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The overexpression of specialized DNA polymerases in cancer

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Abstract

Specialized DNA polymerases are required to bypass DNA damage lesions that would otherwise cause replication arrest and cell death. When operating on non-canonical templates, such as undamaged DNA or on non-cognate lesions, these polymerases exhibit considerably reduced fidelity, resulting in the generation of mutations. Ectopic overexpression of these polymerases can also lead to an increased mutation rate and an enhanced capability of DNA repair, suggesting that they could potentially act as oncogenes if they were overexpressed in cancers. Here, we examine expression patterns of DNA polymerases in matched normal and tumor samples from a diverse range of tissues. As well as investigating the specialized polymerases β, λ, ι and κ, we also investigate the expression of the replicative polymerases α, δ and ε. The data presented provide evidence for the overexpression of specialized polymerases in tumors, with more than 45% of the 68 tumor samples studied demonstrating greater than two-fold enhanced expression of at least one specialized polymerase. Of particular note, DNA polymerase β (pol β) was found to be overexpressed at both the mRNA and protein level in approximately one third of all tumor types studied, with overexpression being particularly frequent in uterus, ovary, prostate and stomach samples. Pols λ, and ι were also found to be overexpressed to a significant extent in a range of tumor types, albeit less frequently than pol β. In contrast, pol κ was rarely found to be overexpressed in tumors but was found to be commonly underexpressed in many samples. Downregulation of pol β expression by siRNA resulted in an increased sensitivity to the chemotherapeutic agent cisplatin, suggesting a role for this polymerase in providing tolerance to cisplatin-induced damage. These observations suggest that specialised DNA polymerases, and particularly pol β, could be considered both as caretaker genes altered during tumorigenesis, and as potential drug targets to sensitise tumors to chemotherapy.
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Keywords: DNA polymerase; Translesion synthesis; Polymerase beta; Iota; Lambda; kappa

1. Introduction

The processes that drive evolution are the same as those that give rise to cancer in man. Genetic diversity generated by mutagenesis provides the basis for natural selection. Likewise, multiple genetic changes [1] followed by clonal selection [2] are required for the generation of tumors. Hanahan and Weinberg [3] suggested that six characteristics need to be acquired for tumorigenesis to take place. With a spontaneous rate of mutation in normal cells estimated to be in the region of 10^{-9} per base pair per cell division [1], the likelihood of a single mammalian cell acquiring all six of these characteristics is negligible. A strong case can therefore be made for the 'mutator phenotype' hypothesis, first proposed some thirty years ago [4]. This hypothesis predicts that a very early event in tumorigenesis is the generation of a mutation that controls the fidelity of DNA replication and/or the efficacy of DNA repair. Cancer susceptibility genes in this class have been described as 'caretakers' by Kinzler and Vogelstein [5], with those involved in regulating cellular proliferation rates being described as 'gatekeepers'. The initial 'mutator' or caretaker mutations can in turn increase the likelihood of alterations in additional genes involved in genome maintenance, thus resulting in a cascade of mutations throughout the genome that under selective pressure may result in the development of malignant cancer cells.

Abbreviations: TLS, translesion synthesis; CPD, cyclobutane pyrimidine dimer; XP-V, xeroderma pigmentosum-variant; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

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An explanation for the molecular mechanism underlying DNA replication fidelity has previously been described in terms of two separate processes, namely DNA polymerization with an associated proof-reading activity and DNA repair involving the mismatch repair pathway [6]. In mammalian cells, genomic replication is performed with high fidelity and rapidity by the B family DNA polymerases (pols) α, δ and ε, with pol α being distributive and associated with primase activity and both pols δ and ε being processive enzymes that possess proof-reading activity [7]. However, this rapid and high fidelity replication comes at a price. Due to the rigid structural constraints required for correctly matched base pairs, any unusual DNA structures caused by DNA damage lesions or adducts are not accepted in the active site of the replicative DNA polymerases and act as replication blocks that must be bypassed if the cell is to survive.

In recent years, a number of specialized DNA polymerases of the X and Y families have been identified [8,9]. These polymerases, which are distributive rather than processive, appear to have greatly reduced steric constraints that allow them to bypass even severely distorted DNA structures in a process termed translesion synthesis (TLS) [10–13]. The specialized DNA polymerases are characterized by their ability to bypass cognate lesions or classes of lesions and maintain a high degree of genetic fidelity by incorporating the nucleotide that would normally pair with the undamaged version of the base [13,14]. A good example of this is provided by the product of the *POLH* gene, polymerase eta (pol η), which can efficiently and accurately copy a DNA template containing a cyclobutane pyrimidine dimer (CPD), a major UV-induced lesion that distorts DNA and blocks the replicative polymerases [15]. Evidence that pol η plays an important role in maintaining genetic fidelity comes from *xeroderma pigmentosum-variant* (XP-V) patients who lack functional pol η and have a substantially increased susceptibility to sunlight-induced cancer [16,17].

Thus, under normal circumstances, specialized polymerases can be considered as agents that promote genomic stability. However, it is clear that trans-lesion synthesis needs to be a highly regulated process because when copying non-cognate lesions or undamaged DNA the specialized polymerases have been shown to exhibit reduced genetic fidelity. For example, both gene inactivation (as in the case of pol η functional loss in XP-V [16]), or modest overexpression (as shown for two-fold or higher levels of pols β and κ [18–20]), result in enhanced mutagenesis that could lead to or aggravate cancer. In addition to a role in mutagenesis, overexpression or increased activity of specialized polymerases could also result in enhanced TLS capability, allowing cancer cells to better cope with the high stress environment that results from increased replication rates and higher levels of oxidative damage. Increased TLS potential could also provide cancer cells with an advantage in coping with the DNA damage resulting from chemotherapeutic assault.

Since both of these phenotypes (increased mutagenic background rate and increased tolerance to DNA damage) could be predicted to provide cancer cells with a selective advantage, we were interested in determining whether or not overexpression of specialized polymerases represented a frequent event in cancer. We investigated this possibility by examining the expression levels of four specialized polymerases, two X family (pols β and λ) and two Y family (ι and κ) members in clinical cancer specimens in order to determine if deregulation of any or all of them was observed. We present data indicating that deregulation of specialized polymerases, and in particular overexpression of pol β, is indeed a common event in tumorigenesis, suggesting that these polymerases could potentially represent a new family of oncogenic proteins.

2. Materials and methods

2.1. cDNA probes

All cDNA probes used were amplified by RT-PCR from HeLa cell RNA as detailed in Table 1. PCR fragments were cloned into pGEM T Easy (Promega) and fully sequenced to confirm that no errors were introduced by the RT-PCR process.

2.2. Matched tumor:normal expression cDNA arrays

Matched tumor:normal expression arrays consist of cDNA representing the transcriptomes of normal or tumor clinical samples, derived from full length mRNAs that have undergone limited rounds of amplification in order to preserve their relative abundances [21]. During this procedure, double stranded cDNA is tailed with a SMART oligonucleotide (sequence given in Table 1). Details of patient records and pathological characterization of tumors on the array is available from Clontech Laboratories Inc. on (http://www.bdbiosciences.com/clontech/techinfo/manuals/PDF/7840-1.pdf). cDNA probes were labeled with α-[^{32}P]-dCTP using a RediPrime II random priming kit (Amersham). Oligonucleotide probes were 5′ end-labeled with γ-[^{32}P]-ATP (Roche). Hybridization with the matched tumor:normal expression array (PT-3424-1, Clontech) was performed in ExpressHyb solution (Clontech) at 65 °C overnight, according to the manufacturer's instructions. Oligonucleotide

Table 1
DNA probes used for hybridization experiments

Gene	Probe	GenBank accession number	Region (nucleotides)
POLB (β)	cDNA	NM_002690	114–1121
POLL (λ)	cDNA	AJ131890	372–2099
POLI (ι)	cDNA	AF140501	65–2212
POLK (κ)	Oligo	NM_016218	1583–1616
POLA (α)	cDNA	NM_016937	3214–4415
POLD (δ)	cDNA	NM_002691	2670–3380
POLE (ε)	cDNA	NM_006231	5447–6902
GAPDH	cDNA	BT006893	554–980

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