DNA Polymerase \( \kappa \), Implicated in Spontaneous and DNA Damage-induced Mutagenesis, Is Overexpressed in Lung Cancer\(^1\)

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Abstract

DNA polymerase \( \kappa \) (Pol \( \kappa \)) is a newly identified low-fidelity polymerase implicated in spontaneous and DNA damage-induced mutagenesis. As an initial study to investigate its possible involvement in tumorigenesis, we compared the expression level of Pol \( \kappa \) in tumors and adjacent nontumorous tissues by Northern blot, semiquantitative RT-PCR, and Western blot analyses. In this study, paired tumor and normal specimens from 29 patients with stages I to IIIb non-small cell lung cancer (NSCLC), including 13 adenocarcinomas, 15 squamous cell cancers, and 1 adenosquamous carcinoma, were analyzed, among which different levels of tumor-associated Pol \( \kappa \) overexpression were observed in 21 of 29 matched specimens. In addition, five matched specimens exhibited elevated Pol \( \kappa \) expression in both tumor and control tissues, whereas only one nontumorous tissue expressed a higher level of Pol \( \kappa \) than its tumor counterpart. The preferential up-regulation of Pol \( \kappa \) expression in tumors was highly significant \((P < 0.001)\). There was no apparent correlation of Pol \( \kappa \) expression levels with tumor histology, grade, and stage or with smoking history. Southern blot analysis did not show amplification of the Pol \( \kappa \) gene, indicating that the elevated Pol \( \kappa \) expression is likely attributable to dysregulated transcription. Our data suggest that Pol \( \kappa \) may contribute to lung tumor development by accelerating the accumulation of mutations.

Introduction

Accumulation of somatic mutations has been causally related to cancer development. Recent studies indicate that mutations can arise during the bypass of DNA damage (1–3). Specialized low fidelity polymerases are required to bypass unrepaired lesions that otherwise block replication by normal polymerases. Recently, a number of such lesion-bypassing polymerases have been identified in mammalian cells, including Pol \( \xi \), Pol \( \eta \), Pol \( \iota \), Pol \( \kappa \), and Rev1 (1–3). Either error-free or error-prone bypass can be accomplished depending on the polymerases used, the type of lesions encountered, and the sequence context of the lesions. Errors, however, seem to occur in most cases and result in mutagenesis. Interestingly, deficiency in Pol \( \eta \), which is able to correctly bypass UV-induced cyclobutane thymine dimers, causes xeroderma pigmentosum variant, a genetically inherited disorder highly susceptible to sunlight-induced skin cancers (4, 5). Although this finding illustrates the significance of this class of polymerases in human disease, the biological functions of the other lesion-bypassing polymerases are still poorly understood.

Pol \( \kappa \) is a low-fidelity polymerase with moderate processivity and is highly inaccurate when replicating undamaged DNA (6–8). Pol \( \kappa \) is the mammalian homologue of the Escherichia coli DinB (9, 10), which is required for \( \lambda \)-phage targeted mutagenesis (11). Overexpression of DinB in \( E. \ coli \) greatly increases mutagenesis in the absence of exogenous DNA-damaging agent (12). Likewise, transient expression of the Pol \( \kappa \) gene in cultured mouse cells caused a 10-fold increase in the incidence of mutations in the endogenous \( Hprt \) locus (10). In addition, Pol \( \kappa \) can carry out error-prone bypass of certain DNA lesions (6, 13), including an abasic site, \( N \)-2-acetylaminofluorene guanine adduct and 8-oxoguanine. Collectively, these results have implicated Pol \( \kappa \) in mutagenesis.

Despite the highly mutagenic properties of Pol \( \kappa \), no evidence has been provided that indicates its possible involvement in human cancer. In the present study, we have examined Pol \( \kappa \) expression in paired tumor and normal specimens from NSCLC.\(^3\) The analysis revealed elevated Pol \( \kappa \) expression in a high percentage of lung tumors in comparison with their nontumorous tissue counterparts. Our data suggest a link between Pol \( \kappa \) expression and lung cancer and implicate a role for Pol \( \kappa \) in tumorigenesis.

Materials and Methods

Tissue Specimen. Surgically resected tumors and their adjacent normal tissue (>3 cm away from the tumor) were obtained from the Chiba Cancer Center Tissue Bank (Chiba, Japan) as fresh-frozen tissues. These included tissues from 29 patients with stages I to IIIb NSCLC, of which 13 patients had adenocarcinomas, 15 had squamous cell cancers, and 1 had adenosquamous carcinoma. These tissues were resected before the patients received chemotherapy and/or radiation therapy.

RNA Extraction and RT-PCR Analysis. Surgically resected tissue (~100 mg) was homogenized mechanically in 1 ml of TRIzol reagent (Life Technologies, Inc., Rockville, MD), and the total RNA was extracted according to the manufacturer’s protocol. First-strand cDNA was synthesized from 5 \( \mu \)g of total RNA using SuperScript II reverse transcriptase (Life Technologies, Inc.) and random primers following the company’s protocol. PCR reaction was performed in 20 \( \mu \)l of buffer containing 10 mm Tris-\( HCl \) (pH 8.3), 50 mm KCl, 1.5 mm MgCl\(_2\), 200 mm each dNTP, 5 pmol each primer, and 1 unit of Taq DNA polymerase (Toyobo, Osaka, Japan). For glyceraldehyde 3-phosphate dehydrogenase gene expression, forward (\( 5' \)-ACCACAGTCCATCCATGAT-3\( ' \)) and reverse (\( 5' \)-TCCACACGGATTGTGTA-3\( ' \)) primers were used, and the amplification was performed at 94°C for 15 s, 60°C for 15 s, and 72°C for 40 s for 25 cycles. For human Pol \( \kappa \) expression, forward (\( 5' \)-GCGATGGACAGTTTTGTA-3\( ' \)) and reverse (\( 5' \)-CTCTTTTGTTG-TGTTTCTCTT-3\( ' \)) primers were used, and the reaction was performed at 95°C for 5 s, 61°C for 10 s, and 72°C for 2 min for 30 cycles. For Pol \( \xi \) expression, forward (\( 5' \)-ACCCAGGCAATCCAAAAC-3\( ' \)) and reverse (\( 5' \)-GGGTCATGTTTCTGATTGG-3\( ' \)) primers were used, and the amplification was carried out at 95°C for 5 s, 61°C for 10 s, and 72°C for 2 min for 30 cycles. For Pol \( \eta \) expression, forward (\( 5' \)-ACCCAGGCAATCCAAAAC-3\( ' \)) and reverse (\( 5' \)-GGGTCATGTTTCTGATTGG-3\( ' \)) primers were used, and the amplification was carried out at 95°C for 5 s, 61°C for 10 s, and 72°C for 1 min for 30 cycles.

Northern and Southern Blot Analyses. For Northern blot analysis, 10 \( \mu \)g of total RNA was resolved in 1% formaldehyde agarose gel and transferred to nylon membrane (Hybond-N, Amersham Pharmacia Biotech). The membrane was baked at 80°C for 1 h under vacuum and hybridized with a \( ^{32} \)P-labeled 1133-bp human Pol \( \kappa \) cDNA fragment (corresponding to nucleotides +451 to +1583 relative to the translational start codon +1). The

\(^1\) The abbreviations used are: NSCLC, non-small cell lung cancer; RT-PCR, reverse transcription-PCR; BaP, benzo(a)pyrene; Pol, DNA polymerase.

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membrane was stripped and rehybridized with an 18S RNA probe to ensure the equal loading of the RNA. For Southern blot analysis, 10 μg of high-molecular weight DNA was digested with HindIII, size-fractionated in 0.7% agarose gel, transferred to a nylon membrane, and hybridized with the same human Pol κ probe. The membrane was similarly rehybridized with a human Rev3 probe corresponding to nucleotides 1772 to 2979 of the published sequence (14).

**Western Blot Analysis.** The tissue (100 mg) was homogenized in 1 ml of lysis buffer [50 mM Tris-HCl (pH 6.8), 2% SDS, 1 mM 2-mercaptoethanol, and 10% glycerol] containing a cocktail of protease inhibitors (Sigma Chemical Co.), 1 mM phenylmethylsulfonyl fluoride, and 1 mM EDTA and then sonicated for 2 min. The lysate proteins were centrifuged at 5000 rpm for 3 min, and the supernatant was heated at 100°C for 5 min before loading to an 8% SDS polyacrylamide gel. The resolved proteins were transferred to a polyvinylidene difluoride membrane with a semidry blotter, blocked with I-block, and the supernatant was heated at 100°C for 5 min before loading to an 8% SDS polyacrylamide gel. The resolved proteins were transferred to a polyvinylidene difluoride membrane with a semidry blotter, blocked with I-block, and reacted with 0.3 g/ml of the rabbit antibodies to Pol κ and then by horseradish peroxidase-labeled goat antibodies to rabbit IgG (Life Technologies, Inc.). The membrane was developed with enhanced chemiluminescence (Amersham Pharmacia Biotech), following the company’s protocol.

**Results**

**Pol κ Expression in Tumors and Their Matched Nontumorous Tissue.** Matched specimens from 29 NSCLC patients were analyzed for Pol κ expression. The clinicopathological features of these cancers are summarized in Table 1. Northern blot analysis was performed with 21 matched specimens from which sufficient amounts of RNA were obtained. In 16 of 21 matched specimens (cases 1 through 10 and cases 12, 13, 15, 17, 18, and 20), Pol κ was overexpressed in tumors but not in their nontumorous tissue counterparts (Fig. 1A). Pol κ expression was elevated both in tumor and nontumorous tissues in case 22, whereas only faint expression could be detected in their nontumorous tissue (Fig. 1B). Pol κ expression could be detected both in tumor and nontumorous tissues in case 22, whereas only faint bands were detectable in case 25 (Fig. 2). We noted that the preferential up-regulation of Pol κ expression in tumor relative to nontumor is statistically significant (P < 0.001; Fisher’s exact test), and the overall expression pattern of the Pol κ gene is summarized in Table 1. We also examined the expression of Pol η, a related translesional DNA polymerase belonging to the same Dinh/Umuc superfamily (9, 10). In contrast to Pol κ, Pol η was expressed at similar levels in both tumor and normal specimens (Fig. 1C), indicating that the up-regulation of Pol κ expression is specific for lung cancer.

Representative matched specimens were analyzed further by Western blot analysis to evaluate the levels of Pol κ protein. Consistent with the results of Northern blot and RT-PCR analysis, the M, 96,000 Pol κ protein was observed in tumors but not in their control tissues in cases 3–5 and was undetectable in case 25 (Fig. 2). We noted that in case 2, the size of the Pol κ protein was reduced to M, 85,000. This smaller product is likely derived from alternatively spliced transcripts (9). Although in general the elevated Pol κ RNA expres-

**Table 1 Clinicopathological features of NSCLC patients**

<table>
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* According to the pTNM classification (19), all tumors being M0.
* Cases 1–21 determined by Northern blot and cases 22–29 by RT-PCR analysis.

SCC, squamous cell carcinoma; AC, adenocarcinoma.
sion was accompanied by an increase in Pol κ protein, the nontumor-
ous tissue of case 11 expressed a higher level of Pol κ mRNA (Fig.
1A) but a lower level of Pol κ protein (Fig. 2) relative to its tumor
counterpart. This suggests that Pol κ expression may be regulated by
both transcriptional and posttranscriptional mechanisms. The Mₙ
96,000 band could also be detected in COS-7 cells transfected with a
human Pol κ cDNA but not an empty vector (Fig. 2), confirming that
it is the Pol κ gene product. There was no apparent correlation
between Pol κ expression levels and specific clinicopathological
features of the NSCLC. A greater number of lung cancer specimens
are now under investigation to determine whether elevated Pol κ
expression might be related to smoking history, metastasis, and clin-
ical outcome.

Pol κ Gene Is Not Amplified. Southern blot analysis was performed
to reveal the copy number of the Pol κ gene. On the basis of the
relative band intensities between the Pol κ gene and Rev3, a
ubiquitously expressed gene (15), we concluded that the Pol κ gene
was not amplified in the tumors (Fig. 3). The Pol κ probe detected
four bands (5, 3.8, 3.3, and 2.1 kb) in all tumor and nontumorous
tissues, as well as in the control placental DNA. There were no
aberrant bands in any of the tumor tissues examined. Therefore, no
chromosomal translocations were evident with the Pol κ probe we
used.

Discussion

Despite the error-prone properties of Pol κ for both undamaged and
damaged DNA, no evidence has been provided thus far to indicate its
relevance in human cancer. The present study is the first documenta-
tion that Pol κ is overexpressed in malignant human tissues compared
with their matched normal tissue counterparts. Because Pol κ is highly
mutagenic when copying undamaged DNA, the elevated Pol κ ex-
pression might cause an increased spontaneous mutation rate. In
addition, the ability of Pol κ to erroneously bypass an abasic site,
N-2-acetylaminofluorene-adduct and 8-oxoguanine, implicates that
mutagenesis induced by these DNA lesions may be greatly enhanced.
Pol κ may thus contribute to tumorigenesis by accelerating the accu-
mulation of both spontaneous and DNA damage-triggered mutations.
Moreover, elevated Pol κ expression in the majority of NSCLCs,
irrespective of their stages, suggests its possible involvement in both
the initiation and progression of tumors.

Using the same matched specimens of NSCLC, we have recently
shown that the human Rev3 gene, which encodes the catalytic subunit
of Pol ζ, is not overexpressed in tumors (15). Whereas human Rev3
appears to be involved in UV-induced mutagenesis (14), its biochem-
ical properties for undamaged and damaged DNA have not been
defined. In addition, we have shown in the present study that the
expression of Pol η is not altered in these lung cancer cells, indicating
that the elevated Pol κ expression does not simply reflect some global
alteration associated with tumorigenesis but is specific for lung can-
cer. The lack of Rev3 and Pol η overexpression in the same NSCLC
indicates that the expression of these polymerases is differentially
regulated. Their potential roles in tumorigenesis may differ depending
on the type, origin, and tissue specificity of cancers.

The most important risk factor for lung cancer is tobacco smoking.
Among the many constituents found in tobacco smoke, BaP is the most
potent carcinogen known (16, 17). In E. coli, the induction of −1 frameshif-
ted mutations by a BaP adduct in one particular template sequence of 5′-GGGG̅BaP-3′ was shown to require DnB (18). In contrast, human Pol κ,
the homologue of E. coli DnB, was reported to bypass a different BaP
adduct in a relatively error-free manner under in vitro conditions (13).
Although additional studies are required to reveal the biochemical prop-
erties of Pol κ, it is possible that error-free or error-prone bypass of BaP
adducts by Pol κ may depend on the type of adducts and the sequence
context. It remains an interesting issue as to whether elevated Pol κ
expression may be relevant to a smoking-associated increase of lung
cancer incidence. In any case, the mutagenic properties of Pol κ for
several other types of DNA lesions, as well as its extreme inaccuracy
when replicating undamaged DNA, strongly suggest that its elevated
expression may result in enhanced mutagenesis, thereby contributing to
tumorigenesis.

It is unclear what causes the up-regulation of Pol κ expression in
such a high percentage of lung tumors and in some nontumorous
tissues. Pol κ is normally expressed at low levels in most tissues
except for the testis (9, 10). Our data indicate that the enhanced Pol κ
expression is not attributable to its gene amplification. One possibility
for the elevated Pol κ transcription is its translocation to the vicinity
of an actively transcribed gene locus. With the Pol κ probe used,
however, we could not detect apparent gene rearrangements within the
Pol κ locus, although the possibility that translocations might occur in
the 5′ or 3′ flanking region cannot be excluded. A more likely
possibility is that Pol κ gene expression may be under the control of
certain transcription factors whose expressions are dysregulated dur-
ing the course of neoplastic transformation. An intriguing possibility
is that Pol κ transcription might be directly or indirectly regulated by
the tumor suppressor p53, which is mutated in ~60% of the lung
cancers, or by the products of the Myc family of oncogenes, which are
frequently amplified and overexpressed in tumors.

Deficiency in DNA repair genes is associated with high suscepti-
bility to cancer. In this regard, DNA repair genes are tumor sup-
pressors. The highly mutagenic properties of Pol κ and its elevated
expression in tumor tissues as revealed in the present study collec-
tively suggest that Pol κ could act as an oncogene. Future studies are
required to determine the function of Pol κ in vivo, in particular its
role in tumorigenesis. It remains to be examined whether other mem-
ers of the recently identified lesion-bypassing DNA polymerases
(1–3) might also be overexpressed in human malignancies.

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obodies to human Pol κ.

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