DNA REPAIR 5 (2006) 347–361

available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/dnarepair

Truncation of the C-terminus of human MLH1 blocks intracellular stabilization of PMS2 and disrupts DNA mismatch repair

Azizah B. Mohd[a], *Brett Palama*[a,b], *Scott E. Nelson*[a], *Guy Tomer*[d,1], *Megan Nguyen*[d], *Xin Huo*[c], *Andrew B. Buermeyer*[a,*]

[a] *Department of Environmental and Molecular Toxicology, Oregon State University, 1007 Agricultural and Life Sciences Bldg., Corvallis, OR 97331, United States*

[b] *Department of Biochemistry and Biophysics, Oregon State University, OR, United States*

[c] *Program in Genetics, Oregon State University, OR, United States*

[d] *Department of Molecular and Medical Genetics, Oregon Health and Sciences University, Portland, OR, United States*

ARTICLE INFO

Article history:
Received 19 August 2005
Received in revised form 27 October 2005
Accepted 3 November 2005
Available online 9 December 2005

Keywords:
DNA mismatch repair
MLH1
PMS2
Heterodimer interaction

ABSTRACT

The human DNA mismatch repair (MMR) protein MLH1 has essential roles in the correction of replication errors and the activation of cell cycle checkpoints and cytotoxic responses to DNA damage that contribute to suppression of cancer risk. MLH1 functions as a heterodimer with the PMS2 protein, and steady state levels of PMS2 are very low in MLH1-deficient cells. Unique to MLH1 among MutL-homolog proteins, and conserved in identified eukaryotic MLH1 proteins, is the so-called C-terminal homology domain (CTH). The function of these C-terminal 20–30 amino acids is not known. We investigated the effect of a C-terminal truncation of human MLH1 (MLH1-L749X) on mammalian MMR by testing its activity in MLH1-deficient cells. We found the CTH to be essential for suppression of spontaneous mutation, activation of a cytotoxic response to 6-thioguanine, and maintenance of normal steady state levels of PMS2. Co-expression in doubly mutant *Mlh1*$^{-/-}$; *Pms2*$^{-/-}$ fibroblasts showed that MLH1-L749X was unable to stabilize PMS2. Over-expression of MLH1-L749X did not reduce stabilization of PMS2 mediated by wild-type MLH1, indicating that truncation of the CTH reduces the ability to compete with wild-type MLH1 for interaction with PMS2. Lack of PMS2 stabilization also was observed with a previously reported pathogenic truncation (MLH1-Y750X), but not with two different point mutations in the CTH. Biochemical assays demonstrated that truncation of the CTH reduced the stability of heterodimers, although MLH1-L749X retained significant capacity for interaction with PMS2. Thus, the CTH of human MLH1 is necessary for error correction, checkpoint signaling, and for promoting interaction with, and the stability of, PMS2. Analysis of the CTH role in stabilizing PMS2 was facilitated by a novel intracellular assay for MLH1–PMS2 interaction. This assay should prove useful for identifying additional amino acids in MLH1 and PMS2 necessary for interaction in cells, and for determining the functional consequences of *MLH1* mutations identified in human cancers.

* *Corresponding author*. Tel.: +1 541 737 8919; fax: +1 541 737 0497.
E-mail address: andrew.buermeyer@oregonstate.edu (A.B. Buermeyer).

[1] Present address: OMRIX Biopharmaceuticals Ltd., Tel Hashomer Hospital, Tel Aviv, Israel.

1568-7864/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2005.11.001

1. Introduction

DNA mismatch repair (MMR) contributes to the maintenance of genomic stability through the correction of replication errors the suppression of recombination between non-identical, but homologous sequences, and the activation of cell cycle arrest and apoptosis in response to DNA damage [8,22,37]. Defects in DNA mismatch repair can greatly increase cancer risk, exemplified by the autosomal dominant Lynch Syndrome (also known as hereditary non-polyposis colorectal cancer, HNPCC) [25,29] and an autosomal recessive childhood hematological malignancy [35,41,45]. Analyses of mice in which MMR genes are deleted [8,14] show the tumor suppressive functions of MMR proteins generally to be conserved in mammals.

MMR-dependent correction of replication errors and responses to DNA damage in eukaryotes requires heterodimeric complexes of the highly conserved MutS Homolog (MSH) and MutL Homolog (MLH) families. MutSα (MSH2/MSH6) or MutSβ (MSH2/MSH3) initially recognizes DNA mismatches [22,37], and MLH heterodimers, principally MutLα (MLH1–PMS2), link mismatch recognition to identification and subsequent excision of the nascent (error containing) DNA strand [22,37]. Although less is known about MMR-dependent responses to DNA damage, recognition and processing of mispairs resulting from replication bypass of damaged bases may result in persistent DNA strand breaks that can signal activation of cellular checkpoints [26,39]. Alternatively, signaling may be accomplished by direct interactions between MSH and MLH proteins and the proteins that regulate cell cycle progression [1,6,15,16,38,42,43]. Loss of either MLH1 or MSH2 completely inactivates the error correction and checkpoint-activation functions of MMR [8,14,37]. Correspondingly, the most common mutations identified in Lynch Syndrome are in *MSH2* or *MLH1* [25,29].

The MLH1 protein (Fig. 1A) can be divided approximately into a highly conserved amino-terminal domain (residues 1–350), necessary for ATP binding and hydrolysis, a linker domain (residues 350–500) that includes a nuclear localization sequence, and a carboxyl-terminal domain (residues 500–756), necessary for MLH1–PMS2 interaction [18]. Interactions mediated by the C-terminal domain additionally appear necessary for recognition of the nuclear localization sequences in MLH1 and PMS2 [4,46]. Furthermore, PMS2 protein abundance is greatly reduced in cells lacking MLH1 [7,9,11,13,47], suggesting that PMS2 might be stabilized by interaction with MLH1. Pathogenic mutations [12,30] along the length of the gene have been reported (Fig. 1A), including more than 30 single amino acid substitutions targeting the C-terminal domain. Predicting the disease risk associated with such mutations requires a thorough understanding of the structure and function of the C-terminal domain of MLH1, in particular the identification of sequences necessary for interaction with PMS2.

Apparently unique to MLH1 is the so-called carboxy terminal homology domain (CTH), identified in a comparison of

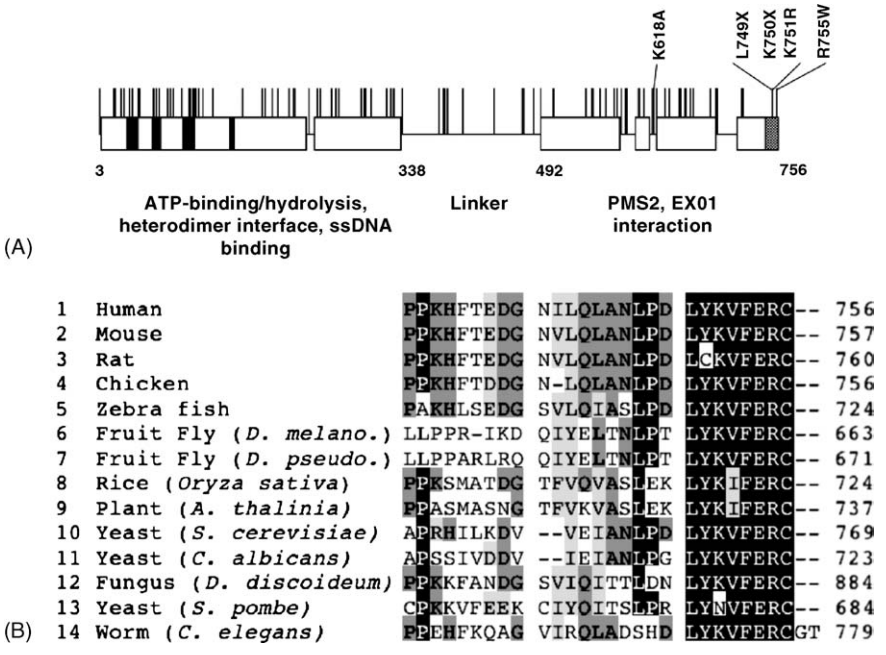

Fig. 1 – Conservation of functional domains of MLH1. (A) A schematic representation of human MLH1 N-terminal, linker and C-terminal domains (delineated approximately by the amino acid residues listed underneath the bar and drawn to scale) is presented. White boxes indicate primary sequence well conserved with yeast (*S. cerevisiae*) MLH1, black boxes the sequences necessary for ATP binding and hydrolysis, and the shaded box at the extreme C-terminus the CTH domain. Vertical lines indicate the location of single amino acid substitutions identified as pathogenic mutations [12,30]. Mutations of interest in the current study are labeled. (B) An alignment of the C-termimal sequences of 14 eukaryotic MLH1 proteins is presented. Shading highlights the degree of conservation within the CTH; residues identical in at least 10 of the listed sequences are shaded in black, residues identical in 7–10 sequences in dark grey and conserved residues in light grey. Numbering indicates the position of the last amino acid in each protein.