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Replicative DNA polymerase mutations in cancer☆

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Three DNA polymerases — Pol α, Pol δ and Pol ε — are essential for DNA replication. After initiation of DNA synthesis by Pol α, Pol δ or Pol ε take over on the lagging and leading strand respectively. Pol δ and Pol ε perform the bulk of replication with very high fidelity, which is ensured by Watson–Crick base pairing and 3′exonuclease (proofreading) activity. Yeast models have shown that mutations in the exonuclease domain of Pol δ and Pol ε homologues can cause a mutator phenotype. Recently, we identified germline exonuclease domain mutations (EDMs) in human *POLD1* and *POLE* that predispose to 'polymerase proofreading associated polyposis' (PPAP), a disease characterised by multiple colorectal adenomas and carcinoma, with high penetrance and dominant inheritance. Moreover, somatic EDMs in *POLE* have also been found in sporadic colorectal and endometrial cancers. Tumors with EDMs are microsatellite stable and show an 'ultramutator' phenotype, with a dramatic increase in base substitutions.

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Introduction

DNA polymerases are responsible for synthesis of DNA and are essential for replication, DNA repair and genetic recombination. DNA replication is a highly complex process and in eukaryotes it involves multiple enzymes including the B family polymerases Pol α, Pol δ, and Pol ε [1,2]. These enzymes catalyse the polymerisation of deoxyribonucleotides into the nascent DNA strand. While Pol α initiates DNA synthesis, Pol δ and Pol ε replace Pol α after primer extension and perform the bulk of DNA replication. Most polymerases lack intrinsic error-checking activity, relying on Watson–Crick base pairing for their fidelity. However, the proofreading (exonuclease) domains of Pol δ and Pol ε ensure that these polymerases have a particularly low error rate, of the order of 10^{-7} substitution mutations per base. A variety of *in vitro* studies has shown that proofreading improves replication fidelity approximately 100-fold [3•,4].

The Pol δ and Pol ε enzymes are heterotetramers in higher eukaryotes. Both Pol δ and Pol ε comprise a catalytic subunit, POLD1 and POLE respectively, and accessory subunits (POLD2/3/4 and POLE2/3/4) that interact with cofactors such as Proliferating Cell Nuclear Antigen (PCNA) [5]. Both genes are ubiquitously expressed and show high levels of evolutionary conservation. The two polymerases differ from each other throughout most of their length, but are homologous (23% identity, 37% similarity) over their exonuclease domains (residues 268–471 of POLE and 304–517 of POLD1).

Based on studies in yeast, it has been shown that Pol δ and Pol ε usually replicate the leading and lagging strand respectively [6,7•]. However, it is still not fully elucidated whether this is always the case at replication forks. Pavlov proposed a model where Pol ε starts replicating the leading strand, but may later dissociate, and Pol δ then takes over to complete the replication [8]. A higher mutation rate in Pol δ exonuclease deficient yeast strains compared to Pol ε exonuclease-deficient strains endorses this hypothesis [8–10].

There is substantial evidence that in addition to DNA synthesis, Pol ε and Pol δ play essential roles in repair of chromosomal DNA [8,11,12]. Pol ε and Pol δ are thought to be involved in several repair pathway including nucleotide excision repair (NER), ismatch repair (MMR) and repair of double strand breaks (DSBR) [12,13].

Polymerase proofreading defects cause mutator phenotypes

Replication fidelity has been extensively studied in yeast and other microbes, though less is known about the impact of proofreading-defective DNA polymerase mutations in higher eukaryotes. The exonuclease domain catalyses the preferential hydrolysis of non-complementary nucleotides at the 3′-terminus, and in

yeast, inactivating missense EDMs of Pol ε and Pol δ cause a base substitution mutator phenotype with variable severity [9,10,14–17]. It has been suggested that in yeast, Pol ε and Pol δ proofread opposite strands at defined replication origins and may proofread for each other [6,18,19]. Data from mice with homozygous germline *Pole* and/or *Pold1* mutations at the exonuclease active site were shown to have distinct, but overlapping tissue-specific tumor phenotypes. *Pole*-mutant animals predominantly had nodal lymphomas and histiocytic sarcomas, whereas *Pold1* mutants had thymic lymphomas and skin papillomas/sarcomas. Both types of mice had intestinal adenomas (more in *Pole*) and lung tumors (more in *Pold1*). Double knockout animals died early from thymic lymphoma. Spontaneous mutations frequencies were higher in *Pole* mutants than *Pold1* mutants [20••]. One explanation could be that the fidelity of lagging strand replication is greater than that of leading strand, because post-replicative DNA mismatch repair (MMR) preferentially corrects lagging strand replication errors [21,22]. However, this in contrast with the data from yeast [14]. Genetic studies in proofreading-deficient, haploid yeast strains which also carried a MMR-defect showed a synthetically lethal phenotype indicating a synergistic effect on the mutation rate of proofreading and MMR [23,24]. This was also confirmed in mouse studies where loss of both proofreading and MMR led to embryonic lethality [20••,25]. Conversely, others have speculated that MMR deficiency may be required for the EDM mutator phenotype to be manifested [26].

Germline mutations in *POLD1* and *POLE* cause polymerase proofreading-associated polyposis (PPAP)

Even if replication fidelity is high, some errors always escape proofreading and are then corrected by MMR [27]. In studies beginning in the late 1980s, it was found that germline mutations in four MMR genes (*MSH2*, *MLH1*, *MSH6* and *PMS2*) were causative for the hereditary colorectal and other cancers that are present in Lynch syndrome (reviewed in [28,29]). Furthermore, somatic silencing of *MLH1* expression occurs in several cancer types, notably CRC and endometrial cancer (EC). In addition, bi-allelic germline *MUTYH* mutations predispose to adenomatous colorectal polyposis and CRC through defective base excision repair. We recently identified specific germline EDMs in *POLD1* and *POLE* that are causative for the development of multiple colorectal adenomas and CRC. Since the phenotype overlaps with those who carry germline mutations in *MUTYH* and the MMR genes, we have called the disease PPAP [30,31••].

Using a combination of whole-genome sequencing of highly selected multiple adenoma patients, linkage analysis, and studies of loss-of-heterozygosity (LOH) in tumors, followed by replication in a large set of familial CRC cases [31••] we identified one germline mutation in *POLE* (p.Leu424Val) and one in *POLD1* (p.Ser478Asn) that were not present in nearly 7000 UK controls or in public databases of controls. In addition, another probably pathogenic mutation, *POLD1* p.Pro327Leu, was found in a further patient with multiple adenomas. Patients who carry EDMs in *POLE* or *POLD1* show variable phenotypes: some have tens of adenomas that do not appear to progress rapidly to cancer, whereas others have a small number of large adenomas or early-onset carcinomas, thus resembling Lynch syndrome. Interestingly, female carriers of *POLD1* p.Ser478Asn have a greatly increased risk of EC. Segregation analysis confirmed a dominant, high-penetrance predisposition to colorectal adenomas. Smith *et al.* have subsequently proposed an additional predisposing *POLE* mutation outside the exonuclease domain [32].

Although there are several single nucleotide polymorphisms (SNPs) located at conserved sites within the polymerase or exonuclease domains of *POLE* and *POLD1*, genome-wide association studies and a few targeted studies have found no associations with cancer risk to date [33–38]. However, a common polymorphism within *POLD3* has been found to be associated with an increased risk of CRC in the general northern European population [39], although the mechanism of action is unknown.

Somatic mutations in *POLD1* and *POLE*

Until recently, several studies had suggested the presence of pathogenic somatic DNA polymerase mutations in cancer, but these studies were too small to show true functionality, many cancers were MMR-deficient (and hence had a high background mutation rate), and EDMs were not distinguished from other polymerase mutations. The relatively-recent Cancer Genome Atlas (TCGA) exome sequencing project has provided the best evidence for *POLE* being the target of recurrent somatic mutations in MMR-proficient, but 'ultramutated' CRCs [40••]. Further analysis showed that the mutations causing the ultramutator phenotype were all EDMs [31••,40••,41]. In the initial TCGA cohort, there were 7 *POLE* non-synonymous EDMs out of a total of 226 CRCs (3%). All of these cancers were microsatellite-stable (i.e. *prima facie* having normal MMR). Although the germline p.Leu424Val change was absent, two recurrent changes were found, p.Val411Leu and p.Ser459Phe. In addition a further recurrent *POLE* EDM, p.Pro286Arg, was found by a different CRC exome sequencing project [42]. No equivalent *POLD1* mutations have been reported for CRC. One possible explanation is that Pol ε and Pol δ act independently in different cells and various cancers might have differential mutational hotspots in oncogenes and tumor suppressors that are replicated from different polymerases [43,44].

Due to the fact that *POLD1* germline mutations predispose to EC, we looked for somatic *POLE* and *POLD1* mutations in sporadic ECs. We found *POLE* EDMs in about 7%