Chapter-26

Genetics of Fanconi Anemia and Failure of ICL DNA Damage Rapair

Author

Dr. Ram Balak Mahto
Assistant Professor
Dept. of Zoology
Vishweshwar Singh Janta College Rajnagar, Madhubani.
L.N.M.U Darbhanga, Bihar.
E.mail: rambalak5@gmail.com

Abstract

Nineteen (19) genes have been identified associated with FA and many more interacting proteins have been discovered. Nineteen (19) genes; FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ/BRIP1, FANCL, FANCM, FANCN/PALB2, and FANCO/RAD51C, FANCP/SLX4, FANCQ/ERCC4, FANCR/RAD51, FANCS/BRACA1, FANCT/UBE2T have been identified to date as being implicated in the Fanconi anemia pathway. Fanconi proteins are mainly involved in interstrand crosslink (ICL) repair which includes cutting out the interstrand crosslink, trans-lesion synthesis (TLS). FA phenotype is also influenced by mitochondrial dysfunction (MDF). FA Not limited to demonstrated that MDF-related symptoms, in addition to oxidative stress (OS), are present in additional syndromes involving DNA damage and repair (such as ataxia-telangiectasia, AT, and Werner syndrome, WS), all of which may have significant roles in these disorders.

Keywords: Fanconi anemia, Mitochondrial Dysfunction, Reactive Oxygen Species, Genomic Instability, Cancer.

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1. INTRODUCTION GENETICS OF FANCONI ANEMIA

FA is recessive hereditary heterogeneous disorder. So far nineteen (19) genes have been identified associated with FA (table.1) and many more interacting proteins have been discovered (http://www.rockefeller.edu/fanconi/). Fanconi proteins are mainly involved in interstrand crosslink (ICL) repair which includes cutting out the interstrand crosslink, trans-lesion synthesis (TLS) (a mode of damage tolerance that uses specialized polymerases to insert a base across from a lesion or abasic site) and homologous recombination (the pathway best known for its role in repairing double strand breaks (1). FA proteins have also been reported to counteract with the proteins involved in the non-homologous end joining (NHEJ) pathway, an error-prone repair pathway that is used to directly replicate DNA ends (2). Owing to the critical importance of the FA pathway in maintaining genome stability, there are currently great limitations in treating Fanconi Anemia. Markedly reduced life expectancy has been observed in FA patients with death due to haematological complications and cancer. Thus, understanding the basis of the bone marrow failure is of critical importance to improve current treatment approaches for patients with FA. The biological function of FA proteins has been the subject of intense investigation in recent years (3). Fanconi anemia is an autosomal recessive genetic disorder that is marked by multiple complicated clinical symptoms, hypersensitivity to DNA cross linking agents, chromosomal instability and increased risk to cancer (4, 5).

Although complementation group B may be attributed to the gene BRCA2. We know that the proteincomplementation group B individuals with Fanconi anemia is a crucial part of the nuclear protein 'core complex' that is accountable for the monoubiquitination of FANCD2, a crucial step in the DNAdamage response pathway linked to both Fanconi anemia and BRCA(6,7,8). Interestingly, the FANCB gene, which codes for this protein, is located at Xp22.31 and is X-chromosome inactivated.

When it comes to genetic counseling Xlinked inheritance has significat implications for Fanconi anemia families in complementation group Fanconi anemia is caused by a single active copy of FANCB, which makes it a potentially vulnerable part of the cellular machinery that protects genomic integrity. It is also necessary for a functional Fanconi anemia–BRCA pathway.

Table 1: Nineteen Genes Involve in Fanconi Anemia**.**

Responsible Gene	Chromosomal Location	Protein Size in KDA
FANCA	16q243	163
FANCB	Xp2231	95
FANCC	9q223	63
FANCD1/BRACA2	13q12.13	380
FANCD2	3p25.3	155,162
FANCE	16p21.22	60
FANCF	11p15	42
FANCG/XRCC9	9p13	68
FANCI/KIAA1794	15q26.1	?
FANCJ/BACH1/BRIP1	17q22-q24	130
FANCL/PHF9/FOG	2p16.1	43
FANCM/Hef	14q213	250
FANCN/PALB2	16p121	131
FANCO/RAD51C	17p25.1	43
FANCP/SLX4	16p133	200
FANCQ/ERCC4/XPF	16p13.12	110
FANCR/RAD51	7q36.1	180
FANCS/BRACA1	17q21.31	220
FANCT/UBE2T	1q31.1	225

FANCA; The Fanconi anemia complementation group A, also referred to as FA, FA1, FAA, FAH, FA-H, FACA, and FANCH, is a genetically heterogeneous recessive disorder. It is characterized by cytogenetic instability, hypersensitivity to DNA cross-linking agents, increased chromosomal breakage, and defective DNA repair. The members of the Fanconi anemia complementation group are not similar in sequence but are connected through their assembly into a common nuclear protein complex. The protein encoded by this gene belongs to complementation group A. Different isoforms of this protein are produced through alternative splicing of multiple transcript variants. Mutations in this gene are the most common cause of Fanconi anemia.

FANCB; The Fanconi anemia complementation group B, also known as FA2, FAB, FACB, FAAP90, and FAAP95, is involved in the repair of DNA lesions. It is assembled into a nucleoprotein complex. Mutations in this gene can lead to chromosome instability and VACTERL syndrome with hydrocephalus.

FANCC; The Fanconi anemia complementation group (FANC), also referred to as FA3, FAC, and FACC, is characterized by cytogenetic instability,

hypersensitivity to DNA cross-linking agents, increased chromosomal breakage, and defective DNA repair. FANCD1; The Fanconi anemia complementation group B, also known as FAD, FACD, FAD1, GLM3, BRCC2, FANCD, PNCA2, FANCD1, XRCC11, and BROVCA2, plays a role in maintaining genome stability. Inherited mutations in BRCA1 and this gene, BRCA2, increase the lifetime risk of developing breast or ovarian cancer. Both BRCA1 and BRCA2 are involved in the homologous recombination pathway for double-strand DNA repair. The BRCA2 gene is located on chromosome 13q12.3 in humans. The BRCA2 protein contains multiple copies of a 70 amino acid motif called the BRC motif, which is important for its function. BRCA2 is classified as a tumour suppressor gene due to the fact that tumours with BRCA2 mutations typically display loss of heterozygosity (LOH) of the wild-type allele. FANCD2, also known as FA4, FAD, FACD, FAD2, or FA-D2, is a protein that undergoes monoubiquitination in response to DNA damage. This leads to its localization to nuclear foci along with other proteins (such as BRCA1 and BRCA2) involved in homology-directed DNA repair. FANCDE is associated with Fanconi anemia, a genetically diverse recessive disorder characterized by cytogenetic instability, heightened sensitivity to DNA cross linking agents, increased chromosomal breakage, and impaired DNA repair. The members of the Fanconi anemia complementation group are not linked by sequence similarity but rather by their assembly into a shared nuclear protein complex.

FANCF, also known as FAF, is part of the Fanconi anemia complementation group F. Like other members of this group, FANCF is connected through its involvement in a common nuclear protein complex.

FANCG, also known as FAG or XRCC9, is characterized by cytogenetic instability, increased sensitivity to DNA cross linking agents, heightened chromosomal breakage, and faulty DNA repair. Similar to other members of the Fanconi anemia complementation group, FANCG is not linked by sequence similarity but rather by its assembly into a shared nuclear protein complex.

FANCI belongs to the FA complementation group I. Like other members of this group, FANCI is connected through its involvement in a common nuclear protein complex.

FANCJ, also known as BACH1, is part of the Fanconi anemia complementation group J. The protein encoded by this gene is a member of the RecQ DEAH helicase family and interacts with the BRCT repeats of breast cancer, type 1 (BRCA1). This interaction is crucial for the normal function of double-strand break repair mediated by BRCA1. It is worth noting that BACH1 is also an alternative name for BRCA1 interacting protein C-terminal helicase 1

(BRIP1), which can sometimes be confused with the official symbol for BTB domain and CNC homolog 1 (BACH1).

FANCL, also known as POG, PHF9, and FAAP43, mediates monoubiquitination of FANCD2 and FANCI. Fanconi anemia is a genetically heterogeneous recessive disorder characterized by cytogenetic instability, hypersensitivity to DNA cross-linking agents, increased chromosomal breakage, and defective DNA repair. FANCM, also known as POF15, SPGF28, FAAP250, and KIAA1596, is related to hypersensitivity to DNA cross-linking agents, increased chromosomal breakage, and defective DNA repair. These proteins assemble into a common nuclear protein complex. This gene encodes the protein for complementation group M, with alternative splicing resulting in multiple transcript variants.

FANCN, also known as PNCA3 and BROVCA5, may function in tumour suppression by binding to and co-localizing with BRCA2 in nuclear foci. This allows for the stable intra-nuclear localization and accumulation of BRCA2.

FANCQ, also known as XPF, RAD1, XFEPS, and ERCC11, forms a complex with ERCC1 and is involved in the 5' incision during nucleotide excision repair. This complex is a structure-specific DNA repair endonuclease that interacts with EME1. Mutations in this gene can cause Xeroderma pigmentosum complementation group F (XP-F) or Xeroderma pigmentosum VI (XP6).

FANCR, also known as RECA, BRCC5, MRMV2, HRAD51, RAD51A, HsRad51, and HsT16930, is a protein belonging to the RAD51 protein family. This protein is highly similar to bacterial RecA and Saccharomyces cerevisiae Rad51 and is involved in the repair and homologous recombination of DNA. It interacts with the Single Strand DNA-binding protein RPA and RAD52, playing a role in homologous pairing and DNA strand transfer. Additionally, it interacts with BRCA1 and BRCA2, which are crucial for the cellular response to DNA damage. BRCA2 regulates the intracellular localization and DNA-binding ability of FANCR. Loss of these controls due to BRCA2 inactivation can lead to genomic instability and tumorigenesis. Multiple transcript variants encoding different isoforms have been identified for this gene.

FANCS, also known as IRIS, PSCP, BRCAI, BRCC1, PNCA4, RNF53, BROVCA1, and PPP1R53, encodes a 190 KDa nuclear phosphoprotein that plays a role in maintaining genomic stability and acts as a tumour suppressor. The BRCA1 gene consists of 22 exons spanning approximately 110 kb of DNA. The encoded protein forms a large multi-subunit protein complex called the BRCA1-associated genome surveillance complex (BASC) by combining with

other tumour suppressors, DNA damage sensors, and signal transducers. It associates with RNA polymerase II and interacts with histone deacetylase complexes through its C-terminal domain. This protein is involved in transcription, DNA repair of double-stranded breaks, and recombination. Mutations in this gene account for around 40% of inherited breast cancers and over 80% of inherited breast and ovarian cancers. Alternative splicing plays a role in modulating the sub cellular localization and physiological function of this gene. There are many alternatively spliced transcript variants. Several mutations associated with diseases have been reported for this gene, but only a few of these variants have been fully characterized. Additionally, a related pseudo gene located on chromosome 17 has been discovered. The Fanconi anemia complementation group T, also referred to as FANCT, PIG50, and HSPC150, is responsible for catalyzing the attachment of Ubiquitin to protein substrates. Mutations in this gene have been linked to Fanconi anemia of complementation group T. There are two transcript variants that encode distinct isoforms of this gene.

2. CURRENT STATUS: EPIDEMIOLOGY

The incidence of FA is approximately three per million and the heterozygote frequency is estimated at 1 in 300 in Europe and the United States. FA has been reported in many ethnic groups and founder mutations have been described in Ashkenazi Jews, who have an approximate carrier frequency of 1 in 89, and Afrikaners where the carrier frequency was estimated at 1 in 83. Though, FA considered as rare disorder, but due to rapid enhancement of FA research, number of patients is increasing throughout the world. Fanconi anemia association in India (REFAIN; http://refain.org) has already identified more than 200 FA children in India.

3. CLINICAL PHENOTYPE

Fanconi anemia (FA) is an inherited bone marrow failure syndrome that is linked to an increased risk of cancer and susceptibility to various stimuli that damage DNA. It also presents with a number of clinical features, including malformations of the upper limbs, an increased incidence of diabetes, and typical abnormalities in skin pigmentation. The functions of the proteins encoded by FA-defective genes (FANC proteins) in DNA damage and repair pathways are well-established. Furthermore, a few other investigations have shown that FA phenotype is also influenced by mitochondrial dysfunction (MDF). Not limited to FA, we have demonstrated that MDF-related symptoms, in addition to oxidative stress (OS), are present in additional syndromes involving DNA damage and repair (such as ataxia-telangiectasia, AT, and Werner syndrome, WS), all of which may have significant roles in these

disorders. The clinical hallmark of FA is bone marrow failure, usually starting in childhood. The anemia is caused by a progressive loss of hematopoietic stem cells and thus affects all blood lineages. Another consistent feature of FA is a high propensity toward malignancy, particularly acute myleogenous leukemia (AML) and squamous cell carcinoma. A wide variety of birth defects can also occur in FA, but are variable even within the same family. The most common defects are listed in Table.2 Together, these clinical manifestations of FA results in a markedly reduced life expectancy with death most frequently due to hematological complications or cancer.

4. CELLULAR PHENOTYPE

Given the established connections between redox pathways and MDF, as well as the correlations between FA phenotype and FA proteins with OS, a a series of independent investigation have demonstrated that mitochondria lay a role in FA phenotype, as evidenced by finding that FANG localizes to mitochondria (9) major mitochondrial functions, including ATP productions mitochondrial membrane potential ($\Delta\Theta$), mitochondrial ultra structure, defective mitochondrial peroxiredoxin 3, and oxygen consumption, were found to be significantly altered in FA cells of genetic subtype A, C, D2, and(10, 11).

These malfunctions were not present in corrected FA cells. Transcripts from bone marrow cells from FA patients compared to healthy donors were examined in another study which discovered that genes related to mitochondrial bioenergetics pathways, such as the electron transport chain and Krebs cycle, were considerably down regulated, roughly by 1.5 to 2-fold (11).

These results, which come from both freshly extracted bone marrow cells and lymphoblastoid or fibroblast cells, suggest that MDF occurs in vivo in FA patients and is not limited to FA cell.

Culture (9,10,11). Many cellular phenotypes have been reported in FA cells, but the most consistent and accepted of these is hypersensitivity to agents which produce interstrand DNA cross-links (ICL) such as mitomycin C (MMC) or Diepoxybutane (DEB). After ICL treatment FA cells display several phenotypes. These include increased chromosome breakage, radial formation and other cytogenetic abnormalities seen in metaphase chromosome spreads. FA cells also are very hypersensitivity to oxygen and ionizing radiation.

FAassociated MDF and OS, the following scenario could be put forth: under normal cell conditions, mitochondria actively manufacture ATP (State 3), and when protons, ADP, and phosphatearetransferred across the inner membrane, electron transport accelerates. In that condition, the respiratory chain

uses up over 90% of the oxygen and converts it to water. It is reasonable to believe that FA cells accrue oxidative damage, which leads to MDF and impairscellular respiration and ATP synthesis. Most FA mitochondria enter a semi resting state, where there is insufficient ATP synthesis and minimal oxygen consumption.

Abnormalities in the mitochondria may arise from all of these occurrences (10). Our most recent data, which comes from six FA patients and is listed in Appendix I, revealed that multiple mitochondrial genes were down-regulated in the cells of FA patients, indicating that MDF is involved in the FA phenotype. Nicotinamide nucleotide transhydrogenase (NNT) is one of the genes that may be involved in detoxifying reactive oxygen species (ROS) because its knockdown was shown to impair redox potential and elevate ROS levels (12). By replenishing GSH antioxidant systems and mitochondrial repair enzymes (thioredoxin, glutaredoxin, peroxyredoxins, and phospholipids hydroperoxidase), NNT may regulate the amount of reactive oxygen species (ROS) and the cellular redox state. Additionally, by creating a proton gradient, NNT may help maintain the potential of the mitochondrial membrane (13, 14). Disorder such as mitochondrial and other genetic disease involving damage to mitochondria in cells of the brain, heart, liver, blood, eye, lungs, kidney (15,16,17,18). They both appear to have faulty DNA repair (DDR) as a common clinical and biochemical hallmark, and there is Direct evidence of MDF/OS present as well, such as change mitochondrial functioning and ultra structure elevated ROS levels, and in imbalance in cellular Bioenergetics pathways, Remarkably, DDR pathway is implicated in a large number of mitochondria related disease (MRD) (either at mtDNA or at nuclear DNA level). Whereas ROS buildup in DDR may also impact and harm mitochondria, deficiencies in mitochondria may simultaneously cause ROS buildup, which is then followed by OS and DNA damage.

Table 2: Clinical Features of FA Children in India

Clinical Features of Fa Children in India (Data from Refain)	
Clinical Features	**%**
Mean Age	7.6yrs
Consanguinity	64
Aplastic Anemia	97
Hyper- Pigmentation	96
"Fanconi Facies"	65
Café Au Lait Spots	78
Thenar Hypoplasia	74
Small for Date	63

Radial Ray Anomaly	56
Renal Anomaly	29
Earanomaly/Impaired Hearing	21
Hypo-Pigmentation	15
Cardiac Anomaly	13
Short Stature (-2SD)	11
Microcephaly (-2SD)	13

5. OXIDATIVE STRESS CAUSE OF DNA DAMAGE.

Metabolically active cells are continuously exposed to various reactive oxygen species [ROS]. ROS comprise of oxygen molecules [O_2], superoxide anion radicals [O_2^-, hydroxyl free radicals [OH], singlet of oxygen molecule [$1/2O_2$], and hydrogen peroxide [H_2O_2], and can be produced by disproportioned endogenous oxygen reduction caused by some cellular enzymes or in mitochondrial respiratory pathway, in addition to that by exogenous exposure of the cells to UV or environmental DNA damaging agents (19). Intracellular ROS levels regulation and central signalling pathways mediated by ROS maintaining the equilibrium between self-renewal, proliferation and differentiation of normal stem cells and hematopoietic progenitor cells in the hematopoietic and neuronal compartments, in addition to that the early embryonic stem [ES] cell compartment. Elevation in ROS levels in the cells above the threshold concentrations can direct to oxidative stress [OS] and that causes DNA damage ultimately leads to genomic instability (20). Oxidative stress is very much considerable to DNA damage approximately 10,000 nucleotide bases in a day per each human cell and that can be one of the major causes of DNA damage and mutation (21). The majority of oxidative stressed DNA damage sites are normally get repaired via base excision repair [BER], even though continuous damage both on two complementary strands of DNA at nearby nucleotides that may results in DNA double-strand breaks [DSBs] that participate in repairing process by non-homologous end-joining [NHEJ] or homologous recombination [HR]. Oxidative stress is also recognized to persuade elevated chromatin single strand DNA nicks [ssDNA] characteristic of those generated during apoptosis. The similarity between ROS and the DNA damage results that both are accomplished to trigger numerous pathways that leads to DNA damage response. As a result of the cellular response and cell signalling against ROS, ranges from cellular proliferation, cell survival to cell growth arrest, senescence, and cellular death depending on the level of ROS and type of the cell under investigation. Continual growth and metabolically active stages of the cell indicates that the incapability to control elevated levels of ROS that leads to modifications of cellular homeostasis or defective DNA damage repair induced by ROS lies at the origin of diseases characterized by both neurodegeneration and bone marrow failure (22). Furthermore, elevated levels

of ROS considered being a distinct feature of Acute Myeloid Leukemia [AML]. These diseases may be considered as reflective of the active functionality of the cells in response to ROS throughout aging persists in the similarities between phenotypes at the cellular level.

6. ROS INDUCES DNA DAMAGE TRIGGERS SIGNALLING AND REPAIRING PATHWAYS

Genomes of all the cells are exposed to both endogenous and exogenous oxidative stresses. It can be refers to an inequity between antioxidant defence mechanism and production of ROS. In cell, ROS generated both by endogenously from cellular metabolisms like oxidative Phosphorylation in mitochondria and oxidation of long-chain fatty acids in peroxisomes, as well as exogenously by toxins from environment like ultra violet [UV] radiation, ionizing radiation [IR], and DNA damaging/chemotherapeutic agents. When ROS generation goes beyond the level, antioxidant defence capacity in cells will be influenced; ROS could react with all varieties of macromolecules in the cell including DNA, RNA, proteins, and lipids. Particularly, DNA damage by oxidative stress might correspond to the major types of DNA damage confirmed that approximately 10,000 alterations in the DNA are produced in a mammalian cell in one day. Oxidative stressed DNA damage consist of oxidized nucleotide bases [both purine and pyrimidine] damage, sugar moiety damaged by oxidation, apurinic/apyrimidinic [AP] sites, single-strand breaks in DNA [SSBs], Double strand breaks [DSBs], intrastrand and interstrand cross links in DNA [ICLs], DNA-protein cross links, damaged bases with mismatched pairs, stalled DNA replication forks, and clustered DNA lesions generated oxidatively [OCDLs]. Oxidative stress also has been concerned in the pathogeneses of numerous diseases, like cancer, bone marrow failure and neurodegenerative disorders.

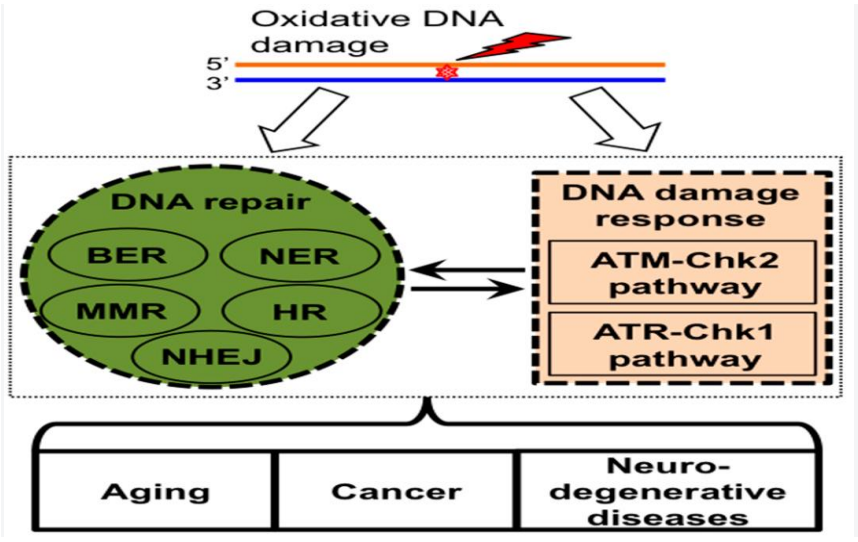

Figure 1: Fate of Oxidative Stressed Cells

The homologous recessive method of genetic inheritance is observed in Fanconi Anemia. With the exception of B, which is located on the X chromosome and accounts for 2% of FA cases, all the genes are autosomal. There have been reports of genetic mosaicism in FA (23). Nineteen (19) genes—FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ/BRIP1, FANCL, FANCM, FANCN/PALB2, and FANCO/RAD51C, FANCP/SLX4, FANCQ/ERCC4, FANCR/RAD51, FANCS/BRACA1, FANCT/UBE2T—have been identified to date as being implicated in the Fanconi anemia pathway. The activation of FANCD2 and FANCI by monoubiquitination in response to DNA damage or during the S phase of the cell cycle is a crucial event in the FA pathway. The formation of a core complex comprising eight proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM) and two FA-Associated Proteins (FAAP100 and FAAP24) in the nucleus is essential for the monoubiquitination of the I-D2 complex. FANCL is most likely to act as the E3 Ubiquitin ligase in this complex (24). After being monoubiquitinated, FANCD2-I is then directed toward nuclear foci and the chromatin complex, where it appears to assist the MRN (MRE11, RAD51, and NBS1) complex in homologous recombination-mediated DNA repair.

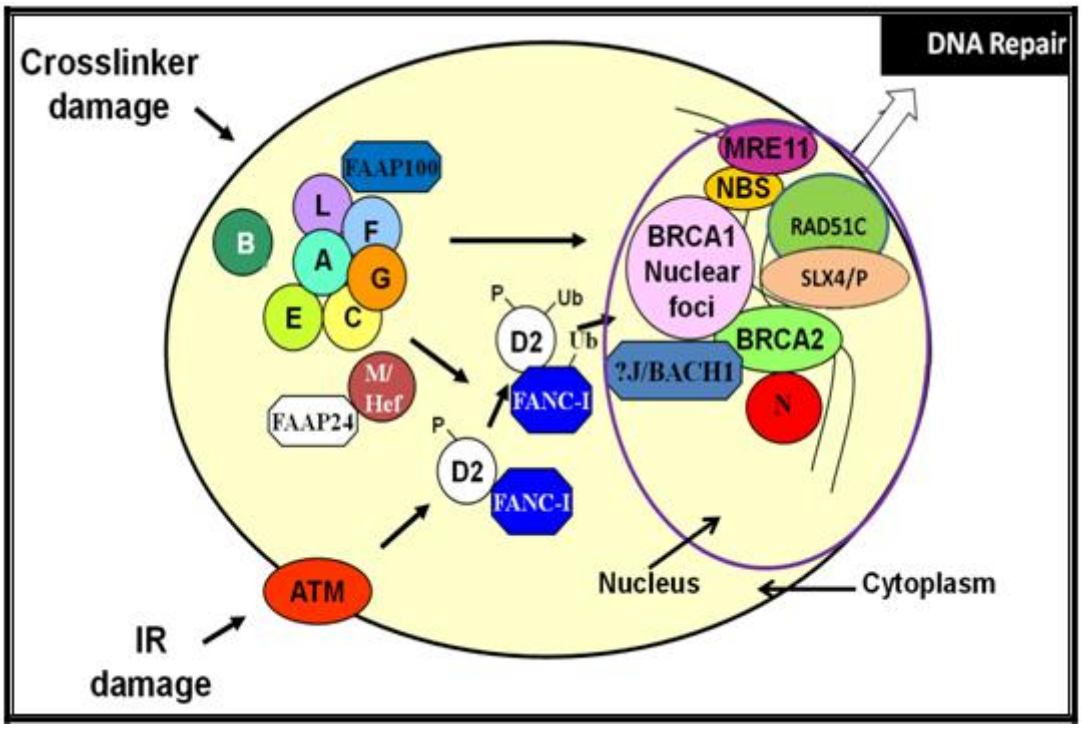

Figure 2: FA Pathway

7. SUMMARY

Fanconi Anemia is very good model for understand the accumulation of multiple mutation and development of cancer. In the lack of FA Pathways Reactive oxygen species generate in mitochondria is the agents cause DNA damage. Mitochondrial dysfunction (MDF) observed in FA. MDF is principal cause of unbalanced production of ROS. In absence of FA pathway increased ROS level root cause of multiple mutation and high probability of development of cancer.

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