Oxidative stress in Fanconi anaemia: from cells and molecules towards prospects in clinical management

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Abstract

Fanconi anaemia (FA) is a genetic disease featuring bone marrow failure, proneness to malignancies, and chromosomal instability. A line of studies has related FA to oxidative stress (OS). This review attempts to evaluate the evidence for FA-associated redox abnormalities in the literature from 1981 to 2010. Among 2170 journal articles on FA evaluated, 162 related FA with OS. Early studies reported excess oxygen toxicity in FA cells that accumulated oxidative DNA damage. Prooxidant states were found in white blood cells and body fluids from FA patients as excess luminol-dependent chemiluminescence, 8-hydroxy-deoxyguanosine, reduced glutathione/oxidized glutathione imbalance, and tumour necrosis factor-α. Some FA gene products involved in redox homeostasis can be summarized as follows: (a) FANCA, FANCC, and FANCG interact with cytochrome P450-related activities and/or respond to oxidative damage; (b) FANCD2 in OS response interacts with forkhead box O3 and ataxia telangiectasia mutated protein; (c) FANCG is found in mitochondria and interacts with PRDX3, and FA-G cells display distorted mitochondria and decreased peroxidase activity; (d) FANCJ (BACH1/BRIP1) is a repressor of haeme oxygenase-1 gene and senses oxidative base damage; (e) antioxidants, such as tempol and resveratrol decrease cancer incidence and haematopoietic defects in Fancd2−/− mice. The overall evidence for FA-associated OS may suggest designing chemoprevention studies aimed at delaying the onset of OS-related clinical complications.

Keywords: bone marrow failure; cancer-prone diseases; chemoprevention; chromosomal instability; DNA damage; oxidative stress.

Introduction

Fanconi anaemia (FA) is a rare genetic disease characterized by bone marrow failure leading to pancytopenia, excess leukaemia, and cancer risk, as well as a broad array of clinical complications and malformations (Auerbach, 2009; Green and Kupfer, 2009). A major cellular defect in FA is chromosomal instability along with excess sensitivity to clastogens, such as mitomycin C (MMC) and 1,2:3,4-diepoxybutane (DEB). FA is caused by mutations in 15 known genes (FANCA through FANCP) (Kee and D’Andrea, 2010; Somyajit et al., 2010; Kim et al., 2011), and the functions of FA genes are mostly attributed to a DNA repair signalling pathway (the FANC pathway), required for protecting the genome from DNA interstrand crosslinks (ICL) (Wang, 2007; Kee and D’Andrea, 2010). Altogether, the functions of FA proteins are currently related to ICL repair in response to the induction of DNA damage exerted by a number of exogenous agents.

Another line of studies has been pursued, based on the scenario that FA phenotype should be related to the onset of redox imbalances and that oxidative stress (OS) is a major feature in FA’s cellular defects and clinical pathogenesis. This line of studies dates back to the early reports by Nordenson (1977) who found that spontaneous chromosomal instability in FA cells was mitigated by superoxide dismutase (SOD), and by Joenje et al. (1981) who reported on excess oxygen toxicity in FA cells. Following a MedLine search that retrieved FA literature published from 1981 to 2010, journal articles were evaluated according to their relationships between FA and OS. A total of 162 OS-related FA papers were found out of a total of 2170 FA papers, with a steadily growing trend of independent reports in the 1981–2010 interval (data not shown).

Thirty years after those early investigations, a body of literature has been accumulated linking OS to FA that prompts a unifying re-appraisal by interfacing the as yet scattered information, namely pointing to in vitro, in vivo, and molecular evidence. Thus, an effort is here attempted in drawing an integrated scenario linking information from early studies to the updated investigations on redox-related functions of several FA-associated (FANC) proteins.
‘Crosslinker sensitivity’ and redox biotransformation

The commonly used definition of FA defects refers to a ‘hypersensitivity of FA cells to interstrand DNA crosslinking agents’ (D’Andrea, 2010). On a merely logical basis, one might wonder about nature’s aim at building up an evolutionary conserved gene set, targeted at defending genome stability against other threats rather than human-mane ‘crosslinkers’. The obvious answer may be that post-Cambrian aerobic biota are all faced with the generalized damages exerted by atmospheric oxygen, the second most reactive non-metallic element after fluorine (Saltzman et al., 2011). Thus, one might reason that the roles of FANC proteins in DNA repair should be at least in part also related to (a) coping with the primigenial oxygen toxicity and (b) disposing of excess endogenous and/or of exogenous molecules by means of oxygen-based redox pathways, as in the case of the P450 enzyme family.

By considering crosslinker toxicity, a long-lasting line of studies has demonstrated, since the early report by Szybalski and Iyer (1964), that the toxicities of several FA-related xenobiotics, i.e., MMC, cisplatin, melphalan, 8-methoxypsoralen+UVA, and cyclophosphamide (CP), are associated with redox-related mechanisms, including biotransformation, redox coupling, glutathione metabolism, and/or induction of oxidative DNA damage (Pritsos and Sartorelli, 1986; Dusre et al., 1990; Clarke et al., 1997; Penketh et al., 2001; Schaaf et al., 2002). It should be recalled that an early study by Joenje and Oostra (1986) found that FA cells, unlike control cells, were sensitive to CP without microsomal metabolic activation. One might infer from that report that FA cells were endowed with the activities effecting CP biotransformation to its active metabolites. In turn, the activities of the cytochrome P450 (CYP) enzyme family in xenobiotic detoxification, and in generating mutagenic metabolites (e.g., of CP), are recognizably related to redox mechanisms (Barouki and Morel, 2001). A different toxicity mechanism is displayed by DEB, suggesting that its activity is due to its potent crosslinking effects and alkylating properties. These stem from the peculiar 1,3-diepoxidation pattern of DEB (Vlachodimitropoulos et al., 1997) in which the ring tension accounts for the marked reactivity towards nucleophiles, and the double epoxide ring allows for the formation of ICLs with many DNA sequences. A substantial body of evidence suggests that individual sensitivity to the genotoxic and cytotoxic effects of DEB depend on oxygen levels and result in a dose-related reduced glutathione (GSH) depletion (Boogaard and Bond, 1996). Two glutathione-related activities, glutathione S-transferase (GST) and GSH peroxidase (GPx), have been implicated in the detoxification of DEB-induced DNA damage (Spanò et al., 1998; Erexson and Tindall, 2000). In addition to conjugation with GSH, a possible transformation route of DEB is hydrolysis (Boogaard and Bond, 1996). A comparative screening of the microsomal hydrolysis of DEB using tissues of rats, mice, and humans was found to give two main metabolites – erythritol and anhydroerythritol. It is tempting to speculate that diol and polyol products of DEB hydrolysis, though expectedly inactive per se, may be sources of cytotoxic aldehydes (e.g., glyoxal) following oxidation and retroaldol reactions under OS conditions (Boogaard and Bond, 1996). Both DEB and MMC exposures were found to induce concentration-related DNA oxidative damage [8-hydroxydeoxyguanosine (8-OHdG)] in sea urchin embryos (Pagano et al., 2001).

This body of literature testifies that the redox-related toxicity mechanisms of these xenobiotics should be related to anomalies, in FA cells, to properly cope with the reactive – and mutagenic – products of drug biotransformation. Altogether, it may be argued that the current term ‘crosslinker sensitivity’ in fact disregards the toxicity mechanisms of FA-specific xenobiotics that are premutagens requiring redox-related bioactivation (Szybalski and Iyer, 1964; Pritsos and Sartorelli, 1986; Dusre et al., 1990; Clarke et al., 1997; Penketh et al., 2001; Schaaf et al., 2002).

Prooxidant state in FA: evidence from cellular and in vivo studies

After the early discovery of oxygen toxicity to FA cells (Joenje et al., 1981), oxygen levels were found to modulate cell growth and cycle in FA fibroblasts, pointing to excess oxygen toxicity with accumulation in S and G2/M phases compared to control cells (Schindler and Hoehn, 1988). Exposure of FA lymphoblastoid cells to hydrogen peroxide resulted in excess oxidative damage both of DNA and RNA (Takeuchi and Morimoto, 1993) that was suggested to relate to a catalase deficiency. Excess luminol-dependent chemiluminescence (LDCL), a direct end point of reactive oxygen species (ROS) formation, was found in freshly drawn leukocytes from FA patients and, to a lesser yet significant extent, in FA heterozygotes (Korkina et al., 1992).

Excess tumour necrosis factor-α (TNF-α) was found by Schulz and Shahidi (1994) in bone marrow plasma and peripheral blood plasma from patients with aplastic anaemia. That report opened the way to a series of investigations ascertaining a major role for TNF-α and other cytokines in FA cells (Castello et al., 1998; Pang et al., 2001; Dufour et al., 2003; Sejas et al., 2007; Lecourt et al., 2010) through multiple interactions with several effectors, including, among others, mitogen-activated protein kinase, nuclear factor-κB (NF-κB) (two major stress-signalling pathways), interleukin-6, and the c-Jun NH2-terminal kinase pathway, which is constitutively activated in FA-A cells (Briot et al., 2008; Ibáñez et al., 2009; Saadatzadeh et al., 2009). The interactions between TNF-α and its receptors (TNF-ARI, TNF-ARII) enhance intracellular events as nuclear factor-κB (NF-κB) activation and transcriptional activity that are enhanced in FA cells compared to normal cells (Ibáñez et al., 2009). Moreover, TNF-α treatment induces FANCA and FANCG through NF-κB (Macé et al., 2007). In turn, TNF-α and its interactors are recognized to be involved in a proinflammatory/prooxidant condition, thus their roles in FA phenotype point, per se, to the relevance of redox imbalances in FA pathogenesis (Obrador et al., 1998; Lindholm et al., 2010).
We reported on two studies of multiple OS end points measured in blood cells and body fluids from FA patients and their parents and siblings (Degan et al., 1995; Pagano et al., 2004). The results showed excess accumulation of white blood cell (WBC) 8-OHdG that correlated both with LDCL and spontaneous chromosomal breaks and, to a lesser extent than WBC 8-OHdG, with urinary 8-OHdG (Pagano et al., 2004). Moreover, increased oxidized glutathione (GSSG)/GSH ratio and plasma methyl-lyxol levels were observed. This multiparameter prooxidant state was detected in untransplanted and, to a lesser extent, in transplanted FA patients, suggesting an association of prooxidant state with FA bone marrow being removed by pretransplant conditioning (Pagano et al., 2004). Another relevant finding of those investigations pointed to age and gender modulation of OS parameters, consistent with a role for puberty and steroid hormone status in redox balance (Elhadd et al., 1998).

Moreover, the immunological assessment in FA patients showed excess T-cell numbers and immunoglobulin levels, a reduced natural killer (NK) cell number with intrinsic defect in cell activity, and an even lower absolute number of B cells with decreased percentage of CD5- and CD10-expressing B cells. This particular B-cell phenotype, not expected in children but in advanced age, may be due to a premature senescence affecting B cells in FA children (Myers et al., 2011).

Beyond the direct information about an in vivo prooxidant state, it should be recalled that FA phenotype is characterized by some clinical features, or complications that find recognized mechanistic explanations on the grounds of redox imbalances, namely type 2 diabetes mellitus (Golubnitschaja et al., 2006; Pácal et al., 2011), a set of malformations (Wells et al., 2009), and cutaneous maculae (café-au-lait spots) (Novellino et al., 1999) that could be hardly explained according to an exclusive theorem of DNA repair deficiency, as shown in Figure 1 (reviewed in Pagano et al., 2005a). In the case of type 2 diabetes mellitus, a prooxidant state and the ensuing oxidative DNA damage were reported to result in the induction of DNA repair systems through the expression of both p21WAF1/CIP1 and p53 response in mice, reported that deficiency in the Fanca gene in mice elicits a p53-dependent growth arrest and DNA damage response to oxidative DNA; OS induces p53 response in Fanca−/− cells, likely due to accumulation of unrepaired DNA damage (Rani et al., 2008).

Other studies reported that FANC proteins and p53 cooperate in apoptosis and cell cycle checkpoint control following DNA damage. Some reports suggested that the activation of p53 leads to an increase in ROS that, by possibly interfering with mitochondrial function and/or integrity, contributes to cell death. In addition, the higher ROS levels appear to be part of the feedback loop that stabilizes p53 resulting in more p53 activity (Du et al., 2008).

### Redox-related functions of FANC proteins

The FANCC protein was found to be associated with redox-related activities, namely NADPH cytochrome P450 reductase (Kruyt et al., 1998) and GST (Cumming et al., 2001). Another report identified 69 proteins as direct interactors of FANCA, FANCC, or FANCG that were associated with transcription regulation, signalling, oxidative metabolism, and intracellular transport (Reuter et al., 2003). Zanier et al. (2004) found a differential expression pattern among FANCC mutated and corrected cells; by using oligonucleotide microarrays, an anti-inflammatory role for the FA proteins was detected, whereas no gene coding for a protein directly involved in DNA repair or DNA damage response was found to be deregulated (Zanier et al., 2004).

The FANCG protein interacts with a P450 protein, cytochrome P450 2E1 (CYP2E1) (Futaki et al., 2002), an activity also known to be involved in redox biotransformation of xenobiotics including, e.g., MMC (Szybulski and Iyer, 1964; Pritsos and Sartorelli, 1986; Dusre et al., 1990; Clarke et al., 1997; Penketh et al., 2001). The FANCA and FANCG proteins were found to respond to redox state in terms of physical structure related to their ability to form disulphide bonds in the FA protein complex; thus, FANCA and FANCG appear to be directly influenced by redox state (Park et al., 2004).

Rani et al. (2008), using Fanca−/− Trp53−/− double knockout mice, reported that deficiency in the Fanca gene in mice elicits a p53-dependent growth arrest and DNA damage response to oxidative DNA; OS induces p53 response in Fanca−/− cells, likely due to accumulation of unrepaired DNA damage (Rani et al., 2008).

### FANC, FANCA-, and FANCC-associated mitochondrial dysfunction

Direct evidence for mitochondrial dysfunction in FA cells was provided by Mukhopadhyay et al. (2006), who reported that the FANC protein is found in mitochondria, and interacts with the mitochondrial peroxidase, peroxiredoxin 3 (PRDX3). In turn, PRDX3 was found to be deregulated in FA-G cells, which displayed distorted mitochondrial structures; moreover, mitochondrial extracts had a significant decrease in thioredoxin-dependent peroxidase activity. Overexpression of PRDX3 suppressed the sensitivity of FA-G cells to H2O2, and a decreased PRDX3 expression increased sensitivity to MMC. Cells from the FA-A and FA-C subtypes also had PRDX3 cleavage and decreased peroxidase activity.
Previous reports had provided evidence for mitochondrial dysfunctions in FA cells (Bogliolo et al., 2002; Rousset et al., 2002). An oxygen-dependent sensitivity of mitochondria was suggested by Rousset et al. (2002), who reported that FA-A fibroblasts underwent mitochondrial matrix densification, unlike control fibroblasts, following 8-methoxypsoralen photoreaction or ultraviolet A irradiation. Mitochondrial function was challenged in FANCA- and FANCC-defective lymphoblastoid cells with rhodamine-1,2,3 and doxycycline (Bogliolo et al., 2002). FA cells, unlike control cells, were not affected by these treatments, which result in acute adenosine triphosphate (ATP) depletion and in a significant enhancement of the fraction of cells undergoing apoptotic cell death. On the other hand, FA cells were very sensitive to 2-deoxy-D-glucose and iodoacetic acid, two inhibitors of the glycolytic metabolism. These findings suggested that FA cells have adapted to withstand mitochondrial stress while relying on glycolytic metabolism (Bogliolo et al., 2002).

Due to the well-established links between mitochondrial functions and oxygen metabolism (Lambert and Brand, 2009), the evidence for mitochondrial dysfunctions in FA-G, FA-A, and FA-C cells represents a crucial standpoint in assessing the fundamental relevance of OS in FA phenotype.

Together, FANCC, FANCQ, and FANCA have shown multifaceted implications with redox-related activities, excess sensitivity to redox state, and mitochondrial dysfunction, as shown in Figure 2 (Szybalski and Iyer, 1964; Prittos and Sartorelli, 1986; Dusre et al., 1990; Clarke et al., 1997; Kruyt et al., 1998; Cumming et al., 2001; Penketh et al., 2001; Bogliolo et al., 2002; Futaki et al., 2002; Rouset et al., 2002; Mukhopadhyay et al., 2006; Du et al., 2008; Rani et al., 2008).

**FANCD2 and FANCJ: multiple identities and redox-related roles**

The FANCD2 protein features a unique role in the FA/BRCA pathway, due to its interactions in activating the FA core complex, in cooperation with FANCI and following monoubiquitination (Thompson and Hinz, 2009). Among several interactors, ataxia telangiectasia mutated protein (ATM) and forkhead box O3 (FOXO3a) were reported to exert OS-specific coordinated actions with FANCD2 (Li et al., 2010; Castillo et al., 2011), consistent with the established functions of both ATM and FOXO3a in response to oxidative DNA damage (Watters, 2003; Yalcin et al., 2008; Huang and Tindall, 2011).

Mono-ubiquitinated FANCD2 was found to be sensitive to DNA damage caused by H$_2$O$_2$, despite an inability to form FANCD2 foci (Willers et al., 2008). The MMC hypersensitivity, but not the H$_2$O$_2$ hypersensitivity, of FANCD2-deficient fibroblasts was associated with an abrogation of RAD51 foci formation, suggesting different cellular responses to these two agents (Willers et al., 2008).

The FANCJ protein was recognized to coincide with two previously known BRCA1-interacting entities, the DNA helicase BRIP1 (BRCA1-interacting protein 1) (Levitus et al., 2005; Levran et al., 2005) and the transcription factor BACH1 (BRCA1-associated C-terminal helicase) (Litman et al., 2005; Cantor and Andreassen, 2006). This protein, currently noted as FANCJ/BACH1/BRIP1, displays functional interaction with BRCA1 in breast and ovary cancer suppression and DNA repair (Cantor et al., 2001, 2004; Xie et al., 2010). FANCJ/BACH1/BRIP1 features a helicase activity and an ATPase activity, also termed ATPase motor (Cantor et al., 2001, 2004), a two-fold activity that is recognized for...
other RECQ helicases that are associated with ATPase activity (Monnat, 2010). Deficiencies in other RECQ helicases cause Bloom syndrome (BS), Werner syndrome (WS), Rothmund-Thomson syndrome (RTS) (Mackintosh and Raney, 2006), and recently the Warsaw breakage syndrome has been attributed to mutations in the helicase DDX11, sharing sequence similarity with FANCI and XPD genes (van der Lelij et al., 2010). Most of these genomic instability syndromes – BS, WS, and RTS – have been related to OS (Pagano et al., 2005b; Szekely et al., 2005; Woo et al., 2006; Zatterale et al., 2007).

Moreover, it may be worth noting that ATPases are variously involved in OS-related mechanisms (Salvemini and Cuzzocrea, 2002; Squier and Bigelow, 2005); thus, it might be suggested that the ATPase component of RECQ helicases plays a direct role in redox-active functions of these DNA repair proteins. This interpretation has been discussed previously for WS (Pagano et al., 2005a; Szekely et al., 2005), and should be elucidated in further investigations. Moreover, one might reason that ATPase activity, hence ATP hydrolysis, is linked by definition to the availability of ATP that is the major product of mitochondrial function. Thus, an FA-associated mitochondrial dysfunction might not be confined to the present knowledge of mitochondrial defects in FA-G, FA-A, and FA-C cells (Bogliolo et al., 2002; Rousseau et al., 2002; Mukhopadhyay et al., 2006); should these defects be found in FA-J cells, one might consider the FANCI/BACH1/BRIP1 deficiency as – at least partly – related to suboptimal ATP levels. This currently open question warrants ad hoc investigations.

Before the recognition of its identity with FANCJ, BACH1 was termed as a transcription repressor and found to regulate redox-related activities, as haeme oxygenase-1 (HO-1) while, in turn, increased levels of haeme resulted in BACH1 inactivation (Kitamura et al., 2003; Reichard et al., 2007; Okada et al., 2010). Moreover, BACH1 was reported to form a p53-containing complex, and was regulated by OS and haeme; thus, BACH1 was related to oxygen metabolism as a negative regulator of p53 (Dohi et al., 2008). Knockdown of BACH1 increased HO-1 by 135-fold but induced the other genes examined to a maximum of only 2.7-fold, while failure to increase glutathione levels or resistance to xenobiotics (MacLeod et al., 2009).

BACH1 was found to exert negative regulation of the antioxidant response element (ARE)-mediated NAD(P)H:quinone oxidoreductase 1 gene expression and induction (Dhakshinamoorthy et al., 2005). ARE-mediated regulation of genes encoding detoxifying/chemopreventive proteins was controlled by Nrf2 and BACH1 factors in response to antioxidants (Dhakshinamoorthy et al., 2005). Recombinant FANCI-A349P protein was reported to have reduced iron and was defective in coupling ATP hydrolysis and pointed to the ability of FANCJ to use the energy from ATP hydrolysis to produce the force required to unwind DNA or destabilize protein bound to DNA is required for its role in DNA repair (Wu et al., 2010).

Suhasini et al. (2009) found that FANCI/BACH1/BRIP1 was inhibited by the oxidative DNA damage thymine glycol in the helicase substrate. In the presence of replication protein A (RPA), FANCI/BACH1/BRIP1 efficiently unwound the DNA substrate harbouring the thymine glycol damage in the non-translocating strand; however, inhibition of FANCI/BACH1/BRIP1 helicase activity by the translocating strand thymine glycol was not relieved. Strand-specific stimulation of human RECQ1 helicase activity was also observed, and RPA bound with high affinity to single-stranded DNA containing a single thymine glycol. The unique ability of FANCI/BACH1/BRIP1 to sense a single thymine glycol oxidative base lesion in either strand of the unwinding duplex and its strand-specific stimulation by RPA to unwind past the lesion may be relevant to a role of the helicase during DNA replication or transcription in conditions of enhanced OS (Suhasini et al., 2009).

Together, as shown in Figure 3, a body of evidence supports a key role for redox-related mechanisms in the functions of the FANCI/BACH1/BRIP1 protein, and further mechanistic links are provided by background information towards deeper insights into the functions of this unique, redox-related FA protein.

### Animal studies linking FA phenotype to OS

A set of murine models for FA are available, including Fanca, Fancc, Fanec, Faneg, Faned2, Fanco-Fanec double, and Fancl knockout mice (Taniguchi and D’Andrea, 2006). Hadjur et al. (2001) found defective haematopoiesis and hepatic steatosis in double knockout mice with Fancc<sup>−/−</sup> and Sod1<sup>−/−</sup>. The authors suggested that the altered redox state likely present in Fancc<sup>−/−</sup>Sod1<sup>−/−</sup> haematopoietic progenitors was responsible for an impairment of cell proliferation or survival, and attributed liver steatosis possibly as a result of OS-induced injury to hepatocyte membranes (Hadjur et al., 2001).

Further investigations utilized FA mouse models. Sejas et al. (2007) found that Fancc<sup>−/−</sup> mice underwent excess inflammatory response as a result of haematopoietic suppression that was corrected by wild-type Fancc gene, suggesting a potential role of the FANCC protein in innate immunity. The lipopolysaccharide-mediated haematopoietic suppression was elicited by TNF-α and triggered ROS formation, along with overexpression of the stress kinase p38 (Sejas et al., 2007).

![Figure 3](image-url)
possibly analogous vs. the above-mentioned intrinsic defect of NK cells in FA children (Macé et al., 2007).

Two recent reports by Zhang et al. (2008, 2010) have provided evidence for counteracting FA-associated OS in Fancd2−/− mice by means of long-term antioxidant administration. Fancd2−/− knockout mice were administered with tempol, a nitroxide antioxidant and a SOD mimetic, resulting in a significant delay in the onset of epithelial tumours (Zhang et al., 2008). A subsequent study of the same group investigated the effects of resveratrol supplementation in Fancd2−/− mice, which were characterized by a significant decrease in haematopoietic stem cell repopulation and spleen colony-forming capacity (Zhang et al., 2010). Treatment with resveratrol improved the marrow microenvironment, partially corrected the abnormal cell cycle status, and significantly improved the spleen colony-forming capacity of Fancd2−/− bone marrow cells (Zhang et al., 2010).

While recognizing the definite relevance of these investigations, one might raise some reservations about the choice of the antioxidants chosen by Zhang et al. (2008, 2010). To our best knowledge, tempol is not recognized as a drug for human use, and scarce clinical trials are currently confined to topic applications (http://clinicaltrials.gov/). In the case of resveratrol, its antioxidant and SIRT1-mimetic properties are well known and several clinical trials are on-going; nevertheless, its bioavailability following oral ingestion is scanty and currently subjected to ad hoc investigations (Subramanian et al., 2010). Thus, one might wonder whether the reports by Zhang et al., though relevant, might be more convincing if more appropriate antioxidants, approved for human use or promptly bioavailable by dietary administration, were utilized (see below).

A long way towards chemoprevention protocols in counteracting FA clinical course

Multifaceted knowledge has been generated that provides evidence for organismal, cellular and molecular redox abnormalities in FA, as summarized in Figures 2 and 3. Directly related to oxygen metabolism, mitochondrial dysfunction found in FA cells appears to provide evidence for the occurrence of a prooxidant state in at least three FA genetic subtypes, G, A, and C (Bogliolo et al., 2002; Rousset et al., 2002; Mukhopadhyay et al., 2006). A major indicator of mitochondrial dysfunction consists of PRDX3 abnormalities in FA cells (Mukhopadhyay et al., 2006), and further investigations on PRDX3 expression and regulation are required to verify the role of its activity – or possible deficiency – in other complementation groups, and in FA clinical phenotype.

Mitochondrial abnormalities and dysfunctions are directly related to the occurrence of OS that, in turn, has widespread implications for many pathological conditions, including a set of cancer-prone and/or ageing-related genetic diseases (reviewed in Pallardó et al., 2010).

A challenging re-appraisal of FA pathogenesis may take advantage from the basic and clinical experience in a set of diseases collectively termed mitochondrial cytopathies (Tarnopolsky, 2008). The pre-existing clinical experience in managing patients with mitochondrial cytopathies by means of combination nutraceuticals (‘mitochondrial cocktail’) may provide useful background in planning animal experiments, and in view of forthcoming clinical trials aimed at counteracting prooxidant state and disease progression (Tarnopolsky, 2008).

For a rare disease like FA, insuperable obstacles to clinical chemoprevention trials may currently be faced due to the scanty numbers of patients susceptible to be recruited, jeopardizing the best efforts in drawing adequate conclusions from too small scale clinical studies. As a possible means for overcoming the restraint imposed by the scarcity of human patients, and on ethical grounds, studies should be designed by utilising knockout mice from different FA complementation groups, at least Fancd2 and, possibly, Fanca−/−, Fancc−/−, and/or Fancg−/−.

In all cases, whether in the design of animal studies or in the prospect of clinical trials, end points should not be confined to changes in lifespan or in some pathological outcomes, such as cancer incidence, or disease-specific complications, such as haematopoietic impairment. Rather, a defined set of OS parameters should be measured, by verifying any time-related alterations vs. initial values, as a prerequisite for implementing any antioxidant treatment. Thereafter, any changes in the in vivo prooxidant states should be monitored at defined time intervals in blood, urine, and in the relevant target tissues, e.g., bone marrow in Fancd2−/− vs. wild-type mice. Thus, the efficacy – if any – of the tested antioxidants in counteracting the initial prooxidant state should allow us to combine the data of biochemical compensation vs. clinical improvement, or a delay in the onset of pathological events, such as malignancies or haematopoietic failure.

A last, yet major issue in study design should rely on the choice of the best appropriate antioxidants. These should be selected in view of the baseline redox imbalances, and of the biochemical targets to be achieved, e.g., glutathione balance. The choice of candidate antioxidants to be used in either animal studies or, even more so, in clinical trials should be targeted to molecules that have been already approved for safe human use with minimal or no adverse effects. Among a number of potential candidate molecules, a few can be identified based on the current knowledge, namely two multifunctional ‘mitochondrial nutrients’ – α-lipoic acid (ALA) and coenzyme Q10 (coQ10) (Tarnopolsky, 2008; Poh and Goh, 2009; Golbidi and Laher, 2010; Hassani et al., 2010; Villalba et al., 2010) – that are well known for their involvement in mitochondrial functions and in maintaining redox balance. Both ALA and coQ10 are endogenous physiologic antioxidants and should be regarded, together and better in combination, as the safest and most effective candidates in the design of chemoprevention protocols both in animal studies and in the prospect of forthcoming pilot studies in FA patients.

This rationale might disclose realistic avenues towards the approval and implementation of chemoprevention studies, with the ultimate goal of mitigating or delaying FA’s clinical progression.
Summary and historical remarks

The early papers around 1980, by Nordenson (1977) and by Joenje et al. (1981), linking FA-associated chromosomal instability to oxygen toxicity were issued in a season of growing interest towards OS as a pathogenetic factor in some disorders (Kahane et al., 1978; Emerit, 1980) and for its role in mutagenesis (Parshad et al., 1980).

In the same years, the reports by Auerbach and Wolman (1976) and by Ishida and Buchwald (1982) placed the major focus of FA defect on excess sensitivity to crosslinking agents, opening the way to the recognized view relating FA defect to crosslinker sensitivity. At that time, a thriving literature was available on the roles of mixed function oxidases in redox bioactivation of xenobiotics (Szybalski and Iyer, 1964; Bartsch et al., 1976; Pegg, 1980). Nevertheless, the crosslinker sensitivity was not related to redox activities in those early studies, nor it seems to be integrated within the mechanistic frame of FA defect after the subsequent investigations that demonstrated the *sine qua non* requirement of redox bioactivation of FA-related xenobiotics (Joenje and Oostra, 1986; Pritsos and Sartorelli, 1986; Dusre et al., 1990; Boogaard and Bond, 1996; Clarke et al., 1997; Vlachodimitropoulos et al., 1997; Spanò et al., 1998; Erexson and Tindall, 2000; Barouki and Morel, 2001; Penketh et al., 2001; Schaff et al., 2002). Thirty years after those early reports, prevailing research efforts have been devoted within the frame of the theory attributing FA defects to the inability to repair DNA ICLs, and convincing evidence supports the view that FA cells are unable to respond to DNA damage.

In the same three decades, a number of independent investigations provided *in vivo*, *in vitro*, and molecular evidence pointing to major roles of OS in FA phenotype (Schindler and Hoehn, 1988; Korkina et al., 1992; Takeuchi and Morimoto, 1993; Schultz and Shahidi, 1994; Degan et al., 1995; Pagano et al., 2004), as well as in the functions of some FANC proteins (Kruyt et al., 1998; Cumming et al., 2001; Futaki et al., 2002; Reuter et al., 2003; Park et al., 2004; Zanier et al., 2004; Du et al., 2008; Rani et al., 2008), with direct implications of some FANC proteins in mitochondrial machinery (Bogliolo et al., 2002; Rouset et al., 2002; Mukhopadhyay et al., 2006).

The present review of this multifaceted, yet self-consistent literature may suggest further investigations aimed at elucidating the relationships of OS in FA, possibly prompting the rationale for adequate actions in chemoprevention of FA clinical progression.

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References


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Giovanni Pagano, ScD, has served as a research worker at the Italian National Cancer Institute in Naples, Italy, active in studies of environmental toxicology. His interest in Fanconi Anaemia (FA) dates back to 1988, when he learned that his son was affected by this disease. Thereafter, he was a founder of the Italian Association for Fanconi Anaemia Research and has been active in FA research, by highlighting the links between FA and oxidative stress.

Anmarita Aiello Talamanca, ScD, is graduated in Pharmacy and majored in clinical biochemistry and molecular biology. She trained at clinical immunology department in National Cancer Institute (Pascale Foundation, Naples) and is fellow at Mercogliano Oncology Research Center (CROM), from 2008 at today. Research Interests: correlation between chronic inflammation and cancer, oxidative stress, cancer microenvironment, hepatocellular cancer, immunometabolism (arginine metabolism) and pharmacogenetic.

Giuseppe Castello, MD, PhD, is the Operative Director of the Cancer Research Centre at Mercogliano (CROM), Italian National Cancer Institute “G. Pascale Foundation” (INCI), Naples, Italy. He is specialized in Allergy and Immunology and in Oncology, with training at the Department of Microbiology and Immunology of the New York Medical College, Val-halla, New York. He served since 1977–2010 at the INCI as Director of the Departments of Immunology, Medical Therapy, Onco-Hematology, and as Scientific Director. He was awarded the title of Emeritus Head Physician at the INCI in 2010. Author of 11 books, 400 papers, 7 patents. Recently, he investigates the relationships between chronic inflammation and cancer.

Federico V. Pallardó, MD, PhD, was trained at the University of Oxford (Metabolic Research Laboratory, UK), in 1989, Harvard Medical School (Boston, USA) and in the Chicago Medical School (North Chicago, Ill., USA). Former Secretary of the SFRR (Europe). Research areas: Regulation of cell cycle and apoptosis by oxidative stress and in the physiopathology of oxidative stress in rare diseases with especial interest on Fanconi Anemia.

Adriana Zatterale, MD, PhD, Master in Medical Genetics and in Clinical Pathology, is the head of the Genetics Dept - ASL Napoli 1 - Naples, Italy, a Section of the Public Health Service providing genetic counselling and chromosomal/molecular analysis, with specific competence in postnatal and cancer cytogenetics and chromosomal instability. The Service is today seat of the “National Fanconi’s Anaemia Register” and one of the main nation-wide reference centre in the field of Chromosomal Instability Syndromes.

Paolo Degan, ScD, had his degree in Biochemistry at the University of Pisa. In 1986 was fellow at the International Agency for Research on Cancer (IARC, Lyon, France) and, in 1990, at the Molecular and Cellular Biology Department at the University of California in Berkeley (CA, USA). He then settled at the National Cancer Institute (IST) in Genova, Italy. Here he pursue the studies on the relationships between oxidative stress, DNA damage in different cancer prone diseases and in cellular models.