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Inflammatory ROS in Fanconi Anemia 
Hematopoiesis and Leukemogenesis 
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1. Introduction

Fanconi anemia (FA) is a genetic disorder characterized by bone marrow failure (BMF), clonal proliferation of hematopoietic stem cells, and transformation to leukemia and other cancers (Ames et al., 1995; Bogliolo et al., 2002; Cohen-Haguenauer et al., 2006; Cumming et al., 2001; Fagerlie et al., 2001; Jonkers et al., 2001; Suematsu et al., 2003). Somatic cell fusion studies show FA is genetically heterogeneous. So far mutations in 15 genes have been identified in FA or FA-like patients (Cohen-Haguenauer et al., 2006; Joenje et al., 1987; Jonkers et al., 2001; Lensch et al., 1999; Stoepker et al., 2011; Yamamoto et al., 2011). The genes encoding the groups A (FANCA), B (FANCB), C (FANCC), D1 (FANCD1/BRCA2), D2 (FANCD2), E (FANCE), F (FANCF), G (FANCG), -I (FANCI/KIAA1794), J (FANCJ/BRIP1), L (FANCL), M (FANCM), N (FANCN/PALB2), O/RAD51C and P/SLX4 proteins have been cloned (de Winter et al., 1998, 2000a, 2000b; Howlett et al., 2002; Joenje et al., 2000; Letitus et al., 2004; Levran et al., 2005; Lo Ten Foe et al., 1996; Meetei et al., 2003, 2004, 2005; Meindl et al., 2010; Reid et al., 2006; Smogorzewska et al., 2007; Somyajit et al., 2010; Strathdee et al., 1992; Timmers et al., 2001; Xia et al., 2006; Yamamoto et al., 2011). The latter two genes are still thought of as tentative as they do not fall within a defined category biologically and the patients carrying these gene mutations are limited. The majority of mutations are found in FANCA, FANCC and FANCG genes in FA patients (Table 1). Recent studies on the biological function of these FA proteins have demonstrated that eight of the FA proteins (namely, FANCA, B, C, E, F, G, L, and M) form a nuclear multiprotein complex (Collins et al., 2005; D’Andrea et al., 2003; de Winter et al., 2000a, 2000b; Howlett et al., 2002; Joenje et al., 2000; Letitus et al., 2004; Levran et al., 2005; Lo Ten Foe et al., 1996; Meetei et al., 2003, 2004, 2005; Meindl et al., 2010; Reid et al., 2006; Smogorzewska et al., 2007; Somyajit et al., 2010; Strathdee et al., 1992; Timmers et al., 2001; Xia et al., 2006; Yamamoto et al., 2011). This complex also interacts with the FAAP100 and FAPP24 proteins, which are also crucial components in the pathway (Ciccia et al., 2007; Horejsi et al., 2009; Collis et al., 2008, Fig 1). FANCM and its interacting proteins, such as FAAP24 and MHF1, MHF2, also play a role in controlling the processing and stabilization of stalled replication forks (Schwab et al., 2010; Luke-Glaser et al., 2010; Singh et al., 2010).
Table 1. Complementation groups and interaction proteins of Fanconi Anemia.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Chromosome Location</th>
<th>Protein Products</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>16q24.3</td>
<td>163 (FAAP95)</td>
<td>FA core complex</td>
</tr>
<tr>
<td>B</td>
<td>Xp22.31</td>
<td>95 (FAAP100)</td>
<td>FA core complex</td>
</tr>
<tr>
<td>C</td>
<td>9q22.3</td>
<td>63 (FAAP152)</td>
<td>FA core complex</td>
</tr>
<tr>
<td>D1</td>
<td>13q12-13</td>
<td>380 (BRCA2)</td>
<td>RAD51 recruitment</td>
</tr>
<tr>
<td>D2</td>
<td>3p25.3</td>
<td>155,162</td>
<td>Involved in DNA repair</td>
</tr>
<tr>
<td>E</td>
<td>6p21-22</td>
<td>60 (FAAP10)</td>
<td>FA core complex</td>
</tr>
<tr>
<td>F</td>
<td>11p15</td>
<td>42 (FAAP10)</td>
<td>FA core complex</td>
</tr>
<tr>
<td>G</td>
<td>9p13</td>
<td>68 (XRCC9)</td>
<td>FA core complex</td>
</tr>
<tr>
<td>I</td>
<td>15q25-16</td>
<td>140 (FANCI/KIAA1794)</td>
<td>Required for maintenance of chromosomal stability</td>
</tr>
<tr>
<td>J</td>
<td>17q22-q24</td>
<td>140 (FANCJ/BACH1/BRIP1)</td>
<td>5'3' DNA helicase/ATPase</td>
</tr>
<tr>
<td>L</td>
<td>2p16.1</td>
<td>43 (FANCL/PHF9/PDG)</td>
<td>FA core complex, FAAP43 ubiquitin ligase</td>
</tr>
<tr>
<td>M</td>
<td>14q21.3</td>
<td>120 (FAAP10)</td>
<td>FA core complex/ATPase/translocase</td>
</tr>
<tr>
<td>N</td>
<td>16p12.1</td>
<td>130 (FANCN/PALB2)</td>
<td>Regulation of BRCA2 location</td>
</tr>
<tr>
<td>O</td>
<td>17q25.1</td>
<td>42 (FANCO/RAD51C)</td>
<td>Involved in HRR of DSB</td>
</tr>
<tr>
<td>P</td>
<td>16p13.3</td>
<td>200 (FANCP/SLX4)</td>
<td>Protect genome stability</td>
</tr>
<tr>
<td>BRCA1</td>
<td>17q21</td>
<td>208 (FAAP10)</td>
<td>E3 ubiquitin ligase</td>
</tr>
<tr>
<td>FAAP100</td>
<td>-</td>
<td>17q25.1</td>
<td>Required for D2 mono-Ub</td>
</tr>
<tr>
<td>FAAP24</td>
<td>-</td>
<td>19q13.11</td>
<td>Required for D2 mono-Ub</td>
</tr>
<tr>
<td>MHF1</td>
<td>-</td>
<td>1p36.22</td>
<td>Interact with FANCM</td>
</tr>
<tr>
<td>MHF2</td>
<td>-</td>
<td>17q25.3</td>
<td>Interact with FANCM</td>
</tr>
</tbody>
</table>

Fig. 1. Function of the FA pathway. Eight FA proteins form a nuclear core complex, which acts as ubiquitin ligase. FANCM interacts with FAAP24, FAAP100 as well as MHF1 and MFH2, resulting in complex chromatin loading and controlling the processing and stabilization of stalled forks, respectively. In response to DNA damage or replication stress, nuclear core complex monoubiquitinates two other FA proteins, FANCD2 and FANCI, which then recruit other downstream FA proteins FANCD1, FANCJ, and FANCN to damaged DNA and involved in DNA repair, cell-cycle control to repair ICL (interstrand crosslink) lesions and to maintain genome stability.

Many studies indicate that FA proteins might play specific roles in hematopoiesis by governing the responses of hematopoietic cells to both genotoxic and cytotoxic stresses. Loss of FA functions causes excessive apoptosis of HSC and progenitor cells (HSC/P) cells leading to BMF in the early stage of FA. As the disease progresses, apoptosis as well as genomic instability impose a selective pressure on FA HSC/P cells and promote the
development of mutant clones, which could be transformed to leukemia (Cumming et al., 1996, 2001; Fagerlie et al., 2001; Haneline et al., 1998, 1999, 2003; Koh et al., 1999; Li X et al., 2004; Li Y et al., 1997; Maciejewski et al., 1995; Nakata et al., 2004; Pang et al., 2001a, 2001b, 2002; Rathbun et al., 1997, 2000; Si et al., 2006; Walsh et al., 1994; Wang et al., 1998; Whitney et al., 1996).

2. FA hematopoiesis

Hematological abnormalities are among the most important clinical features of FA. Children with FA often develop pancytopenia during the first few years of life. Complications of BM failure (BMF) are the major causes of morbidity and mortality of FA, and 80% of FA patients die from BMF (Bagby et al., 2003; Buchwald et al., 1998; Fagerlie et al., 2001; Kutler et al., 2003; Lensch et al., 1999; Liu et al., 2000). In addition, patients with FA have high risk of developing myelodysplasia (MDS) or acute myeloblastic leukemia (AML) (Bagby et al., 2003; Buchwald et al., 1998; D’Andrea et al., 2003; Fagerlie et al., 2001; Kennedy et al., 2005; Tischkowitz et al., 2003). During the BMF-MDS-AML progression, FA patients frequently develop clonal chromosomal abnormalities in the BM HSC/P cells. In fact, secondary occurred clonal cytogenetic abnormalities, such as 3q addition, 5q deletion and monosomy 7, are common in children with FA who have evolved to MDS and AML and non-FA patients with MDS and AML after alkylating agents treatment (Freie et al., 2004; Fridman et al., 2003; Futaki et al., 2002; Giaccia et al., 1998; Lina-Fineman et al., 1995; Rubin et al., West et al., 2000).

Excessive apoptosis and subsequent failure of the HSC compartment led to progressive BMF in FA patients have been documented from in vitro and in vivo studies. However, the molecular etiology of BMF and leukemia in FA remains to be elucidated. Compelling evidence suggest that altered expression of certain growth factors and cytokines, such as reduced expression of interleukin-6 (IL-6) and granulocyte-macrophage colony stimulating factor (GM-CSF) but increased secretion of mitotic inhibitor TNF-α in patient BM cells, may in part be responsible for hematopoietic disease progression in FA (de Cremoux et al., 1996; Dufour et al., 2003; Rosselli et al., 1992; 1994; Schultz et al., 1993; Stark et al., 1993). It is conceivable that these alterations may change the BM microenvironment (for instance, leading to factor deprivation or constant exposure to mitogenic inhibitors) and cause deregulation of cellular homeostasis. It has also been shown that FA BM cells are hypersensitive to a variety of extracellular cytokines, including interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α) (Dufour et al., 2003; Fagerlie et al., 2001; Haneline et al., 1998; Koh et al., 1999; Li X et al., 2004; Li Y et al., 2004; Nakata et al., 2004; Pang et al., 2001a, 2001b, 2002; Rathbun et al., 1997, 2000; Reid et al., 2006; Rosselli et al., 1992; Schultz et al., 1993; Si et al., 2006; Wang et al., 1998; Whitney et al., 1996), which may subsequently lead to cell apoptosis. Indeed, studies of FA patients have demonstrated that BM from FA patients has decreased number of colony-forming progenitors, as well as a reduction in colony size (Doneshbod-Skibba et al., 1980; Gluckman et al., 1989). These data demonstrate defective hematopoiesis in FA (Bagby et al., 2003; Fagerlie et al., 2001; Tischkowitz et al., 2003).

In contrast to FA patients, mouse models deficient for several FA genes, including Fanca, Fancc, Fancd2 and Fancc, do not show spontaneous hematological defects or leukemia development (Cheng et al., 2000; Whitney et al., 1996; Wong & Buchwald, 2002; Yang et al., 2001). Studies in the Fanca and Fancc mouse models show that while blood count and the
number of committed BM progenitors are normal in FA mice as compared to WT mice; however, when subjected to sublethal dose of DNA cross-linking agent mitomycin C (MMC), which does not affect WT mouse cells, to the mutant mice experienced progressive decrease of all peripheral blood parameters, as well as early and committed progenitors, and eventually died within 8 weeks (Chen et al., 1996; Whitney et al., 1996). These results suggest that loss of FA genes in mouse models results in compromised defects in response to environmental insults (Chen et al., 1996; Whitney et al., 1996; Pang et al., 2000; Rathbun et al., 1997; Haneline et al., 1998; Wong & Buchwald, 2002).

Similar to FA-C patients, BM cells from Fancc-/- mice show compromised colony growth capacity following IFN-γ, TNF-α and MIP-1α treatment (Haneline et al., 1998). Literatures suggest that IFN-γ and TNF-α suppress colony growth forming ability of FA mouse BM cells by upregulating other cellular receptors, such as the fas receptor (CD95) (Young et al., 1997). Increase in CD95 expression has been found in CD34+ cells from children with FA as well as the CD34+ fraction of hematopoietic progenitors in Fancc-/- mice, which is associated with increased apoptosis (Cumming et al., 1996; Otsuki et al., 1999). The hypersensitivity of Fancc-/- hematopoietic cells to IFN-γ and TNF-α is also mediated through activation of the RNA-dependent protein kinase (PKR) pathway, which is reported to initiate apoptosis in some instances, as an elevated level of activated PKR was found in Fancc-/- mouse embryonic fibroblasts (Pang et al., 2001, 2002; Zhang et al., 2004). Several groups independently showed compromised hematopoietic engraftment and reconstitution after BM transplantation of FA HSCs (Haneline et al., 2003; Zhang et al., 2007). Deregulation of apoptotic responses in hematopoietic cells may account at least in part for the nearly universal development of BM failure in children with inactivating FA mutations.

3. Inflammation and FA

Inflammation is a biological process orchestrated mainly by myeloid cells and accompanied by infection or phagocytosis (Balwill et al., 2001). Increased oxidative stress in FA patients may be the result of an increased burden of endogenously produced oxidants as well as increased amounts of ROS generated by various inflammatory cytokines. Many studies indicate a correlation between elevated circulating pro-inflammatory cytokines and anemia in patients with leukemia-related BM diseases (Hakim et al., 1993), but direct evidence for the mechanistic link between inflammation and BMF or leukemia is lacking.

There is evidence showing that patients with FA have abnormally high levels of TNF-α (Fagerlie et al., 2001; Fiers et al., 1999; Freie et al., 2003), which is a major mediator of inflammation and ROS production (Liu et al., 2003; Lohrum et al., 1999). Inappropriate induction or activation of TNF-α signaling has been implicated in the pathogenesis of numerous common diseases such as arthritis, heart attacks, and cancer (Ekborn et al., 1990; Jonsson et al., 2005; Mantovani et al., 2002; Marx et al., 2004). It is conceivable that the presence of TNF-α and increased oxidative stress in FA BM may account for profound physiologic changes, including the development of BMF and progression to leukemia.

Similar to TNF-α, IL-1β and IL-6 are also well-known pro-inflammatory cytokines with a wide range of biological activities in immune regulation, hematopoiesis, inflammation and oncogenesis (Ibanez et al., 2009). It has been demonstrated that IL-1β is overexpressed in FA-A patients (de Cremoux et al., 1996). The elevated levels of IL-1β were completely reverted
by complementation of functional FANCA into FA-A lymphocytes. In addition, the constitutive activation of the PI3K-Akt pathway in FA cells upregulates the expression of IL-1β through an NF-κB independent mechanism and this overproduction activates the proliferation of tumour cells (Ibanez et al., 2009). IL-6 is the chief stimulator of the production of most acute phase proteins (Scheller et al., 2011), whereas the other implicated cytokines influence subgroups of acute phase proteins. Recent studies demonstrate the presence of a defect in IL-6 production in FA patients (Coussens et al., 2002; Cumming et al., 1996), suggesting that this cytokine may partly be responsible for pancytopenia associated with BMF, the major clinical feature of FA, in FA patients. In addition, it has been reported that Fancc-/− HSC/P cells had altered growth and apoptosis responses to combinations of stimulatory cytokines, most dramatically in response to a combination of factors that included interleukin-3 (IL-3) and IL-6 (Aubé et al., 2002).

4. FA oxidant hypersensitivity

Even in steady state, hematopoietic cells are exposed to various ROS, which are routinely generated during metabolic or inflammatory process. ROS induce a variety of responses in hematopoietic cells, including cellular proliferation and growth inhibition (Howlett et al., 2002; Ichijo et al., 1997). Like cells from other tissues, hematopoietic cells have developed several mechanisms to prevent the damage induced by oxidative stress. First, antioxidant enzymes, including superoxide dismutases (SODs), catalase, glutathione peroxidases and peroxiredoxins, can directly eliminate ROS. Secondly, other cellular enzymes can function to repair DNA damage induced by ROS in hematopoietic tissues. While FA murine models do not recapitulate some of the major FA clinical manifestations such as BM failure and leukemia, hematopoietic cells from FA knockout mice exhibit extreme oxidant sensitivity. Extensive studies have demonstrated FA oxidant hypersensitivity by using primary and immortalized cell cultures as well as ex vivo materials from patients (Bogliolo et al., 2002; Cohen–Haguenauer et al., 2006; Cumming et al., 1996; Futaki et al., 2002; Hadjur et al., 2001; Kruyt et al., 1998; Pagano et al., 2005; Park et al., 2004; Saadatzadeh et al., 2004). It has also been shown that reoxygenation-generated oxidative stress, which is associated with significant DNA damage and inhibition of colony formation capacity (Ames et al., 1993; Hammond et al., 2003; Chen et al., 2000), induced senescence of bone marrow progenitor cells from Fancc-/− mice compared to their counterparts. While these studies suggest a correlation between oxidative stress and FA disease progression, the mechanism by which oxidative stress influences the function of FA HSC/P cells has not been systematically studied. A number of hypotheses regarding the effect of oxidative stress in FA have been suggested, including the proposal that ROS could damage DNA and inability of FA HSC/P cells to repair such damage would result in exacerbated genomic instability leading to apoptosis and malignant transformation.

Three major FA core complex components, FANCA, FANCC, and FANCG (Bagby et al., 2003; Kennedy et al., 2000; Green et al., 2009), were found to interact with a variety of cellular factors that primarily function in redox-related processes (Table 2), such as FANCC protein interacts with NADPH cytochrome P450 reductase and glutathione S-transferase PI-1 (Cumming et al., 1996; Kruyt et al., 1998), which are involved in either triggering or detoxifying reactive intermediates including ROS. It has also been demonstrated that Fancc-/− mice with deficiency in the anti-oxidative enzyme Cu/Zn superoxide dismutase
demonstrated a defective hematopoiesis (Hadjur et al., 2001). Fancc-/ cells exhibit hyperactivation of ASK1, a serine-threonine kinase that plays an important role in redox apoptotic signaling (Saadatzadeh et al., 2004). Another FA protein, FANCG, interacts with cytochrome P450 2E1, which is associated with the production of reactive oxygen intermediates, and mitochondrial anti-oxidant enzyme peroxiredoxin-3 (Futaki et al., 2002, Mukhopadhyay et al., 2006), which suggested a possible role of FANCG in protection against oxidative DNA damage. Furthermore, FANCA and FANCG interact upon oxidative stress (Park et al., 2004). These findings indicate a crucial role of FA proteins in oxidative stress signaling. We recently found that FANCD2 associated with FOXO3a, a master regulator in response to oxidative stress (Huang et al., 2007; Li et al., 2010; Tsai et al., 2008). While these observations point to the involvement of FA proteins in oxidative stress response, the molecular pathways in which FA proteins function to modulate physiologic oxidative stress have not been defined.

<table>
<thead>
<tr>
<th>FA proteins</th>
<th>Redox-related factors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FANCA</td>
<td>NADPH cytochrome P450 (RED)</td>
<td>Kruyt et al., 1998</td>
</tr>
<tr>
<td></td>
<td>Glutathione S-transferase P1-1 (GSTP1)</td>
<td>Kruyt et al., 2001</td>
</tr>
<tr>
<td></td>
<td>Cu/Zn superoxide dismutase (SOD)</td>
<td>Kruyt et al., 2001</td>
</tr>
<tr>
<td></td>
<td>Apoptosis signal-regulating kinase 1 (ASK1)</td>
<td>Saadatzadeh et al., 2004</td>
</tr>
<tr>
<td>FANCC</td>
<td>Cytochrome P450 2E1 (CYP2E1)</td>
<td>Futaki et al., 2002</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial anti-oxidant enzyme peroxiredoxin-3</td>
<td>Mukhopadhyay et al., 2006</td>
</tr>
<tr>
<td>FANCD2</td>
<td>Forkhead transcription factor FOXO3a</td>
<td>Li et al., 2010</td>
</tr>
</tbody>
</table>

Table 2. Fanconi anemia proteins in redox signaling.

5. Oxidative stress response in FA hematopoietic cells: a FOXO3a connection

Forkhead transcription factors of the FOXO class O including FOXO1, FOXO3a, FOXO4 and FOXO6, are implicated in the regulation of diverse physiologic processes, including cell cycle arrest, apoptosis, DNA repair, stress resistance, and metabolism (Brunet et al., 2004; Huang et al., 2007). It has been established previously that members of the FOXO family are negatively regulated by PKB/c-Akt in response to insulin/IGF signaling, and are involved in regulating cell cycle progression and cell death (Geert et al., 2002; Essers et al., 2004). Among these FOXO proteins, FOXO3a functions as a master regulator of oxidative stress (Huang et al., 2007; Tsai et al., 2008). Several recent studies demonstrate that FOXO3a protects quiescent HSCs from oxidative stress (Tothova et al., 2002, 2007; Miyamoto et al., 2005). Some other studies also indicated that Foxo3a is involved in inflammatory responses, such as inflammatory arthritis, intestinal inflammation, rheumatoid blood and synovial tissue, angiogenesis and postnatal neovascularization etc. (Turrel-Davin et al., 2009; Potente et al., 2005; Jonsson et al., 2005; Walbert et al., 2004).

While strong evidence indicates that FA cells, including hematopoietic cells from FA patients, are intolerant to oxidative stress (Cohen-Haguenauer et al., 2006; Cumming et al., 2001; Du et al., 2008; Futaki et al., 2002; Hadjur et al., 2001; Kruyt et al., 1998; Paganno et al.,
2005; Park et al., 2004; Saadatzadeh et al., 2004; Schindler et al., 1988; Zhang et al., 2005) and certain FA proteins interact with cellular factors involved in redox metabolism (Aggarwal et al., 2003; Ames et al., 1995; Bagby et al., 2003), the molecular pathways in which FA proteins function to modulate physiologic oxidative stress have not been defined. Our recent identification of the FANCD2-FOXO3a complex (Li et al., 2010) and preliminary characterization of impaired anti-oxidant defense in primary BM cells from FA patients opened new research opportunities to extend the functional study on the roles of FA proteins in the context of oxidative stress. We envision a model (Fig 2) in which the FA proteins regulate oxidative stress response through mechanisms involving functional interplay with the major oxidative stress-responsive transcription factor FOXO3a and protection of anti-oxidant genes from oxidative damage. Loss of these FA protein functions leads to elevated levels of ROS. As a consequence, FA HSC/P cells accumulate excessive DNA damage and increased genomic instability. However, further studies remains to be done in this context.

Fig. 2. A model for the role of FA proteins in oxidative stress signaling. In WT cells, the FA pathway helps keep cellular levels of ROS in check through functional interaction with the FOXO3a oxidative stress responsive pathway and safeguarding cellular anti-oxidant genes. In FA cells, both the FOXO3a pathway and the anti-oxidant defense are impaired due to loss of the FA protein functions. As a result, FA cells accumulate high levels of ROS, which damages DNA leading to genomic instability.

6. The FA syndrome links inflammatory ROS to leukemogenesis

Certain chronic inflammatory conditions have long been known to link to cancer. There is compelling evidence that chronic inflammation increases the risk of human cancers such as hepatocellular carcinoma, colon and bladder cancers, B cell lymphomas, and visceral malignancies (Kuper et al., 2000; Mackay et al., 2001; Martin et al., 2011; Suematsu et al., 2003; Umeda et al., 2002; Ziech et al., 2010), probably through the unbalanced machinery between DNA damage and repair (Fig. 3.).
Fig. 3. Possible mechanisms for induction of oxidative stress and DNA damage and the roles in carcinogenesis. Intracellular stress or exogenous insults induces ROS production, which damages DNA, lipids and proteins. Over-produced ROS leads to cell death and activates cell defense machinery, including DNA repair and other cellular signaling pathways to maintain genome stability. Insufficient DNA repair or apoptosis causes mutagenesis, which results in cancer development.

Oxidative stress is considered as an important pathogenic factor in leukemia-prone bone marrow diseases like FA (Bogliolo et al., 2002; Cohen–Haguenauer et al., 2006; Cumming et al., 1996; Futaki et al., 2002; Hadjur et al., 2001; Joenje et al., 1987; Kruyt et al., 1998; Mukhopadhyay et al., 2006; Pagano et al., 2005; Park et al., 2004; Saadatzadeh et al., 2004; Schindler et al., 1988; Zhang et al., 2005a, 2005b). The expression of inflammatory mediators, particularly the pro-inflammatory cytokines TNF-α, interleukin-1beta (IL-1β), and IL-6 in these patients is often associated with increased production of ROS either as a component of their immune response or as a consequence of increased metabolism (Macciò et al., 1998; Mantovani et al., 1997; Mantovani et al., 2002; Tischkowitz et al., 2004). Many studies have shown a correlation between elevated circulating pro-inflammatory cytokines and anemia in patients with leukemia-related BM diseases but direct evidence for the mechanistic link between inflammation and leukemia is lacking.

Normal hematopoiesis is maintained by dynamic interactions between HSCs and the bone marrow microenvironment, which is a complex system consisting of a variety of cell types, including stromal cells of nonhematopoietic, mesenchymal origin as well as hematopoietically derived stromal macrophages producing extracellular matrix components and hematopoietic growth factors (Bhatia et al., 1995; Konopleva & Michael, 2007; Marina et
Alterations of pro-inflammatory cytokine expression such as reduced IL-6 and increased TNF-α, which are often found in FA patient cells, may account for BM microenvironment changes such as growth factor deprivation or constant exposure to mitogenic inhibitors. These alterations may subsequently cause deregulation of cellular homeostasis in FA (de Cremoux et al., 1996; Dufour et al., 2003; Rosselli et al., 1992, 1994; Schultz et al., 1993; Stark et al., 1993) at least partially through upregulation of ROS production.

ROS induce a variety of responses in HSCs, including cellular proliferation and apoptosis (Nakamura et al., 1997; Nakata et al., 2004). ROS can also cause DNA damage and drive HSCs into cell division, which is essential for DNA repair processes (Wilson A et al., 2008). There is strong evidence that HSCs are activated and thus functionally exhausted by oxidative stress. Mice with mutations in the ATM or FOXO genes, as well as various DNA repair genes exhibit premature exhaustion of HSCs due to accumulation of ROS or DNA damage, indicating that cellular balance between ROS and antioxidant defense as well as DNA repair is crucial for the maintenance of HSC self-renewal and hematopoietic function (Rossi et al., 2007; Nijnik et al., 2007).

The inflammatory cytokine TNF-α, which is overproduced in FA patients, has been considered as one important pathological factor involved in the abnormal hematopoiesis in FA. Extensive evidence demonstrated that excessive apoptosis of FA hematopoietic cells induced by TNF-α, may contribute to at least partially the pathophysiology of BM failure in FA. The c-JUN NH2-terminal kinase (JNK) and nuclear factor-kappa B (NF-kB) pathways are two well-established pathway involved in TNF-α-induced ROS production (Nakata et al., 2004; Ma et al., 2009; Ventura et al., 2004). The JNK kinase can be activated by TNF-α-induced ROS. This activation then in turn leads to more ROS production, and sustained JNK activation in NF-kB-deficient cells was suggested to depend on ROS. It has been shown that TNF-α-induced ROS production at inflammatory sites causes DNA damage and therefore cause mutation and cancer (Aggarwal et al., 2003; Kryston et al., 2011; Martin et al., 2011 Sedelnikova et al., 2010; Suematsu et al., 2003; Wajant et al., 2003; Ziech et al., 2010). One possible mechanism is through Oxidation of bases and generation of DNA strand interruptions. However, the accurate measurement of oxidative stress is a hallmark of disease diagnosis as well as treatment. Recently, HPLC associated with tandem mass spectrometry (MS/MS) or electrochemical detector (ECD) together with optimized DNA extraction conditions has been developed as a relevant analytical approach for measuring oxidatively base damage in cellular DNA (Cadet et al., 2006, 2010). Our recent studies demonstrated the inflammatory ROS-mediated hematopoietic suppression and increased chromosomal aberrations in Fancc-/- mice, which is associated with impaired oxidative DNA-damage repair, implicating a role of FA pathway in maintaining genomic stability (Sejas et al., 2007; Zhang et al., 2007). Further studies indicated that TNF-α not only is a pro-apoptotic signal suppressing FA hematopoietic progenitor activity, but also promotes leukemic transformation of FA hematopoietic stem/progenitor cells (Li et al., 2007). Therefore, FA disease progression to leukemia is governed not only by genetic changes intrinsic to the FA cells, but also by epigenetic and environmental factors and that TNF-α-mediated inflammation is one of the most important epigenetic and environmental factors contributing to FA leukemogenesis. Recent study indicate that FA hematopoietic cells are prone to clonal hematopoiesis and malignancy, which is associated with increased
Cytogenetic abnormalities and myeloid malignancies in Fancc-/ BM cells (Haneline et al., 1998, 1999, 2003; Li X et al., 2004; Si et al., 2006). While the role of FA proteins in the regulation of TNF-α-induced ROS production remains to be elucidated, several hypotheses have been proposed, including that FA proteins protect chromosomal DNA from ROS attack or facilitate the repair of oxidative DNA damage, which in turn downstream ROS signaling. It is also possible that FA proteins can regulate the biosynthesis of ROS metabolic molecules, such as glutathione and the expression of antioxidant enzymes (such as glutathione S-transferases and catalase). However, there is no direct evidence for any of these assumptions so far. Another potential target is the redox-sensitive transcription factor NF-κB, a major player involved in transcription regulating during differentiation and inflammation (Dhar et al., 2006). The activation of NF-κB is known to enhance inflammation and promote cancer (Coussens et al., 2002; Fiers et al., 1999; Macdougal et al., 2002). In addition, chronic exposure of FA BM cells to proinflammatory cytokine TNF-α creates an environment selects for somatically mutated preleukemic stem cell clones which are apoptosis-resistant and acquire proliferative advantage (Li et al., 2007). Patients with these TNF-α-resistant BM cells may advance to MDS and AML via a mechanism involving genomic instability, coupled with inflammation driven by high NF-κB transcriptional activity (Fig. 4).

Fig. 4. The pro-inflammatory cytokines and their potential role in FA pathophysiology. Overproduced pro-inflammatory cytokines (TNF-α, IL-6, IL-1β etc.) plays roles in not only pro-apoptotic signal suppressing FA hematopoietic progenitor activity, but also promoting leukemic transformation of FA HSC/P cells, which lead to typical phenotype of FA patients.
7. Functional interaction between the FA proteins and other oxidative stress response pathways

Recent findings of a reduction of the HSC pool and a deficient repopulating capacity in Foxo3a knockout animals (Miyamoto et al., 2007) indicate that FOXO3a plays essential regulatory roles in HSC maintenance through a mechanism of regulating ROS. This is consistent with our recent finding that FANCD2 forms complex with FOXO3a in response to oxidative stress (Li et al., 2010). In addition, we observed several hematopoietic defects in FA mice deficient for Foxo3a (unpublished data). These results suggest that the FA proteins functionally interplay with other oxidative stress response pathways. Indeed, our preliminary results with primary BM cells from FA-A patients show that certain genes functioning in anti-oxidant defense and ROS metabolism fail to respond to oxidative stress (unpublished data). This suggests that one critical function of FA proteins under oxidative stress is to safeguard the expression of these anti-oxidant defense genes through DNA damage repair or gene promoter protection. While these observations indicate that the FA pathway functionally interacts with other cellular oxidative stress response pathways, the molecular mechanisms by which FA proteins function to modulate physiologic oxidative stress remain to be elucidated. Further investigation into the roles of FA proteins in oxidative DNA-damage response and repair, and the functional relationship between inflammatory ROS and genomic instability during FA leukemogenesis not only will advance our understanding of the function of FA proteins in hematopoiesis but also may suggest new targets for therapeutic prevention and treatment of BM failure and cancer progression of the disease.

8. Conclusion

Given other known genomic instability syndromes such as ataxia telangiectasia, Nijmegen breakage syndrome, xeroderma pigmentosum, and Werner syndrome rarely develop BM failure and leukemia, FA has been considered an excellent disease model for studying oxidative stress response in cancer development. Further investigation into the function of FA proteins in oxidative damage response and repair will help shed new light on the role of FA proteins in the maintenance of normal hematopoiesis under conditions of oxidative stress, and yield valuable information on whether targeting components of FA-related oxidative stress signaling pathways may be therapeutically useful in the prevention and treatment of FA BMF and leukemia. In addition, while FA is a rare disease, understanding functional interaction between FA proteins and other critical oxidative stress signaling pathways provides a unique opportunity to mechanistically comprehend and potentially intervene in these physiologically important processes.

9. References


Inflammatory ROS in Fanconi Anemia Hematopoiesis and Leukemogenesis


This unique synthesis of chapters from top experts in their fields targets the unique and significant area of cancer prevention for different types of cancers. Perspective readers are invited to go through novel ideas and current developments in the field of molecular mechanisms for cancer prevention, epidemiological studies, antioxidant therapies and diets, as well as clinical aspects and new advances in prognosis and avoidance of cancer. The primary target audience for the book includes PhD students, researchers, biologists, medical doctors and professionals who are interested in mechanistic studies on cancer prevention and translational benefits for optimized cancer treatment.

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