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Fanconi Anemia Signaling and Cancer

Manoj Nepal[1,2,4], **Raymond Che**[1,2,4], **Jun Zhang**[3], **Chi Ma**[1], and **Peiwen Fei**[1,2,*]

[1]University of Hawaii Cancer Center, University of Hawaii, USA

[2]Graduate Program of Molecular Biosciences and Bioengineering, University of Hawaii, USA

[3]Department of Laboratory Medicine and Pathology, Mayo Clinic Foundation, USA

Abstract

The extremely high cancer incidence associated with patients suffering from a rare human genetic disease, Fanconi Anemia (FA), demonstrates the importance of FA genes. Over the course of human tumor development, FA genes perform critical tumor-suppression roles. In doing so, FA provides researchers with a unique genetic model system to study cancer etiology. Here, we review how aberrant function of the twenty-two FA genes and their signaling network contributes to malignancy. From this perspective, we will also discuss how the knowledge discovered from FA research serves basic and translational cancer research.

Keywords

FA signaling; ATM/ATR; Genome instability; Cancer; and Tumor development & Resistance

I. The FA Pathway: a Tumor Suppressor Signaling Network

Fanconi Anemia (FA) is a rare human genetic disease, originally described by the Swiss pediatrician Guido Fanconi in 1927 [1]. FA occurs following germline mutations in any of FA genes, which is characterized by an early onset of aging, severe bone marrow failure, and an extremely high predisposition to various cancers. To date, researchers have identified germline mutations in 22 specific genes (*FANCA /B /C /D1 /D2 /E /F /G /I /J /L /M /N /O /P /Q /R /S /T /U /V & /W* [2–6], each accounting for an individual FA complementation group. FA patients develop acute myelogenous leukemia (AML) at an incidence 700-fold higher compared to the general population. However, FA patients who overcome severe bone marrow failure following a successful bone marrow transplant are still likely to develop head and neck, esophageal, gastrointestinal, vulvar, and anal cancers at an incidence approximately 50 fold higher [7].

[*]**Correspondence to:** Peiwen Fei, MD PhD, University of Hawaii Cancer Center, 701 Ilalo Street, Honolulu, HI 96813, Phone: 808-356-5786, pfei@cc.hawaii.edu.

[4]Equal contribution

At the cellular level, cells from FA patients are hypersensitive to DNA crosslinking agents and carry a distinct form of chromosomal abnormalities, which has been exploited as a clinical tool to diagnose FA [8, 9]. Together, the similar molecular features along with common clinical symptoms displayed from each FA sub-group suggest that the FA gene encoded products can function in one common signaling pathway. This has been named the *FA pathway* or the *FA-BRCA pathway*, noting that some of the FA proteins are BRCA-related proteins. Activation of the FA pathway occurs as a result of DNA replication or DNA damage, especially the damage triggered from DNA crosslinking agents. Within this process, the FA complex E3 with FANCL as the catalytic subunit [10–13] monoubiquitinates FANCD2 and its paralog FANCI. Following this, the monoubiquitinated FANCD2 and FANCI form nuclear foci and interact with numerous other FA and non-FA proteins to repair the damaged DNA. The monoubiquitination of FANCD2 appears to be at the center of this pathway and has been considered to be the representative event of pathway activation. In addition, recent studies suggested that many FA proteins can also function in a pathway-independent manner [14–16]. In regards to this, FA signaling or FA signaling network now depicts accumulated actions performed by the FA proteins both pathway-dependently and independently.

Mutations in the FA proteins lead to a high tumor incidence. In addition, wild type FA proteins were previously suggested to have tumor suppressor roles several decades ago [17]. As accumulated studies have indicated, malfunctioned FA genes and proteins have been found to be associated with a variety of cancer patients who do not have FA (Table 1). For the first time, our studies have evidently shown that an impaired FA pathway is associated with the development of human cancer, and the altered pathway can promote the development of non-FA human cancer[18–21]. These findings further support the general tumor suppressor roles played by the FA proteins. Whereas, malfunctioned FA proteins have emerged to be a major type of driving force behind the development of non-FA human cancer [6, 22, 23].

In this review, we highlight how FA research has advanced as well as provided perspective in how it will further promote our understanding of human tumorigenesis and cancer treatment. Accordingly, FA signaling acts in the maintenance of genome stability in cooperation with ATM and/or ATR, the major checkpoint master regulators, and in other associated aspects. The latter includes the ancient stress response pathway (SOS) and current topics in gain of function (GOF) and metabolism. To conclude, we will summarize some important perspectives for future studies and hope this review will bring sufficient attention for the contributions of FA studies towards the field of cancer research.

II. FA Signaling and Master Regulators of Cellular Stress Responses

In recent years, tremendous progress has been made to elucidate the various molecular mechanisms controlling the cellular responses to DNA damage, such as checkpoint mechanisms in mammalian cells [24]. Much of our understanding behind the mechanisms involved in the DNA damage responses (DDR) is derived from studies of human cancer susceptibility syndromes wherein the DDR is altered. These syndromes have helped us better understand how ATM (mutated in Ataxia-telangiectasia [25]) and ATR (ataxia

telangiectasia and Rad3-related protein**;** mutated in Seckel syndrome [26]) work as master checkpoint regulators to safeguard genome integrity [27, 28]. Similarly, FA has also contributed to the body of knowledge similar to the aforementioned syndromes. Where studies from our and many other laboratories have used FA as a unique genetic model system to understand how the FA proteins act in the DDR in coordination with these important master checkpoint regulators to refine cellular surveillance on genetic material.

IIa) FA signaling and the DDR

Studies have consistently conferred that when the FA pathway is impaired, cells are hypersensitive to DNA damage, and are unable to successfully repair damaged DNA. As shown in Figure 1 and summarized in the Table 2, FA signaling has been expanded beyond the recognition of the commonly known the FA pathway. To initiate the activation of the FA pathway, FANCM, FA associated protein 24 (FAAP24) and MHF (histone fold proteins) form a complex, which acts at the upstream of the FA pathway. Similar to ATR, the FANCM-FAAP24-MHF complex functions as a key component to detect DNA damage and initiates signal transduction pathways, which promote the monoubiquitination of FANCD2 and FANCI [29–31]. Moreover, earlier studies reported that ATM-dependent phosphorylation of FANCD2 provided further evidence for the role of FANCD2 in the DDR, especially in the S-phase checkpoint response [32]. Within the activation of the FA signaling pathway, numerous players have been recently recognized for their cooperation, including HHR6 [33], hRad18 [33–35], and Blm [36, 37]. Research into FA has continually demonstrated that FA signaling may act as a network, in addition to the primary FA-BRCA pathway axis.

USP1 is known to deubiquitinate FANCD2 and FANCI. However, in addition to this opposition to activate/monoubiquitinate FANCD2/I, USP1 deubiquitination of FANCI has also been associated with promoting core complex recruitment to the site of DNA damage [38]. Whereas, impaired USP1 function leads to genomic instability [39], and cellular senescence with an excessive accumulation of monoubiquitinated FANCI, FANCD2 and PCNA [40]. Therefore, like many other proteins acting in the FA signaling network (Figure 1), USP1 along with the 22 FA proteins critically contributes to the maintenance of genome stability. With an increasing number of FA proteins, FA-associated proteins, and their partners, it is difficult to define the FA signaling network by a definitive number of members.

II-b) FA signaling and ATM

To sufficiently respond to DNA damage, the cellular checkpoints coordinate cell growth arrest with DNA damage repair, or programmed cell death with the elimination of the damaged cells. Double strand DNA breaks (DSBs) may result in the recruitment of the MRN complex [41] at their sites and activation of ATM, and the latter is made through the intermolecular autophosphorylation of Ser1981 and subsequent dissociation of the ATM homodimer [42]. Monomeric ATM can bind to the MRN complex and can be further activated. In turn, activated ATM in turn, phosphorylates the histone variant H2AX at S139 [43, 44]. Subsequently, phosphorylated H2AX (γH2AX) interacts with MDC1 (mediator of DNA damage checkpoint protein-1) at the BRCT region [45], which leads to the expansion

of the similar processes (the recruitment of more ATM–MRN complexes and further H2AX phosphorylation). Activated ATM then phosphorylates downstream targets, in particular, ATM phosphorylates Chk2 at T68 [46–49], p53 at S15 and S20 [50, 51], and HDM2 at S395 [46, 52] to initiate G1 arrest. However, phosphorylation of NBS1 at S343 [53], FANCD2 at S222 [32], FANCS (BRCA1) at S1387 [54], and SMC1at S957 and S966 [55], which also depends upon ATM, leads to an S-phase arrest; accordingly, phosphorylated FANCS (BRCA1) at S1423 [55, 56], hRad17 at S635 and S645 [57] and others cause a G2 arrest. Clearly, the functions of the FA proteins are a necessary part of S and G2 arrest within the ATM signaling. In addition, the regulation of FA signaling by p21 (a cycling-dependent kinase inhibitor) [58] and p53 [59] further supports the role of FA signaling in the downstream of the ATM signaling for regulating cell proliferation. We believe that FA signaling may be involved in coordinating all major events in the checkpoint systems. In particular, ATM not only participates in cell growth arrest & DNA damage repair, but possibly also cell death programs as hinted from the relation with p53 [59]. However, to fully explore the latter, further and more specific studies are needed.

IIc) FA signaling and ATR

ATR is another large kinase with significant sequence homology to ATM. Both target an overlapping set of substrates, in coordination with cell growth arrest, DNA damage repair, and cell death within the cellular checkpoint systems [60]. Conversely, ATR is essential for the viability of mammalian cells, whereas ATM is not [61]. ATM acts upon the occurrences of double-strand breaks (DSBs). In contrast, ATR is activated during the S phase to regulate the firing of replication origins and the repair of stalled replication forks to maintain the faithful DNA replication and subsequently proper segregation of genetic material occurring in the M phase of a cell cycle [62]. As shown in Figure 2, FA signaling is involved in sensing DNA damage, and maintaining normally firing of DNA replication & the stability of replication forks. Therefore, it is commendable to propose the term “ATR-FA signaling” to replace the general term “ATR signaling”. Further, this modification can be strengthened with the roles of FA signaling in DNA damage repair, relevant to nearly all repair mechanisms known so far [11, 12] (Table 2), which is also an essential part of both downstream ATM and ATR initiated signaling events.

ATR signaling primarily responds to the RPA (Replication protein A) -bound single stranded DNA (ssDNA) [61], which triggers the independent recruitment of two protein complexes to the junction of the 5′ ssDNA: the RAD9–RAD1–HUS1 (9-1-1) and ATR–ATRIP (ATR-interacting protein) complex [63–67]. The former involves that RPA directs the loader of the 9-1-1 complexes, the RFC clamp loader (Rad17) [66], which in turn recruits the ATR activator, TOPBP1 (topoisomerase-binding protein-1) [63]. Whereas, the latter is closely involved in FANCM recruitment of ATR-ATRIP to the site of ssDNA. FANCM-FAAP24 was found to bind to the stalled replication forks prior to the binding of RPA [68], indicating FANCM-FAAP24 is crucial for RPA to recruit ATR-ATRIP [29], in addition to being a part of the FA complex. Importantly, FANCM-FAAP24 was also found to interact with ATR-ATRIP and HCLK2 in FA -A, C, and G cells [69], this observation is distinct from the role of FANCM in forming the FA complex E3, which activates FANCD2/I [12]. Aside from FANCM affecting ATR activation, the newly identified FANCW also plays an important role

in ATR functions, at which point can ubiquitinate RPA and modulate the functions of RPA [4, 5]. In addition, the relationship between the FA proteins and ATR may be mutual and of equally importance. The recruitment of ATRIP was reported to be essential for FANCD2 monoubiquitination and FANCI phosphorylation, and the latter is catalyzed by ATR kinase [70]. The FA core complex E3 was found to also enhance the binding of ATRIP to the damaged chromatin site [71]. Together, FANCM, FANCW and other FA players certainly play critical roles in the sensor phase upon stalled replication forks. Furthermore, the importance of the sensing role may be further strengthened as FANCM is one of the mostly conserved FA proteins across species [72], and its functions may be universally required for the checkpoint processes among all living organisms. Here, we believe that the pathway-independent role of FANCM appears to be a critical part in the replicative stress response. Accumulated reported studies have suggested that FAMCM can challenge the commonly known ATR and ATM master regulators as it can stand to be a major member of the master checkpoint regulator family (Figure 2).

Besides ATR-FANCM activity to sense and resolve the stalled replication forks discussed above, Bloom Syndrome RecQ Like Helicase (BLM helicase) has been shown to closely work with the FA proteins in various aspects of cellular life, especially in concert with ATR or “ATR-FA” signaling (Figure 2). Studies that have investigated the functional interactions between FA signaling and BLM have recently demonstrated that BLM can promote the activation of FANCD2, and affect replication forks and the FANCM recruitment upon stalled replication forks [16, 36, 73]. These studies further expand the FA signaling network to govern the stability of replication forks during DNA replication and, thus, genome stability. In addition, ATR/ATM activity might also play a critical role in the modulation of the firing of replication origins by essentially phosphorylating MCMs [74] to couple with the DDR. On the other side, FA signaling via a basal level of FANCD2 monoubiquitination has been identified to play a clear and critical role in regulating the replication origin firing at a proper rate [14]. However, in its absence, cells carrying a compromised basal level of monoubiquitinated FANCD2 would have a slow firing rate, which could explain the early onset of aging associated with FA patients as well as its occurrence in the general population. To date, there were just a few studies demonstrating the importance of FA signaling in the maintenance of genome stability coupling with ATR. However, the role of ATR-FA signaling to regulate a variety of cellular events in DNA replication awaits further study.

III. FA signaling and the Human Homologs of yeast Rad 6 (HHR6) pathway

Aside from ATM and ATR, HHR6/hRad18 also can act as an upstream regulator to monoubiquitinate/activate FANCD2 [33]. These studies were originated from the observation of the similar sensitivity to DNA crosslinking damage revealed in FA cells and Rad6−/− yeast cells. It has been previously shown that FANCD2 monoubiquitination can modulate the activity of translesion DNA synthesis (TLS), at the least through DNA polymerase eta (pol ŋ) [33, 75]. Importantly, FANCD2 has shown to interact with pol ŋ prior to PCNA following DNA damage [76]. PCNA exerts DNA damage error-prone or free repair via recruiting pol eta and other TLS polymerases [77]. As PCNA is the focal point in the HHR6 pathway, this finding stands a critical part to present a more complete picture

regarding the early phase of DNA damage repair. These studies demonstrated roles of FANCD2 as an early responder to DNA damage. For that reason, it may act in a role that is more important than PCNA, a critical scaffold to recruit proteins involved in DNA replication, DNA repair, and chromatin remodeling [78]. Thus, in this manner, FA signaling can act almost immediately to recruit repair proteins to the sites of DNA damage [11, 79]. Although it is still somewhat unclear, studies have shown that FANCD2 and/or the FA core complex may also have a role in coordinating the activity of several other TLS enzymes [80]. Recently, studies supporting this have demonstrated that the interaction of FANCD2 with REV1 promotes the protection of nascent DNA strands in response to replicative stresses [81]. Additionally, the recruitment of the REV1-pol ζ complex requires the FA core complex [82]. Not to mention REV7, a newly identified FA protein (FANCV) [83], is essential in the activity of two TLS enzymes REV1and REV3 [83]. This further widens the scope of FA signaling extending beyond that originally thought. To this end, FA signaling appears to be a commonality in the major stress response-signaling networks (ATM, ATR and HHR6). In addition, the 9-1-1 clamps may stand out as another connection, pursuant to Rad6-Rad18 mediation of the 9-1-1 clamp's ubiquitination [84]. Given the fact that the SOS response is a primitive checkpoint system in prokaryotes [85], the subject relation was concluded to be an evolved form of the SOS response in eukaryotes [84]. However, it remains largely unclear as to how these signaling networks act together in response to a variety of stresses in human cells. FA signaling appears to function as a strong coordinator to tune an advanced form of the SOS response, namely, checkpoint or surveillance mechanisms, to smoothly work in humans.

IV. FA Signaling and Gain of Function

A great deal of research has been focused on unveiling how the FA signaling network acts, particularly to understand how FANCD2 performs as the representative of the FA pathway (Figure 1). In a recent study, designed upon the fact that FA patients are known to have a high incidence of squamous cell carcinoma [86], in which ΔNp63 happens to be highly expressed [87]. The relationship between FANCD2 activation and ΔNp63 was aimed to be tested. Interestingly, the elevation of ΔNp63 is only detected in FA cells carrying inactivated FANCD2, in comparison to both FANCD2 null cells as well as FA cells carrying wtFANCD2 [88]. This observation suggests that a new role for inactivated FANCD2 exists. In particular, it gained a previously unknown role in transactivating ΔNp63 expression, called a Gain-of-Function (GOF), which contrasts LOF (the loss of function). This study further adds an additional layer of complexity to our understanding of the roles of aberrant FA signaling and its contribution to human tumorigenesis. Furthermore, how exactly GOF is displayed at various levels, e.g. genetic, functional, and metabolic (Figure 3) as what FA signaling does, awaits to be further studied, and together advancing our understanding of the pathogenesis of human cancers.

In regards to the rarity of mutations in normal cells and the tremendous numbers of mutations occurred in human tumors, the spontaneous mutation rate observed in normal cells was believed to be incapable of accounting for the number of mutations existed in human cancers. Therefore, a mutator phenotype was acknowledged as the result of mutations in genes encoding proteins that play roles in maintaining genome stability[89].

This can be observed by the increased mutation rate that drives the development of human cancers. Considering that FANCD2 has emerged as an important player in the maintenance of genomic stability, the identified GOF for inactivated FANCD2 might be a specific type of a mutator phenotype.

Transformation of normal cells into cancer cells entails concerted genetic changes in many genes. These changes are generally considered to occur from three major sources: hereditary, somatic, and environmental-factor-caused mutations [90]. “The mutator phenotype” associated with a malfunctioned FANCD2 or any of other FA genes apparently not only amplifies the defective effects of the FA hereditary mutations, but also substantially elevates the spontaneous mutation rate as well as the one that is triggered by environmental factors in mutated FA gene-carriers. The FA population thereby carries a higher mutation rate derived from all three sources of genetic errors in general. This explains why FA patients have an extremely high incidence of cancer compared to the general population who do not have hereditary alterations. Similarly, individuals who are unaffected by FA or other gene mutations possess greater DDRs, and require more time to reach a certain threshold in genetic changes to drive the manifestation of cancer. After all, we cannot help but suggest a theoretic threshold for the sum of genetic alterations, beyond which cancer occurs. With sophisticated bioinformatic tools and developed modern research technologies, we suspect whether it is possible to “quantify” the genetic errors upon assigning “scores” to mutated tumor suppressors, activated oncogenes and many other tumor promotion factors. Whilst entertaining this idea, of course aberrant FA signaling should be scored among the highest.

V. FA Signaling and Metabolism

Metabolomics was the latest addition to the omics’ family to help understand the flow of the biologic information from the level of genomics, transcriptomics, proteomics, extending to the level of metabolomics [91]. FA tumor suppressor signaling has appeared to be instrumental in understanding the human tumorigenesis at both genetic and functional levels, as discussed above. However, this instrumentation was not displayed metabolically in regards to the extremely import roles that metabolites play in presenting biological information. Therefore, metabolic profiling was conducted in human bladder cancer cells carrying an intact or impaired FA pathway. The latter was obtained by ectopically expressing a splice variant of FANCL, FAVL (FAVL-high), Which promotes the degradation of FANCL [19]. As a result, 18 metabolites, which represent the end products of cell proliferation and/or apoptosis, were found to be significantly different between FAVL-high and -low cells. Methionine, phenylalanine and threonine, resulting from a tumorigenic process, were substantially increased in FAVL-high cells (FA signaling compromised cells) [21]. With this study, characterization on the tumor-promotion roles of aberrant FA signaling in the development of human cancer in the general population was achieved at the genomic, functional, as well as the metabolic levels. FA signaling has thus emerged as a very attractive genetic model system for studying the development of non-FA human cancers and adds a challenging layer of complexity to the pathogenesis of human cancer.

Apart from the involvement of FA signaling in the cancer metabolism, the specific FA proteins were recently found to directly participate in the functions of mitochondria [92, 93]

wherein many metabolic processes take place [94]. On the other hand, the altered metabolite concentrations or deregulated metabolic flux may work back to promote FA signaling. Previously, FANCD2 was demonstrated to protect cells from aldehydes; naturally occurring metabolic by-products. This metabolite appears to act more favorably in response to the DNA damaging properties [95–97], such as acetaldehyde, which can activate the FA pathway in a similar manner as inter-strand DNA-crosslinking agent [98]. Despite this, limited studies are present in the literature to explain the relationship between FA signaling and cancer metabolism. With the latest developed specific tools for metabolic studies, the future studies aiming to reveal the roles of FA signaling in cancer metabolism will be another attractive field of investigation. This will further advance our understanding of the clinical impactions of FA signaling and helping develop more effective strategies towards the mission to eradicate human cancer.

VI. FA Signaling and Effective Tools for Cancer Prevention, Diagnosis and Treatment

Usually, it is not a difficult clinical issue to diagnose human cancers when they became symptomatic; unfortunately, often these cancers are well advanced and incurable. This demands cancer researchers to constantly explore and refine the mechanisms, by which tumors progress from each of the assumed “stages”[23]. Subsequently, these mechanisms can be translated into “biomarkers or strategies” possibly for the early diagnosis or an effective cancer treatment accordingly. Potentially, an initial potential link between FA signaling and human cancers can be traced back to 1971 [17], rapid progression had not been made in this aspect of FA research until recently. As summarized in the table one, many studies have demonstrated a relationship between non-FA cancer patients carrying germline FA gene mutations and the development of a subset of human cancers [99–101]. This indicates that FA tumor suppressor signaling is crucial for the initiation of these tumors, as those mutations have been present as early as the beginning of embryogenesis. Understanding this become quite useful in cancer prevention through genetic counseling, e.g. a certain number of mtFANCS or mtFANCD1 carriers got the prophylactic mastectomy and/or oophorectomy to block the BOCS (Breast and Ovary Cancer Susceptibility). Cancer as a genetic disease is believed to be caused by a series of mutations occurring in both germline and somatic cells [22, 90, 102]. Similarly, somatic mutations occurring in at least 22 FA genes are also important for the development of human cancer. The human genome project provided a useful resource in finding a considerable rate of impairment to the FA signaling pathway (then known of only 17 FA genes) with a mean rate of about 30% in non-FA human cancers [20]. Most importantly, this rate is statistically and positively associated with the tumor stages ($p<0.05$). The relevant studies on aberrant FA gene functions have also evidently shown this correlation [18, 19]. This suggests that FA signaling is important in suppressing tumor development across all stages of tumor progression. In light of the very recent publications reporting the involvement of FA signaling in mitochondria [92, 93] and an overlooked form of FANCD2-V2, which is expressed higher in normal cells compared to the corresponding matched malignant cells [103], it is reasonable to suggest that FA signaling protects human cells substantially from as early as the beginning of neoplastic transformation.

Insights into tumorigenesis derived from FA signaling have provided us opportunities to develop new strategies to treat patients. When discussing DNA damage in the context of cancer, it is often depicted as a double-edge sword. On one hand, DNA damage is known to lead to genome instability and cancer. However, on the other hand, many therapeutic plans targeting cancer (Table 3) often rely on a compromised tumor DDR. Therefore, the accumulation of unrepaired damage that leads to cell death is often referred as the cellular sensitivity, and tumor cells carry a malfunctioned FA gene would mostly sensitize to those DNA-damage agents (Table 3). However, the involvement of the TLS family enzymes in the FA signaling pathway appears to present a paradox to our understanding of tumor sensitivity, considering their error-prone (Iota, REV1, REV7, others) or error-free (pol η) effects [104]. Specifically, the mutated pol η (error-free) has been shown to promote cancer development and increases cell sensitivity, as displayed in xeroderma pigmentosum variant (XPV) [105]. However, it is still unclear whether the improper functions of the error-prone TLS polymerases within the impaired FA signaling network may lead to tumor resistance. Due to the multifaceted nature of the extensive FA signaling network, a singular unified linearized effect is difficult to predict. This in turn, adds to the complexity in our understanding of tumor resistance/sensitivity, and the subject dilemma may be inherited in platinum drugs or PARP inhibitors and many other genotoxic cancer therapies (Table 3). Furthermore, this complexity can also be added up by the mutator phenotype, which may be screened, selected, and promoted by the systemic cancer therapies, contributing to the acquired resistance in clinic. Therefore, it could be extremely important to investigate the mutated FA pathway along with the course of tumor development and identify the featured changes, and the latter is critical for understanding the acquired tumor resistance. Nonetheless, we believe the unique genetic model system, which FA presents, will provide worthwhile insight to help deconstruct the complexities associated with tumorigenesis as well as cancer treatment. For example, inactivated FANCD2 symbolizes an impaired FA pathway, which confers to the tumor sensitivity. In this context, cancer cells with impaired FANCD2 activity accompanied by an elevated expression of ΔNp63 [88]. To this point, the cancer cells harboring the elevated ΔNp63 are unlikely to die after an exposure to those genotoxic agents, instead, growing further. Therefore, it appears to be an effective therapeutic strategy as to targeting the elevated ΔNp63, which should regain sensitivity and inhibit tumor growth. This affirmative answer waits for the further specific translational studies, which along with many other similar ones, develop the FA-BRCA-based personalized medicine or precision medicine: a right therapeutic plan for a right patient.

VII. Concluding Remarks

The FA signaling network has now emerged as a unique genetic model system to study simultaneous, sequential, and/or spatially tuning of at least 22 chosen gene functions, which influence cellular life at numerous aspects that none of any other signaling systems is able to reach. Although, FA research began by studying on a relatively obscure chromosomal breakage syndrome with aplastic anemia, much progress has been made as we can now determine roles of the FA proteins in DNA damage repair (Table 2), replication, cell cycle progression, tumorigenesis and cancer treatment. The knowledge gained serves far beyond the rare population of FA patients, as indicated in many types of non-FA human cancers

(Table 1). This significance is also manifested in the coupled questions and/or suggested research niches in the text. For instances, how FA signaling acts in the early responses to a variety of genotoxicants and/or incorporates with a possible evolved mammalian form of SOS responses? Subsequently, how those studies further our understanding of the mechanisms beneath many drugs currently used in clinic, the potential ones on clinical trials (Table 3) as well as the resulting resistance? In addition, FA signaling can also provide frontier insights into cancer prevention and cancer diagnosis as discussed specifically above. All of these are deemed critical, however, have not received sufficient attention (see outstanding questions).

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Abbreviations

AML	Acute Myelogenous Leukemia
ATM	Mutated in Ataxia-telangiectasia
ATR	Ataxia-telangiectasia and Rad3-related Protein; Mutated in Seckel Syndrome
ATRIP	ATR-interacting Protein
BLM	Mutations in the human RecQ helicase
BOCS	Breast and Ovary Cancer Susceptibility
BS	Bloom Syndrome
BRCT	BRCA1 C-Terminal Domain
CHK1 & CHK2	Checkpoint Kinases 1 and 2
DDR	DNA Damage Responses
DSBs	Double Strand DNA Breaks
FA	Fanconi Anemia
FAAP24	FA Associated Protein 24
FANCD2	Fanconi Anemia Complementation group D2
FANCD2-V2	Fanconi Anemia Complementation Group Protein D2 Variant 2
FANCI	Fanconi Anemia Complementation group I
FANCT	The Ubiquitin Conjugating Enzyme E2-UBE2T

FAVL	Variant of FA protein L—FANCL
GOF	Gain of Function
H2AX	A Variant of the H2A Protein Family
HCLK2	Human Homologue of the Caenorhabditis elegans Biological clock protein CLK-2
HDM2	Mouse Double Minute 2 Homolog
HHR6	Human Homologs of Yeast Rad6
hRAD17	Human Homologs of Yeast Rad17
LOF	Loss of Function
MCMs	Mini Chromosomal Maintenance Protein Complex
MDC1	Mediator of DNA damage Checkpoint Protein-1
MHF	Histone folds Proteins
MRN	Mre11-Rad50-Nbs1 Complex
NBS1	Nijmegen Breakage Syndrome Protein 1
p21	A Cycling-dependent Kinase Inhibitor
PARP	Poly (ADP-ribose) Ploymerase
PCNA	Proliferating Cell Nuclear Antigen
Pol η	DNA Polymerase eta
Rad17	RFC Clamp Loader
REV1	DNA Repair Protein REV1
RPA	Replication Protein A
SMC1	Structural Maintenance of Chromosomes Protein 1
SSBs	Single Strand DNA Breaks
ssDNA	Single Stranded DNA
TLS	Translesion DNA Synthesis
TOPBP1	Topoisomerase-binding Protein-1
USP1	Ubiquitin Specific Peptidase 1
XPV	Xeroderma Pigmentosum Variant
ΔNp63	The alternative promoter (P2) of p63 leads to deleted-transactivation domain (TA) isoforms

γH2AX	Phosphorylated Histone Variant

Fanconi Anemia Complementation group

Fanconi Anemia (FA) is a rare genetic disease characterized by chromosomal instability, hypersensitivity to DNA crosslinking agents, defective DNA repair, severe bone marrow failure, cancer susceptibility and many congenital defects. To date, there are 22 Fanconi Anemia genes identified. Germline mutation in any of these 22 genes confers FA, and the corresponding wild type gene can complement the phenotypes shown. Therefore, each FA group resulting from a specific mutated FA gene is called a complementation group.

Fanconi anemia signaling (FA-BRCA) pathway

All groups of FA patients display similar clinical symptoms as well as similar molecular and cellular defects, which suggest all FA gene-encoded proteins that can act in one common signaling pathway, namely the FA pathway or the FA-BRCA pathway, given the fact that several FA portions are Breast Cancer Susceptibility Gene (BRCA)-related proteins.

The FA Signaling Network/FA signaling

We defined that “The FA Signaling Network/FA signaling” is the sum of all singling transduction events, which are performed by any functional unit that contains one or more FA proteins.

ATR-FA Signaling

Accumulated studies indicate that FA singling plays more and more important roles in ATR-initiated cellular responses. In this review, we would like to emphasize the importance, and considered such signaling as ATR-FA signaling.

Translesion DNA Synthesis (TLS)

Translesion Synthesis (TLS) is a DNA damage tolerance process that allows the DNA replication machinery to replicate past DNA lesions. In many case this damage-tolerance mechanism is error-prone, and cell survival is often associated with an increased risk of mutagenesis and carcinogenesis.

Mutator Phenotype (MP)

Mutator phenotype refers to the increase in mutation rate of cancer cells, which results from the mutated genes, and corresponding encoded wild type proteins play important roles in the maintenance of genome stability.

Gain of Function (GOF)

Mutated proteins not only lost the functions performed by the corresponding wild type proteins but also obtained new functions, called Gain of Function.

The Omics Family

Omics family refers to study biology ending in omics, such as genomics, proteomics, metabolomics. Omics aims at the collective characterization and quantification of pools of biological molecules that translate into the structure, function and dynamics of an organism.

Genetic Counseling

Genetic counseling is the process by which patients or relatives at risk of an inherited disorder are advised of the consequences and nature of the disorders, the probability of developing or transmitting it, and the options and treatment and management of the disorders in all supportive aspects.

Mastectomy
Mastectomy is surgery to remove all breast tissue from a breast as a way to treat or prevent breast cancer.

Oophorectomy
Oophorectomy is a surgical procedure to remove one or both parts of ovaries.

Drug Resistance
Drug resistance is the reduction of effectiveness of a medication by developing mechanism against the drug by the body or by Bactria in the process of medication.

Systemic Cancer Therapies
A systemic cancer therapy is the treatment strategy where drugs are spread throughout the body to treat cancer cells wherever they may be.

Acquired Resistant
Acquired resistant is the ability to gain the resistant after treatment of the drugs resulting from mutation of genes involved in normal physiological processes and cellular structures, from the acquisition of foreign resistance genes or from a combination of these two mechanisms.

Tumor sensitivity
Tumor sensitivity means the death of tumor cells after the treatment with chemotherapeutic drugs or others.

Precision medicine
Precision medicine model that proposes the customization of healthcare with medical decisions, practices or products being tailored to the individual patient.

Platinum-drug
Platinum-drug is a term for Cisplatin and its analogues, a common type of chemotherapeutic drugs that can crosslink DNA.

References

1. Bogliolo M, Surralles J. Fanconi anemia: a model disease for studies on human genetics and advanced therapeutics. Curr Opin Genet Dev. 2015; 33:32–40. [PubMed: 26254775]
2. Ceccaldi R, Sarangi P, D'Andrea AD. The Fanconi anaemia pathway: new players and new functions. Nat Rev Mol Cell Biol. 2016; 17(6):337–49. [PubMed: 27145721]
3. Mamrak NE, Shimamura A, Howlett NG. Recent discoveries in the molecular pathogenesis of the inherited bone marrow failure syndrome Fanconi anemia. Blood Rev. 2016
4. Inano S, et al. RFWD3-Mediated Ubiquitination Promotes Timely Removal of Both RPA and RAD51 from DNA Damage Sites to Facilitate Homologous Recombination. Mol Cell. 2017; 66(5): 622, 634 e8. [PubMed: 28575658]

5. Knies K, et al. Biallelic mutations in the ubiquitin ligase RFWD3 cause Fanconi anemia. J Clin Invest. 2017; 127(8):3013–3027. [PubMed: 28691929]
6. Nepal M, et al. FANCD2 and DNA Damage. Int J Mol Sci. 2017; 18(8)
7. Bagby GC Jr. Genetic basis of Fanconi anemia. Curr Opin Hematol. 2003; 10(1):68–76. [PubMed: 12483114]
8. Auerbach AD. Diagnosis of Fanconi anemia by diepoxybutane analysis. Curr Protoc Hum Genet. 2015; 85 p. 8 7 1-17.
9. Oostra AB, et al. Diagnosis of fanconi anemia: chromosomal breakage analysis. Anemia. 2012; 2012:238731. [PubMed: 22693659]
10. Fei P, Yin J, Wang W. New advances in the DNA damage response network of Fanconi anemia and BRCA proteins. FAAP95 replaces BRCA2 as the true FANCB protein. Cell Cycle. 2005; 4(1):80–6. [PubMed: 15611632]
11. Wang W. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. Nat Rev Genet. 2007; 8(10):735–48. [PubMed: 17768402]
12. Kim H, D'Andrea AD. Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Genes Dev. 2012; 26(13):1393–408. [PubMed: 22751496]
13. Meetei AR, et al. A novel ubiquitin ligase is deficient in Fanconi anemia. Nat Genet. 2003; 35(2): 165–70. [PubMed: 12973351]
14. Panneerselvam J, et al. Basal level of FANCD2 monoubiquitination is required for the maintenance of a sufficient number of licensed-replication origins to fire at a normal rate. Oncotarget. 2014; 5(5):1326–37. [PubMed: 24658369]
15. Schlacher K, Wu H, Jasin M. A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. Cancer Cell. 2012; 22(1):106–16. [PubMed: 22789542]
16. Ling C, et al. Bloom syndrome complex promotes FANCM recruitment to stalled replication forks and facilitates both repair and traverse of DNA interstrand crosslinks. Cell Discov. 2016; 2:16047. [PubMed: 28058110]
17. Swift M. Fanconi's anaemia in the genetics of neoplasia. Nature. 1971; 230(5293):370–3. [PubMed: 4927726]
18. Zhang J, et al. FAVL elevation in human tumors disrupts Fanconi anemia pathway signaling and promotes genomic instability and tumor growth. J Clin Invest. 2010; 120(5):1524–34. [PubMed: 20407210]
19. Panneerselvam J, et al. FAVL impairment of the Fanconi anemia pathway promotes the development of human bladder cancer. Cell Cycle. 2012; 11(15):2947–55. [PubMed: 22828653]
20. Shen Y, et al. Mutated Fanconi anemia pathway in non-Fanconi anemia cancers. Oncotarget. 2015; 6(24):20396–403. [PubMed: 26015400]
21. Panneerselvam J, et al. Distinct Metabolic Signature of Human Bladder Cancer Cells Carrying an Impaired Fanconi Anemia Tumor-Suppressor Signaling Pathway. J Proteome Res. 2016; 15(4): 1333–41. [PubMed: 26956768]
22. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000; 408(6810):307–10. [PubMed: 11099028]
23. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144(5):646–74. [PubMed: 21376230]
24. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009; 461(7267):1071–8. [PubMed: 19847258]
25. Boder E. Ataxia-telangiectasia: an overview. Kroc Found Ser. 1985; 19:1–63.
26. Murga M, et al. A mouse model of ATR-Seckel shows embryonic replicative stress and accelerated aging. Nat Genet. 2009; 41(8):891–8. [PubMed: 19620979]
27. Elledge SJ. Cell cycle checkpoints: preventing an identity crisis. Science. 1996; 274(5293):1664–72. [PubMed: 8939848]
28. Zhou Y, et al. Regulation of the DNA Damage Response by DNA-PKcs Inhibitory Phosphorylation of ATM. Mol Cell. 2017; 65(1):91–104. [PubMed: 27939942]

29. Huang M, et al. The FANCM/FAAP24 complex is required for the DNA interstrand crosslink-induced checkpoint response. Mol Cell. 2010; 39(2):259–68. [PubMed: 20670894]
30. Wang Y, et al. FANCM and FAAP24 maintain genome stability via cooperative as well as unique functions. Mol Cell. 2013; 49(5):997–1009. [PubMed: 23333308]
31. Yang H, et al. Structural insights into the functions of the FANCM-FAAP24 complex in DNA repair. Nucleic Acids Res. 2013; 41(22):10573–83. [PubMed: 24003026]
32. Ho GP, et al. Phosphorylation of FANCD2 on two novel sites is required for mitomycin C resistance. Mol Cell Biol. 2006; 26(18):7005–15. [PubMed: 16943440]
33. Park HK, et al. Convergence of Rad6/Rad18 and Fanconi anemia tumor suppressor pathways upon DNA damage. PLoS One. 2010; 5(10):e13313. [PubMed: 20967207]
34. Palle K, Vaziri C. Rad18 E3 ubiquitin ligase activity mediates Fanconi anemia pathway activation and cell survival following DNA Topoisomerase 1 inhibition. Cell Cycle. 2011; 10(10):1625–38. [PubMed: 21478670]
35. Williams SA, et al. The E3 ubiquitin ligase RAD18 regulates ubiquitylation and chromatin loading of FANCD2 and FANCI. Blood. 2011; 117(19):5078–87. [PubMed: 21355096]
36. Panneerselvam J, et al. BLM promotes the activation of Fanconi Anemia signaling pathway. Oncotarget. 2016; 7(22):32351–61. [PubMed: 27083049]
37. Suhasini AN, Brosh RM Jr. Fanconi anemia and Bloom's syndrome crosstalk through FANCJ-BLM helicase interaction. Trends Genet. 2012; 28(1):7–13. [PubMed: 22024395]
38. Castella M, et al. FANCI Regulates Recruitment of the FA Core Complex at Sites of DNA Damage Independently of FANCD2. PLoS Genet. 2015; 11(10):e1005563. [PubMed: 26430909]
39. van Twest S, et al. Mechanism of Ubiquitination and Deubiquitination in the Fanconi Anemia Pathway. Mol Cell. 2017; 65(2):247–259. [PubMed: 27986371]
40. Ogrunc M, et al. USP1 Regulates Cellular Senescence by Controlling Genomic Integrity. Cell Rep. 2016; 15(7):1401–11. [PubMed: 27160904]
41. Jazayeri A, et al. Mre11-Rad50-Nbs1-dependent processing of DNA breaks generates oligonucleotides that stimulate ATM activity. EMBO J. 2008; 27(14):1953–62. [PubMed: 18596698]
42. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature. 2003; 421(6922):499–506. [PubMed: 12556884]
43. Burma S, et al. ATM phosphorylates histone H2AX in response to DNA double-strand breaks. J Biol Chem. 2001; 276(45):42462–7. [PubMed: 11571274]
44. Stiff T, et al. ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. Cancer Res. 2004; 64(7):2390–6. [PubMed: 15059890]
45. Stucki M, Jackson SP. MDC1/NFBD1: a key regulator of the DNA damage response in higher eukaryotes. DNA Repair (Amst). 2004; 3(8–9):953–7. [PubMed: 15279781]
46. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. Nature. 2004; 432(7015):316–23. [PubMed: 15549093]
47. Matsuoka S, Huang M, Elledge SJ. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science. 1998; 282(5395):1893–7. [PubMed: 9836640]
48. Hirao A, et al. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science. 2000; 287(5459):1824–7. [PubMed: 10710310]
49. Matsuoka S, et al. Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. Proc Natl Acad Sci U S A. 2000; 97(19):10389–94. [PubMed: 10973490]
50. Canman CE, et al. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science. 1998; 281(5383):1677–9. [PubMed: 9733515]
51. Banin S, et al. Enhanced phosphorylation of p53 by ATM in response to DNA damage. Science. 1998; 281(5383):1674–7. [PubMed: 9733514]
52. Medina-Medina I, et al. Allosteric Interactions by p53 mRNA Govern HDM2 E3 Ubiquitin Ligase Specificity under Different Conditions. Mol Cell Biol. 2016; 36(16):2195–205. [PubMed: 27215386]

53. Wu X, et al. ATM phosphorylation of Nijmegen breakage syndrome protein is required in a DNA damage response. Nature. 2000; 405(6785):477–82. [PubMed: 10839545]
54. Xu B, et al. Phosphorylation of serine 1387 in Brca1 is specifically required for the Atm-mediated S-phase checkpoint after ionizing irradiation. Cancer Res. 2002; 62(16):4588–91. [PubMed: 12183412]
55. Yazdi PT, et al. SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. Genes Dev. 2002; 16(5):571–82. [PubMed: 11877377]
56. Cortez D, et al. Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. Science. 1999; 286(5442):1162–6. [PubMed: 10550055]
57. Post S, et al. Phosphorylation of serines 635 and 645 of human Rad17 is cell cycle regulated and is required for G(1)/S checkpoint activation in response to DNA damage. Proc Natl Acad Sci U S A. 2001; 98(23):13102–7. [PubMed: 11687627]
58. Rego MA, et al. Regulation of the activation of the Fanconi anemia pathway by the p21 cyclin-dependent kinase inhibitor. Oncogene. 2012; 31(3):366–75. [PubMed: 21685936]
59. Jaber S, et al. p53 downregulates the Fanconi anaemia DNA repair pathway. Nat Commun. 2016; 7:11091. [PubMed: 27033104]
60. Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell. 2010; 40(2):179–204. [PubMed: 20965415]
61. Cortez D, et al. ATR and ATRIP: partners in checkpoint signaling. Science. 2001; 294(5547): 1713–6. [PubMed: 11721054]
62. Shechter D, Costanzo V, Gautier J. Regulation of DNA replication by ATR: signaling in response to DNA intermediates. DNA Repair (Amst). 2004; 3(8–9):901–8. [PubMed: 15279775]
63. Delacroix S, et al. The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. Genes Dev. 2007; 21(12):1472–7. [PubMed: 17575048]
64. Parrilla-Castellar ER, Arlander SJ, Karnitz L. Dial 9-1-1 for DNA damage: the Rad9-Hus1-Rad1 (9-1-1) clamp complex. DNA Repair (Amst). 2004; 3(8–9):1009–14. [PubMed: 15279787]
65. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science. 2003; 300(5625):1542–8. [PubMed: 12791985]
66. Zou L, Liu D, Elledge SJ. Replication protein A-mediated recruitment and activation of Rad17 complexes. Proc Natl Acad Sci U S A. 2003; 100(24):13827–32. [PubMed: 14605214]
67. Zou L, Cortez D, Elledge SJ. Regulation of ATR substrate selection by Rad17-dependent loading of Rad9 complexes onto chromatin. Genes Dev. 2002; 16(2):198–208. [PubMed: 11799063]
68. Collis SJ, et al. FANCM and FAAP24 function in ATR-mediated checkpoint signaling independently of the Fanconi anemia core complex. Mol Cell. 2008; 32(3):313–24. [PubMed: 18995830]
69. Horejsi Z, Collis SJ, Boulton SJ. FANCM-FAAP24 and HCLK2: roles in ATR signalling and the Fanconi anemia pathway. Cell Cycle. 2009; 8(8):1133–7. [PubMed: 19282663]
70. Chen YH, et al. ATR-mediated phosphorylation of FANCI regulates dormant origin firing in response to replication stress. Mol Cell. 2015; 58(2):323–38. [PubMed: 25843623]
71. Tomida J, et al. A novel interplay between the Fanconi anemia core complex and ATR-ATRIP kinase during DNA cross-link repair. Nucleic Acids Res. 2013; 41(14):6930–41. [PubMed: 23723247]
72. Meetei AR, et al. A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. Nat Genet. 2005; 37(9):958–63. [PubMed: 16116422]
73. Zhu J, et al. FANCD2 influences replication fork processes and genome stability in response to clustered DSBs. Cell Cycle. 2015; 14(12):1809–22. [PubMed: 26083937]
74. Shechter D, Gautier J. MCM proteins and checkpoint kinases get together at the fork. Proc Natl Acad Sci U S A. 2004; 101(30):10845–6. [PubMed: 15263078]
75. Zhang J, et al. FANCD2 monoubiquitination provides a link between the HHR6 and FA-BRCA pathways. Cell Cycle. 2008; 7(3):407–13. [PubMed: 18277096]
76. Fu D, et al. Recruitment of DNA polymerase eta by FANCD2 in the early response to DNA damage. Cell Cycle. 2013; 12(5):803–9. [PubMed: 23388460]
77. Gao Y, et al. Mechanisms of Post-Replication DNA Repair. Genes (Basel). 2017; 8(2)

78. Moldovan GL, Pfander B, Jentsch S. PCNA, the maestro of the replication fork. Cell. 2007; 129(4): 665–79. [PubMed: 17512402]

79. Knipscheer P, et al. The Fanconi anemia pathway promotes replication-dependent DNA interstrand cross-link repair. Science. 2009; 326(5960):1698–701. [PubMed: 19965384]

80. Hicks JK, et al. Differential roles for DNA polymerases eta, zeta, and REV1 in lesion bypass of intrastrand versus interstrand DNA cross-links. Mol Cell Biol. 2010; 30(5):1217–30. [PubMed: 20028736]

81. Yang Y, et al. FANCD2 and REV1 cooperate in the protection of nascent DNA strands in response to replication stress. Nucleic Acids Res. 2015; 43(17):8325–39. [PubMed: 26187992]

82. Budzowska M, et al. Regulation of the Rev1-pol zeta complex during bypass of a DNA interstrand cross-link. EMBO J. 2015; 34(14):1971–85. [PubMed: 26071591]

83. Hara K, et al. Crystal structure of human REV7 in complex with a human REV3 fragment and structural implication of the interaction between DNA polymerase zeta and REV1. J Biol Chem. 2010; 285(16):12299–307. [PubMed: 20164194]

84. Fu Y, et al. Rad6-Rad18 mediates a eukaryotic SOS response by ubiquitinating the 9-1-1 checkpoint clamp. Cell. 2008; 133(4):601–11. [PubMed: 18485869]

85. Little JW, Mount DW. The SOS regulatory system of Escherichia coli. Cell. 1982; 29(1):11–22. [PubMed: 7049397]

86. Velleuer E, Dietrich R. Fanconi anemia: young patients at high risk for squamous cell carcinoma. Mol Cell Pediatr. 2014; 1(1):9. [PubMed: 26567103]

87. Deyoung MP, Ellisen LW. p63 and p73 in human cancer: defining the network. Oncogene. 2007; 26(36):5169–83. [PubMed: 17334395]

88. Panneerselvam J, et al. A hidden role of the inactivated FANCD2: upregulating DeltaNp63. Oncotarget. 2013; 4(9):1416–26. [PubMed: 23965832]

89. Loeb LA. A mutator phenotype in cancer. Cancer Res. 2001; 61(8):3230–9. [PubMed: 11309271]

90. Tomasetti C, Li L, Vogelstein B. Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. Science. 2017; 355(6331):1330–1334. [PubMed: 28336671]

91. Patel S, Ahmed S. Emerging field of metabolomics: big promise for cancer biomarker identification and drug discovery. J Pharm Biomed Anal. 2015; 107:63–74. [PubMed: 25569286]

92. Zhang T, et al. Fancd2 in vivo interaction network reveals a non-canonical role in mitochondrial function. Sci Rep. 2017; 7:45626. [PubMed: 28378742]

93. Jayabal P, et al. Involvement of FANCD2 in Energy Metabolism via ATP5alpha. Sci Rep. 2017; 7(1):4921. [PubMed: 28687786]

94. Golczak M, et al. LRAT-specific domain facilitates vitamin A metabolism by domain swapping in HRASLS3. Nat Chem Biol. 2015; 11(1):26–32. [PubMed: 25383759]

95. Garaycoechea JI, et al. Genotoxic consequences of endogenous aldehydes on mouse haematopoietic stem cell function. Nature. 2012; 489(7417):571–5. [PubMed: 22922648]

96. Langevin F, et al. Fancd2 counteracts the toxic effects of naturally produced aldehydes in mice. Nature. 2011; 475(7354):53–8. [PubMed: 21734703]

97. Pontel LB, et al. Endogenous Formaldehyde Is a Hematopoietic Stem Cell Genotoxin and Metabolic Carcinogen. Mol Cell. 2015; 60(1):177–88. [PubMed: 26412304]

98. Joenje H. Metabolism: alcohol, DNA and disease. Nature. 2011; 475(7354):45–6. [PubMed: 21734701]

99. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003; 302(5645):643–6. [PubMed: 14576434]

100. Pal T, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer. 2005; 104(12):2807–16. [PubMed: 16284991]

101. Rahman N,SS, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D, Easton DF, Stratton MR. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat Genet. 2006 Dec 31.

102. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med. 2004; 10(8): 789–99. [PubMed: 15286780]

103. Han B, et al. Overlooked FANCD2 variant encodes a promising, portent tumor suppressor, and alternative polyadenylation contributes to its expression. Oncotarget. 2017; 8(14):22490–22500. [PubMed: 28157704]

104. Vaisman A, Woodgate R. Translesion DNA polymerases in eukaryotes: what makes them tick? Crit Rev Biochem Mol Biol. 2017:1–30.

105. Lange SS, Takata K, Wood RD. DNA polymerases and cancer. Nat Rev Cancer. 2011; 11(2):96–110. [PubMed: 21258395]

Trends

- Fanconi Anemia (FA) is a rare human genetic disease, sometimes called as a chromosomal disorder syndrome (different from Fanconi Syndrome, a kidney disorder).
- Mutations in FA genes are considered as “mutator mutations”, because of their important roles in DNA damage repair. This feature may be a fundamental mechanism underlying “acquired tumor resistance”
- Translesion DNA polymerases appear to be regulated by FA signaling, and may also be an important for tumor sensitivity/resistance.
- To date, it remains unclear how many FA complementation groups exist, because there are FA cases that cannot be assigned to any of the 22 complementation groups presently known.
- Non-monoubiquitinated FANCD2 is a major molecular defect underlying many complementation groups.

Outstanding Questions

1. What is the role of FA signaling in the early response to a variety of genotoxics?
2. How dose FA signaling relates to an evolved mammalian form of SOS responses?
3. What is the role of FA signaling tumor sensitivity/resistance?
4. How does FA signaling contribute to cancer prevention, diagnosis, and prognosis?

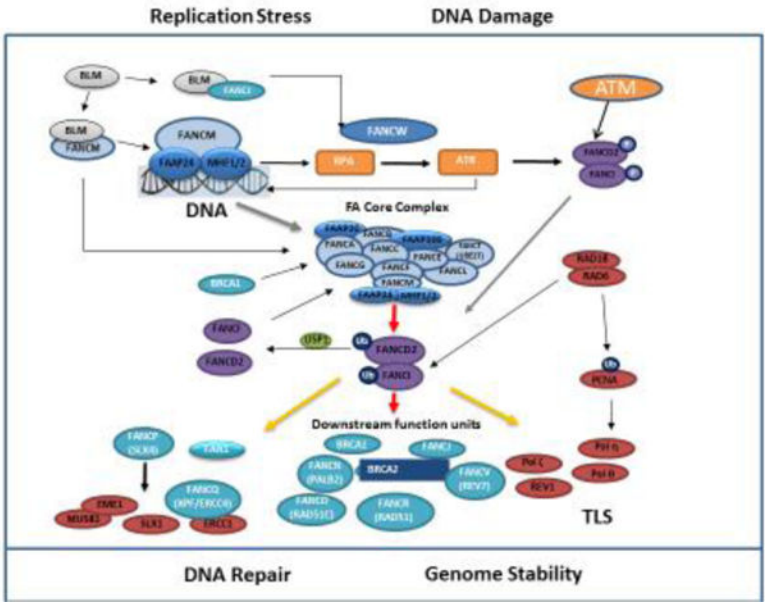

Figure 1. FA Signaling

FA signaling is composed of all signaling transductions involving one or more FA proteins. Within the FA signaling, the FA proteins (FANCA, B, C, E, F, G, L, M, T and possibly I) along with FAAPs (FAAP 20/24/100 and MHF1/2) and others, assure the activity of ubiquitin E3 ligase for the monoubiquitination of FANCD2 and FANCI. Monoubiquitinated FANCD2 and FANCI, in turn, orchestrate the downstream players, including the rest of FA proteins and other non-FA proteins to repair DNA damage via mechanisms of BER, NER, TLS, HR and NHEJ to maintain genome stability. In addition, USP1 can deubiquitinate FANCD2/I, thereby inactivating the FA-BRCA pathway. Most importantly, ATM, ATR and HHR6, together with others known (FANCM and FAAPs) or yet to identify, can act as the upstream regulators for the well-executed checkpoint responses upon DNA damage or replicative stresses. Red arrowheads indicate the canonical FA pathway. FANCM-centered sensing (upstream), possibly including FANCW modulation of RPA functions, would be a typical example in FA signaling to show pathway-independent roles.

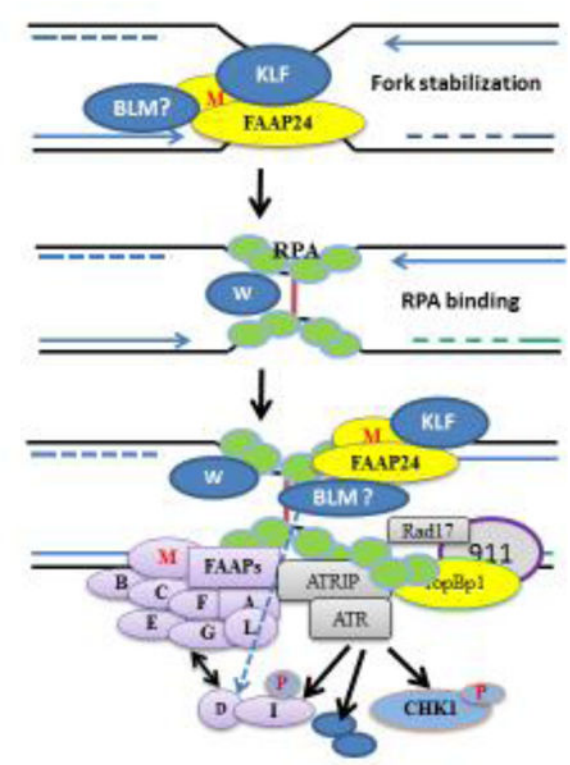

Figure 2. ATR-FA signaling

When replication forks are stalled upon replicative stresses, FANCM-FAAP24-KLM and others can interact with the stalled replication forks to stabilize them for RPA to bind the ssDNA. The RPA complex accumulates and recruits ARTIP, but its function can be modulated by FANCW. The FA core complex facilitates the binding of RPA with ATRIP, which promotes ATR recruitment and followed by many downstream evens initiated by ATR, including the phosphorylation of FANCI that helps FANCD2 monoubiquitination. On the other hand, the RPA complex can also direct Rad17 to load the 9-1-1 complex, which in turn recruits TOPBP1, an activator of ATR. The activated ATR then phosphorylates many downstream targets including CHK1, FANCI, & others. This signaling network is themed with FA and FA-associated proteins from the very beginning to the further downstream of the signaling transduction. In this regard, ATR signaling can be named “ATR-FA signaling”.

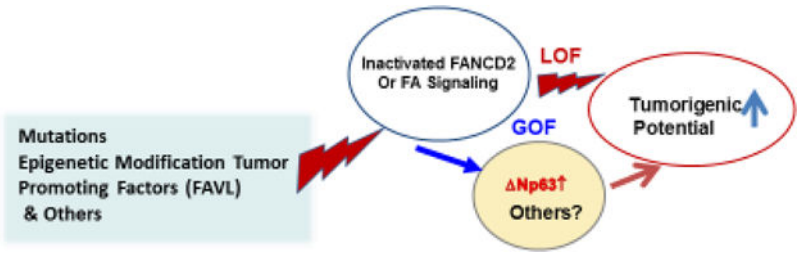

Figure 3. Gain-of-Function of inactivated FANCD2

The activated/monoubiquitinated FANCD2 is the focus of the activated FA pathway, and 80% of FA cases show inability of FANCD2 activation/monoubiquitination. Inactivated FANCD2 not only loses the roles that activated FANCD2 plays, but also exhibits the gain of function phenomenon, and further promotes neoplastic transformation

Table 1

Association of defects in FA genes with non-FA cancers

Defects in FA Genes	Associated Cancer Types	Mutation rate in FA (%)
FANCA	Acute myeloid leukemia (AML)[1] Pancreatic Cancer [2] Solid tumor, Cervical Cancer [3, 4] Oral Cancer [5] Prostate Cancer [6]	64
FANCB	Breast Cancer [7] Child Leukemia [8]	2
FANCC	Bone marrow failure [9] Cervical Cancer [4, 10] Pancreatic cancers[11] Oral Cancer [5] Breast and Ovarian	12
FANCD1	Acute myeloid leukemia, [12] Early Breast Cancer,[13]	2
FANCD2	Acute myeloid Leukemia[14]Breast Cancer [15] Ovarian Cancer [16] Testicular Seminoma [17] Esophageal Squamous Cell Carcinoma (ESCC) [18]Human papillomaviruses (HPVs associated Head, Neck Squamous Cell Carcinomas (HNSCCs) [19] Oral Cancer [5]	4
FANCE	Esophageal Squamous Cell Carcinoma (ESCC)[18] Esophageal cancer and Gastric Cancer [20] Leukemia[21]	1
FANCF	Gastric Cancer, Leukemia[21]Breast Cancer [22] Lung Cancer [23] Oral Cancer [24] Head Neck Squamous Cell Carcinomas [25] Ovarian Cancer [26] Cervical Cancer, Bone marrow failure [27] Prostate Cancer	2
FANCG	Pancreatic Cancers[11] Leukemia[21] AML, Oral Cancer [28] Bladder Cancer [29] Pancreatic Tumor [30] Oral Cancer [5] Ovarian Cancer, Aplastic Anemia [31] Prostate Cancer[6] Breast Cancer [32]	8
FANCI	Breast Cancer, Prostate Cancer [6] Genome Instability [33]	1
FANCJ	Breast Cancer [13] Testicular Seminoma[17]	2
FANCL	Cervical Cancer [4] Esophageal Squamous Cell Carcinoma (ESCC) [18] Leukemia[21] Testicular Seminoma[17]	0.4
FANCM	Leukemia[21]Blooms Syndrome [34] Pancreatic, Ovarian and Breast Cancer [35]	0.1
FANCN	Breast Cancer, Ovarian Cancer, Prostate Cancer, Pancreatic Cancer [36]	0.7
FANCO	Breast Cancer [37] Testicular Seminoma [17]	0.1
FANCP	Breast Cancer [38]	0.5
FANCQ	Leukemia, Breast Cancer [12]	0.1
FANCR	Breast Cancer and Ovarian [39]	0.1
FANCS	Breast and Ovarian Cancer [39]	0.1
FANCT	Breast Cancer [40]	<0.1
FANCU	Risk of Breast Cancer [41]	<0.1
FANCV	Cancer and Bone marrow failure [42]	<0.1
FANCW	Ovarian and testicular atrophy[43]	<0.1

REFERENCE

[1]Tischkowitz, M., et al., *Deletion and reduced expression of the Fanconi anemia FANCA gene in sporadic acute myeloid leukemia.* Leukemia, 2004. **18**(3): p. 420-425.

[2]Rogers, C., et al., *Genetics of the FANCA gene in familial pancreatic cancer.* Journal of medical genetics, 2004. **41**(12): p. e126-e126.

[3] Alter, B.P., *Fanconi anemia and the development of leukemia.* Best Practice & Research Clinical Haematology, 2014.

[4] Juko-Pecirep, I., E.L. Ivansson, and U.B. Gyllensten, *Evaluation of Fanconi anaemia genes FANCA, FANCC and FANCL in cervical cancer susceptibility.* Gynecol Oncol, 2011. **122**(2): p. 377-81.

[5] Tremblay, S., et al., *Young patients with oral squamous cell carcinoma: study of the involvement of GSTP1 and deregulation of the Fanconi anemia genes.* Arch Otolaryngol Head Neck Surg, 2006. **132**(9): p. 958-66.

[6] Mantere, T., et al., *Finnish Fanconi anemia mutations and hereditary predisposition to breast and prostate cancer.* Clin Genet, 2014.

[7] García, M.J., et al., *Analysis of FANCB and FANCN/PALB2 fanconi anemia genes in BRCA1/2-negative Spanish breast cancer families.* Breast cancer research and treatment, 2009. **113**(3): p. 545-551.

[8] Meyer, S., et al., *No evidence of significant silencing of Fanconi genes FANCF and FANCB or Nijmegen breakage syndrome gene NBS1 by DNA hyper-methylation in sporadic childhood leukaemia.* British journal of haematology, 2006. **134**(1): p. 61-63.

[9] Hira, A., et al., *Variant ALDH2 is associated with accelerated progression of bone marrow failure in Japanese Fanconi anemia patients.* Blood, 2013. **122**(18): p. 3206-9.

[10] Huang, F., et al., *The Fanconi anemia group C protein interacts with uncoordinated 5A and delays apoptosis.* PLoS One, 2014. **9**(3): p. e92811.

[11] Rogers, C.D., et al., *The genetics of FANCC and FANCG in familial pancreatic cancer.* Cancer Biol Ther, 2004. **3**(2): p. 167-9.

[12] Alter, B.P., *Fanconi anemia and the development of leukemia.* Best Pract Res Clin Haematol, 2014. **27**(3-4): p. 214-221.

[13] Kitao, H., et al., *Functional interplay between BRCA2/FancD1 and FancC in DNA repair.* J Biol Chem, 2006. **281**(30): p. 21312-20.

[14] Boisvert, R.A. and N.G. Howlett, *The Fanconi anemia ID2 complex: Dueling saxes at the crossroads.* Cell Cycle, 2014. **13**(19): p. 2999-3015.

[15] Krzystolik, K., et al., *Large deletion causing von Hippel-Lindau disease and hereditary breast cancer syndrome.* Hered Cancer Clin Pract, 2014. **12**(1): p. 16.

[16] Colombo, P.E., et al., *Sensitivity and resistance to treatment in the primary management of epithelial ovarian cancer.* Crit Rev Oncol Hematol, 2014. **89**(2): p. 207-16.

[17] Smetsers, S., et al., *Heterozygote FANCD2 mutations associated with childhood T Cell ALL and testicular seminoma.* Fam Cancer, 2012. **11**(4): p. 661-5.

[18] Akbari, M.R., et al., *Mutations in Fanconi anemia genes and the risk of esophageal cancer.* Hum Genet, 2011. **129**(5): p. 573-82.

[19] Park, J.W., et al., *Deficiencies in the Fanconi anemia DNA damage response pathway increase sensitivity to HPV-associated head and neck cancer.* Cancer Res, 2010. **70**(23): p. 9959-68.

[20] Li, W.Q., et al., *Genetic variants in DNA repair pathway genes and risk of esophageal squamous cell carcinoma and gastric adenocarcinoma in a Chinese population.* Carcinogenesis, 2013. **34**(7): p. 1536-42.

[21] Ali, A.M., et al., *Identification and characterization of mutations in FANCL gene: a second case of Fanconi anemia belonging to FA-L complementation group.* Hum Mutat, 2009. **30**(7): p. E761-70.

[22] Zhao, L., et al., *RNAi-mediated knockdown of FANCF suppresses cell proliferation, migration, invasion, and drug resistance potential of breast cancer cells.* Braz J Med Biol Res, 2014. **47**(1): p. 24-34.

[23] Nakajima, T., et al., *Assessment of Chemosensitivity-related Aberrant Methylation of Nonsmall Cell Lung Cancer by EBUS-TBNA.* J Bronchology Interv Pulmonol, 2009. **16**(1): p. 10-4.

[24] Marsit, C.J., et al., *Inactivation of the Fanconi anemia/BRCA pathway in lung and oral cancers: implications for treatment and survival.* Oncogene, 2004. **23**(4): p. 1000-4.

[25] Ameziane, N., et al., *No evidence for FANCF gene silencing in head-and-neck squamous cell carcinomas.* Cell Oncol, 2009. **31**(1): p. 53-6.

[26] Wang, Z., et al., *Promoter hypermethylation of FANCF plays an important role in the occurrence of ovarian cancer through disrupting Fanconi anemia-BRCA pathway.* Cancer Biol Ther, 2006. **5**(3): p. 256-60.

[27] de Winter, J.P., et al., *The Fanconi anaemia gene FANCF encodes a novel protein with homology to ROM.* Nat Genet, 2000. **24**(1): p. 15-6.

[28] Tsai, Y.S., et al., *Areca nut induces miR-23a and inhibits repair of DNA double-strand breaks by targeting FANCG.* Toxicol Sci, 2011. **123**(2): p. 480-90.

[29] Neveling, K., et al., *Disruption of the FA/BRCA pathway in bladder cancer.* Cytogenet Genome Res, 2007. **118**(2-4): p. 166-76.

[30] Kennedy, R.D., et al., *Fanconi anemia pathway-deficient tumor cells are hypersensitive to inhibition of ataxia telangiectasia mutated.* J Clin Invest, 2007. **117**(5): p. 1440-9.

[31] Franco, S., et al., *Telomere dynamics in Fancg-deficient mouse and human cells.* Blood, 2004. **104**(13): p. 3927-35.

[32] Zhu, B., et al., *K63-linked ubiquitination of FANCG is required for its association with the Rap80-BRCA1 complex to modulate homologous recombination repair of DNA interstand crosslinks.* Oncogene, 2015. **34**(22): p. 2867-78.

[33] Colnaghi, L., et al., *Patient-derived C-terminal mutation of FANCI causes protein mislocalization and reveals putative EDGE motif function in DNA repair.* Blood, 2011. **117**(7): p. 2247-56.

[34] Deans, A.J. and S.C. West, *FANCM connects the genome instability disorders Bloom's Syndrome and Fanconi Anemia.* Mol Cell, 2009. **36**(6): p. 943-53.

[35] Hahnen, E., et al., *Germline Mutations in Triple-Negative Breast Cancer.* Breast Care (Basel), 2017. **12**(1): p. 15-19.

[36] Tischkowitz, M. and B. Xia, *PALB2/FANCN: recombining cancer and Fanconi anemia.* Cancer Res, 2010. **70**(19): p. 7353-9.

[37] Somyajit, K., S. Subramanya, and G. Nagaraju, *Distinct roles of FANCO/RAD51C protein in DNA damage signaling and repair: implications for Fanconi anemia and breast cancer susceptibility.* J Biol Chem, 2012. **287**(5): p. 3366-80.

[38] Kim, Y., *Nuclease delivery: versatile functions of SLX4/FANCP in genome maintenance.* Mol Cells, 2014. **37**(8): p. 569-74.

[39] Bogliolo, M. and J. Surralles, *Fanconi anemia: a model disease for studies on human genetics and advanced therapeutics.* Curr Opin Genet Dev, 2015. **33**: p. 32-40.

[40] Virts, E.L., et al., *AluY-mediated germline deletion, duplication and somatic stem cell reversion in UBE2T defines a new subtype of Fanconi anemia.* Hum Mol Genet, 2015. **24**(18): p. 5093-108.

[41] Park, J.Y., et al., *Complementation of hypersensitivity to DNA interstrand crosslinking agents demonstrates that XRCC2 is a Fanconi anaemia gene.* J Med Genet, 2016. **53**(10): p. 672-680.

[42] Bluteau, D., et al., *Biallelic inactivation of REV7 is associated with Fanconi anemia.* J Clin Invest, 2016. **126**(9): p. 3580-4.

[43] Knies, K., et al., *Biallelic mutations in the ubiquitin ligase RFWD3 cause Fanconi anemia.* J Clin Invest, 2017. **127**(8): p. 3013-3027.

Table 2

Roles of FA proteins in DNA damage repair

FA Gene Mutation	DNA repair mechanism	Functional complex	Reference
FANCA	DNA interstrand cross-link (ICL) repair, HR	FA core complex	[1] [2] [3]
FANCB	ICL repair,	FA core complex	[4]
FANCC	ICL repair, HR	FA core complex	[2]
FANCD1	ICL repair, HR	BRCA2 complex	[5]
FANCD2	HR, NER, TLS	ID complex	[6],[2]
FANCE	ICL repair, HR	FA core complex	[2]
FANCF	ICL repair, HR	FA core complex	[2]
FANCG	ICL repair, HR	FA core complex	[2] [3]
FANCI	ICL repair, HR	ID complex	[7]
FANCJ	ICL repair, NER, HR,	Co-operates with FAAP24, FAN1 nuclease, BLM helicase	[8]
FANCL	HR, ICL repair	FA core complex	[9]
FANCM	bind and metabolize a variety of DNA substrates,	FANCM-FAAP complex FA core complex	[10]
FANCN	HR,	BRCA2 complex	[11]
FANCO	HR, ICL repair	A possible HR complex	[12]
FANCP	ICL repair, HR, Holiday junction resolution (HJ)	A possible HR complex	[13]
FANCQ	ICL repair, NER	ERCC1-XPF complex	[14]
FANCR	Resolve stalled replication fork, protect nucleotides from degradation,	RAD51 associates with nuclease FAN1	[15]
FANCS	ICL repair, HR,	BRCA1 complex	[16]
FANCT	ICL repair,	Cooperate with ID complex	[17]
FANCU	ICL repair,	Removing crosslink complex	[18]
FANCV	TLS	Post replication repair complex	[19]
FANCW	HR, ICL repair	RPA complex	[20]

References:

1 Wang, J., et al., *FBW7 regulates DNA interstrand cross-link repair by modulating FAAP20 degradation.* Oncotarget, 2016. **7**(24): p. 35724-35740.

2 Yamamoto, K., et al., *Fanconi anemia protein FANCD2 promotes immunoglobulin gene conversion and DNA repair through a mechanism related to homologous recombination.* Mol Cell Biol, 2005. **25**(1): p. 34-43.

3 van Twest, S., et al., *Mechanism of Ubiquitination and Deubiquitination in the Fanconi Anemia Pathway.* Mol Cell, 2017. **65**(2): p. 247-259.

4 Fei, P., J. Yin, and W. Wang, *New advances in the DNA damage response network of Fanconi anemia and BRCA proteins. FAAP95 replaces BRCA2 as the true FANCB protein.* Cell Cycle, 2005. **4**(1): p. 80-6.

5 Wang, X., P.R. Andreassen, and A.D. D'Andrea, *Functional interaction of monoubiquitinated FANCD2 and BRCA2/FANCD1 in chromatin.* Mol Cell Biol, 2004. **24**(13): p. 5850-62.

6 Nepal, M., et al., *FANCD2 and DNA Damage.* Int J Mol Sci, 2017. **18**(8).

7 Jin, H. and Y. Cho, *Structural and functional relationships of FAN1.* DNA Repair (Amst), 2017. **56**: p. 135-143.

[8] Wilson, D.M., 3rd, et al., *Systematic analysis of DNA crosslink repair pathways during development and aging in Caenorhabditis elegans.* Nucleic Acids Res, 2017. **45**(16): p. 9467-9480.

[9] Jiang, J., et al., *Arsenite Binds to the RING Finger Domain of FANCL E3 Ubiquitin Ligase and Inhibits DNA Interstrand Crosslink Repair.* ACS Chem Biol, 2017. **12**(7): p. 1858-1866.

[10] Ali, A.M., T.R. Singh, and A.R. Meetei, *FANCM-FAAP24 and FANCJ: FA proteins that metabolize DNA.* Mutat Res, 2009. **668**(1-2): p. 20-6.

[11] Tischkowitz, M. and B. Xia, *PALB2/FANCN: recombining cancer and Fanconi anemia.* Cancer Res, 2010. **70**(19): p. 7353-9.

[12] Somyajit, K., S. Subramanya, and G. Nagaraju, *Distinct roles of FANCO/RAD51C protein in DNA damage signaling and repair: implications for Fanconi anemia and breast cancer susceptibility.* J Biol Chem, 2012. **287**(5): p. 3366-80.

[13] Kim, Y., *Nuclease delivery: versatile functions of SLX4/FANCP in genome maintenance.* Mol Cells, 2014. **37**(8): p. 569-74.

[14] McNeil, E.M., et al., *Inhibition of the ERCC1-XPF structure-specific endonuclease to overcome cancer chemoresistance.* DNA Repair (Amst), 2015. **31**: p. 19-28.

[15] Sato, K., et al., *FANCI-FANCD2 stabilizes the RAD51-DNA complex by binding RAD51 and protects the 5′-DNA end.* Nucleic Acids Res, 2016. **44**(22): p. 10758-10771.

[16] Hoang, L.N. and B.C. Gilks, *Hereditary Breast and Ovarian Cancer Syndrome: Moving Beyond BRCA1 and BRCA2.* Adv Anat Pathol, 2017.

[17] Rickman, K.A., et al., *Deficiency of UBE2T, the E2 Ubiquitin Ligase Necessary for FANCD2 and FANCI Ubiquitination, Causes FA-T Subtype of Fanconi Anemia.* Cell Rep, 2015. **12**(1): p. 35-41.

[18] Park, J.Y., et al., *Complementation of hypersensitivity to DNA interstrand crosslinking agents demonstrates that XRCC2 is a Fanconi anaemia gene.* J Med Genet, 2016. **53**(10): p. 672-680.

[19] Bluteau, D., et al., *Biallelic inactivation of REV7 is associated with Fanconi anemia.* J Clin Invest, 2016. **126**(9): p. 3580-4.

[20] Inano, S., et al., *RFWD3-Mediated Ubiquitination Promotes Timely Removal of Both RPA and RAD51 from DNA Damage Sites to Facilitate Homologous Recombination.* Mol Cell, 2017. **66**(5): p. 622-634 e8.

Table 3

Chemotherapy drugs and DNA damage

Chemotherapy drugs on Trials	Targeted Cancer type	Mechanistic Target	REF
Olaparib/Lynparza	Platinum sensitive BRCA-mutated relapsed ovarian	PARP inhibitor	[1]
Veliparib	BRCA-mutated, breast metastatic, TNBC, Ovarian	PARP inhibitor	[1]
Niraparib	Platinum sensitive ovarian, BRCA-mutated, Breast	PARP inhibitor	[1]
Talazoparib	Breast Cancer, Germline BRCA mutations	PARP inhibitor	[1]
Rucaparib	Ovarian, Advanced solid tumors, Breast Cancer	PARP inhibitor	[1]
NMS-P118	Solid tumors	PARP inhibitor	[1]
Iniparib	Metastatic Triple negative breast cancer (TNBC)	PARP inhibitor	[2]
AZD2461	Solid Tumors	PARP inhibitor	[2]
BMN673	AML, Breast, advanced solid tumors, BRCA-mutated	PARP inhibitor	[2]
CEP-9722	Mantle cell Lymphoma, Advanced solid tumors	PARP inhibitor	[2]
E7016	Advanced Solid Tumors, Melanoma	PARP inhibitor	[2]
INO-1001	Melanoma	PARP inhibitor	[2]
MK-4827	Advanced Solid Tumors, Leukemia	PARP inhibitor	[2]
Methoxyamine	Lymphomas	BEK inhibitor	[2]
FDA-approved Chemotherapy drugs	**Targeted Cancer Type**	**Mechanistic Target**	**REF**
Pembrolizumab, Keytruda	Colorectal, Endothelial and gastrointestinal, breast, prostate, bladder cancers Metastatic melanoma, lung cancer, head neck cancer	PDL-1 Blocker	[3]
Romidepsin (FK228)	Ovarian Cancer	FAK, HDAC1 inhibitor	[4]
Bosutinib, Ibrutinib, Cabozantinib	Glioblastoma multiforme (GBM).	Multi kinase, Angiogenesis, BTK inhibitor	[5]
Cisplatin	Ovarian Cancer, Cervical Cancer, Breast Cancer, Head and Neck Cancer, Lungs Cancer, Brain tumor, Mesothelioma	Multiple Target, DNA lesion generation	[6, 7]
Antimetabolite methotrexate	Breast Cancer, Leukemia, Lung, Cancer, Lymphoma, Osteosarcoma	DHFR inhibitor	[7] [6]
5-fluorouracil (5-FU)	Colon Cancer, Esophageal Cancer, Pancreatic Cancer, Breast Cancer, Cervical Cancer, Basal Cell Carcinoma	Thymidylate synthase inhibitor	[6] [8] [7]
Camptothecin	Solid Tumors, Multi functioning with Chemotherapy	Topoisomerase I	[6] [7, 8]
Doxorubicin	Breast Cancer, Bladder Cancer, Lymphoma, Acute Lymphocytic Leukemia	Topoisomerase II	[6] [7]
5′-Aza-2′-deoxycytidine (5-Aza-dC)	Multi-functioning drugs for cancer therapy	DNA Methyltransferase	[9]
Olaparib	Ovarian Cancer	PARP inhibitor	[10]
Riluzole	Breast Cancer	NMDA receptor inhibitor	[11]
LY2606368	Colorectal Cancer	CHK1 inhibitor	[12]
Trametinib	Lung cancer	MEK1/MEK2 inhibitor	[13]
Panobinostat	Ovarian Cancer	HDAC inhibitor	[14]

Chemotherapy drugs on Trials	Targeted Cancer type	Mechanistic Target	REF
Vorinostat, Romidepsin,Belinostat	Lymphomas	HDAC inhibitor	[15]
Fludarabine phosphate (F-AMP)	Gastrointestinal Stromal tumor (GIST)	DNA synthesis inhibitor	[16]
Mitomycin C (MMC)	Anal Cancer, Breast Cancer	Thyrodoxin reductase, redox cycling of rRNA inhibitor	[17]

[1] Gavande, N.S., et al., *DNA repair targeted therapy: The past or future of cancer treatment?* Pharmacol Ther, 2016. **160**: p. 65-83.

[2] Kelley, M.R., D. Logsdon, and M.L. Fishel, *Targeting DNA repair pathways for cancer treatment: what's new?* Future Oncol, 2014. **10**(7): p. 1215-37.

[3] Wang, X., et al., *Effectiveness and safety of PD-1/PD-L1 inhibitors in the treatment of solid tumors: a systematic review and meta-analysis.* Oncotarget, 2017. **8**(35): p. 59901-59914.

[4] Wilson, A.J., et al., *Romidepsin (FK228) combined with cisplatin stimulates DNA damage-induced cell death in ovarian cancer.* Gynecol Oncol, 2012. **127**(3): p. 579-86.

[5] Barrette, A.M., M. Bouhaddou, and M.R. Birtwistle, *Integrating Transcriptomic Data with Mechanistic Systems Pharmacology Models for Virtual Drug Combination Trials.* ACS Chem Neurosci, 2017.

[6] Cheung-Ong, K., G. Giaever, and C. Nislow, *DNA-damaging agents in cancer chemotherapy: serendipity and chemical biology.* Chem Biol, 2013. **20**(5): p. 648-59.

[7] Lo, Y.C., et al., *Computational Cell Cycle Profiling of Cancer Cells for Prioritizing FDA-Approved Drugs with Repurposing Potential.* Sci Rep, 2017. **7**(1): p. 11261.

[8] Parker, W.B. and Y.C. Cheng, *Metabolism and mechanism of action of 5-fluorouracil.* Pharmacol Ther, 1990. **48**(3): p. 381-95.

[9] Putri, J.F., et al., *Induction of senescence in cancer cells by 5′-Aza-2′-deoxycytidine: Bioinformatics and experimental insights to its targets.* Comput Biol Chem, 2017. **70**: p. 49-55.

[10] Yuan, Z., et al., *Olaparib hydroxamic acid derivatives as dual PARP and HDAC inhibitors for cancer therapy.* Bioorg Med Chem, 2017. **25**(15): p. 4100-4109.

[11] Dolfi, S.C., et al., *Riluzole exerts distinct antitumor effects from a metabotropic glutamate receptor 1-specific inhibitor on breast cancer cells.* Oncotarget, 2017. **8**(27): p. 44639-44653.

[12] Manic, G., et al., *CHK1-targeted therapy to deplete DNA replication-stressed, p53-deficient, hyperdiploid colorectal cancer stem cells.* Gut, 2017.

[13] Smida, M., et al., *MEK inhibitors block growth of lung tumours with mutations in ataxia-telangiectasia mutated.* Nat Commun, 2016. **7**: p. 13701.

[14] Wilson, A.J., et al., *Panobinostat sensitizes cyclin E high, homologous recombination-proficient ovarian cancer to olaparib.* Gynecol Oncol, 2016. **143**(1): p. 143-51.

[15] Chun, P., *Histone deacetylase inhibitors in hematological malignancies and solid tumors.* Arch Pharm Res, 2015. **38**(6): p. 933-49.

[16] Pessetto, Z.Y., et al., *Drug repurposing identifies a synergistic combination therapy with imatinib mesylate for gastrointestinal stromal tumor.* Mol Cancer Ther, 2014. **13**(10): p. 2276-87.

[17] Tomasz, M., *Mitomycin C: small, fast and deadly (but very selective).* Chem Biol, 1995. **2**(9): p. 575-9.