Review article

Re-definition and supporting evidence toward Fanconi Anemia as a mitochondrial disease: Prospects for new design in clinical management

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ABSTRACT

Fanconi anemia (FA) has been investigated since early studies based on two definitions, namely defective DNA repair and proinflammatory condition. The former definition has built up the grounds for FA diagnosis as excess sensitivity of patients' cells to xenobiotics as diepoxybutane and mitomycin C, resulting in typical chromosomal abnormalities. Another line of studies has related FA phenotype to a prooxidant state, as detected by both *in vitro* and *ex vivo* studies. The discovery that the FA group G (FANCG) protein is found in mitochondria (Mukhopadhyay et al., 2006) has been followed by an extensive line of studies providing evidence for multiple links between other FA gene products and mitochondrial dysfunction. The fact that FA proteins are encoded by nuclear, not mitochondrial DNA does not prevent these proteins to hamper mitochondrial function, as it is recognized that most mitochondrial proteins are of nuclear origin. This body of evidence supporting a central role of mitochondrial dysfunction, along with redox imbalance in FA, should lead to the re-definition of FA as a mitochondrial disease. A body of literature has demonstrated the beneficial effects of mitochondrial cofactors, such as α-lipoic acid, coenzyme Q10, and carnitine on patients affected by mitochondrial diseases. Altogether, this re-definition of FA as a mitochondrial disease and the prospect use of mitochondrial nutrients may open new gateways toward mitoprotective strategies for FA patients. These strategies are expected to mitigate the mitochondrial dysfunction and prooxidant state in FA patients, and potentially protect transplanted FA patients from post-transplantation malignancies.

1. Introduction

Fanconi Anemia (FA) is a devastating genetic disease characterized by progressive bone marrow failure, birth defects, and predisposition to different types of cancer. At present, the only treatment is hematopoietic stem-cell transplantation (HSCT) and, in a near future, gene therapy. It is an inherited disease associated with 22 defective genes, all – except FANCB and FANCR – recognized by an autosomal recessive inheritance pattern. Early studies in 1970's [1,2] reported on the sensitivity of FA cells to a number of xenobiotics, such as mitomycin C (MMC) or diepoxybutane (DEB). These agents are known to exert a number of toxic effects, including chromosomal abnormalities, such as crosslinks, which

led to the diagnostic procedure of exposing suspected FA cell cultures to DEB (DEB test) [2–4]. This theorem of FA-associated crosslinker sensitivity was defined as the so-called "canonical" definition of FA [4]. Thus, FA is classified as a chromosomal instability disease. In fact, the "Fanconi anemia pathway" has been described as a series of defective DNA repair processes based on the activity of various gene products resulting in the FA phenotype and playing important roles in cancer proneness, which is one of the main features of the disease. Malfunction of this DNA repair pathway has been reported to play also an important role in many primary cancers. It should be noted, however, that both MMC and DEB toxicities are associated with redox mechanisms, as reported since 1980's [5–8] in a body of literature using multiple test systems [9–11],

pointing to redox cycling and apoptotic pathways in MMC- and DEB-related toxicity mechanisms. Thus, it can be argued that the "canonical" FA definition fails to relate the crosslinker sensitivity of FA cells to the established redox-related toxicity mechanisms of crosslinkers.

Another line of studies, also dating back to the 1970's, evaluated the role of redox mechanisms in FA pathogenesis, starting with the pioneering report by Nordenson (1977) [12], who found protective roles of superoxide dismutase and catalase on spontaneous chromosome breaks in cells from FA patients. Joenje and Oostra (1983) [13] evaluated the effects of oxygen tension on chromosomal aberrations of FA cells, and Korkina et al. (1992) [14] found an increased release of reactive oxygen species (ROS) from the FA cells, also reporting on the protective effect of rutin (a ROS scavenger and chelator). Takeuchi and Morimoto (1993) [15] found increased formation of 8-hydroxy-deoxyguanosine (8-OH-dG) in lymphoblasts from FA patients, which was attributed by the authors to catalase deficiency.

Thus, already in the 1990's an early body of evidence supported the view that FA pathogenesis relies on redox-dependent mechanisms. An early *in vivo* support to this view reported that freshly drawn lymphocytes from FA patients displayed the accumulation of 8-OH-dG in DNA and luminol-dependent chemiluminesce [16]. Subsequently, we found that *ex vivo* lymphocytes of FA patients displayed a set of increased prooxidant endpoints, including 8-OH-dG, plasma methylglyoxal, and oxidized:reduced glutathione [17]. A subsequent study confirmed those data in FA patients, compared to patients affected by other oxidative stress (OS)-related genetic diseases [18]. The overall state-of-art from the early studies of the redox mechanisms of several FA gene products (FANCA, FANCC, FANCG and others) led us to a consensus paper in 2003 from a EU-funded project providing rationale for the key-role of oxidative stress in FA pathogenesis [19].

2. Mitochondria as protagonists in FA-associated redox defects

In 2006, Mukhopadhyay et al. reported on the mitochondrial localization of FA group G (FANCG) protein [20]. The authors found that FANCG interacts with the mitochondrial peroxidase peroxiredoxin-3 (PRDX3), which was deregulated in FA-G cells, displaying distorted mitochondrial structures, along with a dramatic decrease in thioredoxin-dependent peroxidase activity. A transient overexpression of PRDX3 was found to suppress the sensitivity of FA-G cells to H_2O_2,

and decreased PRDX3 expression was found to increase MMC sensitivity. That report prompted a series of studies of the association between mitochondrial defects and a number of FA gene products, as summarized in Table 1.

After that pioneering study [20], a series of reports provided a body of literature, which may be summarized as follows:

1) Defective mitochondrial functions [21–33];
2) RNA: altered gene regulation [34];
3) Decreased intracellular calcium concentration [25];
4) Decreased clearance of damaged fibroblast mitochondria and mitochondrial ROS [26];
5) High frequency of mtDNA mutations; downregulation of mtDNA complex-I and complex-III encoding genes [32].

Altogether, an established body of literature points to the key-roles of mitochondrial dysfunction in FA pathogenesis, which is by definition complementary to the pre-existing evidence for a prooxidant condition in FA cells as well as in *ex vivo* cells and plasma from FA patients. Thus, the scientific and clinical community is faced with the task of preserving the so-called "non-canonical" view of this disease [35]. As recently suggested by Milletti et al. (2020), FA genes play basic roles in non-canonical pathways, such as mitochondria homeostasis, inflammation, and virophagy, which act, in some cases, independently of DNA repair processes [36].

Despite the fact that ROS are the main cause of these processes, for the same organ where different sets of mitochondrial substrates are present, specific mechanisms may be different, warranting *ad hoc* investigations.

3. Mitochondrial diseases: current state-of-art

Mitochondrial diseases (MDs) are known as a group of disorders that relate to several clinical features and that share a number of mitochondrial dysfunctions (MDFs). They are typically associated with defects in mitochondrial activities, as assessed in an extensive – and growing – body of literature [37–44]. The genetic basis of MDs is both related to nuclear (nDNA) and to mitochondrial DNA (mtDNA), and defects of nDNA by far exceed those of mtDNA [37–40]. Thus, one has to recognize that a MD condition may rely on the mutation of nDNA and

Table 1

Reported evidence of mitochondrial dysfunction (MDF) in cells of different FA complementation groups.

FA subgroups	Models	Observed MDF	References
FANCG (mutant and corrected)	human lymphoblasts	FANCG protein is found in mitochondria; wild-type but not G546R mutant FANCG physically interacts with the mitochondrial peroxidase peroxiredoxin-3 (PRDX3)	[20]
FANCA	human primary fibroblasts	defective Complex I, ATP production and lymphocytes increased AMP/ATP ratio	[23]
20 FA patients of several subgroups	RNA from low-density bone marrow cells	altered gene regulation of bioenergetic activities and redox-related activities	[34]
FANCA and FANCD2 (mutant and corrected)	immortalized cells and cell extracts	high ROS level and low mitochondrial transmembrane potential ($\Delta\psi m$); altered mitochondrial morphology; decreased ATP production and oxygen uptake	[24]
FANCA (mutant and corrected)	fibroblasts	decreased intracellular calcium concentration; flux of Ca^{2+} mobilized by H_2O_2 significantly lower in FANCA cells vs. controls	[25]
Fancc−/− (mutant and corrected)	murine embryonic fibroblasts	FANCC protein is required for clearance of damaged mitochondria, decreases mitochondrial ROS production and inflammasome activation	[26]
FANCA and FANCC	fibroblasts	impaired autophagy; increased number of autophagic accumulation of dysfunctional mitochondria	[27]
FANCA	fibroblasts	impairment in mitochondrial oxidative phosphorylation countered by an increase in glycolytic flux; prevailing glycolysis as the main energy- producing pathway	[28]

FA subgroups	Models	Observed MDF	References
FANCA	lymphoblasts	the electron transfer between respiring complex I-III is reduced and the ATP/AMP ratio is impaired with defective ATP production and AMP accumulation	[29]
Fanca−/− and *Fancc*−/−	mice hematopoietic stem cells	reduced glucose consumption, lactate production, and ATP production; suppressed glycolysis, leading to enhanced pentose phosphate pathway	[30]
Fancd2 mice	hematopoietic stem and progenitor cells	*Fancd2* deficiency increases mitochondrial protein synthesis and induces mitonuclear protein imbalance; increased mitochondrial respiration and mitochondrial reactive oxygen species	[31]
Mitochondrial DNA	70 FA patients	high frequency of mtDNA variations; downregulation of mtDNA complex-I and complex-III encoding genes	[32]
FANCG mutant and corrected	8 patients	FANCG mutant protein, hFANCGR22P, lost mitochondrial localization and failed to protect mitochondria from oxidative stress; transcriptional downregulation results in iron deficiency of FA protein FANCJ	[33]

does not necessarily require a mtDNA defect. Multiple implications of MDF are recognized for a number of metabolic and neurodegenerative diseases [37]. In the case of FA, one should recall that an extensive number of FA patients bear mtDNA defects, as reported by Solanki et al. (2020), with changes in the mtDNA number in 59% of FA patients studied, a high frequency of mtDNA variations (37.5% of non-synonymous variations and 62.5% synonymous variations) and downregulation of mtDNA complex-I and complex-III encoding genes of OXPHOS (p < 0.05) as strong biomarkers for impairment of mitochondrial functions in FA [32].

In view of counteracting – or mitigating – MDF, a number of studies have focused on the administration of mitochondrial cofactors (also termed "mitochondrial nutrients" – MNs), which play distinct and indispensable roles in mitochondrial metabolism, namely α-lipoic acid (ALA), Coenzyme Q10 (CoQ10) and carnitine (CARN). A limited number of clinical studies – mostly case reports - tested the administration of MNs in patients with MDs with favorable outcomes, as shown in Table 2 [45–49]. Other attempts to treat MD patients with MNs failed to show any significant improvements in clinical conditions or in MDF. As reviewed by El-Hattab et al. (2015), CARN and CoQ10 are commonly used in MELAS syndrome without proven efficacy. Although there is a lack of curative therapies for mitochondrial disorders, the increased number of clinical research evaluating agents targeting different aspects of MDF may be expected to provide more therapeutic options for these diseases in the future [50].

One should note that only the report by Vishwanath et al. (2011) tested the effects of three MNs and a mixture of other mitoprotective agents (a diet with a low content of complex carbohydrates, creatine, folic acid, and ribose) and reported on favorable effects of this treatment [47]. This treatment also followed the rationale suggested by Tarnopolsky (2009) aimed at mitigating MD by means of a "mitochondrial cocktail" [51]. On the other hand, the other reports on this issue were confined to the use of only one MN, thus raising doubts about the effectiveness of this more limited focus in study design. As we have reported previously, the prospect use of MNs in several diseases should foresee the simultaneous administration of MN combinations aimed at corroborating the expected benefits of each MN, and provided the safety of each MN at controlled dosages [52,53].

It is worth noting that Maciejczyk et al. (2017) evaluated three cancer-prone genetic diseases, Ataxia-telangiectasia (A-T), Bloom syndrome (BS) and Nijmegen breakage syndrome (NBS), by focusing on the redox-related and MDF phenotypes of these diseases, suggesting that they be attributed to MDs [54]. According to these authors, their data suggest new mitoprotective strategies for patients suffering from A-T, BS and NBS.

4. Mitochondrial nutrients in prophylaxis of FA post-transplant malignancies

An increased risk of malignancies, namely leukemias, as well as oral and cervical cancers, is a well-established outcome among FA patients, and relates to metabolic changes leading to cancer susceptibility [55]. This risk is enhanced among post-transplant patients [56,57]. The risk of head and neck squamous cell carcinoma is > 500-fold higher among FA patients compared with the general population. Allogeneic hematopoietic cell transplantation (HCT) is the only proven potential curative therapy, enabling to completely correct the progressive bone marrow failure. However, HCT is unable to eliminate the cancer susceptibility of FA patients, which is increased as a consequence of chemo-radiotherapy used in the pre-transplant conditioning therapy.

A body of literature has related neoplastic transformation with the protective actions of several antioxidants in counteracting the redox-related mechanisms involved in carcinogenesis [58,59].

A more specific focus on MNs as potential effectors of protection to malignant transformation has been devoted in a body of literature reporting on the use of each MN in several carcinogenesis models, as summarized in Table 3.

Table 3

Selected reports of testing mitochondrial nutrients (MNs) in carcinogenesis models. MNs: α-lipoic acid (ALA); Coenzyme Q10 (CoQ10), and carnitine (CARN).

Reports/models	MNs	Observed effects	References
Multiple (review)	ALA	use of lipoate and lipoate analogs to therapeutically attack malignant disease	[59]
Multiple (review)	ALA	1) triggers mitochondrial apoptosis pathway 2) antitumor activity *in vivo*	[60]
Multiple (review)	ALA	anti-cancer activity of ALA nanoparticles	[61]
Human gastric cancer cells (*in vitro* study)	ALA	inhibited proliferation and invasion of human gastric cancer cells suppressing MUC4 expression by inhibiting STAT3 binding	[62]
Gastric cancer cells	ALA	inhibited proliferation and invasion of cancer cells by suppression of STAT3-mediated MUC4 gene expression	[63]
Breast cancer (clinical trial)	CoQ10 (100 mg/d)	CoQ10 supplementation ameliorated inflammatory cytokine levels *vs.* controls	[64]
Hepatocellular carcinoma (HCC) (clinical trial)	CoQ10 (300 mg/d)	significantly increased the antioxidant capacity; reduced oxidative stress and inflammation in HCC patients after surgery	[65]
Cancer-related fatigue, and carnitine deficiency (clinical trial)	CARN (up to 3000 mg/d)	relieved fatigue and improved parameters	[66]
	(1000 mg/d)	improved health-related quality of life	[67]

Table 2

Selected case reports or clinical trials testing the administration of mitochondrial nutrients (MNs) in patients with mitochondrial diseases (MDs). MNs: α-lipoic acid (ALA); Coenzyme Q10 (CoQ10), and carnitine (CARN).

MDs	MNs(dosage)	No. Patients(duration)	Observed effects	References
Progressive external ophthalmoplegia	ALA (600 mg/d)	1 (1–7 mo)brain phosphocreatine, 72% increase of	After 1-mo. treatment 55% increase of phosphorylation potential, and decrease of ADP and rate of energy metabolism; further improvements after 7-mo. treatment	[45]
Different mitochondrial cytopathies	CoQ10 (150 mg/d)	6 (6 mo)	brain variables were improved in all patients	[46]
Myopathy presenting with polyarteritis nodosa	CoQ10 + ALA + CARN	1 (several months)	MN mixture and other supplements resulted in clinical improvement	[47]
Myopathy associated with muscle CoQ10 deficiency	CoQ10 (50 μM)	1 (6 mo)	increased complex I, I + III, and II + III activities and clinical improvement	[48]
Chronic progressive external ophthalmoplegia (CPEO)	CARN (3 g/d)	12 CPEO patients +10 controls (2 mo)	improved aerobic capacity and exercise tolerance	[49]

A number of studies have focused on the ALA-associated protective roles in carcinogenesis mechanisms, both in clinical trials [reviewed in 60–62] and in studies of proliferation and invasion of cancer cells [63]. These protective actions are consistent with the recognized functions of ALA both as a powerful antioxidant and for its key-role in mitochondrial function [60].

Two clinical trials were conducted with the administration of CoQ10 aimed at counteracting cancer-associated symptoms such as inflammatory cytokine levels and prooxidant state in cancer patients. The results of these clinical trials showed adjuvant effects of CoQ10 [64,65].

Other clinical trials tested the protective action of CARN on cancer-related fatigue, with adjuvant effects on health-related quality of life, without adverse effects [66,67].

From this literature, one may desume that adjuvant effects were detected using only one MN. On the other hand, given the recognized safety of these cofactors and their complementary role in mitochondrial function [51–53], it is possible to argue about this limitation when testing only one MN. Thus, as previously suggested, it is possible to recommend the adjuvant use of MN combinations in planning mitoprotective strategies aimed at limiting or delaying malignant outcomes in post-transplant FA patients.

It is worth noting that, beyond MNs, a number of other agents have been successfully tested for exerting antioxidant and mitoprotective effects in FA mutant mice or cells, either individually or in combinations. This was the case for N-acetylcysteine, tested in combination with ALA by Ponte et al. [68], or in combination with resveratrol by Usai et al. [69]. A number of other agents displayed protective effects on FA defective physiology in cell or mouse models, such as resveratrol [70–72], metformin [73], tempol [74], or a phytonutrient mixture [75].

Altogether, this background literature may provide precious suggestions in study design aimed at optimizing a combined use of both MN combinations and of other antioxidants aimed at balancing physiology in FA experimental models with the ultimate goal of protecting FA patients or mitigating the course of disease.

5. Conclusion

Two apparently opposite views have been established in defining FA. The former, shared by most of the scientific community, attributes FA to defective DNA repair following exposure to crosslinkers as MMC or DEB. In contrast to this view, termed as "canonical", another, long-established line of studies attributes the basic FA defect to a cellular – and organismal – prooxidant state. This view is somewhere referred to as "non-canonical" in the literature [35].

As discussed in this review, and schematized in Fig. 1, the overall body of literature focused on the FA phenotype should reconcile the two views and definitions. As a fact, chromosomal instability of FA cells, and the cellular and clinical FA phenotype can logically rely on the extensively documented MDF in FA cells. This basic defect both accounts for the redox-dependent activation of cross-linkers and finds a direct relationship with the *in vitro* and *ex vivo* prooxidant state observed in FA cell and patients' cells and plasma.

Thus, in the effort to overcome the present antinomy between the "canonical" and "non-canonical" definitions, the herewith proposed re-definition of FA as a MD is both consistent with FA's phenotype and, most noteworthy, provides suggestive hints for FA's clinical management by the use of MN combinations, possibly in mixture with other effective antioxidants.

Authors' contributions

Conceptualization: Giovanni Pagano, Adriana Zatterale; Methodology: Alex Lyakhovich, Sudit Mukhopadhyay; Writing - original draft preparation: Giovanni Pagano, Federico Pallardó; Writing - review and editing: Luca Tiano, Alex Lyakhovich; Supervision: Marco Trifuoggi.
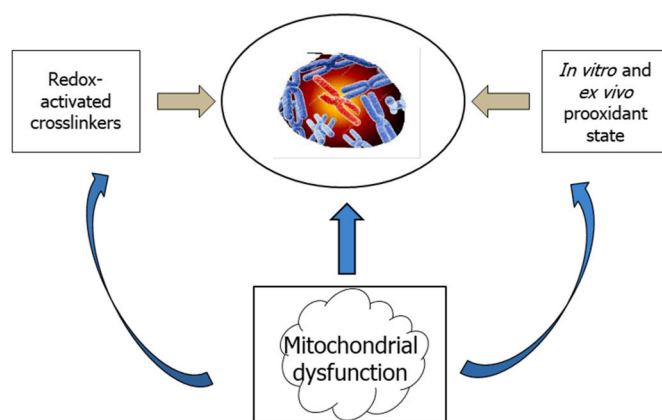

Fig. 1. Chromosomal instability, as a hallmark of FA, is viewed as a multiple outcome of: a) crosslinker activity of redox-related xenobiotics and b) cellular and organismal prooxidant state. A key-role is proposed for mitochondrial dysfunction, both activating crosslinkers to their active derivatives and causing the FA-associated prooxidant condition.

Declaration of competing interest

References

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