We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



12

From the Molecular Biology to the Gene Therapy of a DNA Repair Syndrome: Fanconi Anemia

Paula Río, Susana Navarro and Juan A. Bueren
Hematopoiesis and Gene Therapy Division. Centro de Investigaciones Energéticas Medioambientales y Tecnológicas (CIEMAT) and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) Madrid Spain

1. Introduction

Fanconi anemia (FA) was first described in 1927 by the Swiss pediatrician Guido Fanconi in a family with five children, three of which had various physical abnormalities and hematological defects, in a condition that resembled pernicious anemia [1]. In all the three children the disease was manifested between ages of five to seven years old and had fatal consequences. Studies in peripheral blood cells from these and other patients with the same symptoms made Guido Fanconi to realize that the disorder affected all the hematopoietic lineages, not only erythropoiesis, and that these alterations were usually the main cause of mortality (See review in [2]). From the beginning of his studies, he thought that this disease was too complex to be caused by mutations in one single gene, but neither he, nor the rest of the research community could imagine at that time that mutations in at least fourteen different genes, currently known as FA genes, could account for the same disease. The description of the chromosomal instability of FA cells as a hallmark of the disease in 1964[3] focused the etiology of the disease as a DNA repair failure, but it was almost forty years later when this idea was confirmed.

In this chapter we will make an overview of the implications of the FA pathway in DNA repair and cell survival, and discuss the advances, limitations and perspectives of the therapeutic approaches used for the treatment of the most severe problem that takes place in FA patients, the bone marrow failure (BMF).

2. Fanconi anemia proteins form a complex pathway involved in the repair of DNA inter-strand cross-links

Mutations in at least 14 genes have been associated with FA. Patients with biallelic mutations in any of these FA genes (except in *FANCB,* which is X-linked) are assigned to different complementation groups (Table 1). The identification of the first FA complementation group was conducted by the fusion of cell lines generated from different FA patients[4]. The first FA gene, *FANCC*, was then identified by the transfection of cells from a FA patient with a cDNA expression library, followed by their exposure to mitomycin

C (MMC), a DNA cross-linking drug that is extremely toxic and generates specific chromosomal instability in FA cells[5]. Only those cells complemented with *FANCC* grew after MMC exposure, allowing the identification of the defective gene in these patients. Similar approaches, together with positional cloning and linkage analysis, allowed the identification of other protein members of the so called "FA core complex", which included FANCA, FANCG, FANCF and FANCE[6-10]. Although the description of FANCD2[11] and its activation by monoubiquitination after DNA damage linked FA with DNA repair, the confirmation of the involvement of the FA pathway in DNA repair and its link to homologous recombination occurred in 2002, when *BRCA2* was identified as the *FANCD1* gene[12]. After the discovery of *BRCA2* as a FA gene, several other FA genes were described, initially *FANCL*, containing the ubitiquitin ligase activity of the complex [13]and later on, *FANCB*; the only FA gene linked to the X chromosome[14]. The description of *FANCJ* (*BRIP1*)[15-17], *PALB2*[18] and *RAD51C* (not formally assigned yet as a FA gene)[19, 20] together with the previously described *BRCA2,* definitively linked the FA/BRCA pathway with increased cancer susceptibility[19, 21-24]. In subsequent studies FANCI [25-27] was found to be the partner of FANCD2. Additionally, the finding that FANCM[28] can interact with DNA, and the observation that FANCP (SLX4) had endonuclease activity, is allowing to unravel the role of the FA/BRCA pathway in the repair of DNA interstrand cross-links (ICLs) during replication [29](Table 1).

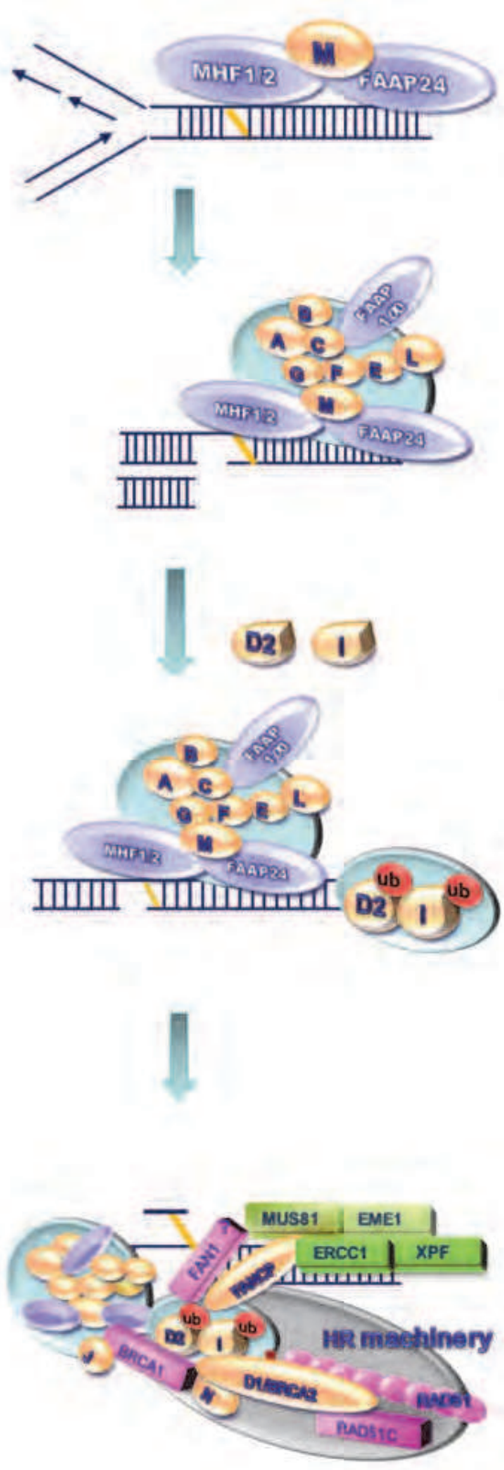
Interstrand cross-linking drugs covalently bind both strands of the DNA helix, blocking the DNA replication and transcription. As a consequence of stalled replication produced by ICLs or at S-phase entry, the FA pathway is activated (See review in [29]). Although the exact role of FA proteins in the repair of ICLs is not clear yet, it is known that they work together in a complex network, where the key event is the monoubiquitination of FANCD2[30] and FANCI [25-27] (D2-I complex). This monoubiquitination requires the presence of the FA core complex, currently known to be formed by FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL and FANCM[31], together with other FA associated proteins(FAAP; see Figure 1). The description of the last members of the FA/BRCA pathway, particularly FANCM, FAN1 (associated nuclease, with no mutations still found in FA patients)[32-35] and FANCP (SLX4)[36, 37] has added new critical elements in this intriguing pathway.

After the generation of an ICL in the DNA, the progression of the DNA replication fork is stalled, and this activates the FA core complex and ATR. The first protein from FA pathway that seems to initiate DNA repair is FANCM. This protein, thanks to its translocase activity [28], together with FAAP24 and MHF1/2[29], moves along the DNA to sense the stalled replication fork[38], facilitating the translocation and anchoring the rest of the FA core complex to damaged DNA. In this way, the FA core complex can monoubiquitinate FANCD2/I, inducing the replication fork pause and the incision/unhooking of the ICL, facilitating the translesion synthesis and finally the HR machinery required for the repair of the DNA damage [39, 40]. Once the D2-I complex is monoubiquitinated, another FA protein - FAN1 - is also recruited to sites of DNA damage, where all these proteins form large nuclear foci. The endonuclease activity of FAN1 suggests that this protein could also pair with MUS81-EME1 to unhook the ICL[32, 35, 40] necessary for DNA repair. In this step, the endonuclease XPF-ERCC1, whose activity is coordinated by the last described member of the FA family SLX4 (FANCP[36, 37]), probably also plays an important role in the repair of the DNA damage (See Figure 1).

C. group	FA genes	Prevalence	Chromosomal location	Protein size (kDa)	Protein characteristics	Required for Ub-FANCD2/I	RAD51 foci
FA-A	*FANCA*	66%	16q24.3	163	Member of the core complex. Phosphorylated by ATR kinase. Two NLS	Yes	Normal
FA-B	*FANCB*	~2%	Xp22.31	95	Member of the core complex. Contains a NLS	Yes	Normal
FA-C	*FANCC*	10%	9q22.3	63	Localized in the nucleus and cytoplasm. Member of the core complex.	Yes	Normal
FA-D1	*BRCA2*	~2%	13q12-13	380	Essential in HR by controlling RAD51. Interacts with FANCG, FANCD2 and PALB2. Cancer susceptibility gene.	No	Reduced
FA-D2	*FANCD2*	~2%	3q25.3	155,162	Monoubiquitinated by FA core complex. Phosphorylated by ATR and probably ATM after DNA damage.	Yes	Normal
FA-E	*FANCE*	~2%	6p21-22	60	Member of the core complex. Directly binds FANCD2. Contains two NLS.	Yes	Normal
FA-F	*FANCF*	~2%	11p15	42	Required for the assembly of FA core complex.	Yes	Normal
FA-G	*FANCG/ XRCC9*	9%	9p13	68	Member of the core complex. Interacts with FANCD1, FANCD2 and XRCC3.	Yes	Normal
FA-I	*FANCI*	~2%	15q25-26	140,147	Monoubiquitinated by FA complex in a FANCD2 dependent manner. It is also phosphorylated after DNA damage.	Yes	Normal
FA-J	*FANCJ/ BRIP1*	~0.2%	17q22-24	140	DNA dependent ATPase and a 5'-3' DNA helicase. Binds the BCRT domain of BRCA1. Cancer susceptibility gene.	No	Normal
FA-L	*FANCL (PHF9)*	~0.2%	2p16.1	43	Core complex member with Ubiquitin ligase activity.	Yes	Normal
FA-M	*FANCM/HEF*	~0.2%	14q21.3	250	Member of the core complex with translocase activity and endonuclease domain. DNA damage sensor.	Yes/ Partially in mice	Normal
FA-N	*FANCN/ PALB2*	~2%	16p12.1	140	Partner and localizer of BRCA2. It links BRCA2 and BRCA1 proteins. Cancer susceptibility gene.	No	Reduced
FA-O	*RAD51C**	~0.2%	17q22	43	Participates in several protein complexes involved in HR. Cancer susceptibility gene.	No	Reduced
FA-P	*SLX4*	~0.2%	16p13.3	200	Scaffold protein for endonucleases MUS81-EME1 and XPF-ERCC1. Involved in resolution of HR intermediates such as Holliday junctions.	No	Normal

* *FANCO/RAD51C* is not formally defined as a FA gene. NLS; nuclear localization signals. HR; Homologous recombination

Table 1. Main characteristics of the Fanconi anemia genes and their proteins.



Step I:

•FANCM-FAAP24-MHF complex move across the DNA

Step II:

•Incision in the DNA and FANCM-FAAP24-MHF complex recruitment of the FA core complex

Step III:

•Monoubiquitination of FANCD2-I complex and accumulation of FANCD2-I in the chromatin

Step IV:

•Recruitment of downstream porteins involved in HR.

•Unhook of ICL by MUS81-EME1 and XPF-ERCC1 and possibly FAN1.

•Repair of the DSB by HR machinery.

Fig. 1. Description of the four essential steps in the repair of DNA interstrand cross-links of the FA/BRCA pathway (Adapted from Niedernhofer [39] , Kee et al.[29], Valeri *et al*. [41] and Cybulski and Howlett[42]).

3. Clinical features of Fanconi anemia

Fanconi anemia is a rare autosomal recessive disease (except for FA-B which is X-linked), with an estimated frequency of 1-5 per 100,000 births. Although the presentation of the disease is very heterogeneous, around 70% of FA patients show physical anomalies, such as skeletal abnormalities, including radial hypoplasia, short stature and microphtalmia. Most FA patients present hypo or hyperpigmentation of the skin, showing the characteristic *café au lait* spots, and with a lower frequency, cardiac, renal, gastrointestinal and other organ malformations [43]. The most common feature of the disease is the bone marrow failure (BMF), which is manifested at a median age of 8 years, being the primary cause of morbidity and mortality in FA patients[44]. Clinical data from the International Fanconi anemia Registry (IFAR) and the German Fanconi anemia Registry (GEFA) have shown that virtually all FA patients would develop BMF by the age of 40 years[45, 46]. At birth, the blood counts are usually normal, macrocytosis usually being the first symptom detected in these patients, followed by thrombocytopenia and neutropenia. Pancytopenia is generally presented between 5 and 10 years of age. The German and American studies showed that the use of a defined abnormality score, based on the analysis of five different congenital abnormalities, can significantly predict the development of the BMF in FA patients[46, 47].

In addition to the BMF, the incidence of cancer in FA patients is also markedly increased compared to the normal population, the incidence of myelodisplasia (MDS) or acute myeloid leukemia (AML) being 33% at the age of 40 years (more than eight hundred fold increased incidence compared to a healthy population). Reports from the IFAR and GEFA have also shown that FA patients are at extraordinary risk of developing specific solid tumors, such as head and neck squamous cell carcinomas (HN-SCCs; with several hundred fold increased frequency), esophagus SCCs (several thousand fold increase) and vulvar cancer in women (also several thousand fold increase)[46, 48, 49].GEFA also observed an increase in breast and brain tumors, while the North American study observed an increased incidence of cervical,ostesarcoma and liver tumors [46].

Myelodisplasia is often presented as a refractory cytopenia with multilineage dysplasia, with or without excess of blasts[49]. Acute myeloid leukemia can be diagnosed primarily or after a MDS phase, with an increasing fraction of blast cells in the BM[50]. The high selective pressure during their teens or early adulthood is probably involved in the development of clonal MDS and AML. The most common abnormalities found in FA patients with MDS/AML are gains of chromosome 1q, monosomy 7, gains of 3q (where *EVI1* is included) [44, 49-51], and abnormalities in *RUNX1/AML1* gene at chromosome 21q[50]. Results by Quentin *et al* also suggest a model of multi-step oncogenesis progression in the BM of FA patients, in which 1q+ (which can be found in the aplastic anemia form of the disease) would possibly constitute the initiating event, while the 3q+, -7/7q and *RUNX1* abnormalities would lead to high grade MDS or AML. In this model 1q+ might clonally rescue the BMF of FA patients, but would not protect against progression towards MDS and leukemia[50].

A remarkable aspect concerning the clinical symptoms of the disease is the observation that some of FA patients can undergo somatic mosaicism by means of a spontaneous reverse mutation or mitotic recombination in one of the FA pathogenic mutations in particular somatic cells[52-54]. If such a reversion occurs in a hematopoietic progenitor or stem cell (HSC), it may confer a proliferation advantage to the reverted cell, leading to the recovery of the BMF in the patient[52-55].

A recent paper described for the first time a characteristic phenotype in a number of FA patients who showed two of the FA hallmark features (high number of chromosomal aberrations after DNA damage and absence of FANCD2 monoubiquitination), compatible with normalized hematological counts[56]. Remarkably, blood cells from these patients did not show the FA characteristic G2/M cell cycle arrest after exposure to DNA cross-linkers[56]. Moreover, in contrast to mosaic FA patients, no reversion in the pathogenic mutations occurred in this new group of FA patients. Based on the above observations, a phenomenon defined as attenuation of the phenotype was described in FA[56]. Attenuation was associated with almost normal blood cell counts, and in some cases with development of MDS or AML. This process was accompanied by clonal hematopoiesis, implying that all peripheral blood cells derive from a single progenitor cell, in which a molecular event reducing CHK1 expression took place, resulting in the attenuated G2 arrest. The conclusions of this work have important implications in the management of FA patients because patients with this phenotype, although having essentially normal blood cell counts, should be followed closely to prevent the development of MDS or AML. Additionally, this study offers new explanations that could account for differences in the severity of the disease between FA siblings with the same mutation. Another important study has recently shown that FA proteins also play a role in mitosis, since FANCD2, FANCI and FANCM are localized to the extremities of ultrafine DNA bridges (UFBs), which link sister chromatids during cell division. FA cells show increase number of UFBs that may inhibit cytokinesis, leading to binucleated or multinucleated cells, a phenomenon that could account for the increased apoptosis and thus, also contribute to BMF [57, 58].

4. Main phenotypic characteristics of Fanconi anemia cells

FA cells are hypersensitive to ICL agents such as mitomycin C (MMC) and diepoxibutane (DEB). Additionally, these drugs specifically induce a high number of chromosomal aberrations in FA cells[59, 60], thus constituting a hallmark for the diagnosis of FA patients[60, 61]. Although normal cells exposed to DNA cross-linkers develop a transient accumulation in the G2/M phase of the cell cycle, the defective ability of FA cells to repair the DNA damage leads to a very significant accumulation of these cells in G2/M after exposure to ICLs[62]. This property has been also used for the diagnosis of FA patients, particularly in skin fibroblasts for the characterization of FA mosaic patients in which no obvious phenotypic markers may be apparent in their peripheral blood cells[63, 64].

FA cells are also characterized by their hypersensitivity to ambient oxygen conditions [65-67], manifested by a poor *ex vivo* growth and clonogenic capacity. This impaired growth properties of FA cells can be significantly restored when the incubation atmosphere is changed to hypoxic conditions (<5% of oxygen)[67], mimicking the low oxygen concentrations present in most tissues, including the BM niche[68, 69].

FA cells also show an increased apoptotic predisposition, something that may account for the BMF of these patients and for the development of malformations during embryonic development. One of the biochemical pathways involved in the increased apoptosis of FA cells is related to the over-production of cytokines such as TNFα and interferon-γ [70, 71]; an observation that has been confirmed in BM from FA patients[72].

FA patient hematopoietic progenitor cells have also shown a defective adhesion and homing activities, associated with an aberrant regulation of Cdc42 activity[73].

5. Identification of complementation groups in Fanconi anemia patients

As described before, fourteen different FA genes have been described so far, whose mutations account for the different FA complementation groups already identified (Table 1). However, there is still a number of patients with clinical symptoms of FA without mutations in any of the 14 FA genes, suggesting that more FA genes will be added to this long list. The identification of the FA complementation group (FA subtyping) in a patient has several advantages in the management of the disease. In this respect FA subtyping 1) confirms the FA diagnosis; 2) facilitates the identification of pathogenic mutations in FA genes, 3) allows to investigate relationships between the phenotype and the genotype in the patients, and 4) it is required for a potential future treatment of the patient by gene therapy.

Once a patient is diagnosed with FA, several alternatives have been used for FA subtyping. Because of the technical difficulties of the cell fusion approaches initially developed by Buschwald[4, 5], Hanenberg *et al.* in 2002 developed a new strategy based on genetic complementation strategies with retroviral vectors harboring the different FA genes[74]. This strategy, combined with Western blot analyses, principally of the monoubiquitinated and non ubiquitinated forms of FANCD2 (to detect if the mutation is upstream or downstream FANCD2 monoubiquitination), and also with the analysis of foci of nuclear proteins, has allowed the assignment of FA patients to the different complementation groups[75]. Sequencing has also been used [76]however, the large number and complexity of some FA genes and their mutations, together with the necessity of verifying the pathogenicity of each new mutation, implies that subtyping of patients with FA by mutational analysis is often time consuming and laborious [75]. Nevertheless, sequencing combined with genetic complementation strategies can be useful to deeply characterize FA patients.

6. Current treatments of the bone marrow failure in Fanconi anemia patients

As discussed above, the BMF is the main cause of mortality in FA patients. Many of the treatments of the BMF are palliative and directed to maintain acceptable numbers of peripheral blood cells. The use of androgens, in some cases combined with corticoids, constitute one of the most common treatments of FA patients in early stages of the disease, when a residual endogenous hematopoiesis remains[43, 77]. However, not all the patients respond to this treatment and, in most cases the response is slow, transient and normally limited to the red blood cells. Although a longer survival has been reported in patients who have been treated with androgens, in comparison with those who have not (20 years *vs* 14 years) [77], it has been reported that their use might constitute an adverse predictor when hematopoietic cell transplantation (HCT) is required [78]. Additionally, side effects such as liver tumors[48, 79] and masculinization may occur when androgens are used[80].

The use of growth factors to activate specific hematopoietic lineages has been also used in FA patients[81, 82], although generally with a limited success due to the transient benefit [82] and risks of leukemia due to the activation of potential pre-leukemic clones already present in the patient[83].

So far, the only curative treatment capable of restoring the hematopoiesis of FA patients in the long-term is allogenic HCT. Many of the obstacles initially found for the HCT of FA patients have been overcome nowadays. The hypersensitivity of FA patients to conditioning regimens was a limiting factor for the success of the first HCTs in FA patients. However,

conditioning is necessary to eliminate the endogenous hematopoiesis and allow the engraftment of donor cells. In 1984, Gluckman *et al*. [84] developed the first successful conditioning regimen for FA patients consisting of a low dose of cyclophosphamide (CY) and a single dose of total body irradiation (TBI). Since then, many different protocols have been developed aiming to limit the radiation exposure in HCT preparative regimens[85], and thus to minimize risks of malignancies in the long-term[77]. As a result of these improvements, current HCTs from HLA(Human Leukocyte Antigen) identical siblings do not generally include irradiation in the conditioning. The use of mild conditioning regimens and the inclusion of fludarabine (FLU) (an antimetabolite with profound immunosuppressive effects) has, therefore, markedly improved the outcome of HCTs with HLA-matched grafts from related donors.

Although the outcome of transplants from alternative donors in FA has also markedly improved in the last decade, the morbidity and mortality associated to these transplants is still significant[86]. Problems like graft failure, acute and chronic graft versus host disease (GVHD) and opportunistic infections are the major obstacles to address. Again, the inclusion of FLU and the use of T cell depletion (TCD) have significantly improved the efficacy of the transplant of FA patients from unrelated donors[87]. Recent clinical trials conducted in the University of Minnesota have shown the relevance of thymic shielding during irradiation with reduced doses of TBI to limit opportunistic infections, and thus to increase overall survivals after unrelated HCTs in FA patients[88]. Ideally, transplantation should be done previously to the development of a myelodysplasic syndrome or leukemia[88]. The success of HCTs in patients that already have developed any of these pathologies is limited in comparison to those who have not. The main risk associated with the HCT of these patients is that the low doses of radio/chemotherapy that must be used in FA patients might not be enough to destroy the endogenous leukemic cells, thus increasing the risk of future relapses [88].

Traditionally the preferential source for the HCT for FA patients was BM or mobilized progenitors from peripheral blood (mPB). However, umbilical cord blood is nowadays also a good alternative for the transplantation of FA patients. This is not only the case for the HCT from HLA-identical siblings - in some instances derived from *in vitro* fertilization and preimplantation genetic diagnosis - but also from unrelated donors[89].

7. Gene therapy as a new strategy for the treatment of Fanconi anemia

Hematopoietic gene therapy, defined as the HCT of genetically corrected autologous HSCs, is considered a good alternative to allogenic HCT in FA. This strategy would avoid GVHD and limit, at least partially, the side effects associated to severe chemo/radiotherapy and immunosupression (See review in [90]).

The previous observation that a number of mosaic FA patients (those who have reverted a pathogenic mutation in a HSC) could progressively improve their hematological status[52-55] opened the possibility of rescuing the BMF of FA patients after the infusion of gene-corrected HSCs, even in the absence of conditioning. As it is the case with HSCs that have reverted a pathogenic mutation, it is expected that *ex vivo* corrected FA HSCs may also develop a proliferation advantage over uncorrected cells, thus restoring progressively hematopoietic system of the patient.

To allow the stable integration of the transgene in the HSCs genome, gamma-retroviral vectors (RVs) have been the most frequently used vectors in clinical gene therapy protocols

[90, 91]. The principle of most of these protocols was based on the purification of $CD34^+$ cells, either from BM or mPB, followed by the transduction with the therapeutic vector, and the re-infusion of the transduced cells in the patient, either pre-conditioned or not[91-94].
Two different gene therapy trials have been already conducted in FA. The first one was developed by Liu and colleagues in FA-C patients [95] and the second one by Kelly and colleagues in FA-A patients [96]. Both protocols used similar conditions to those previously used for the gene therapy of other monogenic diseases, such as X1-SCID. Essentially, in both protocols *in vitro* pre-stimulated $CD34^+$ cells were transduced with RVs for three or four days in culture, and thereafter infused into non conditioned patients. In contrast to the results observed in X1-SCID patients, none of these protocols improved the clinical status of FA patients, indicating the necessity of improving the therapeutic vector and/or the manipulation of the target cells.

8. Towards the development of improved protocols of Fanconi anemia gene therapy

8.1 Lessons from Fanconi anemia mouse models

Mouse models represent an invaluable tool for improving the understanding of the mechanisms responsible of different pathologies, and also for developing new therapies with improved efficacy and reduced side effects. In the case of FA, where intrinsic difficulties exist to engraft inmunodeficient mice with BM from FA patients, the relevance of mouse models is even higher.
Different FA mouse models with disruptions in FA genes such as *Fanca*[97, 98], *Fancc*[99, 100], *Fancd1(Brca2)*[101], *Fancd2*[102], *Fancg*[103], *Fancm*[104] and more recently *Fancp* (*Slx4*)[105] have been generated (See Table 2). Additionally, a FA mouse model based on the deletion of *Usp1* (the enzyme responsible for *FANCD2* deubiquitination) has been described[106]. This gene, however, is not currently considered a FA gene since no FA patients have been so far identified with *Usp1*mutations.
Although all FA mouse models are characterized by their hypersensitivity to DNA-cross-linking agents and in some instances to cytokines such as TNFα and IFN((See Table 2), the severe BMF that takes place in FA patients is far from being reproduced in these models. In fact, only the recently developed mouse model of FA-P ($Btbd12^{-/-}$ mice) is prone to develop marked blood cytopenias, reflected by a reduction in white blood cells (WBCs) and platelets in a significant number of animals[105] (Table 2).
Aiming to generate FA mouse models that resemble more closely the disease observed in FA patients, double knock-outs have been also generated. Among these mouse models, only the double knock-out $Fancc^{-/-}/Fancg^{-/-}$ mice [107] and the $Fancc^{-/-}/Sod1^{-/-}$ mice showed evidences of BMF [108].
Significantly, although some FA mouse models are prone to develop tumors (i.e. FA-D1, FA-D2, FA-M), AML is not spontaneously generated in the FA mouse models generated so far. Studies conducted in $Fancc^{-/-}$ mice have shown, however, that the *ex vivo* culture and/or incubation of $Fancc^{-/-}$ BM cells with TNF-α, whose expression is significantly increased in FA patients, induces leukemic clonal evolution after transplantation [109, 110], suggesting that leukemia development in FA patients could be at least partially related to the deregulated expression of this cytokine.
$Fanca^{-/-}$ as well as $Fancc^{-/-}$ mice, and also mice with a hypomorphic mutation in *Brca2/Fancd1* (FA-D1 mice) constitute the FA mouse models more frequently used both to understand the

role of FA genes in HSCs functionality, and also to evaluate the preclinical efficacy of new therapies in FA. Defects in the HSCs have been observed in all tested FA mouse models, not only in terms of clonogenic potential, but also regarding the engrafting ability and repopulating properties of these cells (see Table 2). Although no aplastic anemia was observed in FA-D1 mice, this mouse model showed a more severe hematopoietic phenotype compared to other models with mutations in FA genes upstream in the FA/BRCA pathway[111]. In this respect, results from our laboratory showed a defective function in the repopulating potential of endogenous FA-D1 HSCs in their own natural microenvironment. This was demonstrated by the observation that BM cells from WT animals could repopulate in the long-term the hematopoietic tissues of FA-D1 unconditioned recipients [111]. This contrasts with studies conducted in other FA mouse models where only after the treatment with IFNγ [112, 113] or DNA damaging drugs, wild-type BM cells could be engrafted in FA recipients (Table 2).

In the field of gene therapy, it was shown for the first time in *Fancc*$^{-/-}$ mice that the retroviral-mediated expression of *Fancc* corrects the defective repopulation ability of FA HSCs[114]. Similar conclusions were obtained in different FA mouse models using RVs, Lentiviral and also Foamyviral vectors (LVs and FVs; Table 1). Significantly, Li *et al*. showed that the *ex vivo* culture of *Fancc*$^{-/-}$ HSCs increases apoptosis and promotes the development of clonal aberrations[109]. Studies conducted with *Fanca*$^{-/-}$ and *Fancc*$^{-/-}$ mice showed, on the other hand, that rapid transductions with LVs or FVs markedly improved the repopulating properties of the HSCs (Table 2). Taken together, these studies suggest the convenience of using similar short-transduction protocols in human FA gene therapy.

Working with the FA-D1 mouse model, our group showed that the infusion of LV-transduced cells in mice pre-treated with a mild conditioning results in a progressive increase in the proportion of genetically-corrected cells, in the absence of any selection treatment. This is in contrast to data obtained in other FA mouse models, where exposures to cytokines or DNA damaging agents were required (Table 2). Moreover, our data showed that in the long term after transplantation, most of the hematopoietic cells of recipient FA-D1 mice were resistant to otherwise cytototoxic doses of MMC and became genetically stable [115], suggesting that a similar proliferation advantage of *ex vivo* corrected HSCs may occur in FA clinical trials.

8.2 Lessons from *in vitro* studies conducted with bone marrow samples from FA patients

Based on the current knowledge on the biology of FA cells, it is now clear that marked differences distinguish FA HSCs from HSCs successfully treated with gene therapy (i.e. X1-SCID HSCs). In this respect, it has been already shown that in the case of FA HSCs, *in vitro* incubation induces apoptosis and genomic instability [109, 116, 135]. Therefore, it is now considered that short transduction strategies would improve the possibilities of engrafting FA patients with genetically corrected cells, as it has been already shown in FA mouse models [119, 125].

Because of the limited number of hematopoietic progenitors and HSCs present in the BM of FA patients [136], we hypothesized that these precursor cells would be actively cycling in the patient and therefore, directly susceptible to transduction with RVs without further *in vitro* stimulation. Consistent with this hypothesis, our studies showed that hematopoietic progenitors from FA patients can be efficiently transduced by Gibbon Ape Leukemia Virus

(GALV)-packaged RVs in protocols that lasted only 12-24 h [136]. Whether or not the most primitive HSCs were also efficiently transduced in these protocols is, however, unknown due to current limitations to engraft immunodeficient animals with BM from FA patients.

DEFECTIVE GENE	HYPERSENSITIVITY		HEMATOPOIESIS		GENE THERAPY	
	MMC	CTKs	BMF	HSC Defects	*In vivo* efficacy	*In vivo* proliferation advantage
Fanca	[97, 116]	IFN-γ [113]	Mild hrombocyt [97,116]	CRA and HSC Mobilization[117]	LVs [118] LVs[119]	+ MMC: [118] + Chemo: [119]
Fancc	[99],[120]	TNFα: - In vitro[110, 121] - In vivo [113] IFN-γ: - In vitro[100, 109] - In vivo [112, 113] - Not sensitive [122]		CRA [123] HSC Growth[124] CRA [125] HSC Engraftment [117]	RVs: [114, 126] [127] [110] [128] LVs: [129] FVs: [125]	+MMC[126] +CPA / IR:[128] + MMC[129]
Fancd1/Brca2	[111]			CRA[111]	[115]	No Treatment [115]
Fancd2	[102],[106],[130]			CRA [130]		
Fancg	[103],[131]	IFN-γ [113]		Microenvironment defects [132]		
Fancm	[104]					
Fancp/Btbd12	[105]		Mild cytopenias [105]			
Usp1	[106]			CRA[130]		
Fancc/Fancc	[133]					
Fanca/Fancg	[107]		Mild cytopenias [107]	CRA [107]		
Fanca/SOD			Anemia and leukop. [108]	CFCs defects [108]		
Fancd2/Mlh1	[134]					

BMF: Bone marrow failure; CTK: Cytokines; CFC: colony forming cell; Chemo: chemotherapy; CPA: cyclophosfamide; CRA: Competitive repopulation ability; FV: foamyviral vector; HSC: hematopoietic stem cell; IR: Ionizing radiation; LV: lentiviral vector; MMC: Mitomycin C; RV: Retroviral vector

Table 2. Principal characteristics of the hematopoietic system of FA mouse models and results from *ex vivo* HSC gene therapy.

Experimental studies [137, 138] and more recently also human trials[139, 140] have demonstrated that LVs currently constitute the most efficient clinical vectors for stably transducing HSCs after very short transduction periods. Additional studies have shown that these vectors facilitate the stable expression of the transgene *in vivo*, while transgene inactivation has been frequently reported in RVs[137]. Finally, but not less importantly, LVs have shown improved safety properties, compared to the RVs already used in human gene therapy [141-145].

Concerning the level of expression that is required for inducing a therapeutic effect in FA cells, we have recently shown in FA-A and FA-D2 cells that a weak expression of FA genes, at least *FANCA* and *FANCD2*, is sufficient to revert the FA phenotype of hematopoietic cells ([146],[147] and unpublished data). Because FA-A is the most frequent FA complementation group [75, 148] our group [147], as well as Hans-Peter Kiem group[149] have proposed independently the same LV construct for the gene therapy of FA patients. In this vector *FANCA* expression is driven by the phosphoglycerate kinase (PGK) promoter and stabilized by a mutated version of the posttranscriptional regulatory element (WPRE) [147, 149](see Figure 2).

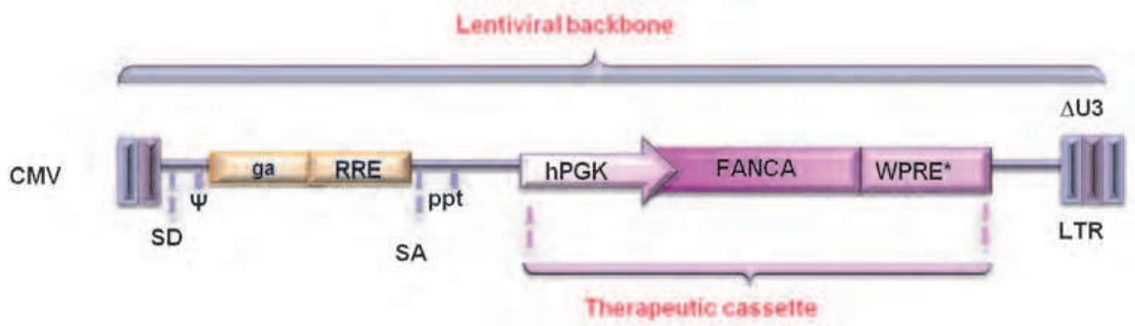


Fig. 2. Illustration of the FANCA-LV proposed independently by González-Murillo *et al* [147] and Becker *et al* [149] for the gene therapy of FA-A patients.

Studies conducted with BM samples from FA patients have also shown that FA progenitor cells are highly sensitive to cytokines such as TNF-α or IFN- γ and also to reactive oxygen species (ROS) [121, 150-154]. Thereafter it was shown that the use of *ex vivo* manipulation conditions that limit the oxidative damage (hypoxia or inclusion of N-acetyl-cysteine) improved the growth of FA progenitor cells[149, 155]. Similar observations were obtained when TNF-α was specifically inhibited with antibodies[72, 136]. Based on these observations and with the aim of improving the repopulation potential of genetically corrected HSCs, many of the experimental protocols aiming the genetic correction of FA HSCs use hypoxia and antibody-mediated inhibition of TNF-α[149, 155].

Concerning the ideal target population to be transduced in FA gene therapy trials, we reasoned that, if possible, the transduction of total BM would be the preferential option[155]. This suggestion derives from the fact that in FA every type of HSC, and also of accessory BM cell, would be directly affected by the genetic defect. Therefore, the genetic correction of each of these populations might be useful for the engraftment of the patient. Using GALV-TR (modified GALV envelope) packaged LVs carrying *FANCA* and/or *EGFP*, we demonstrated the possibility of efficiently transducing hematopoietic and mesenchymal progenitor cells in FA BM samples subjected to a very simple erythrocyte's depletion[155]. Although our data showed that, in contrast to LVs packaged with the G-protein from Vesicular Stomatitis Virus (VSV-G), GALV-TR packaged LVs can efficiently transduce FA BM samples at low multiplicities of infection (around 1-3 infective units/cell), there are still limitations in the production of GMP GALV-TR LVs at high titers. Because high MOIs (Multiplicity of infection) of VSV-G LVs produced in conditions approved for clinical use (GMP) are required to achieve efficient transductions, purified $CD34^+$ or $CD133^+$ cells currently constitute the preferential populations to be used in the next FA gene therapy protocols[156].

9. Perspectives for the future gene therapy of Fanconi anemia patients

The discovery by S. Yamanaka that the transfer of a few transcription factors can reprogram adult somatic cells and generate induced pluripotential cells (iPS cells) [157] has opened new perspectives for the cell and gene therapy of different diseases, particularly in FA. The collaborative study conducted between our group and J.C. Izpisua-Belmonte and J. Surralles groups demonstrated for the first time the possibility of generating disease-free hematopoietic progenitors from genetically corrected fibroblasts from patients with a monogenic disease,

specifically FA patients[158, 159]. Although the technology related to iPS cell generation should be further improved both in terms of efficiency and safety, these strategies have opened an unpredicted applicability in the management of genetic diseases like FA.

10. Concluding remarks

Since the description of the first FA patient by Guido Fanconi in 1927, an extraordinary advance in the understanding of the mechanisms accounting for the disease has occurred. Although more work is still required to elucidate the interactions between the FA/BRCA pathway with the different mechanisms of DNA repair, the relevance of this pathway in the repair of different insults to the DNA is now clear, accounting for the involvement of the FA/BRCA pathway in hereditary and also acquired cancer. Significant advances in the management of the hematopoietic syndromes that FA patients suffer have been produced within the last years, particularly in the field of hematopoietic transplantation. Thanks to these advantages, FA is nowadays not considered a restricted pediatric disease. New challenges have therefore emerged, particularly due to the necessity of developing improved therapies for syndromes that appear in adult FA patients, such as squamous cell carcinomas.

As happened in other diseases, gene therapy was soon considered a good option for the treatment of the BMF in FA patients. Although intrinsic difficulties in the manipulation of FA HSCs have limited the success of FA gene therapy, new vectors and improved FA HSC manipulations have emerged from studies conducted with FA mouse models and with samples from FA patients. All these technical advances have opened new hopes for the application of gene therapy in FA.

Finally, as it was the case with the clinical application of cord blood cells [160] or cells derived from siblings selected by pre-implantation genetic diagnosis[161, 162], FA is the first genetic disease where disease-free blood cells from non hematopoietic tissues, have been generated [158]. It is our hope that all these advances may have a translational clinical impact in our patients in the near future.

11. Acknowledgements

The authors are indebted to FA patients and their families for their kind cooperation with our research in the field of FA. The authors thank Antonio Valeri and José A. Casado for the careful reading of the manuscript and helpful suggestions. Studies conducted at the Division of Hematopoiesis and Gene Therapy, CIEMAT/CIBERER are supported by grants from the European Program "7FWP, Health" (PERSIST; Ref Grant Agreement no: 222878), the Ministry of Science and Innovation: Programa de Fomento de Cooperación Científica Internacional (110-90.1) and Plan Nacional de Salud y Farmacia (SAF 2009-07164) and Fondo de Investigaciones Sanitarias, ISCIII (Programa RETICS-RD06/0010/0015). The authors also thank the Fundación Botín for promoting translational research. CIBERER is an initiative of the Instituto de Salud Carlos III.

12. References

[1] Fanconi G. Familiäre infantile perniziosaartige Anämie (perniciziöses Blutbild und Konstitution). Jahrbuch für Kinderheikunde und Erziehung (Wien). 1927;117:257-80.

[2] Lobitz S, Velleuer E. Guido Fanconi (1892-1979): a jack of all trades. Nat Rev Cancer. 2006 Nov;6(11):893-8.
[3] Schroeder T, Anschutz F, Knopp A. Spontaneous Chromosome aberrations in Familial Panmyelopathy. Human Genetic. 1964;1:194-6.
[4] Duckworth-Rysiecki G, Cornish K, Clarke C, Buchwald M. Identification of two complementation groups in Fanconi anemia. Somat Cell Mol Genet. 1985;11::35-41.
[5] Strathdee CA, Gavish H, Shannon WR, Buchwald M. Cloning of cDNAs for Fanconi's anaemia by functional complementation. Nature. 1992 Apr 30;356(6372):763-7.
[6] consortium FaBc. Positional cloning of the Fanconi anaemia group A gene. Nat Genet. 1996 Nov;14(3):324-8.
[7] de Winter JP, Leveille F, van Berkel CG, Rooimans MA, van Der Weel L, Steltenpool J, et al. Isolation of a cDNA representing the Fanconi anemia complementation group E gene. American journal of human genetics. 2000 Nov;67(5):1306-8.
[8] de Winter JP, Rooimans MA, van Der Weel L, van Berkel CG, Alon N, Bosnoyan-Collins L, et al. The Fanconi anaemia gene FANCF encodes a novel protein with homology to ROM. Nat Genet. 2000 Jan;24(1):15-6.
[9] de Winter JP, Waisfisz Q, Rooimans MA, van Berkel CG, Bosnoyan-Collins L, Alon N, et al. The Fanconi anaemia group G gene FANCG is identical with XRCC9. Nat Genet. 1998 Nov;20(3):281-3.
[10] Lo Ten Foe JR, Rooimans MA, Bosnoyan-Collins L, Alon N, Wijker M, Parker L, et al. Expression cloning of a cDNA for the major Fanconi anaemia gene, FAA. Nat Genet. 1996 Nov;14(3):320-3.
[11] Timmers C, Taniguchi T, Hejna J, Reifsteck C, Lucas L, Bruun D, et al. Positional cloning of a novel Fanconi anemia gene, FANCD2. Mol Cell. 2001 Feb;7(2):241-8.
[12] Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, et al. Biallelic inactivation of BRCA2 in Fanconi anemia. Science. 2002 Jul 26;297(5581):606-9.
[13] Meetei AR, de Winter JP, Medhurst AL, Wallisch M, Waisfisz Q, van de Vrugt HJ, et al. A novel ubiquitin ligase is deficient in Fanconi anemia. Nat Genet. 2003 Oct;35(2):165-70.
[14] Meetei AR, Levitus M, Xue Y, Medhurst AL, Zwaan M, Ling C, et al. X-linked inheritance of Fanconi anemia complementation group B. Nat Genet. 2004 Nov;36(11):1219-24.
[15] Levitus M, Waisfisz Q, Godthelp BC, de Vries Y, Hussain S, Wiegant WW, et al. The DNA helicase BRIP1 is defective in Fanconi anemia complementation group J. Nat Genet. 2005 Sep;37(9):934-5.
[16] Levran O, Attwooll C, Henry RT, Milton KL, Neveling K, Rio P, et al. The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. Nat Genet. 2005 Sep;37(9):931-3.
[17] Litman R, Peng M, Jin Z, Zhang F, Zhang J, Powell S, et al. BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCJ. Cancer Cell. 2005 Sep;8(3):255-65.
[18] Reid S, Schindler D, Hanenberg H, Barker K, Hanks S, Kalb R, et al. Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer. Nat Genet. 2006 Dec 31.

[19] Meindl A, Hellebrand H, Wiek C, Erven V, Wappenschmidt B, Niederacher D, et al. Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. Nat Genet. 2010 May;42(5):410-4.
[20] Vaz F, Hanenberg H, Schuster B, Barker K, Wiek C, Erven V, et al. Mutation of the RAD51C gene in a Fanconi anemia-like disorder. Nat Genet. 2010 May;42(5):406-9.
[21] Seal S, Thompson D, Renwick A, Elliott A, Kelly P, Barfoot R, et al. Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. Nat Genet. 2006 Nov;38(11):1239-41.
[22] Garcia MJ, Benitez J. The Fanconi anaemia/BRCA pathway and cancer susceptibility. Searching for new therapeutic targets. Clin Transl Oncol. 2008 Feb;10(2):78-84.
[23] Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, et al. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat Genet. 2006 Dec 31.
[24] Wong MW, Nordfors C, Mossman D, Pecenpetelovska G, Avery-Kiejda KA, Talseth-Palmer B, et al. BRIP1, PALB2, and RAD51C mutation analysis reveals their relative importance as genetic susceptibility factors for breast cancer. Breast cancer research and treatment. 2011 Mar 16.
[25] Dorsman JC, Levitus M, Rockx D, Rooimans MA, Oostra AB, Haitjema A, et al. Identification of the Fanconi anemia complementation group I gene, FANCI. Cell Oncol. 2007;29(3):211-8.
[26] Sims AE, Spiteri E, Sims RJ, 3rd, Arita AG, Lach FP, Landers T, et al. FANCI is a second monoubiquitinated member of the Fanconi anemia pathway. Nat Struct Mol Biol. 2007 Jun;14(6):564-7.
[27] Smogorzewska A, Matsuoka S, Vinciguerra P, McDonald ER, 3rd, Hurov KE, Luo J, et al. Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. Cell. 2007 Apr 20;129(2):289-301.
[28] Meetei AR, Medhurst AL, Ling C, Xue Y, Singh TR, Bier P, et al. A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. Nat Genet. 2005 Sep;37(9):958-63.
[29] Kee Y, D'Andrea AD. Expanded roles of the Fanconi anemia pathway in preserving genomic stability. Genes Dev. 2010 Aug 15;24(16):1680-94.
[30] Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, et al. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. Mol Cell. 2001 Feb;7(2):249-62.
[31] Wang W. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. Nat Rev Genet. 2007 Oct;8(10):735-48.
[32] Kratz K, Schopf B, Kaden S, Sendoel A, Eberhard R, Lademann C, et al. Deficiency of FANCD2-associated nuclease KIAA1018/FAN1 sensitizes cells to interstrand crosslinking agents. Cell. 2010 Jul 9;142(1):77-88.
[33] Carmona R, Cano E, Grueso E, Ruiz-Villalba A, Bera T, Gaztambide J, et al. Peritoneal repairing cells: A type of bone marrow-derived progenitor cells involved in mesothelial regeneration. J Cell Mol Med. 2010 May 14.
[34] MacKay C, Declais AC, Lundin C, Agostinho A, Deans AJ, MacArtney TJ, et al. Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2. Cell. 2010 Jul 9;142(1):65-76.

[35] Smogorzewska A, Desetty R, Saito TT, Schlabach M, Lach FP, Sowa ME, et al. A genetic screen identifies FAN1, a Fanconi anemia-associated nuclease necessary for DNA interstrand crosslink repair. Mol Cell. 2010 Jul 9;39(1):36-47.
[36] Kim Y, Lach FP, Desetty R, Hanenberg H, Auerbach AD, Smogorzewska A. Mutations of the SLX4 gene in Fanconi anemia. Nat Genet. 2011 Jan 16.
[37] Stoepker C, Hain K, Schuster B, Hilhorst-Hofstee Y, Rooimans MA, Steltenpool J, et al. SLX4, a coordinator of structure-specific endonucleases, is mutated in a new Fanconi anemia subtype. Nat Genet. 2011 Jan 16.
[38] Ciccia A, Ling C, Coulthard R, Yan Z, Xue Y, Meetei AR, et al. Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM. Mol Cell. 2007 Feb 9;25(3):331-43.
[39] Niedernhofer LJ. The Fanconi anemia signalosome anchor. Mol Cell. 2007 Feb 23;25(4):487-90.
[40] O'Donnell L, Durocher D. DNA repair has a new FAN1 club. Mol Cell. 2010 Jul 30;39(2):167-9.
[41] Valeri A, Martinez S, Casado JA, Bueren JA. Fanconi anaemia: from a monogenic disease to sporadic cancer. Clin Transl Oncol. 2011 Apr;13(4):215-21.
[42] Cybulski KE, Howlett NG. FANCP/SLX4: A Swiss Army knife of DNA interstrand crosslink repair. Cell Cycle. 2011 Jun 1;10(11).
[43] Dokal I. Fanconi's anaemia and related bone marrow failure syndromes. Br Med Bull. 2006;77-78:37-53.
[44] Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, Auerbach AD. Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. Blood. 1994 Sep 1;84(5):1650-5.
[45] Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF, et al. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood. 2003 Feb 15;101(4):1249-56.
[46] Rosenberg PS, Alter BP, Ebell W. Cancer risks in Fanconi anemia: findings from the German Fanconi Anemia Registry. Haematologica. 2008 Mar 5.
[47] Rosenberg PS, Huang Y, Alter BP. Individualized risks of first adverse events in patients with Fanconi anemia. Blood. 2004 Jul 15;104(2):350-5.
[48] Alter BP, Greene MH, Velazquez I, Rosenberg PS. Cancer in Fanconi anemia. Blood. 2003 Mar 1;101(5):2072.
[49] Cioc AM, Wagner JE, MacMillan ML, DeFor T, Hirsch B. Diagnosis of myelodysplastic syndrome among a cohort of 119 patients with fanconi anemia: morphologic and cytogenetic characteristics. American journal of clinical pathology. 2010 Jan;133(1):92-100.
[50] Quentin S, Cuccuini W, Ceccaldi R, Nibourel O, Pondarre C, Pages MP, et al. Myelodysplasia and leukemia of Fanconi anemia are associated with a specific pattern of genomic abnormalities that includes cryptic RUNX1/AML1 lesions. Blood. 2011 Mar 3.
[51] Tonnies H, Huber S, Kuhl JS, Gerlach A, Ebell W, Neitzel H. Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients: gains of the chromosomal segment 3q26q29 as an adverse risk factor. Blood. 2003 May 15;101(10):3872-4.

[52] Gregory JJ, Jr., Wagner JE, Verlander PC, Levran O, Batish SD, Eide CR, et al. Somatic mosaicism in Fanconi anemia: evidence of genotypic reversion in lymphohematopoietic stem cells. Proc Natl Acad Sci U S A. 2001 Feb 27;98(5):2532-7.

[53] Gross M, Hanenberg H, Lobitz S, Friedl R, Herterich S, Dietrich R, et al. Reverse mosaicism in Fanconi anemia: natural gene therapy via molecular self-correction. Cytogenet Genome Res. 2002;98(2-3):126-35.

[54] Waisfisz Q, Morgan NV, Savino M, de Winter JP, van Berkel CG, Hoatlin ME, et al. Spontaneous functional correction of homozygous fanconi anaemia alleles reveals novel mechanistic basis for reverse mosaicism. Nat Genet. 1999 Aug;22(4):379-83.

[55] Soulier J, Leblanc T, Larghero J, Dastot H, Shimamura A, Guardiola P, et al. Detection of somatic mosaicism and classification of Fanconi anemia patients by analysis of the FA/BRCA pathway. Blood. 2005 Feb 1;105(3):1329-36.

[56] Ceccaldi R, Briot D, Larghero J, Vasquez N, Dubois d'Enghien C, Chamousset D, et al. Spontaneous abrogation of the G2 DNA damage checkpoint has clinical benefits but promotes leukemogenesis in Fanconi anemia patients. J Clin Invest. 2010 Dec 22.

[57] Mason PJ, Bessler M. Cytokinesis failure and attenuation: new findings in Fanconi anemia. J Clin Invest. 2011 Jan 4;121(1):27-30.

[58] Vinciguerra P, Godinho SA, Parmar K, Pellman D, D'Andrea AD. Cytokinesis failure occurs in Fanconi anemia pathway-deficient murine and human bone marrow hematopoietic cells. J Clin Invest. 2010 Nov 1;120(11):3834-42.

[59] Auerbach AD, Rogatko A, Schroeder-Kurth TM. International Fanconi Anemia Registry: relation of clinical symptoms to diepoxybutane sensitivity. Blood. 1989 Feb;73(2):391-6.

[60] Castella M, Pujol R, Callen E, Ramirez MJ, Casado JA, Talavera M, et al. Chromosome fragility in patients with Fanconi anaemia: diagnostic implications and clinical impact. J Med Genet. 2011 Jan 7.

[61] Auerbach AD, Wolman SR. Susceptibility of Fanconi's anaemia fibroblasts to chromosome damage by carcinogens. Nature. 1976 Jun 10;261(5560):494-6.

[62] Seyschab H, Friedl R, Sun Y, Schindler D, Hoehn H, Hentze S, et al. Comparative evaluation of diepoxybutane sensitivity and cell cycle blockage in the diagnosis of Fanconi anemia. Blood. 1995 Apr 15;85(8):2233-7.

[63] Neveling K, Endt D, Hoehn H, Schindler D. Genotype-phenotype correlations in Fanconi anemia. Mutat Res. 2009 Jul 31;668(1-2):73-91.

[64] Pulsipher M, Kupfer GM, Naf D, Suliman A, Lee JS, Jakobs P, et al. Subtyping analysis of Fanconi anemia by immunoblotting and retroviral gene transfer. Mol Med. 1998 Jul;4(7):468-79.

[65] Joenje H, Arwert F, Eriksson AW, de Koning H, Oostra AB. Oxygen-dependence of chromosomal aberrations in Fanconi's anaemia. Nature. 1981 Mar 12;290(5802):142-3.

[66] Saito H, Hammond AT, Moses RE. Hypersensitivity to oxygen is a uniform and secondary defect in Fanconi anemia cells. Mutat Res. 1993 Oct;294(3):255-62.

[67] Schindler D, Hoehn H. Fanconi anemia mutation causes cellular susceptibility to ambient oxygen. American journal of human genetics. 1988 Oct;43(4):429-35.

[68] Chow DC, Wenning LA, Miller WM, Papoutsakis ET. Modeling pO(2) distributions in the bone marrow hematopoietic compartment. I. Krogh's model. Biophysical journal. 2001 Aug;81(2):675-84.
[69] Parmar K, Mauch P, Vergilio JA, Sackstein R, Down JD. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. Proc Natl Acad Sci U S A. 2007 Mar 27;104(13):5431-6.
[70] Rosselli F, Sanceau J, Gluckman E, Wietzerbin J, Moustacchi E. Abnormal lymphokine production: a novel feature of the genetic disease Fanconi anemia. II. In vitro and in vivo spontaneous overproduction of tumor necrosis factor alpha. Blood. 1994 Mar 1;83(5):1216-25.
[71] Schultz JC, Shahidi NT. Tumor necrosis factor-alpha overproduction in Fanconi's anemia. Am J Hematol. 1993 Feb;42(2):196-201.
[72] Dufour C, Corcione A, Svahn J, Haupt R, Poggi V, Beka'ssy AN, et al. TNF-alpha and IFN-gamma are overexpressed in the bone marrow of Fanconi anemia patients and TNF-alpha suppresses erythropoiesis in vitro. Blood. 2003 Sep 15;102(6):2053-9.
[73] Zhang X, Shang X, Guo F, Murphy K, Kirby M, Kelly P, et al. Defective homing is associated with altered Cdc42 activity in cells from Fanconi anemia group A patients. Blood. 2008 Jun 18.
[74] Hanenberg H, Batish SD, Pollok KE, Vieten L, Verlander PC, Leurs C, et al. Phenotypic correction of primary Fanconi anemia T cells with retroviral vectors as a diagnostic tool. Exp Hematol. 2002 May;30(5):410-20.
[75] Casado JA, Callen E, Jacome A, Rio P, Castella M, Lobitz S, et al. A comprehensive strategy for the subtyping of Fanconi Anemia patients: conclusions from the Spanish Fanconi Anemia research network. J Med Genet. 2007 Apr;44(4):241-9.
[76] Ameziane N, Errami A, Leveille F, Fontaine C, de Vries Y, van Spaendonk RM, et al. Genetic subtyping of Fanconi anemia by comprehensive mutation screening. Hum Mutat. 2008 Jan;29(1):159-66.
[77] Dufour C, Svahn J. Fanconi anaemia: new strategies. Bone Marrow Transplant. 2008 Jun;41 Suppl 2:S90-5.
[78] Guardiola P, Pasquini R, Dokal I, Ortega JJ, van Weel-Sipman M, Marsh JC, et al. Outcome of 69 allogeneic stem cell transplantations for Fanconi anemia using HLA-matched unrelated donors: a study on behalf of the European Group for Blood and Marrow Transplantation. Blood. 2000 Jan 15;95(2):422-9.
[79] Velazquez I, Alter BP. Androgens and liver tumors: Fanconi's anemia and non-Fanconi's conditions. Am J Hematol. 2004 Nov;77(3):257-67.
[80] Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev. 2010 May;24(3):101-22.
[81] Guinan EC, Lopez KD, Huhn RD, Felser JM, Nathan DG. Evaluation of granulocyte-macrophage colony-stimulating factor for treatment of pancytopenia in children with fanconi anemia. The Journal of pediatrics. 1994 Jan;124(1):144-50.
[82] Rackoff WR, Orazi A, Robinson CA, Cooper RJ, Alter BP, Freedman MH, et al. Prolonged administration of granulocyte colony-stimulating factor (filgrastim) to patients with Fanconi anemia: a pilot study. Blood. 1996 Sep 1;88(5):1588-93.
[83] Tischkowitz M, Dokal I. Fanconi anaemia and leukaemia - clinical and molecular aspects. Br J Haematol. 2004 Jul;126(2):176-91.

[84] Gluckman E, Berger R, Dutreix J. Bone marrow transplantation for Fanconi anemia. Seminars in hematology. 1984 Jan;21(1):20-6.
[85] Tan PL, Wagner JE, Auerbach AD, Defor TE, Slungaard A, Macmillan ML. Successful engraftment without radiation after fludarabine-based regimen in Fanconi anemia patients undergoing genotypically identical donor hematopoietic cell transplantation. Pediatr Blood Cancer. 2006 May 1;46(5):630-6.
[86] Wagner JE, Eapen M, MacMillan ML, Harris RE, Pasquini R, Boulad F, et al. Unrelated donor bone marrow transplantation for the treatment of Fanconi anemia. Blood. 2007 Mar 1;109(5):2256-62.
[87] MacMillan ML, Hughes MR, Agarwal S, Daley GQ. Cellular therapy for fanconi anemia: the past, present, and future. Biol Blood Marrow Transplant. 2011 Jan;17(1 Suppl):S109-14.
[88] Macmillan ML, Wagner JE. Haematopoeitic cell transplantation for Fanconi anaemia - when and how? Br J Haematol. 2010 Feb 5;Epub 2010 Feb 5.
[89] Gluckman E, Rocha V, Ionescu I, Bierings M, Harris RE, Wagner J, et al. Results of unrelated cord blood transplant in fanconi anemia patients: risk factor analysis for engraftment and survival. Biol Blood Marrow Transplant. 2007 Sep;13(9):1073-82.
[90] Muller LU, Williams DA. Finding the needle in the hay stack: hematopoietic stem cells in Fanconi anemia. Mutat Res. 2009 Jul 31;668(1-2):141-9.
[91] Naldini L. Ex vivo gene transfer and correction for cell-based therapies. Nat Rev Genet. 2011 May;12(5):301-15.
[92] Aiuti A, Slavin S, Aker M, Ficara F, Deola S, Mortellaro A, et al. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. Science. 2002;296(5577):2410-3.
[93] Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, Gross F, Yvon E, Nusbaum P, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science. 2000;288(5466):669-72.
[94] Gaspar HB, Parsley KL, Howe S, King D, Gilmour KC, Sinclair J, et al. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. Lancet. 2004 Dec 18-31;364(9452):2181-7.
[95] Liu JM, Kim S, Read EJ, Futaki M, Dokal I, Carter CS, et al. Engraftment of hematopoietic progenitor cells transduced with the Fanconi anemia group C gene (FANCC). Hum Gene Ther. 1999 Sep 20;10(14):2337-46.
[96] Kelly PF, Radtke S, Kalle C, Balcik B, Bohn K, Mueller R, et al. Stem cell collection and gene transfer in fanconi anemia. Mol Ther. 2007 Jan;15(1):211-9.
[97] Cheng NC, van de Vrugt HJ, van der Valk MA, Oostra AB, Krimpenfort P, de Vries Y, et al. Mice with a targeted disruption of the Fanconi anemia homolog Fanca. Hum Mol Genet. 2000 Jul 22;9(12):1805-11.
[98] Wong JC, Alon N, McKerlie C, Huang JR, Meyn MS, Buchwald M. Targeted disruption of exons 1 to 6 of the Fanconi Anemia group A gene leads to growth retardation, strain-specific microphthalmia, meiotic defects and primordial germ cell hypoplasia. Hum Mol Genet. 2003 Aug 15;12(16):2063-76.
[99] Chen M, Tomkins DJ, Auerbach W, McKerlie C, Youssoufian H, Liu L, et al. Inactivation of Fac in mice produces inducible chromosomal instability and reduced fertility reminiscent of Fanconi anaemia. Nat Genet. 1996 Apr;12(4):448-51.

[100] Whitney MA, Royle G, Low MJ, Kelly MA, Axthelm MK, Reifsteck C, et al. Germ cell defects and hematopoietic hypersensitivity to gamma-interferon in mice with a targeted disruption of the Fanconi anemia C gene. Blood. 1996 Jul 1;88(1):49-58.

[101] McAllister KA, Bennett LM, Houle CD, Ward T, Malphurs J, Collins NK, et al. Cancer susceptibility of mice with a homozygous deletion in the COOH-terminal domain of the Brca2 gene. Cancer Res. 2002 Feb 15;62(4):990-4.

[102] Houghtaling S, Timmers C, Noll M, Finegold MJ, Jones SN, Meyn MS, et al. Epithelial cancer in Fanconi anemia complementation group D2 (Fancd2) knockout mice. Genes Dev. 2003 Aug 15;17(16):2021-35.

[103] Yang Y, Kuang Y, De Oca RM, Hays T, Moreau L, Lu N, et al. Targeted disruption of the murine Fanconi anemia gene, Fancg/Xrcc9. Blood. 2001 Dec 1;98(12):3435-40.

[104] Bakker ST, van de Vrugt HJ, Rooimans MA, Oostra AB, Steltenpool J, Delzenne-Goette E, et al. Fancm-deficient mice reveal unique features of Fanconi anemia complementation group M. Hum Mol Genet. 2009 Sep 15;18(18):3484-95.

[105] Crossan GP, van der Weyden L, Rosado IV, Langevin F, Gaillard PH, McIntyre RE, et al. Disruption of mouse Slx4, a regulator of structure-specific nucleases, phenocopies Fanconi anemia. Nat Genet. 2011 Feb;43(2):147-52.

[106] Kim JM, Parmar K, Huang M, Weinstock DM, Ruit CA, Kutok JL, et al. Inactivation of murine Usp1 results in genomic instability and a Fanconi anemia phenotype. Dev Cell. 2009 Feb;16(2):314-20.

[107] Pulliam-Leath AC, Ciccone SL, Nalepa G, Li X, Si Y, Miravalle L, et al. Genetic disruption of both Fancc and Fancg in mice recapitulates the hematopoietic manifestations of Fanconi anemia. Blood. 2010 Oct 21;116(16):2915-20.

[108] Hadjur S, Ung K, Wadsworth L, Dimmick J, Rajcan-Separovic E, Scott RW, et al. Defective hematopoiesis and hepatic steatosis in mice with combined deficiencies of the genes encoding Fancc and Cu/Zn superoxide dismutase. Blood. 2001 Aug 15;98(4):1003-11.

[109] Li X, Le Beau MM, Ciccone S, Yang FC, Freie B, Chen S, et al. Ex vivo culture of Fancc -/- stem/ progenitor cells predisposes cells to undergo apoptosis and surviving stem/progenitor cells display cytogenetic abnormalities and an increased risk of malignancy. Blood. 2005 Jan 11;105(9):3465-71.

[110] Li J, Sejas DP, Zhang X, Qiu Y, Nattamai KJ, Rani R, et al. TNF-alpha induces leukemic clonal evolution ex vivo in Fanconi anemia group C murine stem cells. J Clin Invest. 2007 Nov;117(11):3283-95.

[111] Navarro S, Meza NW, Quintana-Bustamante O, Casado JA, Jacome A, McAllister K, et al. Hematopoietic dysfunction in a mouse model for Fanconi anemia group D1. Mol Ther. 2006 Oct;14(4):525-35.

[112] Li X, Yang Y, Yuan J, Hong P, Freie B, Orazi A, et al. Continuous in vivo infusion of Interferon-gamma (IFN-{gamma}) preferentially reduces myeloid progenitor numbers and enhances engraftment of syngeneic wildtype cells in Fancc-/- mice. Blood. 2004 Apr 27.

[113] Si Y, Ciccone S, Yang FC, Yuan J, Zeng D, Chen S, et al. Continuous in vivo infusion of interferon-gamma (IFN-gamma) enhances engraftment of syngeneic wild-type cells in Fanca-/- and Fancg-/- mice. Blood. 2006 Dec 15;108(13):4283-7.

[114] Gush KA, Fu KL, Grompe M, Walsh CE. Phenotypic correction of Fanconi anemia group C knockout mice. Blood. 2000 Jan 15;95(2):700-4.

[115] Rio P, Meza NW, Gonzalez-Murillo A, Navarro S, Alvarez L, Surralles J, et al. In vivo proliferation advantage of genetically corrected hematopoietic stem cells in a mouse model of Fanconi anemia FA-D1. Blood. 2008 Sep 23;112:4853-61.

[116] Rio P, Segovia JC, Hanenberg H, Casado JA, Martinez J, Gottsche K, et al. In vitro phenotypic correction of hematopoietic progenitors from Fanconi anemia group A knockout mice. Blood. 2002 Sep 15;100(6):2032-9.

[117] Milsom MD, Schiedlmeier B, Bailey J, Kim MO, Li D, Jansen M, et al. Ectopic HOXB4 overcomes the inhibitory effect of tumor necrosis factor-{alpha} on Fanconi anemia hematopoietic stem and progenitor cells. Blood. 2009 Mar 6.

[118] Yamada K, Ramezani A, Hawley RG, Ebell W, Arwert F, Arnold LW, et al. Phenotype correction of Fanconi anemia group A hematopoietic stem cells using lentiviral vector. Mol Ther. 2003 Oct;8(4):600-10.

[119] Muller LU, Milsom MD, Kim MO, Schambach A, Schuesler T, Williams DA. Rapid Lentiviral Transduction Preserves the Engraftment Potential of Fanca(-/-) Hematopoietic Stem Cells. Mol Ther. 2008 June;16((6):):1154-60.

[120] Carreau M, Gan OI, Liu L, Doedens M, McKerlie C, Dick JE, et al. Bone marrow failure in the Fanconi anemia group C mouse model after DNA damage. Blood. 1998 Apr 15;91(8):2737-44.

[121] Otsuki T, Nagakura S, Wang J, Bloom M, Grompe M, Liu JM. Tumor necrosis factor-alpha and CD95 ligation suppress erythropoiesis in Fanconi anemia C gene knockout mice. J Cell Physiol. 1999 Apr;179(1):79-86.

[122] Kurre P, Anandakumar P, Grompe M, Kiem HP. In vivo administration of interferon gamma does not cause marrow aplasia in mice with a targeted disruption of FANCC. Exp Hematol. 2002 Nov;30(11):1257-62.

[123] Haneline LS, Gobbett TA, Ramani R, Carreau M, Buchwald M, Yoder MC, et al. Loss of FancC function results in decreased hematopoietic stem cell repopulating ability. Blood. 1999 Jul 1;94(1):1-8.

[124] Aube M, Lafrance M, Charbonneau C, Goulet I, Carreau M. Hematopoietic stem cells from fancc(-/-) mice have lower growth and differentiation potential in response to growth factors. Stem Cells. 2002;20(5):438-47.

[125] Si Y, Pulliam AC, Linka Y, Ciccone S, Leurs C, Yuan J, et al. Overnight transduction with foamyviral vectors restores the long-term repopulating activity of Fancc-/- stem cells. Blood. 2008 Aug 6.

[126] Gush KA, Fu KL, Grompe M, Walsh CE. Phenotypic correction of Fanconi anemia group C knockout mice. Blood. 2000;95(2):700-4.

[127] Haneline LS, Li X, Ciccone SL, Hong P, Yang Y, Broxmeyer HE, et al. Retroviral-mediated expression of recombinant Fancc enhances the repopulating ability of Fancc-/- hematopoietic stem cells and decreases the risk of clonal evolution. Blood. 2003 Feb 15;101(4):1299-307.

[128] Noll M, Bateman RL, D'Andrea AD, Grompe M. Preclinical protocol for in vivo selection of hematopoietic stem cells corrected by gene therapy in Fanconi anemia group C. Mol Ther. 2001;3(1):14-23.

[129] Galimi F, Noll M, Kanazawa Y, Lax T, Chen C, Grompe M, et al. Gene therapy of Fanconi anemia: preclinical efficacy using lentiviral vectors. Blood. 2002 Oct 15;100(8):2732-6.

[130] Parmar K, Kim J, Sykes SM, Shimamura A, Stuckert P, Zhu K, et al. Hematopoietic stem cell defects in mice with deficiency of Fancd2 or Usp1. Stem Cells. 2010 Jul;28(7):1186-95.

[131] Koomen M, Cheng NC, van De Vrugt HJ, Godthelp BC, van Der Valk MA, Oostra AB, et al. Reduced fertility and hypersensitivity to mitomycin C characterize Fancg/Xrcc9 null mice. Hum Mol Genet. 2002;11(3):273-81.

[132] Li Y, Chen S, Yuan J, Yang Y, Li J, Ma J, et al. Mesenchymal stem/progenitor cells promote the reconstitution of exogenous hematopoietic stem cells in Fancg-/- mice in vivo. Blood. 2009 Jan 7.

[133] Noll M, Battaile KP, Bateman R, Lax TP, Rathbun K, Reifsteck C, et al. Fanconi anemia group A and C double-mutant mice: functional evidence for a multi-protein Fanconi anemia complex. Exp Hematol. 2002 Jul;30(7):679-88.

[134] van de Vrugt HJ, Eaton L, Hanlon Newell A, Al-Dhalimy M, Liskay RM, Olson SB, et al. Embryonic lethality after combined inactivation of Fancd2 and Mlh1 in mice. Cancer Res. 2009 Dec 15;69(24):9431-8.

[135] Cumming RC, Lightfoot J, Beard K, Youssoufian H, O'Brien PJ, Buchwald M. Fanconi anemia group C protein prevents apoptosis in hematopoietic cells through redox regulation of GSTP1. Nat Med. 2001 Jul;7(7):814-20.

[136] Jacome A, Navarro S, Casado JA, Rio P, Madero L, Estella J, et al. A simplified approach to improve the efficiency and safety of ex vivo hematopoietic gene therapy in fanconi anemia patients. Hum Gene Ther. 2006 Feb;17(2):245-50.

[137] Guenechea G, Gan OI, Inamitsu T, Dorrell C, Pereira DS, Kelly M, et al. Transduction of human CD34+ CD38- bone marrow and cord blood-derived SCID-repopulating cells with third-generation lentiviral vectors. Mol Ther. 2000;1(6):566-73.

[138] Naldini L, Blomer U, Gallay P, Ory D, Mulligan R, Gage FH, et al. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science. 1996;272(5259):263-7.

[139] Cartier N, Hacein-Bey-Abina S, Bartholomae CC, Veres G, Schmidt M, Kutschera I, et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. Science. 2009 Nov 6;326(5954):818-23.

[140] Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, et al. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature. 2011 Sep 16;467(7313):318-22.

[141] Mitchell RS, Beitzel BF, Schroder AR, Shinn P, Chen H, Berry CC, et al. Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences. PLoS Biol. 2004 Aug;2(8):E234.

[142] Modlich U, Navarro S, Zychlinski D, Maetzig T, Knoess S, Brugman MH, et al. Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors. Mol Ther. 2009 Nov;17(11):1919-28.

[143] Montini E, Cesana D, Schmidt M, Sanvito F, Ponzoni M, Bartholomae C, et al. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. Nat Biotechnol. 2006 Jun;24(6):687-96.

[144] Wu X, Li Y, Crise B, Burgess SM. Transcription start regions in the human genome are favored targets for MLV integration. Science. 2003 Jun 13;300(5626):1749-51.

[145] Biffi A, Bartolomae CC, Cesana D, Cartier N, Aubourg P, Ranzani M, et al. Lentiviral-vector common integration sites in preclinical models and a clinical trial reflect a benign integration bias and not oncogenic selection. Blood. 2011 Mar 14.

[146] Almarza E, Rio P, Meza NW, Aldea M, Agirre X, Guenechea G, et al. Characteristics of lentiviral vectors harboring the proximal promoter of the vav proto-oncogene: a weak and efficient promoter for gene therapy. Mol Ther. 2007 Aug;15(8):1487-94.

[147] Gonzalez-Murillo A, Lozano ML, Alvarez L, Jacome A, Almarza E, Navarro S, et al. Development of lentiviral vectors with optimized transcriptional activity for the gene therapy of patients with Fanconi anemia. Hum Gene Ther. 2010 May;21(5):623-30.

[148] Taniguchi T, D'Andrea AD. Molecular pathogenesis of Fanconi anemia: recent progress. Blood. 2006 Jun 1;107(11):4223-33.

[149] Becker PS, Taylor JA, Trobridge GD, Zhao X, Beard BC, Chien S, et al. Preclinical correction of human Fanconi anemia complementation group A bone marrow cells using a safety-modified lentiviral vector. Gene Ther. 2010 May 20;Epub ahead of print.

[150] Haneline LS, Broxmeyer HE, Cooper S, Hangoc G, Carreau M, Buchwald M, et al. Multiple inhibitory cytokines induce deregulated progenitor growth and apoptosis in hematopoietic cells from Fac-/- mice. Blood. 1998 Jun 1;91(11):4092-8.

[151] Rathbun RK, Christianson TA, Faulkner GR, Jones G, Keeble W, O'Dwyer M, et al. Interferon-gamma-induced apoptotic responses of Fanconi anemia group C hematopoietic progenitor cells involve caspase 8-dependent activation of caspase 3 family members. Blood. 2000;96(13):4204-11.

[152] Zhang X, Li J, Sejas DP, Rathbun KR, Bagby GC, Pang Q. The Fanconi anemia proteins functionally interact with the protein kinase regulated by RNA (PKR). J Biol Chem. 2004 Oct 15;279(42):43910-9.

[153] Sejas DP, Rani R, Qiu Y, Zhang X, Fagerlie SR, Nakano H, et al. Inflammatory reactive oxygen species-mediated hemopoietic suppression in Fancc-deficient mice. J Immunol. 2007 Apr 15;178(8):5277-87.

[154] Vanderwerf SM, Svahn J, Olson S, Rathbun RK, Harrington C, Yates J, et al. TLR8-dependent TNF-(alpha) overexpression in Fanconi anemia group C cells. Blood. 2009 Dec 17;114(26):5290-8.

[155] Jacome A, Navarro S, Rio P, Yanez RM, Gonzalez-Murillo A, Lozano ML, et al. Lentiviral-mediated genetic correction of hematopoietic and mesenchymal progenitor cells from Fanconi anemia patients. Mol Ther. 2009 Jun;17(6):1083-92.

[156] Tolar J, Adair JE, Antoniou M, Bartholomae CC, Becker PS, Blazar BR, et al. Stem Cell Gene Therapy for Fanconi Anemia: Report from the 1st International Fanconi Anemia Gene Therapy Working Group Meeting. Mol Ther. May 3.

[157] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug 25;126(4):663-76.

[158] Raya A, Rodriguez-Piza I, Guenechea G, Vassena R, Navarro S, Barrero MJ, et al. Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. Nature. 2009 Jul 2;460(7251):53-9.

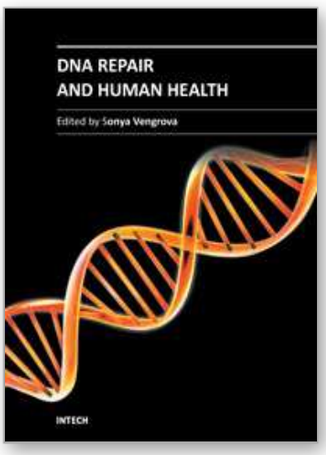
[159] Raya A, Rodriguez-Piza I, Navarro S, Richaud-Patin Y, Guenechea G, Sanchez-Danes A, et al. A protocol describing the genetic correction of somatic human cells and subsequent generation of iPS cells. Nat Protoc. 2010 Apr;5(4):647-60.

[160] Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med. 1989 Oct 26;321(17):1174-8.

[161] Grewal SS, Kahn JP, MacMillan ML, Ramsay NK, Wagner JE. Successful hematopoietic stem cell transplantation for Fanconi anemia from an unaffected HLA-genotype-identical sibling selected using preimplantation genetic diagnosis. Blood. 2004 Feb 1;103(3):1147-51.

[162] Verlinsky Y, Rechitsky S, Schoolcraft W, Strom C, Kuliev A. Designer babies - are they a reality yet? Case report: simultaneous preimplantation genetic diagnosis for Fanconi anaemia and HLA typing for cord blood transplantation. Reproductive biomedicine online. 2000;1(2):31.

IntechOpen



DNA Repair and Human Health
Edited by Dr. Sonya Vengrova

ISBN 978-953-307-612-6
Hard cover, 792 pages
Publisher InTech
Published online 26, October, 2011
Published in print edition October, 2011

Over the past decades, great advances have been made in understanding the cellular DNA repair pathways. At the same time, a wealth of descriptive knowledge of human diseases has been accumulated. Now, the basic research of the mechanisms of DNA repair is merging with clinical research, placing the action of the DNA repair pathways in the context of the whole organism. Such integrative approach enables understanding of the disease mechanisms and is invaluable in improving diagnostics and prevention, as well as designing better therapies. This book highlights the central role of DNA repair in human health and well-being. The reviews presented here, contain detailed descriptions of DNA repair pathways, as well as analysis of a large body of evidence addressing links between DNA damage repair and human health. They will be of interest to a broad audience, from molecular biologists working on DNA repair in any model system, to medical researchers.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Paula Río, Susana Navarro and Juan A. Bueren (2011). From the Molecular Biology to the Gene Therapy of a DNA Repair Syndrome: Fanconi Anemia, DNA Repair and Human Health, Dr. Sonya Vengrova (Ed.), ISBN: 978-953-307-612-6, InTech, Available from: http://www.intechopen.com/books/dna-repair-and-human-health/from-the-molecular-biology-to-the-gene-therapy-of-a-dna-repair-syndrome-fanconi-anemia

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen