Advances in the understanding of Fanconi anemia complementation group D2 protein (FANCD2) in human cancer

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Fanconi anemia (FA) is a rare human genetic disease, resulting from dysfunction in any of 17 known complementation proteins: FANC-A, B, C, D1, D2, E, F, G, I, J, L, M, N, O, P, Q & S, and other unknowns. Besides the severe bone marrow failure, an extremely high incidence of cancer as well as many other clinical symptoms associated with FA patients, FA cells are known of insufficiency in homologous recombination, DNA mismatch repair, nucleotide excision repair, translesion DNA synthesis, and other molecular defects, leading to genome instability. Those similar molecular and cellular/tissue features show that all FA proteins function in one common signaling pathway, namely, the FA pathway. The monoubiquitination of FANCD2 is the central step of the FA pathway activation upon DNA damage or during DNA replication. The molecular functions of FANCD2 emerge as a very attractive filed of investigation in cancer research. Herein, we review the recent progresses in FANCD2 functions at these rapidly progressed aspects.

Keywords: FANCD2; Replication; Gain of Function; DNA damage; Tumor cell sensitivity


Introduction

Fanconi anemia (FA) is a rare hereditary disease, characterized by an extremely high incidence of both hematological and non-hematological malignancies and multiple developmental defects [1-6]. Cells from FA patients display a chromosome breakage and hypersensitivity to DNA crosslinking agents such as mitomycin C (MMC), diepoxybutane (DEB) or cisplatin [7, 8]. Now it has been widely acknowledged that 17 complementation groups [FANC-A, B, C, D1 (BRCA2), D2, E, F, G, I, J (BRIP1), M, N (PALB2), O (RAD51C), P (SLX4), Q (ERCC4) and S (BRCA1)] [1, 5, 6, 9-18] and other unknowns define a multicomponent FA pathway involved in cellular responses to DNA damage and replication.

Sequence database of homologs in different species reveal that FANC2 may determine a highly conserved and central function of a cellular signaling pathway, evolving into a fine-tuning multiple-player one in humans. FANC2 may function either in upstream, downstream or independent of the multi-FA protein complex [19]. So far, the crucial roles of FANC2 playing in the FA pathway are attracting more and more attention. The dysfunction of FANC2 derived from genetic mutation either hetero- or homozygosity has been detected in a variety of cancers [20-23] and concluded to be positively correlated with cancer development [24]. Herein, we review recent studies on the underlying mechanisms of
FANCL expression can represent the functional complexes that perform E3 ubiquitin ligase activity. This complex E3 ubiquitin ligase activity is important after examining the levels of FA complex proteins in cancer cells, we found that FANCL expression was at a low level. Importantly, FAVL impairment of cytoplasmic retention induced by FAVL, enhancing FANCL expression in the nucleus results from its salt bridge with FANCL [30]. With this unique E3-E2 interaction, FANCL's degradation [30] is required to work with replication initiators for the maintenance of a sufficient number of DNA replication origins to fire in time [44]. The loss of the basal level of monoubiquitinated FANCD2, e.g. the loss of this propitious origins to fire in time [44]. The loss of the basal level of monoubiquitinated FANCD2, e.g. the loss of this propitious origin firing, chromosomal abnormality, aging and cancer, selection, FANCD2 can only be mono- but not poly-ubiquitinated in response to DNA damage response.

Ubiquitin modulation for FANCD2 activation

The activation of the FA pathway has been well revealed by the findings that K561 of FANCD2 and K523 of FANCI are monoubiquitinated by the FA complex E3 ubiquitin ligase to form a heterodimer [25-27], which aggregate with the downstream proteins in nuclear foci to exert DNA crosslink and/or double DNA strand break (DSB) repair [28]. The current studies mainly focus on elucidating the modulation of FANCD2 monoubiquitination/activation.

In a defective FA pathway model of non-FA Calu-6 lung cancer cells, we found that FANCL expression was at a low level after examining the levels of FA complex proteins, which perform E3 ubiquitin ligase activity. This complex E3 is required for the monoubiquitination of FANCD2 or the activation of the FA pathway, indicating that the reduced FANCL expression can represent the functional heterozygosity of the FA pathway [29]. Besides FANCL, we also found a novel tumor promotion factor named "FAVL", meaning for a variant of FANCL, was highly expressed in Calu-6 lung cancer cells and in nearly 50% of 130 tested cancer tissue samples. Further, we revealed that a decreased FANCL expression in the nucleus results from its cytoplasmic retention induced by FAVL, enhancing FANCL’s degradation [30]. Importantly, FAVL impairment of the FA pathway promotes a growth advantage for cancer cells and their genome instability in vitro, and thus tumor development represented by a xenograft mouse model. This study, for the first time, indicates that the impaired FA pathway triggered by FAVL contributes to the development of cancers in patients without FA and therefore adds a new challenging layer of complexity to human tumorigenesis [6,30,31].

The biallelic mutation or deficiency of UBE2T, the primary E2 conjugating enzyme contributing to the activation of FA pathway/ the monoubiquitination of FANCD2 has been considered to be a new FA complementation group protein “FANCT”, and reported to cause FA subtype in vivo [32]. The defective UBE2T protein with mutations, such as Ala157Cys, Gln2Glu [32,33] or exon deletions [34] abolishes FANCD2 monoubiquitination and the formation of foci after MMC or other DNA-damage–agent treatment. But these cellular defects can be compensated by wild-type UBE2T overexpression. As one of E2 enzymes for interaction with FANCL, the interface of RING domain of FANCL binding to UBE2T extends longer hydrophobic surface to Tyr311 than other E3 enzymes, and only UBE2T has a specific Arg60 which has the positive charge to form a salt bridge with FANCL [35]. With this unique E3-E2 interaction, FANCD2 plays an anchor role similar to histone's, binding to DNA in the regulation of pol η function. Clearly, in the early response to DNA damage, FANCD2 plays an important role in the recruitment of DNA translesion synthesis enzyme, pol η, to the DNA damage sites for repair [42].

Although monoubiquitination of FANCD2 is essential for FA pathway’s activation and the downstream multistep complex recruitment (BRCA-related FA proteins), one recent study reported that this modification is not required for DNA repair. The complex of FANCD2, RAD51C and RAD18 can also support PCNA monoubiquitination and translesion synthesis in a FANCD2 monoubiquitination independent manner upon hydroxyurea treatment but not other DNA damage agents [43], suggesting an unorthodox function of FANCD2 in response to different types of DNA damage.

Genomic stability maintenance of FANCD2

The similar sensitivity to DNA crosslinking damage revealed from FA cells and yeast rad6-null cells (Rad6-/-) prompted us to explore the potential relationship between the FA and Human Homologs of yeast Rad 6 (HHR6) pathways, in which HHR6-hRad18 activates PCNA, in turn, regulating translesion DNA polymerases including pol η [36]. Now, it is understood that HHR6 and hRad18 (a HHR6 partner), can regulate FANCD2 monoubiquitination [37-39], which bridges the FA and HHR6 pathways in maintaining genome integrity [28,36]. Moreover, monoubiquitinated FANCD2 can modulate the activity of translesion DNA synthesis through binding to pol η at the regions that have been long known for interacting with PCNA [40,41]. Importantly, we found that the interaction of pol η with FANCD2 occurs earlier than that with PCNA in response to DNA damage. We also found the similar MMC sensitivity between cells transfected with either wtFANCD2 or histone-fused pol η [42], suggesting that monoubiquitinated FANCD2 may play an anchor role similar to histone’s, with PCNA 

demonstrated in FA. This finding provides a novel understanding of how FANCD2 functions under normal conditions of cell cycle to maintain genome stability, as well as resulting implications in the strategic improvement for the fight against human cancer. Taken together, FANCD2-centered FA signaling pathway plays a crucial role in the maintenance of genomic stability under both stress and non-stress conditions.

**Gain-of-function of impaired FA pathway in human cancer**

It is generally believed that FA genes lose their tumor suppressor roles as a consequence of encoded abnormal proteins. We discovered that ΔNP63 was not upregulated in FANCD2 null cells nor in wild type FANCD2-containing cells, instead, upregulated only in FANCD2-inactivated cells [45]. Further mechanistic studies indicate that this is, at least partly, resulting from the directly binding to the ΔNP63 promoter region by inactivated FANCD2/non-monoubiquitinated FANCD2. In general, inactivated FANCD2 loses its roles in DNA damage repair and DNA replication, which is not indicative of the up-regulation of any genes. It is very exciting that inactivated FANCD2 was found to possess the feature of "Gain of Function". Therefore, the tumorigenicity resulting from impaired FA signaling/inactivated-FANCD2 can be attributed to the gain-of-function, adding a novel layer of complexity in our understanding of the FA tumor-suppressor signaling pathway.

**FA pathway-targeted agents in cancer therapy**

A considerable cases of cancers arise along with an abnormal FA pathway [24], but a certain percentage of cancer cases with a proficient FA pathway, which conceptually provides a tumor-killing barrier to chemo- and radiotherapy, given its capability in DNA damage repair/maintenance of genome stability. Thus, inhibition of this pathway may sensitize the tumor cells to DNA damage caused by chemoradiotherapies. Besides FAVL compromising the FA pathway [30, 31], some small molecular drugs were also reported recently to reduce the activity of the FA pathway. For instance, PJ34 [46], celastrol [47], mTOR kinase inhibitor [48], Nedd8 conjugation system inhibitor [49], DDN [50], phenylbutyrate [51] and monoketone analogs of curcumin [52] are all against FANCD2 monoubiquitination. Mitoxantrone can promotes the activity of USP11 [53] and inhibit FANCF [54], and both also compromise FANCD2 monoubiquitination. Further, bortezomib can interfere with FA/BRCA1 focus formation [55] and thus sensitize tumor cells. From these recent studies, many chemical substances developed are aimed at FANCD2 monoubiquitination as a tumor target.

**Perspective**

The suggestion that the FA pathway is associated with neoplasm in general population was brought up for more than 40 years [56], and it only recently emerged to be an intensive field of cancer research upon our studies and many of others [1, 5, 9-12, 14-17, 30, 31]. The deficient FA pathway promotes the tumorigenesis by not only facilitating genome instability but also the gain of new roles in the induction of additional oncogenic factors to promote tumor cell growth. In contrast, the normal FA pathway protecting tumor cells from the DNA-damage therapy needs to be suppressed. The abundant function of FANCD2 and other FA genes are under intensively studied. Those studies give a rise of more questions on the roles of the downstream FA protein-associated complexes in facilitating DNA repair and potentially novel effects on tumor suppression and cancer treatment. The protein structures and functional domains of these FA protein associated complexes need to be clarified for the principle of a unique platform proposed/suggested widely to support DNA repair. Moreover, the molecular regulatory network of the FA pathway need to be elucidated for the basis of dynamical responses to DNA damage [57] or during DNA replication [58] in conjunction with proliferation/detention [59], inflammatory reaction [60] and/or apoptosis [61]. Finally, the genetic and epigenetic profiles of FA genes in population for hotspot motifs seemingly need to be completely annotated in the hope of aiding the early cancer diagnosis, and its treatment and prognosis. We are facing a long journey to unveil the complexity of the FA signaling pathway in oncology.

**Conflicting interests**

The authors have declared that no competing interests exist.

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**References**


