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Distinct functional consequences of MUTYH variants associated with colorectal cancer: Damaged DNA affinity, glycosylase activity and interaction with PCNA and Hus1

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ABSTRACT

MUTYH is a base excision repair (BER) enzyme that prevents mutations in DNA associated with 8-oxoguanine (OG) by catalyzing the removal of adenine from inappropriately formed OG:A base-pairs. Germline mutations in the *MUTYH* gene are linked to colorectal polyposis and a high risk of colorectal cancer, a syndrome referred to as MUTYH-associated polyposis (MAP). There are over 300 different *MUTYH* mutations associated with MAP and a large fraction of these gene changes code for missense MUTYH variants. Herein, the adenine glycosylase activity, mismatch recognition properties, and interaction with relevant protein partners of human MUTYH and five MAP variants (R295C, P281L, Q324H, P502L, and R520Q) were examined. P281L MUTYH was found to be severely compromised both in DNA binding and base excision activity, consistent with the location of this variation in the iron-sulfur cluster (FCL) DNA binding motif of MUTYH. Both R295C and R520Q MUTYH were found to have low fractions of active enzyme, compromised affinity for damaged DNA, and reduced rates for adenine excision. In contrast, both Q324H and P502L MUTYH function relatively similarly to WT MUTYH in both binding and glycosylase assays. However, P502L and R520Q exhibited reduced affinity for PCNA (proliferation cell nuclear antigen), consistent with their location in the PCNA-binding motif of MUTYH. Whereas, only Q324H, and not R295C, was found to have reduced affinity for Hus1 of the Rad9–Hus1–Rad1 complex, despite both being localized to the same region implicated for interaction with Hus1. These results underscore the diversity of functional consequences due to MUTYH variants that may impact the progression of MAP.

1. Introduction

Deleterious and mutagenic DNA damage can arise from reactions with reactive oxygen and nitrogen species (RONS) generated during inflammatory responses, and in response to genotoxic agents such as ionizing radiation, chemical mutagens, UV light, and chemotherapeutic drugs [1–3]. Arguably the most studied base oxidation product is 8-oxo-7,8-dihydro-2′-deoxyguanosine (OG) [1], which also serves as a key biomarker for oxidative stress [4,5]. In the absence of repair, OG results in high levels of G:C to T:A transversion mutations due to its ability to mimic T and direct for misincorporation of A to form stable OG:A mismatches [2]. Two base excision repair glycosylases, the human OG glycosylase (hOGG1) and the human MutY homologue (MUTYH) act synergistically to prevent mutations associated with OG in duplex DNA [1,6]. While hOGG1 initiates repair by removing the OG lesion when paired opposite C, MUTYH excises the undamaged A base when mispaired with OG [1]. Subsequent BER enzymes act sequentially at the base-less site to restore the appropriate nucleotide and preserve the originally coded G:C base pair [1,6].

MUTYH-associated polyposis (MAP) is a colorectal cancer predisposition mechanism that was discovered based on the correlation with biallelic inheritance of two germline mutations in *MUTYH* encoding the missense variants Y165C and G382D MUTYH [1,7–9]. Biallelic carriers of the two MAP alleles have increased G to T transversion mutations in the tumor

Abbreviations: 9#3–3#1, Rad9–Hus1–Rad1; araFA, 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine; AST, active site titration; bp, base-pair; BER, base excision repair; CRC, colorectal cancer; ds, double-stranded; Ec, *Escherichia coli*; EMSA, electrophoretic mobility shift assay; FCL, iron-sulfur cluster loop; GFP, green fluorescent protein; IDC, interdomain connector; MAP, MUTYH-associated polyposis; MBP, maltose-binding protein; MOI, multiplicity of infection; PCNA, proliferation cell nuclear antigen; SIFT, sorting intolerant from tolerant; STO, single turnover; TBE, tris-borate-EDTA; TBST, Tris-buffered saline + Tween; TEV, tobacco etch virus; THF, tetrahydrofuranabasic site analog.

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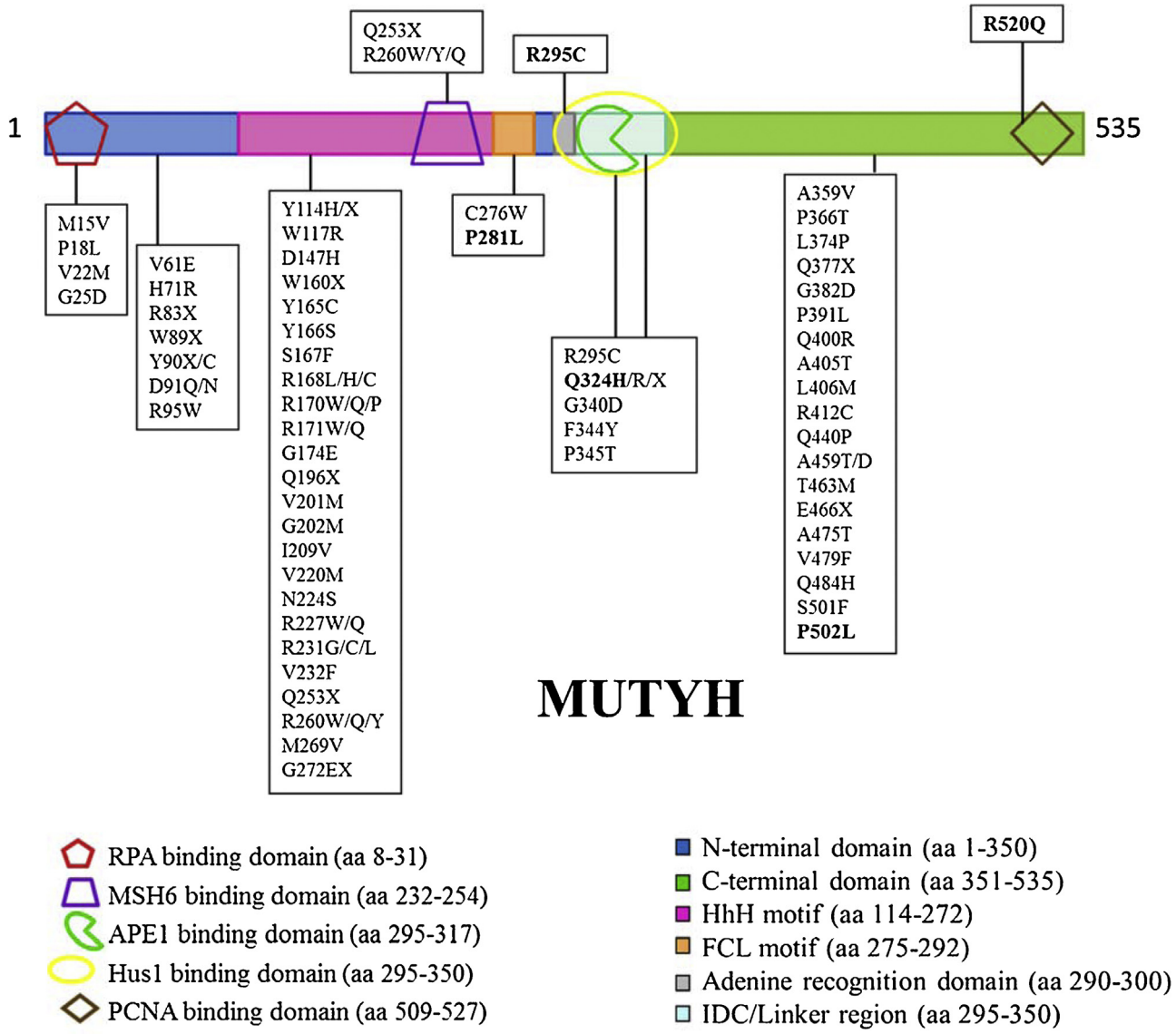

Fig. 1. MUTYH missense and nonsense mutations located in functional domains of the protein. Shown is the α3 transcript of the human MUTYH gene which encodes a 535 amino acid protein. The various binding partners of MUTYH are represented as shapes: replication protein A (RPA), red pentagon; mutS 6 homologue (MSH6), purple trapezoid; Hus1, yellow oval; AP endonuclease 1 (APE1), green pac man symbol; and proliferating cell nuclear antigen (PCNA), brown diamond.

suppressor genes *APC* and *K-ras* in somatic tissues consistent with dysfunctional OG:A repair activity [7,10]. Notably, adenine glycosylase activity and OG:A mismatch affinity are compromised for both variants and most dramatically for Y165C MUTYH [11–13]. Moreover, both variants have been shown to be defective in OG:A repair in a GFP-based cellular assay [12]. Structural and functional studies are consistent with the hypothesis that compromised OG:A repair activity of MUTYH variants is an initiating event leading to mutagenesis and eventual carcinogenesis in MAP [1,14,15].

Since the initial discovery of MAP, over 300 different sequence variants in the *MUTYH* gene have been reported in the Leiden Open Variant Database (LOVD) [16–18]. A significant fraction of these sequence changes code for missense variants of MUTYH [16]. Many MAP-associated variants are localized in the N-terminal catalytic base excision domain (near Y165C) or the C-terminal OG recognition domain (near G382D) (Fig. 1). In such instances, based on homology to the bacterial protein, reduced OG:A recognition or adenine glycosylase activity may be anticipated for some of these variants. However, there are also many *MUTYH* missense variations located within regions implicated in mediating interactions of MUTYH with protein partners (Fig. 1). Indeed, MUTYH variations are found in reported binding sites for AP endonuclease 1 (APE1) [19], proliferating cell nuclear antigen (PCNA) [19–21], MutS homologue 6 (MSH6) [22], replication protein A (RPA) [19], and the Rad9–Hus1–Rad1 (9–1–1) complex [23,24]. The correct associations between MUTYH and its various protein binding partners are necessary to ensure efficient BER of OG:A mismatches and coordination with other cellular processes.

MUTYH variations may compromise key interactions with protein partners, and this may result in more dramatically reduced levels of cellular OG:A repair than anticipated based on the enzyme activity analysis. Moreover, altered interactions with protein partners, such as the 9–1–1 complex, may erode proper signaling responses to DNA damage [25,26]. To provide further insight into how MUTYH variants located in different regions of the protein impact both the intrinsic enzymatic activity and interactions with relevant protein partners, we selected a group of variants for more detailed analysis in this study.

The P502L and R520Q MUTYH variants were selected due to the location of the modified amino acids in the PCNA binding region