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Review

# Human Exonuclease 1 (EXO1) Regulatory Functions in DNA Replication with Putative Roles in Cancer

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**Abstract:** Human exonuclease 1 (EXO1), a 5'→3' exonuclease, contributes to the regulation of the cell cycle checkpoints, replication fork maintenance, and post replicative DNA repair pathways. These processes are required for the resolution of stalled or blocked DNA replication that can lead to replication stress and potential collapse of the replication fork. Failure to restart the DNA replication process can result in double-strand breaks, cell-cycle arrest, cell death, or cellular transformation. In this review, we summarize the involvement of EXO1 in the replication, DNA repair pathways, cell cycle checkpoints, and the link between EXO1 and cancer.

**Keywords:** DNA repair; double strand break repair; exonuclease 1; EXO1; mismatch repair; MMR; NER; nucleotide excision repair; strand displacements; TLS; translesion DNA synthesis

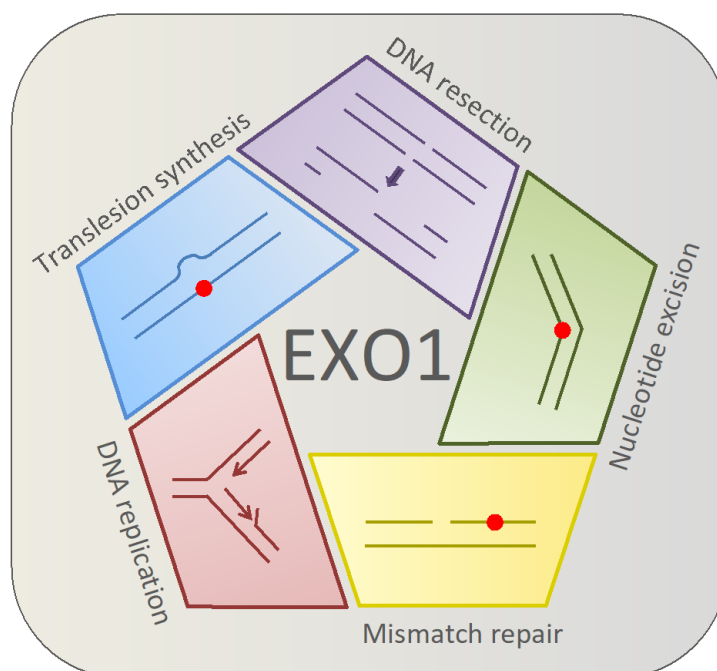
## 1. Introduction

Human exonuclease 1 (EXO1) contributes to checkpoint progression and to several DNA repair pathways involved in reducing DNA replication stress, for example, in mismatch repair (MMR), translesion DNA synthesis (TLS), nucleotide excision repair (NER), double-strand break repair (DSBR), and checkpoint activation to restart stalled DNA forks [1–6]. The multifarious and crucial roles of EXO1 in these DNA repair pathways are summarized in Figure 1.

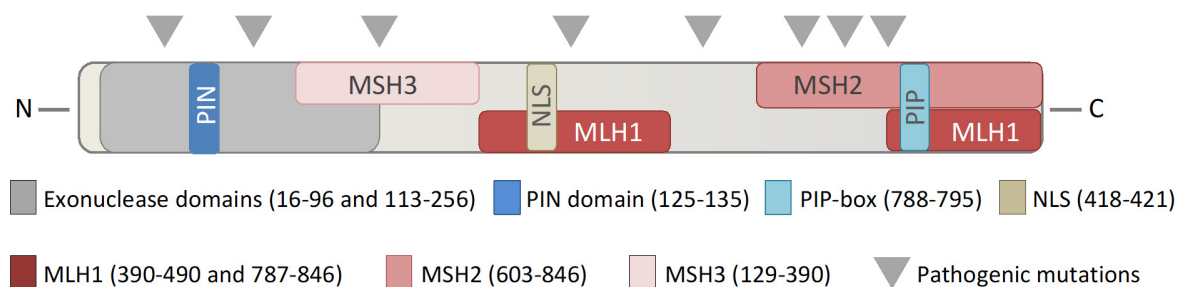
EXO1 is a member of the Rad2/XPG family of nucleases [7], and contains an active domain, located at the N-terminal region of the protein (Figure 2). The EXO1 transcript has 5'→3' exonuclease activity, as well as 5' structure specific DNA endonuclease activity and 5'→3' RNase H activity [7,8]. EXO1 has a high affinity for processing double stranded DNA (dsDNA), DNA nicks, gaps, and DNA fork structures, and is involved in resolving double Holliday junctions [9–12]. During DNA replication in the S-phase of the cell cycle, a polymerase can incorporate a mismatched DNA base or encounter secondary DNA structures, which can stall the replication fork and lead to replication stress. The collapse of a replication fork can have severe consequences, and failure to restart a stalled fork may lead to double-strand breaks, chromosomal rearrangement, cell-cycle arrest, cell death, or malignant transformation [13,14].

The contribution of EXO1 in the safeguarding stability of the genome during DNA replicative and post-replicative processes is well-established. EXO1 activity contributes to several DNA repair processes; however, it is not clear if the absence or malfunction of EXO1 can contribute to cancer

development. We will herein examine the putative wider roles of EXO1 as a guardian of our genome and investigate its possible role in cancer progression and initiation.



**Figure 1.** Human EXO1 participates in both replicative and post-replicative processes. In the replicative process, EXO1 contributes to DNA replication by assisting in the removal of mismatches, bypassing the lesion using translesion synthesis, or by assisting with nucleotide excision repair by activating the NER repair pathway. EXO1 also has a role in DNA resection during the process of homologous recombination.



**Figure 2.** Interaction domains in EXO1. Schematic overview of the relevant interaction domains in the human EXO1 protein, denoting interaction domains with mismatch repair proteins MSH3, MLH1, MSH2, and other significant interaction regions, including with PARP1, PCNA, and the nuclear localization signal (NLS).

## 2. DNA Replication

Enzymes able to metabolize DNA are required for modulating DNA replication. EXO1 is intricately involved in this process both as an enzyme involved in replication and in DNA repair pathways such as homologous recombination, but it is also an essential enzyme in the replication process, such as DNA strand displacement. Strand displacement describes the removal of single stranded RNA or DNA from an RNA:DNA or DNA:DNA duplex, a process required for multiple essential cellular processes, such as DNA replication and DNA repair. Accordingly, flap structure-specific endonuclease 1 (FEN1), EXO1, and polymerase  $\delta$  are the main factors in primer removal and Okazaki fragment maturation at the lagging strand in the process of strand displacement during replication [8,15–19]. In yeast, EXO1 can substitute for RAD27 (FEN1 is the human homolog) in

RNA primer removal [11]. Indeed, in vitro assays suggest that 5' flaps (<5 nt) generated by polymerase  $\delta$  during replication are efficiently removed by FEN1 or EXO1 [9,11,15,16]. The 3'-exonuclease activity of polymerase  $\delta$  avoids excessive strand displacement [19]. Deletion of POL32 (third subunit of polymerase  $\delta$ ) can suppress the lethality of growth defects of RAD27 and polymerase  $\delta$  D520V mutants in yeast (defective for RAD27 and the 3'→5' exonuclease of polymerase  $\delta$ ) [20]. In support of this observation, synthetic lethality is seen in yeast *exo1 $\Delta$* , *rad27 $\Delta$*  double knockout cells [17–19,21]. This suggests significant overlap in the functionality of these enzymes. Accordingly, both human FEN1 and EXO1 have weak flap activity at long 5' flap overhangs (5–20 nucleotides), but efficiently remove mono- or dinucleotide overhangs [8,11,15]. Further, both EXO1 and FEN1 have been demonstrated to have RNA and DNA displacement activity in vitro [8,11,15,21]. In addition, in biochemical assays, it was demonstrated that the human RecQL helicases, RECQ1 and WRN, physically and functionally interact with human EXO1 and increase its exo- and endonucleolytic incision activities catalyzed by EXO1 [22,23]. Both RecQL helicases efficiently unwind the 5' flap DNA substrate [22,23], which is a critical intermediate that arises during the DNA strand displacement process. Therefore, the combined helicase and physical interaction of EXO1 with RECQL1 or WRN may play an important role in the enhancement of DNA strand displacement, such as that occurring during lagging strand DNA synthesis at the replication fork, or during the DNA repair (for example, long patch base excision repair) that also potentially leads to strand displacement. These findings highlight the role of EXO1 in DNA replication and underscore the need for a multitude of enzymatic processes required for human DNA synthesis. Longer DNA flaps with more than 25 nucleotides are processed in the presence of RPA, FEN1, and helicase partner with either the ATP-dependent helicase Petite Integration Frequency 1 (PIF1) or DNA replication helicase/nuclease 2 (DNA2) in vitro [24–28]. However, it was recently demonstrated that DNA2 and RPA can process long flaps independent of RAD27 in yeast [29,30]. In vitro data suggest that POL32 has no effect on the generation of short flaps. Notably, longer flaps only accumulate in the presence of POL32, indicating that polymerase  $\delta$  and FEN1 team up in short flap removal. The role of EXO1 in the removal of long DNA flaps of more than 25 nucleotides has not yet been extensively studied [9,11]. It is possible that EXO1 could potentially act as a back-up to FEN1 during circumstances of cellular stress. However, it has to be taken into account that the actual contribution of EXO1 in humans remains understudied and there is much scope for further work in this area.

### 3. Mismatch Repair

High-fidelity DNA replication is required to maintain an unaltered genetic code during cell division. The MMR pathway is a post-replicative DNA repair system, which mainly corrects DNA polymerase slippage and damaged bases, such as chemically-induced base adducts; base mismatches; and base insertions, deletions, and loops. The MMR pathway consists of several steps, which are detailed below. The initial recognition step of eukaryotic MMR utilizes the MutS $\alpha$  complex made up of mutS homolog 2 (MSH2) and mutS homolog 6 (MSH6) or MutS $\beta$  complex (MSH2 and mutS homolog 3 (MSH3)). The MutS $\alpha$  mainly recognizes single base mismatches, while the MutS $\beta$  complex detects larger lesions, insertion/deletions, or loops [31–33]. The MutS $\alpha$  or  $\beta$  complex operates by binding to the DNA mismatched base or DNA distortion. Following the initial DNA distortion recognition, the MutL $\alpha$  complex (heterodimer of MLH1/Postmeiotic Segregation Increased 2 (PMS2)), proliferating cell nuclear antigen (PCNA), and replication factor C (RFC) are recruited. MutS $\alpha$  or MutS $\beta$  forms a tetrameric complex with MutL $\alpha$  at the site of the replication error. In the presence of PCNA and RFC, the MutL $\alpha$  nicks the DNA at 3' or 5' to the lesion by use of the intrinsic endonuclease activity in PMS2. EXO1's contribution to the MMR was identified in fission yeast (*Schizosaccharomyces pombe*) after it was co-purified with mismatch repair factor MSH2 [2]. EXO1 is the only known nuclease active in the MMR pathway by interacting with the mismatch repair factors mutL homolog 1 (MLH1), MSH2, MSH3, and PCNA (Table 1) [34–42]. EXO1 is recruited to excise the newly synthesized DNA containing the replication error in a MutS $\alpha$  or  $\beta$ , and in a MutL $\alpha$ -dependent manner. Additional factors, such as

replication protein A (RPA), guide the resection of the single stranded DNA (ssDNA) intermediates during the DNA repair process to avoid the formation of secondary DNA structures or excessive DNA degradation [43]. The repair reaction is completed by the joint activities of the PCNA and DNA polymerase  $\delta/\epsilon$  resynthesizing the DNA, and DNA ligase I sealing the nick [44]. Malfunction of MMR is associated with increased microsatellite instability (MSI), a hallmark of certain types of colon cancer, such as hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch Syndrome (Online Mendelian Inheritance in Man (OMIM) #120435) [45–47].

More recently, it was shown that MMR occurs in the absence of EXO1 [48,49], suggesting that a proportion of MMR is EXO1-independent and relies on either strand displacement or involvement of other helicases or nucleases. Indeed, several members of the RecQL family of helicases have been proposed to be involved in MMR. The WRN helicase/exonuclease interacts with MutL1 $\alpha$ , MutS $\alpha$ , MutS $\beta$ , and RPA. However, only MutS $\alpha$ , MutS $\beta$ , and RPA stimulate the DNA helicase activity of WRN on naked DNA [50–52]. Interestingly, it is reported that in some cases, cells from patients with Werner Syndrome (OMIM#277700) show a malfunction in the MMR [32,53–55]. Nonsense mutations in the BLM gene lead to Bloom Syndrome disease (OMIM#210900). Some Bloom Syndrome cases show immunodeficiency and increased MSI [56]. Furthermore, the RECQL helicases, RECQL1 and BLM, physically interact with MutL $\alpha$ , MutS $\alpha$ , and RPA [23,57–60]. Only MutS $\alpha$  and RPA enhance the helicase activity of RECQ1 and BLM [23,58–61]. However, the above is in contrast to *in vitro* assays with human cell extracts of BLM<sup>-/-</sup> and WRN<sup>-/-</sup> that show no defective MMR [62,63]. Altogether, this suggests that the RECQL helicase has some stimulatory role in the MMR pathway, but does not have a significant contribution in the absence of EXO1. Nonetheless, deficiency in the MMR pathway in human cell lines in the absence of helicases WRN or BLM in combination with the depletion of EXO1 has not been reported. In addition, some nucleases have been suggested to back up MMR in the absence of EXO1, including the MRE11 homolog A (MRE11) and FAN1 (FANCD2/FANCI-Associated Nuclease 1) [64]. The contribution of MRE11 to the MMR pathway and to MSI has recently been reviewed [32]. A recent study showed that overexpression of the human polymerase  $\delta$  D316A;E318A mutant resulted in mild MMR deficiency [65]. *In vitro* experiments with cell extracts show that the overexpression or addition of human EXO1 protein complements the mild mutator phenotype of polymerase  $\delta$  D316A;E318A, indicating that EXO1 can provide backup to polymerase  $\delta$  in its MMR activity [65]. It has been suggested that the polymerase  $\delta$  strand displacement activity may indeed depend on the endo-nuclease activity of MutL $\alpha$  in the absence of EXO1 [66]; however, the mechanism is so far unknown. While the role of EXO1 in MMR is well-established, EXO1-independent MMR in eukaryotic cells is still not understood.

#### 4. Translesion DNA Synthesis

Translesion DNA synthesis (TLS) describes the process by which a DNA polymerase can synthesize a DNA strand across a lesion on the template strand. This process is critical to maintaining functional DNA replication in the face of genotoxic stress and may act as a pathway to cope with ultra violet (UV) induced DNA damage [3]. Indeed, in human cell lines, it was demonstrated that EXO1 recruits the TLS DNA polymerases  $\kappa$  and  $\iota$  to sites of UV damage [3]. Interestingly, an inactivating mutation in the aspartate at position 173 to alanine in EXO1 (EXO1-D173A) results in an inability to recruit the TLS polymerase  $\kappa/\iota$  to the damage site, suggesting an active role of EXO1 in TLS [3]. Notably, in yeast, the EXO1 mutant strain (FF447AA) shows defective MMR due to the loss of interaction with MLH1, but is still active in TLS [67]. However, it remains unclear if such an EXO1 variant can assist in UV-induced TLS in mammals. In addition, the yeast 9-1-1 complex (three distinct subunits complex of Ddc1, Mec3, and Rad17 in yeast and RAD9, HUS1, and RAD1 in humans) and EXO1 also contribute to an error-free TLS pathway in a PCNA monoubiquitylation manner that makes use of undamaged sister chromatids as templates for repair [68]. Overall, EXO1 appears to have an emerging role in TLS, requiring further investigation.

## 5. Nucleotide Excision Repair

UV radiation from sunlight mainly damages DNA by causing cyclobutane pyrimidine dimers, and 6–4 photoproducts, lesions typically repaired by the nucleotide excision repair pathway (NER) independent of replication [69]. However, during the S-phase of the cell cycle, UV radiation-induced base lesions block DNA replication. EXO1 belongs to the same family of nucleases as xeroderma pigmentosum complementation group G (XPG), a protein involved in NER. Accordingly, cells damaged by UV exposure and inhibited in translesion synthesis show an accumulation of EXO1 at the DNA damage sites [3]. Indeed, an additive UV-sensitivity effect is observed in yeast when both *rad2* (XPG homolog in human) and *exo1* are knocked out [69]. In addition, yeast EXO1 competes with the translesion synthesis pathways, and converts the NER intermediates to long ssDNA gaps, leading to checkpoint activation [4]. In human cell lines, EXO1 enlarges ssDNA gaps to stretch over 30 nucleotides long to activate the ATR checkpoint [70]. The contribution of EXO1 to NER is likely limited to enlarging the DNA gaps that occur as part of NER leading to checkpoint activation; although this is not well-understood.

## 6. Homologous Recombination and DNA End Resection

Homologous recombination (HR) is an essential process involved in the repair of double strand DNA breaks, mainly in the S and G2-phases of the cell cycle. A possible piece of evidence suggesting the involvement of EXO1 in double strand DNA repair is the observation that *Exo1<sup>null/null</sup>* mice show an increase in chromosomal breaks and base substitution, and predominately develop lymphomas [71]. In addition, human cell lines depleted in EXO1 exert chromosomal instability and demonstrate a hypersensitivity to ionizing radiation (IR), a hallmark of cells defective in homologous recombination [5]. This provides support that EXO1 is required for the HR repair of DSBs in human cells. In contrast, yeast *exo1<sup>-/-</sup>* has no significant defect in recombinational repair, with only minor defects in DNA end processing [16,18,19,72]. Data also suggests that EXO1 is involved in DNA damage signaling upon replication fork stalling [73]. The 5'→3' DNA resection of DSB ends to produce a 3' single stranded DNA overhang is a critical step in the repair of DSBs by HR [74]. In mouse embryonic fibroblasts (MEF), *Exo1<sup>null/null</sup>* cells showed a defect in the DNA damage response [71]. Treatment of *Exo1<sup>null/null</sup>* cells with the topoisomerase inhibitor camptothecin, which creates single strand breaks (SSB) that ultimately lead to DSB during the S-phase, results in a reduction in phosphorylated RPA (pRPA) foci at the DSBs [71]. Recruitment of pRPA is regulated by DNA damage response protein-kinases, such as ataxia telangiectasia mutated (ATM) and ataxia telangiectasia mutated and Rad3 related (ATR) [71]. PARP1, a factor involved in DSB repair, physically interacts with EXO1 at the PAR interaction motif (PIN) at the N-terminus of EXO1 [75,76] and stimulates EXO1 in its 5' excision activity in an in vitro MMR assay [77]. Poly (ADP-Ribose) Polymerase 1 (PARP1) promotes PAR-mediated polyADP-ribosylation (PARylation) recruitment to the DNA damage site, followed by additional DNA repair factors [75,76]. The EXO1-R93G variant, mutated in its PIN domain, is poorly recruited to damaged DNA [76]. This suggests that PARP1 is potentially essential in the early recruitment of EXO1. The interplay between the MRE11-RAD50-NBS1 (MNR)-complex and EXO1 is well-documented [78–81] and deletion in *Mre11*, *Rad50*, or *Nbs1* genes has been shown to be lethal in mice [82]. Mice that carry a hypomorphic allele of *Nbs1* (*Nbs1<sup>ΔB/ΔB</sup>*) are viable, but show severe developmental impairment, embryonic death, and chromosomal instability when *Exo1* is lost [82]. The *Nbs1<sup>ΔB/ΔB</sup>* MEFs depleted in EXO1 strongly influenced DNA replication, DNA repair, checkpoint signaling, and the DNA damage response [82].

The single-stranded DNA binding protein RPA has a central role in DNA replication, DNA repair, recombination, and DNA resection [83]. DNA resection after double strand DNA breaks is proposed to occur via two different routes. In the RPA-BLM-DNA2-MRN mediated route, RPA stimulates DNA unwinding by the DNA helicase BLM in a 5'→3' direction, leading to the formation of single stranded DNA that can be resected by the nuclease DNA2 [79]. The other resection route is mediated by EXO1 and is stimulated by BLM, MRN, and RPA [79]. Indeed, yeast depleted in RPA and loss of *Mre11*

eliminates both SGS1-DNA2 mediated and EXO1-dependent resection pathways [43], suggesting that RPA and MRN are essential for resection. DNA-resection by EXO1 is probably inhibited by the DNA binders RPA, Ku70/80, and/or C-terminal-binding protein interacting protein (CtIP) (the yeast homolog is SAE2) [43,81,84–86]. Accordingly, in nonhomologous end joining, the Ku70/80 heterodimer protects the DNA in a complex with DNA-PKcs for DNA end resection [86–88]. Therefore, EXO1 has a limited role in this pathway. In contrast, EXO1 likely collaborates in an alternative end joining pathway with the WRN in trimming the DNA ends [89–91]. EXO1 interacts with WRN and enhances the exonuclease activity of EXO1 by the C-terminal region of WRN. Biochemical assays suggest that WRN and EXO1 function in replication stress, where WRN enhances EXO1 in processing stalled forks or regressed replication forks [92]. More recently, it was shown that the WRN exonuclease activity prevents unscheduled degradation by MRE11 and EXO1 during replication re-start [93]. Human cells depleted in WRN show an enhanced degradation of the nascent DNA strand by MRE11 and EXO1 after camptothecin treatment [93]. In summary, EXO1 is required for homologous recombination, while it is less essential for nonhomologous end joining.

## 7. Cell Cycle Regulation

Several lines of evidence suggest that EXO1 may be a central regulator of the cell cycle. For example, in S-phase, EXO1 co-localizes with MMR protein MSH2 and cell cycle regulator PCNA [39]. In humans, EXO1 interacts physically with PCNA via the PCNA-interacting protein (PIP box) motif located in the C-terminal region of EXO1 [40,41,94]. Indeed, PCNA stimulates the exonuclease activity of EXO1 on dsDNA substrates [95].

Further evidence for a regulatory function of EXO1 in the cell cycle comes from yeast, where the absence of cell cycle regulator 14-3-3 leads to checkpoint defects [96]. In humans, EXO1 physically interacts with six of the seven 14-3-3 isoforms and is stimulated by isoform 14-3-3 $\eta$  and 14-3-3 $\sigma$  in its exonuclease activity in vitro [96]. The EXO1-dependent resection pathway is restrained by 14-3-3 $\sigma$ , thereby counteracting EXO1 stimulation by PCNA [97,98]. In addition to the 14-3-3 complex, the 9-1-1 complex functions on the crossroads between checkpoint activation and DNA repair, and stimulates DNA resection of yeast EXO1 [99,100]. In total, EXO1 physically and functionally interacts with multiple central proteins involved in cell-cycle regulation and is therefore likely to be important in these processes.

**Table 1.** EXO1 interactor proteins in humans and yeast. Significant interaction partners of EXO1 in humans and yeast during different cellular processes.

Repair Process	EXO1 Interaction Proteins in Human	Reference	EXO1 Interaction Proteins in Yeast	Reference
Mismatch repair	MSH2	[36,38]	MSH2	[2,34]
	MSH3	[32,33]	MSH3	[72]
	MLH1	[38,41]	MLH1	[72]
	PCNA	[40]		
Homologous recombination /DNA replication/DNA end resection	PARP1	[75,76]		
	BLM	[57,79]		
	WRN	[22]	SGS1	[74]
	RECQ1	[23]	SAE2	[74]
	CTIP	[85]		
Cell cycle regulation	PCNA	[40,41,95]	9-1-1	[99,100]
	14-3-3 $\eta$	[97,98]	14-3-3	[96]
	14-3-3 $\sigma$	[97,98]		

## 8. Link to Cancer

EXO1 has been associated with different types of cancers, including Lynch Syndrome, breast, ovarian, lung, pancreatic, and gastric tract cancer (see Table 2) [101–117]. Lynch Syndrome is commonly caused by mutations in the MLH1 and MSH2 genes in humans that give rise to almost two-thirds of all Lynch Syndrome cases [45,118]. A hallmark of MMR deficiency in MSH2<sup>-/-</sup> and MLH1<sup>-/-</sup> cells is the

presence of MSI, leading to increased chromosomal instability, which is believed to be the underlying molecular driver of tumor formation in Lynch syndrome [21,45,118]. Several studies have been conducted on single-nucleotide polymorphisms (SNP) in EXO1 related to MSI in tumors in humans; however, it remains inconclusive if EXO1 defects contribute to MSI. However, in genomic-wide association studies (GWAS), specific mutations in EXO1 have been identified as risk alleles for the development of multiple types of cancer [112,116]. Notably, at least some of these mutations can lead to the loss of protein function. For example, the A153V and N279S mutations are located in the active nuclease domain (as highlighted in both Table 2, and shown graphically in Figure 2) and are likely related to the malfunction of the nuclease activity of EXO1. Other mutations in EXO1, including T439M, E670G, and P757L, are located in the MLH1 and MSH2 binding domains (Figure 2). One of the most studied mutations is the E109K, which was suggested to be dysfunctional in the nuclease domain [71,101]. However, biochemistry studies revealed that EXO1 E109K is functional in its nuclease activity [119,120]. The mutation is located in the EXO1 PAR-binding motif, and therefore potentially not recruited to sites of DNA damage [76]. The clinical data is supported by mouse models, where the loss of *Exo1* leads to an increased incidence of lymphomas, but interestingly not to increased MSI [71]. Pathogenic mutations in both introns, exons and the untranslated regions of EXO1 have been described [112]. Nevertheless, the overexpression of EXO1 has also been reported in several other cancers, which in part is related to increased DNA repair activity [121–124]. However, EXO1 is in general expressed at low levels, independent of the cell-cycle progression or proliferative status of the cell, and increased levels of EXO1 are harmful and lead to genomic instability [6]. Several other nucleases including FEN1 and MRE11 have also been demonstrated to have elevated levels of expression in tumors [125–127]. Clearly, the connection between EXO1 and cancer has been established and could represent a druggable target in cancers where the EXO1 protein is overexpressed.



**Table 2.** Mutations in EXO1 in relation to different cancers. Represents the most commonly reported point mutations in EXO1 in relation to different cancer types. Abbreviations: CRC- colorectal cancer, IC- cancer of the small intestine, BC- breast cancer, PC- pancreatic cancer, GC- gastric cancer, LC- lung cancer, HCC- hepatocellular carcinoma, OC- oral cancer, and CC- cervical cancer.

Mutations in EXO1 Region	Corresponding DNA Sequence Mutation	Reported SNP	Coding and Non-Coding Region	Type of Cancer/Remark	Reference
p.E109K	c.326A>G	rs756251971	exon	CRC	[101]
p.A153V	c.458C>G	rs143955774	exon	CRC, IC Combined with pole c.1373A>T, p.Y458F	[102]
p.N279S	c.836A>G	rs4149909	exon	BC, PC	[103,104]
p.T439M	c.1317G>A	rs4149963	exon	CRC	[105]
p.E589K	c.1765G>A	rs1047840	exon	GC, LC, HCC, Melanoma, Glioblastoma	[106–113]
p.E670G	c.2009A>G	rs1776148	exon	GC, BC, OC, LC, Melanoma, Glioblastoma	[106–109,111–113]
p.R723G/p.R723S	c.2167C>A/c.2167C>T	rs1635498	exon	GC, BC, OC, LC	[107–109,111,112]
p.P757L	c.2270C>T	rs9350	exon	CRC, PC, GC, OC, LC, BC, Melanoma	[105,107–109,111–114]
Non coding region	c.2212-1G>C	rs4150000	Intron, splicing variant	PC	[115]
		rs72755295	Intron, splicing variant		[116]
		rs1776177	UTR region	GC, BC, OC, LC	[107–109,111]
		rs1635517	UTR region	GC, BC, OC, LC	[107–109,111]
		rs3754093	UTR region	GC, BC, OC, LC	[107–109,111]
		rs851797	UTR region	GC, BC, OC, LC	[107–109,111,112,117]
		c.C-908G	rs10802996	UTR region	CC, GC, BC, OC, LC

## 9. Conclusions and Perspectives

Evidently, EXO1 is a central player in DNA metabolic processes. As elucidated herein, EXO1 contributes to several DNA repair pathways, which safeguard DNA replication, including MMR, TLS, HR, and cell cycle regulation (Figure 2). Replication fork collapse and checkpoint failure during DNA replication can lead to chromosomal instability or abnormal DNA repair, leading to translocation, transformation, and cell death, all processes where EXO1 has been implicated.

Nonetheless, several questions remain to be answered. For example, given the putative central role of EXO1, it remains a mystery why the knockout of EXO1 in mice, as well as loss of function, leads to a relatively mild phenotype. Further, the mechanism of EXO1-independent MMR is still unclear, particularly regarding at what point this specific pathway is active. Given the biochemical activity of EXO1, it is possible that an unknown helicase or exonuclease can contribute to MMR repair in the absence of EXO1. DNA polymerase  $\delta$  is a strong candidate, as well as the helicases BLM and/or WRN with minor contributions [50–54,56–58,65]. However, unknown contributors with a more prominent role in MMR may still remain to be discovered.

EXO1 gene variants have been associated with different types of cancers. Interestingly, large GWAS analyses support that specific mutations in domains required for interaction with other

proteins in EXO1 are more commonly occurring in particular types of cancer, as summarized in Table 2 [107–112,116]. The central role of EXO1 in replication and post-replication processes, including checkpoint activation, suggests that EXO1 dysfunction could alter other DNA repair pathways, leading to replication stress followed by genomic instability and the development of cancer. Deregulation of EXO1 protein levels in tumors is commonly reported [121,122]. Furthermore, EXO1 has been addressed as a candidate gene in cancer therapeutics through its increased expression in tumors [123]. Given the large number of processes that involve EXO1, it is not surprising that EXO1 has emerged as a critical protein in cancer research. Nevertheless, several enigmas remain and the EXO1 field is fertile for future explorations.

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