1. Introduction

As part of the special issue on Fanconi anemia (FA), we would like to summarize current understanding on FA and how studies using DT40 cells have contributed to the field. DT40 is an avian B lymphocyte cell line, which has been derived from retrovirally induced lymphoma in the Bursa of Fabricius [1]. In 1991, Drs Jean Marie Buerstedde and Shunichi Takeda reported that this cell line has an extremely high capability in targeted integration of a plasmid DNA into homologous genomic locus [2]. This property is useful in making gene-disrupted cell lines, and DT40 has been exploited in a number of studies in cellular and molecular biology.

Our laboratory has been focusing on FA, a rare hereditary disorder characterized by progressive bone marrow failure, compromised genome stability, and increased incidence of cancer (for clinical aspects of FA, see "Fanconi Anemia and its Diagnosis" Auerbach, this issue) [3–6]. FA is caused by multiple genetic defects, and currently altogether 13 genes have been identified. Mutations in any of these genes result in a similar FA phenotype (except for FA-D1 and FA-N patients; they display severer symptoms), indicating that the FA gene products constitute a common biochemical network often referred to as "FA pathway".

Although we use other cell lines and systems as well, DT40 still remains our favorite model in analyzing molecular mechanisms of FA, since it is a quick and easy system to carry out "clean" genetic experiments. Gene targeting in DT40 cells normally needs screening only 20–30 clones to identify correct targeting events. They grow very fast, and are easy to handle. In contrast, cell lines from FA patients are generally difficult to grow, and even simple stable transfectants might be difficult to obtain. The RNA interference in cultured mammalian cells is certainly highly useful. However, it might not always lead to sufficient reduction of the protein expression, and the remaining protein levels may hamper definite interpretation of the data. In DT40 cells, sophisticated genetic manipulations such as conditional or double/triple knockouts have been achieved. Furthermore, we can introduce a subtle "knock-in" mutation into a locus without touching cis genetic elements. This seems highly useful for dissecting biochemical pathways. However, one potential drawback is that some antibodies against mammalian protein do not cross-react with the chicken protein. "Knock-in" of a fluorescent protein or an epitope tag could overcome this problem. Finally, DT40 is a chicken cell line, and we should be careful about the possibility that there could be some difference between human and chicken system, although fundamental molecular mechanisms are well conserved through evolution.

Based on the known structural and functional properties, the FA proteins may be classified into three groups: (1) components of the FA core complex (FANCA/B/C/E/F/G/L/M), (2) FANCD2 and FANCI, forming I-D complex, (3) breast cancer susceptibility proteins BRCA2/FANCD1, PALB2/FANCN, and BRIP1/FANCJ [5]. In addition, a few gene products have been discovered, which associate with the FA core complex (e.g. FAAP100 and FAAP24 proteins) but without known FA patients lacking these factors [5]. In Table 1, we summarized homologies between human FA genes and chicken counterparts. Fig. 1 shows chromosomal locations of the chicken FA genes as well as FA-related genes.

There is an established link between group (1) and group (2) proteins through the signal transduction pathway leading to FANCD2 monoubiquitination, while group (3) proteins are proposed to function down stream of the (1) and (2) proteins [5]. However, in our view, there is no hard evidence to support this hypothesis, and it is possible that they could function in a distinct manner to the rest of the FA proteins (see below). Therefore hereafter in this review, we will use the term "FA pathway" to indicate the FA core complex–FANCD2/FANCI axis consisting of group (1) and (2) proteins. In case we need to refer all of them including group (3) proteins, we would use the term "FA proteins".

Table 1
Chicken FA and FA-related proteins.

	Gene	Conserved motifs	Conservation to human protein[b]
Group (1)	FANCA		48/65
	FANCB[a]		42/62
	FANCC		49/65
	FANCE		39/52
	FANCF[a]		39/52
	FANCG/XRCC9	Tetratricopeptide repeats (TPR)	35/51
	FANCL	WD40, PHD finger	70/83
	FANCM/hHEF	DEAH helicase, XPF-family nuclease domain	44/60
	FAAP100		53/66
	FAAP24[a]	XPF-family nuclease domain	62/78
Group (2)	FANCD2	Monoubiquitination	56/72
	FANCI	Monoubiquitination S/TQ cluster	66/80
Group (3)	FANCD1/BRCA2	BRC repeat, OB fold	46/60
	FANCN/PALB2[a]		44/62
	FANCJ/BRIP1	DEAH helicase domain	55/70
E2	UBE2T[a]	UBC domain	65/77
Deubiquitinase	USP1		72/81

[a] We have not yet verified sequence data of these chicken genes deposited in the NCBI database.

[b] Percent identity/similarity to human homolog was assessed by BLASTP program at the NCBI website using full-length chicken amino acid sequence. In some of the alignments, there are gaps, resulting in overestimation of the actual overall identity/similarity. Thus the values should be regarded as a rough estimate.

2. FA is a disorder defective in DNA damage response/DNA repair

Genome stability is crucial for maintaining genetic integrity of the organism, and therefore all cells have elaborate systems to repair or tolerate endogenous or exogenous DNA damage. The S phase is a particularly vulnerable period to DNA damage. Presence of DNA lesion in the genome during S phase may cause arrest of DNA replication forks, which must be reinitiated to avoid fork collapse that potentially leads to lethal double-strand breaks (DSBs) or carcinogenic chromosomal rearrangements. Cells carry out this task by two basic mechanisms including (1) template switching to the intact sister chromatid using homologous recombination (HR) strategies, or (2) DNA damage bypass in which the specialized polymerases replicate past DNA lesions and fill single-stranded gaps (termed translesion synthesis, TLS). Masao Sasaki first reported in 1973 an important observation which potentially connects FA with such mechanisms: cells from FA patients exhibit highly elevated levels of chromosome aberrations upon treatment with DNA crosslinker (e.g. diepoxybutane or mitomycin C) [7]. This has led to the proposal that the basic defect in FA is somehow related to the cellular response to interstrand DNA crosslink (ICL) and its repair mechanisms [8]. Although current evidence clearly defines FA as a disorder defective in DNA damage response and/or repair, this has not been substantiated until very recently.

Probably the first concrete evidence that implicate an FA gene in DNA damage response came with the identification of *FANCD2* reported in 2001 [9]. Although FANCD2 protein has no functional motifs known to date, it responds by inducible monoubiquitination and formation of nuclear foci to the treatment with DNA damaging agents such as UV or MMC [10]. Furthermore, the FANCD2 foci

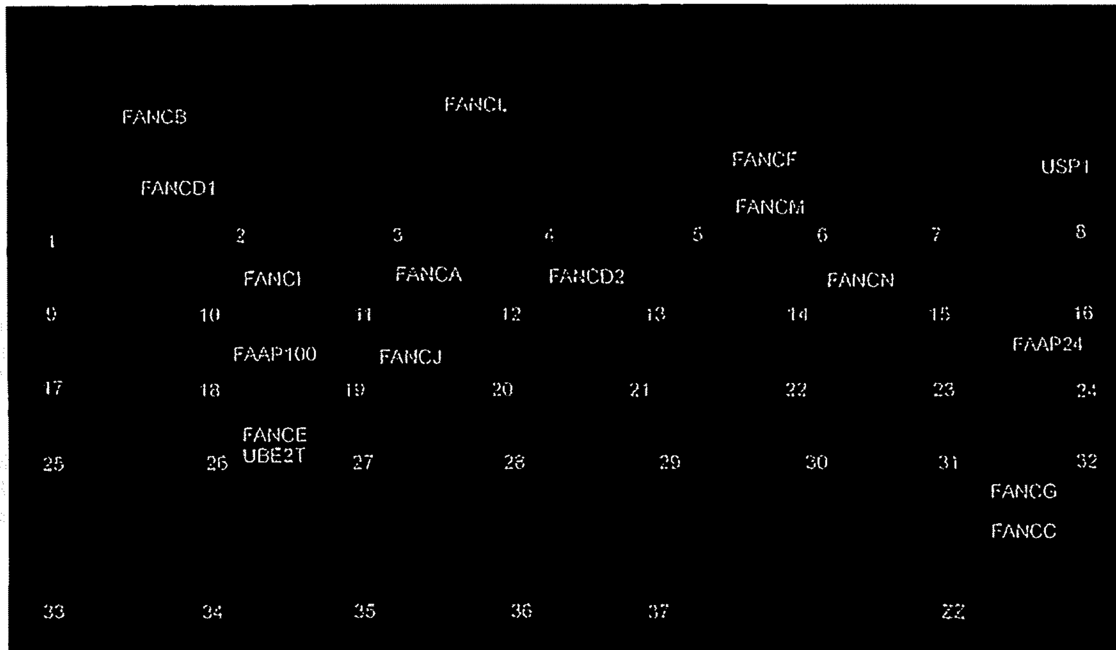

Fig. 1. Chromosomal localization of chicken FA genes. A metaphase spread of male chicken cells is stained with DAPI. Localization of FA and FA-related genes are indicated.

colocalize with BRCA1 as well as Rad51 [11]. Rad51 is a recombinase that plays a central role in HR. In the presence of accessory proteins (e.g. Rad51 paralogs), it polymerizes on 3′-protruded single-stranded DNA overhang such as resected DSB ends, leading to formation of the nucleoprotein filaments. This structure is central to the HR activities like homology search, strand invasion, and strand exchange [12]. On the other hand, BRCA1 provides important mediator function in DNA damage response including HR repair [13]. Heterozygous mutations in BRCA1 as well as BRCA2 account about 60% of the cases of familial breast cancer. Although they have a similar name, there is no structural homology between them.

It is notable that low levels of FANCD2 monoubiquitination occur spontaneously due to endogenous DNA damage as cells progress through S phase [11]. Furthermore, FANCD2 undergoes robust monoubiquitination in response to agents such as hydroxyurea (HU). Since the HU treatment arrests replication fork by depleting nucleotide pool in the cell, it seems likely that the replication fork stalling is the key phenomenon that triggers monoubiquitination.

Then there was the astonishing discovery that some FA cells (they mostly belong to the FA-D1 complementation group) carry biallelic hypomorphic mutations in the familial breast cancer gene BRCA2 [14]. Thus BRCA2 is now classified as the *FANCD1* gene. The BRCA2 protein is an important regulator of Rad51 by facilitating "nucleation" of the nucleoprotein filaments [15,16], corroborating the notion that the FA proteins function in HR repair.

We started working on FA in 2000 to test the hypothesis that FA genes may function in homologous recombination (HR). This idea was based on the observation that DT40 mutants, which lack Rad51 paralogs and thus are defective in HR, display similar phenotype to human FA cells such as MMC sensitivity or higher levels of chromosome breakage [17,18]. It seemed a good model system because we can use a few established HR assays in DT40. We created first *fancg* [19] then *fancd2* [20] knockout DT40 cell lines, and subjected them to the HR assays. For example, we looked at repair capacity of chromosomal DSB in the artificial recombination substrate SCneo carrying an I-SceI site in its defective neo gene (kindly provided by Dr. Maria Jasin, Sloan Kettering Institute). Introduction of the I-SceI expression construct induces DSB, which is repaired by HR using the upstream partial neo sequence as a temple. As a result, the correct HR repair events can be measured by neo-resistant colony formation. We also measured efficiency in gene targeting as ratio of clones that carry targeted to random integration events by Southern blotting following transfection of the targeting constructs. Gene targeting events have been reported to be defective in a number of HR-deficient cell lines [17,21]. In both of these assays, we found significant reduction in the HR events in both *fancd2* [20] or *fancg* [19] knockout mutants. Gene targeting efficiency is reduced also in *fancc* cells [22]. These observations were the first demonstration that cells deficient in FA genes are defective in HR repair. Later, it has been shown that human FA cells are mildly defective in HR using similar, but GFP-based, HR reporter assay [23]. However, whether such mild HR defects can explain extreme ICL sensitivity of FA cells is uncertain.

3. Studying FA gene function in the Ig diversification

In the Bursa of Fabricius, chicken B cells expand immunological repertoire by two mechanisms: gene conversion (GCV) and somatic hypermutation (SHM) (Fig. 2) [24]. GCV is basically achieved by non-reciprocal copying/pasting of genetic information from upstream pseudo gene segments (termed ψV) as a template to the Ig V genes (encoding the variable regions of heavy or light chain) by HR, whereas SHM introduces non-templated single nucleotide substitution due to TLS. TLS employs specialized DNA polymerases, which have in general a relaxed specificity, and are inherently muta-

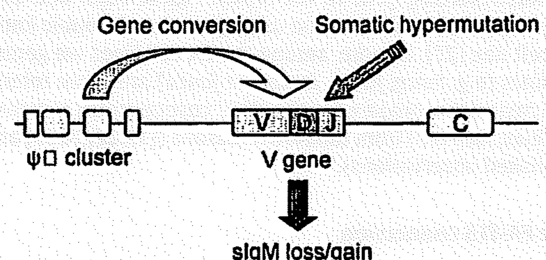

Fig. 2. Ongoing immunoglobulin diversification in DT40 cells. Following V(D)J recombination, Ig V genes are diversified through gene conversion or somatic hypermutation. See text for details. sIgM, surface IgM; V, variable region; C, constant region.

genic. Chicken has only one Ig V gene segment in each of heavy and light chain genes, thus needs such mechanisms to diversify the V genes and to protect against invading organisms. It is now thought that the common initiating event in GCV and SHM in the V gene segments is the conversion of deoxycytidine to uracil, which is mediated by B-cell specific factor activation-induced deaminase (AID) [25]. In turn, the uracil is catalyzed by uracil DNA glycosylase (UDG/UNG), resulting in an abasic site that is processed by either GCV or SHM mechanisms [26].

DT40 cells continue these processes *in vitro*, thus providing a great opportunity to examine how the FA pathway affects Ig diversification in a physiological context. The rate of GCV and SHM events can be estimated by assaying loss or gain of Ig surface expression since GCV and SHM may introduce or correct inactivating mutations (e.g. frame shifts) in the V genes. The exact nature of the events can be clarified by nucleotide sequencing. We looked at the Immunoglobulin (Ig) light chain locus in *fancd2* cells. Cells were subcloned by limiting dilution, and kept for a few weeks in culture. Then the expanded clonal subpopulations were tested for surface Ig expression as well as changes in the IgV sequence. We observed that, in *fancd2* cells, levels of the Ig GCV as well as the SHM were significantly reduced [20]. Patel and coworkers have used *fancc* knockout DT40 and reported similar data, consistent with our conclusion [27]. Collectively, these results implicate the core complex as well as FANCD2 in both HR and TLS processes in diversification of the Ig gene in the chicken B lymphocytes. Interestingly, it has been reported that human and hamster FA cells with or without mutagen treatment have lower levels of mutation frequency in relative to normal human cells, indicating conserved roles of the FA pathway in TLS mechanisms [28,29].

4. Epistasis analysis using FA/HR double knockouts

To further confirm the role of the FA pathway in HR, we wished to study epistasis between FA genes and the core HR machinery. In DT40 (like in yeast *S. cerevisiae*), it is relatively straight forward to make double knockouts, although you may have to first establish a conditional mutant in cases the gene(s) of interest is essential to maintain gene targeting efficiency. Since DT40 has only one copy of *FANCG* or *FANCC* locus – they are on the sex chromosome Z (Fig. 1) – and XRCC3 conditional knockout cell line was available [30], we have deleted *FANCC* and *FANCG* genes in this conditional background. Then the XRCC3 expression cassette was excised by tamoxifen-inducible Cre-loxP system. XRCC3 is one the five Rad51 paralogs, and plays an important role in the early phase HR as a cofactor for Rad51 [17], or possibly as a Holliday Junction resolvase at the late phase HR [31,32].

In colony survival in cisplatin-containing media, both *fancc/xrcc3* [22] and *fancg/xrcc3* [33] double knockouts displayed the same levels of sensitivity to *FANCC*- or *XRCC3*-deficient cells, respectively, revealing an epistatic relationship between the FA pathway and a Rad51paralog gene in ICL repair. Again, Patel and coworkers reported similar observation using *fancc/xrcc2* double knockout DT40 cell line [27]. Of note, among the FA cell lines we generated, some lines (e.g. *fancg*, *fance*, *fancm*, or *fanca*) are more tolerant to the ICL treatment compared to the other core complex mutants (e.g. *fancc*, *fancl*, *faap100*) from unknown reasons ([19,22,34,35] and our unpublished observations).

5. The FA-TLS connection

Analysis on hypermutation in the Ig V gene indicates that the core complex and FANCD2 might be involved in the damage bypass of the abasic sites created by the sequential actions of AID and UDG/UNG. Upon DNA damage, the RAD6/RAD18 heterodimeric complex interacts with ssDNA covered with RPA complex [36], and monoubiquitinates Lys164 of PCNA [37], the sliding clamp for DNA polymerases. RAD6 protein is an E2 enzyme that associates with the E3 ligase RAD18. The monoubiquitination is a critical event for replacing the replicative polymerase with specialized TLS polymerases such as POL η (polymerase switching) [38,39]. Many Y-family TLS polymerases (REV1, POL η, ι, and κ) might be recruited by monoubiquitinated PCNA since they have a motif (so called ubiquitin-binding domain) that specifically binds the ubiquitin moiety on PCNA [40]. Indeed, loss of REV1 or PCNA K164 (*PCNA* K164R mutation) can abrogate levels of non-templated mutations in DT40 [41,42]. However, the direct role of PCNA monoubiquitination in recruitment of the TLS polymerases has been recently challenged (for detailed review, see refs. [43,44]). Furthermore, REV1 protein can interact with multiple TLS polymerases (i.e. POL η, ι, κ, λ, as well as POL ζ comprising of REV3 and REV7) through the C-terminal ~100 aminoacids region of the REV1 protein, suggesting a non-catalytic role of REV1 in polymerase switching. Although TLS activity is under the strict control of RAD6/18 in yeast, in vertebrates there is evidence that defective PCNA monoubiquitination (*RAD18* mutation or *PCNA* K164R mutation) is not epistatic to *REV1* or *POLκ* mutant [42,45–47]. In any case, the mechanisms of TLS regulation is still unclear and under extensive investigation.

To gain insight into how the FA pathway participates in the TLS mechanism, we tested epistasis between *FANCC* and *RAD18* by disrupting *FANCC* gene in *RAD18*-deficient cells [22,48]. The double mutant was more sensitive than either single mutant in the cisplatin sensitivity assay, indicating that a role of the FA pathway in TLS is likely to be RAD18-independent [22]. Consistently, loss of RAD18 does not significantly affect FANCD2 monoubiquitination (our unpublished observation). Further data from two laboratories indicate that *FANCC* is epistatic in ICL sensitivity with the two TLS polymerases *REV1* and *REV3* [27,49]. Thus these data may indicate that the FA pathway act together with REV1 and REV3 in RAD18-independent TLS.

A recent study indicates that the assembly of DNA damage-induced REV1 foci is regulated by the core complex (but not by FANCD2) in a manner dependent on the BRCT domain but not the ubiquitin-binding UBM domain of REV1 [50] (for further discussion see "The Fanconi Anemia Protein Interaction Network: Casting a Wide Net" Rego et al., this issue). Although this finding does not entirely fit with our observation (the lower Ig mutation rate in *fancd2* cells as described above), this study may provide a hint regarding how TLS is regulated through the FA pathway. Although not directly relevant to FA, another recent study that utilized DT40 system has successfully dissected the TLS pathway into two temporally and genetically distinct phases: the TLS upon arrested fork and the postreplicative gap filling [46]. Interestingly, only the latter requires PCNA monoubiquitination, while C-terminal polymerase-interaction domain and the ubiquitin-binding domain of REV1 are needed for the former mechanism.

6. Spontaneous SCEs levels are increased in DT40 FA mutants

Recent studies indicate that sister chromatid exchanges actually represent crossover events between sister chromatids that accompany the HR-mediated restart of stalled replication forks [51]. The levels of the cytologically detectable SCEs are a good measure of cellular HR activity, and therefore it is of interest to look at SCE levels in FA cells. However, it has been reported that human FA cells in general do not have altered levels of SCE (for example, see ref. [52]). This may indicate that only a subset of HR events is affected in FA cells. In mitotic cells, HR generally proceeds in a pathway called synthesis-dependent strand annealing (SDSA) that *does not involve* a crossing over, and only a fraction of HR events accompanies forma-

tion of double Holliday junctions (DHJ) leading to the crossing over events [53]. We and others have noticed that elevated levels of spontaneous SCE are a consistent feature in DT40 FA mutant cell lines [19,20,22,27,54]. These observations may indicate that chicken, but not human, FA proteins are involved in the cross over control, and that function of the FA proteins is fundamentally different between the two species. Alternatively, the human FA pathway does provide minor contribution in regulating levels of the cross over, which is obscured by dominant function of other pathways. If this is the case, the FA pathway may somehow affect two subpathways of HR differently: the SDSA is promoted while the DHJ pathway is down regulated. However, this needs to be tested by further studies.

It is interesting to note that there is a physical association between the FA core complex and BLM helicase, one of the RecQ helicases which is defective in patients with Bloom syndrome [55]. A well-known feature of BLM cells is highly elevated levels of SCE, which is accounted for by the function of BLM helicase in promoting non-crossover events due to Holliday junction dissolution [56]. We observed *FANCC/BLM* double-deficient DT40 displayed the same high levels of SCE as *BLM* single knockout, suggesting that BLM helicase and FANCC function in a common pathway in suppressing SCE [22]. Furthermore, in both human and chicken cells, we found that *FANCD2* and *FANCC* is required for relocalization of GFP-BLM following DNA damage [22]. Thus it is plausible that deregulation of BLM helicase contribute to elevated SCE levels in DT40 FA mutants, and the FA pathway itself has no function in suppressing SCE.

There is another interesting possibility to explain the high levels of SCE. In the TLS-deficient DT40 mutants lacking such genes as *Rad18*, *REV1*, or *REV3*, the spontaneous SCE levels are increased similarly to the FA mutants [48,57]. Since the TLS mutants are less capable of bypassing DNA lesions, it is possible that more stalled replication forks would be channeled into the HR pathway, thus leading to more SCE events. The observed TLS defects in FA cells [20,27] and the epistasis between *FANCC* and *REV1* or *REV3* [27,49] might suggest that the increased SCE is explained by the defects in TLS. RAD18 seems irrelevant, since *RAD18/FANCC* double mutant displayed additive levels of SCE compared to either single mutant [22].

7. Monoubiquitination of FANCD2 is critical for DNA repair function of the FA pathway

The discovery that FANCD2 protein is inducibly monoubiquitinated and forms nuclear foci upon DNA damage is a landmark breakthrough [10]. Monoubiquitinated FANCD2 protein is designated FANCD2-L, while the unmodified form is called FANCD2-S. Importantly, the monoubiquitination and focus formation requires members of the core complex, suggesting that intact FA pathway serves to monoubiquitinate FANCD2 [10]. The monoubiquitination is a key event in the pathway, since FANCD2 mutant protein lacking the monoubiquitination site is unable to form foci, and cannot reverse ICL sensitivity in FA-D2 mutant cells [10].

Initially, BRCA1 was suggested to be the E3 ubiquitin ligase that monoubiquitinates FANCD2 [10]. However, it was soon revealed that FANCD2 monoubiquitination occurs normally in DT40 mutants lacking BRCA1 or its associated ring-finger protein BARD1, although the purified BRCA1/BARD1 dimer displays an *in vitro* ubiquitin ligase activity toward FANCD2 [58]. Instead Weidong Wang's group identified PHF9 as the potential E3 ligase, which associates with the core complex [59]. This protein was found to be defective in some FA patients and termed FANCL. It is now the prevailing view that the core complex is a multi-subunit E3 ligase for FANCD2 (and FANCI, which was discovered later) and its catalytic subunit is FANCL.

Our group independently identified PHF9, which has a signature motif (i.e. PHD/Ring-finger) for the E3 ligase function, as a potential FANCD2-interacting protein by the yeast two-hybrid screening [35]. Indeed, *PHF9/FANCL*-deficient DT40 displayed the FA phenotype as well as defective FANCD2 monoubiquitination. However, co-IP experiments have showed that FANCL–FANCD2 interaction is detectable only in transiently transfected 293T cells, suggesting that the interaction is weak [35]. Of note, FANCE protein is the only known component of the core complex, which is successfully co-immunoprecipitated with FANCD2 at endogenous expression level [60]. These results may suggest that FANCE is the core complex subunit that captures FANCD2 when the core complex monoubiquitinates FANCD2.

To more rigorously test the functional role of FANCD2 monoubiquitination, we generated cells carrying one *FANCD2* allele with the "knock-in" mutation (K563R) of the monoubiquitination site by gene targeting [35]. Since the other *FANCD2* allele was simply disrupted, the cells should express half amount of FANCD2 protein, which could not be monoubiquitinated, compared to the WT level. Western blotting confirmed this was the case, and not surprisingly the cells were exquisitely sensitive to cisplatin in both cell survival and levels of chromosome breakages. We biochemically separated the cells into soluble and chromatin fractions, and looked at the FANCD2 protein by western blotting with or without MMC treatment. In wild type cells, FANCD2-L form or S-form is predominantly detected in chromatin or soluble fraction, respectively. However, in the "knock-in" mutant or *fancl* cells, only S-form was detected mostly in soluble fraction, and we cannot detect FANCD2 focus formation in those cells after DNA damage [35]. We compared *fancd2-null*, *fancd2-K563R*, and *fancl* cells in the HR repair assay of the I-SceI-induced DSB, and found that these three cell lines displayed essentially indistinguishable levels of HR defects [35]. These data support the notion that monoubiquitination of FANCD2 is essential for the focus formation, chromatin loading, and HR repair function.

To monoubiquitinate FANCD2, the FA pathway requires a concerted action of the ubiquitin activating E1 enzyme (UBE1), a conjugating enzyme (E2), and the FA core complex as the E3 ligase. A yeast two-hybrid screen identified FANCL as an interactor of the E2 enzyme UBE2T, suggesting that it may function with the FA core E3 ligase complex [61]. Indeed, human cells depleted of UBE2T protein or DT40 cells rendered deficient in *UBE2T* gene failed to monoubiquitinate FANCD2 both before and after exogenous DNA damage [61,62]. In contrast, cells lacking deubiquitinase USP1 displayed constitutive FANCD2 monoubiquitination even in untreated cells [63,64]. Although a novel component of the USP1 complex UAF1 is recently identified [65], it is still unknown how USP1 activity is coordinated with FANCD2 monoubiquitination. Interestingly, USP1 also catalyzes removal of ubiquitin from monoubiquitinated PCNA [66], which is crucial for the polymerase switching in TLS [39,43].

8. Breast cancer genes: down stream or parallel effectors of the FA pathway?

As stated above, BRCA2 is an important regulator of Rad51 [12]. A heterozygous carrier of BRCA2 mutation has 80% chance in their life time to develops breast cancer [67], while biallelic BRCA2 mutations cause FA with particularly severe phenotype, resulting in highly exaggerated incidence of brain, kidney or hematological malignancies [68].

There are two more genes that cause both familial breast cancer and FA. One is *PALB2/FANCN* identified as a "partner and localizer of BRCA2" that supports BRCA2 function in HR [69–73]. The other is *BRIP1/FANCJ*, a DEAH helicase originally called *BACH1* [74] (this name is confusing with the transcription factor gene *BACH1* which was reported earlier [75], thus should be avoided), which associates with the BRCT domain of BRCA1. Kevin Hiom's group disrupted *BRIP1/BACH1* gene in DT40, and found that *BRIP1*-deficient

cells displayed FA phenotype [54]. This finding helped them identify *BRIP1* as a causative gene in FA-J complementation group [76]. Furthermore, they found that chicken BRIP1 is able to reverse the ICL sensitivity of the *BRIP1*-deficient cells without binding to BRCA1 since the phosphorylation site responsible for binding to BRCA1 BRCT domain is not conserved in chicken BRIP1 [54]. Indeed, Sharon Cantor's group later showed that ICL sensitivity of human *FA-J* cells are reversed by expression of human BRIP1 mutated in the phosphorylation site [77]. These important findings highlighted the utility of chicken system in the characterization of human genetic disorder.

This overlap between familial breast cancer syndrome and FA has led to the speculation that defects in classical FA genes such as *FANCC* or *FANCD2* may also cause breast cancer, given the fact that only ~16% of the patients with familial breast cancer are explained by *BRCA1* or *BRCA2* mutation [78]. However, current evidence has suggested this is not the case. Among established FA genes, only *BRCA2/FANCD1*, *PALB2/FANCN*, and *BRIP1/FANCJ* are found to associate with familial breast cancer [67].

Interestingly, FANCD2 monoubiquitination occurs normally in cells lacking any of these breast cancer genes [10,73,79], leading to the proposal that they function down stream of the core complex–FANCD2 monoubiquitination pathway [4]. Supporting this notion, FANCD2, FANCG or FANCE interacts with BRCA2 [80–82]. Furthermore, there are conflicting reports in the literature regarding whether Rad51 focus formation in FA cells is defective or not [80,83–86].

To gain insight into the relationship between FANCD2 monoubiquitination and role of the BRCA2, we looked at the DNA damage induced chromatin loading of FANCD2 or RAD51 in *brca2* or *fancc* mutant cell lines. *BRCA2* mutant cells are defective only in chromatin loading of RAD51, while RAD51 but not FANCD2 is targeted to chromatin normally in *fancc* cells. These data may suggest the FANCC–FANCD2 or the BRCA2–RAD51 pathways act in an independent manner at least in the early phase (before focus formation) following DNA damage. Consistent with the notion that these pathways are functionally distinct, a recent study examined an actual repair kinetics of psoralen-induced crosslink using a novel assay system, and found that there is a repair defect in human cells deficient in *FANCG*, *FANCA*, or *FANCD2* but not in *FANCD1/BRCA2* [87]. We also found that FANCJ protein constitutively localized in chromatin, and was not remarkably altered by the absence of FANCC or FANCD2 (HK, unpublished observation). Although there is a phenotypic overlap, these results may support a notion that the classical FA pathway (the core complex–FANCD2) is independent of the breast cancer genes. Consistent with this, the classical FA genes do not cause familial breast cancer, while FA patients lacking *FANCD1* or *FANCN* displayed severe developmental abnormalities and early onset of cancer [68,88,89]. There is a precedent in that defects in two different pathways, for example, nucleotide excision repair and TLS, cause one similar syndrome, xeroderma pigmentosum (classical or variant XP) [90]. While *FANCD1*- or *FANCN*-deficient cells are clearly HR-defective, it is still debatable whether *FANCJ*-deficient cells are proficient in recombination or not [54,91]. Furthermore, FA cells mutated in the FA pathway (the core complex–I–D complex axis) are not so severely HR-defective.

9. Monoubiquitin functions as an attachable localization tag for FancD2

FancD2 monoubiquitination is necessary for chromatin targeting, focus formation, and DNA repair function of FANCD2, since FANCD2 lacking the monoubiquitination site cannot get into chromatin fraction efficiently, does not form foci at all, and does not reverse ICL sensitivity in *fancd2* cells [10,35]. To test whether monoubiquitination of FANCD2 is sufficient for its function, we fused chicken FANCD2 lacking the monoubiquitination site (termed D2KR) at its c-terminus with a single ubiquitin moiety (D2KR-Ub), and expressed it into *fancd2* cells [92]. We found that *fancd2* cells expressing D2KR significantly reversed its cisplatin sensitivity to near wild type levels, indicating that D2KR-Ub is functional in DNA repair. This is probably not surprising, since there are a number of examples in the literature in that a c-terminal fusion with ubiquitin functionally mimics its orthotopic monoubiquitination (for example, [93]). Of note, the D2KR-Ub protein appeared not to be ubiquitinated since it is detected as a single band even after DNA damage.

Expression of the D2KR-Ub protein (and its variants) in DT40 mutants allowed us to ask several interesting questions. First we looked at whether D2KR-Ub is localized at chromatin [92]. Consistent with the DNA repair activity, a significant amount of the D2KR-Ub fusion is detectable in chromatin fraction. We noticed that the amount of D2KR-Ub does not change before or after exogenous DNA damage, suggesting that machinery for the chromatin loading of ubiquitinated FANCD2 is constitutively active and no further activation occurs after DNA damage. However, the D2KR-Ub fusion distributes diffusely in the nucleus, and we could not detect its focus formation of even after MMC treatment. Since the cells are protected against killing due to cisplatin by D2KR-Ub, a small amount of the D2KR-Ub protein is sufficient for repair at the DNA damage site. Thus the focus formation is not absolutely required for the repair if a fair amount of FANCD2 is inside chromatin, though the increased local concentration of FANCD2 would probably facilitate the DNA damage repair.

Next, we wanted to get insight about how monoubiquitination localizes the D2KR-Ub protein into chromatin [92]. An obvious possibility is that the monoubiquitin acts as a localization tag for chromatin loading, and a protein that binds ubiquitinated FANCD2 is involved. To gain support for this hypothesis, we introduced a mutation I44A into the monoubiquitin moiety of D2KR-Ub. This mutation is in the hydrophobic patch of the ubiquitin, and is known to disrupt ubiquitin interaction with the ubiquitin-binding domains [94]. We found that the D2KR-Ub-I44A protein was localized to chromatin less efficiently, and could not restore cisplatin tolerance in *fancd2* cells to the levels that the D2KR fused with wild type ubiquitin does. These data support the crucial role of a hypothetical protein that binds monoubiquitinated FANCD2 in DNA repair. This protein should have an ubiquitin-binding domain, however, identity of the protein is still unclear.

If the monoubiquitination acts as an attachable chromatin localization signal, then it might be substituted by another chromatin localization signal. There was a precedent in the literature in that a protein was tethered to chromatin by fusing with histone H2B. Thus it has been shown that chromatin-fixed CHK2 kinase, which is normally diffusible following activation at the DNA damage sites by ATM kinase, was defective in checkpoint signaling [95]. We exploited this methodology, and tested function of D2KR fused with histone H2B in *fancd2* cells. Perhaps surprisingly, expression of D2KR-H2B restored cell survival in cisplatin containing medium similarly to D2KR-Ub. This observation further support the notion that monoubiquitination on FANCD2 functions to localize FANCD2 to chromatin, and for DNA repair function of FANCD2, the monoubiquitination is not essential.

10. Testing function of the core complex independent of FANCD2 monoubiquitination

Since the D2KR-Ub fusion protein is in a way already "monoubiquitinated" and functions in DNA repair without further ubiquitination, we reasoned that D2KR-Ub expression might bypass

the need for the core complex if the sole function of the complex is to mediate FANCD2 monoubiquitination [92]. We tested this idea by introduction of the D2KR-Ub construct to *fancc*, *fancg*, or *fancl* cells. None of the cell lines displayed any reversal of the cisplatin sensitivity, and furthermore, we found that the D2KR-Ub protein is not localized in chromatin fraction in these cells. These data may suggest a role of the core complex in chromatin targeting of monoubiquitinated FANCD2.

If cells lacking the core complex components could not be complemented by D2KR-Ub expression because of its inability to be localized in chromatin, then they should be complemented by the D2KR-H2B expression. However, *fancc*, *fancg*, or *fancl* cells expressing D2KR-H2B were still sensitive to cisplatin even though D2KR-H2B protein is localized in chromatin fraction of these cells. Thus normal DNA repair requires presence of the core complex in addition to chromatin-targeted FancD2.

Collectively, these results have led us to speculate that the core complex has at least three functions [92]. First, it has an E3 ubiquitin ligase function to monoubiquitinate FancD2. Second, it is required for chromatin localization of ubiquitinated FancD2 as revealed by the D2KR-Ub expression. The detailed molecular mechanism for chromatin loading is not clear yet, however, a putative ubiquitin-binding protein may work here as suggested by D2KR-Ub I44A mutant. It is also possible that DNA binding ability of FANCD2 [96] could contribute to the chromatin relocalization. Third, it may have an additional role in DNA repair. This could be direct one, since the core complex has the FANCM subunit, which has an ability of DNA translocase [97], promotes HJ migration [98] and fork reversal [99]. In addition, it is possible that any one of the other components have an unexpected activity.

Recent studies highlighted the role of FANCM and its novel interacting partner FAAP24 in localizing the core complex in chromatin. For example, FAAP24 protein preferentially binds to aberrant DNA structures resembling stalled replication forks [100,101], thus has been suggested to recruit the FANCM-containing core complex to chromatin. Alternatively, it has been reported that FANCM is localized in chromatin throughout the cell cycle, and its phosphorylation in S or G2/M phase regulates FANCM binding or release of the core complex, respectively [102]. In any case, FANCM appears to be crucial in chromatin localization of the core complex. Interestingly, Patel and coworkers detected increased chromatin localization of the core complex following DNA damage using DT40 cells carrying "knocked-in" alleles with multiple epitope tags [62,103]. We could not detect such increased chromatin localization of FANCC using *fancc* cells stably transfected with GFP-FANCC, possibly due to overexpression of the protein. However, we indeed observed MMC-induced GFP-FANCC focus formation that co-localized with FANCD2 foci [92].

11. FAAP100

It seems worthwhile to note the contribution of DT40 system in establishing the crucial role that FAAP100 plays in the FA pathway. Meetei and Wang identified this protein in the core complex preparation purified by anti-FANCA antibody [34]. FAAP100 is an integral component of the core complex, and interacts with FANCB and FANCL, forming a subcomplex. Since they could not find any patients who lacked this protein, the protein is called FAAP100. Although siRNA studies suggested that FAAP100 is important for FANCD2 monoubiquitination, the gene disruption in DT40 was required to definitely show its role in suppressing chromosome aberrations and ICL sensitivity [34]. Although FAAP100 protein is crucial for the core complex assembly like the other core complex members, it may have other functions that are still unknown.

12. FancI: the newest member of the FA pathway

The newest member in the FA pathway, FANCI, has been recently identified through a very clever phospho-proteomic screen in an effort to identify novel ATM/ATR kinase substrates [104], or by positional cloning for the responsible gene in FA-I patients [105], or by search for a FANCD2 homolog [106]. It has been known from the analysis of human FA-I cells that FANCI protein is essential for FancD2 monoubiquitination [79]. It is now clear that FANCI physically associates with FANCD2, resulting in the I–D complex formation. Thus it is plausible that the D2–I interaction is essential for the monoubiquitination.

Identification of FANCI has also revealed that FANCI is also monoubiquitinated by the core complex upon DNA damage or S phase stress in a FANCD2-dependent manner. Thus, monoubiquitination of FANCD2 and FANCI occur in a manner dependent on each other. Furthermore, similarly to the case of FANCD2, USP1 removes ubiquitin from monoubiquitinated FANCI [104,106].

To gain insight into the role of FANCI in the FA pathway, we disrupted *FANCI* gene in DT40 and expressed wild type or monoubiquitination site mutant of GFP-chicken FANCI in these cells [107]. *FANCI*-deficient chicken cells were highly cisplatin sensitive, and lacked FANCD2 monoubiquitination. As expected, we could detect GFP-FANCI monoubiquitination in cells expressing GFP-FANCI WT. Interestingly, GFP-FANCI lacking monoubiquitination site (K525R) expression quite efficiently reversed cell death and chromosome aberrations induced by crosslinking agents in *fanci* cells, indicating that monoubiquitination of FANCI is largely dispensable for the FA pathway. In line with this, human FANCI mutated in the monoubiquitination site also at least partially suppress the phenotypic defects such as MMC sensitivity or chromosome aberrations in human FA-I cells (see Figs. 6E and F in ref. [104]), though defective FANCD2 monoubiquitination is less well reversed than in chicken system.

We also tested whether FANCI is monoubiquitinated in chicken *fancd2* cells reconstituted with D2KR-Ub or H2B fusions, which does not display severe cisplatin sensitivity [107]. Since we do not have a good anti-chicken FancI antibody, we stably introduced GFP-FANCI WT to use anti-GFP to detect FANCI monoubiquitination. These cells did not show any detectable FANCI monoubiquitination even after MMC treatment, indicating that severe cisplatin sensitivity was reversed in the absence of FANCI monoubiquitination.

13. FancI phosphorylation as a molecular switch in the FA pathway activation

In response to DNA damage and replication stress, members of the Phosphatidyl Inositol kinase-like kinase (PIKK) family including ATM, ATR, or DNA-PK are activated, and play essential roles in the DNA damage response such as cell cycle arrest, apoptotic cell death or DNA repair. For example, ATM kinase responds to DSBs and mediates DSB-induced cell cycle arrest at G1/S and G2/M boundaries or intra-S phase [108], and contributes to a fraction of the DSB repair events [109]. These PIKK kinases preferentially phosphorylate serine or threonine in the S/TQ motifs such as Ser15 in human p53 [108].

As discussed above, the FA pathway is efficiently activated leading to FANCD2–FANCI monoubiquitination following treatments with variety of DNA damages or agents that invariably lead to replication fork arrests. Therefore the stalled forks and/or accompanying single-stranded (ss) gaps, rather than DNA lesion itself, likely initiates the triggering signal to activate the FA pathway. The PIKK family kinase ATR is an obvious candidate involved in this step (see also "Fanconi Anemia Proteins and Endogenous Stresses" Pang and Andreassen, this issue), since it

has been known that ATR kinase is activated by ss gap coated with RPA. It has also been suggested that ATR kinase promotes FANCD2 monoubiquitination by directly phosphorylating FANCD2 [110]. Recent reports identified a huge number of potential ATM or ATR substrates following IR or UV DNA damages that included FANCD2, FANCI, or FANCA [111,112]. Interestingly, there are examples in the literature that phosphorylation of the substrate facilitates its interaction with multi-subunit E3 ubiquitin ligase such as SCF, leading to activation of the E3 ligase function [113,114].

It is notable that FANCI has a cluster of conserved six S/TQ sites in a region spanning ~70 aminoacids in its middle part near the monoubiquitination site K563. To test the hypothesis that FANCI phosphorylation in this S/TQ cluster contributes to the FA pathway activation, we expressed chicken FANCI mutants containing multiple AQ substitutions on the S/TQ motifs in *FANCI*-deficient DT40 cells [107]. We found that cells expressing FANCI carrying six but not two or four AQ substitutions in the S/TQ cluster domain are exquisitely cisplatin sensitive and abrogate D2/I monoubiquitination. To visualize FANCI phosphorylation, we employed the Phos-tag reagent that retards migration of phosphoprotein in the gel by binding to phosphorylated aminoacids [115], and successfully detected MMC-induced phosphorylation on the S/TQ cluster in FANCI protein. This phosphorylation was a prerequisite for FANCI monoubiquitination but the reverse was not true. Furthermore, expression of FANCI in the S/TQ cluster carrying six aspartic acid (DQ) mutations, which confer negative charge to the protein and therefore are phospho-mimic, causes constitutive FANCD2 and FANCI monoubiquitination, and reverses cisplatin sensitivity. We have also tested a role of FANCD2 phosphorylation in DT40 *fancd2*-deficient cells by expressing chicken FANCD2 lacking ten potential ATR phosphorylation site (S/TQ motifs), and found minimal effects in cisplatin sensitivity. Thus we conclude that multiple FANCI phosphorylation rather than FANCD2 phosphorylation, probably mediated by ATR kinase, is a molecular switch to turn on the FA pathway [116]. It seems reasonable to assume that the FANCI phosphorylation affects interaction between FANCI/FANCD2 and the core complex, thereby triggering the E3 ligase activity. However, we have not been able to detect such changes in the interaction so far.

14. More unanswered questions

As recent progress in understanding the FA pathway is so rapid, enormous amount of novel information accumulates as summarized in this review. However, many critical questions remain unanswered. Here we would list (only) some of them and give brief comments.

14.1. What is the main biochemical activity of FANCD2 (and FANCI)?

This is a big issue. Monoubiquitinated FANCD2/FANCI appears to associate with each other in chromatin and form foci that colocalize very well. They probably provide a critical function in ICL repair activity. At least recombinant FANCD2 is able to bind to naked DNA including Holliday structure [96]. They may exhibit some enzymatic activity or act as a scaffold to modulate HR and TLS activities. There might be overlap between mechanisms of HR and TLS (for example, some TLS polymerases such as POL η participate both HR and TLS [117]) where the FA pathway may provide a critical function. Also there must be the mechanisms that promote chromatin targeting of the monoubiquitinated FANCD2. This needs to be clarified.

14.2. How does FANCI get phosphorylated? How the phosphorylation activates the core complex to monoubiquitinate FANCD2/FANCI?

It is still unclear how ATR kinase is activated and phosphorylates FANCI in the context of the FA pathway. The FANCI phosphorylation should somehow promote FANCD2–E3 ligase association. The molecular interactions between these factors need to be characterized.

14.3. What is the role of FANCJ in ICL repair? Why are FA-J cells ICL sensitive?

This has not been established. It seems possible that helicase activity of FANCJ is required for efficient replication across the unhooked ICL lesions. Recently, it has been reported that BRIP1/FANCJ helicase has a strong G-quadruplex (G4) unwinding activity in vitro [118]. Consistent with this, *C. elegans* mutated in *FANCJ* homolog *DOG-1* displays frequent loss of G4 tracts during replication (see "*C. elegans*: A Model of Fanconi Anemia and ICL Repair" Youds et al., this issue), indicating the role of FANCJ in removing roadblocks due to the G4 formation for progressing replication forks [119,120]. However, *C. elegans* mutants lacking BRCA2 or FANCD2 homolog do not have a similar phenotype, raising a question about relationship between FANCJ and other FA proteins [121]. It has been reported that *FANCJ* is partially epistatic to *FANCC* as shown by the double knockout analysis in DT40, however, this may actually indicate a non-overlapping component between them.

14.4. What is the endogenous DNA damage the FA pathway is coping with?

It is currently unknown how ICL naturally occurs in the cell or even whether the FA proteins are handling endogenous ICL or not. Since FA cells are sensitive to hyperoxic conditions, and a recent report shows treatment with anti-oxidant tempol is able to retard the initiation of epithelial carcinogenesis in *FANCD2*-deficient mice [122], the FA pathway could be one of the defense mechanisms for the oxygen toxicity. Recently Nakamura and coworkers has shown that both human and chicken cells lacking FA genes are hypersensitive to serum-level concentrations of formaldehyde compared to wild type and other mutant cells deficient in DNA repair pathways [123]. Formaldehyde creates DNA-protein crosslink, which may require the FA proteins for efficient removal.

In summary, we have seen rapid expansion of the knowledge about FA in this ~eight years. Although various methodologies have contributed to this, we believe reverse genetics using DT40 has played significant roles in the field. We expect more discoveries in the near future that potentially lead to better understanding and management for FA and related severe medical conditions.

Conflict of interest

There are no conflicts of interest.

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References

[1] T.W. Baba, B.P. Giroir, E.H. Humphries, Cell lines derived from avian lymphomas exhibit two distinct phenotypes, Virology 144 (1985) 139–151.
[2] J.M. Buerstedde, S. Takeda, Increased ratio of targeted to random integration after transfection of chicken B cell lines, Cell 67 (1991) 179–188.
[3] L.H. Thompson, J.M. Hinz, N.A. Yamada, N.J. Jones, How Fanconi anemia proteins promote the four Rs: Replication recombination, repair, and recovery, Environ. Mol. Mutagen. 45 (2005) 128–142.
[4] A.D. D'Andrea, M. Grompe, The Fanconi anaemia/BRCA pathway, Nat. Rev. Cancer 3 (2003) 23–34.
[5] W. Wang, Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins, Nat. Rev. Genet. 8 (2007) 735–748.
[6] M. Takata, H. Kitao, M. Ishiai, Fanconi anemia: genetic analysis of a human disease using chicken system, Cytogenet. Genome Res. 117 (2007) 346–351.
[7] M.S. Sasaki, A. Tonomura, A high susceptibility of Fanconi's anemia to chromosome breakage by DNA cross-linking agents, Cancer Res. 33 (1973) 1829–1836.
[8] M.S. Sasaki, Is Fanconi's anaemia defective in a process essential to the repair of DNA cross links? Nature 257 (1975) 501–503.
[9] C. Timmers, T. Taniguchi, J. Hejna, C. Reifsteck, L. Lucas, D. Bruun, M. Thayer, B. Cox, S. Olson, A.D. D'Andrea, R. Moses, M. Grompe, Positional cloning of a novel Fanconi anemia gene, FANCD2, Mol. Cell 7 (2001) 241–248.
[10] I. Garcia-Higuera, T. Taniguchi, S. Ganesan, M.S. Meyn, C. Timmers, J. Hejna, M. Grompe, A.D. D'Andrea, Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway, Mol. Cell 7 (2001) 249–262.
[11] T. Taniguchi, I. Garcia-Higuera, P.R. Andreassen, R.C. Gregory, M. Grompe, A.D. D'Andrea, S-phase-specific interaction of the Fanconi anemia protein, FANCD2, with BRCA1 and RAD51, Blood 100 (2002) 2414–2420.
[12] S.C. West, Molecular views of recombination proteins and their control, Nat. Rev. Mol. Cell Biol. 4 (2003) 435–445.
[13] A.R. Venkitaraman, Cancer susceptibility and the functions of BRCA1 and BRCA2, Cell 108 (2002) 171–182.
[14] N.G. Howlett, T. Taniguchi, S. Olson, B. Cox, Q. Waisfisz, C. De Die-Smulders, N. Persky, M. Grompe, H. Joenje, G. Pals, H. Ikeda, E.A. Fox, A.D. D'Andrea, Biallelic inactivation of BRCA2 in Fanconi anemia, Science 297 (2002) 606–609.
[15] L. Pellegrini, D.S. Yu, T. Lo, S. Anand, M. Lee, T.L. Blundell, A.R. Venkitaraman, Insights into DNA recombination from the structure of a RAD51–BRCA2 complex, Nature 420 (2002) 287–293.
[16] H. Yang, Q. Li, J. Fan, W.K. Holloman, N.P. Pavletich, The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA-ssDNA junction, Nature 433 (2005) 653–657.
[17] M. Takata, M.S. Sasaki, S. Tachiiri, T. Fukushima, E. Sonoda, D. Schild, L.H. Thompson, S. Takeda, Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs, Mol. Cell. Biol. 21 (2001) 2858–2866.
[18] M. Takata, M.S. Sasaki, E. Sonoda, T. Fukushima, C. Morrison, J.S. Albala, S.M. Swagemakers, R. Kanaar, L.H. Thompson, S. Takeda, The Rad51 paralog Rad51B promotes homologous recombinational repair, Mol. Cell. Biol. 20 (2000) 6476–6482.
[19] K. Yamamoto, M. Ishiai, N. Matsushita, H. Arakawa, J.E. Lamerdin, J.M. Buerstedde, M. Tanimoto, M. Harada, L.H. Thompson, M. Takata, Fanconi anemia FANCG protein in mitigating radiation- and enzyme-induced DNA double-strand breaks by homologous recombination in vertebrate cells, Mol. Cell. Biol. 23 (2003) 5421–5430.
[20] K. Yamamoto, S. Hirano, M. Ishiai, K. Morishima, H. Kitao, K. Namikoshi, M. Kimura, N. Matsushita, H. Arakawa, J.M. Buerstedde, K. Komatsu, L.H. Thompson, M. Takata, Fanconi anemia protein FANCD2 promotes immunoglobulin gene conversion and DNA repair through a mechanism related to homologous recombination, Mol. Cell. Biol. 25 (2005) 34–43.
[21] O. Bezzubova, A. Silbergleit, Y. Yamaguchi-Iwai, S. Takeda, J.M. Buerstedde, Reduced X-ray resistance and homologous recombination frequencies in a RAD54−/− mutant of the chicken DT40 cell line, Cell 89 (1997) 185–193.
[22] S. Hirano, K. Yamamoto, M. Ishiai, M. Yamazoe, M. Seki, N. Matsushita, M. Ohzeki, Y.M. Yamashita, H. Arakawa, J.M. Buerstedde, T. Enomoto, S. Takeda, L.H. Thompson, M. Takata, Functional relationships of FANCC to homologous recombination, translesion synthesis, and BLM, EMBO J. 24 (2005) 418–427.
[23] K. Nakanishi, Y.G. Yang, A.J. Pierce, T. Taniguchi, M. Digweed, A.D. D'Andrea, Z.Q. Wang, M. Jasin, Human Fanconi anemia monoubiquitination pathway promotes homologous DNA repair, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 1110–1115.
[24] J.M. Buerstedde, H. Arakawa, Immunoglobulin gene conversion or hypermutation: that's the question, Subcell Biochem. 40 (2006) 11–24.
[25] J.E. Sale, D.M. Calandrini, M. Takata, S. Takeda, M.S. Neuberger, Ablation of XRCC2/3 transforms immunoglobulin V gene conversion into somatic hypermutation, Nature 412 (2001) 921–926.
[26] J.M. Di Noia, M.S. Neuberger, Molecular mechanisms of antibody somatic hypermutation, Annu. Rev. Biochem. 76 (2007) 1–22.
[27] W. Niedzwiedz, G. Mosedale, M. Johnson, C.Y. Ong, P. Pace, K.J. Patel, The Fanconi anaemia gene FANCC promotes homologous recombination and error-prone DNA repair, Mol. Cell 15 (2004) 607–620.
[28] J.M. Hinz, P.B. Nham, E.P. Salazar, L.H. Thompson, The Fanconi anemia pathway limits the severity of mutagenesis, DNA Repair (Amst.) 5 (2006) 875–884.
[29] D. Papadopoulo, C. Guillouf, H. Mohrenweiser, E. Moustacchi, Hypomutability in Fanconi anemia cells is associated with increased deletion frequency at the HPRT locus, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 8383–8387.
[30] M. Ishiai, M. Kimura, K. Namikoshi, M. Yamazoe, K. Yamamoto, H. Arakawa, K. Agematsu, N. Matsushita, S. Takeda, J.M. Buerstedde, M. Takata, DNA cross-link repair protein SNM1A interacts with PIAS1 in nuclear focus formation, Mol. Cell. Biol. 24 (2004) 10733–10741.
[31] Y. Liu, J.Y. Masson, R. Shah, P. O'Regan, S.C. West, RAD51C is required for Holliday junction processing in mammalian cells, Science 303 (2004) 243–246.
[32] M. Brenneman, B. Wagener, C. Miller, C. Allen, J. Nickoloff, XRCC3 Controls the fidelity of homologous recombination. Roles for XRCC3 in late stages of recombination, Mol. Cell 10 (2002) 387–395.
[33] J.B. Wilson, K. Yamamoto, A.S. Marriott, S. Hussain, P. Sung, M.E. Hoatlin, C.G. Mathew, M. Takata, L.H. Thompson, G.M. Kupfer, N.J. Jones, FANCG promotes formation of a newly identified protein complex containing BRCA2, FANCD2 and XRCC3, Oncogene 27 (2008) 3641–3652.
[34] C. Ling, M. Ishiai, A.M. Ali, A.L. Medhurst, K. Neveling, R. Kalb, Z. Yan, Y. Xue, A.B. Oostra, A.D. Auerbach, M.E. Hoatlin, D. Schindler, H. Joenje, J.P. de Winter, M. Takata, A.R. Meetei, W. Wang, FAAP100 is essential for activation of the Fanconi anemia-associated DNA damage response pathway, EMBO J. 26 (2007) 2104–2114.
[35] S. Seki, M. Ohzeki, A. Uchida, S. Hirano, N. Matsushita, H. Kitao, T. Oda, T. Yamashita, N. Kashihara, A. Tsubahara, M. Takata, M. Ishiai, A requirement of FancL and FancD2 monoubiquitination in DNA repair, Genes Cells 12 (2007) 299–310.
[36] A.A. Davies, D. Huttner, Y. Daigaku, S. Chen, H.D. Ulrich, Activation of ubiquitin-dependent DNA damage bypass is mediated by replication protein a, Mol. Cell 29 (2008) 625–636.
[37] P. Stelter, H.D. Ulrich, Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation, Nature 425 (2003) 188–191.
[38] P.L. Kannouche, J. Wing, A.R. Lehmann, Interaction of human DNA polymerase eta with monoubiquitinated PCNA: a possible mechanism for the polymerase switch in response to DNA damage, Mol. Cell 14 (2004) 491–500.
[39] K. Watanabe, S. Tateishi, M. Kawasuji, T. Tsurimoto, H. Inoue, M. Yamaizumi, Rad18 guides pol η to replication stalling sites through physical interaction and PCNA monoubiquitination, EMBO J. 23 (2004) 3886–3896.
[40] M. Bienko, C.M. Green, N. Crosetto, F. Rudolf, G. Zapart, B. Coull, P. Kannouche, G. Wider, M. Peter, A.R. Lehmann, K. Hofmann, I. Dikic, Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis, Science 310 (2005) 1821–1824.
[41] L.J. Simpson, J.E. Sale, Rev1 is essential for DNA damage tolerance and non-templated immunoglobulin gene mutation in a vertebrate cell line, EMBO J. 22 (2003) 1654–1664.
[42] H. Arakawa, G.L. Moldovan, H. Saribasak, N.N. Saribasak, S. Jentsch, J.M. Buerstedde, A role for PCNA ubiquitination in immunoglobulin hypermutation, PLoS Biol. 4 (2006) e366.
[43] J.G. Jansen, M.I. Fousteri, N. Wind de, Send in the clamps: control of DNA translesion synthesis in eukaryotes, Mol. Cell 28 (2007) 522–529.
[44] K.Y. Lee, K. Myung, PCNA modifications for regulation of post-replication repair pathways, Mol. Cell 26 (2008) 5–11.
[45] T. Okada, E. Sonoda, Y.M. Yamashita, S. Koyoshi, S. Tateishi, M. Yamaizumi, M. Takata, O. Ogawa, S. Takeda, Involvement of vertebrate polkappa in Rad18-independent postreplication repair of UV damage, J. Biol. Chem. 277 (2002) 48690–48695.
[46] C.E. Edmunds, L.J. Simpson, J.E. Sale, PCNA ubiquitination and REV1 define temporally distinct mechanisms for controlling translesion synthesis in the avian cell line DT40, Mol. Cell 30 (2008) 519–529.
[47] A.L. Ross, L.J. Simpson, J.E. Sale, Vertebrate DNA damage tolerance requires the C-terminus but not BRCT or transferase domains of REV1, Nucleic Acids Res. 33 (2005) 1280–1289.
[48] Y.M. Yamashita, T. Okada, T. Matsusaka, E. Sonoda, G.Y. Zhao, K. Araki, S. Tateishi, M. Yamaizumi, S. Takeda, RAD18 and RAD54 cooperatively contribute to maintenance of genomic stability in vertebrate cells, EMBO J. 21 (2002) 5558–5566.
[49] K. Nojima, H. Hochegger, A. Saberi, T. Fukushima, K. Kikuchi, M. Yoshimura, B.J. Orelli, D.K. Bishop, S. Hirano, M. Ohzeki, M. Ishiai, K. Yamamoto, M. Takata, H. Arakawa, J.M. Buerstedde, M. Yamazoe, T. Kawamoto, K. Araki, J.A. Takahashi, N. Hashimoto, S. Takeda, E. Sonoda, Multiple repair pathways mediate tolerance to chemotherapeutic cross-linking agents in vertebrate cells, Cancer Res. 65 (2005) 11704–117011.
[50] K.D. Mirchandani, R.M. McCaffrey, A.D. D'Andrea, The Fanconi anemia core complex is required for efficient point mutagenesis and Rev1 foci assembly, DNA Repair (Amst.) 7 (2008) 902–911.
[51] E. Sonoda, M.S. Sasaki, C. Morrison, Y. Yamaguchi-Iwai, M. Takata, S. Takeda, Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells, Mol. Cell. Biol. 19 (1999) 5166–5169.
[52] B.C. Godthelp, P.P. van Buul, N.G. Jaspers, E. Elghalbzouri-Maghrani, A. van Duijn-Goedhart, F. Arwert, H. Joenje, M.Z. Zdzienicka, Cellular characterization of cells from the Fanconi anemia complementation group, FA-D1/BRCA2, Mutat. Res. 601 (2006) 191–201.
[53] F. Paques, J.E. Haber, Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae, Microbiol. Mol. Biol. Rev. 63 (1999) 349–404.

[54] W.L. Bridge, C.J. Vandenberg, R.J. Franklin, K. Hiom, The BRIP1 helicase functions independently of BRCA1 in the Fanconi anemia pathway for DNA crosslink repair, Nat. Genet. 37 (2005) 953–957.
[55] A.R. Meetei, S. Sechi, M. Wallisch, D. Yang, M.K. Young, H. Joenje, M.E. Hoatlin, W. Wang, A multiprotein nuclear complex connects Fanconi anemia and Bloom syndrome, Mol. Cell. Biol. 23 (2003) 3417–3426.
[56] L. Wu, I.D. Hickson, The Bloom's syndrome helicase suppresses crossing over during homologous recombination, Nature 426 (2003) 870–874.
[57] T. Okada, E. Sonoda, M. Yoshimura, Y. Kawano, H. Saya, M. Kohzaki, S. Takeda, Multiple roles of vertebrate REV genes in DNA repair and recombination, Mol. Cell. Biol. 25 (2005) 6103–6111.
[58] C.J. Vandenberg, F. Gergely, C.Y. Ong, P. Pace, D.L. Mallery, K. Hiom, K.J. Patel, BRCA1-independent ubiquitination of FANCD2, Mol. Cell 12 (2003) 247–254.
[59] A.R. Meetei, J.P. de Winter, A.L. Medhurst, M. Wallisch, Q. Waisfisz, H.J. van de Vrugt, A.B. Oostra, Z. Yan, C. Ling, C.E. Bishop, M.E. Hoatlin, H. Joenje, W. Wang, A novel ubiquitin ligase is deficient in Fanconi anemia, Nat. Genet. 35 (2003) 165–170.
[60] P. Pace, M. Johnson, W.M. Tan, G. Mosedale, C. Sng, M. Hoatlin, J. de Winter, H. Joenje, F. Gergely, K.J. Patel, FANCE: the link between Fanconi anaemia complex assembly and activity, EMBO J. 21 (2002) 3414–3423.
[61] Y.J. Machida, Y. Machida, Y. Chen, A.M. Gurtan, G.M. Kupfer, A.D. D'Andrea, A. Dutta, UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation, Mol. Cell 23 (2006) 589–596.
[62] A. Alpi, F. Langevin, G. Mosedale, Y.J. Machida, A. Dutta, K.J. Patel, UBE2T, the Fanconi anemia core complex, and FANCD2 are recruited independently to chromatin: a basis for the regulation of FANCD2 monoubiquitination, Mol. Cell Biol. 27 (2007) 8421–8430.
[63] S.M. Nijman, T.T. Huang, A.M. Dirac, T.R. Brummelkamp, R.M. Kerkhoven, A.D. D'Andrea, R. Bernards, The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway, Mol. Cell 17 (2005) 331–339.
[64] V.H. Oestergaard, F. Langevin, H.J. Kuiken, P. Pace, W. Niedzwiedz, L.J. Simpson, M. Ohzeki, M. Takata, J.E. Sale, K.J. Patel, Deubiquitination of FANCD2 is required for DNA crosslink repair, Mol. Cell 28 (2007) 798–809.
[65] M.A. Cohn, P. Kowal, K. Yang, W. Haas, T.T. Huang, S.P. Gygi, A.D. D'Andrea, A UAF1-containing multisubunit protein complex regulates the Fanconi anemia pathway, Mol. Cell 28 (2007) 786–797.
[66] T.T. Huang, S.M. Nijman, K.D. Mirchandani, P.J. Galardy, M.A. Cohn, W. Haas, S.P. Gygi, H.L. Ploegh, R. Bernards, A.D. D'Andrea, Regulation of monoubiquitinated PCNA by DUB autocleavage, Nat. Cell Biol. 8 (2006) 339–347.
[67] T. Walsh, M.C. King, Ten genes for inherited breast cancer, Cancer Cell 11 (2007) 103–105.
[68] J.E. Wagner, J. Tolar, O. Levran, T. Scholl, A. Deffenbaugh, J. Satagopan, L. Ben-Porat, K. Mah, S.D. Batish, D.I. Kutler, M.L. MacMillan, H. Hanenberg, A.D. Auerbach, Germline mutations in BRCA2: shared genetic susceptibility to breast cancer, early onset leukemia, and Fanconi anemia, Blood 103 (2004) 3226–3229.
[69] B. Xia, J.C. Dorsman, N. Ameziane, Y. de Vries, M.A. Rooimans, Q. Sheng, G. Pals, A. Errami, E. Gluckman, J. Llera, W. Wang, D.M. Livingston, H. Joenje, de WinterF J.P., Fanconi anemia is associated with a defect in the BRCA2 partner PALB2, Nat. Genet. 39 (2007) 159–161.
[70] B. Xia, Q. Sheng, K. Nakanishi, A. Ohashi, J. Wu, N. Christ, X. Liu, M. Jasin, F.J. Couch, D.M. Livingston, Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2, Mol. Cell 22 (2006) 719–729.
[71] H. Erkko, B. Xia, J. Nikkilä, J. Schleutker, K. Syrjakoski, A. Mannermaa, A. Kallioniemi, K. Pylkas, S.M. Karppinen, K. Rapakko, A. Miron, Q. Sheng, G. Li, H. Mattila, D.W. Bell, D.A. Haber, M. Grip, M. Reiman, A. Jukkola-Vuorinen, A. Mustonen, J. Kere, L.A. Aaltonen, V.M. Kosma, V. Kataja, Y. Soini, R.I. Drapkin, D.M. Livingston, R. Winqvist, A recurrent mutation in PALB2 in Finnish cancer families, Nature 446 (2007) 316–319.
[72] N. Rahman, S. Seal, D. Thompson, P. Kelly, A. Renwick, A. Elliott, S. Reid, K. Spanova, R. Barfoot, T. Chagtai, H. Jayatilake, L. McGuffog, S. Hanks, D.G. Evans, D. Eccles, D.F. Easton, M.R. Stratton, PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene, Nat. Genet. 39 (2007) 165–167.
[73] S. Reid, D. Schindler, H. Hanenberg, K. Barker, S. Hanks, R. Kalb, K. Neveling, P. Kelly, S. Seal, M. Freund, M. Wurm, S.D. Batish, F.P. Lach, S. Yetgin, H. Neitzel, H. Ariffin, M. Tischkowitz, C.G. Mathew, A.D. Auerbach, N. Rahman, Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer, Nat. Genet. 39 (2007) 162–164.
[74] S.B. Cantor, D.W. Bell, S. Ganesan, E.M. Kass, R. Drapkin, S. Grossman, D.C. Wahrer, D.C. Sgroi, W.S. Lane, D.A. Haber, D.M. Livingston, BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function, Cell 105 (2001) 149–160.
[75] T. Oyake, K. Itoh, H. Motohashi, N. Hayashi, H. Hoshino, M. Nishizawa, M. Yamamoto, K. Igarashi, Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site, Mol. Cell Biol. 16 (1996) 6083–6095.
[76] M. Levitus, Q. Waisfisz, B.C. Godthelp, Y. Vries, S. Hussain, W.W. Wiegant, E. Elghalbzouri-Maghrani, J. Steltenpool, M.A. Rooimans, G. Pals, F. Arwert, C.G. Mathew, M.Z. Zdzienicka, K. Hiom, J.P. De Winter, H. Joenje, The DNA helicase BRIP1 is defective in Fanconi anemia complementation group J, Nat. Genet. 37 (2005) 934–935.
[77] M. Peng, R. Litman, J. Xie, S. Sharma, R.M. Brosh Jr., S.B. Cantor, The FANCJ/MutLalpha interaction is required for correction of the cross-link response in FA-J cells, EMBO J. 26 (2007) 3238–3249.
[78] M.R. Stratton, N. Rahman, The emerging landscape of breast cancer susceptibility, Nat. Genet. 40 (2008) 17–22.
[79] M. Levitus, M.A. Rooimans, J. Steltenpool, N.F. Cool, A.B. Oostra, C.G. Mathew, M.E. Hoatlin, Q. Waisfisz, F. Arwert, J.P. De Winter, H. Joenje, Heterogeneity in Fanconi anemia: evidence for two new genetic subtypes, Blood 103 (2004) 2498–2503.
[80] X. Wang, P.R. Andreassen, A.D. D'Andrea, Functional interaction of monoubiquitinated FANCD2 and BRCA2/FANCD1 in chromatin, Mol. Cell. Biol. 24 (2004) 5850–5862.
[81] S. Hussain, E. Witt, P.A. Huber, A.L. Medhurst, A. Ashworth, C.G. Mathew, Direct interaction of the Fanconi anaemia protein FANCG with BRCA2/FANCD1, Hum. Mol. Genet. 12 (2003) 2503–2510.
[82] S. Hussain, J.B. Wilson, A.L. Medhurst, J. Hejna, E. Witt, S. Ananth, A. Davies, J.Y. Masson, R. Moses, S.C. West, J.P. De Winter, A. Ashworth, N.J. Jones, C.G. Mathew, Direct interaction of FANCD2 with BRCA2 in DNA damage response pathways, Hum. Mol. Genet. 13 (2004) 1241–1248.
[83] Y.G. Yang, Z. Herceg, K. Nakanishi, I. Demuth, C. Piccoli, J. Michelon, G. Hildebrand, M. Jasin, M. Digweed, Z.Q. Wang, The Fanconi anemia group A protein modulates homologous repair of DNA double-strand breaks in mammalian cells, Carcinogenesis 26 (2005) 1731–1740.
[84] M. Digweed, S. Rothe, I. Demuth, R. Scholz, D. Schindler, M. Stumm, M. Grompe, A. Jordan, K. Sperling, Attenuation of the formation of DNA-repair foci containing RAD51 in Fanconi anaemia, Carcinogenesis 23 (2002) 1121–1126.
[85] A. Ohashi, M.Z. Zdzienicka, J. Chen, F.J. Couch, Fanconi anemia complementation group D2 (FANCD2) functions independently of BRCA2- and RAD51-associated homologous recombination in response to DNA damage, J. Biol. Chem. 280 (2005) 14877–14883.
[86] B.C. Godthelp, F. Artwert, H. Joenje, M.Z. Zdzienicka, Impaired DNA damage-induced nuclear Rad51 foci formation uniquely characterizes Fanconi anemia group D1, Oncogene 21 (2002) 5002–5005.
[87] K. Tsuchida, K. Komatsu, Impaired removal of DNA interstrand cross-link in Nijmegen breakage syndrome and Fanconi anemia, but not in BRCA-defective group, Cancer Sci. 99 (2008) 2238–2243.
[88] B. Hirsch, A. Shimamura, L. Moreau, S. Baldinger, M. Hag-Alshiekh, B. Bostrom, S. Sencer, A.D. D'Andrea, Biallelic BRCA2/FANCD1 mutations: association with spontaneous chromosomal instability and solid tumors of childhood, Blood 103 (2003) 2554–2559.
[89] K.J. Patel, Fanconi anemia and breast cancer susceptibility, Nat. Genet. 39 (2007) 142–143.
[90] C. Masutani, R. Kusumoto, A. Yamada, N. Dohmae, M. Yokoi, M. Yuasa, M. Araki, S. Iwai, K. Takio, F. Hanaoka, The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta, Nature 399 (1999) 700–704.
[91] R. Litman, M. Peng, Z. Jin, F. Zhang, J. Zhang, S. Powell, P.R. Andreassen, S.B. Cantor, BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCJ, Cancer Cell 8 (2005) 255–265.
[92] N. Matsushita, H. Kitao, M. Ishiai, N. Nagashima, S. Hirano, K. Okawa, T. Ohta, D.S. Yu, P.J. McHugh, I.D. Hickson, A.R. Venkitaraman, H. Kurumizaka, M. Takata, A FancD2-monoubiquitin fusion reveals hidden functions of Fanconi anemia core complex in DNA repair, Mol. Cell 19 (2005) 841–847.
[93] J. Terrell, S. Shih, R. Dunn, L. Hicke, A function for monoubiquitination in the internalization of a G protein-coupled receptor, Mol. Cell 1 (1998) 193–202.
[94] L. Hicke, H.L. Schubert, C.P. Hill, Ubiquitin-binding domains, Nat. Rev. Mol. Cell Biol. 6 (2005) 610–621.
[95] C. Lukas, J. Falck, J. Bartkova, J. Bartek, J. Lukas, Distinct spatiotemporal dynamics of mammalian checkpoint regulators induced by DNA damage, Nat. Cell Biol. 5 (2003) 255–260.
[96] W.H. Park, S. Margossian, A.A. Horwitz, A.M. Simons, A.D. D'Andrea, J.D. Parvin, Direct DNA binding activity of the fanconi anemia D2 protein, J. Biol. Chem. 280 (2005) 23593–23598.
[97] A.R. Meetei, A.L. Medhurst, C. Ling, Y. Xue, T.R. Singh, P. Bier, J. Steltenpool, S. Stone, I. Dokal, C.G. Mathew, M. Hoatlin, H. Joenje, J.P. de Winter, W. Wang, A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M, Nat. Genet. 37 (2005) 958–963.
[98] K. Gari, C. Decaillet, A.Z. Stasiak, A. Stasiak, A. Constantinou, The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks, Mol. Cell 29 (2008) 141–148.
[99] K. Gari, C. Decaillet, M. Delannoy, L. Wu, A. Constantinou, Remodeling of DNA replication structures by the branch point translocase FANCM, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 16107–16112.
[100] L.J. Niedernhofer, The Fanconi anemia signalosome anchor, Mol. Cell 25 (2007) 487–490.
[101] A. Ciccia, C. Ling, R. Coulthard, Z. Yan, Y. Xue, A.R. Meetei, H. Laghmani el, H. Joenje, N. McDonald, J.P. de Winter, W. Wang, S.C. West, Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM, Mol. Cell 25 (2007) 331–343.
[102] J.M. Kim, Y. Kee, A. Gurtan, A.D. D'Andrea, Cell cycle-dependent chromatin loading of the Fanconi anemia core complex by FANCM/FAAP24, Blood 111 (2008) 5215–5222.
[103] G. Mosedale, W. Niedzwiedz, A. Alpi, F. Perrina, J.B. Pereira-Leal, M. Johnson, F. Langevin, P. Pace, K.J. Patel, The vertebrate Hef ortholog is a component of the Fanconi anemia tumor-suppressor pathway, Nat. Struct. Mol. Biol. 12 (2005) 763–771.
[104] A. Smogorzewska, S. Matsuoka, P. Vinciguerra, E.R. McDonald 3rd, K.E. Hurov, J. Luo, B.A. Ballif, S.P. Gygi, K. Hofmann, A.D. D'Andrea, S.J. Elledge, Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair, Cell 129 (2007) 289–301.
[105] J.C. Dorsman, M. Levitus, D. Rockx, M.A. Rooimans, A.B. Oostra, A. Haitjema, S.T. Bakker, J. Steltenpool, D. Schuler, S. Mohan, D. Schindler, F. Arwert, G. Pals,

C.G. Mathew, Q. Waisfisz, J.P. de Winter, H. Joenje, Identification of the Fanconi anemia complementation group I gene, FANCI, Cell Oncol. 29 (2007) 211–218.
[106] A.E. Sims, E. Spiteri, R.J. Sims 3rd, A.G. Arita, F.P. Lach, T. Landers, M. Wurm, M. Freund, K. Neveling, H. Hanenberg, A.D. Auerbach, T.T. Huang, FANCI is a second monoubiquitinated member of the Fanconi anemia pathway, Nat. Struct. Mol. Biol. 14 (2007) 564–567.
[107] M. Ishiai, H. Kitao, A. Smogorzewska, J. Tomida, A. Kinomura, E. Uchida, A. Saberi, E. Kinoshita, E. Kinoshita-Kikuta, T. Koike, S. Tashiro, S.J. Elledge, M. Takata, FANCI phosphorylation functions as a molecular switch to turn on the Fanconi anemia pathway, Nat. Struct. Mol. Biol. 15 (2008) 1138–1146.
[108] R.T. Abraham, Cell cycle checkpoint signaling through the ATM and ATR kinases, Genes Dev. 15 (2001) 2177–2196.
[109] E. Riballo, M. Kuhne, N. Rief, A. Doherty, G.C. Smith, M.J. Recio, C. Reis, K. Dahm, A. Fricke, A. Krempler, A.R. Parker, S.P. Jackson, A. Gennery, P.A. Jeggo, M. Lobrich, A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci, Mol. Cell 16 (2004) 715–724.
[110] P.R. Andreassen, A.D. D'Andrea, T. Taniguchi, ATR couples FANCD2 monoubiquitination to the DNA-damage response, Genes Dev. 18 (2004) 1958–1963.
[111] M.P. Stokes, J. Rush, J. Macneill, J.M. Ren, K. Sprott, J. Nardone, V. Yang, S.A. Beausoleil, S.P. Gygi, M. Livingstone, H. Zhang, R.D. Polakiewicz, M.J. Comb, Profiling of UV-induced ATM/ATR signaling pathways, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 19855–19860.
[112] S. Matsuoka, B.A. Ballif, A. Smogorzewska, E.R. McDonald 3rd, K.E. Hurov, J. Luo, C.E. Bakalarski, Z. Zhao, N. Solimini, Y. Lerenthal, Y. Shiloh, S.P. Gygi, S.J. Elledge, ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage, Science 316 (2007) 1160–1166.
[113] J.W. Harper, S.J. Elledge, The DNA damage response: ten years after, Mol. Cell 28 (2007) 739–745.
[114] T. Hunter, The age of crosstalk: phosphorylation, ubiquitination, and beyond, Mol. Cell 28 (2007) 730–738.
[115] E. Kinoshita, E. Kinoshita-Kikuta, K. Takiyama, T. Koike, Phosphate-binding tag, a new tool to visualize phosphorylated proteins, Mol. Cell Proteomics 5 (2006) 749–757.
[116] W. Wang, A major switch for the Fanconi anemia DNA damage-response pathway, Nat. Struct. Mol. Biol. 15 (2008) 1128–1130.
[117] T. Kawamoto, K. Araki, E. Sonoda, Y.M. Yamashita, K. Harada, K. Kikuchi, C. Masutani, F. Hanaoka, K. Nozaki, N. Hashimoto, S. Takeda, Dual roles for DNA polymerase eta in homologous DNA recombination and translesion DNA synthesis, Mol. Cell 20 (2005) 793–799.
[118] Y. Wu, K. Shin-Ya, R.M. Brosh Jr., FANCJ helicase defective in Fanconia anemia and breast cancer unwinds g-quadruplex DNA to defend genomic stability, Mol. Cell Biol. 28 (2008) 4116–4128.
[119] I. Cheung, M. Schertzer, A. Rose, P.M. Lansdorp, Disruption of dog-1 in *Caenorhabditis elegans* triggers deletions upstream of guanine-rich DNA, Nat. Genet. 31 (2002) 405–409.
[120] E. Kruisselbrink, V. Guryev, K. Brouwer, D.B. Pontier, E. Cuppen, M. Tijsterman, Mutagenic capacity of endogenous G4 DNA underlies genome instability in FANCJ-Defective *C. elegans*, Curr. Biol. 18 (2008) 900–905.
[121] J.L. Youds, L.J. Barber, J.D. Ward, S.J. Collis, N.J. O'Neil, S.J. Boulton, A.M. Rose, DOG-1 is the *Caenorhabditis elegans* BRIP1/FANCJ homologue and functions in interstrand cross-link repair, Mol. Cell Biol. 28 (2008) 1470–1479.
[122] Q.S. Zhang, L. Eaton, E.R. Snyder, S. Houghtaling, J.B. Mitchell, M. Finegold, C. Van Waes, M. Grompe, Tempol protects against oxidative damage and delays epithelial tumor onset in Fanconi anemia mice, Cancer Res. 68 (2008) 1601–1608.
[123] J.R. Ridpath, A. Nakamura, K. Tano, A.M. Luke, E. Sonoda, H. Arakawa, J.M. Buerstedde, D.A. Gillespie, J.E. Sale, M. Yamazoe, D.K. Bishop, M. Takata, S. Takeda, M. Watanabe, J.A. Swenberg, J. Nakamura, Cells deficient in the FANC/BRCA pathway are hypersensitive to plasma levels of formaldehyde, Cancer Res. 67 (2007) 11117–11122.

Ⅸ. 血液・腫瘍性疾患－20

Fanconi 貧血

Fanconi anemia

矢部みはる*

YABE Miharu

①病　因

Fanconi貧血(FA)は染色体不安定性を特徴とする常染色体劣性の遺伝性疾患であり，造血不全から骨髄異形成症候群(MDS)や白血病などの血液腫瘍や固形癌の発症率も高い。日本小児血液学会の全国登録データによれば，1998～2005年に診断された造血障害性疾患1,411例中89例(6.3%)を占め，男女差はなく，年間発生数は5～10人であった[1]。出生100万人当たり5人前後であり，FA遺伝子の保因者は200～300人に1人と推定される。

②発症機序・病態

1. FA分子経路

FAは遺伝的に多様な疾患であり，DNA架橋剤に対する感受性の体細胞融合による遺伝子相補試験により現在までに13の責任遺伝子が同定されている(表1)[2]。12の相補群遺伝子は常染色体劣性遺伝形式をとるが，B群遺伝子はX連鎖劣性遺伝であることが明らかにされている。FAの各相補群は原因遺伝子が異なるにもかかわらず，患者の臨床症状は類似しており，これらの遺伝子産物は相互作用し，FAコア複合体(8個のFA蛋白FANCA, -B, -C, -E, -F, -G, -L, Mと2つのFA関連蛋白FAAP100, FAAP24を含む)を形成し同一の経路で働いていると考えられる。2001年に同定されたFANCD2蛋白は上記の複合体に依存してモノユビキチン化を受け活性型となり，活性型FANCD2は乳癌感受性遺伝子であるBRCA1と相互作用し，DNA修復を制御する[3]。したがって，これらのどれか1つの遺伝子産物が先天的に欠損すると，この経路の機能不全のためFAとして発症する(図1)[4]。日本人における解析では13の遺伝子群中，A, C, G群の存在が確認されており，A群の頻度がもっとも高いが[5]，遺伝子型と表現型の重症度の関係は単純ではなく，さらなる検討を必要とする。

表1　Fanconi貧血の遺伝子型

相補群	遺伝子	頻度(%)		FANCD2モノユビキチン化への必要性
		欧米	日本	
FA-A	*FANCA*	66	56	＋
FA-B	*FANCB*	～2	－	＋
FA-C	*FANCC*	10	10	＋
FA-D1	*FANCD1/BRCA2*	～2	－	－
FA-D2	*FANCD2*	～2	－	＋
FA-E	*FANCE*	～2	－	＋
FA-F	*FANCF*	～2	－	＋
FA-G	*FANCG/XRCC9*	9	15	＋
FA-I	*FANCI*	～2	－	＋
FA-J	*FANCJ/BRIP1/BACH1*	～2	－	－
FA-L	*FANCL/PHF9*	<0.2	－	＋
FA-M	*FANCM/Hef*	<0.2	－	＋
FA-N	*FANCN/PALB2*	～2	－	－

(Wang[2], 2007より引用一部改変)

2. 細胞表現型

低濃度のマイトマイシンC(MMC)やdiepoxybutane(DEB)などのDNA架橋剤とともにリンパ球を培養すると，多数の染色体断裂やquadriradicalの形成がみられる[6]。PHAなどのマイトジェンを加えたリンパ球の細胞周期のG2が著明に延長するのもFA患者に大きな特徴であり，フローサイトメトリーによる診断が有用である。20%前後のFA患者ではMMCに正常の感受性を示す細胞の混在が報告され，reversionを起こした細胞が増加したモザイク状態であると考えられている。MMCによる染色体断裂試験が偽陰性にでる可能性もあり，また骨髄不全の軽症化や自然寛解例の報告もされており，注意が必要である[7]。こうした体細胞モザイクが疑われるFA患者には皮膚などの線維芽細胞を用いた染色体断裂試験やフローサイトメト

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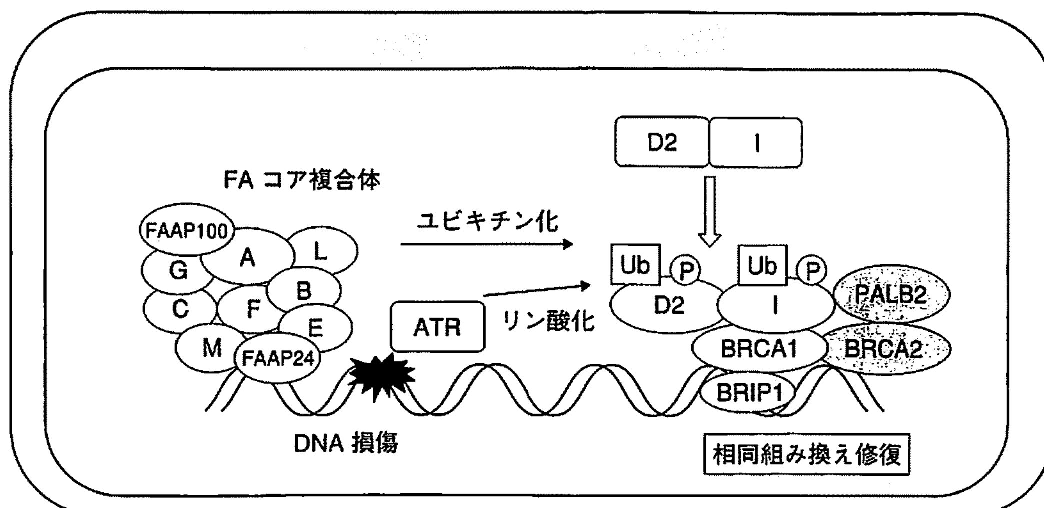

図1 FA/BRCA 経路の模式図(山下ら[4], 2008)
アルファベット A, B, ~M は FANCA, FANCB, ~FANCM 蛋白, FAAP は FA 関連蛋白を示す。Ub：ユビキチン, P：リン酸

リーによる G2 延長の証明が有用かもしれない。

3 臨床症候

1. 身体的特徴

FA の臨床像は多様で種々の合併奇形を伴うが，まったく奇形がみられない症例もある。Fanconi Anemia Resurch Fund の調査では奇形がまったくない症例は 25%を占めたが[8)]，日本の FA 患者 56 例では 4 例(7%)と少なかった[9)]。低身長，カフェオレ斑や皮膚色素沈着，多指症，拇指低形成，前腕の奇形などの外表奇形だけでなく，片腎，消化管や先天性心疾患などの内臓奇形を伴うことも多い(表 2)。

2. 骨髄不全

再生不良性貧血の発症年齢は約 7 歳といわれており，血小板減少が最初の徴候であることが多いが，まれに出生時あるいは成人になってから診断される例もある。日本小児血液学会の再生不良性貧血委員会の全国調査では診断時年齢は 0~14 歳(中央値 5 歳)であった[10)]。後天性再生不良性貧血と同様に汎血球減少に基づく種々の症状が出現してくる。FA では経過中に骨髄異形成症候群(MDS)や急性骨髄性白血病(AML)へと移行する頻度が高く，国際 Fanconi 貧血登録(IFAR)では 10 歳までに 80%，40 歳までに 90%の患者は再生不良性貧血を発症し，30 歳までに 20%，40 歳までに 30%の患者が MDS や白血病に罹患すると報告されている[11)]。MDS や白血病，固形癌を初発症状とする症例もある。

表2 Fanconi 貧血にみられる身体異常の頻度

症状	FA research fund (N=1,206)	日本 (矢部ら，N=56)
皮膚色素沈着	55%	83%
成長障害	51%	76%
上肢	43%	55%
生殖器　男性	32%	9%
生殖器　女性	3%	4%
頭頸部	26%	17%
眼	23%	13%
腎臓・尿路	21%	18%
耳，難聴	9%	16%
下肢ほか骨格系	8%	12%
心・肺	6%	14%
消化管	5%	13%
合併奇形	25%	7%
低身長/色素沈着のみ	11%	18%

(Owen ら[8)], 2003 より引用一部改変)

3. 固形癌

FA 患者では高頻度に固形癌がみられ，IFAR の 2002 年までの 20 年間の登録 754 例のうち 120 例が血液腫瘍であったが，79 例は非血液系の固形癌であった[11)]。頭頸部扁平上皮癌，膣扁平上皮癌，肝細胞癌の占める割合が多く，血液腫瘍の発症が 10 代に多いのに対して，固形癌の発症は 25 歳を

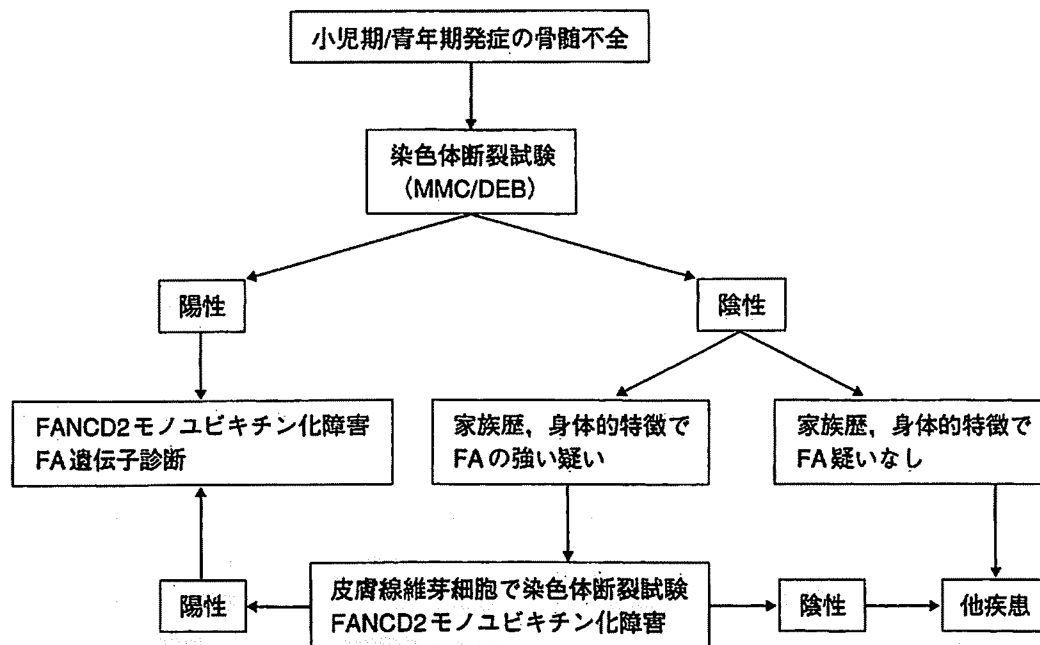

図2　Fanconi貧血の診断のためのフローチャート

超えた比較的年齢の高い患者に発症する傾向にあった。

4 臨床検査

再生不良性貧血の重症度は後天性再生不良性貧血で用いられている基準に従って判断する。

臨床像のみでFAを確定診断することは困難である。小児や青年期に発症した再生不良性貧血患者に対しては全例に染色体断裂試験を行うことが望ましい。一部のFA細胞ではMMCで染色体脆弱を検出しにくい症例もありIFARではDEBを用いた染色体検査を推奨している。また，FANCD2産物に対する抗体を用い，ウエスタンブロット法でモノユビキチン化を確認する方法も有用である。FANCD2のモノユビキチン化が障害されていれば，A，C，G群のいずれかに属する可能性が高く，FAが疑われるにもかかわらずFANCD2のモノユビキチン化が正常の場合はD1群(BRCA2)の異常が考えられる。診断のフローチャートを図2に示す。

FA患者の一部の症例には異常ヘモグロビン症がないにもかかわらず胎児ヘモグロビン(HbF)の高値を伴う例がある。また，MDSやAMLへ移行する頻度が高いため，定期的な骨髄検査が必要である。白血化に伴う染色体異常として7番染色体(−7, 7q−)や1番染色体の異常が多い。3q26q29の染色体の異常では高率にMDS/AMLへ移行することが報告されている[12)]。

5 治療目標および効果判定

FAの治療目標は正常造血を得ることと，固形癌の発症を予防することにある。再生不良性貧血の治療は特発性造血障害に関する調査研究班から参照ガイドとして提案されている[5)]。

1．保存的治療

FAは幹細胞レベルの障害に基づく造血障害であり，抗リンパ球グロブリンなどの免疫抑制療法の効果は期待できない。蛋白同化ホルモンが約半数の患者で一時的な効果を示すことがあるが，肝障害などの副作用があり，造血幹細胞移植の成績を下げるとの報告があるため，長期の使用は避ける。輸血は後天性再生不良性貧血と同様の基準で開始し，ヘモグロビンは6.0 g/dlを，血小板は5,000/μlを維持することが望ましい。好中球減少時に重症感染症を発症した場合は顆粒球コロニー刺激因子(G-CSF)の投与も考慮する。

2．造血幹細胞移植

FA患者にとって造血幹細胞移植は唯一根治が期待できる治療法である。通常の再生不良性貧血で用いられる放射線照射や大量シクロホスファミド

(CY)の投与では，重篤な粘膜障害や急性移植片対宿主病(GVHD)による治療関連毒性が強かった。少量 CY と局所放射線照射(TAI)に抗胸腺グロブリン(ATG)を加え，GVHD に対するシクロスポリン A (CyA)の投与により，HLA 一致同胞間移植は向上したが，一致同胞以外の代替ドナーからの移植の成績は，生着不全と急性 GVHD のためきわめて不良であった[13]。最近 FA 患者に対してフルダラビン(Flu)を中心とした前処置が開発され，飛躍的に成績が向上した。わが国でも 27 例の代替ドナーから Flu を含む前処置を用い，T 細胞除去を用いない移植を施行し(うち 7 例は MDS 症例，12 例は HLA 不一致)，26 例に生着が得られ，生存している[14]。Flu レジメンは免疫抑制効果だけではなく，抗腫瘍効果にも期待がもたれる。

1）移植幹細胞ソース

幹細胞ソースは原則として骨髄とする。FA に対する造血幹細胞移植後の二次癌の発症は，慢性 GVHD が大きな危険因子となるため，末梢幹細胞移植は選択しない。HLA 一致の血縁臍帯血移植は骨髄と同等の成績が得られるが，非血縁臍帯血移植は生着不全のリスクが高く推奨しない。

2）移植適応

FA 患者では 10 歳を過ぎると MDS や AML への移行の頻度が高まることと移植後の慢性 GVHD の合併頻度が高まることより，10 歳を移植適応年齢の目安とするが軽症例では絶対適応ではない。再生不良性貧血では汎血球減少の重症度に応じて移植時期を選択する。クローナルな染色体異常の発現例や MDS や AML に進展した場合は早期の移植実施を考える。FA から移行した急性白血病に対する確立した化学療法のプロトコールはない。その多くは AML であり，減量したアントラサイクリン系の薬剤やフルダラビン，中等量キロサイドなどが報告されているが，化学療法のみでの根治は期待できず，早期に移植療法を実施する。

3）移植前処置と GVHD 予防

再生不良性貧血と MDS・AML 移行例，HLA 一致同胞間移植と代替ドナーからの移植とでは前処置や GVHD 予防はそれぞれ異なり，専門施設への紹介が望ましい。

6 合併症

FA 患者では自然経過での固形癌の発生率が高いうえに，移植前処置で使用する放射線やアルキル化剤の感受性も高く，また慢性 GVHD の合併に伴いより固形癌の発生が高まるため長期的な観察が必要である。

身体合併奇形は小児外科，整形外科，耳鼻科などと連絡をとり手術を施行する。

FA 患者では多くの内分泌異常を伴う症例が多い[8]。低身長は約半数に認められ，一部の患者では成長ホルモンの分泌不全が報告されているが，貧血や移植に伴う影響もあり，不明な点も多い。糖尿病については耐糖能の不良と高インスリン血症を示す症例もあるが，輸血によるヘモクロマトーシスの影響や蛋白同化ホルモン，移植で使用されるプログラフなどの影響もあり種々の要因が考えられる。そのほか，甲状腺機能低下症も約 40％の患者に合併する。原発性性腺機能不全もみられ，女性では月経の発来の遅れ，月経期間が不規則，妊娠率の低下，閉経が早いなどの異常がみられる。男性では精子形成不良が高度な症例が多い。

7 臨床経過および予後

小児再生不良性貧血委員会の報告では FA の予後は診断年度で大きく異なる[1]。診断年度 1988～1993 年に比べて 1994 年診断以後の症例は明らかに改善している。フルダラビンの使用により FA 患者の非血縁者間移植は飛躍的に向上し，2001～2005 年診断 15 例中 8 例が同種移植を受けているが全員生存しており，非移植例も含めてこの群の生存率は 100％となっている。FA は小児に限らず，とくに血液腫瘍や固形癌の合併などの自然歴を解明するには成人も含めた全国調査が必要である。正常造血を得ることに関しては短期的には改善が得られたものの，固形癌の発症を含めた長期的予後は不明な点が多い。

文献

1）小原　明：日本における小児再生不良性貧血など造血障害性疾患の現状：日本小児血液学会再生不良性貧血委員会疫学調査 1988～2005 年．日小血会誌 **22**：53-62，2008

2) Wang W : Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. Nat Rev Genet **8** : 735-748, 2007
3) Timmners C, Taniguchi T, Hejna J, et al : Positional cloning of a novel Fanconi anemia gene, FANCD2. Mol Cell **7** : 241-248, 2001
4) 山下孝之, 小田 司, 関本隆志 : Fanconi 貧血 ; ゲノム損傷ストレスと造血幹細胞の老化. 日本臨牀 **66** : 477-482, 2008
5) 小島勢二, 谷ヶ崎博, 山下孝之 : Fanconi 貧血診療の参照ガイド. 臨床血液 **47** : 791-802, 2005
6) Sasaki MS, Tomomura A : A high susceptibility of Fanconi's anemia to chromosome breakage by DNA cross-linking agents. Cancer Res **33** : 1829-1836, 1973
7) Soulier J, Leblanc T, Larghero J, et al : Detection of somatic mosaicism and classification of Fanconi anemia patients by analysis of the FA/BRCA pathway. Blood **105** : 1329-1336, 2005
8) Owen J, Owen Frohnmayer L, Ellen M : Fanconi anemia ; Standards for clinical care, Fanconi Anemia Research Fund Inc, Oregon, p6, 2003
9) Yabe M, Yabe H, Hamanoue S, et al : *In vitro* effect of fludarabine, cyclophosphamide, and cytosine arabinoside on chromosome breakage in Fanconi anemia patients : relevance to stem cell transplantation. Int J Hematol **85** : 354-361, 2007
10) 矢部みはる, 谷ヶ崎博, 迫 正廣 : Fanconi 貧血の全国調査 ; 二次調査報告. 日小血会誌 **17** : 554-556, 2003
11) Kulter DI, Singh B, Satagopan J, et al : A 20-year perspective on the International Fanconi Anemia Registry. Blood **101** : 1249-1256, 2003
12) Tonnies H, Huber S, Kuhl JS, et al : Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients : gains of the chromosomal segment 3q26q29 as an adverse risk factor. Blood **101** : 3872-3874, 2003
13) Guardiola Ph, Pasquini R, Dokal I, et al : Outcome of 69 allogeneic stem cell transplantation for Fanconi anemia using HLA-matched unrelated donors : a study on behalf of the European group for blood and marrow transplantation. Blood **95** : 422-429, 2000
14) Yabe H, Inoue H, Matsumoto M, et al : Allogeneic haematopoietic cell transplantation from alternative donors with a conditioning regimen of low-dose irradiation, fludarabine and cyclophosphamide in Fanconi anemia. Br J Haematol **134** : 208-212, 2006

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Fanconi貧血の造血幹細胞移植

矢部みはる*

Fanconi貧血(FA)は染色体不安定性と身体奇形を特徴とし，ほとんどが常染色体劣性の遺伝性疾患であり，骨髄不全から白血病への移行や，固形腫瘍の発症率も高い．血液学的異常は40歳までに90％以上の症例に生じると考えられており，現時点では造血幹細胞移植が唯一治癒の期待できる治療法である．リン酸フルダラビン(Flu)を前処置に用い，タクロリムスを移植片対宿主病(GVHD)の予防に使用することにより，わが国のFAの非血縁間移植の成績は飛躍的に向上した．FAの移植後の二次がんを含めた長期的予後は不明な点も多く，今後の検討課題である．

はじめに

Fanconi貧血(FA)患者にとって造血幹細胞移植は唯一根治が期待できる治療法である．通常の再生不良性貧血で用いられる放射線照射や大量シクロホスファミド(cyclophosphamide：CY)の投与では，重篤な粘膜障害や急性移植片対宿主病(graft-versus-host disease：GVHD)による治療関連毒性が強く，満足な成績は得られなかった．少量CYと局所放射線照射すなわち，胸腹部照射(thoraco-abdominal irradiation：TAI)あるいは全リンパ節照射(total lymphoid irradiation：TLI)に抗胸腺グロブリン(anti-thymocyte globulin：ATG)を加え，GVHD予防に対するシクロスポリン(cyclosporine：CyA)の投与により，ヒト組織適合抗原(human leukocyte antigen：HLA)一致同胞間移植は向上したが，一致同胞以外の代替ドナーからの移植の成績は，生着不全と急性GVHDのためきわめて不良であった[1][2]．最近FA患者に対してリン酸フルダラビン(Fludarabine phosphate：Flu)を中心とした前処置が開発され，飛躍的に成績が向上し，造血能の改善が得られている．わが国でも27例の代替ドナーからFluを含む前処置を用い，T細胞除去を用いない移植を施行し(うち12例はHLA不一致)，26例に生着が得られ，生存している[3]．FAは小児血液学会の再生不良性貧血の登録においても年間登録数5例前後と患者数もかぎられるため，前方視的治療研究は世界でも少なく，情報も乏しい[4]．疾患登録事業で得られたデータや海外の文献をもとに，われわれも参加し，専門家が共同で，わが国のFA患者に対する診療の参照ガイドを作成した[5]．治療の中心となるのは造血幹細胞移植であり，自施設症例を中心に，欧米諸国などの移植方法と比較検討を交えて紹介する．

Key Words
Fanconi貧血
造血幹細胞移植
フルダラビン
代替ドナー
タクロリムス

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表❶ Fanconi貧血の移植適応・東海大学医学部附属病院の場合(小島ら，2006[5]より改変引用)

1．再生不良性貧血	
Stage 1(軽症)	経過観察
Stage 2(中等症)	10歳未満では経過観察． 10歳以上ではHLA一致血縁ドナーがいれば同種骨髄移植
Stage 3(やや重症)	HLA一致血縁ドナーがいれば同種骨髄移植
Stage 4，5(重症：最重症)	HLA1座不一致血縁ドナー，HLA一致～HLA1座不一致非血縁ドナーからの移植を含めて適応とする．
2．骨髄異形成症候群・白血病	
RA，クローナルな染色体異常	重症再生不良性貧血に準じる
RAEB・白血病	HLA1座不一致血縁ドナー，HLA一致～HLA1座不一致非血縁ドナーからの移植も含めて適応とする．生命予後がきわめて不良と予想される例ではHLA2，3座不一致血縁ドナーからの移植も考慮する．

RA：refractory anemia, RAEB：refractory anemia of excess of blasts

1．移植幹細胞ソース

幹細胞ソースは原則として骨髄とする．FAに対する造血幹細胞移植後の二次がんの発症は，慢性GVHDが大きな危険因子となるため，末梢幹細胞移植は選択しない．HLA一致の血縁臍帯血移植は骨髄とほぼ同等の成績が得られる可能性が高いが，非血縁臍帯血移植は生着不全のリスクが高く，推奨しない．最近Gluckmanら[6]はヨーロッパでのFAに対する非血縁臍帯血移植の成績を報告している．93例の3年生存率は約40％であり，56例が死亡している．死因の多くは感染症，生着不全，GVHDなどであるが，Fluを用いた前処置やHLA 6/6一致例，移植細胞数が＞4.9×10^7/kgの症例では50～60％の生存率が期待できうるとしており，今後条件のよい臍帯血が得られれば，適応拡大となるかもしれない．

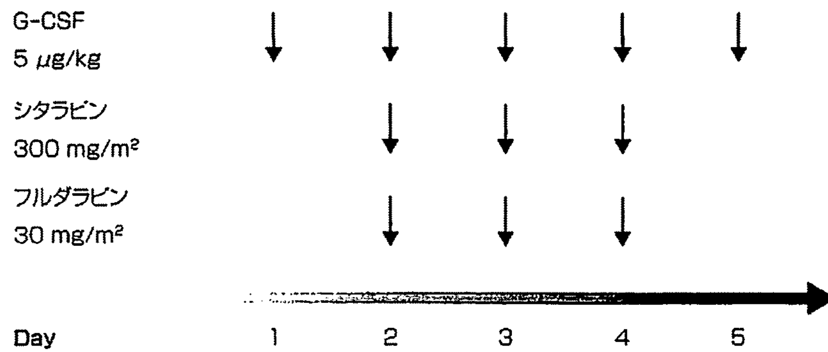
2．移植適応

FA患者では10歳を過ぎると骨髄異形成症候群(myelodysplastic syndrome：MDS)や急性骨髄性白血病(acute myeloblastic leukemia：AML)への移行の頻度が高まることと移植後の慢性GVHDの合併頻度が高まることより，10歳を移植適応年齢の目安とするが軽症例では絶対適応ではない．再生不良性貧血では汎血球減少の重症度に応じて移植時期を選択する．クローナルな染色体異常の発現例やMDSやAMLに進展した場合は早期の移植実施を考える．FA患者に対する診療の参照ガイドからのFAの移植適応を示す(表❶)[5]．FAから移行した急性白血病に対する確立した化学療法のプロトコールはない．その多くはAMLであり，減量したアントラサイクリン系の薬剤やフルダラビン，少量～中等量シタラビンなどが報告されているが，化学療法のみでの根治は期待できず，早期に造血幹細胞移植を実施する．Mehtaら[7]が報告したreduced intensity FLAG(fludarabine, cytosine arabinoside：Ara-C, granulocyte-colony stimulating factor：G-CSF)療法はわれわれも使用したが，移植までの病勢をコントロールするには比較的無理なく使用できる(図❶)[7]．

3．移植前処置

通常の移植前処置で用いられる放射線照射や大量CY投与では移植関連毒性が強く，少量CYとTAI，ATGの併用によりHLA一致同胞間移植では安定した成績が得られるようになった[8)~10)]．しかしながら，放射線照射を含む前処置と移植後二次がんの関連が指摘されたことから，照射量の減量化，あるいはFluを基盤とした，放射線治療を含まない前処置へ取り組みが強まっている．

HLA一致同胞ドナーからの移植と代替ドナーからの移植では移植前処置法は異なる．一致同胞ドナーからの同種骨髄移植の治療成績を示す(表❷)．少量のCYとTAI，ATGの併用から，CY単剤投与[11)12)]あるいはFlu



図❶ Reduced intensity FLAG 療法(Mehta PA *et al*, 2007[7]より改変引用)

表❷ Fanconi 貧血に対する HLA 一致同胞(家族内)移植による治療成績(筆者作成)

著者	症例数	前処置	GVHD予防	拒絶(%)	急性GVHD II~IV度(%)	慢性GVHD(%)	生存率(%)
Socie *et al*[8]	50	CY(20~40 mg/kg)/TAI(5 Gy)	CyA	6	56	70	74(4年) 59(8年)
Yabe *et al*[15]	5	CY(20~150 mg/kg)/TAI/TBI(6~8 Gy)/ALG	CyA/MTX	20	0	20	100(2年)
Dufour *et al*[9]	22 5	CY(20 mg/kg)/TAI/TBI(5 Gy) CY(120 mg/kg)	CyA または CyA/MTX	8	36	13	81(3年)
Farzin *et al*[10]	30 5	CY(20 mg/kg)/TAI(4 Gy)/ATG CY(40 mg/kg)/TBI(4.5 Gy)/ATG	CyA/ATG /±コルチコステロイド	7 0	20 40	7 40	89(10年)
Bonfim *et al*[11]	43	CY(60 mg/kg)	CyA/MTX	12	17	29	93(3年)
Ayas *et al*[12]	22 34	CY(20 mg/kg)/TAI(4 Gy)/ATG CY(60 mg/kg)	CyA/ATG	0	9 9	9 6	73(7年) 97(3年)
Tan *et al*[13]	11	Flu/CY/ATG	CyA/MP /T細胞除去	0	0	0	100(2年)
Pasquini *et al*[14]	77 71	照射群　種々 非照射群	CyA/MTX	3 4	23 21	18 24	78(5年) 81(5年)

Flu：fludarabine, CY：cyclophosphamide, ATG：antithymocyte globulin, ALG：antilymphocyte globulin,
TAI：thoraco-abdominal irradiation, TBI：total body irradiation, CyA：cyclosporine, MTX：methotrexate, MP：methylprednisolone

を含む非照射レジメンへと移植方法の開発が試みられている[13][14]．FA 患者のなかにはマイトマイシン C(MMC)やディエポキシブタン(diepoxybutane：DEB)に正常の感受性を示す細胞の混在が報告され，モザイク状態を反映すると考えられる．モザイクの患者では上記薬剤に正常の感受性を示すリンパ球が存在し，これらのリンパ球は前処置に用いる CY に対しても正常の感受性であるため，少量の CY では生着不全の可能性が高くなる．われわれ[15]は移植前に患者リンパ球に CY 代謝物を加え，染色体脆弱性を個別に検査し，前処置に使用する CY 投与量を調節することにより良好な移植成績をあげることができた．わが国の FA 患者ではこうしたモザイク状態を示す患者が高頻度であると推測されるため，一律の CY 単剤投与では拒絶のリスクが高まると予測される[16]．Flu の登場は，このような CY 投与量の調節を不要にし，HLA 一致同胞間移植での非照射レジメンを可能とした．われわれも 2000 年以降の移植では表❸[5]に示したような Flu を含んだ一律の非照射前処置に替えることにより，7 例全例に生着が得られ生存している．

これに対して，代替ドナーからの移植の成績はきわめて不良で，少量 CY と TAI，ATG の併用療法では拒絶，急性 GVHD や移植関連毒性などの死亡が多く，30 %前後の生存率しか得られなかった[1][2][17]．代替ドナーからの同種骨髄移植の治療成績を示す(表❹)．Flu を含んだ前

表❸　Fanconi貧血に対する移植前処置法・東海大学医学部附属病院の場合(小島ら，2006[5]より改変引用)

	HLA一致同胞ドナー	代替ドナー
再生不良性貧血(RAを含む)		
Flu	25 mg/m²×6 days	25 mg/m²×6 days
CY	10 mg/kg×4 days	10 mg/kg×4 days
ATG	1.25 mg/kg×4 days	1.25 mg/kg×4days
TLI/TAI	なし	3 Gy
骨髄異形成症候群および急性白血病(RAEB以上の進行例)		
Flu	25 mg/m²×6 days	25 mg/m²×6 days
CY	10 mg/kg×4 days	10 mg/kg×4 days
ATG	1.25 mg/kg×4days	1.25 mg/kg×4 days
TBI	4.5 Gy(1.5 Gy×3 times)	4.5 Gy(1.5 Gy×3 times)

RA：refractory anemia, RAEB：refractory anemia of excess of blasts, Flu：fludarabine, CY：cyclophosphamide, ATG：antithymocyte globulin, TAI：thoraco-abdominal irradiation, TLI：total lymphoid irradiation, TBI：total body irradiation

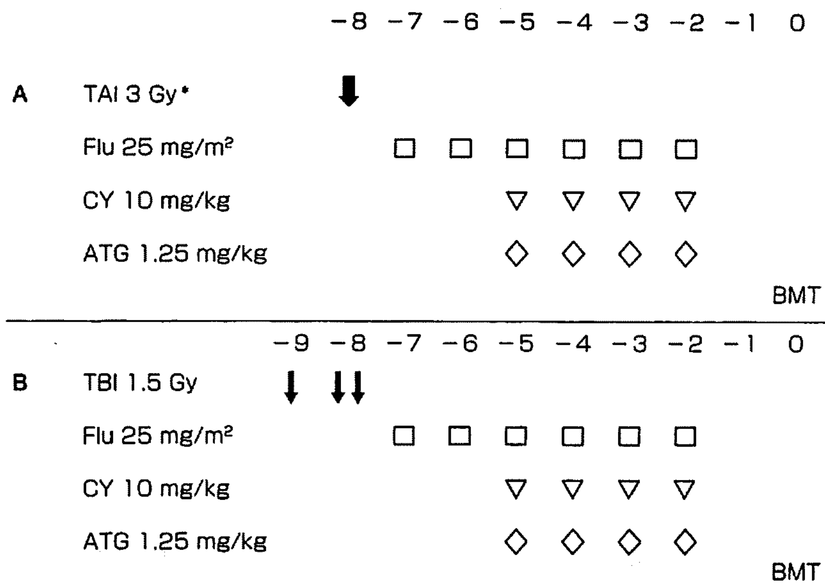
表❹　Fanconi貧血に対する代替ドナーからの移植による治療成績(筆者作成)

著者	症例数	前処置	GVHD予防 T細胞除去症例(%)	拒絶(%)	急性GVHD II~IV度(%)	慢性GVHD(%)	生存率(%)
Gluckman *et al*[2]	48	CY20 ->100 mg/kg/TBI/LFI	CyA/MTX 18	24	51	46	29(2年)
MacMillan *et al*[17]	29	CY40 mg/kg/TBI 4.5~6 Gy/ATG	CyA/MTX 100	37	32	0	34(1年)
Guariola *et al*[1]	69	CY20~60>=mg/kg/TAI/TBI 種々	CyA/MTX/コルチコステロイド /anti-T therapy 38	20	42	42	33(3年)
Boyer *et al*[18]	8	CY20 mg/kg/Flu/TBI4.5 Gy /ATG	なし 100	29	29	0	58±18 (1年)
Yabe *et al*[3]	27	Cy40 mg/kg/Flu/TAI/TBI 3~4.5 Gy/ATG	Tacrolimus/MTX/MMF 0	3.7	12	31	96(1年)
Wagner *et al*[19]	52 43	非Flu群　種々 Flu群　種々	CyA/MTX/ Tacrolimus種々 71(両群あわせて)	22	31	30	13~52 (3年)
Chaudhury *et al*[20]	18	CY40 mg/kg/Flu/TBI4.5 Gy /ATG	Tacrolimus 100	0	6	0	67~72 (5年)

Flu：fludarabine, CY：cyclophosphamide, Bu：busulfan, ATG：antithymocyte globulin, ALG：antilymphocyte globulin, TAI：thoraco-abdominal irradiation, TBI：total body irradiation, LFI：limited field irradiation, CyA：cyclosporine, MTX：methotrexate, MMF：mycophenolate mofetil

処置の開発により，代替ドナーからの移植成績は飛躍的に向上した．欧米諸国ではFluを含んだ前処置にT細胞除去を加えたGVHD予防を用いることにより，生着を向上させ，GVHDを回避することができるようになり，成績の向上に結びついた[18]~[20]．わが国のFA患者は，先に述べたようにモザイクを呈する患者が多く，拒絶のリスクが高いため，T細胞除去はおこなわない．代替ドナーからの移植前処置では，HLA一致同胞間移植の前処置にTAI 3Gyを加えることにより良好な成績が得られている(表❸[5]，図❷A)．非照射レジメンへと移行できるかが今後の課題である．

再生不良性貧血と，骨髄異形成症候群や急性白血病に進展した症例とでは移植前処置は異なる．白血病細胞の増殖の少ない不応性貧血(refractory anemia：RA)までは再生不良性貧血に準じた前処置で対応が可能と思われる．白血病細胞の増殖がみられる芽球増加型不応性貧血



図❷ Fanconi貧血に対する移植前処置の投与スケジュール(筆者作成)
A：再生不良性貧血(不応性貧血を含む)
*HLA一致同胞間移植ではTAI 3 Gyを省く
B：骨髄異形成症候群(RAEB以上の進行例)および急性白血病

RA：refractory anemia, RAEB：refractory anemia of excess of blasts, Flu：fludarabine, CY：cyclophosphamide, ATG：antithymocyte globulin, TAI：thoraco-abdominal irradiation, TBI：total body irradiation

(RA with excess of blasts：RAEB)以上の進行例ではTAIを全身照射(total body irradiation：TBI)に変更し，3 Gyから4.5 Gyへと照射量を増大することで対応している(表❸[5]，図❷B)．Chaudhuryら[20]はわれわれと同様の前処置で，T細胞除去を加えた10例のRAEB以上の進行例での移植で，うち8例が生存と良好な成績を報告している．これらの症例のなかで，予後不良といわれる7番染色体異常を含む7症例中5例が再発なく生存中であり，Fluの抗腫瘍効果にも期待が持たれる．

4．GVHD予防

FAではHLA一致同胞間移植でもgradeⅡ以上の急性GVHDが発症する可能性が高く，1998年のヨーロッパの報告でも46例中26例(56.5％)に合併していた[8]．Guardiolaら[21]の報告では少量CY＋TAIの前処置で，HLA一致同胞間移植を受けたFA患者のgradeⅡ以上の急性GVHDの発症率は，スタンダード(CY 200 mg/kg)な治療を受けた再生不良性貧血の患者の2倍であり，12歳以下の患児での頻度は7倍で，慢性GVHDへの移行も高かった．CyAの併用や前処置の軽減によりHLA一致同胞間移植での急性GVHDの頻度は減少し，さらに，Fluを中心とした非照射レジメンの開発とともに同胞間移植の急性GVHDのコントロールは容易となってきている．

一方，代替ドナーからの移植における急性GVHDの頻度は高く，Fluレジメンに変更後も欧米諸国ではT細胞除去がGVHDのコントロールの主体になっている．先に述べたようにわが国のFA患者では，モザイクを呈する患者が多く，拒絶のリスクが高いため，T細胞除去はおこなわない．わが国では，欧米諸国にくらべ，明らかに中等症以上の急性GVHDの合併頻度は低く，代替ドナーからの移植においてはタクロリムスの持続点滴により，GVHDの重症化はほとんどみられていない[3]．当施設でのFA患者に対するGVHD予防法を紹介する(表❺)．

5．移植後二次がん

FA患者では自然経過での固形がんの発生率が高いう