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The human *POLH* gene is not mutated, and is expressed in a cohort of patients with basal or squamous cell carcinoma of the skin

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Abstract. Skin cancer, the most common cancer in the general population, is strongly associated with exposure to the ultraviolet component of sunlight. To investigate the relationship between DNA damage processing and skin tumour development, we determined the *POLH* status of a cohort of skin cancer patients. The human *POLH* gene encodes DNA polymerase η (polη), which normally carries out accurate translesion synthesis past the major UV-induced photoproduct, the dithymine cyclobutane dimer. In the absence of active polη in xeroderma pigmentosum variant (XPV) patients, mutations accumulate at sites of UV-induced DNA damage, providing the initiating step in skin carcinogenesis. Forty patients diagnosed with skin cancer were genotyped for polymorphisms in the *POLH* protein-coding sequence, using glycosylase-mediated polymorphism detection (GMPD) and direct DNA sequencing of *POLH* PCR products derived from white blood cell genomic DNA. All individuals carried the wild-type *POLH* sequence. No *POLH* mutations were identified in genomic DNA from skin tumours derived from 15 of these patients. As determined by RT-PCR, *POLH* mRNA was expressed in all normal and skin tumour tissue examined. Polη protein was also detectable by Western blotting, in two matched normal and skin tumour extracts. An alternatively spliced form of *POLH* mRNA, lacking exon 2, was more readily detected in skin tissue than in white blood cells from the same patient. Real-time PCR was used to quantify *POLH* expression in matched normal and skin tumour-derived mRNA from a series of patients diagnosed with either basal or squamous cell carcinoma. Compared to matched normal skin tissue from the same patient, 1 of 7 SCC, and 4 of 10 BCC tumours examined showed at least a 2-fold reduction in *POLH* expression, while 1 of 7 SCC, and 3 of 10 BCC tumours showed at least a 2-fold increase in *POLH* expression. Differences in gene expression, rather than sequence changes may be the main mechanism by which *POLH* status varies between normal and skin tumours in the population under investigation. Knowledge of the *POLH* status in skin tumours could contribute to an understanding of the role of this gene in the development of the most common cancer in the general population.

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Introduction

Both genetic and environmental factors play a role in the complex etiology of cancer (1). Genes involved in repair and processing of damaged DNA play an important role in cancer susceptibility (2). In the rare genetic disease xeroderma pigmentosum (XP), mutations in genes required for repair or replication of ultraviolet light-damaged DNA lead to a greatly increased incidence of basal and squamous cell carcinoma, as well as malignant melanoma, following exposure to ultraviolet light (3). In XP complementation groups A through G, inactivating mutations in genes encoding proteins in the nucleotide excision repair pathway, lead to an increased frequency of mutations in the genome, and ultimately to cancer. In the variant form of XP (XPV), skin cancer susceptibility results from a mutation in the *POLH* gene (4,5). The human *POLH* gene, located on chromosome 6p21.1-6p12, consists of 11 exons, of which exon 1 is untranslated (4-6). *POLH* encodes DNA polymerase η (polη), a member of the Y family of specialised DNA polymerases, that also includes pol ι, κ, and Rev1. Polη normally carries out accurate replication (translesion synthesis) of UV-damaged DNA; by insertion of two adenines opposite the major UV-induced lesion, the dithymine cyclobutane pyrimidine dimer, the frequency of UV-induced mutations in skin cells is reduced (7). Polη may also be involved in bypass of the other major UV-induced lesion, the [6-4] photoproduct (8). Polη reduces the frequency

of mutations that occur during replication of UV-damaged DNA. In the absence of active polη in XPV patients, replication of UV-damaged DNA is carried out by additional DNA polymerases, such as polymerase ς (REV3) or pol ι, that frequently misinsert a base at the lesion site, greatly increasing the incidence of mutations in XPV cells (9-11). Mouse strains lacking polη are also skin cancer-prone (12).

Most of the mutations in *POLH* identified in XPV patients result in production of a truncated, non-functional, form of polη (4,5), although a number of point mutations have also been described (13). *POLH* mRNA consists of a full length transcript, as well as an alternatively spliced transcript, lacking exon 2 that is expressed most prominently in human testis, and in foetal liver (14,15). The alternatively spliced transcript does not produce functional protein (14). Given the role of polη in preventing mutagenesis and the extreme skin cancer susceptibility of XPV patients lacking this protein, alterations in the *POLH* sequence or in gene expression that reduce but do not eliminate polη activity, could contribute to the development of skin cancer.

Reduced polη expression could influence the extent of translesion synthesis and mutation fixation in sun-exposed skin cells. Conversely, given the error-prone polymerase activity of polη on undamaged DNA (16), overexpression of polη in skin tumours could contribute to genome instability and to the accumulation of mutations. There is evidence that the status of error-prone DNA polymerases is altered in cancer. For example, expression of polß is increased in a number of tumours and tumour cell lines (17-20). Polß mRNA was found be elevated in almost half of tumour tissues compared to matched normal tissue (21). DNA pol κ is overexpressed in non-small cell lung cancer (NSCLC) but not in matched normal lung tissue (22). DNA pol ι, which shows strong homology to the *POLH* gene product polη, is overexpressed in a number of human lung cancer cell lines (23). In addition, breast cancer cells that overexpress pol ι show increased UV-induced mutagenesis, and extracts of such cells show reduced replication fidelity (24).

However, despite its association with skin cancer, relatively little is known about the status of *POLH* in skin tumours (25). In the present study, the *POLH* genotype and mRNA expression pattern was analysed in a cohort of skin cancer patients. To determine whether sequence changes in the *POLH* gene are associated with skin cancer development, white blood cell genomic DNA isolated from 40 skin cancer patients was screened for polymorphisms in all 10 protein-coding exons of *POLH*. Genomic DNA isolated from 15 individual skin tumours was analysed for mutations in *POLH*. We also demonstrate, using RT-PCR that *POLH* mRNA is expressed in all normal and skin tumour tissue examined, and use real-time PCR to compare mRNA expression between matched normal and skin tumour tissue.

Materials and methods

Patient cohort and tissue collection. Surgically removed skin tumour, adjacent normal skin tissue and white blood cells were obtained from patients attending an outpatient clinic of the Department of Plastic, Reconstructive and Hand Surgery, University College Hospital, Galway (UCHG), Ireland. Collection of human tissues was based on informed consent, under a protocol approved by the Research Ethics Committee, UCHG. A portion (5-20 mg) of tumour tissue was isolated by punch biopsy and immediately frozen, from patients undergoing surgery to remove the skin lesion. Where indicated, matched normal skin tissue was isolated from 0.5 to 1 cm away from the tumour. Normal skin was diagnosed as being free of skin cancer.

Analysis of POLH genomic DNA sequence. For *RAD30* genotyping, blood samples were taken from 40 patients (26 male, average age 70.3 years, range 45-94 years; 14 female, average age 57.9 years, range 19-85 years). Diagnoses, confirmed by the Department of Pathology, UCHG, were: 13 SCC, 12 BCC, 9 seborrhoeic keratosis (SK), 2 actinic keratosis (AK), 2 Bowen's disease, 1 keratocanthoma (KA), 1 lentigo maligna (LM). Skin tumour tissue was also obtained from 15 of these patients (12 male, 3 female). The diagnoses of this subset of patients were: 8 SCC, 5 BCC, 1 SK and 1 KA. Genomic DNA was extracted from white blood cells and skin tumour tissue using the Wizard genomic DNA purification kit (Promega, Madison, WI, USA). Exons 2 through 11 of *POLH* were amplified from genomic DNA, using primer sequences derived from (26), or designed based on *POLH* DNA sequence (Table I). The *POLH* gene was analysed for mutations using the glycosylase-mediated polymorphism detection (GMPD) protocol (27,28), and by direct sequencing. GMPD is based on the incorporation of uracil opposite adenine during the PCR. Sequence changes are observed as extra or missing bands upon cleavage of the PCR products at uracil residues using uracil-DNA glycosylase, and separation of the resulting fragments by denaturing gel electrophoresis (27,28). Using this method the majority of sequence changes can be detected as fragment length polymorphisms, since 10 of 12 possible base changes involve either adenine or thymine residues (27). Prior to the use of GMPD in the analysis of genomic DNA from skin tumours, the protocol was validated by demonstrating that this approach could be used to detect known *POLH* mutations, in exons 2, 4 and 5 in genomic DNA derived from the XPV cell lines XP30RO, XP2CH and XP7TA, respectively. Each of the 10 protein coding exons of *POLH* was amplified using primer pairs designed to include amplification of the entire exon, including the intron-exon borders. Exon 11 was amplified using 3 separate primer pairs. Forward primers were labelled with 5' TET, and reverse primers with 5' HEX. PCR amplification was carried out in the presence of dUTP, followed by GMPD using uracil-DNA glycosylase cleavage to detect sequence changes (27). Fragments were separated on an ABI377 DNA sequencer, and fragment lengths were analysed using GeneScan™ analysis software. Following GMPD, any PCR products that showed even minor alterations in the fluorescent peak pattern, as detected using GeneScan software, were re-analysed using commercial sequencing services (Lark Technologies, Essex, UK). Independently, PCR products corresponding to exons 2, 4 and 8 derived from matched tumour and WBC DNA from 10 individual patients were analysed by direct DNA sequencing (Lark Technologies) without prior GMPD analysis. *POLH* sequences were analysed by alignment with published *POLH* sequences (GenBank accession numbers: AB037999, AB038001 and AB038005).

Table I. Primer sequences for amplification of *POLH* exons from genomic DNA.

Primer name	Exon	Oligonucleotide sequence (5' to 3')	Product size (bp)
C2 forward[a]	2	CTGGCATTTTGGATTAGGTG	312
D2 reverse[a]		TGGGACAACAGAGAAAGGC	
BF3 forward[b]	3	TTATGAAGCTCGTGCATTTGG	191
AF3 reverse[b]		ACCAGTTCCTAGCCTCATGG	
C4 forward[a]	4	GCAGACAATCTCAAGGTTGC	314
D4 reverse[a]		CAGGAAGCAGAAGCAGTATG	
C5 forward[a]	5	GGTCTTCTAAGCTGGCTGCC	427
D5 reverse[a]		ACATAGGCGTGCGAAACTGC	
C6 forward[a]	6	TGGGGATGTTGTGGGATACC	398
D6 reverse[a]		TATGTATTTCCCCTGGCTC	
C7 forward[a]	7	TCCTGAACCTTTTGGAGAG	391
AF7 reverse[b]		GGGCCATAGTGACCCAAGAT	
BF8 forward[b]	8	GCTGGTCTTATTAGGTCTTG	183
AF8 reverse[b]		GACCCCTTTAAAAGAGGTAG	
C9 forward[a]	9	TATGGGGAGTCTTTGGTGC	267
D9 reverse[a]		TTGTTCCTGCTCCATAAGG	
BF10 forward[b]	10	CCATTGTCACCCTGGTTCTT	388
D10 reverse[a]		AATTTGAGTGATTAGACACAATGAGG	
C11 forward[a]	11a	CATCTATGGATTAAATCTGTCCTATGG	413
AF11 reverse[b]		AGCCTGAGTGGGAGCAGTAA	
BF11 forward[b]	11b	AGGCTCCCATGAGCAATTC	396
DF11 reverse[b]		ACTTCTCACAGGGCACTTGG	
CCF11forward[b]	11c	CCAAGTGCCCTGTGAGAAGT	430
RF11 reverse[b]		ATGGGACCGTAACTCAGCAC	

[a]Primer sequences derived from Yuasa *et al* (6). [b]Primers designed based on *POLH* DNA sequence (GenBank accession numbers: AB037998; AY388614; AB038000; AB038005; AB038008).

Total RNA extraction and RT-PCR. Total RNA from skin tumour and normal tissue was extracted using the RNeasy Protect™ mini kit (Qiagen Corp.). RNA extraction from blood samples was performed using the PreAnalytix PAXgene system (Qiagen) according to the manufacturer's instructions. The quality of the total RNA was confirmed based on the integrity of 28s and 18s rRNA following agarose gel electrophoresis. PCR primers spanning exon 1 to exon 4 of *POLH* and incorporating an alternative splice site (14) were used to amplify 2 PCR products of 358 bp and 218 bp, derived from the full-length and alternatively spliced transcripts, respectively. PCR amplification was carried out in a 50 μl reaction volume containing 1 μl cDNA, 1.25 U *Taq* polymerase, 50 pmol of each primer [forward 'PolF' 5' GCCAG GTGTTTGTTACCTTGA 3', reverse 'PolR' 5' GCCAGGT GTTTGTTACCTTGA 3'], 2.5 mM $MgCl_2$, and 0.2 mM each dNTP. PCR conditions were: 94˚C for 40 sec, 48˚C for 40 sec, 72˚C for 40 sec, for 35 cycles. To confirm that the PCR products were derived from *POLH*, the 358-bp and 218-bp amplified products were isolated from an agarose gel, and analysed by direct DNA sequencing (Lark Technologies, UK).

Real-time PCR. Real-time PCR reactions were carried out in 20 μl of 1X Lightcycler-FastStart enzyme/reaction mix (Roche Diagnostics GmbH, Germany), containing 10 pmol of each primer (forward 5' ATCATGGAAGGGTGGTGGAAT 3', reverse 5' TGGCTTCCCGGTACTTGG 3'), 4 pmol *POLH* Taqman probe (6FAM-TTAGCTTTCCCACGGGACTCA CGA). Primer and probe sequences were designed by TIB Molbiol (Germany). The primers spanned *POLH* exons 3 and 4, and generated a single PCR product of 167 bp. The PCR conditions were: 95˚C for 10 sec, 55˚C for 10 sec and 72˚C for 5 sec, for 45 cycles. ß-actin expression was used as a standard to normalise *POLH* expression. Reaction conditions remained the same except that 1.7 pmol of each ß-actin primer (forward 5' AGCCTCGCCTTTGCCGA 3', reverse 5' CTG GTGCCTGGGGCG 3') and ß-actin Taqman probe (6FAM-CCGCCGCCCGTCCACACCCGC) was added to the reaction. One cDNA preparation was generated from each matched

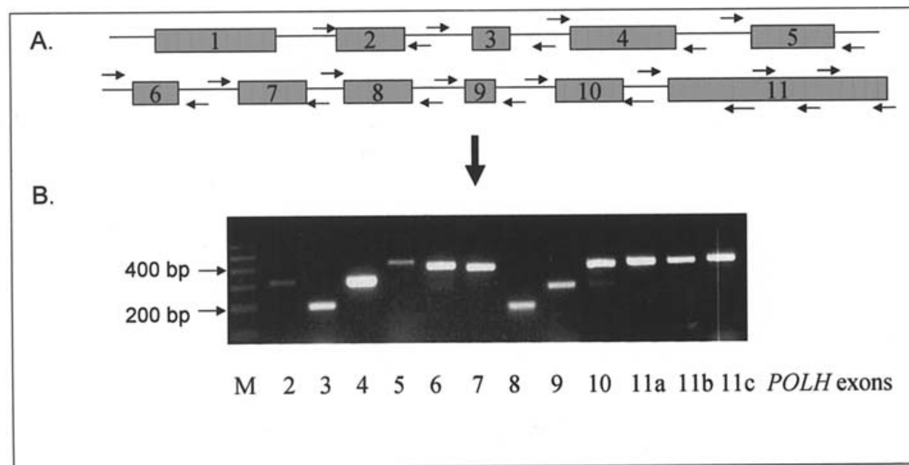

Figure 1. (A) Schematic diagram of the amplification of 10 protein coding exons of human *POLH*. (B) Agarose electrophoresis of individual *POLH* exons amplified by PCR from human genomic DNA isolated from GM00637 cells. Left lane, 100 bp DNA marker; individual *POLH* exons numbered 2, 3, 4, 5, 6, 7, 8, 9, 10, 11a, 11b and 11c, respectively. Exon 11 was completely amplified in three separate PCR reactions.

normal and tumour tissue sample, and duplicate assays were carried out. Standard curves for *POLH* and ß-actin were generated by serial dilutions of external standards. Results were analysed using Lightcycler version 3.5 software, and Lightcycler Relative Quantification™ (RelQuant™) software version 1 (Roche Diagnostics).

Immunoblotting. Proteins were extracted from tumour and normal tissue, and from normal (GM00637) and XP30RO fibroblasts using the PARIS protein extraction system (Ambion), used according to the manufacturer's instructions. Protein (20 μg) was resolved on 8% SDS-polyacrylamide gels and transferred onto nitrocellulose membrane. Polη expression was detected as described (29) using anti-polη antibody B-7 (1:100 dilution, Santa Cruz Biotechnology).

Results

POLH genotyping in skin cancer patients. In order to determine the *POLH* genotype of the skin cancer patient cohort, both tumour tissue and blood samples were collected from 15 skin cancer patients, while blood samples alone were collected from an additional 25 patients. A portion (5-20 mg) of tumour tissue was isolated by punch biopsy and immediately frozen, from patients undergoing surgery to remove the skin lesion. Using 5-TET, HEX or FAM fluorescently-labelled primers in exon-specific PCR reactions (Table I), all 10 coding exons of *POLH* were individually amplified from purified genomic DNA (Fig. 1). Single base changes in *POLH* sequence can be detected using glycosylase-mediated polymorphism detection (GMPD; 25,27), a fragment length polymorphism assay where sequence changes are revealed as gain or loss of fluorescent peaks on an automated sequencer. This was confirmed by the presence of an additional peak in the profile derived from exon 4 of the XP1CH cell line (Fig. 2) which contains a C to T transition at nucleotide position 376 of the *POLH* gene (4,5). The mutations present in *POLH* in XP30RO and XP7TA cells (4,5) were also readily detected using GMPD (data not shown). Individual *POLH* exons were therefore screened for sequence changes using (GMPD). Direct DNA sequencing was carried out on any PCR products that showed even minor alterations in the GMPD peak profile. In addition, PCR products from exons 2, 4 and 8, derived from both white blood cells and skin tumour tissue from 10 patients, were sequenced directly, without pre-screening using GMPD. Sequences were aligned with published *POLH* sequences (Genbank accession numbers: AB037999, AB038001 and AB038005) using ClustalW. The *POLH* gene was found to be wild-type in all patients with sporadic skin tumours examined. Overall, ~164,000 nucleotides of human genomic DNA were analysed for sequence changes in *POLH*, and neither polymorphisms nor sporadic mutations in the tumours were detected.

Expression of POLH mRNA in skin tumours. To date, there have not been any investigations of *POLH* expression in skin tumours. We have analysed *POLH* mRNA expression in skin tumours using RT-PCR. *POLH* transcripts include an alternatively spliced mRNA that lacks exon 2 (14). To determine whether the alternately spliced form of *POLH* is expressed in skin tumours, the primers Pol F and Pol R, spanning exon 2, were used to amplify a full-length 358 base-pair (bp) product, and the alternatively spliced 218 bp PCR product, lacking exon 2. Full-length *POLH* mRNA is expressed in all WBCs, skin tumours and normal skin tissue examined (Fig. 3). However, the alternatively spliced form of the *POLH* transcript are more readily detectable in skin tumours and normal skin tissue (where examined) than in WBCs from the same patient (Fig. 3). DNA polη protein is also expressed in matched normal and tumour tissue samples, as shown by Western blotting of two skin tissue extracts, using an anti-polη antibody. A band corresponding to 78 kDa was detectable in both normal and tumour tissue extracts; this represents endogenous human DNA polη as the corresponding 78 kDa band was not present in cell extracts derived from the XP30RO cell line that lacks polη (Fig. 4).

Quantification of POLH mRNA expression using real-time PCR. Since end-point RT-PCR is not sensitive to small

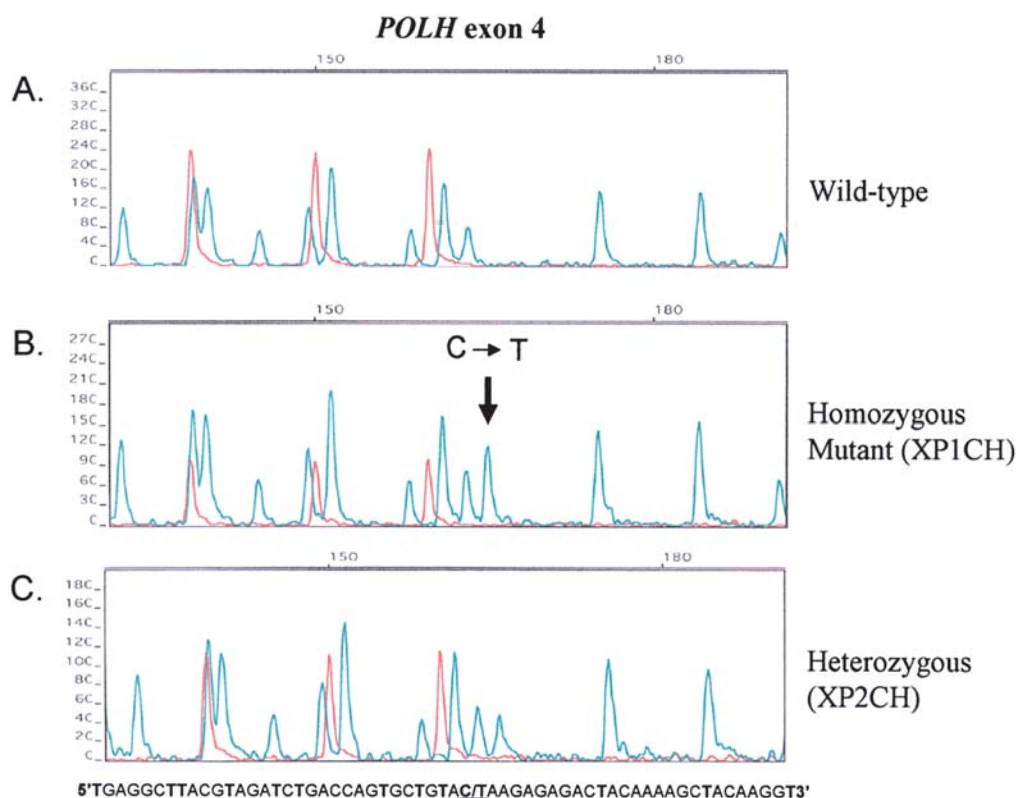

Figure 2. GMPD profiles of 59 bp of exon 4, forward strand from: (A) wild-type, XP7TA cells; (B) homozygote for C→T transition, XP2CH cells, and (C) heterozygote, XPHICH cells. The relevant DNA sequence in the profile is shown below the panels. The positions of thymine bases generating peaks in the GMPD profile are highlighted in bold in the sequence. The additional peak generated as a result of the C→T transition in XP2CH cells is arrowed (middle panel).

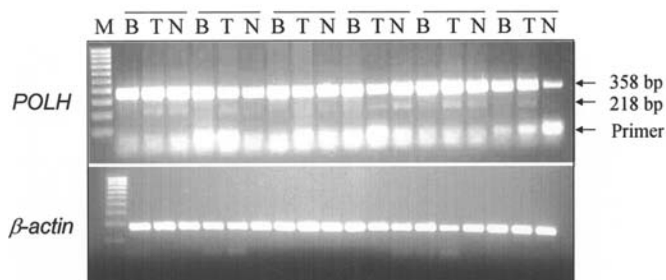

Figure 3. (A) RT-PCR amplification of cDNA generated from matched samples (B, WBCs; T, tumour tissue; N, normal tissue) from 6 patients. Lane M, 100 bp DNA marker; lanes 1, 2, 3, *POLH* amplified from WBCs, tumour tissue and normal tissue respectively from same patient (BCC); lanes 4, 5 and 6, WBCs, tumour tissue and normal tissue respectively from patient (sebaceous hyperplasia); lanes 7 to 18 (SCC, malignant melanoma, BCC and SCC, respectively). (B) RT-PCR amplification of ß-actin cDNA (174 bp) generated from WBCs, tumour and normal tissue, as in A.

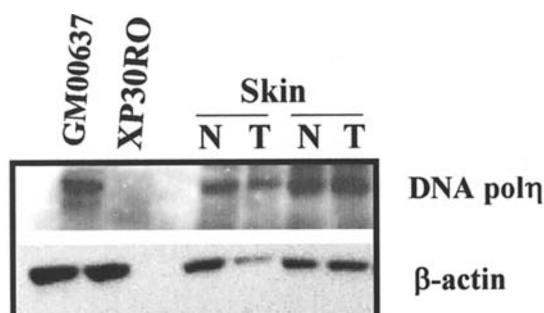

Figure 4. Polη expression in human skin tissue. Total cell extract (20 μg) from matched normal and skin tumour tissue was electrophoresed in an 8% SDS-PAGE gel. GM00637, normal human fibroblast; XP30RO, XP variant cell line lacking polη. Western blotting was carried out using anti-polη antibody (upper panel), and anti-ß-actin antibody (lower panel).

differences in the levels of mRNA, *POLH* mRNA expression was further analysed using real-time PCR. Using primers optimised for amplification of a PCR product spanning *POLH* exons 3 and 4 (Pol S, forward primer, and Pol A, reverse primer) and *POLH* Taqman probe (Pol P, labelled with LC-Red 640), *POLH* expression was quantified following real-time PCR using Roche Diagnostics Lightcycler Relative Quantification software version 1. ß-actin expression was used as a standard to normalise *POLH* expression between samples. The identities of the *POLH* and ß-actin PCR products (Fig. 5) were confirmed using direct DNA sequencing (Lark Technologies). The real-time PCR reaction was linear over a wide range of concentrations of the target sequences (Fig. 5), and was sensitive to differences in *POLH* mRNA expression, as shown by differences in *POLH* expression between several human cell lines relative to GM00637 human fibroblasts (Fig. 6). Consistent with previous studies where *POLH* mRNA was analysed by Northern blotting (4), *POLH* expression was found to be greatly reduced in the XP variant cell line XP7TA (Fig. 6).

To determine whether *POLH* expression was increased or decreased in skin cancer, *POLH* expression was compared

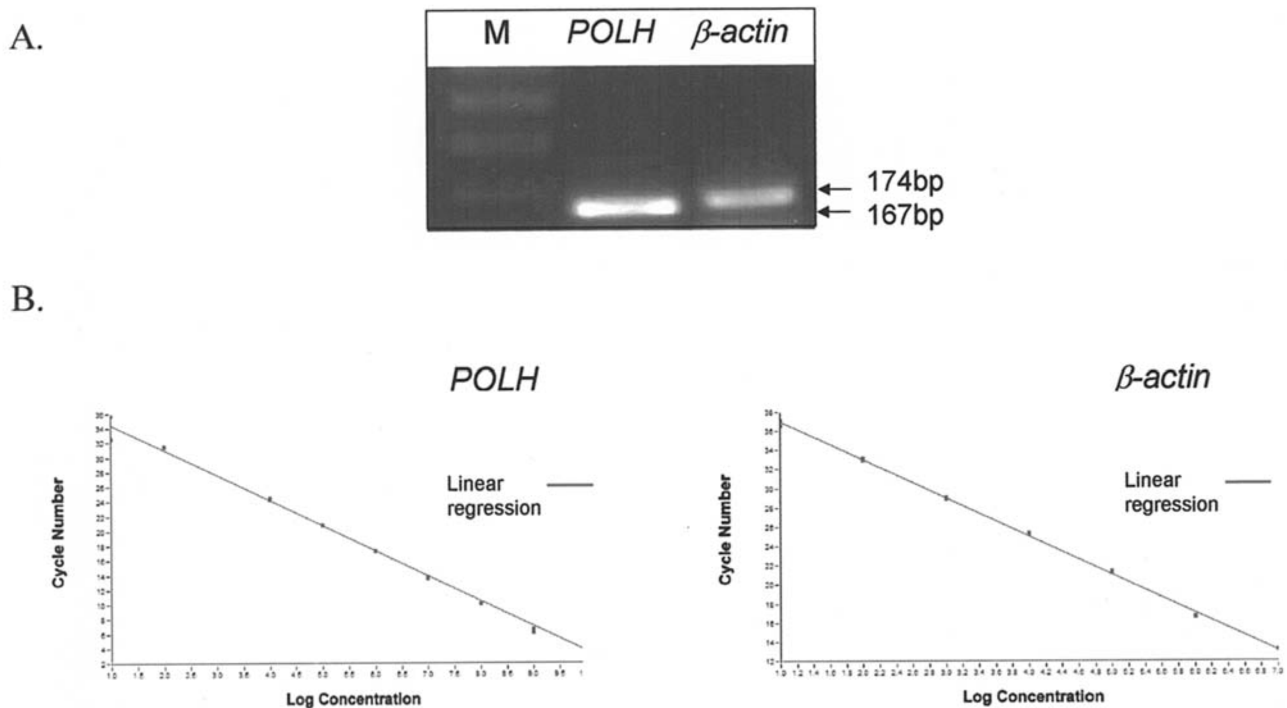

Figure 5. (A) Real-time PCR products generated from cDNA amplified from normal fibroblast RNA. Lane M, 100 bp DNA marker; lane 2, *POLH*, lane 3, ß-actin. (B) Standard curves of human *POLH* and ß-actin generated using real-time PCR, using 10-fold serial dilutions of a 167-bp and 174-bp fragment, respectively.

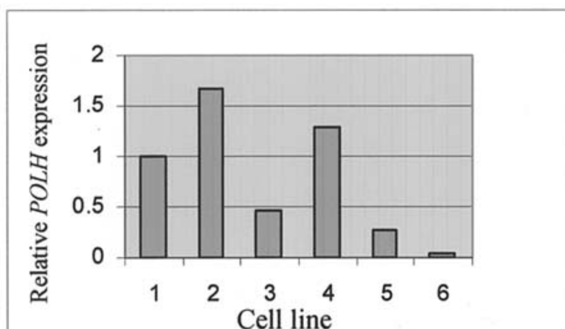

Figure 6. Relative expression of *POLH* mRNA in a series of human cell lines. Expression was normalised to the expression in human GM00637 normal fibroblasts, determined using real-time PCR. Cell lines are numbered: 1, GM00637; 2, HeLa; 3, XP1CH; 4, XP30RO; 5, XP2CH; 6, XP7TA. Data derived from a single experiment.

between a series of matched normal and tumour tissue, derived from 19 patients (comprising 10 BCC, 7 SCC, 1 malignant melanoma, and 1 benign sebaceous hyperplasia (Table II). In the case of the BCC cohort, average *POLH* expression is 1.53-fold higher in BCC compared to matched normal tissue from the same patient. However, at the level of individual patients, 3 showed at least a 2-fold increase in *POLH* expression in the tumour tissue compared to the matched normal tissue, while 4 showed at least a 2-fold decrease in *POLH* expression (Table II). Thus, *POLH* is clearly expressed in all cases of BCC examined, but expression may be either decreased or increased relative to matched adjacent normal tissue. In the case of SCC, average *POLH* expression is increased 1.45-fold compared to the matched adjacent normal tissue from the same patient. However, in this case, 1 patient showed a 2-fold increase, and 1 patient showed a 2-fold decrease in *POLH* expression (Table II). Thus, individual tumours vary in the level of *POLH* expression compared to the matched normal skin tissue.

Discussion

To characterise the role of the skin cancer susceptibility gene, *POLH*, in skin cancer incidence in the general population, we have developed methods to reproducibly amplify all 10 coding exons of *POLH* from skin tumour and white blood cell genomic DNA. Glycosylase-mediated polymorphism detection has been validated by demonstrating that a known single-base substitution in *POLH* can be readily detected using this approach, and has been used, along with direct DNA sequencing to screen for sequence changes in *POLH* in the present skin cancer cohort. No sequence changes were detected in *POLH* DNA in 40 WBCs or in 15 skin tumours examined. In this patient cohort, the *POLH* gene is not polymorphic, and no somatic mutations occur in the skin tumours. Any minor peak alterations observed in the GMPD profile represented background changes in the cleavage pattern that were revealed by the use of the high sensitivity fluorescence detection system. This result is consistent with the recent study that mutations were not detected in the *POLH* coding sequence from 17 squamous cell carcinomas from a US patient cohort (30). Thus, mutations in *POLH* appear to be infrequent in skin tumours, although this does not rule out the possibility that in rare cases, mutations in *POLH* play a role in tumour development. While a number of polymorphisms

Table II. Expression of *POLH* mRNA in matched tumour and normal skin tissues.

Tumour	Age	Gender	Relative *POLH* mRNA expression (tumour/matched normal skin)
BCC	75	M	2.37
BCC	93	F	5.87
BCC	74	M	0.49
BCC	83	M	0.35
BCC	84	F	2.31
BCC	66	M	0.25
BCC	63	M	0.70
BCC	85	M	1.55
BCC	66	F	0.50
BCC	97	F	0.93
SCC	90	F	0.21
SCC	76	M	0.60
SCC	77	M	0.82
SCC	92	M	1.23
SCC	78	M	5.42
SCC	80	M	0.86
SCC	59	M	1.00
MM	88	M	0.67
SHben	63	F	1.25

have been identified in *POLH* in a smaller patient cohort (30), none were detected in the current patient cohort; this may reflect the likely genetic homogeneity of the sample population.

POLH mRNA was expressed in all tissues, including white blood cells, BCC, SCC and MM, and matched normal skin tissues from the same patients. This is consistent with the previous study that *POLH* is expressed in all normal and cancerous lung tissues examined using end-point PCR (22). Analysis of *POLH* expression in specific cell types in skin tumours may be more informative than the total level of expression. Since many cell types are analysed in the tumours used in the present study, determination of *POLH* expression in purified cell types isolated by, for example laser capture microdissection (LCM) may also be informative. For example, while the alternatively spliced *POLH* mRNA product (14) was not as readily detected in WBC as in skin, it is possible that the alternatively spliced form detected using RT-PCR is derived from a specific cell type present in the skin sample, rather than being present in low amounts in all cells in the tissue. Using Western blotting we also found that polη protein is expressed in skin tissue, and further investigation of the levels and distribution of the protein in skin tumours may be of interest. To further analyse *POLH* mRNA expression in skin, a real-time PCR-based method was developed. Consistent with previous studies (4,5), when *POLH* expression was analysed in a series of cell lines, expression was found to be greatly reduced in XP7TA cells, compared to other XPV and non-XPV lines. Individual skin tumours showing either increased or decreased *POLH* expression relative to matched normal skin tissue were identified. There is evidence that differences in expression of specialised DNA polymerases may be important in cancer development (21). DNA pol ß, which is normally involved in repair synthesis in base excision repair (BER) and single strand break repair (SSBR) pathways (31) was over-expressed in ~50% of human tumours examined (21). Over-expression of pol ß by only 2-fold has been demonstrated to lead to genetic instability and accelerated tumorigenesis in mouse model systems, suggesting that a rigorous regulation of its expression may be essential *in vivo* (19,32,33). The expression of pol ι, another Y-family DNA polymerase, varied between cell lines, from low expression in normal cells and three lung cancer cell lines, to relatively high expression in two lung cancer cell lines, which may suggest a role in the development of lung cancers (23). Breast cancer cell lines that overexpress pol ι show increased UV-induced mutagenesis, and extracts of such cells show reduced replication fidelity (24). Furthermore, pol κ has been reported to be overexpressed in lung tumours (22) while DNA polymerase θ is upregulated in lung and colon tumours (34). Thus, further characterisation of the status of this important class of proteins in tumours, and investigation of the contribution of imbalances in the level of individual DNA polymerases to the loss of genome stability that occurs during tumour development, is warranted.

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