

Review paper**FANCONI ANEMIA GENES AND REACTIVE OXYGEN SPECIES IN CANCER****DEVELOPMENT****ABSTRACT**

Fanconi Anemia (FA), an autosomal recessive disease of childhood. However, the FA pathway is responsible For the development of leukemia and the other cancers. It has been also demonstrated that Fanconi anemia, an only human genomic instability syndrome is very sensitive to oxidative stress and reactive oxygen species (ROS) overproduction. In present work, we consider major mechanisms of antioxidant protection in Fanconi anemia cells. We showed that there are two types of such mechanisms: the suppression of reactive oxygen species overproduction by Fanconi anemia genes through the activation of basic Fanconi anemia proteins under the conditions of oxidative stress and the application of free radical scavengers able to react with iron-dependent reactive oxygen species such as flavonoids rutin and quercetin. The last nontoxic compounds of vitamin P group might be recommended for the treatment of Fanconi anemia patients. Then, we discussed the role of Fanconi anemia proteins in cancer development.

Key words: Fanconi anemia, ROS, antioxidants, rutin

Fanconi anemia (FA) is an autosomal recessive disease of childhood characterized by progressive pancytopenia, developmental abnormalities, bone marrow failure, and high disposition to leukemia and the other cancers. It has been demonstrated that the FA pathway is an important way in the development of such deadly adult pathologies as breast and cervical cancer [1,2]. These findings sharply increase an interest to studying FA molecular mechanisms. The special interest is attracted to Fanconi anemia because FA is the only human genomic instability syndrome uniquely sensitive

25 to oxidative stress. Earlier, an importance of reactive oxygen species (ROS) overproduction i.e.
26 oxidative stress has been demonstrated. Joenje *et al.* has shown that erythrocyte superoxide
27 dismutase (SOD) was decreased in FA [3]. These authors also proposed that the formation of
28 chromosomal aberrations in FA anemia might be explained by the genetic toxicity of oxygen [4]
29 underlining an important role of oxidative stress in FA development.

30 **OUR EARLIER STUDIES**

31 In 1960, medicine doctors from Russian Institute of Hematology for Children (Moscow) asked our
32 group working in the field of free radical-associated diseases to study the possible application of
33 antioxidants for the treatment of FA patients. We hoped that antioxidants and free radical scavengers
34 could act positively on behalf of FA patients. To study the effects of antioxidants on FA cells, we
35 measured the ROS production especially superoxide in these cells. Major results of this *in vitro*
36 study have been summarized as follows [5,6]: ROS formation was measured by lucigenin- and
37 luminol-dependent chemiluminescence (CL) in non-stimulated and stimulated blood and bone
38 marrow leukocytes of FA patients. It was found that the FA blood leukocytes produced the
39 enhanced level of luminol CL in comparison with leukocytes from normal donors. Lucigenin CL
40 was also higher in FA blood leukocytes, although its effect was not very significant. Similar but
41 smaller effects were observed for bone marrow FA leukocytes. Earlier, we have already
42 demonstrated that flavonoids rutin and its aglycone quercetin possess both free radical scavenging
43 and chelating properties. We have shown that both flavonoids were the most effective inhibitors of
44 iron-dependent microsomal lipid peroxidation comparing to lipid peroxidation initiated by carbon
45 tetrachloride [7]. (This work had a good response; at present, it has been cited more than 800 times).

46 We proposed that ROS formation in FA leukocytes was the iron-catalyzed process because it
47 was characterized by the enhanced luminol CL typical for the formation of iron-dependent ROS.
48 This proposal was supported by studying the effects of various free radical inhibitors on luminol CL

49 of FA leukocytes [5]: Superoxide dismutase (SOD) inhibited only slightly luminol CL, while
50 mannitol (the typical hydroxyl radical scavenger) and rutin were the strongest inhibitors. (Later on,
51 we also demonstrated that rutin was a strong inhibitor of iron-dependent lipid peroxidation of rat
52 brain homogenates [8] and the free radical formation in iron-overloaded rats [9]. Moreover, we
53 found that rutin efficiently inhibited free radical formation and oxyhemoglobin oxidation in β -
54 thalassemic red blood cells [10]). These findings reassured us to apply the non-toxic flavonoid rutin
55 (vitamin P) for the treatment of FA patients.

56 The results of rutin therapy for a small group of FA patients were encouraging. Rutin (vitamin P)
57 was permitted for application to patients (FA patients were under the supervision of our co-authors
58 medical doctors). No toxic side effects were observed in patients. ROS production by FA leukocytes
59 sharply decreased, patients' health was essentially improved [6]. Unfortunately, we have no
60 possibility to continue our study despite the numerous requests of medical doctors.

61

62 **THE NATURE OF REACTIVE OXYGEN SPECIES IN FA CELLS**

63 Our findings demonstrated that ROS formation in FA cells was connected with the iron species. In
64 1992, in accord with the views of that time we proposed that the major damage produced by ROS in
65 cells is the direct interaction of ROS with biomolecules. As the superoxide, a major precursor of
66 ROS in cells is unreactive in free radical processes [11,12], we proposed that the reactive iron-
67 contained species could be responsible for free radical damage in FA cells. We suggested that the
68 iron-catalyzed superoxide conversion into reactive hydroxyl radicals (the Fenton reaction) was
69 responsible for their formation in FA cells. Indeed, there are some evidences of hypersensitivity of
70 FA cells toward oxygen and iron [13]. Although reactive hydroxyl radicals are probably unable to
71 achieve the target biomolecules, they can be formed during the contacts of superoxide with DNA
72 "iron fingers" [14].

73 A major conclusion from our previous work was that FA characterized by the enhanced
74 production of reactive iron-dependent species, which might be the source of fatal disorders in this
75 hereditary disease. At present, many authors agreed that FA cells existed under the conditions of
76 ROS overloading. Thus, Hadjur *et al.* concluded that the abnormality of FA cells depended on ROS
77 overproduction [15]. Du *et al.* pointed out that FA is an only human genomic instability syndrome,
78 which was uniquely sensitive to oxidative stress [16]. Therefore, there is no doubt about an
79 importance of ROS in Fanconi anemia. However, the damaging mechanisms of ROS activity remain
80 to be investigated.

81

82 **THE FA GENETIC PATHWAY**

83 The most important development of FA molecular mechanisms was the identification of Fanconi
84 anemia genes responsible for synthesis of special FA proteins FANC (among them FANCA, B, C,
85 E, F, G, L, M). It was found that eight major FA proteins FANCA, B, C, E, F, G, L, and M) are
86 formed a nuclear complex [16]. In response to DNA damage or DNA replication stress FA complex
87 monoubiquitinated into the two FA proteins FANCD2 and FANCI, which then recruited the other
88 downstream FA proteins including FANCD1 (which is also named the breast cancer protein
89 BRCA2), FANCI, and FANCN to enter nuclear loci containing the damaged DNA.

90 It has been shown that FA proteins play the critical role in the regulation of oxidative stress.
91 Thus, deficiency in FA genes apparently affected mitochondrial ROS [17]. The redox-sensitive
92 proteins FANCA and FANCF exist as monomers under non-oxidizing conditions but form a new
93 nuclear complex through the intermolecular disulfide bonds in response to oxidative damage [18].
94 FANCA, FANCC, and FANCG participate in redox processes in mice with combined deficiencies of
95 the genes encoding *Fancc* and Cu/Zn superoxide dismutase [15].

96 FA proteins function through the interaction with some enzymes. Saadatzadeh *et al.* showed that
97 *Fancca*^{-/-} cells were highly sensitive to oxidants (hydrogen peroxide) and underwent enhanced
98 apoptosis [19]. Antioxidative compounds enhanced the survival of these cells. Thus, the redox-
99 dependent ASK1 kinase was hyperactive in hydrogen peroxide-treated *Fancca*^{-/-} cells. Another FA
100 protein FANCG interacted with mitochondrial antioxidant enzyme peroxiredoxin-3 and cytochrome
101 P450 2E1 (CYP2E1) [20]. This member of P450 superfamily was responsible for ROS production
102 and the activation of carcinogens. These findings suggested that the interaction of FANCG with
103 CYP2E1 might increase DNA oxidation.

104 It is known that the tumor protein p53 plays an important role in the prevention of cancer.
105 Furthermore, some findings demonstrate that p53 deficiency might enhance cancer development in
106 FA patients and FA mice. Therefore, it was suggested that FA proteins could interact with p53
107 under the conditions of oxidative stress. Freie *et al.* showed that ionizing radiation (IR) induced p53
108 elevated levels of in cells from *Fancc* mutant mice and that the inactivation of p53 enhanced TNF-
109 induced apoptosis in myeloid cells from *Fancc*^{-/-} mice [21]. Rani *et al.* demonstrated that FA
110 proteins protected cells from the stress-induced proliferative arrest and tumor evolution
111 through the modulation of signaling pathways, which connected FA proteins to p53 [22]. Du *et*
112 *al.* proposed that two major FA proteins FANCA and FANCC might coordinate with p53 in the
113 regulation of oxidative stress response [16].

114 It has been shown that the *Foxo3a* gene might be involved in ROS formation [23]. For
115 example, Tothova *et al.* showed that ROS levels increased in Foxo-deficient hematopoietic stem
116 cells that correlated with the changes in expression of ROS-regulated genes [24]. Correspondingly,
117 *Foxo3a* plays an important role in ROS regulation in FA cells. Thus, Li *et al.* showed that the
118 treatment of FA cells with hydrogen peroxide stimulated the formation of a complex between
119 FANCD2 and FOXO3a with subsequent monoubiquitination of FANCD2 [25]. It was suggested

120 that the overexpression of *Foxo3a* reduced abnormal accumulation of ROS, enhanced cellular
121 resistance to oxidative stress, and increased antioxidant gene expression only in cells corrected by a
122 FANCD2 protein capable of interacting with FOXO3a.

123 It has been shown that ROS accelerated the development of hydrocephalus (abnormal
124 accumulation of cerebrospinal fluid in the brain) in mouse model of FA [26]. The deletion of
125 *Foxo3a* in FA mice increased the ROS accumulation and subsequently deregulated mitosis and
126 ultimately apoptosis in the neural stem and progenitor cells NSPCs, leading to hydrocephalus
127 development. The antioxidants NAC and quercetin reduced ROS formation in both neural stem
128 cell NSCs and in the brain of double knockout (DKO) offspring mice. Importantly, quercetin
129 greatly diminished the synthetic lethality imposed by DKO and completely eliminated
130 hydrocephalus in DKO mice.

131

132 **MECHANISMS OF ANTIOXIDANT PROTECTION IN FANCONI ANEMIA**

133 **ANTIOXIDANTS AND FREE RADICAL SCAVENGERS**

134 As it was aforementioned, we previously showed that oxidative stress (ROS overproduction) in FA
135 cells might be diminished by the application of flavonoid rutin, an antioxidant and free radical
136 scavenger [5,6]. We suggested that rutin might be able to scavenge reactive iron-dependent ROS.
137 Rutin is a nontoxic compound (vitamin P) permitted for the treatment of patients. Therefore, our
138 colleagues, medical doctors were allowed to use rutin for the treatment of FA patients. They
139 observed certain positive effects in patients. (It should be mentioned that several FA families asked
140 us to prolong the treatment of FA children but we were unable to continue our work).

141 Now we were encouraged to find out that in subsequent studies flavonoids were used for the
142 suppression of oxidative stress in FA. We were happy to find out that quercetin (the aglycone of
143 rutin) turned out to be an effective antioxidant in FA cells. Antioxidant effect of quercetin was also

144 showed in FA animals. Li *et al.* found that mice deficient for the *Fanca* or *Fancc* genes were
145 diabetes-prone when fed with a high-fat diet [27]. Treatment of FA mice with quercetin
146 diminished diabetes and obesity. It was already demonstrated that quercetin reduced ROS
147 formation and eliminated hydrocephalus in double knockout mice [26]. Ponte *et al.* proposed that
148 the cocktail of antioxidants lipoic acid and NAC might be applied as a prophylactic approach to delay
149 progressive clinical symptoms in FA patients [28].

150 **ANTIOXIDANT EFFECTS OF FA GENES**

151 In 2001, Hadjur *et al.* showed that encoding *Fancc* and Cu/Zn superoxide dismutase genes might be
152 useful for the treatment of defective hematopoiesis and hepatic steatosis in mice [13]. These findings
153 suggested an important role of FA genes in protection of FA cells from oxidative stress. In
154 subsequent works, it was confirmed that FA genes were able to suppress ROS overproduction. Du *et*
155 *al.* showed that major antioxidant defense genes were downregulated in FA patients due to the
156 increased oxidative DNA damage in the promoters of antioxidant genes [29]. They showed that FA
157 proteins together with the chromatin-remodeling factor BRG1 protected the promoters of
158 antioxidant defense genes. Oxidative stress activated FA pathway through monoubiquitination of
159 FANCD2. After this, FANCA or FANCD2 proteins formed the ternary complex with BRG1 at the
160 promoters of antioxidant genes. It has been suggested that this complex played essential role for the
161 protection of promoters from ROS damage. As it has been mentioned, ROS influenced the
162 development of hydrocephalus in mouse model of FA [26]. Combined deficiency of two FA genes
163 *Fancc* and *Fancd2* led to the inactivation of *Foxo3a* gene, the enhancement of ROS level, and
164 apoptosis of neural stem and progenitor cells. Antioxidants quercetin and NAC reduced ROS
165 formation in the brain of mice, while quercetin completely deleted hydrocephalus. Mukhopadhyay
166 *et al.* identified the FA group G (FANCG) protein in mitochondria, which interacted with the

167 mitochondrial peroxidase peroxiredoxin-3 (PRDX3) [30]. The formation of this complex prevented
168 the destruction of PRDX3 peroxidase and diminished ROS formation.

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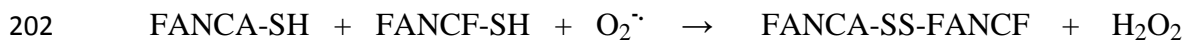
170 **DISCUSSION**

171 Fanconi anemia is a rare chromosome instability syndrome, which is characterized by aplastic
172 anemia in childhood. It is known that FA possesses high disposition to leukemia and other cancers.
173 Now, there are new findings, which make us to reconsider more carefully the connection between
174 FA and cancer. Some new contemporary data demonstrate that the FA pathway is an important route
175 for the development of such deadly adult pathologies as the breast and cervical cancer. Thus,
176 D'Andrea pointed out that abnormalities in the FA pathway are found not only in childhood
177 Fanconi
178 anemia but also in sporadic cancers in adults [1]. Narayan *et al.* investigated the molecular
179 genetic basis and the role of (FA)-BRCA pathway in cervical cancer (CC) [2]. These authors showed
180 that the *fancf* gene was disrupted by either the promoter hypermethylation or the deregulated gene
181 expression in a majority of cervical cancer. They also found that gene inactivation in the FA-BRCA
182 pathway by epigenetic alterations pointed out at its major role in the development of cervical cancer.
183 Chen *et al.* demonstrated that the regulation of FANCD2 by m-TOR pathway led to the resistance of
184 cancer cells to DNA double-strand breaks [31]. It is no surprising that among thirteen FA genes one
185 of genes is the well-known breast cancer susceptibility gene *brca2*.

186 In normal cells, the FA pathway is not constitutively active but it is turned on by the following
187 DNA damage. As DNA damage depends on oxidative stress, ROS overproduction should be an
188 important factor of FA pathway activation. Mechanism of response of FA genes to DNA damage is
189 not fully established, but it has been shown that FA genes exist as the nuclear complex before
190 activation. In response to the DNA damage or the DNA replication stress FA complex

191 monoubiquitinated into the two FA proteins FANCD2 and FANCI, which then recruited the other
192 downstream FA proteins including the breast cancer protein BRCA2, FANCI, and FANCN to enter
193 nuclear loci containing the damaged DNA. Then FANCA and FANCF form a new complex through
194 the intermolecular disulfide bonds after exposure to oxidative stress [16]. Thus, FA genes
195 accomplish important defense function, which is confirmed by high sensitivity of *Fanc*^{-/-} cells to
196 ROS.

197 We now can visualize the complete defense scheme of ROS inhibition in FA cells. It has been
198 shown that the oxidative stress (ROS overproduction) initiates the activation of FA genes, which
199 remained inactive under unstressed conditions. After activation, FANCA and FANCF proteins
200 formed a complex through the intermolecular disulfide bonds, and we might propose that the
201 disulfide complex was formed by the reaction of superoxide with these proteins:



203 This proposal was supported by the effect of SOD on the redox processes of FANCA, FANCC, and
204 FANCG in mice [13].

205 Another mode of antioxidant activity of FA proteins is the protection of antioxidant gene
206 promoters from ROS overproduction. Du *et al.* pointed out that oxidative stress is an important
207 pathogenic factor in leukemia-prone home marrow diseases such as Fanconi anemia [29]. It has
208 been proposed that the FA pathway plays a crucial role in protecting major antioxidant defense
209 genes from oxidative damage. This protection was probably accomplished in response to oxidative
210 stress by the interaction with the chromatin-remodeling machinery. Indeed, it has been shown that
211 the oxidative stress-induced activation of FA pathway (FANCD2 ubiquitination) was required for
212 the formation of the FA-BRG1-promoter complex. This complex is essential for the protection of the
213 antioxidant gene promoters from oxidative damage.

214 Thus, the activation of FA pathway under the conditions of oxidative stress led to cellular
215 protection through various ways. Taking into account the above-mentioned consideration, these
216 ways might be presented as follows:

217

218 **Table 1**

219 **FA PATHWAYS OF CELLULAR PROTECTION**

220

221 **1. Protection of antioxidant gene promoters**

222

223 FANCD2 ubiquitination

224 ↓

225 FA-BRG1-promoter complex

226 ↓

227 Protection of antioxidant gene promoters

228

229

230 **2. Formation of the disulfide complex**

231

232 $\text{FANCA} + \text{FANCF} + \text{O}_2^{\cdot-} \rightarrow \text{FANCA-SS-FANCF}$

233

234

235 **3. Suppression of DNA double-strand breaks**

236

237 $\text{FANCD2} \rightarrow \text{m-TOR pathway} \rightarrow \text{DNA double-strand breaks}$

238

239

240 **4. Suppression of ROS overproduction**

241

242 Antioxidants (quercetin and NAC) \rightarrow ROS↓ (diabetes and hydrocephalus in FA patients)

243

244 **5. Enhancement of gene antioxidant activity**

245

246 The interaction of genes with enzymes (Foxo3a, p50, p450)

247

248

249 **CONCLUSIONS**

- 250 (1) Direct interaction of FANCA and FANCF with ROS (superoxide) to form a complex through the
251 intermolecular disulfide bonds.
- 252 (2) The regulation of FANCD2 by the m-TOR pathway might lead to the resistance of cells to DNA
253 double-strand breaks as in cervical cancer.
- 254 (3) FANCD2 ubiquitination following the formation of FA-BRG1-promoter complex capable of
255 protection of antioxidant gene promoters.
- 256 (4) Antioxidant activity of FA genes enhanced by the interaction with various enzymes such as
257 Foxo3a, p50, p450.
- 258 (5) Application of antioxidants and free radical scavengers (quercetin and NAC) to decrease ROS
259 overproduction in such diseases as diabetes or hydrocephalus in FA patients.

260

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