Is hEXO1 a Cancer Predisposing Gene?

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Introduction

Changes in the efficiency of DNA repair and recombination activities can cause predisposition to cancer. The finding that hereditary nonpolyposis colorectal cancer (HNPPC) families frequently harbor mutations in DNA mismatch repair (MMR) genes has generated widespread interest in this research area (1-5). At the time of writing, germ line mutations in at least four genes, hMSH2, hMSH6, hMLH1, and hPMS2, all homologues of bacterial components involved in MMR, have been found in HNPPC patients (6). However, because some HNPPC families fail to display mutations in the known MMR genes, changes in other components of the MMR pathway may be responsible. One such new candidate gene is the human exonuclease 1 (hEXO1). The exact functional and biological roles of hEXO1 remain unclear, but this exonuclease has been implicated in DNA replication, recombination, and repair. The recent discovery that hEXO1 plays a role in MMR and the identification of germ line mutations in hEXO1 in patients with atypical HNPPC suggest a possible nexus between hEXO1 mutations and HNPPC. hEXO1 may also be linked to carcinogenesis through its role in recombination events such as repair of DNA double-strand breaks (DSB) and telomere stabilization.

Exonuclease 1

The hEXO1 gene is located at 1q42-43 and consists of 14 exons that yield a ~3 kb transcript (7-9). Two different forms of hEXO1 exist (HEX1/hEXO1a and hEXO1b) that arise from alternatively spliced mRNA transcripts, involving only the COOH-terminal content outside the nuclease domain of the protein. Thus far, functional differences between the two splice variants have not been observed. EXO1 belongs to class III of the RAD2 family of endonucleases and exonucleases and is generally described as a 5'-3' exonuclease 1 (9-12). Recent work suggests that it also plays a role in 3'-5' exonucleolytic degradation of DNA (12). The strong sequence homologies within the RAD2 family are restricted to two discrete regions, designated the N (NH2-terminal) and I (internal) regions, which comprise the catalytic domain responsible for nuclease activities. The RAD2 class III subfamily consists of EXO1-like enzymes reported in yeast, fly, and mammals and have been ascribed roles in DNA repair, replication, and recombination (7-12, 14-16). The RAD2 domain of hEXO1 exhibits a 5'-3' nuclease activity on both ssDNA and dsDNA substrates. The specific 5'-3' exonuclease activity is higher on blunt-end and 5' recessed dsDNA substrates compared with duplex DNA containing a 5' overhang or ssDNA (12). The exonuclease activity of hEXO1 at nicked and gapped duplex DNA substrates is similar or slightly higher than that observed for blunt-end duplexes. hEXO1 does not discriminate between RNA and DNA substrates in vitro (17). Whereas hEXO1 clearly possesses a 5'-3' activity, its involvement in 3' nick-directed repair suggests that the enzyme has a cryptic 3'-5' activity or that it is necessary for activation of a distinct activity that is responsible for excision in the 3'-5' direction (13). In addition to an exonucleolytic function, hEXO1 has a flap structure specific endonuclease activity analogous to FEN1 (12). hEXO1 does not incise endonucleolytically at bubble-like structures, G/T mismatches, or uracil residues but degrades these duplex substrates exonucleolytically (12). Both 5'-3' exonuclease and flap exonuclease activities require a divalent metal cofactor, with Mg2+ being the preferred metal ion (12). hEXO1 has been shown to display a RNase H activity (17). This observation indicates that the enzyme may also be involved in DNA replication, in agreement with studies in yeast suggesting a backup function of EXO1 to FEN1 in RNA primer removal. Overexpression of yeast EXO1 or hEXO1 protein complements the defects of RAD27 (FEN1) mutants, suggesting that these two RAD2 family members have analogous functions (8, 11, 17). Moreover, a study of yeast mutants shows that EXO1 RAD27 double mutants are nonviable, unlike the corresponding single mutants. The lethality could be caused by an inability to remove primer RNA during lagging strand DNA synthesis (11). Localization of hEXO1 is restricted to the nucleus and it colocalizes with proliferating cell nuclear antigen (PCNA; refs. 18, 19). The colocalization of hEXO1 and PCNA during S phase suggests that this enzyme could be involved in events associated with DNA replication, perhaps as a backup to FEN1 in the removal of RNA primers, similar to that proposed for the yeast orthologues. However, the colocalization of hEXO1 and PCNA during S phase suggests that this enzyme could be involved in events associated with DNA replication, perhaps as a backup to FEN1 in the removal of RNA primers, similar to that proposed for the yeast orthologues. However, the colocalization of hEXO1 and PCNA during S phase suggests that this enzyme could be involved in events associated with DNA replication, perhaps as a backup to FEN1 in the removal of RNA primers, similar to that proposed for the yeast orthologues. However, the colocalization of hEXO1 and PCNA during S phase suggests that this enzyme could be involved in events associated with DNA replication, perhaps as a backup to FEN1 in the removal of RNA primers, similar to that proposed for the yeast orthologues.
polymerase proofreading activity (22). The MMR system is responsible for maintaining the genomic integrity, and the activity of the repair system lowers the spontaneous mutation frequency up to a 1,000-fold (23). The mutator phenotype of EXO1-deficient yeast is not as significant as that observed in MSH2 strains, but the mutation spectrum suggests a MMR defect (11, 24-26). In bacteria, redundant exonucleases have been shown to be active in MMR (27-29), and the fact that EXO1-deficient yeast strains are weak mutators indicate that alternate activities may support the excision step in this lower eukaryote as well. To date, hEXO1 is the only exonuclease identified as part of the human MMR system and it is believed to play an essential role by functioning both as a 5′-3′ and as a 3′-5′ nuclease as well as contributing to the overall stability of the MMR complex (13, 30-34).

Regulation of Exonuclease Activity in DNA MMR
The yeast and the hEXO1 have been shown to interact with MMR proteins (7, 11, 18, 34-36). Both hMSH2 and hMLH1 bind to the COOH-terminal domain of hEXO1, whereas hMSH3 interacts with a hEXO1 domain in the NH2-terminal part of the protein. Furthermore, experimental evidence suggests the formation of a ternary complex between hEXO1-hMLH1-hPMS2 (hEXO1-hMutLo; ref. 19) as well as between hEXO1-hMSH2-hMSH6 (hEXO1-hMutSo; ref. 37).

The influence of MMR proteins on the activity and function of hEXO1 has been studied. It was shown that hMutSo activates the 5′-3′ activity of hEXO1 in a mismatch-dependent manner, an effect that is best explained by protein-protein interactions on a heteroduplex (13). Furthermore, the hEXO1 activity on heteroduplex DNA is significantly enhanced in the presence of hMutLo (13). Whereas hMutSo is sufficient for hEXO1 activation on substrates nicked 5′ to a mismatch, both hMutSo and hMutLo are required for excision on substrates containing a nick 3′ to the mismatch (5′-3′ nuclease activity; ref. 13). hMutSo and hMutLo not only activate excision on a G/T heteroduplex but also suppress hEXO1 action on homoduplex DNA (19, 30). The observation that hEXO1 is also required for excision directed by a strand break located 3′ to the mismatch (13) indicates that the 5′-3′ hydrolytic activity of hEXO1 must be regulated in a manner that depends on the relative orientation of strand signal and mismatch. This implies the existence of additional factors that regulate hEXO1 activity when mismatch-provoked excision is directed by a 3′ strand break. Such factors have not yet been identified but could include PCNA, replication factor A, and ATP, which have been shown to have significant impact on the efficiency and regulation of excision in eukaryotic MMR (30).

Genetic Recombination
DSBs are a very disruptive form of DNA damage and, if left unrepaired, can lead to broken chromosomes and cell death. On the other hand, if repaired improperly, DSBs can lead to chromosome translocation and cancer (38). Two independent pathways carry out repair of DSBs: homologous recombination and nonhomologous DNA end joining. EXO1 does not seem to be involved in repair of DSBs through nonhomologous DNA end joining (39-42) but functions via the homologous recombination pathway (42-44).

Genetic recombination is involved in several different cellular processes, such as meiotic recombination, repair of DSBs, antibody generation, and telomere maintenance (45). EXO1 is involved in recombinational events, although the exact function of EXO1 during recombination remains uncertain. Proposed activities include generation of 5′ resected ends needed for strand exchange (10, 15, 46), resolution of DNA intermediates formed during recombination (12), and repair of mismatches in heteroduplex DNA that lead to gene conversion events (25, 47). Furthermore, hEXO1 interacts with the Werner syndrome protein, and this interaction stimulates the nucleolytic activities of hEXO1 (48). EXO1 has also been proposed a role in meiotic recombination (24, 44, 46, 47, 49), and transcription of the gene encoding EXO1 is highest in cell types and tissues associated with meiosis (8, 9, 14, 16, 36). Along these lines, it was shown that both male and female Exo1 mutant mice are sterile presumably because of a meiotic defect (49).

Gene Expression of EXO1 in Mammalian Tissues
hEXO1 is highly expressed in fetal tissues of liver and thymus, whereas expression in adult liver, spleen, and kidney has not been detected, indicating a role for hEXO1 in development of these tissues (9, 36). The high expression of hEXO1 in fetal liver and adult bone marrow, areas of active hematopoiesis, suggests that hEXO1 could act in DNA metabolic processes that occur during differentiation of various stem cell lineages (9, 36). This hypothesis is supported by results demonstrating that transcription factors Sp1/Sp3 and cAMP-responsive element binding protein-1 are required for hEXO1-promoter activity (50). The high expression of hEXO1 in thymus and bone marrow also indicates that this exonuclease might participate in V(D)J rearrangement and the generation of antibody diversity. Experimental evidence to support this hypothesis is provided by studies of Exo1 mutant mice (51), which display decreased class-switch recombination and changes in the characteristics of somatic hypermutation similar to those observed in Msh2 mutant mice (51, 52).

Telomeres
Telomeres have important roles in cellular processes including chromatin organization and control of cell proliferation (53-55). The common pathway for telomere maintenance in human cells involves the ribonucleoprotein telomerase (53, 56), but alternative mechanisms for lengthening of telomeres, which is dependent on homologous recombination, are functional in human cells under selective pressure. Such alternative mechanisms are detectable in a small percentage of tumors, including bone and soft tissue sarcomas, glioblastomas, and carcinomas of the lung, kidney, breast, and ovary (57-60). A role for EXO1 has been proposed in end resection at telomeres (61-63), and deletion of EXO1 reduced the mutation rate in telomerase deficient cells, suggesting that EXO1 contributes to the end resection that generates terminal deletions in cells with dysfunctional telomeres (64). The observation that EXO1 promotes genetic instability in telomerase-deficient cells might have interesting implications for carcinogenesis and emphasizes...
the importance of cellular mechanisms, which regulate exonuclease activity such as inhibition of hEXO1 activity on homoduplex DNA by the hMutSα and hMutLα MMR complexes.

Cancer

Inactivation of the human MMR pathway has been implicated in onset of both hereditary and sporadic cancers (23, 65, 66). HNPCC is caused by mutations in MMR genes, and HNPCC individuals are heterozygous for a MMR gene mutation. An acquired somatic mutation in or chromosomal loss of the wild-type allele (loss of heterozygosity) is believed to promote tumorigenesis as a consequence of MMR deficiency, increasing the likelihood of inactivation of key regulatory genes involved in growth suppression, apoptosis, or signal transduction (67). Estimated incidences of HNPCC vary between 0.5% and 13% of all colorectal cancers (CRC; refs. 68-70) and are characterized by an early onset of malignancy (age <50 years) and high penetrance (71). Furthermore, HNPCC individuals have an increased frequency of certain extracolorectal malignancies in the endometrium, uterus, ovary, breast, and small intestine (72). The International Collaborative Group on HNPCC has defined criteria to provide a basis for uniformity of the diagnosis for HNPCC, known as the Amsterdam criteria (73, 74). Families that fulfill several but not all of these criteria are defined as having atypical HNPCC. Mutations in hMSH2 and hMLH1 account for the majority of clinically defined HNPCC mutations, whereas mutations in hMSH6 and hPMS2 are rare. Germ line mutations in these genes have been identified in only a proportion of families with atypical HNPCC (75, 76). In a significant number of the cases, mutations in known MMR genes have not been identified, which suggests that essential components in the MMR pathway remain to be identified (70, 77).

hEXO1 Mutations in HNPCC Patients

Microsatellite instability (MSI) often indicates that a tumor has developed as a result of MMR deficiency, and the majority of sporadic MSI cancers are caused by inactivation of hMLH1 by promoter hypermethylation rather than somatic mutations or loss of heterozygosity (70, 78). Because hEXO1 is involved in MMR, it is expected that hEXO1 mutations contribute to HNPCC and/or sporadic CRC. Germ line variants of hEXO1 were detected in a proportion of patients with atypical HNPCC but were absent from >200 healthy control individuals, suggesting a role for hEXO1 in CRC. All hEXO1 variants were identified in families in which no germ line hMLH1, hMSH2, hMSH6, or hMLH1 mutations had been detected and all tumor samples displayed various degrees of MSI, suggesting a MMR compromised background (79). The identification of both high and low MSI tumors with hEXO1 mutations indicates a difference in the pathogenic background between the various individuals carrying potential hEXO1 mutations. Eight of the germ line variants identified by Wu et al. (79) were examined using functional assays. It was shown that the variants E109K and L410R were defective in exonuclease activity, whereas P640S, G759E, and P770L showed reduced ability to interact with hMSH2 (80). Therefore, the pathogenic effect of certain hEXO1 variants could be explained by reduced MMR activity. However, another study reported that five of nine hEXO1 variants identified by Wu et al. (79) were detected in both CRC and control individuals with equal frequency, indicating that these are naturally occurring polymorphisms unrelated to cancer predispositions (81). Interestingly, four hEXO1 variants, V27A, E109K, L410R, and P770L, were only detected in samples from CRC patients and not in control individuals. Based on these results, the authors concluded that hEXO1 variants either do not predispose to HNPCC or account for a very small proportion of such cases (81).

EXO1-Deficient Mice

Knockout mice provide a useful model system to study the function of individual mammalian genes and to model a range of human inherited disorders. The Exo1 mutant mice are characterized by reduced survival, increased susceptibility to the development of lymphomas, and higher mutation rates, although the mutation frequency is lower than in Msh2-deficient mice cells (49, 82). Furthermore, Exo1 mutant cells show elevated MSI at mononucleotide repeats and incomplete in vitro MMR of base-base mismatches and single-base insertion/deletion loops but normal repair of dinucleotide insertion/deletion loops (49). Comparable observations come from Msh6 mutant mice, which develop lymphomas or epithelial tumors of the skin and uterus (82). Extracts from Msh6-deficient cells are defective in repair of base-base mismatches but are capable of repairing insertion/deletion loops (83). Germ line mutations in hMSH6 have been identified at a high incidence in patients with atypical HNPCC, and these cases are characterized by a late onset of cancer as well as low rates of MSI in the tumors (84, 85). In addition, hMSH6 mutations have been linked to a high incidence of endometrial cancers and incomplete penetrance compared with classic HNPCC (86). It is therefore possible that a similar pathology may explain the relation between hEXO1 mutations and HNPCC resulting in late onset of disease, deviating tumor spectrum, and/or incomplete penetrance. If so, a significant number of individuals carrying hEXO1 mutations would not be recognized as HNPCC but instead diagnosed as sporadic cancer. Furthermore, it is possible that the weak mutator phenotype of hEXO1 mutations is only pathogenic in combination with other mutator alleles. This multimutation hypothesis is substantiated by studies in yeast, demonstrating that the weak mutator phenotype of EXO1 mutant cells becomes robust when combined with other weak mutator alleles in genes encoding MLH1, PMS1, MSH2, PCNA (POL30), or DNA polymerase δ (POL3; refs. 26, 33). Interestingly, tumor samples from two HNPCC families with the same hEXO1 mutation (L410R) showed different MSI status (79). This difference in phenotypes may well reflect the presence of unidentified secondary mutations that in combination with the hEXO1-L410R mutation cause cancer progression in these patients. Along these lines, the hEXO1-L410R mutation has been identified in an individual from a suspected HNPCC family carrying a missense mutation in hMSH2 of unknown pathology (81).

The contribution of mutations in hEXO1 to cancer in a set of 21 families that fulfill the diagnostic criteria for HNPCC was recently examined (87). These families carried no mutations in...
hMSH2 or hMLH1, but hEXO1 had four changes that were identified previously and one that is a novel mutation (79, 81, 87). The new missense mutation identified was not considered causative because it was observed in three unrelated patients and did not involve a highly conserved amino acid. Based on these observations, it was concluded that hEXO1 is not linked to HNPCC (87). However, the transgenic mouse model system suggests that mutations in hEXO1 are likely linked to late onset of CRC or atypical HNPCC. All families included in the study by Thompson et al. (87) fulfill the Amsterdam criteria for HNPCC. The selection of families based on these criteria would exclude patients carrying pathogenic hEXO1 mutations, because these would not be expected to fulfill the Amsterdam criteria if indeed hEXO1 is linked to late onset CRC or atypical HNPCC.

Polymorphisms in the hEXO1 gene, which are not recognized as actual mutations, may have relevance for the development of sporadic cancers. It can be speculated that certain gene variations in combination with individual genetic backgrounds may promote carcinogenesis at an advanced state of life. This phenomenon is known to apply for some weak mutations in hMLH1, which result in a late onset of cancers that are diagnosed as sporadic cancers and characterized by a high MSI status (78). One way to explain the multimutation hypothesis is to invoke that hEXO1 physically interacts with other MMR proteins to stabilize the repair complex. Destabilization of this complex and the resulting onset of cancer will depend on the combination and the nature of hEXO1 and other MMR gene variants.

Is hEXO1 an Oncogene?

The surprising observation that 12 of 13 tumors examined from atypical HNPPC patients with hEXO1 mutations had lost the mutant allele and retained the normal one (79) suggests that a functional active hEXO1 provides an advantage for cancer progression. This speculation agrees with expression analysis demonstrating that hEXO1 is expressed in some human cancer tissues but not in the corresponding nonneoplastic tissues (36). Overexpression of hEXO1 might increase genetic instability either by changing the MMR activity or by affecting recombinational events at telomeres or at DSBs. The MMR proteins have been shown to regulate the nuclease activity of hEXO1, and any change in the interaction between hEXO1 and other MMR proteins may result in genetic instability. This may occur if the nuclease is not inhibited from acting on homoduplex DNA during MMR. The hEXO1 could also cause genetic instability through its involvement in recombinational events, promoting carcinogenesis by increasing chromosome rearrangements, amplifications, or deletions. Recently, nine individuals from two British families with skin and uterine leiomyomata were analyzed for tumor MSI status and family history of HNPCC spectrum cancers (88). Leiomyomata is a benign condition resulting from heterozygous germ line deletions of 1q42.3-q43 encompassing several genes including hEXO1. None of these tumors displayed MSI, and none of the nine individuals from the two families had any personal or family history of HNPCC spectrum cancers (88). These results indicate that hEXO1 deficiency does not confer a genetic advantage.
instability phenotype, nor does it contribute to the development of HNPPC. However, if hEXO1 provides a growth advantage to neoplastic cells, hEXO1 deficiency might actually delay the development of cancer.

Summary

The link between hEXO1 and cancer is not obvious, and the literature reveals contradicting and ambiguous results. However, two different hypotheses might explain these contradicting conclusions (Fig. 1). Germ line mutations in hEXO1 are likely to result in a weak mutator phenotype and may predispose the carriers to develop cancer at an advanced age and to possibly display phenotypes not associated with classic HNPPC. Such affected individuals would be diagnosed with sporadic CRC. Alternatively, the weak mutator phenotype of hEXO1 mutations becomes robust and thereby pathogenic when combined with either strong or weak mutator alleles of other MMR genes. This multiamutation hypothesis for the pathology of hEXO1 mutations may explain the contradicting results described in the literature regarding the relation between hEXO1 and cancer. Finally, hEXO1 may be involved in carcinogenesis through pathways not directly associated with the MMR pathway. One such pathway could be genetic recombination; altered hEXO1 activity may cause genetic instability through perturbation of homologous recombination, repair of DSBs, and/or telomere resection.

References

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