Molecules and Cells

Minireview

Role of RUNX Family Transcription Factors in DNA Damage Response

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Cells are constantly exposed to endogenous and exogenous stresses that can result in DNA damage. In response, they have evolved complex pathways to maintain genomic integrity. RUNX family transcription factors (RUNX1, RUNX2, and RUNX3 in mammals) are master regulators of development and differentiation, and are frequently dysregulated in cancer. A growing body of research also implicates RUNX proteins as regulators of the DNA damage response, often acting in conjunction with the p53 and Fanconi anemia pathways. In this review, we discuss the functional role and mechanisms involved in RUNX factor mediated response to DNA damage and other cellular stresses. We highlight the impact of these new findings on our understanding of cancer predisposition associated with RUNX factor dysregulation and their implications for designing novel approaches to prevent cancer formation in affected individuals.

Keywords: cancer, cell cycle arrest, DNA damage response, Fanconi anemia, p53, RUNX1, RUNX2, RUNX3, tumor suppressor

INTRODUCTION

Cells are constantly exposed to stresses that cause genomic DNA damage leading to depurination, deamination, DNA crosslinks, single strand breaks (SSBs), double stand breaks (DSBs), and chromosomal translocations. Endogenous factors include DNA replicative stress as well as reactive oxygen species (ROS) and aldehydes generated during normal cellular metabolism. Exogenous factors include ionizing radiation, chemical agents, and inflammation.

Cells have evolved intricate mechanisms to respond to and recover from DNA damage. This involves a hierarchical orchestration of proteins that act as sensors, transducers and effectors (Fig. 1). After sensing DNA damage, cells normally arrest their cell cycle, repair the damage, and/or undergo apoptosis or senescence if the damage is beyond repair. Two main sensors are the Mre11/Rad50/Nbs1 (MRN) complex, which acts in response to DSBs (Lamarche et al., 2010) and the Replication Protein A (RPA) complex, which acts in response to SSBs (Zou et al., 2006). The MRN complex recruits ATM (ataxia telangiectasia mutated) and the RPA complex recruits ATR (ATM and rad3-related) (Maréchal and Zou, 2013). Subsequent steps involve the recruitment of Chk1 and Chk2 kinases. These kinases act at the apex of a cascade of phosphorylation events involving a multitude of proteins. These link the DNA damage response (DDR) to transcription, cell cycle regulation, DNA repair, and apoptosis.

p53, THE GUARDIAN OF THE GENOME

p53 in response to DNA damage

The transcription factor p53 is a central regulator of the DDR. Loss-of-function mutations in its gene, *TP53*, are one of the

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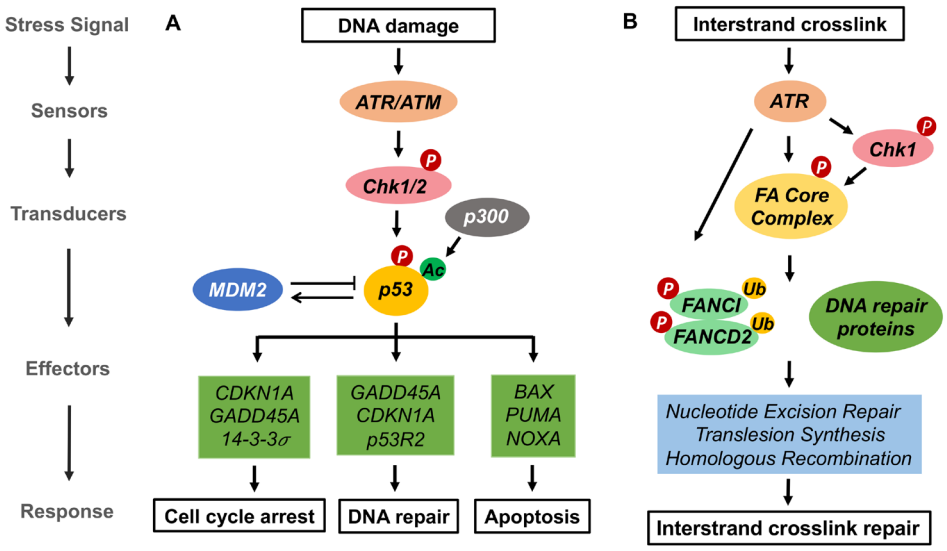

Fig. 1. p53-dependent DNA damage response and the Fanconi anemia (FA) pathway. (A) p53-dependent pathway. DNA damage activates ATR/ATM and CHK1/CHK2 kinases leading to p53 phosphorylation. p53 undergoes p300 dependent acetylation which transactivates specific p53 target genes resulting in either cell cycle arrest and DNA repair or apoptosis. (B) FA pathway. The FA pathway is activated during the S-phase of the cell cycle upon DNA replication fork stalling at ICLs. ATR/CHK1 is activated and in turn activates FA core complex, which promotes the monoubiquitylation of the FANCI-FANCD2 heterodimer. The ubiquitylated FANCD2-FANCI heterodimer at ICLs recruits DNA repair proteins involved in nucleotide excision repair, translesion synthesis and homologous recombination to stabilize the fork and repair ICLs.

most frequent occurrences in cancer. The importance of p53 in maintaining genomic integrity and removing damaged cells has earned it the name "guardian of the genome". p53 is involved in many different types of DNA damage and repair mechanisms (Fig. 1A).

p53 regulation

p53 protein is maintained at a low level under normal physiological conditions. However, upon genotoxic stress, p53 protein levels markedly increase. This occurs primarily through post-transcriptional mechanisms allowing cells to respond rapidly to DNA damage (Kastan et al., 1991). p53 undergoes a wide array of modifications including acetylation, ubiquitination, phosphorylation, and methylation (Liu et al., 2019). Such modifications change its ability to interact with partner proteins and alter its stability and cellular localization. One key modification is polyubiquitination, which occurs on several lysine residues on the carboxy terminus (Lys-370, Lys-371, Lys-373, Lys-381, Lys-382, and Lys-386). This is mediated primarily by the E3 ubiquitin ligase mouse double minute 2 (MDM2) and leads to p53 degradation (Lohrum et al., 2001; Rodriguez et al., 2000). Many of the same lysine residues can be acetylated by p300 and TIP60, which blocks ubiquitination and thereby stabilizes p53 protein (Reed and Quelle, 2014). p53 is also a substrate for ATR and ATM, which phosphorylate serine 15 leading to p53 activation (Cheng and Chen, 2010).

The activity of p53 as a transcription factor depends on its nuclear localization, which is also tightly regulated. This is driven by three nuclear localization signals and two nuclear export signals (NES) (O'Keefe et al., 2003). In response to DNA damage, p53 undergoes modifications on several of its residues (Fig. 1A). Phosphorylation of serine and threonine residues on the amino terminal dampens its interaction with MDM2 stabilizing p53 and enhancing its nuclear localization. Stress-induced tetramerization of p53 interferes with the interaction between the NES and its receptor thereby facilitating p53 nuclear retention (Marchenko et al., 2010).

Once active and in the nucleus, p53 acts as a positive regulator of genes involved in DNA repair, cell cycle arrest, and apoptosis, including DNA damage-binding protein 2 (*DDB2*), *XPC*, *CDKN1A* (which encodes p21), *GADD45A*, *PUMA*, and *BAX* (Fischer, 2017). In addition, p53 positively regulates its own regulators such as *MDM2* and *p53-induced phosphatase 1* creating complex feedback loops (Zhou et al., 2017).

FANCONI ANEMIA PATHWAY IN DNA DAMAGE RESPONSE

The Fanconi anemia (FA) pathway plays an important role in sensing and repairing interstrand crosslinks (ICLs) (Fig. 1B) (Moldovan and D'Andrea, 2009). ICLs result from exposure to reactive chemical compounds such as certain chemotherapeutic agents (e.g., cis-platin), mitomycin C, nitrous oxide, endogenous ROS, peroxide intermediates, and reactive aldehydes (Lopez-Martinez et al., 2016). ICLs can also occur in context of damaged nucleotide bases, DNA–protein complexes, DNA-RNA hybrids (R-loops), and DNA G quadraplexes (Rodríguez and D'Andrea, 2017). Unrepaired ICLs can lead to DNA DSBs. Accumulation of ICLs are highly toxic to cells with as few as 20 to 40 cross links causing cell death (Dronkert and Kanaar, 2001; Lawley and Phillips, 1996; McHugh et al.,

2001).

Twenty-two FA pathway genes have been discovered to date (Niraj et al., 2019). Germline mutations of any of these causes FA, which is characterized by bone marrow failure, congenital abnormalities and a predisposition to cancer. The FA pathway is activated by ICLs during the S phase of the cell cycle (Wang, 2007). ICLs are recognized by ATR and CHK1, which phosphorylate FA proteins including FANCE, FANCM, and FANCG (Qiao et al., 2004; Singh et al., 2013; Wang, 2007; Wilson et al., 2010). Phosphorylated FA proteins assemble with other DNA repair proteins to form the FA core complex. Ubiquitylation of the FANCD2-FANCI heterodimer by the FA core complex recruits DDR proteins to damaged foci to resolve ICLs and resume replication/transcription (Meetei et al., 2003; Rickman et al., 2015).

FA proteins also protect single stranded nascent DNA and stabilize stalled replication forks. In addition, FA pathway proteins play roles in non-homologous end joining (NHEJ) (Renaud et al., 2016), base excision repair (BER) (Kelsall et al., 2012), alternative end joining (Nguyen et al., 2014), chromosome segregation (Chan et al., 2009; Naim and Rosselli, 2009), and cytoprotection from ROS and proinflammatory driven apoptosis (Haneline et al., 1998; Schindler and Hoehn, 1988; Whitney et al., 1996).

RUNX FAMILY OF PROTEINS

RUNX family transcription factors play essential roles in a wide range of biological processes including embryonic development, cell proliferation, differentiation, lineage determination and apoptosis. There are three RUNX family members in mammals (RUNX1, RUNX2, and RUNX3), all of which heterodimerize with a common non-DNA binding core binding factor beta (CBF-β) subunit. They act as both transcriptional activators and repressors. The three family members have distinct tissue expression patterns with some overlap particularly between RUNX1 and RUNX3. Knockout of each *RUNX* gene in mice leads to specific phenotypes indicating non-redundant roles. *RUNX1* knockout mice have vascular defects and a failure to establish definitive hematopoiesis during embryogenesis (Wang et al., 1996). *RUNX2* knockout mice have malformed bone and cartilage tissues (Komori et al., 1997; Otto et al., 1997). *RUNX3* deficient mice have impaired lymphopoiesis and neurogenesis, and develop gastrointestinal hyperplasia as they age (Levanon and Groner, 2009).

Structure of RUNX proteins

The main conserved domain of RUNX family proteins is the runt domain, which is located in the amino terminal region of all three proteins and mediates sequence-specific DNA binding and dimerization with CBF-β. RUNX proteins undergo a conformational shift upon binding to CBFβ (Yan et al., 2004), which allows high affinity binding to the consensus DNA sequence (Py)G(Py)GGT(Py). The carboxyl half of the proteins contains the transactivating and autoinhibitory domains, PPxY motif, nuclear matrix targeting signal (NMTS) and VWRPY repressor motif (Imai et al., 1998; Ito et al., 2015; Mangan and Speck, 2011).

RUNX proteins as tumor suppressors and oncogenes

RUNX1 is one of the most frequently mutated genes in hematological malignancies including acute myelogenous leukemia (AML) M0 subtype (15-35%), myelodysplastic syndrome (MDS) (10-20%), chronic myelomonocytic leukemia (CML)(37%), and MDS/myeloproliferative neoplasm (MPN) (14%) (Blyth et al., 2005; Ernst et al., 2010; Harada and Harada, 2011; Kuo et al., 2009; Osato, 2004). *RUNX1* translocations also occur in human leukemia. The t(8;21) translocation, which generates the RUNX1-ETO fusion protein, is one of the most frequent chromosomal translocations in AML patients (10-20%) (Blyth et al., 2005) and RUNX1-ETV6 is the most common translocation in pediatric B-cell acute leukemia (Sun et al., 2017).

Inherited monoallelic germline *RUNX1* mutations cause familial platelet disorder with predisposition to leukemia (FPD/AML) (Godley, 2014; Jongmans et al., 2010; Osato, 2004; Owen et al., 2008; Song et al., 1999). These are typically loss-of-function or dominant negative mutations (Osato, 2004). Affected individuals have about a 44% lifetime risk of developing MDS or AML (Godley, 2014). Bi-allelic *RUNX1* mutations occur during progression to leukemia, highlighting RUNX1 as a classic tumor suppressor. Conversely, RUNX1 can act as an oncogene in T-cell leukemia (Choi et al., 2017).

Amplification of chromosome 6p21, which contains the *RUNX2* gene, is an early event in osteosarcoma development (Forus et al., 1995; Lau et al., 2004; Martin et al., 2011). Increased *RUNX2* expression is also found in breast and prostate cancer cells and is associated with greater invasion and metastasis implying that RUNX2 has pro-oncogenic and pre-metastatic properties (Akech et al., 2010; Ito et al., 2015; Pratap et al., 2008).

RUNX3 has been reported to act as a tumor suppressor in gastric, colon, and other solid tumors (Bae and Choi, 2004; Chen et al., 2014; Chuang and Ito, 2010; Manandhar and Lee, 2018).

ROLE OF RUNX FACTORS IN DNA DAMAGE AND STRESS RESPONSE

Work over the past decade has provided growing evidence that RUNX proteins play roles in response to DNA damage and other cellular stresses. Interestingly, RUNX1/RUNX3 and RUNX2 appear to have opposite activities.

RUNX1/RUNX3 in p53-dependent DDR pathways

RUNX1 or RUNX3 deficient cells show defects in DNA repair, including base excision, homologous recombination and interstrand DNA crosslink repair (Bellissimo and Speck, 2017). One mechanism involves p53-dependent pathways (Fig. 2). RUNX1-deficient murine hematopoietic stem and progenitor cells (HSPCs) have lower p53 protein levels and a markedly blunted increase in p53 protein levels and attenuated activation of p53 target genes following radiation (Cai et al., 2015). Similarly, hematopoietic progenitor cells differentiated from FPD/AML patient-derived human induced pluripotent stem cells (hIPSCs) have signs of impaired DDR as measured by increased γ-H2AX and 53BP1 nuclear foci and reduced mRNA transcript levels of the p53 direct target genes *CDKN1A*,

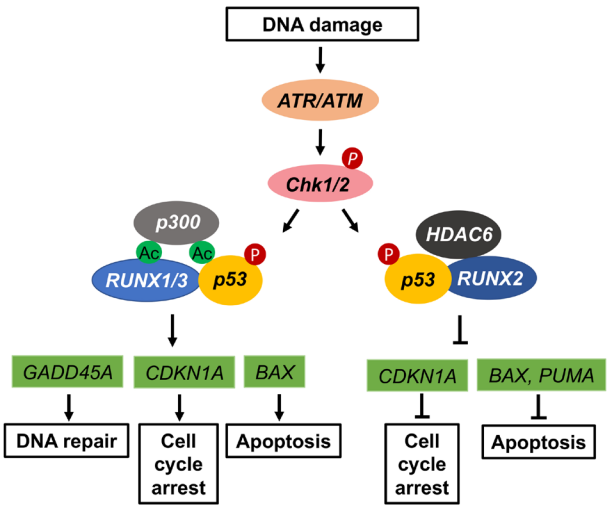

Fig. 2. Role of *RUNX* proteins in *p53*-dependent DNA damage response. In response to DNA damage *p53* gets phosphorylated at serine-15 in a phosho-*ATM/ATR* dependent manner. RUNX1 and RUNX3 play similar roles in promoting *p53* acetylation and recruiting it to gene promoters of downstream target genes involved in DNA repair, cell cycle arrest and apoptosis. Association of *RUNX2* with *HDAC6* dampens acetylation to repress apoptosis and maintain cell cycle progression.

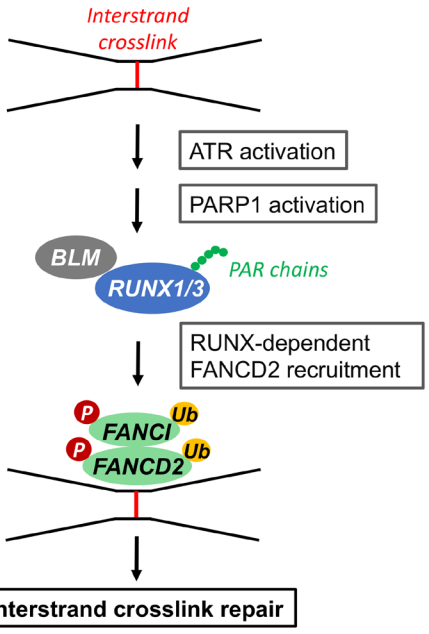

Fig. 3. Role of RUNX proteins in the FA pathway. In response to DNA fork stalling at ICLs during the S phase of cell cycle, ATR and PARP1 are activated, which induces RUNX poly(ADP-ribosyl)ation and its interaction with BLM helicase. This leads to efficient recruitment of FANCD2-FANCI onto the ICLs for repair. Data from the article of Tay et al. (2018) (Cell Rep. *24*, 1747-1755) in accordance with the Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) license.

NOXA, *BAX*, and *GADD45* (Antony-Debré et al., 2015). This occurs even in the absence of exogenous genotoxic stress such as irradiation or chemotherapeutics. Importantly, the defective DDR can be rescued by expressing wild type RUNX1 in these cells. Likewise, Satoh et al. (2012) showed that a RUNX1 C-terminal deletion mutant attenuates the DDR to DSBs and suppresses *GADD45A* expression in murine HSPCs cells. Wu et al. (2013) showed that both p53 and RUNX1 are strongly upregulated at the protein level following adriamycin exposure of human colon carcinoma and human osteosarcoma cells. Moreover, RUNX1 forms a physical complex with p53 and is recruited to p53 target genes which are subsequently activated.

Like RUNX1, RUNX3 acts as a positive regulator of the DDR in p53 dependent pathways. In response to DNA damage, RUNX3 translocates to the nucleus and colocalizes with p53 (Yamada et al., 2010). RUNX3 also recruits phosphorylated ATM to p53 facilitating phosphorylation of p53 Ser-15 (Wu et al., 2013). It also enhances p300 mediated acetylation at K373/382 (Chi et al., 2009) leading to p53 activation. Consistent with these findings, RUNX3 deficient backgrounds have reduced p53 downstream target gene expression (Wang et al., 2014). Collectively, these studies indicate that RUNX1 and RUNX3 act as important co-activators of p53 in the DDR. Interestingly, RUNX proteins and p53 share a common s-type Ig fold 3-dimensional structure in their DNA binding domains (Berardi et al., 1999).

RUNX1/RUNX3 in the FA pathway

There is emerging evidence that RUNX1 and RUNX3 also function in the FA pathway (Fig. 3). Double knockout of RUNX1 and RUNX3 within the murine hematopoietic compartment causes lethal phenotypes due to bone marrow failure and a myeloproliferative disorder (Wang et al., 2014). Impaired recruitment of monoubiquitinated FANCD2 protein to ICL foci leads to hypersensitivity to the crosslinking agent mitomycin C in these mice. Subsequent work showed that RUNX proteins undergo PARP-dependent poly(ADP-ribosyl)ation following DNA damage enabling them to efficiently interact with the Bloom syndrome protein (BLM) and control FANCD2 focus formation (Tay et al., 2018).

RUNX1 oncofusion proteins and DDR

Several studies have examined the effects of RUNX1 fusion proteins on DDR pathways. Human hematopoietic cells expressing RUNX1-ETO display an activated p53 pathway, increased apoptosis and increased sensitivity to DNA damage (Krejci et al., 2008). p53 knockdown increased the resistance of RUNX1-ETO cells to ionizing radiation and chemotherapy, indicating that high levels of p53 in RUNX1-ETO cells are responsible for the increased sensitivity to DNA damage. RUNX1-ETO downregulates genes associated with base excision DNA repair pathways including 8-oxoguanine DNA glycosylase (*OGG1*) (Alcalay et al., 2003; Forster et al., 2016; Krejci et al., 2008; Liddiard et al., 2010). The homologous recombination pathway has been reported to be inefficient in RUNX1-ETO expressing cells (Esposito et al., 2015). RUNX1-ETO cells are extremely sensitive to poly (ADP-ribose) polymerase (PARP) inhibitors, which potently inhibit cells that

have defective homologous recombination repair. RUNX1 overexpression induces senescence-like growth arrest in primary MEFs with an intact p19(ARF)-p53 pathway, but in the absence of p53, RUNX1 shows pro-oncogenic activity cell growth *in vivo* (Wotton et al., 2004).

RUNX1 and RUNX3 in cellular stress responses

In addition to roles in DDR, RUNX1 and RUNX3 participate in other cellular stress pathways by modulating ribosomal biogenesis (Cai et al., 2015), promoting the unfolded protein response (Cai et al., 2015), reacting to hypoxia (Lee et al., 2017), and regulating the Restriction (R) Point when cells decide to undergo proliferation or cell death (Chi et al., 2017; Lee et al., 2019).

RUNX2 as a negative regulator of DDR

In response to genotoxic stress, RUNX2, like RUNX1 and RUNX3, forms a complex with p53, which gets recruited to p53 target gene promoters. However, in contrast to RUNX1 and RUNX3, RUNX2 significantly downregulates p53 target gene expression (Fig. 2). RUNX2 knockdown results in elevated p53 target gene expression and enhanced apoptosis in response to adriamycin (Ozaki et al., 2013b). RUNX2's activity in repressing p53 target genes depends on its ability to recruit the histone deacetylase HDAC6 (Ozaki et al., 2013a; Westendorf et al., 2002).

p73 and p63 are p53 homologues that also accumulate in the nucleus following DNA damage. RUNX2 forms a complex with p73 and attenuates pro-apoptotic p53/p73-dependent DDR in human osteosarcoma-derived U2OS cells (Ozaki et al., 2013b). It also represses DNA damage-mediated upregulation of p73. Depletion of RUNX2 enhances chemotherapy sensitivity of p53-deficient human pancreatic cancer cells through induction of p63-mediated cell death (Ozaki et al., 2016; Sugimoto et al., 2015). Loss of RUNX2 also sensitizes osteosarcoma to chemotherapy-induced apoptosis (Roos et al., 2015; Shin et al., 2016). RUNX2-expressing lymphomas show consistently low apoptosis. RUNX2 and MYC collaborate to activate resistance to growth inhibitory responses thereby inducing tumor-specific survival (Blyth et al., 2006). RUNX2 also suppresses the cell cycle regulator pRB therefore promoting cell cycle progression (Calo et al., 2010; Wysokinski et al., 2015). Taken together, these data suggest that RUNX2 primarily plays an oncogenic role with respect to DNA damage and cell cycle responses.

FUTURE PERSPECTIVES AND THERAPEUTIC STRATEGIES

In addition to their classic roles in development and differentiation, the studies highlighted in this mini review provide growing evidence that RUNX family transcription factors play important roles responding to cellular stress, maintaining genomic stability and ensuring cellular quality control. This activity likely contributes to the cancer predisposition associated with dysregulation of their expression. These studies also reveal a highly interconnected relationship between RUNX factors and the p53 pathway. This includes their activity in stabilizing p53 protein and modulating p53 direct target genes. Emerging data also suggest a role for RUNX proteins in the FA pathway and replicative stress. These findings have a number of important clinical implications. First, they suggest that patients with mutant RUNX factors should limit DNA-damaging exposures as much as possible. Second, they may provide insights into selective pressures and mechanisms that promote progression to cancer in patients with RUNX protein dysfunction. Third, they can potentially be exploited for new therapeutic approaches, for example: (1) MDM2 inhibitors may be useful in reversing the p53 protein instability associated with RUNX1/RUNX3 deficiency; (2) PARP inhibitors could be utilized to leverage the increased sensitivity to DNA damage of cells containing certain somatic RUNX protein alterations; (3) HDAC6 inhibitors could potentially be used in cancers associated with RUNX2 overexpression; and (4) immunotherapy may be effective given the production of potential neoantigens associated with genomic instability. Important future directions will be the development of RUNX genetic model systems to test these potential new therapies and the further elucidation of the molecular mechanisms by which RUNX proteins sense and respond to cellular stress.

Disclosure

The authors have no potential conflicts of interest to disclose.

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