REVIEW

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Fanconi anemia pathway—the way of DNA interstrand cross-link repair

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The study of rare genetic diseases usually inspires the research of cancer biology. Fanconi anemia (FA), is a rare cancer susceptibility syndrome with an incidence of only 1 per 350,000 births. FA is an autosomal recessive disease with three main features: chromosome instability, hypersensitivity to DNA cross-linking agents such as mitomycin C (MMC), cisplatin and so on, and susceptible to a number of cancer types, mainly leukemia and squamous cell carcinomas of the head and neck or gynecologic system. DNA cross-linking agents may led to DNA cross-linking lesion, and Fanconi anemia pathway plays a key role in repairing its cross-linking. However, FA pathway is closely linked with carcinogenesis and tumor drug resistance. This paper mainly focuses on the FA pathway and its progress in cancer research.

1. Introduction

Fanconi anemia (FA) is a heritable disease characterized by various clinical features including bone marrow failure, congenital abnormalities, and cancer predisposition. Now scientists have identified 15 FA or FA-like genes: FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, FANCN, FANCP and RAD51C (FANCK was skipped because it was difficult to discriminate from FANCA in oral conversations (de Winter and Joenje 2009). These genes encode Fanconi anemia complementation group proteins with other related proteins. They constitute the FA pathway to repair the DNA cross-linking lesion. The FA pathway was also called as the FA/BRCA pathway, in which the FA proteins repair the DNA lesion with breast cancer type 1 susceptibility protein (BRCA1) and breast cancer type 2 susceptibility protein (BRCA2). FA/BRCA pathway has three key points: FA core complex synthesis, the mono-ubiquitinylation of FANCD2 and FANCI, and the formation of FANCI–FANCD2 (ID) complex and FANCD2-I nuclear foci.

2. Fanconi anemia complementation group proteins

Fanconi anemia complementation group protein A is the most commonly mutated protein in Fanconi anemia. It is mutated in two-thirds of all FA cases. It undergoes phosphorylation by protein kinase B and forms a complex with FANCC protein in the cell nucleus. FANCA has been described as being hypermutable due to the abundance of Alu elements, homopolymeric tracts and direct repeats. The prevalence of large genomic rearrangement in the BRCA1 gene, also with high density of Alu sequences, and their contribution to breast cancer susceptibility has already been demonstrated in several populations (Mazoyer 2005). So FANCA, a protein partner of BRCA1, might also hold such inactivating alterations, conferring increased risk for breast cancer (Solyom et al. 2011a).

FANCG undergoes phosphorylation by cell division control protein 2 (CDC2) protein kinase during mitosis. It forms a complex with other Fanconi anemia proteins, especially FANCA, FANCC, FANCG, and FANCM, and protects cells from DNA damage by genotoxic agents. It functions as scaffold mediating interactions with the other FA proteins. In the FA core complex, FANCA and FANCG form a subunit. Recent research shows that overall the phenotype of a−/−/g−/− double knockout mice and cells appeared highly similar to the phenotype of Fanca or Fancg single knockouts. The lack of an augmented phenotype suggests that null mutations in Fanca or Fancg are fully epistatic, making additional important functions outside of the FA core complex highly unlikely. Since no redundant or divergent functions were identified for FANCA and FANCG, it is likely that both proteins act as scaffolds and regulate FA core complex assembly and ubiquitin ligase activity (Solyom et al. 2011).

FANCB was called Fanconi anemia associated protein 95 (FAAP95) first, then renamed as FANCB. The special feature of this gene was not in its functional domains (it only contained a bipartite nuclear localization signal), but in its location on the X-chromosome (Meetei et al. 2004). As a consequence, FA-B patients are exclusively male. Mutations in the gene FANCB can cause X-linked VACTERL-hydrocephalus syndrome (X-linked VACTERL-H), a rare disorder which own to truncate the FANCB open reading frame and results in highly skewed X-inactivation in unaffected carrier females (McCauley et al. 2011). Deleting FancB exon 2 can reduce proliferation of mouse ES cell and increase its' sensitivity to MMC (Kim et al. 2011). FANCB, FANCL and FAAP100 to form a stable subcomplex. Formation of this subcomplex protects each component from proteolytic degradation and also allows their coregulation by FANCA and FANCM during nuclear localization (Ling et al. 2007).

FANCC regulates the activities of cytochrome P450 reductase and glutathione S-transferase. It is found predominately in the cytoplasm, but moves to the cell nucleus in response to FANCE protein. FANCC was the first identified FA gene, by Manuel

Buchwald's group in 1992 (Strathdee et al. 1992). FANCE interacts with FANCC protein and FANCD2 protein. It promotes the accumulation of FANCC protein in the cell nucleus. FANCE is predominantly localized in the nucleus and acts as a molecular bridge between the FA core complex and FANCD2, through direct binding of both FANCC and FANCD2. The endogenous FANCE protein was not detected in nuclear extracts of FA-C cells lacking FANCC, but was present in FA cells from other complementation groups. Previous studies showed that FANCC needs the FANCE protein to accumulate in the nucleus of the cell, so FANCE and FANCC are reciprocally essential for their nuclear accumulation (Leveille et al. 2006). Lower FANCE expression levels were found in the nucleus of FA-G, -I, -L and -M cells, suggesting that several FA proteins are needed for stabilization of FANCE in the nucleus.

FANCD1 is also known as BRCA2. BRCA2 is a protein that in humans encoded by the BRCA2 gene. BRCA2 orthologs have been identified in most mammals for which complete genome data are available. BRCA2 belongs to the tumor suppressor gene family and the protein encoded by this gene is involved in the repair of chromosomal damage with an important role in the error-free repair of DNA double strand breaks. Alan D'Andrea and co-workers discovered that FA-D1 patients carried biallelic mutations in BRCA2 and expressed truncated BRCA2 proteins (de Winter and Joenje 2009). BRCA2 supports the formation of RAD51 filaments, which are essential for strand invasion during the homologous recombination process. FA patients with biallelic BRCA2 mutations have a much more severe clinical phenotype (de Winter and Joenje 2009). Any of the eight truncated BRC repeats of BRCA2 would weaken RAD51 binding. Removal of the C-terminal BRC repeat from some truncation mutants (e.g., BRC1–3, BRC1–5 and BRC1–8) increased Rad51 binding strength. BRCA2 may affect homologous recombination through the regulation of Rad51 binding strength (Ochiai et al. 2011).

FANCL is an E3 ubiquitin ligase. It plays a key role in the DNA damage response pathway of Fanconi anemia proteins. It is associated with mono-ubiquitination of FANCD2 protein and the redistribution of FANCD2 to nuclear foci that containing BRCA1 protein. FANCD2 undergoes monoubiquitination by FANCL protein in response to DNA damage. Also, in response to ionizing radiation it can undergo phosphorylation by ataxia telangiectasia mutated protein. This protein is monoubiquitinated in response to DNA damage, resulting in its localization to nuclear foci with other proteins (BRCA1 and BRCA2) involved in homology-directed DNA repair. This monoubiquitination is required for interaction with the nuclease FAN1. Various forms of DNA, such as single-stranded, double-stranded and branched DNA, robustly stimulated the FANCD2 monoubiquitylation *in vitro* up to a level comparable to its *in vivo* monoubiquitylation. This stimulation of the FANCD2 monoubiquitylation occurs in the FANCI–FANCD2 complex (Sato et al. 2012). FANCD2 is also a target for caspase-mediated apoptotic pathway, which may be an early indicator for apoptotic cell death (Park et al. 2011).

The FANCI protein associates with FANCD2 to form the FANCI-FANCD2 (ID) complex, localizes to chromatin in response to DNA damage. FANCI is identified as an ataxia telangiectasia mutated (ATM)/ataxia-telangiectasia Rad3-related (ATR) kinase substrate required for resistance to mitomycin C. FANCI shares sequence similarity with FANCD2, and is likely evolving from a common ancestral gene.

FANCF is an essential component of a nuclear complex core that protects the genome against chromosomal instability. FANCF acts as a flexible adaptor protein. It interacts directly with FANCG protein and helps stabilize a complex FANCA protein and FANCC protein through its C-terminal. FANCF also interacts with FANCC–FANCE sub-complex through N-terminal region (de Winter et al. 2000). The N-terminus of FANCF interacts with partner of Sld five 2 (PSF2), a member of the GINS complex essential for both the initiation and elongation steps of DNA replication, and FANCM binds the same domain of FANCF. The FANCF–FANCM interaction is essential to monoubiquitylate FANCD2, the FANCF–PSF2 is not (Tumini et al. 2011). A FANCF cis element is activated by interferon consensus sequence-binding protein (ICSBP), the interferon consensus sequence binding protein, in differentiating myeloid cells. ICSBP-induced FANCF expression protects myeloid cells from DNA cross-link damage during the genotoxic stress of differentiation (Saberwal et al. 2009).

FANCJ/BRIP1 was first identified as a novel BRCA1-interacting protein in a pull-down assay with the BRCA1 carboxyl-terminal (BRCT) motifs of BRCA1. FANCJ was identified by genetic linkage analysis and microcell-mediated chromosome transfer. It was on chromosome 17. The protein contained a DEAH helicase domain and was called BRCA1-associated C-terminal helicase (BACH1). FANCJ interacts with REV1 at the fork to facilitate the replication of a subset of G-quadruplex-forming sequences, and the collaboration between FANCJ and Werner syndrome/Bloom syndrome defends epigenetic stability by ensuring continuous replication at G-quadruplex-forming DNA sequences (Sarkies et al. 2012).

FANCM was called FAAP250 first, renamed as FANCM, which contains a DEAH-like helicase domain at its N-terminus and an excision repair cross-complementing rodent repair deficiency complementation group 4 (ERCC4)/XPF like nuclease domain at its C-terminus. FANCM probably acts as a replication fork remodeler that promotes fork reversal and creates chicken-foot structures upon stalling of the replication fork (Gari et al. 2008). This function is associated with its ATP-dependent branch-migration activity (Suhasini and Brosh 2012). FANCM with its translocase activity, may move away the core complex from the lesion that stalled the replication fork, so that other could repair factors to access the DNA. This activity also protects cells from accumulating 53BP1-OPT domains, which mark lesions resulting from problems arising during replication (Blackford et al. 2012). FANCM forms an evolutionarily conserved DNA-processing complex with MHF1/MHF2 (histone-fold-containing proteins), which is essential for DNA repair in response to genotoxic stress. A fragment of FANCM (FANCM (661–800), designated FANCM-F) binds MHF1 and MHF2 through a 'dual-V' shaped structure (Tao et al. 2012).

FANCN, partner and localizer of BRCA2, are also known as partner and localizer of BRCA2 (PALB2), a BRCA2 binding protein. This protein binds to and colocalizes with BRCA2 in nuclear foci and is likely to permit the stable intranuclear localization and accumulation of BRCA2. PALB2 binds the single strand DNA and directly interacts with the recombinase RAD51 to stimulate strand invasion, a vital step of homologous recombination (Buisson et al. 2010). PALB2 can function synergistically with a BRCA2 chimera (termed piccolo, or piBRCA2) to further promote strand invasion (Buisson et al. 2010). FANCN regulates cellular redox homeostasis through its interaction with kelch-like ECH-associated protein 1 (KEAP1), an oxidative stress sensor that binds and represses the master antioxidant transcription factor nuclear respiratory factor-2 (NRF2) (Ma et al. 2012).

FANCP (also known as BTB (POZ) domain containing 12 (BTBD12) and synthetic lethal of unknown function protein4 (SLX4)) is a protein involved in DNA repair, where it has important roles in the final steps of homologous recombination (Klein and Symington 2009). The version of SLX4 present in humans and other mammals acts as a sort of scaffold upon which other proteins form several different multiprotein complexes. The

SLX1-SLX4 complex acts as a Holliday junction resolvase. As such, the complex cleaves the links between two homologous chromosomes that form during homologous recombination. This allows the two linked chromosomes to resolve into two unconnected double-strand DNA molecules (Svendsen et al. 2009). SLX4 also associates with RAD1, RAD10 and Single-strand annealing weakened protein 1 (SAW1) in the single-strand annealing pathway of homologous recombination (Mimitou and Symington 2009).

RAD51C is a protein which is encoded by the RAD51C gene. This gene is a member of the RAD51 family of related genes, which encode strand-transfer proteins which are thought to be involved in recombinational repair of damaged DNA and in meiotic recombination. This gene product interacts with two other DNA repair proteins, are encoded by RAD51B and X-ray repair cross-complementing protein 3 (XRCC3), but not with itself. The protein copurifies with XRCC3 protein in a complex, reflecting their endogenous association and suggesting a cooperative role during recombinational repair. This gene is one of four localized to a region of chromosome 17q23 where amplification occurs frequently in breast tumors. Overexpression of the four genes during amplification has been observed and suggests a possible role in tumor progression. RAD51C participates in interstrand cross-link (ICL), double strand break-induced DNA damage signaling and controls intra-S-phase checkpoint through CHK2 activation, and its pathological mutants has identified in FA and breast and ovarian cancers (Somyajit et al. 2012).

3. FA core complex

FA core complex with the E3 ubiquitin ligase activity, mediated the mono-ubiquitinylation of FANCD2 and FANCI. It consists of eight kinds of FA proteins: FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL and FANCM. Now we also found the other two genes FAAP24 and FAAP100 which encoded proteins involved in FA core complex, but the mutations of these genes were not directly related with FA (de Winter and Joenje 2009). FAAP24 with FANCM together form heterodimers, relatively independent of the other FA core complex components, plays the role of recognition and binds DNA damage sites. FANCM-FAAP24 complex could bind the FA core complex to chromosomes and then locate in the DNA injury sites, so that FA core complex is directly involved in DNA damage repairing (Huang et al. 2010). Replication fork stalling stimulates FANCM to recruit a stable complex consisting of FANCA,-B, -C, -E, -F, -G, and -L (de Winter and Joenje 2009). FA core complex can be divided into multiple sub-units in the structure, including a sub-unit of FANCA and FANCG, another subunit of FANCC and FANCE,FANCF as a link protein, the C-terminal is connected directly with FANCG to stable the interaction between FANCA, FANCC and FANCG. The N-terminal interact with the FANCC/FANCE subunit, enabling FANCA/FANCG subunit more stable. Due to FANCF the FA core complex is more firmly (Leveille et al. 2004). FANCL, FANCB and FAAP100 form a subunits, in which FANCL is an E3 ubiquitin ligase, mediate the mono-ubiquitinylation of FANCD2 and FANCI, FANCB makes FANCL stable (Moldovan and D'Andrea 2009). FAAP20, an integral subunit of the multisubunit Fanconi anemia core complex. FAAP20 binds to FANCA subunit and is required for stability of the complex and monoubiquitination of FANCD2. FAAP20 contains a ubiquitin-binding zinc finger 4 domain and binds to the monoubiquitinated form of Rev1. FAAP20 binding stabilizes Rev1 nuclear foci and promotes interaction of the Fanconi anemia core with PCNA-Rev1 DNA damage bypass complexes (Kim et al. 2012).

4. Mono-ubiquitinylation of FANCD2 and its downstream

FANCD2 together with FANCI form a dynamic complex. FA core complex, with FANCL as the catalytic subunit, acts as an E3-ubiquitin ligase to monoubiquitinate FANCD2 and FANCI in conjunction with the ubiquitin-conjugating enzyme E2T (UBE2T). Ubiquitination of FANCD2 and FANCI is important for the maintenance of ubiquitin on the other, indicating the existence of a dual ubiquitin-locking mechanism required for ID complex function (Smogorzewska et al. 2007). Ubiquitination of FANCD2-I leads to its localization to chromatin foci. These foci are considered to be DNA repairing structures because they contain repair factors such as Rad51, BRCA1, BRCA2, NBS1, PCNA, or γH2AX (Moldovan and D'Andrea 2009). The monoubiquitination of FANCD2 is considered as an activating step in FA pathway. Ubiquitylated FANCD2 recruits SLX4 to DNA damage sites, where it mediates the resolution of recombination and intermediates the generated during the processing of interstrand cross-links (Yamamoto et al. 2011). Two nucleases necessary for crosslink repair have been identified, Fanconi anemia-associated nuclease 1 (FAN1) and exonuclease 3'-5' domain-like 2 (EXDL2). FAN1 colocalizes at sites of DNA damage with the ID complex in a manner dependent on FAN1's ubiquitin-binding domain (UBZ), the ID complex, and monoubiquitination of FANCD2. FAN1 possesses intrinsic 5'-3' exonuclease activity and endonuclease activity that cleaves nicked and branched structures (Smogorzewska et al. 2010).

5. Regulation of the FA/BRCA pathway

DNA damage such as interstrand cross-links can stall the replication fork, and create single-stranded regions coated by replication A related protein (RPA). This leads to activation of the checkpoint kinase ATR and its downstream effector kinase CHK1. ATR and CHK1 phosphorylate several components of the FA core complex and the ID complex. Phosphorylation of FANCI may act as a switch, making the ID complex ready for ubiquitination. Phosphorylation of FANCD2 and Fanconi anemia core components affects the efficiency of, but is not essential for, ID ubiquitination by the FA core complex, together with E1 and UBE2T. Analogously, ubiquitination of FANCD2 is essential for DNA repair, activating the ID complex for chromatin binding; on the other hand, FANCI ubiquitination affects the efficiency of, but is not essential for, DNA repair. FANCM also has a direct role in DNA repair, as a branch point translocase for stalled replication forks and four-way junctions (Wang 2008a).

Phosphorylation of FANCI by ATR makes the ID complex a substrate for efficient ubiquitination by the FA core; phosphorylation of FANCI may also turn the ubiquitinated ID complex into a poor substrate for deubiquitination by the ubiquitin specific protease 1 (USP1)–USP1-associated factor 1 (UAF1) complex, resulting in increased levels of monoubiquitinated FANCD2. These two mechanisms are not mutually exclusive. Molecular interactions between WD40 repeats and phosphorylated FANCI may regulate the ubiquitination status of FANCD2. Both FANCL and UAF1 contain WD40 repeats that bind phospho-Ser/Thr, and thus could potentially bind phosphorylated FANCI, stimulating the ubiquitination reaction on FANCD2 or turning off the deubiquitinating enzyme complex UAF1–USP1. FANCE may contribute to the first reaction, as it can bind directly to FANCD2 (Wang 2008a).

USP1 is a deubiquitinated enzyme, UAF1 and USP1 form a stable complex, UAF1 proteins contain the WD40 repeats which can activate the the deubiquitination of FANCD2. FANCD2

ubiquitinated in S phase and then participate in DNA repair in downstream, when the cells are out of S phase, deubiquitinating enzyme UPS1 deubiquitinats the FANCD2, the FANCD2 again engage in the FANCD2 cycle (Nijman et al. 2005). UAF1 is key adjustment of deubiquitination, it not only can be combined with USP1, but also participate in deubiquitination of two new USP12 and USP46 (Cohn et al. 2009). Studies have found that USP1-UAF1 compound is effective in promoting homologous recombination to repair DNA double-strand damage (Murai et al. 2011). P21 is the the USP1 transcription inhibitor, via adjusting the FA pathway to influence the USP1 (Rego et al. 2012).

UBE2T is E2 ubiquitin conjugating enzyme, FANCL has E3 ubiquitin ligase activity. E2 ubiquitin conjugating enzyme-UBE2T ubiquitinates FANCD2 together with E3 which contains FANCL. The FANCL can monoubiquitinate UBE2T, this ubiquitination occurs in the K91 on UBE2T and reduce its E2 activity. If the ubiquitination of UBE2T is connected the FANCL stable will restrain the FA-E3 complex and thus cause a negative adjustment in FA pathway (Machida et al. 2006). Human homologs of the yeast ubiquitin-conjugating enzyme Rad6 (HHR6)'s going up and down can also cause changes the FANCD2's ubiquitination level, but the mechanism is different from UBE2T (Zhang et al. 2008). Ubiquitin-conjugating enzyme E2W (UBE2W) is a new E2 ubiquitin-conjugating enzyme. The FANCD2 ubiquitination regulation mechanism is different from UBE2T and HHR6. Increase of UBE2W promotes FANCD2's ubiquitination, when lowered it can significantly reduce the influence of the UV radiation on FANCD2 ubiquitination, but it has no effect in MMC ubiquitination (Zhang et al. 2011).

ATR checkpoint kinase and CHK1 participate in the regulation of the FANCD2 monoubiquitination. FANCA is phosphorylated after DNA damage and its localization and chromatin. In the FA pathway FANCA's phosphorylation site is ser1449 mediated by the ATR, but this process does not occur in S phase (Collins et al. 2009). FANCD2 has two phosphorylation sites, thr691 and ser717. ATR and ATM can phosphorylate FANCD2. ATR-mediated FANCD2 phosphorylation occurs in S phase checkpoint, and promotes the ubiquitination of FANCD2. ATM can be involved in the regulation of ionizing radiation-induced S phase checkpoint and normal S phase thr691-phosphorylation (Ho et al. 2006). The FANCG the ser7 of ATR phosphorylation contribute to stability, FANCA and FANCC (Qiao et al. 2004). FANCE subunit of the FA complex has two phosphorylation sites, Thr346 and Ser374. Chk1 phosphorylates Thr346 and Ser374, at the same time promotes FANCD2 ubiquitination and nuclear foci formation. However, this ubiquitination regulation by Chk1 and cannot participate in the repair of ICL. It can only be said to be non-functional regulation of CHK1 participation (Wang et al. 2007).

6. FA/BRCA pathway and drug resistence

Crosslinking of DNA includes three different types. This can either occur in the same strand (intrastrand crosslink) or in the opposite strands of the DNA (interstrand crosslink). Crosslinks also occur between DNA and protein.

DNA crosslinking agents include two types: exogenous crosslinking agents and endogenous crosslinking agents. Exogenous cross linking agents are together with alkylating agents (carmustine and nitrogen mustard), cisplatin, and its derivatives. Some alkylating agents such as nitrogen mustard which is used in chemotherapy can cross link with DNA at N7 position of guanine on the opposite strands forming an interstrand crosslink (Ali-Osman et al. 1995). Cisplatin can form DNA cross links as monoadduct, interstrand crosslink, intrastrand crosslink or DNA protein crosslink. Mostly it acts on the adjacent N-7 guanine forming an 1,2 intrastrand crosslink (Poklar et al. 1996). Endogenous cross linking agents are HNO, aldehydes such as malondialdehyde, acrolein, formaldehyde, and psoralens (Wu et al. 2005). In addition, ionizing radiation can also cause DNA cross-linking.

Many DNA crosslinking agents are chemotherapy drugs in treatment of tumors. DNA Crosslinking can break replication of DNA, block replication fork, and if not repaired timely may lead to cell death. Because of this characteristic many DNA crosslinking agents are used in cancer chemotherapy, such as nitrogen mustard, cisplatin, mitomycin C, and so on. During cancer treatment drug resistance may occur, even treatment failure. The study showed that the cells, through the FA/BRCA pathway, repair DNA cross-linking by homologous recombination, so that the FA / BRCA pathway might be related to the drug resistance of tumor cells.

In the study of multiple myeloma we found that enhanced ICL repair via the Fanconi anemia FA/BRCA pathway contributes to drug resistance in melphalan-resistant myeloma cell lines. Disruption of this pathway reverses drug resistance. Using siRNA to knock down FANCF drug-resistant cells can reverse drug resistance. Overexpression of FANCF in drug-sensitive cells partially reproduced the drug-resistant phenotype (Chen et al. 2005). Combination of melphalan with *curcumin* had stronger effects on the proliferation inhibition, inducement of apoptosis, G2/M phase arrest, and enhancement of intracellular drug concentration than melphalan alone in those cells. This effect of curcumin is achieved by downregulation of FANCD2 protein ubiquitination. The ubiquitination of FANCD2 is a critical step of the FA/BRCA pathway, when this process is inhibited, the FA/BRCA pathway is also blocked. This also shows that the FA/BRCA pathway plays an important role in drug resistance in multiple myeloma (Xiao et al. 2010).

In the treatment of glioma cells are found to be resistant to temozolomide (TMZ) and carmustine (BCNU). FANCD2 monoubiquitination and FANCD2 nuclear foci formation are increased in these glioma cells. Moreover, inhibition of FA pathway activated by a small molecule inhibitor (curcumin) or by small interference of RNA suppression caused increased sensitivity to TMZ/BCNU in glioma cells (Chen et al. 2007).

Irofulven belongs to a new class of anticancer agents that are analogues of mushroom-derived illudin toxins. It induces DNA double-strand breaks and FANCD2 which may play an important role in modulating cellular responses and chemosensitivity in response to irofulven treatment. Irofulven induces FANCD2 monoubiquitination and nuclear foci formation (Wang et al. 2006). ATR is important in mediating irofulven-induced FANCD2 monoubiquitination. Thus it is clear that the FA/BRCA pathway can repair irofulven induced ICL.

Ovarian carcinomas with mutations in the BRCA2 are particularly sensitive to platinum compounds. However, such cancers ultimately develop cisplatin resistance. This acquired cisplatin-resistance can be mediated by secondary intragenic mutations in BRCA2 that restore the wild-type BRCA2 reading frame (Sakai et al. 2008). BRCA2 is the FANCI of FA pathway. The FANCI protein associates with FANCD2 and forms an ID complex to participate in the repair of DNA cross-linking.

The interaction between FA/BRCA pathway and tumor cell's drug resistance requires further studies. As the Fanconi anemia pathway is essential for cells to resist killing by DNA cross-linking agents, molecules that can turn off the Fanconi anemia pathway may be used to sensitize cancer cells to improve the efficacy of chemotherapy, whereas those that can turn on the pathway may be used to boost healthy cells to resist killing by anticancer drugs (Wang 2008b).

7. FA/BRCA pathway and cancer

FA gene mutations and silence are associated with various human tumors. FA patients have a high risk to develop tumors, including various solid tumors and non-solid tumors, acute myeloid leukemia is the most common of them. Most FA patients bear acute myelogenous leukemia or bone marrow failure in early time of life. Current research concerned is about FA-related tumors include breast cancer, ovarian cancer, prostate cancer and lung cancer, head and neck squamous cell carcinoma.

Breast cancers are immunohistochemically classified into 3 major groups depending on expression of ER, PR, and HER. The first class contains three negative (TN) expressions, ER, PR and HER2 for negative. In the second class, the HER2 overexpression type, ER, and PR are negative and HER2 are positive. The third, luminal type include luminal-a type and luminal-b type, luminal-a type, ER and (or) PR positive, HER2 negative or Ki67 low expression; luminally-b type, ER and (or) PR-positive, HER2-positive and (or) the Ki67 overexpression. Approximately 15% of all invasive breast cancers are of the TN subtype, this kind of breast cancer is coupled with a paucity of approved therapeutic targets. Both sporadic and hereditary TN cancers appear to have recurrent defects in double-strand break (DSB) repair, largely due to genetic or epigenetic perturbations of the BRCA1 gene (Stecklein et al. 2012). The efficacy of DNA damage chemotherapy treatment, in BRCA1/2 related breast cancer is better than in sporadic breast cancer. BRCA1/2 related breast cancer's clinical complete response (cCR) is 91% and pathologic complete response (pCR) is 44%. Noncarriers show only 30% cCR and 4% pCR (Chappuis et al. 2002). This phenomenon may be due to the fact that FA/BRCA pathway's defects lead to DNA damage and repair barriers, so as to make to cancer cells sensitive to chemotherapy. BRCA1 and BRCA2 related breast cancer types are different. BRCA2-related breast cancer mostly hormone receptor-positive and BRCA1-related breast cancer is mostly hormone receptor-negative, TN breast cancer (Bordeleau et al. 2010). Downregulation of BRCA1 in sporadic breast cancer is very common (Thompson et al. 1995). BRCA1 together with RAD51 during s and G2 colocalized with nuclear foci, RAD51 involved in the regulation of homologous recombination, will increase the risk of breast cancer (Somyajit et al. 2010). Recent studies show that FANCA deficiency can also increase susceptibility to breast cancer (Solyom et al. 2011). BRCA1/BRCA2-associated ovarian cancers may be associated with a more favorable clinical course. BRCA1/BRCA2 mutation-associated epithelial ovarian cancer (EOC) patients had longer disease-free survival (49 vs 19 months) and overall survival times (91 months vs 54 months) than non-BRCA-associated EOC epithelial ovarian cancers (EOC) (Cass et al. 2003). Compared with sporadic BRCA1/BRCA2 mutation-associated EOC patients have higher survival rates. The study of platinum sensitive ovarian cancer cells showed that these cancer cells have methylated the FANCF promoter, thus impairing monoubiquitination of FANCD2, and DNA crosslinking damage cannot be effectively repaired. By studying the BRCA1 mRNA expression in epithelial ovarian cancer we found that after the platinum-based chemotherapy, patients with low or intermediate expression of BRCA1 mRNA had a mean 57.2-month overall survival compared to 18.2 months in those with high levels of BRCA1 mRNA expression (Quinn et al. 2007). A homozygous mutation in the RAD51C gene was recently found to cause Fanconi anemia-like disorder and improved the risk susceptibility for ovarian cancer (Pelttari et al. 2011).

The somatic inactivation of BRCA1 may play a role in the pathogenesis of prostate cancer. Studies have demonstrated that the risk of prostate cancer in BRCA2 mutation patiens will increase 2.5 to 7.3 times (van Asperen et al. 2005). Prostate cancers arising in carriers of deleterious BRCA2 mutations exhibited more aggressive histology than sporadic cancers (Mitra et al. 2008). Many lung cancer chemotherapies including cisplatin compounds and FA/BRCA pathways can repair DNA damage caused by this drug, so by blocking the FA/BRCA pathway can increase therapeutic effects of chemotherapy in lung cancer. Lung cancer patients with medium or low expression of the BRCA1 mRNA have lower death risk than those with higher BRCA1 mRNA expression (Boukovinas et al. 2008). USP1 can regulate FANCD2 ubiquitination, and the USP1/UAF1 inhibitors act synergistically with cisplatin in inhibiting cisplatin-resistant non-small cell lung cancer (NSCLC) cell proliferation (Chen et al. 2011).

8. Conclusions

Although research for mechanisms of FA pathway has made a lot of progress and breakthroughs, with new FA-related genes and proteins have been found, there are still many problems that have not been clarified. Downstream mechanism of ID complex, interactions between FA proteins and other related proteins, and the role of FA gene in tumors need to be further studied.

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