Fanconi anemia is a highly penetrant cancer susceptibility syndrome

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First described by Guido Fanconi in 1927, Fanconi anemia (FA) is now one of the best defined inherited bone marrow failure syndromes. It is usually inherited as an autosomal recessive trait, but in a small subset of FA cases it can be an X-linked recessive disorder. FA patients show marked clinical heterogeneity. Characteristic features include the progressive development of bone marrow failure (BMF) and an increased predisposition to malignancy.[1-3] Affected individuals may also have one or more congenital/developmental abnormalities including abnormal skin pigmentation (e.g. café au lait spots), skeletal (e.g. radial ray anomalies), genitourinary (e.g. horseshoe kidney), and gastrointestinal (e.g. duodenal atresia) abnormalities. A significant subset (~30%) of FA patients have no apparent somatic abnormalities. The majority of patients present towards the end of the first decade of life. However, an increasing number of patients are being diagnosed for the first time in adulthood. At the same time, many patients diagnosed in childhood are now surviving into adulthood. This means that awareness of this disorder on the part of both adult and pediatric physicians is becoming increasingly important.

Over the last 20 years, major advances have been made in our understanding of the biology of FA. FA cells characteristically display a high frequency of spontaneous chromosomal breakage and hypersensitivity to DNA cross-linking agents, such as diepoxybutane (DEB). This genomic instability led to the development of a diagnostic test (i.e. increased chromosomal breakage in FA cells compared with normal controls after exposure to DEB) over two decades ago and this is still a useful test today. This *FA cell hallmark* has also facilitated many advances in our understanding of FA, including clarification of the complex genetics of this disease with 13 subtypes/complementation groups identified at present.[4] The genes (*FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM* and *FANCN*) responsible for these subtypes have all been identified.

Studies from many research groups around the world have demonstrated that the proteins encoded by the FA genes participate in a complicated network which is important for DNA repair.[5] Specifically, eight of the FA proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL and FANCM) interact with each other and form a nuclear complex called the *FA core complex* (Figure 1). The *FA core complex* is required for the activation of the FANCD2 protein to a monoubiquitinated isoform (FANCD2-Ub). In normal (non-FA) cells, FANCD2 is monoubiquitinated in response to DNA damage and is targeted to chromatin containing the DNA damage (e.g. DNA cross-link). FANCD2-Ub then interacts with DNA repair proteins (including BRCA2 and RAD51) leading to repair of the DNA damage. In cells from FA-A, FA-B, FA-C, FA-E, FA-F, FA-G, FA-L or FA-M patients, FANCD2 monoubiquitination is not observed. It has recently been established that FANCI[6] (the protein mutated in the FA-I subtype) is a paralogue of FANCD2. FANCI associates with FANCD2 as the FANCI-FANCD2 (I-D2) complex. Like FANCD2, FANCI is also monoubiquitinated. FA-D1 patients have biallelic mutations in BRCA2.[7] These observations have linked the FA proteins (FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, and FANCM) with BRCA1 and BRCA2 (FANCD1) in a DNA damage response pathway called *the FA/BRCA pathway*. The *BRCA2* protein is important for the repair of DNA damage by homologous recombination (HR). Cells lacking *BRCA2* inaccurately repair damaged DNA and are hypersensitive to DNA cross-linking agents. It has also been established that FANCJ is BRIP1 (partner of *BRCA1*) and that FANCN[8] is PALB2 (partner of *BRCA2*). These findings further strengthen the connection between the FA and BRCA proteins and DNA repair.

The FA/BRCA pathway is activated in response to DNA damage (e.g. replication fork arrest) and involves ATR (Ataxia–Telangiectasia and RAD3 related protein) (Figure 1). The pathway is inactivated by the de-ubiquitinating enzyme, USP1.[9] ATR appears to directly regulate the FA pathway as it is required for the monoubiq-

uitination of *FANCD2* and *FANCI*. Disruption of this FA/BRCA pathway, as occurs when there are biallelic mutations in any of the 13 FA genes, results in an abnormal DNA damage response. Many of the steps involved in the FA/BRCA pathway have now, therefore, been clarified. However, the precise role of all the FA proteins in DNA repair still remains to be fully defined. It is equally clear that FA cells also display other abnormalities in addition to genomic instability. This includes hypersensitivity to oxygen, accelerated telomere shortening, abnormal cell cycle kinetics, and overactivation of the mitogen-activated protein kinase pathways (MAPKs which leads to overproduction of tumor necross factor–α).[10,11] These observations suggest that our understanding of the molecular events responsible for all the FA biological phenotypes is currently incomplete. They also suggest that it is too simplistic to think of FA as a disorder of purely defective DNA repair. As in many other clinical problems, an improved understanding of FA requires an integrated approach drawing upon both clinical and basic research investigations. Over the years, the various clinical features of FA first observed by Fanconi have been refined with the identification of new clinical cases of FA. As discussed above, FA has been shown to be associated with an increased risk of malignancy. But because of its rarity, estimates of the various associated risks need to be validated as more data are obtained through analysis of larger, well characterized groups of FA patients.

In this issue of the journal, Rosenberg *et al*. report[12] on the detailed analysis of the German Fanconi Anemia Registry in a cohort of 181 patients. These were studied to determine the risks of BMF, acute myeloid leukemia (AML) and solid tumor (ST) according to age. The first adverse event was BMF in 66, AML in 14 and ST in 10 patients. The O/E ratio (observed/expected) was 44 for all cancers, 26 for all solid tumors, and 868 for AML. These increased risks were all statistically significant. Detailed subset analysis demonstrated significantly elevated O/E ratios for the following cancers; 6281 for esophageal, 2411 for vulva, 240 for head and neck, 34 for breast and 23 for brain tumors. These figures are

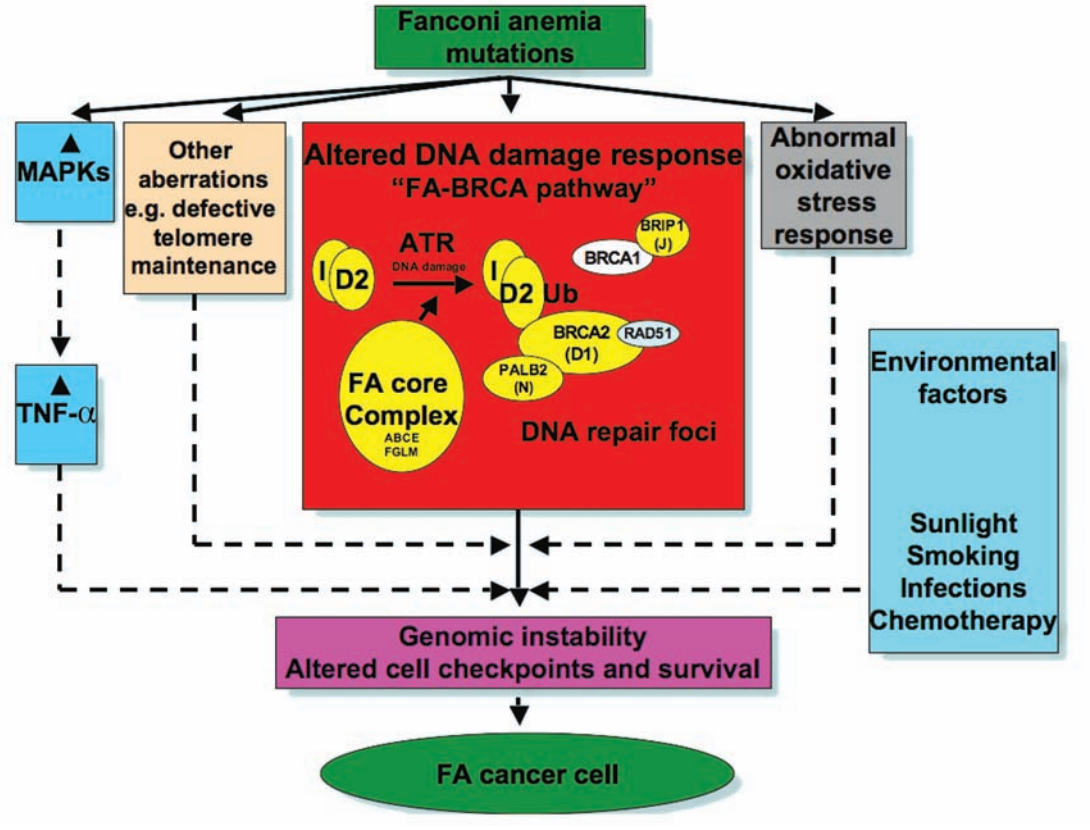

Figure 1. Schematic representation of the factors predisposing to malignancy in Fanconi anemia. Constitutional mutations in FA cells lead to aberration of the "FA-BRCA pathway", aberrant activation of the mitogen activated protein kinases (MAPKs), abnormal handling of oxidative stress, defective telomere maintenance, and possibly other biological aberrations. These result in increased genomic instability and altered cell survival/checkpoints. Collectively these aberrations generate a *cell environment* that predisposes to the development of *FA cancer cells*. Environmental factors, such as smoking and sunlight, might also add to the effect of the FA mutations. Within the "FA-BRCA pathway", the proteins shown in yellow are those mutated in different FA patients. The FA core complex consists of eight FA proteins (A, B, C, E, F, G, L and M) and this together with ATR (Ataxia-Telangiectasia and RAD3 related protein) is essential for the ubiquitination-activation of I-D2 complex after DNA damage. Activated I-D2-Ub translocates to DNA repair foci where it associates with other DNA damage response proteins including BRCA2 and RAD51, and participates in DNA repair. TNF-α: tumor necrosis factor-α; Ub: ubiquitination.

broadly similar to the North American Survey published previously[13] and confirm that FA patients are at a significantly increased risk of developing a range of hematologic and non-hematologic malignancies.

Rosenberg *et al.*[12] also confirmed that absent/abnormal radii, and a 5-item (developmental delay, cardiopulmonary abnormality, abnormal kidney, abnormal hearing/deafness and abnormal head size) congenital/developmental abnormality score were significant risk factors. They established that the cumulative incidence of BMF by age 10 yrs. varied between 12.6% in the lowest BMF risk group to 84% in the highest. Furthermore, the relative hazard (RH) of BMF was significantly higher in patients of complementation group FA-G versus FA-A (RH=2.2) and in FA-C versus FA-A (RH=5.4). These observations are consistent with previous reports from the European Fanconi Anemia Registry (EUFAR)[14] and the International Fanconi Anemia Registry (IFAR).[15] Furthermore, they found that patients transplanted prior to any malignancy may develop cancers. However, probably due to the small numbers involved, the elevated risk did not reach statistical significance. The findings of Rosenberg *et al.* from the German Registry therefore validate prior risk estimates for BMF and malignancy in FA patients making it clear that FA is a pleiotropic syndrome with a marked susceptibility to develop both bone marrow failure and cancer. The major cause of premature mortality in FA patients is the development of BMF. Definitive treatment for BMF is offered by hemopoietic stem cell transplantation (SCT). From the *in vitro* and *in vivo* studies it has become clear that cells from FA patients are hypersensitive to agents such as cyclophosphamide and irradiation compared with non-FA patients. Following the poor results of early protocols, SCT conditioning regimens have been modified by reducing the dose of cyclophosphamide and radiation. Results using Fludarabine based protocols which avoid[16,17] or use lower doses[18] of radiotherapy seem to be encouraging for both sibling and unrelated stem cell transplants with two year survival rates of between 65-90%. This represents a major improvement in outcome following SCT in FA patients, although follow-up is short.

In the future it is therefore likely that the main complications which will present a major clinical challenge in FA patients will be the development of cancer and other problems related to premature aging. Identifying patients most at risk of cancer will help improve patient management. As highlighted by Rosenberg *et al.*[12] and previous studies, because of competing risks, patients at a lower risk of BMF are more likely to develop AML or ST. Therefore, in the group at lowest predicted risk of BMF, the cumulative incidence by age 49 yrs. was 33.6% for ST and 23.7% for AML. It is noteworthy that Rosenberg *et al.* found that some FA-subgroups have a greater risk of cancer compared with others (e. g. FA-G compared with FA-A). Unfortunately, in this study the complementation status of 69 out of the 181 patients studied was not known. Elsewhere it has been observed that the subtypes FA-D1 and FA-N are associated with high risks of solid childhood malignancies (e.g. Wilm's tumour and medullobalstoma) which are not usually seen in the other FA subtypes.[8, 19] Furthermore, heterozygous mutations in *FANCD1* (*BRCA2*), *FANCN* (*PALB2*) and *FANCJ* (*BRIP1*) give an elevated risk of breast cancer yet this is not the case for the other FA-genes. These differences not only highlight the varying risks of malignancy in FA subtypes but also that the relationship between the FA proteins and their interactions with other molecules is complex at both cellular and molecular levels.

In addition to the constitutional biallelic FA mutations, it is very likely that other factors are important in the development of cancer in FA patients. Given the genomic instability inherent in all FA cells, it is highly probable that environmental insults such as exposure to sunlight and smoking are important additional risk factors in the development of malignancy in FA, although these are difficult to quantitate (Figure 1). There are also data regarding the possible role of viral infections (e.g. human papillomavirus).[20] It seems sensible to advise FA patients to avoid these environmental insults as far as possible. It also seems appropriate to reduce/eliminate the dose of irradiation as well as reducing the dose of chemotherapy in SCT protocols as far as practically feasible in an attempt to reduce the risk of post-SCT cancer development. Treatment for patients who develop cancer is usually difficult although in the case of hematologic malignancies some patients can be rescued by SCT. The best treatment strategy for ST in FA patients remains to be defined and represents a major clinical challenge for the future.

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