting various enzymatic machineries and ATP-dependent remodelers and are functionally responsible for protein stability, activity and localization [10]. While mediated primarily through relatively fast posttranslational modifications, the DDR also triggers a broad range of gene expression program in the damaged cells, leading to the expression of those genes involved in the DNA repair, cell cycle control or apoptosis. Importantly, a large portion of this reprogramming is mediated by non-coding RNAs (ncRNAs), including small microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) [11]. In this review, we provide an overview of various regulators in the ATM/ATR-p53 DNA damage signaling pathway and define the crucial functions of these factors in the fine-tuning DDR.

2. Post-translational modifications (PTMs)

PTMs are a critical layer of regulation in the DDR because they provide a means of altering the functional features of a given protein without the necessity of de novo protein synthesis. PTMs are enzyme-catalyzed alterations of a particular protein, which include the addition or removal of chemical groups to one or more amino acid residues of the protein. The most common protein modifications following DNA damage are phosphorylation, acetylation, methylation, ubiquitination, sumoylation and neddylation [12,13]. In most cases, these modifications are reversible and, accordingly, regulated by two types of counteracting enzymes. For example, phosphorylation is performed by protein kinases that covalently link a phosphate group onto a serine, threonine or tyrosine of the target protein, whereas phosphatases reverse this alteration by removing the phosphate group. Phosphorylation usually results in a conformational change of the target protein due to the introduction of a negative charge, leading to the activation or inactivation of an enzyme.

2.1. Protein kinases

A well-characterized PTM mechanism in the DDR is protein phosphorylation executed by kinases (Fig. 1). The phosphatidylinositol 3-kinase-related kinases (PIKKs), including ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3 related) and DNA-dependent protein kinase catalytic subunit (also known as DNA-PKcs), are at the heart of the DNA damage signaling cascade [14,15]. ATM and ATR are highly conserved through evolution with the homologs Mec1p/Tel1p in yeast *Saccharomyces cerevisiae*. Upon DNA damage, the binding of Mre11-Rad50-Nbs1 (MRN) complex to the DSBs triggers the auto-phosphorylation of ATM at S1981 and switch from inactive dimers to active monomers [16,17]. Similarly, the localization of ATR to the damage site and its subsequent activation is dependent on the binding of Rad9-Hus1-Rad1 (9-1-1) clamp complex, primarily in response to replication stress and collapsed DNA replication forks [18]. The activation of ATM or ATR in turn leads to the phosphorylation of many downstream substrates that are involved in DNA repair, cell cycle progression and apoptosis.

ATR shares a spectrum of substrates with ATM and was, therefore, thought to be functional redundant: both preferentially phosphorylate the serine or threonine residues that precede glutamines (called SQ/TQ motifs) in their substrates. Indeed, Matsuoka and colleagues have identified more than 900 ATM/ATR phosphorylation motifs encompassing over 700 proteins in response to DNA damage [19]. One of the well-studied substrates of ATM/ATR in the DDR is histone H2A variant H2AX, which is phosphorylated on S139, yielding γH2AX [20]. The formation of γH2AX occurs rapidly in response to DNA damage and is required for recruiting DDR factors onto the damaged chromatin. γH2AX is then recognized by MDC1, which is also phosphorylated and activated in an ATM-dependent manner [21]. The BRCT domain of MDC1 directly recognize the phosphoserine 139 in the carboxyl end of γH2AX and recruitment of MDC1 to γH2AX foci is required for the formation of MRN, BRCA1 and 53BP1 foci [22].

The two initiating kinases ATM/ATR transduce the DNA damage signal through the checkpoint kinases, Chk1 and Chk2, which relay and amplify the DDR signal [23]. Chk2 is phosphorylated and activated by ATM at T68 primarily in response to DSB, subsequently oligomerized and autophosphorylated at T383 and T387 [24]. Chk1 is active even in unperturbed cells, and further activated by ATR through phosphorylation at S317 and S345, which in turn lead to

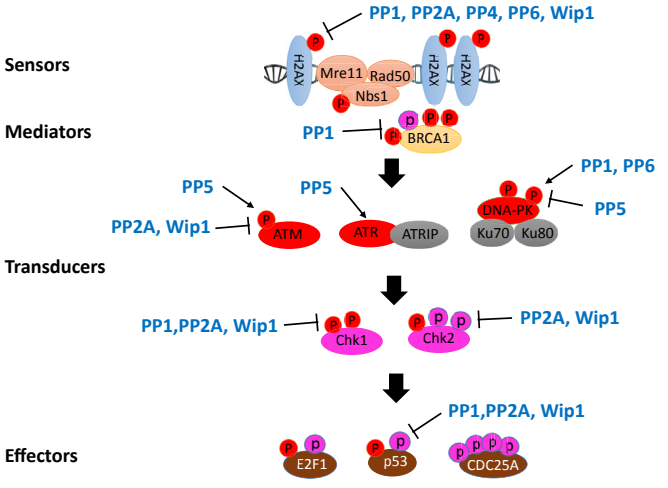

Fig. 1. Roles of Ser/Thr kinases and phosphatases in DNA damage response. Proteins phosphorylated by ATM, ATR or DNA-PKcs are marked with red phosphate groups and proteins phosphorylated by Chk1 or Chk2 are marked with pink phosphate groups. Phosphatases are also shown to regulate the activity of DDR proteins positively or negatively.