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Chapter

The Function of FEN1 is Regulated by Post-Translational Modification

Zhenxing Wu, Xiaofen Mo, Chengbo Lang and Jinjing Luo

Abstract

Flap endonuclease 1 (FEN1) is a multifunctional DNA branching nuclease. Post-translational modifications (PTMs) exist in this protein widely, including phosphorylation, methylation, acetylation, ubiquitination and small ubiquitination modification (SUMO). Here, we make a summary for those PTMs studies on FEN1, to illustrate relationships between mutations of those amino acids and their functions alteration of FEN1. Numerous evidences have confirmed that dysfunction of FEN1 would lead to genome instability, and then induce a variety of chromosome-related diseases ultimately, including tumors. On one hand, interaction partner also stimulates FEN1 nuclease activity, to further ensure an effective role in the processing of different DNA structures; on the other hand, PTMs may regulate protein-protein interactions and FEN1's cellular localization.

Keywords: FEN1, post-translational modification, mutation

1. Introduction

Flap endonuclease 1 (FEN1) is one of the member of the 5′ nuclease superfamily with specific structure [1]. It could participate in Okazaki fragment maturation, removal of RNA primers in delayed strand replication, maintenance of telomeres, long base excision repair etc [2–8]. Therefore, we believe that the nuclease activity of FEN1 is to maintain genome stability, and it is a necessary condition for normal cell cycle progression. However, if FEN1 is activated on inappropriate time, it would result in interrupting the cell cycle and then cause DNA damage. Therefore, function of FEN1 must be performed at a precise location within a suitable protein complex in appropriate time. Dysregulation of FEN1 activity may lead to destruction of genetic information encoded in DNA and disrupt cell cycle. Studies have found that the expression of endogenous FEN1 increases in the G1 phase, reaches a peak in the S phase, and then drops sharply in the subsequent G2/M phase [9]. A large number of experiments have shown that FEN1 could undergo methylation [10], phosphorylation [11], SUMOylation [12, 13] and ubiquitination, a series of post-translational modifications (PTMs), finally FEN1 may degrade through the proteasome pathway in the G2/M phase [14].

2. PTMs of FEN1

2.1 Methylation and phosphorylation of FEN1

In order to complete lagging strand DNA synthesis, mammals need to efficiently and accurately process up to 50 million Okazaki fragments in each cell cycle, and researchers have found that FEN1 plays an important role [15, 16]. Henneke et al. found that methylation and phosphorylation are important signals for the binding and dissociation of FEN1 and proliferating cell nuclear antigen (PCNA) replicas [11, 17]. Methylation promotes FEN1 binding to PCNA. Interacting with PCNA, Pol-δ, replication protein A and DNA ligase I, FEN1 removes RNA primers during DNA replication [18], and then be phosphorylated, resulting in disassociation from DNA complex and entering the next step [19].

2.2 SUMO3 modification of phosphorylated FEN1

After completing the DNA synthesis of the lagging strand, phosphorylated FEN1 is dissociated from the DNA complex [19]. Hietakangas et al. and Mohideen et al. [20, 21] speculated that it was modified by SUMO3, and they used SUMO3 to measure the SUMOylation for phosphorylated FEN1 *in vitro*, and compared it with the unphosphorylated FEN1. Results showed that SUMOylation efficiency of phosphorylated FEN1 was significantly higher than that of unphosphorylated. Guo et al. co-transfected HeLa cells with a plasmid encoding His-SUMO3 and WT FEN1, S187A mutant (phosphorylated deficient) or S187D mutant (sustained phosphorylated) for FEN1's phosphorylation study [19]. Compared with WT FEN1, S187A prevented FEN1 from undergoing SUMOylation, while S187D was the opposite. Henneke et al. treated the cells with Olomoucine, SUMO3 modification of WT FEN1 and S187D FEN1 was inhibited significantly [20]. Those indicated that SUMO3 modification of FEN1 is regulated by the phosphorylation of FEN1 at S187 (**Figure 1A**).

2.3 Degradation of FEN1 mediated by SUMO3

Small ubiquitin-related modifier (SUMO) molecule is a newly discovered ubiquitin-like molecule, which participates in protein PTMs and plays an important role [22]. Mammals have SUMO1, SUMO2 and SUMO3, these 3 paralogs passed through 2 different enzymes, SUMO activating enzyme E1 and E2 [23, 24]. To test whether FEN1 degradation is related to SUMOylation or not, SUMO1, 2 and 3 overexpressing level for FEN1 was determined. It has been found that overexpression of SUMO3 led to a sharply decrease in FEN1, which indicated that SUMO3 modification is related to FEN1 degradation. SUMO3 modification site on FEN1 was identified as lysine 168 (K168). To confirm this, mutated K168R and WT FEN1 were subjected to SUMOylation *in vitro*. WT was SUMOylated, while K168R was not (**Figure 1A**). Then it was found that c-Myc-WT FEN1 was SUMOylated in cells, while SUMOylation of K168R was not. These showed that K168 is the main SUMO3 modification site of FEN1. Further research found that K168R resists its degradation of FEN1 in G2/M phase, further confirming that degradation of FEN1 requires SUMO3 modification at K168.

2.4 UBE1/UBE2M/PRP19 complex ubiquitinated FEN1

Ubiquitination is a universal signal pathway for ubiquitin-proteasome degradation for proteins [25]. After its ubiquitinated, FEN1 could be recognized as a

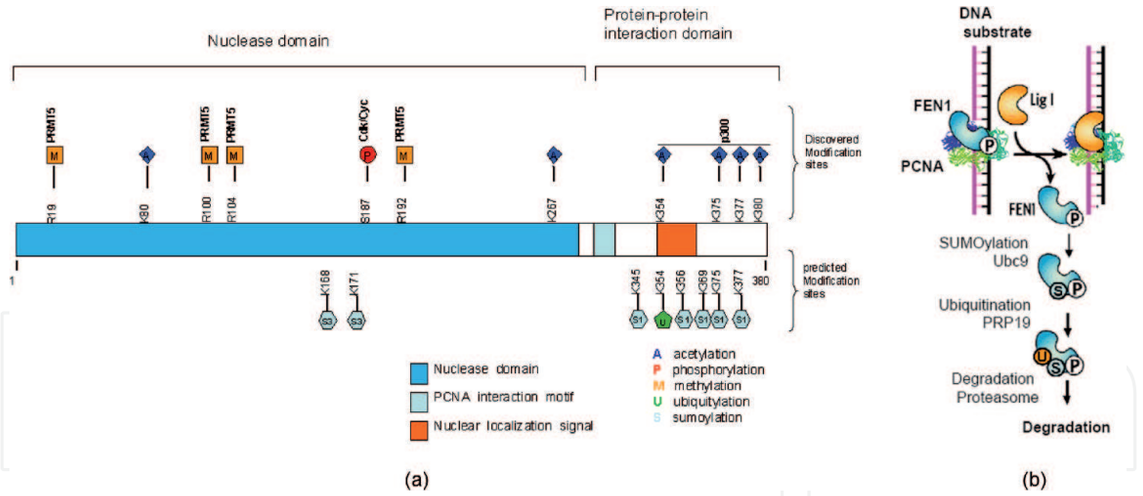

Figure 1.
(a) PTMs programmed mutation sites. Up is discovered modification sites. Down is predicted modification sites. A stands for acetylation; P stands for phosphorylation; M stands for methylation; U stands for ubiquitination; S stands for SUMOylation. (b) Model of sequential modifications to degrade FEN1. FEN1 is phosphorylated in late S phase, then dissociation from PCNA and the DNA replication fork. Once released from the DNA replication fork, phosphorylated FEN1 is then SUMOylated, which triggers ubiquitination by PRP19 and its degradation ultimately.

degradation target. Researchers determined that FEN1 is ubiquitinated *in vitro* and *in vivo*. Mass spectrometry showed that mutation of K354R abolished the ubiquitination of FEN1 (**Figure 1A**), which confirmed K354 is the ubiquitination site indeed. Ubiquitination is similar to SUMOylation, both processes involve sequential reaction steps mediated by a set of enzymes [25, 26]. Only E1 (SAE1/SAE2) and E2 (Ubc9) have been found for SUMOylation. However, it was reported that there are dozens of proteins involved in ubiquitination [27], and 138 interacting proteins then have been identified, including UBE1 [28], UBE2M [29], PRP19 [30] and UBQLN4 [31], which have been previously determined to play a role in ubiquitination pathway. It was reported that UBE1 has E1 ubiquitin-activation activity, UBE2M has E2 ubiquitin-binding activity, PRP19 has E3 ligase-activity, and UBLNQ4 may play a role in de-ubiquitination. It has also been found that the consumption of UBQLN4 had no effect on its ubiquitination, while consumption of UBE1 and UBE2M eliminated its ubiquitination, while consumption of PRP19 showed that FEN1's ubiquitination was significantly reduced. Purified UBE1, UBE2 and PRP19 were also tested, indicating that they could lead to FEN1's ubiquitination *in vitro*.

2.5 FEN1 is degraded through the ubiquitin-proteasome pathway during G2/M

It has been showed that endogenous FEN1's expression increased during G1 phase, reached a peak in S phase, and then dropped sharply in G2/M phase, which indicated that FEN1 was differentially regulated during cell cycle progression. MG132 (Carbobenzoxyl-leucinyl-leucinyl- leucinal), the proteasome inhibitor, was used for study. Results showed that the level of FEN1 in G2/M phase increased, while the level in S phase did not change significantly, suggesting that degradation is regulated by the proteasome pathway (**Figure 1B**) [32].

2.6 Acetylation of FEN1

Hasan et al. found that the transcriptional co-activator P300 histone acetyltransferase could acetylate Lys354, Lys375, Lys377 and Lys380 residues of FEN1 [33].

Acetylation of FEN1 reduced the DNA binding ability and nuclease activity significantly, and then inhibited the interaction between FEN1 and 9-1-1 complex (Rad9/Rad1/Hus1 cell cycle checkpoint complex) [34], however, binding ability to PCNA was not affected [35]. Lys80, Lys267, Lys375, have been found as 3 acetylating sites [36]. Strangely, Lys80 and Lys267 were not found in previous studies, and functions of these new sites were still unclear. New acetylation sites, such as Lysl25, Lys252, Lys254 and Lys314, have also been identified then [37]. It showed that acetylation levels of K125A and K252/K254A mutants decreased significantly, indicating that these sites were key sites for FEN1's acetylation. In addition, the proportion of G1 phase for K125A and K252/K254A increased significantly. K252/K254A mutant cells were more sensitive to UV induced DNA damage [37]. These indicated that acetylating modification of those sites plays a role in maintaining the normal physiological state of cells and their genomic stability.

2.7 Succinylation of FEN1

It has been found some succinylation modifications sites in FEN1 [38]. Lys200, Lys354 overlapped with some of identified acetylation sites, indicating that both were succinylation and acetylation modification sites for FEN1. As succinylation of lysine is more complex than acetylation, this modification may induce greater changes in chemical properties, structure and function of FEN1 [39]. Acetylation and succinylation of lysine are very important in gene transcription, cell metabolism, and DNA damage response. In addition, overlap of these modification sites for acetylation and succinylation indicated that there may be some complementary, synergistic or antagonistic effect between those modifications.

3. Summary

PTMs occur sequentially in the degradation process of FEN1, and each PTM is necessary for the next reaction. The absence or inhibition of any step would prevent FEN1 from degrading successfully. Researchers have also found that overexpression of WT FEN1 caused 25% cells closed to tetraploidy, while 65% in K354A; just only 5% in parental HeLa cells. It could be concluded that overexpression of FEN1 makes the chromosomal number in disorder. High FEN1 levels were usually observed in cancer cells. In addition, FEN1's overexpression has been observed in a variety of cancers, which is a sign for poor prognosis.

Sequential PTMs of FEN1 in the cell cycle process, is the separation of methylated FEN1 from PCNA, first undergoes phosphorylation, though demethylation reaction after methylated FEN1 has still not been illustrated. Phosphorylation induces FEN1 to undergo SUMO3 modification, and SUMO3 modification further stimulates ubiquitination. Finally, FEN1 is degraded through the proteasome pathway. Any residues of these PTMs have been mutated, degradation of FEN1 would be hindered. Therefore, there must be a precise regulation mechanism to ensure that FEN1 could function at suitable time. Numerous evidences have confirmed that the dysfunction of FEN1 would lead to instability of the genome, and induce a variety of chromosome-related diseases ultimately, including tumors. Interaction partners stimulate nuclease activity to further ensure an effective role of FEN1 in the processing of DNA structures, and PTMs also may regulate protein-protein interactions and FEN1's localization in cells. In the future, it is believed that more mechanisms of FEN1 PTMs' function would be discovered, to understand cancers development better, and it also would benefit mankind clinically.

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