FORUM REVIEW ARTICLE

Oxidative Stress-Associated Protein Tyrosine Kinases and Phosphatases in Fanconi Anemia

Jie Li¹ and Qishen Pang²

Abstract

Significance: Fanconi anemia (FA) is a genetic disorder featuring chromosomal instability, developmental defects, progressive bone marrow failure, and predisposition to cancer. Besides the predominant role in DNA damage response and/or repair, many studies have linked FA proteins to oxidative stress. Oxidative stress, defined as imbalance in pro-oxidant and antioxidant homeostasis, has been considered to contribute to disease development, including FA. **Recent Advances:** A variety of signaling pathways may be influenced by oxidative stress, particularly the equilibrium between protein kinases and phosphatases, consequently leading to an aberrant phosphorylation state of cellular proteins. Dysfunction of kinases/phosphatases has been implicated in the pathophysiology of human diseases. In FA, evidence is emerging that links abnormal phosphorylation/dephosphorylation of signaling molecules to clinical complications and malformations. **Critical Issues:** In this study, we review the recent findings on the oxidative stress-related kinases and phosphatases, particularly tyrosine phosphatases in FA. **Future Directions:** Understanding the role of oxidative stress-related kinases and phosphatases in FA may provide unique and generic possibilities for the future development of therapeutic strategies by targeting the dysregulated protein kinases and phosphatases in a clinical setting. *Antioxid. Redox Signal.* 20, 2290–2301.

Introduction

FANCONI ANEMIA (FA) is a rare genetic disease associated with chromosomal instability, bone marrow failure, developmental defects, cancer risk, and a broad array of clinical complications and malformations (20, 78, 129). To date, 15 FA complementation groups and the corresponding genes have been identified, including FANCA, B, C, D1, D2, E, F, G, I, J, L, M, N, O, and P, which may function through the FA-BRCA pathway (20, 22, 53, 55, 78, 108, 129). Among them, eight of the FA proteins (composed of FANCA, B, C, E, F, G, L, and M) form a nuclear complex and act as a ubiquitin ligase in response to DNA damage or DNA replication stress by monoubiquitinating two substrates FANCD2 and FANCI (Fig. 1) (20, 78, 91, 129).

Ever since early research reported excessive oxygen toxicity in FA cells, a great number of studies have related FA to oxidative stress, which has been considered a critical factor in

the pathogenesis of FA (1, 26, 28, 37, 52, 67, 69, 80, 84, 88, 90, 91, 113, 136, 137). Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense, is associated with many human disease states, including FA, which is arguably a unique disease model characterized by abnormal accumulation of ROS and a dysfunctional response to oxidative damage (26, 91). Evidence has been accumulated that ROS, including hydrogen peroxide, superoxide, and the hydroxyl radical, could regulate the transduction of signals from the membrane to the nucleus *via* the modulation of cellular enzymatic activity by oxidation and reduction (98, 127). Among the enzymes modified by oxidative stress, protein kinases and phosphatases serve as important regulators of cellular signaling pathways (60, 98). Excessive generation of ROS has been shown to activate multiple protein kinases, such as extracellular signal-regulated kinase (ERK)1/2, protein kinase B (PKB), and protein tyrosine kinases (PTKs) (25, 31, 43, 50, 118,

¹Division of Neurosurgery, Center for Theoretic and Applied Neuro-Oncology, Moores Cancer Center, University of California, San Diego, La Jolla, California.

²Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, Ohio.

FIG. 1. A simplified model of the Fanconi anemia (FA)/BRCA pathway. Eight FA proteins (FANCA, B, C, E, F, G, L, and M) along with FAAP100 and FAAP20 form a nuclear protein complex (FA core complex), which acts as ubiquitin ligase by mono-ubiquitinating two substrates FANCD2 and FANCI, followed by recruiting other downstream FA proteins, including FANCD1, FANCI, and FANCN.

125, 127). Additionally, a variety of phosphatases are the sensitive targets of oxidative stress and may be inactivated under oxidizing conditions, which would amplify the effect of redox-linked activation of key protein kinases (60, 110, 111, 131). Given that the balance between protein kinases and phosphatases dictates the overall phosphorylation state of cellular phosphoproteins, aberrant protein phosphorylation may exacerbate the pathophysiology of multiple human diseases and targeting them by small molecules therefore would be anticipated to ameliorate clinical symptoms (34, 38, 76, 79).

In this review, we will describe the abnormal protein kinases and phosphatases influenced by oxidative stress, in particular, aberrant protein tyrosine phosphatases (PTPs) in FA. Furthermore, we will discuss the involvement of abnormal protein phosphorylation during the development of clinical complications and the potential drug targets to improve the FA phenotype.

Impaired Tyrosine Phosphatases in Oxidative Stress

The reversible phosphorylation of protein residues, regulated by protein kinases and phosphatases, modulates many important aspects of cell life, including metabolism, transcription, cell cycle progression, cytoskeletal rearrangement, differentiation, and apoptosis (1, 13, 33, 111, 120). Protein phosphorylation status also plays a critical role in the intercellular communication during development, in physiological responses, and in homeostasis, and in the functioning of the nervous and immune systems (15, 24, 47, 81, 123). Impaired protein phosphorylation triggered by aberrant functions of protein kinases and phosphatases therefore contributes to a number of disorders (1, 13, 77, 86, 111). Protein kinases and phosphatases themselves may be direct targets of oxidative stress (1, 13, 40, 43, 77, 111, 117, 120). In particular, tyrosine phosphorylation of proteins controlled by PTKs and PTPs plays a key role in the pathogenesis of multiple disorders (63, 77, 93, 135). The regulation of PTPs is thus of vital importance for cell signaling.

The PTP superfamily is encoded by ~100 genes in the human genome, which shares an overall structure with a core catalytic domain composed of four parallel β -strands surrounded by α -helices on both sides (1, 7). The active site sequence Cys(X)5Arg is referred to as "PTP signature motif,"

which connects a center β -strand to an α -helix at the center of the catalytic site. In addition, the diversity of PTPs is conferred by the regulatory domains (1, 13, 77, 85, 104, 117, 135). Furthermore, PTPs can be categorized by the sensitivity to vanadate, the insensitivity to okadaic acid, the ability to hydrolyze the p -nitrophenyl phosphate, and lack of metal ion dependence for catalysis (7, 13). Functionally, PTPs can act both positively and negatively during the regulation of signal transduction through their specific dephosphorylation activity (120).

Based on phosphamino acid specificity, PTPs can be classified into four groups: the classical receptor (RPTPs), the classical nonreceptor PTP (nrPTPs), the dual specificity PTP (dsPTPs), and the low-molecular weight PTPs (low M_r PTPs) (1). Furthermore, RPTPs and nrPTPs fall into several subtypes according to their noncatalytic domain structures (1, 3, 95, 111, 120). RPTPs are predominantly found in the plasma membrane, whereas nrPTPs are localized to a variety of intracellular compartments, including the cytosol, plasma membrane, and endoplasmic reticulum (ER) (Fig. 2).

A series of studies have indicated that the activities of PTPs can be regulated by a variety of mechanisms, including the binding of both agonistic or antagonistic ligands (RPTPs), dimerization followed by autophosphorylation (CD45 and PTP- α), and phosphorylation at tyrosine residues to provide the binding sites for Src-homology 2-containing proteins (PTP- α) (1, 111, 117). Increasing evidence indicated that PTP activities can also be regulated by the redox state within the cell (Fig. 3) (1, 40, 60, 111, 117). The catalytic site of the majority of PTPs contains a cysteine residue with pKa values in the range of 4–6, which makes these sites hypersusceptible to oxidation. It has been shown that a wide range of oxidants could interact with PTP-active sites to regulate the enzymatic activities. The redox regulation of tyrosine phosphatases depends on different oxidative stress conditions and is tissue specific, which frequently occurs within the catalytic site of the enzyme (13, 14).

Oxidation of the catalytic cysteine of PTPs occurs *in vivo* in response to intracellular ROS production, which may be stimulated by a variety of factors, including platelet-derived growth factor, epidermal growth factor, insulin, and several other cytokines and integrins (23, 63, 99, 119). Cysteine residues are also affected by extracellular ROS or by an

FIG. 2. Schematic representation of the protein tyrosine phosphatase (PTP) family. The PTP family is broadly divided into non-transmembrane (nonreceptor, nrPTPs) and receptor-like PTPs (RPTPs), which can be further classified into subfamilies. RPTPs are transmembrane proteins consisting of an extracellular part and the intracellular domains. The extracellular part typically contains domains implicated in cell adhesion. The intracellular part consists of one PTP catalytic domain and, in some subfamilies, a PTP domain with little or no catalytic activity. nrPTPs only have a single PTP catalytically active domain, which is linked to domains that mediate protein-protein interactions, including Src-homology 2 (SH2).

imbalance of the redox potential within the cell (Fig. 3). Therefore, hydrogen peroxide or other radical species may oxidize cysteine residues in proteins to form cysteine sulfenic acid (Cys-SOH), which can be further stabilized by the formation of interior intramolecular disulfide (S-S) or sulfenyl-amide bonds. The last conformation is characterized by a five-member ring that is formed by the covalent linkage of an atom of the catalytic cysteine to amide nitrogen in a neighboring residue.

Most RPTPs contain two intracellular enzymatic domains (D1 and D2). Oxidative stress could inhibit RPTPs by changing the conformation (9, 66, 72). It was shown that H_2O_2

treatment induced a conformational change in the cytoplasmic region that was dependent on a cysteine residue in D2, which enhanced RPTP- α dimerization and inactivated phosphatase activity. Moreover, SHP-1 and SHP-2 activity could be inhibited by oxidative stress generated by ionizing radiation or low H_2O_2 concentrations in a Ca^{2+} -dependent NOS and an S-nitrosylation-dependent manner (9, 66, 72). Further investigation indicated that oxidative stress-induced inhibition on PTP activity is reversible (46, 122, 131). ROS accumulation resulting from cytokine treatment decreases the PTP1B activity by oxidative inactivation, which can be restored by the treatment with NAC, an ROS scavenger (46, 57).

FIG. 3. Reactive oxygen species (ROS) mediate cell signaling to regulate the protein phosphatase in response to the various extracellular stimuli. Cellular ROS can be generated in response to multiple external stimuli, including ionizing radiation, H_2O_2 , growth factors, cytokines, and ligands for G protein-coupled receptor (GPCR). As shown in the diagram, phosphatase may be inhibited by ROS via oxidation of cysteine residues in proteins to form cysteine sulfenic acid (Cys-SOH). Moreover, SHP-1 and SHP-2 activity could be inhibited by oxidative stress generated by ionizing radiation or low H_2O_2 concentrations in a Ca^{2+} -dependent NOS and an S-nitrosylation-dependent manner. ROS levels also regulate the protein phosphorylation status through modulating the kinases and/or phosphatase reversibly.

H₂O₂ treatment also oxidized and inactivated several PTPs *in vitro* (63, 77, 135).

Oxidative Stress Contributes to the FA Cellular and Clinical Phenotype

Early studies by Nordenson and Joenje first reported excess oxygen toxicity in FA cells that accumulated oxidative DNA damage (52, 84). Nordenson revealed that spontaneous chromosomal instability in FA cells was mitigated by superoxide dismutase (SOD) (84). Joenje *et al.* later reported that chromosomal aberrations in FA were oxygen dependent (52). Since then, oxidative stress was found to modulate cell growth (26, 90, 91) and cycle in FA cells (26, 90–92, 105) and linked to FA-associated chromosomal instability (26, 52, 68, 90, 91, 100). Both *in vitro* and *in vivo* evidence indicated that FA cells were in a prooxidant state (1, 8, 11, 17, 18, 20–22, 26, 28, 37, 68, 69, 71–77, 87, 88, 90–92, 100, 103, 134). Exposure to hydrogen peroxide caused extra oxidative damage on both DNA and RNA in FA cells compared with the damage in normal cells (116). Excess luminol-dependent chemiluminescence (LDCL), a direct endpoint of ROS formation, was found in freshly drawn leucocytes from FA patients and, to a lesser yet significant extent, in FA heterozygotes (58). Pagano *et al.* observed that excessive accumulation of white blood cell 8-OHdG correlated both with LDCL and spontaneous chromosomal breaks, as well as increased oxidized glutathione (GSSG)/GSH ratio and plasma methyl-glyoxal levels (89). Altered production of the proinflammatory cytokine, tumor necrosis factor α (TNF- α) may also contribute to the prooxidant state in FA cells. It has been reported that TNF- α could enhance intracellular and extracellular O₂⁻ production and induce DNA breakage and cell death (8, 10, 32, 68, 112, 132). Moreover, in the presence of genotoxic agents, such as radiation or chemotherapeutic drugs, the cytotoxic effects of TNF- α can be potentiated. Overproduction of TNF- α in FA cells

enhances intracellular events such as nuclear factor- κ B (NF- κ B) activation and transcriptional activity, and leads to constitutive activation of multiple signaling pathways, including mitogen-activated protein kinase (MAPK), NF- κ B (two major stress-signaling pathways), and c-Jun NH2-terminal kinase (JNK) pathway (26, 88, 90, 105). Furthermore, TNF- α treatment upregulated the expression of FANCA and FANCG, and increased the FANCA/FANCG complex in the nucleus as well through the NF- κ B pathway (71, 72). Indeed, TNF- α and its interactors have been implicated in FA pathogenesis (26, 88, 90, 105). Several FA gene products have been shown to play roles in redox homeostasis directly or indirectly (Fig. 4) (26, 37, 52, 67, 69, 90–92, 100, 105, 114, 116). Both FANCA and FANCG proteins were found to be directly influenced by the redox state in terms of physical structure (94). In response to the redox state, FANCA and FANCG proteins could form disulfide bonds in the FA protein complex (27, 94). The FANCC protein was reported to bind with NADPH cytochrome-P450 reductase and glutathione S-transferase P1-1 (37, 61). Futaki *et al.* revealed that FANCG interacts with a P450 protein, cytochrome P450 2E1 (CYP2E1) (35). Rani *et al.* reported that deficiency in the *Fanca* gene in mice elicits a p53-dependent growth arrest and DNA damage response to oxidative DNA damage and oncogenic stress (97). FANCD2 was found to interact with the ataxia-telangiectasia mutated protein (ATM) and forkhead box O3 (Foxo3a) in response to oxidative DNA damage (67), which is consistent with the established functions of both ATM and FOXO3a during oxidative stress (67, 121). FANCI was recognized to coincide with the DNA helicase BRCA1-interacting protein 1 (BRIP1) and the transcription factor BRCA1-associated C-terminal helicase (BACH1) and that FANCI/BACH1/BRIP1 is a repressor of heme oxygenase-1 (HO-1) gene and senses oxidative base damage (114).

Furthermore, some FA gene products correlated to oxygen metabolism are also associated with mitochondrial dysfunction. Mukhopadhyay *et al.* observed that FANCG localizes to

FIG. 4. Overall scenario of the relationships of FA proteins with oxidative stress-related interactors. Eight FA proteins form a nuclear complex, which acts as ubiquitin ligase by monoubiquitinating two substrates FANCD2 and FANCI, followed by recruiting other downstream FA proteins, including FANCD1, FANCI, and FANCN. FA gene products involved in redox homeostasis can be summarized as follows: FA proteins and/or FA protein complexes and subcomplexes may interact with or regulate AKT, Cyt450, nuclear factor- κ B (NF- κ B), GST, Cyp2E1; FANCI is a repressor of heme oxygenase (HO)-1 gene and senses oxidative base damage; FANCG interacts directly with peroxiredoxin 3 (PRDX3) in mitochondria; FANCD2 in response to oxidative stress may interact with Foxo3a and ataxia-telangiectasia mutated protein (ATM).

mitochondria and interacts with mitochondrial peroxide, peroxyredoxin 3 (PRDX3) (80). In turn, PRDX3 was deregulated in FANCG-deficient cells, which may due to distorted mitochondrial structures, and mitochondrial extract showed significant decrease in thioredoxin-dependent peroxidase activity. Overexpression of PRDX3 overcomes the sensitivity of FA-G cells to hydrogen peroxide, and a decreased PRDX3 expression leads to hypersensitivity to mitomycin C. FANCA- and FANCC-deficient cells also had PRDX3 cleavage and decreased peroxidase activity (80, 102).

A set of murine FA models also link FA phenotypes to oxidative stress (42, 105, 136, 137). Hadjur *et al.* revealed defective hematopoiesis and hepatic steatosis in *Fancc*^{-/-} *Sod1*^{-/-} mice and the altered redox state was responsible for an impairment of cell proliferation or survival, and attributed to hepatocyte membranes (42). Sejas *et al.* found that *Fancc*^{-/-} mice exhibited excess inflammatory response as a result of hematopoietic suppression. The lipopolysaccharide-mediated hematopoietic suppression was elicited by TNF- α and triggered ROS formation, along with overexpression of the stress kinase p38 (105). Zhang *et al.* observed that long-term antioxidant administration could counteract FA-associated oxidative stress in *Fancc*^{-/-} mice (136, 137). These studies demonstrated that *Fancc*^{-/-} mice with tempol treatment, a nitroxide antioxidant and a SOD mimetic, showed a significant delay in the onset of epithelial tumors; and treatment with resveratrol improved the spleen colony-forming capacity of *Fancc*^{-/-} bone marrow cells.

Altogether, these findings provide both direct and indirect evidence that oxidative stress is at least a component of FA cellular and clinical phenotype (Fig. 5) (5, 11, 17, 20, 26, 54, 68, 69, 88, 90, 94, 97, 129, 130).

Oxidative Stress-Associated Protein Phosphorylation and Dephosphorylation Abnormalities in FA

Oxidative stress due to excessive accumulation of ROS was reported to induce double-stranded RNA-dependent protein kinase (PKR)-dependent apoptosis *via* interferon- γ (IFN- γ) activation signaling (96). Pang *et al.* revealed that FANCC could bind to and facilitate the activation of STAT1 by IFN- γ and hematopoietic growth factors (92). Saadatzaadeh *et al.* revealed that hyperactivation of ASK1 triggered by oxidative stress contributed to the H₂O₂- and TNF- α -induced apoptosis, which was ablated by ASK1-specific siRNA and a dominant negative ASK1 mutant to inhibit the ASK1 kinase activity (103). Further study from the Haneline group indicated that antioxidants or a p38 inhibitor could protect *Fancc*^{-/-} MEFs and c-Kit⁺ cells from TNF- α apoptosis by inhibiting ASK1 activity (8). Altogether, altered redox status may contribute to the apoptosis prone in FA cells by protein phosphorylation dysregulation (8, 92, 96, 103).

More direct evidence to elucidate the tyrosine kinases involved in the oxidative stress response or triggered by extra accumulation of ROS was obtained by human phosphoreceptor tyrosine kinase (RTK) arrays reported by Li *et al.* (Fig. 6) (69). After H₂O₂ treatment, multiple tyrosine kinases, including insulin receptor (IR), insulin-like growth factor 1 receptor (IGF-1R), Janus kinase 2 (JAK2), anaplastic lymphoma kinase (ALK), TET tyrosine kinase, and endothelial (Tie 2), were activated differentially in human FA-C cells compared with the ones in normal human lymphoblastic

FIG. 5. Consequence of FA defects. The FA pathway serves during the DNA damaging sensing/signaling, DNA damage repair, and eliminating ROS by cooperating with various interactors, such as ATM/ATR, Foxo3a/STAT1, separately. Mutations of FA complementation groups lead to the FA phenotypes such as congenital abnormalities, cancer, and bone marrow failure.

cells, suggesting that FA deficiency impairs multiple tyrosine kinase signaling pathways.

Oxidative Stress-Associated Insulin Resistance in FA

One of the clinical characteristics of FA is the metabolic disorder, which is manifested by diabetes and other abnormalities of glucose metabolism (30, 39, 115). A recent clinical investigation showed that abnormalities of glucose homeostasis in FA patients were frequent (up to 81% of FA patients), including hyperglycemia (impaired glucose tolerance or diabetes mellitus) and hyperinsulinemia (30, 39, 115). However, the underlying molecular etiology of the FA diabetes remains unclear. Insulin resistance, a state that precedes many clinical manifestations of the metabolic syndrome by inhibiting the action of insulin, is a key contributor to the pathogenesis of obesity and type 2 diabetes (T2D) mellitus (2, 41). The literature indicated that mutations in the FANCA and FANCC genes among 15 complementation groups have been identified in more than 70% of FA patients worldwide (5, 21, 54, 74, 83, 100). We previously employed two FA knockout mouse models, *Fanca*^{-/-} and *Fancc*^{-/-}, to study the mechanisms contributing to the abnormalities of glucose homeostasis and insulin resistance in FA (Fig. 6) (69). Our results indicated that mice deficient for the *Fanca* or *Fancc* gene seem to be prone to diabetes and obesity, consistent with the clinical complication in FA patients. Specifically, both *Fanca*^{-/-} and *Fancc*^{-/-} mice showed higher fasting blood glucose and insulin resistance, as determined by the standard glucose tolerance test and insulin tolerance test (2, 30, 39, 69, 115).

Insulin signaling is critical for the regulation of glucose levels and the avoidance of diabetes mellitus, whereas defective insulin signaling may cause insulin resistance, leading to multiple diseases, including T2D and obesity (62, 128). The IR is activated through phosphorylation at multiple tyrosine residues of the β -subunit, which then phosphorylates and recruits different substrate adaptors, including members of the insulin receptor substrate (IRS) family and leads to the activation of downstream signaling, such as PI3K/AKT/PKB

FIG. 6. Aberrant signaling triggered by H_2O_2 in FANCC-deficient patient-derived lymphoblastic cell compared with the one in human normal lymphoblastic cell. To identify tyrosine kinases involved in oxidative stress response in FA cells, human phosphoreceptor tyrosine kinase (RTK) arrays were employed to screen 78 different RTKs for differentially activated kinases in response to oxidative stress induced by H_2O_2 treatment in human lymphoblastic cell lines (LCLs) derived from a normal donor (HSC93) (A, B) or a FA patient assigned to the complementation group C (FANCC; HSC536) (C, D). After H_2O_2 treatment, multiple tyrosine kinases, including insulin receptor (IR), insulin-like growth factor 1 receptor (IGF-1R), Janus kinase 2 (JAK2), anaplastic lymphoma kinase (ALK), TET tyrosine kinase, and endothelial (Tie 2), were activated differentially in human FANCC-deficient cells compared with the ones in normal human lymphoblastic cells.

and PKCzeta cascades (12, 40, 124). Our results indicated that FA insulin resistance may have resulted from the reduction of IR tyrosine phosphorylation and an increase in the inhibitory serine phosphorylation of IRS-1 (Fig. 7) (69).

Numerous studies indicated that oxidative stress may contribute to the pathogenesis and clinical complications in FA. The IR signaling pathway is regulated tightly at multiple levels, including receptor phosphorylation and feedback of downstream signals, such as IRS-1, triggered by ROS (12, 44, 65, 82, 126, 127). Our investigation on the role of ROS in FA insulin resistance indicated that high levels of ROS contribute to the FA insulin resistance, which could be restored by the

natural antioxidant quercetin, suggesting potential therapeutic treatment to improve the FA phenotype (69).

FA patients have abnormally high levels of $TNF-\alpha$, which is a major mediator of inflammation and ROS production, leading to persistent PKR activation in FA mice (82, 91, 93, 101, 138). It was known that PKR phosphorylation induced by ROS could inhibit IRS-1 by phosphorylation at inhibitory site Ser312 in human liver cells HepG2 (IRS-1Ser307 in mouse cells) (82, 133). Consistent with the inhibitory effect of PKR on the insulin signaling triggered by oxidative stress, our inhibitor screen identified PKR kinase as one of the two factors that mediate the ROS effect of FA insulin resistance. Furthermore,

FIG. 7. Schematic model for ROS-induced insulin resistance in FA. Overproduction of tumor necrosis factor α ($TNF-\alpha$) leads to the accumulation of ROS and consequently influences two critical steps of IR signaling: decrease IR tyrosine phosphorylation through PTP- α ; increase the inhibitory phosphorylation of insulin receptor substrate-1 (IRS-1) by activation of double-stranded RNA-dependent protein kinase (PKR) kinase, leading to insulin resistance. Inhibition of PTPalpa or PKR failed to restore glucose metabolism. The natural antioxidant Quercetin ameliorates the negative effect of ROS on insulin signaling and maintains metabolic homeostasis.

using the shRNA screen, we identified the tyrosine phosphatase PTP- α as another critical factor that mediates the ROS effect on FA insulin resistance.

The identification of PKR kinase and PTP- α phosphatase that mediates the inhibitory effect of ROS on insulin signaling provides a potential mechanistic link between ROS and insulin resistance in FA. Given, targeting PKR or PTP- α alone failed to restore insulin signaling in FA cells, the amelioration of FA insulin resistance may require simultaneously targeting both molecular events, which can both restore IR tyrosine phosphorylation and suppress inhibitory IRS-1Ser307 phosphorylation. Indeed, the combined inhibition of both PKR and PTP- α rescues FA insulin resistance caused by TNF- α in FANCA knockdown cells (Fig. 7).

Proposal to Target PTP and PKR Kinase to Improve FA Phenotype

FA can cause a wide range of medical problems. There is no cure for this disease so far, however, many medical therapies, procedures, and surgeries can now treat the symptoms and improve the FA phenotype, including blood transfusions, blood and marrow stem cell transplant, androgen therapy, synthetic growth factors, and gene therapy (18, 19, 54, 134).

Scientific implication that oxidative stress may contribute to the pathogenesis and clinical complications in FA provides attractive therapeutic strategies by blocking the production of ROS or enhancing the DNA damage repair ability (70, 136, 137). *In vivo* experiments conducted with *Fancd2*^{-/-} mice revealed that antioxidants administered long-term could delay significantly the onset of epithelial tumors and improve the spleen colony-forming capacity by counteracting FA-associated oxidative stress (136, 137). Quercetin, a naturally occurring flavonoid found in a variety of fruits and green vegetables, has a wide range of biological activity such as free radical scavenging, iron chelating, anti-inflammation, and anticancer (56,

107). We have shown that quercetin effectively antagonized the negative effect of ROS on insulin signaling and maintaining metabolic homeostasis, and thus may be useful for therapies against insulin resistance-associated metabolic syndrome, as demonstrated in the FA disease model.

Redox abnormalities in FA may lead to abnormal activities of kinases and phosphatases, especially tyrosine kinases and phosphatases. Given the important roles of these enzymes in the modulation of cellular proliferation, differentiation, and viability, tyrosine kinases and phosphatases therefore become attractive and promising targets for therapies (Fig. 8) (4, 6, 29, 59, 64, 75, 86, 109). Tyrosine kinases can be inhibited pharmacologically through multiple mechanisms (Fig. 7). The small molecules may function through directly inhibiting the catalytic activity of the kinase by interfering with the binding of ATP or substrates, blocking the dimerization of fusion kinases or preventing the ligand binding (4, 6, 59, 75). The development of suitable inhibitors for PTPs is still under investigating although the emerging oncogenic function of members of the PTP family has led to their consideration as drug targets (29, 64, 86, 109). There are significant technical challenges for the PTP inhibitor design due to high polarity of the active compounds and the specificity of compounds to distinguish the closely related catalytic domains of functionally different PTPs (29, 64, 71, 73, 86, 109), and currently no specific PTP inhibitors have been clinically approved yet (45). Encouragingly, these problems have, in part, been addressed by the use of prodrug strategies and structural information techniques (29, 64, 71, 73, 86, 109).

It has been shown that inhibition of PKR represents an interesting strategy for neuroprotection, and preventing the cell death induced by ER stress in cultured human neuroblastoma cells (16, 36, 48, 49, 51, 106, 139). PKR was constitutively activated in FA-C cells, which contributed to the hypersensitivity of bone marrow progenitor cells to growth repression mediated by the inhibitory cytokines IFN- γ and

FIG. 8. PKR may serve as the therapeutic target in FA. FANCC associates with PKR and HSP70 (a PKR activation inhibitor) exposed to either combination of TNF- α and interferon- γ (IFN- γ) or dsRNA and IFN- γ . Constitutive activation of PKR in FA cells, caused by overproduced cytokines or accumulation of ROS due to FA complementation mutation, contributes to the FA clinical phenotype, including hypersensitivity to IFN- γ /TNF- α , which may be abrogated by PKR inhibition.

TNF- α (69, 92, 105, 138). Inhibition of PKR activation by overexpression of a nonphosphorylated form of eukaryotic translation initiation factor- α reversed PKR-mediated block of messenger RNA translation and partly abrogated PKR-mediated apoptosis in response to IFN- γ , TNF- α , and dsRNA (69, 92, 105, 138). Further investigation indicated that overactivation of PKR may also trigger the inhibitory phosphorylation of IRS-1, which partly contributes to the insulin resistance and abnormal glucose metabolism (69). Therefore, it is reasonable to consider the PKR inhibitor as an interesting therapeutic candidate in FA patients. However, aberrant accumulation of cytokines in FA cells may trigger multiple signaling pathways besides PKR, particularly the bias caused by phosphatases may lead to an insufficient therapeutic benefit (69, 92, 105, 138).

Oxidative stress in FA leads to the aberrant phosphorylation status *via* disrupting the balance between kinases and phosphatases. Targeting phosphatase therefore may also serve as the potential therapeutic target to improve FA phenotype. PTP- α is reported to affect transformation and tumorigenesis, inhibition of proliferation and cell cycle arrest, neuronal differentiation and outgrowth. In FA, PTP- α also modulated insulin signaling through regulating the IR phosphorylation. Thus, PTP- α has promise as a target for drug discovery. However, inhibition of PTP- α alone may also fail to restore the functional insulin signaling, indicating that a combined therapeutic strategy may be an optimal way to improve FA phenotype.

Innovation

Hypersensitivity to oxidative stress has been considered to contribute to the syndrome progression in Fanconi anemia (FA). Recent studies indicated that redox status may cause the aberrant phosphorylation states of cellular proteins *via* regulating the balance between protein kinases and phosphatases, which may contribute to disease development, including FA. Current review summarized the latest understanding on the roles of FA proteins in modulating redox functions and highlighted the phosphorylation state of proteins, including protein tyrosine kinases and protein tyrosine phosphatases in FA cells, and provided a review of the evidence for molecular and clinical involvement of oxidative stress in the FA phenotype.

Acknowledgments

This work was supported, in part, by National Institutes of Health (grants R01 CA109641 and R01 HL076712). Q.P. is supported by a Leukemia & Lymphoma Scholar award.

References

- Alonso A, Sasin J, Bottini N, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, and Mustelin T. Protein tyrosine phosphatases in the human genome. *Cell* 117: 699–711, 2004.
- Andrikopoulos S, Blair AR, Deluca N, Fam BC, and Proietto J. Evaluating the glucose tolerance test in mice. *Am J Physiol Endocrinol Metab* 295: E1323–E1332, 2008.
- Arimura Y and Yagi J. Comprehensive expression profiles of genes for protein tyrosine phosphatases in immune cells. *Sci Signal* 3: rs1, 2010.
- Arora A and Scholar EM. Role of tyrosine kinase inhibitors in cancer therapy. *J Pharmacol Exp Ther* 315: 971–979, 2005.
- Bagby GC, Jr. Genetic basis of Fanconi anemia. *Curr Opin Hematol* 10: 68–76, 2003.
- Baselga J. Targeting tyrosine kinases in cancer: the second wave. *Science* 312: 1175–1178, 2006.
- Bhaduri A and Sowdhamini R. A genome-wide survey of human tyrosine phosphatases. *Protein Eng* 16: 881–888, 2003.
- Bijangi-Vishehsaraei K, Saadatzaheh MR, Werne A, McKenzie KA, Kapur R, Ichijo H, and Haneline LS. Enhanced TNF- α -induced apoptosis in Fanconi anemia type C-deficient cells is dependent on apoptosis signal-regulating kinase 1. *Blood* 106: 4124–4130, 2005.
- Blanchetot C, Tertoolen LG, Overvoorde J, and den Hertog J. Intra- and intermolecular interactions between intracellular domains of receptor protein-tyrosine phosphatases. *J Biol Chem* 277: 47263–47269, 2002.
- Briot D, Mace-Aime G, Subra F, and Rosselli F. Aberrant activation of stress-response pathways leads to TNF- α oversecretion in Fanconi anemia. *Blood* 111: 1913–1923, 2008.
- Ceccaldi R, Parmar K, Mouly E, Delord M, Kim JM, Regairaz M, Pla M, Vasquez N, Zhang QS, Pondarre C, Pefault de Latour R, Gluckman E, Cavazzana-Calvo M, Leblanc T, Larghero J, Grompe M, Socie G, D'Andrea AD, and Soulier J. Bone marrow failure in Fanconi anemia is triggered by an exacerbated p53/p21 DNA damage response that impairs hematopoietic stem and progenitor cells. *Cell Stem Cell* 11: 36–49, 2012.
- Chakraborty A, Koldobskiy MA, Bello NT, Maxwell M, Potter JJ, Juluri KR, Maag D, Kim S, Huang AS, Dailey MJ, Saleh M, Snowman AM, Moran TH, Mezey E, and Snyder SH. Inositol pyrophosphates inhibit Akt signaling, thereby regulating insulin sensitivity and weight gain. *Cell* 143: 897–910, 2010.
- Chiarugi P and Buricchi F. Protein tyrosine phosphorylation and reversible oxidation: two cross-talking post-translation modifications. *Antioxid Redox Signal* 9: 1–24, 2007.
- Chiarugi P and Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci* 28: 509–514, 2003.
- Chico LK, Van Eldik LJ, and Watterson DM. Targeting protein kinases in central nervous system disorders. *Nat Rev Drug Discov* 8: 892–909, 2009.
- Couturier J, Morel M, Pontcharraud R, Gontier V, Faucconneau B, Paccalin M, and Page G. Interaction of double-stranded RNA-dependent protein kinase (PKR) with the death receptor signaling pathway in amyloid beta (A β)-treated cells and in APPSLPS1 knock-in mice. *J Biol Chem* 285: 1272–1282, 2010.
- Cumming RC, Lightfoot J, Beard K, Youssoufian H, O'Brien PJ, and Buchwald M. Fanconi anemia group C protein prevents apoptosis in hematopoietic cells through redox regulation of GSTP1. *Nat Med* 7: 814–820, 2001.
- D'Andrea AD. The Fanconi Anemia/BRCA signaling pathway: disruption in cisplatin-sensitive ovarian cancers. *Cell Cycle* 2: 290–292, 2003.
- D'Andrea AD. Susceptibility pathways in Fanconi's anemia and breast cancer. *N Engl J Med* 362: 1909–1919, 2010.
- D'Andrea AD and Grompe M. The Fanconi anaemia/BRCA pathway. *Nat Rev Cancer* 3: 23–34, 2003.

21. de Winter JP and Joenje H. The genetic and molecular basis of Fanconi anemia. *Mutat Res* 668: 11–19, 2009.
22. Deans AJ and West SC. DNA interstrand crosslink repair and cancer. *Nat Rev Cancer* 11: 467–480, 2011.
23. Demple B and Amabile-Cuevas CF. Redox redux: the control of oxidative stress responses. *Cell* 67: 837–839, 1991.
24. Dolton GM, Sathish JG, and Matthews RJ. Protein tyrosine phosphatases as negative regulators of the immune response. *Biochem Soc Trans* 34: 1041–1045, 2006.
25. Dridi S, Hirano Y, Tarallo V, Kim Y, Fowler BJ, Ambati BK, Bogdanovich S, Chiodo VA, Hauswirth WW, Kugel JF, Goodrich JA, Ponican SL, Hinton DR, Kleinman ME, Baffi JZ, Gelfand BD, and Ambati J. ERK1/2 activation is a therapeutic target in age-related macular degeneration. *Proc Natl Acad Sci U S A* 109: 13781–13786, 2012.
26. Du W, Adam Z, Rani R, Zhang X, and Pang Q. Oxidative stress in Fanconi anemia hematopoiesis and disease progression. *Antioxid Redox Signal* 10: 1909–1921, 2008.
27. Du W, Li J, Sipple J, Chen J, and Pang Q. Cytoplasmic FANCA-FANCC complex interacts and stabilizes the cytoplasm-dislocalized leukemic nucleophosmin protein (NPMc). *J Biol Chem* 285: 37436–37444, 2010.
28. Du W, Rani R, Sipple J, Schick J, Myers KC, Mehta P, Andreassen PR, Davies SM, and Pang Q. The FA pathway counteracts oxidative stress through selective protection of antioxidant defense gene promoters. *Blood* 119: 4142–4151, 2012.
29. Easty D, Gallagher W, and Bennett DC. Protein tyrosine phosphatases, new targets for cancer therapy. *Curr Cancer Drug Targets* 6: 519–532, 2006.
30. Elder DA, D'Alessio DA, Eyal O, Mueller R, Smith FO, Kansra AR, and Rose SR. Abnormalities in glucose tolerance are common in children with fanconi anemia and associated with impaired insulin secretion. *Pediatr Blood Cancer* 51: 256–260, 2008.
31. Evans JL, Goldfine ID, Maddux BA, and Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 23: 599–622, 2002.
32. Fehsel K, Kolb-Bachofen V, and Kolb H. Analysis of TNF alpha-induced DNA strand breaks at the single cell level. *Am J Pathol* 139: 251–254, 1991.
33. Force T, Krause DS, and Van Etten RA. Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition. *Nat Rev Cancer* 7: 332–344, 2007.
34. Force T, Kuida K, Namchuk M, Parang K, and Kyriakis JM. Inhibitors of protein kinase signaling pathways: emerging therapies for cardiovascular disease. *Circulation* 109: 1196–1205, 2004.
35. Futaki M, Igarashi T, Watanabe S, Kajigaya S, Tatsuguchi A, Wang J, and Liu JM. The FANCG Fanconi anemia protein interacts with CYP2E1: possible role in protection against oxidative DNA damage. *Carcinogenesis* 23: 67–72, 2002.
36. Gale MJ, Jr., Korth MJ, and Katze MG. Repression of the PKR protein kinase by the hepatitis C virus NS5A protein: a potential mechanism of interferon resistance. *Clin Diagn Virol* 10: 157–162, 1998.
37. Garcia-Higuera I, Kuang Y, Naf D, Wasik J, and D'Andrea AD. Fanconi anemia proteins FANCA, FANCC, and FANCG/XRCC9 interact in a functional nuclear complex. *Mol Cell Biol* 19: 4866–4873, 1999.
38. Geraldine P and King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circ Res* 106: 1319–1331, 2010.
39. Giri N, Batista DL, Alter BP, and Stratakis CA. Endocrine abnormalities in patients with Fanconi anemia. *J Clin Endocrinol Metab* 92: 2624–2631, 2007.
40. Gual P, Le Marchand-Brustel Y, and Tanti JF. Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. *Biochimie* 87: 99–109, 2005.
41. Guilherme A, Virbasius JV, Puri V, and Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 9: 367–377, 2008.
42. Hadjur S, Ung K, Wadsworth L, Dimmick J, Rajcan-Separovic E, Scott RW, Buchwald M, and Jirik FR. Defective hematopoiesis and hepatic steatosis in mice with combined deficiencies of the genes encoding Fancx and Cu/Zn superoxide dismutase. *Blood* 98: 1003–1011, 2001.
43. Hanks SK and Hunter T. Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *FASEB J* 9: 576–596, 1995.
44. Hayes GR and Lockwood DH. Role of insulin receptor phosphorylation in the insulinomimetic effects of hydrogen peroxide. *Proc Natl Acad Sci U S A* 84: 8115–8119, 1987.
45. Heneberg P. Use of protein tyrosine phosphatase inhibitors as promising targeted therapeutic drugs. *Curr Med Chem* 16: 706–733, 2009.
46. Heneberg P, Draberova L, Bambouskova M, Pompach P, and Draber P. Down-regulation of protein-tyrosine phosphatases activates an immune receptor in the absence of its translocation into lipid rafts. *J Biol Chem* 285: 12787–12802, 2010.
47. Herve JC and Sarrouilhe D. Modulation of junctional communication by phosphorylation: protein phosphatases, the missing link in the chain. *Biol Cell* 94: 423–432, 2002.
48. Ingrand S, Barrier L, Lafay-Chebassier C, Fauconneau B, Page G, and Hugon J. The oxindole/imidazole derivative C16 reduces *in vivo* brain PKR activation. *FEBS Lett* 581: 4473–4478, 2007.
49. Jammi NV, Whitby LR, and Beal PA. Small molecule inhibitors of the RNA-dependent protein kinase. *Biochem Biophys Res Commun* 308: 50–57, 2003.
50. Jayakumar AR, Panickar KS, Murthy Ch R, and Norenberg MD. Oxidative stress and mitogen-activated protein kinase phosphorylation mediate ammonia-induced cell swelling and glutamate uptake inhibition in cultured astrocytes. *J Neurosci* 26: 4774–4784, 2006.
51. Jha BK, Polyakova I, Kessler P, Dong B, Dickerman B, Sen GC, and Silverman RH. Inhibition of RNase L and RNA-dependent protein kinase (PKR) by sunitinib impairs antiviral innate immunity. *J Biol Chem* 286: 26319–26326, 2011.
52. Joenje H, Arwert F, Eriksson AW, de Koning H, and Oostra AB. Oxygen-dependence of chromosomal aberrations in Fanconi's anaemia. *Nature* 290: 142–143, 1981.
53. Kee Y and D'Andrea AD. Expanded roles of the Fanconi anemia pathway in preserving genomic stability. *Genes Dev* 24: 1680–1694, 2010.
54. Kennedy RD and D'Andrea AD. The Fanconi Anemia/BRCA pathway: new faces in the crowd. *Genes Dev* 19: 2925–2940, 2005.
55. Kim Y, Lach FP, Desetty R, Hanenberg H, Auerbach AD, and Smogorzewska A. Mutations of the SLX4 gene in Fanconi anemia. *Nat Genet* 43: 142–146, 2011.
56. Kobori M, Masumoto S, Akimoto Y, and Takahashi Y. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. *Mol Nutr Food Res* 53: 859–868, 2009.

57. Koch T, Seifert A, Wu DF, Rankovic M, Kraus J, Borner C, Brandenburg LO, Schroder H, and Holtt V. mu-opioid receptor-stimulated synthesis of reactive oxygen species is mediated via phospholipase D2. *J Neurochem* 110: 1288–1296, 2009.
58. Korkina LG, Samochatova EV, Maschan AA, Suslova TB, Cheremisina ZP, and Afanas'ev IB. Release of active oxygen radicals by leukocytes of Fanconi anemia patients. *J Leukoc Biol* 52: 357–362, 1992.
59. Krause DS and Van Etten RA. Tyrosine kinases as targets for cancer therapy. *N Engl J Med* 353: 172–187, 2005.
60. Krejsa CM and Schieven GL. Impact of oxidative stress on signal transduction control by phosphotyrosine phosphatases. *Environ Health Perspect* 106 Suppl 5: 1179–1184, 1998.
61. Krut FA, Hoshino T, Liu JM, Joseph P, Jaiswal AK, and Youssoufian H. Abnormal microsomal detoxification implicated in Fanconi anemia group C by interaction of the FAC protein with NADPH cytochrome P450 reductase. *Blood* 92: 3050–3056, 1998.
62. Kubota T, Kubota N, Kumagai H, Yamaguchi S, Kozono H, Takahashi T, Inoue M, Itoh S, Takamoto I, Sasako T, Kumagai K, Kawai T, Hashimoto S, Kobayashi T, Sato M, Tokuyama K, Nishimura S, Tsunoda M, Ide T, Murakami K, Yamazaki T, Ezaki O, Kawamura K, Masuda H, Moroi M, Sugi K, Oike Y, Shimokawa H, Yanagihara N, Tsutsui M, Terauchi Y, Tobe K, Nagai R, Kamata K, Inoue K, Kodama T, Ueki K, and Kadowaki T. Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. *Cell Metab* 13: 294–307, 2011.
63. Kwon J, Lee SR, Yang KS, Ahn Y, Kim YJ, Stadtman ER, and Rhee SG. Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors. *Proc Natl Acad Sci U S A* 101: 16419–16424, 2004.
64. Labbe DP, Hardy S, and Tremblay ML. Protein tyrosine phosphatases in cancer: friends and foes! *Prog Mol Biol Transl Sci* 106: 253–306, 2012.
65. Lammers R, Moller NP, and Ullrich A. The transmembrane protein tyrosine phosphatase alpha dephosphorylates the insulin receptor in intact cells. *FEBS Lett* 404: 37–40, 1997.
66. Leach JK, Black SM, Schmidt-Ullrich RK, and Mikkelsen RB. Activation of constitutive nitric-oxide synthase activity is an early signaling event induced by ionizing radiation. *J Biol Chem* 277: 15400–15406, 2002.
67. Li J, Du W, Maynard S, Andreassen PR, and Pang Q. Oxidative stress-specific interaction between FANCD2 and FOXO3a. *Blood* 115: 1545–1548, 2010.
68. Li J, Sejas DP, Zhang X, Qiu Y, Nattamai KJ, Rani R, Rathbun KR, Geiger H, Williams DA, Bagby GC, and Pang Q. TNF-alpha induces leukemic clonal evolution *ex vivo* in Fanconi anemia group C murine stem cells. *J Clin Invest* 117: 3283–3295, 2007.
69. Li J, Sipple J, Maynard S, Mehta PA, Rose SR, Davies SM, and Pang Q. Fanconi anemia links reactive oxygen species to insulin resistance and obesity. *Antioxid Redox Signal* 17: 1083–1098, 2012.
70. Li X, Sipple J, Pang Q, and Du W. Salidroside stimulates DNA repair enzyme Parp-1 activity in mouse HSC maintenance. *Blood* 119: 4162–4173, 2012.
71. Liu G, Xin Z, Pei Z, Hajduk PJ, Abad-Zapatero C, Hutchins CW, Zhao H, Lubben TH, Ballaron SJ, Haasch DL, Kaszubska W, Rondinone CM, Trevillyan JM, and Jirousek MR. Fragment screening and assembly: a highly efficient approach to a selective and cell active protein tyrosine phosphatase 1B inhibitor. *J Med Chem* 46: 4232–4235, 2003.
72. Lorenz U. SHP-1 and SHP-2 in T cells: two phosphatases functioning at many levels. *Immunol Rev* 228: 342–359, 2009.
73. Lund IK, Andersen HS, Iversen LF, Olsen OH, Moller KB, Pedersen AK, Ge Y, Holsworth DD, Newman MJ, Axe FU, and Moller NP. Structure-based design of selective and potent inhibitors of protein-tyrosine phosphatase beta. *J Biol Chem* 279: 24226–24235, 2004.
74. MacKay C, Declais AC, Lundin C, Agostinho A, Deans AJ, MacArtney TJ, Hofmann K, Gartner A, West SC, Helleday T, Lilley DM, and Rouse J. Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2. *Cell* 142: 65–76, 2010.
75. Madhusudan S and Ganesan TS. Tyrosine kinase inhibitors in cancer therapy. *Clin Biochem* 37: 618–635, 2004.
76. Mangialasche F, Solomon A, Winblad B, Mecocci P, and Kivipelto M. Alzheimer's disease: clinical trials and drug development. *Lancet Neurol* 9: 702–716, 2010.
77. Meng TC, Fukada T, and Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases *in vivo*. *Mol Cell* 9: 387–399, 2002.
78. Moldovan GL and D'Andrea AD. How the fanconi anemia pathway guards the genome. *Annu Rev Genet* 43: 223–249, 2009.
79. Moller DE. New drug targets for type 2 diabetes and the metabolic syndrome. *Nature* 414: 821–827, 2001.
80. Mukhopadhyay SS, Leung KS, Hicks MJ, Hastings PJ, Youssoufian H, and Plon SE. Defective mitochondrial peroxiredoxin-3 results in sensitivity to oxidative stress in Fanconi anemia. *J Cell Biol* 175: 225–235, 2006.
81. Mustelin T, Vang T, and Bottini N. Protein tyrosine phosphatases and the immune response. *Nat Rev Immunol* 5: 43–57, 2005.
82. Nakamura T, Furuhashi M, Li P, Cao H, Tuncman G, Sonnenberg N, Gorgun CZ, and Hotamisligil GS. Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. *Cell* 140: 338–348, 2010.
83. Neveling K, Endt D, Hoehn H, and Schindler D. Genotype-phenotype correlations in Fanconi anemia. *Mutat Res* 668: 73–91, 2009.
84. Nordenson I. Effect of superoxide dismutase and catalase on spontaneously occurring chromosome breaks in patients with Fanconi's anemia. *Hereditas* 86: 147–150, 1977.
85. Ostman A, Frijhoff J, Sandin A, and Bohmer FD. Regulation of protein tyrosine phosphatases by reversible oxidation. *J Biochem* 150: 345–356, 2011.
86. Ostman A, Hellberg C, and Bohmer FD. Protein-tyrosine phosphatases and cancer. *Nat Rev Cancer* 6: 307–320, 2006.
87. Pagano G. Mitomycin C and diepoxybutane action mechanisms and FANCC protein functions: further insights into the role for oxidative stress in Fanconi's anaemia phenotype. *Carcinogenesis* 21: 1067–1068, 2000.
88. Pagano G, Degan P, d'Ischia M, Kelly FJ, Nobili B, Pallardo FV, Youssoufian H, and Zatterale A. Oxidative stress as a multiple effector in Fanconi anaemia clinical phenotype. *Eur J Haematol* 75: 93–100, 2005.
89. Pagano G, Degan P, d'Ischia M, Kelly FJ, Pallardo FV, Zatterale A, Anak SS, Akisik EE, Beneduce G, Calzone R, De Nicola E, Dunster C, Lloret A, Manini P, Nobili B, Saviano A, Vuttariello E, and Warnau M. Gender- and age-related distinctions for the *in vivo* prooxidant state in Fanconi anaemia patients. *Carcinogenesis* 25: 1899–1909, 2004.
90. Pagano G, Talamanca AA, Castello G, Pallardo FV, Zatterale A, and Degan P. Oxidative stress in Fanconi anaemia:

- from cells and molecules towards prospects in clinical management. *Biol Chem* 393: 11–21, 2012.
91. Pang Q and Andreassen PR. Fanconi anemia proteins and endogenous stresses. *Mutat Res* 668: 42–53, 2009.
 92. Pang Q, Fagerlie S, Christianson TA, Keeble W, Faulkner G, Diaz J, Rathbun RK, and Bagby GC. The Fanconi anemia protein FANCC binds to and facilitates the activation of STAT1 by gamma interferon and hematopoietic growth factors. *Mol Cell Biol* 20: 4724–4735, 2000.
 93. Pang Q, Keeble W, Diaz J, Christianson TA, Fagerlie S, Rathbun K, Faulkner GR, O'Dwyer M, and Bagby GC, Jr. Role of double-stranded RNA-dependent protein kinase in mediating hypersensitivity of Fanconi anemia complementation group C cells to interferon gamma, tumor necrosis factor-alpha, and double-stranded RNA. *Blood* 97: 1644–1652, 2001.
 94. Park SJ, Ciccone SL, Beck BD, Hwang B, Freie B, Clapp DW, and Lee SH. Oxidative stress/damage induces multimerization and interaction of Fanconi anemia proteins. *J Biol Chem* 279: 30053–30059, 2004.
 95. Paul S and Lombroso PJ. Receptor and nonreceptor protein tyrosine phosphatases in the nervous system. *Cell Mol Life Sci* 60: 2465–2482, 2003.
 96. Pyo CW, Lee SH, and Choi SY. Oxidative stress induces PKR-dependent apoptosis via IFN-gamma activation signaling in Jurkat T cells. *Biochem Biophys Res Commun* 377: 1001–1006, 2008.
 97. Rani R, Li J, and Pang Q. Differential p53 engagement in response to oxidative and oncogenic stresses in Fanconi anemia mice. *Cancer Res* 68: 9693–9702, 2008.
 98. Rhee SG. Cell signaling. H_2O_2 , a necessary evil for cell signaling. *Science* 312: 1882–1883, 2006.
 99. Rhee SG, Chang TS, Bae YS, Lee SR, and Kang SW. Cellular regulation by hydrogen peroxide. *J Am Soc Nephrol* 14: S211–S215, 2003.
 100. Rosselli F, Briot D, and Pichierri P. The Fanconi anemia pathway and the DNA interstrand cross-links repair. *Biochimie* 85: 1175–1184, 2003.
 101. Rosselli F, Sanceau J, Gluckman E, Wietzerbin J, and Moustacchi E. Abnormal lymphokine production: a novel feature of the genetic disease Fanconi anemia. II. *In vitro* and *in vivo* spontaneous overproduction of tumor necrosis factor alpha. *Blood* 83: 1216–1225, 1994.
 102. Rousset S, Nocentini S, Rouillard D, Baroche C, and Moustacchi E. Mitochondrial alterations in fanconi anemia fibroblasts following ultraviolet A or psoralen photoactivation. *Photochem Photobiol* 75: 159–166, 2002.
 103. Saadatzaheh MR, Bijangi-Vishehsaraei K, Hong P, Bergmann H, and Haneline LS. Oxidant hypersensitivity of Fanconi anemia type C-deficient cells is dependent on a redox-regulated apoptotic pathway. *J Biol Chem* 279: 16805–16812, 2004.
 104. Salmeen A and Barford D. Functions and mechanisms of redox regulation of cysteine-based phosphatases. *Antioxid Redox Signal* 7: 560–577, 2005.
 105. Sejas DP, Rani R, Qiu Y, Zhang X, Fagerlie SR, Nakano H, Williams DA, and Pang Q. Inflammatory reactive oxygen species-mediated hemopoietic suppression in Fancc-deficient mice. *J Immunol* 178: 5277–5287, 2007.
 106. Shimazawa M and Hara H. Inhibitor of double stranded RNA-dependent protein kinase protects against cell damage induced by ER stress. *Neurosci Lett* 409: 192–195, 2006.
 107. Skaper SD, Fabris M, Ferrari V, Dalle Carbonare M, and Leon A. Quercetin protects cutaneous tissue-associated cell types including sensory neurons from oxidative stress induced by glutathione depletion: cooperative effects of ascorbic acid. *Free Radic Biol Med* 22: 669–678, 1997.
 108. Somyajit K, Subramanya S, and Nagaraju G. Distinct roles of FANCO/RAD51C protein in DNA damage signaling and repair: implications for Fanconi anemia and breast cancer susceptibility. *J Biol Chem* 287: 3366–3380, 2012.
 109. Sorio C, Melotti P, D'Arcangelo D, Mendrola J, Calabretta B, Croce CM, and Huebner K. Receptor protein tyrosine phosphatase gamma, Ptp gamma, regulates hematopoietic differentiation. *Blood* 90: 49–57, 1997.
 110. Stoker AW. Receptor tyrosine phosphatases in axon growth and guidance. *Curr Opin Neurobiol* 11: 95–102, 2001.
 111. Stoker AW. Protein tyrosine phosphatases and signalling. *J Endocrinol* 185: 19–33, 2005.
 112. Suematsu N, Tsutsui H, Wen J, Kang D, Ikeuchi M, Ide T, Hayashidani S, Shiomi T, Kubota T, Hamasaki N, and Takeshita A. Oxidative stress mediates tumor necrosis factor-alpha-induced mitochondrial DNA damage and dysfunction in cardiac myocytes. *Circulation* 107: 1418–1423, 2003.
 113. Sun J, Hoshino H, Takaku K, Nakajima O, Muto A, Suzuki H, Tashiro S, Takahashi S, Shibahara S, Alam J, Taketo MM, Yamamoto M, and Igarashi K. Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. *EMBO J* 21: 5216–5224, 2002.
 114. Suzuki H, Tashiro S, Sun J, Doi H, Satomi S, and Igarashi K. Cadmium induces nuclear export of Bach1, a transcriptional repressor of heme oxygenase-1 gene. *J Biol Chem* 278: 49246–49253, 2003.
 115. Swift M, Sholman L, and Gilmour D. Diabetes mellitus and the gene for Fanconi's anemia. *Science* 178: 308–310, 1972.
 116. Takeuchi T and Morimoto K. Increased formation of 8-hydroxydeoxyguanosine, an oxidative DNA damage, in lymphoblasts from Fanconi's anemia patients due to possible catalase deficiency. *Carcinogenesis* 14: 1115–1120, 1993.
 117. Tiganis T and Bennett AM. Protein tyrosine phosphatase function: the substrate perspective. *Biochem J* 402: 1–15, 2007.
 118. Tirosh A, Potashnik R, Bashan N, and Rudich A. Oxidative stress disrupts insulin-induced cellular redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. A putative cellular mechanism for impaired protein kinase B activation and GLUT4 translocation. *J Biol Chem* 274: 10595–10602, 1999.
 119. Tonks NK. Redox redux: revisiting PTPs and the control of cell signaling. *Cell* 121: 667–670, 2005.
 120. Tonks NK. Protein tyrosine phosphatases: from genes, to function, to disease. *Nat Rev Mol Cell Biol* 7: 833–846, 2006.
 121. Tsai WB, Chung YM, Takahashi Y, Xu Z, and Hu MC. Functional interaction between FOXO3a and ATM regulates DNA damage response. *Nat Cell Biol* 10: 460–467, 2008.
 122. Tsou PS, Talia NN, Pinney AJ, Kendzicky A, Piera-Velazquez S, Jimenez SA, Seibold JR, Phillips K, and Koch AE. Effect of oxidative stress on protein tyrosine phosphatase 1B in scleroderma dermal fibroblasts. *Arthritis Rheum* 64: 1978–1989, 2012.
 123. Ubersax JA and Ferrell JE, Jr. Mechanisms of specificity in protein phosphorylation. *Nat Rev Mol Cell Biol* 8: 530–541, 2007.
 124. Um SH, D'Alessio D, and Thomas G. Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1. *Cell Metab* 3: 393–402, 2006.
 125. Vepa S, Scribner WM, and Natarajan V. Activation of protein phosphorylation by oxidants in vascular endothe-

- lial cells: identification of tyrosine phosphorylation of caveolin. *Free Radic Biol Med* 22: 25–35, 1997.
126. Vepa S, Scribner WM, Parinandi NL, English D, Garcia JG, and Natarajan V. Hydrogen peroxide stimulates tyrosine phosphorylation of focal adhesion kinase in vascular endothelial cells. *Am J Physiol* 277: L150–L158, 1999.
 127. Verweij CL and Gringhuis SI. Oxidants and tyrosine phosphorylation: role of acute and chronic oxidative stress in T- and B-lymphocyte signaling. *Antioxid Redox Signal* 4: 543–551, 2002.
 128. Virkamaki A, Ueki K, and Kahn CR. Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. *J Clin Invest* 103: 931–943, 1999.
 129. Wang W. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. *Nat Rev Genet* 8: 735–748, 2007.
 130. Woo HI, Kim HJ, Lee SH, Yoo KH, Koo HH, and Kim SH. Acute myeloid leukemia with complex hypodiploidy and loss of heterozygosity of 17p in a boy with Fanconi anemia. *Ann Clin Lab Sci* 41: 66–70, 2011.
 131. Wright VP, Reiser PJ, and Clanton TL. Redox modulation of global phosphatase activity and protein phosphorylation in intact skeletal muscle. *J Physiol* 587: 5767–5781, 2009.
 132. Yan B, Wang H, Rabbani ZN, Zhao Y, Li W, Yuan Y, Li F, Dewhirst MW, and Li CY. Tumor necrosis factor- α is a potent endogenous mutagen that promotes cellular transformation. *Cancer Res* 66: 11565–11570, 2006.
 133. Yang X, Nath A, Opperman MJ, and Chan C. The double-stranded RNA-dependent protein kinase differentially regulates insulin receptor substrates 1 and 2 in HepG2 cells. *Mol Biol Cell* 21: 3449–3458, 2010.
 134. Yarde DN, Oliveira V, Mathews L, Wang X, Villagra A, Boulware D, Shain KH, Hazlehurst LA, Alsina M, Chen DT, Beg AA, and Dalton WS. Targeting the Fanconi anemia/BRCA pathway circumvents drug resistance in multiple myeloma. *Cancer Res* 69: 9367–9375, 2009.
 135. Yu JS, Chen HC, and Yang SD. Reversible tyrosine phosphorylation/dephosphorylation of proline-directed protein kinase FA/glycogen synthase kinase-3 α in A431 cells. *J Cell Physiol* 171: 95–103, 1997.
 136. Zhang QS, Eaton L, Snyder ER, Houghtaling S, Mitchell JB, Finegold M, Van Waes C, and Grompe M. Tempol protects against oxidative damage and delays epithelial tumor onset in Fanconi anemia mice. *Cancer Res* 68: 1601–1608, 2008.
 137. Zhang QS, Marquez-Loza L, Eaton L, Duncan AW, Goldman DC, Anur P, Watanabe-Smith K, Rathbun RK, Fleming WH, Bagby GC, and Grompe M. *Fancd2*^{-/-} mice have hematopoietic defects that can be partially corrected by resveratrol. *Blood* 116: 5140–5148, 2010.
 138. Zhang X, Li J, Sejas DP, Rathbun KR, Bagby GC, and Pang Q. The Fanconi anemia proteins functionally interact with the protein kinase regulated by RNA (PKR). *J Biol Chem* 279: 43910–43919, 2004.
 139. Zhu PJ, Huang W, Kalikulov D, Yoo JW, Placzek AN, Stoica L, Zhou H, Bell JC, Friedlander MJ, Kmjevic K, Noebels JL, and Costa-Mattioli M. Suppression of PKR promotes network excitability and enhanced cognition by interferon- γ -mediated disinhibition. *Cell* 147: 1384–1396, 2011.

Address correspondence to:

Dr. Jie Li

Division of Neurosurgery

Center for Theoretic and Applied Neuro-Oncology

Moore's Cancer Center

University of California, San Diego

3855 Health Sciences Drive

La Jolla, CA 92093

E-mail: jil015@ucsd.edu

Date of first submission to ARS Central, October 24, 2013; date of acceptance, November 9, 2013.

Abbreviations Used

ALK = anaplastic lymphoma kinase
 ATM = ataxia-telangiectasia mutated protein
 BACH1 = BRCA1-associated C-terminal helicase
 BRIP1 = BRCA1-interacting protein 1
 dsPTP = the dual specificity protein tyrosine phosphatase
 ER = endoplasmic reticulum
 ERK = extracellular signal-regulated kinase
 FA = Fanconi anemia
 FANCA = Fanconi anemia complementation group A
 FANCC = Fanconi anemia complementation group C
 FANCD2 = Fanconi anemia complementation group D2
 FANCG = Fanconi anemia complementation group G
 FANCI = Fanconi anemia complementation group I
 GPCR = G protein-coupled receptor
 HO = heme oxygenase
 IFN- γ = interferon- γ
 IGF-1R = insulin-like growth factor 1 receptor
 IR = insulin receptor
 IRS = insulin receptor substrate
 JAK2 = Janus kinase 2
 JNK = c-Jun NH₂-terminal kinase
 LDCL = luminol-dependent chemiluminescence
 low M_r PTPs = low-molecular weight PTPs
 MAPK = mitogen-activated protein kinase
 NF- κ B = nuclear factor- κ B
 nrPTP = the classical nonreceptor protein tyrosine phosphatase
 PKB = protein kinase B
 PKR = double-stranded RNA-dependent protein kinase
 PRDX3 = peroxiredoxin 3
 PTK = protein tyrosine kinase
 PTP = protein tyrosine phosphatase
 ROS = reactive oxygen species
 RPTP = receptor protein tyrosine phosphatase
 RTK = receptor tyrosine kinase
 SH2 = Src-homology 2
 T2D = type 2 diabetes
 TNF = tumor necrosis factor